

Anonymous Reviewer #1:

This research group should be commended for attempting an experiment comparing the effects of inundation on decomposition rates. The idea of the experiment is laudable, but aspects of the experiment design are inadequately explained and the statistical analysis is lacking. Specific comments follow.

1. Introduction Page 3, Line 5. Rates of productivity are maximized at an optimum flooding frequency, but this statement should be confined to a specific region. The actual rate of productivity related to an ecosystem type depends on geographical region, and this is particularly true of coastal marshes over their worldwide distributional range. *The three references in the previous sentence all consider the factors controlling geographic variation in salt marsh productivity. The sentence in question is strictly related to how productivity varies with inundation frequency. The existence of an optimum flooding frequency appears to be widespread, and therefore does not deserve a regional caveat. In the revised manuscript, we will add references from other regions and plant species to show that the existence of an optimum is indeed a general pattern (Voss et al., 2012; Kirwan and Guntenspergen, 2012; Langley et al., in press,). Nevertheless, we will also make reference to a couple papers that suggest only weak evidence (Kirwan et al., 2012) or no evidence for an optimum (Marani et al., 2004).*

Marani, M., Lanzoni, S., Silvestri, S. & Rinaldo, A. (2004) Tidal landforms, patterns of halophytic vegetation and the fate of the lagoon of Venice. *Journal of Marine Systems*, 51, 191–210.

Voss, C.M., Christian, R.R., and Morris, J.T., 2012. Marsh macrophyte responses to inundation anticipate impacts of sea-level rise and indicate ongoing drowning of North Carolina marshes. *Mar Biol.* DOI 10.1007/s00227-012-2076-5

2. Methods How was inundation estimated? A reference is given to Weiss et al., but this calculation is a fundamental part of the paper and should be explained fully in this paper. Also, total annual inundation may or may not reflect overall hydrology. These areas must be tidal? Also, the decomposition rate is likely to be highly dependent on drawdown or flood periods, and this needs to be described in the paper. Mean values of salinity are given, but decomposition is very sensitive to salinity level. If there are periods of time during the year with salinity levels outside the mean and/or fluctuation in inundation (flood or high tide), these periods must be considered in the analysis of the data.

The existing manuscript contains the following sentence describing water level measurements: “At each site we recorded water levels in the tidal creek with pressure transducers and calculated the frequency and duration of inundation for each mesocosm elevation.” To be clear, we will add a sentence to the revised manuscript stating “Inundation in a given row occurs when the water level in the creek is higher than the elevation of the mesocosm, and we report only the duration of inundation averaged over the entire experiment.”

2. Page 5, Line 13. Correct the spelling of *Schoenoplectus*.
Thank you, we will correct this in the revised manuscript.

3. Page 6, Line 21.
Should explain why bags might gain weight (e.g., sediments, microbes).

This is a good point. We will add the phrase “presumably from analytical error and/or the introduction of exogenous material” to the sentence in question so that it now reads, “Eight ripped SOMbags and two bags with apparent weight gain (presumably from analytical error and/or the introduction of exogenous material) were discarded, leaving a total of 87 bags used in the analysis.”

4. Page 6, Line 5. Peat was used for the decomposition? Peat is not just one thing, and is comprised of wood, leaves and roots. of different ages. These components decompose at different rates. Was there an attempt to separate out wood pieces, which is very recalcitrant. Lack of homogeneity in the decomposition material could have led to a great deal of variability in the results.

The peat used at each site was all collected from a common location (Rhode River marsh) and homogenized before making litter bags. The peat is ultimately derived from the native sedges and grasses that occur at all sites. There is no woody material in this marsh peat, and the integration of different aged materials makes our litter bags more representative of natural decay. In any case, the bags were homogenous so not a source of between-site variation.

5. Page 7. The analytic methods are particularly lacking. There are 3 sites with various numbers of platforms and years of study. It is tough to come up with a simple and powerful method to analyze these data, and it is not surprising that the regression methods chosen did not find any patterns. I see patterns when I look at the regressions in Fig. 3, and wonder why these were not captured in the statistical analysis. An ANOVA approach to compare Rhodes and Blackwater may be the way to detect differences related to inundation. An ANOVA approach would allow you to test for the variability related to site differences and time. Transquaking may have too little data to do this, although it should be considered. For Rhodes and Blackwater, if some of the analysis shows that some factor is not an important (e.g., year), it may be possible to collapse the annual data for one of the sites, and then to do a balanced comparison of Rhodes and Blackwater with means of years. If some aspects of the data set are unbalanced, in may be possible to use some distributionless tests to do the analysis. At any rate, an ANOVA approach may be the only way that you can account for the variability that needs to be accounted for to try to make the main point of this paper, which is that "inundation does matter". It is likely that the situation here is a case in which inundation and decomposition were not adequately dealt with in the analysis. Also keep in mind that k values are not linear, so an ANOVA on k values would reflect the spontaneous rates of change of these negative exponentials. Again, using k values should add to the power of an ANOVA approach to analyze these data. If the ANOVA cannot be done, then you are left with the regression approaches that you are currently using in the paper, and you may not be able to account convincingly for variability related to site and time differences. That is a real weakness of the regression analysis used currently in the paper. See additional details related to regression approaches for data analysis under "Fig. 3".

We have now conducted an ANOVA on the Blackwater and Rhode River data from 2011, so that the year of the experiment is not a confounding variable. As the reviewer states, the decomposition response may not be linear. Therefore, we binned the sea level treatments into groups and ran a two-way ANOVA with sea level as a categorical variable, so that the test would not be constrained to a particular relationship. The results of the ANOVA confirm our original

conclusion, that inundation does not have a strong effect on decomposition rate. We have also removed the Transquaking River data from all panels in Figure 3 for simplicity, and since the number of flooding treatments at this site are so few.

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Location	1	1	0.03098046	11.0996	0.0033*
3 flood groups	2	2	0.00156122	0.2797	0.7589
Location*3 flood groups	2	2	0.01080473	1.9355	0.1704

Results of two-way ANOVA (location x flooding) where flooding treatment is split into 3 groups (<33% inundated, 33-67% inundated, >67% inundated). RR was significantly faster than at BW. There was no effect of flooding or any interaction with site.

6. Page 9. Sea level rise would also change salinity levels, so this study is more of a test of inundation than sea level rise. For that matter, you have used means of salinity, and discounted salinity as a factor. Ignoring salinity may be a mistake because of event-based, seasonal or tidal conditions related to salinity. The same may be true of inundation. Nutrients may also differ between the sites, but salinity is likely to be important here.

We agree that this experiment looked primarily at the effect of inundation on the rate of decomposition. Other factors, such as salinity, may also change with sea level rise. However, even the direction of change is unclear. Salt water intrusion may cause fresh and brackish marshes to become saltier (e.g. Perry and Hershner, 1999). On the other hand, more frequent flooding associated with sea level rise typically dilutes soil salinities in salt marshes (Morris, 2000), and expected increases in precipitation may also lead to fresher soils. Thus, the direction of salinity change is unknown so our experiment appropriately attempts to isolate the effects of a single variable (inundation) associated with sea level rise. Nevertheless, the review raises an important point and we will add the following sentences to the experimental approach section. "Although we have attempted to isolate inundation as the primary variable, soil salinity and temperature likely co-vary with flooding frequency. Trends in these variables likely approximate conditions in natural marshes. For example, soil salinity in the frequently flooded, low elevation mesocosms is controlled by the salinity of flood waters, whereas the salinity of high elevation mesocosms varies with evaporation and precipitation." We will also explicitly state that we did not measure salinity in the experiment, and emphasize that salinity is an important influence on decay rate.

Perry, J.E. and Hershner, C.H., 1999. Temporal changes in the vegetation pattern in a tidal freshwater marsh. *Wetlands* 19, 90-99.

Morris JT (2000) Effects of sea-level anomalies on estuarine processes. In: Hobbie J (ed) *Estuarine science: a synthetic approach to research and practice*. Island Press, Washington, pp 107-127

7. Page 11, Line 5. Another source of error may be differences in the constituents of the peat material itself.

We disagree- as described in Response #4, the same peat was used for all three sites and homogenized before making bags.

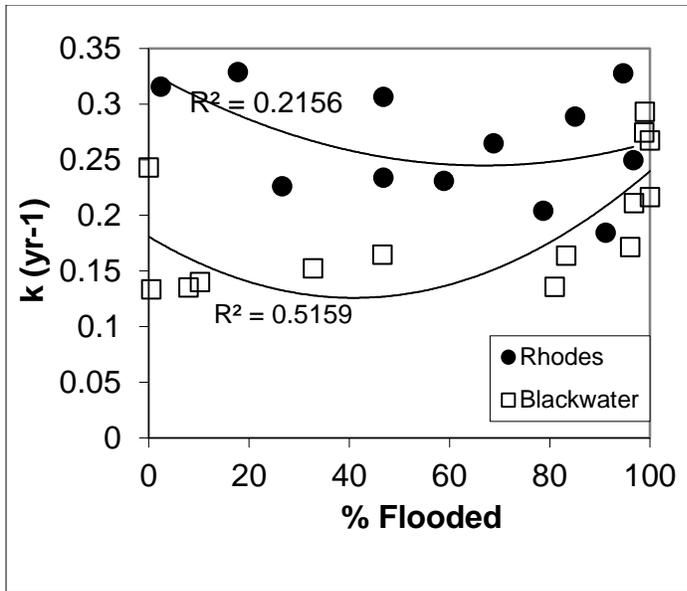
8. Fig. 3. Page 19. The k values should be statistically analyzed using ANOVA and nested by site. Some of the sites also were done in more than one year (Rhode River), and this needs to be accounted for in the model. Inundation periods might be grouped into categories. Differences are

examined by site in the regression (but the shapes of curves not compared), and this approach is not likely to pick up differences related to inundation. Using this type of analysis, at the very least the shapes of the curves should be compared statistically. Even so, a regression approach will not identify the main sources of variability in the way that an ANOVA can.

We appreciate this suggestion, and as described in response 5, followed advice. We binned sea level treatments into groups and used sea level as a categorical variable in a two-way ANOVA (sea level \times site), so that our assumption of the relationship between flooding (linear or quadratic or otherwise) and decomposition would not limit our ability to detect an effect of inundation. Regardless of how many flooding treatment groups we designated (we tried three, four and five- with six we lost degrees of freedom), we found no significant effect of flooding. Site was highly significant in all cases. We now mention the results of the three-group ANOVA in the text.

9. Fig. 3. Percentage of mass loss should be log transformed before analysis (if used). I assume that the values in Fig. 3a are the same values are used in Fig. 3b and 3d, so that only one of these analysis procedures should be used. It's not really valid to give various analysis procedures for the same data set in a paper. K values reflect the spontaneous rate of change peat decay over time, so that this value may be the one to use in the data analysis. Following Fig. 3c, I can see that the curves of k values by site seem to be 2nd order polynomials with lowest values in medium levels of inundation. I doubt that the Transquaking site can be analyzed with only 3 inundation levels. It should be possible to compare the shapes of the curves of Rhodes and Blackwater though to test the difference across inundations % levels. The patterns are not likely to be linear so second order polynomials and exponentials should be fitted to the data. Keep in mind that a k value reflects a negative exponential. Also, site means might be used to fit these curves, which would reduce the variation contributed by site conditions or unidentifiable factors, and capture the variation related to inundation (See Underwood 1997). In the end, I'm not sure which data analysis approach should be used. The problem is that I can see patterns in the data, and it is not convincing that the statistical analysis did not detect these patterns.

We agree that Figure 3a and 3b are redundant, and have therefore deleted Figure 3a. The deletion of the mass loss data in Figure 3a also appeases the reviewer's first concern (that mass loss data should be log transformed if used). We have also taken the reviewer's advice and removed the Transquaking River data from Figure 3 altogether. However, we disagree that Figure 3c argues for a convincing 2nd order polynomial relationship between flooding and decomposition. We have included such a fit in the figure below. A 2nd order polynomial fit is insignificant at the Rhode River site, and marginally significant at the Blackwater site ($p < 0.10$). More importantly, we are unaware of any mechanistic explanation for lowest decomposition at an intermediate flooding frequency, and our C and N concentration data suggests the opposite (Figure 4).



10. Fig. 4. The same problem may be true of the C and N regressions in Fig. 4. The means may be fairly clear, and show differences between sites across inundations. These are percentages losses of C and N, so the values likely need to be arcsine square root transformed before analysis.

Because the percentages are all very low (because decomposition is slow) our data did not display the sigmoid distribution characteristic of other wider-ranging percentage data, and therefore do not require a transformation. A Shapiro-Wilk W test showed the residuals were distributed normally.