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Alice Blanckaert

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Sorbonne Université

École doctorale 129 : Sciences de l'Environnement

Centre Scientifique de Monaco – Équipe d'Écophysiologie

**Effets de la disponibilité en macro- et micro-nutriments sur la physiologie
des coraux tropicaux**

Effects of macro- and micro-nutrient availability on tropical coral physiology

Alice Blanckaert

Thèse de doctorat des Sciences de l'Environnement

Dirigée par Dr. Christine Ferrier-Pagès

Co-encadrée par Dr. Renaud Grover

Présentée et soutenue publiquement le 16 Janvier 2023 à Monaco

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AVANT-PROPOS

Note sur la traduction française

Ce manuscrit est essentiellement écrit en anglais. Toutefois, un résumé détaillé en français est écrit à la fin du manuscrit.

Contenu

Cette thèse se présente sous la forme de quatre articles publiés formant les différents chapitres de la thèse.

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Liste des principales abréviations

Abréviations	Traduction anglaise	Traduction française
AcPA	Acid phosphatase activity	Activité des phosphatase acide
AFDW	Ash free dry weight	Poids sec sans cendre
APA	Alkaline phosphatase activity	Activité des phosphatase alcaline
ATP	Adenosine triphosphate	Adénosine triphosphate
BCA	Bicinchoninic acid	Acide bicinchoninic
C	Carbon	Carbone
CAT	Catalase	Catalase
Cd	Cadmium	Cadmium
Chl	Chlorophyll	Chlorophylle
Cu	Copper	Cuivre
DIC	Dissolved inorganic carbon	Carbone inorganique dissout
DIN	Dissolved inorganic nitrogen	Azote inorganique dissout
DINut	Dissolved inorganic nutrient	Nutriment inorganique dissout
DIP	Dissolved inorganic phosphorus	Phosphore inorganique dissout
DON	Dissolved organic nitrogen	Azote organique dissout
DOP	Dissolved organic phosphorus	Phosphore organique dissout
DW	Dry weight	Poids sec
ETR	Electron transport rate	Taux de transfert des électrons
Fe	Iron	Fer
FSW	Filtered seawater	Eau de mer filtrée
GoA	Gulf of Aquaba	Golf d'Aquaba
LPO	Lipid peroxidation	Peroxydation des lipides
MAAs	Mycosporine like amino acids	Acides aminés analogue de la mycosporine
N	Nitrogen	Azote
NH ₄	Ammonium	Ammonium
Ni	Nickel	Nickel
NO ₃	Nitrate	Nitrate
P	Phosphorus	Phosphore
PA	Phosphatase activity	Activité des phosphatases
PAR	Photosynthetically active radiation	Rayonnement photosynthétiquement actif

Pg	Gross photosynthesis	Photosynthèse brut
PIP	Particulate inorganic phosphorus	Phosphore organique particulaire
Pn	Net photosynthesis	Photosynthèse nette
PO ₄	Phosphate	Phosphate
PON	Particulate organic nitrogen	Azote organique particulaire
POP	Particulate organic phosphorus	Phosphore organique particulaire
PSII	Photosystem II	Photosystème II
R	Respiration	Respiration
RNS	Reactive nitrogen species	Espèces réactives de l'azote
ROS	Reactive oxygen species	Espèces réactives de l'oxygène
UVA/B	Ultraviolet radiation A/B	Rayon ultraviolet A/B
UVR	Ultraviolet radiation	Rayon ultraviolet
Zn	Zinc	Zinc

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RÉSUMÉ / ABSTRACT

RÉSUMÉ

Les coraux tropicaux se sont adaptés à vivre dans des eaux pauvres en nutriments en établissant une relation symbiotique avec des dinoflagellés photosynthétiques (Symbiodiniaceae). En effet, les dinoflagellés utilisent les macronutriments (azote et phosphore inorganiques dissous, DIN et DIP) et métaux-traces de l'eau pour former des molécules organiques essentielles à la croissance du corail hôte. Cependant, pour une croissance et un fonctionnement optimal, l'association corail-dinoflagellé nécessite une qualité et des concentrations optimales de ces nutriments. Ce n'est pas le cas dans les récifs exposés à des perturbations anthropiques ou à des conditions environnementales particulières (upwellings par exemple). Dans ces récifs, où les concentrations en nutriments peuvent être particulièrement faibles ou élevées ou avec des rapports DIN:DIP déséquilibrés, il peut y avoir une rupture de la symbiose.

L'objectif principal de cette thèse est d'étudier les effets de la disponibilité des nutriments et des changements dans le rapports DIN:DIP de l'eau de mer sur la physiologie des coraux. Cette thèse fournit de nouvelles informations sur la réponse de différentes espèces de coraux à des rapports DIN:DIP équilibrés et déséquilibrés. Globalement, les coraux restent en bonne santé tant qu'ils sont exposés à un rapport DIN:DIP équilibré dans l'eau de mer, même à des concentrations élevées de DIN et de DIP. Cependant, les résultats montrent une réponse complexe des coraux à des rapports DIN :DIP déséquilibrés, en fonction des facteurs environnementaux et des espèces étudiées. En effet, les octocoralliaires et les coraux scléactiniaires réagissent différemment à un rapport DIN:DIP déséquilibré. Les octocoralliaires ont des taux d'absorption de DIN et de DIP très faibles qui sont insuffisants pour modifier leur composition tissulaire en C:N:P et altérer la physiologie du corail. En revanche, chez les coraux scléactiniaires, les taux élevés d'absorption de DIN et de DIP induisent rapidement un apport important de nutriments dans le tissu corallien. Lorsque l'apport d'azote n'est pas équilibré par un apport proportionnel de phosphore, des modifications de la composition élémentaire et de la stœchiométrie des tissus sont observées, ce qui entraîne une dégradation de la physiologie du corail. De plus, les résultats montrent que l'azote a un impact différent sur les coraux selon sa forme, ammonium ou nitrate. L'ammonium favorise l'absorption du phosphore via l'hydrolyse du phosphore organique dissous (DOP) par les phosphatases, alors que le contraire est observé pour le nitrate. Enfin, les résultats indiquent également que les coraux peuvent être limités en métaux-traces, notamment en fer, ce qui peut réduire leur résilience au stress thermique.

Cette thèse fournit des informations essentielles pour une meilleure gestion des récifs à l'ère du changement climatique. Les résultats obtenus montrent qu'il est important de maintenir un rapport DIN:DIP équilibré dans l'eau de mer pour assurer la bonne santé des coraux et soulignent l'importance de la disponibilité de métaux notamment le fer pour la résistance et la résilience des coraux au stress thermique.

Keywords : nutriment, stœchiométrie, symbiose, azote, phosphore, métaux-traces

ABSTRACT

Tropical corals have adapted to life in nutrient-poor waters by establishing a symbiotic relationship with photosynthetic dinoflagellates (Symbiodiniaceae). Indeed, dinoflagellates use macronutrients (dissolved inorganic nitrogen and phosphorus, DIN and DIP) and trace metals from the seawater to form organic molecules essential for coral growth. However, for optimal growth and function, the coral-dinoflagellates association requires optimal quality and concentrations of these nutrients. This is not the case in reefs exposed to anthropogenic disturbances or specific environmental conditions (e.g. upwellings). In these reefs, particularly low or high nutrient concentrations or imbalanced DIN:DIP ratios may disrupt the symbiosis.

The main objective of this thesis is to investigate the effects of nutrient availability and changes in seawater DIN:DIP ratios on coral physiology. In particular, this thesis provides new insights into the response of different coral species to balanced and imbalanced DIN:DIP ratios. Overall, corals remain healthy as long as they are exposed to a balanced DIN:DIP ratio in seawater, even at high DIN and DIP concentrations. In contrast, the results show a complex response to imbalanced DIN:DIP ratios that depends on environmental factors and coral species. Octocorals have very low uptake rates of DIN and DIP, which are not sufficient to alter their tissue composition and stoichiometry (C:N:P) and impair coral physiology. In contrast, in scleractinian corals, a high uptake rates of DIN and DIP rapidly induce a large supply of nutrients into the coral tissue. If nitrogen input is not balanced by a proportional input of phosphorus, changes in cellular elemental composition and stoichiometry are observed, leading to an impairment of coral physiology. In addition, nitrogen identity (ammonium or nitrate) impacts corals differently. Ammonium promotes phosphorus uptake via hydrolysis of dissolved organic phosphorus (DOP) by phosphatases, while the opposite is observed with nitrate.

Finally, the results also suggest that corals may be limited in trace metals, particularly iron, which may reduce coral resilience to thermal stress.

This thesis provides important information for better management of reefs in the era of climate change. The results show that maintaining a balanced DIN:DIP ratio in seawater is essential for coral health and highlight the importance of metal availability, particularly iron, for coral resistance and resilience to thermal stress.

Keywords: nutrient, stoichiometry, symbiosis, nitrogen, phosphorus, metals

INTRODUCTION



INTRODUCTION

I Brief overview of coral reefs

1.1. Origin of the reefs

Coral reefs are one of the oldest ecosystems on Earth, dating back to the Palaeozoic era (Stanley, 2001). Although the most well-known coral reefs thrive in warm and shallow tropical waters, they also exist in many cold and deep waters of all oceans, including Arctic and sub-Antarctic waters. The focus of this thesis is on tropical coral reefs, which can form atolls, fringing reefs and barrier reefs, as first described by Charles Darwin (Darwin, 1837). **Fringing reefs** are nearshore formations found on the side of a volcanic island (Fig 1a). **Barrier reefs** are separated from the island by a lagoon. They are formed after geological (subduction/subsidence, e.g. French Polynesia, Mayotte) or hydrological (sea level rise; New Caledonia) events (Fig 1b). Over time, the island may disappear completely, leaving only the reef, which is now called an **atoll** (e.g. Maldives (Fig 1c)).

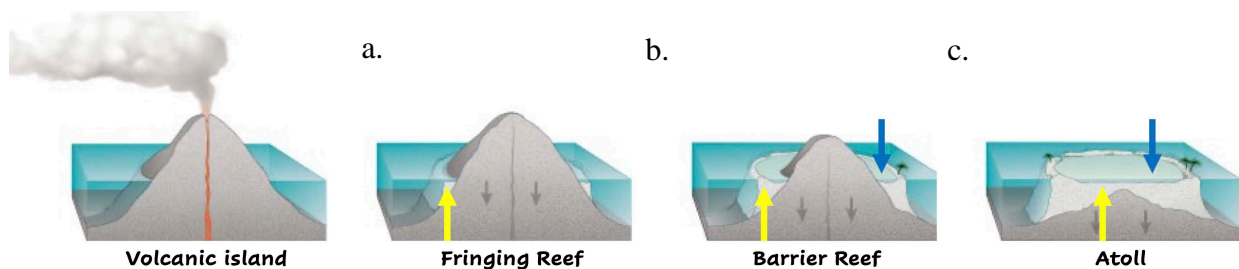


Figure 1. Illustration of the three main types of coral reefs, starting with the volcanic island stage
 (a) Fringing reefs develop on the side of a volcanic island. Geological and hydrological events lead to the formation of
 (b) a barrier reef and finally (c) an atoll.

Yellow arrows mark the coral reef, while blue arrows mark the lagoon.

Modified from USGS: <https://pubs.usgs.gov/fs/2002/fs025-02/>

Tropical coral reefs are mostly located in the equatorial belt, between 30 °N and 30 °S (Fig 2).

They are distributed in three main regions (Fig 2):

Middle East region: reefs are distributed all along the Red Sea.

Indo-Pacific region: the largest reef area, including Malaysia, Indonesia and Australia.

Atlantic region: reefs are mainly located around Bermuda and in the Caribbean.

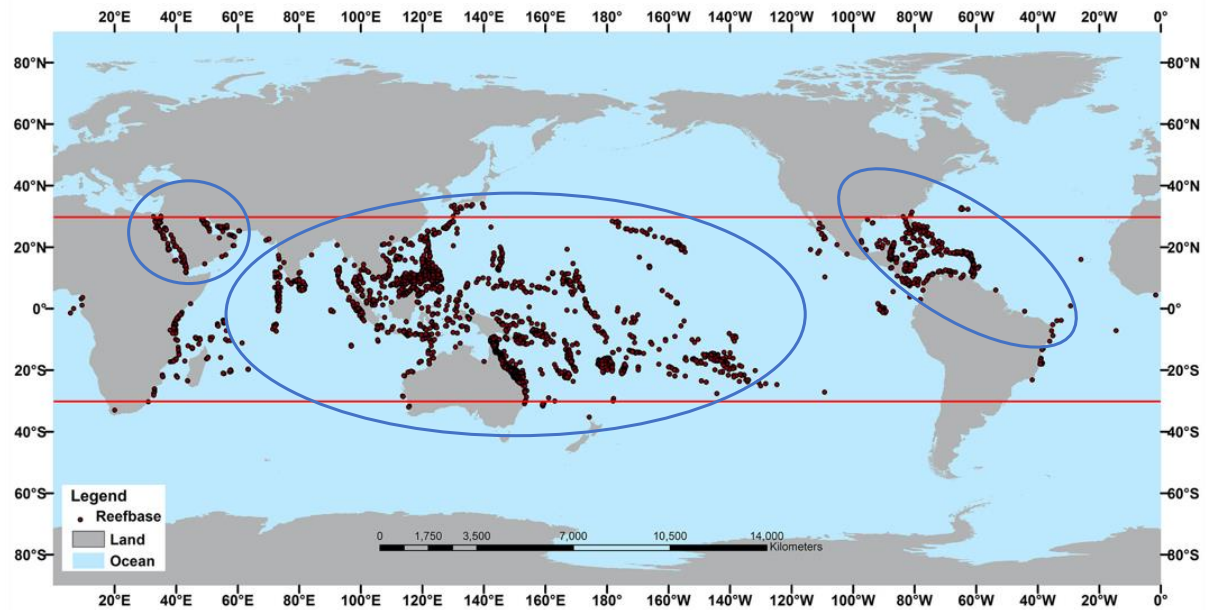


Figure 2. **Location of coral reefs worldwide**

Red lines represent latitudes 30° N and 30° S. Blue circles, from left to right, represent the Middle East, the Indo-Pacific, and the Atlantic region. Red dots represent coral reefs
Modified from NOAA

1.2. Coral reefs: their biological and economic importance

All together, coral reefs cover about 300 000 km² and account for only 0.1 % of the ocean surface (Spalding et al., 2001). However, they host more than 25 % of marine biodiversity (Knowlton *et al.*, 2010), and provide shelter and food for more than 93,000 recorded species, from a wide range of phyla (Annelida, Arthropoda, Chlorophyta, Echinochordata, Porifera...Reaka-Kudla, 1997). Coral reefs and their associated mangroves are therefore among the most productive ecosystems, and are referred to as the “rainforests of the ocean” (Connell, 1978). In addition to being biodiversity hotspots, they provide a variety of services, including nutrition through fisheries products and fish stocks, recreation through tourism and diving, and coastal protection through absorption of wave energy (Ferrario et al., 2014; Spalding et al., 2016). Reef organisms are also used in the pharmaceutical industry for their antimicrobial, anti-inflammatory or anticoagulant properties (Leal *et al.*, 2012). Overall, the economic value of reefs is estimated to be around 25 billion € per year (Spalding et al., 2017) with around 500 million people benefiting from reefs (Wilkinson & Souter, 2008). The complex 3D reef structure, which is essential for maintaining biodiversity and economic services, is built by calcifying organisms such as scleractinian corals.

II Description of the reef-building scleractinian corals

II.1. Taxonomy and anatomy

Corals are diploblastic organisms, with two embryonic tissue layers: an endoderm and an ectoderm. They have a simple plane of organisation with a six- or eightfold radial symmetry. They have no specific respiratory, excretory, or circulatory system, but only an ectodermal pharynx, the stomodeum, which connects the “mouth” and the gastric cavity of the organism (Fig 3). They are often surrounded by a layer of mucus, secreted by the coral itself. Corals also form a ‘holobiont’, i.e. a combination of a host and other species that live in or around it and together form a discrete ecological unit. The coral holobiont is formed by the animal polyp and all its partners, including dinoflagellate algae (Symbiodiniaceae), but also other microorganisms such as bacteria, viruses and fungi found in the mucus layer, coral tissue and skeleton (Lema *et al.*, 2012).

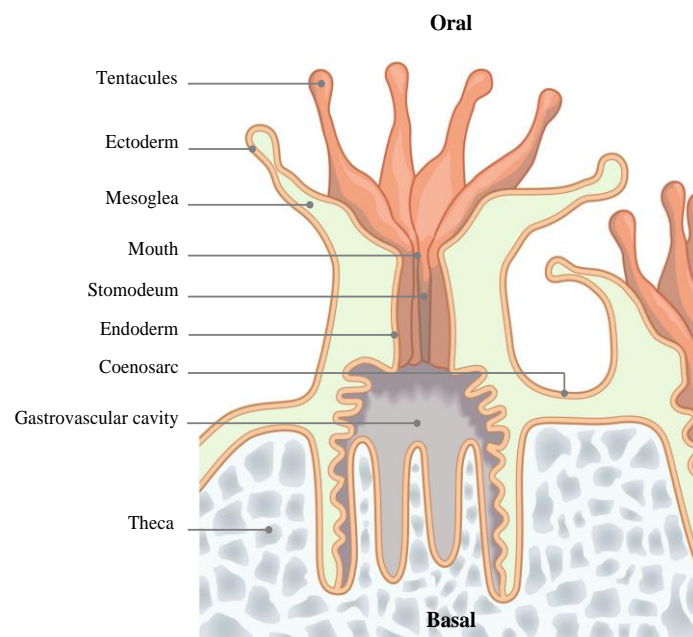


Figure 3. Cross section of a colonial scleractinian coral
Modified from Dorling Kindersley

Corals belong to the phylum Cnidaria and the subphylum Anthozoa (Fig 4). They date back to the Mesozoic, the middle of Triassic (Stanley, 1988). Corals are distributed between two classes of Anthozoa: Octocorallia and Hexacorallia (in the Scleractinia order). Octocorallia, also called soft corals, have an eightfold symmetry, and usually lack a calcium carbonate skeleton. Instead, they form spicules, small needle-like crystals, in their tissues. Scleractinian corals, on the other hand, also called “stony corals”, have a sixfold symmetry and

all secrete an aragonite (calcium carbonate) exoskeleton. They are the building blocks of coral reefs, with about 1,500 species recorded in this order (Appeltans *et al.*, 2012).

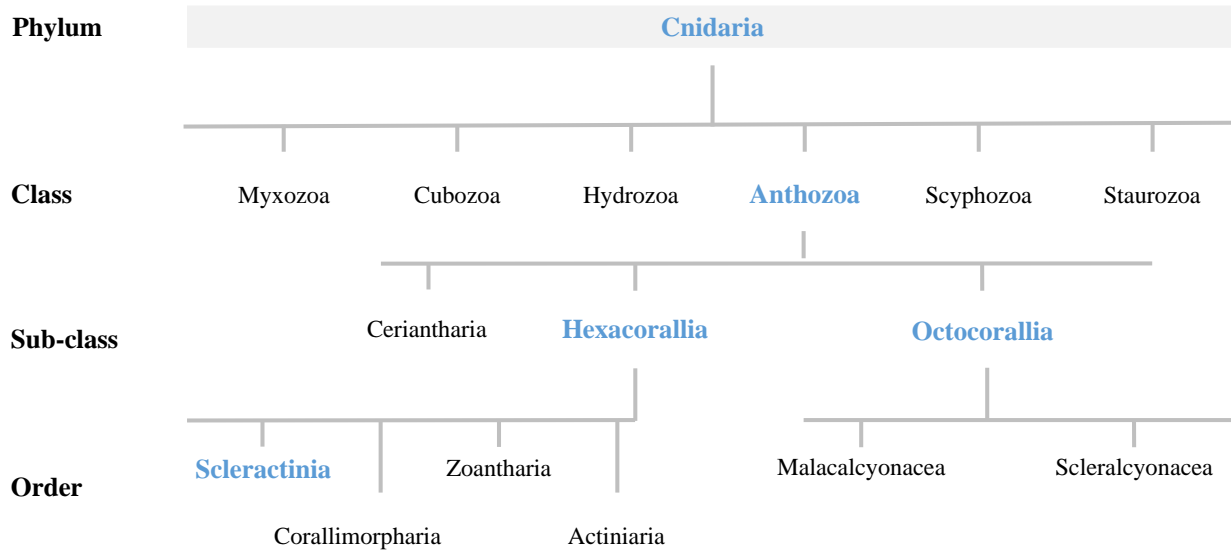


Figure 4. Simplified taxonomy of the Anthozoans (WoRMS)

Scleractinian corals can be distinguished according to their ability to form reefs: whether **hermatypic**, for reef-building corals, or **ahermatypic**, for single organisms and non reef-building corals (Schuhmacher & Zibrowius, 1985).

Another way to classify corals is by the presence of symbiotic Symbiodiniaceae dinoflagellates, formerly called ‘zooxanthellae’ (Schuhmacher & Zibrowius, 1985). Hence, the old classification defines:

- **Zooxanthellate** corals: Corals living in symbiosis with dinoflagellates
- **Azooxanthellate** corals: Corals without symbiotic dinoflagellates
- **Apozooxanthellate** corals: Corals temporally living without symbiotic dinoflagellates, mainly after a stress event.

Nowadays, the term ‘zooxanthellae’ is no longer used. We therefore refer to symbiotic versus asymbiotic and aposymbiotic corals, depending on whether they have symbiotic dinoflagellates or not.

II.2. Presentation of the corals used in this thesis

In this thesis we focused on three species of tropical hermatypic symbiotic scleractinian corals: *Stylophora pistillata*, *Pocillopora damicornis* and *Turbinaria reniformis* and one tropical symbiotic octocoral: *Sarcophyton glaucum* (Fig 5). All coral species maintained at the Centre Scientifique de Monaco originated from the Red Sea (Gulf of Aqaba, Israel).

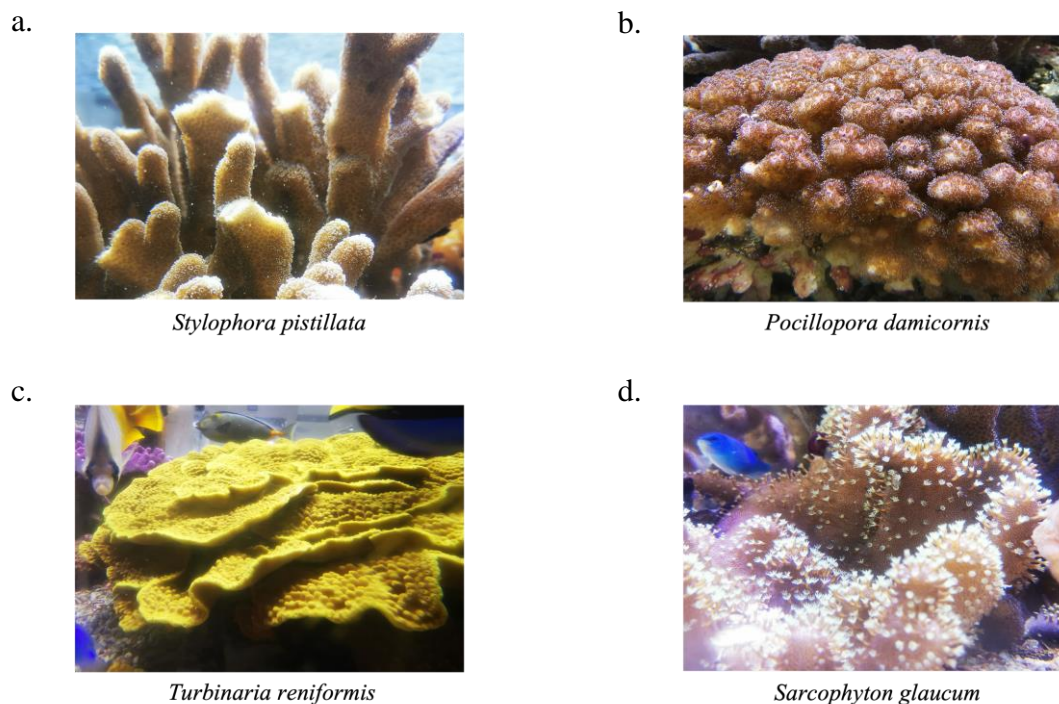


Figure 5. Scleractinian corals and octocorals used in the thesis

Photos of the three hermatypic symbiotic scleractinian corals: (a) *Stylophora pistillata*, (b) *Pocillopora damicornis* and (c) *Turbinaria reniformis*, the ahermatypic symbiotic octocoral: (d) *Sarcophyton glaucum*.

Credit: Alice Blanckaert CSM.

Stylophora pistillata (Esper 1792) is a branching coral belonging to the family Pocilloporidae. It has a wide distribution in the tropics. It is a brooding coral that reproduces by internal fertilisation and releases planula larvae into the water column. Colonies can be either hermaphroditic (with male and female polyps) or male-only and thus sterile (Rinkevich & Loya, 1987; Shefy *et al.*, 2018). They also have a mode of asexual reproduction by fragmentation. *S. pistillata* has been widely used as a scientific model in coral studies since the mid 20th century. As a result, numerous data are available on its morphology, physiology and genetics (Shefy & Rinkevich, 2021; Meziere *et al.*, 2022).

Pocillopora damicornis (Linnaeus 1758) is another branching coral species belonging to the family Pocilloporidae and is widely distributed in the tropical region. Like *S. pistillata*, *P. damicornis* can reproduce either sexually (brooding corals) or by asexual reproduction by

fragmentation (Richmond, 1987; Chou *et al.*, 2019). *P. damicornis* has also been used extensively as a model in coral research.

Turbinaria reniformis (Bernard 1896) is a laminar, plate-like coral belonging to the family Dendrophylliidae and is widely distributed. Unlike the previous two coral species, *T. reniformis* reproduces sexually through mass spawning events in which female and male colonies (gonochorous corals) release their gametes synchronously into the water column (Shlesinger & Loya, 2019). It can also reproduce asexually by fragmentation.

Sarcophyton glaucum (Quoy & Gaimard 1833) is the only tropical octocoral species used in this thesis. It is a soft coral, belonging to the family Alcyoniidae in the order Alcyonacea (Fig 3). It is a common species found in tropical and subtropical waters. Like *T. reniformis*, *S. glaucum* also reproduces sexually through mass spawning events in which female and male colonies (gonochorous corals) release their gametes synchronously into the water column (Benayahu & Loya, 1986). It also reproduces asexually by fragmentation.

III Coral symbiosis

III.1. Definition of symbiosis

Symbiosis comes from the Greek (*συν* “with”, *βίωσις* “living”) and literally means “living together”. It defines two or several different species that “live together” in a long-term relationship (Louie, 2010). Symbiosis is ubiquitous in marine and terrestrial environments. In most symbiotic relationships, the host is the largest partner (e.g. the coral), while the symbiont is the smallest (e.g. the dinoflagellates, and other microorganisms).

The nature of this relationship falls into one of the three categories described below, and can shift from one category to the other depending on environmental conditions:

- **Mutualism:** The relationship is beneficial for both partners, e.g. sea anemones and clownfish or bees and flowers.

- **Commensalism:** The relationship is beneficial for one of the partners, while it is neutral for the second partner, e.g. remora and sharks.

- **Parasitism:** The relationship is beneficial for one of the partners, while it is detrimental for the second partner, e.g. fish and parasitic isopods.

Symbiosis is facultative if the organisms can live independently or obligate if one cannot survive without the other. It can be considered either as endosymbiosis (the symbiont lives inside the host partner, in its cells - intracellular - or tissue - extracellular) or ectosymbiosis

(the symbiont lives on the surface of the host tissue). The coral-dinoflagellate association is defined as a mutualistic intracellular endosymbiosis, as both the host polyp and the endosymbiotic dinoflagellates benefit from this association (see following paragraphs). In addition, coral animal form close relationships with a variety of other microorganisms, but the nature of these association is not well defined and depends on the microorganism considered. For example, corals contain nitrogen-fixing bacteria, also called diazotrophs (Lema *et al.*, 2012; Ainsworth *et al.*, 2015). Diazotrophs can convert dinitrogen N_2 into another form of nitrogen available to the coral host (Bednarz *et al.*, 2021). In this thesis, the relation between corals and microorganisms is not explored and we will focus on the dinoflagellate symbiosis.

III.2. Taxonomy of symbiotic dinoflagellates

Dinoflagellates are unicellular algae that date back to the late Triassic, about 200 million years ago (Morden & Sherwood, 2002). They exhibit different trophic strategies, from obligate heterotrophs or autotrophs to mixotrophs (Barton *et al.*, 2013). They can be either free-living or symbiotic algae. The taxonomy of the dinoflagellate is complex. The dinoflagellates associated with corals belong to the family Symbiodiniaceae, within the kingdom Chromista (Fig 6).

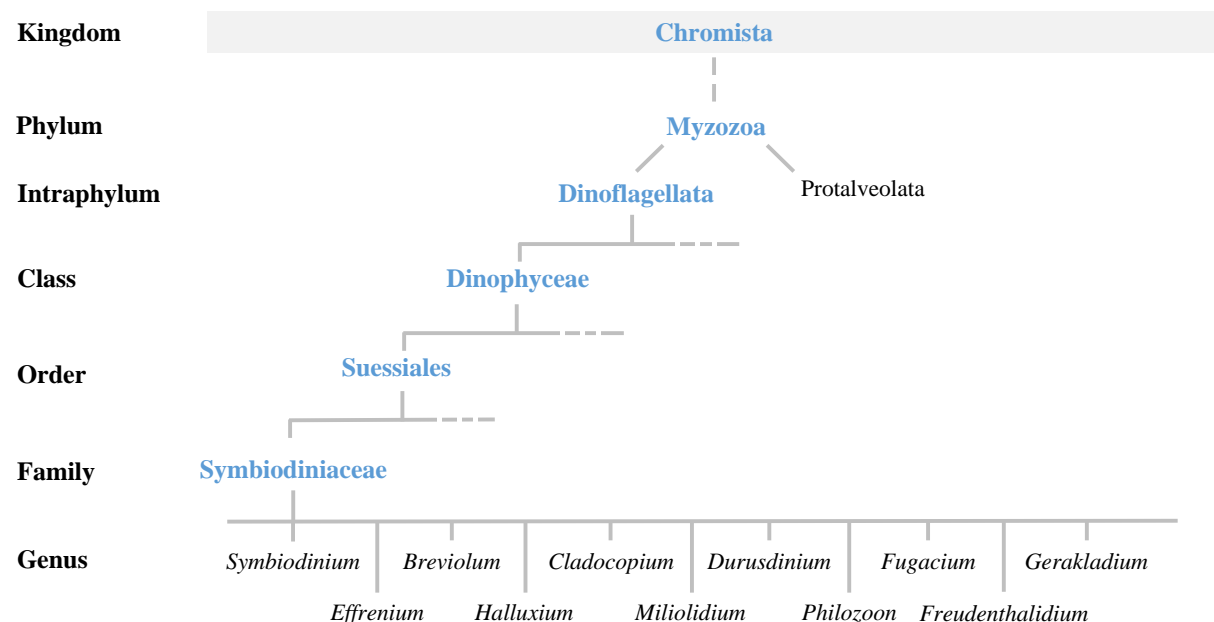


Figure 6. Simplified classification of the Symbiodiniaceae family (WoRMS)

Recently, the taxonomy of the Symbiodiniaceae family has been revised based on molecular analyses and classified into eleven taxonomically accepted genera, previously referred to as “clades” (LaJeunesse *et al.*, 2018; 2021; Nitschke *et al.*, 2020; Pochon & LaJeunesse, 2021) : *Symbiodinium*, *Brevolium*, *Cladocopium*, *Durusdinium*, *Fugacium*, *Gerakladium*, *Effrenium*,

Hallaxium, *Miliolidium*, *Philozoon* and *Freudenthalidium*. Of the eleven genera, *Effrenium*, *Mililolidium*, are not associated with cnidarians. These symbiotic dinoflagellates (Jørgensen, 2009) are acquired by the coral either by vertical transmission (parental transmission, maternally inherited symbiont) or horizontal transmission (from the environment) (Abrego *et al.*, 2009; Baird *et al.*, 2009).

The symbionts are located in the endoderm of the host, in a vacuole, called a “symbiosome” (Fig 7). In most species, the animal cell contains a single dinoflagellate, although there may be up to six symbionts per animal cell (Muscatine *et al.*, 1998). Symbiont density is usually expressed as dinoflagellate cells per surface area of the coral skeleton, in cm^2 . Under standard conditions, this symbiont density is about 1.5 million dinoflagellate cells per cm^2 (Muscatine *et al.*, 1998). Symbiont density is regulated by various factors such as light or nutrient availability (Jones & Yellowlees, 1997). Different dinoflagellate clades also respond differently to changes in light, temperature or nutrient availability (Baker, 2003; Gilbert *et al.*, 2010; Richier *et al.*, 2010). Therefore, within a coral species, the associated clade may vary according to geographical location and depth (Baker, 2003; LaJeunesse *et al.*, 2004). Corals can also host different clades simultaneously, although one clade is always dominant.

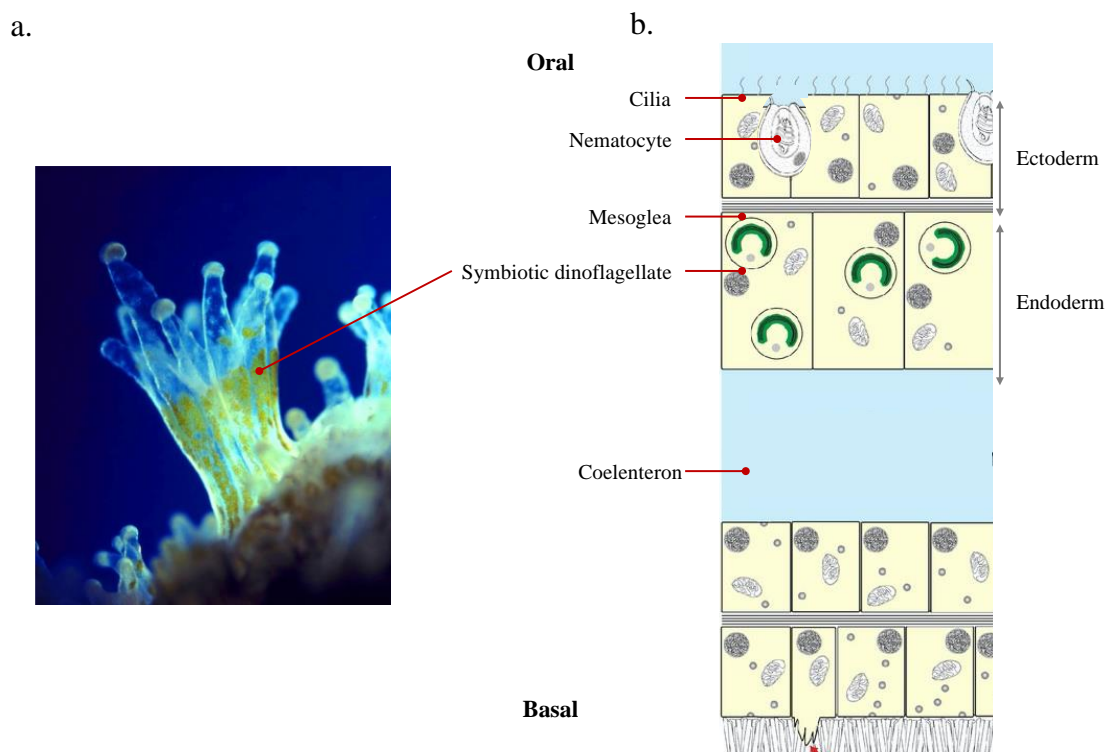


Figure 7. Location of the symbiotic dinoflagellates in a polyp
 (a.) Picture of a polyp with the symbiotic dinoflagellates visible in green and (b.) representation of a polyp tissue cross-section
 Modified from Drake *et al* (2020)

III.3. Coral-dinoflagellate symbiotic association

The symbiotic association between corals and Symbiodiniaceae is based on the exchange of nutrients between partners. Coral holobionts are thus mixotrophic organisms able to obtain nutrients from heterotrophic (organic nutrients, animal polyp) and autotrophic (inorganic nutrients, endosymbiotic dinoflagellates) strategies. By optimising the flow of nutrients between partners (Fig 8) the coral holobiont can thrive in oligotrophic, nutrient-poor waters (nitrogen <math>< 0.5 \mu\text{M}</math> – phosphorus <math>< 0.2 \mu\text{M}</math>) (Muscatine & Porter, 1977).

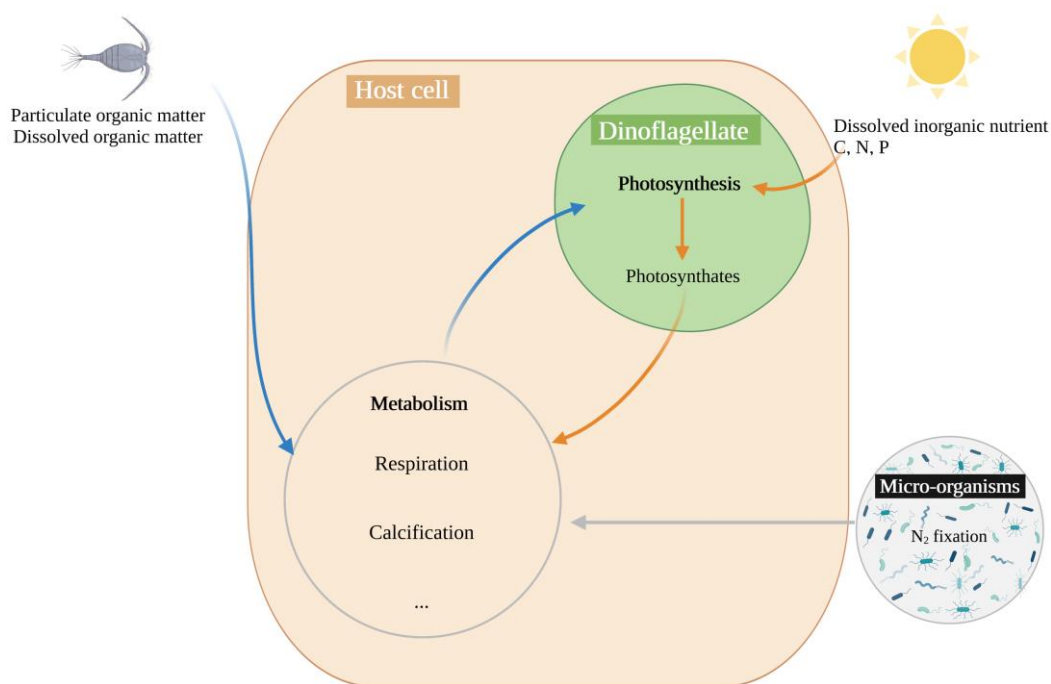


Figure 8. **Simplified overview of the coral-dinoflagellate-microorganisms symbiosis**

Orange arrows stand for autotrophic nutrition, **blue arrows** for heterotrophic nutrition and **gray arrows** for nutrition by microorganisms.

Created with BioRender

Corals polyp are able to feed on living or detrital particulate organic material (POM), from pico- (0.2 μm) to meso-plankton (1 mm) (Muscatine, 1973). The animal host may also consume dissolved organic material (DOM) such as carbohydrates and dissolved free amino acids. Corals share with their symbionts nutrients derived from their heterotrophic diet (Rahav *et al.*, 1989; Tremblay *et al.*, 2014), so that the essential elements are not lost by the symbiotic association.

The autotrophic dinoflagellates fix dissolved inorganic carbon (DIC) into organic molecules (photosynthates) during photosynthesis. These photosynthates are carbon-rich organic compounds, mainly sugars such as glucose (Burriesci *et al.*, 2012) and lipids (Treignier *et al.*, 2008). In shallow waters, 95 % of the photosynthates products are transferred to the coral host

to meet its respiratory and energy needs (Muscatine *et al.*, 1984; Rosset *et al.*, 2015). In addition, dinoflagellates are also able to assimilate dissolved inorganic and organic nitrogen (DIN/DON) and phosphorus (DIP/DOP) (Ferrier-Pagès *et al.*, 2016; Grover *et al.*, 2002, 2003, 2006, 2008) into organic compounds, as well as other nutrients such as metals (see further description below). N and P-enriched organic compounds are partially transferred to the host (Muscatine & Porter, 1977; Rahav *et al.*, 1989; Tremblay *et al.*, 2014; Wang & Douglas, 1999).

IV Nutrient acquisition in coral-dinoflagellates symbiosis

IV.1. *The concept of elementomics*

Elementomics is a relatively new term that appeared in a 2008 editorial of the *Journal of Analytical Atomic Spectrometry* and was defined as the study of “biological element speciation” (Jakubowski & Hieftje, 2008). Elements are the building blocks of living organisms. They are generally divided into two categories: **macro-** and **microelements**. Macroelements (carbon (C), nitrogen (N), phosphorus (P), oxygen (O), hydrogen (H) and sulphur (S)) are required by organisms in large quantities, with C, N and P making the majority of organism’s biomass (Da Silva & Williams, 2001). Among other things, nitrogen is a key element for the synthesis of proteins and amino acids, while phosphorus is needed for the synthesis of adenosine triphosphate (ATP), nucleic acid and phospholipids (Sterner & Elser, 2002). Nitrogen and phosphorus are both associated with organismal growth (Elser *et al.*, 2000) and primary production (Redfield, 1958; Ryther & Dunstan, 1971; Larned, 1998; Karl *et al.*, 2001; Mather *et al.*, 2008; Godinot *et al.*, 2011). Microelements such as metals (iron, zinc, nickel, cobalt, chromium ...) are needed in small quantities or in trace amounts. They play a role as enzymes cofactors in many catalytic or electron transfer reactions in cells.

Elements can be acquired by corals through the heterotrophic feeding or through the uptake of dissolved compounds.

The thesis focuses on the assimilation of macro (N and P) and microelements and their effects on coral physiology.

IV.2. *Nitrogen*

IV.2.a. *Nitrogen forms and cycling in reef waters*

In the reefs, nitrogen comes from a variety of sources: from the ocean by lateral advective flux, from the deep by vertical advective flux and upwelling events, from land runoff and wind erosion and atmospheric nitrogen deposition, from the migration of large organisms such as

fish schools and from bacterial regeneration of nitrogen particles (D'Elia, 1988). A study by Garrison et al (2003) highlighted the importance of atmospheric deposition (dust) as a major source of nitrogen. These various sources provide reef organisms with particulate organic nitrogen (PON), dissolved organic (DON) and inorganic nitrogen (DIN), and dinitrogen (N_2). **PON** consists of N-containing organic material retained on a 0.2 to 0.7 μm filter (Jørgensen, 2009; Wang et al., 2008), and thus includes both living organisms and dead organic materials (Jørgensen, 2009). It makes up a relatively small part of the total fixed nitrogen pool in the ocean, less than 0.3 % (Gruber, 2008). In reef waters, PON is found in concentrations of 0.4 to 1.6 $\mu\text{mol L}^{-1}$ (Wang et al., 2008). **DON** is usually defined as N-containing organic material that is not retained on a 0.2 μm filter. It includes a variety of nitrogen-containing compounds such as urea, nucleotides, free amino acids, proteins, peptides and small viruses and bacteria (Aluwihare & Meador, 2008; Jørgensen, 2009). With concentrations ranging from 3.8 to 5.6 $\mu\text{mol L}^{-1}$, it is a major source of N for many organisms, including corals (Tanaka *et al.*, 2011; Sipler & Bronk, 2015). **DIN** is the other pool of dissolved nitrogen, composed of ammonium, nitrite and nitrate. Ammonium, NH_4^+ , is a natural element derived from animal waste or N_2 fixation. In reef waters, ammonium is found in concentrations of 0.2 to 0.5 μM (Bythell, 1990). In seawater, ammonium is the most reduced form of DIN, and is therefore highly susceptible to oxidation. Nitrite, NO_2^- , is usually a transitional form of nitrogen. It is in turn rapidly oxidised to nitrate by microorganisms. In oligotrophic reef waters, it is present in trace amounts, about 0.05 μM (Bythell, 1990). Nitrate is the form of nitrogen with the highest degree of oxidation and the most stable in an aqueous medium. In reef waters, the concentration of nitrate ranges from 0.2 to 1.8 μM (Bythell, 1990). However, in some eutrophic areas, nitrate concentrations can reach 31 μM (Vaiare, French Polynesia; Rouzé *et al.*, 2015). Finally, although N_2 is the prevalent N source (90 %, Gruber, 2008)), it is only accessible to diazotrophic organisms capable of transforming N_2 into available dissolved nitrogen, such as ammonium.

The nitrogen cycle in the ocean is quite complex and involves different organisms and processes (Fig 9). Dinitrogen (N_2) is reduced to bioavailable ammonium by diazotrophic organisms through **N_2 fixation** (Larkum *et al.*, 1988). Ammonium, in turn, is taken up by autotrophic organisms, while it is excreted by heterotrophic organisms. Ammonium can also be oxidised by chemoautotrophic oxic bacteria to nitrite and then to nitrate (nitrification process, Wiebe *et al.*, 1975). Nitrate is also a major source of nitrogen for autotrophic organisms. PON can sink into the anoxic layer, be remineralised to ammonium and nitrate (remineralisation process, D'Elia, 1988; D'Elia & Wiebe, 1990) and re-enter the oxic layer

through vertical mixing and upwelling (Howard *et al.*, 2014). In the anoxic layer, ammonium can be converted to N_2 by planktomycetes through **anaerobic ammonium oxidation**. N_2 can also be produced by **nitrate reduction** and **denitrification** by anaerobic bacteria that use nitrate for their respiration. These bacteria can also use nitrate to produce ammonium via the **dissimilatory nitrate reduction to ammonium (DNRA)** pathway via two enzymes: nitrate reductase and nitrite reductase (D'Elia & Wiebe, 1990).

IV.2.b. Assimilation of nitrogen by the coral holobiont

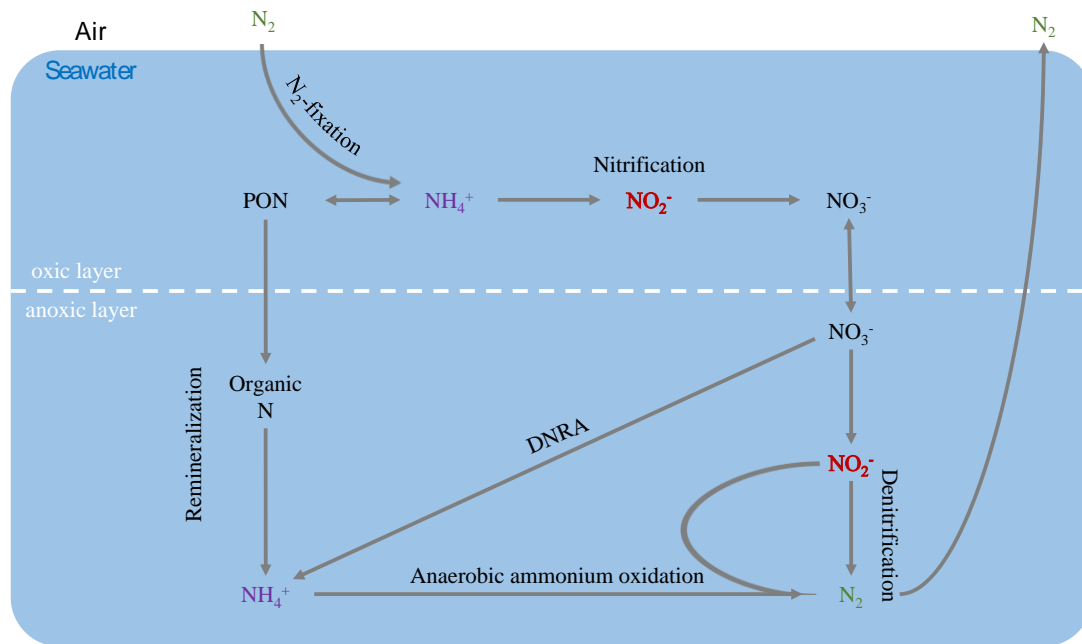


Figure 9. **Simplified overview of the nitrogen cycle at the oxic/anoxic interface in the water column**
 N_2 : dinitrogen, NH_4^+ : ammonium, NO_2^- : nitrite, NO_3^- : nitrate, PON: particulate organic nitrogen, Organic N: organic nitrogen, DNRA: dissimilatory nitrate reduction to ammonium.
 Modified from Francis *et al.* (2007)

Coral polyp can assimilate PON by grazing on planktonic cells ranging from bacteria to microzooplankton depending on the polyp size (Houlbreque & Ferrier-Pagès, 2009). Both the coral host and symbionts can take up DON by direct transport and diffusion (Grover *et al.*, 2006, 2008), while DIN is mainly assimilated by the symbiotic dinoflagellates (Grover *et al.*, 2002; Pernice *et al.*, 2012; Kopp *et al.*, 2013). The assimilation of ammonium requires the glutamine synthetase and glutamate dehydrogenase enzymes (Rahav *et al.*, 1989), then ammonium is incorporated into amino acids mainly by transamination processes (Roberts *et al.*, 1999). Although ammonium is present at lower concentrations compared to nitrate, its assimilation rate is higher, ranging from 2 to 30 $ng\ N\ h^{-1}\ cm^{-2}$ at a natural seawater concentration below 0.5 μM and up to 519 $ng\ N\ h^{-1}$ at a concentration of 5 μM (Grover *et al.*, 2002). The assimilation of ammonium is thus positively correlated with the ammonium

concentration in seawater, but also depend on the nutritional status of the holobiont or the photosynthetic activity (Grover *et al.*, 2002).

Unlike ammonium, the assimilation of nitrate requires both nitrate reductase and nitrite reductase, which have so far only been found in plants and algae (Crossland & Barnes, 1977). Therefore, only the symbiotic dinoflagellates and few other bacteria can assimilate nitrate. The assimilation rate of nitrate by corals is lower than that of ammonium, probably due to the high energy required for its assimilation (Dagenais-Bellefeuille & Morse, 2013) in addition to its assimilation being limited to only one partner of the coral holobiont. Uptake rates vary between 1.2 and 6.1 ng N h⁻¹ cm⁻² for 0.3 and 3 µM nitrate concentrations (Grover *et al.*, 2003). Thus, again, the assimilation rate is positively correlated with nitrate concentration in seawater, but also with the photosynthetic activity of the symbionts (Grover *et al.*, 2003). Finally, corals have access to N₂ through the presence of diazotrophs in their tissue, mucus layer and skeleton (Bednarz *et al.*, 2021). However, this is not the most important form of N used by the coral holobiont, accounting for less than 10 % of the N requirements of many corals (Bednarz *et al.*, 2021).

IV.3. Phosphorus

IV.3.a. Phosphorus forms and cycling in reef waters

The supply of phosphorus to the reef can be from a variety of sources: atmospheric (dust deposition), terrestrial (wastewater discharge, land runoff and erosion of igneous rocks), oceanic (vertical and lateral advective flux), or from the reef itself (sediment resuspension). These sources supply the reef with particulate inorganic P (**PIP**), which makes up between 20 and 70 % of the phosphorus pool (Yoshimura *et al.*, 2007; Lomnitz *et al.*, 2016), but can only be used by corals if it dissolves first into inorganic phosphorus in seawater (DIP, Van Cappellen *et al.*, 2014). Corals can instead use particulate organic P (POP), dissolved organic (DOP) and inorganic phosphorus (DIP). **POP** consists of living organisms, such as plankton and bacteria, retained on a 0.2 to 0.45 µm filter. In the water column, it is found in relatively low concentrations around 0.10 µmol L⁻¹ (Furnas *et al.*, 1992; Charpy, 2001; Lomnitz *et al.*, 2016). However, in the sediment and benthic community, concentrations can reach 100 mmol m⁻² (Atkinson, 2011). POP can be transformed into **DOP** (Van Cappellen *et al.*, 2014), which consists of phosphoesters, phosphonates, phospholipids, nucleic acids, nucleotides and vitamins (B₁ and B₁₂) (Karl & Björkman, 2015). This phosphorus pool is found in concentrations of 0.12 to 0.5 µmol L⁻¹ in the euphotic layer (Furnas *et al.*, 1992; Charpy, 2001)

and decreases with depth to below $0.05 \mu\text{mol L}^{-1}$ (Karl & Björkman, 2015). DOP is mainly used when DIP is scarce. **DIP** consists mainly in orthophosphate ions (HPO_4^{2-} or H_2PO_4^- , depending on the seawater pH). In seawater, at a pH of 8, HPO_4^{2-} accounts for 87 % of orthophosphate ions, followed by $\text{H}_2\text{PO}_4^{3-}$ at 12 % (Kester & Pytkowicz, 1967; Ruttenberg, 2003). Another type of DIP is condensed phosphate polymers, which are called meta-, pyro- or polyphosphate depending on their structure. Overall, DIP is found in reef waters in concentrations of 0.05 to $0.5 \mu\text{mol L}^{-1}$ (Kinsey & Davies, 1979).

IV.3.b. Phosphorus assimilation by the coral holobiont

In the ocean, DIP is the most bioavailable form of phosphorus for marine organisms, as its assimilation has a low metabolic cost (Björkman & Karl, 1994). DIP assimilation in the coral holobiont occurs both in the symbiont by active transport (Jackson & Yellowlees, 1990; Muller-Parker *et al.*, 1990) and in the coral host by phosphorus/sodium cotransporters (Godinot, 2012). Some small DOP molecules, such as adenosine monophosphate (AMP) can also be taken up directly by microorganisms (Ruby *et al.*, 1985; Weiss *et al.*, 1991; Karl, 2014; Karl & Björkman, 2015). Larger DOP compounds must first be hydrolysed into inorganic phosphorus by specific enzymes, such as phosphatase, phytase, phosphodiesterase and nuclease (Karl & Björkman, 2015). Phosphatases are considered the most important class of enzymes for DOP hydrolysis in oligotrophic environments (Taga & Kobori, 1978; Karl & Craven, 1980). Corals possess extracellular and intracellular phosphatases capable of hydrolysing monophosphoester (DOP) and internal DOP into orthophosphate ions (DIP) (Godinot *et al.*, 2013; Wiedenmann *et al.*, 2013).

In this thesis, one chapter will focus on the capacity of scleractinian corals and octocorals to produce phosphatases under different environmental conditions and use DOP as a source of phosphorus. Such P source has indeed been overlooked until now but is important to explain and understand coral's health.

IV.4. Importance of the nitrogen and phosphorus stoichiometry for the stability of the symbiosis

Meunier *et al.* (2017) defined ecological stoichiometry as 'a framework linking the metabolic requirements of an organism to the relative supply of elements in the environment' (Sterner & Elser, 2002; Hessen *et al.*, 2013). It postulates a crucial relationship between the balance of elements, typically, but not limited to, carbon (C), nitrogen (N) and phosphorus (P), and their role in determining growth and reproduction of organisms as well as in ecological interactions. Recognition of the importance of stoichiometric constraints between consumer needs and prey

nutrient content has greatly improved our understanding of trophic interactions. For example high C:P diet are often of low quality for a variety of organisms such as molluscs (Stelzer & Lamberti, 2002; Fink & Von Elert, 2006), crustaceans (Boersma & Kreutzer, 2002; Meunier *et al.*, 2016), insects (Perkins *et al.*, 2004) and fish (Borlongan & Satoh, 2001; Vrede *et al.*, 2011). Ecological stoichiometry has been proven to be a highly suitable framework in community ecology to explain consumer responses to prey quality - food intake, growth, as well as competition between species and consumer effects on prey nutrient composition (Sturner, 1990; Sturner *et al.*, 1992; Sturner & Hessen, 1994). When nutrients are not limiting, the molar element ratio C:N:P ratio of particulate matter is 106:16:1, the Redfield ratio (Redfield, 1958). This is the balanced reference ratio, that meets the requirements of living organisms. Also, in pristine oligotrophic seawater, the natural N:P ratio of dissolved inorganic nutrients (DIN:DIP ratio) ranges approximately from 4:1 to 7:1, which is lower than the canonical Redfield ratio of particulate matter (Crossland *et al.*, 1984; Furnas *et al.*, 1995; Rosset *et al.*, 2017; Smith *et al.*, 1981). We will consider this natural DIN:DIP ratio of dissolved elements as a balanced DIN:DIP ratio in this thesis.

Corals can however, be exposed to changes in seawater DIN:DIP ratios, either naturally (e.g. upwelling, etc...) or human activity (Bell *et al.*, 1999; D'Angelo & Wiedenmann, 2014; Houk *et al.*, 2020; Lourey *et al.*, 2001; Radenac *et al.*, 2001). Indeed, nowadays, however, more and more reefs are affected by eutrophication, i.e. over-enrichment of seawater with one or more nutrients, due to anthropogenic activities, such as land runoff, agriculture and municipal waste (Fabricius, 2011). Eutrophication can lead to an imbalanced DIN:DIP ratio, which affects the health and physiology of reef organisms. Higher nutrient concentrations and availability can, for example, lead to the reef ecosystem shifting from a coral-dominated to an algae-dominated reef (Littler *et al.*, 2006; Vermeij *et al.*, 2010), as macroalgae require a large supply of nutrients to grow. In the absence of herbivores, macroalgal proliferation can lead to competition for nutrients, space and light with corals (Littler *et al.*, 2006; Garren, 2016) (Fig 10). Such shifts have occurred all over the world, from the Gulf of Eilat in the Red Sea (Genin *et al.*, 1995) and the Great Barrier Reef in Australia (McCook, 1999; Cheal *et al.*, 2010) to the Caribbean (Mumby, 2009) and New Caledonia (Vieira *et al.*, 2015). Therefore, investigating the effects of eutrophication, particularly through nitrogen and phosphorus enrichment, on the physiology of coral holobionts is a priority.

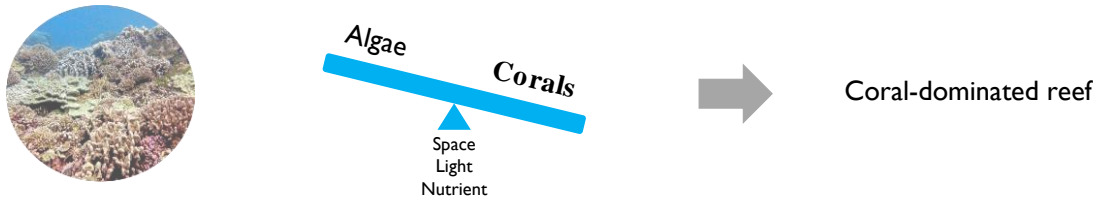
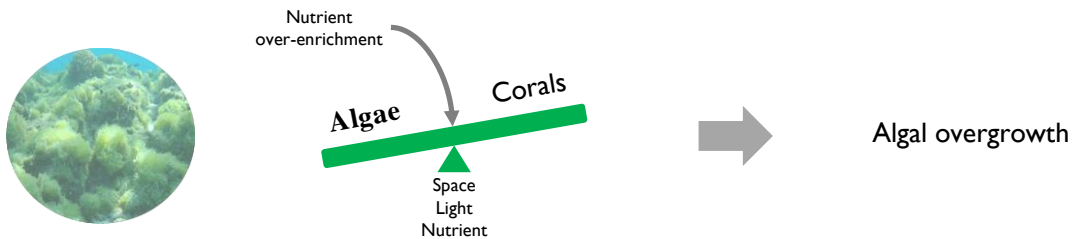
Balance DIN:DIP ratio**Imbalance DIN:DIP ratio**

Figure 10. Simplified representation of phase shifts in a reef at different DIN:DIP ratios of dissolved nutrients

At a balanced DIN:DIP ratio reefs are coral-dominated ecosystems. However, over-enrichment with nutrients leads to an imbalanced DIN:DIP ratio and the proliferation of macroalgae, which compete with corals for light and space. Eventually, the macroalgae take over the reef, which is then an algae-dominated ecosystem

IV.5. Effect of an imbalanced DIN:DIP ratio on coral physiology

The effects of nutrient enrichment have been investigated in numerous studies. However, the results are often contradictory, with studies showing a positive, neutral, or detrimental effect of nutrient enrichment on coral physiology (Table 1). For example, studies have found a decrease in coral growth under DIP or DIN enrichment alone (Kinsey & Davies, 1979; Tomascik & Sander, 1985; Stambler *et al.*, 1991; Ferrier-Pagès *et al.*, 2000, 2001; Koop *et al.*, 2001; Renegar & Riegl, 2005; Marangoni *et al.*, 2020). Other studies found the opposite under similar enrichment conditions: an increase in coral calcification and growth rate under phosphate enrichment (Atkinson *et al.*, 1995; Koop *et al.*, 2001; Dunn *et al.*, 2012) or nitrogen enrichment (Atkinson *et al.*, 1995; Chauvin *et al.*, 2011). However, such increase in coral growth may also be accompanied by a decrease in skeletal density making the coral more porous (Koop *et al.*, 2001; Dunn *et al.*, 2012) and thus more susceptible to physical injury. Nutrient enrichment, especially nitrate, also has controversial effects on coral physiology. Indeed, some studies showed either an increase (Marubini & Davies, 1996; Ezzat *et al.*, 2015) or a decrease (Schlöder & D’Croz, 2004) in symbiont density. Similarly, contradictory effects on photosynthetic rates have been observed (Ezzat *et al.*, 2015, 2016b; Liu *et al.*, 2020). In

addition, studies have shown that ammonium and nitrate may have different impacts on coral physiology, with a beneficial effect of ammonium and a detrimental effect of nitrate (Ezzat *et al.*, 2015; Burkepile *et al.*, 2020; Marangoni *et al.*, 2020).

Recently, Wiedenmann *et al.* (2013) tried to reconcile these opposite results by hypothesizing that it is not the nutrient per se or its concentration that is detrimental to corals, but more the DIN:DIP ratio.

Indeed, they showed that a balanced DIN:DIP ratio was not detrimental to corals, while an imbalanced DIN:DIP ratio, with a lack of phosphorus compared to nitrogen reduced the photosynthetic efficiency of the symbionts and led to bleaching during thermal stress (Wiedenmann *et al.*, 2013). Under phosphorus limitation, membrane phospholipids were replaced with sulfolipids that are less resistant to thermal stress (Rosset *et al.*, 2017). These results suggest that simultaneous enrichment with nitrogen and phosphorus in a balanced ratio could mitigate the deleterious effects of each nutrient. This hypothesis is supported by the observations that:

- 1) phosphorus enrichment mitigated the negative effects of nitrogen enrichment on coral growth and photosynthesis (Koop *et al.*, 2001; Ezzat *et al.*, 2016b)

- 2) nitrogen enrichment mitigated the negative effects of phosphorus enrichment on coral recovery after stress (Ferrier-Pagès *et al.*, 2000).

Overall, further studies are required to fully assess coral response to nutrient enrichment via balanced or imbalanced DIN:DIP ratios.

In this thesis we investigated the effects of balanced and imbalanced DIN:DIP ratios, following nitrate enrichment, on the coral holobiont physiology and phosphorus requirements.

Table 1. **Non-exhaustive list of studies on nutrients enrichment and their impact on coral physiology**

Orange results indicate a negative effect of nutrient enrichment, while **green** results indicate a positive effect of nutrient enrichment on coral physiology. **Purple** results indicate that no effect of nutrient enrichment was observed on the parameters studied.

Modified and updated from Ezzat (2016)

Nutrient	Study	Concentration	N:P ratio	Context	Coral species	Results											
						Coral Growth	Dinoflagellate density	Chlorophyll content	Photosynthesis rates	Tissue composition	Carbon	Nitrogen	Phosphorus	Recovery rates	Reproduction rates	Survival	Coral health
Nitrate	(Marubini & Davies, 1996)	1.2 μ M	24	Exp	<i>Porites porites</i>	- skeleton density	+ symbiont density	/	/	/							
	<i>Montastrea annularis</i>				- skeleton density	+ symbiont density	/	/	/								
	(Ferrier-Pagès <i>et al.</i> , 2001)	2 μ M	10	Exp	<i>Stylophora pistillata</i>	/	/		/								
	(Voss & Richardson, 2006)				<i>Siderastrea siderea</i>												
	(Tanaka <i>et al.</i> , 2017)	2 μ M	90	Exp	<i>Montipora digitata</i>	/		+ chlorophyll content			/	+ N in symbiont					
					<i>Porites cylindrica</i>	/		+ chlorophyll content			/	- CN in symbiont					
	(Ezzat <i>et al.</i> , 2016b)	2.5 μ M	25	Exp	<i>Turbinaria reniformis</i>	/		/	/								
	(Ezzat <i>et al.</i> , 2015)				<i>Stylophora pistillata</i>		+ symbiont density	+ chlorophyll content	- photosynthesis rates	/							
	(Marangoni <i>et al.</i> , 2020)	3 μ M	15	Exp	<i>Stylophora pistillata</i>	- calcification	/	/	- F_v / F_m	/ (protein content)	+ ROS : Nitric oxide, lipid peroxidation damage, protein tyrosine nitration						- bleaching resistance during thermal stress
(Dobson <i>et al.</i> , 2021b)	<i>Turbinaria reniformis</i>				/		/	/	- protein content	/ (lipid content)							

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Nutrient	Study	Concentration	N:P ratio	Context	Coral species	Results														
						Coral Growth	Dinoflagellate density	Chlorophyll content	Photosynthesis rates	Tissue composition	Carbon	Nitrogen	Phosphorus	Recovery rates	Reproduction rates	Survival	Coral health			
Nitrate	(Wiedenmann <i>et al.</i> , 2013)	3 µM	42	Exp	<i>Montipora foliosa</i>		- symbiont density (compare to High N/High P – HN/HP)		F _v /F _m < 0.5 - F _v /F _m (compared to HN/HP)	+ SQGD + phosphatase activity (compare to High N/Ambiant P)								Bleaching		
					<i>Euphyllia paradivisia</i>		- symbiont density (compared to HN/HP)	- chlorophyll a per symbiont (compared to HN/HP)	F _v /F _m > 0.5											Bleaching
					<i>Acropora polystoma</i>				- F _v /F _m (compared to HN/HP)											
	(Fabricius <i>et al.</i> , 2013)	4 µM	80	Exp	<i>Acropora millepora</i>	/		/						/			/	No bleaching observed		
					<i>Montipora tuberculosa</i>	/		/						/		/	No bleaching observed			
	(Marubini & Davies, 1996)	5 µM	104	Exp	<i>Porites porites</i>	- skeleton density	+ symbiont density and cell volume	+ chlorophyll content	/	/										
					<i>Montastrea annularis</i>	- skeleton density	+ symbiont density and cell volume	+ chlorophyll content	+ gross photosynthetic rate	/										
	(Renegar & Riegl, 2005)	5 µM	71	Exp	<i>Acropora cervicornis</i>	- coral growth														
	(Burkepile <i>et al.</i> , 2020)				<i>Pocillopora</i>												- colony survival	- bleaching resistance		
					<i>Acropora</i>												/	- bleaching resistance		
	(Liu <i>et al.</i> , 2020)	6 µM	-	Exp	<i>Porites</i>													/	/	
	<i>Pocillopora damicornis</i> (larvae)							+ net photosynthetic rate + P _{net} /R	/	but changes in fatty acid composition										
	(Renegar & Riegl, 2005)	10µM	142	Exp	<i>Acropora cervicornis</i>	- coral growth														
	(Higuchi <i>et al.</i> , 2015)				<i>Montipora digitata</i>		/			(F _v /F _m)	/	(Super oxide dismutase and catalase activity)								- resistance to bleaching under high light or thermal stress
	(Liu <i>et al.</i> , 2020)				<i>Pocillopora damicornis</i> (larvae)						/	/	but changes in fatty acid composition							
(Nordemar <i>et al.</i> , 2003)	15 µM	-	Exp	<i>Porites cylindrica</i>		/	/	- primary production rates												
(Marubini & Davies, 1996)	20 µM	404	Exp	<i>Porites porites</i>	- skeleton density	+ symbiont density and cell volume	+ chlorophyll content	/	+ protein content in symbiont											
				<i>Montastrea annularis</i>	- skeleton density	+ symbiont density and cell volume	+ chlorophyll content	+ gross photosynthetic rate	+ protein content in symbiont											

Nutrient	Study	Concentration	N:P ratio	Context	Coral species	Results												
						Coral Growth	Dinoflagellate density	Chlorophyll content	Photosynthesis rates	Tissue composition	Carbon	Nitrogen	Phosphorus	Recovery rates	Reproduction rates	Survival	Coral health	
Nitrate	(Schlöder & D'Croz, 2004)	20 µM	100	Exp	<i>Porites lobata</i>		- symbiont density										/	
	<i>Pocillopora damicornis</i>					- symbiont density												- survival rate
	(Liu <i>et al.</i> , 2020)		-	Exp	<i>Pocillopora damicornis</i> (larvae)				+ respiration rate	- fatty acids Changes in fatty acid composition								
	(Liu <i>et al.</i> , 2020)	40 µM	-	Exp	<i>Pocillopora damicornis</i> (larvae)				- net photosynthetic rate - P _{net} /R + respiration rate	- fatty acids Changes in fatty acid composition								
Ammonium	(Harrison & Ward, 2001)	1 µM	8	Exp	<i>Goniastrea aspera</i> <i>Acropora longicyathus</i>											/	- fertilization rates - embryo development	
	(Béraud <i>et al.</i> , 2013)	3 µM	6	Exp	<i>Turbinaria reniformis</i>	/	- symbiont density	+ chlorophyll content per symbiont - other pigment	/	/	(protein and lipid) - δ ¹⁵ N in symbionts							
	(Ezzat <i>et al.</i> , 2015)		60	Exp	<i>Stylophora pistillata</i>		+ symbiont density	+ chlorophyll content	+ carbon fixation	+ protein biomass								
	(Marangoni <i>et al.</i> , 2020)		15	Exp	<i>Stylophora pistillata</i>	+ calcification	+ symbiont density	+ chlorophyll content	/	/ (protein content)	- ROS - lactate content							Maintain coral metabolism during thermal stress
	(Harrison & Ward, 2001)	5 µM	28	Exp	<i>Goniastrea aspera</i>												- embryo development	
	(Stambler <i>et al.</i> , 1991)	7 µM	70	Exp	<i>Pocillopora damicornis</i>	- growth rate	+ symbiont density	+ chlorophyll content										
	(Ferrier-Pagès <i>et al.</i> , 2000)	10 µM	25	Exp	<i>Stylophora pistillata</i>	/												
	(Harrison & Ward, 2001)		53	Exp	<i>Acropora longicyathus</i>													- fertilization rates - embryo development
	(McCauley & Goulet, 2019)		12.2	Exp	<i>Pseudoplexaura porosa</i> <i>Eunicea tourneforti</i>		+ symbiont density + mitotic index	+ chlorophyll content	/	/	(Dry tissue %, carbohydrate, lipid, protein and refractory content in both the organic matter and sclerite fraction)	/	/					
							+ symbiont density + mitotic index	+ chlorophyll a content	+ F _v /F _m (at noon only)	/	(Dry tissue %, carbohydrate, lipid, protein and refractory content in both the organic matter and sclerite fraction)	/	/					

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Nutrient	Study	Concentration	N:P ratio	Context	Coral species	Results													
						Coral Growth	Dinoflagellate density	Chlorophyll content	Photosynthesis rates	Tissue composition	Carbon	Nitrogen	Phosphorus	Recovery rates	Reproduction rates	Survival	Coral health		
Nitrate	(Schlüder & D'Croz, 2004)	20 μ M	100	Exp	<i>Porites lobata</i>		- symbiont density										/		
	<i>Pocillopora damicornis</i>					- symbiont density												- survival rate	
	(Liu <i>et al.</i> , 2020)		-	Exp	<i>Pocillopora damicornis</i> (larvae)				+ respiration rate		- fatty acids Changes in fatty acid composition								
	(Liu <i>et al.</i> , 2020)	40 μ M	-	Exp	<i>Pocillopora damicornis</i> (larvae)				- net photosynthetic rate - P_{net}/R + respiration rate		- fatty acids Changes in fatty acid composition								
Ammonium	(Harrison & Ward, 2001)	1 μ M	8	Exp	<i>Goniastrea aspera</i>												/		
	<i>Acropora longicyathus</i>																	- fertilization rates - embryo development	
	(Béraud <i>et al.</i> , 2013)	3 μ M	60	Exp	<i>Turbinaria reniformis</i>	/	- symbiont density	+ chlorophyll content per symbiont - other pigment	/		/ (protein and lipid) - $\delta^{15}N$ in symbionts								
	(Ezzat <i>et al.</i> , 2015)				<i>Stylophora pistillata</i>	+ symbiont density	+ chlorophyll content	+ carbon fixation	+ protein biomass										
	(Marangoni <i>et al.</i> , 2020)	15	Exp	<i>Stylophora pistillata</i>	+ calcification	+ symbiont density	+ chlorophyll content	/		/ (protein content) - ROS - lactate content								Maintain coral metabolism during thermal stress	
	(Harrison & Ward, 2001)	5 μ M	28	Exp	<i>Goniastrea aspera</i>													- embryo development	
	(Stambler <i>et al.</i> , 1991)	7 μ M	70	Exp	<i>Pocillopora damicornis</i>	- growth rate	+ symbiont density	+ chlorophyll content											
	(Ferrier-Pagès <i>et al.</i> , 2000)		25	Exp	<i>Stylophora pistillata</i>	/													
	(Harrison & Ward, 2001)		53	Exp	<i>Acropora longicyathus</i>														- fertilization rates - embryo development
	(McCauley & Goulet, 2019)	10 μ M	12.2	Exp	<i>Pseudoplexaura porosa</i>		+ symbiont density + mitotic index	+ chlorophyll content	/		/ (Dry tissue %, carbohydrate, lipid, protein and refractory content in both the organic matter and sclerite fraction)	/	/						
<i>Eunicea tourneforti</i>		+ symbiont density + mitotic index			+ chlorophyll a content	+ F_v/F_m (at noon only)	/ (Dry tissue %, carbohydrate, lipid, protein and refractory content in both the organic matter and sclerite fraction)	/	/										

Nutrient	Study	Concentration	N:P ratio	Context	Coral species	Results													
						Coral Growth	Dinoflagellate density	Chlorophyll content	Photosynthesis rates	Tissue composition	Carbon	Nitrogen	Phosphorus	Recovery rates	Reproduction rates	Survival	Coral health		
Ammonium	(Stambler <i>et al.</i> , 1991)	15 µM	150	Exp	<i>Pocillopora damicornis</i>	- growth rate	+ symbiont density	+ chlorophyll content											
	(Snidvongs & Kinzie, 1994)		78	Exp	<i>Pocillopora damicornis</i>			+ chlorophyll content			/	+ N content in symbiont	/						
	(Muscatine <i>et al.</i> , 1989)	20 µM	-	Exp	<i>Stylophora pistillata</i>		+ symbiont density			+ protein content in symbiont		- C:N in coral tissue							
	(Muller-Parker <i>et al.</i> , 1994)		-	Exp	<i>Pocillopora damicornis</i>		+ symbiont density	+ chlorophyll content		+ protein content in animal tissue									
	(Cox & Ward, 2002)		-	Exp	<i>Montipora capitata</i>	/ (linear extension)		/			/ C in tissue	+ N in tissue				- spawning.	- egg size		
	(Bassim & Sammarco, 2003)		-	Exp	<i>Pocillopora damicornis</i>									planulation recovery after stress		- planulation	/ fecundity		
			-	Exp	<i>Diploria strigosa</i>											- larval development	/ settlement	/ larvae survival	
	(Koop <i>et al.</i> , 2001)	36.2 µM	7	Exp	<i>Acropora longicyathus</i>	/					- lipid content								
					<i>Acropora aspera</i>	/					- lipid content								
					<i>Acropora palifera</i>	- growth rate													
					<i>Pocillopora damicornis</i>	- calcification rate													
					<i>Stylophora pistillata</i>	- calcification rate													
					<i>Sarcophyton ehrenbergi</i>				/ (lipid)										
					<i>Pocillopora damicornis</i>				+ symbiont density	/			/						
	(Harrison & Ward, 2001)	50 µM	253	Exp	<i>Goniastrea aspera</i>											- embryo development			
(McCauley & Goulet, 2019)	56		Exp	<i>Pseudoplexaura porosa</i>		- mitotic index	/		- F _v /F _m (at dusk only)	/	(Dry tissue %, carbohydrate, lipid, protein and refractory content in both the organic matter and sclerite fraction)	/	/						
				<i>Eunicea tourneforti</i>		- mitotic index	/	/	/	(Dry tissue %, carbohydrate, lipid, protein and refractory content in both the organic matter and sclerite fraction)	/	/							
(Harrison & Ward, 2001)	100 µM		503	Exp	<i>Acropora longicyathus</i>										- fertilization rates	- embryo development			
(Burkpile <i>et al.</i> , 2013)	35.7 mg.m ⁻² d ⁻¹		7	In situ (fish excretion)	no specific species											- coral abundance through macroalgae proliferation			

Nutrient	Study	Concentration	N:P ratio	Context	Coral species	Results												
						Coral Growth	Dinoflagellate density	Chlorophyll content	Photosynthesis rates	Tissue composition	Carbon	Nitrogen	Phosphorus	Recovery rates	Reproduction rates	Survival	Coral health	
Phosphorus	(Koop <i>et al.</i> , 2001)	5 µM	0.1	Exp	<i>Acropora longicyathus</i>	- skeletal density					+ lipid content							
					<i>Acropora aspera</i>	/				+ lipid content								
					<i>Acropora palifera</i>	+ growth rate												
					<i>Pocillopora damicornis</i>	- calcification rate												
					<i>Stylophora pistillata</i>	- calcification rate												
					<i>Sarcophyton ehrenbergi</i>				/ (lipid)									
	(Harrison & Ward, 2001)	10 µM	0.06	Exp	<i>Acropora longicyathus</i>											- fertilization rates		
(Harrison & Ward, 2001)	50 µM	0.01	Exp	<i>Goniastrea aspera</i>												- embryo development		
(Harrison & Ward, 2001)	100 µM	0.006	Exp	<i>Acropora longicyathus</i>												- fertilization rates		
NO ₃ / PO ₄	(Renegar & Riegl, 2005)	5 / 2 µM	2.5	Exp	<i>Acropora cervicornis</i>	- coral growth												
	(Ezzat <i>et al.</i> , 2015)	2.5 / 0.6 µM	4	Exp	<i>Stylophora pistillata</i>	Maintain calcification rate under thermal stress			Maintain photosynthetic rate under thermal stress								Maintain coral metabolism during thermal stress	
	(Ezzat <i>et al.</i> , 2016b)				<i>Turbinaria reniformis</i>	/		+ chlorophyll content	/									
	(Hoadley <i>et al.</i> , 2016)	3.5 / 0.3 µM	11.5	Exp	<i>Turbinaria reniformis</i>	/	/	/	/ (net photosynthetic rates)	- F _v /F _m (under thermal stress and/or elevated pCO ₂)	/ (protein)							Limit deleterious effect of thermal stress and elevated pCO ₂
	(Tanaka <i>et al.</i> , 2007)	5 / 0.3 µM	16.5	Exp	<i>Acropora pulchra</i>			+ chlorophyll content	+ net production rate	+ coral tissue biomass	+ C in symbiont	+ C in coral tissue	+ N in symbiont	+ N in coral tissue				
	(Tanaka <i>et al.</i> , 2017)	2 / 0.1 µM	20	Exp	<i>Montipora digitata</i>	/		+ chlorophyll content			+ C in symbiont		+ N in symbiont	- CN in symbiont	- CN in coral tissue			
<i>Porites cylindrica</i>					/		+ chlorophyll content			/		+ N in symbiont	- CN in symbiont					

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Nutrient	Study	Concentration	N:P ratio	Context	Coral species	Results													
						Coral Growth	Dinoflagellate density	Chlorophyll content	Photosynthesis rates	Tissue composition	Carbon	Nitrogen	Phosphorus	Recovery rates	Reproduction rates	Survival	Coral health		
NO ₃ / PO ₄	(Holcomb <i>et al.</i> , 2010)	6 / 0.3 μM	20	Exp	<i>Astrangia poculata</i>	/													
	(Wiedenmann <i>et al.</i> , 2013)	6.5 / 0.3 μM		Exp	<i>Montipora foliosa</i>			F _v /F _m > 0.6									/		
				Exp	<i>Euphyllia paradivisia</i>			F _v /F _m > 0.5								/			
(Tanaka <i>et al.</i> , 2010)	10 / 0.5 μM	Exp	<i>Acropora polystoma</i>													/			
				Exp	<i>Montipora digitata</i>			+ chlorophyll content					- CN in coral holobiont	- CP in coral holobiont					
Depletion NO ₃ / PO ₄	(Wiedenmann <i>et al.</i> , 2013)	0.7 / 0.006 μM	116	Exp	<i>Montipora foliosa</i>		- symbiont density		F _v /F _m > 0.6										
NH ₄ / PO ₄	(Harrison & Ward, 2001)	1 / 1 μM	1	Exp	<i>Acropora longicyathus</i>												- fertilization rates - embryo development		
		5 / 5 μM													- fertilization rates - embryo development				
		100 / 100 μM													- fertilization rates - embryo development				
		0.5 / 0.5 μM												- embryo development					
		1 / 1 μM				<i>Goniastrea aspera</i>													- embryo development
		5 / 5 μM																- embryo development	
	50 / 50 μM															- fertilization rates - embryo development			
	(Ezzat <i>et al.</i> , 2015)	3 / 1 μM	3	Exp	<i>Stylophora pistillata</i>		/	/	- photosynthesis rates										
	(Ferrier-Pagès <i>et al.</i> , 2000)	10 / 2 μM	5	Exp	<i>Stylophora pistillata</i>	- growth rate											Observed recovery		
	(Bongiorni <i>et al.</i> , 2003)	2.5 / 0.4 μM	6	In situ (fish farm)	<i>Stylophora pistillata</i>	+ growth rate in vertical nubbins													
(Koop <i>et al.</i> , 2001)	36.2 / 5.1 μM	7	Exp	<i>Acropora longicyathus</i>	/														
				<i>Acropora palifera</i>	+ growth rate														
					<i>Sarcophyton ehrenbergi</i>					/ (lipid)									
(Stambler <i>et al.</i> , 1991)	15 / 2 μM	7.5	Exp	<i>Pocillopora damicornis</i>	- growth rate	+ symbiont density	+ chlorophyll content												
(Langdon & Atkinson, 2005)	109 / 13 μM	8.4	Exp	<i>Porites compressa</i>	- calcification rate				+ carbon fixation										
				<i>Montipora verucosa</i>	- calcification rate				+ carbon fixation										

Nutrient	Study	Concentration	N:P ratio	Context	Coral species	Results												
						Coral Growth	Dinoflagellate density	Chlorophyll content	Photosynthesis rates	Tissue composition	Carbon	Nitrogen	Phosphorus	Recovery rates	Reproduction rates	Survival	Coral health	
NH ₄ / PO ₄	(Muscatine <i>et al.</i> , 1989)	20 / 2 μM	10	Exp	<i>Stylophora pistillata</i>		+ symbiont density											
	(Ferrier-Pagès <i>et al.</i> , 2000)			Exp	<i>Stylophora pistillata</i>	- growth rate								Observed recovery				
	(Kinsey & Davies, 1979)			In situ	no specific species	- calcification rate												
	(Meyer & Schultz, 1985)	2.4 / 0.2 mmol m ⁻² d ⁻¹	12	In situ (fish excretion)	<i>Acropora palmata</i>	/	/			/		+ N in the holobiont	+ P content in the holobiont					
	(Snidvongs & Kinzie, 1994)	15 / 1.2 μM	12.5	Exp	<i>Pocillopora damicornis</i>			/			/	/	- P in symbiont					
	(Stambler <i>et al.</i> , 1991)	15 / 0.5 μM	30	Exp	<i>Pocillopora damicornis</i>	- growth rate	+ symbiont density	+ chlorophyll content										
	(Meyer & Schultz, 1985)	7.3 / 0.1 mmol m ⁻² d ⁻¹	73	In situ (fish excretion)	<i>Porites furcata</i>	+ growth rate	+ symbiont density			+ tissue thickness		+ N in the holobiont						
NH ₄ + NO ₃ / PO ₄	(Bruno <i>et al.</i> , 2003)	11 + 6.4 / 4.6 μM	3.8	In situ	<i>Montastrea annularis</i> <i>Montastrea fransii</i>												- resistance to Yellow band disease	
	(Loya <i>et al.</i> , 2004)	(1 + 0.5) / 0.1 μM	12	In situ (fish farm)	<i>Stylophora pistillata</i>												- oocyte size - fertilization - maturation rate - larvae development	
	(Atkinson <i>et al.</i> , 1995)	(2 + 5) / 0.5 μM	14	Exp	57 coral species	+ growth rate												
	(Vega Thurber <i>et al.</i> , 2014)	3.9 / 0.3 μM	14.5	In situ Exp (Garden fertilizer)	<i>Siderastrea siderea</i> <i>Agaricia spp.</i>													- resistance to Dark Spot Syndrome - bleaching resistance
	Meyer <i>et al.</i> 1983	6.7 / 0.4 mmol m ⁻² d ⁻¹ Particulate dissolved N and P	17	In situ	<i>Porites furcata</i>	+ growth rate												
NH ₄ + NO ₃	Tanaka <i>et al.</i> 2014	(0.14 + 6) / 0.28 μM	18	Exp	<i>Acropora tenuis</i>		+ symbiont density No change in algal release rate	+ chlorophyll content										
	Chauvin <i>et al.</i> 2011	5 + 0.6	40	In situ (groundwater discharge)	<i>Acropora muricata</i>	+ calcification rates (acidic condition)			+ photosynthesis									
	Hadjioannou <i>et al.</i> 2019	6 + 5	55	In situ	<i>Cladocora caespitosa</i>	/	- symbiont density	/	- gross photosynthetic rates	/	- total organic carbon flux	/ (Total nitrogen flux)						

V Microelements

V.1. Trace elements in seawater

Microelements are present in an organism in small or trace amounts and often relate to metals. In the marine environment, metals can come from a variety of natural sources, which are listed below (Table 2) (Libes, 2011):

- **River runoff:** The mechanical and chemical weathering of rocks in river-beds is an important source of trace elements in the oceans.
- **Atmospheric deposition:** Wind can pick up metals either in gaseous form, or as small aeolian particles ($< 10 \mu\text{m}$) called aerosols, especially in desert areas, and transport them to the oceans. Particles come from weathered rocks, soils, plants, bacteria, phytoplankton, fungi, and insects. They are then deposited in the ocean, either by dry deposition (i.e. particles falling by gravity when the wind dies down) or by wet deposition (where aerosol and gas combine in raindrops). This atmospheric deposition can be locally and punctually enhanced by volcanic eruptions (Olgun *et al.*, 2011).
- **Hydrothermal activity:** Although a local source, hydrothermal fluids are considered important sources of metals in reduced form.
- **Groundwater seeps:** This is the discharge of terrestrial water accumulated in aquifers into the ocean. Groundwater is usually anoxic and contains metals in reduced form, especially ferrous ion (Fe^{2+}) (Moore, 2010).
- **Sediment:** Particles containing trace elements eventually sink and accumulate in sediment, where they can then be remobilised.

Table 2. Net flux of trace metal naturally released in the ocean from the three major sources per year
Modified from Libes *et al* (2011)

Metal	Fluvial discharge (particulate + dissolved) (mol y^{-1})	Atmospheric deposition (particulate + dissolved) (mol y^{-1})	Hydrothermal axial flux (dissolved) (mol y^{-1})
Aluminium	5.4×10^{12}	2.5×10^{11}	$1.2 - 6 \times 10^8$
Iron	1.3×10^{12}	6.5×10^{12}	$2.3 - 19 \times 10^{10}$
Manganese	3.4×10^{10}	1.4×10^9	$1.1 - 3.4 \times 10^{10}$
Nickel	2.8×10^9	1×10^9	-
Cobalt	6.2×10^8	4×10^9	$6.6 - 68 \times 10^5$
Chromium	3.4×10^9	1×10^9	-
Copper	$3.4 - 7.4 \times 10^9$	6×10^{11}	$3 - 13 \times 10^8$
Zinc	$6.4 - 20 \times 10^9$	3.1×10^9	$0.12 - 0.32 \times 10^{10}$
Lead	$7.7 - 9 \times 10^8$	1.3×10^9	$2.7 - 110 \times 10^5$
Cadmium	4.4×10^7	3×10^9	-

These fluxes can be enriched by anthropogenic influences leading to a doubling of metal input to the reefs (Fabricius, 2005; Libes, 2011). For example, exhaust fumes from vehicles, coal-fired power plants and waste incineration plants increase the emission of particles into the atmosphere (Fabricius, 2005). The weathering of soils by wind and water due to intensive agriculture, deforestation, strip mining and urbanisation also increases the supply of metals into the ocean. In developing countries, where most tropical reefs are located, untreated urban and industrial wastewater further increases the input of trace metals to reef waters.

In the water column, trace metals can be mineralised and dissolved, flocculated and hydrolysed or adsorbed and desorbed. In this cycle, trace metals can occur either as particulate compounds ($> 0.45 \mu\text{m}$) in association with organic matter, or as dissolved elements ($< 0.45 \mu\text{m}$), in association with micromolecular inorganic complexes. The dissolved fraction of trace metals is usually relatively small, as they are prone to forming complexes with particles (Hoffmann *et al.*, 2012). However, in order to be taken up or absorbed by organisms, trace elements must be bioavailable. Bioavailable trace metals are found in pico- to nanomolar concentrations in the surface layer (Pinedo-González *et al.*, 2015). The concentration profile can vary with depth.

V.2. *Trace elements in plant and animal physiology*

Depending on their ability to participate in biological functions, trace metals are classified as “essential” or “non-essential”. Trace metals classified as essential are known to interfere with various enzyme systems (Libes, 2011; Reich *et al.*, 2020).

A deficiency of an “essential” trace metal can be detrimental to an organism, while its enrichment can have positive effects by enhancing metabolic pathways (Adriano, 2001). On the other hand, the absence or enrichment of a “non-essential” trace metal may have no effect on an organism (Adriano, 2001). Above a certain concentration threshold, however, any trace metal is likely to become toxic and eventually lethal to an organism. The dose-response relationship between organisms and assimilated trace metals thus depends on their biological function in the organism (Fig 11). In this thesis the metals are always at concentrations below their toxicity threshold.

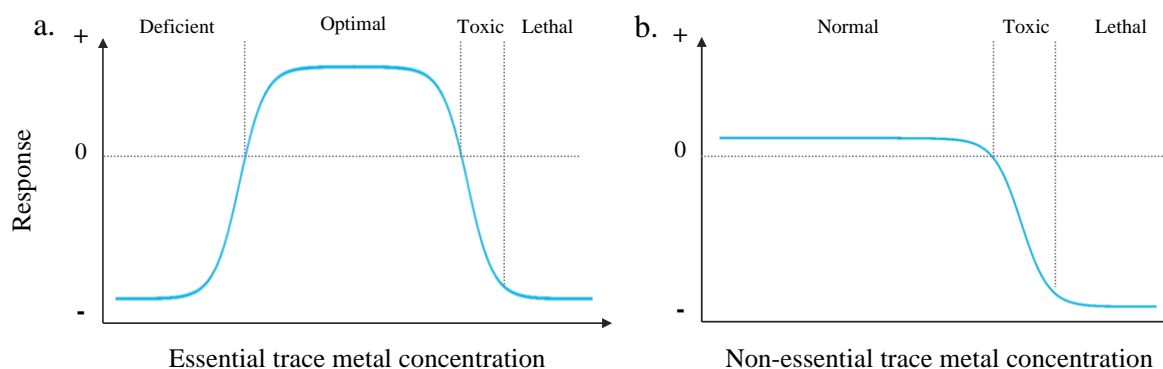


Figure 11. **Dose-response relationship of trace metal in organisms**

Response of organisms to increased concentration of (a) essential trace metals and (b) non-essential trace metals

Modified from Adriano (2001)

V.3. Trace elements in corals

In the coral holobiont, the uptake of metals can occur either by heterotrophic feeding for the particulate fraction (Ferrier-Pagès *et al.*, 2018), or by diffusion and active transport for the dissolved fraction (Ferrier-Pagès *et al.*, 2005). Corals accumulate metals differentially depending on their taxonomy, with octocorals accumulating a higher quantity of metals than scleractinian corals (Denton & Burdon-Jones, 1986). In the holobiont, metals tend to accumulate in different places: in the skeleton, in the symbiotic dinoflagellate or in the host polyp (tissues and gametes) (Reichelt-Brushett & McOrist, 2003). Some metals, particularly iron, copper, manganese and zinc are preferentially stored in the symbiotic dinoflagellates, as they are involved in photosynthetic processes (Ferrier-Pagès *et al.*, 2018). They can then either be detoxified - by sequestration in the skeleton and possibly even excreted during gametes and symbionts release (Reichelt-Brushett & McOrist, 2003; Bastidas & Garcia, 2004) – or used by the holobiont in different metabolic processes (Table 3).

In addition to being used in physiological processes, the availability of metals might be key to the resistance and resilience of corals to environmental stress. Indeed, studies have shown that a slight increase in trace metals below their toxicity level enhances the resistance of the coral holobiont to environmental stress (Shick *et al.*, 2011; Ferrier-Pagès *et al.*, 2018; Reich *et al.*, 2021). For example, symbiotic dinoflagellates are exposed to environmental stressors such as high light intensities that exceed the photosynthetic capacities of the symbionts and high temperatures that can significantly impair the photosynthetic system (Suggett & Smith, 2020). In the symbiotic dinoflagellate, the photosynthetic process generates reactive oxygen species (ROS) that can be detrimental to the holobiont (Roberty *et al.*, 2014). These ROS can be removed by antioxidant molecules such as superoxide dismutase (Fe, Mn, Cu/Zn -SOD),

catalase (Fe) and ascorbate peroxidases (Fe) (Raven *et al.*, 1999). Metal uptake can thus promote the production and activity of antioxidants and increase the resistance of coral holobionts to environmental stress.

Table 3. Example of the role of some essential trace metals in various biogeochemical pathways

Metals	Role in biological pathway	Concentration in the reef	References
Iron	Photosynthesis (PS I and II, cytochrome b ₆ f, ferredoxin) Respiration Antioxidant (catalase, peroxidase and superoxide dismutase -FeSOD) Macronutrient assimilation (nitrite and nitrate reductase) Cellular processes (e.g. DNA replication/reparation)	0.25 nmol L ⁻¹	Boye et al, 200 Raven et al, 1999 Reich et al, 2020
Zinc	Antioxidant (Superoxide dismutase -Cu/Zn-SOD) Macronutrient assimilation (carbonic anhydrase, phosphatase) Cellular processes Co-factor in around 300 enzymes	3 nmol L ⁻¹	Aparicio-González et al, 2012 Christianson & Fierke, 1996 Plocke et al, 1962 Reich et al, 2020 Vallee & Auld, 1990
Copper	Photosynthesis (plastocyanin) Respiration Antioxidant (Superoxide dismutase -Cu/Zn-SOD) Cellular processes (e.g. DNA replication/reparation)	2 nmol L ⁻¹	Aparicio-González et al, 2012 Peers & Price, 2006 Raven et al 1999 Reich et al, 2020
Manganese	Photosynthesis (Oxygen-evolving complex) Antioxidant (superoxide dismutase - MnSOD)	5 nmol L ⁻¹	Kessler et al 1957 Morgan, 2000 Sauer, 1980 Wolfe-Simon et al, 2006
Nickel	Antioxidant (Superoxide dismutase - NiSOD) Macronutrient assimilation (urease)	4 nmol L ⁻¹	Aparicio-González et al, 2012 Dupont et al, 2008 Price & Morel, 1991
Cobalt	Macronutrient assimilation Vitamin b₁₂	0.6 nmol L ⁻¹	Agostini et al, 2009 Aparicio-González et al, 2012 Nolan et al, 1992 Reich et al, 2020
Molybdenum	Macronutrient assimilation (nitrite and nitrate reductase)	100 nmol L ⁻¹	Aparicio-González et al, 2012 Reich et al, 2020
Cadmium	Macronutrient assimilation (carbonic anhydrase)	0.3 nmol L ⁻¹	Aparicio-González et al, 2012 Lane & Morel, 2000
Chromium	-	3 nmol L ⁻¹	Sirinawin et al, 2000

VI Hypothesis and aims

The overall aim of this thesis is to study

The effects of macro- and micronutrient availability on coral physiology

The thesis is further divided into two sub-objectives.

VI.1. Axis I : Coral response to balanced and imbalanced seawater DIN:DIP ratios

The availability of DIN and DIP in reef environments can greatly influence coral metabolism and susceptibility to bleaching. However, the question of whether DIN or DIP enrichment is beneficial or detrimental to corals is still controversial. On the one hand, enrichment with DIN or DIP has been shown to reduce bleaching and enhance coral metabolism under non-stressful and stressful conditions (see e.g. Atkinson *et al.*, 1995; Dunn *et al.*, 2012; Béraud *et al.*, 2013). On the other hand, DIN enrichment can also stimulate symbiont cell division, leading to higher retention of photosynthates in symbiont cells and increased bleaching (see e.g. Wooldridge, 2013). Wiedenmann *et al.* (2013) finally summarized the information and proposed a conceptual model in which the DIN:DIP ratio in seawater is the most important factor explaining coral bleaching. In the future, significant changes in the DIN:DIP ratio in seawater will occur, due to ocean warming and/or eutrophication of coastal waters (van de Waal *et al.*, 2010; Santos *et al.*, 2014).

Nevertheless, little is still known about the effects of the DIN:DIP ratio on the physiology of different coral species maintained under different environmental conditions. The ability of corals to regulate the assimilation of DIN and DIP as a function of seawater DIN:DIP ratio has also been insufficiently studied and further studies on this topic are needed.

Research questions:

- 1) **First question:** Does DIN enrichment at normal/low DIP concentrations (imbalanced DIN:DIP ratios) have always adverse effects on coral health? On the contrary, do corals remain healthy under balanced DIN and DIP enrichment?
- 2) **Second question:** Do we observe a change in cellular C:N:P content and stoichiometry under imbalanced DIN:DIP ratios? In other words, does DIN enrichment affect corals because it induces phosphorus limitation in coral tissue?
- 3) **Third question:** Does the ability of corals to feed on dissolved nutrients (DIN, DIP and DOP) determine their response to DIN enrichment?

To answer these questions, three experiments were conducted.

VI.1.a. *The effects of nitrogen enrichment on the physiology of Pocilloporidae*

VI.1.a.i. The effects nitrate enrichment and global stressors on *Pocillopora damicornis*

We tested the effects of balanced (DIN:DIP 2.5 - 0.5:0.2) and imbalanced (DIN:DIP 17.5 – 3.5:0.2) DIN:DIP ratios, due to nitrate enrichment, a local stressor, on coral physiology. These conditions were tested alone or in combination with two global stressors (ocean warming and UVR exposure). For this purpose, we maintained nubbins of *Pocillopora damicornis* in aquaria with or without nitrate enrichment (3 μ M) and with or without UVR exposure, at normal and elevated temperatures of 26 °C and 30 °C. We then investigated the physiology of the holobiont and its oxidative status. This study addresses three questions. First, what are the effects of an imbalanced DIN:DIP ratio due to nitrate enrichment on the physiological and oxidative status of *P. damicornis* in the absence of other stress factors? Second, how does exposure to combined stressors affect the physiological and oxidative status of *P. damicornis*? Third, what is the worst combination of factors for coral health? Following the hypothesis and theory of Wiedenmann et al. (2013), we hypothesised that nitrate enrichment alone would increase the oxidative status of *P. damicornis*, without inducing bleaching, due to the ability of corals to increase their antioxidant capacity. We also predicted that thermally or UVR stressed corals maintained under nitrate enrichment would exhibit higher oxidative stress, exceeding the coral's antioxidant capacity, leading to bleaching. Finally, we hypothesised that the triple stress condition – UVR exposure, thermal stress, and nitrate enrichment – could be the most detrimental to coral health.

VI.1.a.ii. The effects of nitrogen enrichment on the phosphorus requirement of *Stylophora pistillata*

This experiment was performed with the tropical scleractinian coral *Stylophora pistillata*. We tested the DOP requirement of *S. pistillata* colonies as a function of seawater DIN:DIP ratio and nitrogen source. We tested the following conditions: Nutrient deficiency (balanced ratio, NP 2.5 0.5:0.2), 1 to 5 days of continuous enrichment with nitrate or ammonium (long term imbalance ratio, NP 17.5 – 3.5:0.2) and nutrient deficiency with a short nitrate or ammonium pulse (short term imbalance ratio, DIN:DIP 150 – 3:0.02). The assimilation of DOP-derived P was assessed by the activity of two enzymes - acid and alkaline phosphatases. This study addresses 4 questions. First, what is the phosphorus requirement of corals under control (balanced DIN:DIP ratio) and DIP starvation conditions? Second, does a short or long-term increase in nitrogen availability (imbalance DIN:DIP ratio) alter the DOP requirements of

corals? Third, does ammonium and nitrate have a different effect on the DOP requirements of corals? Finally, does internal AcPA respond differently than external APA to changes in seawater N and P concentrations? We hypothesised that a lack of P in seawater will increase the phosphatase activities when the coral is provided with DOP. We also hypothesise that nitrogen enrichment will enhance the phosphatase activities when the coral is provided with DOP, to maintain a balanced N:P ratio in its tissue. In addition, we expect that nitrate and ammonium will have different effects on coral phosphorus requirements, since they have different effects on coral physiology. Finally, we hypothesize that external phosphatases will have a quicker response to changes in seawater DIN and DIP, than internal phosphatases.

VI.1.b. The effects of nitrate enrichment on diverse coral families

VI.1.b.i. The effects of balanced and imbalanced DIN:DIP ratios on the physiology of a scleractinian coral (*Dendrophyllidae*) and an octocoral (*Alcyoniidae*)

Some octocorals are known to replace scleractinian corals in eutrophic or nutrient-enriched reefs (Rodriguez *et al.*, 2020). A previous experiment has also shown that they have a lower capacity of DIN assimilation (Pupier *et al.*, 2021), which could protect them from the effects of an imbalanced DIN:DIP ratio. We therefore tested the effects of balanced and imbalanced DIN:DIP ratios on the physiology of a tropical scleractinian coral *Turbinaria reniformis* and a tropical octocoral *Sarcophyton glaucum*. We subjected both coral species to the following conditions: imbalanced DIN:DIP ratio either by nitrate (DIN:DIP 17.5 – 3.5:0.2) or phosphorus enrichment (DIN:DIP 0.23 – 0.5:2.2) alone, or balanced DIN:DIP ratio either under control condition (DIN:DIP 2.5 – 0.5:0.2) or simultaneous nitrate and phosphorus enrichment of corals. This study addresses 2 questions. First, what are the effects of balanced and imbalanced ratios on coral physiology? Second, do scleractinian corals and octocorals respond in the same way to these DIN:DIP ratios? We hypothesised that an imbalanced DIN:DIP ratio would have different effects than a balanced DIN:DIP ratio, regardless of the nutrient concentration in seawater. We also predicted that the effects of imbalanced DIN:DIP ratio would be less detrimental to octocorals physiology, as octocorals are more abundant in eutrophic reefs.

VI.2. Axis II: Coral response to microelements supply

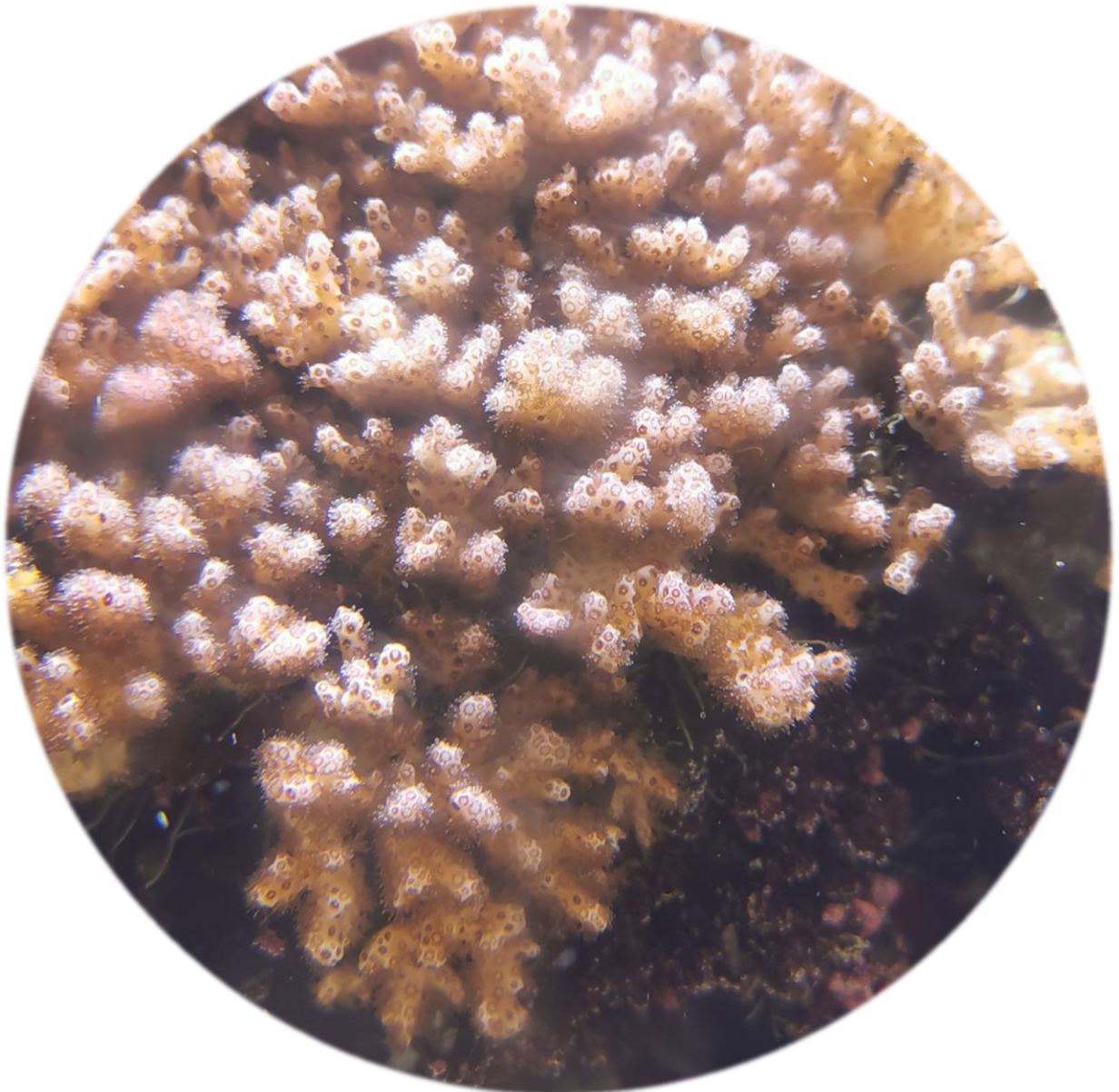
As mentioned in the general introduction, trace metals are essential for several functions in corals, including calcification, photosynthesis and antioxidant processes. However, very few studies have investigated the coral requirement for metals.

Research question: Are corals limited only in macronutrients, or can they also be limited in trace metals? Can the availability of trace metals also determine their ability to withstand heat stress?

VI.2.a. Desert dust deposition and metal content in scleractinian corals

In the northern Red Sea, corals are particularly resistant to thermal stress, probably due to their evolutionary history. In addition, corals in this region are exposed to frequent dust storms coming from the surrounding desert, which bring nutrients, especially trace metals. We therefore investigated the type and amount of trace metals assimilated by *S. pistillata* during desert dust deposition. For this purpose, nubbins of *Stylophora pistillata* were maintained with or without dust deposition at 25 °C, 30 °C and 34 °C. We measured their physiological and oxidative status as well as their metal content. This study addresses two questions. First, how does the deposition of desert dust affect the general physiology of scleractinian corals at low and elevated temperatures? Second, does metal supply help corals recover after thermal stress? Since Red Sea corals are particularly resistant to thermal stress, we hypothesised that dust deposition might be beneficial to coral health. We predicted that they could also recover faster after bleaching caused by thermal stress.

CHAPITRE 1:
IMPACT OF LOCAL AND
GLOBAL STRESSORS ON CORAL
PHYSIOLOGY



CHAPTER 1: IMPACT OF LOCAL AND GLOBAL STRESSORS ON CORAL PHYSIOLOGY

I Overview of the publication

Coral reefs are under increasing pressure from environmental stressors, such as increased seawater temperatures and intense ultraviolet radiation (UVR), which can disrupt the symbiosis between corals and dinoflagellates and induces bleaching. In addition to global stressors, corals are also subjected to pressures from local stressors, such as nitrate pollution from agricultural activities. Overall, thermal stress, UVR and nitrate enrichment can cause oxidative damage through the overproduction of reactive oxygen species (ROS: superoxide anions, hydroxyl radicals...) and reactive nitrogen species (RNS: nitric oxide radicals and peroxyxynitrite) (Lesser, 2006; Weis, 2008; Moniczewski *et al.*, 2015). Oxidative damage can be mitigated by an increased antioxidant capacity in corals, i.e. the production of ROS-RNS scavenging compounds, enzymatic (catalase, superoxide dismutase) and non-enzymatic (urease, glutathione) (Krueger *et al.*, 2014).

However, the effects of global and local stressors can have divergent outcomes on coral physiology. Indeed, bleaching does not always occur under the combination of high temperatures and UVR. These two stressors can have either synergistic (Rosic *et al.*, 2020) or antagonistic (McCauley *et al.*, 2018) effects on coral physiology, likely depending on the dose of UVR. Similarly, experimental studies have shown that calcification rates, skeletal density, photosynthate fluxes, or reproductive success can be reduced under nitrate (Marubini & Davies, 1996; Nordemar *et al.*, 2003; Renegar & Riegl, 2005; Wiedenmann *et al.*, 2013; Ezzat *et al.*, 2015; Liu *et al.*, 2020; Marangoni *et al.*, 2020) or phosphate enrichment alone (Ferrier-Pagès *et al.*, 2000; Harrison & Ward, 2001; Koop *et al.*, 2001; Renegar & Riegl, 2005).

In contrast, in situ and laboratory observations have also reported healthy corals exposed to high nutrient concentrations (Lirman & Fong, 2007; Sawall *et al.*, 2011; Ezzat *et al.*, 2015, 2016b; Liu *et al.*, 2020). Wiedenmann *et al.* (2013), hypothesized that it is the imbalanced DIN:DIP ratios rather than the nutrient concentrations in seawater that are detrimental to coral health.

All together, these observations suggest that more studies are needed to better understand the effects of these stressors on coral physiology.

We tested the effects of a balanced (DIN:DIP = 2.5 - 0.5:0.2) and an imbalanced (DIN:DIP = 17.5 - 3.5:0.2) DIN:DIP ratio, alone or in combination with seawater warming (30°C) and UVR exposure, on coral physiology and oxidative status. To this end, we maintained nubbins of *Pocillopora damicornis* in aquaria at 26 °C, with or without UVR exposure and with a balanced or imbalanced N:P ratio for 6 weeks. The UVR dose received daily by the corals was equivalent to that received by corals at 10 m depth in a tropical reef and was therefore relatively low. The nitrate enrichment used to achieve an imbalance N:P ratio of 17.5 was 3 µM, a common nitrate concentration found in eutrophic reefs. After this first phase, the temperature was gradually increased from 26 °C to 30 °C in half of the aquaria. At the end of the two weeks, several parameters were evaluated:

- **Physiology:** photosynthetic and respiration rate, protein biomass, chlorophyll content and symbiont density.
- **Oxidative status:** ROS (directly or indirectly through lipid peroxidation damage), RNS (indirectly, protein tyrosine nitration) and non-enzymatic antioxidant capacity (uric acid, glutathione).

This study addresses three questions.

What are the effects of an imbalanced N:P ratio due to nitrate enrichment on the physiological and oxidative status of P. damicornis in the absence of other stress factors?

We observed a significant decrease in photosynthetic rates, normalised to skeletal area, without changes in oxidative stress parameters. This impairment of photosynthesis can be explained by the competition between nitrate and carbon assimilation, which reduces the efficiency of photosynthesis. Indeed, the necessary reduction of nitrate to ammonium before it can be incorporated into the coral metabolism is energetically costly and diverts electrons from the photosynthetic chain, reducing carbon assimilation (Nunes-Nesi *et al.*, 2010).

How does exposure to combined stressors affect the physiological and oxidative status of P. damicornis?

In this study, it was found that exposure to low UVR enhanced or maintained photosynthetic rates at their maximum level, even in the presence of an imbalanced N:P ratio and thermal stress. This maintenance of photosynthesis is associated with an increase in non-enzymatic antioxidant compounds, possibly by stimulating the production of mycosporine-like amino acids (MAAs) (Shick *et al.*, 1999). These compounds play a dual role: they can absorb UVR and are simultaneously involved in coral antioxidant metabolism by scavenging ROS (Rosic & Dove, 2011). In addition, UVB has been shown to act as a signal in photosystem restoration

by preventing photoinhibition, and thus increasing photosynthetic efficiency (Hanelt *et al.*, 2006). UVA also appears to increase the activity of two enzymes, carbonic anhydrase and nitrate reductase, which are involved in various processes of the photosynthetic pathway (Figueroa & Viñepla, 2001).

What is the worst combination of factors for coral health?

In our study, thermal stress and an imbalanced DIN:DIP ratio, whether in combination or alone, had the worst effects on coral health, characterised by a collapse in photosynthetic rates. In addition, under thermal stress the production of ROS as well as the production of non-enzymatic antioxidant compounds increased.

In summary, in the absence of UVR, an imbalance DIN:DIP ratio (17.5 - 3.5:0.2) due to nitrate enrichment, combined with thermal stress had detrimental effects on coral physiology and increased oxidative stress. However, low UVR exposure improved the overall metabolism of corals, especially photosynthesis and antioxidant capacity. Thus, UVR exposure is a crucial factor influencing the response of corals to environmental stress. The results of this study have helped to understand the inconsistencies observed in coral response to an imbalanced DIN:DIP ratio.

II Publication

Low levels of ultra-violet radiation mitigate the deleterious effects of nitrate and thermal stress on coral photosynthesis

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II.1. Abstract

Reef ecosystems are under increasing pressure from global and local stressors. Rising seawater temperature and high ultraviolet radiation (UVR) levels are the main drivers of the disruption of the coral-dinoflagellate symbiosis (bleaching). Bleaching can also be exacerbated by nitrate contamination in coastal reefs. However, the underlying physiological mechanisms are still poorly understood. Here, we assessed the physiological and oxidative state of the scleractinian coral *Pocillopora damicornis*, maintained eight weeks in a crossed-factorial design including two temperatures (26 °C or 30 °C), and two nitrate (0.5 and 3 µM-enriched), and UVR (no UVR and 25/1.5 Wm⁻² UVA/B) levels. Nitrate enrichment, and high temperature, significantly impaired coral photosynthesis. However, UVR alleviated the nitrate and temperature-induced decrease in photosynthesis, by increasing the coral's antioxidant capacity. The present study contributes to our understanding of the combined effects of abiotic stressors on coral bleaching susceptibility. Such information is urgently needed to refine reef management strategies.

II.2. Introduction

Coral reefs are among the most diverse and productive marine ecosystems on the planet despite thriving in oligotrophic waters (i.e. low levels of nitrogen and phosphorus; Crossland et al., 1991). They provide a range of important economic services to local population, such as tourism and recreation, fisheries and coastal protection (Moberg & Folke, 1999). Reefs rely on the nutritional and optimized symbiosis between corals and autotrophic dinoflagellates of the Symbiodiniaceae family (LaJeunesse et al., 2018). Symbionts efficiently take up and assimilate dissolved inorganic nutrients from the oligotrophic seawater (Muscatine et al., 1984; Godinot et al., 2009; Rådecker et al., 2015) and are able to recycle the host's metabolic wastes (Rahav et al., 1989). They also transfer most of their photosynthates to the coral host, which relies on this major source of nutrients to sustain its respiratory demand, growth and reproduction (Muscatine et al., 1984; Tremblay et al., 2014).

Despite their ecological and economical value, reef ecosystems are under increasing pressure from multiple global stressors, which disrupt the stability of the coral-dinoflagellate symbiosis (Ellis et al., 2019). The loss of symbionts (i.e. bleaching; Weis 2008) significantly impairs the coral nutritional state and may ultimately lead to significant mortality rates. Coral bleaching is thus one of the greatest threats to reef ecosystems nowadays (Eakin et al., 2019; Suggett & Smith, 2020). Large-scale bleaching events have increased in frequency over the

last decades mainly due to heatwaves, and elevated sea surface temperatures (SST) maintained over several weeks (Couch *et al.*, 2017; Hughes *et al.*, 2017). Coral bleaching susceptibility and severity, however, depends on other environmental parameters such as the irradiance levels, both photosynthetically active radiation (PAR) and ultra-violet radiation (UVR), either UVA (400-320 nm) or UVB (320-280 nm). UVR can penetrate the water column down to 150 m depth in very oligotrophic waters (Tedetti & Sempéré, 2006; Barron *et al.*, 2009; Kahng *et al.*, 2019). While the thermotolerance threshold of corals is generally decreased under exposure to high PAR or UVR levels (Lesser & Farrell, 2004; Torregiani & Lesser, 2007; Downs *et al.*, 2013), the contrary has also been observed (Halac *et al.*, 2010; McCauley *et al.*, 2018; Rosic *et al.*, 2020). For example, Rosic *et al.* (2020) showed a synergetic effect of low PAR (no UVR exposure) and thermal stress that caused higher bleaching levels of the scleractinian coral *Acropora millepora* compared to thermal stress and high PAR. Antagonistic interactions of temperature and UVR were also observed on Caribbean octocorals (McCauley *et al.*, 2018). Finally, two experiments showed an enhancement of the photosynthetic performances of coral larvae under UVR exposure (Zhou *et al.*, 2016, 2017). This discrepancy in the effects of UVR on coral physiology is most likely due to the different doses of UVR to which organisms are exposed (Barron *et al.*, 2009; Overmans & Agustí, 2020). Therefore, UVR levels may be a tipping point towards postponing or speeding up DNA damage and coral bleaching (Ben-Zvi *et al.*, 2019).

In addition to global change stressors, coral reefs also face local anthropogenic impacts. These include upwelling events (Richardson *et al.*, 2020), nutrient enrichment of coastal seawater through land run off, agriculture and urban wastes (Fabricius, 2005, 2011). Under these conditions, inorganic nitrogen (N) and phosphorus (P) concentrations can be much higher (up to 4 μM N and 0.5 μM P, (Brodie *et al.*, 2011; Govers *et al.*, 2014; Naumann *et al.*, 2015; Rouzé *et al.*, 2015)) than the usual levels measured on reefs (ca. 0.5 μM N and 0.1 μM P (Kinsey & Davies, 1979; Charpy, 2001; Lomas & Lipschultz, 2006; Meeder *et al.*, 2012)). Recent findings have shown that concentrations of 2 to 5 μM of nitrate, which is the main nitrogen compound resulting from human activities, can enhance coral bleaching during thermal stress, (Wiedenmann *et al.*, 2013; Ezzat *et al.*, 2016a; Rosset *et al.*, 2017; Burkepile *et al.*, 2020; Marangoni *et al.*, 2020). However, as for UVR exposure, the effect of nitrate on thermal stress-induced bleaching largely varies, depending for example on coral species, symbiont densities, nitrate and other nutrient concentrations (Schlöder & D’Croz, 2004; Fabricius *et al.*, 2013; Serrano *et al.*, 2018).

Variations in coral bleaching susceptibility under either UVR exposure or nitrate enrichment, may depend on changes in UVR or nitrate levels, and more specifically on corals' antioxidant capacity (Ezzat *et al.*, 2015; Krueger *et al.*, 2015; Muller-Parker *et al.*, 2015; Marangoni *et al.*, 2020). Under thermal stress, the concentrations of reactive oxygen species (ROS) and nitrogen species (RNS) within the host and symbiont cells drastically increase (Weis, 2008). These compounds cause cellular damage through DNA degeneration, protein oxidation and lipid peroxidation (Freeman & Crapo, 1982; Suggett & Smith, 2020). In order to cope with high levels of ROS and RNS, corals produce various protecting molecules, such as ascorbate, catalase (CAT), glutathione peroxidases and super-oxide dismutases (Liñán-Cabello *et al.*, 2010; Krueger *et al.*, 2014). Damages to coral tissue and bleaching occur when ROS and RNS production exceed the antioxidant capacity of the coral holobiont. While UVR and nitrate can respectively be sources of ROS and RNS (Lesser, 2006; Moniczewski *et al.*, 2015), the oxidative status of corals under the combination of the two stressors, has never been investigated and thus needs further attention.

The present study aims to investigate the effects of nitrate enrichment on coral bleaching susceptibility, when combined with different temperatures and/or UVR conditions. For this purpose, we used the widespread scleractinian coral species *Pocillopora damicornis*. We measured the changes in the physiological and oxidative status of the corals following exposure to the individual and combined stressors. We used a UVR level that can be received by tropical corals living at 10-15 m depth (daily dose of 125 Wd⁻¹ UVA and 7.5 Wd⁻¹ UVB), instead of the three times higher doses measured in shallow reefs. We hypothesized that thermally-stressed or UVR-stressed corals exposed to nitrate enrichment would experience increased levels of oxidative stress compared to corals maintained with no nitrate enrichment. Given the increasing exposure of reefs to human pollution and recurrent heat waves events, this study aims to identify the worse combination of factors for the survival of reef building corals such as *Pocillopora damicornis*. A deeper understanding of the impacts of multiple stressors on reef building corals will help identify adaptive strategies to better protect corals and associated coral reefs.

II.3. Material and Methods

II.3.a. Experimental design

Ten colonies of the scleractinian coral *Pocillopora damicornis* were used to generate 160 nubbins (16 nubbins from each colony), which were then equally divided in 16 aquaria of

25 L (one nubbin per colony, 10 per aquaria). Nubbins were left for three weeks in similar conditions (see below) for healing and were fed once a week with *Artemia salina* nauplii. During this period, aquaria were continuously supplied (at a rate of 8 L.h⁻¹) with oligotrophic seawater (0.5 µM nitrate, 0.2 µM phosphorus) and corals were maintained at 25 °C under a photosynthetically active radiation (PAR) of 200 ± 10 µmol photons.m⁻².s⁻¹ (12:12h photoperiod). PAR was delivered by 400 W metal halide lamps (HPITS, Philips) above the aquaria and PAR intensity was measured using a LI-COR data logger (LI-100) connected to a quantum sensor (LI-193). Temperature was controlled by heaters connected to an Elliwell PC 902/T controller. Nutrient levels were measured once a week using an AA3 Seal autoanalyzer according to Aminot et al. (2009).

The 16 aquaria were then divided into 4 sets of 4 aquaria, each set being maintained under a different nitrate and UVR level (Fig 12). The first set was a control condition (C26), which was not enriched with nitrate (0.5 µM nitrate) and not exposed to UVR. A second set of 4 aquaria (UV26) was not enriched with nitrate, but received 25 Wm⁻² of UVA and 1.5 Wm⁻² of UVB during 5 hours per day (between 10:00 and 15:00) provided by two Q-panel UVA 340 lamps. The spectral distribution of these lamps is shown in Shick et al (1999). The two other sets of aquaria were enriched with 3 µM nitrate and maintained with or without the levels of UVR described above (N26 or UVN26 respectively). Nitrate enrichment was performed using stock solutions of 300 µM NaNO₃. The solutions were delivered to the aquaria with a peristaltic pump and were renewed every week. The enrichment condition chosen was similar to the nitrate concentration observed in reefs experiencing nutrient enrichment (Costa *et al.*, 2000; Brodie *et al.*, 2011; Govers *et al.*, 2014; Naumann *et al.*, 2015; Rouzé *et al.*, 2015). The intensity of UVR provided to the two sets of 4 aquaria was recorded using an International Light ILT1400 portable radiometer equipped with two detectors: SEL033/UVA and SEL240/UVB and the coefficient of attenuation of UVR irradiance in water was taken into account. UVA and UVB values, applied during only 5 h, corresponded to a daily dose of 125 Wd⁻¹ UVA and 7.5 Wd⁻¹ UVB). Such doses can be considered low compared to those received by corals on shallow reefs, which can reach more than 350 Wd⁻¹ UVA and 19 Wd⁻¹ UVB (Barron *et al.*, 2009; Overmans & Agustí, 2020). In these reefs, in summer, peaks of 60 to 70 Wm⁻² UVA and 3 Wm⁻² UVB can occur at midday (Kaneohe Bay, Hawaii and Heron Island, Australia) (Torregiani & Lesser, 2007; Rosic *et al.*, 2020). Our conditions, therefore, correspond to tropical corals living at 15 m depth, where UVR levels are significantly reduced. The factorial design with UVR and nutrient levels was maintained for six weeks, before a thermal stress was applied to half of the aquaria (Fig 12).

Temperature was increased by 0.5 °C every two days until reaching 30 °C. This temperature was maintained for one week until bleaching was visually observed, and the measurements described below were performed.

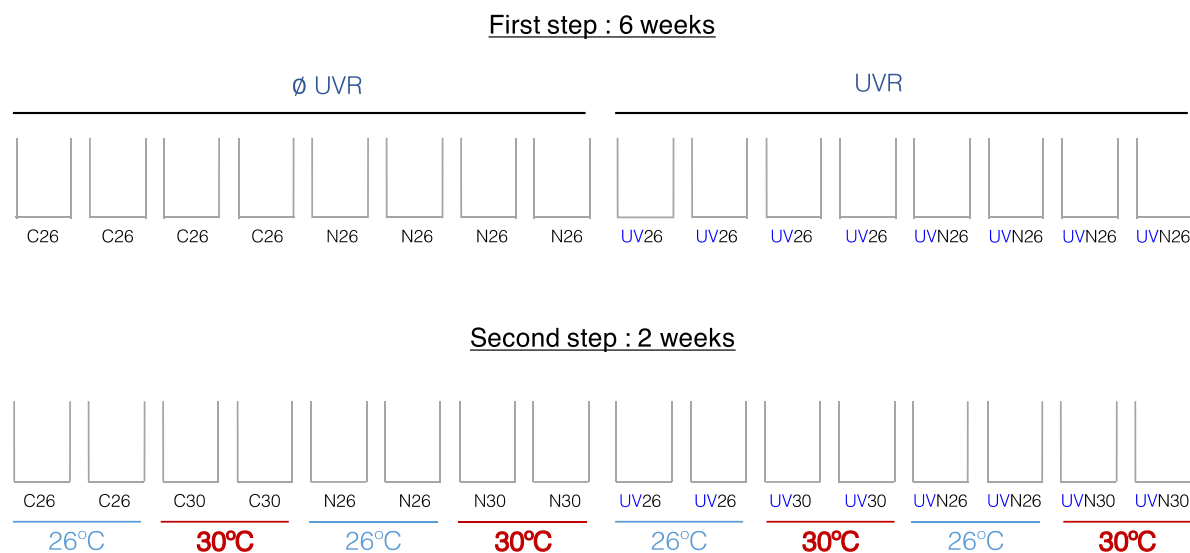


Figure 12. Cross-factorial experimental design

16 aquaria were used with 10 nubbins per aquaria. All tanks were supplied with continuous fresh seawater (0.5 μM nitrate) and kept under PAR irradiance of $200 \pm 10 \mu\text{mol photons.m}^{-2}.\text{s}^{-1}$. Corals were not fed.

During six weeks, 8 aquaria were kept under UVR exposure (25 Wm^{-2} UVA and 1.5 Wm^{-2} UVB) and 8 aquaria without UVR exposure. Out of the 8 aquaria, 4 were kept in control seawater and 4 were enriched with nitrate (3 μM nitrate). Then, for 2 weeks, for each nitrate (N)-UVR condition 2 aquaria were kept at 26 °C and 2 aquaria were raised to 30 °C.

The experimental conditions are the following: **C26-C30**: control corals maintained at 26 °C or 30 °C without nitrate enrichment and UVR exposure, **N26-N30**: corals maintained under nitrate enrichment (3 μM nitrate) at 26 °C or 30 °C, **UV26-UV30**: corals exposed to UVR (25 Wm^{-2} UVA and 1.5 Wm^{-2} UVB) at 26 °C or 30 °C, **UVN26-UVN30**: corals maintained under UVR exposure and nitrate enrichment (3 μM nitrate) at 26 °C or 30 °C.

II.3.b. Physiological measurements

II.3.b.i. Photosynthesis parameters

Rates of net photosynthesis (Pn) and respiration (R) were estimated on 6 nubbins per condition (from different colonies, and 3 nubbins per aquarium). For this purpose, nubbins were placed in 60 mL Plexiglass chambers filled with 0.45 μm -filtered seawater, maintained at 26 °C or 30 °C, and stirred. Each chamber was equipped with an oxygen sensor (Polymere Optical Fiber, PreSens, Regensburg, Germany) connected to an Oxy-4 (Channel fiber-optic oxygen meter, PreSens, Regensburg, Germany). Oxygen concentration was recorded for 30 min with the Oxy4v2-30fb software, in the dark for R and at $200 \mu\text{mol photons.m}^{-2}.\text{s}^{-1}$ for Pn. Two calibrations were done at 0% O_2 with nitrogen saturated seawater and at 100% O_2 with air saturated seawater. The gross photosynthesis rate was obtained by adding the absolute value of the respiration rate to the corresponding net photosynthesis rate. This obtained gross photosynthesis rate is likely to be an underestimation of the actual gross photosynthesis rate (Schrammeyer *et al.*, 2014). At the end of the measurements, nubbins were frozen for the later

determination of the symbiont density, proteins and chlorophyll a and c₂ (Chl) content. Pn, Pg, and R rates were expressed as $\mu\text{mol O}_2\cdot\text{h}^{-1}\cdot\text{cm}^{-2}$. The surface area was obtained with the single dip wax technique (Stimson & Kinzie III, 1991).

II.3.b.ii. Symbiont density, chlorophyll and proteins content

Samples used in the previous measurements were thawed and the tissues were separated from the skeleton using a Water-Pick and 10-15 mL of filtered seawater. A 100 μL sub-sample was used for the determination of the symbiont density with a Z1 Coulter Particle Counter (Beckman Coulter, US). For each sample, five technical replicates were performed. Another 5 ml sub-sample was used for the chlorophyll content analysis. For this purpose, the sub-sample was centrifuged at 5530 g for 15 min at 4 °C to separate the animal tissue (supernatant) from the dinoflagellates (pellet). Then the supernatant was discarded, and the pellet resuspended in 5 ml of acetone 100%, to extract chlorophyll in the dark at 4 °C for 24 hours. Finally, the extract was centrifuged at 5530 g for 15 minutes at 15 °C. The absorbance of the supernatant was recorded at 630 nm, 663 nm and 750 nm using a UVmc² Spectrophotometer (Safas, Monaco). The concentration of chlorophyll a and c₂ was then calculated using the equations by Jeffrey and Humphrey (1975). Finally, 500 μL were used for the proteins content of the total holobiont and incubated for 5 hours at 60 °C in sodium hydroxide. Measurements were done with the Bicinchoninic acid (BCA) assay kit according to Smith (1985). All measurements were normalized to the skeletal surface area of the nubbins.

II.3.c. *Oxidative stress analysis*

48 nubbins – 6 per conditions (from different colonies and 3 per aquaria) – were snap-frozen in liquid nitrogen and kept at -80 °C for oxidative stress analysis. For each analysis, a small coral fragment of 5-10 mm was collected from the main fragment.

II.3.c.i. Reactive oxygen species (ROS) levels

The ROS level in the tissues was quantified using the fluorescent probe 5-(and-6)-carboxy-2',7'-dichlorofluorescein diacetate (H₂DCFDA, Molecular Probes) as described by Ruiz-Leal and George (2004), with some modifications. In presence of ROS, H₂DCFDA emits fluorescence quantified by spectrofluorometry. Coral fragments were fresh collected, sonicated on ice (Frequency 70 kHz, Vibra-Cell™, Bioblock Scientific, France) in a homogenizing buffer (pH 7.75) containing Tris-HCl 100 mM, EDTA 2 mM and MgCl₂.6H₂O 5 mM in MilliQ Water. After sonication, the holobiont homogenates were centrifuged at 10000 g for 10 min at 4 °C, and the protein content was quantified following the Bradford Protein Assay (Bradford, 1976). Samples were standardized to the protein concentration of 0.5 $\mu\text{g}\cdot\mu\text{L}^{-1}$ and 10 μL were then added in triplicates on a black 96-wells microplate with reaction buffer (pH 7.2) containing

HEPES 30 mM, KCl 200 mM and MgCl₂·6H₂O 1 mM in MilliQ water. Finally, 10 µL of H₂-DCFDA (16 µM in ethanol) was added in each well. The fluorescence (excitation: 488 nm; emission: 525 nm) was read every 5 minutes for up to 50 minutes using a spectrofluorometer (Xenius[®], SAFAS, Monaco). The area beneath the kinetic curve was considered the level of ROS in the sample. The results were expressed as fluorescence units per minute (F.U. x min).

II.3.c.ii. Lipid peroxidation (LPO)

LPO was assessed following the Thiobarbituric Acid Reactive Substance protocol (TBARS method), in line with Oakes and Van Der Kraak (2003). This method quantifies damage to lipids through the reaction between malondialdehyde (MDA), a byproduct of lipid peroxidation, and thiobarbituric acid (TBA). Coral fragments were sonicated (Frequency 70 kHz, Vibra-Cell[™], Bioblock Scientific, France) in a homogenizing buffer (KCl (1,15%) solution containing 35 µM butylatedhydroxytoluene (BHT)) on ice, centrifuged at 10000 g for 10 min at 4 °C, and the protein content was quantified following the Bradford Protein Assay (Bradford, 1976). Samples were standardized to the protein concentration of 0.35 µg·µL⁻¹ and incubated at 95 °C for 30 min in flat-bottomed Eppendorf with acetic acid solution (20%), thiobarbituric acid (TBA) solution (0.8%), SDS solution (8.1%). After cooling, 100 µl of MilliQ water and 500 µl of n-butanol were added with through vortexing. The tubes were then centrifuged at 10000 g for 10 minutes at 15 °C. Finally, 150 µl of the organic phase (supernatant) was placed, in duplicate, in 96-well black plates and the fluorescence was read at 553 nm and 515 nm each minute for up to three minutes using a spectrofluorometer (Xenius[®], SAFAS, Monaco). Results were normalized considering the total protein content in the sample homogenates in each well and expressed as nmol MDA.mg protein⁻¹, which were calculated from a standard curve built using hydrolysed tetramethoxypropane (TMP).

II.3.c.iii. Protein tyrosine nitration (PTN)

The level of 3-nitrotyrosine modified proteins in corals, a product of PTN resulting from oxidative damage to proteins by peroxyxynitrite, was assessed using the Nitrotyrosine ELISA Kit (ab113848, Abcam, Cambridge, UK) following manufacturer's instructions. The coral fragments were sonicated (Frequency 70 kHz, Vibra-Cell[™], Bioblock Scientific) in phosphate buffered saline (PBS) (pH 7.4) on ice, centrifuged at 10,000 g for 10 minutes at 4 °C. The protein content was quantified following the Bradford Protein Assay (Bradford, 1976). Protein content of each sample was standardized to 0.6 µg·µL⁻¹ and incubated on ice for 20 min in extraction buffer kit solution (1:1). Standards were made with 3-Nitrotyrosine-Bovine Serum Albumin (3-NT-BSA) (reconstituted in 1 mL of 1x Incubation Buffer kit solution). Samples and standards were added on a 96-well plate coated with an antibody specific for 3-

nitrotyrosine. The plate was wrapped in foil and left to incubate for two hours at room temperature, before each well was washed twice with Wash Buffer. Liquid excess was completely removed. Then a biotin labelled anti-3-nitrotyrosine detector antibody was added to each well and left to incubate for one hour before washing twice with Wash Buffer. Each well was then incubated for one hour with HorseRadish Peroxidase (HRP)-labeled kit solution and thoroughly washed three times with Wash Buffer. Finally, the HRP Development kit solution was added in each well and the absorbance was immediately read at 600 nm every minute up to 15 minutes. Results were normalized considering the total protein content in the sample homogenates in each well and expressed as $\text{ng 3NT-BSA.mg protein}^{-1}$.

II.3.c.iv. Non-enzymatic total antioxidant capacity (TAC)

The non-enzymatic total antioxidant capacity was assessed using the “OxiSelect Total Antioxydant Capacity (TAC) Assay Kit” (STA-360, Cell Biolabs Inc., San Diego USA) according to manufacturer’s instructions. This assay measures antioxidant capacity of biomolecules via single electron transfer (SET) mechanism. The coral fragments were sonicated (Frequency 70 kHz, Vibra-Cell™, Bioblock Scientific) in PBS (pH 7.4) on ice, centrifuged at 10000 g for 10 min at 4 °C, and the protein content was quantified following the Bradford protein assay (Bradford, 1976). Samples protein content were standardized to $0.9 \mu\text{g}.\mu\text{L}^{-1}$. Standards were made with a serial dilution of uric acid from 1mmol.L^{-1} to $0.00390 \text{mmol.L}^{-1}$. Samples and standard were placed on a 96-well plate with reaction buffer (Kit solution buffer and PBS). The absorbance was recorded at 490 nm using a spectrofluorometer (Xenius®, SAFAS, Monaco). Samples were compared with a known uric acid standard curve, with absorbance values being proportional to the sample’s total reductive capacity. Results were normalized considering the total protein content in the sample homogenates in each well and expressed as $\mu\text{M Copper Reducing Equivalents (CRE).mg protein}^{-1}$.

II.3.d. *Statistical analysis*

Statistical analyses were processed using the program RStudio Version 3.5.1. Data were checked for normality using the Shapiro-Wilk test and for homoskedasticity using Levene’s test on the residuals from the linear model (Edmunds & Gates, 2002). When the assumptions were not met, data were transformed with a log transformation or if necessary, a Box Cox transformation. Mixed effects models were used to test the effect of the three fixed factors: “Nutrient”, “UV” and “Temperature”, with the random factor “Aquarium” on the different physiological and oxidative stress parameters measured: holobiont protein biomass, dinoflagellates density, total chlorophyll a and c₂ content, net and gross photosynthesis rate per skeletal surface area and per symbiont cell, respiration rate per skeletal surface area and per

symbiont cell, ROS level, lipid peroxidation and protein nitration. The Mixed Effect model showed that the effect of the random factor “Aquarium” was negligible for each parameter tested; this random factor was, therefore, removed from the models and three-way ANOVA were used with the factors: “Nutrient”, “UV” and “Temperature”. When the homoskedasticity assumption was not met, a White-adjusted ANOVA was run, using a heteroskedasticity-consistent covariance matrix estimator (White, 1980). Analyses were followed by a pairwise comparison test when factors effect was significant. Differences were assumed significant when $p < 0.05$.

II.4. Results

II.4.a. Effect of UVR exposure and nitrate enrichment at normal temperature (26 °C)

The result of the statistical tests are reported in the supplementary Appendix I in Table S1, for physiological parameters, Table S2, S3 for photosynthesis parameters normalized to skeletal surface area and to symbiont cell and Table S4, for oxidative stress parameters.

At 26 °C, no significant effect of UVR exposure and/or nitrate enrichment was observed on the tissue parameters (Fig 13a, b, c) compared to the control (C26). No significant change was observed on the respiration rates per skeletal surface area (ANOVA, $p > 0.91$, Fig 14c) or per symbiont cell (ANOVA, $p > 0.27$, Fig S1c, Appendix I). However, there was a 80% decrease in net photosynthesis, in the N26 condition compared to C26 condition, when photosynthesis was expressed both per skeletal surface area (pairwise comparison, $p < 0.004$, Fig 14a) or symbiont cell (pairwise comparison, $p < 0.0095$, Fig S1a, Appendix I). However, net photosynthesis increased by 60% in the combined nitrate + UVR (UVN26), compared to the N26 condition (pairwise comparison - per surface: $p < 0.003$, Fig 14a - per symbiont: $p < 0.0004$, Fig S1a, Appendix I). Gross photosynthesis rates per skeletal surface area, also, increased under UVR exposure (ANOVA, $p < 0.006$, Fig 14b). Finally, the non-enzymatic total antioxidant capacity (TAC) significantly increased in all UVR condition (ANOVA, $p < 0.0001$, Fig 15a) as well as the protein nitration in the UVN26 compared to N26 condition (pairwise comparison, $p < 0.004$, Fig 15b).

II.4.b. Effect of UVR exposure and nitrate enrichment under thermal stress (30 °C)

The results of the statistical tests are reported in the supplementary Appendix I in Table S1, for physiological parameters, Table S2, S3 for photosynthesis parameters normalized per skeletal

surface area and per symbiont cell and Table S4, for oxidative stress parameters. The linear models (ANOVA) indicate that thermal stress had both independent and combined effects (with UVR exposure and nutrient enrichment) on coral physiology.

Compared to the same conditions at 26°C, thermal stress alone drove a significant decrease in symbiont density in all conditions (ANOVA, $p < 0.0001$, Fig 13b). It also induced, an increase in Pg (ANOVA, $p < 0.0001$, Fig S1b, Appendix I), in respiration rates per symbiont (ANOVA, $p < 0.0001$, Fig S1c, Appendix I), and an increase in ROS levels (ANOVA, $p < 0.03$, Fig 15c), and in lipid peroxidation damages (ANOVA, $p < 0.00011$, Fig 15d). In addition, temperature interacted with nitrate enrichment, since the Pn rates per symbiont (White-adjust ANOVA, $p < 0.04$) were lower in the N30 condition compared to the N26 condition (pairwise comparison, $p < 0.0004$, Fig S1a, Appendix I).

Temperature also interacted with UVR exposure for the protein nitration level, which is higher in the UV30 than in UV26 conditions (pairwise comparison, $p < 0.02$, Fig 15b, T. S4b).

UVR exposure increased the antioxidant capacity of corals in all conditions (ANOVA, $p < 0.0001$, Fig 15a). In addition, at high temperature (30°C), UVR exposure led to an increased the protein biomass in coral tissue (pairwise comparison, C30/UV30 - N30/UVN30, $p < 0.047$, Fig 13a) and in total chlorophyll a and c₂ content (ANOVA, $p < 0.045$, Fig 13c) compared to corals non exposed to UVR. It also increased the Pg normalized to skeletal surface area (ANOVA, $p < 0.006$, Fig 14b) and symbiont cell (pairwise comparison, C30/UV30 - N30/UVN30, $p < 0.024$, Fig S1b, Appendix I). Finally, the level of protein nitration in the UV30 was significantly higher than in the C30 condition (pairwise comparison, $p < 0.035$, Fig 15b). The interaction between nitrate and UVR exposure impacted ROS levels in coral tissue (ANOVA, $p < 0.012$), which significantly increased in the UVN30 compared to N30 (pairwise comparison, $p < 0.0013$, Fig 15c).

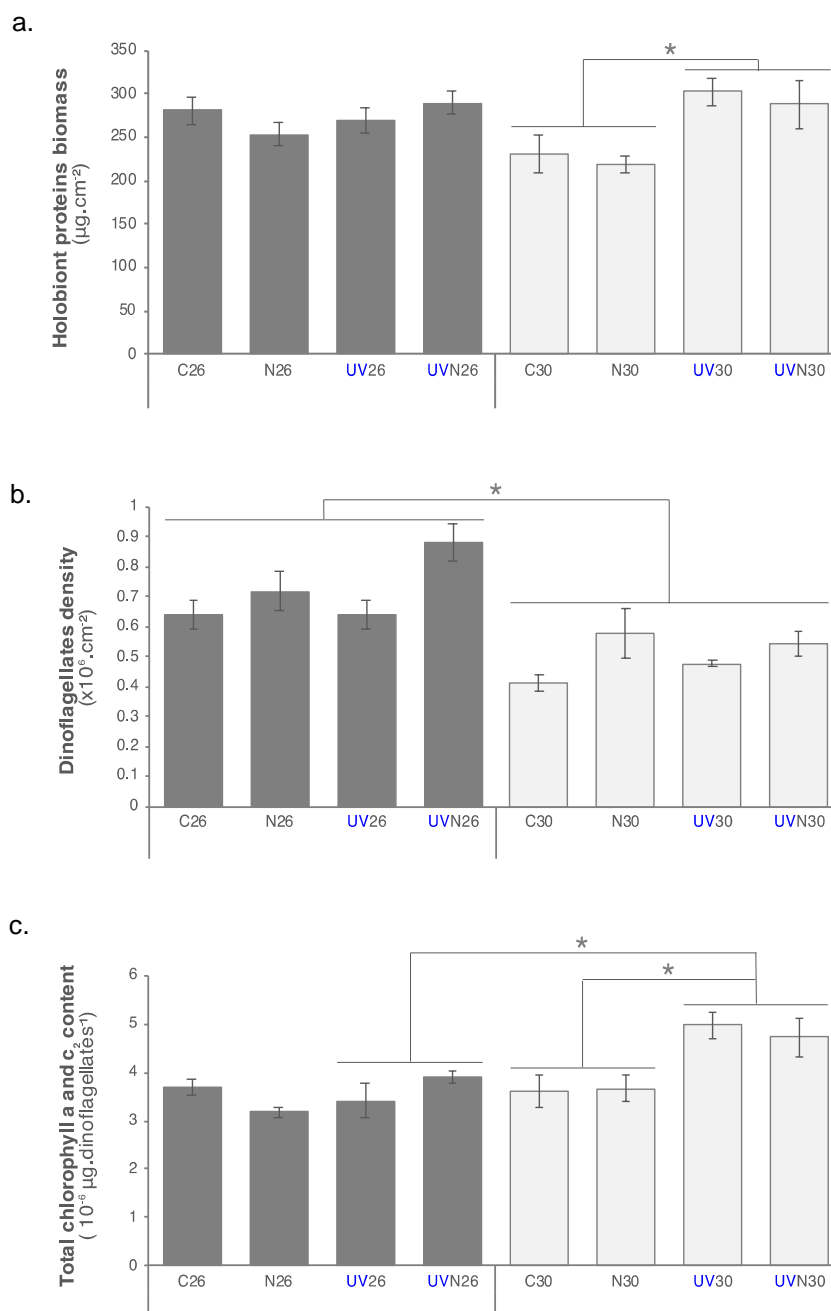


Figure 13. Effects of temperature, UVR and nitrate levels on the physiological parameters of the coral *Pocillopora damicornis*

The barplots represent proteins biomass normalized by surface area ($\mu\text{g}\cdot\text{cm}^{-2}$) (a.), dinoflagellates density normalized by surface area ($10^6\cdot\text{cm}^{-2}$) (b.) and total chlorophyll a and c₂ content normalized by dinoflagellate cell ($10^{-6}\cdot\mu\text{g}\cdot\text{dinoflagellates}^{-1}$) (c.), under different nitrate (N)-UVR conditions. Dark grey bars represent corals maintained at 26°C (26) while light grey bars are corals maintained at 30°C (30) - **C26-C30**: control corals maintained at 26°C or 30°C without nitrate enrichment and UVR exposure, **N26-N30**: corals maintained under nitrate enrichment (3 μM nitrate) at 26 °C or 30°C, **UV26-UV30**: corals exposed to UVR (25 Wm^{-2} UVA and 1.5 Wm^{-2} UVB) at 26 °C or 30°C, **UVN26-UVN30**: corals maintained under UVR exposure and nitrate enrichment (3 μM nitrate) at 26 °C or 30°C.

Data represent mean and standard error of 6 replicates. Stars represent significantly different values (p value < 0.05).

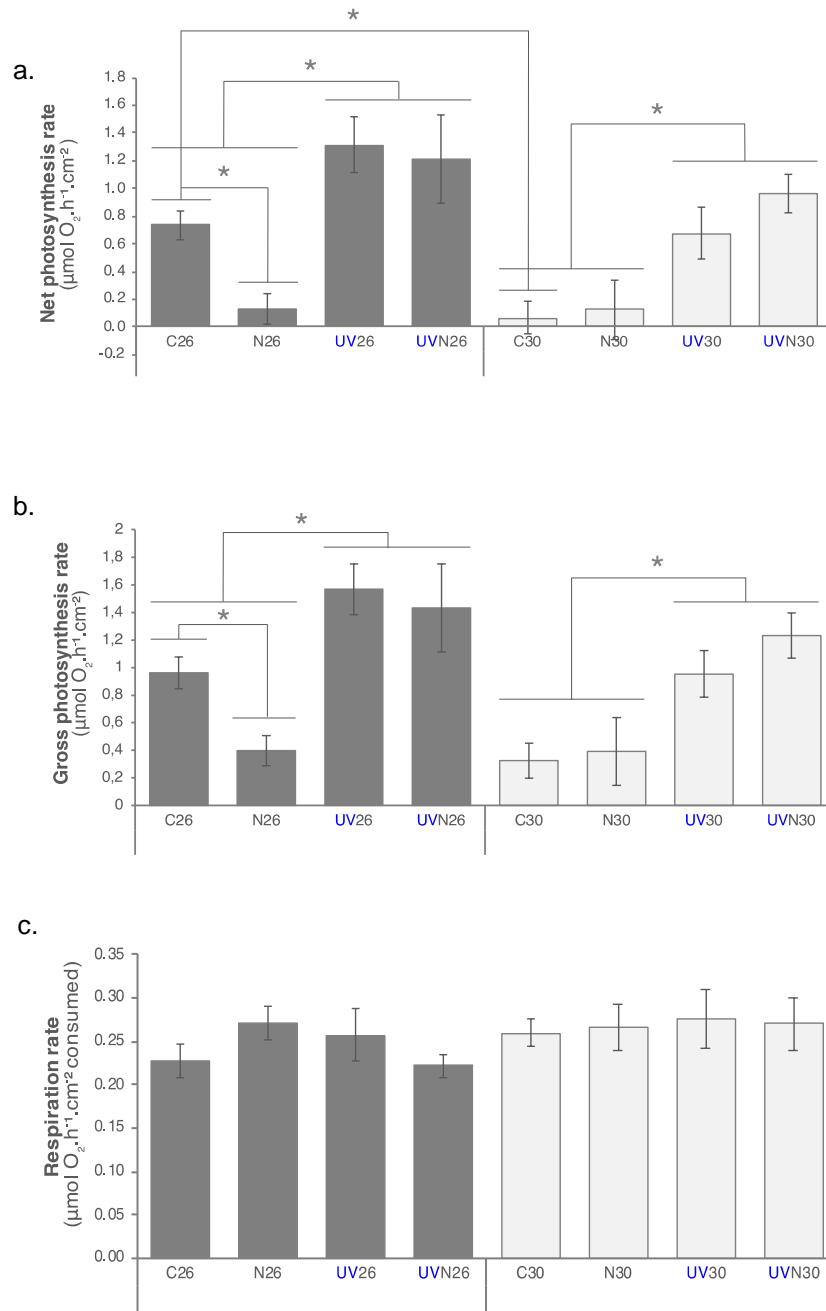


Figure 14. Effects of temperature, UVR and nitrate levels on the oxygen fluxes (per skeletal surface area) measured for the coral *Pocillopora damicornis* maintained under different environmental conditions

The barplots represent net (a.) and gross (b.) photosynthesis rates and respiration (c.) rates ($\mu\text{mol O}_2 \cdot \text{h}^{-1} \cdot \text{cm}^{-2}$) normalized per surface area under different nitrate (N)-UVR conditions. Dark grey bars represent corals maintained at 26°C (26) while light grey bars are corals maintained at 30°C (30) - **C26-C30**: control corals maintained at 26°C or 30°C without nitrate enrichment and UVR exposure, **N26-N30**: corals maintained under nitrate enrichment (3 μM nitrate) at 26°C or 30°C, **UV26-UV30**: corals exposed to UVR (25 Wm^{-2} UVA and 1.5 Wm^{-2} UVB) at 26°C or 30°C, **UVN26-UVN30**: corals maintained under UVR exposure and nitrate enrichment (3 μM nitrate) at 26°C or 30°C. Data represent mean and standard error of 6 replicates. Stars represent significantly different values (p value < 0.05).

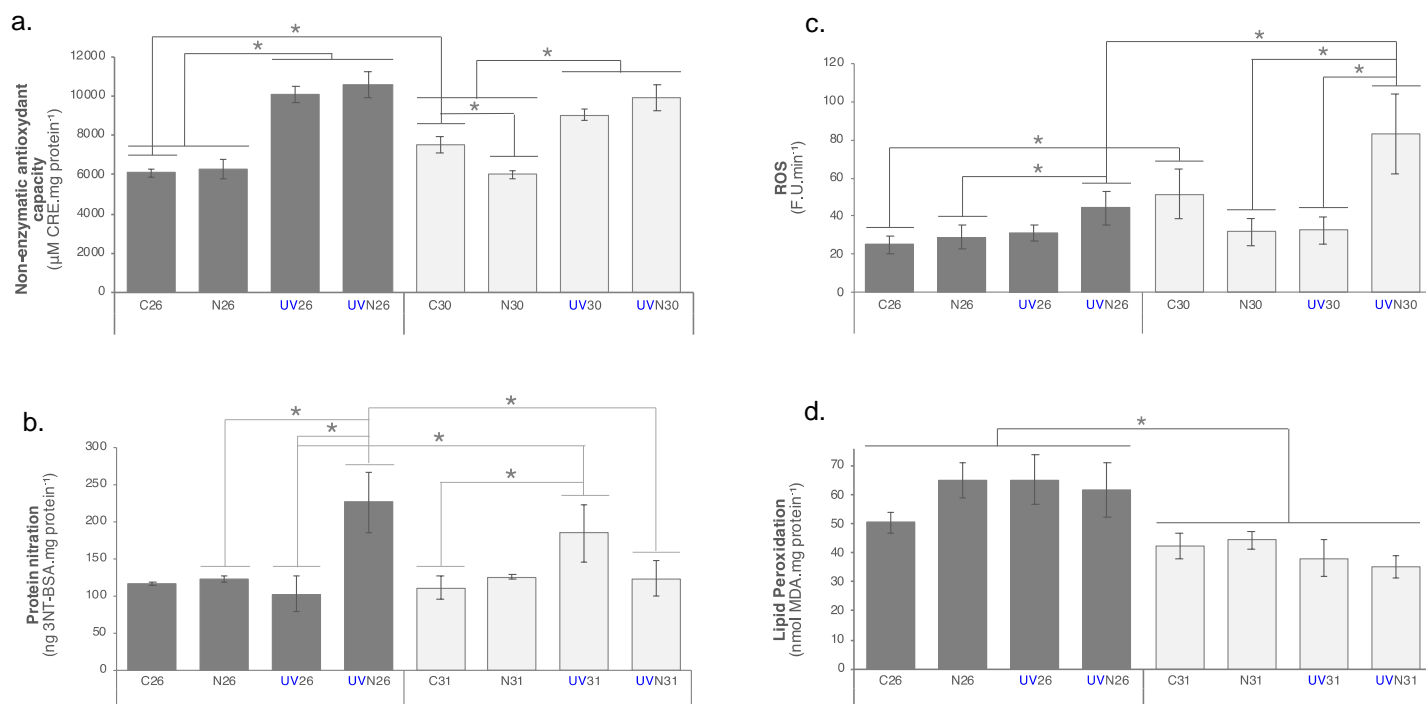


Figure 15. Effects of temperature, UVR and nitrate levels on the oxidative stress parameters of the coral holobiont *Pocillopora damicornis*

The barplots represent the non-enzymatic antioxidant capacity ($\mu\text{M CRE}\cdot\text{mg protein}^{-1}$) (a.), protein nitration level ($\text{ng } 3\text{NT-BSA}\cdot\text{mg protein}^{-1}$) (b.), reactive oxygen species levels ($\text{F}\cdot\text{U}\cdot\text{min}^{-1}$) (c.) and lipid peroxidation level ($\text{nmol MDA}\cdot\text{mg protein}^{-1}$) (d.) under different nitrate (N)-UVR conditions. Dark grey bars represent corals maintained at 26°C (26) while light grey bars are corals maintained at 30°C (30) - **C26-C30**: control corals maintained at 26°C or 30°C without nitrate enrichment and UVR exposure, **N26-N30**: corals maintained under nitrate enrichment (3 μM nitrate) at 26°C or 30°C, **UV26-UV30**: corals exposed to UVR (25 Wm^{-2} UVA and 1.5 Wm^{-2} UVB) at 26°C or 30°C, **UVN26-UVN30**: corals maintained under UVR exposure and nitrate enrichment (3 μM nitrate) at 26°C or 30°C. Data represent mean and standard error of six replicates. Stars represent significantly different values (p value < 0.05).

II.5. Discussion

Reef ecosystems are under increasing pressure from interacting multiple stressors (e.g. rising temperatures, and excessive irradiance and nutrient levels). Stressors can have synergistic, antagonistic or additive effects on coral physiology, but these interactions are, however, not well understood (Chumun *et al.*, 2013; Higuchi *et al.*, 2015). By quantifying the combined effects of UVR exposure, nitrate enrichment and thermal stress on the physiology and the oxidative stress response of the widespread scleractinian coral *Pocillopora damicornis*, this study brings a deeper understanding of the response of the coral symbioses to multiple environmental changes. Specifically, our results demonstrate that low UVR intensities (and low doses) can mitigate the negative effects of thermal stress by enhancing the antioxidant capacity of coral colonies. In addition, low UVR intensities can prevent the negative effects of nitrate on symbiont photosynthesis, most likely, by regulating the complex links between carbon and nitrogen metabolism.

II.5.a. Nitrate enrichment without UVR exposure impaired symbiont photosynthesis

Nitrate enrichment in absence of UVR exposure induced a significant decrease in net photosynthesis (normalized to surface area or symbiont cell) of *P. damicornis*. Nitrate-induced decrease in carbon fixation or photosynthetic efficiency has already been observed in several coral species (Nordemar *et al.*, 2003; Chumun *et al.*, 2013; Ezzat *et al.*, 2015; Courtial *et al.*, 2017). This was coupled with a decline in the amount of photosynthates transferred from the symbionts to the host tissue (Ezzat *et al.*, 2015). A bleaching with nitrate enrichment has sometimes been observed (Burkepile *et al.*, 2020) and could explain the lower rates of photosynthesis observed here with the nitrate-enriched corals. However, this is unlikely as no significant reduction in symbiont density and/or chlorophyll a and c_2 content occurred. The deleterious effects of nitrate on coral physiology may also be due to an imbalanced nitrogen to phosphorus ratio (N:P ratio). In this case, symbiont growth is promoted by an increase in nitrogen availability, but the lack of phosphorus weakens the lipid membranes of the symbionts, especially during thermal stress (Wiedenmann *et al.*, 2013; Ezzat *et al.*, 2016a; Rosset *et al.*, 2017). Although this might happen under an important phosphorus deficiency, here, phosphorus concentrations were around 0.2 μM and symbiont growth was not enhanced by nitrogen supplementation. In addition, corals under UVR exposure and nitrate enrichment (UVN26) did not decrease their photosynthetic rates. All together, these observations suggest that the N:P ratio was not imbalanced in our study and has thus not driven the decrease in photosynthesis observed in both the N26 and N30 conditions.

The photosynthesis impairment under nitrate enrichment is likely due to a competition, between carbon and nitrogen assimilation for ATP and reductants generated by photosynthetic electron transport (Nunes-Nesi *et al.*, 2010). Conversion of one nitrate molecule into ammonium indeed requires ATP and consumes one NADPH and six reduced ferredoxins. Therefore, in plants, 25% of the ATP and NADPH produced from light energy by the electron transfer complex is used for nitrate assimilation, and only 75% remains available for carbon fixation (Bloom, 2015). In plants, when light is limited, or when nitrogen metabolism is not paired with carbon metabolism, nitrate assimilation and carbon dioxide fixation will directly compete for both ATP and reductants generated by the photosynthetic electron transport (Nunes-Nesi *et al.*, 2010). In this case, nitrate can cause a reduction in carbon assimilation rates. It can also disrupt the C:N balance and alter essential metabolites (Saiz-Fernández *et al.*, 2017). In turn, shortage of carbon skeletons may delay the assimilation of nitrate into amino acids (Bloom, 2015). This can cause an oxidative stress condition, by an accumulation of nitrite

inside the coral tissue. Although in this experiment there was no significant increase in ROS under nitrate enrichment, there was a slight increase in LPO, a proxy for cellular damage (Gutteridge, 1995). This suggests that corals might have experienced a slight damage from nitrate exposure, possibly through the generation of nitric oxide (Lundberg *et al.*, 2008). In addition, TAC values decreased in the N30 treatment compared to control (C26), further indicating an oxidative imbalance. Overall, competition between carbon and nitrogen for both ATP and reductants is the most plausible explanation for the decrease in photosynthesis observed in the coral nubbins (Fig 16). Any increase in carbon fixation might therefore reduce the nitrate effect on photosynthesis, as observed with corals exposed to low doses of UVR (see below).

II.5.b. Low doses of UVR are beneficial to corals, both under thermal stress or nitrate enrichment

In shallow reefs, corals receive high levels of UVR. However, they synthesize UV-absorbing compounds such as mycosporine-like amino acids (MAAs) which partially protect them from UVR (Shick *et al.* 1999). Some of these MAAs can also act as antioxidant compounds (Yakovleva *et al.*, 2004; Rosic & Dove, 2011). In this experiment, exposure of *P. damicornis* to UVR increased TAC in coral tissue at 30 °C, which likely helped avoid an accumulation of ROS and, thus, a potential damage to biomolecules (as shown by LPO). On the contrary, corals which were not exposed to UVR, experienced decreased TAC level at 30°C and significant bleaching. Alternatively, UVR might have also protected corals from photoinhibition during exposure to high temperature and light. It has indeed been shown that UVR trigger photoreceptors that control extraction-contraction of coral polyps (Ben-Zvi *et al.*, 2019). Contraction of the polyps under UVR may have prevented photodamage and bleaching in the thermal stressed corals.

In this experiment, UVR exposure also significantly enhanced photosynthesis at elevated temperature notably through an increased chlorophyll level in symbiont cells. A similar effect had previously been observed, with UVA, on the photosynthetic performances of either free algae (Gao *et al.*, 2007; Gao & Xu, 2008; Xu & Gao, 2009) or of Symbiodiniacea in symbiosis with the larvae of *Pocillopora damicornis* and *Seriatopora calendrum* (Zhou *et al.*, 2016, 2017). Such positive effect of UVA on photosynthesis might be due to the fact that the activity of the carbonic anhydrase (which transports inorganic carbon used for photosynthesis) is enhanced by UVA radiation. Enhanced photosynthesis was even recorded in free algae when exposed to UVB (Chen *et al.*, 2020), which were shown to protect them against photoinhibition (Hanelt *et al.*, 2006). The positive effect of UVR on coral photosynthesis contradicts the usual

observation of increased bleaching under UVR exposure and thermal stress (Lesser & Farrell, 2004). However, bleaching is mainly observed under high UVR doses. On the contrary, the daily dose of UVR used in this study is lower and corresponds to that received by coral colonies around 15 m depth (Barron *et al.*, 2009; Overmans & Agustí, 2020; Rosic *et al.*, 2020). Taken all together, these observations suggest that the effect of UVR on coral physiology is dose-dependent and that low levels of UVR may enhance coral photosynthesis by increasing cellular chlorophyll content or enhancing the carbonic anhydrase activity.

In addition to its photoprotective role at a high temperature, low doses of UVR protect symbionts against the negative effect nitrate has on photosynthesis. Indeed, the impairment of coral photosynthesis under nitrate enrichment was not observed when corals received UVR. Many studies have shown the effect of nutrient enrichment or depletion on the UV-induced decrease in photosynthesis (Rojo *et al.*, 2019). However, this is the first time the contrary is observed - the alleviation of nitrate-induced decrease in photosynthesis by UVR. In macroalgae, activities of both nitrate reductase (involved in nitrate assimilation) and carbonic anhydrases (involved in carbon fixation) are stimulated by exposure to UVR (Kumar *et al.*, 1996; Viñepla *et al.*, 2006). They show a peak in the evening, whereas the peak is delayed without UVR. In addition, Figueroa & Viñepla (2001) observed in two marine algae that UVR acts as an environmental signal involved in the control of carbon and nitrogen cycles, and regulates feedback processes that control N assimilation as a function of carbon content. Therefore, in corals, UVR may have prevented the nitrate-induced inhibition of photosynthesis by regulating the complex links between carbon and nitrogen metabolism (Fig 16). In addition, since UVR promotes the synthesis of MAAs, rich in nitrogen (Peinado *et al.*, 2004; Korbee *et al.*, 2005; Shick *et al.*, 2005; Zheng & Gao, 2009), it might have also stimulated the reduction of nitrate into ammonium and its subsequent incorporation into MAAs.

II.6. Conclusion

In conclusion, this multifactorial study shows that light conditions, and specifically UVR levels may influence the sensitivity of coral species to thermal stress and nutrient pollution. We, indeed, demonstrated that UVR plays an important role in mitigating the effects of thermal stress and nitrate enrichment on coral photosynthesis. However, since UVR can also be detrimental to coral's photosynthesis under high doses, future studies should aim at investigating the dose-effect response of corals to UVR exposure. Overall, our results indicate that UVR may be a critical factor, which not only affects the distribution of corals, but also their response to environmental stress.

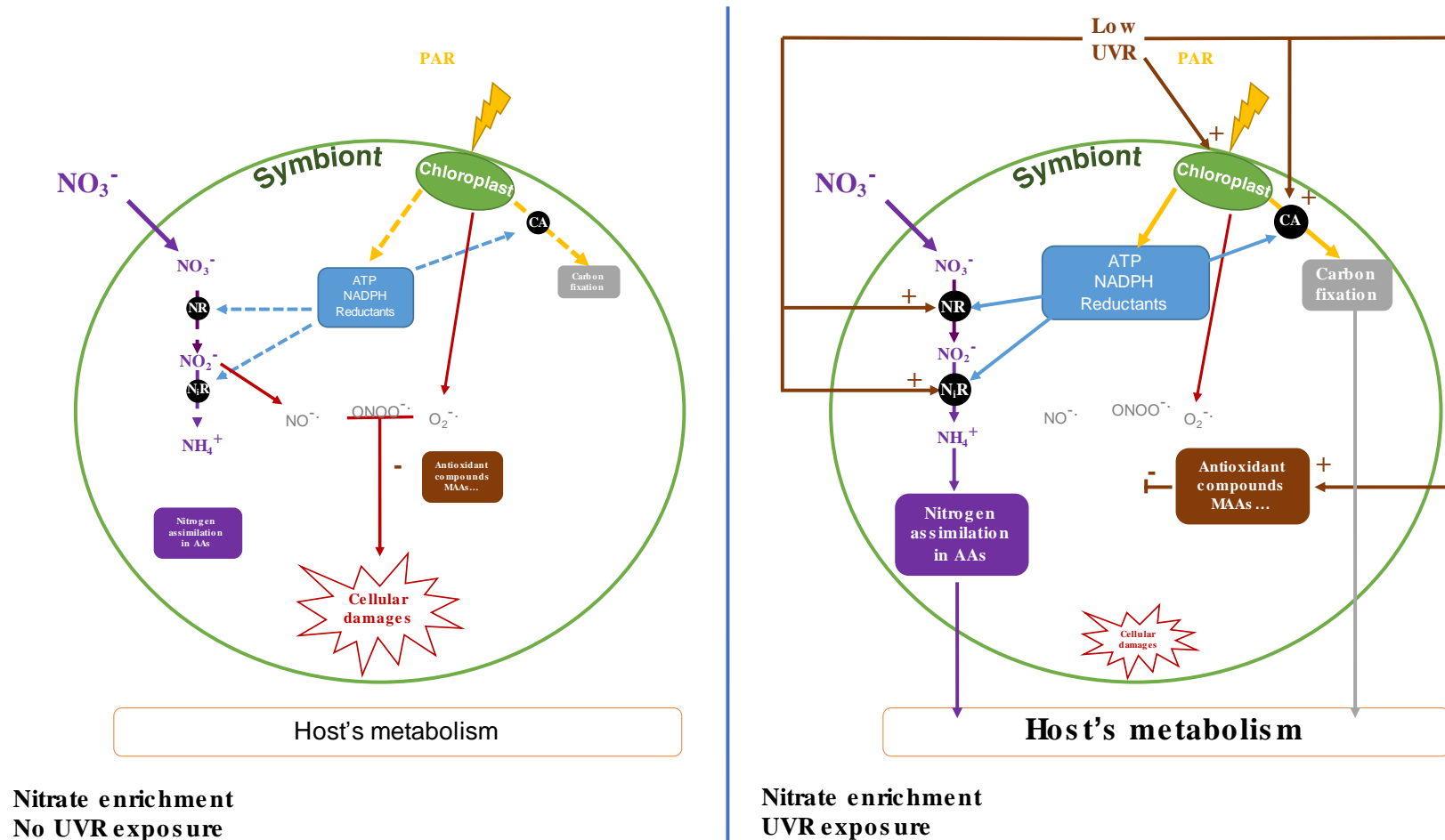


Figure 16. Summary of the main results obtained with nitrate-enriched corals with or without exposure to ultraviolet radiation
 The figure briefly summarizes the results obtained on the effect of nitrate enrichment on the dinoflagellate symbionts of the scleractinian coral *Pocillopora damicornis* in absence of ultraviolet radiation (UVR) (left panel) and with exposure to low UVR levels (right panel). Dashed arrows represents reduced processes while plain arrows (and increased text-box size) represent UVR-enhanced processes.
PAR: photosynthetically active radiation; **NR** and **NiR:** nitrate and nitrite reductase respectively; **NO₃⁻**, **NO₂⁻** and **NH₄⁺**: nitrate, nitrite and ammonium respectively, **NO⁻** and **ONOO⁻** are the reactive species of nitrogen: nitric oxide and peroxynitrite; **O₂⁻** is a reactive species of oxygen: superoxide anion; **CA:** carbonic anhydrase. **MAAs** and **AAs:** Mycosporine-like amino acids and amino acids respectively; **ATP** and **NADPH:** Adenosine triphosphate and Nicotinamide adenine dinucleotide phosphate
 Carbon fixation and the reduction of nitrate into ammonium require ATP and other energetic molecules produced by photosynthesis. Low UVR exposure enhances photosynthesis and the production of energetic molecules (especially at 30°C), by increasing chlorophyll content. It is also known to stimulate the signaling pathway of the NR, NiR and CA, enhancing their activity. Therefore, nitrate reduction and carbon assimilation are enhanced in presence of low UVR levels. Finally, UVR stimulates the synthesis of antioxidant compounds such as MAAs, which offer a protection against oxidative stress.

CHAPTER 2:
SPECIES SPECIFIC RESPONSE TO
BALANCED AND IMBALANCED
DIN:DIP RATIOS



CHAPTER 2: SPECIES SPECIFIC RESPONSES TO BALANCED AND IMBALANCED DIN:DIP RATIOS

In the first chapter, we found that exposure of a scleractinian coral to nitrate enrichment alone (imbalanced DIN:DIP ratio) significantly decreased rates of net and gross photosynthesis, both under normal, controlled, conditions and under thermal stress when corals were not exposed to UV radiation. However, no deleterious effects of imbalanced DIN:DIP ratio were observed under a low dose of UVR, which can be attributed to a positive effect of UVR on coral physiology. These results have partially helped to understand the contradictions observed in the response of corals to an imbalanced DIN:DIP ratio. Other factors however induce a different response of corals to nutrients.

I Overview of the article

The response of corals to an imbalanced DIN:DIP ratio may also be due to changes in the elemental stoichiometry (C:N:P) of coral tissue. Symbiodiniaceae are in fact very sensitive to a change in nutrient availability in seawater. Their uptake rates of DIN and DIP generally increase proportionally to the nutrient concentrations in seawater (Grover et al., 2002, 2003). At high DIN or DIP concentrations, there is therefore a strong influx of nutrients within the symbiosis, which can disrupt cellular stoichiometry in coral tissue.

The aim of this study was therefore to determine how the DIN:DIP ratio and the nutrient availability alter the physiology and the elemental stoichiometry of coral tissue and symbionts. We maintained nubbins of the octocoral *Sarcophyton glaucum* and the scleractinian *Turbinaria reniformis* under balanced or imbalanced DIN:DIP ratios for five weeks. The different conditions were achieved with natural seawater (balanced DIN:DIP ratio: 2.5) or by enrichment with either DIP at 1 μ M (imbalanced DIN:DIP ratio: 0.4), DIN at 3 μ M (imbalanced DIN:DIP ratio: 17.5) or a combination of DIN and DIP (balanced DIN:DIP: 2.9). After five weeks, nubbins were sampled and various parameters were assessed: symbiont density, protein content, total chlorophyll a and c₂ content, photosynthetic rates, photosystem efficiency, lipid and carbohydrate content, and elemental composition with carbon, nitrogen and phosphorus content in the coral holobiont.

This study addressed two questions:

- i) What are the effects of balanced and imbalanced DIN:DIP ratios on the nutrient uptake rates, physiology and C:N:P stoichiometry of the tissues and symbionts of two understudied corals: the octocoral *Sarcophyton glaucum* and the scleractinian coral *Turbinaria reniformis*?
- ii) Do scleractinian corals and octocorals respond in the same way to changes in the DIN:DIP ratio?

We observed significant differences in the response of scleractinian and octocoral species to a balanced and imbalanced DIN:DIP ratio, mainly due to a species-specific difference in the uptake rates of nutrients.

T. reniformis display high nutrient uptake rates, which also increased with nutrient availability in seawater. Thus, an imbalance DIN:DIP ratio, via DIN enrichment with nitrate alone led to a large influx of nitrogen in the holobiont and an increase in the cellular N:P ratio. This increase in cellular N:P ratio may eventually lead to phosphorus limitation in the coral and affect its physiology. Here, we observed a decrease in the photosynthetic capacities of the coral under DIN:DIP ratio, similar to what has been previously observed in other scleractinian corals (Nordemar *et al.*, 2003; Wiedenmann *et al.*, 2013; Ezzat *et al.*, 2015; Marangoni *et al.*, 2020). However, a balanced DIN:DIP ratio with high nutrient content leads to balanced nutrient uptake, maintaining a balance in the cellular elemental composition of the coral and preventing the induction of phosphorus limitation in the coral. Indeed, we showed that a balanced DIN:DIP ratio with high nutrients helped maintain the photosynthetic abilities of the coral and even enhanced its metabolism through increased lipid and carbohydrate content.

In contrast, the nutrient uptake rate of *S. glaucum*, was relatively low. Furthermore, nutrient uptake is only increased when the DIN:DIP ratio was balanced, preventing an unilateral massive influx of nutrients in the octocoral. The limited and balanced supply of nutrients both at a balanced and imbalanced DIN:DIP ratio, has maintained the elemental composition and stoichiometry of the octocoral. As a result, the physiology of *S. glaucum* was not affected by changes in the DIN:DIP ratio of seawater. These results may explain why octocorals are less affected by reef nutrification than scleractinian corals (Schleyer & Celliers, 2003; Baum *et al.*, 2016).

In conclusion, this study found that the response of corals to changes in the DIN:DIP ratio is species-specific, with octocorals appearing to be less affected than scleractinian corals. Furthermore, we found that the coral response to balanced and imbalanced DIN:DIP ratios depends on changes in the elemental composition and stoichiometry of corals, with imbalanced

cellular N:P ratio adversely affecting coral physiology. The results of this study have helped to understand how coral species respond to nutrient enrichment. They also demonstrate the importance of investigating cellular stoichiometry when assessing how corals respond to a balanced and imbalanced DIN:DIP ratio.

II Publication

Species-specific response of corals to imbalanced ratios of inorganic nutrients

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II.1. Abstract

Dissolved inorganic phosphorus (DIP) is a limiting nutrient in the physiology of scleractinian corals. Anthropogenic addition of dissolved inorganic nitrogen (DIN) to coastal reefs increases the seawater DIN:DIP ratio and further increases P limitation, which is detrimental to coral health. The effects of imbalanced DIN:DIP ratios on coral physiology require further investigation in coral species other than the most studied branching corals. Here we investigated the nutrient uptake rates, elemental tissue composition and physiology of a foliose stony coral, *Turbinaria reniformis*, and a soft coral, *Sarcophyton glaucum*, exposed to four different DIN: DIP ratios (0.5:0.2, 0.5:1, 3:0.2, 3:1). The results show that *T. reniformis* had high uptake rates of DIN and DIP, proportional to the seawater nutrient concentrations. DIN enrichment alone led to an increase in tissue N content, shifting the tissue N:P ratio towards P limitation. However, *S. glaucum* had 5 times lower uptake rates and only took up DIN when the seawater was simultaneously enriched with DIP. This double uptake of N and P did not alter tissue stoichiometry. This study allows us to better understand the susceptibility of corals to changes in DIN:DIP ratio and predict how coral species will respond under eutrophic conditions in the reef.

II.2. Introduction

Coral reefs are one of the most diverse and productive ecosystems in the world even though they thrive in oligotrophic waters, that have low levels of dissolved inorganic nitrogen (DIN, $< 0.5 \mu\text{M}$) and phosphorus (DIP, $< 0.2 \mu\text{M}$). This paradox, commonly referred to as the “Darwin Paradox” (Darwin, 1842), is explained by a highly efficient nutrient cycling between corals, which are the building blocks of the reefs, and their symbiotic micro-organisms, including Symbiodiniaceae dinoflagellates (Muscatine & Porter, 1977; Rådecker *et al.*, 2015). Dinoflagellates metabolize DIN and DIP from seawater or from the host’s metabolic waste products into organic nutrients which are then translocated to the coral host to meet most of its energy needs (Ferrier-Pagès *et al.*, 2016). In turn, the host captures planktonic prey and shares this nutrient source with its dinoflagellates (Tremblay *et al.*, 2014). Finally, other coral-associated microbes, such as bacteria and fungi, can potentially be involved in carbon fixation, nitrogen metabolism and sulfur cycling, among many other functions (Rådecker *et al.*, 2015).

The close nutritional relationship between corals and Symbiodiniaceae responds strongly to the nutrient environment. On the one hand, nutrient deficiencies, especially in phosphorus which is often a limiting nutrient in reef waters (D’Angelo & Wiedenmann, 2014; Larned *et al.*, 1998), impairs coral health and exacerbates coral bleaching (i.e., the loss of symbionts from coral host tissues) under heat stress conditions (Ezzat *et al.*, 2016a; Ezzat *et al.*, 2019; Rosset *et al.*, 2017). On the other hand, experimental exposure to elevated seawater nutrient concentrations, such as those found in reefs exposed to agricultural and urban wastes (Fabricius, 2005; 2011), may also affect coral physiology (Table S1, Appendix II). Studies have shown that calcification rates, skeletal density, photosynthate fluxes, or reproductive success are reduced under nitrate (Ezzat *et al.*, 2015; Liu *et al.*, 2020; Marangoni *et al.*, 2020; Marubini & Davies, 1996; Nordemar *et al.*, 2003; Renegar & Riegl, 2005; Wiedenmann *et al.*, 2013) or phosphate enrichment (Renegar & Riegl, 2005; Ferrier-Pagès *et al.*, 2000; Harrison & Ward, 2001; Koop *et al.*, 2001). However, this impairment in coral physiology under high concentrations in dissolved inorganic nutrients is not systematic, as in situ and laboratory observations have also reported healthy corals exposed to high nutrient concentrations (Ezzat *et al.*, 2015; 2016b; Lirman & Fong, 2007, Liu *et al.*, 2020, Sawall *et al.*, 2011). In some cases, nutrient enrichment may even improve coral growth or reduce their susceptibility to bleaching (Atkinson *et al.*, 1995; Chauvin *et al.*, 2011; Ezzat *et al.*, 2015; Hoadley *et al.*, 2016; Marangoni *et al.*, 2020; Meyer & Schultz, 1985; Tanaka *et al.*, 2007; 2014; Wiedenmann *et al.*, 2013). Wiedenmann *et al.* (2013) was the first to suggest that it is not the nutrient concentration per

se, but rather an imbalanced DIN:DIP ratio that is detrimental to coral physiology. DIN:DIP ratios are considered balanced between 3 and 7, which are the ratios commonly recorded in pristine reef (Rosset *et al.*, 2017; Furnas *et al.*, 1995). Significant deviations from these values might indicate potential nutrient limitation, with low DIN:DIP ratios suggesting nitrogen limitation and high DIN:DIP ratios suggesting phosphorus limitation.

The observed physiological changes of corals exposed to imbalanced DIN:DIP ratios are not well explained. A high DIN:DIP ratio (nitrogen enrichment) was shown to enhance symbiont growth and induce phosphorus starvation. In turn, phosphorus deficiency reduces the photosynthetic capacity of corals and can impair the structure of phospholipid membranes of the symbionts, lowering temperature thresholds for coral bleaching (D'Angelo & Wiedenmann, 2014; Ezzat *et al.*, 2016; Morris *et al.*, 2019; Rosset *et al.*, 2017). Therefore, imbalanced DIN:DIP ratios can induce significant changes in the stoichiometry (Carbon (C):N:P) of coral tissue, itself dependent on DIN and DIP availability in seawater, as observed in phytoplankton (Sauterey & Ward, 2022, Zhou *et al.*, 2021). Because P is essential for ribosomal RNA and N is a major component of nucleic acids and proteins, coral growth and health are directly related to tissue N and P content (Blanckaert *et al.*, 2020; Lovelock *et al.*, 2014). Measuring elemental stoichiometry of coral tissues and symbionts may therefore be used to determine nutrient limitation and assess how the seawater DIN:DIP ratio might alter coral physiology. However, to date, changes in coral tissue composition with varying DIN:DIP ratios have been poorly studied. Of the 47 studies reported in in Table S1 (Appendix II) on the effects of nutrient enrichment and imbalanced DIN:DIP ratios on coral physiology, only four studies have measured the C:N content of coral tissue and symbionts under N enrichment (ammonium: Muscatine *et al.* 1989; Snidvongs and Kinzie 1994; McCauley and Goulet 2019; and nitrate: Tanaka *et al.* 2017) and three under combined N and P enrichment (Tanaka *et al.* 2007, 2010, 2017) (Table S2, Appendix II). The N:P or C:P ratio of coral tissue and symbionts was not considered, although P can be an important limiting compound for coral physiology (Ezzat *et al.*, 2016a). In addition, most studies focused on branching coral species (*Acropora*, *Stylophora*, *Pocillopora*) while other coral morphologies or groups were hardly studied (Table S1, Appendix II).

This study therefore investigated the effect of balanced and imbalanced DIN:DIP ratios on the physiology and elemental stoichiometry of two understudied coral groups: the scleractinian coral *Turbinaria reniformis*, which is a foliose coral, and the octocoral *Sarcophyton glaucum*. These species are different from scleractinian corals, that are particularly abundant in

oligotrophic, pristine environments. *T. reniformis* is indeed known to thrive in turbid, particle-rich environments (Anthony, 2006), while *S. glaucum* belongs to the octocorals known to dominate the benthic community in nutrient-rich environments (Baum *et al.*, 2016). It addresses two questions: First, are there different effects of balanced and imbalanced DIN:DIP ratios on the nutrient uptake rates, physiology, and cellular C:N:P stoichiometry of the corals? Second, do the scleractinian coral and the octocoral respond in the same way to changes in DIN:DIP ratios? We hypothesize that an imbalanced DIN:DIP ratio should be less detrimental to octocoral physiology, as octocorals appear to be dominant over scleractinian corals in eutrophic reefs (Baum *et al.*, 2016). To answer these questions, corals were exposed to two balanced DIN:DIP ratios with different nutrient concentrations: DIN:DIP_{2.5} (0.5:0.2) and DIN:DIP₃ (3:1) and two imbalanced DIN:DIP ratios: DIN:DIP_{0.5} (0.5:1) and DIN:DIP₁₅ (3:0.2). The DIN:DIP_{2.5} corresponds to the natural seawater control condition and will be referred to as the control condition in the results and discussion sections. The results of this study will provide useful insight on the future of corals submitted to eutrophication and inform on the different managerial strategies that can be implemented depending on the dominant corals species.

II.3. Materials & Methods

II.3.a. Experimental design

Two widespread Red Sea coral species were used for this experiment, the octocoral *Sarcophyton glaucum* and the scleractinian coral *Turbinaria reniformis*. These two species were maintained under oligotrophic, nutrient-poor conditions, similar to the Red Sea reef conditions in shallow waters. Therefore, total dissolved inorganic nitrogen (DIN) and phosphorus (DIP) concentrations were around 0.5 μM and 0.2 μM (Wafar *et al.*, 2016). In the Red Sea, these concentrations remain low throughout the years in shallow waters (Wafar *et al.*, 2016). For each coral species, 96 nubbins were produced from 12 different colonies (8 nubbins per colony) and then equally divided in 8 aquaria (one nubbins per colony, 12 nubbins per species and aquaria). Nubbins were left for three weeks in an open system continuously supplied with filtered seawater, pumped at 40m depth and illuminated by 400W metal halide lamps (HPIT, Philips) at an irradiance of $200 \pm 10 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ (12h:12h photoperiod). Temperature was controlled at 25 °C by heaters connected to Elli-Well PC 902/T controllers. Nubbins were fed twice a week with *Artemia salina* nauplii, to help with coral healing. After

healing, feeding was stopped, and nubbins were maintained in this condition for a week before the following experimental design was applied.

The 8 aquaria were divided in 4 sets of 2 aquaria, each set maintained at a different concentration of dissolved inorganic nitrogen (DIN) and phosphorus (DIP) (Fig. S1, Appendix II). The first set was the control condition with a balanced DIN:DIP ratio of 2.5 (named “Control, balanced DIN:DIP_{2.5}”), which received natural seawater (undetectable amounts of ammonium, 0.5 μM nitrate and 0.2 μM phosphate). The second set of aquaria with an imbalanced DIN:DIP ratio of 0.5 (named “DIN:DIP_{0.5}”) was enriched with 1 μM DIP delivered in the form of phosphates. The third set of aquaria with an imbalanced DIN:DIP ratio of 15 (named “DIN:DIP₁₅”) was enriched with 3 μM DIN delivered in the form of nitrate. Finally, the last set of aquaria with a balanced DIN:DIP ratio of 3 (named “DIN:DIP₃”) was enriched with both 3 μM nitrate and 1 μM phosphate to reach an equivalent DIN:DIP ratio to the control condition, DIN:DIP_{2.5}. These concentrations were chosen as they are commonly measured in reefs under agricultural and urban pollution (Costa *et al.*, 2000). DIN and DIP were delivered to each aquarium by two peristaltic pumps (Ismatec) from two concentrated stock solutions of sodium nitrate (NaNO_3) and di-sodium phosphate (Na_2HPO_4). To ensure water mixing and a homogeneous nutrient concentration, submersible pumps (Aquarium system, micro-jet MC 320, Mentor, OH, USA) were set up in each aquarium. Nutrient concentrations were controlled using an Autoanalyser 3 HR (SEAL Analytical, USA) according to Aminot *et al.* (2009). Nubbins were maintained in these conditions for five weeks. Four sets of measurements were performed with 6 to 4 nubbins each, sampled from different colonies and different aquaria (Table S5, Fig S1, Appendix II). Six nubbins were used for the measurements of nutrient uptake rates, photosynthetic efficiency, oxygen fluxes, symbiont density, chlorophyll and protein concentrations. A second set of six nubbins was used for the determination of the lipid and carbohydrate content. Oxidative stress (lipid peroxidation and antioxidant capacity) was obtained from the third set of six nubbins, while the C,N,P tissue content was measured on a fourth set of six nubbins.

II.3.b. Dissolved inorganic nutrients uptake rate

Nutrient uptake rates under different DIN:DIP ratios were measured on six nubbins per condition. Nubbins were incubated in individual beakers filled with 200 mL of 0.45 μm filtered seawater, maintained at 25 °C and stirred. No DIN and DIP were added in the control beakers. On the contrary, 3 μM of nitrate were added to the DIN:DIP₁₅ and DIN:DIP₃ conditions from a solution of sodium nitrate and 1 μM of phosphate was added to the conditions DIN:DIP_{0.5} and DIN:DIP₃ from a solution of disodium phosphate. Immediately after the addition of nitrate

and phosphate to the respective beakers and every hour for three hours, subsamples of 10 mL of seawater were taken. They were filtered through a 0.45 μm filter syringe in a 15 mL Falcon tube and stored at 4 °C until analysis the following day. Nutrient concentrations in the seawater subsamples were analysed using an Autoanalyser 3 HR (SEAL Analytical, USA) according to Aminot et al. (2009). Nitrate and phosphate uptake rates under the different conditions were measured by their depletion in the seawater between the different timepoints.

II.3.c. Measurement of photosynthetic parameters

Rates of respiration (R) and net photosynthesis (Pn) were measured on the same six nubbins as in 4.2. Nubbins were transferred into a 60 mL closed transparent plexiglass chamber filled with 0.45 μm filtered seawater, maintained at 25 °C and stirred. Chambers were equipped with an oxygen-sensor (Polymere Optical Fiber, PreSens, Regensburg, Germany) connected by an optical fiber to an Oxy-4 (Channel fiber-optic oxygen meter, PreSens, Regensburg, Germany). The oxygen concentration was recorded with the Oxy4v2-30fb software, in the dark for R and at 200 $\mu\text{mol photons}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ for Pn. Calibrations were done at 0 % O₂ with nitrogen saturated seawater and at 100 % O₂ with air saturated seawater. Incubations were stopped when a variation of at least 10 % of the dissolved oxygen concentration was reached. Gross photosynthesis (Pg) rate was obtained by adding Pn and the absolute value of R. This calculated Pg is likely to be an underestimation of the actual gross photosynthesis rate (Schrameyer *et al.*, 2014). Nubbins were used for the nutrient uptake rates measurements, then frozen at -80 °C for later analysis of tissue parameters.

The efficiency of the photosystem II of the dinoflagellate (Fv/Fm) was also measured on the same six nubbins. The nubbins were first placed in darkness for 10 minutes. Then, using an optical fiber connected to a Dual Pam/F (Fiber version, Heinz Waltz, Germany) a saturated pulse of photosynthetically active radiation (PAR) was sent and the Fv/Fm recorded. Nubbins were not sacrificed after this measurement and used for the different analysis.

II.3.d. Tissue parameters

All data were normalised to ash free dry weight (AFDW) according to Pupier *et al.* (2018) or protein biomass, since skeletal surface area was impossible to retrieve from soft corals. After being sampled, *S. glaucum* nubbins were directly lyophilized for 72 h (Christ Martin™ Alpha™ Freeze dryer, Fisher Scientific), while *T. reniformis* nubbins were first water picked to remove the tissue from the skeleton before being lyophilized. The dry weight of the nubbins was then recorded, and the AFDW was determined by burning a known weight of powder at 450 °C for 4 h and subtracting the ash weight (inorganic fraction) from the dry weight.

II.3.d.i. Symbiodinium density, total protein and chlorophyll content

These three parameters were assessed from nubbins used for the photosynthetic measurements. Freeze-dried samples were re-suspended into 10 mL of filtered seawater and homogenized with a Potter tissue grinder. From each nubbin, a 5 mL sub-sample was used for the determination of the total chlorophyll a and c₂ concentration. It was first centrifuged at 3,000g for 10 min at 4 °C to pellet the dinoflagellates. The pellet was then re-suspended into 5 mL of 100% acetone to extract chlorophyll a and c₂ at 4°C in the dark for 24 h. Finally, the extract was centrifuged at 15 °C, 530 g for 15 min before reading the absorbance at 630, 663 and 750 nm with a spectrofluorometer (UVmc² Safas, Xenius, Monaco). Chlorophyll a and c₂ concentrations were calculated according to the equations of Jeffrey and Humphrey (1975).

The protein content of the coral holobiont was quantified in a 500 µL sub-sample of each nubbin. The sub-sample was incubated 5 h at 60 °C in 0.5 M sodium hydroxide (1:1). It was then centrifuged 1min at 1000g and the supernatant was distributed in a 96-well microplate in triplicate. The bicinchoninic acid (BCA) assay kit solution (Interchim) was then added to each well according to Smith *et al.* (1985) before incubating the microplate for 30 min at 60 °C. Finally, the absorbance was read at 562 nm with a spectrofluorometer (UVmc² SAFAS, Xenius, Monaco). The standard curve was done with known concentrations of bovine serum albumin (BSA). Finally, a 100 µL subsample from each nubbin was used to estimate the dinoflagellate density with a Z1 Coulter Particle Counter (Beckman Coulter). Each sample was counted in triplicate and each measurement was done twice.

II.3.d.ii. Lipid and carbohydrate content

6 nubbins per treatment and species were flash frozen in liquid nitrogen and lyophilized, for the subsequent measurement of lipid and carbohydrate content. Holobiont lipid content was measured using pre-burned (450 °C for 5 h) Pyrex glass tubes and the phospho-sulpho-vanillin reaction as described in Barnes and Blackstock (1973). Briefly, from each nubbin, a 20 mg sub-sample was suspended in a solution of chloroform/methanol (2:1), mixed for 20 min, and then centrifuged at 3,000 g for 5 min at 4 °C. The supernatant was collected in a new Pyrex glass tube and placed in a dry bath at 90 °C until complete evaporation. The residue was treated with 500 µL concentrated sulfuric acid before adding vanillin reagent for 30 minutes in the dark. Absorbance was then read at 520 nm with a spectrofluorometer (Xenius®, SAFAS, Monaco). The standard curve was prepared with known concentrations of cholesterol (Knight *et al.*, 1972).

The carbohydrate content was assessed using the Total Carbohydrate Assay kit (Sigma Aldrich), based on the phenol-sulfuric acid method, following the manufacturer instruction.

Briefly, from each nubbin, a sub-sample was sonicated (20 pulses at 70Hz, Vibra-Cell™, Bioblock Scientific, France) on ice in cold 1X PBS (Phosphate-Buffered Saline), then centrifuged at 10,000 g for 5 minutes. The supernatant was deposited in 96 well plate with sulfuric acid, and incubated in the dark for 15 minutes at 90 °C, before receiving the Developer solution from the manufacturer kit. After 5 min, the absorbance was read at 490 nm with a spectrofluorometer (Xenius®, SAFAS, Monaco). The standard curve was prepared with known concentrations of D-glucose.

II.3.e. Oxidative status

Oxidative stress analysis were done on 6 nubbins per condition and species, snap-frozen in liquid nitrogen and kept at -80 °C before analysis. Each analysis was done on a 0.5 - 1 cm fragment cut from the main nubbin.

II.3.e.i. Non-enzymatic total antioxidant capacity (NETAC)

Fragments were sonicated on ice in cold PBS 1X and centrifuged at 10,000 g for 10 min at 4 °C. Protein content was measured as described above. All samples were diluted to the same protein concentration, before NETAC was assessed with the OxiSelect™ Total Antioxidant Capacity Assay kit (Cell Biolabs Inc.), based on the reduction of copper (II) to copper (I) by antioxidants. Final samples were added to a 96-well plate with the reaction buffer and the absorbance was read at 490 nm with a spectrofluorometer (Xenius®, SAFAS, Monaco). Results were expressed as μM Copper Reducing Equivalents (CRE). mg protein^{-1} . The standard curve was prepared with known concentrations of uric acid.

II.3.e.ii. Lipid peroxidation (LPO)

Fragments were sonicated on ice in a homogenizing buffer composed of a KCL solution (1.15%) with 35 μM butylatedhydroxytoluene (BHT). Samples were then centrifuged at 10,000g at 4 °C for 10 minutes and protein content was measured as described above and standardized to 0.8 $\mu\text{g}\cdot\mu\text{L}^{-1}$. The lipid peroxidation damage was assessed according to the ThioBarbituric Acid Reactive Species (TBARS) method (Oakes & Van Der Kraak, 2003), with malonaldehyde as an end product. Final samples were deposited on a black 96-well plate in duplicates and absorbance was read every minute for 3 minutes at 533 nm (emission) and 515 nm (excitation). The standard curve was prepared with known concentrations of tetrametoxyp propane.

II.3.f. Carbon, Nitrogen, Phosphorus content

6 nubbins per species and condition were flash-frozen in liquid nitrogen and stored at -80 °C for later analysis of their CNP content. They were freeze-dried before analysis. In each

condition, two nubbins did not have enough tissue to allow for accurate results, thus statistical analysis were done on 4 nubbins.

II.3.f.i. Phosphorus content

Sub-samples from each nubbin were weighed and digested in a persulfate potassium solution autoclaved at 121 °C (Menzel & Corwin, 1965). Samples were then placed in triplicate on a 96-well microplate with a solution of ammonium molybdate and incubated at room temperature for 10 minutes, before addition of a solution of malachite green. After 30 minutes, the absorbance was measured at 630 nm using a spectrofluorometer (Xenius®, SAFAS, Monaco) to quantify the orthophosphate content. The standard curve was prepared with known concentrations of potassium phosphate.

II.3.f.ii. Carbon and nitrogen content

Sub samples from each nubbin (1 mg) were placed in a tin cap and analysed with an EA-IRMS Integra2 (Sercon, England).

II.3.g. *Statistical analyses*

Statistical tests were performed using the free open-source software R. Data were checked for homoskedasticity using Levene's test on the residual from the linear model (Levene, 1960) and for normality using the Shapiro-Wilk Test. One-way ANOVA were used to test for the statistical differences in each species between the conditions: DIN:DIP_{2.5} (0.5:0.2) and DIN:DIP₃ (3:1) and two imbalanced DIN:DIP ratios: DIN:DIP_{0.5} (0.5:1) and DIN:DIP₁₅ (3:0.2). If the assumption of normality and homoskedasticity were not validated the non-parametric Kruskal Wallis test was performed. Analysis was followed by a post hoc test, pairwise comparison with "fdr" correction, when factors effect was significant. The statistical outcomes are recorded in a table in the appendix (Table S3, Appendix II). Differences were considered as significant when the calculated p value was below 0.05.

II.4. *Results*

II.4.a. *Effect of DIN:DIP ratios on the physiology and tissue composition of Turbinaria reniformis*

Significant changes in *T. reniformis* physiology occurred primarily under the balanced DIN:DIP₃ (combined enrichment in DIN and DIP) and are presented first in this result section (Table S3a, for median and standard error values; Table S4a,b,c for statistical results). This nutrient condition increased the rates of DIN (nitrate) and DIP (phosphate) uptake by fifteen (Fig. 17a, p value 0.001) and twenty-fold (Fig. 17b, p value 0.001) respectively compared to

the control condition, DIN:DIP_{2.5}. Although, both the density of symbiotic dinoflagellates (Fig. 18a, p value 0.06) and the protein content (Fig. 18b, p value 0.4) remained unchanged, the chlorophyll a and c₂ content (Fig. 19, p value 0.02) and net photosynthetic rates (Fig. 20a, p value 0.002) doubled compared to the control condition, as a result of higher nutrient availability. This increase in carbon assimilation was followed by an increase in carbon reserves with a doubling of lipid (Fig. 21a, p value 0.03) and carbohydrate content (Fig. 21b, p value 0.01) as well as an increase in the total carbon content (Fig. 22a, p value 0.05). Also, due to increased nitrate there was a subsequent increase in the total nitrogen content of *T. reniformis* (Fig. 22b, p value 0.04), so that the C:N ratio remained unchanged compared to the control condition (Fig. 22c, p value 0.5). On the other hand, despite enhanced DIP uptake under DIN:DIP₃, the phosphorus content remained unchanged (Fig. 23a, p value 0.15), likely due to the large variability in the phosphorus content in the control condition. Therefore, the C:P ratio was not statistically different from the control level, although it almost doubled on average (Fig. 23b, p value 0.06).

The imbalanced in DIN:DIP₁₅ (DIN enrichment alone) significantly increased nitrate uptake rates in *T. reniformis* by sevenfold (Fig. 17a, p value 0.005) and thus the nitrogen content in coral tissue (Fig. 22b, p value 0.03) compared to the control condition. As a result, the cellular N:P ratio under imbalanced DIN:DIP₁₅ was twice that of the control condition (Fig. 23c, p value 0.01). Although some other parameters (chlorophyll content, net photosynthetic rates, carbohydrate, lipid and carbon content) tended to increase with DIN:DIP₁₅, this increase was not significant (Fig. 19, 20a, 21 and 22a, p value > 0.1). The other physiological parameters (P content, C:N and C:P ratios, symbiont density and protein content) remained unchanged compared to the control condition (Fig. 18, 22c, 23a, b, p value > 0.05).

The imbalanced DIN:DIP_{0.5} (DIP enrichment alone) enhanced the uptake rates of phosphate (Fig. 17b, p value 0.001) and reduced the symbiotic dinoflagellates density by half (Fig. 18b, p value 0.001), without any change in total chlorophyll content and photosynthetic rates (Fig. 19, 20a, p value > 0.6). On the other hand, DIN:DIP_{0.5} doubled the carbohydrate content (Fig. 21a, p value 0.01) as well as the total carbon (Fig. 22a, p value 0.003) and nitrogen content (Fig. 22b, p value 0.01) compared to the control condition. However, the P content remained unchanged despite a tendency to increase (Fig. 23a, p value 0.15). Both the C:P and N:P ratios also remained unaffected in DIN:DIP_{0.5} compared to the control, likely due to the variability in P content among samples (Fig. 23b, c, p values > 0.7).

II.4.b. *Effect of DIN:DIP ratios on the physiology and tissue composition of Sarcophyton glaucum.*

Overall, the different DIN:DIP ratios had a limited effect on *S. glaucum* physiology (Table S3b, Table S4). The nitrate uptake rate doubled in DIN:DIP₃ (DIN and DIP enrichment) compared to the control condition (Fig. 17a, p value 0.02), while phosphate uptake rate remained unchanged (Fig. 17b, p value 0.46). In addition, carbohydrate content decreased by half under DIN:DIP₁₅ (DIN enrichment) compared to the control condition DIN:DIP_{2.5} (Fig. 21b, p value 0.004). However, under DIN:DIP_{0.5} (DIP enrichment), the carbohydrate content increased (Fig. 21a, p value 0.02) along with the protein content (Fig. 18b, p value 0.01). The other parameters remained at the control level under the different balanced and imbalanced DIN:DIP ratios (Figs. 19-23, p values > 0.2).

II.4.c. *Comparison in the physiological parameters between Turbinaria reniformis and Sarcophyton glaucum under a control control balanced DIN:DIP_{2.5} without enrichment.*

Despite similar dinoflagellate density (Fig. 18b, p value 0.14) and total chlorophyll content (Fig. 19, p value 0.7) in both coral species, the net photosynthetic rate and respiration rate of *S. glaucum* were significantly lower than those of *T. reniformis* (Fig. 20a, b, p values 0.002). While carbohydrate content was similar between coral species (Fig. 21a, p value 0.06), lipid content was ten times higher in *S. glaucum* than in *T. reniformis* (Fig. 21b, p value 0.001). However, the protein content was divided by two in *S. glaucum* (Fig. 18a, p value 0.002). Overall, the carbon (Fig. 22a, p value 0.03) and nitrogen (Fig. 22b, p value 0.03) contents were significantly higher in *S. glaucum* than in *T. reniformis*, while phosphorus content was similar in both coral species (Fig. 23a, p value 0.85). Thus, the C:N (Fig. 22c, p value 0.03), C:P (Fig. 23b, p value 0.03) and N:P (Fig. 23c p value 0.004) ratios were significantly higher in *S. glaucum* than in *T. reniformis*.

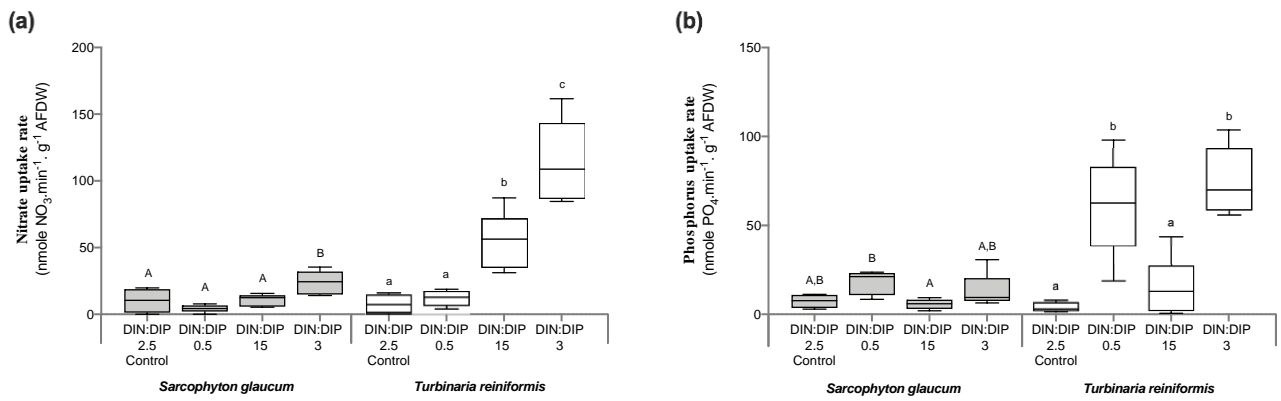


Figure 17. Nutrient uptake rates in the octocoral *Sarcophyton glaucum* and the scleractinian *Turbinaria reiniformis*. Boxplots represent the nitrate (a) and phosphorus (b) uptake rates of *S. glaucum* (grey) and *T. reiniformis* (white) under two balanced (DIN:DIP = 2.5 and 3) and two imbalanced (DIN:DIP = 0.5 and 15) ratios. The natural seawater control condition corresponds to the DIN:DIP ratio of 2.5. The box delimits the first and third quartile, the black bar represents the median and whiskers represent minimum and maximum values, n = 6. Statistically significant differences within species are represented by different letters: uppercase letters (A and B) for *S. glaucum* and lowercase letters (a, b and c) in *T. reiniformis*.

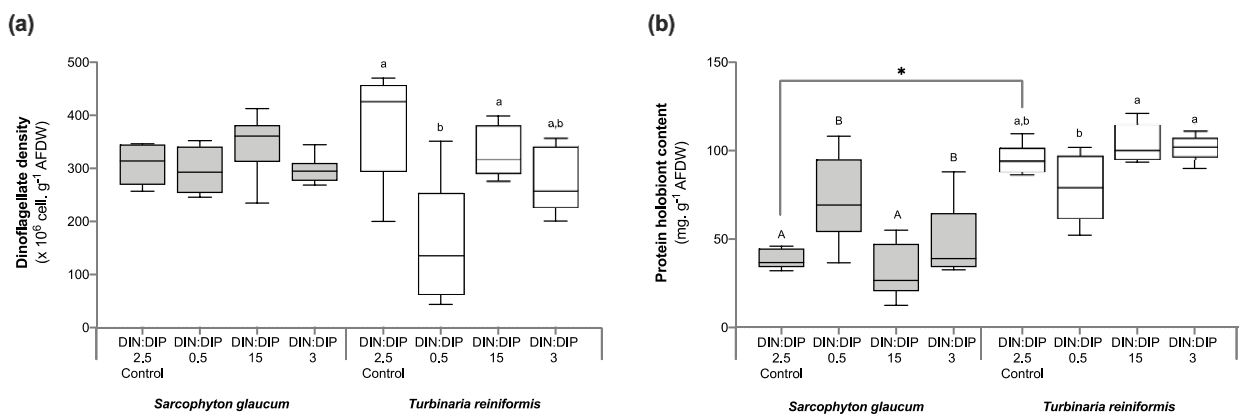


Figure 18. Dinoflagellate density and protein biomass of the octocoral *Sarcophyton glaucum* and the scleractinian *Turbinaria reiniformis*.

Boxplots represent the symbiotic dinoflagellate density (a) and the total protein content (b) of *S. glaucum* (grey) and *T. reiniformis* (white) under two balanced (DIN:DIP = 2.5 and 3) and two imbalanced (DIN:DIP = 0.5 and 15) ratios. The natural seawater control condition corresponds to the DIN:DIP ratio of 2.5. The box delimits the first and third quartile, the black bar represents the median and whiskers represent minimum and maximum values, n = 6. Statistically significant differences within species are marked by different letters: uppercase letters (A and B) for *S. glaucum* and lowercase letters (a, b and c) in *T. reiniformis*. Statistically significant differences between species, only for the control condition, are marked by an asterisk.

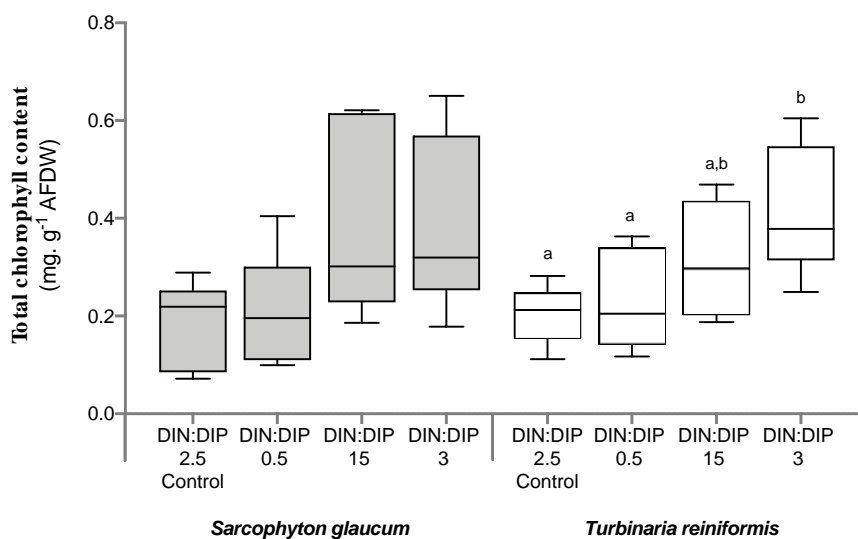


Figure 19. Total chlorophyll a and c₂ content in the octocoral *Sarcophyton glaucum* and the scleractinian *Turbinaria reiniformis*.

Boxplot represent the total chlorophyll a and c₂ content of *S. glaucum* (grey) and *T. reiniformis* (white) under two balanced (DIN:DIP = 2.5 and 3) and two imbalanced (DIN:DIP = 0.5 and 15) ratios. The natural seawater control condition corresponds to the DIN:DIP ratio of 2.5. The box delimits the first and third quartile, the black bar represents the median and whiskers represent minimum and maximum values, n = 6. Statistically significant differences within species are marked by different letters: uppercase letters for *S. glaucum* and lowercase letters (a, b and c) in *T. reiniformis*.

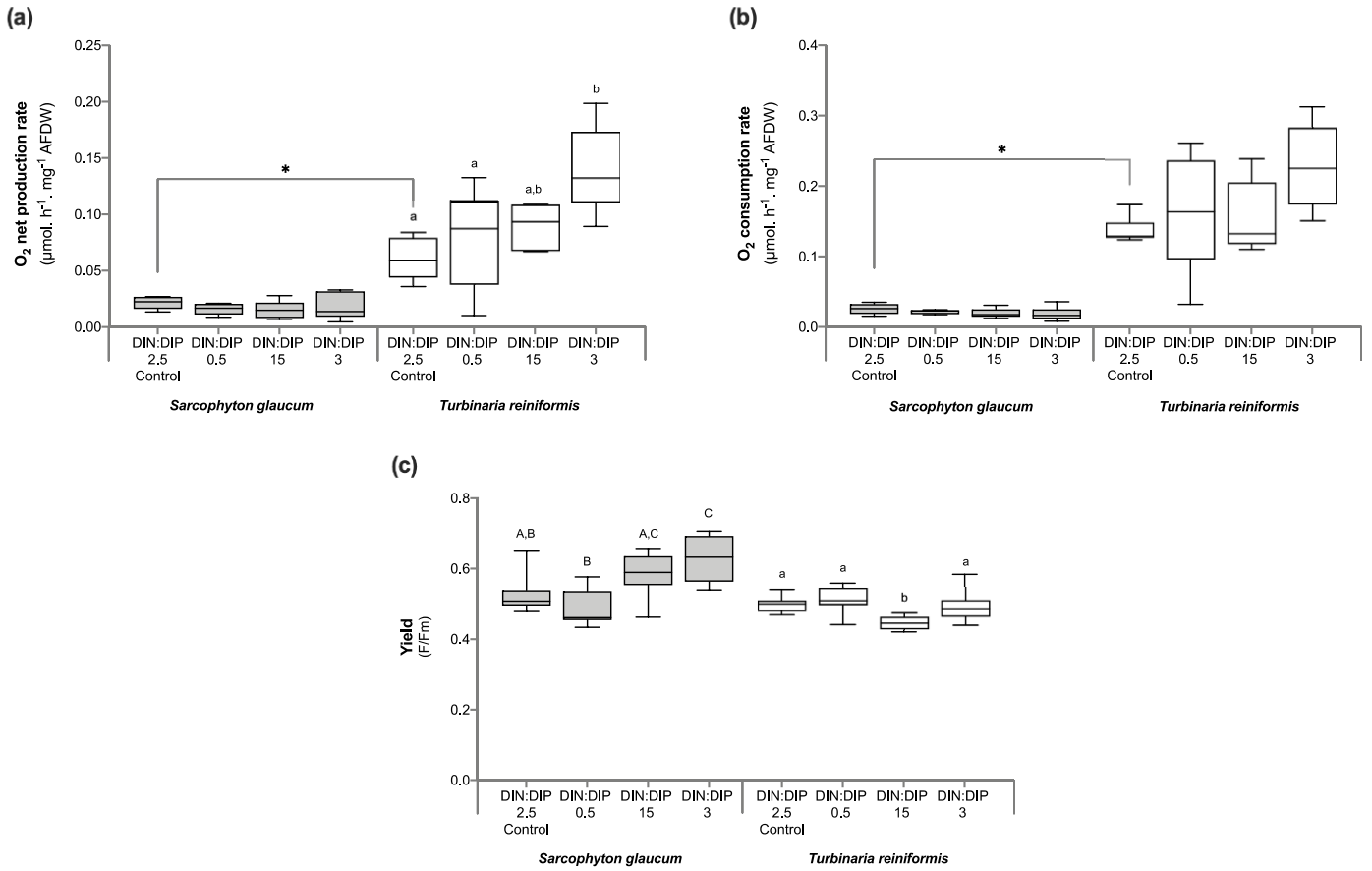


Figure 20. Photosynthetic capabilities in the octocoral *Sarcophyton glaucum* and the scleractinian *Turbinaria reniformis*.

Boxplots represent the net photosynthetic (a) and respiration (b) rates and the photosynthetic efficiency of the photosystem II (c) of *S. glaucum* (grey) and *T. reniformis* (white) under two balanced (DIN:DIP = 2.5 and 3) and two imbalanced (DIN:DIP = 0.5 and 15) ratios. The natural seawater control condition corresponds to the DIN:DIP ratio of 2.5. The box delimits the first and third quartile, the black bar represents the median and whiskers represent minimum and maximum values, n = 6. Statistically significant differences within species are marked by different letters: uppercase letters (A, B and C) for *S. glaucum* and lowercase letters (a, b and c) in *T. reniformis*. Statistically significant differences between species, only for the control condition, are marked by an asterisk

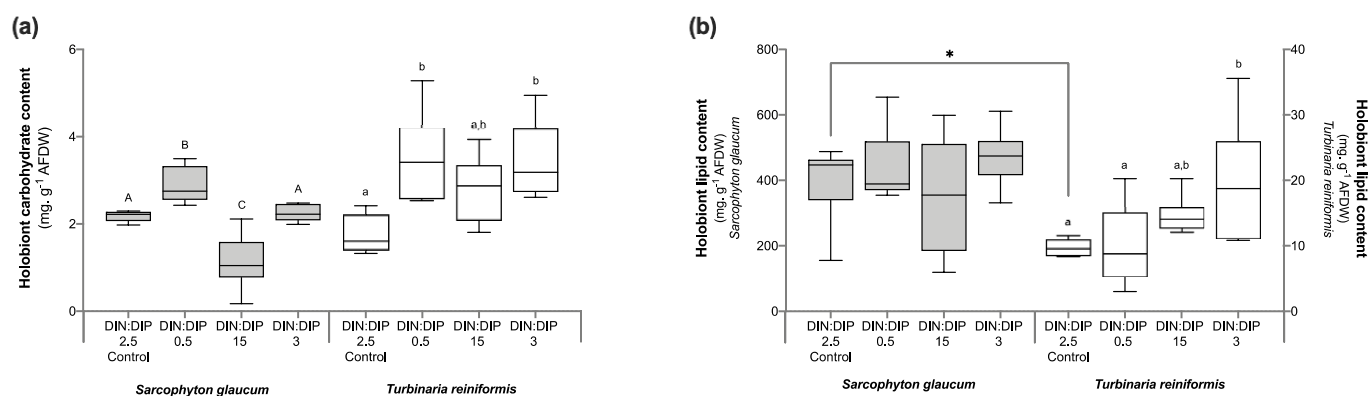


Figure 21. Nutrient uptake rates in the octocoral *Sarcophyton glaucum* and the scleractinian *Turbinaria reiniformis*. Nutrient uptake rates in the octocoral *Sarcophyton glaucum* and the scleractinian *Turbinaria reiniformis*. Boxplots represent the nitrate (a) and phosphorus (b) uptake rates of *S. glaucum* (grey) and *T. reiniformis* (white) under two balanced (DIN:DIP = 2.5 and 3) and two imbalanced (DIN:DIP = 0.5 and 15) ratios. The natural seawater control condition corresponds to the DIN:DIP ratio of 2.5. The box delimits the first and third quartile, the black bar represents the median and whiskers represent minimum and maximum values, n = 6. Statistically significant differences within species are represented by different letters: uppercase letters (A and B) for *S. glaucum* and lowercase letters (a, b and c) in *T. reiniformis*.

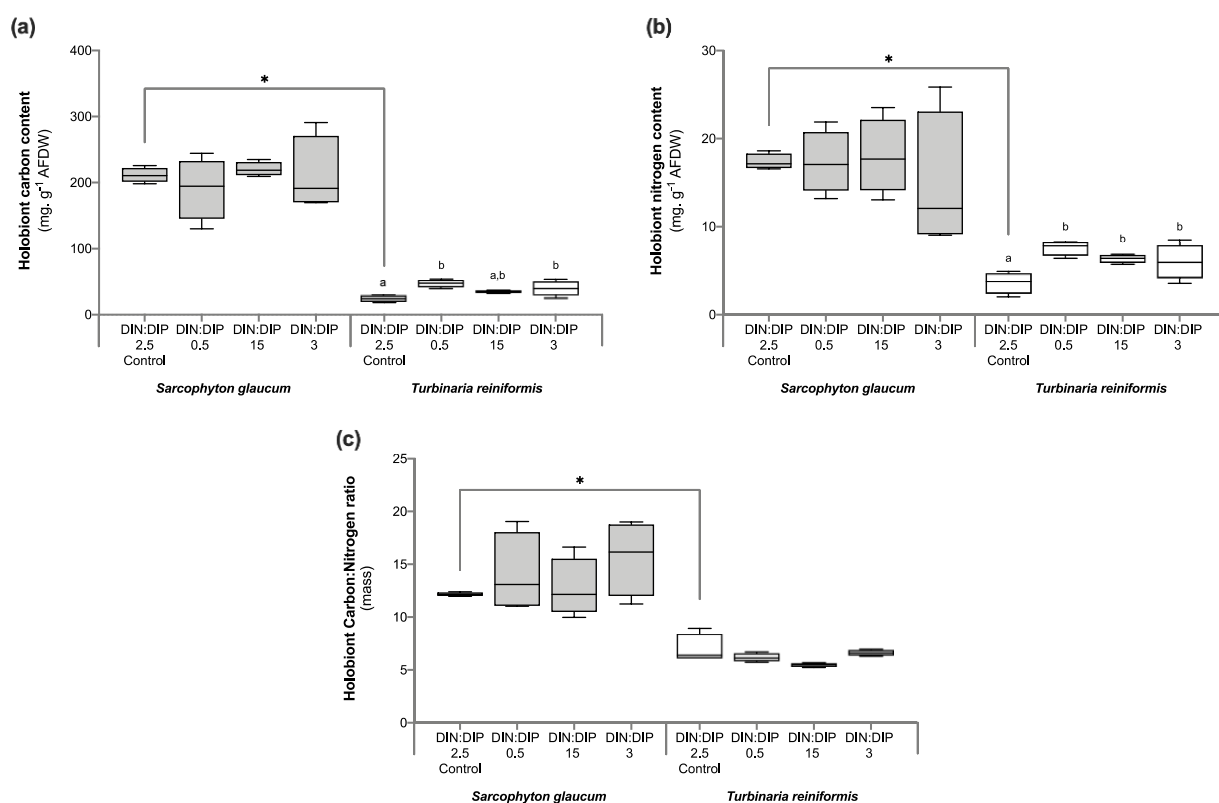


Figure 22. Composition in carbon, nitrogen and the carbon-to-nitrogen (C:N) ratio in the octocoral *Sarcophyton glaucum* and the scleractinian *Turbinaria reiniformis*.

Boxplots represent the carbon (a) and nitrogen (b) content and the C:N ratio (c) of *S. glaucum* (grey) and *T. reiniformis* (white) under two balanced (DIN:DIP = 2.5 and 3) and two imbalanced (DIN:DIP = 0.5 and 15) ratios. The natural seawater control condition corresponds to the DIN:DIP ratio of 2.5. The box delimits the first and third quartile, the black bar represents the median and whiskers represent minimum and maximum values, n = 6. Statistically significant differences within species are marked by different letters: uppercase letters for *S. glaucum* and lowercase letters (a, b and c) in *T. reiniformis*. Statistically significant differences between species, only for the control condition, are marked by an asterisk.

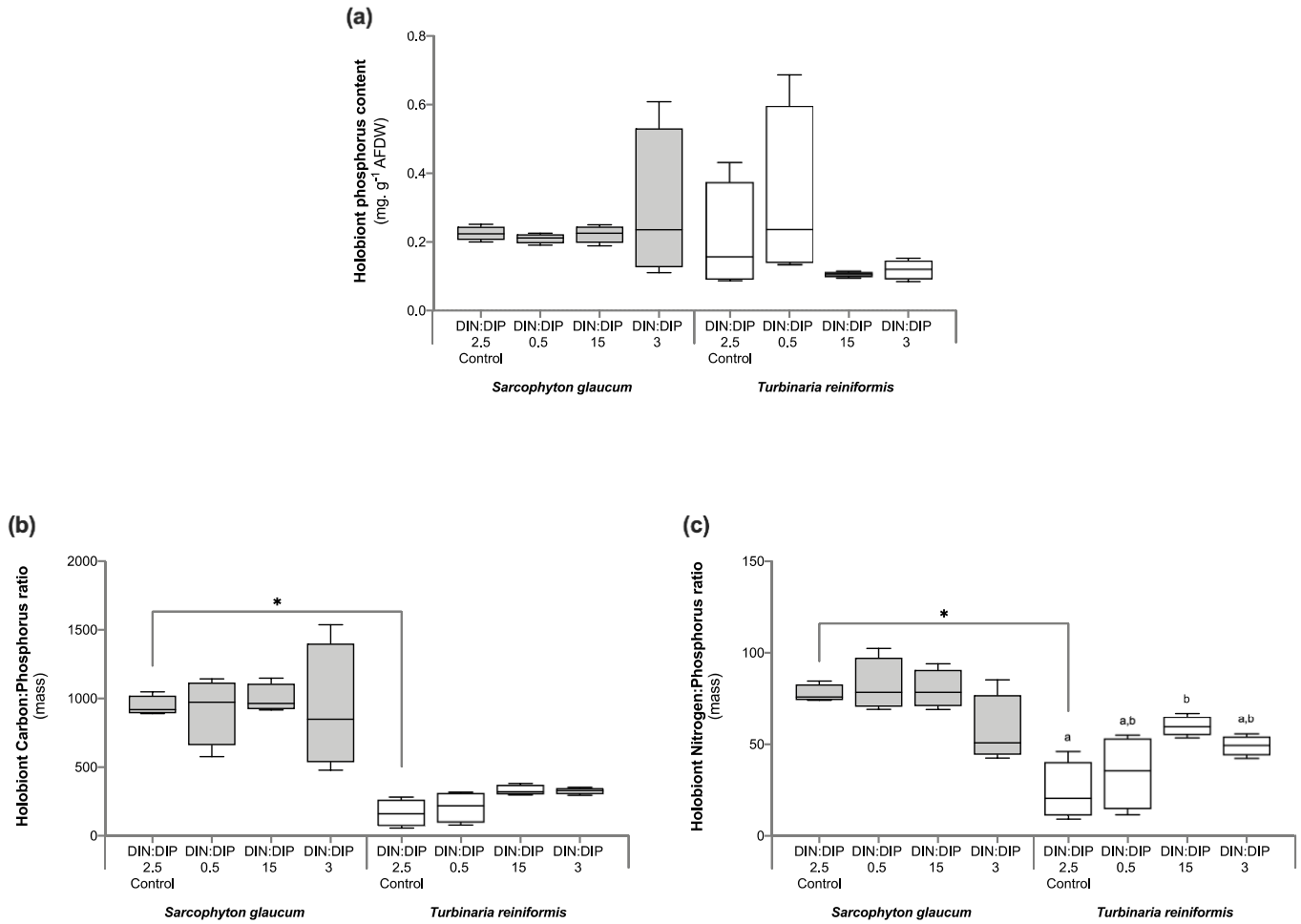


Figure 23. Composition in phosphorus and the carbon-to-phosphorus (C:N) and nitrogen-to-phosphorus (C:P) ratios in the octocoral *Sarcophyton glaucum* and the scleractinian *Turbinaria reiniformis*.

Box-plots represent the phosphorus (a) content and the C:P (b) and N:P (c) ratios of *S. glaucum* (grey) and *T. reiniformis* (white) under two balanced (DIN:DIP = 2.5 and 3) and two imbalanced (DIN:DIP = 0.5 and 15) ratios. The natural seawater control condition corresponds to the DIN:DIP ratio of 2.5. The box delimits the first and third quartile, the black bar represents the median and whiskers represent minimum and maximum values, n = 6. Statistically significant differences within species are marked by different letters: uppercase letters for *S. glaucum* and lowercase letters (a, b and c) in *T. reiniformis*. Statistically significant differences between species, only for the control condition, are marked by an asterisk.

II.5. Discussion

Declining water quality in many reefs is causing phase shifts in the composition of the benthic community, from a dominance of scleractinian corals to a dominance of macroalgae (De'ath & Fabricius, 2010), and other benthic organisms such as soft corals (Baum *et al.*, 2016), which may therefore be the dominant benthic organisms in the future. Numerous studies have therefore examined the effects of organic and inorganic nutrient pollution on scleractinian corals, which are the main reef builders. These studies have shown that both types of pollution

affect the metabolism and resistance of scleractinian corals to thermal stress (Burkepile *et al.*, 2020; Marangoni *et al.*, 2020; Pogoreutz *et al.*, 2018; Wiedenmann *et al.*, 2013). This is because nutrient enrichment can lead to the disruption of the symbiosis between corals and their associated microbiota (Boilard *et al.*, 2020; Krediet *et al.*, 2013; Pogoreutz *et al.*, 2018). In contrast to scleractinian corals, studies have shown that organic and inorganic contaminants do not always have detrimental effects on soft corals or even increase their resistance to thermal stress (Koop *et al.*, 2001; Klinke *et al.*, 2022), which may facilitate their ecological dominance over scleractinian corals. Therefore, further studies comparing the response of soft corals and scleractinian corals to nutrient enrichment are needed to better predict future reef development. The results of this study show that the physiology and tissue stoichiometry of *S. glaucum* did not change under the different DIN:DIP ratios scenarios because this species, as many soft corals, had very low nutrient uptake rates. Therefore, little N and P entered the symbiosis under varying DIN:DIP ratios. In contrast, *T. reniformis*, as all hard corals, took up nutrients proportionally to their concentration in seawater. The massive input of N under nitrogen imbalanced condition (DIN:DIP₁₅) led to significant changes in tissue stoichiometry and may explain the impairment of coral physiology observed in many previous studies. However, a balanced enrichment in DIN and DIP (DIN:DIP₃) resulted in improved physiology and metabolism of *T. reniformis*, suggesting that the physiology of scleractinian corals is co-limited by N and P.

II.5.a. Effect of DIN:DIP ratio on the physiology of T. reniformis

Results of this study first show that changes in seawater DIN:DIP ratios can induce changes in nutrient uptake rates by the scleractinian coral *T. reniformis*. Uptake of DIN and DIP at different DIN:DIP ratios have been poorly studied in photosynthetic organisms and never with coral species to our knowledge (see (Lubsch & Timmermans, 2020; Perini & Bracken, 2014) for macroalgae, (Aubriot & Bonilla, 2018) for cyanobacteria, and (Treseder & Vitousek, 2001) for trees). This study first confirms the findings of previous studies that uptake rates are dependent on the nutrient concentration in seawater, with increased uptake rates at high nutrient concentrations (DIN:DIP₃) (Aubriot & Bonilla, 2018; Lubsch & Timmermans, 2020; Perini & Braken, 2014). Furthermore, our results demonstrate that the availability in one nutrient increases the uptake rate of the other nutrient, as it has been observed in phytoplankton species, but not in corals (Aubriot & Bonilla, 2018). The highest uptake rates of DIN were indeed obtained with the combined enrichment (DIN:DIP₃). In contrast, *T. reniformis* took up DIP whenever its concentration in seawater was elevated (DIN:DIP₃ and DIN:DIP_{0.5}), indicating that P was a limiting nutrient for this coral species under all conditions. Overall, P is known to be

an essential nutrient for corals (Elser & Bracken, 2007; Ezzat *et al.*, 2016), and is found in limited concentrations in the environment. A deficiency in P leads to alteration of the thylakoid membranes of the symbiont and increases susceptibility to bleaching (Rodriguez-Casariiego *et al.*, 2018; Wiedenmann *et al.*, 2013).

In this study, two conditions (DIN:DIP_{0.5} and DIN:DIP₁₅) were imbalanced compared to what is normally observed in reefs (DIN:DIP between 4 and 7: (Furnas *et al.*, 1995; Rosset *et al.*, 2017)). Such situation may induce a subsequent change in element concentrations and stoichiometry in the coral tissue, and lead to imbalanced cellular N:P ratios. A significant effect of imbalanced DIN:DIP ratios was indeed observed on the physiology and/or elemental stoichiometry of the scleractinian coral *T. reniformis*, depending on whether seawater was enriched with DIP or DIN. Overall, DIP enrichment (DIN:DIP_{0.5}) increased the total carbon (as carbohydrates) and nitrogen content of coral tissue without altering internal elemental stoichiometry. These results are consistent with the notion that the availability of a limiting nutrient (especially phosphorus) promotes the uptake and/or assimilation of the other nutrients (Aubriot & Bonilla, 2018; Lubsch & Timmermans, 2020). This is also consistent with a previous study showing that the carbon content in *Montipora digitata* symbionts increased significantly when DIP was increased in seawater (Tanaka *et al.*, 2017). On the contrary, enrichment with DIN alone (DIN:DIP₁₅) did not enhance the production of organic carbon or the assimilation of phosphorus, neither in this study nor in the previous study by Tanaka *et al.* (2017). Instead, enrichment with DIN significantly increased the N content and the N:P ratio in the coral tissue, as previously observed by Wiedenmann *et al.* (2013). In this study, the N:P ratio increased from about 20 in the control condition (balanced DIN:DIP_{2.5}) to about 60 in the imbalanced DIN:DIP₁₅. Such a situation is known to be detrimental to coral growth and health in many branching species (Ezzat *et al.*, 2015; Koop *et al.*, 2001; Marangoni *et al.*, 2020; Nordemar *et al.*, 2003; Tanaka *et al.*, 2007), in particular under thermal stress (Wiedenmann *et al.*, 2013). No physiological changes were measured in *T. reniformis* during this relatively short-term incubation with DIN enrichment, as previously observed (Béraud *et al.*, 2013; Dobson *et al.*, 2021; Ezzat *et al.*, 2016). The results might have been different for a long-term enrichment incubation, or if a thermal stress would have been applied. On the contrary, other scleractinian species showed an impairment of their physiology after the same incubation time with similar nitrogen concentrations (Marangoni *et al.*, 2020; Wiedenmann *et al.*, 2013). All together, the results obtained suggest that *T. reniformis* belongs to the scleractinian species that may be the most resistant to changes in seawater stoichiometry. Accordingly, Tanaka *et al.* (2017) observed species-specific susceptibility to unbalanced DIN:DIP ratios. The stronger

effects were observed in symbionts of *M. digitata* compared to *Porites cylindrica*. The authors hypothesized that this was due to the lower N content per skeletal surface of *M. digitata*, making the symbionts more dependent on seawater and host-derived N (Tanaka *et al.*, 2017). In contrast to the imbalanced DIN:DIP conditions, increased DIN and DIP concentrations with a balanced ratio (DIN:DIP₃) did not change the cellular stoichiometry of *T. reniformis* because uptake rates increased for both nutrients. All measured physiological and tissue parameters were even increased under this balanced nutrient enrichment. Overall, given the significant increase in net and gross photosynthetic rates, as well as in carbohydrate and lipid content, the dual enrichment can be considered beneficial to coral physiological performances, as previously suggested (Dobson *et al.*, 2021; Ezzat *et al.*, 2016a; Shantz & Burkepile, 2014). Although calcification rates were not assessed in this study, moderate nutrient enrichment, as used in this study, has been shown to be beneficial to corals by mitigating the negative effects of thermal stress or acidification (Chauvin *et al.*, 2011; Dobson *et al.*, 2021; Ezzat *et al.*, 2016a; 2019; Holcomb *et al.*, 2010) without negatively affecting calcification (Bongiorni *et al.*, 2003; Dizon *et al.*, 2005; Tanaka *et al.*, 2007). Although there is a beneficial direct effect of DIN and DIP enrichment on coral performance, we must keep in mind that many indirect effects, such as increased bacterial, phytoplankton, macroalgal growth and corallivorous predators, might occur under natural reef conditions (D'Angelo & Wiedenmann, 2014).

II.5.b. Effect of DIN:DIP ratio on the physiology of S. glaucum

The soft coral species *S. glaucum* responded differently than *T. reniformis* to a change in DIN:DIP ratio. First of all, *S. glaucum* exhibited much lower nitrogen and phosphorus uptake rates than *T. reniformis*, even under high nutrient concentrations in seawater (DIN:DIP₃). These results are in agreement with a previous study (Ezzat *et al.*, 2017), which found lower nutrient uptake and assimilation rates in soft corals compared to scleractinian corals. Several reasons were proposed for this. Different Symbiodiniaceae species and densities may have different nutrient acquisition efficiency (Ezzat *et al.*, 2017). The coral microbiome can also lead to variations in nutrient supply, for example through the presence of diazotrophic bacteria in scleractinian corals (Bednarz *et al.*, 2021; Pupier *et al.*, 2021). Morphological characteristics may also explain the observed differences in nutrient uptake. In soft corals, the presence of a thick mesoglea may act like a barrier and prevent the diffusion of molecules such as DIC (Furla *et al.*, 1998). On the other hand, in scleractinian corals, the presence of a calcium carbonate skeleton generates protons (McConnaughey & Whelan, 1997) which are buffered in presence of ammonium (Crossland & Barnes, 1974). Nutrient uptake in corals is also enhanced in the light by photosynthesis (Grover *et al.*, 2002). Thus, the lower nutrient uptake rate in soft corals

could be explained by their consistently lower photosynthetic rates compared to scleractinian corals observed in both this study and Pupier *et al.* (2021). It might also be due to the thick mesoglea and tissue layer of soft corals, which may act as a barrier to the diffusion of inorganic nutrients. The low nutrient uptake rates in *S. glaucum* compared to *T. reniformis* may induce a continuous P and N limitation in this species and may also explain the higher tissue C:N:P ratio. While the N:P ratio in *T. reniformis* is slightly higher than the Redfield ratio (20 vs 16), it indeed reached up to 80 in *S. glaucum*. The high carbon content in *S. glaucum* is evidenced by 10 times higher lipid content (per mg AFDW) than in *T. reniformis*. In contrast, the protein content, requiring N and P, is two times lower in *S. glaucum*. However, it should be noted that for *S. glaucum*, traces of calcium carbonate spicules could also drive the measured high carbon content in the tissues.

The low nutrient uptake rates of *S. glaucum* may explain why no major changes in tissue stoichiometry (C:N:P) or element concentrations were observed under imbalanced DIN:DIP ratios. Low nutrient uptake rates may have prevented a massive input of nutrients into the coral tissue and subsequent changes in host-symbiont interactions or elemental stoichiometry. Under a combined and balanced DIN and DIP enrichment (DIN:DIP₃), there was a significant increase in nutrient uptake rates, albeit relatively low compared to *T. reniformis*. As a consequence, no effects on *S. glaucum* physiology, element concentration or tissue stoichiometry were observed.

The lack of large physiological changes under imbalanced or enriched DIN:DIP conditions could explain why soft corals are less affected by reef nutrification than scleractinian corals (Baum *et al.*, 2016; Schleyer *et al.*, 2003). Future studies should aim at understanding the effects of imbalanced DIN:DIP ratios on the trophic food chain and the consequences for coral nutrition. Soft corals are indeed supposed to rely more on heterotrophy and on phytoplankton grazing than scleractinian corals (Fabricius *et al.*, 1995; 2000). Therefore, any increase in seawater DIN or DIP may promote phytoplankton growth, which in turn benefits the growth of soft corals through increased feeding.

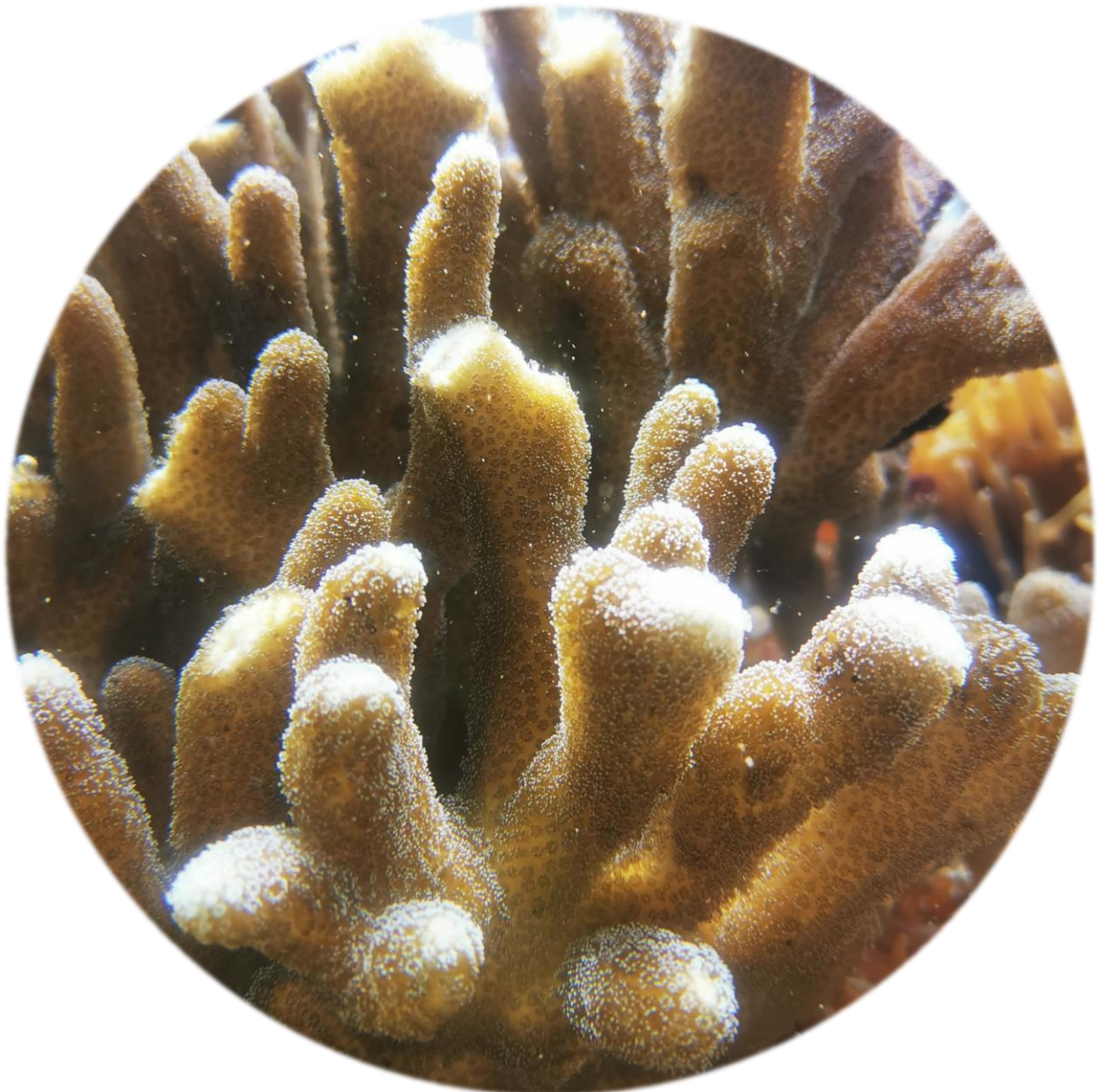
All together, these results suggest that soft corals such as *S. glaucum*, which have low nutrient uptake rates, can be relatively unaffected under imbalanced DIN:DIP ratios of the seawater, because nutrients do not massively enter the coral tissue and do not lead to imbalanced cellular elemental ratios. On the contrary, species with high uptake rates, such as all scleractinian corals, will be more affected under imbalanced DIN:DIP ratios of the seawater. Indeed, they will experience a large influx of nutrients within the coral tissue, which will lead to imbalanced

cellular elemental ratios. The response to imbalanced DIN:DIP ratios is however species specific in scleractinian corals.

II.6. Conclusion

This study brings key information on the response of corals to nutrient enrichment. The results first show that changes in seawater DIN:DIP ratio may induce large changes in tissue stoichiometry and elemental composition of coral species with high nutrient uptake rates. On the contrary, species with low nutrient uptake rates might be less responsive to changes in seawater DIN:DIP ratio. In addition, an imbalanced DIN:DIP ratio due to nitrogen enrichment may also induce an imbalanced N:P ratio in coral tissue, and therefore lead to potentially detrimental effects on coral physiology due to P limitation. Such situation, in combination with thermal stress, can exacerbate bleaching (Donovan *et al.* 2020; (Wiedenmann *et al.*, 2013). High nitrate and low phosphate concentrations are found in coastal reefs subjected to river and/or agricultural and effluent discharges (Stimson *et al.*, 2001; Szmant, 2002; Tanaka *et al.*, 2011). Although only one soft coral and one scleractinian coral species were tested in this study, soft corals appear to be more resistant to a change in seawater nutrient concentrations than scleractinian corals. This is due to their lower ability to take up and assimilate dissolved nutrients in seawater (Pupier *et al.*, 2021), as well as their higher tissue C and N content per AFDW. However, this needs further investigation as species-specific differences between scleractinian corals have also been observed (Tanaka *et al.*, 2017). Such differential responses to an unbalanced DIN:DIP ratio may help to understand the differential bleaching susceptibility of corals during thermal stress. Therefore, corals with a higher ability to control their internal N:P ratio and maintain a balanced N:P ratio may be more resistant to bleaching.

CHAPTER 3:
IMPACT OF DIN:DIP RATIOS ON
THE PHOSPHORUS REQUIREMENT
OF SCLERACTINIAN CORALS



CHAPTER 3: IMPACT OF DIN:DIP RATIOS ON THE PHOSPHORUS REQUIREMENT OF SCLERACTINIAN CORALS

In the two previous chapters, we found that corals can be negatively affected by an imbalanced DIN:DIP ratio following nitrate enrichment:

- depending on environmental conditions, where the lack of low doses of UVR does not protect coral physiology and particularly its photosynthetic capacities
- depending on nutrient uptake rates, where a high uptake rate of DIN induces a massive input of nitrogen inside the coral tissue and disrupts the cellular N:P stoichiometry. However, when DIP is also available in the surrounding environment, the simultaneous input of phosphorus inside the symbiosis balances the cellular N:P ratio and even enhances coral physiology.

I Overview of the article

Corals can maintain a balanced cellular N:P ratio under DIN enrichment not only by taking up DIP from seawater, but also dissolved organic phosphorus (DOP). While DIP occurs in nanomolar concentrations in reef waters (Bell et al., 2007; Morrison et al., 2013), DOP compounds can account for 70–90% of the total dissolved phosphorus pool (Ruttenberg & Dyrman, 2012). However, DOP must be hydrolysed by phosphatase enzymes to DIP before corals can use it. High phosphatase activity is considered a good indicator of P scarcity in the environment (Annis & Cook, 2002), as it occurs either at low DIP concentrations (< 20 nM for phytoplankton) or at elevated ratios of DIN:DIP (Cembella *et al.*, 1984; Rees *et al.*, 2009; Suzumura *et al.*, 2012). Coral holobionts have intracellular and extracellular acid (AcPA) and alkaline phosphatase (APA) activities (Atkinson, 1987; Godinot *et al.*, 2013; Wiedenmann *et al.*, 2013), but there is still limited knowledge on the regulation of their activity. A few studies have shown that extracellular APA is enhanced under DIP deficiency or during bleaching (Godinot *et al.* 2013). Thus, studies are urgently needed to better understand the conditions under which phosphatases are expressed in corals. Particular attention should be paid to phosphatase activities in corals thriving in DIN-enriched conditions to assess possible P limitation or P starvation of N-enriched corals.

In this study, we assessed the cellular CNP composition and the activities of intra- and extracellular alkaline (APA) and intracellular acid phosphatases (AcPA) of the coral host and symbionts associated with *Stylophora pistillata* exposed to different DIP and DIN conditions. Conditions were the following: 1) nutrient depletion, with undetectable level of DIN and DIP, 2) long-term (3 days) DIN enrichment with 2 μ M ammonium or nitrate, 3) short-term (1h pulse) of DIN enrichment with 2 μ M ammonium or nitrate.

This study addresses two questions:

What is the effect of seawater DIP depletion on the cellular P content and phosphatase activities of the corals?

We observed that DIP depletion enhanced the extracellular APA after one day of depletion, suggesting that corals have to rely on DOP to fulfil their P requirements. However, after three days of phosphorus depletion, a significant collapse in the extracellular APA was observed. Similarly, the cellular phosphorus content, as well as the nitrogen content in the symbiotic dinoflagellate collapsed after three days of nutrient depletion. This suggests that corals are very sensitive to P shortage in seawater as they do not have sufficient internal P reserves to cope with prolonged nutrient deficiency.

Does ammonium enrichment have a different effect than nitrate on the cellular P content and phosphatase activities?

We observed different outcomes on extracellular APA and elemental composition depending on the form of DIN enrichment. Ammonium enrichment prevented cellular phosphorus depletion in coral tissue by promoting the assimilation of phosphorus via increased extracellular APA. This can explain why ammonium enrichment was found to be rather beneficial to scleractinian coral physiology (Ezzat *et al.*, 2015; Marangoni *et al.*, 2020). On the other hand, nitrate induced cellular phosphorus depletion in the coral tissue due to a lack of enhancement of the extracellular APA. Such lack of APA enhancement may be due to the fact that the energy needed to produce and release phosphatase enzymes is rather used to reduce nitrate into ammonium. This led to an imbalance cellular N:P ratio that may explain the impairment of coral physiology under nitrate enrichment.

In summary, this study has shown that although DIN enrichment increases the phosphorus demand of corals, the nitrogen identity can affect the coral ability to obtain phosphorus. Ammonium enrichment enhances phosphorus assimilation via DOP hydrolysis by

phosphatases, maintaining a balanced cellular N:P ratio. In contrast, nitrate enrichment restricts the coral access to the DOP pool of phosphorus via limited phosphatase activity, resulting in an increased imbalanced cellular N:P ratio. Thus, unlike ammonium, nitrate enrichment tends to promote coral phosphorus starvation, which explains the differential coral response to nitrate (negative) and ammonium (neutral/positive) enrichment (Ezzat *et al.*, 2015; Burkepile *et al.*, 2020; Marangoni *et al.*, 2020). Overall, our results contribute to the critical understanding of the importance of phosphorus demand in symbiotic corals, especially with an imbalanced DIN:DIP ratio following DIN enrichment.

II Publication

Nutrient starvation and nitrate pollution impairs the assimilation of dissolved organic phosphorus in coral-Symbiodiniaceae symbiosis

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II.1. Abstract

Phosphorus (P) is an essential but limiting nutrient for coral growth due to low concentrations of dissolved inorganic concentrations (DIP) in reef waters. P limitation is often exacerbated when concentrations of dissolved inorganic nitrogen (DIN) increase in the reef. To increase their access to phosphorus, corals can use organic P dissolved in seawater (DOP). They possess phosphatase enzymes that transform DOP into DIP, which can then be taken up by coral symbionts. Although the concentration of DOP in reef waters is much higher than DIP, the dependence of corals on this P source is still poorly understood, especially with different concentrations of DIN in seawater. As efforts to predict the future of corals increase, improved knowledge of the P requirements of corals living under different DIN concentrations may be

key to predicting coral health. In this study, we investigated P content and phosphatase activities (PAs) in *Stylophora pistillata* maintained under nutrient starvation, long-term nitrogen enrichment (nitrate or ammonium at 2 μM) and short-term (few hours) nitrogen pulses. Results show that under nutrient depletion and ammonium-enriched conditions, a significant increase in PAs was observed compared to control conditions, with no change in the N:P ratio of the coral tissue. On the contrary, under nitrate enrichment, there was no increase in PAs compared to control conditions, but an increase in the N:P ratio of the coral tissue. These results suggest that under nitrate enrichment, corals were unable to increase their ability to rely on DOP and replenish their cellular P content. An increase in cellular N:P ratio is detrimental to coral health as it increases the susceptibility of coral bleaching under thermal stress. These results provide an overall view of the P requirements of corals exposed to different nutrient conditions and improves our understanding of the effects of nitrogen enrichment on corals.

II.2. Introduction

Nitrogen (N) and phosphorus (P) are key nutrients for plant and animal growth in terrestrial and aquatic ecosystems (Elser *et al.*, 2007), as they are involved in cell structure, transfer of genetic information or energy transduction, among other processes (Hecky & Kilham, 1988; Lehninger *et al.*, 1993; Canfield *et al.*, 2005). However, in marine ecosystems such as coral reefs, dissolved inorganic and organic N and P (DIN/DON and DIP/DOP respectively) are present in low concentrations (Stambler, 1999), limiting the potential for growth and reproduction of animals such as reef-building corals. To overcome nutrient limitation, corals have evolved as metaorganisms or holobionts (Rohwer *et al.*, 2002; Bosch & McFall-Ngai, 2011). They have established symbiotic relationships with microorganisms such as bacteria and photosynthetic dinoflagellates from the Symbiodiniaceae family (LaJeunesse *et al.*, 2004, 2018), allowing the coral holobiont to benefit from all nutrient sources. While some bacteria are involved in nitrification, denitrification, nitrogen fixation and dissimilatory nitrate reduction to ammonium (DNRA) (Neave *et al.*, 2016; Benavides *et al.*, 2017; Glaze *et al.*, 2021), Symbiodiniaceae drive the uptake and assimilation of DIN and DIP (Grover *et al.*, 2002; Kopp *et al.*, 2013). In turn, the coral host can take up the particulate and dissolved organic forms (Grover *et al.*, 2002, 2008). The Symbiodiniaceae also supply the host with photosynthetic products, which can provide up to 90% of the coral's energy requirement (Falkowski *et al.*, 1984).

A healthy symbiotic relationship between corals and their algal symbionts depends on adequate nutrient supply and, in particular, on a balanced stoichiometry (relative abundance) of DIN:DIP in seawater (Wiedenmann *et al.*, 2013). Yet, phosphorus inputs in reefs are generally low compared to N inputs, leading to a strong stoichiometric imbalance between dissolved N and P in many reef ecosystems (reviewed in D'Angelo & Wiedenmann, 2014). In particular, DIN can accumulate in coastal reefs following natural events (upwellings, nitrogen fixation, nitrification, wind mixing or mobilization from sediments) and anthropogenic (agricultural and river runoff) disturbances (Bell *et al.*, 1999; Lourey *et al.*, 2001; Radenac *et al.*, 2001; Houk *et al.*, 2020). An imbalanced DIN:DIP ratio in seawater, following nitrogen enrichment, is a major threat to scleractinian corals (Fabricius, 2005; D'Angelo & Wiedenmann, 2014; Duprey *et al.*, 2016; Rosset *et al.*, 2017; Ezzat *et al.*, 2019). Indeed, P deficiency reduces the photosynthetic capacity of corals and can impair the structure of phospholipid membranes of dinoflagellates, lowering temperature and light thresholds for coral bleaching (D'Angelo & Wiedenmann, 2014; Ezzat *et al.*, 2016a; Rosset *et al.*, 2017; Morris *et al.*, 2019). Prolonged bleaching, i.e. the loss of symbiotic dinoflagellates and/or photosynthetic pigments (Douglas, 2003), leads to coral starvation, as dinoflagellates are the main source of nutrients and energy for corals. As efforts to predict the future of coral ecosystems increase, better knowledge of the phosphorus requirement and assimilative capacity of phosphorus by corals living under different DIP and DIN concentrations may be key to predicting coral health.

In addition to their heterotrophic diet, corals have access to DIP and DOP mainly through their symbiotic dinoflagellates and associated microbes. While DIP occurs in nanomolar concentrations in reef waters (Bell *et al.*, 2007; Morrison *et al.*, 2013), DOP compounds can account for 70–90% of the total dissolved phosphorus pool (Ruttenberg & Dyrman, 2012). However, DOP must be hydrolysed by phosphatase enzymes to DIP before corals can use it. High phosphatase activity is considered a good indicator of P scarcity in the environment (Annis & Cook, 2002), as it occurs either at low DIP concentrations (< 20 nM for phytoplankton) or at elevated ratios of DIN:DIP (Cembella *et al.*, 1984; Rees *et al.*, 2009; Suzumura *et al.*, 2012). Coral holobionts have intracellular and extracellular acid (AcPA) and alkaline phosphatase (APA) activities (Atkinson, 1987; Godinot *et al.*, 2013; Wiedenmann *et al.*, 2013), but there is still limited knowledge on the regulation of their activity. A few studies have shown that extracellular APA is enhanced under DIP deficiency or during bleaching (Godinot *et al.* 2013). One study also measured an increase in intracellular APA of the Symbiodiniaceae under nitrate enrichment, suggesting that nitrate assimilation may trigger P deficiency (Wiedenmann *et al.*, 2013). This is consistent with soil studies where it was

observed that total N was the strongest factor explaining variations in phosphatase activities (Margalef *et al.*, 2017). Thus, studies are urgently needed to better understand the conditions under which phosphatases are expressed in corals. Particular attention should be paid to phosphatase activities in corals thriving in DIN-enriched conditions to assess possible P limitation or P starvation of N-enriched corals.

In this study, we reported the activity of intra- and extracellular alkaline (APA) and intracellular acid phosphatases (AcPA) of the coral host and Symbiodiniaceae exposed to different DIP and DIN conditions. Our study addresses two questions. First, what are the effects of seawater P depletion on the cellular P content and phosphatase activities of the corals? Second, does ammonium enrichment have a different effect than nitrate on the cellular P content and phosphatase activities? We hypothesise that corals maintained under P depletion or N enrichment would present a deficiency in cellular P content and would increase their phosphatase activities compared to natural seawater conditions. We also hypothesise that N enrichment in the form of nitrate or ammonium would have a different effect on phosphatase activities, as they also induce a different physiological response in corals (Ezzat *et al.*, 2015; Marangoni *et al.*, 2020). This study provides an overall view of the P requirements of corals maintained under different nutritional conditions and improves our understanding of the effects of nitrification on corals.

II.3. Material & Method

Ten colonies of the scleractinian coral *Stylophora pistillata* were used to generate 300 nubbins (30 nubbins per colony), which were identified and maintained in six large open-flow aquaria for three weeks to heal. Aquaria received a continuous supply of natural seawater (0.5 μM DIN and 0.2 μM DIP) pumped at a depth of 50 m in front of the laboratory. During healing, nubbins were fed *Artemia* nauplii twice weekly, and maintained under photosynthetically active radiation (PAR) of $200 \pm 10 \mu\text{mol photons m}^{-2} \text{s}^{-1}$. PAR was provided by 400 W halide metal lamps (HPITS, Philips) mounted above the aquaria. Intensity was measured with a ULM-500 (Heinz Walz GmbH, Germany) data logger equipped with a quantum PAR sensor (US-SQS/L). Temperature was monitored with heaters connected to an Elliwell PC 902/T controller and seawater mixing was ensured with water pumps. After healing, feeding was stopped and nubbins were kept in this condition for two weeks before being used in the different experiments detailed below.

In the following experiments, nubbins from different mother colonies were divided among three aquaria per condition, under similar light level and temperatures as described above. They were rotated between the three aquaria every day to ensure homogeneity within a condition.

II.3.a. Experimental conditions

For the first experiment (summarised in Fig 24), we tested whether extracellular APA was produced by the mucus-associated microorganisms or by the coral tissue. For this purpose, ten nubbins were selected from ten different colonies and aquaria to be incubated in the light (as above) and 25 °C in individual beakers: 1) Five nubbins were incubated for 2 hours before starting the measurements of APA production by the whole holobiont 2) Five other nubbins were incubated for 2 hours during which they released mucus. Mucus and nubbins were separated by transferring nubbins in five new beakers, and APA production by the mucus or coral alone was then measured. Five control beakers with only seawater were also run in parallel. Phosphatase assay was done, as described below.

For the second experiment (summarised in Fig 24), we tested the effect- of nutrient depletion on the APA and AcPA. To this end, 75 coral nubbins were selected from 10 different colonies and aquaria and equally distributed into 6 small aquaria (same light and temperature as above). Three aquaria were maintained under control conditions with a continuous supply of natural seawater (0.5 μM DIN and 0.2 μM DIP). The three other aquaria were maintained under nutrient-depleted condition (undetectable levels of DIN and DIP). Nubbins thus received a continuous supply of seawater passed through an initial aquarium containing several large colonies of *Stylophora pistillata*. These colonies were able to quickly consume nutrients contained in the seawater. DIN and DIP concentrations were measured every day using an AA3 Seal Autoanalyzer according to Aminot et al. (2009). After 3 days of incubation, 6 nubbins per condition (control/depleted) were sampled for the CNP measurements. 10 nubbins per condition were also sampled after 1, 3 and 5 days and incubated in individual beakers for the phosphatase assay (APA and AcPA) as described below.

For the third experiment (summarised in Fig 24), we tested the effect of a long-term DIN enrichment on the APA and AcPA. To this end, 80 coral nubbins were selected from 10 different colonies and equally distributed into 9 small aquaria (same light and temperature as above). They were maintained in 3 control aquaria (continuous supply of natural seawater, 0.5 μM DIN and 0.2 μM DIP) or in 3 nitrate or ammonium-enriched aquaria. Nutrient enrichment was achieved by enriching seawater with 2 μM nitrate (NO_3^-) or 2 μM ammonium (NH_4^+), continuously supplied to the aquaria with a peristaltic pump. The chosen levels of nitrate and

ammonium enrichment were consistent with those found in eutrophic reefs (Costa *et al.*, 2000; Brodie *et al.*, 2011; Govers *et al.*, 2014). Nitrogen concentrations were measured every day as described above. After 3 days of incubation, 6 nubbins per condition (control, ammonium and nitrate) were sampled for the CNP measurement. 10 nubbins per condition were also sampled after 1 and 3 days and incubated in individual beakers for the phosphatase assay (APA and AcPA) as described below.

For the fourth experiment (summarised in Fig 24), we tested the effect of a short-term DIN enrichment on the APA and AcPA.

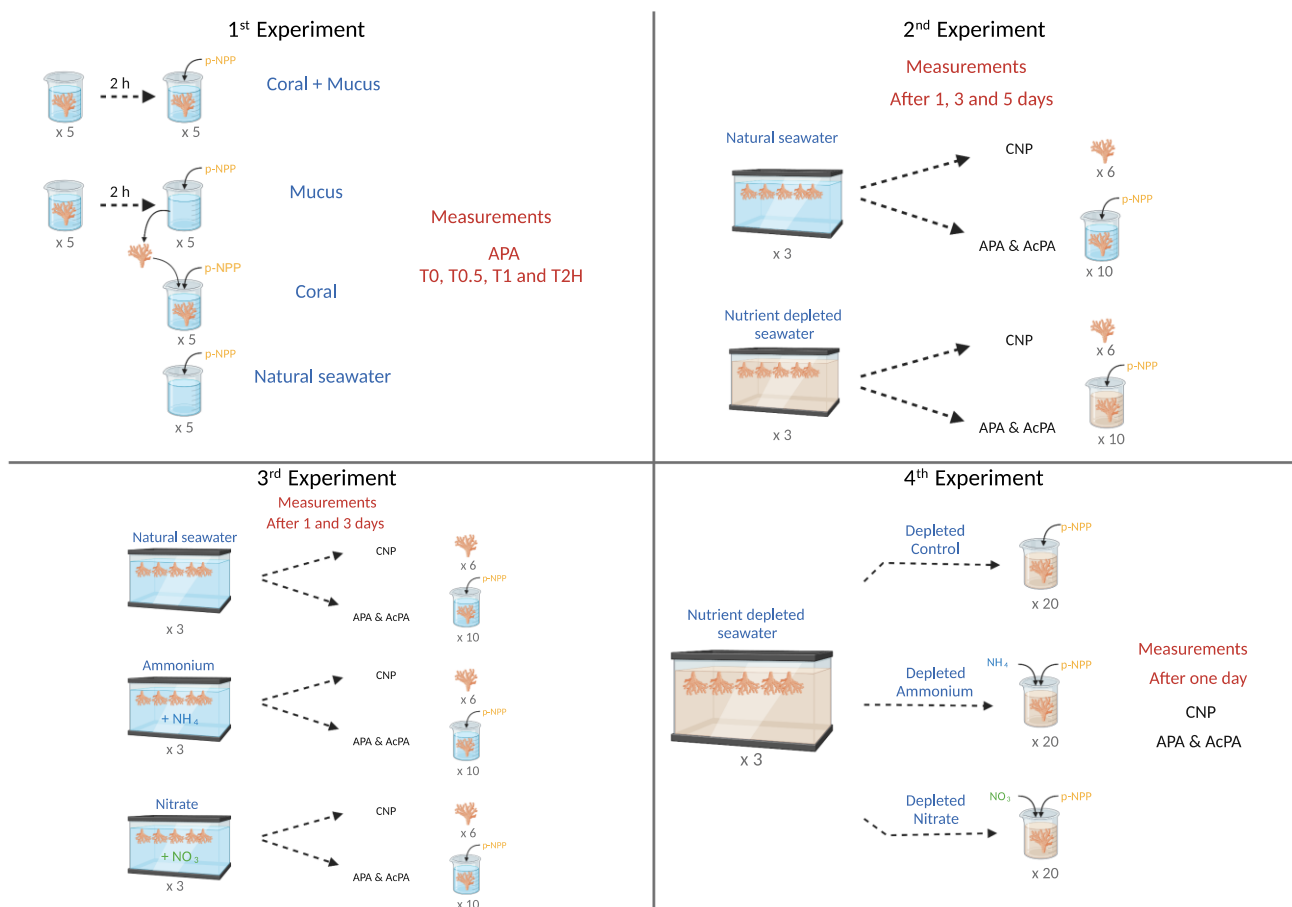


Figure 24. Experimental design for extracellular alkaline phosphatase activity (APA) measurement

First experiment: Location of the production of extracellular APA in the coral holobiont in 4 conditions with 5 replicates each: **Coral + Mucus**, **Mucus alone**, **Coral alone** and **Natural seawater** alone as a control

Second experiment: Impact of nutrient depletion on the APA and AcPA in corals and their elemental composition

Third experiment: Impact of nitrogen enrichment the APA and AcPA in corals and their elemental composition, with either ammonium (NH_4) or nitrate (NO_3)

Fourth experiment: Impact of nitrogen enrichment pulse in nutrient depleted seawater on the APA and AcPA in corals and their elemental composition

The activity of the extracellular alkaline phosphatase was assessed by a colorimetric assay. Para-nitrophenyl phosphate (**p-NPP**) was added at a final concentration of 250 μM in each beaker. The phosphatase will hydrolyse p-NPP into inorganic phosphorus (orthophosphate) and para-nitrophenol (p-NP) that will give the solution a yellow taint. The absorbance of the solution is read on a spectrophotometer at 400 nm and represent the production of p-NP, proxy for extracellular APA rate.

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To this end, 60 coral nubbins were selected from 10 different colonies and equally distributed into 3 small aquaria (same light and temperature as above). They were maintained in nutrient-depleted seawater for one day. After nutrient depletion, one-third of the nubbins (20) were incubated in individual beakers with seawater (control) for phosphatase assay, as described below. The other two-third were incubated for 1 h in seawater enriched with 2 μM nitrate (20) or ammonium (20) before phosphatase activities were measured.

II.3.b. Phosphatase assay

Phosphatase activities were measured using para-Nitrophenyl phosphate (pNpp) as DOP substrate according to Hernandez et al. (1992). In this method, pNpp is hydrolysed by phosphatases into hydrogen phosphate (HPO_4^{2-}) and para-Nitrophenol (pNp). After the pH has been raised to 11 by adding NaOH, pNp displays a yellow coloration, the absorbance of which can be measured at 400 nm with a spectrophotometer. The production of pNp is proportional to the consumption of pNpp, which allows the evaluation of phosphatase activity. In this study, preliminary experiments were performed (full description in SI, Appendix III) to determine the pNpp concentration and incubation time to obtain maximum APA and AcPA. With a substrate concentration of 250 μM pNpp and an incubation time of either 8h for the extracellular phosphatases and 2h for the intracellular phosphatases, a significant and reliable measurement of phosphatase activities was obtained (Fig S1, Appendix III). The following experiments were therefore performed under the above conditions.

II.3.b.i. Extracellular alkaline phosphatase assay

Coral nubbins (10 nubbins per condition) were pre-incubated for 30 minutes in individual beakers filled with 200 mL of filtered seawater (FSW), with the correct nutrient concentrations as described above (control, nutrient enriched or nutrient depleted). Seawater was stirred continuously with a stir plate. The temperature was kept constant at 25°C using a water bath and the irradiance was adjusted to 200 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ using HQI lamps. After 30 minutes and once the coral polyps were fully expanded, substrate pNpp was added to each beaker at a final concentration of 250 μM . Immediately after the addition of pNpp and every 2 hours for 8 hours, subsamples of 1 mL seawater were taken. They were filtered through a 0.45 μm filter syringe, placed in an Eppendorf® tube containing NaOH at a final concentration of 80 mM, vortexed and centrifuged at 11 000g for 5 minutes. Absorbance was read at 400 nm in 96-well plates using a spectrophotometer (Xenius®, SAFAS, Monaco). APA was calculated from the production of pNp during the 8-hour incubation and according to a standard curve generated with a known concentration of pNp. Corals were then used for the intracellular phosphatase assay, as described below.

II.3.b.ii. Intracellular acid and alkaline phosphatase assay

Corals were air brushed in a Tris buffer at pH 5 for acid phosphatase (5 nubbins per condition) or pH 9 for alkaline phosphatase (5 nubbins per condition). The host and dinoflagellate fractions were separated by centrifugation for 10 minutes at 5 000g. Dinoflagellate pellets were washed 3 times with 5 mL of the appropriate Tris buffer (pH 5 or 9), sonicated for 4 minutes and centrifuged at 5000g for 5 minutes. Host and symbiont fractions were transferred to Falcon® tubes to which pNpp was added at a final concentration of 250 µM. The tubes were then incubated at 25°C in a dry bath. Subsamples were taken immediately after the addition of pNpp and after 0.5 h, 1 h and 2 h and then treated as described above. Coral skeletons were preserved for normalization by surface area determination using the single dip wax technique (Stimson & Kinzie III, 1991).

II.3.c. Carbon, Nitrogen, and Phosphorus content

6 nubbins per experimental condition (corals maintained 3 days in nutrient depleted, nutrient enriched and control condition, Fig 24) were flash-frozen in liquid nitrogen and stored at -80°C for later analysis of their CNP content. For this purpose, nubbins were thawed, and air brushed in Milli-Q water. Samples were centrifuged at 5 530 g for 15 minutes at 4 °C to separate the dinoflagellate fraction (pellet) and the animal tissue (supernatant). The dinoflagellate pellet was washed three times with 5 mL Milli-Q water. The dinoflagellate and coral tissue fractions were frozen at -80 °C before being freeze-dried using an Alpha 2-4 LD Plus Freeze Dryer (Chris Martin Scientific, UK).

II.3.c.i. Phosphorus content

The P content was analysed using the persulphate digestion method according to Van Veldhoven and Mannaerts (1987). 3 mg of the freeze-dried animal tissue and 1 mg of the dinoflagellate fraction were diluted in 2M NaCl. The organic compounds in the samples were digested with a persulphate potassium solution by autoclaving at 121 °C (Menzel & Corwin, 1965). The orthophosphate released during digestion was determined with a colorimetric assay by adding ammonium molybdate and ten minutes later malachite green. Absorbance was measured at 630 nm using a spectrofluorometer (Xenius®, SAFAS, Monaco). The standard curve was prepared with known concentrations of potassium phosphate.

II.3.c.ii. Carbon and Nitrogen content

The same nubbins used for P determination were also used for C and N analysis. For this purpose, 1 mg of the freeze-dried samples was placed in a tin cap and analysed with an EA-IRMS Integra2 (Sercon, England).

II.3.d. Statistical analysis

Statistical tests were performed using the free open-source software R. Due to the relatively small number of samples – 9 to 10 samples for extracellular APA and 4 to 5 samples for intracellular phosphatase activities – the non-parametric Wilcoxon-Mann Withney test was used to compare phosphatase activities between conditions.

II.4. Results

II.4.a. APA activity in the mucus and coral tissue

The extracellular alkaline phosphatase activity produced by the coral alone and the coral holobiont (coral + mucus) condition are similar (Fig S2, Table S1 p value: 0.78, Appendix III), while the enzyme activity in the mucus condition is null (Fig S2, Table S1, Appendix III).

II.4.b. Effect of nutrient depletion on phosphatase activity

The results of the statistical tests are reported in Table S2a, b, Appendix III.

Overall, the APA (p value: 0.06) and AcPA (p value: host 0.48, dinoflagellate 0.14) measurements were similar in all control conditions performed in the different assays, suggesting that it is a conserved basal activity in our culture conditions (Fig 25, Table S2a, Appendix III). After 1 day in nutrient depleted seawater, extracellular APA was 50% higher than in the control condition (Fig 25a, p value: 0.01). It then decreased to the control level after 3 days of nutrient depletion (Fig 25a, p value: 0.47) and 80 % below control level after 5 days of depletion (Fig 25a, p value: < 0.00001).

The intracellular AcPA of dinoflagellates followed a similar trend as the extracellular APA. It was 50 % higher than in the control level after 1 day under nutrient-depleted conditions (Fig 25b, p value: 0.02), but showed a 70% decrease below control level after 3 days (Fig 25b, p value < 0.04). In animal tissue, intracellular AcPA was at least 50 % lower under nutrient-depletion than under control conditions after 1 and 5 days (Fig 25c, p value < 0.03). Overall, there was no major change in intracellular APA (Fig S3, Appendix III).

After three days of nutrient depletion, the phosphorus content of the symbiotic dinoflagellate was reduced by 30 % compared to the control condition (Fig 26a, p value 0.01), while its nitrogen content (Fig 26b, p value 0.09) remained relatively constant. It leads to a non-significant increase in the internal N:P (Fig 26c, p value: 0.06). The N and P stoichiometry of the host tissue did not change with nutrient depletion (Fig S4, Table S3, Appendix III).

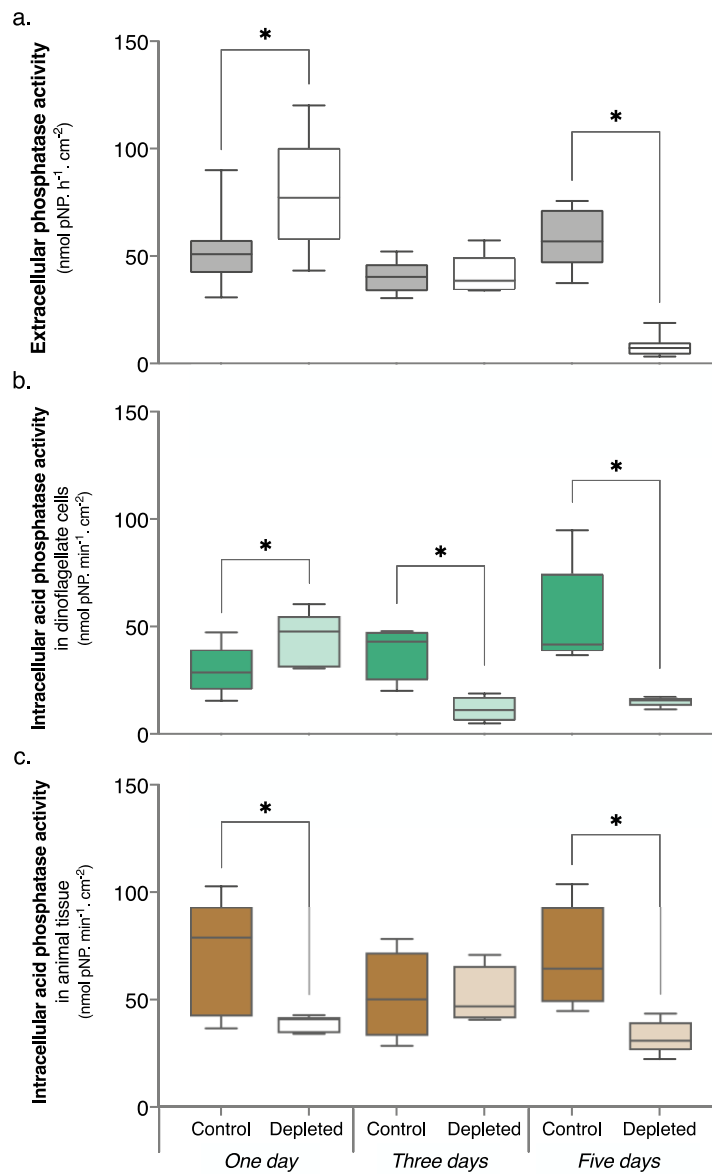


Figure 25. Activity of the phosphatases under nutrient depletion condition

Boxplots show the activity of extracellular alkaline phosphatase in grey (a.) and intracellular acid phosphatase in dinoflagellate cells in green (b.) and in animal tissue in brown (c.) normalised to skeletal surface area. Measurements were taken after one, three and five days either under natural seawater (**Control**, darker box) or under nutrient deficiency (**Depleted**, lighter box, undetected amount of dissolved inorganic phosphorus and nitrogen). The box delimits the first and third quartile, the black bar represents the median and whiskers represent minimum and maximum values, extracellular $n = 6 - 10$, intracellular $n = 5$. Statistically significant differences are marked with an asterisk.

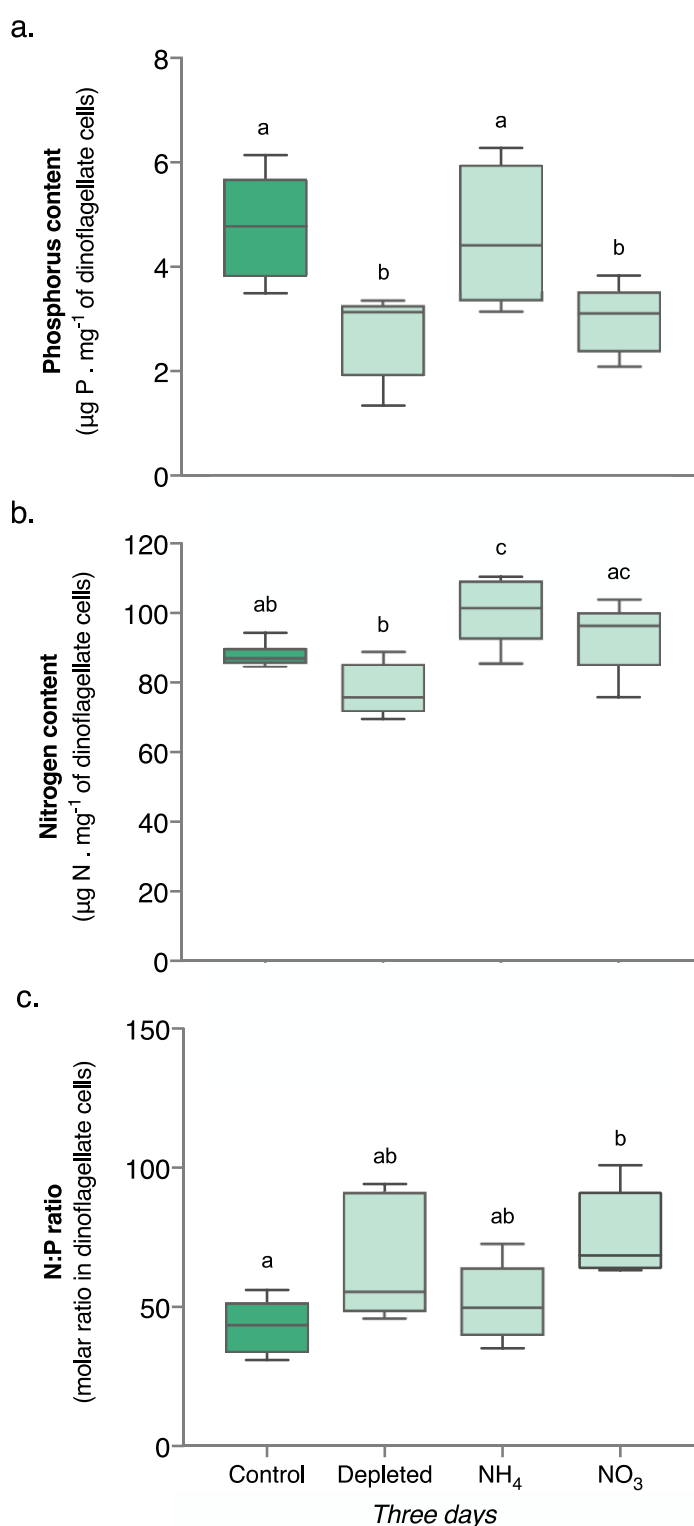


Figure 26. Composition of the dinoflagellate in phosphorus and nitrogen and its nitrogen to phosphorus ratio Boxplots show dinoflagellate phosphorus (a.) and nitrogen (b.) content and their nitrogen-phosphorus ratio (c.) under natural seawater (**Control**, dark green), nutrient depletion (**Depleted**, undetected amount of dissolved inorganic phosphorus and nitrogen), ammonium enrichment (**NH_4** , at $2 \mu\text{M}$) and nitrate enrichment (**NO_3** , at $2 \mu\text{M}$). Measurements were taken after three days under the different experimental conditions. The box delimits the first and third quartile, the black bar represents the median and whiskers represent minimum and maximum values, $n = 6$. Statistically significant differences are represented by different letters (a, b and c).

II.4.c. Effect of long-term nitrogen enrichment on the phosphatase activity

The results of the statistical tests are reported in Tables S4 and Table S5, Appendix III.

Overall, the APA (p value: NH_4 0.06 and NO_3 0.67) and AcPA (p value: host 0.11 and 0.41, dinoflagellate 0.12 and 0.24) measurements were similar in all control conditions during this experiment (Fig 27, Table S4a and S5a, Appendix III). Ammonium enrichment induced a doubling of the extracellular APA after 1 day (p value: 0.004) and a 50 % increase after 3 days (p value: 0.01) (Fig 27a). After a 3-day ammonium enrichment, intracellular AcPA remained unchanged in the dinoflagellate cells (Fig 27b, p value: 0.44) but decreased by half in the animal tissues compared to the control (Fig 27c, p value: 0.014).

Nitrate enrichment did not induce any change in extracellular APA (Fig 27d, p value: 1 day 0.08, 3 days 0.21). After 3 days of nitrate enrichment, intracellular AcPA decreased by 50 % in both dinoflagellate cells (Fig 27e, p value: 0.04), and host tissue (Fig 27f, p value: 0.04) compared to control conditions. There were no significant changes in intracellular APA under both ammonium and nitrate enrichment (Fig S5, Appendix III).

While ammonium enrichment did not change the phosphorus content of the dinoflagellate (Fig 26a, p value 0.78) it significantly increased its nitrogen content (Fig 26b, p value 0.02). However, the N:P ratio did not increase significantly (Fig 26c, p value 0.39). Under nitrate enrichment the phosphorus content of the dinoflagellates was significantly decreased (Fig 26b, p value 0.02), while its nitrogen content remained unchanged (Fig 26a, p value 0.26). These changes led to a significant 50 % increase in N:P ratio of the dinoflagellate (Fig 26c, p value 0.03).

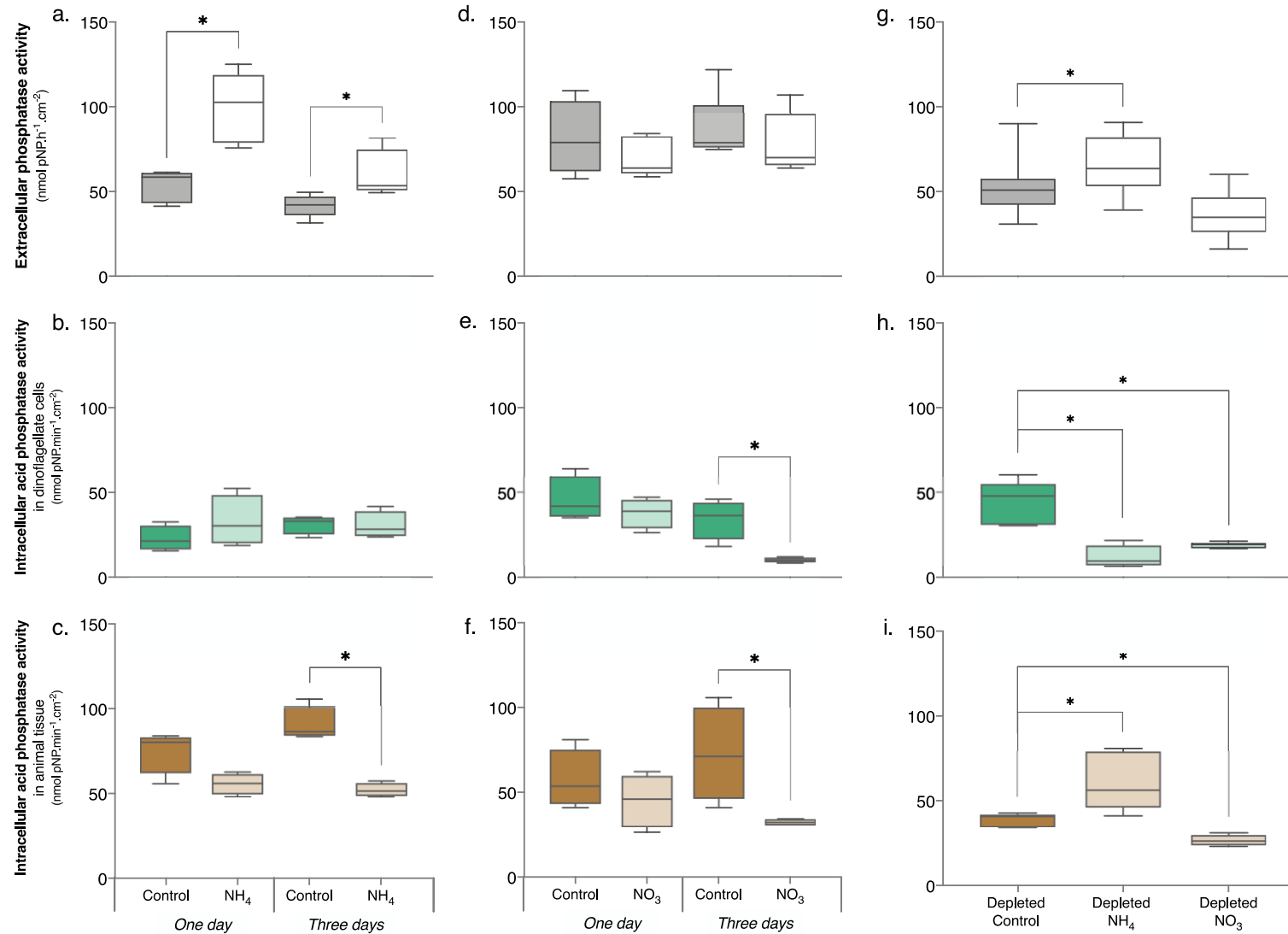


Figure 27. Activity of the extracellular APA and intracellular AcPA under nitrogen enrichment

Boxplots show the activity of extracellular alkaline phosphatase in grey (a., d., g.) and intracellular acid phosphatase in dinoflagellate cells in green (b., e., h.) and in animal tissue in brown (c., f., i.) normalised to skeletal surface area. Measurements were done after one and three days in natural seawater (**Control**) or under enrichment in 2 μM ammonium (**NH₄**) (a., b., c.) and nitrate (d., e., f.) and after one day either under nutrient-depleted seawater alone (**Depleted Control**) or under nutrient-depleted seawater in combination with a 2 μM nitrogen enrichment pulse with either ammonium (**Depleted NH₄**) or nitrate (**Depleted NO₃**) normalised to skeletal surface area. The box delimits the first and third quartile, the black bar represents the median and whiskers represent minimum and maximum values, extracellular n = 5, intracellular n = 4. Statistically significant differences are marked with an asterisk.

II.4.d. Effect of a nitrogen pulse on phosphatase activity

The results of the statistical tests are reported in Table S6, Appendix III.

In nutrient-depleted corals, a 2 μ M ammonium pulse significantly increased the extracellular APA compared to the control level (nutrient-depleted seawater) (Fig 27a, p value: 0.032). The ammonium pulse significantly decreased intracellular AcPA below control level in dinoflagellate cells (Fig 27b, p value: 0.004), while it increased AcPA in animal tissues (Fig 27c, p value: 0.006).

In nutrient-depleted corals, a 2 μ M nitrate pulse significantly decreased extracellular APA compared to the control level (Fig 27a, p value: 0.007). In both dinoflagellate cells and animal tissues, the nitrate pulse significantly decreased intracellular AcPA below control levels (Fig 27b, c, p value: dinoflagellates 0.004 and animal tissues 0.006). The intracellular APA showed the same pattern under both ammonium and nitrate enrichment. In animal tissue, nitrogen enrichment significantly decreased APA below control levels (Fig S6, Appendix III) whereas in dinoflagellate cells, nitrogen enrichment significantly increased APA above control levels (Fig S6, Appendix III).

II.5. Discussion

The aim of this study was to investigate the phosphorus requirements of a model scleractinian coral exposed to different nitrogen concentrations and forms in seawater by analysing intracellular and extracellular phosphatase activities. Overall, we found that the extracellular APA was due to the coral - dinoflagellate association and not to the microbes associated with the mucus. The increase in extracellular APA and intracellular AcPA of the symbionts during short-term nutrient deficiency suggests that the corals were lacking phosphorus, and that phosphatase activity is a good proxy for P depletion in corals, in agreement with phytoplankton studies. In turn, the higher APA and AcPA with short and long-term ammonium enrichment suggest that corals have higher P requirements when they are N-repleted. However, our study also demonstrates that phosphatase activities are not regulated in a similar way by ammonium and nitrate enrichment, since this later did not increase APA and AcPA compared to control conditions, although it induced a significant decrease in cellular P content. Overall, the changes in phosphatase activity reflect changes in coral phosphorus requirements that can have significant implications for coral health.

The results of the first experiment have shown that most of the extracellular APA was due to the activity of the coral-dinoflagellate association and not to the mucus-associated

microbes. It is therefore an indicator of the coral's nutritional status and varies with the coral's nutrient requirements, as previously observed (Godinot *et al.*, 2013). Under control conditions, we measured significant extracellular APA when corals are provided with dissolved organic phosphorus (about 60 nmol pNp h⁻¹ cm⁻², or 100-120 nmol h⁻¹ mg⁻¹ protein), which is in the same range as previously measured APA values (Godinot *et al.*, 2013). Such activity suggests that the coral holobiont can efficiently use organic P when it is present in seawater. Although natural DOP concentrations in reefs are relatively low, ranging from 0.06 to 0.54 μM (Karl & Björkman, 2015), corals may be exposed to high DOP concentrations during sediment resuspension (Miyajima *et al.*, 1998), plankton decomposition, or exposure to river discharges (Baird *et al.*, 2021). Furthermore, phosphatases are involved in the degradation of prey in the gastric cavities of corals, where concentrations of dissolved organic P can thus be very high, above 40 μmol L⁻¹ (Agostini *et al.*, 2012). Under control conditions, a basic level of intracellular AcPA was also observed. This enzymatic activity suggests that transport and intracellular hydrolysis of small organophosphate molecules occurs in both coral host cells and symbionts.

Nutrient deficiency (i.e. undetectable levels of DIP and DIN in seawater) induced a significant and rapid decrease in the cellular P content of the symbionts after only 3 days, suggesting that the corals can become P limited very quickly (Fig 28). P starvation was shown to inhibit cell division (Li *et al.*, 2016), to accelerate ATP cycling and repress sulfolipid degradation in free-living dinoflagellates, among other processes (Vanucci *et al.*, 2010; Shi *et al.*, 2017; Zhang *et al.*, 2019). In this experiment, nutrient deficiency had different effects on phosphatase activities depending on the duration of starvation. A one-day nutrient starvation resulted in an increase in external APA and internal AcPA in the symbionts. An increase in extracellular APA is an indicator of P deficiency in marine organisms such as cyanobacteria (Yentsch *et al.*, 1972; Harke *et al.*, 2012) or planktonic (Hoppe, 2003; Zhang *et al.*, 2019; Lavrinovičs *et al.*, 2021) and symbiotic algae (Annis & Cook, 2002; Wiedenmann *et al.*, 2013). Under these conditions, the organic phosphorus molecules dissolved in seawater become an important P source for the organisms. The increase in intracellular AcPA of symbiont cells after one-day starvation also indicates that the symbionts are able to use their internal pool of organic phosphorus when nutrients are scarce, as previously observed in several species of phytoplankton subjected to P limitation (Dyhrman & Palenik, 1999; Rengefors *et al.*, 2003). The internal pool of organic phosphorus in symbionts consists mainly of organic mono- and diester phosphate and phosphonates, which serve as P storage molecules (Godinot *et al.*, 2016). In host cells, however, we observed a significant decrease in AcPA, which may be due to the

small content of P storage molecules in this compartment. Indeed, the pool of phosphorus in host tissue consists mainly of phosphate and phosphonates (Godinot *et al.*, 2016), on which phosphatases have no impact. It has to be noticed that the increase in both extracellular and intra cellular phosphatases occurred for DIP lower than 0.2 μM , which is the concentration in our control treatment. This finding suggests that corals are adapted to oligotrophic environments and to very low N and P concentrations in seawater.

Long-term depletion (3 to 5 days) of inorganic nutrients in seawater significantly impaired coral metabolism, lead to a decline in both external APA and internal AcPA (Fig 28). The impairment of coral metabolism under nutrient deficiency has been demonstrated in previous studies (Rosset *et al.*, 2017; Ezzat *et al.*, 2019). In this study, coral colonies even died after 10 days of nutrient starvation (results not shown). Thus, the observed decrease in external APA could be due to a lack of sufficient energy for the synthesis of this enzyme, while the decrease in internal AcPA could reflect the rapid depletion of internal P reserves, as suggested by the significant decrease observed in cellular P content of the symbionts. Overall, these results suggest that corals are very sensitive to DIP shortage because they do not have sufficient internal P reserves to cope with prolonged nutrient deficiency. In this experiment, we observed that the autotrophic feeding capacities of the coral holobiont is impaired within 3 days of nutrient deficiency, while corals died from tissue loss after 10 days of nutrient deficiency. As nutrient deficiency in pristine reef ecosystems are expected to increase in the future (Moore *et al.*, 2013), coral loss may also increase.

Nitrogen enrichment, which can occur naturally (upwelling, nitrogen fixation, nitrification, wind mixing or mobilization from sediments) or through wastes from urbanisation and agricultural activities (Fabricius, 2011), had different effects on phosphatase activities depending on which form of N was provided to the corals (Fig 28). In recent years, evidence has accumulated on the fundamentally different physiological responses of corals - in terms of photosynthesis, calcification, and symbiotic stability – to ammonium and nitrate enrichment (Shantz & Burkepile, 2014; Ezzat *et al.*, 2015; Marangoni *et al.*, 2020). While ammonium enrichment is beneficial for coral health, nitrate enrichment is more detrimental (Shantz and Burkepile 2014; Ezzat *et al.* 2015; Marangoni *et al.* 2020). This is partly explained by the different metabolic costs associated with the assimilation of ammonium and nitrate. Ammonium can be directly incorporated into amino acids and proteins by the coral host and dinoflagellates through the action of glutamine synthetase (Pernice *et al.*, 2012; Dagenais-Bellefeuille & Morse, 2013). On the other hand, nitrate must be reduced to ammonium by the nitrate and nitrite reductases, which are found in dinoflagellates and some symbiotic

microorganisms, before it can be assimilated (Miller & Yellowlees, 1989; Grover *et al.*, 2003; Kimes *et al.*, 2010). This reduction is energetically costly and deprives the photosynthetic chain of electrons, impairing the photosynthetic activity of dinoflagellates (Ezzat *et al.*, 2015). Furthermore, due to the presence of oxygen molecules, the assimilation of nitrate increases the oxidative status of the coral holobiont, while ammonium tends to reduce it (Marangoni *et al.*, 2020).

The results obtained in this study show that ammonium enrichment (3 days of continuous enrichment or pulse addition) increases the alkaline phosphatase activity, prevents P depletion in symbiont tissue and maintains a balanced N:P ratio within the coral tissue and Symbiodiniaceae. All together, these results suggest that ammonium promotes the assimilation of phosphorus by corals through increased extracellular phosphatase activity and may explain why ammonium was found to be beneficial to coral physiology in previous study (Ezzat *et al.*, 2015; Marangoni *et al.*, 2020). However, after 3 days of ammonium enrichment, intracellular AcPA in host tissues decreased, indicating that internal P reserves were depleted, as was also observed with long-term nutrient depletion, although the N:P ratio remained unchanged. In contrast to ammonium, nitrate enrichment significantly decreased AcPA and did not increase extracellular APA, probably due to a lack of energy to simultaneously assimilate nitrate and synthesise phosphatases. Indeed, in the algae *Phaeocystis*, it has been shown that the energy and organic carbon required by the algae to synthesise alkaline phosphatase comes from photosynthesis in the light and photosynthetically fixed carbon in the dark (Lancelot *et al.*, 1986; Van Boekel & Veldhuis, 1990). As coral photosynthesis is significantly impaired under nitrate enrichment (Ezzat *et al.*, 2015), the synthesis of phosphatases is likely impaired, and DOP cannot be a significant source of P for corals. The decrease in cellular P content under nitrate enrichment, together with the imbalanced N:P ratio of coral tissue confirms the limitation in P availability in seawater and could also explain the impairment of coral physiology observed in previous studies (Wiedenmann *et al.*, 2013; Ezzat *et al.*, 2016a; Rosset *et al.*, 2017). Different effects of ammonium and nitrate on phosphatase activity have also been observed in sediment, soil and phytoplankton studies. While ammonium increased APA in lake sediments (Ma *et al.*, 2018) and both APA and AcPA in floodplain soils, nitrate had no effect on these activities (Arenberg & Arai, 2021). This difference was also due to the lower energy cost of ammonium uptake for soil bacteria. In phytoplankton, alkaline phosphatases were observed to respond to the form of available N rather than to changes in DIN:DIP ratios (Fitzsimons *et al.*, 2020).

II.6. Conclusion

A deficiency of dissolved inorganic nutrients (DINut), especially phosphorus, in reef waters has potentially broad ecological effects on coral reefs. It can disrupt reef ecosystems, increase the severity of coral disease (Vega Thurber *et al.*, 2014), and decrease coral resistance to thermal stress (Vega Thurber *et al.*, 2014; Zhao *et al.*, 2021). Nutrient deficiency occurs when heat waves stratify the water column and remove DINut from surface waters. This study shows that the lack of DINut in seawater also affects the ability of corals to break down dissolved organic matter to take up available N and P. Combined with the findings of a previous study (Ezzat *et al.*, 2019) that showed impairment of the heterotrophic feeding capacity of corals under DINut deficiency, we can expect severe coral starvation in the future, along with the increasing frequency and intensity of heat waves. Furthermore, phosphorus deficiency can occur when anthropogenic nitrogen inputs to reef ecosystems from agricultural activities, erosion, and wastewater discharges alter the N:P ratio. Phosphorus deficiency has previously been shown to disrupt the photosynthetic capacity of coral symbionts and increase coral susceptibility to bleaching (Wiedenmann *et al.*, 2013). This study further suggests that nitrate enrichment increases phosphorus starvation in corals by inducing a depletion in their cellular P reserves and by suppressing their capacity to increase phosphatase production. Overall, long-term nitrogen enrichment, either by ammonium or nitrate, reduces the corals' internal P reserves and promotes P deficiency. Because coral species differ in their ability to store and utilize P, they could have different levels of P limitation. This remains to be investigated to fully understand the differences in coral susceptibility to bleaching following thermal stress and imbalanced N:P ratios. Overall, our results contribute to the critical understanding of the importance of phosphorus in the functioning of symbiotic corals.

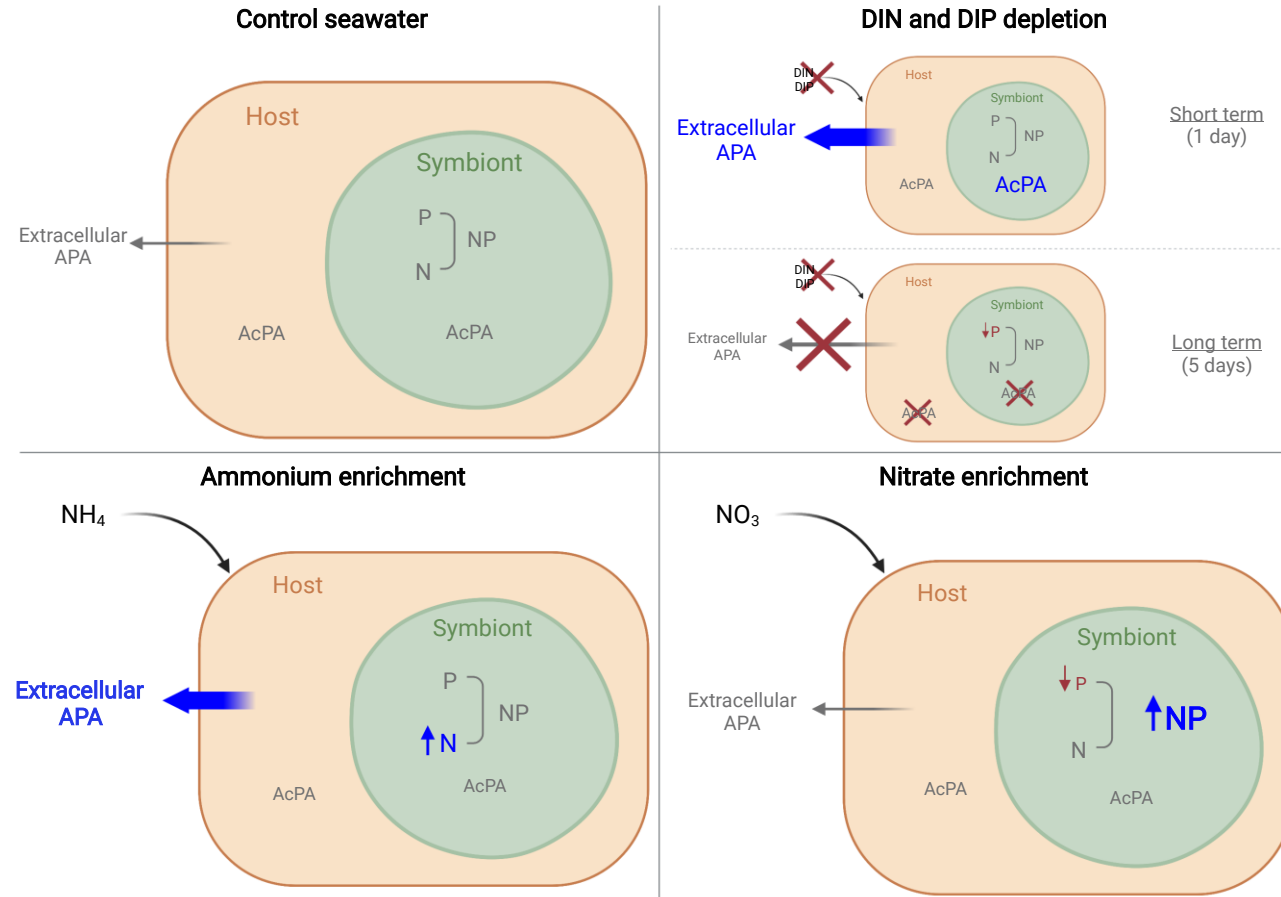


Figure 28. Overview of the effects of nutrients on phosphatase activity and elemental composition

The figure summarised the changes in extracellular alkaline phosphatase activity (APA) and the intracellular acid phosphatase activity (AcPA) within the host animal cell (Host, brown) and its endosymbiotic dinoflagellate (symbiont, green) under different conditions: Natural seawater (Control), nutrient depletion (undetectable amount of dissolved inorganic nitrogen (DIN) and phosphorus (DIP), **DIN and DIP depletion**) either during one day (**short term**) or five days (**long term**), **ammonium enrichment** at 2 μM and **nitrate enrichment** at 2 μM . Changes in elemental phosphorus (P) and nitrogen (N) content and the internal nitrogen to phosphorus (N:P) ratio in symbiotic dinoflagellate cells.

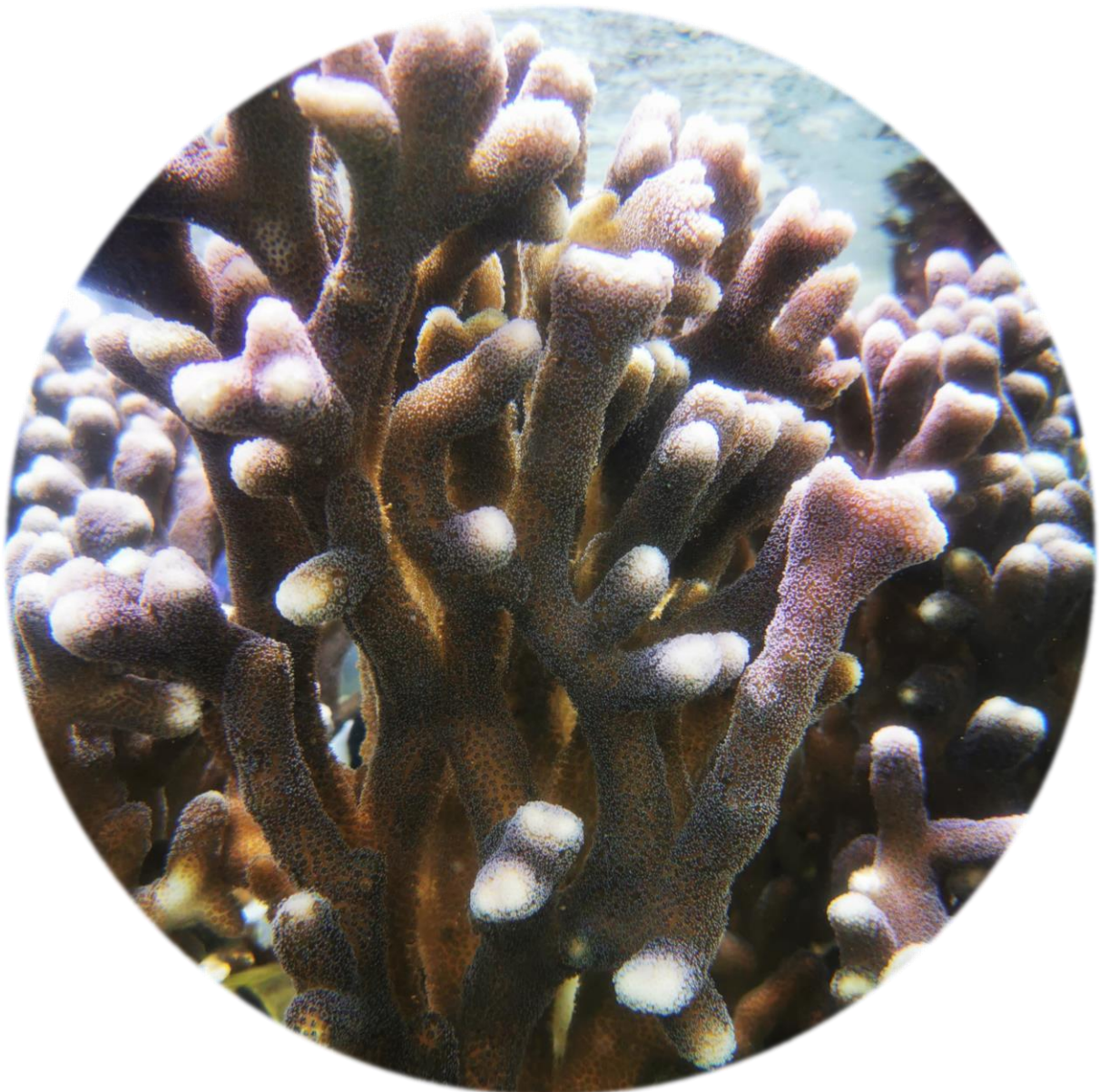
The **grey** text represents the baseline activities of extracellular APA and intracellular AcPA/APA in both host and symbiont cells measured in the control condition. The **blue** text in the different conditions represents an increase in extracellular and intracellular APA, N content and NP ratio, while the **red** text and cross represent a decrease in extracellular and intracellular APA/AcPA and P content compared to the control condition.

Phosphatase activity is used as an indicator of dissolved organic phosphorus (DOP) uptake in corals.

Short-term nutrient deficiency increases P requirement of the coral through DOP uptake and consumption of internal resources in the dinoflagellate cells. However, if nutrient starvation persists, the coral metabolism is impaired. Finally, nitrogen enrichment has a different effect on the corals depending on the nitrogen source. While ammonium enrichment increased P assimilation and maintained the internal NP ratio in the symbiont, nitrate enrichment did not promote P assimilation and led to an imbalance internal N:P ratio.

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CHAPTER 4:
IMPACT OF DESERT DUST
DEPOSITION AND METAL SUPPLY
ON THE PHYSIOLOGY OF A
SCLERACTINIAN CORAL



CHAPTER 4: IMPACT OF DESERT DUST DEPOSITION AND METAL SUPPLY ON THE PHYSIOLOGY OF A SCLERACTINIAN CORAL

The previous chapters investigated the effects of balanced and imbalanced DIN:DIP ratios, and particularly the effects of DIN enrichment on coral physiology and phosphorus requirement. In this chapter, we investigated the effect of microelements on coral physiology, via trace metals supplied by desert dust deposition.

I Overview of the article

Coral reefs worldwide are facing frequent mass bleaching events that result in massive coral loss. However, in the northern Red Sea, the Gulf of Aqaba, few cases of mass bleaching have been recorded, despite corals living above the usual bleaching temperature threshold, suggesting that this region may serve as a coral refuge (Fine *et al.*, 2013). This observation can be partly explained by the evolutionary history of corals, as they went through the warmer southern Red Sea, and acquired thermal resistance, during the recolonisation from the Arabian Sea (Fine *et al.*, 2013; Savary *et al.*, 2021). Other factors, such as environmental history may also contribute to coral response to thermal stress (Hall *et al.*, 2018). Therefore, it is important to understand which environmental conditions may increase coral tolerance to thermal stress. In the Gulf of Aqaba, corals are exposed to frequent and intense dust storms originating from the surrounding arid deserts. This dust is an important source of both macronutrients (nitrate, Chen *et al.*, 2007) and micronutrients, particularly iron, zinc, cadmium, and copper (Torfstein *et al.*, 2017). Dust deposition has both positive and negative effects on the water column. Indeed, some of the trace metals released by dust (iron, zinc) can increase the primary productivity of phytoplankton, while some other trace metals (i.g. copper) can be toxic. In any case, high metal concentrations are always toxic to most organisms. The effects of desert dust on the physiology of scleractinian corals have not been studied before, even though Red Sea corals are continuously exposed to dust. The aim of this study was thus to investigate the effects of desert dust deposition on a scleractinian coral and on its resistance to thermal stress.

We maintained nubbins of *Stylophora pistillata* for three weeks at 25 °C with or without dust. The dust concentration in each aquarium was similar to that received by the corals during a dust storm: 1 mg L⁻¹. Then the temperature in half of the aquaria was gradually increased from

25 °C to 30 °C and maintained for three weeks. At the end of the three weeks, we took samples and evaluated several parameters:

- **Physiology:** photosynthetic and respiration rate, protein biomass, chlorophyll content and symbiont density, metal content (ICP MS), C and N content, Stable isotope analysis $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$.
- **Oxidative status:** ROS (indirect - lipid peroxidation damage and protein carbonylation), RNS (indirect - protein tyrosine nitration) and non-enzymatic antioxidant capacity (uric acid, glutathione).

Additional nubbins maintained at 30 °C in the non-dust enriched condition were gradually increased to 34 °C and maintained at this stress temperature for one week until bleaching was visually observed. The temperature was then lowered again to 30 °C and kept with or without dust enrichment for three weeks before physiological measurements of photosynthesis and respiration rates, symbiont density and chlorophyll content were carried out.

This study addresses two questions.

First, how does the deposition of desert dust affect the general physiology of scleractinian corals at low and elevated temperatures?

The deposition of desert dust in corals had different effects depending on the seawater temperature. At low temperature, the level of iron, zinc and nickel were higher in the dinoflagellate cells with the dust (compared to control corals), leading to an increase in photosynthetic rates and chlorophyll content of the corals. This increase is due to the fact that zinc and iron, among other metals, are used for the production of photosynthetic compounds such as ferredoxin, cytochrome b_6 and photosystems I and II, as well as various enzymes (Raven *et al.*, 1999). At high temperature, the iron, zinc and nickel levels of dinoflagellate cells were higher in the dust-exposed corals compared to the control corals, but this did not lead to changes in the coral physiology. This is because iron in the dinoflagellate cells and photosynthetic rates in the control corals were naturally enhanced by high seawater temperature. It is therefore possible that the dust-enriched corals reached their maximum carbon assimilation rates at 25 °C already. These physiological changes occurred even though dust enrichment increased oxidative damage to corals through lipid peroxidation. Exposure to metals such as chromium, iron or nickel is known to increase oxidative stress (Sunderman Jr, 1987; Welch *et al.*, 2002; Sinha *et al.*, 2005), but this did not impact coral physiology in this study.

Second, does the metal supply help corals recover after thermal stress?

We observed that corals kept under dust enrichment during the recovery phase (at 30°C) after a thermal stress (at 34 °C) had higher photosynthetic rates than non-enriched corals. Thus, the supply of desert dust may help corals recover faster after a thermal stress event.

In summary, this study found that metal, and particularly iron, supplied by desert dust deposition helps to promote coral photosynthetic capacities at low temperatures. Furthermore, this study has also shown that temperature is a factor in the bioaccumulation of metals. Overall, results suggest that corals are frequently facing metal limitation, particularly in iron.

II Publication

Desert dust deposition supplies essential bioelements to Red Sea corals

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II.1. Abstract

Climate change-related increase in seawater temperature has become a leading cause of coral bleaching and mortality. However, corals from the northern Red Sea show high thermal tolerance and no recorded massive bleaching event. This specific region is frequently subjected to intense dust storms, coming from the surrounding arid deserts, which are expected to increase in frequency and intensity in the future. The aerial dust deposition supplies essential bioelements to the water column. Here, we investigated the effect of dust deposition on the

physiology of a Red Sea coral, *Stylophora pistillata*. We measured the modifications in coral and Symbiodiniaceae metallome (cellular metal content), as well as the changes in photosynthesis and oxidative stress status of colonies exposed during few weeks to dust deposition. Our results show that 1 mg L⁻¹ of dust supplied nanomolar amounts of nitrate and other essential bioelements, such as iron, manganese, zinc, and copper, rapidly assimilated by the symbionts. At 25 °C, metal bioaccumulation enhanced the chlorophyll concentration and photosynthesis of dust-exposed corals compared to control corals. These results suggest that primary production was limited by metal availability in seawater. A 5 °C increase in seawater temperature enhanced iron assimilation in both control and dust-enriched corals. Temperature rise increased the photosynthesis of control corals only, dust-exposed ones having already reached maximal photosynthesis rates at 25 °C. Finally, we observed a combined effect of temperature and bioelement concentration on the assimilation of molybdenum, cadmium, manganese, and copper, which were in higher concentrations in symbionts of dust-exposed corals maintained at 30 °C. All together these observations highlight the importance of dust deposition in the supply of essential bioelements, such as iron, to corals and its role in sustaining coral productivity in Red Sea reefs.

II.2. Introduction

Coral reefs are in decline worldwide due to a combination of local (e.g. pollution and overfishing) and global (e.g. ocean warming and acidification) anthropogenic pressures (Kennedy *et al.*, 2013; Heron *et al.*, 2016). Among all threats, those associated with global ocean warming are the most severe, as rising seawater temperatures triggers repeated mass coral bleaching events in almost all of the world's reefs (Van Hooidonk *et al.*, 2016; Hughes *et al.*, 2017). Bleaching occurs when warmer than normal sea temperatures disrupt the mutualistic relationship between corals and algal symbionts (Symbiodiniaceae, (LaJeunesse *et al.*, 2018)) that reside within their tissues (Suggett & Smith, 2020). Bleached corals are physiologically damaged, and prolonged bleaching leads to coral starvation and death, as photosynthates produced by the Symbiodiniaceae are no longer sufficient to cover their nutrient requirements (Ezzat *et al.*, 2019).

Corals, however, display geographical variation in their susceptibility to global warming. In some locations, such as the Northern Red Sea (Gulf of Aqaba, GoA), mass coral bleaching was never recorded, suggesting that reefs in this region may serve as coral refugia, and may persist through climate change (Fine *et al.*, 2013). Red Sea corals, such as *Stylophora*

pistillata, appear to be living at least 5 °C below their bleaching threshold in the GoA and can resist heat exposure of 11 degree-heating weeks (DHW) compared to 4 DHW for this species in other regions, such as the Great Barrier Reef (Bellworthy & Fine, 2017; Krueger *et al.*, 2017). Such physiological resistance to ocean warming is partly explained by evolutionary selection, as corals passed through the warmer Southern Red Sea during re-colonization from the Arabian Sea (Fine *et al.*, 2013; Savary *et al.*, 2021). In addition to biological adaptations, the environmental history can also contribute to divergence in coral response to heat stress (Hall *et al.*, 2018). It is thus of vital importance to unveil which environmental parameters may enhance or reduce the thermal tolerance of Red Sea corals.

GoA reefs are surrounded by arid deserts (Sinai, Negev and Arabian) and are subjected either to scarce and patchy rain events causing flashfloods (Katz *et al.*, 2015) or to intense and frequent dust storms (Torfstein & Kienast, 2018). These events are both expected to increase in frequency and intensity with climate change (desertification and land-use changes) (Prospero & Lamb, 2003; Woodward *et al.*, 2005; Guieu *et al.*, 2014). Airborne dust is a primary source of iron (Fe) (Chase *et al.*, 2006, 2011; Chen *et al.*, 2008; Shaked, 2008; Polyviou *et al.*, 2018), with an estimated deposition rate of 10 $\mu\text{mol m}^{-2} \text{d}^{-1}$ (Chase *et al.*, 2006). Other metals such as zinc (Zn), cadmium (Cd), copper (Cu) and nickel (Ni) are also supplied to seawater in the bio-available phases (Torfstein *et al.*, 2017). The ratios of dissolved Fe, Cu and Cd to phosphate are well in excess of those typically found in phytoplankton cells (Chase *et al.*, 2011). Dust deposition can also potentially contribute a substantial fraction of dissolved inorganic nitrogen (nitrate, NO_3^-) to the euphotic zone during the summer stratification period (Chen *et al.*, 2007). Thus, it regularly modifies the chemical composition of seawater by providing important bioelements for the primary producers as well as toxic elements that may affect the marine biodiversity (Wankel *et al.*, 2010; Benaltabet *et al.*, 2020). Although GoA reefs regularly receive large amounts of dust (up to 6 Mt, Jish Prakash *et al.*, 2015), the effects of this dust on living organisms have mainly focused on free-living microorganisms and algae. It was shown to enhance primary and bacterial production (Rahav *et al.*, 2018), as well as N_2 fixation rates through the coupling between the N_2 -fixer *Trichodesmium* sp. and a bacteria capable of solubilizing Fe (Basu *et al.*, 2019). Finally, changes in bacterial lipid composition (Meador *et al.*, 2017) or in zooplankton and picoplankton community composition (Wu *et al.*, 2018; Feliú *et al.*, 2020) were recorded after dust addition.

The effect of dust deposition on the main reef builders scleractinian corals has, to our knowledge, never been assessed. On the one hand, dust may contribute to maintaining a healthy symbiotic relationship between corals and their algae by supplying bioelements necessary for

biological processes, such as chlorophyll synthesis, nitrate reduction and photoprotection (Andresen *et al.*, 2018; Li *et al.*, 2020). Some metals, such as manganese, iron and zinc, may also mitigate the susceptibility of corals to thermal stress and bleaching (Biscéré *et al.*, 2018; Ferrier-Pagès *et al.*, 2018; Shick *et al.*, 2011). Dust can, therefore, be one of the factors, in addition to the evolutionary selection, that contributes to the enhanced resistance and resilience of GoA corals to high seawater temperatures. On the other hand, toxic elements present in the dust, as well as pathogenic bacteria, may reduce the heat tolerance of the corals, by increasing their oxidative status (Garrison *et al.*, 2003; Hunter & Cervone, 2017). Although a correlation between occurrence of coral diseases, dust deposition and thermal stress, has been observed for Caribbean reefs (Garrison *et al.*, 2003; Hunter & Cervone, 2017), such correlation has never been reported for GoA reefs. Overall, the scarcity of studies on the effect of dust deposition on scleractinian corals calls for more investigations on the benefits and risks of corals exposed to this additional nutrient supply.

Here, we have investigated whether bioelements contained in the dust are beneficial or detrimental to the health of the common reef building scleractinian, *Stylophora pistillata* in the northern Red Sea. For this purpose, we measured the changes in coral and Symbiodiniaceae metallome (bioelement content of the cells), as well as the changes in photosynthesis and oxidative stress status of colonies exposed during few weeks to relevant concentrations of dust deposition. Changes in coral physiology under dust exposure were also evaluated on already heat-induced bleached corals. These results shed light on the importance of desert dust deposition for coral health.

II.3. Material and Methods

II.3.a. Origin and characterization of the dust

Aerian dust samples were collected at the Interuniversity Institute (IUI) for Marine Science in Eilat, (Israel), as in Basu *et al.* (2019). The dust was sampled using traps placed on the roof of the IUI. It was then kept in metal-free tubes (Labcon) completely sealed to avoid any contamination. The coral reefs of Eilat are situated less than 100 m from the IUI buildings, ensuring that the dust collected was identical to the one reaching the reef.

To assess the supply of nitrate and phosphate by dust, we prepared five beakers filled with 1L of 0.22 μm filtered seawater and 20 mg of dust in each beaker. The seawater was pumped at 40m depth in the Mediterranean Sea close to the laboratory. The large amount of dust used was necessary to detect a significant change in nutrients. Seawater was sampled after

48h using pre-cleaned (with acid and distilled water) syringes and tubes. Nitrate and phosphate concentrations were immediately analysed on a AA3 Seal autoanalyzer according to Aminot et al (2009). Nitrate and phosphate were also measured in control seawater samples (without dust addition)

To assess the supply of metals by dust, all material was pre-cleaned with suprapur nitric acid (HNO_3 , ROTIPURAN® *Supra*, Carl Roth) and distilled water- except the minisart filters, which were washed with distilled seawater and several seawater samples. Five beakers were filled with 1L of 0.22 μm filtered seawater in which 1 mg of dust was dissolved. Seawater was sampled after 48h incubation using a syringe mounted with a teflon tube at the end to avoid touching water with the syringe. It was then filtered on 0.22 μm minisart filter and kept in tightly closed 50 mL tubes. The concentration of trace elements in these seawater samples (and in control water samples, without dust addition) was measured using high resolution inductively coupled plasma mass spectrometry (HR ICP-MS; Element 2, Thermo Finnigan) (see Layglon et al (2021) for detailed analysis). Quantification was performed by using external matrix matching calibration. Briefly, the stock solutions with increasing element concentrations (0, 0.1, 1 and 10 ppb) for external calibration were prepared in 10 times diluted CASS-5 (Nearshore seawater reference material for trace metals, NRC, Canada) certified sample. Internal In standard (10 ppb) was added to both calibration solutions and sample solutions in order to minimise matrix effect affecting accuracy of the measurement. The same certified sample was also used for the quality control (QC) and measured every 10th sample in the sequence. The obtained recoveries agreed within 10% with the certified value. For determination of element concentrations, seawater samples were diluted 10 times with 2 % high purity nitric acid (69% HNO_3 ROTIPURAN® *Supra*, Carl Roth). Working standards, as well as blank solutions were prepared with addition of 2 % high purity HNO_3 .

II.3.b. Experimental design

Fifteen colonies of the scleractinian coral *Stylophora pistillata* were used to generate 180 nubbins (12 nubbins from each colony), which were then equally divided in 12 aquaria of 40 L (15 nubbins per aquaria). The mother colonies were cultured several years in aquaria under controlled conditions, without any influence of dust deposition. Nubbins were maintained for three weeks at 25 °C under a photosynthetically active radiation (PAR) of $200 \pm 10 \mu\text{mol photons.m}^{-2}.\text{s}^{-1}$ (12:12h photoperiod) for healing and were fed twice a week with *Artemia salina* nauplii. PAR was delivered by 400 W metal halide lamps (HPITS, Philips) suspended above the aquaria. PAR intensity was measured using a LI-COR data logger (LI-100) connected to a quantum sensor (LI-I93). Temperature was controlled by heaters connected

to an Elliwell PC 902/T controller and water pumps ensured seawater mixing. After healing, feeding was stopped and nubbins were maintained another week before the implementation of the following experimental design (Fig 29a).

The 12 aquaria were divided in 2 sets of 6 aquaria, maintained at 25 °C (average winter temperature in the Gulf of Aqaba: 25.93 ± 0.69 , (Shaltout, 2019) and $200 \mu\text{moles photons m}^{-2} \text{ s}^{-1}$. The first set was a control condition (Control 25°C), which was not enriched with dust. The second set (Dust 25°C) was enriched with dust at 1 mg L^{-1} . The dust enrichment chosen was similar to the dust concentration measured in the upper mixed layer of GoA, during a dust-storm (Mescioglu *et al.*, 2019). It was higher than the mean dust deposition measured in late winter and early spring ($\sim 150\text{--}250 \mu\text{g m}^{-3}$) (Al-Taani *et al.*, 2015; Torfstein *et al.*, 2017). However, Red Sea corals are continuously exposed to dust, which was not the case in this experiment. Dust was pre-dissolved 48h in advance in 50 mL seawater, which was added each morning, and five times a week, to the aquaria. The amounts of metals and macronutrients to which corals were exposed were thus identical to those measured in the preliminary experiments described above for the characterization of the dust. To fully expose the corals to the dust, the water supply was turned off in all tanks (including controls) for 6h/day. However, all aquaria were equipped with underwater pumps ensuring a water movement around the corals. Seawater in the aquaria was renewed in ca 3 hours once seawater supply was turned back on.

This experimental design was maintained for 3 weeks, after which three aquaria in each dust/non-dust condition were still maintained at 25 °C, whereas the temperature in the other half was increased to 30 °C representing the upper level of the summer temperature in the GoA (Shaltout, 2019). This temperature is slightly higher than the optimal temperature for *S. pistillata* (29 °C : Krueger *et al.*, 2017). It does not induce bleaching or stress in this Red Sea species but is sufficient to induce bleaching in many other species of the world. Temperature was increased slowly by 0.5 °C every 2 days and once at 30 °C, temperature was maintained for 3 additional weeks, before measurements described below were performed. In total, corals were exposed 5 times per week during 7 weeks to dust deposition. Four conditions were achieved, control and dust-exposed at 25 °C, or at 30 °C.

In addition to this first set of aquaria, 16 additional nubbins were generated from 4 of the above colonies and dispatched into 4 additional aquaria (1 nubbins per colony per tank) to implement the following experimental design (Fig 29b). All nubbins were maintained in control conditions (no dust, $200 \mu\text{moles photons m}^{-2} \text{ s}^{-1}$) and treated as described above (3

weeks at 25 °C, followed by an increase in temperature to 30 °C). At the end of the 7 weeks incubation, temperature was increased to 34 °C (1 °C per day over 3 days) and maintained for a week until visual bleaching was observed. Such temperature is indeed known to be the bleaching threshold for *S. pistillata* (Fine *et al.*, 2013; Woolstra *et al.*, 2020; Evensen *et al.*, 2021). Temperature was then returned to 30 °C in 4 days and half of the aquaria were enriched with dust (as described above) while the other half was maintained without dust exposure during 3 additional weeks, to assess the recovery rates of *S. pistillata* in the two conditions. At the end, nubbins were sampled for measurements of photosynthesis, respiration, symbiont density and chlorophyll content as described below.

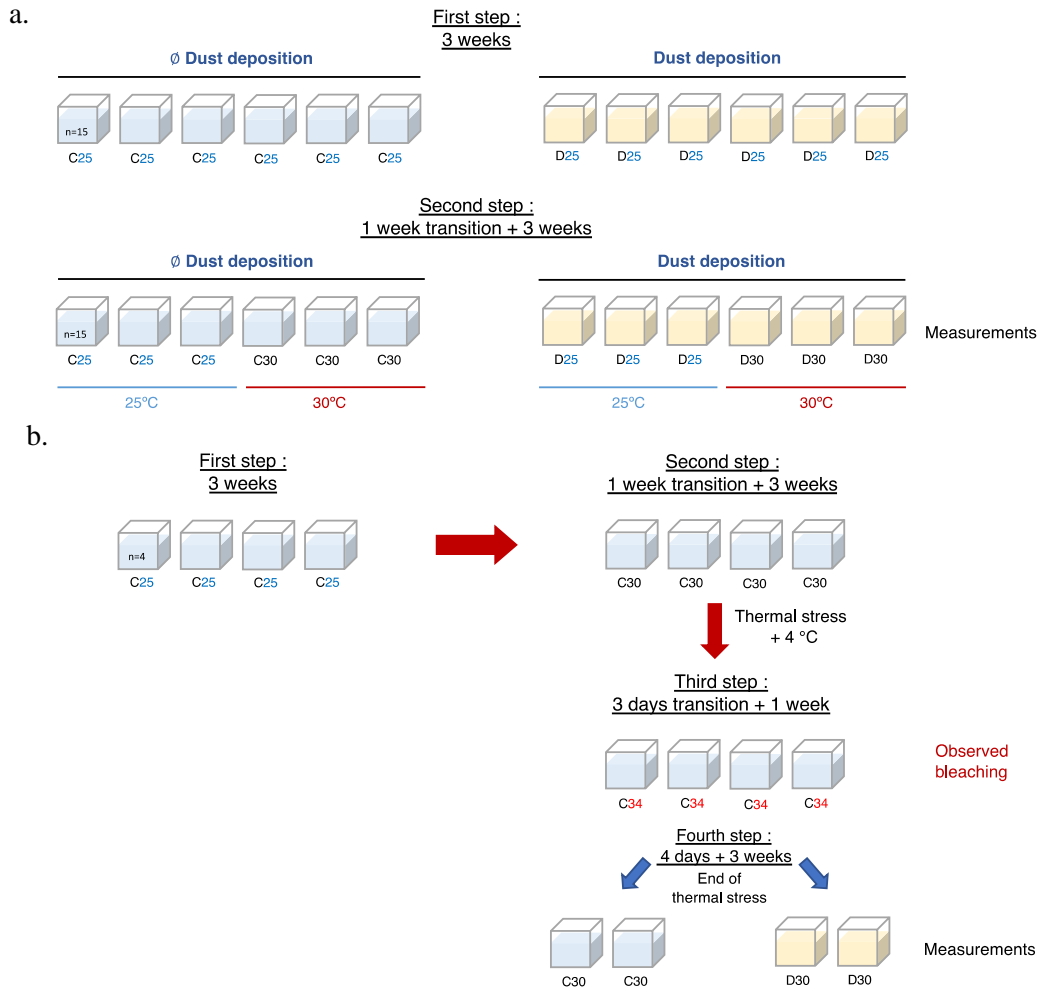


Figure 29. Cross factorial experimental design (a.) and recovery experiment (b.)

n represent the number of nubbins placed in each aquaria.

Nubbins of *Stylophora pistillata* were not fed for the duration of the experiment and kept under PAR irradiance of $200 \pm 10 \mu\text{mol photon.m}^{-2}.\text{s}^{-1}$.

The following experimental condition were applied: **C25 - C30**: control corals maintained at 25 °C or 30 °C without aerial dust deposition, **D25 - D30**: corals maintained at 25 °C or 30 °C supplied with aerial dust at 1 mg.L^{-1} , **C34**: control corals maintained at 34 °C without aerial dust deposition for one week, until bleaching is visually observed. Sampling was done at the end of each experiment, indicated by **Measurement**.

II.3.c. *Physiological measurements*

II.3.c.i. Photosynthesis parameters

Six nubbins per condition (two nubbins per aquarium from different colonies) were used to estimate rates of net (Pn) and gross (Pg) photosynthesis, as well as respiration rates (R).

Nubbins were placed in Plexiglass chambers (60 mL) filled with 0.45 μm -filtered seawater maintained at the corresponding experimental temperature (25 °C or 30 °C) and stirred. An oxygen sensor (Polymere Optical Fiber, PreSens, Regensburg, Germany) connected to an Oxy-4 (Channel fiber-optic oxygen meter, PreSens, Regensburg, Germany) was placed in each chamber. Oxygen concentration was recorded with the Oxy4v2-30fb software, for 45 min, at 200 $\mu\text{mol photons}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ for Pn and in the dark for R. Calibration was done at 100 % with O₂-saturated seawater and at 0 % with nitrogen saturated seawater. Pg was calculated by adding the absolute values of Pn and R. However, this calculated value of Pg is probably an underestimation of the actual Pg (Schrameyer *et al.*, 2014). After measurements, all nubbins were frozen (-20 °C) for later analysis of protein holobiont content, symbiont density and chlorophyll *a* and *c*₂ content (Chl). Photosynthesis rates were expressed as $\mu\text{mol O}_2\cdot\text{h}^{-1}\cdot\text{cm}^{-2}$. Surface was determined using the single dip wax technique (Stimson & Kinzie III, 1991).

II.3.c.ii. Protein holobiont content, symbiont density, and chlorophyll

Nubbins used in photosynthesis measurements were thawed and tissues were separated from the skeleton using a Water-pick, 10-15 mL of filtered seawater and a dounce tissue grinder.

Protein holobiont content of each nubbin was determined in a 500 μL sub-sample using the Bicinchoninic acid (BCA) assay kit according to Smith (1985). Another 100 μL sub-sample was used to determine symbiont density using a Z1 Coulter Particle Counter (Beckman Coulter, US). Finally, chlorophyll content was measured using a 5 mL sub-sample. First, animal tissue and dinoflagellates were separated by centrifugation at 5530 g for 15 min at 4 °C. The animal tissue (supernatant) was discarded, and the dinoflagellate fraction (pellet) was resuspended in 5 mL of pure acetone and kept in the dark for 24 hours at 4 °C to extract chlorophyll. The chlorophyll extract was then centrifugated at 15 °C for 15 minutes at 5530 g. The supernatant absorbance was read at 750 nm, 663 nm and 630 nm with a UVmc² Spectrophotometer (Safas, Monaco). Chlorophyll *a* and *c*₂ concentration was calculated following Jeffrey and Humphrey (1975) equations. Measurements were normalized with the skeletal surface area of the nubbins. The carbon (C) and nitrogen (N) content as well as the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of the animal tissue and symbiotic dinoflagellates were also analysed, and the methods and results are presented in the Supplementary Information (SI, Appendix IV) for clarity.

II.3.d. *Oxidative stress analysis*

Oxidative stress analyses were done on 6 nubbins per condition (2 nubbins per aquaria, taken from different colonies) – 24 nubbins – snap-frozen in liquid nitrogen and kept at 80 °C. Each analysis was done on a 0.5 - 1 cm fragment cut from the main nubbin.

II.3.d.i. Lipid Peroxidation (LPO)

LPO was assessed, according to Oakes and Van Der Kraak (2003), following their Thiobarbituric Acid Reactive Substance (TBARS) protocol. The reaction between a byproduct of lipid peroxidation - malondialdehyde (MDA) – and thiobarbituric acid (TBA) quantifies the damage to lipids. Coral fragments were immersed in a homogenizing buffer - KCl (1,15%) solution containing 35 μM butylatedhydroxytoluene (BHT) - and sonicated on ice (Frequency 70 kHz, Vibra-Cell™, Bioblock Scientific, France). Samples were centrifugated at 4 °C for 10 min at 10000 g, and protein content was assessed in line with Bradford Protein Assay (Bradford, 1976) and were standardized to 0.8 $\mu\text{g}\cdot\mu\text{L}^{-1}$. Standards were made with tetramethoxypropane (TMP) hydrolysed into MDA. Samples were then incubated in flat-bottomed Eppendorf for 30 min at 95 °C with thiobarbituric acid (TBA) solution (0.8%), acetic acid solution (20%) and SDS solution (8.1%). Tubes were left to cool down at room temperature. Then, 500 μL of n-butanol and 100 μL of MilliQ water were added with careful vortexing to mix the two phases. Samples were then centrifuged at 15 °C for 10 min at 10000 g. The organic phase (supernatant) of the samples were added to a 96-well black plate in duplicate and the fluorescence was read at 515 nm and 553 nm every minute for 3 minutes with a spectrofluorometer (Xenius®, SAFAS, Monaco). Results were normalized with the sample homogenates protein content in the wells, expressed as nmol MDA.mg protein⁻¹.

II.3.d.ii. Protein tyrosine nitration (PTN)

Level of 3-Nitrotyrosine (3-NT)-modified proteins, produced by the oxidative damage to proteins caused by peroxynitrite, was assessed with the Nitrotyrosine ELISA Kit (ab113848, Abcam, Cambridge, UK) following manufacturer's instructions. Coral fragments were immersed in a phosphate buffered saline (PBS) at pH 7.4 and sonicated on ice (Frequency 70 kHz, Vibra-Cell™, Bioblock Scientific). Samples were centrifuged at 4 °C for 10 min at 10000 g, and protein content was assessed in line with Bradford Protein Assay (Bradford, 1976) and were standardized to 1 $\mu\text{g}\cdot\mu\text{L}^{-1}$. Samples were incubated on ice for 20 min in the extraction buffer kit solution (1:1). 3-NT-Bovine Serum Albumin (3-NT-BSA) were used for the standards. Standards and samples were added to a 3-NT antibody-specific 96-well plate. The plate was left to incubate for 2 hours at room temperature, wrapped in foil. Wells were washed with Wash Buffer and liquid excess was completely removed. An anti-3-NT detector biotin was added to

each well and the plate was incubated for one hour. Wells were washed twice with Wash Buffer. A HorseRadish Peroxidase (HRP) -labeled kit solution was added on the plate and incubated for one hour before being washed thrice with Wash Buffer. Then, HRP Development kit solution was added and absorbance was read every minute for 15 min at 600 nm. Results were normalized with the sample homogenates protein content in the wells, expressed as 3NT-BSA.mg protein⁻¹.

II.3.d.iii. Non-enzymatic total antioxidant capacity (NETAC)

NETAC was assessed with the “OxiSelect Total Antioxydant Capacity (TAC) Assay Kit” (STA-360, Cell Biolabs Inc., San Diego USA) according to the manufacturer’s instructions. This kit measures biomolecules’ antioxidant capacity via single electron transfer (SET) mechanism. Coral fragments were immersed in PBS at pH 7.4 and sonicated on ice (Frequency 70 kHz, Vibra-Cell™, Bioblock Scientific). Samples were centrifuged at 4 °C for 10 min at 10000 g, and protein content was assessed in line with Bradford Protein Assay (Bradford, 1976) and were standardized to 0.9 µg.µL⁻¹. Standards were made with uric acid. Standards and samples were added to a 96-well plate with reaction buffer (Buffer kit solution and PBS). The absorbance was read at 490 nm with a spectrofluorometer (Xenius®, SAFAS, Monaco). Absorbance values were proportional to the sample’s NETAC. Results were normalized with the sample homogenates protein content in the wells, expressed as µM Copper Reducing Equivalentents (CRE).mg protein⁻¹.

II.3.d.iv. Protein carbonylation

Protein carbonylation level was assessed using the “OxiSelect Protein Carbonyl ELISA kit” (STA-310, Cell Biolabs Inc., San Diego USA) according to manufacturer’s instruction. This assay measures the quantity of protein carbonyl derivatives of proline, lysine, arginine and threonine, markers of ROS damages. The coral fragments were sonicated (Frequency 70 kHz, Vibra-Cell™, Bioblock Scientific) in PBS (pH 7.4) on ice, centrifuged at 10000 g for 10 minutes at 4 °C, and the protein content was quantified following the Bradford protein assay (Bradford, 1976). Samples’ protein content was standardized to 0.8 mg.mL⁻¹. Standards were made with known ratios of oxidized and reduced BSA with final protein carbonyl concentrations ranging from 0 (reduced BSA) to 7.5 (oxidized BSA) nmol.mg⁻¹. Samples and standards were incubated in triplicate on a 96-wells protein binding plate for 2 hours at 37 °C, before they were washed 3 times with PBS. They were then incubated 45 minutes in the dark with a DNPH kit solution, before they were washed 5 times with Ethanol/PBS (1:1) and incubated for 5 minutes on orbital shaker between each wash. Samples and standard were washed again 2 times with PBS and incubated for 1.5 hours with Blocking kit solution on an

orbital shaker and washed 3 times with Wash Buffer kit solution. Samples and standards were incubated with anti-DNP antibody for 1 hour on an orbital shaker and washed 3 times with Wash Buffer kit solution. They were, then, incubated with HRP conjugated secondary antibody for 1 hour on an orbital shaker and washed 5 times with Wash Buffer kit solution. Finally, Substrate kit solution was added to each well and once coloration appeared, after 3 minutes, Stop solution from the kit was added to the wells. The absorbance was recorded at 450 nm using a spectrofluorometer (Xenius[®], SAFAS, Monaco). Results were normalized with the sample homogenates protein content in the wells, expressed as protein carbonyl nmol.mg protein⁻¹.

II.3.e. Metal analysis in coral tissue

All devices used for metal analysis were cleaned with suprapure nitric acid (Merck) and then washed three times with MilliQ water, as described above. The machine and sample replication were tested on five independent nubbins of *S. pistillata* (not included in the experiment), before the experimental samples were analysed. The treatment applied to these nubbins was identical to the one applied to the experimental samples.

For the experiment, 24 nubbins- 6 per conditions (from different colonies and 2 per aquaria), were snap-frozen in liquid nitrogen and kept at -80 °C until analysis in a trace metal clean lab. Before the analyses, nubbins were thawed, and the tissue was separated from the skeleton through water-picking with artificial seawater made with MilliQ water and metal-free sodium chloride. Algal symbionts were separated from the host tissue and then each fraction was freeze-dried. For the elemental determination, the freeze-dried samples (~10-100 mg) were digested in 3 mL of concentrated high purity nitric acid (69% HNO₃ ROTIPURAN[®] Supra, Carl Roth) using a microwave oven (Multiwave Eco, Anton Paar). No additional sample drying was performed before weighting. The procedural/acid/MilliQ blanks (n = 6) were also determined using the same protocol. For most of the elements/samples, the blank contribution was negligible (< 1%), while only for Fe and Cr it was in average up to ~12%. The quality control was checked by determining the elements content (n = 6) in a certified sample IAEA 461 (*Trace Elements and Methyl Mercury Mass Fractions in IAEA-461 Clam (Gafrarium tumidum) Sample*), with relative standard deviation (RSD) <4%. The obtained recoveries ranged from 81% to 100%. The concentration of elements in a digested samples were determined in two-fold diluted samples using HR ICP-MS (Element 2, Thermo Finnigan), with 10 ppb In added as an internal standard. The quantification was performed by means of an external calibration (0, 0.1, 1, 10 and 100 ppb standards).

Surface area was determined for normalization using the single dip wax technique (Stimson & Kinzie III, 1991).

II.3.f. Statistical analysis

Statistical analyses were processed using the program RStudio Version 3.5.1. Data were checked for normality using the Shapiro-Wilk test and for homoskedasticity using Levene's test on the residuals from the linear model (Levene, 1960). A Principal Component analysis (PCA) was done on the metal content of the holobiont (host tissues and dinoflagellate cells) (Fig S1, Appendix IV). Two-way ANOVA models were used to test the effect of the two factors: "Treatment" (Control/Dust) and "Temperature" (25/30) on the different physiological and oxidative stress parameters as well as stable isotope and metal composition measured: dinoflagellates density, holobiont protein content, total chlorophyll *a* and *c*₂ content, net and gross photosynthesis rate per skeletal surface area and per symbiont cell, respiration rate per skeletal surface area and per symbiont cell, lipid peroxidation, protein nitration, protein carbonylation, non-enzymatic antioxidant capacity, and the $\delta^{15}\text{N}$, $\delta^{13}\text{C}$, Mo, Cd, Mg, Cr, Mn, Fe, Co, Cu and Zn in the animal tissue and symbiotic dinoflagellate. Analyses were followed by a post hoc test, pairwise comparison with *fdr* correction, when factors effect was significant. Differences were assumed significant when $p < 0.05$. For the bleaching recovery experiment, the effect of Control and Dust were analysed using a Welch t-test, after verifying normality. Differences were assumed significant when $p < 0.05$. For the metal content of the seawater the Control and Dust treatment were analysed using a Wilcoxon-Mann-Whitney (WMW) test. Differences were assumed significant when $p < 0.05$.

II.4. Results

The results of the statistical tests are reported in the supplementary Appendix IV in: Table S1 for the metal composition in the seawater, Table S2, for physiological parameters, Table S3 and Table S4 for photosynthesis parameters normalized respectively to skeletal surface area and to symbiont cell, Table S5, for oxidative stress parameters, Table S6 and Table S7 for metal and stable isotope composition in the coral holobiont, and Table S8 for the physiological parameters of the recovered corals.

II.4.a. Macronutrient and metal composition of seawater with dust addition

The seawater content in iron (WMW, $p < 0.033$, Fig 30a), zinc (WMW, $p < 0.0001$, Fig 30b), manganese (WMW, $p < 0.0001$, Fig 30c), cobalt (WMW, $p < 0.02$, Fig 30d), chromium (WMW, $p < 0.022$, Fig 30e) and copper (post hoc, $p < 0.0001$, Fig 30f) was significantly higher

with 1 mg L⁻¹ dust supplementation than in the controls. The nitrate and phosphate concentrations were also determined using an AA3 Seal autoanalyzer according to Aminot et al (2009). The concentrations were equal to $0.5 \pm 0.3 \mu\text{M NO}_3^-$ and $0.2 \mu\text{M PO}_4^{3+}$ in seawater, with no difference between the control and dust-enriched aquaria. This is because 1 mg L⁻¹ of dust generated only an enrichment of ca. 75 nM of NO₃. This enrichment was calculated according to the fact that the dissolution of 20 mg of dust during 48h in 1 L of filtered seawater generated 1.5 μM NO₃⁻.

II.4.b. *Impact of dust on Stylophora pistillata*

II.4.b.i. Tissue parameters

At 25 °C, symbiont density and total protein content were not significantly different between the dust and control conditions (ANOVA, $p > 0.67$ and $p > 0.33$ respectively, Fig 31a, b). However, dust-enriched corals presented a significantly higher content in chlorophyll *a* and *c*₂ normalized to skeletal surface area and to symbiont cell doubled (post-hoc, $p < 0.037$ and $p < 0.012$ respectively, Fig 31c, d), a higher lipid damage (ANOVA, $p < 0.0015$, Fig 31e), and a 2 to 9-fold higher photosynthesis rates – both net photosynthesis normalized to skeletal surface area and symbiont cell (post hoc, $p < 0.0002$ and $p < 0.0004$ respectively, Fig 32a,b)), and gross photosynthesis, normalized to skeletal surface area and to symbiont cell (post hoc, $p < 0.0035$ and $p > 0.0183$ respectively, Fig 32a, b). The following parameters did not significantly changed with dust supplementation compared to controls: respiration rates normalized to surface area and symbiont cell (ANOVA, $p > 0.3$, Fig 32e, f), $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ value (Fig S2, Appendix IV), C and N content of coral tissue and symbiont cells (Fig S3, Appendix IV), and oxidative stress parameters other than lipid peroxidation (Fig. S4, Appendix IV).

At 30 °C, the addition of dust had a limited impact on coral's physiological parameters. Only the total chlorophyll content per symbiont cells decreased by 30 % in the dust enriched corals compared to control corals (post hoc, $p < 0.026$, Fig 31d).

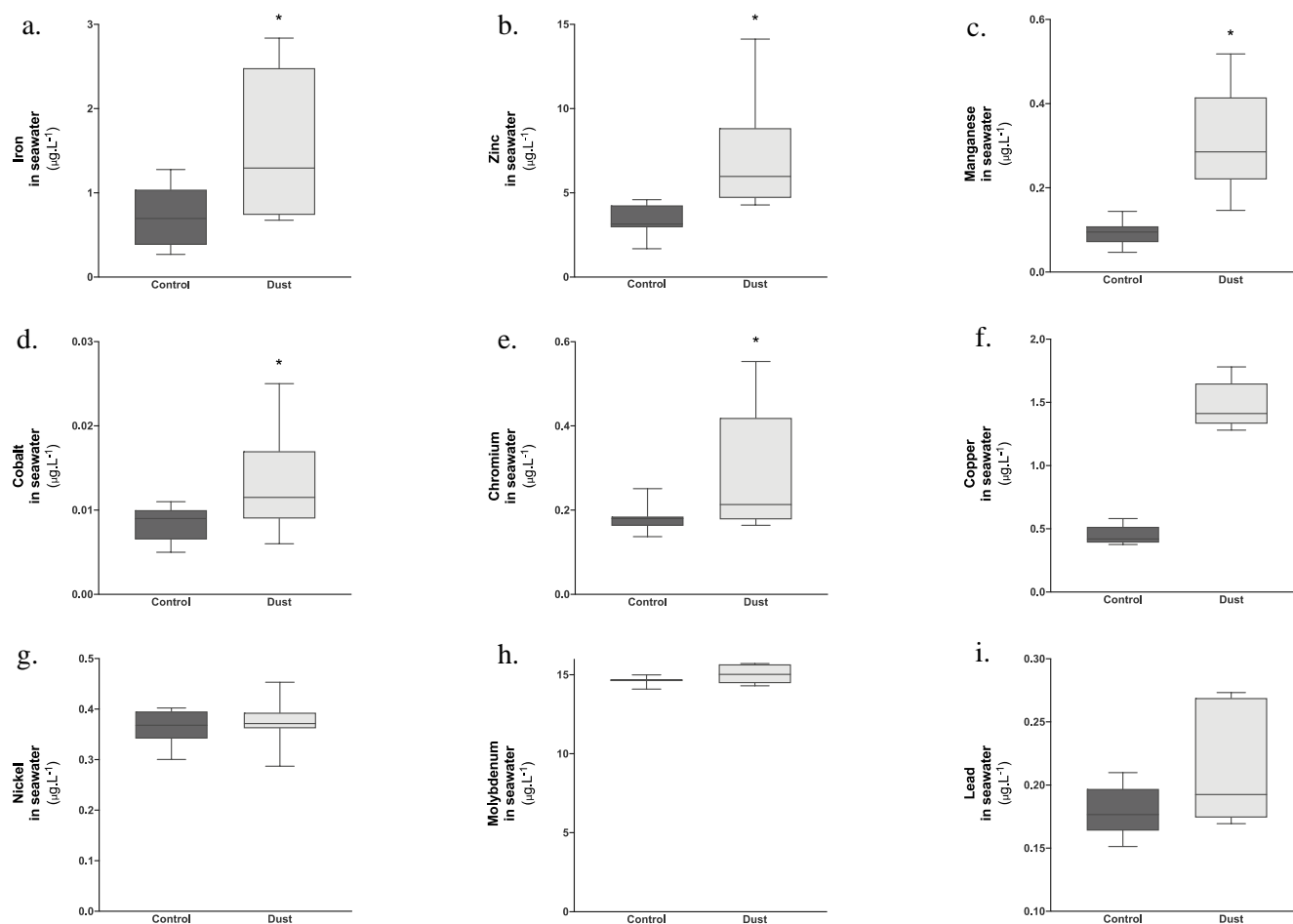


Figure 30. Metal composition of dust enriched seawater

Boxplots represent the concentration of iron (a.), zinc (b.), manganese (c.), cobalt (d.), chromium (e.), copper (f.), nickel (g.), molybdenum (h.) and lead (i.) supplied in seawater without dust deposition (**Control**, dark grey) and in dust-enriched seawater (**Dust**, light grey). Aerial dust, at 1 mg.L^{-1} , was incubated in fresh seawater for 48 hours to obtain the maximum metal supply. The box delimits the 1st and 3rd quartile, the black bar represents the median and whiskers represent min and max, $n=10$. Statistically significant differences are represented by an asterisk.

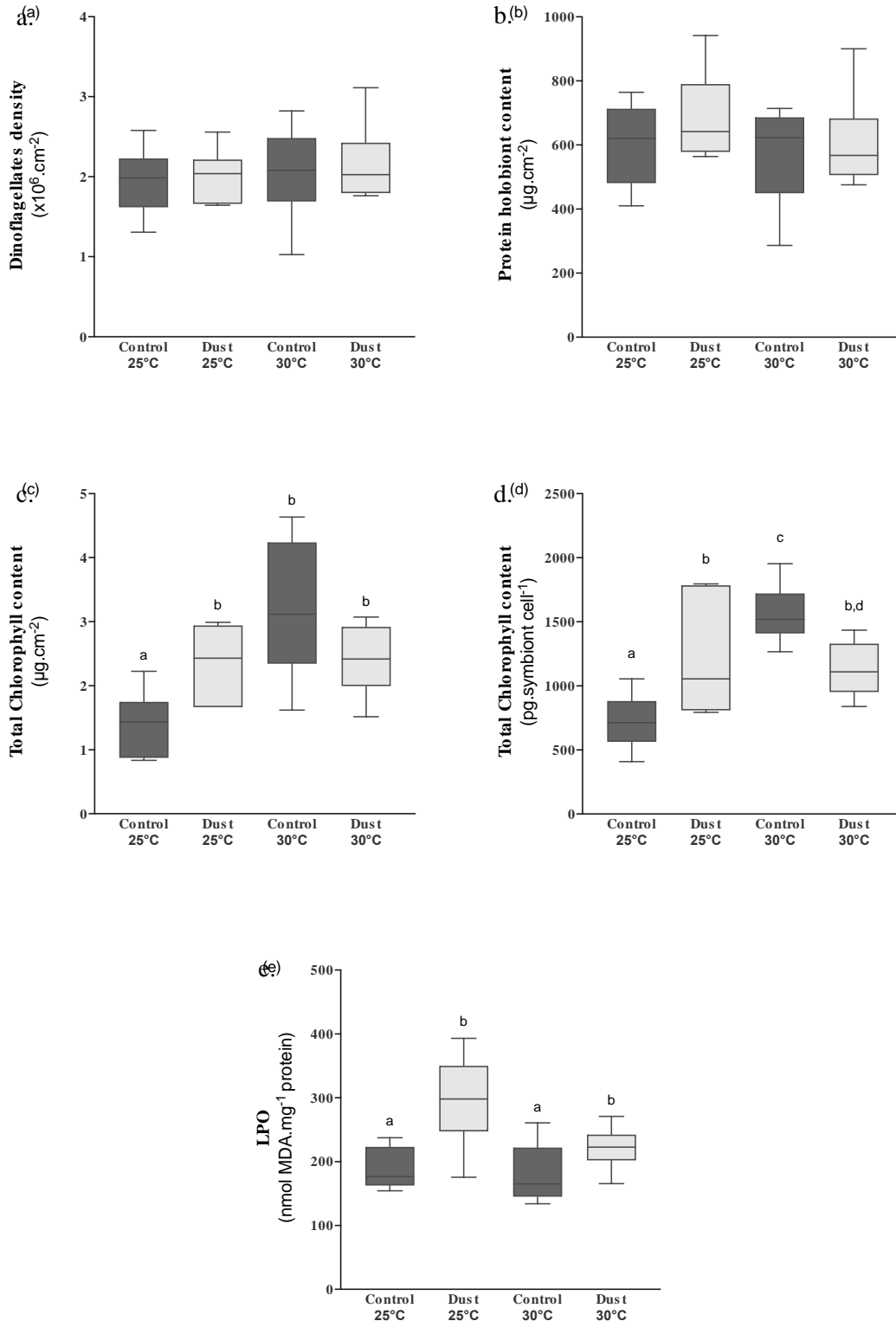


Figure 31. **Physiology and oxidative status of *S. pistillata***

Boxplots represent the dinoflagellates density normalized per skeletal surface area (a.), protein content normalized per skeletal surface area (b.), Chlorophyll a and c2 content normalized per skeletal surface area (c.) and per dinoflagellates cell (d.), as well as the level of membrane damage by lipid peroxidation normalized per protein (e.) under different condition of dust deposition: **Control 25°C – Control 30°C**: control corals maintained at 25 °C or 30 °C without aerial dust deposition (dark grey), **Dust 25°C – Dust 30°C**: corals maintained at 25 °C or 30 °C supplied with aerial dust at 1 mg.L^{-1} (light dark)

The box delimits the 1st and 3rd quartile, the black bar represents the median and whiskers represent min and max, $n=6$
Statistically significant differences are represented by different letters (a, b, c and d)

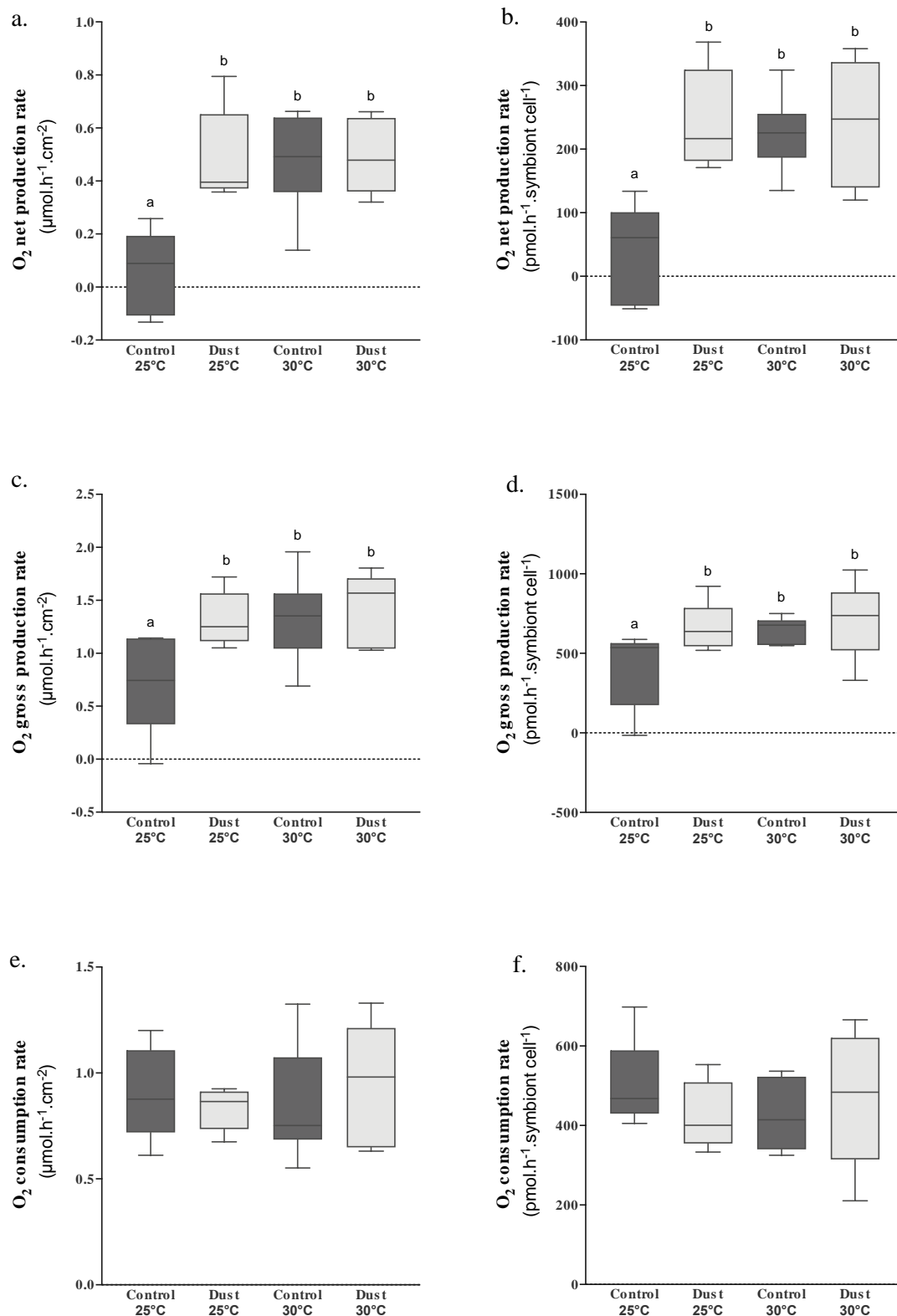


Figure 32. **Photosynthesis rates of *S. pistillata***

Boxplots represent the net photosynthesis rates normalized per skeletal surface area (a.) and per symbiotic dinoflagellate cell (b.), the gross photosynthesis rates normalized per skeletal surface area (c.) and per symbiotic dinoflagellates cell (d.) and respiration rates normalized per skeletal surface area (e.) and per symbiotic dinoflagellates cell (f.) under different condition of dust deposition.

Control 25°C – Control 30°C: control corals maintained at 25 °C or 30 °C without aerial dust deposition (dark grey), **Dust 25°C – Dust 30°C:** corals maintained at 25 °C or 30 °C supplied with aerial dust at 1 mg.L⁻¹ (light dark).The box delimits the 1st and 3rd quartile, the black bar represents the median and whiskers represent min and max, n=6.

Statistically significant differences are represented by different letters (a or b)

II.4.b.ii. Metal content

The metallome of the coral tissue and symbiont cells expressed in $\mu\text{g}\cdot\text{mg}^{-1}$ DW or cm^{-2} is presented in the Supplementary material (Table S10, S11, Appendix IV), while the figures present the results per cm^2 only. Metal content in the host tissues was different from the metal content of the symbionts, but not different across treatments, suggesting that no major changes occurred either with increased temperature or dust exposure. On the contrary, the metal content, particularly iron, cobalt and zinc, in the dinoflagellates cells, showed larger changes with the treatment. We thus further looked at the differences between treatments with an ANOVA. The metallome of the coral tissue and symbiont cells was impacted by dust deposition. In the coral tissue, dust addition increased the magnesium (by 20-150 % - ANOVA, $p < 0.012$, Fig 33a), as well as the zinc content (by 20-70 % - ANOVA, $p < 0.049$, Fig 33b) regardless of temperature. It also increased the cadmium content at 30 °C (by two-fold - post hoc, $p < 0.04$, Fig 33c), but decreased the manganese content at 25 °C (by 40 % - post hoc, $p < 0.01$, Fig 33d). In the symbiont cells, regardless of the temperature, dust-enrichment enhanced the zinc (by 40-70 % - ANOVA, $p < 0.034$, Fig 34a), nickel (by 40-100 % - ANOVA, $p < 0.0012$, Fig 34b) and chromium content (by 50-80 % - ANOVA, $p < 0.044$, Fig 34c). In addition, dust enrichment significantly increased the iron content at 25 °C (by 80 % - post hoc, $p < 0.03$, Fig 34d), while at 30 °C, it increased manganese (by 100 % - post hoc, $p < 0.002$, Fig 34e), copper (post hoc, $p < 0.006$, Fig 34f), and magnesium content (by 60 % - post hoc, $p < 0.05$, Fig 34g).

II.4.c. *Impact of elevated temperature on Stylophora pistillata*

In control corals, elevated temperature enhanced by two-fold the total chlorophyll content, normalized to skeletal surface area and symbiont cells (post hoc, $p < 0.0005$ and $p < 0.0003$ respectively, Fig 31c,d) as well as the photosynthesis rates normalized to skeletal surface area and symbiont cell (post hoc, $p < 0.0002$ and $p < 0.0009$ respectively, Fig 32a,b). In the dust-enriched corals, temperature rise had no impact on the coral's physiological parameters or the oxidative status.

Temperature increase did not change the metal composition of the animal tissue exposed to dust enrichment, and only reduced the manganese content of control corals compared to 25°C (Fig 33). However, several metals increased at 30°C in the dust-enriched symbionts, such as manganese (three-fold - post hoc, $p < 0.005$, Fig 34e), copper (two-fold - post hoc, $p < 0.007$, Fig 34f), magnesium (two-fold - post hoc, $p < 0.003$, Fig 34g), molybdenum (three-fold - post hoc, $p < 0.01$, Fig 34h), and cadmium (three-fold - post hoc, $p < 0.006$, Fig 34i). Also, iron content significantly increases in the control corals at 30°C compared to 25 °C ($p < 0.03$, Fig 34d).

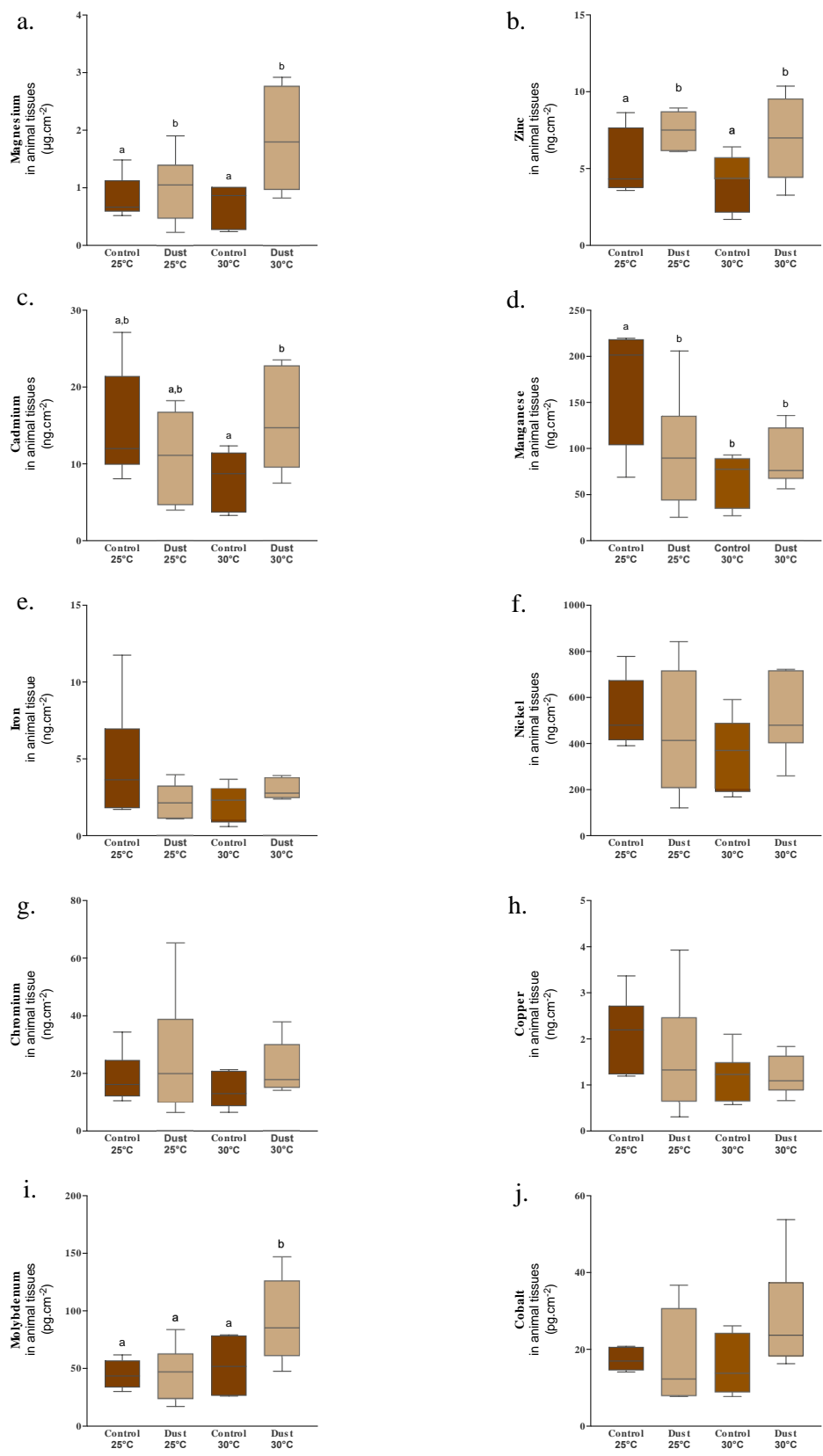


Figure 33. Metal composition of the animal tissue of *S. pistillata*

Boxplots represent the metal composition in magnesium (a.), zinc (b.), cadmium (c.), manganese (d.), iron (e.), nickel (f.), chromium (g.), copper (h.), molybdenum (i.) and cobalt (j.) in the animal tissue of the coral under different condition of dust deposition. **Control 25°C – Control 30°C**: control corals maintained at 25 °C or 30 °C without aerial dust deposition. (dark brown), **Dust 25°C – Dust 30°C**: corals maintained at 25 °C or 30 °C supplied with aerial dust at 1 mg.L⁻¹ (light brown). The box delimits the 1st and 3rd quartile, the black bar represents the median and whiskers represents min and max, n=6. Statistically significant differences are represented by different letters (a or b)

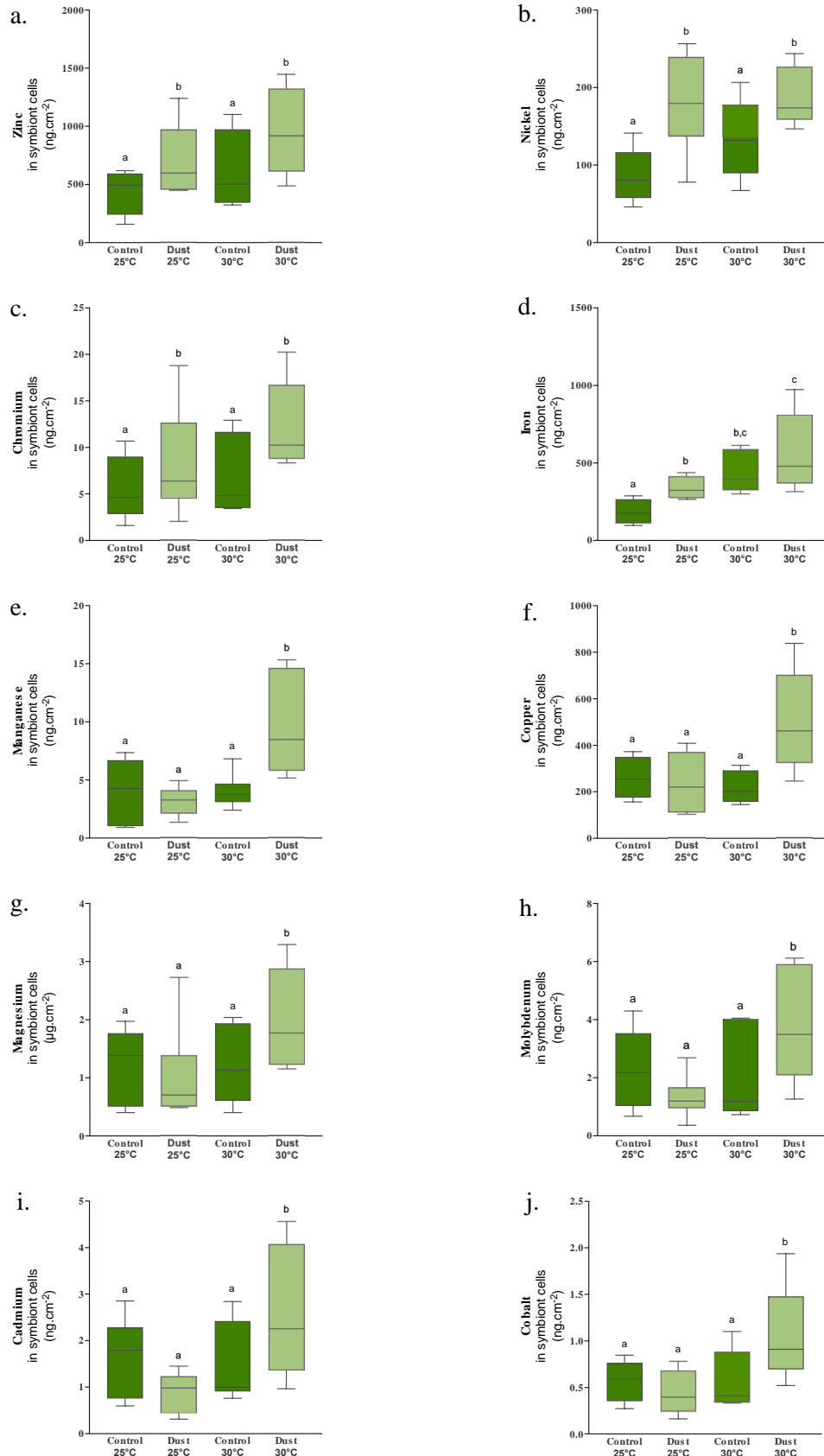


Figure 34. Metal composition of the symbiotic dinoflagellates in *S. pistillata*

Boxplots represent the metal composition of zinc (a.), nickel (b.), chromium (c.), iron (d.), manganese (e.), copper (f.), magnesium (g.), molybdenum (h.), cadmium (i.) and cobalt (j.) in the symbiotic dinoflagellates under different condition of dust deposition: **Control 25°C – Control 30°C**: control corals maintained at 25 °C or 30 °C without aerial dust deposition. (dark green), **Dust 25°C – Dust 30°C**: corals maintained at 25 °C or 30 °C supplied with aerial dust at 1 mg.L⁻¹ (light green).

The box delimits the 1st and 3rd quartile, the black bar represents the median and whiskers represent min and max, n=6. Statistically significant differences are represented by different letters (a or b)

II.4.d. Impact of dust on *S. pistillata* recovery after bleaching

The recovery of bleached corals was assessed after three weeks with and without dust supplementation. The dinoflagellate density and protein content of the holobiont was significantly lower with the addition of dust compared to the non-enriched corals (25 %, Fig S5, Appendix IV). The chlorophyll *a* and *c*₂ content normalized to skeletal surface area or symbiont cell remained unchanged with the addition of aerial dust (Fig S5, Appendix IV). However, dust-enrichment significantly increased the net photosynthesis rate normalized to surface area and symbiont cell (Welch t.test, $p < 0.026$, Fig 35a, b). Respiration rates and gross photosynthesis rates were not significantly different (Fig S5, Appendix IV).

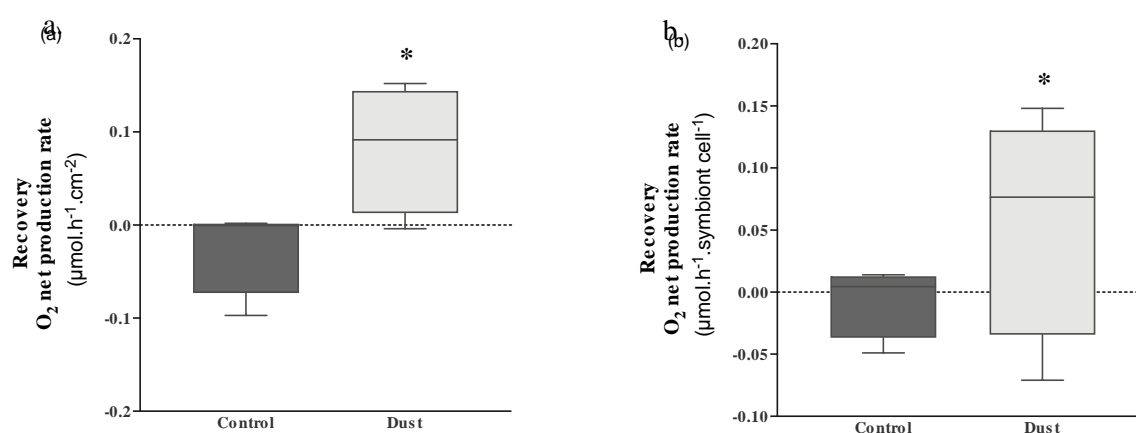


Figure 35. Recovery experiment: Photosynthesis rates of *S. pistillata*

Nubbins were bleached at high temperature, 34 °C, then left to recover at 30 °C, either in fresh seawater (**Control**, dark grey) or in dust enriched seawater, at 1 mg.L⁻¹ (**Dust**, light grey).

Boxplots represent the net photosynthesis rates normalized per skeletal surface area (a.) and per dinoflagellate cells (b.) after three weeks of recovery.

The box delimits the 1st and 3rd quartile, the black bar represents the median and whiskers represent min and max, n=4. Statistically significant differences are represented by an asterisk

II.5. Discussion

In this study, we analysed the bioelement composition and physiology of the widespread coral species *S. pistillata*, associated to *Symbiodinium* sp. (Clade A) under control and dust-exposed conditions to elucidate the beneficial or detrimental effect of dust on Red Sea corals. *S. pistillata* is an interesting species as it originates from the GoA, is well-known for its particular resistance to heat stress, has a culturable symbiont and is a well described system. Figure 36 summarizes the main results obtained in this study and the importance of dust as a metal source for corals exposed to different environmental conditions. Overall, the results indicate that desert dust delivers nanomolar amounts of nitrate and essential metals in seawater, which are assimilated mainly in the algal symbionts and can enhance their photosynthetic capacity. Moreover, the results reveal that access to dust-derived supplements can bolster coral

resistance to thermal challenges. Therefore, when in moderation, land derived metal sources can be critical to the persistence of reef-building corals. Finally, the results show that both seawater temperature and metal availability had a significant effect on metal accumulation in the symbionts and on the overall physiology of *S. pistillata*.

II.5.a. Analysis of dust confirm a seawater enrichment in nitrate and metals

The supplementation of nitrate and some other essential bioelements such as iron, manganese, zinc and copper in seawater, observed in this experiment (Fig 29) is in agreement with previous in situ measurements of the Red Sea dust (Chase *et al.*, 2006, 2011; Chen *et al.*, 2007, 2008; Al-Taani *et al.*, 2015; Torfstein *et al.*, 2017). Delivery of nutrients is rapid, since it occurred within 24-48h. Considering a total annual dust deposition of 6 Mt in the Red Sea (Jish Prakash *et al.*, 2015), dust clouds are a major source of nutrients for the benthic and pelagic organisms of this region. Nitrate can be detrimental to corals at concentrations above 2 μM , which are most often observed in disturbed areas (Shantz & Burkepile, 2014; Marangoni *et al.*, 2020). It can disrupt coral-dinoflagellate symbiosis by enhancing symbiont growth and oxidative stress (Marubini, 1996; Marangoni *et al.*, 2020; Zhao *et al.*, 2021) and decreasing inorganic carbon assimilation (Ezzat *et al.*, 2015). However, at low doses, such as those supplied by the dust (75 nM in this experiment), nitrate is a fundamental element for coral health, supplying sufficient nitrogen to oligotrophic reef waters to enhance or maintain coral metabolism without disrupting the symbiosis (Ezzat *et al.*, 2019). The very low $\delta^{15}\text{N}$ signatures of plankton (Kürten *et al.*, 2016) and coral tissue (Alamaru *et al.*, 2009; Bednarz *et al.*, 2021) indeed suggest a significant supply of dust-derived nitrate to the reef organisms (Wankel *et al.*, 2010). In our study, however, there was no change in the $\delta^{15}\text{N}$ signature of the dust-exposed corals compared to control corals, nor in their elemental nitrogen content (Appendix IV, Fig S2), likely due to the relatively short-time exposure to dust compared to the coral tissue renewal time.

II.5.b. Aerial dust deposition enhances coral photosynthesis of at 25°C and after bleaching

Metals from dust deposition were rapidly accumulated in the symbionts rather than in the host, in agreement with previous observations on the active role of symbionts as nutrient sink (Reichelt-Brushett & McOrist, 2003; Ferrier-Pagès *et al.*, 2005; Houlbrèque *et al.*, 2012). Two metals were however consistently accumulated in the tissue of the dust-enriched corals: magnesium and zinc. Magnesium plays an important role in biomineralization for calcifying organisms (Von Euw *et al.*, 2017; Martino *et al.*, 2019), while zinc enters the composition of numerous metalloproteins (Maret, 2002; Lionetto *et al.*, 2016). The dust-induced enhancement

of metal uptake in the host tissue suggests that the animal is limited in these two essential bioelements when grown in typical oligotrophic seawater.

Both seawater temperature and bioelement availability significantly changed the metallome of the algal symbionts. At 25°C, symbionts of dust-exposed corals were enriched in iron and zinc which were supplied in significant amounts by the dust. The corresponding increase in chlorophyll concentration (Fig 31d) and in net and gross photosynthesis of dust-exposed corals compared to control corals (Fig 32) suggests that primary production was limited by the availability in those nutrients. A comparable enrichment of the symbionts in iron was observed when corals were fed with *Artemia salina* nauplii, suggesting that symbionts are consistently limited in iron (Ferrier-Pagès et al., 2018). Nitrate, but especially iron and zinc are known to enhance the primary productivity of photosynthetic organisms (Raven *et al.*, 1999). Iron is involved in the composition of the photosystems I and II, as well as the cytochrome b_6 and ferredoxin complexes of the photosynthetic chain (Raven *et al.*, 1999). Zinc acts as an important cofactor for several metalloenzyme, such as carbonic anhydrase, essential in the uptake of inorganic carbon from seawater (Lionetto *et al.*, 2016). In coral reef ecosystems, iron and other trace metals are often deficient (Entsch *et al.*, 1983; Reich *et al.*, 2020), while photosynthetic dinoflagellates have among the highest iron requirements of microalgae (Rodriguez *et al.*, 2016; Reich *et al.*, 2020), followed by copper, manganese, zinc and then nickel (Rodriguez *et al.*, 2016). Therefore, it is not surprising that the rates of photosynthesis were enhanced in dust-exposed corals. This study however defines the cellular concentration of trace metals necessary to enhance the productivity of in-hospite symbionts, since this occurred with a 60% increase in iron (Control 25°C: mean = 11.81 fg.symbiont cell⁻¹ ; Dust 25°C: mean = 64.24 fg.symbiont cell⁻¹), and a 50% increase in zinc (Control 25°C: mean = 33.65 fg.symbiont cell⁻¹ ; Dust 25°C: mean = 51.95 fg.symbiont cell⁻¹). These values are however restricted to *Symbiodinium* clade A, associated with *S. pistillata* (Ezzat *et al.*, 2017), since Reich et al. (2020) showed a species-specific response to trace metal limitation. Thus, further studies are needed to evaluate the impact of dust addition on corals associated with different symbiont clades and/or different coral species fulfilling different ecological specialties. In addition, the dust-enhancement of several metal concentrations in seawater might have alleviated the limitation of one metal in the uptake of another one. In particular, it has been demonstrated that the uptake of iron can be enhanced by the addition of zinc (Santana-Casiano *et al.*, 1997), while the assimilation of nitrate, via the nitrate reductase, requires metals such as iron, zinc, molybdenum and nickel among others (Raven, 1988; Glass *et al.*, 2009). Thus, it is the availability of several metals in dust-enriched seawater that might have induced

such increase in symbiont's primary production. This highlights the importance of the metallome in the assessment of coral health and nutritional status. It occurred without any change in the $\delta^{13}\text{C}$ signature of both the animal tissue and the symbiotic dinoflagellate cells. This is again due to the slow renewal rate of coral tissue compared to the short exposition time to the nutrients. All together, these results highlight the importance of taking into account the metallome in the assessment of coral health and nutritional status. Further, they highlight how the examination of metallome reveals more intricate responses to environmental fluctuations that could not be detected via evaluating reserves of major nutrients.

Results obtained with bleached colonies of *S. pistillata* confirm that nutrients released from the dust are beneficial to coral photosynthesis, since dust-exposed corals presented a higher net photosynthesis compared to control bleached corals, despite having a lower density of symbionts and photosynthetic pigments. All together, these results confirm that the photosynthetic machinery of the symbionts needs metals such as iron and manganese, which are also essential for the stability of the association between corals and their endosymbiotic dinoflagellates (Shick *et al.*, 2011; Biscéré *et al.*, 2018a; Ferrier-Pagès *et al.*, 2018; Reich *et al.*, 2020). These results also strongly suggest that coral bleaching can be mitigated by the supply of essential metals (specifically iron and manganese) to the Symbiodiniacea, either through dust deposition as in this study or through zooplankton supply. Ferrier-Pagès *et al.* (2018) indeed observed that symbionts of heterotrophically-fed corals were enriched in iron, manganese and magnesium and presented a greater resistance to bleaching compared to the symbionts of unfed corals. In another study, the direct supply of manganese dissolved in seawater to corals also mitigated thermal stress induced bleaching (Biscéré *et al.*, 2018a). It must be noted that dust-exposed corals showed an increase in lipid peroxidation, and thus damages to membranes. An increased metal uptake can indeed lead to an increase in oxidative stress in marine organisms (Valko *et al.*, 2005). In particular, literature showed that damages due to lipid peroxidation increases with exposure to chromium (Susa *et al.*, 1996; Sinha *et al.*, 2005), iron (Welch *et al.*, 2002) and nickel (Sunderman Jr, 1987). In this study, these metals increased with dust deposition in both the animal tissue (iron) and the symbiotic dinoflagellates (chromium, iron and nickel) (Fig 33, 34). However, no other parameters of oxidative stress (ROS, protein nitration, protein carbonylation and non-enzymatic total antioxidant capacity) were changed by the deposition of aerial dust. This suggests that the damages to lipids might have been due to a higher renewal of symbiont membranes. In addition, it also suggests that metals supplied by dust were not allocated to ROS scavenging, although further studies, at the

molecular level, are needed to conclude on the implication of metals in the oxidative stress response of corals.

II.5.c. High temperature enhances metal uptake in Symbiodiniaceae

Results obtained at 30 °C highlight a temperature enhancement of iron assimilation by Symbiodiniaceae, both in control and dust-exposed corals (Fig 34d), as observed previously for symbionts of *S. pistillata* (Ferrier-Pagès *et al.*, 2018). The assimilation of several other metals, such as molybdenum, cadmium, manganese and copper were also enhanced in dust-enriched corals, suggesting a combined effect of temperature and bioelement concentration on the uptake of these metals (Fig 34). Studies on plants and animal have indeed demonstrated that metal bioaccumulation depends on temperature among several other environmental factors (Fritioff *et al.*, 2005; Jezierska & Witeska, 2006). because high temperatures lead to increased metabolism (Baker *et al.*, 2018) and a greater dependency on metal-requiring processes for photosynthesis, nutrient assimilation, or for the synthesis of antioxidant molecules (Raven *et al.*, 1999; Ferrier-Pagès *et al.*, 2018; Reich *et al.*, 2021). Dinoflagellate photosynthesis is therefore reduced under high temperatures and low iron availability (Reich *et al.*, 2021). In this study, while the increase in intracellular iron and manganese at 30 °C enhanced the chlorophyll content and rates of net and gross photosynthesis of control corals, no more enhancement of these parameters occurred in dust-enriched corals. All together, these results suggest that dust-enriched corals already reached the maximal carbon fixation rate. By comparing the results obtained at 30 °C with those of 25 °C, we can even suggest that iron- and not zinc or manganese, was the limiting factor for photosynthesis at 25 °C, although we cannot preclude any beneficial effects from the other nutrients.

II.6. Conclusion

Climate change severely disrupts the mutualistic relationship between reef building corals and their endosymbiotic dinoflagellates, and thus jeopardizes coral health and the future of reef ecosystems (Ellis *et al.*, 2019). It has been demonstrated that corals can better cope with thermal stress when they have access to greater amounts of macro (both nitrogen and phosphorus) and micronutrients, such as iron, manganese and zinc (Béraud *et al.*, 2013; Rosset *et al.*, 2017; Biscéré *et al.*, 2018a; Ferrier-Pagès *et al.*, 2018; Reich *et al.*, 2021). In many reefs, however, low nutrient and low metal availability hinder the growth and photosynthesis of coral symbionts and compromise their capacity to cope with thermal stress. This study offers clear evidence that aerial dust enriches the water in essential nutrients and promotes coral photosynthesis at low temperature or in bleached corals. This likely one of the reasons of the

resistance of GoA corals to seawater warming. Our results also demonstrates that iron uptake in marine organisms is not only modulated by light or seawater pH, as shown in previous studies (Sunda & Huntsman, 1997; Fujii *et al.*, 2011; Hoffmann *et al.*, 2012), but also by iron availability and seawater temperature. All together, these observations highlight the complex regulation of metal uptake by coral symbionts, that need to be further investigated (Fig 36). Indeed, future environmental changes, such as ocean acidification and warming will result in significant decreases in nutrient availability, that will have profound impact on coral health.

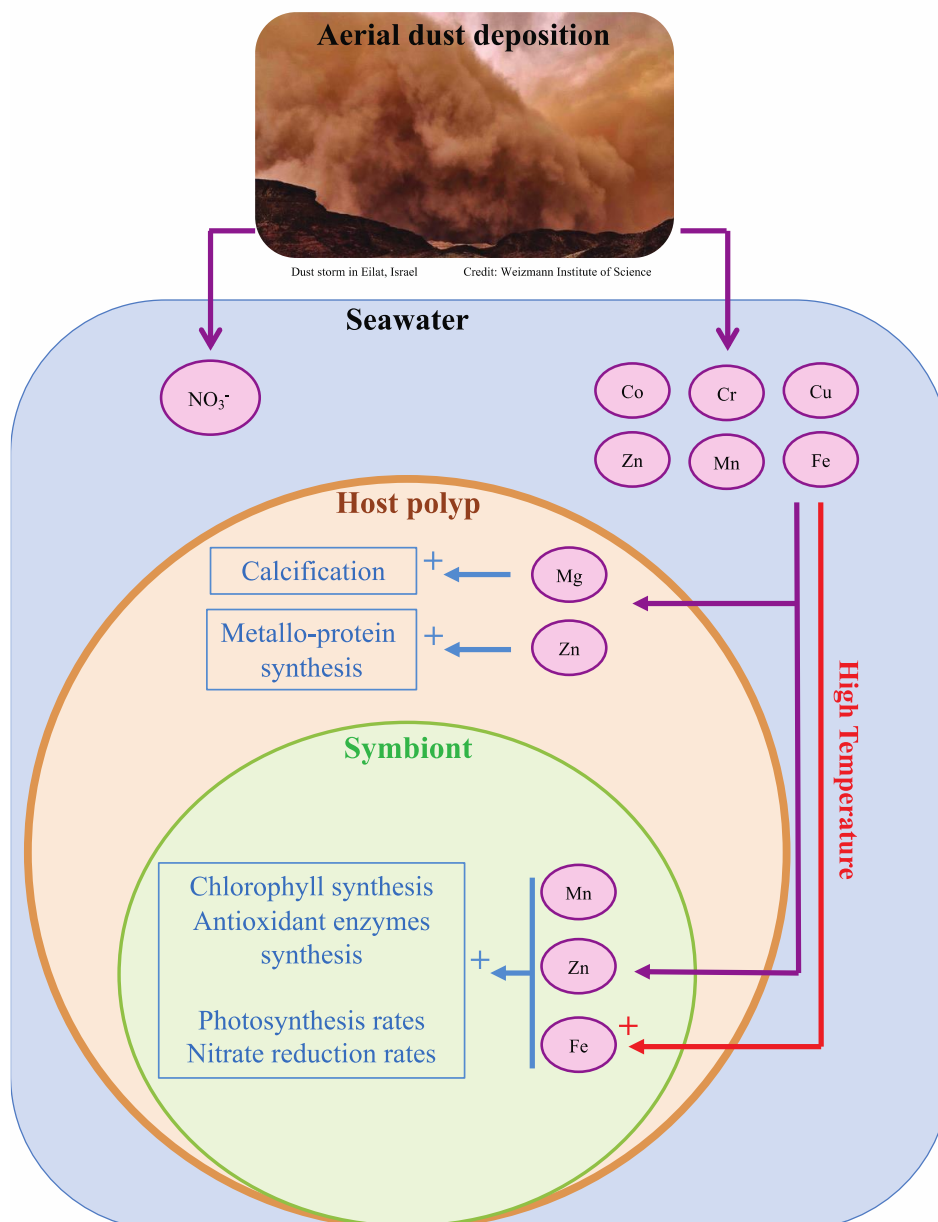


Figure 36. **Summary of the effect of nutrients supplied by dust deposition on *S. pistillata***

Aerial dust deposition supplies essential bioelements (purple) to the seawater, such as NO_3^- : nitrate, **Co**: cobalt, **Cr**: chromium, **Cu**: copper, **Zn**: zinc, **Mn**: manganese, **Fe**: iron. Metal uptake in the animal polyp (brown) and the symbiotic dinoflagellate (green) help to promote different metabolic processes (blue) in the holobiont. Temperature rise (red arrow) enhanced the uptake of iron in the symbiotic dinoflagellates. Results obtained in this experiment as well as in Biscéré et al. (2018) for calcification and Montalbetti et al. (2021) for metallo-protein synthesis.

GENERAL DISCUSSION CONCLUSION AND PERSPECTIVES



GENERAL DISCUSSION, CONCLUSION AND PERSPECTIVES

I General discussion

The main objective of this thesis was to investigate the response of corals to changes in the availability of macronutrients (dissolved inorganic nitrogen and phosphorus, DIN and DIP) and metals in seawater. Many studies (presented in the introduction, Table 1) have already assessed the effects of DIN and DIP availability on coral metabolism. All together, these studies have suggested that the DIN:DIP ratio is a main driver of the coral response to nutrient enrichment, with some corals being impacted by a high DIN:DIP ratio (excess DIN compared to DIP). However, the effects of imbalanced DIN:DIP ratios are unclear and need further investigations. This thesis has therefore tackled this subject and is divided into two main aims: i) assess the effects of balanced and imbalanced seawater DIN:DIP ratios on coral physiology and 2) assess the importance of metals for coral physiology and resistance to thermal stress. For this purpose, we worked with an octocoral species *Sarcophyton glaucum* and three scleractinian coral species – the branching species *Stylophora pistillata* and *Pocillopora damicornis* and the foliose species *Turbinaria reniformis*. The results obtained in the individual chapters are discussed independently in the respective sections. In this general discussion, we first summarize the main results obtained in each chapter, before presenting some answers to the questions below:

- 4) **First question:** Does DIN enrichment at normal/low DIP concentrations (imbalanced DIN:DIP ratios) have always adverse effects on coral health? On the contrary, do corals remain healthy under balanced DIN and DIP enrichment?
- 5) **Second question:** Do we observe a change in cellular C:N:P content and stoichiometry under imbalanced DIN:DIP ratios? in other words, does DIN enrichment affect corals because it induces phosphorus limitation in coral tissue?
- 6) **Third question:** Does the ability of corals to feed on dissolved nutrients (DIN, DIP and DOP) determine their response to DIN enrichment?
- 7) **Fourth question:** Are corals limited only in macronutrients, or can they also be limited in trace metals? Can the availability of trace metals also determine their ability to withstand heat stress?

I.1. Summary of the results

In Chapter 1, we investigated the cross effects of two temperatures, UVR levels and imbalanced DIN:DIP ratio on the physiology and bleaching response of *Pocillopora damicornis*. The imbalanced DIN:DIP ratio (17.5 instead of 2.5 for a natural balanced DIN:DIP) was achieved by adding 3 μM nitrate. We observed that the photosynthesis of the symbionts of *P. damicornis* was impaired under thermal stress or nitrate enrichment (imbalanced N:P), but only when corals were shaded from UVR. However, exposure to low levels of UVR counterbalanced the negative effects of both thermal stress and imbalanced DIN:DIP ratio on symbiont photosynthesis. Overall, this work highlights the importance of taking into account the environment when assessing the response of corals to a chemical stressor.

In Chapter 2, we investigated the effects of different balanced and imbalanced DIN:DIP ratios on the physiology and elemental composition of the octocoral *Sarcophyton glaucum* and the scleractinian *Turbinaria reniformis*. The different DIN:DIP ratios were achieved by adding 3 μM nitrate and 1 μM phosphate, either separately (imbalanced DIN:DIP ratio of 17.5 and 0.4 respectively) or in combination (balanced DIN:DIP ratio of 2.9). We observed that imbalanced DIN:DIP ratio changed the elemental composition of *T. reniformis*. Nitrate enrichment led to an imbalanced N:P ratio and a P limitation in the coral tissue, together with detrimental effects on symbiont photosynthetic efficiency. This change in cellular stoichiometry was linked to high uptake rates of nitrate from seawater, and a large inflow of nitrogen into the coral tissue. On the contrary, no changes were observed in *S. glaucum*, likely due to much lower nitrate uptake rates. This study thus suggests that the detrimental effects of nitrate on coral physiology are partly due to a change in cellular stoichiometry of the coral tissue. These changes occur only if a large amount of nitrogen enters the symbiosis.

In Chapter 3, we investigated the effects of balanced and imbalanced DIN:DIP seawater ratio on the capacity of the scleractinian coral *Stylophora pistillata* to use dissolved organic phosphorus (DOP) in seawater. This capacity was assessed through the activity of the extracellular and intracellular phosphatases, which are involved in the degradation of organic into inorganic phosphorus. The different DIN:DIP ratios were obtained by nutrient depletion (undetectable nutrient levels, balanced DIN:DIP ratio of 2), long-term enrichment with 2 μM nitrate or ammonium (imbalanced DIN:DIP ratio of 12.5) and short-term enrichment with 2 μM nitrate or ammonium (imbalanced DIN:DIP ratio of 250). We observed that depletion, or nitrate and ammonium enrichment had different effects on the cellular P content, N:P ratio and

phosphatase activity. Nutrient depletion enhanced phosphatase activities. However, such enhancement failed at maintaining a maximal cellular P content, although the N:P ratio of the coral tissue was still balanced. The decrease in cellular P content was due to the complete lack of phosphate in seawater, following nutrient depletion. Ammonium also increased phosphatase activity. Such enhancement maintained a maximal cellular P content, as well as a balanced N:P ratio. On the contrary, phosphatase activity was not enhanced under nitrate enrichment. As a consequence, we observed a significant decrease in cellular P content, leading to an imbalanced N:P ratio in coral tissue. This chapter shows that nitrate disrupts the cellular N:P ratio of the coral tissue by impacting the capacity of corals to take up phosphorus from seawater.

In Chapter 4, we investigated the effects of increased trace metal availability on the physiology of the scleractinian coral *S. pistillata* at two temperatures, 25 °C (winter) and 30 °C (summer). We found that metal accumulation in the coral symbionts depended on metal concentration in seawater and seawater temperature. Both factors increased the cellular content of the symbionts in iron and zinc, which in turn led to an increase in chlorophyll content and photosynthetic rates. Metal availability in seawater also increased the rates of photosynthesis of bleached corals following a thermal stress. This study therefore highlights the importance of metals for coral metabolism, especially under thermal stress.

1.2. Axis I: Coral response to balanced and imbalanced seawater DIN:DIP ratios

1.2.a. Does DIN enrichment at normal/low DIP concentrations (imbalanced DIN:DIP ratios) always have adverse effects on coral health

The effects of an imbalanced DIN:DIP ratio following nitrate enrichment on coral physiology has been the focus of several studies, which have shown both positive, null, and negative effects on the photosynthetic parameters, the calcification and the heat stress resistance of corals (Table 1). We found the same discrepancy in this thesis. We indeed investigated the response of three coral species belonging to different orders and families and having different shapes: *Pocillopora damicornis* (Scleractinian, Pocilloporidae, branching coral) *Turbinaria reniformis* (Scleractinian, Dendrophylliidae, foliose coral) and *Sarcophyton glaucum* (Octocoral, Alcyoniidae) to imbalanced DIN:DIP ratio caused by nitrate enrichment.

We first observed a species-specific response, with a significant effect on *P. damicornis*, a milder effect on *T. reniformis* and no effect on *S. glaucum*. This is in line with the literature, which observed that an imbalanced DIN:DIP ratio impairs the physiology of branching corals,

as evidenced in this thesis by the decrease in photosynthetic rates of *P. damicornis* (Table 1, Wiedenmann *et al.* 2013; Ezzat *et al.* 2015; Marangoni *et al.* 2020). However, foliose corals were shown to be less responsive to an imbalanced DIN:DIP ratio, as evidenced in this thesis by the decrease in photosystem efficiency but maintenance of photosynthetic rates in *T. reniformis* (Table 1, Ezzat *et al.* 2016b; Dobson *et al.* 2021b). Finally, and although they have been less investigated, all studies on octocorals showed that they are insensitive to imbalanced DIN:DIP ratio (Table 1). This is evidenced in this thesis by the lack of changes in the physiology of *S. glaucum*. All together, these observations suggest that coral species react differently to imbalanced DIN:DIP ratio in the seawater, probably by displaying a different metabolism or different strategies in terms of nutrient uptake and assimilation.

Although there is a species-specific response to nutrient enrichment, the results of this thesis also show that environmental factors such as UV radiation, can influence the coral response to an imbalance DIN:DIP ratio. We investigated the response of *P. damicornis* to imbalanced DIN:DIP ratio (nitrate enrichment) under thermal stress and/or UV radiation. We observed that exposure to low levels of UVR counterbalanced the negative effects of nitrate and also thermal stress on the coral's photosynthetic rates. The different ways in which low doses of UVR can be beneficial to corals have been detailed in chapter 1. Mainly, exposure to low doses of UVR was shown a) to increase the concentration of photosynthetic pigments in phytoplankton (Wang *et al.*, 2022), b) to stimulate the activity of the carbonic anhydrase, which is responsible for providing CO₂ for photosynthesis in phytoplankton (Wu & Gao, 2009; Xu & Gao, 2009); c) to promote the synthesis of nitrogen-rich UV-absorbing compounds, removing the excess nitrate from coral tissue (Rai *et al.*, 1998; Peinado *et al.*, 2004; Korbee *et al.*, 2005; Shick *et al.*, 2005; Zheng & Gao, 2009). Thus, enhancement of both carbon and nitrogen cycling by exposure to low UVR may have prevented nitrate-induced inhibition of photosynthesis by regulating the complex link between carbon and nitrogen metabolism. Our study used low levels of UVR. It has to be kept in mind that high doses of UVR can be harmful to corals (e.g. Gleason *et al.* 2006; Torregiani and Lesser 2007; Ferrier-Pagès *et al.* 2007; Torres-Pérez and Armstrong 2012).

1.2.b. Do corals remain healthy under balanced DIN and DIP enrichment?

We investigated the response of *T. reniformis* and *S. glaucum* to a balanced supply of DIN and DIP. The response was also species-dependent. While an imbalanced DIN:DIP ratio decreased the photosynthetic efficiency of *T. reniformis*, a balanced supply of nutrient had positive effects, with increased photosynthetic rates and tissue biomass. Thus, a balanced DIN:DIP ratio

promoted the metabolism of *T. reniformis*. This is in agreement with previous studies which showed that a balanced and moderate nutrient enrichment, as used in this study, benefit corals by mitigating the negative effects of thermal stress or acidification (Holcomb *et al.*, 2010; Chauvin *et al.*, 2011; Ezzat *et al.*, 2016a, 2019; Dobson *et al.*, 2021a) without negatively affecting calcification (Bongiorni *et al.*, 2003; Dizon & Yap, 2005; Tanaka *et al.*, 2007). In contrast to *T. reniformis*, the physiology of *S. glaucum* was not enhanced by the dual DIN:DIP enrichment.

Overall, the response of corals to nutrient enrichment, either provided in a balanced or imbalanced ratio was species-specific. While the physiology of the octocoral *S. glaucum* was not affected by changes in nutrient availability in seawater, scleractinian always responded by changing their physiology. Environmental factors must also be considered in the response of corals to changes in nutrient availability, since exposure to low levels of UVR mitigated the negative effects of DIN enrichment.

1.2.c. Do we observe a change in C:N:P content and stoichiometry with changes in seawater DIN:DIP ratio?

We investigated the elemental composition of three coral species belonging to different orders and families and having different shapes: *Stylophora pistillata* (Scleractinian, Pocilloporidae, branching coral) *Turbinaria reniformis* (Scleractinian, Dendrophylliidae, foliose coral) and *Sarcophyton glaucum* (Octocoral, Alcyoniidae) to imbalanced DIN:DIP ratio caused by nitrate enrichment. We observed that nitrate supply increased the cellular N:P ratio of the scleractinian corals *S. pistillata* and *T. reniformis*. In *S. pistillata*, the increase in cellular N:P ratio was driven by a decrease in internal phosphorus content, characteristic of a phosphorus deficiency. A study by Rosset *et al.* (2017) similarly highlighted an internal phosphorus starvation triggered by nitrogen addition. In *S. pistillata*, previous studies have shown that nitrate enrichment enhanced oxidative stress and decreased the photosynthesis of the symbionts as well as the carbon translocation from the symbionts to the host (Ezzat *et al.*, 2015; Marangoni *et al.*, 2020). In contrast to *S. pistillata*, the alteration of the cellular N:P ratio in *T. reniformis* was driven by an increase in internal nitrogen content, while phosphorus content remained stable. This is likely to have the same negative effects on the physiology of *T. reniformis*, as a previous study showed that photosynthesis and calcification were impaired under the combined effects of nitrate enrichment and thermal stress (Ezzat *et al.*, 2016b). Finally, unlike the scleractinian

corals studied, the elemental composition of the octocoral *S. glaucum* remained stable even with an imbalanced DIN:DIP ratio, and no physiological changes were observed in this thesis or in previous studies (Koop *et al.*, 2001). All together, these observations suggest that the observed physiological response of the corals to imbalanced DIN:DIP ratio is due to changes in their cellular stoichiometry.

In *S. pistillata*, we also investigated the changes in elemental composition with an imbalanced DIN:DIP ratio caused by ammonium. We only observed an imbalance cellular N:P ratio with nitrate enrichment, not with ammonium enrichment. The differential effect of nitrogen form on the internal composition of the coral holobiont may explain while ammonium has always been found to be less detrimental to coral physiology than nitrate (Ezzat *et al.*, 2015; Burkepile *et al.*, 2020; Marangoni *et al.*, 2020). Indeed, nitrate reduced the thermal tolerance and photosynthesis efficiency while increasing oxidative stress in the coral holobiont (Ezzat *et al.*, 2015; Marangoni *et al.*, 2020). On the contrary, ammonium helped to maintain coral metabolism even under thermal stress (Ezzat *et al.*, 2015; Marangoni *et al.*, 2020).

In summary, nitrate enrichment can alter the elemental composition and stoichiometry of the corals, either by reducing the internal P content or increasing the internal N content. The results however reveal differences between scleractinian corals and octocorals. While the cellular N:P stoichiometry and the physiology were affected by nitrate enrichment in scleractinian corals, no changes were observed in the octocoral studied. The results also highlight a difference between nitrate and ammonium enrichment on the cellular N:P ratio since ammonium enrichment did not disrupt the cellular N:P ratio. A similar difference was observed at the physiological level in previous studies.

1.2.d. Does the ability of corals to feed on dissolved nutrients determine their response to DIN enrichment?

1.2.d.i. Differences in the uptake of dissolved inorganic nutrients (DINut) at DIN enrichment

The results obtained in this thesis show that the capacity of corals to feed on dissolved inorganic nutrients affect their cellular elemental stoichiometry and indirectly their physiology.

The scleractinian coral *T. reniformis* showed high rates of nitrate uptake under nitrate enrichment. Such high rates of nitrogen uptake following nitrogen enrichment have already been observed in scleractinian corals (Grover *et al.*, 2002, 2003; Badgley *et al.*, 2006; Pupier

et al., 2021) . Under low DIP availability (imbalanced DIN:DIP ratio), this massive input of nitrogen in the coral tissue induced an imbalanced cellular N:P ratio. However, when DIP concentrations were increased in seawater (balanced DIN:DIP ratio), DIP was also taken up rapidly by *T. reniformis*. This dual uptake of DIN and DIP did not disrupt the cellular stoichiometry of *T. reniformis*.

In contrast to *T. reniformis*, *S. glaucum* showed a 5 times lower nitrate uptake rates under nitrate enrichment alone, similar to the control. Low DIN uptake rates were also previously observed in several species of soft corals (Pupier *et al.*, 2021), suggesting that it is a general feature of soft corals. Such low input of nitrogen inside the tissue of *S. glaucum* likely did not disrupt the cellular N:P ratio. Nitrate uptake rates slightly increased under the combined DIN:DIP enrichment, so that no P limitation was possible. Such control of cellular N:P ratio may explain the adaptation and dominance of soft corals in nutrient-rich reefs (Schleyer & Celliers, 2003; Baum *et al.*, 2016). Indeed, soft corals were shown to outcompete and overgrow scleractinian corals in nutrient-rich reefs (Alino *et al.*, 1992; Norström *et al.*, 2009).

Overall, these results highlight fundamental differences between scleractinian and soft corals in their nutritional capacity, which may in turn explain their different susceptibility to nutrient enrichment and eutrophication in general.

I.2.d.ii. Differences in phosphorus demand under DIN enrichment

The study performed on the activity of the extracellular and intracellular phosphatase activity brings additional insights into how nitrate enrichment can disrupt the cellular N:P ratio of the coral tissue. In addition to inducing a massive input of nitrogen inside the coral tissue (as described above), nitrate enrichment impairs the capacity of corals to exploit the external sources of phosphorus, particularly the dissolved organic phosphorus (DOP). All together, this explains the increase in cellular N:P ratio and P limitation observed under nitrate enrichment. Corals can overcome their P limitation by degrading DOP sources in seawater via the release of extracellular phosphatases. They also possess intracellular phosphatases, which are able to degrade intracellular DOP reserves when P is needed. Phosphatase activities are therefore known to increase when corals are grown under very low levels of dissolved inorganic P and are P limited (Godinot *et al.*, 2013, Chapter 3). The results obtained in this thesis also show that phosphatase activities increase under ammonium enrichment- so when corals have an excess of nitrogen and need P to compensate for this excess and can thus use both nutrients for their growth. However, the results also show that phosphatase activities are not increased under nitrate enrichment. A likely explanation is that the synthesis of phosphatase requires energy and organic carbon from photosynthesis (Van Boekel & Veldhuis, 1990). Since nitrate is

known to require energy for its reduction into ammonium, and is also known to impair the coral's photosynthetic abilities (Chapter 1 and Ezzat *et al.*, 2015), corals may not have enough energy to assimilate nitrate and synthesise phosphatase enzymes at the same time.

In summary, different coral species have different nutrient uptake strategies depending on the seawater DIN:DIP ratio. While scleractinian corals show a high nutrient uptake when nutrients are available in seawater, octocorals show an increased nutrient uptake only under balanced DIN:DIP.

Finally, nitrate, unlike ammonium, impairs the corals' ability to hydrolyse DOP, and thus promotes phosphorus deficiency in corals.

Therefore, the corals' ability to use DIN, DIP and DOP will determine how corals respond to imbalanced and balanced DIN:DIP enrichment.

1.3. Axis II: Coral response to microelements, trace metals, supply

Here, we investigated the effects of the supply of trace metals by dust originating from the deserts around the Gulf of Aqaba (Sinai, Negev and Arabian desert). We assessed the effects of this dust, rich in trace metals, on the physiology of the branched scleractinian *S. pistillata*.

1.3.a. Are corals limited only in macronutrients, or can they also be limited in trace metals?

We found that the supply of trace metals, especially iron and zinc, through dust leaching, increased the photosynthetic capacities of the symbionts of *S. pistillata*, as evidenced by an increase in total chlorophyll content and photosynthetic rate. Iron is essential for the synthesis of several compounds of the photosynthetic chain such as photosystem I and II, cytochrome b_6 and the ferredoxin complexes (Raven *et al.*, 1999). Zinc, for its part, is a known co-factor for over 300 metalloenzymes, including carbonic anhydrase, which is necessary for the uptake of inorganic carbon required for the photosynthetic process (carbon cycle) (Lionetto *et al.*, 2016), and alkaline phosphatase (phosphorus cycle) (Shaked *et al.*, 2006; Reich *et al.*, 2020). As the supply of metals enhanced primary production of *S. pistillata*, this suggests that trace metals, especially iron, are limiting nutrients for corals. Such limitation has already been highlighted for different coral species (Entsch *et al.*, 1983; Shick *et al.*, 2011; Reich *et al.*, 2020). Iron limitation can induce a decrease in nitrate and phosphorus assimilation, symbionts division as well as photosynthesis efficiency (Entsch *et al.*, 1983; Shick *et al.*, 2011; Reich *et al.*, 2020).

The results of this study also suggest that the uptake of one metal can be regulated by the availability of other metals. For example, Santana-Casiano *et al.* (1997) have shown that the uptake of iron is enhanced when zinc is available, and Reich *et al.* (2020) have shown that the uptake of nickel, zinc, cobalt, molybdenum and vanadium is enhanced by the supply of iron. Furthermore, metabolic processes usually require several metals to be fully functional. For example, the carbon cycle requires iron, zinc, copper, cadmium and cobalt (Morel *et al.*, 1994; Twining & Baines, 2013), the phosphorus cycle requires zinc (Shaked *et al.*, 2006) while the nitrogen cycle requires iron, zinc, nickel and molybdenum for nitrate reductase activity (Raven, 1988; Glass *et al.*, 2009; Twining & Baines, 2013). Therefore, depletion of one metal can affect a variety of processes, especially in the assimilation of macroelements. In iron-deficient symbiotic dinoflagellates, impairment of nitrogen assimilation leads to a decrease in cell division (Reich *et al.*, 2020). Further studies should therefore aim at investigating the combined effects of metal availability on coral physiology.

1.3.b. Can the availability of trace metals also determine their ability to withstand heat stress?

We investigated the effects of a temperature increase of 25 °C and 30 °C on the metal content of *S. pistillata* with or without a supply of trace metals. In this study, the temperature of 30 °C was not sufficient to trigger bleaching in *S. pistillata*. Thus, we were not able to assess the role of trace metals on the coral ability to withstand heat stress.

However, our result did show that increase in temperature boosted the photosynthetic rates of the symbiotic dinoflagellates in control non-metal enriched corals. This increase in photosynthetic rates at high temperature was induced by a bioaccumulation of iron in the symbiont cells. This phenomenon of bioaccumulation of metals with temperature has also been observed in plants and animals, due to a higher metabolism at high temperature (Fritioff *et al.*, 2005; Jezierska & Witeska, 2006; Baker *et al.*, 2018). In addition, in metal-enriched corals, there was also a bioaccumulation of metals (manganese, copper, magnesium, molybdenum, cadmium and cobalt). However, the photosynthetic rates of corals were not further enhanced, likely because photosynthetic rates were already at their maximum at 25°C in the metal-enriched corals.

We also investigated the effect of metal availability in *S. pistillata* recovering from thermal stress and bleaching. We found that metal availability helped to boost the photosynthetic rates of recovering corals. This further suggest that the coral holobiont, and particularly the photosynthetic machinery is potentially limited in metals. Though, while we did not directly

assess the role of metal availability on the coral thermal stress resistance, its beneficial role in coral recovery suggests that metal supply could mitigate coral bleaching under thermal stress.

In summary, corals can not only be limited in macroelements, but also in microelements, particularly in iron. The metal deficiency in corals can severely alter their nutritional status and eventually jeopardise the stability of the coral-dinoflagellate symbiosis.

The role of trace metals availability under thermal stress has not been completely assessed in the thesis. However, we did observe that corals increased their metal requirement at high temperature. In addition, metal availability also helped corals to recover after a thermal stress.

II Conclusion

Coral response to balanced and imbalanced DIN:DIP enrichment is complex and environmental dependent. Overall, we found in this work that the deleterious effects of nitrate enrichment on coral physiology is species-specific and partly due to a change in the cellular stoichiometry of the coral holobiont. Nitrate enrichment can disrupt the cellular N:P ratio of the coral holobiont, mainly by inducing P limitation. In addition to macroelements, microelements particularly iron are essential for coral metabolism and resilience to thermal stress. This information is important to properly assess the response of corals to increasing stress from nutrient enrichment, as management strategies should be specifically tailored to the environmental characteristics and composition of coral species in managed reefs.

III Perspectives

This work has provided important insights on 1) the coral response to nutrient enrichment and 2) the role of trace elements in the coral holobiont but has also paved the way for future experiments.

III.1. Axis I: Coral response to balanced and imbalanced seawater DIN:DIP ratios

III.1.a. Does DIN enrichment at normal/low DIP concentrations (imbalanced DIN:DIP ratios) always have an adverse effects on coral health? On the contrary, do corals remain healthy under balanced DIN and DIP enrichment?

In the literature, more than half of the studies have focused on scleractinian corals, especially branching corals (Table 1). However, we highlighted that the coral response to DIN enrichment was species-specific, with scleractinian corals being more sensitive to nutrient imbalance than octocorals. Thus, the next step would be to expand the range of species used in studies. A focus

on octocorals is particularly important as they seem to be insensitive to nutrient enrichment and be thus quite successful in growing in nutrient-rich waters.

Moreover, most studies on nutrient enrichment are conducted in the laboratory under control conditions, and while natural PAR irradiance is replicated with lamps, the UV spectrum is usually missing. However, this work highlighted the important influence of environmental factors, especially UVR exposure, on the coral response to nutrient enrichment. Therefore, the inclusion of natural UVR exposure and other combination of environmental factors is essential to fully assess the coral response to nutrient enrichment.

III.1.b. Do we observe a change in C:N:P content and stoichiometry with changes in seawater DIN:DIP ratio? In other words, does DIN enrichment affect corals because it induces phosphorus limitation in coral tissue?

In this work, we found that the change in coral physiology under DIN enrichment was induced by changes in elemental composition and especially in the cellular N:P ratio. We also found that changes in elemental composition can occur before significant changes of coral physiology were observed. However, there are not many studies in the literature examining the effects of nutrient enrichment on coral elemental composition (Meyer & Schultz, 1985; Muscatine *et al.*, 1989; Stambler *et al.*, 1991; Snidvongs & Kinzie, 1994; Cox & Ward, 2002; Tanaka *et al.*, 2007, 2010, 2017; Godinot *et al.*, 2011; McCauley & Goulet, 2019). Furthermore, of these ten studies, the phosphorus content was only assessed in less than half (4) of the studies (Meyer & Schultz, 1985; Snidvongs & Kinzie, 1994; Tanaka *et al.*, 2010; Godinot *et al.*, 2011). Therefore, it is important to include elemental composition with a focus on nitrogen and phosphorus content and subsequent cellular N:P ratios in further studies as this may reveal complex responses to nutrient enrichment that cannot be detected by evaluating coral physiology alone.

III.1.c. Does the ability of corals to feed on dissolved nutrients (DIN, DIP and DOP) determine their response to DIN enrichment?

In this work, we found that the ability of corals to use DOP determines coral response to DIN enrichment via phosphatase activity. Phosphatase activity in corals has been studied mainly in scleractinian corals so far (Jackson *et al.*, 1989; Godinot *et al.*, 2013; Wiedenmann *et al.*, 2013; Houlbrèque *et al.*, 2015). However, as corals species differ in their ability to store and utilise phosphorus, they might have different levels of phosphorus limitation. Thus, assessing the phosphorus limitation and requirement in a wider range of coral species is essential to fully understand the differences in coral susceptibility to bleaching following thermal stress and imbalanced N:P ratios.

III.2. Axis II: Coral response to trace metals

The effect of trace metals on coral physiology have been poorly studied, at the exception of some studies which have investigated their assimilation by corals and their allocation to the different compartments of the symbiotic association (e.g.: Harland & Brown, 1989; Reichelt-Brushett & McOrist, 2003; Metian *et al.*, 2015; Hédouin *et al.*, 2016). Therefore, many investigations remain to be done.

Overall, it may be interesting to link changes in the metallome with changes in the elementome and physiology of different coral species under a range of environmental conditions, including seawater warming, to assess which trace elements can drive the coral response to the environment. Also, literature on plants or phytoplankton has shown that the uptake of macronutrients may be driven by the availability of trace metals (Morel *et al.*, 1994; Shaked *et al.*, 2006; Reich *et al.*, 2020). The assimilation of carbon is dependent on the carbonic anhydrase activity, requiring zinc and cobalt. Similarly, the assimilation of phosphorus depends on alkaline phosphatase activity, requiring zinc, while the assimilation of nitrogen depends on the nitrate reductase activity, requiring molybdenum and vanadium. The field of trace metal interactions- which trace metal promotes the assimilation of other trace metals, such as iron uptake enhanced by zinc addition in algae (Santana-Casiano *et al.*, 1997)- in corals is also completely unknown. Furthermore, studies also showed that phytoplankton could use alternate trace metals as substitutes in certain processes, such as using cadmium instead of zinc in carbonic anhydrase (Morel *et al.*, 2020). Thus, further studies are needed on how to understand how trace metals availability can affect the nutritional capacity of corals.

Finally, corals are also holobionts, associated with microbes, which have high capacities in taking up metals. For example, numerous bacteria have siderophores to scavenge iron in seawater. The role of these microbes in acquiring and transferring trace metals to their host is very poorly known and need further investigations, as this is the basis of any symbiosis. In addition, we have demonstrated that trace metal content increases in coral tissue following dust addition. However, the desert dust is not only rich in trace metals, it also consists of a variety of organisms, from bacteria to fungi and other microeukaryotes. These microorganisms can interact with corals and be both beneficial such as diazotrophic bacteria or detrimental (Bednarz *et al.*, 2021; Uyl *et al.*, 2016; reviewed in: van Oppen & Blackall, 2019). Therefore, it is essential to assess the role of the microorganisms caused by the desert dust to understand how desert dust deposition impact corals.

RÉSUMÉ DÉTAILLÉ EN FRANÇAIS



RÉSUMÉ DÉTAILLÉ EN FRANÇAIS

I Introduction

I.1. Présentation des récifs coralliens

Les récifs coralliens couvrent une faible portion de la surface des océans, environ 0.1 % avec une surface de 300 000 km² (Spalding et al., 2001). Cependant, ces écosystèmes abritent plus de 25 % de la biodiversité marine (Knowlton *et al.*, 2010) et sont parmi les plus productifs sur Terre (Connell, 1978). Ils remplissent différentes fonctions autant biologiques, en tant que niche écologique, protection côtière et source de composés pharmaceutiques, qu'économique en tant qu'attraction touristique (Ferrario et al., 2014; Leal et al., 2012; Spalding et al., 2016). Globalement, les services rendus par les récifs coralliens sont estimés à 25 milliards d'euros par an, avec plus de 500 millions de personnes qui en dépendent (Spalding et al., 2017; Wilkinson & Souter, 2008). Ces services rendus par les récifs coralliens sont étroitement liés à leur structure tridimensionnelle complexe, formés par divers organismes calcifiants, en particulier les coraux scléactiniaires.

I.2. Description des coraux

Les coraux sont des organismes diploblastiques composés de deux couches de tissus : l'endoderme et l'ectoderme (Fig 3). Leur plan d'organisation est relativement simple et suit

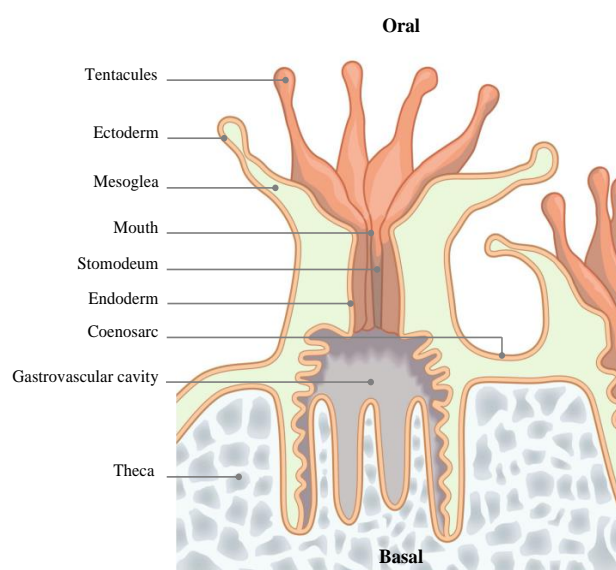


Figure 3. Coupe transversale d'un corail scléactiniaire colonial
Modifié à partir de Dorling Kindersley

une symétrie radiale d'ordre six ou huit. Les coraux peuvent également être entourés d'une couche de mucus qu'ils sécrètent.

Ils font parties des Anthozoaires dans le phylum des Cnidaires (Fig 4). Chez les Anthozoaires, il y a deux classes principales : celle des Octocoralliaires, communément appelés les « coraux mous », qui présentent une symétrie octogonale et sont généralement caractérisés par une absence de squelette en carbonate de calcium, et celle des Hexacoralliaires qui comprend les Scléractiniaires, communément appelés les « coraux durs », ils présentent une symétrie hexagonale et possèdent un exosquelette de carbonate de calcium (aragonite). Ces derniers sont considérés comme les bâtisseurs des récifs coralliens, avec près de 1500 espèces connues (Appeltans *et al.*, 2012).

Les coraux forment un **holobionte**, qui est l'association d'un organisme hôte, animal et d'autres organismes symbiotiques vivant au contact ou à l'intérieur de l'hôte. Chez le corail symbiotique, ces organismes sont des algues dinoflagellés implantés dans les cellules de l'animal hôte, ainsi que divers micro-organismes telles que des bactéries, virus et champignons, présents dans le mucus, les tissus ou le squelette.

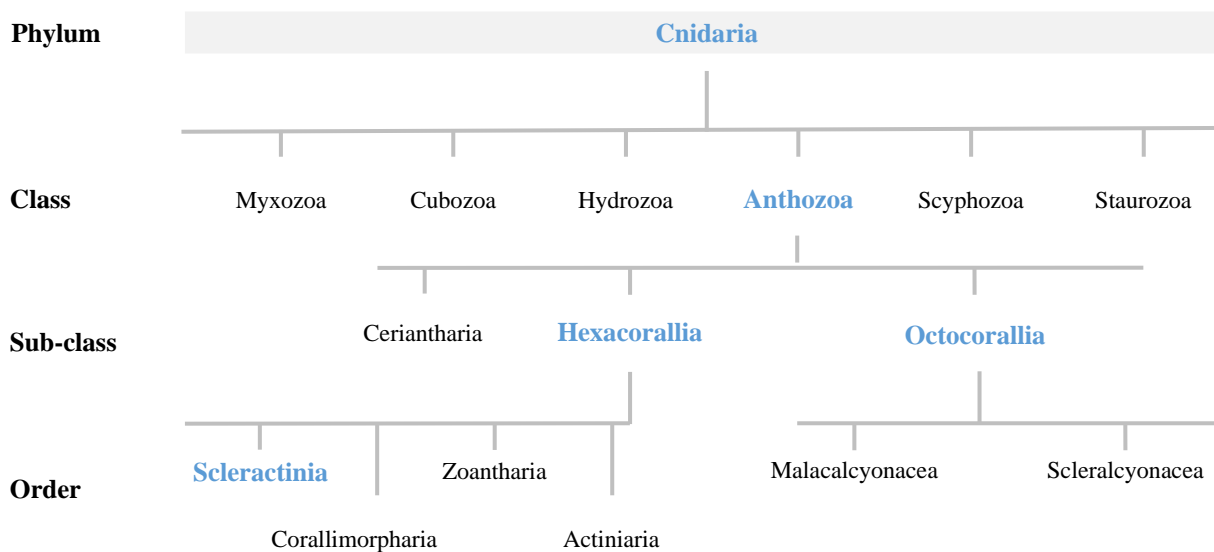


Figure 4. Taxonomie simplifiée des Anthozoaires

Pour cette thèse, quatre espèces de coraux tropicaux ont été étudiés : trois espèces de scleractiniens hermatiques symbiotiques : *Stylophora pistillata*, *Pocillopora damicornis* et *Turbinaria octocoralliaire* et une espèce d'octocoralliaire symbiotique : *Sarcophyton glaucum* (Fig 5). Ces coraux proviennent de la Mer Rouge (Golf d'Aqaba, Israël) et sont maintenus au Centre Scientifique de Monaco.

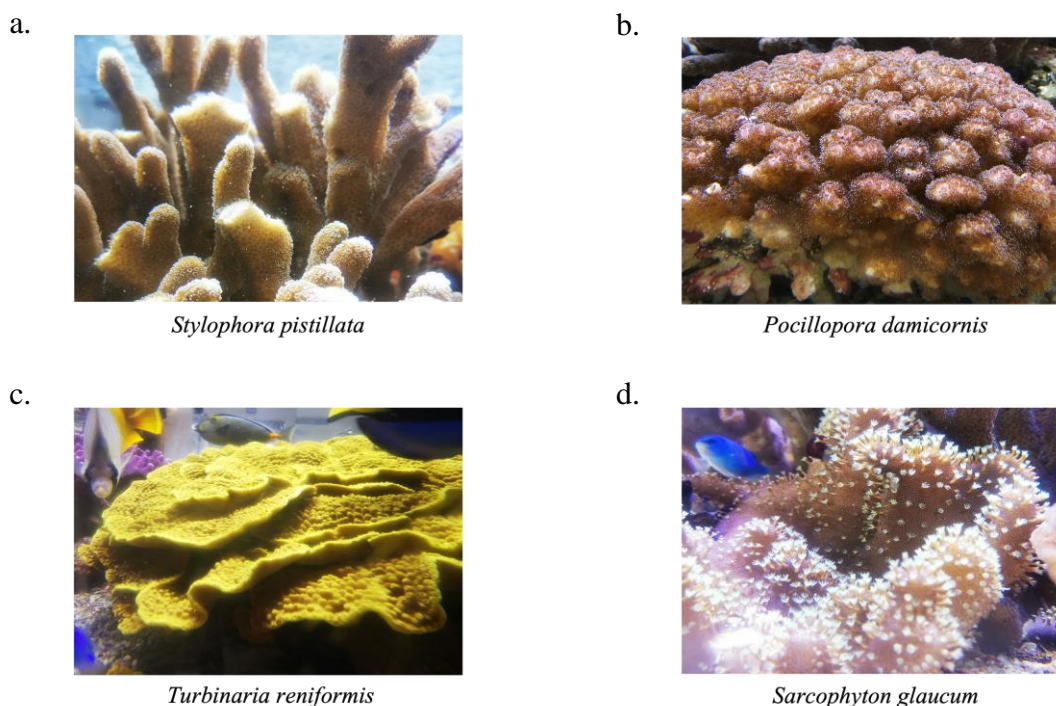


Figure 5. **Coraux scléactiniaires et octocoralliaires utilisés dans la thèse**

Photos des trois coraux scléactiniaires symbiotique hermatypiques : (a) *Stylophora pistillata*, (b) *Pocillopora damicornis* et (c) *Turbinaria reniformis*, et de l'octocoralliaire symbiotique ahermatypique : (d) *Sarcophyton glaucum*.

Credit: Alice Blanckaert CSM.

1.3. Description et fonctionnement de la symbiose corail-dinoflagellés

La symbiose est définie comme deux ou plusieurs espèces qui vivent ensemble (Louie, 2010). C'est un phénomène omniprésent dans le monde vivant. Dans la plus part des cas, dans une relation symbiotique, le partenaire le plus grand est appelé hôte, ici le corail animal, et le plus petit est appelé le symbionte, ici les dinoflagellés et autre microorganismes. Il existe trois types de relation symbiotiques :

- **Mutualiste** : La relation est bénéfique pour tout les partenaires, ex : les anémones et les poissons clowns.
- **Commensaliste** : La relation est bénéfique pour un des partenaires, tout en étant neutre pour les autres partenaires, ex : les poissons remora et les requins.
- **Parasitique** : La relation est bénéfique pour un des partenaires, au détriment des autres, ex : les poissons et les isopodes.

On peut parler d'endosymbiose, lorsque le symbionte vit à l'intérieur de l'hôte. Plus précisément, elle est qualifiée d'intracellulaire si les symbiontes se trouvent à l'intérieur des cellules, et d'extracellulaire s'ils sont présents dans les tissus. Il s'agira d'une ectosymbiose, lorsque le symbionte vit à la surface de l'hôte. La relation entre le corail et les dinoflagellés est définie comme une endosymbiose intracellulaire mutualiste. Les dinoflagellés sont des algues

unicellulaires qui peuvent avoir un mode de vie libre ou en symbiose. Les dinoflagellés qui sont associées aux coraux font parties de la famille des Symbiodiniaceae (Fig 6).

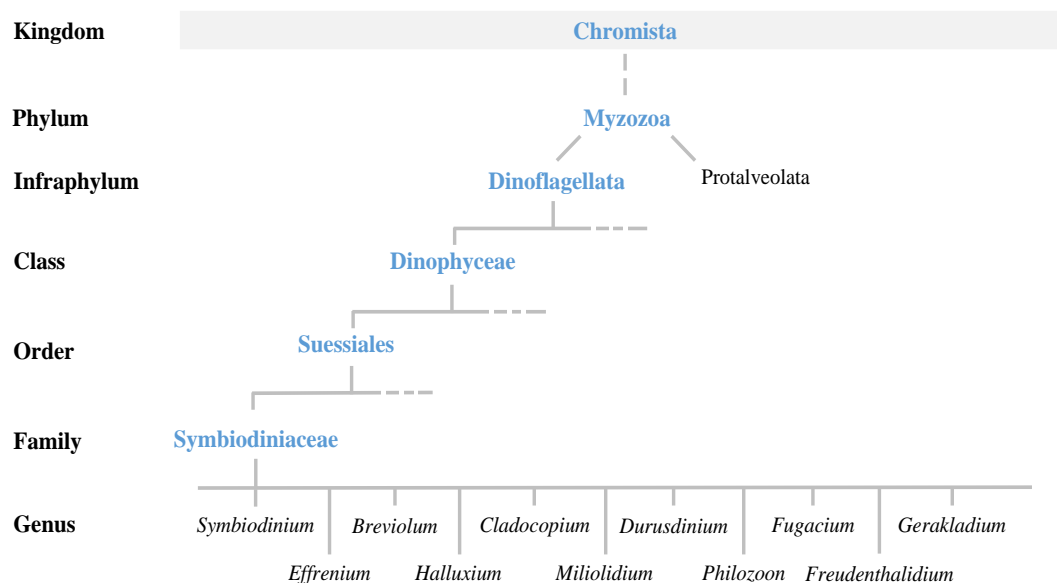


Figure 6. Taxonomie simplifiée des Symbiodiniaceae

Ils sont répartis dans sept genres, anciennement appelé clades (LaJeunesse *et al.*, 2018): *Symbiodinium* - clade A, *Brevolium* - clade B, *Cladocopium* - clade C, *Durusdinium* - clade D, *Effrenium* - clade E, *Fugacium* - clade F and *Gerakladium* - clade G. *Effrenium*, le clade E, est le seul genre à ne pas être associé aux cnidaires. Les dinoflagellés symbiotiques peuvent être acquis par transmission verticale (transmission parentale) ou horizontale (venant de l'environnement) (Abrego *et al.*, 2009; Baird *et al.*, 2009). Ils sont situés à l'intérieur des cellules endodermiques du corail hôte, dans une vacuole appelée symbiosome (Fig 7).

Dans la majorité des cas, une cellule animale, contient un seul symbionte mais il peut en contenir jusqu'à six (Muscatine *et al.*, 1998). La densité de dinoflagellés symbiotiques dans un corail est généralement exprimée en nombre d'individus par cm² de squelette. Cette valeur est, en moyenne, égale à 1.5 million de dinoflagellés par cm² (Muscatine *et al.*, 1998). La densité de dinoflagellés peut être influencée par différents facteurs environnementaux tels que la lumière, la température ainsi que le disponibilité en nutriments (Baker, 2003; Gilbert *et al.*, 2010; Richier *et al.*, 2010). De plus, les différents genres de dinoflagellés réagissent différemment aux changements environnementaux.

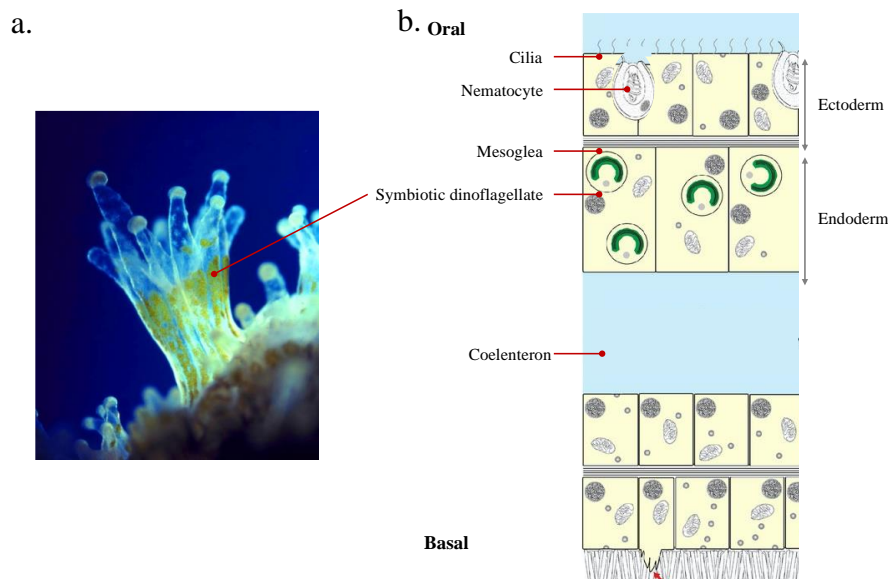


Figure 7. Localisation des dinoflagellés symbiotiques dans un polype (a.) Photo d'un polype avec les dinoflagellés symbiotiques visibles en vert et (b.) représentation d'une coupe transversale de tissu de polype Modifié à partir de Drake et al (2020)

La symbiose entre le corail et les dinoflagellés est basée sur l'échange de nutriment entre les deux partenaires (Fig 8). L'holobionte est un organisme mixotrophe, capable d'obtenir des éléments nutritifs par hétérotrophie (consommation de proies et de composés organiques) et par autotrophie (absorption de nutriments inorganiques, photosynthèse).

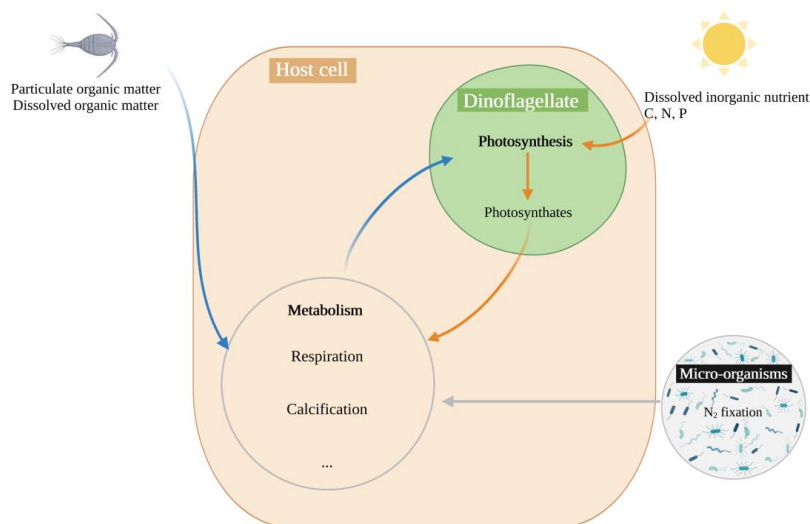


Figure 8. Représentation simplifiée des flux de nutriments dans la symbiose corail-dinoflagellé-microorganismes Les flèches orange représentent la nutrition autotrophe, les flèches bleues la nutrition hétérotrophe et les flèches grises la nutrition acquise par les microorganismes. Créé avec BioRender

Cette optimisation du flux de nutriments entre les partenaires permet à l'holobionte de se développer et prospérer dans des eaux oligotrophes, pauvres en nutriments (azote $< 0.5 \mu\text{M}$

et phosphore $< 0.2 \mu\text{M}$) (Muscatine & Porter, 1977). D'un côté, les polypes, hétérotrophes, sont capables de capturer la matière particulaire organique détritique ou vivante, allant du pico- ($0.2 \mu\text{m}$) au méso-plancton (1 mm) (Muscatine, 1973). Le corail est également capable d'absorber des molécules organiques dissoutes dans l'eau de mer tels que les carbohydrates et les acides aminés libres. Certains constituants de la matière ingérés par l'hôte pourront ensuite être transférés à ses symbiontes (Rahav et al., 1989; Tremblay et al., 2014). D'un autre côté, les dinoflagellés, autotrophes, peuvent fixer le carbone inorganique dissous dans des molécules organiques produites lors de la photosynthèse, appelées photosynthétats. Ces photosynthétats, riches en carbone, sont essentiellement du glucose (Burriesci *et al.*, 2012), mais également des lipides (Treignier *et al.*, 2008). Dans les eaux peu profondes, 95 % des photosynthétats sont transférés vers le corail pour répondre à ses besoins respiratoire et énergétique (Muscatine *et al.*, 1984; Rosset *et al.*, 2015). De plus, les dinoflagellés sont également capable d'assimiler de l'azote et du phosphore organique et inorganique dissous afin de les métaboliser par le biais de processus enzymatiques (Grover *et al.*, 2002, 2003, 2006, 2008; Ferrier-Pagès *et al.*, 2016). Ces composés néoformés sont ensuite partiellement transférés vers l'hôte également (Muscatine & Porter, 1977; Rahav et al., 1989; Tremblay et al., 2014; Wang & Douglas, 1999).

I.4. Acquisition des nutriments dans la symbiose

I.4.a. Concept d'élémentome

La notion d'élémentome est relativement récente, elle est définie comme étant l'étude de la spéciation des éléments biologiques dans un éditorial du *Journal of Analytical Atomic Spectrometry* en 2008 (Jakubowski & Hieftje, 2008). Les éléments sont à la base de la composition des organismes, divisés en macro- et microéléments. Les macroéléments sont ceux nécessaires en grandes quantités, particulièrement le carbone (C), l'azote (N) et le phosphore (P) (Da Silva & Williams, 2001). L'azote est essentiel pour la synthèse d'acide aminés/protéines et d'acides nucléiques, tandis que le phosphore est essentiel pour la synthèse d'adénosine triphosphate (ATP), acides nucléiques et phospholipides (Sternner & Elser, 2002). L'azote et le phosphore sont donc associés à la croissance des organismes et à leur production primaires (Elser *et al.*, 2000; Karl *et al.*, 2001).

Les microéléments, tels que les métaux (fer, zinc, nickel ...), eux sont nécessaires en faibles voir très faibles quantités. Ils sont constitutifs de bon nombre d'enzymes et de cofacteurs.

Cette thèse s'intéresse à l'assimilation de macro (N et P) et microéléments ainsi qu'à leur impact sur la physiologie corallienne.

I.4.a.L'azote

I.4.a.i. Les différentes formes azotées et leur cycle dans les récifs coraliens

L'azote qui parvient jusqu'aux récifs coralliens a des origines très variées (D'Elia, 1988) :

- Du milieu marin en provenance du large, par transport advectif latéral ou des profondeurs par upwelling et transport advectif vertical, mais également de l'excrétion ou la décomposition des organismes marins ainsi que de la régénération de particules azotées par les bactéries.
- Du milieu terrestre par lessivages ou transports éoliens.
- D'origine atmosphérique.

L'azote est apporté au récif sous différentes formes :

- **Azote organique particulaire (PON)** : Particules de taille supérieure à 0.2 μm , incluant les organismes vivants et la matière détritique (Jørgensen, 2009). Cette forme azotée est trouvée à des concentrations comprises entre 0.4 et 1.6 μM , elle représente moins de 0.3 % de l'azote dans les océans (Gruber, 2008).

- **Azote organique dissous (DON)** : Micro-organismes (virus et bactéries) et molécules en solution, tels que l'urée, les nucléotides, les acides aminés libres, les protéines et les peptides (Aluwihare & Meador, 2008; Jørgensen, 2009). Cette forme azotée est trouvée à des concentrations généralement comprises entre 3.8 et 5.6 μM . C'est une des sources majeures d'azote pour les coraux (Tanaka *et al.*, 2011; Sipler & Bronk, 2015).

- **Azote inorganique dissous (DIN)** : Cette autre forme d'azote dissous est composée d'ammonium, nitrite et nitrate et comprend également dans sa définition le diazote atmosphérique. L'ammonium NH_4^+ provient principalement des déchets animaux ou de la fixation de N_2 . Il est présent dans les récifs à des concentrations comprises entre 0.2 à 0.5 μM . C'est la forme de DIN la plus réduite, et par conséquent la plus susceptible d'être oxydée (Bythell, 1990). Le nitrite, NO_2^- , est une forme d'azote transitionnelle, rapidement oxydée en nitrate par les microorganismes. Il est présent en très faible quantité, avec une concentration proche de 0.05 μM (Bythell, 1990). Le nitrate, NO_3^- , est la forme d'azote la plus oxydée et donc la plus stable en milieu aqueux. Sa concentration dans les récifs oligotrophe est comprise entre 0.2 et 1.8 μM (Bythell, 1990). Cependant, dans certaines eaux eutrophisées la concentration en nitrate peut monter jusqu'à 31 μM (Vaiare, Polynésie Française; Rouzé *et al.*, 2015). Finalement, le diazote N_2 est la forme dominante de DIN, représentant près de 90% de l'azote dans l'océan (Gruber, 2008). Cependant le diazote n'est utilisable que par une petite

fraction d'organisme unicellulaires, que sont les diazotrophes, qui sont les seuls capables de réduire le diazote en ammonium.

Dans l'océan, le cycle de l'azote est relativement complexe, impliquant divers organismes et processus (Fig 9). Dans un premier temps, le diazote est réduit en ammonium biodisponible par les diazotrophes par **fixation du diazote** (Larkum *et al.*, 1988). L'ammonium est absorbé à son tour par les organismes autotrophes, puis excrété par les organismes hétérotrophes. L'ammonium peut également être oxydé en nitrite puis en nitrate par des bactéries aérobies chimioautotrophes, c'est le **processus de nitrification** (Wiebe *et al.*, 1975). Le **PON** peut descendre dans la couche anoxique, où il sera reminéralisé en ammonium puis en nitrate, c'est le **processus de reminéralisation** (D'Elia, 1977; D'Elia & Wiebe, 1990) avant de réintégrer la couche oxique par mélange vertical et upwelling (Howard *et al.*, 2014). Dans la couche anoxique, l'ammonium peut être converti en N_2 par les planctomycètes par **oxydation anaérobie de l'ammonium**. N_2 peut également être formé par un processus de réduction des nitrates appelé **dénitrification** par des bactéries anaérobies qui utilisent le nitrate pour leur respiration. Un ultime processus bactérien permet la réduction du nitrate en ammonium. Ce processus appelé « **réduction dissimilaire des nitrates en ammonium** » permet de conserver l'azote dans le milieu environnant (D'Elia & Wiebe, 1990).

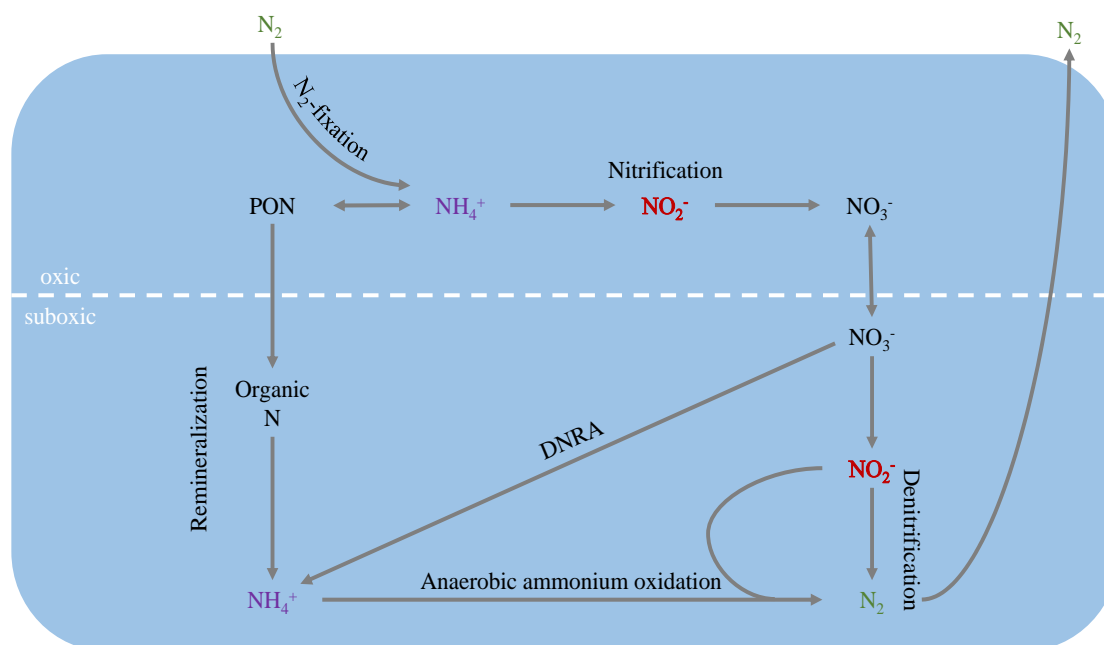


Figure 9. Représentation simplifiée du cycle de l'azote

N_2 : diazote, NH_4^+ : ammonium, NO_2^- : nitrite, NO_3^- : nitrate, **PON**: azote organique particulaire, **Organic N**: azote organique, **DNRA**: réduction dissimilaire des nitrates en ammoniums.

I.4.a.ii. Assimilation de l'azote par l'holobionte corallien

Les polypes assimilent le PON en se nourrissant de planctons, à une échelle allant des bactéries au microzooplancton selon la taille du polype (Houlbreque & Ferrier-Pagès, 2009).

L'hôte animal et les symbiotes peuvent également absorber le DON par transport direct et diffusion membranaire (Grover *et al.*, 2006, 2008), le DIN, quant à lui, est principalement assimilé par les symbiotes (Grover *et al.*, 2002; Pernice *et al.*, 2012; Kopp *et al.*, 2013). La métabolisation de l'ammonium en glutamate peut être réalisée selon deux voies enzymatiques : la glutamate déshydrogénase (GDH), qui est présente dans l'hôte animal, et la glutamate synthase/ glutamine oxoglutarate amidotransférase (GS/GOGAT) spécifique aux symbiotes et beaucoup plus efficace que la GDH (Rahav *et al.*, 1989). L'ammonium peut ensuite être incorporé dans les autres acides aminés par transamination (Roberts *et al.*, 1999). L'assimilation de nitrate, quant à lui, nécessite l'action combinée de la nitrate réductase et de la nitrite réductase. Ces deux enzymes n'ont été trouvées jusqu'à présent que chez les plantes et les algues (Crossland & Barnes, 1977). Par conséquent, contrairement à l'assimilation d'ammonium, l'assimilation de nitrate n'est possible que par les symbiotes et quelques autres bactéries dans l'holobionte corallien. Le taux d'assimilation du nitrate est également plus faible que celui de l'ammonium (Grover *et al.*, 2002, 2003), probablement en raison de l'énergie élevée requise pour son assimilation (Dagenais-Bellefeuille & Morse, 2013) et du fait que cette assimilation est limitée à un partenaire. Les taux d'assimilation de ces deux formes azotées sont positivement corrélés à leur concentration dans l'eau de mer ainsi qu'à l'activité photosynthétique des symbiotes (Grover *et al.*, 2002, 2003).

Les coraux peuvent également avoir accès au diazote atmosphérique à travers la présence de diazotrophes dans leur squelette, tissus et mucus (Bednarz *et al.*, 2021). Toutefois, ce n'est pas la forme d'azote la plus utilisée par l'holobionte. Il a été montré que ce processus de fixation de diazote couvre moins de 10% des besoins en azote des coraux (Bednarz *et al.*, 2021).

I.4.b. Le phosphore

I.4.b.i. Les différentes formes phosphorées et leur cycle dans les récifs coraliens

Comme pour l'azote, l'apport de phosphore dans les récifs peut provenir de différentes sources : atmosphérique (dépôt de poussières), terrestre (rejet d'eaux usées), océanique (flux advectif vertical et latéral) ou du récif lui-même (remise en suspension des sédiments). Le phosphore est apporté au récif sous forme de :

- **Phosphore inorganique particulaire (PIP)** : Cette forme phosphorée est composée des ions phosphores adsorbés sur des particules (biotiques ou abiotiques) et les

produits de stockage intracellulaire dans les organismes. Elle représente entre 20 et 70 % du phosphore total dans les récifs (Yoshimura *et al.*, 2007; Lomnitz *et al.*, 2016). Cependant le PIP doit d'abord être dissous en phosphore inorganique (DIP) dans l'eau de mer afin de pouvoir être utilisé par les coraux (Van Cappellen *et al.*, 2014).

- **Phosphore organique particulaire (POP)** : Cette forme phosphorée est constituée d'organismes vivants tels que le plancton et les bactéries, retenus sur un filtre de 0.2 à 0.45 μm . Il est présent à des concentrations relativement faibles dans le récif, autour de 0.10 $\mu\text{mol L}^{-1}$ (Furnas *et al.*, 1992; Charpy, 2001; Lomnitz *et al.*, 2016). Cependant, dans les sédiments, les concentrations de POP peuvent atteindre 100 mmol m^{-2} (Atkinson, 2011).

- **Phosphore organique dissous (DOP)** : Cette forme phosphorée est composée de phosphoesters, phosphonates, phospholipides, acides nucléiques, nucléotides et vitamines (B1 et B12) (Karl & Björkman, 2015). Les concentrations en DOP varient entre 0.12 et 0.5 μM dans la zone euphotique (Furnas *et al.*, 1992; Charpy, 2001), diminuant avec la profondeur jusqu'à moins de 0.05 μM (Karl & Björkman, 2015).

- **Phosphore inorganique dissous (DIP)** : Cette forme phosphorée est constituée d'ions orthophosphates (HPO_4^{2-} ou $\text{H}_2\text{PO}_4^{3-}$, en fonction du pH de l'eau de mer) et de polymères de phosphate condensés, appelés méta-, pyro- ou polyphosphate selon leur structure. Le DIP est présent dans les récifs à des concentrations de 0.05 à 0.5 $\mu\text{mol L}^{-1}$ (Kinsey & Davies, 1979).

I.4.b.ii. Assimilation du phosphore par l'holobiont du corail

Dans l'océan, le DIP est la forme de phosphore la plus disponible pour les organismes marins, du fait de son faible coup métabolique (Björkman & Karl, 1994). L'assimilation du DIP se fait à la fois par l'hôte animal, au moyen de cotransporteurs phosphore/sodium (Godinot, 2012), et par les symbiontes, par transports actifs (Jackson & Yellowlees, 1990; Muller-Parker *et al.*, 1990). Les molécules constitutives du DOP de larges tailles doivent d'abord être hydrolysés en phosphore inorganique par des enzymes spécifiques, telles que les phosphatases, phytases, phosphodiesterases et nucléases afin de pouvoir bénéficier aux organismes marins (Karl & Björkman, 2015). Les phosphatases sont considérées comme la classe d'enzymes la plus importante pour l'hydrolyse du DOP dans les environnements oligotrophes (Taga & Kobori, 1978; Karl & Craven, 1980). Les coraux synthétisent des phosphatases intra et extracellulaires capables d'hydrolyser les monophosphoesters (DOP) présents dans l'organisme ou dans le milieu extérieur en ions orthophosphate (DIP) (Godinot *et al.*, 2013; Wiedenmann *et al.*, 2013).

Dans cette thèse, un chapitre sera consacré à la capacité des coraux scléactiniaires et des octocoraux à produire des phosphatases dans différentes conditions environnementales et à utiliser le DOP comme source de phosphore additionnel. Cette source de phosphore constitue un apport essentiel pour le maintien de l'état de santé des coraux.

1.4.c. Importance de la stœchiométrie de l'azote et du phosphore pour la stabilité de la symbiose corallienne

En écologie, la stœchiométrie a été défini par Meunier et al. (2017) comme « un cadre reliant les exigences métaboliques d'un organisme à la disponibilité en éléments dans l'environnement (Sturner & Elser, 2002; Hessen *et al.*, 2013) ». Elle postule une relation cruciale entre l'équilibre des éléments essentiels, tels que le carbone (C), l'azote (N) et le phosphore (P), et leur rôle dans la détermination de la croissance et de la reproduction des organismes, ainsi que dans les interactions écologiques. La reconnaissance de l'importance des contraintes stœchiométriques entre les besoins des consommateurs et la teneur en nutriments des proies a grandement amélioré notre compréhension des interactions trophiques. La stœchiométrie écologique s'est avérée être un cadre très utilisé en écologie des communautés pour expliquer les réponses des prédateurs face à la qualité des proies - prise alimentaire, croissance, ainsi que la compétition entre les espèces et les effets des prédateurs sur la composition en nutriments des proies (Sturner, 1990; Sturner *et al.*, 1992; Sturner & Hessen, 1994). Lorsque les nutriments ne sont pas limitants, le rapport molaire C:N:P théorique de la matière particulaire est de 106:16:1, le rapport Redfield (Redfield, 1958). Ce rapport est considéré comme le rapport de référence répondant aux besoins des organismes vivants. De plus, dans une eau de mer oligotrophe, le rapport DIN:DIP naturel des nutriments dissous varie approximativement de 4:1 à 7:1, inférieur au rapport Redfield (Smith *et al.*, 1981; Crossland *et al.*, 1984; Furnas *et al.*, 1995; Rosset *et al.*, 2017). Ce rapport DIN:DIP naturel des éléments dissous sera considéré comme un rapport DIN:DIP équilibré dans cette thèse.

De nos jours, de plus en plus de récifs sont affectés par la nutrification/eutrophisation, c'est-à-dire un surenrichissement de l'eau de mer en un ou plusieurs nutriments, dû aux activités anthropiques, telles que le ruissellement des terres, l'agriculture et les déchets municipaux (Fabricius, 2011). La nutrition peut déséquilibrer le rapport DIN:DIP, qui affecte la santé et la physiologie des organismes des récifs. Des concentrations et une disponibilité accrues de nutriments peuvent, par exemple, faire évoluer l'écosystème récifal d'un récif dominé par les coraux vers un récif dominé par les algues (Littler *et al.*, 2006; Vermeij *et al.*, 2010), car les macroalgues ont besoin d'un apport important de nutriments pour se développer. En l'absence

d'herbivores, la prolifération des macroalgues peut entraîner une compétition avec les coraux pour les nutriments, l'espace et la lumière (Littler *et al.*, 2006; Garren, 2016) (Fig 10). De tels changements se sont produits dans le monde entier, du golfe d'Eilat en mer Rouge (Genin *et al.*, 1995) et de la Grande Barrière de Corail en Australie (McCook, 1999; Cheal *et al.*, 2010) aux Caraïbes (Mumby, 2009) et à la Nouvelle-Calédonie (Vieira *et al.*, 2015). Par conséquent, l'étude des effets de la nutrification, notamment par l'enrichissement en azote et en phosphore, sur la physiologie des holobiontes coralliens est une priorité.

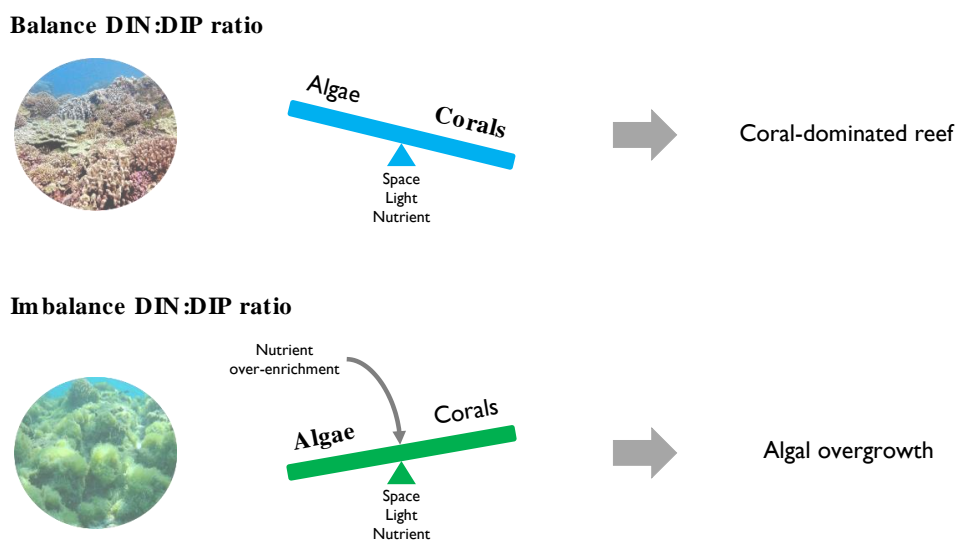


Figure 10. Représentation simplifiée des changements de phases dans un récif sous différents rapports DIN:DIP

Lorsque le rapport DIN:DIP est équilibré, les récifs coralliens sont des écosystèmes dominés par les coraux. Cependant, un enrichissement excessif en nutriments entraîne un déséquilibre du rapport DIN:DIP et la prolifération de macroalgues, qui entrent en compétition avec les coraux pour la lumière et l'espace. Finalement, les macroalgues prennent le dessus, et le récif devient un écosystème dominé par les algues.

1.4.d. Impact d'un rapport DIN:DIP déséquilibré sur la physiologie des coraux

Dans les récifs, l'enrichissement de l'eau de mer en azote ou en phosphore provient principalement de la fertilisation agricole et du lessivage des terres. De nombreuses études ont étudié les effets de l'enrichissement en nutriments. Cependant, les résultats sont souvent contradictoires, avec des études qui montrent un effet positif, neutre ou néfaste de l'enrichissement en nutriments sur la physiologie des coraux (Tableau 1). Par exemple, des études ont trouvé une diminution de la croissance des coraux sous un enrichissement en phosphore ou en azote seul (Kinsey & Davies, 1979; Tomascik & Sander, 1985; Stambler *et al.*, 1991; Ferrier-Pagès *et al.*, 2000, 2001; Koop *et al.*, 2001; Renegar & Riegl, 2005; Marangoni *et al.*, 2020). D'autres études ont trouvés des résultats opposés dans des conditions d'enrichissement similaires : une augmentation de la calcification et du taux de croissance des

coraux sous enrichissement en phosphate (Atkinson *et al.*, 1995; Koop *et al.*, 2001; Dunn *et al.*, 2012) ou en azote (Atkinson *et al.*, 1995; Chauvin *et al.*, 2011). Cependant, une telle augmentation de la croissance du corail peut également s'accompagner d'une diminution de la densité du squelette qui le corail plus fragile (Koop *et al.*, 2001; Dunn *et al.*, 2012) et donc moins résistant aux incidents météorologiques.

L'enrichissement en nutriments, notamment en nitrate, a également des effets controversés sur la physiologie des coraux. En effet, certaines études ont montré une augmentation (Marubini & Davies, 1996; Ezzat *et al.*, 2015) ou une diminution (Schlöder & D'Croz, 2004) de la densité des symbiotes. De même, des effets contradictoires sur les taux de photosynthèse ont été observés (Ezzat *et al.*, 2015, 2016b; Liu *et al.*, 2020). En outre, des études ont montré que l'ammonium et le nitrate peuvent avoir des impacts différents sur la physiologie des coraux, avec un effet bénéfique de l'ammonium et un effet néfaste du nitrate (Ezzat *et al.*, 2015; Burkepile *et al.*, 2020; Marangoni *et al.*, 2020).

Récemment, Wiedenmann *et al.* (2013) ont tenté de concilier ces résultats en émettant l'hypothèse que ce n'est pas le nutriment en soi ou sa concentration qui est néfaste pour les coraux, mais plutôt le rapport DIN:DIP. En effet, ils ont montré qu'un rapport DIN:DIP équilibré n'était pas préjudiciable aux coraux, alors qu'un rapport DIN:DIP déséquilibré, avec un manque de phosphore par rapport à l'azote, réduisait l'efficacité photosynthétique des symbiotes et conduisait à un blanchissement lors d'un stress thermique (Wiedenmann *et al.*, 2013). En cas de limitation en phosphore, les phospholipides membranaires sont remplacés par des sulfolipides qui sont moins résistants au stress thermique. Ces résultats suggèrent que l'enrichissement simultané en azote et en phosphore dans un rapport équilibré pourrait atténuer les effets délétères de chaque nutriment. Cette hypothèse est soutenue par les observations selon lesquelles 1) l'enrichissement en phosphore a atténué les effets négatifs de l'enrichissement en azote sur la croissance et la photosynthèse des coraux (Koop *et al.*, 2001; Ezzat *et al.*, 2016b) ou selon lesquelles - 2) l'enrichissement en azote a atténué les effets négatifs de l'enrichissement en phosphore sur la récupération des coraux après un stress (Ferrier-Pagès *et al.*, 2000).

Dans l'ensemble, l'étude des macro-éléments et en particulier de l'azote et du phosphore, ont besoin d'être poursuivies afin de comprendre pleinement leur interaction et leur effet sur la physiologie des holobiontes coralliens.

Dans cette thèse, nous avons étudié les effets de rapports DIN:DIP équilibrés et déséquilibrés, suite à un enrichissement en nitrate, sur la physiologie et les besoins en phosphore des holobiontes coralliens.

I.5. Microéléments

I.5.a. Les métaux-traces dans l'eau de mer

Les microéléments, principalement les métaux, sont présents dans un organisme en petites quantités ou à l'état de traces. Dans le milieu marin, les métaux peuvent provenir de diverses sources naturelles, (Libes, 2011): les ruissellement fluviaux, les dépôts atmosphériques, l'activité hydrothermale, les eaux souterraines ou encore les sédiments.

Ces échanges naturels peuvent être enrichis par des facteurs anthropiques conduisant à un doublement de l'apport de métaux aux récifs (Fabricius, 2005; Libes, 2011). Par exemple, les gaz d'échappement des véhicules, des centrales électriques au charbon et des usines d'incinération des déchets augmentent l'émission de particules dans l'atmosphère (Fabricius, 2005). L'altération des sols par le vent et l'eau en raison de l'agriculture intensive, de la déforestation, de l'exploitation minière à ciel ouvert et de l'urbanisation augmente également l'apport de métaux dans l'océan. Dans les pays en développement, où se trouvent la plupart des récifs tropicaux, les eaux usées urbaines et industrielles non traitées augmentent encore l'apport de métaux-traces dans les eaux des récifs.

Dans la colonne d'eau, les métaux traces peuvent être minéralisés et dissous, flocculés et hydrolysés ou adsorbés et désorbés. Dans ce cycle, les métaux-traces peuvent se présenter soit sous forme de composés particuliers ($> 0,45 \mu\text{m}$) en association avec la matière organique, soit sous forme d'éléments dissous ($< 0,45 \mu\text{m}$), en association avec des complexes inorganiques micromoléculaires. La fraction dissoute des métaux-traces est généralement relativement faible, car ils sont enclins à former des complexes avec les particules (Hoffmann *et al.*, 2012). Cependant, pour être absorbés par les organismes, les éléments traces doivent être biodisponibles. Les métaux-traces biodisponibles se trouvent à des concentrations pico- à nanomolaires dans la couche de surface (Pinedo-González *et al.*, 2015). Le profil de concentration peut varier avec la profondeur.

I.5.b. Le rôle des métaux-traces dans la physiologie végétale et animale

Les métaux-traces sont classés comme « essentiels » ou « non essentiels » en fonction de leur capacité à participer aux fonctions biologiques. Les métaux traces classés comme essentiels sont connus pour interférer avec divers systèmes enzymatiques (Libes, 2011; Reich *et al.*, 2020). Une carence en un métal-trace « essentiel » peut être préjudiciable à un organisme, tandis que son enrichissement peut avoir des effets positifs en améliorant les voies métaboliques (Adriano, 2001). En revanche, l'absence ou l'enrichissement en métaux « non essentiels » n'aura aucun effet sur un organisme (Adriano, 2001). Au-delà d'un certain seuil

de concentration, tout métal trace est susceptible de devenir toxique et éventuellement létal pour un organisme. Dans cette thèse, les métaux sont toujours à des concentrations inférieures à leur seuil de toxicité.

1.5.c. Le rôle des métaux-traces dans les coraux

Dans l'holobionte corallien, l'absorption des métaux peut se faire soit par hétérotrophie pour la fraction particulaire (Ferrier-Pagès *et al.*, 2018), soit par diffusion et transport actif pour la fraction dissoute (Ferrier-Pagès *et al.*, 2005). Les coraux accumulent les métaux de manière différente selon leur taxonomie, les octocoraux accumulant une plus grande quantité de métaux que les coraux scléactiniaires (Denton & Burdon-Jones, 1986). Dans l'holobionte, les métaux ont tendance à s'accumuler à différents endroits : dans le squelette, dans le dinoflagellé symbiotique ou dans le polype hôte (tissus et gamètes) (Reichelt-Brushett & McOrist, 2003). Certains métaux, notamment le fer, le cuivre, le manganèse et le zinc sont préférentiellement stockés dans les dinoflagellés symbiotiques, car ils sont impliqués dans les processus de photosynthèse (Ferrier-Pagès *et al.*, 2018). Ils peuvent ensuite être soit détoxifiés - par séquestration dans le squelette et peut-être même excrétés lors de la libération des gamètes et des symbiotes (Reichelt-Brushett & McOrist, 2003; Bastidas & Garcia, 2004) - soit utilisés par l'holobionte dans différents processus métaboliques (Tableau 3). En plus d'être utilisés dans les processus physiologiques, la disponibilité des métaux pourrait être la clé de la résistance et de la résilience des coraux au stress environnemental. En effet, des études ont montré qu'une légère augmentation des métaux-traces en dessous de leur niveau de toxicité améliore la résistance de l'holobionte corallien au stress environnemental (Shick *et al.*, 2011; Ferrier-Pagès *et al.*, 2018; Reich *et al.*, 2021). Par exemple, les dinoflagellés symbiotiques sont exposés à des facteurs de stress environnementaux tels que des intensités lumineuses élevées qui peuvent inhiber les capacités photosynthétiques des symbiotes et des températures élevées qui peuvent altérer considérablement le système photosynthétique (Suggett & Smith, 2020). Chez le dinoflagellé symbiotique, le processus de photosynthèse génère des espèces réactives de l'oxygène (ROS) qui peuvent être néfastes pour l'holobionte (Roberty *et al.*, 2014). Ces ROS peuvent être éliminées par des enzymes antioxydantes telles que la superoxyde dismutase (Fe, Mn, Cu/Zn -SOD), la catalase (Fe) et les ascorbate peroxydases (Fe) (Raven *et al.*, 1999). L'absorption de métaux peut donc favoriser la production et l'activité des antioxydants et augmenter la résistance de l'holobionte corallien au stress environnemental

Tableau 3. Exemples du rôle de quelques métaux traces essentiels dans diverses voies biochimiques

Metals	Rôle dans les voies biologiques	Concentration dans les récifs	Références
Fer	Photosynthèse (PS I et II, cytochrome b ₆ f, ferredoxin) Respiration (Transport d'électron) Capacité antioxydante (catalase, peroxydase et superoxyde dismutase -FeSOD) Assimilation des macronutriments (nitrite et nitrate reductase) Divers processus cellulaire (ex. Réplication/réparation de l'ADN)	0.25 nmol L ⁻¹	Boye et al, 2000 Raven et al, 1999 Reich et al, 2020
Zinc	Photosynthèse (PSII) Capacité antioxydante (Superoxyde dismutase -Cu/Zn-SOD) Assimilation des macronutriments (anhydrase carbonique, phosphatase) Divers processus cellulaire (acid nucléique) Co-facteur de 300 enzymes	3 nmol L ⁻¹	Aparicio-González et al, 2012 Baishnab & Mohanty, 1980 Christianson & Fierke, 1996 Plocke et al, 1962 Reich et al, 2020 Vallee & Auld, 1990
Cuivre	Photosynthèse (plastocyanin) Respiration (Transport d'électron) Capacité antioxydante (Superoxyde dismutase -Cu/Zn-SOD) Divers processus cellulaire (ex. Réplication/réparation de l'ADN)	2 nmol L ⁻¹	Aparicio-González et al, 2012 Peers & Price, 2006 Raven et al 1999 Reich et al, 2020
Manganèse	Photosynthèse (Oxygen-evolving complex) Capacité antioxydante (superoxyde dismutase - MnSOD)	5 nmol L ⁻¹	Kessler et al 1957 Morgan, 2000 Sauer, 1980 Wolfe-Simon et al, 2006
Nickel	Capacité antioxydante (Superoxyde dismutase - NiSOD) Assimilation des macronutriments (urease)	4 nmol L ⁻¹	Aparicio-González et al, 2012 Dupont et al, 2008 Price & Morel, 1991
Cobalt	Assimilation des macronutriments Vitamin b₁₂	0.6 nmol L ⁻¹	Agostini et al, 2009 Aparicio-González et al, 2012 Nolan et al, 1992 Reich et al, 2020
Molybdène	Assimilation des macronutriments (nitrite et nitrate reductase)	100 nmol L ⁻¹	Aparicio-González et al, 2012 Reich et al, 2020
Cadmium	Assimilation des macronutriments (anhydrase carbonique)	0.3 nmol L ⁻¹	Aparicio-González et al, 2012 Lane & Morel, 2000
Chrome	-	3 nmol L ⁻¹	Sirinawin et al, 2000

1.6. Hypothèse et objectifs

L'objectif général de cette thèse est d'étudier :

Les effets de la disponibilité en macro- et micro-nutriments sur la physiologie des coraux

1.6.a. Effets des rapports DIN:DIP équilibrés et non-équilibrés – i.e Quels sont les effets de l'enrichissement en azote sur la physiologie des coraux et leur besoin en phosphore ?

La disponibilité en DIN et DIP dans les récifs peut grandement influencer le métabolisme des coraux et leur susceptibilité au blanchissement. Cependant, la question de savoir si l'enrichissement en DIN ou DIP est bénéfique ou néfaste pour les coraux est encore controversée. D'une part, il a été démontré que l'enrichissement en DIN ou DIP réduit le blanchissement et améliore le métabolisme des coraux dans des conditions stressantes ou non (Atkinson *et al.*, 1995; Dunn *et al.*, 2012; Béraud *et al.*, 2013). D'autre part, l'enrichissement en DIN peut également stimuler la division cellulaire des symbiotes, entraînant une rétention plus élevée des photosynthétats dans les cellules des symbiotes et une augmentation du blanchissement (Wooldridge, 2013). Wiedenmann *et al.* (2013) ont finalement résumé les informations et proposé un modèle conceptuel dans lequel le rapport DIN:DIP dans l'eau de mer est le facteur le plus important expliquant le blanchissement des coraux. À l'avenir, des changements importants du rapport DIN:DIP se produiront, via le réchauffement des océans et/ou de l'eutrophisation des eaux côtières (van de Waal *et al.*, 2010; Santos *et al.*, 2014).

Néanmoins, on sait encore peu de choses sur les impacts des changements dans le rapport DIN:DIP sur la physiologie de différentes espèces de coraux maintenues dans des conditions environnementales différentes. La capacité des coraux à réguler l'assimilation du DIN et du DIP en fonction du rapport DIN:DIP de l'eau de mer a également été insuffisamment étudié et des travaux supplémentaires sur ce sujet sont nécessaires.

Questions:

- 1) **Première question** : L'enrichissement en DIN à des concentrations normales/faibles en DIP (rapports DIN:DIP déséquilibrés) a-t-il toujours des effets négatifs sur la santé des coraux ? Au contraire, les coraux restent-ils en bonne santé en cas d'enrichissement équilibré en DIN et DIP ?
- 2) **Deuxième question** : Observons-nous un changement dans le contenu cellulaire C:N:P et la stœchiométrie dans le cas de rapports DIN:DIP déséquilibrés ? En d'autres termes, l'enrichissement en DIN affecte-t-il les coraux parce qu'il induit une limitation du phosphore dans les tissus coralliens ?
- 3) **Troisième question** : La capacité des coraux à se nourrir de nutriments dissous (DIN, DIP et DOP) détermine-t-elle leur réponse à l'enrichissement en DIN ?

Pour répondre à ces questions, trois expériences ont été réalisées.

I.6.a.i. Les effets de l'enrichissement en azote sur la physiologie des Pocilloporidae

- Les effets d'un enrichissement en nitrate et des facteurs de stress globaux sur *Pocillopora damicornis*. Nous avons exposé des boutures de *P. damicornis* à différents rapports DIN:DIP (équilibré (DIN:DIP : 2.5 - 0.5:0.2) et déséquilibré avec un enrichissement en nitrate (DIN:DIP : 17.5 – 3.5 :0.2), sans ou en combinaison avec un stress thermique (30 °C), avec ou sans exposition aux UV, sur la physiologie et le statut oxydatif des coraux.

- Les effets d'un enrichissement en nitrates sur les besoins en phosphore de *Stylophora pistillata* : Nous avons évalué la composition cellulaire en CNP et les activités des phosphatases alcalines intra et extracellulaires (APA) et acides intracellulaires (AcPA) du corail hôte et des symbiotes associés à *Stylophora pistillata* exposés à différentes conditions de DIP et DIN. Les conditions étaient les suivantes : 1) épuisement des nutriments, avec un niveau indétectable de DIN et de DIP, 2) enrichissement à long terme (3 jours) en DIN avec 2 µM d'ammonium ou de nitrate, 3) enrichissement à court terme (impulsion de 1h) en DIN avec 2 µM d'ammonium ou de nitrate.

I.6.a.ii. Les effets de l'enrichissement en nitrate sur diverses familles de coraux

- Les effets du rapport DIN:DIP équilibrés et déséquilibrés sur la physiologie d'un corail scléactiniaire (*Dendrophyllidae*, *Turbinaria reniformis*) et d'un octocoralliaire (*Alcyoniidae*, *Sarcophyton glaucum*). Nous avons évalué la composition cellulaire et la physiologie de ces deux coraux sous des rapports DIN:DIP équilibrés ou déséquilibrés. Les différentes conditions ont été obtenues avec de l'eau de mer naturelle (rapport DIN:DIP équilibré : 2.5) ou par enrichissement avec du DIP à 1 µM (rapport DIN:DIP déséquilibré : 0.4), du DIN à 3 µM (rapport DIN:DIP déséquilibré : 17.5) ou une combinaison de DIN et de DIP (DIN:DIP équilibré : 2.9).

I.6.b. *Effet de l'apport de microéléments sur la physiologie des coraux*

Les métaux-traces sont essentiels pour de nombreuses fonctions des coraux, notamment la calcification, la photosynthèse et la capacité antioxydante. Cependant, très peu d'études ont étudié les besoins des coraux en métaux.

Questions: Les coraux sont-ils limités uniquement en macronutriments, ou peuvent-ils également être limités en métaux traces ? La disponibilité des métaux traces peut-elle également déterminer leur capacité à résister au stress thermique ?

I.6.b.i. Déposition de poussière du désert et teneur en métaux dans les coraux scléactiniaires

Dans le nord de la mer Rouge, les coraux sont particulièrement résistants au stress thermique, probablement en raison de leur histoire évolutive. En outre, les coraux de cette région sont exposés à de fréquentes tempêtes de poussière provenant du désert environnant, apportant des nutriments, notamment des métaux-traces. Nous avons donc étudié le type et la quantité de métaux-traces assimilés par *S. pistillata* pendant le dépôt de poussière du désert.

II **Chapitre 1 : Impact des facteurs de stress locaux et globaux sur la physiologie des coraux**

Les récifs coralliens sont soumis à une pression croissante liée à des facteurs environnementaux, tels que l'augmentation de la température de l'eau de mer et le rayonnement ultraviolet (UV), qui peut perturber la symbiose entre les coraux et les dinoflagellés et induire le blanchissement. Outre les facteurs de stress globaux, les coraux sont également soumis à des facteurs de stress locaux, tels que la pollution par le nitrate. Globalement, le stress thermique, les UV et l'enrichissement en nitrate peuvent provoquer des dommages oxydatifs par la surproduction d'espèces réactives de l'oxygène (ROS : anions superoxydes, radicaux hydroxyles...) et d'espèces réactives de l'azote (RNS : radicaux oxyde nitrique et peroxyde nitrite) (Lesser, 2006; Weis, 2008; Moniczewski *et al.*, 2015). Les dommages oxydatifs peuvent être atténués par une capacité antioxydante accrue chez les coraux, c'est-à-dire la production de composés piègeurs de ROS-RNS, enzymatiques (catalase, superoxyde dismutase) et non enzymatiques (uréase, glutathion) (Krueger *et al.*, 2014).

Cependant, les effets des facteurs de stress globaux et locaux peuvent avoir des résultats divergents sur la physiologie des coraux. En effet, le blanchissement ne se produit pas toujours sous la combinaison de températures élevées et de rayons UV. Ces deux facteurs peuvent avoir des effets synergiques (Rosic *et al.*, 2020) ou antagonistes (McCauley *et al.*, 2018) sur la physiologie des coraux, probablement en fonction de la dose de rayons UV. De même, des études expérimentales ont montré que les taux de calcification, la densité du squelette, les flux de photosynthèse ou le succès reproducteur peuvent être réduits sous l'effet d'un enrichissement en nitrates (Marubini & Davies, 1996; Nordemar *et al.*, 2003; Renegar & Riegl, 2005; Wiedenmann *et al.*, 2013; Ezzat *et al.*, 2015; Liu *et al.*, 2020; Marangoni *et al.*, 2020) ou en phosphate seul (Ferrier-Pagès *et al.*, 2000; Harrison & Ward, 2001; Koop *et al.*, 2001; Renegar & Riegl, 2005).

En revanche, des observations in situ et en laboratoire ont également fait état de coraux sains exposés à de fortes concentrations de nutriments (Lirman & Fong, 2007; Sawall *et al.*, 2011; Ezzat *et al.*, 2015, 2016b; Liu *et al.*, 2020). Wiedenmann *et al.* (2013), ont émis l'hypothèse que ce serait le déséquilibre des rapports DIN:DIP plutôt que les concentrations en nutriments dans l'eau de mer qui seraient préjudiciables à la santé des coraux.

L'ensemble de ces observations indique que des études supplémentaires sont nécessaires pour mieux comprendre les effets de ces facteurs de stress sur la physiologie des coraux.

Nous avons testé les effets d'un rapport DIN:DIP équilibré (DIN:DIP = 2,5 - 0,5:0,2) et déséquilibré (DIN:DIP = 17,5 - 3,5:0,2), seul ou en combinaison avec un réchauffement de l'eau de mer (30°C) et une exposition aux UV, sur la physiologie et le statut oxydatif de *Pocillopora damicornis*.

Cette étude répond à trois questions :

Quels sont les effets d'un rapport N:P déséquilibré dû à un enrichissement en nitrates sur le statut physiologique et oxydatif de P. damicornis en l'absence d'autres facteurs de stress ?

Nous avons observé une diminution significative des taux de photosynthèse, normalisés à la surface du squelette, sans changement des paramètres de stress oxydatif. Cette altération de la photosynthèse peut être expliquée par la compétition entre le nitrate et l'assimilation du carbone, qui réduit l'efficacité de la photosynthèse. En effet, la réduction nécessaire du nitrate en ammonium avant qu'il puisse être incorporé dans le métabolisme du corail est énergétiquement coûteuse et détourne les électrons de la chaîne photosynthétique, réduisant ainsi l'assimilation du carbone (Nunes-Nesi *et al.*, 2010).

Comment l'exposition à des facteurs de stress combinés affecte-t-elle le statut physiologique et oxydatif de P. damicornis ?

Dans cette étude, il a été constaté que l'exposition à de faibles rayons UV améliorait ou maintenait les taux de photosynthèse à leur niveau maximal, même en présence d'un rapport NP déséquilibré et d'un stress thermique. Ce maintien de la photosynthèse est associé à une augmentation des composés antioxydants non enzymatiques, peut-être en stimulant la production d'acides aminés de type mycosporine (MAAs) (Shick *et al.*, 1999). Ces composés jouent un double rôle : ils peuvent absorber les UVR et sont simultanément impliqués dans le métabolisme antioxydant corallien en piégeant les ROS (Rosic & Dove, 2011). En outre, il a été démontré que les UVB agissent comme un signal dans la restauration du photosystème en empêchant la photoinhibition et en augmentant ainsi l'efficacité photosynthétique (Hanelt *et al.*, 2006). Les UVA semblent également augmenter l'activité de deux enzymes, l'anhydrase

carbonique et la nitrate réductase, qui sont impliquées dans divers processus de la voie photosynthétique (Figuerola & Viñeola, 2001).

III Chapitre 2 : Effets des rapports DIN:DIP équilibrés ou non sur différentes espèces de coraux.

La réponse des coraux à un rapport DIN:DIP déséquilibré peut également être due à des changements dans la stœchiométrie élémentaire (C:N:P) du tissu corallien. Les Symbiodiniaceae, qui vivent dans les tissus coralliens, sont en effet très sensibles à un changement de la disponibilité des nutriments dans l'eau de mer. Les taux d'absorption de DIN et de DIP par les Symbiodiniaceae augmentent généralement de manière proportionnelle aux concentrations de nutriments dans l'eau de mer (Grover *et al.*, 2002, 2003). À des concentrations élevées de DIN ou de DIP, il y a donc un fort afflux de nutriments au sein de la symbiose, ce qui peut perturber la stœchiométrie cellulaire dans les tissus coralliens.

L'objectif de cette étude était donc de déterminer comment le rapport DIN:DIP de l'eau de mer et la disponibilité des nutriments modifient la stœchiométrie élémentaire des tissus coralliens et des symbiotes.

Cette étude répond à deux questions :

i) *Quels sont les effets de rapports DIN:DIP équilibrés et déséquilibrés sur les taux d'absorption des nutriments, la physiologie et la stœchiométrie C:N:P des tissus et des symbiotes de deux coraux peu étudiés : l'octocoralliaire *Sarcophyton glaucum* et le corail scléactiniaire *Turbinaria reniformis* ?*

ii) *Les coraux scléactiniaires et les octocoraux répondent-ils de la même manière aux changements du rapport DIN:DIP ?*

Nous avons observé des différences significatives dans la réponse des espèces de scléactiniaires et d'octocoraux à un rapport DIN:DIP équilibré et déséquilibré, principalement dues à une différence spécifique à l'espèce dans les taux d'absorption des nutriments.

Chez *T. reniformis*, les nutriments sont absorbés lorsqu'ils sont présents en concentrations élevées. Ainsi, un rapport DIN:DIP déséquilibré, via l'enrichissement en DIN avec du nitrate seul, conduit à une absorption unilatérale de DIN dans l'holobiont, caractérisée par une augmentation du rapport N:P cellulaire. Cette augmentation du rapport N:P cellulaire peut éventuellement conduire à une limitation du phosphore dans le corail et affecter sa physiologie. Ici, nous avons observé une diminution des capacités photosynthétiques du corail sous un rapport DIN:DIP, similaire à ce qui a été précédemment observé chez d'autres coraux scléactiniaires (Nordemar *et al.*, 2003; Wiedenmann *et al.*, 2013; Ezzat *et al.*, 2015;

Marangoni *et al.*, 2020). Cependant, un rapport DIN:DIP équilibré avec une concentration élevée en nutriments conduit à une absorption équilibrée des nutriments, qui maintient la composition cellulaire du corail. La promotion d'un rapport N:P cellulaire équilibré a empêché l'induction d'une limitation en phosphore chez le corail, limitant ainsi les effets néfastes sur sa physiologie. En effet, un rapport DIN:DIP équilibré avec des nutriments élevés a permis de maintenir les capacités photosynthétiques du corail et même d'améliorer son métabolisme par une augmentation du contenu en lipides et en hydrates de carbone.

En revanche, le taux d'absorption des nutriments de *S. glaucum* est relativement faible. De plus, l'absorption des nutriments n'est augmentée que lorsque le rapport DIN:DIP est équilibré, ce qui empêche un afflux massif unilatéral de nutriments dans l'octocoralliaire. L'apport limité et équilibré de nutriments, à la fois à un rapport DIN:DIP équilibré et déséquilibré, a maintenu la composition élémentaire et la stœchiométrie de l'octocoralliaire. Par conséquent, la physiologie de *S. glaucum* n'a pas été affectée par les changements du rapport DIN:DIP de l'eau de mer. La stœchiométrie élémentaire maintenue chez *S. glaucum* peut expliquer pourquoi les octocoraux sont moins affectés par la nutrification des récifs que les coraux scléactiniaires (Schleyer & Celliers, 2003; Baum *et al.*, 2016).

En conclusion, cette étude a montré que la réponse des coraux aux changements du rapport DIN:DIP est spécifique à chaque espèce, les octocoralliaires semblant être moins affectés que les coraux scléactiniaires. De plus, nous avons découvert que la réponse des coraux aux rapports DIN:DIP équilibrés et déséquilibrés dépend des changements dans la composition élémentaire et la stœchiométrie des coraux, avec un rapport N:P cellulaire déséquilibré affectant négativement la physiologie des coraux. Les résultats de cette étude ont permis de comprendre comment les espèces de coraux répondent à l'enrichissement en nutriments. Ils démontrent également l'importance d'étudier la stœchiométrie cellulaire lors de l'évaluation de la réponse des coraux à un rapport DIN:DIP équilibré et déséquilibré.

IV Chapitre 3 : Impact de la nutrition sur les besoins en phosphore des coraux scléactiniaires

Les coraux peuvent maintenir un rapport N:P cellulaire équilibré sous enrichissement en DIN non seulement en absorbant le DIP de l'eau de mer, mais aussi le phosphore organique dissous (DOP). Alors que le DIP est présent en concentrations nanomolaires dans les eaux des récifs (Bell *et al.*, 2007; Morrison *et al.*, 2013), les composés DOP peuvent représenter 70-90% du pool total de phosphore dissous (Ruttenberg & Dyhrman, 2012). Cependant, le DOP doit être hydrolysé en DIP par des enzymes phosphatases pour que les coraux puissent l'utiliser. Une

activité élevée des phosphatases est donc considérée comme un bon indicateur de la limitation en P dans l'environnement (Annis & Cook, 2002), car elle se produit soit à de faibles concentrations de DIP (< 20 nM pour le phytoplancton), soit à des rapports élevés de DIN:DIP (Cembella *et al.*, 1984; Rees *et al.*, 2009; Suzumura *et al.*, 2012). Les coraux possèdent une activité intracellulaire et extracellulaire de phosphatase acide (AcPA) et alcaline (APA) (Atkinson, 1987; Godinot *et al.*, 2013; Wiedenmann *et al.*, 2013), mais les connaissances sur la régulation de leur activité sont encore limitées. Quelques études ont montré que l'APA extracellulaire est renforcée en cas de carence en DIP ou pendant le blanchissement (Godinot *et al.*, 2013). Ainsi, des études sont urgemment nécessaires pour mieux comprendre les conditions dans lesquelles les phosphatases sont exprimées chez les coraux. Une attention particulière doit être accordée aux activités des phosphatases chez les coraux qui prospèrent dans des conditions enrichies en DIN afin d'évaluer une éventuelle limitation ou famine en P des coraux enrichis en N.

Dans cette étude, nous avons évalué la composition cellulaire en CNP et les activités des phosphatases alcalines intra- et extracellulaires (APA) et acides intracellulaires (AcPA) du corail hôte et des symbiotes associés à *Stylophora pistillata* exposés à différentes conditions de DIP et de DIN.

Cette étude répond à deux questions :

Quel est l'effet de la déplétion en DIP de l'eau de mer sur la teneur en P cellulaire et les activités phosphatases des coraux ?

Nous avons observé que la déplétion en DIP a augmenté l'APA extracellulaire après un jour de déplétion, ce qui suggère que les coraux doivent compter sur le DOP pour combler leurs besoins en phosphore. Cependant, après trois jours de déplétion en phosphore, un effondrement significatif de l'APA extracellulaire a été observé. De même, la teneur en phosphore cellulaire, ainsi que la teneur en azote dans le dinoflagellé symbiotique se sont effondrées après trois jours d'épuisement des nutriments. Cela suggère que les coraux sont très sensibles à la pénurie de phosphore dans l'eau de mer car ils ne disposent pas de réserves internes de phosphore suffisantes pour faire face à une carence prolongée en nutriments.

L'enrichissement en ammonium a-t-il un effet différent de celui du nitrate sur la teneur en phosphore cellulaire et les activités des phosphatases ?

Nous avons observé différents résultats sur l'APA extracellulaire et la composition élémentaire selon la forme d'enrichissement en DIN. L'enrichissement en ammonium a empêché l'épuisement du phosphore cellulaire dans les tissus coralliens en favorisant l'assimilation du phosphore via une augmentation de l'APA extracellulaire. Cela peut expliquer pourquoi

l'enrichissement en ammonium s'est révélé plutôt bénéfique pour la physiologie des coraux scléactiniaires (Ezzat *et al.*, 2015; Marangoni *et al.*, 2020). D'autre part, le nitrate a induit un appauvrissement en phosphore cellulaire dans le tissu corallien en raison d'un manque de stimulation de l'APA extracellulaire. Ce déficit de stimulation de l'APA peut être dû au fait que l'énergie nécessaire pour produire et libérer les enzymes phosphatases est plutôt utilisée pour réduire le nitrate en ammonium. Cela a conduit à un déséquilibre du rapport N:P cellulaire qui peut expliquer l'altération de la physiologie du corail sous enrichissement en nitrate.

En résumé, cette étude a montré que bien que l'enrichissement en DIN augmente la demande en phosphore des coraux, la nature de la source d'azote peut affecter la capacité du corail à obtenir du phosphore. L'enrichissement en ammonium améliore l'assimilation du phosphore via l'hydrolyse du DOP par les phosphatases, maintenant un rapport N:P cellulaire équilibré. En revanche, l'enrichissement en nitrate restreint l'accès du corail au réservoir de DOP via une activité limitée des phosphatases, ce qui entraîne un déséquilibre accru du rapport N:P cellulaire. Ainsi, contrairement à l'ammonium, l'enrichissement en nitrate tend à favoriser la carence en phosphore des coraux, ce qui explique la réponse différente des coraux à l'enrichissement en nitrate (négatif) et en ammonium (neutre/positif) (Ezzat *et al.*, 2015; Burkepille *et al.*, 2020; Marangoni *et al.*, 2020). Dans l'ensemble, nos résultats contribuent à la compréhension critique de l'importance de la demande en phosphore chez les coraux symbiotiques, notamment avec un rapport DIN:DIP déséquilibré suite à un enrichissement en DIN.

V Chapitre 4 : Impact du dépôt de la poussière du désert sur le récif et de l'apport en métal sur la physiologie d'un corail scléactiniaire.

Les récifs coralliens du monde entier sont confrontés à de fréquents épisodes de blanchissement massif qui entraînent une perte massive de coraux. Cependant, au nord de la mer Rouge, dans le golfe d'Aqaba, peu de cas de blanchissement de masse ont été enregistrés, bien que les coraux vivent au-dessus du seuil habituel de température qui génère ce blanchissement, ce qui suggère que cette région pourrait servir de refuge pour les coraux (Fine *et al.*, 2013). Cette observation peut s'expliquer en partie par l'histoire de l'évolution des coraux qui ont recolonisé la mer Rouge lors de la remontée des eaux après la fin de la précédente période glaciaire (8000 ans). Lors du contact avec les eaux de la Mer Rouge demeurées très chaudes ($> 32^{\circ}\text{C}$), ils ont acquis une résistance thermique qui a permis la recolonisation depuis la mer d'Arabie (Fine *et al.*, 2013; Savary *et al.*, 2021). D'autres facteurs, tels que l'histoire environnementale, peuvent également contribuer à la réponse des coraux au stress thermique (Hall *et al.*, 2018). Par

conséquent, il est important de comprendre quelles conditions environnementales peuvent augmenter la tolérance des coraux au stress thermique. Dans le golfe d'Aqaba, les coraux sont exposés à des tempêtes de poussière fréquentes et intenses provenant des déserts arides environnants. Cette poussière est une source importante de macronutriments (nitrate, Chen et al., 2007) et de micronutriments, notamment de fer, de zinc, de cadmium et de cuivre (Torfstein *et al.*, 2017). Les dépôts de poussières ont des effets à la fois positifs et négatifs sur la colonne d'eau. En effet, certains des métaux-traces libérés par les poussières (fer, zinc) peuvent augmenter la productivité primaire du phytoplancton, tandis que d'autres métaux-traces (par exemple le cuivre) peuvent être toxiques. Dans tous les cas, de fortes concentrations de métaux sont toujours toxiques pour la plupart des organismes. Les effets de la poussière du désert sur la physiologie des coraux scléactiniaires n'ont pas été étudiés auparavant, même si les coraux de la mer Rouge sont continuellement exposés à la poussière. L'objectif de cette étude était donc d'examiner les effets du dépôt de poussière du désert sur un corail scléactiniaire et sur sa résistance au stress thermique.

Cette étude répond à deux questions :

Comment le dépôt de poussière affecte-t-il la physiologie générale des coraux scléactiniaires à des températures basses et élevées ?

Le dépôt de poussière du désert dans les coraux a des effets différents selon la température de l'eau de mer. À basse température, le niveau de fer, de zinc et de nickel était plus élevé dans les cellules de dinoflagellés avec la poussière (par rapport aux coraux témoins), ce qui a entraîné une augmentation des taux de photosynthèse et de la teneur en chlorophylle des coraux. Cette augmentation est due au fait que le zinc et le fer, entre autres métaux, sont utilisés pour la production de composés photosynthétiques tels que la ferredoxine, le cytochrome b6 et les photosystèmes I et II, ainsi que diverses enzymes (Raven *et al.*, 1999). À température élevée, les niveaux de fer, de zinc et de nickel des cellules de dinoflagellés étaient plus élevés dans les coraux exposés à la poussière que dans les coraux témoins, sans toutefois entraîner de changements dans la physiologie du corail. Cela s'explique par le fait que le fer dans les dinoflagellés et les taux de photosynthèse dans les coraux témoins étaient naturellement plus importants en raison de la température élevée de l'eau de mer. Il est donc possible que les coraux enrichis en poussière aient déjà atteint leur taux maximal d'assimilation du carbone à 25 °C. Ces changements physiologiques se sont produits même si l'enrichissement en poussière a augmenté les dommages oxydatifs des coraux par peroxydation des lipides. L'exposition à des métaux tels que le chrome, le fer ou le nickel est connue pour augmenter le stress oxydatif

(Sunderman Jr, 1987; Welch *et al.*, 2002; Sinha *et al.*, 2005), mais cela n'a pas eu d'impact sur la physiologie des coraux dans cette étude.

L'apport de métal aide-t-il les coraux à récupérer après un stress thermique ?

Nous avons observé que les coraux maintenus sous enrichissement en poussière pendant la phase de récupération (à 30 °C) après un stress thermique (à 34 °C) avaient des taux de photosynthèse plus élevés que les coraux non enrichis. Ainsi, l'apport de poussière du désert peut aider les coraux à récupérer plus rapidement après un événement de stress thermique.

En résumé, cette étude a révélé que le métal, et en particulier le fer, apporté par le dépôt de poussière du désert contribue à promouvoir les capacités photosynthétiques des coraux à basse température. En outre, cette étude a également montré que la température est un facteur de bioaccumulation des métaux. Dans l'ensemble, les résultats suggèrent que les coraux sont fréquemment confrontés à une limitation en métaux, notamment en fer.

VI Discussion générale et conclusion

VI.1. Discussion générale

L'objectif principal de cette thèse est d'étudier la réponse des coraux aux changements de la disponibilité des macronutriments (DIN et DIP) et des métaux dans l'eau de mer. De nombreuses études (présentées dans l'introduction, Tableau 1) ont déjà évalué les effets de la disponibilité du DIN et du DIP sur le métabolisme des coraux. Dans l'ensemble, ces études ont suggéré que le rapport DIN:DIP est un facteur principal de la réponse des coraux à l'enrichissement en nutriments, certains coraux étant affectés par un rapport DIN:DIP élevé (excès de DIN par rapport au DIP). Cependant, les effets des rapports DIN:DIP déséquilibrés ne sont pas clairs et nécessitent des investigations supplémentaires. Cette thèse s'est donc attaquée à ce sujet et est divisée en deux objectifs principaux : i) évaluer les effets des rapports DIN:DIP équilibrés et déséquilibrés de l'eau de mer sur la physiologie des coraux et 2) évaluer l'importance des métaux pour la physiologie des coraux et leur résistance au stress thermique. À cette fin, nous avons travaillé avec une espèce d'octocoralliaire *Sarcophyton glaucum* et trois espèces de coraux scléactiniaires - les espèces branchues *Stylophora pistillata* et *Pocillopora damicornis* et l'espèce laminaire *Turbinaria reniformis*.

VI.1.a. Axe I : Effets des rapports DIN:DIP équilibrés et non-équilibrés

VI.1.a.i. L'enrichissement en DIN à des concentrations normales/faibles en DIP (rapports DIN:DIP déséquilibrés) a-t-il toujours des effets négatifs sur la santé des coraux ?

Les effets d'un rapport DIN:DIP déséquilibré suite à un enrichissement en nitrate sur la physiologie des coraux ont fait l'objet de plusieurs études, avec des effets positifs, nuls ou négatifs sur les paramètres photosynthétiques, la calcification et la résistance au stress thermique des coraux (Tableau 1). Nous avons constaté la même divergence dans cette thèse. Nous avons en effet étudié la réponse de trois espèces de coraux appartenant à différents ordres et familles et ayant des formes différentes : *Pocillopora damicornis* (Scléactiniaire, Pocilloporidae, corail branchu) *Turbinaria reniformis* (Scléactiniaire, Dendrophylliidae, corail laminaire) et *Sarcophyton glaucum* (Octocoralliaire, Alcyoniidae) à un déséquilibre du rapport DIN:DIP causé par un enrichissement en nitrate.

Nous avons d'abord observé une réponse spécifique aux espèces, avec un effet significatif sur *P. damicornis*, un effet plus léger sur *T. reniformis* et aucun effet sur *S. glaucum*. Ceci est en accord avec la littérature, qui a observé qu'un rapport DIN:DIP déséquilibré nuit à la physiologie des coraux branchus, comme en témoigne dans cette thèse la diminution des taux de photosynthèse de *P. damicornis* (Tableau 1, Wiedenmann et al. 2013 ; Ezzat et al. 2015 ; Marangoni et al. 2020). Cependant, les coraux laminaires se sont avérés moins sensibles à un rapport DIN:DIP déséquilibré, comme en témoigne dans cette thèse la diminution de l'efficacité du photosystème mais le maintien des taux de photosynthèse chez *T. reniformis* (Tableau 1, Ezzat et al. 2016b ; Dobson et al. 2021b). Enfin, et bien qu'elles aient été moins étudiées, toutes les études sur les octocoralliaires ont montré qu'ils sont insensibles au déséquilibre du rapport DIN:DIP (Tableau 1). Ceci est mis en évidence dans cette thèse par l'absence de changements dans la physiologie de *S. glaucum*. L'ensemble de ces observations suggère que les différentes espèces de coraux réagissent différemment au déséquilibre du rapport DIN:DIP dans l'eau de mer, probablement en affichant un métabolisme différent ou des stratégies différentes en termes d'absorption et d'assimilation des nutriments.

Bien qu'il y ait une réponse spécifique de l'espèce à l'enrichissement en nutriments, les résultats de cette thèse montrent également que des facteurs environnementaux tels que le rayonnement UV, peuvent influencer la réponse du corail à un rapport DIN:DIP déséquilibré. Nous avons étudié la réponse de *P. damicornis* à un rapport DIN:DIP déséquilibré (enrichissement en nitrate) sous stress thermique et/ou rayonnement UV. Nous avons observé que l'exposition à de faibles niveaux de rayons UV contrebalançait les effets négatifs du nitrate et du stress

thermique sur les taux de photosynthèse du corail. Les différentes façons dont de faibles doses de rayons UV peuvent être bénéfiques aux coraux ont été détaillées dans le chapitre 1. L'amélioration des cycles du carbone et de l'azote par l'exposition à de faibles rayons UV peut avoir empêché l'inhibition de la photosynthèse induite par les nitrates en régulant le lien complexe entre le métabolisme du carbone et de l'azote. Il faut garder à l'esprit que notre étude a utilisé de faibles niveaux d'UV, en revanche de fortes doses d'UVR peuvent être nocives pour les coraux (par exemple, Gleason et al. 2006 ; Torregiani et Lesser 2007 ; Ferrier-Pagès et al. 2007 ; Torres-Pérez et Armstrong 2012).

Dans l'ensemble, la réponse des coraux à l'enrichissement en nutriments, qu'ils soient fournis dans un rapport équilibré ou déséquilibré, était spécifique à chaque espèce. Alors que la physiologie de l'octocoralliaire *S. glaucum* n'était pas affectée par les changements de la disponibilité des nutriments dans l'eau de mer, les scléactiniaires ont toujours répondu en modifiant leur physiologie. Les facteurs environnementaux doivent également être pris en compte dans la réponse des coraux aux changements de la disponibilité des nutriments, puisque l'exposition à de faibles niveaux de rayons UV a atténué les effets négatifs de l'enrichissement en DIN.

VI.1.a.ii. Observe-t-on un changement dans la teneur en C:N:P et la stœchiométrie avec des changements dans le rapport DIN:DIP de l'eau de mer ?

Nous avons étudié la composition élémentaire de trois espèces de coraux appartenant à différents ordres et familles et ayant des formes différentes : *Stylophora pistillata*, *Turbinaria reniformis* et *Sarcophyton glaucum* à un rapport DIN:DIP déséquilibré causé par un enrichissement en nitrate. Nous avons observé que l'apport de nitrate augmentait le rapport N:P cellulaire des coraux scléactiniaires *S. pistillata* et *T. reniformis*. Chez *S. pistillata*, l'augmentation du rapport N:P cellulaire était due à une diminution de la teneur en phosphore interne, caractéristique d'une carence en phosphore. Une étude de Rosset et al (2017) a de même mis en évidence une famine interne en phosphore déclenchée par l'ajout d'azote. Chez *S. pistillata*, des études précédentes ont montré que l'enrichissement en nitrate augmentait le stress oxydatif et diminuait la photosynthèse des symbiotes ainsi que la translocation du carbone des symbiotes vers l'hôte (Ezzat et al., 2015 ; Marangoni et al., 2020). Contrairement à *S. pistillata*, l'altération du rapport N:P cellulaire chez *T. reniformis* a été induite par une augmentation de la teneur en azote interne, tandis que la teneur en phosphore est restée stable. Ceci est susceptible d'avoir les mêmes effets négatifs sur la physiologie de *T. reniformis*, car une étude précédente a montré que la photosynthèse et la calcification étaient altérées sous les

effets combinés de l'enrichissement en nitrate et du stress thermique (Ezzat et al., 2016b). Enfin, contrairement aux coraux scléactiniaires étudiés, la composition élémentaire de l'octocoralliaire *S. glaucum* est restée stable même avec un rapport DIN:DIP déséquilibré, et aucun changement physiologique n'a été observé dans cette thèse ou dans des études précédentes (Koop et al., 2001). L'ensemble de ces observations suggère que la réponse physiologique observée des coraux à un rapport DIN:DIP déséquilibré est due à des changements dans leur stœchiométrie cellulaire.

Chez *S. pistillata*, nous avons également étudié les changements de la composition élémentaire avec un rapport DIN:DIP déséquilibré causé par l'ammonium. Nous n'avons observé un déséquilibre du rapport N:P cellulaire qu'avec un enrichissement en nitrate, mais pas avec un enrichissement en ammonium. L'effet variable de la forme d'azote sur la composition interne de l'holobionte corallien peut expliquer pourquoi l'ammonium s'est toujours avéré moins nuisible à la physiologie du corail que le nitrate (Burkpile et al., 2020 ; Ezzat et al., 2015 ; Marangoni et al., 2020).

En résumé, l'enrichissement en nitrate peut modifier la composition élémentaire et la stœchiométrie des coraux, soit en réduisant la teneur interne en P, soit en augmentant la teneur interne en N. Les résultats révèlent toutefois des différences entre les coraux scléactiniaires et les octocoralliaires. Alors que la stœchiométrie cellulaire N:P et la physiologie ont été affectées par l'enrichissement en nitrate chez les coraux scléactiniaires, aucun changement n'a été observé chez l'octocoralliaire. Les résultats mettent également en évidence une différence entre l'enrichissement en nitrate et en ammonium sur le rapport N:P cellulaire puisque l'enrichissement en ammonium n'a pas perturbé le rapport N:P cellulaire. Une différence similaire a été observée au niveau physiologique dans des études précédentes.

VI.1.a.iii. La capacité des coraux à utiliser les nutriments dissous détermine-t-elle leur réponse à l'enrichissement en DIN ?

Différences dans l'absorption des nutriments inorganiques dissous (DINut) lors de l'enrichissement en DIN

Les résultats obtenus dans cette thèse montrent que la capacité des coraux à se nourrir de nutriments inorganiques dissous affecte leur stœchiométrie élémentaire cellulaire et indirectement leur physiologie.

Le corail scléactiniaire *T. reniformis* a montré des taux élevés d'absorption de nitrate sous enrichissement en nitrate. De tels taux élevés d'absorption d'azote, suite à un enrichissement en azote ont déjà été observés chez les coraux scléactiniaires (Badgley et al., 2006 ; Grover et al.,

2002, 2003 ; Pupier et al., 2021). Sous une faible disponibilité en DIP (rapport DIN:DIP déséquilibré), cet apport massif d'azote dans le tissu corallien a induit un rapport N:P cellulaire déséquilibré. Cependant, lorsque les concentrations de DIP sont augmentées dans l'eau de mer (rapport DIN:DIP équilibré), le DIP est également absorbé rapidement par *T. reniformis*. Cette double absorption de DIN et de DIP n'a pas perturbé la stœchiométrie cellulaire de *T. reniformis*.

Contrairement à *T. reniformis*, *S. glaucum* a montré un taux d'absorption de nitrate 5 fois plus faible sous enrichissement en nitrate seul, similaire au contrôle. De faibles taux d'absorption de DIN ont également été observés précédemment chez plusieurs espèces de coraux mous (Pupier et al., 2021), ce qui suggère qu'il s'agit d'une caractéristique générale des coraux mous. Un apport aussi faible d'azote dans le tissu de *S. glaucum* n'a probablement pas perturbé le rapport N:P cellulaire. Les taux d'absorption de nitrate ont légèrement augmenté sous l'enrichissement combiné DIN:DIP, de sorte qu'aucune limitation de phosphore n'était possible. Un tel contrôle du rapport N:P cellulaire peut expliquer l'adaptation et la dominance des coraux mous dans les récifs riches en nutriments (Baum et al., 2016 ; Schleyer & Celliers, 2003). En effet, il a été démontré que les coraux mous concurrencent et dominent les coraux scléactiniaires dans les récifs riches en nutriments (Alino et al., 1992 ; Norström et al., 2009). Dans l'ensemble, ces résultats mettent en évidence des différences fondamentales entre les coraux scléactiniaires et les coraux mous au niveau de leur capacité nutritionnelle, ce qui peut à son tour expliquer leur sensibilité différente à l'enrichissement en nutriments et à l'eutrophisation en général.

Différences dans les besoins en phosphore sous enrichissement en DIN

L'étude réalisée sur l'activité de la phosphatase extracellulaire et intracellulaire apporte un éclairage supplémentaire sur la manière dont l'enrichissement en nitrates peut perturber le rapport N:P cellulaire du tissu corallien. En plus d'induire un apport massif d'azote dans le tissu corallien (comme décrit ci-dessus), l'enrichissement en nitrates nuit à la capacité des coraux à exploiter les sources externes de phosphore, en particulier le phosphore organique dissous (DOP). L'ensemble de ces éléments explique l'augmentation du rapport N:P cellulaire et la limitation en phosphore observée en cas d'enrichissement en nitrates.

Les coraux peuvent surmonter leur limitation en phosphore en dégradant les sources de DOP dans l'eau de mer via la libération de phosphatases extracellulaires. Ils possèdent également des phosphatases intracellulaires qui sont capables de dégrader les réserves intracellulaires de DOP lorsque le phosphore est requis. L'activité des phosphatases est donc connue pour

augmenter lorsque les coraux sont cultivés sous de très faibles niveaux de P inorganique dissous et sont limités en P (Godinot et al., 2013, chapitre 3). Les résultats obtenus dans cette thèse montrent également que les activités des phosphatases augmentent en cas d'enrichissement en ammonium - donc lorsque les coraux ont un excès d'azote et ont besoin de P pour compenser cet excès et ainsi pouvoir utiliser les deux nutriments pour leur croissance. Cependant, les résultats montrent également que l'activité des phosphatases n'augmentent pas en cas d'enrichissement en nitrate. Une explication probable est que la synthèse des phosphatases nécessite de l'énergie et du carbone organique provenant de la photosynthèse (Van Boekel & Veldhuis, 1990). Comme le nitrate est connu pour nécessiter de l'énergie pour sa réduction en ammonium, et qu'il est également connu pour altérer les capacités photosynthétiques du corail (chapitre 1 et Ezzat et al., 2015), les coraux peuvent ne pas avoir suffisamment d'énergie pour assimiler le nitrate et synthétiser les enzymes phosphatases en parallèle.

En résumé, les différentes espèces de coraux ont des stratégies d'absorption des nutriments différentes selon le rapport DIN:DIP de l'eau de mer. Alors que les coraux scléactiniaires montrent une absorption élevée de nutriments lorsque ceux-ci sont disponibles dans l'eau de mer, les octocoraux montrent une absorption accrue de nutriments uniquement lorsque le rapport DIN:DIP est équilibré.

Enfin, le nitrate, contrairement à l'ammonium, nuit à la capacité des coraux à hydrolyser le DOP, et favorise donc la carence en phosphore chez les coraux.

Par conséquent, la capacité des coraux à utiliser le DIN, le DIP et le DOP déterminera la façon dont les coraux répondent à un enrichissement déséquilibré et équilibré en DIN:DIP.

VI.1.b. Axe II : Effets de l'apport de microéléments sur la physiologie des coraux

Nous avons étudié les effets de l'apport de métaux-traces par la poussière provenant des déserts autour du Golfe d'Aqaba (Sinaï, Néguev et désert d'Arabie). Nous avons évalué les effets de cette poussière, riche en métaux-traces, sur la physiologie du scléactiniaire ramifié *S. pistillata*.

VI.1.b.i. Les coraux sont-ils limités uniquement en macronutriments, ou peuvent-ils également être limités en métaux-traces ?

Nous avons constaté que l'apport de métaux-traces, en particulier de fer et de zinc, par lessivage de la poussière, augmentait les capacités photosynthétiques des symbiotes de *S. pistillata*, comme en témoigne une augmentation de la teneur totale en chlorophylle et du taux de

photosynthèse. Le fer est essentiel à la synthèse de plusieurs composés de la chaîne photosynthétique tels que les photosystèmes I et II, le cytochrome b6 et les complexes ferrédoxines (Raven et al., 1999). Le zinc, quant à lui, est un cofacteur connu pour plus de 300 métalloenzymes dont l'anhydrase carbonique, nécessaire à l'absorption du carbone inorganique requis pour le processus de photosynthèse (cycle du carbone) (Lionetto et al., 2016), et la phosphatase alcaline (cycle du phosphore) (Reich et al., 2020 ; Shaked et al., 2006). Comme l'apport de métaux a augmenté la production primaire de *S. pistillata*, cela suggère que les métaux-traces, en particulier le fer, sont des nutriments limitants pour les coraux. Une telle limitation a déjà été mise en évidence pour différentes espèces de coraux (Entsch et al., 1983 ; Reich et al., 2020 ; Shick et al., 2011). La limitation en fer peut induire une diminution de l'assimilation du nitrate et du phosphore, de la division des symbiotes ainsi que de l'efficacité de la photosynthèse (Entsch et al., 1983 ; Reich et al., 2020 ; Shick et al., 2011).

Les résultats de cette étude suggèrent également que l'assimilation d'un métal peut être régulée par la disponibilité d'autres métaux. Par exemple, Santana-Casiano et al. (1997) ont montré que l'absorption de fer est augmentée lorsque le zinc est disponible, et Reich et al. (2020) ont montré que l'absorption de nickel, zinc, cobalt, molybdène et vanadium est également augmentée par l'apport de fer. En outre, les processus métaboliques nécessitent généralement plusieurs métaux pour être pleinement fonctionnels. Par exemple, le cycle du carbone nécessite du fer, du zinc, du cuivre, du cadmium et du cobalt (Morel et al., 1994 ; Twining & Baines, 2013), le cycle du phosphore nécessite du zinc (Shaked et al., 2006) tandis que le cycle de l'azote nécessite du fer, du zinc, du nickel et du molybdène pour l'activité de la nitrate réductase (Glass et al., 2009 ; Raven, 1988 ; Twining & Baines, 2013). Par conséquent, l'appauvrissement d'un métal peut affecter une variété de processus, en particulier dans l'assimilation des macroéléments. Chez les dinoflagellés symbiotiques déficients en fer, l'altération de l'assimilation de l'azote entraîne une diminution de la division cellulaire (Reich et al., 2020). Des études complémentaires devraient donc viser à étudier les effets combinés de la disponibilité des métaux sur la physiologie des coraux.

VI.1.b.ii. La disponibilité des métaux-traces peut-elle également déterminer leur capacité à résister au stress thermique ?

Nous avons étudié les effets d'une augmentation de température de 25 °C et 30 °C sur la teneur en métaux de *S. pistillata* avec ou sans apport de métaux-traces. Dans cette étude, la température de 30 °C n'a pas été suffisante pour déclencher le blanchissement de *S. pistillata*. Ainsi, nous n'avons pas pu évaluer le rôle des métaux-traces sur la capacité du corail à résister au stress thermique.

Cependant, nos résultats montrent que l'augmentation de la température a stimulé les taux de photosynthèse des dinoflagellés symbiotiques chez les coraux témoins non enrichis en métaux. Cette augmentation des taux de photosynthèse à haute température a été induite par une bioaccumulation de fer dans les cellules du symbiote. Ce phénomène de bioaccumulation des métaux avec la température a également été observé chez les plantes et les animaux, en raison d'un métabolisme plus élevé à haute température (K. G. Baker et al., 2018 ; Fritioff et al., 2005 ; Jezierska & Witeska, 2006). En outre, chez les coraux enrichis en métaux, on a également constaté une bioaccumulation des métaux (manganèse, cuivre, magnésium, molybdène, cadmium et cobalt). Cependant, les taux de photosynthèse des coraux n'étaient pas plus élevés, probablement parce que les taux de photosynthèse étaient déjà à leur maximum à 25°C dans les coraux enrichis en métaux.

Nous avons également étudié l'effet de la disponibilité des métaux chez les *S. pistillata* en phase de récupération d'un stress thermique et d'un blanchissement. Nous avons constaté que la disponibilité des métaux contribuait à stimuler les taux de photosynthèse des coraux en voie de rétablissement. Cela suggère en outre que l'holobionte corallien, et en particulier la machinerie photosynthétique, est potentiellement limitée en métaux. Bien que nous n'ayons pas évalué directement le rôle de la disponibilité des métaux sur la résistance des coraux au stress thermique, son rôle bénéfique dans la récupération des coraux suggère que l'apport de métaux pourrait atténuer le blanchissement des coraux en cas de stress thermique.

En résumé, les coraux peuvent être limités non seulement en macroéléments, mais également en microéléments, notamment en fer. La carence en métaux chez les coraux peut gravement altérer leur statut nutritionnel et éventuellement mettre en péril la stabilité de la symbiose corail-dinoflagellé.

Le rôle de la disponibilité des métaux-traces sous stress thermique n'a pas été complètement évalué dans la thèse. Cependant, nous avons observé que les coraux augmentaient leurs besoins en métaux à haute température. De plus, la disponibilité des métaux a également aidé les coraux à récupérer après un stress thermique.

VI.2. Conclusion

La réponse des coraux à un enrichissement équilibré ou déséquilibré en DIN:DIP est complexe et dépend de l'environnement. Globalement, nous avons constaté, dans ce travail, que les effets délétères de l'enrichissement en nitrate sur la physiologie du corail sont spécifiques à chaque espèce et sont en partie dus à un changement de la stœchiométrie cellulaire de l'holobionte

corallien. L'enrichissement en nitrate peut perturber le rapport N:P cellulaire de l'holobionte corallien, principalement en induisant une limitation en phosphore. En plus des macroéléments, les microéléments, en particulier le fer, sont essentiels au métabolisme des coraux et à leur résilience au stress thermique. Ces informations sont importantes pour évaluer correctement la réponse des coraux au stress croissant dû à l'enrichissement en nutriments, car les stratégies de management doivent être spécifiquement adaptées aux caractéristiques environnementales et à la composition des espèces de coraux dans les récifs gérés.

VI.3. Perspectives

Ce travail a fourni des informations importantes sur 1) la réponse du corail à l'enrichissement en nutriments et 2) le rôle des éléments traces dans l'holobionte corallien, mais a également ouvert la voie à de futures expériences.

VI.3.a. Axe I : Réponse du corail à des rapports DIN:DIP équilibrés et déséquilibrés dans l'eau de mer

VI.3.a.i. L'enrichissement en DIN à des concentrations normales/faibles en DIP (rapports DIN:DIP déséquilibrés) a-t-il toujours des effets négatifs sur la santé des coraux ? Au contraire, les coraux restent-ils en bonne santé en cas d'enrichissement équilibré en DIN et DIP ?

Dans la littérature, plus de la moitié des études se sont concentrées sur les coraux scléactiniaires, en particulier les coraux branchus (Tableau 1). Cependant, nous avons souligné que la réponse des coraux à l'enrichissement en DIN était spécifique à chaque espèce, les coraux scléactiniaires étant plus sensibles au déséquilibre des nutriments que les octocoraux. Ainsi, la prochaine étape consisterait à élargir la gamme d'espèces utilisées dans les études. Il est particulièrement important de se concentrer sur les octocoraux, qui semblent insensibles à l'enrichissement en nutriments et réussissent donc très bien à se développer dans des eaux riches en nutriments.

De plus, la plupart des études sur l'enrichissement en nutriments sont menées en laboratoire dans des conditions de contrôle, et si l'irradiation PAR naturelle est reproduite à l'aide de lampes, le spectre UV est généralement absent. Cependant, ce travail a mis en évidence l'influence importante des facteurs environnementaux, notamment l'exposition aux UV, sur la réponse des coraux à l'enrichissement en nutriments. Par conséquent, l'inclusion de l'exposition naturelle aux rayons UV et d'autres combinaisons de facteurs environnementaux est essentielle pour évaluer pleinement la réponse des coraux à l'enrichissement en nutriments.

VI.3.a.ii. Observe-t-on un changement dans le contenu et la stœchiométrie C:N:P avec des changements dans le rapport DIN:DIP de l'eau de mer ? En d'autres termes, l'enrichissement en DIN affecte-t-il les coraux parce qu'il induit une limitation du phosphore dans les tissus coralliens ?

Dans ce travail, nous avons constaté que le changement dans la physiologie du corail sous l'enrichissement en DIN était induit par des changements dans la composition élémentaire et surtout dans le rapport N:P cellulaire. Nous avons également constaté que des changements dans la composition élémentaire peuvent se produire avant que des changements significatifs de la physiologie du corail ne soient observés. Cependant, il n'existe pas beaucoup d'études dans la littérature examinant les effets de l'enrichissement en nutriments sur la composition élémentaire des coraux (Cox & Ward, 2002 ; Godinot et al., 2011 ; McCauley & Goulet, 2019 ; Meyer & Schultz, 1985 ; Muscatine et al., 1989 ; Snidvongs & Kinzie, 1994 ; Stambler et al., 1991 ; Tanaka et al., 2007, 2010, 2017). En outre, sur ces dix études, la teneur en phosphore n'a été évaluée que dans moins de la moitié (4) des études (Godinot et al., 2011 ; Meyer & Schultz, 1985 ; Snidvongs & Kinzie, 1994 ; Tanaka et al., 2010). Par conséquent, il est important d'inclure la composition élémentaire en mettant l'accent sur la teneur en azote et en phosphore et les rapports N:P cellulaires ultérieurs dans les études ultérieures, car cela peut révéler des réponses complexes à l'enrichissement en nutriments qui ne peuvent pas être détectées en évaluant uniquement la physiologie des coraux.

VI.3.a.iii. La capacité des coraux à se nourrir de nutriments dissous (DIN, DIP et DOP) détermine-t-elle leur réponse à l'enrichissement en DIN ?

Dans ce travail, nous avons découvert que la capacité des coraux à utiliser le DOP détermine la réponse des coraux à l'enrichissement en DIN via l'activité phosphatase. L'activité des phosphatases chez les coraux a été étudiée principalement chez les coraux scléactiniaires jusqu'à présent (Godinot et al., 2013 ; Houlbrèque et al., 2015 ; Jackson et al., 1989 ; Wiedenmann et al., 2013). Cependant, comme les espèces de coraux diffèrent dans leur capacité à stocker et à utiliser le phosphore, elles pourraient avoir différents degrés de limitation en phosphore. Ainsi, l'évaluation de la limitation et des besoins en phosphore dans un plus grand nombre d'espèces de coraux est essentielle pour comprendre pleinement les différences dans la sensibilité des coraux au blanchissement suite à un stress thermique et à des rapports N:P déséquilibrés.

VI.3.b. Axe II : Réponse des coraux aux métaux-traces

L'effet des métaux-traces sur la physiologie des coraux a été peu étudié, à l'exception de quelques études qui ont porté sur l'assimilation de ces métaux par les coraux et leur allocation aux différents compartiments de l'association symbiotique. Par conséquent, de nombreuses recherches restent à faire.

Dans l'ensemble, il peut être intéressant de relier les changements dans le métallome avec les changements dans l'élémentome et la physiologie de différentes espèces de coraux dans une gamme de conditions environnementales, y compris le réchauffement de l'eau de mer, pour évaluer quels éléments-traces peuvent déterminer la réponse du corail à l'environnement. En outre, la littérature sur les plantes ou le phytoplancton a montré que l'assimilation des macronutriments peut être déterminée par la disponibilité des métaux-traces (Morel et al., 1994 ; Reich et al., 2020 ; Shaked et al., 2006). L'assimilation du carbone dépend de l'activité de l'anhydrase carbonique, nécessitant du zinc et du cobalt. De même, l'assimilation du phosphore dépend de l'activité des phosphatases alcalines, nécessitant du zinc, tandis que l'assimilation de l'azote dépend de l'activité de la nitrate réductase, nécessitant du molybdène et du vanadium. Le domaine des interactions entre les métaux-traces - quel métal-trace favorise l'assimilation d'autres métaux-traces, comme par exemple l'assimilation du fer augmentée par l'ajout de zinc dans les algues (Santana-Casiano et al., 1997) - dans les coraux est également totalement inconnu. En outre, des études ont également montré que le phytoplancton pouvait utiliser d'autres métaux-traces comme substituts dans certains processus, comme l'utilisation du cadmium à la place du zinc dans l'anhydrase carbonique (Morel et al., 2020). Ainsi, des études supplémentaires sont nécessaires pour comprendre comment la disponibilité des métaux-traces peut influencer la capacité nutritionnelle des coraux.

Enfin, les coraux sont également des holobiontes, associés à des microbes, qui ont de grandes capacités de captation des métaux. Par exemple, de nombreuses bactéries possèdent des sidérophores qui piègent le fer dans l'eau de mer. Le rôle de ces microbes dans l'acquisition et le transfert des métaux-traces à leur hôte est très mal connu et doit être approfondi, car c'est la base de toute symbiose. En outre, nous avons démontré que la teneur en métaux traces augmente dans les tissus coralliens après l'ajout de poussière. Cependant, la poussière du désert n'est pas seulement riche en métaux traces, elle est également constituée d'une variété d'organismes, des bactéries aux champignons et autres micro-eucaryotes. Ces microorganismes peuvent interagir avec les coraux et être à la fois bénéfiques, comme les bactéries diazotrophes, ou nuisibles (Bednarz et al., 2021 ; Uyl et al., 2016 ; revu dans : van Oppen & Blackall, 2019).

Par conséquent, il est essentiel d'évaluer le rôle des micro-organismes causés par la poussière du désert pour comprendre comment les dépôts de poussière du désert impactent les coraux.

APPENDICES



APPENDICES

I Appendix I – Supplementary material Chapter 1

Publication: **Low levels of ultra-violet radiation mitigate the deleterious effects of nitrate and thermal stress on coral photosynthesis**

1.1. Results

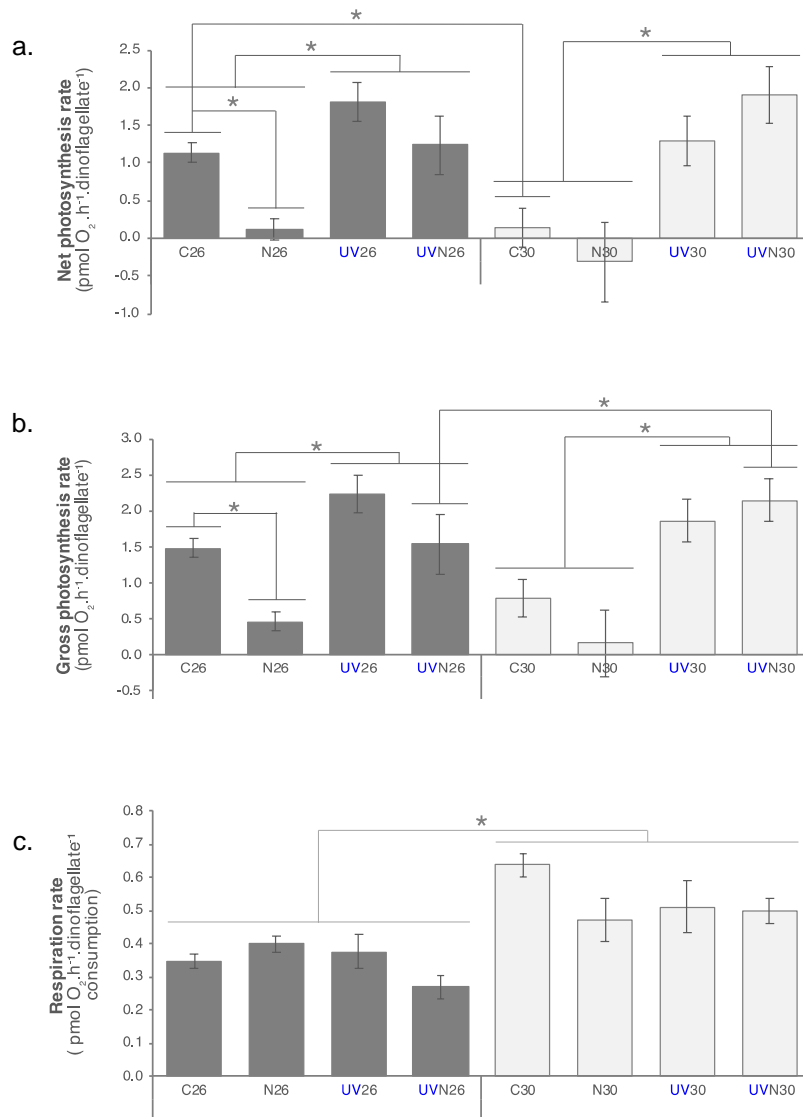


Figure S1. Oxygen fluxes (per dinoflagellate cell) measured for the coral *Pocillopora damicornis* maintained under different environmental conditions.

The barplots represent net (a.) and gross (b.) photosynthesis rates and respiration (c.) rates ($\mu\text{mol O}_2 \cdot \text{h}^{-1} \cdot \text{dinoflagellate}^{-1}$) under different nitrate (N)-UVR conditions. Dark grey bars represent corals maintained at 26°C (26) while light grey bars are corals maintained at 30°C (30) – **C26-C30**: control corals maintained at 26°C or 30°C without nitrate enrichment and UVR exposure, **N26-N30**: corals maintained under nitrate enrichment (3 μM nitrate) at 26 °C or 30°C, **UV26-UV30**: corals exposed to UVR (25 Wm^{-2} UVA and 1.5 Wm^{-2} UVB) at 26 °C or 30°C, **UVN26 – UVN30**: corals maintained under UVR exposure and nitrate enrichment (3 μM nitrate) at 26 °C or 30°C –Data represents mean and standard error of 6 replicates. Stars represent significantly different values (p value < 0.05).

Table S1: Statistical tests (ANOVA and pairwise comparison) testing the effects of temperature, UVR and nitrate levels on the physiological parameters of the coral *Pocillopora damicornis*.

The statistical tests were performed on 6 coral nubbins per condition. Pairwise comparisons were done when the ANOVA test showed significant interaction between parameters. The significant p-value (< 0.05) are highlighted in green.

The tables present the outcomes for (a.) the protein biomass normalized to surface area ($\mu\text{g}\cdot\text{cm}^{-2}$) (b.) dinoflagellate density normalized to surface area ($10^6\cdot\text{cm}^{-2}$), (c.) total chlorophyll a and c₂ content normalized to dinoflagellates cell ($10^{-6}\mu\text{g}\cdot\text{dinoflagellates}^{-1}$), under different nutrient-UVR conditions at 26 °C (26) and 30°C (30) - **C26-C30**: control corals maintained at 26°C or 30°C without nitrate enrichment and UVR exposure, **N26-N30**: corals maintained under nitrate enrichment (3 μM nitrate) at 26 °C or 30°C, **UV26-UV30**: corals exposed to UVR (25 Wm^{-2} UVA and 1.5 Wm^{-2} UVB) at 26 °C or 30°C, **UVN26 – UVN30**: corals maintained under UVR exposure and nitrate enrichment (3 μM nitrate) at 26 °C or 30°C

Holobiont protein content : ANOVA Output					
	Df	Sum Sq	Mean Sq	F value	Pr (> F)
Nutrient	1	26	26	0.008	0.9311
UV	1	17611	17611	5.098	0.0284
Temperature	1	1243	1243	0.36	0.5514
Nutrient:UV	1	2613	2613	0.756	0.3887
Nutrient:Temperature	1	43	43	0.013	0.9112
UV:Temperature	1	11680	11680	3.381	0.072
Nutrient:UV:Temperature	1	2017	2017	0.584	0.4484
Residuals	49	169264	3454		

Pairwise comparison							
	C26	C30	UV26	UV30	N26	N30	UVN26
C30	0.118						
UV26	0.689						
UV30		0.04	0.302				
N26	0.414						
N30		0.748			0.377		
UVN26			0.495		0.288		
UVN30				0.624		0.046	0.953

Dinoflagellates density : ANOVA Output					
	Df	Sum Sq	Mean Sq	F value	Pr (> F)
Nutrient	1	0.1077	0.1077	5.379	0.0246
UV	1	0.0461	0.0461	2.304	0.1354
Temperature	1	0.8902	0.8902	4.45E+01	2.17E-08
Nutrient:UV	1	0.001	0.001	0.052	0.8214
Nutrient:Temperature	1	0.0034	0.0034	0.169	0.6824
UV:Temperature	1	0.0375	0.0375	1.871	0.1777
Nutrient:UV:Temperature	1	0.0285	0.0285	1.423	0.2386
Residuals	49	0.9811	0.02		

Pairwise comparison							
	C26	C30	UV26	UV30	N26	N30	UVN26
C30	0.00414						
UV26	0.26133						
UV30		0.43551	0.0041				
N26	0.34642						
N30		0.05212			0.13491		
UVN26			0.02695		0.04612		
UVN30				0.5508		0.64592	3.90E-06

Total chlorophyll a and c ₂ content : ANOVA Output					
	Df	Sum Sq	Mean Sq	F value	Pr (> F)
Nutrient	1	0.73	0.728	0.979	0.32735
UV	1	5.91	5.913	7.958	0.00694
Temperature	1	7.51	7.51	10.106	0.00259
Nutrient:UV	1	0.76	0.76	1.023	0.31693
Nutrient:Temperature	1	0	0	0	0.98345
UV:Temperature	1	3.17	3.171	4.267	0.04428
Nutrient:UV:Temperature	1	1.33	1.331	1.791	0.18707
Residuals	48	35.67	0.743		

Pairwise comparison							
	C26	C30	UV26	UV30	N26	N30	UVN26
C30	0.8689						
UV26	0.5348						
UV30		0.0116	0.0025				
N26	0.3145						
N30		0.9347			0.3864		
UVN26			0.2793		0.1574		
UVN30				0.5757		0.0241	0.0381

Table S2: Statistical tests (ANOVA and pairwise comparison) testing the effects of temperature, UVR and nitrate levels on oxygen fluxes normalized to surface area of *Pocillopora damicornis*.

The statistical tests were performed on 6 coral nubbins per condition. Pairwise comparisons were done when the ANOVA test showed significant interaction between parameters. The significant p-value (< 0.05) are presented in green.

The tables present the outcomes for the net (a.) and gross (b.) photosynthesis rates and respiration (c.) rates ($\mu\text{mol O}_2\cdot\text{h}^{-1}\cdot\text{cm}^{-2}$) normalized per surface area under different nutrient-UVR conditions at 26 °C (26) and 30°C (30)- **C26-C30**: control corals maintained at 26°C or 30°C without nitrate enrichment and UVR exposure, **N26-N30**: corals maintained under nitrate enrichment (3 μM nitrate) at 26 °C or 30°C, **UV26-UV30**: corals exposed to UVR (25 Wm^{-2} UVA and 1.5 Wm^{-2} UVB) at 26 °C or 30°C, **UVN26 – UVN30**: corals maintained under UVR exposure and nitrate enrichment (3 μM nitrate) at 26 °C or 30°

a. Net photosynthesis rates normalized per surface area : ANOVA Output

	Df	Sum Sq	Mean Sq	F value	Pr (> F)
Nutrient	1	0.00296	0.00296	0.715	0.401614
UV	1	0.06422	0.06422	15.515	0.000245
Temperature	1	0.03107	0.03107	7.505	0.008407
Nutrient:UV	1	0.01339	0.01339	3.236	0.07786
Nutrient:Temperature	1	0.01722	0.01722	4.16	0.046481
UV:Temperature	1	0.00019	0.00019	0.046	0.831747
Nutrient:UV:Temperature	1	0.00387	0.00387	0.936	0.337793
Residuals	52	0.21525	0.00414		

Pairwise comparison

	C26	C30	UV26	UV30	N26	N30	UVN26
C30	0.00396						
UV26	0.54256						
UV30		0.10529	0.07432				
N26	0.00644						
N30		0.87458			0.99435		
UVN26			0.7578		0.00295		
UVN30				0.37905		0.01188	0.4028

b. Gross photosynthesis rates normalized per surface area : ANOVA Output

	Df	Sum Sq	Mean Sq	F value	Pr (> F)
Nutrient	1	0	0.00002	0.004	0.951
UV	1	0.0536	0.05364	8.208	0.006
Temperature	1	0.0034	0.00341	0.521	0.474
Nutrient:UV	1	0	0.00002	0.003	0.957
Nutrient:Temperature	1	0.0148	0.01478	2.261	0.139
UV:Temperature	1	0.0001	0.00011	0.016	0.899
Nutrient:UV:Temperature	1	0.0001	0.00006	0.01	0.922
Residuals	52	0.3398	0.00654		

c. Respiration rates normalized per surface area : ANOVA Output

	Df	Sum Sq	Mean Sq	F value	Pr (> F)
Nutrient	1	0.00229	0.002287	0.503	0.4816
UV	1	0.00005	0.000048	0.011	0.9186
Temperature	1	0.01583	0.01583	3.479	0.07
Nutrient:UV	1	0.0046	0.004601	1.011	0.3193
Nutrient:Temperature	1	0.00061	0.000609	0.134	0.716
UV:Temperature	1	0.00305	0.003049	0.67	0.4168
Nutrient:UV:Temperature	1	0.0021	0.002096	0.461	0.5003
Residuals	51	0.23204	0.00455		

Table S3: Statistical tests (ANOVA and pairwise comparison) testing the effects of temperature, UVR and nitrate levels on oxygen fluxes normalized to dinoflagellate cells of *Pocillopora damicornis*.

The statistical tests were performed on 6 coral nubbins per condition. Pairwise comparisons were done when the ANOVA test showed significant interaction between parameters. The significant p-value (< 0.05) are presented in green.

The tables present the outcomes for the net (a.) and gross (b.) photosynthesis rates and respiration (c.) rates ($\mu\text{mol O}_2\cdot\text{h}^{-1}\cdot\text{dinoflagellate}^{-1}$), normalized to dinoflagellate cell, under different nutrient-UVR conditions at 26 °C (26) - and 30°C (30)-**C26-C30**: control corals maintained at 26°C or 30°C without nitrate enrichment and UVR exposure, **N26-N30**: corals maintained under nitrate enrichment (3 μM nitrate) at 26 °C or 30°C, **UV26-UV30**: corals exposed to UVR (25 Wm^{-2} UVA and 1.5 Wm^{-2} UVB) at 26 °C or 30°C, **UVN26-UVN30**: corals maintained under UVR exposure and nitrate enrichment (3 μM nitrate) at 26 °C or 30°C

a. Net photosynthesis rates normalized per dinoflagellate cell : ANOVA Output

	Df	Sum Sq	Mean Sq	F value	Pr (> F)
Nutrient	1	0.23	0.232	0.276	0.6019
UV	1	23.76	23.764	28.237	2.38E-06
Temperature	1	0.5	0.499	0.593	0.4447
Nutrient:UV	1	2.62	2.617	3.109	0.0838
Nutrient:Temperature	1	3.77	3.769	4.479	0.0392
UV:Temperature	1	2.15	2.147	2.552	0.1164
Nutrient:UV:Temperature	1	0.33	0.335	0.397	0.5312
Residuals	51	42.92	0.842		

Pairwise comparison

	C26	C30	UV26	UV30	N26	N30	UVN26
C30	0.00944						
UV26	0.03898						
UV30		0.02181	0.24721				
N26	0.00013						
N30		0.46339			0.46852		
UVN26			0.0287		0.00034		
UVN30				0.24575		8.39E-03	0.46129

b. Gross photosynthesis rates normalized per dinoflagellate cell : ANOVA Output

	Df	Sum Sq	Mean Sq	F value	Pr (> F)
Nutrient	1	0.0722	0.0722	4.477	0.039265
UV	1	0.2131	0.2131	13.213	0.000646
Temperature	1	0.5581	0.5581	34.602	3.13E-07
Nutrient:UV	1	0.0163	0.0163	1.013	0.318854
Nutrient:Temperature	1	0.0086	0.0086	0.533	0.468738
UV:Temperature	1	0.0242	0.0242	1.5	0.226342
Nutrient:UV:Temperature	1	0.0637	0.0637	3.95	0.052253
Residuals	51	0.8225	0.0161		

Pairwise comparison

	C26	C30	UV26	UV30	N26	N30	UVN26
C30	0.11366						
UV26	0.07238						
UV30		0.02364	0.40078				
N26	0.02322						
N30		0.20401			0.52935		
UVN26			0.40078		0.02009		
UVN30				0.48447		3.00E-05	1.20E-01

c. Respiration rates normalized per dinoflagellate cell : ANOVA Output

	Df	Sum Sq	Mean Sq	F value	Pr (> F)
Nutrient	1	0.0166	0.0166	1.17	0.2849
UV	1	0.0172	0.0172	1.212	0.2765
Temperature	1	0.5502	0.5502	38.673	1.16E-07
Nutrient:UV	1	0.0021	0.0021	0.145	0.7053
Nutrient:Temperature	1	0.0113	0.0113	0.797	0.3764
UV:Temperature	1	0.0009	0.0009	0.061	0.8054
Nutrient:UV:Temperature	1	0.0664	0.0664	4.664	0.0358
Residuals	48	0.6829	0.0142		

Pairwise comparison

	C26	C30	UV26	UV30	N26	N30	UVN26
C30	3.50E-05						
UV26	0.62233						
UV30		0.17557	0.03489				
N26	0.43375						
N30		0.02577			0.33071		
UVN26			0.08273		0.06227		
UVN30				0.61651		0.68814	0.00011

Table S4: Outcome of the statistical tests (ANOVA and pairwise comparison) testing the effects of temperature, UVR and nitrate levels on the oxidative stress parameters of *Pocillopora damicornis*.

The statistical tests were performed on 6 coral nubbins per condition. Pairwise comparisons were done when the ANOVA test showed significant interaction between parameters. The significant p-value (< 0.05) are presented in green.

The tables present the outcomes for (a.) the non-enzymatic antioxidant capacity ($\mu\text{M CRE.mg protein}^{-1}$), (b.) protein nitration level ($\text{ng3NT-BSA.mg protein}^{-1}$), (c.) reactive oxygen species levels (F.U.min^{-1}), (d.) and lipid peroxidation level ($\text{nmol MDA.mg protein}^{-1}$), under different nutrient-UVR conditions at 26 °C (26) and 30°C (30)- **C26-C30**: control corals maintained at 26°C or 30°C without nitrate enrichment and UVR exposure, **N26-N30**: corals maintained under nitrate enrichment (3 μM nitrate) at 26 °C or 30°C, **UV26-UV30**: corals exposed to UVR (25 Wm^{-2} UVA and 1.5 Wm^{-2} UVB) at 26 °C or 30°C, **UVN26-UVN30**: corals maintained under UVR exposure and nitrate enrichment (3 μM nitrate) at 26 °C or 30°C

a.

Total non-enzymatic antioxidant capacity : ANOVA Output

	Df	Sum Sq	Mean Sq	F value	Pr (> F)
Nutrient	1	0	0	0.001	0.9801
UV	1	71175	71175	113.934	2.85E-13
Temperature	1	130	130	0.208	0.6506
Nutrient:UV	1	2734	2734	4.376	0.0428
Nutrient:Temperature	1	698	698	1.117	0.2969
UV:Temperature	1	3148	3148	5.039	0.0304
Nutrient:UV:Temperature	1	1608	1608	2.574	0.1165
Residuals	40	24988	625		

Pairwise comparison

	C26	C30	UV26	UV30	N26	N30	UVN26
C30	0.0318						
UV26	2.40E-07						
UV30		0.02289	0.11217				
N26	0.76792						
N30		0.02302			0.6649		
UVN26			0.43721		4.90E-08		
UVN30				0.19031		3.90E-07	0.28803

b.

Protein nitration : White Adjusted ANOVA (Type III)

	Df	F	Pr(>F)
(Intercept)	1	44.4383	5.48E-08
Nutrient	1	0.7141	0.4031
Temperature	1	0.1085	0.74361
UV	1	2.5893	0.11545
Nutrient:Temperature	1	0.1939	0.66205
Nutrient:UV	1	2.0419	0.16078
Temperature:UV	1	2.7626	0.10431
Nutrient:Temperature:UV	1	6.8357	0.01254
Residuals	40		

Pairwise comparison

	C26	C30	UV26	UV30	N26	N30	UVN26
C30	0.86934						
UV26	0.67414						
UV30		0.03485	0.0191				
N26	0.84993						
N30		0.67231			0.94444		
UVN26			0.00068		0.00382		
UVN30				0.0783		0.9615	0.00405

c.

Reactive oxygen species level : ANOVA Output

	Df	Sum Sq	Mean Sq	F value	Pr (> F)
Nutrient	1	1543	1543	2.413	0.1288
UV	1	2232	2232	3.491	0.0696
Temperature	1	3314	3314	5.183	0.0287
Nutrient:UV	1	4446	4446	6.953	0.0122
Nutrient:Temperature	1	199	199	0.311	0.5807
UV:Temperature	1	171	171	0.267	0.6083
Nutrient:UV:Temperature	1	2571	2571	4.02	0.0523
Residuals	37	23660	639		

Pairwise comparison

	C26	C30	UV26	UV30	N26	N30	UVN26
C30	0.1259						
UV26	0.70997						
UV30			0.22196				
N26	0.80183						
N30		0.2031				0.85987	
UVN26			0.37418			0.30696	
UVN30				0.00146		0.00125	0.01213

d.

Lipid peroxidation : ANOVA Output

	Df	Sum Sq	Mean Sq	F value	Pr (> F)
Nutrient	1	0.059	0.0594	0.535	0.468987
UV	1	0.051	0.0505	0.455	0.503993
Temperature	1	2.071	2.0707	18.653	0.000104
Nutrient:UV	1	0.018	0.0181	0.163	0.688558
Nutrient:Temperature	1	0.008	0.008	0.072	0.790239
UV:Temperature	1	0.067	0.0669	0.603	0.4423
Nutrient:UV:Temperature	1	0.038	0.0375	0.338	0.564334
Residuals	39	4.33	0.111		

II Appendix II – Supplementary material Chapter 2

Publication: **The effects of seawater nutrient imbalance on coral physiology is species-specific**

II.1. Introduction

Table S1: **Summary of the impact of different nutrient enrichment (nitrate, ammonium, phosphorus alone or in combination) on different physiological parameters of corals.**

Table was based on the results presented by 47 studies. n represents the number of studies that measured this parameter. Nutrient impacts were evaluated as positive (1, green), negative (-1, red) or neutral (0, white), when diverging results were found in the literature an average were calculated.

The purpose of this table was only to have a quick overview of the parameters studied in the literature and the overall impact of nutrient, it is only meant as an informative table.

	Coral growth	Symbiont density	Chlorophyll content	Photosynthetic rates	Tissue parameters	Carbon content	Nitrogen content	Phosphorus content	Reproduction	Survival	Coral Health
NO ₃	-0.38 (n=8)	0 (n=6)	0.29 (n=7)	-0.17 (n=12)	0 (n=3)	0 (n=1)	1 (n=1)			-0.25 (n=4)	-0.83 (n=6)
NH ₄	-0.3 (n=10)	0.5 (n=8)	1 (n=6)	0.5 (n=4)	0.4 (n=5)	0 (n=1)	0.5 (n=2)	0 (n=1)	-1 (n=2)		0 (n=2)
PO ₄	-0.45 (n=11)	0.4 (n=5)	-0.25 (n=4)	0.67 (n=3)	0.67 (n=3)	0.25 (n=4)	0.33 (n=3)	0 (n=2)			
NO ₃ / PO ₄	0 (n=6)	0 (n=1)	0.5 (n=4)	0.33 (n=3)	0 (n=1)	0 (n=1)	1 (n=1)				
NH ₄ / PO ₄	-0.3 (n=6)	0.4 (n=5)	0.5 (n=2)	0 (n=2)	1 (n=1)	0 (n=1)	0.5 (n=2)	0 (n=2)			
NO ₃ / NH ₄	0.5 (n=2)	-1 (n=1)	0 (n=1)	0 (n=2)	0 (n=1)	-1 (n=1)					
NO ₃ / NH ₄ / PO ₄	0.25 (n=4)	1 (n=1)	1 (n=1)						-1 (n=1)		-1 (n=2)

Table S2: Summary of the carbon, nitrogen and phosphorus content and carbon-to-nitrogen, carbon-to-phosphorus and nitrogen-to-phosphorus ratios under different nutrient enrichment condition.

Nutrient enrichment	Partner	Study	Context	Coral species	Concentration	Carbon content ($\mu\text{g cm}^{-2}$)	Nitrogen content ($\mu\text{g cm}^{-2}$)	Phosphorus content ($\mu\text{g cm}^{-2}$)	C:N (atomic)	C:P (atomic)	N:P (atomic)	
Control	Coral holobiont	Meyer & Shultz 1985	In situ (fish excretion)	<i>Acropora palmata</i>			208 \pm 30	31 \pm 4			21.8 \pm 0.7	
				<i>Porites furcata</i>			451 \pm 0.12	44 \pm 2			26.3 \pm 0.7	
		Tanaka et al 2010	Exp	<i>Montipora digitata</i>		3432 \pm 540	392 \pm 42	26.4 \pm 2.5	10.2	336	33	
		Cox and Ward 2002	Exp	<i>Montipora capitata</i>		40.8 \pm 10.9 (%)	4.1 \pm 0.23 (%)					
		Stambler et al 1991	Exp	<i>Stylophora pistillata</i>					6.1	233	38	
	Animal tissue	Muscatine et al. 1989	Exp	<i>Stylophora pistillata</i>		704.7 \pm 52.8	112.7 \pm 5.9		7.31 \pm 0.16			
		Tanaka et al 2007	Exp	<i>Stylophora pistillata</i>		4116 \pm 348	644 \pm 56		7.4 \pm 0.1			
		Godinot et al. 2011	Exp	<i>Stylophora pistillata</i>		824.4 \pm 34.8	145.6 \pm 9.8	4.9 \pm 0.7	6.6	432.1	65.4	
		McCauley & Goulet 2019	Exp	<i>Pseudoplexura porosa</i>					6.8			
				<i>Eunicea tourneforti</i>					4			
		Tanaka 2017	Exp	<i>Montipora digitata</i>		396			7.9			
	<i>Porites cylindrica</i>				600			6.9				
	Symbiont	Muscatine et al. 1989	Exp	<i>Stylophora pistillata</i>		204 \pm 46.8	29.9 \pm 6.4		7.7 \pm 0.7			
		Snidvong & Kinzie 1994	Exp	<i>Pocillopora damicornis</i>		307 (pg.cell ⁻¹)	33 (pg.cell ⁻¹)	2.2 (pg.cell ⁻¹)	11	360	33	
		Tanaka et al 2007	Exp	<i>Stylophora pistillata</i>		312 \pm 24	49 \pm 5.6		7.5 \pm 0.3			
		Godinot et al. 2011	Exp	<i>Stylophora pistillata</i>		343.2 \pm 22.8	63 \pm 9.8	1.6 \pm 0.09	6.4	550.0	86.5	
McCauley & Goulet 2019		Exp	<i>Pseudoplexura porosa</i>					11.9				
			<i>Eunicea tourneforti</i>					10.3				
Tanaka 2017	Exp	<i>Montipora digitata</i>		132			7.9					
<i>Porites cylindrica</i>				309			6.6					
NO ₃	Host	Tanaka 2017	Exp	<i>Montipora digitata</i>		360			7.2			
				<i>Porites cylindrica</i>		600			6.7			
	Symbiont	Tanaka 2017	Exp	<i>Montipora digitata</i>		132			5.2			
				<i>Porites cylindrica</i>		309			5.9			

NH ₄	Coral holobiont	Cox and Ward 2002	Exp	<i>Montipora capitata</i>	20 µM	48.8 ± 11.9 (%)	5.7 ± 0.87 (%)					
	Animal tissue	McCauley & Goulet 2019	Exp	<i>Pseudopleaxura porosa</i>	10 µM				7			
				<i>Eunicea tourneforti</i>	10 µM				5.8			
		Muscatine et al. 1989	Exp	<i>Stylophora pistillata</i>	20 µM	766 ± 118.2	152.2 ± 3.4		5.88 ± 0.78			
		McCauley & Goulet 2019	Exp	<i>Pseudopleaxura porosa</i>	50 µM					6.7		
	<i>Eunicea tourneforti</i>			50 µM					5.3			
	Symbiont	McCauley & Goulet 2019	Exp	<i>Pseudopleaxura porosa</i>	10 µM					11.6		
				<i>Eunicea tourneforti</i>	10 µM					10.7		
		Snidvong & Kinzie 1994	Exp	<i>Pocillopora damicornis</i>	15 µM	311 (pg.cell ⁻¹)	70 (pg.cell ⁻¹)	1.3 (pg.cell ⁻¹)	5	618	119	
Muscatine et al. 1989		Exp	<i>Stylophora pistillata</i>	20 µM	286.2 ± 24.7	75.5 ± 5.9		4.4 ± 0.2				
	McCauley & Goulet 2019		Exp	<i>Pseudopleaxura porosa</i>	50 µM				10.4			
			Exp	<i>Eunicea tourneforti</i>	50 µM				10.6			
PO ₄	Coral holobiont	Stambler et al 1991	Exp	<i>Stylophora pistillata</i>	0.5 µM				5.8	416	71	
					2 µM				5.1	375	73	
	Animal tissue	Muscatine et al. 1989	Exp	<i>Stylophora pistillata</i>	2 µM	493.1 ± 141.2	86.2 ± 0.8		6.69 ± 1.86			
					Godinot et al. 2011	Exp	<i>Stylophora pistillata</i>	0.5 µM	744 ± 31.2	135.8 ± 4.2	4.6 ± 0.4	6.4
		McCauley & Goulet 2019	Exp	<i>Pseudopleaxura porosa</i>	2.5 µM	782.4 ± 42	134.4 ± 8.4	5.2 ± 0.8	6.8	388.1	57.1	
					<i>Eunicea tourneforti</i>	4 µM				5.8		
				Exp	<i>Eunicea tourneforti</i>	4 µM				5.4		
	Symbiont	Muscatine et al. 1989	Exp	<i>Stylophora pistillata</i>	2 µM	207.1 ± 39.9	33.4		6.3			
					Snidvong & Kinzie 1994	Exp	<i>Pocillopora damicornis</i>	1.2 µM	235 (pg.cell ⁻¹)	27 (pg.cell ⁻¹)	1.2 (pg.cell ⁻¹)	10
		Godinot et al. 2011	Exp	<i>Stylophora pistillata</i>	0.5 µM	398.4 ± 34.8	65.8 ± 15.4	2 ± 0.09	7.1	518.8	73.4	
2.5 µM					490.8 ± 79.2	78.4 ± 12.6	3.1 ± 0.16	7.3	409.0	56.0		
McCauley & Goulet 2019		Exp	<i>Pseudopleaxura porosa</i>	4 µM				13.1				
	Exp	<i>Eunicea tourneforti</i>	4 µM				9.1					
NO ₃ / PO ₄	Coral holobiont	Tanaka et al 2010	Exp	<i>Montipora digitata</i>	10.4 µM 0.49 µM N:P 21	3108 ± 252	420 ± 14	28.5 ± 1.2	8.6	282	33	

NO ₃ / PO ₄	Animal tissue	Tanaka et al 2007	Exp (5 days)	<i>Stylophora pistillata</i>	5 µM	5940 ± 480	882 ± 84		7.9 ± 0.1		
			Exp (10 days)	<i>Stylophora pistillata</i>	0.3 µM N:P : 16	7056 ± 672	1008 ± 98		8.1 ± 0.1		
		Tanaka 2017	Exp	<i>Montipora digitata</i> <i>Porites cylindrica</i>		456 657			7.1 6.6		
	Symbiont	Tanaka et al 2007	Exp (5 days)	<i>Stylophora pistillata</i>	N : 5 µM P : 0.3 µM N:P : 16	456 ± 36	62 ± 5.6		8.8 ± 0.3		
			Exp (10 days)			624 ± 60	84 ± 8.4		8.7 ± 0.2		
		Tanaka 2017	Exp	<i>Montipora digitata</i> <i>Porites cylindrica</i>	N : 1.5 µM P : 0.1 µM N:P 15	192 402			6.4 6.1		
NH ₄ / PO ₄	Coral holobiont	Meyer & Shultz 1985	In situ (fish excretion)	<i>Acropora palmata</i>	N : 2.4 mmol m ⁻² d ⁻¹ P : 0.2 mmol m ⁻² d ⁻¹		440 ± 50	50 ± 5			20.7 ± 2.1
				<i>Porites furcata</i>	N : 7.3 mmol m ⁻² d ⁻¹ P : 0.1 mmol m ⁻² d ⁻¹		564 ± 16	48 ± 2		24.4 ± 1	
	Animal tissue	Muscatine et al. 1989	Exp	<i>Stylophora pistillata</i>	N:P : 10	844.2 ± 60.2	172.4 ± 21.4		5.80 ± 1.13		
	Symbiont	Muscatine et al. 1989	Exp	<i>Stylophora pistillata</i>	N:P : 10	303.3 ± 15.8	74.6 ± 0.9		4.7 ± 0.2		
Snidvong & Kinzie 1994		Exp	<i>Pocillopora damicornis</i>	N:P 12.5	184 (pg.cell ⁻¹)	42 (pg.cell ⁻¹)	0.9 (pg.cell ⁻¹)	5	528	103	

III Results

Table S3: Summary of the median and standard error (SE) for each parameter in *Turbinaria reniformis* (a.) and *Sarcophyton glaucum* (b.)a. *Turbinaria reniformis*

	Control balanced DIN:DIP _{2.5}		Balanced DIN:DIP ₃		Imbalanced DIN:DIP _{0.5}		Imbalanced DIN:DIP ₁₅	
	Median	± SE	Median	± SE	Median	± SE	Median	± SE
Nitrate uptake (nmole NO ₃ .min ⁻¹ .g ⁻¹ AFDW)	7.35	3.66	108.84	12.61	12.60	2.98	56.55	8.71
Phosphorus uptake (nmole PO ₄ .min ⁻¹ .g ⁻¹ AFDW)	3.17	1.41	70.39	7.77	62.79	11.41	13.19	6.48
Total chlorophyll a and c2 (mg.g ⁻¹ AFDW)	0.21	0.024	0.38	0.054	0.21	0.046	0.30	0.049
Net photosynthetic rates (mmol.h ⁻¹ .g ⁻¹ AFDW)	0.06	0.007	0.14	0.016	0.09	0.018	0.09	0.007
Respiration rates (mmol.h ⁻¹ .g ⁻¹ AFDW)	-0.13	0.01	-0.16	0.01	-0.16	0.03	-0.21	0.02
Yield	0.50	0.01	0.49	0.02	0.51	0.01	0.45	0.01
Holobiont carbohydrate content (mg.g ⁻¹ AFDW)	1.61	0.18	3.19	0.41	3.42	0.41	2.89	0.31
Holobiont lipid content (mg.g ⁻¹ AFDW)	9.70	0.51	18.90	3.74	8.82	2.55	14.13	1.18
Carbon (mg.g ⁻¹ AFDW)	24.07	2.44	39.29	5.85	47.19	2.98	35.35	1.10
Nitrogen (mg.g ⁻¹ AFDW)	3.77	0.60	5.95	1.00	7.78	0.41	6.40	0.23
Phosphorous (mg.g ⁻¹ AFDW)	0.16	0.08	0.12	0.01	0.24	0.13	0.11	0.004
C:N (mass)	6.39	0.67	6.60	0.13	6.15	0.19	5.52	0.10
C:P (mass)	161.96	49.69	325.66	11.56	218.71	57.54	318.33	18.75
N:P (mass)	20.36	7.90	49.31	2.72	35.61	10.16	59.35	2.74
Dinoflagellate density (x10 ⁶ cells.g ⁻¹ AFDW)	425.88	42.12	258.50	25.06	135.83	46.25	317.84	19.87
Holobiont protein content (mg.g ⁻¹ AFDW)	94.24	3.54	102.50	3.02	79.32	8.70	100.29	4.57

b. *Sarcophyton glaucum*

	Control balanced DIN:DIP _{2.5}		Balanced DIN:DIP ₃		Imbalanced DIN:DIP _{0.5}		Imbalanced DIN:DIP ₁₅	
	Median	± SE	Median	± SE	Median	± SE	Median	± SE
Nitrate uptake (nmole NO ₃ .min ⁻¹ .g ⁻¹ AFDW)	10.49	4.65	24.38	4.02	4.33	1.28	12.44	2.02
Phosphorus uptake (nmole PO ₄ .min ⁻¹ .g ⁻¹ AFDW)	7.67	1.94	9.52	3.76	21.24	3.01	5.95	1.11
Total chlorophyll a and c2 (mg.g ⁻¹ AFDW)	0.22	0.04	0.32	0.07	0.20	0.05	0.30	0.08
Net photosynthetic rates (mmol.h ⁻¹ .g ⁻¹ AFDW)	0.02	0.002	0.01	0.005	0.02	0.002	0.01	0.003
Respiration rates (mmol.h ⁻¹ .g ⁻¹ AFDW)	-0.03	0.003	-0.02	0.004	-0.02	0.001	-0.02	0.003
Yield	0.51	0.02	0.63	0.02	0.46	0.02	0.59	0.02
Holobiont carbohydrate content (mg.g ⁻¹ AFDW)	2.22	0.06	2.22	0.08	2.75	0.17	1.05	0.26
Holobiont lipid content (mg.g ⁻¹ AFDW)	446.93	50.01	474.39	36.65	389.04	46.10	355.07	76.07
Carbon (mg.g ⁻¹ AFDW)	210.51	5.64	191.34	28.29	194.49	23.46	218.67	5.37
Nitrogen (mg.g ⁻¹ AFDW)	17.14	0.45	12.08	3.92	17.06	1.79	17.68	2.15
Phosphorous (mg.g ⁻¹ AFDW)	0.22	0.01	0.24	0.11	0.21	0.01	0.23	0.01
C:N (mass)	12.15	0.09	16.16	1.79	13.09	1.91	12.15	1.40
C:P (mass)	918.89	36.50	848.54	228.29	973.19	122.93	964.33	52.22
N:P (mass)	75.78	2.45	50.80	9.51	78.46	7.30	78.43	5.26
Dinoflagellate density (x10 ⁶ cells.g ⁻¹ AFDW)	314.18	16.30	294.76	10.69	292.96	18.03	361.03	24.55
Holobiont protein content (mg.g ⁻¹ AFDW)	36.78	2.27	39.00	8.72	69.23	10.23	26.63	6.36

Table S4: Summary of the statistical outcomes of the different measurements done on *Sarcophyton glaucum* and *Turbinaria reniformis*

a. Nutrient uptake rates

Nitrate uptake per AFDW															
<i>Sarcophyton glaucum</i>						<i>Turbinaria reniformis</i>					Comparison <i>S. glaucum</i> vs <i>T. reniformis</i> in control condition				
Condition	Df	Sum Sq	Mean Sq	F value	P value	Condition	Df	Kruskal-Wallis	Chi-squared	P value	t	df	Welch two sample t-test		
Residuals	15	696.82	46.45	7.10	0.003	Residuals	3	16.70		0.001	0.44	5	p-value		
	Estimate	SE	Df	t.ratio	P value (fdr)		Estimate	SE	Df	t.ratio	P value (fdr)				
Control - NO ₃	-0.3	4.6	15	-0.06	0.95	Control - NO ₃	-48.1	13.8	16	-3.48	0.005				
Control - NO ₃ PO ₄	-13.4	4.6	15	-2.93	0.02	Control - NO ₃ PO ₄	-107.3	13.8	16	-7.77	< 0.001				
Control - PO ₄	5.8	4.6	15	1.28	0.26	Control - PO ₄	-4.5	15.1	16	-0.30	0.77				
NO ₃ - NO ₃ PO ₄	-13.1	4.3	15	-3.04	0.02	NO ₃ - NO ₃ PO ₄	-59.2	12.3	16	-4.80	0.00				
NO ₃ PO ₄ - PO ₄	19.3	4.3	15	4.47	0.003	NO ₃ PO ₄ - PO ₄	102.8	13.8	16	7.45	< 0.001				
NO ₃ - PO ₄	6.1	4.3	15	1.42	0.26	NO ₃ - PO ₄	43.6	13.8	16	3.16	0.01				
Phosphate uptake per AFDW															
<i>Sarcophyton glaucum</i>						<i>Turbinaria reniformis</i>					Comparison <i>S. glaucum</i> vs <i>T. reniformis</i> in control condition				
Condition	Df	Sum Sq	Mean Sq	F value	P value	Condition	Df	Sum Sq	Mean Sq	F value	P value	t	df	Welch two sample t-test	
Residuals	17	686.73	40.396	4.0547	0.024	Residuals	18	7001.7	389	15.9	< 0.001	1.45	5.48	p-value	
	Estimate	SE	Df	t.ratio	P value (fdr)		Estimate	SE	Df	t.ratio	P value (fdr)				
Control - NO ₃	1.6	4.1	17	0.40	0.98	Control - NO ₃	-11.9	12.7	18	-0.94	0.79				
Control - NO ₃ PO ₄	-6.1	4.1	17	-1.49	0.46	Control - NO ₃ PO ₄	-71.0	12.7	18	-5.58	< 0.001				
Control - PO ₄	-10.5	4.3	17	-2.46	0.10	Control - PO ₄	-57.0	12.7	18	-4.47	0.001				
NO ₃ - NO ₃ PO ₄	-7.7	3.7	17	-2.11	0.19	NO ₃ - NO ₃ PO ₄	-59.1	12.7	18	-5.19	< 0.001				
NO ₃ PO ₄ - PO ₄	-4.4	3.9	17	-1.14	0.67	NO ₃ PO ₄ - PO ₄	-14.1	12.7	18	1.24	0.61				
NO ₃ - PO ₄	-12.1	3.9	17	-3.15	0.03	NO ₃ - PO ₄	-45.1	12.7	18	-3.96	0.01				

Sarcophyton glaucum						Turbinaria reniformis					Comparison S. glaucum vs T. reniformis in control condition		
Photosystem efficiency Yield (Fv/Fm)													
Condition	Df	Sum Sq	Mean Sq	F value	P value	Condition	Df	Sum Sq	Mean Sq	F value	P value	Wilcoxon rank sum test	
Residuals	28	0.09	0.003	9.10	0.0002	Residuals	28	0.021	0.007	6.612	0.002	W	p-value
												44	0.23
	Estimate	SE	Df	t.ratio	P value (fdr)		Estimate	SE	Df	t.ratio	P value (fdr)		
Control - NO ₃	-0.06	0.03	28	-2.02	0.20	Control - NO ₃	0.05	0.02	28	3.33	0.01		
Control - NO ₃ PO ₄	-0.10	0.03	28	-3.49	0.01	Control - NO ₃ PO ₄	0.01	0.02	28	0.43	0.97		
Control - PO ₄	0.04	0.03	28	1.33	0.56	Control - PO ₄	-0.01	0.02	28	-0.87	0.82		
NO ₃ - NO ₃ PO ₄	-0.04	0.03	28	-1.47	0.47	NO ₃ - NO ₃ PO ₄	-0.05	0.02	28	-2.90	0.03		
NO ₃ PO ₄ - PO ₄	0.14	0.03	28	4.82	< 0.001	NO ₃ PO ₄ - PO ₄	-0.02	0.02	28	-1.29	0.58		
NO ₃ - PO ₄	0.10	0.03	28	3.35	0.01	NO ₃ - PO ₄	-0.07	0.02	28	-4.19	0.00		

Sarcophyton glaucum						Turbinaria reniformis					Comparison S. glaucum vs T. reniformis in control condition			
Carbohydrate per Ash free dry weight														
Condition	Df	Sum Sq	Mean Sq	F value	P value	Condition	Df	Sum Sq	Mean Sq	F value	P value	Welch two sample t-test		
Residuals	19	3.13	0.16	19.19	< 0.0001	Residuals	19	12.25	0.64	5.92	0.00	t	df	p-value
												2.29	6	0.06
	Estimate	SE	Df	t.ratio	P value (fdr)		Estimate	SE	Df	t.ratio	P value (fdr)			
Control - NO ₃	1.05	0.25	19	4.29	0.008	Control - NO ₃	-1.05	0.46	19	-2.27	0.14			
Control - NO ₃ PO ₄	-0.07	0.25	19	-0.26	0.79	Control - NO ₃ PO ₄	-1.66	0.49	19	-3.41	0.01			
Control - PO ₄	-0.70	0.25	19	-2.84	0.02	Control - PO ₄	-1.76	0.46	19	-3.80	0.01			
NO ₃ - NO ₃ PO ₄	-1.12	0.23	19	-4.77	0.004	NO ₃ - NO ₃ PO ₄	-0.60	0.49	19	-1.24	0.61			
NO ₃ PO ₄ - PO ₄	-0.63	0.23	19	-2.71	0.02	NO ₃ PO ₄ - PO ₄	-0.10	0.49	19	-0.21	1.00			
NO ₃ - PO ₄	-1.75	0.23	19	-7.48	< 0.001	NO ₃ - PO ₄	-0.71	0.46	19	-1.53	0.44			

Sarcophyton glaucum						Turbinaria reniformis					Comparison S. glaucum vs T. reniformis in control condition		
Lipid per Ash free dry weight													
Condition	Df	Sum Sq	Mean Sq	F value	P value	Condition	Df	Kruskal-Wallis Chi-squared		P value	Welch two sample t-test		
Residuals	20	352694	17635	0.89	0.46	Residuals	3	11.1		0.02	t	df	p-value
											7.77	5	0.001
							Estimate	SE	Df	t.ratio	P value (fdr)		
						Control - NO ₃	-4.88	3.33	20	-1.47	0.22		
						Control - NO ₃ PO ₄	-9.90	3.33	20	-2.98	0.03		
						Control - PO ₄	-0.31	3.33	20	-0.09	0.93		
						NO ₃ - NO ₃ PO ₄	-5.02	3.33	20	-1.51	0.22		
						NO ₃ PO ₄ - PO ₄	9.59	3.33	20	2.88	0.03		
						NO ₃ - PO ₄	4.56	3.33	20	1.37	0.22		

c. Elemental composition and stoichiometry

<i>Sarcophyton glaucum</i>				Carbon content per ash free dry weight <i>Turbinaria reniformis</i>				Comparison <i>S. glaucum</i> vs <i>T. reniformis</i> in control condition	
Condition	Df	Kruskal-Wallis Chi-squared	P value	Condition	Df	Kruskal-Wallis Chi-squared	P value	W	p-value
	3	2.27	0.52		3	10.70	0.01	16	0.03
				Estimate	SE	Df	t.ratio	P value (fdr)	
				Control - NO ₃	-10.79	5.013	12	-2.15	0.19
				Control - NO ₃ PO ₄	-15.12	5.013	12	-3.02	0.05
				Control - PO ₄	-22.72	5.013	12	-4.53	0.003
				NO ₃ - NO ₃ PO ₄	-4.329	5.013	12	-0.86	0.82
				NO ₃ PO ₄ - PO ₄	-7.599	5.013	12	-1.52	0.46
				NO ₃ - PO ₄	-11.927	5.013	12	-2.38	0.13

<i>Sarcophyton glaucum</i>				Nitrogen content per ash free dry weight <i>Turbinaria reniformis</i>				Comparison <i>S. glaucum</i> vs <i>T. reniformis</i> in control condition	
Condition	Df	Kruskal-Wallis Chi-squared	P value	Condition	Df	Kruskal-Wallis Chi-squared	P value	W	p-value
	3	1.74	0.63		3	8.63	0.03	16	0.03
				Estimate	SE	Df	t.ratio	P value (fdr)	
				Control - NO ₃	-2.72	0.89	12	-3.06	0.03
				Control - NO ₃ PO ₄	-2.36	0.89	12	-2.65	0.04
				Control - PO ₄	-3.93	0.89	12	-4.41	0.01
				NO ₃ - NO ₃ PO ₄	0.36	0.89	12	0.41	0.69
				NO ₃ PO ₄ - PO ₄	-1.57	0.89	12	-1.76	0.16
				NO ₃ - PO ₄	-1.21	0.89	12	-1.35	0.24

<i>Sarcophyton glaucum</i>				Carbon : Nitrogen ratio per ash free dry weight <i>Turbinaria reniformis</i>				Comparison <i>S. glaucum</i> vs <i>T. reniformis</i> in control condition	
Condition	Df	Kruskal-Wallis Chi-squared	P value	Condition	Df	Kruskal-Wallis Chi-squared	P value	W	p-value
	3	1.57	0.67		3	9.55	0.02	16	0.03
				Estimate	SE	Df	t.ratio	P value (fdr)	
				Control - NO ₃	1.46	0.51	12	2.88	0.08
				Control - NO ₃ PO ₄	0.34	0.51	12	0.67	0.52
				Control - PO ₄	0.76	0.51	12	1.50	0.29
				NO ₃ - NO ₃ PO ₄	-1.12	0.51	12	-2.21	0.14
				NO ₃ PO ₄ - PO ₄	0.42	0.51	12	0.83	0.51
				NO ₃ - PO ₄	-0.70	0.51	12	-1.37	0.29

Phosphorus content																																															
<i>Sarcophyton glaucum</i>			<i>Turbinaria reniformis</i>																																												
Condition	Df	Kruskal-Wallis Chi-squared	P value	Condition	P value																																										
Condition	3	0.71	0.87	3	0.15																																										
<table border="1"> <thead> <tr> <th colspan="3">Welch two sample t-test</th> </tr> <tr> <th>t</th> <th>df</th> <th>p-value</th> </tr> </thead> <tbody> <tr> <td>0.21</td> <td>3</td> <td>0.85</td> </tr> </tbody> </table>						Welch two sample t-test			t	df	p-value	0.21	3	0.85																																	
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t	df	p-value																																													
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Carbon : Phosphorus ratio per ash free dry weight																																															
<i>Sarcophyton glaucum</i>			<i>Turbinaria reniformis</i>																																												
Condition	Df	Sum Sq	Mean Sq	F value	P value																																										
Condition	3	15704	5235	0.07	0.97																																										
Residuals	12	855414	71284																																												
<table border="1"> <thead> <tr> <th colspan="3">Wilcoxon rank sum test</th> </tr> <tr> <th>W</th> <th colspan="2">p-value</th> </tr> </thead> <tbody> <tr> <td>16</td> <td colspan="2">0.029</td> </tr> </tbody> </table>						Wilcoxon rank sum test			W	p-value		16	0.029																																		
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	Estimate	SE	Df	t.ratio	P value (fdr)																																										
Control - NO ₃	-163.37	55.97	12	-2.92	0.05																																										
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Condition	Df	Kruskal-Wallis Chi-squared	P value	Condition	P value																																										
Condition	3	3.0221	0.3882	3	0.02																																										
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	Estimate	SE	Df	t.ratio	P value (fdr)																																										
Control - NO ₃	-35.74	9.5	12	-3.76	0.01																																										
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d. Dinoflagellate density and protein content

Dinoflagellate density

<i>Sarcophyton glaucum</i>						<i>Turbinaria reniformis</i>						Comparison <i>S. glaucum</i> vs <i>T. reniformis</i> in control condition		
Condition	Df	Sum Sq	Mean Sq	F value	P value	Condition	Df	Sum Sq	Mean Sq	F value	P value	t	df	p-value
Residuals	20	39234	1961.7			Residuals	19	0.22	7403			-1.66	6	0.14
	Estimate	SE	Df	t.ratio	P value (fdr)									
Control - NO ₃	53.61	49.68	20	1.08	0.29	Control - NO ₃	53.61	49.68	20	1.08	0.29			
Control - NO ₃ PO ₄	108.84	49.68	20	2.19	0.06	Control - NO ₃ PO ₄	108.84	49.68	20	2.19	0.06			
Control - PO ₄	224.14	49.68	20	4.51	0.001	Control - PO ₄	224.14	49.68	20	4.51	0.001			
NO ₃ - NO ₃ PO ₄	55.23	49.68	20	1.11	0.29	NO ₃ - NO ₃ PO ₄	55.23	49.68	20	1.11	0.29			
NO ₃ PO ₄ - PO ₄	115.3	49.68	20	2.32	0.06	NO ₃ PO ₄ - PO ₄	115.3	49.68	20	2.32	0.06			
NO ₃ - PO ₄	170.53	49.68	20	3.43	0.01	NO ₃ - PO ₄	170.53	49.68	20	3.43	0.01			

Protein holobiont content

<i>Sarcophyton glaucum</i>						<i>Turbinaria reniformis</i>						Comparison <i>S. glaucum</i> vs <i>T. reniformis</i> in control condition	
Condition	Df	Sum Sq	Mean Sq	F value	P value	Condition	Df	Sum Sq	Mean Sq	F value	P value	W	p-value
Residuals	20	6789.9	339.49	5.6226	0.005809	Residuals	19	2789.3	146.81	4.43	0.02	0	0.002
	Estimate	SE	Df	t.ratio	P value (fdr)		Estimate	SE	Df	t.ratio	P value (fdr)		
Control - NO ₃	6.99	10.6	20	0.66	0.52	Control - NO ₃	-8.7	7	19	-1.24	0.34		
Control - NO ₃ PO ₄	-9.94	10.6	20	-0.93	0.43	Control - NO ₃ PO ₄	-6.56	7	19	-0.94	0.43		
Control - PO ₄	-33.84	10.6	20	-3.18	0.01	Control - PO ₄	15.8	7.34	19	2.15	0.09		
NO ₃ - NO ₃ PO ₄	-16.93	10.6	20	-1.59	0.19	NO ₃ - NO ₃ PO ₄	2.14	7	19	0.31	0.76		
NO ₃ PO ₄ - PO ₄	-23.90	10.6	20	-2.25	0.07	NO ₃ PO ₄ - PO ₄	22.36	7.34	19	3.05	0.02		
NO ₃ - PO ₄	-40.23	10.6	20	-3.84	0.01	NO ₃ - PO ₄	24.5	7.34	19	3.34	0.02		

IV Materials and Methods

Table S5. Example of nubbin repartition for the different measurements in one condition and one coral species

We used 6 nubbins per condition/species, with three nubbins from each aquarium

Measurement 1: Nutrient uptake rate, Photosynthesis rates, PAM, chlorophyll content, dinoflagellate density and protein content

Measurement 2: Lipid and carbohydrate content

Measurement 3: Lipid peroxidation (LPO) and non-enzymatic total antioxidant capacity (NETAC)

Measurement 4: Carbon, nitrogen and phosphorus content

	Nubbin number (each from a different colony)											
	1	2	3	4	5	6	7	8	9	10	11	12
Aquarium 1	1	3	4	2	3	1		4	2	1	3	2
Aquarium 2	2	4	1	3		3	2	1	4	3	2	1

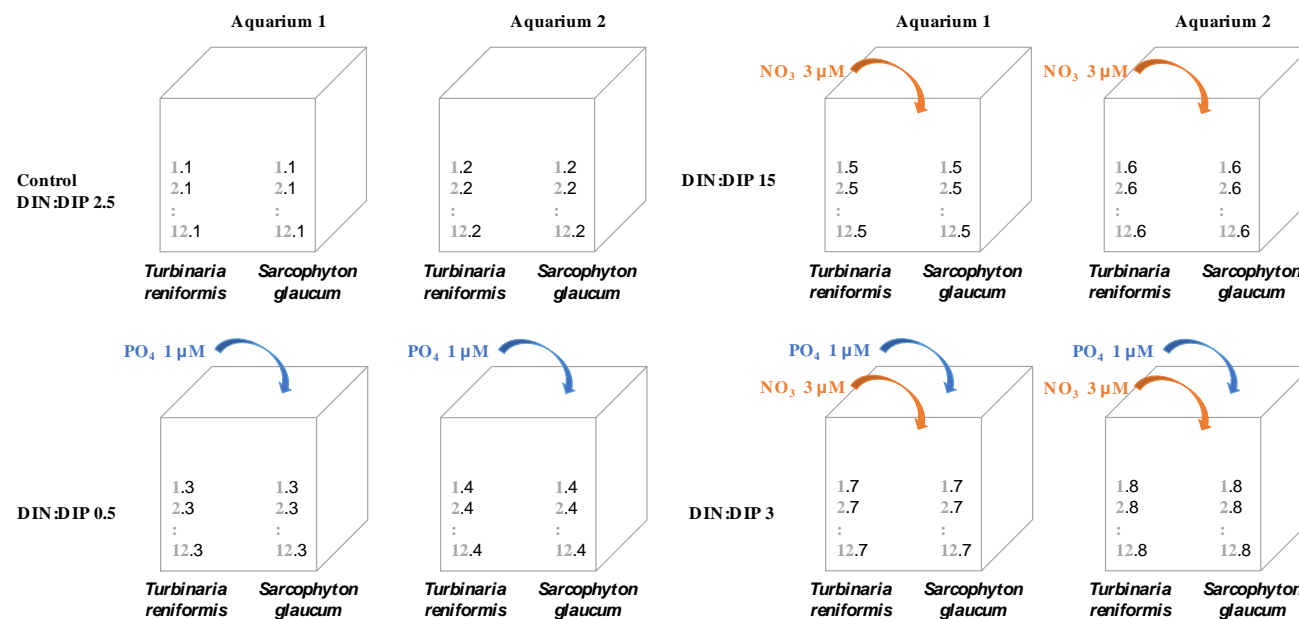


Figure S1. Summary of the experimental design and repartition of nubbins across treatment and aquaria.

Each aquarium has 24 nubbins in total, with 12 nubbins per species (*Turbinaria reniformis* and *Sarcophyton glaucum*). The numbers from 1 to 12 in grey represent the ID number of the mother colony. The numbers from 1 to 8 in black represent the id number of the nubbin cut from the mother colony. In each aquaria there is one nubbin from each colony.

V Appendix III – Supplementary material Chapter 3

Publication: Nutrient starvation and nitrate pollution impairs the assimilation of dissolved organic phosphorus in coral-Symbiodiniaceae symbiosis

V.1. Material and Method - Preliminary experiments on the best concentration of pNpp and the best incubation time needed for the phosphatase measurements.

In a first experiment, we assessed the extracellular alkaline phosphatase activity (APA) during a 3-hour assay with five different initial concentrations of pNpp: 20, 50, 100, 150 and 250 μM . For each concentration three nubbins were placed in individual beakers, with three additional beakers containing seawater without coral nubbin, and left to acclimate for 30 min before starting the phosphatase assay as described in the Material and Method section.

In a second experiment, we assessed the extracellular alkaline phosphatase activity (APA) every 2 hours for 8 hours with the previously determined pNpp concentration of 250 μM . To this end, five nubbins of *Stylophora pistillata* were placed in individual beakers and left to acclimate for 30 min before adding 250 μM of pNpp and starting the phosphatase assay.

The results of these two preliminary experiments are reported in Figure S1. APA was linearly correlated to the pNpp concentration added to the beakers (Fig S1a) and to the incubation time (Fig S1b).

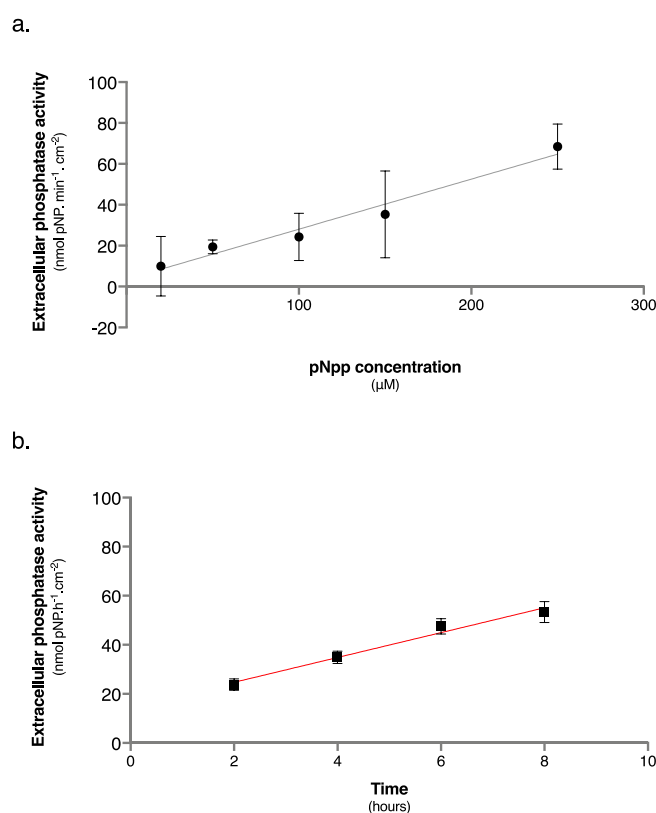


Figure S1: Outcomes of the preliminary tests to optimize extracellular alkaline phosphatase activity measurement.

Evolution of the extracellular phosphatase activity, represented as the pNp production rate, depending on the initial pNpp concentration (a) and incubation time (b).

V.2. Results

V.2.a. Testing the phosphatase activity in the mucus, coral alone and coral holobiont.

As reported in the results, there was no phosphatase activity in the mucus, while APA was detected in the coral and coral holobiont (Fig S2, Table S1).

Table S1: Outcome of the statistical tests on the production site of extracellular activity

	Df	Sum Sq	Mean Sq	F value	P value
Condition	2	6.0157	3.008	28.079	< 0.0001
Residuals	12	1.2855	0.107		

Condition-pairwise	Estimate	SE	Df	t.ratio	p value
Corail - Mucus	2.039	0.608	19	3.355	0.0132
Corail - Corail + Mucus	1.75	0.608	19	2.881	0.0192
Corail + Mucus - Mucus	-0.167	0.608	19	-0.275	0.7864

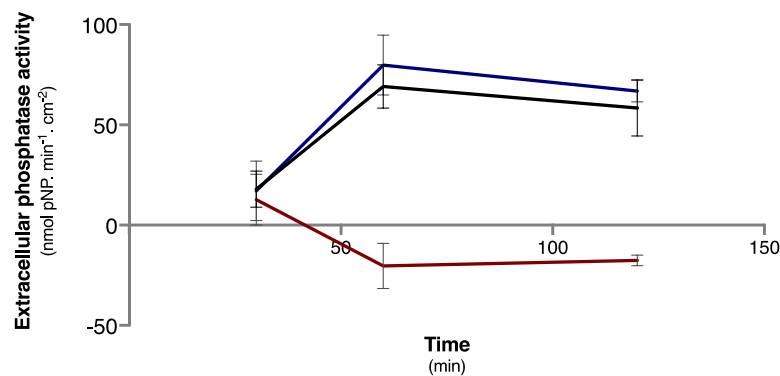


Figure S2. Production site of extracellular phosphatase in the coral

Expression of the extracellular phosphatase activity in the coral holobiont (black), the coral alone (blue) and the mucus alone (red)

V.2.b. Effect of nutrient depletion on phosphatase activity

Table S2. Outcome of the statistical tests performed to assess the differences of nutrient depletion in APA and AcPA.

a) Outcome of the statistical differences between the controls

Controls : Extracellular activity under a 1, 3 or 5 days of depletion

	Df	Sum Sq	Mean Sq	F value	P value
Condition	2	1114.9	557.47	3.118	0.063
Residuals	23	4112.6	178.81		

Controls : Intracellular activity under a 1, 3 or 5 days of depletion

Host fraction

Acid phosphatase activity

	Df	Sum Sq	Mean Sq	F value	P value
Condition	2	926.7	463.37	0.79	0.48
Residuals	11	6440.2	585.47		

Alkaline phosphatase activity

	Df	Sum Sq	Mean Sq	F value	P value
Condition	2	5055.1	2527.54	2.67	0.11
Residuals	11	10431.4	948.310		

Dinoflagellate fraction

	Df	Sum Sq	Mean Sq	F value	P value
Condition	2	1446.4	723.20	2.39	0.14
Residuals	11	3324.1	302.19		

	Df	Sum Sq	Mean Sq	F value	P value
Condition	2	112.22	56.11	0.20	0.82
Residuals	11	3094.71	281.33		

b) Outcome of the statistical tests comparing the impact of 1, 3 and 5 days of nutrient depletion on APA and AcPA

Extracellular activity of the alkaline phosphatase enzymes with nutrient depletion		
	W	p value
Depleted 1 day	16	0.0045
Depleted 3 days	17	0.47
Depleted 5 days	100	< 0.00001

Intracellular activity of phosphatase enzymes with nutrient depletion					
Host fraction			Dinoflagellate fraction		
Acid phosphatase activity			Alkaline phosphatase activity		
	W	p value		W	p value
Depleted 1 day	22	0.028	Depleted 1 day	4	0.048
Depleted 3 days	9	0.443	Depleted 3 days	16	0.015
Depleted 5 days	25	0.004	Depleted 5 days	25	0.004

Alkaline phosphatase activity			Alkaline phosphatase activity		
	W	p value		W	p value
Depleted 1 day	1	0.008	Depleted 1 day	15	0.345
Depleted 3 days	2	0.057	Depleted 3 days	3	0.100
Depleted 5 days	6	0.111	Depleted 5 days	15	0.345

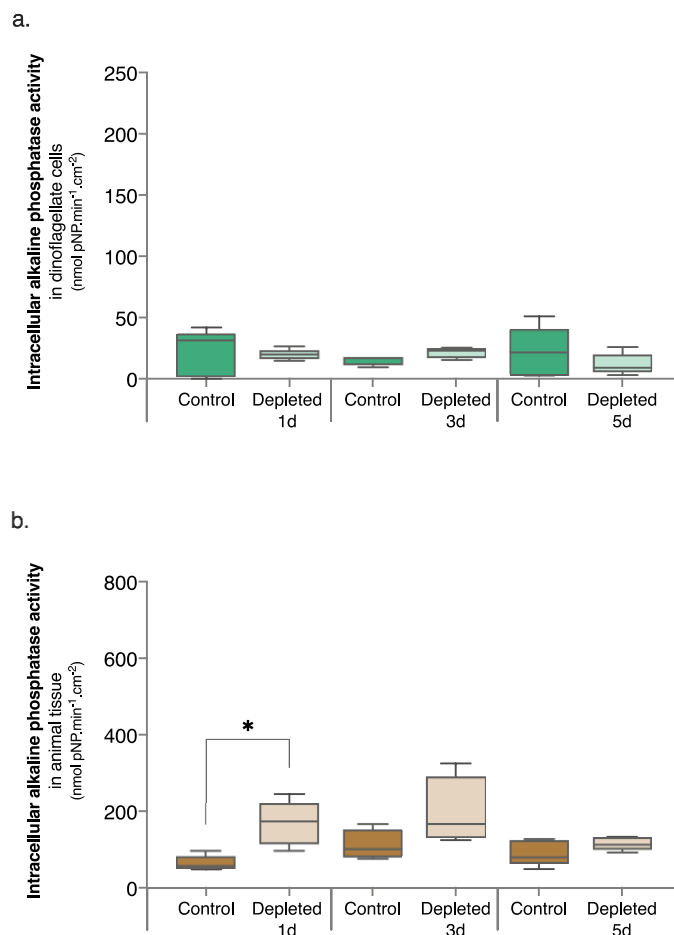


Figure S3. Activity of the intracellular alkaline phosphatases under nutrient depletion condition

Boxplots show the activity of intracellular alkaline phosphatase in dinoflagellate cells in green (a.) and in animal tissue in brown (b.) normalised to skeletal surface area. Measurements were taken after one, three and five days either under natural seawater (**Control**, darker box) or under nutrient deficiency (**Depleted**, lighter box, undetected amount of dissolved inorganic phosphorus and nitrogen). The box delimits the first and third quartile, the black bar represents the median and whiskers represent minimum and maximum values, intracellular $n = 5$. Statistically significant differences are marked with an asterisk.

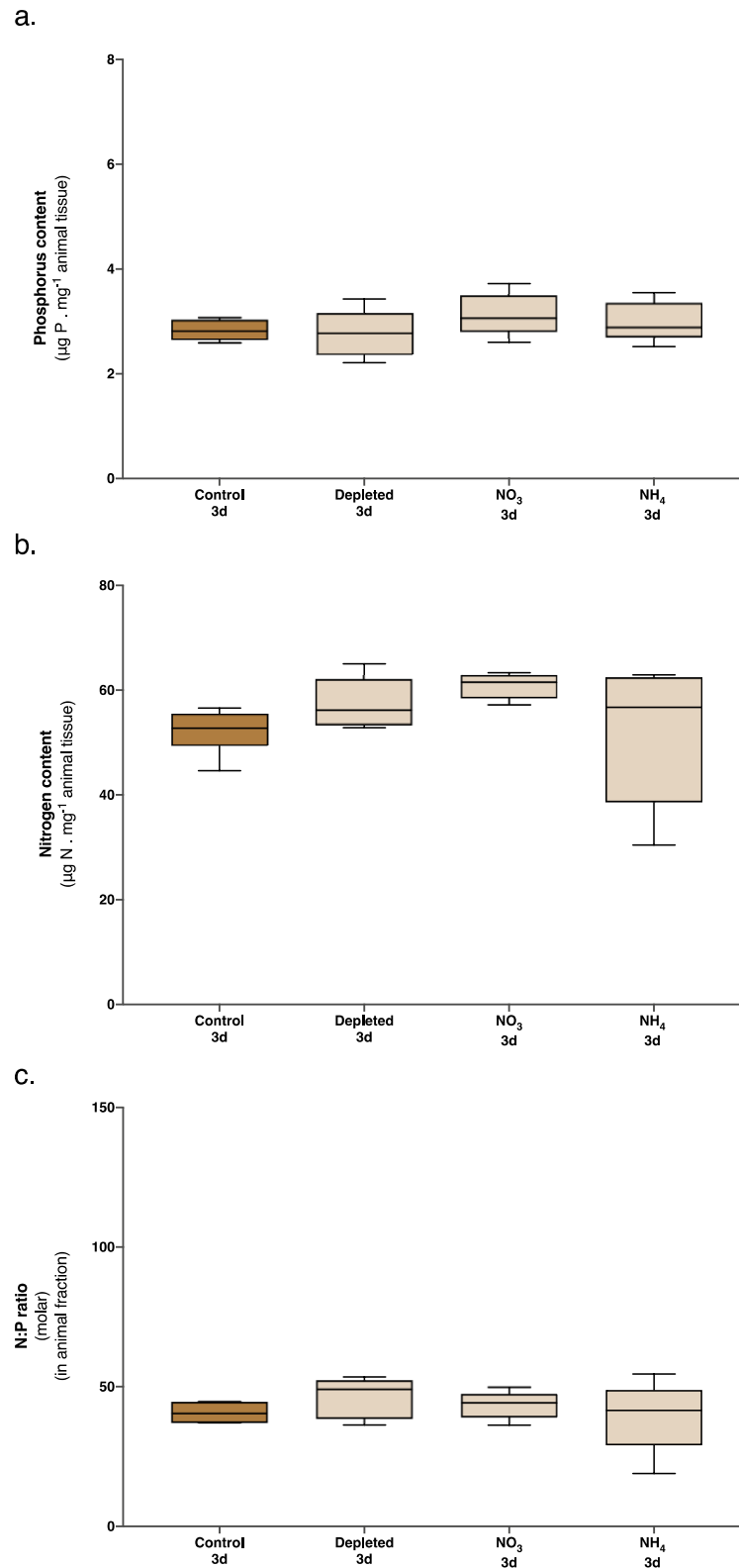


Figure S4. **Composition of the animal tissue in phosphorus and nitrogen and its nitrogen to phosphorus ratio** Boxplots show the animal tissue phosphorus (a.) and nitrogen (b.) content and their nitrogen-phosphorus ratio (c.) under natural seawater (**Control**, dark brown), nutrient depletion (**Depleted**, undetected amount of dissolved inorganic phosphorus and nitrogen), nitrate enrichment (NO_3 , at $2 \mu\text{M}$) and ammonium enrichment (NH_4 , at $2 \mu\text{M}$). Measurements were taken after three days under the different experimental conditions. The box delimits the first and third quartile, the black bar represents the median and whiskers represent minimum and maximum values, $n = 6$.

Table S3. Outcome of the statistical test on the animal tissue and dinoflagellate cells elemental composition in carbon, nitrogen and phosphorus and their associated elemental ratios, CN, CP and NP

Phosphorus content											
Host fraction						Dinoflagellate fraction					
	Df	Sum Sq	Mean Sq	F value	P value	Condition	Df	Sum Sq	Mean Sq	F value	P value
Condition	3	0.43	0.14	1.06	0.39	Condition	3	18.99	6.33	6.29	0.004
Residuals	20	2.72	0.14			Residuals	19	19.13	1.01		
Condition-pairwise	Estimate	SE	Df	t.ratio	p value						
Control - Depleted	2.04	0.61	19	3.36	0.013						
Control - Nitrate	1.75	0.61	19	2.88	0.019						
Control - Ammonium	-0.17	0.61	19	-0.28	0.79						
Nitrate - Ammonium	1.58	0.58	19	2.73	0.02						
Ammonium - Depleted	1.87	0.58	19	3.23	0.013						
Nitrate - Depleted	-0.29	0.58	19	-0.50	0.75						
Nitrogen content											
Host fraction						Dinoflagellate fraction					
	Df	Sum Sq	Mean Sq	F value	P value	Condition	Df	Sum Sq	Mean Sq	F value	P value
Condition	3	20373	115.94	2.1402	0.13	Condition	3	1451.7	483.91	7.493	0.0016
Residuals	20	1083.46	54.173			Residuals	19	1227	64.58		
Condition-pairwise	Estimate	SE	Df	t.ratio	p value						
Control - Depleted	9.82	4.87	19	2.02	0.09						
Control - Nitrate	-5.35	4.64	19	-1.15	0.26						
Control - Ammonium	12.55	4.64	19	2.71	0.028						
Nitrate - Ammonium	7.2	4.64	19	1.55	0.16						
Ammonium - Depleted	22.38	4.87	19	4.6	0.001						
Nitrate - Depleted	-15.17	4.87	19	-3.12	0.017						
Carbon content											
Host fraction						Dinoflagellate fraction					
	Df	Sum Sq	Mean Sq	F value	P value	Condition	Df	Sum Sq	Mean Sq	F value	P value
Condition	3	20373	6790.90	5.01	0.0095	Condition	3	17144.5	5714.8	18.075	< 0.0001
Residuals	20	27114	1355.70			Residuals	19	6007.3	316.200		
Condition-pairwise	Estimate	SE	Df	t.ratio	p value	Condition-pairwise	Estimate	SE	Df	t.ratio	p value
Control - Depleted	-55.75	21.3	20	-2.62	0.032	Control - Depleted	-72.7	10.8	19	-6.75	< 0.0001
Control - Nitrate	-50.56	21.3	20	-2.38	0.041	Control - Nitrate	-58.5	10.3	19	-5.69	0.0001
Control - Ammonium	-9.26	21.3	20	-0.44	0.80	Control - Ammonium	34.8	10.3	19	3.39	0.0046
Nitrate - Ammonium	-59.82	21.3	20	-2.81	0.032	Nitrate - Ammonium	-23.7	10.3	19	-6.75	< 0.0001
Ammonium - Depleted	-65.01	21.3	20	-3.06	0.032	Ammonium - Depleted	-37.9	10.8	19	-3.52	0.0046
Nitrate - Depleted	5.19	21.3	20	0.24	0.81	Nitrate - Depleted	14.2	10.8	19	1.32	0.20
Carbon to nitrogen ratio											
Host fraction						Dinoflagellate fraction					
	Df	Sum Sq	Mean Sq	F value	P value	Condition	Df	Sum Sq	Mean Sq	F value	P value
Condition	3	1.85	0.615	10.81	0.0002	Condition	3	14.86	4.95	10.04	0.0003
Residuals	20	1.14	0.057			Residuals	19	9.38	0.49		
Condition-pairwise	Estimate	SE	Df	t.ratio	p value	Condition-pairwise	Estimate	SE	Df	t.ratio	p value
Control - Depleted	-0.585	0.14	20	-4.25	0.0012	Control - Depleted	-1.88	0.43	19	-4.42	0.0009
Control - Nitrate	-0.148	0.14	20	-1.08	0.29	Control - Nitrate	-0.47	0.41	19	-1.16	0.31
Control - Ammonium	-0.16	0.14	20	-1.16	0.29	Control - Ammonium	-0.30	0.41	19	-0.75	0.46
Nitrate - Ammonium	-0.308	0.14	20	-2.24	0.06	Nitrate - Ammonium	-0.78	0.41	19	-1.91	0.11
Ammonium - Depleted	-0.745	0.14	20	-5.41	0.0002	Ammonium - Depleted	-2.18	0.43	19	-5.13	0.0004
Nitrate - Depleted	0.437	0.14	20	3.17	0.0096	Nitrate - Depleted	1.41	0.43	19	3.31	0.007
Carbon to phosphorus ratio											
Host fraction						Dinoflagellate fraction					
	Df	Sum Sq	Mean Sq	F value	P value	Condition	Df	Sum Sq	Mean Sq	F value	P value
Condition	3	19190	6396.50	3.3745	0.039	Condition	3	358541	119514.0	5.8252	0.0058
Residuals	20	37911	1895.50			Residuals	18	369299	20517.00		
Condition-pairwise	Estimate	SE	Df	t.ratio	p value	Condition-pairwise	Estimate	SE	Df	t.ratio	p value
Control - Depleted	-59.6	25.1	20	-2.37	0.08	Control - Depleted	-329.3	90.6	18	-3.64	0.01
Control - Nitrate	-21.8	25.1	20	-0.87	0.47	Control - Nitrate	-200.3	86.7	18	-2.31	0.066
Control - Ammonium	-15.7	25.1	20	-0.63	0.54	Control - Ammonium	40.4	86.7	18	0.47	0.65
Nitrate - Ammonium	-37.5	25.1	20	-1.49	0.23	Nitrate - Ammonium	-159.9	82.7	18	-1.93	0.10
Ammonium - Depleted	-75.3	25.1	20	-3.00	0.043	Ammonium - Depleted	-288.9	86.7	18	-3.331	0.01
Nitrate - Depleted	37.9	25.1	20	1.51	0.23	Nitrate - Depleted	129.1	86.7	18	1.488	0.18

Nitrogen to phosphorus ratio											
Host fraction						Dinoflagellate fraction					
Condition	Df	Sum Sq	Mean Sq	F value	P value	Condition	Df	Sum Sq	Mean Sq	F value	P value
Residuals	20	1200.88	60.044	1.0335	0.399	Residuals	17	4382.8	257.81	4.3036	0.01972
Condition-pairwise		Estimate	SE	Df	t.ratio	p value					
Control - Depleted		-23.98	10.15	17	-2.361	0.061					
Control - Nitrate		-32.85	10.15	17	-3.234	0.029					
Control - Ammonium		8.8	9.72	17	0.905	0.39					
Nitrate - Ammonium		-24.05	9.72	17	-2.473	0.061					
Ammonium - Depleted		-15.18	9.72	17	-1.562	0.21					
Nitrate - Depleted		-8.87	10.15	17	-0.873	0.39					

V.2.c. *Effect of long-term nitrogen enrichment on the phosphatase activity*

Table S4. **Outcome of the statistical tests on the effect of ammonium enrichment on phosphatase activity**

Comparisons after 1 and 3 days of ammonium enrichment at 2 μ M were all done with the control condition

a) Outcome of the statistical differences between the controls

Controls : Extracellular activity under a 1 or 3 days of ammonium enrichment						
	t	df	P value			
	2.2734	7.1428	0.056			
Controls : Intracellular activity under a 1 or 3 days of ammonium enrichment						
Host fraction			Dinoflagellate fraction			
Acid phosphatase activity						
t	df	P value	t	df	P value	
-1.8623	5.65	0.115	-1.8237	5.5661	0.122	
Alkaline phosphatase activity						
t	df	P value	t	df	P value	
-8.5958	4.05	0.001	1.1688	5.958	0.287	

b) Outcome of the statistical tests comparing the impact of 1 and 3 days of ammonium enrichment on APA and AcPA

Extracellular activity of the alkaline phosphatase enzymes with ammonium enrichment					
	W	p value			
Ammonium 1 day	0	0.004			
Ammonium 3 days	1	0.008			
Intracellular activity of phosphatase enzymes with ammonium enrichment					
Host fraction			Dinoflagellate fraction		
Acid phosphatase activity					
	W	p value			
Ammonium 1 day	14	0.057	Ammonium 1 day	4	0.171
Ammonium 3 days	16	0.014	Ammonium 3 days	9	0.443
Alkaline phosphatase activity					
	W	p value			
Ammonium 1 day	9	0.443	Ammonium 1 day	3	0.100
Ammonium 3 days	14	0.057	Ammonium 3 days	16	0.014

Table S5. Outcome of the statistical tests on the effect of nitrate enrichment on phosphatase activity
Comparisons after 1 and 3 days of nitrate enrichment at 2 μ M were all done with the control condition

a) Outcome of the statistical differences between the controls

Controls : Extracellular activity under a 1 or 3 days of nitrate enrichment						
	t	df	P value			
	0.4409	7.7581	0.671			
Controls : Intracellular activity under a 1 or 3 days of nitrate enrichment						
Host fraction			Dinoflagellate fraction			
Acid phosphatase activity						
	t	df	P value	t	df	P value
	-0.9025	5.0453	0.408	1.3079	5.915	0.239
Alkaline phosphatase activity						
	t	df	P value	t	df	P value
	-0.69762	5.9768	0.512	1.7005	3.3898	0.177

b) Outcome of the statistical tests comparing the impact of 1 and 3 days of nitrate enrichment on APA and AcPA

Extracellular activity of the alkaline phosphatase enzymes with nitrate enrichment					
	W	p value			
Nitrate 1 day	20	0.075			
Nitrate 3 days	17	0.210			
Intracellular activity of phosphatase enzymes with nitrate enrichment					
Host fraction		Dinoflagellate fraction			
Acid phosphatase activity					
	W	p value			
Nitrate 1 day	11	0.243	Nitrate 1 day	10	0.343
Nitrate 3 days	16	0.014	Nitrate 3 days	0	0.014
Alkaline phosphatase activity					
	W	p value			
Nitrate 1 day	6	0.331	Nitrate 1 day	5	0.500
Nitrate 3 days	12	0.171	Nitrate 3 days	7	0.443

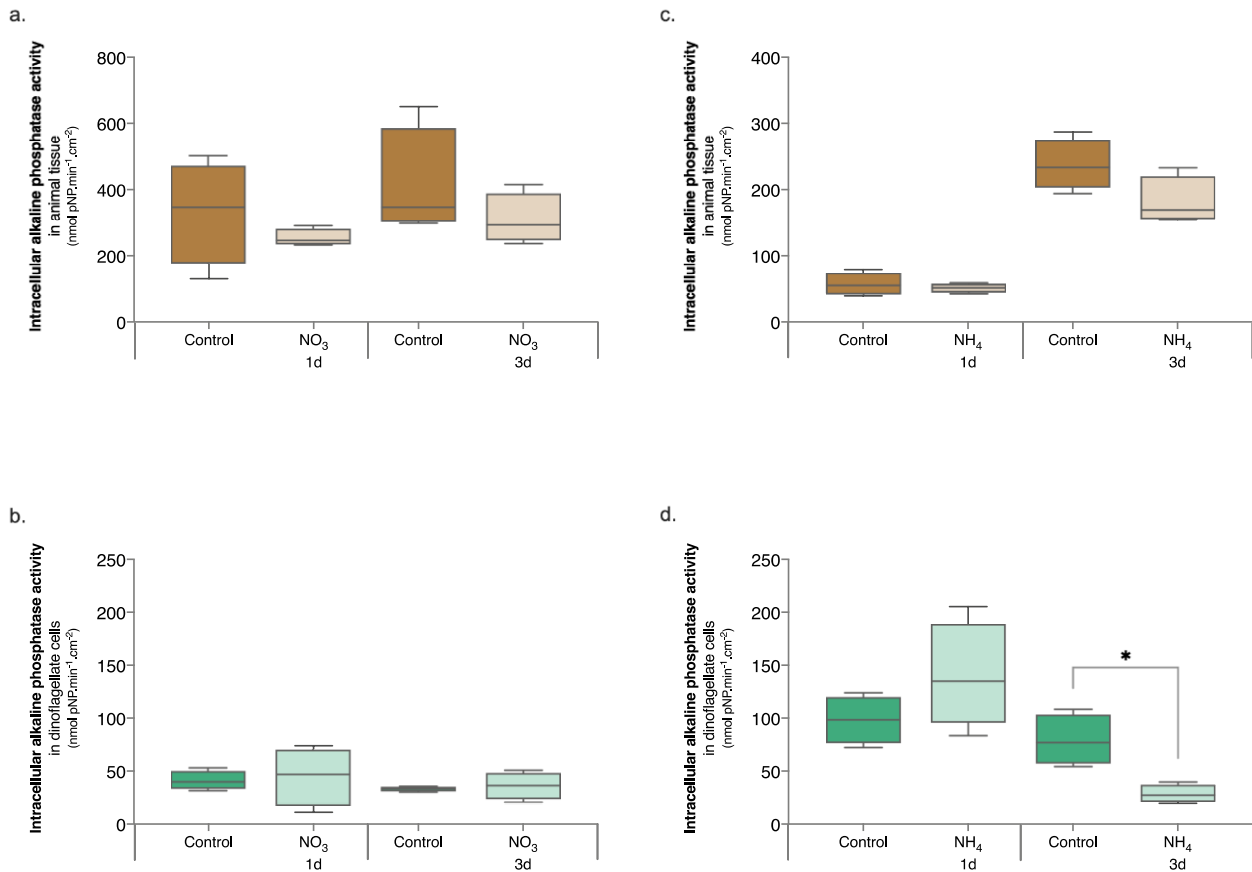


Figure S5. Activity of the intracellular alkaline phosphatases under nitrogen enrichment

Boxplots show the activity of intracellular alkaline phosphatase under nitrate enrichment in the animal tissue in brown (a.) and in the dinoflagellate cells in green (b.) and under ammonium enrichment in the animal tissue in brown (c.) and in the dinoflagellate cells in green (d.) normalised to skeletal surface area.

Measurements were taken after one and three days after enrichment. The box delimits the first and third quartile, the black bar represents the median and whiskers represent minimum and maximum values, intracellular n = 5. Statistically significant differences are marked with an asterisk.

V.2.d. Effect of a nitrogen pulse on phosphatase activity

Table S6. Outcome of the statistical tests on the effect of a pulse of nitrogen in nutrient-depleted seawater on phosphatase activity

Comparisons after a pulse of nitrogen at 2 μ M of nitrate or ammonium were all done with the control condition

Extracellular activity of the alkaline phosphatase enzymes with a pulse of nitrogen in nutrient depleted water				
	W	p value		
Pulse of nitrate	82	0.007		
Pulse of ammonium	25	0.032		
Intracellular activity of phosphatase enzymes with a pulse of nitrogen in nutrient depleted water				
	Host fraction		Dinoflagellate fraction	
Acid phosphatase activity	W	p value	W	p value
Pulse of nitrate	25	0.004	25	0.004
Pulse of ammonium	1	0.008	25	0.004
Alkaline phosphatase activity	W	p value	W	p value
Pulse of nitrate	25	0.004	0	0.004
Pulse of ammonium	25	0.004	0	0.004

VI Appendix IV – Supplementary material Chapter 4

Publication: **Desert dust deposition brings essential macro and micronutrients to Red Sea corals**

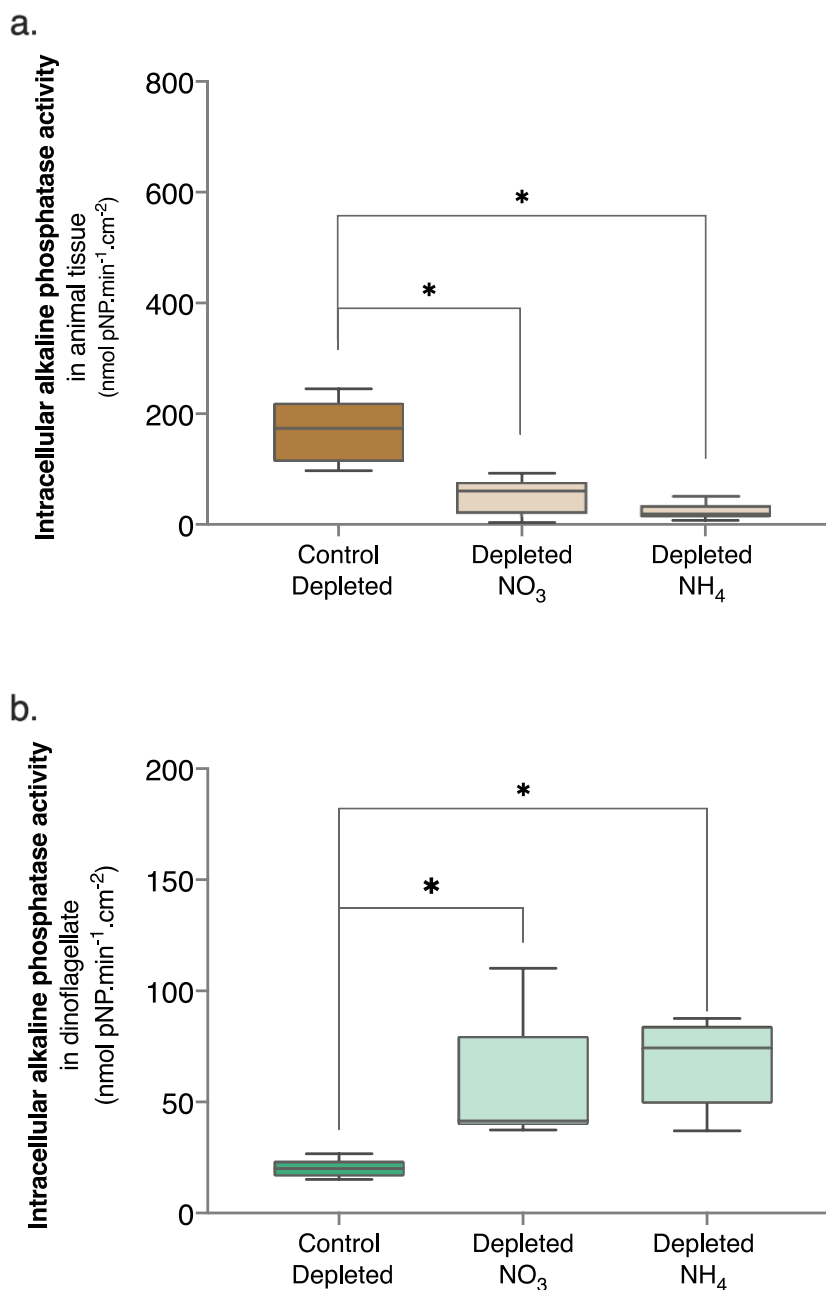


Figure S6. Activity of the intracellular alkaline phosphatases under a nitrogen pulse in nutrient-depleted seawater Boxplots show the activity of intracellular alkaline phosphatase in the animal tissue in brown (a.) and in the dinoflagellate cells in green (b.) normalised to skeletal surface area in nutrient-depleted without enrichment (**Control Depleted**), or with a pulse of nitrate (**Depleted NO₃**) or ammonium (**Depleted NH₄**).

The box delimits the first and third quartile, the black bar represents the median and whiskers represent minimum and maximum values, intracellular n = 6. Statistically significant differences are marked with an asterisk.

VI.1. Material and method

Stable isotope analysis and C N content

24 nubbins- 6 per conditions (from different colonies and 2 per aquaria), were snap-frozen in liquid nitrogen then kept at -80 °C for later analysis. Prior to the analyses, nubbins were thawed and the tissues were separated from the skeleton using a Water-Pick and 10-15 mL of Milli Q water. Samples were then centrifugated at 5530 g for 15 min at 4 °C to separate the animal tissue (supernatant) from the symbionts (pellet). The symbiont pellet was washed several times with 3 mL of Milli Q water as described above. Animal and symbiont fractions were frozen at -80 °C, before being freeze-dried using the Alpha 2-4 LD plus Freeze drier (Christ Martin Scientific, UK) and sent for analysis. The C and N content as well as the $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ analyses were done at the Marine Biological Laboratory, Stable Isotope Laboratory, University of Chicago, USA on a Europa 20-20 Continuous Flow-Isotope Ratio Mass Spectrometry (CF-IRMS) with the Europa ANCA-SL elemental analyser (PDZ, Europa, UK).

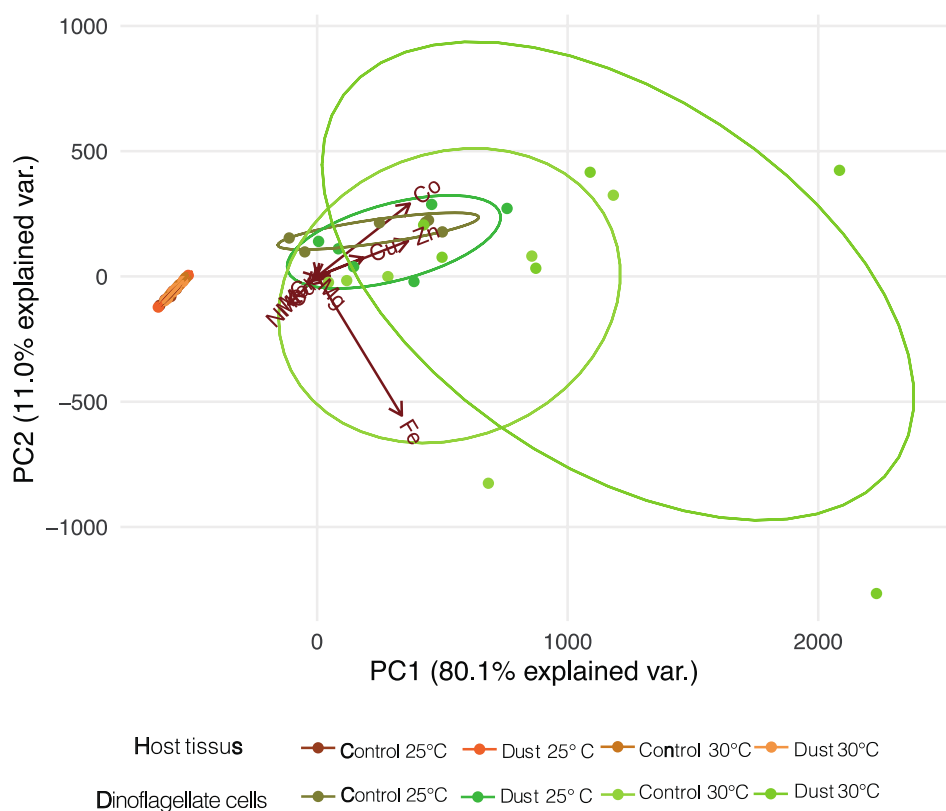


Figure S7. **Principal Component Analysis of the metal content in the host and dinoflagellate fraction**

PCA of the metal content (Mg: magnesium, Cu: copper, Co: Cobalt, Zn: Zinc, Fe: Iron, Ni: nickel, Mo: molybdenum, Cd: Cadmium, Cr: Chromium, Mn: Manganese) in the host tissues (orange) and dinoflagellates cells (green) under different enrichment conditions.

Control 25°C – Control 30°C: control corals maintained at 25°C or 30°C without aerial dust deposition, **Dust 25°C – Dust 30°C:** corals maintained at 25°C or 30°C supplied with aerial dust at 1mg.L⁻¹. 6 nubbins were used in each condition.

VI.2. Results

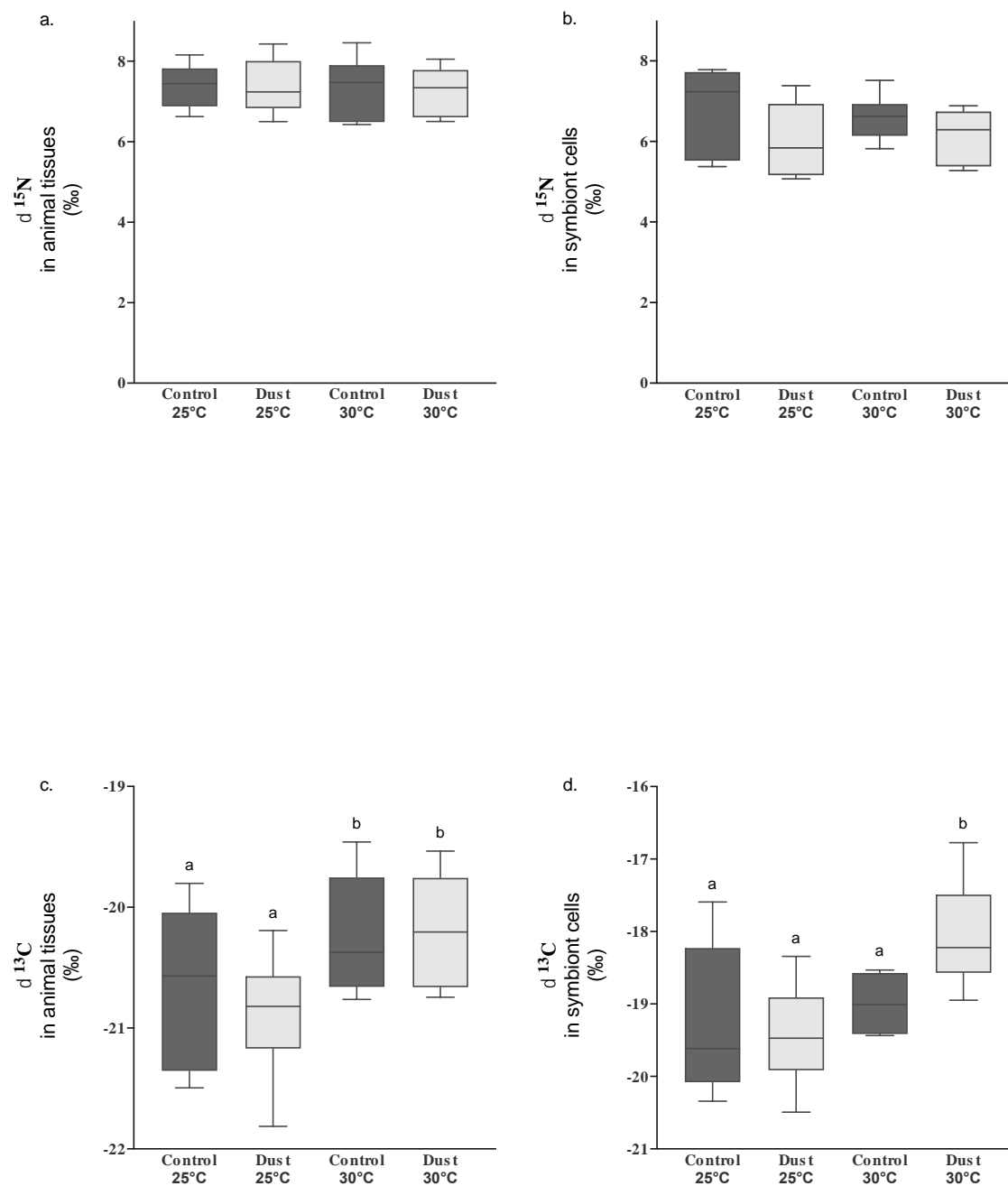


Figure S2. Stable isotope value of *S. pistillata*

Boxplots represent the $\delta^{15}\text{N}$ values of the coral animal tissue (a) and the dinoflagellate cells (b) and the $\delta^{13}\text{C}$ values of the coral animal tissue (c) and the dinoflagellate cells (d) under different conditions of temperature and dust enrichment

Control 25°C – Control 30°C: control corals maintained at 25°C or 30°C without aerial dust deposition, **Dust 25°C – Dust 30°C:** corals maintained at 25°C or 30°C and supplied with aerial dust at $1\text{mg}\cdot\text{L}^{-1}$

The box delimits the 1st and 3rd quartile, the black bar represents the median and whiskers represents min and max, $n=6$. Statistically significant differences are represented by different letters (a or b)

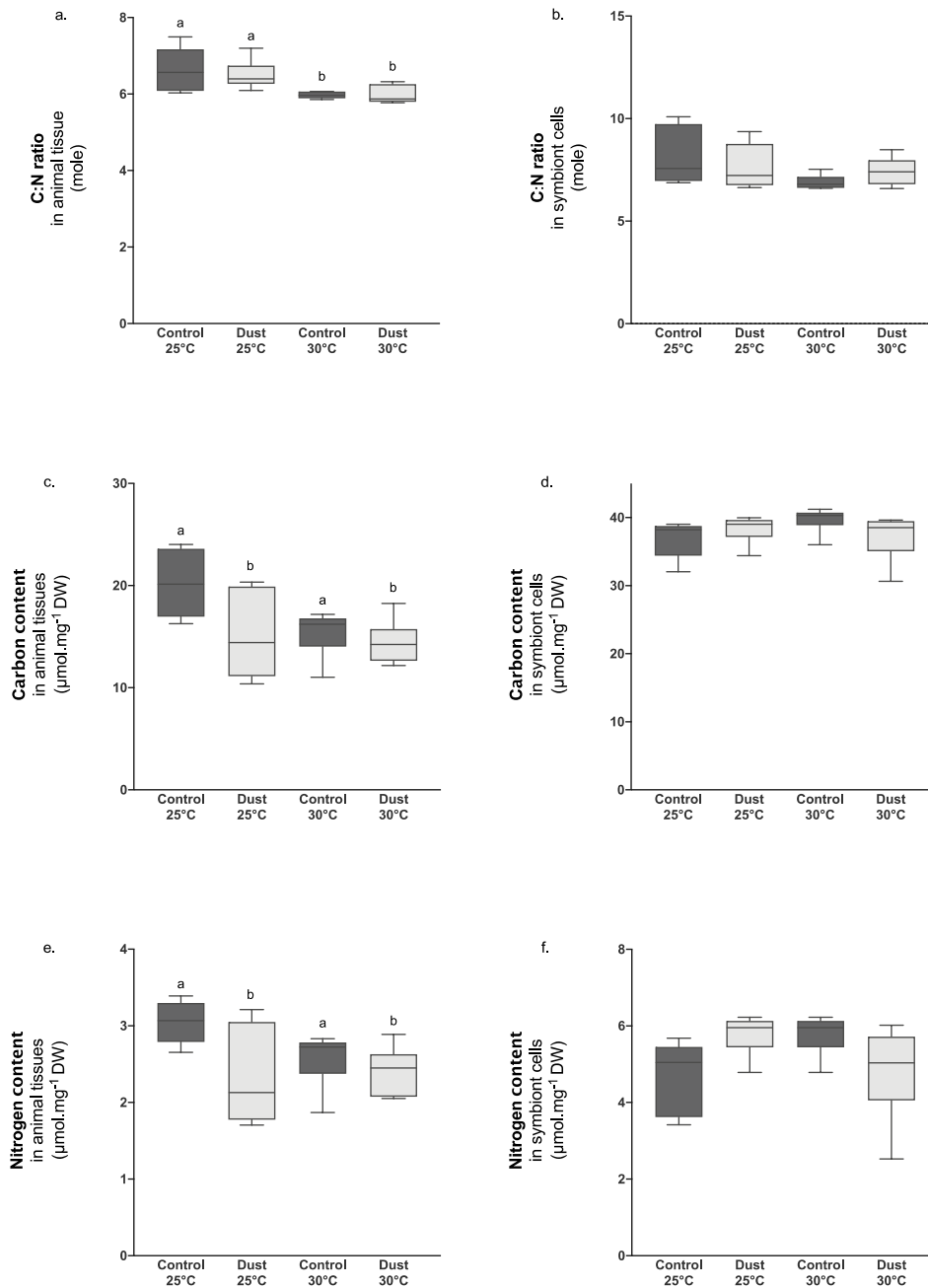


Figure S3. Carbon (C) and Nitrogen (N) content of *S. pistillata*

Values of the molar C:N ratio in the animal tissue (a) and the dinoflagellates cells (b)

Carbon content in the animal tissue (c) and the dinoflagellates cells (d)

Nitrogen content in the animal tissue (e) and the dinoflagellate cells (f) under different conditions of temperature and dust enrichment

Control 25°C – Control 30°C: control corals maintained at 25°C or 30°C without aerial dust deposition, **Dust 25°C – Dust 30°C:** corals maintained at 25°C or 30°C supplied with aerial dust at $1\text{mg}\cdot\text{L}^{-1}$

The box delimits the 1st and 3rd quartile, the black bar represents the median and whiskers represents min and max, $n=6$.

Statistically significant differences are represented by different letters (a or b)

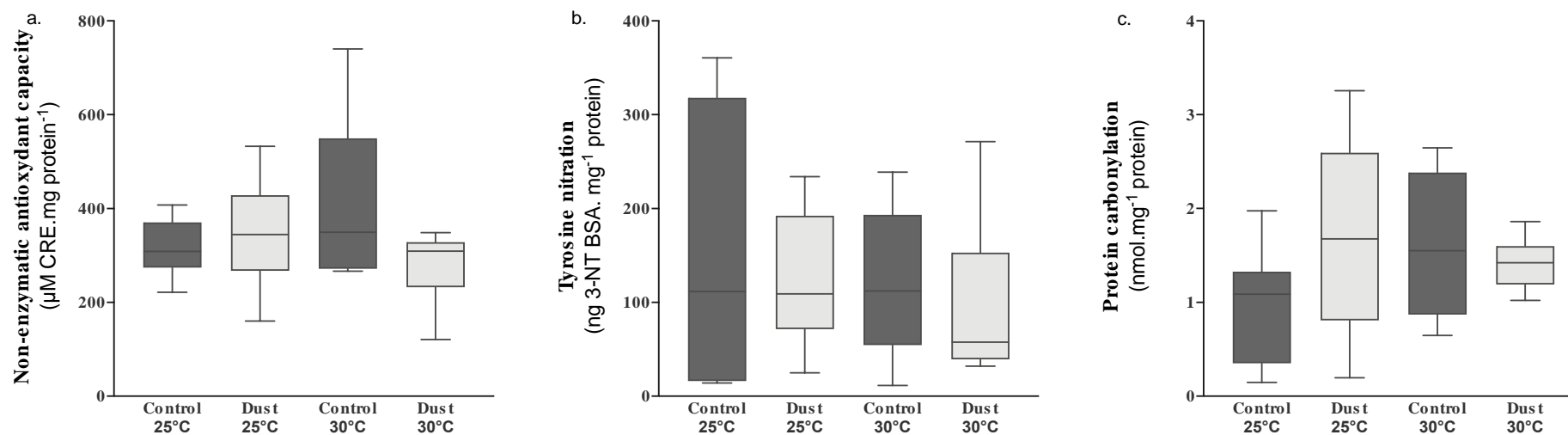


Figure S4. Oxidative status of *S. pistillata*

The boxplots represent the total non-enzymatic antioxidant capacity (a), the tyrosine nitration level (b) and the protein carbonylation level (c) under different conditions of temperature and dust enrichment.

Control 25°C – Control 30°C: control corals maintained at 25°C or 30°C without aerial dust deposition, **Dust 25°C – Dust 30°C:** corals maintained at 25°C or 30°C and supplied with aerial dust at $1 \text{ mg} \cdot \text{L}^{-1}$

The box delimits the 1st and 3rd quartile, the black bar represents the median and whiskers represents min and max, $n=6$

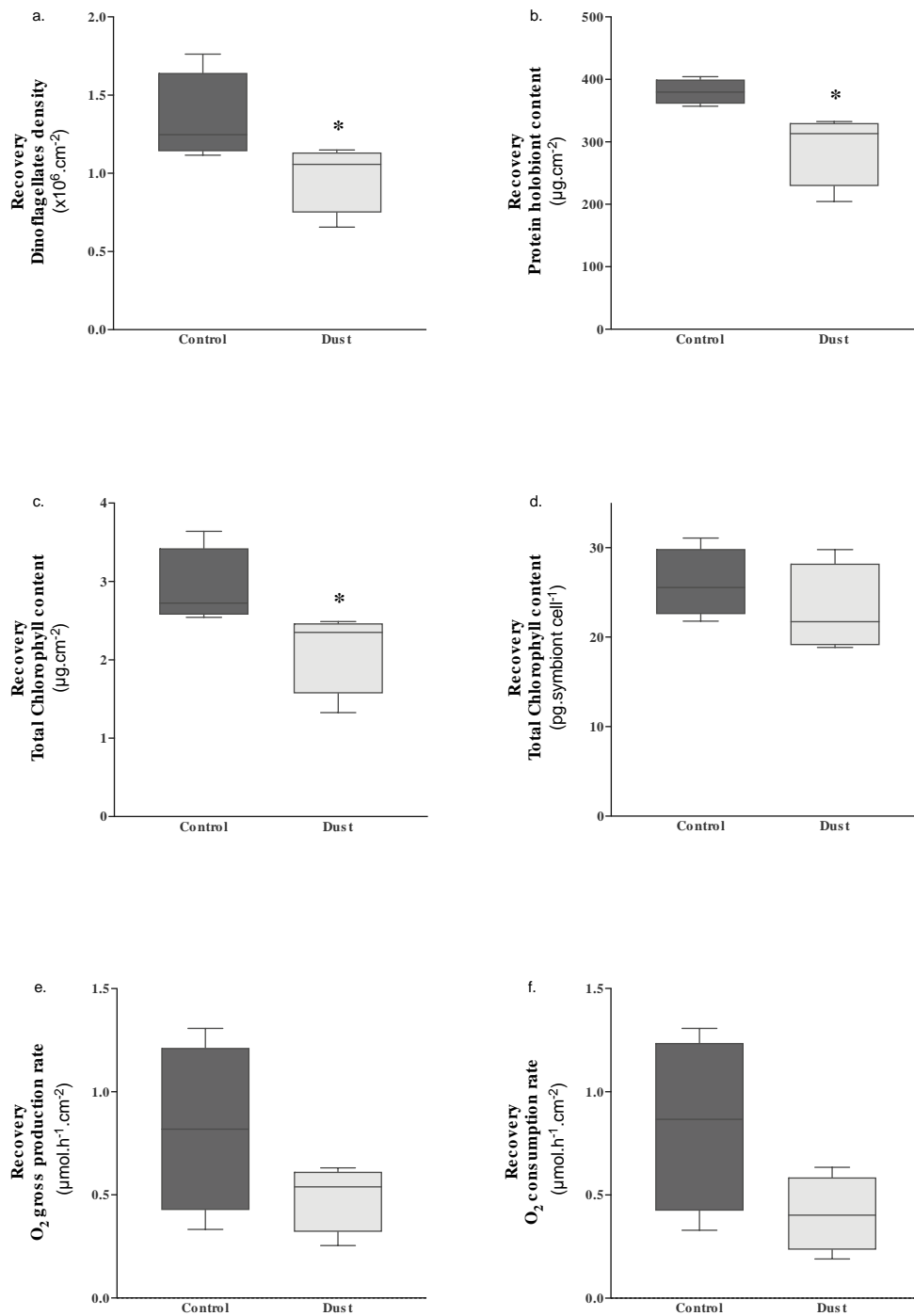


Figure S5. Recovery experiment: Physiology and photosynthesis rates of *S. pistillata*

Nubbins were bleached at the high temperature of 34°C, then left to recover at 30°C, either in normal seawater (**Control**) or in dust enriched seawater, at 1 mg L⁻¹ (**Dust**). After three weeks, the dinoflagellates density (a), protein holobiont content (b), total chlorophyll a and c₂ content normalized for skeletal surface area (c) and dinoflagellates cell (d) were measured. The gross photosynthesis rates (e) and the respiration rates (f), both normalized to skeletal surface area were also analyzed. The black bar represents the mean and error bars represent standard error, n=4. Statistically significant differences are represented by a star (*)

Table S1. Outcome of the statistical tests (Wilcoxon test) analyzing the effects of dust deposition on the metal composition of seawater

The significant p-value (< 0.05) are presented in green.

The tables present the outcomes for (a.) molybdenum, (b.) lead, (c.) chromium, (d.) manganese, (e.) iron, (f.) cobalt, (g.) nickel, (h.) copper and (i.) zinc.

a. Molybdenum in seawater			f. Cobalt in seawater		
Treatment	W	P value	Treatment	W	P value
Treatment	39	0.2179	Treatment	19	0.0199

b. Lead in seawater			g. Nickel in seawater		
Treatment	W	P value	Treatment	W	P value
Treatment	31	0.08275	Treatment	29	0.1701

c. Chromium in seawater			h. Copper in seawater		
Treatment	W	P value	Treatment	W	P value
Treatment	20	0.02174	Treatment	0	0.003968

d. Manganese in seawater			i. Zinc in seawater		
Treatment	W	P value	Treatment	W	P value
Treatment	0	< 0.00001	Treatment	4	< 0.00001

e. Iron in seawater		
Treatment	W	P value
Treatment	14	0.03248

Table S2: Outcome of the statistical tests (ANOVA and pairwise comparison) analyzing the effects of dust deposition and temperature on the physiological parameters of the coral *Stylophora pistillata*.

Pairwise comparisons were done when the ANOVA test showed significant interaction between parameters. The significant p-values (< 0.05) are highlighted in green.

The tables present the outcomes for (a.) the protein biomass normalized to surface area ($\mu\text{g}\cdot\text{cm}^{-2}$) (b.) dinoflagellate density normalized to surface area ($10^6\cdot\text{cm}^{-2}$), (c.) total chlorophyll a and c_2 content normalized to dinoflagellates cell ($10^{-6}\mu\text{g}\cdot\text{dinoflagellates}^{-1}$), under different conditions: **C25-C30**: control corals maintained at 25°C or 30°C without aerial dust deposition, **D25-D30**: corals maintained at 25°C or 30°C supplied with aerial dust at $1\text{mg}\cdot\text{L}^{-1}$

a. Holobiont protein biomass					
	Df	Sum Sq	Mean Sq	F value	P value
Treatment	1	20789	20788.9	0.9823	0.3335
Temperature	1	18401	18401	0.8695	0.3622
Treatment:Temperature	1	3418	3418.1	0.1615	0.692
Residuals	20	423280	21164		

b. Dinoflagellates density					
	Df	Sum Sq	Mean Sq	F value	P value
Treatment	1	0.0414	0.0414115	0.1858	0.671
Temperature	1	0.0923	0.092272	0.414	0.5273
Treatment:Temperature	1	0.0034	0.003368	0.0151	0.9034
Residuals	20	4.4577	0.222883		

c. Chlorophyll content per skeletal surface area						Pairwise comparison					
	Df	Sum Sq	Mean Sq	F value	P value		Estimate	SE	Df	t.ratio	P value (fdr)
Treatment	1	0.039	0.039	0.072	0.791133	C25 - D25	-0.95	0.425	20	-2.237	0.0369
Temperature	1	5.1388	5.1388	9.4986	0.00588	C30 - D30	0.789	0.425	20	1.857	0.0781
Treatment:Temperature	1	4.5333	4.5333	8.3794	0.0008959	C25 - C30	-1.7947	0.425	20	-4.226	0.0004
Residuals	20	10.8201	0.541			D25 - D30	-0.0562	0.425	20	-0.132	0.896

d. Chlorophyll content per symbiotic dinoflagellates						Pairwise comparison					
	Df	Sum Sq	Mean Sq	F value	P value		Estimate	SE	Df	t.ratio	P value (fdr)
Treatment	1	5711	5711	0.063	0.80431	C25 - D25	-493	174	20	-2.837	0.0102
Temperature	1	847581	847581	9.3568	0.006196	C30 - D30	431	174	20	2.482	0.0221
Treatment:Temperature	1	1281493	1281493	14.1469	0.001229	C25 - C30	-838	174	20	-4.823	0.0001
Residuals	20	1811692	90585			D25 - D30	86.3	174	20	0.497	0.6249

Table S3. Outcome of the statistical tests (ANOVA and pairwise comparison) analyzing the effects of dust deposition and temperature on oxygen fluxes normalized to surface area of *Stylophora pistillata*

Pairwise comparisons were done when the ANOVA test showed significant interaction between parameters. The significant p-values (< 0.05) are presented in green.

The tables present the outcomes for the net (a.) and gross (b.) photosynthesis rates and respiration (c.) rates ($\mu\text{mol O}_2\cdot\text{h}^{-1}\cdot\text{cm}^{-2}$) normalized to surface area, under different conditions: **C25-C30**: control corals maintained at 25°C or 30°C without aerial dust deposition, **D25-D30**: corals maintained at 25°C or 30°C supplied with aerial dust at $1\text{mg}\cdot\text{L}^{-1}$

a.

Net photosynthesis per skeletal surface area						pairwise comparison					
	Df	Sum Sq	Mean Sq	F value	P value		Estimate	SE	Df	t.ratio	P value (fdr)
Treatment	1	0.24465	0.24465	8.6754	0.008306	C25 - D25	-0.4354	0.102	19	-4.282	0.0004
Temperature	1	0.23571	0.23571	8.3583	0.00936	C30 - D30	-0.0159	0.097	19	-0.164	0.8711
Treatment:Temperature	1	0.25136	0.25136	8.9134	0.007601	C25 - C30	-0.42259	0.102	19	-4.156	0.0005
Residuals	19	0.53581	0.0282			D25 - D30	-0.00312	0.097	19	-0.032	0.9747

b.

Gross photosynthesis per skeletal surface area					
	Df	Sum Sq	Mean Sq	F value	P value
Treatment	1	0.61449	0.61449	4.4082	0.04936
Temperature	1	0.69251	0.69251	4.9678	0.03809
Treatment:Temperature	1	0.30192	0.30192	2.1658	0.15748
Residuals	19	2.64858	0.1394		

c.

Respiration per skeletal surface area					
	Df	Sum Sq	Mean Sq	F value	P value
Treatment	1	0.04364	0.04364	0.5622	0.4621
Temperature	1	0.06342	0.06342	0.817	0.3768
Treatment:Temperature	1	0.00314	0.003142	0.0405	0.8426
Residuals	20	1.55251	0.077625		

Table S4. Statistical tests (ANOVA and pairwise comparison) testing the effects of dust deposition and temperature on oxygen fluxes normalized to dinoflagellate cells in *Stylophora pistillata*

Pairwise comparisons were done when the ANOVA test showed significant interaction between parameters. The significant p-values (< 0.05) are presented in green.

The tables present the outcomes for the net (a.) and gross (b.) photosynthesis rates and respiration (c.) rates ($\mu\text{mol O}_2\cdot\text{h}^{-1}\cdot\text{cm}^{-2}$) normalized to dinoflagellate cell, under different conditions: **C25-C30**: control corals maintained at 25°C or 30°C without aerial dust deposition, **D25-D30**: corals maintained at 25°C or 30°C supplied with aerial dust at $1\text{mg}\cdot\text{L}^{-1}$

a.

Net photosynthesis per symbiotic dinoflagellates						pairwise comparison					
	Df	Sum Sq	Mean Sq	F value	P value		Estimate	SE	Df	t.ratio	P value (fdr)
Treatment	1	63267	63267	9.911	0.005294	C25 - D25	-210.4	48.4	19	-4.349	0.0003
Temperature	1	45423	45423	7.1157	0.015217	C30 - D30	-16.9	46.1	19	-0.367	0.718
Treatment:Temperature	1	53480	53480	8.3779	0.009291	C25 - C30	-190.4	48.4	19	-3.936	0.0009
Residuals	19	121286	6383			D25 - D30	3.08	46.1	19	0.067	0.9475

b.

Gross photosynthesis per symbiotic dinoflagellates					
	Df	Sum Sq	Mean Sq	F value	P value
Treatment	1	129956	129956	3.6943	0.06972
Temperature	1	110018	110018	3.1276	0.09303
Treatment:Temperature	1	61638	61638	1.7522	0.20131
Residuals	19	668362	35177		

c.

Respiration per symbiotic dinoflagellates					
	Df	Sum Sq	Mean Sq	F value	P value
Treatment	1	2555	2.5547	0.1127	0.7406
Temperature	1	3021	3021.3	0.1333	0.7189
Treatment:Temperature	1	2478	2478.5	0.1093	0.7444
Residuals	20	453463	22673.1		

Table S5. Outcome of the statistical tests (ANOVA and pairwise comparison) analyzing the effects of dust deposition and temperature on the oxidative stress parameters of *Stylophora pistillata*.

Pairwise comparisons were done when the ANOVA test showed significant interaction between parameters. The significant p-values (< 0.05) are presented in green.

The tables present the outcomes for (a.) the non-enzymatic antioxidant capacity, (b.) protein carbonylation level, and (c.) lipid peroxidation level, under different conditions: **C25-C30**: control corals maintained at 25°C or 30°C without aerial dust deposition, **D25-D30**: corals maintained at 25°C or 30°C supplied with aerial dust at 1mg.L⁻¹

a. Non enzymatic antioxidant capacity						c. Lipid peroxidation					
	Df	Sum Sq	Mean Sq	F value	P value		Df	Sum Sq	Mean Sq	F value	P value
Treatment	1	4608	460.7	0.4283	0.5211	Treatment	1	4608	460.7	0.4283	0.5211
Temperature	1	5301	5301.3	0.4927	0.4917	Temperature	1	5301	5301.3	0.4927	0.4917
Treatment:Temperature	1	54	54.3	0.005	0.9442	Treatment:Temperature	1	54	54.3	0.005	0.9442
Residuals	18	193659	10758.8			Residuals	20	193659	10758.8		

b. Protein carbonylation					
	Df	Sum Sq	Mean Sq	F value	P value
Treatment	1	0.3702	0.37016	0.6912	0.4161
Temperature	1	0.2255	0.22553	0.4212	0.5241
Treatment:Temperature	1	1.2036	1.20355	2.2475	0.1503
Residuals	19	10.1746	0.53551		

Table S6. Outcome of the statistical tests (ANOVA and pairwise comparison) analyzing the effects of dust deposition and temperature on the metal composition of corals

Pairwise comparisons were done when the ANOVA test showed significant interaction between parameters. The significant p-values (< 0.05) are presented in green. The tables present the outcomes for (a-b) molybdenum, (c-d) cadmium, (e-f) magnesium, (g-h) chromium, (i-j) manganese, (k-l) iron, (m-n) cobalt, (o-p) nickel, (q-r) copper, (s-t) zinc respectively in the animal host tissues and dinoflagellate cells.

a. Molybdenum content in animal tissue					
	Df	Sum Sq	Mean Sq	F value	P
Treatment	1	2548.8	2548.8	3.9107	0.06193
Temperature	1	4210.4	4210.4	6.4601	0.01942
Treatment:Temperature	1	2178.9	2178.9	3.3431	0.08244
Residuals	20	13035.1	651.8		

b. Molybdenum content in dinoflagellate cells						pairwise comparison					
	Df	Sum Sq	Mean Sq	F value	P		Estimate	SE	Df	t.ratio	P value (fdr)
Treatment	1	0.919	0.9189	0.4214	0.5236	C25 - D25	0.969	0.852	20	1.136	0.2693
Temperature	1	6.977	6.9768	3.2	0.08879	C30 - D30	-1.751	0.852	20	-2.054	0.0532
Treatment:Temperature	1	11.098	11.0976	5.0901	0.03541	C25 - C30	0.282	0.852	20	0.33	0.7445
Residuals	20	43.604	2.1802			D25 - D30	-2.438	0.852	20	-2.86	0.0097

c. Cadmium content in animal tissue						pairwise comparison					
	Df	Sum Sq	Mean Sq	F value	P		Estimate	SE	Df	t.ratio	P value (fdr)
Treatment	1	19.73	19.733	0.554	0.46535	C25 - D25	3.91	3.45	20	1.135	0.2698
Temperature	1	7.59	7.59	0.2131	0.64935	C30 - D30	-7.54	3.45	20	-2.188	0.0407
Treatment:Temperature	1	196.64	196.639	5.5203	0.02918	C25 - C30	6.85	3.45	20	1.988	0.0607
Residuals	20	712.42	35.621			D25 - D30	-4.6	3.45	20	-1.335	0.1969

d. Cadmium content in dinoflagellate cells						pairwise comparison					
	Df	Sum Sq	Mean Sq	F value	P		Estimate	SE	Df	t.ratio	P value (fdr)
Treatment	1	0.1822	0.1822	0.2035	0.65675	C25 - D25	0.757	0.546	20	1.386	0.189
Temperature	1	3.3428	3.3428	3.7343	0.06759	C30 - D30	-1.106	0.546	20	-2.024	0.0565
Treatment:Temperature	1	5.2071	5.2071	5.8169	0.0256	C25 - C30	0.185	0.546	20	0.339	0.7382
Residuals	20	17.9033	0.8952			D25 - D30	-1.678	0.546	20	-3.072	0.006

e. Magnesium content in animal tissue					
	Df	Sum Sq	Mean Sq	F value	P
Treatment	1	2.5587	2.55866	7.6546	0.0119
Temperature	1	0.8022	0.80217	2.3998	0.13703
Treatment:Temperature	1	1.3775	1.37752	4.1211	0.05587
Residuals	20	6.6.6853	0.33426		

f.

Magnesium content in dinoflagellate cells

	Df	Sum Sq	Mean Sq	F value	P
Treatment	1	0.1729	0.17291	0.4142	0.52755
Temperature	1	2.216	2.21602	5.3081	0.03269
Treatment:Temperature	1	2.5727	2.57268	6.1624	0.02256
Residuals	19	7.9321	0.41748		

pairwise comparison

	Estimate	SE	Df	t.ratio	P value (fdr)
C25 - D25	0.558	0.319	19	1.425	0.1703
C30 - D30	-0.784	0.373	19	-2.103	0.0491
C25 - C30	0.017	0.373	19	0.046	0.9641
D25 - D30	-1.325	0.391	19	-3.387	0.0031

g.

Chromium content in animal tissue

	Df	Sum Sq	Mean Sq	F value	P
Treatment	1	330.6	330.63	2.0261	0.17
Temperature	1	100	100.04	0.6131	0.4428
Treatment:Temperature	1	1.3	1.26	0.0077	0.9308
Residuals	20	3263.7	163.18		

h.

Chromium content in dinoflagellate cells

	Df	Sum Sq	Mean Sq	F value	P
Treatment	1	99.82	99.817	4.6266	0.04389
Temperature	1	41.81	41.81	1.9379	0.17919
Treatment:Temperature	1	11.58	11.577	0.5366	0.47234
Residuals	20	431.5	21.575		

i.

Manganese content in animal tissue

	Df	Sum Sq	Mean Sq	F value	P
Treatment	1	4253	4252.7	1.7345	0.20273
Temperature	1	18363	18362.8	7.4896	0.01271
Treatment:Temperature	1	14313	14313	5.8378	0.02537
Residuals	20	49036	2451.8		

pairwise comparison

	Estimate	SE	Df	t.ratio	P value (fdr)
C25 - D25	75.5	28.6	20	2.64	0.0157
C30 - D30	-22.2	28.6	20	-0.77	0.4461
C25 - C30	104.16	28.6	20	3.644	0.0016
D25 - D30	6.48	28.6	20	0.227	0.823

j.

Manganese content in dinoflagellate cells

	Df	Sum Sq	Mean Sq	F value	P
Treatment	1	33.453	33.453	4.4722	0.047202
Temperature	1	62.801	62.801	8.3956	0.008903
Treatment:Temperature	1	65.066	65.066	8.6984	0.007929
Residuals	20	149.605	7.48		

pairwise comparison

	Estimate	SE	Df	t.ratio	P value (fdr)
C25 - D25	0.932	1.58	20	0.59	0.5617
C30 - D30	-5.654	1.58	20	-3.581	0.0019
C25 - C30	0.0578	1.58	20	0.037	0.9711
D25 - D30	-6.5283	1.58	20	-4.134	0.0005

k.

Iron content in animal tissue

	Df	Sum Sq	Mean Sq	F value	P
Treatment	1	3.494	3.4938	0.8168	0.3769
Temperature	1	4.875	4.8747	1.1397	0.2984
Treatment:Temperature	1	16.1	16.0997	3.764	0.0666
Residuals	20	85.547	4.2773		

l.

Iron content in dinoflagellate cells

	Df	Sum Sq	Mean Sq	F value	P
Treatment	1	3.494	3.4938	0.8168	0.3769
Temperature	1	4.875	4.8747	1.1397	0.2984
Treatment:Temperature	1	16.1	16.0997	3.764	0.0666
Residuals	20	85.547	4.2773		

pairwise comparison

	Estimate	SE	Df	t.ratio	P value (fdr)
C25 - D25	-154	116	14	-2.7795	0.03228
C30 - D30	-124	104	14	-1.193	0.2527
C25 - C30	-260	110	14	-2.364	0.033
D25 - D30	-230	110	14	-2.091	0.0552

m.

Cobalt content in animal tissue

	Df	Sum Sq	Mean Sq	F value	P
Treatment	1	236.27	236.27	2.289	0.1459
Temperature	1	117.06	117.06	1.1341	0.2996
Treatment:Temperature	1	214.84	214.84	2.0815	0.1646
Residuals	20	2064.32	103.22		

n.

Cobalt content in dinoflagellate cells

	Df	Sum Sq	Mean Sq	F value	P
Treatment	1	199138	199138	1.7102	0.2058
Temperature	1	568088	568088	4.8787	0.03902
Treatment:Temperature	1	557518	557518	4.7879	0.0407
Residuals	20	2328868	116443		

pairwise comparison

	Estimate	SE	Df	t.ratio	P value (fdr)
C25 - D25	123	197	20	0.623	0.5406
C30 - D30	-487	197	20	-2.472	0.0225
C25 - C30	-2.88	197	20	-0.015	0.9885
D25 - D30	-612.53	197	20	-3.109	0.0055

O.

Nickel content in animal tissue

	Df	Sum Sq	Mean Sq	F value	P
Treatment	1	8960	8960	0.2294	0.6372
Temperature	1	16573	16573	0.4243	0.5222
Treatment:Temperature	1	86440	86440	2.2132	0.1524
Residuals	20	781146	39057		

p.

Nickel content in dinoflagellate cells

	Df	Sum Sq	Mean Sq	F value	P
Treatment	1	33201	33201	14.5454	0.001172
Temperature	1	3803	3803	1.6662	0.21225
Treatment:Temperature	1	2342	2342	1.0259	0.32386
Residuals	19	43369	2283		

q.

Copper content in animal tissue

	Df	Sum Sq	Mean Sq	F value	P
Treatment	1	0.3589	0.35893	0.5094	0.48365
Temperature	1	2.6478	2.64783	3.7577	0.06681
Treatment:Temperature	1	0.4194	0.4194	0.5952	0.44944
Residuals	20	14.093	0.70465		

r.

Copper content in dinoflagellate cells

	Df	Sum Sq	Mean Sq	F value	P
Treatment	1	75399	75399	3.0486	0.09785
Temperature	1	145268	145268	5.8736	0.02613
Treatment:Temperature	1	112893	112893	4.5646	0.04662
Residuals	18	445184	24732		

pairwise comparison

	Estimate	SE	Df	t.ratio	P value (fdr)
C25 - D25	26.3	101	19	0.261	0.7968
C30 - D30	-209.7	96	19	-2.185	0.0416
C25 - C30	-31.7	96	19	-0.331	0.7446
D25 - D30	-267.8	101	19	-2.659	0.0155

s.

Zinc content in animal tissue

	Df	Sum Sq	Mean Sq	F value	P
Treatment	1	23.958	23.9582	4.4109	0.0486
Temperature	1	0.83	0.8304	0.1529	0.6999
Treatment:Temperature	1	4.186	4.1859	0.7707	0.3904
Residuals	20	108.632	5.4316		

t.

Zinc content in dinoflagellate cells

	Df	Sum Sq	Mean Sq	F value	P
Treatment	1	482847	482847	5.2173	0.0394
Temperature	1	274119	274119	0.8861	0.1115
Treatment:Temperature	1	8228	8228	0.0694	0.7752
Residuals	18	1761504	97861		

Table S7. Outcome of the statistical tests (ANOVA and pairwise comparison) analysing the effects of dust deposition and temperature on the stable isotope and carbon and nitrogen composition of corals

Pairwise comparisons were done when the ANOVA test showed significant interaction between parameters. The significant p-values (< 0.05) are presented in green.

The tables present the outcomes for (a-b) $\delta^{13}\text{C}$, (c-d) $\delta^{15}\text{N}$, (e-f) C:N ratio, (g-h) carbon content and (i-j) nitrogen respectively in the animal tissue host and the dinoflagellate cells.

a.

$\delta^{13}\text{C}$ in animal tissue					
	Df	Sum Sq	Mean Sq	F value	P
Treatment	1	0.0581	0.5811	0.1977	0.66162
Temperature	1	1.8454	1.84542	6.2773	0.02149
Treatment:Temperature	1	0.0951	0.09509	0.3235	0.5762
Residuals	19	5.5857	0.29398		

b.

$\delta^{13}\text{C}$ in dinoflagellate cells					
	Df	Sum Sq	Mean Sq	F value	P
Treatment	1	0.7624	0.7624	1.3516	0.25939
Temperature	1	4.0468	4.0468	7.1745	0.01486
Treatment:Temperature	1	1.8202	1.8202	3.2269	0.08836
Residuals	19	10.7171	0.5641		

c.

$\delta^{15}\text{N}$ in animal tissue					
	Df	Sum Sq	Mean Sq	F value	P
Treatment	1	0.0094	0.00944	0.0212	0.8857
Temperature	1	0.0256	0.02558	0.0576	0.813
Treatment:Temperature	1	0.0087	0.00869	0.0196	0.8902
Residuals	19	8.4421	0.44432		

d.

$\delta^{15}\text{N}$ in dinoflagellate cells					
	Df	Sum Sq	Mean Sq	F value	P
Treatment	1	1.944	1.94435	2.7765	0.1121
Temperature	1	0.0003	0.00034	0.0005	0.9827
Treatment:Temperature	1	0.1088	0.10877	0.1553	0.6979
Residuals	19	13.3057	0.7003		

e.

C:N ratio in animal tissue					
	Df	Sum Sq	Mean Sq	F value	P
Treatment	1	0.00258	0.00258	0.0201	0.88741
Temperature	1	1.91226	1.91226	14.8811	0.001061
Treatment:Temperature	1	0.02181	0.02181	0.1697	0.684953
Residuals	19	2.44154	0.1285		

f.

C:N ratio in dinoflagellates cells					
	Df	Sum Sq	Mean Sq	F value	P
Treatment	1	1.014	1.0145	0.5386	0.472
Temperature	1	0.627	0.627	0.333	0.5707
Treatment:Temperature	1	4.78	4.7796	2.5376	0.1277
Residuals	19	35.786	1.8835		

g.

Carbon content in animal tissue					
	Df	Sum Sq	Mean Sq	F value	P
Treatment	1	45.73	45.73	4.7098	0.04287
Temperature	1	40.836	40.836	4.2058	0.05434
Treatment:Temperature	1	25.069	25.069	2.5819	0.12458
Residuals	19	184.479	9.709		

h.

Carbon content in dinoflagellate cells

	Df	Sum Sq	Mean Sq	F value	P
Treatment	1	2.508	2.508	0.3702	0.5501
Temperature	1	3.108	3.108	0.4588	0.50636
Treatment:Temperature	1	22.218	22.218	3.2795	0.08599
Residuals	19	128.72	6.7747		

i.

Nitrogen content in animal tissue

	Df	Sum Sq	Mean Sq	F value	P
Treatment	1	1.0159	1.0159	5.298	0.03284
Temperature	1	0.1887	0.18871	0.9841	0.33364
Treatment:Temperature	1	0.4426	0.44261	2.3082	0.14516
Residuals	19	3.6433	0.19175		

j.

Nitrogen content in dinoflagellate cells

	Df	Sum Sq	Mean Sq	F value	P
Treatment	1	0.498	0.498	0.5685	0.4601
Temperature	1	0.7885	0.78854	0.9001	0.35466
Treatment:Temperature	1	3.0895	3.0895	3.5268	0.07582
Residuals	19	16.6443	0.87602		

Table S8. Outcome of the statistical tests (Wilcoxon test) analyzing the effects of dust deposition on the recovery of *Stylophora pistillata* after a thermal stress

Nubbins were bleached at the high temperature of 34°C, then left to recover at 30°C, either in normal seawater (**Control**) or in dust enriched seawater, at 1 mg. L⁻¹ (**Dust**). After three weeks, physiological parameters were measured: (a,b) net photosynthesis rates normalized respectively to skeletal surface area and dinoflagellate cells, (c.) gross photosynthesis rates per skeletal surface area, (d.) respiration rates, (e.) dinoflagellate density, (f,g) chlorophyll content normalized respectively to skeletal surface area and dinoflagellate cells and (h.) protein biomass per skeletal surface area.

The significant p-values (< 0.05) are presented in green.

a.

Net photosynthesis per skeletal surface area

	t	df	P value
Treatment	-2	5	0.0241

b.

Net photosynthesis per symbiotic dinoflagellate

	t	df	P value
Treatment	-3	3	0.0248

c.

Gross photosynthesis per skeletal surface area

	t	df	P value
Treatment	1.5	4	0.11

d.

Respiration per skeletal surface area

	t	df	P value
Treatment	-3	3	0.0248

e.

Symbiont density

	t	df	P value
Treatment	2	5	0.04

f.

Chlorophyll per skeletal surface area

	t	df	P value
Treatment	2	5	0.03

g.

Chlorophyll per symbiotic dinoflagellate

	t	df	P value
Treatment	1	5	0.2

h.

Protein biomass per skeletal surface area

	t	df	P value
Treatment	1	5	0.2

Table S9: Summary of the P:R values in *Stylophora pistillata*

Tables give the average P:R in the thermal stress experiment (a) in each condition: non-exposed control corals (**Control**) and dust-exposed corals (**Dust**) at both 25°C and 30°C and in the recovery experiment (b) in the two conditions: non exposed-control corals (**Control**) and dust exposed (**Dust**) corals.

The average is calculated based on the content of 6 nubbins for each condition in the thermal stress experiment and 4 nubbins for each condition in the recovery experiment. **SE** represents the standard error.

a.

25°C				30°C			
Control		Dust		Control		Dust	
Average	SE	Average	SE	Average	SE	Average	SE
1.0979	0.1648	1.5797	0.0664	1.5619	0.0918	1.5389	0.0629

b.

Control		Dust	
Average	SE	Average	SE
0.9776	0.0241	1.2542	0.0913

Table S10. Summary of the nutrient (carbon and nitrate) and metal content normalized both to skeletal surface area and dry weight in the host tissue

Metal average values are given for each condition: non-exposed control corals (**Control**) and dust-exposed corals (**Dust**) at 25°C and 30°C. Data are normalized to skeletal surface (ng.cm⁻²) and dry weight (µg.g⁻¹ DW).

Nutrient average values are given for each condition for non-exposed control corals (**Control**) and dust-exposed corals (**Dust**) at 25°C and 30°C. Data are normalized to dry weight (µmol.mg⁻¹ DW) and percentage (%).

The average is calculated based on the content of 6 nubbins for each condition. **SE** represents the standard error.

		Host tissue content							
		25°C				30°C			
		Control		Dust		Control		Dust	
		Average	SE	Average	SE	Average	SE	Average	SE
Molybdenum	ng.cm ⁻²	0.0450	0.0053	0.0460	0.0098	0.0520	0.0096	0.0920	0.0148
	µg.g ⁻¹ DW	0.0642	0.0045	0.0552	0.0065	0.0731	0.0092	0.0909	0.0127
Cadmium	ng.cm ⁻²	0.0149	0.0029	0.0110	0.0024	0.0080	0.0015	0.0156	0.0027
	µg.g ⁻¹ DW	0.0210	0.0030	0.0130	0.0019	0.0111	0.0015	0.0153	0.0022
Magnesium	ng.cm ⁻²	825.1600	148.6547	999.033	237.1299	711.651	147.2512	1843.8300	350.4727
	µg.g ⁻¹ DW	1159.6837	118.5465	1154.6930	178.5769	973.1990	152.1687	1801.8410	292.8396
Chromium	ng.cm ⁻²	0.0185	0.0036	0.0255	0.0087	0.0140	0.0026	0.0219	0.0038
	µg.g ⁻¹ DW	0.0274	0.0058	0.0305	0.0099	0.0196	0.0025	0.0225	0.0041
Manganese	ng.cm ⁻²	0.1708	0.0258	0.0953	0.0260	0.0666	0.0115	0.0888	0.0127
	µg.g ⁻¹ DW	0.2554	0.0476	0.1147	0.0284	0.0933	0.0108	0.0905	0.0130
Iron	ng.cm ⁻²	4.6600	1.5267	2.2592	0.4615	2.1210	0.4832	2.9960	0.2729
	µg.g ⁻¹ DW	4.5560	0.9193	2.7870	0.4381	2.9100	0.5153	3.0800	0.3597
Cobalt	ng.cm ⁻²	0.0174	0.0012	0.0176	0.0049	0.0158	0.0033	0.0280	0.0057
	µg.g ⁻¹ DW	0.0255	0.0020	0.0211	0.0043	0.0221	0.0034	0.0281	0.0054
Nickel	ng.cm ⁻²	0.5320	0.0613	0.4506	0.1129	0.3594	0.0658	0.5181	0.0722
	µg.g ⁻¹ DW	0.7667	0.0551	0.5362	0.1123	0.2200	0.0621	0.5100	0.0540
Copper	ng.cm ⁻²	2.1160	0.3341	1.6070	0.5271	1.1870	0.2230	1.2070	0.1749
	µg.g ⁻¹ DW	3.1030	0.4890	1.9190	0.6052	1.6740	0.2293	1.2290	0.1777
Zinc	ng.cm ⁻²	5.3280	0.8696	6.4910	1.0630	4.1210	0.7478	6.9540	1.0843
	µg.g ⁻¹ DW	7.4710	0.5995	7.9140	0.5870	5.7030	0.6912	6.8090	0.8169
Carbon	µmol.mg ⁻¹ DW	24.83	4.75	15.11	1.73	15.38	0.92	14.43	0.88
	%	24.30	1.81	18.13	2.08	18.46	1.11	17.32	1.06
Nitrogen	µmol.mg ⁻¹ DW	2.70	0.36	2.33	0.27	2.58	0.15	2.41	0.13
	%	4.27	0.18	3.26	0.38	3.61	0.21	3.38	0.18

Table S11: Summary of the metal and nutrient content in the dinoflagellate cells

Metal average values are given for each condition: non-exposed control corals (**Control**) and dust-exposed corals (**Dust**) at **25°C** and **30°C**. Data are normalized to skeletal surface ($\text{ng}\cdot\text{cm}^{-2}$) and dry weight ($\mu\text{g}\cdot\text{g}^{-1}$ DW).

Nutrient average values are given for non-exposed control corals (**Control**) and dust-exposed corals (**Dust**) at **25°C** and **30°C**. Data are normalized to dry weight ($\mu\text{mol}\cdot\text{mg}^{-1}$ DW) and percentage (%).

The average is calculated based on the content of 6 nubbins for each condition. **SE** represents the standard error.

		Dinoflagellate content							
		25°C				30°C			
		Control		Dust		Control		Dust	
		Average	SE	Average	SE	Average	SE	Average	SE
Molybdenum	$\text{ng}\cdot\text{cm}^{-2}$	2.2906	0.5484	1.3219	0.3083	2.0090	0.6437	3.7600	0.8022
	$\mu\text{g}\cdot\text{g}^{-1}$ DW	0.4220	0.0749	0.2940	0.0482	0.3529	0.0660	0.5140	0.0903
Cadmium	$\text{ng}\cdot\text{cm}^{-2}$	1.6580	0.3420	0.9000	0.1755	1.4720	0.3538	2.5780	0.5692
	$\mu\text{g}\cdot\text{g}^{-1}$ DW	0.3080	0.0455	0.1990	0.0208	0.2750	0.0294	0.3460	0.0484
Magnesium	$\text{ng}\cdot\text{cm}^{-2}$	1231.0000	257.8964	1015.0000	351.3428	1214.0000	267.6246	1998.0000	346.2365
	$\mu\text{g}\cdot\text{g}^{-1}$ DW	229.7234	0.0028	241.4670	68.6846	242.4380	55.4982	277.3560	26.5388
Chromium	$\text{ng}\cdot\text{cm}^{-2}$	5.5444	1.3991	8.2340	2.4093	6.7950	1.7097	12.2628	1.9231
	$\mu\text{g}\cdot\text{g}^{-1}$ DW	1.0260	0.1973	1.8220	0.4441	1.2640	0.1553	1.7840	0.2749
Manganese	$\text{ng}\cdot\text{cm}^{-2}$	4.0550	1.1051	3.1230	0.5101	3.9970	0.6099	9.6510	1.7703
	$\mu\text{g}\cdot\text{g}^{-1}$ DW	0.7410	0.1776	0.7228	0.0798	0.8010	0.0610	1.3230	0.1273
Iron	$\text{ng}\cdot\text{cm}^{-2}$	183.7910	40.7714	337.4030	37.3096	443.6590	61.3314	567.2660	114.8732
	$\mu\text{g}\cdot\text{g}^{-1}$ DW	45.2700	6.0599	62.3660	10.1613	89.5710	8.6848	81.8440	8.1165
Cobalt	$\text{ng}\cdot\text{cm}^{-2}$	0.5700	0.0896	0.4470	0.0951	0.5729	0.1287	1.0600	0.2098
	$\mu\text{g}\cdot\text{g}^{-1}$ DW	0.1090	0.0118	0.1020	0.0157	0.1100	0.0133	0.1490	0.1490
Nickel	$\text{ng}\cdot\text{cm}^{-2}$	86.3376	13.9851	180.5040	25.6895	133.3140	22.9950	186.9950	15.4135
	$\mu\text{g}\cdot\text{g}^{-1}$ DW	16.7440	2.4197	49.0830	13.2717	36.1394	7.7926	27.3670	2.5137
Copper	$\text{ng}\cdot\text{cm}^{-2}$	260.5160	38.4107	234.2230	59.6569	219.8830	31.7300	501.9920	88.0655
	$\mu\text{g}\cdot\text{g}^{-1}$ DW	50.4090	5.9454	54.1510	9.4649	55.9790	8.8995	70.0110	7.4871
Zinc	$\text{ng}\cdot\text{cm}^{-2}$	432.7130	85.2596	686.6380	146.0923	618.0510	131.5598	949.6530	149.4458
	$\mu\text{g}\cdot\text{g}^{-1}$ DW	97.7647	14.9903	133.1700	26.9141	117.2400	10.3690	131.1540	9.3652
Carbon	$\mu\text{mol}\cdot\text{mg}^{-1}$ DW	36.9000	1.2700	38.3400	0.8300	39.7000	0.7600	37.2000	1.3900
	%	44.2800	1.5300	46.0100	1.0000	47.6400	0.9200	44.6400	1.6700
Nitrogen	$\mu\text{mol}\cdot\text{mg}^{-1}$ DW	4.6400	0.4300	5.1289	0.3600	5.7800	0.2200	4.7997	0.5000
	%	6.4900	0.6100	7.1800	0.5100	8.0888	0.3000	6.7200	0.7000

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