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LETTER

Fungicide-induced declines of freshwater biodiversity modify ecosystem functions and services

Taegan A. McMahon,[1,*,†] Neal T. Halstead,[1] Steven Johnson,[2] Thomas R. Raffel,[3] John M. Romansic,[1] Patrick W. Crumrine,[4] and Jason R. Rohr[1,†]

Abstract

Although studies on biodiversity and ecosystem function are often framed within the context of anthropogenic change, a central question that remains is how important are direct vs. indirect (via changes in biodiversity) effects of anthropogenic stressors on ecosystem functions in multitrophic-level communities. Here, we quantify the effects of the fungicide chlorothalonil on 34 species-, 2 community- and 11 ecosystem-level responses in a multitrophic-level system. At ecologically relevant concentrations, chlorothalonil increased mortality of amphibians, gastropods, zooplankton, algae and a macrophyte (reducing taxonomic richness), reduced decomposition and water clarity and elevated dissolved oxygen and net primary productivity. These ecosystem effects were indirect and predictable based on changes in taxonomic richness. A path analysis suggests that chlorothalonil-induced reductions in biodiversity and top-down and bottom-up effects facilitated algal blooms that shifted ecosystem functions. This work emphasises the need to re-evaluate the safety of chlorothalonil and to further link anthropogenic-induced changes in biodiversity to altered ecosystem functions.

Keywords

Agrochemical, biodiversity, chlorothalonil, community, contaminant, ecosystem function, ecosystem services, freshwater ecosystem, mesocosm, pesticide.

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INTRODUCTION

Interest in the relationship between biodiversity and ecosystem function stems at least partly from the concern that anthropogenically driven declines and changes in biodiversity will reduce or alter the goods and services offered by ecosystems. Despite this underlying motivation, most biodiversity–ecosystem functioning studies manipulate species richness or composition rather than anthropogenic factors (Hooper *et al.* 2005; Balvanera *et al.* 2006; Hillebrand & Matthiessen 2009; Reiss *et al.* 2009). There are several reasons why it is important to manipulate anthropogenic factors themselves and subsequently quantify their effects on ecosystem functions in multitrophic-level systems (Duffy *et al.* 2007; Hillebrand & Matthiessen 2009). First, the relationship among anthropogenic stressors, biodiversity and ecosystem functions is often dependent on species composition and abiotic factors that can vary across studies, space and time (Hooper *et al.* 2005; Rohr & Crumrine 2005; Reiss *et al.* 2009; Rohr *et al.* 2011). Hence, predictions about the relationships among stressors, biodiversity and function might be unreliable unless they are based on a single study that considers all three under the same conditions. Second, many anthropogenic stressors might only affect species that contribute little to ecosystem functions or that are functionally redundant with species that are not sensitive to the stressor (Hooper *et al.* 2005); thus, it is possible for stressors to have little effect on ecosystem functions even when they cause significant declines in biodiversity.

Third, stressors could have direct effects on function, as well as indirect effects mediated through changes to biodiversity (Hillebrand & Matthiessen 2009). For example a chemical contaminant could bind to important elements, such as nitrogen, phosphorus or carbon, directly affecting the cycling of these nutrients or it could indirectly affect these cycles by affecting biota. Indeed, the importance of indirect (via diversity) vs. direct (via abiotic constraints) effects of anthropogenic stress on ecosystem functioning is considered an important but unexamined question in biodiversity-functioning research (Hillebrand & Matthiessen 2009).

A fourth reason to consider the effects of anthropogenic factors on ecosystem functions in multitrophic-level systems is that there appears to be some consistency in biodiversity-functioning relationships based on simple community manipulations, but the loss or addition of species at multiple trophic levels, which is more consistent with present biodiversity losses and additions, remains less well explored (Duffy *et al.* 2007; Reiss *et al.* 2009). The limited available research suggests that multitrophic interactions produce a wider and less predictable array of diversity-functioning relationships than those predicted for single trophic levels, emphasising the need for empirical research on how top-down and bottom-up effects of stressors on biodiversity affect ecosystem functioning (Duffy *et al.* 2007; Reiss *et al.* 2009).

One of the challenges to addressing these gaps in the biodiversity-function literature is selecting among the many possible ecosystems and stressors to study. Of all the ecosystems on the planet, freshwater ecosystems support the greatest concentration of bio-

[1]*Department of Integrative Biology, University of South Florida, Tampa, FL, 33620, USA*
[2]*Gulf Coast Research and Education Center, University of Florida, Wimauma, FL, 33598, USA*
[3]*Biology Department, Dickinson College, Carlisle, PA, 17013, USA*
[4]*Department of Biological Sciences, Rowan University, Glassboro, NJ, 08028, USA*
**Correspondence: E-mail: tamcmaho@mail.usf.edu*
†Equal co-authorship.

diversity (Dudgeon *et al.* 2006) and provide humans with a multitude of goods and services, including drinking water, food, decomposition of waste and habitat for animals and plants (Baron *et al.* 2002). Most of these goods and services are provided directly or indirectly by the biota of freshwater ecosystems (Covich *et al.* 2004; Hooper *et al.* 2005; Loreau 2010). Yet freshwater ecosystems are among the most imperiled, with biodiversity losses occurring much faster in freshwater than terrestrial or marine environments (Ricciardi & Rasmussen 1999; Dudgeon *et al.* 2006).

Although there are many causes to the losses of freshwater biodiversity and associated ecosystem functions, pollution is regarded as a potent threat to aquatic species in the USA, second only to habitat loss (Wilcove & Master 2005). Moreover, there are over 100 000 registered chemicals in the USA and European Union (EU 2001), and each year in the USA alone, over one billion pounds of pesticides are applied and 97% of streams in urban and agricultural landscapes have detectable levels of pesticides (Gilliom *et al.* 2007). Hence, chemical contaminants are undoubtedly one of the most diverse and common abiotic stressors. Nevertheless, pollution is documented as one of the most understudied stressors in conservation science (Lawler *et al.* 2006), and manipulative research on the indirect effects of contaminants on ecosystem functions mediated by changes in biodiversity is essentially nonexistent for freshwater ecosystems (but see Carlisle & Clements 2005 for a correlative example).

We postulate that broad-spectrum pesticides (i.e. pesticides with modes of action that target physiological mechanisms common to many taxa) will have a high probability of affecting ecosystem functions and services because they affect a wider array of taxa than more targeted pesticides. The more taxa affected by a stressor, the greater the likelihood that it will affect species that strongly contribute to functions and/or overcome the stability provided by functionally redundant species in food webs. As an example, the fungicide chlorothalonil would be considered a broad-spectrum pesticide because its mode of action is to disrupt cellular respiration (by binding to glutathione; Caux *et al.* 1996; USEPA 1999), which is essential for almost all eukaryotic organisms.

To address the gap in our understanding of the importance of direct vs. indirect effects of anthropogenic stressors on ecosystem functions in multitrophic-level systems, we established freshwater mesocosms containing four trophic levels and quantified the effects of ecologically relevant concentrations of chlorothalonil on 34 species-level, 2 community-level and 11 ecosystem-level responses. We hypothesised that chlorothalonil would not directly affect ecosystem properties. Rather, we predicted that chlorothalonil would adversely affect many freshwater taxa and that these subsequent changes to community composition would in turn affect indicators of ecosystem functions. We used path analysis to (1) provide support for indirect effects of this chemical on community composition, (2) link changes in biodiversity to modifications of ecosystem functions and (3) evaluate whether the alteration of ecosystem functions was predominantly driven by top-down or bottom-up effects of chlorothalonil.

Background of chlorothalonil

Chlorothalonil, an organochlorine compound, is the most commonly used fungicide in the USA (US EPA 2004), but its impacts on freshwater communities and ecosystem properties have not been reported. Chlorothalonil has a short half-life in water, approximately 44 h (Caux *et al.* 1996; USEPA 1999), but even the technical formulation is commonly contaminated with the more hazardous and persistent hexachlorobenzene (Hung *et al.* 2010), which was banned in the USA because of its carcinogenic potential (The International Programme on Chemical Safety (IPCS) 1998). Chlorothalonil concentrations of 290 and 272 $\mu g\ L^{-1}$ have been detected in runoff and groundwater respectively near golf courses (Shuman *et al.* 2000). Nevertheless, the peak estimated environmental concentration (EEC) of chlorothalonil in ponds based on applications in cropping systems is ~ 164 $\mu g\ L^{-1}$ (calculated using the US EPA GENEEC v2 software, see Table S1 in Supporting Information for parameters), whereas the peak EEC associated with chlorothalonil application on turf is as high as 462 $\mu g\ L^{-1}$ (USEPA 1999). Effects of an agrochemical near or below the EEC can affect the decision to approve its use. To ensure that our concentrations were relevant to those estimated in nature and to policy, tanks receiving chlorothalonil received either 164 or 328 $\mu g\ L^{-1}$ (nominal concentrations), concentrations well below the potential peak EEC values for turf applications.

MATERIAL AND METHODS

Mesocosm experiment

This experiment was conducted at a mesocosm facility approximately 20 miles southwest of Tampa, FL, for 4 weeks from July to August 2008. Mesocosms consisted of cattle water tanks containing 800 L of water covered with 60% shade cloth to reduce sun exposure and prevent entry or escape of animals. Three weeks before the start of the experiment, each tank received aliquots of zooplankton and algae (collected from four local ponds and homogenised before addition), 300 g of leaf litter (predominantly *Quercus virginiana*) to provide refugia and nutritional detritus, a pre-weighed leaf packet (5 g of *Q. virginiana* leaves encased in nylon) to quantify decomposition and vertical clay tiles (8 cm^2) for periphyton quantification. Just prior to chlorothalonil or solvent additions, each tank received two tadpole (*Osteopilus septentrionalis*, *Rana sphenocephala*), six macroarthropod (nymph *Anax junius*, nymph Libellulidae, adult *Belostoma fluminеum*, *Ranatra* sp., Corixidae sp adults, juvenile *Procambarus clarkii*), four gastropod (all adults: *Viviparus georgianus*, *Planorbella trivolvis*, *P. scalaris*, *Melanoides tuberculata*) and two macrophyte species (*Hydrilla verticillata*, *Utricularia macrorhiza*; initial abundances provided in Table S2). Hence, these mesocosms contained many of the major taxa, at similar abundances, as found in Florida ponds (Reiss & Brown 2005). All organisms were collected from ponds within approximately 1 km of N28°06.759′ W082°23.014′. All tadpoles were Gosner stage 25 (Gosner 1960) at the start of the experiment. The macrophyte, *U. macrorhiza*, is not emphasised here because it did not successfully establish in any cattle tanks and was absent from all cattle tanks by the end of the second week of the experiment.

Tanks received one of four treatments: 164 or 328 $\mu g\ L^{-1}$ of chlorothalonil (dissolved in 500 $ng\ L^{-1}$ acetone), solvent (500 $ng\ L^{-1}$ acetone) control or water control. There were four replicates of each treatment (16 tanks total) arranged in a randomised block design. Chlorothalonil was applied as a single application of technical-grade compound (purity > 98%; Chemservice, West Chester, PA, USA). In the environment, exposures are typically episodic and occur with runoff from rain events. Water

samples from each tank were collected approximately 1 h after dosing and analysed by the Mississippi State Chemical Laboratory. These measured chlorothalonil concentrations were determined to be 172 and 351 $\mu g\ L^{-1}$ for the two chlorothalonil treatments respectively. Water quality measurements in the tanks before dosing were (in $\mu g\ L^{-1}$) calcium: 39 000, nitrate: 77, nitrite: 66, total nitrogen: 370 and phosphorous: 60.

We took repeated measurements of dissolved oxygen (DO), pH, temperature at dawn and dusk, light penetration through the water column, chlorophyll *a* in periphyton and phytoplankton, photosynthetic efficiency of periphyton and phytoplankton, macrophyte abundance and zooplankton abundance and diversity (Early: weeks 1–2; Late: weeks 3–4; see Appendix S1). Net primary productivity was calculated as the difference in daily DO measurements between dawn and dusk (Noel *et al.* 2010). Tanks were drained at the conclusion of the experiment (week 5), at which time amphibians, gastropods and macroarthropods were enumerated; macrophytes were weighed and leaf packets were dried and weighed.

Direct effects on abiotic factors

To test for direct effects of chlorothalonil on pH, DO and light, the ecosystem properties incorporated into the path analysis described below, we filled glass jars with 500 mL of ultrapure water and then applied a single application of solvent (acetone), or 172 or 351 $\mu g\ L^{-1}$ of technical-grade chlorothalonil (four replicates/treatment). We used ultrapure water to ensure that no living organisms were present and thus only direct effects were possible. For 8 days, we recorded pH and DO approximately every other day and light daily using the same meters used in the mesocosms experiment.

Statistical analyses

All proportions were arcsine-square-root transformed and counts were log transformed (see Table S3 for details). Given that many of our response variables were in different units, all response variables were standardised after transformation (mean = 0, standard deviation = 1) so that all responses had equal weight in the ordination analyses.

We conducted a Principal Coordinates Analysis (PCoA) and a distance-based Redundancy Analysis (db-RDA), both based on Bray–Curtis distances, to evaluate the multivariate relationships between predictors and response variables (see Table S3 for the 45 response variables used in these analyses). PCoA is a linear, unconstrained ordination analysis (which extracts synthetic axes summarising patterns of variation in the data) and db-RDA is the direct ordination analogue, which relates such variation to predictor variables (McArdle & Anderson 2001). The predictor variables for the db-RDA were spatial block and chlorothalonil concentration. Consistency between the PCoA and db-RDA would suggest that the most important predictor variables were quantified from the experiment. Linear ordination analyses were selected because the average response to the extracted hypothetical axis was linear rather than unimodal. We also conducted the ordination analyses using Euclidean and Hellinger distances (Figures S1 and S2) to evaluate whether the analyses were dependent on the selected distance measure. Ordination analyses were conducted with CANOCO 4.5, and triplots, which display the ordination results, were generated with CanoDraw 4.12 (ter Braak & Smilauer 2002).

We used Monte Carlo permutation tests (9999 randomisations) to evaluate the multivariate and univariate effects of chlorothalonil concentration and spatial block. Randomisation tests were preferred for multivariate analyses because of the challenges of meeting the assumptions of multivariate normality (McArdle & Anderson 2001). Permutation tests were also used to compare the two chlorothalonil treatments with the control treatments, but no alpha adjustment for multiple tests was made. Repeated measures analyses were also conducted for variables quantified through time, testing for effects of block, chlorothalonil concentration, time and a chlorothalonil-by-time interaction. We also tested whether chlorothalonil concentration affected taxonomic richness and evenness. These analyses included the two amphibian species, the four snails species, the six macroarthropod taxa, the four zooplankton taxa (at the first sampling period; excluding nauplii) and *H. verticillata*. Not all biota were identified to the species level. To address this, we conducted the analyses on both morphospecies and generic richness.

Some taxa increased with chlorothalonil concentration but only late in the experiment. We hypothesised that these increases, and subsequent changes to ecosystem properties, might be a function of indirect effects of chlorothalonil. We used path analysis, based on maximum likelihood and log-likelihood ratio tests, to evaluate the level of support for models and to test the significance of the model paths (see Results). Path analysis is a form of multiple regression focusing on causality among a series of variables (Grace 2006). Factor analyses for latent variables were conducted first and then a path analysis was conducted on the structural model. We selected among candidate models using Akaike information criterion with a correction for a finite sample size (AIC_c). Specifically, we used path analysis and associated AIC_c values to (1) test among hypothesised indirect effects, (2) evaluate the strength of top-down (loss of algal herbivores) and bottom-up effects (increase in light) of chlorothalonil on algae and ecosystem properties and (3) determine if both the animals and plants contributed to changes in ecosystem properties. Finally, to more explicitly test for associations between losses to biodiversity and ecosystem functions, we conducted a path analysis with chlorothalonil concentration as a predictor of taxonomic richness and richness as a predictor of ecosystem properties late in the experiment. Path analyses were conducted using Statistica 9.0 (Statsoft, Tulsa, OK, USA).

We did not use path analysis to compare the strength of direct and indirect effects of chlorothalonil on ecosystem functions. The reason is that a pathway directly from chlorothalonil to ecosystem properties would describe any residual variation accounted for by chlorothalonil, which could include direct effects of chlorothalonil on ecosystem properties as well as indirect effects of chlorothalonil mediated by aspects of the community that were not quantified, such as bacteria and fungi. Hence, a pathway from chlorothalonil to ecosystem properties would not isolate direct effects, which is why we used an experiment to test whether there were any direct effects of chlorothalonil on the ecosystem properties highlighted in the path analysis.

RESULTS

There were no multivariate differences between the solvent and water control tanks and thus they were pooled for all analyses. The PCoA and db-RDA produced very similar ordination triplots (Fig. 1a, b; Figures S1 and S2), suggesting that the primary gradient

(*x*-axis) was indeed chlorothalonil rather than a variable that was not quantified (because db-RDA constrains the axis to quantified predictors, whereas PCoA extracts a hypothetical axis). Thus, arrows pointing right and left in these ordination diagrams (Fig. 1a and b) represent factors that increased and decreased respectively, with increasing chlorothalonil concentrations.

Species- and community-level effects

We first focus on species-level variables that were quantified only at the end of the experiment (Table 1; Fig 1b). For these variables, increasing chlorothalonil concentrations reduced the survival of amphibian and gastropod species (Figures S3 and S4), but had no detectable effects on the macroarthropod community (Figure S5; Table 1; Fig. 1b, univariate results are available in Table S3). Furthermore, these effects on amphibians and gastropods (Table S3) were generally observed with exposure to the lowest chlorothalonil concentration (Table S3; Figures S3 and S4).

We quantified several variables both early and late in the experiment. Increasing chlorothalonil concentrations reduced *H. verticillata* abundance at both time points (Table 1; Fig. 1b), and the lowest concentration was enough to cause a significant reduction in *H. verticillata* (Table S3; Figure S6). For the remaining response variables, there was a significant multivariate interaction between chlorothalonil and time, indicating a time-dependent response to the chemical (Table 1). Increasing chlorothalonil concentrations were associated with reductions in zooplankton and periphyton abundance early in the experiment (Fig. 1b; Figures S7 and S8), and the lowest concentration alone was sufficient to reduce zooplankton abundance (Table S3). In contrast, chlorothalonil exposure had no significant effect on phytoplankton early in the experiment (Tables S3 & S4; Fig. 1b; Figure S8). Later in the experiment, however, chlorothalonil concentration was associated positively with periphyton and phytoplankton, but no longer had a significant effect on zooplankton (Fig. 1b; Figures S7 and S8), suggesting recovery of the zooplankton community and eventual indirect positive effects of chlorothalonil on algae.

Exposure to increasing chlorothalonil concentrations was associated with a significant reduction in taxonomic richness ($F_{1,11} = 49.37$, $P < 0.001$), and both concentrations of chlorothalonil had lower richness than the controls (Fig. 2a). Although evenness decreased with increasing chlorothalonil concentrations (Fig. 2b), the relationship was not significant ($F_{1,11} = 1.05$, $P = 0.328$). These results did not change when the analyses were conducted at the generic level.

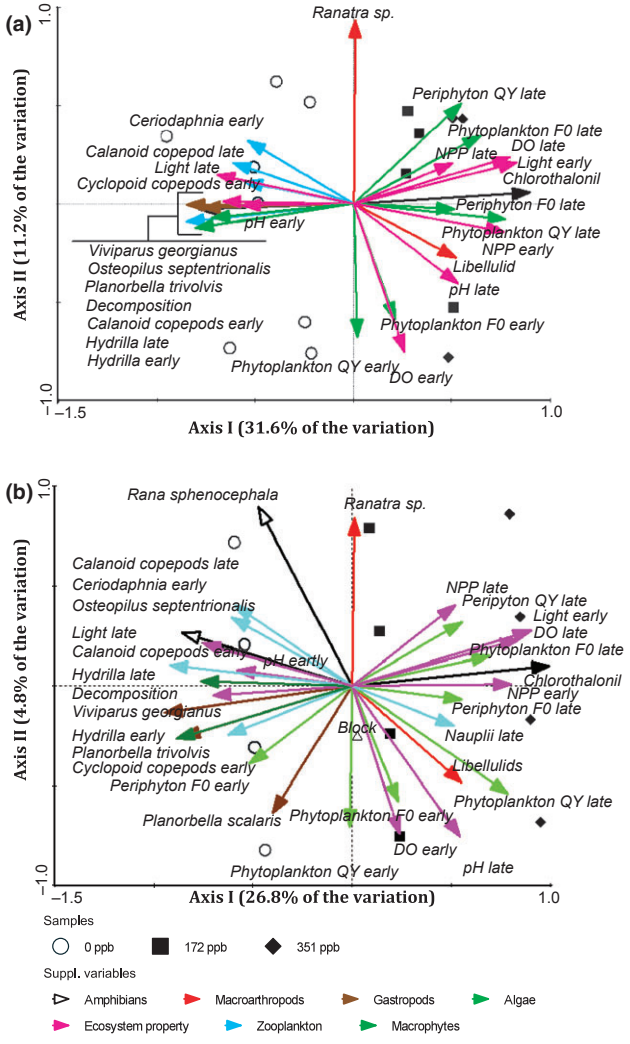

Figure 1 Principal coordinates analysis (PCoA; (a) and distance-based redundancy analysis (db-RDA; constrained to chlorothalonil concentration and block; (b), based on Bray–Curtis distances, of community and ecosystem responses (projected post hoc into ordination space). Arrows pointing right and left represent factors that increased and decreased respectively with increasing chlorothalonil concentrations. Response (supplementary) variables with correlation coefficients outside −0.5–0.5 and −0.45–0.45 are displayed for the PCoA and db-RDA respectively. See Table S2 for the variables used. The angle between responses is negatively proportional to the correlation of those variables, and distance among samples approximates the dissimilarity in their community and ecosystem responses. F0 and QY are estimates of chlorophyll a and photosynthetic efficiency respectively. DO = Dissolved oxygen, NPP = Net primary productivity.

Table 1 Results of Monte Carlo permutation tests (9999 randomisations, $n = 16$) to evaluate how chlorothalonil concentration (continuous predictor) affects various taxonomic groups and ecosystem properties early (first 2 weeks) and late (last 2 weeks) in the experiment

	Statistics			
	Chlorothalonil		Chlorothalonil × time	
Responses (number of variables)*	F	P	F	P
All responses (45)	4.51	**< 0.001**	–	–
Non-repeated measures responses				
Amphibian community (2)	8.39	**0.005**	–	–
Gastropod community (4)	14.32	**< 0.001**	–	–
Macroarthropod community (7)	0.71	0.662	–	–
Decomposition rate (1)	5.83	**0.030**	–	–
Repeated-measures responses				
All repeated-measures responses (14)	4.06	**0.001**	3.16	**0.001**
Zooplankton community (5)	4.66	**0.004**	4.57	**0.039**
Algal community (4)	2.19	0.108	3.44	**0.038**
Hydrilla verticillata (1)	25.63	**< 0.001**	0.03	0.929
Ecosystem properties (5)	3.28	**0.038**	3.60	**< 0.001**

*See Table S2 for a list of the variables in each response group. Bold values are statistically significant.

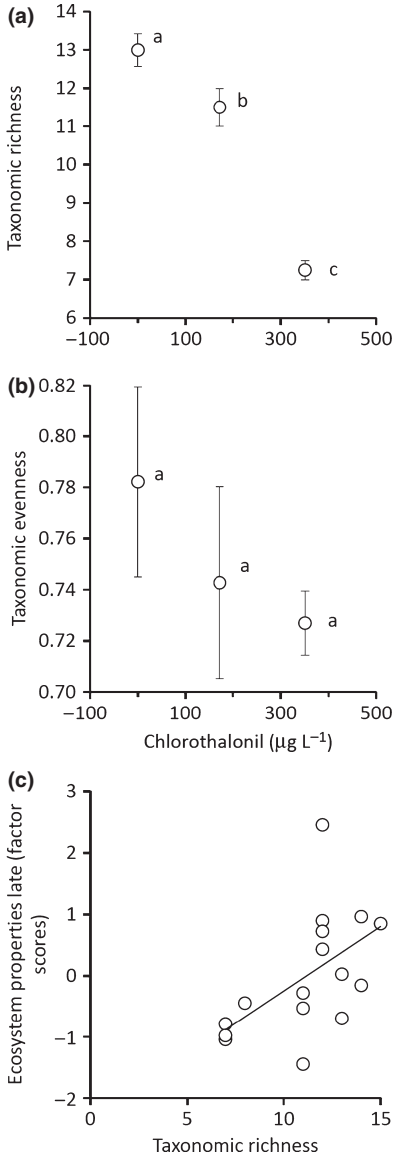

Figure 2 Effects of chlorothalonil exposure on the number of taxa per mesocosms (taxonomic richness, (a) and evenness of taxa per mesocosms (Simpson's index, (b), and the association between taxonomic richness and ecosystem properties late in the experiment (c). Shown are the means and standard errors (n = 8 for controls, n = 4 for 172 and 351 μg L^{-1}) and best-fit lines. Different lowercase letters within each panel reflect significant differences ($P < 0.05$) among chlorothalonil concentrations, according to a Fisher's LSD multiple comparison test.

Ecosystem-level effects

Time-dependent effects of chlorothalonil were also observed for ecosystem properties. Early in the experiment, increasing chlorothalonil concentrations were generally associated with reductions in pH, and increases in dissolved oxygen and light intensity in the water column (Table S4; Fig. 1b; Figure S9). Later in the experiment, however, increasing chlorothalonil concentrations were associated with increased pH, decreased light availability and even stronger increases in dissolved oxygen (Table S4; Fig. 1b; Figure S9). Even the lowest concentration of chlorothalonil was also associated with reduced decomposition rates, an important ecosystem service (Fig. 1b; Figure S10; Table S3). Interestingly, chlorothalonil concentration was associated positively, rather than negatively, with net primary productivity because of algal blooms late in the experiment (Fig. 1b; Table S4).

In our Direct Effects on Abiotic Factors Experiment, chlorothalonil had no significant effect on light levels, pH or DO when it was applied to ultrapure water (Main effects: $P = 0.22$, 0.98, 0.99 respectively; ecosystem property-by-time interactions: $P > 0.271$; Figures S11–S13). Given these results and the fact that chlorothalonil has a half-life of 44 h (Caux *et al.* 1996; USEPA 1999), there is little evidence that chlorothalonil could have had any direct effect on light levels, pH or DO in the mesocosm experiment.

Links between biodiversity & ecosystem properties

Chlorothalonil, an agrochemical designed to disrupt cellular respiration, would not be expected to promote the growth of attached and suspended algae directly or directly affect most ecosystem properties (e.g. light, dissolved oxygen, pH and decomposition rates). Thus, we hypothesised that the observed increase in algae and changes in ecosystem properties were a result of indirect effects of chlorothalonil. We postulated that by reducing the dominant algal herbivores (e.g. tadpoles, snails and zooplankton) and by increasing light early in the experiment (by reducing the shading effects of the floating macrophyte *H. verticillata*; $R^2 = 0.640$, $F_{1,14} = 24.90$, $P < 0.001$), chlorothalonil promoted algal growth later in the experiment. We also hypothesised that this increase in net primary productivity and the loss of invertebrates and vertebrates drove the changes in ecosystem properties (e.g. light, pH and dissolved oxygen) later in the experiment.

The path model with the greatest support based on AIC_c included both top-down and bottom-up effects of chlorothalonil on factors affecting algae early in the experiment (Table 2; Fig. 3). According to this model, the chlorothalonil-induced decrease in dominant herbivores and increase in light early in the experiment increased algae later in the experiment. This in turn elevated pH and dissolved oxygen and reduced light availability in the water column (ecosystem properties; Fig. 3; Table 2). Both top-down (herbivores) and bottom-up (light) effects were significant predictors of phytoplankton ($P < 0.001$, $P < 0.001$ respectively) and periphyton ($P = 0.007$, $P = 0.019$ respectively) abundance late in the experiment. We could not discriminate between the top-down only and bottom-up only models based on AICc (Table 2), but the model containing top-down and bottom-up effects had more than 1.5 and 2 times the weight as the top-down only and bottom-up only models, suggesting that both top-down and bottom-up effects of chlorothalonil were important. Adding a path directly from the dominant herbivores to the ecosystem properties did not significantly improve model AIC_c, suggesting that the direct effect of animals on the measured ecosystem properties late in the experiment was small relative to the effect of the primary producers on these ecosystem properties (Table 2; Fig. 3).

A path model with chlorothalonil concentration as a predictor of taxonomic richness and richness as a predictor of ecosystem properties late in the experiment revealed that richness was indeed a significant predictor of ecosystem properties late in the experiment ($\beta = 0.180$, $X^2 = 5.40$, $P = 0.020$, $R^2 = 0.302$, Fig. 2c; see '*Community-level Effects*' and Fig. 2a for effects of chlorothalonil on taxonomic richness). Furthermore, the relationship between richness

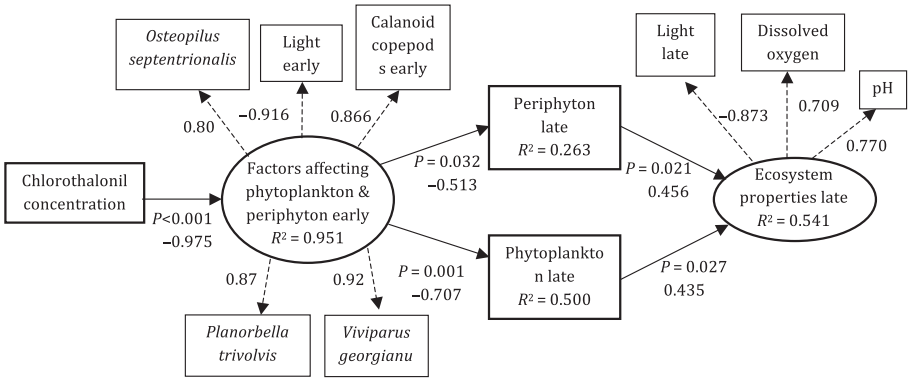

Figure 3 Best path model (based on AIC_c) suggesting that effects of chlorothalonil on ecosystem properties late in the experiment were mediated by chlorothalonil effects on light and algal grazers early in the experiment. Probability values and standardised coefficients are next to each path. Given the sample size (n = 16) and statistical power, 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). The Steiger-Lind RMSEA index for the path model was 0.0 (90% CI: 0.000–0.322) and the Population Gamma Index was 1.0 (90% CI: 0.828–1.000), indicating a good fit of the model. See Table 2 for models that were considered.

Table 2 Comparison of various path models examining the indirect effects of chlorothalonil on ecosystem properties mediated by changes in biodiversity

Model	Free parameters	AIC_c	Δ_i	w_i
Top-down and bottom-up effects of chlorothalonil (a–c, m, n)	9	56.55	0.00	0.46
Top-down effects only (d–f, m, n)	9	57.46	0.90	0.29
Bottom-up effects only (g–i, m, n)	9	58.23	1.67	0.20
Top-down and bottom-up effects plus an additional path (a–c, j, m, n)	10	69.31	12.75	< 0.0001
Top-down effects only plus an additional path (d–f, k, m, n)	10	69.57	13.02	< 0.0001
Bottom-up effects only plus an additional path (g–i, l–n)	10	73.26	16.71	< 0.0001

The paths included in each model are provided in the parentheses. Given the relatively short half-life of chlorothalonil and its positive association with algae late in the experiment, we assumed that it did not directly affect algae or ecosystem properties late in the experiment.

a, Chlorothalonil concentration to top-down and bottom-up factors affecting algae early (T&B_AE); b, T&B_AE to phytoplankton late in the experiment; c, T&B_AE to periphyton late in the experiment; d, Chlorothalonil concentration to top-down factors affecting algae early in the experiment (T_AE); e, T_AE to phytoplankton late in the experiment; f, T_AE to periphyton late in the experiment; g, Chlorothalonil concentration to bottom-up factors affecting algae early in the experiment (B_AE); h, B_AE to phytoplankton late in the experiment; i, B_AE to periphyton late in the experiment; j, T&B_AE to ecosystem properties late in the experiment; k, T_AE to ecosystem properties late in the experiment; l, B_AE to ecosystem properties late in the experiment; m, Phytoplankton late in the experiment to ecosystem properties late in the experiment; n, Periphyton late in the experiment to ecosystem properties late in the experiment.

and ecosystem properties was linear over the tested levels of richness (Fig. 2c).

DISCUSSION

Species- and community-level effects

Chlorothalonil, at ecologically relevant concentrations (USEPA 1999), had no detectable direct effects on indicators of ecosystem function, but indirectly affected functions by reducing biodiversity. Chlorothalonil caused significant mortality of amphibians, gastropods, zooplankton, algae and macrophytes, resulting in significant reductions in freshwater biodiversity (measured as taxonomic richness). In most cases, significant mortality occurred at the lower chlorothalonil concentration, which is an ecologically relevant concentration for freshwater systems (USEPA 1999). Although our study is the only reported community-level experiment on chlorothalonil, our results are consistent with several direct toxicity studies conducted in the laboratory and with observations in the field. For example ~ 164 μg L^{-1} of chlorothalonil killed 100% of four different species of amphibians in a series of laboratory experiments (McMahon *et al.* 2011), and amphibian die-offs have been documented after chlorothalonil applications to cranberry bogs (Winkler *et al.* 1996). Chlorothalonil also has been documented to cause mortality of several plant species in the laboratory and field (Caux *et al.* 1996), and LC_{50} values for *Daphnia* (zooplankton), isopods and freshwater shrimp have been reported as 70, 40 and 3.6 μg L^{-1} of chlorothalonil respectively (Caux *et al.* 1996; Grabusky *et al.* 2004). Moreover, the results of pesticide studies conducted in mesocosms regularly match patterns in the field (e.g. Rohr *et al.* 2008).

The only quantified taxon that chlorothalonil did not significantly kill was macroarthropods (insects and crayfish). Most of the prey of the macroarthropods were adversely affected by chlorothalonil (e.g. tadpoles, snails and zooplankton), which might have made it easier for macroarthropods to attain resources through predation and/or scavenging, minimising any direct effects of chlorothalonil on this taxon. However, it is also possible that macroarthropods are less susceptible to chlorothalonil. Although attached algae significantly declined early in the experiment with chlorothalonil exposure, suspended algae did not. This was likely due to suspended algae experiencing an early release from competition, as chlorothalonil was acutely toxic to *H. verticillata* that shaded the phytoplankton. Attached algae were shaded by both *H. verticillata* and phytoplankton and thus might not have experienced as large of a benefit from the loss of the macrophyte as the suspended algae.

Several taxa with short generation times, specifically zooplankton and algae, seemed to recover from the chlorothalonil exposure by

the end of the 4-week experiment. This highlights the importance of considering temporal dynamics and recovery processes in response to anthropogenic stressors (Clements & Rohr 2009). In fact, although chlorothalonil caused significant reductions in attached algae early in the experiment, it was associated with algal blooms by the end of the experiment, not unlike the effects of eutrophication.

Although it should be disconcerting that the estimated field concentration of the most commonly used fungicide in the USA is toxic to such a broad range of taxa, it might not be surprising given that its mode of action is to disrupt cellular respiration, a process vital to the survival of most eukaryotic organisms on the planet. In fact, chlorothalonil is one of only a few organochlorine pesticides that are still registered for use in the USA, European Union and Australia. Most other organochlorines, such as DDT, dieldrin, chlordane, hepatochlor and lindane, are banned because of their toxic properties. In fact, the results of this study emphasise the need to re-evaluate the safety of chlorothalonil to biodiversity and, given recent reviews highlighting that most fungicides are general biocides (e.g. Maltby *et al.* 2009), to assess whether fungicides generally induce indirect community- and ecosystem-level effects similar to those caused by chlorothalonil.

Ecosystem-level effects

In addition to reducing biodiversity, chlorothalonil altered ecosystem functions. The lowest tested concentration of chlorothalonil significantly reduced decomposition rates, probably because it was toxic to fungi. Decomposition of organic material is an important ecosystem service provided by freshwater ecosystems that has major impacts on ecosystem energetics (Baron *et al.* 2002; Dudgeon *et al.* 2006). In addition, by the end of the experiment, chlorothalonil was associated with increased net primary productivity and dissolved oxygen and decreased water clarity and light availability.

Biodiversity–ecosystem functioning relationship

Many anthropogenic stressors might cause declines in biodiversity but not affect ecosystem functions if they reduce species that contribute little to function or that are functionally redundant in communities. Chlorothalonil, however, affected several ecosystem functions. Our experiment revealed that its effects on net primary productivity, light, pH and DO were not direct and thus must be mediated by its effects on biodiversity. This conclusion was supported by the significant association between chlorothalonil-induced declines in taxonomic richness and ecosystem properties late in the experiment (Fig. 2c). Despite considerable emphases on associations between biodiversity and ecosystem functions (Hooper *et al.* 2005; Balvanera *et al.* 2006; Hillebrand & Matthiessen 2009; Reiss *et al.* 2009), to the best of our knowledge, this is the first manipulative study to demonstrate that contaminant-induced changes to ecosystem functions were driven by contaminant-induced declines in biodiversity and not direct effects of the anthropogenic factor on ecosystem properties.

Whereas most researchers infer indirect effects of contaminants (e.g. Boone *et al.* 2004; Relyea *et al.* 2005; Relyea 2009), we used path analysis and model selection to evaluate the level of support for the hypothesised indirect effects. These analyses suggest that the algal blooms late in the experiment were a function of chlorothalonil reducing predation from algal herbivores (top-down effect) and shading from the macrophyte *H. verticillata* (bottom-up effect). Although there has been a longstanding debate regarding whether top-down or bottom-up mechanisms are more important in structuring communities (Hunter & Price 1992; Gripenberg & Roslin 2007), our best statistical model (based on AICc) included both top-down and bottom-up effects on algae. However, we could not discriminate between models with only top-down and only bottom-up effects. These results suggest that both top-down and bottom-up effects were likely contributors to the observed algal blooms. This is consistent with recent reviews and studies highlighting that both top-down and bottom-up effects structure communities (Menge 2000; Frank *et al.* 2007; Gruner *et al.* 2008). Importantly, algae were positively associated with ecosystem properties late in the experiment, suggesting that the effects of chlorothalonil on ecosystem functions were mediated by changes in biodiversity that facilitated algal blooms. Indeed, several studies have documented the tight links between aquatic ecosystem functions and algal and grazer dynamics (Worm *et al.* 2000; Carlsson *et al.* 2004; Lohrer *et al.* 2004).

Species declines at various trophic levels can affect ecosystem functions in complex synergistic or antagonistic ways and thus there have been calls to study the effects of vertical (multiple trophic levels) and horizontal (within a tropic level) losses of species to ecosystem functioning (Duffy *et al.* 2007; Reiss *et al.* 2009). Chlorothalonil initially affected three trophic levels (light resources, algae and algal grazers) and multiple competing species within trophic levels (e.g. multiple algal grazers), but we still detected a clear linear relationship between biodiversity losses and the quantified ecosystem properties (Fig. 2c). Despite these complex changes within and across trophic levels, our path analysis revealed that the effects on ecosystem functions were predictable *a posteriori* based on basic knowledge of associations among functional groups within the food web and which functional groups contribute most to the focal ecosystem properties (Fig. 3). Hence, a promising corollary of this research is that complex effects of anthropogenic factors within and across multiple trophic levels still might have predictable effects on ecosystem properties if there is at least a basic understanding of (1) the effects of the stressor on functional groups, (2) the food web architecture and (3) associations between functional groups and the focal ecosystem properties. This is consistent with recent emphases on the value of linking trait- and functional group-based approaches to food web theory to predict the effects of species losses or additions on ecosystem functions (Petchey & Gaston 2006; Rohr *et al.* 2006; Suding *et al.* 2008; Clements & Rohr 2009; Hillebrand & Matthiessen 2009; Reiss *et al.* 2009). We encourage further research on the importance of direct and indirect (via biodiversity) effects of anthropogenic stressors on ecosystem functions in systems with complex trophic interactions.

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STATEMENT OF AUTHORSHIP

All authors agreed to submission of the manuscript and accept the responsibility for the accuracy and integrity of the manuscript. JRR conceived and designed the experiments. TAM, NTH, TRR, JMR, PWC, SJ and JRR conducted the experiments. JRR conducted the statistics, TAM and JRR wrote the paper and all authors contributed to the editing.

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