Impacts of aquaculture nutrient sources: ammonium uptake of commercially important eucheumatoids depends on phosphate levels [1]

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Research Article

Abstract

In an integrated multitrophic aquaculture (IMTA) system, seaweeds serve as extractive species that utilize excess nutrients thereby reducing the risk of eutrophication and promoting sustainable aquaculture. However, the use of excessive fish feeds and the resultant fecal waste as nutrient streams can contribute to variations in nitrogen and phosphorus levels (e.g., primarily NH_4^+ and PO_4^{-3}) in the surrounding area, and this may impact the physiology of the integrated seaweeds particularly on how these species take up inorganic nutrients. In this study, the effect of different PO_4^{-3} levels on NH_4^+ uptake of the three commercially important eucheumatoids *Kappaphycus alvarezii*, *Kappaphycus striatus* and *Eucheuma denticulatum* was examined under laboratory conditions. Seaweed thalli (n = 4) were incubated in seawater media containing 30 µM NH_4^+, representing eutrophic conditions, and 0, 0.5, 1.0, 1.5, 3.0 or 5.0 µM PO_4^{-3} for 1 h under a saturating light level of 116 ± 7.13 µmol photons m^{-2} s^{-1} inside a temperature-controlled laboratory. Species-specific responses to PO_4^{-3} levels were observed. For *K. alvarezii*, maximum NH_4^+ uptake (17.8 ± 1.6 µmol gDW^{-1} h^{-1}) was observed at 0.5 µM PO_4^{-3} and the uptake rate declined at higher PO_4^{-3} levels. For *K. striatus*, the NH_4^+ uptake increases with increasing PO_4^{-3} levels, with maximum N-uptake (6.35 ± 0.9 µmol gDW^{-1} h^{-1}) observed at 5.0 µM PO_4^{-3}. For *E. denticulatum*, maximum NH_4^+ uptake (14.6 ± 1.4 µmol gDW^{-1} h^{-1}) was observed at 1.0 µM PO_4^{-3}. Our results suggest that, among the three eucheumatoid species, the NH_4^+ uptake of *K. striatus* persist even at high levels of PO_4^{-3}. However, our results also showed that *K. striatus* had the lowest range of NH_4^+ uptake rates. These results should be taken into consideration when incorporating eucheumatoids in IMTA system where PO_4^{-3} levels significantly vary in space and time.

Introduction

Seaweeds require carbon dioxide (CO_2), water (H_2O) and light to photosynthesize and produce organic compounds. In addition, seaweeds also require nutrients from the surrounding seawater to maintain their health and sustain their physiological and metabolic functions. Two of the most essential nutrients required by macroalgae are nitrogen (N) and phosphorus (P). Generally, macroalgae utilize inorganic nitrogen such as ammonium (NH_4^+), nitrate (NO_3^-) or nitrite (NO_2^-), although some macroalgae can also use organic nitrogen-based compounds such as urea as their nitrogen source (Probyn and Chapman 1982; Phillips and Hurd 2004; Smith et al. 2021). On the other hand, seaweeds typically absorb phosphorus in its orthophosphate (PO_4^{-3}) form. Due to the significant amounts of phosphorus and nitrogen that seaweeds require, these two elements are considered as macronutrients (Harrison and Hurd 2001). Moreover, these two are essential because they are used by macroalgae to synthesize biochemical compounds (e.g., pigments, proteins, amino acids, phospholipids, nucleotides, sugar-phosphates, etc.) that are required for their proper functioning (Douglas et al. 2014; Roleda and Hurd 2019). Other nutrients such as iron (Fe), zinc (Zn) and copper (Cu) are used in smaller quantities and therefore regarded as micronutrients (Harrison and Hurd 2001).

The ability of macroalgae to take up and assimilate inorganic nutrients from the seawater makes them a good candidate extractive species in an integrated multi-trophic aquaculture (IMTA). In this set-up, the excess nutrients are absorbed by seaweeds leading to lowered eutrophication risks while concurrently promoting sustainable aquaculture (Chopin et al. 2001). However, not all seaweeds can be incorporated into an IMTA system. The eco-physiological characteristics and market value of the candidate seaweed must always be taken into consideration when incorporating it in a seaweed-based integrated aquaculture (Kang et al. 2013). Seaweeds to be integrated in an IMTA system must have fast growth rates and high nutrient uptake rates to effectively remove and assimilate nutrients from the mariculture effluents (Chopin et al. 2001; Neori et al. 2004; Kang et al. 2013). The integrated seaweeds should maintain good health for considerable periods of time and withstand various types of environmental stresses that can be encountered during cultivation (Neori et al. 2004; Kang et al. 2013). Lastly, seaweeds in an IMTA system should be easy to cultivate and have high demand and market value (Buschmann et al. 1996; Neori et al. 2004).

The growth and efficiency of seaweeds as biofilters in an IMTA are dependent on several culture conditions (Buschmann et al. 2001). For instance, seaweeds in an IMTA system are subject to fluctuating nutrient levels. In mariculture farms, the variability of nutrient levels can result from different waste streams, e.g., excretion of reared organisms, direct enrichment by applied feeds, and remineralization through microbial degradation of organic compounds (Burford and Williams 2001; Bouwman et al. 2013). In Bolinao-Anda, Pangasinan, Philippines, the degradation of uneaten fish feeds in an intensive mariculture site had resulted to high P levels (up to 4 μM during dry season) and sustained eutrophic conditions around the area (Ferrera et al. 2016). The impacts of high P levels on invertebrates (e.g., Uddin et al. 2016), corals (e.g., Klinges et al. 2022; Mezger et al. 2022), and phytoplankton (e.g., Smith 2006; Eker-Develi et al. 2006) had been thoroughly investigated. On the contrary, studies on how PO_4^{-3} affects the physiology of commercially important seaweeds are limited. Specifically, little information is available on how elevated PO_4^{-3} concentrations affect the uptake of other nutrients. This needs to be investigated because this may have significant implications when incorporating seaweeds in an IMTA system, where nutrient levels fluctuate both spatially and temporally.

Eucheumatoids are a group of macroalgae that are considered good candidate species in an IMTA set-up due to their biofiltration potentials and established market (Rodrigueza and Montaño 2007; Melendres 2021; Narvarte et al. 2022). These macroalgae are farmed globally due to their phycocolloid (carrageenan) content, which is used in a wide array of economic and industrial applications (Bixler and Porse 2011). Thus, the incorporation of eucheumatoids in an IMTA system can provide additional source of income for farmers (da Silva et al. 2022). The potentials of eucheumatoids as co-culture species in an IMTA system has been investigated in a number of studies (e.g., Hayashi et al. 2008; Kambey et al. 2020; Pires et al. 2021). However, these studies have only focused on the growth and instantaneous nutrient uptake performance of these seaweeds. In-depth research, e.g., mechanistic response with ecological and economic implications, should also be done on the nutrient uptake responses of commercially important eucheumatoids to environmental changes observed in an IMTA set up, like fluctuating nutrient levels.

The objective of this study is to evaluate the effects of different PO_4^{-3} levels on the NH_4^+ uptake of commercially important carragenophytes *Kappaphycus alvarezii*, *Kappaphycus striatus* and *Eucheuma denticulatum*. The uptake rates were determined by incubating samples under different concentrations of PO_4^{-3} under laboratory conditions and measuring the amount of NH_4^+ absorbed by seaweed samples. The results of our study have important implications on selecting which species are to be incorporated in an IMTA.

Materials and Methods

Collection, identification, acclimatization of eucheumatoids

Samples of *K. alvarezii* var. *tambalang*, *K. striatus* var. *sacol* and *E. denticulatum* var. *spinosum* (Fig. 1) were obtained from the land-based hatchery cultures of Algal Ecophysiology Laboratory (Algae Lab) located in the University of the Philippines Marine Science Institute- Bolinao Marine Laboraory (UPMSI-BML), Bolinao, Pangasinan, Philippines. Healthy samples, characterized by the absence of ice-ice disease and epiphyte infestation, were collected using sterile blades. All seaweed samples were previously identified using DNA barcoding (Roleda et al. 2021). The collected samples were acclimated, given sufficient time to heal wounds, and then starved overnight in a nutrient-deplete artificial seawater (ASW, prepared by dissolving commercial sea salt in distilled water as instructed by the manufacturer) inside a temperature controlled (25°C) laboratory. The samples were placed in a transparent jar, bubbled with air to maintain water movement, and exposed to 116 ± 7.13 µmol photons $m^{-2} s^{-1}$ irradiance.

Preparation of incubation media

Nutrient-deplete natural seawater (NDSW) was used in the preparation of experimental media. NDSW was prepared by stripping inorganic nutrients (NH_4^+, NO_3^-, NO_2^- and PO_4^{-3}) from natural, UV- sterilized seawater. Approximately, 470 g of clean *Ulva* sp. (blade) was incubated in a 20 L tank filled with natural seawater inside a temperature-controlled room (25°C) under 147 ± 4.27 µmol photons $m^{-2} s^{-1}$ irradiance provided by 4×20 W daylight LED lamps (LT8S-20W-DL, Omni, Manila, Philippines). The tank was also continuously bubbled with air to reduce the diffusion boundary layer, thereby enhancing the nutrient uptake rate of *Ulva* sp. After 18 h of incubation, the seawater was filtered using a 200 µm polyester filter bag, and seawater samples were obtained to measure the inorganic nutrient concentrations spectrophotometrically. All nutrients were found to have undetectable concentrations.

The media for the experiment proper consisted of seawater with 6 different PO_4^{-3} levels (0, 0.5, 1.0, 1.5, 3.0 and 5.0 µM PO_4^{-3}) and a constant NH_4^+ concentration (30 µM representing eutrophic conditions). These media were prepared by dissolving appropriate amount of potassium dihydrogen phosphate (KH_2PO_4) and ammonium chloride (NH_4Cl), respectively, in NDSW.

NH_4^+ uptake of eucheumatoids

To measure the NH_4^+ uptake rates of eucheumatoids at different PO_4^{-3} levels, 2−3 g of fresh seaweed samples (n = 4 for each species) were incubated in an Erlenmeyer flask containing 70 ml of each of the above-mentioned media. The incubation was carried out inside a temperature-controlled (25°C) laboratory with a saturating irradiance of 116 ± 7.13 µmol photons m^{-2} s^{-1} (measured using LiCOR, LI-1400 light meter with cosine sensor) supplied by 3 × 20 W daylight LED lamps (LT8S-20W-DL, Omni, Manila, Philippines). All experimental units were haphazardly placed in an orbital shaker (KJ201BD, Wincom Company Ltd, Changsha Hunan, China) set at 140 rpm to reduce the boundary layer. A blank sample (i.e., experimental unit without the seaweed sample) was also incubated to determine uptake other than that caused by the eucheumatoid samples. After 1 h incubation, 10 mL of the seawater was pipetted, stored in 15 mL polyethylene centrifuge tubes, and analyzed for remaining NH_4^+ ions. The analysis was done spectrophotometrically using 1240 mini UV-Vis spectrophotometer (Shimadzu, Japan) following the standard methods of Strickland and Parsons (1972). After the experiment, all seaweed samples were dried inside a 60°C oven until a constant dry weight (DW) was obtained.

Data analyses

The percent reduction in NH_4^+ concentration was calculated based on the difference in NH_4^+ concentration prior to and after the incubation. The uptake rate was calculated using the formula:

$$V = \frac{(N_i - N_f) \times \text{vol}}{DW \times t}$$

Here, N_i is the initial NH_4^+ concentration, N_f is the concentration of the remaining NH_4^+ after incubation, 'vol' is the volume of seawater, DW is the dry weight of the seaweed sample and t is the duration of incubation.

All NH_4^+ uptake data were expressed as mean ± standard error (mean ± SE). For each species, significant variations in NH_4^+ uptake rates among different PO_4^{-3} levels were assessed using one-way analysis of variance (ANOVA). This was followed by Tukey's post hoc test when significant variations were observed. The ANOVA was performed after the normality (Shapiro-Wilk test) and homoscedasticity (Levine's test) of data were satisfied. All significance levels were set at α = 0.05. All statistical analyses and data visualization were done using the software SPSS v23 (IBM Corp).

Results

The NH_4^+ concentration in the blank samples did not significantly vary before and after the incubation period. Therefore, the decrease in NH_4^+ concentration in all other experimental units can only be attributed to the uptake of the seaweed samples.

All three eucheumatoid species were able to reduce the amount of NH_4^+ in the medium under different PO_4^{-3} levels. *K. alvarezii*, *K. striatus* and *E. denticulatum* were able to reduce the NH_4^+ concentration by

40−66%, 21−59%, and 39−59%, respectively.

The NH_4^+ uptake rates of the three eucheumatoid species under different PO_4^{-3} levels were observed to be species-specific (Table 1 and Fig. 2). Among the three eucheumatoid species, *K. striatus* had the lowest range of NH_4^+ uptake rate. This is followed by *E. denticulatum* and *K. alvarezii* (Table 1).

Table 1

Range of NH_4^+ uptake (n = 4) of the 3 eucheumatoid species under laboratory conditions

Eucheumatoid species	NH_4^+ uptake rate, µmol gDW^{-1} h^{-1}
Kappaphycus alvarezii	8.36−17.8
Kappaphycus striatus	3.36−6.35
Eucheuma denticulatum	6.60−14.6

Interestingly, all eucheumatoid species had a positive NH_4^+ uptake rate even at 0 µM PO_4^{-3} level in the medium (Fig. 2). The NH_4^+ uptake rates at 0 µM PO_4^{-3} were also significantly different among the three species examined (one-way ANOVA, $F_{2,9}$ = 32.9, $p < 0.05$). The NH_4^+ uptake rate at 0 µM PO_4^{-3} of *K. striatus* (3.43 ± 0.77 µmol gDW^{-1} h^{-1}) was significantly lower compared to that of *K. alvarezii* (12.7 ± 0.99 µmol gDW^{-1} h^{-1}, Tukey's post hoc, $p < 0.05$) and *E. denticulatum* (9.71 ± 0.66 µmol gDW^{-1} h^{-1}, Tukey's post hoc, $p = 0.001$). However, no significant difference was observed between the NH_4^+ uptake rate at 0 µM PO_4^{-3} of *K. alvarezii* and *E. denticulatum* (Tukey's post hoc, $p = 0.075$).

The NH_4^+ uptake rate of *K. alvarezii* varied among the PO_4^{-3} levels (Fig. 2a, one-way ANOVA, $F_{5,18}$ = 16.3, $p < 0.05$). The maximum NH_4^+ uptake rate observed at 0.5 µM PO_4^{-3} (17.8 ± 1.6 µmol gDW^{-1} h^{-1}) was comparable to the NH_4^+ uptake rate at 1.0 (14.5 ± 0.64 µmol gDW^{-1} h^{-1}; Tukey's post hoc, $p = 0.717$) and 1.5 µM PO_4^{-3} (13.6 ± 0.79 µmol gDW^{-1} h^{-1}; Tukey's post hoc, P = 0.970). However, at PO_4^{-3} levels ≥ 3.0 µM, the NH_4^+ uptake rate significantly dropped by 52−53% (Fig. 2a; Tukey's post hoc, $p > 0.05$).

The NH_4^+ uptake rate of *K. striatus* increased with increasing PO_4^{-3} level, although the increase was not statistically significant (Fig. 2b; one way ANOVA, $F_{5,18}$ = 2.87, $p > 0.05$). The lowest NH_4^+ uptake rate (3.43 ± 0.77 µmol gDW^{-1} h^{-1}) was observed at 0 µM PO_4^{-3} while the highest NH_4^+ uptake (6.35 ± 1.0 µmol gDW^{-1} h^{-1}) was observed at 5.0 µM PO_4^{-3}.

For *E. denticulatum*, the NH_4^+ uptake rates significantly varied among the PO_4^{-3} levels (Fig. 2c; one way ANOVA, $F_{5,18}$ = 4.18, $p < 0.05$). The maximum uptake rate (14.6 ± 1.4 µmol gDW^{-1} h^{-1}) observed at 1.0 µM PO_4^{-3} was statistically higher than the uptake rates at 0 and 0.5 µM PO_4^{-3}. At PO_4^{-3} levels ≥ 1.5

µM, the NH_4^+ uptake of *E. denticulatum* significantly decreased by 40–43% (Fig. 2c, Tukey's post hoc, *p* < 0.05).

Discussion

The three eucheumatoid species examined in our study were able to significantly reduce NH_4^+ concentration under different PO_4^{-3} levels. This corroborates the studies of other authors (e.g., Rodrigueza and Montaño 2007; Hayashi et al. 2008; Narvarte et al. 2022) who demonstrated the efficiency of eucheumatoids in absorbing NH_4^+ ions. Furthermore, *K. alvarezii* and *E. denticulatum* had also been shown to be efficient in absorbing other nutrients like PO_4^{-3}, NO_3^- and NO_2^- (Hayashi et al. 2008; Kambey et al. 2020). These findings, along with those of our current study, indicate that the use of eucheumatoids as biofilters in an IMTA can successfully reduce the detrimental effects of eutrophication.

The NH_4^+ uptake rates of eucheumatoids under different PO_4^{-3} levels were species-specific, pointing to diverse ammonium uptake machineries found among eucheumatoids. Several ammonium transporters (AMTs) have been reported in seaweeds. For example, the chlorophyte *Ulva linza* possesses the AMT1, AMT2 and AMT3 subfamilies of ammonium transporters while the AMT1 subfamily predominates in the rhodophyte *Pyropia yezoensis* (Li et al. 2019; Fan et al. 2020). These diverse AMTs could also occur in eucheumatoids which enables them to exhibit distinct responses to PO_4^{-3} levels. Our study also showed that among the three species examined, *K. striatus* had the lowest range of NH_4^+ uptake rate. This difference might be attributable to the low surface area to volume (SA: V) ratio of the seaweed samples used in the incubation experiment (Fig. 1). Theoretically, seaweeds with high SA: V ratio would have faster nutrient uptake rates because nutrients are absorbed across the entire surface area of the seaweed thallus (Taylor et al 1998, Rosenberg and Ramus 1984). This is also supported by several published literatures showing a strong positive correlation between nutrient (NH_4^+, NO_3^-, PO_4^{-3}) uptake rate and SA: V ratio (e.g.: Littler and Littler 1980; Rosenberg and Ramus 1984; Taylor et al. 1998; Narvarte et al. 2022).

All eucheumatoid species showed a positive NH_4^+ uptake rate even when the medium has 0 µM PO_4^{-3}. This may imply that either the presence of PO_4^{-3} ion in the bulk water is not a requirement for the uptake of NH_4^+ or there is still sufficient pool of reserve tissue P after the seaweed samples were starved overnight prior to the uptake experiment, if internal P is essential for N uptake. However, when the reserved nutrients in their internal pools were exhausted to cope with the NDSW overnight, then the positive NH_4^+ uptake rates at 0 µM PO_4^{-3} may therefore have resulted from the passive transport of NH_4^+ when the samples were exposed to high NH_4^+ levels, filling in the formerly empty nutrient pools. The results of our study also showed that the NH_4^+ uptake rate at 0 µM PO_4^{-3} varied among the three eucheumatoid species.

The NH_4^+ uptake rate of eucheumatoids increased with increasing PO_4^{-3} levels. However, a decline in NH_4^+ uptake rate was observed for *K. alvarezii* and *E. denticulatum* at ≥ 3.0 and ≥ 1.0, respectively, μM PO_4^{-3}. Similarly, the nitrogen uptake of *Gracilaria lamaneiformis* markedly increased at high PO_4^{-3} levels (Xu et al. 2010). The increase in NH_4^+ uptake with increasing PO_4^{-3} level may suggest that PO_4^{-3} ions are possibly being converted to energy sources such as ATP, which could then be used to power the higher assimilation of nitrogen (i.e., production of pigments, proteins, amino acids, and other N-based compounds). This would subsequently result to more NH_4^+ ions that are passively transported across cells. Moreover, a high PO_4^{-3} level is known to promote the regeneration of ribulose-1-5-bisphosphate (RuBP) resulting in enhanced photosynthetic efficiency (Rao and Terry 1989). This fast photosynthetic rate would generally be accompanied by faster nutrient uptake rates (Suárez-Álvarez et al. 2012). On the other hand, the decline in the NH_4^+ uptake rates at higher PO_4^{-3} levels observed in *K. alvarezii* and *E. denticulatum* may indicate that high PO_4^{-3} levels may have hindered the uptake of NH_4^+ ions. It is known that NH_4^+ is primarily passively absorbed through transport proteins (i.e., through facilitated diffusion; Ninnemann et al. 1994; Hurd et al. 2014). Thus, PO_4^{-3} ions may have blocked these transport proteins preventing the uptake of NH_4^+. Alternatively, the serine residues of the transport proteins might have been phosphorylated at high PO_4^{-3} levels. In terrestrial plants, the phosphorylation of serine residues is known to inhibit the activity of the enzyme nitrate reductase (Bachmann et al. 1996; Lillo et al. 2004; Grossman and Aksoy 2015). The same mechanism might also be at work in the transport proteins of seaweeds. At high levels of PO_4^{-3}, the transport proteins of *K. alvarezii* and *E. denticulatum* might have been phosphorylated and this subsequently triggered conformational changes in its protein structure, thereby preventing the uptake of NH_4^+ ions.

In general, a high nutrient level tends to improve growth and nutrient uptake in seaweeds. For instance, *K. alvarezii* had higher growth and nutrient uptake at high levels of nutrient (Luhan et al. 2015; Narvarte et al. 2022). Moreover, *Fucus vesiculosus* samples that were enriched with N displayed the highest P uptake efficiency at biologically relevant P levels (Perini and Bracken 2014). Nevertheless, surpassing the optimal nutrient level might lead to adverse effects. In our study, the reduced NH_4^+ uptake at high PO_4^{-3} levels observed in *K. alvarezii* and *E. denticulatum* might indicate that the seaweeds were being negatively impacted by the toxic level of PO_4^{-3} in the medium. Conversely, elevated PO_4^{-3} levels did not affect the NH_4^+ uptake of *K. striatus*, suggesting that this species had unique N requirement. Rodrigueza and Montaño (2007) suggested that N assimilation in *K. striatus* was concentrated not only toward the synthesis of cell wall structures but also toward the formation of protoplasmic constituents. Thus, the uninhibited N uptake of *K. striatus* at high PO_4^{-3} level might imply that this species requires more N to synthesize such cellular components.

The maximum NH_4^+ uptake rate of the three eucheumatoid species also varied among the PO_4^{-3} levels. *K. alvarezii* had maximum NH_4^+ uptake at 0.5 μM PO_4^{-3} while *K. striatus* and *E. denticulatum* had

maximum NH_4^+ uptake rate at 5.0 and 1.0 μM PO_4^{-3}, respectively. This observation may reflect the nutrient requirements of seaweeds and their ability to respond with varying PO_4^{-3} levels, both of which are known to vary from species to species. In addition, these results have significant implications when incorporating these macroalgae in an IMTA system. Our results suggest that the uptake machineries of *K. striatus* are more tolerant to high PO_4^{-3} levels compared to that of *K. alvarezii* and *E. denticulatum*. However, our results also showed that *K. striatus* had the lowest range of NH_4^+ uptake (Table 1) among the three eucheumatoid species, suggesting that the overnight starvation may have not been enough to empty the internal N pool and that *K. striatus* might have lots of nutrient reserves. This can be verified by e.g., analyzing the tissue N and P contents. For *Ulva lactuca*, it has been shown that the internal nutrient (N and P) storage was sufficient for approximately 10 days (Lubsch and Timmermans 2018). On the other hand, the internal storage capacity of eucheumatoids is yet to be investigated. Although the data presented in our study should be taken into consideration when incorporating eucheumatoids in IMTA, the results of our present study should not be the sole criterion in selecting which species works best in an IMTA set-up. It is important to consider other factors such as growth rate, tolerance to environmental stress, resistance to diseases and pest, and biochemical performance, when choosing species to be integrated in IMTA.

Declarations

Authorship Contribution Statement

BCV Narvarte: Investigation, Data Curation, Formal analysis, Visualization, Writing-Original Draft. LAR Hinaloc: Investigation, Visualization, Writing – review & editing. SMC Gonzaga: Investigation, Writing – review & editing. MY Roleda: Project administration, Supervision, Methodology, Writing- Review & Editing.

Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Figures

Figure 1

Cultures of *K. alvarezii* (left), *K. striatus*(center) and *E. denticulatum* (right) of AlgaE Laboratory in Bolinao, Pangasinan, Philippines. The photos above are the representative samples of the actual thalli used in the nutrient uptake experiment. Scale bar = 2.0 cm
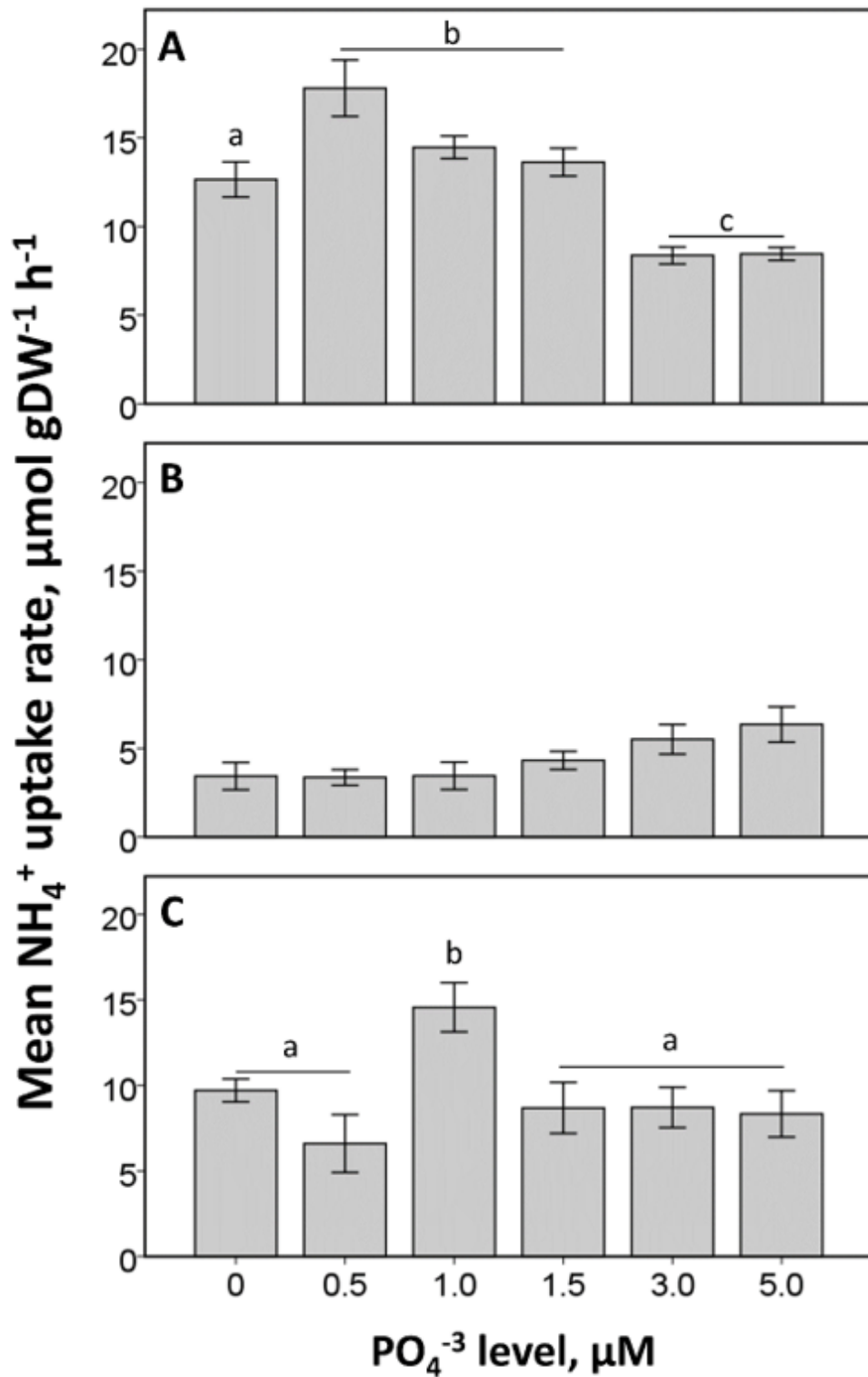

Figure 2

Mean ±SE (n = 4) NH_4^+ uptake rates of the eucheumatoids *K. alvarezii* (A), *K. striatus* (B), and *E. denticulatum* (C) under different PO_4^{-3} levels. The subscripts *a, b* and *c* represent significant variations in NH_4^+ uptake among PO_4^{-3} levels ($p < 0.05$).