

Dear Dr Slomp, dear reviewers,

we would like to thank all reviewers for taking the time and effort to provide us with such detailed feedback on our manuscript 'Carbon degradation in agricultural soils flooded with seawater after managed coastal realignment'. We have carefully considered all comments and our responses and suggestions on how we will address these can be found below each individual reviewer's comments. Implementing these changes based on the comments of the reviewers will greatly improve our manuscript. We would like to draw attention to the refinements of the argumentation for organic carbon preservation in soils flooded with seawater, especially by incorporating comparisons to relevant carbon degradation values described in existing literature. Furthermore, the reviewer comments have led us to clarify our description of the experimental conditions and methodology used in the study. We feel that our suggested revisions will improve our manuscript beyond the level necessary to be considered for publication in *Biogeosciences*.

Kind regards,

Kamilla Sjøgaard, Alexander Treusch and Thomas Valdemarsen

Author response comments

Interactive comment on "Carbon degradation in agricultural soils flooded with seawater after managed coastal realignment" by Kamilla S. Sjøgaard et al.

Anonymous Referee #1

Received and published: 20 January 2017

General comments:

authors: Sjøgaard et al. This manuscript evaluates the effect of flooding soils with seawater on the carbon mineralisation pathways and rates in these soils. This is clearly a relevant topic with respect to planned managed coastal realignment projects to improve coastal defences against sea level rise. The

experiment tackles an environmental issue and seems to be well designed and executed. The manuscript is well written and to the point. However, the major hypothesis (hypothesis 3: does the flooding of soils promote organic carbon preservation?), which is the core and carries the impact of this paper is not well supported (see section below). Furthermore, there are a few more issues and some technical corrections that need revision before this manuscript is ready to be accepted in BGS. These issues need to be addressed before the manuscript is ready for publication. I recommend major revisions.

Major specific comments:

1.1

- Paragraph 4.3: This paragraph is, according to me, the most important conclusion of this manuscript. If coastal soils are re-exposed to marine conditions, will they promote carbon burial and this form a negative feedback on atmospheric CO₂ concentrations? Unfortunately, this is also the least documented paragraph, and it does not provide enough evidence to valid such a strong conclusion as posed on P13L11-12 (this study suggests that the majority of soil OC will be permanently preserved . . .).

There is not data or values of pre-flooding mineralisation rates, nor a comparison to normal marine conditions. Furthermore, the TCO₂ flux of 67 mmol m⁻² d⁻¹ in the uncultivated soil measured by the end of the experiment (and the value of 239 mmol m⁻² d⁻¹ on day 13) (see section 3.2) are indications for an extremely high mineralization rate. The effluxes in the cultivated soil (29 mmol m⁻² d⁻¹) indicate normal rates for marine sediments. It is highly likely these rates are transient, and are driven by the DOC production, but this would mean that the standard soil conditions do not produce this DOC, and thus that reinstating marine conditions actually inhibits carbon burial.

Author response:

We acknowledge that pre-flooding mineralization rates would have been good to have, but we did not have the resources to conduct such measurements. In

the revised version of the manuscript we will strengthen this part of the manuscript by comparing measured mineralization rates to values available from the literature for comparable marine sediments and agricultural soils. We will add a table to make this argument clear. TCO₂ fluxes from Danish coastal marine sediments for comparison are described in Valdemarsen et al. (2010) and Valdemarsen et al. (2014) investigated mineralization rates from a Danish fjord using the same methods as in this experiment. Danish agricultural soils had CO₂ effluxes between 42 and 167 mmol m⁻² d⁻¹ (Chirinda et al. 2014), which is higher than the fluxes from our experiment at steady state.

We see a major acceleration in mineralization at UC, however this is only a short-term effect of leaching and degradation of the labile carbon constituting only a minor fraction of the TOC pool (6-7%). Our experiment indicates that the vast majority of the TOC pool will be preserved long-term after flooding with seawater. Furthermore, we believe that the final CO₂ efflux rates for UC are influenced by porewater CO₂ diffusing out of the sediment – porewater CO₂ that has accumulated in the initial phases of the experiment when mineralization rates were high. When considering the mineralization rates measured in the anoxic incubations at station UC, the final rates are lower than the TCO₂ fluxes (40-55 mmol m⁻² d⁻¹).

1.2

Hence, there seems to be no direct evidence that newly flooded coastal habitats will be hotspots for carbon burial. I propose that the authors give a stronger foundation for this paragraph, and show that re-exposure to marine conditions actually decreases the carbon mineralisation (e.g. by providing an estimate of pre-flooding mineralization rates, or by comparing the carbon burial to pre-flooding carbon burial, and normal marine carbon burial rates).

Author response:

Author response:

For reasons mentioned above we disagree with the statement that our study does not provide direct evidence for the fact “that flooded soils will become hotspots for OC burial”. In the revised manuscript we will strengthen our argumentation for this matter by including more comparisons to the literature

regarding mineralization rates in soils and marine sediments and temporal degradation patterns.

1.3

- Paragraph 4.4: FeIII is indeed efficient as being a sulphide buffer in flooded soils, however, figure 6 shows that virtually all FeIII is converted to FeII by the end of the experiment. This indicates that the FeIII sulphide-buffer was exhausted and sulphide will start accumulating after ~1 year. This should be mentioned in this paragraph, and I would also reconsider the term 'efficient buffer' when this would only be active for the time span of 1 year.

Author response:

We believe that Fe will continue buffering the sulphide production, as sulphide reacts with both oxidized and reduced forms of Fe. At the end of the experiment enough FeII was left to buffer sulphide beyond 1 year. Reviewer #2 also agreed with this (Section 4.4). We will amend the manuscript with the appropriate references, e.g. Rickard and Morse (2005) and the ones suggested by Reviewer #2, to clarify this in the relevant paragraphs of the revised manuscript.

1.4

- Paragraph 2.3: I have a few remarks/questions for the data analysis that was applied:

When calculating the slopes of the rates in the jar experiments, did you apply any outlier check?

Author response:

Yes, we conducted a check for obvious outliers.

1.5

I don't agree with the linear data interpolation that you used to correct for missing data points. In my experience, reactions rates tend to follow exponential trends rather than linear ones. If you want to use this linear interpolation, I would advise to include a small section on the possible errors you make while doing this interpolation.

Author response:

The linear data interpolation was used to estimate mineralization rates at 10-15 cm depth, based on measured mineralization rates at depths above and below. It is true that mineralization rates tend to decrease exponentially from the surface and downwards – the exponential pattern is typically very evident from the surface to a few cm depth, while the variation in mineralization rates with depth appears almost linear below. We therefore think that in this case linear interpolation in between two measured data points is a reasonable way to estimate missing data. The error of using linear contra exponential interpolation between data points will be minor since mineralization rates decrease almost linearly at the relevant depths.

1.6

The correlation you used to convert organic matter to OC units is based on only two points? How did you estimate the significance? Can you show a plot that shows the OM vs the OC, and what model you used?

Author response:

The conversion equation to convert organic matter (OM) into OC was based on 20 data points, that showed a highly significant linear OM – OC relationship in the soils [$OC(\%) = 0.0649 \times OM(\%) + 0.0936$, $r^2=0.9824$, $n=20$]

Minor specific comments:

1.7

- P1L11, P3L18: I seem to get a bit confused with the sentence structure. Was station C not in the area that was reflooded? And is the sampling area reflooded, planned to be reflooded or not planned to be reflooded?

Author response:

Both stations are in the reclaimed area, and also the area that has been flooded in the managed realignment. What we tried to explain is that station C, which is agricultural soil, is representative for the majority of the area, while station UC only represent a minority of the area (reedswamp). This is also visualized in figure 1.

As this did not become clear, we will re-phrase the sentence on P3L18 to “Station C however, resembled the majority of the re-flooded area that was

farmed since the land reclamation (fertilized, ploughed and used for monoculture, also illustrated in Fig. 1)”

1.8

- P7L14-21: you mention that the experimental period was not long enough to achieve full saturation of SO₄ at 20 cm depth. However, in the C cores, sulphate reaches that depth after the first week, and the concentration at depth decreases over time. This shows that sulphate consumption increases over time (most likely when the FeIII inventory decreases). The UC cores show an increase of sulphate over time and have indeed not achieved saturation at depth.

Author response:

By full saturation we mean the same concentration (or close to) as the overlying water as is typically observed in marine sediments with moderate metabolic activity. In both soil types sulfate concentrations decreased steeply with depth throughout the entire experiment and porewater sulfate in the deeper soils were far from equilibrium with respect to sulfate. We will amend the text to clarify this.

1.9

- P8L11-14: You say that TCO₂ production could not be determined below 5 cm depth. You then estimate this TCO₂ production by assuming that SR was the dominating pathway at depth. However, when I look at Table 3, you show that the contribution of other anaerobic pathways was 19% for UC and 54% in C, so SR was clearly not the dominant pathway. Also, considering the high FeIII concentrations in the sediment, I would assume that dissimilatory iron reduction is also an important pathway. Considering this, I have some problems with Figure 5, where you show that all TCO₂ production from 4 months onwards is due to SR. This is a consequence of your assumption, and I don't feel that this is well founded. Can you provide more justification for this?

Author response:

Regarding table 3, please see the next comment below. In our experiment there was an almost 2:1 relationship between CO₂-production and sulfate

consumption. This will be mentioned in the revised manuscript. We will also add a reference for the SRx2 conversion (Jørgensen 2006).

1.10

- P11L30: I think you can make an estimate of the time evolution of the relative importance of the mineralisation pathways, which could provide more information than the integrated budget over 1 year (since SR will always end up being the dominant pathway if you wait long enough). It would also improve the impact of the manuscript.

Author response:

We thank the reviewer for the suggestion and acknowledge that the budget in table 3 doesn't illustrate this point. In the revised manuscript we will change table 3 to indicate the total values, as well as temporal development in contribution of mineralization pathways. This will also contribute to the argumentation for carbon preservation.

1.11

- P12L5: Based on the results from the FeIII – measurement of Lovely and Philips, I believe you can estimate the importance of dissimilatory iron reduction (at least, that is what they teach at the AMME summerschool in Odense every year).

Author response:

It is true that there are some tentative correlations between the FeIII content in marine sediments and relative contribution of Fe-reduction to total OC-mineralization – see fig. 6 in Jensen et al. (2003). But this relationship only holds for marine sediments under steady state conditions – not in this case where we are far from steady state.

Technical corrections:

1.12

- Abstract: I find the paper well written, but I don't feel the same about the abstract, it does not flow very well (e.g. 'So far' at the beginning of a sentence). I would advise revising the abstract in order to improve attraction.

Author response:

We will revise the abstract as suggested by the reviewer.

1.13

- P2L18: "Further it is" -> Furthermore it is - P3L25: is the water in the tanks from the same site? If so, please indicate. -P10L9: aerobic OC degradation contributed to 18 and 10 % to of the total . . . - P11L21 anaerobic TCO₂ production, was detected -> remove the comma - Figure 3: I would use different symbols for the different months (when printed in black and white, the colors will be difficult to distinguish). - Figure 4: same remark as for figure 3, and I would consider changing the axes of the right panels (it is impossible to see the different SR rates).

Author response:

We will include the above corrections in the revised manuscript.

Interactive comment on "Carbon degradation in agricultural soils flooded with seawater after managed coastal realignment" by Kamilla S. Sjøgaard et al.

Anonymous Referee #2

Received and published: 7 February 2017

General Comments: Sjøgaard et al. investigate the effect of seawater flooding on the metabolism of soil organic carbon in soils from a reclaimed wetland that had either been cultivated for 140 years or allowed to become a reedswamp. This is an important issue globally as coastal land managers turn to a program of "depoldering" to restore the functions and ecosystem services of intertidal coastal habitats. The author's use bottle and core incubations to explore patterns of carbon mineralization both through time and with soil depth. They conclude that seawater sulfates rapidly accelerate carbon degradation upon flooding, but soils quickly regain a new equilibrium as mineralization slows over time, resulting in only 6-7% of the original soil organic carbon being lost, which they conclude indicates seawater flooding will result in a negative feedback on atmospheric CO₂ concentrations by preserving C. While the analysis conducted were generally well executed and

the results comprehensive, they were not designed to test the hypotheses posed in the introduction, specifically (H1) that soil carbon degradation is related to the lability of organic matter, which is not assessed in the current study, (H2) that flooding preserves organic carbon or (H3) there is a negative feedback with soil flooding and atmospheric CO₂ concentrations. The results presented are sufficient to support a comprehensive study of the effects of seawater reintroduction to reclaimed coastal lands, but the hypotheses and conclusions must be significantly re-framed to be acceptable for publication. I recommend major revisions.

Major specific comments:

2.1

-The abstract is the first mention of coastal realignment but through the manuscript it is discussed as a relatively novel concept about which little is known. There is an extensive body of literature on “managed realignment” also call dike-breach restoration, or depoldering. While I believe the author’s data is amongst the most detailed laboratory study of carbon degradation in this body of literature, making it a unique and important addition, they have not used this literature to their advantage and have neglected some key publications, among them the studies of Portnoy and Giblin (Eco. Apps. 1997 pp1054), recent publications by Ardon et al. (GCB 2013 pp296 and Biogeochemistry 2016 411), for a review of biogeochemical changes due to salinization see Herbert et al (2015 Ecosphere) and for reviews of dike-breach restoration see Burdick & Roman (2012) Tidal Marsh Restoration: A Synthesis of Science and Management (Springer)

Author response:

We thank the reviewer for considering our study ‘amongst the most detailed laboratory study of carbon degradation in this body of literature’. While it is always difficult to find and incorporate all available knowledge into manuscripts, we will certainly read and incorporate the studies suggested by the reviewer into the revised version of the manuscript.

2.2

- There are two problems with the authors' central argument that flooding soils enhances carbon preservation and therefore has a negative feedback with atmospheric CO₂ concentrations.

(1) The authors do not show that flooding soils enhances carbon preservation (over what?). The reader may assume that the authors intend to say that flooding the soils preserves more carbon than would be preserved if the land was not subjected to flooding. In fact, the data they present shows flooding increases carbon mineralization, at least initially. While the authors' supposition is not unfounded (intertidal soils on average accumulate carbon at 5-10x the rate of terrestrial soils e.g. McLeod et al 2012) the authors analyses cannot show this because they did not measure mineralization rates in the cultivated and uncultivated soils in the absence of seawater flooding. At the very least the authors could provide a comparison of published rates for similar marine sediments, similar reed swamp sediments, and similar agricultural soils, however this would only be sufficient to suggest, not confirm, enhanced carbon sequestration.

Author response:

As described in the first response comment to reviewer 1#, we will refine the argumentation and provide comparisons to published rates.

2.3

(2) The authors have confused preserving stored carbon with negative carbon-climate feedbacks. Preventing carbon from entering the atmosphere (i.e. through flooding of soils) at best has a null impact on atmospheric CO₂ concentrations. To have a negative feedback on atmospheric CO₂ concentrations a system must remove CO₂ from the atmosphere which is a process not explored in the current MS. Mackey et al (Nature Climate Change 2013) provide an excellent perspective on this. This may well be the case if intertidal vegetation is established etc. but is not the case in the current study.

Author response:

We have used the term "negative feedback on atmospheric CO₂ concentrations" to describe the fact that, due to the preservation of the organic C present in the soils at the time of flooding, on a longer term less

CO₂ will be emitted than under a scenario where the area would not have been flooded. We believe that this is the correct understanding of the term 'negative feedback'. In the revised version of the manuscript we will consult the manuscript suggested by the reviewer and assess if our argumentation should be refined.

2.4

- Hypothesis 1 & 2: while the dependence of mineralization on content is investigated, the authors do not make any measurements of lability or the origin of organic matter, thus these are weak points of argument that should not be the focus of the manuscript. Instead, the bulk of the analysis are targeted toward bulk organic matter degradation and the pathways of degradation.

Author response:

There's a considerable amount of literature discussing the importance of labile and refractory organic matter from bulk C-degradation rates (Westrich and Berner 1984, Burdige 1991, Valdemarsen et al. 2014). The exponentially decreasing trend in C-degradation can only be explained by a gradual depletion of the most labile components of soil organic C. This will be clarified in the revised manuscript.

2.5

-The strongest way to re-frame the data in hand would be a comparison of the effects of seawater flooding on cultivated versus what seem to be freshwater wetland soils focusing on the rates of carbon loss, the proportion of initial carbon lost, and the pathways of mineralization. There are obvious differences in the two sites that lend themselves to this discussion and the topic is still highly relevant to efforts to re-flood former agricultural land (cultivated) as well as restore artificial freshwater impoundments (Portnoy & Giblin, Bouldoc & Afton etc.) or the migration of saltwater into historically freshwater areas (absent of restoration).

Author response:

We thank the reviewer for his/her suggestion to rewrite the manuscript with an alternative angle. However, rephrasing the manuscript would be

counterproductive in relation to the main motivation for the manuscript, which was to assess the total impact of flooding on soil C degradation in the specific area that was flooded.

2.6

-Section 4.1 It is the production of small polymers/monomers small enough for microbial uptake that is considered the rate limiting step for carbon degradation (e.g. the enzymatic latch hypotheses) not the generation of DOC which can be highly recalcitrant. DOC is not equivalent to easily degradable materials.

Author response:

The reviewer is right that it is ‘the production of small polymers/monomers small enough for microbial uptake that is considered the rate limiting step for carbon degradation’, but in most cases by far, most of the DOC produced per time unit is ‘small polymers/monomers small enough for microbial uptake’ while only a small proportion is recalcitrant DOC. However, over time recalcitrant DOC may accumulate to high (and quantitatively important) levels in soil porewater. We will make sure that this point is adequately described in the revised manuscript.

2.7

-The authors do not sufficiently address the caveats of long core/bottle incubations and the various experimental artifacts introduced.

Author response:

We will include a section discussing the potential influences that our experimental setup had on our results, e.g. day/night cycles of light and temperature, daily water exchange due to tides, very undisturbed soils in the experiment due to e.g. lacking of fauna.

2.8

Minor specific comments: Abstract o Ln. 6 delete “continue for centuries and”
o Ln. 8: delete “So far”; what kind of soils?
o Ln. 9: delete “In this study”

Author response:

We will adjust the abstract to the changes after revision of the manuscript and revise it accordingly.

2.9

Section 1 o Ln. 20: This paragraph invokes far too many specificities related to SLR. Suggest compressing into a single sentence. This paper is about managed realignment with SLR as one of the justifications, not specificities of SLR or scenarios. o Please revisit this argument. It is the hydrolytic enzymes in conjunction with radicals that can depolymerize refractory compounds. There are also multiple other arguments for accelerated decomposition in aerobic environments, including free energy of alternate terminal electron acceptor pathways and other metabolic constraints. o The last two sentences of the first full paragraph starting on page 2 are confusing. Where is “here”? Do the authors intend to say soil organic matter of terrestrial origin may be difficult for marine organisms? o Soil organic carbon is generally abbreviated as SOC

Author response:

We will reduce the paragraph on SLR (sea level rise) to one or two sentences and fuse it with the following paragraph that introduces managed coastal realignment. All the other minor points mentioned by the reviewer related to this part of the manuscript will also be addressed in the revised manuscript

2.10

Section 2.1 o Give details about the reclamation: was the area diked and drained?

Author response:

This is correct. In the revised manuscript “reclaimed” will be substituted with “diked and continuously drained”.

2.11

o Because the authors are using so many acronyms for different carbon compounds, the use of “C” and “UC” for the sites can make for difficult reading. Suggest switch to agricultural field (AF=C) and reedswamp (RS=UC) as they are more descriptive. o Was the reedswamp freshwater?

Author response:

We thank the reviewer for an alternative suggestion for abbreviations, but we prefer to keep our original terminology.

2.12

Section 2.2.3 o Were vials flushed to remove oxygen prior to the incubations?

Author response:

Vials were not flushed prior to incubations.

2.13

Section 2.3 o The budget calculation is unclear. Please clearly describe which data sources are utilized for the carbon budget.

Author response:

We will improve the description of budget calculations.

2.14

Section 4.4 o Reduced iron (FeII) responsible for buffering sulfide accumulation (Reddy and DeLaune 2008) appears to increase through most of the study and show no substantial declines (particularly in station C) over the course of the year, indicating there should be sufficient Fe buffer for sulfide generated over longer time scales (>1 year) since metabolic rates appear to decline over time (See Schoepfer et al. 2014. JGR: Biogeosciences).

Author response:

In the revised manuscript, the argument related to sulfide buffering will be revised according to the reviewer's suggestion, with e.g. the incorporation of the suggested references.

Technical corrections:

2.15

2.2.1 Give simple details of flow injection analysis (model of instrument). Was Zinc added to prevent H₂S interference for CO?

Author response:

The instrument for flow injection analysis is exactly as described in the reference provided (Hall and Aller 1992), so we feel that no additional description is needed. Saturated HgCl₂ was added, which also precipitates sulfide through the formation of HgS.

2.16

Figure 2b. Re-scale y-axis to fit data.

Author response:

We will perform the suggested change in the revised manuscript.

Interactive comment on “Carbon degradation in agricultural soils flooded with seawater after managed coastal realignment” by Kamilla S. Sjøgaard et al.

E. Metzger (Referee)

edouard.metzger@univ-angers.fr

Received and published: 10 February 2017

Dear Editor and co-Authors,

I was happy to have a chance to read this manuscript that provides interesting data about coastal soils that are about to be flooded by seawater under sea level rise. I intended to give constructive comments and suggestions and apologize if some comments seem too harsh, my stylistic skills are quite limited in English.

Edouard Metzger Associated professor at the University of Angers, France

Overall comments:

3.1

The study depicted and discussed in the present manuscript represents considerable experimental and analytical work that deserves to be published. In my opinion, the most interesting feature is the almost total replacement of iron oxides by iron sulphides after one year of incubation of a soil with seawater. Such rapid mineralogical transformation suggests rapid anaerobic mineralisation processes that affect the carbonate system and carbon

recycling that should be less efficient since the soil tends to become anoxic due to sulfate reduction. Therefore, author main hypothesis is that it should represent a significant negative feedback on atmospheric CO₂ concentrations. There is a major objection to such statement due to the lack of elements of comparison. The authors do not show any mineralisation rate of soils before seawater flooding.

Author response:

Reviewer #1 and #2 have also mentioned that we lack a comparison to mineralization rates in soils before flooding. As stated in the response to reviewer #1's comment, the revised manuscript will include comparisons to typical mineralization rates in soils as well as improved arguments related to this matter.

3.2

Returning to the mineralogical transformation (i.e. iron oxides turned into iron sulphides), nothing is said about potential release of phosphorus and eutrophication that should also have as important feedback even more important to atmospheric CO₂. Samples exist, I would suggest to analyse P and address its remobilisation.

Author response:

While we acknowledge that P-release from newly flooded sediments is an extremely important process in relation to eutrophication issues, we have tried to write a focused manuscript dealing with C-, Fe- and S-cycling. Adding P-cycling would make it impossible to keep the story tight and focused, as we would have to discuss many more processes in detail related to P-cycling, as well as broader eutrophication issues. We therefore prefer to not include P at this point.

3.3

The considerable amount of data should permit authors to make a temporal mass balance in order to better precise the relative importance of anaerobic respiration processes and secondary reactions that limit reduced iron and free sulphide diffusion within the sediment and toward the soil water interface.

Author response:

We agree with the comment and temporal mass balances for C, S and Fe are already included in the manuscript – see figures 5, 6 and 7. Furthermore, we already will address a similar comment of reviewer #1 at “- P11L30:” under “Minor specific comments” regarding table 3, which will also clarify the temporal mass balance.

3.4

A secondary point but still important is a better discussion about HCl extractions and iron speciation. There is an extensive literature about selectivity of phases extracted by HCl, dithionite and ascorbic acid that should be considered here (e.g. Kotska and Luther, GCA, 1994; Hyacinthe et al., GCA, 2006).

Author response:

We do not think that a detailed discussion of Fe-extraction methodology is relevant for the story. However, in the revised manuscript we will improve the description of Fe-extractions and include relevant references to illustrate which Fe-pools are extracted by the chosen method.

3.5

Then, a quick discussion about the limitations of ex situ long term incubations that cut down hydrosedimentary processes should be addressed.

Author response:

Reviewer #2 has already recommended this above (the comment after “Section 4.1”), and we will address the potential influences that our experimental setup had on the results in a new paragraph.

Summarizing, I recommend a major revision of the manuscript by stepping down on conclusions about carbon preservation and atmospheric CO₂ feedback and by examining in more detail the importance of iron cycling.

Abstract:

3.6

Should be rewritten in a new version of the manuscript

Author response:

The abstract of the revised manuscript will be thoroughly revised.

Introduction:

3.7

L64-68 advection processes induced by tidal currents in such porous environment with higher level of connectivity between pores and burrows is not considered here

Author response:

We do not think including information about tidal currents and soil porosity would add to the overall story and improve the indicated passage.

3.8

L83-88 check on in situ experiments by Yucel and Lebris about lignin degradation

Author response:

Thank you for making us aware of this, we will check the suggested reference.

Materials and methods:

3.9

L152 were cores sliced, centrifuged and conditioned under nitrogen flux?

Author response:

Cores were sliced under normal atmosphere.

3.10

L162-163 was chloride analysed as well? As a conservative species, chloride is necessary to evaluate sulfate consumption from sulfate profiles in environments of variable salinity. This could refine SR calculations from bulk incubated sediment.

Author response:

Chloride was measured as a proxy for the progress of the intrusion of seawater into the core. However, in this highly unusual case, with virtually all dissolved components (including Cl) far from being at steady state, it is, to our knowledge, very difficult, if not impossible, to use chloride data to correct for sulfate consumption.

Results

3.11

L265-283 Difficult to use TCO₂ data since they are potentially compromised. I always recommend to analyse TCO₂ or alkalinity as soon as the sample was extracted from the core slice to avoid such disagreement. At this point those data seem invalid for publication.

Author response:

As mentioned in the manuscript (P8 L12-16) we only use the CO₂ data that are not compromised by experimental artefacts.

3.12

L284-291 The authors mention that incubation time is too short to achieve full saturation over the entire core. I would agree with that and this can be quite well predicted using diffusive models. For instance the Einstein equation ($x = (Dt)^{1/2}$, Boudreau, 1996) suggest that in 12 month a molecule of sulfate would diffuse in free water at 20°C of about 12 cm ($D = 5 \cdot 10^{-6} \text{ cm}^2 \cdot \text{s}^{-1}$, Krom and Berner, 1980). For one week, diffusion allow sulfate to travel only 2 cm. This would suggest that not only diffusion can explain sulfate data and that during pouring of marine water most of it flowed downward through burrows or gaps in the soil. In order to avoid transport processes and to show sulfate consumption from profiles in variable salinity you could, as mentioned above, to normalise sulfate by chloride: a decrease of the ratio would indicate sulfate consumption that could be quantified in terms of rate to be compared to SR calculated from anoxic incubations. From IC spectra you should be able to retrieve chloride concentrations at least for low salinity samples. I am afraid samples at the higher salinity have to be diluted and reanalysed. . .

Author response:

True, the initial water infiltration during flooding was also a transport mechanism for sulfate. This information will be added in the revised manuscript. While we agree that it could be interesting to evaluate if sulfate reduction could also be measured by considering chloride/sulfate ratios in porewater, we believe that the suggested method would be subject to large errors as we are dealing with an experimental system far from steady state. We therefore prefer to base our discussion on direct sulfate consumption measurements, which is usually a very precise method to obtain sulfate reduction measurements – see e.g. (Kristensen and Hansen 1995, Valdemarsen et al. 2012)

3.13

L327-328 add a reference for the SRx2 conversion. Do you achieve a ratio of 2:1 in measured samples? Plotting both measurements should give a nice line with a slope of 2. What about methane oxidation affecting sulfate consumption (1:1 ratio)?

Author response:

We will add a reference for the SRx2 conversion (Jørgensen 2006). We observed an almost 2:1 relationship between CO₂-production and sulfate consumption in our experiment, indicating that the influence of methane oxidation was negligible. This will be mentioned in the revised manuscript.

3.14

L405-407 You suggest other processes than sulfate reduction to explain carbon mineralisation. You should consider more carefully iron reduction. Data are there to show how important this process is in your soils. This can flaw your main hypothesis that SR is the main mineralisation process going on in your soils after marine water flooding. In a recent study our team showed in intertidal estuarine mudflats that iron reduction remains a major process among sulfate reduction whatever the salinity due to regular replenishment of iron rich particles from the river (Thibault de Chanvalon et al, JSR, 2016). This points out the fact that such long term incubation experiments have somehow to take into account hydrosedimentary processes that can greatly affect organic matter mineralisation.

Author response:

As mentioned by the reviewer, systems with high importance of Fe-reduction are systems where Fe-oxides are continuously replenished e.g. by either sedimentation of Fe-oxide-rich particles or intense vertical mixing of the sediment matrix due to bioturbation. Fe-reduction may also be of high relative importance in Fe-rich sediments with low metabolic activity. However, in our experimental system where organic matter of high lability is present in excess initially, sulfate reduction will become the dominating pathway. This becomes very clear from our direct measurements of CO₂ production and sulfate consumption, showing that sulfate reduction was responsible for 63 and 36% of total organic carbon degradation (P10 L6). We do not see how we can use our results to quantify Fe-reduction, as oxidized Fe may have been reduced due to both bacterial and chemical reduction and we have no method to distinguish between the two.

Discussion

3.15

L448-449 Have a look into in situ experiments of wood degradation in marine waters realised by Nadine Lebris team in the Mediterranean (e.g. Yucel et al, Chemosphere, 2013).

Author response:

Thank you for bringing this to our attention, we will include this reference and compare the SR rates.

3.16

L470 The authors claim that SR accounted up to 100% of TCO₂ production. How they explain dissolved iron profiles that still show an effective source of reduced iron that should account for a significant part of anaerobic mineralisation processes.

Author response:

The high importance of SR was evaluated based on direct measurements of sulfate consumption and CO₂-production, and we do not see how this method

can be questioned. We agree that any proportion of TCO₂ production not explained by SR, may have been due to Fe-reduction.

3.17

478-481 there is a bias in the statement since it is made from anaerobic incubations. Data show tha important sulfate reduction occurs near the surface, station UC and is about to be near zero in the other station. What about sulfate reduction if oxygen still diffuses from the surface? You could assess such question looking at porewater profiles. I would be glad to see TCO₂ time series and how they fit to linear regressions. Maybe there you can find clues about the relative importance of other mineralisation processes than SR.

Author response:

We are not sure how to understand the reviewer's comment. In our experimental setup the water overlying the cores was oxygenated and oxygen continuously diffused from the overlying water into the soil cores. The reason that sulfate reduction was occurring close to the sediment surface is that all oxygen was consumed in the uppermost soil layers due to extremely high metabolic rates of aerobic microorganisms initially.

3.18

L494-495 this final statement underlines the importance of having robust co₂ consumption rates: if the sum of iron and sulfate reduction does not achieve mass balance, it becomes to consider other reducing processes for iron in a way and other mineralisation processes in the other

So far, my concerns seem to suggest that a tentative of achievement of mass balnce calculation for C, S and Fe could greatly help interpretations. This could be possible from solid phase speciation and dissolved iron, CO₂ and sulfate profiles

Author response:

No doubt Fe-reduction may have been a metabolic pathway of minor importance in our experiment. However, we cannot estimate the importance of microbial Fe-reduction since Fe may have been reduced by two competing

processes – microbial reduction or spontaneous chemical reduction by sulphide – and we do not have a way to estimate the relative importance of the two.

3.19

502-504 not a sink, at most a zero source. The whole paragraph lacks of evidence. Especially that there are no unflooded cores as reference.

Author response:

This has also been mentioned by reviewer #2. We acknowledge this and will address this matter in the revised manuscript.

3.20

Section 4.4. could be developed by discussing in more detail the switch from FeIII to FeII of the solid phase. It would benefit of mass balance calculations as well. Is there any chance of adding some mineral images or analyses? It would be interesting to look at the crystallinity of iron sulphide minerals formed during the experiment. In marine sediment framboidal pyrite is formed. I wonder what would be the impact of refractory organic matter on pyrite formation.

Author response:

The presented data clearly documents a switch in Fe-speciation from the domination of oxidized Fe initially, to almost exclusively reduced Fe by the end. Unfortunately we do not have mineral images of any kind and will therefore not be able to add information obtained from image analysis in the revised manuscript.

3.21

Conclusion

Conclusions should be re-drawn according to discussion's evolution.

Author response:

The conclusions will be re-written after the revisions according to all reviewer comments have been implemented.

3.22

Figures

Figures 3 and 4: I would suggest a change in colours for different profiles overtime. The grey scale print is very difficult to read. It would be perfect if colours and grey scale evolve progressively with time and with more contrast.

Author response:

We will implement the suggestion of the reviewer in the updated version of the manuscript.

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Carbon degradation in agricultural soils flooded with seawater after managed coastal realignment

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Abstract. Permanent flooding of low-lying coastal areas is a growing threat due to climate change induced sea level rise. An increasingly common solution to protect coastal areas is their intentional flooding by ‘managed coastal realignment’. However, the biogeochemical implications of flooding agricultural soils with seawater are still not well understood. We conducted a 1-year mesocosm experiment to investigate microbial carbon degradation processes in soils flooded with seawater. Agricultural soils were sampled on the northern coast of the island Fyn (Denmark) at Gyldensteen Strand, an area that was subsequently flooded in a coastal realignment project. We found rapid carbon degradation almost immediately after experimental flooding and microbial sulfate reduction established quickly as an important mineralization pathway. Nevertheless, no free sulfide was observed as it precipitated as Fe-S compounds with Fe acting as a natural buffer, preventing toxic effects of free sulfide in soils flooded with seawater. The refractory nature of the terrestrial organic carbon, in combination with the anoxic conditions created in the soil after flooding, caused significantly decreased organic carbon degradation after 6 months. During the experiment only 6-7 % of the initial soil organic carbon pools were degraded. On this basis we suggest that flooding of coastal soils through sea level rise or managed coastal realignment, will cause significant preservation of soil organic carbon and create an overall negative feedback on atmospheric carbon dioxide concentrations.

1 Introduction

20 Sea level rise driven by global climate change is expected to continue for centuries, impacting about 70 % of the global coastlines (Church et al., 2013). Rising sea level causes higher and more frequent storm surges, which lead to more incidences of floodwaters overtopping and breaking coastal defenses (FitzGerald et al., 2008). Reclaimed coastal areas with low elevation are especially vulnerable to flooding. A low cost strategy of coastal protection is ‘managed coastal realignment’, whereby old coastal defenses are deliberately breached, and new ones are constructed further inland (Cooper, 25 2003; French, 2008; Roman and Burdick, 2012). The flooded areas created by managed coastal realignment act as buffer zones, protecting populated areas or valuable assets against flooding. Projects where coastal soils are flooded with seawater, by managed coastal realignment and similar techniques, occur at an increasing rate (Herbert et al., 2015; Pethick, 2002; Wolters et al., 2005).

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Comment [1]: Revised according to comment 1.12, 2.8, 3.6

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Comment [2]: Revised according to comment 2.1

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Comment [3]: Revised according to comment 2.9

Many studies have been performed on freshwater wetlands experiencing salinization and less on diked and drained agricultural soil systems (Ardon et al., 2016; Ardon et al., 2013; Herbert et al., 2015; Portnoy, 1999; Portnoy and Giblin, 1997), where flooding with seawater has dramatic consequences for soil biogeochemistry. Depending on soil porosity and moisture content, soil environments can have deep oxygen penetration (75-100 cm) (Dziejowski et al., 1997; MacDonald et al., 1993; Neira et al., 2015), since oxygen (O₂) can rapidly be supplied from the overlying atmosphere via diffusion. Therefore, surface soils are predominantly oxic environments where soil organic matter is degraded by a wide variety of microorganisms, fungi and fauna (Boer et al., 2005; Kalbitz et al., 2000). Aerobic degradation is catalysed by hydrolytic enzymes and reactive oxygen radicals that can break bonds in refractory organic compounds such as lignin and cellulose, and facilitate complete degradation of soil organic carbon (SOC) to CO₂ (Canfield, 1994). However, when soils are flooded, O₂ penetration is dramatically reduced, since O₂ solubility in water is low and O₂ diffusion in water is 10⁴ times slower than in air (Neira et al., 2015). O₂ will therefore be depleted by microbial and abiotic O₂ consuming processes in surface soils, which become anoxic below a few millimeters depth. In anoxic environments mutualistic consortia of microorganisms degrade organic macromolecules into smaller moieties by the excretion of exoenzymes and extracellular hydrolysis, which are then fermented into smaller organic molecules, mainly acetate (Valdemarsen and Kristensen, 2010). The fermentation products are taken up by microorganisms and terminally oxidized to carbon dioxide (CO₂) by the consumption of alternative electron acceptors (e.g. nitrate, Mn oxides, Fe oxides and sulfate) (Arnosti, 2011; Glud, 2008). Furthermore, soils flooded with seawater are enriched with sulfate that can be utilized as an alternative electron acceptor, and microbial sulfate reduction (SR) is expected to become a major mineralization pathway (Sutton-Grier et al., 2011; Weston et al., 2011).

While some studies have looked at SOC mineralization pathways in different types of soils introduced to saltwater (Ardon et al., 2016; Chambers et al., 2013; Herbert et al., 2015; Neubauer et al., 2013; Weston et al., 2006; Weston et al., 2011), a lot is still unknown about how the dynamics between initial SOC degradation to DOC and the terminal mineralization are affected by the introduction of saltwater (Herbert et al., 2015). Many soils subject to managed coastal realignment contain considerable amounts of SOC (Franzluebbers, 2010; Wolters et al., 2005). The degradation of SOC after flooding will depend on the rate of establishment of heterotrophic microbial communities and their ability to degrade SOC (Schmidt et al., 2011). Labile organic carbon may be easily degraded by marine microorganisms, while more complex organic carbon, and especially structurally complex organic compounds such as cellulose and lignin, may be virtually non-degradable in anoxic environments (Kim and Singh, 2000; Kristensen and Holmer, 2001). Flooding of coastal soils by sea level rise and coastal realignment may therefore cause significant preservation of stored SOC, implying an overall negative feedback on atmospheric CO₂ concentrations.

In this study the fate of SOC after flooding with seawater was investigated in soils collected at Gyldensteen Strand on the northern coast of Fyn, Denmark, an area that was designated to be flooded in a coastal realignment project. We were especially interested in following the temporal establishment of dominating microbial pathways and quantifying the rates and temporal trajectories of SOC degradation in newly flooded soils. We hypothesized that (1) total SOC degradation activity in soils after flooding depends on SOC content and lability, and that (2) most SOC at the time of flooding will, due

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Comment [4]: Revised according to comment 2.9

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Comment [5]: Revised according to comment 1.13

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Comment [6]: According to comment 2.3

to its terrestrial origin, be non-degradable and hence preserved under the anoxic conditions formed after the flooding. Therefore it is plausible that (3) the majority of SOC will be preserved in the flooded soils, creating a negative feedback on atmospheric CO₂ concentrations. To test these hypotheses we performed parallel mesocosm experiments with two different types of soils that were experimentally flooded with seawater. SOC-degradation and other biogeochemical developments in the mesocosms were traced with high temporal and spatial resolution for the next 12 months. The results showed how flooding with seawater impacts C-degradation and soil biogeochemistry and formed the basis for an initial evaluation of potential feedbacks of flooding on atmospheric CO₂ concentrations.

2 Materials and methods

2.1 Study site

This study was conducted in relation to the nature restoration project at Gyldensteen Strand funded by the Danish Aage V. Jensen Nature Foundation. The sampling site (55°34'26.4"N 10°08'17.0"E) was a shallow intertidal habitat until 1871 (size of ~600 ha), where it was diked and continuously drained to create new land for agriculture. The reclaimed area was for the following 140 years mainly used for production of different crops such as onions and grains (Stenak, 2005). As a part of the nature restoration project, selected sections of the dikes were removed in March 2014 and 211 ha of the area were permanently flooded with seawater and turned into a shallow marine lagoon.

2.2 Experimental design and Sampling

Sampling for the mesocosm experiment was performed in November 2013, half a year before the flooding of the site, at two different stations representing uncultivated (UC) and cultivated (C) soils (Fig. 1). Station UC was located in an area with low elevation, which never could be properly drained. Station UC was therefore abandoned for agriculture and became a reed swamp that accumulated plant material and litter. Station C, however, resembled the majority of the re-flooded area that was farmed since the land reclamation (fertilized, ploughed and used for monoculture, also illustrated in Fig. 1). From each station, 15 soil cores were sampled in 30 cm long, 8 cm internal diameter stainless steel core liners. The core liners were hammered 25 cm down into the soil, dug up with a spade and closed in both ends with rubber stoppers.

In the laboratory, the headspaces of individual soil cores were gently flooded with 22-26 salinity seawater collected from the shore face directly north of station UC (Fig. 1). Soil cores were then transferred to 70 L incubation tanks filled with seawater. During the whole experiment the flooded cores were maintained at 15 °C and kept in darkness. The water in the tanks was rigorously aerated through air diffuser stones and 10-20 L of the seawater in the tanks was exchanged with fresh seawater (also collected from the shore face) every 14 days. Thus soil cores were incubated under constant environmental conditions, while factors such as diurnal temperature variations, tidal exchange, benthic primary production and bioturbation were omitted by the experimental setup.

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Comment [7]: According to comment 2.10

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Comment [8]: According to comment 1.7

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Comment [9]: Revised according to comment 1.13

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Comment [10]: Added according to comment 2.7 and 3.5

The flooded soil cores were incubated for 12 months. Flux experiments were conducted with 3 random soil cores from each station at various times (weekly in the first month, biweekly for the next 3 months and monthly hereafter). Core sectionings were performed on 3 randomly selected soil cores from each station at different times during the experiment (before the flooding, 1 week after and after 2, 4, 6 and 12 months).

5 2.2.1 Flux experiments

Fluxes of O₂, dissolved organic carbon (DOC) and TCO₂ (= CO₃²⁻ + HCO₃⁻ + H₂CO₃) between soil and overlying water were measured regularly as described above. Cores were equipped with stirring magnets, closed with rubber stoppers and placed around a central magnet rotating at 60 rpm and hereafter incubated for about 4 hours in darkness. O₂ was measured and water samples were taken in the headspace of the soil cores at the beginning and end of incubations. O₂ was measured with an optical dissolved oxygen meter (YSI ProODO). DOC samples were stored at -20 °C until analysis using a Shimadzu TOC-5000 Total Organic Analyzer. Samples for TCO₂ analysis were kept in 3 mL gas-tight exetainers for a maximum of 1 week until analysis by flow injection (Hall and Aller, 1992).

2.2.2 Core sectioning

Core sectioning was performed by slicing each soil core into 6 depth intervals (0-1, 1-2, 3-5, 5-10, 10-15 and 15-20 cm). Porewater was extracted from each depth interval by centrifugation and GF/C filtration in double centrifuge tubes (500 g, 10 min). The porewater was sampled for various parameters; 500 µL porewater were preserved with 30 µL saturated HgCl₂ for TCO₂, 250 µL porewater were preserved with 50 µL 1 M zinc acetate (ZnAc) for total dissolved sulfide (TH₂S = H₂S + HS⁻ + S₂⁻) analysis, 250 µL porewater were preserved with 100 µL 0.5 M HCl for Fe²⁺ analysis and remaining porewater was stored at -20 °C until analysis for sulfate (SO₄²⁻) and DOC. TCO₂ and DOC samples were stored and analyzed as described above. TH₂S samples were analyzed by the method of Cline (1969). Fe²⁺ samples were analyzed by the Ferrozine method (Stookey, 1970). SO₄²⁻ was analyzed by liquid ion chromatography on a Dionex ICS-2000 system.

Reactive iron, RFe, was extracted from soil subsamples from every depth interval with 0.5 M HCl for 30 min while shaking (Lovley and Phillips, 1987). After centrifugation (500 g, 10 min) the supernatant was transferred to sampling vials and stored at room temperature until analysis for reactive Fe(II) and Fe(III) [RFe(II) and RFe(III), respectively]. The supernatant was analyzed for Fe²⁺ and RFe by the ferrozine method (Stookey 1970) before and after reduction with hydroxylamine (Lovley and Phillips, 1987). RFe(II) was calculated directly, while RFe(III) was calculated from the difference between RFe and RFe(II). An estimate of total Fe content was obtained by boiling combusted soil subsamples in 1 M HCl for 1 hour at 120 °C. The supernatant was stored at room temperature until analysis by the ferrozine method.

Acid volatile sulfides (AVS) (Rickard and Morse, 2005) and chromium reducible sulfur (CRS) were determined on soil subsamples preserved with 1 M ZnAc and stored at -20 °C until analysis. AVS and CRS were extracted by 2-step distillation as described in Fossing and Jørgensen (1998). Sulfide concentrations in the distillates were analyzed by the method described by Cline (1969).

Soil characteristics were also determined for every depth interval during every core sectioning. Soil density was determined gravimetrically and soil subsamples were dried (24 h, 105 °C) to determine water content and porosity. Soil organic matter content was measured as the weight loss of dry sediment after combustion (520 °C, 5 hours). SOC on selected soil samples (samples obtained after 1 week and 6 months) was also measured by elemental analysis on Carlo Erba CHN EA1108 Elemental Analyzer according to Kristensen and Andersen (1987).

2.2.3 Anoxic incubations (Jar experiments)

Depth distribution of microbial TCO₂ and DOC production and SR were estimated from anoxic soil incubations (Kristensen and Hansen, 1995; Quintana et al., 2013). The excess soil from core sectionings was pooled into 4 depth intervals (0-2, 2-5, 5-10 and 15-20 cm), thoroughly homogenized and tightly packed into 6-8 glass scintillation vials (20 mL). The vials were closed with screw caps, buried head-down in anoxic mud and incubated at 15 °C in darkness. 2 jars from each jar series were sacrificed every week for porewater extraction in the following 4 weeks. The screw caps were changed to a perforated lid containing a GF/C filter and the jars were centrifuged upside-down in a centrifuge tube (10 min at 500 g). The extracted porewater was sampled and analyzed for TCO₂, DOC and SO₄²⁻ as described above.

2.3 Data analysis

Fluxes of TCO₂, DOC and O₂ were calculated from the concentration differences between start and end samples. Microbial rates in jar experiments (DOC and TCO₂ production and SR) were calculated for 0-2, 2-5, 5-10, 15-20 cm depth intervals by fitting the time dependent concentration changes by linear regressions with checks for obvious outliers. When the slopes were significant ($p < 0.05$), the volume specific reaction rates ($\text{nmol cm}^{-3} \text{d}^{-1}$) in individual depth layers were calculated from the regression slopes corrected for sediment porosity. Microbial reaction rates, porewater and solid pools were depth integrated over 0-20 cm and converted to area specific units. Linear data interpolation was used to correct for missing data points, e.g. for the depth interval 10-15 cm where microbial rates were not measured. There was a significant linear correlation between organic matter content and SOC for both sampling stations $[\text{OC}(\%) = 0.0649 \times \text{LOI}(\%) + 0.0936, r^2=0.9824, n=20]$. This correlation was used to convert organic matter into SOC for the time points where SOC was not directly measured. A one-way ANOVA was performed on area specific SOC pools at the different time points to test for significant changes in the SOC pools over time. Depth integrated SR rates were normalized to C-units since an almost 2:1 relationship between TCO₂ production and SR (Jørgensen, 2006) was observed throughout the experiment. For soil characteristics, fluxes, porewater and solid pools errors were calculated as standard errors of the mean (SEM). For depth-integrated values of microbial rates and solid pools errors were calculated as standard errors propagation (SEP) of standard deviation (SD) values following Eq. (1):

$$SEP = \sqrt{SD_{0-1 \text{ cm}}^2 + \dots + SD_{15-20 \text{ cm}}^2} \quad (1)$$

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Comment [11]: According to comment 1.4

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Comment [12]: According to comment 1.6

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Comment [13]: According to comment 1.9 and 3.13

In a carbon budget estimating SOC degradation during the experiment, total degradation of SOC (mol m^{-2}) was calculated as the sum of the time integrated TCO_2 efflux, time integrated DOC efflux and area specific TCO_2 and DOC in porewater by the end of the experiment. The percentage of the initial SOC pool degraded during the experiment was calculated from the estimated total degradation of SOC and mean bulk SOC pool. In a time specific carbon degradation budget, total degradation to TCO_2 was calculated as the sum of time integrated TCO_2 efflux and accumulated porewater TCO_2 at different time points after flooding (1 week and 2, 4, 6 and 12 months). Based on the jar experiments, total anaerobic TCO_2 production and TCO_2 production by SR (according to a 2:1 relationship between TCO_2 production and SR) was calculated by time integration at different time points after flooding (1 week and 2, 4, 6 and 12 months). Relative contributions of SR to anaerobic degradation to TCO_2 were estimated from TCO_2 production and TCO_2 production by SR measured in jar experiments.

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Comment [14]: Revised according to comment 2.13

10 3 Results

3.1 Soil characteristics

The two sampled stations had very different soil appearance, as a result of different use after the land reclamation (i.e. no cultivation and cultivation). Station UC was overgrown with mosses and grasses, and a dense layer of roots and litter characterized the upper 5 cm of the soil, while the deeper parts of the soil (>10 cm depth) consisted of clay. At station C only relatively small amounts of grass and root material were evident in the upper 5 cm. Some of the vegetation was still alive 2 months after the flooding, as indicated by long green grass leaves seeking light, but it slowly died out thereafter. The soil at both stations contained partially degraded shell material from gastropods and bivalves remaining from when the area was a marine lagoon before 1871.

There was very little variation in soil characteristics between successive core sectionings, so results were averaged for the whole experiment (Table 1). The water content at station UC decreased with depth from 83 % at the top to 35 % in the bottom, while water content only decreased from 32 % to 20 % at station C. The same depth trend was observed for porosity. The high water content and porosity at station UC was caused by high amounts of plant material (e.g. roots), while the soil at station C was sandy, homogenous and poor in organic debris.

Soil organic content varied greatly with depth at station UC, and the topsoil was enriched with SOC (16 % compared to the bottom (1 %)) (Table 1). SOC varied between 0.8 and 1.4 % at station C with no depth variation. A one-way ANOVA showed no significant difference between the SOC contents at the different time points at either station UC or C ($df = 17$, $F = 1.9$, $p = 1.16$ for both stations).

3.2 CO_2 and DOC efflux, and O_2 consumption

TCO_2 effluxes in UC soil were highest in the beginning of the experiment with a maximum of $239 \pm 30 \text{ mmol m}^{-2} \text{ d}^{-1}$ measured on day 13 (Fig. 2a). Subsequently it decreased to about $130 \text{ mmol m}^{-2} \text{ d}^{-1}$ 31-199 days after flooding and stabilized

around $67 \text{ mmol m}^{-2} \text{ d}^{-1}$ from day 220 to the end. The TCO_2 effluxes in C soil were relatively constant around an average of $29 \text{ mmol m}^{-2} \text{ d}^{-1}$.

High DOC efflux was evident 1 day after flooding at station UC ($108 \pm 3 \text{ mmol m}^{-2} \text{ d}^{-1}$) (Fig. 2b), while it decreased to around $60 \text{ mmol m}^{-2} \text{ d}^{-1}$ 6-20 days after flooding and to $17 \text{ mmol m}^{-2} \text{ d}^{-1}$ after approximately 2 months to the end. DOC effluxes at station C showed a similar pattern, averaging $25 \text{ mmol m}^{-2} \text{ d}^{-1}$ in the first 2 months after flooding, and decreasing to an average of $5 \text{ mmol m}^{-2} \text{ d}^{-1}$ for the remaining experiment.

O_2 consumption decreased almost linearly during the 1-year experiment on both stations (Fig. 2c). At station UC initial O_2 consumption was $57 \pm 3 \text{ mmol m}^{-2} \text{ d}^{-1}$, 1-45 days after flooding, and then it steadily decreased to $19 \pm 3 \text{ mmol m}^{-2} \text{ d}^{-1}$ by the end. At station C there was a less pronounced temporally decreasing trend. O_2 consumption was highest initially with about $26 \text{ mmol m}^{-2} \text{ d}^{-1}$ at day 1-13 and then decreased to $9 \pm 0.6 \text{ mmol m}^{-2} \text{ d}^{-1}$ by the end.

3.3 Porewater chemistry

Porewater DOC was high 1 week after flooding at both stations (on average 10.4 and 3.8 mM at stations UC and C, respectively; Fig. 3a). Over the experiment porewater DOC decreased slightly in UC soil, while it increased slightly in C soil.

Porewater TCO_2 concentrations in UC soil were in the range of 5-13 mM between 1 week and 2 months after flooding, and profiles showed a slightly increasing pattern with depth (Fig. 3b). Afterwards an unexpected drop in TCO_2 concentrations, especially in the deep soil (>2 cm depth), was observed. This was likely an experimental artifact, however, caused by extremely high Fe^{2+} concentrations >2 mM in the porewater. During sample storage the Fe^{2+} got oxidized to Fe-oxhydroxides and formed an orange-brown precipitate at the bottom of the sample containers, probably leading to sample-acidification and TCO_2 degassing (Moses et al. 1987; Hedin 2006). Porewater TCO_2 concentrations in UC soil after 4 months were affected by this artifact. In C soil, porewater Fe^{2+} did not accumulate at the same rate as in UC soil and only exceeded 2 mM in the 10-20 cm depth layer after 6 months. Here porewater TCO_2 accumulated gradually over time as expected (Fig. 3b). Rapid TCO_2 accumulation occurred in the first 2 months, where TCO_2 increased from 3-5 mM to 11 mM below 3 cm depth. After 2 months to the end, TCO_2 increased further in the 2-10 cm depth interval, while a decrease occurred below 10 cm depth, which was probably related to Fe^{2+} exceeding 2 mM.

High concentrations of SO_4^{2-} were introduced to the soil when flooded with seawater. Yet the initial water infiltration and diffusion was the only transport mechanism for dissolved SO_4^{2-} in the mesocosm setup and the experimental period was evidently not sufficiently long to achieve equilibrium in SO_4^{2-} in porewater concentrations down to 20 cm depth. As a result, porewater SO_4^{2-} decreased steeply with depth at both stations (Fig. 3c). By the end of the experiment in UC soil, SO_4^{2-} decreased from ~17 mM at the surface to zero below 10 cm depth. In C soil SO_4^{2-} decreased linearly from ~17 mM at the surface to 0-2 mM at the bottom.

After 7 days of flooding the Fe^{2+} depth distribution in porewater was constant with depth, with on average 0.02 and 0.2 mM at station UC and C, respectively (Fig. 3d). Afterwards a progressive increase in porewater Fe^{2+} was observed at

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Comment [15]: According to comment 3.12

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Comment [16]: According to comment 1.8

both stations. At station UC Fe^{2+} increased to up to 1.3 ± 0.6 mM at 5-15 cm depth after 2 months and stabilized after 6 months, where Fe^{2+} exceeded 4 mM below 5 cm depth. The same trend was observed at station C, where Fe^{2+} accumulated to up to 3.7 mM at 15-20 cm depth after 12 months.

3.4 Anaerobic net DOC production in jar experiments

5 Net DOC production after 1 week of flooding was high in the surface 0-2 cm at station UC (2666 ± 695 $\text{nmol cm}^{-3} \text{d}^{-1}$; Fig. 4a) and decreased exponentially with depth to 203 ± 23 $\text{nmol cm}^{-3} \text{d}^{-1}$ at 15-20 cm depth. A gradually decreasing net DOC production was observed in all depth layers over the experiment, and by the end significant net DOC production (121 - 172 $\text{nmol cm}^{-3} \text{d}^{-1}$) was only detected in the upper 0-5 cm. A similar pattern in net DOC production was observed at station C, although rates were much lower than at station UC. After 1 week of flooding, net DOC production at station C was

10 1155 ± 158 $\text{nmol cm}^{-3} \text{d}^{-1}$ in the upper 0-2 cm of the soil but only 66 - 83 $\text{nmol cm}^{-3} \text{d}^{-1}$ below. After 4 months it had decreased to 135 $\text{nmol cm}^{-3} \text{d}^{-1}$ in the top 0-2 cm and no net DOC production was detected below 5 cm depth. Very low rates (21 - 25 $\text{nmol cm}^{-3} \text{d}^{-1}$) were detected in the top 0-5 cm by the end.

Depth integrated net DOC production at station UC was initially 118 - 133 $\text{mmol m}^{-2} \text{d}^{-1}$ in the first 2 months after flooding and then gradually declined to 8 $\text{mmol m}^{-2} \text{d}^{-1}$ after 12 months (Fig. 5). Initial depth integrated net DOC production at station C was 4-fold lower than at station UC. Net DOC production in C soil decreased by 75 % in the first 2 months after flooding and almost no net DOC production occurred after 6 months.

3.5 Anaerobic TCO_2 production in jar experiments

Initial depth trends in TCO_2 production were generally similar to those observed for DOC, but temporal trends were markedly different (Fig. 4b). At station UC, TCO_2 production was initially almost 1000 $\text{nmol cm}^{-3} \text{d}^{-1}$ in the top 0-2 cm and decreased to 380 $\text{nmol cm}^{-3} \text{d}^{-1}$ at 15-20 cm depth. After 2 months, TCO_2 production had increased in the surface 0-2 cm to 6250 $\text{nmol cm}^{-3} \text{d}^{-1}$, while rates below 10 cm depth remained relatively low. After 4 months, TCO_2 production decreased to about 2500 $\text{nmol cm}^{-3} \text{d}^{-1}$ in the top 0-2 cm, while it was not possible to determine TCO_2 production rates directly for soil deeper than 5 cm due to the problem with extremely high porewater Fe^{2+} described above. As seen below, porewater SO_4^{2-} concentrations were not affected by the high porewater Fe^{2+} concentrations. For the affected data points TCO_2 production was calculated as rate of SR x 2, assuming that SR was the dominating CO_2 producing process in the anoxic soil (Jørgensen, 2006). The calculations showed that TCO_2 production had decreased further after 6 and 12 months in the top 5 cm (600 - 1000 $\text{nmol cm}^{-3} \text{d}^{-1}$) and was quite stable below (0 - 85 $\text{nmol cm}^{-3} \text{d}^{-1}$). TCO_2 production rates were generally much lower in C soil, while relative trends for TCO_2 production and their development over time were quite similar between stations. Maximum TCO_2 production rates occurred at 0-2 cm depth, where TCO_2 production varied from 400 to 780 $\text{nmol cm}^{-3} \text{d}^{-1}$ between 1

25 week and 2 months and then gradually decreased to 110 $\text{nmol cm}^{-3} \text{d}^{-1}$ by the end. Similar trends were observed in the deeper soil, where TCO_2 production decreased from 180 - 310 $\text{nmol cm}^{-3} \text{d}^{-1}$ after 7 days to 7 - 53 $\text{nmol cm}^{-3} \text{d}^{-1}$ after 12 months.

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Area specific TCO₂ production at station UC was initially 115-200 mmol m⁻² d⁻¹ in the first 2 months, and decreased to 40 mmol m⁻² d⁻¹ after 6 months (Fig. 5). At station C area specific TCO₂ production was relatively stable around 44 mmol m⁻² d⁻¹ for the first 4 months and decreased to 21 and 10 mmol m⁻² d⁻¹ after 6 and 12 months, respectively.

3.6 SR in jar experiments

5 Significant SR was measured in the top 0-5 cm (470 nmol cm⁻³ d⁻¹) in UC soil 1 week after flooding, while no SR was detected below (Fig. 4c). After 2 months, high SR was only measured in the top 0-2 cm (3128±190 nmol cm⁻³ d⁻¹). After 4 months SR was still highest in the topsoil (1217±147 nmol cm⁻³ d⁻¹), while significant SR was detected down to 10 cm depth. From 4 months to the end, SR gradually decreased at all depths to 338±147 and 43±6 nmol cm⁻³ d⁻¹ at 0-2 and 5-10 cm depth, respectively. Since SO₄²⁻ did not reach the bottom (15-20 cm) during the experiment at station UC, no SR occurred here. In C soil SR occurred at considerably lower rates than in UC soil. After 1 week SR was 177±25 nmol cm⁻³ d⁻¹ at 0-2 cm depth and decreased exponentially with depth to zero at 15-20 cm depth. By month 2 and 4, SR occurred at all depths (20-159 nmol cm⁻³ d⁻¹). Afterwards SR decreased in the upper 15 cm while no SR was detected in the 15-20 cm depth interval.

10 Depth integrated SR at station UC increased from 24 to 63 mmol m⁻² d⁻¹ between week 1 and month 2, corresponding to 48 and 126 mmol m⁻² d⁻¹ carbon mineralization, respectively (Fig. 5). SR had decreased to 27.7 mmol m⁻² d⁻¹ after 12 months. SR increased during the first 4 months in C soil (6 to 12 mmol m⁻² d⁻¹) and then decreased to 4 mmol m⁻² d⁻¹ after 12 months.

3.7 Solid pools of Fe and S

Before flooding, RFe(II) in UC soil increased with depth from 4 μmol cm⁻³ at 0-1 cm depth to 13 μmol cm⁻³ at 15-20 cm depth, while a corresponding increase in RFe(III) occurred from 19 to 44 μmol cm⁻³ (Fig. 6). The RFe pools at station C were relatively constant with depth, on average 2.5 and 23 μmol cm⁻³ for RFe(II) and RFe(III), respectively. Twelve months after flooding, RFe(II) in UC soil had increased to 34-59 μmol cm⁻³, while RFe(III) had accumulated to 134.5±85 μmol cm⁻³ in the top and decreased to an average of 4 μmol cm⁻³ below. A similar trend was obtained in C soil with RFe(III) accumulating to 51.9±1.4 μmol cm⁻³ on the surface. In UC and C soil, total RFe initially consisted of 78 and 92 % Fe(III), respectively, while it was reduced to 19 and 10 % by the end. Clearly, RFe(III) became reduced to RFe(II) during the experiment due to the anoxic conditions created by flooding.

25 The RFe content was quite heterogeneous at the study sites and there were large variations between soil cores. Based on all the depth profiles obtained over the experiment, average total Fe content in UC and C soil was 19.3±2.8 mol m⁻² and 26.7±1.8 mol m⁻², respectively.

30 Although jar experiments suggested high SR in both soil types, dissolved sulfide (TH₂S) was never detected in the porewater. Instead, a large fraction of the sulfide produced during SR accumulated as AVS and CRS in both soil types (Fig. 7). One week after flooding, AVS and CRS in UC soil were low (0.2-2.7 μmol cm⁻³), except at 2-5 cm depth where AVS content was slightly elevated. 12 months after flooding, AVS and CRS had increased to 25±10 and 41±11 μmol cm⁻³ at 2-5

cm depth, respectively, while no accumulation occurred below 10 cm depth. A similar pattern was observed in C soil, where AVS and CRS were initially constant with depth averaging 0.1 and 3.5 $\mu\text{mol cm}^{-3}$, respectively, and accumulated to 6.4 \pm 1 and 8.4 \pm 0.7 $\mu\text{mol cm}^{-3}$ after 12 months of flooding, respectively. Over the whole experiment total sulfide accumulated as AVS and CRS gradually increased, from 0.5 mol m⁻² before flooding to 4.7 mol m⁻² after 12 months in UC soil, and from 0.63 to 2 mol m⁻² in C soil.

3.8 Budgets for SOC degradation

Area specific SOC pools were 710.9 \pm 54 and 232.5 \pm 22 mol m⁻² (n = 18) in UC and C soil, respectively (Table 2). Total SOC degradation estimated as the sum of TCO₂ and DOC effluxes, and porewater accumulation over the 1-year experiment was 49.6 and 14.8 mol m⁻² at station UC and C, respectively, corresponding to 7 and 6 % of the SOC pools.

Total SOC mineralization to TCO₂ was estimated as the sum of TCO₂ efflux and porewater accumulation during the whole experiment (Table 3), which was 40.0 and 12.0 mol m⁻² at station UC and C respectively. The importance of anaerobic SOC degradation for total TCO₂ mineralization could be calculated from jar experiments, and a total of 32.6 and 10.8 mol m⁻² SOC was converted to TCO₂ anaerobically, corresponding to 82 and 90 % of flux-based total TCO₂ production at station UC and C, respectively. The SR measured in jar experiments corresponded to 25.3 and 4.3 mol m⁻² CO₂ production at station UC and C during the experiment. Thus 63 and 36 % of the flux-based total TCO₂ production was driven by SR in UC and C soil, respectively, starting at 30-40 % after 1 week and gradually increasing up to 100 % by the end of the experiment. This means that the remaining 19 and 54 % of the flux-based total TCO₂ production was produced by other anaerobic processes than SR in UC and C soil, respectively (e.g. nitrate or Fe reduction).

4 Discussion

4.1 Temporal trends in SOC degradation

The UC and C soil had very different organic content. UC soil had not been used for agriculture and organic matter consisting of dead and alive plant matter had accumulated in the topsoil (Table 1), while lower organic matter content was evident in C soil due to lower plant cover and regular mechanical soil reworking during agricultural cultivation (Benbi et al., 2015; Six et al., 1998). Consequently, the bulk SOC pool was 3 times higher in UC soil than in C soil. The source of soil organic matter at both stations was terrestrial plants such as grasses and herbs rich in cellulose and lignified tissues (Arndt et al., 2013; Sullivan, 1955). Such organic matter is refractory towards degradation in anaerobic marine sediments (Kristensen, 1990, 1994) compared to structurally simple phytoplankton, microphytobenthos and macroalgae, which are common organic carbon sources in coastal marine sediments (Dubois et al., 2012; Fry et al., 1977). It was therefore uncertain to which extent the SOC at Gyldensteen Strand could serve as substrate for developing microbial communities after the flooding with

seawater. Nevertheless, we observed high heterotrophic activity (e.g. O₂ uptake and TCO₂ production) right after the flooding, indicating that at least part of the SOC in both soil types was readily available for microbial degradation.

Cleavage of particulate organic carbon to DOC by extracellular enzymes is the primary degradation step in waterlogged anoxic soils and sediments (Arnosti, 2011; Weiss et al., 1991). The produced DOC is hereafter converted into short chain fatty acids and acetate, by microbially mediated fermentation and hydrolysis, which then are terminally oxidized to CO₂ by e.g. SR (Canfield et al., 2005; Valdemarsen and Kristensen, 2010). DOC production can therefore generally be considered the rate-limiting step for organic carbon degradation. However, a small proportion of produced DOC is recalcitrant and may accumulate in soil pore water over time in an experimental setup without advective porewater transport. In this experiment we observed high DOC concentrations in porewater and highest DOC production in jar experiments already 7 days after flooding with seawater (Fig. 3a & 5). Part of this DOC may have leached to the porewater as a result of flooding (Kalbitz et al., 2000), while the rest was produced by microbial degradation of particulate SOC (Kim and Singh, 2000). Microbial degradation of soil organic matter to DOC was initiated immediately after flooding irrespective of the shift to anoxic conditions. Differences in DOC production rates indicated that the availability of degradable SOC was clearly highest in UC soil compared to C soil following the overall difference in total SOC content. However, total DOC production ceased rapidly in both soil types and was close to zero after 1 year. Ardon et al. (2016) also observed reduced DOC concentration and export due to introduction of saltwater. It therefore appears that only a minor portion of SOC (6-7 %; Table 2) is available for microbial degradation under the present conditions (flooded with seawater and anoxic conditions). The low degradability of SOC after flooding probably reflects limitations of the anaerobic microbial communities to degrade complex organic matter of terrestrial origin (Fors et al., 2008; Yucel et al., 2013).

Heterotrophic DOC oxidizing microbes were also active immediately after flooding as shown by initial TCO₂ effluxes and high TCO₂ production in the jar experiments 7 days after flooding (Fig. 2a & 5). Rapid microbial CO₂ production has previously been observed in experiments with experimentally flooded soils (Chambers et al., 2011; Neubauer et al., 2013; Weston et al., 2011). In both soil types, TCO₂ production in the surface soil increased over the first 2 months, peaked, and then decreased gradually towards the end. These temporal dynamics were out of phase with DOC availability, indicating that terminally oxidizing microbes may adapt slower to flooded conditions than hydrolyzing microbes. Similar cases of initial substrate hydrolysis outpacing fermentation and SR has been observed before (Arnosti et al., 1994), maybe due to lag response in the microbial community (Bruchert and Arnosti, 2003). Nevertheless, the majority (~80 %; Table 2) of produced DOC over the whole experiment was oxidized completely to TCO₂, while the rest effluxed to the overlying water (~19 %) or accumulated in porewater (~1 %).

4.2 SOC degradation pathways

SO₄²⁻ was an important electron acceptor in both soils and SR accounted for 63 and 36 % of the total TCO₂ production during the experiment in UC and C soil, respectively (Table 3). One week after flooding, active SR corresponding to 30-40 % of anaerobic TCO₂ production was detected in the jar experiment. The relative importance of SR increased gradually over

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the experiment and by the end accounted for up to 100 % of the anaerobic TCO₂ production in both soil types. This is in accordance with Weston et al. (2006) who measured SR in freshwater marsh soil exposed to saltwater in anoxic flow through reactors, and found that the relative importance of SR for total TCO₂ production increased from 18 % initially to >95 % after 4 weeks. The delay in SR probably reflects a lag phase for the community of SO₄²⁻ reducing microbes to respond to elevated SO₄²⁻ levels. The delay in SR could also reflect initial competition with other TCO₂ producing pathways (e.g. NO₃⁻ and Fe reduction) in the time right after flooding when NO₃⁻ and oxidized Fe might have been abundant. However, as the soil became reduced due to increased SOC degradation activity and limited O₂ supply, electron acceptors other than SO₄²⁻ were rapidly depleted and SR became the dominant respiration pathway.

By combining results from flux and jar experiments it was possible to confine the relative importance of different microbial respiration pathways in flooded soils. The difference between TCO₂ effluxes (aerobic + anaerobic processes) and TCO₂ production in jar experiments (anaerobic processes) suggested that aerobic respiration only played a minor role in the flooded soils (18 and 10 % in UC and C soil, respectively). On the other hand, SR was quantitatively a very important pathway, constituting 63 and 36 % of total C-mineralization to TCO₂ in UC and C soil, respectively. Hence 19 (UC) to 54 % (C) of TCO₂ production occurred by respiration processes not directly accounted for. Weston et al. (2006) found that Fe reduction was responsible for about 60 % of CO₂ production in the first 4 days after saltwater intrusion in coastal soils. When considering the high initial concentrations and the rapid decrease in soil RFe(III) in our experiment (Fig. 6), respiratory Fe-reduction was probably an important respiration process initially. However, based on this experiment it was not possible to distinguish between biological and chemical Fe-reduction.

4.3 Will newly flooded coastal habitats be hotspots for SOC burial?

In this study we observed that only 6-7 % of the total SOC pool in coastal soils was degraded by microbial processes in the first year after flooding with seawater. The low final SOC degradation rates (especially the very low DOC production) suggested that remaining SOC would be permanently buried due to the limited ability of anaerobic microbial communities to degrade complex organic matter of terrestrial origin (Burdige, 2007; Canfield, 1994; Hedges and Keil, 1995). Neubauer et al. (2013) similarly found long-term reduction of degradation rates and lability of SOC pools in a tidal freshwater marsh experiencing saltwater intrusion, which also support preservation of the SOC pool. Considering that terrestrial non-flooded vegetated soils generally have CO₂ effluxes in the order of 0.1 to >1 mol m⁻² d⁻¹ (Chirinda et al., 2014; Fang and Moncrieff, 2001; Hursh et al., 2017; Rustad et al., 2001), which is much higher than measured in the flooded soils in this study (Table 4), it appears that flooding of coastal soils with seawater, due to either sea level rise or mitigation techniques such as coastal realignment, will cause reduced CO₂ efflux and a negative feedback on atmospheric CO₂ concentrations. This negative feedback may constitute a marked C-sink. For instance, a detailed investigation of the soil characteristics in the topsoils (down to 20 cm) at Gyldensteen Strand suggests 48±6·10³ kg SOC ha⁻¹ (average ± SEM, n = 30, T. Valdemarsen, unpublished results). Hence, when assuming that about 10 % of the soil organic matter will be degraded after flooding, the

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nature restoration project at Gyldensteen Strand (211 ha) constitutes an immediate C-sink of about $9 \cdot 10^6$ kg SOC (or about 0.02 % of the total annual CO₂ emission of Denmark).

4.4 Efficient Fe-driven sulfide buffering in flooded soils

5 Accumulation of free H₂S is often seen in metabolically active organic enriched marine sediments, where it has toxic effects on benthic fauna (Hargrave et al., 2008; Valdemarsen et al., 2010). It was therefore a concern if free H₂S would accumulate in the soils from Gyldensteen after flooding, since this could hamper the succession of benthic fauna as well as overall ecological developments. However, despite the extremely high initial SR rates in the flooded soils, comparable to SR measured beneath fish farms (Bannister et al., 2014; Holmer et al., 2003) no accumulation of free H₂S occurred in any of the soil types. Dent (1986); Portnoy and Giblin (1997); Weston et al. (2011) also observed a similar lack of H₂S accumulation in soils introduced to saltwater, suggesting that newly flooded soils have a high capacity to buffer H₂S. Budget considerations suggest that most of the produced H₂S was immediately re-oxidized, e.g. with O₂ in the surface soils, while a significant proportion (37 and 93 % in UC and C soil, respectively) precipitated as different Fe-S compounds, for instance FeS and Fe₃S₄ in AVS and FeS₂ and S⁰ in CRS (Reddy and DeLaune, 2008; Rickard and Morse, 2005; Valdemarsen et al., 2010). The depth profiles of solid Fe and S showed that sulfide precipitation occurred at the same depths where active SR was measured, i.e. in the upper 10 cm in UC soil and down to 20 cm depth in C soil. The decreasing microbial activity and increasing Fe(II) over time will create a long term sulfide buffering capacity in the soil (Schoepfer et al., 2014).

5 Conclusions

In this study a rapid stimulation of heterotrophic microbial degradation of SOC was observed in two different soils (uncultivated or cultivated) following flooding with seawater. Degradation rates peaked in the first 2 months after flooding, and hereafter gradually declined to low levels after 1 year. Microbial SR was rapidly established in both soil types and was the dominating respiration pathway. Nevertheless, despite extremely high SR rates, H₂S did not accumulate in the soils as it was re-oxidized with O₂ at the soil-water interphase or precipitated with Fe to form AVS and CRS. All three hypotheses stated initially were confirmed. Total SOC degradation activity in the tested soils clearly did depend on SOC content (hypothesis 1) and was 3-fold higher in organic rich uncultivated soil compared to the organic poor cultivated soil. However, only a small proportion of SOC (6-7 %) was degraded in the first year after flooding, and when considering the low final SOC degradation rates, it appears that most SOC is non-degradable under anoxic marine conditions and will be preserved after flooding (hypothesis 2). Hence this study suggests that in soils flooded with seawater the majority of SOC will be permanently preserved in comparison to non-flooded soils, therefore creating an overall negative feedback on atmospheric CO₂ concentrations (hypothesis 3).

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Comment [22]: Revisions according to comment 1.3 and 2.14

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Table 1 Mean values of water content, porosity and soil organic carbon (SOC) for all core sectionings. Error indicated as SEM (n = 15).

| | Depth (cm) | Water content (%) | Porosity | SOC (%) |
|---------------|------------|-------------------|-------------|------------|
| Station UC | 0.5 | 82.9 ± 0.7 | 0.82 ± 0.04 | 16.2 ± 0.8 |
| | 1.5 | 75.5 ± 1.6 | 0.97 ± 0.02 | 16.1 ± 1.2 |
| | 3.5 | 60.5 ± 1.8 | 0.79 ± 0.01 | 11.0 ± 0.8 |
| | 7.5 | 39.3 ± 0.9 | 0.60 ± 0.01 | 5.2 ± 0.2 |
| | 12.5 | 33.0 ± 0.7 | 0.54 ± 0.01 | 3.5 ± 0.2 |
| | 17.5 | 34.5 ± 0.8 | 0.56 ± 0.01 | 3.5 ± 0.2 |
| Station C | 0.5 | 32.0 ± 0.6 | 0.58 ± 0.02 | 1.4 ± 0.0 |
| | 1.5 | 24.8 ± 0.5 | 0.53 ± 0.01 | 1.1 ± 0.0 |
| | 3.5 | 21.6 ± 0.3 | 0.40 ± 0.01 | 1.0 ± 0.0 |
| | 7.5 | 18.9 ± 0.4 | 0.35 ± 0.01 | 0.8 ± 0.1 |
| | 12.5 | 17.9 ± 0.3 | 0.34 ± 0.00 | 0.9 ± 0.0 |
| | 17.5 | 19.8 ± 0.4 | 0.37 ± 0.01 | 1.0 ± 0.0 |

Table 2 Carbon budget table showing mean soil organic carbon (SOC) \pm SEP (n = 18) in uncultivated (UC) and cultivated (C) soil. Total time integrated efflux and accumulation of total carbon dioxide (TCO₂) and dissolved organic carbon (DOC) in porewater are also shown.

| Carbon budget (mol m ⁻²) | Station UC | Station C |
|---|----------------|----------------|
| Initial SOC pool | 710.9 \pm 54 | 232.5 \pm 22 |
| TCO ₂ efflux | 39.9 | 11.2 |
| DOC efflux | 8.9 | 2.4 |
| TCO ₂ porewater accumulation | 0.1 | 0.8 |
| DOC porewater accumulation | 0.7 | 0.5 |
| Total SOC degradation | 49.6 | 14.8 |
| Percentage of SOC pool degraded | 7 % | 6 % |

Table 3 Budget table based on cumulative time integrated total degradation to carbon dioxide (TCO₂), and TCO₂ production and sulfate reduction (SR) measured in jar experiments (anaerobic rates) at different times after flooding (1 week [1W] and 2, 4, 6 and 12 months [2M, 4M, 6M and 12M, respectively). Relative contributions of SR and other anaerobic respiration processes to total anaerobic TCO₂ production (jar experiments) at different time points after flooding.

10

| | | Station UC | | | | | Station C | | | | |
|---|---|------------|------|------|------|------|-----------|-----|-----|-----|------|
| | | 1W | 2M | 4M | 6M | 12M | 1W | 2M | 4M | 6M | 12M |
| Cumulative degradation to TCO ₂ (mol m ⁻²) | Total degradation to TCO ₂ | 2.07 | 10.4 | 18.8 | 27.4 | 40.0 | 0.5 | 2.7 | 5.0 | 6.6 | 12.0 |
| | Anaerobic degradation to TCO ₂ | 0.8 | 8.7 | 19.9 | 24.2 | 32.6 | 0.3 | 2.5 | 6.0 | 8.0 | 10.8 |
| | TCO ₂ production by SR | 0.3 | 4.7 | 12.9 | 16.9 | 25.3 | 0.1 | 0.8 | 2.2 | 3.1 | 4.3 |
| Relative contributions (%) | SR | 42 | 63 | 91 | 100 | 100 | 29 | 30 | 54 | 23 | 93 |
| | Other anaerobic respiration | 58 | 37 | 9 | 0 | 0 | 71 | 70 | 46 | 77 | 7 |

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Comment [23]: Revised according to comment 1.9, 1.10 and 3.3

Table 4

| | CO ₂ efflux (mmol m ⁻² d ⁻¹) | Study |
|---------------------------------|--|---------------------------|
| Danish coastal marine sediments | 33 | Valdemarsen et al. (2010) |
| | 24-45 | Valdemarsen et al. (2014) |
| Agricultural soils | 46-167 | Chirinda et al. (2014) |
| | 69-111 | Fang and Moncrieff (2001) |
| | 386-1374 | Rustad et al. (2001) |
| | 182±96 | Hursh et al. (2017) |
| Soils flooded with seawater | 29-67 | This study |

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Comment [24]: Added according to comment 1.1, 1.2, 2.2 3.1, 3.19

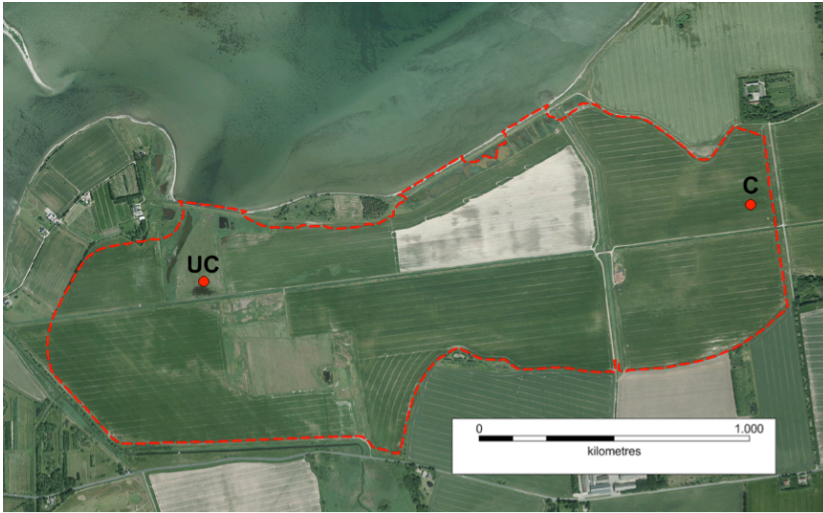
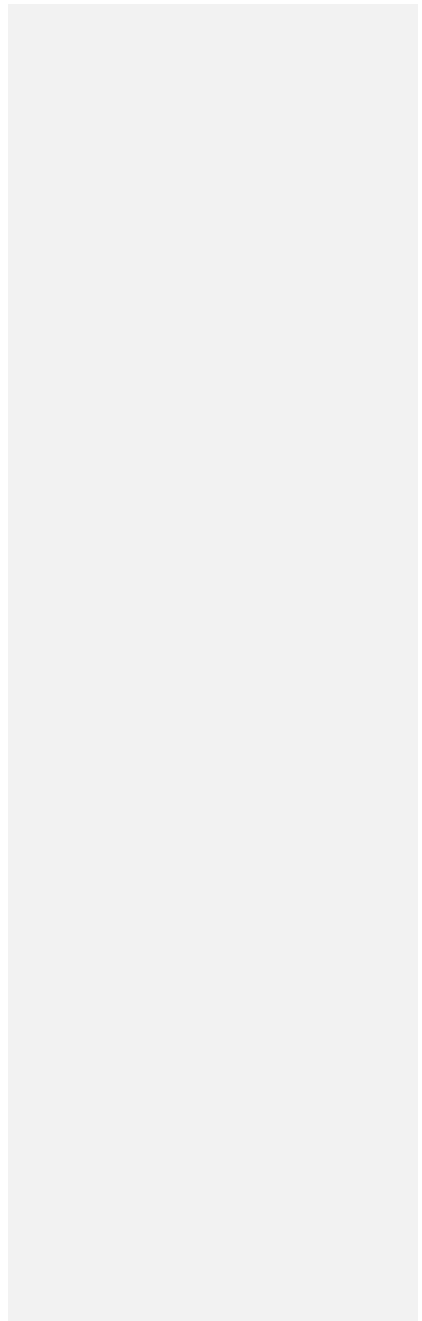


Figure 1 Map of Gyldensteen Strand with the location of the 2 sampling stations for collecting uncultivated (UC) and cultivated (C) soil cores. The dashed red line indicates the area flooded with seawater in March 2014.



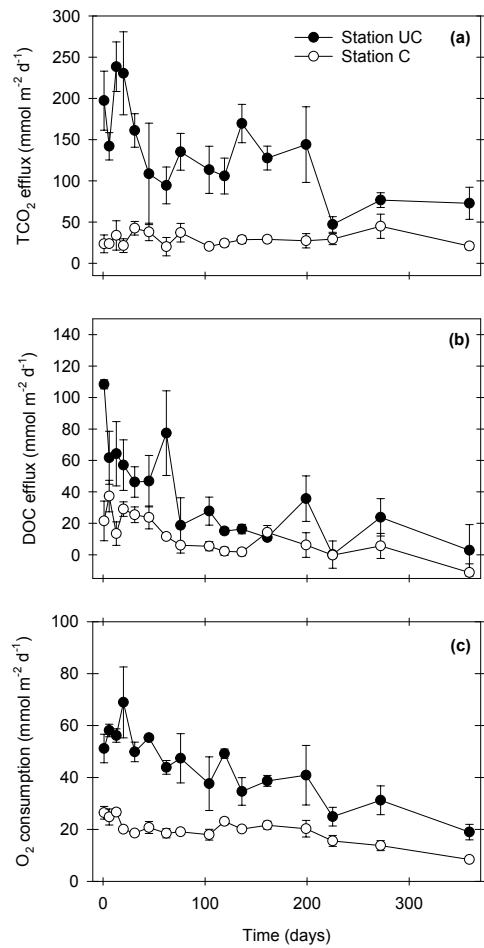


Figure 2 Fluxes of total carbon dioxide (TCO₂, A), dissolved organic carbon (DOC, B) and oxygen (O₂) consumption (C) in soil cores with uncultivated (UC) and cultivated (C) soil after flooding. Error bars indicate SEM (n = 3).

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Comment [25]: Revised according to comment 2.16

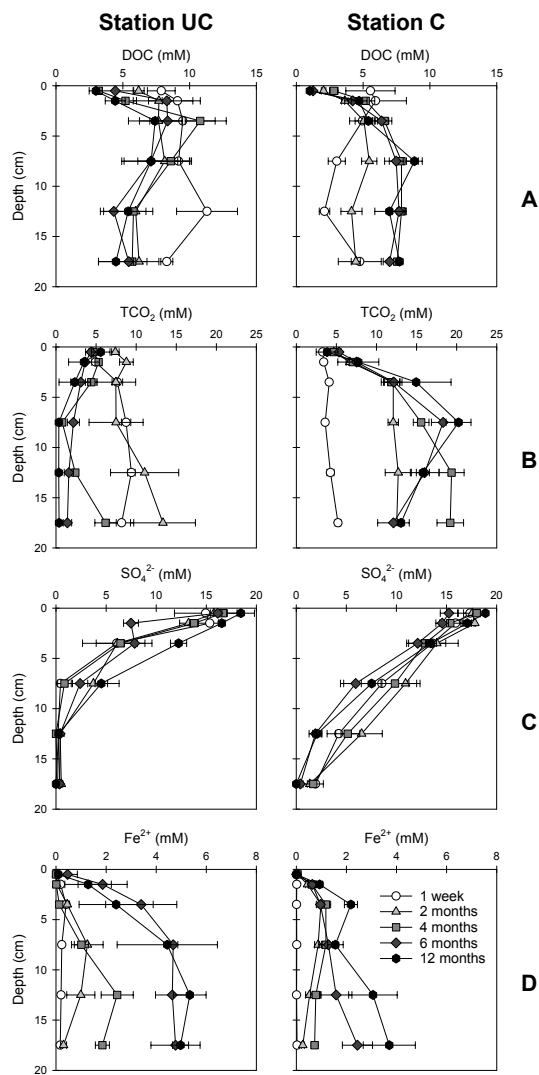


Figure 3 Porewater profiles for dissolved organic carbon (DOC, A), total carbon dioxide (TCO₂, B), sulfate (SO₄²⁻, C) and Fe²⁺ (D) in uncultivated (UC) and cultivated (C) soil flooded with seawater. Error bars indicate SEM (n = 3).

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Comment [26]: Revised according to comment 1.13 and 3.22

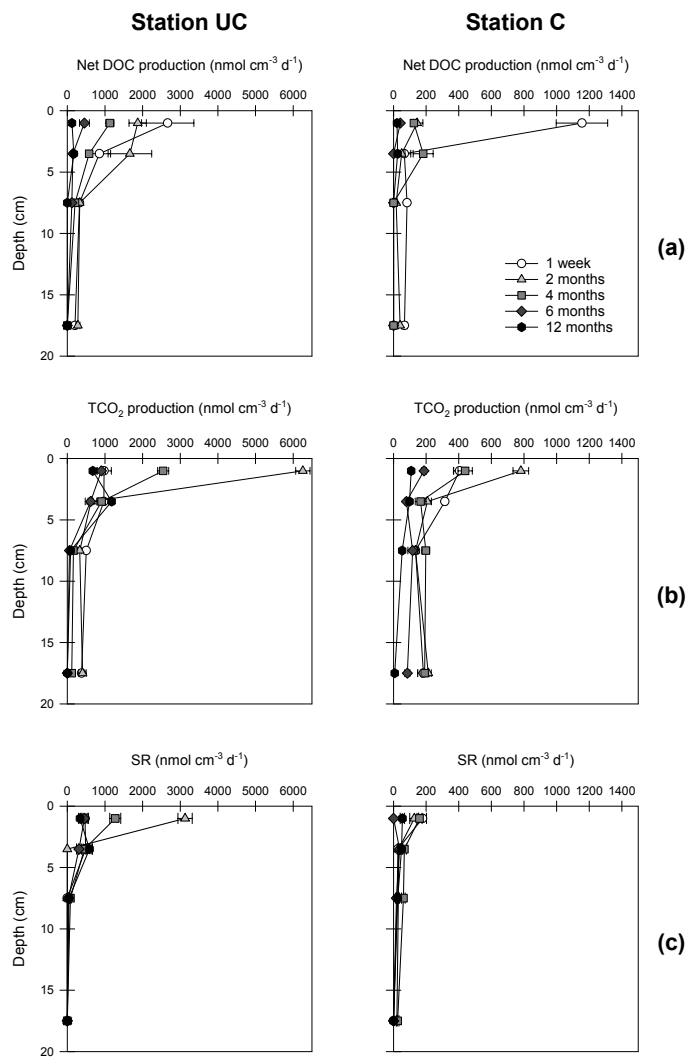
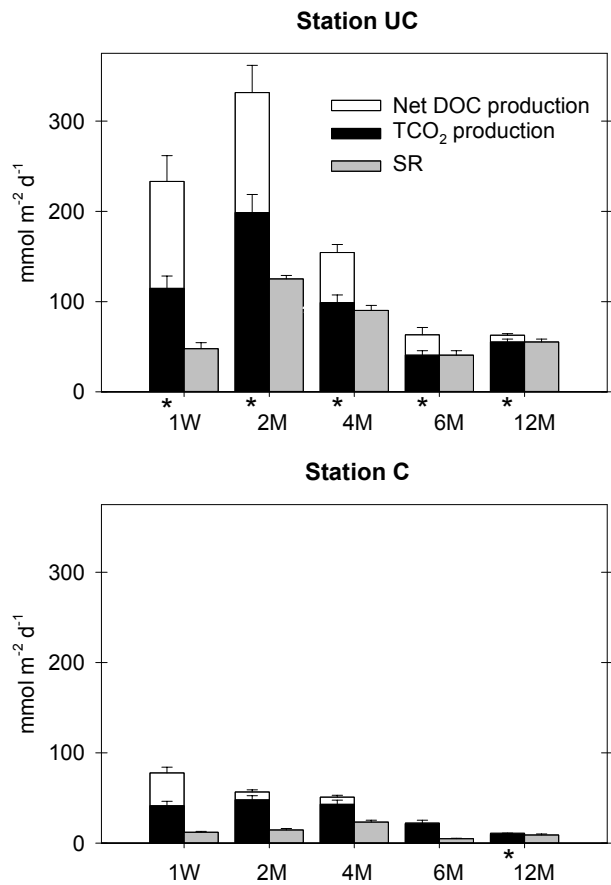


Figure 4 Temporal and spatial variability in production of dissolved organic carbon (DOC, A) and carbon dioxide (TCO₂, B) and sulfate reduction (SR) measured in jar experiments with uncultivated (UC) and cultivated (C) soils flooded with seawater. Note the different x-axis scaling for station UC and C measurements. Error bars indicate SEM.

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Comment [27]: Revised according to comment 1.13 and 3.22



5 Figure 5 Results from jar experiments showing area specific net production of dissolved organic carbon (DOC) and total carbon dioxide (TCO₂), and sulfate reduction (SR, based on SR rate measurements converted to C-units) in uncultivated (UC) and cultivated (C) soil at different times after flooding (1 week [1W] and 2, 4, 6 and 12 months [2M, 4M, 6M and 12M, respectively]). In columns marked with *, TCO₂ production was corrected with 2 x SR. Error bars indicate SEP (n = 4).

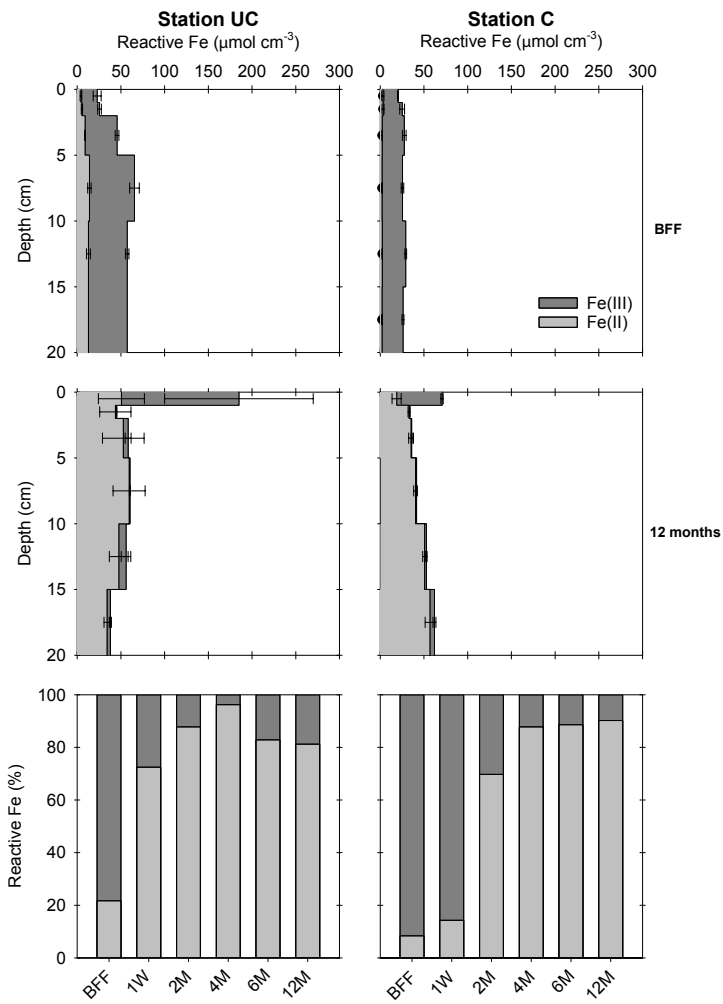
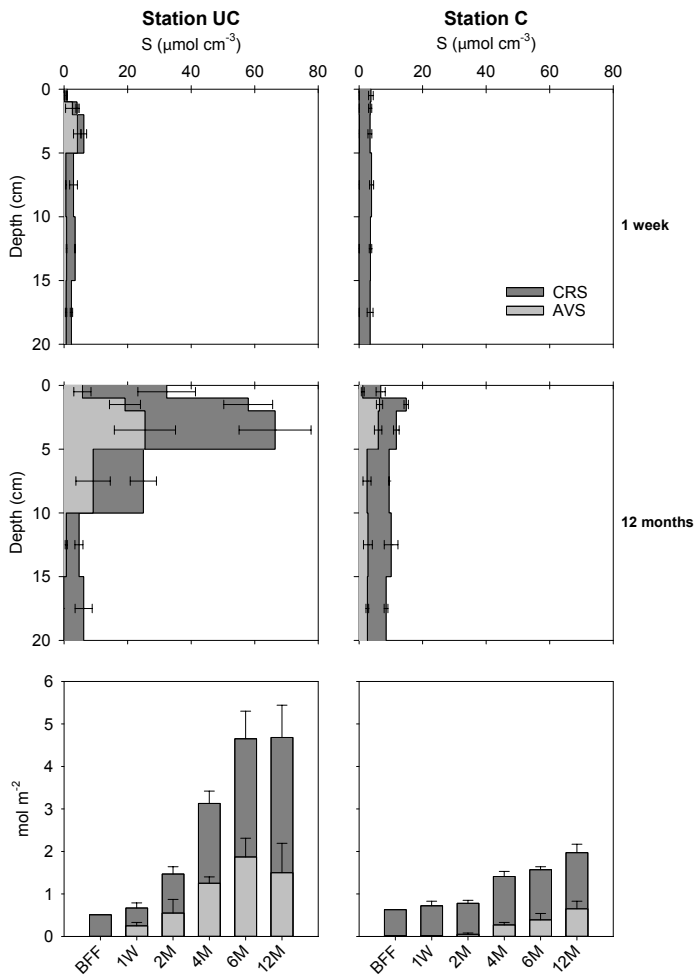


Figure 6 Upper panels show concentration of reactive Fe(II) and Fe(III) in uncultivated (UC) and cultivated (C) soils before flooding (BFF) and 12 months after flooding. Lower panels show the relative contributions of reactive Fe(II) and Fe(III) in the upper 20 cm at various times after flooding (1 week [1W] and 2, 4, 6 and 12 months [2M, 4M, 6M and 12M], respectively). Error bars indicate SEM ($n = 3$).

5



5 **Figure 7** Upper panels show concentration of chromium reducible sulfides (CRS) and acid volatile sulfides (AVS) in uncultivated (UC) and cultivated (C) soils before flooding (BFF) and 12 months after flooding. Lower panels show the depth integrated pools of AVS and CRS in the upper 20 cm at various times after flooding (1 week [1W] and 2, 4, 6 and 12 months [2M, 4M, 6M and 12M], respectively). Error bars indicate SEM ($n = 3$).