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Abiotic conditions drive significant variability in nutrient processing by a common Caribbean sponge, *Ircinia felix*

Stephanie K. Archer ,[1]* **Julia L. Stevens,**[1,2] **Ryann E. Rossi,**[1] **Kenan O. Matterson,**[3] **Craig A. Layman**[1]
[1]Department of Applied Ecology, North Carolina State University, Raleigh, North Carolina
[2]Genomics and Microbiology, North Carolina Museum of Natural Sciences, Raleigh, North Carolina
[3]Department of Biology, University of Alabama at Birmingham, Birmingham, Alabama

Abstract

Coral reefs typically occur in oligotrophic waters, where tight recycling of energy and nutrients is essential in order to support their high productivity. Sponges are efficient filter feeders that host diverse and abundant microbial communities that often contain members capable of carrying out complex nutrient transformations. Consequently, sponges often act as significant sources of bioavailable forms of nitrogen and phosphorus while acting as sinks for dissolved organic carbon (DOC). However, little attention has focused on variability of nutrient release by sponges and no studies have reported how abiotic conditions may impact sponge-driven changes in nutrient concentrations. Here, we show that a common Caribbean sponge, *Ircinia felix*, is capable of being both a source and a sink for DOC, ammonium, nitrate/nitrite (NO_x^-), and phosphate (PO_4^{3-}). Additionally, we show that abiotic conditions, particularly ambient nutrient availability, seem to explain a significant amount of the variability (R^2 range from 0.40 to 0.65). Interestingly, as ambient nutrient concentrations increased, *I. felix* transitioned from acting as a source to serving as a sink for all nutrient forms measured. We also found *I. felix*-associated bacteria exhibit a significantly higher abundance of predicted nitrogen metabolism, carbon fixation, and photosynthetic genes relative to ambient water and sediment. These results suggest that sponges play an important and dynamic role in biogeochemical cycling on reefs, particularly as human activities alter natural nutrient dynamics in coastal systems.

Coral reefs provide a wide range of services and are thus among the world's most valued ecosystems (Costanza et al. 1997, 2014; de Groot et al. 2012). Many of these services (e.g., food production) are linked to high productivity resulting from efficient recycling of energy and nutrients within the coral reef (hereafter reefs). Sponges, a common component of the benthic community in Caribbean reef ecosystems, contribute to this tight recycling of energy and nutrients through their efficient filter feeding (Reiswig 1971*b*). Additionally, sponges host large microbial communities which are capable of capturing and transforming dissolved forms of energy and nutrients (de Goeij et al. 2008; Fiore et al. 2010; Webster and Taylor 2012; Freeman et al. 2013). As a result, sponges play an important role in maintaining the productivity of reef systems (Diaz and Rutzler 2001; de Goeij et al. 2013).

Sponge holobionts (the sponge and their associated microbiome) contribute to the high productivity and efficient recycling of energy on reefs in two ways. First, many sponges host photosynthetic cyanobacteria in their tissues, with some sponges receiving the majority of their nutrition from these symbionts (Erwin and Thacker 2007, 2008; Thacker and Freeman 2012). Second, although corals and their symbiotic zooxanthellae, macroalgae, and phytoplankton are critical for reef productivity, they inefficiently leach sugars into the water column in the form of dissolved organic carbon (DOC) (Haas et al. 2010). Sponge holobionts can take up DOC and do so at high rates, facilitating the retention of energy within the reef ecosystem (de Goeij et al. 2013).

Sponges also transform limiting nutrients on reefs into forms that can stimulate primary production (Maldonado et al. 2012). High microbial abundance (HMA) sponges typically host microbial communities 2–4 orders of magnitude denser than the surrounding seawater (Reiswig 1981; Hentschel et al. 2012). These microbial communities are also significantly different than those of seawater, including microbes associated with complex nitrogen (N) and phosphorus (P) transformations (This study, Hentschel et al. 2006; Fiore et al. 2010; Sabarathnam et al. 2010). Nutrient fluxes through sponges are partially regulated by the

*Correspondence: skraftarcher@gmail.com

Additional Supporting Information may be found in the online version of this article.

exchange of resources (e.g., nutrients, photosynthate) between the sponge and their microbial symbionts (Thacker and Freeman 2012; Freeman et al. 2013; Fiore et al. 2015). Nutrient transfer symbioses are often influenced by the abiotic conditions in which they occur (Kiers et al. 2010; Johnson et al. 2013; Shantz and Burkepile 2014), and there is evidence to suggest sponge-microbe symbioses are no different (Freeman et al. 2013). As such, it is reasonable to suspect that DOC and inorganic N and P fluxes through the sponge holobiont will vary along with abiotic conditions. However, past studies have not quantified nutrient flux across broad spatial and temporal scales (but see Fiore et al. 2013).

In this study, we quantified the change in DOC, N (NH_4^+ and NO_3^- and NO_2^-), and phosphate (PO_4^{3-}) concentrations in water passing through a common species of sponge, *Ircinia felix*, at a total of nine reefs distributed across three Caribbean and sub-tropical Atlantic Islands. We examined correlations between the change in nutrient concentrations attributable to this sponge and a suite of abiotic variables including: ambient nutrient availability, water temperature, and light intensity. In a follow-up study, we collected both water samples and sponge tissue samples. Then, using bacterial amplicon sequencing we employed predicted metagenome algorithms to correlate the change in inorganic N with predicted microbial nitrogen metabolism genes. Predicted metagenomes were also analyzed to compare nitrogen metabolism, carbon fixation, and photosynthetic genes between sponge, sediment, and water.

Methods

Study species

Ircinia felix is a common ball shaped sponge on reefs throughout the subtropical and tropical Atlantic and Caribbean (Diaz 2005; Loh and Pawlik 2014). *I. felix* is classified as a HMA sponge (Weisz et al. 2008) and is known to host photosynthetic cyanobacterial symbionts (Erwin and Thacker 2007) and act as a source of nitrogen (ammonium (NH_4^+) and nitrate/nitrite (NO_3^- and NO_2^-, hereinafter referred to as NO_x^-)) (Southwell et al. 2008). To our knowledge DOC and PO_4^{3-} processing has not been measured previously in *I. felix*.

Sampling sites

Sponges were sampled at nine reefs surrounding three islands: Great Abaco Island, The Bahamas (26°28′N 77°05′W; 25 June 2014–28 June 2014), New Providence Island, The Bahamas (25°02′N 77°24′W; 12 June 2014–14 June 2014), and Curaçao (12°7′N 68°56′W; 01 August 2014–09 August 2014) (Fig. 1). The islands vary greatly in their geology and location. The Bahamian islands are limestone platforms with large expanses of shallow seas, while Curaçao is an old volcanic island with fringing reefs close to shore (Bak 1977; Buchan 2000). Great Abaco Island is the largest of the three islands at approximately 1681 km^2, Curaçao is the next largest at 444 km^2, and New Providence is the smallest at 207 km^2; the islands follow an opposite pattern of human density at 10, 339 and 1190 people km^{-2}, respectively (Department of Statistics of the Bahamas 2012; Curaçao Central Bureau of Statistics 2014).

InEx sampling

Six sponges were sampled at each reef, with the exception of one reef on Great Abaco Island where three sponges were sampled (total $n = 51$). At each site, HOBO® Data Loggers (Onset Computer Corp.) were placed next to each sponge to record water temperature every hour for 24 h. At least one data logger at each site was also equipped to record irradiance at depth. Both patch and fringing reefs were sampled at depths between 2 m and 15 m.

The InEx sampling method accurately samples changes in nutrient concentrations as water passes through a sponge by simultaneously collecting ambient water entering the sponge (in-current) and exiting the sponge (ex-current) (Yahel et al. 2005). Full exchange of the ex-current sampling tube was ensured by first holding an identical tube filled with fluorescein dye just above the sponge's osculum and recording the total time required to clear the tube of the dye. The ex-current sampling tube was then held just above the same osculum for 1.5X the time it took to clear the dye from the tube, while a second person slowly drew back the plunger of a syringe held near the base of the sponge to collect water entering the sponge (in-current). The difference between the in-current and ex-current values is attributable to processes occurring within the sponge (*see* Yahel et al. 2005 for additional details). In-current water samples provided ambient nutrient concentrations. Three in-current and three ex-current samples were collected from each sponge. From these samples, we measured DOC, dissolved inorganic nitrogen (NH_4^+ and NO_x^-), and PO_4^{3-}. Samples for ammonium, NO_x^-, and PO_4^{3-} were filtered with a 0.45 μm Whatman nylon-membrane filter into scintillation vials which had been soaked in a 10% HCl acid solution for 24 h then triple rinsed with deionized water (B-pure Pressure Cartridge System (Thermo Scientific) Series 583). Both the filter and scintillation vial were twice rinsed with $\sim$ 2 mL of sample water. DOC samples were filtered through a 0.7 μm Whatman GF/F glass fiber filter into pre-combusted glass ampoules with 1–2 drops of concentrated H_3PO_4 (80%). Both filters and glass ampoule were pre-combusted at 450°C for 4 h. All filtration equipment was acid washed as described above and twice rinsed with sample water prior to sample retention. All samples were placed on ice and frozen (or refrigerated in the case of DOC) for transport to North Carolina State University (NCSU). Ammonium was determined using the indophenol blue method. Phosphate was analyzed using the method described by Murphy and Riley (1962). DOC and NO_x^- samples were sent to the NCSU Environmental and Agricultural Testing Service and the University of Georgia Analytical Chemistry Lab, respectively, for analysis.

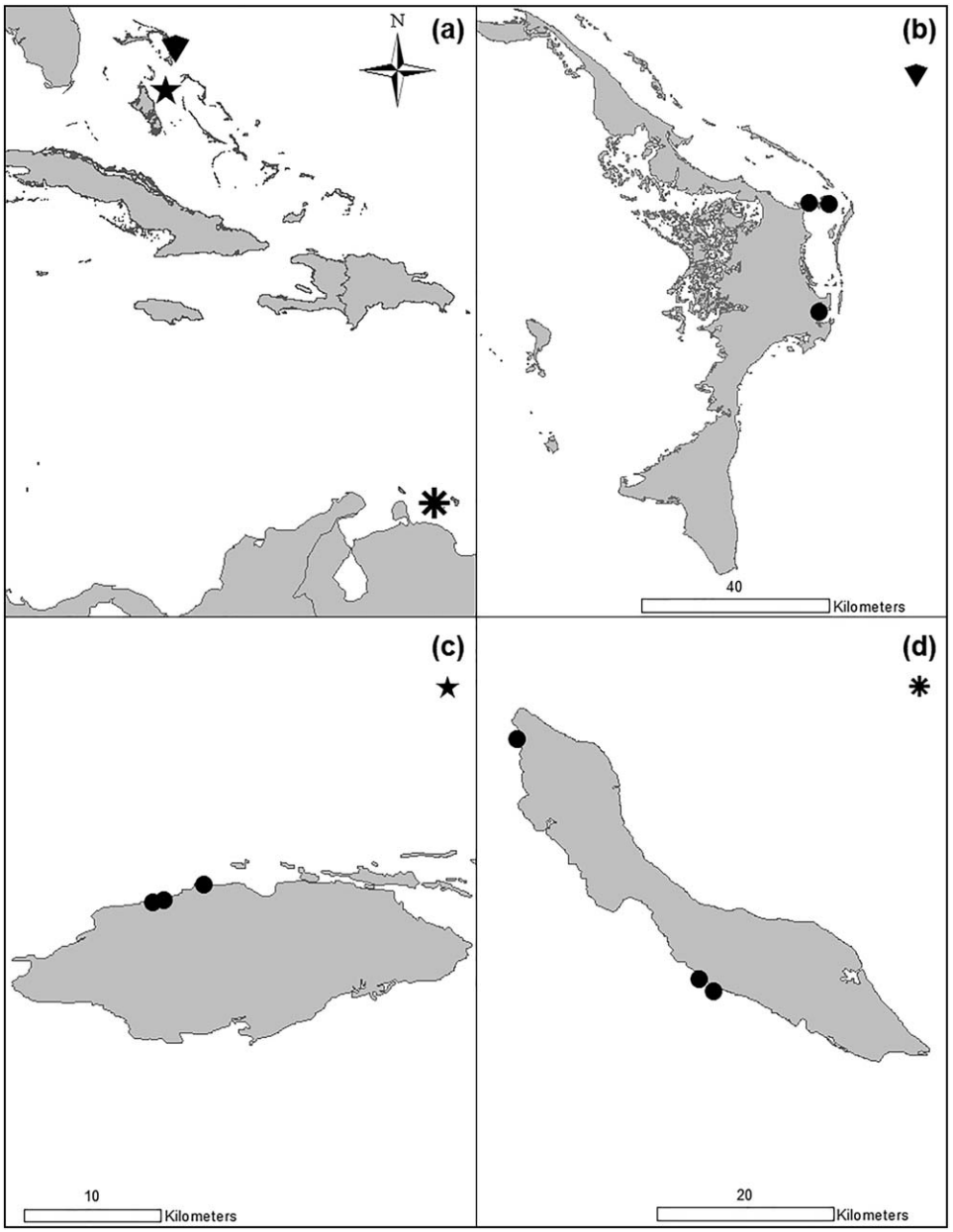

Fig. 1. Caribbean overview (**a**) and island specific map of sampling locations on Abaco (**b**), New Providence (**c**), and Curaçao (**d**).

Bacterial assemblage and nitrogen processing

In order to correlate inorganic nitrogen changes with sponge bacterial assemblage, 20 sponges were sampled separately from Great Abaco Island, The Bahamas. Following InEx sampling of these sponges, an approximately 1 cm^3 portion of sponge was collected. Sediment directly adjacent to each sponge was collected into plastic bags and transferred immediately into 2 mL cryovials filled with RNAlater. To obtain a sample of the water column bacteria the filter used for processing the in-current sample was preserved for microbial extraction. Samples remained frozen for transport to the laboratory for DNA extraction. The water samples collected from these sponges were analyzed for ammonium and NO_x^- concentrations using the methods described above.

Total community DNA was extracted from each sponge, sediment, and water sample using the MoBio PowerSoil DNA Isolation kit according to manufacturer's recommendations. The following adjustments were made to the isolation protocol: 25 mg of starting material was used followed by a maceration step of sponge and filter material. The V4/V5 region of the 16S rRNA gene was amplified using the 515F/806R primer pair (Caporaso et al. 2012). PCR was conducted in triplicate using the following reagent concentrations: 0.2 μM of each primer, 1.25 U 5-Prime PerfectTaq DNA polymerase,

5 μL 10X PCR buffer, 2.5 μL $MgCl_2$, 0.4 μM dNTPs, 1 μL template DNA, and PCR grade water to 25 μL. Amplification conditions were as follows: 3 min initial denaturation at 94°C, followed by 25 cycles of 94°C for 45 s, 58°C for 1 min, and 72°C for 90 s with a final extension at 72°C for 10 min. Triplicate PCR products were pooled and remaining enzymes degraded using ExoSap-IT (Affymetrix) following manufacturer's protocol.

Cleaned PCR products were indexed using Nextera XT sample prep kit (Illumina) with Phusion Taq. A final PCR clean up was conducted using AxyPrep Mag PCR clean-up kit (Axygen) and final concentration quantified using the Qubit 2.0 Fluorometer. Individual, indexed samples were pooled in equimolar ratios and prepared for sequencing following the Illumina guide for preparing 16S rRNA gene amplicons and sequenced on the Illumina MiSeq platform. Raw sequences are archived at the Short Read Archive (PRJNA342549).

Sequences were processed and quality controlled with QIIME (Caporaso et al. 2010) and MOTHUR (Kozich et al. 2013). A total of 3,070,183 sequences were processed (Supporting Information Table S1) through quality control, chimera checking using vsearch, OTU clustering with the uclust algorithm, and pynast alignment to the Ribosomal Database Project (Wang et al. 2007). The final OTU table was rarefied to 6062 sequences $sample^{-1}$ for downstream analyses. Predicted metagenomes to characterize potential bacterial community function against sequenced KEGG pathway orthologs were generated using the PICRUSt pipeline (Langille et al. 2013) on the Galaxy online interface. While PICRUSt provides a predictive gene annotation, its interpretation is limited by the quality and depth of sequencing. Namely, the program relies on 16S rRNA sequencing which does not report strain level variation. Despite these limitations, predicted gene annotations have been shown to significantly correlate with whole genome shotgun sequencing (Langille et al. 2013). A script of the complete, annotated sequence process pipeline can be downloaded from https://github.com/JuliaLStevens/abaco-sponges.

Statistical analysis

For all nutrient metrics, we evaluated the change in nutrient concentrations attributable to metabolic or microbial processes occurring within the sponge. For the remainder of the manuscript we will refer to this as the change in the nutrient (e.g., change in DOC). Change in nutrient concentration was calculated as the difference in ex-current and in-current concentrations (i.e., ex-current – in-current for DOC, NH_4^+, NO_x^-, PO_4^{3-}). As a result, positive values of the response variables indicated that the sponge was a source of that nutrient while a negative value indicated that the sponge acted as a sink. Individual sponges were assigned as either sources, sinks, or no measurable change by comparing the values of the triplicate samples against zero in a two-tailed *t*-test. If the change in a nutrient was significantly different than zero it was assigned as either a source (a positive change) or a sink (a negative change). If the change in the nutrient was not significantly different than zero the sponge was considered to show no measurable change for that nutrient.

Any two predictor variables (Table 1) with a coefficient of correlation higher than 0.50 were considered correlated. All possible combinations of predictor variables (excluding combinations including correlated variables) were evaluated and the best fit model was selected using corrected Akaike's Information Criteria (AICc) and model weights. The best fit model was evaluated for overall fit using adjusted R^2. The effects of individual predictor variables were evaluated using both the coefficients from the linear model as well as partial R^2 values, designated throughout the remainder of the manuscript as η^2.

To compare overall bacterial community structure between sponges, sediment, and water permutational analysis of varience (PERMANOVA) based on the Bray–Curtis dissimilarity metric was employed using the vegan package in R. Linear regression was employed to evaluate potential correlation between the nitrogen metabolism gene abundance from predicted metagenomes of 20 *I. felix* and the change in dissolved inorganic nitrogen (ammonium + NO_x^-) of water moving through the sponges. Prior to analysis predicted gene abundance was log10 transformed. To compare predicted abundance of nitrogen metabolism, carbon fixation, and photosynthetic genes between sponges, water, and sediment, one-way analyses of variance (ANOVA) with Bonferroni correction for multiple comparison.

Results

Within sponge variation was small with an average of 2.53% (range: 0.18–5.12%) difference between the triplicate samples. The mean value for each sponge was used in data analyses. When averaging the values for all islands, *I. felix* was a sink for DOC and NH_4^+ and a source of NO_x^- and PO_4^{3-} across all sites, although only the change in DOC was significantly different than zero ($t_{50} = -3.06$, $p = 0.004$). However, the changes in nutrient concentrations were extremely variable for all nutrients measured (Table 2) and sponges acting as either a source or a sink were observed on all islands for all nutrients (Table 3; Supporting Information Fig. S1).

The best fit models adequately explained variance in the change in all nutrients; adjusted R^2 values ranged from 0.40 for PO_4^{3-} to 0.65 for NO_x^- (Table 4; top 20 models for each response variable can be found in Supporting Information Tables S2–S5). No single predictor variable was important for all nutrients measured. However, for all nutrients the ambient availability of the nutrient was the strongest predictor for the change in that nutrient. Also, in all cases, *I. felix* transitioned from being a source to a sink of the nutrient as ambient nutrient concentrations increased (Table 4; Supporting Information Figs. S2–S5). Interestingly, light and

Table 1. Potential explanatory variables included in model selection. Variables with matching symbols were correlated. No models were run containing both variables. Light and temperature summary statistics were calculated from values recorded over 24 h surrounding sampling, unless otherwise indicated. All variables (other than the sponge-specific variables) were correlated with both Island and Reef, therefore the only model considered with either Island or Reef as a predictor variable was the model with location as the only predictor variable

Light (Lux)	Ambient nutrients (μmol L^{-1})	Temperature (°C)	Sponge	Location
Maximum§	DOC	Maximum*	Tube clearance rate	Island
Median§	NH_4^+	Median*	(proxy for pumping rate)	Reef
Light at time of sampling	NO_x^-	Minimum*	Volume	
	PO_4^{3-}	Variance§		
		Temp at time of sampling*		

Table 2. Mean and standard deviation (SD) (μmol L^{-1}) for the change in nutrients attributable to processes occurring within the sponge. A negative value indicates the sponge is a sink for the nutrient while a positive value indicates the sponge is a source. Values significantly different than 0 (as determined using a *t*-test and Bonferroni correction for multiple comparisons) are indicated by a *.

	DOC	NH_4^+	NO_x^-	PO_4^{3-}
Overall				
Mean	−36.68*	−0.07	0.06	0.08
SD	85.74	0.80	0.26	0.91
Abaco				
Mean	−40.46	−0.02	−0.001	0.41
SD	114.61	1.17	0.28	1.08
Curaçao				
Mean	−1.94	−0.36*	0.17*	0.04
SD	41.69	0.64	0.29	0.96
New Providence				
Mean	−68.27*	0.17	0.002	−0.17
SD	81.96	0.43	0.18	0.61

temperature at the time of sampling did not correlate with any of the observed variation in all nutrients we evaluated. Rather median light (NO_x^-), minimum temperature (NH_4^+), and temperature variance (DOC and NH_4^+) measured over a 24 h period including the time of sampling were correlated with nutrient processing (Table 4). Sponge-specific variables were not universally correlated with change in nutrient concentrations. Sponge volume was an important predictor of both DOC and NO_x^- with larger sponges acting as larger sources of these nutrients. Sampling tube clearance time, which we used as a proxy for sponge pumping rate, was included in the best fit model for both PO_4^{3-} and NO_x^- but was only significant for PO_4^{3-}. As tube clearance time increased (slower sponge pumping) *I. felix* went from being a source to a sink for PO_4^{3-}. Although it was not significant, the same pattern holds for NO_x^-.

Table 3. The number of sponges acting as a source, a sink, or with no measurable change (NMC) for each nutrient by island. Individual sponges were assigned as either sources, sinks, or no measurable change by comparing the values of the triplicate samples against zero in a two-tailed *t*-test. If the change in a nutrient was significantly different than zero it was assigned as either a source (a positive change) or a sink (a negative change). If the change in the nutrient was not significantly different than zero the sponge was considered to show NMC for that nutrient.

	DOC	NH_4^+	NO_x^-	PO_4^{3-}
Abaco				
Source	4	9	10	7
Sink	10	6	5	4
NMC	1	0	0	4
Curaçao				
Source	8	3	16	9
Sink	9	14	2	9
NMC	1	1	0	0
New Providence				
Source	3	10	10	6
Sink	15	6	7	11
NMC	0	2	1	1

Bacterial assemblage and nitrogen processing

The bacterial diversity in *I. felix* samples was significantly different from that found in both the ambient seawater and adjacent sediment ($R^2 = 0.61$, $p < 0.001$). While overall diversity was higher in the sediment samples, the abundance of functional genes associated with nutrient processing were predicted to be significantly higher in sponge samples relative to both sediment and water ($F_{2,41} = 46.44$, $p < 0.0001$, Fig. 2). Similarly, carbon fixation and photosynthetic gene abundance was higher in sponges than water and sediment (Fig. 2).

While not a significant correlation, the abundance of predicted nitrogen metabolism genes tended to increase with an increasing change in DIN attributable to the sponge

Table 4. Overall model fit statistics and parameter coefficients, *p*-values, and η^2 in the best fit model for each response variable.

Response Variable		Light	Ambient nutrients				Temperature		Sponge		Model Statistics			
		Median	DOC	NH_4^+	NO_x^-	PO_4^{3-}	Min	Var	Pumping	Volume	DF	*F*	*p*-value	R^2
	Coefficient		−0.88					114.85		12.35				
DOC	*p*-value		<0.0001					0.02		0.01	3,47	12.38	<0.0001	0.41
	η^2		0.39					0.10		0.13				
	Coefficient		−0.003	−1.19			0.39	1.05						
NH_4^+	*p*-value		0.04	<0.0001			<0.0001	0.01			4,46	20.91	<0.0001	0.61
	η^2		0.09	0.60			0.47	0.13						
	Coefficient	−0.0001			−0.98				−0.001	0.02				
NO_x^-	*p*-value	0.002			<0.0001				0.11	0.05	4,46	24.3	<0.0001	0.65
	η^2	0.19			0.65				0.04	0.07				
	Coefficient					−0.86			−0.01					
PO_4^{3-}	*p*-value					0.0001			0.003		2,48	17.35	<0.0001	0.40
	η^2					0.27			0.17					

($R^2 = 0.13$). In other words, as sponges transitioned from acting as a sink of DIN to a source there was a trend toward relatively higher abundances of genes associated with nitrogen metabolism (Fig. 3). Consistent with the first study, *I. felix* transitioned from being a source to a sink of both ammonium and as NO_x^- ambient nutrient concentrations increased (Supporting Information Figs. S6–S7).

Discussion

This study demonstrates that there can be significant variability in nutrient processing in sponges and that to truly understand sponges' role in reef ecosystem function we must gain a better understanding of the conditions governing the outcome of sponge-microbe symbioses. Many sponges host abundant and diverse microbial communities that include members capable of complex chemical transformations (Fiore et al. 2010; Thacker and Freeman 2012). This, in addition to sponges' ability to pump large amounts of water (Reiswig 1971*a*), results in sponges having a large impact on nutrient dynamics (Southwell et al. 2008; Maldonado et al. 2012; Kahn et al. 2015). Despite recording large variances, previous studies have concentrated on mean sponge nutrient fluxes when discussing sponges' impact on nutrient cycling on reefs (Corredor et al. 1988; Diaz and Ward 1997; Southwell et al. 2008 but see Fiore et al. 2013). However, we suggest that variability in nutrient processing in sponges is an extremely important process to understand, as individuals within the same species of sponge are capable of acting as both a source and a sink for a wide range of nutrients. For the first time we show that variability in sponge nutrient processing is correlated with both abiotic conditions and sponge specific variables.

Although many of our abiotic variables differed among sites, location was not a primary predictor of sponge nutrient processing for any of the nutrients measured (Table 4). Instead, abiotic variables including ambient nutrient availability, light, and temperature were significantly correlated with the change in nutrient concentrations attributable to *I. felix*. Moreover, it is important to note that our study is observational. While significant correlative associations are evident between the abiotic environment and sponge nutrient processing, it does not imply a causal relationship. However, our study does reveal some interesting patterns that warrant further investigation.

Our results show a similar pattern for all forms of nutrients we investigated; as an ambient nutrient concentration increased, *I. felix* transitioned from being a source of that nutrient to acting as a sink (Supporting Information Figs. S1–S4). Additionally, we found that as sponges become sources of DIN the predicted nitrogen metabolism of their microbial community also increases. Together, these results suggest a context dependency in either the composition of the active members of the sponge microbiome or in sponge-microbial interactions (Fiore et al. 2010; Maldonado et al. 2012; Thacker and Freeman 2012). Traditionally the microbial community within sponges has been considered spatially and temporally stable (Erwin et al. 2012; Simister et al. 2012). However, recent work has shown that the active members of sponges' microbial communities can change, sometimes over short temporal scales (Zhang et al. 2014; Fiore et al. 2015). Our results support both of these observations, as predicted nitrogen metabolism within the microbial community increased as ambient DIN availability decreased despite the overall community structure remaining stable. Further work is needed to determine if the patterns observed in this study represent a true cause and effect relationship. Additionally, subsequent investigations are necessary to determine if this pattern is consistent across other sponges.

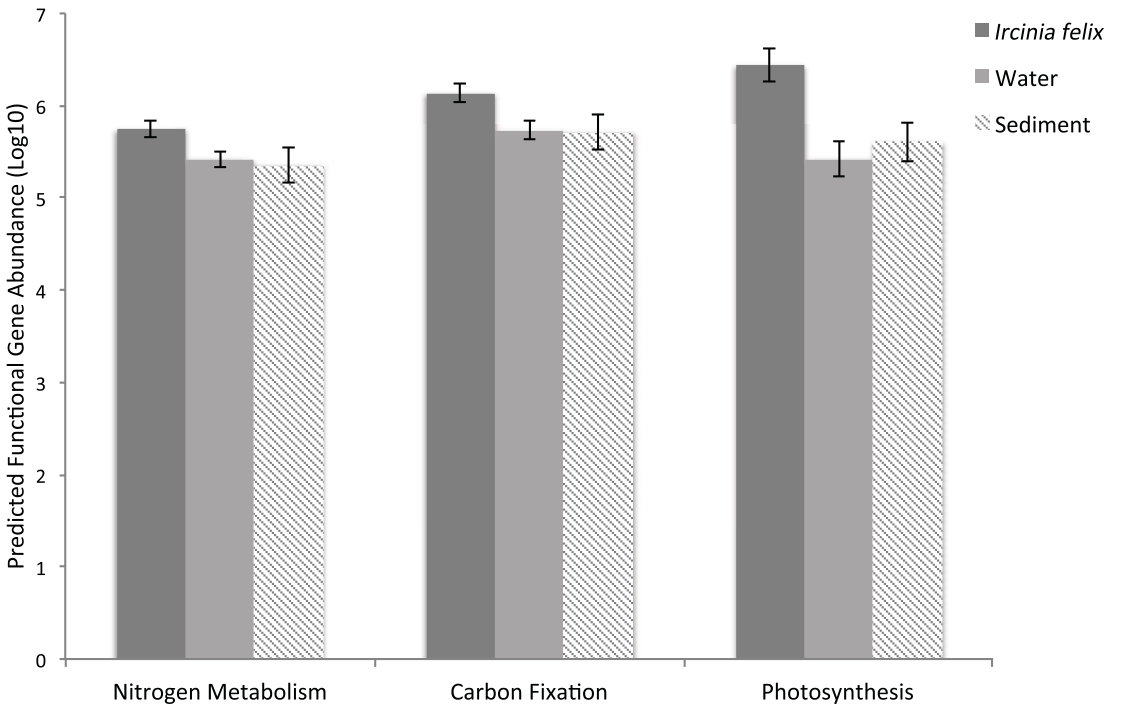

Fig. 2. Predicted abundances for functional genes associated with nutrient processing compared among sponges, water, and sediment. For all three functional groups sponges have a significantly greater abundance of nutrient processing genes relative to ambient water and adjacent sediment.

There is some evidence to suggest that our findings are part of a consistent pattern, e.g., Fiore et al. (2013) found a similar patter with another HMA sponge common to Caribbean reefs, *Xestospongia muta*. Additionally, McMurray et al. (2016) found *X. muta* increased uptake of DOC as ambient DOC concentrations increased, mirroring our results with *I. felix*. If widespread, the results of this study could have important implications for reef biogeochemistry, as well as for ecosystem responses to anthropogenic nutrient loading (Vitousek et al. 1997).

The changes in both DOC and PO_4^{3-} observed were largely explained by ambient nutrient concentrations (DOC and PO_4^{3-}, respectively) with less variation explained by sponge volume (DOC) and pumping rate (PO_4^{3-}). Temperature variability was also an important predictor of the change in DOC, although a mechanism for this is unclear. Both changes in DOC and PO_4^{3-} within the sponge can be driven by similar processes, sponge and microbial consumption (increase uptake) and metabolic waste (increase output) (de Goeij et al. 2008; Rix et al. 2016*a*,*b*). Although from our data it is impossible to determine the ultimate cause of increased DOC and PO_4^{3-} uptake by sponges, a plausible hypothesis is that the microbial community capable of utilizing these nutrients is stimulated by their availability. For example, we found that the microbial community associated with *I. felix* has a high potential for carbon fixation and photosynthesis, consistent with the findings of Erwin and Thacker (2007). If ambient phosphorus concentrations are low enough to limit primary production, any increase in PO_4^{3-} could stimulate primary production by the sponge's photosymbionts and ultimately result in increased utilization of nutrients by the microbial community within the sponge. If the pattern of increased DOC uptake with increasing DOC availability

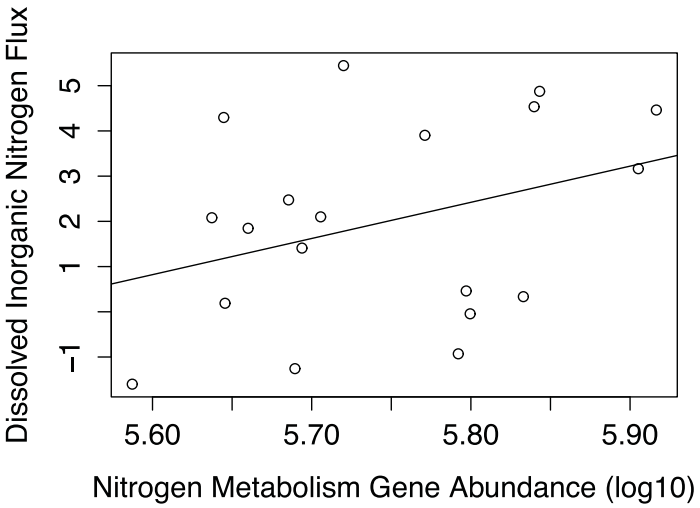

Fig. 3. Correlation between predicted abundance of genes associated with nitrogen metabolism and the total change of inorganic nitrogen following sponge processing. Multiple R^2: 0.13; $F_{1,17}$: 2.52; p-value: 0.13.

documented here in *I. felix* and by McMurray et al. (2016) in *X. muta* is a true pattern, this could have interesting implications for reef energy cycling and productivity.

Although both *I. felix* and *X. muta* are HMA sponges and transfer of DOC from microbial symbionts to the host sponge has been shown (Freeman and Thacker 2011; Freeman et al. 2013), both HMA and LMA sponges can directly utilize dissolved organic matter (DOM), in particular DOC (de Goeij et al. 2008; Rix et al. 2016*a*,*b*). This suggests that direct uptake of DOC by sponge cells may play an important role in carbon cycling on reefs. Because LMA sponges typically pump at much higher rates than HMA sponges (Weisz et al. 2008), their role in DOC cycling on reefs may be considerable. Therefore, further work clarifying if the context dependent patterns shown here are driven by the response of the microbial community, sponge cells, or both will have large implications for our understanding of the drivers governing carbon cycling on reefs.

Ammonium and NO_x^- processing in sponges are inextricably linked. Both NH_4^+ and NO_x^- can be used by photosynthetic symbionts or converted to N_2 gas through anaerobic ammonium oxidation (anammox) or denitrification, respectively. Ammonium can be converted to NO_x^- by microbial symbionts through nitrification. Finally, ammonium can be generated by microbes within the sponge via nitrogen fixation and through ammonification during regular metabolic activity. Although all of these processes have been documented within sponge holobionts (Hoffmann et al. 2009; Fiore et al. 2010; Schlappy et al. 2010*a*; Han et al. 2013; Zhang et al. 2013; Zhang et al. 2014), oxygenation of sponge tissue at any given time will determine the relative rates of the various nitrogen transformation. Nitrification, ammonification, and uptake by photosymbionts typically (or obligately) occur in oxic environments while nitrogen fixation, denitrification, and anammox must occur in anoxic environments. There is evidence that sponges can simultaneously have oxygenated and anoxic portions of their mesohyl (where the majority of microbial symbionts reside) creating diverse microhabitats that favor different nitrogen transformations (Schlappy et al. 2010*b*). However, the sponge's pumping rate is also correlated with oxygen levels with tissues rapidly becoming anoxic as pumping ceases (Hoffmann et al. 2008). Therefore, we expected to see a decrease in NO_x^- released by *I. felix* as pumping rates slowed. This pattern was observed in all sponges sampled, as the time taken to clear the sampling tube increased (slower pumping rate) the change in NO_x^- decreased with *I. felix* transitioning from source to sink.

We did not observe a relationship between sponge pumping rate and ammonium output. This is not surprising, as processes contributing to increased (ammonification, fixation) and decreased (nitrification, anammox) ammonium output occur in both oxygenated and anoxic conditions. The two strongest predictors of the change in ammonium concentrations were minimum temperature and ambient NH_4^+ concentration. As minimum temperature increased *I. felix* released increasing amounts of ammonium. A source of ammonium within the sponge, ammonification, is the result of sponge metabolic processing, which should increase with temperature. There are several potential drivers of the observed negative correlation between ambient ammonium and the release of NH_4^+ by *I. felix*. These include increased activity by photosymbionts (nitrogen is often limiting for primary production in oligotrophic environments) (Vitousek and Howarth 1991; Allgeier et al. 2010), increased nitrification or anammox, or a decrease in nitrogen fixation. These potential drivers are not mutually exclusive and, unfortunately, here we cannot conclusively distinguish among them.

Because of the conversion of ammonium to NO_x^- that occurs during nitrification, and the fact that *I. felix* had been previously reported as a nitrifying sponge, we expected to observe a correlation between ambient ammonium concentrations and the change in NO_x^- concentrations. Bayer et al. (2008) reported such a correlation for *Aplysina aerophoba*; when they increased ambient ammonium values, NO_x^- release increased considerably. However, they found large seasonable variation in NO_x^- release and were only able to stimulate NO_x^- production via ammonium additions when sponges were already producing NO_x^-. Interestingly, restricting analysis to only *I. felix* individuals acting as sources of NO_x^- we also see a strong relationship between ambient ammonium and the change in NO_x^- concentrations (Fig. 4). However, this correlation only extends up to ambient ammonium concentrations of ~ 1.2 μmol L^{-1}. We recorded a minimum number of observations with ambient ammonium concentrations above 1.2 μmol L^{-1}, therefore it is difficult to tell if this is a true threshold or an artifact of our data. Regardless, the similarity of our results with those from Bayer et al. (2008) are intriguing. It appears as though something other than ammonium availability controls whether or not the community of nitrifying microbial symbionts within sponges are active and when the nitrifying community is active increasing ammonium also increases NO_x^- output. Future research should focus on determining the drivers of activity in the nitrifying community within sponges in order to accurately understand sponges' true impact on nitrogen cycling.

Our results show that the change in nutrient concentrations attributable to sponges are extremely variable and correlated with abiotic and biotic conditions. Here we contribute to a growing body of work demonstrating that sponges play a critical and underappreciated role in nutrient cycling on reefs (Maldonado et al. 2012). We showed that the microbial community within *I. felix* has a significantly higher potential for nitrogen metabolism, carbon fixation, and photosynthesis than the microbial communities in either the sediment or the water column. This result supports the conclusions of Southwell et al. (2008) who found

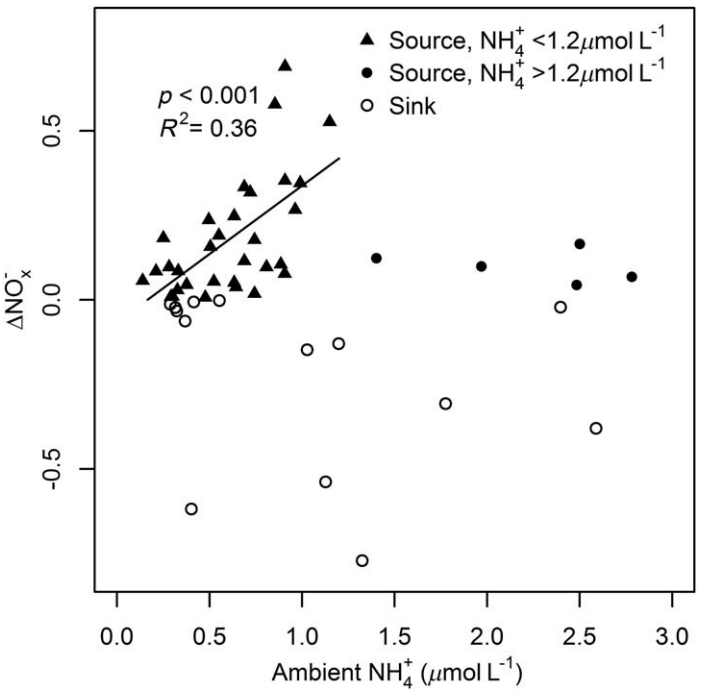

Fig. 4. Correlation between ambient ammonium (μmol L^{-1}) and the change in NO_x^-. When analysis is restricted to *Ircinia felix* individuals acting as sources of NO_x^- there is a strong correlation between change in NO_x^- and ambient ammonium concentrations up 1.2 NH_4^+ μmol L^{-1}.

that the sponge community contributes more to nitrogen cycling on reefs than sediments. However, we show that using mean nutrient flux measurements greatly oversimplifies the potential impact of sponge nutrient processing on reef biogeochemical cycles. Most importantly our results suggest sponge nutrient processing may be strongly context dependent with ambient conditions influencing the composition of the active component of the microbial community. Understanding the relationship between observed changes in nutrient concentrations and abiotic conditions is imperative if we hope to understand how reef functioning will respond to global environmental change, particularly as sponges are predicted to become a more prevalent component of reef ecosystems (Bell et al. 2013; McMurray et al. 2015).

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Conflict of Interest

None declared.

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