Freshwater Biology

Freshwater Biology (2015) **60,** 551–562 doi:10.1111/fwb.12503

Nutrient enrichment scarcely affects ecosystem impacts of a non-native herbivore in a spring-fed river

CRYSTAL W. DATRI, COREY L. PRAY, YIXIN ZHANG AND WESTON H. NOWLIN
Department of Biology, Aquatic Station, Texas State University, San Marcos, TX, U.S.A

SUMMARY

1. Non-native invasive species (NNIS) are a global issue whose introduction into novel ecosystems can fundamentally alter nutrient recycling and storage. It is therefore important to understand factors that affect the abundance and distribution of NNIS and their effects on ecosystems.
2. We investigated how nutrient enrichment and herbivorous armoured catfish separately and in combination affect ecosystem processes in a subtropical spring-fed river (San Marcos, River, TX, U.S.A.). A replicated stream channel experiment in which nutrient enrichment was cross-classified with the presence and the absence of armoured catfish was conducted to evaluate how nutrient enrichment may affect the ecosystem and nutrient cycling effects of catfish.
3. The presence of catfish reduced periphyton biomass and altered detrital decomposition rates and, contrary to predictions, decreased periphyton N:P. In addition, the presence of catfish increased the severity of periphyton P-limitation. We found little evidence that adding nutrients altered the effects of armoured catfish on ecosystem dynamics.
4. Armoured catfish likely play an important role in the nutrient dynamics of the San Marcos River, but nutrient enrichment has little influence on determining the magnitude of ecosystem and nutrient cycling effects of this invasive and stoichiometrically unique consumer.

Keywords: ecological stoichiometry, ecosystem function, eutrophication, invasive species, loricariid catfish

Introduction

The invasion of exotic or non-native invasive species (NNIS) presents one of the largest threats to aquatic ecosystems (Sala *et al.*, 2000; Malmqvist & Rundle, 2002). The successful establishment of NNIS in ecosystems depends on characteristics of both the habitat (Lonsdale, 1999; Stachowicz *et al.*, 2002) and the invading species (Rejmanek & Richardson, 1996; Kolar & Lodge, 2001). Some non-native species are more successful invaders than others (Rejmanek & Richardson, 1996; Kolar & Lodge, 2001), and those that succeed typically have competitive superiority over native species (Seabloom *et al.*, 2003) through post-invasion adaptation (Ellstrand & Schierenbeck, 2000; Siemann & Rogers, 2001). Populations of non-native species released into novel environments may escape regulation by native competitors and predators (Keane & Crawley, 2002), leading to the competitive exclusion of trophically similar species (Douglas, March & Minckley, 1994; Gido & Franssen, 2007). In addition, introduction of non-native species into aquatic ecosystems alters community dynamics and ecosystem processes (Flecker & Townsend, 1994; Hall, Tank & Dybdahl, 2003; Scott *et al.*, 2012).

Invasion of ecosystems by herbivorous and detritivorous fish is a substantial concern for the conservation of native fish and the preservation of ecosystem function. Moyle & Light (1996a) hypothesised that using rarely limiting food resources, benthic-feeding fish are likely to establish populations in novel habitats, potentially becoming invasive. These predictions are supported by field observations that benthivorous fish are commonly found as non-native taxa in fish assemblages (Gido & Franssen, 2007). Invasion by these fish is particularly concerning because invading algivores and detritivores can outcompete native fish in the same trophic guild, alter nutrient cycling and modify trophic pathways (Simon, Townsend & Biggs, 2004; Pound *et al.*, 2011; Scott *et al.*, 2012).

Spring-influenced lotic ecosystems often exhibit high diversity and levels of endemism, but also face

Correspondence: Weston H. Nowlin, Department of Biology, Aquatic Station, Texas State University, 601 University Drive, San Marcos, TX 78666, U.S.A. E-mail: wn11@txstate.edu

anthropogenic stressors (Bowles & Arsuffi, 1993; Crowe & Sharp, 1997; Earl & Wood, 2002). Because spring-influenced ecosystems exhibit low variability in physicochemical conditions, they are particularly sensitive to the successful invasion of non-native species (Moyle & Light, 1996b). In addition, many spring ecosystems face considerable disturbance through eutrophication and subsequent loss of water quality (Bowles & Arsuffi, 1993). Eutrophication of aquatic ecosystems has an array of consequences including the alteration of productivity, nutrient cycling and species diversity, and the loss of ecosystem services (Carpenter *et al.*, 1998). In addition, increased resource availability has been identified as a pivotal factor that increases invasion success (Vitousek *et al.*, 1997; Davis, Grime & Thompson, 2000; Thompson, Hodgon & Grime, 2001; Romanuk & Kolasa, 2005). If eutrophication leads to higher invasibility, nutrient enrichment may make spring ecosystems more invasible and change how invasive species affect ecosystem function and nutrient dynamics.

Detritivorous and herbivorous armoured catfish in the family Loricariidae (hereafter referred to as catfish) are native to Central and South America, but have invaded subtropical and spring-fed systems in and around North America, including Hawaii, Florida, Puerto Rico, Texas and Mexico (Page, 1994; Hoover, Killgore & Confrancesco, 2004; Capps & Flecker, 2013). Their presence in novel habitats is particularly concerning because of their effects on nutrient and trophic dynamics (Scott *et al.*, 2012; Capps & Flecker, 2013). Catfish directly affect periphyton biomass through consumption and increase benthic sediment redistribution and transport (Scott *et al.*, 2012). Catfish also indirectly affect algae by altering nutrient dynamics (Hood, Vanni & Flecker, 2005; Knoll *et al.*, 2009; Capps & Flecker, 2013). Loricariid catfish are stoichiometrically unique consumers, containing relatively high body phosphorus (P) content because their bodies are armoured with bony scutes (Vanni *et al.*, 2002). Consequently, catfish assimilate and retain a substantial proportion of P from their food (Hood *et al.*, 2005), thereby excreting dissolved inorganic nutrients with high nutrient:P ratios (Vanni *et al.*, 2002; Capps & Flecker, 2013). In invaded ecosystems, altered nutrient recycling and supply of dissolved inorganic nutrients by catfish may affect periphyton P content, thereby affecting periphyton P:carbon (C) and P:nitrogen (N) ratios (Knoll *et al.*, 2009; Scott *et al.*, 2012).

The purpose of our study is to address the interaction between invasive loricariid catfish (*Hypostomus plecostomus*) and nutrient loading in the spring-influenced San Marcos River, Texas, U.S.A. Armoured catfish were first noted in the upper spring-influenced portion of the San Marcos River in the late 1990s and are now abundant (Perkin & Bonner, 2011; Scott *et al.*, 2012). They have changed trophic dynamics of the river (Pound *et al.*, 2011) and have the potential to alter periphyton biomass and nutrient ratios, and sediment transport (Scott *et al.*, 2012). In addition, the upper portion of the San Marcos River is under increasing pressure from urban development and nutrient loading from non-point and point (i.e. effluent from the San Marcos Sewage Treatment Plant; Groeger *et al.*, 1997) sources. Therefore, there is clearly a need to understand the interactions and implications of catfish and nutrient loading in the San Marcos River.

In this study, we examined how the effects of catfish and nutrient enrichment individually and interactively affect ecosystem dynamics in a replicated stream channel experiment. We also assessed whether armoured catfish exhibited higher growth rates and better condition under nutrient enrichment. We hypothesised that the presence of catfish would lead to a decrease in periphyton biomass and increased sediment disturbance and transport. We used a stoichiometric approach and hypothesised that catfish would alter periphyton C:nutrient and N:P ratios through grazing and excretion. We hypothesised that adding nutrients would stimulate periphyton biomass, but the presence of catfish would largely negate these stimulatory effects because catfish are efficient grazers. We also hypothesised that catfish would be in better condition under nutrient-enriched conditions at the end of the experiment.

Methods

The San Marcos River emerges from *c.* 200 springs in the Edwards Aquifer that discharge into the headwaters (Spring Lake). Water flows over two waterfalls at the outlet of the lake and forms the San Marcos River, which continues to its confluence with the Blanco River 7.2 km downstream. The upper San Marcos River, located between Spring Lake and the Blanco River, is clear, has abundant macrophytes and nearly constant temperatures (*c.* 22 °C) and physicochemistry (Groeger *et al.*, 1997). However, nutrient concentrations, especially P, increase substantially below the Sewage Treatment Plant, which lies *c.* 7 km downstream from the headwaters. Total phosphorus (TP) concentration increases from *c.* 10 –μg L^{-1} at the headwaters to *c.* 300 μg L^{-1} below the Sewage Treatment Plant discharge (Groeger *et al.*, 1997; W.H. Nowlin, unpubl. data).

Stream channel experiment

To examine the separate and combined impacts of invasive catfish and nutrient enrichment, an experiment was conducted involving stream channel mesocosms at Texas State University (see Scott *et al.*, 2012 for detailed description of stream channels). Briefly, stream channels consisted of 10 cement channels supplied with water from an artesian well fed from the Edwards Aquifer. Each channel was fed from individual well heads and divided in half using a PVC frame lined with heavy (6-mil) plastic, yielding a total of 20 stream channels. The experiment consisted of a 2×2 fully factorial design of four treatment combinations: (i) catfish absent, unenriched; (ii) catfish present, unenriched; (iii) enriched, catfish absent; and (iv) catfish present and enriched. Each treatment combination was replicated five times and randomly applied to stream channels ($n = 20$). Catfish treatments consisted of three catfish per channel; these densities are within the range of densities observed in the river (Scott *et al.*, 2012). Catfish were caught from the San Marcos River and were between 19 and 29 cm total length (TL). Each catfish was individually marked using identifying fin clips, and weight (g) was determined. Three catfish died early in the experiment and were immediately removed and replaced with catfish of similar size. Nutrient enrichment consisted of adding dissolved N (as KNO_3 and NH_4Cl) and P (as $NaH_2PO_4{\cdot}H_2O$) using slow-drip Nalgene bottles. Bottles dripped nutrients in channels over 3-day periods, were replaced with new bottles when empty and yielded loading rates of 530.9 mg N m^{-2} day^{-1} and 21.8 mg P m^{-2} day^{-1} (N:P molar = 54 : 1) over the course of experiment. The experiment began with the introduction of catfish and nutrients and lasted for 77 days.

Forty-watt full-spectrum fluorescent lights (light intensity *c.* 150 μmol cm^{-2}) were hung *c.* 0.5 m above channels, and lighting was provided 16 : 8 light:dark cycle. Substrata consisting of sand, pebble, gravel and cobble in proportions similar to the San Marcos River (50% cobble, 50% sand, pebbles and gravel) were added to each channel. Rocks were collected from the San Marcos River each week for 21 days prior to the start of the experiment and scrubbed for periphyton. The scrubbed rocks were distributed among channels. Equal aliquots of the periphyton slurry were added to channels to facilitate periphyton colonisation. Aquatic macroinvertebrates were collected weekly for 21 days prior to the experiment using a kick net from the upper portion of the San Marcos River and placed in a bucket of river water. Bucket contents were then equally divided and distributed among channels. Because macroinvertebrates were allowed to 'drift' downstream and out of channels, this procedure was repeated weekly.

Unglazed ceramic tiles (15.2 × 15.2 cm) were added to channels to measure periphyton biomass, benthic organic matter (OM) and benthic inorganic matter (IM). To assess indirect effects of each factor (catfish and nutrients), half of the tiles were placed in 2-cm aperture wire mesh cages; each channel received four uncaged and four caged tiles. One caged and one uncaged tile were removed from each stream channel after 21, 42, 63 and 77 days. Removed tiles were scrubbed with a nylon brush and rinsed with Milli-Q H_2O into acid-washed plastic cups. A portion of the slurry was filtered onto Pall A/E filters to determine periphyton biomass as chlorophyll *a* (chl *a*). Filters were frozen until chl *a* was extracted with acetone for a minimum of 4 h in the dark and measured on a Turner Designs Trilogy fluorometer. OM and IM on tiles were determined by filtering a portion of the slurry onto pre-ashed and pre-weighed Pall A/E filters. Filters were dried at 60 °C for 48 h, weighed, ashed at 450 °C and weighed to calculate OM and IM concentrations (mg cm^{-2}). Slurry subsamples were filtered on to pre-ashed Whatman GF/F filters to determine C and N (Flash EA 1112 Series NC Soil Analyzer) and P concentration (HCl digestion followed by the molybdenum blue method) (American Public Health Association, 1992; Wetzel & Likens, 2000) on tiles.

Leaf litter breakdown rate was assessed by adding 12 leaf packs to each channel 21 days prior to beginning the experiment. Leaf packs consisted of 4.4 ± 0.88 g ($\bar{x} \pm$ SE) of sycamore (*Plantanus* sp.) leaves tied together at the petiole with monofilament fishing line. All leaf packs were pre-weighed and numbered with a plastic tag. To assess indirect effects of each factor (catfish and nutrients), half of the leaf packs were enclosed in 3-mm aperture plastic mesh, so they were inaccessible to catfish (bagged). Leaf packs were weighted to submerge leaves. Two packs (one open and one bagged) were removed from each channel at days 21, 35, 49, 63 and 77. Packs were removed using a 25-μm sieve and the material rinsed with Milli-Q H_2O into a plastic bag and placed on ice until analysis. Leaf packs were pulled apart and rinsed with Milli-Q H_2O to remove fine materials and organisms. Leaf material was dried at 60 °C for 7 day and weighed to determine per cent mass lost from each leaf pack. Material from each pack was

homogenised and analysed for C, N and P using the methods described for periphyton.

To assess effects on nutrient limitation of periphyton, we used nutrient-diffusing substrata (NDS) (Francoeur *et al.*, 1999; Flecker *et al.*, 2002) on days 28 and 63 of the experiment. NDS were constructed using 20 mL plastic vials filled with nutrient-amended agar and topped with Pall A/E filters. Filters were held in place by caps with holes drilled through the tops. Each channel received two replicates of each nutrient-amended NDS treatment (+N or +P) and two replicates of a non-amended (control) NDS treatment. Agar (23 g L^{-1} H_2O) was infused with KNO_3 for N and Na_2HPO_4 for P enrichment (final nutrient molarity was 0.5 M for both N and P) (Tank, Bernot & Rosi-Marshall, 2006). We did not include an N+P treatment because N concentrations in the stream water were relatively high (800–1500 μg NO_3^-N L^{-1}), and we were primarily interested in examining the limitation status of each individual nutrients (especially P) in response to experimental treatments and not potential co-limitation or sequential limitation responses. After incubating in stream channels for 14–18 days, NDS were removed and filters were frozen until analysis for chl *a*, as previously described. For each stream channel, the chl *a* responses of each nutrient-amendment treatment (no nutrients, +N, +P) were averaged, and the responses to nutrient amendment were determined by calculating the response ratio as *ln* (chl a_E/chl a_C), where chl a_E is the chl *a* concentration of the nutrient-enriched NDS and chl a_C is the chl *a* concentration of the unenriched NDS (Gough, Osenberg & Gross, 2000).

At the end of the experiment, catfish were caught using dip nets, and wet weights (g) were recorded. We quantified nutrient recycling rates and body nutrient composition of catfish using method of Schaus *et al.* (1997). Animals were placed in separate acid-washed plastic containers containing 2 L of filtered stream water. After 1 h, water was filtered through Pall A/E filters and analysed for NH_4^+-N and PO_4^{3-}-P using the phenate method (Solorzano, 1969) and the molybdenum blue method (Wetzel & Likens, 2000), respectively. Excretion rates were calculated as the change in NH_4^+-N or PO_4^{3-}-P per unit wet mass of individual catfish per unit time (μmol g wet weight^{-1} h^{-1}). To quantify body nutrient content, fish were immediately pithed after excretion trials, dried at 60 °C and ground to a fine powder. Samples were analysed for C and N as described for periphyton slurries. We also assessed condition by determining lipid content (Arrington *et al.*, 2006). Lipid content of catfish was estimated following Folch, Lees & Sloane-Stanley (1957) and Post & Parkinson (2001). A 0.5 ± 0.0001 g sample of dried, homogenised tissue was weighed and placed in a 30-mL screw-top test tube, and 8 mL of chloroform and 8 mL of methanol were added. The mixture was heated to boiling in a 61 °C water bath. Test tubes were removed, cooled to room temperature, and the liquid was decanted and brought to 25 mL with chloroform. This volume was filtered through No. 1 Whatman filter paper into a 125-mL separatory funnel, followed with 10 mL of 0.9% saline solution, and the entire mixture shaken and allowed to separate. The bottom liquid layer was drained into a pre-weighed aluminium weigh boat. Dish contents were evaporated at 70 °C, allowed to cool to room temperature and weighed to the nearest 0.0001 g on a Mettler Toledo MS104S analytical balance.

Data analysis

To assess the main effects of each factor (catfish or nutrients) and their interaction on chl *a* concentration for open and caged tiles, leaf litter decomposition for open and bagged leaf packs and C:N, C:P and N:P of periphyton and leaf litter over the course of the experiment, we used a two-way repeated measures ANOVA. Because periphyton nutrient limitation was assessed only on two dates, a separate two-way ANOVA was performed for each date comparing responses to each nutrient amendment for all treatment combinations. Catfish lipid content, mass-specific excretion rates and catfish elemental composition at the end of the experiment were averaged by channel because the 'channel' was considered the unit of replication. Nutrient excretion rates, body nutrient ratios (C:N, C:P and N:P) and per cent lipid of catfish were analysed using paired *t*-tests to compare fish in channels with versus without nutrient enrichment. All statistical analyses were performed with R software (R Core Team, 2012). Statistical significance for all analyses was inferred at $P \leq 0.05$.

Results

The presence of catfish significantly reduced periphyton biomass on open tiles (Fig. 1a; Table 1), but nutrient enrichment did not significantly affect periphyton biomass. However, there was a significant nutrient × time interaction, indicating that chl *a* on open tiles varied significantly through time under nutrient-enriched conditions. Neither catfish nor enrichment significantly affected periphyton biomass of caged tiles (Fig. 1b; Table 1). The presence of catfish significantly decreased IM on open tiles (Fig. 1c; Table 1) and increased IM on

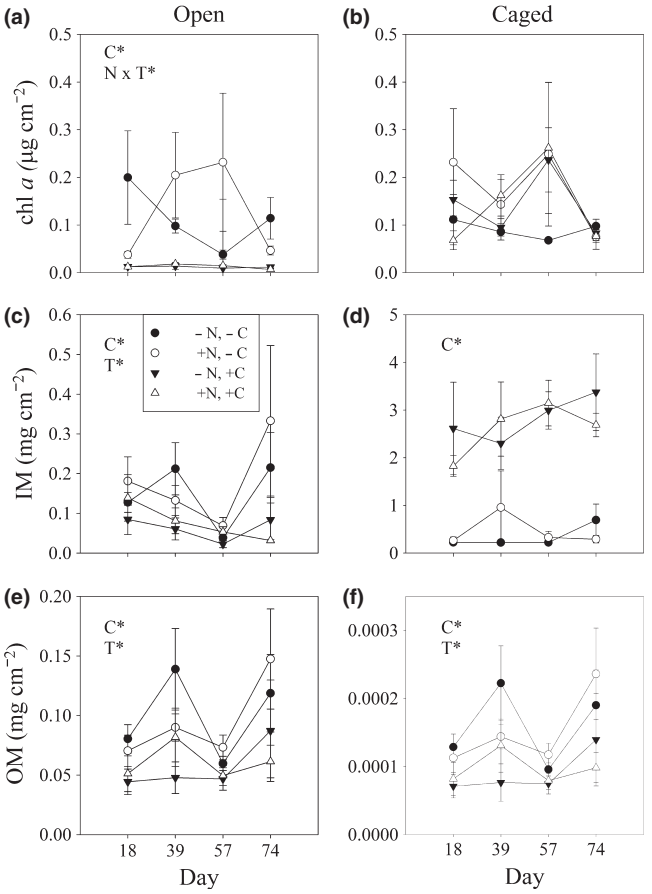

Fig. 1 Responses of periphyton biomass (chl *a*), inorganic matter (IM) and organic matter (OM) on open (a, c, and e) tiles (accessible to catfish) and caged tiles (b, d and f) (inaccessible to catfish). Only significant effects of each factor and interaction term are indicated on the graphs for each response variable (C = catfish, N = nutrients and T = time). Note differences in scale of the *y*-axes of panels. Error bars are ± 1 SE.

caged tiles (Fig. 1d; Table 1). In addition, both catfish and time significantly affected OM on open and caged tiles, with the presence of catfish decreasing the concentration of OM (Fig. 1e & f; Table 1). In contrast, enrichment had no effect on IM and OM on open and caged tiles.

Neither catfish nor enrichment significantly affected periphyton nutrient ratios (C:N, C:P or N:P) of open tiles (Fig. 2a, c & e; Table 1). Due to problems with analytical equipment, we excluded the first sampling date for periphyton C:N, C:P and N:P on caged tiles from analyses. We analysed the remaining three sampling dates and found that neither catfish nor nutrients significantly affected caged tile C:N or C:P (Fig. 2b & d; Table 1). However, the presence of catfish significantly reduced periphyton N:P on caged tiles (Fig. 2f; Table 1).

Both catfish and nutrients significantly increased the rate of leaf litter decomposition in open leaf packs (Fig. 3a; Table 2). In addition, a significant catfish × time interaction effect was detected, indicating that leaf litter mass initially did not differ among treatments with and without catfish, but the difference among treatments increased over time. Neither catfish nor nutrients significantly affected mass loss of bagged leaf packs (Fig. 3b; Table 2).

Catfish significantly increased C:N of open leaf packs (Fig. 4a; Table 2), but did not have a significant effect on any other leaf pack nutrient ratios, regardless of whether packs were bagged or open (Fig. 4b–f; Table 2). In contrast, nutrient enrichment led to significantly lower leaf litter C:P and N:P in both bagged and open leaf packs, indicating enrichment of leaf litter in P (Fig. 4a–f; Table 2).

Periphyton growth responses on NDS on Day 28 indicated that amendments of both N and P reduced periphyton growth relative to controls (Fig. 5a & b; Table 3). Nutrient enrichment exacerbated the inhibition of N on periphyton growth on NDS (i.e. a greater inhibition of periphyton growth responses to N in stream channels receiving nutrient additions). In contrast, the presence of catfish lessened the inhibition of P-amended periphyton growth. By Day 63, N-enrichment again reduced periphyton growth response on N-enriched substrata for all treatments, but there were no significant treatment effects (Fig. 5a). However, the presence of catfish led to significant P-limitation of periphyton (Fig. 5b).

At the end of the experiment, the mass-specific NH_4^+ excretion rate of catfish in stream channels with and without nutrient additions did not differ significantly (Fig. 6a; Table 4). However, catfish from enriched channels exhibited significantly lower mass-specific P excretion rates (Fig. 6b). At the end of the experiment, catfish lipid content and body nutrient ratios (C:N, C:P and N:P) did not differ significantly between nutrient treatments. In all stream channels, catfish body lipid content, C:N, C:P and N:P (molar) were on average *c.* 6%, 4 : 1, 80 : 1 and 19 : 1, respectively.

Discussion

In our stream channel experiment, the presence of catfish generally affected more ecosystem components than did nutrient enrichment. In addition, catfish more strongly influenced the 'green' portions of the food web (i.e. periphyton-based components), while nutrient enrichment largely affected detrital or 'brown' ecosystem components. Although both the presence of catfish and

Table 1 Summary statistics from repeated measures two-way ANOVA examining the effects of catfish and nutrients on chl *a*, inorganic matter (IM), organic matter (OM) and nutrient ratios on open tiles and tiles enclosed in cages in the stream channel experiment

		chl *a*		IM		OM		C:N		C:P		N:P	
Tile Type	Effect	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Open Tiles	Date	2.571	0.066	4.324	**0.010**	3.842	0.016	4.971	**0.005**	2.038	0.124	5.220	**0.004**
	Catfish	93.108	**<0.001**	18.219	**0.001**	21.327	**<0.001**	0.112	0.742	1.096	0.311	0.914	0.353
	Nutrients	0.254	0.621	0.827	0.377	0.156	0.698	0.714	0.411	0.296	0.594	0.871	0.365
	Date × catfish	0.261	0.853	1.273	0.296	0.376	0.771	1.280	0.295	0.464	0.709	0.258	0.855
	Date × nutrients	4.997	**0.005**	0.965	0.418	0.091	0.965	0.277	0.842	0.219	0.883	0.320	0.811
	Catfish × nutrients	0.145	0.709	0.417	0.528	0.434	0.520	0.001	0.980	0.583	0.456	0.402	0.535
	Date × catfish × nutrients	1.671	0.187	0.834	0.483	1.221	0.314	0.072	0.975	0.604	0.616	0.498	0.686
Caged Tiles	Date	2.118	0.111	1.075	0.3687	6.244	**0.001**	10.274	**<0.001**	0.921	0.409	1.883	0.170
	Catfish	0.989	0.335	53.415	**<0.001**	177.861	**<0.001**	1.670	0.215	2.129	0.164	16.742	**0.001**
	Nutrients	0.253	0.622	0.003	0.959	0.672	0.425	0.008	0.931	1.059	0.319	3.358	0.086
	Date × catfish	1.147	0.340	0.861	0.468	1.185	0.326	0.440	0.649	2.721	0.083	1.064	0.358
	Date × nutrients	0.983	0.409	1.485	0.2308	0.892	0.453	0.027	0.974	1.672	0.206	1.938	0.162
	Catfish × nutrients	0.785	0.389	0.206	0.6561	0.360	0.557	0.253	0.622	0.813	0.381	1.536	0.233
	Date × catfish × nutrients	1.282	0.292	0.141	0.935	0.324	0.808	0.199	0.821	1.569	0.225	0.976	0.389

Bold *P*-values indicate significance at the $P \leq 0.05$ level.

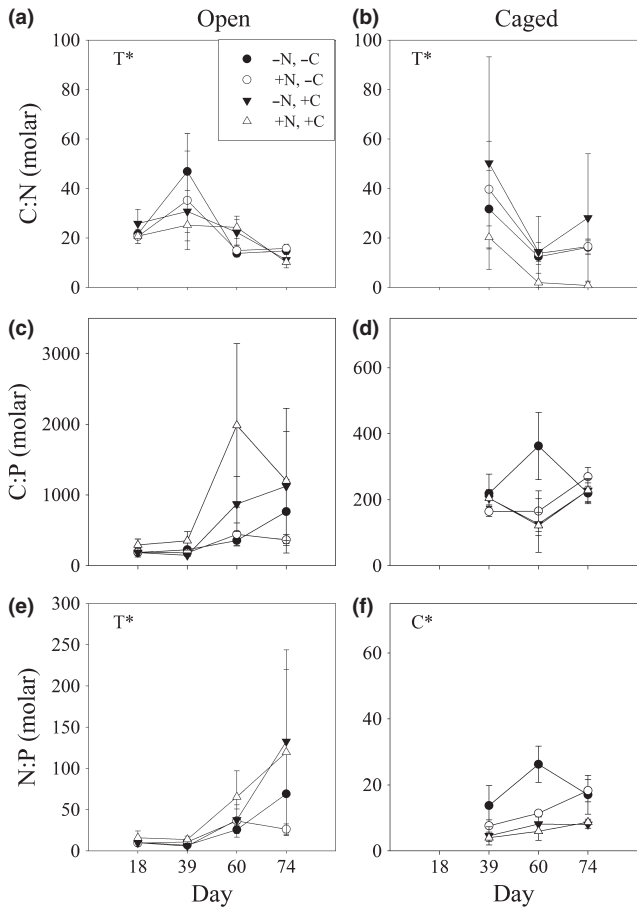

Fig. 2 Responses of periphyton nutrient ratios (C:N, C:P and N:P) on open (a, c, e) and caged (b, d, f) tiles. Figure symbols as in Fig. 1. Only significant effects of each factor and interaction term are indicated on the graphs for each response variable (C = catfish, N = nutrients, and T = time). Note differences in scale of the *y*-axes of panels. Error bars are ± 1 SE.

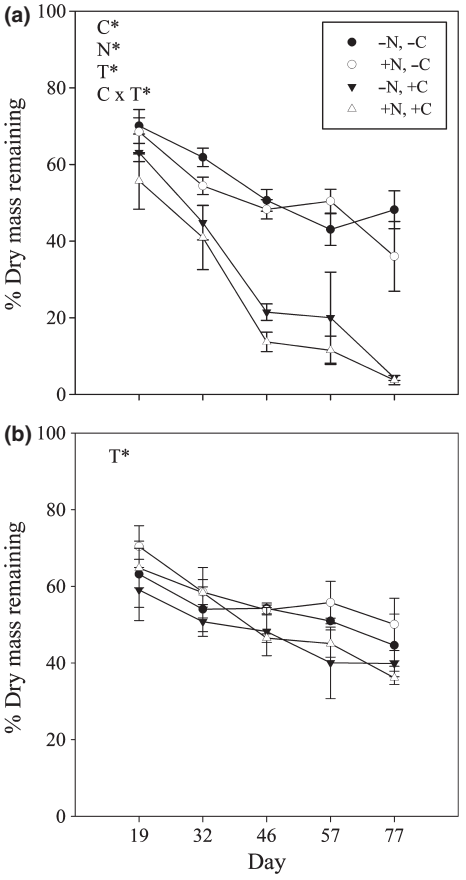

Fig. 3 Responses of leaf litter decay to armoured catfish and nutrients. Per cent mass remaining of (a) open and (b) bagged leaf litter. Only significant effects of each factor and interaction term are indicated on the graphs for each response variable (C = catfish, N = nutrients and T = time). Error bars are ± 1 SE.

Table 2 Summary statistics from repeated measures two-way ANOVA examining the effects of catfish and nutrients on per cent mass remaining and nutrient ratios of open leaf packs and bagged leaf packs in the stream channel experiment

		% Mass Remaining		C:N		C:P		N:P	
Leaf pack type	Effect	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Open	Date	38.637	**<0.001**	2.819	**0.033**	6.380	**<0.001**	7.701	**<0.001**
	Catfish	114.010	**<0.001**	6.492	**0.022**	1.050	0.321	0.171	0.684
	Nutrients	4.452	**0.051**	2.807	0.113	7.896	**0.013**	7.602	**0.014**
	Date × Catfish	5.111	**0.001**	5.165	**0.001**	0.758	0.557	0.701	0.594
	Date × Nutrients	0.068	0.991	0.848	0.500	0.265	0.899	0.366	0.832
	Catfish × Nutrients	0.456	0.509	1.488	0.240	0.540	0.473	0.179	0.678
	Date × Catfish × Nutrients	1.325	0.272	0.598	0.666	0.545	0.704	0.496	0.739
Bagged	Date	5.819	**0.001**	1.826	0.135	14.355	**<0.001**	14.522	**<0.001**
	Catfish	4.001	0.063	1.758	0.204	1.346	0.263	0.220	0.645
	Nutrients	0.576	0.459	0.174	0.682	9.050	**0.008**	6.844	**0.019**
	Date × Catfish	0.324	0.861	1.302	0.280	1.155	0.340	0.794	0.534
	Date × Nutrients	0.703	0.593	0.025	0.999	1.366	0.256	1.332	0.268
	Catfish × Nutrients	0.630	0.439	0.009	0.924	0.566	0.463	0.506	0.487
	Date × Catfish × Nutrients	0.644	0.633	0.094	0.984	0.812	0.522	0.449	0.773

Bold *P*-values indicate significance at the $P \leq 0.05$ level.

nutrients affected multiple ecosystem properties and processes, we found little evidence of an interaction between these two factors.

The presence of catfish had both direct and indirect effects on multiple ecosystem characteristics which influence resource availability and habitat structure. Catfish significantly decreased periphyton, IM and OM on tiles they could directly access and increased the amount of IM and OM on caged tiles. Catfish consume periphyton and their foraging and movements can redistribute sediments (Power, 1984; Scott *et al.*, 2012), and our results suggest that catfish were actively grazing periphyton and removing OM and IM on open tiles and redepositing OM and IM onto caged tiles. Catfish also led to significantly greater losses of leaf litter mass in open leaf packs. Previous studies have also found that benthic grazing fish can increase fragmentation and mass loss of leaf litter by directly grazing on litter-associated biofilms and/or through their movement on sediments (Evans-White, Dodds & Whiles, 2003; Scott *et al.*, 2012). However, catfish did not affect rates of litter decomposition or nutrient ratios of bagged leaf litter, indicating that indirect effects of catfish excretion had little impact on the detrital components of the stream channel food web.

Contrary to our initial predictions, catfish did not significantly affect periphyton nutrient stoichiometry of open tiles, but decreased periphyton N:P on closed tiles, indicating P enrichment of periphyton. The lack of effects on grazer-accessible substrata differs from a number of other studies. For example, Scott *et al.* (2012) found that armoured catfish reduced periphyton C:P, C:N and N:P on benthic substrata which catfish could directly access. Stoichiometric theory predicts that catfish, with their relatively high body P content, excrete dissolved nutrients at relatively high N:P, leading to depleted periphyton P content and increased periphyton N:P (Sterner, 1990; Vanni *et al.*, 2002; Knoll *et al.*, 2009). Our current study does not agree with predictions from stoichiometric theory, and Hillebrand, Frost & Liess (2008), in a meta-analysis of 119 experiments, similarly found that the presence of grazers with high body P content generally increased P content. Although this general trend across experiments contradicts stoichiometric predictions, Hillebrand *et al.* (2008) hypothesised that it may be the result of a complex but poorly understood interaction between grazer growth rates, flexibility in grazer body P content and P requirements for grazer growth.

The presence of catfish had limited effect on periphyton stoichiometry, but affected the severity of periphyton P-limitation and this effect intensified as the experiment progressed. Although periphyton growth was inhibited on P-enriched NDS across all treatments on the first sampling date, periphyton growth responses on P-enriched NDS were less negative in the presence of catfish. By the second sampling date, however, the presence of catfish led to a positive growth response of periphyton communities on P-enriched NDS, indicating P-limitation of periphyton. These findings generally agree with stoichiometric predictions, but are not consistent with our periphyton nutrient composition results (i.e. little to no effect on

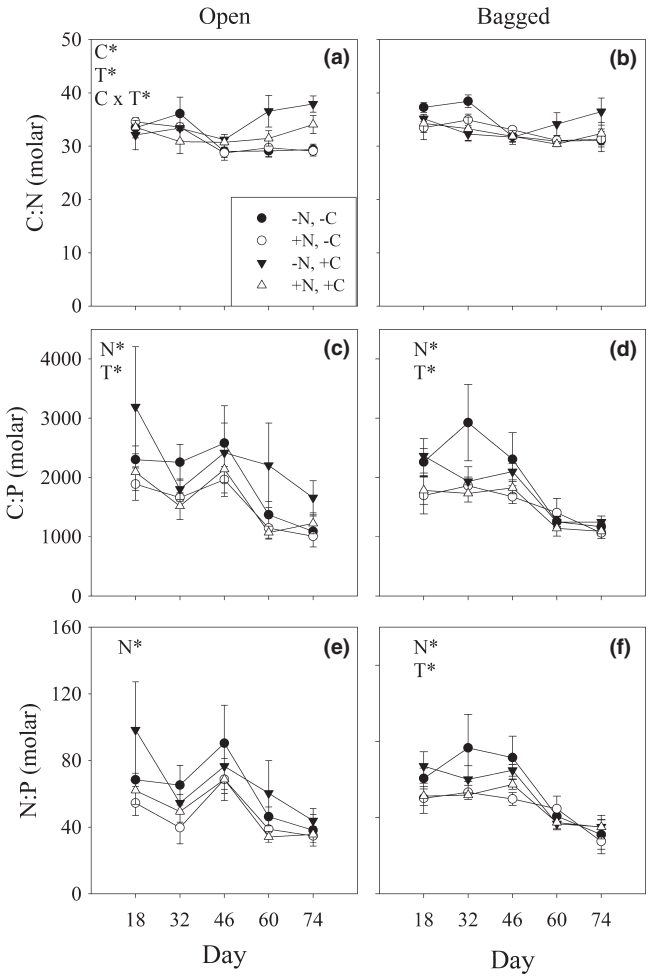

Fig. 4 Responses of leaf litter nutrient ratios to armoured catfish and nutrient enrichment. Leaf litter C:N, C:P and N:P in open (a, c, e) and bagged (b, d, f) leaf packs over the experimental period. Symbols as in Fig. 3. Only significant effects of each factor and interaction term are indicated on the graphs for each response variable (C = catfish, N = nutrients and T = time). Error bars are ± 1 SE.

periphyton P content). Although there was no response of periphyton C:P to the presence of armoured catfish, periphyton C:P was >200 : 1 across all treatments, suggesting P-limitation of periphyton (Hillebrand & Sommer, 1999). It is possible that catfish sequestered enough P over the course of the experiment to exacerbate periphyton P-limitation. Indeed, variation in periphyton stoichiometry within replicates of the same treatment was relatively high, which may have additionally obscured any relationship between periphyton C:P and P-limitation status. Alternatively, differences between tile periphyton C:P and growth responses of periphyton on NDS may be due to differences in the algal taxa that colonised these different substratum types, ceramic tiles versus glass-fibre filters (Von Schiller *et al.*, 2007). However, the likelihood of this scenario is unknown because we did not assess differences in algal species composition between substratum types.

In contrast to the effects of catfish, nutrient enrichment in the stream channel experiment did not change the biomass or nutrient composition of periphyton. However, it did affect the detrital portion of the food web by increasing leaf litter decomposition rates and changing leaf litter stoichiometry. These results indicate that decomposition rates of terrestrial detritus in the stream channels were likely nutrient limited (Cross, Wallace & Rosemond, 2007) and that periphyton production was not as constrained by inorganic nutrient availability. We also observed that nutrient enrichment significantly decreased C:P and N:P of bagged and open leaf packs. Increased P content of leaf litter in stream channels receiving nutrient enrichment was likely associated with increased litter-associated microbial biomass and production. In detrital-based systems, nutrient enrichment can reduce stoichiometric constraints on organic matter use, leading to greater incorporation of these energy sources into food webs (Cross *et al.*, 2007). The San Marcos River food web depends largely on algal-based C (Pound *et al.*, 2011), but the present study suggests that nutrient enrichment of the river may increase decomposition and incorporation of terrestrial detritus into the river food web.

There was no evidence of an interaction between the effects of catfish and nutrient additions on ecosystem properties. Other studies have found that the magnitude of effects of herbivorous and benthivorous fish depends on nutrients (Drenner *et al.*, 1998; Flecker *et al.*, 2002; Evans-White & Lamberti, 2006). However, meta-analyses of experiments examining separate and interactive effects of nutrients and grazers on primary producers have found little support for a consistent interaction between these two factors (Hillebrand, 2002; Gruner *et al.*, 2008). Although primary producers can be controlled both by nutrient supply and grazers (Hillebrand *et al.*, 2002; Gruner *et al.*, 2008), the effects of each of these factors on primary producers can differ both spatially and temporally, limiting the potential for interactive effects (Hillebrand, 2002). In the present study, treatments receiving catfish were always exposed to catfish, but nutrient additions were made with slow-drip bottles over 3-day intervals. Thus, the addition of nutrients was more pulsed than the presence of catfish, leading to the impacts of enrichment to be more temporally variable than the effects of catfish. In addition, periphyton assemblages may have experienced spatial differ-

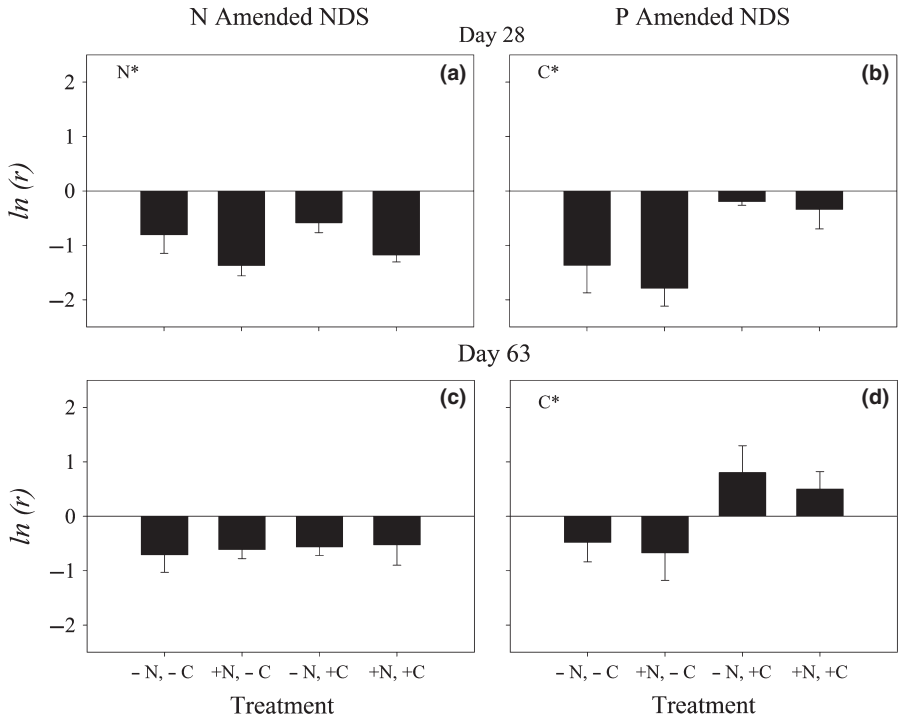

Fig. 5 Responses of periphyton growing on NDS on agar amended with N (a, c) or P (b, d), relative to control agar (no added nutrients). Only significant effects of each factor and interaction term are indicated on the graphs for each response variable (C = catfish, N = nutrients). Error bars are +1 SE.

Table 3 Summary statistics from two-way ANOVAs examining the effects of catfish and nutrients on periphyton nutrient deficiency (Note: dates were analysed with separate ANOVAs; see Methods)

NDS Amendment	Effect	$F_{1, 16}$	P
Day 28			
N	Nutrients	6.576	**0.021**
	Catfish	0.849	0.371
	Nutrients × Catfish	0.004	0.948
P	Nutrients	0.646	0.433
	Catfish	13.753	**0.002**
	Nutrients × Catfish	0.153	0.701
Day 63			
N	Nutrients	0.063	0.806
	Catfish	0.180	0.677
	Nutrients × Catfish	0.012	0.914
P	Nutrients	0.336	0.570
	Catfish	8.276	**0.011**
	Nutrients × Catfish	0.017	0.898

Bold *P*-values indicate significance at the $P \leq 0.05$ level.

ences in grazing pressure, leading to a lack of interaction between periphyton and grazers (Steinman, 1996; Hillebrand, 2002). It is possible that catfish did not consistently occupy and graze from all areas equally within channels, leading to spatial variability in their effects within each channel.

We saw no indication that nutrient enrichment led to greater growth and fitness of catfish. At the end of the experiment, catfish lost weight in both enriched and unenriched stream channels and catfish lipid and

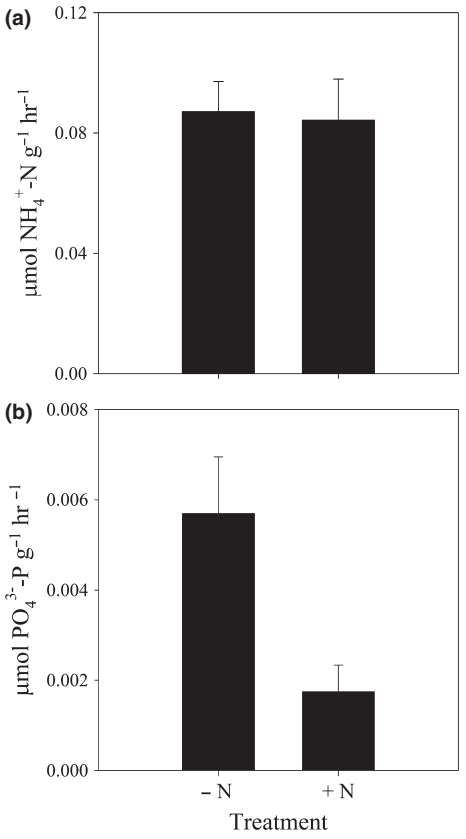

Fig. 6 Mass-specific excretion rates of catfish at the end of the stream channel experiment in unenriched (−N) and enriched (+N) stream channels. (a) Mass-specific NH_4^+-N excretion rates and (b) mass-specific PO_4^{3-} - P excretion rates. Error bars are + 1 SE.

Table 4 Summary statistics from *t*-tests examining the effects of nutrient enrichment on catfish mass-specific NH_4^+ and PO_4^{3-} excretion rates, per cent lipid content and body nutrient ratios (C:N, C:P, and N:P) at the end of the stream channel experiment

Response	*t*	*P*
NH_4^+ excretion rate	−0.132	0.900
PO_4^{3-} excretion rate	−2.642	**0.036**
% lipid (whole body)	1.345	0.217
C:N (whole body)	1.170	0.294
C:P (whole body)	0.444	0.669
N:P (whole body)	0.224	0.828

Bold *P*-values indicate significance at the $P \leq 0.05$ level.

nutrient content did not differ between enriched and unenriched stream channels. However, catfish in nutrient-enriched channels exhibited significantly lower mass-specific P excretion rates than catfish in unenriched stream channels. Decreased P excretion by catfish in enriched channels may be due to increased allocation of P to growth (e.g. Liess & Hillebrand, 2006). Thus, it is possible that fish in enriched channels allocated more P to bone maintenance and growth (albeit not much) than catfish in unenriched channels, leading to significantly lower mass-specific P excretion rates.

Introduction of NNIS and the modification or degradation of habitats affects ecosystems globally (Vitousek *et al*., 1997; Malmqvist & Rundle, 2002). Increased nutrient loading may contribute to the successful invasion and establishment of NNIS (Vitousek *et al*., 1997; Davis *et al*., 2000; Romanuk & Kolasa, 2005). Results from our study lend support to a growing body of evidence that, although nutrients and grazers are both fundamentally important in structuring primary producer communities, the interaction of these two factors is less common than once thought (Hillebrand, 2002; Gruner *et al*., 2008). In the present study, nutrient enrichment effects were largely limited to the detrital portion of the food web, but the effects of invasive catfish were more far-reaching, affecting periphyton biomass, benthic sediment distribution, rates of terrestrial OM decomposition and the nutrient limitation status of periphyton. Our results suggest that invasion of the San Marcos River ecosystem by catfish has the potential for a greater impact than nutrient enrichment on ecosystem processes and that their impacts on ecosystem functioning are largely independent of nutrient loading.

Acknowledgments

We would like to thank Chad Thomas, Susanna Scott, Pete Diaz, John Bryant, Danielle Livingston, Robby Maxwell, Jesse Becker, Chekka Lash, Clara Folb, Josh Perkin, Kristy Kollaus and Nathan Dammeyer for assistance in the field and in the laboratory. Funding for this project was provided to YZ and WHN by a Texas Parks and Wildlife State Wildlife Grant.

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(Manuscript accepted 13 October 2014)