Received: 19 May 2018 | Revised: 18 March 2020 | Accepted: 19 March 2020

DOI: 10.1111/mec.15417

ORIGINAL ARTICLE

MOLECULAR ECOLOGY WILEY

Gene correlation networks reveal the transcriptomic response to elevated nitrogen in a photosynthetic sponge

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Funding information
Australian Research Council, Grant/Award Number: FT120100480; Charles Darwin University

Abstract

Nutrient levels in coastal environments have been increasing globally due to elevated inputs of sewage and terrigenous sediments carrying fertilizers. Yet, despite their immense filtering capacities, marine sponges appear to be less affected by elevated nutrients than sympatric benthic organisms, such as corals. While the molecular-level stress response of sponges to elevated seawater temperatures and other toxicants has been defined, this study represents the first global gene expression analysis of how sponges respond to elevated nitrogen. Gene correlation network analysis revealed that sponge gene modules, coded by colours, became either highly upregulated (Blue) or downregulated (Turquoise, Black, Brown) as nitrogen treatment levels increased. Gene Ontology enrichment analysis of the different modules revealed genes involved in cell signalling, immune response and flagella motility were affected by increasing nitrogen levels. Notably, a decrease in the regulation of NF-kappaB signalling and an increase in protein degradation was identified, which is comparable to metabolic pathways associated with the sponge thermal stress response. These results highlight that *Cymbastela stipitata* can rapidly respond to changes in the external environment and identifies pathways that probably contribute to the ability of *C. stipitata* to tolerate short-term nutrient pulses.

KEYWORDS

nutrients, sponge, Tag-Seq, transcriptomics

1 | INTRODUCTION

Sponges are the oldest extant Metazoans, with fossils dating back to the Precambrian (Li et al., 2014) and recent work supporting sponges as sister to all other animals (Feuda et al., 2017). They form an abundant and diverse component of benthic communities, with 8,866 formally described species (20,000 estimated species), that occupy both marine and freshwater habitats from the poles to the tropics (Van Soest et al., 2017). Sponges also play integral roles in ecosystem processes such as bioerosion and consolidation, benthic–pelagic coupling through their immense filtering capabilities, as well as nutrient cycling through their complex symbiosis with microorganisms (Bell, 2008; de Goeij et al., 2013; Diaz & Rüetzler, 2001). Sponges undertake this nutrient cycling using exceptionally diverse microbial communities, with over 40 microbial phyla (including candidate phyla) known to associate with sponges (Thomas et al., 2016).

Nutrient levels in coastal areas have been increasing due to inputs from both point (e.g., sewage effluent) and nonpoint (e.g., agricultural and urban run-off) sources (Carpenter et al., 1998), and this has contributed to the degradation of reef ecosystems worldwide (D'Angelo & Wiedenmann, 2014; Fabricius et al., 2011). Various aspects of coral physiology (e.g., reproductive success, calcification rates and growth) are negatively impacted by elevated nutrients (Dunn, Sammarco, & LaFleur, 2012; Fabricius, 2005; Koop et al.,

2001; Loya, Lubinevsky, Rosenfeld, & Kramarsky-Winter, 2004). The balance of nutrients is also important, with elevated nitrogen in combination with low phosphorous contributing to coral starvation (D'Angelo & Wiedenmann, 2014). Corals exposed to high nutrients show an upregulation of antioxidant genes indicative of an oxidative stress response (Rosic et al., 2011), as well as genes associated with cell apoptosis and innate immunity (Lin et al., 2016). Despite their immense filtering capabilities, which should increase their exposure to contaminants in the water column, sponges seem comparatively less affected by high nutrient levels. For instance, both *Rhopaloeides odorabile* and *Cymbastela stipitata* were visually unaffected and maintained stable symbiont communities at the highest nitrogen treatments, 10 and 240 µm respectively (Luter, Gibb, & Webster, 2014; Simister, Taylor, Tsai, & Webster, 2012). Similarly, nutrient enrichment did not alter the microbial community or increase disease lesion progression in *Aplysina cauliformis* (Gochfeld, Easson, Freeman, Thacker, & Olson, 2012). These studies all focused on microbial community dynamics rather than exploring impacts on host gene expression. The molecular-level stress response of sponges has been assessed in samples exposed to elevated temperature (Guzman & Conaco, 2016; Koziol, Batel, Arinc, Schröder, & Müller, 1997; López-Legentil, Song, McMurray, & Pawlik, 2008; Müller et al., 1995; Pantile & Webster, 2011; Webster et al., 2013) and toxicants, such as polychlorinated biphenyls (PCBs) (Wiens et al., 1998) and heavy metals (Müller et al., 1998; Schröder et al., 1999, 2006), although only one study utilized a transcriptome-wide approach (see Guzman & Conaco, 2016).

Here we measured the sponge gene expression response to short-term elevated nitrogen exposure, typical of sewage effluent and flood plumes, to understand the potential cause-and-effect pathway of elevated nutrients. We employed Tag-based RNA-sequencing (RNA-seq), a cost-effective method to measure expression in eukaryotic protein coding genes (Lohman, Weber, & Bolnick, 2016; Meyer, Aglyamova, & Matz, 2011), combined with gene coexpression network analysis (Langfelder & Horvath, 2008) to evaluate global gene expression responses to eight levels of nitrogen in a time-course experimental design.

2 | MATERIALS AND METHODS

2.1 | Experimental samples

Individuals of *Cymbastela stipitata* (n = 24) were collected from Channel Island, Northern Territory, Australia, and exposed to four different nitrogen treatment levels (n = 3 tanks per treatment), ranging from 2 to 240 µm dissolved inorganic nitrogen (DIN) (Table S1), using Thrive water-soluble fertilizer as previously described (Luter et al., 2014). Nitrogen levels were manipulated to reflect a short-term sewage discharge event (48 hr), followed by reduced nitrogen levels simulating a flood-plume event (7 days). Three replicate samples were collected from each treatment at both time points (e.g., 2, 120, 160, 240 µm DIN at 48 hr and 2, 7, 10, 27 µm DIN at 7 days) yielding a total of 24 samples which were snap frozen in liquid nitrogen and stored at −80°C for further analysis.

2.2 | Reference transcriptome: extraction, sequencing and processing

Total RNA was extracted from three biological replicates in both the ambient (2 µm) and high treatment (240 µm) using the PowerPlant RNA Isolation Kit (MoBio Laboratories) following the manufacturer's protocol. RNA was immediately treated to remove contaminating DNA using a TURBO DNA-freeTM Kit (Ambion) following the manufacturer's protocol. To concentrate and improve the quality of the RNA, DNase-treated samples were processed with RNA Clean & Concentrator (Zymo Research). RNA quantity and purity were determined using gel electrophoresis (1.1%, w/v) with agarose gel containing GelRed (Biotium) and a NanoDrop 2000 spectrophotometer (Thermo Scientific). High-quality RNA (260/280 nm ratio of 2.0) from the six individual samples was sent to the Australian Genome Research Facility (AGRF) for cDNA library construction with the TruSeq RNA Library Prep Kit version 2 and downstream paired-end (2 × 250 bp) sequencing on the Illumina MiSeq platform.

Sequence adapters were removed from the raw reads using FASTX _ TOOLKIT (http://hannonlab.cshl.edu/fastx toolkit). Reads with < 50 bp or with a homopolymer run of "A" ≥9 were discarded, and reads with a PHRED quality of at least 20 over 90% of the read were subjected to assembly.

A reference transcriptome for downstream Tag RNA-seq analysis combined reads from the six individual samples and was assembled using TRINITY version 2.0.6 (Grabherr et al., 2011) using default parameters for de novo assembly and in silico normalization (Haas et al., 2013). Assembled contigs (>400 bp) were assigned putative gene names and gene ontologies based on BLASTX pairwise sequence homology searches against the UniProt Knowlegebase Swis-Prot database (Uniprot, 2015). BLASTX comparisons to the CEGMA core eukaryotic gene set (Parra, Korf, & Bradnam, 2007) and Web-MGA server (http://weizhong-lab.ucsd.edu/webMGA/server/kog/) (Wu, Zhu, Fu, Niu, & Li, 2011) were used to assign EuKaryotic Orthologous Groups (KOG) annotations (orthologous or paralogous proteins). The KAAS server was used to assign the Kyoto Encyclopedia of Genes and Genomes (KEGG) identifications (http://www.genome.jp/kegg/kaas/) (Moriya, Itoh, Okuda, Yoshizawa, & Kanehisa, 2007). The BBMAP package (Bushnell, 2015) was used to calculate GC content and transcriptome completeness was assessed using BUSCO (Simão, Waterhouse, Ioannidis, Kriventseva, & Zdobnov, 2015) with the *metazoan_odb9* data set and default settings.

2.3 | Tag RNA-seq: extraction, sequencing and processing

Total RNA was extracted from 24 experimental samples using the same procedures and kits described for the reference transcriptome.

One microgram of RNA per sample was prepared for Tag-based RNA-seq as in Meyer et al. (2011), with modifications for sequencing on the Illumina HiSeq2500 platform at the University of Texas at Austin Genome Sequencing and Analysis Facility (full protocols can be obtained from: https://github.com/ckenkel/tag-based_RNAseq). Briefly, Tag-based RNA-seq quantifies gene expression by sequencing random fragments obtained from the 3′ end of polyadenylated transcripts, generating more accurate estimates of protein-coding transcript abundances than standard RNA-seq, at a fraction of the cost (Lohman et al., 2016).

The full bioinformatic pipeline used in this study can be found online (https://github.com/ckenkel/tag-based_RNAseq). Briefly, 142 million raw reads were generated, with individual counts ranging from 1.5 to 12 million reads per sample. Of these, reads without the 5′-Illumina leader sequence and PCR (polymerase chain reaction) duplicates (defined as reads mapping to the same starting position in the reference and aligning with 100% identity along the length of the shorter read) were discarded, and this leader was trimmed from remaining reads. The FASTX_TOOLKIT (version 0.0.13; http://hannonlab.cshl.edu/fastx_toolkit) was then used to trim the reads after a homopolymer run of "A" ≥8 bases was encountered, retain reads with minimum sequence length of 20 bases, and quality-filter them requiring PHRED quality of at least 20 over 90% of the sequence. The remaining 17 million filtered reads were mapped to the reference transcriptome by sample using BOWTIE 2 (version 2.2.6) (Langmead & Salzberg, 2012) and read counts were assembled for each contig (Table S1). On average, 593,554 reads per library (range: 240,568–1,024,347; 77%–87%) were mapped to 21,675 unique contigs (Table S2). Raw Illumina sequence data were deposited in NCBI's SRA under the BioProject ID PRJNA383089.

2.4 | Statistical analyses

All statistical analyses were carried out in the R (version 3.1.2) statistical environment (R Core Development Team, 2017). Count data were normalized using the package DESEQ (version 1.20) as follows (Anders & Huber, 2010). Contigs with zero counts in more than 50% of samples were removed, leaving 12,875 contigs across 24 samples in the data set. Dispersion estimates of raw counts were obtained by maximizing a Cox–Reid-adjusted profile likelihood of a model specifying treatment and time point for each sample and the empirical dispersion value was retained for each gene. Count data were rlog-transformed using DESEQ2 (version 1.8.2) (Love, Huber, & Anders, 2014) and used in subsequent Weighted Gene Co-expression Network Analyses (WGCNA; version 5.1).

R scripts for network construction were modelled after tutorials for undirected WGCNA (Langfelder & Horvath, 2008). Construction of co-expression networks consisted of four steps: (a) Pearson correlations for all genes across all treatments were used to construct a similarity matrix of gene expression, retaining the sign of the expression change ("signed networks"); (b) expression similarities were transformed into connection strengths (connectivities) through a power adjacency function, using a soft thresholding power of 9, which best satisfied the assumption of a scale-free network topology; (c) linkage hierarchical clustering was coupled with a topological overlap matrix to identify groups of genes (network modules) whose expression was highly positively correlated, retaining only modules with at least 30 genes and merging modules with ≥75% similar expression profiles (Figure S1); and (d) network properties (e.g., module eigengenes) were related to experimental conditions and sampling time points.

Functional enrichment analyses were conducted to identify over-representation of particular functional groups within modules, based on Gene Ontology (GO) classification (Ashburner et al., 2000). For each GO term, the number of annotations assigned to genes within a module was compared to the number of annotations assigned to the rest of the data set to evaluate whether any ontologies were more highly represented within the module than expected by chance using the package GO-MWU (Fisher's exact test, false discovery rate [FDR] <0.05) as per Wright, Aglyamova, Meyer, and Matz (2015). The GO categories were hierarchically clustered based on the number of shared genes within the data set to identify categories probably driven by the same genes.

3 | RESULTS AND DISCUSSION

3.1 | Visual inspection of sponges between treatments

Sponge tissue appeared visibly healthy over the entire duration of the 7-day experiment, with individuals in the highest nitrogen treatment visibly indistinguishable from those in the ambient controls (Figure 1; Luter et al., 2014). This is consistent with reports for other

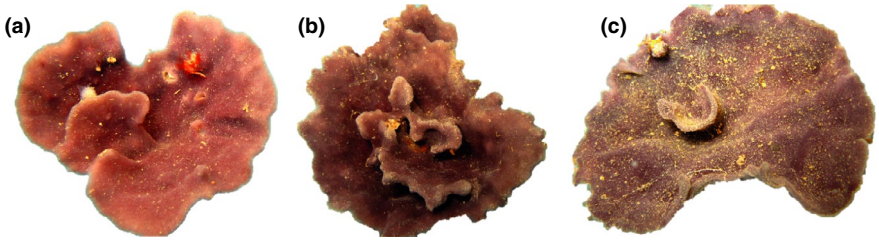

FIGURE 1 Photographs of *Cymbastela stipitata* from nitrogen exposure experiments. Representatives from the ambient treatment (a), 7 μm (b) and 240 μm (c), which signify the highest nitrogen treatment from mimicking the flood plume event (48 hr) and sewage pulse (7 days), respectively [Colour figure can be viewed at wileyonlinelibrary.com]

sponge species subjected to elevated nitrogen levels, such as the Great Barrier Reef sponge *Rhopaloeides odorabile* (Simister et al., 2012), and the Caribbean sponge *Aplysina cauliformis* (Gochfeld et al., 2012).

3.2 | Reference transcriptome assembly

The reference transcriptome, built from pooling reads from three ambient samples and three samples from the 240 µm treatment, produced a total of 45,639 contigs, or isogroups, with an average length of 708 bp, an N50 of 1,172 bp and a mean GC content of 50% (Table S3). GO annotations based on sequence homology comparisons to the Swiss-Prot database were made for 14,146 isogroups (31%). KEGG pathway annotations were assigned to 19,426 isogroups, comprising 4,356 unique KEGG annotations. Analysis of the core eukaryotic gene set using the CEGMA pipeline identified that 98.8% of eukaryotic KOGs were represented within the reference assembly distributed across 22,188 isogroups. Of the 978 genes present in the Metazoan BUSCO lineage, 93.4% of the *Cymbastela stipitata* isogroups were represented by complete transcripts, 3.2% were fragmented and 3.4% were missing. This level of transcriptome completeness exceeds a recent study that identified 77.9% of *Halisarca caerulea* contigs were represented by complete transcripts (Kenny et al., 2018).

3.3 | Gene correlation networks

WGCNA was used to examine differences in sponge gene expression between nitrogen treatment levels over the 7-day experiment. The 12,875 isogroups included in the analysis were assigned to 13 co-expression modules, ranging in size from 119 to 4,075 genes, with seven significantly correlated with nitrogen treatment level. Each module was colour-coded (Figure 2) and genes in the Magenta (n = 347), Blue (n = 3,131) and Tan (n = 290) modules were all up-regulated, while the Black (n = 491), Green (n = 637), Turquoise (n = 4,075) and Brown (n = 1,134) modules were downregulated with respect to nitrogen treatment level (Figure 2). Two modules were increasingly up- or downregulated (Blue and Turquoise, respectively) as nitrogen treatment levels increased, whereas the other two modules (Black and Brown) showed a slightly different pattern comprising an initial increase followed by a subsequent decrease at the highest nitrogen levels (Figure 2). The module

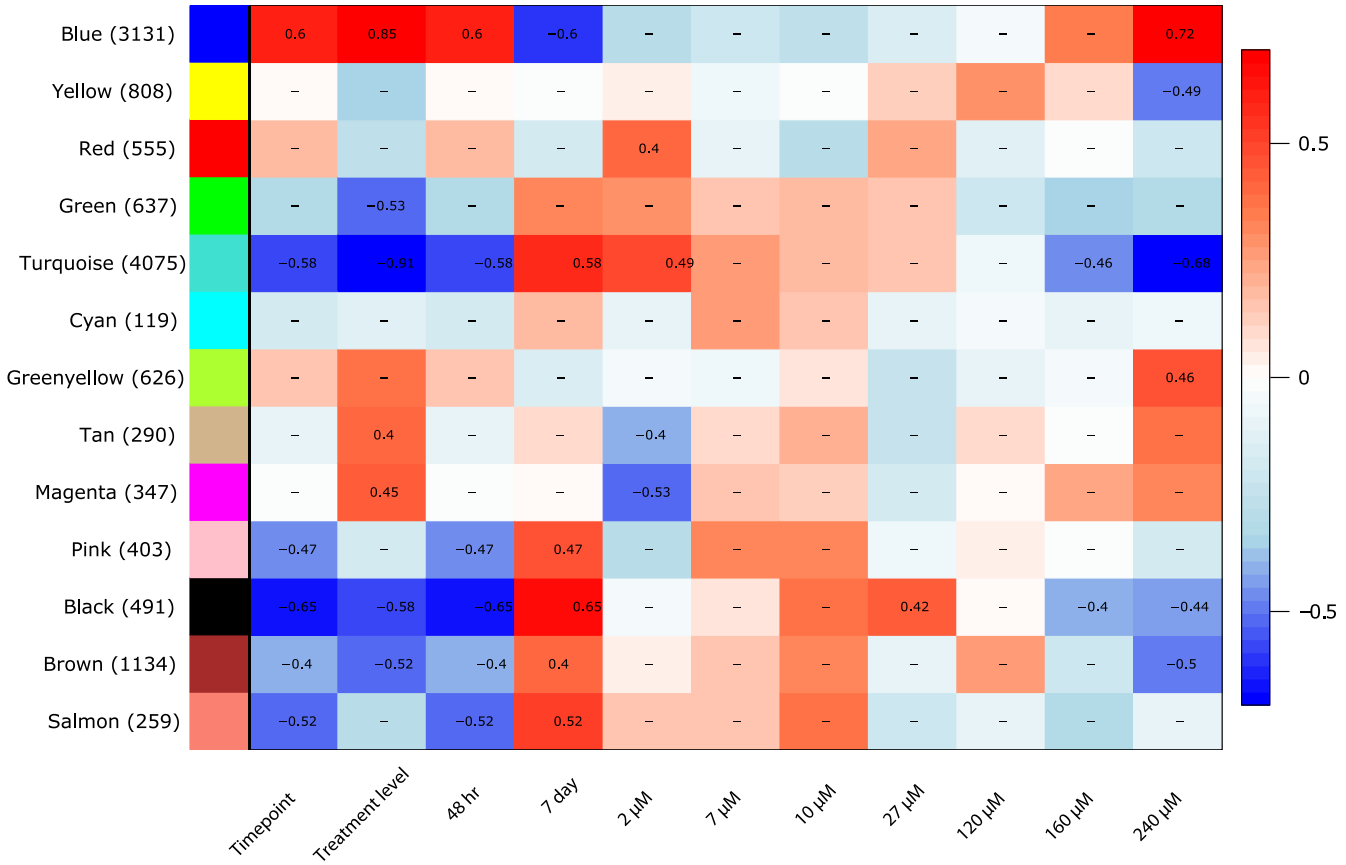

FIGURE 2 Heatmap of module–trait correlations. Each row corresponds to a module eigengene and each column corresponds to either a time point, treatment or specific nitrogen concentration. The number of genes corresponding to each module is given in parentheses. In the heatmap matrix, red indicates a positive correlation, whereas blue indicates a negative correlation. Correlations are based on Pearson's R with only significant correlations depicted. Individual time points (e.g., 48 hr and 7 days) and specific nitrogen treatment levels (e.g., 2–240 µm) were defined as categorical traits, coded in binary [Colour figure can be viewed at wileyonlinelibrary.com]

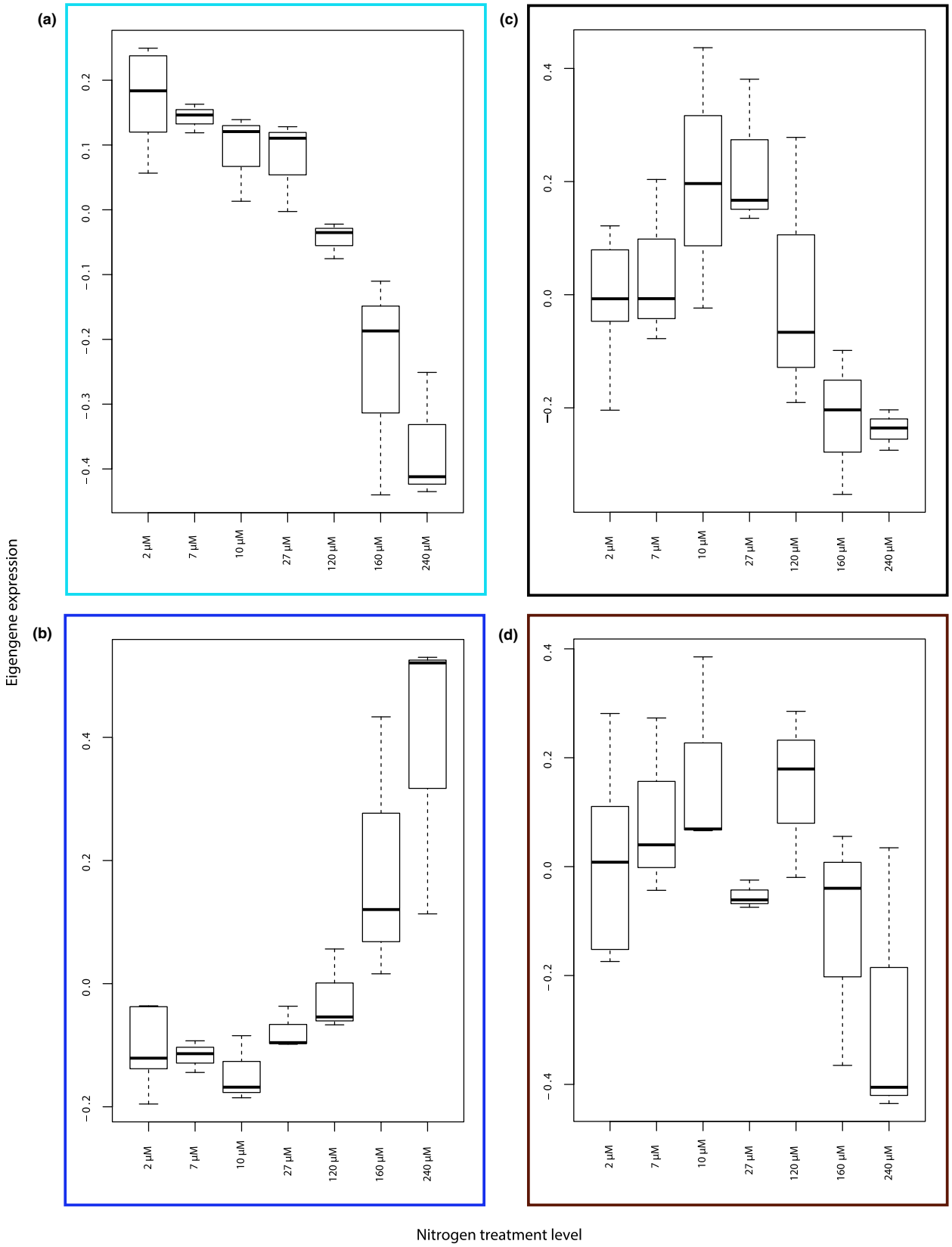

FIGURE 3 Module-specific boxplots of gene expression. Each box plot depicts the median and interquartile range of module eigengenes with respect to nitrogen treatment level for the Turquoise (a), Blue (b), Black (c) and Brown (d) modules. Box plot whiskers extend 1.5 times beyond the interquartile range. The ambient (2 µm) treatment from 48 hr and 7 days was combined while 120, 160 and 240 µm correspond to 48 hr and 7, 10, 27 µm correspond to 7 days [Colour figure can be viewed at wileyonlinelibrary.com]

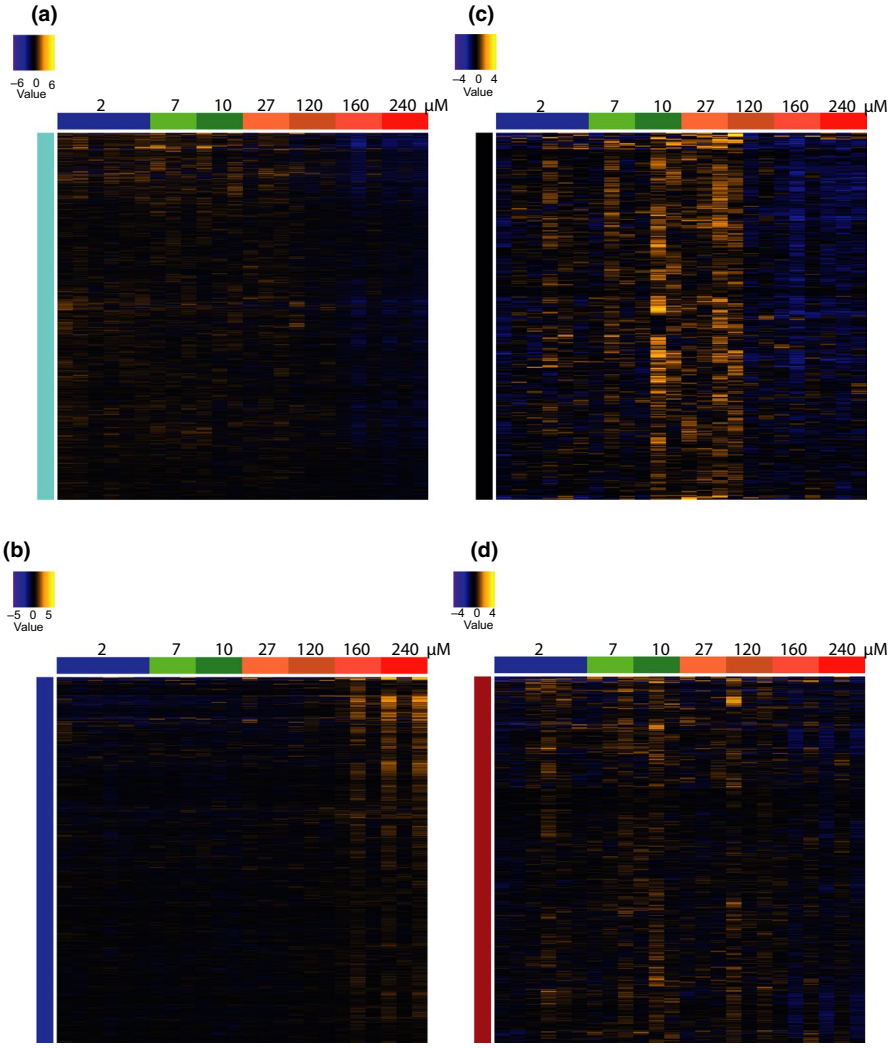

FIGURE 4 Module-specific heatmaps of gene expression. Columns represent individual samples, which are organized by increasing nitrogen treatment level, with the second three replicates (e.g., SM332, SM333 and SM334) of the 2 µm treatment corresponding to the 7-day time point: Turquoise (a), Blue (b), Black (c) and Brown (d) modules [Colour figure can be viewed at wileyonlinelibrary.com]

eigengene (the first principal component of the expression matrix) expression patterns confirmed this trend, with highly upregulated genes in the Turquoise module being observed at ambient nitrogen levels (Figure 3a), whereas genes from the Blue module were all upregulated at the highest nitrogen level of 240 µm (Figure 3b). In contrast, the Black and Brown modules displayed more variable expression, with observed gene expression increasing at the lowest nitrogen levels, while also being downregulated at the highest nitrogen level (Figure 3c,d). The WGCNA approach enabled us to identify genes responding to nitrogen treatment level directly, regardless of time point. For example, while the lower nitrogen treatment levels (7, 10 and 27 µm) were sampled from the later time point (7 days), the response to the nitrogen treatment level revealed a similar trend to the 48-hr time point either increasing (Blue) or decreasing (Turquoise) in expression as nitrogen levels increased (Figure 3a,b). While clear patterns of expression existed between treatments, considerable variation between the three individual sample replicates was also apparent within all modules (Figure 4). This variation is probably attributable to the use of sponge individuals as biological replicates and is consistent with interindividual variation in gene expression reported in other marine invertebrates (Császár, Seneca, & Oppen, 2009; Souter et al., 2011). It is also possible that three biological replicates per treatment was too low to explain the variation (Todd, Black, & Gemmell, 2016); however, despite the relatively low replication, treatment effects were still identified.

3.4 | Functional enrichment of modules

Genes in the WGCNA Turquoise module (e.g., genes highly upregulated in ambient and low nitrogen treatment levels) were enriched in functions associated with a change in the immune response (Figure 5a). In particular, genes with the assigned GO terms "negative regulation of NIK/NF-kappaB signaling" and "negative regulation of defense response" (e.g., NRL family CARD domain-containing proteins) decreased as nitrogen levels increased, suggesting an overall increase in defence and immune response genes as nitrogen levels increase. Sponges are known to possess a well-developed innate immune system (Hentschel, Piel, Degnan, & Taylor, 2012; Pita, Rix, Slaby, Franke, & Hentschel, 2018; Srivastava et al., 2010), including NRL genes, which may help to distinguish symbionts from food bacteria (Degnan,

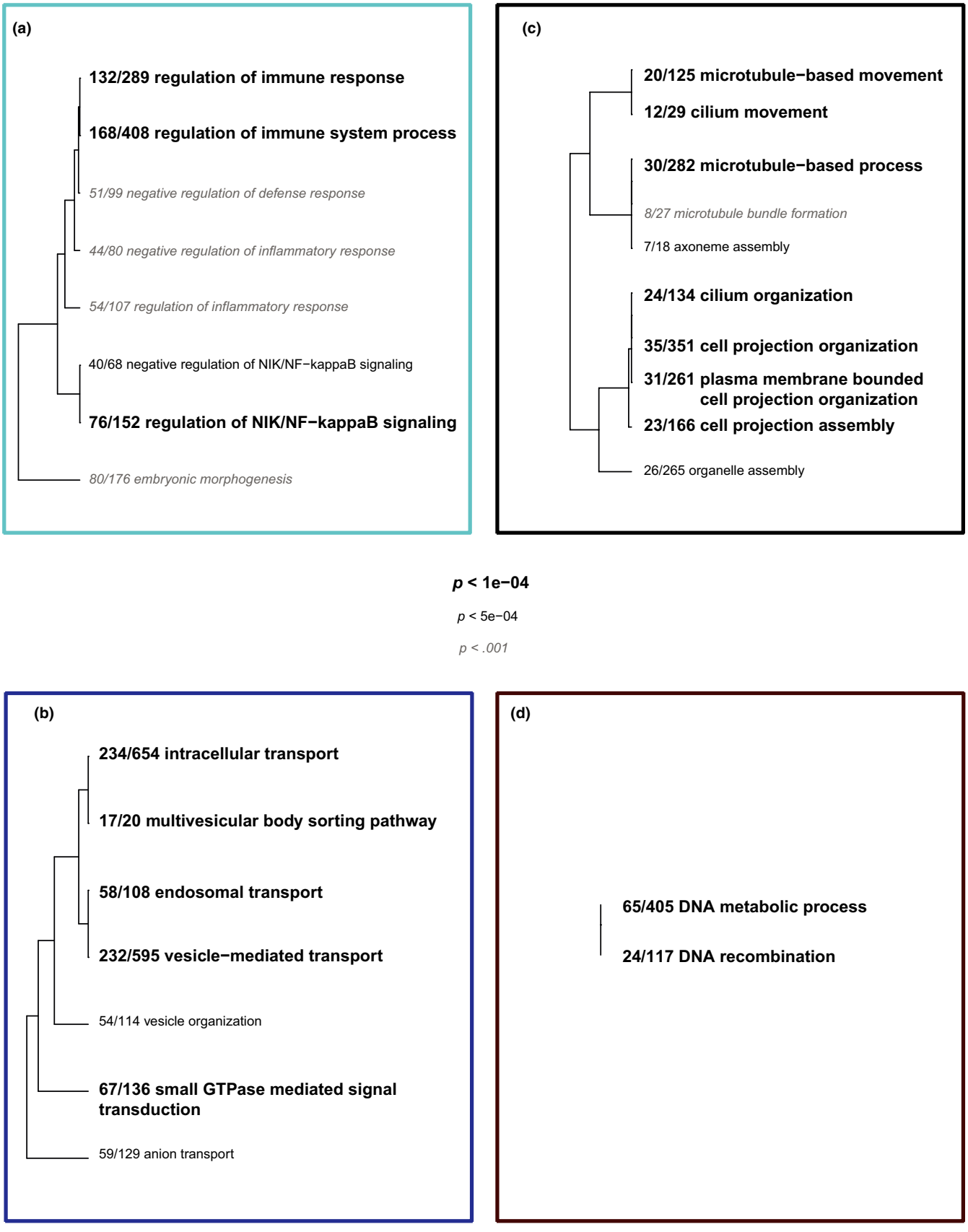

FIGURE 5 Gene Ontology (GO) categories enriched for each module. GO terms are from the Biological Processes domain. Font size and bold formatting correspond to the FDR-adjusted *p*-value derived from Fisher's exact test. The dendrogram shows the sharing of genes between categories and the fractions correspond to the number of genes significant within that category of genes. Individual genes from each GO term/module are detailed in Appendix S1 [Colour figure can be viewed at wileyonlinelibrary.com]

2015). Changes in innate immunity have also been identified in corals (DeSalvo, Sunagawa, Voolstra, & Medina, 2010) and sponges (Guzman & Conaco, 2016) in response to increased temperature, as well as in oil-contaminated corals (DeLeo et al., 2018). Most notable, however, is a similar change in gene expression of immunity-related factors in *Galaxea fascicularis* from eutrophic environments (Lin et al., 2016). Several genes upregulated in *G. fascicularis* in response to coastal eutrophication were also upregulated in this study, including Ras-related proteins, apoptosis regulators, tumour necrosis factor receptor proteins, and NF-kappaB signalling (Appendix S1; Lin et al., 2016). Conversely, several upregulated genes associated with *G. fascicularis* exposure to eutrophic environments, including heat shock proteins, were not present in any of the significant modules from the current study. Investigation of gene expression in the coral *Acropora tenuis* collected from a site characterized by high turbidity and dissolved nutrients also identified an enrichment of immunity genes, compared to control samples (Rocker, Kenkel, Francis, Willis, & Bay, 2019).

Genes that were upregulated at the higher nitrogen treatment levels (Blue module) were enriched in functions related to intracellular transport and cell signalling (Figure 5b). In particular, an increase in exocytosis of membrane-bound proteins and genes associated with cell to cell signalling was evident, as well as genes involved in protein degradation (Appendix S1). The Blue module also displayed an enrichment of genes within the GO term "anion transport," which included ABC transporters known for their role in transporting metal ions, inorganic ions, amino acids and metabolites across intracellular membranes (Vasiliou, Vasiliou, & Nebert, 2009). NPK fertilizers as used in this study generally contain low amounts of trace elements such as zinc, copper, manganese and molybdenum (Senesi & Polemio, 1981), and in order to maintain the DIN concentrations required for the high treatment levels, a large amount of Thrive fertilizer was required. Samples exposed to the highest treatment level received >100 times more trace elements than ambient controls, which may have contributed to an imbalance of metals within the cells and led to an upregulation of ABC transporter genes to establish homeostasis.

The Black module, containing genes downregulated at the highest nitrogen levels, was enriched in functions relating to cilia and flagella motility (Figure 5c). Sponges possess flagellated cells called choanocytes that are responsible for pumping water essential for capturing food, for eliminating waste and for gas exchange (Bergquist, 1978). Most studies reporting a reduction or arrest in pumping have been in response to suspended sediments (Grant et al., 2019; Strehlow, Jorgensen, Webster, Pineda, & Duckworth, 2016; Tompkins-MacDonald & Leys, 2008); however, a downregulation of genes in this module suggests *C. stipitata* is responding in a similar way to elevated nutrients. Investigations into pumping rates in sponges have shown that tissue anoxia is a direct function of pumping behaviour (Hoffmann et al., 2008). Although not directly assessing the impacts of DIN on the sponge host, a chaser experiment found no significant difference in pumping rates between control and DIN-treated *Xestospongia muta* individuals (Fiore, Baker, & Lesser, 2013); however, there is potential for the formation of microhabitats in sponge tissue where anaerobic and aerobic nitrogen transformation can occur (Fiore, Jarett, Olson, & Lesser, 2010). It remains to be seen whether the formation of these microhabitats changes the abundance or composition of choanocyte chambers, but our results suggest a connection between increased nitrogen and a decrease in genes that may be involved in choanocyte chamber function.

Similar to the Turquoise and Black modules, genes within the Brown module were also downregulated in the highest nitrogen treatment and were enriched in functions relating to "DNA metabolic processes" and "DNA recombination" (Figure 5d). These GO terms included several DNA repair proteins (including RAD51, DNA endonucleases and Tonsoku-like) and multiple transcription elongation factors that suggest a decrease in both DNA replication and DNA repair capability at the highest two nitrogen levels (Appendix S1). In corals, increased nitrogen availability results in an overall decrease in growth rate, while fostering an increased photosynthetic rate via their photosymbionts (Shantz & Burkepile, 2014), particularly when combined with low phosphorous levels (D'Angelo & Wiedenmann, 2014). Our gene expression results suggest that gene expression and DNA replication and repair is reduced as a result of elevated nitrogen exposure. An under-representation of genes involved in DNA synthesis and repair has also been identified in deep-sea corals exposed to oil and dispersants (DeLeo et al., 2018).

Overall, a much higher number of modules were downregulated with respect to increasing nitrogen treatment level in this study (five modules comprising 6,362 genes versus three modules comprising 3,768 genes). Similarly, other transcriptome-wide studies of sponges (Guzman & Conaco, 2016), corals (Moya et al., 2012) and oysters (Zhao, Yu, Kong, & Li, 2012) have reported a higher number of genes downregulated in response to temperature, acidification and salinity, respectively. The consistent downregulation of genes across taxa indicates a similar response to various environmental stressors.

4 | CONCLUSIONS

Changes in *Cymbastela stipitata* gene expression following exposure to flood plume levels of nitrogen highlights the ability of sponges to sense and respond to fluctuating levels of nutrients in the environment. Specifically, sponges from the highest nitrogen treatment had differential expression of genes involved in cell signalling, changes to the immune response and decrease in flagella motility. The gene expression response in *C. stipitata* was comparable to thermal stress responses seen in other reef invertebrates, including an increased immune response, a decrease in the regulation of NF-kappaB signalling and an increase in protein degradation. Additional experiments encompassing different sponge species and higher biological replication are needed to determine if sponge communities will respond to elevated nitrogen in similar ways and if the genes identified here

could be used as markers of nitrogen stress and/or tolerance in other reef invertebrates. While nitrogen transformation in sponges is primarily attributed to their microbial symbionts, this study has shown that the host sponge cells also respond to elevated nitrogen associated with short-term nutrient pulses and that these molecular changes may contribute to the apparent tolerance of sponges to eutrophication.

ACKNOWLEDGEMENTS

This study was supported by a NAMRA Postdoctoral Fellowship and a Charles Darwin University Faculty of Engineering, Health, Science and the Environment project grant awarded to H.M.L. N.S.W. was funded through an Australian Research Council Future Fellowship (FT120100480). The funding bodies played no role in the design of the study, analysis, interpretation of the data and writing the manuscript. We acknowledge Derek Sarovich for his support accessing and using the high-performance computer run by Genomics Northern Australia.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

AUTHORS CONTRIBUTIONS

H.M.L., K.G. and N.S.W. designed the experiment. H.M.L. performed laboratory work, data analysis and drafted the manuscript. C.D.K., M.T., T.P. and P.W.L. contributed to data analysis. All authors read and approved the final manuscript.

DATA AVAILABILITY STATEMENT

The raw sequence data generated during the current study are available in the NCBI's SRA under BioProject ID PRJNA383089 (Tag seq data) and PRJNA565855 (reference transcriptome). The Transcriptome Shotgun Assembly project has been deposited at DDBJ/EMBL/GenBank under accession GHWA00000000. The version described in this paper is the first version, GHWA01000000. The input files and scripts for WGCNA have been provided as supplementary data.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Luter HM, Kenkel CD, Terzin M, et al. Gene correlation networks reveal the transcriptomic response to elevated nitrogen in a photosynthetic sponge. *Mol Ecol*. 2020;29:1452–1462. https://doi.org/10.1111/mec.15417