Nutrient gradients simulate different adjustments of coral-algal symbiosis

CURRENT STATUS: UNDER REVIEW

Microbiome  BMC

Haoya Tong
The Hong Kong University of Science and Technology
htongaa@connect.ust.hk
Corresponding Author
ORCiD: https://orcid.org/0000-0003-3044-6698

Guowei Zhou
South China Sea Institute of Oceanology Chinese Academy of Sciences

Fang Zhang
South China Sea Institute of Oceanology Chinese Academy of Sciences

Jin Sun
Hong Kong University of Science and Technology

Weipeng Zhang
Hong Kong University of Science and Technology

Hui Huang
South China Sea Institute of Oceanology Chinese Academy of Sciences

Pei-Yuan Qian
Hong Kong University of Science and Technology

DOI:
10.21203/rs.3.rs-17147/v1

SUBJECT AREAS
General Microbiology

KEYWORDS
coral, symbiosis, microbiome, nutrient pollution
Abstract

Background: Eutrophication is one of the major causes of coral reef degradation but the effect of eutrophication on coral and its symbiont algae remains unclear, particularly for the larval stage of coral. In the present study, the physiological and transcriptomic responses of the larvae of an ecologically important scleractinian coral *Pocillopora damicornis* were analyzed after a 5-day exposure to elevated nitrate in order to assess the survival and adaptation of coral-algal symbiosis under elevated nutrients.

Results: The results showed that multiple larval transcripts were significantly correlated with Symbiodiniaceae transcripts. The major differentially expressed transcripts in coral/Symbiodiniaceae included those responsible for energy synthesis/consumption, nitrogen metabolism and stressor response. Slightly elevated nitrate concentration could in fact promote the health of coral meta-organism. With increase in nitrate concentrations, coral larvae showed significant stress response to maintain the coral-algal symbiosis and coral-algal symbiosis was impaired, while Symbiodiniaceae switched photosynthetic states for ATP synthesis, material transport and nitrogen metabolism for symbiosis maintenance under the control of the coral hosts.

Conclusions: Our results suggest that adjustment of coral-algal symbiosis via coral control and a shift in Symbiodiniaceae photosynthetic states serves as the basis of coral meta-organism adaptation under eutrophication stresses. The larvae of *P. damicornis* and Symbiodiniaceae displayed different transcriptomic responses to nitrate enrichment. Coral larva meta-organism can adapt to moderately elevated nutrient concentration while extreme eutrophication can impair coral-algal symbiosis and affect coral larvae survival ultimately.

Background

Coral reefs, which maintain high biodiversity and provide abundant natural resources, are declining rapidly in recently years anthropogenically triggered by global climate change, pollution from coastal cities, overfishing and other human activities [1, 2]. Nutrient pollution (especially excessive nitrogen) has been recognized as one of the main stressors leading to coral reefs degradation by reducing coral resistance, reproduction, recruitment and increasing mass mortality [3–6]. However, while several
studies did not detect obvious difference in physiology of coral under high nutrient concentrations [3, 7], slight increase of nutrients did increase growth rate of coral in other cases [8, 9]. Thus, how corals response to eutrophication remains as a controversy in general.

Coral meta-organism consists of Symbiodiniaceae, bacteria and other microbes, which are fundamental to coral reef ecosystem thriving in oligotrophic ocean [10, 11]. Coral can acquire photosynthates from Symbiodiniaceae while Symbiodiniaceae use the metabolites from coral [12]. Most dissolved inorganic nitrogen was uptaken by Symbiodiniaceae in coral meta-organisms even though both corals and Symbiodiniaceae can acquire nitrogen from the environment [13]. The uptaken nitrogen was fixed by symbiotic algae, metabolized by coral associated microbes, and transferred to corals [14, 15]. Overall, the symbiosis is essential for coral meta-organisms to utilize nitrogen in form of inorganic compound from environment and enables the potential adaptive mechanism under eutrophication. Therefore, the intimate relationships make the response of coral to nutrient pollution are complex.

Most of coral adaptation studies have focused on global stressors, such as global warming and ocean acidification [16, 17]. Several mechanisms were proposed to explain decline in coral coverage under nutrient pollution. One common mechanism is that the decline is caused by macroalgal overgrowth [18–20]). The overgrowth of macroalgae caused poor light penetration and anoxic environment which choke corals ultimately [21–23]. Another widely acceptable theory is that high nutrients can destabilize coral-algal symbiosis, making coral vulnerable under thermal stress [24]. Symbiodiniaceae can keep more photosynthates under high nitrogen concentrations [25] which may bring in limitation of CO₂ and cause coral bleaching eventually. Besides, high nitrogen concentration followed by elevated nitrogen fixation in coral meta-organisms led to a phosphate starvation and raised cell division rate although coral control Symbiodiniaceae with a nitrogen limited internal environment [26], affecting the susceptibility of Symbiodiniaceae to thermal stress and thus reforming the coral-algal symbiosis [24]. Furthermore, nitrogen concentration can affect the photosynthesis efficiency as well as status in algae, which may finally affect coral-algal symbiosis [4].

Dispersal and recruitment of coral larvae play critical roles in establishing coral reefs [27], but the
impact of eutrophication on coral larvae remains largely unknown. It was reported that coral larvae retained high energy requirement and less physiology capacity, leading to high susceptibility to environmental changes [28, 29]. For example, larvae of Pocillopora damicornis reduced oxygen consumption under high partial pressure of CO$_2$ (pCO$_2$) but elevated pCO$_2$ did not affect adults significantly [30, 31].

P. damicornis is a hermaphroditic brooder and is one of the most widespread corals in the world [27, 32]. In the present study, we exposed P. damicornis larvae to four different nitrate concentrations along with ambient seawater as the control and then examined the photosynthetic physiology and the transcriptome changes of coral meta-organisms, with an aim to explore the potential mechanism of coral adaptation under eutrophication.

Results

**De novo assembly of reference transcriptome for coral and Symbiodiniaceae**

The reference transcriptome with a total of 122 GB clean reads was generated from all the samples from all the treatments and control to estimate differential gene expression under enriched nutrient conditions. In total, 677,207 transcripts were assembled with alignments of all the samples over 98%. Statistics based on all the transcripts and the longest isoform per gene including N50, median and average contig length were listed in Table S2 of the Additional File 1. After filtration, 110,546 transcripts of coral and 35,044 transcripts of Symbiodiniaceae were kept for downstream analysis, displayed a BUSCO transcriptome completeness of 89% and 37%, respectively (Supplementary Table S3, Additional File 1). The completeness of coral transcriptome was similar with previous research but not the Symbiodiniaceae transcriptome [33, 34]. This is because coral larvae were used for RNA extraction, not Symbiodiniaceae pure culture and some Symbiodiniaceae transcripts were discarded if they can be found in coral database. The numbers of transcripts in the present study was much bigger than those of the predicted genes and transcripts in a previous study [27]. Because one gene may have different transcripts and we didn’t exclude high similar transcripts to avoid missing any information. Only limited number of bacterial transcripts were detected, maybe due to sequencing depth; and these bacteria included cyanobacteria and some other nitrogen cycling related bacteria,
suggesting that bacteria also played certain roles in coral adaptation under nutrient enrichment (Supplementary Table S4, Additional File 1) but further analysis was not conducted in the present study.

**Different expressed transcripts in coral and Symbiodiniaceae**

For coral transcripts, the samples in 5, 10, 20, and 40 treatment groups had 24, 32, 37, and 48 up-regulated genes and 20, 26, 26, and 48 down-regulated genes in comparison to those in the control, respectively (FDR < 0.05, |logFC| > 1). Referring to Symbiodiniaceae transcripts, the samples in 5, 10, 20, and 40 treatment groups showed 1, 58, 2, and 16 up-regulated genes and 1, 300, 4, and 17 down-regulated genes in comparison to those in the control, respectively (FDR<0.05, |logFC| > 1). The transcript differentially expressed in at least one treatment was regarded as differential expressed transcript. In total, 176 coral transcripts and 378 Symbiodiniaceae transcripts were retained as the differentially expressed ones for following analysis. Figure 1 shows that nMDS of coral differentially expressed transcripts in different samples were well separated by nitrate concentrations. The samples from the highest nitrate concentration treatment showed large distances with the samples in the control. The samples in 5, 10, and 20 groups lay between 40 group and the control. There was no significant difference among samples in the same group. However, Symbiodiniaceae differentially expressed transcripts did not show a clear gradient through nitrate concentrations. The samples from 10 group were well separated from other samples. Among 5, 20, and 40 and the control groups, 40 and the control still had the longest distance, while the samples from 5 and 20 groups cluster together. Heatmap of coral and Symbiodiniaceae transcripts showed similar pattern with nMDS (Supplementary Fig. S2, Additional File 1), even though many transcripts displayed different expression levels across the samples within the same group.

**Function partitioning and diversity of coral and Symbiodiniaceae from different treatments**

Most differentially expressed transcripts in corals were related to energy consumption, membrane transform and stressor response. In Symbiodiniaceae, however, the highly differentially expressed transcripts were related to photosynthesis, nitrogen cycling and stressor response (Supplementary
Table S5, Additional File 1). Veen diagram exhibited the core differentially expressed transcripts in coral and Symbioniaecaeae under nitrate enrichment (Fig. 2). Fifteen coral transcripts were found to differentially expressed in four treatments. Most of them were related to development and reproduction, suggesting development of coral larvae were affected under nutrient stress (Supplementary Fig. S3a, Additional File 1). Nineteen Symbioniaecaeae transcripts were found to differentially express in at least two comparisons. Transcripts for transport are majority of these Symbioniaecaeae transcripts (Supplementary Fig. S3b, Additional File 1), indicating coral-algal symbiosis were affected under eutrophication and Symbiodiaceae played critical role in coral larval adaptation. Nitrogen compound metabolic transcripts were found in both 15 coral and 19 Symbioniaecaeae transcripts.

When mapping the differentially expressed transcripts to KEGG pathways, both coral and Symbioniaecaeae differentially expressed transcripts had large numbers assigned to purine/thiamine metabolism and antibiotics biosynthesis pathways. Symbioniaecaeae had more differentially expressed transcripts assigned to nitrogen compound metabolism pathway (Table 2). These indicated that nutrient enrichment can affect energy metabolism of coral meta-organism, coral meta-organism establish defense mechanism for potential pathogens.

According to GSEA results of coral transcripts, 54, 147, 126, and 88 gene sets were identified as up-regulated and 45, 228, 36, and 99 gene sets as down-regulated in 5, 10, 20 and 40 groups, respectively. For Symbioniaecaeae transcripts, 337, 408, 324, and 398 gene sets were up-regulated and 17, 156, 127, and 161 gene sets were down-regulated in 5, 10, 20 and 50 groups, respectively. The dissimilarity through nitrate gradients of coral and Symbioniaecaeae transcripts depicted by GSEA was different from that by pairwise analysis because GSEA took more genes into consideration, forming a more complete picture of functional differences.

The main ATP generation related transcripts were more increased in Symbioniaecaeae than those in corals in all treatments, especially 5 and 40 group (Fig. 3a), indicating the increasing energy demand of coral meta-organism under eutrophic conditions might more rely on Symbioniaecaeae. Coral and Symbioniaecaeae enriched different sets of stress response genes in the treatments (Fig. 3c) with
similar functions of stress response transcripts found in their total transcriptome. Coral genes related to immune processes were down-regulated in 10 group but up-regulated in 5 and 40 groups. Coral genes related to defense response to virus were down-regulated in 10 and 40 groups whereas genes related to defense response to bacteria were up-regulated in these two groups, indicating that coral becomes vulnerable and may suffer from attacks from potential pathogens and virus due to increased nutrients. Most Symbiodiniaceae stressor-related transcripts were activated through nitrate concentrations yet some transcripts potentially related to stressors were depressed in some groups. Coral stress response genes were mostly related to bacteria/virus defense and inflammatory response, while Symbiodiniaceae stress response genes were mainly related to detoxification, chemical stimulus and oxidative stress, implying they potentially worked together to undertake various stressors.

The sets of functional genes that differentially expressed in at least two pairs were shown in differential expression heatmap of gene sets, exhibiting more details of coral meta-organism functions in different treatments (Supplementary Fig. S4, Additional File 1). Among the coral transcripts, carbohydrate and carbohydrate derivative transport gene sets were up-regulated in 10 and 20 groups, but showed no significant difference in 5 and 40 groups, suggesting coral tends to require more energy under high nitrate concentrations. No nitrogen metabolic related functional gene sets in coral were enriched in those treatments.

For Symbiodiniaceae transcripts, transcripts belonging to different photosystems differentially expressed in response to nitrate enrichment, suggesting Symbiodiniaceae were at different metabolic states of photosynthesis under different nitrate conditions with different proportion of increased photosystem I/II (PSI/PSII) related transcripts: in 5, 10 and 40 groups, PSII related transcripts were more overexpressed than the transcripts related to PSI, while in 20 group, PSI transcripts were more overexpressed (Fig. 3b, Supplementary Fig. S4, Additional File 1). And PSII in 5 group had the highest proportion in increased PSI/PSII transcripts. These demonstrating photosystem adjustments of Symbiodiniaceae under different nitrate concentrations. However, light harvesting related transcripts decreased in 10, 20 and 40 groups and only highly expressed in 5 group, suggesting Symbiodiniaceae
worked better under slightly increased nitrate concentration while impaired under eutrophic conditions. Consistent with results of photosystem related transcripts, carbon fixation and carbohydrate biosynthetic processes were especially highly overexpressed in 5 group, decreased in 10 group, and either overexpressed or showed no differences in other groups. Carbohydrate derivative biosynthetic process increased in all groups compared with the control. Carbohydrate derivative catabolic process was activated in 5 and 10 groups but depressed in 20 and 40 groups. These results suggest Symbiodiniaceae physiological functions were affected under high nitrate concentrations. Nitrogen compound transport and metabolic process were induced in 5, 10 and 40 groups but deactivated in 20 group. Catabolic process of organonitrogen compound was highly expressed in 5 and 10 group but decreased in 20 and 40 groups when compared to the control, suggesting that Symbiodiniaceae can help with nitrogen metabolism and transport for coral meta-organism adaptation under nutrient stress.

**Coral-algal symbiosis under different nitrate concentrations**

The average Symbiodiniaceae density in *P. damicornis* larvae increased in all treatments compared with the control but there were no significant differences among treatment groups (*p* = 0.05) (Fig. 4a). The $P_G$ was increased when larvae were exposed to nitrate enrichment in general and was the highest in 5 group, slightly increased in 10 and 20 groups, and moderately increased in 40 group (Fig. 4b). In 5 group, the average Symbiodiniaceae density slightly increased but the $P_G$ and $P_N$ were the highest, suggesting the photosynthesis efficiency of Symbiodiniaceae in 5 group was substantially enhanced when nitrate concentration was high. However, further increase in nitrate concentration led to Symbiodiniaceae overgrowth but did not increased its photosynthesis efficiency (Fig. 4b). These were consistent with transcriptome changes that Symbiodiniaceae worked best in 5 group. The correlation between coral transcripts and Symbiodiniaceae photobiological parameters differed in different nitrate concentrations (Fig. 4c). Coral transcripts from different group distributed along dbRDA axis 1 over nitrate concentration gradient. Axis 1 was positively correlated with Chl c and negatively correlated with Symbiodiniaceae density, implying that with increasing nitrate, Chl c and
Symbiodiniaceae density were two main factors contributing to coral transcriptome variation. Co-expression network of coral-Symbiodiniaceae differentially expressed transcripts showed a compact organized network, driven by coral transcripts (Fig. 5). In total, 15,179 out of 66,704 significant correlations ($p \leq 0.1$) were detected in the network. All the top 30 highest betweenness centrality of the network were coral transcripts and betweenness centrality of Symbiodiniaceae also exhibited lower than corals’ considering the quantity effects, indicating coral transcripts played more important roles in the network. GO map of core transcripts in coral showed that the coral transcripts that were highly correlated with Symbiodiniaceae transcripts were related to stressors response (GO0051716, GO0050896 etc.), nitrogen compound metabolic process (GO0034641, GO0006807 etc.) and ATP/carbohydrate binding (GO0005524, GO0097367 etc.) (Supplementary Fig. S5a-c, Additional File 1). Symbiodiniaceae core transcripts exhibited nitrogen compound metabolism/transport (GO0006807, GO0015696 etc.) and ATP binding (GO0005524) (Supplementary Fig. S5d-f, Additional File 1), suggesting that coral-algal symbiosis works for coral nitrogen metabolism and adaptation under high nitrate concentrations. Based on KEGG pathway analysis, the core coral transcripts were assigned to purine and thiamine metabolism pathways responsible for energy metabolism (Table 2).

Discussion

Transcriptomic response of coral during nutrient stress

Previous studies revealed that eutrophication can cause coral reproduction depress, reduce calcification and skeleton density [35, 36]. On contrary, several studies argued that nutrient enrichment would not affect coral physiology directly and that exceed nutrients promote coral growth in certain circumstance [7, 8, 37]. In the present study, nitrate treatment did not affect larval survival while elevated nitrogen concentration induced several changes in protein metabolism and increased stressor responses of coral larvae. And larval development transcripts decreased in all treatments with not significant decrease in 5 group. Meanwhile, transcripts involved in antibiotic biosynthesis pathway expressed differently among the treatments. Considering the limited duration of treatments in the present study, extreme eutrophication may threat coral larvae’s long-term survival via developmental inhibition.
Photosynthetic adaptation of Symbiodiniaceae to nutrient enrichment

A number of studies have shown that algae regulated their photosynthetic pathways and had state transitions of photosynthesis under nutrient stress [38, 39]. When exposed to continuous stress, stoichiometry of photosystem in algae can be adjusted for photosynthetic acclimation [40]. Consistent with these researches, PSI/PSII transcripts of Symiodiniaceae in the present study showed divergent expression levels in different treatments. Nitrogen starvation and nutrient stress mainly affected PSII and expression level of PSII varied more than that of PSI [41, 42]. There are mainly two proposed mechanisms for PSI/PSII adjustment under nutrient stress: 1) state 2 of photosynthesis is induced for high ATP generation to satisfy the energy requirements under nitrogen stress [40]. For instance, Turpin and Bruce [40] discovered that ammonia assimilation could induce state transitions of photosynthesis, and non-photochemical quenching of PSII was associated with fluorescence yield of PSI increase during ammonia assimilation due to high energy requirement. PSII is surrounded by cyclic electron flow which can also generate ATP for potential nutrient assimilation [43]. 2) The high expression of PSII under nutrient stress is due to its self-repairment and re-assembly to guarantee its function [44, 45]. However, as gene expression may not be directly related to enzyme activity [46], it is uncertain if PSII was over excited in the treatments according to its gene expression level. Photosystem adjustment occurred under different nitrate concentrations according to different photosystem transcripts expression levels in different treatments. ATP generation was found to be largely improved in state 2 of photosynthesis, combined with evidence of PSI fluorescence yield under nutrient stress [40]. In the present study, ATP biosynthetic process was more overexpressed in 5, 10 and 40 together with higher overexpressed PSII in these three groups, suggesting Symbiodiniaceae in 5, 10 and 40 groups appeared to be at different photosynthetic states from 20 group and this was consistent with variation trend of PSII expression level. Meanwhile, the elevated expression levels of stressor response genes increased over nitrate concentration gradient, may also suggesting enhanced damage repairing occurred. Thus, increased PSII expression of Symbiodiniaceae in the present study could not only contribute to damage repairing but also balance ATP generation via switching photosynthetic states. Furthermore, most of differentially expressed Symbiodiniaceae
transcripts were related to energy generation/consumption and antibiotic generation. To conclude, Symbiodiniaceae regulates photosystem states and active stressor response pathways for adaptation under nutrient stress.

**Coral-algal symbiosis works for coral meta-organism adaptation under eutrophication**

Coral-algal symbiosis is fundamental to extend the capacity of coral meta-organism to face various environmental stressors [4]. Energy related transcripts of coral and Symbiodiniaceae were closely correlated and accompanied with adjustment of Symbiodiniaceae photosystem, indicating that Symbiodiniaceae adaptation is critical for coral hosts’ high energy requirement during nitrogen assimilation under nutrient stress. No nitrogen metabolism related genes of coral larvae were enriched in all the treatments; and the differentially expressed coral nitrogen metabolism related transcripts were significantly correlated with Symbiodiniaceae transcripts. Furthermore, several nitrogen metabolism related genes of Symbiodiniaceae were enriched in the treatments. These results indicate that coral larvae rely on Symbiodiniaceae to metabolize nitrogen compounds, which is consistent with previous studies [12, 47].

Previous studies have revealed that coral get can control Symbiodiniaceae in three ways: 1) coral stimulates Symbiodiniaceae to release their photosynthates [48, 49]; 2) coral digests/degrades Symbiodiniaceae to control Symbiodiniaceae density [50]; and 3) coral limits the nutrients uptake of Symbiodiniaceae [26, 51]. In the present study, Symbiodiniaceae density did not increase significantly in 5 group, indicating that coral larvae might have regulated the Symbiodiniaceae density by digesting algae or limiting the nitrogen availability to avoid overgrowth of Symbiodiniaceae and to enhance the photosynthetic rate for high energy requirement. In 5 group, most coral larval transcripts for protein regulation did not enrich and coral larval development transcripts did not decrease significantly, together with the highest photosynthetic efficiency of Symbiodiniaceae in this group, suggesting an optimal growth status of coral larva meta-organism under this nitrate concentration. When nitrate concentration was raised to 10 µM, Symbiodiniaceae density increased but photosynthetic rate decreased in comparison to the number in 5 group. Symbiodiniaceae density in 20 group was the highest among all treatments, while the photosynthetic rate was lower than that in
5 group. Besides, Symbiodiniaceae in 20 group might at a different state of photosynthesis from other groups and ATP biosynthesis process transcripts were at the lowest expression level compared with among all the treatments. Many coral protein regulation transcripts were extremely over expressed and many stressor response transcripts were absent in 20 group, suggesting that the nitrogen concentration might be beyond the control limitation of coral larvae. Under this nitrogen concentration, the stabilization of coral-algal symbiosis might have been broken, leading to Symbiodiniaceae overgrowth, coral physiology altered, and ATP generation declined. When nitrate concentration increased to 40 µM, the density of Symbiodiniaceae was lower than that in 20 group, together with tremendous over expression of many stressor response transcripts in Symbiodiniaceae, suggesting that the exceed nitrogen may be harmful to Symbiodiniaceae.

Based on previous genomic research on coral and Symbiodiniaceae [52, 53], we identified 15 kinds of gene sets, maintaining coral-algal symbiosis potentially, in the total coral/Symbiodiniaceae transcriptome, including material transporting and stress response, and propose a conceptual model to decipher coral meta-organism adaptation under eutrophication condition (Fig. 6a). More transporting genes showed enriched in Symbiodiniaceae, especially no negative transporting response were found in 5 group, inferring an optimal survival condition of coral larva meta-organism under slightly increasing nitrate concentration and coral larva meta-organism mostly relied on Symbiodiniaceae for adaptation under eutrophication, consistent with results from other analysis (Fig. 6b). Metal ion transport, contributing to Symbiodiniaceae growth and coral-algal symbiosis maintenance, decreased in 10, 20 and 40 groups suggesting coral-algal symbiosis impairment under eutrophication. Combined with the results from GSEA and correlation analysis, these indicated that coral-algal symbiosis was affected under eutrophication, and coral larvae regulated the coral-algal symbiosis with Symbiodiniaceae adjusting photosystems and transport for maintaining the symbiosis. Thus, the model assumes that control/protection from coral hosts and adjustment of Symbiodiniaceae photosystem/transport are key strategies for coral-algal symbiosis to adapt to nutrient stress. Coral-algal symbiosis serves as the basis to balance the energy and nitrogen cycling under eutrophication.

When nitrate concentration excesses control limits of coral larvae, coral-algal symbiosis becomes
unstable and coral meta-organism becomes susceptible to environmental stressors.

Conclusion

The present study showed that the larvae of *P. damicornis* and Symbiodiniaceae displayed different transcriptomic responses to nitrate enrichment. We proposed that coral larvae can adapt to moderately elevated nutrient concentration via coral-algal symbiosis adjustment, especially photosystem/transporting adjustment and potential photosynthetic state transition of Symbiodiniaceae for maintaining coral-algal symbiosis under nutrient stress. Under eutrophication, coral protects Symbiodiniaceae from extreme nutrient stress and Symbiodiniaceae regulate the photosystems for efficient energy synthesis and nitrogen metabolism. These results provide knowledge base for coastal management and coral reef conservation and suggest that management of nutrient enrichment is significant for coral recruitment.

Methods

**Study area and sample collection**

Luhuitou fringing reef, located in southeast Sanya Bay of Hainan Island in the South China Sea (Supplementary Fig S1, Additional File 1). It is about 3 km long and 250–500 m wide, and receives sewage discharges and runoffs from both the Sanya Bay and Sanya River and thus, it is largely influenced by anthropogenic activities. The nutrient level in Luhuitou fringing reef has been enriched and the nitrate concentration is much higher than those in typically oligotrophic waters of coral reefs [54]. The live coral coverage has been lost since the 1960s due to anthropogenic impacts and global climate change [2].

Ten healthy colonies of the adult *P. damicornis* were collected from the Luhuitou fringing reef and immediately transferred to the nearby CAS-HKUST Sanya Joint Laboratory of Marine Science Research. Colonies were placed in individual larval collection apparatus with running sand-filtered seawater until larvae were released before new moon. Planula larvae from different colonies were pooled and randomly assigned to experimental treatments.

**Experimental setup**

The nitrate enrichment experiment was conducted in small aquaria (350 ml) equipped with
recirculating pump. There were five treatments with each containing four replicates. The control aquarium received only 0.5 µm filtered natural seawater while for four nutrient-enriched treatments, aquaria were supplied with potassium nitrate at the target concentration of 5 µM, 10 µM, 20 µM and 40 µM. The nitrate concentration in the control is 2.5 µM, reflecting the reef environment from which corals were collected. The nitrate of the seawater in the aquaria was determined by nutrient autoanalyser (Seal Analytical AA3, Germany) during the experiment. The actual nitrate concentration during the experiment is given in Table S1 in the Additional File 1. Aquaria were illuminated with T5 fluorescent bulbs (Giesemann, German) on a 12 hr/12 hr light/dark cycle, which provided a mean irradiance of 300 µmol photons m\(^{-2}\) s\(^{-1}\). The seawater temperature of the system was kept constant at 29°C using temperature controllers (WEIPRO, China). The experiment was maintained under above conditions for 5 days.

At the end of the experiment, 20 larvae for each replicate were randomly selected for physiological measurements as described below. The remaining larvae were aliquoted and preserved at −80°C for transcriptomic analyses (with about 20 larvae per aliquot).

**Photosynthesis and respiration**

Rates of net photosynthesis \((P_N)\) and dark respiration \((R_D)\) were assessed per treatment in 2 ml glass chambers. Chambers were equipped with magnetic stir bars and a temperature-compensated oxygen minisensor (Ocean optics, USA) connected to the 4-Channel Microsensor Oxygen Meter (Presens, Germany). \(P_N\) was measured during the midday, whereas \(R_D\) was measured in darkness. Chambers with seawater as blank controls for each treatment. \(P_N\) and \(R_D\) were then calculated by regressing oxygen production/consumption against incubation time and expressed as nmol O\(_2\) larva\(^{-1}\) min\(^{-1}\).

Gross photosynthesis \((P_G)\) was expressed by adding \(R_D\) to \(P_N\). After measurements, larvae were persevered at −80°C before symbiont densities and pigment concentrations were measured.

**Symbiont density and pigment**

Algal symbiont density and pigment were determined following the protocol described in Wiedenmann et al. [24]. Briefly, larvae were homogenized using a pestle and resuspended in 200 µl
autoclaved filtered seawater (AFSW). Each 40 ml homogenates were loaded on a haemocytometer to quantify algal symbiont density. The actual algae counts were averaged to sample size as cells larva⁻¹. The remaining homogenates were used for determining Chl a, Chl c and Carotenoids concentrations by measuring the absorbance of methanol extracts at 480, 510, 630, 664 and 750 nm using microplate reader (BioTek, USA) according to [55]. Pigment concentrations were then normalized to corresponding symbiont density to yield pigment concentration cell⁻¹.

**Transcriptome sequencing**

Total RNA was extracted from the aliquots of 20 P. damicornis larvae in each replicate, using TRizol (Invitrogen) according to manufacturer’s instructions. RNA quantity and integrity were assessed with electrophoretic and Agilent 2100 bioanalyzer (Agilent Technologies, USA), respectively. cDNA libraries were prepared from 3 µg of RNA per sample using NEBNext® UltraTM RNA Library Prep Kit for Illumina® (NEB, USA) following manufacturer’s recommendation. Pooled and barcoded libraries were sequenced on the HiSeq PE150 Illumina sequencing platform by Novogene Bioinformatics Technology Co., Ltd., Beijing, China (www.novogene.cn).

**Transcriptome assembly**

Data from all samples were used to generate a total coral holobiont reference transcriptome by Trinity 2.8.5 after low quality reads removal [56]. The coral holobiont transcriptome was blasted using blastx against NCBI scleractinian database and Symbiodiniaceae database, respectively, with expection value of 1.0E-50 and 33bp amino acids length cutoff, to create P. damicornis transcriptome and Symbiodiniaceae transcriptome. The resulting scleractinian reference transcriptome was then balsted against Symbiodiniaceae database with same parameters and removed the balsted reads to generate the final pure P. damicornis transcriptome ensuring all the transcripts were from P. damicornis. Symbiodiniaceae transcriptome was performed similarly to remove the ambiguous transcripts and establish the pure Symbiodiniaceae transcriptome. The completeness of final P. damicornis and Symbiodiniaceae transcriptomes was assessed using BUSCO [57]. And then performed with interPro analysis and GO mapping and annotation via Blast2GO under default
parameters to retrieve GO terms of the transcripts [58].

**Statistical analysis**

Each sample was mapped and calculated counts against *P. damicornis* and Symbiodiniaceae final transcriptomes, respectively, by RSEM to estimate the response of coral meta-organism to the nutrient stress [59]. The pairwise differential expression analysis of each treatment against the control was established using edgeR with logFC more than 1 or less than -1 as well as FDR of 0.05 to identify a differentially expressed coral/Symbiodiniaceae transcript. The log base 2 of counts per million reads (CPM) for each transcript, which was differentially expressed in at least one pairwise analysis, was used to perform non-parametric multidimensional scaling (nMDS) in Primer-e, generate venn diagrams and create coral/Symbiodiniaceae heatmap of differentially expressed transcripts in OriginLab 2019 (OriginLab, USA). Gene set enrichment analysis was performed for each pairwise differential expression result to measure the coral/Symbiodiniaceae functional alterations in each treatment in comparison with the control [60]. The enriched functions of coral/Symbiodiniaceae related to ATP generation and photosystem were squared transformed and generating column charts. Stress response functions, functions potentially related to coral-algal symbiosis and enriched functions detected in at least two pairs were kept to create functional heatmaps in OriginLab to measure the physiological performance of coral meta-organism in the treatments and control. The variations of Symbiodiniaceae density, net photosynthesis and gross photosynthesis were analyzed and performed in OriginLab. To estimate the correlations between Symbiodiniaceae and coral functional variations, Pearson coefficient was calculated between differentially expressed genes of coral and Symbiodiniaceae. Distance-based redundancy analysis (dbRDA) was conducted in Primer-e among coral differentially expressed genes, pigment, density and net photosynthesis of Symbiodiniaceae. Co-expression network was generated with significant correlations between coral and Symbiodiniaceae (*p* ≤ 0.1), and analyzed in Cytoscape to visualize the correlations between coral and Symbiodiniaceae. The coral-Symbiodiniaceae transcripts with betweenness centrality higher than 0.005 were regarded as core transcripts and were used to conduct combined GO graphs to visualize the function annotations of these transcripts.
Declarations

**Ethics approval and consent to participate**
Not applicable

**Consent for publication**
Not applicable

**Availability of data and material**
All raw sequence data has been submitted to NCBI under SRA accession number PRJNA611041

**Competing interests**
The authors declare no competing interests.

**Funding**
This work was supported by the grants from the Hong Kong Branch of Southern Marine Science and Engineering Guangdong Laboratory (Guangzhou) (SMSEGLSC01) and from UGC (JLFS/M-602/18) of HKSAR to PY Qian and a grant from the National Natural Science Foundation of China (41876192) to GW Zhou.

**Authors’ contributions**
HYT, GWZ, FZ, PYQ and HH contributed to the study design, culture experiments bioinformatics analysis, data interpretation, and manuscript preparation. JS, WPZ and all authors read and approved the manuscript.

**Acknowledgments**
We thank the Administration of Sanya Coral Reef National Nature Reserve for providing sampling permits.

**References**
1. Bellwood DR, Hughes TP, Folke C, Nyström M: *Confronting the coral reef crisis*. *Nature* 2004, **429**:827-833.

2. Hughes TP, Huang H, Young MAL: *The wicked problem of China's disappearing coral reefs*. *Conserv Biol* 2013, **27**:261-269.

3. Fabricius KE: *Effects of terrestrial runoff on the ecology of corals and coral...*
reefs: review and synthesis. *Mar Pollut Bull* 2005, 50:125-146.

4. D’Angelo C, Wiedenmann J: Impacts of nutrient enrichment on coral reefs: new perspectives and implications for coastal management and reef survival. *Curr Opin Environ Sustain* 2014, 7:82-93.

5. Duprey NN, Yasuhara M, Baker DM: Reefs of tomorrow: eutrophication reduces coral biodiversity in an urbanized seascape. *Glob Chang Biol* 2016, 22:3550-3565.

6. Vega Thurber RL, Burkepile DE, Fuchs C, Shantz AA, McMinds R, Zaneveld JR: Chronic nutrient enrichment increases prevalence and severity of coral disease and bleaching. *Glob Chang Biol* 2014, 20:544-554.

7. Szmant AM: Nutrient enrichment on coral reefs: Is it a major cause of coral reef decline? *Estuaries* 2002, 25:743-766.

8. Dunn JG, Sammarco PW, LaFleur G: Effects of phosphate on growth and skeletal density in the scleractinian coral Acropora muricata: A controlled experimental approach. *J Exp Mar Biol Ecol* 2012, 411:34-44.

9. Lubarsky KA, Silbiger NJ, Donahue MJ: Effects of submarine groundwater discharge on coral accretion and bioerosion on two shallow reef flats. *Limnol Oceanogr* 2018, 63:1660-1676.

10. Cunning R, Baker AC: Excess algal symbionts increase the susceptibility of reef corals to bleaching. *Nat Clim Chang* 2012, 3:259.

11. Bourne DG, Morrow KM, Webster NS: Insights into the coral microbiome: Underpinning the health and resilience of reef ecosystems. *Annu Rev Microbiol* 2016, 70:317-340.

12. Radecker N, Pogoreutz C, Voolstra CR, Wiedenmann J, Wild C: Nitrogen cycling in corals: the key to understanding holobiont functioning? *Trends Microbiol* 2015,
13. Pernice M, Meibom A, Van Den Heuvel A, Kopp C, Domart-Coulon I, Hoegh-Guldberg O, Dove S: A single-cell view of ammonium assimilation in coral-dinoflagellate symbiosis. *ISME J* 2012, **6**:1314-1324.

14. Reynaud S, Martinez P, Houlbrèque F, Billy I, Allemand D, Ferrier-Pagès C: Effect of light and feeding on the nitrogen isotopic composition of a zooxanthellate coral: role of nitrogen recycling. *Mar Ecol Prog Ser* 2009, **392**:103-110.

15. Kopp C, Pernice M, Domart-Coulon I, Djediat C, Spangenberg JE, Alexander DTL, Hignette M, Meziane T, Meibom A: Highly dynamic cellular-level response of symbiotic coral to a sudden increase in environmental nitrogen. *mBio* 2013, **4**:e00052-00013.

16. ANTHONY KRN, MAYNARD JA, DIAZ-PULIDO G, MUMBY PJ, MARSHALL PA, CAO L, HOEGH-GULDBERG O: Ocean acidification and warming will lower coral reef resilience. *Glob Chang Biol* 2011, **17**:1798-1808.

17. McCulloch M, Falter J, Trotter J, Montagna P: Coral resilience to ocean acidification and global warming through pH up-regulation. *Nat Clim Chang* 2012, **2**:623-627.

18. Lapointe BE: Nutrient thresholds for bottom-up control of macroalgal blooms on coral reefs in Jamaica and southeast Florida. *Limnol Oceanogr* 1997, **42**:1119-1131.

19. Britta S, David WK: Nutrient-limited growth of the coral reef macroalga Sargassum bacculareia and experimental growth enhancement by nutrient addition in continuous flow culture. *Mar Ecol Prog Ser* 1998, **164**:199-211.

20. Fabricius KE, Cooper TF, Humphrey C, Uthicke S, De’Ath G, Davidson J, Legrand H, Thompson A, Schaffelke B: A bioindicator system for water quality on inshore
20. Hallock P, Schlager W: Nutrient excess and the demise of coral reefs and carbonate platforms. *PALAIOS* 1986, 1:389-398.

21. Bruno JF, Petes LE, Drew Harvell C, Hettinger A: Nutrient enrichment can increase the severity of coral diseases. *Ecol Lett* 2003, 6:1056-1061.

22. Voss JD, Richardson LL: Nutrient enrichment enhances black band disease progression in corals. 2006, 25:569-576.

23. Wiedenmann J, D’Angelo C, Smith EG, Hunt AN, Legiret F-E, Postle AD, Achterberg EP: Nutrient enrichment can increase the susceptibility of reef corals to bleaching. *Nat Clim Chang* 2013, 3:160-164.

24. Wooldridge SA: Breakdown of the coral-algae symbiosis: towards formalising a linkage between warm-water bleaching thresholds and the growth rate of the intracellular zooxanthellae. *Biogeosciences* 2013, 10:1647-1658.

25. Falkowski PG, Dubinsky Z, Muscatine L, McCloskey L: Population control in symbiotic corals. *BioScience* 1993, 43:606-611.

26. Rivest EB, Kelly MW, DeBiasse MB, Hofmann GE: Host and symbionts in *Pocillopora damicornis* larvae display different transcriptomic responses to ocean acidification and warming. *Front Mar Sci* 2018, 5.

27. Okubo N, Yamamoto HH, Nakaya F, Okaji K: Oxygen consumption of a single embryo/planula in the reef-building coral *Acropora intermedia*. *Mar Ecol Prog Ser* 2008, 366:305-309.

28. Rivest EB, Hofmann GE: Responses of the metabolism of the larvae of *Pocillopora damicornis* to ocean acidification and warming. *PLOS ONE* 2014,
31. Putnam HM, Gates RD: Preconditioning in the reef-building coral Pocillopora damicornis and the potential for trans-generational acclimatization in coral larvae under future climate change conditions. *J Exp Biol* 2015, **218**:2365.

32. Tung-Yung Fan J-JL, Sheng-Xian Ie and Lee-Shing Fang: Lunar periodicity of larval release by pocilloporid corals in southern Taiwan. *Zool Stud* 2002, **41**:288-294.

33. Maor-Landaw K, van Oppen MJH, McFadden GI: Symbiotic lifestyle triggers drastic changes in the gene expression of the algal endosymbiont Breviolum minutum (*Symbiodiniaceae*). *Ecol Evol* 2020, **10**:451-466.

34. Kenkel CD, Bay LK: Novel transcriptome resources for three scleractinian coral species from the Indo-Pacific. *GigaScience* 2017, **6**.

35. Noga Stambler NP, Zvy Dubinsky, and John Stimson: Effects of nutrient enrichment and water motion on the coral Pocillopora damicornis. *Pac Sci* 1991, **45**:299-307.

36. Loya Y, Lubinevsky H, Rosenfeld M, Kramarsky-Winter E: Nutrient enrichment caused by in situ fish farms at Eilat, Red Sea is detrimental to coral reproduction. *Mar Pollut Bull* 2004, **49**:344-353.

37. Lucia B, Shai S, Dror A, Baruch R: Survival, growth and gonad development of two hermatypic corals subjected to in situ fish-farm nutrient enrichment. *Mar Ecol Prog Ser* 2003, **253**:137-144.

38. Berges JA, Charlebois DO, Mauzerall DC, Falkowski PG: Differential effects of nitrogen limitation on photosynthetic efficiency of photosystems i and ii in microalgae. *Plant Physiol* 1996, **110**:689.

39. Wollman F-A: State transitions reveal the dynamics and flexibility of the photosynthetic apparatus. *EMBO J* 2001, **20**:3623-3630.
40. Turpin DH, Bruce D: Regulation of photosynthetic light harvesting by nitrogen assimilation in the green alga Selenastrum minutum. *FEBS Lett* 1990, **263**:99-103.

41. Kolber Z, Zehr J, Falkowski P: Effects of growth irradiance and nitrogen limitation on photosynthetic energy conversion in photosystem II. *Plant Physiol* 1988, **88**:923.

42. Gruszecki WI, Veeranjaneyulu K, Zelent B, Leblanc RM: Energy transfer process during senescence: fluorescence and photoacoustic studies of intact pea leaves. *BBA Bioenergetics* 1991, **1056**:173-180.

43. Ananyev G, Gates C, Kaplan A, Dismukes GC: Photosystem II-cyclic electron flow powers exceptional photoprotection and record growth in the microalga Chlorella ohadii. *BBA Bioenergetics* 2017, **1858**:873-883.

44. Giardi MT, Masojidek J, Godde D: Effects of abiotic stresses on the turnover of the D1 reaction centre II protein. 1997, **101**:635-642.

45. Murata N, Takahashi S, Nishiyama Y, Allakhverdiev SI: Photoinhibition of photosystem II under environmental stress. *BBA Bioenergetics* 2007, **1767**:414-421.

46. Yin J, Zhang D, Zhuang J, Huang Y, Mu Y, Lv S: Study on the correlation between gene expression and enzyme activity of seven key enzymes and ginsenoside content in ginseng in over time in Ji’an, China. *Int J Mol Sci* 2017, **18**:2682.

47. Tanaka Y, Suzuki A, Sakai K: The stoichiometry of coral-dinoflagellate symbiosis: carbon and nitrogen cycles are balanced in the recycling and double translocation system. *ISME J* 2018, **12**:860-868.

48. Gates RD, Hoegh-Guldberg O, McFall-Ngai MJ, Bil KY, Muscatine L: Free amino acids exhibit anthozoan “host factor” activity: they induce the release of
photosynthate from symbiotic dinoflagellates in vitro. PNAS 1995, 92:7430.

49. Cook CB, Davy SK: Are free amino acids responsible for the `host factor' effects on symbiotic zooxanthellae in extract. Hydrobiologia 2001, 461:71-78.

50. Titlyanov EA, Titlyanova TV, Leletkin VA, Tsukahara J, van Woesik R, Yamazato K: Degradation of zooxanthellae and regulation of their density in hermatypic corals. Mar Ecol Prog Ser 1996, 139:167-178.

51. Xiang T, Lehnert E, Jinkerson RE, Clowes S, Kim RG, DeNofrio JC, Pringle JR, Grossman AR: Symbiont population control by host-symbiont metabolic interaction in Symbiodiniaceae-cnidarian associations. Nat Commun 2020, 11:108.

52. Lin S, Cheng S, Song B, Zhong X, Lin X, Li W, Li L, Zhang Y, Zhang H, Ji Z, et al: The Symbiodinium kawagutii genome illuminates dinoflagellate gene expression and coral symbiosis. Science 2015, 350:691-694.

53. Shinzato C, Shoguchi E, Kawashima T, Hamada M, Hisata K, Tanaka M, Fujie M, Fujiwara M, Koyanagi R, Ikuta T, et al: Using the Acropora digitifera genome to understand coral responses to environmental change. Nature 2011, 476:320-U382.

54. Cao D, Cao W, Yu K, Wu G, Yang J, Su X, Wang F: Evaluation of anthropogenic influences on the Luhuitou fringing reef via spatial and temporal analyses (from isotopic values). J Geophys Res: Oceans 2017, 122:4431-4443.

55. Strickland JD, Parsons TR: A practical handbook of seawater analysis. Ottawa: Fisheries Research Board of Canada 1972.

56. Grabherr MG, Haas BJ, Yassour M, Levin JZ, Thompson DA, Amit I, Adiconis X, Fan L, Raychowdhury R, Zeng Q, et al: Full-length transcriptome assembly from RNA-Seq data without a reference genome. Nat Biotechnol 2011, 29:644-652.

57. Simão FA, Waterhouse RM, Ioannidis P, Kriventseva EV, Zdobnov EM: BUSCO:
assessing genome assembly and annotation completeness with single-copy orthologs. *Bioinformatics* 2015, 31:3210-3212.

58. Conesa A, Götz S, García-Gómez JM, Terol J, Talón M, Robles M: **Blast2GO: a universal tool for annotation, visualization and analysis in functional genomics research.** *Bioinformatics* 2005, 21:3674-3676.

59. Li B, Dewey CN: **RSEM: accurate transcript quantification from RNA-Seq data with or without a reference genome.** *BMC Bioinform* 2011, 12:323.

60. Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, Paulovich A, Pomeroy SL, Golub TR, Lander ES, Mesirov JP: **Gene set enrichment analysis: A knowledge-based approach for interpreting genome-wide expression profiles.** *PNAS* 2005, 102:15545-15550.

Tables

Table 1. KEGG pathways related to differentially expressed transcripts
| Transcript                                    | Pathways                                                                 |
|----------------------------------------------|--------------------------------------------------------------------------|
| Coral                                        | Purine metabolism, Thiamine metabolism                                   |
| TRINITY_DN14229_c0_g1_i9                     |                                                                          |
| TRINITY_DN13556_c0_g1_i5                     |                                                                          |
| TRINITY_DN4043_c0_g1_i16                     | Phenylpropanoid biosynthesis, Tryptophan metabolism, Glyoxylate and dicarboxylate metabolism, Biosynthesis of antibiotics |
| TRINITY_DN6898_c0_g2_i4                      | Glycosphingolipid biosynthesis - ganglio series, Glycosaminoglycan degradation, Amino sugar and nucleotide sugar metabolism, Glycosphingolipid biosynthesis - globo and isoglobo series, Other glycan degradation, Various types of N-glycan biosynthesis |
| TRINITY_DN34708_c0_g1_i4                      | Nitrogen metabolism                                                      |
| TRINITY_DN32750_c0_g1_i20                     | Selenocompound metabolism, Aminoacyl-tRNA biosynthesis                   |
| TRINITY_DN7390_c0_g1_i21                     | Terpenoid backbone biosynthesis, Biosynthesis of antibiotics             |
| TRINITY_DN3703_c0_g1_i10                     | Cutin, suberine and wax biosynthesis, Glycerolipid metabolism            |
| TRINITY_DN5858_c0_g1_i21                     | Purine metabolism                                                        |
| TRINITY_DN24186_c0_g1_i5                      | N-Glycan biosynthesis                                                    |
| TRINITY_DN5858_c0_g1_i7                      | Purine metabolism                                                        |
| Symbiodiniaceae                              | Terpenoid backbone biosynthesis, Biosynthesis of antibiotics             |
| TRINITY_DN7791_c0_g2_i7                      |                                                                          |
| TRINITY_DN1389_c1_g2_i3                      | Alanine, aspartate and glutamate metabolism, Taurine and hypotaurine metabolism, Arginine biosynthesis, Nitrogen metabolism, D-Glutamine and D-glutamate metabolism |
| TRINITY_DN252939_c0_g2_i1                     | Thiamine metabolism, Purine metabolism                                   |
| TRINITY_DN148303_c0_g1_i2                     | Thiamine metabolism, Purine metabolism                                   |
| TRINITY_DN2449_c0_g3_i1                      | Thiamine metabolism, Purine metabolism                                   |
| TRINITY_DN144370_c0_g1_i1                     | Oxidative phosphorylation                                                |
| TRINITY_DN30301_c0_g2_i5                      | Alanine, aspartate and glutamate metabolism, Inositol phosphate metabolism, Streptomycin biosynthesis, Purine metabolism, Phosphatidylinositol signaling system, Biosynthesis of antibiotics |
| TRINITY_DN15645_c0_g1_i1                      | Thiamine metabolism, Purine metabolism, Biosynthesis of antibiotics      |
| TRINITY_DN16680_c1_g1_i3                      | Lipopolysaccharide biosynthesis                                           |
| TRINITY_DN30548_c0_g1_i2                      | Drug metabolism - other enzymes, Ether lipid metabolism                  |
| TRINITY_DN41973_c0_g1_i1                      | Thiamine metabolism, Purine metabolism                                   |
| TRINITY_DN1971_c0_g1_i3                      | Thiamine metabolism, Purine metabolism                                   |
| TRINITY_DN19477_c0_g1_i1                      | Starch and sucrose metabolism                                            |
| TRINITY_DN9266_c0_g1_i2                      | Carbon fixation in photosynthetic organisms                              |
| TRINITY_DN458_c0_g1_i4                        | Fatty acid biosynthesis                                                  |
| TRINITY_DN7549_c0_g1_i8                        | Nitrogen metabolism                                                     |
| TRINITY_DN45498_c0_g1_i2                      | Drug metabolism - other enzymes                                          |
| TRINITY_DN4215_c0_g1_i5                      | Thiamine metabolism, Purine metabolism                                   |
| TRINITY_DN9510_c0_g1_i1                      | Thiamine metabolism, Purine metabolism                                   |
| TRINITY_DN19429_c0_g1_i1                      | Thiamine metabolism, Purine metabolism                                   |
Table 2. KEGG pathways related to core transcripts

|      | Seq                                      | Pathway                                         |
|------|------------------------------------------|-------------------------------------------------|
| Coral| TRINITY_DN14229_c0_g1_i9                 | Purine metabolism, Thiamine metabolism          |
|      | TRINITY_DN13556_c0_g1_i5                | Purine metabolism, Thiamine metabolism          |
|      | TRINITY_DN5858_c0_g1_i7                 | Purine metabolism                               |

Figures

![Figure A](image_url)

![Figure B](image_url)
Figure 1

nMDS ordinations. nMDS plots of differentially expressed transcripts of coral (a) and Symbiodiniaceae (b) based on the square root transformed log base 2 of counts per million reads (CPM) by the Bray-Curtis measure of dissimilarity. Each symbol represents a replicate.

Kruskal’s stress values are provided.
Figure 2

Venn diagram. The venn diagram resulting from comparison of differentially expressed transcripts of coral (a) and Symbiodiniaceae (b) in different treatments compared with control.
Figure 3

GSEA results of coral/Symbiodiniaceae transcriptome in different treatments. GSEA displaying the major enriched gene sets related to ATP generation (a), photosystem (b) and stress response (c) of coral/Symbiodiniaceae transcriptome through nitrate concentrations.
Correlations between coral transcripts and Symbiodiniaceae physiology. a, b present algal density and photosynthetic rate in larvae from different groups, respectively. Each dot in A
represent a single larva. Each box in A and B represents the range of Q1-Q3 in each group, and the whisker in each box represents Q1-1.5SD, Q3+1.5SD of each group, respectively. Q1 and Q3 are the first and third quartile, and SD is standard deviation. The line in each box represents the mean value for each group. PN and PG in B represent net photosynthetic rate and gross photosynthetic rate, respectively. dbRDA indicates correlations between variance of coral differentially expressed transcripts and physiological conditions of Symbiodiniaceae (c). Each symbol represents a replicate from different groups.

Figure 5

Correlation of coral and Symbiodiniaceae transcripts under nutrient stress. Organic correlation network visualizing significant correlations between transcripts of coral and Symbiodiniaceae. Red and green represent coral and Symbiodiniaceae transcripts, respectively. Node size reflects the betweenness centrality of the variable. Line types (solid = positive and dashed = negative) indicate the Pearson correlation coefficient.
Figure 6

Coral-algal symbiosis related pathways and concept model for coral adaptation under eutrophic conditions. Representing the primary potential coral-algal symbiosis related pathways in coral meta-organisms (a) and enrichments of related gene sets in different treatments (b). Red, blue and light blue represent increase, decrease and no difference, respectively in B. Abbreviation: AA, amino acids; DOP, dissolved organic phosphorus; Glc,
glucose; Glu, glutamate; ROS, reactive oxygen; TCA, tricarboxylic acid cycle; VB2, vitamin B2 (riboflavin); VB9, vitamin B9 (folate).

Supplementary Files
This is a list of supplementary files associated with this preprint. Click to download.
Supplementary.docx