1	1. Point-by-point reply to the reviewer comments
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3	2. Marked-up manuscript version (text only)
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1. Point-by-point reply to the reviewer comments:

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

42

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).

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48 **REVIEWER 1, anonymous**

50 General comments

This article deals with an important aspect of carbon's fate in coastal wetlands in relation to 51 52 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 53 carbon. Ecosystems considered in this "global" scale study are mainly tidal marches but some 54 55 mangroves sites were counted in the selected sites. Authors are assessing OM degradation and 56 transformation, as a proxy of Carbon sequestration using the TBI approach. Thus, authors claim 57 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 58 publish although as authors cautioned, there are limits with the used method (obvious quality 59 60 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 61 methodology was clear however, some choices were not judicious in the context of this study 62 and may need to be revaluated (see specific comments). The adding of TIDE experimental site 63 64 was a very interesting. The discussion is well organised but it needs to be shortened. 65

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.

69

70 Specific comments

I am not a specialist of meta-analysis, therefore I will not comment on the validity or not of the 71 72 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 73 related to the quality and the fate of the litter-bags contents (here Tea-bags) which are strongly 74 75 related to sedimentation dynamic and water velocity. In absence of a clear indication on how 76 sediments (and OM) are behaving in each site, I am concerned about the amalgam in the same 77 meta-analysis different systems in term of hydrological functioning: Salt Marches vs. 78 Mangroves, High tide vs. low tide (in salt marches). For instance, estuarine mangroves receive 79 loads of sediments from rivers whereas Europeans salt marches in open Bays get sediments 80 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 81 considering the main origin of sediments (not to confound with OM), without impacted TIDE 82 sites, river presence or not, water velocity, human activities: These factors, of ecological 83

importance, might be those missing to explain some global, or local, differences. If these datacannot be compiled they should at least be discussed.

86

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.

- 91
- 92 <u>Response concerning sedimentary factors:</u>

We agree with the reviewer that the different systems we compiled in a single meta-analysis are 93 94 characterized by potentially important differences in both sediment load and origin. We did not 95 explicitly assess sediment loads of our study sites. However, by distinguishing between 96 minerogenic and organogenic systems (i.e. sediment rich vs. sediment poor systems) in our 97 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 98 99 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 100 zones) are consistent within both minerogenic and organogenic systems. 101

102

103 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, 104 105 brackish, salt) on our response variables, with fresh systems far up in the estuary experiencing 106 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, 107 108 this should have been reflected in our meta-analyses (i.e. Table 2). That being said, salinity of 109 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 110 noted that those were not estuarine (as assumed by the reviewer) but coastal systems in the 111 112 present study.

113

114 **REVIEWER 2, Dr J. Keuskamp**

115 General comments

This paper discusses the control that the soil matrix exerts on the decomposition of organic 116 matter in tidal wetlands. Their large carbon stocks and sensitivity to global change make this a 117 highly relevant topic for scientists and policy makers alike. The paper is well-written and easy to 118 read, while presenting novel data with important conclusions on the relation between 119 decomposition and global change. The usage of a standardised method over a wide range of 120 121 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 122 123 some unavoidable methodological weaknesses. Many of the environmental parameters which 124 are discussed in relation to decomposition are often strongly correlated with tidal regime (i.e. 125 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 126

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.

130

131 We thank Dr Keuskamp for his constructive comments on our study. We agree that correlations 132 between the assessed environmental parameters should be carefully considered in the 133 interpretation of our results. Accordingly, we put more emphasize on this throughout the ms; 134 some examples from different sections:

- 134 some examples from different sections:
- 135
- 136 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)."

141

149

142 Results:

143 "Temperature was highly correlated with latitude and tidal amplitude, and temperature was not independent of soil 144 type (mineral/organic) and ecosystem type (marsh/mangrove) (Table 2). The effect of latitude was similarly 145 pronounced as the temperature effect on S – and consequently –effects of these two parameters on S cannot be 146 separated (Table 2). By contrast, tidal amplitude and soil type did not significantly affect S, and the difference in S 147 between mangroves and marshes was only marginally significant (Table 2). These findings suggest that the presented 148 temperature effect on S occurs to be mainly independent of tidal amplitude and soil type."

150 Discussion:

151 "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."

155 The reviewer is specifically concerned about interactions with the parameters tidal regime and 156 latitude.

157 →In terms of describing the tidal regime, we assessed tidal amplitude and, by comparing high 158 and low elevated zones within sites, a relative measure for flooding frequency (i.e. low zones 159 more frequently flooded than high zones). Tidal amplitude did not affect k and S (Table 3). It 160 showed up as a potentially important predictor in our CART, probably because of its strong 161 correlation with other parameters. However, this result needs to be considered cautiously 162 because splits based on tidal amplitude suggest mixed effects (Fig. S1a).

- 163 →In terms of flooding frequency (high vs. low elevated zones), we discuss a number of 164 potentially relevant interactions that were mentioned by the reviewer: redox -> 423-434; 165 salinity -> 444; nutrient status -> 452-465. The reviewer makes an important point by 166 mentioning soil temp interactions with tidal regime. We will address this point further below 167 where soil vs air temp differences are discussed.
- 168 \rightarrow 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
- 171
- 172 The current description of the data-analysis does not describe how the authors have 173 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.

- 176 The reviewer is correct. We revised the description of the statistics and also corrected some 177 statistical analyses:
- 178

179 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 180 181 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 182 183 independent variable (as assessed visually) assured robustness of paired t-tests. To assess the consistency of potential 184 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 185 186 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). 187 188 We tested for effects of nutrient enrichment on k and S in the data from the TIDE project site (Massachusetts, US) 189 using two-way ANOVA with enrichment treatment and marsh zone as predictors. When Levene's test indicated 190 heterogeneous variance (true for k), data were log-transformed, which stabilized variance. Normal distribution of 191 residuals was assessed visually."

192

193 <u>Corrected statistics/data structure:</u>

194 Statistics in Table 2 (Spearman correlations and U tests) were based on mean values of each 195 site-by-zone combination (n = 51). Sites with observations in two zones were thus 196 overrepresented. In the revised version these statistics are based on site means (n = 30; 197 compare 2.2).

198

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 colocated in larger estuarine/coastal regions (line 148 and revised Table 1).

206

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.

211

The reviewer is correct. A linear effect of temp is not expected. The intention for showing the 212 213 linear fit was only to better illustrate the significant temp effect on S (as tested/identified with 214 non-parametric Spearman correlation). However, we should not have used linear regression as an additional hypothesis test. \rightarrow We only use Spearman correlation for this in the revised 215 version. Yet, to better illustrate the temp effects, we still present scatterplots and use curve 216 fitting to illustrate significant temp effects. Indeed, the model with both highest R2 and lowest 217 standard error of estimate describing the significant temp effect on S is not linear but 218 logarithmic (Figure 2) 219

220

221 Lastly, I would like to add that the strength if the TBI lays in its standardisation. I would 222 therefore recommend to mention the S/k calculated with the standard approach alongside with 223 the re-scaled values calculated with the more aggressive extraction method. This would allow 224 for easy comparison with other data such as the TBI-values from mangroves mentioned in the methods paper. See also below. 225 226 We agree with the reviewer. The same point has been raised by Dr Sarneel in an interactive 227 comment (below). We have prepared a table with all site x zone values for k and S, giving both 228 229 the original TBI-values and the modified (Table S3; referenced in the ms: L513). 230 231 **Specific comments** 232 L79 and L83-L84 seem largely redundant to me 233 234 Deleted old lines 79-80 235 236 L85-L86 'OM decomposition' is somewhat ambitious as it is not clear whether this refers to 237 decomposition rate (k) or extend (S), please revise. 238 The sentence was changed to "Consequently, global changes that might decrease OM preservation in 239 240 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, 241 we do not intend to specify the processes at this stage of the Introduction, but do this further 242 down in the text (i.e. 115-119). 243 244 245 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 246 247 describes the decomposition rate of the hydrolysable fraction and is not calculated over the 248 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 249 250 avoid confusion this should be made explicit here. 251 We avoid reference to classical litter bag experiments here and instead make the meaning of k252 clearer in the respective section of the Methods. 253 254 L120 The recalcitrant fraction is also decomposable, albeit a lot slower 255 256 257 This was poor wording of course \rightarrow changed to "rapidly decomposable". 258 259 L127 ' thereby improving our process-level understanding on how global warming affects 260 carbon turnover' Not sure what this means exactly 261 262 Deleted "process-level". 263

- 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?
- 267

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

272

277

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, Ncontent) 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
- 284

282

285 We specified as follows:

286 "In 21 sites, we compared high and low elevated zones, which were characterized by distinct plant species 287 compositions (i.e. different communities in high vs. mid vs. low marshes) or by different stature of 288 mangroves (i.e. dwarf vs. fringe phenotypes). We used relative elevation (i.e. high vs. low elevated zone) 289 as a site-specific proxy for relative sea level. By doing so, we did not capture the actual variability in the 290 tidal inundation regime across our study sites as these vary in absolute elevation and in elevation relative 291 to mean high water."

292

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?

300

301 Dr Keuskamp brings a valid point here that indeed needs more consideration. Air temperature 302 would obviously diverge from soil temperature depending on factors such as canopy shading or 303 tidal regime and water temperature. As a consequence, air temperature can only approximate 304 the temperature conditions of the actual decomposition environment. However, considering 305 that we stretch a temp gradient of approx. 20°C, we are confident that this would also translate 306 into a profound soil-temperature gradient across our study sites.

307

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).

314

315 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. 316 However, in several of our sites, soil temp was assessed at the time point of insertion and 317 318 retrieval of bags. We plotted these data against the mean air temp of the day as acquired from 319 the accuweather service in Figure S3. \rightarrow We see that generally air temp is a good proxy for soil 320 temp across sites. Yet, there is considerable variability in soil temp not explained by air temp, 321 which would result from the fact that soil temp was assessed in one time point as opposed to 322 mean air temp of a single day and of course from other factors, such as distance of weather 323 station from site, shading, influence of water temp etc..

324

325 \rightarrow We agree with the reviewer that this needs to be stated and discussed in the manuscript. 326 Accordingly, we put more emphasize on this throughout the ms; some examples from different 327 sections:

328 329 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)."

- 333
- 334 **Discussion**:

³³⁵ "The present study used air temperature as a proxy for top-soil temperature. Thus, the temperature regime of the ³³⁶ decomposition environment was only approximated, which certainly would have weakened a significant relationship ³³⁷ between temperature and k. However, following typical Q10 values for biological systems of 2-3 (Davidson and ³³⁸ Janssens, 2006), k should have at least doubled over the gradient of $\Delta T > 15^{\circ}$ C; yet our data do not even show a ³³⁹ tendency of an effect (rs = 0.02; Table 2). We therefore propose that other parameters exerted overriding influence ³⁴⁰ on k, mainly masking temperature effects, and have not been captured by our experimental design. This notion is in ³⁴¹ line with the fact that studies conducted at ..."

342

Lastly, we want to stress a related point here: "low" and "high" in the figure legend of Fig.S3 343 344 refer to the low and high elevated zones within the systems. A paired t-test comparing the 345 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 346 347 were not consistently more pronounced in either the low or the high elevated zones. 348 Additionally, soil temp was not significantly affected by zone (p = 0.342). One of our main 349 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 350 none of the sites (Table S2). 351

352

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

354

355 Unilever belongs to PepsiCo, but of course the tea is produced by Unilever. PepsiCO was deleted356 in order to avoid confusion.

357 358 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 360 empty nylon bag itself without contents. I do not know if that one has always been dried, 361 however, empty-bag weights were very consistent among labs. \rightarrow By contrast, initial tea-content 362 weights showed some variability across the involved labs. I also noticed that some labs, after 363 drying at 70°C, used desiccators, in which the material could cool down without sucking 364 moisture, before weighing and some didn't. I therefore assessed if potential moisture 365 366 differences of the initial tea material or differences in the amount of the initial material could 367 have affected S or k. However, there was no relationship between green initial weight and S 368 $(r_2=0.0003; p=0.936)$ and no between rooibos initial and k $(r_2=0.005; p=0.728)$.

- 369 370 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 371 372 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 373 TBI S/k values calculated according to protocol alongside the obtained S/k values obtained by 374 the revised protocol. 375
- 376

359

- 377
- We agree. We added a table with the original TBI values accordingly (see comment further up). 378

L220-L250 Would you be able to indicate whether potential violations of the assumptions 379 underlying the statistical tests were assessed? For example, were the residuals of the ANOVA 380 381 procedure tested for normality / homogeneity of variance?

- 382
- 383 This was indeed missing. We added these missing details to the Methods, see comment further 384 up.
- 385

386 L250 It is critical to this conclusion that air temperature is a good proxy of soil temperature

387 (see earlier remark). The interaction between temperature effect and tidal position reinforces 388 the suspicion that this is not the case.

389

390 We agree with the first half of this remark (see addressed further up), but not with the second. 391 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 392 393 >14.5°C; however, this apparent temp effect is inconsistent within this group of mesotidal 394 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, 395 396 neither did the temp difference between soil and air.

397

398 L314 As also noted in L313, the absence of a temperature effect is very unusual. Could the 399 authors rule out the possibility that this is due to a mismatch between soil and air temperature? 400

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).

407

408 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 409 410 wetlands, we took a separate look at the data of the North American East coast latitudinal 411 gradient along which previous studies have shown clear temp effects on decomposition 412 processes and microbial biomass (Blum et al. 2004; Kirwan et al. 2014; Mozdzer et al. 2014). Species composition of these marshes is guite constrained (i.e. Spartina alterniflora dominated) 413 414 reducing confounding effects induced by differences in vegetation. Along this gradient, we 415 clearly see an increase in S and also the expected decrease in k, although temp explains more 416 variability for S (Fig. S2). We added this figure to the manuscript in order to illustrate that temp 417 effects on k can be identified on the regional scale, but not on the global scale with more 418 confounding factors.

419

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.

426

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.

430

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

433

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)

438

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).

441

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? 446

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.

450 →No, I am not aware of loss of particles from the bags in situ. In fact, in a recent study 451 (microcosm study, Wadden Sea) we used the new tea bags (those without nylon mesh) that 452 wouldn't allow for loss of material through the mesh. Also with these bags, we had ~11% 453 negative values (Hao Tang, Peter Mueller et al. unpublished data), comparable to what we 454 found in some Wadden Sea marshes in the present study using nylon mesh bags.

- 455 \rightarrow Comparing our results to those reported in Djukic et al. (2018), it becomes clear that negative 456 S values occur less frequently across terrestrial systems, however, are not negligible either.
- 457
- 458 Technical corrections L74 Earth? Not sure if this should be with a capital E
- 459 L77 Separate SRL from citations
- 460 L94-98 This sentence is very hard to read. Split.
- 461 L346 add 'in' before 'tidal wetlands'
- 462 Thanks, technical corrections have been made.
- 463

464 **REVIEWER 3, anonymous**

465 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 466 467 order to cover a gradient in temperature, inundation regime, etc. While such cross-ecosystem 468 studies have a high potential, I feel the impact of this dataset in terms of new insights is 469 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 470 dataset should be publishable, but it needs a more critical discussion and should provide the 471 readers with a more complete overview of the caveats and assumptions used in the TBI 472 approach, so that the readers can better assess what can and cannot be deduced from these 473 474 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, 475 476 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 477 478 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.

- 484
- 485 Specific suggestions

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

489

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.

492

493 L60-61: data from the eutrophication experiment: would not extrapolate this to 'high sensitivity 494 to global change'. Eutrophication will also affect the nutrient content of locally produced 495 biomass, this aspect is not taken into account when standardized material is used in the 496 experiments.

497

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:

502

503 "Standardized approaches like this, or also the cotton-strip assay (e.g. Latter and Walton, 1988), are useful 504 to separate the effects of environmental factors other than OM quality on decomposition processes and to 505 assess their relative importance. Otherwise, complex interaction effects of the abiotic environment and 506 OM quality make it difficult to predict the relevance of certain environmental factors for decomposition 507 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 508 509 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 510 decomposition processes suggested here. Future research therefore needs to address OM quality feedbacks 511 on decomposition processes in tidal wetlands in order to gain a more complete understanding of global-512 change effects on tidal-wetland stability and carbon-sequestration capacity." 513

514

515 We agree with the reviewer that this sentence needs to be toned down in the abstract, because 516 there is no space for further elaboration on the assumptions and methodological 517 considerations. We will go with "potentially high sensitivity of OM stabilization to global change." 518

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.

522

523 We agree; this is important for the interpretation of our results. However, conventional litter 524 bag experiments are also restricted in their choice of material; here actually lies an advantage of 525 the standardized approach, although we acknowledge that the quality of the TBI materials is 526 obviously closer to that of wetland plant litter than to the marine derived, labile allochthonous 527 organic input a tidal wetland receives. We have elaborated on this in 4.4:

528

"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."

536

538

537 Additionally, we toned down our Implications (4.5):

- 539 "This study addresses the influence of temperature, relative sea level, and coastal eutrophication on the 540 initial transformation of biomass to SOM, and it does not encompass their effects on the existing SOM 541 pool. However, aspects of S and k are key components of many tidal wetland resiliency models (Schile et 542 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 543 approach, our data identify strong negative effects of temperature, relative sea level, and coastal 544 545 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, 546 thus increasing their vulnerability to accelerated SLR, and we highlight the need for experimental studies 547 assessing the extent to which the here identified effects translate into native OM dynamics." 548
- 549

550 section 2.2: it is important for the readers not familiar with the Keuskamp et al. paper to reiterate and stress the assumptions on which this approach is based, e.g. that k2 (decomposition 551 552 constant of the non-labile fraction) is assumed to be 0, and that S is assumed to be similar for 553 both types of tea. I still find this major shortcomings- we know the first assumption not to be 554 valid, and I have not seen strong arguments to support the second assumption. The main reason 555 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 556 to be mentioned explicitly and the limitations of the approach should be discussed more 557 558 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? 559

560

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):

⁵⁶⁷ "Decomposition rate (k) and stabilization (S) were assessed following the TBI protocol (Keuskamp et al., ⁵⁶⁸ 2013). The TBI approach can be considered as a simplified litter-bag approach, allowing a time- and cost-⁵⁶⁹ efficient characterization of the decomposition environment, because k and S can be estimated without ⁵⁷⁰ repeated sampling of the decomposing material as in conventional approaches. This implies the ⁵⁷¹ assumptions that (1) S is equal for the two types of material used in the approach and (2) that ⁵⁷² decomposition of non-hydrolyzable materials during the 3 months of deployment is negligible. We refer ⁵⁷³ the reader to Keuskamp et al. (2013) for further detail and validity assessments of assumptions."

574

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 583 assessing decomposition processes tidal wetlands, we will separately present our data on k and 584 585 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 586 587 instance, Kirwan et al. (2014; Biogeosciences) demonstrated a strong increase in cellulose decay 588 with both temperature and latitude, and Mozdzer et al. (2014; Ecology) showed a marked 589 decrease in sulfate reduction with latitude along this transect. The TBI parameters assessed 590 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 591 592 the decomposition environment of tidal wetland soils.



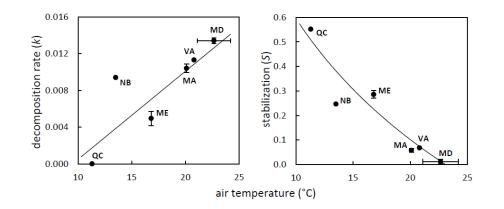


Figure S2 Site means of decomposition rate (left) and stabilization (right) versus mean air temperature of the deployment period shown for the ten sites situated along the latitudinal gradient of the North American Atlantic coast; state acronyms are shown (compare Table 1). Regression lines illustrate significant relationships^; regression models with lowest standard error of estimate (SEE) and highest R² are shown. Decomposition rate: y = 0.001x - 0.0091; R² = 0.692; SEE = 0.003; stabilization: $y = -0.712\ln(x) + 2.2331$; R² = 0.860; SEE = 0.070

594 595

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

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⁵⁹⁸ "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."

605 606

607 L212-214: provide the data from Keuskamp et al. as well, we cannot compare or assess how 608 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) 650

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Global--change effects on early-stage decomposition processes in tidal 653

- wetlands: implications Implications from a global survey using 654
- standardized litter 655
- 656

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698 Manuscript type: Primary research

699 Abstract

700 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 701 over the global carbon cycle and at the same time gain elevation to keep pace with sea-level rise 702 703 (SLR). The present study provides assessed the first global-scale field-based experimental 704 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 705 706 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 707 708 negative effects of temperature and relative sea level on OM-stabilization, as based on the 709 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 710 711 frequently flooded zones. OM stabilization Stabilization declined by ~9075% over the studied temperature gradient from 10.9 to 28.5°C, corresponding to a decline of ~5% over a 1°C-712 713 temperature increase. Additionally, data from the Plum Island long-term ecological research site 714 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 715 to global change. We therefore provide evidence that rising temperature, accelerated SLR, and 716 coastal eutrophication may decrease the future capacity of tidal wetlands to sequester carbon by 717 affecting the initial transformations of recent OM inputs to soil organic matterOM. 718 719

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722 Tidal wetlands, such as marshes and mangroves, provide a wide array of ecosystem services that 723 have been valued at approximately US\$ 10,000 per hectare and year, making them some of the 724 most economically valuable ecosystems on Earth (Barbier et al., 2011; Kirwan and Megonigal, 725 2013). Yet, tidal wetlands are threatened and vulnerable ecosystems, experiencing pronounced 726 loss though global-change impacts, such as land use (Pendleton et al., $2012_{\frac{1}{2}}$) and accelerated 727 sea-level rise (SLR+) (Craft et al., 2009; Crosby et al., 2016). In recent years, carbon 728 sequestration has increasingly been recognized as an ecosystem service of tidal wetlands (e.g. 729 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 730 731 both autochthonous and allochthonous production) co-occur with reducing soil conditions and 732 thus slow rates of decomposition, leading to organiclong-term carbon--sequestration rates that 733 exceed those of most other ecosystem types by orders an order of magnitude (Mcleod et al., 734 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 735 and Megonigal, 2013). Consequently, global changes that might increased ecrease OM 736 737 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 738 that affect the transformation of organic inputs to stable soil OM (SOM) in tidal wetlands and to 739 740 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

745	since the 1960s (Prescott, 2010). Global-scale assessments of litter decomposition have been
746	conducted as both meta-analyses (e.g. Zhang et al., 2008) and as inter-site studies along
747	latitudinal gradients (Berg et al., 1993; Torfymow et al., 2002; McTiernan et al., 2003;
748	Cornelissen et al., 2007; McTiernan et al., 2003; Powers et al., 2009; Trofymow et al., 2002) in
749	order to assess effects of climate parameters, $-$ mostly with focus on temperature and moisture
750	gradients, on decomposition rate. Besides abiotic or climate effects, these studies could also
751	identify litter quality itself as an important predictor for decomposition rate (Zhang et al., 2008).
752	Relationships between single climate or litter-quality parameters and decomposition rate
753	often are not linear. Instead, complex interactions between litterquality and climate parameters
754	seem to control litter decomposition (Zhang et al., 2008), creating challenges in separating
755	climate from litter-quality effects and predicting the relevance of potential global-change drivers
756	for decomposition rate. In order to separately assess environmental or climate effects on litter
757	decomposition at a global scale, it is therefore necessary to standardize litter quality in inter-site
758	studies. However, implications of litter-decay data for carbon sequestration need to be considered
759	cautiously, as the link among litter-decomposition rate, SOM formation, and ultimately carbon
760	sequestration is not straightforward (Prescott, 2010; Cotrufo et al., 2013; Prescott, 2010):
761	Because plant tissues are not resistant to decay per se, it is critical to understand their
762	biogeochemical transformation into stable compounds that leads to the formation of SOM (i.e.
763	stabilization) rather than understanding the pace at which early stages of stage decomposition
764	proceeds (Prescott, 2010; Castellano et al., 2015; Haddix et al., 2016; Prescott, 2010).
765	Keuskamp and others (2013) developed an efficient approach for studying litter
766	decomposition and OM transformation at a global scale, using commercially available tea as
767	standardized material. Their Tea Bag Index (TBI) approach is based on the deployment of two
768	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 769 770 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 771 772 becomes stabilized during deployment. 773 In the present study, we assessed effects of the impacts of multiple global-change factors 774 -global warming, sea-level rise (accelerated SLR), salt-water intrusion, and coastal 775 eutrophication -- on both OM decomposition rate and stabilization in tidal--wetland soils by 776 conducting a worldwide field studysurvey 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 777 778 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 779 conducting paired measurements in both high- and low-elevated zones of tidal wetlands 780 781 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 782 flooding, the existing literature on hydrology and SLR effects on OM decomposition in tidal 783 wetlands yields equivocal results, which is often due to the overriding effect of OM quality on 784 785 decomposition rate (Hemminga and Buth, 1991; Kirwan et al., 2013; Mueller et al., 2016). 786 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 787 decomposition processes in tidal wetlands (Morrissey et al., 2014). Specifically, high 788 789 concentrations of dissolved sulfate in seawater, acting as an alternative terminal electron acceptor, can enhance anaerobic microbial metabolism in systems with lower salinity (Megonigal 790 791 et al., 2004; Sutton Grier et al., 2011). Lastly, we used the long term ecological research site of 792 the TIDE project Lastly, we used the TIDE project plots of the Plum Island long-term ecological

793	research site in Massachusetts, US (Deegan et al., 2012) to experimentally assess both the effects
794	of coastal eutrophication and – with respect to SLR-driven increases in flooding frequency – the
795	relevance of nutrient delivery through floodwater for the early stages of OM decomposition in
796	tidal wetlands.
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812 2 Methods

813 2.1 Study sites and experimental design

The studyworldwide survey was conducted in 26 tidal wetlands during the 2015 growing season 814 815 (Figureand included a total of 30 tidal-wetland sites. Sites were partly co-located within larger 816 coastal and estuarine regions (Fig. 1, Table 1). NineEleven sites were situated along the European 817 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, 818 819 Chesapeake Bay, and San Francisco Bay, and four mangrove sites were situated along the 820 Caribbean coast of Central America in Belize and Panama. Additionally, one Chinese site 821 (Yangtze Estuary) and one Argentinian site were included in our study. Sixteen of the sites were 822 salt marshes, sixten were tidal freshwater and brackish sites, and four sites were mangroves. At 22In 21 sites, we compared high and low elevated zones, which were characterized by distinct 823 824 plant species compositions (i.e. different communities in high vs. mid vs. low marshes) or by 825 different stature of mangroves (i.e. dwarf vs. fringe phenotypes). We used relative elevation (i.e. 826 high vs. low elevated zone) as a site-specific proxy for relative sea level. By doing so, we did not 827 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-828 829 term experimental site of the TIDE project in Massachusetts, US to assess effects of nutrient 830 enrichment on litter-decomposition rate and stabilization. Through nitrate additions to the 831 incoming tides on at least 120 days per year, nutrient enriched areas at the TIDE project site 832 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 833 834 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).

837	Decomposition rate and stabilization were measured by deploying tea bags in ten points
838	per zone or treatment within a site (n=10). Spacing between replicates within a zone or treatment
839	was ≥ 2 m. However, as sites differed considerably in their areal extent, the distribution and thus
840	spacing between points had to be adjusted to be representative for the given system. Temperature
841	Air temperature for the period of deployment was measured at site, or temperature data was
842	obtained from the online service of AccuweatherAccuWeather (accuweather.com; accessed
843	12/25/2016) for locations within a distance of 15 km to the site for most sites, but not further than
844	60 km for some remote sites. It needs to be noted here, that top-soil temperature would differ
845	from air temperature depending on factors such as canopy shading or tidal regime and water
846	temperature. As a consequence, air temperature can only approximate the temperature conditions
847	of the actual decomposition environment (Fig. S3).
848	2.2 Decomposition rate and stabilization measurements
849	Decomposition rate (k) and stabilization $\frac{factor}{(S)}$ were assessed following the $\frac{Tea Bag}{(S)}$
850	Index TBI protocol (Keuskamp et al., 2013). Briefly, at The TBI approach can be considered as a
851	simplified litter-bag approach, allowing a time- and cost-efficient characterization of the
852	decomposition environment, because k and S can be estimated without repeated sampling of the
853	decomposing material as in conventional approaches. This implies the assumptions that (1) S is
854	equal for the two types of material used in the approach and (2) that decomposition of non-
855	hydrolyzable materials during the 3 months of deployment is negligible. We refer the reader to
856	Keuskamp et al. (2013) for further detail and validity assessments of assumptions.

857	7 <u>At</u> each point <u>of measurement</u> , two n	ylon tea bags (200 µm mesh size), one containing
858	8 green tea (EAN: 8 722700 055525; Lipton, U	nilever + PepsiCo, UK) and one containing rooibos
859	9 (8 722700 188438, Lipton, Unilever + Pepsic	e, UK), were deployed as pairs in ~8 cm soil depth,
860	0 separated by ~5 cm. The initial weight of the	contents was determined by subtracting the mean
861	1 weight of $\frac{10}{\text{ten}}$ empty bags (bag + string + la	bel) from the weight of the intact tea bag prior to
862	2 deployment (content + bag + string + label).	The tea bags were retrieved after an incubation time
863	3 of $\sim 9092 \pm 6$ (SD) days, with three sites have	ng an incubation period >100 days and one site <80
864	4 <u>days.</u> Upon retrieval, tea bags were opened, <u>a</u>	nd tea materials were carefully separated from clay
865	5 particles and fine roots and soil, dried for 48	n at 70°C, and weighed.
866	6 Calculations for k and S followed Keuskamp	et al. (2013):
867	7	
868	8 Eq 1) $Wr(t) = ar e-kt$	+ (1-ar)
869	9	
870	0 Eq 2) $S = 1 - ag / Hg$	
871	1	
872	2 Eq 3) $ar = Hr (1-S)$	
873	3	
874	4 Wr(t) describes the substrate weight of rooibo	os after incubation time (t in days), ar the labile and
875	5 1-ar the recalcitrant fraction of the substrate,	and k is the decomposition rate constant. S describes
876	6 the stabilization factor, ag the decomposable	fraction of green tea (based on the mass loss during

877 incubation) and Hg the hydrolysablehydrolyzable fraction of green tea. The decomposable

878	fraction of rooibos tea is calculated in Eq 3 based on its hydrolysablehydrolyzable fraction (Hr)
879	and the stabilization factor S. With $Wr(t)$ and ar known, k is calculated using Eq 1.
880	In accordance with Keuskamp et al. (2013), extractions for determination of the
881	hydrolysablehydrolyzable fractions of green and rooibos tea followed Ryan et al. (1990).
882	However, instead of using Ryan's forest products protocol we conducted the alternative forage
883	fiber protocol for the determination of the hydrolysablehydrolyzable fraction. Briefly, 1 g of dried
884	tea material (70°C for 24 h) was boiled in cetyltrimethyl ammonium bromide (CTAB) solution (1
885	g CTAB in 100 ml 0.5 M H2SO4) for 1 h (Ryan et al., 1990; Brinkmann et al., 2002 <u>; Ryan et al.,</u>
886	<u>1990</u>). The extract was filtered through a 16-40- μ m sinter filter crucible (Duran, Wertheim,
887	Germany) using a water-jet vacuum pump and washed with 150 ml of hot water followed by
888	addition of acetone until no further de-coloration occurred (Brinkmann et al., 2002). The
889	remaining material was left in the sinter, dried for 12 h at 70°C, cooled in a desiccator and
890	weighed. 20 mL of 72% H2SO4 was added to the sinter and filtered off after an incubation of 3 h,
891	followed by washing with hot water to remove remaining acid. The sinter was dried at 70°C for
892	12 h, cooled in a desiccator, and weighed to determine the non-hydrolysablehydrolyzable
893	fraction. Finally, the sinter containing the remaining sample was ignited at 450°C for 3 h in order
894	to determine the ash content of the material.
895	In addition to the determination of the hydrolysable hydrolyzable fraction, we measured
896	total C and N contents of the tea material using an elemental analyzer (HEKAtech, Wegberg,
897	Germany). The hydrolysablehydrolyzable fraction of both green and rooibos tea was higher than
000	managered in Koushamment al. (2012) (Table 2) However, the determined C and N contents of the

- 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).
- 900 Therefore, deviations from the <u>hydrolysablehydrolyzable</u> fraction as reported previously are

901 likely due to the less conservative extraction assessment in the present study and not due to actual902 changes in the quality of the materials.

903 2.3 Data Analysesmining

904 For all across-site analyses, mean values of each site by elevation zone (or site by salinity class) 905 combination were used (N=51). Relationships between single parameters and litter 906 decomposition are often not linear. Instead, critical thresholds seem to exist at which a certain 907 predictor (e.g. mean annual temperature) becomes influential (Rothwell et al., 2008; Prescott, 908 2010Prescott, 2010; Rothwell et al., 2008). In the first step of data mining, we therefore used 909 classification and regression tree analysis (CART) to identify potential thresholds and important 910 predictors for k and S (Fig. S1). Data mining was conducted using STATISTICA 10 (StatSoft 911 Inc., Tulsa, OK, USA).

In the first step of our data analysis, we therefore used classification and regression tree 912 913 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 914 915 and correlated or uncorrelated predictor variables (Breiman et al., 1984; Rothwell et al., 2008), 916 and it has been recommended to identify thresholds and to handle large-scale decomposition data 917 sets (Rothwell et al., 2008; Prescott, 2010). We conducted CRTA separately for k and S using 918 temperature, salinity class, tidal amplitude, ecosystem type, soil type, and relative elevation as 919 predictor variables (Table 1). V-fold cross validation was set at 5 (as commonly conducted, 920 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. 921

922 <u>2.4 Statistical analyses</u>

923	To test for correlations between the variables salinity class, effects of temperature on k and S,
924	Spearman rank correlations were conducted using site means $(n = 30)$. As we did not expect
925	temperature to be independent of other parameters in this observational study, we constructed a
926	Spearman correlation matrix including the parameters temperature, latitude, tidal amplitude, k
927	and S, Spearman rank correlations were used (Table 3). Mann Whitney U tests were conducted to
928	test for salinity class, k, and S. Additionally, we tested for differences in k and Sthese parameters
929	between marshes and mangroves and betweensites with mineral and organic soil types.
930	We tested for linear effects of temperature on k and S across sitessoils, using simple linear
931	regression analyses (Fig. 2). Two-tailed paired t-tests were used to Mann-Whitney U tests (Table
932	3). Curve fitting was used to further explore relationships between temperature, k, and S, and
933	regression models with lowest standard error of estimate and highest R2 are displayed in Figure 2
934	and S2.
935	To test for effects of relative elevation (as proxy for relative sea level) on k and S (Fig. 3).
936	Subsequent one, two-tailed paired t-tests were conducted to test for. Mean values of high and low
937	elevated zones of the same effect within mineral, organic, marsh, and mangrove systems
938	separately.
939	In 21 of our 22 sites where tea bags were deployed in both high and low elevation zones,
940	replication was sufficient to conduct were used ($n = 21$). The absence of outliers and normal
941	distribution of the difference in the independent variable (as assessed visually) assured robustness
942	of paired t-tests. To assess the consistency of potential effects of relative elevation on k and S,
943	one-way ANOVA to test for differences in k and S between zones forwas used in each site
944	separately (Fig. S2). replication was sufficient in 20 sites). Normal distribution of residuals was
945	assessed visually, Levene's test was used to test for homogeneity of variance, and data were log-
946	transformed if assumptions were not met. Mann-Whitney U tests were conducted as a non-

947	parametric alternative when log-transformed data did not meet ANOVA assumptions (Table S1).
948	We tested for effects of nutrient enrichment on k and S in the data from the TIDE project
949	site (Massachusetts, US) using two-way ANOVA with enrichment treatment and marsh zone as
950	predictors. All analyses were conducted using STATISTICA 10 (StatSoft Inc., Tulsa, OK,
951	USA). When Levene's test indicated heterogeneous variance (true for k), data were log-
952	transformed, which stabilized variance. Normal distribution of residuals was assessed visually.
953	Lastly, in order to assess the applicability of the TBI approach in tidal wetlands, we
954	separately investigated the temperature response of k and S for the ten sites situated along the
955	North American Atlantic coast (Fig. S2). Previous studies have shown clear
956	temperature/latitudinal effects on decomposition and microbial activity along this well-studied
957	transect (Kirwan et al. 2014; Mozdzer et al. 2014), allowing us to compare the TBI approach with
958	other methods. Regional-scale transects with sufficient temperature/latitudinal range along other
959	coastlines could not be identified (Fig. 1; Table 1). Statistical analyses were conducted using
960	STATISTICA 10 (StatSoft Inc., Tulsa, OK, USA).
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968 **3 Results**

969 3.1 Temperature effects

970 We found no linear (Fig. 2a) or monotonic (Table 3) relationshipsrelationship between

- 971 temperature and k-<u>across study sites (Fig. 2a; Table 3).</u> Also, <u>CRTACART</u> revealed temperature
- 972 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;
- 974 node 5) with sites $\geq 14.5^{\circ}$ C during deployment supporting considerably higher rates of
- 975 decomposition than sites characterized by lower temperatures. However, this apparent
- temperature effect was inconsistent within the group of observations with tidal amplitude >2.1m
- 977 (Fig. S1a; nodes 13-15). In contrast to the results of the global-scale assessment, k is strongly and
- 978 positively related with temperature across the ten sites situated along the North American
- 979 Atlantic coast, with temperature explaining approx. 70% of variability in k (Fig. <u>S2)</u>. Furthermore,

980 the majority of sites (65%) are characterized by tidal amplitudes <2.1 m and show no temperature
 981 effect on k.

982 In contrast to the temperature response of k, SStabilization was strongly affected by 983 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 CRTACART (Fig. S1b). However, 984 985 **CRTACART** also identified a narrow temperature range (21.9-23.6°C) in which increasing 986 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 987 988 sites (Ebro Delta and Venice Lagoon) of our survey. The positive relationship between 989 temperature and S was even clearer when focusing on the ten sites along the North American 990 Atlantic coast, with temperature explaining >85% of variability in S (Fig. 2b.S2).

991	Temperature was highly correlated with latitude and tidal amplitude, and temperature was
992	not independent of soil type (mineral/organic) and ecosystem type (marsh/mangrove) (Table 3).
993	The effect of latitude was similarly pronounced as the temperature effect on S – and consequently
994	-effects of these two parameters on S cannot be separated (Table 3). By contrast, tidal amplitude
995	and soil type did not significantly affect S, and the difference in S between mangroves and
996	marshes is only marginally significant (Table 3). These findings suggest that the presented
997	temperature effect on S occurs to be mainly independent of tidal amplitude and soil type.
998	3.2 Effects of relative sea level <u>and nutrient enrichment</u>
999	Paired comparisons of high vs. low elevated zones indicate no consistent effect of relative sea
1000	level on k across sites (p > 0.1; Fig. $3a + e$), whereas S was significantly reduced by 29% in low
1001	compared to high elevated zones ($p < 0.01$; Fig. 3b). This significant reduction of S in low vs.
1002	high elevated zones was consistent across mineral and organic, as well as marsh and mangrove
1003	systems (Fig. 3d). Testing for effects of relative sea level within each site separately revealed that
1004	S is significantly reduced by 28-87% in the lower elevated zone in $\frac{1514}{2120}$ sites. A;
1005	whereas a significant increase of S in low vs. high elevated zones was found in none of these
1006	21the 20 sites (Fig. Table S1). This finding demonstrates the consistency of the sea-level effect
1007	on S irrespective of ecosystem type (marsh/mangrove) soil type (mineral/organic) and site
1008	salinity (brackish/salt). S2). In tennine of the sites, we also found a significant effect of relative
1009	sea level on k ; with. However, in six sites k was significantly higher k-in low vs. high zones, and
1010	in seventhree sites and k was significantly lower k-in low vs. high zones in three sites (Fig. S2.
1011	The direction of effects on k seems to be independent of ecosystem type, soil type, and site
1012	salinity (Table S1).
1013	3.3 Effects of salinity and nutrient enrichment

1014 We found no significant relationship between salinity class and k or S (Table 3). Also, CRTA did
 1015 not reveal salinity class as an important factor for k and S (Fig. S1), and no consistent salinity
 1016 effect on k and S was found when comparing sites of different salinities within single estuarine
 1017 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 fertilizedenriched high
marsh and not further reduced by fertilization nutrient enrichment (Fig. 43d). In contrast, k was
not responsive to the fertilization nutrient enrichment treatment in neither low nor high marsh
(Fig. 43c).

1023 3.4 Other factors influencing decomposition rate and stabilization

<u>CRTACART</u> revealed tidal amplitude as an important predictor for k (Fig. S1a). However, this 1024 1025 result needs to be interpreted considered cautiously because splits based on tidal amplitude 1026 suggest mixed effects (Fig. S1a). Accordingly, no linear (p > 0.68; $r^2 = 0.004$) or 1027 monotonic significant relationship (Table 3) existed between tidal amplitude and k, and effects of 1028 tidal amplitude are not independent from other factors because strong correlations existed with 1029 ecosystem and soil type, temperature, and latitude (Table 3). across sites (Table 3). Soil type 1030 (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, 1031 Fig.). Also, CART did not reveal salinity class S1a). In comparison, S was lower in mangroves 1032 1033 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 CRTAan 1034 important factor for k and S (Fig. S1bS1). 1035

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1038	The findings of the present study cannot demonstrate consistent effects of either temperature or
1039	relative sea level on the decomposition rate of recent OM inputs (commonly assessed as k in litter
1040	bag studies) in tidal wetlands. With respect to C sequestration, however, litter decay data need to
1041	be considered cautiously, as the link among decomposition rate, SOM formation, and ultimately
1042	C sequestration is not straightforward. That is, plant tissues and other fresh OM inputs into an
1043	ecosystem are not resistant to decay per se, and as a consequence, it is critical to understand their
1044	biogeochemical transformation into stable compounds that leads to the formation of SOM (i.e.
1045	stabilization) rather than understanding the pace at which decomposition proceeds (Prescott,
1046	2010; Castellano et al., 2015; Haddix et al., 2016). Here, we also assessed OM stabilization, and
1047	in contrast to decomposition rate, stabilization decreased with temperature and was consistently
1048	lower in low vs. high elevated zones of tidal wetlands. Our study therefore provides indirect
1049	evidence that rising temperature and accelerated SLR could decrease the capacity of tidal
1050	wetlands to sequester C by affecting the initial transformations of recent OM inputs to SOM (i.e.
1051	stabilization).

1052 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.

1060	The present. Following study used air temperature as a proxy for top-soil temperature.
1061	Thus, the temperature regime of the decomposition environment was only approximated, which
1062	certainly would have weakened a significant relationship between temperature and k. However,
1063	following typical Q10 values for biological systems of 2-3 (Davidson & and Janssens, 2006), k
1064	should have at least doubled over at the gradient of $\Delta T > 15^{\circ}C$. However, findings from; yet our
1065	data do not even show a tendency of an effect ($rs = 0.02$; Table 2). We therefore propose that
1066	other parameters exerted overriding influence on k, mainly masking temperature effects, and
1067	have not been captured by our experimental design. This notion is in line with the fact that
1068	studies conducted at single-marsh to regional scales are not conclusive eitherreport equivocal
1069	results on the temperature response of k, ranging from no or smallmoderate (Charles & and
1070	Dukes, 2009; Kirwan et al., 2014; Janousek et al., 2017; Kirwan et al., 2014) to strong
1071	seasonally-driven temperature effects with a Q10 >3.4 as found within a single site (Kirwan $\&$
1072	Blum, 2011). Although temperature sensitivity of OM types is variable (Craine et al., 2010;
1073	Hines et al., 2014; Wilson et al., 2016), temperature sensitivity of the used TBI materials was
1074	sufficiently demonstrated (Keuskamp et al., 2013). We therefore conclude that other parameters
1075	exerted overriding influence on k, mainly masking temperature effects and have not been
1076	captured by our experimental design. For instance, we do not have data on plant biomass
1077	parameters that are thought to exert strong control on decomposition in tidal wetlands through
1078	priming effects (Wolf et al., 2007; Mueller et al., 2016; Bernal et al., 2017). Likewiseand Blum,
1079	2011). For instance, large differences in site elevation and hydrology could have induced high
1080	variability in k across sites and masked potential temperature effects. Indeed, we demonstrate
1081	significant but mixed effects of relative sea level on k for some sites; (Table S2); however, we do
1082	not have sufficient data on actual site elevation or hydrology to control for these factors as
1083	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 1087 1088 strongly affected by temperature. Overall, S decreased by ~9075% over our temperature gradient from 10.9 to 28.5°C, corresponding to a decline of ~5% over a 1°C-temperature increase (Figure 1089 (Fig. 2b). Thus, we demonstrate a considerable temperature effect on the initial steps of biomass 1090 decomposition in tidal wetlands. This effect, however, However, as also demonstrated for k, the 1091 temperature effect on S was much clearer at the regional scale when focusing on the sites along 1092 the North American Atlantic coast (Fig. S2), suggesting high variability in S across regions 1093 irrespective of the temperature regime. In accordance, we also demonstrate a clear divergence of 1094 the four Mediterranean sites from the regression model (Fig. 2; S1), which could be related to 1095 1096 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 1097 stabilization and to separate temperature from latitudinal and other interacting effects (e.g. as 1098 outlined above for k) that are difficult to control for in observational studies. 1099 The temperature effect on the initial steps of biomass decomposition we identified in the 1100 present study is not driven by changes in decomposition rate per se, but – more importantly – by 1101 affecting the transformation of fresh and rapidly decomposable organic matter into stable 1102 compounds, with potentially important implications for Carbon sequestration (e.g. Cotrufo et al., 1103 1104 2013). In their global-scale assessment, Chmura et al. (2003) indeed report a negative 1105

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

1108	higher temperatures to be the responsible driver forof this relationship. Plant production and thus
1109	OM input is known to increase with latitude and temperature in tidal wetlands (Baldwin et al.,
1110	2014; Charles & and Dukes, 2009; Gedan & and Bertness, 2009; Kirwan et al., 2009; Baldwin et
1111	al., 2014), but this increase seems to be more than compensated by higher microbial
1112	decomposition. Working onat the same spatial scale as Chmura et al. (2003), our study strongly
1113	supports this hypothesis and provides the mechanistic insight into the temperature control of OM
1114	decomposition as an important a potential driver of C arbon sequestration in tidal wetlands.
1115	4.2 Relative-sea-level effects on decomposition processes
1116	Flooding and thus progressively lower oxygen availability in soil is supposed to be a strong
1117	suppressor of decomposition (Davidson and Janssens, 2006). In tidal wetlands, differences in
1118	flooding frequency along elevational gradients often induce sharp gradients in oxygen availability
1119	and redox conditions (Davy et al., 2011; Kirwan et al., 2013; Langley et al., & Janssens, 2006).
1120	Despite this dominant paradigm, we clearly demonstrate that k is not reduced in low vs. high
1121	elevated zones of tidal wetlands (Fig. 2013), with potentially strong influence on OM
1122	decomposition and carbon cycling. However, the effect of redox conditions on OM break-down
1123	is determined by the chemical quality of the decomposing material: Decomposition of aged or
1124	recalcitrant OM can indeed be slower and incomplete in the absence of oxygen, whereas the
1125	break-down of fresh and labile OM can be largely unaffected by oxygen availability (Benner et
1126	al., 1984; Kristensen et al., 1995). Thus, also decomposition rate and stabilization of labile,
1127	hydrolyzable OM, as assessed in the present study, is not necessarily affected by redox
1128	conditions. Here, we demonstrate that k is not reduced in low (more frequently flooded) vs. high
1129	elevated (less frequently flooded) zones of tidal wetlands (Fig. 3a). This finding is in accordance
1130	with an increasing number of studies demonstrating negligible direct effects of sea level on

1131	decomposition rate in tidal wetland soils (Janousek et al., 2017; Kirwan et al., 2013; Mueller et
1132	al., 2016; Janousek et al., 2017). A SLR-induced reduction in decomposition rate with positive
1133	feedback on tidal wetland stability seems therefore to be an unlikely scenario.). Furthermore, we
1134	show that S is strongly reduced in low vs. high elevation zones, suggesting that the conversion of
1135	recent OM inputs to stable compounds and eventually SOM-is in fact lower in more flooded
1136	zones of tidal wetlands. Accelerated As the stabilization of labile OM inputs is a major driver of
1137	SOM formation (Cotrufo et al., 2013, 2015; Haddix et al., 2016), one important implication of
1138	this finding is that accelerated SLR consequently seems to yield yields the potential to decrease
1139	SOM formation and with that C-the carbon-sequestration potential of tidal wetlands. This finding
1140	and its implication may occur counterintuitive with respect to the often sharp redox gradients
1141	along tidal wetland zonations and with flooding The(Davy et al., 2011; Kirwan et al., 2013;
1142	Langley et al., 2013), and the mechanism by which S is decreased in the more flooded zones of
1143	the present study is unknown. Because we did not observe consistent salinity effects on S and k
1144	in our data (Figs. S1, S3),, we do not suppose that regular exposure of litter to salt water explains
1145	the unexpected finding. Instead, Likewise, soil temperature was not consistently affected by
1146	relative elevation across sites ($p > 0.3$; paired t-test based on data shown in Fig. S3). Instead, we
1147	argue that more favorable soil moisture conditions in low vs. high elevated zones could have
1148	decreased OM stabilization if higher flooding frequencies did not induce redox conditions low
1149	enough to suppress microbial activity in the top soil. In support of this, flooding-frequency
1150	induced changes in moisture conditions have been reported as primary driver of surface litter
1151	breakdown, leading to more than four-fold increased litter mass loss in low vs. high marsh
1152	zones of a New Jersey salt marsh (Halupa <u>& and</u> Howes, 1995).
1153	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).

1160 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 1161 1162 worldwide, we included the long-term ecological research site of the TIDE project in 1163 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. 1164 Important for our argument that decomposition may be favored by higher nutrient availability in 1165 1166 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 1167 1168 fertilized high marsh and not further reduced by fertilization (Fig. 4<u>3d</u>). Johnson et al. (2016) 1169 demonstrate that nutrient enriched high-marsh plots of the TIDE project receive 19±2 g N m-2 1170 yr1, approximately 10-times the N load of reference high-marsh plots $(2\pm 1 \text{ g N m-2 yr-1})$; mean±SE), thus explaining the strong treatment effect observed in the high marsh. In accordance 1171 with low stabilization in the reference low marsh, which is equally low as the nutrient enriched 1172 1173 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 1174 1175 enriched low-marsh plots do not result in additional reduction of S compared to the reference low 1176 marsh (Fig. 43d). These findings suggest that microbial communities of the high marsh are N 1177 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 1184 based on the findings of a single field experiment only, our study adds to a growing number of 1185 reports illustrating the impact of coastal eutrophication on tidal wetland C cycling (Morris & 1186 Bradley, 1999; Deegan et al., 2012; Keuskamp et al., 2015a; Kirwan & Megonigal, 2013; 1187 Keuskamp et al., 2015bMorris and Bradley, 1999). At the same time, however, we highlight the 1188 need to improve our understanding of coastal eutrophication in interaction with other global 1189 1190 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. 1191

1192 4.4 <u>Methodological The Tea Bag Index – methodological</u> considerations

The Interpretation of results obtained from standardized approaches like the present needs to be 1193 1194 made cautiously because OM quality of OM (i.e. its chemical composition) is a key parameter 1195 affecting its decomposition. -As the quality of the TBI materials differ from that of wetland plant 1196 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 1197 1198 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 1199 1200 decompose and stabilize the deployed standardized material. Standardized approaches like this,

1201	or also the cotton-strip assay (e.g. Latter and Walton, 1988), are useful to separate the effects of
1202	environmental factors other than OM quality on decomposition processes and to assess their
1203	relative importance. Otherwise, complex interaction effects of the abiotic environment and OM
1204	quality make it difficult to predict the relevance of certain environmental factors for
1205	decomposition processes, potentially masking the effects of important -global-change drivers
1206	(reviewed in Prescott, 2010).global-change drivers (Prescott, 2010). At the same time, however,
1207	the global-change factors considered in the present study are likely to induce changes in the
1208	quality of the OM accumulating in tidal wetlands, for instance through shifts in plant-species
1209	composition and plant-tissue quality, that can potentially counterbalance or amplify the effects on
1210	decomposition processes suggested here. Future research therefore needs to address OM quality
1211	feedbacks on decomposition processes in tidal wetlands in order to gain a more complete
1212	understanding of global-change effects on tidal-wetland stability and carbon-sequestration
1213	capacity. Stabilization is thought to be a key parameter for capturing changes in decomposition
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1213	with consequence for C sequestration. Indeed, Keuskamp et al. (2013) demonstrate that S, as
1214	with consequence for C sequestration. Indeed, Keuskamp et al. (2013) demonstrate that S, as
1214 1215	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
1214 1215 1216	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
1214 1215 1216 1217	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
1214 1215 1216 1217 1218	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
1214 1215 1216 1217 1218 1219	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 (Meleod et al., 2011). Based on the S values obtained from initial calculations using the hydrolysablehydrolyzable fractions suggested by Keuskamp et al. (2013), a large
1214 1215 1216 1217 1218 1219 1220	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 (Meleod et al., 2011). Based on the S values obtained from initial calculations using the hydrolysablehydrolyzable fractions suggested by Keuskamp et al. (2013), a large number of observations in fact-yielded a negative S (data not shownTable S3). S becomes
1214 1215 1216 1217 1218 1219 1220 1221	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 (Meleod et al., 2011). Based on the S values obtained from initial calculations using the hydrolysablehydrolyzable fractions suggested by Keuskamp et al. (2013), a large number of observations in fact-yielded a negative S (data not shownTable S3). S becomes negative when the mass loss from green tea is greater than the predicated maximum loss based on
1214 1215 1216 1217 1218 1219 1220 1221 1222	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 (Meleod et al., 2011). Based on the S values obtained from initial calculations using the hydrolysablehydrolyzable fractions suggested by Keuskamp et al. (2013), a large number of observations in fact-yielded a negative S (data not shownTable S3). S becomes negative when the mass loss from green tea is greater than the predicated maximum loss based on its hydrolysablehydrolyzable fraction. At least two processes could have caused or contributed to

1225	least often not low enough to hamper decomposition processes.of the hydrolyzable fraction of the
1226	TBI materials. As a consequence, the relatively high top-soil moisture of tidal wetlands could
1227	provide favorable conditions for decomposition, following typical moisture-decomposition
1228	relationships as demonstrated for terrestrial ecosystems (e.g. Curiel Yuste et al., 2007), and S
1229	should at least not expected to be high in the top soil environment of tidal wetlands.). Potentially,
1230	moisture <u>conditions</u> and nutrient supply are even high enough to allow for considerable break
1231	down of non-hydrolysablehydrolyzable compounds within three months of deployment, such as
1232	lignin (Berg <u>∧</u> McClaugherty, 2003; Knorr2014; Duboc et al., 20052014; Feng et al., 2010;
1233	DubocKnorr et al., 20142005). Second, different protocols and methods to determine
1234	hydrolysable and non-hydrolysable fractionsthe hydrolyzable fraction of plant materials exist and
1235	lead to variable results. The hydrolysablehydrolyzable fraction of plant materials can
1236	consequently be over- or underestimated depending on method, protocol, and type of sample
1237	material. The use of the slightly higher hydrolysablehydrolyzable fractions we determined for
1238	calculations of the TBI parameters effectively eliminated negative S values. In that regard, using
1239	the values obtained from the alternative protocol given in Ryan et al. (1990) seemed more
1240	reasonable in our study. Although However, it needs to be stressed here that direction and size of
1241	reported effects on S and k in the present study are almost independent fromof the
1242	hydrolysablehydrolyzable fraction used for calculations, further research is required to improve
1243	our estimates of the hydrolysable fractions in TBI materials.
1244	Future research will have to test the applicability of the TBI approach in different
1245	ecosystems and test the validity of its assumptions (i.e. S is equal for both types of material used,
1246	and mass loss of non-hydrolyzable material is negligible over 3 months of deployment). The
1247	results of our regional scale assessment along the North American Atlantic coast transect are in
1248	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.

1252 4.5 Implications

1253 While awareness about potential global warming impacts on OM preservation and their resulting 1254 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 1255 1256 and their biophysical feedbacks (Kirwan et al., 2016). Potentially negative effects of accelerated 1257 SLR on OM preservation were thus far overlooked, probably because stimulation of 1258 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 1259 1260 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. 1261

1262 This study addresses the influence of temperature, relative sea level, and coastal eutrophication 1263 on the initial transformation of biomass to SOM, and it does not encompass their effects on the 1264 existing SOM pool. However, aspects of S and k are key components of many tidal wetland 1265 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 <u>Although actual rates of S</u> 1266 and k cannot be inferred from this study using a standardized approach, our data identify strong 1267 1268 negative effects of temperature, relative sea level, and coastal eutrophication on OMthe 1269 stabilization mayof 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 1270 wetland accelerate carbon turnover in tidal wetlands, thus increasing their vulnerability to 1271

1272	accelerated SLR, and we highlight the need for experimental studies assessing the extent to which
1273	the here identified effects translate into native OM dynamics.
1274	Our findings imply that particularly the vulnerability of organic systems might increase with
1275	global change because in these systems soil volume is almost exclusively generated by the
1276	preservation of OM. At the same time, however, mineral dominated systems, such as temperate
1277	European salt marshes, experience large amounts of easily decomposable allochthonous-OM
1278	input that relies on substantial stabilization in order to become sequestered (Middelburg et al.,
1279	1997; Allen, 2000; Khan et al., 2015). Thus, future rates of C sequestration could be substantially
1280	reduced in mineral dominated tidal wetland systems.
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1300 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 LMSB, 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.

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1322 Figure captions

1323 Figure 1 Overview map of study <u>sitesregions</u>. Notes: See Table 1 for <u>region and</u> site details.

1324 Figure 2 (a) Decomposition rate (ksite means; n = 30) and (b) stabilization factor (S(site means; n))

1325 = 30) versus mean atmosphericair temperature during deployment period. Shown are results of

 1326
 linearRegression line illustrates significant relationship between temperature and stabilization

 1327
 (Table 2): regression analyses across and within elevation zones and organic model with lowest

- 1328 standard error of estimate (SEE) and mineral soils. highest R2 is shown: y = -0.27ln(x) + 0.99; R2 1329 = 0.239; SEE = 0.131; excluding the Mediterranean sites (21.9-23.6°C; n = 4) from the regression
- 1329 = 0.239, SEE = 0.151, excluding the Mediterranean sites (21.9-25.0 C, II = 4) from the I 1330 yields: $y = -0.344 \ln(x) + 1.233$; R2 = 0.510; SEE = 0.101

1331Figure 3 (a+c) Decomposition rate (k)-and (b+d) stabilization factor (S)-in high (orange) and1332low (blue) elevated zones of tidal marsh and mangrove sites (compare Table 1). High and low1333elevated zones are characterized by distinct plant-species assemblages or by different stature of1334mangroves along the flooding gradient within each site. Shown are means \pm SE for all sites (a +1335b) and for mineral, organic, marsh, and mangrove systems separately (c + d). P values refer to1336results of paired t-tests (ns, P > 0.05; * P \leq 0.05; ** P \leq 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
- 1343 comparisons (Tukey's HSD test). panels c + d): ns = not significant; * = $p \le 0.05$; ** = $p \le 0.01$
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