Received: 1 December 2017 | Revised: 7 March 2018 | Accepted: 7 March 2018

DOI: 10.1111/gcb.14141

PRIMARY RESEARCH ARTICLE

WILEY Global Change Biology

Coral bleaching is linked to the capacity of the animal host to supply essential metals to the symbionts

Christine Ferrier-Pagès[1] | Lucie Sauzéat[2] | Vincent Balter[2]

[1]Centre Scientifique de Monaco, Equipe Ecophysiologie corallienne, Monaco, Monaco

[2]CNRS UMR 5276 "Laboratoire de Géologie de Lyon", Ecole Normale Supérieure de Lyon, Lyon Cedex 07, France

Correspondence
Christine Ferrier-Pagès, Centre Scientifique de Monaco, Monaco, Monaco.
Email: ferrier@centrescientifique.mc

Abstract

Massive coral bleaching events result in extensive coral loss throughout the world. These events are mainly caused by seawater warming, but are exacerbated by the subsequent decrease in nutrient availability in surface waters. It has therefore been shown that nitrogen, phosphorus or iron limitation contribute to the underlying conditions by which thermal stress induces coral bleaching. Generally, information on the trophic ecology of trace elements (micronutrients) in corals, and on how they modulate the coral response to thermal stress is lacking. Here, we demonstrate for the first time that heterotrophic feeding (i.e. the capture of zooplankton prey by the coral host) and thermal stress induce significant changes in micro element concentrations and isotopic signatures of the scleractinian coral *Stylophora pistillata*. The results obtained first reveal that coral symbionts are the major sink for the heterotrophically acquired micronutrients and accumulate manganese, magnesium and iron from the food. These metals are involved in photosynthesis and antioxidant protection. In addition, we show that fed corals can maintain high micronutrient concentrations in the host tissue during thermal stress and do not bleach, whereas unfed corals experience a significant decrease in copper, zinc, boron, calcium and magnesium in the host tissue and bleach. In addition, the significant increase in δ^{65}Cu and δ^{66}Zn signature of symbionts and host tissue at high temperature suggests that these isotopic compositions are good proxy for stress in corals. Overall, present findings highlight a new way in which coral heterotrophy and micronutrient availability contribute to coral resistance to global warming and bleaching.

KEYWORDS
copper isotope, coral bleaching, essential metals, global warming, heterotrophy, zinc isotope

1 | INTRODUCTION

Mutualistic nutritional symbioses between animals and microorganisms are widespread in aquatic and terrestrial ecosystems, because they convey ecological advantages to the partners by allowing them to exploit a large panel of food sources (Douglas, 1998; Saffo, 1992; Venn, Loram, & Douglas, 2008). For example cyanobacteria in symbiosis with lichens, or bacteria associated to insects, supply their host with essential amino acids (Douglas, 1998; Usher, Bergman, & Raven, 2007). Prominent and widely recognized nutritional symbioses in the marine environment are those formed between dinoflagellate algae and sea anemones, jellyfish, sponges, clams or corals (Muscatine & Porter, 1977; Norton, Shepherd, Long, & Fitt, 1992). In particular, the association between corals and dinoflagellates of the genus *Symbiodinium* is at the basis of tropical reef ecosystems, which are as biodiverse as the rain forest, gathering more than 25% of all known marine species (Moberg & Folke, 1999).

Dinoflagellates in symbiosis with corals transform inorganic into organic nutrients via photosynthesis and translocate most of their photosynthates to the animal host for its own nutritional needs

(Muscatine, Falkowski, Porter, & Dubinsky, 1984; Tremblay, Grover, Maguer, Legendre, & Ferrier-Pagès, 2012). In turn, the host provides shelter to the dinoflagellates, but also nutrients from its metabolic waste products and/or its heterotrophic nutrition, i.e. the capture of zooplankton prey (Tremblay, Maguer, Grover, & Ferrier-Pagès, 2015; Yellowlees, Rees, & Leggat, 2008). Since photosynthates cover almost 100% of the energetic needs of the symbiotic association in optimal living conditions, most studies until now have focused on better understanding the many services provided by dinoflagellates to corals under varying environmental conditions (reviewed in Davy, Allemand, & Weis, 2012; Tremblay, Grover, Maguer, Hoogenboom, & Ferrier-Pagès, 2014). The contrary, i.e. the amount and nature of heterotrophic nutrients translocated from the host to the symbionts have been very poorly studied (Hughes, Grottoli, Pease, & Matsui, 2010; Piniak, Lipschultz, & McClelland, 2003; Tremblay et al., 2015). Heterotrophy can, however, maintain the growth and metabolism of both symbionts and hosts whenever conditions are not favourable to autotrophy, such as in low-light environments (Anthony, 2000), or during seawater warming, which leads to oxidative stress, symbiont expulsion (bleaching) and nutritional starvation (Ferrier-Pagès, Rottier, Beraud, & Levy, 2010; Grottoli, Rodrigues, & Palardy, 2006). The processes by which heterotrophy sustains coral metabolism during bleaching are not all known, except that particle capture is a major source of macronutrients (carbon, nitrogen and phosphorus), which enter into the composition of energetic reserves (Grottoli et al., 2006; Hughes & Grottoli, 2013). In addition, heterotrophy promotes the re-establishment of photosynthate translocation after heat stress, suggesting that the symbionts are one of the main beneficiaries of this nutrient source (Tremblay, Gori, Maguer, Hoogenboom, & Ferrier-Pagès, 2016). Recently, genomic analyses also showed, in corals fed with zooplankton, an up-regulation of genes involved in the antioxidant response (Levy et al., 2016). Such response may be mediated through the heterotrophic supply of micronutrients (trace metals), which are constituents of antioxidant enzymes (Richier, Furla, Plantivaux, Merle, & Allemand, 2005), or which enter into the composition of photosynthetic pigments of the coral symbionts and are essential during bleaching (Shick et al., 2011).

Although the acquisition and allocation of autotrophic and heterotrophic macronutrients in corals start to be well studied via the use of stable isotope ratios such as $^{13}C/^{12}C$ and $^{15}N/^{14}N$ (Hughes et al., 2010; Tremblay et al., 2015), the trophic ecology of trace elements remains almost unknown, both under normal growth conditions and during thermal stress. To date, the wide majority of studies have focused on metal and trace element concentrations in coral skeletons to follow pollution events (Barnes, Taylor, & Lough, 1995; Bastidas & Garcıa, 1999; David, 2003; Fallon, White, & McCulloch, 2002). Those performed at the tissue level showed that algal symbionts actively take up and accumulate trace elements dissolved in seawater (Ferrier-Pagès et al., 2005; Harland & Nganro, 1990; Reichelt-Brushett & McOrist, 2003), suggesting that coral bleaching may result in micronutrient limitation through symbiont loss. However, the changes in micronutrient concentrations or isotopic signature of symbionts and host tissue with feeding and environmental stress are still unknown. As shown in other organisms such as mice and humans, isotopic signature of copper and zinc can be significantly affected by pathological conditions (e.g. Balter et al., 2015) as well as by dietary conditions (e.g. Costas-Rodríguez, Van Heghe, & Vanhaecke, 2014; Jaouen, Pons, & Balter, 2013; Jaouen, Szpak, & Richards, 2016). This suggests that a change from autotrophic to heterotrophic feeding caused by bleaching conditions could also be marked by isotopic deregulations. In this study, we have investigated the trophic ecology of trace elements in the scleractinian coral *Stylophora pistillata* as well as the changes in the isotopic signature of copper and zinc under normal and thermal stress conditions. The first aim was to assess whether heterotrophy brings essential micronutrients to the symbiotic association, and contributes, by this process, to increase the resistance of corals to thermal-stress induced bleaching. The second aim was to evaluate whether heterotrophy, and/or thermal stress, can be traced through a change in the isotopic signature of copper and zinc. Investigating those aspects allows for a better understanding of the processes leading to coral bleaching. The results will show whether bleaching is exacerbated by the lack of trace elements in coral tissue and whether zooplankton capture can supply these micronutrients to corals.

2 | MATERIALS AND METHODS

2.1 | Maintenance of coral colonies

Five colonies of the scleractinian coral *Stylophora pistillata* (Esper 1797), originating from the Red Sea (Aqaba, Jordan), were used to generate 40 large nubbins of ca. 10 cm long (8 nubbins from each of the five colonies). Nubbins were then equally divided into four treatments and two 20 L aquaria per treatment (8 aquaria in total). During the first 5 weeks, four aquaria were fed during 2 hr and three times a week with 4,000 *artemia salina* nauplii, whereas the other four aquaria were kept unfed. All aquaria were maintained in an open flow system, with a water renewal rate of 10 L/hr and at a constant temperature of 25°C ± 0.5°C. Light was provided by hydrargyrum quartz iodide (HQI) lights at a photosynthetic active radiation level (PAR) of 200 μmol m^{-2} s^{-1} (measured using a spherical quantum sensor; LiCor LI-193, Lincoln, NE, USA), with a 12 hr:12 hr dark:light cycle. After these first 5 weeks, and for each feeding conditions, two aquaria were kept at 25°C while temperature was slowly raised (1°C every 2 days) in the two other aquaria to reach 30°C. Temperature and trophic conditions were maintained for three additional weeks prior measurements of trace metal concentrations and symbiont density were performed as described below.

2.2 | Treatments

Five nubbins in each condition were sampled for the determination of the symbiont density. Coral tissue was extracted from the skeleton using an air pick and homogenized with a Potter tissue grinder.

The symbiont density was quantified with three sub-samples of 100 μl using a Z1 Coulter Particle Counter (Beckman Coulter). The five remaining nubbins in each condition were sampled, rinsed using artificial seawater (ASW) made with ultrapure sodium chloride (NaCl) (Trace Select for trace analysis ≥99.999%, Sigma Aldrich). They were then individually placed in 50 ml beakers (precombusted at 480°C for 4 hr in a ThermolyneH 62700 oven and rinsed with ASW) containing 5 ml of ASW. For each sample, tissue was completely removed from the skeleton with an air pick and homogenized with a Potter tissue grinder (treated as beakers). The homogenate was divided into a host and a symbiont fraction. For the host fraction, the homogenate was centrifuged (Biofuge 17RS Heraeus) at 3,000 *g* for 10 min to pellet most of the symbionts. The supernatant was re-centrifuged twice to eliminate the remaining symbionts, then flash frozen with liquid nitrogen, and freeze-dried using a Heto (model CT 60) drier. For the symbiont fraction, the pellet was washed several times in ASW, flashed frozen and freeze-dried.

2.3 | Analyses of trace and major element concentrations

All chemical analyses were carried out in clean laminar flow hoods using double-distilled acids to avoid any exogenous contaminations. Freeze-dried coral and symbiont samples were dissolved in a concentrated HNO_3-H_2O_2 mixture, in Savillex beakers, at 120°C for at least 72 hr. Major and trace element concentrations were first measured in a small aliquot on an ICP-AES (iCAP 6000 Radial) and a quadrupole ICP-MS Thermo iCap-Q, respectively, at the Ecole Normale Supérieure (ENS) of Lyon following the method described in Garçon et al. (2017). Oxide interference and analytical drift were corrected using indium (In) and scandium (Sc) addition as internal standards for trace and major elements respectively (Sigma Aldrich, France). In-house (sheep plasma, OEP) and international standards (bovine liver, 1577c, Sigma Aldrich) as well as complete duplicate and re-run analyses were measured to ensure the validity and assess the precision of our results. Metal concentrations are all reported in μg/g (ppm) dry weight.

2.4 | Analyses of copper and zinc isotopic compositions

Copper and zinc isotopic compositions were then measured following the procedure described by Maréchal, Télouk, and Albarède (1999). Briefly, before each isotopic measurement, samples were purified by ion-exchange chromatography using quartz columns filled with 1.8 ml of Bio-Rad AGMP-1 (100–200 mesh) anion-exchange resin. Copper was first eluted with 20 ml of HCl (7 mol/L) + 0.001% H_2O_2 followed by zinc with 10 ml of HNO_3 (0.5 mol/L). The purification step helps removing elements (e.g. Ni, Ti, Al or Ba), which may interfere with copper and zinc during the instrumental measurement (Chen et al., 2016; Sossi, Halverson, Nebel, & Eggins, 2014). Indeed, it has been shown that small amounts of Ni and Ti residues, for example can produce isobaric interferences and shift the measured $\delta^{66}Zn$ values by more than 0.07‰ if the Ni/Zn and Ti/Zn ratios are higher than 0.001 and 0.01 respectively (Chen et al., 2016).

On the day of the analyses, an aliquot of the Zn and Cu purified solutions, previously evaporated to dryness, are dissolved in a Cu (Cu SRM 976, National Institute of Standards and Technology, Gaithersburg, MD, USA) or Zn-doped solution (Zn JMC 3-0749L, Johnson Matthey Royston, UK), respectively, to reach sample concentration of about 300 μg/L that is similar to the concentration of the standard solution that was run between each sample. Both copper and zinc isotopic compositions, expressed as

$$\delta^{65}Cu_{sample}\,(\text{in}\,‰) = \left[\frac{\left(^{65}Cu/^{63}Cu\right)_{sample}}{\left(^{65}Cu/^{63}Cu\right)_{standard}} - 1\right] * 1,000$$

$$\delta^{66}Zn_{sample}\,(\text{in}\,‰) = \left[\frac{\left(^{66}Zn/^{64}Zn\right)_{sample}}{\left(^{66}Zn/^{64}Zn\right)_{standard}} - 1\right] * 1,000$$

were measured on a Nu Plasma (Nu 500) MC-ICP-MS in wet plasma conditions. Instrumental mass fractionation was corrected with an exponential law using an elemental-doping method as recommended by Maréchal et al. (1999). $\delta^{66}Zn$ and $\delta^{65}Cu$ were then calculated by standard bracketing using Zn JMC 3-0749L (Johnson Matthey Royston, UK) and Cu SRM 976 (NIST, Gaithersburg, MD, USA) as reference standards. Note that these standards were repeatedly measured between each sample to correct for the instrumental drift throughout the analytical sequence; a method called standard bracketing. The accuracy of the isotopic compositions was assessed based on the analyses of an in-house (sheep plasma; OEP) and international (bovine liver, 1577c, Sigma Aldrich, France) standard solution at the beginning and during the analytical sequence. The average $\delta^{66}Zn$ measured for the in-house standard solution was +0.75‰ ± 0.07 and −1.19‰ ± 0.07 for $\delta^{65}Cu$ which is in good agreement with our previous reference in-house values ($\delta^{65}Cu$ = −1.15‰ ± 0.20 (standard deviation (*SD*), *n* = 35) and $\delta^{66}Zn$ = +0.73‰ ± 0.09 (*SD*, *n* = 20). Our results for the international standard (1577c) are also in good agreements with previous results: $\delta^{66}Zn_{this\ study}$ = −0.20‰ ± 0.06 (*SD*, *n* = 10) compared to $\delta^{66}Zn_{1577c}$ = −0.16‰ ± 0.15 (*SD*, *n* = 45) and $\delta^{65}Cu_{this\ study}$ = +0.30‰ ± 0.13 (*SD*, *n* = 10) compared to $\delta^{65}Cu_{1577c}$ = +0.37‰ ± 0.14 (*SD*, *n* = 43). On the basis of re-run samples and complete duplicate analyses, we estimate the precision of our measurements at ± 0.1 (*SD*) for both $\delta^{65}Cu$ and $\delta^{66}Zn$. The long-term precision based on the repeated measurements of standard Zn JMC 3-0749L and Cu SRM 976 alone is similar (±0.07‰ (*SD*, *n* = 480)).

2.5 | Statistics

A correlation-based principal component analysis (PCA) was used to quantify the effect of thermal stress and heterotrophic feeding on the chemical composition of symbionts and host tissue of colonies of *Sylophora pistillata*. The method consists in identifying new variables called principal components (PCs), which are linear combination of the original variables and along which data variation is maximal. For each principal component (PC), linear regressions, using standard

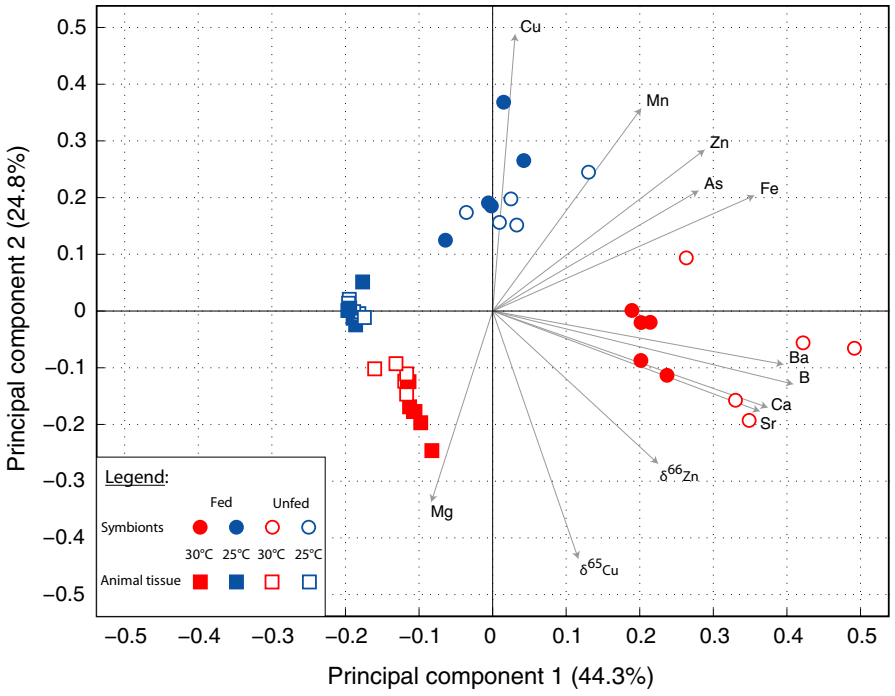

FIGURE 1 Principal Component Analysis (PCA) taking into account all data (micronutrient concentrations in symbionts and host tissue, as well as the isotopic signature of zinc and copper in each compartments, $n = 5$ samples for each condition) at 25°C (normal growth temperature) and 30°C (stress)

least-squares techniques, were thus used to estimate the relationship between parameters. In this study, the variables include the chemical concentrations of the trace elements measured in host tissue and symbionts (manganese, iron, magnesium, zinc, strontium, arsenic, barium, calcium, boron and copper), as well as copper (δ^{65}Cu) and zinc (δ^{66}Zn) isotopic compositions. All data were log transformed and normalized, and samples with incomplete data were excluded. PCA was implemented in MATLAB™.

2.5.1 | Statistical analyses

All parameters were expressed as mean ± standard deviation (*SD*) of five measurements. Statistical analyses were performed using SYSTAT 13 software (Systat Software, Chicago, IL, USA). Data were checked for normality using the Kolmogorov–Smirnov test with Lilliefors correction and for homoscedasticity using Levene test. Data were transformed with a natural logarithm transformation when required (i.e. Fe, Sr, Ca and B in symbionts; Fe, Mg, Zn, Ba, Ca and B in coral host). Factorial ANOVA with two factors (feeding an temperature) were performed and when there were significant differences between treatments, analyses were followed by a posteriori testing (Tukey's test). Differences between factors were considered significant for p-values <.05.

3 | RESULTS

The PCA yielded two principal components (PC1 and PC2) that explain ca. 70% of the total variance (Figure 1). In this figure, grey arrows graphically represent the loading factors (i.e. weight of each variable on PC1 and PC2, and Table S1). The first component (PC1), which explains 44% of the total variance, is defined by positive correlations with several trace elements such as iron (Fe), barium (Ba) or boron (B). This principal component demonstrates that symbionts contained higher micronutrient concentrations (μg/g dry weight) than the animal tissue, suggesting that nutrients were not distributed equally in these two compartments. Principal component 2 (PC2) explains 25% of the total variance and is defined by relatively high weights from Cu, but negative correlation with magnesium (Mg), δ^{65}Cu and δ^{66}Zn. This principal component separates both symbionts and animal tissues maintained at 25 and 30°C, showing that thermal stress was associated with variation in both their micronutrient content and isotopic composition. As demonstrated by the PCA and the results summarized in Table 1, for both temperatures investigated (25 and 30°C), and in all feeding conditions, symbionts presented higher concentrations (μg/g dry weight) than the animal tissue, except for Mg, whose concentration tended to be slightly higher in the host tissue (Figure 1).

The two factors ANOVA (Table 2) shows significant effect of both feeding and temperature, alone or in combination, on the micronutrient concentrations of symbionts and animal tissue. At the normal growth temperature (25°C), symbionts of fed colonies presented higher concentrations of Mg (post hoc, p-value = .002), Fe (p-value = .003) and Mn (p-value <.001), but lower concentrations of strontium (Sr), and arsenic (As) (p-value = .01 and .004 respectively) than symbionts of unfed colonies (Table S2). Inversely, there was no significant difference in micronutrient concentrations between fed and unfed coral tissue at 25°C (Table S3, post hoc, p value >.1). Host tissue of fed corals had, however, a higher δ^{65}Cu signature (−0.42‰ ± 0.12, ANOVA, p value = .0455) than the host tissue of unfed corals (−0.67‰ ± 0.05 respectively) (Figure 2).

Thermal stress (30°C) induced a significant bleaching in unfed colonies (t-test, p-value = .0016), with a 50% reduction in symbiont

TABLE 1 Micronutrient concentrations (μg/g dry weight) in symbionts and host tissue of fed and unfed *S. pistillata* maintained under normal (25°C) and thermal stress (30°C) conditions

	Higher concentrations in fed symbionts at 25°C			Lower concentrations in fed symbionts at 25°C								
	Mn	Fe	Mg	Zn	Sr	As	Ba	Ca	B	Cu	δ^{65}Cu (‰)	δ^{66}Zn (‰)
Symbionts 25°C												
Fed	6.12*	81.73	659.15	156.61*	160.35*	4.48	0.59*	12.02*	12.86*	14.02*	−0.70*	0.36*
SD	1.22	13.89	168.28	23.70	64.89	0.86	0.17	4.72	2.27	3.13	0.18	0.04
Symbionts 25°C												
Unfed	0.22*	45.83*	275.15*	198.14*	358.77*	10.77	1.22*	23.39*	16.24*	16.76*	−0.75*	0.46
SD	0.25	4.89	58.82	31.84	94.19	4.07	0.61	8.55	4.79	1.38	0.09	0.03
Symbionts 30°C												
Fed	1.19*	83.61	810.45	209.40*	1,992*	5.99	2.98*	96,242*	60.01*	3.56*	0.25*	0.56*
SD	0.33	35.15	132.33	20.13	840.41	1.17	1.18	73,835	8.28	0.93	0.11	0.03
Symbionts 30°C												
Unfed	2.32*	100.84*	864.49*	111.33*	3,984*	6.48	4.89*	116,904*	59.53*	3.57*	0.22*	0.54
SD	1.52	57.38	360.29	72.34	1,975	3.91	2.16	65,108	25.66	1.40	0.09	0.05
Host 25°C												
Fed	0.07*	4.22*	924.45*	11.87*	8.02*	1.83*	0.04	0.73*	3.02*	6.59	−0.42*	0.35*
SD	0.07	0.74	178.86	2.46	0.67	0.28	0.01	0.05	1.25	7.52	0.12	0.02
Host 25°C												
Unfed	0.04	3.63	881.99	13.14*	9.24*	1.74*	0.09	0.80*	2.62*	3.55*	−0.67*	0.56
SD	0.08	0.44	273.63	1.68	3.59	0.27	0.10	0.25	1.07	0.19	0.05	0.02
Host 30°C												
Fed	0.35*	11.11*	5,670*	31.24*	46.62*	3.32*	0.19	3,581*	16.63*	1.36	0.38*	0.56*
SD	0.10	2.44	2,002	3.37	8.49	1.36	0.18	583.86	4.53	0.30	0.18	0.02
Host 30°C												
Unfed	0.10	4.45	1,360	6.28*	27.06*	3.21*	0.04	1,393*	5.84*	0.63*	0.31*	0.52
SD	0.03	2.06	461.09	0.74	3.51	1.29	0.01	446.35	1.79	0.31	0.12	0.08

Data are mean and standard deviation (*SD*) of five samples per condition.
*Represents significant differences in micronutrient concentrations between the two temperature conditions for both symbionts and host under fed or unfed conditions.

density (Figure 2). On the contrary, it had no effect on the symbiont density of fed colonies, which also presented two times more symbionts (Figure 2). Thermal stress significantly changed the micronutrient concentrations (Tables 1, 2 and S2, S3) of the symbiotic association. There was a significant increase of Sr, Ca, Zn and B concentrations in both symbionts and host of fed and unfed colonies (Tables 1 and 2). High temperature also significantly increased Mg concentrations in fed host tissue and unfed symbionts, Ba in fed and unfed symbionts, as well as Mn in the host tissue of fed corals (Tables 2, S2, S3). On the contrary, the Cu content of fed and unfed colonies was significantly decreased (Table 2). Finally, thermal stress significantly increased the δ^{65}Cu and δ^{66}Zn values of both host and symbionts, independently of the feeding status (Figure 2).

The binary plot of Figure 3a shows that there was an overall increase in both the δ^{65}Cu and δ^{66}Zn values with thermal stress in corals and symbionts. The other binary plots performed with micronutrient concentrations (Figure 3b–f) exhibit two thermal stress patterns on fed and unfed coral colonies: data corresponding to fed and unfed host or fed and unfed symbionts are tightly clustered at 25°C, suggesting that at this temperature there is no effect of the dietary regime. The same pattern is observed for all symbionts at 30°C, suggesting that they obtain the same amount of micronutrients from their respective fed and unfed hosts. On the contrary, micronutrient concentrations in the host show a distillation process at 30°C, associated to a depletion in unfed hosts compared to fed ones (red arrows bleaching, Figure 3b–f). The amplitude by which hosts are depleted increases as the micronutrient concentrations decrease.

4 | DISCUSSION

Understanding the nutritional requirements of scleractinian corals is critical to assess their health status and predict how reefs will change in response to rising seawater temperatures (Connolly, Lopez-Yglesias, & Anthony, 2012; Ferrier-Pagès et al., 2010). The main finding of this study is that heterotrophic feeding brings

TABLE 2 Results of the two factors ANOVA testing the effects of temperature and feeding on the nutrient concentrations and isotopic signatures

	Symbionts			Tissue		
	df	*p*	*F*	*df*	*p*	*f*
Manganese (Mn)						
Feeding	1	**.0010**	17.27	1	**.0010**	16.04
Temperature	1	**.0273**	6.07	1	**.0002**	22.89
Feeding × temperature	1	**<.001**	61.84	1	**.0075**	9.36
Error	14					
Iron (Fe)						
Feeding	1	**.0467**	4.65	1	**.0004**	20
Temperature	1	**.0004**	19.61	1	**.0003**	21.75
Feeding × temperature	1	**<.0001**	65.32	1	**.0044**	10.95
Error	16			16		
Magnesium (Mg)						
Feeding	1	.3671	0.86	1	**.0014**	14.84
Temperature	1	**<.0001**	48	1	**<.0001**	43.72
Feeding × temperature	1	**<.0001**	28.43	1	**.0039**	11.38
Error	16			16		
Zinc (Zn)						
Feeding	1	.0419	4.89	1	**<.0001**	45.67
Temperature	1	.2934	1.18	1	.4327	0.64
Feeding × temperature	1	**<.0001**	30.4	1	**<.0001**	38.65
Error	16			16		
Strontium (Sr)						
Feeding	1	**.0105**	8.39	1	**.0005**	18.89
Temperature	1	**<.0001**	90.03	1	**<.0001**	119.91
Feeding × temperature	1	.3487	0.93	1	**.0002**	22.85
Error	16			16		
Arsenic (As)						
Feeding	1	**.0048**	10.7	1	.5212	0.43
Temperature	1	.6682	0.17	1	**.0008**	18.83
Feeding × temperature	1	.1279	2.58	1	.6728	0.19
Error	16			16		
Barium (Ba)						
Feeding	1	**.0021**	13.49	1	.5284	0.18
Temperature	1	**<.0001**	52.96	1	.2393	0.34
Feeding × temperature	1	**.0438**	4.79	1	**.0357**	5.66
Error	16			16		
Calcium (Ca)						
Feeding	1	**.0223**	7.02	1	**.0075**	8.75
Temperature	1	**<.0001**	744.93	1	**<.0001**	650.5
Feeding × temperature	1	.82	0.05	1	**.0035**	12.11
Error	11			16		
Boron (B)						
Feeding	1	**.0052**	10.43	1	**.0042**	11.15
Temperature	1	**<.0001**	132.15	1	**<.0001**	56.67
Feeding × temperature	1	**.0294**	5.71	1	**.0275**	5.89

(Continues)

TABLE 2 (Continued)

	Symbionts			Tissue		
	df	*p*	*F*	*df*	*p*	*f*
Error	16			16		
Copper (Cu)						
Feeding	1	**.0336**	5.4	1	.2796	1.25
Temperature	1	**<.0001**	222.68	1	**.0286**	5.78
Feeding × temperature	1	.141	2.4	1	.5027	0.47
Error	16			16		
δ^{65}Cu						
Feeding	1	.8399	0.04	1	**.0455**	4.7
Temperature	1	**<.0001**	72.23	1	**<.0001**	44.26
Feeding × temperature	1	.8398	0.04	1	.3745	0.83
Error	16			16		
δ^{66}Zn						
Feeding	1	.6455	0.22	1	.5186	0.44
Temperature	1	**<.0001**	33.11	1	**<.0001**	103.31
Feeding × temperature	1	.1159	2.76	1	**.0331**	5.44
Error	16			16		

Analyses were performed with five replicates per condition. Significant differences are in bold.

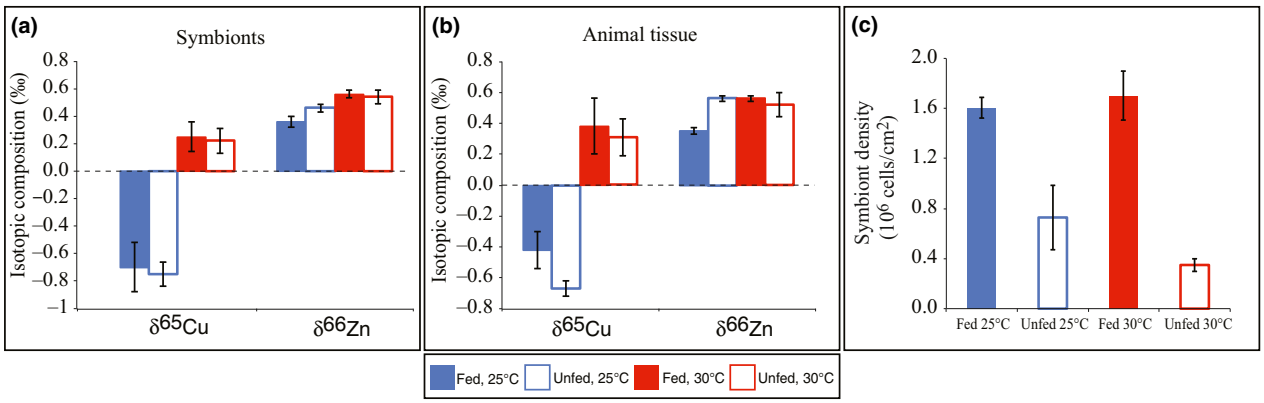

FIGURE 2 Isotopic signature of copper (δ^{65}Cu) and zinc (δ^{66}Zn) in (a) symbionts and (b) host tissue of colonies maintained fed and unfed at 25°C or 30°C; (c) Symbiont density (10^6 cell/cm^2) (data are mean and standard deviation of 5 samples per experimental conditions)

essential micronutrients to corals, which are mainly accumulated in the symbionts, and help avoid thermal stress damages and subsequent bleaching. During thermal stress, symbionts retain micronutrients at the expense of the coral host, thus being more parasites than symbionts. We also demonstrate a significant disruption in the Ca, Mg, Sr and B homeostasis in coral tissue during thermal stress, as well as a significant increase in the δ^{65}Cu and δ^{66}Zn values of coral host and symbionts. These isotopic compositions thus constitute good proxies for thermal stress in corals.

4.1 | Effect of the dietary regime at 25°C

Symbionts were the main sink for micronutrients within the symbiotic association (Figure 1), although these nutrients were provided to corals as particulate organic food, first digested by the animal. This observation is contrary to the usual view that symbionts are the main food suppliers of the symbiotic association, and provides one of the rare evidence of inverse nutrient translocation, from the animal host to the symbionts (Piniak et al., 2003; Tremblay et al., 2015). Although the above studies showed that symbionts profited from heterotrophic macronutrients (carbon, nitrogen) entering the symbiotic association, the transfer was restricted to less than 20% of the total gain, against 100% in this study.

Plankton feeding significantly increased, in symbiont cells, the concentrations of three main micronutrients, namely Mn, Fe and Mg (Table 1). Among other functions, Mn, Fe and Mg are all involved in the composition of antioxidant enzymes (Krueger et al., 2015). These enzymes, such as catalases, peroxidases and Fe/Mn or Mn-superoxide dismutases (SOD), are present in *Symbiodinium* (Lin et al., 2015; Zhang, Zhuang, Gill, & Lin, 2013), and are used to scavenge reactive

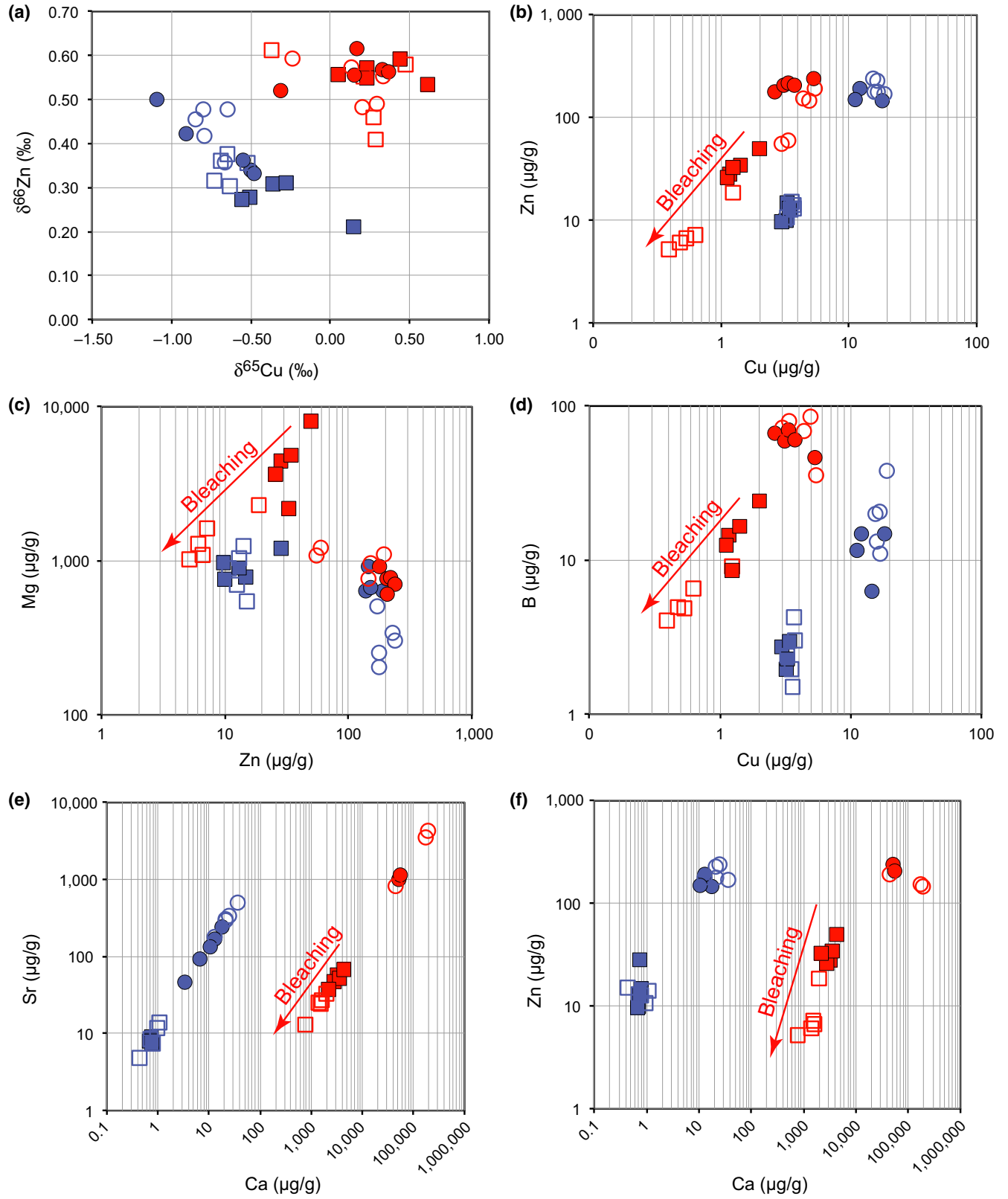

FIGURE 3 Relationship between the δ^{65}Cu and δ^{66}Zn values, or between the concentration (µg/g) in Cu and Zn, Mg and Zn, B and Cu, Ba and Ca, Zn and Ca (n = 5 samples for each experimental condition). Open circles and squares represent unfed symbionts and host tissue at 25°C (blue) or 30°C (red) respectively. Filled circles and squares represent fed symbionts and host tissue at 25°C (blue) or 30°C (red) respectively. The arrow indicates the direction of the distillation process during bleaching

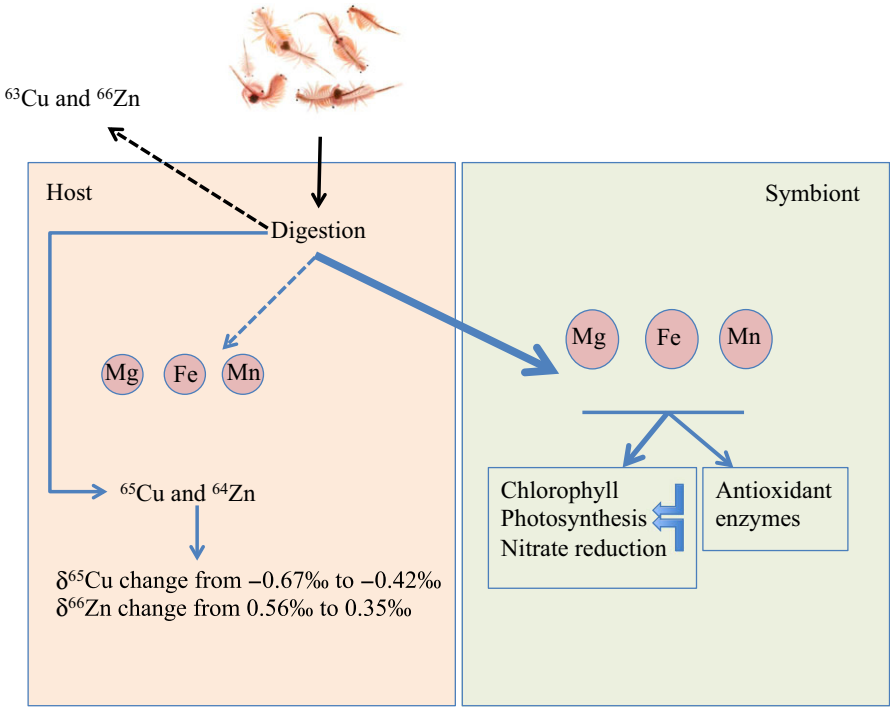

FIGURE 4 Heterotrophic supply of micronutrients within the coral-dinoflagellate association. Zooplankton predation increases the amount of Mg, Mn and Fe in symbionts. These metals are involved in photosynthesis processes, in nitrate reduction and in the structure of antioxidant enzymes. Prey digestion preferentially releases light copper isotope (i.e. ^{63}Cu) and inversely heavy zinc isotope (^{66}Zn), increasing the δ^{65}Cu signature and decreasing the δ^{66}Zn signature of coral tissue. Blue arrows: positive effect

oxygen species (ROS) continuously produced through photosynthesis and over-produced during thermal stress (Weis, 2008). Mn and Fe also play a major role in photosynthesis, nitrate reduction, as well as in chlorophyll and amino acid synthesis (Twining & Baines, 2013). This involvement of Fe and Mn in photosynthetic processes is one of the explanations why symbiont density, chlorophyll content, and photosynthetic rates are significantly enhanced in heterotrophically fed coral colonies compared to unfed ones, both in normal growth and stress conditions (this study and reviewed in Houlbrèque & Ferrier-Pagès, 2009). On the contrary, a lack of Fe during thermal stress increases the bleaching susceptibility of corals (Shick et al., 2011). Overall, these results highlight the importance of Fe and other key micronutrient availability for coral photosynthesis and health. Whereas concentrations of Fe, Mn, Mg were enhanced in fed symbionts under normal growth conditions, concentrations of Ca and Sr, which are main components of coral skeletons, were significantly decreased (Table 1). Such changes may be linked to a higher translocation of these elements to the skeleton, since fed corals present higher calcification rates than unfed corals (Houlbrèque & Ferrier-Pagès, 2009). The schematic diagram of Figure 4 summarizes the findings observed in this study and the potential role of metals in symbionts, under normal growth conditions of *S. pistillata*.

The δ^{65}Cu and δ^{66}Zn values were significantly different between fed and unfed hosts and symbionts (Figure 2), suggesting, for the first time, that corals fractionate Cu and Zn isotopes depending on their dietary regime. The higher δ^{65}Cu values in fed colonies is linked to the consumption of Cu in the form of particulate organic matter, whereas unfed colonies only rely on the uptake of dissolved inorganic Cu by the symbionts. The δ^{65}Cu signature in fed corals indeed follows the rule that organic matter is enriched in the heavier Cu isotope compared to dissolved inorganic Cu (Petit et al., 2013). The lower δ^{66}Zn signature of fed compared to unfed colonies is also in agreement with the observation that carnivores are slightly ^{66}Zn-depleted relative to herbivores (Jaouen et al., 2016). Overall, the significant effect of feeding on the δ^{66}Zn and δ^{65}Cu signatures demonstrates that metal isotopes represent useful proxies to trace nutrient flows within a symbiotic association, although they have poorly been studied in marine food webs in general (Jaouen et al., 2016).

4.2 | Effect of thermal stress

After 3 weeks of thermal stress, which induced bleaching in unfed corals (Figure 2), significant changes occurred in micronutrient concentrations and Cu-Zn isotopic compositions of symbionts and host tissue under both fed and unfed conditions. We observed a clear increase in the δ^{65}Cu and δ^{66}Zn values of both hosts and symbionts (Figure 3a). Such isotopic variations can be related to an increase in reactive oxygen species (ROS) generated by thermal stress in coral tissue (Cunning & Baker, 2013). As light isotopes create weaker links with molecules compared to heavy isotopes (Schauble, 2004), these bonds are more easily disrupted by ROS (Demidov, 2007; Shchepinov & Pestov, 2010). Light isotopes will thus preferentially be released in the medium. Although this assumption still needs to be verified, we can already suggest that δ^{65}Cu and δ^{66}Zn signatures represent good proxies for thermal stress in corals. In addition, the correlation which existed between δ^{65}Cu and δ^{66}Zn at 25°C was lost at 30°C (Figure 3), suggesting that animals and symbionts were not in equilibrium any more at 30°C.

For all corals, thermal stress also induced a very large increase of the Mg, Sr, Ca and B concentrations compared to normal growth conditions, in particular in symbionts of unfed colonies. Although

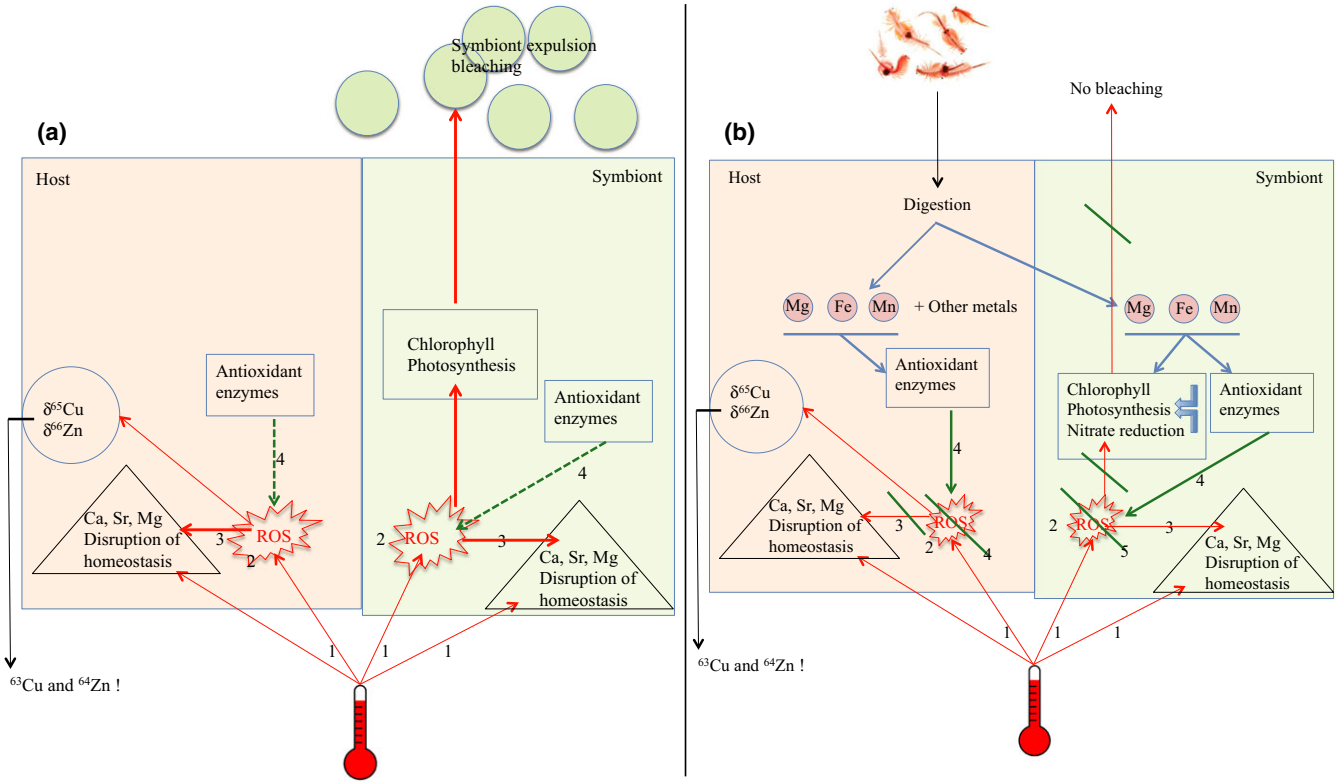

FIGURE 5 Schematic diagram summarizing the effect of thermal stress on micronutrient concentrations in unfed (a) and fed (b) corals. Thermal stress (1) increases ROS (reactive oxygen species) in coral tissue (2), and induces the disruption of Ca, Sr, Mg and B homeostasis (3). In unfed corals (a), micronutrient concentrations and antioxidant enzymes are not produced in sufficient amount to significantly reduce ROS (4). Photosynthesis is impaired and symbionts are expulsed (bleaching). In fed corals (b), feeding increases the amount of micronutrients in host tissue and symbionts. Antioxidant enzymes are produced in sufficient amount to significantly reduce ROS (4). Photosynthesis is not impaired and symbionts are not expulsed (no bleaching). Red arrows: negative effect, green arrows: positive effect

such changes have never been investigated in corals, an increase in intracellular Ca concentration has been monitored in insects (Teets, Yi, Lee, & Denlinger, 2013), chicken lymphocytes (Han et al., 2010) or terrestrial plants (Zhu, 2001) during thermal stress, whereas an increase in Mg was observed in the shrimp *Crangon crangon* (Sartoris & Pörtner, 1997). Genomic studies, which have investigated the molecular basis of cnidarian bleaching, also suggested that Ca homeostasis was disrupted in heat-stressed corals (DeSalvo et al., 2008; Levy et al., 2016; Rodriguez-Lanetty, Harii, & Hoegh-Guldberg, 2009). Finally, Dishon et al. (2015) demonstrated a decline of $\delta^{11}B$ as a result of coral bleaching, likely due, according to our results, to the observed large increase in B concentration, which should have changed the intracellular pH. All these micronutrients play important cellular roles. Ca is an intracellular messenger, whereas Mg has a protective role against oxidative damage (Freedman et al., 1992; Sartoris & Pörtner, 1997), which suggests that the significant Mg increase in coral cells followed the temperature-induced ROS production (Weis, 2008). Finally, B is needed, among others, for membrane structural integrity and carbohydrate metabolism (reviewed in Pilbeam & Kirkby, 1983), two important processes in corals.

Despite the large internal changes induced by thermal stress, mixotrophic corals (fed with artemia) did not bleach while autotrophic corals did. The lack of bleaching, as well as the maintenance of high rates of photosynthesis and calcification in mixotrophic corals have been observed several times in previous studies (Borell & Bischof, 2008; Connolly et al., 2012; Ferrier-Pagès et al., 2010). The binary plots of micronutrient concentrations in fed and unfed coral colonies (Figure 3b–f) highlight a new mechanism by which heterotrophy sustains coral metabolism during thermal stress, e.g. via the supply of essential micronutrients to the host and symbionts. These plots show similar micronutrient concentrations in symbionts of both fed and unfed colonies during thermal stress. In other words, symbionts sequestered the same amount of nutrients irrespective of the feeding status of their host. As micronutrients are mostly translocated from the host to the symbionts, this led to two different conditions in host tissue depending on the feeding status of the colonies. In fed corals, heterotrophy supplied enough micronutrients to the host to sustain symbiont's needs and no bleaching is observed. In unfed corals, symbiont repletion lead to a significant depletion of micronutrients in host tissue (Figure 3b–f), and a cost for the symbiotic association, which underwent bleaching and nutrient starvation. The amplitude by which hosts were depleted increased as the micronutrient concentrations decreased. These results thus demonstrate the significant effect of feeding in providing essential trace and major elements to the animals during thermal stress. The significant bleaching in unfed colonies further suggests

that, contrary to fed corals, micronutrient concentrations in unfed host tissue were not sufficient to sustain high densities of symbionts. Finally, they also suggest that thermal stress has promoted symbiont parasitism in unfed corals. A similar selfish behaviour of symbionts during thermal stress has very recently been observed for macronutrients (Baker, Freeman, Wong, Fogel, & Knowlton, 2018). Figure 5 summarizes the findings and hypotheses on the role of metals in coral bleaching.

In summary, our results indicate that the δ^{65}Cu and δ^{66}Zn values represent good proxies for stress in corals. They also show that heterotrophy and thermal stress induce significant changes in micronutrient concentrations and isotopic signatures of the scleractinian coral *Stylophora pistillata*. The results obtained first reveal that symbionts are the major sink for trace elements in the symbiotic association, and accumulate Mn, Mg and Fe from the heterotrophic feeding of the host. They also demonstrate that bleaching is exacerbated by the shortage of trace elements in coral tissue (especially Cu, Zn, B, Mg, Ca) and that heterotrophic feeding can supply these essential metals to corals. Such results thus highlight a new way by which heterotrophy contributes to coral resistance to bleaching, and have major implications for the resilience of coral reefs under threat of global change.

ACKNOWLEDGEMENTS

The authors thank Cecile Rottier and Magali Boussion for help in coral extraction. We also thank two reviewers, and Prof. Oren Levy, for their helpful comments, which have greatly improved the manuscript. This work is part of RTPI Nutress (Réseau Thématique Pluridisciplinaire International sur l'Ecophysiologie de la Nutrition).

ORCID

Christine Ferrier-Pagès http://orcid.org/0000-0002-0357-4486

REFERENCES

Anthony, K. R. N. (2000). Enhanced particle-feeding capacity of corals on turbid reefs (Great Barrier Reef, Australia). *Coral Reefs*, *19*(1), 59–67. https://doi.org/10.1007/s003380050227

Baker, D. M., Freeman, C. J., Wong, J. C. Y., Fogel, M. L., Knowlton, N. (2018). Climate change promotes parasitism in a coral symbiosis. *The ISME Journal*, *12* (3), 921–930. https://doi.org/10.1038/s41396-018-0046-8

Balter, V., da Costa, A. N., Bondanese, V. P., Jaouen, K., Lamboux, A., Sangrajrang, S., … Heynaud, P. (2015). Natural variations of copper and sulfur stable isotopes in blood of hepatocellular carcinoma patients. *Proceedings of the National Academy of Sciences*, *112*(4), 982–985. https://doi.org/10.1073/pnas.1415151112

Barnes, D. J., Taylor, R. B., & Lough, J. M. (1995). On the inclusion of trace materials into massive coral skeletons. Part II: Distortions in skeletal records of annual climate cycles due to growth processes. *Journal of Experimental Marine Biology and Ecology*, *194*(2), 251–275. https://doi.org/10.1016/0022-0981(95)00091-7

Bastidas, C., & Garcıa, E. (1999). Metal content on the reef coral *Porites astreoides*: An evaluation of river influence and 35 years of chronology. *Marine Pollution Bulletin*, *38*(10), 899–907. https://doi.org/10.1016/S0025-326X(99)00089-2

Borell, E. M., & Bischof, K. (2008). Feeding sustains photosynthetic quantum yield of a scleractinian coral during thermal stress. *Oecologia*, *157*(4), 593. https://doi.org/10.1007/s00442-008-1102-2

Chen, S., Liu, Y., Hu, J., Zhang, Z., Hou, Z., Huang, F., & Yu, H. (2016). Zinc isotopic compositions of NIST SRM 683 and Whole-Rock reference materials. *Geostandards and Geoanalytical Research*, *40*(3), 417–432. https://doi.org/10.1111/j.1751-908X.2015.00377.x

Connolly, S. R., Lopez-Yglesias, M. A., & Anthony, K. R. (2012). Food availability promotes rapid recovery from thermal stress in a scleractinian coral. *Coral Reefs*, *31*(4), 951–960. https://doi.org/10.1007/s00338-012-0925-9

Costas-Rodríguez, M., Van Heghe, L., & Vanhaecke, F. (2014). Evidence for a possible dietary effect on the isotopic composition of Zn in blood via isotopic analysis of food products by multi-collector ICP-mass spectrometry. *Metallomics*, *6*, 139–146. https://doi.org/10.1039/C3MT00244F

Cunning, R., & Baker, A. C. (2013). Excess algal symbionts increase the susceptibility of reef corals to bleaching. *Nature Climate Change*, *3*(3), 259–262. https://doi.org/10.1038/nclimate1711

David, C. P. (2003). Heavy metal concentrations in growth bands of corals: A record of mine tailings input through time (Marinduque Island, Philippines). *Marine Pollution Bulletin*, *46*(2), 187–196. https://doi.org/10.1016/S0025-326X(02)00315-6

Davy, S. K., Allemand, D., & Weis, V. M. (2012). Cell biology of cnidarian-dinoflagellate symbiosis. *Microbiology and Molecular Biology Reviews*, *76*(2), 229–261. https://doi.org/10.1128/MMBR.05014-11

Demidov, V. V. (2007). Heavy isotopes to avert ageing? *Trends in Biotechnology*, *25*(9), 371–375. https://doi.org/10.1016/j.tibtech.2007.07.007

DeSalvo, M. K., Voolstra, C. R., Sunagawa, S., Schwarz, J. A., Stillman, J. H., Coffroth, M. A., … Medina, M. (2008). Differential gene expression during thermal stress and bleaching in the Caribbean coral *Montastraea faveolata*. *Molecular Ecology*, *17*(17), 3952–3971. https://doi.org/10.1111/j.1365-294X.2008.03879.x

Dishon, G., Fisch, J., Horn, I., Kaczmarek, K., Bijma, J., Gruber, D. F., … Tchernov, D. (2015). A novel paleo-bleaching proxy using boron isotopes and high-resolution laser ablation to reconstruct coral bleaching events. *Biogeosciences*, *12*(19), 5677–5687. https://doi.org/10.5194/bg-12-5677-2015

Douglas, A. E. (1998). Nutritional interactions in insect-microbial symbioses: Aphids and their symbiotic bacteria Buchnera. *Annual Review of Entomology*, *43*(1), 17–37. https://doi.org/10.1146/annurev.ento.43.1.17

Fallon, S. J., White, J. C., & McCulloch, M. T. (2002). Porites corals as recorders of mining and environmental impacts: Misima Island, Papua New Guinea. *Geochimica et Cosmochimica Acta*, *66*(1), 45–62. https://doi.org/10.1016/S0016-7037(01)00715-3

Ferrier-Pagès, C., Houlbrèque, F., Wyse, E., Richard, C., Allemand, D., & Boisson, F. (2005). Bioaccumulation of zinc in the scleractinian coral *Stylophora pistillata*. *Coral Reefs*, *24*(4), 636–645. https://doi.org/10.1007/s00338-005-0045-x

Ferrier-Pagès, C., Rottier, C., Beraud, E., & Levy, O. (2010). Experimental assessment of the feeding effort of three scleractinian coral species during a thermal stress: Effect on the rates of photosynthesis. *Journal of Experimental Marine Biology and Ecology*, *390*(2), 118–124. https://doi.org/10.1016/j.jembe.2010.05.007

Freedman, A. M., Mak, I. T., Stafford, R. E., Dickens, B. F., Cassidy, M. M., Muesing, R. A., & Weglicki, W. B. (1992). Erythrocytes from magnesium-deficient hamsters display an enhanced susceptibility to oxidative stress. *American Journal of Physiology*, *262*, C1371–C1375. https://doi.org/10.1152/ajpcell.1992.262.6.C1371

Garçon, M., Sauzéat, L., Carlson, R. W., Shirey, S. B., Simon, M., Balter, V., & Boyet, M. (2017). Nitrile, latex, neoprene and vinyl gloves: A primary source of contamination for trace element and zn isotopic analyses in geological and biological samples. *Geostandards and Geoanalytical Research*, *41*(3), 367–380.

Grottoli, A. G., Rodrigues, L. J., & Palardy, J. E. (2006). Heterotrophic plasticity and resilience in bleached corals. *Nature*, *440*(7088), 1186–1189. https://doi.org/10.1038/nature04565

Han, A. Y., Zhang, M. H., Zuo, X. L., Zheng, S. S., Zhao, C. F., Feng, J. H., & Cheng, C. (2010). Effect of acute heat stress on calcium concentration, proliferation, cell cycle, and interleukin-2 production in splenic lymphocytes from broiler chickens. *Poultry Science*, *89*(10), 2063–2070. https://doi.org/10.3382/ps.2010-00715

Harland, A. D., & Nganro, N. R. (1990). Copper uptake by the sea anemone *Anemonia viridis* and the role of zooxanthellae in metal regulation. *Marine Biology*, *104*(2), 297–301. https://doi.org/10.1007/BF01313271

Houlbrèque, F., & Ferrier-Pagès, C. (2009). Heterotrophy in tropical scleractinian corals. *Biological Reviews*, *84*(1), 1–17. https://doi.org/10.1111/j.1469-185X.2008.00058.x

Hughes, A. D., & Grottoli, A. G. (2013). Heterotrophic compensation: A possible mechanism for resilience of coral reefs to global warming or a sign of prolonged stress? *PLoS ONE*, *8*(11), e81172. https://doi.org/10.1371/journal.pone.0081172

Hughes, A. D., Grottoli, A. G., Pease, T. K., & Matsui, Y. (2010). Acquisition and assimilation of carbon in non-bleached and bleached corals. *Marine Ecology Progress Series*, *420*, 91–101. https://doi.org/10.3354/meps08866

Jaouen, K., Pons, M. L., & Balter, V. (2013). Iron, copper and zinc isotopic fractionation up mammal trophic chains. *Earth and Planetary Science Letters*, *374*, 164–172. https://doi.org/10.1016/j.epsl.2013.05.037

Jaouen, K., Szpak, P., & Richards, M. P. (2016). Zinc isotope ratios as indicators of diet and trophic level in arctic marine mammals. *PLoS ONE*, *11*(3), e0152299. https://doi.org/10.1371/journal.pone.0152299

Krueger, T., Fisher, P. L., Becker, S., Pontasch, S., Dove, S., Hoegh-Guldberg, O., … Davy, S. K. (2015). Transcriptomic characterization of the enzymatic antioxidants FeSOD, MnSOD, APX and KatG in the dinoflagellate genus *Symbiodinium*. *BMC Evolutionary Biology*, *15*(1), 48. https://doi.org/10.1186/s12862-015-0326-0

Levy, O., Karako-Lampert, S., Ben-Asher, H. W., Zoccola, D., Pagès, G., & Ferrier-Pagès, C. (2016). Molecular assessment of the effect of light and heterotrophy in the scleractinian coral *Stylophora pistillata*. *Proceedings Royal Society B*, *283*, 20153025. https://doi.org/10.1098/rspb.2015.3025

Lin, S., Cheng, S., Song, B., Zhong, X., Lin, X., Li, W., … Cai, M. (2015). The *Symbiodinium* Kawaguti genome illuminates dinoflagellate gene expression and coral symbiosis. *Science*, *350*, 691–694. https://doi.org/10.1126/science.aad0408

Maréchal, C. N., Télouk, P., & Albarède, F. (1999). Precise analysis of copper and zinc isotopic compositions by plasma-source mass spectrometry. *Chemical Geology*, *156*(1), 251–273. https://doi.org/10.1016/S0009-2541(98)00191-0

Moberg, F., & Folke, C. (1999). Ecological goods and services of coral reef ecosystems. *Ecological Economics*, *29*(2), 215–233. https://doi.org/10.1016/S0921-8009(99)00009-9

Muscatine, L., Falkowski, P. G., Porter, J. W., & Dubinsky, Z. (1984). Fate of photosynthetic fixed carbon in light-and shade-adapted colonies of the symbiotic coral *Stylophora pistillata*. *Proceedings of the Royal Society of London B: Biological Sciences*, *222*(1227), 181–202. https://doi.org/10.1098/rspb.1984.0058

Muscatine, L., & Porter, J. W. (1977). Reef corals: Mutualistic symbioses adapted to nutrient-poor environments. *BioScience*, *27*(7), 454–460. https://doi.org/10.2307/1297526

Norton, J. H., Shepherd, M. A., Long, H. M., & Fitt, W. K. (1992). The zooxanthellal tubular system in the giant clam. *The Biological Bulletin*, *183*(3), 503–506. https://doi.org/10.2307/1542028

Petit, J. C., Schäfer, J., Coynel, A., Blanc, G., Deycard, V. N., Derriennic, H., … Mattielli, N. (2013). Anthropogenic sources and biogeochemical reactivity of particulate and dissolved Cu isotopes in the turbidity gradient of the Garonne River (France). *Chemical Geology*, *359*, 125–135. https://doi.org/10.1016/j.chemgeo.2013.09.019

Pilbeam, D. J., & Kirkby, E. A. (1983). The physiological role of boron in plants. *Journal of Plant Nutrition*, *6*(7), 563–582. https://doi.org/10.1080/01904168309363126

Piniak, G. A., Lipschultz, F., & McClelland, J. (2003). Assimilation and partitioning of prey nitrogen within two anthozoans and their endosymbiotic zooxanthellae. *Marine Ecology Progress Series*, *262*, 125–136. https://doi.org/10.3354/meps262125

Reichelt-Brushett, A. J., & McOrist, G. (2003). Trace metals in the living and nonliving components of scleractinian corals. *Marine Pollution Bulletin*, *46*(12), 1573–1582. https://doi.org/10.1016/S0025-326X(03)00323-0

Richier, S., Furla, P., Plantivaux, A., Merle, P. L., & Allemand, D. (2005). Symbiosis-induced adaptation to oxidative stress. *Journal of Experimental Biology*, *208*(2), 277–285. https://doi.org/10.1242/jeb.01368

Rodriguez-Lanetty, M., Harii, S., & Hoegh-Guldberg, O. (2009). Early molecular responses of coral larvae to hyperthermal stress. *Molecular Ecology*, *18*(24), 5101–5114. https://doi.org/10.1111/j.1365-294X.2009.04419.x

Saffo, M. B. (1992). Coming to terms with a field: Words and concepts in symbiosis. *Symbiosis*, *14*(1–3), 17–31.

Sartoris, F. J., & Pörtner, H. O. (1997). Increased concentrations of haemolymph Mg2 + protect intracellular pH and ATP levels during temperature stress and anoxia in the common shrimp. *Journal of Experimental Biology*, *200*, 785–792.

Schauble, E. A. (2004). Applying stable isotope fractionation theory to new systems. *Reviews in Mineralogy and Geochemistry*, *55*(1), 65–111. https://doi.org/10.2138/gsrmg.55.1.65

Shchepinov, M. S., & Pestov, N. B. (2010). Isotope effect, essential diet components and prospects of aging retardation. *Russian Journal of General Chemistry*, *80* (7), 1514–1522. https://doi.org/10.1134/S1070363210070480

Shick, J. M., Iglic, K., Wells, M. L., Trick, C. G., Doyle, J., & Dunlap, W. C. (2011). Responses to iron limitation in two colonies of *Stylophora pistillata* exposed to high temperature: Implications for coral bleaching. *Limnology and Oceanography*, *56*(3), 813–828. https://doi.org/10.4319/lo.2011.56.3.0813

Sossi, P. A., Halverson, G. P., Nebel, O., & Eggins, S. M. (2014). Combined separation of Cu, Fe and Zn from rock matrices and improved analytical protocols for stable isotope determination. *Geostandards and Geoanalytical Research*, *39*(2), 129–149.

Teets, N. M., Yi, S. X., Lee, R. E., & Denlinger, D. L. (2013). Calcium signaling mediates cold sensing in insect tissues. *Proceedings of the National Academy of Sciences*, *110*(22), 9154–9159. https://doi.org/10.1073/pnas.1306705110

Tremblay, P., Gori, A., Maguer, J. F., Hoogenboom, M., & Ferrier-Pagès, C. (2016). Heterotrophy promotes the re-establishment of photosynthate translocation in a symbiotic coral after heat stress. *Scientific Reports*, *6*, 38112. https://doi.org/10.1038/srep38112

Tremblay, P., Grover, R., Maguer, J. F., Hoogenboom, M., & Ferrier-Pagès, C. (2014). Carbon translocation from symbiont to host depends on irradiance and food availability in the tropical coral Stylophora pistillata. *Coral Reefs*, *33*(1), 1–13. https://doi.org/10.1007/s00338-013-1100-7

Tremblay, P., Grover, R., Maguer, J. F., Legendre, L., & Ferrier-Pagès, C. (2012). Autotrophic carbon budget in coral tissue: A new 13C-based model of photosynthate translocation. *Journal of Experimental Biology*, *215*(8), 1384–1393. https://doi.org/10.1242/jeb.065201

Tremblay, P., Maguer, J. F., Grover, R., & Ferrier-Pagès, C. (2015). Trophic dynamics of scleractinian corals: Stable isotope evidence. *Journal of Experimental Biology*, *218*(8), 1223–1234. https://doi.org/10.1242/jeb.115303

Twining, B. S., & Baines, S. B. (2013). The trace metal composition of marine phytoplankton. *Annual Review of Marine Science*, *5*, 191–215. https://doi.org/10.1146/annurev-marine-121211-172322

Usher, K. M., Bergman, B., & Raven, J. A. (2007). Exploring cyanobacterial mutualisms. *Annual Review of Ecology, Evolution, and Systematics*, *38*, 255–273. https://doi.org/10.1146/annurev.ecolsys.38.091206.095641

Venn, A. A., Loram, J. E., & Douglas, A. E. (2008). Photosynthetic symbioses in animals. *Journal of Experimental Botany*, *59*(5), 1069–1080. https://doi.org/10.1093/jxb/erm328

Weis, V. M. (2008). Cellular mechanisms of Cnidarian bleaching: Stress causes the collapse of symbiosis. *Journal of Experimental Biology*, *211* (19), 3059–3066. https://doi.org/10.1242/jeb.009597

Yellowlees, D., Rees, T. A. V., & Leggat, W. (2008). Metabolic interactions between algal symbionts and invertebrate hosts. *Plant, Cell & Environment*, *31*(5), 679–694. https://doi.org/10.1111/j.1365-3040.2008.01802.x

Zhang, H., Zhuang, Y., Gill, J., & Lin, S. (2013). Proof that dinoflagellate spliced leader (DinoSL) is a useful hook for fishing dinoflagellate transcripts from mixed microbial samples: *Symbiodinium* kawagutii as a case study. *Protist*, *164*(4), 510–527. https://doi.org/10.1016/j.protis.2013.04.002

Zhu, J. K. (2001). Cell signaling under salt, water and cold stresses. *Current Opinion in Plant Biology*, *4*(5), 401–440. https://doi.org/10.1016/S1369-5266(00)00192-8

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

How to cite this article: Ferrier-Pagès C, Sauzéat L, Balter V. Coral bleaching is linked to the capacity of the animal host to supply essential metals to the symbionts. *Glob Change Biol*. 2018;00:1–13. https://doi.org/10.1111/gcb.14141