


Widespread agrochemicals differentially affect zooplankton biomass and community structure

MARIE-PIER HÉBERT ^{1,2,3,10} VINCENT FUGÈRE,^{1,2,3,4,5} BEATRIX E. BEISNER,^{2,3,4} NAÍLA BARBOSA DA COSTA,^{2,6}
 ROWAN D. H. BARRETT,^{1,2,4,7} GRAHAM BELL,^{1,4} B. JESSE SHAPIRO,^{2,6,8} VIVIANE YARGEAU,⁹
 ANDREW GONZALEZ,^{1,4} AND GREGOR F. FUSSMANN^{1,2,4}

¹Department of Biology, McGill University, Montréal, Québec H3A 1B1 Canada

²Groupe de Recherche Interuniversitaire en Limnologie (GRIL), Montréal, Québec H2V 0B3 Canada

³Department of Biological Sciences, University of Québec at Montreal, Montréal, Québec H3C 3V8 Canada

⁴Québec Centre for Biodiversity Science (QCBS), Montréal, Québec H3A 1B1 Canada

⁵Département des Sciences de L'environnement, Université du Québec à Trois-Rivières, Trois-Rivières, Québec G9A 5H7 Canada

⁶Département des Sciences Biologiques, Université de Montréal, Montréal, Québec H2V 0B3 Canada

⁷Redpath Museum, McGill University, Montréal, Québec H3A 0C4 Canada

⁸Department of Microbiology and Immunology, McGill Genome Centre, Montréal, Québec H3A 0G1 Canada

⁹Department of Chemical Engineering, McGill University, Montréal, Québec H3A 0C5 Canada

Citation: Hébert, M.-P., V. Fugère, B. E. Beisner, N. Barbosa da Costa, R. D. H. Barrett, G. Bell, B. J. Shapiro, V. Yargeau, A. Gonzalez, and G. F. Fussmann. 2021. Widespread agrochemicals differentially affect zooplankton biomass and community structure. *Ecological Applications* 31(7):e02423. 10.1002/eap.2423

Abstract. Anthropogenic environmental change is causing habitat deterioration at unprecedented rates in freshwater ecosystems. Despite increasing more rapidly than many other agents of global change, synthetic chemical pollution—including agrochemicals such as pesticides—has received relatively little attention in freshwater community and ecosystem ecology. Determining the combined effects of multiple agrochemicals on complex biological systems remains a major challenge, requiring a cross-field integration of ecology and ecotoxicology. Using a large-scale array of experimental ponds, we investigated the response of zooplankton community properties (biomass, composition, and diversity metrics) to the individual and joint presence of three globally widespread agrochemicals: the herbicide glyphosate, the neonicotinoid insecticide imidacloprid, and nutrient fertilizers. We tracked temporal variation in zooplankton biomass and community structure along single and combined pesticide gradients (each spanning eight levels), under low (mesotrophic) and high (eutrophic) nutrient-enriched conditions, and quantified (1) response threshold concentrations, (2) agrochemical interactions, and (3) community resistance and recovery. We found that the biomass of major zooplankton groups differed in their sensitivity to pesticides: ≥ 0.3 mg/L glyphosate elicited long-lasting declines in rotifer communities, both pesticides impaired copepods (≥ 3 $\mu\text{g/L}$ imidacloprid and ≥ 5.5 mg/L glyphosate), whereas some cladocerans were highly tolerant to pesticide contamination. Strong interactive effects of pesticides were only recorded in ponds treated with the combination of the highest doses. Overall, glyphosate was the most influential driver of aggregate community properties of zooplankton, with biomass and community structure responding rapidly but recovering unequally over time. Total community biomass showed little resistance when first exposed to glyphosate, but rapidly recovered and even increased with glyphosate concentration over time; in contrast, taxon richness decreased in more contaminated ponds but failed to recover. Our results indicate that the biomass of tolerant taxa compensated for the loss of sensitive species after the first exposure, conferring greater community resistance upon a subsequent contamination event; a case of pollution-induced community tolerance in freshwater animals. These findings suggest that zooplankton biomass may be more resilient to agrochemical pollution than community structure; yet all community properties measured in this study were affected at glyphosate concentrations below common water quality guidelines in North America.

Key words: agricultural pollution; ecological stability; freshwater ecosystems; herbicide glyphosate; multiple stressors; neonicotinoid insecticide imidacloprid; pollution-induced community tolerance; resistance and recovery; synthetic pesticides; water quality guidelines.

INTRODUCTION

Manuscript received 13 October 2020; revised 8 February 2021; accepted 3 March 2021. Corresponding Editor: Shelley E. Arnott.

¹⁰E-mail: mphebert4@gmail.com

Freshwater ecosystems have been extensively altered by human-induced global change and environmental degradation. A critical, yet relatively understudied

dimension of global change is synthetic chemical pollution (Rockström et al. 2009, Steffen et al. 2015, Mazor et al. 2018), including the release of agrochemical contaminants such as pesticides in ecosystems (Malaj et al. 2014, Schäfer et al. 2016). Pesticide manufacturing has risen massively since agricultural industrialization, expanding more rapidly than other well-recognized anthropogenic drivers of global change (Bernhardt et al. 2017). With a global annual application of pesticides exceeding 4.1 million tons (FAO 2020), their occurrence in surface and ground waters is increasingly reported (Wittmer et al. 2010, Ippolito et al. 2015, Stehle and Schulz 2015), in addition to the recurrent presence of other agrochemicals such as fertilizers (Vörösmarty et al. 2010). Despite these global trends, there are a limited number of studies addressing how the rising load and diversity of synthetic agrochemicals entering freshwaters affect the ecological functioning of communities and ecosystems (Gessner and Tlili 2016, Mazor et al. 2018, Reid et al. 2019; but see Rumschlag et al. 2020).

Ecotoxicological assessments of agrochemical effects on freshwater biota often rely on laboratory assays conducted in simplified settings, facilitating the identification of modes of action and threshold concentrations causing impairment. Although crucial to establishing baseline knowledge of synthetic chemicals, laboratory toxicity tests typically focus on single species and agrochemicals, ignoring the possible influence of many indirect and interacting factors on multispecies assemblages (Fleeger et al. 2003, Rohr et al. 2006). Long-standing needs to better transpose observations from ecotoxicological studies to our understanding and management of ecosystems include several key considerations: (1) a broader appreciation of how species and trophic interactions may modulate responses to pollutants (Relyea and Hoverman 2006, 2008); (2) determining interactions of co-occurring agrochemicals (Coors and De Meester 2008, Relyea 2009); (3) incorporation of temporal dynamics to assess time-dependent effects of agrochemicals or community recovery (Rohr and Crumrine 2005, Halstead et al. 2014); (4) accounting for physicochemical factors (e.g., exposure to natural light; Fenoll et al. 2015) that may influence the flow, toxicity, and degradation of agrochemicals, especially pesticides. Field experiments, such as whole-ecosystem manipulations or outdoor mesocosms, are complementary in this regard, as they can demonstrate how the structure and function of biological systems may be influenced under realistic conditions, expanding the scale of inquiry in ecotoxicology and tackling processes operating at higher levels of organization (Rohr et al. 2006, Peters et al. 2013). Echoing earlier calls, recent studies stressed the need to better integrate ecology and ecotoxicology to develop effective risk assessments and conservation strategies adapted to complex environments confronted with rising agrochemical pollution (Gessner and Tlili 2016, Bernhardt et al. 2017).

A diversity of pesticides and fertilizers co-occur in surface waters, in part because it is common practice to apply agrochemicals as mixtures (Altenburger et al. 2013). Within the United States alone, pesticides are nearly ubiquitous in lotic systems, with >90% of the streams located in agricultural, urban, and mixed land use areas having traces of at least two pesticides (Gilliom et al. 2006). The concomitant presence of agrochemicals can generate interactive effects that may weaken or strengthen individual effects of agrochemicals on biota, resulting in nonadditive outcomes (synergies or antagonisms) that are hard to predict from single agrochemical studies (Relyea 2009, Geyer et al. 2016). Previous syntheses of multiple stressor effects on freshwater biota indicate that cumulative effects are more often interactive than additive (Jackson et al. 2016, Birk et al. 2020). For example, Chará-Serna et al. (2019) found that imidacloprid, a neonicotinoid insecticide, mitigated the positive effect of nutrients on freshwater invertebrate richness. The study of multiple stressors, however, has thus far primarily focused on characterizing interaction types across organisms, not along stress gradients; in fact, the paucity of regression-style designs has been recognized as a hindrance to assess effect sizes and potential response thresholds (Cottingham et al. 2005, Kreyling et al. 2018, Orr et al. 2020). Although stressor interactions represent an important issue for aquatic conservation (Côté et al. 2016, Reid et al. 2019), interactions of pesticides remain rarely addressed, with very few studies of synthetic chemicals and xenobiotics other than fertilizers (Jackson et al. 2016, Birk et al. 2020).

Adding further complexity to the study of agrochemicals is that different biotic properties may show distinct responses to multiple pollutants. Examining temporal variation in aggregate community properties, such as standing biomass, species assemblages, or functional roles, aids in identifying the underlying processes allowing communities to resist or recover from stressors (Halstead et al. 2014, Hillebrand et al. 2018). Faced with a disturbance, community properties may show resistance (i.e., ability to remain unaffected upon disturbance) or recovery (i.e., ability to return to initial state after disturbance). In some cases, the resistance or recovery of a given property can be achieved via the lack of resistance or recovery in another property; a trade-off that has been observed between community structure and biomass. For instance, community structure may rearrange under stress (e.g., turnover or loss of taxa; Murphy and Romanuk 2014), enabling biomass stocks and production to be maintained, and thus preserving a key ecosystem function (Gonzalez and Loreau 2009, Allan et al. 2011, Hoover et al. 2014). In such a scenario, the resistance of community biomass is achieved through species sorting, implying a relatively weaker resistance of community structure. This trade-off, whereby biomass resistance is accomplished at the expense of community structure, may occur if tolerant taxa compensate for more sensitive community members; a process that may

be influenced by the diversity and (co-)tolerance patterns within the original species pool (Cottingham et al. 2001, Vinebrooke et al. 2004, Arnoldi et al. 2019). Analogous trade-offs can also occur between the recovery of community structure and biomass over time (Hillebrand and Kunze 2020). Furthermore, if stress-induced species sorting results in the replacement of sensitive taxa with tolerant ones, community-wide tolerance may increase, leading to greater resistance upon subsequent stress exposure, i.e., stress- or pollution-induced community tolerance (Vinebrooke et al. 2004, Tlili et al. 2016). However, if too few taxa are tolerant, communities may collapse entirely, leading to cascading food web and ecosystem effects (Dunne and Williams 2009), such as secondary extinctions (Ives and Cadinale 2004); similarly, declines in few but functionally distinct taxa may also result in marked cascading effects. For example, the increased use of neonicotinoid insecticides in agricultural watersheds can induce a decline in zooplankton and the subsequent collapse in the yield of a fishery (Yamamuro et al. 2019). Overall, assessing resistance and recovery of aggregate community properties may reveal mechanisms by which biota cope with stress, providing in the context of this study a clearer picture of immediate vs. long-lasting effects of agrochemical pollution.

In this study, we address how complex zooplankton communities respond to the individual and cumulative effects of fertilizers (nutrient pollution) and two widely used, globally relevant pesticides: the herbicide glyphosate and the neonicotinoid insecticide imidacloprid. Glyphosate- and imidacloprid-based pesticides are extensively used in agricultural and urban landscapes (Simon-Delso et al. 2015, Maggi et al. 2020, Satiroff et al. 2021) and can reach aquatic ecosystems in different ways (Struger et al. 2017, Hébert et al. 2019, Medalie et al. 2020), leading to their widespread occurrence (Aparicio et al. 2013, Morrissey et al. 2015, Montiel-Léon et al. 2019) and concerns over their toxicity to aquatic life (Anderson et al. 2015, van Bruggen et al. 2018). Zooplankton have been extensively used as model organisms in toxicological assays with non-target aquatic biota. Table S1 (Appendix S1) provides a non-exhaustive compilation of (>40) experimental studies addressing the effects of the herbicide glyphosate and the insecticide imidacloprid on freshwater zooplankton; reflecting the long-standing contrast between the many short-term, single-species laboratory tests and the more limited field-based evaluations of communities exposed to multiple agrochemicals. Although extensive laboratory research has demonstrated the adverse effects of these pesticides on zooplankton, effects may be undetectable or different through other, more realistic testing approaches (Mikó et al. 2015; Appendix S1: Table S1). Further, the joint presence of the insecticide and the herbicide could potentially elicit interactive effects, in part owing to their different mode of action; the former being designed to extirpate insect pests (neural transmission

disruption; Anderson et al. 2015), while the latter to control weeds (inhibition of amino acid synthesis; van Bruggen et al. 2018). Finally, the wide range of sensitivity across zooplankton taxa, as well as the discrepancy between model species used in laboratory tests and those found to be responsive in natural assemblages (Appendix S1: Table S1) further highlight the need to examine how these pesticides may affect complex communities in agroecosystems.

Using an outdoor array of 48 experimental ponds, we performed a 43-d study to track temporal variation in zooplankton (crustacean and rotifer) community properties (i.e., biomass, composition, and three diversity metrics) along single and combined gradients of glyphosate and imidacloprid concentrations, under low (mesotrophic) and high (eutrophic) nutrient-enriched conditions. We contrasted responses of zooplankton biomass and community structure (hereby measured via composition and diversity), and quantified for all aggregate community properties: (1) response threshold concentrations, (2) interactive effects of agrochemicals, and (3) resistance and recovery. For each objective, we formulated general predictions in Table 1. Aligned with recent calls to bridge ecotoxicology and ecology, our results also contribute to the broader field of ecological stability and ecosystem responses to global change, while having practical implications for freshwater zooplankton exposed to globally relevant agricultural pollutants at concentrations both below and above common water quality guidelines in North America.

METHODS

Experimental design and treatments

We conducted a field experiment at the Large Experimental Array of Ponds (LEAP), a pond mesocosm facility built at McGill University's Gault Nature Reserve (Mont St-Hilaire, Québec, Canada), which is a protected, forested area. Large Experimental Array of Ponds is connected via a 1-km pipe to the headwater Lake Hertel from which water and organisms can flow by gravity and accumulate in a large reservoir; once mixed, water and organisms can be evenly distributed across 100 experimental ponds, each having a capacity of ~1,000 L.

On 11 May 2016, we filled all experimental ponds with lake water and planktonic organisms, followed by a three-month acclimation period. We removed fish using a coarse sieve when filling ponds, and periodically removed tadpoles and debris with a net. Every two weeks, we replaced 10% of the pond volume with a fresh lake inoculum to track seasonal changes in Lake Hertel's plankton community, maximize the diversity of the initial pool of species, and minimize ecological drift across ponds prior to the experiment. We recorded physicochemical variables and water level weekly to track homogeneity across ponds. On 10 August 2016, we

TABLE 1. Summary of initial predictions for each objective.

Treatment	Prediction
<i>General effects of agrochemicals</i>	
Glyphosate (herbicide)	Negative (direct and resource-mediated) effects on zooplankton (based on Appendix S1: Table S1).
Imidacloprid (insecticide)	Negative (direct) effects on zooplankton (based on Appendix S1: Table S1).
Nutrient fertilizers	Positive (resource-mediated) effects on zooplankton.
<i>Objective I: Response thresholds</i>	
Glyphosate	Negative effects on communities observed at 0.2 mg/L or higher (\geq dose 4) (based on Appendix S1: Table S1).
Imidacloprid	Negative effects on communities observed at 0.3 mg/L or higher (\geq dose 3) (based on Appendix S1: Table S1).
<i>Objective II: Additive vs. interactive effects of agrochemicals†</i>	
Combination: Gly + Nu	Nu will mitigate the negative effects of Gly; possible positive interaction.
Combination: Imi + Nu	Nu will mitigate the negative effects of Imi; possible positive interaction.
Combination: Gly + Imi	Gly and Imi will result in greater negative effects; possible negative interaction.
Combination: Gly + Imi + Nu	Nu will mitigate the combined negative effects of Gly and Imi; possible positive or negative interaction.
<i>Objective III: Community resistance and recovery</i>	
Single and joint presence of pesticides	Biomass will show greater resistance and recovery than community structure (composition and diversity metrics).

†Use of abbreviations for clarity purposes: Gly, glyphosate; Imi, imidacloprid; Nu, nutrient fertilizers.

selected 48 ponds for a collaborative experiment to assess the responses of planktonic communities, including phytoplankton and bacterioplankton; see Fugère et al. (2020) and Barbosa da Costa et al. (2021), respectively. Here we only report observations made over the first 43 d, as zooplankton communities were no longer sampled at the same resolution beyond this time point.

Agrochemical treatments consisted of two nutrient-enriched levels, mimicking mesotrophic (ambient Lake Hertel state) and eutrophic conditions, and three pesticide gradients each spanning eight levels: glyphosate alone, imidacloprid alone, and a combination of both pesticides; see schematic representation in Fig. 1a. A regression design for pesticide application enabled the quantification of effect strengths while identifying threshold concentrations affecting zooplankton. Target doses of glyphosate (acid equivalent) were 0 (control), 0.04, 0.10, 0.30, 0.70, 2.00, 5.50, and 15.0 mg/L; while imidacloprid were 0 (control), 0.15, 0.40, 1.00, 3.00, 8.00, 22.0, and 60.0 μ g/L (Fig. 1b). Pesticides covered a range of concentrations in line with those from ecotoxicological studies (Appendix S1: Table S1), while spanning benchmarks considered safe for aquatic life (Canadian Water Quality Guidelines; CCME 2007, 2012, Fig. 1b). Target concentrations were set to maintain a constant (logarithmic) increment across doses. For glyphosate, none of the doses exceeded short-term (acute exposure) criteria for aquatic life in Canada (CCME 2012; Fig. 1b) or for freshwater invertebrates in the United States (Office of Pesticide Program; EPA 2019).

To mimic the natural flow of agrochemicals to surface waters, we applied nutrients as a press treatment, and pesticides in the form of two pulses. We manipulated

nutrient enrichment while maintaining the same nitrogen (N) to phosphorus (P) molar ratio as our source lake (N: P \sim 33). Target P concentrations were 15 μ g P/L (mesotrophic; referred to as low nutrient) and 60 μ g P/L (eutrophic; high nutrient); we first added nutrient solutions (prepared with KNO₃, KH₂PO₄, and K₂HPO₄) on 10 August 2016. To maintain a press treatment, this step was repeated every two weeks. We started our 43-d experiment on 17 August (day 1). On days 6 and 34, we applied pesticide treatments. We prepared glyphosate solutions with Roundup Super Concentrate (Monsanto); calculations to reach target concentrations were based on the glyphosate acid content of the formulation. We prepared imidacloprid-based solutions by dissolving imidacloprid powder (Sigma-Aldrich) in ultrapure water.

Sampling

We sampled all ponds on six occasions: days 1, 7, 15, 30, 35, and 43. Our sampling schedule included one instance prior to the first pesticide pulse on day 6, three time points between the two pulses, and two after the second pulse on day 34. The relatively long interval between the two pulses (28 d) was intended to permit the potential recovery of communities prior to the second pulse. On each sampling day, we collected water with 35-cm long integrated tube samplers at multiple locations within ponds and stored samples in dark Nalgene bottles for nutrient and biotic measurements other than zooplankton. To avoid cross-contamination, we assigned each pond its own sampler and bottles. We immediately transferred samples to our on-site indoor

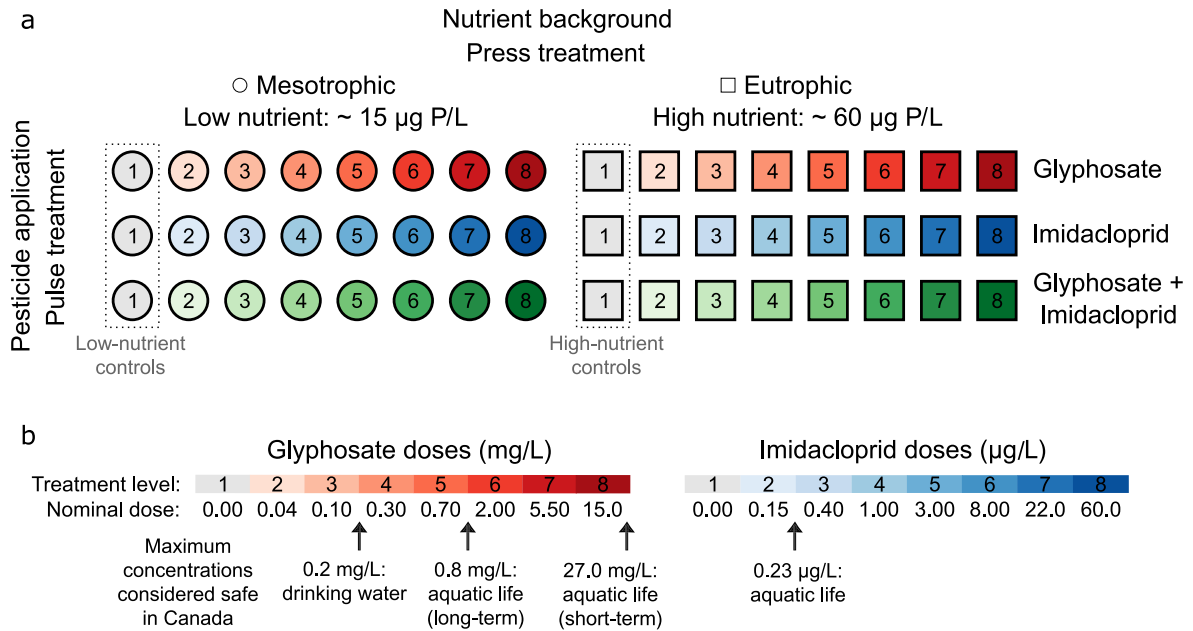


FIG. 1. Simplified scheme of the experimental design. (a) Nutrient treatment (two levels) and pesticide gradients (eight doses for each of the three gradients) applied to the 48 experimental ponds. Nutrients were applied as a press treatment whereas pesticides were applied twice in the form of pulses on days 6 and 34. Symbols correspond to low- and high-nutrient treatments. Color indicates pesticide treatment type (red, glyphosate only; blue, imidacloprid only; green, both pesticides); color saturation and numbers refer to target pesticide doses upon the application of the first pulse. Control ponds, wherein only nutrient background was manipulated, are denoted in gray. (b) Nominal and chemical doses of pesticides are represented with respect to water quality threshold concentrations in Canada (CCME 2007, 2012).

laboratory and kept them in the dark at 4°C. For zooplankton, we collected water at multiple locations in each pond using our integrated samplers and sieved 2 L with a 64-µm mesh. We anesthetized zooplankton with carbonated water and fixed samples with ethanol (final concentration ~75%) on site. We measured a standard suite of physicochemical parameters using a YSI multi-parameter sonde (YSI, Yellow Springs, Ohio, USA). To measure pesticides concentrations, we collected samples outside of our regular sampling schedule, as pesticides are subject to partial degradation in water. We sampled ponds immediately after pesticide application, on days 6 and 34; we collected additional samples on days 14 and 29 in a subset of the ponds to track degradation over time. We acidified pesticide samples (pH below 3) and kept them frozen at -20°C until analysis. A full list of physicochemical and biological measurements taken in this experiment can be found in Appendix S1: Table S3.

Laboratory analyses

We carried out a thorough analysis of zooplankton community composition for all 48 experimental ponds of the six sampling occasions ($N = 288$). For the focal 288 samples of this study, we counted and identified every crustacean and rotifer individual (spanning 24 taxa; see Appendix S1: Table S4 for a species list) using

an Olympus dissecting scope and an Olympus inverted microscope. We referred to three core taxonomic keys adapted to North American species: Thorp and Covich (2001), Haney et al. (2013), and Hudson and Lesko (2003).

We analyzed nutrient samples in the GRIL (Interuniversity Research Group in Limnology) laboratory at the University of Québec at Montréal. We oxidized TP and TN samples with persulfate and alkaline persulfate digestions, respectively. We measured TP as orthophosphate using the spectrophotometric molybdenum method (890 nm; Ultrospec 2100 pro, Biochrom), and TN as nitrites (reduced via a cadmium reactor) with a flow analyzer (OI-Analytical Flow Solution 3100). We measured pesticides concentrations via liquid chromatography coupled to mass spectrometry (LC-HRMS) using an Accela 600-Orbitrap LTQ XL (LC-HRMS, Thermo Scientific) in the Department of Chemical Engineering at McGill University. The methods used are more extensively described in Fugère et al. (2020; glyphosate) and Barbosa da Costa et al. (2021; imidacloprid). Briefly, we measured glyphosate via heated electrospray ionization in negative mode (mass range = 50–300 m/z [mass m per charge number z]), and imidacloprid in positive mode (mass range = 50–700 m/z). We used an ion trap to perform targeted data acquisition for the product ion spectra (MS2) and generate identification fragments.

Data manipulation and statistical analyses

To enable comparison across crustaceans and rotifers, we converted abundance data to biomass using taxon-specific individual dry mass estimates compiled by Gsell et al. (2016), Hébert (2014), and Hébert et al. (2016). We then applied a $\log_{10}(1 + X)$ -transformation on biomass data. In analyses, we treated nutrient treatment as a binary factor variable, and used nominal levels of treatment doses for pesticides. We performed all statistical analyses in R version 4.0.0 (R Development Core Team 2020).

We explored relationships with physicochemical factors but included none as predictors in models as there was little variation across ponds. We also examined the potential role of chlorophyll *a* (chl *a*) as a driver of zooplankton biomass. However, chl *a* was highly correlated with glyphosate and TP after pulse 1 (presumably due to fertilizing effects of glyphosate-derived P; see *Discussion*). To avoid the inclusion of highly correlated predictors, we decided against including chl *a* in our main models. The rationale behind this decision was that our ultimate goal was to assess the causal, potentially cascading effects of agrochemicals on zooplankton, not to disentangle direct from indirect effects (e.g., mediated via changes in chl *a*). As such, regardless of whether zooplankton biomass increased with chl *a* as a result of glyphosate fertilizing effects over time, this indirect effect was triggered by the presence of glyphosate in water, which is ultimately the effect that we aimed to quantify. We nonetheless present relationships between chl *a* and zooplankton biomass in Appendix S1: Table S5, Fig. S3.

Community biomass.—We quantified the effects of time, agrochemicals, and all interactions on the biomass of total and group-specific (cladoceran, copepod, and rotifer) zooplankton communities, using linear mixed effect models (LMM). To account for pseudoreplication (non-independence) across temporally repeated measurements from the same ponds, we set “individual pond” as a random effect. Predictors were standardized as per Gelman (2008; i.e., centered and divided by two standard deviations) prior to running LMMs so as to adequately compare effect sizes between continuous (pesticide dose) and binary (nutrient level) predictors. To fit LMMs, we used the function `lmer` in the R package `lme4`. We report model marginal R^2 and conditional R^2 , representing proportions of variance explained by fixed factors alone (i.e., treatment and time) and both fixed and random factors, respectively. We also report all parameter estimates (effect size) and intraclass correlation coefficients (ICC; estimate of within-pond correlation across temporally repeated measures) in Appendix S1: Tables S5–S9.

Given the time-dependence of effects (i.e., direction of effect reversing over time), we quantified the effects of each agrochemical (nutrients, glyphosate, imidacloprid) and their interactions (nutrients \times glyphosate, nutrients \times imidacloprid, glyphosate \times imidacloprid) for each sampling day, fitting all effects as interaction effects with

time (converted to a factor). We also tested higher-order interactions but in none of the model were they significant; thus, they are not presented. We quantified parameter estimates for day 1 (see Appendix S1) but excluded them from main figures, as pesticides were only applied as of day 6. We validated LMMs through the examination of residuals (distribution and homoscedasticity). We graphically represented effect sizes (measured as model parameter estimates) in forest plots using the `sjplot` and `ggplot2` packages in R.

To identify threshold concentrations affecting zooplankton communities, we used univariate regression trees (URT). Through recursive partitioning, URTs repeatedly divide data to identify predictor values associated with abrupt changes in response data; predictor values are retained as “thresholds” (breaking points or splits) when data are divided such that the sums of squares of the groups created by the tree are minimized. Univariate regression trees provide complementary information to LMMs, as the former can detect nonlinear effects and specifically identify doses causing biotic responses (unlike LMMs that quantify overall effects across sites/doses). We included nominal pesticide levels, nutrient status, and time as predictors in URTs, and used the `ctree` function in the R package `party`. To preclude overfitting, we restricted models to a maximum of four splits, only allowed when $P < 0.01$; P values were estimated by permutation tests as per Hothorn et al. (2006).

Community structure: composition and diversity metrics.—To assess how total and group-specific zooplankton community structure varied across ponds and over time, we measured changes in diversity metrics and taxonomic composition, using a series of univariate (diversity metrics) and multivariate (ordinations; composition) analyses.

We examined variation in community diversity using three indices: alpha diversity (exponent of the Shannon index), richness (taxon number), and evenness (Pielou's index). We calculated diversity metrics for crustaceans, rotifers, and the whole zooplankton community. We quantified effects of treatments using LMMs, built with the same structure as the biomass models described previously, and identified breaking points using URTs. Note that, in the context of our experiment, the application of biocides temporarily led to very low densities (e.g., after the first pulse), precluding the use of rarefied estimates of diversity.

Prior to compositional analyses, we discarded data from two low-nutrient, glyphosate-treated sites from day 7 (doses 7 and 8; i.e., one day after the first application of the strongest doses), as those samples did not contain any zooplankton, making them unfit for traditional community analyses. Note, however, that the lack of zooplankton in those samples is likely representative of strong declines, not of the collapse of entire communities, as subsequent biomass increases were observed shortly after. We log-transformed biomass data as per

Anderson et al. (2006) to reduce asymmetry. To visualize compositional changes over time with respect to treatment, we used principal component analysis (PCA). We built PCAs using the *rda* function of the R package *vegan* (Oksanen et al. 2019). To better illustrate temporal dynamics, we constructed the multidimensional space of the ordination using pond data from all sampling days; then, we represented time-specific compositional data in six different panels. By doing so, we incorporated temporal signals in PCA scores. We then used PCA scores to quantify the effect of treatments and time on community composition with a LMM, and identify breaking points in compositional shifts using URTs.

To more clearly visualize temporal shifts in species assemblages, and identify which taxa were most responsive to treatment and thus responsible for compositional changes, we used principal response curves (PRC; Van den Brink et al. 2008). Principal response curves are a type of redundancy analyses contrasting divergence in composition between reference (control) and perturbed (treated) sites in a chronological fashion. The graphical output of PRCs illustrate the degree to which treated communities deviate (left *y*-axis) from controls (horizontal line where $Y = 0$) over time (*x*-axis). We averaged replicates of control communities based on their nutrient treatment (see Fig. 1a) to quantify a mean community matrix for each nutrient level. Using the *prc* function in the R package *vegan* (Oksanen et al. 2019), we built six PRCs for each agrochemical combination, and an additional one using only low- and high-nutrient control ponds to quantify the effect of nutrient enrichment alone. Using the first constrained axis, we examined the proportion of the variance explained by (1) time alone (conditional coefficient), and (2) the interaction between treatment and time (constrained coefficient). Species scores are projected on the right *y*-axis as a taxon-specific measure of responsiveness to treatment and contribution to overall compositional changes. Score signs indicate the direction of response (positive: increases in density; negative: decreases in density) relative to control communities; only scores higher than 0.5 (in absolute value) were retained.

Resistance and recovery measures.—Using the framework developed in Hillebrand et al. (2018), we explored resistance and recovery of four community properties (biomass, composition, richness, and alpha diversity), with the aim of comparing responses between biomass and community structure (i.e., composition and diversity metrics). We hereby define community resistance as the ability of a community to withstand a perturbation (i.e., similarity in community properties between control and treated sites immediately after perturbation), and recovery as the ability to return to the state in which the community would be in the absence of a perturbation (i.e., similarity in community properties between control and treated sites some time after perturbation). For each pesticide dose, we estimated resistance to treatment using

measurements made on sampling days 7 and 35 (i.e., 24–36 h after pesticide application), and used data from subsequent days (15, 30, 43) to track recovery after pulses. We averaged replicates of control communities based on their nutrient level (consistently with our approach to estimate a mean control community in PRCs). To quantify the resistance and recovery of community properties, we used effect size measures between controls and pesticide-treated ponds as per Hillebrand et al. (2018). For biomass, richness, and diversity, we calculated the log response ratio (LRR) between controls and treated communities. In this framework, $LRR = 0$ indicates full resistance or recovery; $LRR < 0$ indicates low resistance or incomplete recovery; and $LRR > 0$ indicates low resistance or recovery via overcompensation/stimulation. For composition, we calculated the similarity between controls and treated ponds using the Bray-Curtis dissimilarity index, resulting in values ranging from 0 and 1, whereby 1 indicates full resistance/recovery.

For each pesticide pulse, we examined resistance and recovery trends across community properties via three types of relationships: (1) between (resistance/recovery) measures of community properties and pesticide dose (Appendix S1: Fig. S7), (2) between (resistance/recovery) measures of two different community properties (Fig. 7), and (3) between resistance and recovery within community properties (Appendix S1: Fig. S9). We used Spearman nonparametric rank correlation coefficients to assess the strength of relationships. As mentioned, two zooplankton samples were empty, resulting in LRRs of $-\infty$ for biomass and richness; we graphically illustrated those measures (identified via gray layers) but excluded them from the calculation of correlation coefficients.

RESULTS

Effects of agrochemicals on biomass

Over the 43 d of this experiment, the total biomass of zooplankton communities ranged from 0 to 1,667.3 $\mu\text{g/L}$ (dry mass; 0–1,142.5 organisms/L), with a mean of 128.8 $\mu\text{g/L}$ (98.3 organisms/L). On day 1, one week prior to the first pulse of pesticides, zooplankton biomass did not differ between low- and high-nutrient ponds (Fig. 2). A LMM revealed that nutrient enrichment alone did not affect total zooplankton biomass throughout the experiment, nor did it affect the biomass of major taxonomic groups, i.e., cladocerans, copepods, and rotifers (group-specific communities; Fig. 3a–d).

One day after the first pulse of pesticides (day 7), glyphosate triggered a rapid decline in total zooplankton biomass (Figs. 2a, 3a). While the application of glyphosate (alone or in combination) resulted in clear biomass reductions in copepod and rotifer communities (Fig. 2c, d), no such decrease occurred in cladocerans (Fig. 2b). At low and moderate glyphosate concentrations, total

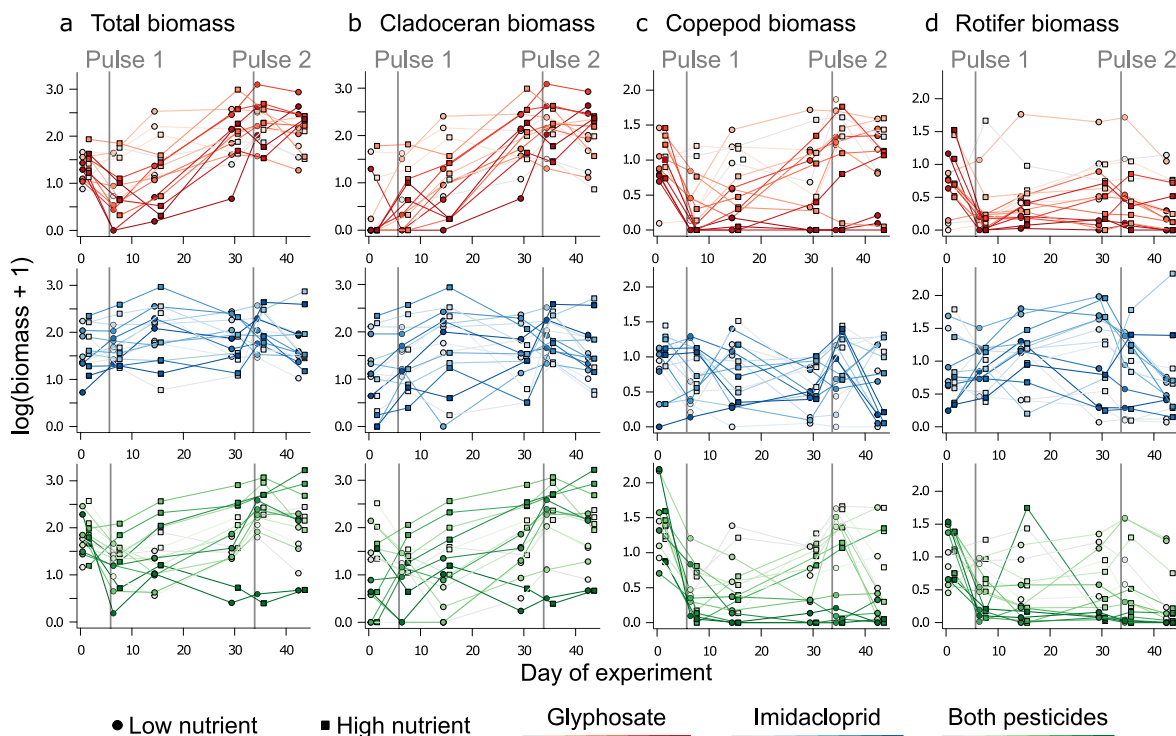


FIG. 2. Temporal dynamics of (a) total zooplankton, (b) cladoceran, (c) copepod, and (d) rotifer biomass ($\mu\text{g/L}$) over the course of the experiment. Symbols correspond to low- and high-nutrient treatments; color and saturation indicate the nature and dose of pesticide treatment, respectively. A small horizontal offset between low- and high- nutrient ponds was used to facilitate visualization.

copepod biomass remained relatively low but partly recovered over time, in contrast to communities exposed to high doses of glyphosate (Fig. 2c); a trend that was even more pronounced in immature copepods (Appendix S1: Fig. S2c–e). Rotifer communities drastically declined in ponds exposed to glyphosate (alone or in combination; >90% biomass loss across ponds) and failed to recover (Fig. 2d). For cladocerans, the negative effect of glyphosate was transient, only visible on days 7 and 15 (Figs. 2b, 3b). Cladocerans then markedly increased over time, with no apparent decline even after the second pesticide pulse. By day 43, a strong, positive effect of glyphosate was detected (Fig. 3b), with increasing cladoceran biomass along the glyphosate gradient (Fig. 2b; Appendix S1: S2b). Because cladocerans constituted a large proportion of zooplankton biomass, the time-dependent effect of glyphosate on Cladocera, shifting from a negative to a positive influence over time, was also visible for total biomass (Figs. 2a, 3a). Importantly, total zooplankton biomass showed clear recovery from pulse 1 and no apparent decline following pulse 2.

Imidacloprid did not affect the biomass of total zooplankton, cladoceran, or rotifer communities. However, the insecticide reduced copepod biomass over time after pulse 2 (Fig. 3c; especially in copepodites; Appendix S1:

Fig. S11). A weak positive effect was detected on total zooplankton on day 15, likely due to marginally significant positive (indirect) effects on rotifers and cladocerans (Fig. 3a–d).

Overall, the joint presence of glyphosate and imidacloprid resulted in responses akin to those observed in ponds treated with glyphosate alone (Fig. 2). One notable exception were ponds exposed to the highest dose of both pesticides, where cladocerans, and thus total zooplankton biomass, declined dramatically and never recovered (Fig. 2a, b), reflecting strong pesticide interaction. A LMM revealed that pesticide interactions on total zooplankton and cladoceran biomass were, however, only significant on day 43 (Fig. 3a, b). This result constitutes the only evidence of strong agrochemical interaction in our study; only a few weak interactions were found otherwise.

Overall, glyphosate was the strongest driver of zooplankton biomass (Fig. 3). A URT identified threshold concentrations of glyphosate causing substantial biomass decreases in communities of rotifers (dose 4 = 0.3 mg/L), cladocerans (dose 5 = 0.7 mg/L; transient negative effect only), and copepods (dose 7 = 5.5 mg/L; Fig. 3f–h). Dose 5 (0.7 mg/L) was also the threshold exposure at which total zooplankton biomass started to strongly decrease (transient effect; Fig. 3e). A breaking point was

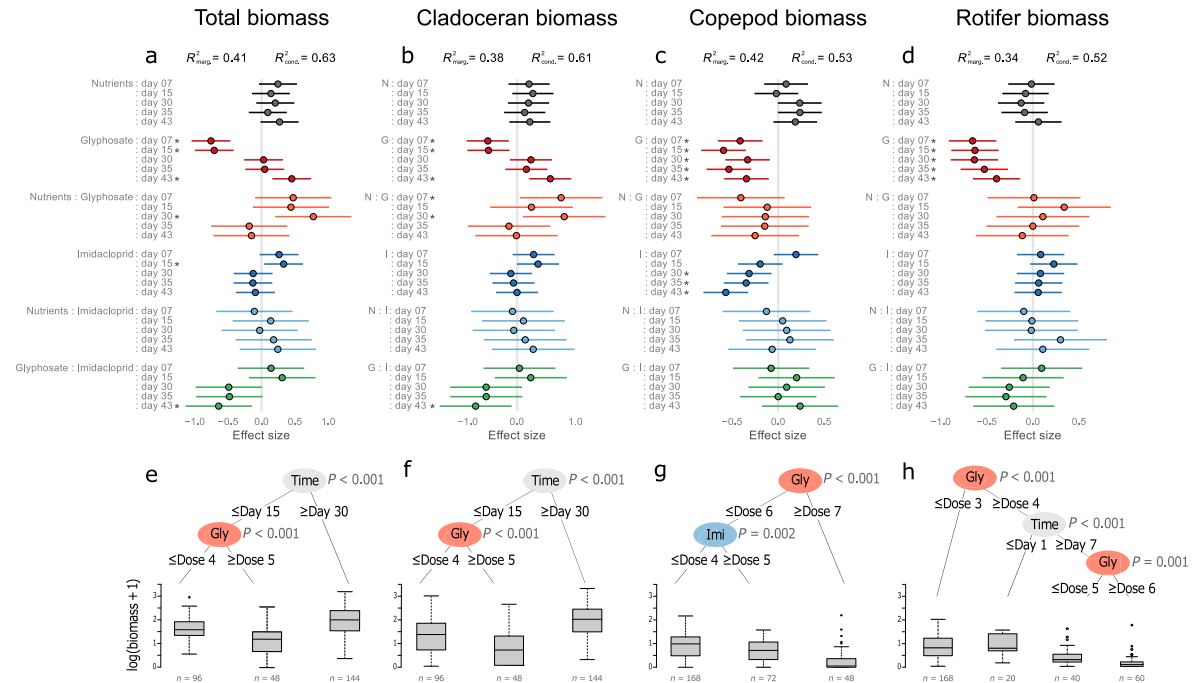


Fig. 3. Effects of agrochemical treatments on zooplankton biomass. (a–d) Forest plots illustrating the time-dependent effects of nutrients, glyphosate, and imidacloprid and their interactions on (a) total zooplankton, (b) cladoceran, (c) copepod, and (d) rotifer biomass. In forest plots, each point represents an effect size quantified as the parameter estimates of linear mixed effect models (LMMs). Error bars represent 95% confidence intervals; effects are considered significant ($P < 0.05$) when there is no overlap with zero (denoted with asterisks). (e–h) Univariate regression tree (URT) models identifying response thresholds (agrochemical concentrations or day of experiment) associated with declines in (e) total zooplankton, (f) cladoceran, (g) copepod, and (h) rotifer biomass.

also identified for imidacloprid, with concentrations $\geq 3 \mu\text{g/L}$ (dose 5) eliciting declines in copepod biomass.

In a separate analysis, a LMM indicated that chl *a* concentration was also a driver of zooplankton biomass, but with limited predictive power. Chl *a* enhanced total zooplankton and cladoceran community biomass, but had little to no effect on copepods and rotifers (see Appendix S1: Table S5, Fig. S3). By the end of the experiment, ponds treated with glyphosate or both pesticides had the highest biomass of algae and zooplankton, except for the combination of the highest pesticide doses (Appendix S1: Fig. S3). Given that chl *a* was (1) highly correlated with glyphosate and TP after pulse 1 (Fugère et al. 2020) and (2) a weaker driver of zooplankton biomass than agrochemicals, chl *a* was excluded from the set of predictors (see *Methods*).

Effects of agrochemicals on community structure

A total of 24 zooplankton taxa were identified in this experiment, reflecting the complexity of our seminatural pond communities.

Diversity.—Pesticide type and dose affected total zooplankton taxon richness and, thus, alpha diversity (measured here as the exponent of the Shannon index), but

had no apparent effect on community evenness (Appendix S1: Fig. S5). LMMs using richness as a response variable provided a better fit than models using alpha diversity for all of total zooplankton, crustacean, and rotifer communities (Fig. 4a–c vs. Appendix S1: Fig. S6). Thus, we primarily focus on effects of agrochemicals on richness, while reporting analogous but weaker effects on Shannon diversity in Appendix S1.

Glyphosate strongly affected zooplankton community richness (Fig. 4a–c), especially in rotifers. Unlike its effect on biomass, glyphosate maintained an adverse effect on community richness throughout the experiment (Fig. 4a vs. Fig. 3a). Declines in richness were also stronger with glyphosate doses, applied alone or in combination; however, the richness of crustaceans exposed to low doses of glyphosate alone (not in the presence of imidacloprid) slightly recovered over time. A URT determined that $\geq 0.3 \text{ mg/L}$ glyphosate (dose 4) resulted in a two-fold decrease in rotifer richness (Fig. 4f); an even lower breaking point (0.1 mg/L glyphosate) was identified for (Shannon) diversity (Appendix S1: Fig. S6b). Imidacloprid ($\geq 0.15 \mu\text{g/L}$) contributed to reducing crustacean richness upon the second pulse, but only under low-nutrient conditions (Fig. 4e). The insecticide was also a driver of crustacean (Shannon) diversity loss over time (Appendix S1: Fig. S6b).

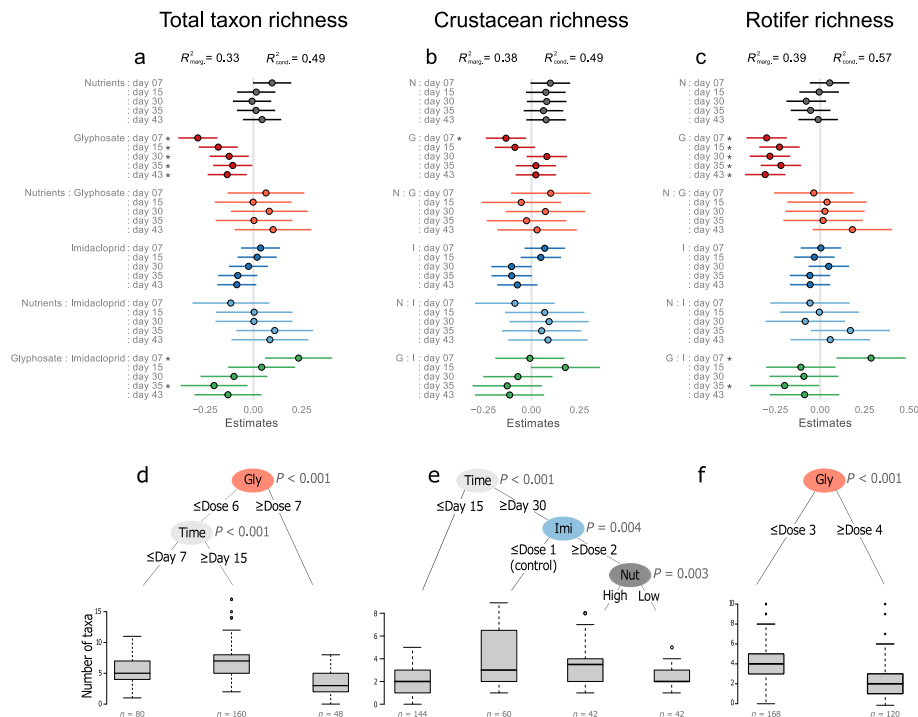


FIG. 4. Effects of agrochemical treatments on the number of zooplankton taxa. (a–c) Forest plots illustrating the time-dependent effects of agrochemicals and their interactions on (a) total zooplankton, (b) crustacean, and (c) rotifer richness over time. Effect sizes are quantified as the parameter estimate of LMMs (significance as detailed in Fig. 3). (d–f) URT models identifying response thresholds (agrochemical concentrations or day of experiment) associated with declines in (d) total zooplankton, (e) crustacean, and (f) rotifer taxon richness.

Community composition.—A series of six ordinations (PCA) uncovered clear effects of time and treatment on zooplankton community composition. On day 1, communities were similar across ponds (Fig. 5a). Shortly after the first pesticide pulse, communities diverged in composition, with ponds exposed to glyphosate vs. imidacloprid being diametrically opposed in the ordinations of days 7 and 15 (Fig. 5b, c). Community assemblages remained different across ponds until the end of the experiment (Fig. 5d–f). Using PC1 scores as a proxy for compositional shifts, both the LMM and URT indicated that glyphosate and time were the strongest drivers (Fig. 5g, h), with the greatest species shift occurring at 2 mg/L glyphosate (dose 6).

Taxon-level responses.—PRCs illustrated compositional divergence between treated and control ponds (deviations of colored lines from the gray horizontal line, where $y = 0$), while indicating which taxa were most responsive (right y -axis). The first pulse of glyphosate led to the decline of several species (Fig. 6a, b and Appendix S1: Fig. S1). By day 30, communities faced with low to moderate treatments showed an increase in *Scapholeberis mucronata* and other cladocerans. Ponds exposed to high glyphosate doses lost most species present in less contaminated ponds (e.g., copepods, rotifers; Fig. 6a, b), and remained primarily composed of *Alona* (in distinctly high density) and *Chydorus sphaericus*.

Overall, members of *Alona*, *Chydorus*, and *Scapholeberis* proliferated the most in glyphosate-treated ponds (Appendix S1: Fig. S1a, d, g). Imidacloprid also led to compositional changes (Fig. 6c, d); however, apart from a greater representation of some rotifers and an increased presence of *Scapholeberis*, no clear pattern of species turnover emerged along the insecticide gradient; in contrast to the herbicide.

Communities confronted with both pesticides showed marked compositional shifts, with stronger turnover at higher doses (Fig. 6e, f). Certain taxa responded similarly when faced with glyphosate alone and both pesticides (Fig. 6a, b vs. e, f); e.g., declines in rotifers. In low-nutrient ponds, assemblages deviated distinctly from controls after each pulse, with *Alona* becoming dominant while rotifers and copepods declined (Fig. 6e). In high-nutrient ponds, communities were characterized by an increase in cladocerans, especially *Scapholeberis*, and a clear loss of rotifers and immature copepods (Fig. 6f). The most notable trend was the increasing dominance of *Alona*, *Chydorus*, and *Scapholeberis* across ponds treated with any pesticide (Appendix S1: Fig. S1a, d, g), reflecting a (co-)tolerance to both the herbicide and insecticide.

The interaction between time and nutrient enrichment alone explained relatively little variation in species assemblages (36%; Appendix S1: Fig. S4), indicating that pesticide treatment was a stronger driver of species turnover than nutrient enrichment.

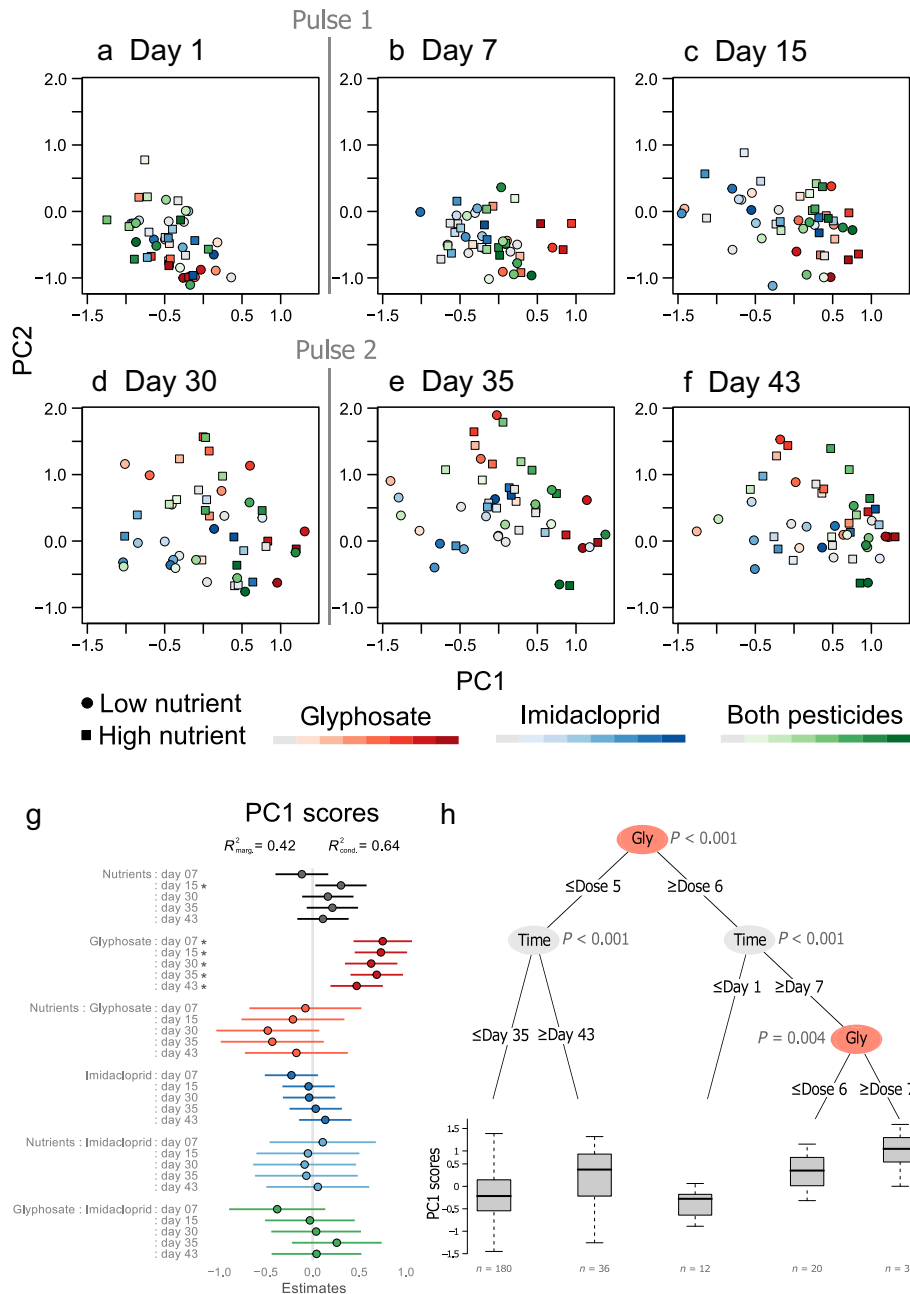


FIG. 5. Temporal variation in zooplankton community composition across ponds and agrochemical treatments. (a–f) Principal component analysis (PCA) illustrates changes in community composition across sampling days. (g) Forest plot indicating the time-dependent effects of agrochemicals and their interactions on changes in community composition (measured as PC scores of the first axis shown in panels a–f; PC1). Effect sizes are quantified as the parameter estimate of LMMs (significance as detailed in Fig. 3); however, direction of effects should not be interpreted. (h) URT model identifying response thresholds (agrochemical concentrations or day of experiment) associated with compositional shifts (measured as PC1).

Resistance and recovery of community properties

For the first pesticide pulse, community resistance and recovery were both lower with increasing pesticide treatments in all of the community properties (Appendix S1: Fig. S7). For the second pulse, resistance and recovery were relatively stronger overall (LRRs closer to 0;

composition similarity closer to 1). For biomass only, the long-term recovery was stronger with increasing pesticide doses, except for the highest combination of both pesticides (Appendix S1: Fig. S7a). By the end of the experiment, while pond richness and composition showed low recovery, biomass had exceeded full recovery (surpassing biomass levels in control ponds), indicating

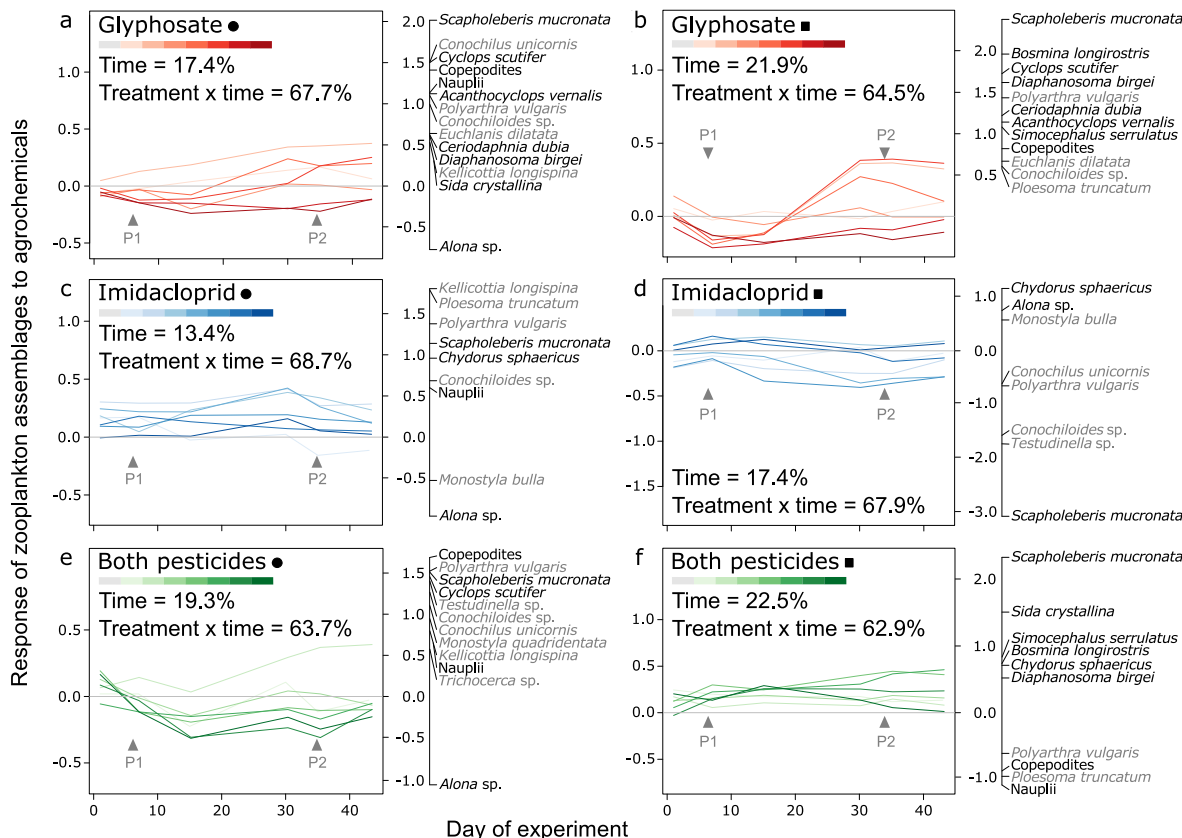


FIG. 6. Temporal variation in zooplankton taxon assemblages illustrated by principal response curves (PRCs). PRCs graphically represent how composition diverge between control ponds and ponds exposed to (a, b) glyphosate, (c, d) imidacloprid, and (e, f) both pesticides, under low- (a, c, e; circle) or high- (b, d, f; square) nutrient conditions. The left y-axis reflects deviations in composition (curves) relative to control communities over time, whereas the right y-axis indicates the relative contribution of species to compositional changes (responsive species scores). Species score signs indicate the direction of response relative to individual community curves. Crustacean vs. rotifer taxa are represented in black and gray, respectively. P1 and P2 indicate the timing of the first and second pesticide pulse. Proportions of the variance explained by time alone (conditional coefficients) and by the interaction between treatment and time (constrained coefficients) are provided for each type of agrochemical combination.

clear stimulatory effects of glyphosate. Additional diversity relationships are reported in Appendix S1.

Aside from reflecting a relatively smaller effect of the second pulse on zooplankton communities, the analysis of resistance and recovery within community properties was inconclusive (Appendix S1: Fig. S9). In particular, none of the community properties showed a correlated resistance or recovery to both pulses, indicating that initial responses to pesticide stress could not predict responses to subsequent exposure events (Appendix S1: Fig. S9a).

The examination of relationships among community properties revealed that the responses in biomass and composition were positively correlated upon and after pulse 1 (days 7 and 15; Fig. 7a); by day 30, however, biomass markedly recovered, unlike composition. Similarly, richness and biomass responses were correlated for pulse 1, but not pulse 2 (Fig. 7b); reflecting the greater recovery of biomass relative to richness over time. Composition and richness remained tightly coupled over the experiment, indicating that compositional shifts were primarily owed to changes in the number of taxa (Fig. 7c).

At the end of the experiment (day 43), the relationship between biomass and composition had become visibly negative (Fig. 7a). No correlation was detected due to the absence of biomass recovery in the two pond communities faced with strong pesticide interaction at the highest combination of treatments (isolated in the lower left quadrant); when these two ponds are removed, however, the negative correlation becomes significant ($r = -0.35$; $P = 0.05$). The emergence of this negative relationship over time indicates a potential trade-off between the recovery of biomass and composition across all other 46 ponds.

DISCUSSION

Combining approaches from community ecology and ecotoxicology in the context of agricultural pollution, we quantified the effects of three widespread synthetic agrochemicals on zooplankton biomass and community structure. Of all possible single and interactive effects, we found that glyphosate, applied alone or in combination, was the most influential driver of community-wide

biomass, composition, and diversity, with a marked time-dependent effect on biomass. Although imidacloprid impaired copepods, the insecticide did not affect zooplankton community-wide biomass. Our results indicate that community properties respond rapidly to glyphosate exposure but do not recover equally over time. Importantly, at the community scale, zooplankton biomass showed little resistance to the first pulse of glyphosate, but rapidly recovered and even increased as a function of the glyphosate dose received; in ponds exposed to higher concentrations of glyphosate (alone or with imidacloprid), however, the number of taxa declined and failed to recover. We found that some cladocerans can be highly tolerant to pesticide contamination and their biomass can compensate for the decline of more sensitive community members; this sorting process conferred greater resistance to zooplankton communities upon the second pesticide pulse. Below, we position the pesticide concentrations inducing biotic responses within the ecotoxicological literature (reviewed in Appendix S1: Table S1) and discuss our findings with regards to underlying community processes, as well as the implications for freshwater zooplankton in agricultural areas.

Community responses to agrochemicals

Zooplankton biomass and structural responses varied with agrochemical treatment type and severity, and among major groups: cladocerans, copepods, and rotifers. Unlike other studies (Alexander et al. 2013, 2016, Baker et al. 2016, Geyer et al. 2016), nutrient enrichment, alone or combined with pesticide contamination, had surprisingly little effect on zooplankton in our ponds. Of the few nutrient-related signals detected, the positive interactive effect with glyphosate on cladoceran biomass, and thus total zooplankton, after the first pesticide pulse was the strongest, indicating that nutrients partly contributed to the subsequent cladoceran proliferation and overall zooplankton community recovery.

Although imidacloprid exerted milder effects on zooplankton communities as compared to glyphosate, the insecticide distinctly impaired copepods. Given that only two copepod species were identified in our ponds, this result should not be generalized across Copepoda. Copepod biomass declined at doses $\geq 3 \mu\text{g/L}$, in agreement with other mesocosm-based assessments reporting harmful effects at concentrations between 3 and 4 $\mu\text{g/L}$ (Schrama et al. 2017, Sumon et al. 2018, Chará-Serna et al. 2019). A compelling case study by Yamamuro et al. (2019) showed that decadal increases in the use of neonicotinoids caused the collapse of freshwater zooplankton, and in turn, of fisheries yield; notably, the once dominant and most sensitive taxon of the zooplankton community was a copepod, whose biomass decline coincided with the introduction of imidacloprid. Unlike copepods, cladocerans and rotifers appeared to be tolerant to imidacloprid in our experiment. Yet, adverse effects of imidacloprid have been documented at

similar or lower concentrations for a wide array of aquatic invertebrates (Van Dijk et al. 2013, Morrissey et al. 2015, Raby et al. 2018), including some cladoceran and rotifer taxa found in our ponds (e.g., *Polyarthra*; Sumon et al. 2018; Appendix S1: Table S1). No clear pattern of species sorting emerged along the imidacloprid gradient; however, the decline in copepods likely benefitted cladocerans and rotifers after the first pulse, possibly as a result of relaxed competition.

The presence of glyphosate in ponds resulted in differential short- and long-lasting effects across major zooplankton groups. Indeed, the first pulse elicited immediate declines in copepods and rotifers but not cladocerans, while subsequent biomass increases were only observed in cladocerans. This pattern was clearly visible in ponds contaminated with the herbicide alone and in conjunction with the insecticide, highlighting the relatively strong influence of glyphosate in our experiment, but more importantly, the co-tolerance of some cladocerans to both pesticides. However, the prolonged declines in all major groups, and thus of overall zooplankton, in ponds treated with the combination of the highest pesticide doses reveal evidence of negative interactive effects of glyphosate and imidacloprid on zooplankton, which has thus far not been documented, to our knowledge. Although dose-dependent, the suppression of all glyphosate-induced positive effects by imidacloprid could be indicative of a reversal effect, often referred to as an "ecological surprise" in multiple stressor research (Jackson et al. 2016).

The general patterns of species turnover in communities exposed to glyphosate (alone or in combination) highlighted a stark contrast between sensitive rotifer (long-lasting declines at concentrations $\geq 0.3 \text{ mg/L}$) and copepod zooplankton (partial recovery over time, but none $\geq 5.5 \text{ mg/L}$), and highly tolerant cladocerans, with *Alona*, *Chydorus*, and *Scapholeberis* still thriving under severe glyphosate contamination, even in the presence of imidacloprid. Information on rotifers is relatively limited (Appendix S1: Table S1), but our results are consistent with other studies of glyphosate toxicity on copepods, with notably greater sensitivity found in immature stages as compared to adults (Lim et al. 2019). While compelling evidence has accrued for acute and chronic toxicity in cladocerans at similar concentrations of glyphosate (e.g., ranges of LC_{50} for *Daphnia* and *Ceriodaphnia*: 0.45–10.6 and 4.8–6.05 mg/L , respectively; Tsui and Chu 2003, Cuhra et al. 2013), such observations mostly rely on single-species cultures in a laboratory setting, with few to no studies using the most tolerant species found in our ponds. Several multispecies assessments recorded only minor or transient community-wide density effects of glyphosate (Vera et al. 2012, Baker et al. 2016, Gutierrez et al. 2017, Lu et al. 2020), and concluded that the herbicide was unlikely to cause the collapse of entire zooplankton communities under normal-use circumstances (that is, glyphosate concentrations up to $\sim 2.25 \text{ mg/L}$ in water, as per Relyea 2005, 2006). In this regard, our results also

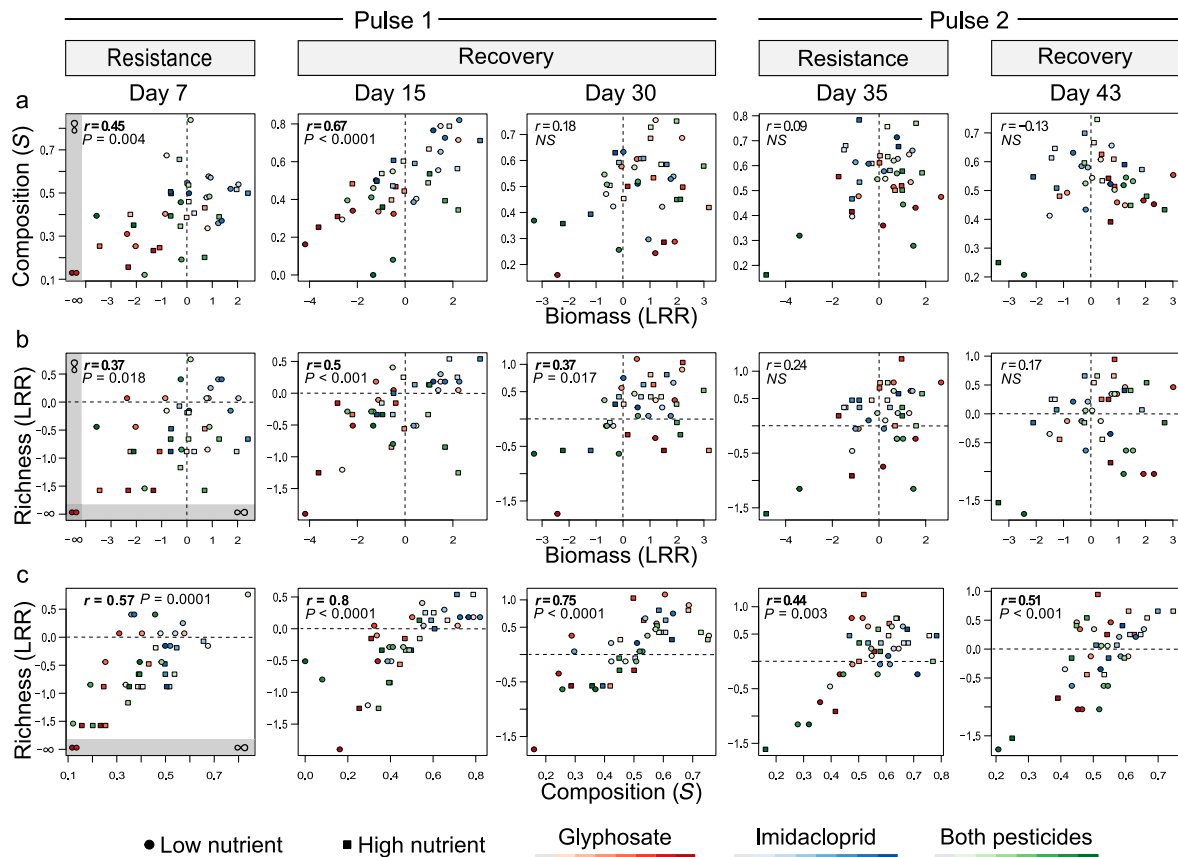


FIG. 7. Relationships between the resistance or recovery of zooplankton community properties over time: (a) composition vs. biomass; (b) richness vs. biomass; (c) richness vs. composition. Resistance measures are estimated on days 7 (pulse 1) and 35 (pulse 2), whereas recovery measures are estimated on days 15, 30 (i.e., 9 and 24 d after pulse 1), and 43 (9 d after pulse 2). Spearman rank correlation coefficients are indicated for each bivariate relationship; significant coefficients ($P < 0.05$) are highlighted in boldface type. Resistance and recovery measures of biomass and richness are expressed as log response ratio (LRR) between treated and control communities: 0, maximum resistance or recovery, as indicated via dash lines; < 0 , low resistance via alteration/underperformance or incomplete recovery; > 0 , low resistance via overperformance/stimulation or recovery via overcompensation/stimulation. For composition, measures are expressed as a similarity index (S) between treated and (nutrient-specific) control communities: 0, low; 1, maximum resistance/recovery. Note that in the absence of zooplankton individuals (i.e., no individuals found in samples; only two low-nutrient high-glyphosate ponds on day 7), LRRs based on null values of biomass and richness = $-\infty$; such measures are graphically represented but excluded from correlation coefficients.

suggest that, on a longer-term basis, glyphosate may have limited adverse effects on total zooplankton biomass. Nonetheless, the sensitivity and long-lasting declines of rotifers may have important implication for food web processes (Arndt 1993, Miracle et al. 2007), warranting further investigation.

The rapid and marked proliferation of tolerant cladocerans following the first application of glyphosate was likely attributable to a stimulatory, bottom-up effect induced by the nutrient content of glyphosate. Glyphosate acid contains 18.3% P, implying that its presence in water represents an additional source of P that may be used by microbial and algal communities, either in the form of glyphosate or degraded products (Hove-Jensen et al. 2014, Wang et al. 2016, 2017, Brock et al. 2019, Lu et al. 2020). Other studies have reported increases in phytoplankton in the presence of glyphosate, attributing

this fertilizing effect to glyphosate-derived P (Pérez et al. 2007, Forlani et al. 2008, Saxton et al. 2011, Harris and Smith 2016); that is, even in observational studies of natural ecosystems (Berman et al. 2020). The parallel study by Fugère et al. (2020) showed that glyphosate led to dose-dependent increases in TP and chl *a* concentrations in our ponds, presumably as a result of phytoplankton P limitation (initial N:P molar ratio ~ 33). Taken in context with our results, it appears that glyphosate-mediated increases in algal resources enhanced the growth of tolerant cladocerans. By the end of our experiment, positive (indirect) effects of glyphosate on cladoceran and overall zooplankton biomass were indeed visible at all treatment concentrations, with the highest biomass levels recorded in most contaminated ponds, demonstrating that the dose-dependent fertilizing effect of glyphosate on phytoplankton can transfer to higher trophic levels; i.e.,

cascading stimulatory effects. Though less documented than toxic effects, bottom-up effects of glyphosate on zooplankton have been previously observed (Vera et al. 2012). Thus, our results reveal a two-step effect of glyphosate on zooplankton biomass: negative in the short-term due to the loss of sensitive taxa, but positive over longer timescales owing to the increased growth of tolerant taxa. This positive effect will, however, be conditional upon the presence of at least one or a few tolerant taxa in the community, a condition that may not always be satisfied in nature.

As a result of differential sensitivity across taxa, the rise in (co-)tolerant cladoceran zooplankton permitted the recovery of community biomass in ponds treated with glyphosate or both pesticides; this sorting process also prompted greater resistance during the second pulse. This result is consistent with the concept of stressor-pollution-induced community tolerance (Bérard and Benninghoff 2001, Vinebrooke et al. 2004, Tlili et al. 2016), whereby initial exposure to stress may eliminate sensitive taxa from a community, leading to increased community tolerance upon subsequent exposure events. Indeed, species sorting in favor of tolerant cladocerans induced by the first pulse of glyphosate in our ponds likely conferred community tolerance (and thus greater biomass resistance), allowing the maintenance of zooplankton biomass after the second pulse. This result constitutes evidence of pollution-induced community tolerance in zooplankton faced with pesticide contamination, providing a rare example of such tolerance in metazoans, given that most ecotoxicological studies of pollution-induced community tolerance focus on microbial, algal, or periphytic communities (Blanck 2002, Boivin et al. 2002, Tlili et al. 2016).

We also found that community-wide biomass showed greater recovery than composition and diversity indices, as have previous studies of functional and structural responses to environmental disturbances (Hoover et al. 2014, Hillebrand and Kunze 2020). In fact, the recovery and increase of biomass after exposure to glyphosate (alone or with imidacloprid) was achieved through species sorting, all while community richness declined. The striking contrast in temporal patterns of biomass vs. richness, whereby effects of glyphosate were time-dependent for the former but remained negative for the latter (Fig. 3a vs. Fig. 4a), have clear implications for biodiversity loss and ecosystem functioning in freshwaters; that is, even when total zooplankton biomass appears unaffected. Further, the concomitant increase in biomass and decrease in richness clearly confirm that glyphosate-induced taxon loss was not attributable to low density effects in our experiment. When excluding the two pond communities that failed to recover in biomass (i.e., those faced with strong pesticide interaction at the highest treatment combination), we also found a significant negative relationship between the recovery of community biomass and composition, pointing to a trade-off between the recovery of biomass and

community structure. In sum, our study indicates that the long-term effect of glyphosate contamination on zooplankton varies among community properties, with increasing concentrations causing species loss, compositional shifts, but greater biomass production in the remaining tolerant taxa.

Implications and concluding remarks

Comprising a total of 24 zooplankton taxa representative of the local species pool (Thompson et al. 2015), our 48 pond communities revealed complex processes that may have only been observable under the realistic conditions of our field-based experiment. Together, these results contribute to addressing the community-level effects of synthetic contaminants, while also providing insight into the study of ecological stability and multiple stressors in the context of agrochemical pollution. Nevertheless, our experimental system was inoculated with communities from a single lake; a deeper understanding of how freshwater environments respond to the rising prevalence of diverse contaminants will require further investigations of multispecies assemblages across ecosystem types.

Although community biomass may be resilient to severe pesticide contamination, species loss and compositional shifts in favor of a few distinct tolerant taxa can have implications for freshwater food web processes and ecosystem stability in agricultural areas (Pennekamp et al. 2018, Frank and Tooker 2020). Losing taxon-specific functions while enhancing those provided by tolerant taxa could destabilize ecosystems (Arnoldi et al. 2019). In environments prone to glyphosate pollution, the proliferation of herbivorous cladocerans at the expense of omnivorous or carnivorous cyclopoids or less effective filter-feeding rotifers could modulate trophic interactions and top-down pressure (Sommer et al. 2001, 2003). Another potential consequence of glyphosate is nutrient enrichment cascading up the food chain. While often overlooked as a source of anthropogenic P, glyphosate-derived P inputs in intensive agricultural areas are now comparable in magnitude to other past P-sources (e.g., detergents) that once required legislation (Hébert et al. 2019), and its excessive usage warrants more attention in watershed management.

In this experiment, concentrations at which pesticides caused biotic effects differed among zooplankton community properties, with most response thresholds falling within the range of previously recorded measurements in agricultural water bodies. Though pervasive in surface waters, imidacloprid and glyphosate concentrations are highly variable, in part owing to variability in land use intensity and biodegradation potential (Hladik et al. 2014, Medalie et al. 2020). Globally, imidacloprid and glyphosate concentrations can range between $\sim <0.01$ – $320 \mu\text{g/L}$ and $\sim <0.001$ – 5.2 mg/L , respectively (Struger et al. 2008, Annett et al. 2014, Morrissey et al. 2015, Hénault-Ethier et al.

2017, Satiroff et al. 2021); but concentrations on the order of ng/L are common for both pesticides (Montiel-Léon et al. 2019). In our ponds, glyphosate adversely affected overall zooplankton biomass at 0.7 mg/L (≥ 0.3 mg/L in rotifers), but concentrations ≥ 0.04 mg/L were sufficient to enhance total biomass over time; and alterations of community structure were observed at 0.1 mg/L. For imidacloprid, concentrations ≥ 3 $\mu\text{g/L}$ affected copepod biomass and ≥ 0.15 $\mu\text{g/L}$ slightly reduced crustacean richness and diversity. While some of these threshold exposure concentrations may be on the high end of environmentally relevant glyphosate concentrations, most effects were observed under the benchmark of 2.25 mg/L that is often used as the worst-case scenario in ecotoxicology (Relyea 2005, 2006, Baker et al. 2016, Geyer et al. 2016).

Most crucially, our study demonstrates that community properties could be affected at pesticide concentrations below common North American water quality guidelines. This is especially the case for glyphosate criteria for the protection of aquatic life, both in Canada (0.8 mg/L and 27 mg/L for long- and short-term exposure, respectively; CCME 2007, 2012) and the United States (26.6 mg/L and 49.9 mg/L for long- and short-term exposure of freshwater invertebrates, respectively; EPA 2019). Although Canadian benchmarks for glyphosate remain more conservative as compared to those of the United States, these criteria appear too permissive to ensure protection. In light of the global expansion in glyphosate use, we believe that national guidelines should be revised, especially in agricultural areas.

ACKNOWLEDGMENTS

We are thankful to Michelle Gros, Katherine Velghe, Marilyne Robidoux, and Marco Castro for their assistance with laboratory work; David Maneli, Charles Normandin, Alexandre Arkilanian, and Tara Jagadeesh for their help with field work; Capucine Lechartre and Olympe Durrenberger for their assistance with the literature review. We also thank two anonymous reviewers for their constructive comments. M.-P. Hébert was supported by the Natural Sciences and Engineering Research Council (NSERC), Fonds de Recherche du Québec Nature-Technologies (FRQNT), and NSERC-CREATE via the GRIL. V. Fugère was supported by NSERC. N. Barbosa da Costa was supported by FRQNT and NSERC-CREATE via the GRIL. R. D. H. Barrett and B. J. Shapiro were supported by Canadian Research Chairs. The LEAP facility was funded by the Canadian Foundation for Innovation and Liber Ero Chair in Biodiversity Conservation attributed to A. Gonzalez. This work was also supported by a National Geographic grant for early-career researchers awarded to M. P. Hébert and NSERC Discovery Grants attributed to B. E. Beisner, A. Gonzalez, and G. F. Fussmann.

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SUPPORTING INFORMATION

Additional supporting information may be found online at: <http://onlinelibrary.wiley.com/doi/10.1002/eap.2423/full>

OPEN RESEARCH

Environmental variables and phytoplankton data (Fugère 2020) are available on Figshare: <https://doi.org/10.6084/m9.figshare.11717361.v2>. Zooplankton community data (Hébert 2021) are available on Figshare: <https://doi.org/10.6084/m9.figshare.14977092.v1>