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High temperature and eutrophication alter biomass allocation of black mangrove (*Avicennia germinans* L.) seedlings

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ABSTRACT

Mangrove restoration is underway along tropical coastlines to combat their rapid worldwide decline. However, restoration success is limited due to local drivers such as eutrophication, and global drivers such as climate change, yet their interactions remain unclear. We conducted a mesocosm experiment to assess the impact of increased nutrients and temperature on the photosynthetic efficiency and development of black mangrove seedlings. Seedlings exposed to high temperature and eutrophication showed reduced root growth and disproportionally long stems, with lower net assimilation rates. This architectonical imbalance between root and stem growth may increase susceptibility to physical disturbances and dislodgement. Notably, none of the experimental seedlings displayed signs of photophysiological stress, and those exposed to increased nutrients and temperature exhibited robust photosynthetic performance. The disbalance in biomass allocation highlights the importance of considering local nutrient status and hydrodynamic conditions in restoration projects, ensuring the effective anchorage of mangrove seedlings and restoration success under a warming climate.

1. Introduction

Mangrove forests are located at the interphase between land and sea of tropical and subtropical coastlines. They provide a wide range of resources and often occur near high human population densities (Friess et al., 2019). These ecosystems not only provide climate change mitigation through the capture and storage of carbon (Lagomasino et al., 2019), but they also play a role in flood protection and erosion control (Blankespoor et al., 2017; Horstman et al., 2014), as well as acting as buffers of nutrients and sediment run-off from land sources (Agraz-Hernández et al., 2018; Moroyoqui-Rojo et al., 2015; Wadnerkar et al., 2019). Over the past decades, however, increasing local human pressures and climate change, including rapid-onset events such as hurricanes, have led to a sustained loss of mangrove forests (Goldberg et al., 2020). A recent study on the extent of change mangroves indicates that 3.4% of mangrove forests were lost between 1996 and 2020 worldwide (Bunting et al., 2022).

Once mangroves have been lost, their reestablishment is difficult unless the proper environmental conditions are met (Kamali and Hashim, 2011; Macy et al., 2021; Pérez-Ceballos et al., 2017), which makes restoration a vital tool to counteract the global loss of mangroves (Macy et al., 2021; McKee and Faulkner, 2000; Ram et al., 2021; Toledo et al., 2001). However, mangrove restoration is currently prone to failure due to lack of understanding of the cause for decline (thus the cause was not tackled), poor site selection (Hai et al., 2020), choosing areas with unsuitable topography or hydrology (Kodikara et al., 2017), failing to implement positive ecosystem interactions (Gillis et al., 2014, 2017), and potentially the interactive effects between local (e.g. nutrients) and global (e.g. increasing temperature) drivers. Increasing air and sea surface temperatures by global warming can impact newly restored mangroves by raising leaf temperatures and consequently inhibiting photosynthesis (Arifanti, 2020). High temperature also leads to larger evaporation rates, which increases salinity and affects mangrove structure and growth (Hai et al., 2020). Additionally, it causes the seedlings and propagules to dry out faster (Elster, 2000). A study that encompassed all mangrove restoration sites in Vietnam found that dry and arid zones had the lowest survival rates of restored seedlings, highlighting the importance of climate and global drivers in restoration (Kodikara

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et al., 2017). The same study determined that nutrient imbalance and availability in the soil are also causes for poor seedling survival (Kodikara et al., 2017), indicating that local drivers also play a role in restoration success. Furthermore, eutrophication in mangrove soils makes them more vulnerable to physical disturbances such as tropical storms (Feller et al., 2015).

Mangrove restoration projects mostly use nursery-reared seedlings grown to a suitable age for replanting (Toledo et al., 2001). When these seedlings are deployed to the natural environment, they have already overcome the seed and emergence phases, and usually consist of a small root system, a stem and a few leaves. The transplanted seedlings must then overcome the establishment phase in their new environment, which is often limited by physicochemical stress factors such as nutrient regimes (McKee, 1995). The successful establishment of seedlings in the new environment increases their resilience against dislodgement and is therefore key to restoration success. However, this crucial phase could be slowed or even hindered by stressors present in the new environment such as high temperature and eutrophication. Despite the importance of using seedlings in restoring/re-planting mangroves, information on the establishment phase of nursery-reared mangroves is generally lacking. In addition, most scientific studies focus on adult, well-established mangrove trees or early life stages (seed survival and germination).

Previously, the impacts of temperature and eutrophication on mangroves were investigated separately. Mangrove seedling development can be affected by excess nutrients (Agraz-Hernández et al., 2018; Moroyoqui-Rojo et al., 2015), often resulting in lower root/shoot biomass ratios (McKee, 1995; Reef et al., 2016). High temperature causes inhibition of root growth (Krauss et al., 2008). The interactive effects of temperature and eutrophication were tested for the first time in the orange mangrove *Bruguiera sexangula* (Gillis et al., 2019). This study found antagonistic effects between these two stressors on the above-ground growth, i.e. stem length, and limited root architecture. When exposed to high nutrients and temperature, orange mangrove seedlings grew less than when exposed to the two stressors separately (Gillis et al., 2019). Limited root development as a consequence of high nutrients and temperature suggested a lower resistance of seedlings to withstand their dynamic environment, which could potentially impact mangrove resilience to physical disturbance and, thereby, anchorage. Since these findings are only reported for the orange mangrove, the effects of high nutrients and temperature on other mangrove species remain unknown. Thus, it is important to investigate the response of other mangrove species to multiple stressors such as temperature and eutrophication as these responses are likely to differ based on species characteristics and ecological niches.

The widespread black mangrove *Avicennia germinans* commonly inhabits intertidal zones and is considered an important pioneer tree. Pioneer species, such as the black mangrove, can colonise bare sediment and establish positive plant interactions that facilitate later colonisation by other plant species (Friess et al., 2012). Restoration projects use black mangrove seedlings around the world, with varying temperatures and nutrient conditions (Macy et al., 2021; McKee and Faulkner, 2000; Ram et al., 2021), although mangrove restoration, in general, has not yielded high success (Lovelock et al., 2022). To increase restoration success and improve site selection, a key yet unanswered question is how nutrients and temperature impact black mangrove seedlings' health and development, and whether they exhibit a similar growth response as the orange mangrove. Moreover, previous studies ran for relatively short periods of time i.e. up to six weeks, thus failing to detect long-term responses or did not consider below-ground biomass, which is closely linked to above-ground biomass dynamics.

Growth and development responses are often linked to changes in photosynthetic efficiency, and these responses are valuable indicators of the health of restored mangroves (Rovai et al., 2013). Temperature changes can cause stress (Duke et al., 2017; Liu and Wang, 2020) and marked reductions in the photosynthetic efficiency of several mangrove species (Chen et al., 2017). Furthermore, excess nutrients result in higher photosynthetic electron transport (Feller et al., 2003). Assessing the photosynthetic efficiency of mangroves provides a deeper insight into their tolerance and adaptation capacity to temperature and eutrophication pressures. Moreover, it is unknown to what extent black mangrove seedlings can experience changes in response to increased nutrients and temperature.

In this study, we aimed to assess the effect of temperature and nutrient conditions on the photosynthetic efficiency and development of black mangrove seedlings. We addressed the following questions: How do increased nutrient loads and temperature impact 1) biomass allocation, growth, and nutrient ratios in tissue, 2) photosynthetic efficiency, and 3) net assimilation rates of black mangrove seedlings? To answer these questions, we conducted an *ex-situ* experiment using black mangrove seedlings that were four months old, a typical age for outplanting. The seedlings were subjected to two different levels of nutrients and temperature and their combined effect under controlled conditions for a period of ten weeks.

2. Materials and methods

Black mangrove seedlings were reared from propagules produced by a single adult tree at Burgers' Zoo, Arnhem, collected in July 2021. The propagules (1–4 days old from the time of detachment) were transported to the nursery at Nergena greenhouse, Unifarm, WUR. They were horizontally placed on a nursery tray containing water-logged quartz sand under a 12 h natural light regime and ~25 °C air temperature. No nutrients were provided at this stage. When the seedlings reached four months of age, those that appeared in good health were selected based on a visual examination of the colour and shape of their stem and leaves. Stem length was 19.5 ± 3.2 cm high, and the average number of leaves was 5.58 ± 1.0. Six weeks prior to the experiment, seedlings had been planted into 8 × 8 × 8 cm nursery pots containing ~390 cm^3 of quartz sand previously washed and ashed, of 0.1–0.3 mm grain size. The sediment was fertilised with 0.5 g of an NPK 15:9:11 slow-release fertiliser for aquatic plants (Nutri Caps with Osmocote ®, Colombo B. V., the Netherlands) by placing the fertiliser granules at root level during reporting. The fertiliser composition was ~6.6 % NO_3–N, 8.4 % NH_4^+N, 9 % P_2O_5, 11 % K_2O, 2 % MgO, 0.03 % B, 0.05 % Cu, 0.045 % Fe, 0.06 % Mn, 0.02 % Mo, and 0.015 % Zn. One week prior to the start of the experiment, seedlings were transported from the nursery to climate-controlled rooms set to 25 °C and 30 °C.

2.1. Experimental design

To test the interactive effects of nutrients and temperature on black mangrove seedlings, a 10-week mesocosm experiment was conducted in two climate-controlled rooms without natural daylight set at different temperatures at Wageningen University & Research (WUR) from November 26, 2021 to February 4, 2022. Pots with seedlings were placed in individual containers filled with 1.7 l of artificial seawater (Aqua Medic Reef Salt, AB Aqua Medic GmbH, Germany) and a salinity of 32 g l^{-1} (Fig. 1), following a completely randomised design. Each container (mesocosm) contained one experimental unit (mangrove seedling). The experimental setup consisted of four treatments, resulting from the combination of 2 factors with 2 levels each (Fig. 1). Thus, the experiment included the following treatments: nutrient-enriched 25 °C (n = 6), non-enriched 25 °C (n = 6), nutrient-enriched 30 °C (n = 6), and non-enriched 30 °C (n = 6). The lower temperature level represents the mean air temperature in mangrove forests (Fazlioglu et al., 2020). The upper-temperature level resembles a hypothetical global warming scenario of 5 °C that would be reached, for instance, in Australia and the Red Sea region under the SSP5-8.5 scenario by 2081–2100 (Gutiérrez et al., 2021). Nutrient-enriched conditions were achieved by adding 0.4 g of slow-release fertiliser by pushing a thin stick down into the sediment and inserting the granules into the resulting hole at the root level to resemble a continued supply of nutrients over time. The experiment

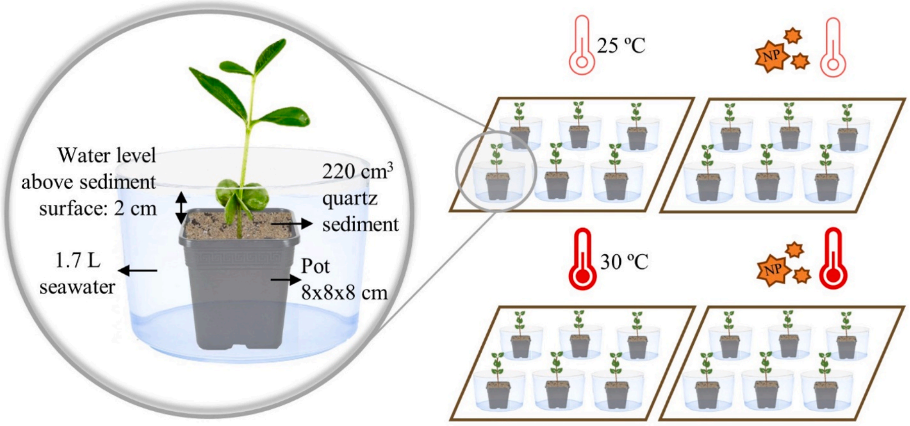

Fig. 1. Black mangrove seedlings were grown in individual containers submerged in artificial seawater under different temperature and nutrient conditions.

included negative control treatments, i.e. without a seedling to monitor nutrient concentrations in the porewater over time without the seedling's influence. The water level in the containers was set approximately 2 cm above the sediment surface and was restored every two days by adding demineralised water to compensate for evaporation, thus avoiding salinity fluctuations. Lighting was provided by a 400 W metal halide grow light (Hortilux, Monster, the Netherlands) 80 cm above the canopies and set on a 12-h light cycle, with a light intensity of 300 μmol m^{-2} s^{-1} measured at leaf height. To reduce potential spatial variations within the experimental area, mesocosms were redistributed randomly on a weekly basis across the experimental surface. In our experience, roots grow out of the drainage holes on the bottom of the pot when the pot size is too small. In this experiment, roots stayed inside the pot, therefore it was assumed that pot size did not restrain seedling root growth. Furthermore, the time span of the experiment would suffice for the roots to grow (Gillis et al., 2019). Seedlings were regularly misted with demineralised water to provide moisture and increase air humidity.

2.2. Growth, biomass, and elemental content

The first measurements took place after one week of acclimation, at the start of the experiment and were repeated weekly for leaf number, leaf length, leaf width, stem length, and photosynthetic efficiency (F_v/F_m). At the end of the experiment, leaves were sampled for total nitrogen (TN), total phosphorous (TP), and total iron (TFe) analysis, and a subsample of three randomly selected seedlings per treatment was harvested for above-ground biomass and below-ground biomass measurements. TN and TP were also determined from above-ground and below-ground tissues to get a clear overview of nutrient allocation within the different plant parts. Leaf length, leaf width, and leaf area were measured from 30 randomly selected leaves of varying sizes across all treatments to fit an equation to predict leaf area. Leaf length and width (predictors) were measured by hand using an analogue calibre, and leaf area (response) was determined using ImageJ, version 1.52 (Schneider et al., 2012) from a white-background photograph of the leaves, which served as input to calculate leaf area. Above- and below-ground parts were segmented at the hypocotyl and dried at 60 °C for 48 h, or until they had reached a constant weight. Leaf samples followed the same procedure. Dry weight was recorded for above- and below-ground parts, and samples were grounded into powder using a ball mill (MM200, Retsch GmbH, Haan, Germany). Finely ground samples were transformed into single digests following the sulfuric acid–selenium digestion method at 330 °C in a Kjeldatherm block digestion system and transferred to a Segmented Flow Analyzer (Skalar Analytical B. V., the Netherlands) for TN and TP determination (Novozamsky et al., 1983, 1984). Furthermore, only leaf digests were used for Tfe determination using ICP-OES (Thermo iCAP-6500, Thermo Fisher Scientific) at Soil Chemistry Laboratory (CBLB, Wageningen, the Netherlands).

To monitor nutrient concentrations supplied by the slow-release fertiliser, weekly porewater samples were taken using a Rhizon for porewater sampling (Rhizon SMS, Rhizosphere Research Products B.V, the Netherlands) of 0.15 μm pore size and a length of 5 cm, connected to a 30 ml Luer-lock syringe. Porewater NH_4, PO_4, and $NO_3 + NO_2$ were determined from each porewater sample using a Segmented Flow Analyzer (Skalar Analytical B.V., the Netherlands) at Soil Chemistry Laboratory (CBLB, Wageningen, the Netherlands).

2.3. Photosynthetic efficiency

Plant health was assessed by measuring mangrove seedlings' photosynthetic efficiency (F_v/F_m) using the Pulse Amplitude Modulated (PAM) fluorometry technique. The photosynthetic efficiency of mangroves, so-called F_v/F_m, is a widely recognised indicator of photophysiological activity and is measured as the chlorophyll fluorescence on a localized surface of their leaves. This parameter is defined as the maximum quantum yield of PSII, the most commonly used chlorophyll fluorescence parameter for studying stress photophysiology in mangroves (Bhagooli et al., 2021) and a powerful indicator of the plant's photosynthetic performance (Guidi et al., 2019). Seedlings were dark-adapted for 30 min prior to non-destructive F_v/F_m measurements on the four fully expanded healthy leaves per plant closest to the apical meristem (Panda et al., 2006) using a PAR-FluorPen FP 110 (Photon System Instruments, Czech Republic). These four measurements were subsequently used to calculate the mean F_v/F_m value for each plant, which provided a more accurate estimate than a single measurement. The F_v/F_m is calculated as $F_v/F_m = (F_m - F_0)/F_m$, where F_0 and F_m are the minimum and maximum fluorescence after dark acclimation, respectively.

2.4. Statistical analysis

Data analysis was conducted in R, version 4.0.2 (R Core Team, 2021). Variables were tested for normality and homogeneity of variance. When the relevant assumptions were not met, logarithmic or square root transformations were conducted prior to analysis, and statistical comparisons were only made between treatments unless stated otherwise. When homoscedasticity was not achieved throughout the normality transformations, variables were analysed using a generalised linear model (GLM). To account for the temporal dynamics of continuous variables such as the F_v/F_m, a generalised linear mixed model was used (GLMM), where nutrient enrichment and temperature were treated as fixed factors, and time was a random factor. End-point variables were analysed using two-way analysis of variance (ANOVA). Along with *glm* and *glmer* procedure, the *Anova* function from the *car* package was used to report Chi-squared and p-values. Results with a significance value of $\alpha \leq 0.05$ were considered statistically significant. To account for differences in nutrient concentration between leaves, above-ground biomass, and below-ground biomass, the plant part was included in the TN and TP ANOVA tests. N:P, N:Fe, and P:Fe ratios from the three different plant parts were tested separately. When presenting descriptive statistics, they indicate the mean value ± the standard error (SE). In addition to the measured variables, each seedling's Net Assimilation Rate (NAR) was also calculated to assess their efficiency in using resources under contrasting nutrient loads. Other authors referred to the net assimilation rate as *E* (Goodman, 1973; Vernon and Allison, 1963), and the formula used for its calculation has changed across studies and time (Anten and Ackerly, 2001; Eagles, 1971). In this study, NAR (g m^{-2} day^{-1}) was calculated according to the following formula:

$$NAR = W / (L*t)$$

where W is the seedling's total dry weight (g), L is leaf area (m^2), and t is the time span of the experiment (days) (Hayes et al., 2017).

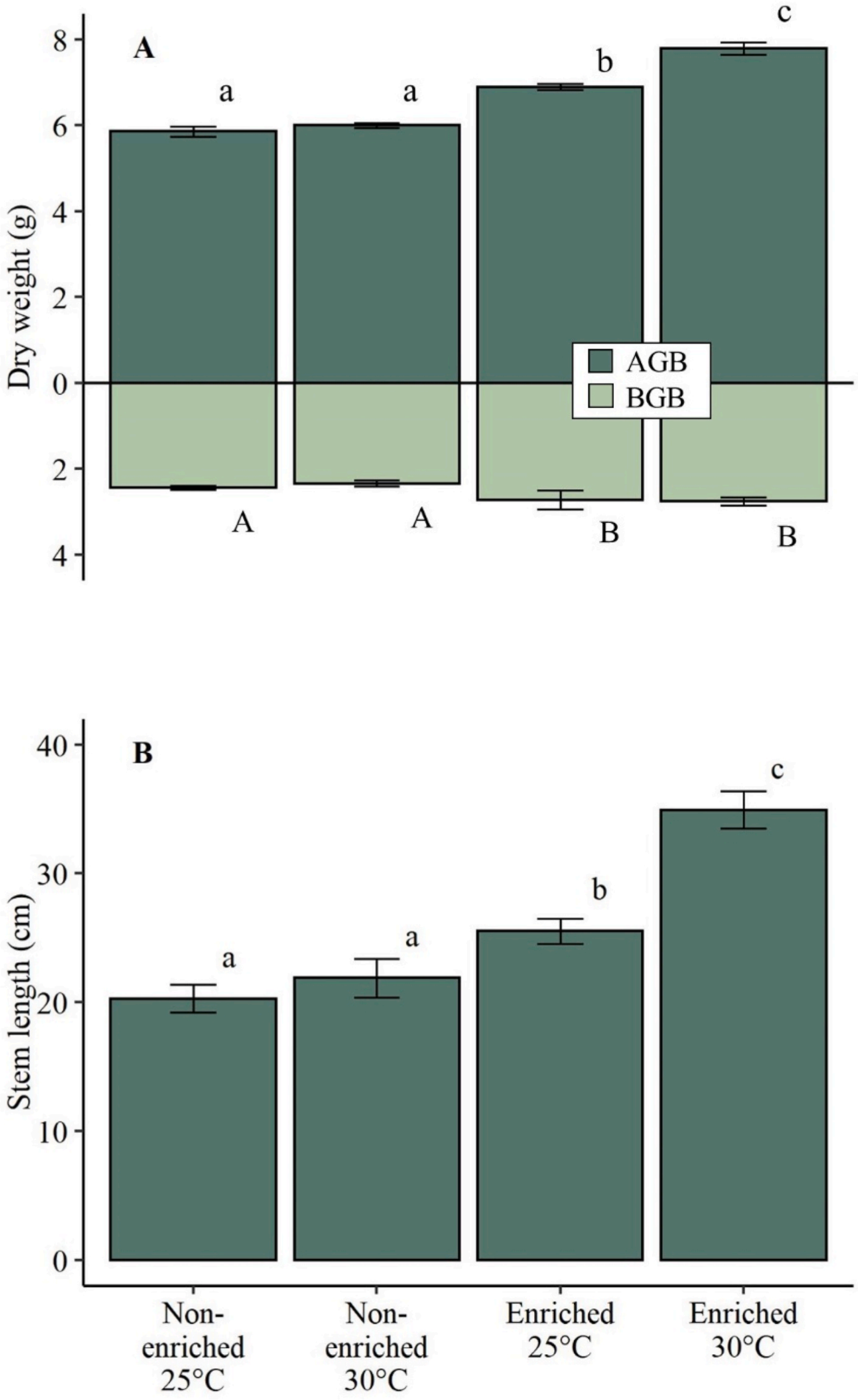

Fig. 2. (A) Dry weight of above (ABG) and below-ground (BGB) parts, and (B) stem length at the end of the experimental period. Means are presented with standard errors. Compact letter display next to bars indicates significant differences between treatments.

3. Results

Nutrients and temperature caused pronounced differences in seedling biomass allocation and photosynthetic efficiency over the 10-week experimental period. The two-way ANOVA revealed a positive main effect of both nutrient enrichment (F (1, 20) = 50.6, $p < 0.001$) and temperature (F (1, 20) = 18.4, $p < 0.001$), resulting in longer stems across all treatments. The interaction effect between nutrient enrichment and temperature was also significant (F (1, 20) = 9.3, $p < 0.01$), specifically, by the end of the experiment the mean stem length in the enriched 30 °C treatment was 1.7-fold higher compared to the control (non-enriched 25 °C) treatment (Fig. 2). Around week seven, we observed bending in the seedling stems of the enriched 30 °C treatment. The main effects of both nutrient enrichment (F (1, 20) = 70.1, $p < 0.001$) and temperature (F (1, 20) = 23.9, $p < 0.001$) on leaf area were also significant, indicating that nutrient enriched conditions or a temperature of 30 °C led to an increased mean leaf area. Although the interaction effect of nutrient enrichment and temperature was not significant (F (1, 20) = 1.9, $p = 0.18$), leaf area increased 2.7-fold in the enriched 30 °C compared to the control treatment (non-enriched 25 °C) (Fig. A1). Temporal trends in growth parameters, such as leaf area and stem length, indicated that seedling growth stabilized after week eight in all treatments, except for the enriched 30 °C treatment, where growth continued to increase at a relatively constant rate until the end of the experiment. Seedling survival was 100 % during the entire experimental period.

Results indicated that the mean above-ground biomass of seedlings was higher under nutrient enrichment (F(1, 8) = 183,5, $p < 0.001$) and temperature (F(1, 8) = 24.5, $p < 0.01$), but only the main effect of nutrient enrichment led to higher below-ground biomass (F(1, 8) = 7.6, $p < 0.05$). Furthermore, the interaction effect between nutrients and temperature (F(1, 8) = 12.8, $p < 0.01$) indicated that the impact of temperature on above-ground biomass was more pronounced under nutrient-enriched conditions, resulting in a 33 % increase in above-ground biomass (Fig. 2). Although the main effect of nutrient enrichment on below-ground biomass was found to be significant, it only resulted in a 12 % increase in biomass compared to the control (non-enriched 25 °C) treatment.

Overall, TN was highest in leaves (13.23 ± 1.13 mg g^{-1}), followed by above-ground biomass (10.28 ± 1.25 mg g^{-1}) and below-ground biomass (8.08 ± 0.41 mg g^{-1}), and TN results were markedly increased by nutrient enrichment in the sediment porewater (Fig. A2). The three independent two-way ANOVA tests, one for each plant part, showed a significant effect of nutrient enrichment on TN in leaves (F(1, 20) = 27.8, $p < 0.001$), TN in above-ground biomass (F(1, 8) = 71.7, $p < 0.001$), and TN in below-ground biomass (F(1, 8) = 23.2, $p < 0.01$). The main effect of temperature and the interaction between both factors were non-significant. Similarly, TP was found to be highest in leaves (1.04 ± 0.07 mg g^{-1}) followed by above-ground biomass (0.82 ± 0.06 mg g^{-1}) and below-ground biomass (0.76 ± 0.10 mg g^{-1}). TP in leaves was significantly higher under nutrient enriched conditions (F(1, 20) = 6.13, $p < 0.05$), but neither the main effect of temperature nor the combination between the two factors was found significant with regard to TP in leaves, but the pairwise comparisons with Tukey adjustment did not show any further differences between treatments (Fig. A3). Furthermore, TP in above-ground biomass was significantly increased by the main effects of nutrient enrichment (F(1, 8) = 18.4, $p < 0.01$) and temperature (F(1, 8) = 9.6, $p < 0.05$), but the interaction effect was not significant. The two-way ANOVA for TP in below-ground biomass did not yield a main effect for nutrient enrichment or temperature, and the interaction effect was also not significant. Overall, the Fe concentration in leaves ranged from 0.12 to 1.30 mg g^{-1}, with the lowest mean values observed in the enriched 30 °C treatment (0.23 ± 0.04 mg g^{-1}) and the highest in the non-enriched 25 °C treatment (0.45 ± 0.18 mg g^{-1}). The main effects of nutrient enrichment and temperature on Fe concentrations were found non-significant, as was the interaction effect between nutrient enrichment and temperature.

With regard to nutrient ratios, a two-way ANOVA yielded a main effect of nutrient enrichment (F(1, 8) = 89.3, $p < 0.001$) and temperature (F(1, 8) = 35.9, $p < 0.001$) such that N:P in above-ground biomass was higher under 30 °C and nutrient-rich conditions. However, results indicated a non-significant effect of neither nutrient enrichment nor temperature on N:P of leaves and below-ground biomass (Fig. 3A). The interaction effect of nutrient enrichment and temperature was not significant for N:P in any of the plant parts, i.e. leaves, above-ground biomass, and below-ground biomass. Leaf N:Fe was significantly higher in nutrient enriched treatments (F(1, 20) = 13.83, $p < 0.01$), with the highest values found under enriched 30 °C (94.76 ± 15.77) and the lowest in non-enriched 30 °C (33.72 ± 10.05). In terms of foliar P:Fe, the enriched treatments exhibited significantly higher P:Fe ratios, with the highest value in the enriched 30 °C treatment (6.03 ± 0.83) and the lowest value in the non-enriched 30 °C treatment (2.89 ± 0.83). Overall, data dispersion was high for P:Fe ratios. Pairwise comparisons using a Tukey adjustment found no further differences between treatments due to its more conservative approach (Fig. 3C).

The mixed model (GLMM) applied to assess changes in the photosynthetic efficiency (F_v/F_m) of seedlings over time revealed a significant main effect of nutrient enrichment ($p < 0.001$) and temperature ($p < 0.001$), as well as a significant interaction between the two main factors and time ($p < 0.001$). F_v/F_m values ranged from 0.71 to 0.845 μmol m^{-2} s^{-1} (Fig. 4A). The F_v/F_m of black mangrove seedlings increased by 4.1 %, 7.6 % and 5.2 % over the ten weeks in the non-enriched 30 °C treatment, enriched 25 °C treatment, and enriched 30 °C treatment, respectively, compared to the non-enriched 25 °C treatment. Nutrient addition caused a steeper increase in F_v/F_m values during the first half of the experiment, but all treatments reached a relatively stable trend after week seven. Despite the enriched 30 °C treatment showing the sharpest initial increase, its final F_v/F_m values were lower than the enriched 25 °C treatment by the end of the experiment (Fig. 4A), indicating that such high photosynthetic performance could not be sustained in the long-term.

A generalised linear model (GLM) on the calculated NAR values showed that neither the main effect of nutrient enrichment nor temperature were significant. The interaction effect between both main factors, however, was significant ($z = -2.6$, $p < 0.01$), resulting in 48% lower NAR in the enriched 30 °C compared to the control (non-enriched 25 °C) treatment. This result indicates that the effects of nutrient enrichment on NAR were stronger under 30 °C of temperature. In contrast, the main effects of temperature or nutrient enrichment alone only caused a reduction in NAR by 5 % and 8 %, respectively (Fig. 4B).

4. Discussion

High temperature in combination with nutrient enrichment enhanced black mangrove seedling growth but altered biomass allocation between above- and below-ground structures. The seedlings exposed to high nutrients and temperature showed a disproportional increase in above-ground biomass compared to their roots (33 % vs. 12 % dry weight gain). Additionally, this was accompanied by a decrease in Net Assimilation Rate (NAR), indicating lower carbon incorporation in photosynthesis per unit leaf area (Lambers et al., 2008). These results suggest that under high nutrients and high temperature seedlings allocated more carbon compounds to photosynthetically active organs such as leaves resulting in rapid growth of stems but potentially weaker cell walls and low fibre content (Lambers et al., 2008).

This study is the first to investigate the combined effect of high temperature and nutrient enrichment on black mangroves, examining impacts on both below and aboveground plant growth and development. Previous studies have focussed on the effects of single drivers on either above or belowground plant parts (with the exception of Gillis et al., 2019), with limited research on their combined effects. Lower biomass allocation to roots than shoots (Mckee, 1995) and increased leaf

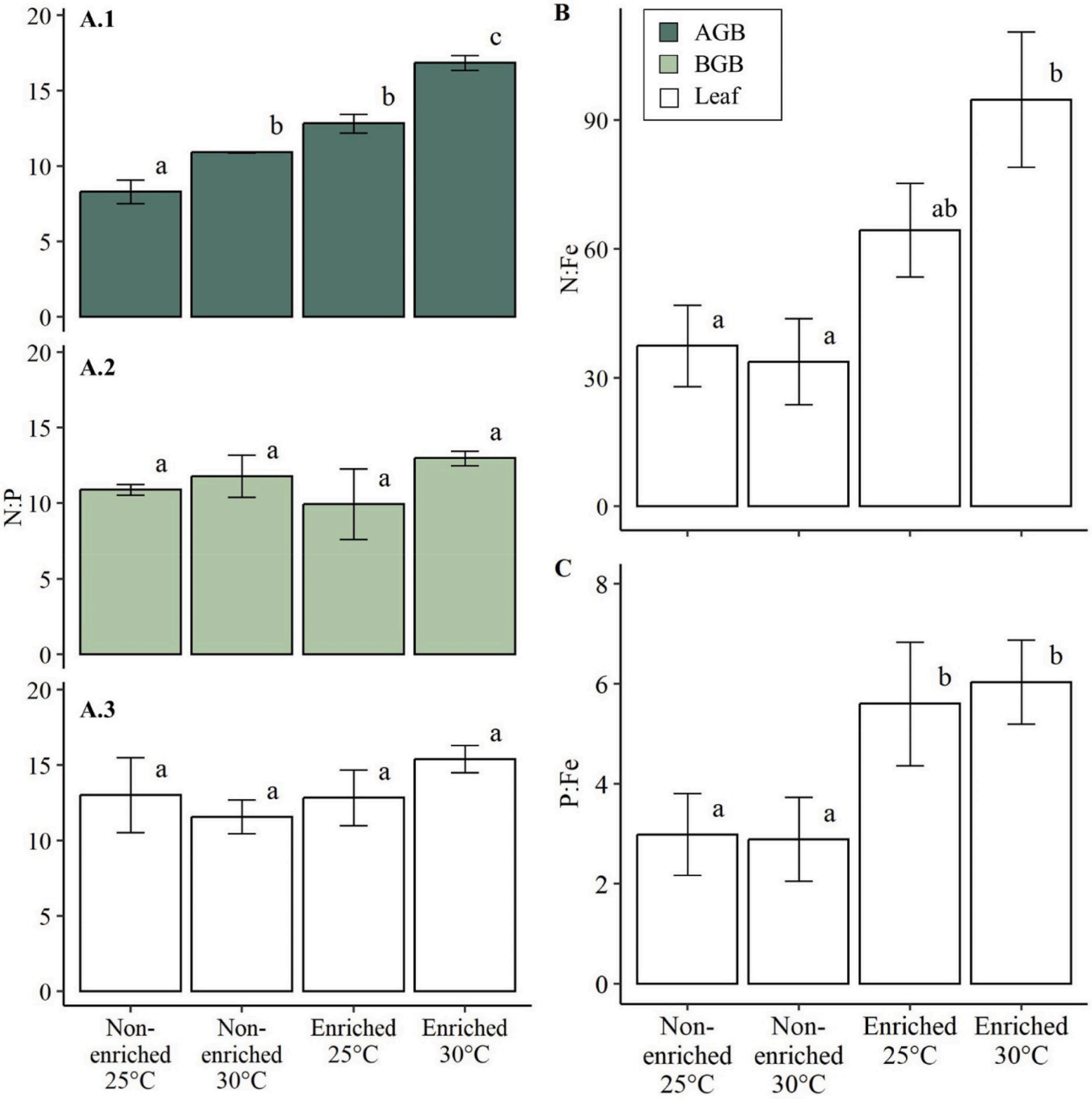

Fig. 3. Seedling tissue nutrient ratios measured at the end of the experimental period; (A) N:P ratio measured from above-ground biomass (dark green bars), below-ground biomass (light green bars), and leaves (white bars); (B) N:Fe leaf tissue ratios; and (C) P:Fe leaf tissue ratios. Columns represent the mean values of the different treatments with standard error bars. Compact letter display depicts significant differences between treatments.

production (Simpson et al., 2013) had been previously reported separately as an effect of nutrient enrichment in black mangrove seedlings. Moreover, a lower biomass allocation to roots is generally common in plants when they approach their optimum temperature (Lambers et al., 2008). By studying both drivers and plant parts together, we gain a more comprehensive understanding of their synergistic impacts. In this experiment, both temperature levels used (25 °C and 30 °C) appear to fall within the species' optimal temperature range, as indicated by the minor root growth observed.

Notably, the response of black mangroves to high temperature and nutrient enrichment differs from that of orange mangroves. Previous research on the above-ground growth of orange mangrove *B. sexangula* showed antagonistic interactions between high temperature and nutrient enrichment, negatively impacted root architecture (Gillis et al., 2019). These contrasting responses are likely caused by differences in the temperature optimum of the two species, with the orange mangrove exceeding the optimum while the black mangrove does not reach it. When exposed to high temperature and nutrient enrichment, *A. Germinans* seedlings in our experiment do not experience substantial root growth, potentially impacting mangrove ecosystem traits such as carbon stock potential (Gillis et al., 2023). Furthermore, the bending observed in their stems indicates that the seedlings were becoming top-heavy, which may be attributed to their inability to support their weight due to poor structural composition (Lambers et al., 2008).

Both temperature and nutrient enrichment influenced N:P in above-ground tissue (Fig. 3), where only an effect of nutrient enrichment was to be expected, as it has been previously reported from short-term growth experiments (Güsewell, 2004). Thus, our findings first revealed an effect of temperature on N:P ratios in above-ground black mangrove tissues. Overall, the N:P values measured in this study were above 10, which is also the worldwide accepted value for N:P ratio in mangroves (Berrenstein et al., 2013). When looking only at the absolute N and P values, our leaf nutrient contents are generally higher compared to other plant parts, and their concentration increases under nutrient enrichment (Fig. A3). This nutrient allocation pattern may be due to the role of leaves in photosynthesis, protein synthesis, and chlorophyll

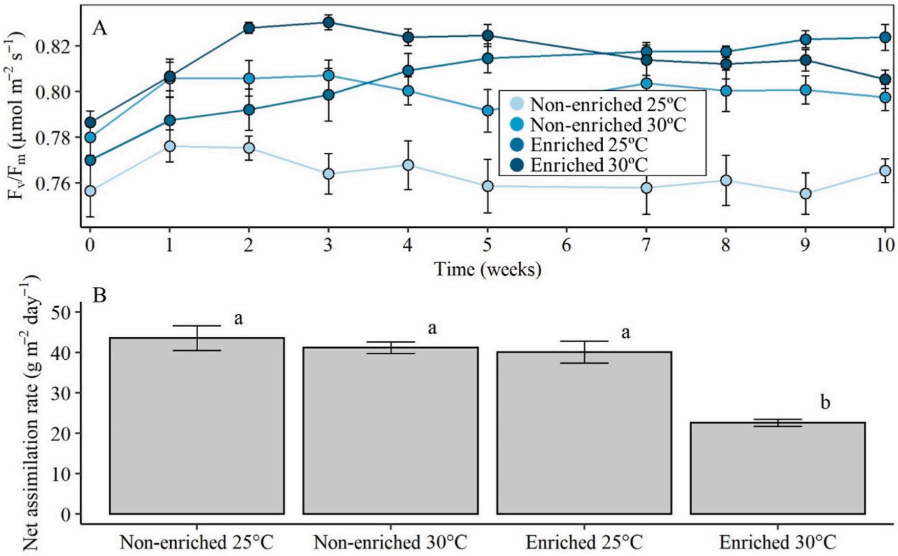

Fig. 4. (A) Photosynthetic efficiency (F_v/F_m) of seedlings over the 10-week experimental period. Marker shapes indicate mean values per treatment, and error bars represent standard error to the mean. Standard error bars are smaller than symbols in some cases. (B) Net Assimilation Rate (NAR). Columns represent mean values with standard error bars. The letters above the columns indicate significant differences between group means.

production, processes that require significant amounts of nutrients. However, the combination of high nutrients and temperature had a negative impact on N and P concentrations in both above and below-ground tissue, likely due to the effects of temperature on photosynthesis and nutrient allocation.

Our results also indicate that the iron content in mangrove leaves is consistent with those reported in previous studies for other mangrove species. For instance, Bakshi et al. (2017) found that the iron concentration in *Avicennia marina*, *Avicennia alba*, and *Avicennia officinalis* leaves ranged between 0.15 and 0.3 mg g^{-1} of dry weight, which aligns with our findings of 0.12–1.3 mg g^{-1}. Similarly, Machado et al. (2005) reported leaf concentrations of iron in *Laguncularia racemosa* and *Rhizophora mangle* up to 0.15 mg g^{-1}, on the lower end of the concentrations found in this study (Fig. A3). Much higher concentrations were found by Thanh-Nho et al. (2019) in mangroves exposed to inputs from a heavily industrialized mega-city. Thanh-Nho et al. reported that the iron content in saplings of *Avicennia alba* leaves was around 2.28 mg g^{-1}, a 6.5-fold higher than the average value in the present study. Furthermore, we observed a decreasing trend in foliar Fe with treatment, exactly the opposite as what occurs with foliar N and P (Fig. A3). One possible explanation is that nutrient enrichment reduced the availability and mobility of iron in sediment, e.g. by increasing pH, which may result in lower solubility and availability of iron. Another possible explanation is that nutrient enrichment, and P in particular, altered the element and nutrient balance in mangrove tissues, which affected the uptake, transport, and accumulation of iron in leaves. Unlike other mangrove species prone to excluding metals at the root level, black mangroves are iron-excreting species, capable of taking up more metals and translocating them to their leaves where they are excreted (Maldonado-Román et al., 2012). Thus, nutrient enrichment may have affected Fe uptake, which was reflected as lower Fe concentrations in leaves (Fig. A3).

The photosynthetic efficiency of the seedlings indicated a high plant performance throughout the experiment (Fig. 4A), i.e. above a maximum quantum yield of 0.71, and reaching up to ~0.83 (Murchie and Lawson, 2013). The enriched 30 °C treatment showed the highest photosynthetic efficiency over time, although this trend was reversed at six weeks, reflected by a slight decline of F_v/F_m. This decline is believed to be an artifact of the experimental conditions, as the supply rate of nutrients by the slow-release capsule was lower than the seedlings' nutrient demand. This theory was supported by the accumulation of nutrients in the negative control treatment (without a seedling), showing that the slow-release granules continued to release nutrients over the entire experimental period (Fig. A2 D). Still, these were automatically taken up by the seedlings (Fig. A2 H). These results indicate that, at this life stage, *A. germinans* seedlings under the given experimental conditions did not experience stress to the extent that it would impact their photosynthetic efficiency. Therefore, if *A. germinans* can maintain its photosynthetic efficiency under such conditions as indicated by the F_v/F_m value (>0.79), it may contribute significantly to the success of coastal restoration projects using mangrove seedlings.

While all the mangrove plants in our study originated from the same parent tree, it is important to acknowledge that this may impact the generalisability and applicability of our findings to other black mangrove varieties. However, this aspect does not affect the significance of our experimental results. We recommend further experimental work on the responses of black mangroves to the combined effects of high temperature and eutrophication, involving seedlings with diverse genetic backgrounds.

5. Implications for ecological restoration

The disproportionately long and bent stems resulting from nutrient enrichment may increase vulnerability of mangroves to waves, wind and hurricane disturbance and hinder their recovery (Feller et al., 2015) or successful establishment. A study by Balke et al. (2011) showed that young mangroves make use of "windows of opportunity" in space and time for root growth and anchorage, and the penetration of roots several centimetres into the sediment could still lead to their dislodgement. Therefore, successful anchorage to soil by efficiently developing roots and moderate growth, e.g. stature and stem elongation have been proven crucial for mangrove resilience to physical disturbance (Balke et al., 2011; Feller et al., 2015). Recent experimental studies on the optimal seedling morphology to withstand physical disturbance revealed that small, short shoots and long, sturdy roots are the key factors for *Avicennia marina* seedlings' stability (van Hespen et al., 2022). These findings are likely also be applicable to *A. germinans* since both species belong to the same genus. Thus, the fast-growing seedlings in our study, with long and unstable stems, may face challenges in withstanding hydrodynamic forces and experiencing establishment failure. Lastly, this phenomenon is not only applicable to mangrove ecosystems. It has been found that in aquatic plants exposed to eutrophication, their long, low-density stems are prone to breakage when exposed to mechanical stressors (Lamberti-Raverot and Puijalon, 2012).

Overall, our experimental results showed that mangrove seedlings exposed to high nutrients and temperature exhibited biomass allocation patterns that may increase plant vulnerability to dislodgement. Restoring mangrove populations in coastal areas under high eutrophication and climate warming may come at the cost of a higher vulnerability to physical disturbances. Our mesocosm experimental results cannot be directly extrapolated to natural mangrove communities, however, they indicate how black mangroves respond to two timely relevant pressures that might play a key role in restoration success, highlighting the potential risks of mangrove restoration under eutrophication and global warming.

6. Conclusions

The combination of high temperature and nutrient enrichment accelerated black mangroves' above-ground growth and improved their photosynthetic yield. However, reduced root growth and disproportionally long, bent stems, with lower net assimilation rates, may introduce instability and suggest greater vulnerability to physical disturbance, e.g. waves and wind, that may eventually lead to mangrove dislodgement. Our results also point towards a potential synergistic effect caused by high temperature and nutrient enrichment. These outcomes may serve as an example that the negative consequences of global-scale stressors such as global warming can be dampened by implementing local policies, such as preventing localized coastal eutrophication. Our findings also highlight the importance of considering the local nutrient status and hydrodynamic conditions when executing mangrove restoration projects. Further work should verify whether these effects occur under *in-situ* mangrove restoration conditions and whether our results apply to other mangrove species.

Credit author statement

Sara P. Cobacho: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Visualization, Writing – original draft. Sjoerd A. R. Janssen: Data curation, Formal analysis, Investigation, Methodology. Mabel A. C. P. Brekelmans: Methodology, Visualization. Ingrid A. van de Leemput: Supervision, Writing – review & editing. Milena Holmgren: Supervision, Writing – review & editing. Marjolijn J. A. Christianen: Conceptualization, Resources, Supervision, Writing – review & editing

Declaration of competing interest

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

Data availability

The data that support the findings of this study are available on the data repository 4TU.Researchdata, with the identifier https://doi.org/10.4121/9da99710-10b9-4221-a6d1-553f754a522f.

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Appendices.

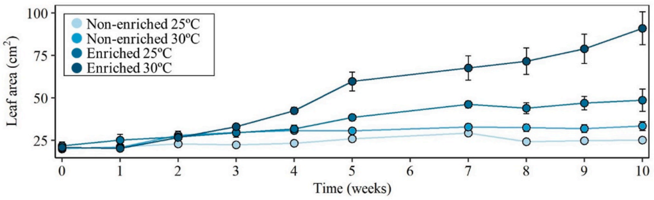

Fig A1. Leaf area of seedlings over the 10-week experimental period. Marker shapes indicate mean values per treatment, and error bars represent standard error to the mean.

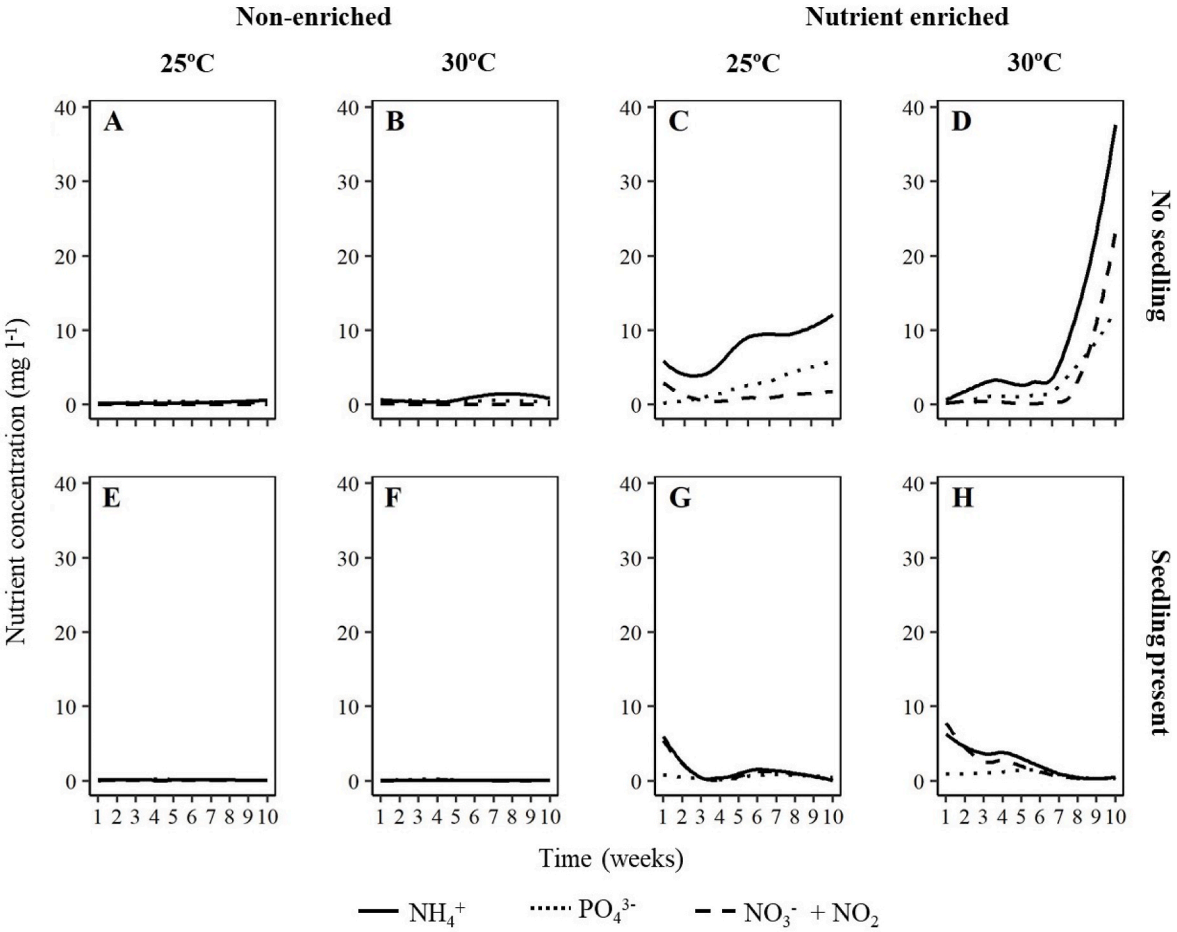

Fig A2. Porewater NH_4^+,PO_4^{3-}, and NO_3^- + NO_2^- concentrations (see legend) over time for the four different treatments indicated above the graphs. Top row shows nutrient concentrations for experimental units without a seedling (A to D), and units containing a seedling are shown in the bottom row (E to H). Nutrient concentrations in the porewater were not statistically analysed and were only displayed for visual assessment.

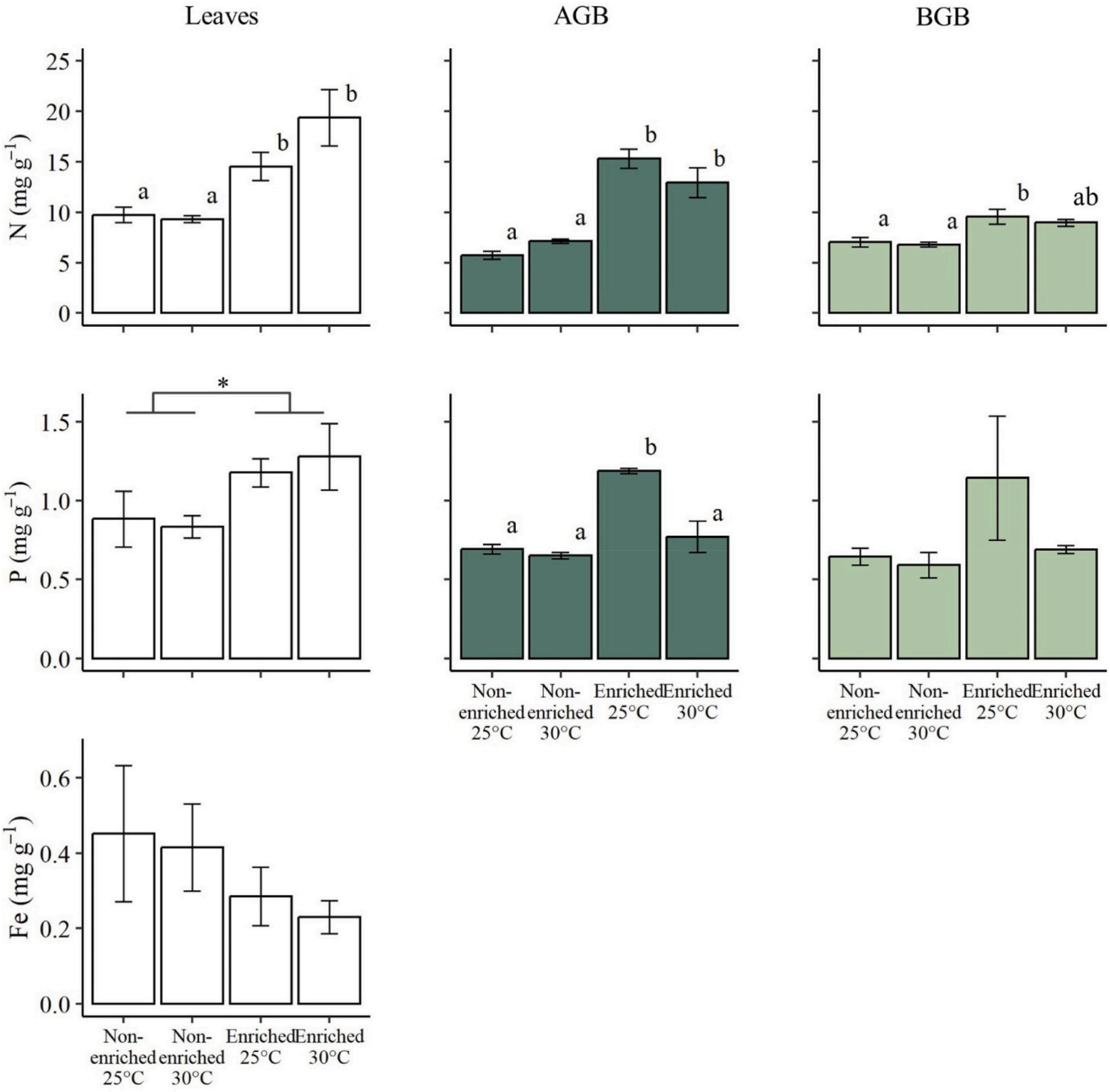

Fig A3. Total nitrogen (N), total phosphorus (P), and total iron (Fe) in seedling tissue from the different plant parts: leaves, above-ground biomass, and below-ground biomass. Columns represent mean values with standard error bars. The letters above the columns indicate significant differences between group means. When the overall F test comparing all four group means was significant but the more conservative Tukey test was not, asterisk signs were used to indicate significance values (*: $p < 0.05$, **: $p < 0.01$).

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