J Appl Phycol (2016) 28:3075–3082
DOI 10.1007/s10811-016-0848-x

Bioremediation of aquaculture wastewater: evaluating the prospects of the red alga *Palmaria palmata* (Rhodophyta) for nitrogen uptake

Britta Grote[1]

Received: 17 December 2015 / Revised and accepted: 31 March 2016 / Published online: 19 April 2016

Abstract The bioremediation capacity of the red macroalga *Palmaria palmata* was assessed by two experiments. First, uptake rates of *P. palmata* cultured in four treatments with varying levels and ratios of the N sources ammonium (NH_4^+) and nitrate (NO_3^-) (18/15, 0/30, 30/45, 50/65 μM) were measured over a 3-h period to evaluate N source preference. Secondly, *P. palmata* were cultured in five treatments with varying levels and ratios of ammonium and nitrate (300/12, 0/312, 500/12, 0/512, 250/262 μM) for 3 weeks to evaluate specific growth rates, protein content, and ammonia toxicity. *Palmaria palmata* had a higher affinity for NH_4^+ than for NO_3^- as N source. However, in the single N source trials, NO_3^- uptake was higher than that of NH_4^+. The maximum specific growth rate of 11.99 % day^{-1} was observed in the 0/512 μM ammonium/nitrate treatment after 3 weeks, whereas the minimum specific growth rate of 2.21 % day^{-1} was observed in the 500/12 μM ammonium/nitrate treatment after 3 weeks. NO_3^- supported higher growth rates, whereas NH_4^+ increased tissue N, and therefore protein content. Total protein content of the algal tissue was significantly higher in *P. palmata* of the NH_4^+ treatments, reaching up to 20.6 % DW, than of those from the NO_3^-treatments. *Palmaria palmata* showed signs of poisoning after 3 weeks in the highest NH_4^+ treatment. This study indicates that *P. palmata* is a suitable species for ecological engineering in integrated multitrophic aquaculture systems as it shows a relatively high growth performance, high nutrient uptake rates, and elevated protein content under NH_4^+ supply.

✉ Britta Grote
britta.grote@awi.de

[1] Alfred Wegener Institute Helmholtz Center for Polar and Marine Research (AWI), Bremerhaven, Germany

Keywords Eco-intensification · Ecological engineering · Nutrient uptake · Dulse · Protein content

Introduction

Worldwide, nearly 50 % of the total finfish and invertebrate production and 96 % of the total seaweed production are generated by the growing aquaculture sector (Chopin 2014, FAO 2014). While the industry is expected to grow, there is also the need for eco-intensification of aquaculture. The cultivation of seaweed together with fed species in integrated multi-trophic aquaculture (IMTA) is improving the overall ecological efficiency, sustainability, and economics of the business (Neori and Nobre 2012). Thus, the prospects of seaweed culture for bioremediation and its cost-efficient production in IMTA systems turn it into an important tool in environmental management (Troell et al. 2003). Consequently, seaweed cultivation should be intensified and more seaweed species of temperate regions need to be studied regarding their capacity for bioremediation.

Total ammonia nitrogen (TAN) (unionized ammonia (NH_3) and ionized ammonia (NH_4^+)) is a main excretory product of the N metabolism of fish and is toxic to most fish species at concentrations above 1.5 mg NH_3-N L^{-1} (Dosdat et al. 1996, Hagopian and Riley 1998). Empirical studies showed differences between macroalgae species in the biofiltration of dissolved nitrogen from aquaculture wastewater (Hernandez et al. 2002). There is some evidence that these varying efficiencies for N uptake are a result of a complex interplay between nutrient availability and the position in the zonation of an ecosystem (Sánchez de Pedro et al. 2013). Suitable seaweed candidate species for IMTA systems should have high growth rates, high nutrient uptake efficiencies, and practical methods of cultivation (Neori et al. 2004). Several studies

have demonstrated the ability and effectiveness of seaweed as biofilter integrated in fed finfish/shrimp culture (Neori et al. 1991, 1996; Nelson et al. 2001, Schuenhoff et al. 2003). Seaweed biofilters were even found to be more effective than the traditional method of bacterial bio-filtration (Cahill et al. 2010). Most studies on seaweed from temperate regions as biofilters in IMTA used the green algae *Ulva* spp. and the red algae *Gracilaria* spp., which are well-established aquaculture species and whose nutrient uptake abilities are high compared to most other seaweeds (e.g., Martinez-Aragon et al. 2002; Neori et al. 2000, 2004; Msuya and Neori 2008; Abreu et al. 2013). Furthermore, species of the genus *Porphyra* (*Porphyra* and *Pyropia*; Sutherland et al. 2011) are expected to be suitable candidates for IMTA, as they have high nutrient uptake and growth rates (Kang et al 2014). Both genera of red seaweeds, *Gracilaria* and *Porphyra*, are characterized by a high surface area to volume ratio, due to their fine branched or rather thin appearance. However, in the case of eco-intensification of offshore aquaculture operations, the macroalgae species additionally needs to be robust enough to withstand a high energy environment (Buck and Buchholz 2005). Therefore, the more robust red seaweed dulse, *Palmaria palmata* (Linnaeus) Weber & Mohr, is a possible candidate as seaweed biofilter for North Atlantic IMTA systems in high energy environments.

Dulse is an edible red alga growing in the intertidal or shallow subtidal of the North Atlantic, and its culture methods are well established (Le Gall et al. 2004; Pang and Lüning 2004, 2006). Knowledge of the biomitigation capacity of dulse will have practical implications for intended eco-intensification of mass cultivation in IMTA systems at exposed marine sites. However, when comparing the results of the few existing studies on dulse, the outcomes regarding N source preference and N uptake efficiency are to some extent inconsistent (Morgan and Simpson 1981; Martinez and Rico 2004; Corey et al. 2013). The influence of N source on N storage and therefore protein content was not studied in dulse. Furthermore, *P. palmata* was described as an ammonium (NH_4^+)-sensitive species (Morgan and Simpson 1981), which could reduce its biofilter suitability for IMTA systems. Hence, the purpose of this study was to evaluate the bioremediation capacity of dulse to clarify its suitability for IMTA operations.

Therefore, adjusted nutrient concentrations were used in two experiments to fill the gap in knowledge on *P. palmata* in order to validate its prospects for IMTA cultivation. In the short-term experiment, N source preference and N uptake efficiencies were investigated, whereas the hebdomadal nutrient uptake experiment, running for 3 weeks, examined the influence of N source on growth rates, protein content, and the NH_4^+ sensitivity.

Materials and methods

Prior to the experiments, clean, apical tips of *P. palmata* blades were cut into 1-cm lengths and placed into transparent glass bottles filled with 5 L of filtered, autoclaved seawater containing 100 mL Provasoli nutrient solution (34 psu, pH 8, Provasoli 1968). The seaweed blades were acclimated by culturing for up to 3 weeks in tumbling suspension by continuous aeration in a 10 °C temperature controlled room on a 16:8 light/dark (L/D) cycle under approximately 60 μmol photons m^{-2} s^{-1} of irradiance. All glassware used was rinsed with 10 % HCl followed by autoclaving prior to use. Postacclimation tissue samples were collected in triplicate and frozen at −20 °C for further analysis.

Design of experiments

For the two experiments, the first one a short experiment and the second a hebdomadal experiment, two to three blades of *P. palmata*, 10 to 14 cm in length and equivalent to ca. 2.0 g fresh weight per 1 L transparent glass beaker, were kept in filtered (0.2 μm), autoclaved North Sea water and aerated with pressurized air. Provasoli nutrient solution without nitrogen (N) was added in 10 mL portions to each of the 20 1 L beakers, containing sufficient phosphate, vitamins, and micronutrients. Temperature was maintained at 10 °C under saturating light intensity of 125 μmol photons m^{-2} s^{-1} (Martinez and Rico 2004), at a photoperiod of 16:8 (L/D, Sagert and Schubert 2000). Light was supplied by 36 W, 965 Biolux fluorescent lamps (Osram, Germany). Irradiance was measured using a light meter (Li-Cor Li-189 with a flat Li-Cor Quantum sensor, USA).

In the short experiment, the seaweed blades were cultured with varying levels of ammonium chloride (NH_4Cl) and sodium nitrate ($NaNO_3$) in four different treatments (Table 1), each conducted in triplicate. For each treatment, a control beaker without seaweed was maintained for the duration of the experiment. Nutrient uptake was monitored, following stocking of algal blades to the beakers, after 15, 30, 60, 120, and 180 min by taking water samples of 15 mL with sterile syringes, which were filtered (sterile 0.2 μm) into sterile centrifuge tubes and stored at −80 °C until further analyses. The concentrations of ammonium, nitrate, nitrite, and phosphate of samples were measured with an Alliance Instruments Evolution III continuous flow autoanalyzer (Salzburg, Austria).

In the hebdomadal experiment, the seaweed blades were cultured with five levels of the nutrients NH_4Cl and $NaNO_3$ (Table 1), each conducted in triplicate for 3 weeks. For each treatment, a control beaker without seaweed was maintained for the duration of the experiment, enabling measurement of possible ammonium loss by volatilization or bacteria. Semi-weekly nutrients and Provasoli solution without N were

Table 1 Nutrient concentration (NH_4^+ and NO_3^-, μM) and N:P ratio of the different treatments in the short and the hebdomadal experiments

Short experiment			Hebdomadal experiment		
Treatment	NH_4^+/NO_3^- [μM]	N:P	Treatment	NH_4^+/NO_3^- [μM]	N:P
1	18/15	2:1	1	300/12	18:1
2	0/30	2:1	2	0/312	18:1
3	30/45	4:1	3	500/12	30:1
4	50/65	7:1	4	0/512	30:1
			5	250/262	30:1

exchanged, and once a week, the seaweed biomass was dried on the surface with paper towels and weighed (fresh weight (FW)). Specific growth rate (SGR [% day^{-1}]) was determined weekly by weighing the samples to the nearest 0.001 g within 2 min, and it was calculated using the following formula:

$$\mathrm{SGR} = \frac{ln\,\mathrm{FW}_b - ln\,\mathrm{FW}_a}{t_b - t_a} \times 100$$

where FW_a and FW_b are the fresh weight (g) at days t_a and t_b, respectively. Tissue samples were taken by reducing the seaweed biomass to the initial density of ca. 2.0 g L^{-1} at each weighing. Tissue samples were frozen at −20 °C until further analyses. The dry weight (DW)/fresh weight (FW) ratio of 7.9 was determined by drying tissue samples to constant weight at 60 °C.

Dried tissues were ground to powder using pestle and mortar, weighed into tin cups and then analyzed for total nitrogen (N) and carbon (C) using a EURO EA Elemental Analyzer (EURO VECTOR Instruments, Milan, Italy). Total protein content of *P. palmata* was calculated according to Lourenço et al. (2002). The calculation of protein content of red algae using a nitrogen-to-protein conversion factor of 4.59 has proven to be a strong proxy for whole biomass protein quantification (Lourenço et al. 2002). Analyzing total elemental nitrogen is based on high-temperature combustion and is less liable to interferences.

Nitrogen removal (g N g^{-1} DW day^{-1}) of dulse was calculated according to Kim et al. (2007):

$$\mathrm{N\ removal} = \frac{B_t - B_0 \times \mathrm{tissue}\,N}{\left(\frac{B_t + B_0}{2}\right) \times t} \times \frac{DW}{FW} \times 10^3$$

where B_t and B_0 are the biomass (g) at day t (final) and day 0 (initial), respectively.

In both experiments, phosphate (P) was not limiting for *P. palmata* nitrogen uptake or growth as it was around 16–18 μM mL^{-1} in the beginning of the different trials and it was not depleted until the water exchange or the end of the experiments (data not shown). In the hebdomadal experiment, N/P ratios were 18:1 and 30:1, representing concentrations near the Redfield ratio and the average optimal ratio for seaweed growth (Atkinson and Smith 1983). Nutrient concentration of the control beakers without seaweed did not change significantly for the duration of the experiments.

Statistical analysis

Data were analyzed for normality, homogeneity of variance, and the difference between treatments and weeks were analyzed by using ANOVA followed by a Tukey's HSD post hoc analysis ($p = 0.05$). Statistical analyses were performed using STATISTICA 9 (StatSoft, USA).

Results

In the short experiment, mean nutrient uptake by *P. palmata* was 93.7 % in the treatment enriched with 30 μM NO_3^-, while in the 18 μM NH_4^+, only 69.9 % were taken up after 180 min (Fig. 1a). When both nutrients were supplied in higher concentrations of 30 μM NH_4^+ and 45 μM NO_3^- and 50 μM NH_4^+ and 65 μM NO_3^-, mean uptake after 180 min was higher for NH_4^+ than for NO_3^- (Fig. 1b). For the 30 μM NH_4^+ and 45 μM NO_3^- nutrient treatment, 71.9 and 53 %, respectively, were taken up by the red algae. In the treatment with the highest concentration of 50 μM NH_4^+ and 65 μM NO_3^-, uptake was 75.2 % NH_4^+ and 34.2 % NO_3^-. Furthermore, uptake of nutrients within the first 15 min of the experiment was 3 to 4.5 times higher for NH_4^+ than for NO_3^- (Fig. 1b).

In the hebdomadal experiment, N source and concentration had a significant effect on growth rate of *P. palmata* ($p < 0.05$). In general, *P. palmata* from the nitrate treatments showed significantly higher mean SGRs than those from the ammonium treatments after the 3-week experiment ($p < 0.05$; Fig. 2). However, the observed variances in mean SGR of the red alga were not significantly different during the first week of the experiment. Dulse in the treatment with 250/262 μM ammonium/nitrate grew at a similar mean SGR as in the pure ammonium treatments during the three weeks. Highest mean SGR of 11.99 % day^{-1} was observed in the 0/512 μM ammonium/nitrate treatment after 3 weeks, whereas the lowest mean SGR of 2.21 % day^{-1} was observed in the 500/

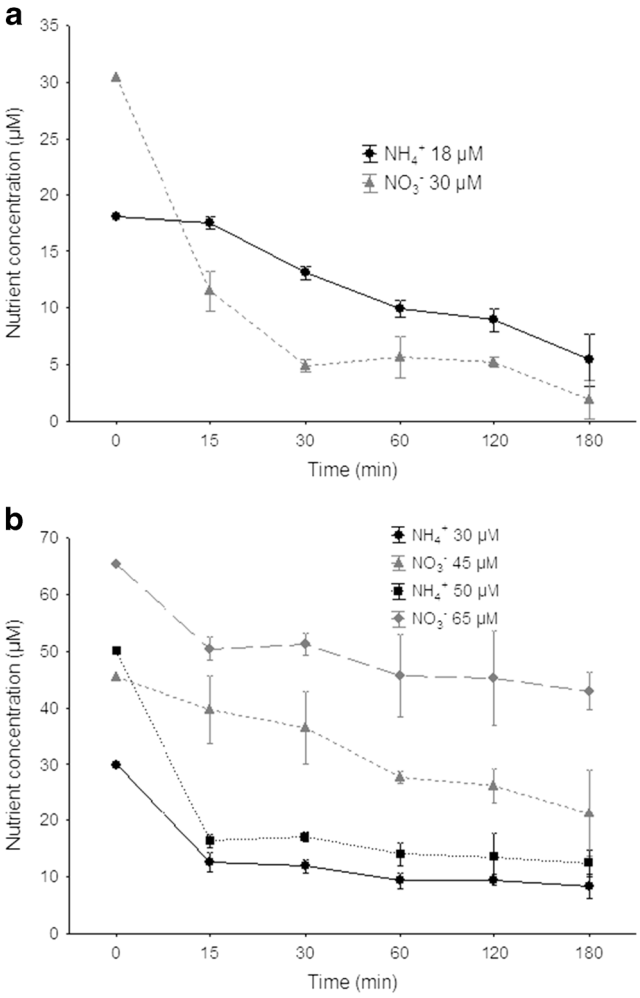

Fig. 1 Short experiment: mean concentration ± SD of NH_4^+ and NO_3^- (μM) over time (min) for (a) single N source experiments and (b) both N sources

12 μM ammonium/nitrate treatment after 3 weeks (Fig. 2). Within the same treatments, there was a significant increase

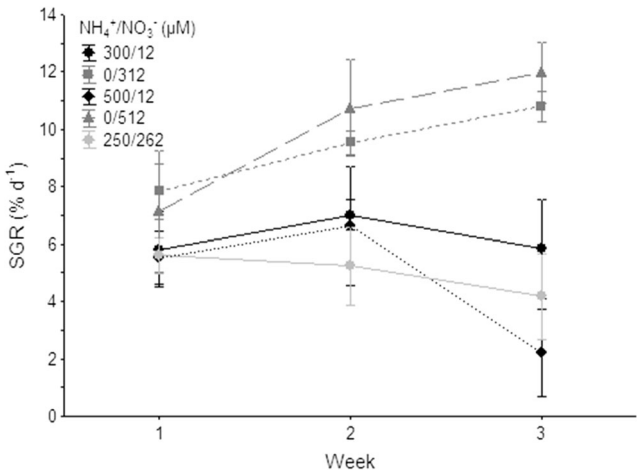

Fig. 2 Hebdomadal experiment: mean SGR ± SD (% day^{-1}) of *P. palmata* in five different nutrient treatments during the three experiment weeks

in mean SGR at 0/512 μM ammonium/nitrate ($p < 0.05$) and a significant decrease in mean SGR from week 2 to week 3 at 500/0 μM ammonium/nitrate ($p < 0.05$). Furthermore, the fronds of *P. palmata* in the latter treatment showed signs of bleaching after 3 weeks.

Nitrate availability also had a significant effect ($p < 0.05$) on tissue N of *P. palmata* and therefore on protein content (Table 2). Total protein content of the algal tissue was significantly higher in *P. palmata* of the ammonium treatments than of those from the nitrate treatments ($p < 0.05$) (Table 2). This could be observed especially in weeks 2 and 3 of the experiment. The C/N ratios of the ammonium and nitrate treatments were significantly different during all 3 weeks of the experiment (Table 2), being lower in those with added ammonium. The highest mean C/N ratio of 15.98 was observed in the tissue of *P. palmata* at 0/312 ammonium/nitrate after the first week of the experiment, whereas the lowest mean C/N ratio of 6.51 was detected in seaweed tissue at 500/12 ammonium/nitrate after 3 weeks (Table 2). Tissue C % was not affected by nutrient supply. In general, mean protein content of *P. palmata* increased significantly in 300/12, 500/12 and 250/262 μM ammonium/nitrate from week 1 to week 3 ($p < 0.05$). The C/N ratio significantly decreased in these nutrient treatments during the 3-week experiment ($p < 0.05$).

Mean N removal rate was between 0.29 and 0.89 mg N g^{-1} DW day^{-1} during experiment A (Fig. 3). In general, N removal rate increased from week 1 to week 2 in all treatments, except at 250/262 μM NH_4^+/NO_3^-, where it remained around 0.63 mg N g^{-1} DW day^{-1} for 3 weeks. In week 3, only the NO_3^- treatments showed a slight increase in N removal rate. The only significant decrease in N removal rate was observed at 500/12 μM NH_4^+/NO_3^- in week 3 ($p < 0.05$; Fig. 3).

Discussion

This study focused on the suitability of *P. palmata* for IMTA, in which the main N source is NH_4^+. *Palmaria palmata* showed clearly a higher affinity for NH_4^+ than for NO_3^- as N source, when both nutrients were available. However, in the single N source trials, nitrate uptake was higher than that of NH_4^+, indicating a suppression of NO_3^- uptake in presence of NH_4^+. Inhibition of NO_3^- uptake by NH_4^+ was observed in some macroalgae in the three major seaweed divisions, Chlorophyta, Rhodophyta, and Phaeophyta (Hanisak and Harlin 1978; D'Elia and DeBoer 1978; Haines and Wheeler 1978). NH_4^+ is already a reduced type of N and theoretically, less energy is needed for its incorporation into amino acids and proteins than for NO_3^- (Syrett 1962). However, Thomas and Harrison (1987) concluded that the level of suppression of NO_3^- uptake by NH_4^+ varies depending on the extent of NO_3^- depletion in the algal tissue. However, N tissue deficiency seems not to be a major factor in nutrient preference of

Table 2 Hebdomadal experiment: mean (± SD) of *P. palmata* tissue calculated protein content (% DW), C/N ratio, carbon (% DW), and nitrogen (% DW) at the start and at the end of each week of the experiment

week	NH_4^+/NO_3^- [μM]	Protein content %	C:N	C%	N%
0	start	8.67 ± 0.90	15.66 ± 0.55	29.20 ± 1.43	1.89 ± 0.24
1	300/12	13.78 ± 0.65 a	10.13 ± 0.10 a	30.33 ± 0.55 a	3.00 ± 0.17 a
	0/312	9.14 ± 1.19 b	15.98 ± 0.21 b	31.24 ± 0.58 a	1.99 ± 0.32 b
	500/12	14.30 ± 0.51 a	10.09 ± 0.04 a	31.32 ± 2.13 a	3.12 ± 0.14 a
	0/512	10.26 ± 0.98 a	14.16 ± 0.31 b	31.48 ± 2.17 a	2.24 ± 0.26 a
	250/262	13.73 ± 0.87 a	10.29 ± 0.04 a	30.78 ± 2.92 a	2.99 ± 0.23 a
2	300/12	17.58 ± 1.30 a	7.93 ± 0.14 a	30.69 ± 1.73 a	3.89 ± 0.35 a
	0/312	10.98 ± 0.53 b	13.24 ± 0.24 b	31.58 ± 1.01 a	2.39 ± 0.14 b
	500/12	17.68 ± 1.52 a	7.71 ± 0.17 a	29.55 ± 1.77 a	3.85 ± 0.41 a
	0/512	11.10 ± 0.80 b	12.44 ± 0.32 b	29.97 ± 1.51 a	2.42 ± 0.21 b
	250/262	15.73 ± 1.08 a	8.51 ± 0.10 a	29.06 ± 0.92 a	3.43 ± 0.29 a
3	300/12	19.18 ± 1.06 a	6.72 ± 0.15 a	28.04 ± 2.04 a	4.18 ± 0.28 a
	0/312	11.23 ± 1.06 b	13.21 ± 0.55 b	32.02 ± 0.74 a	2.45 ± 0.28 b
	500/12	20.10 ± 1.23 a	6.51 ± 0.03 a	28.31 ± 1.51 a	4.38 ± 0.33 a
	0/512	11.93 ± 0.72 b	11.14 ± 0.08 b	28.82 ± 0.48 a	2.60 ± 0.19 b
	250/262	20.62 ± 0,58 a	7.10 ± 0.17 a	28.48 ± 0.75 a	4.01 ± 0.09 a

Values per week with different letters are significantly different ($p = 0.05$)

P. palmata, as low initial N content (N, 1.8 ± 0.11 % DW Martinez and Rico 2004; 1.89 ± 0.24 % DW, this study) in the tissue lead to a similar affinity for both N sources (21.5 μM NH_4^+, 65.2 μM NO_3^-) in the study of Martinez and Rico (2004), whereas a greater affinity for NH_4^+ was found in this study. The suppression of NO_3^- uptake by NH_4^+ in *P. palmata* is supported by the study of Morgan and Simpson (1981) using much higher nutrient concentrations (0.5–2 mM), whereas Corey et al. (2013) reported that low concentrations of 30 μM NH_4^+ enhanced NO_3^- uptake (270 μM NO_3^- added). In this study, low concentrations of NH_4^+ were used in order to trace a threshold level of enhancement or inhibition of NO_3^- uptake. This threshold level could not be located and the results of this study suggest that the suppression of NO_3^- uptake is rather an indirect result of NH_4^+ uptake. Presumably, the relationship between the uptake rates of the two N sources is more complex than previously thought.

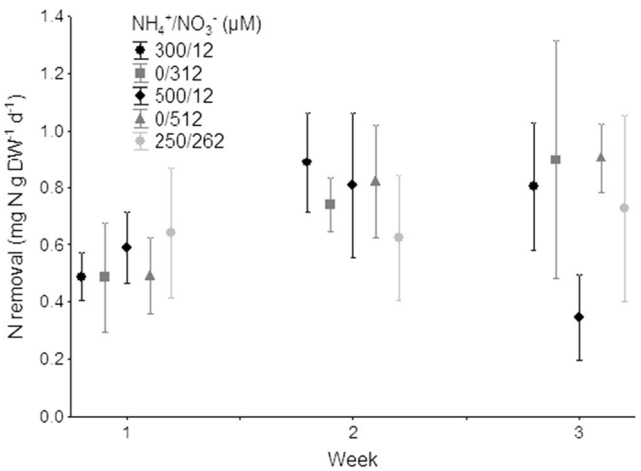

Fig. 3 Hebdomadal experiment: mean N removal ± SD (mg N g^{-1} DW day^{-1}) of *P. palmata* in five different nutrient treatments during the three experiment weeks

Despite the higher affinity for NH_4^+, uptake of NO_3^- supported higher growth rates of *P. palmata* in this study. Similar results were found by Morgan and Simpson (1981) and Demetropolous and Langdon (2004), where *P. palmata* and *P. mollis* were more productive under NO_3^- supply than with NH_4^+ in the long term at higher nutrient concentrations than used in this study. Macrophytes are able to store surplus nutrients in their tissue (Thomas and Harrison 1985). If internal nutrients are depleted, growth rates usually decline. Generally, the C/N ratio of macroalgae lies between 5 and 40 (Niell 1976; Hanisak 1979). C/N ratios of 10–15 equal a status of sufficient nutrient supply, whereas a lower ratio indicates N storage, and a higher ratio reflects N limitation (Fredriksen and Rueness 1989). Corey et al. (2013) found only minor differences in growth rates of *P. palmata* depending on N source. The initial C/N ratio of the red alga was 7.3 (Corey et al. 2013), already indicating a N surplus, which might have led to these result. The initial C/N ratio of *P. palmata* in this study indicated a slight N limitation of fronds at the beginning of the experiment. In the NH_4^+ trials, C/N ratio reached values below 10 after the second week, demonstrating the storage of excess N in the tissue. However, in the NO_3^- trials, C/N ratios stayed above 10 even after the third week, despite increasing values of N % DW, indicating no storage of N, but its support of the higher productivity.

In this study, due to the storage of N, protein content of *P. palmata* increased up to 20–20.6 % DW in the NH_4^+ trials after 3 weeks. Protein content in cultured *P. palmata* was

found to reach up to 35–36 % DW under higher nutrient supply than in this study (Morgan et al. 1980, Demetropoulus and Langdon 2004). In wild *P. palmata* from the French Atlantic coast, protein levels between 9 % DW in summer and 25 % DW in winter were calculated (Galland-Irmouli et al. 1999). In the previous studies, the protein content was calculated with the Kjeldahl method (tissue N × 6.25). However, the relative portion of nonprotein N is greater in red algae compared to other plants and consequently a lower nitrogen-to-protein conversion factor (4.59) was calculated for red macroalgae (Lourenço et al. 2002) and used in this study. Using the Kjeldahl method, the highest protein content of *P. palmata* would have been 27.4 % DW after 3 weeks supplied with high NH_4^+. In contrast, in the NO_3^- trials, *P. palmata* tissue contained less protein but had higher SGRs than in the NH_4^+ trials. Supporting this finding, high NO_3^- supply to *P. mollis* in tank culture resulted in increased SGRs instead of high protein content, as tissue N was not statistically different over the range of NO_3^- additions at different light levels (Demetropoulus and Langdon 2004). Therefore, N source and N tissue history have an effect on growth rate and biochemical composition in the red alga *P. palmata*. Coculture of *P. palmata* with fed organisms in an IMTA system would lead to higher protein content in the red alga due to high NH_4^+ supply. Depending on the intended application, the culture method of *P. palmata* can be modified in terms of main N source, either focusing on growth and biomass production or on quality of tissue, such as protein content.

However, excessive supply of NH_4^+ led to first signs of NH_4^+ sensitivity in dulse, which were visible at 500 μM NH_4^+ exposure for 3 weeks, not only directly through bleached tissue but also indirectly by significantly reduced SGRs. Morgan and Simpson (1981) found signs of toxication during long-term exposure at 500 μM NH_4^+ four times a week with flushing of three to four tank volumes day^{-1}. In *P. mollis*, signs of toxication were visible after 2-week exposure to concentrations of 7059 μM NH_4NO_3 four times a week and flushing of one tank volume day^{-1} (Demetropolous and Langdon 2004). The concentrations used in this study of 300 and 500 μM NH_4^+ correspond to 5.4 and 9 mg L^{-1} TAN, respectively, which would relate to a concentration of 1.03 and 1.71 mg L^{-1} NH_3^+, respectively, at pH 7 and 10 °C (Goddard 1996). These are unfavorable concentrations in recirculating aquaculture systems (RAS), as NH_3^+ influences growth and performance of fish at a concentration of 0.02–0.1 mg L^{-1} and at 0.09 to 3.35 mg L^{-1} NH_3^+ becomes toxic to fish depending on the species, fish size, exposure time, and other environmental factors (Handy and Poxton 1993; Person-Le Ruyet et al. 1995). However, TAN released by intensive aquaculture varies substantially depending on species, fish size, stocking density, feed and feeding rate used, type of aquaculture system and temperature (Handy and Poxton 1993). The TAN concentrations in intensive RAS hatcheries are usually below 110 μM for the more susceptible young fish stages (Howell and Baynes 2004), but can reach for Atlantic halibut up to 2225 μM day^{-1}, depending on feeding regime, temperature, and fish size (Kim et al. 2013). However, there would only be a punctual high nutrient supply after fish feeding and this would only be relevant, where aquaculture effluents are discharged directly into seaweed tanks. In RAS, nutrient concentrations would be kept lower due to protection of fish health and growth, whereas in open water, such high concentrations would not be reached due to dilution.

In conclusion, this study provides important data of *P. palmata* performance, nutrient uptake rates, and protein content, presenting its prospects for IMTA and ecological engineering. *Palmaria palmata* is a promising extractive candidate for temperate water IMTA due to its N uptake efficiency and its relatively high productivity. In a medium-scale RAS, the excess N of 1 kg turbot supported the growth of more than 6.5 kg of dulse (Grote and Buck, in review). These results do not automatically extrapolate to commercial-scale operations because the nutrient uptake can be nonlinear, influenced by many different variables, such as temperature. A modeling approach as the first step to understand these interrelations, enabled the calculation of dimension of the seaweed culture needed in relation to the culture size of the fed organism (Grote and Buck, in review). However, more research is needed to provide more information on bioremediation of *P. palmata* in open cultures on commercial scale with varying environmental conditions in order to show the economic and technical viability of such operations. Therefore, studies in large-scale RAS and offshore systems are required to clearly demonstrate the feasibility of *P. palmata* mass culture in commercial- scale IMTA systems.

Acknowledgments The German Federal Office for Agriculture and Food (Bundesanstalt für Landwirtschaft und Ernährung, BLE) funded this study, which is a part of the project *Offshore Site Selection* (OSS) (313-06.01-28-1-73.010-10). Many thanks go to Sabine Strieben, Alfred Wegener Institute, Helmholtz Centre for Polar and Marine Research (AWI), for her dedicated help and to Prof. Dr. C. Wiencke and the working group "Seaweed Biology," AWI, for scientific and logistic assistance, especially Claudia Daniel and Andreas Wagner for technical support. I like to thank the two reviewers for improving the manuscript by critical comments and suggestions.

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