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Original Article

Algal response to nutrient enrichment; insights into growth and chemical concentrations in two algae, *Halimeda macroloba* Decaisne and *Turbinaria conoides* (J. Agardh) Kützing

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Abstract

The effect of nutrient enrichment on the growth and chemical concentrations of two different algal life strategies, *Halimeda macroloba* and *Turbinaria conoides* were assessed to test the predictions of the Carbon-Nutrient Balance Hypothesis (CNBH). We concluded that nutrient enrichment had no strong effects on the growth but had a direct effect on the polyphenol concentration of both *H. macroloba* and *T. conoides*. These results support the CNBH that nutrient plays an important role in the polyphenol concentrations of these two species. Polyphenol concentrations of *H. macroloba* and *T. conoides* were higher at the ambient site than the enriched site. However, the growth of both species did not change significantly and did not vary throughout the time. When comparing the responses of *H. macroloba* and *T. conoides* to the nutrient enrichment, we found that *Halimeda* allocated resources to growth and defense while *Turbinaria* allocated more resources into defense than growth.

Keywords: carbon-nutrient balance hypothesis, *Halimeda macroloba*, Nutrient enrichment, Polyphenol, *Turbinaria conoides*

1. Introduction

In recent years, increased nutrient availability has an increasing impact on coastal waters worldwide (Bokn *et al*., 2002). Nutrient enrichment has affected macroalgal abundance and community structures (Thacker *et al*., 2001) and can dramatically increase macroalgal growth and biomass (Lapointe, 1999). Nutrients also may affect other aspects of resource allocation among such important functions as growth, reproduction, and chemical defense (Bazzaz *et al*., 1987; Pfister and Van Alstyne, 2003). It may modify the trade-off between growth and defense. The allocation to defense can be costly in terms of growth, especially when nutrients are enriched (Yates and Peckol, 1993; Jormalainen and Honkanen, 2004). For example, when nutrients are limited, algae may allocate resources to defense that make it less attractive to herbivores (Cronin and Lodge, 2003; Dethier *et al*., 2005). When nutrients are added, more resources might be allocated to growth than defenses (Yates and Peckol, 1993; Cronin and Hay, 1996).

These responses to nutrient availability are related to

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the carbon/nutrient balance hypothesis (CNBH). The CNBH predicts that the resource allocation to growth and defense in plants is regulated by nutrient concentrations (Bryant *et al.*, 1983). When growth is nutrient limited, plants will allocate photosynthate to carbon-based secondary metabolites rather than to growth (Bryant *et al.*, 1983). In marine systems, there has been limited research on the direct effects of nutrient enrichment on secondary metabolites of marine macroalgae. There are a few studies that have addressed the CNBH, and showed that nutrient availability may or may not to be related to the concentration of secondary metabolites (Yates and Peckol, 1993; Cronin and Hay, 1996; Pavia and Toth, 2000). These studies examined the polyphenol-producing algae (Cronin and Hay, 1996; Pavia and Toth, 2000) but have not been tested and compared with algae of different life strategies.

From the idea of *r*- and *K*- selection, one would expect that life strategies of different algae may involve different allocation of resources (Lawrence and McClintock, 1988). A higher resource allocation towards growth would be expected in *r*-selected species than in *K*-selected species, which are expected to allocate more resources into defense. In addition, there are no experiments testing the carbon/nutrient balance hypothesis for *Halimeda* and *Turbinaria*. These two subtidal algal species; *Halimeda macroloba* and *Turbinaria conoides* were used in this study because the subtidal algal species live in a discrete range of depths, with the lower limit set by light limitation, and they provide an ideal system for examining the predictions of the CNBH regarding carbon limitation (Van Alstyne *et al.*, 2001).

Responses of algae with different life strategies to environmental changes may help to get a better understanding about resource allocation patterns in each life strategy and how nutrients affect these patterns. So, the aims of this study were to assess the effect of nutrient enrichment on growth and chemical defense and test the predictions of CNBH with two algae with different life strategies: *Halimeda macroloba* and *Turbinaria conoides*. An experiment was designed to address the following questions: 1) Are selected algae behaving differently at resource allocation when algae encounter nutrient enrichment? 2) Does nutrient enrichment influence resource allocation to chemical defense?

2. Materials and Methods

This study was carried out in the shallow (2-3 m in depth) coastal waters at Ko Rab and Ko Tan, Mu Ko Thale Tai National Park (9° 192 203 N, 99° 462 803 E), Gulf of Thailand, Southern Thailand. The climate of this area is tropical and under monsoonal influence. There are two dominant seasons, a dry season dominated by Southwest Monsoon (February-April) and a rainy season predominated by Northeast Monsoon (May-January) with a major rain peak in November. The average rainfall varies from 8.1-302 mm and the sea surface water temperature varies from 27-30°C. The salinity varies between 29-32 ppt. The maximum and minimum tidal ranges for the Ko Samui coast are about 3 and 0.8 m, respectively. The tides are semi-diurnal. The study sites that were chosen depended on the unique distribution of each of the 2 taxon; *Halimeda macroloba* and *Turbinaria conoides*. Depending on their life strategy, *Halimeda* can be defined as an *r*-selection with fast growth, early reproduction and short life span. *Turbinaria* can be classified as a *K*-selection with a slower growth rate, delayed reproduction and a longer life span.

The study was conducted during May-June, 2008, when *H. macroloba* and *T. conoides* were observed and taken at 3 fixed sampling times, after 7, 21 days and 2 months. Twelve ambient and 12 nutrient enriched plots (50 cm x 50 cm) of each species were permanently marked using thread and labelled with plastic tags. Each plot was placed at a maximum of 1 or 2 m apart. Algae inside each plot were pruned by removing primarily the larger / older algae to eliminate variance in density and age between plots and leaving about 30 and 20 young mature thalli of *Halimeda* and *Turbinaria*, of mean length 9.4±0.87 cm and 12±0.2 cm (mean ± SE), respectively. To determine the effect of nutrients, nutrients levels were manipulated into 2 levels: enriched and ambient levels with three replicates of each. Enrichment involved adding Osmocote® a slow-release fertilizer; 60 g of Osmocote fertilizer (14-14-14 [N-P-K]) were enclosed in three mesh bags constructed from nylon hosiery. The mesh bags were placed in the center and two diagonal corners of each plot. The bags were changed biweekly. The fertilizer treatment could have potentially increased nutrient concentrations about 2 times in the enriched treatment comparing with the ambient treatment. The ambient nitrate and phosphate concentrations were 0.07±0.02 mg/L and 0.47±0.04 mg/L, respectively (Mayakun, unpublished data).

For growth measurements, biomass (wet and dry weight), the thallus length (holdfast to frond apex), the width of the base of the stipe, number of segments, holdfast and stipe diameter of all plants were measured.

To measure the nutrient uptake ability, tissue nutrient concentrations (carbon, nitrogen, phosphorus content) in the vegetative tissues of algae were measured. To assess their carbon, nitrogen, and phosphorus content the macroalgae were collected from each plot and placed in Zip-loc bags, returned to the laboratory, cleaned of sediment and epiphytes, then wet-weighed and dried in an oven (65°C) to constant weight. The dried samples were ground to a fine powder with a mortar and pestle and subsequently analyzed for C:N:P ratios using Fluorescence Spectrometer at the Central Analytical Center, Faculty of Natural Resources, Prince of Songkla University.

To determine polyphenol concentrations, the algae were collected from each plot and placed in Zip-loc bags, returned to the laboratory under dark and cold conditions. Algae were sorted and cleaned of sediment and epiphytes and frozen in -85°C until used. The algae were then minced by a food cutter to a fine powder. The ground tissues were extracted in 80% aqueous ethanol in a 500 mL flask and then placed on an ultrasonic shaker for 1 h. The ethanol extract was filtered and dried under reduced pressure using a rotary evaporator. The polyphenol concentrations were measured using a modified Folin-Ciocalteu method (Folin and Ciocalteu, 1927) for quantification of the total polyphenol concentration. For the assay, 500 µL of Folin-ciocalteu's phenol reagent was added to the extracted samples and samples kept at room temperature for 3 min. After 3 min, 100 µL of saturated sodium carbonate solution was added and kept in the dark at room temperature for 1 h. The absorbance of the samples was read at 725 nm. Phloroglucinol (1, 3, 5-trihydroxybenzene) was used as a standard.

3. Data Analysis

A 2-way analysis of variance was used to examine the effects of the fixed factors, nutrient treatments and sampling times on algal growth, and chemical concentrations. Cochran's *C*-test was used to determine the homogeneity of variances. In the case of a violation, a log (x+1) transformation was made prior to analysis. Differences among the mean effects of nutrients at each sampling time and between treatments were tested with Tukey's HSD test. All statistical analyses were made using the computer program SPSS for Windows version 11.5.

4. Results

The nutrient enrichment had a significant influence on the mean diameter of the holdfast and stipe of *Halimeda macroloba* ($p=0.032$, $p=0.035$, Table 1A). All other terms such as thallus length and dry weight were not significantly different. After one week of enrichment, the diameter of the holdfast and stipe was greater and this trend continued over the 3 weeks and 2 months (Figure 1A, Table 2). After 3 weeks and 2 months of enrichment, the thallus length and dry weight in *H. macroloba* were longer and had a greater dry weight (Figure 1A, Table 2). However, there was no significant effect on the growth of *Turbinaria conoides* ($p>0.05$, Table 1B, Figure 1B) and its growth did not vary significantly through time (Table 1B). However, after 3 weeks, the thallus length had increased and the dry weight was greater (20.5%, 46.2%, Table 2). In addition, we observed some epiphytes such as the red algae, *Jania* sp. and *Champia* sp. were present on every collected thallus of *T. conoides*. For the enriched plots, there was no significant effect on the

Table 1A. The effect of nutrient enrichment on thallus length, dry weight, holdfast diameter and stipe diameter of *Halimeda macroloba*. * $P<0.05$; ** $P<0.01$; *** $P<0.001$; ns not significant.

Species	*Halimeda macroloba*											
	Thallus length			Dry weight			Holdfast diameter			Stipe diameter		
Source	df	MS	F	df	MS	F	df	MS	F	df	MS	F
Time	2	0.272	10.247**	2	0.045	1.489***	2	0.353	7.234**	2	0.438	7.569**
Nutrient	1	0.00007	0.003ns	1	0.002	0.072ns	1	0.296	6.058*	1	0.336	5.810*
Time x nutrient	2	0.058	2.172ns	2	0.048	1.608ns	2	0.001	0.013ns	2	0.083	0.278ns
Error	11	0.027		11	0.03		11	0.049		11	0.058	
Total	17			17			17			17		

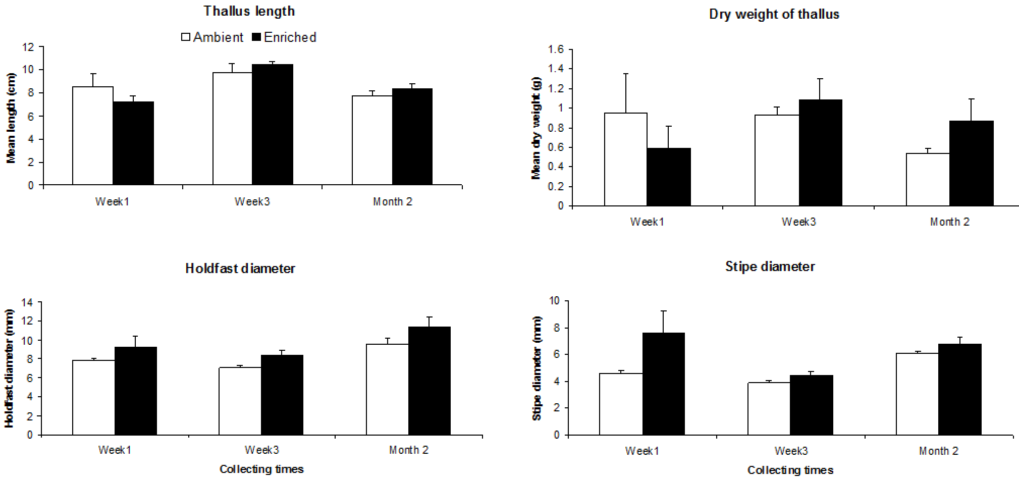

Figure 1A. The effect of nutrient enrichment on thallus length, dry weight, holdfast and stipe diameter of *Halimeda macroloba*. (Data are means ± SE of 90 algal thallus of each treatment and sampling time).

Table 1B. The effect of nutrient enrichment on thallus length, dry weight, holdfast diameter and stipe diameter of *Turbinaria conoides*. * $P<0.05$; ** $P<0.01$; *** $P<0.001$; ns not significant.

Species	*Turbinaria conoides*											
	Thallus length			Dry weight			Holdfast diameter			Stipe diameter		
Source	df	MS	F	df	MS	F	df	MS	F	df	MS	F
Time	2	0.088	0.347 ns	2	0.142	2.580 ns	2	0.032	2.239 ns	2	0.010	1.892 ns
Nutrient	1	0.074	0.291 ns	1	0.025	0.450 ns	1	0.014	0.997 ns	1	0.001	0.117 ns
Time x nutrient	2	0.069	0.273 ns	2	0.014	0.257 ns	2	0.034	2.355 ns	2	0.007	1.344 ns
Error	14	0.253		14	0.055		14	0.014		14	0.005	
Total	20			20			20			20		

Table 2. The effect of nutrient enrichment on thallus length, dry weight, holdfast and stipe diameter changes in *Halimeda macroloba* and *Turbinaria conoides*. (+) positive changes; (–) negative changes

Species		*Halimeda macroloba*				*Turbinaria conoides*			
Treatment	Times	Thallus length	Dry weight	Holdfast diameter	Stipe diameter	Thallus length	Dry weight	Holdfast diameter	Stipe diameter
	Week 1	-15.5%	-36.9%	+18.2%	+63.8%	-0.31%	+35.7%	+0.53%	+10.4%
Enrich	Week 3	+6.80%	+17.40%	+18.9%	+13.4%	+20.5%	+46.2%	-9.68%	-2.94%
	Month 2	+7.50%	+62.2%	+18.8%	+11.1%	-15.8%	+12.8%	-21.6%	-4.76%

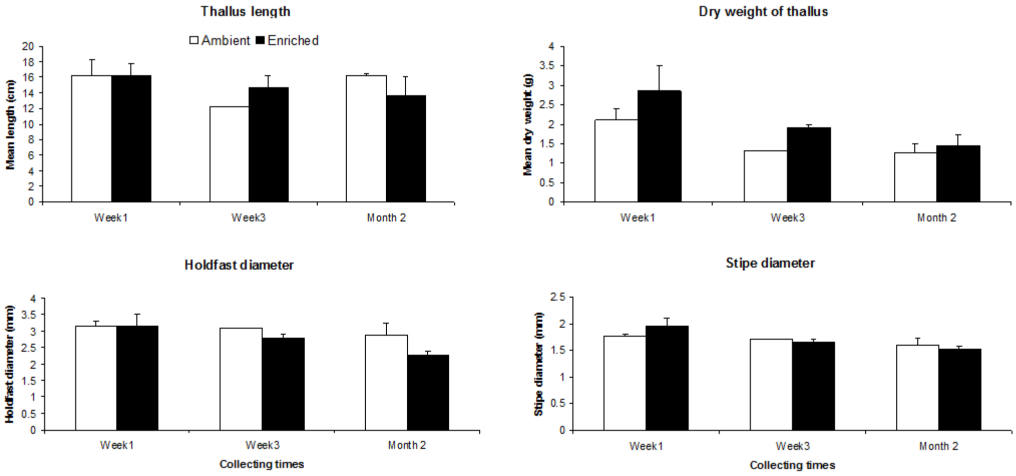

Figure 1B. The effect of nutrient enrichment on thallus length, dry weight, holdfast and stipe diameter of *Turbinaria conoides*. (Data are means ± SE of 60 algal thallus of each treatment and sampling time).

C:N, C:P, and N:P ratios of *H. macroloba* (p=0.066, p=0.834, p=0.260, respectively) however the C:P and N:P of *H. macroloba* changed through time (p<0.001, p<0.001). After one week of enrichment, the C:N:P of *Halimeda* increased, and indicated that *Halimeda* quickly took up and stored nutrient. For *T. conoides*, enrichment had a significant effect on only the C:N (p=0.001) and there was an interaction between time x nutrient (p=0.005). In addition, the C:N:P ratios of *T. conoides* varied through time (p=0.004, p=0.009, p=0.009, respectively) (Table 3). The C:N:P ratios of *Turbinaria* at the end of the first week were not changed when compared to the initial ratios.

Nutrient enrichment had a significant effect on the polyphenol concentrations in *Halimeda macroloba* and *Turbinaria conoides* (p=0.029, p=0.031, Table 4). The polyphenol concentrations of the two species were significantly lower when the nutrient was enriched (Figure 2 A-B, Table 4). This is consistent with some predictions of the CNBH; that polyphenols decreased when nutrients were enriched. There was a significant interaction between time and nutrient on the polyphenol concentrations in *H. macroloba* (p<0.001). In contrast, there was no significant interaction between time

Table 3. Tissue levels (% dry wt.) of C:N, C:P, and N:P of *Halimeda macroloba* and *Turbinaria conoides* from enriched plots (Values in parentheses = SE of 3 replicates for each sampling time).

Species	*Halimeda macroloba*			*Turbinaria conoides*		
Times	C:N	C:P	N:P	C:N	C:P	N:P
Initial	18.9 (1.56)	639 (218)	33.7 (10.6)	50.7 (1.03)	1697 (720)	33.8 (14.6)
Week 1	19.4 (1.23)	726 (31.8)	37.9 (3.21)	48.3 (2.41)	1697 (36.6)	35.4 (2.53)
Week 3	21.3 (3.66)	362 (28.6)	18.2 (3.53)	29.8 (0.00)	247 (0.00)	8.31 (0.00)

and nutrient with respect to the polyphenol concentrations of *T. conoides* (p=0.471) (Table. 4). Polyphenol concentrations were not significantly correlated with changes in thallus length, dry weight, holdfast and stipe diameter of both *H. macroloba* and *T. conoides* (linear regressions: p>0.05). However, there was a significant positive correlation between the polyphenol concentrations of *H. macroloba* and their C:P (R^2 = 37.2 %, p = 0.043). The polyphenols of *T. conoides* were not significantly correlated with C:N, C:P, and N:P (linear regression: p>0.05).

When comparing the responses of *r*-species, *Halimeda macroloba* and *K*-species, *Turbinaria conoides*, to the nutrient enrichment, we found that *Halimeda* allocated extra resources to growth and defense while *Turbinaria* allocated more resources into defense related systems than the growth. This supports the idea that the *r*-and *K*-species, having different life strategies also have different responses to the allocations of resources.

5. Discussion

These results indicate that nutrient enrichment affected the growth measurements (holdfast and stipe diameters) and polyphenol concentrations of *Halimeda macroloba* and affected only the polyphenol concentrations of *Turbinaria conoides*.

The tissue nutrient study indicated that the C:N:P ratios of *H. macroloba* and *T. conoides* were not affected by nutrient enrichment. The tissue nutrients of *H. macroloba*, showed that the C:N, C:P, and N:P (18.9, 639, and 33.7) ratios were similar to the values of the marine macrophytes (22, 700, and 35) reported by Atkinson and Smith (1983). In contrast the C:N, C:P, and N:P ratios of *T. conoides*, initially were 50.7, 1697, and 33.8, respectively. A decrease of the C:N:P ratios indicates that there was a nitrogen and phosphorus limitation on the growth of *Halimeda* and *Turbinaria*.

The results of nutrient enrichment on the polyphenol

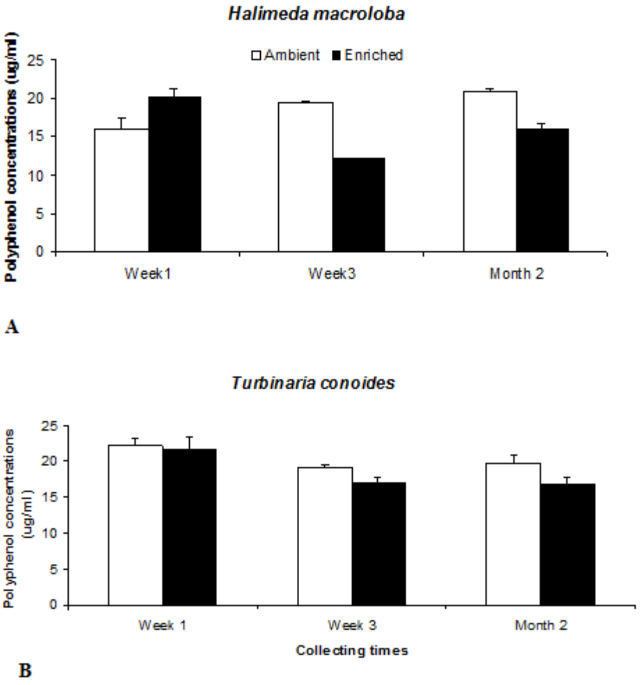

Figure 2A-B. The effect of nutrient enrichment on polyphenol concentrations in two different life strategies of algae, *Halimeda macroloba* (2A) and *Turbinaria conoides* (2B). (Data are means ± SE of 90 thallus of *Halimeda* and 60 thallus of *Turbinaria* of each treatment and sampling time, respectively).

Table 4. The effect of nutrient enrichment on polyphenol concentrations in two different life strategies of algae, *Halimeda macroloba* and *Turbinaria conoides*. * P<0.05; ** P<0.01; *** P<0.001; ns not significant.

	Halimeda macroloba			*Turbinaria conoides*		
Source of variation	df	MS	*F*	df	MS	*F*
Between subjects						
Time	2	17.744	2.168 ns	2	86.137	9.597 ***
Nutrient	1	43.556	5.321 *	1	44.463	4.954 *
Time x nutrient	2	109.171	13.336 ***	2	6.870	0.765 ns
Error	27	8.186		48	8.975	

concentrations of *H. macroloba* supported the predictions of the carbon/nutrient balance hypothesis (CNBH). The CNBH predicts that nutrient concentrations regulate resource allocation to growth and defense in plants. When nutrient is added, plants will allocate carbon to growth rather than to C-based secondary metabolites (Bryant *et al*., 1983). So, nutrient enrichment decreases the concentration of secondary metabolites.

Nutrient enrichment had no effect on the growth of *T. conoides*, but did affect polyphenol concentrations. Polyphenol concentrations in *Turbinaria* were higher from the ambient site than from the enriched site. However, growth did not change significantly and did not vary through the time of this study. This was a similar result to the previous studies in the brown alga, *Dictyota ciliolata*; nutrient enrichment had no effect on the growth in the field but had an effect in the laboratory (Cronin and Hay, 1996). In addition, Van Alstyne and Pelletreau (2000) found that increased phosphorus and iron decreased polyphenol concentrations in *Fucus gardneri* embryos but had a negative effect on growth.

The possible explanations for the unchanged growth of *Turbinaria* in the enriched situation site are 1) the initial nutrients were not limiting the growth of *Turbinaria* in this study site, so there were no changes in growth when nutrients were enriched, 2) *Turbinaria* had greater internal nutrient storages. When the external nutrient was enriched, there was no changes in C:N:P ratios in the first week of enrichment, 3) epiphytic algae, that do grow faster ,with higher nutrient uptake rates than compared with macroalgae, may affect the availability of nutrients to the macroalgae's after nutrient enrichment (Hemmi *et al*., 2005). From our results, we assume that the epiphytes such as red algae, *Jania* sp. and *Champia* sp. on *Turbinaria*, may take up nutrient quickly and may have an affect on the *Turbinaria*'s responses to the nutrient enrichment.

From our results, the effect of nutrient enrichment on the polyphenol concentrations of *T. conoides* is consistent with some predictions of the CNBH. Polyphenols in *Turbinaria* decreased when nutrients were enriched. Our results support the work of Yates and Peckol (1993). They found that *Fucus* had higher polyphenol concentrations at a low nutrient site than at a high nutrient site.

In this study, we found that selected algae, *H. macroloba* and *Turbinaria conoides*, behaved differently at resource allocation when nutrient is enriched. This is an expectation of *r*- and *K*- selection, and clearly indicates that different life strategy of algae involve different allocations of resources (Lawrence and McClintock, 1988). Higher resource allocation towards growth would be expected in *r*-selected species, *H. macroloba* than in *K*-selected species, *T. conoides*, which are expected to allocate more resources into defense. These results may help to explain the distribution and abundance of *H. macroloba* and *T. conoides.* Also, their responses to stresses and environmental changes such as nutrient and herbivory can be predicted.

From this study, we conclude that the nutrient enrichment had no strong effect on growth but had direct effect on the polyphenol concentrations of both *H. macroloba* and *T. conoides* and our results support the CNBH that nutrient play an important role in the polyphenol concentrations of both different life strategies.

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