Influence of light quality and nitrogen on colouration, biochemical composition and physiology of Chondracanthus teedei var. lusitanicus

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Abstract

This study explores how light quality and nutrient availability affect the colouration, internal compounds and physiological performance of *Chondracanthus teedei* var. *lusitanicus*, a red alga with potential applications in the food industry and European aquaculture. Seaweeds were exposed to four different light qualities (Blue, Green, Amber, and Red) and two nutrient conditions (nitrogen and phosphate -NP- enrichment, and phosphate -P- enrichment). Growth rates, pigment composition, protein content, photosynthetic performance and internal carbon and nitrogen content were determined. Results indicated that nitrogen availability was the most significant factor determining colouration and biological performance over the short-term (11 days). Nitrogen deficiency led to depigmentation and impaired photosynthetic performance. Light quality also influenced colouration and physiology, but the effects were slower and less pronounced. The interaction between nitrogen availability and light quality resulted in three distinct morphotypes: bluish-green under all NP enrichment treatments, dark green under blue light and P enrichment, and pale green under green, amber, and red lights with P enrichment. Blue light combined with NP enrichment increased the content of chlorophyll *a*, carotenoids, and biliproteins (phycoerythrin and phycocyanin) content whereas red light yielded the highest growth rates under both nutrient conditions. This study emphasizes the importance of environmental factors in macroalgal cultivation and provides insights for developing short-term cultivation protocols to produce high-quality, visually appealing seaweed biomass for gastronomic use.

Introduction

The use of macroalgae in the food industry has greatly increased in recent years (Mouritsen et al., 2019). The search for new species with unique characteristics has played a fundamental role in the expansion of this sector (McHugh, 2003). In 2022, production of algae reached 38 million tonnes, of which 97 percent originated from aquaculture. (FAO 2024). However, very few macroalgae species are currently cultivated successfully on a large-scale system. Thus, new species should be domesticated and made more appealing to support the diversification of large-scale production and avoiding massive exploitation of natural populations, which could lead to harmful effects on coastal ecosystems (Fricke et al., 2024; Araújo et al., 2021). Many species of red algae are widely used in food and phycocolloids industries, as well as in cosmetics and pharmaceutical products (Bermejo et al., 2020; Rejeki et al., 2018; Vega et al., 2021). In some South American countries, the Gigartinaceae species *Chondracanthus chamissoi* (C. Agardh) Kützing 1843 is highly regarded in gastronomy for its unique flavour and texture (Baltazar Guerrero et al., 2024). Studies have explored ways to control its life cycle and develop consistent cultivation systems (Avila et al., 2011; Oyarzo et al., 2021). In Europe, there has been an increasing interest in fine cuisine using species from the same genus, particularly the *Chondracanthus teedei* var. *lusitanicus* (J.E. De Mesquita Rodrigues) Bárbara & Cremades, which is found along the Atlantic coast and in parts of the Mediterranean Sea (Orfanidis, 1993). Several studies have been conducted in recent decades to optimize its cultivation (Guiry et al. 1987; Zinoun 1993; Pereira 2020). In Spain, it has also been cultivated using raft systems in traditional salt ponds -i.e., Salinas- (Bermejo et al., 2019).

Macroalgae biomass collected directly from the environment can be extremely heterogeneous due to the diversity and dynamism of the environmental conditions associated with coastal and estuarine ecosystems (Varela-Álvarez et al., 2019). In order to thrive in these fluctuating environmental conditions macroalgae have developed long- and short-term acclimation strategies. These strategies include modifications in thallus morphology (Monro & Poore, 2005) or alterations in cell wall composition (Carmona et al., 1998) as long-term physiological responses; or rapid changes in pigmentation and adjustments to the composition and functionality of photosynthetic membranes (Moreira et al., 2024; Talarico & Maranzana, 2000) as short-term acclimations. Even within individual thalli, pigment composition can change due to rapid photoacclimation (Beach & Smith, 1996). In littoral waters, the spectral composition and intensity of solar radiation change along depth. In coastal waters, red and blue wavelengths of sunlight are selectively absorbed by chlorophylls, leaving algae at depths beyond several meters primarily exposed to green and blue-green light of diminishing intensity. Due to their ability to synthesize accessory pigments which absorbs green-orange light, such as phycoerythrin, red algae can grow in deep or turbid waters (Haxo & Blinks, 1959; Kumar & Singh, 1979). Several studies have reported on the influence of light quality on the pigment content, growth and photosynthetic activity in red algae (Leukart & Lüning, 1994). Variable spectral proportions (e.g., red, amber, green and blue) affect the relative pigment composition and may act as photomorphogenic signals regulating algal metabolism and growth (Lüning, 1992; Rüdiger & López Figueroa, 1992). Blue light generally promotes phycobiliprotein synthesis and other N-compounds, while red light boosts growth rates (Figueroa et al., 1995, Korbee et al., 2005, Nguyen et al., 2017). Other studies reported that red light promotes chlorophyll *a* production in *Porphyra umbilicalis* (López-Figueroa & Niell 1989), while a significant increase was also found as well in *Gracilaria fisheri* when exposed to green light (Nguyen et al., 2017). Likewise, red and blue light promoted phycobiliproteins synthesis, while green light encouraged lutein production and promoted growth in cultivated *Halymenia floresii* (Godínez-Ortega et al., 2008). Additionally, the application of supplemented green light at low irradiances over saturated amber light for photosynthesis stimulated the accumulation of phycoerythrin in the red alga *Gracilaria gracilis* (Ghedifa et al., 2021).

Another key factor influencing macroalgae growth, their pigment composition or their biochemical composition is the nutrient availability (Hanisak 1983, Bermejo et al., 2022; Gao et al., 2019). In temperate marine and estuarine ecosystems, nitrogen (N) is typically the limiting nutrient (Howarth et al., 2000; Bermejo et al., 2022). Its availability is vital for protein synthesis and for most light-harvesting pigments (Figueroa et al., 1995b; Pliego-Cortés et al., 2017). Consequently, nitrogen depletion can lead to the rapid decay of these organisms, resulting in decreased pigmentation, bioactive compounds, growth rates, and photosynthetic performance (Figueroa et al., 2022). Soluble proteins and amino acids serve as an internal nitrogen reservoir that supports growth in specific phases of cultivation. In red algae, phycobiliproteins are suggested to act as nitrogen storage proteins, as well as playing a role in photosynthetic pigments (Bird et al. 1982; Vergara & Niell 1993; Figueroa 1995a). Phosphorus is also commonly a limiting nutrient, crucial for the structure of molecules such as ATP or ribulose-1,5 biphosphate. Its deficiency affects metabolic processes such as photosynthesis, nitrogen uptake o carbon fixation (Lapointe et al. 1987, Bermejo et al., 2020). Due to the rapid acclimation responses of

macroalgae, their appearance and quality can be modified by altering the physicochemical parameters of cultivation in short protocols (Bermejo et al., 2020, Figueroa et al., 2022, Vega et al., 2024,). Consequently, macroalgae biomass can be adapted for different target markets, such as the food industry and the production of bioactive compounds. In the food industry, seaweeds are valued for their visual appearance in a dish, so the existence of different colour morphotypes of the same seaweed would be highly valued. Examples include “tosaka-nori” (*Meristotheca papulosa* (Montagne) J. Agardh) or “hana-tsunomata” (*Chondrus crispus* Stackhouse), which are both available and commercially produced in different colours.

Therefore, understanding how environmental variables such as light and nutrient availability modulate the appearance of macroalgae is key to increasing the value of seaweed products. This study aims to optimize a short cultivation protocol to modify the appearance and biomass quality of *C. teedei*, while assessing the physiological implications of light spectra and nutrient availability on its internal composition, growth, and photosynthetic performance.

Materials & Methods

Collection and processing

Specimens of *C. teedei* var. *lusitanicus* were collected from a tidal creek in the Cadiz Bay area, Spain (36.457789, -6.229971). Thalli were placed in plastic bags containing wet tissue to prevent desiccation and were transported to the laboratory inside a cool box within 24 hours of collection. Upon the arrival, healthy thalli were selected and rinsed using artificial seawater (35‰) to remove sediments, epiphytes, and associated fauna. Fertile gametophytes were excluded to minimise physiological variability in the biomass used for the experiment.

Acclimation

Prior to the experiment, selected thalli were acclimated for approximately one month in 10 L tanks with artificial seawater (35 PSU; Aquarium Systems Instant Ocean sea salt, France) in a culture density of 3 g L^{-1}. Culture chamber temperature was set at 18˚ C and the algae were maintained under white light using cool white LED tubes (Systion 4000K, 30W) at an irradiance of 110 ± 10 µmol photons m^{-2} s^{-1} in a 16:8 light:dark cycle. Water was replaced weekly and supplemented with 1 mL of a 0.4 M KNO_3 solution and 1 mL of a 0.04 M KH_2PO_4 solution per litre of seawater, resulting in final concentrations of 400 µM and 40 µM, respectively. Air pumps provided constant bubbling to maintain consistent water motion and facilitate nutrient uptake, as well as preventing the accumulation of oxygen around the thalli.

Experimental set-up

A factorial experimental design was employed to assess the combined effects of light quality and nitrogen enrichment on the colour, growth, pigment composition, protein content and tissue carbon and

nitrogen content of *Chondracanthus teedei*. Four light quality treatments (blue = B, green = G, amber = A and red = R) (Fig. 1) and two nutrient regimes (enrichment with KNO_3 and KH_2PO_4, and enrichment with KH_2PO_4 only) were considered.

Each light treatment was set at 100 ± 10 µmol photons m^{-2} s^{-1} using LED lamps. In some cases, it was necessary to partially cover the lamps with meshes to adjust the irradiance. Irradiance was measured using a spherical underwater quantum sensor ((US-SQS/L, Walz GmbH, Germany), which was connected to a LI-250 Radiometer (Licor, Nebraska, USA). Specimens were incubated at 18˚C under the different experimental conditions for 11 days. Each treatment consisted in four replicates with 3 g of seaweed placed in a cylindrical methacrylate tank containing 1L of artificial seawater (35 PSU). All cylinders (n = 32) were continuously aerated to maintain water movement and were partially covered with parafilm to reduce evaporation. Salinity was monitored daily using a salinity meter Laqua PC220 (HORIBA Advanced Techno, Co., Ltd., Japan) and distilled water was added as necessary to adjust salinity. Artificial seawater was replaced every three days and the tanks were cleaned to remove any possible biofouling from the inner surface. Regarding nutrient conditions, nitrogen enrichment treatments were supplemented with 1 mL of a 0.4 M potassium nitrate (KNO_3) solution and 1 mL of a 0.04 M potassium hydrogen phosphate (KH_2PO_4) solution per litre of seawater, to provide nitrate and phosphate. Final nitrate and phosphate concentrations were close to 400 µM and 40 µM, respectively (10N:1P ratio). In contrast, non-N enriched treatments were supplemented with only 1 mL of a 0.04 M KH_2PO_4 solution per litre of seawater (0N:1P ratio).

After 11 days of exposure to different experimental conditions, the growth rate of thalli, their pigment composition, protein content, photosynthetic performance, and their tissue Carbon and Nitrogen content were analysed. The different morphotypes were identified by monitoring thallus colour throughout the experiment.

Pigment composition

Prior to the pigment extraction, samples were lyophilized and macerated using either a mortar or a mixer mill. For each pigment analysis, 25 mg of dry weight (DW) of seaweed tissue per mL of extraction solvent were used. To avoid unwanted biases, different parts of the thallus were selected in every sample.

Chlorophyll *a* (mg g^{-1} DW) and carotenoids (mg g^{-1} DW) were extracted using methanol (90%) as the organic solvent and analysed spectrophotometrically according to the protocol of Gheysen et al. (2019). Maceration was performed using a mortar, with the addition of sterile sand to increase cell wall disruption and promote pigment extraction. Carotenoids (Antheraxanthin, Fucoxanthin and β-carotene) were determined using a high-performance liquid chromatography (HPLC) system (1260 Agilent InfinityLab Series, USA) equipped with a diode-array detector (DAD). Prior to chromatographic analysis, 500 µL of the algal extract was filtered through a 0.2 µm membrane. An 80 µL aliquot of the filtered

extract was injected into a Zorbax SB-C8 column (5 µm; 150 mm × 4.6 mm, Agilent), which was maintained at 30°C, while the sample tray was kept at 5°C. The mobile phases consisted of methanol:ammonium acetate buffer (80:20, pH 7.2, 0.5 M), acetonitrile:ultra-pure water (90:10), and ethyl acetate. Pigments were detected at wavelengths of 444, 450, and 664 nm.

Phycobiliproteins (PE, phycoerythrin and PC, phycocyanin; mg g^{-1} DW) were quantified spectrophotometrically using the equations of Beer & Eshel (1985) (Eqs. 1 and 2). Maceration was performed using a MM400 ball mixer mill (Retsch GmbH, Germany) with three 30-second pulses. Samples were then incubated for 24 hours with 1 mL of phosphate buffer solution (1 M, pH 6.5) in the dark at 8°C to extract the pigments. Finally, samples were centrifuged for 15 minutes at 1000 rpm prior to the spectrophotometric analysis.

$$\mathrm{PE} = [(A_{564} - A_{592}) - (A_{455} - A_{592}) * 0.2] * 0.12$$

1

$$\mathrm{PC} = [(A_{618} - A_{645}) - (A_{692} - A_{645}) * 0.51] * 0.15$$

2

Soluble protein content

To improve the protein extraction under cold, dark conditions, 25 mg of lyophilized and macerated samples were weighed, immersed in 1 mL of phosphate buffer solution (1M; pH = 6.5) and left for 24 hours. The samples were then centrifuged for 15 minutes at 1000 rpm. Total protein content was subsequently quantified by spectrophotometry following the Bradford method (1976). Briefly, 50 µL of the supernatant was added to a BioRad solution containing a phosphate buffer mixture. After 15 minutes, the total protein content was determined by measuring the absorbance at 595nm.

Total internal C and N

A small fragment of the cultured seaweed was freeze dried and ground using a MM400 ball mixer mill (Retsch GmbH, Germany). The ground samples were stored in Eppendorf tubes in a desiccator with silica gel until they were sent to the Research Support Central Services (SCAI) at the University of Malaga (Malaga, Spain), where the C and N content of the tissue was determined using a CNHS LECO-932 elemental analyser (St. Joseph, MI, USA).

Growth

Biomass of each replicate was assessed before and after the incubation period using an analytical balance with a 0.1 mg resolution. Any excess of water was removed using a manual centrifuge prior to

weighing. Then, the relative growth rate (RGR) was estimated by considering the initial and final biomass and assuming exponential thallus growth (Eq. 3).

$$\mathrm{RGR}\ \left(\%\ day^{-1}\right) = 100 \times\ (\ln(\frac{FW_f}{FW_0})/\ t$$

3

where RGR is the relative daily growth rate; FW_f is the final fresh weight after 11 days of culture; FW_0 is the initial fresh weight; and t is the number of culture days. After the last weighting, each replicated were placed in a labelled plastic bag and stored at − 80 ◦C in a freezer until further analysis.

Photosynthetic activity

Photosynthetic activity was estimated using *in vivo* chlorophyll *a* fluorescence of photosystem II (PSII) with a MiniPAM-II Flurometer (Walz, Germany), which uses Red-light (λ_{max} = 625 nm light) to measure actinic and saturated light pulses. To determine the maximal quantum yield (F_v/F_m), the thalli were incubated in the dark for 15 minutes. Basal fluorescence (F_0) was determined using a measuring light, and maximal fluorescence (F_m) was determined in dark adapted algae after a saturated light pulse (3000 µmol photons m^{-2} s^{-1}). Maximum quantum yield was calculated as specified in Eq. 4.

$F_v/F_m = (F_m - F_0)/F_m$ (4)

Rapid light curves (RLCs) were also conducted to assess the photosynthetic performance of the seaweed in depth. RLC represent how *in vivo* chlorophyll *a* fluorescence parameters vary with the increasing irradiance (*ex situ* measurements). After determining the maximal quantum yield, algae were exposed to twelve increasing irradiances of Actinic red light (from 0 to 1500 µmol photons m^{-2} s^{-1}) for 20 seconds. Saturating pulses were performed after each incubation period to allow calculation of the effective quantum yield (Y(II)) and the electron transport rate (ETR).

Y(II) was quantified using the following Eq. 5, where *F*t is the basal fluorescence, and *F*m' the maximal fluorescence measured after application a saturation light pulse was applied to the different light qualities. The ETR was calculated using Eq. 6, where E_{PAR} is the PAR irradiance (µmol photons m^{-2} s^{-1}), A is the absorptance which was calculated as specified in Eq. 7. Here, Et is the irradiance passing through the thallus, E0 is the emitted irradiance, and FII is the fraction of chlorophyll *a* associated to PSII. The value of 0.15 is used for red algae and cyanobacteria (Grzymski et al., 1997, Figueroa et al., 2003, Johnsen & Sakshaug, 2007). The ETR vs. irradiance curves obtained were fitted according to Eilers & Peeters (1988) model, yielding the following parameters: photosynthetic efficiency of ETR (α_{ETR}), maximal ETR (ETR_{max}) and saturated irradiance (E_k).

Y(II) = $(F_m - F_t) / F_m'$ (5)

ETR = Y(II) * E * A * FII (6)

A = 1 - (Et / E0) (7)

Identify colour morphotypes

To analyse colour changes in *C. teedei* thalli, images were taken every two or three days. Each replicate was placed between two transparent plastic sheets and scanned using a Brother LT-340CL printer scanner. This method helps to avoid differences in brightness and colour properties between pictures. During the process, culture media was replaced and supplemented with the corresponding nutrients for each treatment. After scanning, thalli were returned to their tanks.

The resulting pictures were analysed with the Adobe Photoshop CS6 software to obtain RGB (Red, Green, Blue) values from different parts of each thallus (Bermejo et al., 2020). The digital colour depends on the proportion between the three RGB components. Final morphotypes were identified after the incubation period, along with the time required for each treatment to produce them.

Statistical analysis

Prior to any statistical test, data were tested for normality and homoscedasticity using the Shapiro-Wilk and Levene tests, respectively. A two-factorial ANOVA was conducted to determine the effect of light quality (4 levels) and nitrogen availability (2 levels) on growth, photosynthetic performance, pigment composition, total soluble protein and internal C and N content. Then, a post hoc Tukey test was performed to compare between levels of influential factors. Alternatively, when variables showed unequal variances (Fuc, %C and C/N), a Scheirer-Ray-Hare test for non-parametric data was performed to determine the effects of the two factors assessed. In these cases, a Dunnet-Tukey-Kramer Pairwise test was used to compare between levels of influential factors.

A two-way PERmutational Multivariable Analysis of VAriance (PERMANOVA) was used to assess the effects of light (fixed; 4 levels) and nutrient addition (fixed, 2 levels) on algal colour over 11 days of incubation (i.e., RGB signal obtained from a picture). This analysis was based on the euclidean distance between RGB values of each replicate along the experiment. Additionally, a distance-based test for homogeneity of multivariate dispersion (PERMDISP) and a Principal component analysis (PCA) were performed to interpret and visualize data patterns. A posteriori pairwise comparisons were done to identify differences between treatments along the experiment. Final morphotypes were identified with a PERMANOVA test considering the final RGB values. Similarly, eight PERMANOVA tests were developed to determine how long takes each treatment to obtain a different colour.

All statistical analyses were performed using the software R-programme (R Core Team 2017). Probability of significance for the null hypothesis was set at 5%, and when necessary (i.e. PERMANOVA, PERMDISP) analyses were based on 9999 permutations.

Results

Pigment composition

Pigment composition was significantly affected by light quality and enrichment condition (Suppl. Tables 1 and 2). ANOVA results showed a significant effect of both factors interaction for Chl *a* and Phycoerythrin (p-value = 0.005 and 0.001, respectively). Regarding Antheraxanthin and Phycocyanin were significantly affected by both light and enrichment condition (p-value = 0.001), but no interaction was found between factors, and Fucoxanthin was significantly affected only by enrichment condition (Scheirer Ray-Hare test; p-value = 0.001). All pigments presented the highest values under N enrichment and blue light conditions, excluding β-carotene, which presented maximal values under green light conditions, though no significant differences were observed between light qualities (p-value > 0.05) (Fig. 2f). Chl *a*, Fuc, Ax, PE and PC presented up to twice the value under blue light compared to other light qualities under N enrichment (Fig. 2a-e). The lowest values of Chl *a*, Fuc and Ax values were found in thalli cultivated under red light, while minimum PE and PC contents were recorded under green light (Suppl. Table 3).

Soluble protein content

The soluble protein content was significantly affected by both light quality (p-value = 0.001) and nutrient enrichment conditions (p-value = 0.001), but the interaction between factors was not significant. The difference between the maximum and minimum observed values was 3.5-fold (Fig. 3). The soluble protein content ranged from 239.79 ± 20.08 mg· g^{-1} DW, under N enrichment and red light incubation, to 66.65 ± 9.91 mg · g^{-1} DW in thalli incubated under non N enrichment and green light conditions (Suppl. Table 4). N enrichment significantly increased the soluble protein content and, regarding light quality, blue and red light treatments resulted in the highest soluble protein content.

Internal total C and N

Nitrogen content was clearly affected by enrichment conditions (p-value = 0.001), with N enriched treatments, showing a N content more than twice that of non N enriched treatments. Light quality did not significantly affect N content (Suppl. Table 1), but the interaction between the two factors was marginally significant (p-value = 0.076). In this regard, under N enrichment, red light treatment presented the highest N content, while under non N enrichment, the lowest N content was found in red light treatments and the highest in blue light treatments (Suppl. Table 4). C content was significantly affected by light quality (Scheirer Ray-Hare test; p-value = 0.02) (Suppl. Table 2). After incubation, the highest C content was observed under red light with N enrichment, while the lowest value was observed under blue light without N enrichment (32.4 ± 1.5% and 27.6 ± 3.2% C, respectively) (Suppl. Table 4). Consequently, C/N ratio was only significantly affected by N enrichment (Scheirer Ray-Hare test; p-value = 0.001; Suppl. Table 2), with values 1.5 to 2.5-fold lower under N enriched conditions (Fig. 4). However, analysing each enrichment condition separately using an ANOVA test showed significant differences between light qualities under non N enrichment conditions (p-value = 0.02). In this regard, blue light treatments

presented a mean C/N value that was up to 1.5-fold lower compared to other light qualities. The highest mean value was observed under red light with non N enrichment, and the lowest under blue light with N enrichment (19.6 ± 2.8 and 7.8 ± 0.98, respectively) (Suppl. Table 4).

Growth

Growth rate was positively affected by nitrogen enrichment. However, no significant effects were observed in the different light quality treatments tested (Suppl. Table 1). N enrichment significantly increased the mean growth rate by 25% ($p = 0.04$) compared to non N enrichment, when all light treatments within each enrichment condition were pooled (Fig. 5). Nevertheless, red light treatments showed the highest growth rates at both nutrient levels. Maximum RGR values were found in thalli incubated with N enrichment under red light conditions (3.2 ± 1.09% FW day^{-1}), and the minimum RGR was recorded under blue light with non N enrichment (1.38 ± 0.95% FW day^{-1}) (Suppl. Table 4).

Photosynthetic **activity**

Rapid light curve (RLC) graphs (Fig. 6) showed ETR values for each light quality in both enrichment conditions, as well as the initial values before incubation. N enriched treatments had higher ETR values than non N enriched treatments, while the initial thalli had the lowest ETR values and clear evidence of photoinhibition, as reflected by the downward trend in the light curve.

Maximum quantum yield (F_v/F_m) was higher in the initial photosynthetic performance analysis, but there were no significant differences between light treatments or enrichment conditions after the incubation period (p-value > 0.05). ETR_{max} increased significantly by up to 3-fold compared to the initial value (2.67 ± 1.01 µmol $e^- m^{-2} s^{-1}$) after the incubations, and a significant effect of the enrichment condition was observed (p-value = 0.002).

Blue light N enriched treatments presented 1.8-fold higher ETR_{max} mean values than blue light without N enrichment (8.9 ± 1.3 and 4.97 ± 1.98 µmol $e^- m^{-2} s^{-1}$, respectively). Photosynthetic efficiency (α_{ETR}) did not differ significantly between treatments or compared to the initial value. Meanwhile, Ek values increased up to 4.3-fold after the incubation (Suppl. Table 5). ANOVA analysis revealed a significant effect of the enrichment condition on E_k (p-value = 0.04), with full enriched treatments showing 1.3-fold higher values when all light qualities within each enrichment condition were pooled together. However, post-hoc analysis did not show any differences between light quality treatments (Suppl. Table 1).

Colour analysis.

After 11 days incubation, three different morphotypes were achieved (Fig. 7): bluish green, dark green and pale green. Bluish green on every N enriched treatment notwithstanding the light quality, dark green on blue-light with non N enrichment, and pale green for green-light, amber-light and red-light in non N enrichment conditions.

Despite the three final morphotypes being identified using the Euclidean distances of the final thalli´s RGB values, only two groups were represented in the cluster analysis, which was strongly associated with the enrichment condition (Fig. 8). In this regard, the two groups presented different levels of dispersion: non N enrichment group (P) was clearly wider and more dispersed than the N enriched group (N + P), due to the greater influence of the light quality on non N enriched treatments. This could explain the interaction between the two factors showed in the PERMANOVA (Suppl. Table 6, p-value = 0.03).

The PCA (Fig. 9) shows the colour evolution for each light quality treatment over the course of the incubation period. In non N enrichment treatments, the colour of all thalli changed significantly after 5 to 7 days of incubation. However, the three final colour morphotypes were only assessed on day 11. Pale green morphotype appeared more rapidly under red light, whereas blue light did not produce the pale green morphotype after 11 days of incubation.

Discussion

From a culinary perspective, obtaining thalli of different colours is interesting because it creates more visually appealing food products for consumers. For instance, the Canadian company Acadian Seaplants Ltd. markets a blend of *Chondrus crispus* thalli in three different colours (green, yellow, and red), known as Hana Tsunomata®, which is highly valued in the food industry (Craigie et al., 2019). Colour of *Porphyra* blades depends on their pigment composition, and they are valued differently by consumers (Moritsen, 2009; Blouin et al., 2011). The aim of this study is to produce *C. teedei* specimens with different colours and biochemical properties by using different light qualities and nutritional conditions, with the intention of offering them as a culinary product.

Macroalgae can rapidly adapt their appearance and chemical composition, making them more attractive or suitable for different applications (Bermejo et al., 2019; Figueroa et al., 2022; Godínez-Ortega et al., 2008; Rejeki et al., 2018; Varela-Álvarez et al., 2019). Therefore, designing short cultivation protocols is highly relevant for producing homogeneous and high-quality macroalgal biomass.

This study monitored the biochemical content, colour changes and photosynthetic performance of *C. teedei* during an 11-days incubation period under four different light qualities (Blue, Green, Amber, and Red) and two nutrient conditions (with or without nitrogen enrichment). The biochemical content of *C. teedei* was strongly affected by both nutrient availability and light quality. Several studies have demonstrated that nitrogenous molecules, such as proteins and most photosynthetic pigments, are directly affected by nitrogen availability (Algarra & Rüdiger, 1993; Jones et al., 1996; López-Figueroa & Rüdiger, 1991). Coulteri & Macler (1986) reported increased levels of amino acids, biliproteins, and chlorophyll in *Gelidium coulteri*, following inorganic nitrogen enrichment. Figueroa et al., (2022) reported thallus depigmentation in the red alga *Gracilaria cornea* under prolonged N deficiency and UV radiation, since macroalgae use the accumulated N in their pigments to meet vital physiological needs. In terms of light quality, blue light stimulated the synthesis of proteins and photosynthetic pigments (Chl a, PE, PC, Fuc, Ax) in *C. teedei*, as it has been observed in other red algae. The way in which organisms adapt to

different wavelengths of light has been studied extensively for decades (Barufı et al., 2015; Beach & Smith, 1996; Ghedifa et al., 2021; Yocum & Blinks, 1958).

In *Porphyra umbilicalis*, Figueroa et al., (1994, 1995b) demonstrated that red light promoted thallus expansion and cell size, while blue light stimulated the accumulation of nitrogenous compounds in the form of photosynthetic pigments (Chl *a*, PC, and PE) and other proteins (Korbee et al., 2005). In *Halimeda floresii*, Godínez-Ortega et al., (2008) also reported a blue light stimulation of biliprotein synthesis. Green light increased the carotenoids lutein and its precursor, α-carotene. This indicates the activation of the xanthophyll cycle to optimize photosynthesis by increasing the light-harvesting function of lutein (Andersson et al., 2006). In *C. teedei*, β-carotene showed no significant differences in response to different light qualities, but maximal values were consistently observed under green light incubation, suggesting that the activation of the xanthophyll cycle is promoted by green light. Yocum & Blinks, 1958 determined that *Porphyra* specimens exposed to blue light for 10 days contained more PE and less chlorophyll than specimens exposed to green light. López-Figueroa & Niell, 1990 observed higher PE synthesis under green light in several red algae (*Porphyra umbilicalis*, *Ellisolandia elongata*, and *Plocamium cartilagineum*), whereas PC synthesis was induced by red light. In another study, *E. elongata* showed increased PE and PC levels when incubated in green light, and a decreased levels when incubated in red light. Conversely, Chl *a* increased in specimens incubated in red light and decreased in those incubated in green light (Algarra et al., 1991). This “inverse chromatic adaptation” was also observed in *Porphyra laciniata* in its natural environment (López-Figueroa, 1992). However, the mechanism by which Chl *a* is synthesised in response to different light qualities is complex and not yet fully understood. The interaction between phytochrome and a blue-light photoreceptor in controlling Chl *a* synthesis in *Ulva rigida* and *Porphyra* sp. has been reported (Lopez-Figueroa & Niell, 1989, Figueroa et al., 1995b).

The lowest growth rates were consistently observed in non N enriched treatments, demonstrating the detrimental impact of N deficiency on the development of photosynthetic organisms. The C:N ratio demonstrated lower N accumulation than C accumulation in non N enrichment treatment due to the absence of N in the medium. The maximum internal C content found in red light N enriched treatments are in aligns with previous studies (Figueroa et al. 1995a and b). While research has extensively examined the favourable effects of blue light on protein synthesis and enzyme activation, red light has been showed to support carbohydrate accumulation in unicellular green algae and higher plants (Senger, 1987; Ruyters, 1987; Wada & Kadota, 1989; Senger & Senger, 1991; Korbee et al., 2005).

In non N enriched treatment, specimens incubated in blue light had a higher N content due to the stimulating effect of blue light on organic nitrogen accumulation (Calero et al. 1980, Azuara & Aparicio 1983, Figueroa et al. 1995b). However, no differences in intracellular N were found between light qualities with N enrichment in *C. teedei*. This could be due to an increase in structural proteins in thalli exposed to red light, as this promotes cell growth and thallus expansion (Figueroa et al., 1994). In terms of colour changes, the combination of different light qualities with N enrichment resulted in three distinct morphotypes: bluish-green thalli under all N enriched incubations, dark green thalli under blue light

incubation without N supplementation, and pale green thalli under green, amber, and red light incubations without N supplementation. Therefore, the availability of N was the most determining factor in the observed colour change. N depletion caused depigmentation in thalli without N supplementation, whereas both growth and photosynthetic rate, estimated as ETR, increased in N enrichment.

The photosynthetic performance in macroalgae is influenced by nutrient availability and light quality. On the one hand, N deficiency reduces the photosynthetic activity (Figueroa et al., 2022), as observed in *C. teedei*. Conversely, Lüning & Dring, (1985) and Dring & Lüning, (1985) demonstrated the low photosynthetic efficiency of red algae under blue light. Photosynthesis profiles of red algae (*Delesseria*, *Phyllophora*, *Porphyra*, and *Chondrus*) showed low rates of photosynthesis in blue light (400–500 nm). According to the reported photosynthetic action spectra, therefore, photosynthetic efficiency in red algae is generally lower in blue light than in red, yellow or green (Haxo & Brinks, 1950; Lüning and Dring, 1985; Grzymski et al., 1997).

Contrary to what might be expected, this study found that *C. teedei* incubated in blue light presented the highest electron transport rates (ETR_{max}). This may be related to the increase in biliprotein content caused by the blue light incubation, as it was observed by Figueroa et al. (1995) in *Porphyra umbilicalis*. Since the PC absorbs light in the orange-red region (600 to 640 nm), thalli incubated in blue light will use red light more efficiently. Haxo & Brinks (1950) found a similar fact when studying the light action spectra of different macroalgae with different pigment compositions. In red algae that primarily contain PE, the action spectrum exhibits distinct peaks at 495, 540, and 565 nm. However, in *Porphyra*, as PC levels rise and PE diminishes, the action spectrum shows increased activity in the 600–640 nm range, where PC absorbs light. The lowest ETR_{max} and Ek values showed by the initial *C. teedei* in the experiment might be due to the shaded acclimation, since all the biomass used for the experiment was acclimated in the same tank, which had a considerable self-shading effect. Despite the broad study of photosynthetic metabolism in recent decades, discrepancies and gaps in knowledge regarding light-harvesting processes and the rapid adaptation of pigment composition in macroalgae remain.

In terms of appearance, thalli incubated under green, amber and red light in non N enriched treatments became pale-green morphotypes due to depigmentation caused by N depletion. This depigmentation combined with an increasing synthesis of accessory pigments, induced by blue light, generated the dark-green morphotype. Finally, in all N enriched treatments only the bluish-green morphotype was observed, despite the increasing synthesis of accessory pigments, induced by blue light.

In conclusion, this study has demonstrated that the colour and biomass quality of *C. teedei* can be altered using short, easily reproducible cultivation protocols that manipulate light and nutrient availability. Generally, a lighter colour (pale green) indicates lower nutritional value than a darker colour. As it is the case of algae grown under supplementation without N in all light qualities except in blue light. A dark green colour is reached under blue light due to a higher level of biliproteins compared to the other light qualities. However, algae grown with N enrichment were all darker in colour (Bluish green) than those tested under non N enrichment and had higher nutritional value (i.e. total proteins and pigment

content). PCA analysis of RGB values extracted from *C. teedei* pictures successfully determined the colour change and the time required to achieve it. The observed morphotypes reflected this species' physiological response to N deficiency and the induction of blue-light pigment synthesis.

Declarations

Competing interests:

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Author Contribution

Authors' contributions: I.M. : Conceptualization, Methodology, Investigation, Writing, Review, Editing - original draft; M.J.R.P.: Conceptualization, Methodology, Investigation, Review; F.S.G.: Conceptualization, Investigation, Review, Editing; M.L.P.: Conceptualization, Investigation, Review, Editing; M.M.: Conceptualization, Investigation, Review, Editing; N.K.: Conceptualization, Investigation, Review, Editing; F.L.F.: Conceptualization, Methodology, Investigation, Writing, Review, Editing - original draft, Supervision; R.B.: Conceptualization, Methodology, Investigation, Review, Editing - original draft, Supervision, Project administration. All authors have read and agreed to the published version of the manuscript.

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Data Availability

All data generated or analyzed during this study are included in this published article.

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Figures

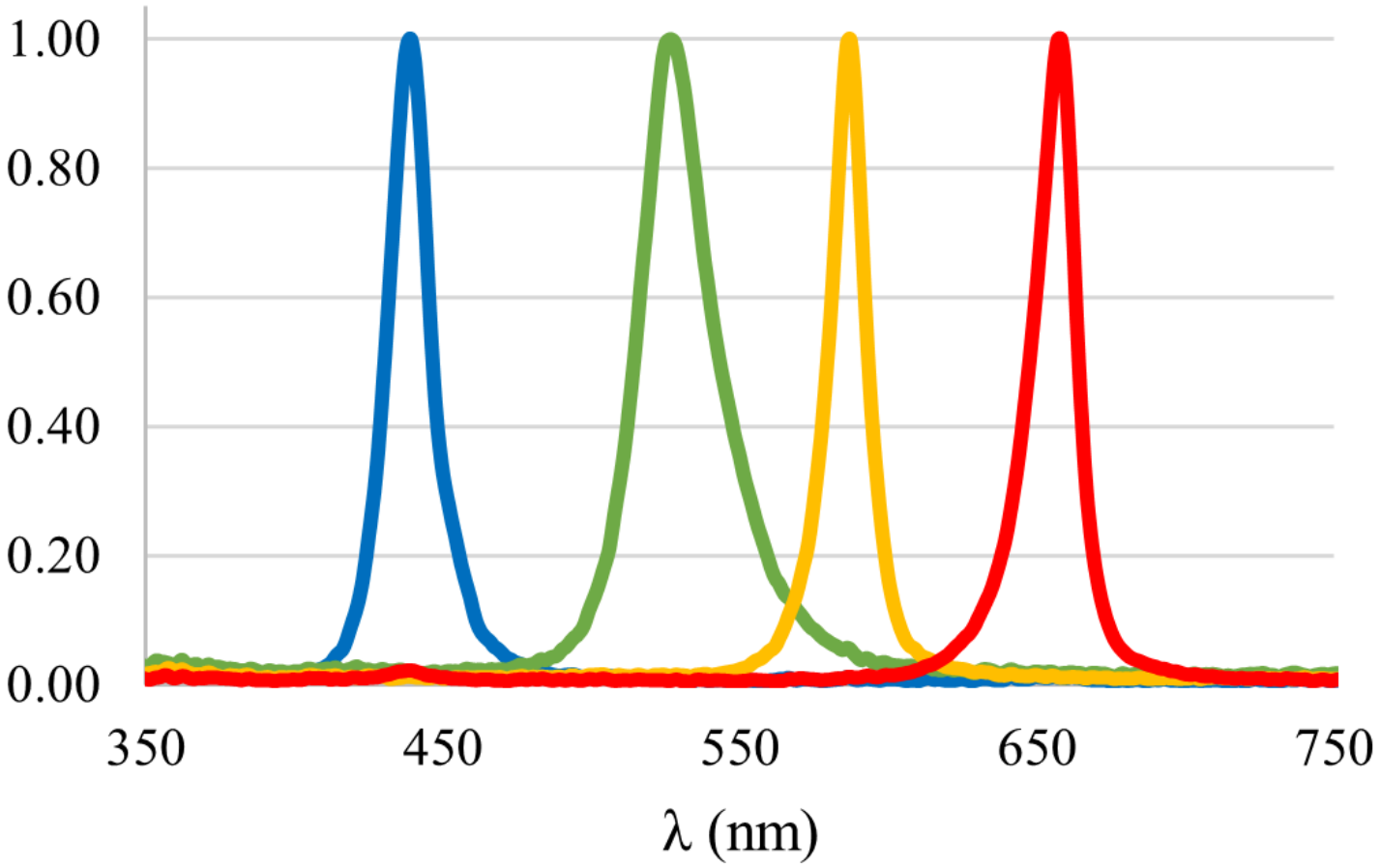

Figure 1

Relative spectral photon fluence rate (RSPFR) of the different light wavelengths (λ) used: Blue (450 nm), Green (525 nm), Amber (590 nm) and Red (660 nm) light using LED lamps.

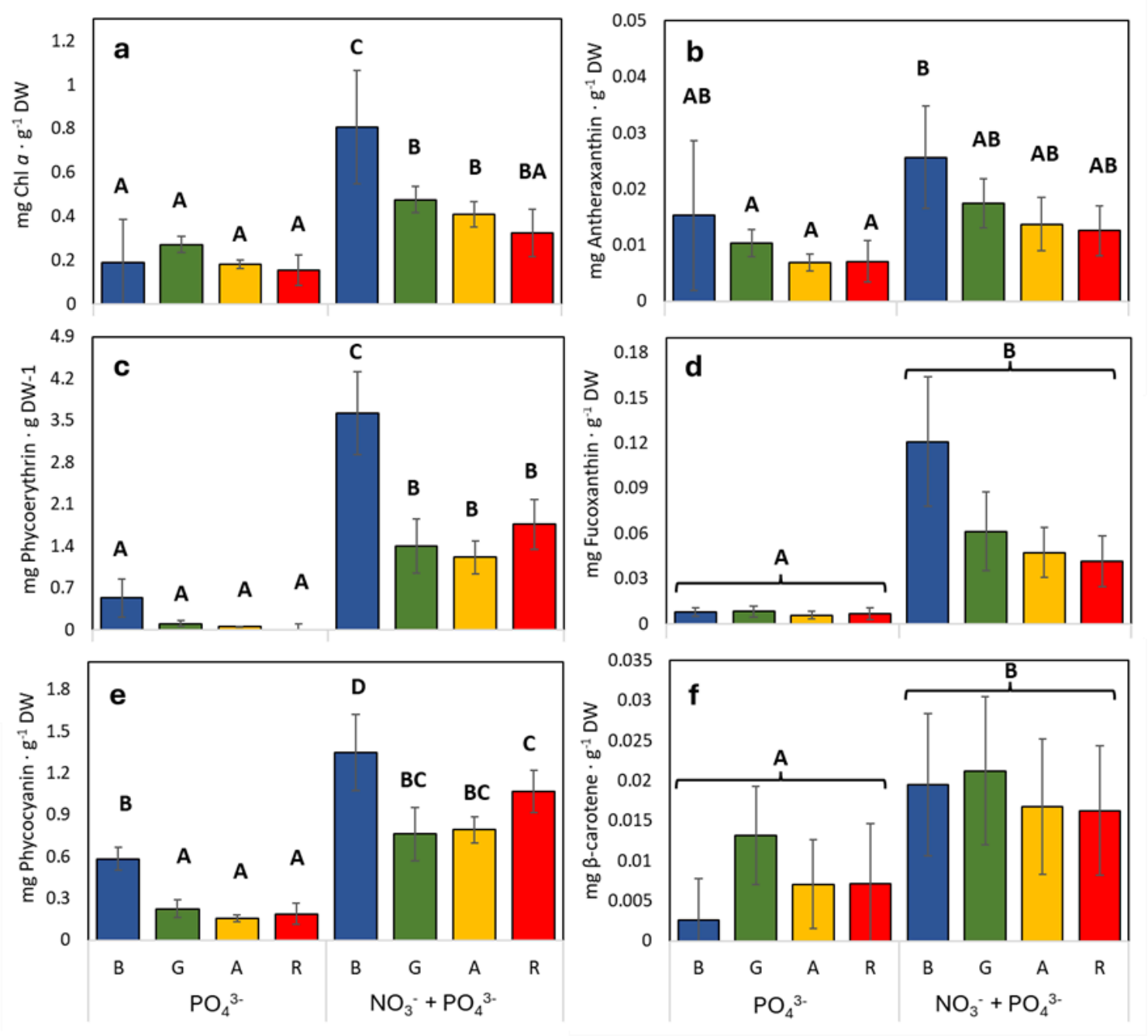

Figure 2

Pigment contents (a-f) of *C. teedei* incubated under different light qualities (Blue; Green, Amber; Red) and supplemented with or without N. Data is represented as mean ± SD (n = 4). Different letters above the bars represent significant differences ($p < 0.05$).

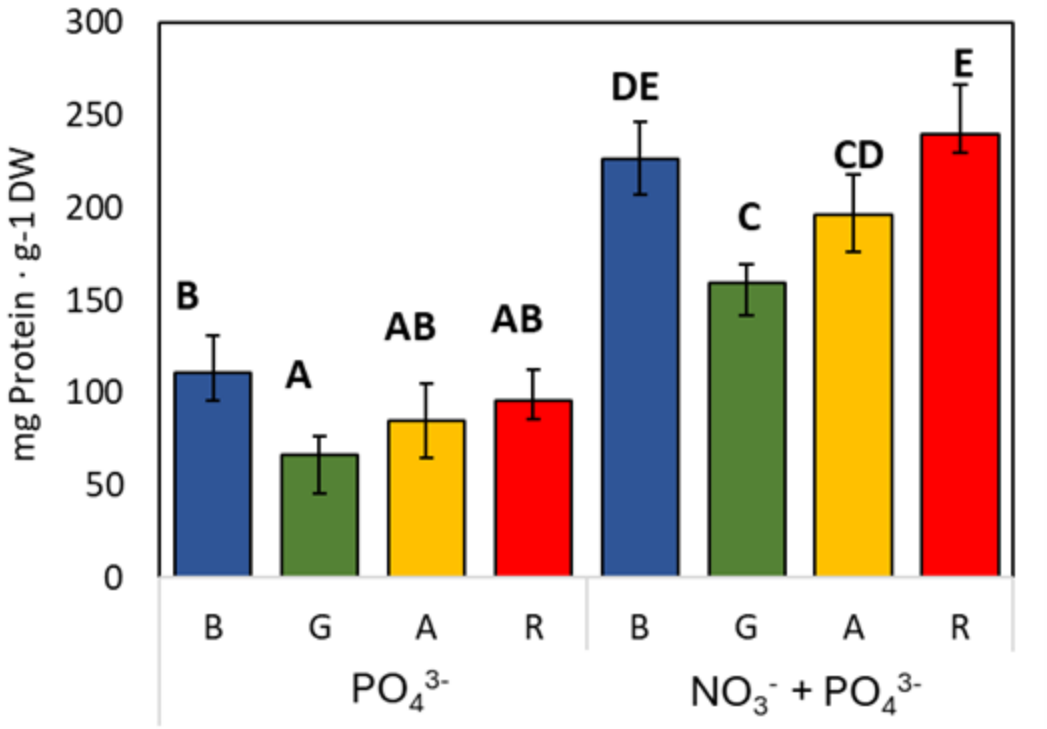

Figure 3

Soluble protein content of *C. teedei*incubated under different light qualities (Blue; Green, Amber; Red) and supplemented with or without N. Data is represented as mean ± SD (n = 4). Different letters above the bars represent significant differences ($p < 0.05$).

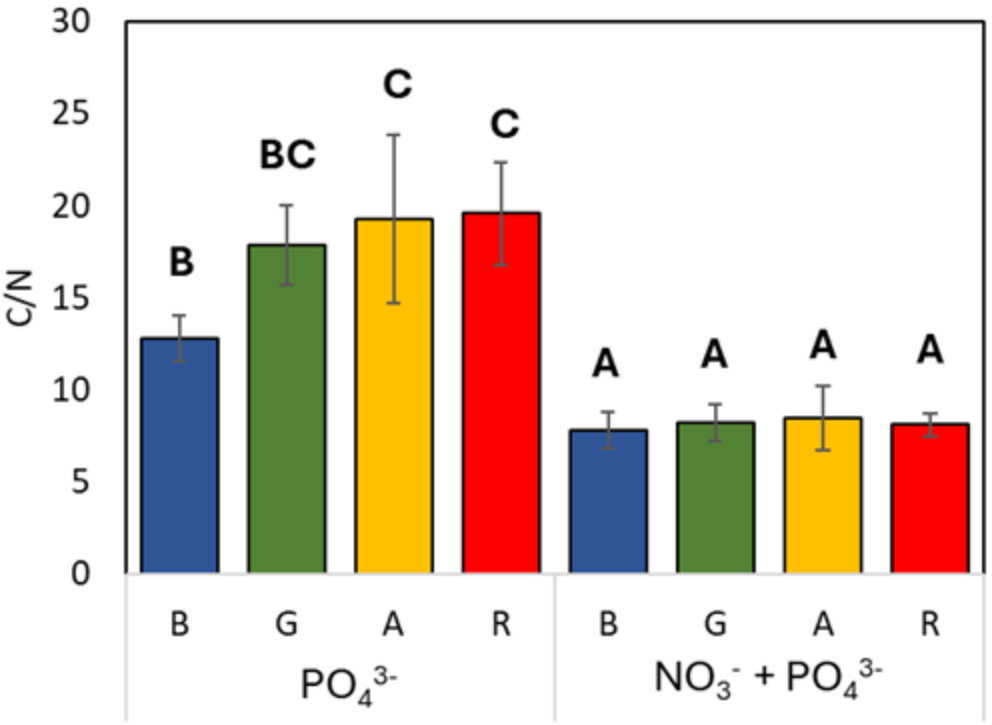

Figure 4

C/N ratio of *C. teedei* incubated under different light qualities (Blue; Green, Amber; Red) and supplemented with or without N. Data is represented as mean ± SD (n = 4). Different letters above the bars represent significant differences ($p < 0.05$).

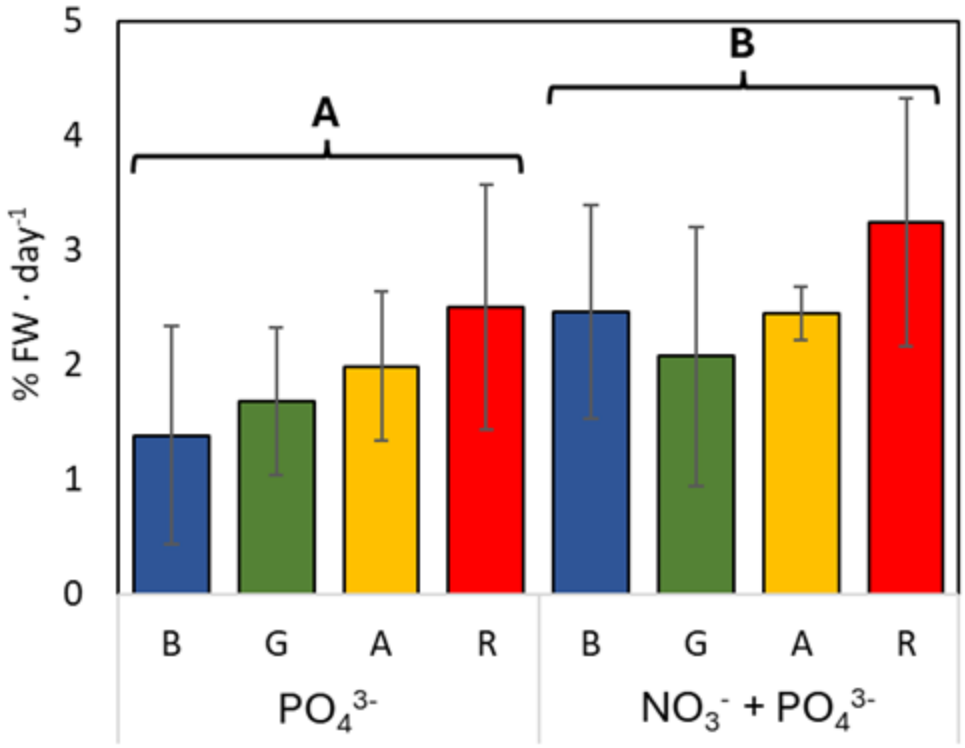

Figure 5

Relative growth rate of *C. teedei* incubated under different light qualities (Blue; Green, Amber; Red) and supplemented with or without N. Data is represented as mean ± SD (n = 4). Different letters above the bars represent significant differences ($p < 0.05$).

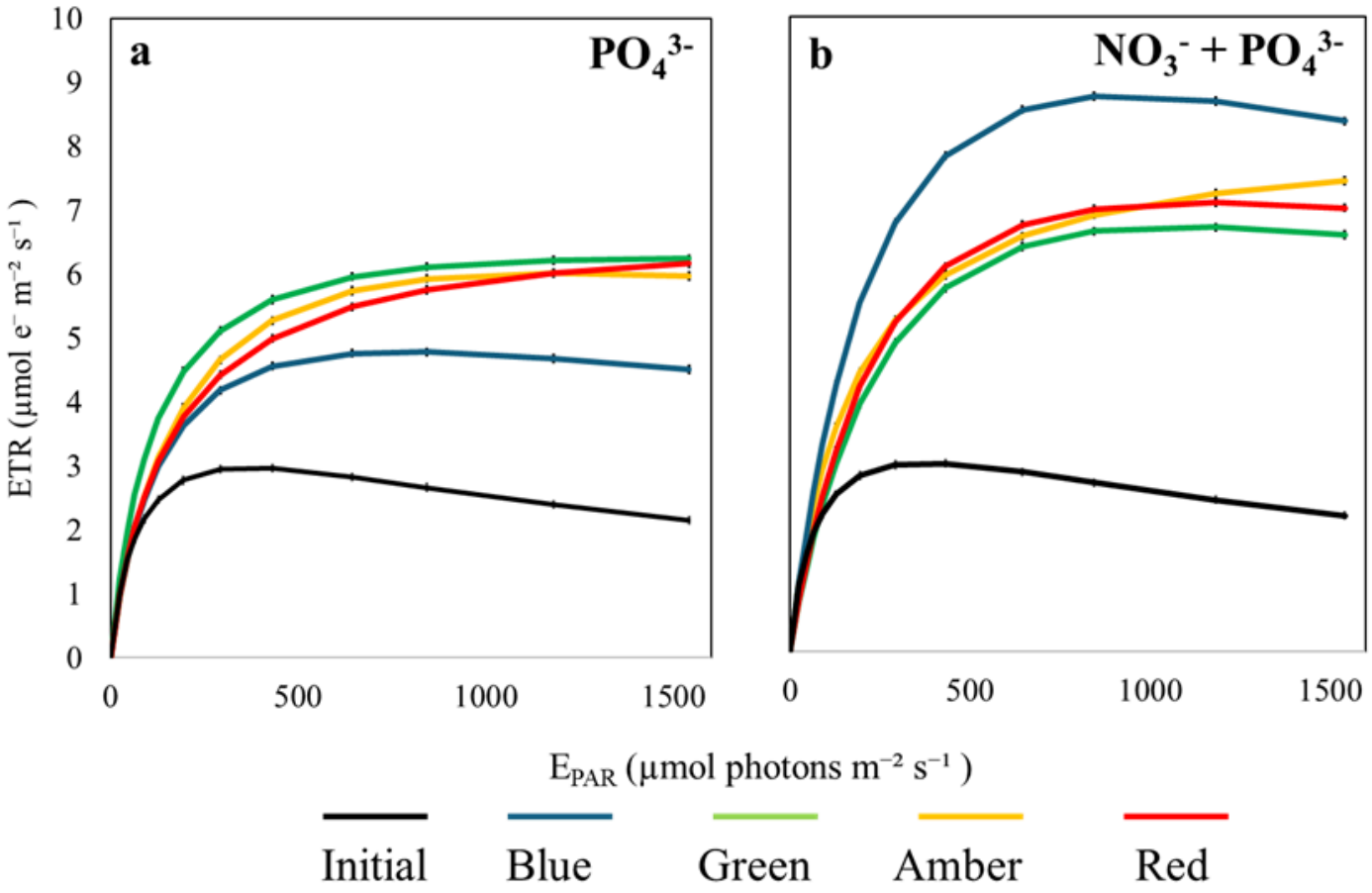

Figure 6

RLC of *C. teedei* (n=4) i.e. Electron transport rate (ETR) versus irradiance of photosynthetic active radiation (E_{PAR}) supplemented without (a) or with N (b). Light quality treatments are represented as blue, green, amber and red line in each graph. Black line represents the initial RLC before incubation. Data is represented as mean ± SD (n = 4).

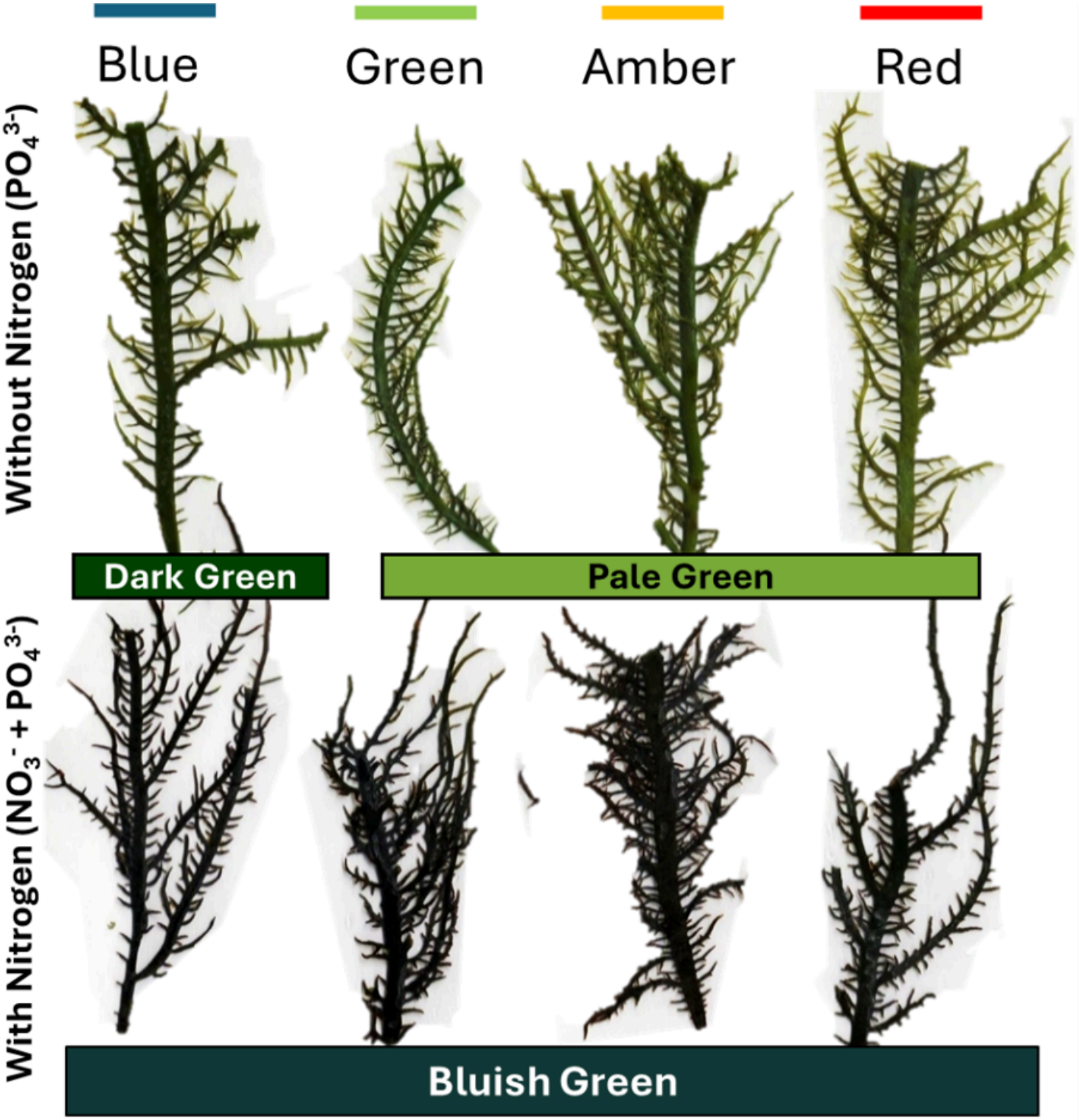

Figure 7

Final morphotypes of *C. teedei* after 11 days of incubation under different light qualities (Blue; Green; Amber; Red) and supplemented with or without N.

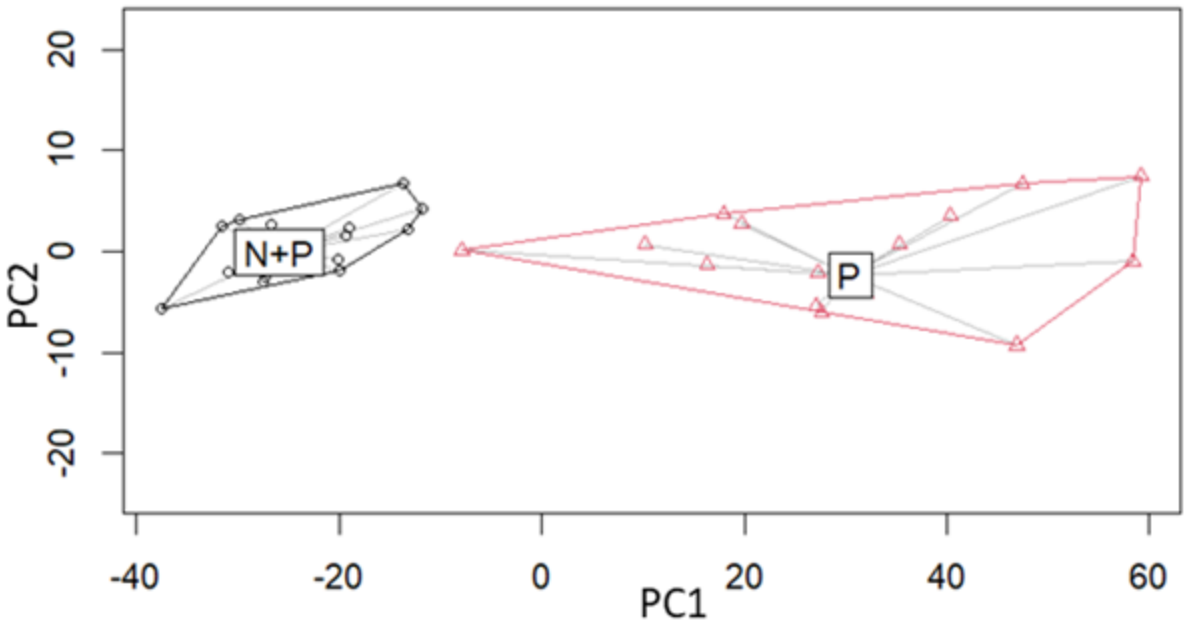

Figure 8

PCA depicting final colour types of *C. teedei* after 11 days of incubation at different light qualities (Blue; Green, Amber; Red) with (N+P) or without N (P) (n=4).

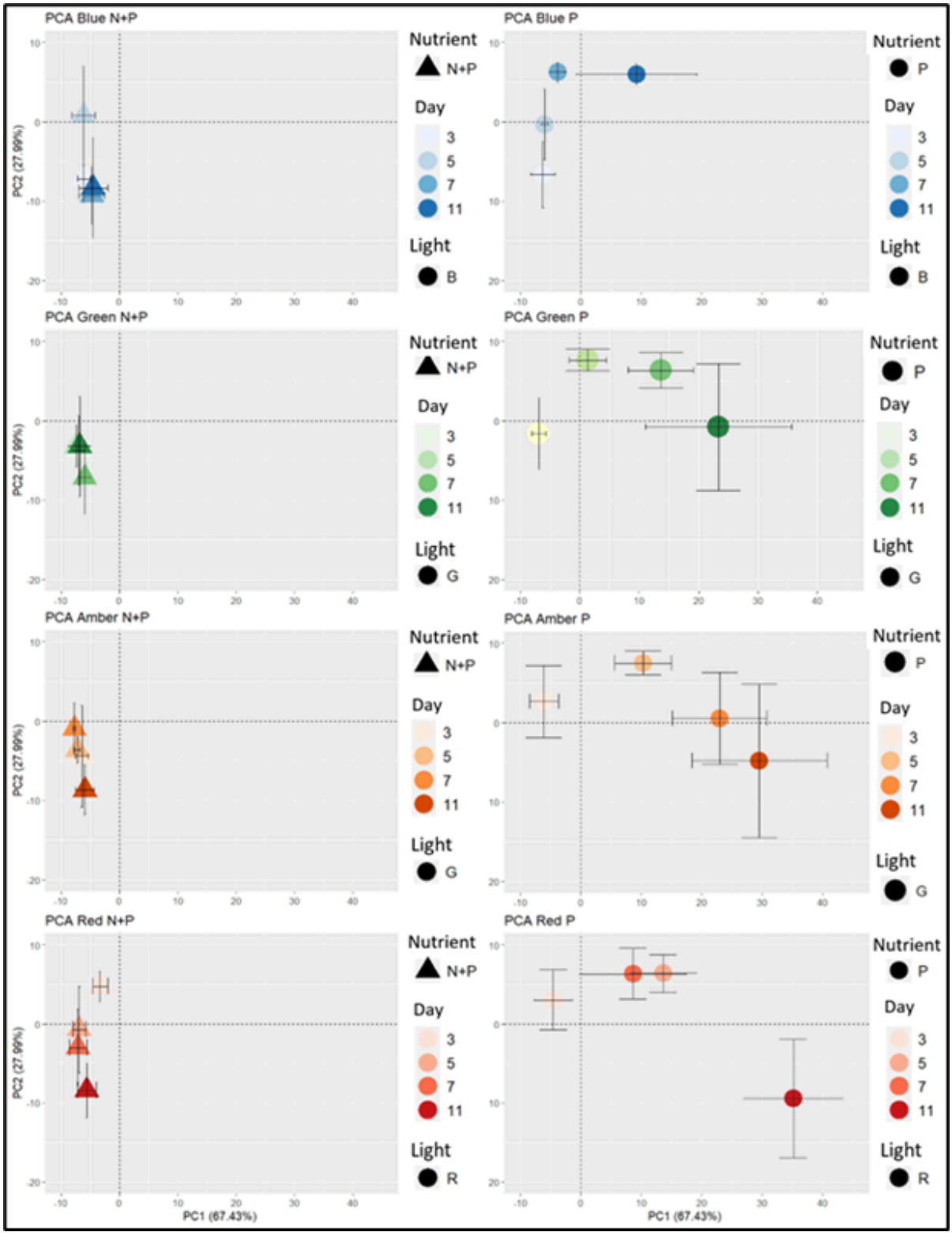

Figure 9

PCA for colour changes along the experiment of *C. teedei* incubated at different light qualities (B; G, A; R) with (N+P) or without N (P) enrichment (n=4).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Moreuetal.2025JApplPhycolSupplMat.docx