

# Trophic downgrading results in complex ecosystem dynamics in experimental tropical floodplain food webs

Richard M. Pendleton · David J. Hoeinghaus ·  
Luiz C. Gomes · Angelo A. Agostinho

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**Abstract** Species or functional guild loss from upper trophic positions, i.e., trophic downgrading, will likely have important consequences for ecosystem functioning due to cascading direct and indirect effects. Using 1000 l mesocosms, we examined how sequential loss of species occupying upper trophic positions influenced ecosystem functioning of experimental floodplain lagoon food webs. Treatments were developed based on fish assemblage data from long-term field surveys of tropical floodplain lagoons,

and response variables represented multiple components of ecosystem functioning. Sequential loss of species occupying upper trophic positions significantly influenced multiple ecosystem responses including changes in fish assemblage structure, nutrient concentrations, and zooplankton density. Although loss of species from specific functional roles is expected to facilitate predictive understanding of ecosystem consequences, we observed complex and dynamic responses to trophic downgrading that did not follow expectations of strong predicted top-down effects. The highly connected food web structure in our system and relative balance between top-down and bottom-up processes likely suppressed cascading effects. Consequences of biodiversity loss in highly connected multitrophic ecosystems may be difficult to predict as ecosystem responses will likely deviate from simplified food chain dynamics or from patterns that emerged from single trophic level studies.

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R. M. Pendleton (✉) · D. J. Hoeinghaus  
Department of Biological Sciences and the Institute of Applied Sciences, University of North Texas, Denton, TX 76203, USA  
e-mail: rich.pendleton@gmail.com

*Present Address:*

R. M. Pendleton  
Illinois River Biological Station, Illinois Natural History Survey, Prairie Research Institute, University of Illinois Urbana-Champaign, Havana, IL 62644, USA

L. C. Gomes · A. A. Agostinho  
Departamento de Biologia, Curso de Pós-Graduação em Ecologia de Ambientes Aquáticos Continentais, Universidade Estadual de Maringá, Maringá, PR 7020-900, Brazil

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## Introduction

Trophic downgrading, or loss of upper trophic position consumers, can result in dramatic changes to community structure and ecosystem functioning across a wide variety of ecosystems (Estes et al., 2011). Ecosystem

responses resulting from these losses can range from alterations in nutrient cycling, carbon sequestration, and changes in disease dynamics to more subtle shifts in community composition or the distribution of biomass (Carpenter et al., 2001; Schmitz et al., 2010; Estes et al., 2011). In addition to loss of upper trophic position consumers, loss or decline in abundance of other species occupying intermediate trophic positions can influence the structure and functioning of a given ecosystem (Hensel & Silliman, 2013; Connelly et al., 2014). Although these studies are far less numerous, the biodiversity of intermediate trophic position consumers can play important roles in maintaining ecosystem functions and processes.

In simplified food chains, species loss from upper trophic positions can often result in predictable responses among lower trophic levels in the form of trophic cascades (e.g., Power et al., 1985; Carpenter et al., 2001). However, ecosystems typically have high biodiversity within and across trophic levels, with numerous species and functional guilds integrating multiple trophic pathways at upper trophic positions (Duffy et al., 2007). The ability to predict ecosystem responses resulting from species loss in complex food webs may be difficult as a result of altered species interactions (i.e., direct and indirect) and alternate flows of energy (e.g., Downing, 2005; Srivastava & Vellend, 2005; O’Gorman et al., 2008; Pendleton et al., 2014). More research is needed to understand the potential ecosystem consequences that may arise from species loss among trophic positions, but relatively few studies have experimentally manipulated diversity loss in multitrophic systems (Balvanera et al., 2006).

Investigating loss of species occupying higher trophic positions is particularly relevant as these species are often more vulnerable to extinction or extirpation due to their dependency on the production of lower trophic levels, smaller population sizes, long generation times, and human exploitation (Duffy, 2002; Duffy, 2003; Petchey et al., 2004). This is especially the case for freshwater ecosystems that are globally faced with accelerating rates of habitat loss and degradation (Millennium Ecosystem Assessment 2005). Tropical freshwater ecosystems in particular are characterized by high diversity within and across trophic levels and are increasingly threatened, yet remain understudied in regards to the influence of species loss on community structure and ecosystem

functioning. A recent experimental study by Pendleton et al. (2014) found that loss of rare fish species from tropical floodplain food webs generally decreased nutrient concentrations, primary and secondary production, and whole ecosystem metabolism. Although it is clear that rare species can significantly contribute to ecosystem multifunctionality (Pendleton et al., 2014), it remains to be seen whether the effect of biodiversity is similar when species are excluded based on other realistic scenarios, such as trophic downgrading.

Here, we present results from a mesocosm experiment complementary to Pendleton et al. (2014) in which we investigate the effects of trophic downgrading on ecosystem functioning of tropical floodplain food webs. We further investigate direct trophic interactions among our measured response variables and how these interactions relate to species loss from multiple trophic positions. Long-term field data collected from isolated lagoons were used to identify realistic experimental assemblages. Biodiversity of experimental fish assemblages was reduced based on ordered trophic guilds (i.e., piscivore, zooplanktivore/insectivore, detritivore/algivore) to represent patterns of trophic downgrading across treatments. We hypothesize loss of entire trophic guilds will elicit strong cascading effects between primary and secondary production as observed in other ecosystems. Partial reduction of a trophic guild, in which some species of a given trophic guild are present, is expected to result in a trophic cascade of lower magnitude observed among ecosystem responses.

## Methods

### Human and animal rights statement

All species were properly collected and handled in an ethical manner and with all required permissions from the Brazilian Environmental Ministry (Ministério do Meio Ambiente (MMA), Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio), Sistema de autorização e informação em Biodiversidade (SISBIO)) under protocol number 22442-1, authentication code: 3263346. No other permissions were required for completion of this research, and this study does not include endangered or otherwise protected species.

## Description of study system and foundation for the experimental design

This research was conducted in the Upper Paraná River floodplain, Brazil. The Upper Paraná River is heavily impounded, and has only one free-flowing stretch located between Porto Primavera Reservoir and Itaipu Reservoir (Agostinho et al., 2004). This 230 km reach has a wide floodplain ( $\leq 20$  km) on the western margin that is seasonally inundated from December through April. Basin-wide impoundments and other human activities have strongly affected the Upper Paraná floodplain, including alterations of inundation dynamics, community structure, and ecosystem processes (Agostinho et al., 2004; Thomaz et al., 2004; Hoeninghaus et al., 2007, 2008). More than 4,500 species utilize the floodplain, and its functioning is critical for maintenance of regional biodiversity (Agostinho et al., 2013).

The Upper Paraná River Floodplain Long-Term Ecological Research (LTER) program has intensively studied biotic, abiotic, and social aspects of the floodplain for more than a decade (Thomaz et al., 2004). The LTER program database of fish assemblage structure from seasonally isolated floodplain lagoons (standardized surveys between 2000 and 2007) was used to provide the foundation for the experimental design. Only data from austral spring were used to coincide with our study period and seasonal isolation of lagoons from the main channel (i.e., low water season). Species richness of individual lagoons ( $n = 11$ ) across all years ranged from 3 to 19 ( $\bar{X} = 10$ ). The realism of biodiversity experiments can be enhanced by using the full biodiversity gradient for a given ecosystem (Naeem, 2008). Therefore, 2 species and 18 species were used as our low- and high-diversity endpoints with the midpoint of 10 species to match the range and mean species richness observed in seasonally isolated lagoons. See Pendleton et al. (2014) for more details on study location, seasonality of lagoons, standardized fish assemblage sampling, species composition, and food web structure.

Species were ranked and ordered by abundance for each lagoon for each year to identify patterns of species relative abundances. The presence of a species varied among lagoons and years in this dynamic system, therefore, when a species was absent from a

particular lagoon it was assigned the median rank between the number of species present in that lagoon and the total number of species observed during the collection period (2000–2007;  $n = 59$ ). This prevented a highly abundant species in a single lagoon from inflating its abundance across all lagoons over time by accounting for lagoons and years in which it was absent. The rank of each species was then summed across lagoons over time to identify species that consistently had high relative abundances and occurred in most or all lagoons over time. A rank-abundance curve was then generated based on abundances of all species across all lagoons and time (see Pendleton et al., 2014). Using this rank-abundance curve, sequentially nested subsets of the high-diversity endpoint (18 species) were used as experimental assemblages representing sequential loss of species based on trophic position, with the middle experimental richness level equal to the mean species richness observed from the LTER data (i.e., 10 species; see Experimental design below and Zavaleta & Hulvey (2004) and Pendleton et al. (2014) for comparable designs).

## Experimental venue

To represent littoral habitats of isolated lagoons, twenty-four 1000 l cylindrical mesocosms (1.0 m high, 1.4 m diameter) located at the Upper Paraná River Floodplain LTER field station were used as experimental units and were randomly assigned to treatments. Mesocosms were stocked with river water, sandy substrate, floating macrophytes, and benthic structure and inoculated with nutrients, phytoplankton, and zooplankton to better simulate natural conditions (Pendleton et al., 2014). Following these additions, average initial conditions of variables of interest among mesocosms better resembled concentrations observed in natural isolated lagoons during austral spring, though with far lower variability. The following results reflect the mean and standard deviations for concentrations in mesocosms at the start of the experiment and values obtained from natural lagoons, respectively): total phosphorus (TP) ( $15 \mu\text{g/l} \pm 10$ ;  $88 \mu\text{g/l} \pm 92$ ), total nitrogen (TN) ( $850 \mu\text{g/l} \pm 92$ ;  $458 \mu\text{g/l} \pm 198$ ), phytoplankton (measured as chlorophyll *a* concentration ( $3.9 \mu\text{g/l} \pm 2.8$ ;  $15 \mu\text{g/l} \pm 22$ ), and zooplankton

(6,739 individuals/m<sup>3</sup> ± 4,769; field data reported separately for major taxonomic groups, e.g., approximate mean copepod density of 5,000 individuals/m<sup>3</sup> and cladoceran density of 7,000 individuals/m<sup>3</sup>) (Thomaz et al., 2004). Shade cloth (50% light penetration) covered all mesocosms and simulated natural riparian reduction of solar irradiance. Mesocosms were otherwise exposed to climatic conditions and potential aerial colonization by macroinvertebrates, which were not quantified in this study. Fishes were collected using multiple methods and were placed in holding tanks prior to stocking to ensure survival and acclimation to experimental conditions. For each species, individuals of similar size were stocked in experimental mesocosms over a 48 h period, and individuals that succumbed to handling stress were replaced for the first 7 days of the experiment. Any subsequent mortality that was observed was attributed to interactions (i.e., predation or competition) occurring within the experiment and not from handling during stocking.

#### Experimental design

Experimental assemblages were structured based on species loss by ordered trophic position, such that the high- and low-diversity endpoints and mean observed species richness from field surveys comprise the bounds and midpoint for the treatments (18, 14, 10, 6, and 2 species; Table 1). Species were classified into trophic guilds based on reviews of the trophic ecology of fishes from the Upper Paraná River and its floodplain and supplemented with accounts from the primary literature (see Pendleton et al., 2014). Using ordered trophic guilds (e.g., piscivore, zooplanktivore/insectivore, detritivore/algivore), species richness was sequentially reduced by four species for each treatment. When more than four species occurred within a trophic guild, rarer species based on their summed rank abundance were excluded first. Therefore, experimental reductions based on trophic position represent an “expected” trophic downgrading scenario based on the trophic structure of an average lagoon and predictions of extinction/extirpation susceptibility of upper trophic position consumers. To isolate the effect of biodiversity loss, abundance was held constant at 65 individuals per mesocosm and evenness of relative abundances was maintained constant across treatments by proportionally distributing individuals across species such that the slope of the rank–

abundance curve (i.e., evenness) was maintained constant (see Pendleton et al., 2014). For example, when determining the abundances for the 6-species assemblage, the 26 individuals (totaled from the four species with higher trophic positions in the 10-species assemblage) were proportionally distributed among the six species in this assemblage (Table 1). Thus, our experimental assemblages maintain natural patterns of dominance and rarity observed in lagoons, while maintaining the effect of sequential loss of species by trophic position. Additionally, a treatment without fishes was included as a control. We were unable to standardize biomass among treatments at the same time as abundance and evenness due to the high morphological diversity of species included. Therefore, we included fish biomass [from estimates in Pendleton et al. (2014)] as a covariate in analyses to account for potential effects of biomass among treatments. Response variables were regressed against fish biomass and residuals were saved and plotted to represent response variables among treatments and over time after controlling for differences in fish biomass. The experiment ran for 18 days and each treatment was replicated four times.

#### Community and ecosystem response variables

Eight components of ecosystem structure and function were measured as response variables: fish assemblage structure (i.e., species composition and relative abundance), nutrient concentrations (TP and TN), phytoplankton density, periphyton density, zooplankton density, benthic organic matter (BOM), and macrophyte biomass. Day-0 samples (excluding fish assemblage structure and macrophyte biomass measured at the end of the experiment) were collected after all fishes were stocked, and all subsequent weekly samples were collected on 7-day intervals based on that date, with the exception of the last sample collection (i.e., day 18). Periphyton density was measured as chlorophyll *a* concentration from accumulated algae on semi-porous clay bricks. See Pendleton et al. (2014) for detailed methodology.

An index of ecosystem multifunctionality was calculated based on average Z-scores for each response variable, excluding fish assemblage structure (Maestre et al., 2012). Z-scores were calculated for each time step in each mesocosm to allow for all response variables to be measured on a common scale

**Table 1** Species composition and trophic guild assignment across experimental diversity treatments ordered by trophic position

Species	Diversity level						Trophic guild	Rank abundance
	0	2	6	10	14	18		
No. species	0	2	6	10	14	18		
No. trophic guilds	0	2	4	6	6	7		
<i>Serrapinnus</i> sp 1		45	27	18	13	10	A	1
<i>Steindachnerina insculpta</i>		20	10	3	5	4	D-A	6
<i>Astyanax altiparanae</i>			16	12	10	8	I	2
<i>Loricariichthys platymetopon</i>			5	2	3	4	D	8
<i>Serrapinnus notomelas</i>			4	2	2	2	A	12
<i>Steindachnerina brevipinna</i>			3	2	1	1	D-A	16
<i>Moenkhausia forestii</i>				10	9	8	Z-I	3
<i>Hyphessobrycon eques</i>				7	7	6	Z	4
<i>Aphyocharax anisitsi</i>				6	5	6	Z-I	5
<i>Psellogrammus kennedyi</i>				3	4	4	Z-I	7
<i>Roeboides descalvadensis</i>					2	3	Z	9
<i>Characidium aff. zebra</i>					2	2	I	11
<i>Moenkhausia bonita</i>					1	2	I	13
<i>Aphyocharax dentatus</i>					1	1	Z-I	15
<i>Pyrhulina australis</i>						1	I	17
<i>Hoplias aff. malabaricus</i>						1	P	10
<i>Acestrorhynchus lacustris</i>						1	P	14
<i>Serrasalmus marginatus</i>						1	P	18

Values for each species at each diversity level are stocking abundances in experimental mesocosms. In the highest diversity treatment ( $n = 18$ ), the species rank–abundance curve is proportional to field data from isolated lagoons scaled to 65 individuals (see Pendleton et al. 2014). For subsequent treatments, species are excluded based on trophic position, and where applicable, rarity based on species-summed rank abundance (i.e., when more than four species have similar trophic positions, rank abundance was used as the second factor determining exclusion). Abundance is held constant (65 individuals) by proportionally distributing individuals from those species that were excluded from the prior assemblage (i.e., species with higher trophic positions) to remaining species in the subsequent assemblage such that the slope of the rank–abundance curve (i.e., evenness) was maintained constant. Trophic guild assignment: (A) algivore, (D) detritivore, (D-A) detritivore/algivore, (I) insectivore, (P) piscivore, (Z) zooplanktivore, and (Z-I) zooplanktivore/insectivore [see Table S1 in Pendleton et al. (2014)]

of standard deviation units for a given time step. The response variables quantified in this experiment relate to the maintenance of primary production, secondary production, and nutrient cycling, therefore, higher values for these response variables are representative of higher ecosystem multifunctionality (Maestre et al., 2012; Pendleton et al., 2014).

#### Data analyses

Fish assemblages were subject to multitrophic interactions, therefore, we used non-metric multidimensional scaling (NMDS) to compare final fish assemblage structure among mesocosms at the end

of the experiment. The analysis was conducted using Bray–Curtis similarities calculated from the species relative abundance by replicate matrix. Macrophyte biomass was measured only at the end of the experiment, and treatment effects were compared using one-way ANOVA. The remaining response variables were analyzed using mixed-model, repeated-measures ANOVA (rmANOVA) in SPSS 17.0. Mesocosms were treated as a random effect, treatment was the fixed effect, and fish biomass was included as a time-varying covariate. An unstructured covariate matrix was used based on the lowest Akaike's Information Criterion (AIC) value when testing against other covariate matrices. A Shapiro–Wilk

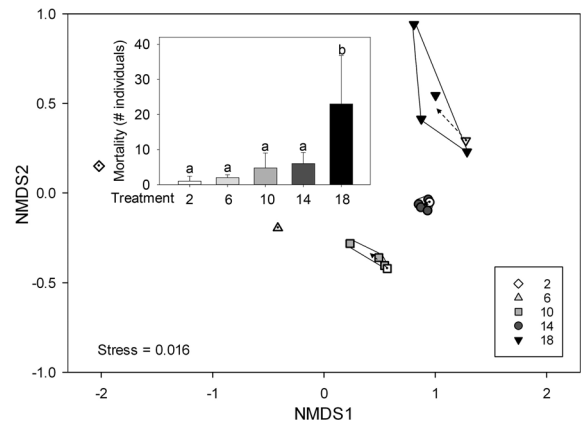
( $\alpha = 0.05$ ) test confirmed normality of residuals to meet the assumptions of ANOVA (Kéry & Hatfield, 2003). When normality was violated, response variables were transformed (i.e.,  $TP = \log_{10}$ ; zooplankton = square root) to approach normality. The magnitude and direction of change varied among treatments and over time resulting in violation of normality of residuals for several response variables when viewed over the entire study period. Therefore, normality of residuals was assessed at each time step and confirmed for all response variables, with few exceptions.

Direct trophic interactions link many of the response variables in this study. To identify possible mechanisms driving variation in response variables over time, complementary rmANOVAs testing effects of direct trophic interactions were conducted using all possible covariate combinations. The biomass of each trophic guild was included in each analysis to test for possible treatment effects due to differences in biomasses among trophic guilds. Backward selection was used to exclude models with non-significant covariates. Best-fitting models were selected and interpreted based on the lowest  $\Delta_i$  AIC ( $\Delta_i$  AIC =  $AIC_i - AIC_{\min}$ ) with  $\Delta_i$  AIC values < 2 indicating substantial empirical support (Burnham & Anderson, 2002). To allow for inclusion of macrophyte biomass as a time-varying covariate, we estimated values at intermediate time steps using initial (i.e., combined average dry weight for each species, similar in size to those stocked in the mesocosms) and final dry mass and a linear model (Henry-Silva et al., 2008).

## Results

### Community and ecosystem responses over time

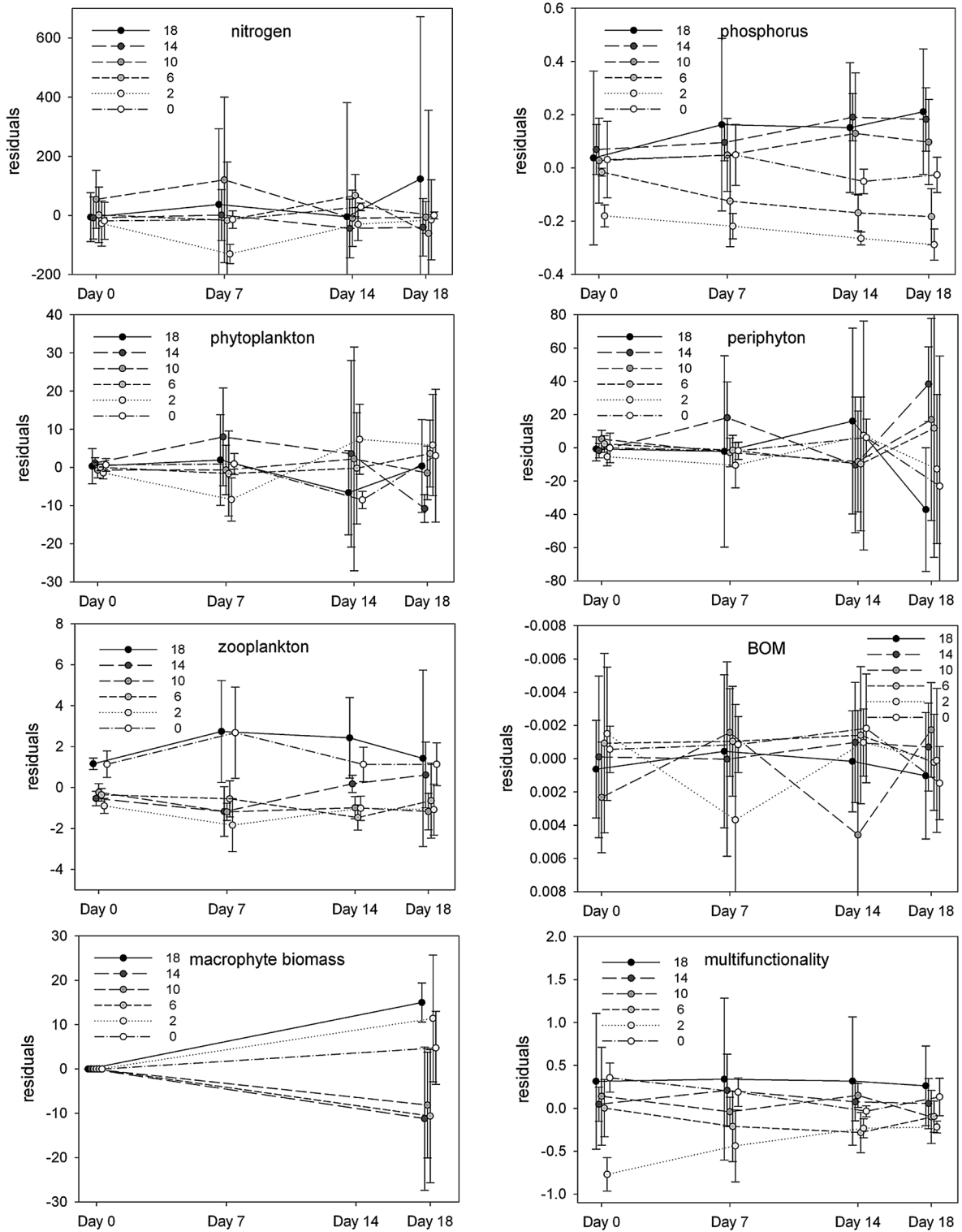
Mortality among 2, 6, 10, and 14 species assemblages were not statistically different, whereas mortality was significantly greater in the 18-species assemblage that contained all three piscivores (Tukey's HSD;  $P < 0.05$ ; Fig. 1). In addition to consumption of smaller-bodied species at lower trophic positions, we observed intraguild predation by the piranha *Serrasalmus marginatus*. In two of the four 18-species replicates, the piranha consumed the caudal fin of the pike characid *Acestrorhynchus lacustris*, resulting in



**Fig. 1** Non-metric multidimensional scaling of fish assemblages at the beginning and end of the experiment. Initial assemblage structures for each treatment are indicated by *open symbols*, and the *dot* and *dashed line* denote the direction of change from initial to final assemblage structure for each treatment. Note that due to minimal mortality, initial and final fish assemblage structure of 2 and 6 species treatments did not change. *Top left* mean ( $\pm 1$  SD) mortality at the end of the experiment

mortality. Some mortality (primarily among algivores and zooplanktivores/insectivores) was observed in treatments that lacked piscivores (Fig. 1). Ordination of the final species relative abundance by replicate matrix using NMDS indicated fish assemblage structure of replicates among treatments remained distinct, and in fact the 18-species treatment became more distinct despite within-treatment variation resulting from mortality (Fig. 1).

Without accounting for fish biomass, nutrient concentrations, phytoplankton density, and periphyton density generally increased over time while BOM generally decreased over time (Online Resource 1). However, after controlling for the effects of fish biomass, general increases in nutrient concentrations, phytoplankton density, and periphyton density were no longer apparent, and the magnitude and direction of change varied among treatments and over time (Fig. 2). Zooplankton density for the control and 18-species treatments exhibited higher values when compared to the other treatments (Fig. 2). Increases over time were observed for macrophyte biomass in the control and 2- and 18-species treatments whereas biomass declined for the remaining treatments. Ecosystem multifunctionality tended to decrease slightly throughout the experiment, and the 18-species



**Fig. 2** Mean and standard deviation of unstandardized residuals of response variables by treatment and over time. Residuals are from simple linear regression of the response

variable and fish biomass, and therefore are measures of the response variable among treatments that control the potentially confounding effect of fish biomass

treatment tended to exhibit higher scores when compared to other assemblages.

After controlling for the effects of fish biomass, significant treatment effects were observed for nutrient concentrations and zooplankton densities but not for phytoplankton densities, periphyton densities, and BOM (Table 2). TN and TP concentrations were significantly different among treatments ( $P = 0.001$  and  $P < 0.001$ , respectively) and TP differed over time ( $P = 0.017$ ; Table 2). Time effects were observed for phytoplankton and periphyton densities (both  $P < 0.001$ ) and periphyton had a significant treatment by time interaction ( $P = 0.038$ ; Table 2). Zooplankton densities were significantly different among treatments and time (both  $P < 0.001$ ; Table 2). No treatment effects were observed for BOM, and macrophyte biomass did not differ among treatments for *E. crassipes* ( $F_{5,17} = 1.832$ ,  $P = 0.160$ ) but significant treatment effects were observed for *P.*

*stratiotes* and the combined weight of both macrophyte species ( $F_{5,17} = 4.298$ ,  $P = 0.010$  and  $F_{5,17} = 3.811$ ,  $P = 0.017$ , respectively).

#### Effects of direct trophic interactions on response variables

The model including total fish biomass, phytoplankton density, and zooplankton density best explained the differences in TN among treatments with significant treatment and time effects and a time by treatment interaction ( $P < 0.001$ ,  $P = 0.005$ , and  $P = 0.007$ , respectively; Table 2). For TP, zooplankton density as a covariate was the best-fitting model with significant treatment and time effects (both  $P < 0.001$ ; Table 2). The next best-fitting models with similar main effects included no covariates and total fish biomass alone as a covariate (Table 2). The best-fitting model for phytoplankton density included TP and zooplankton density

**Table 2** Best-fitting models for each response variable calculated using mixed model, repeated measures ANOVA

Model	Main effects			Covariates						AIC	$\Delta_i$ AIC
	SR	Time	SR $\times$ time	fb	Phyto	p	Zoo	Alg	Detrit/alg		
$N_1$	<b>&lt;0.001</b>	<b>0.005</b>	<b>0.007</b>	<b>&lt;0.001</b>	<b>0.002</b>		<b>&lt;0.001</b>			864.7	0
$N_2$	<b>0.001</b>	<b>0.070</b>	0.063	<b>&lt;0.001</b>			<b>0.027</b>			870.8	6.1
$N_3$	<b>0.001</b>	0.228	0.095	<b>&lt;0.001</b>						895.3	30.7*
$P_1$	<b>&lt;0.001</b>	<b>&lt;0.001</b>	0.825				<b>&lt;0.001</b>			-44.5	0
$P_2$	<b>&lt;0.001</b>	<b>&lt;0.001</b>	0.435							-43.3	1.2
$P_3$	<b>&lt;0.001</b>	<b>0.017</b>	0.774	<b>0.001</b>						-42.9	1.7
$P_4$	<b>&lt;0.001</b>	<b>0.014</b>	0.846	<b>0.017</b>			<b>0.005</b>			-38.3	6.3
Peri <sub>1</sub>	<b>0.032</b>	<b>&lt;0.001</b>	<b>0.038</b>				<b>0.027</b>	<b>0.011</b>	<b>0.037</b>	646.9	0
Peri <sub>2</sub>	0.860	<b>&lt;0.001</b>	<b>0.038</b>	0.570						675.3	28.3*
Phyto <sub>1</sub>	0.092	<b>&lt;0.001</b>	0.096			<b>&lt;0.001</b>	<b>0.004</b>			484.9	0
Phyto <sub>2</sub>	0.269	<b>&lt;0.001</b>	0.157	<b>0.022</b>						525.2	40.3*
Zoo <sub>1</sub>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	0.054							232.4	0
Zoo <sub>2</sub>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	0.513	0.059						234.6	2.2
BOM <sub>1</sub>	0.918	<b>&lt;0.001</b>	0.098							-515.1	0
BOM <sub>2</sub>	0.919	<b>0.003</b>	0.103	0.725						-497.8	17.3*
Macro <sub>1</sub>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	0.132						76.7	0

Models investigated direct trophic interactions using every possible covariate combination as well as the effect of fish biomass (treatment covariate). Each row represents an individual model grouped for each response variable ( $N$  total nitrogen,  $P$  total phosphorus, *peri* periphyton density, *phyto* phytoplankton density, *zoo* zooplankton density, *BOM* benthic organic matter, *macro* macrophyte biomass). For a given response variable, models are ranked by goodness of fit according to  $\Delta_i$  AIC value.  $P$  values for the main effects (*SR* species richness) and covariate(s) (*fb* total fish biomass, *phyto* phytoplankton, *p* phosphorus, *zoo* zooplankton, *alg* algivore, *detrit/alg* detritivore/algivore) from ANOVA models are given within cells and bold values represent significant results ( $\alpha = 0.05$ ). Only models with  $\Delta_i$  AIC  $< 7$  are shown, except that models where fish biomass exceeded the  $< 7$   $\Delta_i$  AIC threshold are still presented and denoted in italics and \* next to  $\Delta_i$  AIC value



as covariates and had a significant time effect ( $P < 0.001$ ; Table 2). Differences in periphyton density were best explained by algivore biomass, detritivore/algivore biomass, and zooplankton density with significant treatment and time effects and a time by treatment interaction ( $P = 0.032$ ,  $P < 0.001$ ,  $P = 0.038$ , respectively; Table 2). The model with no covariates best explained differences in zooplankton density and although only marginally significant as a covariate, fish biomass was the next best-fitting model with the same main effects ( $P < 0.001$  for both treatment and time effects; Table 2). BOM and macrophyte biomass either had no significant covariates or the best-fitting model did not include covariates. For these response variables, differences among treatments could not be attributed to specific direct trophic interactions.

## Discussion

Trophic downgrading influenced several aspects of ecosystem structure and function within our experimental mesocosms. Fish mortality was significantly higher in the most specious treatment (i.e., piscivores present) and resulted in further dissimilarity in assemblage structure when compared with the other treatment assemblages. After accounting for the potential influence of fish biomass, significant species richness effects for nutrient concentrations (i.e., TN and TP) and zooplankton density were observed, in addition to significant time effects among response variables. Despite biodiversity losses occurring among treatments in an ordered top-down manner, we did not observe the anticipated strong cascading effects of species loss from upper trophic positions on ecosystem function that may be considered a hallmark consequence of trophic downgrading (Estes et al., 2011). Instead, loss of biodiversity within our experimental food web led to complex ecosystem dynamics.

Trophic cascades have been well documented in a variety of biological communities and ecosystem types (Pace et al., 1999), often with predictable and strong top-down effects in the presence or absence of a predator or consumer (Power et al., 1985; Carpenter et al., 2001; Schmitz et al., 2010; Estes et al., 2011). We anticipated that the reduction or complete exclusion of a trophic guild with each subsequent diversity

level would alter remaining trophic levels via top-down release or modify the strength of possible top-down control. Instead, the observed interactions among trophic levels provided minimal evidence of strong top-down release or top-down control, with few exceptions. For instance, high zooplankton densities in the 18-species treatment were observed and may have resulted from greater proportional mortality of zooplanktivorous fishes (i.e., release of zooplankton in the presence of piscivores) when compared to the 10- and 14-species treatments. However, zooplankton densities were similar among treatments in the absence of zooplanktivores (i.e., possible release of zooplankton) when compared to treatments containing zooplanktivores in the absence of piscivores (i.e., possible suppression of zooplankton). Compositional or size distributional changes may have occurred within the zooplankton communities while maintaining similar densities due to zooplanktivore selectivity or capture efficiency, but the taxonomic resolution of this study does not allow for more detailed examination of potential effects of zooplanktivores on zooplankton communities. Additionally, our analyses using different covariates to test for direct trophic interactions also indicated possible top-down effects on periphyton (algivores, detritivore/algivores, and zooplankton densities were the best predictors for differences in periphyton densities). However, the directionality of this relationship was difficult to determine due to potential bottom-up processes via nutrient remineralization from consumer excretion. It is therefore likely that top-down processes were not solely responsible for patterns observed in the functioning of these complex food webs.

Consumers across all trophic levels can also produce strong nutrient-mediated bottom-up effects. For instance, both fishes and zooplankton can provide substantial nutrient sources through excretion, egestion, and translocation of nutrients (Vanni, 2002; Schmitz et al., 2010). The aforementioned release of zooplankton in the 18-species treatment likely also coincided with strong bottom-up effects via remineralization of nutrients by piscivores (i.e., highest fish mortality observed in this treatment) and subsequent increased zooplankton excretion due to higher zooplankton densities. This interpretation is supported by our covariate analysis (fish biomass and zooplankton densities were significant covariates for nutrients) and elevated TP in the 18-species treatment. Collectively,

ecosystem responses within our study likely reflect the net consequence of bottom-up effects (i.e., nutrient remineralization) in addition to top-down effects (i.e., consumption). The varied means by which upper trophic levels may affect ecosystems by top-down (Estes et al., 2011) and bottom-up (Schmitz et al., 2010) processes emphasizes the difficulties in predicting ecosystem responses to trophic downgrading in complex ecosystems.

As multitrophic biodiversity and ecosystem function studies become more prevalent, food web ecology and associated food web properties may help explain observed patterns (Woodward, 2009). For example, high food web connectance may dampen cascading effects as multiple pathways of direct (i.e., consumption) and indirect effects are prevalent throughout complex food webs (Finke & Denno, 2004; Borer et al., 2005). Early food web studies suggested that connectance should decrease with increasing species richness (Pimm, 1982). However, recent tests found that this generalization is not necessarily supported; connectance may be independent or even be positively correlated with species richness (Winemiller, 1989; Warren, 1994), and the nature of this relationship may play an important role in mediating ecosystem responses to biodiversity loss. In our study, expected connectance decreased with increasing species richness (Online Resource 2), although the rate of decrease slowed as richness increased, and increased for the 18-species treatment relative to the 6-, 10-, and 14-species treatments. High variability in mortality among prey species and trophic guilds across replicates (standard deviations for species: 0.5–3.3 and trophic guilds: 0.10–0.21) supports the classification of piscivores in this study as generalist consumers in our experimental food web. High connectance and the broad diet breadth of piscivores may be expected to dampen cascading effects as a single prevalent top-down pathway was not evident. Furthermore, we observed intraguild predation between piscivores and this antagonistic interaction has been shown to weaken trophic cascades (Finke & Denno, 2004). These results may explain the lack of clearly distinguishable responses among adjacent trophic levels and the lack of diversity effects for phytoplankton and periphyton densities. However, trophic cascades have been observed in other diverse food webs (e.g., Byrnes et al., 2006). Quantifying interaction strengths among species in light of connectivity may facilitate

understanding of cascading responses as more specific comparisons could be made among other ecosystems in which cascades are observed. For example, O’Gorman and Emmerson (2009) found removal of strongly interacting species produced a dramatic cascade, while removal of weakly interacting species had no significant effects on primary and secondary production.

In addition to the balance of top-down and bottom-up processes, functional redundancy among fish species across treatments may explain the lack of fish diversity effects observed for phytoplankton/periphyton densities and BOM. Studies have suggested that functional redundancy among organisms may provide “biological insurance” to environmental perturbation through compensatory dynamics (Naeem, 1998; Joner et al., 2011). As species richness was reduced and species were lost among trophic guilds, two of the three primary consumer guilds (i.e., algivore and detritivore/algivore) were conserved across all diversity treatments (excluding the no fish control). Specifically, the genera *Serrapinnus* and *Steindachnerina* were present in all diversity treatments. Species within these genera (*Serrapinnus* sp. 1, *S. notomelas*, and *Steindachnerina insculpta*, *S. brevipinna*) are morphometrically and ecologically very similar (Oliveira et al., 2002; Santi-Rampazzo et al., 2007; N.S. Hahn, personal communication). Thus, in treatments where *Serrapinnus notomelas* and *Steindachnerina brevipinna* were excluded, we believe *Serrapinnus* sp. 1 and *Steindachnerina insculpta* were able to functionally compensate (and vice versa when considering losses due to predation on both species in each genera). Species-specific contributions to ecosystem processes cannot be fully disentangled in our experiment; we purposefully chose a complex multitrophic design for the above-mentioned reasons knowing that this prohibited a fully factorial design. That limitation aside, we suggest primary consumers in this study may have utilized resources in a similar manner or were able to functionally compensate for loss of individuals within or among species of the same guild, which would limit diversity effects on basal resources (i.e., phytoplankton/periphyton densities and BOM; Blake & Duffy, 2010; Colón-Gaud et al., 2010). Yet for stable coexistence among species, perfect functional redundancy is rare (Loreau, 2004) and species likely influence other ecosystem processes differently over varying temporal and spatial scales despite functional similarity (e.g., Duffy et al., 2001).

For example, P excretion rate and N:P excretion ratio can vary greatly among Neotropical fish families and potentially have strong influences on nutrient cycling (e.g., low P excretion exhibited by Loricariidae; Vanni et al., 2002). Primary consumers within this experiment encompass three different families and potential stoichiometric differences among these species may have contributed to the observed differences in nutrient concentrations among treatments. Thus, using multiple ecosystem responses is essential in complex food webs as the overall multifunctionality of an ecosystem likely depends on multiple species contributing to different functions over time (Garnfeldt et al., 2008; Zavaleta et al., 2010).

Differences in macrophyte biomass were observed as the 2- and 18-species treatments and the control tended to increase over time, whereas biomass tended to decrease in remaining treatments. These patterns may be attributed to the presence or relative abundance of the fish species *Astyanax altiparanae* in each of the treatments. *A. altiparanae* is primarily insectivorous but also has the tendency to consume higher plants and algae (Peretti & Andrian, 2008; Crippa et al., 2009). Treatments that lacked *A. altiparanae* (i.e., 2-species and control) tended to have higher average macrophyte biomass at the end of the experiment when compared to the 6-, 10-, and 14-species treatments. Although the 18-species treatment contained *A. altiparanae*, initial stocking densities were lower than other treatments and consumption by piscivores resulted in relatively low final survivorship (~44% survivorship across replicates). Although dietary analyses were not conducted, it is possible that direct consumption of macrophytes by *A. altiparanae* resulted in the observed differences in macrophyte biomass over time.

Interestingly, multifunctionality tended to slightly decrease over time after accounting for fish biomass, with the exception of the 2-species treatment. Multifunctionality is a composite metric of all measured ecosystem responses, and given the stable trends in most variables over time, we can identify phytoplankton densities and macrophyte biomass as primary drivers of the increases over time observed for the 2-species treatment (i.e., comprised by two primary consumer species). Grazers have the ability to control basal resources by consumption but can also stimulate production via nutrient remineralization (Gido et al., 2010). Low initial phytoplankton/periphyton densities

may indicate low nutrient availability or possible early grazing control by the two primary consumer species. Over time, consumption and subsequent nutrient turnover likely compensated grazing effects leading to increases in primary production, notably phytoplankton densities and macrophyte biomass. Despite these trends, multifunctionality did not show a strong correlation with species richness, in contrast with the positive correlation between plant species richness and ecosystem multifunctionality in previous research on dryland ecosystems (Maestre et al., 2012) and a similar pattern observed for Neotropical fishes by Pendleton et al. (2014). The most specious treatment did tend to exhibit higher multifunctionality, but a holistic metric of ecosystem function may be less amenable to interpretation in complex multitrophic ecosystems.

Some mortality (primarily among algivores and zooplanktivores/insectivores) was observed in treatments that lacked piscivores. Based on our experimental design we cannot determine the cause of that mortality. One possible explanation could be competition; dead individuals often appeared emaciated and resource levels were lower at the beginning of the experiment when fish densities were highest. Low primary and secondary production may be expected in natural lagoons directly following isolation from the main river (analogous to the start of the experiment) as production is positively related to water residence time (time since isolation from the main river; Schagerl et al., 2009). Therefore, a lag period may be expected before sustainable phytoplankton and zooplankton populations are reached. Alternatively, mortality in these treatments may be a result of initial handling stress. However, we believe handling mortality would be minimal as we replaced dead individuals at the beginning of the experiment, minimized handling time, and held fishes in holding tanks prior to the experiment to ensure survival and acclimation to experimental conditions. Furthermore, throughout the experiment, fishes were observed performing foraging, schooling, and habitat-associated behaviors as expected based on knowledge of these species in the natural environment.

Although our mesocosms were open to aerial colonization of macroinvertebrates, we expect that macroinvertebrates likely played a minor role on measured ecosystem variables. The only macroinvertebrates that we observed to colonize the mesocosms were case-spinning caddisfly larvae, which were

present on the walls of all mesocosms (colonization and survival rates appeared similar among treatments). In contrast to our mesocosms, macroinvertebrate communities are more diverse in natural lagoons, just like fish assemblages in natural lagoons are more diverse than in our experiment. Importantly, Neotropical fishes perform ecological roles that are dominated by macroinvertebrates in temperate ecosystems; therefore, we expect fishes functionally performed many of these ecological roles within the experiment.

The duration of this experiment may have influenced our ability to detect strong cascading effects if they only become apparent over longer temporal scales (see references within Estes et al., 2011). For example, higher zooplankton densities observed in the 18-species treatment may be indicative of the onset of a trophic cascade and an extension of the experiment may have revealed stronger cascading interactions via consumption of zooplanktivores by piscivores. Other experiments manipulating fish consumer species have observed rapid responses among lower trophic levels in relatively short duration studies (e.g., Power et al., 1985; Bengtson et al., 2008; Carey & Wahl, 2011a). However, unlike the aforementioned experiments, this study contained greater diversity within and among trophic levels which we believe greatly influenced the potential for strong cascading effects via the relative balance of top-down and bottom-up processes and functional redundancy. Our interpretations of the acute effects observed in this study do not preclude the possibility of trophic cascades developing at some later point in time had the experiment been allowed to continue, especially given the dynamic nature of the observed responses. On the other hand, previous studies in freshwater streams have found that macro-consumer effects are transient and disappear after a few weeks (Murdock et al., 2010). The observed effects from this 18-day study provide a foundation for future experiments that could be performed *in situ* in natural isolated lagoons that would be more amenable to longer study durations. Specifically, do acute effects persist through time perhaps as strong founder effects, are consumer effects transient, or would abundance/biomass across trophic levels more closely approximate a trophic cascade? Such experiments in our floodplain ecosystem would still be temporally restricted (i.e., a few months) as lagoons are only isolated from the main river during the dry season.

Several multitrophic studies have provided mechanistic interpretation of the effects of species loss on ecosystem functioning (e.g., Bruno & O'Connor, 2005; Griffin et al., 2008; Carey & Wahl, 2011b). However, identifying general patterns emerging from studies of biodiversity loss from multitrophic ecosystems has challenged ecologists because consequences of species loss in food webs can be highly context dependent and often difficult to predict without full understanding of the multitude of direct and indirect effects within the food web (Srivastava & Vellend, 2005; Duffy et al., 2007). A previous study by Pendleton et al. (2014) examined ecosystem consequences of loss of rare species using similar initial fish assemblages and levels of species richness as in this study and found that biodiversity significantly influenced ecosystem metabolism, nutrients, and primary and secondary production. Here, loss of diversity under a different non-random extinction scenario (i.e., via trophic downgrading), led to complex and dynamic responses among ecosystem processes. Similar to previous biodiversity-ecosystem function research that tested for effects of random versus ordered experimental assemblages (Zavaleta & Hulvey, 2004; Bracken et al., 2008), the results from this study considered alongside those from Pendleton et al. (2014) suggest that differences in the order or composition of predicted non-random species loss can result in divergent ecosystem dynamics for the same ecosystem and diversity levels. Thus, small differences in forecasted biodiversity changes may be compounded at the ecosystem level and projected ecosystem outcomes of biodiversity loss should be interpreted with broad bounds to accommodate such uncertainty.

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