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SUPPLEMENTARY INFORMATION

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Community rescue in experimental phytoplankton communities facing severe herbicide pollution

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Supplementary Information for:

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Supplementary Results

Effect of imidacloprid

Sixteen ponds were part of two experimental arrays of 8 ponds (one of each nutrient treatment) which received two pulse applications of imidacloprid in addition to the glyphosate gradient (Fig. 1). Imidacloprid is a neonicotinoid insecticide that inhibits nicotinic acetylcholine receptors in the central nervous system of insects. This neurotoxin is not expected to have toxic effects on phytoplankton, but it could indirectly influence the response of algae to glyphosate via its potential toxic effects on crustacean zooplankton. The imidacloprid gradient included 8 doses at equal intervals on a logarithmic scale, namely 0, 0.15, 0.41, 1.1, 3, 8.16, 22.19, and 60.31 μg/L. Pure analytical grade imidacloprid (Sigma-Aldrich: Oakville, ON) was dissolved in de-ionized water and added to mesocosms to reach target concentrations, assuming a pond volume of 1000 L. The two pulse applications occurred on the same day as the two glyphosate pulses of Phase I. No third pulse was applied, i.e. there was no 'Phase II' for the imidacloprid treatment since we did not observe any response to the treatment in Phase I, either in these two arrays with both imidacloprid and glyphosate (included in this study) or in arrays contaminated with imidacloprid only (data not show here).

For both algal biomass (Fig. 2) and physico-chemical parameters (Figs. S1-S2), limited variation within glyphosate and nutrient levels suggest that the imidacloprid gradient had little impact on responses to treatments (i.e. all four data points of a given colour show a consistent response even though two of them also included an imidacloprid treatment). Likewise, high zooplankton density in the high-glyphosate ponds (which include two high-imidacloprid ponds) suggest that the insecticide treatment did not induce high mortality in crustaceans. To test effects of imidacloprid statistically, we refitted all additive models described in the main text while also adding a smooth term for imidacloprid (log 1 + the targeted dose). For the model of biomass dynamics over Phase I, this effect included an interaction with time, as for the glyphosate effect. In all models with an extra imidacloprid effect, the statistical significance of glyphosate and nutrient effects reported in the main text remained unchanged, even when the smoothing parameter (k) of the GA(M)M had to be lowered to accommodate the extra smooth term.

Future imidacloprid treatment had no effect on algal biomass on day 2, before treatments were applied (GAM, effect of 'future imidacloprid dose': $p = 0.931$). Imidacloprid exposure then had no effect on algal biomass during Phase I (GAMM, tensor product interaction between time and imidacloprid: $p = 0.649$), nor did it predict biomass at the end of Phase II (GAM, effect of Phase I imidacloprid: $p = 0.096$). A regression tree analysis also confirmed that imidacloprid had no effect on chlorophyll *a* concentration during Phase I (Fig. S3). In the subset of ponds with community composition data, we also found no effect of imidacloprid on genus number (GAM, effect of imidacloprid: $p = 0.183$), alpha diversity ($p = 0.382$), community divergence (Bray-Curtis dissimilarity) during Phase I ($p = 0.472$), community synchrony during Phase I ($p = 0.562$), or ordination axes (NMDS1: $p = 0.476$; NMDS2: $p = 0.532$). Finally, imidacloprid treatment during Phase I also had no effect on zooplankton density at the end of Phase II (GAM, effect of imidacloprid: $p = 0.122$). We thus conclude that the imidacloprid treatment had no measurable effect on any of the response variables discussed in this manuscript. Hence, pairs of ponds receiving the same nutrient and glyphosate treatment in Phase I were considered replicates even if they differed in their imidacloprid concentration.

Supplementary Table 1. Additional information on (generalized) additive (mixed) models discussed in the main text. For each (G)A(M)M, we report the response variable, the day(s) of the experiment on which the modelled data were collected, the sample size (*n*), the predictors and their R (mgcv) syntax, the error structure for the residuals (along with the link function in case of generalized models), the adjusted R^2 of the model, the number of basis dimensions for smooth terms (*k*), and the figure panels in which model results are illustrated. Note that day 2 was before the first glyphosate dose was applied, days 8 and 43 correspond to the beginning and end of Phase I, and day 57 corresponds to the end of Phase II. Predictors included in a 's()' or 'ti()' function are thin plate regression splines and tensor product interactions, respectively. Unquoted predictors are parametric terms. Factor-smooth interactions using 'nutrient.ordered' as a factor are 'difference smooths', testing whether a given smooth function differs between high and low-nutrient ponds. gly. = glyphosate.

response	**day**	***n***	**predictors** *(significant effects in bold)*	**error**	R^2	***k***	**Figure**
log chlorophyll *a*	2	34	**nutrient** + s(future gly. dose)	Gaussian	0.22	8	2b
log chlorophyll *a*	8-43	238	**nutrient** + **s(date)** + **ti(date, log gly.)** + **ti(date, log gly., by=nutrient.ordered)** + **s(date, site, bs="fs")**	Gaussian	0.88	6	2c-e
log chlorophyll *a*	57 vs. 43	32	nutrient + s(log Phase I chl. a) + **s(log Phase I gly.)**	Gaussian	0.88	9	2f-g
log chlorophyll *a*	57	32	**s(log Phase II gly.)**	Gaussian	0.31	9	S5
genus number	43	18	nutrient + **s(log gly.)** + s(log gly., by=nutrient.ordered)	Poisson (log link)	0.11	6	3b
alpha diversity	43	18	nutrient + **s(log gly.)** + s(log gly., by=nutrient.ordered)	Gaussian	0.31	6	3e
Bray-Curtis dissimilarity	2 vs. 43	18	nutrient + s(log gly.) + s(log gly., by=nutrient.ordered)	beta (logit link)	0.02	6	4b
synchrony	8-43	18	nutrient + s(log gly.) + s(log gly., by=nutrient.ordered)	Gaussian	0.36	6	4c
log chlorophyll *a*	57	16	**s(log Phase I gly.)**	Gaussian	0.89	7	5b
log chlorophyll *a*	57	16	s(1 of 11 other community variables)	Gaussian	< 0.4	7	5b

Supplementary Table 2. Abundance of the two dominant crustacean zooplankton species at the end of Phase II (P2), in the 18 ponds for which phytoplankton biovolume data were collected. Rare individuals from other species were seen but not counted. We also indicate Phase I (P1) treatments, Phase II glyphosate treatment (yes or no), and final phytoplankton biomass. Note that variation in the abundance of the herbivorous cladoceran *Chydorus sphaericus* does not correlate with phytoplankton biomass (Pearson r = -0.09). Phase II control ponds (E1 and H1) differed from treated ponds in that they also had abundant *Cyclops scutifer,* an omnivorous copepod. Nonetheless, control ponds and ponds exposed to high doses of glyphosate in Phase I have comparable phytoplankton biomass despite the low abundance of *Cyclops* in the later.

pond	P1 glyphosate (mg/L)	P1 nutrient	treated in P2?	*Chydorus sphaericus*	*Cyclops scutifer*	*Cyclopoid* nauplii	total	P2 chlorophyll *a* (µg/L)
C1	0	low	yes	6	2	0	8	2.5
C4	0.3	low	yes	2	1	0	3	0.2
C7	5.4	low	yes	269	0	0	269	3.4
C8	14.8	low	yes	12	0	0	12	56.1
D1	0	high	yes	17	3	0	20	0.5
D4	0.3	high	yes	8	1	0	9	0.5
D7	5.4	high	yes	5	2	0	7	5.7
D8	14.8	high	yes	5	1	0	6	77.5
J1	0	high	yes	7	1	0	8	0.6
J4	0.3	high	yes	28	1	0	29	0.7
J7	5.4	high	yes	49	1	0	50	30.5
J8	14.8	high	yes	31	0	0	31	84.1
K1	0	low	yes	1	2	0	3	0.6
K4	0.3	low	yes	9	0	0	9	0.4
K7	5.4	low	yes	302	2	0	304	3.8
K8	14.8	low	yes	18	0	0	18	144.9
E1	0	low	no	378	68	44	490	40.1
H1	0	high	no	4	34	67	105	77.6