



# Community rescue in experimental phytoplankton communities facing severe herbicide pollution

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**Community rescue occurs when ecological or evolutionary processes restore positive growth in a highly stressful environment that was lethal to the community in its ancestral form, thus averting biomass collapse in a deteriorating environment. Laboratory evidence suggests that community rescue is most likely in high-biomass communities that have previously experienced moderate doses of sublethal stress. We assessed this result under more natural conditions, in a mesocosm experiment with phytoplankton communities exposed to the ubiquitous herbicide glyphosate. We tested whether community biomass and prior herbicide exposure would facilitate community rescue after severe contamination. We found that prior exposure to glyphosate was a very strong predictor of the rescue outcome, while high community biomass was not. Furthermore, although glyphosate had negative effects on diversity, it did not influence community composition significantly, suggesting a modest role for genus sorting in this rescue process. Our results expand the scope of community rescue theory to complex ecosystems and confirm that prior stress exposure is a key predictor of rescue.**

Human-induced global change has led to unprecedented rates of population extirpation and species extinction<sup>1–3</sup>, a ‘biodiversity crisis’ that affects ecosystem functions and services<sup>4,5</sup>. However, populations can sometimes persist in degraded environments through plastic or evolutionary trait changes that increase resistance to stress, defined here as a foreign perturbation that causes population decline. A population is said to have undergone ‘rescue’ when either plastic or genetic changes avert population collapse in an environment that would otherwise (without plastic or genetic changes) be lethal to all individuals. This rescue process involves an increase in mean stress resistance within the population, leading to a reversal from population decline to growth in a constant, initially stressful environment. Although ‘plastic rescue’ has received relatively little attention<sup>6,7</sup>, evolutionary rescue has been studied more extensively both theoretically and empirically to identify factors that increase its incidence<sup>8–11</sup>. Two such factors include population size before environmental degradation and pre-exposure to moderate doses of stress. The former influences the risk of stochastic extinction, while the population experiences a decline in abundance at the onset of stress<sup>12–15</sup>. The latter creates selection that increases the frequency of stress-resistant individuals in the population, allowing it to withstand more severe doses of stress thereafter<sup>13,16,17</sup>.

Increases in stress resistance can also maintain aggregate community properties such as biomass and diversity under stressful conditions. ‘Community rescue’ occurs when ecological or evolutionary processes restore positive growth in a highly stressful environment that was lethal to the community in its ancestral form, averting biomass collapse in a deteriorating environment<sup>18–20</sup>. Community rescue differs from community resilience and resistance<sup>21–23</sup>, two concepts that are normally used to describe the

response of communities to tolerable perturbations. In contrast, community rescue implies recovery of a viable, productive community despite no recovery/amelioration of environmental conditions, and at a level of stress expected to cause complete biomass collapse without some form of adaptive or plastic response. Community rescue is demonstrated with a two-phase experimental design<sup>18,19</sup>. In Phase I of the experiment, some communities are allowed to adapt to a low to moderate dose of stress, while other communities are maintained in benign conditions. In Phase II of the experiment, all communities are confronted with a dose of stress lethal to all populations of the ancestral community. Communities that avert biomass collapse in Phase II are said to have undergone community rescue (two or more species have recovered positive growth in the stressful environment), while the collapse of naive communities confirms that the stress was lethal to the community in its antecedent form. ‘Lethal stress’ in previous rescue experiments was defined as a dose of stress that produces a very low measure of population or community abundance, comparable to what is measured in sterile growth medium<sup>12,13,18</sup>.

As with population rescue, community rescue involves an increase in absolute fitness in the stressful environment but, in this case, averaged across all individuals of all species in the focal community. This increase in mean absolute fitness may be achieved via phenotypic plasticity, evolution (selection acting on standing variation or de novo mutations) and/or taxon sorting (changes in community composition in favour of resistant species). In the first empirical study of community rescue, conducted with soil microbes exposed to the herbicide Dalapon under laboratory conditions, rescue of community biomass occurred through a combination of ecological and evolutionary processes<sup>18</sup>. Furthermore, the likelihood of community rescue depended on some of the same factors that

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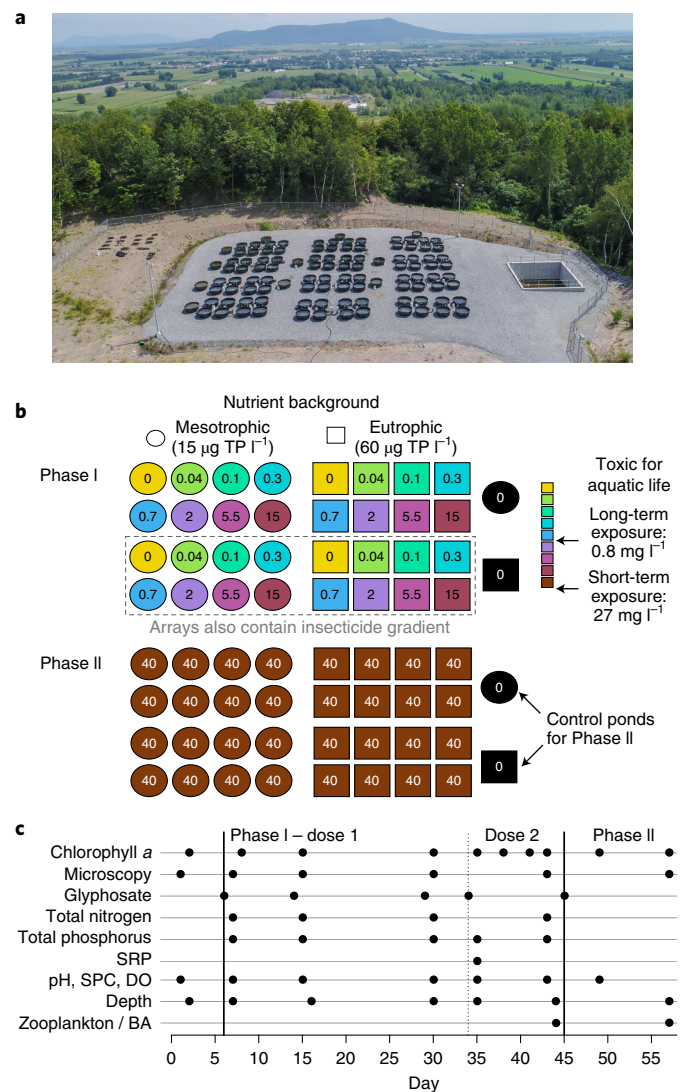
predict rescue in single-species experiments; for example, community abundance (summed across populations/species) and the history of stress of the community (prior exposure to moderate levels of stress). The latter mechanism effectively increases community-averaged stress resistance: episodes of moderate stress can induce plasticity and/or increase the frequency of stress-tolerant genotypes and species, thus averting biomass collapse once a community is subsequently exposed to a severe dose of stress that would otherwise have been lethal without prior exposure.

To assess these results under more natural conditions, we used experimental plankton communities sourced from a pristine lake, fed into replicate 1,000-l mesocosms at the Large Experimental Array of Ponds (LEAP) in Southern Québec (Fig. 1a). LEAP mesocosms are exposed to natural environmental fluctuations and contain complex communities with trophic structure and a diversity of naturally co-occurring species of bacteria, algae and zooplankton<sup>19</sup>. We confronted these communities with high doses of the herbicide glyphosate, known to have toxic effects on several species of phytoplankton<sup>24–27</sup>. Glyphosate is currently the most widely used herbicide (and pesticide) worldwide, with an applied tonnage rising continuously since the development of glyphosate-resistant crops in the 1990s<sup>28–30</sup>. Widespread application of glyphosate has led to the rapid evolution of glyphosate resistance in many plants species<sup>31,32</sup>, enabling evolutionary rescue of pest populations and causing weed management problems<sup>33,34</sup>. The ubiquitous presence of glyphosate in the environment has also sparked concerns over potential health and ecotoxicological effects<sup>35–39</sup>. In our study region, where intensive agriculture of glyphosate-resistant crops is a dominant form of land use, traces of glyphosate have been found in all agricultural water bodies monitored by government authorities<sup>40</sup>. In contrast, it is unlikely that our source lake (Lac Hertel) has been contaminated with glyphosate, as it is located on a mountain within a forested protected area with no history of agriculture.

We tested whether naive phytoplankton communities from Lac Hertel could be rescued from severe glyphosate pollution, and if so, whether rescue would be facilitated by higher community biomass and pre-exposure to moderate stress<sup>18</sup>. The experiment had two phases (Fig. 1b). In Phase I, we manipulated community biomass (with a press nutrient treatment of 15 or 60  $\mu\text{g l}^{-1}$  of phosphorus) and pre-exposure to moderate stress (with two pulse applications of Roundup—a common formulation of glyphosate—varying in concentration from 0  $\text{mg l}^{-1}$  in the six control ponds to 15  $\text{mg l}^{-1}$  in the most contaminated ponds). Then, in Phase II, all ponds excepting two control ponds were exposed to a dose of Roundup expected to be lethal to all phytoplankton species in our communities after short-term exposure. Note that, because this experiment was embedded in a multiple stressor study with various contaminants, some ponds were also treated with an insecticide, imidacloprid (Fig. 1b). However, as explained in the Methods, this insecticide had no detectable effect on our communities (see Supplementary Results). Throughout the experiment, we tracked phytoplankton biomass (based on chlorophyll *a* concentration), community composition (genus-level biovolume) and water chemistry, including glyphosate and nutrient concentrations (Fig. 1c). We also report zooplankton and bacterial densities at key time points. Phytoplankton community biomass at the end of Phase II indicates the potential of a community to persist in a severely degraded environment (expected to be lethal) and reflects the outcome of community rescue, which we relate to the two factors manipulated in Phase I.

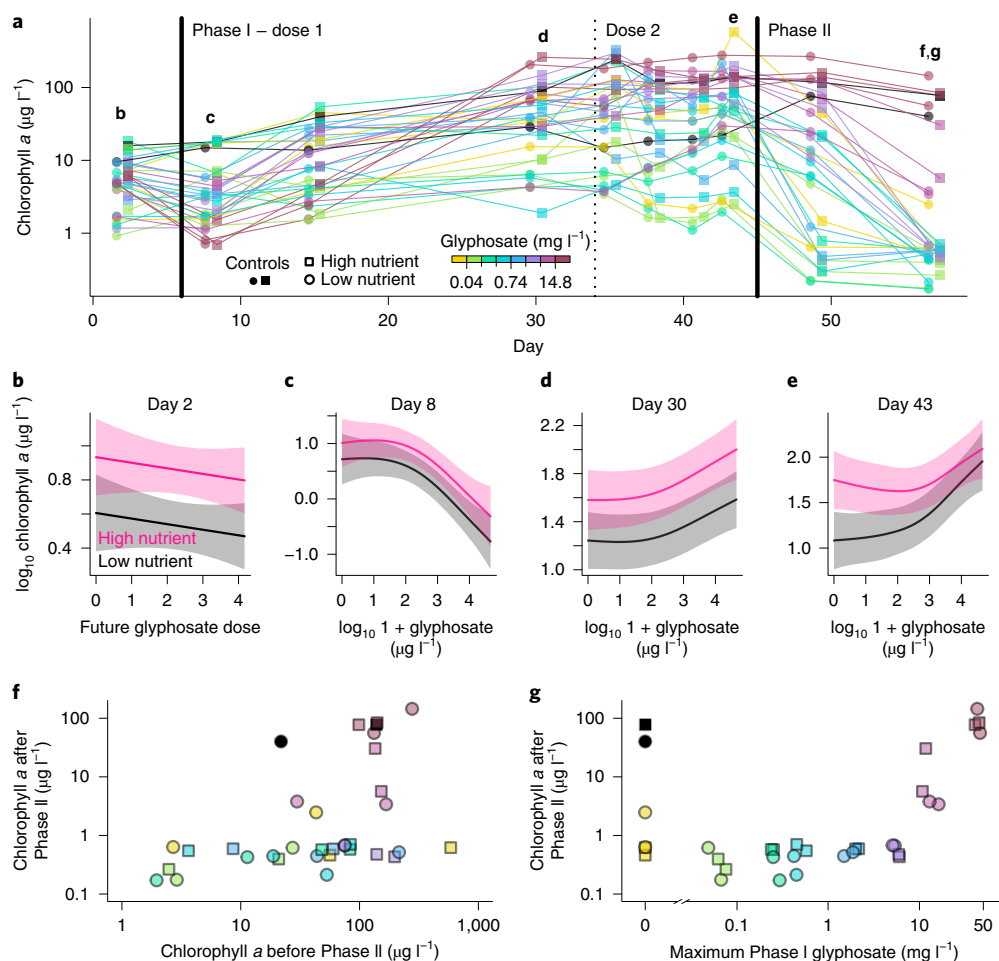
## Results

On the first sampling day, one week after the first nutrient application but before glyphosate application, high-nutrient ponds had a greater phytoplankton biomass than low-nutrient ponds (generalized additive model (GAM), nutrient effect:  $P=0.003$ ;  $R^2=0.22$ ; Fig. 2a,b). This positive effect of nutrient enrichment on



**Fig. 1 | Experimental site, design and timeline.** **a**, Aerial photograph of the LEAP facility at Gault Nature Reserve, located near an area of intensive agriculture. **b**, Schematic representation of experimental treatments. Colours and numbers within symbols indicate target glyphosate concentrations after application of one dose. The nutrient treatment was a press treatment maintained with biweekly nutrient addition. The glyphosate treatment involved the following: in Phase I, two pulse applications (doses) of Roundup ranging in concentration from 0 to 15  $\text{mg l}^{-1}$  of glyphosate acid; and in Phase II, one dose of 40  $\text{mg l}^{-1}$  in all experimental ponds. Yellow and black ponds are pesticide-free in Phase I, while yellow ponds (but not black ponds) receive the lethal dose in Phase II. As discussed in the Methods, half of the mesocosms were also treated with an insecticide gradient. Note that treatment combinations were allocated randomly in space, such that the spatial clustering shown here is for illustrative purposes only. **c**, Timeline of the experiment. Symbols indicate measurement dates for variables listed on the left. Temperature was also recorded in all ponds with automated sensors. Thick vertical lines indicate the beginning of Phases I and II, while the dotted line indicates the second dose of Phase I. TP, total phosphorus; SRP, soluble reactive phosphorus; SPC, specific conductance; DO, dissolved oxygen; BA, bacterial abundance.

phytoplankton biomass remained significant throughout Phase I of the experiment (generalized additive mixed model (GAMM), nutrient effect:  $P=0.012$ ; Fig. 2a,c–e; see Supplementary Table 1 for additional model details). In contrast, and as expected, ponds

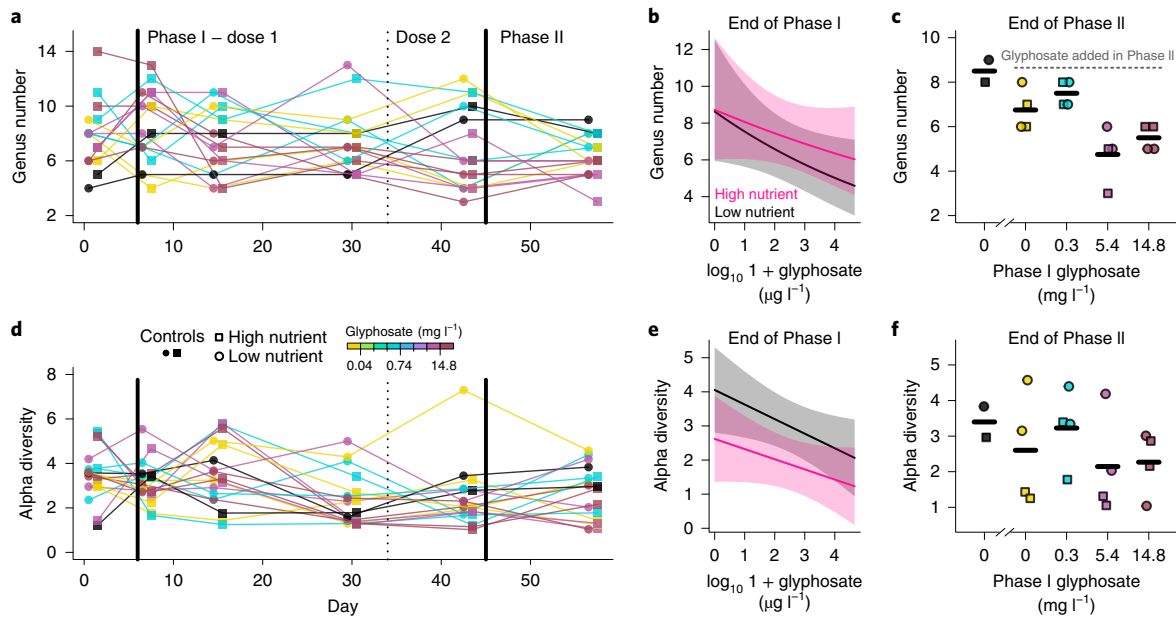


**Fig. 2 | Phytoplankton biomass dynamics during the experiment.** **a**, Time series of chlorophyll *a* concentration (a proxy for phytoplankton biomass) in all ponds over the course of the experiment. Symbols and colours indicate nutrient and glycosate treatments, respectively. A small offset on *x*-axis values was introduced to better distinguish low- and high-nutrient ponds. Both yellow and black lines/symbols are control (glycosate-free) ponds for Phase I, while only black lines/symbols are control ponds for Phase II. **b–e**, Results of additive mixed models predicting chlorophyll *a* concentration from measured glycosate concentration and nutrient treatment. Model results are shown for various key time points of Phase I: before the first dose (**b**), shortly after the first dose (**c**), before the second dose (**d**), and after the second dose (**e**). Shaded polygons illustrate 95% confidence intervals. **f,g**, Chlorophyll *a* concentration at the end of Phase II as a function of chlorophyll *a* at the end of Phase I (**f**) or maximum recorded glycosate concentration during Phase I (**g**). Black symbols are control ponds for Phase II.

assigned to different glycosate treatments did not originally differ in phytoplankton biomass before the first glycosate pulse (GAM:  $P=0.393$ ; Fig. 2a,b). The two pulse applications of glycosate during Phase I then had a strong, time-dependent effect on biomass (GAMM, interaction effect of time and glycosate concentration:  $P<0.0001$ ;  $R^2=0.88$ ; Fig. 2a,c–e), an effect that was in part modulated by the nutrient treatment (GAMM, interaction effect of time, glycosate concentration and nutrient treatment:  $P=0.05$ ). When we applied the first pulse (day 6), glycosate had a negative impact on phytoplankton biomass in both high- and low-nutrient ponds receiving high doses of glycosate (Fig. 2a,c). However, even the most affected communities recovered quickly and effects of glycosate on phytoplankton biomass were no longer evident by day 15 (Fig. 2a). This recovery of biomass occurred even though glycosate concentration remained constant during this period (Extended Data Fig. 1a).

Between days 15 and 30, after recovery from the first pulse and before a second pulse was applied, effects of glycosate on phytoplankton biomass reversed from negative to positive, with biomass increasing sharply in the high-glycosate ponds (Fig. 2a,d). Four weeks after the first pulse, we applied a second dose of glycosate

on day 34, assuming that all glycosate from the first pulse would have degraded by then. Instead, this second pulse led to in-pond glycosate concentrations approximately twice as high as targeted due to negligible degradation of the first pulse (Extended Data Fig. 1a) and evaporation causing a gradual decline in water level during Phase I (Extended Data Fig. 2a). Despite glycosate concentration exceeding  $30\text{ mg l}^{-1}$  in some ponds, this second (unintentionally more severe) dose did not have a negative effect on biomass. Rather, the glycosate–biomass relationship remained positive after the second pulse (Fig. 2e) and chlorophyll *a* concentration reached values  $>100\text{ }\mu\text{g l}^{-1}$  in all high-glycosate ponds by the end of Phase I (Fig. 2a). This long-term, positive effect of glycosate on biomass was strongest in low-nutrient (but high-glycosate) ponds (Fig. 2e). Chlorophyll *a* concentration also increased in high-nutrient, glycosate-free ponds over the course of Phase I, with phytoplankton biomass reaching its maximum value ( $585\text{ }\mu\text{g l}^{-1}$ ) in one of these ponds (yellow squares in Fig. 2a). Regression tree analysis confirmed the time-dependent effect of high doses of glycosate on biomass (a negative effect at doses  $>3\text{ mg l}^{-1}$  before day 15, and a positive effect at doses  $>4\text{ mg l}^{-1}$  after day 15), and the positive effect of the nutrient treatment on



**Fig. 3 | Effect of glyphosate on phytoplankton biodiversity.** **a,d**, Time series of richness (**a**) and alpha diversity (effective number of genera, **d**) in the 18 ponds for which we collected composition data. Symbols and colours are as in Fig. 2. **b,e**, Results of additive models predicting richness (**b**) or diversity (**e**) at the end of Phase I as a function of glyphosate concentration and nutrient treatment. **c,f**, Richness (**c**) and alpha diversity (**f**) of communities at the end of Phase II in relation to Phase I glyphosate treatment (target concentration after one dose). Data are shown for both glyphosate-treated ponds (yellow, cyan and purple symbols) and Phase II control ponds that do not receive the high dose of glyphosate (black symbols). Thick horizontal lines indicate group means. A small offset on x-axis values was introduced for visualization purposes.

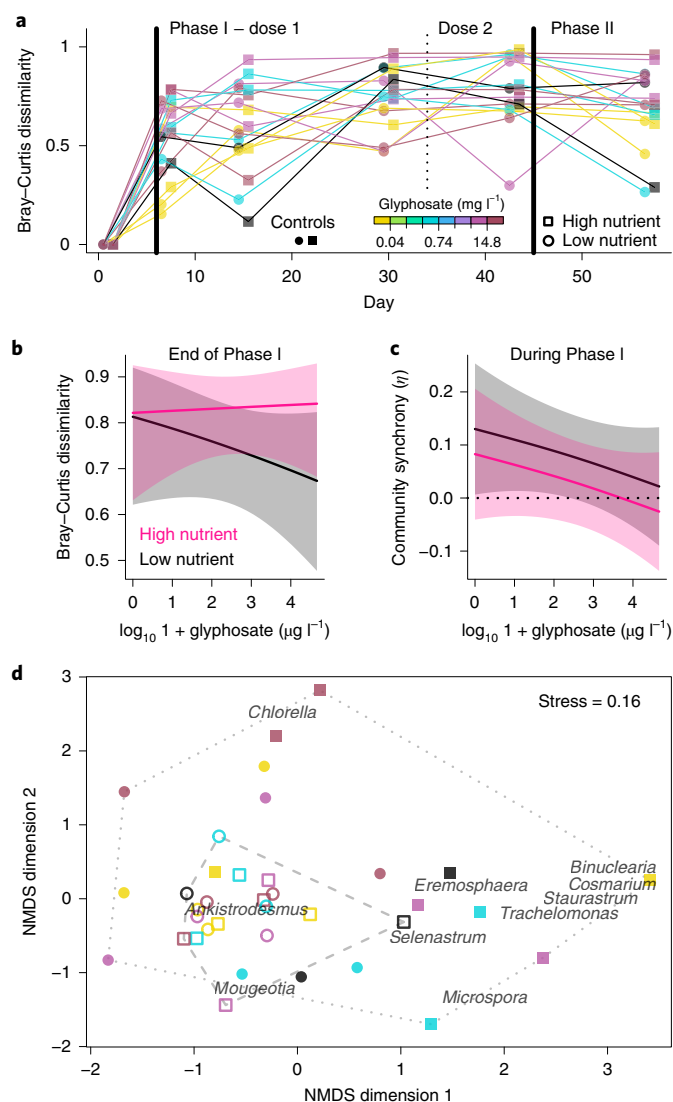
biomass but only in ponds receiving no or low doses of glyphosate (Extended Data Fig. 3).

We attribute the longer-term, positive effect of glyphosate during Phase I to the nutrient content of the glyphosate molecule (8.3% nitrogen and 18.3% phosphorus). Glyphosate application led to a strong, dose-dependent increase in total nitrogen (TN) and total phosphorus (TP) concentrations during Phase I (Extended Data Fig. 1b,c). Given the high N:P molar ratio of the ponds (>30), phytoplankton biomass production is expected to be limited by phosphorus (P), such that positive effects of glyphosate on biomass could occur when glyphosate degradation releases bioavailable P-containing compounds<sup>41,42</sup>. In high-glyphosate ponds, TP concentrations reached very high values (>1 mg l<sup>-1</sup>; Extended Data Fig. 1c), although most of this P probably consisted of biologically unavailable glyphosate molecules measured by the TP assay. Indeed, at the resolution of our analytical methods, there was no measurable glyphosate degradation over Phase I (Extended Data Fig. 1a); yet even a small degradation percentage could release a substantial amount of bioavailable P. For example, assuming that 0.5% of glyphosate degraded, this would correspond to an addition of ~30 μg l<sup>-1</sup> of bioavailable P in the ponds receiving the highest glyphosate dose (a nutrient input comparable to our high-nutrient treatment). Measurement of soluble reactive phosphorus on day 35 suggested that bioavailable P was slightly higher in ponds receiving the highest glyphosate dose (Extended Data Fig. 4). We suggest that a small percentage of glyphosate degraded during Phase I, which released bioavailable P and stimulated phytoplankton biomass production in high-glyphosate ponds. This ‘fertilizing’ effect was greatest in (presumably more P-limited) ponds receiving the low-nutrient treatment (Fig. 2e and Extended Data Fig. 3). Glyphosate and nutrient treatments had little influence on physicochemical parameters other than nutrient concentrations (Extended Data Fig. 2).

In Phase II of the experiment, when all communities (except two controls) were exposed to a severe dose of glyphosate expected to be

lethal (target in-pond concentration = 40 mg l<sup>-1</sup>), biomass collapsed (chlorophyll *a* <1 μg l<sup>-1</sup>) in most communities (Fig. 2a). However, some exposed communities retained as much biomass as the control communities, indicating community rescue. We then related the outcome of community rescue in Phase II to Phase I treatments; note that, despite positive effects of glyphosate on biomass, there was still ample independent variation across ponds in both biomass and glyphosate exposure by the end of Phase I, allowing us to distinguish these two influences on final phytoplankton biomass. We found that biomass at the end of Phase II was unrelated to both community biomass before Phase II (GAM, effect of Phase I chlorophyll *a*:  $P=0.613$ ; Fig. 2f) and to nutrient treatment (GAM, nutrient effect:  $P=0.357$ ; squares versus circles in Fig. 2f,g). In contrast, the extent of glyphosate exposure during Phase I was a very strong predictor of final phytoplankton biomass (GAM, effect of Phase I glyphosate:  $P<0.0001$ ;  $R^2=0.88$ ; Fig. 2g). High-biomass ponds that had not been exposed to glyphosate showed biomass collapse in Phase II, while communities pre-exposed to high doses of glyphosate did not. No obvious change in phytoplankton biomass or water chemistry was noted for the two control ponds during Phase II (Fig. 2a,f,g and Extended Data Fig. 2), suggesting that seasonal environmental changes cannot explain biomass collapse during Phase II.

Although the biomass response of all ponds within a given glyphosate treatment was very consistent (Fig. 2g), we noted substantial variability in glyphosate concentrations reached during Phase II, in part due to residual glyphosate from Phase I applications (Extended Data Fig. 1a). We tested for an effect of measured Phase II glyphosate concentration on Phase II phytoplankton biomass and found a positive relationship driven entirely by the response of high-glyphosate ponds (Extended Data Fig. 5). Because glyphosate added during Phase I hardly degraded, some high-glyphosate communities that retained high phytoplankton biomass in Phase II were also those that were exposed to the most extreme concentrations. For example, in two high-glyphosate ponds,



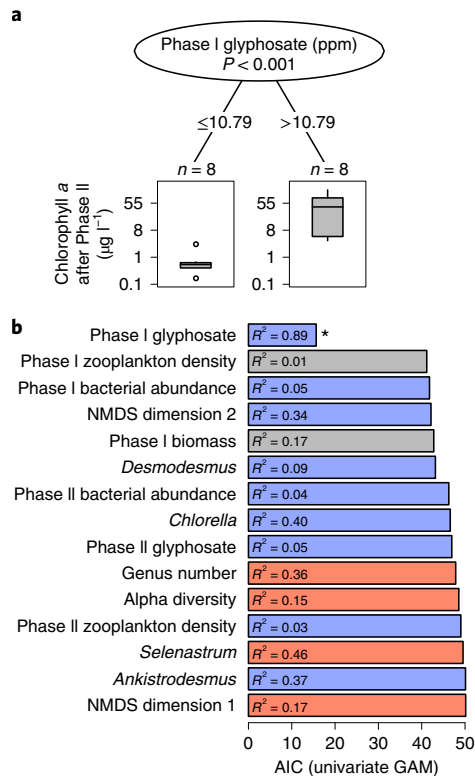
**Fig. 4 | Effect of glyphosate on phytoplankton community composition.**

**a**, Time series of Bray–Curtis dissimilarity of each pond relative to its starting composition. Higher values indicate greater community divergence over the course of the experiment. Symbols and colours are as in Figs. 2 and 3. **b,c**, Results of additive models predicting Bray–Curtis dissimilarity at the end of Phase I (**b**) or community synchrony ( $\eta$ ) during Phase I (**c**) as a function of glyphosate concentration and nutrient treatment. For the synchrony index, more negative values indicate more asynchronous dynamics, while a value of 0 indicates independent taxon fluctuations. **d**, NMDS representation of community composition at the beginning (open symbols) and end (full symbols) of Phase I based on Bray–Curtis dissimilarity. Convex hulls illustrate variation in community composition across ponds at the beginning (dashed line) and end (dotted line) of Phase I. The positions in two-dimensional space of the ten most abundant taxa are also shown.

Phase II glyphosate concentration exceeded  $80 \text{ mg l}^{-1}$  (Extended Data Fig. 1a), yet biomass remained as high in these ponds as in the two control ponds (Fig. 2g). The other six ponds treated with the highest or second highest glyphosate dose in Phase I reached a glyphosate concentration closer to target ( $40 \text{ mg l}^{-1}$ ), as did most ponds that did not receive glyphosate in Phase I. Yet, only ponds exposed to high doses in Phase I retained their biomass in Phase II, indicating that variation in Phase II glyphosate concentration (and its potential influence on nutrient concentrations) does not predict Phase II biomass responses.

We then characterized phytoplankton community composition and diversity in the 18 ponds for which we obtained biovolume data. These analyses suggested a gradual loss of diversity in high-glyphosate ponds over the course of Phase I (Fig. 3a,d). At the end of Phase I, glyphosate had a negative effect on both genus number and alpha diversity measured as the effective number of genera (GAM, effect of glyphosate:  $P=0.05$  for both response variables;  $R^2=0.11$  for genus number and 0.31 for the effective number of genera; Fig. 3b,e). In contrast, the nutrient treatment had no effect on genus number (GAM nutrient effect:  $P=0.411$ ; Fig. 3b); a marginally significant effect on the effective number of genera (GAM, nutrient effect:  $P=0.07$ ; Fig. 3e); and no effect on the relationship between glyphosate and diversity (GAM, interaction effect of glyphosate and nutrients:  $P>0.5$  for both richness and alpha diversity; Fig. 3b,e). At the end of Phase II, all glyphosate-treated communities had lower genus richness than control communities; for example, communities exposed to the highest dose in Phase I had, on average, 43% fewer genera than control communities despite their comparable final biomass (Fig. 3c). For alpha diversity, more variability was visible among ponds of a given glyphosate treatment but ponds exposed to the highest two doses in Phase I also had, on average, roughly 40% lower alpha diversity at the end of Phase II than control ponds (Fig. 3f). In spite of these negative effects on diversity, glyphosate exposure had little influence on overall phytoplankton community composition because of the presence of a few highly dominant taxa (*Selenastrum*, *Ankistrodesmus* and *Chlorella*) across all ponds. When comparing community composition at the beginning versus end of Phase I using the Bray–Curtis dissimilarity index, we noted that all ponds diverged from their starting composition regardless of their nutrient or glyphosate treatment (Fig. 4a). Dissimilarity at the end of Phase I, that is the extent of community divergence over the first 44 days of the experiment, was not significantly related to treatments (GAM, effects of glyphosate, nutrient treatment and their interaction:  $P>0.2$  for all effects; Fig. 4b). Community synchrony ( $\eta$ ) over Phase I was also unaffected by treatments (GAM, effects of glyphosate, nutrient treatment and their interaction:  $P>0.16$  for all effects; Fig. 4c). We expected greater temporal correlations in genus biovolume in high-glyphosate ponds, assuming that the herbicide would induce strong genus sorting<sup>42</sup>; instead, synchrony values were all close to zero, indicating that dynamics of different genera were mostly uncorrelated, even in high-glyphosate, high-nutrient ponds. Glyphosate exposure also had no significant effect on community composition at the genus level (Fig. 4d). Indeed, although composition was initially similar across ponds (Fig. 4d, open symbols), communities diverged in directions not predicted by their experimental treatments (Fig. 4d, full symbols). Distance-based multivariate analysis of variance (ANOVA) confirmed that neither glyphosate treatment ( $P=0.2$ ) nor nutrient treatment ( $P=0.65$ ) predicted community composition at the end of Phase I.

To determine which factors best predicted community biomass at the end of Phase II (the outcome of community rescue), we conducted two analyses in which Phase I glyphosate exposure, biomass, diversity and composition variables were all included as predictors of final phytoplankton biomass, in the 16 ponds for which data were available for all variables. We also included the density of crustacean zooplankton and bacteria as predictors, as grazing by the former could have influenced phytoplankton responses and as potential glyphosate degradation by the latter could modulate the toxicity of the glyphosate added in Phase II. In a regression tree analysis, we found that glyphosate exposure in Phase I was the only variable necessary to predict final phytoplankton biomass; a threshold exposure concentration of  $10.8 \text{ mg l}^{-1}$  during Phase I determined whether final biomass was below or above  $1 \mu\text{g l}^{-1}$  of chlorophyll *a* (Fig. 5a). Then, when fitting and comparing independent GAMs with one of 15 factors as the predictor variable and biomass at the end of the experiment as the response, we found that glyphosate concentration



**Fig. 5 | Predictors of final phytoplankton biomass. a**, Regression tree model of phytoplankton biomass at the end of Phase II as a function of various community properties. Results ( $P$  value) of a permutation test of a correlation between the response and the one significant predictor (glyphosate exposure during Phase I) is indicated. **b**, Model fit (AIC) of univariate GAMs with phytoplankton biomass at the end of Phase II as the response variable and one of the community properties used in **a** as the predictor variable. A lower AIC indicates better fit. Blue bars indicate positive monotonic trends, red bars negative monotonic trends and grey bars non-monotonic trends. Numbers inside bars correspond to adjusted  $R^2$ . Genus names represent relative biovolumes of a given taxon at the end of Phase I. \*Indicates that the GAM smooth term was statistically significant (in this case, at  $P < 0.001$ ); ppm, parts per million ( $\text{mg l}^{-1}$ ).

at the end of Phase I was by far the best predictor of final biomass (Fig. 5b). Not only was glyphosate treatment a poor predictor of community composition, as described above, but community composition at the end of Phase I was itself a poor predictor of final biomass in Phase II. Zooplankton densities at the end of Phase I and Phase II were also poor predictors of phytoplankton biomass at the end of the experiment (Fig. 5b); in fact, zooplankton density was highest in some high-glyphosate ponds with substantial phytoplankton biomass (Extended Data Fig. 6). The cladoceran herbivore *Chydorus sphaericus* was highly dominant in all glyphosate-treated ponds, irrespective of Phase I treatments or final phytoplankton biomass such that changes in zooplankton composition are unlikely to explain phytoplankton responses (see Supplementary Table 2 for additional information on zooplankton). Finally, bacterial abundance was also not a significant predictor of phytoplankton responses in Phase II. Therefore, the only significant driver of final phytoplankton biomass was glyphosate exposure during Phase I.

## Discussion

We found that a history of glyphosate exposure prevents biomass collapse when communities are subsequently confronted with a concentration that is normally lethal to naive communities.

Pre-exposure to glyphosate in Phase I was a very strong predictor of the outcome of community rescue in Phase II. This result is consistent with laboratory microcosm studies finding an influence of prior exposure on the likelihood of population and community rescue<sup>18,20</sup>, and with a mesocosm experiment in which we observed that community rescue from severe acidification was facilitated by prior exposure to low pH<sup>19</sup>. The collapse of glyphosate-naive communities to biomass values comparable to phytoplankton-free water validates that the dose of glyphosate used in Phase II was lethal to all constituent populations of the ancestral (source) community, which confirms that viable communities by the end of Phase II have undergone community rescue.

This result is also consistent with the ecotoxicological literature on pollution-induced community tolerance (PICT), which finds that prior exposure to a contaminant generally renders microbial communities more tolerant to that contaminant<sup>44,45</sup>. One key distinction between PICT and community rescue is that PICT usually tests tolerance to sublethal stress whereas community rescue applies to situation where the stress is expected to cause extirpation (hence the need for ‘rescue’). This distinction is akin to the distinction between rapid (contemporary) adaptation and evolutionary rescue; only some cases of contemporary adaptation are also examples of evolutionary rescue, that is when a stressor is severe enough to cause extirpation<sup>10</sup>. The theory of evolutionary rescue and its focus on absolute, rather than relative, fitness provided a new perspective to studies of human-induced evolution, asking whether adaptation can prevent extinction<sup>8,11</sup>. Likewise, the literature on PICT (for example, refs. 46–48) shall provide an empirical foundation upon which a theory of community rescue can be built, to determine the conditions under which induced tolerance can prevent community extirpation.

In contrast to previous studies and to our initial hypothesis, community biomass was not a significant predictor of biomass responses in Phase II. High-nutrient, glyphosate-naive ponds with a biomass similar or greater than high-glyphosate ponds by the end of Phase I lost their biomass in Phase II. This result contrasts with the only other community rescue experiment that manipulated community biomass (the laboratory experiment described in ref. 18) and suggests that high biomass alone was insufficient to ensure rescue in this experiment. One key difference between the two experiments which may explain this discrepancy is that the range of biomass variation in field (mesocosm) conditions might have been narrower than in the laboratory, such that no pond had a biomass so low as to hamper rescue. Nonetheless, in glyphosate-exposed communities, greater biomass may still favour rescue but we could not assess this interaction given the lack of glyphosate-exposed, low-biomass communities (due to the positive long-term effect of glyphosate on biomass).

The contrasting Phase II biomass response of communities with and without a history of glyphosate exposure indicates that Phase I exposure increased mean population fitness in a glyphosate-rich environment, when averaging across all species in the community. Various processes could bring about this increased community-averaged ‘glyphosate resistance’, including phenotypic plasticity (for example, induced tolerance<sup>49</sup>), evolution (sorting of standing genetic variation and de novo mutations) and ecological processes such as taxon sorting (species or higher taxonomic levels). We suggest that our results are inconsistent with a strong role for taxon sorting, at least at the genus level. Indeed, glyphosate treatment only induced weak sorting; the same genera could dominate control (glyphosate-susceptible) and exposed (glyphosate-resistant) ponds at the end of Phase I (see also ref. 50). Community composition did not predict the rescue outcome, nor did the relative biovolume of taxa common in (some) resistant communities. Although we cannot rule out that some unmeasured chemical- or community-level property of mesocosms treated with glyphosate in Phase I was responsible for

the maintenance of their biomass in Phase II, we quantified many physicochemical parameters as well as the density of other trophic levels (bacteria and zooplankton) and none of these variables predicted community rescue. For example, we can reject the hypothesis that greater bacterial abundance and faster glyphosate break-down in high-glyphosate ponds underlies their resistance in Phase II since high-glyphosate ponds are also those ponds that reached the highest concentrations due to prior accumulation of glyphosate (final glyphosate concentration is positively, not negatively, related to final phytoplankton biomass). Therefore, we suggest that the most parsimonious explanation for our results is that plasticity or evolution increased glyphosate resistance in exposed communities regardless of which genera were originally present in these communities.

Another study with the same lake community exposed to a different stressor (acidification) showed that a press perturbation induced rapid genotype sorting within species<sup>51</sup>. Quantifying the relative importance of plasticity, evolution and taxon sorting on the community-averaged trait 'glyphosate resistance' (regardless of its mechanistic basis) would require population pedigrees<sup>52</sup> or a reciprocal transplant experiment<sup>53</sup>; however, both approaches are challenging to implement in this experimental system where different taxa dominated glyphosate-resistant ponds and are thus the focus of ongoing research. For example, molecular analyses outside the scope of this study will help us clarify the role of clonal selection within species (an evolutionary process) and species sorting within genera (an ecological process) in response to glyphosate.

Our results also highlight the dual effect of glyphosate on a naive lake phytoplankton community: herbicidal at first but fertilizing over a longer period. Importantly, herbicidal effects were only observed at the highest experimental doses, in the  $\text{mg l}^{-1}$  range. Such concentrations exceed, by orders of magnitude, concentrations typically measured in water bodies in agricultural areas, which are generally in the  $\text{ng to } \mu\text{g l}^{-1}$  range<sup>35,37</sup>. Moreover, we used Roundup, reputed to be even more toxic than pure glyphosate due to its surfactant<sup>24,54,55</sup> and still recorded modest toxicity in phytoplankton (and zooplankton). Thus, in lakes with a plankton composition similar to our source community, runoff of glyphosate from agricultural fields is unlikely to cause a notable loss of plankton biodiversity and biomass. However, the longer-term, fertilizing effect of Roundup on phytoplankton biomass would be expected to occur at all doses. Indeed, all nutrients contained in commercial formulations of glyphosate applied to fields constitute a nutrient input that persists in the environment even after the herbicide degrades (unlike ecotoxicological effects, which eventually vanish once degradation is complete). In some areas with intensive agriculture of glyphosate-resistant crops, glyphosate application can now constitute a substantial source of anthropogenic P—an input that is comparable in magnitude to other P sources that have been previously regulated<sup>30</sup>. Other experimental studies, focused on cyanobacteria or diatoms instead of green algae, have also observed a fertilizing effect of glyphosate and attributed it to the nutrient content of the herbicide<sup>25,50,56,57</sup>. Thus, a key environmental impact of glyphosate pollution might be via its effect on nutrient loading<sup>25,57–59</sup>, an issue that warrants further investigation given the increasing use of this pesticide.

An alternative explanation for the 'fertilizing effect' of glyphosate would be weaker top-down control<sup>19</sup>; that is, if glyphosate toxicity cleared herbivorous zooplankton from high-glyphosate ponds. However, we can reject this hypothesis as high-glyphosate ponds also have more, not less, zooplankton at the end of Phase II. Yet it is possible that treatment effects on zooplankton influenced phytoplankton composition during Phase I, if not biomass. We cannot explain the variation that we observed in phytoplankton composition, which was not influenced by treatments. However, this would not invalidate our argument that final phytoplankton composition does not matter for the rescue outcome in Phase II.

Indeed, regardless of what drove changes in phytoplankton composition during Phase I (zooplankton or otherwise), the Phase II biomass response of all ponds within glyphosate treatment levels was remarkably consistent despite clear differences in composition.

To conclude, we observed community rescue in phytoplankton communities pre-exposed to glyphosate stress but not in communities naive to glyphosate. Remarkably, communities exposed to a glyphosate-rich environment for only a few weeks could retain their biomass when later confronted with a very high dose. Nonetheless, the highest concentrations of glyphosate necessary for subsequent rescue caused a loss of diversity, as glyphosate-resistant communities had ~40% fewer genera than uncontaminated ponds. This loss of diversity may hamper adaptation to other stressors. Therefore, one key avenue for future research will be to determine whether the loss of variation induced by rescue from one stressor influences the likelihood of rescue from another stressor<sup>60–63</sup>. This would better define the limits of community rescue in human-dominated landscapes where multiple stressors typically co-occur. Evidence of population rescue in nature is accumulating<sup>64–67</sup>; the next challenge will be to determine which constituents of affected communities can undergo rescue and whether they can sustain the recovery of ecosystem functions and services in degraded environments.

## Methods

**Experimental design.** The experiment was conducted at the LEAP facility at McGill University's Gault Nature Reserve in Québec, Canada (45° 32' N, 73° 08' W). This facility comprises >100 mesocosms (1,136-l Rubbermaid plastic tanks) that can be filled with water and planktonic organisms piped down from a lake (Lac Hertel) located 1 km upstream of the facility (Fig. 1a). Lac Hertel is a fully forested (and protected) watershed with no history of agriculture, so its community should be naive to glyphosate. All mesocosms were filled on 11 May 2016 with lake water sieved to remove fish but not plankton. Biweekly water changes of 10% total mesocosm volume (with lake water and organisms) were performed until the experiment began. Major terrestrial inputs (pollen and leaves) and tadpoles were removed periodically with a leaf skimmer to reduce initial heterogeneity among ponds. Our 34-pond experiment ran from 17 August (day 1) to 12 October (day 57), after which all mesocosm water was pumped into a sewage system that outflows into a large retention basin. Several months later, after glyphosate had degraded to a low concentration considered safe for aquatic life<sup>68</sup> and for human consumption<sup>69</sup>, the water was released in a field outside the protected area.

Figure 1b illustrates our experimental design. In Phase I of the experiment (days 1–44), we manipulated community biomass and pre-exposure to sublethal stress. Then, Phase II (days 45–57) of the experiment represented our rescue assay, when all ponds (except two controls) were exposed to a high dose of glyphosate expected to be lethal (see below). We manipulated community biomass in Phase I via a nutrient treatment applied to all 34 ponds, attributing 17 ponds to a 'mesotrophic' (low nutrient) treatment with a target TP concentration of  $15 \mu\text{g l}^{-1}$  (similar to Lac Hertel) and 17 ponds to a 'eutrophic' (high nutrient) treatment with a target TP concentration of  $60 \mu\text{g l}^{-1}$  (Fig. 1b). We prepared a concentrated nutrient solution of  $\text{KNO}_3$  ( $107.66 \text{ g l}^{-1}$ ),  $\text{KH}_2\text{PO}_4$  ( $2.17 \text{ g l}^{-1}$ ) and  $\text{K}_2\text{HPO}_4$  ( $2.82 \text{ g l}^{-1}$ ) with the same N:P molar ratio (33:1) as Lac Hertel in August 2016. Every 2 weeks for 8 weeks, 5 or 20 ml of that stock solution were applied to low- and high-nutrient ponds, respectively. The first nutrient addition took place on 10 August, 1 week before sampling started, to ensure that phytoplankton communities would have passed their exponential growth phase when applying the first pesticide pulse.

The glyphosate treatment of Phase I involved two pulses of Roundup Super Concentrate (Monsanto), applied on days 6 and 34. We used Roundup rather than pure glyphosate salt because local agricultural fields are sprayed with commercial formulations of glyphosate, not with the pure compound. Importantly, we used this herbicide as a general stressor to induce environmental degradation; the precise mechanism of toxicity was not the focus of our study. Across mesocosms, Roundup doses varied in their target concentration of glyphosate acid, the active ingredient in Roundup. A total of eight concentrations were used: control ponds ( $0 \text{ mg l}^{-1}$ ) and seven doses of glyphosate ( $0.04$ – $15 \text{ mg l}^{-1}$ ) separated by equal intervals on a logarithmic scale to cover a broad gradient (Fig. 1b; Phase I). Some doses used were greater than the Canadian aquatic toxicity criterion (environmental concentrations considered safe for aquatic life) for long-term glyphosate exposure but the range of concentrations used falls below the criterion for short-term exposure (Fig. 1b)<sup>68</sup>. These toxicity criteria are based on ecotoxicological assays with phytoplankton, plants, invertebrates, fish and amphibians. The glyphosate gradient was repeated four times; twice at each nutrient level (totalling 32 ponds; Fig. 1b). We also included one additional pesticide-free pond at each nutrient level to serve as controls for Phase II; thus, there were six control (glyphosate-free) ponds in Phase I (shown as yellow and black symbols in Fig. 1b) but two control

ponds for Phase II (black symbols in Fig. 1b). Roundup was added to the mesocosms to reach target concentrations, assuming a mean pond volume of 1,000 l. On the basis of existing literature<sup>56,70,71</sup>, we expected glyphosate to degrade quickly in water (for example half-life of 6–7 d in ref.<sup>70</sup>), such that both doses, separated by a 28-d interval, had the same target concentration. We applied two doses in Phase I to ensure sufficient exposure time for community adaptation.

Phase II began on day 45, when all ponds except the two controls were treated with glyphosate to reach a target in-pond concentration of 40 mg l<sup>-1</sup>, which exceeds the Canadian aquatic toxicity criterion for short-term exposure by 13 mg l<sup>-1</sup> (ref.<sup>68</sup>). In a laboratory pilot experiment with water samples from the mesocosms, a glyphosate concentration of 40 mg l<sup>-1</sup> also reduced chlorophyll *a* concentration to 0.2–0.4 µg l<sup>-1</sup>, which is comparable to chlorophyll *a* values observed in microfiltered lake water measured with the same instrument. In line with previous laboratory experiments that quantified 'rescue' by comparing the optical density of microbial populations with that of sterile medium<sup>12,13,18</sup>, we consider that 40 mg l<sup>-1</sup> of glyphosate represents a 'lethal stress' to our mesocosm communities as it leads to chlorophyll *a* concentrations comparable to water without phytoplankton. Community biomass at the end of Phase II (day 57), namely the capacity of a community to remain viable under severely deteriorated conditions that are normally lethal, was our measure of community rescue. Finally, because the 34 ponds used in this study were part of a larger (ecotoxicological) experiment with multiple agricultural stressors, two arrays of eight ponds (one at each nutrient level) receiving a glyphosate gradient in Phase I also received a gradient of imidacloprid, a neonicotinoid insecticide. This insecticide gradient had no detectable effect on any of the response variables that we report in this study, including zooplankton density at the end of the experiment (see Supplementary Results). Thus, both glyphosate gradients for each nutrient treatment were grouped and considered replicates.

**Sampling.** The sampling schedule for each response variable is shown in Fig. 1c. Mesocosm water was sampled with integrated samplers made from 2.5-cm diameter PVC tubing. Samples were collected at five random locations in the upper 35 cm of the water column and combined in a 1-l dark Nalgene bottle, previously triple-washed with pond water. Each pond had a dedicated sampler and bottle to minimize cross-pond contamination. While sampling, bottles were kept in coolers and then transferred to an on-site laboratory. The 1-l samples were used to measure nutrient concentrations and phytoplankton biomass and composition (glyphosate samples were collected separately; see later). To estimate phytoplankton biomass, 50 ml were poured into a dark microcentrifuge tube. Chlorophyll *a* concentration, a proxy for phytoplankton biomass, was then determined fluorometrically with a FluoroProbe (Moldaenke). The FluoroProbe determines both total phytoplankton biomass (pigment concentration) and the biomass of four major groups that differ in their pigment colouration and fluorescence: green algae (chlorophytes and euglenophytes), golden/brown algae (diatoms, chrysophytes and dinoflagellates), blue-green algae (cyanobacteria) and cryptophytes.

To measure phytoplankton community composition at a finer taxonomic resolution in a subset of ponds (receiving glyphosate dose 1 (controls), 4, 7 or 8), we preserved 45-ml samples with Lugol's iodine solution for later microscopic enumeration. Samples were identified to genus level using the Utermöhl method<sup>72</sup>. Subsamples were sedimented in a 10-ml settling chamber and then screened using an inverted phase contrast microscope (Zeiss). A minimum of 200 cells and ten fields were counted at both ×100 and ×400 magnifications, to include both large and small cells. Ten fields at ×40 magnification were also counted to identify large colonies. Colony number was multiplied by a genus-specific average number of cells per colony and then added to the cell count at higher magnification. Counts were converted to biovolume using a genus-specific mean cell volume obtained from a trait database for phytoplankton genera of Southern Québec (B.E. Beisner, unpublished observations). Missing values for some taxa were obtained from a larger, published database<sup>73</sup> accessed through the R package 'phytotraitr' (available from: <https://github.com/andrewdolman/phytotraitr>), using the median value reported for a given genus. For three (rare) taxa missing from this database, we used the value of a morphologically similar, closely related genus.

For nutrient concentrations, we retained 40-ml whole-water samples in acid-washed glass tubes, in duplicate, for TN and TP. Samples were refrigerated until processed in the GRIL (Groupe de Recherche Interuniversitaire en Limnologie) analytical laboratory at the Université du Québec à Montréal. Samples for TN were analysed with a continuous flow analyser (OI Analytical) using an alkaline persulfate digestion method, coupled with a cadmium reactor, following a standard protocol<sup>74</sup>. Phosphorus concentration was determined spectrophotometrically by the molybdenum blue method after persulfate digestion<sup>75</sup>. Pond TN and TP concentrations were estimated as the mean of the two duplicates. On day 36 of the experiment, one day after applying the second glyphosate dose, we measured TP and soluble reactive phosphorus (SRP) in 16 ponds (eight glyphosate doses × two nutrient treatments; in the two arrays without insecticide), to determine whether glyphosate applications increased SRP concentration. SRP was measured with the same protocol as TP but water samples were prefiltered with 0.45-µm syringe filters to exclude particulate phosphorus.

To measure in-pond glyphosate concentration and validate that we established the target gradient, 1-l water samples were collected in clear plastic bottles

immediately after applying Roundup. Samples were acidified to a pH < 3 with sulfuric acid and frozen at -20°C until analysis. Samples were collected in all ponds after each application of Roundup, as well as in a subset of ponds (dose 1 (control), 4 and 8; that is 0, 0.3 and 15 mg l<sup>-1</sup>) 8 and 23 d after the first dose, to estimate the rate of glyphosate degradation in our mesocosms. Glyphosate concentration was later determined in the Department of Chemical Engineering at McGill University with liquid chromatography heated electrospray ionization tandem mass spectrometry using an Accela 600-Orbitrap LTQ XL (LC-MS, Thermo Scientific). Acquisition was conducted in full scan mode (50–300 m/z) at high resolution (FTMS = 30,000 m/Dz), with an ion trap used to perform targeted data acquisition for the product ion spectra (MS2) and generate identification fragments. The accuracy of the method was 100.64%, while its limits of detection and quantification were 1.23 and 4.06 µg l<sup>-1</sup>, respectively. Data were analysed with Xcalibur 2.1.0 (Thermo Scientific). We also collected a sample of lake water and confirmed that it had no glyphosate.

Water pH, dissolved oxygen (DO) and specific conductance (SPC) were measured in situ in each mesocosm with a hand-held probe (YSI Inc.) placed in the volumetric centre of the pond. Measurements were taken at sunrise and sunset; the mean of both measurements was used to quantify the daily average. Depth in the centre of the pond was recorded with a metre stick; we only measured depth in glyphosate-free ponds as little variation was observed across the array. Water temperature was recorded every 15 min over the course of the experiment with HOB0 pendant autonomous temperature data loggers (Onset) used in all ponds. We also collected zooplankton samples at the end of Phases I and II of the experiment. A total 2 l of water collected with the integrated samplers in ten random locations were combined and filtered with a 64-µm sieve. Zooplankton were anaesthetized using carbonated water and then preserved in 95% ethanol to a final concentration of 75% ethanol. Abundance and density of crustaceans (cladocerans and copepods) were determined microscopically. Finally, we quantified bacterial abundance (BA) at the end of Phases I and II. From the 1-l water samples described above, a 1-ml aliquot was fixed with glutaraldehyde (1% final concentration), flash frozen in liquid nitrogen, and preserved at -80°C until analysis. In the laboratory, samples were thawed, diluted (1:25) with Tris-EDTA buffer (pH 8), aliquoted into two duplicate tubes and stained with SYTO 13 (Thermo Fisher) for 10 min at room temperature. Stained samples were ran on a BD Accuri C6 flow cytometer at an event rate of 100–1,000 events s<sup>-1</sup>. Cell count was then determined within a predefined 'gate' on a green fluorescence versus side-scatter cytogram to exclude background noise and large particles (1-µm standard fluorescent beads were used to delineate the gate). Cell counts were converted to bacterial density on the basis of flow duration, flow rate and sample dilution. The two analytical duplicates were averaged to get a single value of bacterial density per water sample.

**Statistical analyses.** All analyses were conducted in R v.3.5.0 (ref.<sup>76</sup>). Phytoplankton analyses only included green algae because FluoroProbe data indicated that this group contributed 98.6% of phytoplankton biomass when considering all ponds and sampling dates together. Rare golden/brown algae were detected at the onset of the experiment but went extinct quickly in all ponds irrespective of nutrient and glyphosate treatments. Other groups (for example, cyanobacteria and cryptophytes) were rare, with pigment concentrations generally below the limit of detection of the FluoroProbe.

Time series of chlorophyll *a* concentration (log transformed) in Phase I were modelled using GAMs fitted with the function 'gam' in the R package 'mgcv'<sup>77</sup>. We used GAMs for most analyses to account for the nonlinearity of many relationships, even when variables were log transformed. We validated GAMs using the 'gam.check' function in 'mgcv' to inspect the distribution of model residuals, to compare fitted and observed values, and to verify that the basis dimension of smooth terms (*k*) was large enough. Model fit (adjusted *R*<sup>2</sup>) and basis dimension of smooth terms for all GAMs are reported in Supplementary Table 1 for all models. Statistical significance of predictors was assessed using the summary.gam() function of mgcv. All smooth terms in GAMs were thin plate regression splines (bs='tp' in mgcv) unless noted otherwise.

To confirm that ponds from different glyphosate treatments did not initially differ in biomass, we first tested for an effect of nutrient treatment (a binary factor) and 'future glyphosate dose' (a smooth term corresponding to the log-transformed glyphosate treatment assigned to a given pond) on chlorophyll *a* on day 2, before the first glyphosate dose was applied. We then modelled chlorophyll *a* on all sampling occasions of Phase I (days 8–43) as a function of nutrient treatment, time (a smooth term), glyphosate concentration measured in the pond (log transformed; a tensor product interaction with time), a nutrient by glyphosate by time interaction (a 'difference smooth' based on the ordered factor 'nutrient' and modulating the time by glyphosate tensor product interaction) and 'pond' (a random effect/factor-smooth interaction with time; the mgcv syntax of this model and all other GAMs are provided in Supplementary Table 1). This model required a glyphosate concentration for all sampling occasions. Because we found no evidence of glyphosate degradation after the first pulse (see Results), glyphosate concentration in ponds that we did not sample on any given date was assumed to correspond to the concentration when the pond was sampled last (that is, after a Roundup addition). We also used regression tree analysis to



determine threshold concentrations of pesticides leading to significant changes in biomass during Phase I. We constructed a conditional inference regression tree with log-transformed chlorophyll *a* as the response, and date, nutrient treatment, and glyphosate and imidacloprid concentrations as predictors. This tree was fitted with the 'ctree' function in the R package 'party'<sup>78</sup>, using Monte Carlo permutation tests to assess the significance of correlations. Finally, to test the hypothesis that community biomass and pre-exposure to sublethal stress influence the likelihood of community rescue, we fitted a GAM with log-transformed chlorophyll *a* at the end of Phase II as the response variable and nutrient treatment (a factor) and log-transformed chlorophyll *a* and glyphosate concentration at the end of Phase I as predictors (two smooth terms). We only modelled Phase II biomass in ponds that received the lethal dose.

We then conducted a number of community analyses in the subset of ponds (18) with biovolume data, after confirming that biovolume and biomass data correlated well (see Extended Data Fig. 7). We calculated genus number and alpha diversity (effective number of genera<sup>79</sup>, the exponent of Shannon's diversity index) and used GAMs to test for an effect of glyphosate concentration, nutrient treatment and their interaction on these two variables, on the last time point of Phase I (for genus number, we used a Poisson GAM). Diversity at the end of Phase II was also examined but no statistical test was performed since all ponds received the same glyphosate dose. Divergence in community composition (relative biovolume of genera) during the experiment was quantified with the Bray–Curtis dissimilarity index. For each pond, we calculated dissimilarity at each time point relative to initial composition on day 2. We also quantified community synchrony during Phase I (between day 2 and day 44), to determine whether glyphosate exposure led to compensatory dynamics across genera. We estimated synchrony ( $\eta$ ) with the R package 'codyn'<sup>80</sup>, whereby  $\eta$  is the average temporal correlation between the biovolume of each genus and the total biovolume of all other genera in the community<sup>81</sup>. An  $\eta$  value of 1 indicates perfect synchrony (all taxa fluctuate in synchrony), a value of  $-1$  indicates perfect asynchrony among taxa (with biovolume remaining constant) and a value close to 0 indicates independent fluctuations among genera. We then tested whether treatments influenced community divergence (dissimilarity at the beginning versus end of Phase I) and  $\eta$  (community synchrony) by fitting GAMs with either variable as the response, and with nutrient treatment, glyphosate concentration at the end of Phase I (log transformed; a smooth term) and their interaction as predictors. The GAM of Bray–Curtis dissimilarity used a beta error structure. To visualize divergence in community composition during Phase I, we constructed non-metric multidimensional scaling (NMDS) representations of community composition in two dimensions, including data from day 2 (before treatments) and day 44 (end of Phase I). NMDS analysis was performed with the 'metaMDS' function in the R package 'vegan'<sup>81</sup>, using the Bray–Curtis dissimilarity index. Finally, we used distance-based permutational multivariate ANOVA to determine whether nutrient and glyphosate treatments influenced community composition at the end of Phase I. This analysis was implemented using the 'adonis' function in vegan and used a Bray–Curtis distance matrix as the response and either glyphosate or nutrient treatment as the predictor (our sample size was too small to include both factors in a single MANOVA).

To quantify which community variable best predicted rescue in Phase II, we then used univariate regression tree analysis and Akaike information criterion (AIC)-based model comparison of univariate GAMs. Both analyses used log-transformed chlorophyll *a* at the end of Phase II as the response and a number of (scaled) predictor variables hypothesized to influence community response to the lethal dose of glyphosate, namely glyphosate concentrations at the end of Phase I and Phase II (log transformed), community composition at the end of Phase I (the two NMDS axes), zooplankton and bacterial density at the end of Phases I and II (log transformed), and chlorophyll *a* (log transformed), genus number, alpha diversity and the biovolume (log transformed) of four taxa at the end of Phase I. These taxa were *Selenastrum*, *Ankistrodesmus*, *Desmodesmus* and *Chlorella*, which collectively accounted for 96.5% of total biovolume at the end of Phase II (and thus constitute the only taxa that could influence rescue). A conditional inference regression tree with these predictors was fitted as described above for Phase I chlorophyll *a*. A separate univariate GAM was also fitted for each of the 15 predictor variables and model fit (the extent to which each predictor is linked to rescue) was compared using AIC and adjusted  $R^2$ . The directionality of each relationship (increasing, decreasing or non-monotonic) was assessed visually with the 'plot.gam' function of 'mgcv'. These two analyses focused on the 16 ponds for which all data requirements were met.

**Reporting Summary.** Further information on research design is available in the Nature Research Reporting Summary linked to this article.

## Data availability

The data necessary to reproduce figures and results in this study are publicly archived in Figshare: <https://doi.org/10.6084/m9.figshare.11717361.v2>.

## Code availability

The R code necessary to reproduce figures and results in this study is available on GitHub: [https://github.com/VFugere/LEAP2016\\_NEE](https://github.com/VFugere/LEAP2016_NEE).

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## References

- Ceballos, G., Ehrlich, P. R. & Dirzo, R. Biological annihilation via the ongoing sixth mass extinction signaled by vertebrate population losses and declines. *Proc. Natl Acad. Sci. USA* **114**, E6089–E6096 (2017).
- Wake, D. B. & Vredenburg, V. T. Are we in the midst of the sixth mass extinction? A view from the world of amphibians. *Proc. Natl Acad. Sci. USA* **105**, 11466–11473 (2008).
- Thomas, C. D. Rapid acceleration of plant speciation during the Anthropocene. *Trends Ecol. Evol.* **30**, 448–455 (2015).
- Cardinale, B. J. et al. Biodiversity loss and its impact on humanity. *Nature* **486**, 59–67 (2012).
- Hooper, D. U. et al. A global synthesis reveals biodiversity loss as a major driver of ecosystem change. *Nature* **486**, 105–108 (2012).
- Chevin, L.-M., Gallet, R., Gomulkiewicz, R., Holt, R. D. & Fellous, S. Phenotypic plasticity in evolutionary rescue experiments. *Phil. Trans. R. Soc. Lond. B* **368**, 20120089 (2013).
- Kovach-Orr, C. & Fussmann, G. F. Evolutionary and plastic rescue in multitrophic model communities. *Phil. Trans. R. Soc. B* **368**, 20120084 (2013).
- Bell, G. Evolutionary rescue. *Annu. Rev. Ecol. Evol. Syst.* **48**, 605–627 (2017).
- Alexander, H. K., Martin, G., Martin, O. Y. & Bonhoeffer, S. Evolutionary rescue: linking theory for conservation and medicine. *Evol. Appl.* **7**, 1161–1179 (2014).
- Carlson, S. M., Cunningham, C. J. & Westley, P. A. H. Evolutionary rescue in a changing world. *Trends Ecol. Evol.* **29**, 521–530 (2014).
- Gomulkiewicz, R. & Holt, R. D. When does evolution by natural selection prevent extinction? *Evolution* **49**, 201–207 (1995).
- Bell, G. & Gonzalez, A. Evolutionary rescue can prevent extinction following environmental change. *Ecol. Lett.* **12**, 942–948 (2009).
- Bell, G. & Gonzalez, A. Adaptation and evolutionary rescue in metapopulations experiencing environmental deterioration. *Science* **332**, 1327–1330 (2011).
- Ramsayer, J., Kaltz, O. & Hochberg, M. E. Evolutionary rescue in populations of *Pseudomonas fluorescens* across an antibiotic gradient. *Evol. Appl.* **6**, 608–616 (2013).
- Samani, P. & Bell, G. Adaptation of experimental yeast populations to stressful conditions in relation to population size. *J. Evol. Biol.* **23**, 791–796 (2010).
- Gonzalez, A. & Bell, G. Evolutionary rescue and adaptation to abrupt environmental change depends upon the history of stress. *Phil. Trans. R. Soc. Lond. B* **368**, 20120079 (2013).
- Lachapelle, J. & Bell, G. Evolutionary rescue of sexual and asexual populations in a deteriorating environment. *Evolution* **66**, 3508–3518 (2012).
- Low-Décarie, E. et al. Community rescue in experimental metacommunities. *Proc. Natl Acad. Sci. USA* **112**, 14307–14312 (2015).
- Bell, G. et al. Trophic structure modulates community rescue following acidification. *Proc. R. Soc. B* **286**, 20190856 (2019).
- Fussmann, G. F. & Gonzalez, A. Evolutionary rescue can maintain an oscillating community undergoing environmental change. *Interface Focus* **3**, 20130036 (2013).
- Hillebrand, H. et al. Decomposing multiple dimensions of stability in global change experiments. *Ecol. Lett.* **21**, 21–30 (2018).
- Pimm, S. L. The complexity and stability of ecosystems. *Nature* **307**, 321–326 (1984).
- McCann, K. S. The diversity–stability debate. *Nature* **405**, 228–233 (2000).
- Tsui, M. T. K. & Chu, L. M. Aquatic toxicity of glyphosate-based formulations: comparison between different organisms and the effects of environmental factors. *Chemosphere* **52**, 1189–1197 (2003).
- Saxton, M. A., Morrow, E. A., Bourbonniere, R. A. & Wilhelm, S. W. Glyphosate influence on phytoplankton community structure in Lake Erie. *J. Great Lakes Res.* **37**, 683–690 (2011).
- Christy, S. L., Karlander, E. P. & Parochetti, J. V. Effects of glyphosate on the growth rate of *Chlorella*. *Weed Sci.* **29**, 5–7 (1981).
- Wong, P. K. Effects of 2,4-D, glyphosate and paraquat on growth, photosynthesis and chlorophyll—a synthesis of *Scenedesmus quadricauda* Berb 614. *Chemosphere* **41**, 177–182 (2000).
- Benbrook, C. M. Trends in glyphosate herbicide use in the United States and globally. *Environ. Sci. Eur.* **28**, 3 (2016).
- Duke, S. O. & Powles, S. B. Glyphosate: a once-in-a-century herbicide. *Pest Manag. Sci.* **64**, 319–325 (2008).
- Hébert, M.-P., Fugère, V. & Gonzalez, A. The overlooked impact of rising glyphosate use on phosphorus loading in agricultural watersheds. *Front. Ecol. Environ.* **17**, 48–56 (2019).

31. Gilbert, N. A hard look at GM crops. *Nature* **497**, 24–26 (2013).
32. Hicks, H. L. et al. The factors driving evolved herbicide resistance at a national scale. *Nat. Ecol. Evol.* **2**, 529–536 (2018).
33. Green, J. M. The rise and future of glyphosate and glyphosate-resistant crops. *Pest Manag. Sci.* **74**, 1035–1039 (2018).
34. Kreiner, J. M., Stinchcombe, J. R. & Wright, S. I. Population genomics of herbicide resistance: adaptation via evolutionary rescue. *Annu. Rev. Plant Biol.* **69**, 611–635 (2018).
35. Van Bruggen, A. H. C. et al. Environmental and health effects of the herbicide glyphosate. *Sci. Total Environ.* **616–617**, 255–268 (2018).
36. Motta, E. V. S., Raymann, K. & Moran, N. A. Glyphosate perturbs the gut microbiota of honey bees. *Proc. Natl Acad. Sci. USA* **115**, 10305–10310 (2018).
37. Annett, R., Habibi, H. R. & Hontela, A. Impact of glyphosate and glyphosate-based herbicides on the freshwater environment. *J. Appl. Toxicol.* **34**, 458–479 (2014).
38. Helander, M., Saloniemi, I. & Saikkonen, K. Glyphosate in northern ecosystems. *Trends Plant Sci.* **17**, 569–574 (2012).
39. Relyea, R. A. The impact of insecticides and herbicides on the biodiversity and productivity of aquatic communities. *Ecol. Appl.* **15**, 618–627 (2005).
40. Giroux, I. *Présence de pesticides dans l'eau au Québec: Portrait et tendances dans les zones de maïs et de soya – 2011 à 2014* (MELCC, 2015); <https://go.nature.com/2SfqYGc>
41. Dill, G. M. et al. in *Glyphosate Resistance in Crops and Weeds: History, Development, and Management* (ed. Nandula, V.) 1–33 (Wiley, 2010).
42. Hove-Jensen, B., Zechel, D. L. & Jochimsen, B. Utilization of glyphosate as phosphate source: biochemistry and genetics of bacterial carbon-phosphorus lyase. *Microbiol. Mol. Biol. Rev.* **78**, 176–197 (2014).
43. Gross, K. et al. Species richness and the temporal stability of biomass production: a new analysis of recent biodiversity experiments. *Am. Nat.* **183**, 1–12 (2014).
44. Tlili, A. et al. Pollution-induced community tolerance (PICT): towards an ecologically relevant risk assessment of chemicals in aquatic systems. *Freshw. Biol.* **61**, 2141–2151 (2016).
45. Blanck, H. & Wängberg, S.-Å. Induced community tolerance in marine periphyton established under Arsenate stress. *Can. J. Fish. Aquat. Sci.* **45**, 1816–1819 (1988).
46. Bérard, A. & Benninghoff, C. Pollution-induced community tolerance (PICT) and seasonal variations in the sensitivity of phytoplankton to atrazine in nanocosms. *Chemosphere* **45**, 427–437 (2001).
47. Gustavson, K. et al. Pollution-induced community tolerance (PICT) in coastal phytoplankton communities exposure to copper. *Hydrobiologia* **416**, 125–138 (1999).
48. Millward, R. N. & Grant, A. Assessing the impact of copper on nematode communities from a chronically metal-enriched estuary using pollution-induced community tolerance. *Mar. Pollut. Bull.* **30**, 701–706 (1995).
49. Hua, J., Morehouse, N. I. & Relyea, R. Pesticide tolerance in amphibians: induced tolerance in susceptible populations, constitutive tolerance in tolerant populations. *Evol. Appl.* **6**, 1028–1040 (2013).
50. Pizarro, H. et al. Glyphosate input modifies microbial community structure in clear and turbid freshwater systems. *Environ. Sci. Pollut. Res.* **23**, 5143–5153 (2016).
51. Thibodeau, G., Walsh, D. A. & Beisner, B. E. Rapid eco-evolutionary responses in perturbed phytoplankton communities. *Proc. R. Soc. B* **282**, 20151215 (2015).
52. van Benthem, K. J. et al. Disentangling evolutionary, plastic and demographic processes underlying trait dynamics: a review of four frameworks. *Methods Ecol. Evol.* **8**, 75–85 (2017).
53. Govaert, L., Pantel, J. H., De Meester, L. & Coulson, T. Eco-evolutionary partitioning metrics: assessing the importance of ecological and evolutionary contributions to population and community change. *Ecol. Lett.* **19**, 839–853 (2016).
54. Cuhra, M., Traavik, T. & Böhn, T. Clone- and age-dependent toxicity of a glyphosate commercial formulation and its active ingredient in *Daphnia magna*. *Ecotoxicology* **22**, 251–262 (2013).
55. Lipok, J., Studnik, H. & Gruyaert, S. The toxicity of Roundup® 360 SL formulation and its main constituents: glyphosate and isopropylamine towards non-target water photoautotrophs. *Ecotoxicol. Environ. Saf.* **73**, 1681–1688 (2010).
56. Vera, M. S. et al. New evidences of Roundup® (glyphosate formulation) impact on the periphyton community and the water quality of freshwater ecosystems. *Ecotoxicology* **19**, 710–721 (2010).
57. Austin, A. P., Harris, G. E. & Lucey, W. P. Impact of an organophosphate herbicide (Glyphosate®) on periphyton communities developed in experimental streams. *Bull. Environ. Contam. Toxicol.* **47**, 29–35 (1991).
58. Gaupp-Berghausen, M., Hofer, M., Rewald, B. & Zaller, J. G. Glyphosate-based herbicides reduce the activity and reproduction of earthworms and lead to increased soil nutrient concentrations. *Sci. Rep.* **5**, 12886 (2015).
59. Harris, T. D. & Smith, V. H. Do persistent organic pollutants stimulate cyanobacterial blooms? *Inland Waters* **6**, 124–130 (2016).
60. Brennan, G. & Collins, S. Growth responses of a green alga to multiple environmental drivers. *Nat. Clim. Change* **5**, 892–897 (2015).
61. Zhang, C., Jansen, M., De Meester, L. & Stoks, R. Thermal evolution offsets the elevated toxicity of a contaminant under warming: a resurrection study in *Daphnia magna*. *Evol. Appl.* **11**, 1425–1436 (2018).
62. Kelly, M. W., DeBiasse, M. B., Villela, V. A., Roberts, H. L. & Cecola, C. F. Adaptation to climate change: trade-offs among responses to multiple stressors in an intertidal crustacean. *Evol. Appl.* **9**, 1147–1155 (2016).
63. Bell, G. Evolutionary rescue and the limits of adaptation. *Phil. Trans. R. Soc. Lond. B* **368**, 20120080 (2013).
64. Schiebelhut, L. M., Puritz, J. B. & Dawson, M. N. Decimation by sea star wasting disease and rapid genetic change in a keystone species, *Pisaster ochraceus*. *Proc. Natl Acad. Sci. USA* **115**, 7069–7074 (2018).
65. Whitehead, A., Clark, B. W., Reid, N. M., Hahn, M. E. & Nacci, D. When evolution is the solution to pollution: key principles, and lessons from rapid repeated adaptation of killifish (*Fundulus heteroclitus*) populations. *Evol. Appl.* **10**, 762–783 (2017).
66. Matz, M. V., Tremblay, E. A., Aglyamova, G. V. & Bay, L. K. Potential and limits for rapid genetic adaptation to warming in a Great Barrier Reef coral. *PLoS Genet.* **14**, e1007220 (2018).
67. Epstein, B. et al. Rapid evolutionary response to a transmissible cancer in Tasmanian devils. *Nat. Commun.* **7**, 12684 (2016).
68. Canadian water quality guidelines for the protection of aquatic life: Glyphosate. *Canadian Environmental Quality Guidelines* (Canadian Council of Ministers of the Environment, 2012); <http://st-ts.cme.ca/en/index.html>
69. *Guidelines for Canadian Drinking Water Quality—Summary Table* (Health Canada, 2017).
70. Pérez, G. L. et al. Effects of the herbicide Roundup on freshwater microbial communities: a mesocosm study. *Ecol. Appl.* **17**, 2310–2322 (2007).
71. Khadra, M., Planas, D., Girard, C. & Amyot, M. Age matters: submersion period shapes community composition of lake biofilms under glyphosate stress. *FACETS* **3**, 934–951 (2018).
72. Lund, J. W. G., Kipling, C. & Le Cren, E. D. The inverted microscope method of estimating algal numbers and the statistical basis of estimations by counting. *Hydrobiologia* **11**, 143–170 (1958).
73. Kremer, C. T., Gillette, J. P., Rudstam, L. G., Brettum, P. & Ptacnik, R. A compendium of cell and natural unit biovolumes for >1200 freshwater phytoplankton species. *Ecology* **95**, 2984–2984 (2014).
74. Patton, C. J. & Kryskalla, J. R. *Methods of analysis by the U.S. Geological Survey National Water Quality Laboratory: evaluation of alkaline persulfate digestion as an alternative to Kjeldahl digestion for determination of total and dissolved nitrogen and phosphorus in water* Water-Resources Investigations Report 2003-4174 (USGS, 2003); <http://pubs.er.usgs.gov/publication/wri034174>
75. Wetzel, R. G. & Likens, G. *Limnological Analyses* (Springer Science & Business Media, 2000).
76. R Core Team. *R: A Language and Environment for Statistical Computing* (R Foundation for Statistical Computing, 2018).
77. Wood, S. N. *Generalized Additive Models: An Introduction with R* (Chapman and Hall/CRC, 2017).
78. Hothorn, T., Hornik, K. & Zeileis, A. Unbiased recursive partitioning: a conditional inference framework. *J. Comput. Graph. Stat.* **15**, 651–674 (2006).
79. Jost, L. Entropy and diversity. *Oikos* **113**, 363–375 (2006).
80. Hallett, L. M. et al. codyn: An R package of community dynamics metrics. *Methods Ecol. Evol.* **7**, 1146–1151 (2016).
81. Oksanen, J. et al. *vegan: Community Ecology Package* R package version 2.5-6 (2019); <https://CRAN.R-project.org/package=vegan>

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**Author contributions**

V.F., M.-P.H., R.D.H.B., B.E.B., G.B., G.F.F., B.J.S. and A.G. designed the study. V.F., M.-P.H. and N.B.C. collected the data. C.C.Y.X. and V.Y. contributed to the development of laboratory methods. B.E.B. provided a phytoplankton trait database for biovolume estimation. V.F. analysed data, made the figures and drafted the manuscript. All authors contributed significantly to data interpretation and commented on manuscript drafts.

**Competing interests**

The authors declare no competing interests.

**Additional information**

**Extended data** is available for this paper at <https://doi.org/10.1038/s41559-020-1134-5>.

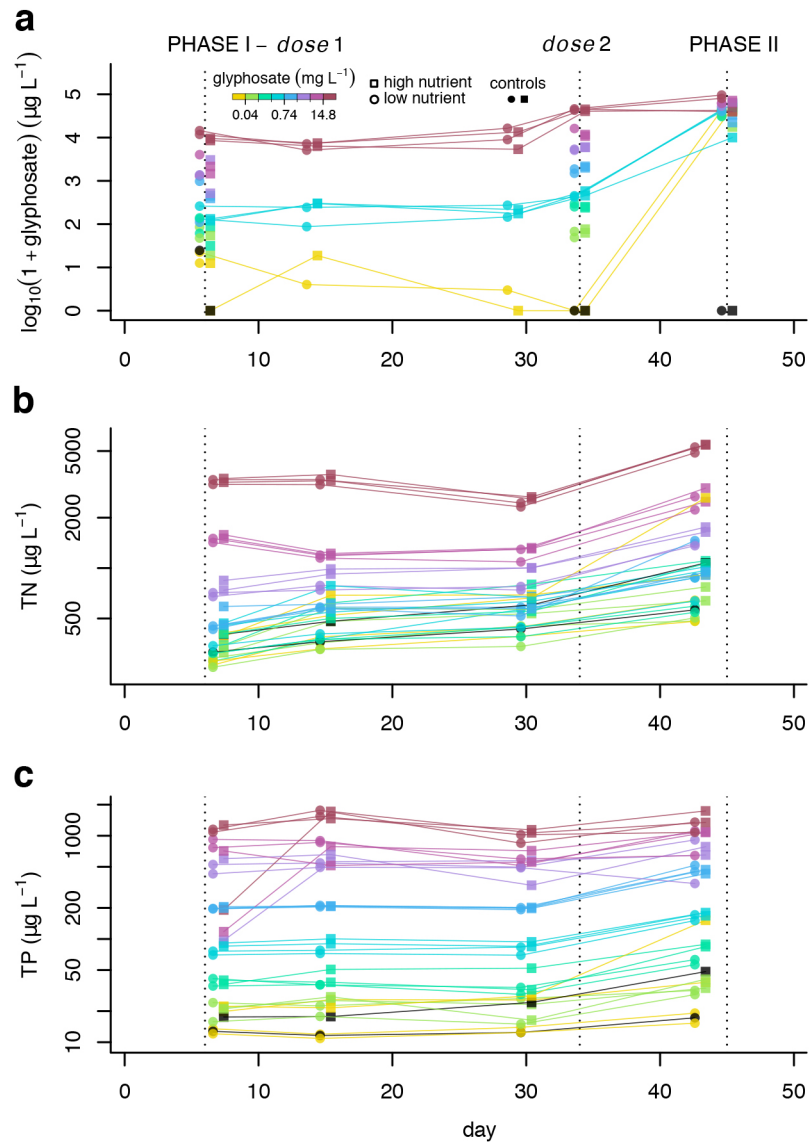
**Supplementary information** is available for this paper at <https://doi.org/10.1038/s41559-020-1134-5>.

**Correspondence and requests for materials** should be addressed to V.F. or A.G.

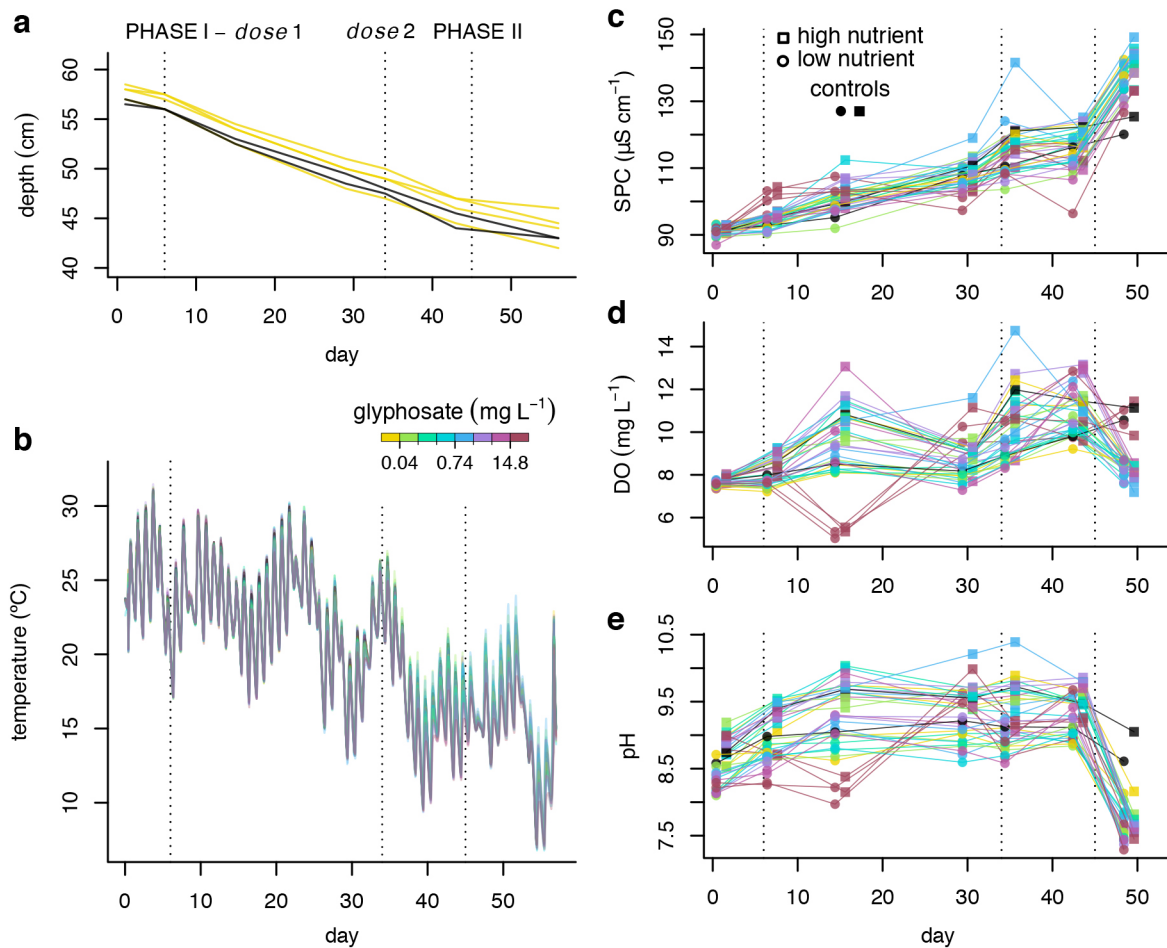
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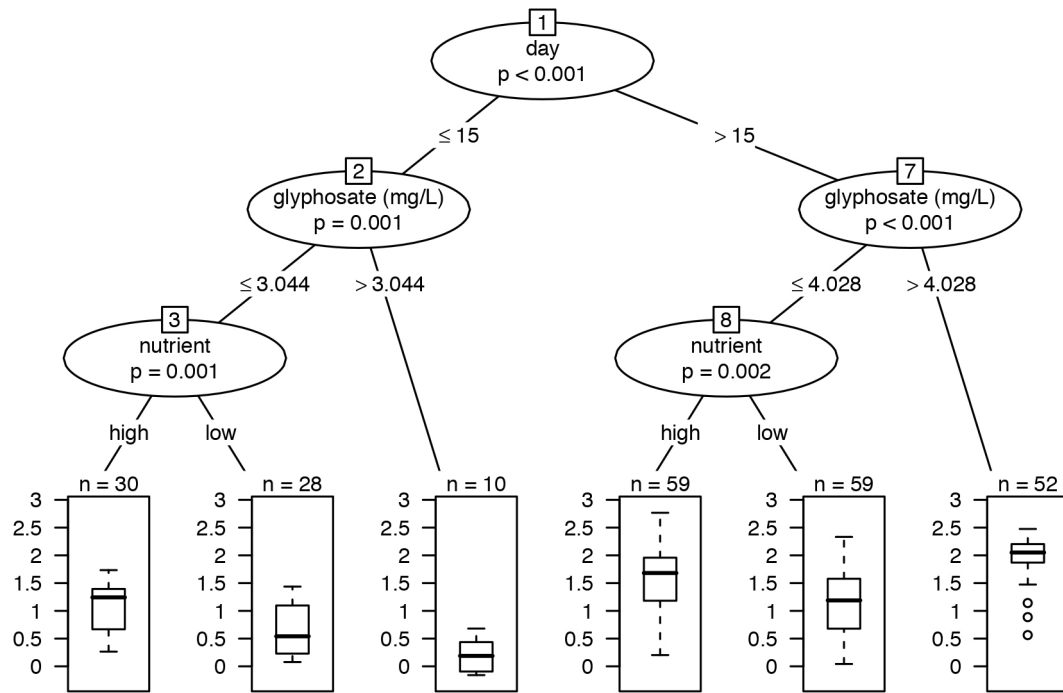
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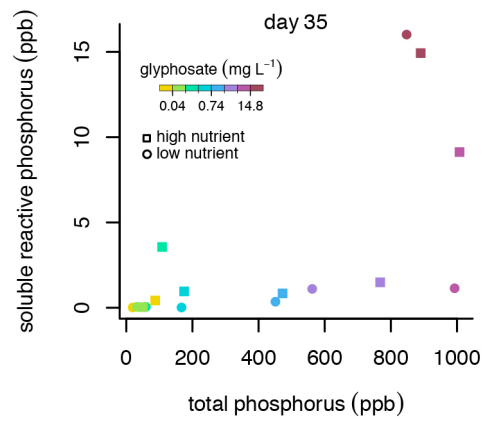
**Extended Data Fig. 1 | Glyphosate and nutrient concentrations.** Time series of glyphosate concentration (**a**), total nitrogen (TN; **b**), and total phosphorus (TP; **c**) during the experiment. Lines are colour-coded by glyphosate treatment while symbols indicate nutrient treatment (**a** small offset on x axis values was introduced to better distinguish low and high nutrient ponds). In (**a**), low glyphosate concentrations in some control ponds in early Phase I are most likely the product of field contamination.



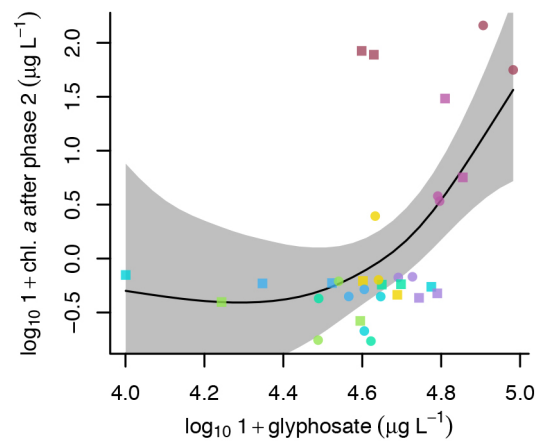
**Extended Data Fig. 2 | Other physicochemical parameters.** Time series of water depth (**a**), temperature (**b**), specific conductance (SPC; **c**), dissolved oxygen (DO; **d**), and pH (**e**) during the experiment. Lines are colour-coded by glyphosate treatment while symbols indicate nutrient treatment, as in Extended Data 1. (**a,b**) Depth and temperature varied over time but not across mesocosms. (**c**) Mean specific conductance increased slightly over Phase I (from 91 to 116  $\mu\text{S}/\text{cm}$ ), indicative of solute accumulation in the mesocosms due to evaporation. (**d**) Dissolved oxygen concentration tracked changes in phytoplankton biomass and was negatively affected by the first glyphosate pulse in the ponds exposed to the highest dose. (**e**) pH was mostly stable over time, although the highest glyphosate doses temporarily lowered pH by < 1 unit. In Phase II, biomass collapse in most communities decreased dissolved oxygen concentration (**d**), while specific conductance and pH respectively increased and decreased in all ponds that received the lethal dose irrespective of the response of their phytoplankton community (**c,e**).



**Extended Data Fig. 3 | Drivers of Phase I biomass dynamics.** Regression tree model of phytoplankton biomass over the course of Phase I as a function of date, nutrient treatment, and glyphosate and imidacloprid concentrations. Boxplots show the distribution of log-transformed chlorophyll a values in each group. Results (p value) of permutation tests of a correlation between the response and significant predictors are indicated. The tree demonstrates that at dates <math>< 15</math> days, biomass is negatively affected only in ponds receiving more than 3 mg/L of glyphosate, while after day 15, biomass increases with glyphosate treatment but only in ponds receiving doses > 4 mg/L. This regression tree also shows that the positive effect of the nutrient treatment on biomass is only significant in low-glyphosate ponds, and it confirms that imidacloprid had no discernible effect on phytoplankton biomass.

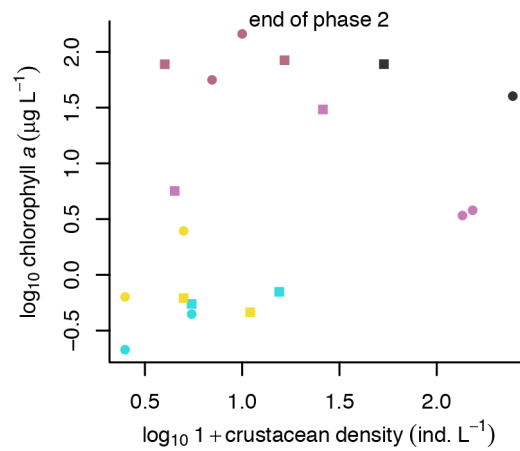


**Extended Data Fig. 4 | Phosphorus data.** Relationship between total phosphorus (TP) and soluble reactive phosphorus (SRP) in 16 ponds on day 35 of the experiment, shortly after the second glyphosate dose was applied. Symbols indicate nutrient treatment while colours indicate glyphosate treatment. Note that ponds receiving the highest glyphosate dose also show the highest concentration of SRP. However, given that SRP was measured immediately after a glyphosate application but several weeks after the onset of the nutrient treatment, and given that SRP is often assimilated very quickly by phytoplankton, these measurements likely under-estimate the effect of the nutrient treatment on SRP. ppb = parts per billion ( $\mu\text{g/L}$ ).

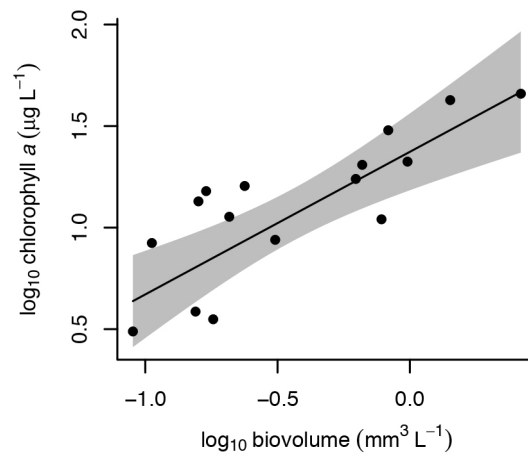


**Extended Data Fig. 5 | Final biomass vs. final glyphosate concentration.** Phytoplankton biomass at the end of Phase II as a function of Phase II glyphosate concentration. Although all ponds had a target in-pond concentration of 40 mg/L for the lethal dose in Phase II, residual glyphosate from past exposure, and/or potential error associated with glyphosate measurements for some ponds, led to unintended variance in measured concentrations at the beginning of Phase II. The effect of glyphosate concentration on phytoplankton biomass ( $\log 1+x$  transformed) was statistically significant in a GAM ( $p = 0.007$ ). However, the modelled relationship was positive (thus excluding the possibility that high-biomass ponds received less glyphosate in Phase II), driven by the high biomass of high-glyphosate ponds, and failed to capture the response of the two high-nutrient ponds that received the highest glyphosate dose in Phase I. Symbols indicate nutrient and glyphosate treatment as in Extended Data 4. chl. = chlorophyll.





**Extended Data Fig. 6 | Final biomass vs. zooplankton density.** Phytoplankton biomass at the end of Phase II as a function of crustacean zooplankton density at the end of Phase II. Symbol shape indicates nutrient treatment while symbol colour indicates Phase I glyphosate treatment, following the same nomenclature as all other figures. Note that the GAM testing for an effect of zooplankton density ( $\log 1+x$  transformed) on phytoplankton biomass ( $\log$  transformed) described in the main text (Fig. 5b) excluded the two control ponds (black symbols in this figure), as for all other predictor variables—hence the low explanatory power of the zooplankton GAM in Fig. 5b.



**Extended Data Fig. 7 | Correspondence between biomass and biovolume.** Relationship between community biovolume and biomass measured as chlorophyll a concentration. Each symbol represents a pond for which biovolume and biomass data were averaged across time points. The line and polygon indicate fitted values with confidence intervals from a GAM with biomass as the response and biovolume as the predictor. Biovolume was a significant predictor of biomass ( $p = 0.0004$ ), and the adjusted  $R^2$  of this GAM was 0.54. The Pearson correlation coefficient between the two variables was 0.75 for the time-averaged data and 0.56 for the raw (unaveraged) data considering all pond by sampling occasion combinations as independent data points.

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### 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](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                                 | Involvement in the study  |
|-------------------------------------|---|
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Antibodies                             |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Eukaryotic cell lines                  |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Palaeontology                          |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> Animals and other organisms |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Human research participants            |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Clinical data                          |

### Methods

- | n/a                                 | Involvement in the study                        |
|-------------------------------------|---|
| <input checked="" type="checkbox"/> | <input type="checkbox"/> ChIP-seq               |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Flow cytometry         |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> MRI-based neuroimaging |

## Animals and other organisms

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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 $\mu\text{m}$ 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.