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PROCEEDINGS B

Species interactions and environmental context affect intraspecific behavioural trait variation and ecosystem function

Camilla Cassidy, Laura J. Grange, Clement Garcia, Stefan G. Bolam and Jasmin A. Godbold

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Review timeline

Original submission:	16 September 2019
1st revised submission:	19 November 2019
2nd revised submission:	5 January 2020
Final acceptance:	6 January 2020

Note: Reports are unedited and appear as submitted by the referee. The review history appears in chronological order.

Review History

RSPB-2019-2143.R0 (Original submission)

Review form: Reviewer 1

Recommendation
Accept with minor revision (please list in comments)

Scientific importance: Is the manuscript an original and important contribution to its field?
Good

General interest: Is the paper of sufficient general interest?
Good

Quality of the paper: Is the overall quality of the paper suitable?
Good

Is the length of the paper justified?
Yes

Should the paper be seen by a specialist statistical reviewer?
No

Do you have any concerns about statistical analyses in this paper? If so, please specify them explicitly in your report.
No

It is a condition of publication that authors make their supporting data, code and materials available - either as supplementary material or hosted in an external repository. Please rate, if applicable, the supporting data on the following criteria.

Is it accessible?
Yes

Is it clear?
Yes

Is it adequate?
Yes

Do you have any ethical concerns with this paper?
No

Comments to the Author
Review – Species interactions and environmental context affect intraspecific behavioural trait variation and ecosystem function Cassidy et al., PROC B.
The authors present the results of a well-designed mesocosm experiment, which explores how environmental and ecological context affect intraspecific trait expression and variation and subsequently alter community mediated ecosystem functioning. The study reveals that (behavioural and morphological) trait expression and variation vary with environmental and ecological context. They ultimately conclude that traits and ecosystem process are determined by current and historic conditions, and as such typologies, which assume constant trait expression and ignore context dependencies, could undermine predictive approaches of ecosystem functioning and services.

There has been a recent shift towards functional trait-based approaches to capture and help predict how ecosystems respond to environmental change; however there are some shortfalls to the use of functional traits. As such, I was pleased to see the subject being covered by the authors and felt that it represents a topic of reasonable interest for those working in the ecological fields of global environmental change, and believe it is appropriate for this journal. The research ideas are not completely novel, studies quantifying intraspecific-variation in functional traits have emerged over the last decade or so as their importance is recognised but this study explores how biotic and abiotic variables can underpin this variation, and the consequences of such variation for ecosystem processes.

In general, the work is scientifically sound and the conclusions of the paper are mostly supported by the data.
They use a factorial experimental design to test what at first glance seems like quite simple concepts, but incorporate numerous variables within their models to isolate mechanisms driving trait variability and go further to consider how variability contributes to elements of ecosystem functioning. The manuscript is generally well written but could be improved slightly by clarification of some of the statistical methods, and projection and discussion of the results into a wider framework, remarking their global relevance.

Comments

Introduction
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Lines 232 - 238, 257 - 261 – Did models test for interactions or just main effects? I believe it is the former but this is not specified.

Lines 248 – 250 – I appreciate the use of multivariate approaches when using multiple functional traits, however I do question its use for just two traits. Especially considering results are presented and discussed separately for each trait. This approach makes more sense if the traits are uncorrelated. If the behaviours are uncorrelated then this result could be included as justification.

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Fig 328-329 – I'm slightly confused why the authors mention the complex interactions between trait variation x population, and density x species treatment, but then do not describe them, and instead go on to make broad statements about main effects. Why test for interactions in the first place?

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It would be useful to have more specific explanation of how variability could be integrated with current functional approaches or what needs to be done for successful implementation.

Henn, J. J., Buzzard, V., Enquist, B. J., Halbritter, A. H., Klanderud, K., Maitner, B. S., … Vandvik, V. (2018). Intraspecific Trait Variation and Phenotypic Plasticity Mediate Alpine Plant Species Response to Climate Change . Frontiers in Plant Science . Retrieved from https://www.frontiersin.org/article/10.3389/fpls.2018.01548
Mauffrey, A. R. L., Cappelatti, L., & Griffin, J. N. (2019). Traditional functional groups capture limited variation in the trait space of macroalgae. BioRxiv, 803965. https://doi.org/10.1101/803965

Review form: Reviewer 2

Recommendation
Reject – article is not of sufficient interest (we will consider a transfer to another journal)

Scientific importance: Is the manuscript an original and important contribution to its field?
Acceptable

General interest: Is the paper of sufficient general interest?
Marginal

Quality of the paper: Is the overall quality of the paper suitable?
Poor

Is the length of the paper justified?
Yes

Should the paper be seen by a specialist statistical reviewer?
No

Do you have any concerns about statistical analyses in this paper? If so, please specify them explicitly in your report.
Yes

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Is it accessible?
Yes

Is it clear?
Yes

Is it adequate?
Yes

Do you have any ethical concerns with this paper?
No

Comments to the Author
The manuscript by Cassidy et al. aims to investigate how changes in the species identity, faunal density and differences in the environmental context of species affects their expression of biological traits associated with bioturbation and ecosystem functioning. This paper has the potential to provide an interesting and timely insight into the role of intraspecific interactions and historical environmental context into the functioning of marine benthic ecosystems. However, in its current form the manuscript is almost impenetrable to the reader. The authors need to drastically rewrite this manuscript, based around a clear hypothesis and significantly reduce their use of Biodiversity-Ecosystem Functioning jargon. As the paper currently stands, I am afraid I have to recommend its rejection from Proceeding of the Royal Society B. I do, however, think the experimental work carried out was quite elegant and I believe that a revised version would be suitable for publication in another journal.

Key issues of concern within the paper are as follows:
1. No clear definitions of what a functional trait is, and how changes in the suite of functional

traits expressed by a community affects the way an ecosystem functions.
2. The assumption that the reader will understand the concept of an ecosystem function / ecosystem functioning.
3. No clearly articulated hypothesis under test
4. No justification for why PERMANOVA was used to analyse differences between species identities and contexts. Why was a distance-based multivariate test used here. I think it may have been clearer to use univariate statistical models to test for these effects.
5. The use of very long sentences with multiple arguments. This made the discussion very difficult and confusing to read.

I also feel that the term ecosystem functioning would probably be better replaced with nutrient fluxes within the manuscript. In effect the response to changes in biological traits under test is the flux in ammonia, nitrate and phosphate. I think it is more appropriate to discuss these as geochemical responses to changes in bioturbation and outline that this is a proxy for functioning of an ecosystem. I have not provided a list of specific changes because I believe the authors need to rewrite the manuscript from scratch, with the exception of the figures which are clear and elegantly summarise the results. I am sorry that I cannot be more positive.

Decision letter (RSPB-2019-2143.R0)

30-Oct-2019

Dear Ms Cassidy:

Your manuscript has now been peer reviewed and the reviews have been assessed by an Associate Editor. As you will see, the reviewers and the Associate Editor have raised some concerns with your manuscript and we would like to invite you to revise your manuscript to address them. In particular, Reviewer 2 notes, and I completely agree, that as the manuscript is written it is difficult for non-experts to follow. This is a shame, as I think your work has a potentially large impact beyond your immediate area. When you are revising, I encourage you to consider how to either re-phrase or include definitions or parenthetical explanations to make your paper accessible to a broader range of readers. Of course, please be sure to address each of the reviewers' comments carefully. The reviewers' comments (not including confidential comments to the Editor) and the comments from the Associate Editor are included at the end of this email.

We do not allow multiple rounds of revision so we urge you to make every effort to fully address all of the comments at this stage. If deemed necessary by the Associate Editor, your manuscript will be sent back to one or more of the original reviewers for assessment. If the original reviewers are not available we may invite new reviewers. Please note that we cannot guarantee eventual acceptance of your manuscript at this stage.

To submit your revision please log into http://mc.manuscriptcentral.com/prsb and enter your Author Centre, where you will find your manuscript title listed under "Manuscripts with Decisions." Under "Actions", click on "Create a Revision". Your manuscript number has been appended to denote a revision.

When submitting your revision please upload a file under "Response to Referees" - in the "File Upload" section. This should document, point by point, how you have responded to the reviewers' and Editors' comments, and the adjustments you have made to the manuscript. We require a copy of the manuscript with revisions made since the previous version marked as 'tracked changes' to be included in the 'response to referees' document.

Your main manuscript should be submitted as a text file (doc, txt, rtf or tex), not a PDF. Your figures should be submitted as separate files and not included within the main manuscript file.

When revising your manuscript you should also ensure that it adheres to our editorial policies (https://royalsociety.org/journals/ethics-policies/). You should pay particular attention to the following:

Research ethics:
If your study contains research on humans please ensure that you detail in the methods section whether you obtained ethical approval from your local research ethics committee and gained informed consent to participate from each of the participants.

Use of animals and field studies:
If your study uses animals please include details in the methods section of any approval and licences given to carry out the study and include full details of how animal welfare standards were ensured. Field studies should be conducted in accordance with local legislation; please include details of the appropriate permission and licences that you obtained to carry out the field work.

Data accessibility and data citation:
It is a condition of publication that you make available the data and research materials supporting the results in the article. Datasets should be deposited in an appropriate publicly available repository and details of the associated accession number, link or DOI to the datasets must be included in the Data Accessibility section of the article (https://royalsociety.org/journals/ethics-policies/data-sharing-mining/). Reference(s) to datasets should also be included in the reference list of the article with DOIs (where available).

In order to ensure effective and robust dissemination and appropriate credit to authors the dataset(s) used should also be fully cited and listed in the references.

If you wish to submit your data to Dryad (http://datadryad.org/) and have not already done so you can submit your data via this link
http://datadryad.org/submit?journalID=RSPB&manu=(Document not available), which will take you to your unique entry in the Dryad repository.

If you have already submitted your data to dryad you can make any necessary revisions to your dataset by following the above link.

For more information please see our open data policy http://royalsocietypublishing.org/data-sharing.

Electronic supplementary material:
All supplementary materials accompanying an accepted article will be treated as in their final form. They will be published alongside the paper on the journal website and posted on the online figshare repository. Files on figshare will be made available approximately one week before the accompanying article so that the supplementary material can be attributed a unique DOI. Please try to submit all supplementary material as a single file.

Online supplementary material will also carry the title and description provided during submission, so please ensure these are accurate and informative. Note that the Royal Society will not edit or typeset supplementary material and it will be hosted as provided. Please ensure that the supplementary material includes the paper details (authors, title, journal name, article DOI). Your article DOI will be 10.1098/rspb.[paper ID in form xxxx.xxxx e.g. 10.1098/rspb.2016.0049].

Please submit a copy of your revised paper within three weeks. If we do not hear from you

within this time your manuscript will be rejected. If you are unable to meet this deadline please let us know as soon as possible, as we may be able to grant a short extension.

Thank you for submitting your manuscript to Proceedings B; we look forward to receiving your revision. If you have any questions at all, please do not hesitate to get in touch.

Best wishes,
Dr Sarah Brosnan
Editor, Proceedings B
mailto: proceedingsb@royalsociety.org

Associate Editor
Board Member: 1
Comments to Author:
In line with the comments of R2, the authors should include a few more definitions of terms that may be less transparent to those not working directly in the biodiversity-ecosystem function area as suggested by this reviewer. However, they cannot use language that is not consistent with this research area, so while they could tone down the jargon, it cannot be entirely changed or removed. The reviewer also felt that the work was missing hypotheses and I suggest that the authors consider formulating their work in such a context. R1 was far more positive but has some critical points that will need to be addressed in a revision. They recommend some clarification of Methods as well as a greater projection of the work into a wider framework with respect to their global relevance, including an intra vs. inter-specific variation context. Both reviewers request justification of the statistical methods used (PERMANOVA in particular). The authors also need to be careful of their discussion of main effects when interaction is significant as pointed out by R2. Please address all comments completely in a revision.

Reviewer(s)' Comments to Author:
Referee: 1

Comments to the Author(s)
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Referee: 2

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Author's Response to Decision Letter for (RSPB-2019-2143.R0)

See Appendix A.

RSPB-2019-2143.R1 (Revision)

Review form: Reviewer 1

Recommendation
Accept as is

Scientific importance: Is the manuscript an original and important contribution to its field?
Good

General interest: Is the paper of sufficient general interest?
Good

Quality of the paper: Is the overall quality of the paper suitable?
Good

Is the length of the paper justified?
Yes

Should the paper be seen by a specialist statistical reviewer?
No

Do you have any concerns about statistical analyses in this paper? If so, please specify them explicitly in your report.
No

It is a condition of publication that authors make their supporting data, code and materials available - either as supplementary material or hosted in an external repository. Please rate, if applicable, the supporting data on the following criteria.

Is it accessible?
Yes

Is it clear?
Yes

Is it adequate?
Yes

Do you have any ethical concerns with this paper?
No

Comments to the Author
I agree with Reviewer 2 that the paper was lacking somewhat in background knowledge on functional traits and ecosystem functioning. However, The authors have improved this by expanding on certain sections in the introduction, and have clarified arguments in the discussion to make it easier to follow. Overall I feel the manuscript has been improved by revisions in response to comments from myself and reviewer 2, such that it is suitable for publication within this journal.

Review form: Reviewer 2

Recommendation
Accept with minor revision (please list in comments)

Scientific importance: Is the manuscript an original and important contribution to its field?
Good

General interest: Is the paper of sufficient general interest?
Excellent

Quality of the paper: Is the overall quality of the paper suitable?
Acceptable

Is the length of the paper justified?
Yes

Should the paper be seen by a specialist statistical reviewer?
Yes

Do you have any concerns about statistical analyses in this paper? If so, please specify them explicitly in your report.
Yes

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Is it accessible?
Yes

Is it clear?
Yes

Is it adequate?
Yes

Do you have any ethical concerns with this paper?
No

Comments to the Author
The revised manuscript by Cassidy et al. is a significant improvement over the first draft, and I now feel better able to understand and comment on the underlying science. The key findings are interesting and clearly within the scope of the Proceedings of the Royals Society B. I appreciate the authors attempt to clarify their reasoning behing the use of permutational ANOVA as a data analysis tool. I remain, however, unconvinced that distance-based multivariate approach is the best way to test for differences in this case. Perhaps though, if the authors could provide some visualisation of the multivariate analysis, using ordination plots it would allow me and other interested researchers to see how the suite of traits and response variables change visually. Within the vegan package in R, the constrained analysis of principle components function provides a good analogue for nPERMANOVA, which allows ordination plots to be generated. I also would refer the authors back to Anderson's various papers on nPERMANOVA as a method and asked whether the assumptions of homogenous multivariate dispersion of the data have been satisfied?

With regard to the experimental design, I believe the authors need to provide some justification for the complexity. Their analysis involves a four-way analysis of variance, but each treatment is only replicated between 4 and 6 times. Given the level of replication, I am unconvinced that the study has the statistical power to adequately detect a potentially large number of important treatment effects. The authors need to consider this, and provide some justification for their design, in light of the relatively limited replication.

Finally, I would ask the authors to spend some more time proof-reading their manuscript and try to reduce the lengths of some of their sentences, and the use of adverbs like 'therefore', 'however', 'further' etc.

Overall, I believe the paper is worthy of publication and makes an important contribution to our understanding of how biological traits control ecosystem functioning. I therefore recommend its publication following further minor revisions.

Specific Corrections.

Page 2 Line 30-31: 'We use PERMANOVA to quantify…' please revise to 'We use PERMANOVA to test…' You are referring to an inferential statistical test, to quantify you would be reporting the effect size, not simply if there is a difference.

Page 3 Line 53: Replace 'However,' with 'The'.

Page 3 Line 63: Delete 'A majority of'.

Page 3 Line 37: Rearrange 'Therefore, for management purposes, …' to 'For Management purposes, therefore, …' This reads more elegantly.

Page 4 Line 77: Replace 'and' with 'with'.

Page 4 Line 92 – Page 5 Line 97: This sentence is too long. It needs to be broken up into a couple of shorter sentences.

Page 4 Line 100: 'Further, trait expression shifts dramatically…' Please can you revise this to 'Trait expression, furthermore, dramatically shifts…'.

Page 4 Lines 103 – 106. I it possible to break this sentence up, it is long and contains a number of separate clauses.

Page 4 Line 109: Delete 'Indeed'.

Page 5 Line 116: Replace 'we seek to highlight the' with 'we investigate' or some other less verbose derivative.

Page 6 Line 122: Replace 'hypothesised' with 'hypothesise' and be consistent with tenses.

Page 6 Line 136: Delete 'As such'.

Page 6 Line 140: Delete 'for example'.

Page 7 Line 143: 'significantly higher in Total Organic Carbon' please cite the p-value and test used to identify the significance.

Page 7 Line 157-157 and throughout: 'Replicate faunal assemblages (hereafter referred to as 'communities')' I realise this is pedantic but these are assemblages and not communities. So why not refer to them as 'assemblages'? This would be more appropriate for the experiment.

Page 8 Line 172: Typo 'replicates' should be 'replicated'.

Page 9 Line 213: Replace 'while' with 'whilst.'

Page 10 Line 224: Delete 'in part.'

Page 12 Lines 272-281: A four-way ANOVA puts in place lot of possible interactions. I am concerned that with 6 replicates of each treatment (and in some cases 5 or 4 replicates) I am not convinced that you have the statistical power to adequately test these multiple interaction terms.

Page 13 Line 290: Delete 'Further'.

Page 13 Line 297-299: I don't fully understand the rationale for analysing the aspects of

movement behaviour in a multivariate framework. I think there needs to be some justification for why this has been done in the methods. The use of PERMANOVA needs to be explained in the paper.

Page 15 Line 342: Should read 'Ecosystem Functioning'

Page 16 Line 361-364: This first sentence of the discussion is somewhat generic. It should clearly state the key finding of the paper. Also, if you are describing your findings, why do you cite some other papers?

Page 17 Line 392: Please stop using 'Further' to start a sentence. In most cases this is superfluous and unnecessary. Like 'therefore', 'however', 'therefore' and 'thus' it is not required in the majority of cases where it is used.

Page 17 Line 395-396: Please revise this sentence for clarity.

Page 18 Line 411: Replace 'This demonstrable' with 'The'. There is no need to add additional verbosity to every sentence.

Page 18 Line 425 – Page 19 Line 429: Long sentence, needs revised. Also, how would you recommend intraspecific variation is measured. This is not clear, and providing a clear outline of how it would be achieved would turn this into a useful intervention. At present this is rather generic statement.

Page 19 Line 432-433: '… likely less essential…' inelegant phrasing, please revise.

Page 19 Line 434: Delete 'Nonetheless'.

Page 19 Line 439: Delete 'especially'.

Page 19 Line 442: Delete 'seek to' and 'under'.

Page 19 Line 443-446: 'Failure to do so…' Revise this sentence for clarity.

Page 19 Line 449: Delete 'such'.

Page 20 Line 457-465: The conclusions are rather generic sounding, and repetitive of the final paragraph of the discussion. What is the key take home message from your study, and what are its implications for how we understand the functioning of ecosystems? Try to express this clearly and directly.

Decision letter (RSPB-2019-2143.R1)

17-Dec-2019

Dear Ms Cassidy

Thank you for a very nice revision of your manuscript. I am pleased to inform you that your Review manuscript RSPB-2019-2143.R1 entitled "Species interactions and environmental context affect intraspecific behavioural trait variation and ecosystem function" has been accepted for publication in Proceedings B pending some minor revisions. I agree with the AE and reviewer 2 that a visualization of your data would be a really good addition to help readers interpret your

results. In addition, please see the other suggestions made by this reviewer to help improve the clarify and flow of your manuscript.

To upload your manuscript, log into http://mc.manuscriptcentral.com/prsb and enter your Author Centre, where you will find your manuscript title listed under "Manuscripts with Decisions." Under "Actions," click on "Create a Revision." Your manuscript number has been appended to denote a revision.

You will be unable to make your revisions on the originally submitted version of the manuscript. Instead, upload a new version through your Author Centre.

Before uploading your revised files please make sure that you have:

1) A text file of the manuscript (doc, txt, rtf or tex), including the references, tables (including captions) and figure captions. Please remove any tracked changes from the text before submission. PDF files are not an accepted format for the "Main Document".

2) A separate electronic file of each figure (tiff, EPS or print-quality PDF preferred). The format should be produced directly from original creation package, or original software format. Please note that PowerPoint files are not accepted.

3) Electronic supplementary material: this should be contained in a separate file from the main text and the file name should contain the author's name and journal name, e.g authorname_procb_ESM_figures.pdf
All supplementary materials accompanying an accepted article will be treated as in their final form. They will be published alongside the paper on the journal website and posted on the online figshare repository. Files on figshare will be made available approximately one week before the accompanying article so that the supplementary material can be attributed a unique DOI. Please see: https://royalsociety.org/journals/authors/author-guidelines/

4) Data-Sharing and data citation
It is a condition of publication that data supporting your paper are made available. Data should be made available either in the electronic supplementary material or through an appropriate repository. Details of how to access data should be included in your paper. Please see https://royalsociety.org/journals/ethics-policies/data-sharing-mining/ for more details.

If you wish to submit your data to Dryad (http://datadryad.org/) and have not already done so you can submit your data via this link
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If you have already submitted your data to dryad you can make any necessary revisions to your dataset by following the above link.

5) For more information on our Licence to Publish, Open Access, Cover images and Media summaries, please visit https://royalsociety.org/journals/authors/author-guidelines/.

Once again, thank you for submitting your manuscript to Proceedings B and I look forward to receiving your final version. If you have any questions at all, please do not hesitate to get in touch.

Sincerely,
Dr Sarah Brosnan
Editor, Proceedings B
mailto:proceedingsb@royalsociety.org

Associate Editor Board Member: 1
Comments to Author:
I am happy to now accept this manuscript with minor revisions. R2 has continued to raise concerns with the statistical approach and I agree with their suggestion of including the multivariate plots from the PERMANOVA. Otherwise, there are just a few minor tweaks that need to be dealt with, including revision of particularly long sentences.

Reviewer(s)' Comments to Author:

Referee: 1

Comments to the Author(s)
I agree with Reviewer 2 that the paper was lacking somewhat in background knowledge on functional traits and ecosystem functioning. However, The authors have improved this by expanding on certain sections in the introduction, and have clarified arguments in the discussion to make it easier to follow. Overall I feel the manuscript has been improved by revisions in response to comments from myself and reviewer 2, such that it is suitable for publication within this journal.

Referee: 2

Comments to the Author(s)
The revised manuscript by Cassidy et al. is a significant improvement over the first draft, and I now feel better able to understand and comment on the underlying science. The key findings are interesting and clearly within the scope of the Proceedings of the Royals Society B. I appreciate the authors attempt to clarify their reasoning behing the use of permutational ANOVA as a data analysis tool. I remain, however, unconvinced that distance-based multivariate approach is the best way to test for differences in this case. Perhaps though, if the authors could provide some visualisation of the multivariate analysis, using ordination plots it would allow me and other interested researchers to see how the suite of traits and response variables change visually. Within the vegan package in R, the constrained analysis of principle components function provides a good analogue for nPERMANOVA, which allows ordination plots to be generated. I also would refer the authors back to Anderson's various papers on nPERMANOVA as a method and asked whether the assumptions of homogenous multivariate dispersion of the data have been satisfied?

With regard to the experimental design, I believe the authors need to provide some justification for the complexity. Their analysis involves a four-way analysis of variance, but each treatment is only replicated between 4 and 6 times. Given the level of replication, I am unconvinced that the study has the statistical power to adequately detect a potentially large number of important treatment effects. The authors need to consider this, and provide some justification for their design, in light of the relatively limited replication.

Finally, I would ask the authors to spend some more time proof-reading their manuscript and try to reduce the lengths of some of their sentences, and the use of adverbs like 'therefore', 'however', 'further' etc.

Overall, I believe the paper is worthy of publication and makes an important contribution to our understanding of how biological traits control ecosystem functioning. I therefore recommend its publication following further minor revisions.

Specific Corrections.

Page 2 Line 30-31: 'We use PERMANOVA to quantify…' please revise to 'We use PERMANOVA to test…' You are referring to an inferential statistical test, to quantify you would be reporting the effect size, not simply if there is a difference.

Page 3 Line 53: Replace 'However,' with 'The'.

Page 3 Line 63: Delete 'A majority of'.

Page 3 Line 37: Rearrange 'Therefore, for management purposes, …' to 'For Management purposes, therefore, …' This reads more elegantly.

Page 4 Line 77: Replace 'and' with 'with'.

Page 4 Line 92 – Page 5 Line 97: This sentence is too long. It needs to be broken up into a couple of shorter sentences.

Page 4 Line 100: 'Further, trait expression shifts dramatically…' Please can you revise this to 'Trait expression, furthermore, dramatically shifts…'.

Page 4 Lines 103 – 106. I it possible to break this sentence up, it is long and contains a number of separate clauses.

Page 4 Line 109: Delete 'Indeed'.

Page 5 Line 116: Replace 'we seek to highlight the' with 'we investigate' or some other less verbose derivative.

Page 6 Line 122: Replace 'hypothesised' with 'hypothesise' and be consistent with tenses.

Page 6 Line 136: Delete 'As such'.

Page 6 Line 140: Delete 'for example'.

Page 7 Line 143: 'significantly higher in Total Organic Carbon' please cite the p-value and test used to identify the significance.

Page 7 Line 157-157 and throughout: 'Replicate faunal assemblages (hereafter referred to as 'communities')' I realise this is pedantic but these are assemblages and not communities. So why not refer to them as 'assemblages'? This would be more appropriate for the experiment.

Page 8 Line 172: Typo 'replicates' should be 'replicated'.

Page 9 Line 213: Replace 'while' with 'whilst.'

Page 10 Line 224: Delete 'in part.'

Page 12 Lines 272-281: A four-way ANOVA puts in place lot of possible interactions. I am concerned that with 6 replicates of each treatment (and in some cases 5 or 4 replicates) I am not convinced that you have the statistical power to adequately test these multiple interaction terms.

Page 13 Line 290: Delete 'Further'.

Page 13 Line 297-299: I don't fully understand the rationale for analysing the aspects of movement behaviour in a multivariate framework. I think there needs to be some justification for

why this has been done in the methods. The use of PERMANOVA needs to be explained in the paper.

Page 15 Line 342: Should read 'Ecosystem Functioning'

Page 16 Line 361-364: This first sentence of the discussion is somewhat generic. It should clearly state the key finding of the paper. Also, if you are describing your findings, why do you cite some other papers?

Page 17 Line 392: Please stop using 'Further' to start a sentence. In most cases this is superfluous and unnecessary. Like 'therefore', 'however', 'therefore' and 'thus' it is not required in the majority of cases where it is used.

Page 17 Line 395-396: Please revise this sentence for clarity.

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Page 19 Line 449: Delete 'such'.

Page 20 Line 457-465: The conclusions are rather generic sounding, and repetitive of the final paragraph of the discussion. What is the key take home message from your study, and what are its implications for how we understand the functioning of ecosystems? Try to express this clearly and directly.

Author's Response to Decision Letter for (RSPB-2019-2143.R1)

See Appendix B.

Decision letter (RSPB-2019-2143.R2)

06-Jan-2020

Dear Ms Cassidy

I am pleased to inform you that your manuscript entitled "Species interactions and environmental

context affect intraspecific behavioural trait variation and ecosystem function" has been accepted for publication in Proceedings B.

You can expect to receive a proof of your article from our Production office in due course, please check your spam filter if you do not receive it. PLEASE NOTE: you will be given the exact page length of your paper which may be different from the estimation from Editorial and you may be asked to reduce your paper if it goes over the 10 page limit.

If you are likely to be away from e-mail contact please let us know. Due to rapid publication and an extremely tight schedule, if comments are not received, we may publish the paper as it stands.

If you have any queries regarding the production of your final article or the publication date please contact procb_proofs@royalsociety.org

Your article has been estimated as being 10 pages long. Our Production Office will be able to confirm the exact length at proof stage.

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Electronic supplementary material:
All supplementary materials accompanying an accepted article will be treated as in their final form. They will be published alongside the paper on the journal website and posted on the online figshare repository. Files on figshare will be made available approximately one week before the accompanying article so that the supplementary material can be attributed a unique DOI.

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Thank you for your fine contribution. On behalf of the Editors of the Proceedings B, we look forward to your continued contributions to the Journal.

Sincerely,
Proceedings B
mailto: proceedingsb@royalsociety.org

Appendix A

We provide below a breakdown of how all reviewers comments have been addressed in the revised manuscript. The comments provided in review are **bolded**, our response directly below is in plain text, and the specific changes made are provided and inset. Line numbers and quoted text from the previously submitted manuscript are provided under '*Previously*', while the changed text and updated line numbers from the manuscript with tracked changes are provided under '*Revised*'. The manuscript with all tracked changes is provided with the file name 'Cassidyetal_Manuscript_TrackedChanges.docx'.

We believe that the changes made strengthen the substantial scope and timely nature of our findings that was recognised by both reviewers. We greatly appreciate the opportunity to resubmit this manuscript for further consideration based on the strength of these changes, and thank the reviewers and editors alike for their time and input.

We look forward to hearing from you in due course.

Yours sincerely,

Camilla Cassidy

Reviewer 1

Introduction

1) Lines 82 – 89, this section contains lots of information but is slightly vague, I'm aware that submissions to this journal should be concise but I feel this section could be expanded slightly giving an example or two.

The first two sentences of this section (Previously lines 80-85, Revised lines 103-106) have been condensed, while the latter sentence (Previously lines 85-89, Revised lines 106-112) has been expanded for clarity and to include more, relevant information.

> *Previously (Lines 80-89)*: Such physiological differences, including associated differences in the mean and variance of body size, may influence an individual's contribution to ecosystem functioning (Norkko et al., 2013). Morphological traits can be used to determine both species responses to the environment, and their ecological relationships and roles, as metabolic theories provide a mechanistic basis for the scaling relationships between body size and ecosystem properties (Fritschie & Olden, 2016). However, intraspecific variation occurs beyond demographic influences, and is primarily driven by two processes: genetic adaptation to historic conditions across generations, creating distinct genetic ecotypes, and acclimation across shorter temporal scales in response to

prevailing biotic and abiotic conditions (Mitchell & Bakker, 2014a; Wohlgemuth et al., 2017).

Revised (Lines 103-112): Such physiological differences, including associated morphological differences in the mean and variance of body size, determine the scale of an individual's contribution to ecosystem functioning (Norkko et al., 2013; Fritschie & Olden, 2016). However, intraspecific variation occurs beyond demographic influences (Mitchell & Bakker, 2014a). Some site-specific differences originate as a genetic component, stemming from long-term adaptation to historic conditions that creates distinct genetic ecotypes through multi-generational selection processes (Calosi et al., 2013; Robins et al., 2013). In addition, variation also arises over shorter temporal scales in the form of acclimation responses to prevailing biotic and abiotic conditions (Wohlgemuth et al., 2017).

Methods

2) Line 138 – How do you know that the species do not differ genetically – is this an assumption, based on distance, or is there some reference to a study?

As Reviewer 1 inferred, this is an assumption based on distance. Invertebrate taxa which broadcast spawn and/or possess pelagic larval stages, such as our study species, have substantial distribution potential (Robins et al., 2013) and lack genetic differentiation even over large (>20 km) distances (Alp et al., 2012). This justification has now been made clearer, and supporting citations have been included.

Previously (Lines 136-140): Given the separation (~ 12 km) and presence of hydrogeographical barriers (Gage, 1972; Friedrich et al., 2014) between them, these sites contain individuals of both species that, though not distinct with respect to genetics, are assumed to have experienced differing ecological and environmental histories throughout their post-larval lifetimes.

Revised (Lines 192-199): Taxa with pelagic larvae, such as these species, have substantial distribution potential and are exchanged across landscape-scale distances and hydrographical barriers only in these early ontogenetic stages (Robins et al., 2013; Ershova et al., 2019). As such, given the proximate distance (~12 km) and presence of substantial changes in seabed terrain and flow conditions between sites (Gage, 1972; Friedrich et al., 2014), we infer that individuals from each site are likely not genetically distinct but will have been exposed throughout their post-larval lifetimes to differing ecological and environmental conditions (Alp et al., 2012).

Alp, M., Keller, I., Westram, A. M., & Robinson, C. T. (2012). How river structure and biological traits influence gene flow: a population genetic study of two stream invertebrates with differing dispersal abilities. *Freshwater Biology*, *57*, 969-981. doi: 10.1111/j.1365-2427.2012.02758.x
Robins, P. E., Neill, S. P., Giménez, L., Jenkins, S. R., & Malham, S. K. (2013). Physical and biological controls on larval dispersal and connectivity in a highly energetic shelf sea. *Limnology and Oceanography*, *58*(2), 505-524. doi: 10.4319/lo.2013.58.2.0505

3) Lines 156-162 – The experimental design could be clarified slightly, stating clearly the number of factor levels for each variable (it currently doesn't state that there are 2 sites), giving the total number of treatment combinations. Then the reader can easily visualise the experimental design.

The experimental design has been clarified as suggested with the number of factor levels specified. This revised paragraph now matches the full explanation of treatment levels also provided in the '2.5 Statistical Methods' section (Revised lines 325-342).

> *Previously (Lines 156-162)*: Our experiment required 102 aquaria arranged in a full factorial design. Replicate faunal assemblages (hereafter referred to as 'communities') from each sampling site (hereafter referred to as 'population', which represent historic exposure to discrete abiotic conditions) contained *A. filiformis* and *A. chiajei* in one of three species treatments (monoculture of *A. filiformis*, monoculture of *A. chiajei*, or both species in mixture), across a range of three naturally observed densities (low, medium and high, between 250 - 1000 ind. m^{-2}, Supporting information, Table S1 & S2).
>
> *Revised (Lines 234-241):* Our experiment required 102 aquaria arranged in a full factorial design (Supporting information, Table S2 & S3). Replicate faunal assemblages (hereafter referred to as 'communities') from each sampling site (2 levels; Loch Etive and Loch Linnhe, which represent historic exposures to discrete abiotic conditions hereafter referred to as 'populations') contained *A. filiformis* and *A. chiajei* in one of three species treatments (3 levels; monoculture of *A. filiformis*, monoculture of *A. chiajei*, or both species in mixture), across three naturally observed densities (3 levels; low, medium and high, between 250 - 1000 ind. m^{-2}, Supporting information, Table S3).

4) I found Table S2 slightly confusing, the reader is drawn to interpret a table row by row (Like table S3), but here the rows are not aligned they are simply separating the treatments into a list. I think each column could have just two rows, one for the treatment name (stating n = 2/3) and one for the list of treatments.

These changes have been made to Table S2 as suggested, which is now a 3 x 2 table which presents the number of treatment levels in the first row, and the conditions of these treatments in

the second. Some information as to the specific composition of individuals in the treatments has been removed to improve the ease of interpreting the table, as this information is included in Table S3. Lines referring to Tables S2 and S3 (Revised lines 235 and 241) signpost to Supporting information accordingly.

5) Line 175 – References to morphological and behavioural traits should be consistent – the section is named behavioural trait expression but refers to both behavioural and morphological, I recommend deleting the word behavioural. And be specific when referring to individual trait expression throughout the manuscript.

As suggested, section headings have been renamed from 'Individual behavioural trait expression' to the broader and more applicable 'Individual trait expression' in Methods (Revised lines 258 and 346) and Results (Revised line 412), and a use of the phrase 'individual trait expression' which specifically refers to behavioural traits has been clarified.

> *Previously (Line 366)*: Density and species identity influence individual trait expression…
>
> *Revised (Line 588):* Further, density and species identity influence intraspecific behavioural trait expression…

6) Lines 209 – 211 – Just to clarify; dyed sediment was imaged 8 days after its introduction? So was it introduced or imaged on day 23? Please make this clear.

Sediment was introduced 8 days prior to the end of the experiment (Day 23) and imaged on its final day (Day 31); this has been reworded.

> *Previously (Lines 209-211)*: To visualize particle movement, 24 g dry weight aquaria^{-1} of dyed sediment that fluoresces in UV light (green colour; < 125µm; Brianclegg Ltd., UK) was imaged 8 days after introduction (day 23).
>
> *Revised (Lines 297-300):* To visualize particle movement 24 g dry weight aquaria^{-1} of dyed sediment that fluoresces in UV light (green colour; < 125 µm; Brianclegg Ltd., UK) was introduced to the sediment surface on Day 23 and imaged 8 days later (Day 31).

7) Lines 232 - 238, 257 - 261 – Did models test for interactions or just main effects? I believe it is the former but this is not specified.

Interactions were tested for during both PERMANOVA and ANOVA; this has now been specified throughout the Methods.

Previously (Line 232): Permutational multivariate analysis of variance (PERMANOVA) and ANOVA were used to determine the effect of…

Revised (Line 325): Permutational multivariate analysis of variance (PERMANOVA) and ANOVA were used to determine the independent and interacting effects…

Previously (Line 257): PERMANOVA models were developed, to test; i) the effect of…

Revised (Line 361): PERMANOVA models were developed to test the independent and interacting effects of; i)…

8) Lines 248 – 250 – I appreciate the use of multivariate approaches when using multiple functional traits, however I do question its use for just two traits. Especially considering results are presented and discussed separately for each trait. This approach makes more sense if the traits are uncorrelated. If the behaviours are uncorrelated then this result could be included as justification.

The multivariate approach PERMANOVA was selected for several reasons. As stated in text (Revised lines 350-352) PERMANOVA itself has been recommended as a technique highly appropriate for detection of intraspecific trait variation (Mitchell & Bakker, 2014), as it is highly robust to heterogeneity of variance, non-normality, and differences in correlation structure (Anderson & Walsh, 2013).

More broadly, however, we believe a multivariate approach is the most appropriate analytical direction for our purposes. The two traits represent differing aspects of intraspecific variability, with one being a response trait (time to begin movement, being those traits that determine how an organism responds to environmental condition) and the other an effect trait (time to complete burial, being those functional traits that determine how an organism affects ecosystem properties) (Degen et al., 2018). By having used multivariate analyses in line with that of other trait-based approaches focussing on only small numbers of traits (e.g. Pansch et al., 2014; Bregman et al., 2016; Deveautour et al., 2017), we integrate both traits and so trait-types together to better represent changes in the overall 'personality' or behavioural syndrome of the organism (Moran et al., 2017). This allows differences in whole-organism behaviour to be more readily linked to differences in community behaviour across variable environmental conditions. Cumulative change in an individual's functional role cannot be understood using univariate techniques. This justification has now been included.

Revised (Lines 347-349): Multivariate analyses were used to represent overall differences in the behavioural 'personalities' of individuals between species identities and contexts

(Moran et al., 2017), integrating response (time to begin movement) and effect (time to complete burial) traits.

Further, given how highly dependent the relationship between traits is on the treatment conditions, we do not believe these variables can be considered closely correlated across the scope of the experiment. As suggested by Reviewer 1, the two behavioural traits display differing correlation patterns between (**a**) interspecific differences, (**b**) intraspecific differences with species mixture, (**c**) population, and (**d**) density). There was no significant correlation for *A. filiformis* at a species level (t = 0.5779, df = 93, p-value = 0.5647) (**a**), and no correlation intraspecifically for *A. filiformis* in monoculture (t = 0.17523, df = 60, p-value = 0.8615), *A. filiformis* in mixture (t = 1.406, df = 31, p-value = 0.1697), and *A. chiajei* in monoculture (t = 1.3917, df = 64, p-value = 0.1688) (**b**).

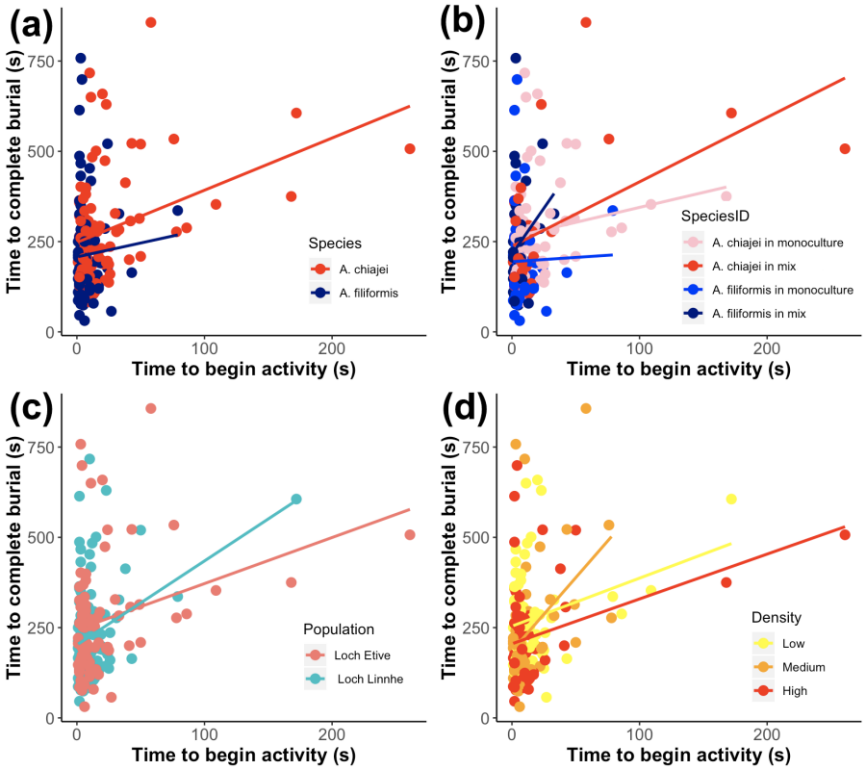

Figure of the time to begin activity and the time to complete burial (s) for all individuals in the experiment, coloured depending on their (**a**) species, (**b**) species identity, (**c**) population of origin, or (**d**) community density. Lines show linear regressions, highlighting the context-dependency of the strength of correlation between the two variables.

Anderson, M. J., & Walsh, D. C. (2013). PERMANOVA, ANOSIM, and the Mantel test in the face of heterogeneous dispersions: what null hypothesis are you testing?. *Ecological Monographs*, *83*(4), 557-574. doi: 10.1890/12-2010.1

Bregman, T. P., Lees, A. C., MacGregor, H. E., Darski, B., de Moura, N. G., Aleixo, A., ... & Tobias, J. A. (2016). Using avian functional traits to assess the impact of land-cover change on ecosystem processes linked to resilience in tropical forests. *Proceedings of the Royal Society B: Biological Sciences*, *283*(1844), 20161289. doi: 10.1098/rspb.2016.1289

Degen, R., Aune, M., Bluhm, B. A., Cassidy, C., Kędra, M., Kraan, C., ... & Bremner, J. (2018). Trait-based approaches in rapidly changing ecosystems: A roadmap to the future polar oceans. *Ecological Indicators*, *91*, 722-736. doi: 10.1016/j.ecolind.2018.04.050

Deveautour, C., Donn, S., Power, S. A., Bennett, A. E., & Powell, J. R. (2018). Experimentally altered rainfall regimes and host root traits affect grassland arbuscular mycorrhizal fungal communities. *Molecular Ecology*, *27*(8), 2152-2163. doi: 10.1111/mec.14536

Mitchell, R. M. & Bakker, J. D. (2014). Quantifying and comparing intraspecific functional trait variability: a case study with *Hypochaeris radicata*. *Functional Ecology, 28*, 258-269. doi: 10.1111/1365-2435.12167

Moran, N.P., Mossop, K.D., Thompson, R.M., Chapple, D.G. & Wong, B.B. (2017). Rapid divergence of animal personality and syndrome structure across an arid-aquatic habitat matrix. *Oecologia*, *185*, pp.55-67.

Pansch, C., Schaub, I., Havenhand, J., & Wahl, M. (2014). Habitat traits and food availability determine the response of marine invertebrates to ocean acidification. *Global Change Biology*, *20*(3), 765-777. doi: 10.1111/gcb.12478

9) Lines 250 - 252 – For PERMANOVA I believe homogeneity of dispersions should be checked using the PERMDISP function (Anderson, 2006).

As suggested, results of PERMDISP have now been included. PERMDISP was used to test the homogeneity of dispersions prior to PERMANOVA; there was no significant difference in the dispersions between groups for both behavioural traits between populations (F1,190 = 0.57, P = 0.45), species identities (F1,188 = 1.20, P = 0.31) and densities (F1,189 = 1.22, P = 0.30). These results suggest that all significant differences detected by PERMANOVA were caused by a shift in the extent of trait variation in terms of magnitude (i.e. a context-dependent effect on trait value), and not by variation around the mean within groups (i.e. a context-dependent effect on trait variation) (Assis et al., 2013).

> *Revised (Lines 352-357):* Permutational analysis of multivariate dispersion (PERMDISP) was used to test for homogeneity of variance between populations ($F_{1,190}$ = 0.57, P = 0.45), species identities ($F_{1,188}$ = 1.20, P = 0.31) and densities ($F_{1,189}$ = 1.22, P = 0.30). These results support that any significant differences in PERMANOVA between treatments are due to changes in the values of trait expression, not shifts in the overall extent of variation itself.

Assis, J., Claro, B., Ramos, A., Boavida, J., & Serrão, E. A. (2013). Performing fish counts with a wide-angle camera, a promising approach reducing divers' limitations. *Journal of Experimental Marine Biology and Ecology,* 445, 93-98. doi: 10.1016/j.jembe.2013.04.007

10a) Lines 257-261, 269-275 – I am slightly confused why the authors construct models separately with a) 3 independent variables and b) 2 or 3 independent variables + morphological trait expression (PERMANOVA) or variation (ANOVA)?

With regards to ANOVA for factors affecting community-level net behaviours, we have now removed the 3-term model and present only the 4-term model in both the Methods (Revised Lines 400-405) and Results (476-520).

> *Previously (Lines 269-275)*: 3-way ANOVA models were developed to investigate the independent and interactive effects of context (species treatment, density, population) on each community-level behaviour (Δ [Br^-], $^{f\text{-}SPI}L_{max}$, SBR) and nutrient concentration ([NH_4-N], [NO_3-N], [NO_2-N], [PO_4-P]). For each community-level behaviour (Δ [Br^-], $^{f\text{-}SPI}L_{max}$, SBR), ANOVA models were also developed to determine the effect of all context factors (species treatment, density, population), and of intraspecific variation in morphological trait expression (CV of mean arm length), on net community-level effect behaviour.

> *Revised (Lines 400-405):* 4-way ANOVA was used to investigate the independent and interactive effects of context (population, species treatment, density) and intraspecific variation in morphological trait expression (CV of mean arm length) on each community-level behaviour (Δ [Br^-], $^{f\text{-}SPI}L_{max}$, SBR), and a 3-way ANOVA to test the independent and interactive effects of context (population, species treatment, density) on nutrient concentration ([NH_4-N], [NO_3-N], [NO_2-N], [PO_4-P]).

> *Previously (Lines 313-336)*: Community-level bioturbation and bioirrigation behaviours were differentially affected by abiotic and biotic context (species mixture treatment, density, population). The maximum depth of particle redistribution, $^{f\text{-}SPI}L_{max}$, was significantly affected by the independent effects of density (ANOVA: $F_{2,90} = 6.14$, $P < 0.01$) and population (ANOVA: $F_{1,90} = 9.11$, $P < 0.01$) (Fig. 2a). $^{f\text{-}SPI}L_{max}$ increased with density, while remaining shallower in mesocosms with individuals from Loch Etive in comparison to Loch Linnhe (coefficient ± SE = 0.40 ± 0.51, t = 0.78, $P = 0.44$) (Fig. 2b). SBR was significantly influenced by the interactive effect of density x species treatment (ANOVA: $F_{4,78} = 2.68$, $P = 0.038$) (Fig. 2c). The magnitude of differences in SBR between species treatments were increased at greater densities.

In addition, SBR was the only community behaviour affected by the trait variation of component individuals (CV mean arm length) in addition to biotic and abiotic context (species mixture treatment, density, population). SBR was significantly affected by the interactive effects of morphological trait variation x population of origin (ANOVA: $F_{1,74}$ = 4.81, P = 0.031) (Fig. 2d), in addition to the interactive effect of density x species treatment (ANOVA: $F_{4,74}$ = 3.16, P = 0.018) (Fig. 2c). SBR was generally greater in those communities of greater densities, and those originating in Loch Linnhe. Though the extent of variation for average arm length did not differ significantly between densities or populations (ANOVA: $F_{1,94}$ = 0.02, P = 0.88, variation in the morphology of individuals appears elevated for individuals originating from Loch Linnhe or maintained under medium density (Supporting information, Fig. S8). Bioirrigation activity ($\Delta[Br^-]$) did not vary with abiotic or biotic context as results showed that, although the density x population interaction was included in the minimal adequate mode, its effects were non-significant (ANOVA: $F_{2,89}$ = 2.24, P = 0.11, Supporting information, Fig. S9).

Revised (Lines 476-520): Community-level bioturbation and bioirrigation behaviours were differentially affected by abiotic and biotic context (species mixture treatment, density, population) and morphological trait variation. The maximum depth of particle redistribution, $^{f\text{-}SPI}L_{max}$, was significantly affected by the independent effects of density (ANOVA: $F_{2,60}$ = 5.85, P < 0.01) and population (ANOVA: $F_{1,60}$ = 8.68, P < 0.01). $^{f\text{-}SPI}L_{max}$ increased with density (Fig. 2a), while remaining shallower in mesocosms with individuals from Loch Etive in comparison to Loch Linnhe (coefficient ± SE = 0.40 ± 0.51, t = 0.78, P = 0.44) (Fig. 2b). SBR differed significantly with the interactive effects of density x species treatment (ANOVA: $F_{4,74}$ = 3.16, P = 0.018), and population of origin x morphological trait variation (ANOVA: $F_{1,74}$ = 4.81, P = 0.031). The magnitude of differences in SBR between species treatments were increased at greater densities (Fig. 2c), with higher surface boundary roughness found in Loch Linnhe communities with greater morphological trait variation (CV mean arm length) (Fig. 2d). Though the extent of variation for average arm length did not differ significantly between densities (ANOVA: $F_{2,78}$ = 1.76, P = 0.18), species treatments (ANOVA: $F_{2,78}$ = 0.61, P = 0.55) or populations (ANOVA: $F_{1,78}$ = 0.02, P = 0.88), variation in the morphology of individuals was elevated for individuals originating from Loch Linnhe or maintained under medium density (Supporting information, Fig. S8). Bioirrigation activity ($\Delta[Br^-]$) did not vary with abiotic or biotic context as results showed that, although the density x population interaction was included in the minimal adequate mode, its effects were non-significant (ANOVA: $F_{2,90}$ = 1.11, P = 0.34, Supporting information, Fig. S9).

With regards to PERMANOVA for individual-level trait expression, we constructed two separate three-term models rather than one single four-term model to allow clearer testing of two aspects of the intraspecific response. Under the first (three terms; population x species identity x density)

we test community-level effects on intraspecific behavioural trait expression. Under the second (three terms; population x species identity x individual morphology) we instead focus the test on the individual-level differences induced by individuals' variable morphological trait expression, which itself may interact with population or species. Significant morphological differences between populations (ANOVA: $F_{1,184} = 4.57$, $P = 0.034$) and species (ANOVA: $F_{1,184} = 14.99$, $P < 0.001$) suggest that morphological trait expression and its implications in affecting behaviour may be site-specific or interspecific. By contrast, density was unrelated to morphology as it was a factor created during experimental manipulation. As such, it was not included in the latter model. The explanation of this approach has been made clearer in text.

> *Previously (Line 257-261)*: PERMANOVA models were developed, to test; i) the effect of all context factors (species identity, density, population), and ii) the effect of context factors which represent permanent aspects of individuals' identity (population, species identity), and of each individual's morphological trait expression (mean arm length), on multivariate intraspecific behavioural trait expression.

> *Revised (Line 361-392):* PERMANOVA models were developed test the independent and interacting effects of; i) community-level effects (population, species identity, density), and ii) individual-level differences in morphological trait expression (mean arm length) between communities (population, species identity), on multivariate intraspecific behavioural trait expression. Data exploration showed there were differences in morphological trait expression between populations (ANOVA: $F_{1,184} = 4.57$, $P = 0.034$) and species (ANOVA: $F_{1,184} = 14.99$, $P < 0.001$) which may contribute to observed site-specific and interspecific effects.

10b) Would backward selection from the full model not result in the best model regardless, and you could check whether including the trait factor significantly improves the model or not?

As Reviewer 1 inferred, backward selection was indeed used to obtain the minimum adequate model for ANOVA, and this has now been made clearer in text.

> *Previously (Lines 241-243)*: … for all ANOVA models, the minimal adequate effects structure was determined using backward selection informed by Akaike Information Criteria (AIC) (Godbold et al., 2017).

> *Revised (Lines 400-409):* 4-way ANOVA was used to test the independent and interactive effects of context (population, species treatment, density) and intraspecific variation in morphological trait expression (CV of mean arm length) on each community-level

behaviour (Δ [Br^-], $^{f\text{-}SPI}L_{max}$, SBR), and a 3-way ANOVA was used to test the independent and interactive effects of context (population, species treatment, density) on nutrient concentration ([NH_4-N], [NO_3-N], [NO_2-N], [PO_4-P]). Model assumptions were assessed visually for normality (Q-Q plot), heterogeneity of variance (plotted residuals vs. fitted values), and the presence of outliers or overly influential data points (Cook's Distance) and the minimal adequate effects structure was determined using backward selection informed by Akaike Information Criteria (AIC) (Zuur et al., 2009).

11) Considering morphological trait expression differed significantly between species and populations I do worry about it's inclusion within models as an independent variable – as in fact there is covariance with the other factors.

As noted in Comment 10a, morphological trait expression is included in a second PERMANOVA model to address this concern; we seek to demonstrate that there is a significant interaction between morphological trait values and population which appears to influence behavioural trait expression, and the potential role of morphology in contributing to population or interspecific differences *per se* cannot be teased apart. We have amended the Discussion to make these aspects clearer.

Revised (Lines 554-577): We found site-specific and interspecific differences in morphological trait expression. By consequence, it is difficult to interrogate the role of population or species *per se* in determining behavioural trait expression. Body size determines the scaling relationship between the traits expressed by a species and their ecosystem role, and larger individuals are often liable to have stronger effects on ecosystem functioning (Larsen et al., 2005). Given this relationship, intraspecific morphological variability has already been incorporated into some functional trait approaches via a community average (Solan et al., 2004). Body size traits are a complex and potentially transient response to genetic influences, age, food and other resources (Liao et al., 2016). Even where two organisms are allegedly found within the same functional group, larger individuals are expected to have proportionally larger effects to ecosystem functioning (e.g. displace more sediment and pump more water (Norkko et al., 2013), and intraspecific morphological expression may be a significant influence on the functional roles of species. However, even beyond the contributions of morphological differences, individuals with shared local histories are likely to consistently express similar traits…

12) Also Figure S6, it looks like there is an interaction but I assume this must have been non-significant?

This interaction is indeed non-significant; this result has been added to the figure legend, for clarity.

Previously: Analysis showed a significant difference in average arm length between species (ANOVA: $F_{1,188} = 14.996$, $P < 0.001$) and populations (ANOVA: $F_{1,188} = 4.033$, $P = 0.046$).

Revised: Analysis showed a significant difference in average arm length between species (ANOVA: $F_{1,188} = 14.99$, $P < 0.001$) and populations (ANOVA: $F_{1,188} = 4.03$, $P = 0.046$), but no significant interaction (ANOVA: $F_{1,184} = 2.79$, $P = 0.096$).

Results

13) I like that movement behaviours were visualised separately to highlight differences between treatments, thus avoiding post-hoc tests. But I do wonder if some of the results should be interpreted with caution.

Lines 296 – 298 - The statement that *A. chiajei* individuals in a mixture take longer to begin movement and complete burial than those in monoculture seems a bit exaggerated considering the overlap of error bars (Figs 1a,b).

Please see below (Comment 14).

14) Lines 298 – 300 - Same comment as above applies to the statement that time taken to begin movement increases with density (Fig 1c).

Both the sentence above (Comment 13) and this sentence have been reworded. An additional sentence (underlined below) has been added to preface these interpretations and note that the strength of directional effects differs between behavioural traits.

Previously (Lines 293-300): Overall, *A. chiajei* took significantly longer before beginning or completing burial than *A. filiformis*, however intraspecific differences are present in both species and between individuals maintained in monoculture or in a mixed community. For *A. chiajei* the time taken to begin movement, and for *A. chiajei* and *A. filiformis* the time taken to fully complete burial, was increased in mixed species treatments in comparison to the monocultures (Fig. 1a – 1b). For both species, the time taken to begin moving increased with density, whilst the time taken to complete burial decreased with density (Fig. 1c – 1d).

Revised (444-453): Overall, *A. chiajei* took significantly longer before beginning or completing burial than *A. filiformis*, however intraspecific differences are present in both species and between individuals maintained in monoculture or in a mixed community. The extent of these context-dependent differences varied depending on the trait, and patterns between treatment conditions were consistently less prominent for the time taken to begin movement. For both *A. chiajei* and *A. filiformis,* the time taken to fully complete

burial was increased in mixed species treatments in comparison to monoculture, with a similar if weaker pattern suggested for *A. chiajei and* the time taken to begin movement (Fig. 1a – 1b). For both species, the time taken to complete burial decreased with density (Fig. 1c – 1d).

15) Lines 304-310 – I assume this should state 'Did not differ significantly between variables or their interactions'.

This sentence has been changed in line with this suggestion, as it is more conclusive than stating at length the conditions which were non-significant.

Previously (Lines 304-307): The coefficient of variation of both behavioural traits (time to begin activity and time to complete burial) within communities did not differ significantly between densities, species identities, or populations…

Revised (Lines 468-470): The coefficient of variation of both behavioural traits (time to begin activity and time to complete burial) within communities did not differ significantly between variables or their interactions…

16) Lines 314 – 319 - Be consistent with reference to figures, Fig 2a is referenced directly after the test statistics that relate to Fig 2b, rather than its own.

The reference to Fig. 2a has been moved a sentence later to ensure consistency with all other figure references in the text; the figure panels are referenced only when describing any significant patterns which are visible in the figure, not immediately following the sentence in which the test statistic is given.

Previously (Lines 314-319): The maximum depth of particle redistribution, $^{f\text{-}SPI}L_{max}$, was significantly affected by the independent effects of density (ANOVA: $F_{2,90} = 6.14$, $P < 0.01$) and population (ANOVA: $F_{1,90} = 9.11$, $P < 0.01$) (Fig. 2a). $^{f\text{-}SPI}L_{max}$ increased with density, while remaining shallower in mesocosms with individuals from Loch Etive in comparison to Loch Linnhe (coefficient ± SE = 0.40 ± 0.51, t = 0.78, $P = 0.44$) (Fig. 2b).

Revised (Lines 478-482): The maximum depth of particle redistribution, $^{f\text{-}SPI}L_{max}$, was significantly affected by the independent effects of density (ANOVA: $F_{2,60} = 5.85$, $P < 0.01$) and population (ANOVA: $F_{1,60} = 8.68$, $P < 0.01$). $^{f\text{-}SPI}L_{max}$ increased with density (Fig. 2a), while remaining shallower in mesocosms with individuals from Loch Etive in comparison to Loch Linnhe (coefficient ± SE = 0.40 ± 0.51, t = 0.78, $P = 0.44$) (Fig. 2b).

17) Additionally there is an error in the figure 2 legend – the legend doesn't correspond to the correct panels and 5 panels are referred to despite there being only 4.

The figure legend for Fig. 2 has been updated from that supplied previously - which was submitted in error, and described a previous version of the figure which referred to the use of standard deviation rather than the coefficient of variation (CV) as the representation of trait variance - and now specifies the four panels correctly.

> *Previously*: **Fig. 2:** The effects of biotic and abiotic context on (**a**) and (**b**) ${}^{f\text{-}SPI}L_{max}$ (mean ± SE, n= 6) (cm) and (**c**), (**d**) and (**e**) surface boundary roughness (mean ± SE, n= 6) (cm) in mesocosms containing *A. filiformis* and *A. chiajei* in monoculture or mixture, showing the (**a**) independent effect of density and (**b**) the independent effect of population, and (**c**) the interactive effect of standard deviation of mean arm length and density, (**d**) the interactive effect of standard deviation of mean arm length and population and (**e**) the interactive effect of density x species treatment.

> *Revised:* **Fig. 2:** The effects of biotic and abiotic context on (**a**) and (**b**) ${}^{f\text{-}SPI}L_{max}$ (mean ± SE, n= 6) (cm) and (**c**) and (**d**) surface boundary roughness (mean ± SE, n= 6) (cm) in mesocosms containing *A. filiformis* and *A. chiajei* in monoculture or mixture, showing the (**a**) independent effect of density and (**b**) the independent effect of population, (**c**) the interactive effect of density x species treatment, and (**d**) the interactive effect of morphological trait variation (CV of mean arm length) and population.

18) Also Fig 2c and 2d are not referred to chronologically in the text

This paragraph has been clarified, in line with the removal of the three-term model (Comment 10).

> *Previously (Lines 323-328)*: In addition, SBR was the only community behaviour affected by the trait variation of component individuals (CV mean arm length) in addition to biotic and abiotic context (species mixture treatment, density, population). SBR was significantly affected by the interactive effects of morphological trait variation x population of origin (ANOVA: $F_{1,74} = 4.81$, $P = 0.031$) (Fig. 2d), in addition to the interactive effect of density x species treatment (ANOVA: $F_{4,74} = 3.16$, $P = 0.018$) (Fig. 2c).

> *Revised (Lines 482-487):* SBR differed significantly with the interactive effects of density x species treatment (ANOVA: $F_{4,74} = 3.16$, $P = 0.018$), and population of origin x morphological trait variation (ANOVA: $F_{1,74} = 4.81$, $P = 0.031$). The magnitude of differences in SBR between species treatments were increased at greater densities (Fig.

2c), with higher surface boundary roughness found in Loch Linnhe communities with greater morphological trait variation (CV mean arm length) (Fig. 2d).

19) Lines 328-329 – I'm slightly confused why the authors mention the complex interactions between trait variation x population, and density x species treatment, but then do not describe them, and instead go on to make broad statements about main effects. Why test for interactions in the first place?

This sentence has been replaced to describe both the species treatment x density interaction and the trait variation x population interaction.

Previously (Lines 328-329): SBR was generally greater in those communities of greater densities, and those originating in Loch Linnhe.

Revised (Lines 484-487): The magnitude of differences in SBR between species treatments were increased at greater densities (Fig. 2c), with higher surface boundary roughness found in Loch Linnhe communities with greater morphological trait variation (CV mean arm length) (Fig. 2d).

Discussion

20) The manuscript would be improved by some discussion of intra- vs interspecific variation – This can vary considerably between traits (Henn et al., 2018) and for some systems/groups of species it has been shown that interspecific variation always outweighs intra (Mauffrey et al – preprint). I'm aware that due to the limited number of species its not possible to compare inter- and intra- in this study, however if intraspecific variation is negligible compared to inter- then perhaps there is still some merit for traditional approaches using mean values. Intraspecific variation was not equal across the traits measured in this study, might high variation be more common for some types of traits than others? Differences between measured traits could also be mentioned.

We have incorporated these aspects into the Discussion section as suggested (Revised lines 712-769). We first discuss the consequences of intraspecific differences between treatments on ecosystem functioning, and then move on to the relative importance of these consequences given the comparative magnitude of intra- vs interspecific variation. The consideration of intra- and interspecific variation also influences the contexts in which we suggest the incorporation of intraspecific variation into trait-based study be viewed as a valuable priority (Revised lines 717-761). Intrinsically, however, both intraspecific and interspecific variation integrate to underpin responses to ecological change (Revised lines 766-769).

21) Only a small number of traits are measured (albeit they include behavioural and morphological), I suggest the authors expand on how results might transfer to other traits and the significance of that for ecosystem functioning, e.g. are some traits more prone to variability, thus mean values would severely misrepresent trait expression, and are there some traits that might be more consistent, thus accounting for variation would have little impact on overall assessment of some element of ecosystem functioning?

We have incorporated this aspect as suggested (Revised lines 714-717). Where we, as above (Comment 20), note that the relative importance of intraspecific differences is variable, we also highlight that this relationship will be affected by the relative extent of variation in a trait, and the strength of its relationship with the ecosystem function of interest. Some traits are liable to be more effective functional effect descriptors than others, as they may have a stronger causal or 'mechanistic' relationship with the biotic control of functioning (Hale et al., 2014).

Hale, R., Mavrogordato, M. N., Tolhurst, T. J., & Solan, M. (2014). Characterizations of how species mediate ecosystem properties require more comprehensive functional effect descriptors. *Scientific reports*, *4*, 6463. doi: 10.1038/sdata.2015.52

22) It would be useful to have more specific explanation of how variability could be integrated with current functional approaches or what needs to be done for successful implementation.

As suggested, we have noted the specific aspects raised during this study that should specifically be integrated into current functional trait approaches (Revised lines 783-851). We have also included evaluation of those circumstances under which this incorporation of intraspecific variation is likely to return the most valuable information (Revised lines 717-761).

Reviewer 2

23) The manuscript by Cassidy et al. aims to investigate how changes in the species identity, faunal density and differences in the environmental context of species affects their expression of biological traits associated with bioturbation and ecosystem functioning. This paper has the potential to provide an interesting and timely insight into the role of intraspecific interactions and historical environmental context into the functioning of marine benthic ecosystems. However, in its current form the manuscript is almost impenetrable to the reader. The authors need to drastically rewrite this manuscript, based around a clear hypothesis and significantly reduce their use of Biodiversity-Ecosystem Functioning jargon. As the paper currently stands, I am afraid I have to recommend its rejection from Proceeding of the Royal Society B. I do, however, think the experimental work carried out was quite

elegant and I believe that a revised version would be suitable for publication in another journal.

We find the stance and comments raised by Reviewer 2 to be highly instructive, and appreciate their suggestions that help to make our experimental findings to be more broadly understandable and applicable.

A majority of Reviewer 2's reservations centre on the extensive use of functional trait and ecosystem functioning language, and related terminology. As addressed below, we have introduced examples of these concepts to make their implications to a broader range of biological contexts more apparent. The usage of these phrases has also been reduced throughout the Discussion where appropriate, such that the inherent meaning of our findings can be emphasised in plain terms. We believe that these efforts alleviate the reservations raised by Reviewer 2, and allows our manuscript to be even more accessible and the consequences of our findings more readily inferred.

Key issues of concern within the paper are as follows:

24) 1. No clear definitions of what a functional trait is, and how changes in the suite of functional traits expressed by a community affects the way an ecosystem functions.

Please see below (Comment 25).

25) 2. The assumption that the reader will understand the concept of an ecosystem function / ecosystem functioning.

At their first use, we provide specific examples of functional traits and ecosystem functions. We do so to define their nature as, respectively, the characteristics of organisms dictating effects and responses, and the cumulative processes sustaining ecosystems. By providing examples in this manner, we highlight the broad potential implications of our findings to a range of other systems, functions and traits. As this vocabulary has become more widely referenced across biological and ecological fields, fewer authors explicitly define terms such as 'functional trait', 'ecosystem functioning' and 'ecosystem service' (e.g. Wohlgemuth et al., 2017; Siqueuira et al., 2019; Start, 2018; Thompson et al., 2018), and our usage is in line with this convention. We have made an effort not simply to supply a definition, but rather we seek to use the terminology of functional traits as a way of encapsulating broad ecological phenomena and so succinctly represent concepts with potentially widespread relevance.

> *Previously (Lines 50-60)*: After two decades of empirical study, motivated by unprecedented species loss and environmental change, there is now unequivocal evidence that altering biodiversity affects ecosystem functioning and, ultimately, the provision of ecosystem services (Adair et al., 2018). However, current research emphasizes that the biological traits expressed within a community, rather than the number of species *per se*, mediate ecosystem functioning (Gagic et al., 2015; Read et al., 2017). As a result, functional trait-based approaches are increasingly adopted as predictive tools by

ecosystem managers (Rijnsdorp et al., 2015; Bolam et al., 2017), as they allow incorporation of species performance into projections of environmental change and provide a mechanistic understanding of the biotic control over ecosystem functioning or service delivery (Laughlin, 2014; Funk et al., 2017; Thomsen et al., 2019).

Revised (Lines 50-61): Decades of empirical study, motivated by unprecedented species loss and environmental change, have provided unequivocal evidence that altering biodiversity affects ecosystem functioning (e.g. primary production, nutrient cycling, sediment stability) and, ultimately, the provision of ecosystem services (Adair et al., 2018). However, current research emphasizes that rather than the number of species, ecosystem functioning is instead mediated by the functional traits (e.g. behavioural, morphological or life history characteristics) expressed within a community (Gagic et al., 2015; Read et al., 2017). As a result, functional trait-based approaches are increasingly adopted as predictive tools by ecosystem managers (Rijnsdorp et al., 2015; Bolam et al., 2017) as they incorporate species performance into projections of environmental change. In doing so, they confer understanding of the biological mechanisms underpinning faunal mediation of ecosystem functioning (Laughlin, 2014; Funk et al., 2017; Thomsen et al., 2019).

Thompson, P. L., Isbell, F., Loreau, M., O'Connor, M. I., & Gonzalez, A. (2018). The strength of the biodiversity–ecosystem function relationship depends on spatial scale. *Proceedings of the Royal Society B: Biological Sciences*, *285*(1880), 20180038. doi: 10.1098/rspb.2018.0038
Siqueira, A. C., Bellwood, D. R., & Cowman, P. F. (2019). The evolution of traits and functions in herbivorous coral reef fishes through space and time. *Proceedings of the Royal Society B: Biological Sciences*, *286*(1897), 20182672. doi: 10.1098/rspb.2018.2672
Start, D. (2018). Predator macroevolution drives trophic cascades and ecosystem functioning. *Proceedings of the Royal Society B: Biological Sciences*, *285*(1883), 20180384. doi: 10.1098/rspb.2018.0384
Wohlgemuth, D., Solan, M., & Godbold, J. A. (2017). Species contributions to ecosystem process and function can be population dependent and modified by biotic and abiotic setting. *Proceedings of the Royal Society B: Biological Sciences*, *284*(1855), 20162805. doi: 10.1098/rspb.2016.2805

26) 3. No clearly articulated hypothesis under test

Our aims and objectives have been explicitly rephrased as hypotheses.

Previously (Lines 119-126): We seek to highlight the importance of incorporating intraspecific and individual-level trait variation into trait-based study, illustrating that faunally-mediated community processes and ecosystem functions with which these traits

are associated will be subject to context-dependent change. Here, we interrogate the role of biotic context and differing abiotic history in influencing intraspecific trait expression, using two co-occurring species of infaunal marine invertebrate (brittlestars *Amphiura filiformis* and *A. chiajei*). We show the role of biotic and site-specific environmental context in influencing individual- and community-level behaviour, and by extension ecosystem functioning.

Revised (Lines 156-184): In this study, we seek to highlight the importance of incorporating intraspecific and individual-level trait variation into trait-based study, illustrating that faunally-mediated community processes and ecosystem functioning with which these traits are associated are subject to context-dependent change. To achieve these aims, we interrogated the effect of biotic context and differing abiotic history on communities of two co-occurring species of infaunal marine invertebrate (brittlestars *Amphiura filiformis* and *A. chiajei*). We hypothesised that i) biotic and site-specific environmental context influence the expression of individual traits and community-level behaviour, and that ii) this variability would aid in understanding concurrent differences in biogeochemical proxies (nutrient concentration) for ecosystem function.

27) 4. No justification for why PERMANOVA was used to analyse differences between species identities and contexts. Why was a distance-based multivariate test used here. I think it may have been clearer to use univariate statistical models to test for these effects.
> Please see above, in response to Reviewer 1 (Comment 8, Page 3).

28) 5. The use of very long sentences with multiple arguments. This made the discussion very difficult and confusing to read.
> Sentences with multiple clauses have been reworked throughout the Discussion to improve readability.

Previously (Line 98-101): Further, trait expression shifts dramatically in response to biotic influences, primarily from direct interactions with neighbouring individuals and/or species (Hawlena et al., 2011; Wohlgemuth et al., 2017; Calder-Potts et al., 2018; Thomsen et al., 2019) which may be competitive…

Revised (Line 140-143): Further, trait expression shifts dramatically in response to biotic influences, primarily from neighbouring individuals and/or species (Hawlena et al., 2011; Wohlgemuth et al., 2017; Calder-Potts et al., 2018; Thomsen et al., 2019). These interactions may be competitive…

Previously (Line 359-360): … and that the expression of traits by individuals, and the net behaviour of their communities and ecosystems, vary with context.

Revised (Line 548-549): … as the expression of traits by individuals and so the net behaviour of their communities varies with context.

Previously (Line 361-362): We find these differences in response to both prevailing biotic and historic abiotic conditions, which result in complex…

Revised (Line 549-550): Differences in response to both prevailing biotic and historic abiotic conditions result in complex…

Previously (Lines 336-370): Density and species identity influence individual trait expression as community composition dictates the neighbour-effects that drive behaviour including space and resource use (De Backer et al., 2011; Kraft et al., 2015; Calder-Potts et al., 2018), with implications for understanding the role of shifting biodiversity as a driver for altered ecosystem functioning (Cadotte et al., 2011; Thomsen et al., 2019).

Revised (Lines 588-592): Further, density and species identity influence intraspecific behavioural trait expression as community composition determines the neighbour-effects that dictate behaviours including space and resource use (De Backer et al., 2011; Kraft et al., 2015; Calder-Potts et al., 2018). These effects carry implications for understanding the role of shifting biodiversity as a driver for altered ecosystem functioning (Thomsen et al., 2019).

Previously (Lines 405-409): Indeed, patterns of sediment reworking relate to measured dissolved nutrient concentrations in a consistent manner, coherent with prevailing understanding as to how the construction of sediment architecture represents the burrowing and irrigation behaviours exerting biogenic influence on the function of benthic habitats (Kristensen et al., 2014; Wohlgemuth et al., 2017).

Revised (Lines 708-712): Differences in sediment reworking between treatments mechanistically underpin the differences in dissolved nutrient release observed between the same conditions, demonstrating that change in behavioural trait expression influences biogeochemical processes and so mediates the functioning of benthic habitats (Kristensen et al., 2014; Wohlgemuth et al., 2017).

Previously (Lines 427-431): Given especially that natural systems are increasingly subject to drivers of environmental and ecological change, we highlight the need to determine the contexts in which intraspecific variability arises (Moran et al., 2017; Matesanz & Ramírez-Valiente, 2019) and, within this framework, to isolate under which circumstances it contributes to the functional integrity of ecosystems (Wright et al., 2016; Zuo et al., 2017).

Revised (Lines 771-775): Given especially that natural systems are increasingly subject to drivers of ecological change, we highlight the need to determine the contexts in which intraspecific variability arises (Moran et al., 2017; Matesanz & Ramírez-Valiente, 2019). Within this framework, we must also seek to isolate under which circumstances it contributes to the functional integrity of ecosystems (Wright et al., 2016; Zuo et al., 2017).

29) I also feel that the term ecosystem functioning would probably be better replaced with nutrient fluxes within the manuscript. In effect the response to changes in biological traits under test is the flux in ammonia, nitrate and phosphate. I think it is more appropriate to discuss these as geochemical responses to changes in bioturbation and outline that this is a proxy for functioning of an ecosystem.

In studies using benthic models of traits and functioning, nutrient concentration is a widely accepted proxy that provides a comparative 'snapshot' of biogeochemical processes contributory to ecosystem functioning such as sediment nutrient cycling (e.g. Ieno et al., 2006; Wohlgemuth et al., 2017). This usage has now been made clear.

Previously (Line 125-126): … influencing individual- and community-level behaviour, and by extension ecosystem functioning.

Revised (Lines 182-184): … this variability would aid in understanding concurrent differences in biogeochemical proxies (nutrient concentration) for ecosystem function.

Further, where quantified effects to nutrient flux as a proxy for ecosystem functioning are discussed with explicit reference to our results, the implied strength of this apparent relationship is softened appropriately.

Previously (Lines 358): … in the way in which species mediate important aspects of ecosystem functioning…

Revised (Line 546): … on the way in which species mediate the processes contributory to ecosystem functioning…

Previously (Lines 407-409): … coherent with prevailing understanding as to how the construction of sediment architecture represents the burrowing and irrigation behaviours exerting biogenic influence on the function of benthic habitats (Kristensen et al., 2014; Wohlgemuth et al., 2017).

Revised (Lines 710-712): … demonstrating that change in behavioural trait expression influences biogeochemical processes and so mediates the functioning of benthic habitats (Kristensen et al., 2014; Wohlgemuth et al., 2017).

In line with convention and to ensure readability, quantified changes in nutrient concentration in response to bioturbation are still termed 'Ecosystem functioning' in section headings during the Methods (Revised line 315 and 399) and Results (Revised line 522).

Ieno, E. N., Solan, M., Batty, P., & Pierce, G. J. (2006). How biodiversity affects ecosystem functioning: roles of infaunal species richness, identity and density in the marine benthos. *Marine Ecology Progress Series*, *311*, 263-271. doi: 10.3354/meps311263

Wohlgemuth, D., Solan, M., & Godbold, J. A. (2017). Species contributions to ecosystem process and function can be population dependent and modified by biotic and abiotic setting. *Proceedings of the Royal Society B: Biological Sciences*, *284*(1855), 20162805. doi: 10.1098/rspb.2016.2805

End of response.

Appendix B

Camilla Cassidy
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Dear Dr. Sarah Brosnan, Editor,

We thank you for your offer of publication pending minor revisions for our manuscript, entitled “Species interactions and environmental context affect intraspecific behavioural trait variation and ecosystem function”.

All suggestions raised by reviewers and the Associate Editor have been addressed, as we outline in detail below. These encompass the addition of multivariate visualisation plots to the supplementary material, and small changes of wording for brevity throughout. Line numbers from the previously submitted manuscript are provided following each of Reviewer’s 2’s suggested corrections under ‘*Previously’*, while the changed text and updated line numbers from the revised manuscript are under ‘*Revised’*.

We believe these changes improve the clarity and flow of our manuscript, and the requested visualisation of our statistical test strengthens our findings and aids interpretation by readers.

We look forward to hearing from you in due course.

Yours sincerely,

Camilla Cassidy

Revised changes

Associate Editor Board Member: 1

I am happy to now accept this manuscript with minor revisions. R2 has continued to raise concerns with the statistical approach and I agree with their suggestion of including the multivariate plots from the PERMANOVA. Otherwise, there are just a few minor tweaks that need to be dealt with, including revision of particularly long sentences.

We thank you for your acceptance; we address Reviewer 2's specific corrections below by including multivariate plots, as suggested, and have revised sentences throughout for brevity.

Referee: 1

I agree with Reviewer 2 that the paper was lacking somewhat in background knowledge on functional traits and ecosystem functioning. However, the authors have improved this by expanding on certain sections in the introduction, and have clarified arguments in the discussion to make it easier to follow. Overall I feel the manuscript has been improved by revisions in response to comments from myself and reviewer 2, such that it is suitable for publication within this journal.

All authors wish to extent their thanks for Reviewer 1 for their highly instructive comments, which strongly aided in improving the scope and clarity of the manuscript.

Referee: 2

The revised manuscript by Cassidy et al. is a significant improvement over the first draft, and I now feel better able to understand and comment on the underlying science. The key findings are interesting and clearly within the scope of the Proceedings of the Royals Society B. I appreciate the authors attempt to clarify their reasoning behind the use of permutational ANOVA as a data analysis tool. I remain, however, unconvinced that distance-based multivariate approach is the best way to test for differences in this case. Perhaps though, if the authors could provide some visualisation of the multivariate analysis, using ordination plots it would allow me and other interested researchers to see how the suite of traits and response variables change visually. Within the vegan package in R, the constrained analysis of principle components function provides a good analogue for nPERMANOVA, which allows ordination plots to be generated. I also would refer the authors back to Anderson's various papers on nPERMANOVA as a method and asked whether the assumptions of homogenous multivariate dispersion of the data have been satisfied?

We thank Reviewer 2 for their recommendations on how to clarify any final queries that may arise regarding the implementation or interpretation of our statistical method. We incorporate visualisation with ordination plots as suggested (addressed in Comment 21), and ensure that our manuscript clearly justifies our use of the test against its statistical assumptions. We have tested for homogeneity of multivariate dispersions using PERMDISP, and explain our rationale within the Methods (Revised manuscript, Lines 250-255).

With regard to the experimental design, I believe the authors need to provide some justification for the complexity. Their analysis involves a four-way analysis of variance, but each treatment is only replicated between 4 and 6 times. Given the level of replication, I am unconvinced that the study has the statistical power to adequately detect a

potentially large number of important treatment effects. The authors need to consider this, and provide some justification for their design, in light of the relatively limited replication.
We address this comment below (Comment 19).

Finally, I would ask the authors to spend some more time proof-reading their manuscript and try to reduce the lengths of some of their sentences, and the use of adverbs like 'therefore', 'however', 'further' etc.

Overall, I believe the paper is worthy of publication and makes an important contribution to our understanding of how biological traits control ecosystem functioning. I therefore recommend its publication following further minor revisions.
We address all specific corrections regarding sentence length and the use of adverbs below, and have made changes throughout to improve clarity.

Specific Corrections.
1) Page 2 Line 30-31: 'We use PERMANOVA to quantify…' please revise to 'We use PERMANOVA to test…' You are referring to an inferential statistical test, to quantify you would be reporting the effect size, not simply if there is a difference.

> *Previously (Line 30)*: We use PERMANOVA to quantify the effect of species identity….
>
> *Revised (Line 30):* We use PERMANOVA to test the effect of species identity…

2) Page 3 Line 53: Replace 'However,' with 'The'.

> *Previously (Line 53)*: However, current research emphasizes that rather than the number of species, ecosystem functioning is instead mediated by the functional traits (e.g. behavioural, morphological or life history characteristics) expressed within a community (Gagic et al., 2015; Read et al., 2017).
>
> *Revised (Line 53):* Current research emphasizes that rather than the number of species, ecosystem functioning is instead mediated by the functional traits (e.g. behavioural, morphological or life history characteristics) expressed within a community (Gagic et al., 2015; Read et al., 2017).

3) Page 3 Line 63: Delete 'A majority of'.
'A majority of current' has been revised to 'conventional' to improve brevity, yet convey that some trait-based frameworks do incorporate a small degree of intraspecific variation (e.g. through fuzzy coding methods, or average biomass).

> *Previously (Line 63)*: A majority of current trait-based approaches and proposed frameworks implicitly assume that the expression of traits remains constant between conspecifics, irrespective of biotic or environmental context (Albert et al., 2010; Hevia et al., 2017).

Revised (Line 63): Conventional trait-based approaches and proposed frameworks implicitly assume that the expression of traits remains constant between conspecifics, irrespective of biotic or environmental context (Albert et al., 2010; Hevia et al., 2017).

4) Page 3 Line 37: Rearrange 'Therefore, for management purposes, …' to 'For Management purposes, therefore, …' This reads more elegantly.

Previously (Line 67): Therefore, for management purposes, authors may rely on trait values from literature or databases to characterise the functional importance of species (Gogina et al., 2016; Bolam et al., 2017; Solan et al., 2019).

Revised (Line 67): For management purposes, therefore, authors may rely on trait values from literature or databases to characterise the functional importance of species (Gogina et al., 2016; Bolam et al., 2017; Solan et al., 2019).

5) Page 4 Line 77: Replace 'and' with 'with'.

Previously (Line 77): Individual organisms are non-identical, and differing forms of trait expression are distributed unevenly throughout communities (Carmona et al., 2016; Roscher et al., 2018).

Revised (Line 77): Individual organisms are non-identical, with differing forms of trait expression distributed unevenly throughout communities (Carmona et al., 2016; Roscher et al., 2018).

6) Page 4 Line 92 – Page 5 Line 97: This sentence is too long. It needs to be broken up into a couple of shorter sentences.

Previously (Line 92-97): Mechanisms of phenotypic plasticity result in widespread and often substantial trait variability (Roscher et al., 2018); the trait expression of an individual may be transient over time and space, altering their activities and potential contributions to ecosystem processes in response to habitat features (Törnroos et al. 2015; Read et al., 2017), climatic drivers (Baranov et al., 2016; Nagelkerken & Munday, 2016; Landeira-Dabarca et al., 2018; Peterson et al., 2019), and resource availability (Hawlena et al., 2011; Murray et al., 2017).

Revised (Line 92-97): Mechanisms of phenotypic plasticity result in widespread and often substantial trait variability over time and space (Roscher et al., 2018). Transient trait expression in individuals alters their activities and potential contributions to ecosystem processes in response to habitat features (Törnroos et al. 2015; Read et al., 2017), climatic drivers (Baranov et al., 2016; Nagelkerken & Munday, 2016; Landeira-Dabarca et al., 2018; Peterson et al., 2019), and resource availability (Hawlena et al., 2011; Murray et al., 2017).

7) Page 4 Line 100: 'Further, trait expression shifts dramatically…' Please can you revise this to 'Trait expression, furthermore, dramatically shifts…'.

Previously (Line 100-103): Further, trait expression shifts dramatically in response to biotic influences, primarily from neighbouring individuals and/or species (Hawlena et al., 2011; Wohlgemuth et al., 2017; Calder-Potts et al., 2018; Thomsen et al., 2019).

Revised (Line 100-103): Trait expression, furthermore, also shifts dramatically in response to biotic influences, primarily from neighbouring individuals and/or species (Hawlena et al., 2011; Wohlgemuth et al., 2017; Calder-Potts et al., 2018; Thomsen et al., 2019).

8) Page 4 Lines 103 – 106. I it possible to break this sentence up, it is long and contains a number of separate clauses.

Previously (Line 103-106): These interactions may be competitive or complementary in nature, and so may determine species coexistence or exclusion (Turcotte & Levine, 2016; Pérez-Ramos et al., 2019), and potentially facilitate enhanced productivity, ecosystem functioning and service delivery (Finerty et al., 2016).

Revised (Line 103-105): Competitive or complementary interactions determine species coexistence and exclusion (Turcotte & Levine, 2016; Pérez-Ramos et al., 2019), and so potentially facilitate enhanced productivity, ecosystem functioning and service delivery (Finerty et al., 2016).

9) Page 4 Line 109: Delete 'Indeed'.

Previously (Line 109): Indeed, it is increasingly recognised that…

Revised (Line 109): It is increasingly recognised that…

10) Page 5 Line 116: Replace 'we seek to highlight the' with 'we investigate' or some other less verbose derivative.

Previously (Line 116): In this study, we seek to highlight the importance of incorporating intraspecific…

Revised (Line 115): In this study, we investigate the importance of incorporating intraspecific …

11) Page 6 Line 122: Replace 'hypothesised' with 'hypothesise' and be consistent with tenses.

Previously (Line 122): We hypothesised that i) biotic and site-specific…

Revised (Line 120): We hypothesise that i) biotic and site-specific…

12) Page 6 Line 136: Delete 'As such'.

Previously (Line 136): As such, given the proximate distance (~12 km) and presence of substantial changes in seabed terrain and flow conditions between sites (Gage, 1972; Friedrich et al., 2014), we infer …

Revised (Line 135): Given the proximate distance (~12 km) and presence of substantial changes in seabed terrain and flow conditions between sites (Gage, 1972; Friedrich et al., 2014), we infer…

13) Page 6 Line 140: Delete 'for example'.

Previously (Line 140-142): Loch Etive, for example, is subject to greater stratification and more frequent episodic flushing relative to Loch Linnhe that affects nutrient and organic material dynamics (Friedrich et al., 2014).

Revised (Line 139-141): Loch Etive is subject to greater stratification and more frequent episodic flushing relative to Loch Linnhe that affects nutrient and organic material dynamics (Friedrich et al., 2014).

14) Page 7 Line 143: 'significantly higher in Total Organic Carbon' please cite the p-value and test used to identify the significance.

The statistical test, previously only quoted in the referenced supporting information, has now been brought into the manuscript.

Previously (Line 142-144): Sediment at Loch Etive is finer and contains a significantly higher total organic carbon (TOC) content in comparison to the Loch Linnhe site (Supporting information, Table S1 and Fig. S2 & S3).

Revised (Line 141-143): Sediment at Loch Etive is finer and contains a significantly higher total organic carbon (TOC) content in comparison to the Loch Linnhe site (ANOVA: $F_{2,10}$= 30.78, $P < 0.001$, Supporting information, Table S1 and Fig. S2 & S3).

15) Page 7 Line 157-157 and throughout: 'Replicate faunal assemblages (hereafter referred to as 'communities')' I realise this is pedantic but these are assemblages and not communities. So why not refer to them as 'assemblages'? This would be more appropriate for the experiment.

We acknowledge, given the experimental nature of our study, the validity of the term 'assemblages'. However, our findings discuss net behaviours such as bioturbation and bioirrigation at what is widely termed the 'community level' (Schmitz et al., 2015; Laughlin et al., 2018), and functional trait-based approaches refer to the values or variability of traits expressed within a population or assemblage as the 'community functional potential', 'community-weighted trait mean' or similar, even during experimental manipulation (e.g. Solan et al., 2004; Rodriquez et al., 2016; Grossman et al., 2017). In this context, 'community' encompasses the sum of component organisms with influence on quantified functioning (Schmitz et al., 2015).

As such, we have used the single term 'community' here to avoid confusion when discussing behaviours or implications at this 'assemblage/community level', and to ensure the implications of our findings are clear to biologists working *in situ*.

Rodriguez, S., Martín, A. P., Sousa-Pinto, I., & Arenas, F. (2016). Biodiversity effects on macroalgal productivity: exploring the roles of richness, evenness and species traits. *Marine Ecology Progress Series*, *562*, 79-91.
Grossman, J. J., Cavender-Bares, J., Hobbie, S. E., Reich, P. B., & Montgomery, R. A. (2017). Species richness and traits predict overyielding in stem growth in an early-successional tree diversity experiment. *Ecology*, *98*(10), 2601-2614.
Laughlin, D. C., Chalmandrier, L., Joshi, C., Renton, M., Dwyer, J. M., & Funk, J. L. (2018). Generating species assemblages for restoration and experimentation: A new method that can simultaneously converge on average trait values and maximize functional diversity. *Methods in Ecology and Evolution*, *9*(7), 1764-1771.
Schmitz, O. J., Buchkowski, R. W., Burghardt, K. T., & Donihue, C. M. (2015). Functional traits and trait-mediated interactions: connecting community-level interactions with ecosystem functioning. In *Advances in Ecological Research* (Vol. 52, pp. 319-343). Academic Press.
Solan, M., Cardinale, B. J., Downing, A. L., Engelhardt, K. A., Ruesink, J. L., & Srivastava, D. S. (2004). Extinction and ecosystem function in the marine benthos. *Science*, *306*(5699), 1177-1180.

16) Page 8 Line 172: Typo 'replicates' should be 'replicated'.

Previously (Line 172-173): Each combination of factors was replicates six times, with the exception of two treatments (n = 4 and n = 5) (total n = 102, Supporting information, Table S3).

Revised (Line 171-172): Each combination of factors was replicated six times, with the exception of two treatments (n = 4 and n = 5) (total n = 102, Supporting information, Table S3).

17) Page 9 Line 213: Replace 'while' with 'whilst.'

Previously (Line 212-213): This length of time is sufficient to allow visualisation of particle movement while avoiding vertical homogenization of the tracers.

Revised (Line 211-212): This length of time is sufficient to allow visualisation of particle movement whilst avoiding vertical homogenization of the tracers.

18) Page 10 Line 224: Delete 'in part.'

Previously (Line 223-225): Ecosystem functioning was represented through the proxy of sediment nutrient release, which is in part mediated by the sediment movement behaviours of benthic fauna (Kristensen et al., 2014; Wohlgemuth et al., 2017).

Revised (Line 222-224): Ecosystem functioning was represented through the proxy of sediment nutrient release, which is mediated by the sediment movement behaviours of benthic fauna (Kristensen et al., 2014; Wohlgemuth et al., 2017).

19) Page 12 Lines 272-281: A four-way ANOVA puts in place lot of possible interactions. I am concerned that with 6 replicates of each treatment (and in some cases 5 or 4 replicates) I am not convinced that you have the statistical power to adequately test these multiple interaction terms.

The experimental design adopted for this study balanced the need between providing detailed understanding of the complex responses of the system, whilst also ensuring that the size of the experiment remained feasible. Therefore, 4-6 replicates per treatment combination was deemed appropriate to satisfy the research question.

In our initial submission, the community-level behaviours were analysed using both a 3-term model (each behaviour tested against the variables Population, Density and Species treatment) and a 4-term model (each behaviour tested against Population, Density, Species treatment and Morphological trait variation, as represented by the coefficient of variation (CV) of mean arm length); the 3-term model was later removed in line with suggestions from Reviewer 1. Both the previous three-term model and the current four-term model detect a two-way interaction driving sediment boundary roughness (SBR differing with the interactive effect of Density x Species treatment). However, the four-term model also detects an interaction including the additional term (SBR differing with the interactive effect of Population x Morphological trait variation). This supports that it is necessary to include all 4 terms simultaneously, as differences in morphological trait variation affect the interpretation of the other treatment variables.

20) Page 13 Line 290: Delete 'Further'.

Previously (Line 290): Further, when considered alongside only those aspects…

Revised (Line 291): When considered alongside only those aspects…

21) Page 13 Line 297-299: I don't fully understand the rationale for analysing the aspects of movement behaviour in a multivariate framework. I think there needs to be some justification for why this has been done in the methods. The use of PERMANOVA needs to be explained in the paper.

The previous explanation of PERMANOVA has been expanded with an additional sentence (underlined) to accommodate reference to the newly incorporated multivariate visualisation plots, which are located in ESM (Supporting information, Fig. S6). As suggested by Reviewer 2, we use Principal Component Analysis (PCA) in the *vegan* package. We provide three biplots, each showing the relative relationship of the multivariate behavioural traits to each other and, respectively, to the treatment groups for Population, Density, and Species identity. These biplots provide insight into 'what the PERMANOVA sees', and so support the reader's interpretation of the independent effects reported in Results. They also highlight the differing relationships of each trait within these treatments, and so aid understanding of why a multivariate framework has been employed. Overall, this revised justification now

more clearly articulates the rationale of using PERMANOVA for analysis of behavioural traits.

> *Previously (Line 244-249)*: Multivariate analyses were used to represent overall differences in the behavioural 'personalities' of individuals between species identities and contexts (Moran et al., 2017), integrating response (time to begin movement) and effect (time to complete burial) traits. PERMANOVA (iterations = 999) was used, as it is robust to non-normality and differing correlation structures and so is particularly suited for the detection of differences in intraspecific trait expression (Mitchell & Bakker, 2014b).
>
> *Revised (Line 243-250):* Multivariate analyses were used to represent overall differences in the behavioural 'personalities' of individuals between species identities and contexts (Moran et al., 2017), integrating response (time to begin movement) and effect (time to complete burial) traits. PERMANOVA (iterations = 999) was used, as it is robust to non-normality and differing correlation structures and so is particularly suited for the detection of differences in intraspecific trait expression (Mitchell & Bakker, 2014b). Patterns of intraspecific trait expression differ between the behavioural traits, and between context treatments (Supporting information, Fig. S6).

22) Page 15 Line 342: Should read 'Ecosystem Functioning'

> *Previously (Line 342)*: **3.3 Ecosystem function**
>
> *Revised (Line 343):* **3.3 Ecosystem functioning**

23) Page 16 Line 361-364: This first sentence of the discussion is somewhat generic. It should clearly state the key finding of the paper. Also, if you are describing your findings, why do you cite some other papers?

Citations had previously been included to support this broad summary of our conclusions. This statement has now been reworded to reference only the findings of this study, and citations have been removed.

> *Previously (Line 361-367)*: We demonstrate that intraspecific variation can have substantial influence on the way in which species mediate processes contributory to ecosystem functioning (Des Roches et al., 2018; Roscher et al., 2018), as the expression of traits by individuals and so the net behaviour of their communities varies with context. Differences in response to both prevailing biotic and historic abiotic conditions result in complex influences throughout the ecosystem structure and create a substantial diversity of trait expressions within species (Törnroos et al., 2015; Peterson et al., 2019).
>
> *Revised (Line 362-365):* Overall, our results demonstrate significant influence of context on the trait expression of individuals. We show that this context-dependency then affects the functional roles and contributions of species by mechanistically

underpinning concurrent change in community behaviour and ecosystem functioning.

24) Page 17 Line 392: Please stop using 'Further' to start a sentence. In most cases this is superfluous and unnecessary. Like 'therefore', 'however', 'therefore' and 'thus' it is not required in the majority of cases where it is used.

Changes have been made throughout as suggested to remove these words. In this specific instance, the use of 'further' is unchanged to aid clarity, as we refer to additional mediation based on species identity/density of an overall effect determined by population.

> *Revised (Line 386-392):* Origin in the distinct conditions of either loch contributes to differences in trait expression at an individual-level, and in the community-level net effects which these traits in part underpin (Wohlgemuth et al., 2017). [...] Further, density and species identity influence intraspecific behavioural trait expression as community composition determines the neighbour-effects that dictate behaviours including space and resource use (De Backer et al., 2011; Kraft et al., 2015; Calder-Potts et al., 2018).

25) Page 17 Line 395-396: Please revise this sentence for clarity.

> *Previously (Line 395-396)*: These effects carry implications for understanding the role of shifting biodiversity as a driver for altered ecosystem functioning (Thomsen et al., 2019).

> *Revised (Line 393-394):* These effects in turn underpin the role of shifting biodiversity in driving altered ecosystem functioning (Thomsen et al., 2019).

26) Page 18 Line 411: Replace 'This demonstrable' with 'The'. There is no need to add additional verbosity to every sentence.

> *Previously (Line 411-413)*: This demonstrable potential for intraspecific variation should not be overlooked, given that it can strongly determine the functional identity and context-dependent contributions of each species (Des Roches et al., 2018).

> *Revised (Line 409-411):* The potential for intraspecific variation should not be overlooked, given that it can strongly determine the functional identity and context-dependent contributions of each species (Des Roches et al., 2018).

27) Page 18 Line 425 – Page 19 Line 429: Long sentence, needs revised. Also, how would you recommend intraspecific variation is measured. This is not clear, and providing a clear outline of how it would be achieved would turn this into a useful intervention. At present this is rather generic statement.

This sentence has been reworked into two sentences, and now contains actionable suggestion of how intraspecific variation can be incorporated into trait-based methods.

Previously (Line 425-429): We suggest that measuring intraspecific variation should be a particular priority for comparisons of taxa between differing conditions or in changing environments, as our findings show the functional roles of individuals and communities cannot be assumed to be consistent or substitutable between contexts.

Revised (Line 423-427): We suggest that quantifying the extent of intraspecific variation should be a particular priority where environmental conditions are changing, or where taxa are compared across gradients. Mesocosm experimental studies or sub-sampling of trait expression *in situ* offers ability to establish the realised functional contributions or variability of species in complement to conventional trait-based study (Henn et al., 2018).

28) Page 19 Line 432-433: '… likely less essential…' inelegant phrasing, please revise.

Previously (Line 432-434): …and that quantification of intraspecific variability is likely less essential for projections of functioning and service delivery at ecosystem-scales with high species richness (Wright et al., 2016).

Revised (Line 429-431): … and that quantification of intraspecific variability will be less likely to alter projections of functioning and service delivery at ecosystem-scales with high species richness (Wright et al., 2016).

29) Page 19 Line 434: Delete 'Nonetheless'.

We maintain the use of 'nonetheless' in this instance, as we believe it is beneficial to draw attention to the contrast between the conventional view established the previous sentence, and the potential insights that our findings show could be gained through quantification of intraspecific trait variation.

Revised (Line 429-433): … and that quantification of intraspecific variability will be less likely to alter projections of functioning and service delivery at ecosystem-scales with high species richness (Wright et al., 2016). Nonetheless, to do so characterises the sources, pathways, and potential consequences of altered conditions (Albert et al., 2010; Fisher et al., 2015).

30) Page 19 Line 439: Delete 'especially'.

Previously (Line 439): Given especially that natural systems are increasingly subject to drivers of ecological change…

Revised (Line 436): Given that natural systems are increasingly subject to drivers of ecological change…

31) Page 19 Line 442: Delete 'seek to' and 'under'.

Previously (Line 441-442): Within this framework, we must also seek to isolate under which circumstances it contributes to the functional integrity of ecosystems (Wright et al., 2016; Zuo et al., 2017).

Revised (Line 438-440): Within this framework, we must isolate the circumstances where it contributes to the functional integrity of ecosystems (Wright et al., 2016; Zuo et al., 2017).

32) Page 19 Line 443-446: 'Failure to do so…' Revise this sentence for clarity.

Previously (Line 443-446): Failure to do so risks jeopardising accurate understanding and projection of ecosystem functioning due to inadequate characterisation of traits and, with them, biodiversity (Wohlgemuth et al., 2017; Adair et al., 2018; Des Roches et al., 2018).

Revised (Line 440-442): Failure to do so jeopardises understanding and prediction of ecosystem functioning due to inadequate characterisation of traits and, by result, biodiversity (Wohlgemuth et al., 2017; Adair et al., 2018; Des Roches et al., 2018).

33) Page 19 Line 449: Delete 'such'.

Previously (Line 449-451): Our findings demonstrate that such trait-based approaches to ecosystem study require more detailed functional metrics than has previously been assumed.

Revised (Line 445-447): Our findings demonstrate that trait-based approaches to ecosystem study require more detailed functional metrics than has previously been assumed.

34) Page 20 Line 457-465: The conclusions are rather generic sounding, and repetitive of the final paragraph of the discussion. What is the key take home message from your study, and what are its implications for how we understand the functioning of ecosystems? Try to express this clearly and directly.

As suggested, the Conclusions have been reworked to more clearly emphasise the overall findings and implications of the manuscript, without repeating the latter messages of the Discussion.

Previously (Line 457-465): Our findings illustrate that the trait expression of individuals and communities is influenced by aspects of abiotic and biotic context, and concisely demonstrate that the resultant identity and composition of these communities influence the functioning of ecosystems. Our study highlights the need to consider the context-dependency of species trait expression when quantifying ecosystem function, and demonstrates the utility of incorporating individual-level trait expression into trait-based approaches as a means by which to understand biological responses to changing environments. Such information will be essential to understand the functional diversity of systems, and guide efforts which seek to maintain good ecosystem function and service delivery in the face of ecological change.

Revised (Line 454-462): Our findings show that the expression of traits by individuals and so the net behaviour of their communities differs with biotic and abiotic context. Such changes in individual functional contributions have important implications for mediation of ecosystem functioning. Our study highlights that trait-based approaches which do not consider the context-dependency of trait expression are at risk of misrepresenting the functional roles of taxa. Quantification of intraspecific variability will offer ecologists better insight into biological responses to environmental conditions, and aid ecosystem management approaches seeking to maintain good ecosystem function and service delivery in the face of environmental change.