Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

In vitro assays with Kappaphycus alvarezii for the bioremediation of coastal eutrophic systems in Southeast Brazil

Raquel Oliveira-Moreira
University Federal Fluminense, Niterói – RJ
Teresa Cristina Mello-Guimarães
University Federal Fluminense, Niterói – RJ
Michele Maria Silva
Institute of Radioprotection and Dosimetry (CNEN)
Manuella Borges-Barreto
Institute of Radioprotection and Dosimetry (CNEN)
Tatiane Rocha Pereira
Institute of Radioprotection and Dosimetry (CNEN)
Maria Angélica Wasserman
Institute of Radioprotection and Dosimetry (CNEN)
Julio Cesar Wasserman
julio.wasserman@gmail.com

University Federal Fluminense, Niterói – RJ

Research Article

Keywords: Coastal ecosystem, Eutrophication, Bioremediation, Nutrient absorption rate, Kappaphycus alvarezii

Posted Date: April 1st, 2024

DOI: https://doi.org/10.21203/rs.3.rs-4151223/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Additional Declarations: No competing interests reported.

Abstract

The commercial alga *Kappaphycus alvarezii* (Doty) L.M.Liao 1996 may constitute a good bioremediator for eutrophicated coastal environments. In the present research, the ability of *K. alvarezii* to remove nutrients from the water was tested via *in vitro* experiments under various conditions. Cultivated *K. alvarezii* samples were obtained from commercial aquaculture in Angra dos Reis, Brazil, together with large volumes of water from the same area and Rodrigo de Freitas Lagoon, Rio de Janeiro, Brazil. Eight experimental sets were prepared with variable concentrations of nutrients, and pre-weighed pieces of algae, subjected to artificial illumination. The physical-chemical parameters were continuously monitored. Water samples were periodically analyzed for ammonium, nitrite, nitrate, and phosphate. Algal samples were collected at the end of the experiment to quantify biomass growth/decay, nitrogen and phosphorus tissue contents. The ammonium concentration in the water decreased due to preferential consumption of this nutrient by the algae, as well as oxidation. The algae were able to incorporate nutrients in their tissues; however, they were not able to promote a reduction in dissolved concentrations. Nutrient removal rates were elevated and seemed to occur in microcosms at the very beginning of the experiments, but release was observed in the end. However, phosphorus removal was attained in the microcosms that did not receive artificial fertilizers. It was concluded that the use of *K. alvarezii* as a bioremediator in coastal lagoons is possible, but more complex *in vitro* and *in situ* experiments are still necessary for a better understanding of removal processes.

1. Introduction

Marine macroalgae, especially red algae, are considered an important renewable marine resource for the production of various natural products, such as food, animal feed, biostimulants, and hydrocolloids (Rakhasiya et al. 2023). *Eucheuma* spp. and *Kappaphycus alvarezii* (Doty) L.M.Liao 1996 are among the most cultivated seaweeds used for carrageenans, and *Gracilaria* spp. are used for agar production(Debbarma et al. 2022). The production of *K. alvarezii* has been consistently increasing since the beginning of its commercial cultivation due to the growing demand (Narvarte et al. 2022). In addition to their use in the food, cosmetic, and pharmaceutical industries, macroalgae can be applied in the bioremediation of aquatic environments and can serve as a natural fertilizer in agriculture. The relevance of algae as a source of renewable energy in the production of biofuels should also be emphasized (Biris-Dorhoi et al. 2020).

According to FAO (2022), in the year 2020, the total production of agriculture was 87.5 million tons of aquatic animals and 35.1 million tons of algae for both food and non-food products, representing a 1.4% increase from 2019. In 2020, production increased in countries such as China and Japan, while cultivation decreased in Southeast Asia and the Republic of Korea. Asian countries were the largest producers, accounting for 97% of the total algae production in 2020. China contributed 58% of the total, becoming the leading producer, followed by Indonesia with 27% and the Republic of Korea with 5%. Among marine macroalgae, *K. alvarezii* is one of the most well-known and economically valuable for carrageenan production. Native to the Philippines, it was introduced in various countries for commercial

purposes (Das et al. 2021). In Brazil, specifically in Santa Catarina, the production of *K. alvarezii* between 2022 and 2023 (300.35 tons) increased 194% compared to that in 2021/2022 (102.3 tons). A total of 31.3 tons per hectare were produced in a rather small area of 9.59 hectares. On average, each producer yielded 13.65 tons in an area with an average size of 0.44 hectares (Santos 2023).

For the optimal biomass production of algae, it is important to understand the physiology of the nutrients they absorb and the environmental factors that influence their metabolism. The chemical composition of algae can vary significantly due to environmental conditions (light, temperature, habitat, salinity) and genetic differences (Biris-Dorhoi et al. 2020). Light and temperature impact the nutrient uptake capacity of algae, thereby influencing their growth and productivity (Roleda and Hurd 2019). Red macroalgae contain polysaccharides (carrageenan or agar) that make up 40–50% of their dry matter (Carpena et al. 2022); in addition, they contain proteins, amino acids, sterols, carotenoids, bromophenols, and other natural bioactive compounds.

In addition to constituting an important commercial product, macroalgae are part of the food web in coastal marine environments and are increasingly cultivated for bioremediation in the aquaculture sector, helping to mitigate environmental impacts (Guillén et al. 2022). Several studies have assessed the behavior of the macroalga *Ulva lactuca* in the presence of various pollutants in aquatic environments (Areco et al. 2021). This species is resistant to salinity variations, exhibits a high growth rate, and displays strong rates of nutrient absorption, particularly ammonium (NH_4^+), showing good development in eutrophic environments (Nielsen et al. 2012; Bonanno et al. 2020). The alga *Gracilaria gracilis* has also been considered effective at reducing dissolved nutrients such as nitrogen and phosphorus. Its cultivation tends to contribute significantly to both eutrophication control and biotechnology due to its use for antibiotic and agar production (Spanò et al. 2022).

K. alvarezii has been studied for its potential use as a bioremediator. In some studies, it has been observed that its cultivation enhances carbon dioxide sequestration, and it acts as a nutrient sink when it is grown with other organisms under various salinities, improving water quality (Hayashi et al. 2010; Reis et al. 2011). The potential for removing nitrogen and phosphorus compounds from seawater has been demonstrated, with reductions of 24% in nitrite and nitrate concentrations and 6% in phosphate concentration (Doty et al. 1987).

In this context, algae cultivation can promote an improvement in water quality due to its potential for nutrient removal (Hayashi et al. 2008) and provide shelter for various species of fish, crustaceans, and other aquatic organisms (Paula et al. 1998). The aim of this study was to assess, through *in vitro* experiments, the nutrient removal rate following the cultivation of the macroalgae *K. alvarezii* in environments with the addition of fertilizer, in natural conditions, and eutrophicated by anthropogenic activities.

2. Materials and Methods

The assay was carried out in the laboratory of the Institute of Radioprotection and Dosimetry (IRD/CNEN), where microcosms were installed. The samples collected during the assay were transported to the Institute of Geosciences at the University Federal Fluminense (UFF) for the determination of dissolved nutrients (ammonium, nitrite, nitrate and phosphate), total nitrogen and total phosphorus (in algae tissue). The analyses were carried out at the Natural Water Analysis Laboratory (LAAN) of the UFF Environment and Sustainable Development Network (REMADS-UFF) at the same institute.

The first water collection was carried out near the shore of Conceição de Jacareí Beach at coordinates 23°01'59.81"S (latitude) and 44°09'42.89"W (longitude). The algae collection site was close to Sororoca Island, with coordinates 23°02'16.69"S (latitude) and 44°09'40.38"W (longitude). Notably, in Rio de Janeiro, *K. alvarezii* is cultivated in floating rafts (Fig. 1). The second water collection site was Rodrigo de Freitas Lagoon at coordinates 22°58'20.50"S (latitude) and 43°12'56.97"W (longitude), a choked coastal system that receives large quantities of untreated sewage from the drainage basin.

Eight microcosms with a recirculating water system were used for this assay according to Guimarães *et al.* (Fig. 2). The setup of the experiments is summarized in Table 1. Commercial fertilizer (inorganic fertilizer applied in hydroponics) was added to six of the microcosms (the composition is described in Table 2) to test the effect of different concentrations on the growth of *K. alvarezii*. To assess the behavior of the algae in different environments, microcosm 1 (lagoon) and 2 (control) did not receive fertilizer, as they were filled with water from Rodrigo de Freitas Lagoon (naturally contaminated) and Mangaratiba uncontaminated seawater, respectively.

Reservoirs 1 in all microcosms (Fig. 2) were carefully filled with seawater or lagoon water to a height of 15 cm. Within each microcosm, two 40 cm long ropes (10 mm thick) were placed (as shown in Figs. 2 and 3), supporting algae pieces fixed with twine. Each rope held 6 pieces of approximately 3 cm of *K. alvarezii*, totaling 12 algae pieces in each microcosm. To enhance algae growth, besides adding fertilizer to the water, artificial lighting was provided by LED SMD2835 lamps (red and blue) and Grow Power brand panels, with 1000 W and a luminosity of 9500 lm, which were emitted in the wavelength range between 400–700 nm, featuring 81 light points and measuring 180x180x20 mm. The illumination system was connected to a 12-hour timer to simulate daylight/night dark periods (Fig. 3).

Table 1
– Setup of the microcosm experiments in the present research.

Microcosm	Environmental setup	Fertilization
Microcosm 1	K alvarezii cultivation in the Rodrigo de Freitas lagoon water	Natural
Microcosm 2	K alvarezii cultivation in the seawater	No
Microcosms 3 and 4	K alvarezii cultivation in the seawater – Microcosm A	40 grams of fertilizer in 40.5 L seawater
Microcosms 5 and 6	K alvarezii cultivation in the seawater – Microcosm B	30 grams of fertilizer in 40.5 L seawater
Microcosms 7 and 8	K alvarezii cultivation in the seawater – Microcosm C	20 grams of fertilizer in 40.5 L seawater

Table 2
Fertilization applied in each of the microcosms, assays A, B and C. The values were calculated based on the recipe enclosed in the commercial product MLB-1008787979 (hydroponics fertilizer). The values in grams for each compound correspond to the value added to each of the 40.5 L microcosms.

Compounds	%	Microcosm A (3 and 4)	Microcosm B (5 and 6)	Microcosm C (7 and 8)
NO_3 (g)	7.8	3.104	2.328	1.552
NH_4 (g)	0.6	0.242	0.182	0.121
P_2O_5 (g)	3.0	1.200	0.900	0.600
K_2O (g)	9.3	3.733	2.800	1.867
Mg (g)	1.1	0.451	0.338	0.225
S (g)	1.3	0.533	0.400	0.267
B (g)	0.02	0.008	0.006	0.004
Cu (g)	0.003	0.0013	0.0010	0.0007
Mn (g)	0.02	0.007	0.005	0.003
Mo (g)	0.02	0.010	0.007	0.005
Zn (g)	0.01	0.003	0.002	0.001
Fe (g)	2.0	0.800	0.600	0.400
CaO (g)	8.8	3.533	2.650	1.767
Insoluble Minerals and organic compounds (g)	65.9	26.375	19.781	13.187

At the beginning of the assays, seawater, lagoon water, and macroalgae were characterized in terms of their physicochemical parameters and nutrient concentrations, and the initial values of the microcosm experiments were recorded. Water and algae sampling, preparation, and the initiation of microcosm experiments took place on July 10th, 2022, with microcosm sampling measurements starting the following day (Fig. 4). The *in vitro* assays were conducted under uncontrolled laboratory temperature conditions. Water samples collected during the assays were stored in capped centrifuge tubes (50 ml) and frozen for subsequent analyses. Water samples were collected at T0, T1 (2 days), T2 (9 days), and T3 (15 days), as described in Fig. 4. The sampled macroalgae were transported to the laboratory in plastic bags, briefly rinsed with tap water to remove epiphytes and sediments, and introduced to the microcosms. Aliquots of the plant material were placed in an oven for evaluation of the dry weight, which was determined after drying at 50°C until constant weight.

Water column measurements were carried out according to Fig. 4, and macroalgae biomass sampling was carried out weekly in the microcosms. To check the pH and Eh, a Hanna HI8424 portable meter was used, with specific electrodes for each parameter. Dissolved oxygen and temperature were measured with a Mettler-Toledo Seven 2Go Pro optical oximeter. Water samples were collected, filtered through preweighed Whatman GF/C filters, and poured into 50 mL Falcon tubes (for nutrient determination). The concentrations of dissolved inorganic nitrogen (sum of ammonium, nitrite and nitrate concentrations) and dissolved inorganic phosphorus (orthophosphate) were determined in the filtered samples. Spectrophotometric analyses were performed according to the procedures described by Strickland and Parsons (1972), Grasshoff et al. (1983) and Zhang and Fischer (2006).

Based on the results of the analyses, the removal rates (*RRs*) were calculated to verify the biofilter potential of *K. alvarezii* with the equation used by Hayashi et al. (2008) (Eq. 1).

$$RR\,(\%deremo\mathrm{val}) = [(\,Ri - Rf)/(Ri\,)]\,x100 \text{ (Eq. 1)}$$

where RR = Removal Rate

Ri = Initial nutrient concentration (µM)

Rf = final concentration relative to the days of cultivation (µM)

RR values were calculated only for total nitrogen because we understand that conversions among chemical forms of nitrogen (nitrate-nitrite-ammonium and vice-versa) may occur due to biochemical processes not linked to algae removal.

3. Results

The results of the present research are presented in Table 1 and Figs. 5–9. Physico-chemical parameters measured during the assays and the raw results of nutrient concentrations were presented in Supplementary Materials 1 and 2.

3.1. Concentrations in the microcosms water

Seawater and lagoon water were quite different, with salinities of 34.09 (marine condition) and 12.83 (brackish) and pH values of 8.37 (marine) and 7.16 (lagoon). The introduction of fertilizers likely affected the physicochemical parameters, with a significant drop in pH (ranging between 5.5 and 6.5 in all microcosms; the raw data are presented in the Supplementary Materials 1). The dissolved oxygen concentration was relatively stable throughout the assay at approximately 6 mg L^{-1}, indicating that the water circulation in the system ensures aeration (Supplementary Materials 1). There was no system to control the temperature in these microcosm assays, but measurements showed slight fluctuations between 23°C and 24°C throughout the assay (Supplementary Materials 1).

Table 3 shows significant variations in nutrient concentrations of the water during *K. alvarezii* cultivation (raw data are presented in Supplementary Materials 1). It is possible to observe that concentrations of nitrogen forms considerably oscillate.

Figures 5 and 6 show the *removal rates (RRs*) in each experimental microcosm for dissolved inorganic nitrogen (molar summation of dissolved nitrogen forms) and dissolved inorganic phosphate, respectively. As shown in Fig. 5, microcosm 2 (only seawater and algae) showed a small release in the first 9 days, then a strong release in the following period, which promoted a strong overall (T1-T3) release of nitrogen. The enrichment of the cultivation water with nutrients did not yield very strong removal of nitrogen (Fig. 5), but there was a greater efficiency in the second period in the enriched microcosms, which barely compensated for the initial release of microcosms A and C. In low salinity naturally eutrophicated water (microcosm 1), a strong release of nutrients in the first period is compensated for by strong removal in the second period, but yields an overall release of nitrogen.

Table 3
Ammonium, nitrite, nitrate, and phosphate concentrations obtained throughout the assays.

	Assays	NH_4^+ (mg L^{-1})	NO_2^- (mg L^{-1})	NO_3^- (mg L^{-1})	PO_4^{3-} (mg L^{-1})
T0	Lagoon	0.20	0.03	0.58	0.16
	Sea	< 0.01	0.03	1.13	0.10
T1 (2 days)	Microcosm 1 (lagoon)	0.39	0.02	0.60	0.67
	Microcosm 2 (sea)	< 0.01	0.02	0.40	0.04
	Microcosm A	0.95	0.13	14.26	7.74
	Microcosm B	0.79	0.23	14.53	7.81
	Microcosm C	0.57	0.67	7.36	8.31
T2 (9 days)	Microcosm 1 (lagoon)	0.51	0.91	1.33	0.32
	Microcosm 2 (sea)	< 0.01	0.01	0.40	0.05
	Microcosm A	0.93	1.60	16.94	9.72
	Microcosm B	0.74	1.51	12.58	10.04
	Microcosm C	0.54	1.34	8.06	10.58
T3 (15 days)	Microcosm 1 (lagoon)	0.01	0.67	1.07	0.14
	Microcosm 2 (sea)	0.01	0.02	0.75	0.05
	Microcosm A	0.96	1.39	15.22	10.87
	Microcosm B	0.74	1.38	11.96	10.97
	Microcosm C	0.61	1.39	6.40	11.58

Figure 6 shows that in microcosm 1 algae promoted a high removal rate throughout the entire experimental period. In the seawater (microcosm 2), the concentrations of phosphate are low (0.10 mg L^{-1}), and regardless of plant growth, the algae did not seem to remove phosphate. Although the *RR* was very negative (-25%), the concentration in the water increased by only 0.01 mg L^{-1}. Microcosms A, B, and C presented very similar behaviors, with increasing concentrations in the water.

3.2. Biomass, nitrogen, and phosphorus in algae tissues

Figure 7 shows that the algal biomass tended to decrease toward the end of the assay, indicating senescence and decay of the organic matter. Measurements of biomass were performed at the beginning

(the wet weight of algae introduced in each microcosm) and at the end of the experiments (the remaining algae were weighed); therefore, it was not possible to verify oscillations during cultivation.

Regardless of the fact that biomass generally decayed during the assays (Fig. 7), total nitrogen and total phosphorus in the algal tissues tended to get enriched at the very beginning of the experiments (T1, 2 days), decreasing from the first to the second and last samplings (Figs. 7 and 8; Fig. 4 indicates the sampling periods).

It is noteworthy the fact that nitrogen and phosphorus concentrations in the natural environment collected algae (T0) were always lower than concentrations in the assays, even in its end, after a strong decrease. Although we cannot claim that this enrichment is attributed to the removal of nutrients, at least the rapid increase in T1 may indicate removal of nutrients from the water column. We didn't calculate a balance of nutrients in the water because there are many parameters affecting nutrients that were not followed, like consumption by phytoplankton and bacteria, denitrification, and nitrification (chemical and biological).

4. Discussion

Regardless of the variations of the physical-chemical parameters in the water column in the different experimental microcosms, the development of algae in both systems was similar. This is different from what was observed by Reis et al. (2011), who verified a twofold growth performance in seawater compared with brackish water (15 PSU) with the brown variant of *K. Alvarezii*. With other variants (green and red), these authors observed worse performances in lower salinities. It was expected that values of pH, temperature, and dissolved oxygen wouldn't oscillate, but it was verified that adding fertilizers promoted acidification of the systems. Even primary production (normally an alkalinizing process; Cunha and Wasserman, 2003) was not able to increase pH.

Variations in chemical forms of nitrogen (Table 3 and Supplementary Materials 2) can be associated with a few factors that control dissolved nutrients, including oxidation, which converts ammonium into nitrite and nitrate, and macroalgae consumption and excretion. Phytoplankton which is present in the microcosms may also play a role in the removal or excretion of nutrients (Herbert 1999). For dissolved phosphate, primary producers (including algae and phytoplankton) significantly affected its concentrations (Williamson et al. 2018). It cannot be excluded that part of the added fertilizers could have remained undissolved in the marine waters of the assays.

The removal rates of dissolved inorganic nitrogen (Fig. 5) show strong release (negative values) for nitrogen in the beginning of all assays (T1 – T2) and a mild removal in the second period (T2 – T3). The behavior of phosphorus was different (Fig. 6), showing that algae are strongly affected by the availability of nutrients, and poor or excess nutrients may change their metabolic state and therefore their removal rates from the water column (Pedersen 1994; Beardall and Raven 2021). Dissolved inorganic phosphorus in mesocosm 1 (Fig. 6) is an example where the availability of natural phosphate (not fertilized) promoted a more intense removal during all phases of the assay. Lenzi et al. (2003) showed that the

provision of nutrients (in their case, from the sediment) in a eutrophicated system may promote an increase in algal biomass. These authors state that the regeneration of phosphate from the sediment was constant and the algal biomass had to be managed; otherwise, its decomposition would release nutrients back into the sediment. In our case, considering that *K. alvarezii* is a commercially important plant, its production in sediment-contaminated areas is interesting.

In microcosms A, B, and C, significant removal or release of nutrients from plants could be discarded because the amount of dissolved phosphorus in the water is too large (due to the addition of fertilizer) to be affected by these inputs. On the other hand, the absorption of nutrients by algae in these enriched microcosms may not be noticeable from water concentrations (Fig. 6). On the other hand, dissolved inorganic phosphorus concentrations in the mesocosm 2 (natural seawater) were very low and could not be significantly affected by removal, nor by release (just a small release was observed).

From our results, it cannot be asserted that algae can remove ammonium to supply their nitrogen needs, however, the literature states that this is the preferred chemical form of nitrogen for algae (Kim et al. 2007). Although Hayashi et al. (2008) reported an ammonium removal rate of 70% in integrated algae/fish farming, in our work this evaluation would be conjectural because oxidation due to aeration and bacterial nitrification/denitrification were not accountable (Kuenen and Robertson 1994).

The observed increase in DIN observed in microcosm 1 in the first 9 days (Fig. 5) may be attributed to the decomposition of organic matter in the water, considering that during collection the water was observed to be very turbid. However, within a few days, the water recirculation system in the mesocosms seemed to have degraded the organic matter (probably BOD), and the water then became transparent (such as in a sewage treatment plant). This degradation of organic matter likely released some soluble nitrogen into the water column. However, at the end of the assay, it appears that the algae were able to remove much of this nitrogen from the water column.

For phosphate concentrations (corresponding to DIP) there was a removal of 79% in microcosm 1 (lagoon water) between T1 and T3, and 48% in microcosm 2 (control/seawater) that were far better than reported by Doty et al. (1987) who verified reductions of 6% in phosphate and 24% in nitrite and nitrate concentrations in experiments with *K. alvarezii* cultivated in the Philippines.

The cultivation of seaweed in microcosms is a consistent way of testing biological responses to strictly controlled environmental conditions; however, many studies have shown that these assays should not last very long (Peng et al. 2022; Hayashi et al. 2011). For many reasons, algae tend to decay after a few weeks, and in many cases, some authors have observed the incidence of ice-ice disease (Pedra et al. 2017). In our experiments, the decay of the algae started in the mean course and measurements were only carried out in the end, so we could not follow any relevant growth in the first few days. Pires et al. (2021), in a 70-day growth assay with *K. alvarezii*, measured biomass every 7 days, and the growth rates increased and decreased alternately until the end of the assay. This should also be occurring in our work; however, we could not depict these alternations that should promote growth (and more intense nutrient removal) in the beginning and then, senescence in the end.

Although the biomass measurements indicate only the decay in the end, the analyses of the algal tissue nitrogen allowed to verify whether the absorption rates varied in relation to the concentrations in the water and to better understand the removal process. For nitrogen and phosphorus, there is enrichment of the tissues in the beginning of the assay (Figs. 8 and 9), probably due to the strong change in environmental conditions. Luxury absorption of nutrients (Hurd et al. 2014) was reported in the Antarctic algae *Himantothallus grandifolius*, *Laminaria solidungula* subject to strong environmental changes and oscillations in nutrient availability (Korb and Gerard 2000). This behavior has been reported for *K. alvarezii* by Kambey et al. (2020) in experiments carried out in fish floating net cages in Indonesia. At the end of the assay, after 15 days of incubation the nitrogen and phosphorus were released back into the water column, but as shown in Figs. 5 and 6, no release of nutrients was detected in the water column, probably because they were consumed by phytoplankton or bacteria.

5. Conclusion

The follow up of the physical-chemical parameters in the assays indicate that it was possible to reproduce environmental conditions. Although the incorporation of fertilizers promoted a severe reduction in the pH, this parameter remained constant throughout the experiment, and the growth rates of the algae were not impacted by this parameter. As we observed, the algae could not survive for more than 15 days in the assay's environmental conditions.

In the first period of the experiment, *K. alvarezii* was able to grow in all experimental microcosms (even in lower salinities); however, the response in terms of nutrient removal is not clear, because there were several processes, including oxidation, consumption by phytoplankton that interfered in the evaluation. The oxidation process that was forced in the microcosms did not allow to distinguish removal of the different nitrogen forms, including ammonium which is reputed to be the preferential form consumed by algae. The analysis of removal/release (*RR*) of total nitrogen facilitated interpretations.

It was observed that the behavior of *K. alvarezii* in naturally eutrophicated and fertilized waters was quite distinct. A strong removal of phosphorus was observed in the natural eutrophicated assay, while in the fertilized water this behavior was not observed, indicating that the response of algae to fertilization is not direct, but it depends on various metabolic parameters. This can be confirmed by the strong enrichment in algal tissue nutrient content at the beginning of all assays, which should be interpreted as a luxurious accumulation.

The removal/release rates (*RR*) indicate that many processes take place simultaneously, including the release of nutrients through the decomposition of organic matter, removal, and release by macroalgae, removal and release by microalgae. Therefore, the expected removal of nutrients from the water column as algae grow was not clearly observed. It would be interesting to remove organic matter and phytoplankton from the assays by previous filtrations and oxidations, but then, microcosms would not accurately reproduce environmental conditions. In future research, control of these parameters by measuring chlorophyll a, and total nitrogen, phosphorus, and carbon in filtered samples is a better idea to

better understand the algal removal/release rates of nutrients in assays water. On the other hand, *in situ*, large-scale experiments should also be carried out to better understand the use of these algae as bioremediators.

Declarations

Funding: This research received funds from the following governmental agencies: Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) Grant # 310425/2020-4) and PCI/MCTI CNPq Program for the Scholarship grant (# 301233/2022-5 and 300418/2024-8).

Competing Interest: The authors have no competing interests to declare that are relevant to the content of this article.

Availability of data and material: Raw data were made available as Supplementary Materials.

Authors Contribution

Raquel de Oliveira Moreira: Methodology; Formal Analysis and investigation; Writing – original draft preparation

Teresa Cristina Mello-Guimarães: Methodology; Formal Analysis and investigation; Writing review and editing

Michele Maria da Silva: Methodology; Formal Analysis and investigation; Writing – original draft preparation

Manuella Borges-Barreto: Methodology; Formal Analysis and investigation; Writing – original draft preparation

Tatiane Rocha Pereira: Methodology; Formal Analysis and investigation; Writing – original draft preparation

Maria Angélica Wasserman: Formal analysis and investigation; Writing - review and editing; Resources; Supervision;

Julio Cesar Wasserman: Conceptualization; Methodology; Formal analysis and investigation; Writing - review and editing; Supervision

Acknowledgements

JCW is grateful to Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for the financial support (Grant # 310425/2020-4). MMS, MBB are thankful to Programa de Capacitação Institucional – Ministério de Ciência Tecnologia e Inovação - Conselho Nacional de Desenvolvimento

Científico e Tecnológico (PCI/MCTI CNPq) for Scholarship grants (# 301233/2022-5 and 300418/2024-8, respectively).

References

1. Areco MM, Salomone VN, Afonso MdS (2021) *Ulva lactuca*: A bioindicator for anthropogenic contamination and its environmental remediation capacity. Marine Environmental Research 171:105468. doi:https://doi.org/10.1016/j.marenvres.2021.105468
2. Beardall J, Raven JA (2021) Algal Metabolism. In: Encyclopedia of Life Sciences. John Wiley & Sons, Ltd, pp 17–25. doi:https://doi.org/10.1002/9780470015902.a0029261
3. Biris-Dorhoi ES, Michiu D, Pop CR, Rotar AM, Tofana M, Pop OL, Socaci SA, Farcas AC (2020) Macroalgae-A Sustainable Source of Chemical Compounds with Biological Activities. Nutrients 12 (10). doi:10.3390/nu12103085
4. Bonanno G, Veneziano V, Piccione V (2020) The alga *Ulva lactuca* (Ulvaceae, Chlorophyta) as a bioindicator of trace element contamination along the coast of Sicily, Italy. Science of The Total Environment 699:134329. doi:https://doi.org/10.1016/j.scitotenv.2019.134329
5. Calijuri MdC, Cunha DGF (2013) Engenharia Ambiental: conceitos, tecnologia e gestão. Elsevier,
6. Carpena M, Garcia-Perez P, Garcia-Oliveira P, Chamorro F, Otero P, Lourenço-Lopes C, Cao H, Simal-Gandara J, Prieto MA (2022) Biological properties and potential of compounds extracted from red seaweeds. Phytochemistry Reviews. doi:10.1007/s11101-022-09826-z
7. Cunha LC, Wasserman JC (2003) Relationship between nutrients and macroalgal biomass in a Brazilian Coastal Lagoon: The impact of a lock construction. Chem Ecol 19 (4):283–298. doi:https://doi.org/10.1080/0275754031000095732
8. Das AK, Sequeira RA, Maity TK, Prasad K (2021) Bio-ionic liquid promoted selective coagulation of k-carrageenan from *Kappaphycus alvarezii* extract. Food Hydrocolloids 111:106382. doi:https://doi.org/10.1016/j.foodhyd.2020.106382
9. Debbarma J, Viji P, Rao BM, Ravishankar CN (2022) Seaweeds: Potential Applications of the Aquatic Vegetables to Augment Nutritional Composition, Texture, and Health Benefits of Food and Food Products. In: Ranga Rao A, Ravishankar GA (eds) Sustainable Global Resources of Seaweeds Volume 2: Food, Pharmaceutical and Health Applications. Springer International Publishing, Cham, pp 3–54. doi:10.1007/978-3-030-92174-3_1
10. Doty MS, Caddy JF, G BS, Santelices B (1987) Case Studies of Seven Commercial Seaweed Resources (FAO Fisheries Technical Papers). Organização das Nações Unidas para Agricultura e Alimentação (FAO),
11. FAO (2022) The State of World Fisheries and Aquaculture 2022. Towards Blue Transformation. FAO, Rome. doi:https://doi.org/10.4060/cc0461en
12. Grasshoff K, Manfred E, Klaus K (1983) Methods of seawater analysis. 2 edn. Verlage Chemie, Florida

13. Guillén PO, Motti P, Mangelinckx S, De Clerck O, Bossier P, Van Den Hende S (2022) Valorization of the chemical diversity of the tropical red seaweeds *Acanthophora* and *Kappaphycus* and their applications in aquaculture: A review. 9. doi:10.3389/fmars.2022.957290
14. Guimarães TCSM, Montenegro KS, Wasserman MAV, Wasserman JC (2021) Innovative microcosm experiments for the evaluation of the regeneration rates of nutrients in sediments of a hypersaline lagoon. Mar Poll Bull 166:112252. doi:https://doi.org/10.1016/j.marpolbul.2021.112252
15. Hayashi L, Faria GSM, Nunes BG, Zitta CS, Scariot LA, Rover T, Felix MRL, Bouzon ZL (2011) Effects of salinity on the growth rate, carrageenan yield, and cellular structure of *Kappaphycus alvarezii* (Rhodophyta, Gigartinales) cultured in vitro. Journal of Applied Phycology 23 (3):439–447. doi:10.1007/s10811-010-9595-6
16. Hayashi L, Hurtado AQ, Msuya FE, Bleicher-Lhonneur G, Critchley AT (2010) A Review of Kappaphycus Farming: Prospects and Constraints. In: Seaweeds and their Role in Globally Changing Environments. Cellular Origin, Life in Extreme Habitats and Astrobiology. pp 251–283. doi: http://doi.org/10.1007/978-90-481-8569-6_15.
17. Hayashi L, Yokoya Ns, Ostini S, Pereira R, Braga E, Oliveira E (2008) Nutrients removed by *Kappaphycus alvarezii* (Rhodophyta, Solieriaceae) in integrated cultivation with fishes in re-circulating water. Aquaculture 277:185–191. doi:10.1016/j.aquaculture.2008.02.024
18. Herbert RA (1999) Nitrogen cycling in coastal marine ecosystems. FEMS Microbiology Reviews 23 (5):563–590. doi:10.1111/j.1574-6976.1999.tb00414.x %J FEMS Microbiology Reviews
19. Hurd CL, Harrison PJ, Bischof K, Lobban CS (2014) Seaweed Ecology and Physiology - Chap. 6: Nutrients. Seaweed Ecology and Physiology, 2 edn. Cambridge University Press, Cambridge. doi:DOI: 10.1017/CBO9781139192637.007
20. Kambey CSB, Sondak CFA, Chung I-K (2020) Potential growth and nutrient removal of *Kappaphycus alvarezii* in a fish floating-net cage system in Sekotong Bay, Lombok, Indonesia. Journal of the World Aquaculture Society 51 (4):944–959. doi:https://doi.org/10.1111/jwas.12683
21. Kim JK, Kraemer GP, Neefus CD, Chung IK, Yarish C (2007) Effects of temperature and ammonium on growth, pigment production and nitrogen uptake by four species of *Porphyra* (Bangiales, Rhodophyta) native to the New England coast. Journal of Applied Phycology 19 (5):431–440. doi:10.1007/s10811-006-9150-7
22. Korb RE, Gerard VA (2000) Nitrogen assimilation characteristics of polar seaweeds from differing nutrient environments. Marine Ecology Progress Series 198:83–92
23. Kuenen JG, Robertson LA (1994) Combined nitrification-denitrification processes. FEMS Microbiology Reviews 15 (2–3):109–117. doi:10.1111/j.1574-6976.1994.tb00129.x
24. Lenzi M, Palmieri R, Porrello S (2003) Restoration of the eutrophic Orbetello lagoon (Tyrrhenian Sea, Italy): water quality management. Mar Poll Bull 46 (12):1540–1548
25. Narvarte BCV, Hinaloc LAR, Genovia TGT, Gonzaga SMC, Tabonda-Nabor AM, Roleda MY (2022) Physiological and biochemical characterization of new wild strains of *Kappaphycus alvarezii*

(Gigartinales, Rhodophyta) cultivated under land-based hatchery conditions. Aquatic Botany 183:103567. doi:https://doi.org/10.1016/j.aquabot.2022.103567

26. Nielsen MM, Bruhn A, Rasmussen MB, Olesen B, Larsen MM, Møller HB (2012) Cultivation of *Ulva lactuca* with manure for simultaneous bioremediation and biomass production. Journal of Applied Phycology 24 (3):449–458. doi:10.1007/s10811-011-9767-z
27. Paula EJd, Pereira RTL, Ostini S (1998) Introdução de espécies exóticas de *Eucheuma* e *Kappaphycus* (Gigartinales, Rhodophyta) para fins de maricultura no litoral brasileiro: abordagem teórica e experimental. Congresso Latino-Americano
28. Pedersen MF (1994) Transient ammonium uptake in the macroalga Ulva lactuca (Chlorophyta): Nature, regulation, and the consequences for choice of measuring technique Journal of Phycology 30 (6):980–986. doi:10.1111/j.0022-3646.1994.00980.x
29. Pedra AGL, Ramlov F, Maraschin M, Hayashi L (2017) Cultivation of the red seaweed *Kappaphycus alvarezii* with effluents from shrimp cultivation and brown seaweed extract: Effects on growth and secondary metabolism. Aquaculture 479:297–303. doi:10.1016/j.aquaculture.2017.06.005
30. Peng GG, Fu JX, Luo X, Pang BJ, Huang LF, Lan WL (2022) Determining the optimum ratio of polycultured shellfish and seaweed: A microcosm study. Appl Ecol Environ Res 20 (2):1241–1250. doi:10.15666/aeer/2002_12411250
31. Pires CM, Bazzo GC, Barreto PLM, Santo CMD, Ventura TFB, Pedra AGL, Rover T, McGovern M, Hayashi L (2021) Cultivation of the red seaweed *Kappaphycus alvarezii* using biofloc effluent. Journal of Applied Phycology 33 (2):1047–1058. doi:10.1007/s10811-020-02335-6
32. Rakhasiya B, Munisamy S, Mathew DE, Tothadi S, Yadav A, Mantri VA (2023) Potential utility of industrially unwanted constituent under the framework of waste to wealth: Edible salt from commercial marine red seaweed *Kappaphycus alvarezii* (Doty) L.M. Liao. Bioresource Technology Reports 23:101529. doi:https://doi.org/10.1016/j.biteb.2023.101529
33. Reis RP, Loureiro RR, Mesquita FS (2011) Does salinity affect growth and carrageenan yield of *Kappaphycus alvarezii* (Gigartinales/Rhodophyta)? Aquaculture Research 42 (8):1231–1234. doi:https://doi.org/10.1111/j.1365-2109.2010.02699.x
34. Roleda M, Hurd C (2019) Seaweed nutrient physiology: application of concepts to aquaculture and bioremediation. Phycologia 58:552–562. doi:10.1080/00318884.2019.1622920
35. Santos AA (2023) Produção da macroalga *Kappaphycus alvarezii* em Santa Catarina, safra 2022/2023. Agropecuária Catarinense 36 (2):7–9
36. Spanò N, Di Paola D, Albano M, Manganaro A, Sanfilippo M, D'Iglio C, Capillo G, Savoca S (2022) Growth performance and bioremediation potential of *Gracilaria gracilis* (Steentoft, L.M. Irvine & Farnham, 1995). International Journal of Environmental Studies 79 (4):748–760. doi:10.1080/00207233.2021.1954775
37. Strickland JDH, Parsons TR (1972) A practical handbook of seawater analysis. 2 edn. Fisheries Research Board of Canada, Ottawa

38. Williamson TJ, Vanni MJ, González MJ, Renwick WH, Bremigan MT, Conroy JD (2018) The importance of nutrient supply by fish excretion and watershed streams to a eutrophic lake varies with temporal scale over 19 years. Biogeochemistry 140 (2):233–253. doi:10.1007/s10533-018-0490-6
39. Zhang J-Z, Fischer C (2006) A simplified resorcinol method for direct spectrophotometric determination of nitrate in seawater. Marine Chemistry 99:220–226. doi:10.1016/j.marchem.2005.09.008

Figures



Figure 1

K. alvarezii macroalgae cultivation raft in Ilha Grande Bay, Rio de Janeiro, Brazil.

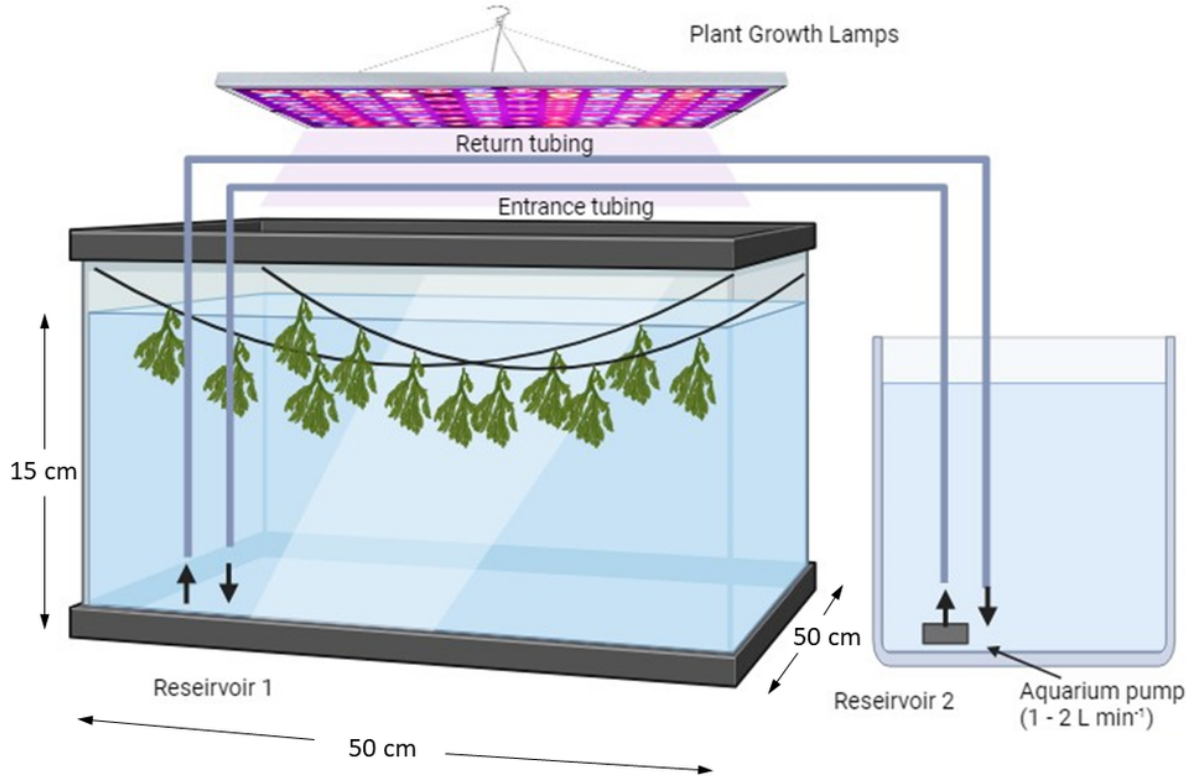


Figure 2

Scheme of the microcosm system used for the in vitro assays.



Figure 3

On the left, the algae were installed in the microcosms. On the right, the microcosms and their water exchange compartments are shown. Microcosms are colored by plant growth lamps.

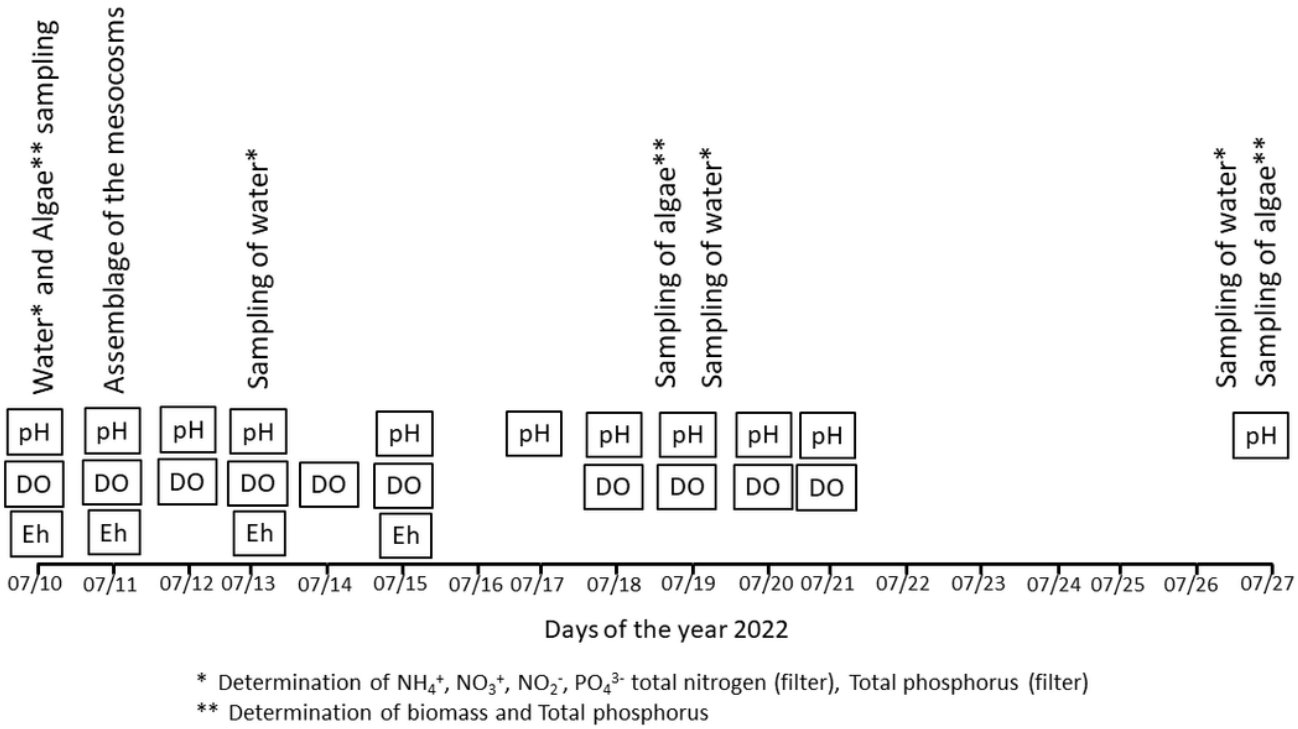


Figure 4

Indications of the parameters measured in the water and particulate matter in the 16 days of assays in the microcosms.

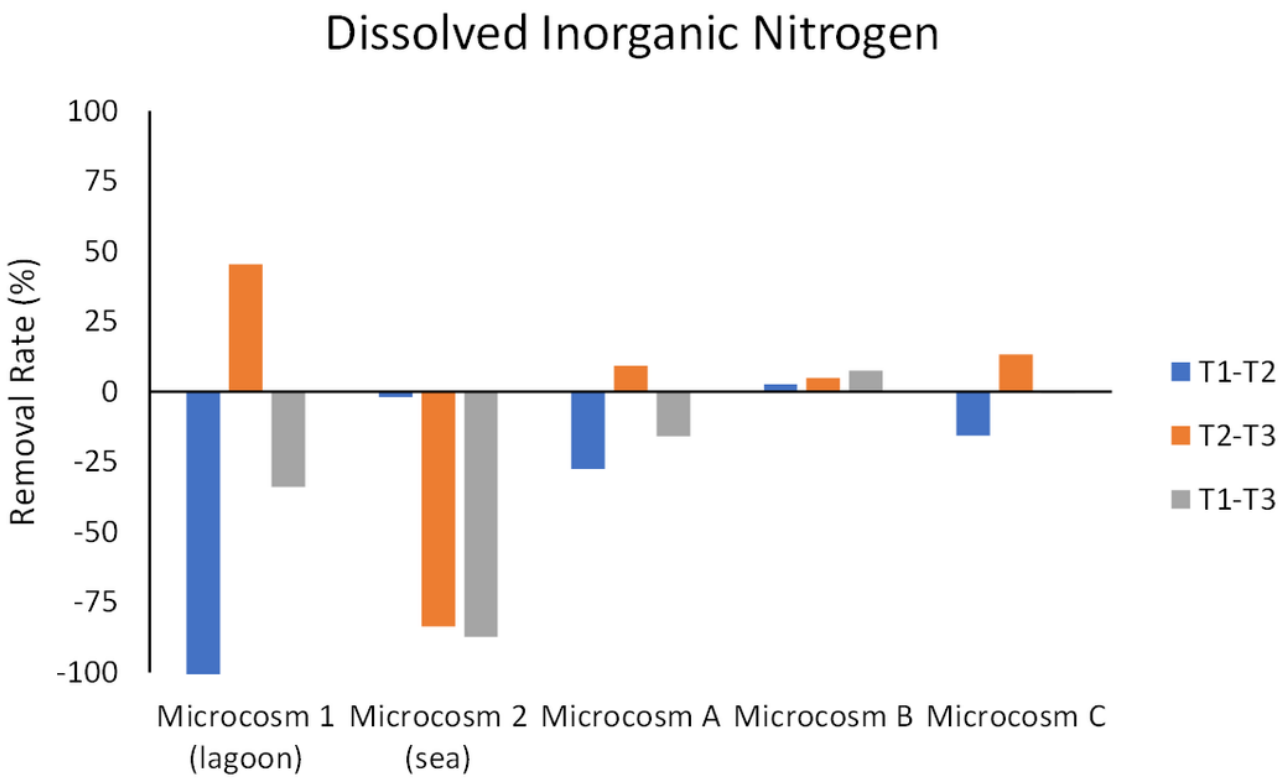


Figure 5

Removal Rates of DIN in microcosms, calculated according to the formula of Hayashi et al. (2010). Positive values indicate removal, while negative values indicate the release of nutrients. T1-T2 represents values from the beginning until day 9, T2-T3 indicates the values between the ninth and fifteenth days,and T1-T3 is the overall *RRs*of the assays.

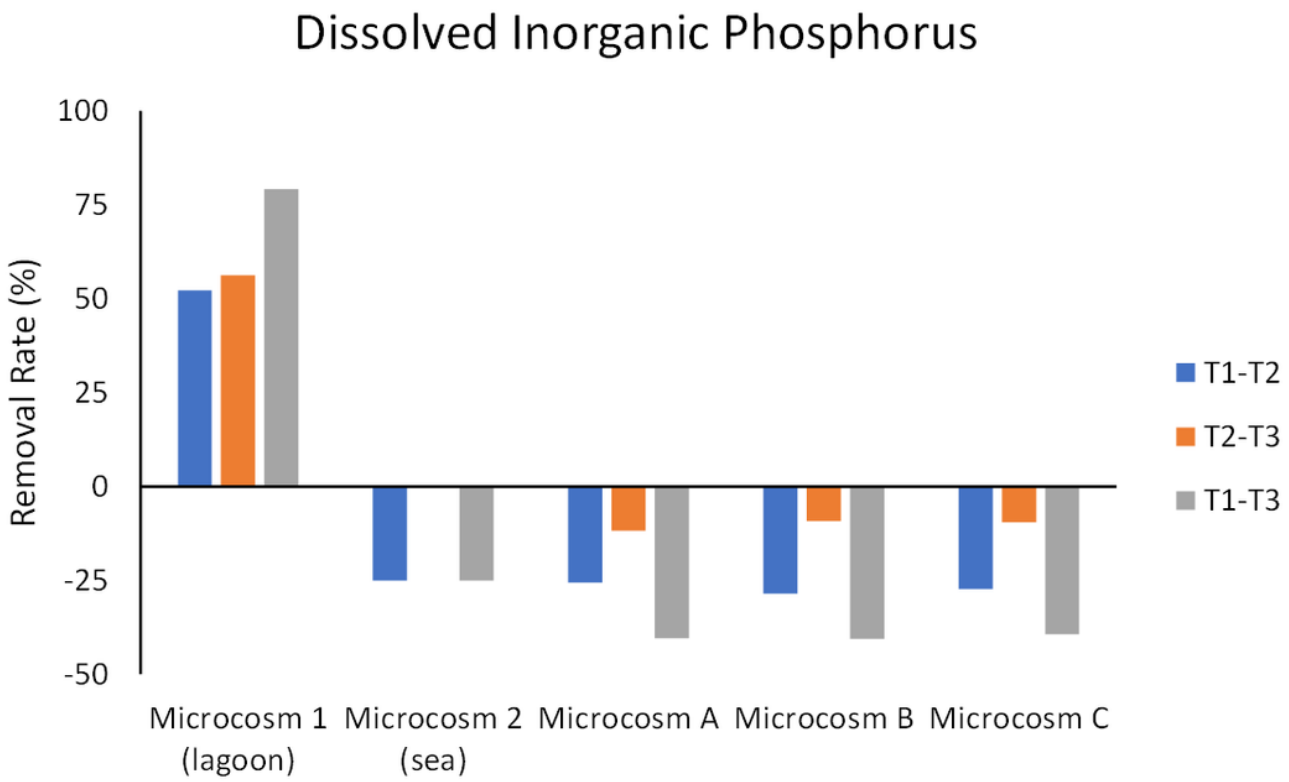


Figure 6

Removal Rates of DIP in microcosms, calculated according to the formula of Hayashi et al. (2010). Positive values indicate removal, while negative values indicates the release of nutrients. T1-T2 represents values from the beginning until day 9, T2-T3 indicate the values between the ninth and fifteenth days,and T1-T3 is the overall *RR* of the assays.

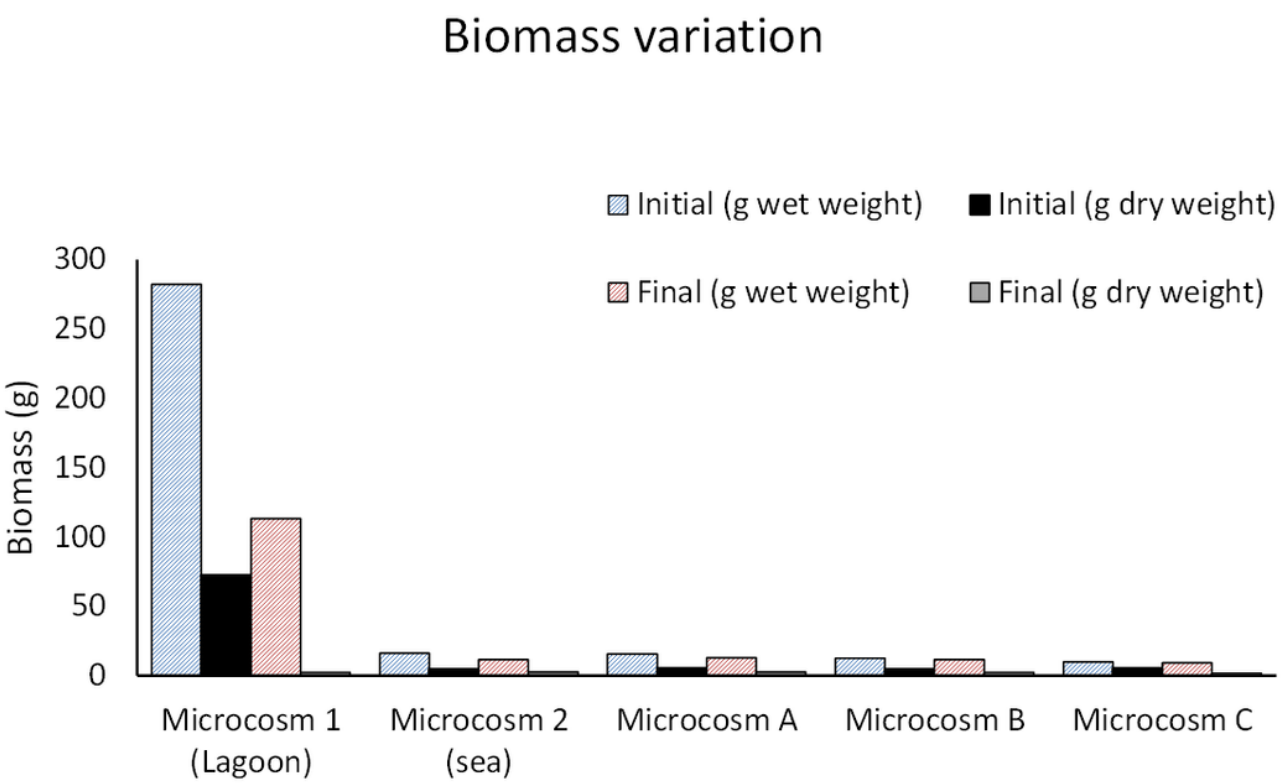


Figure 7

Algal biomass at the beginning and end of the microcosm assays.

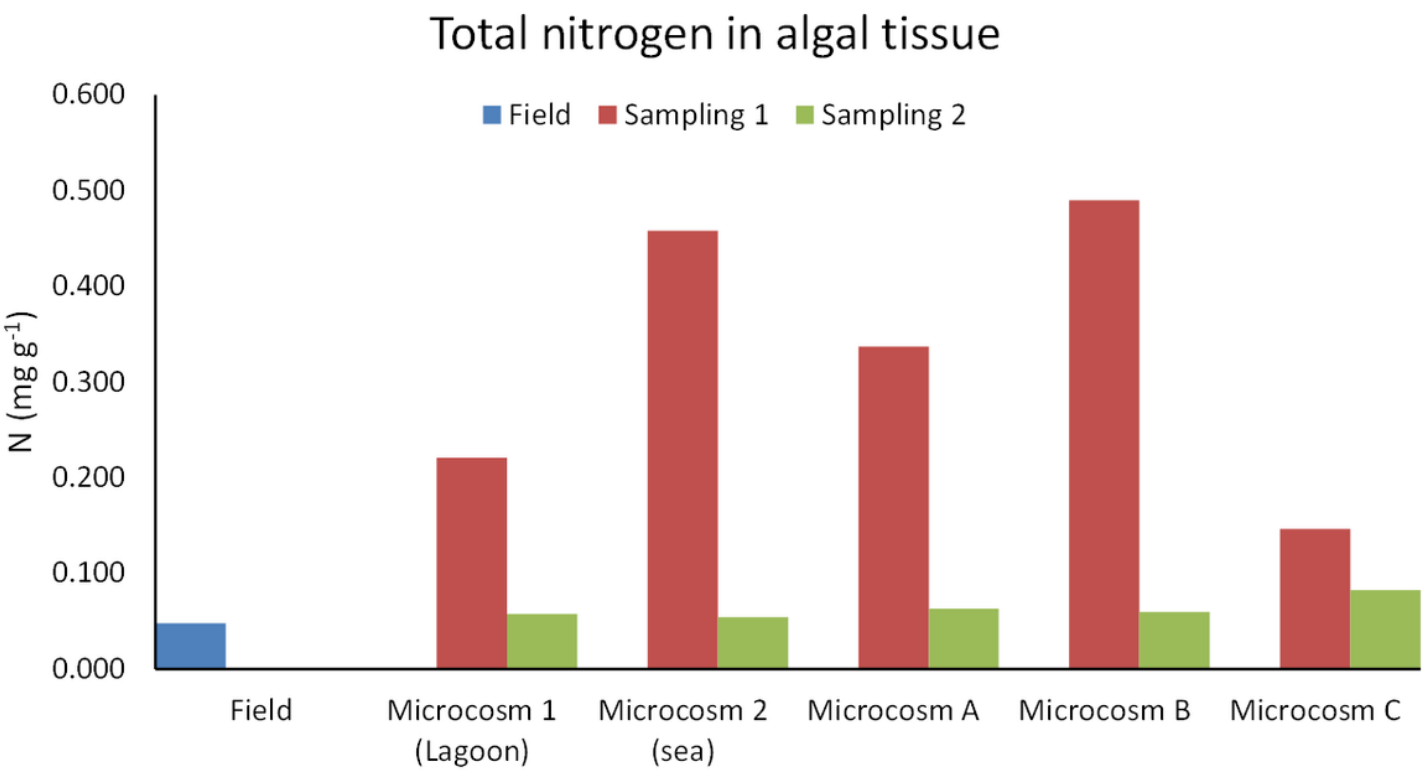


Figure 8

Total nitrogen concentration in algae tissues. “Field” refers to freshly collected natural algae.

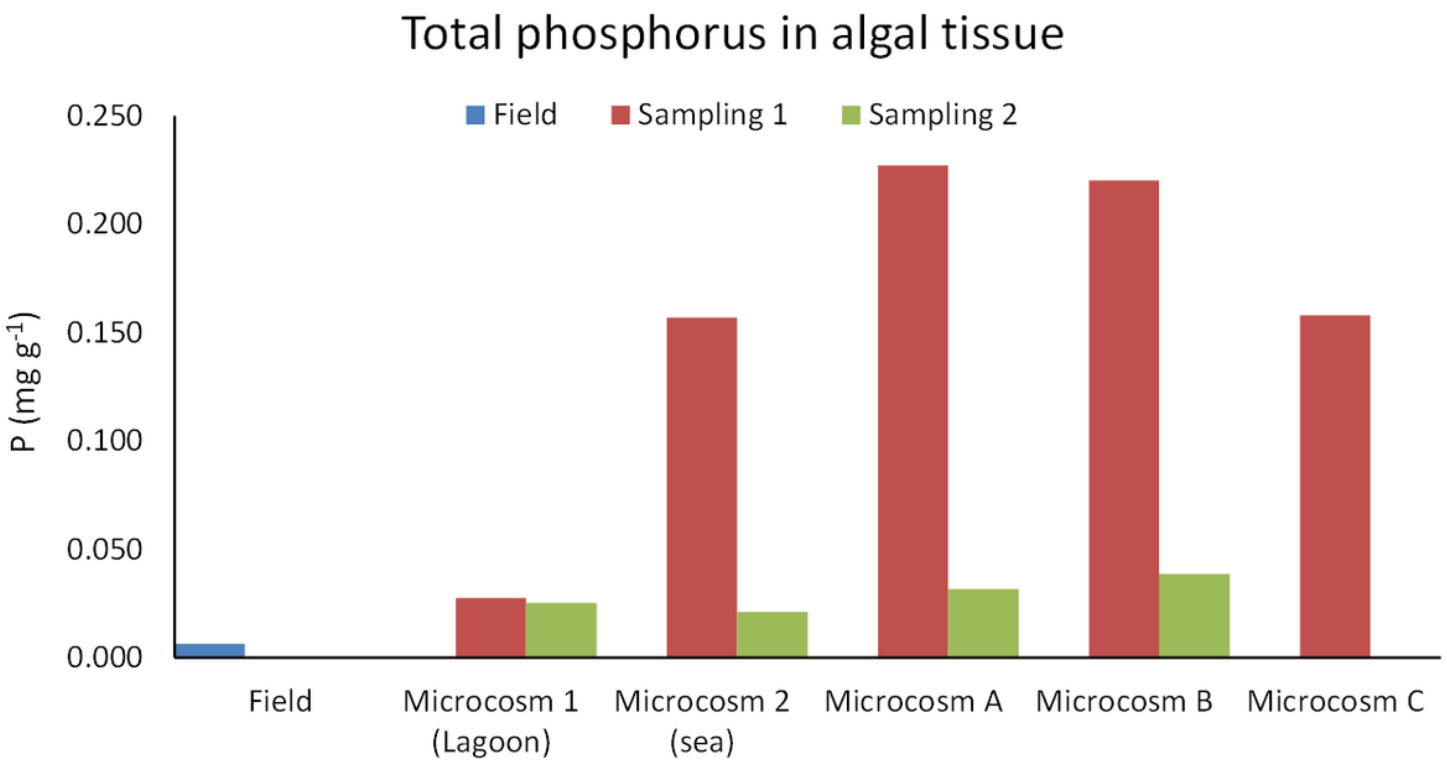


Figure 9

Total phosphorus concentration in algae tissues. “Field” refers to freshly collected natural algae.

Supplementary Files

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

- SupplementaryMaterials.docx