Relative Diazotroph Abundance in Symbiotic Red Sea Corals Decreases With Water Depth

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Microbial dinitrogen (N_2) fixation (diazotrophy) is a trait critical for coral holobiont functioning. The contribution of N_2 fixation to holobiont nitrogen (N) supply likely depends on the ecological niche of the coral holobiont. Consequently, coral-associated diazotroph communities may exhibit distinct activity patterns across a water depth gradient. We thus compared relative abundances of diazotrophs in the tissues of two common hard coral species, *Podabacia* sp. and *Pachyseris speciosa*, along their water depth distribution (10–30 m and 30–50 m, respectively) in the Central Red Sea. The relative gene copy numbers of the *nifH* gene (i.e., referenced against the eubacterial 16S rRNA gene), as a proxy for N_2 fixation potential, were assessed via quantitative PCR. We hypothesized that relative *nifH* gene copy numbers would decrease with water depth, assuming a related shift from autotrophy to heterotrophy. Findings confirmed this hypothesis and revealed that *nifH* gene abundances for both corals decreased by ~97% and ~90% from the shallowest to the deepest collection site. However, this result was not significant for *Pachyseris speciosa* due to high biological variability. The observed decrease in *nifH* gene abundances may be explained by the relative increase in heterotrophy of the coral animal at increasing water depths. Our results underline the importance of interpreting microbial functions and associated nutrient cycling processes within the holobiont in relation to water depth range reflecting steep environmental gradients.

Keywords: coral reefs, diazotrophy, heterotrophy, autotrophy, nitrogen fixation, depth gradient

INTRODUCTION

Scleractinian corals are associated with a diverse microbial community. This microbiome is comprised of dinoflagellate algae of the family Symbiodiniaceae (LaJeunesse et al., 2018) and a multitude of other eukaryotic and prokaryotic microbes that together form a meta-organism, the coral holobiont (Rohwer et al., 2002). Coral-associated microbiomes are host-specific, and provide key functions to the holobiont, such as provision and cycling of essential nutrients (among others) (Falkowski et al., 1984; Rohwer et al., 2002; Wegley et al., 2007; Rädecker et al., 2015; Hernandez-Agreda et al., 2018). Among these, diazotrophy or biological fixation of dinitrogen (N_2), a functional trait associated with diverse Bacteria and Archaea, i.e., diazotrophs, is associated with a broad range of scleractinian corals (Shashar et al., 1994; Lesser et al., 2007; Lema et al., 2012, 2014; Rädecker et al., 2014; Pogoreutz et al., 2017a,b). Diazotrophy, along with other nitrogen (N)

cycling pathways, was proposed central to the exceptional adaptation of scleractinian corals to oligotrophic environments (Shashar et al., 1994; Lesser, 2004; Lesser et al., 2007; Rädecker et al., 2015). Indeed, coral-associated N_2 fixation provides an important source of N for Symbiodiniaceae (Lesser et al., 2007; Cardini et al., 2015; Benavides et al., 2016; Bednarz et al., 2017), thereby helping to sustain holobiont productivity in shallow water corals when nutrients are scarce (Lesser et al., 2007; Cardini et al., 2015; Bednarz et al., 2017).

Microbial dinitrogen fixation is catalyzed by the nitrogenase enzyme complex, the activity of which is affected by its abiotic environment. Elevated levels of dissolved oxygen (O_2) for instance can irreversibly deactivate dinitrogenase reductase, one of the two subunits of the enzyme complex (Compaoré and Stal, 2010), thereby hampering N_2 fixation activity. Further, the enzymatic activity of the nitrogenase complex increases with temperature (Compaoré and Stal, 2010) and is hypothesized to decrease with increased environmental availability of bioavailable N [commonly referred to as "ammonia switch-off"; (Kessler et al., 2001)]. As O_2 availability within the coral tissues can exhibit considerable diurnal variation due to high rates of photosynthesis by Symbiodiniaceae during the day (Sorek et al., 2013), irradiance may be a critical driver of coral-associated N_2 fixation (Bednarz et al., 2017). These environmental parameters are subject to temporal and spatial change on a coral reef. Thus, to meet the metabolic carbon (C) and N requirements of the holobiont, scleractinian corals exhibit shifts in resource partitioning and may display variable levels of heterotrophy (Muscatine and Kaplan, 1994; Bednarz et al., 2017; Williams et al., 2018).

Acquisition of N through N_2 fixation in the coral holobiont is an energetically costly process that is likely fueled by photosynthetically fixed C (photosynthate) of the Symbiodiniaceae (Cardini et al., 2015). Given the attenuation of solar irradiance with depth in the water column (Lesser et al., 2009; Mass et al., 2010; Bednarz et al., 2018), autotrophic potential of the coral holobiont potentially decreases with depth as well, making the holobiont rely more on heterotrophy to fulfill its energy requirements (Ferrier-Pagès et al., 2003; Lesser et al., 2010). As heterotrophic food sources have a higher N content than photosynthate provided by Symbiodiniaceae, the N demand of the coral holobiont may decrease with increasing heterotrophy (Dubinsky and Jokiel, 1994). In this light, Pogoreutz et al. (2017b) showed a negative correlation of N_2 fixation activity with heterotrophic potential of scleractinian corals by using relative *nifH* gene copy numbers as a proxy for N_2 fixation potential. Thus, we hypothesized that corals at the shallower end compared to their deeper vertical distribution ranges will exhibit differential relative N_2 fixation activity, i.e., lower relative N_2 fixation activity with increasing depths. In order to test this hypothesis, we (i) assessed diazotroph abundances in two Red Sea corals, the mushroom coral *Podabacia* sp. (Fungiidae) and *Pachyseris speciosa* (Agariciidae), along a depth gradient spanning 10–50 m, and (ii) assessed the correlation of environmental parameters along the corals' depth gradient with the observed depth-dependent patterns of diazotroph abundance. To assess the relative abundance of diazotrophs in the coral microbiome, relative *nifH* gene copy numbers were obtained via quantitative PCR of the *nifH* gene normalized to the 16S rRNA gene (Pogoreutz et al., 2017a,b).

MATERIALS AND METHODS

Coral Collection

Two species of scleractinian coral, *Podabacia* sp. and *Pachyseris speciosa*, were sampled using SCUBA and technical diving at the midshore reef Al Fahal (N22°18′19.98″, E38°57′46.08″) in the Saudi Arabian central Red Sea in November 2012 (Ziegler et al., 2015). The Saudi Arabian Coastguard Authority issued sailing permits to the site that include coral collection. Both corals are listed as "least concern" according to the IUCN Red List of Threatened Species (Hoeksema et al., 2014a,b). One fragment from the central upward-facing surface per colony ($n = 3–5$) of both species were sampled in 10 m intervals from 10 to 50 m water depth. The depths at which the corals were sampled reflects the main distribution of each coral species at our sampling site (see sample distribution in **Table 1**).

Quantification of Diazotroph Communities

Coral tissue was removed from snap-frozen fragments with ice cold 4% NaCl solution using a standard airgun and an airbrush (Airbrush-starter-set, Conrad Electronic SE, Germany). Tissue slurries were homogenized and stored at −20°C until further processing. DNA was extracted from coral tissue slurries using the Qiagen DNeasy Plant Mini Kit (Qiagen, Germany) according to manufacturer's instructions. For DNA extraction, 100 µl aliquots of each tissue slurry sample were used in 300 µl AP-1 buffer. Extracted DNA was quantified using a Qubit fluorometer (Thermo Fisher Scientific, United States) and adjusted to a DNA concentration of 5 ng µl^{-1}.

To determine coral tissue-associated diazotroph abundances [as a proxy for N_2 fixation potential (Pogoreutz et al., 2017b)], relative gene copy numbers were quantified for the prokaryotic *nifH* gene (as the target gene) and the bacterial 16S rRNA (as a reference gene and a proxy for the total bacterial community) as reported previously (Pogoreutz et al., 2017a). The 16S rRNA gene was selected as a reference gene in the present study as it exhibited stable relative gene copy numbers in coral tissues across the entire water depth distribution range (see **Supplementary Figure S1**). Relative quantification was achieved with quantitative PCR (qPCR) in triplicate reactions with the Platinum SYBR Green qPCR SuperMix-UDG (Invitrogen, United States) according to manufacturer's instructions. Amplifications were performed with 5 µl SuperMix, 0.2 µL ROX reference dye,

TABLE 1 | Distribution and number of samples used to assess diazotroph abundances associated with the tissues of two species of reef-building corals along a water depth gradient in the central Red Sea.

Water depth (m)	10	20	30	40	50
Podabacia sp. (n)	3	3	3	–	–
Pachyseris speciosa (n)	–	–	5	3	4

0.2 µL of each 10 µM primer, 1 µL of input DNA template, and RNAse-free water to adjust the reaction volume to 10 µL. To amplify the bacterial 16S rRNA gene, the primers 781F (5′-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG -3′) and 1061R (5′- GTCTCGTGGGCTCGGAGATGTGTATAAGAGACA -3′) were used (Andersson et al., 2008). This primer pair includes the region V6 and covers the position 781 to 1,060. For amplification of the *nifH* gene, the degenerate primer pair F2 (5′-TGYGAYCCIAAIGCIGA -3′) and R6 (5′-TCIGGIGARATGATGGC -3′) was used (Gaby and Buckley, 2012). This primer pair covers the position 115 to 473. The qPCR was run under the following thermal profile: 2 min at 50°C, 1 min at 94°C, followed by 50 cycles of 30 s at 94°C, 1 min at 51°C, 1 min at 72°C, and an extension cycle of 1 min at 72°C (Pogoreutz et al., 2017b). The specificity of the amplifications was confirmed by melting curve analysis. Standard calibration curves were run simultaneously covering 6 orders of magnitude (10^4–10^9 copies of template per assay for the 16S rRNA and *nifH* gene). The qPCR efficiency (E) was >85% for both primers, calculated according to the equation $E = [10^{(-1/slope)} -1]$. Relative fold change of *nifH* gene copy numbers was calculated for all depths and both species as $2^{(-\Delta\Delta Ct)}$ using samples from the deepest sampling location of the respective species as a reference. Different depths at which both corals were collected (overlap only at 30 m) precluded direct comparisons of relative *nifH* gene abundances between both species.

Environmental Parameters

Environmental parameters at the collection site were collected on 3 days (within 10 days of each other) around noon in September 2012. All measuring procedures and environmental data were previously described and published in Ziegler et al. (2015). Briefly, a conductivity-temperature-depth recorder (CTD; SBE 16plusV2, Seabird Electronics, United States) was deployed to 50 m depth in close proximity to the sampling location measuring water temperature, PAR, O_2 saturation, salinity, turbidity, and chlorophyll concentration approximately every 10 m. Measurements at each depth lasted approximately 1 min.

Statistical Analyses

Data were analyzed using non-parametric permutational multivariate analysis of variance (PERMANOVA) using PRIMER-E version 6 software (Clarke and Gorley, 2006) with the PERMANOVA+ add on (Anderson, 2001). To test for differences in relative *nifH* gene copy numbers per species and individual environmental parameters across depths, 1-factorial PERMANOVAs were performed, based on Euclidean distances of normalized and/or square-root transformed data. Type I (sequential) sum of squares was used with unrestricted permutation of raw data (999 permutations) and PERMANOVA pairwise tests with parallel Monte Carlo tests were carried out when significant differences occurred.

To identify the environmental parameter that "best explains" the multivariate physiological pattern of the coral samples, a biota-environmental matching (BIOENV) routine was computed with 999 permutations based on Euclidean distances. This analysis maximizes Spearman rank correlations between the

resemblance matrices through permutation of all trial variables. Salinity and turbidity were omitted from the analysis due to their small-scale differences along the depth gradient. Input data were normalized prior to analysis by subtracting the variable's mean from each value and dividing it by the standard deviation using PRIMER normalization option. Finally, a correlation with depth for Symbiodiniaceae cell density of the investigated coral species [data obtained from Ziegler et al. (2015)] and relative *nifH* gene copy numbers was determined via linear regression using SigmaPlot 12 (Systat software).

RESULTS

Coral Diazotroph Abundances Along a Depth Gradient

The qPCR results confirmed the presence of the *nifH* gene in the microbial communities associated with both investigated Red Sea coral species and across the entire distribution range of sampling depths (**Figure 1**). Despite belonging to two different coral families (Fungiidae and Agariciidae) and exhibiting differences in water depth distribution range, the two coral species exhibited a similar pattern: relative *nifH* gene copy numbers for each coral species were highest at the two shallowest water depths and decreased at the greatest water depth (**Figure 1**). Significant differences with water depth were found for the mushroom coral *Podabacia* sp. (PERMANOVA, pseudo-F = 53.2, $p < 0.001$; **Table 2**), which exhibited significantly higher numbers of relative *nifH* gene copies at 10 and 20 m water depth (38-fold and 23-fold, respectively), compared to their conspecifics sampled at 30 m (pair-wise PERMANOVA, 10–30 m: $t = 8.61$, $p = 0.004$; 20–30 m: $t = 10.82$, $p < 0.001$; **Figure 1A**). These fold-changes correspond to a decrease in relative *nifH* gene copy numbers of 97% with water depth. *Pachyseris speciosa* exhibited 9-fold and 11-fold higher numbers of relative *nifH* gene copies at 30 and 40 m, respectively, compared to 50 m, but these differences were statistically not significant (PERMANOVA, pseudo-F = 1.69, $p = 0.230$; **Figure 1B** and **Table 2**). These fold-changes correspond to a decrease in relative *nifH* gene copy numbers of 90% with water depth. Overall, variation of biological replicates was greater in *Pachyseris speciosa* than in *Podabacia* sp. (**Figure 1**).

Environmental Parameters

All environmental parameters, except for turbidity, showed significant differences with depth (PERMANOVA, $p < 0.001$; for full details, see **Table 2**). Temperature decreased by 3.8°C from 10 to 50 m water depth (30.1 and 26.3°C, respectively; **Figure 2A**) (pair-wise PERMANOVA, $t = 40.21$, $p < 0.001$). PAR exhibited an exponential decrease with water depth. Approximately 6% of the light available at 10 m water depth around noon (561 µmol photons m^{-2} s^{-1}) penetrated down to 50 m water depth (35 µmol photons m^{-2} s^{-1}) (**Figure 2B**). Both temperature and PAR decreased with each successive depth measurement (pair-wise PERMANOVA, $p < 0.001$). Dissolved O_2 concentration decreased from 5.48 mg L^{-1} at 10 m to 5.28 mg L^{-1} at 50 m (pair-wise PERMANOVA, $t = 10.51$, $p < 0.001$; **Figure 2C**). Salinity decreased between 10 and 30 m from 39.4 PSU to 39.3 PSU

FIGURE 1 | Fold changes in relative *nifH* gene copy numbers normalized to 16S rRNA gene copy numbers in the tissues of two Red Sea scleractinian corals **(A)** *Podabacia* sp. and **(B)** *Pachyseris speciosa* (n = 3–5) depending on water depth. Fold changes were calculated in relation to the greatest collection depth of the respective species; error bars indicate upper confidence intervals (+ 1 SE). Black circles represent data points. Different letters above bars indicate significant differences within species ($p < 0.01$). As both corals were collected at different depths (limited overlap only at 30 m), direct comparisons of relative *nifH* gene abundances between both species were precluded.
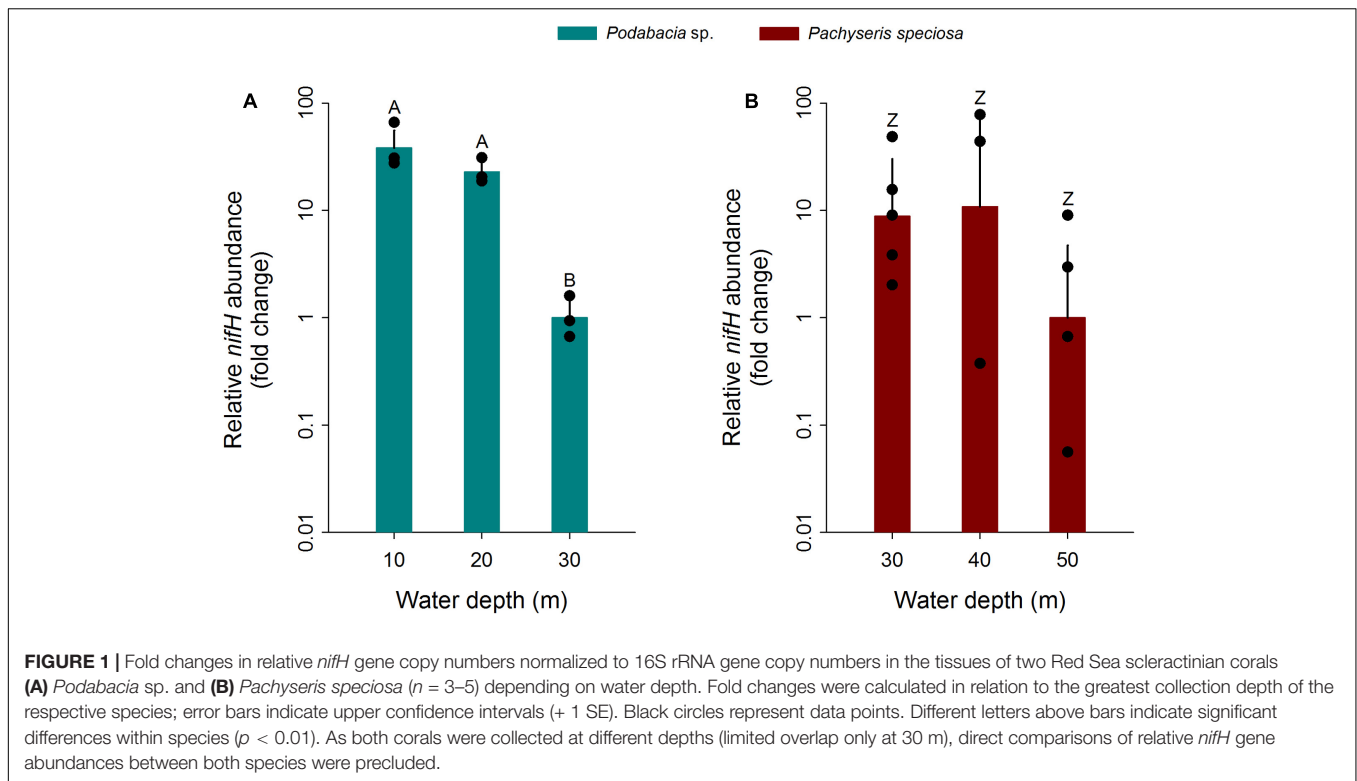
TABLE 2 | Summary of depth effects on relative *nifH* gene copy numbers associated with the tissues of two Red Sea coral species and for environmental factors based on permutational multivariate analysis of variance (PERMANOVA).

Parameter	Effect	df	Pseudo-F	p
Relative *nifH* gene copy numbers *Podabacia* sp.	Depth	2	53.2	0.001
Relative *nifH* gene copy numbers *Pachyseris speciosa*	Depth	2	1.7	0.230
Temperature	Depth	5	441.7	0.001
Photosynthetic active radiation (PAR)	Depth	5	343.2	0.001
Dissolved oxygen (O_2)	Depth	5	19.3	0.001
Salinity	Depth	5	15.9	0.001
Turbidity	Depth	5	0.9	0.457
Chlorophyll concentration	Depth	5	29.0	0.001

(pair-wise PERMANOVA, $t = 6.01$, $p < 0.001$; **Figure 2D**), but increased again between 30 and 50 m from 39.3 PSU to 39.4 PSU (pair-wise PERMANOVA, $t = 5.12$, $p < 0.001$; **Figure 2D**). Turbidity remained stable along the water depth gradient (**Figure 2E**). Chlorophyll concentration increased significantly from 0.14 mg m^{-3} at 10 m to 0.72 mg m^{-3} at 50 m (pair-wise PERMANOVA, $t = 16.90$, $p < 0.001$; **Figure 2F**). See **Table 3** for full details on pair-wise comparisons between all depths per environmental parameter.

In general, environmental parameters changed more drastically along the depth distribution of *Podabacia* sp. compared to those along the depth distribution of *Pachyseris speciosa*: temperature decreased with 2.7 and 0.8°C, respectively;

PAR decreased with 444 μmol m^{-2} s^{-1} and 82 μmol m^{-2} s^{-1}, respectively; and chlorophyll concentrations increased by 0.33 mg m^{-3} and 0.25 mg m^{-3}, respectively. Oxygen increased by 0.06 mg L^{-1} along the depth distribution of *Podabacia* sp., while it decreased with 0.26 mg L^{-1} along the depth distribution of *Pachyseris speciosa*.

Relationships of Relative *nifH* Gene Copy Numbers With Environmental Parameters

No correlation was found between depth and Symbiodiniaceae cell density for *Podabacia* sp. (Linear Regression, $F = 0.059$, $r^2 = 0.008$, $p = 0.82$; **Figure 3A**) and *Pachyseris speciosa* (Linear Regression, $F = 0.006$, $r^2 = 0.001$, $p = 0.94$; **Figure 3B**). A negative correlation was found between depth and relative *nifH* gene copy numbers (Linear Regression, $F = 12.1$, $r^2 = 0.388$, $p = 0.003$; **Figure 4**), i.e., decreasing relative *nifH* gene copy numbers with increasing depth. The environmental parameter that best explained this pattern in the BIOENV routine was temperature ($r = 0.317$, $p < 0.01$; **Supplementary Figure S2**), followed by chlorophyll concentration ($r = 0.289$), PAR ($r = 0.269$) and dissolved O_2 ($r = 0.105$).

DISCUSSION

In order to explore patterns of N_2 fixation activity along a depth gradient, we assessed relative *nifH* gene copy numbers in the tissues of two scleractinian corals species on a central Red Sea
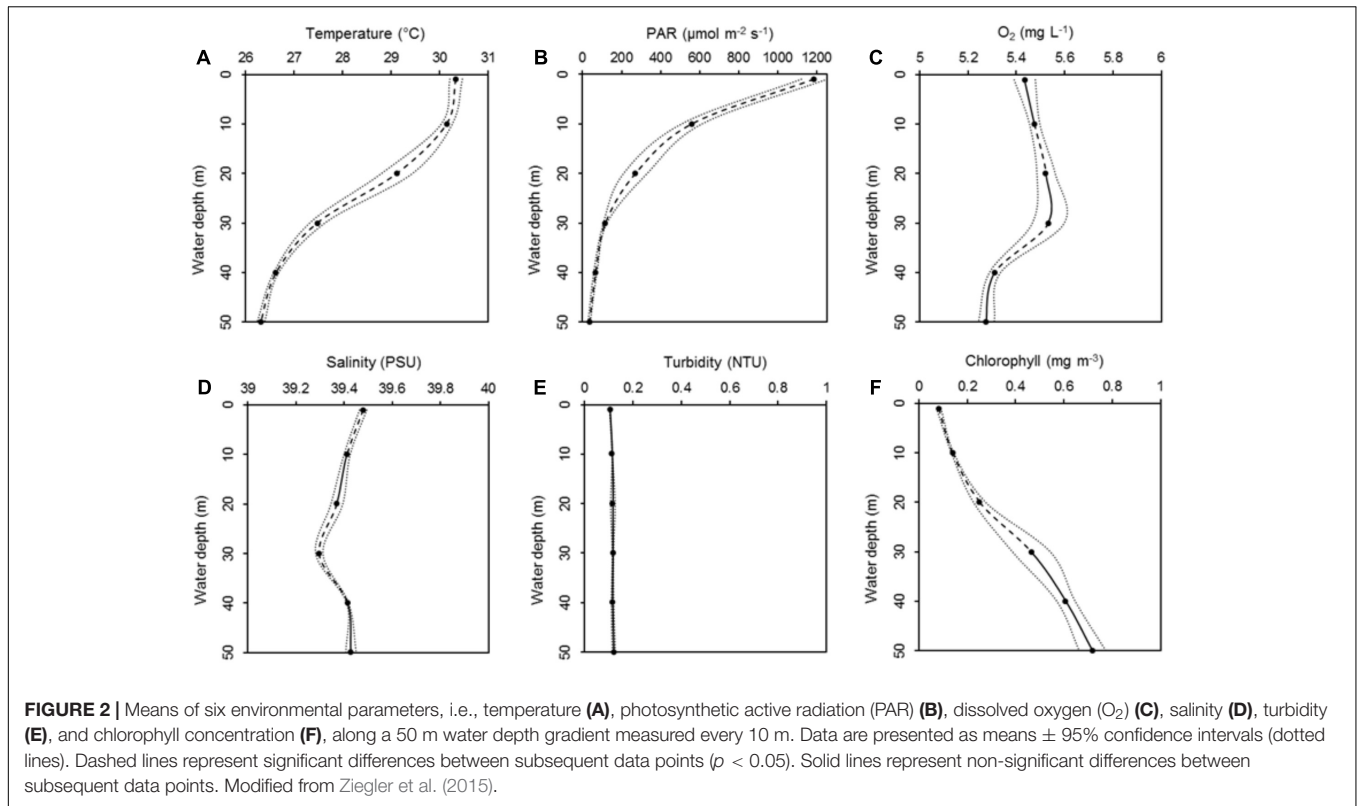
FIGURE 2 | Means of six environmental parameters, i.e., temperature **(A)**, photosynthetic active radiation (PAR) **(B)**, dissolved oxygen (O$_2$) **(C)**, salinity **(D)**, turbidity **(E)**, and chlorophyll concentration **(F)**, along a 50 m water depth gradient measured every 10 m. Data are presented as means ± 95% confidence intervals (dotted lines). Dashed lines represent significant differences between subsequent data points ($p < 0.05$). Solid lines represent non-significant differences between subsequent data points. Modified from Ziegler et al. (2015).

TABLE 3 | Pairwise comparison of 6 environmental parameters between water depths (m).

A								B							
1	X							1	X						
10	*	X						10	***	X					
20	***	***	X					20	***	***	X				
30	***	***	***	X				30	***	***	***	X			
40	***	***	***	***	X			40	***	***	***	***	X		
50	***	***	***	***	***	X		50	***	***	***	***	***	X	
	1	10	20	30	40	50			1	10	20	30	40	50	

C								D							
1	X							1	X						
10	ns	X						10	**	X					
20	**	*	X					20	***	ns	X				
30	*	ns	ns	X				30	***	***	*	X			
40	***	***	***	***	X			40	***	ns	ns	***	X		
50	***	***	***	***	ns	X		50	ns	ns	ns	***	ns	X	
	1	10	20	30	40	50			1	10	20	30	40	50	

E								F							
1	X							1	X						
10	ns	X						10	***	X					
20	ns	ns	X					20	***	***	X				
30	ns	ns	ns	X				30	***	***	*	X			
40	ns	ns	ns	ns	X			40	***	***	***	ns	X		
50	ns	ns	ns	ns	ns	X		50	***	***	***	ns	ns	X	
	1	10	20	30	40	50			1	10	20	30	40	50	

*Temperature (A), photosynthetic active radiation (PAR) (B), dissolved oxygen (O$_2$) (C), salinity (D), turbidity (E), and chlorophyll concentration (F). Obtained by pair-wise PERMANOVA. ns, not significant; *, significant (* <0.05; ** <0.01; and *** <0.001).*
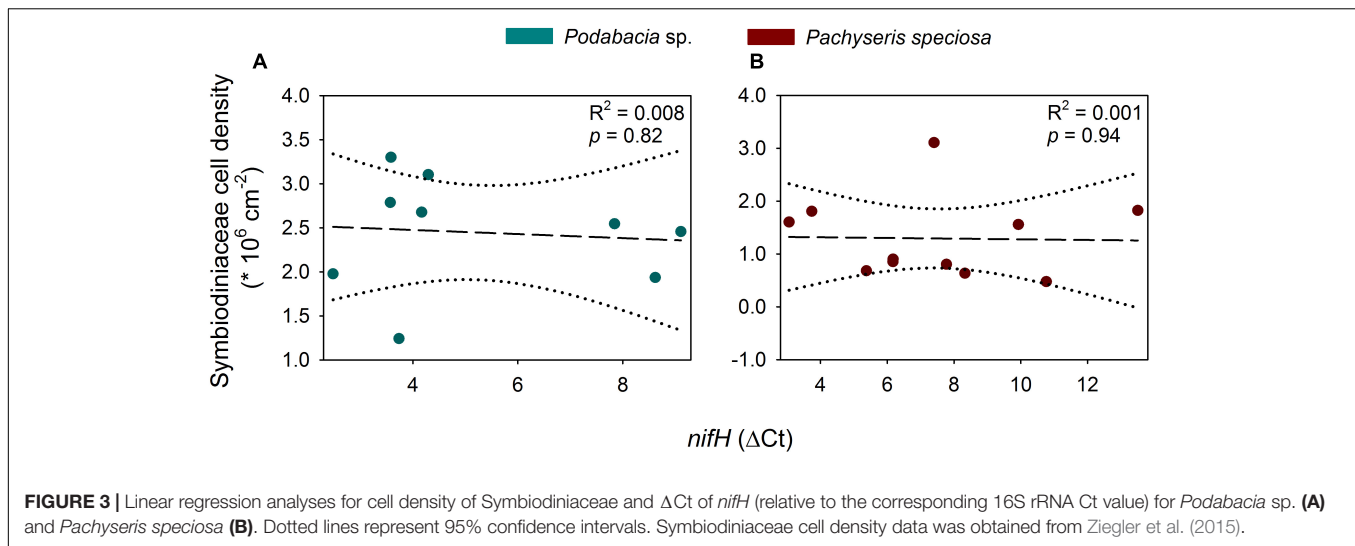
FIGURE 3 | Linear regression analyses for cell density of Symbiodiniaceae and ΔCt of *nifH* (relative to the corresponding 16S rRNA Ct value) for *Podabacia* sp. **(A)** and *Pachyseris speciosa* **(B)**. Dotted lines represent 95% confidence intervals. Symbiodiniaceae cell density data was obtained from Ziegler et al. (2015).
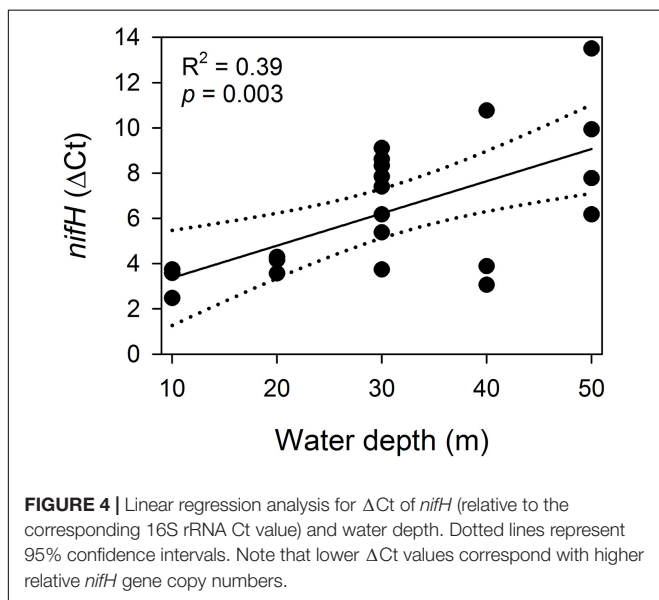

FIGURE 4 | Linear regression analysis for ΔCt of *nifH* (relative to the corresponding 16S rRNA Ct value) and water depth. Dotted lines represent 95% confidence intervals. Note that lower ΔCt values correspond with higher relative *nifH* gene copy numbers.

coral reef. Independent of their respective depth distribution range, which overlapped little, both coral species exhibited decreased relative *nifH* gene copy numbers at their greatest sampling depth. Temperature decreased from 30.1°C at 10 m water depth to 26.3°C at 50 m water depth and was, of the parameters tested, the environmental variable that best explained the observed patterns.

At the deepest sampling depths, *nifH* gene abundances for *Podabacia* sp. and *Pachyseris speciosa* were ∼97% and ∼90% lower, respectively, relative to conspecifics collected from shallower depths. This suggests a differential ecological dependence of these coral species on diazotrophy along their depth distribution range, as observed previously in other corals (Bednarz et al., 2017). This may be related to a shift from autotrophic to heterotrophic metabolism of the coral holobiont

with increasing water depths. Indeed, coral species with higher heterotrophic potential may harbor lower abundances of diazotrophs in their tissue (Pogoreutz et al., 2017b). Furthermore, translocation of photosynthate from Symbiodiniaceae to the coral host, potentially fueling the energy-expensive activity of coral-associated diazotrophs (Pogoreutz et al., 2017a), are reported to decrease from >90% to around 30% along a (mesophotic) depth gradient in the coral *Stylophora pistillata* due to changes in holobiont resource partitioning (Muscatine et al., 1989). Thus, the observed decrease in relative *nifH* gene copy numbers, i.e., relative diazotroph abundances, with increasing water depth may indeed be linked to reduced photosynthate translocation by Symbiodiniaceae (Lesser et al., 2007; Bednarz et al., 2018). Other studies have reported increased dependence on heterotrophy by coral holobionts with increasing water depth, decreasing temperature and/or light availability (Anthony and Fabricius, 2000; Palardy et al., 2008; Einbinder et al., 2009; Ferrier-Pagès et al., 2011; Godinot et al., 2011; Mies et al., 2018; Williams et al., 2018). Thereby, heterotrophy could provide the coral holobiont with a large part of its nutrient demand at these greater depths (Houlbrèque and Ferrier-Pagès, 2009; Lesser et al., 2010; Wijgerde et al., 2011; Burmester et al., 2018). However, this depth-dependent rate of N$_2$ fixation may be less pronounced when the supply of heterotrophic resources is not limited (Fox et al., 2018; Williams et al., 2018). It is therefore noteworthy to mention that data obtained here were collected in the highly oligotrophic Red Sea, with potentially limited heterotrophic resource abundance.

The results obtained here support the observation of low diazotroph abundances that align with increased heterotrophic potential. While previously investigated in a comparative taxonomic framework (Pogoreutz et al., 2017b), we here provide a spatial context. Both temperature and PAR decreased with depth at rates that are comparable to measurements in other coral reef systems (Frade et al., 2008; Mass et al., 2010; Schramek et al., 2018). Out of the measured parameters, temperature was the environmental variable that best explained the observed patterns in the present study. Indeed, temperature is a key environmental

driver of N_2 fixation activity and diazotroph abundance in scleractinian corals (Santos et al., 2014; Cardini et al., 2016; Pogoreutz et al., 2017a). Notably, seawater temperature decreased more strongly over the depth distribution range of *Podabacia* sp. than over the distribution range of *Pachyseris speciosa*. Thus, more pronounced differences in environment along the vertical depth distribution of *Podabacia* sp. compared to *Pachyseris speciosa* may explain the more pronounced differences in relative *nifH* relative gene copy numbers between sampling depths and coral species. Chlorophyll concentration was the second best environmental variable to explain the relative diazotrophic abundance pattern with depth. While no direct link between ambient chlorophyll concentrations and N_2 fixation is known, the increasing chlorophyll concentration with depth could be due to increased nutrient availability (Tilstra et al., 2018), potentially leading to "ammonia switch-off" (Kessler et al., 2001), and/or increased abundance of zooplankton that graze on phytoplankton. Thus, potentially more N is available for heterotrophic uptake for the corals. This may, in part, explain the decreasing relative *nifH* gene copy numbers and supports the hypothesis of increased heterotrophic potential at greater depths.

Pachyseris speciosa appears to prefer a low light regime (DiPerna et al., 2018). Indeed, *Pachyseris speciosa* at our study site was only found in deeper (upper mesophotic) waters. Previously, Ziegler et al. (2015) demonstrated that the acclimatization potential of Red Sea corals with mesophotic distribution is related to their algal symbiont physiologies, which is first and foremost driven by PAR (and interactions of other environmental parameters). In the present study, relative *nifH* gene copy numbers decreased with decreasing PAR despite a lack of correlation with cell densities of their photosynthetic algal symbiont. PAR positively correlates with N_2 fixation activity in benthic coral reef organisms (Bednarz et al., 2015; Tilstra et al., 2017), while increased O_2 may negatively affect the nitrogenase enzyme complex (Compaoré and Stal, 2010). While the latter may in part explain the pattern observed in *Podabacia* sp. between 10 and 20 m water depth, the opposite effect is found at all other depths for both species. Additionally, many diazotrophs have mechanisms in place to protect them from the potentially harmful effect of O_2 on the nitrogenase enzyme (Berman-Frank et al., 2001; Bednarz et al., 2018). Ambient dissolved O_2 measured may therefore not be a main driver of relative *nifH* gene copy numbers in the present study.

It is worth mentioning that the central Red Sea experiences pronounced seasonal changes in environmental parameters (Sawall et al., 2014; Roik et al., 2016). The period of study (i.e., fall) is characterized by stratification with fairly stable environmental parameters (Roik et al., 2016), which is followed in winter by mixing and thus less pronounced differences in environmental parameters with depth. Thus, the results obtained here may not be representative for this site and these species throughout the entire year but merely during this season. Furthermore, the high variability of relative *nifH* gene copy numbers in *Pachyseris speciosa* may be due to the limited number of replicates used in the present study. For future research, this could be overcome by collecting a higher number of replicates.

In summary, our study suggests that corals in shallow water may rely more heavily on N acquisition through N_2 fixation. In contrast, deep(er) water conspecifics likely rely more heavily on heterotrophy for nutrient acquisition, potentially conveying high intraspecific trophic plasticity. Results obtained here contribute to a growing body of knowledge highlighting fine-scale ecological differences of corals along their water depth distribution range (Rocha et al., 2018), and underline the importance of interpreting microbial functions and associated nutrient cycling processes within the holobiont in relation to water depth.

AUTHOR CONTRIBUTIONS

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2019.00372/full#supplementary-material

FIGURE S1 | Ct values from quantitative PCR of 16S rRNA gene amplicons at three different water depths for *Podabacia* sp. **(A)** and *Pachyseris speciosa* **(B)**. Black circles represent data points.

FIGURE S2 | Results of BIOENV analysis performed with PRIMER 6.

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