

## RESEARCH ARTICLE

# Tropical bloom-forming mesoalgae *Cladophoropsis* sp. and *Laurencia* sp.—responses to ammonium enrichment and a simulated heatwave

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## Funding information

Stipend funded by the Leibniz Centre for Tropical Marine Research (ZMT) and the Centre for Science and Technology of the Non-Aligned and Other Developing Countries (NAM S&T Centre) (NAM-ZMT), Grant/Award Number: NAM-05/69/2018; Deutsche Forschungsgemeinschaft, Grant/Award Number: SEAMAC; TE 1046/3-1; Brazilian-German Aquaculture Network BluEcoNet

Editor: A.H. Buschmann

## Abstract

Algal blooms are increasing worldwide, driven by elevated nutrient inputs. However, it is still unknown how tropical benthic algae will respond to heatwaves, which are expected to be more frequent under global warming. In the present study, a multifactorial experiment was carried out to investigate the potential synergistic effects of increased ammonium inputs (25 μM, control at 2.5 μM) and a heatwave (31°C, control at 25°C) on the growth and physiology (e.g., ammonium uptake, nutrient assimilation, photosynthetic performance, and pigment concentrations) of two bloom-forming algal species, *Cladophoropsis* sp. and *Laurencia* sp. Both algae positively responded to elevated ammonium concentrations with higher growth and chlorophyll *a* and lutein concentrations. Increased temperature was generally a less important driver, interacting with elevated ammonium by decreasing the algae's %N content and N:P ratios. Interestingly, this stress response was not captured by the photosynthetic yield (*Fv/Fm*) nor by the carbon assimilation (%C), which increased for both algae at higher temperatures. The negative effects of higher temperature were, however, buffered by nutrient inputs, showing an antagonistic response in the combined treatment for the concentration of VAZ (violaxanthin, antheraxanthin, zeaxanthin) and thalli growth. Ammonium uptake was initially higher for *Cladophoropsis* sp. and increased for *Laurencia* sp. over experimental time, showing an acclimation capacity even in a short time interval. This experiment shows that *both algae* benefited from increased ammonium pulses and were able to overcome the otherwise detrimental stress of increasingly emerging temperature anomalies, which provide them a strong competitive advantage and might support their further expansions in tropical marine systems.

## KEYWORDS

ammonium uptake, benthic bloom, eutrophication, heatwave, photosynthetic performance, seaweed

**Abbreviations:** DPS, de-epoxidation state of the xanthophyll cycle; *Fv/Fm*, maximum quantum efficiency of photosystem II, variable fluorescence divided by maximum fluorescence; SGR, specific growth rate, showing percentage change per day; VAZ, summed up term for violaxanthin, antheraxanthin, and zeaxanthin.

Anna Fricke and Felix Bast equal first author contribution.

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## INTRODUCTION

Anthropogenic nutrient inputs are of increasing concern in coastal regions worldwide (Wurtsbaugh et al., 2019). Elevated nutrient concentrations have resulted in eutrophication and often in the formation of dense benthic algal blooms (Lapointe et al., 2018; Teichberg et al., 2010), which could provide a significant threat to shallow-water ecosystems (Heisler et al., 2008; Valiela et al., 2016), for example, causing hypoxic events, loss of marine biodiversity, and ultimately could also impact the coastal economy (Rabalais et al., 2009).

In addition, global warming (IPCC, 2022) is often interacting with eutrophication (Fricke et al., 2018; Ontoria et al., 2019), and its impacts in tropical areas have started emerging only recently (Chaudhary et al., 2021). Different studies have documented shifts in seaweed communities in response to rising temperatures, including increases in the distribution range of tropical seaweeds and decreases in temperate species (Geppi & Riera, 2022; Tanaka et al., 2012; Wernberg et al., 2011). In addition to the observable long-term alterations, there have been short-term events (durations of about 5 days to several months), called marine heatwaves, which have resulted in water temperatures rising to abnormally high levels (temperatures warmer than the 90 percentile based on a 30-year historical baseline), which have caused severe damage to coastal ecosystems (Frölicher & Laufkötter, 2018; Gouvêa et al., 2017; Hobday et al., 2016).

Testing the response of bloom-forming algae to combined stressors is essential for better understanding bloom dynamics under future environmental conditions. It has become a critical aim under UN Sustainable Developmental Goal number 13 (SDG 13: climate action; UN General Assembly, 2015), for which methods of control or mitigation of algal blooms are an important research avenue. Temperature, light, and nutrient levels are three key factors limiting algal growth (Lüning, 1991). Temperature response in algae depends on several factors over varying time scales: (i) physiological response is immediate within seconds to minutes, (ii) phenotypic acclimatization occurs within hours to days, and (iii) genotypic adaptations to changed environments occur over much longer timescales, from a few thousand to millions of years (Eggert, 2012).

Studies on inorganic nitrogen uptake kinetics have been done on several dominant and bloom-forming macroalgae located in temperate and tropical regions (Fernández et al., 2020; Pérez-Mayorga et al., 2011; Wang et al., 2014). However, compared to the high macroalgal diversity, data on uptake rates are still relatively scarce from tropical species with the exception of a few well-known studies (Cohen & Fong, 2004; Lapointe, 1981; Lapointe & Duke, 1984). Specifically, evaluation of the temperature sensitivity to nutrient

uptake is vital for various reasons, including (i) to study the potential impacts of global change (e.g., heatwaves) on algae, (ii) to increase algal biomass production for industries including food and biofuel, (iii) to optimize the removal of ammonia from intensive fish/shrimp tank cultivation systems as a form of “phycoremediation” (phytoremediation using algae; Samocha et al., 2015), and (iv) to predict and control algal blooms driven by eutrophication (Fan et al., 2020). Studies conducted on microalgae such as *Chaetoceros* and *Dunaliella* have shown that specific affinity for nitrate over ammonium is strongly dependent on temperature, whereas a temperature dependence was less apparent for ammonium uptake (Reay et al., 1999). This basic biological information is missing for most algae species.

Next to different conspicuous bloom-forming macroalgal genera like *Sargassum*, *Gracilaria*, or *Ulva*, there are a number of reports on blooming, somewhat cryptic taxa (Fricke et al., 2017; Littler et al., 2010; Lovelock et al., 2008; Vermeij et al., 2010). These so-called mesoalgae, a term first introduced by Fricke et al. (2017), bridge the classical size-related terms macroalgae (visible by naked eye) and microalgae (visible under a microscope), and the term refers to all macroscopically visible algal elements (e.g., tufts, turfs, mats, and crusts), which can hardly be determined on a generic level without a microscope (Fricke et al., 2017; Jurán & Kaštovský, 2019). In this respect, mesoalgae can comprise different minute macroalgal stages (e.g., propagules, germlings, uniseriate, and fine filamentous thalli), as well as dense-growing colonies of microalgae (e.g., cyanobacteria and diatoms). Mesoalgae have been reported to play an important role in various coastal habitats, including coral reefs and seagrass beds (Karcher et al., 2020; Vermeij et al., 2010; Wear et al., 1994). Given their small sizes and observed invasive potential, further knowledge of species acclimatization potential is urgently needed.

The present study addressed the responses and phenotypic acclimatization potential to increased ammonia inputs and a simulated heat wave of two important bloom-forming tropical mesoalgal genera: the chlorophyte *Cladophoropsis* sp. (Cladophorales, Ulvophyceae) and the rhodophyte *Laurencia* sp. (Ceramiales, Florideophyceae). The genus *Cladophoropsis* comprises abundant pantropical species, such as *C. membranacea* (Pakker et al., 1995), and has been observed growing attached to benthos, seagrass, and coral reef habitats in the infralittoral fringe in the Western Indian Ocean region of East Africa (Zanzibar, Tanzania; Leliaert & Coppejans, 2006). Members of the genus *Laurencia* have been observed growing epiphytic on different macrophytes (Leliaert et al., 2001), where they can contribute to biofouling and affect the commercial seaweed cultivation in Zanzibar (Largo et al., 2020). Species such as *L. papillosa* have been reported as

forming blooms in southern Taiwan coral reef ecosystems in response to seasonal changes in nutrient availability (Tsai et al., 2005). There are many reports of the blooms of these two genera from fragile coral reef ecosystems around the world (Hay et al., 1987; McCabe et al., 2016; Van Der Strate et al., 2002). Moreover, recently the invasive rhodophyte *Chondria tumulosa*, morphologically similar and taxonomically closely related to *Laurencia* spp., has been described from an uninhabited, remote, and pristine island chain in Hawaii (Sherwood et al., 2020). With its comparable small thallus size (individual thalli about 2–7 cm in length) and cryptogenic appearance, this mesoalga has been observed to form dense mats (about 18 cm thick and up to 100 cm in extension) all over the coral reef. In Zanzibar, Tanzania, both mesoalgal genera, *Cladophoropsis* and *Laurencia*, have been observed in association with seagrass and have increasingly been observed overgrowing seagrasses, which may be a result of several factors, including increasing nutrient inputs and rising temperatures. This study highlights the importance of a better understanding of the so-far widely overlooked mesoalgal components in the benthic system. The key research questions we investigated were:

1. How are growth, nutrient uptake and assimilation, and photosynthetic performance of the green and red tropical bloom-forming mesoalgae *Cladophoropsis* sp. and *Laurencia* sp. impacted by short-term elevation in nutrients and temperature?
2. What are the responses and phenotypic acclimatization potentials of these mesoalgae toward the altered abiotic conditions?
3. What are the implications of continued elevation in ammonium inputs and temperature on these bloom-forming benthic species?

To answer these questions, we carried out a multifactorial in-vitro experiment with the two mesoalgae under increased ammonium (25 μM, control at 2.5 μM) and temperature (31°C, control at 25°C) treatments. To the best of our knowledge, this is the first study ever conducted on the physiological performance of these two critical bloom-forming benthic species under controlled environmental conditions.

## MATERIALS AND METHODS

### Algal material

Both mesoalgae were collected, together with seagrass, in Zanzibar, Tanzania (6°7'42" S, 39°10'47" E) in 2016 and were afterward jointly cultivated at a water temperature of 25 ± 1°C, salinity of 35 ± 1, and pH of 8 in the tanks of the MaRine Experimental Ecology

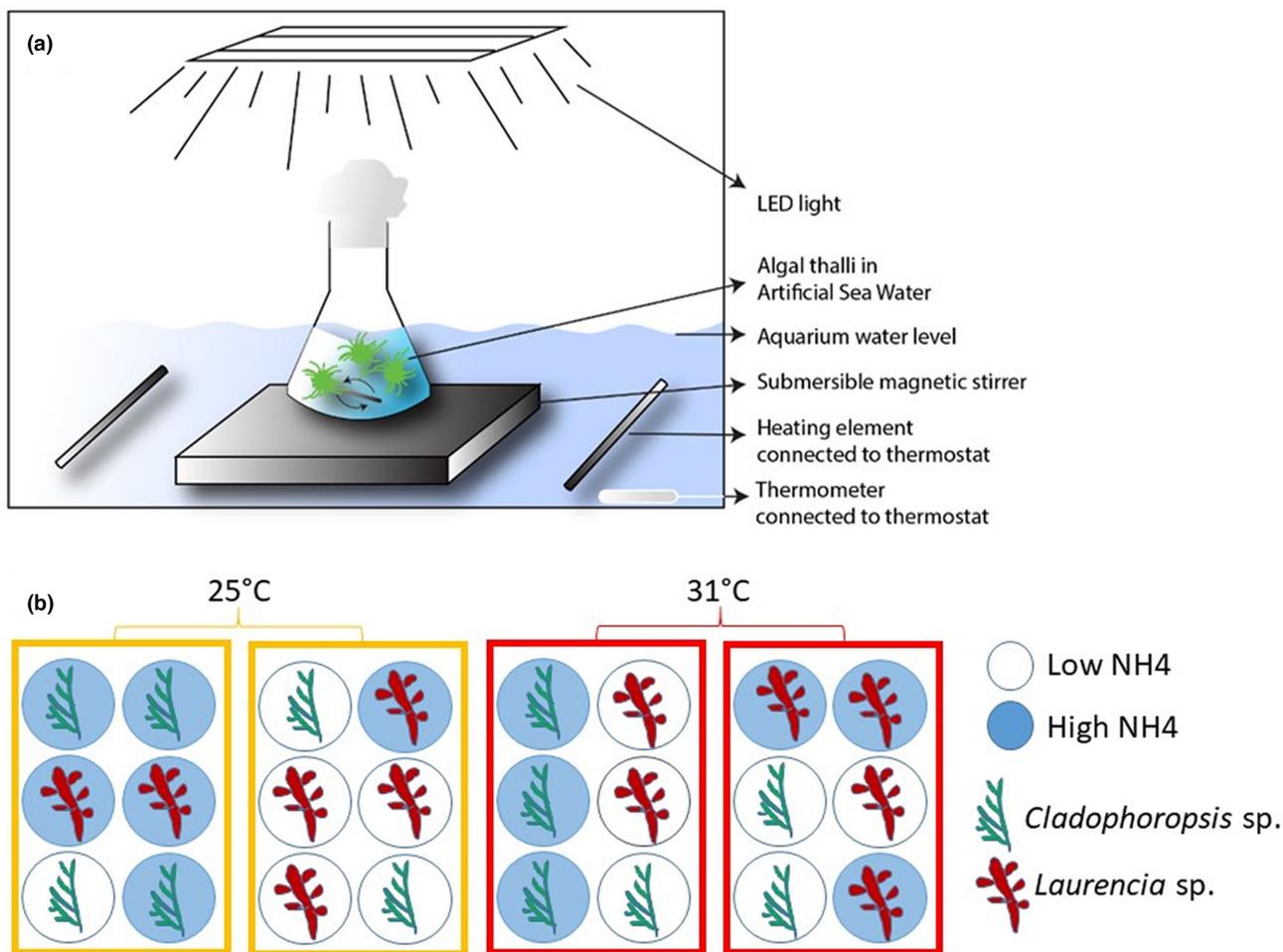
(MAREE) facility at Leibniz Centre for Tropical Marine Research (ZMT), Bremen, Germany.

Shortly before the experiment, the mesoalgal species *Cladophoropsis* sp. and *Laurencia* sp. were collected and isolated from the tanks. One-liter conical glass flasks served as experimental units, with three replicates for each treatment combination, resulting in 12 flasks per mesoalgal species (1 g fresh weight inoculum per flask). Each flask was filled with 500 mL artificial seawater (ASW, Red Sea Salt, Red Sea Europe Company, filtered through 0.22 μm filter) and illuminated with LED lamps with a 12:12 h light:dark photoperiodicity (200 ± 30 μmol photons · m<sup>-2</sup> · s<sup>-1</sup>, Hydra illumination, Aqua Illumination) in larger aquarium tanks to maintain a constant temperature. Both mesoalgae were starved for 3 days (grown in 0.22 μm filtered, low nutrient seawater with nutrient concentrations: NO<sub>3</sub><sup>-</sup> 2.8 ± 1.5 μM, NH<sub>4</sub><sup>+</sup> < 0.3 μM, PO<sub>4</sub><sup>3-</sup> < 0.002 μM) and pre-acclimatized to the respective experimental light conditions.

### Experimental set-up

To investigate the potential interacting impacts of varying ammonium concentrations and elevated water temperature on the algal physiology, we conducted a split-plot design with the two-level factors: (i) ammonium enrichment nested in (ii) water temperature for a period of 12 days. For ammonium enrichment, we chose a low concentration dose of 2.5 μM and a high concentration dose of 25 μM NH<sub>4</sub>Cl. In addition, to avoid phosphorus limitation, 2.5 μM of phosphate were provided to all treatments. These concentrations were chosen based on the nutrient regime at the study site, which showed low and even undetectable concentrations in the water column (Teichberg, unpublished), whereas pore waters had high DIN concentrations ranging from 7.28 to 14.82 μM and N:P ratios of 4.8 to 9.1 (Moreira-Saporiti et al., 2021). To maintain nutrient concentrations over the experimental period, corresponding media was exchanged every 3 days, and flasks were placed on stir plates throughout the experiment to ensure uniform mixing (Figure 1a).

For the water temperature treatment, we applied two levels: 25°C, which is within the range of average water temperatures in Zanzibar where the algae were collected and was the temperature at which the organisms were cultivated (Nyandwi & Mwaipopo, 2000), and 31°C, which corresponds to a scenario of elevated water temperature in Zanzibar expected under climate change (de Jong Cleynert et al., 2021) and lies within the scale of recent observed marine heatwave events (Pak et al., 2022). Although current water temperatures in shallow water systems in Zanzibar can reach over 31°C during daytime hours in low tide (Teichberg, pers. obs.), these conditions generally do not last for long periods of time. Temperatures were simulated with two



**FIGURE 1** Experimental set-up, presented as diagram (a) and top-down view (b) within each experimental tank (ET) set as a water bath.

water baths per temperature treatment using the experimental tanks (ETs) in the MAREE, in which experimental flasks were distributed randomly (Figure 1b). To monitor and maintain the experimental conditions, temperature, pH, and salinity were controlled at each sampling event using a multi-parameter probe (Multi 3630 WTW by Xylem, Germany) and temperature logger (tidbit, Onset, USA). Minimum, maximum, and average water temperatures were calculated for each ET to verify experimental conditions (File S1: Table S1 in the Supporting Information).

In total, the study involved low and high levels of two factors (ammonium additions and temperature) for two mesoalgal species (*Cladophoropsis* sp. and *Laurencia* sp.) with three replicates each (2 factors  $\times$  2 level  $\times$  2 species  $\times$  3 replicates = 24 flasks). The flasks were exposed to one of four treatments: L25 (low  $\text{NH}_4^+$  at 25°C, control), H25 (high  $\text{NH}_4^+$  at 25°C), L31 (low  $\text{NH}_4^+$  at 31°C), and H31 (high  $\text{NH}_4^+$  at 31°C). The experiment ran for 12 days, and the response variables were (i)

algal growth, (ii) ammonium uptake, (iii) tissue nutrient content, (iv) photosynthetic yield and pigments concentration, and (v) microphotometry of preserved slides.

## Algal growth

For determination of algal growth, algal wet weight was determined at day 0 (D0), day 6 (D6), and day 11 (D11). For this purpose, the alga was removed and carefully blotted dry before weighing. Specific growth rates (SGRs, % increase per day) were calculated using the following equation of Pérez-Mayorga et al. (2011):

$$\text{SGR} \left( \% \cdot \text{d}^{-1} \right) = \frac{(\ln X_2 - \ln X_1)}{(t_2 - t_1)} \times 100$$

where  $X_1$  and  $X_2$  are the values of wet weight at experimental start at  $t_1$  (D0) and experimental end at  $t_2$  (D11). Additionally, to compare outcomes with existing literature,

data daily growth rate (*DGR*) was calculated following Yong et al. (2013):  $DGR = \left[ \left( \frac{W_t}{W_0} \right)^{\frac{1}{d}} - 1 \right] \times 100$

## Thallus morphology

Microscopic analyses of the algal thalli at the end of the experiment revealed differences in branching frequency in *Laurencia* sp., which was further investigated. To investigate potential changes in branching patterns and epiphyte load, branch segments were investigated and counted per cm of algal thalli, performed at D12 of the experiment, using a stereomicroscope (10 and 20 times magnification; Axioscope; Zeiss; Germany) equipped with a camera system.

## Ammonium uptake

Right after the media exchange and determination of algal biomass, ammonium concentrations were measured at 60 min on D0, D6, and D11. For the uptake experiments, media was sampled and filtered through 0.22 μm CA-filters (GF/A sterile filters; Whatman International Ltd, Maidstone, UK), and ammonium concentrations were immediately determined spectrophotometrically using the salicylate-dichloroisocyanurate method (Yu et al., 1994) in a plate reader (TECAN, calibrated according to German DIN 38402-51:2017-05). To investigate the change in ammonium uptake over time, the relative ammonium uptake rate was calculated following the equation:

$$Uptake \text{ NH}_4 = \frac{\mu\text{M NH}_{4,t0} - \mu\text{M NH}_{4,t60}}{\text{Time} \times W_t}$$

where  $\mu\text{M NH}_{4,t0}$  is the ammonium concentration at the start of the uptake experiment and  $\mu\text{M NH}_{4,t60}$  is the concentration after 60 min (end of the uptake experiment). Time is the uptake experiment duration, which in this case was 60 min, and  $W_t$  is the algae wet weight (g). Due to the observed rapid depletion of ammonium in the media, this analysis was only feasible for the high ammonium treatments.

## Nutrient assimilation

Tissue carbon (C), nitrogen (N), and phosphorus (P) content were determined at the end of the experiment to compare the assimilation of nutrients among the different treatments via the stored nutrient pools. For total C and N, subsamples of the two mesoalgae from each treatment were dried at 50°C in a forced-air oven for 48 h, ground to a fine powder with mortar and pestle, and weighed into tin capsules using an analytical scale prior to analysis. Total N and C contents of the tissue

samples were measured in the Euro EA3000 Elemental Analyzer. Acetanilid 5 (Hexatech) was used as a standard. Repeated measurements of internal standards with known C and N concentrations (Low Soil Standard OAS 5; IVA) ensured measurement accuracy. Carbon and nitrogen contents were expressed as a percentage of dry weight. Percent tissue phosphorus (P) was analyzed using the wet alkaline persulphate digestion technique method on a TECAN M200Pro plate reader after Hansen and Koroleff (1999). Fichtennadel (1.69 mg P · g<sup>-1</sup>) and SRM1515 Apple leaves (1.59 mg P · g<sup>-1</sup>) were used as standard reference materials. Ratios of C:N, C:P, N:P, and C:N:P were determined based on the molecular weight of each element.

## Photosynthetic performance

To investigate potential changes in the photosynthetic performance of the algae over the experimental time, maximum quantum yield (*Fv/Fm*) was determined using a Maxi-Imaging-PAM with an integrated CCD camera (Heinz Walz GmbH, Effeltrich, Germany; WALZ GmbH, Effeltrich, Germany) at D6 and D11. Each mesoalgae replicate was transferred to a 24 well-plate, moistened in ambient seawater, and dark-adapted for a minimum period of 30 min at 25°C. This allowed the parallel assessment of different samples under identical conditions. An image of minimum fluorescence of the dark-adapted state ( $F_0$ , 1 μmol · m<sup>-2</sup> · s<sup>-1</sup>) was recorded followed by an excitation pulse (intensity 2400 μmol · m<sup>-2</sup> · s<sup>-1</sup> and duration 0.8 s) to yield maximum fluorescence of the dark-adapted state ( $F_m$ ) of 470 nm actinic light of a LED and simultaneous recording of a fluorescence image. To allow measurements of the filamentous thallus structure, five areas of interest (AOIs) were set and averaged for each individual.

## Pigment concentrations

In addition to photosynthetic yield, we quantified concentrations of photosynthetic pigments, including chlorophyll *a* and *b*, β-carotene, lutein, violaxanthin, antheraxanthin, and zeaxanthin, using reversed-phase high-performance liquid chromatography (HPLC). Algal samples were immediately frozen at -80°C after the experiment. Prior to analysis samples were freeze-dried and pulverized in a Fast-Prep®-24 homogenizer (MP Biomedicals, Solon, OH, USA) to a fine powder. Pigments were extracted from approximately 20–50 mg dry weight per sample in 1 mL of ice-cold 90% Acetone for 24 h at -20°C in the dark. After centrifugation for 5 min at 4°C (13,000 rpm), samples were filtered through a 45 μm nylon syringe filter (Nalgene®, Nalge Nunc International, Rochester, NY, USA). HPLC analysis was performed on a LaChrom Elite system

equipped with a chilled autosampler L-2200 and a DAD detector L-2450 (VWR Hitachi International GmbH, Darmstadt, Germany). A Spherisorb® ODS-2 column (25 cm × 4.6 mm, 5 μm particle size; Waters, Milford, MA, USA) with a LiChropher® 100-RP-18 guard cartridge was used for the separation of pigments, applying a gradient according to Wright et al. (1991). Peaks were detected at 440 nm and identified and quantified by co-chromatography with standards for chlorophyll *a* and *b*, β-carotene, lutein, violaxanthin, antheraxanthin, and zeaxanthin (DHI Lab Products, Hørsholm, Denmark) using software EZChrom Elite ver 3.1.3. (Agilent Technologies, Santa Clara, CA, USA). Pigment contents were expressed as μg per mg dry weight, and concentrations for violaxanthin, antheraxanthin, and zeaxanthin were summed up under the term VAZ to provide an overview of the total xanthophyll cycle. In addition, to evaluate the photoprotective state of *Cladophoropsis* sp., the de-epoxidation state (DPS) of the Xanthophyll cycle was calculated following Colombo-Pallotta et al. (2006):

$$\text{DPS} = ([\text{zeaxanthin}] + 0.5 [\text{antheraxanthin}]) / \sum \text{XC}$$

where XC is the xanthophyll cycle, composed of the sum of violaxanthin, zeaxanthin, and antheraxanthin.

## Statistical analyses

For the analysis of the responses of the mesoalgae *Cladophoropsis* sp. and *Laurencia* sp. to the experimental treatments (Nutrients and Temperature), we used mixed-effects models (Model 1). For the variables SGR, Chl *a*, Chl *b*, β-carotene, lutein, VAZ, DPS, %N, %C, %P, CN ratio, CP ratio, NP ratio, branching, and *Fv/Fm*, we used the nutrient and temperature treatments and their interaction as explanatory variables in the model (fixed effects) and the experimental units (ETs) in which the experiment took place as a random effect (random intercept of the nesting factor ET; Table 1; File S1: Tables S2 and S3). We analyzed whether the uptake rate of the algae differed among the temperature treatments at each sampling time (D0, D6, and D11) only in the high nutrients treatment (Model 2; Table 2; File S1: Table S4). For this model, we used temperature treatment, sampling time, and their interaction as explanatory variables (fixed effects) and the algae ID as a random effect to account for the repeated measurements on the same algae at three sampling times. Significance of the explanatory variables was tested using model comparison through the likelihood ratio test. All models were validated for homoscedasticity, normality, and independence of the residuals. Data were scouted for outliers, which were identified as data exceeding 1.5 times the interquartile range of variation

of the dataset. Outliers were only eliminated from the model when they did not allow to meet the model assumptions. Due to general homoscedasticity and independence problems due to different variances at each level of the treatments, a variance structure was used (VarIdent) for model validation. For Model 1, a variance structure was applied at each level of the Temperature: nutrient interaction, except for DPS, in which the variance structure was applied at the two temperature levels. For Model 2, a variance structure was applied at each level of the Temperature: sampling time interaction. For the response variable VAZ in *Cladophoropsis* sp., one outlier was eliminated. For the analysis of the uptake rate, some dependence to the algae ID could not be eliminated for both *Cladophoropsis* sp. and *Laurencia* sp.; these results should, therefore, be interpreted with caution. The software R was used for performing all the statistical analysis (R Core Team, 2022). The R package nlme was used for mixed-effects modeling (Pinheiro et al., 2022). The R package emmeans was used for pairwise comparisons (Lenth, 2022). GraphPad Prism v 8 software was used for graphical presentation.

## RESULTS

### Mesoalgal growth

Algal wet mass plotted as a function of time indicated a higher biomass accumulation with ammonium enrichment in both taxa (Figure 2a,b), but particularly in *Cladophoropsis* sp., which had a higher biomass accumulation overall. This also led to higher specific growth rates (SGRs) in both mesoalgal species under higher ammonium enrichment (Table 1). For *Cladophoropsis* sp., SGR significantly increased under enriched ammonium conditions, reaching  $8.48 \pm 0.51\% \cdot \text{d}^{-1}$  at 25°C (H25) and  $6.3 \pm 7.21\% \cdot \text{d}^{-1}$  at 31°C (H31; Figure 2c; Table 1). In contrast to *Cladophoropsis* sp., a negative SGR was measured for *Laurencia* sp. under low ammonium treatments, with the lowest SGR measured in the low ammonium-high temperature (L31) treatment, reaching an average of  $-8.92 \pm 4.42\% \cdot \text{d}^{-1}$  (Figure 2d; Table 1).

### Algal morphology

To study any potential effects of temperature and altered ammonium concentrations on thallus morphology, we investigated the branching pattern of *Laurencia* sp. After 12 days of exposure to different treatments, we observed a significant increase in branching number with high ammonium concentrations, whereas no temperature effect was observed (Table 1). An average

**TABLE 1** Analysis of the responses of *Cladophoropsis* and *Laurencia* to the experimental treatments (Nutrients and Temperature) using mixed-effects models (Model 1) with Nutrients and Temperature as fixed terms and the experimental unit (ET) as random intercept.

Species	Response variable	Explanatory variables	Chi-square	df	p-value	Pairwise comparison
<i>Cladophoropsis</i>	SGR (%d <sup>-1</sup> )	<b>Nutrient</b>	<b>8.997</b>	<b>1</b>	<b>0.002</b>	<b>H &gt; L</b>
		Temperature	3.724	1	0.053	
		Nutrient:Temperature	0.249	1	0.617	
	Chlorophyll a	<b>Nutrient</b>	<b>13.622</b>	<b>1</b>	<b>0.0002</b>	<b>H &gt; L</b>
		Temperature	0.800	1	0.370	
		Nutrient:Temperature	1.916	1	0.1663	
	Chlorophyll b	<b>Nutrient</b>	<b>12.689</b>	<b>1</b>	<b>0.0004</b>	<b>H &gt; L</b>
		Temperature	0.928	1	0.335	
		Nutrient:Temperature	0.551	1	0.457	
	β-Carotene	<b>Nutrient</b>	<b>12.723</b>	<b>1</b>	<b>0.0004</b>	<b>H &gt; L</b>
		Temperature	1.889	1	0.169	
		<b>Nutrient:Temperature</b>	<b>4.778</b>	<b>1</b>	<b>0.028</b>	<b>H25 &gt; L25; H31 &gt; L31</b>
	Lutein	<b>Nutrient</b>	<b>6.915</b>	<b>1</b>	<b>0.008</b>	<b>H &gt; L</b>
		Temperature	1.603	1	0.205	
		Nutrient:Temperature	2.410	1	0.120	
	VAZ	<b>Nutrient</b>	<b>8.761</b>	<b>1</b>	<b>0.003</b>	
		Temperature	0.341	1	0.558	
		<b>Nutrient:Temperature</b>	<b>6.310</b>	<b>1</b>	<b>0.012</b>	<b>H31 &gt; L31</b>
	DPS	Nutrient	0.431	1	0.511	
		<b>Temperature</b>	<b>8.829</b>	<b>1</b>	<b>0.003</b>	<b>25 &gt; 31</b>
		Nutrient * Temperature	0.228	1	0.632	
	% N	<b>Nutrient</b>	<b>13.209</b>	<b>1</b>	<b>0.0003</b>	<b>H &gt; L</b>
		Temperature	0.333	1	0.563	
		Nutrient:Temperature	0.861	1	0.353	
	% C	Nutrient	2.196	1	0.138	
		<b>Temperature</b>	<b>4.851</b>	<b>1</b>	<b>0.027</b>	<b>31 &gt; 25</b>
		Nutrient:Temperature	0.857	1	0.354	
% P	Nutrient	0.131	1	0.717		
	Temperature	2.677	1	0.101		
	<b>Nutrient:Temperature</b>	<b>7.898</b>	<b>1</b>	<b>0.004</b>	<b>L25 &lt; L31; H25 &gt; L31; H31 &gt; L31</b>	
CN ratio	<b>Nutrient</b>	<b>17.534</b>	<b>1</b>	<b>&lt;0.0001</b>	<b>L &gt; H</b>	
	Temperature	1.987	1	0.158		
	Nutrient:Temperature	0.317	1	0.572		
CP ratio	Nutrient	0.987	1	0.320		
	<b>Temperature</b>	<b>11.506</b>	<b>1</b>	<b>0.0007</b>	<b>25 &gt; 31</b>	
	Nutrient:Temperature	1.332	1	0.248		
NP ratio	Nutrient	3.185	1	0.074		
	<b>Temperature</b>	<b>13.780</b>	<b>1</b>	<b>0.0002</b>	<b>25 &gt; 31</b>	
	Nutrient:Temperature	0.224	1	0.635		

(Continues)

TABLE 1 (Continued)

Species	Response variable	Explanatory variables	Chi-square	df	p-value	Pairwise comparison
<i>Laurencia</i>	SGR (%d <sup>-1</sup> )	<b>Nutrient</b>	<b>11.413</b>	<b>1</b>	<b>0.0007</b>	<b>H &gt; L</b>
		Temperature	0.061	1	0.803	
		<b>Nutrient:Temperature</b>	<b>6.074</b>	<b>1</b>	<b>0.013</b>	<b>H25 = H31 &gt; L31, H25 &gt; L25</b>
	Chlorophyll a	<b>Nutrient</b>	<b>15.314</b>	<b>1</b>	<b>0.0001</b>	<b>H &gt; L</b>
		<b>Temperature</b>	<b>4.080</b>	<b>1</b>	<b>0.043</b>	<b>25 &gt; 31</b>
		Nutrient:Temperature	0.380	1	0.537	
	β-Carotene	Nutrient	0.323	1	0.569	
		Temperature	0.003	1	0.955	
		Nutrient:Temperature	0.123	1	0.725	
	Lutein	<b>Nutrient</b>	<b>5.562</b>	<b>1</b>	<b>0.018</b>	<b>H &gt; L</b>
		Temperature	1.046	1	0.306	
		Nutrient:Temperature	3.251	1	0.071	
	VAZ (Zeaxanthin)	<b>Nutrient</b>	<b>7.425</b>	<b>1</b>	<b>0.006</b>	<b>H &gt; L</b>
		Temperature	2.237	1	0.134	
		Nutrient:Temperature	0.003	1	0.953	
	% N	Nutrient	0.262	1	0.608	
		Temperature	1.052	1	0.304	
		<b>Nutrient:Temperature</b>	<b>5.695</b>	<b>1</b>	<b>0.017</b>	<b>H25 &gt; L25</b>
	% C	Nutrient	0.473	1	0.491	
		<b>Temperature</b>	<b>6.348</b>	<b>1</b>	<b>0.011</b>	<b>31 &gt; 25</b>
		Nutrient:Temperature	3.641	1	0.056	
% P	Nutrient	0.151	1	0.697		
	<b>Temperature</b>	<b>9.521</b>	<b>1</b>	<b>0.002</b>		
	<b>Nutrient:Temperature</b>	<b>5.480</b>	<b>1</b>	<b>0.019</b>	<b>L25 &lt; H31, L31</b>	
CN ratio	<b>Nutrient</b>	<b>4.123</b>	<b>1</b>	<b>0.042</b>	<b>L &gt; H</b>	
	Temperature	1.858	1	0.172		
	Nutrient:Temperature	3.396	1	0.065		
CP ratio	Nutrient	0.958	1	0.327		
	<b>Temperature</b>	<b>6.971</b>	<b>1</b>	<b>0.030</b>	<b>25 &gt; 31</b>	
	<b>Nutrient:Temperature</b>	<b>4.028</b>	<b>1</b>	<b>0.044</b>	<b>H31 &lt; L31</b>	
NP ratio	Nutrient	0.971	1	0.324		
	<b>Temperature</b>	<b>4.172</b>	<b>1</b>	<b>0.041</b>	<b>25 &gt; 31</b>	
	<b>Nutrient:Temperature</b>	<b>5.629</b>	<b>1</b>	<b>0.017</b>	<b>H31 &lt; L31; H25 &gt; L25</b>	
Branching	<b>Nutrient</b>	<b>6.188</b>	<b>1</b>	<b>0.012</b>	<b>H &gt; L</b>	
	Temperature	1.018	1	0.312		
	Nutrient:Temperature	1.674	1	0.195		

Note: The outcome of the random effects is provided in Table S2. Significant differences indicated by p values < 0.05 are printed in bold. Effect directions and outcome of subsequent pairwise comparisons are provided for significant fixed effects and can be found in detail in Table S3.

of  $25 \pm 7$  and  $31 \pm 4$  branches  $\cdot \text{cm}^{-2}$  were observed in L25 and in L31 respectively, whereas higher branching numbers of  $42 \pm 11$  and  $39 \pm 6$  branches  $\cdot \text{cm}^{-2}$  were observed in H25 and in H31 respectively. In addition, microscopic observations also revealed various epiphytes (*Hydrolithon* sp., *Ulvea* sp., and unidentifiable green filamentous algae).

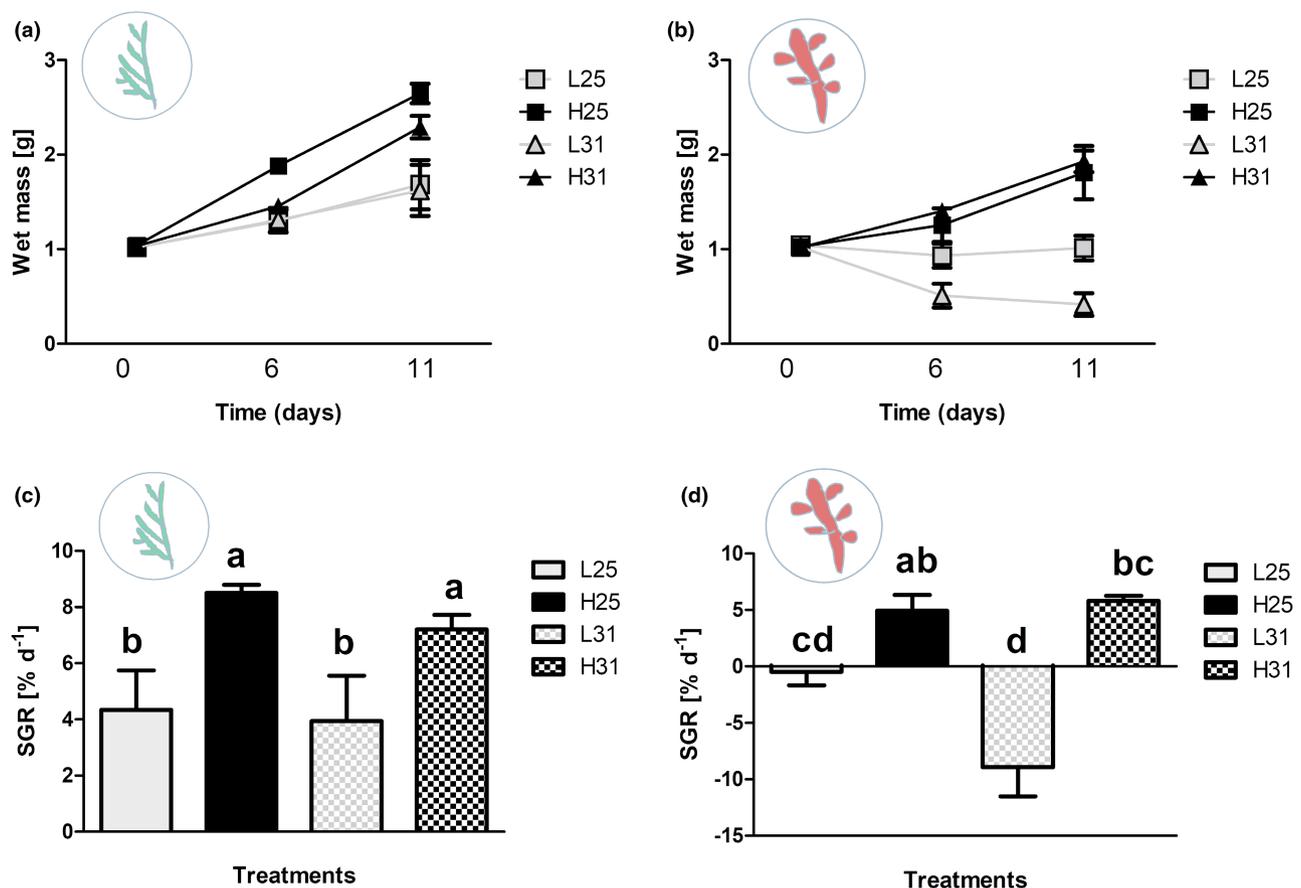
## Ammonium uptake

We observed strong differences in the uptake pattern for the different species. The incubations showed that *Cladophoropsis* sp. took up ammonium faster at 31°C and decreased significantly in its overall uptake at the end of the study at D11 (Table 2; Figure 3a). For *Laurencia*

**TABLE 2** Output of the mixed-effects model (Model 2) testing the differences of Ammonium uptake in the high Ammonium (HL) treated mesoalgae *Cladophoropsis* sp. and *Laurencia* sp. in relation to temperature (25°C and 31°C) and exposure time (D0, D6, and D11). Temperature treatment, sampling time, and their interaction were the explanatory variables (fixed effects), and the algae ID was the random effect accounting for the repeated measurements on the same algae at three sampling times.

Species	Explanatory variables	Chi-square	df	p-value	Pairwise comparison
<i>Cladophoropsis</i> sp.	Temperature	8.291	1	0.004	31 > 25
	Time	22.537	1	<0.0001	D0 = D6 > D11
	Temperature × time	5.357	1	0.068	
<i>Laurencia</i> sp.	Temperature	0.950	1	0.329	
	Time	17.052	1	0.0002	D0 < D6 = D11
	Temperature × time	6.655	1	0.035	See Figure 3 for detailed pairwise comparisons

Note: The outcome of the random effects are provided in Table S2. Significant differences indicated by p values < 0.05 are printed in bold. Effect directions and outcome of subsequent pairwise comparisons are provided for significant fixed effects and can be found in detail in Table S3.

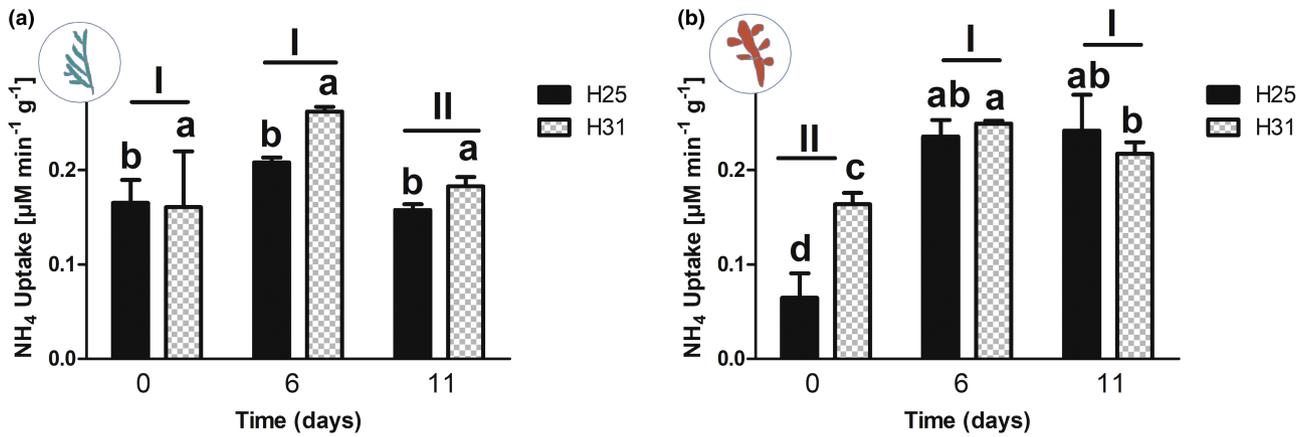


**FIGURE 2** Differences in biomass growth of *Cladophoropsis* sp. (left) and *Laurencia* sp. (right) exposed to low (L) and high (H) ammonium additions at 25 or 31°C (L25, H25, L31, H31). Top panel (a, b): Algal wet mass (g) plotted as a function of time (days). Bottom panel (c, d): Specific growth rates (% · d<sup>-1</sup>) of mesoalgae under each treatment. Small letters and numbers indicate significant differences identified by pairwise comparisons following mixed effect Model 1, provided in Table 1 and Table S2. Data showing mean ± SE, n = 3.

sp., a significant temperature × sampling time interaction was observed (Table 2), as it also incorporated ammonium faster at elevated temperature (31°C) at the start of the experiment, but this effect vanished over experimental time (Figure 3b). In addition, uptake rates increased significantly over the initial 6 days (Table 2).

## Tissue nutrient content

Post-experimental analysis of the tissue %C, %N, and %P contents showed differences among nutrient and temperature treatments (Tables 1 and 3). In both species, %C significantly increased with



**FIGURE 3** Ammonium ( $\text{NH}_4^+$ ) uptake calculated for the mesoalgae *Cladophoropsis* sp. (a) and *Laurencia* sp. (b), exposed to high  $\text{NH}_4^+$  concentrations at different temperatures (25°C, 31°C) and times (D0, D6, D11). Small letters and numbers indicate significant differences identified by pairwise comparisons following mixed effect Model 2, provided in Table 1 and Table S3, whereas numbers indicate differences in time. Data showing mean  $\pm$  SE,  $n=3$ .

elevated temperature. In contrast, %N was highly sensitive to increased ammonium concentrations with species-specific differences in response. Although *Cladophoropsis* sp. had consistently higher %N in the high nutrient treatment, the nutrient effect vanished under elevated temperature in *Laurencia* sp. (Table 3). The C:N ratio of the tissue significantly decreased under elevated ammonium concentrations in both species (Tables 1 and 3). The %P was highly temperature dependent, significantly increasing its concentrations under elevated temperature for both species (Tables 1 and 3). Consequently, C:P values decreased with elevated temperature for both species. Similarly, N:P significantly decreased for *Cladophoropsis* sp. at 31°C, while *Laurencia* sp. showed an interaction between the nutrient and temperature treatments, peaking at H25 and showing its lowest level at H31 (Table 3).

Considering the C:N:P ratios, *Cladophoropsis* sp. showed the highest ratios of 1311:33:1 under low ammonium conditions at low temperature (L25), decreasing to minimum levels of 310:7:1 in high temperature (L31). For *Laurencia* sp. both extreme ratios were observed under ammonium-enriched conditions, ranging from 2634:149:1 at 25°C to 1165:51:1 at 31°C (Table 3).

## Photosynthetic performance

Maximum quantum yield ( $F_v/F_m$ ) varied across treatment and time for both species (Figure 4). After 6 days of exposure to the experimental treatments, we measured significant higher yields under elevated temperature for both species (Table 4; Figure 4). While this pattern remained consistent for *Laurencia* sp. until the end of the experiment, *Cladophoropsis* sp. showed a weak nutrient:temperature interaction on D11 (Table 4),

which translated into non-significant pairwise differences among the treatments. For both species, maximum quantum yield decreased with time.

## Pigment analysis

*Cladophoropsis* sp. showed higher content of chlorophyll *a*, chlorophyll *b*, and lutein under the high nutrient treatment, whereas the concentrations of  $\beta$ -carotene and VAZ showed significant interactions with temperature (Figure 5a; Table 1). Considering the photoprotective state of the xanthophyll cycle, temperature significantly decreased the DPS (from  $0.7 \pm 0.01$  to  $0.4 \pm 0.02$  in low ammonium conditions), whereas no differences was measured between the different nutrient treatments (Figure 5b; Table 1) for the green mesoalga (Figure 5c).

In *Laurencia* sp., chlorophyll *a* content significantly increased under the elevated ammonium treatment but decreased with elevated temperature (Figure 5d; Table 1). Lutein and the VAZ increased significantly with increasing ammonium levels. Although phycobilins in red algae were not measured through the HPLC method, red coloration of *Laurencia* sp. was visibly darker in the high ammonium treatments, indicating a higher content of these pigments in those treatments (Figure 5e).

## DISCUSSION

The ammonium enrichment, as well as the heat-wave, significantly affected the two mesoalgae *Cladophoropsis* sp. and *Laurencia* sp. in this study. Moreover, taxa-specific differences were recognized, as shown in the summary overview (Figure 6).

**TABLE 3** Percent carbon (%C), nitrogen (%N) and phosphorous (%P) and C:N, C:P and N:P molar ratios of the mesoalgae exposed to different ammonium (Nut: low and high) and temperature (T: 25 and 31°C) levels.

Species	Nut	T	%C	%N	%P	C:N	C:P	N:P
<i>Cladophoropsis</i> sp.	L	25	24.5 ± 0.8 <sup>b</sup>	0.70 ± 0.15 <sup>b</sup>	0.050 ± 0.014 <sup>b</sup>	42 ± 9 <sup>a</sup>	1311 ± 701 <sup>a</sup>	33 ± 19 <sup>a</sup>
	H	25	25.3 ± 0.6 <sup>b</sup>	1.08 ± 0.16 <sup>a</sup>	0.049 ± 0.011 <sup>ab</sup>	28 ± 4 <sup>b</sup>	1177 ± 251 <sup>a</sup>	43 ± 8 <sup>a</sup>
	L	31	25.9 ± 0.4 <sup>a</sup>	0.71 ± 0.02 <sup>b</sup>	0.163 ± 0.032 <sup>a</sup>	43 ± 1 <sup>a</sup>	310 ± 121 <sup>b</sup>	7 ± 3 <sup>b</sup>
	H	31	26.0 ± 0.8 <sup>a</sup>	0.98 ± 0.1 <sup>a</sup>	0.065 ± 0.020 <sup>a</sup>	31 ± 2 <sup>b</sup>	676 ± 426 <sup>b</sup>	22 ± 16 <sup>b</sup>
<i>Laurencia</i> sp.	L	25	21.8 ± 1.4 <sup>b</sup>	1.04 ± 0.1 <sup>b</sup>	0.024 ± 0.003 <sup>b</sup>	25 ± 3 <sup>a</sup>	2704 ± 633 <sup>a</sup>	110 ± 25 <sup>b</sup>
	H	25	21.0 ± 1.1 <sup>b</sup>	1.38 ± 0.18 <sup>a</sup>	0.028 ± 0.004 <sup>ab</sup>	18 ± 2 <sup>b</sup>	2634 ± 245 <sup>a</sup>	149 ± 26 <sup>a</sup>
	L	31	26.2 ± 1.1 <sup>a</sup>	1.25 ± 0.13 <sup>ab</sup>	0.045 ± 0.005 <sup>a</sup>	25 ± 3 <sup>a</sup>	2211 ± 651 <sup>b</sup>	93 ± 39 <sup>c</sup>
	H	31	22.7 ± 1.3 <sup>a</sup>	1.16 ± 0.19 <sup>ab</sup>	0.038 ± 0.002 <sup>a</sup>	23 ± 3 <sup>b</sup>	1165 ± 343 <sup>c</sup>	51 ± 17 <sup>d</sup>

Note: Differences between the treatments are indicated with superscript letters. Data are showing mean ± SD of n=3.

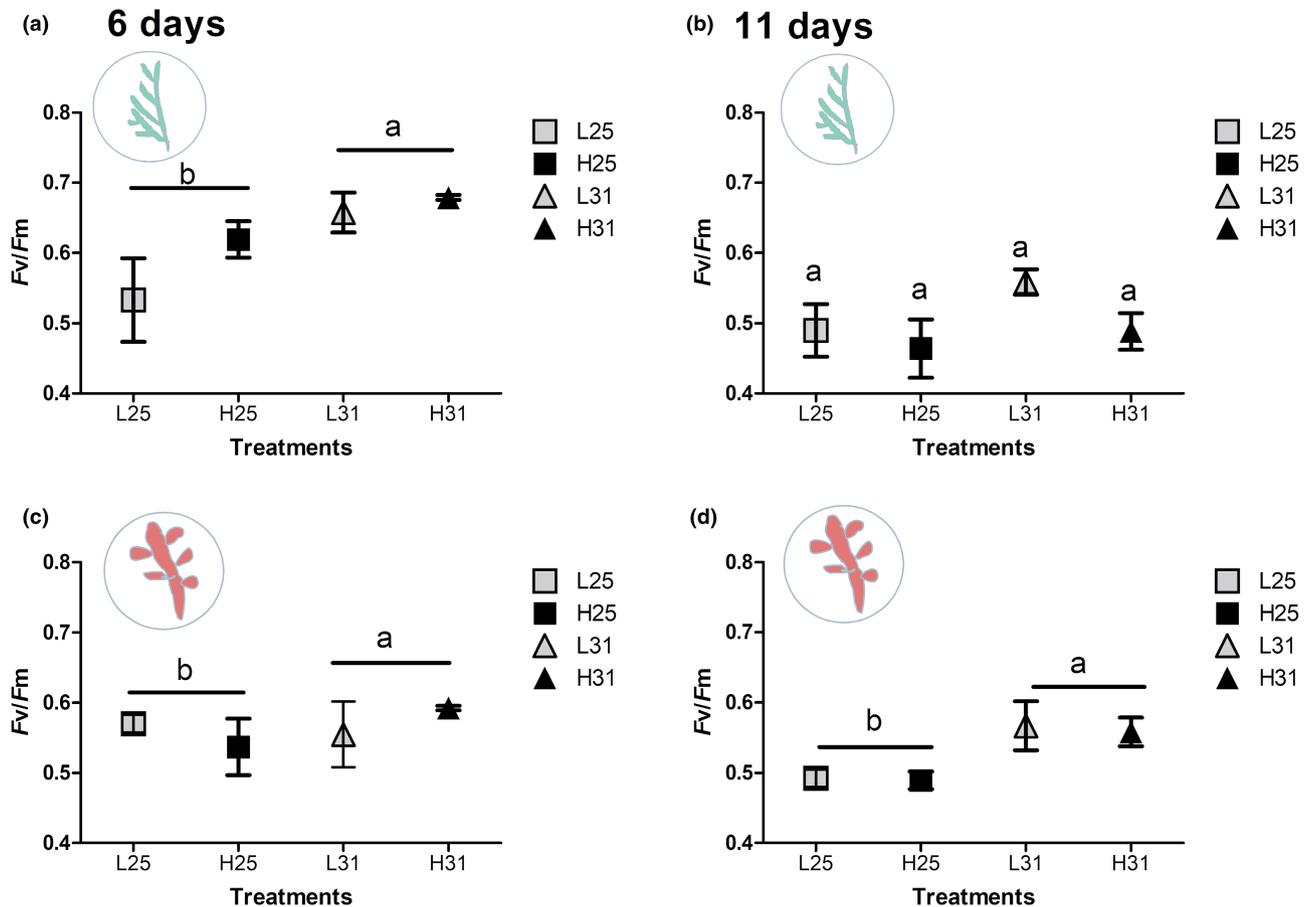
## Responses to increased ammonium concentrations

The mesoalgae were able to rapidly respond to the altered ammonium conditions, acclimatizing their physiology and morphology within days. This observed fast and effective acclimatization provides a clear competitive advantage against other primary producers, such as seagrasses (Leliaert et al., 2001; Wear et al., 1994), and might explain the reported increasing dominance of the otherwise rather cryptic mesoalgae in different coastal ecosystems, including coral reefs (Karcher et al., 2020; Stuercke & Mcdermid, 2004; Vermeij et al., 2010).

The mesoalgae responded positively to the elevated ammonium concentrations, with taxa-specific responses. In both species, ammonium uptake happened quickly over the 60 minutes, when provided in high concentrations. As most of the marine and estuarine habitats are N-limited (Paerl, 2018), this rapid uptake might indicate an adaptive strategy by the mesoalgae to make the best use of ammonium when available. *Cladophoropsis* sp. peaked in its ammonium uptake after 6 days of the experiment, whereas the observed decrease during the end of the study might either reflect an inner saturation of the quickly incorporated nutrients or a change in the algal metabolic activity. The incorporated N significantly increased the %N tissue content and lowered the correspondent C:N ratio, elevated the relative growth rates, and supported the photosynthetic apparatus with higher pigment concentrations. This pattern has also been observed in other enrichment studies for green algae (e.g., *Ulva australis*; Reidenbach et al., 2017), in which an ammonium-dependent increase in the photosynthetic yield ( $F_v/F_m$ ) was also reported.

The daily growth rates (DGR; calculated after Yong et al., 2013) under ammonium enrichment of *Cladophoropsis* sp. ranged from 6.49 to 9.43%·d<sup>-1</sup>, which corresponded to the values of other Cladophorales, including *Cladophora parriaudii* (DGR=4.75–11.2%) and *C. coelothrix* (DGR=3.98–7.37%; Ross et al., 2018). The high growth rate under the ammonium enrichment of *Cladophoropsis* sp. can be explained due to its high surface area to volume ratio (Zainee & Rozaimi, 2020) and simple thallus structure (Littler & Littler, 1980), typical of opportunistic and early successional taxa (Shepherd & Edgar, 2013).

*Laurencia* sp. reacted positively to the elevated ammonium levels, but in contrast to *Cladophoropsis* sp., it increased its uptake over time, which promoted growth. This measured time lag might reveal a potential acclimatization process triggered by the elevated external N concentrations affecting the uptake kinetics and internal N storage capacity (Gouvêa et al., 2017). Similar to the response of *Cladophoropsis* sp., *Laurencia* sp. increased its %N tissue content and, consequently,



**FIGURE 4** Maximum quantum efficiency ( $F_v/F_m$ ) of PSII at various treatments (L25, H25, L31, H31;  $n=3$ ) and time points (D6 and D11). Upper panels (a, b) summarize data for *Cladophoropsis* sp., while lower panels (c, d) summarize data for *Laurencia* sp. after 6- and 11-day exposure, respectively. Small letters and numbers indicate significant differences identified by pairwise comparisons following mixed effect Model 1, provided in Table 1 and Table S2.

reduced its C:N ratio. This is indicative of algae being N-limited at ambient nutrient levels (Gouvêa et al., 2017; Lapointe et al., 2004) and in its local environment (Delgado & Lapointe, 1994; Tsai et al., 2005) and was further confirmed by the increase in growth at the high ammonium treatment. Its tissue composition under the H31 treatment closely resembles reported tissue levels (%C: 20.1, %N: 1.71, %P: 0.05) and C:N:P ratios (1080:75:1) of *L. intricata* from the carbonate and N-rich waters of the Florida Keys (Lapointe, 1992). Ammonium enrichment also had positive effects on the concentrations of chlorophyll *a* and carotenoids, which is a common response of the genus *Laurencia* to these conditions (Bender-Champ et al., 2017; Gouvêa et al., 2017) due to the algae's capacity to store N as pigments when nutrients are available (Lapointe & Ryther, 1979). However, no positive effects in the photosynthetic performance were observed for *Laurencia* sp. This result contrasts with previous reports on the species *L. intricata* and *L. catarinensi*, for which photosynthetic rates were positively correlated to N and P enrichment (Gouvêa et al., 2017; Reef et al., 2012). In addition to the increase in the measured chlorophyll

and carotenoids, we also observed a change in the reddish thallus coloration, indicating an increase in the phycobilin content under elevated ammonium levels. The phycobilisome content (complex of chromatophores, the phycobilins, and the phycobiliproteins) is known to adjust to light and N-availability (Ruan et al., 2018) and might be consequently used as internal N storage in red algae (Lapointe, 1981). In addition, the elevated ammonium levels also induced a change in the thallus morphology of *Laurencia* sp., expressed by a higher number of branchlets. Branching morphology in the genus *Laurencia* has been reported to be a reliable indicator of environmental disturbances (Kilar & McLachlan, 1986). This change in branching pattern might indicate morphological acclimatization, comparable to root traits of aquatic plants to increase N uptake by altered surfaces (Huang et al., 2019). The increase in branching could be interpreted both as an increase in growth due to higher nutrient availability and an acclimatization process in which *Laurencia* sp. increased its surface area, promoting higher ammonium uptake. The quick acclimatization by *Laurencia* sp. explains its abundance in different trophic regimes, from mesotrophic

**TABLE 4** Analysis of the photosynthetic responses (*Fv/Fm*) of *Cladophoropsis* sp. and *Laurencia* sp. to the experimental treatments (nutrients and temperature) using mixed-effects models (Model 1) with nutrients and temperature as fixed terms and the experimental unit (ET) as a random effect.

Species	Day	Explanatory variables	Chi-square	df	p-value	Pairwise comparison
<i>Cladophoropsis</i> sp.	Day 6	Nutrient	0.724	1	0.394	<b>31 &gt; 25</b>
		<b>Temperature</b>	<b>6.392</b>	<b>1</b>	<b>0.011</b>	
		Nutrient:Temperature	0.129	1	0.719	
	Day 11	Nutrient	1.050	1	0.305	<b>No differences</b>
		Temperature	0.107	1	0.742	
		<b>Nutrient:Temperature</b>	<b>3.917</b>	<b>1</b>	<b>0.047</b>	
<i>Laurencia</i> sp.	Day 6	Nutrient	0.115	1	0.733	<b>31 &gt; 25</b>
		<b>Temperature</b>	<b>6.880</b>	<b>1</b>	<b>0.008</b>	
		Nutrient:Temperature	0.003	1	0.949	
	Day 11	Nutrient	0.091	1	0.762	<b>31 &gt; 25</b>
		<b>Temperature</b>	<b>8.814</b>	<b>1</b>	<b>0.003</b>	
		Nutrient:Temperature	0.023	1	0.878	

Note: The outcome of the random effects is provided in Table S2. Effect directions and outcome of subsequent pairwise comparisons are provided for significant fixed effects in detail in Table S3. Significant differences indicated by p values < 0.05 are printed in bold.

estuaries (Potter et al., 2021) to DIN-enriched ones (Lapointe et al., 2004), and why *Laurencia* is considered one of several opportunistic algae taxa (Howarth et al., 2000) in coastal ecosystems.

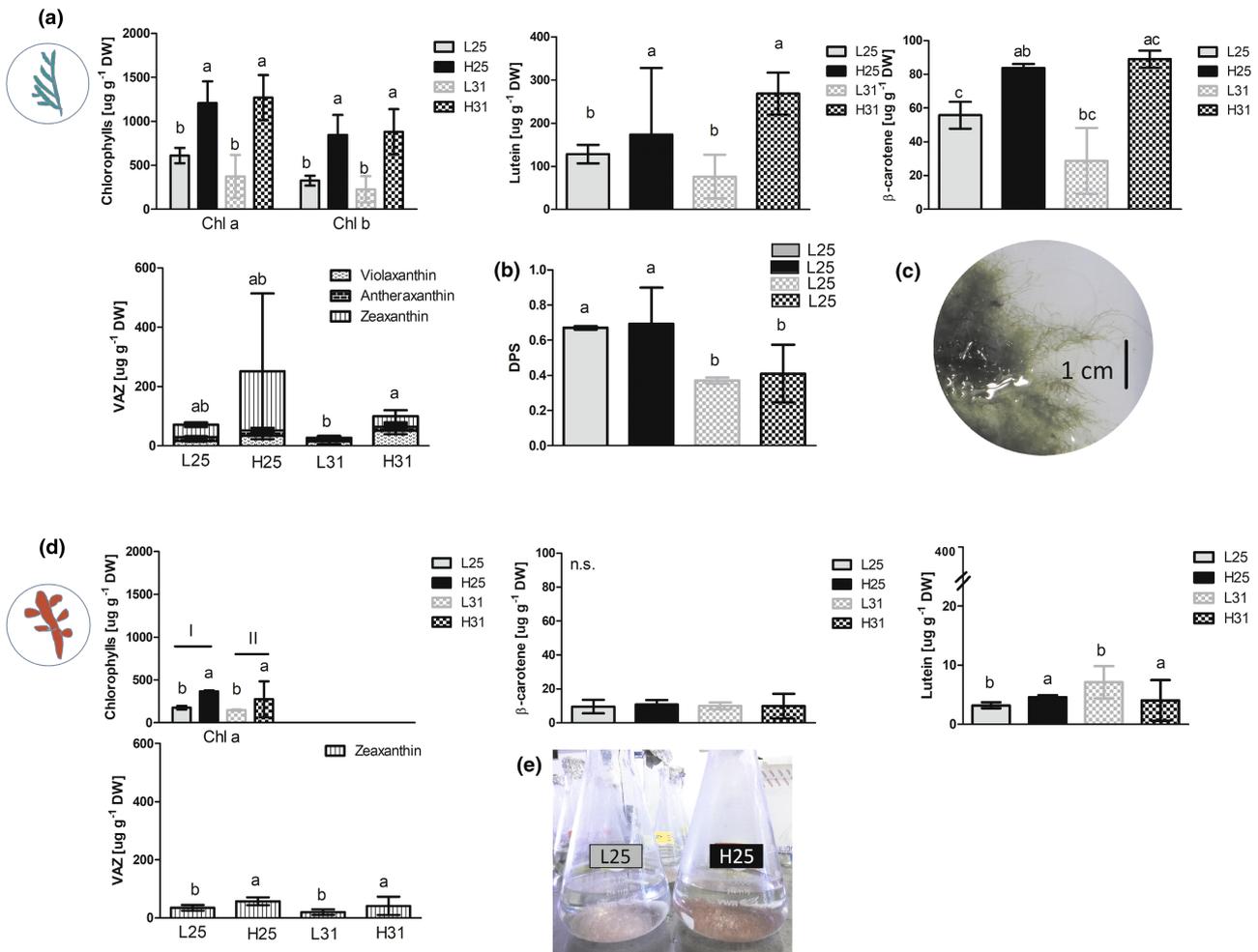
Overall, the two studied mesoalgae benefitted from ammonium enrichment, allowing them to store excess N in their tissues and pigments, while also supporting their growth. However, the two mesoalgae did not respond identically to the altered nutrient regime. In contrast to *Cladophoropsis* sp., which showed a higher initial nutrient uptake, *Laurencia* had a longer acclimatization time but exceeded *Cladophoropsis* sp. with a 3.5 higher N:P ratio at control temperature (in H25). Moreover, the maximum N:P values reached in the experiment exceeded both by 4.7–16.4 times the values of the natural environment of 4.8–9.1, measured in pore water (Moreira-Saporiti et al., 2021). These findings indicate that the oligotrophic conditions under which the mesoalgae naturally live are limiting their growth and that they probably would benefit from further coastal nutrient pollution (e.g., sewage disposal and agricultural runoff). Moreover, the measured physiological acclimatization and phenotypic adaptation (e.g., increasing branching pattern) toward the altered environmental conditions could provide these mesoalgae important competitive advantages over other benthic organisms such as seagrasses or corals (Stewart & Carpenter, 2003).

## Responses to the heatwave

Compared to ammonium enrichment, the increased temperature in the form of a heatwave appeared to be less of a driving factor for the mesoalgae.

*Cladophoropsis* sp. showed biomass growth in all treatments, which indicates that even during the heatwave, it was not experiencing a temperature beyond its thermal limit. Members of the genus *Cladophoropsis* have a strict tropical origin (Pakker et al., 1994), which explains why they have high thermal optima and could withstand or even benefit from heatwaves. This is further illustrated by the fact that several species of the genus *Cladophoropsis* can survive temperatures exceeding their local range, such as *C. sundanensis* at 35°C for 2 weeks (Pakker et al., 1995) and *C. membranacea* at 36°C (Pakker et al., 1994). Although no changes were measured in the pigment concentrations in *Cladophoropsis* sp., the de-epoxidation state (DPS) of the xanthophyll cycle decreased with elevated temperature, whereas the photosynthetic yield (*Fv/Fm*) significantly increased. Changes in the *Fv/Fm*, estimating the maximum quantum efficiency of PSII, have been widely used for stress detection, with decreasing values indicating increasing photoprotective activities such as non-photochemical quenching (NPQ; Baker & Rosenqvist, 2004). Likewise, the photoprotective DPS is suspected to increase in response to elevated stressors, like temperature alterations (Monteiro et al., 2020). Neither were the case for *Cladophoropsis* sp. in the experiment, further indicating that this taxon was within its thermal optimum during the simulated heatwave.

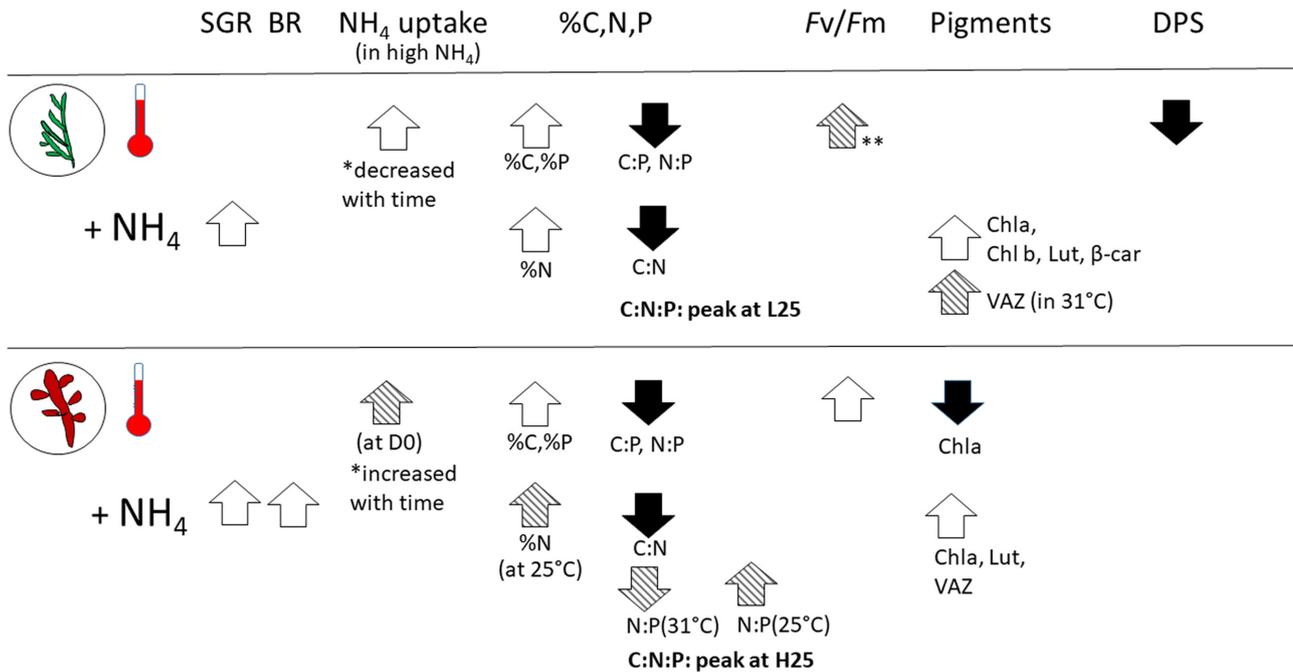
In contrast, the red mesoalga *Laurencia* sp. was more affected by the elevated temperature, reflected in a sharp decrease in growth (L31). Temperature preference in the *Laurencia* genus seems to be highly local and variable, with previous reports showing a preference of *L. papillosa* for temperatures above 30°C, which leads to blooming in the summer months (Tsai et al., 2005), *L. dendroidea* and *L. okamurae* having their maximum



**FIGURE 5** Differences in (a) photosynthetic pigment concentrations, (b) de-epoxidation state of the xanthophyll cycle (DPS) measured in (c) *Cladophoropsis* sp., and of (d) photosynthetic pigment concentrations of *Laurencia* sp., both mesoalgae exposed to various treatments (L25, H25, L31, H31;  $n=3$ ). Data showing mean  $\pm$  SD. Letters indicate significant differences based on mixed effect Model 1, provided in Table 1. (e) shows different coloration of *Laurencia* sp. observed in the L25 and H25 treatment at the end of the experiment.

growth rate at 25°C (Kuwano et al., 1998; Sudatti et al., 2011, 2021), *L. brongniartii* being negatively affected by increased temperatures typical of future climate change scenarios (Phelps et al., 2017), and *L. decussata* reducing its growth at 28°C (Graba-Landry et al., 2018). Interestingly, *Laurencia* sp. was able to counterbalance the temperature stress in the high ammonium environment (H31), in which it showed growth comparable to that under low temperatures (H25). This explains why both nutrient availability and temperature are important drivers of seasonal abundance in the genus *Laurencia* (Tsai et al., 2005) and how nutrient enrichment can buffer negative effects of thermal stress. The additional rise in temperature due to the heatwave seemed to exacerbate a negative physiological state, measured in a decreasing %N tissue content and corresponding decreasing N:P ratio. However, on the first day of the experiment, ammonium uptake was higher in the high-temperature treatment, indicating a certain benefit to the temperature increase in this physiological mechanism. This has been reported for nitrate

uptake in *L. brongniartii* (Nishihara et al., 2004), which showed increased uptake with higher temperature and irradiance. Gouvêa et al. (2017) suggest that this response could be caused by an increase in the activity of enzymes related to the metabolism of nitrogen, that is, enzymes related to carbon metabolism (RUBISCO) or nitrate reductase (Rosenberg & Ramus, 1982; Wheeler & Weidner, 1983). In *Laurencia* sp., the contradicting pattern of increasing photosynthetic yield and decreasing chlorophyll a concentration might be connected to a potential change in the temperature sensitive phycobilins (Steven, 2014). Given the absence of ammonium-driven increase in %N tissue content in the high temperature-high ammonium treatment, it might be probable that a part of the incorporated N was used for the construction of protective compounds. In fact, different types of heat stress responses are reported for algae, including the synthesis of heat shock proteins (chaperone) or regulation of internal carbohydrate concentrations or structures (Barati et al., 2019). This pattern happens also with algae growth, with higher



**FIGURE 6** Overview of the different responses measured in the two mesoalgae *Cladophoropsis* sp. (above) and *Laurencia* sp. (below) exposed to elevated temperature (marked by thermometer symbol) and high ammonium level (marked by + NH<sub>4</sub>). Arrows indicate the response direction of the parameter investigated: specific growth rate (SGR), branching (BR), NH<sub>4</sub> uptake, percentage of C, N, and P; Fv/Fm; pigment concentrations; and the de-epoxidation state of the xanthophyll cycle (DPS). Whereas the white arrow indicates an overall positive response, the black arrow indicates an overall negative response and the dashed arrow indicates a response limited to certain treatment (interactive effects, which are provided in brackets). \*For NH<sub>4</sub> uptake: timewise changes in uptake rates were indicated. \*\*no differences were found in pairwise comparison investigating the significant interactive effect in Fv/Fm data for *Cladophoropsis* sp. as indicated in Table 4.

photosynthetic yield and lower growth at the heatwave treatment. These two variables generally follow the same direction, as shown by Nishihara et al. (2004) when they measured a decrease in photosynthetic yield and growth when *Cladophoropsis* sp. was exposed to a temperature beyond its thermal optimum. This pattern can be explained due to the fact that the algae goes through an acclimatization process (Padilla-Gamiño & Carpenter, 2007), and there is a lag between the acclimatization of the photosynthetic apparatus and the general fitness of the algae, as reflected by algae growth. Considering that both algae showed increased photosynthetic efficiency under increased temperature, we can conclude that they were still within their thermal optima (Padilla-Gamiño & Carpenter, 2007) during the heatwave. The decrease in the growth of *Laurencia* sp. under the heatwave treatment may be due to a need for a longer process of acclimation for the physiology of the algae. However, *Laurencia* sp. shows how nutrient excess can buffer the negative effects of temperature stress, indicating that eutrophication can help ameliorate the threat of heatwaves. The acclimation of both mesoalgae to the short-term exposure to warmer temperatures reflects their capacity to withstand heatwaves, which do not seem to pose an immediate threat to their survival, especially under nutrient enrichment conditions.

## Ecological implications

Eutrophication is one of the major current threats to coastal ecosystems worldwide, deteriorating water quality (Adams et al., 2020), causing phase shifts (McManus & Polsenberg, 2004), and impacting coastal species negatively. Eutrophication is mainly caused by large inputs of nitrogen and phosphorus from agricultural lands (Hodgkin & Hamilton, 1993) and aquaculture (Thomsen et al., 2020) being flushed to the coastal areas. In addition to eutrophication, heatwaves exceeding the maximum summer temperatures pose a regular and periodical threat to coastal ecosystems (Strydom et al., 2020). The results of this experiment shed light on how coastal mesoalgae will behave in future eutrophication and short-term temperature increase scenarios.

Nutrient additions facilitate eutrophication and the colonization of estuarine ecosystems (Potter et al., 2021), coral reefs (McClanahan et al., 2002), and seagrass (Valesini et al., 2019) by opportunistic algae. Opportunistic bloom-formers have been reported to have higher N uptake rates compared with more specialized algae, enabling these algae to out-compete the local species (Pedersen, 1994; Pedersen & Borum, 1997). Despite the fact that marine angiosperms can benefit from nutrient enrichment (Viana

et al., 2020), their acclimation process seems to be slower than that of mesoalgae. Additionally, their light requirements are higher than that of algae, and considering the epiphytic nature of *Laurencia* sp. and *Cladophoropsis* sp., these mesoalgae could overgrow, shade, and ultimately outcompete seagrasses in eutrophication scenarios where top-down control is limited.

Although excess nutrients favor mesoalgae growth, there is also a tipping point at which N and P can become toxic (Reef et al., 2012; Tsai et al., 2005). This happens when the capacity to safely store them as pigments, proteins, or other compounds is exhausted (Bird et al., 1982; Lobban & Harrison, 1994). Excessive increases of DIN in seawater can, therefore, shift the dominance of diverse, multilayered algae communities to structurally simple communities dominated by one species (Skriptsova et al., 2018). So, while eutrophication is beneficial for mesoalgae, uncontrolled nutrient enrichment of coastal ecosystems can even go beyond the safe limit for opportunistic algae. There are already reports indicating that *Laurencia* spp. cannot survive under extreme nutrient enrichment (Potter et al., 2021) and that there are tipping point concentrations of DIN at which the growth of *L. papillosa* turns negative (Tsai et al., 2005).

Heatwaves can push organisms above their thermal optimum and negatively affect their physiology. In the case of the mesoalgae *Cladophoropsis* sp. and *Laurencia* sp., we did not find severe negative effects of the simulated heatwave, indicating that these taxa can withstand temperatures above the typical local conditions. This stands in opposition to other taxa, like corals or marine plants, which have been shown to bleach (Leggat et al., 2019) and die-off (Strydom et al., 2020) due to severe temperature increases. Additionally, these other benthic organisms tend to be negatively affected by eutrophication (McManus & Polsenberg, 2004), further decreasing their possibilities of survival, whereas mesoalgae benefit from eutrophied conditions that could even buffer any negative effect observed by temperature increases. This indicates that, if these conditions become the norm in coastal tropical ecosystems, a phase shift is to be expected from the long-lived, benthic corals and marine plants to the more opportunistic and ephemeral mesoalgae, as has already been reported from different sites (Karcher et al., 2020; Vermeij et al., 2010; Wear et al., 1994). The implications of this change are not only a shift in the dominant taxon but also in the ecosystem functions and services provided.

## CONCLUSIONS

The main conclusions of this study are that increased ammonium levels generally increased the performance of both mesoalgae, while elevated temperature alone

decreased their performance. It is likely that these mesoalgae will be more limited by nutrients at higher temperatures and will perform well only under enriched conditions. The observed species-specific responses revealed how additional ammonium increase could improve the growth and, thus, the survival of the otherwise temperature-stressed mesoalgae. In the long run, this N-rendered enhancement in growth could help to sustain more than one species in the mesoalgal community. A combination of ocean warming (e.g., temperature anomalies and heatwaves) and ammonium input (e.g., via agricultural run-off to the ocean) could in fact be beneficial not only for the growth but also for the diversity of the bloom-forming tropical mesoalgae. Such multi-species algal assemblages could further expand over the reefs, thus functioning as a biologically mediated habitat for the support and sustenance of the associated community. Inversely, in the case that the bloom-forming mesoalgae is considered as invasive or as a harmful species, this could have substantial negative impacts on the native community structure and even impact commercial seaweed cultivation by overgrowth. This study highlights the importance of undertaking multi-factor approaches for a more holistic understanding of the impacts of climate change in combination with other local environmental drivers.

## AUTHOR CONTRIBUTIONS

**Anna Fricke:** Conceptualization (equal); data curation (equal); formal analysis (equal); investigation (equal); methodology (equal); visualization (equal); writing – original draft (equal). **Felix Bast:** Conceptualization (equal); formal analysis (equal); funding acquisition (equal); investigation (equal); methodology (equal); resources (equal); validation (equal); visualization (equal); writing – original draft (equal). **Agustin Moreira Saporiti:** Data curation (equal); formal analysis (equal); writing – review and editing (equal). **Giovanni Martins Bussanello:** Investigation (supporting); methodology (supporting); resources (supporting). **Flower Msuya:** Funding acquisition (equal); project administration (equal); resources (equal); writing – review and editing (equal). **Mirta Teichberg:** Conceptualization (equal); funding acquisition (lead); investigation (equal); methodology (equal); project administration (lead); resources (equal); software (equal); supervision (equal); validation (equal).

## ACKNOWLEDGMENTS

This project was under the framework of the DFG project “Seagrass and macroalgal community dynamics and performance under environmental change (SEAMAC; TE 1046/3-1)” awarded to M. Teichberg. Authors thank Matthias Birkicht and Dr. Donata Monien for their help with plate reader calibration, Dr. Fay Belshe for support with the statistics, Prof. Dr. Kai Bischof and Britta Meyer-Schlosser

(University of Bremen) for help with nutrient and pigment analyses and the ZMT-MAREE team for their support in cultivation. GB internship was supported by the Brazilian-German Aquaqualture Network BluEcoNet. FB's research stay at the host laboratory was supported by an international fellowship from Non-Aligned Movement-Leibniz-Zentrum für Marine Tropenforschung (NAM-ZMT: NAM-05/69/2018). Open Access funding enabled and organized by Projekt DEAL.

## DATA AVAILABILITY STATEMENT

The data supporting the findings of this study are available from the corresponding author upon request.

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## SUPPORTING INFORMATION

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**File S1.** Containing Tables S1–S4.

**How to cite this article:** Fricke, A., Bast, F., Moreira-Saporiti, A., Martins Bussanello, G., Msuya, F. E., & Teichberg, M. (2024). Tropical bloom-forming mesoalgae *Cladophoropsis* sp. and *Laurencia* sp.—responses to ammonium enrichment and a simulated heatwave. *Journal of Phycology*, 00, 1–20. <https://doi.org/10.1111/jpy.13435>