LETTER

Community ecology theory predicts the effects of agrochemical mixtures on aquatic biodiversity and ecosystem properties

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Abstract

Ecosystems are often exposed to mixtures of chemical contaminants, but the scientific community lacks a theoretical framework to predict the effects of mixtures on biodiversity and ecosystem properties. We conducted a freshwater mesocosm experiment to examine the effects of pairwise agrochemical mixtures [fertiliser, herbicide (atrazine), insecticide (malathion) and fungicide (chlorothalonil)] on 24 species- and seven ecosystem-level responses. As postulated, the responses of biodiversity and ecosystem properties to agrochemicals alone and in mixtures was predictable by integrating information on each functional group's (1) sensitivity to the chemicals (direct effects), (2) reproductive rates (recovery rates), (3) interaction strength with other functional groups (indirect effects) and (4) links to ecosystem properties. These results show that community ecology theory holds promise for predicting the effects of contaminant mixtures on biodiversity and ecosystem services and yields recommendations on which types of agrochemicals to apply together and separately to reduce their impacts on aquatic ecosystems.

Keywords

Contaminant, ecosystem function, ecosystem services, freshwater ecosystem, mesocosm, pesticide.

INTRODUCTION

The majority of surface waters in areas of agricultural, urban or mixed land use have detectable levels of two or more biocides (Gilliom *et al.* 2006), which is not surprising given that many agrochemicals are applied in mixtures (Relyea 2009; Altenburger *et al.* 2013). Because chemical mixtures in wetlands are the norm rather than the exception, there has been an increased focus on developing chemical risk assessment methods that incorporate the predicted effects of mixtures (Belden *et al.* 2007; Backhaus & Faust 2012; Altenburger *et al.* 2013). The field of toxicology has developed some understanding of the effects of chemical mixtures on individual species (Fairchild *et al.* 1994; Anderson & Lydy 2002; Boone & James 2003; Hayes *et al.* 2006; Relyea 2009) and has spawned models for predicting the effects of mixtures of chemicals with similar or different modes of action on the growth, reproduction and mortality of individuals (Altenburger *et al.* 2000, 2013; Backhaus *et al.* 2000; Belden *et al.* 2007; Backhaus & Faust 2012). However, less is known about the effects of mixtures on aquatic communities and ecosystem properties (Relyea 2009; Altenburger *et al.* 2013). Predicting when and how contaminant mixtures will influence communities and ecosystem properties poses many more challenges than predicting the responses of individual taxa in isolation, requiring integration of (1) both direct and indirect density- and trait-mediated effects of contaminants (Brock *et al.*

2000a,b; Fleeger *et al.* 2003; Relyea & Hoverman 2006), (2) starting community composition (Hooper *et al.* 2005; Rohr & Crumrine 2005) and (3) non-additive effects (synergisms and antagonisms), which are more likely with species interactions (Relyea & Hoverman 2006).

With thousands of biocides in use globally, it is logistically impossible to study the responses of aquatic communities to each individual chemical, not to mention all possible chemical mixtures. Predicting the effects of pesticide mixtures is increasingly important for risk assessment in light of increasing evidence of pesticide exposure driving changes in community composition, ecosystem properties and the loss of regional biodiversity (Schäfer *et al.* 2007; Beketov *et al.* 2013). Hence, we need a framework that can clarify this complexity by offering a null model or expectation for mixtures and, ideally, predict the effects of contaminant mixtures on aquatic communities. Despite this need, much of the research examining the effects of contaminant mixtures on communities, biodiversity and ecosystem properties has been descriptive rather than mechanistic or predictive (but see Koelmans *et al.* 2001; Traas *et al.* 2004). Expanding upon the hypotheses of Rohr and colleagues (Rohr *et al.* 2006; Clements & Rohr 2009), we submit that food web theory offers such a predictive framework, providing null hypotheses regarding antagonistic, additive or synergistic interactions between chemicals. By integrating information on which functional groups (1) are generally sensitive to particular chemical classes (i.e. direct effects), (2)

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their reproductive rates (i.e. recovery after chemical exposure) and (3) the strong interactions those groups have with other species in food webs (i.e. indirect effects), and then (4) coupling this information with the effects functional groups have on ecosystems properties, community ecology theory should be able to predict the effects of contaminant mixtures on biodiversity and ecosystem functions and services. To test this general hypothesis, we conducted an outdoor mesocosm experiment quantifying the effects of a fertiliser, herbicide (atrazine), insecticide (malathion) and fungicide (chlorothalonil), in isolation and in all pairwise combinations, on 24 species- and seven ecosystem-level responses.

This predictive framework, if supported by empirical data, could provide a platform from which the general risks of various agrochemical mixtures could be assessed. This framework could allow investigators to use the more well-known responses of important functional groups to agrochemical contamination to identify particular agrochemical combinations that may pose particularly severe threats to aquatic biodiversity and ecosystem services. Furthermore, if responses of taxa to agrochemicals can be predicted by either chemical structure (De Roode *et al.* 2006) or phylogeny (Guénard & Ohe 2011), the combined risks of new agrochemicals and mixtures in which they might occur could be estimated before they are approved for use. Ultimately, this framework will allow us to generate specific policy recommendations, such as altering the timing of agrochemical applications to minimise the adverse impacts of chemical contaminants on aquatic communities.

Predictions

We predicted that fertilisers would increase the biomass of primary producers and both primary and secondary consumers through bottom-up effects of increased nutrient availability (Fig. 1a; Chase 2003). Herbicides were predicted to act antagonistically to fertilisers, limiting primary production and thus decreasing primary and secondary consumers (Fig. 1b; Brock *et al.* 2000a). By directly decreasing the abundance of zooplankton (phytoplanktivores) and arthropod consumers, we expected insecticides to induce positive indirect effects on non-arthropod herbivores and phytoplankton via reductions in competitive and consumer-resource interactions (Fig. 1c; Brock *et al.* 2000b). Fungicides typically have non-specific modes of action (e.g. inhibiting cell division; Maltby *et al.* 2009). For example, chlorothalonil (the fungicide used in this study), reduces glutathione within cells and thus disrupts cellular metabolism (Tillman *et al.* 1973). Thus, we expected negative direct effects of fungicides across all trophic levels (Fig. 1d).

We expected the responses of aquatic communities to agrochemical mixtures to be predictable based on the direction and magnitude of the combined direct and indirect effects of each chemical in isolation. Thus, when direct or indirect effects of agrochemicals in a mixture are antagonistic, aquatic community composition is expected to be more similar to controls than treatments with either chemical alone. Conversely, when the effects of chemicals in a mixture are in the same direction, we expect communities to be more distinct from controls.
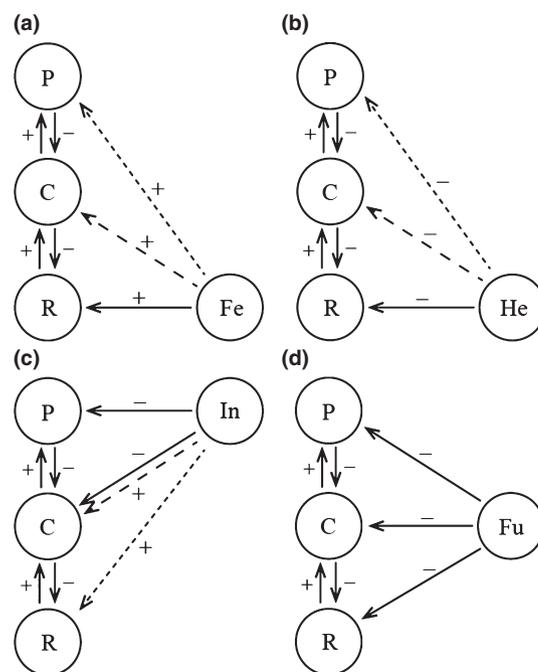
Figure 1 Predicted direct (solid arrows) and indirect (dashed arrows) effects of (a) fertiliser, (b) herbicide, (c) insecticide and (d) fungicide on a tri-trophic community. Relative strength of indirect effects are indicated by length of dashes in the arrow (long dashes = stronger indirect effect). R, resource; C, consumer; P, predator.

More specifically, we predicted that fertiliser would generally mitigate the negative effects of biocides via antagonistic direct and indirect effects (Fig. 1), resulting in communities more similar to the controls than those exposed to the biocide alone. A notable exception to this general prediction might occur in fertiliser and insecticide mixtures where the combined positive direct and indirect effects on primary producers might initially result in algal blooms before arthropod consumers begin to recover (Fig. 1a,c; Traas *et al.* 2004); this might delay recovery to an uncontaminated state. In contrast, we predicted that biocide mixtures would generally have greater effects than the individual biocides alone, with mixture-exposed communities being more distinct from the controls than their respective biocide-only treatments. However, we predicted the herbicide–insecticide mixture would not follow this general rule because the direct negative effects of herbicides on phytoplankton were expected to at least partially counteract the indirect increase in phytoplankton caused by insecticide exposure (Fig. 1b,c; Boone & James 2003). Finally, given the well-established relationship between biodiversity and ecosystem functions (Hooper *et al.* 2005), we predicted that contaminant-induced changes to biodiversity, either through changes in species richness or relative abundance, would translate to detectable changes in ecosystem properties. In particular, we expected dissolved oxygen and pH to respond to changes in primary production, and light and temperature to respond to changes in phytoplankton density. Here, we provide support for most of these predictions, providing a promising approach for predicting and mitigating the deleterious effects of multiple contaminants on freshwater ecosystems.

METHODS

The mesocosm experiment was conducted over 7 weeks from July to August 2008 at a facility in southeastern Hillsborough County, FL, USA (27°45.530′ N, 082°13.650′ W). This timing is near the beginning of the rainy season when the probability of agrochemical runoff events increases. Freshwater aquatic communities were established for 3 weeks before the start of the experiment; 1200 L plastic tanks were filled with 800 L of well water, inoculated with local zooplankton, periphyton and phytoplankton, and covered with 60% shade cloth. Water temperatures 10 cm below the surface ranged from 27 to 29 °C. At these temperatures, we estimated generation times of all zooplankton and algae species to be ≤ 7 days (Gillooly 2000). Additional organisms were collected from ponds in Hillsborough County, FL, USA, and added just before the agrochemical additions to generate aquatic communities representative of temporary wetlands in peninsular Florida (Evans *et al.* 1999), consisting of two species of amphibian larvae, four species of snails, five species of macroarthropods, zooplankton, periphyton and phytoplankton [Table S1; see Supporting Information (SI) for specific methodological details]. We conceptualised this community into six trait-based functional groups divided between two tri-trophic compartments. The first tri-trophic compartment consisted of phytoplankton, zooplankton and zooplankton predators (Corixidae), and the second consisted of periphyton, grazers (snails and tadpoles) and predators of grazers (crayfish, hemipterans and odonate larvae). Organisms were collected from local ponds within ~1 km of 27°06.579′ N, 082°23.014′ W. Phytoplankton, light availability and water temperature were measured weekly, and periphyton, zooplankton, dissolved oxygen and pH were sampled biweekly, using standard sampling procedures (see SI). At the conclusion of the experiment, all tanks were drained, amphibians, snails and macroarthropods were counted, and leaf litter packets were dried and weighed. This research was approved by animal care and use committee protocols W3228 at the University of South Florida and 023-08WEC at the University of Florida.

To standardise relative agrochemical exposure concentrations, we used US EPA GENEEC v2 software (US EPA, Washington, DC, USA), which uses the physicochemical properties of agrochemicals in conjunction with manufacturer application recommendations to estimate the peak exposure concentration (EEC) for each biocide (parameters reported in Table S5). Experimental treatments consisted of four agrochemicals in isolation at one of two concentrations (1× and 2× EEC; fertiliser: nitrogen, 1× = 4400 µg/L, phosphorus, 1× = 440 µg/L; fungicide: chlorothalonil, EEC = 164 µg/L; herbicide: atrazine, EEC = 102 µg/L; insecticide: malathion, EEC = 101 µg/L), all six possible pairwise mixtures of agrochemicals (1× EEC of each chemical) and water and solvent (50 mg/L acetone) controls. Total nitrogen and phosphorus concentrations were similar to those found in high productivity ponds reported by Chase (2003). Nitrogen and phosphorus concentrations in control tanks were 370 and 60 µg/L, respectively, but we did not determine which was the limiting nutrient. We included the 2× EEC treatments of each agrochemical alone to account for mixture treatments containing twice the number of

EEC complements. Four replicate tanks of each treatment (64 mesocosms total) were arranged in a randomised block design. Agrochemicals were applied as single applications of technical grade compounds (chlorothalonil = 99.0% purity, atrazine = 98.9% purity and malathion = 98.4% purity, Chemservice, West Chester, PA, USA; fertiliser added as $NaNO_3$ and $NaH_2PO_4 \cdot H_2O$, Thermo Fisher Scientific, Waltham, MA, USA) dissolved in acetone. To quantify actual concentrations, water samples were collected from each tank approximately 1 h after application of agrochemicals, were pooled into a single sample because of the high cost of these analyses (~$150/sample), and were analysed by the Mississippi State Chemical Laboratory.

Data analysis

All data were natural log-transformed prior to analysis, and Bray–Curtis similarity was used as the dissimilarity measure for analyses of the full community. Water and solvent control tanks had similar communities ($P = 0.474$), so these treatments were pooled for subsequent analyses. Similarly, there were no effects of agrochemical concentration on the community-level response within agrochemical treatments, so 1× and 2× EEC treatments were pooled for each agrochemical in subsequent analyses (Table S2).

Our predictions were presented as *a priori* expectations about whether the communities exposed to agrochemical mixtures would be either significantly more or less similar to the control treatments, relative to the communities exposed to the respective biocides alone. To test the specific hypotheses associated with our predictions, we calculated the Bray–Curtis dissimilarity between the centroids of the agrochemical treatments and the control centroid ($\overline{BC}_{Treatment,Co}$). For each comparison (e.g. fertiliser–fungicide mixture vs. fungicide-only), we subtracted the dissimilarity between the centroids of the mixture treatment and the control from the dissimilarity between the centroids of the biocide-only treatment and the control. Thus, a positive value would indicate that the mixture treatment was relatively more similar to the control treatment than the biocide alone, and vice versa. For example, the Bray–Curtis dissimilarity between fungicide-only treatments and control treatments ($\overline{BC}_{Fu,Co}$) was 10.99, whereas the dissimilarity between the fertiliser-fungicide treatment and the control treatment ($\overline{BC}_{Fe+Fu,Co}$) was 7.07, and therefore $\overline{BC}_{Fu,Co} - \overline{BC}_{Fe+Fu,Co} = 3.92$. We then compared this observed value to a null distribution of simulated test statistics in which all treatment designations were randomly reshuffled among the tanks of the treatments being compared. The dissimilarity between each tank and the control centroid and the dissimilarities between the randomised treatment centroids and the control centroid were calculated following the methods of Anderson (2006). We performed this randomisation procedure 10 000 times and determined significance as the proportion of simulated test statistic values that were either greater than or equal to (or less than or equal to, depending on the *a priori* prediction) the observed test statistic.

Community-level and individual species responses to agrochemicals 4 weeks post-exposure were further explored using permutation-based analysis of variance (PERMANOVA;

see SI) and permutation-based tests of homogeneity of multivariate dispersions from the spatial median of each treatment (PERMDISP; Anderson *et al.* 2008). To visualise the community response to treatments, we performed a distance-based redundancy analysis (dbRDA) to generate a constrained ordination diagram, using the significant main effects and interaction terms determined from the PERMANOVA analysis as categorical predictor variables (Anderson *et al.* 2008). Distance-based redundancy analysis is a direct analogue to traditional redundancy analysis (RDA), but is more flexible in that it can be used with non-Euclidean measures of distance (such as Bray–Curtis dissimilarity) that are often more appropriate for the analysis of ecological data (Legendre & Anderson 1999). All community and individual species responses were analysed using PERMANOVA+ for PRIMER (v6; PRIMER-E Ltd, Plymouth, UK).

To test the hypothesis that the changes in aquatic community composition associated with agrochemical mixtures resulted in subsequent changes in ecosystem properties, we performed a combined factor-path analysis using methods described previously (McMahon *et al.* 2012). To reduce the number of potential pathways in the path analysis, we identified latent variables representing the responses of various functional groups (e.g. – herbivores of periphyton) and ecosystem properties (dissolved oxygen, pH, etc.). Latent variables were constructed by performing a confirmatory factor analysis in Statistica (v11; StatSoft, Tulsa, OK, USA; with varimax rotation) to extract the underlying correlational structure among the dependent variables associated with each functional group or class of ecosystem properties. We then conducted a path analysis using the lavaan package in R (Rosseel 2012; R Core Team 2013) to determine the significance of hypothesised causal pathways among agrochemical mixtures and the latent functional groups and ecosystem properties.

RESULTS

Direct and indirect effects of individual agrochemicals

Multivariate dispersion was not different among treatments ($F_{15,48} = 1.474$, $P = 0.153$). Each agrochemical had a significant main effect on aquatic community composition (Table 1; Figs 2, S1). Snail abundance was generally higher in the presence of fertiliser, particularly for *Planorbella trivolvis* and *Viviparus georgianus*, whereas crayfish (*Procambarus alleni*) abundance was lower (Fig. 2b; Table S2). Fungicide decreased leaf litter decomposition and generally decreased the abundance of herbivores, particularly calanoid copepods, larval amphibians and snails (Fig. 2b; Table S2). Phytoplankton (Fig. S2a) and periphyton chlorophyll *a* (Fig. S2d) increased in treatments with fungicide. Herbicide reduced phytoplankton (Fig. S2b), periphyton abundance (when herbivores were present; Fig. S2e) and crayfish survival, and increased *P. trivolvis* abundance (Fig. 2b; Table S2). Insecticide treatments did not significantly affect macroarthropods, but had strong effects on the abundance of cladoceran zooplankton; more specifically, *Ceriodaphnia* sp. abundance was reduced throughout the experiment, but *Diaphanosoma* sp. abundance

Table 1 Results of PERMANOVA analysis of full community response to agrochemical mixtures

Source	d.f.	SS	MS	Pseudo-F	P*
Block	3	856.04	285.35	2.653	0.001
Fertiliser	1	274.96	274.96	2.556	0.022
Fungicide	1	1444.4	1444.4	13.428	0.001
Herbicide	1	220.17	220.17	2.047	0.041
Insecticide	1	452.02	452.02	4.202	0.001
Fe × Fu	1	244.29	244.29	2.271	0.037
Fe × He	1	293.10	293.10	2.725	0.010
Fe × In	1	219.26	219.26	2.038	0.054
Fu × He	1	122.59	122.59	1.140	0.334
Fu × In	1	64.62	64.617	0.601	0.766
He × In	1	142.98	142.98	1.329	0.229
Pooled	50	5378.2	107.56		
Total	63	10269			

P-values determined by permutation.

was higher at the end of the experiment in insecticide treatments (Table S2; Figs 2b, S3).

Testing predictions for agrochemical mixtures

Fertiliser–biocide mixtures

No fertiliser–biocide mixture was significantly different from the control treatments (Table S3), despite significant main effects of each biocide (Table 1). At the end of the experiment, communities exposed to fertiliser–fungicide mixtures were more similar to the control treatment than were the communities exposed to the fungicide-only treatments (Figs 2, S1). This was particularly the case for species that could reproduce within the tanks, such as zooplankton (Fig. S3) and snails (Fig. S4). Indeed, when considering only those taxa that could exhibit population recovery within the tanks (i.e. reproduce), community composition in fertiliser–fungicide mixtures was significantly more similar to the control than fungicide alone communities ($\overline{BC}_{Fu,Co} - \overline{BC}_{Fe+Fu,Co} = 3.92$, $P = 0.0073$).

Similarly, the fertiliser–insecticide mixture treatment resulted in a community more similar to that of the control than insecticide alone communities ($\overline{BC}_{In,Co} - \overline{BC}_{Fe+In,Co} = 3.24$, $P = 0.0139$). Consistent with our predictions, phytoplankton abundance was initially higher in fertiliser–insecticide mixtures than with either chemical alone (Fig. S2c) and zooplankton communities in the mixture treatment were initially similar to those of insecticide-only treatments (Fig. S3c,d). However, by the end of the experiment, cladoceran zooplankton and phytoplankton abundances in fertiliser–insecticide mixture treatments were similar to control treatments, unlike those of the insecticide-only treatments (Figs S2c, S3c,d).

The combination of herbicide and fertiliser resulted in communities more distinct from the control than were those exposed to herbicide alone ($\overline{BC}_{He,Co} - \overline{BC}_{Fe+He,Co} = -6.20$, $P = 0.0081$). The abundances of snails and crayfish in the fertiliser–herbicide mixture were higher and lower, respectively, than predicted by the additive effects of each chemical alone (fertiliser*herbicide; snails: Pseudo-$F_{1,63} = 3.343$, $P = 0.0335$; Fig. S4c,d; Table S2). The mixture community was characterised by greater initial periphyton growth (in the absence of
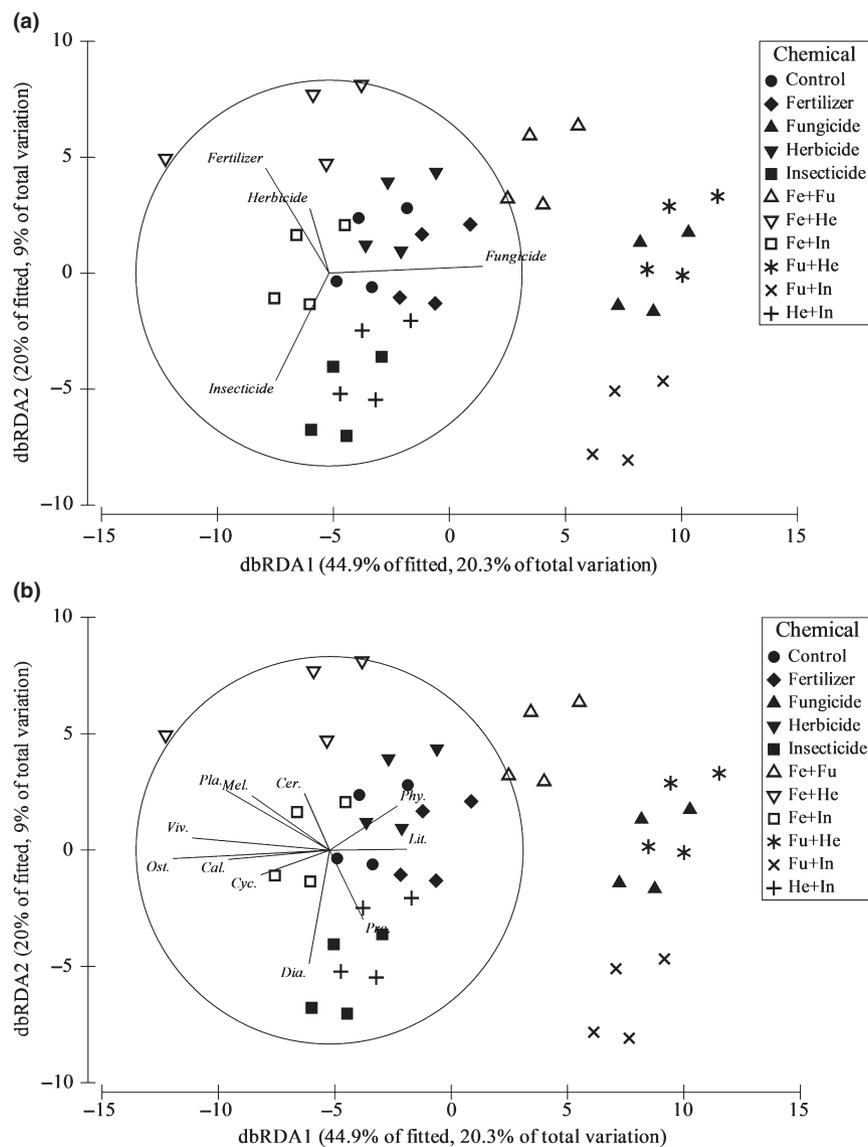
Figure 2 Distance-based redundancy analysis of community-level responses to agrochemical treatments showing (a) vector overlays of predictor variables and (b) vector overlays of species responses. Species abbreviations are *Cal*., calanoid copepods; *Cer*., *Ceriodaphnia* sp.; *Cyc*., cyclopoid copepods; *Dia*., *Diaphanosoma* sp.; *Lit*., percent leaf litter mass remaining; *Ost*., *Osteopilus septentrionalis*; *Phy*., phytoplankton F0; *Pla*., *Planorbella trivolvis*; *Pro*., *Procambarus alleni*.

herbivores; Table S2) and greater abundances of periphyton-consuming herbivores (snails) at the end of the experiment. Phytoplankton abundance in the fertiliser–herbicide mixture treatment was generally low early in the experiment and then increased late (Fig. S2b), consistent with fertiliser-facilitated recovery.

Biocide–biocide mixtures
Aquatic communities exposed to pairs of biocides were representative of the sum of the main effects of each chemical in isolation (Table 1, Figs 2, S1). Thus, when each chemical reduced the abundance of a given taxa, the mixture community exhibited a lower abundance than in either biocide-only treatment. Likewise, when the two chemicals had opposing effects in isolation, the mixture effect was intermediate between the respective single-biocide treatments. Fungicides

were toxic to several taxa, and mixtures of fungicide with either herbicide or insecticide shifted communities further from controls, as predicted (Figs 2, S1). Indeed, each of these two mixtures had communities that were significantly different from the controls (Table S3). The fungicide–insecticide mixture community was significantly less similar to the control community than were those of either fungicide ($\overline{BC}_{Fu,Co} - \overline{BC}_{Fu+In,Co} = -3.96$, $P = 0.0123$) or insecticide alone ($\overline{BC}_{In,Co} - \overline{BC}_{Fu+In,Co} = -7.60$, $P = 0.0017$). In fact, combined fungicide-insecticide exposure resulted in aquatic communities that were the most distinct from communities that did not receive agrochemicals, consistent with these two agrochemicals having the largest main effects out of the four agrochemicals tested (Table 1; Figs 2, S1). Community composition in tanks exposed to fungicide–herbicide mixtures was significantly more distinct from the controls relative to that of

herbicide alone ($\overline{BC}_{He,Co} - \overline{BC}_{Fu+He,Co} = -10.31$, $P = 0.0020$), but not fungicide alone ($\overline{BC}_{Fu,Co} - \overline{BC}_{Fu+He,Co} = -3.34$, $P = 0.1463$). This is consistent with herbicide having a relatively small main effect on community composition compared to that of fungicide (Table 1; Figs 2, S1). Also as predicted, the mixture of herbicide and insecticide resulted in communities intermediate between those exposed to either biocide alone (Figs 2, S1), and of similar distance to the control communities relative to either biocide alone (Table S3; $\overline{BC}_{He,Co} - \overline{BC}_{He+In,Co} = -2.09$, $P = 0.2781$; $\overline{BC}_{In,Co} - \overline{BC}_{He+In,Co} = -1.25$, $P = 0.8660$).
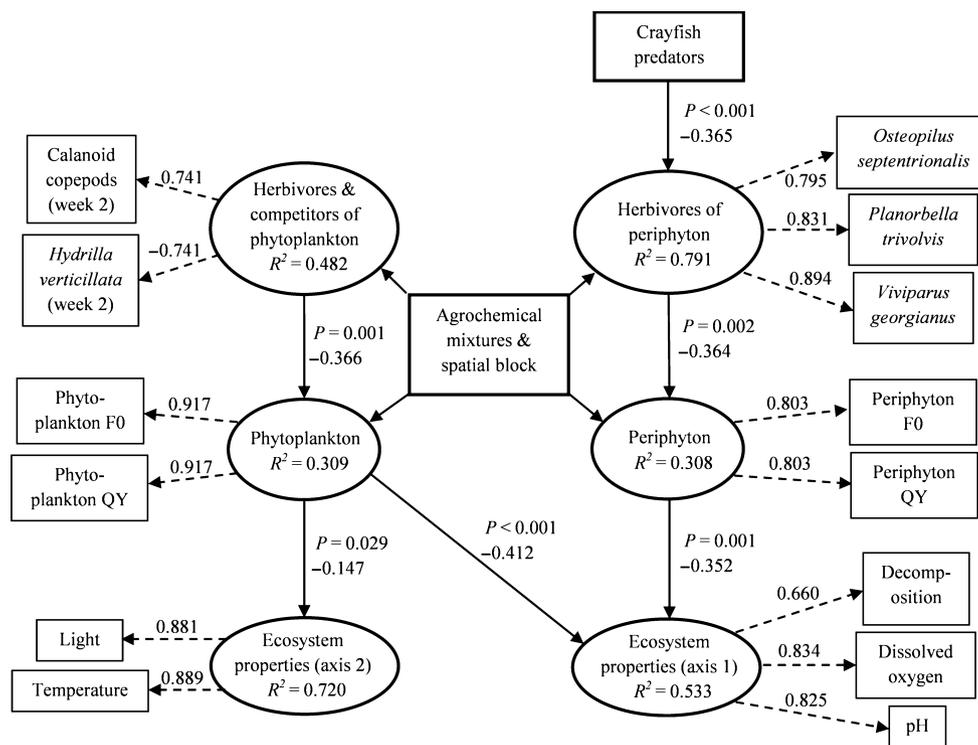
Effects on ecosystem properties

Ecosystem properties were best represented by two principal factors: (1) axis 1, correlated with leaf litter decomposition, dissolved oxygen and pH, and (2) axis 2, correlated with light availability and water temperature (Fig. 3). Neither ecosystem axis was directly influenced by agrochemicals, with the exception of fungicide having a direct influence on axis 1 (Fig. 3; Table S4). This effect was presumably mediated by direct effects of chlorothalonil on fungal-associated decomposition (Table S2), as there were no direct effects of chlorothalonil on DO or pH in a separate experiment (McMahon *et al.* 2012). Instead, these ecosystem properties were indirectly and predictably affected by agrochemicals through changes in periphyton and phytoplankton abundances (Fig. 3). The agrochemicals affected predators of herbivores and herbivores. These effects then cascaded down to the primary producers that drove much of the effects on the measured ecosystem properties (Table S4; Fig. 3). More specifically, when agrochemicals directly decreased herbivore abundances, there were indirect positive effects on periphyton and phytoplankton, which subsequently increased dissolved oxygen (through photosynthesis) and pH, and decreased decomposition. Similarly, these increases in phytoplankton reduced light penetration through the water column and thus water temperature.

DISCUSSION

Fertiliser–biocide mixtures

We predicted that fertiliser would generally reduce the adverse effects of biocides by limiting direct toxicity of the biocide (by providing increased energetic resources necessary for detoxification) or facilitating reproduction-mediated recovery through increased primary productivity. Consequently, we predicted that communities exposed to fertiliser–biocide mixtures would be more similar to the controls than either agrochemical alone. Consistent with our predictions, the community composition of each fertiliser–biocide mixture was generally more similar to

Figure 3 Path model demonstrating that effects of pesticide mixtures on ecosystem properties were mediated by the impacts of the mixtures on biodiversity. Given the sample size ($n = 64$), factor analyses for latent variables (ellipses and dashed arrows) were conducted before the path analysis was conducted on the structural model (bold shapes and solid arrows). Probability values and standardised coefficients are next to each path and factor loadings are provided next to dashed lines. To reduce visual complexity, the coefficients and probability values for the effects of the agrochemical mixtures and spatial block on herbivores, algae and ecosystem properties are not provided. See Table S4 for these coefficients and probability values. F_0 is a measure of chlorophyll *a* and QY is a measure of photosynthetic efficiency. The root mean square error of approximation (RMSEA) for the model was 0.1, indicating a good fit of the model to the data.

that of the control treatment than were the communities of the respective biocide-only treatments (Figs 2, S1). The negative main effects of fungicide and insecticide were eliminated or decreased when fertiliser was paired with either agrochemical. The observed early peak in phytoplankton abundance followed by subsequent zooplankton recovery in the fertiliser–insecticide mixture treatment provides evidence of fertiliser-facilitated recovery. This is consistent with other experimental data in which the observed recovery of zooplankton to combined nutrient and insecticide exposure was faster than the rate of recovery suggested by simulations (Traas *et al.* 2004). Similarly, phytoplankton abundance in fertiliser–herbicide mixtures was initially low but recovered late in the experiment. In contrast, the repeated measures data from the fertiliser–fungicide mixture exhibit reduced mortality relative to the fungicide-only treatment early in the experiment, suggesting that fertiliser either reduced toxicity to the fungicide, or that recovery occurred more rapidly than in the other mixture treatments.

The fertiliser–herbicide mixture did not respond as we predicted. Instead, we observed initial increases in periphyton and increased snail abundance at the end of the experiment. These results are consistent with previous research demonstrating that the negative direct effects of atrazine on phytoplankton increase light availability, which indirectly increases periphyton (because light can be a limiting resource) and subsequently increases snail populations (Rohr *et al.* 2008b, 2012; Baxter *et al.* 2011; Staley *et al.* 2011). Similar indirect effects on algal dynamics may occur with other herbicides (Brock *et al.* 2000a). Nevertheless, it remains unclear whether this shift toward increased snail abundance is specific to the atrazine–fertiliser mixtures or whether it would also occur with other herbicides mixed with fertiliser. Importantly, most of the interactions between fertiliser and biocides were observed for species that could reproduce in the mesocosms during the course of the experiment (snails, zooplankton and algae), emphasising the importance of reproduction-mediated recovery processes for predicting short- and long-term effects of contaminant mixtures (Rohr *et al.* 2006). Caution should be exercised in the generalisation of fertilisers as mediators of the negative impacts of biocides because the net effects will depend on the magnitude and duration of exposure to each chemical. Indeed, chronic exposures to fertiliser can lead to large-scale shifts in community composition (Scheffer & Carpenter 2003; Slavik *et al.* 2004).

Biocide–biocide mixtures

We predicted that community responses to mixtures of biocides would generally be less similar to controls than treatments exposed to either biocide in isolation, with the exception of herbicides and insecticides, which should mitigate one another because herbicides should counteract algal blooms caused by insecticide-induced mortality of phytoplanktivorous zooplankton. As predicted, pairwise mixtures of biocides resulted in communities that exhibited an additive response to the main effects of each chemical. Mixtures of fungicide with either herbicide or insecticide shifted communities further from controls (Figs 2, S1). Consistent with the relatively large main effects of fungicide and insecticide, the

fungicide–insecticide mixture resulted in communities that were not only the most distinct from the controls but also distinct from either chemical alone (Table S3). The main effect of herbicide was small relative to that of fungicide, and thus communities exposed to fungicide–herbicide mixtures were distinct from those exposed only to herbicide but not the fungicide-only treatments. Likewise, the main effects of herbicide and insecticide were generally opposing, and community composition in herbicide-insecticide treatments was therefore intermediate relative to the treatments exposed to each biocide in isolation (Figs 2, S1). This result is consistent with previous studies exploring the effects of herbicide–insecticide mixtures on amphibians and snails (Boone & James 2003; Rohr & Crumrine 2005).

Effects on ecosystem properties

Consistent with our predictions, ecosystem properties did not respond directly to agrochemicals. Rather, ecosystem properties responded to changes in the abundance of primary producers, which responded to agrochemicals in isolation or as mixtures either directly (through direct toxicity or increased nutrient availability) or indirectly (through changes in herbivore abundance). Herbivory can be an important mediator of ecosystem processes in aquatic ecosystems, by changing the composition of primary producers. However, the consequences of herbivory on ecosystem properties depend on the architecture of the food web. For example, invasive snails can increase phytoplankton biomass, water turbidity and nutrient levels by selectively removing macrophytes (Carlsson *et al.* 2004), yet, in other systems, snails mediate the impacts of nutrient additions by reducing epiphytic algae and indirectly increasing macrophyte primary productivity (Verhoeven *et al.* 2012). This suggests that understanding the combined direct and indirect effects of agrochemicals on herbivores may be particularly important for predicting net effects on ecosystem properties.

The ability to successfully predict the responses of ecosystem properties to agrochemical mixtures is of particular importance because freshwater ecosystems provide many goods and services to humans that are related to these properties (Costanza *et al.* 1997; Baron *et al.* 2002). Dissolved oxygen, pH and decomposition are important correlates with the rate of energy flow through ecosystems, suggesting that the responses of other unmeasured ecosystem services might also be predictable using this theoretical framework. Natural resource managers are often tasked with management of ecosystem services, in addition to individual species or biological communities (De Groot *et al.* 2010), and better models to predict contaminant effects on ecosystem functions would help them make better decisions about what management solutions to employ.

The presence of simultaneous effects across trophic levels and functional redundancies within trophic levels presents challenges to predicting ecosystem-level responses to changes in biodiversity (Covich *et al.* 2004; Hooper *et al.* 2005; Duffy *et al.* 2007; Reiss *et al.* 2009). Our experiment provided scenarios in which most pairwise agrochemical mixtures had direct effects on multiple trophic levels in communities where functional redundancies were present in each level (e.g. multiple grazers and predators). Despite this complexity, we were

generally able to successfully predict changes in community composition, and thus changes in ecosystem-level properties, by integrating knowledge on (1) the direct effects of contaminants on functional groups, (2) the recovery rates of functional groups, (3) food web architecture (indirect effects) and (4) the relationship between functional groups and specific ecosystem functions (Rohr *et al.* 2006; Suding *et al.* 2008; Clements & Rohr 2009). Although the magnitude of indirect contaminant effects in diverse natural ecosystems might be reduced by greater functional redundancies or different interaction strengths among species, our ability to predict these indirect effects for relatively diverse communities in large-scale mesocosms suggests that this food web-based framework might be capable of predicting indirect effects on natural ecosystems, as well.

Caveats

With thousands of different biocides in use globally, this work has great potential to simplify risk assessment for agrochemicals. However, further work is needed to determine whether these results are general and thus representative of other agrochemicals within these broad agrochemical types. Our study examined pairwise mixtures of simultaneously introduced agrochemicals of different types and responses were observed over a relatively short time frame. Chronic exposure to and relative differences in the environmental persistence of agrochemical contaminants may result in community- and ecosystem-level responses that are only apparent over longer periods (Leibold *et al.* 1997; Slavik *et al.* 2004).

In closed micro- and mesocosm community experiments, observed recovery dynamics can be limited if either the length of the experiment is insufficient to allow multiple generations of all species, or when species with non-aquatic life stages (e.g. amphibians and odonates) are unable to recolonise. This experiment was limited to a relatively short time frame to avoid unrealistic responses that would have resulted from stochasticity in the reproduction of highly fecund arthropod predators (i.e. crayfish). In such cases, simulations modelling recolonisation of species have been used to better extrapolate from experimental community-level dynamics to natural conditions (Traas *et al.* 2004).

The responses we observed to pairwise chemical exposures might also be altered by mixtures of three or more agrochemical types and the initial concentrations of the constituent chemicals. We did not observe evidence of a community-level dose response to the $1\times$ and $2\times$ EEC treatments for any of the agrochemicals. Generally, the $1\times$ EEC concentrations seemed to be sufficiently high to produce the maximum population-level responses on the target taxa. Therefore, the addition of more chemical did not significantly increase mortality of these taxa, and hence did not cause greater indirect effects on the community. However, significant responses to mixtures have been observed when individual chemical concentrations were sufficiently low to produce no or little effect in isolation (Faust *et al.* 2000). It is possible that significant effects of mixtures on community- or ecosystem-level responses could occur at lower environmental concentrations of the constituent chemicals. Recent evidence of catastrophic shifts in communities exposed to low levels of multiple stressors, often over long periods of time (Scheffer & Carpenter 2003; Slavik *et al.* 2004), highlights the need for further empirical research in these contexts. We submit that although the logistics of testing more complex mixtures over longer time scales becomes more difficult, our framework provides a null expectation against which observed experimental effects can be tested.

Policy and management implications

Provided that our results are representative, we can suggest potential management recommendations to reduce potential impacts of agrochemicals to nearby surface waters. We stress that we are not advocating for increased use of agrochemicals. Rather, given that agrochemicals will continue to be applied to turf and crops, we suggest that timing of agrochemical applications could be altered, to the extent possible, to mitigate risk. Specifically, our results suggest that coupling applications of fertiliser with any biocide, coupling applications of insecticides with herbicides and avoiding simultaneous applications of all other biocides could reduce risk to aquatic communities relative to present agrochemical application practices. Modifying the spatial arrangement of crops to minimise the environmental risks from runoff events that might mix agrochemicals applied separately on adjacent fields may prove to be a particularly feasible strategy. One important exception is the mixture of fertiliser with herbicides, which was observed to increase snail abundance. Snail population dynamics are of particular concern because of the importance of snails as intermediate hosts for human and wildlife diseases (Rohr *et al.* 2008a,b; Moran *et al.* 2009), and as mediators of indirect effects on ecosystem functions (Carlsson *et al.* 2004; Verhoeven *et al.* 2012). Although implementation of these management practices is likely to reduce adverse effects on aquatic ecosystems, we advocate using an adaptive management approach to assess potential effects that are outside the scope our experimental design.

Despite the complexity of responses of aquatic ecosystems to agrochemical pollution, our experiment suggests that we can use food web theory and our knowledge of the responses of taxa susceptible to isolated agrochemicals to predict the effects of agrochemical mixtures on both community composition and ecosystem functions. With knowledge of food web architecture and the relative strength of interactions among functional groups, responses of more complex natural systems (including actual streams, ponds, lakes or terrestrial habitats) and mixtures (e.g. three or more chemicals), might also be predictable.

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