

Reporting Summary

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Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

- | | |
|-------------------------------------|--|
| n/a | Confirmed |
| ☐ | ☒ The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement |
| ☐ | ☒ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly |
| ☒ | ☐ The statistical test(s) used AND whether they are one- or two-sided
<i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i> |
| ☐ | ☒ A description of all covariates tested |
| ☒ | ☐ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons |
| ☐ | ☒ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals) |
| ☐ | ☒ For null hypothesis testing, the test statistic (e.g. F , t , r) with confidence intervals, effect sizes, degrees of freedom and P value noted
<i>Give P values as exact values whenever suitable.</i> |
| ☒ | ☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings |
| ☐ | ☒ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes |
| ☒ | ☐ Estimates of effect sizes (e.g. Cohen's d , Pearson's r), indicating how they were calculated |

Our web collection on [statistics for biologists](#) contains articles on many of the points above.

Software and code

Policy information about [availability of computer code](#)

Data collection

n/a

Data analysis

All analyses were conducted in R. All packages are described in the methods & listed in the references. All software used for analysis is publicly available in open access. See code availability section.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research [guidelines for submitting code & software](#) for further information.

Data

Policy information about [availability of data](#)

All manuscripts must include a [data availability statement](#). This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

The following figures have associated raw data: Main text Figures 2 to 5, and Extended Data Figures 1 to 7. These data and the R codes for drawing the figures and statistical tests have been made available online, as described in the data availability and code availability sections.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☐ Life sciences ☐ Behavioural & social sciences ☒ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Ecological, evolutionary & environmental sciences study design

All studies must disclose on these points even when the disclosure is negative.

Study description

We report an experiment testing the theory of 'community rescue' in semi-natural conditions. We exposed model freshwater ecosystems (34 pond mesocosms inoculated with a plankton community from a pristine lake) to various doses of the herbicide glyphosate. Based on results obtained from microcosm experiments, we determined whether pre-exposure to sublethal stress and high population/community abundance would facilitate community rescue. We conducted a two-phase experiment. In Phase I, we applied nutrient and glyphosate treatments following a factorial design, with 8 glyphosate doses X 2 nutrient levels, replicated twice (32 ponds/experiment units + 2 controls for phase II = 34 ponds). The nutrient treatment manipulated community abundance (phytoplankton biomass) while the glyphosate treatment manipulated the extent of pre-exposure to sublethal stress. Then, in Phase II, all 32 ponds treated in Phase I received a 'normally-lethal' dose of glyphosate. We determined whether biomass was maintained in some ponds, and if so, whether this was predicted by the treatments of Phase I. We also had two control ponds to account for seasonal effects on biomass in the absence of treatment. We predicted that high-nutrient ponds and ponds having received a high (but sublethal) dose of glyphosate in Phase I would be most likely to avert biomass collapse in Phase II. We confirmed a role for pre-exposure to sublethal stress but not community biomass. These results expand the scope of community rescue theory to more complex communities, and also identify a dual effect of glyphosate on lake phytoplankton: herbicidal in the short-term, then fertilizing in the longer-term (at high doses).

Research sample

Our samples are phytoplankton and zooplankton samples from the ponds, as well as key physico-chemical parameters such as depth, nutrient concentration, temperature, dissolved oxygen, pH, and conductance. The ponds are filled with water and organisms from a nearby lake; we thus sampled the ponds as one would sample the water column of a lake. Phytoplankton samples comprised mostly green algae, i.e. chlorophytes of various genera such as *Chlorella*, *Desmodesmus*, and *Selenastrum*. Zooplankton samples comprised microcrustaceans such as *Bosmina longirostris* and *Chydorus sphaericus*. Samples from control ponds are meant to represent the plankton community from our source lake in the absence of anthropogenic stressors. Samples from glyphosate and nutrient-treated ponds are meant to represent the hypothesized effect of these stressors on our source lake, if it was contaminated.

Sampling strategy

All ponds were sampled multiple times using various instruments. Water samples were collected with integrated tube samplers deployed at multiple random locations across the pond surface. Water samples were homogenized and split into subsamples to measure algal biomass, community composition, and nutrient concentration, with standard protocols. Water pH, conductance, and dissolved oxygen were measured with handheld YSI probes. Temperature was measured continuously with data loggers. Depth was measured with a meter stick. We used 34 ponds for this experiment. In mesocosm studies, it is quite typical to have 4 replicates (ponds/bags) per treatment combination in factorial experiments. This is normally sufficient to detect effects given that all experimental units begin with the same conditions, communities, etc., and thus usually respond similarly to the treatments. We could have used this strategy and have 2-4 glyphosate treatment X 2 nutrient treatment X 4 replicates, but we instead employed a regression design with more (8) glyphosate doses X 2 nutrient levels X 2 replicates. This was meant to cover a broad glyphosate gradient and identify the concentrations at which impacts are visible, as opposed to use few doses of glyphosate and treat this treatment as a factor. This regression design was powerful in that we can say within what range of glyphosate concentrations one would expect negative effects on plankton communities, an approach that is also common in the ecotoxicological literature. Because starting conditions among ponds were relatively homogeneous and responses to treatments were consistent across ponds (as is typical in mesocosm experiments), our sample size was sufficient to uncover strong effects of glyphosate.

Data collection

Our field team was composed of one postdoc (VF), two PhD students (MPH, NBdC), and three undergraduate field assistants. All field data were recorded by at least two persons: one person measuring the parameter of interest in the pond, and one person recording the measured value in a notebook. The initials of who recorded the data on any given day was also noted on a virtual lab notebook. All data were entered on computer spreadsheets and backed up on an online server the day of collection. Spreadsheets and the virtual lab notebook were then uploaded on the Open Science Framework server at the end of the experiment for consultation and usage by all collaborators on the project.

Timing and spatial scale

Our experiment ran from August 17th to October 12th 2016. The treatments involved three pulse applications of glyphosate distributed over this study period. Our multiple sampling occasions involved, for each glyphosate pulse, a sample before the treatment, a sample shortly after the treatment (1-2 days), and a sample 7-10 days after the treatment. There was no gap in the schedule; on any sampling day, all ponds from all treatments were always sampled. In this case the spatial scale of the study is our mesocosm infrastructure and our source lake, which is a small woodland lake with a water chemistry and plankton community representative of undisturbed (forested) lakes in the Saint-Lawrence lowlands. We used experimental evolution with model communities to test a theory; our objective was not to extend our results to many natural lakes at a large spatial scale.

Data exclusions

For one response variable (phytoplankton biomass), we excluded data for groups other than chlorophytes. As indicated in the manuscript, over 99% of biomass was contributed by chlorophytes. Other algal groups (diatoms, cryptophytes) went quickly extinct in all ponds (control and treatment), indicating that the pond/mesocosm environment is not ideal for their proliferation. Keeping these rare taxa in the analysis would not influence our results. We chose to focus only on chlorophytes because it makes it clearer for the reader that total biomass represents biomass of green algae, which then corresponds well with the community composition analyses. For some variables that are expensive to measure (e.g. phytoplankton community composition), we used a subset of

collected samples. We chose ponds in the middle and at both ends of the logarithmic glyphosate gradient (doses 1, 4, 7 and 8), and focused on key time points of the experiment (beginning and end of Phases I and II, and the 3 time points of the first glyphosate dose when we observed community rescue in the biomass data). We had identified a priori this subset of ponds and time points for targeted, more detailed community analyses, following the same approach that some of our co-authors (GB and AG) had used in a previous community rescue experiment conducted in microcosms (Low-Decarie et al 2015 PNAS, cited in the manuscript).

Reproducibility

Given the scale of the experiment (a costly, large-scale mesocosm experiment over two months), we did not replicate the experiment. However, the 2020 experiment at LEAP (our mesocosm infrastructure) will build on our 2016 results and involve a community rescue experiment with glyphosate, this time using 96 ponds (i.e. adding an extra treatment to extend our results to the metacommunity scale). We should thus be able to verify that our core finding (community rescue at very high doses of glyphosate predicted by pre-exposure to sublethal stress) holds in a different year.

Randomization

Blocks of 8 ponds receiving one of two possible nutrient treatments were allocated randomly in space across the infrastructure of 96 ponds. Within each block with a single nutrient level, a gradient of 8 glyphosate doses was established. This was meant to replicate the glyphosate gradient in subunits of 8 ponds close in proximity. We did not observe spatial effects on any response variable; values varied in time (e.g. seasonal effects on temperature) or based on treatments, but not in space based on proximity. All ponds were close to one another (they were laid out on a 30 X 40 m flat gravel platform) and received the same amount of light and precipitation. All ponds were filled at the same time using a piping system with multiple outlets.

Blinding

Members of the field team sampling the ponds other than VF and MPH were unaware of the treatment allocation across the platform, to minimize potential biases when collecting integrated water samples. External laboratories processing algal, glyphosate, and nutrient samples did not know about our experimental design or treatments. We did not use blinding during statistical analyses but see no reason for this to influence our results. As mentioned above, all data and code were archived online in open access, which in principle should allow statistical analyses to be replicated.

Did the study involve field work? ☒ Yes ☐ No

Field work, collection and transport

Field conditions

The experiment was conducted at the 'Large Experimental Array of Ponds' facility at McGill University's Gault Nature Reserve in Québec, Canada. This reserve is a protected deciduous forest in the Saint-Lawrence lowlands. The mesocosm infrastructure is located in an open area at the border of the reserve, on a gravel platform. Weather data for this area can be accessed on the Canadian government website at: http://climat.meteo.gc.ca/climate_data/daily_data_e.html?StationID=48374. Over the course of our experiment, total rainfall was 34.4 mm. Maximum, minimum, and mean daily air temperature was 23.3, 11.3, and 17.3 degrees C, respectively. Pond water temperature and depth are reported in the supplementary material.

Location

The mesocosms and field laboratory are located at 45°32'N, 73°08'W, at an elevation of 121 masl.

Access and import/export

The infrastructure can be accessed via a road. No permit was required to collect plankton in our experimental ponds, or to fill the ponds with water and plankton from our source lake. Our proposal to conduct an experiment with glyphosate was reviewed and approved by the director and by the scientific manager of Gault Nature Reserve.

Disturbance

We expect the study to have caused minimal disturbance to the local environment. All water used in the experiment was contained in an on-site, 10000L retention basin until glyphosate concentration had reached levels similar to the measurement error of our instrument. These concentrations are orders of magnitude lower than concentrations considered safe for aquatic life and safe for drinking in Canada, and than concentrations used by farmers spraying fields adjacent to the reserve. All water used in the experiment was then released in a sparsely-vegetated area/field between the conservation sector of the nature reserve and private properties/agricultural fields outside of the nature reserve. Our source lake was for several decades the municipal source of tap water in the region and is thus already well connected to downstream areas and fields.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☒	☐ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology
☐	☒ Animals and other organisms
☒	☐ Human research participants
☒	☐ Clinical data

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Animals and other organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research

Laboratory animals	The study did not involve laboratory animals
Wild animals	We collected zooplankton samples (microscopic crustaceans) in our experimental ponds. A total of 2 L of water collected with integrated samplers at 10 random locations were combined and filtered with a 64 µm sieve. Zooplankton were anesthetized using carbonated water and then preserved in 95% ethanol to a final concentration of 75 % ethanol. No permit was required to collect zooplankton in our experimental ponds.
Field-collected samples	The study did not involve samples collected from the field
Ethics oversight	No ethical approval was required for this experiment as we used invertebrate samples, and did not perform manipulations on these animals.

Note that full information on the approval of the study protocol must also be provided in the manuscript.