

Dear editor,

We have now carefully addressed the comments from the two reviewers concerning the revised version of the manuscript. Our responses to individual comments are outlined in red in the text below. We are happy to see that both reviewers are generally positive towards the manuscript.

We have given special concern to the critical comments from Reviewer#2 regarding the wider implications of this study. We agree that we may initially have been too bold when describing the implications of our study. In the revised manuscript we have markedly down-played the climate aspects of the story.

We hope that the revised manuscript is acceptable for publication in Biogeosciences!

Sincerely,

Kamilla S. Sjøgaard

Response to comments from Reviewer#1

Dear dr. Sjøgaard,

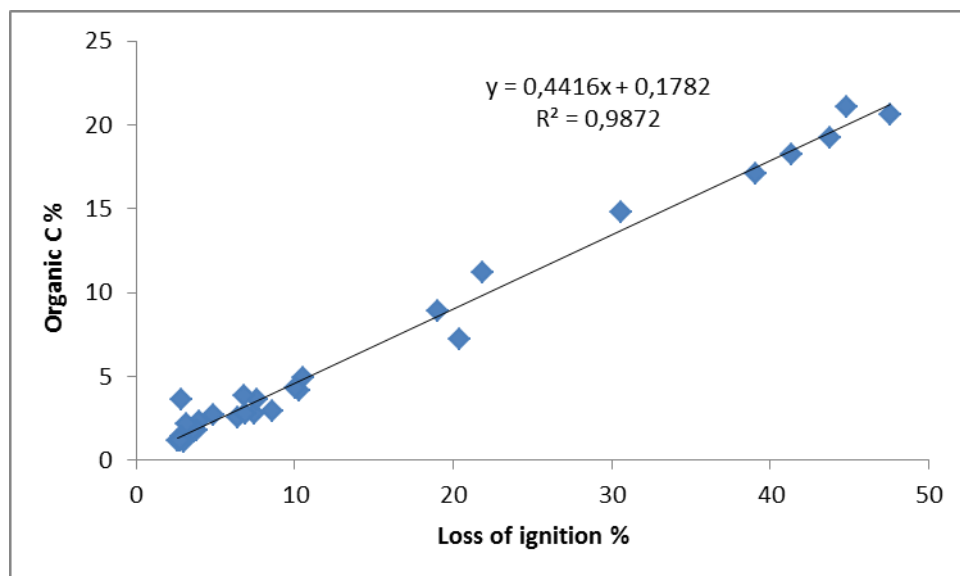
Thank you for this substantial revision of the manuscript, it does look much better now, and I only have a few (minor) comments and suggestions left.

Author response: *We thank Reviewer#1 for constructive criticism. We hope we have managed to respond to her/his comments and suggestions in a satisfactory manner.*

Reply to comment 1.6:

I was not very clear in this comment, my apologies. My question was: If you analyzed OC of only 2 time points, did that proved a big enough range to warrant a linear regression (i.e., if the OC vs. OM contents cluster at the two end-points of the regression, you will have a significant regression, but the equation will not necessarily be correct as the slope will be very dependent on the endpoints). Could you perhaps add a plot of the OM vs OC as Supplementary Information?

Author response: *The datapoints were more or less evenly distributed within the range of the dataset and clustering was not a problem. The regression can be seen below. We do not think these data are so important that they should be added as supplementary material, but will do so if needed.*



P4L14: were the cores sliced in an anoxic atmosphere?

Author response: *No*

P5L17: did you use a statistical test to check for outliers? Or did you remove them on sight (which is acceptable, if they are obvious, but that has to be mentioned)

Author response: *No, no formal statistical outlier tests were performed. Line was rephrased to: "...changes by linear regressions after removing obvious outliers (visual check)."*

P6L5: 'accumulated porewater TCO₂ at different time points ...' -> I assume that this is the accumulation, correct for the accumulation at the time before (i.e. integrated PW TCO₂ at month 2 - integrated PW TCO₂ at week 1 = produced TCO₂ between week 1 and month 2)?

Author response: *Correct.*

P6L16-18: Do you have any idea of the accumulation rate of the sediment? Can you estimate which depth part of the soil is still marine? Considering that you can still find shell material, some part will be the old marine sediment. And thus, how much of the organic carbon is actually soil organic carbon?

Author response: *This is very difficult as the area has been used for agriculture and has been mechanically reworked. We will at a later stage attempt to do deep cores in the area to check if we can find a "marine signal" deep in the soil.*

Section 3.2 and lower: give standard deviations if you show average values

Author response: *Done*

Section 4.2: the two paragraphs are kind of repetitive (first one talks about SR and mentions O₂ and other electron acceptors, the second one talks about O₂ and others and mentions SR); you could shorten this by combining them and streamlining the text.

Author response: *Thanks for the suggestion. We prefer to keep the two paragraphs as they are. We think mixing the information in the two paragraphs will result in confusion.*

Section 4.4: it might be nice to try and make a rough estimation of the time scale over which this buffering will stay active (assuming the rate of sulfate reduction at the end + the percentage that precipitates + the content of reactive non-sulfurized FeII and FeIII left).

Author response: *We think it is very difficult to come up with a credible estimate based on simple considerations. Especially since more Fe is available for precipitation with sulfide than we can account for – total Fe extractions require much harsher extraction methods than utilized here.*

P12L25 could these high effluxes of CO₂ also be due to the absence of CaCO₃ precipitation, or is this a negligible effect?

Author response: *This part was revised in response to a comment by Reviewer#2 and comment is no longer relevant.*

Table 3: the relative contribution numbers do not seem to add up, so I am not sure if I understand the table correctly. First row:

TCO₂ measured based on the whole core incubations.

Second row: TCO₂ production in anaerobic jar incubations.

Third row: Sulfate reduction in carbon units (so converted 2:1).

Fourth row: relative contribution of SR to anaerobic respiration (so third row/second row * 100).

Fifth row: relative contribution of other anaerobic pathways (so (1- third row/second row)*100)

If this is correct, then your percentages do not make sense (if I estimate the relative contribution of SR to total anaerobic respiration, I get these values): $0.3/0.8 = 0.375$, $4.7/8.7 = 0.54$, $12.9/19.9=0.64$, etc.

Also, I would suggest to maybe change the table: Keep the first two row, and then show the relative contributions of aerobic respiration – sulfate reduction – other anaerobic pathways.

Author response: We have changed table 3 in accordance with this comment.

Response to major comments from Reviewer#2

Sjøgaard et al. present an impressive data set exploring the effects of seawater flooding on carbon biogeochemistry in (1) agricultural and (2) a freshwater reedswamp with high temporal resolution over an extended 1 year time period (again, impressive). While the experiment itself was clearly well executed and the authors do a very nice job constructing a metabolic budget given the experimental constraints, the study design does not address the original hypotheses and cannot support the type of strong conclusions drawn by the authors.

Author response:

- *We thank Reviewer#2 for constructive criticism and are glad that he/she acknowledges that the study is based on a substantial experimental effort and a solid data-set that deserves to be published. We also acknowledge that Reviewer#2 feels very strongly that we are over-interpreting the data in some instances. In some instances we agree with the reviewer and have revised the text accordingly (see text below). In other cases, we argue our case and hope that the reviewer can see our point and accept our revisions.*

The authors have responded constructively to the original reviewer comments regarding methodology and literature, but have not addressed the key concerns from the original reviewers regarding the bulk of their interpretation of the data. Because there are no measurements from unflooded soils (C) or freshwater flooded soils (UC), there is no ambient condition with which to compare the effects of seawater flooding. Furthermore, there is no methodological control (i.e. cores from a seawater lagoon) to assess whether the laboratory handling (not seawater flooding) were responsible for the observed patterns. This does not doom the study, but severely restricts the type of solid conclusions that can be drawn to those regarding the effect of seawater on agricultural versus reedswamp soils only. In fact, the authors barely discuss the implications of antecedent conditions in the two sites (C & UC) (besides organic content) but it seems that the fact UC was a saturated (anaerobic) reedswamp is very important for framing and interpreting the results.

Author response:

- *The experimental set-up and vigorous sampling scheme prevented us from including additional experimental series in the experiment (for instance a series with unflooded soil, which would require a completely different sampling strategy or ambient controls from a seawater lagoon). However, the last author of the manuscript (TBV) has previously run similar, extensive long term experiments with different sediments from Odense Fjord – a lagoon very close to the sampling sites described in this manuscript (see Valdemarsen et al. 2014 (MEPS) and 2015 (Biogeosciences)) – we therefore have a kind of a methodological control as requested by the reviewer, and relatively good experience with interpreting results of this kind. We do not understand the critique in the last sentence – we very clearly state that the two soils under investigation have different origin and different characteristics, i.e. agricultural soil vs. reedswamp soils, and the whole point of the study is to see how the biogeochemistry develop in the different soils after flooding.*

While some speculation regarding impacts on broader C cycling is appropriate, the current manuscript extends speculation to strongly-worded conclusions regarding coastal carbon sinks that convey far more certainty than is appropriate. All three original referees objected to the author's conclusions as they are based on a fundamental misunderstanding of carbon-climate feedbacks and it is disappointing that the authors continue to emphasize this point over more evidence-based conclusions from their rich data set.

Author response:

- *We have removed or significantly down-played the strongly-worded conclusions regarding impacts of the current study in relation to global C-cycling, in order to accommodate the reviewer (see text below).*

Therefore the paper must undergo major revisions before it can be considered for publication. The following hypotheses and discussion pertinent to them must be significantly re-written:

H1 & H2: The authors cannot assess whether the origin or lability of organic matter plays any role in SOC degradation because lability is not measured and there is no control provided to show that recently flooded soils have a higher C loss than marine sediments under the same experimental conditions. While the chemical structure of material certainly impacts degradation, this is simply not measured in the current study and should not be the main focus.

Author response:

- *We disagree that the first hypothesis is not relevant and valid. To recap: H1 (page 2, line 34) states that total SOC degradation activity in soils after flooding depends on SOC content and lability.*
- *The reviewer seems to partially object to these hypotheses because 'organic matter lability' was not measured. We agree that organic matter lability was not measured directly, as in quantifying organic matter structure by biochemical analysis. However, the term lability in H1 should be perceived as a relative term related to the overall degradation patterns observed in the two soil types and compared to existing knowledge about carbon degradation processes in contemporary marine sediments. There is a whole body of literature regarding organic matter degradation in marine sediments supporting this use of the terms 'labile' and 'refractory' organic matter based on differences in degradation rates (e.g. Westrich and Berner 1984, Canfield 1994, Hedges and Keil 1995, Kristensen and Holmer 2001, Valdemarsen et al. 2009 etc. etc.).*
- *In the manuscript we compare the organic carbon degradation rates in a relatively organic rich soil (e.g. visibly rich in roots, organic debris) to a relatively organic poor soil (e.g. almost no visible organic debris). We find that the C-degradation rates are much higher in the organic rich soil and that in both soil types C-degradation appears to attenuate towards a much lower level. Ergo "We find that flooding of soils with differing soil organic content results in different, post-flooding degradation patterns, which can*

only be explained by organic higher content and/or lability in the organic rich soil.” We therefore confirm H1 (page 13, line 28).

- *With regards to H2 (page 3, line 1) stating that “most SOC at the time of flooding will, due to its terrestrial origin, be non-degradable and hence preserved under the anoxic conditions formed after the flooding.” We agree that this hypothesis was too boldly stated in the original version of the manuscript. We have revised it to: “a large proportion of SOC will be non-degradable due to the anoxic soil conditions forming after the flooding.” Similarly we have rephrased the conclusion based on this hypothesis (page 13, line 13) to: “it appears that a large proportion of SOC is non-degradable under anoxic marine conditions and will essentially be preserved after flooding (hypothesis 2).” The reason for maintaining the hypothesis/conclusion is described below.*
- *Degradation in aquatic sediments follows exponential decay kinetics, meaning that rates decreases gradually with time as degradable organic C is depleted (e.g. Westrich and Berner 1984). In the current experiment we also found decreasing degradation rates in both soil types, although the temporal attenuation was much higher in the organic rich soil than in the organic poor soil. In both soil types, only about 6 to 7% of the initial soil organic C was degraded during the first year, and when considering the temporal decay patterns – and ESPECIALLY the DOC production close to 0 after 1 year - it is highly unlikely that the soil organic matter in any soil type would have been degraded within foreseeable time if the experiment had continued. We therefore conclude that: “a large proportion of SOC is non-degradable under anoxic marine conditions and will essentially be preserved after flooding.” This statement follows the conclusions from a similar experiment lasting for 2 years, with 8 marine sediments collected in a nearby lagoon (Valdemarsen et al. 2014), where exponential decay patterns were explored in greater detail. Based on marine sediments showing similar decay patterns, generally <60-89% of organic matter present at the time of sampling appeared to be degradable under anoxic conditions. All though not directly comparable, we believe that a similar conclusion holds for the current case.*

H3: the study cannot support the key conclusions of the paper, namely that seawater flooding will preserve C, because the authors do not measure SOC degradation prior to flooding and their comparisons to agricultural rates rely on rates from disparate systems (some tropical, some global averages) measured largely in-situ (they therefor include autotrophic (root) respiration and are not comparable to the present study. Furthermore, respiration is meaningless if we don't know what gross primary productivity is. What is important is the net exchange of C, which is not addressed in this comparison with other studies. We can assume GPP it is 0 for the cores, so respiration is the only number in the equation ($GPP-R=NEE$) and the net exchange is negative (i.e. always out of the system). This is not the case for the other ag systems from the review. While it is clear that flooded soils preferentially preserve carbon in general, the authors should support this with outside literature as there results do not directly address this.

The conclusion that flooded site will constitute and immediate C-sink/negative carbon-climate feedback is highly objectionable and has great potential for misuse and misunderstanding. This is not a matter of data

interpretation or an over-extension of data, it is incorrect. I implore the authors to consult the IPCC or Verified Carbon Standards (VCS) for finite definitions of the terms sink, stock, and source as they apply to greenhouse gas feedbacks. These terms are well established and clearly defined by the global change community.

Why the conclusion that flooded site will constitute an immediate C-sink/negative carbon-climate is inaccurate:

Agricultural soils can and do sequester C through the accumulation of crop residue (C sink), albeit at a lower rate than natural systems. As is the case with some drained agricultural land, it is also possible that SOC from reclaimed marine/intertidal/marsh sediments is still being lost to aerobic oxidation at a higher rate than crop residue is accumulating (C source). The authors present no evidence for either case as the antecedent (pre flood) condition was not measured. Thus there is no baseline to conclude how the direction of C flux has changed.

While it is not clear what the end-point of this particular coastal managed realignment is (subtidal or intertidal mudflat? subtidal seagrass bed? intertidal wetland?), the current study supposes it is a subtidal flat (always flooded, no vegetation) which can lead to 1 of 3 outcomes:

(1) assume ag land (C) was a C source (carbon emissions as respiration > crop C uptake) and flooding preserved C (100% preservation in 1 year = C stock) this is not the case in this study and even if it was a system simply cannot be a C sink/negative feedback unless primary production is removing C from the atmosphere. Prevented emissions do not equal negative carbon-climate feedbacks because C is NOT being removed from the atmosphere. Zero emission scenario.

(2) assume ag land (C) was a C source, even at the measured 93% preservation in 1 year, the site is small C source and is a candidate for reduced emissions only, again not a sink, no negative climate feedback.

(3) ag land was a small sink (carbon uptake from crops > carbon emissions from ecosystem respiration) and flooding preserves 93% of the SOM. Flooding (without vegetation establishment) now makes the site a net source of C and thus there is a positive climate feedback. Furthermore, in the reedswamp (UC) soil, all indications are that saltwater increases respiration in freshwater anaerobic environments (Weston, Neubauer, and many other citations), this represents the potential for positive feedback, not to mention the death of vegetation.

If the soils were vegetated (subtidal seagrass or intertidal wetland) then we have a candidate for a negative feedback.

I will reiterate that the data the authors have produced is interesting and impressive and should be published. It will be of great interest to the coastal community. As is, I have no qualms regarding the methods or data, only the interpretation. I encourage the authors to consider re-writing the hypotheses and discussion/conclusions in a way that emphasizes a direct connection to their results.

Author response:

- *We acknowledge that none of the three authors of the manuscript are experts in climate research, and we may therefore not be fully familiar with the terms and definitions*

related to the work of IPCC. In the original version of the manuscript we concluded that since C-degradation appears to have almost ceased after 1 year of flooding and only 6-7% of initial soil organic C was degraded during the first year after flooding, then most of the soil organic C present at the time of flooding will most likely be permanently buried. We called this a "C-sink", and quantified it to constitute $9 \cdot 10^6$ kg organic C when considering the average organic content in soils at the study site. The basic assumptions for this calculation are OK – after all if 93-94% of SOC remains after one year and net degradation of particulate SOC has ceased then the conclusion that "most SOC will be preserved" must be valid. However, we did one major error: we used the term C-sink without checking the correct use of the word "sink". According to the IPCC and the comments of Reviewer#2 a sink has to be permanent, i.e. be a lasting benefit to the global C-budget with annual benefits of more or less the same magnitude. In our case, however, the benefits related to our conclusion is temporary and only relates to the soil organic C being buried in the soils at the time of flooding. Our conclusion was never meant in relation to the long-term C-balance of the area – we agree that we do not have the data to support such a statement.

- *To accommodate Reviewer#2's criticism we have made a number of changes to the manuscript:*
 - *Last sentence of the abstract was revised. Before it was: "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". The revised sentence reads: "On this basis we suggest that most of the organic carbon present in coastal soils exposed to flooding through sea level rise or managed coastal realignment will be permanently preserved."*
 - *We have deleted hypothesis 3 from the manuscript.*
 - *The headline of section 4.3 (page 12) was "Will newly flooded coastal habitats be hotspots for SOC burial?". It was changed to "Fate of SOC".*
 - *Refinement of argument (page 12, line 26): "The low final SOC degradation rates, and especially the very low final DOC production in both soil types, suggest that the majority of SOC present in soils at the time of flooding will be permanently buried..."*
 - *We have deleted the following sentence from section 4.3 (page 12) as it relates to the long-term C-balance of the area of which we have no knowledge: "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.”

- We have rephrased the concluding sentence of section 4.3. It is now clearly stated that we do not think our results indicate that the study area will constitute a C-sink. Concluding sentence now reads: “Hence flooding of coastal soils due to sea level rise or intentional flooding by managed realignment may lead to significant C-preservation. At Gyldensteen Strand SOC burial will be in the order of $48 \pm 6 \cdot 10^3$ kg SOC ha⁻¹ (average \pm SEM, n = 30) when considering a detailed investigation of the soil characteristics down to 20 cm depth (T. Valdemarsen, unpublished results). Nevertheless this C-preservation does not constitute a permanent C-sink as it only relates to the SOC buried in the soils at the time of flooding.”*

- We have revised the concluding sentence in the manuscript. It was: “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).” The revised sentence: “Hence this study suggests that in soils flooded with seawater the majority of SOC will be permanently preserved.”*

Minor comments are included in attached PDF.

Referee Report:

bg-2016-417-referee-report.pdf

Response to minor comments from reviewer#2 (comments extracted from pdf-file)

Page 1, line 7: please provide a more descriptive phrasing that acknowledge manage coastal realignment applies to lands that are would naturally be within the range of tides

Author response: Sentence was revised to: “...protect coastal areas lying below sea-level is intentional...”

Page 1, line 11: Give exact time line (e.g. number of days)

Author response: Sentence was revised to: “We found rapid carbon degradation to TCO₂ one day after experimental flooding and onwards and...”

Page 1, line 14: For the first year of the study

Author response: It is mentioned in line 9 that the study lasted 1 year.

Page 1, line 14: This is misleading as no measure was made of carbon composition. Please remove or restate so it is clear the authors did not measure chemical composition of OM.

Author response: Sentence was rephrased to: “Organic carbon degradation decreased significantly after 6 months, indicating that most of the soil organic carbon was refractory towards microbial degradation under the anoxic conditions created in the soil after flooding.

Page 1, line 16: give duration of expt.

Author response: It is mentioned in line 9 that the study lasted 1 year.

Page 1, line 18: Reduced emissions do not equal a negative carbon-climate feedback. A negative feedback MUST include a mechanism for removing C from the atmosphere. At best, emissions are reduced (still non-zero), so the positive feedbacks are reduced, but the direction does not change.

Author response: Sentence was deleted.

Page 1, line 22: Not all will be familiar with "reclaimed" and "managed coastal realignment" terminology as they are region specific. Make sure to give a brief description of reclaimed as done below for MCR.

Author response: We do not agree that the term “Reclaimed coastal areas” needs to be explained in more detail.

Page 1, line 26: Citation? Gedan et al. 2012 etc.

Author response: The reference has been inserted, from 2011 though

Page 2, line 2: Review paper

Author response: The reference has been deleted

Page 2, line 15: This phrasing is very awkward... particularly "terminally oxidized"

Author response: and yet “terminally oxidized” is grammatically correct and frequently used in scientific texts. No revisions were made in response to this comment.

Page 2, line 16: Furthermore does not fit here. Consider re-organizing paragraph to discuss the basics of OM degradation common to all systems (enzyme hydrolysis, oxidation) and then contrast changes under anaerobic conditions. O₂ is a terminal electron acceptor so flooding introduces a series of ALTERNATIVE terminal electron acceptors and relies more heavily on fermentation

Author response: “Furthermore” was deleted. Sentence was revised to: “Sulfate is abundant in seawater, and microbial sulfate reduction (SR) is therefore expected to become a major mineralization pathway in soils flooded with seawater (Sutton-Grier et al., 2011; Weston et al., 2011).”

Page 2, line 28: This negative feedback only occurs if flooding increases carbon uptake by the system (i.e. via the establishment of seagrass or emergent marsh). The scenario discussed in the paper is emissions reduction ONLY.

Author response: Sentence was revised to “Flooding of coastal soils by sea level rise and coastal realignment may therefore cause significant preservation of the SOC contained in the soils at the time of flooding.”

Page 3, line 15: Depth? Subtidal or intertidal?

Author response: The tidal range in the area is only about ± 30 cm, so most of the lagoon is permanently subtidal with some of the areas closest to the coast being impacted by periodic exposure. Sentence was revised to: “...into a shallow and mostly subtidal marine lagoon.”

Page 10, line 25: Wetland plants such as those from UC are aquatic plants, not terrestrial plants. Please revise terminology as vascular plants and plankton/algae

Author response: We have revised sentence. Sentence now read: “...stations was terrestrial and wetland plants such as grasses, reed and herbs rich in cellulose and lignified tissues...”

Page 11, line 4: The author's assumption that DOC is a proxy for microbial degradation of SOM is highly problematic. DOC can be generated by leaching of dry sediments (abiotic), change in ionic strength or other physicochemical changes, or by the death of microbial communities and leaching of cellular components. The authors must at least acknowledge that DOC can be a poor proxy for enzymatic hydrolysis, particularly as they did not include a control set of cores that were flooded only.

Author response: The first part of the sentence is referring to C-degradation in general and it is not appropriate to mention lysis and leaching here. We agree with the reviewer that initially DOC is not only produced as a result of degradation. Initially leaching as a result of flooding due to cell lysis etc. may be a significant DOC source, and this is mentioned in Page 11, line 10: “Part of this DOC may have leached to the porewater as a result of flooding...” Over the whole experimental duration, most DOC was produced as a result of microbial C-degradation.

Page 11, line 15: This result was attributed to increased flocculation of dissolved OM, not microbial processing

Author response: We have deleted reference to Ardon et al. 2016 here. We have added the sentence: “Valdemarsen et al. 2014 similarly observed gradually decreasing DOC production over 2 years in 8 different sediment types from Odense Fjord, indicating gradual depletion of degradable organic matter despite high sediment organic content and abundance of energetically favorable electron acceptors.”

Page 11, line 25: All microbes require a terminal electron acceptor. Please revise text throughout.

Author response: Sentence was revised to: “indicating that microbes oxidizing DOC to CO₂ adapt slower to flooded conditions than fermenting and hydrolyzing microbes.”

Page 11, line 26: No evidence it is hydrolysis not abiotic process.

Author response: Sentence was revised to: “Similar cases of initial DOC-production due to leaching and/or substrate hydrolysis..”

Page 12, line 23: This Neubauer study would be analogous to the UC site (reedswamp) in the present study and Neubauer and the present study show that the introduction of seawater increases the metabolism of soil carbon. Neubauer showed that saltwater intrusion would enhance SOC loss, not store more carbon.

Author response: We are referring to the conclusion in the Neubauer paper about the effect long term exposure to seawater on SOC degradation rates.

Page 12, line 26: These numbers include autotrophic respiration as well as heterotrophic and are not useful for comparison unless the soil carbon stock is taken into account (i.e. what % of soil C stock is lost to heterotrophic respiration.)

Author response: line and table 4 were deleted. Comment no longer relevant.

Page 12, line 29: Please consider that many agricultural soils do sequester C (remove carbon from the atmosphere via photosynthesis and preserve it as SOM), albeit at a lower rate than natural systems. The authors are only showing C loss from the sediments, which will be a C source unless colonized by some primary producer that will add C.

Page 13, line 1: This is not a sink. This is a stock.

Author response: sentence was revised

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

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5 Correspondence to: Kamilla S. Sjøgaard (kamillasjogaard@biology.sdu.dk)

Abstract. Permanent flooding of low-lying coastal areas is a growing threat due to climate change and related sea level rise. An increasingly common solution to protect coastal areas lying below sea-level is 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 to TCO₂ one day after experimental flooding and onwards 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. Organic carbon degradation decreased significantly after 6 months, indicating that most of the soil organic carbon was refractory towards microbial degradation under the anoxic conditions created in the soil after flooding. During the experiment only 6-7 % of the initial soil organic carbon pools were degraded. On this basis we suggest that most of the organic carbon present in coastal soils exposed to flooding through sea level rise or managed coastal realignment will be permanently preserved.

1 Introduction

20 Sea level rise driven by global climate change is expected to continue for centuries, and will in the near future impact about 70 % of the global coastlines (Church et al., 2013). Rising sea level causes higher and more frequent storm surges and 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 (Gedan et al., 2011). There are an increasing number of projects where coastal soils are flooded with seawater, by managed coastal realignment and similar techniques (Herbert et al., 2015; Pethick, 2002; Wolters et al., 2005).

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Many studies have been performed on freshwater wetlands experiencing salinization [from seawater intrusion](#) and less on diked and drained agricultural soil systems [exposed to flooding](#) (Ardon et al., 2016; Ardon et al., 2013; Portnoy, 1999; Portnoy and Giblin, 1997). [Existing studies show that 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 [soils flooded with seawater, and](#) become anoxic [except for the upper few millimeters](#). In [aquatic anoxic soils and sediments](#) 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 [other](#) microorganisms and [oxidized](#) to carbon dioxide (CO₂) by [the reduction of](#) alternative electron acceptors (e.g. nitrate, Mn oxides, Fe oxides and sulfate) (Arnosti, 2011; Glud, 2008). [Sulfate is abundant in seawater, and microbial sulfate reduction \(SR\) is therefore expected to become a major mineralization pathway in](#) soils flooded with seawater, (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; 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 the SOC contained in the soils at the time of flooding](#).

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) [a large proportion of SOC will be non-](#)

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degradable [due to](#) the anoxic [soil](#) conditions forming after the flooding. To [investigate the response in](#) 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 [and mostly subtidal](#) 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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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 [after removing obvious outliers \(visual check\)](#). 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 [$\text{OC}(\%) = 0.442 \times \text{LOI}(\%) + 0.178$, $r^2=0.987$, $n=36$]. 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. [Errors for soil characteristics, fluxes, porewater and solid pools](#) were calculated as standard errors of the mean (SEM). [Errors for depth-integrated values of microbial rates and solid pools](#) 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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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.

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-oxyhydroxides 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

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 [and wetland](#) plants such as grasses, [reed](#) 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 (Armosti, 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 e.g. cell lysis due to 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. Valdemarsen et al. 2014 similarly observed gradually decreasing DOC production over 2 years in 8 different sediment types from Odense Fjord, indicating gradual depletion of degradable organic matter despite high sediment organic content and abundance of energetically favourable electron acceptors. 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 microbes oxidizing DOC to CO₂ adapt slower to flooded conditions than fermenting and hydrolyzing microbes. Similar cases of initial DOC-production due to leaching and/or substrate hydrolysis outpacing fermentation and SR has been observed before (Armosti et al., 1994), maybe due to lag response in the microbial community (Bruchert and Armosti, 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 %).

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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 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 Fate of SOC

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, and especially the very low final DOC production in both soil types, suggest that the majority of SOC present in soils at the time of flooding will 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). For comparison 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 SOC. Hence flooding of coastal soils due to sea level rise or intentional flooding by managed realignment may lead to significant C-preservation. At Gyldensteen Strand SOC burial will be in the order of 48±6·10³ kg SOC ha⁻¹ (average ± SEM, n = 30) when considering a detailed investigation of the soil characteristics down to 20 cm depth

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(T. Valdemarsen, unpublished results). [However, this C-preservation does not constitute a permanent C-sink as it only relates to the SOC buried in the soils at the time of flooding.](#)

4.4 Efficient Fe-driven sulfide buffering in flooded soils

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 [a large proportion of SOC](#) is non-degradable under anoxic marine conditions and will [essentially](#) be preserved after flooding (hypothesis 2). Hence this study suggests that in soils flooded with seawater the majority of SOC will be permanently preserved.

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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 showing cumulated time integrated total degradation to carbon dioxide (TCO₂) in flooded uncultivated (UC) and cultivated (C) soil, based on TCO₂-fluxes and total anaerobic TCO₂ production based on jar experiments. Estimated partitioning between aerobic respiration, sulfate reduction and other anaerobic respiration processes is also shown. Different times after flooding are indicated by 1W [1 week] and 2M, 4M, 6M and 12 M [2, 4, 6 and 12 months, respectively].

	Station UC					Station C				
	1W	2M	4M	6M	12M	1W	2M	4M	6M	12M
Degradation to TCO ₂ (mol m ⁻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 (mol m ⁻²)	0.8	8.7	19.9	24.2	32.6	0.3	2.5	6.0	8.0	10.8
Aerobic respiration (% of total)	61	16	0	12	18	40	7	0	0	10
Sulfate reduction (% of total)	15	45	65	62	62	20	30	37	39	36
Other anaerobic respiration processes (% of total)	24	39	35	26	20	40	63	63	61	54

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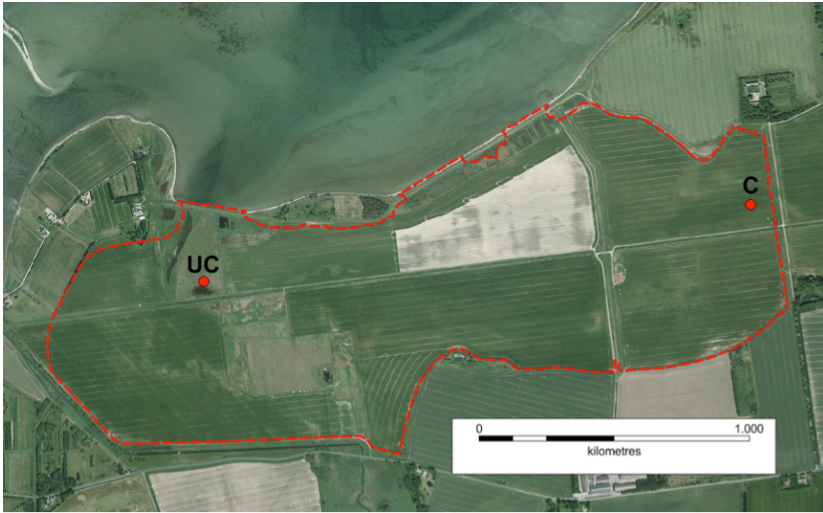
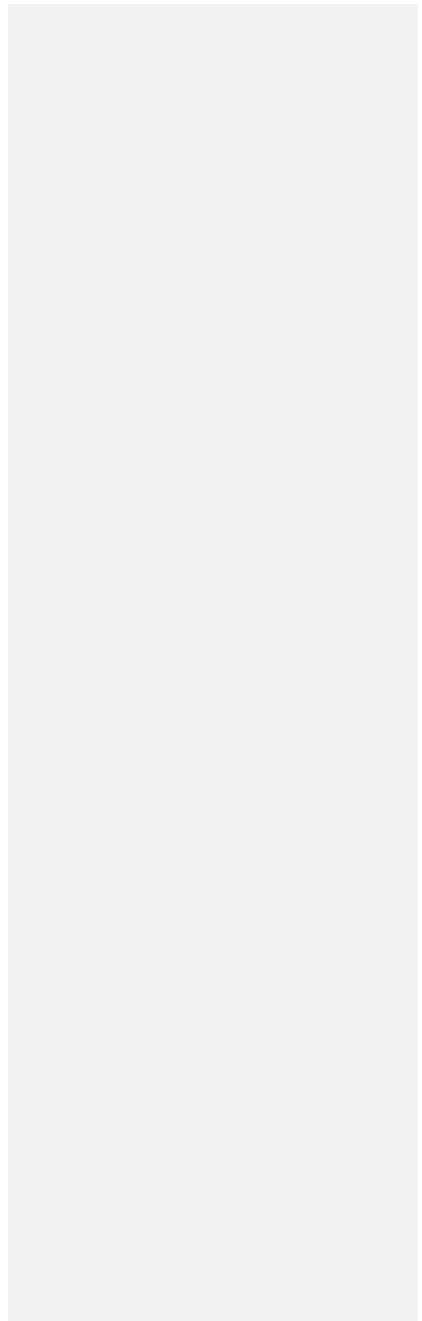


Figure 1 Map of Gyldensteen Strand with the location of the 2 sampling stations for collecting uncultivated (UC) and cultivated (C) soil cores. The dashed red line indicates the area flooded with seawater in March 2014.



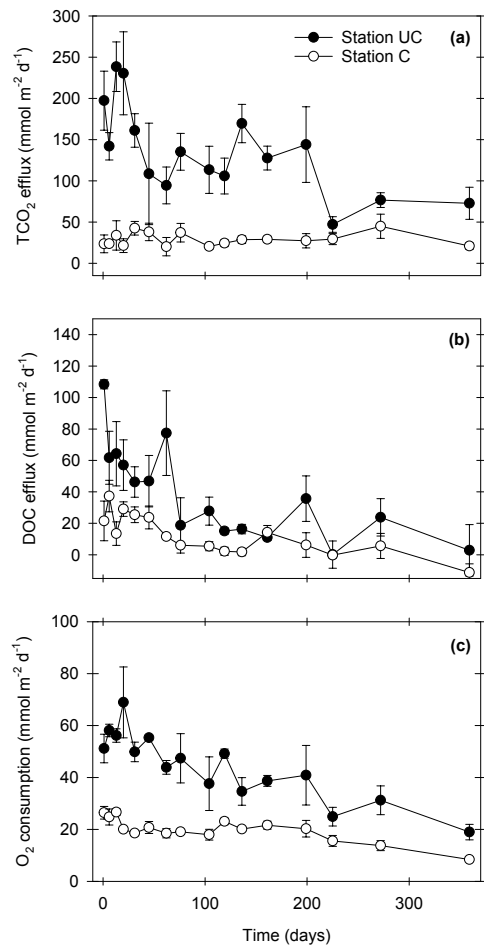


Figure 2 Fluxes of total carbon dioxide (TCO₂, A), dissolved organic carbon (DOC, B) and oxygen (O₂) consumption (C) in soil cores with uncultivated (UC) and cultivated (C) soil after flooding. Error bars indicate SEM (n = 3).

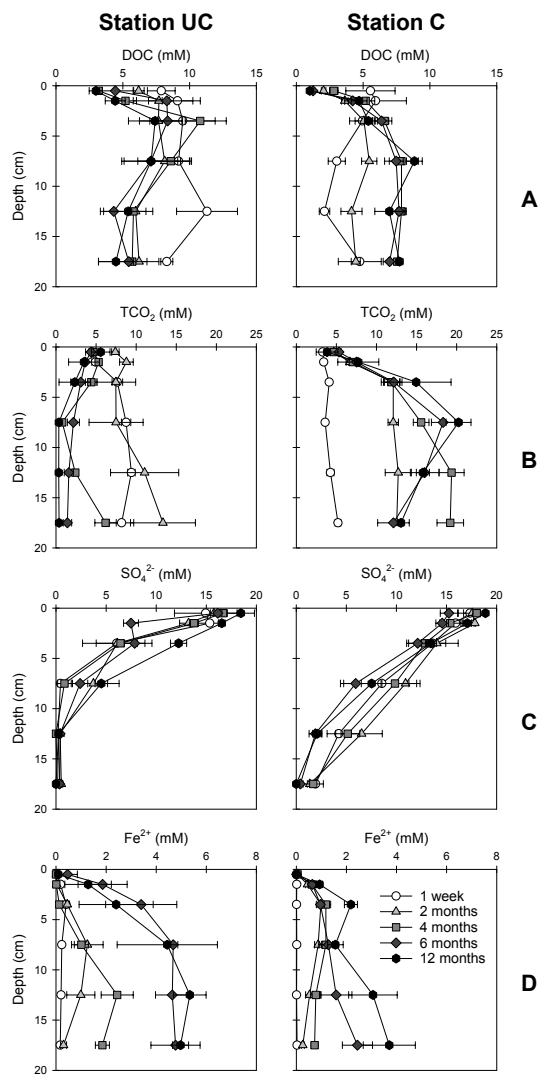


Figure 3 Porewater profiles for dissolved organic carbon (DOC, A), total carbon dioxide (TCO₂, B), sulfate (SO₄²⁻) (C) and Fe²⁺ (D) in uncultivated (UC) and cultivated (C) soil flooded with seawater. Error bars indicate SEM (n = 3).

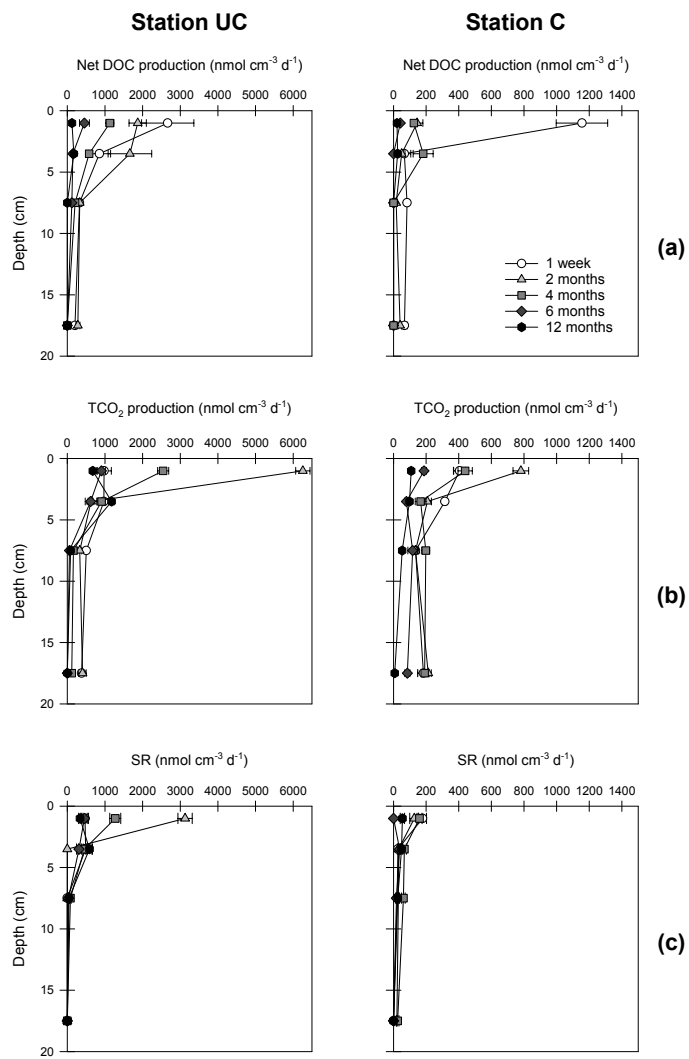
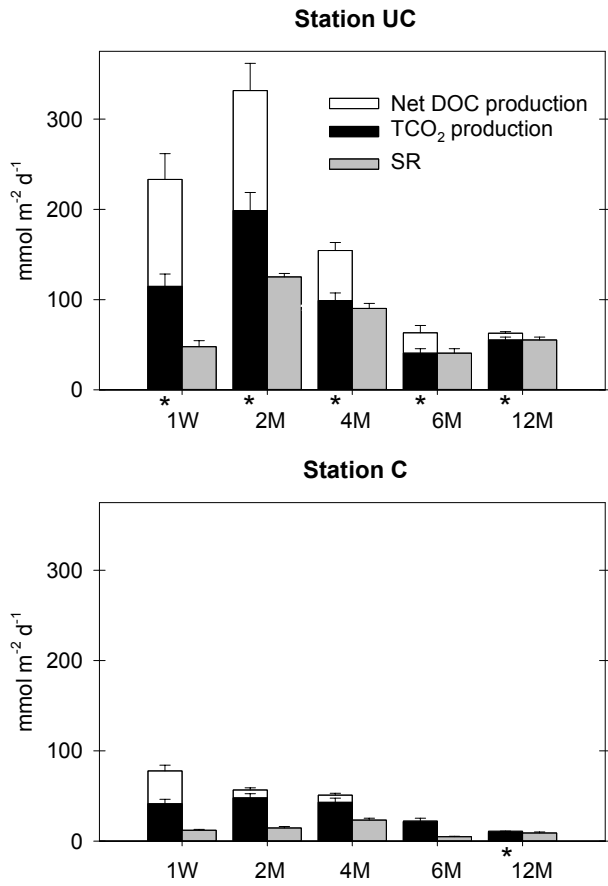


Figure 4 Temporal and spatial variability in production of dissolved organic carbon (DOC, A) and carbon dioxide (TCO₂, B) and sulfate reduction (SR) measured in jar experiments with uncultivated (UC) and cultivated (C) soils flooded with seawater. Note the different x-axis scaling for station UC and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



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).

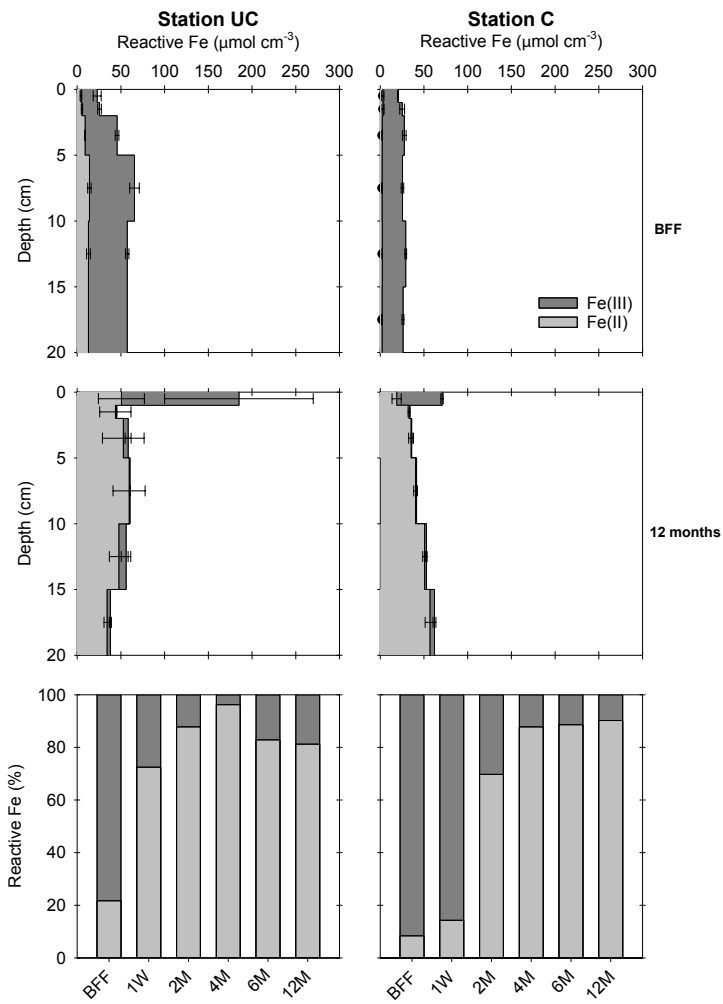
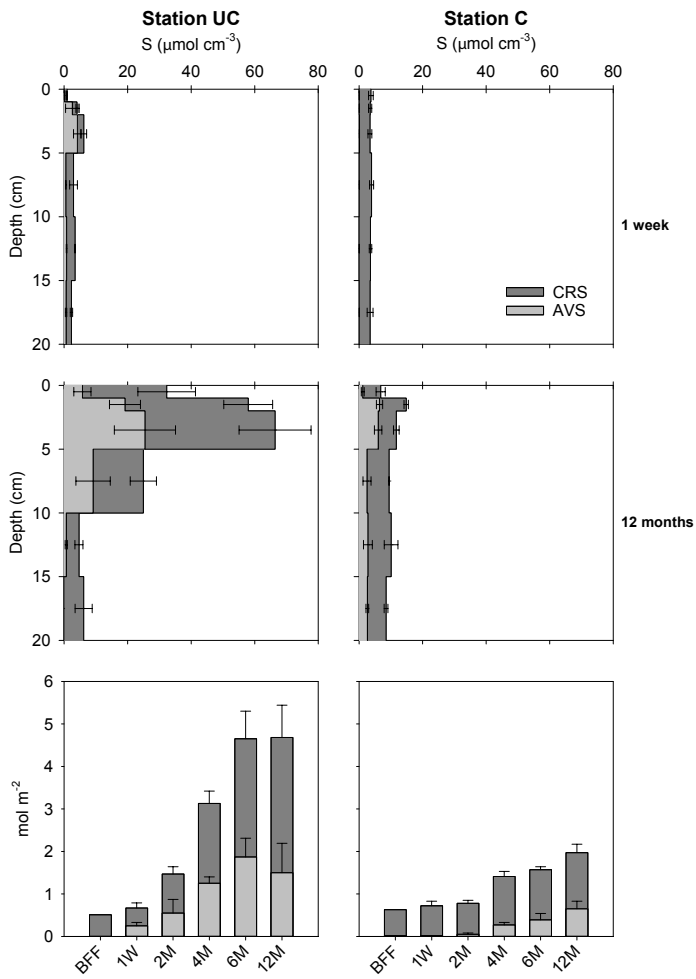


Figure 6 Upper panels show concentration of reactive Fe(II) and Fe(III) in uncultivated (UC) and cultivated (C) soils before flooding (BFF) and 12 months after flooding. Lower panels show the relative contributions of reactive Fe(II) and Fe(III) in the upper 20 cm at various times after flooding (1 week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