This pdf file contains point-by-point replies and marked-up ms versions of
- the first round of reviews (major revisions)
- the editor response after revisions (minor revisons)

1. Point-by-point reply to the reviewer comments (first round of reviews, major revision)

2. Marked-up manuscript version (text only) (first round of reviews, major revision)

1. Point-by-point reply to the reviewer comments:

We thank all three reviewers for their constructive comments and suggestions. In the pages below we respond to each of these separately.

In the following, reviewer comments are shown in in black, author responses in red font. Line numbers given below refer to the revised version of the ms (separate file, not the marked-up version below).

REVIEWER 1, anonymous

General comments

This article deals with an important aspect of carbon's fate in coastal wetlands in relation to global changes and their impacts on these ecosystems. Indeed, wetlands are receiving a growing attention in the climate change debate in relation to their high capacity to sequester blue carbon. Ecosystems considered in this "global" scale study are mainly tidal marches but some mangroves sites were counted in the selected sites. Authors are assessing OM degradation and transformation, as a proxy of Carbon sequestration using the TBI approach. Thus, authors claim that they provided indirect evidences that rising Temperature and Sea Level and eutrophication will impact the capacity of tidal wetlands to sequester carbon. This work is worthwhile to publish although as authors cautioned, there are limits with the used method (obvious quality differences of Tea-bag OM with "real" plants) and also that they may have missed some influent factors that control OM degradation and sequestration. Introduction was well thought and the methodology was clear however, some choices were not judicious in the context of this study and may need to be revaluated (see specific comments). The adding of TIDE experimental site was a very interesting. The discussion is well organised but it needs to be shortened.

We thank the reviewer for his constructive feedback on our study. As requested, we streamlined the discussion where appropriate; however, several required additions have also been made, so that the overall length of the Discussion did not substantially change.

Specific comments

I am not a specialist of meta-analysis, therefore I will not comment on the validity or not of the numerical methods, but one thing is sure, analyses need always to rely on field knowledge even if results are "counterintuitive". The discussion is based on two characteristics (k, S) that are related to the quality and the fate of the litter-bags contents (here Tea-bags) which are strongly related to sedimentation dynamic and water velocity. In absence of a clear indication on how sediments (and OM) are behaving in each site, I am concerned about the amalgam in the same meta-analysis different systems in term of hydrological functioning: Salt Marches vs. Mangroves, High tide vs. low tide (in salt marches). For instance, estuarine mangroves receive loads of sediments from rivers whereas Europeans salt marches in open Bays get sediments mainly from the oceans. One way to tackle this concern is to process the same calculations/test s/figures without adding the mangrove sites to the pool of data. Same thing can be done by considering the main origin of sediments (not to confound with OM), without impacted TIDE sites, river presence or not, water velocity, human activities: … . These factors, of ecological importance, might be those missing to explain some global, or local, differences. If these data cannot be compiled they should at least be discussed.

We agree with the reviewer that factors other than those assessed in this study might have been influential and could have masked expected results (i.e missing temp effect on k). In accordance with the reviewer's suggestion, we elaborated on this in several sections of the discussion: e.g. 392-395; 399-407.

Response concerning sedimentary factors:
We agree with the reviewer that the different systems we compiled in a single meta-analysis are characterized by potentially important differences in both sediment load and origin. We did not explicitly assess sediment loads of our study sites. However, by distinguishing between minerogenic and organogenic systems (i.e. sediment rich vs. sediment poor systems) in our analyses, we are confident to have already captured the relative importance of sediment load on our response variables. Please note that this categorical factor did not show up to be important in our classification- & regression-tree data mining (CART). Furthermore, our two most important findings (i.e. S decreases with temperature; S is lower in low vs. high elevated zones) are consistent within both minerogenic and organogenic systems.

We indirectly also addressed sediment origin (riverine vs. marine) by including both estuarine and coastal systems in our study. Specifically, we tested for effects of salinity class (fresh, brackish, salt) on our response variables, with fresh systems far up in the estuary experiencing the lowest marine influence and salt-water systems experiencing the highest marine influence. If sediment origin (riverine vs. marine) had an important influence on our response variables, this should have been reflected in our meta-analyses (i.e. Table 2). That being said, salinity of floodwater and sediment origin can of course not easily be separated in an observational study. Concerning the reviewer's remark on the sediment origin of our mangrove sites, it needs to be noted that those were not estuarine (as assumed by the reviewer) but coastal systems in the present study.

REVIEWER 2, Dr J. Keuskamp

General comments
This paper discusses the control that the soil matrix exerts on the decomposition of organic matter in tidal wetlands. Their large carbon stocks and sensitivity to global change make this a highly relevant topic for scientists and policy makers alike. The paper is well-written and easy to read, while presenting novel data with important conclusions on the relation between decomposition and global change. The usage of a standardised method over a wide range of tidal systems allows for a generalisation to the global scale, making this paper relevant to the broad readership of Biogeosciences. The explorative nature of the experiment also introduced some unavoidable methodological weaknesses. Many of the environmental parameters which are discussed in relation to decomposition are often strongly correlated with tidal regime (i.e. soil temperature, salinity, nutrient status, microbial biomass, and redox status), or latitude (i.e. nutrient limitation, vegetation type). In its current version, the manuscript does not always

acknowledge the potentially spurious relation between these factors. While this does not invalidate the main conclusions I would recommend to consider non-causality more carefully when attributing effects to specific environmental parameters.

We thank Dr Keuskamp for his constructive comments on our study. We agree that correlations between the assessed environmental parameters should be carefully considered in the interpretation of our results. Accordingly, we put more emphasize on this throughout the ms; some examples from different sections:

Methods/statistics:

"As we did not expect temperature to be independent of other parameters in this observational study, we constructed a Spearman correlation matrix including the parameters temperature, latitude, tidal amplitude, salinity class, *k*, and *S*. Additionally, we tested for differences in these parameters between marshes and mangroves and sites with mineral and organic soils, using Mann-Whitney U tests (Table 2)."

Results:

"Temperature was highly correlated with latitude and tidal amplitude, and temperature was not independent of soil type (mineral/organic) and ecosystem type (marsh/mangrove) (Table 2). The effect of latitude was similarly pronounced as the temperature effect on *S* – and consequently –effects of these two parameters on *S* cannot be separated (Table 2). By contrast, tidal amplitude and soil type did not significantly affect *S*, and the difference in *S* between mangroves and marshes was only marginally significant (Table 2). These findings suggest that the presented temperature effect on *S* occurs to be mainly independent of tidal amplitude and soil type."

Discussion:

"Future experimental work is therefore required in order to further assess the effects of temperature on OM stabilization and to separate temperature from latitudinal and other interacting effects (e.g. as outlined above for k) that are difficult to control for in observational studies."

The reviewer is specifically concerned about interactions with the parameters tidal regime and latitude.

→In terms of describing the tidal regime, we assessed tidal amplitude and, by comparing high and low elevated zones within sites, a relative measure for flooding frequency (i.e. low zones more frequently flooded than high zones). Tidal amplitude did not affect k and S (Table 3). It showed up as a potentially important predictor in our CART, probably because of its strong correlation with other parameters. However, this result needs to be considered cautiously because splits based on tidal amplitude suggest mixed effects (Fig. S1a).

→In terms of flooding frequency (high vs. low elevated zones), we discuss a number of potentially relevant interactions that were mentioned by the reviewer: redox -> 423-434; salinity -> 444; nutrient status -> 452-465. The reviewer makes an important point by mentioning soil temp interactions with tidal regime. We will address this point further below where soil vs air temp differences are discussed.

→Changes in nutrient status/limitation and vegetation type with latitude are relevant for the interpretation of the temperature effects on S and k. We accordingly extended discussion of temperature effects on k and S: 392-395; 399-407

The current description of the data-analysis does not describe how the authors have ascertained themselves that underlying assumptions of the statistical tests used were not violated. Where applicable, tests of heterogeneity, normality, and independence should be included, or other tests considered.

The reviewer is correct. We revised the description of the statistics and also corrected some statistical analyses:

Specified assumption checks:

"To test for effects of relative elevation (as proxy for relative sea level) on k and S, two-tailed paired t-tests were conducted. Mean values of high and low elevated zones of the 21 sites where tea bags were deployed in both high and low elevation zones were used (n = 21). The absence of outliers and normal distribution of the difference in the independent variable (as assessed visually) assured robustness of paired t-tests. To assess the consistency of potential effects of relative elevation on k and S, one-way ANOVAs were used in each site separately (replication was sufficient in 20 sites). Normal distribution of residuals was assessed visually, Levene's test was used to test for homogeneity of variance, and data were log-transformed if assumptions were not met. Mann-Whitney U tests were conducted as a non-parametric alternative when log-transformed data did not meet ANOVA assumptions (Table S2). We tested for effects of nutrient enrichment on k and S in the data from the TIDE project site (Massachusetts, US) using two-way ANOVA with enrichment treatment and marsh zone as predictors. When Levene's test indicated heterogeneous variance (true for k), data were log-transformed, which stabilized variance. Normal distribution of residuals was assessed visually."

Corrected statistics/data structure:

Statistics in Table 2 (Spearman correlations and U tests) were based on mean values of each site-by-zone combination (n = 51). Sites with observations in two zones were thus overrepresented. In the revised version these statistics are based on site means (n = 30; compare 2.2).

A related point is that the three sites at the Ebro delta (and the three Maine sites) were considered as different zones of the same site, characterized by different salinities (fresh/brackish/salt marsh). However, we noticed that they are actually as far apart as the two sites in Massachusetts or the three mangrove sites in Panama. For reasons of consistency, they are now considered separate sites. This, however, does not change any of the findings/conclusions previously drawn. We clarify in the Methods that many of our sites are co-located in larger estuarine/coastal regions (line 148 and revised Table 1).

For example a linear fitting is performed between k and S with temperature, without mentioning testing for residual patterns to uncover non-linearity. As the authors note the relation between decomposition and single parameters are often not linear (L221), in which case the result of a linear model is unreliable.

The reviewer is correct. A linear effect of temp is not expected. The intention for showing the linear fit was only to better illustrate the significant temp effect on S (as tested/identified with non-parametric Spearman correlation). However, we should not have used linear regression as an additional hypothesis test. → We only use Spearman correlation for this in the revised version. Yet, to better illustrate the temp effects, we still present scatterplots and use curve fitting to illustrate significant temp effects. Indeed, the model with both highest R2 and lowest standard error of estimate describing the significant temp effect on S is not linear but logarithmic (Figure 2)

Lastly, I would like to add that the strength if the TBI lays in its standardisation. I would therefore recommend to mention the S/k calculated with the standard approach alongside with the re-scaled values calculated with the more aggressive extraction method. This would allow for easy comparison with other data such as the TBI-values from mangroves mentioned in the methods paper. See also below.

We agree with the reviewer. The same point has been raised by Dr Sarneel in an interactive comment (below). We have prepared a table with all site x zone values for k and S, giving both the original TBI-values and the modified (Table S3; referenced in the ms: L513).

Specific comments

L79 and L83-L84 seem largely redundant to me

Deleted old lines 79-80

L85-L86 ’OM decomposition’ is somewhat ambitious as it is not clear whether this refers to decomposition rate (k) or extend (S), please revise.

The sentence was changed to “Consequently, global changes that might decrease OM preservation in tidal wetland soils not only affect carbon sequestration, but also decrease ecosystem stability against SLR.” Obviously, preservation is also affected by decomposition rate and stabilization; however, we do not intend to specify the processes at this stage of the Introduction, but do this further down in the text (i.e. 115-119).

L117 Although this should have been more explicit in the TBI method paper (Keuskamp et al, 2013), the k estimated by TBI is not exactly equivalent to the classical litter bag experiment as it describes the decomposition rate of the hydrolysable fraction and is not calculated over the entire mass. We have therefore adapted k1 to indicate that this is the k of the most labile fraction, as opposed to k2 which refers to the decomposition rate of the recalcitrant fraction. To avoid confusion this should be made explicit here.

We avoid reference to classical litter bag experiments here and instead make the meaning of *k* clearer in the respective section of the Methods.

L120 The recalcitrant fraction is also decomposable, albeit a lot slower

This was poor wording of course →changed to “rapidly decomposable”.

L127 ’ thereby improving our process-level understanding on how global warming affects carbon turnover’ Not sure what this means exactly

Deleted “process-level”.

L137 I am somewhat surprised that the oxidation of organic matter would be limited by the supply of SO4 in brackish tidal wetlands. Wouldn't the constant flushing with water replenish SO4 to saturating levels in brackish/salt water systems?

Well, it probably depends on how much seawater input the brackish system experiences. Anyhow, our dataset does not actually allow to accurately describe salinity effects on k and S (too imbalanced, low number of fresh systems). We simplified accordingly and only test for effects of temperature, relative sea level, and eutrophication. Compare 120-135, 240-265

L154 '(i.e. dwarf vs. fringe phenotypes)' Aren't these also Rhizophora vs Avicennia? In that case phenotypes would not be the appropriate description. These mangroves belong to different genera, each with their own properties (soil oxygenation, phenolic compound production, N-content) that are known to influence decomposition.

In most cases you would assume so, but here both fringe and dwarf are indeed Rhizophora with very few individuals of Avicennia: Please compare: Mckee et al. (2007) Global Ecology and Biogeography, **16**, 545–556; Lovelock et al. (2005) Caribbean Journal of Science, Vol. 41, No. 3, 456-464, 2005

L154 'Relative elevation' as relative to what? mean lower tide, mean mean tide? Please specify

We specified as follows:

"In 21 sites, we compared high and low elevated zones, which were characterized by distinct plant species compositions (i.e. different communities in high vs. mid vs. low marshes) or by different stature of mangroves (i.e. dwarf vs. fringe phenotypes). We used relative elevation (i.e. high vs. low elevated zone) as a site-specific proxy for relative sea level. By doing so, we did not capture the actual variability in the tidal inundation regime across our study sites as these vary in absolute elevation and in elevation relative to mean high water."

L169-170 Decomposition rates depend on soil temperature rather than on air temperature. Others have shown (e.g Piccolo et al. 1993, Reckless et al. 2011) that in tidal wetlands, the soil temperature is strongly determined by inundation regime in which case the accuweather temperature are not an accurate reflection of the decomposition environment. Moreover, inundation regime and temperature effects would be confounded. Could it be shown accuweather estimated temperatures vs measured temperatures so that the reader can see for themselves whether the accuweather approximation suffices?

Dr Keuskamp brings a valid point here that indeed needs more consideration. Air temperature would obviously diverge from soil temperature depending on factors such as canopy shading or tidal regime and water temperature. As a consequence, air temperature can only approximate the temperature conditions of the actual decomposition environment. However, considering that we stretch a temp gradient of approx. 20°C, we are confident that this would also translate into a profound soil-temperature gradient across our study sites.

The two studies mentioned by the reviewer, Piccolo et al. 1993 and Ricklefs et al. 2012, present data for un-vegetated tidal flat systems. For marsh systems, we would rather refer to Kirwan et al. (2014). The authors show, that in marshes along the well-studied latitudinal gradient of the US East coast (and we do share a number of sites), soil temp and air temp are highly correlated, while the relationship between soil temp and water temp is far weaker (Kirwan et al. 2014, *Temperature sensitivity of organic-matter decay in tidal marshes*; biogeosciences: **Fig. 2a**).

In our study sites, we did not continuously measure soil temp over the 3 months of deployment, and thus it is difficult to assess how well soil and air temp were correlated in this study. However, in several of our sites, soil temp was assessed at the time point of insertion and retrieval of bags. We plotted these data against the mean air temp of the day as acquired from the *accuweather* service in Figure S3. → We see that generally air temp is a good proxy for soil temp across sites. Yet, there is considerable variability in soil temp not explained by air temp, which would result from the fact that soil temp was assessed in one time point as opposed to mean air temp of a single day and of course from other factors, such as distance of weather station from site, shading, influence of water temp etc..

→We agree with the reviewer that this needs to be stated and discussed in the manuscript. Accordingly, we put more emphasize on this throughout the ms; some examples from different sections:

Methods:

"It needs to be noted here, that top-soil temperature would differ from air temperature depending on factors such as canopy shading or tidal regime and water temperature. As a consequence, air temperature can only approximate the temperature conditions of the actual decomposition environment (Fig. S3)."

Discussion:

"The present study used air temperature as a proxy for top-soil temperature. Thus, the temperature regime of the decomposition environment was only approximated, which certainly would have weakened a significant relationship between temperature and k. However, following typical Q10 values for biological systems of 2-3 (Davidson and Janssens, 2006), k should have at least doubled over the gradient of $\Delta T > 15°C$; yet our data do not even show a tendency of an effect ($r_s = 0.02$; Table 2). We therefore propose that other parameters exerted overriding influence on k, mainly masking temperature effects, and have not been captured by our experimental design. This notion is in line with the fact that studies conducted at …"

Lastly, we want to stress a related point here: "low" and "high" in the figure legend of Fig.S3 refer to the low and high elevated zones within the systems. A paired t-test comparing the difference of air temp and soil temp between the paired high and low elevated zones indicates no significant effect of zone ($p = 0.563$). This shows that differences between air and soil temp were not consistently more pronounced in either the low or the high elevated zones. Additionally, soil temp was not significantly affected by zone ($p = 0.342$). One of our main findings, that S is consistently lower in low vs. high zones, is consequently not temperature affected (i.e. *S* was significantly reduced in 14 of 20 sites, and the opposite was observed in none of the sites (Table S2).

L176 Pepsico, to my knowledge the bags are produced by Lipton, which is a Unilever brand.

Unilever belongs to PepsiCo, but of course the tea is produced by Unilever. PepsiCO was deleted in order to avoid confusion.

L180 Were the reference bags dried at 70oC prior to mass determination?

This may be a misunderstanding: reference bags were used to determine a mean value of the empty nylon bag itself without contents. I do not know if that one has always been dried, however, empty-bag weights were very consistent among labs. →By contrast, initial tea-content weights showed some variability across the involved labs. I also noticed that some labs, after drying at 70°C, used desiccators, in which the material could cool down without sucking moisture, before weighing and some didn't. I therefore assessed if potential moisture differences of the initial tea material or differences in the amount of the initial material could have affected S or k. However, there was no relationship between green initial weight and S (r2=0.0003; p=0.936) and no between rooibos initial and k (r2=0.005; p=0.728).

L198-L200 It could well be that the method described is a more accurate operationalisation of the labile (non-hydrolysable) fraction. Redefining the labile fraction and the consequential shift in S, and rescaling of k, may however lead to misunderstandings when the results of this study are used in comparisons with other TBI experiments. I would therefore suggest to provide the TBI S/k values calculated according to protocol alongside the obtained S/k values obtained by the revised protocol.

We agree. We added a table with the original TBI values accordingly (see comment further up).

L220-L250 Would you be able to indicate whether potential violations of the assumptions underlying the statistical tests were assessed? For example, were the residuals of the ANOVA procedure tested for normality / homogeneity of variance?

This was indeed missing. We added these missing details to the Methods, see comment further up.

L250 It is critical to this conclusion that air temperature is a good proxy of soil temperature (see earlier remark). The interaction between temperature effect and tidal position reinforces the suspicion that this is not the case.

We agree with the first half of this remark (see addressed further up), but not with the second. That is, there is no clear interaction between tidal position and temperature: Temperature seems to affect k in mesotidal systems (tidal amp >2.1m) with k higher in sites with temp >14.5°C; however, this apparent temp effect is inconsistent within this group of mesotidal systems. That is, sites with temp >18.2°C show lower k than those sites with temp <18.1°C. Please note comment further up: temp did not differ between high and low elevated zone, neither did the temp difference between soil and air.

L314 As also noted in L313, the absence of a temperature effect is very unusual. Could the authors rule out the possibility that this is due to a mismatch between soil and air temperature?

We stretch large gradients of approx. 20°C for both soil and air temp, and there is not even the slightest tendency of a temp effect on k (Spearman's rank coefficient = 0.02; Figure 2), while S is strongly affected. It therefore seems that that other factors exert overriding control over k and more strongly mask temp effects on k than on S. Yet, we agree on the need to discuss the methodological inaccuracy in determining temp of the decomposition environment, and we addressed this point (see comment further up).

We want to stress a related point here concerning the missing temp effect on k: In order to address remarks by Reviewer 3 and demonstrate the usefulness of the TBI method for tidal wetlands, we took a separate look at the data of the North American East coast latitudinal gradient along which previous studies have shown clear temp effects on decomposition processes and microbial biomass (Blum et al. 2004; Kirwan et al. 2014; Mozdzer et al. 2014). Species composition of these marshes is quite constrained (i.e. Spartina alterniflora dominated) reducing confounding effects induced by differences in vegetation. Along this gradient, we clearly see an increase in S and also the expected decrease in k, although temp explains more variability for S (Fig. S2). We added this figure to the manuscript in order to illustrate that temp effects on k can be identified on the regional scale, but not on the global scale with more confounding factors.

The effect of temp on k at the regional scale but the missing effect at global scale is also in agreement with the just recently published article on *Early stage litter decomposition across biomes* by Ika Djukic and others. (Although they did not assess specifically k and S in their study using the TBI tea materials, they simply assessed mass loss of the two materials). Across biomes, climate (temp and precipitation) had no effect on break down; however, within biomes the effect was strong.

L332 I would recommend discussing potential confounding of temperature effects with other changes in decomposition matrix (e.g. nutrient availability, redox status, vegetation, salinity). With respect to k, such reservations are made in L323/L329, but are absent here.

We agree with the reviewer and added similar considerations for the discussion on temp effects on S (400-407).

L351 Can this be generalised to continuously submerged parts of the soil? The TBI is at a relatively low depth, where tidal pumping may cause increased influx of oxygen during tidal subsidence. Especially in tannin-rich mangrove systems, temporal oxygenation may make a large difference by allowing breakdown of phenolic compounds (see also Freeman et al, 2001)

We agree with the reviewer. We elaborated our discussion on expected redox effects, also with respect to comments by Reviewer 3 (427-435, 515-521).

L445 In mangrove TBI experiments that I have conducted S values have always been positive, and I am somewhat puzzled by the large difference. Negative S values could also be caused by loss of recalcitrant particles as I have observed when using teabags in open water. Did you have any indications that this has taken place here?

We were puzzled as well when realizing that so many values were lower than they should be. Indeed, the FL mangrove values you report in Keuskamp et al 2013 are considerably higher. That's also when I decided to check whether the quality of the material had changed.

→No, I am not aware of loss of particles from the bags in situ. In fact, in a recent study (microcosm study, Wadden Sea) we used the new tea bags (those without nylon mesh) that wouldn't allow for loss of material through the mesh. Also with these bags, we had ~11% negative values (Hao Tang, Peter Mueller et al. unpublished data), comparable to what we found in some Wadden Sea marshes in the present study using nylon mesh bags.

→Comparing our results to those reported in Djukic et al. (2018), it becomes clear that negative S values occur less frequently across terrestrial systems, however, are not negligible either.

Technical corrections L74 Earth? Not sure if this should be with a capital E
L77 Separate SRL from citations
L94-98 This sentence is very hard to read. Split.
L346 add 'in' before 'tidal wetlands'

Thanks, technical corrections have been made.

REVIEWER 3, anonymous

Mueller et al. conducted decomposition experiments using tea bags based on a standardized approach developed by Keuskamp et al. (2013), across different marsh and mangrove sites in order to cover a gradient in temperature, inundation regime, etc. While such cross-ecosystem studies have a high potential, I feel the impact of this dataset in terms of new insights is relatively limited. The dataset can be published but I feel the impact of the conclusions should be toned down somewhat – the manuscript does not really deliver what the title suggests. The dataset should be publishable, but it needs a more critical discussion and should provide the readers with a more complete overview of the caveats and assumptions used in the TBI approach, so that the readers can better assess what can and cannot be deduced from these data. My main point is that the TBI index – both the original and the modified protocol suggested here – has plenty of limitations and it remains an operationally defined procedure, with several assumptions that are open to discussion. In addition, we are not looking at mineralization of in situ produced material hence some interactive effects will be missed in this approach; results should not be over-interpreted or generalized.

We thank the reviewer for his critical and constructive feedback on our work. We have revised our ms, particularly the discussion part (4.4 Methodological considerations; 4.5 Implications), in order to provide the reader with a more complete overview of the assumptions and limitations involved with the TBI approach. We have provided more detailed responses below regarding the specific comments raised by the reviewer.

Specific suggestions

L55: “stabilization was 29% lower”: this does not mean much if you do not define stabilization here, it can be interpreted in different ways. For me this remains a somewhat problematic proxy (see further comments).

We agree with the reviewer’s concern and specified the parameter in the abstract (53-55). The second part of the comment will be addressed further below.

L60-61: data from the eutrophication experiment: would not extrapolate this to ‘high sensitivity to global change’. Eutrophication will also affect the nutrient content of locally produced biomass, this aspect is not taken into account when standardized material is used in the experiments.

The reviewer is of course correct to state that with eutrophication, also the quality of the biomass produced in the system would change with potentially important consequences for the decay process. Thus, interpretation of the results obtained with standardized litter need to be conducted cautiously. In the discussion it now reads as follows:

“Standardized approaches like this, or also the cotton-strip assay (e.g. Latter and Walton, 1988), are useful to separate the effects of environmental factors other than OM quality on decomposition processes and to assess their relative importance. Otherwise, complex interaction effects of the abiotic environment and OM quality make it difficult to predict the relevance of certain environmental factors for decomposition processes, potentially masking the effects of important global-change drivers (Prescott, 2010). At the same time, however, the global-change factors considered in the present study are likely to induce changes in the quality of the OM accumulating in tidal wetlands, for instance through shifts in plant-species composition and plant-tissue quality, that can potentially counterbalance or amplify the effects on decomposition processes suggested here. Future research therefore needs to address OM quality feedbacks on decomposition processes in tidal wetlands in order to gain a more complete understanding of global-change effects on tidal-wetland stability and carbon-sequestration capacity.”

We agree with the reviewer that this sentence needs to be toned down in the abstract, because there is no space for further elaboration on the assumptions and methodological considerations. We will go with “potentially high sensitivity of OM stabilization to global change.”

L90-95: an important caveat here is that you only study the decomposition of one type of source material (well, in two versions), but not other sources that contribute to the OM pool e.g. marine or other aquatic inputs into the intertidal system.

We agree; this is important for the interpretation of our results. However, conventional litter bag experiments are also restricted in their choice of material; here actually lies an advantage of the standardized approach, although we acknowledge that the quality of the TBI materials is obviously closer to that of wetland plant litter than to the marine derived, labile allochthonous organic input a tidal wetland receives. We have elaborated on this in 4.4:

“Interpretation of results obtained from standardized approaches like the present needs to be made cautiously because OM quality (i.e. its chemical composition) is a key parameter affecting its decomposition. As the quality of the TBI materials differ from that of wetland plant litters, and likely even more from the quality of the imported allochthonous OM (Khan et al., 2011), we did not expect to capture actual rates of early-stage OM break-down in this study. Instead, we used the TBI to characterize the decomposition environment by obtaining a measure for the potential to decompose and stabilize the deployed standardized material."

Additionally, we toned down our Implications (4.5):

"This study addresses the influence of temperature, relative sea level, and coastal eutrophication on the initial transformation of biomass to SOM, and it does not encompass their effects on the existing SOM pool. However, aspects of S and k are key components of many tidal wetland resiliency models (Schile et al., 2014; Swanson et al., 2014) that have highlighted the critical role of the organic contribution to marsh elevation gain. Although actual rates of S and k cannot be inferred from this study using a standardized approach, our data identify strong negative effects of temperature, relative sea level, and coastal eutrophication on the stabilization of fresh organic inputs to tidal-wetland soils. We argue that these unanticipated combined effects yield the potential to strongly accelerate carbon turnover in tidal wetlands, thus increasing their vulnerability to accelerated SLR, and we highlight the need for experimental studies assessing the extent to which the here identified effects translate into native OM dynamics."

section 2.2: it is important for the readers not familiar with the Keuskamp et al. paper to re-iterate and stress the assumptions on which this approach is based, e.g. that k2 (decomposition constant of the non-labile fraction) is assumed to be 0, and that S is assumed to be similar for both types of tea. I still find this major shortcomings- we know the first assumption not to be valid, and I have not seen strong arguments to support the second assumption. The main reason to make these assumptions is to allow to estimate k and S using only one time point of measurements instead of having to measure at different points in time. These aspects deserve to be mentioned explicitly and the limitations of the approach should be discussed more critically. -What is the added value of this approach compared to simply measuring the decay of the biomass over a limited number of time steps, and using a more realistic decay model?

Please note that Keuskamp et al. (2013) show that the TBI is quite robust against deviations from the assumption that S is the same for the two materials. The assumption that k2 could be considerably higher than 0 during 3 months of deployment has already been questioned by us (520-522). However, the reviewer brings important points here. In accordance, we elaborated on the description of the TBI calculations in the respective section of the Methods (2.2):

"Decomposition rate (*k*) and stabilization (*S*) were assessed following the TBI protocol (Keuskamp et al., 2013). The TBI approach can be considered as a simplified litter-bag approach, allowing a time- and cost-efficient characterization of the decomposition environment, because *k* and *S* can be estimated without repeated sampling of the decomposing material as in conventional approaches. This implies the assumptions that (1) *S* is equal for the two types of material used in the approach and (2) that decomposition of non-hydrolyzable materials during the 3 months of deployment is negligible. We refer the reader to Keuskamp et al. (2013) for further detail and validity assessments of assumptions."

The advantage of the TBI approach over a longer-term litter experiment is the time efficiency that allowed us to assess decomposition in a large number of sites during the same growth season and find enough collaborators capable to contribute with their work. Obviously, as outlined by the inventers of the method (Keuskamp et al. 2013), the TBI can't substitute the precision of classic litter bag methods, but it considerably reduces the effort necessary to fingerprint local decomposition. It is a trait-off between precision and effort that helps gathering decomposition data across ecosystems and biomes.

In order to demonstrate the usefulness of the method and its comparability to other methods assessing decomposition processes tidal wetlands, we will separately present our data on k and S for the sites along the North American East coast latitudinal gradient, along which previous studies have shown clear temperature and latitudinal effects on decomposition processes. For instance, Kirwan et al. (2014; Biogeosciences) demonstrated a strong increase in cellulose decay with both temperature and latitude, and Mozdzer et al. (2014; Ecology) showed a marked decrease in sulfate reduction with latitude along this transect. The TBI parameters assessed along the same transect are in tight agreement with the previously reported results, particularly the findings by Kirwan et al. (2014), demonstrating the usefulness of the method to characterize the decomposition environment of tidal wetland soils.

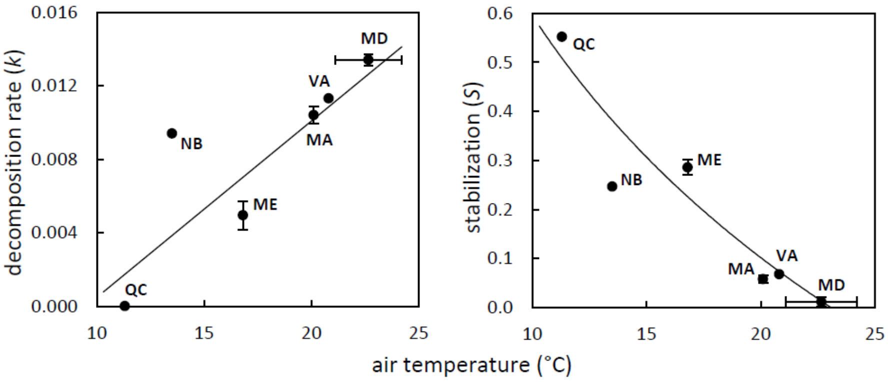

Figure S2 Site means of decomposition rate (left) and stabilization (right) versus mean air temperature of the deployment period shown for the ten sites situated along the latitudinal gradient of the North American Atlantic coast; state acronyms are shown (compare Table 1). Regression lines illustrate significant relationships^; regression models with lowest standard error of estimate (SEE) and highest R^2 are shown. Decomposition rate: y = 0.001x - 0.0091; R^2 = 0.692; SEE = 0.003; stabilization: y = -0.712ln(x) + 2.2331; R^2 = 0.860; SEE = 0.070

Lastly, we discuss the applicability of the TBI approach in 4.4:

"Future research will have to test the applicability of the TBI approach in different ecosystems and test the validity of its assumptions (i.e. S is equal for both types of material used, and mass loss of non-hydrolyzable material is negligible over 3 months of deployment). The results of our regional scale assessment along the North American Atlantic coast transect are in tight agreement with previously reported results on cellulose break-down and soil microbial activity along this well studied transect (Kirwan et al., 2014; Mozdzer et al., 2014). We can thereby demonstrate the usefulness of the TBI approach to assess early-stage decomposition in tidal-wetland soils."

L212-214: provide the data from Keuskamp et al. as well, we cannot compare or assess how much higher your data are.

Good idea, both are included now (Table S1).

L 427-434: This is somewhat problematic also. It demonstrates the disadvantages of using these operationally defined indices; to which extent is this caused by the assumption that S is identical for the two types of substrate?

We agree that the operational definition can cause problems and its implications have been addressed as mentioned further up. The assumption that S is identical for the two types of substrate is irrelevant in this context: S is determined only based on what is left of the green tea material after incubation. The problem discussed in this section is that more material was decomposed from the green tea material than theoretically possibly based on its hydrolysable fraction. So either non-hydrolysable material was also decomposed to a considerable degree (as also mentioned by the reviewer further up) or the hydrolysable fraction is in fact higher than previously described.

Secondly, keep in mind that anaerobic decomposition processes are important in tidal wetlands, and can occur at high rates (similar order of magnitude as aerobic decomposition) up to substantial depths.

This is a valid point that needed more consideration. We specified in section 4.2:

“In tidal wetlands, differences in flooding frequency along elevational gradients often induce sharp gradients in oxygen availability and redox conditions (Davy et al., 2011; Kirwan et al., 2013; Langley et al., 2013), with potentially strong influence on OM decomposition and carbon cycling. However, the effect of redox conditions on OM break-down is determined by the chemical quality of the decomposing material: Decomposition of aged or recalcitrant OM can indeed be slower and incomplete in the absence of oxygen, whereas the break-down of fresh and labile OM can be largely unaffected by oxygen availability (Benner et al., 1984; Kristensen et al., 1995). Thus, also decomposition rate and stabilization of labile, hydrolyzable OM, as assessed in the present study, is not necessarily affected by redox conditions. Here, we demonstrate …”

2. Marked-up manuscript version (text only)

Global~~-~~-change effects on early-stage decomposition processes in tidal wetlands~~: implications~~ – Implications from a global survey using standardized litter

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Abstract

Tidal wetlands, such as tidal marshes and mangroves, are hotspots for carbon sequestration. The preservation of organic matter (OM) is a critical process by which tidal wetlands exert influence over the global carbon cycle and at the same time gain elevation to keep pace with sea-level rise (SLR). The present study ~~provides~~assessed the ~~first global-scale field-based experimental evidence~~ effects of temperature and relative sea level ~~effects~~ on the decomposition rate and stabilization of OM in tidal wetlands~~. The study was conducted in 26 marsh and mangrove sites across four continents~~ worldwide, utilizing commercially available standardized ~~OM~~litter. While effects on decomposition rate per se were minor, we show ~~unanticipated and combined~~strong negative effects of temperature and relative sea level on ~~OM~~ stabilization, as based on the fraction of labile, rapidly hydrolyzable OM that becomes stabilized during deployment. Across study sites, OM stabilization was 29% lower in low, more frequently flooded vs. high, less frequently flooded zones. ~~OM stabilization~~Stabilization declined by ~~~90~~75% over the studied temperature gradient from 10.9 to 28.5°C~~, corresponding to a decline of ~5% over a 1°C temperature increase.~~. Additionally, data from the Plum Island long-term ecological research site in Massachusetts, US show a pronounced reduction in OM stabilization by >70% in response to simulated coastal eutrophication, confirming the potentially high sensitivity of OM stabilization to global change. We therefore provide evidence that rising temperature, accelerated SLR, and coastal eutrophication may decrease the future capacity of tidal wetlands to sequester carbon by affecting the initial transformations of recent OM inputs to soil ~~organic matter~~OM.

1 Introduction

Tidal wetlands, such as marshes and mangroves, provide a wide array of ecosystem services that have been valued at approximately US$ 10,000 per hectare and year, making them some of the most economically valuable ecosystems on Earth (Barbier et al., 2011; Kirwan and Megonigal, 2013). Yet, tidal wetlands are threatened and vulnerable ecosystems, experiencing pronounced loss though global-change impacts, such as land use (Pendleton et al., 2012~~),~~) and accelerated sea-level rise (SLR~~;~~) (Craft et al., 2009; Crosby et al., 2016). In recent years, carbon sequestration has increasingly been recognized as an ecosystem service of tidal wetlands (~~e.g.~~ Chmura et al., 2003; Mcleod et al., 2011).~~ Tidal wetlands are efficient long-term carbon sinks, preserving organic matter (OM) for centuries to millennia.~~ Here, high rates of OM input (from both autochthonous and allochthonous production) co-occur with reducing soil conditions and thus slow rates of decomposition, leading to ~~organic~~long-term carbon~~-~~ sequestration rates that exceed those of most other ecosystem types by ~~orders~~an order of magnitude (Mcleod et al., 2011). At the same time, suppressed decomposition and the preservation of OM is a primary process by which many tidal wetlands gain elevation and keep pace with rising sea level (Kirwan and Megonigal, 2013). Consequently, global changes that ~~might increase~~decrease OM ~~decomposition~~preservation in tidal~~-~~ wetland soils not only affect carbon sequestration, but also decrease ecosystem stability against SLR. It is therefore critical to identify global~~-~~ change factors that affect the transformation of organic inputs to stable soil OM (SOM) in tidal wetlands and to assess the magnitude of their effects.

There are multiple methods for assessing factors that influence carbon sequestration, including direct measurements of plant production, ~~organic~~ carbon stocks, accretion, and decomposition rates. Litter-bag techniques assessing the weight loss of plant material over time are probably the easiest way to measure decomposition rates in situ and have been widely used since the 1960s (Prescott, 2010). Global-scale assessments of litter decomposition have been conducted as both meta-analyses (e.g. Zhang et al., 2008) and as inter-site studies along latitudinal gradients (Berg et al., 1993; ~~Torfymow et al., 2002; McTiernan et al., 2003;~~ Cornelissen et al., 2007; McTiernan et al., 2003; Powers et al., 2009; Trofymow et al., 2002) in order to assess effects of climate parameters~~,~~ – mostly with focus on temperature and moisture gradients~~,~~ – on decomposition rate. Besides abiotic or climate effects, these studies could also identify litter quality itself as an important predictor for decomposition rate (Zhang et al., 2008).

Relationships between single climate or litter-quality parameters and decomposition rate often are not linear. Instead, complex interactions between litter~~-~~-quality and climate parameters seem to control litter decomposition (Zhang et al., 2008), creating challenges in separating climate from litter-quality effects and predicting the relevance of potential global-change drivers for decomposition rate. In order to separately assess environmental or climate effects on litter decomposition at a global scale, it is therefore necessary to standardize litter quality in inter-site studies. However, implications of litter-decay data for carbon sequestration need to be considered cautiously, as the link among litter-decomposition rate, SOM formation, and ultimately carbon sequestration is not straightforward (~~Prescott, 2010;~~ Cotrufo et al., 2013; Prescott, 2010): Because plant tissues are not resistant to decay per se, it is critical to understand their biogeochemical transformation into stable compounds that leads to the formation of SOM (i.e. stabilization) rather than understanding the pace at which early~~ stages of~~ stage decomposition proceeds (~~Prescott, 2010;~~ Castellano et al., 2015; Haddix et al., 2016; Prescott, 2010).

Keuskamp and others (2013) developed an efficient approach for studying litter decomposition and OM transformation at a global scale, using commercially available tea as standardized material. Their Tea Bag Index (TBI) approach is based on the deployment of two types of tea that considerably differ in their OM quality. The method allows for the determination of the decomposition rate constant (in the following referred to as decomposition rate or k~~), as in classic litter-bag approaches,~~) and a stabilization factor (in the following referred to as stabilization or S), which describes the fraction of labile and rapidly decomposable OM that becomes stabilized during deployment.

In the present study, we assessed effects of the ~~impacts of multiple~~ global-change factors ~~–~~global warming, ~~sea-level rise (~~accelerated SLR~~), salt-water intrusion~~, and coastal eutrophication —on both OM decomposition rate and stabilization in tidal~~-~~-wetland soils by conducting a worldwide ~~field study~~survey using standardized litter. First, by covering a large temperature gradient of ΔT >15 °C across sites, we aimed to capture temperature effects on OM decomposition rate and stabilization, thereby improving our~~ process-level~~ understanding on how global warming affects carbon turnover and ultimately sequestration in tidal wetlands. Second, by conducting paired measurements in both high- and low-elevated zones of tidal wetlands worldwide, we were aiming to gain insight into potential effects of accelerated ~~relative~~ SLR on carbon turnover. Despite the dominant paradigm that decomposition is inversely related to flooding, the existing literature on hydrology and SLR effects on OM decomposition in tidal wetlands yields equivocal results, which is often due to the overriding effect of OM quality on decomposition rate (Hemminga and Buth, 1991; Kirwan et al., 2013; Mueller et al., 2016). ~~Additionally, by expanding our study to include fresh and brackish sites, we anticipated to capture the effects of salt-water intrusion into brackish and fresh systems, which is likely to affect decomposition processes in tidal wetlands (Morrissey et al., 2014). Specifically, high concentrations of dissolved sulfate in seawater, acting as an alternative terminal electron acceptor, can enhance anaerobic microbial metabolism in systems with lower salinity (Megonigal et al., 2004; Sutton-Grier et al., 2011). Lastly, we used the long-term ecological research site of the TIDE project~~Lastly, we used the TIDE project plots of the Plum Island long-term ecological research site in Massachusetts, US (Deegan et al., 2012) to experimentally assess both the effects of coastal eutrophication and – with respect to SLR-driven increases in flooding frequency – the relevance of nutrient delivery through floodwater for the early stages of OM decomposition in tidal wetlands.

2 Methods

2.1 Study sites and experimental design

The ~~study~~worldwide survey was conducted ~~in 26 tidal wetlands~~ during the 2015 growing season ~~(Figure~~and included a total of 30 tidal-wetland sites. Sites were partly co-located within larger coastal and estuarine regions (Fig. 1, Table 1). ~~Nine~~Eleven sites were situated along the European coasts of the North Sea, Mediterranean, and Baltic, ~~ten~~Thirteen sites were located along the East and West coasts of North America including the St. Lawrence Estuary, Bay of Fundy, Chesapeake Bay, and San Francisco Bay, and four mangrove sites were situated along the Caribbean coast of Central America in Belize and Panama. Additionally, one Chinese site (Yangtze Estuary) and one Argentinian site were included in our study. Sixteen of the sites were salt marshes, ~~six~~ten were tidal freshwater and brackish sites, and four sites were mangroves. ~~At 22~~In 21 sites, we compared high and low elevated zones, which were characterized by distinct plant species compositions (i.e. different communities in high vs. mid vs. low marshes) or by different stature of mangroves (i.e. dwarf vs. fringe phenotypes). We used relative elevation (i.e. high vs. low elevated zone) as a site-specific proxy for relative sea level. By doing so, we did not capture the actual variability in the tidal inundation regime across our study sites as these vary in absolute elevation and in elevation relative to mean high water. Finally, we included the long-term experimental site of the TIDE project in Massachusetts, US to assess effects of nutrient enrichment on litter-decomposition rate and stabilization. Through nitrate additions to the incoming tides on at least 120 days per year, nutrient enriched areas at the TIDE project site receive floodwater with 10-15 fold increased nitrogen (N) concentrations compared to reference areas since 2004. From 2004-2010 also phosphate was added to the floodwater~~,~~; however, this has been discontinued because creek water P concentrations are high enough to prevent secondary P limitation through N enrichment (details in Deegan et al., 2012; Johnson et al., 2016).

Decomposition rate and stabilization were measured by deploying tea bags in ten points per zone or treatment within a site (n=10). Spacing between replicates within a zone or treatment was ≥2 m. However, as sites differed considerably in their areal extent, the distribution and thus spacing between points had to be adjusted to be representative for the given system. ~~Temperature~~ Air temperature for the period of deployment was measured at site, or temperature data was obtained from the online service of ~~Accuweather~~AccuWeather (accuweather.com; accessed 12/25/2016) for locations within a distance of 15 km to the site for most sites, but not further than 60 km for some remote sites. It needs to be noted here, that top-soil temperature would differ from air temperature depending on factors such as canopy shading or tidal regime and water temperature. As a consequence, air temperature can only approximate the temperature conditions of the actual decomposition environment (Fig. S3).

2.2 Decomposition rate and stabilization measurements

Decomposition rate (k) and stabilization ~~factor~~ (S) were assessed following the ~~Tea Bag Index~~TBI protocol (Keuskamp et al., 2013). ~~Briefly, at~~The TBI approach can be considered as a simplified litter-bag approach, allowing a time- and cost-efficient characterization of the decomposition environment, because k and S can be estimated without repeated sampling of the decomposing material as in conventional approaches. This implies the assumptions that (1) S is equal for the two types of material used in the approach and (2) that decomposition of non-hydrolyzable materials during the 3 months of deployment is negligible. We refer the reader to Keuskamp et al. (2013) for further detail and validity assessments of assumptions.

At each point of measurement, two nylon tea bags (200 µm mesh size), one containing green tea (EAN: 8 722700 055525; Lipton, Unilever~~ + PepsiCo~~, UK) and one containing rooibos (8 722700 188438, Lipton, Unilever~~ + PepsiCo~~, UK), were deployed as pairs in ~8 cm soil depth, separated by ~5 cm. The initial weight of the contents was determined by subtracting the mean weight of ~~10~~ten empty bags (bag + string + label) from the weight of the intact tea bag prior to deployment (content + bag + string + label). The tea bags were retrieved after an incubation time of ~~~90~~92 ± 6 (SD) days~~.~~, with three sites having an incubation period >100 days and one site <80 days. Upon retrieval, tea bags were opened, and tea materials were carefully separated from ~~clay particles and~~ fine roots and soil, dried for 48 h at 70°C, and weighed.

Calculations for k and S followed Keuskamp et al. (2013):

Eq 1) $$Wr(t) = ar\ e-kt + (1-ar)$$

Eq 2) $$S = 1 – ag / Hg$$

Eq 3) $$ar = Hr\ (1-S)$$

Wr(t) describes the substrate weight of rooibos after incubation time (t in days), ar the labile and 1-ar the recalcitrant fraction of the substrate, and k is the decomposition rate constant. S describes the stabilization factor, ag the decomposable fraction of green tea (based on the mass loss during incubation) and Hg the ~~hydrolysable~~hydrolyzable fraction of green tea. The decomposable fraction of rooibos tea is calculated in Eq 3 based on its ~~hydrolysable~~hydrolyzable fraction (Hr) and the stabilization factor S. With Wr(t) and ar known, k is calculated using Eq 1.

In accordance with Keuskamp et al. (2013), extractions for determination of the ~~hydrolysable~~hydrolyzable fractions of green and rooibos tea followed Ryan et al. (1990). However, instead of using Ryan's forest products protocol we conducted the alternative forage fiber protocol for the determination of the ~~hydrolysable~~hydrolyzable fraction. Briefly, 1 g of dried tea material (70°C for 24 h) was boiled in cetyltrimethyl ammonium bromide (CTAB) solution (1 g CTAB in 100 ml 0.5 M H2SO4) for 1 h (~~Ryan et al., 1990;~~ Brinkmann et al., 2002; Ryan et al., 1990). The extract was filtered through a 16-40-µm sinter filter crucible (Duran, Wertheim, Germany) using a water-jet vacuum pump and washed with 150 ml of hot water followed by addition of acetone until no further de-coloration occurred (Brinkmann et al., 2002). The remaining material was left in the sinter, dried for 12 h at 70°C, cooled in a desiccator and weighed. 20 mL of 72% H2SO4 was added to the sinter and filtered off after an incubation of 3 h, followed by washing with hot water to remove remaining acid. The sinter was dried at 70°C for 12 h, cooled in a desiccator, and weighed to determine the non-~~hydrolysable~~hydrolyzable fraction. Finally, the sinter containing the remaining sample was ignited at 450°C for 3 h in order to determine the ash content of the material.

In addition to the determination of the ~~hydrolysable~~hydrolyzable fraction, we measured total C and N contents of the tea material using an elemental analyzer (HEKAtech, Wegberg, Germany). The ~~hydrolysable~~hydrolyzable fraction of both green and rooibos tea was higher than reported in Keuskamp et al. (2013) (Table 2). However, the determined C and N contents of the tea materials are in agreement with those reported in Keuskamp et al. (2013) (Table 2). Therefore, deviations from the ~~hydrolysable~~hydrolyzable fraction as reported previously are likely due to the less conservative extraction assessment in the present study and not due to actual changes in the quality of the materials.

2.3 Data ~~Analyses~~mining

~~For all across-site analyses, mean values of each site by elevation zone (or site by salinity class) combination were used (N=51).~~ Relationships between single parameters and litter decomposition are often not linear. Instead, critical thresholds seem to exist at which a certain predictor (e.g. mean annual temperature) becomes influential (~~Rothwell et al., 2008; Prescott, 2010~~Prescott, 2010; Rothwell et al., 2008). In the first step of data mining, we therefore used classification and regression tree analysis (CART) to identify potential thresholds and important predictors for k and S (Fig. S1). Data mining was conducted using STATISTICA 10 (StatSoft Inc., Tulsa, OK, USA).

~~In the first step of our data analysis, we therefore used classification and regression tree analysis (CRTA) to identify important predictors for k and S. CRTA is a non-parametric procedure for the step-wise splitting of the data set with any number of continuous or categorical and correlated or uncorrelated predictor variables (Breiman et al., 1984; Rothwell et al., 2008), and it has been recommended to identify thresholds and to handle large-scale decomposition data sets (Rothwell et al., 2008; Prescott, 2010). We conducted CRTA separately for k and S using temperature, salinity class, tidal amplitude, ecosystem type, soil type, and relative elevation as predictor variables (Table 1). V-fold cross validation was set at 5 (as commonly conducted, compare Rothwell et al. (2008)), and the minimum number for observations per child node was set at n = 4, corresponding to at least two sites or 8% of the total data set.~~

2.4 Statistical analyses

To test for ~~correlations between the variables salinity class,~~effects of temperature on k and S, Spearman rank correlations were conducted using site means (n = 30). As we did not expect temperature to be independent of other parameters in this observational study, we constructed a Spearman correlation matrix including the parameters temperature, latitude, tidal amplitude, ~~k and S, Spearman rank correlations were used (Table 3). Mann-Whitney U tests were conducted to test for~~ salinity class, k, and S. Additionally, we tested for differences in ~~k and S~~these parameters between marshes and mangroves and ~~between~~sites with mineral and organic ~~soil types.~~

~~We tested for linear effects of temperature on k and S across sites~~soils, using ~~simple linear regression analyses (Fig. 2). Two-tailed paired t-tests were used to~~Mann-Whitney U tests (Table 3). Curve fitting was used to further explore relationships between temperature, k, and S, and regression models with lowest standard error of estimate and highest R2 are displayed in Figure 2 and S2.

To test for effects of relative elevation (as proxy for relative sea level) on k and S ~~(Fig. 3). Subsequent one~~, two-tailed paired t-tests were conducted ~~to test for~~. Mean values of high and low elevated zones of the ~~same effect within mineral, organic, marsh, and mangrove systems separately.~~

~~In~~ 21 ~~of our 22~~ sites where tea bags were deployed in both high and low elevation zones~~,~~ ~~replication was sufficient to conduct~~ were used (n = 21). The absence of outliers and normal distribution of the difference in the independent variable (as assessed visually) assured robustness of paired t-tests. To assess the consistency of potential effects of relative elevation on k and S, one-way ANOVA ~~to test for differences in k and S between zones for~~was used in each site separately (~~Fig. S2).~~ replication was sufficient in 20 sites). Normal distribution of residuals was assessed visually, Levene’s test was used to test for homogeneity of variance, and data were log-transformed if assumptions were not met. Mann-Whitney U tests were conducted as a non-parametric alternative when log-transformed data did not meet ANOVA assumptions (Table S1).

We tested for effects of nutrient enrichment on k and S in the data from the TIDE project site (Massachusetts, US) using two-way ANOVA with enrichment treatment and marsh zone as predictors. ~~All analyses were conducted using STATISTICA 10 (StatSoft Inc., Tulsa, OK, USA).~~When Levene's test indicated heterogeneous variance (true for k), data were log-transformed, which stabilized variance. Normal distribution of residuals was assessed visually.

Lastly, in order to assess the applicability of the TBI approach in tidal wetlands, we separately investigated the temperature response of k and S for the ten sites situated along the North American Atlantic coast (Fig. S2). Previous studies have shown clear temperature/latitudinal effects on decomposition and microbial activity along this well-studied transect (Kirwan et al. 2014; Mozdzer et al. 2014), allowing us to compare the TBI approach with other methods. Regional-scale transects with sufficient temperature/latitudinal range along other coastlines could not be identified (Fig. 1; Table 1). Statistical analyses were conducted using STATISTICA 10 (StatSoft Inc., Tulsa, OK, USA).

3 Results

3.1 Temperature effects

We found no ~~linear (Fig. 2a) or monotonic (Table 3) relationships~~relationship between temperature and k~~.~~ across study sites (Fig. 2a; Table 3). Also, ~~CRTA~~CART revealed temperature only as a ~~minor predictor~~subordinate splitting variable for k (~~Figure~~Fig. S1a). Specifically, temperature seems to positively affect k in meso-tidal systems only (amplitude >2.1m; Fig. S1a; node 5) with sites ≥14.5°C during deployment supporting ~~considerably~~ higher rates of decomposition than sites characterized by lower temperatures. However, this apparent temperature effect was inconsistent within the group of observations with tidal amplitude >2.1m (Fig. S1a; nodes 13-15). In contrast to the results of the global-scale assessment, k is strongly and positively related with temperature across the ten sites situated along the North American Atlantic coast, with temperature explaining approx. 70% of variability in k (Fig. S2).~~Furthermore, the majority of sites (65%) are characterized by tidal amplitudes <2.1 m and show no temperature effect on k.~~

~~In contrast to the temperature response of k, S~~Stabilization was strongly affected by temperature (Fig. 2b; Table 3). The significant negative correlation between S and temperature (~~$p < 0.01$; r2 = 0.287;~~ Fig. 2b; Table 3) agrees well with the ~~CRTA~~CART (Fig. S1b). However, ~~CRTA~~CART also identified a narrow temperature range (21.9-23.6°C) in which increasing temperature led to higher stabilization (Fig. S1b; node 11). This group of observations ~~(n = 5)~~ from the general pattern is also clearly visible in Figure 2b and represents the 4 Mediterranean sites (Ebro Delta and Venice Lagoon) of our survey. The positive relationship between temperature and S was even clearer when focusing on the ten sites along the North American Atlantic coast, with temperature explaining >85% of variability in S (Fig. ~~2b.~~S2).

Temperature was highly correlated with latitude and tidal amplitude, and temperature was not independent of soil type (mineral/organic) and ecosystem type (marsh/mangrove) (Table 3). The effect of latitude was similarly pronounced as the temperature effect on S – and consequently –effects of these two parameters on S cannot be separated (Table 3). By contrast, tidal amplitude and soil type did not significantly affect S, and the difference in S between mangroves and marshes is only marginally significant (Table 3). These findings suggest that the presented temperature effect on S occurs to be mainly independent of tidal amplitude and soil type.

3.2 Effects of relative sea level and nutrient enrichment

Paired comparisons of high vs. low elevated zones indicate no consistent effect of relative sea level on k across sites ($p > 0.1$; Fig. 3a~~ + c~~), whereas S was significantly reduced by 29% in low compared to high elevated zones ($p < 0.01$; Fig. 3b). ~~This significant reduction of S in low vs. high elevated zones was consistent across mineral and organic, as well as marsh and mangrove systems (Fig. 3d).~~ Testing for effects of relative sea level within each site separately revealed that S is significantly reduced by 28-87% in the lower elevated zone in ~~15~~14 of ~~21~~20 sites~~. A~~; whereas a significant increase of S in low vs. high elevated zones was found in none of ~~these 21~~the 20 sites (~~Fig.~~ Table S1). This finding demonstrates the consistency of the sea-level effect on S irrespective of ecosystem type (marsh/mangrove) soil type (mineral/organic) and site salinity (brackish/salt).~~S2).~~ In ~~ten~~nine of the sites, we also found a significant effect of relative sea level on k~~; with~~. However, in six sites k was significantly higher ~~k~~ in low vs. high zones, and in ~~seven~~three sites ~~and~~k was significantly lower ~~k~~ in low vs. high zones~~ in three sites (Fig. S2~~. The direction of effects on k seems to be independent of ecosystem type, soil type, and site salinity (Table S1).

~~3.3 Effects of salinity and nutrient enrichment~~

~~We found no significant relationship between salinity class and k or S (Table 3). Also, CRTA did not reveal salinity class as an important factor for k and S (Fig. S1), and no consistent salinity effect on k and S was found when comparing sites of different salinities within single estuarine regions (Chesapeake, Ebro Delta, Long Marsh, San Francisco Bay; Fig. S3).~~

The nutrient enrichment treatment at the TIDE project site decreased S by 72% in the high marsh (Fig. 3d).~~.~~ S in the low marsh likewise was similarly low as in the ~~fertilized~~enriched high marsh and not further reduced by ~~fertilization~~nutrient enrichment (Fig. ~~4~~3d). In contrast, k was not responsive to the ~~fertilization~~nutrient enrichment treatment in neither low nor high marsh (Fig. ~~4~~3c).

3.4 Other factors influencing decomposition rate and stabilization

~~CRTA~~CART revealed tidal amplitude as an important predictor for k (Fig. S1a). However, this result needs to be ~~interpreted~~considered cautiously because splits based on tidal amplitude suggest mixed effects (Fig. S1a). Accordingly, no ~~linear ($p > 0.68$; $r2 = 0.004$) or monotonic~~significant relationship ~~(Table 3)~~ existed between tidal amplitude and k~~, and effects of tidal amplitude are not independent from other factors because strong correlations existed with ecosystem and soil type, temperature, and latitude (Table 3).~~ across sites (Table 3). Soil type (mineral~~ vs. ~~/organic) and ecosystem type (marsh~~ vs. ~~/mangrove) did not affect k and S across sites (Table 3). We found no significant relationship between salinity class and k or S (Table 3~~, Fig. ~~). Also, CART did not reveal salinity class~~S1a). In comparison, S was lower in mangroves than in marshes and lower in organic than in mineral systems (Table 3), presumably caused by temperature effects because ecosystem and soil type did not show up~~ as ~~predictors in CRTA~~an important factor for k and S (Fig. ~~S1b~~S1).

4 Discussion

~~The findings of the present study cannot demonstrate consistent effects of either temperature or relative sea level on the decomposition rate of recent OM inputs (commonly assessed as k in litter bag studies) in tidal wetlands. With respect to C sequestration, however, litter-decay data need to be considered cautiously, as the link among decomposition rate, SOM formation, and ultimately C sequestration is not straightforward. That is, plant tissues and other fresh OM inputs into an ecosystem are not resistant to decay per se, and as a consequence, it is critical to understand their biogeochemical transformation into stable compounds that leads to the formation of SOM (i.e. stabilization) rather than understanding the pace at which decomposition proceeds (Prescott, 2010; Castellano et al., 2015; Haddix et al., 2016). Here, we also assessed OM stabilization, and in contrast to decomposition rate, stabilization decreased with temperature and was consistently lower in low vs. high elevated zones of tidal wetlands. Our study therefore provides indirect evidence that rising temperature and accelerated SLR could decrease the capacity of tidal wetlands to sequester C by affecting the initial transformations of recent OM inputs to SOM (i.e. stabilization).~~

4.1 Temperature effects on decomposition processes

~~Surprising~~A positive relationship between temperature and decomposition rate was found only at the regional scale across the ten sites along the North American Atlantic coast (Fig. S2), but not across all sites at the global scale (Fig. 2). Even though this finding occurs surprising in the context of basic biokinetic theory, ~~the temperature response of decomposition rate was weak or not~~ it is in agreement with findings of Djukic and others (2018), demonstrating climate effects on the break-down of the TBI materials across terrestrial ecosystems at the biome scale, but not at the global scale across biomes.

The present~~. Following~~ study used air temperature as a proxy for top-soil temperature. Thus, the temperature regime of the decomposition environment was only approximated, which certainly would have weakened a significant relationship between temperature and k. However, following typical Q10 values for biological systems of 2-3 (Davidson ~~&~~and Janssens, 2006), k should have at least doubled over ~~a~~the gradient of ΔT >15°C~~. However, findings from~~; yet our data do not even show a tendency of an effect (rs = 0.02; Table 2). We therefore propose that other parameters exerted overriding influence on k, mainly masking temperature effects, and have not been captured by our experimental design. This notion is in line with the fact that studies conducted at single-marsh to regional scales ~~are not conclusive either~~report equivocal results on the temperature response of k, ranging from no or ~~small~~moderate (Charles ~~&~~and Dukes, 2009; ~~Kirwan et al., 2014;~~ Janousek et al., 2017; Kirwan et al., 2014) to strong seasonally-driven temperature effects with a Q10 >3.4 as found within a single site (Kirwan ~~& Blum, 2011). Although temperature sensitivity of OM types is variable (Craine et al., 2010; Hines et al., 2014; Wilson et al., 2016), temperature sensitivity of the used TBI materials was sufficiently demonstrated (Keuskamp et al., 2013). We therefore conclude that other parameters exerted overriding influence on k, mainly masking temperature effects and have not been captured by our experimental design. For instance, we do not have data on plant-biomass parameters that are thought to exert strong control on decomposition in tidal wetlands through priming effects (Wolf et al., 2007; Mueller et al., 2016; Bernal et al., 2017). Likewise~~and Blum, 2011). For instance, large differences in site elevation and hydrology could have induced high variability in k across sites and masked potential temperature effects. Indeed, we demonstrate significant but mixed effects of relative sea level on k for some sites~~;~~ (Table S2); however, we do not have sufficient data on actual site elevation or hydrology to control for these factors as covariates affecting the temperature effect on k. Likewise, we do not have data on nutrient availability, plant productivity, or various anthropogenic impacts that could have exerted strong control over decomposition processes in the studied sites (Deegan et al., 2012; Keuskamp et al., 2015a; Macreadie et al., 2017; Mueller et al., 2016).

In contrast to the missing ~~or subtle effects~~effect of temperature on k, OM stabilization was strongly affected~~ by temperature.~~. Overall, S decreased by ~~~90~~75% over our temperature gradient from 10.9 to 28.5°C~~, corresponding to a decline of ~5% over a 1°C temperature increase (Figure~~ (Fig. 2b). Thus, we demonstrate a considerable temperature effect on the initial steps of biomass decomposition in tidal wetlands. ~~This effect, however,~~However, as also demonstrated for k, the temperature effect on S was much clearer at the regional scale when focusing on the sites along the North American Atlantic coast (Fig. S2), suggesting high variability in S across regions irrespective of the temperature regime. In accordance, we also demonstrate a clear divergence of the four Mediterranean sites from the regression model (Fig. 2; S1), which could be related to differences in precipitation or nutrient availability across study regions. Future experimental work is therefore required in order to further assess the effects of temperature on OM stabilization and to separate temperature from latitudinal and other interacting effects (e.g. as outlined above for k) that are difficult to control for in observational studies.

The temperature effect on the initial steps of biomass decomposition we identified in the present study is not driven by changes in decomposition rate per se, but – more importantly – by affecting the transformation of fresh and rapidly decomposable organic matter into stable compounds, with potentially important implications for ~~C~~carbon sequestration (e.g. Cotrufo et al., 2013).

In their global-scale assessment, Chmura et al. (2003) indeed report a negative relationship of soil organic C density and mean annual temperature within both salt marshes and mangroves. ~~Indeed,~~ Chmura and colleagues hypothesized stimulated microbial decomposition at higher temperatures to be the responsible driver ~~for~~of this relationship. Plant production and thus OM input is known to increase with latitude and temperature in tidal wetlands (Baldwin et al., 2014; Charles ~~&~~and Dukes, 2009; Gedan ~~&~~and Bertness, 2009; Kirwan et al., 2009~~; Baldwin et al., 2014~~), but this increase seems to be more than compensated by higher microbial decomposition. Working ~~on~~at the same spatial scale as Chmura et al. (2003), our study ~~strongly~~ supports this hypothesis and provides ~~the~~ mechanistic insight into the temperature control of OM decomposition as ~~an important~~a potential driver of ~~C~~carbon sequestration in tidal wetlands.

4.2 Relative-sea-level effects on decomposition processes

Flooding and thus progressively lower oxygen availability in soil is supposed to be a strong suppressor of decomposition (Davidson and Janssens, 2006). In tidal wetlands, differences in flooding frequency along elevational gradients often induce sharp gradients in oxygen availability and redox conditions (Davy et al., 2011; Kirwan et al., 2013; Langley et al., ~~& Janssens, 2006). Despite this dominant paradigm, we clearly demonstrate that k is not reduced in low vs. high elevated zones of tidal wetlands (Fig.~~2013), with potentially strong influence on OM decomposition and carbon cycling. However, the effect of redox conditions on OM break-down is determined by the chemical quality of the decomposing material: Decomposition of aged or recalcitrant OM can indeed be slower and incomplete in the absence of oxygen, whereas the break-down of fresh and labile OM can be largely unaffected by oxygen availability (Benner et al., 1984; Kristensen et al., 1995). Thus, also decomposition rate and stabilization of labile, hydrolyzable OM, as assessed in the present study, is not necessarily affected by redox conditions. Here, we demonstrate that k is not reduced in low (more frequently flooded) vs. high elevated (less frequently flooded) zones of tidal wetlands (Fig. 3a). This finding is in accordance with an increasing number of studies demonstrating negligible direct effects of sea level on decomposition rate in tidal wetland soils (Janousek et al., 2017; Kirwan et al., 2013; Mueller et al., 2016~~; Janousek et al., 2017). A SLR-induced reduction in decomposition rate with positive feedback on tidal wetland stability seems therefore to be an unlikely scenario.~~). Furthermore, we show that S is strongly reduced in low vs. high elevation zones, suggesting that the conversion of recent OM inputs to stable compounds ~~and eventually SOM~~ is in fact lower in more flooded zones of tidal wetlands. ~~Accelerated~~As the stabilization of labile OM inputs is a major driver of SOM formation (Cotrufo et al., 2013, 2015; Haddix et al., 2016), one important implication of this finding is that accelerated SLR ~~consequently seems to yield~~yields the potential to decrease ~~SOM formation and with that C~~ the carbon-sequestration potential of tidal wetlands. ~~This finding and its implication may occur counterintuitive with respect to the often sharp redox gradients along tidal wetland zonations and with flooding~~ The~~(Davy et al., 2011; Kirwan et al., 2013; Langley et al., 2013), and the~~ mechanism by which S is decreased in the more flooded zones of the present study is unknown. Because we did not observe consistent salinity effects on S and k in our data~~ (Figs. S1, S3),~~, we do not suppose that regular exposure of litter to salt water explains the unexpected finding. ~~Instead,~~Likewise, soil temperature was not consistently affected by relative elevation across sites (p > 0.3; paired t-test based on data shown in Fig. S3). Instead, we argue that more favorable soil moisture conditions in low vs. high elevated zones could have decreased OM stabilization if higher flooding frequencies did not induce redox conditions low enough to suppress microbial activity in the top soil. In support of this, flooding-frequency induced changes in moisture conditions have been reported as primary driver of surface litter break~~-~~-down, leading to more than four-fold increased litter mass loss in low vs. high marsh zones of a New Jersey salt marsh (Halupa ~~&~~and Howes, 1995).

Additionally, greater nutrient availability and less nutrient-limited microbial communities in more frequently flooded zones could have contributed to this effect (Deegan et al., 2012;

Kirwan et al., 2013). Strong effects of both high quality marine-derived OM and nutrient amendments on microbial structure and activity have been reported (Deegan et al., 2012; ~~Keuskamp et al., 2015a;~~ Kearns et al., 2016; Keuskamp et al., 2015b; Mueller et al., 2017), suggesting that regular marine OM and nutrient inputs in more frequently flooded zones can positively affect decomposition~~.~~ (see further discussed below in 4.3).

4.3 Nutrient enrichment reduces stabilization – insights from the TIDE project

In addition to our global survey of early-stage decomposition processes in tidal wetlands ~~worldwide~~, we included the long-term ecological research site of the TIDE project in Massachusetts, US to experimentally assess both the effects of coastal eutrophication and the relevance of nutrient delivery through floodwater for OM decomposition in tidal wetlands. Important for our argument that decomposition may be favored by higher nutrient availability in low elevated, more frequently flooded zones, we observed a strong reduction (>70%) of S by nutrient enrichment in the high marsh, whereas S in the low marsh likewise was low as in the fertilized high marsh and not further reduced by fertilization (Fig. ~~4~~3d). Johnson et al. (2016) demonstrate that nutrient enriched high-marsh plots of the TIDE project receive 19±2 g N m-2 yr1, approximately 10-times the N load of reference high-marsh plots (2±1 g N m-2 yr-1; mean±SE), thus explaining the strong treatment effect observed in the high marsh. In accordance with low stabilization in the reference low marsh, which is equally low as the nutrient enriched high marsh, reference plots of the low marsh receive 16±4 g N m-2 yr1, the same high N load as the enriched high-marsh plots. Surprisingly, however, N loads of 171±19 g N m-2 yr1 in the enriched low-marsh plots do not result in additional reduction of S compared to the reference low marsh (Fig. ~~4~~3d). These findings suggest that microbial communities of the high marsh are N limited, and that N additions to a certain level can stimulate early OM decomposition and thus reduce stabilization. The missing effect of N loads exceeding 16 g m-2 yr1 on stabilization in the low marsh indicates that microbial communities are less or not N limited due to the naturally greater nutrient availability. The findings of the TIDE project therefore support our concept that higher nutrient availability and less nutrient-limited microbial communities in more frequently flooded zones could have contributed to the observed reduction of OM stabilization in low vs. high elevated zones of tidal wetlands in our global assessment.

Although our conclusions on effects of nutrient enrichment on OM decomposition are based on the findings of a single field experiment only, our study adds to a growing number of reports illustrating the impact of coastal eutrophication on tidal wetland C cycling (~~Morris & Bradley, 1999;~~ Deegan et al., 2012; Keuskamp et al., 2015a; Kirwan ~~&~~and Megonigal, 2013; ~~Keuskamp et al., 2015b~~Morris and Bradley, 1999). At the same time, however, we highlight the need to improve our understanding of coastal eutrophication in interaction with other global changes, particularly accelerated SLR and concomitant changes in flooding frequency, on the cycling of both labile and refractory C pools in order to predict future stability of tidal wetlands.

4.4 ~~Methodological~~The Tea Bag Index – methodological considerations

~~The~~ Interpretation of results obtained from standardized approaches like the present needs to be made cautiously because OM quality ~~of OM~~ (i.e. its chemical composition) is a key parameter affecting its decomposition. As the quality of the TBI materials differ from that of wetland plant litters, and likely even more from the quality of the imported allochthonous OM (Khan et al., 2011), we did not expect to capture ~~precise and absolute values for wetland litter~~actual rates of early-stage OM break~~-~~down in this study. Instead, we used the ~~Tea Bag Index~~TBI to characterize the decomposition environment by obtaining a measure for the potential to decompose and stabilize the deployed standardized material. Standardized approaches like this, or also the cotton-strip assay (e.g. Latter and Walton, 1988), are useful to separate the effects of environmental factors other than OM quality on decomposition processes and to assess their relative importance. Otherwise, complex interaction effects of the abiotic environment and OM quality make it difficult to predict the relevance of certain environmental factors for decomposition processes, potentially masking the effects of important ~~global-change drivers (reviewed in Prescott, 2010).~~global-change drivers (Prescott, 2010). At the same time, however, the global-change factors considered in the present study are likely to induce changes in the quality of the OM accumulating in tidal wetlands, for instance through shifts in plant-species composition and plant-tissue quality, that can potentially counterbalance or amplify the effects on decomposition processes suggested here. Future research therefore needs to address OM quality feedbacks on decomposition processes in tidal wetlands in order to gain a more complete understanding of global-change effects on tidal-wetland stability and carbon-sequestration capacity. ~~Stabilization is thought to be a key parameter for capturing changes in decomposition with consequence for C sequestration. Indeed, Keuskamp et al. (2013) demonstrate that S, as determined by the TBI, is significantly related with the C sequestration potential of an ecosystem as defined by FAO (2000). In the present study, however, a large percentage of observations showed relatively low values for S, although tidal wetlands are known to act as particularly effective C sinks (Mcleod et al., 2011).~~ Based on the S values obtained from initial calculations using the ~~hydrolysable~~hydrolyzable fractions suggested by Keuskamp et al. (2013), a large number of observations ~~in fact~~ yielded a negative S (~~data not shown~~Table S3). S becomes negative when the mass loss from green tea is greater than the predicated maximum loss based on its ~~hydrolysable~~hydrolyzable fraction. At least two processes could have caused ~~or contributed to~~ this result: First, ~~we demonstrate that S is indeed reduced in low vs. high elevated zones across~~ our ~~study sites, indicating~~data indicate that redox conditions in the top soil of tidal wetlands are ~~at~~

~~least often~~ not low enough to hamper decomposition ~~processes.~~of the hydrolyzable fraction of the TBI materials. As a consequence, ~~the relatively~~ high top-soil moisture of tidal wetlands could provide favorable conditions for decomposition, following typical moisture-decomposition relationships as demonstrated for terrestrial ecosystems (e.g. Curiel Yuste et al., 2007~~), and S should at least not expected to be high in the top-soil environment of tidal wetlands.~~). Potentially, moisture conditions and nutrient supply ~~are~~ even ~~high enough to~~ allow for considerable break~~-~~-down of non-~~hydrolysable~~hydrolyzable compounds within three months of deployment, such as lignin (Berg ~~&~~and McClaugherty, ~~2003; Knorr~~2014; Duboc et al., ~~2005~~2014; Feng et al., 2010; ~~Duboc~~Knorr et al., ~~2014~~2005). Second, different protocols ~~and methods~~ to determine ~~hydrolysable and non-hydrolysable fractions~~the hydrolyzable fraction of plant materials exist and lead to variable results. The ~~hydrolysable~~hydrolyzable fraction ~~of plant materials~~ can consequently be over- or underestimated depending on ~~method,~~ protocol~~,~~ and type of sample material. The use of the slightly higher ~~hydrolysable~~hydrolyzable fractions we determined for calculations of the TBI parameters effectively eliminated negative S values. In that regard, using the values obtained from the alternative protocol given in Ryan et al. (1990) seemed more reasonable in our study. ~~Although~~However, it needs to be stressed here that direction and size of reported effects on S and k in the present study are almost independent ~~from~~of the ~~hydrolysable~~hydrolyzable fraction used for calculations~~, further research is required to improve our estimates of the hydrolysable fractions in TBI materials~~.

Future research will have to test the applicability of the TBI approach in different ecosystems and test the validity of its assumptions (i.e. S is equal for both types of material used, and mass loss of non-hydrolyzable material is negligible over 3 months of deployment). The results of our regional scale assessment along the North American Atlantic coast transect are in tight agreement with previously reported results on cellulose break-down and soil microbial activity along this well studied transect (Kirwan et al., 2014; Mozdzer et al., 2014). We can thereby demonstrate the usefulness of the TBI approach to assess early-stage decomposition in tidal-wetland soils.

4.5 Implications

~~While awareness about potential global-warming impacts on OM preservation and their resulting threat to future tidal wetland stability has been raised (Kirwan & Mudd, 2012), concepts on the vulnerability of tidal wetlands to accelerated SLR mainly focus on plant-productivity responses and their biophysical feedbacks (Kirwan et al., 2016). Potentially negative effects of accelerated SLR on OM preservation were thus far overlooked, probably because stimulation of decomposition processes through increasing flooding is counterintuitive (Mueller et al., 2016). Here, we provide evidence that accelerated SLR is unlikely to slow down the decomposition rate of fresh OM inputs and additionally may strongly decrease OM stabilization and thus potentially the fraction of net primary production and other OM inputs to stable SOM.~~

This study addresses the influence of temperature, relative sea level, and coastal eutrophication on the initial transformation of biomass to SOM, and it does not encompass their effects on the existing SOM pool. However, aspects of S and k are key components of many tidal wetland resiliency models (Schile et al., 2014; Swanson et al., 2014) that have highlighted the critical role of the organic contribution to marsh elevation gain. ~~Thus, combined~~ Although actual rates of S and k cannot be inferred from this study using a standardized approach, our data identify strong negative effects of temperature, relative sea level, and coastal eutrophication on ~~OM~~the stabilization ~~may~~of fresh organic inputs to tidal-wetland soils. We argue that these unanticipated combined effects yield the potential to strongly ~~reduce OM accumulation rates and increase wetland~~ accelerate carbon turnover in tidal wetlands, thus increasing their vulnerability to accelerated SLR, and we highlight the need for experimental studies assessing the extent to which the here identified effects translate into native OM dynamics.

~~Our findings imply that particularly the vulnerability of organic systems might increase with global change because in these systems soil volume is almost exclusively generated by the preservation of OM. At the same time, however, mineral dominated systems, such as temperate European salt marshes, experience large amounts of easily decomposable allochthonous-OM input that relies on substantial stabilization in order to become sequestered (Middelburg et al., 1997; Allen, 2000; Khan et al., 2015). Thus, future rates of C sequestration could be substantially reduced in mineral dominated tidal wetland systems.~~

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Author contributions

PM, SN, KJ, and LMS-B designed the overall study. PM analyzed and interpreted the data. PM wrote the initial version of the manuscript with regular comments and editing provided by LMS-B, TJM, and SN. PM, LMS-B, TJM, GLC, TD, YK, AVdG, PE, CS, AD'A,CI, ML, UN, BJJ, AHB, SAY, DIM, ZY, and JW designed and conducted the field studies in the respective sites and commented on an earlier version of the manuscript.

Figure captions

Figure 1 Overview map of study ~~sites~~regions. Notes: See Table 1 for region and site details.

Figure 2 (a) Decomposition rate (~~k~~site means; n = 30) and (b) stabilization ~~factor (S~~(site means; n = 30) versus mean ~~atmospheric~~air temperature during deployment period. ~~Shown are results of linear~~Regression line illustrates significant relationship between temperature and stabilization (Table 2); regression ~~analyses across and within elevation zones and organic~~model with lowest standard error of estimate (SEE) and ~~mineral soils.~~highest R2 is shown: y = -0.27ln(x) + 0.99; R2 = 0.239; SEE = 0.131; excluding the Mediterranean sites (21.9-23.6°C; n = 4) from the regression yields: y = -0.344ln(x) + 1.233; R2 = 0.510; SEE = 0.101

Figure 3 (a~~ + c~~) Decomposition rate ~~(k) ~~and (b~~ + d~~) stabilization ~~factor (S) ~~in high ~~(orange) ~~and low ~~(blue) ~~elevated zones of tidal marsh and mangrove sites (~~compare Table 1). High and low elevated zones are characterized by distinct plant species assemblages or by different stature of mangroves along the flooding gradient within each site. Shown are means ± SE for all sites (a + b) and for mineral, organic, marsh, and mangrove systems separately (c + d). P-values refer to results of paired t-tests (ns, P > 0.05; * P ≤ 0.05; ** P ≤ 0.01).~~

~~Figure 4 Effects of marsh elevation (zone) and ~~n = 21; compare Tables 1, S1). (c) Decomposition rate and (d) stabilization in nutrient ~~enrichment on both decomposition rate (k) and stabilization factor (S) in long-term ~~enriched ~~(filled bars) and~~versus reference~~ areas (open bars) in the~~ high marsh (Spartina patens zone) and low marsh (Spartina alterniflora zone) of the TIDE project site at the Plum Island Sound Estuary, Massachusetts, US. Shown are means ± SE and results of paired t-tests (panels a + b) and two-way ANOVAs ~~and ~~plus Tukey's HSD test for pairwise comparisons (~~Tukey's HSD test). ~~panels c + d): ns = not significant; * = p ≤ 0.05; ** = p ≤ 0.01

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Contents:

1.) **Point-by-point reply** (to editor response after revision)

2.) **Marked-up version** (to editor response after revision)

1.) Point-by-point reply

EDITOR COMMENT: Dear authors, after reading your revised MS, I find it can be published in Biogeosciences after you consider the following technical point: In its present form, your MS is very short and the supplementary material relatively long. The MS that is supposed to be published contains only 3 figures. However, some figures in the supplementary material are cited a lot in the text: Fig S1 is cited 9 times and Fig S2 is cited 5 times. I wonder what are the motivations for this choice and if the paper would not benefit from the insertion some of this additional figures in the MS rather than in the supplementary material.
Could you please explain the motivation for this choice, unusual for Biogeosciences, and if relevant, provide a revised MS that include the most cited figures and tables in the main text.

REPLY: Dear Editor, we considered figures 1 and 3 as well as table 1 to be quite large and therefore decided to move less relevant information to the supplement. In fact, we thought our MS would be rather too long than too short; so thanks for the rectification! We agree that some of the supplementary material is indeed cited quite often throughout the MS.

Concerning Fig. S1: This figure presents (only) the results of a data mining approach, and it is used only in addition/to support the results presented in the other figures and tables. We don't want the reader to think that it displays the primary output of our statistical analyses to test for temperature/sea level/eutrophication effects. Although insightful, it is less relevant for our story line than the other figurers/tables. That being said, we think it has been cited more often than necessary in the previous version of the MS. We addressed this, reducing the number of citations from 9 to 4.

Instead of moving Fig S1 to the main text, we would prefer to include Fig S2. Its results are indeed quite central, and inclusion would help the reader follow our story more easily. In the revised version, it is included as the new Fig 3 (of 4).

2.) MARKED-UP VERSION:

Global-change effects on early-stage decomposition processes in tidal wetlands – Implications from a global survey using standardized litter

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Manuscript type: Primary research

Abstract

Tidal wetlands, such as tidal marshes and mangroves, are hotspots for carbon sequestration. The preservation of organic matter (OM) is a critical process by which tidal wetlands exert influence over the global carbon cycle and at the same time gain elevation to keep pace with sea-level rise (SLR). The present study assessed the effects of temperature and relative sea level on the decomposition rate and stabilization of OM in tidal wetlands worldwide, utilizing commercially available standardized litter. While effects on decomposition rate per se were minor, we show strong negative effects of temperature and relative sea level on stabilization, as based on the fraction of labile, rapidly hydrolyzable OM that becomes stabilized during deployment. Across study sites, OM stabilization was 29% lower in low, more frequently flooded vs. high, less frequently flooded zones. Stabilization declined by ~75% over the studied temperature gradient from 10.9 to 28.5°C. Additionally, data from the Plum Island long-term ecological research site in Massachusetts, US show a pronounced reduction in OM stabilization by >70% in response to simulated coastal eutrophication, confirming the potentially high sensitivity of OM stabilization to global change. We therefore provide evidence that rising temperature, accelerated SLR, and coastal eutrophication may decrease the future capacity of tidal wetlands to sequester carbon by affecting the initial transformations of recent OM inputs to soil OM.

1 Introduction

Tidal wetlands, such as marshes and mangroves, provide a wide array of ecosystem services that have been valued at approximately US$ 10,000 per hectare and year, making them some of the most economically valuable ecosystems on earth (Barbier et al., 2011; Kirwan and Megonigal, 2013). Yet, tidal wetlands are threatened and vulnerable ecosystems, experiencing pronounced loss though global-change impacts, such as land use (Pendleton et al., 2012) and accelerated sea-level rise (SLR) (Craft et al., 2009; Crosby et al., 2016). In recent years, carbon sequestration has increasingly been recognized as an ecosystem service of tidal wetlands (Chmura et al., 2003; Mcleod et al., 2011). Here, high rates of organic matter (OM) input (from both autochthonous and allochthonous production) co-occur with reducing soil conditions and thus slow rates of decomposition, leading to long-term carbon-sequestration rates that exceed those of most other ecosystem types by an order of magnitude (Mcleod et al., 2011). At the same time, suppressed decomposition and the preservation of OM is a primary process by which many tidal wetlands gain elevation and keep pace with rising sea level (Kirwan and Megonigal, 2013). Consequently, global changes that decrease OM preservation in tidal-wetland soils not only affect carbon sequestration, but also decrease ecosystem stability against SLR. It is therefore critical to identify global-change factors that affect the transformation of organic inputs to stable soil OM (SOM) in tidal wetlands and to assess the magnitude of their effects.

There are multiple methods for assessing factors that influence carbon sequestration, including direct measurements of plant production, carbon stocks, accretion, and decomposition rates. Litter-bag techniques assessing the weight loss of plant material over time are probably the easiest way to measure decomposition rates in situ and have been widely used since the 1960s (Prescott, 2010). Global-scale assessments of litter decomposition have been conducted as both meta-analyses (e.g. Zhang et al., 2008) and as inter-site studies along latitudinal gradients (Berg et al., 1993; Cornelissen et al., 2007; McTiernan et al., 2003; Powers et al., 2009; Trofymow et al., 2002) in order to assess effects of climate parameters on decomposition rate. Besides abiotic or climate effects, these studies could also identify litter quality itself as an important predictor for decomposition rate (Zhang et al., 2008).

Relationships between single climate or litter-quality parameters and decomposition rate often are not linear. Instead, complex interactions between litter-quality and climate parameters seem to control litter decomposition (Zhang et al., 2008), creating challenges in separating climate from litter-quality effects and predicting the relevance of potential global-change drivers for decomposition rate. In order to separately assess environmental or climate effects on litter decomposition at a global scale, it is therefore necessary to standardize litter quality in inter-site studies. However, implications of litter-decay data for carbon sequestration need to be considered cautiously, as the link among litter-decomposition rate, SOM formation, and ultimately carbon sequestration is not straightforward (Cotrufo et al., 2013; Prescott, 2010): Because plant tissues are not resistant to decay per se, it is critical to understand their biogeochemical transformation into stable compounds that leads to the formation of SOM (i.e. stabilization) rather than understanding the pace at which early-stage decomposition proceeds (Castellano et al., 2015; Haddix et al., 2016; Prescott, 2010).

Keuskamp and others (2013) developed an efficient approach for studying litter decomposition and OM transformation at a global scale, using commercially available tea as standardized material. Their Tea Bag Index (TBI) approach is based on the deployment of two types of tea that considerably differ in their OM quality. The method allows for the determination of the decomposition rate constant (in the following referred to as *decomposition rate* or *k*) and a stabilization factor (in the following referred to as *stabilization* or *S*), which describes the fraction of labile and rapidly decomposable OM that becomes stabilized during deployment.

In the present study, we assessed effects of the global-change factors global warming, accelerated SLR, and coastal eutrophication on both OM decomposition rate and stabilization in tidal-wetland soils by conducting a worldwide survey using standardized litter. First, by covering a large temperature gradient of $\Delta T > 15$ °C across sites, we aimed to capture temperature effects on OM decomposition rate and stabilization, thereby improving our understanding on how global warming affects carbon turnover and ultimately sequestration in tidal wetlands. Second, by conducting paired measurements in both high- and low-elevated zones of tidal wetlands worldwide, we were aiming to gain insight into potential effects of accelerated SLR on carbon turnover. Despite the dominant paradigm that decomposition is inversely related to flooding, the existing literature on hydrology and SLR effects on OM decomposition in tidal wetlands yields equivocal results, which is often due to the overriding effect of OM quality on decomposition rate (Hemminga and Buth, 1991; Kirwan et al., 2013; Mueller et al., 2016). Lastly, we used the TIDE project plots of the Plum Island long-term ecological research site in Massachusetts, US (Deegan et al., 2012) to experimentally assess both the effects of coastal eutrophication and – with respect to SLR-driven increases in flooding frequency – the relevance of nutrient delivery through floodwater for the early stages of OM decomposition in tidal wetlands.

2 Methods

2.1 Study sites and experimental design

The worldwide survey was conducted during the 2015 growing season and included a total of 30 tidal-wetland sites. Sites were partly co-located within larger coastal and estuarine regions (Fig. 1, Table 1). Eleven sites were situated along the European coasts of the North Sea, Mediterranean, and Baltic, Thirteen sites were located along the East and West coasts of North America including the St. Lawrence Estuary, Bay of Fundy, Chesapeake Bay, and San Francisco Bay, and four mangrove sites were situated along the Caribbean coast of Central America in Belize and Panama. Additionally, one Chinese site (Yangtze Estuary) and one Argentinian site were included in our study. Sixteen of the sites were salt marshes, ten were tidal freshwater and brackish sites, and four sites were mangroves. In 21 sites, we compared high and low elevated zones, which were characterized by distinct plant species compositions (i.e. different communities in high vs. mid vs. low marshes) or by different stature of mangroves (i.e. dwarf vs. fringe phenotypes). We used relative elevation (i.e. high vs. low elevated zone) as a site-specific proxy for relative sea level. By doing so, we did not capture the actual variability in the tidal inundation regime across our study sites as these vary in absolute elevation and in elevation relative to mean high water. Finally, we included the long-term experimental site of the TIDE project in Massachusetts, US to assess effects of nutrient enrichment on litter-decomposition rate and stabilization. Through nitrate additions to the incoming tides on at least 120 days per year, nutrient enriched areas at the TIDE project site receive floodwater with 10-15 fold increased nitrogen (N) concentrations compared to reference areas since 2004. From 2004-2010 also phosphate was added to the floodwater; however, this has been discontinued because creek water P concentrations are high enough to prevent secondary P limitation through N enrichment (details in Deegan et al., 2012; Johnson et al., 2016).

Decomposition rate and stabilization were measured by deploying tea bags in ten points per zone (or treatment) within a site (n = 10). Spacing between replicates within a zone (or treatment) was ≥2 m. However, as sites differed considerably in their areal extent, the distribution and thus spacing between points had to be adjusted to be representative for the given system. Air temperature for the period of deployment was measured at site, or temperature data was obtained from the online service of *AccuWeather* (accuweather.com; accessed 12/25/2016) for locations within a distance of 15 km to the site for most sites, but not further than 60 km for some remote sites. It needs to be noted here, that top-soil temperature would differ from air temperature depending on factors such as canopy shading or tidal regime and water temperature. As a consequence, air temperature can only approximate the temperature conditions of the actual decomposition environment (Fig. ~~S3~~S2).

2.2 Decomposition-rate and stabilization measurements

Decomposition rate (*k*) and stabilization (*S*) were assessed following the TBI protocol (Keuskamp et al., 2013). The TBI approach can be considered as a simplified litter-bag approach, allowing a time- and cost-efficient characterization of the decomposition environment, because *k* and *S* can be estimated without repeated sampling of the decomposing material as in conventional approaches. This implies the assumptions that (1) *S* is equal for the two types of material used in the approach and (2) that decomposition of non-hydrolyzable materials during the 3 months of deployment is negligible. We refer the reader to Keuskamp et al. (2013) for further detail and validity assessments of assumptions.

At each measuring point, two nylon tea bags (200 µm mesh size), one containing green tea (EAN: 8 722700 055525; Lipton, Unilever, UK) and one containing rooibos (8 722700 188438, Lipton, Unilever, UK), were deployed as pairs in ~8 cm soil depth, separated by ~5 cm. The initial weight of the contents was determined by subtracting the mean weight of ten empty bags (bag + string + label) from the weight of the intact tea bag prior to deployment (content + bag + string +

label). The tea bags were retrieved after an incubation time of 92 ± 6 (SD) days, with three sites having an incubation period >100 days and one site <80 days. Upon retrieval, tea bags were opened, and tea materials were carefully separated from fine roots and soil, dried for 48 h at 70°C, and weighed.

Calculations for *k* and *S* followed Keuskamp et al. (2013):

Eq 1) $$W_r(t) = a_r e^{-kt} + (1-a_r)$$

Eq 2) $$S = 1 - a_g / H_g$$

Eq 3) $$a_r = H_r (1-S)$$

$W_r(t)$ describes the substrate weight of rooibos after incubation time (*t* in days), a_r the labile and $1-a_r$ the recalcitrant fraction of the substrate, and *k* is the decomposition rate constant. *S* describes the stabilization factor, a_g the decomposable fraction of green tea (based on the mass loss during incubation) and H_g the hydrolyzable fraction of green tea. The decomposable fraction of rooibos tea is calculated in Eq 3 based on its hydrolyzable fraction (H_r) and the stabilization factor *S*. With $W_r(t)$ and a_r known, *k* is calculated using Eq 1.

In accordance with Keuskamp et al. (2013), extractions for determination of the hydrolyzable fractions of green and rooibos tea followed Ryan et al. (1990). However, instead of using Ryan's *forest products protocol* we conducted the alternative *forage fiber protocol* for the determination of the hydrolyzable fraction. Briefly, 1 g of dried tea material (70°C for 24 h) was boiled in cetyltrimethyl ammonium bromide (CTAB) solution (1 g CTAB in 100 ml 0.5 M H_2SO_4) for 1 h (Brinkmann et al., 2002; Ryan et al., 1990). The extract was filtered through a 16-40-µm sinter filter crucible (Duran, Wertheim, Germany) using a water-jet vacuum pump and washed with

150 ml of hot water followed by addition of acetone until no further de-coloration occurred (Brinkmann et al., 2002). The remaining material was left in the sinter, dried for 12 h at 70°C, cooled in a desiccator and weighed. 20 mL of 72% H_2SO_4 was added to the sinter and filtered off after an incubation of 3 h, followed by washing with hot water to remove remaining acid. The sinter was dried at 70°C for 12 h, cooled in a desiccator, and weighed to determine the non-hydrolyzable fraction. Finally, the sinter containing the remaining sample was ignited at 450°C for 3 h in order to determine the ash content of the material.

In addition to the determination of the hydrolyzable fraction, we measured total C and N contents of the tea material using an elemental analyzer (HEKAtech, Wegberg, Germany). The hydrolyzable fraction of both green and rooibos tea was higher than reported in Keuskamp et al. (2013) (Table S1). However, the determined C and N contents of the tea materials are in agreement with those reported in Keuskamp et al. (2013) (Table S1). Therefore, deviations from the hydrolyzable fraction as reported previously are likely due to the less conservative extraction assessment in the present study and not due to actual changes in the quality of the materials.

2.3 Data mining

Relationships between single parameters and litter decomposition are often not linear. Instead, critical thresholds seem to exist at which a certain predictor (e.g. mean annual temperature) becomes influential (Prescott, 2010; Rothwell et al., 2008). In the first step of data mining, we therefore used classification and regression tree analysis (CART) to identify potential thresholds and important predictors for k and S (Fig. S1). Data mining was conducted using STATISTICA 10 (StatSoft Inc., Tulsa, OK, USA).

2.4 Statistical analyses

To test for effects of temperature on *k* and *S*, Spearman rank correlations were conducted using site means (n = 30). As we did not expect temperature to be independent of other parameters in this observational study, we constructed a Spearman correlation matrix including the parameters temperature, latitude, tidal amplitude, salinity class, *k*, and *S*. Additionally, we tested for differences in these parameters between marshes and mangroves and sites with mineral and organic soils, using Mann-Whitney U tests (Table 2). Curve fitting was used to further explore relationships between temperature, *k*, and *S*, and regression models with lowest standard error of estimate and highest R^2 are displayed in Figure 2 and ~~S2~~3.

To test for effects of relative elevation (as proxy for relative sea level) on *k* and *S*, two-tailed paired t-tests were conducted. Mean values of high and low elevated zones of the 21 sites where tea bags were deployed in both high and low elevation zones were used (n = 21). The absence of outliers and normal distribution of the difference in the independent variable (as assessed visually) assured robustness of paired t-tests. To assess the consistency of potential effects of relative elevation on *k* and *S*, one-way ANOVAs were used in each site separately (replication was sufficient in 20 sites). Normal distribution of residuals was assessed visually, Levene's test was used to test for homogeneity of variance, and data were log-transformed if assumptions were not met. Mann-Whitney U tests were conducted as a non-parametric alternative when log-transformed data did not meet ANOVA assumptions (Table S2).

We tested for effects of nutrient enrichment on *k* and *S* in the data from the TIDE project site (Massachusetts, US) using two-way ANOVA with enrichment treatment and marsh zone as predictors. When Levene's test indicated heterogeneous variance (true for *k*), data were log-transformed, which stabilized variance. Normal distribution of residuals was assessed visually.

Lastly, in order to assess the applicability of the TBI approach in tidal wetlands, we separately investigated the temperature response of *k* and *S* for the ten sites situated along the North American Atlantic coast (Fig. ~~S2~~3). Previous studies have shown clear temperature/latitudinal effects on decomposition and microbial activity along this well-studied transect (Kirwan et al. 2014; Mozdzer et al. 2014), allowing us to compare the TBI approach with other methods. Regional-scale transects with sufficient temperature/latitudinal range along other coastlines could not be identified (Fig. 1; Table 1). Statistical analyses were conducted using STATISTICA 10 (StatSoft Inc., Tulsa, OK, USA).

3 Results

3.1 *Temperature effects*

We found no relationship between temperature and *k* across study sites (Fig. 2a; Table 2). Also, CART revealed temperature only as a subordinate splitting variable for *k*~~ (Fig. S1a)~~. Specifically, temperature seems to positively affect *k* in meso-tidal systems only (amplitude >2.1m~~; Fig. S1a; node 5)~~ with sites ≥14.5°C during deployment supporting higher rates of decomposition than sites characterized by lower temperatures. However, this apparent temperature effect was inconsistent within the group of observations with tidal amplitude >2.1m (Fig. S1a~~; nodes 13-15~~). In contrast to the results of the global-scale assessment, *k* was strongly and positively related with temperature across the ten sites situated along the North American Atlantic coast, with temperature explaining approx. 70% of variability in *k* (Fig. ~~S2~~3).

Stabilization was strongly affected by temperature (Fig. 2b; Table 2). The significant negative correlation between *S* and temperature (Fig. 2b; Table 2) agrees well with the CART~~ (Fig. S1b)~~. However, CART also identified a narrow temperature range (21.9-23.6°C) in which increasing temperature led to higher stabilization (Fig. S1b; node 11). This group of observations diverging from the general pattern is also clearly visible in Figure 2b and represents the four Mediterranean sites (Ebro Delta and Venice Lagoon) of our survey. The positive relationship between temperature and *S* was even clearer when focusing on the ten sites along the North American Atlantic coast, with temperature explaining >85% of variability in *S* (Fig. ~~S2~~3).

Temperature was highly correlated with latitude and tidal amplitude, and temperature was not independent of soil type (mineral/organic) and ecosystem type (marsh/mangrove) (Table 2). The effect of latitude was similarly pronounced as the temperature effect on *S* – and consequently –

effects of these two parameters on *S* cannot be separated (Table 2). By contrast, tidal amplitude and soil type did not significantly affect *S*, and the difference in *S* between mangroves and marshes was only marginally significant (Table 2). These findings suggest that the presented temperature effect on *S* occurs to be mainly independent of tidal amplitude and soil type.

3.2 *Effects of relative sea level and nutrient enrichment*

Paired comparisons of high vs. low elevated zones indicate no consistent effect of relative sea level on *k* across sites ($p > 0.1$; Fig. ~~3a~~4a), whereas *S* was significantly reduced by 29% in low compared to high elevated zones ($p < 0.01$; Fig. ~~3b~~4b). Testing for effects of relative sea level within each site separately revealed that *S* is significantly reduced by 28-87% in the lower elevated zone in 14 of 20 sites; whereas a significant increase of *S* in low vs. high elevated zones was found in none of the 20 sites (Table S2). This finding demonstrates the consistency of the sea-level effect on *S* irrespective of ecosystem type (marsh/mangrove), soil type (mineral/organic), and site salinity (brackish/salt). In nine of the sites, we also found a significant effect of relative sea level on *k*. However, in six sites *k* was significantly higher in low vs. high zones, and in three sites *k* was significantly lower in low vs. high zones. The direction of effects on *k* seems to be independent of ecosystem type, soil type, and site salinity (Table S2).

The nutrient enrichment treatment at the TIDE project site decreased *S* by 72% in the high marsh (Fig. ~~3d~~4d). *S* in the low marsh likewise was similarly low as in the enriched high marsh and not further reduced by nutrient enrichment (Fig. ~~3d~~4d). In contrast, *k* was not responsive to the nutrient enrichment treatment in neither low nor high marsh (Fig. ~~3c~~4c).

3.4 *Other factors influencing decomposition rate and stabilization*

CART revealed tidal amplitude as an important predictor for *k*~~ (Fig. S1a)~~. However, this result needs to be considered cautiously because splits based on tidal amplitude suggest mixed effects

(Fig. S1a). Accordingly, no significant relationship existed between tidal amplitude and *k* across sites (Table 2). Soil type (mineral/organic) and ecosystem type (marsh/mangrove) did not affect *k* and *S* across sites (Table 2). We found no significant relationship between salinity class and *k* or *S* (Table 2). Also, CART did not reveal salinity class as an important factor for *k* and *S* ~~(Fig. S1)~~.

4 Discussion

4.1 Temperature effects on decomposition processes

A positive relationship between temperature and decomposition rate was found only at the regional scale across the ten sites along the North American Atlantic coast (Fig. ~~S2~~3), but not across all sites at the global scale (Fig. 2). Even though this finding occurs surprising in the context of basic biokinetic theory, it is in agreement with findings of Djukic and others (2018), demonstrating climate effects on the break-down of the TBI materials across terrestrial ecosystems at the biome scale, but not at the global scale across biomes.

The present study used air temperature as a proxy for top-soil temperature. Thus, the temperature regime of the decomposition environment was only approximated, which certainly would have weakened a significant relationship between temperature and *k*. However, following typical Q10 values for biological systems of 2-3 (Davidson and Janssens, 2006), *k* should have at least doubled over the gradient of ΔT >15°C; yet our data do not even show a tendency of an effect ($r_s = 0.02$; Table 2). We therefore propose that other parameters exerted overriding influence on *k*, mainly masking temperature effects, and have not been captured by our experimental design. This notion is in line with the fact that studies conducted at single-marsh to regional scales report equivocal results on the temperature response of *k*, ranging from no or moderate (Charles and Dukes, 2009; Janousek et al., 2017; Kirwan et al., 2014) to strong seasonally-driven temperature effects with a Q10 >3.4 as found within a single site (Kirwan and Blum, 2011). For instance, large differences in site elevation and hydrology could have induced high variability in *k* across sites and masked potential temperature effects. Indeed, we demonstrate significant but mixed effects of relative sea level on *k* for some sites (Table S2); however, we do not have sufficient data on actual site elevation or hydrology to control for these factors as covariates affecting the temperature effect on *k*. Likewise, we do not have data on nutrient availability, plant productivity, or various anthropogenic impacts that could have exerted strong control over decomposition processes in the studied sites (Deegan et al., 2012; Keuskamp et al., 2015a; Macreadie et al., 2017; Mueller et al., 2016).

In contrast to the missing effect of temperature on *k*, OM stabilization was strongly affected. Overall, *S* decreased by 75% over our temperature gradient from 10.9 to 28.5°C (Fig. 2b). Thus, we demonstrate a considerable temperature effect on the initial steps of biomass decomposition in tidal wetlands. However, as also demonstrated for *k*, the temperature effect on *S* was much clearer at the regional scale when focusing on the sites along the North American Atlantic coast (Fig. ~~S2~~3), suggesting high variability in *S* across regions irrespective of the temperature regime. In accordance, we also demonstrate a clear divergence of the four Mediterranean sites from the regression model (Fig. 2~~; S1~~), which could be related to differences in precipitation or nutrient availability across study regions. Future experimental work is therefore required in order to further assess the effects of temperature on OM stabilization and to separate temperature from latitudinal and other interacting effects (e.g. as outlined above for *k*) that are difficult to control for in observational studies.

The temperature effect on the initial steps of biomass decomposition we identified in the present study is not driven by changes in decomposition rate per se, but – more importantly – by affecting the transformation of fresh and rapidly decomposable organic matter into stable compounds, with potentially important implications for carbon sequestration (e.g. Cotrufo et al., 2013). In their global-scale assessment, Chmura et al. (2003) indeed report a negative relationship of soil organic C density and mean annual temperature within both salt marshes and mangroves. Chmura and colleagues hypothesized stimulated microbial decomposition at higher temperatures to be the responsible driver of this relationship. Plant production and thus OM input is known to increase with latitude and temperature in tidal wetlands (Baldwin et al., 2014; Charles and Dukes, 2009; Gedan and Bertness, 2009; Kirwan et al., 2009), but this increase seems to be more than compensated by higher microbial decomposition. Working at the same spatial scale as Chmura et al. (2003), our study supports this hypothesis and provides mechanistic insight into the temperature control of OM decomposition as a potential driver of carbon sequestration in tidal wetlands.

4.2 Relative-sea-level effects on decomposition processes

Flooding and thus progressively lower oxygen availability in soil is supposed to be a strong suppressor of decomposition (Davidson and Janssens, 2006). In tidal wetlands, differences in flooding frequency along elevational gradients often induce sharp gradients in oxygen availability and redox conditions (Davy et al., 2011; Kirwan et al., 2013; Langley et al., 2013), with potentially strong influence on OM decomposition and carbon cycling. However, the effect of redox conditions on OM break-down is determined by the chemical quality of the decomposing material: Decomposition of aged or recalcitrant OM can indeed be slower and incomplete in the absence of oxygen, whereas the break-down of fresh and labile OM can be largely unaffected by oxygen availability (Benner et al., 1984; Kristensen et al., 1995). Thus, also decomposition rate and stabilization of labile, hydrolyzable OM, as assessed in the present study, is not necessarily affected by redox conditions. Here, we demonstrate that k is not reduced in low (more frequently flooded) vs. high elevated (less frequently flooded) zones of tidal wetlands (Fig. ~~3a~~4a). This finding is in accordance with an increasing number of studies demonstrating negligible direct effects of sea level on decomposition rate in tidal wetland soils (Janousek et al., 2017; Kirwan et al., 2013; Mueller et al., 2016). Furthermore, we show that S is strongly reduced in low vs. high elevation zones, suggesting that the conversion of recent OM inputs to stable compounds is in fact lower in more flooded zones of tidal wetlands. As the stabilization of labile OM inputs is a major driver of SOM formation (Cotrufo et al., 2013, 2015; Haddix et al., 2016), one important implication of this finding is that accelerated SLR yields the potential to decrease the carbon-sequestration potential of tidal wetlands.

The mechanism by which S is decreased in the more flooded zones of the present study is unknown. Because we did not observe consistent salinity effects on S and k in our data, we do not suppose that regular exposure of litter to salt water explains the unexpected finding. Likewise, soil temperature was not consistently affected by relative elevation across sites ($p > 0.3$; paired t-test based on data shown in Fig. ~~S3~~S2). Instead, we argue that more favorable soil moisture conditions in low vs. high elevated zones could have decreased OM stabilization if higher flooding frequencies did not induce redox conditions low enough to suppress microbial activity in the top soil. In support of this, flooding-frequency induced changes in moisture conditions have been reported as primary driver of surface litter break-down, leading to more than four-fold increased litter mass loss in low vs. high marsh zones of a New Jersey salt marsh (Halupa and Howes, 1995). Additionally, greater nutrient availability and less nutrient-limited microbial communities in more frequently flooded zones could have contributed to this effect (Deegan et al., 2012; Kirwan et al., 2013). Strong effects of both high quality marine-derived OM and nutrient amendments on microbial structure and activity have been reported (Deegan et al., 2012; Kearns et al., 2016; Keuskamp et al., 2015b; Mueller et al., 2017), suggesting that regular marine OM and nutrient inputs in more frequently flooded zones can positively affect decomposition (see further discussed below in 4.3).

4.3 Nutrient enrichment reduces stabilization – insights from the TIDE project

In addition to our global survey of early-stage decomposition processes in tidal wetlands, we included the long-term ecological research site of the TIDE project in Massachusetts, US to experimentally assess both the effects of coastal eutrophication and the relevance of nutrient delivery through floodwater for OM decomposition in tidal wetlands. Important for our argument that decomposition may be favored by higher nutrient availability in low elevated, more frequently flooded zones, we observed a strong reduction (>70%) of *S* by nutrient enrichment in the high marsh, whereas *S* in the low marsh likewise was low as in the fertilized high marsh and not further reduced by fertilization (Fig. 3d). Johnson et al. (2016) demonstrate that nutrient enriched high-marsh plots of the TIDE project receive 19±2 g N m^{-2} yr^{1}, approximately 10-times the N load of reference high-marsh plots (2±1 g N m^{-2} yr^{-1}; mean±SE), thus explaining the strong treatment effect observed in the high marsh. In accordance with low stabilization in the reference low marsh, which is equally low as the nutrient enriched high marsh, reference plots of the low marsh receive 16±4 g N m^{-2} yr^{1}, the same high N load as the enriched high-marsh plots. Surprisingly, however, N loads of 171±19 g N m^{-2} yr^{1} in the enriched low-marsh plots do not result in additional reduction of *S* compared to the reference low marsh (Fig. ~~3d~~4d). These findings suggest that microbial communities of the high marsh are N limited, and that N additions to a certain level can stimulate early OM decomposition and thus reduce stabilization. The missing effect of N loads exceeding 16 g m^{-2} yr^{1} on stabilization in the low marsh indicates that microbial communities are less or not N limited due to the naturally greater nutrient availability. The findings of the TIDE project therefore support our concept that higher nutrient availability and less nutrient-limited microbial communities in more frequently flooded zones could have contributed to the observed reduction of OM stabilization in low vs. high elevated zones of tidal wetlands in our global assessment.

Although our conclusions on effects of nutrient enrichment on OM decomposition are based on the findings of a single field experiment only, our study adds to a growing number of reports illustrating the impact of coastal eutrophication on tidal wetland C cycling (Deegan et al., 2012; Keuskamp et al., 2015a; Kirwan and Megonigal, 2013; Morris and Bradley, 1999). At the same time, however, we highlight the need to improve our understanding of coastal eutrophication in interaction with other global changes, particularly accelerated SLR and concomitant changes in flooding frequency, on the cycling of both labile and refractory C pools in order to predict future stability of tidal wetlands.

4.4 The Tea Bag Index – methodological considerations

Interpretation of results obtained from standardized approaches like the present needs to be made cautiously because OM quality (i.e. its chemical composition) is a key parameter affecting its decomposition. As the quality of the TBI materials differ from that of wetland plant litters, and likely even more from the quality of the imported allochthonous OM (Khan et al., 2011), we did not expect to capture actual rates of early-stage OM break-down in this study. Instead, we used the TBI to characterize the decomposition environment by obtaining a measure for the potential to decompose and stabilize the deployed standardized material. Standardized approaches like this, or also the cotton-strip assay (e.g. Latter and Walton, 1988), are useful to separate the effects of environmental factors other than OM quality on decomposition processes and to assess their relative importance. Otherwise, complex interaction effects of the abiotic environment and OM quality make it difficult to predict the relevance of certain environmental factors for decomposition processes, potentially masking the effects of important global-change drivers (Prescott, 2010). At the same time, however, the global-change factors considered in the present study are likely to induce changes in the quality of the OM accumulating in tidal wetlands, for instance through shifts in plant-species composition and plant-tissue quality, that can potentially counterbalance or amplify the effects on decomposition processes suggested here. Future research therefore needs to address OM quality feedbacks on decomposition processes in tidal wetlands in order to gain a more complete understanding of global-change effects on tidal-wetland stability and carbon-sequestration capacity.

Based on the S values obtained from initial calculations using the hydrolyzable fractions suggested by Keuskamp et al. (2013), a large number of observations yielded a negative S (Table S3). S becomes negative when the mass loss from green tea is greater than the predicated maximum loss based on its hydrolyzable fraction. At least two processes could have caused this result: First, our data indicate that redox conditions in the top soil of tidal wetlands are not low enough to hamper decomposition of the hydrolyzable fraction of the TBI materials. As a consequence, high top-soil moisture of tidal wetlands could provide favorable conditions for decomposition, following typical moisture-decomposition relationships as demonstrated for terrestrial ecosystems (e.g. Curiel Yuste et al., 2007). Potentially, moisture conditions and nutrient supply even allow for considerable break-down of non-hydrolyzable compounds within three months of deployment, such as lignin (Berg and McClaugherty, 2014; Duboc et al., 2014; Feng et al., 2010; Knorr et al., 2005). Second, different protocols to determine the hydrolyzable fraction of plant materials exist and lead to variable results. The hydrolyzable fraction can consequently be over- or underestimated depending on protocol and type of sample material. The use of the slightly higher hydrolyzable fractions we determined for calculations of the TBI parameters effectively eliminated negative *S* values. In that regard, using the values obtained from the alternative protocol given in Ryan et al. (1990) seemed more reasonable in our study. However, it needs to be stressed here that direction and size of reported effects on *S* and *k* in the present study are almost independent of the hydrolyzable fraction used for calculations.

Future research will have to test the applicability of the TBI approach in different ecosystems and test the validity of its assumptions (i.e. *S* is equal for both types of material used, and mass loss of non-hydrolyzable material is negligible over 3 months of deployment). The results of our regional scale assessment along the North American Atlantic coast transect are in tight agreement with previously reported results on cellulose break-down and soil microbial activity along this well studied transect (Kirwan et al., 2014; Mozdzer et al., 2014). We can thereby demonstrate the usefulness of the TBI approach to assess early-stage decomposition in tidal-wetland soils.

4.5 Implications

This study addresses the influence of temperature, relative sea level, and coastal eutrophication on the initial transformation of biomass to SOM, and it does not encompass their effects on the existing SOM pool. However, aspects of S and k are key components of many tidal wetland resiliency models (Schile et al., 2014; Swanson et al., 2014) that have highlighted the critical role of the organic contribution to marsh elevation gain. Although actual rates of S and k cannot be inferred from this study using a standardized approach, our data identify strong negative effects of temperature, relative sea level, and coastal eutrophication on the stabilization of fresh organic inputs to tidal-wetland soils. We argue that these unanticipated combined effects yield the potential to strongly accelerate carbon turnover in tidal wetlands, thus increasing their vulnerability to accelerated SLR, and we highlight the need for experimental studies assessing the extent to which the here identified effects translate into native OM dynamics.

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Author contributions

PM, SN, KJ, and LMS-B designed the overall study. PM analyzed and interpreted the data. PM wrote the initial version of the manuscript with regular comments and editing provided by LMS-B, TJM, and SN. PM, LMS-B, TJM, GLC, TD, YK, AVdG, PE, CS, AD'A,CI, ML, UN, BJJ, AHB, SAY, DIM, ZY, and JW designed and conducted the field studies in the respective sites and commented on an earlier version of the manuscript.

Figure captions

Figure 1 Overview map of study regions. *Notes:* See Table 1 for region and site details.

Figure 2 ~~(a) D~~**Site means of d**ecomposition rate **(a)** ~~(site means; n = 30)~~ and ~~(b)~~ stabilization **(b)** ~~(site means; n = 30)~~ versus mean air temperature during deployment period. Regression line illustrates significant relationship between temperature and stabilization (Table 2); regression model with lowest standard error of estimate (SEE) and highest R^2 is shown: y = -0.27ln(x) + 0.99; R^2 = 0.239; SEE = 0.131; excluding Mediterranean sites (21.9-23.6°C; n = 4) from the regression yields: y = -0.344ln(x) + 1.233; R^2 = 0.510; SEE = 0.101

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Figure 3 Site means of decomposition rate **(a)** and stabilization **(b)** versus mean air temperature of the deployment period shown for the ten sites situated along the latitudinal gradient of the North American Atlantic coast; state abbreviations are shown (compare Table 1). Regression lines illustrate significant relationships; regression models with lowest standard error of estimate (SEE) and highest R^2 are shown. Decomposition rate: y = 0.001x - 0.0091; R^2 = 0.692; SEE = 0.003; stabilization: y = -0.712ln(x) + 2.2331; R^2 = 0.860; SEE = 0.070

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Figure ~~3~~ **4** **(a)** Decomposition rate and **(b)** stabilization in high and low elevated zones of tidal marsh and mangrove sites (n = 21; compare Tables 1, S1). **(c)** Decomposition rate and **(d)** stabilization in nutrient enriched versus reference high marsh (*Spartina patens* zone) and low marsh (*Spartina alterniflora* zone) of the TIDE project site at the Plum Island Sound Estuary, Massachusetts, US. Shown are means ± SE and results of paired t-tests (panels a + b) and two-way ANOVAs plus Tukey's HSD test for pairwise comparisons (panels c + d): ns = not significant; * = $p \leq 0.05$; ** = $p \leq 0.01$

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