

Long-term changes in structure and function of a tropical headwater stream following a disease-driven amphibian decline

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SUMMARY

1. Taxonomic and functional diversity in freshwater habitats is rapidly declining, but we know little about how such declines will ultimately affect ecosystems. Neotropical streams are currently experiencing massive losses of amphibians, with many losses linked to the chytrid fungus, *Batrachochytrium dendrobatidis* (*Bd*).
2. We examined the ecological consequences of the disease-driven loss of amphibians from a Panamanian stream. We quantified basal resources, macroinvertebrates, N uptake and fluxes through food-web components and ecosystem metabolism in 2012 and 2014 and compared them to pre-decline (2006) and 2 year post-decline (2008) values from a prior study.
3. Epilithon biomass accrued after the decline, more than doubling between 2006 and 2012, but then decreased fivefold from 2012 to 2014. In contrast, suspended particulate organic matter (SPOM) concentrations declined continuously after the amphibian decline through 2014.
4. Biomass of filter-feeding, grazing and shredding macroinvertebrates decreased from 2006 to 2014, while collector–gatherers increased during the same time period. Macroinvertebrate taxa richness decreased from 2006 (52 taxa) to 2012 (30 taxa), with a subsequent increase to 51 taxa in 2014.
5. Community respiration, which initially decreased after the amphibian decline, remained lower than pre-decline in 2012 but was greater than pre-decline values in 2014. Gross primary production remained low and similar among years, while NH_4^+ uptake length in both 2012 and 2014 was longer than pre-decline. Nitrogen flux to epilithon increased after the decline and continued to do so through 2014, but N fluxes to fine particulate organic matter and SPOM decreased and remained low.
6. Our findings underscore the importance of studying the ecological consequences of declining biodiversity in natural systems over relatively long time periods. There was no evidence of functional

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redundancy or compensation by other taxa after the loss of amphibians, even after 8 years.

Keywords: conservation/biodiversity, food webs, invertebrates, nutrient cycling, running water/rivers/streams

Introduction

Biodiversity on Earth is rapidly declining, with current extinction rates approaching those of the five known mass extinction events (Barnosky *et al.*, 2011). While the ultimate consequences of declining biodiversity, including the loss of functional groups and decreases in the abundance of previously common taxa (Vaughn, 2010), remain to be seen, there is growing evidence that declining biodiversity leads to significant changes in ecosystems, in particular the efficiency at which ecosystems process essential resources such as limiting nutrients (Hooper *et al.*, 2012). However, questions of how experimental studies of biodiversity and ecosystem function scale, both temporally and spatially, remain unanswered (Petchey *et al.*, 2004; Duffy, 2009).

Biodiversity is declining most quickly in freshwater habitats. Extinction rates in freshwater habitats are *c.* 5x faster than in terrestrial habitats, with extinctions proceeding at the rate of *c.* 4% per decade (Dudgeon *et al.*, 2006; Vaughn, 2010). These losses are driven by multiple stressors, including habitat loss and degradation, pollution and climate change (Dudgeon, 2010; Vaughn, 2010). While declining biodiversity alters freshwater ecosystem structure and function in experimental studies (Jonsson & Malmqvist, 2000; McIntyre *et al.*, 2007; Kominoski & Pringle, 2009), less is known about the consequences of these losses from natural settings. Whole-ecosystem experiments are particularly important in understanding true responses to many perturbations (Carpenter, 1989; Schindler, 1998; Taylor, Flecker & Hall, 2006).

Neotropical headwater streams are undergoing catastrophic amphibian declines associated with the infectious chytrid fungus, *Batrachochytrium dendrobatidis* (*Bd*) (Lips *et al.*, 2006; Whiles *et al.*, 2006). This pathogenic fungus has moved through Central America in a predictable, wave-like fashion (Lips *et al.*, 2006; Cheng *et al.*, 2011). This predictability allowed for comparisons of ecosystem structure and function before and after amphibian declines (see Table 1 for an overview). Small-scale manipulations (e.g. Flecker, Feifarek & Taylor, 1999; Ranvestel *et al.*, 2004; Rugenski, Múrria & Whiles, 2012) and larger-scale, but relatively short-term examina-

tions (e.g. Colón-Gaud *et al.*, 2010a,b; Whiles *et al.*, 2013), suggest that stream-dwelling amphibians are important components of Neotropical food webs and their losses can have ecosystem-level consequences. However, long-term effects (e.g. >2 years) are poorly known (but see Connelly *et al.*, 2014).

We built on prior studies of the Tropical Amphibian Declines in Streams (TADS) project by examining long-term ecological responses to an amphibian decline (occurring in late 2006) in a tropical headwater stream. Previous studies in this stream documented changes in benthic organic matter, algal resources and macroinvertebrates, along with changes in nutrient cycling and stream metabolism 2 years after the decline (Barnum *et al.*, 2013; Whiles *et al.*, 2013). Furthermore, these prior studies found no evidence for compensation or functional redundancy by other groups (e.g. increases in algal grazing macroinvertebrates in response to loss of tadpoles and increases in algal biomass).

Given some documented time lags in ecosystem responses following disturbances (e.g. Lindenmayer *et al.*, 2010; Sponseller *et al.*, 2010; Dodds *et al.*, 2012), our objective was to quantify the same elements of ecosystem structure and function 6 and 8 years after the decline to assess whether changes were still occurring, if the ecosystem had stabilised over time or if any recovery had occurred. We hypothesised that tadpole populations would fail to recover to pre-decline levels and that their ongoing absence would have continued effects. Specifically, we predicted that epilithon and fine particulate organic matter (FPOM) mass would continue to increase, suspended particulate organic matter (SPOM) concentrations would continue to decrease and there would be no compensation for the loss of tadpoles by other taxonomic groups. We also predicted that reach-scale respiration would continue to decrease, whereas gross primary production (GPP) and NH_4^+ spiralling length would continue to increase with time since the amphibian decline. Although previous studies did not closely examine macroinvertebrate community structure, we used changes in basal food resources to predict macroinvertebrate responses. Specifically, we predicted that the biomasses of filter-feeding macroinvertebrates would decrease, collector-gatherer and

Table 1 Studies of the effects of amphibian declines on stream ecosystem structure and function in Panamanian streams

Study	Major findings
Barnum <i>et al.</i> (2013)	Used stable isotopes to investigate isotopic feeding niches of grazers after an amphibian decline. Although some grazing insect taxa shifted isotopic niches, none filled the niche of tadpoles.
Colón-Gaud <i>et al.</i> (2010a)	Estimated secondary production and consumption of grazing insects and tadpoles for 2 years including a year before and during an amphibian decline. They found that some insect grazers responded with increased production and consumption, while others declined or showed no response.
Colón-Gaud <i>et al.</i> (2010b)	Quantified secondary production and community assemblage of stream macroinvertebrates in two streams for 2 years. The tadpole decline began in one stream during the second year of the study. They found production was lower in year 2 in the stream where amphibians declined, especially production of shredding invertebrates. Patterns of abundance and production were linked to availability of basal resources.
Connelly <i>et al.</i> (2008)	Experimentally excluded tadpoles from substrata in two streams, one with tadpoles and one without. They found that the stream with tadpoles had higher levels of benthic chlorophyll- <i>a</i> , organic matter and inorganic sediment, and lower levels of ash-free dry mass and chlorophyll- <i>a</i> weighted net primary production (NPP) in the exclusion treatments. The stream without tadpoles showed no differences between treatments. Following the experiment, the stream with tadpoles experienced disease-driven decline in tadpoles and subsequent increases in benthic chlorophyll- <i>a</i> , organic matter, inorganic sediment and reach-scale NPP.
Connelly <i>et al.</i> (2011)	Examined the effects of tadpoles on leaf litter decomposition by experimentally excluding tadpoles from leaf packs in two streams (one with tadpoles, one without tadpoles). They found no difference in breakdown rates between streams or between treatments.
Connelly <i>et al.</i> (2014)	Quantified the effects of a tadpole decline by measuring algal biomass, benthic biofilm biomass, benthic chlorophyll- <i>a</i> concentrations, algal food quality, stable isotope ratios of basal resources and production of grazing invertebrates using a long-term data set that included 2 years of pre-decline data, a period of tadpole decline and 3 years of post-decline data. They found that initial increases (first 5 months) of algal and benthic biofilm biomass were reduced after 3 years but were still higher than pre-decline levels. The $\delta^{15}\text{N}$ of biofilms in pool habitats was depleted 5 months post-decline and remained so 3 years later, indicating that tadpoles play a major role in N cycling in these streams when they are present.
Frauentorf <i>et al.</i> (2013)	Used the trophic basis of production approach to examine energy flow through a food web prior to an amphibian decline. They found that detrital food resources were heavily utilised and contributed significantly to energy flux in the system. Algal consumption was highest during the dry season, and omnivory was ubiquitous.
Ranvestel <i>et al.</i> (2004)	Used tadpole enclosures to experimentally examine the effects of tadpoles on algal and macroinvertebrate communities and sediment dynamics. They found that tadpoles decreased algal abundance and biovolume, total sediments and both organic and inorganic fractions of sediments on stream substrata. They also found that grazing mayflies preferred areas with tadpoles, suggesting tadpoles facilitate these small grazers.
Rugenski <i>et al.</i> (2012)	Used mesocosms to examine the effects of tadpoles, shredding invertebrates and a combination of the two on leaf litter breakdown rates. They found that leaf area loss was greatest when tadpoles and invertebrates were combined. They also found that respiration rates of leaves incubated with tadpoles and both tadpoles and invertebrates were greater than control treatments and tadpoles altered the C : N of leaf biofilm and particulate organic matter in the mesocosms. Their results indicate that tadpoles indirectly enhance leaf breakdown by influencing shredder feeding and microbial assemblages.
Whiles <i>et al.</i> (2013)	Examined the effects of amphibian declines on ecosystem function and macroinvertebrate communities by comparing pre-decline data to data collected 2 years after the decline. They found that fine particulate organic matter, epilithon biomass, gross primary production and NH_4^+ uptake length increased. Seston concentrations, N uptake rate and community respiration decreased. They found no changes in macroinvertebrate communities or evidence of functional redundancy to compensate for loss of grazing tadpoles in the system.

grazing macroinvertebrates would increase and shredders would show no change.

Methods

Site description

Río Maria is a high-gradient headwater stream in the eastern Cordillera Central of Panama near the town of El Valle (8°38'N, 80°02'W). The study reach is a 180-m reach of a 2nd-order stream, located c. 900 m

above sea level, in a heavily shaded wet-tropical forest. There are distinct wet and dry seasons in this region, with the dry season usually from January to mid-May.

We sampled in February of 2012 and 2014 during dry-season baseflow conditions and used data from February 2006 (pre-amphibian decline) and February 2008 (2 years post-decline) presented in Whiles *et al.* (2013) to examine long-term, post-decline patterns. During the 2006 sampling, c. 20 species of tadpoles were present in the stream. Shortly thereafter, a wave of *Bd* moved through the area,

largely decimating amphibian populations (Whiles *et al.*, 2013).

A small concrete dam (c. 2 m high) was constructed c. 23 m above the study reach in September 2008 by local developers. By February 2012, the small reservoir behind the dam had filled in with sediment, water was <0.5 m deep behind the dam and this small reservoir acted as a wide, shallow pool, with minimal apparent physical impact downstream.

Tadpole biomass

We sampled tadpole density and biomass in five haphazardly selected pools. We placed block nets at both the upstream and downstream ends of the pools and removed tadpoles from the pool using aquarium nets until depletion following the methods described in Whiles *et al.* (2013). We identified (to genus) and measured (total body length) each tadpole to the nearest mm. We estimated tadpole biomass (g dry mass [DM] m⁻²) using length–mass relationships developed following Benke *et al.* (1999). We measured area of pools to estimate areal biomass and abundance.

Macroinvertebrates

We sampled macroinvertebrates and benthic organic matter in five riffles and five pools/runs using a Surber sampler (0.0929 m², 250 µm mesh) and a stovepipe corer (0.0314 m²), respectively. We preserved samples in 8% formalin. Samples were divided into coarse (>1 mm) and fine (<1 mm and >250 µm) fractions. All macroinvertebrates were removed from coarse fractions; fine fractions containing large amounts of material were subsampled (1/2–1/8) using a Folsom plankton sample splitter. We identified macroinvertebrates to the lowest practical taxonomic level, usually genus (Chironomidae were identified as either Tanypodinae or non-Tanypodinae) using Merritt, Cummins & Berg (2008) and regional guides (Roldán Pérez, 1996; Springer, Ramírez & Hanson, 2010). We measured individuals to the nearest mm and used length–mass relationships to estimate biomass (Benke *et al.*, 1999).

Organic matter

We sorted organic matter from coarse fractions of benthic samples into leaf, wood, algae, macrophyte and miscellaneous coarse particulate organic matter (CPOM). Categories of CPOM and all organic matter from fine fractions (FPOM) were dried to constant mass at 50 °C, weighed, combusted at 500 °C and

reweighed to estimate dry mass (DM) (Wallace, Hutchens & Grubaugh, 2006).

We sampled sestonic organic matter by filtering samples ($n = 7$ in 2012 and $n = 8$ in 2014) of 6 L of stream water from the upstream end of the reach through pre-ashed 0.47-mm glass fibre filters (GFF, 0.7 µm retention, Whatman). We stored filters in a dry box until return to the laboratory, where they were dried at 50 °C to constant mass and processed in the same manner as CPOM and FPOM to estimate DM.

We sampled epilithon from the top surfaces of rocks to estimate biomass. We randomly chose 10 rocks (five in runs, five in pools/runs), taking care not to disturb material on the rock surfaces. We sampled the epilithon using a sampler modified after Loeb (1981). In 2012, we carefully removed rocks from the stream and scrubbed a 4-cm² area of the rock using a template and a wire brush and then rinsed the material into a sample container using stream water. We then filtered the slurry through a pre-ashed GFF and stored the filters in a dry box. Filters were processed as for other organic matter samples to estimate DM (Steinman, Lamberti & Leavitt, 2006).

Ecosystem metabolism

We used a one-station, open-channel method to measure ecosystem metabolism at two sites in the stream reach (Bott, 2006). We measured dissolved oxygen every 10 min for c. 50 h at each site using a Hydrolab MS-5 datalogging sensor (Hach Hydromet, Loveland, CO, U.S.A.). Concurrently, we measured light using an Odyssey irradiance metre (Dataflow System Pty Limited, Christchurch, New Zealand). We modelled GPP and community respiration using the methods and equations of Holtgrieve *et al.* (2010), although we fit the model by minimising the least squares instead of using a Bayesian approach (Riley & Dodds, 2012) as modified for single-station measurements (Dodds *et al.*, 2013). We used the reaeration rates measured in 2008 by Whiles *et al.* (2013), as the stream was the same size and discharge was similar in 2012 and 2014. In addition to reaeration, other parameters in the model included light, discharge and mean stream width. We used days as replicates within years.

Ammonium spiralling

We followed the methods of Tank, Bernot & Rosi-Marshall (2006) to measure ammonium uptake length using a short-term ammonium release. Briefly, we continuously pumped ammonium chloride (NH₄Cl, 1 g L⁻¹) and a conservative tracer (NaCl, 227 g L⁻¹) into the top

of the study reach to increase NH_4^+ to *c.* 5x background concentration (goal concentration $10 \mu\text{g L}^{-1}$). We continuously measured conductivity at a station downstream (120 and 140 m in 2012 and 2014, respectively) of the pump until plateau (at 99 and 92 min after the beginning of the addition in 2012 and 2014, respectively) using a conductivity metre (Yellow Springs Instruments 85). We then collected triplicate water samples at stations -5 (background), 10, 20, 40, 80 and 120 m from the pump. In 2014, we collected three additional samples 140 m downstream from the pump. Water samples were analysed for ammonium using a fluorometer (Turner Designs Aquafluor) and the orthophthaldialdehyde method (Holmes *et al.*, 1999). We calculated ammonium uptake length using an exponential decay model where:

$$\ln N_x = \ln N_0 - kx$$

where N_x is the concentration of NH_4^+ corrected for background and dilution at plateau at x metres downstream of the injection point, N_0 is NH_4^+ concentration corrected for background concentrations at the injection point and k is the decay rate (Stream Solute Workshop, 1990). Uptake length S_w is the inverse of k

$$S_w = k^{-1}$$

Conservative tracer data were used to correct for dilution along the stream reach.

We also used the Tracer Addition for Spiral Curve Characterization (TASCC) method (Covino, McGlynn & McNamara, 2010) to estimate NH_4^+ uptake length in 2014. This method allowed estimation of uptake length at ambient conditions which is comparable to estimates from 2006 and 2008, which were performed at ambient concentrations of NH_4^+ , with ^{15}N NH_4^+ releases. Methods that elevate background ammonium concentration overestimate uptake lengths relative to background (Mulholland *et al.*, 2002). For the TASCC method, we added a slug of 5.0 g of NH_4Cl and 984.0 g of NaCl as our conservative tracer at the same station we used for the NH_4^+ addition described above. We then continuously measured conductivity 140 m downstream. We took background samples for tracer and nutrient in triplicate prior to the addition of the slug at 140 m and 5 m upstream of the location of the slug addition. We took 47 samples at 140 m, noting time and conductivity at the time each sample was collected. The samples were analysed for NH_4^+ as described above. The ambient S_w was then extrapolated from the concentration-dependent S_w values (Covino *et al.*, 2010).

Nitrogen fluxes

Fluxes of nitrogen (N) between food-web compartments were calculated based on the addition of $^{15}\text{NH}_4\text{Cl}$ additions to the stream in February 2006 and February 2008 (Whiles *et al.*, 2013). Briefly, a $^{15}\text{NH}_4\text{Cl}$ solution was added to the stream continuously for 14 days to act as a tracer through the food web as it was taken up by basal compartments and consumed by animals in the stream. The basic protocol of the Lotic Intersite Nitrogen Experiment (LINX) was followed (<http://www.faculty.biol.vt.edu/webster/linx/protocol.html>), and food-web compartments were sampled nine times in 2006 and 11 times in 2008 (both prior to, during and after ^{15}N release both years). Details of the field sampling and analytical methods are presented in Whiles *et al.* (2013). The concentrations of ^{15}N in both animals and their food resources were measured periodically for *c.* 50 days after ^{15}N was released. The rate of increase in ^{15}N in the animals was then used to determine the flux of N into each food-web compartment. We used the 2008 (post-decline) N uptake rates to estimate the amount of N transferred between food-web compartments for the 2012 and 2014 data, as these were all post-decline years. Flux rates were modelled with a linked box model approach (Wollheim *et al.*, 1999; Dodds *et al.*, 2000; Whiles *et al.*, 2013). We included taxa we considered 'dominant' (biomass $\geq 10 \text{ mg DM m}^{-2}$) in models.

Statistical analyses

We used a one-tailed Fisher's exact test to compare differences in macroinvertebrate biomass and organic matter pools between our 2012 and 2014 measurements and 2006 (pre-decline) conditions. This procedure allows for comparisons of parameters with low sample sizes (Zar, 2010). We compared our 2012 and 2014 values to just the 2006 pre-decline values from Whiles *et al.* (2013) to limit the number of multiple comparisons and used a Bonferroni correction when multiple comparisons were made. We considered $P < 0.1$ marginally significant.

We analysed macroinvertebrate community data from all years using non-metric multidimensional scaling (MDS) analysis performed on matrices of macroinvertebrate biomass. We tested for differences among years and habitats using analysis of similarity (ANOSIM), and taxa driving those differences were identified using the SIMPER (similarity percentage) test in PRIMER (PRIMER-E Ltd., Luton, U.K.). We used rarefaction to estimate taxa richness from samples collected each year and

to calculate 95% confidence intervals for annual richness estimates (EstimateS; Colwell, 2013). Differences in annual richness estimates between years were considered significant when 95% unconditional confidence intervals did not overlap.

Results

Ecosystem structure

Tadpole biomass and diversity were lower in 2012 and 2014 than pre-decline values in 2006. *Lithobates warszewitschii* and *Colostethus* spp. were the dominant taxa in Río Maria before the decline, while only *Smilisca* spp. were found in 2012. In 2014, we found mostly *L. warszewitschii* and one specimen of *Colostethus* sp. (Fig. 1). Total tadpole biomass was three orders of magnitude lower in 2012 than 2006 and rebounded slightly in 2014 (Fig. 1).

We observed mixed long-term patterns in individual organic matter pools (Fig. 2). Mean epilithon mass was higher in both 2012 and 2014 than in 2006 (pre-decline) ($P < 0.05$), while SPOM concentrations were lower in 2012 and 2014 than 2006 ($P < 0.05$). CPOM standing

stock decreased in 2014 compared to 2006 (Fig. 2, $P < 0.05$), while FPOM in 2012 and 2014 was similar to the 2006 pre-decline value.

Habitat-weighted biomass of shredding and filter-feeding macroinvertebrates was lower in 2012 and 2014 than in 2006 (Fig. 3, $P < 0.05$ and $P < 0.10$, respectively). Biomass of grazing macroinvertebrates was lower in 2014 than 2006 (Fig. 3, $P < 0.05$), while biomass of collecting-gathering macroinvertebrates was higher in 2012 than 2006 (Fig. 3, $P < 0.05$).

Macroinvertebrate taxa richness decreased significantly between 2008 and 2012 and was significantly higher in 2014 than in 2012 (Fig. 4, $P < 0.05$). The structure of the grazing macroinvertebrate compartment of the food web was altered, with only one dominant (biomass >10 mg dry mass m^{-2}) grazing mayfly (*Farrodes*) in the stream during February 2012, compared to two in 2008 (*Thraulodes* and *Baetis*) and four in 2006 (*Thraulodes*, *Farrodes*, *Stenonema* and *Hagenulopsis*). In 2014, there was no single genus fitting our definition of a dominant taxon, but the Leptophlebiidae assemblage (*Thraulodes*, *Farrodes* and *Hagenulopsis*) had a collective biomass of 10.0 mg DM m^{-2} . The

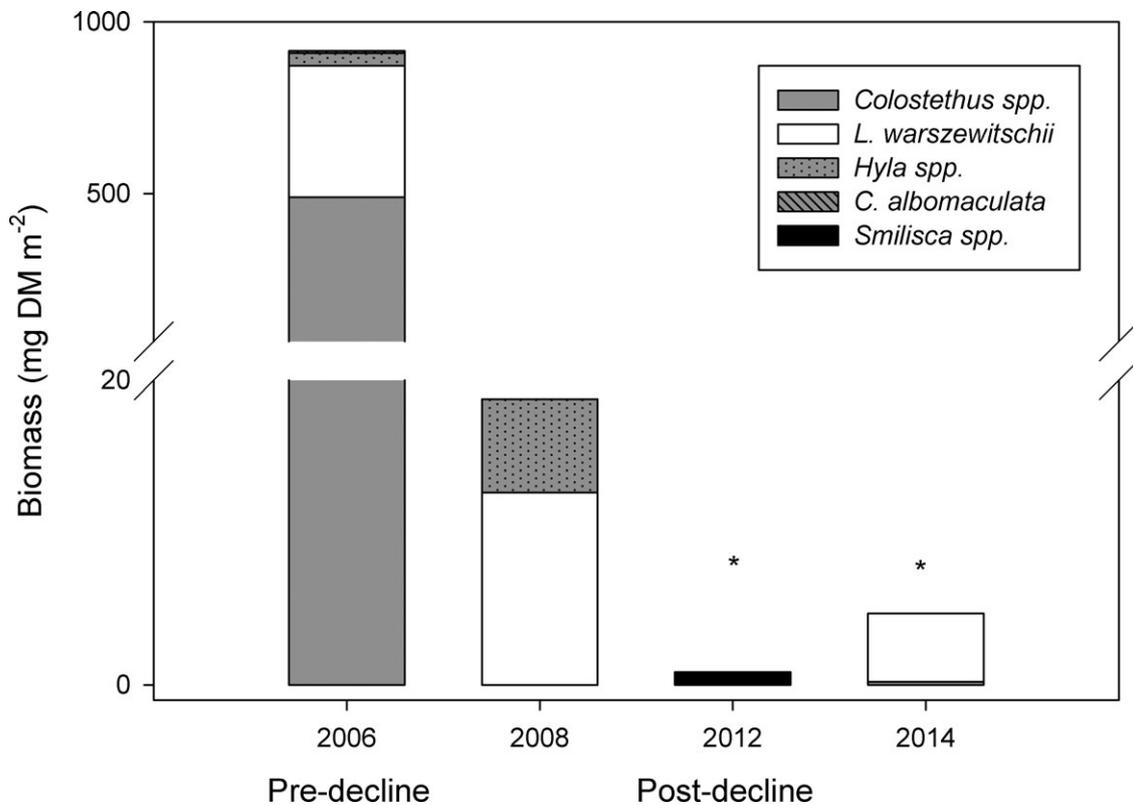


Fig. 1 Habitat-weighted biomass of dominant tadpole taxa in Río Maria in 2006 (pre-amphibian decline), 2008 (2 years post-decline), 2012 (6 years post-decline) and 2014 (8 years post-decline). Asterisks indicate significant differences in 2012 and 2014 compared to 2006 ($P < 0.05$). The 2008 value was significantly different than 2006 (Whiles *et al.*, 2013). Note the log scale on the Y-axis. For amphibian taxa, L = *Lithobates*, C = *Cochranella*.

Fig. 2 Mean (\pm SE) standing stocks of (a) epilithon, (b) SPOM, (c) CPOM and (d) FPOM for organic matter compartments in 2006 (pre-amphibian decline, open), 2008 (2 years post-decline, grey), 2012 (6 years post-decline, grey) and 2014 (8 years post-decline, grey). Asterisks indicate significantly different 2012 and 2014 means compared to 2006 ($P < 0.05$). The 2008 epilithon, SPOM and FPOM means were significantly different than 2006 values (Whiles *et al.*, 2013). Definitions for abbreviations are as follow: DM, dry mass; SPOM, suspended organic matter; CPOM, coarse particulate organic matter (>1 mm); FPOM, fine particulate organic matter (<1 mm).

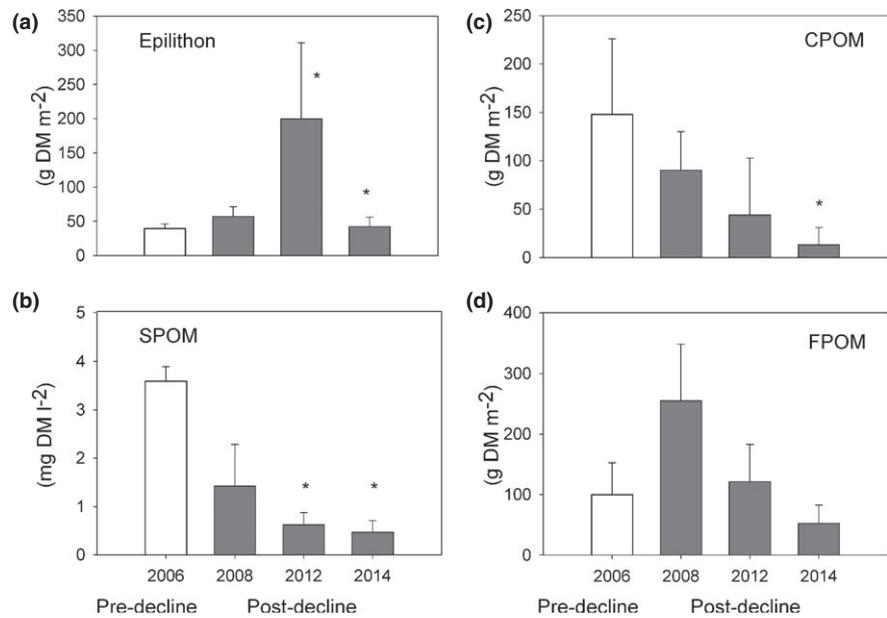
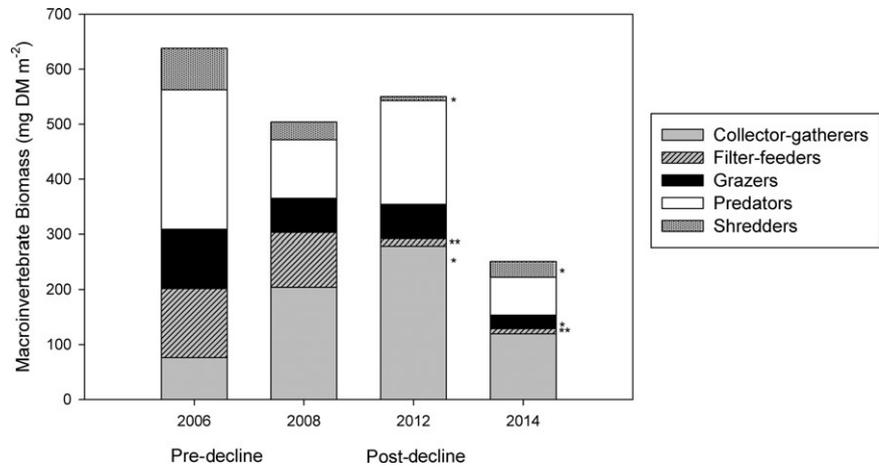


Fig. 3 Habitat-weighted mean biomass of macroinvertebrate functional feeding groups in Río Maria in 2006 (pre-decline), 2008 (2 years post-decline), 2012 (6 years post-decline) and 2014 (8 years post-decline). DM is dry mass. Asterisks indicate significantly different mean biomass estimates compared to 2006 (* $P < 0.05$, ** $P < 0.10$).



increase in macroinvertebrate taxa richness between 2012 and 2014 was largely due to the presence of a few individuals of some Odonata (three taxa), Elmidae (five taxa) and Baetidae (three taxa).

The NMDS ordination indicated that the macroinvertebrate assemblage changed over time (Fig. 5). While riffle communities differed among all years (ANOSIM, $P < 0.05$), communities in pools did not change between 2006 and 2014, although the 2012 community was different from pool communities in all other years (ANOSIM, $P < 0.05$).

Ecosystem processes

Components of ecosystem metabolism changed substantially over time in Río Maria, although the directions of change were contrary to our hypotheses. While GPP in 2012 and 2014 was similar to 2006 (Fig. 6), community

respiration decreased from 2006 to 2012 and then increased in 2014 (Fig. 6, $P = 0.083$).

Ambient ammonium concentrations increased from 2006 to 2012, and then decreased in 2014, while ammonium uptake length increased from 2006 to 2012 and remained high in 2014 (Fig. 6, $P = 0.075$). Nitrogen fluxes between food-web compartments continued to change, with more available N incorporated into epilithon and less into SPOM in 2012 than 2008 (Fig. 7). The flux of N into epilithon and CPOM decreased in 2014, with a decrease in the standing stock of those compartments (Fig. 7). Fluxes of N into animals remained low during 2012 and 2014 in the absence of grazing tadpoles.

Discussion

Our study links the loss of an abundant and diverse consumer group with changes in ecosystem function

and community structure of remaining animals over a relatively long time period in a natural ecosystem. While some of the ecosystem changes we observed (e.g. N fluxes, epilithon standing stock) were evident 2 years post-decline, others were not evident until 6 or 8 years post-decline, or became more pronounced over time (e.g. significant decreases in SPOM). These results add to growing evidence that loss of key consumers strongly affects ecosystems (e.g. Taylor *et al.*, 2006; Hooper *et al.*, 2012) and demonstrate that in natural ecosystems changes may persist, increase in intensity over time and/or may not be immediately evident after a loss of species diversity.

The loss of key consumers, and possibly declines in consumer biodiversity, may result in overall reduced

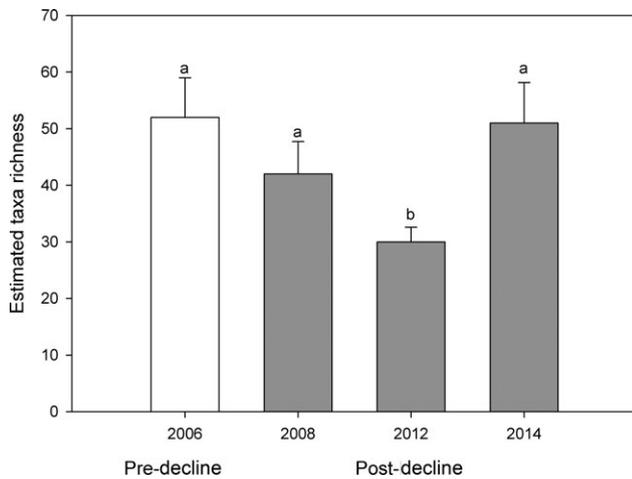


Fig. 4 Estimated taxa richness based on rarefaction curves and 95% confidence intervals generated from macroinvertebrate samples collected in Río Maria in 2006 (pre-decline), 2008 (2 years post-decline), 2012 (6 years post-decline) and 2014 (8 years post-decline). There was no difference in the number of taxa among 2006, 2008 and 2014, but fewer taxa were found in the stream in 2012 than other years ($P < 0.05$).

consumer abundance and biomass when there is no functional redundancy. Studies examining relationships between biodiversity and ecosystem function frequently maintain constant abundance or biomass while altering diversity in treatments (e.g. Jonsson & Malmqvist, 2000; Cardinale, Palmer & Collins, 2002; Tilman, Reich & Knops, 2006; Kominoski & Pringle, 2009; although see McIntyre *et al.*, 2007). Our work provides an example of how a decline in consumer diversity can be coupled with declines in abundance and biomass, suggesting that the assumption of constant abundance or biomass may be questionable. This result is notable because similar losses of diversity and biomass have occurred with pathogenic *Bd* in many streams in Central America and are ongoing. However, it is important to note that we cannot separate the effects of taxa loss from the effects of the loss of a functional group; we documented decreases in the biomass, abundance and diversity of tadpoles, with no compensatory increases in abundance or biomass of the remaining tadpole or macroinvertebrate taxa.

Secondary Losses of Biodiversity and Biomass

We observed losses of macroinvertebrate biomass and diversity from non-consumptive effects (i.e. non-predatory effects). The mechanisms behind the decreases in macroinvertebrate richness and biomass following the amphibian decline are not clear, but may be linked to changes in basal resources associated with the loss of tadpoles. For grazing insects, declines may be related to community shifts in epilithon. The diatom community shifts to larger species and epilithon accrues in systems without tadpoles (Ranvestel *et al.*, 2004; Connelly *et al.*, 2008), which may render food resources inaccessible for small grazing macroinvertebrates. In Río Maria,

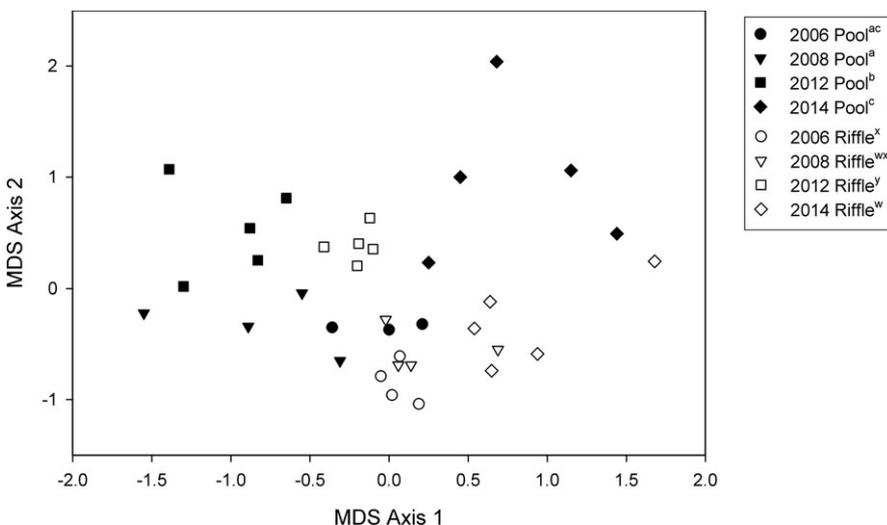


Fig. 5 Non-metric multidimensional scaling ordination of macroinvertebrates (based on biomass of individual taxa) in Río Maria in February 2006 (pre-decline), 2008, 2012 and 2014 (2-, 6- and 8-years post-decline, respectively) (2D stress = 0.18). Samples from the same year and habitat have the same symbol. Communities in both riffles and pools were compared among years. Within each habitat type, years with significant differences (ANOSIM, $P < 0.05$) are indicated by different superscripts.

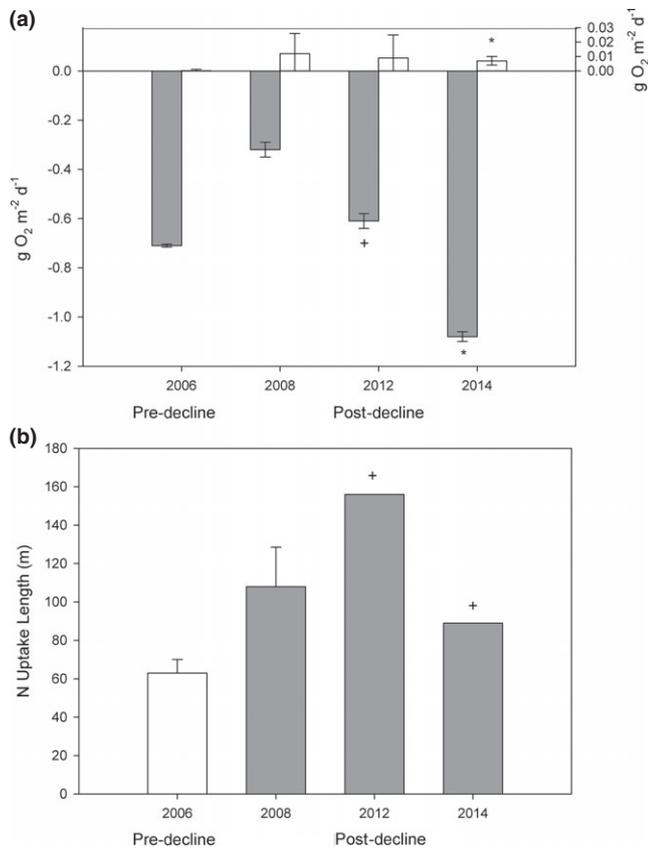


Fig. 6 (a) Mean gross primary production (GPP, open bars) and community respiration (CR, grey bars) in Río Maria in 2006 (pre-amphibian decline) and 2008, 2012 and 2014 (2-, 6- and 8-years post-amphibian decline, respectively) and (b) ammonium uptake length pre-amphibian decline (open bar) and 2-, 6- and 8-years post-decline (grey bars). Asterisks indicate significant differences from 2006 ($P < 0.05$); pluses indicate marginally significant differences from 2006 ($P < 0.10$). The 2008 GPP, CR and NH_4^+ uptake length estimates were significantly different than 2006 (Whiles *et al.*, 2013). Error bars represent ± 1 SE.

dominant grazing mayflies declined from three taxa to one taxon after the amphibian decline. In 2006 and 2008, two genera of leptophlebiid mayflies, *Farrodes* and *Thraulodes*, were abundant. However, 6 years post-decline, we collected only *Farrodes*. While the abundance and habitat-weighted biomass of *Farrodes* did not increase with the loss of *Thraulodes*, the mean mass of individual *Farrodes* was 3.6–8.3 times greater in 2012 than 2006 and 2008, respectively ($P < 0.05$), suggesting that larger-bodied individuals were better adapted to the post-decline conditions in the stream. The patterns we observed with grazing mayflies after the amphibian decline in Río Maria were consistent with a similar, much shorter-term study in similar streams, where *Farrodes* biomass increased following amphibian declines (Colón-Gaud *et al.*, 2010a). These patterns suggest there is homogenisation of resources used by grazing mayflies after an amphibian

decline and eventual competitive exclusion of some grazing mayfly taxa.

Losses in macroinvertebrate richness were primarily from taxa feeding at the same trophic level as the tadpoles, indicating indirect effects of tadpoles may be important in structuring macroinvertebrate communities. These findings support those from a concurrent, shorter-term study in a nearby headwater stream where the number of taxa in multiple trophic levels declined between 15 and 25%, depending on the habitat, after an amphibian decline (Barnum *et al.*, *in review*). This pattern is also consistent with modelling approaches that have been used to predict the community-level effects of the loss of species with varying degrees of connectedness within the food web (Dunne, Williams & Martinez, 2002; Dunne & Williams, 2009). Furthermore, ecosystems with less functional redundancy are expected to experience more secondary extinctions, as the loss of an individual species represents a larger proportion of the species pool (Ebenman, Law & Borrvall, 2004). The increase in macroinvertebrate richness we documented in 2014 suggests some degree of recovery for the system; yet, this pattern was driven by the presence of a few individuals of a limited number of taxa. Importantly, this increase in macroinvertebrate diversity did not result in subsequent increases in biomass.

Río Maria exhibited less functional redundancy than similar-sized streams in other regions (e.g. streams in the southeast and the arid southwest U.S.; Schofield *et al.*, 2008; Boersma *et al.*, 2014). Similar cascading effects of biodiversity loss on ecosystem structure and function have been documented in marine and terrestrial ecosystems (Paine, 1966; Estes & Palmisano, 1974; Berger *et al.*, 2001), but examples from freshwater systems are limited. While losses of taxa from direct interactions (e.g. competition for food resources) are straightforward, our findings suggest tadpoles are likely affecting macroinvertebrates through indirect interactions (e.g. bioturbation, altering algal community structure). Non-consumptive interactions have been noted to result in secondary extinctions in other systems, including Costa Rican bromeliad systems and plant–aphid–parasitoid communities, although the mechanisms behind those extinctions were different (Srivastava & Bell, 2009; Sanders, Sutter & Frank van Veen, 2013).

Grazing insects in Río Maria have yet to compensate for the functional roles of tadpoles in N dynamics. Although the primary food source for grazing macroinvertebrates, epilithic algae, increased after the amphibian decline, macroinvertebrate populations did not increase. Tadpoles influence algal communities by physical

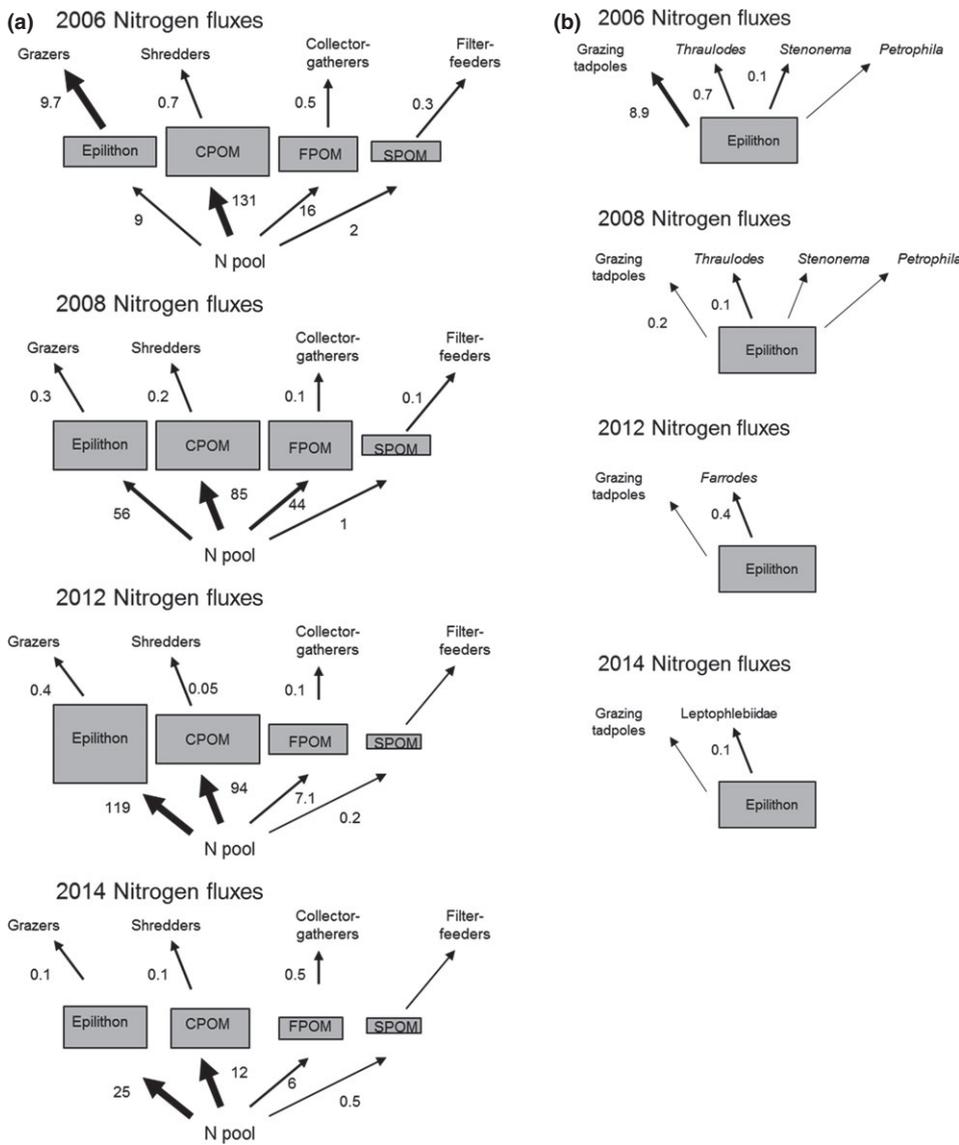


Fig. 7 (a) Nitrogen fluxes ($\text{mg N m}^{-2} \text{ day}^{-1}$) between food-web compartments in 2006 (pre-amphibian decline), 2008 (2 years post-amphibian decline), 2012 (6 years post-amphibian decline) and 2014 (8 years post-amphibian decline) on left and (b) from epilithon to grazers on right. Arrows without flux values are $<0.1 \text{ mg N m}^{-2} \text{ day}^{-1}$. Definitions for abbreviations are as follows: CPOM, coarse particulate organic matter; FPOM, fine particulate organic matter; SPOM, suspended particulate organic matter. The Leptophlebiidae assemblage in the 2014 model refers to the genera *Thraulodes*, *Farodes* and *Hagenulopsis*; their collective biomass was $10.0 \text{ mg DM m}^{-2}$.

disturbance and through feeding; therefore, the loss of tadpoles from the system may have altered the ability of macroinvertebrates to access food resources (Flecker *et al.*, 1999; Ranvestel *et al.*, 2004; Connelly *et al.*, 2008). Hence, the flux of N from epilithon to higher trophic levels declined with time. Lack of functional redundancy was also evidenced by a companion study in our site demonstrating the isotopic diet niche of tadpoles remained unfilled 2 years post-decline (Barnum *et al.*, 2013).

Construction of a low-head dam *c.* 25 m upstream of our sampling reach did not appear to influence our results. The small impoundment was built in the stream after the 2008 sampling date. By the time we sampled in 2012, however, the structure had filled with sediments, and water depth behind the structure was similar to that in our sampling reach. While the sediments retained behind the dam could have acted as a source of sus-

pended materials during high-water events in the wet season, stream velocity was not likely high enough to entrain accumulated sediments in the dry season when we sampled (Allan, 1995). It is possible that during construction, and for some time afterwards, the structure may have influenced stream communities (e.g. Coll *et al.*, 2011), but this relatively small structure appeared to be at equilibrium 2 years before we sampled in 2012.

Changes in ecosystem processes and function

Animals can influence nutrient cycling in freshwater ecosystems through a variety of pathways (see Vanni, 2002 for a review). These can include direct effects through consumption of resources, secondary production and excretion/egestion (Grimm, 1988; Vanni, Layne & Arnott, 1997; Hall, Tank & Dybdahl, 2003). Indirect effects on

nutrient cycling include bioturbation (Flecker *et al.*, 1999) and competition with, facilitation of or consumption of other animals important in nutrient cycles (Moore *et al.*, 2007; Rugenski *et al.*, 2012). Non-native taxa can have a strong influence on nutrient cycling in some stream ecosystems (Hall *et al.*, 2003), but less is known about how losses of taxa affect nutrient cycling (but see Taylor *et al.*, 2006; Whiles *et al.*, 2013; Capps, Atkinson & Rugenski, 2015). We examined how the loss of a dominant amphibian group influenced N fluxes, storage and uptake length through time. Our study built upon work conducted by Whiles *et al.* (2013) that documented alterations to the N cycle 2 years after the amphibian decline. Notably, a number of the changes observed 2 years after the decline became more pronounced 6 years after the decline, including the flux of N to the epilithon, the increasing role of the epilithon pool as a N sink and the shift in the dominant grazing taxon (*Thraulodes* to *Farrodes*). The time lag in some of the responses we measured may be due to the continued decline in the abundance and diversity of amphibians in the stream, the lack of nearby sources for colonising animals or the continuing change in the quality and/or quantity of basal resources in Río Maria. We speculate tadpoles increase the heterogeneity and diversity of resources available to other consumers, creating a more functionally complex and species-rich animal assemblages.

As we examined long-term patterns of the quality and quantity of epilithon and NH_4^+ uptake in Río Maria, we found an emerging positive relationship between epilithon biomass and NH_4^+ spiralling length (Fig. 8). This relationship contrasts with other studies that have shown that increased biomass of primary producers (or benthic organic matter) decreases nutrient spiralling length (Mulholland *et al.*, 1985; Valett, Crenshaw & Wagner, 2002). Our results may be explained by changes in the epilithon in Río Maria after the decline; epilithon is now composed primarily of senescent diatom material (Whiles *et al.*, 2013). We hypothesise this senescent material acts as a barrier between the water column and the active components of the epilithon on the substrata. Accordingly, there appeared to be less overlying senescent material on substrata in 2014 when NH_4^+ uptake length decreased (Fig. 8).

Although the high NH_4^+ uptake we documented in 2012 is consistent with our hypotheses and with the overall patterns we observed, it should be interpreted with some caution because we used a different uptake measurement method in 2012. Ammonium uptake was calculated in 2006 and 2008 using ^{15}N -release data, which did not result in appreciable increases in ambient concentrations of NH_4^+ . We did not perform an ^{15}N

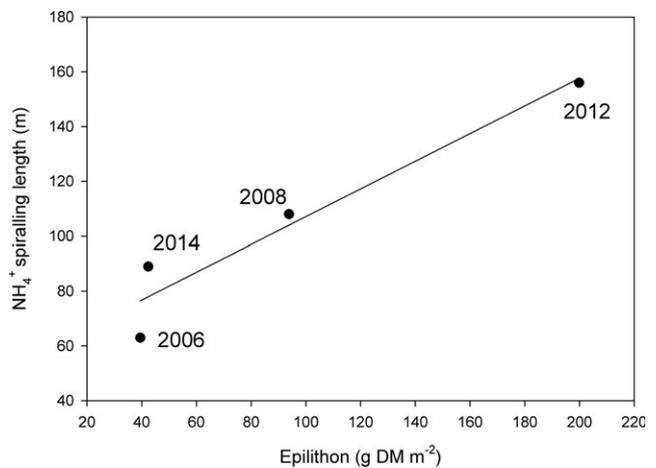


Fig. 8 Habitat-weighted epilithon dry mass (g DM m^{-2}) versus NH_4^+ spiralling length for 2006 (pre-amphibian decline), 2008 (2 years post-amphibian decline), 2012 (6 years post-amphibian decline) and 2014 (8 years post-amphibian decline) ($R^2 = 0.93$, $P = 0.037$). The year that each data point was collected is indicated on the plot.

release in 2012 or 2014; therefore, we used a short-term NH_4^+ enrichment to measure uptake. This standard method can overestimate spiralling length (Dodds *et al.*, 2002; Payn *et al.*, 2005), suggesting that the increase in spiralling length that we observed from 2008 to 2012 may not have been as pronounced as we report. However, the TASCC method we used in 2014 allowed us to back-calculate spiralling length to ambient ammonium concentrations, making estimates more comparable to the values obtained with the ^{15}N addition methods used in 2006 and 2008 (Covino *et al.*, 2010).

The amphibian decline in Río Maria resulted in a less productive stream ecosystem, and this response is consistent with observations of productivity/biodiversity relationships in terrestrial and marine systems (Loreau *et al.*, 2001; Worm *et al.*, 2006). Eight years after the amphibian decline in Río Maria, the ecosystem continues to change in tadpole community structure, the quality and quantity of organic matter and rates of ecosystem processes. Without further sampling, we cannot predict whether Río Maria is stable at this point (e.g. has entered an alternate stable state), or will continue to change. Some variables we measured suggest possible recovery to pre-decline levels (e.g. epilithon and FPOM mass), while others show no change or continued movement away from pre-decline conditions (e.g. seston concentrations).

Natural, long-term, multifunction data sets

Other long-term studies have shown unexpected or delayed responses of both community structure and

ecosystem processes following disturbance (e.g. Lindenmayer *et al.*, 2010; Sponseller *et al.*, 2010; Dodds *et al.*, 2012; Strayer *et al.*, 2014). The additional sampling in 2012 and 2014 allowed us to further document the changes in taxa that were initially detected in 2008, including instability in the macroinvertebrate community that did not become evident until 2012. Likewise, the replacement of the leptophlebiid mayfly *Thraulodes* by *Farrodes* as the dominant grazer in the system in 2012, and then lack of a single dominant grazing mayfly taxon in 2014, would have been overlooked if sampling had stopped in 2008.

Our study is the only one that we know of to examine the effects the loss of a taxon of key consumers on multiple ecosystem processes in a natural ecosystem over a relatively long time period (8 years). Humans have caused similar diversity losses over time (e.g. extinction of marsupials in Australia, loss of megafauna in North America, extinction of many bird species in New Zealand). In these cases, scientists were not present to study how much function was lost with the removal of large numbers of dominant animals from a single group (e.g. mammals, birds or amphibians). While little is known about how biodiversity loss and ecosystem function respond over long time periods, our results support the notion that these relationships could be dynamic (Cardinale *et al.*, 2007; Eisenhauer *et al.*, 2010; Reich *et al.*, 2012; Allan *et al.*, 2013). Our work also underscores the importance of measuring indicators of multiple ecosystem functions, as some functional measures may change at different rates or remain unchanged after the loss of taxa. Thus, it is important to consider multiple ecosystem processes over time when examining potential ecosystem changes in response to changes in diversity (Gamfeldt, Hillebrand & Jonsson, 2008; Maestre *et al.*, 2012). For example, while GPP did not change from 2006 to 2012, other functions (e.g. ammonium spiralling length, SPOM standing stock and N flux to epilithon) did continue to change over this period, underscoring the value of long-term data sets for understanding the ecological consequences of declining biological diversity.

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