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## To replicate, or not to replicate: Should we shift to unreplicated multi-level designs in ecological experimentation?

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Ecological responses to environmental changes are often non-linear due to the interactive effect of several drivers on the response. Earth-system modelling is increasingly asking for empirically based, mechanistic understanding about the ecological responses to multiple (interacting) environmental stressors. To deliver this, novel, logistically and financially feasible designs are needed for ecological experimentation to constrain the number of experimental units whilst being able to capture non-linearities in ecological responses.

Here we propose, that unreplicated multilevel designs of experiments might provide the required information, potentially leading to a paradigm shift in ecological experimentation. To test this, we combined artificial data simulations with empirical data from purpose built, real-world experiments.

Consistently, unreplicated sampling at a maximum number of treatment levels maximized prediction success (R<sup>2</sup> to known truth) in artificial data based on common relationships (linear, unimodal, saturating) of two interacting drivers. These results were consistent irrespective of noise level and underlying response surface. Consistent results were also obtained from the two purpose built real-world experiments with unreplicated designs outperforming replicated designs at all possible sampling intensities.

Our findings from reproducible simulations combined with realistic empirical data suggest that a paradigm shift to unreplicated multilevel designs of experiments would be a major step towards a mechanistic understanding of non-linear ecological responses to continuous environmental drivers.