LETTER

Nutrient loading associated with agriculture land use dampens the importance of consumer-mediated niche construction

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Abstract

The linkages between biological communities and ecosystem function remain poorly understood along gradients of human-induced stressors. We examined how resource provisioning (nutrient recycling), mediated by native freshwater mussels, influences the structure and function of benthic communities by combining observational data and a field experiment. We compared the following: (1) elemental and community composition (algal pigments and macroinvertebrates) on live mussel shells and on nearby rocks across a gradient of catchment agriculture and (2) experimental colonisation of benthic communities on live vs. sham shells controlling for initial community composition and colonisation duration. We show that in near pristine systems, nutrient heterogeneity mediated by mussels relates to greater biodiversity of communities, which supports the notion that resource heterogeneity can foster biological diversity. However, with increased nutrients from the catchment, the relevance of mussel-provisioned nutrients was nearly eliminated. While species can persist in disturbed systems, their functional relevance may be diminished or lost.

Keywords

Agricultural land use, aquatic ecosystem, biodiversity, ecosystem function, ecosystem services, environmental context, environmental gradient, food web, niche construction, nitrogen, resource provisioning.

INTRODUCTION

Almost all ecosystems suffer some degradation from agricultural, industrial and residential development (Wardle *et al.* 2004; Pereira *et al.* 2010). Agriculture is particularly damaging due to the removal of native vegetation, disturbance of soils and heavy reliance on fertilisers (Tilman *et al.* 2002). This type of land use alone now encompasses ~40% of the Earth's ice-free surface area (Foley *et al.* 2005). The effects of agricultural land use extend far beyond the areas under cultivation, primarily as a result of elevated export of nutrients (Turner & Rabalais 1994; Caraco & Cole 1999; Foley *et al.* 2005), which accelerate and intensify eutrophication in downstream aquatic ecosystems. While eutrophication, derived from agricultural activities, is known to increase primary production in downstream habitats, less is known about how it affects consumer-mediated niche construction (Chapin *et al.* 2006; Cardinale 2011; but see Davis *et al.* 2010).

An ecological function refers to a process or service that the environment (living and non-living) provide or perform within an ecosystem. In addition to predation or herbivory, a particularly important function mediated by consumers is niche construction, e.g. through the provisioning of resources and habitat for other species within the community. Niche construction via the provisioning of space or resources can promote species co-existence and foster key linkages between biological diversity and the functioning of ecosystems (Erwin 2008). While the importance of engineering physical space as habitat is well established (Wright & Jones 2006), the provisioning of nutritional resources remains less understood, particularly along gradients of resource availability and anthropogenic disturbance.

Consumer-mediated (i.e. animal, microbial and fungal) nutrient release facilitates linkages among abiotic and biotic compartments and can, in some cases, significantly influence energy and material fluxes within ecosystems (Flecker *et al.* 2002; Vanni 2002; Wilson & Xenopoulos 2011). These subsidies can increase supply rates and alter ratios of key nutrients that limit the population growth of primary producers and consumers; creating conditions favourable for species co-existence and increased biological diversity (Elser & Urabe 1999; Frost *et al.* 2002; Sterner & Elser 2002).

In terms of nutritional resources, the extent to which animals (i.e. non-microbial or fungal, hereafter referred to simply as consumers) foster heterogeneous niche opportunities for others depends on three factors; all of which can vary across levels of disturbance and resource availability: (1) the abundance, physiological and nutritional status of the consumer, (2) the extent to which the consumer contributes a unique set of resources relative to what is available in the environment and (3) the extent to which the provisioned resources are in demand by other organisms within the ecosystem. Often, the importance of such provisioning is demonstrated by documenting or extrapolating the hypothetical consequence associated with the introduction (Hall *et al.* 2003), extirpation (McIntyre *et al.* 2007) or reduction in biomass (Baxter *et al.* 2005) of ecologically distinct species. Few if any studies, however, consider alterations in the environmental contexts that govern the importance of the provisioned resources (Richardson *et al.* 2010). Further, even when resource-

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provisioning species persist, increased nutrient supply resulting from anthropogenic activities may modify or eliminate the provisioned resource mosaic (e.g. nutrient heterogeneity). As such, it is conceivable for an ecosystem to exhibit significant functional losses despite maintaining an intact community structure.

We examined how the functional importance of consumer-mediated nutrient release on benthic communities varied among freshwater streams altered by different amounts of agriculture in the catchment. Specifically, we assessed the functional role of native freshwater mussels as nutrient providers in streams altered by elevated nutrient concentrations stemming from upstream agricultural land use. While freshwater mussels, like rocks, serve as a hard substrate for colonising organisms and could be considered ecosystem engineers (Wright & Jones 2006), they also provide nutritional subsidies to local environments by releasing nutrients and bio-depositing organic material (Spooner & Vaughn 2008). In the strictest sense, nutrients are not created, but modified and provided via physiological activities related to mussel metabolism. As such, we here consider niche construction performed by mussels to be a function of nutrient release instead of ecological engineering *per se*. We predicted that this important ecological role would be diminished in streams with high catchment agriculture where elevated stream nutrient concentrations could overwhelm the subsidies produced by mussel excretion and bio-deposition. We assessed the physiological condition, released nutrients and ecological responses; created by two widely distributed and dominant species of freshwater mussels (*Elliptio complanata* and *Lasmigona costata*) in rivers that vary in land cover and water quality characteristics.

METHODS

Site selection, environmental characterisation and experimental design

The study area spanned 14 individual streams (one site per stream) throughout southwestern Ontario, Canada. All streams drain into the Great Lakes and are reflective of the eastern forest-boreal transition ecoregion. The topography of the watersheds is rolling to relatively flat and drains through post-glacial sediments. The land cover and use in the catchments is a mix of forests, wetlands and agriculture and to a lesser extent developed (urban or suburban) land (see Supplemental Table 1 and Wilson & Xenopoulos 2008; Williams *et al.* 2012 for detailed watershed characteristics). Freshwater mussel populations, while somewhat ubiquitously distributed, are often observed as patches (~100 m long) called 'mussel beds.' Therefore, using an unpublished Fisheries and Oceans Canada database, site selection was initially filtered according to the presence of either or both focal mussel species *E. complanata* (Lightfoot 1786) and *L. costata* (Rafinesque 1820) (~80–90 sites), and then refined according to the desired range of agriculture intensity, watershed area and proximity to laboratory.

We used ArcMap 9.0 to delineate and quantify land use within each watershed using geospatial data obtained through the Ontario Ministry of Natural Resources (OMNR 2002, 2007). We established land use classifications for the 100-m riparian buffer zone in the entire watershed area above each site and categorised them in the following groups:% continuous row cropland,% rotational cropland, % rural land,% wooded,% wetland and% developed areas (Wilson & Xenopoulos 2008). For each sampling location, we measured ambient nutrient concentrations ($n = 3$ per site) and extracellular enzyme activities in the water column ($n = 3$ per site). All measurements were taken once under base flow conditions in late July to August. Water column extracellular enzyme activities of alkaline phosphatase (APA) and aminopeptidase (AMA) and their ratios were determined as in Williams *et al.* (2012) and used as a surrogate evaluation of phosphorus (APA) and nitrogen (AMA) limitation. Water quality parameters measured included total dissolved nitrogen, total dissolved phosphorus, ammonia and dissolved organic carbon (as in Wilson & Xenopoulos 2008).

Because anthropogenic disturbances are often multiplicative and rarely influence a single component of the environment, we used a principal component analysis to collapse variation and further refine the agriculture gradient and environmental variability (Fig. 1, Supplemental Table 1). Two axes were extracted which cumulatively explained 75% of the environmental variation across sites (52 and 25% respectively). Negative values associated with PC axis 1 had less agriculture influence, more wetland and woodland land cover, and lower concentrations of total dissolved nitrogen and ammonia compared to positive PC 1 sites, which were characterised by a greater catchment agriculture land use and higher levels of dissolved nitrogen (Fig. 1). As such, we here define our environmental gradient of agriculture land use according to PC axis 1 scores which were used in subsequent analyses.

Mussel physiological condition and resource provisioning

We considered the spatial distance of our sampling regime to be governed by the local influence of mussel nutrient release, which dissipates with distance, and the extent to which mussels and rocks experience similar algal and macroinvertebrate colonisation patterns. Mussel searches were performed using viewboxes and wading. When a mussel was detected, a nearby rock (within ~0.5–1 m) of similar size was haphazardly sampled either adjacent or upstream of the live mussel. Mussels and rocks ($n = 10$ for each species of mussel) were placed into individual Ziploc® bags (SC Johnson Company, Racine, Wisconsin, USA) filled with 125 ml of distilled water, and gently scrubbed of all surface material. A subset ($n = 5$) of these slurries were passed through a filter (250 μm mesh) to collect benthic macroinvertebrates and then placed into a 250 ml Nalgene plastic bottle on ice for laboratory processing (described below). Mussels were then placed into 500 ml glass respirometers filled with Combo media (a nutritionally balanced water to minimise stress and facilitate accurate nutrient estimates; see Kilham *et al.* 1998) and initial oxygen concentration and temperature measurements were recorded using a Hach LDO model HQD10 meter. Non-mussel control respirometers ($n = 5$) were used to assess changes in oxygen and nutrient composition unrelated to mussel activity. Respirometers were incubated for 1 h upon which final oxygen measurements taken, water volume measured and 100 ml of water filtered through a 0.47 μm glass fibre filter (GFF) and placed on ice for nutrient determination. Mussels were placed on ice and brought back to the laboratory where they were dissected, meristics quantified (length, wet and dry weight with and without shell) and foot tissue freeze dried for isotope, elemental content and lipid determination.

Excretion samples (ammonia and soluble reactive phosphorus) were quantified using phenol hypochlorite and the ascorbic acid methods, respectively, on a Beckman spectrophotometer (APHA, Awwa & WEF 2005). Mussel total lipid content was quantified gravimetrically (as in McMeans *et al.* 2012). Mussel tissue %N, %C,

Table 1 Effects of species identity and agricultural impact on mussel condition, mussel provided ecosystem services and ecological consequence of rendered services

	A. Source of variation			B. Correlation to PC$_1$ axis			
Dependent variable	Species (F$_{1,21}$)	Agricultur (F$_{1,21}$)	Sp. × Ag. (F$_{1,21}$)	Elliptio.	Lasm. (r)	Rock	Water
Physiological condition							
O : N¶	0.007	7.859*	0.33	−0.42*	−0.825‡		
Lipid content	7.35*	5.74*	0.649	−0.4*	−0.729‡		
Tissue δ^{15}N	0.25	32.21‡	0.032	0.815‡	0.782†		
Tissue δ^{13}C	0.056	0.274	0.513	−0.08	−0.433*		
Tissue N : P¶	5.42†	12.73†	1.83	0.303	0.243		
Resource provision							
Ammonia excretion rate	0	6.51*	0.682	0.598*	0.727*		
Phosphorus excretion rate	1.6	1.23	0.67	−0.373	−0.452		
N : P excretion¶	1.29	16.81‡	2.88	0.694†	0.783†		
Nutrient context							
Dissolved N : P water column							0.821†
Excretion-water column D$_{(N : P)}$	0.64	14.76‡	1.281	−0.717†	−0.760†		
Nitrogen ectoenzyme (AMA)		10.18*					−0.345
Phosporus ectoenzyme (APA)		2.85					0.611‡
AMA : APA¶		6.054*					−0.594‡
Ecosystem response							
Periphyton N : P	0.312	0.734	1.366	0.003	−0.108	0.214	
Periphyton δ^{15}N	0.614	8.07‡	0.585	0.757*	0.580*	0.536*	
Periphyton δ^{13}C	0.057	0.167	0.001	0.115	−0.044	0.170	
D$_{N : P}$	0.3	50.82‡	0.281	0.581*	0.647*		
D$_{δ^{15}N}$	0.346	8.016*	1.587	0.757†	0.595*		
D$_{δ^{13}C}$	0.068	0.009	0.331	0.360	0.201		
Community consequence							
Pigment diversity	0.070	7.81†	0.575	−0.694*	−0.625*	−0.452	
Macroinvertebrate diversity	0.470	9.61†	0.004	−0.657*	0.745*	−0.496	
Pigment dissimilarity	0.634	6.211*	0.163	−0.704*	−0.634*		
Macroinvertabrate dissimilarity	0.466	9.474*	0.157	−0.412	0.825†		
D$_{pigment diversity}$	1.207	26.839‡	0.157	−0.777†	−0.576		
D$_{macroinvertebrate diversity}$	0.449	17.572‡	0.386	−0.626*	−0.858‡		

Panel A represents Two-way ANOVA results for the mixed effects of species identity {*Elliptio*, *Lasmigona*) and agricultural impact (Low, High). Panel B represents linear regression results for response metrics across the agricultural gradient (PC$_1$).
*$P < 0.05$.
†$P < 0.01$.
‡$P < 0.001$.
¶Data were square root transformed to meet the assumption of normality.

^{15}N and ^{13}C isotopes ratios were assayed using an EuroEA3028-HT EuroVector Elemental Analyzer (EuroVector SpA, Milan, Italy) coupled with a Micromass IsoPrime Continuous Flow Isotope Ratio Mass Spectrometer (Micromass, UK; International standards: $\delta^{15}N = -4.56$; $\delta^{13}C = -26.39$; USGS41 ($\delta^{15}N = +47.6$; $\delta^{13}C = +37.63$). Mussel tissue phosphorus was quantified using persulfate digestion followed by the ascorbic acid method (APHA, Awwa & WEF 2005). Dissolved O : N ratios reflect a balance between net anabolism and catabolism, and were calculated as the molar ratio of oxygen consumed relative to ammonia excreted (Spooner & Vaughn 2008). All nutrient excretion and oxygen consumption rates were standardised to 20 °C according to the Q$_{10}$ temperature correction method (White & Seymour 2003).

Ecosystem response and community metrics

Rocks and shells were photographed, and surface area quantified using Image J software (Rasband 2012). Mussels burrow vertically with a posterior portion of the shell exposed to the water column.

We quantified shell exposure by measuring the scarring area on the posterior, which, can be inferred as a long-term (lifetime) integrator of shell exposure available as habitat for other species. We then evaluated if the extent of burrowing (i.e. fraction of exposed shell surface area) varied across the agricultural gradient.

Algal slurries were diluted to 500 ml and a series of 50 ml aliquots were filtered onto GFF filters for pigment determination, carbon, nitrogen and phosphorus elemental composition, and $\delta^{15}N$ and $\delta^{13}C$ isotopes. Algal community structure was biochemically estimated using taxon-specific pigments. Filters dedicated to pigment analysis were frozen at −80 °C until processing. Filters were then placed into a 80 : 20 methanol:acetone mixture for extraction overnight and dried down using nitrogen gas. Seventeen different pigments were quantified using pigment concentrations derived from the Reverse-Phase HPLC method (Alliance Waters 2695 Separations Module, Waters 2475 Multi λ Fluorescence Detector). All pigments were standardised relative to chlorophyll *a* concentration and characterised to algal types (diatoms, diatoms and chrysophytes, cryptophytes, chlorophytes, and cyanophytes) according to Leavitt
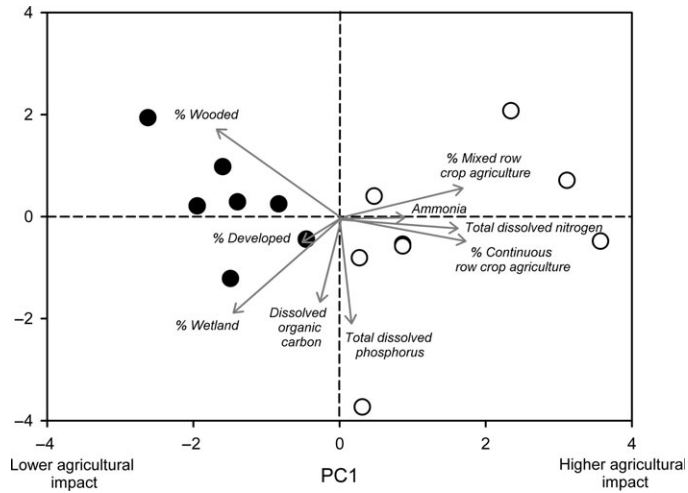
Figure 1 Results of principle components analysis synthesising land use (%) and water quality data for 14 sites. Eigenvectors and associated explanatory variables in italics and grey lines. Solid points represent sites with low and open points higher agricultural impacts respectively. PC axis 1 explains 53% and PC axis 2 explains 25% variation in the environmental data.

& Hodgson (2001). Algal elemental (%C, N, P) and isotopic ($\delta^{15}N$ and $\delta^{13}C$) composition were quantified according to the methods previously described for mussel tissue. Macroinvertebrates were sorted and identified to 27 major taxonomic groups according to the Ontario Benthic Biodiversity Network protocol (Supplemental Table 2). Both macroinvertebrate and algae communities for each rock and mussel were standardised according to total surface area (entire mussel) and characterised by calculating the Gini-Simpson's diversity index for each individual mussel or rock and community dissimilarity among rocks and mussels at each site (Vegan package R) (Dixon 2003).

To evaluate the importance of mussel provisioning across the agriculture gradient we used two separate biologically relevant approaches (nutrients in the water column as a comparison for nutrient excretion and rocks as a comparison for non-mussel-provisioned habitat). We considered rocks as a non-provision control because (1) we assume rocks do not release, or release very little nutrients, (2) previous work by Spooner & Vaughn (2006) suggest that nutrients are quickly sequestered by organisms on the shell and (3) at our grain of measurement, we expect stream flow and dilution to minimise neighbouring influences of mussel-provisioned nutrients to nearby rocks.

We calculated standardised effect sizes among treatments (mussel species) and null measurements (water column or rock) according to Hillebrand & Kahlert (2001). This approach calculates a mean difference between treatments and null measurements correcting for the standard error and sample size among groups. Thus, in addition to comparing among treatments, we were also able to generalise across response measures (i.e. $D_{dissolved\ nutrients\ N\ :\ P}$ and $D_{periphyton\ N\ :\ P}$).

$$D = \frac{\bar{X}_E - \bar{X}_C}{S} J$$

$$S = \sqrt{\frac{(n_e - 1)(SD_n)^2 + (n_c - 1)(SD_c)^2}{(n_e + n_c - 2)}}$$

$$J = 1 - \frac{3}{4(n_e + n_c - 2) - 1}$$

where D is the effect size for a given comparison of a test (E) vs. a null measurement (C) (e.g. excretion vs. water column N : P; mussel vs. rock periphyton N : P; mussel vs. rock macroinvertebrate diversity, etc.) S is the pooled variance among the groups. J is a correction factor for small sample sizes. Effect sizes greater than zero indicate a greater importance of mussel functioning over ambient conditions.

Because organisms on mussels should directly benefit from excreted nutrients compared to rocks (i.e. no excreted nutrients), we estimated the dissolved elemental nutrient imbalance ($D_{dissolved\ nutrient\ N\ :\ P}$), i.e. water column nutrient ratios vs. mussel nutrient excretion ratios. This evaluates the extent to which mussels provide resources in ratios different from what is available in the water column. Here, mussels provided nutrients in ratios different to what was available from the water column when the imbalance value was greater or less than zero. Similarly, to assess how communities responded to the availability of these provisioned resources, we then compared the elemental ($D_{N\ :\ P}$) and isotopic ($D_{\delta13C}$ and $D_{\delta15N}$) imbalances of periphyton on mussels compared to rocks. We also compared pigment ($D_{periphyton}$) and macroinvertebrate ($D_{macroinvertebrate}$) diversity on mussel shells to those on rocks. Although mathematically different, the concept of $D_{diversity}$ is biologically analogous to that of overyielding in traditional biodiversity studies, where positive values represent a yield in a treatment greater than in control (in our case diversity on mussel shells is greater than rocks). This allowed us to evaluate the functional importance of mussels by comparing communities on substrates (mussels and rocks) that were within metres of each other and thus should have equal probabilities for local colonisation of algae and macroinvertebrates from similar species pools.

Because we were interested in mussel condition, the extent to which they excreted nutrients, and their relative importance to ecosystem functioning along the gradient of agriculture influence, we used a suite of statistical approaches. We evaluated mussel condition (lipid content, tissue $\delta^{13}C$ and $\delta^{15}N$, elemental composition) and rendered nutrients (excreted ammonia, phosphorus and molar N : P) by regressing the average mass-corrected condition or excretion value for each species, or average site-specific effect size (effect on macroinvertebrate or periphyton) against the corresponding PC axis 1 score for that site. To more broadly assess the importance of land use and mussel species identity, we grouped sites according to their PC axis 1 scores (i.e. positive valued sites = high agriculture; negative valued sites = low agriculture). We then performed 2-way ANOVAs using agricultural impact (high, low), species (*Elliptio*, *Lasmigona*) and the interaction of the two on both species condition- and excretion-sets of response variables. All analyses were performed using R software (R Development Core Team 2012).

Gradient studies and the use of effect size metrics among observations have long been used in ecology to evaluate ecological dynamics that otherwise would be difficult to replicate in the laboratory or manipulate in the field (Hillebrand & Kahlert 2001; Fukami & Wardle 2005; Fitter & Hillebrand 2009). Indeed, simultaneously replicating 14 levels of agriculture intensity that encompasses natural variability in nutrient regimes, physiological dynamics of a long-lived consumer (lifespan of freshwater mussels range from ~30 to 100 years), and local/regional components of algal, microbial and macroinvertebrate communities is not practically or logistically

possible. Nonetheless, as an effort to mechanistically ground truth our field study and to test the hypothesis that nutrient release and not solely the mere presence of physical habitat govern our observations, we performed a manipulative experiment at two of the study sites with low catchment agriculture.

We compared nutrient release, elemental and community composition of benthic algae, and macroinvertebrate community composition on live mussels (*E. complanata*) to shucked shells (hereafter referred to as sham mussels). At each site, recently dead mussels were collected, shucked, filled with agar, and the two valves were then sealed together with silicone. We then collected an additional five live mussels. Live and sham mussels (*n* = 5 per treatment per site) were scrubbed clean and returned to the stream bed in their own sediment filled plastic 1 L containers. After 2 weeks, mussels were removed and processed according to the methods previously outlined for nutrient excretion, algal elemental composition, and pigment and macroinvertebrate community composition. At one site, three of the live mussels were lost to predation.

RESULTS

Mussel physiological processes were significantly different along the gradient of agriculture land use. Mussel oxygen to nitrogen (O : N)

ratio (Fig. 2a, Table 1) and tissue lipid content (Fig. 2b, Table 1), both declined indicating poorer physiological condition in catchments with more agriculture. Following this trend, mussel tissue $\delta^{15}N$ content in both species increased with agriculture despite no change in elemental (C, N, P) or $\delta^{13}C$ composition (Fig. 2c, Table 1, Supplemental Fig. 1).

Both dissolved nitrogen and N : P ratios increased in the water column with greater agriculture in the stream catchment (Fig. 3a). Similarly, excreted ammonia (Fig. 2d) and dissolved nitrogen to phosphorus (N : P) ratios also increased with greater catchment agriculture (Fig. 3a, Table 1). Excreted N : P, however, was higher than that measured in the water column ($D_{dissolved \; nutrient \; (N \; : \; P)}$) in streams having less agriculture, whereas the opposite pattern was observed in streams having more agriculture in their catchments (Fig. 3a, Table 1). On the basis of this, we estimate that on a volumetric basis, one litre of mussel excretia was ~6 times more nitrogen concentrated than surrounding ambient water in low agricultural sites and ~1.3 times more dilute in agriculture streams.

Microbial extracellular activity was greatest at low agriculture sites indicating that localised nutrient stress may be a factor limiting bacteria and algal growth, shortcomings that would be locally alleviated by mussel-excreted nutrients (Fig. 2g-i, Table 1). Indeed, while periphyton N : P content was nonlinear on both mussels and rocks
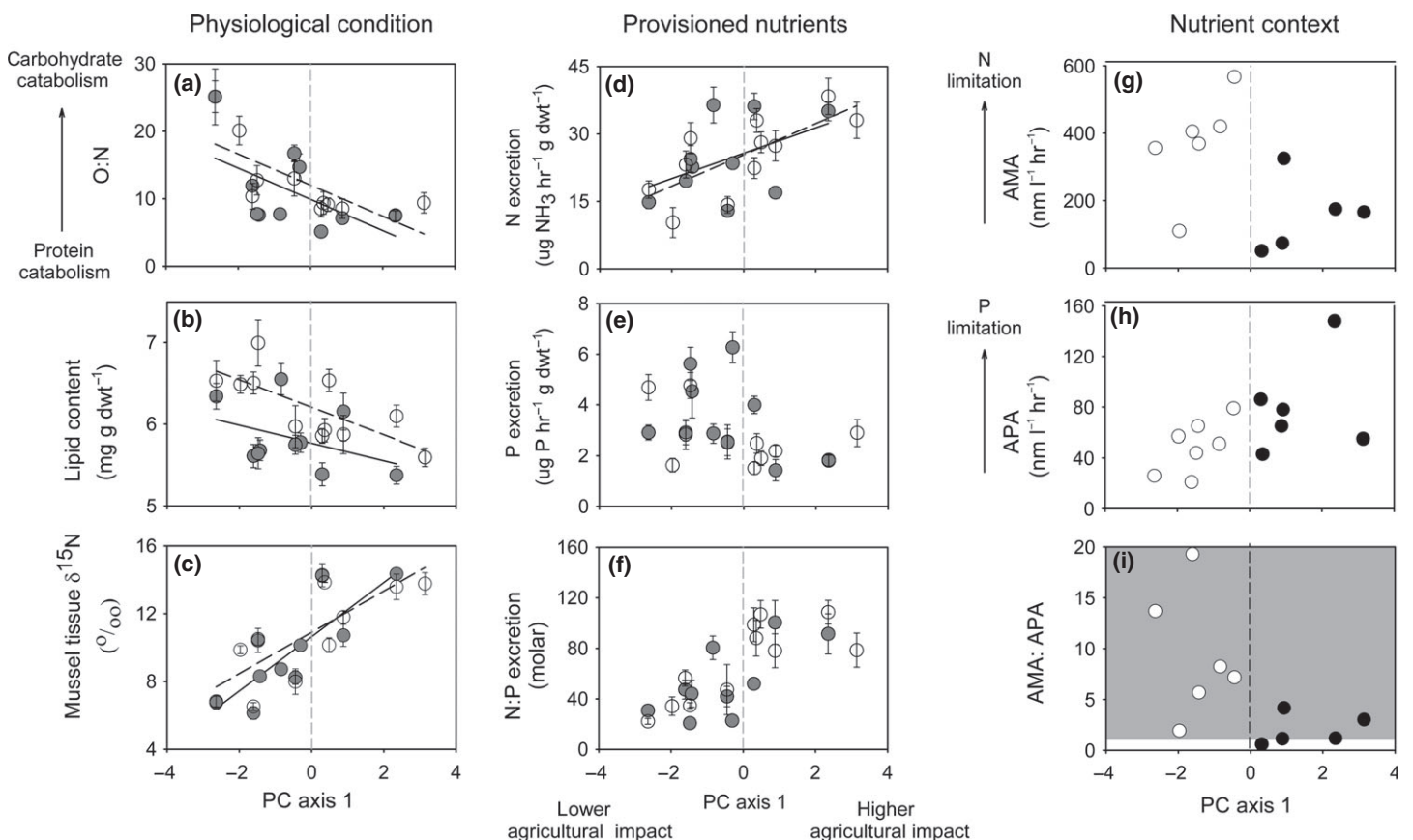

Figure 2 Relationships between site-specific agricultural impact intensity (principal components axis 1) and mean mussel physiological condition: (a) O : N; (b) Lipid content; (c) Mussel tissue $\delta^{15}N$; provisioned nutrients: (d) Ammonia excretion; (e) Phosphorus excretion; and (f) N : P excretion; and nutrient context: (g) nitrogen limitation (AMA activity), r = −0.34, P > 0.05; (h) Phosphorus limitation (APA activity), r = 0.611, P < 0.001; and (i) ratio between nitrogen and phosphorus limitation (AMA: APA) r = −0.594, P < 0.001. Closed grey and open points represent mean site-specific values ± 1 SE for *Elliptio* and *Lasmigona* respectively (a-f); Open and closed circles represent low and high agricultural sites, (g-h) respectively. Shaded grey region represents sites where AMA: APA < 1 (nitrogen limitation).
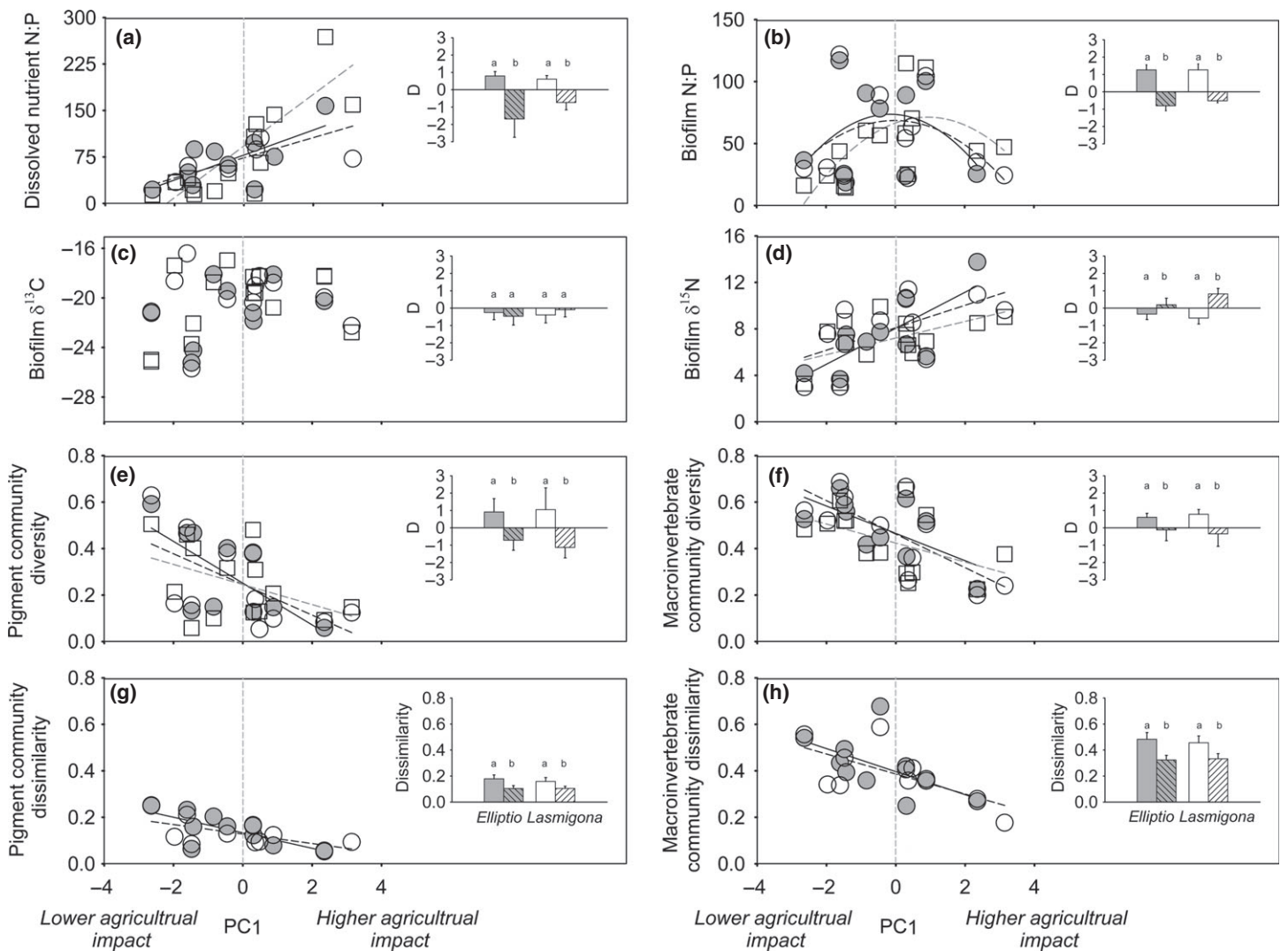
Figure 3 Mean site-specific species ecological response metrics across the gradient of agricultural land use: (a) Dissolved nutrient N : P ratios for *Elliptio* excretion (grey circles), *Lasmigona* excretion (white circles) and ambient water column (white squares); (b) Periphyton nutrient N : P ratios on shells and rocks white squares; (c) Mean periphyton $\delta^{13}C$ content; (d) periphyton $\delta^{15}N$ content; (e) Simpson's diversity index for pigments; (f) Simpson's diversity index for macroinvertebrates; (g) Pigment community dissimilarity; and (h) Macroinvertebrate community dissimilarity. Inset bar charts represent mean effect sizes (D ± 1 SE) (a-f), and mean community dissimilarity (g-h) for each species across grouped land-cover types; open bars represent low and hashed bars high agricultural impact respectively. Letters represent significant differences among treatments (see Table 1).

with increasing catchment agriculture; periphyton measured on mussels was relatively more nitrogen-rich than rocks in the low agriculture sites (Fig. 3b, Table 1). Furthermore, despite no observed differences in periphyton elemental (C or P) or $\delta^{13}C$ composition, periphyton on mussel shells became increasingly $\delta^{15}N$ enriched compared to those found on rocks at sites with greater agriculture (Fig. 3c-d, Table 1).

The ecological importance of nutrients provided by mussels to benthic communities was highly dependent upon ambient nutrient conditions. First, in general, the diversity of periphyton pigments and macroinvertebrate communities on mussel shells and rocks decreased with increasing agriculture (Fig. 3e,f). We also found a strong, consistent pattern whereby both periphyton pigment and macroinvertebrate community dissimilarity among mussel shells and rocks declined linearly, by 51 and 43%, respectively, along the

increasing agriculture gradient (Fig. 3g,h). The relative effect sizes for both pigment ($D_{pigment\ diversity}$) and macroinvertebrate ($D_{macroinvertebrate\ diversity}$) diversity were greater than zero in the low and negative or non-significant in the high agriculture sites (Fig. 3e, f). The extent of burrowing activity did not differ for either species across the gradient ($r_{Elliptio} = 0.19$; $r_{Lasmigona} = -0.23$; both $P > 0.05$). Furthermore, burrowing did not relate to the extent of dissimilarity or effect sizes of macroinvertebrate and pigment communities on rocks vs. mussels (See Supplemental Table 3).

The magnitude of both dissolved nutrient ($D_{dissolved\ nutrient\ N\ :\ P}$) and periphyton elemental ($D_{periphyton\ N\ :\ P}$) effect sizes was positively associated with the extent of extracellular enzyme activity, indicating a greater importance of mussel-derived nutrients when available nitrogen is limiting (Fig. 4a,b). In addition, pigment community dissimilarity and diversity effect size were positively associ-
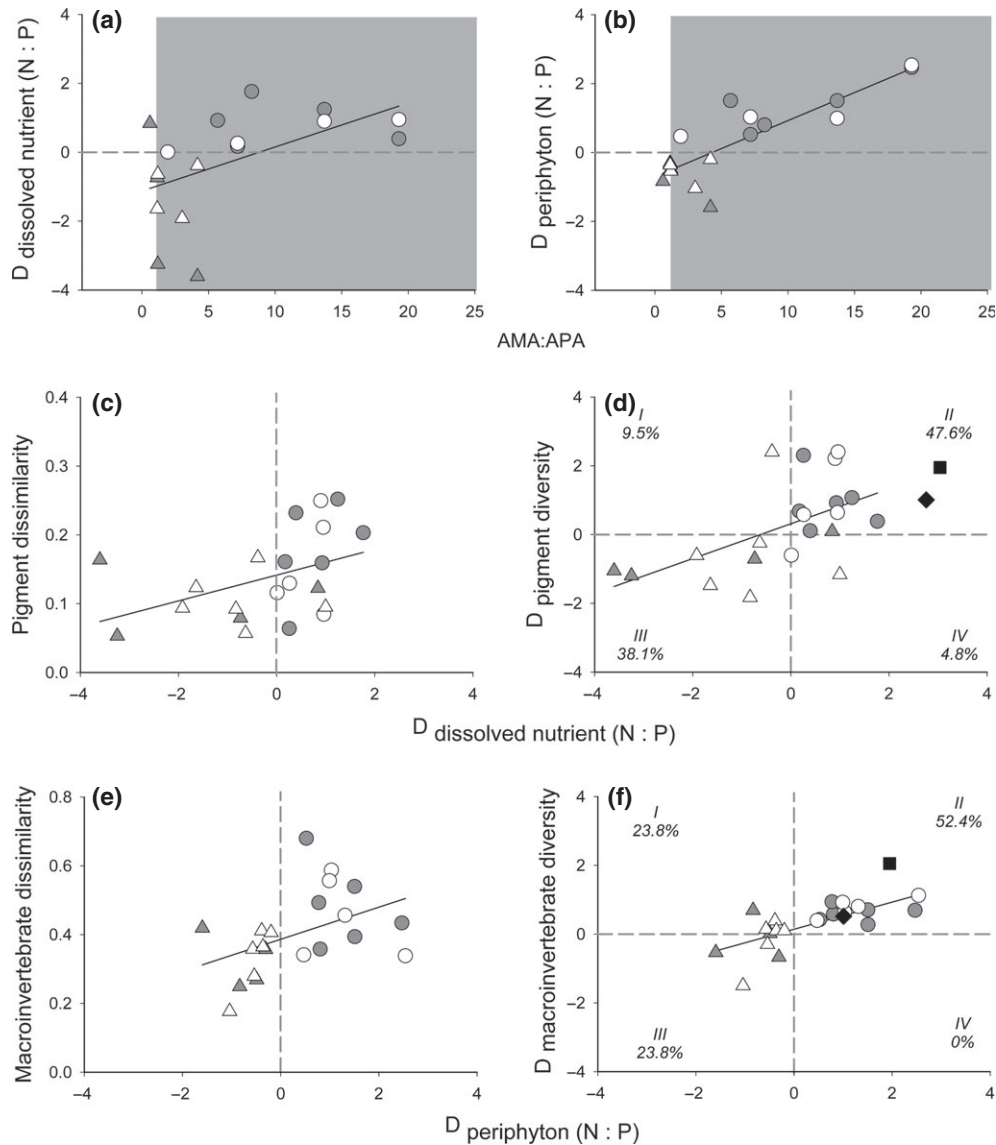
Figure 4 Linear regressions between site-specific effect size metrics among trophic levels and resource availability: ambient nutrient context (AMA: APA) and (a) $D_{dissolved\ nutrient\ (N\ :\ P)}$ ($r = 0.608$, $P = 0.004$); and (b) $D_{periphyton\ (N\ :\ P)}$ ($r = 0.814$, $P < 0.001$); Nutrient elemental context $D_{dissolved\ nutrient\ (N\ :\ P)}$ and (c) pigment community dissimilarity; (d) pigment diversity, $D_{Pigment\ diversity}$; Periphyton elemental context D periphyton (N : P) and (e) macroinvertebrate community dissimilarity; and (f) macroinvertebrate diversity, $D_{Macroinvertebrate\ diversity}$. Grey and white symbols represent mean values for *Elliptio* and *Lasmigona* respectively; circles and triangles represent low and high agriculturally impacted sites respectively. Black squares (Indian River) and diamonds (Fleetwood River) reflect results quantified from the manipulative field experiment comparing live mussels and sham shells. Black trend lines reflect significant relationships among variables for both species combined (field experiment excluded). Values in parentheses represent the percentage of points within a given quadrant (field experiment excluded).

ated with mussel excretion effect sizes ($D_{dissolved\ N\ :\ P}$) (Fig. 4c,d). Similarly, macroinvertebrate community dissimilarity and diversity effect size (Fig. 4e,f) were also positively associated with periphyton elemental imbalances among rocks and mussel shells ($D_{periphyton\ N\ :\ P}$). Chi-square analysis revealed that the distribution of data points among measures was non-random (E, $X^2 = 8.0$, $P < 0.005$; and F, $X^2 = 4.7$, $P < 0.05$), and that the importance of mussels at one trophic level is typically followed by a significant corresponding relationship at another trophic level (Fig. 4d,f).

Our manipulative experiment demonstrated that the N : P excreted by live mussels more than doubled the magnitude released by sham shells ($F_{indian} = 47.56$; $P < 0.05$; $F_{fleetwood} = 29.8$; $P < 0.05$)

(Fig. 5a). Algal elemental composition on live mussels also differed from sham shells at the Indian River site ($F_{1,9} = 11.15$; $P < 0.05$), but was marginally non-significant at the Fleetwood River site ($F_{1,7} = 4.66$; $P = 0.09$) (Fig. 4b). Mean macroinvertebrate and pigment diversity were significantly higher (18 and 23% respectively) on live mussels compared to sham shells at Indian River (Pigment: $F_{1,9} = 3.57$; $P < 0.05$; Macroinvertebrate: $F_{1,9} = 4.12$; $P < 0.05$). Both were also greater on live mussels at Fleetwood River (20 and 17% respectively), but marginally non-significant (Pigment: $F_{1,9} = 2.23$; $P > 0.05$; Macroinvertebrate: $F_{1,9} = 2.49$; $P > 0.05$), likely due to the decreased power resulting from loss of live mussels from predation (Fig. 5c,d).
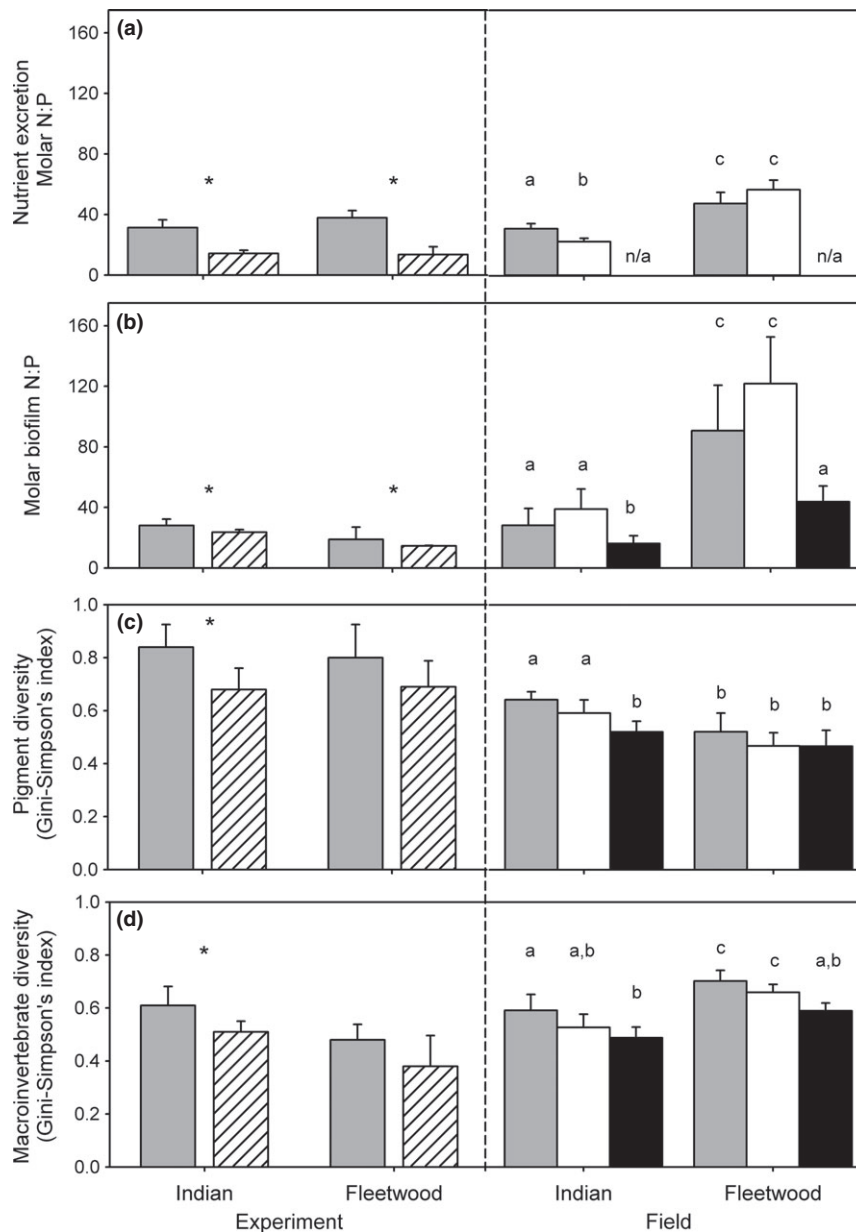
Figure 5 Comparison of experimental (left panel) and measured field (right panel) results at two sites minimally impacted by agriculture. (a) Mean release rates of nutrients (molar N : P) from live and sham *Elliptio complanata* shells. (b–d) Nutrient composition of attached biofilm (b); diversity of attached algal pigments (c); and diversity of attached benthic macroinvertebrates (d) on shells or rocks. The left portion of the panels reflects differences among live *E. complanata* (grey) and sham shells (hashed) (± 1 SE). The right portion of the panels reflect differences among live *Elliptio* sp. (grey) and *Lasmigona* sp. (white) mussels and rocks (black) quantified at the corresponding sites in the field (± 1 SE). Asterisks represent significant differences among sham and live mussels; letters denote significant differences ($P < 0.05$) among the two species of live mussels and rock.

DISCUSSION

We show that in stream ecosystems with low catchment agriculture, mussel nutrient excretion creates locally heterogeneous resource patches fostering a greater diversity of algae and macroinvertebrates. In contrast, with increasing catchment agriculture, the links between mussel-derived nutrients and community structure of benthic communities become less apparent; suggesting that mussel-derived niche construction is diminished by excessive nutrient loading.

This apparent reduction in importance at high agriculture sites could be explained from the perspective of the mussel (i.e. shifting physiology or behaviour of the mussels in ways that reduce their impact); or from the surrounding environment (i.e. the way or extent to which nutrients are cycled through the ecosystem); or both. We found that physiological metrics of mussel health declined with agriculture. For example, O : N, an indicator of anabolic vs. catabolic processes, declined across the gradient which may reflect a shift in either dietary (food source) or internal use (catabolism) of proteins over carbohydrates. However, since carbon isotopes often reflect food sources and mussel tissue $\delta^{13}C$ varied little across sites, it is unlikely that novel food items alone contributed to the altered signal of physiological condition. Rather, greater fractionation of mussel tissue

$\delta^{15}N$ under higher agriculture likely resulted from either nitrogen enrichment of common food sources, mussel-specific physiological responses to greater dietary nitrogen, or a combination of other factors associated with agriculture (i.e. pesticides, suspended solids). Nevertheless, most mussel-specific biochemical metrics (metabolism, nutrient excretion and lipid content) shifted across the gradient suggesting that both the physiological response and the extent of resource provisioning can be altered by landscape-level agricultural intensity. Despite declining physiological condition under greater agriculture influence, mussels provided *more* nutrients when the demand for nitrogen (as measured by extracellular enzymes) was lower and the supply rate from the watershed was higher; thus, physiological responses alone do not explain the patterns observed in our study.

Both algal and macroinvertebrate community structure on mussel shells differed from nearby rocks but only in the low agriculture sites; assemblages on shells and rocks at high agriculture sites became increasingly indistinguishable from each other. Differences in community structure at such small scales (~1 m) across the agricultural gradient could be explained by three non-exclusive mechanisms: (1) differential colonisation frequency of macroinvertebrate and algae propagules among mussels and rocks at low agriculture sites but equal colonisation at high agriculture sites, which is unlikely, (2) differences in post-colonisation development of the communities between substrates (population growth rates, species interactions, shuttling among patches), which emerge only at low agriculture sites due to resource/niche availability, or (3) differences in mussel burrowing behaviour among sites. This physical explanation would be applicable if greater burrowing activity at either the high or low agriculture sites resulted in disparate disturbance regimes on shells vs. rocks (e.g. Gribben *et al*. 2009; mussel response to an invasive algae). In our study, however, the fraction of burrowing did not appear to differ across sites and was unrelated to effects sizes that compared algal or macroinvertebrate communities among mussels and rocks. In contrast, a mechanism based on community development (scenario #2) is supported by the fact that effect sizes reflecting mussel-derived elemental heterogeneity (i.e. mussel-excreted − vs. ambient-dissolved nutrients and periphyton elemental composition on rocks vs. mussels) all significantly declined with increasing agriculture.

It seems more likely that resource differences among rocks and mussel shells accounted for differential success of colonisers at low agriculture sites and the nutrient 'swamping' in streams with high agriculture decoupled this pattern by collapsing resource heterogeneity. For example, while we found increased release rates of dissolved nitrogen and higher N : P release from mussels (Fig. 2d-f), the stream biotic demand for N and P decreased with increasing agriculture (Fig. 2g-i). Thus, even though the mussel's physiological response to increasing agriculture was to effectively supply more nutrients, such subsidies were only important in conditions where low nutrient concentrations occurred (Fig. 4a). Similarly, the extent to which periphyton elemental content differed on mussels and rocks ($D_{periphyton\ (N\ :\ P)}$) was also highly associated with relative stream biotic demand for N and P (Fig. 4b). Periphyton on mussel shells was more nitrogen-rich compared to rocks at sites where (1) ambient nitrogen supply was lowest and (2) mussels excreted the greatest amount of nutrients relative to ambient conditions.

The decoupling between mussel-derived nutrients and community structure in high agriculture sites extended across multiple trophic levels. For example, the degree to which algal pigments on rocks and mussels differed was positively associated with the extent to which excreted nutrients differed from ambient conditions ($D_{dissolved\ N\ :\ P}$) (Fig. 4c-d). This pattern was weakest in the high agriculture sites where nitrogen was abundant and less limited, likely reflecting a strong dominance of a single primary producer (in our case, filamentous algae such as *Cladophora*) on both rocks and mussels. Cardinale (2011) found similar dominance patterns in benthic algal community structure, with corresponding decreases in ecosystem function (measured as nitrogen uptake), when physical habitat heterogeneity was experimentally decreased. Following this trend, macroinvertebrate communities among mussels and rocks were more dissimilar when periphyton elemental composition on mussels differed from ambient rocks. These patterns were non-random, with more than half of all cases representing greater macroinvertebrate diversity on mussels when elemental content was also greater than rocks (Fig. 4f). Thus in terms of resource availability, pigment diversity was greater on mussels when they excreted more nutrients in all cases but one (Fig. 4d); and macroinvertebrate diversity on mussels always exceeded rocks when periphyton elemental ratios on mussels differed most from those than nearby rocks (Fig. 4f).

Our manipulative field experiment comparing live mussels to sham shells supported this pattern. At each site, both treatments were colonised from the same initial point (shells scrubbed clean), over the same duration of time (2 weeks), and in close spatial proximity (a few metres away from each other). At both sites, live mussels released more nutrients and had greater periphyton elemental content and diversity of pigments and macroinvertebrates compared to sham shells (Fig. 5). Moreover, when plotted alongside their corresponding field observations and effect size estimates for their respective sites, we found that they were similar in magnitude (Fig. 5) and within the same Cartesian space (Fig. 4f,h; quadrant 2). Admittedly, these are bottom-up interpretations, future studies that incorporate grazing effects or a combination of both bottom-up and top-down forces should also be considered.

We found a greater influence of mussel nutrient provisioning on benthic communities residing in resource depauperate, low agriculture streams, which, in terms of niche construction, demonstrates the importance of consumer-mediated resources on patch-level biodiversity (sensu McIntyre *et al*. 2008). Concepts that link resource heterogeneity to community structure and ecosystem functioning are rich in ecological theory (Chase 2005; Harpole & Tilman 2007). The majority of these, including those that also integrate spatial aspects of colonising species pools (Snyder & Chesson 2004; Tilman 2004; Harpole & Tilman 2007; Cardinale *et al*. 2009), are often considered from the perspective of resource limitation. In resource poor environments, greater biodiversity appears to be partly a function of resource subsidies beyond ambient conditions. These subsidies, whether generated from the environment (e.g. watershed, groundwater, atmosphere) and processed via microbes or from a particular species (be it an engineer, facilitator or mediator), can elevate resources beyond some threshold required for new species to survive, persist and co-exist with others.

Our results are generally consistent with these concepts; we found greatest dissimilarity in biodiversity between rocks and mussels at low agriculture streams where mussels created both stoichiometrically distinct and more abundant (6 × more concentrated) nutrients compared to the water column. While observed mere decimetres apart, our study suggests a mechanistic framework whereby local patch heterogeneity created by freshwater mussels can translate to observed patterns at greater spatial scales ranging from a few to sev-

eral hundred meters (Vaughn & Spooner 2006; Spooner *et al.* 2012). Our study also illustrates a larger point that cultural eutrophication and other stressors can short-circuit the ecological mechanisms through which communities relate to the functioning of ecosystems.

Annual human nitrogen use has increased from ~15 Tg yr^{-1} to 187 Tg yr^{-1} over the past century (Foley *et al.* 2005; Galloway *et al.* 2008) creating conditions of nutrient availability that are dramatically different from those under which species co-evolved over the past thousands to millions of years. Much like the globally changing balance of climate gasses, temperature and pH commonly associated with climate change, the effects of increased nitrogen are now being detected in far-flung ecosystems and are altering relationships between consumers and producers in pelagic ecosystems (Elser *et al.* 2009), coastal marine regions (Galloway *et al.* 2008) and terrestrial grasslands (Harpole & Tilman 2007). While other ecological functions performed by consumers may still be significant (e.g. biofiltration of suspended sediment, predation, physical engineering of habitat), our results broadly demonstrate that the relevance of consumer-driven nutrient recycling, which is an important mechanism that promotes local biodiversity, is diminished by nutrient loading in agriculturally influenced ecosystems. Much emphasis has been placed on species extinctions, biomass declines, and the time-lags, before which, these are experienced in ecosystems. Our results indicate that despite maintaining intact community structure, key ecological processes may still be lost. Given that nutrient cycling accounts for over half of the estimated value of global ecosystem services (Costanza *et al.* 1997), we assert that decoupling of these fundamental processes is significant in the streams and rivers that connect landscapes with the coasts and oceans.

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AUTHORSHIP

DES and MAX co-conceived the conceptual and methodological development of the study with input from PCF, DES collected, processed, interpreted and analysed the data, produced the figures and wrote the first draft. OP assisted with the collection and processing of field data and collected and analysed the experimental data. MAX, PCF, MTA and HH contributed to the data interpretation and editing of the drafts. All authors edited the final drafts. MAX coordinated the study.

CONFLICT OF INTEREST

The authors declare no competing financial interests.

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