Reply to ‘Pseudoreplication and greenhouse-gas emissions from rivers’

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We did not account for week of incubation (i.e. batch) within the statistical model as we do not consider that there is a reasonable mechanism by which sample incubation week was impacted by this experimental approach and the associated consistent sample storage over the course of batch incubations. We find Tiegs et al.’s argumentation that batch-specific conditions “likely differed in unknown ways” from batches tested in other weeks highly unconvincing. Moreover, we do not consider isolative segregation of batch-specific controls to have any discernible impact on the experimental results, as discussed below. In fact, we are convinced that the results of our experiments are more robust by exposing all experimental temperature treatments to the same incubation environment, rather than introducing unnecessary uncertainty and risk of technical failure or variance in performance through the use of different incubators, as suggested by Tiegs et al. Notably, the differences between the temperature at the top and bottom of the incubator were very small (0.0 to 0.6 °C, Table 1), within the typical error range of standard electronic temperature measurement devices, further indicating that the incubator provided uniform environmental conditions.

Tiegs et al. furthermore highlight the lack of replication of geology as a treatment and posit that no conclusions can be drawn with respect to geological effects in Comer-Warner et al. We would like to emphasise that our conclusions at no point claim to draw interpretations for the entirety of the two example geologies used in Comer-Warner et al. Instead, we followed a paired catchment approach as has been used for more than 100 years in hydrological and environmental sciences, using the observed differences between multiple samples from different locations in each stream to highlight the differences between the two rivers, which varied predominantly by geology.

While we agree with Tiegs et al. about the potential uncertainties arising from the storage of sediments at 4 °C during the course of the experiments as discussed in detail in Comer-Warner et al., we consider the alternative of repeated sampling closer to the time of respective batch incubations to impose considerably larger experimental uncertainties due to the temporally highly

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dynamic nature of river and streambed chemical and microbial conditions, with biogeochemical turnover ranging from minutes to days. Based on our sampling strategy and similar starting points for the treatment effects, our statistical analysis showed significant differences between the two streams over the course of independent batch incubations.

Additionally, Tiegs et al.\(^1\) state that the conclusion of non-linear and threshold responses observed in our data are predominantly based on the reduction in microbial activity observed from 21 to 26 °C. We would like to highlight that this statement is not accurate as a decrease in microbial activity was not observed between 21 and 26 °C in all sediment classes and was not observed in the case of CO\(_2\) production in any sediment classes. The interpretation of non-linear and threshold responses was, therefore, not solely reliant on the observation of lower microbial activity at 26 than 21 °C, as suggested by Tiegs et al.\(^1\). Tiegs et al.\(^1\) express scepticism about the supposed linear relationship between methane fluxes and temperature determined from the meta-analysis of Yvon-Durocher et al.\(^12\). Tiegs et al.\(^1\) account of the results presented in Yvon-Durocher et al.\(^11\) is not accurate though, as their meta-analysis found exponential and non-linear relationships between methane fluxes and temperature (as highlighted in the Addendum for Comer-Warner et al.\(^2\)). Furthermore, non-linearity and threshold responses of greenhouse-gas fluxes to temperature have previously been found in a variety of ecosystems, e.g., refs. 12-14.

### Data availability

The dataset generated during the current study is available from the corresponding author on reasonable request.

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### Table 1 The variation in temperature between the top and bottom of the incubator used.

| Temperature treatment (°C) | Mean difference (°C) | Standard deviation (°C) |
|-----------------------------|----------------------|------------------------|
| 5                           | 0.6                  | 0.3                    |
| 9                           | 0.6                  | 0.2                    |
| 15                          | 0.4                  | 0.2                    |
| 21                          | 0.1                  | 0.1                    |
| 26                          | 0.0                  | 0.2                    |

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### References

1. Tiegs, S. D. & Raffel, T. Pseudoreplication and greenhouse-gas emissions from rivers. *Nat. Commun.* https://doi.org/10.1038/s41467-019-13303-1 (2019).
2. Comer-Warner, S. et al. Thermal sensitivity of CO\(_2\) and CH\(_4\) emissions varies with streambed sediment properties. *Nat. Commun.* 9, 2803 (2018).
3. Hurlbert, S. Pseudoreplication and the design of ecological field experiments. *Ecol. Monogr.* 54, 187–211 (1984).
4. Davies, M. J. & Gray, A. Don’t let spurious accusations of pseudoreplication limit our ability to learn from natural experiments (and other messy kinds of ecological monitoring). *Ecol. Evol.* 5, 5295–5304 (2015).
5. Oksanen, L. Logic of experiments in ecology: is pseudoreplication a pseudoissue? *OIKOS* 94, 27–38 (2001).
6. Oksanen, L. The devil lies in details: reply to Stuart Hurlbert. *OIKOS* 104, 598–605 (2004).
7. Colegrave, N. & Ruxton, G. Using biological insight and pragmatism when thinking about pseudoreplication. *Trends Ecol. Evolution.* 33, 28–35 (2018).
8. Millar, R. B. & Anderson, M. J. Remedies for pseudoreplication. *Fish. Res.* 70, 397–407 (2004).
9. Brown, A. E., Zhang, L., McMahon, T. A., Western, A. W. & Veretsey, R. A. A review of paired catchment studies for determining changes in water yield resulting from alterations in vegetation. *J. Hydrol.* 310, 28–61 (2005).
10. Van Loon, A. F. et al. Using paired catchments to quantify the human influence on hydrological droughts. *Hydrological Earth Syst. Sci.* 23, 1725–1739 (2019).
11. Yvon-Durocher, G. et al. Methane fluxes show consistent temperature dependence across microbial to ecosystem scales. *Nature* 507, 488–491 (2014).
12. Carey, J. C. et al. Temperature response of soil respiration largely unaltered with experimental warming. *PNAS* 113, 13797–13802 (2016).
13. Gill, A. L., Giasson, M., Yu, R. & Finzi, A. C. Deep peat warming increases surface methane and carbon dioxide emissions in a black spruce-dominated ombrotrophic bog. *Glob. Change Biol.* 23, 5398–5411 (2017).
14. Xing, Y. et al. Methane and carbon dioxide fluxes from a shallow hypereutrophic subtropical Lake in China. *Atmos. Environ.* 39, 5532–5540 (2005).

### Author contributions

S.C.-W. wrote the manuscript, P.R., D.C.G., S.U., N.K., B.M., D.M.H. and S.K. contributed to the discussion of the content and the editing of the manuscript.

### Competing interests

The authors declare no competing interests.

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