

Interactive comment on “Two-dimensional distribution of living benthic foraminifera in anoxic sediment layers of an estuarine mudflat (Loire Estuary, France)” by A. Thibault de Chanvalon et al.

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Interactive comment on

“Two-dimensional distribution of living benthic foraminifera in anoxic sediment layers of an estuarine mudflat (Loire Estuary, France)” by A. Thibault de Chanvalon et al.

Dear editor(s)

Reviewers 2 and 3 both indicated that in the first version, the discussion and conclusion were not enough focused on the main topic of the manuscript, which is the new

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sampling strategy, allowing us to study simultaneously the distribution of foraminifera and chemical species on a cm scale. They also indicated that parts of the discussion (and even conclusion) were far too speculative, because we don't dispose of enough high quality data to go that far in our hypotheses.

After considering these remarks very carefully, we came to the conclusion that the reviewers are absolutely right. Consequently, we took care to focus the paper more on the major contribution (new sampling methods), and we carefully screened the discussion and conclusion. We deleted the most speculative hypotheses, and carefully distinguished everywhere between factual evidence and (the remaining) hypothetical explanations.

As a consequence, we were able to reduce the length of the discussion from 263 to 244 lines. On the following pages, you'll find our responses to the more important remarks of the 3 reviewers. In most cases we largely agreed with their comments, and made the necessary changes in the manuscript. Concerning suggestions for minor changes, they were all followed in the text, and are not detailed here. The revised manuscript and figures have been upload as supplement of this comment.

Answer to reviewer 1:

R#1: 1. Effect of the thickness of sediments :The authors need to discuss more on the fact that the cubic sediments collected with jaw device has a 1cm thickness. The comparison between foraminiferal distribution and other environmental factors, namely, dissolved iron concentration, dissolved reactive phosphorous, polychaete tube distribution, must be considered with this thickness effect. The Moran's index analysis indicated that the foraminiferal distribution has a patchiness of 1cm scale. This suggests that the foraminiferal distributions on the sectioned side, which the iron, phosphorous and polychaete tube distributions were examined, may differ from that the other side (1cm behind).

Answer: As better explained in the new version of the manuscript (section 4.1, third

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paragraph), we believe that the fact that the characteristic patch length of ≥ 1 cm, for both foraminifera 2D distribution and iron or phosphorus 2D concentration distribution, clearly shows that our comparison of a sampling plan (chemistry) and a 1 cm thick sediment layer (foraminifera) is pertinent.

R#1: 2. Lowest TOC values at 1 to 2 cm depth. The authors interpret that the lower *A. tepida* abundances at the depth of 1 to 2 cm are caused by the upward migration of *A. tepida* to oxygenated surface layers. On the other hand, interestingly, the TOC concentrations in sediments also showed lowest values at the 1 to 2 cm depth in sediments. Although there was only one TOC profile in this study, if we assume that the profile is common at this area, the profile suggests characteristic sedimentation/mixing/production of organic matters in sediments at the site. The distribution of organic matter may also explain foraminiferal distribution in the sediments. However, there was no discussion on this TOC profile in the manuscript.

Answer: TOC indeed shows a minimum at 1 cm depth. However, the minimum is very punctual; a few mm lower TOC again has a similar value as at the surface. It appears therefore that there is no direct relation between foraminiferal density and TOC.

R#1: 3. Vertical distribution of *H. germanica* There is no discussion on the distribution of *H. germanica*, which showed deeper distribution than *A. tepida* (Fig 5a). The deeper distribution means either they have low mobility or low sensitivity to go back toward surface, or they have low productivity at the surface, based on the interpretation on *A. tepida* (Fig. 11). The authors could add some discussion on this, or at least describe the results, otherwise the authors can omit the *H. germanica* from the Fig. 5a .

Answer: *H. germanica* has been deleted from the Figure because of its very low density. This is now explained in the text (section 3.4., first paragraph).

R#1: 4. Figures re-organization Figure 1 can be omitted. Figure 4 can be presented with Figure 7, together with Figure 5b. If the authors will not mention about the *H. germanica*, data in Fig. 5a is sufficient to be presented in Fig. 8a, so the Fig. 5 can

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be arranged into new Fig. 7 and Fig. 8. Figure 10 could be omitted.

Answer: Figs. 1 and 10 have been deleted; Figs. 4, 5, 7 and 8 have been reorganized according to the reviewer's comment.

âĀĀ R#1: In the introduction, the authors referred to some studies that describe controlling factors of foraminiferal patchiness such as organic carbon, grain size, etc. It is pity that these parameters were not quantified from the jaw samples (I know that the cubic cm is not sufficient to perform all these parameters, though).

Answer: We agree with this comment, but we are no longer able to add this info.

R#1: Page 10334, line 11 The patchiness of the foraminiferal density, and input of organic matter, may be caused by the same events.

Answer: if the reviewer suggest by these "same events" are burrowing events, we would partially disagree since in our results no evidence of foraminiferal concentration around burrows was observed.âĀĀ

Anonymous Referee #2

R#2: The methodological approach of this study, although valid and interesting is overshadowed by the presentation of conjecture as observed results of this study.

Answer: We carefully went through the entire discussion, and better separated facts and interpretation, making it very clear when we are presenting hypothetical scenarios.

R#2: The experimental approach seems to be sound although comparison of only a single sample from "the jaw device" and a traditional sediment core seems to be a rather small sample size which would not be appropriate for publication in a study not presenting preliminary results from a new methodological approach.

Answer: Indeed, the main goals of this study were 1) to present a sampling protocol allowing us to simultaneously investigate centimeter scale variability of foraminifera and chemical species, and 2) to show what kind of information can be obtained in

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this way. To convince the reader that the vertical part of the observed 2D patterns of foraminiferal distribution is representative for a larger area, we added faunal data for a second traditional core sampled at the same site and time. Both replicates are in very good agreement (Fig. 6)

R#2: Abstract- The patterns of Fe are interesting and outlining the importance of high resolution dissolved iron profiles and what they have the potential to illustrate, especially in regards to microfaunal habitats, should be better addressed in the abstract.

Answer: the abstract (and conclusions) were modified to better highlight the relation between iron enrichment and high foraminiferal concentrations.

R#2: Pg 10318 Lines 1&2 contain "data not shown". This information should be presented in an appendix.

Answer: we added in Fig. 3A and referred to a publication in preparation.

R#2: Pg 10318 Section 2.2 1-D sampling and processing section: In this section Figures 3a and b can be cited. This would result in their coming before Figure 2 and therefore being out of order. Alternatively sections 2.2 and 2.3 could be switched.

Answer: Figure 2b about 2D sampling can be hardly cited in the section 2.2 and Figure 2a is pertinent only because it allows comparison with Figure 3b. We don't think there is a real necessity to have an illustration to understand core slicing, thus we chose to conserve the actual organization.

R#2: Pg 10319 Line 19. Is there any potential of the anoxic gel adversely altering the results of this experiment? For example could it act as a deterrent to organisms in the immediate vicinity encouraging them to move in the opposite direction over the 5hr sampling period?

Answer: there are many arguments to believe that acrylamide gels have no incidence on biota. Firstly, the extensive literature using this approach never mentioned such an effect. Secondly, once the acrylamide polymerized, no leaking or redissolution occurs

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and only direct digestion would be toxic. A membrane is present to prevent damages to gel and migration of microbes to the inner side. Finally, the good agreement between 1D and 2D in chemistry and foraminifera suggests no significant effect at the scale of the study.

R#2: Pg 10320 Lines 5-9. Error in volumetrics could be avoided if sediments sampled were determined volumetrically (e.g. Rathburn and Corliss 1994).

Answer: The volume calculated from the mass as proposed in Rathburn and Corliss (1994) should have a comparable uncertainty due to the high porosity variation of intertidal sediment (from 0.9 to 0.7).

R#2: Pg 10322 Lines 9-10. Are the polychaetes observed in the sediment thought to be the creators of all the burrows in the sample? Are these any characteristics in the burrows that may suggest an inhabitant or creator?

Answer: According to direct observations, most of the burrows are inhabited by polychaetes. The sediment surface was covered by worm imprints, sign of intense polychaete activity. The frequent ventilation of the worms explains the decrease of dissolved iron visible on the DET 2D.

R#2: Pg 10325 Line 25 -Pg 10326 Line 1. This is an interesting statement that merits further discussion. For this methodology to become commonly accepted its usefulness and necessity should be demonstrated and discussed. If this methodology shows roughly similar results, in terms of densities, why should it be adopted rather than traditional foraminiferal and geochemical sampling techniques? A section focusing on this would provide a much more powerful and interesting argument than the inferred reasons for the *A. tepida* depth maximum between 3-5cm

Answer: Again, the goal of the present study is not to propose a new sampling technique for foraminifera that would replace the classical one but to provide a technique that simultaneously gives information on lateral variability of foraminiferal densities at

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a centimeter scale and a precise description of chemical gradients in porewaters on a similar scale.

R#2: Pg 10326 Line 1. Correct. A larger sample size would be much more desirable. Yet, the small sample size does not keep you from drawing wide conclusions not observed but only hypothesized from the small amount of material examined.

Answer: We agree with the reviewer that in the previous version, some parts were too speculative in view of the small sample size. Nevertheless, the comparison between the samples taken with the new device and two traditional cores show very comparable foraminiferal densities and vertical distribution. Concerning patchiness at a cm scale, the densities for the 1cm³ samples were high enough to yield statistically significant results. In response to this comment (and similar comments of reviewer 3), we carefully screened the text, deleted all very speculative parts, and now indicate always very carefully when proposed scenario's are hypothetical.

R#2: Pg 10330 Section 4.3. Here it may be helpful to discuss any observed vertical distribution patterns of foraminifera, especially *A. tepida*, to environmental parameters. How quickly would you expect *A. tepida* to react to environmental changes? Minutes? Hours? Days? (this is discussed a little later but only in terms of oxygen).

Answer: Foraminifera should respond in hours to days, as their observed speed is roughly 1mm h⁻¹ (between 5 mm h⁻¹ (Wetmore, 1988) and 0.24mm h⁻¹ (Gross, 2000)). As such, foraminifera may be expected to react within days to major events taking place in the sediment, such as the formation of macrofaunal burrows.

R#2: How quickly, if at all, do you expect geochemical parameters to shift in these areas? One may expect rising tide and changing salinity to alter at least surface water geochemistry. Is there any evidence it changes porewaters as well? Does your sampling scheme reflect the ability to capture this? It would also be helpful to discuss the scale and timing of environmental changes in this region. Samples were taken at low tide on a cloudy day. Would you expect different distributions at high tide? On a sunny

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day?

Answer: Tidal variation will mainly affect the upper centimeter, since the permeability of the muddy sediment is very low. Conversely, a new burrow, introducing oxygen in a reduced environment would change the geochemical signal in a few minutes, whereas the residence time of dissolved iron in the sediment is about 2 days. Summarizing, we assume that both geochemical parameters and foraminiferal distribution in the suboxic part of the sediment should not be impacted by tidal cycles or luminosity.

R#2: The distributions you observe here have been observed before. Are there similarities between these study regions and parameters? Were burrows present in these studies as well? The distribution of *A. tepida* was not discussed in these studies but are there any others that discuss a population maxima at depths of 3cm or more?

Answers: The two cited publications (Alve and Murray (2001) and Bouchet et al. (2009)), studied comparable intertidal and highly bioturbated environments. For example, density was around 100 *H. diversicolor* m⁻² in Aiguillon Cove (Bouchet, 2009). Numerous studies reported subsurface maxima of foraminifera since the 1960's., some new examples have been added in the manuscript.

R#2: Also, a definition of the 8 vertical replicates should be discussed either here or presented in the results

Answer: The term “replicate” is generally used to identify groups of samples for which the variation is considered as (statistical) noise. Conversely the terms “station” or “site” identify groups of samples for which the variation is considered as a signal we have to understand. Since we use the different cubes to investigate the presence of potential heterogeneity, and the processes causing it, the term “replicate” was not well chosen. In this new version of the manuscript we don't use it any more. Moreover we performed a pairwise comparison between the different columns using paired t test. Using a standard t-test, no significant statistical differences were found between the 8 columns (see comment figure).

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R#2: Pg 10332 Lines 12-16. The statement for bioirrigation reviving *A. tepida* and that there was no correlation between burrows and living *A. tepida* and burrows seem conflicting.

Answer: The lack of correlation between *A. tepida* and burrows (at a cm-scale) suggests that only foraminifera living less than 1 cm away from burrows are re-activated. Alternatively, part of the revived foraminifera could migrate to the surface sediment, further diminishing concentrations around the burrow. We preferred not to expand the discussion on these very speculative aspects.

R#2: Pg10334 Line 1. What is the average burrow depth? How can we calculate that? Does it correlate with the abundance peak of *A. tepida*?

Answer: In most cases, the visible length of burrows does not represent their total length. There is no clearly defined maximum or average depth. As far as we can judge, there is no correlation between foraminiferal densities and burrow density.

R#2: A note for this section: This study demonstrates no direct observation of vertical or lateral migration of *A. tepida*. Therefore, statements of foraminiferal migration should be limited to potential occurrences (conjecture) and not presented as your own observations.

Answer: We carefully went through the entire discussion, and better separated facts and interpretation, making it clear whenever we present hypothetical scenarios.

R#2: The samples obtained in this study are from a 5 hour time period. It would be beneficial for the authors to discuss the fact that this represents only a fraction of time during the lifespan of foraminifera. Given where these samples were taken one would think that geochemical parameters, even in pore waters, would be likely to change on the scale of days to months. The authors themselves discuss the potential mobility of *A. tepida* in the discussion. A better sampling plan over a longer time period may provide insights into how these organisms react to pore water geochemical changes.

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As it is the "snapshot" view of *A. tepida* over a 5hr period cannot provide great insights into this at this stage.

Answer: as stated in a precedent remark, the temporal variation of geochemical parameters due to tidal cycles is assumed to be negligible in the suboxic part of the sediment. The lateral variability of foraminifera is compared to the heterogeneity of geochemical species, which is probably mainly caused by macrofaunal bioturbation. Since these processes (burrows, redox zones and foraminiferal habitat selection) operate at similar time scales, we are convinced that our data are very relevant. However, we fully agree with the reviewer that another sampling scheme is necessary to study the influence of tidal and seasonal changes on foraminiferal assemblages living at the sediment surface. This is beyond the scope of the present study.

R#2: This study can simply state abundances, distributions and geochemistry within these samples. All other observations drawn from these relationships are conjecture and should be treated as such. The 6 "conclusions" presented in the conclusions section were not demonstrated by this study. They are conjecture derived from observations of this study. It may be possible that this methodological approach could provide insights into these possible reactions of *A. tepida*, however, this manuscript does not demonstrate them directly. The conclusion should be adjusted to better convey this. Re-writing the conclusion to focus on the new methodology demonstrated here and its usefulness in oceanographic, modern analogue, paleoceanographic and paleoclimatic studies and/or interpretations would be better served. If retained the conjecture in the existing conclusions should be moved to the discussion. [...]. This discussion could and should be shortened up for greater impact.

Answer : The hypothetical interpretation presented in the conclusion of the initial version of the manuscript was shifted to the discussion. We believe that in order to better appreciate the benefits of the new sampling method, it is useful to interpret our data, and to present a scenario that can subsequently be tested in further studies. However, the discussion now is limited to observations and more robust interpretations. The

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remaining hypotheses have always been clearly indicated as such.

Anonymous Referee #3

R#3: Nevertheless, the discussion is very long and in many parts chaotic going far beyond the data (and the approach) presented in the manuscript. Prior to publication the discussion section need to be rewritten. I suggest that the paper will take more of a methodological approach/theme rather than try to hypothesize about range of possible factors, not included in the current study that may explain foraminiferal distribution in sediment.

Answer: As answered to reviewer 2, the discussion was rewritten; the most speculative parts were removed, and all remaining hypotheses are clearly indicated as such.

R#3: Lines16-17 would you then also conclude that the distribution of *A. tepida* at depth is not linked to worm burrows?

Answer: indeed. We tried to make this clearer in the discussion and conclusions

R#3: Finally authors suggest that they will attempt to confront all mechanism listed on lines 22-26 (p. 10316). I would like to point out that authors have not directly measured presence of labile organic matter in their samples, rather that their interpretations are based on presence of dissolved Fe²⁺ from iron oxide reduction. Of course micro-organism that reduce iron will also be associated with organic matter break down but I would also like to argue that if the sediment indeed would contain very high amounts of organic matter the peak in the iron reduction would not take place at around 8 cm depth in sediment (Fig 7) but much shallower.

Answer : Even with high amounts of organic matter, bioirrigation and biomixing are likely to extend the depth of iron remobilization down to several cm depth as illustrated by our dataset (numerous study have reported such impact of macrofaunal activity, see references in the manuscript). Moreover, in estuarine mudflats, low salinity reduces the importance of sulfato-reduction. In deeper and more saline environments, this process

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is likely to force a much shallower iron peak.

R#3: Authors should keep this in mind when interpreting their results especially as they do not have direct measurements on quality nor quantity of organic matter in their sediment samples with corresponding dissolved iron.

Answer: Indeed, no direct description of organic quality or quantity is available in two dimensions, and the TOC vertical profile indicates little change in quantity and quality at a core scale. However, the rich literature (most relevant papers cited in the manuscript, 4.2.2.) about the influence of fecal pellets or other biogenic particles on iron remobilization allows us to conclude that local decay of a biogenic particles causes a local enrichment in reduced iron in the porewaters .

R#3: p. 10325 lines 1-7 could it be that some burrows contain more or less dissolved Fe²⁺ and P depending on how active these burrows are and how frequently these are flushed? Longer flushing time/inactive burrows would allow dissolved species accumulate I would speculate?

Answer: Indeed different burrows show different concentrations depending of the flushing frequency and on the delay since the last flushing event. However, in the case of an active burrow (i.e. in case of oxygen replenishment) the concentration of dissolved Fe inside the burrow is expected to be always below the concentration of the surrounding sediment.

R#3: Discussion 4.1 heading: I do not think currently this section really focuses on what the heading suggest.

Answer: the first paragraph of the discussion was deleted and the section was slightly changed in order to be more straightforward and focus on the heading of the section.

R#3: p. 10325 lines 10-22 I recommend rewriting the start of the discussion. Now it reads like an abstract and just seems to add unnecessary length to the paper. Done

R#3: p. 10325 lines 23-26 The start of this paragraph should be moved to results. Also

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I suggest that Figure 8 should be first introduced in the results section. Done

R#3: p.10326/27 line29/1-4 I would be careful in suggesting that foraminiferal distribution is not linked to sediment geochemistry. Could it not be the case that dissolved iron and iron redox chemistry is not so relevant for explaining foraminiferal distribution in sediment? We know that some foraminifera denitrify so their distribution may be more closely driven by oxygen and nitrate dynamics. As denitrification is not directly related to iron availability, iron reduction correlation is not seen in this study. Furthermore iron kinetics are generally regarded as “slow processes”, and as reduction of iron takes place from solid phase, where as nitrate is present as a gas in sea water, their dynamics in this setting can be different. I would speculate that in heterogeneous, tidally influenced environment where oxygen may periodically enter burrows, causing nitrification and relatively sudden changes in oxygen supply and demand, and subsequent changes in denitrification, iron oxidation and reduction occur on slightly different time scales.

Answer: There is no overall correlation between dissolved iron and *A. tepida* density but a strongly localized influence of iron patches on *A. tepida* density. The ambiguous sentence in the initial text was rewritten. The discussion on denitrification has been slightly extended in the new version, indicating that all available data suggest that it is highly unlikely that *A. tepida* are capable to denitrify.

R#3: 4.2 (and all subsections 4.2.1, 4.2.2.) This whole section (and the whole discussion) should be more focused on the topic of this manuscript, which is about the new method for studying foraminifera in two dimensions and correlating this with sediment geochemistry. Now the section discusses sediment heterogeneity very broadly, and the tackling issues like influence of long-term macrofaunal bioturbation, which have clearly not been studied here. This paper shows a snap shot in time.

Answer: We agree with the reviewer, and carefully screened and rewrote the text, the focus is more on the main issue now, the new method allowing us to obtain to-

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gether foraminiferal and geochemical data on a cm scale. Consequently, all more general considerations about macrofaunal impact on sediment heterogeneity have been deleted. A more focused paragraph, discussing the influence of macrofaunal activity on foraminifera, has been added.

R#3: A different type of experiment/ study is needed to truly examine macrofaunal impact on sediment heterogeneity, and associated changes in foraminiferal distribution.

Answer: We partly agree with the reviewer. However, methodological approach proposed here gives information about the relationship between foraminiferal distribution and sediment heterogeneity, which will be mainly caused by bioturbation. Therefore, it appears that our method provides valuable indirect information about macrofaunal bioturbation.

R#3: 4.3. As for previous section I think it is important that the authors will focus their study on data that they have and keep the context of the paper in the new method plus foraminiferal distribution. It is ok to speculate on some issues but you cannot make confirm conclusions about controlling ecological parameters if you do not have data to back this up.

Answer: We completely agree with the reviewer, and consequently, focused the paper more on the main topic, deleted the most speculative interpretations, and always clearly distinguished between observations and hypotheses.

R#3: p. 10332 line 5-8 if the low iron at 1-3 cm depth would correspond with frequent oxygen supply by bioirrigation would you not also think that there would be more *A. tepida* then? It would be then more preferable habitat for them; in contrast this is where the numbers are lowest?

Answer: Oxygen supply by burrows will always be an intermittent process, which can probably not sustain permanent living faunas. But as suggested in the discussion (clearly indicated as a hypothesis), in case of active burrows, foraminifera with a low-

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ered metabolism in anoxic sediments could be temporarily re-activated. This could allow them to profit for short periods of time from the organic-rich conditions related to burrows, and eventually, top migrate back to the sediment surface.

R#3: p. 10334 line 6-7 how is the length of the biogenic particles identified by dissolved iron?

Answer: As mentioned in the manuscript (section 4.2.) patches A/7-8, G-H/8-9 and F-G17 in Fig. 5A and 5B are centimeter-wide.

R#3: p.10334 25-27 and p. 10335 lines 1-9 please rewrite this part of conclusions so that it will be consistent with revised, more focussed discussion. Also it is not necessary to point out in which section a particular conclusion is discussed. Done

R#3: Fig 5 Data on *H. germanica* is presented in the figure but not presented in text or discussed in any way. I can see it is less common than *A. tepida* but if it is presented in figure it should also be at least mentioned in the text. Furthermore, its abundance seems to increase with depth?

Answer: Since the species is too scarce for any reliable observations, we decided to delete all figures related to it.

R#3: Fig 6 left hand column with Corg data also has on bottom O2 scale. I would delete the O2 scale as it is given in the O2 pore water profile and as O2 is below detection limit at few mm it is not visible at all on the left hand side diagram. Also why was O2 not also measured in the bottom water. How did the authors evaluate the sediment water interface?

Answer: We modified Fig. 6 following the suggestion of the reviewer. O2 was calibrated using the overlying water supposed to be saturated in O2. The sediment water interface was roughly visually estimated during profiling. During data treatment, the interface was repositioned according to the break visible in the O2 profile after the start of the concentration decrease. The resulting diffusive boundary layer has a length estimated

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between 0.1 and 0.4mm. All this is explained in the supplementary material section S2 now.

R#3: Fig 8 b somewhere in the figure or the caption it should be mentioned that the DET-1D equivalent mean and extrema is derived from 2D plot. This was quite confusing when trying to link the discussion and figure together. Fig 10. As the aim of this study was not to explain the impact of macrofauna on distribution of foraminifera, and authors have no real data on this. I would perhaps delete this figure and at the same time make the discussion more focused on the topic of the new method.

Answer: Figs. 8b and 10 have been removed

R#3: Fig 11. Consider leaving out.

Answer: Fig. 11 shows the conceptual model that we use to summarise the hypotheses we propose to explain the foraminiferal distribution at the end of the discussion. Since this gives the reader a good idea about the insights which can be obtained by our new method, we prefer to keep it.

Please also note the supplement to this comment:

<http://www.biogeosciences-discuss.net/12/C6591/2015/bgd-12-C6591-2015-supplement.pdf>

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	1	2	3	4	5	6	7
2	0.69	-	-	-	-	-	-
3	0.93	0.69	-	-	-	-	-
4	0.69	0.93	0.69	-	-	-	-
5	0.59	0.69	0.59	0.69	-	-	-
6	0.69	0.75	0.59	0.69	0.80	-	-
7	0.71	0.93	0.59	0.96	0.69	0.69	-
8	0.93	0.69	0.69	0.69	0.59	0.59	0.59

Legend: Pairwise t test between the *A.tepida* density from the columns of the sediment slice. For each pair of columns the distribution similarity is tested using a paired t test. P value corrected according to Benjamini & Hochberg (1995) are reported in the matrix.

Fig. 1.

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