

How well do endemic wetland plant species perform in water purification?

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Abstract

Rising anthropogenic-induced nutrient enrichment of surface waters is of great concern globally as it jeopardizes the ecological integrity and functioning of freshwater ecosystems. Floating wetlands have been successfully used to treat nutrient enriched wastewater in developing nations, and provide additional co-benefits. We aimed to quantify the nutrient removal efficiency of high-potential, locally endemic wetland species on floating wetlands in different conditions and to understand whether the nutrient uptake process was characterised by key plant functional traits. Two experiments were run under Mediterranean-climate conditions of the Western Cape of South Africa: (1) a closed, oligotrophic mesocosm experiment representing local conditions and (2) a real-life (*in-situ*) eutrophic application. The mesocosm experiment conducted under oligotrophic local conditions yielded low nitrate, phosphate and ammonium removal rates ($34.8\text{--}35.2 \text{ mgNO}_3\text{-Nm}^{-2}\cdot\text{d}^{-1}$, $10.4\text{--}10.7 \text{ mgPO}_4\text{-Pm}^{-2}\cdot\text{d}^{-1}$ and $3.6\text{--}3.8 \text{ mgNH}_4\text{-Nm}^{-2}\cdot\text{d}^{-1}$) in comparison to other floating wetland studies globally, yet high removal efficiencies ($>90\%$). However the eutrophic *in-situ* experiment demonstrated the potential for these same locally endemic plants to remove up to $312 \text{ g}\cdot\text{m}^{-2}$ of nitrogen and $47 \text{ g}\cdot\text{m}^{-2}$ of phosphorus per year—which is relatively high compared to similar global research. *Cyperus textilis* had the highest daily nutrient uptake and content followed by *Prionium serratum* and *Juncus lomatophyllus*, while *J. lomatophyllus* had the greatest nutrient uptake efficiency. Two of the three species (*C. textilis* and *P. serratum*) stored significantly more total nutrients in their shoot tissue compared to their root tissue, suggesting that the permanent removal of nutrients from the system is possible through shoot harvesting. Floating wetlands planted with endemic plant species have the potential to remove nutrients effectively and sustainably from eutrophic water and can thus be implemented as low-cost nature-based solutions to mitigate pollution of lentic systems.

1 INTRODUCTION

Increasing eutrophication of surface waters is of great concern globally as it jeopardizes freshwater ecosystems. This trend has necessitated the development of technologies for water purification. Typically, conventional wastewater treatment facilities are still widely used to improve water quality internationally, although they are costly to maintain and upgrade (Keizer-Vlek et al., 2014; Kivaisi, 2001; Wang et al., 2015). Due to increasing demand for wastewater treatment, there is a need for more innovative, affordable and sustainable decentralised technologies to treat wastewater – particularly for developing nations (Kivaisi, 2001). Extensive research into design considerations and optimization has resulted in various types of constructed wetlands being developed for different wastewater types (Babatunde et al., 2008; Batchelor and Loots, 1997; Jacklin et al., 2021b; Wood and Pybus, 1992; Wu et al., 2015; S. Wu et al., 2014; Yeh et al., 2015). Floating wetlands, a type of constructed wetland, are manufactured buoyant structures designed to support emergent wetland plants. They are installed for the purpose of nutrient removal (Vymazal, 2007), with co-benefits such as aesthetic appeal, biodiversity enhancement (Biggs et al., 2006) and environmental education (Ahn, 2016). Floating wetlands also do not require highly qualified technicians to install and maintain, have no energy consumption, are able to

fluctuate with water levels and require low capital costs (Abed et al., 2017; Pavlineri et al., 2017; Kivaisi, 2001).

Studies have demonstrated that floating wetlands are capable of successfully removing nutrients and pollutants from various wastewater types (Headley and Tanner, 2008; Hubbard et al., 2004; Keizer-Vlek et al., 2014; Wang et al., 2015; White and Cousins, 2013). A study showed that floating wetlands planted with *Typha latifolia* removed 534 g.m⁻² Nitrogen (N), 79 g.m⁻² Phosphorus (P) and 563 g.m⁻² Potassium (K) over a 16 month period from swine-polluted lagoon wastewater in the USA (Hubbard et al., 2004). *Panicum hematomon* removed 323 gN.m⁻², 48 gP.m⁻² and 266 gK.m⁻² from the same. Urban wastewaters have been purified using floating wetlands planted with *Iris pseudacorus* and *Typha angustifolia* and which were found to remove 25 gN.m⁻² and 0.8 gP.m⁻², and 2.5 gN.m⁻² and 0.006 gP.m⁻² respectively over a three month period in the Netherlands (Keizer-Vlek et al., 2014). Another study focussed on heavy metals found that *Juncus effusus* floating wetlands accumulated 0.023 mg.g⁻¹ Nickel and 0.080 mg.g⁻¹ Zinc in the shoots, and 0.131 mgNi.g⁻¹ and 0.21 mgZn.g⁻¹ in the roots over a four month period in France (Ladislav et al., 2015). In the same study, *Carex riparia* accumulated 0.031 mgNi.g⁻¹ and 0.045 mgZn.g⁻¹ in the shoots, compared to 0.113 mgNi.g⁻¹ and 0.045 mgZn.g⁻¹ in the roots over the same time period (Ladislav et al., 2015). Floating wetlands have also been found to be successful in remediating organic contaminants such as pesticides, pharmaceuticals, personal care products and per- and polyfluoroalkyl substances (Awad et al. 2022). Therefore, floating wetland technology demonstrates successful, non-specific application across various wastewater and pollutant types.

Plant uptake is one pathway in which nutrients are removed in floating wetland systems. Plants facilitate the removal of nutrients through direct assimilation (absorption or adsorption), or indirectly by creating habitat for microbial communities on the root infrastructure – which assist in nutrient removal through processes such as denitrification (Lynch et al., 2015; Olguín et al., 2017). Various studies have highlighted the ability of plants to assist in phytoremediation of surface waters (Schachtschneider et al., 2017; Jacklin et al 2021a,b,c,d). The uptake capacity of plants varies considerably across species and is largely dependent on their anatomical and physiological properties, or their functional traits, which, in turn, allow different plants to have different tolerances to various nutrient thresholds (Moor et al., 2017; Pavlineri et al., 2017; Pérez-Harguindeguy et al., 2013; Wang et al., 2015; Wanielist et al., 2012). There is no universal trend as to where plants predominantly store their nutrients (i.e. disproportionately in their roots or shoots, or equally) (Wang et al., 2015; Wanielist et al., 2012). This potential for variation in uptake rates and storage location emphasises that plant selection is crucial in optimizing nutrient removal for specific use-cases and needs. If nutrient removal from the system via stem harvesting (e.g. mowing or cutting) is desired, then selecting plants with dominant storage in shoots is important.

Species commonly selected for use in constructed wetlands tend to be ubiquitous generalists, for example *Phragmites* spp. and *Typha* spp. (Wu et al., 2015). Despite plant selection being a key element in designing constructed wetlands, very few wetland plant species have been thoroughly researched for

this purpose globally (Vymazal, 2013). At the time of this research, very few wetland plant species had been investigated in the South African context, but more recently, a phyto-guide to species selection has been developed (Jacklin et al. 2021a). Nevertheless, a large research gap remains to assess the potential of various indigenous plant species for nutrient removal (Wang and Sample, 2014). South Africa has no shortage of potentially useful wetland species, particularly in the Cape Region where there is high floral diversity (Hoveka et al., 2020). Moreover, in South Africa's Cape region where this study took place, surface waters are typically oligotrophic (Ward & Winter, 2016) and thus native species are adapted to this typically low nutrient environment. However, large-scale landscape transformation in the Cape has resulted in significant nutrient enrichment of water bodies (Ward & Winter, 2016). Against this backdrop, the primary focus of this research was to investigate the nutrient removal capacities of local wetland plant species under near natural and nutrient enriched scenarios.

In this study, we aimed to quantify the nutrient removal efficiency of high-potential, locally endemic wetland species on floating wetlands in a low nutrient mesocosm and high nutrient *in-situ* experiment. We investigated: (i) the nitrogen and phosphorus removal capacity of floating wetlands planted with three locally endemic species: *Cyperus textilis*, *Juncus lomatophyllus* and *Prionium serratum*, (ii) the contribution of plant nutrient uptake to the overall removal capacity of these floating wetlands, (iii) the dominant location of plant nutrient storage (roots or shoots), and (iv) the influence of plant functional traits in the process of nutrient uptake. We then reflect on the potential for practical implementation given the results of the experiments.

2 METHODS

2.1 Study species

Plant selection has been identified as a crucial design consideration in constructed wetlands (Brisson and Chazarenc, 2009). Therefore, the criteria used to select species for experimentation is very important. Species selection for this study was based on the following criteria (adapted from Tanner 1996):

1. "Ecological acceptability" i.e. no significant weed or disease risks or danger to the ecological or genetic integrity of surrounding natural ecosystems.
2. Tolerance of local climatic conditions, pests and diseases.
3. Tolerance of pollutants and hypertrophic waterlogged conditions.
4. Ready propagation, and rapid establishment, spread and growth.
5. Ability to co-exist with other wetland plants; not too large (i.e. should not sink the frame structure).
6. Interesting/promising indigenous species that have not yet been studied in a wetland context.

A short-list of eleven potential wetland species was compiled based on local industry experience (Table S1, Supplementary Material). From these, three high potential, locally endemic wetland species were selected for the experiment based on an evaluation using the six criteria outlined above (Table S2,

Supplementary Material): *Cyperus textilis*, *Juncus lomatophyllus*, and *Prionium serratum*. These three species were used in both sets of experiments and the young plants were obtained from a local nursery.

2.2 Mesocosm experiment: oligotrophic conditions

2.2.1 Experimental set-up

This mesocosm experiment was designed as a closed mass balance experiment with the aim to isolate the effects of floating wetlands on water quality. The experiment was conducted over one-month in a greenhouse (240 m²) at Stellenbosch University from 27 January to 27 February 2017 i.e. during the summer season (Table S3, Supplementary Material). The experiment was performed in twenty-four 90 L plastic tanks (0.45 m x 0.75 m). The tanks were positioned in a randomised block design with six replicates each (Fig. 1). Each tank contained a standard floating wetland planted with ten plants of the same species (along with a small amount of their associated soil), except for the control which was without vegetation and soil (Keizer-Vlek et al., 2014). A standard floating wetland was constructed of high-density foam, mesh, hessian and a soil saver layer and was fastened together with cable ties. A set of four small garden fountain pumps were rotated daily between tanks, after being cleaned, to circulate the water. This was to avoid anoxia while keeping costs down.

The seedlings were acquired four months prior, in September 2016, and the floating wetlands were constructed, and tanks filled with 70 L of municipal water (containing insignificant nutrient concentrations). The floating wetlands established over these four months whilst plant health, growth and survival were monitored closely using the framework of Brisson & Chazarenc (2009). Additions of Pokon (fertilizer for pot plants, Universeel plantenvoedsel, manufacturer: Pokon Naturado) were made during this period to ensure sufficient nutrients for growth as well as the presence of trace elements (B, Cu, Fe, Mn, Mo, Zn, and K).

2.2.2 Nutrient additions

On 27 January 2017, the tanks were emptied and refilled with new municipal water. Three water samples were taken from the municipal water supply and tested, prior to any fertiliser additions, to establish baseline NH₄-N, NO₃-N and PO₄-P concentrations. After this, 31.5 mL of NO₃-N, 8.4 mL of NH₄-N and 7.0 mL of PO₄-P were added in 1000 mg.L⁻¹ concentrate forms to establish oligotrophic conditions relative to local ecosystems (Table S4 and Table S5, Supplementary Material). Three days later, a 100 mL water sample was taken for nutrient analysis from each tank to confirm the calculated concentrations of bioavailable nutrients (NH₄-N, NO₃-N, and PO₄-P).

During the experiment, concentrations of bioavailable nutrients were tested each week in two random tanks selected from each different plant species (Keizer-Vlek et al., 2014) as well as the control to determine whether more nutrients needed to be added in order to remain within the predetermined range. Only two tanks were sampled due to budget constraints; ideally each tank would have been tested. The water in the tanks was topped up weekly to 70 L using municipal water and the amount of

water added was recorded for each tank. Two composite samples of the municipal water were tested for bioavailable nutrients each week. If the nutrient concentrations fell below minimum concentrations ($0.16 \text{ mgNH}_4\text{-N.L}^{-1}$, $0.45 \text{ mgNO}_3\text{-N.L}^{-1}$, $0.10 \text{ mgPO}_4\text{-P.L}^{-1}$), more nutrients were added to all the tanks and these amounts were recorded. All water quality tests were performed by AL Abbott & Associates Ltd, which are accredited to the ISO 17025:2005 standard (registration number 1982/004379/07).

In addition, each week, various physico-chemical variables (dissolved oxygen, conductivity, temperature and pH) were recorded at a depth of 0.30 m using a handheld multi-parameter water quality meter in each tank (Model: YSI 556 Multi Probe System; YSI Environmental). Overall, water quality did not differ significantly among treatments except for pH, which was significantly lower in the *J. lomatophyllus* treatments, and significantly higher in the *C. textilis* treatments (Table S6, Supplementary Material).

2.2.3 Plant sampling and analysis

At the start and end of the experiment, randomly chosen plant specimens were removed from each of the 18 planted floating wetlands for biomass measurements (wet and dry root and shoot biomass) and tissue nutrient analysis (for TN and TP concentrations). One plant per floating wetland (total of 18 plants) was removed at the start of the experiment, whilst two plants (total of 36 plants) were removed at the end of the experiment to increase the representativeness due to differential growth over the experiment. Before specimens were dried, several key plant functional traits were measured (10 measurements per plant), including plant height, root and shoot lengths, root surface area, leaf surface area, leaf and root mass. The surface areas were calculated using the method of Verschoren et al., (2017). Indices, such as specific root length, specific leaf area, root dry matter content and leaf dry matter content, were also calculated. Specimens were then dried to a constant mass at 70°C for a minimum of 48 hours. Root and shoot dry mass were determined for each specimen after which they were ground to 0.5 mm particle size using a *Retsch Mill*. Total nitrogen and phosphorus concentrations were measured in the roots and shoots of each specimen. All the plant tissue analyses were performed by *BEMLAB Ltd*, which are accredited to the ISO 17025:2005 standard (registration number 1996/006836/07). Total nitrogen content was determined using the Dumas Method and total phosphorus content was determined using a dry ash method whereby the sample was combusted in a muffle furnace at 480°C for 8 hours, acidified, digested by heating, diluted, and analysed by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) (Spangler et al., 2019a). Any floating algae were removed daily using a fish net, where necessary, from the tanks to establish a standard for nutrient removal with minimal interference of algae and therefore minimizing bias (e.g. between experiment and control).

2.2.4 Nutrient removal ability

The nutrient removal ability of floating wetlands was calculated in three ways. Firstly, **total removal** of bioavailable nutrients ($\text{NO}_3\text{-N}$, $\text{NH}_4\text{-N}$ and $\text{PO}_4\text{-P}$) in the tanks was measured throughout the experiment through mass balance calculations following the water testing. Total removal was calculated by subtracting the bioavailable nutrients remaining in the tanks ($\text{NO}_3\text{-N}$, $\text{NH}_4\text{-N}$ and $\text{PO}_4\text{-P}$ concentrations

multiplied by the water volume) from the total nutrients added to the system throughout the experiment. Secondly, the **removal rate** was calculated, which used the total experiment duration to calculate a daily removal rate. Thirdly, **removal efficiency** was calculated, which is the total removal of bioavailable nutrients over the duration of the experiment relative to the total available nutrients in the system, expressed as a percentage. Data are made available in Table S7, Supplementary Material.

2.3 In-situ experiment: eutrophic conditions

2.3.1 Experimental set-up

This experiment was designed to mimic the eutrophic conditions of many small farm dams in the region. This experiment was carried out in a large concrete wastewater retention pond at the Water Hub, located in Franschhoek in the Western Cape of South Africa; a remnant of the old Franschhoek Wastewater Treatment Works. The pond has a volume of 63.6 m³ and measures 4.8 m (width) X 5 m (length) X 2.65 m (height) (Fig. 2).

This experiment took place over three months from 12 March 2021 to 12 June 2021, in the austral autumn. Highly eutrophic water from the Stibeuel River, which flows past the Langrug informal settlement near Franschhoek before passing the Water Hub, was pumped into an old concrete wastewater retention pond in which the floating wetlands were established. The tank was filled to approximately 50% capacity. Eighteen floating wetlands of 0.5 m x 0.4 m (i.e. 0.2 m²) in size were constructed (Fig. 2) using high-density foam, mesh, hessian, wooden dial sticks, and cable ties. Six replicates (wetlands) were used for each of the three treatments (plant species) and each floating wetland was planted with ten seedlings of the relevant species. The seedlings were carefully removed from their potting soil and as much soil as possible was detached without causing damage to the root system. Given that this was not a mesocosm experiment with individual wetlands in individual water bodies to investigate mass balance impacts on water quality, no control was necessary.

Young seedlings were sourced from a local restoration nursery, Green Intaba, prior to the start of the experiment and were planted onto the constructed floating wetlands on 4 January 2021. The plants were allowed to establish over the following two months, during which plant health, growth, and survival were periodically monitored. Several *J. lomatophyllus* plants did not survive (plant health of these few seedlings was poor prior to being planted onto wetland structures, likely due to water stress during transport) and were replaced during the establishment phase. Due to the clonal growth of this species, plastic separators were placed between plants where necessary to distinguish the original individuals. The experiment commenced once the plants had successfully established. As there was a concrete bridge that cast a partial shadow over the retention pond, the position of each wetland (sun, shade, or mixed) was recorded during site visits (every two weeks during the establishment phase and weekly during the experiment) and the wetlands were moved to new locations on a rotational basis to ensure a randomised experimental design and prevent light exposure from becoming a bias.

2.3.2 Plant sampling and analysis

The experiment commenced on 12 March 2021 and ran for a period of three months. At 28-day intervals during the experiment, three randomly selected plants were sampled (removed) from each floating wetland. Plant roots and shoots were separated for independent analysis, and the same method applied for grinding and drying as described for the mesocosm experiment was applied. The ground samples were thoroughly mixed, and a representative composite sample of root and shoot tissue of the three plants sampled from each wetland was sent to BEMLAB for tissue nutrient analysis of total nitrogen and total phosphorous. The same laboratory methods were used as described for the mesocosm experiment. The use of composite sampling was essential due to budget constraints; however, ANOVA power analyses indicated that the sample size was sufficient to detect significant differences if they are present (Table S8 and Table S9, Supplementary Material). The power analyses were based on the data from the mesocosm experiment, however, as the *in-situ* experiment was conducted under more eutrophic conditions, greater effect sizes were expected. Plant functional traits such as leaf dry matter content (LDMC), and root dry matter content (RDMC) were determined by dividing dry mass (mg) by wet mass (g) of leaves and roots respectively.

Water quality samples were taken at the start of the experiment period as well as at monthly intervals during the experiment and sent to the laboratory at the South African Council for Scientific and Industrial Research (CSIR) for analysis of bioavailable nutrients (phosphates ($\text{PO}_4\text{-P}$), nitrates ($\text{NO}_3\text{-N}$), and ammonia + ammonium ($\text{NH}_3 + \text{NH}_4$). The CSIR are accredited to the ISO 17025:2017 standard (registration number T0093). This method cannot differentiate between the ammonium and ammonia. In addition to these samples, physicochemical variables (temperature, pH, total dissolved solids, and dissolved oxygen) were monitored weekly during the experiment using a handheld multi-parameter water quality meter to ensure nutrient levels did not reach toxic levels (Table S10, Supplementary Material).

2.3.3 Plant nutrient content and uptake capacity

Plant uptake capacity, defined here as total nitrogen and total phosphorus uptake in plant tissues, was measured for both roots and shoots, by plant tissue analysis. Uptake was calculated by multiplying plant dry weight at the start and end of the experiment by the respective TN and TP concentrations, and subtracting the two (Keizer-Vlek et al., 2014). Plant nutrient concentration was used as an indication of **nutrient storage efficiency** (a measure of how well plants can store nutrients in their tissue). The ratio of the **shoot to root nutrient content** was used to test for differences in nutrient storage location among species.

Nutrient uptake rates were estimated by calculating the mean nutrient uptake over 28-day periods and standardising these values to a daily rate (Table S11, Supplementary Material). To enable comparison with literature values, nutrient uptake values ($\text{mg}\cdot\text{d}^{-1}$) per floating wetland (0.2 m^2) were standardised to values per square meter. The floating structures used in this experiment were 0.2 m^2 in size and were planted with 10 plants each, thus 5 wetlands and 50 plants would be needed to cover 1 m^2 of surface water. To estimate the uptake of each species in terms of $\text{mg}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$, the mean daily uptake rate was multiplied by 50. To get an estimate of the nutrient uptake ability of these systems over a longer period,

the daily values ($\text{mg.m}^{-2}.\text{d}^{-1}$) were also converted to an annual estimate ($\text{g.m}^{-2}.\text{y}^{-1}$); this estimate does however not take the plant growth cycle into account. This experiment was in the austral autumn and therefore during the spring and summer it is possible that uptake rates may be higher, and in winter slightly lower. It is important to emphasize that these plants are evergreen and that there are still high levels of solar radiation in the autumn in South Africa. Despite this, it is likely that the results from this experiment provide a slightly lower estimate than the plants full potential. It is important to note that three plants were removed from each wetland monthly, thus the remaining plants had more space to grow and less competition. Consequently, the uptake rates ($\text{mg.m}^{-2}.\text{d}^{-1}$) in this experiment represent values that would be obtained at relatively high planting densities (50 plants per m^2) accompanied by regular biomass harvesting.

2.4 Data analysis

For the mesocosm and *in-situ* experiment, most statistical analyses were performed in Statistica and R version 3.6.2 (R Core Team, 2019).

For the mesocosm experiment, **one-way ANOVAs** were performed to investigate significant difference in total removal, removal rates and removal efficiencies of $\text{NO}_3\text{-N}$, $\text{NH}_4\text{-N}$ and $\text{PO}_4\text{-P}$ between all the floating wetland treatments because the data were found to be normally distributed and variances were homoscedastic. For significant differences, the **Fisher least significant difference (LSD) post hoc** test was used. To investigate significant differences in traits LDMC, RDMC, SLA and SRL, a **one-way ANOVA** was run. For SRL and RDMC data, a **Welch** test was run because the variances were heteroscedastic and the data were normally distributed. For significant differences, a **Games-Howel** post hoc was run. For the SLA and LDMC, the data were normally distributed, and the variances were homoscedastic. For significant differences, a **LSD post hoc** test was run.

For the *in-situ* experiment, differences in dry mass, nutrient content, and nutrient storage efficiency, **linear mixed models** were fitted using the 'lmer' function within the 'lme4' package (Bates et al., 2015). The "wetland" and "plant" sampled were entered as random effects to account for dependence between observations from the same wetland. "Species", "sampling day", and the interaction between species and sampling day were entered as fixed effects. First, the effect of the interaction between species and sampling day was tested by comparing the fit of the model to a reduced model that only contained the two main effects. Where the interaction term was significant, separate models were run to test the significance of species on each sampling day individually. If a significant difference was not detected, the interaction term was excluded from the model and the significance of the main effects were tested. The significance was tested by running a **Kenward-Roger F-test** using the "pbkrtest" package in R (Halekoh and Højsgaard, 2014). Where a significant influence of species or sampling day was detected, a **post hoc** test was performed on the relevant model using the 'emmeans' function from the 'emmeans' package in R (Lenth, 2021). **Correlation analyses** were performed to determine if the physicochemical variables were correlated with the water quality data as well as to determine whether there was an association between plant growth (dry matter at the three timesteps) and nutrient content. Pearson's

correlation analysis was used for parametric data while Spearman's Rank correlation analysis was used for non-parametric data. To investigate significant differences between shoot:root ratios across both experiments, a **generalised linear mixed model** (with binomial distribution) was used. A multiple comparison **Tukey test** was used to test for significant differences.

3 RESULTS

3.1 Nutrient uptake ability under oligotrophic conditions

The total removal (mg) of $\text{NO}_3\text{-N}$, $\text{PO}_4\text{-P}$ and $\text{NH}_4\text{-N}$ from the water was very low across planted and unplanted floating wetland treatments. The total $\text{NO}_3\text{-N}$ and $\text{NH}_4\text{-N}$ removal of floating wetlands did not differ significantly among treatments (Fig. 3A). However, the floating wetlands planted with *Cyperus textilis*, *Juncus lomatophyllus* and *Prionium serratum* had significantly higher total $\text{PO}_4\text{-P}$ removal relative to the control ($F_{(3,20)} = 6.52$; $\text{df} = 20$; $p < 0.01$) (Fig. 3A).

Low removal rates ($\text{mg.m}^2.\text{d}^{-1}$) from the water were observed for all parameters and treatments (Fig. 3B). No significant difference was observed between treatments for $\text{NO}_3\text{-N}$ and $\text{NH}_4\text{-N}$ removal rates, however there was a significant difference in the $\text{PO}_4\text{-P}$ removal rate between floating wetlands planted with *C. textilis*, *J. lomatophyllus* and *P. serratum* relative to the control ($F_{(3,20)} = 6.52$; $\text{df} = 20$; $p < 0.01$) (Fig. 3B).

When comparing nutrient removal efficiencies, high removal efficiencies ($> 90\%$) were observed across all treatments (Fig. 3C). This was also occasionally the case for the control; however, results were more variable. There was a significant difference in $\text{PO}_4\text{-P}$ removal efficiency between floating wetlands planted with *C. textilis*, *J. lomatophyllus* and *P. serratum* relative to the control ($F_{(3,20)} = 6.42$; $\text{df} = 20$; $p < 0.01$) (Fig. 3C).

3.2 Nutrient uptake potential under eutrophic conditions

Cyperus textilis accumulated a mean total of 477.9 ± 254.45 mg TN and 72.3 ± 40.36 mg TP over a one-month period under eutrophic conditions – which is significantly higher than *P. serratum* and *J. lomatophyllus* which accumulated a mean total of 175.3 ± 43.60 mg TN and 14.5 ± 6.42 mg TP, and 30.9 ± 80.99 mg TN and 6.7 ± 14.27 mg TP respectively over the same period (Fig. 4). Two species, namely *C. textilis* and *P. serratum*, stored significantly more nutrients (both nitrogen and phosphorus) in their shoot tissue compared to their root tissue (Nitrogen: $F_{(4,30)} = 19.44$; $\text{df} = 30$; $p < 0.01$) (Phosphorus: $F_{(4,30)} = 17.65$; $\text{df} = 30$; $p < 0.01$) (Fig. 4). Specifically, *C. textilis* stored significantly more TN and TP in its shoots than both *J. lomatophyllus* and *P. serratum*. *Cyperus textilis* also had the highest nutrient uptake and content per day followed by *P. serratum* and *J. lomatophyllus* (Fig S 1, Fig S 2, Fig S 3, Supplementary Material), while *J. lomatophyllus* had the greatest nutrient uptake efficiency (Fig S 4, Supplementary Material).

3.3 What is the role of plant functional traits in the process of nutrient uptake?

3.3.1 Dominant location of plant nutrient storage

The nitrogen shoot:root ratio for all treatments was significantly higher in the *in-situ* experiment compared to the mesocosm experiment ($F_{(5,36.3)} = 83.35; p < 0.01$). However, this differed to the phosphorus shoot:root ratios for *C. textilis* and *P. serratum* which were significantly higher in the *in-situ* experiment (Fig. 5). For both Total Nitrogen and Total Phosphorus shoot:root ratios, *C. textilis* was significantly higher than the other treatments within the *in-situ* experiment (Fig. 5).

Interestingly, these trends did not concur with the concentrations of nutrient storage within the roots and shoots of the study species under different nutrient environments. *Juncus lomatophyllus* stored a significantly higher concentration of nitrogen and phosphorus when compared to the other two plant species (Fig. 6). Generally, *J. lomatophyllus* had significantly higher concentrations of both Nitrogen and Phosphorus stored within the shoots in comparison to the roots – except for the *in-situ* experiment, where the roots stored significantly more Phosphorus than the shoots (Fig. 6). Also, most plants species, regardless of the experiment type, stored a significantly higher concentration of nutrients in the shoots than their roots – particularly *J. lomatophyllus* and *C. textilis* (Fig. 6).

3.3.2 Plant functional traits

When comparing traits of the three endemic wetland plant species, the LDMC of *C. textilis* was significantly higher across all species, while *J. lomatophyllus* was significantly lower ($F_{(2,33)} = 166.88; df = 33; p < 0.01$) (Fig. 7). The RDMC was significantly higher for *P. serratum* and significantly lower for *J. lomatophyllus* ($F_{(2,33)} = 143.51; df = 33; p < 0.01$) (Fig. 7). The mean SRL and mean SLA of *J. lomatophyllus* was significantly higher than that of *P. serratum* and *C. textilis* (Fig. 7). The traits measured in the *in situ* experiment were very similar and are not presented here (Table S15, Supplementary Material).

3.3.3 Relationship between plant growth and nutrient content

Relatively similar significantly positive relationships between nutrient content (both nitrogen and phosphorus) and plant growth (i.e. an increase in dry matter) were observed for all three species for both roots and shoots in the *in-situ* experiment (Fig. 8). However, *J. lomatophyllus* appeared to store slightly higher concentrations of TN and TP per unit dry matter in both shoots and roots, but especially roots, relative to the other two species. However, *J. lomatophyllus* remains relatively small over the three months relative to the other two species, leading to lower overall TN and TP contents. The mean ratio of

plant dry matter (g) to nitrogen content (g) for all three species was 55:1, while that of phosphorus was 421:1 (Fig. 8).

4 DISCUSSION

4.1 Nutrient uptake ability

In the mesocosm experiment, the total removal amount and removal rates of bioavailable nutrients across treatments were very low overall due to the naturally oligotrophic conditions applied. Oligotrophic Cape freshwater systems are nutrient poor primarily due to the nutrient deficient parent geology (Lamont 1983). Other floating wetland studies are found to have high removal rates – ranging between removal rates as high as Headley and Tanner (2012) and as low as Saeed et al., (2016) i.e. from 210 to 114 000 $\text{mgNO}_3\text{-N.m}^{-2}.\text{d}^{-1}$, 559 to 4600 $\text{mgPO}_4\text{-P.m}^{-2}.\text{d}^{-1}$ and, 1480 to 36000 $\text{mgNH}_4\text{-N.m}^{-2}.\text{d}^{-1}$ (Headley and Tanner, 2012; Saeed et al., 2016; Stewart et al., 2008). This range of removal rates is still high in comparison to the low removal rates found in this mesocosm experiment.

The high removal efficiencies (> 90%) observed for all nutrient parameters ($\text{NO}_3\text{-N}$, $\text{PO}_4\text{-P}$ and $\text{NH}_4\text{-N}$) across planted floating wetland treatments in the mesocosm experiment is likely due to the adaptation of these species to naturally oligotrophic conditions. These efficiencies were not significantly different for the control (except for $\text{PO}_4\text{-P}$), but the controls exhibited much higher variability and greater ranges for $\text{NH}_4\text{-N}$. These removal efficiencies are very high in comparison to other studies, such as Van De Moortel et al. (2010) who observed that the presence of plants (i.e. *Carex* sp.) on floating wetlands resulted in a significantly higher $\text{NH}_4\text{-N}$ removal efficiency (35%) than the control (3%), which consisted of an unplanted mat. Keizer-Vlek et al. (2014) also showed this trend where an *Iris*-planted floating wetland had a much higher TN removal efficiency of 98%, in comparison to the unplanted control of only 14%. Nevertheless, Keizer-Vlek et al. (2014) and Van De Moortel et al. (2010) emphasised the large variability of removal efficiencies between studies. The low nutrient removal amounts, but high removal efficiencies observed in our experiment suggests that most nutrients added to the treatments were removed by the floating wetlands.

The similar removal efficiencies and amounts observed between floating wetlands treatments and the control suggest that microbes associated with the surface area provided by the wetland infrastructure may be performing nutrient uptake in the absence of plants, or may play an important role even in the presence of plants (Stewart et al., 2008; Wu et al., 2016). However, where plants were present on floating wetlands, it is likely that the nutrients were accumulated within the plant matter as evidenced by an increase in plant biomass. Whilst additional nutrient removal pathways (i.e. other than the role of plants) are not the focus of this paper, various other pathways could explain the trends observed in the mesocosm experiment (Stewart et al., 2008; Wu et al., 2016). This experiment suggested that these floating wetlands and endemic plants had high potential that required testing in a real-life eutrophic application.

4.2 Nutrient uptake potential

Testing the potential for these endemic plant species for water purification in a eutrophic *in-situ* experiment revealed high nutrient uptake capacities across all species. However, *Cyperus textilis* outperformed both *Juncus lomatophyllus* and *Prionium serratum* in terms of nutrient uptake, storing most of this in its shoots, largely due to its greater size and faster growth rates. Therefore, considering water quality alone (i.e. not other factors, such as biodiversity), *C. textilis* had the highest benefit out of the three study species. All species grew well and appeared to tolerate and thrive in the eutrophic conditions despite being adapted to oligotrophic conditions. Clearly no thresholds were exceeded, but a follow-up study on the tolerance levels and ranges of optimal performance of these endemic species to eutrophic conditions would be useful.

A review of eight studies, conducted mainly in humid subtropical and sub-humid continental climates, found that nitrogen uptake abilities for plant species, including common species such as *Phragmites australis*, *Juncus effusus* and *Typha* species, varied between 13.2 and 390 mgN.m⁻².d⁻¹ (Choudhury et al., 2019). A review of another eight studies, conducted mainly in China, found that the water purification abilities of species such as *Canna indica*, *P. australis*, *J. effusus* and *Typha* species varied between 17 and 912 mgN.m⁻².d⁻¹ with mean uptake rates of 430 ± 350 mgN.m⁻².d⁻¹ (Wang et al., 2020). Compared to these studies, both *C. textilis*, with nitrogen uptake of 853 mgN.m⁻².d⁻¹, and *P. serratum* with nitrogen uptake of 313 mgN.m⁻².d⁻¹ performed comparatively well.

Temperature plays an important role in plant growth and thus nutrient uptake. A study looking at the applicability of *P. australis* and *Zizania caduciflora* floating wetlands to treat agricultural runoff in China found that nitrogen uptake ranged between 206.5 and 628.5 mgN.m⁻².d⁻¹ with the highest uptake rates occurring during the summer months (Lu et al., 2009). The water temperatures of the current study were similar to those observed during winter, autumn and spring in Lu et al., (2009), yet the nitrogen uptake rate for *C. textilis* outperformed the maximum uptake rates found in this study, perhaps indicating that at higher water temperatures, such as in summer, maximum potential uptake may occur. Under the low temperatures found in northern Sweden (mean summer temperature of 10°C), floating wetlands had a nitrogen uptake rate of between 7.56–24.75 mgN.m⁻².d⁻¹ (Choudhury et al., 2019). Thus, all three plant species outperformed species grown in colder climates, highlighting the potential value of floating treatment wetlands particularly in warmer regions with longer growing seasons such as South Africa.

The phosphorus uptake rates of *J. lomatophyllus* and *P. serratum* in our study were also higher than several other species studied (Wang et al., 2020). A study conducted in south Australia on the use of *Paspalum paspalodes*, *Myriophyllum aquaticum*, and *Ranunculus repens* planted floating mats found that these three species had mean phosphorus uptake rates ranging between 43 and 86 mgP.m⁻².d⁻¹ (Wen and Recknagel, 2002), and in our study *C. textilis* performed comparatively well with 129 mgP.m⁻².d⁻¹ unlike *J. lomatophyllus* and *P. serratum*. Likewise *C. textilis* performed well relative to results of a review of eight studies, conducted mainly in China, which found that phosphorus uptake rates for

numerous plant species, including *Canna indica*, *P. australis*, *J. effusus* and *Typha* species, varied between 2 and 163 mgP.m⁻².d⁻¹ (Wang et al., 2020). A study conducted after ours, investigating the use of nine South African endemic plant species for use in biofilters, also confirmed that *C. textilis* and *P. serratum* consistently showed some of the best results in terms of nutrient removal relative to the other seven species (Jacklin et al., 2021b).

The nitrogen removal ability of *C. textilis* was similar to that of *C. indica* measured at varying ratios of ammonium and nitrate concentrations in China while *P. serratum* had slightly lower nutrient removal abilities (Zhang et al., 2018). *Canna indica*, *Thalia dealbata*, and *Lythrum salicaria* were found to have accumulated a mean of 1523 mg, 1619 mg, and 908 mg of nitrogen per plant and 144 mg, 102 mg, and 71 mg of phosphorus per plant respectively over 84 days in an experiment in China (Ge et al., 2016). Over the same time in our *in situ* experiment, *C. textilis* accumulated a mean of 1855 mg of nitrogen and 262 mg of phosphorus per plant, thus performing better than all three species. The review of eight studies, conducted mostly in China, found that the nutrient content measured for several species ranged between 32 and 5803 mg of nitrogen per plant, and between 2 and 437 mg of phosphorus per plant (Wang et al., 2020). *Cyperus textilis* and *P. serratum* performed better in the *in-situ* experiment than the majority of the species in the review (Wang et al., 2020). In contrast, the nutrient storage of *P. cordata* and *J. effusus* plants surpassed the nutrient storage measured in our study, likely due to the greater plant biomass production (Spangler et al., 2019b). Nitrogen and phosphorus removal of floating wetlands planted with *Carex stricta*, *Agrostis alba*, *Iris ensata*, *Panicum virgatum*, and *Canna generalis* together with a generalist species after eight weeks were all greater than the nutrient removal measured in our study, however, the nutrient concentrations used were higher than that in the current study (Spangler et al., 2019a).

4.3 Role of key plant functional traits in nutrient removal

Cyperus textilis performed best in terms of nutrient uptake under eutrophic conditions, followed by *Prionium serratum* and then *Juncus lomatophyllus*. The traits that seemed to characterise this high nutrient uptake were biomass, leaf dry matter content (LDMC) and specific leaf areas (SLA). Both biomass and LDMC were higher for the two species that performed better at nutrient uptake, whereas SLA was lower. This may indicate that wetland species with high LDMC, low SLA and that are able to grow relatively large, may be highly suitable for floating treatment wetlands.

Juncus lomatophyllus had the highest nutrient uptake efficiency, i.e. highest N and P concentrations in its tissues, which was concomitant with higher SLA. Although higher SLA in turn suggests a higher relative growth rate and a more acquisitive strategy in terms of the plant economic spectrum (Garnier et al., 2001; Grassein et al., 2015; Moor et al., 2017; Pérez-Harguindeguy et al., 2013), *J. lomatophyllus* is a small wetland plant, which does not attain high biomass, but rather spreads laterally via vegetative growth. Therefore its potential for nutrient uptake is lower in eutrophic conditions compared to the other two species. The other two species were more conservative in terms of their resource use, but able to attain greater size and biomass and therefore take up more nutrients overall. The life history strategies of *C. textilis* and *P. serratum* are likely to be more that of competitors (Rebelo et al. 2022), whereas *J.*

lomatophyllus appears to me more of a ruderal or stress tolerant wetland species (Grime, 1977). Species that are more competitive and amass more biomass would need to be frequently harvested to keep biomass down and avoid self-shading and senescence on floating treatment wetlands.

4.4 Caveats

There were several limitations to this study due to budget or logistical constraints, since this research was conducted in a resource constrained country: (1) less water quality sampling of bio-available nutrients than would be desired in the mesocosm experiment, (2) the taking of composite samples in both experiments, and (3) relatively small sample sizes in both experiments. The relatively small sample sizes meant that if in some cases larger plants were randomly sampled at the start of the experiment (e.g. in the mesocosm experiment), and smaller plants at the end, and *vice versa*, the attempts to quantify nutrient uptake would be biased. The only way to overcome this, since sampling is destructive, would be to have a much larger sample size, which would be much more costly. Lastly, the role of plants in nutrient uptake relative to microbes is debated (Brix, 1993; Pavlineri et al., 2017; Shutes, 2001). In the mesocosm experiment, overall nutrient uptake was similar to the control, which may suggest that microbial activity plays an important role, or alternatively that it can compensate for the absence of plants when a substrate with large surface area (e.g. floating wetland frame) is provided. No conclusions can be made without directly studying the contribution of microbes, and this is an interesting avenue for future research.

4.5 Practical considerations and conservation implications

Biomass was found to increase over the experiment period, indicating that plants continued to grow and accumulate nutrients, however, the growth and nutrient removal rates were found to decrease over time. Two primary factors may have contributed to the decreased growth and nutrient uptake rate, namely competition and/or self-shading and temperature decline towards winter.

While competition for space between plants could have reduced the rate of biomass accumulation, the impact thereof was likely minimal, as plants were removed at intervals for sampling, providing room for growth. Self-shading could have played a role in reducing the rate of biomass production for *C. textilis*, as this species had prolific shoot growth (Suding et al., 2005). However, plants also have intrinsic growth limitations, meaning that they are able to grow rapidly and take up nutrients until a certain size is reached whereafter growth rate decreases (Lasfar et al., 2007). Competition, self-shading and intrinsic growth limitations all suggest that biomass harvesting could be a good solution to maintain the system in a state that allows for optimal growth and nutrient removal (Sun et al., 2019). Similar to other studies, our results for both experiments indicate that shoots are generally the dominant nutrient storage location for these three species, thus shoot harvesting and disposal is recommended to maximise nutrient uptake, and permanently remove nutrients from the system (Barco et al., 2021; Garcia Chanc et al., 2019; Huth et al., 2021; Schwammberger et al., 2020; White and Cousins, 2013). Harvesting shoot biomass stimulates new, accelerated growth, reduces internal nutrient cycling, and thus increases nutrient removal (Colares et al., 2020; Ng and Chan, 2021; Zhou et al., 2017).

With high temperatures positively influencing plant growth (typically more rapid growth), nutrient removal is accelerated during summer months (Bi et al., 2019). Furthermore, actively growing shoots can have higher nutrient concentrations than mature shoots (Sun et al., 2019). If plants slow uptake as winter approaches, this would suggest that having floating wetlands installed and established before the austral summer would be important for best results.

To understand the potential impact of these uptake rates at a farm-scale, a quick calculation using floating wetlands theoretically planted with *C. textilis* was performed. This calculation was based on a similarly polluted farm dam with 10 floating wetlands, each with 10 *C. textilis* plants on each wetland. The potential uptake was estimated at 0.6 kg of nitrogen and 0.1 kg of phosphorus per year (Table S17, Supplementary Material).

4.6 Additional benefits of floating wetlands

This study has demonstrated that certain endemic South African wetland plant species show potential to remove excess nutrients from eutrophic water and thrive in these conditions. In addition to their nutrient uptake abilities, floating treatment wetlands offer numerous co-benefits, such as biodiversity, aesthetic and educational benefits (Stefanakis, 2019). Floating wetlands provide habitats for fauna, act as refugia for aquatic species in urban and agricultural areas, could potentially act as steppingstone habitats, and provide aesthetic benefits to people (Stefanakis, 2019). In the current study, the floating wetlands were found to be home to at least two frog species, *Strongylopus grayii* and *Sclerophrys capensis*, a snake, *Lycodonomorphus rufulus*, and numerous dragonfly species were seen in the vicinity of the wetlands. Artificial ponds have been found to have great conservation value for aquatic insect diversity by forming part of a diverse pondscape and conservation corridors in degraded or transformed landscapes (Deacon et al., 2018). As such, the use of native wetland species in artificial ponds to improve water quality will benefit the overall ecosystem health and biodiversity of the region.

5 CONCLUSION

Floating wetlands, a cost-effective green technology, present an opportunity to purify agricultural wastewater in lentic systems, like small farm dams, in South Africa. Using endemic South African wetland plant species, especially those with fast growth rates such as *Cyperus textilis*, has high potential to effectively remove nutrients as well as provide other co-benefits. This research fills a gap in the literature in terms of using endemic wetland species for floating treatment wetlands in the Global South, and specifically Africa.

Declarations

Competing interests

The authors have no relevant financial or non-financial interests to disclose.

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Author contributions

AJR, PF, OB contributed to the study conception and design. Material preparation, data collection and analysis were performed by PF, OB, AJR. The first draft of the manuscript was written by AJR, PF, OB and PF, OB, AJR, KJE, DLM commented on previous versions of the manuscript. PF, OB, AJR, KJE, and DLM read and approved the final manuscript.

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Data availability

The datasets generated during and/or analysed during the current study are available in the supplementary material.

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Figures

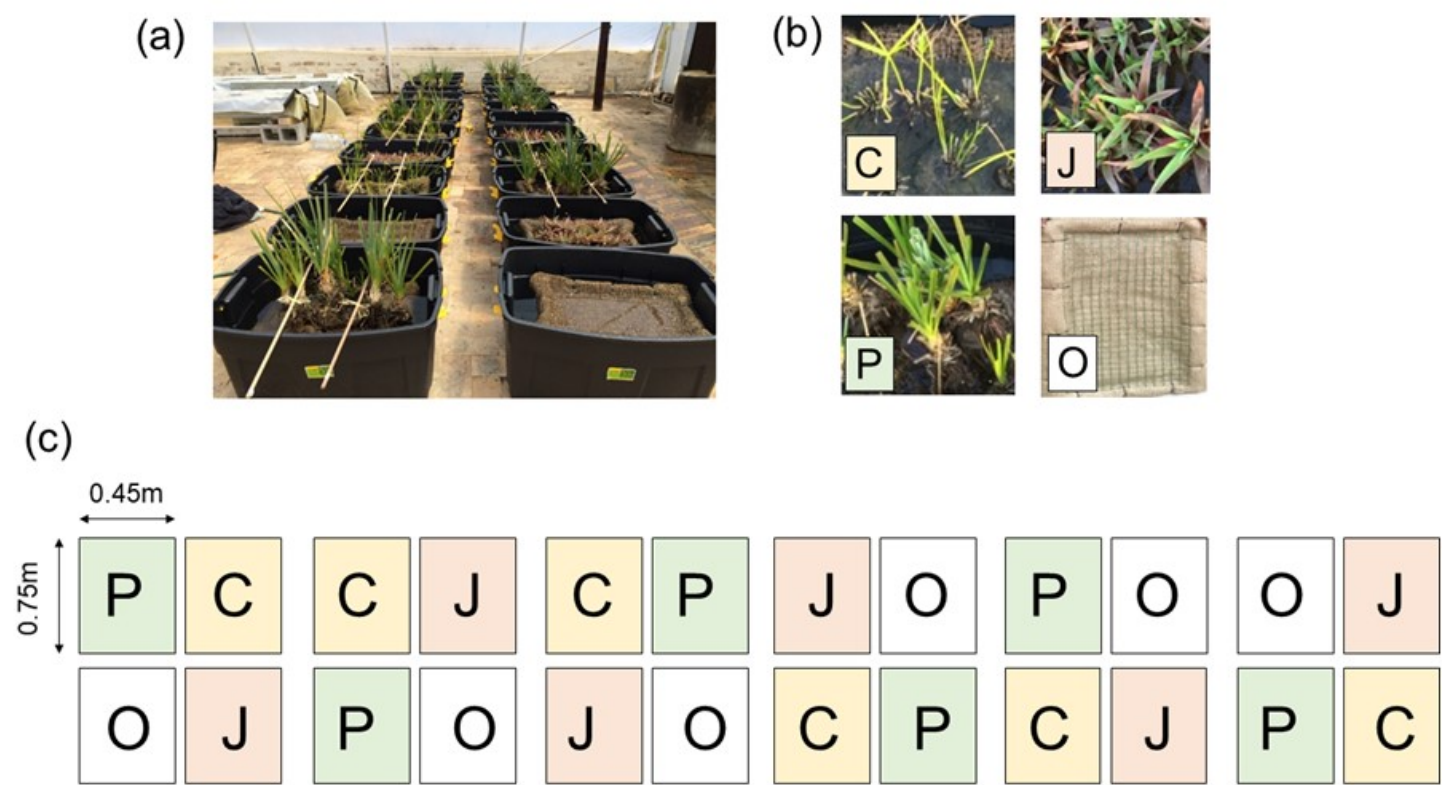


Figure 1

Design of the mesocosm experiment in the greenhouse (a), showing the floating wetlands: “P” is *Prionium serratum*, “J” is *Juncus lomatophyllus*, and “C” is *Cyperus textilis*, (b) and the configuration (c), in Stellenbosch, South Africa (n=24)

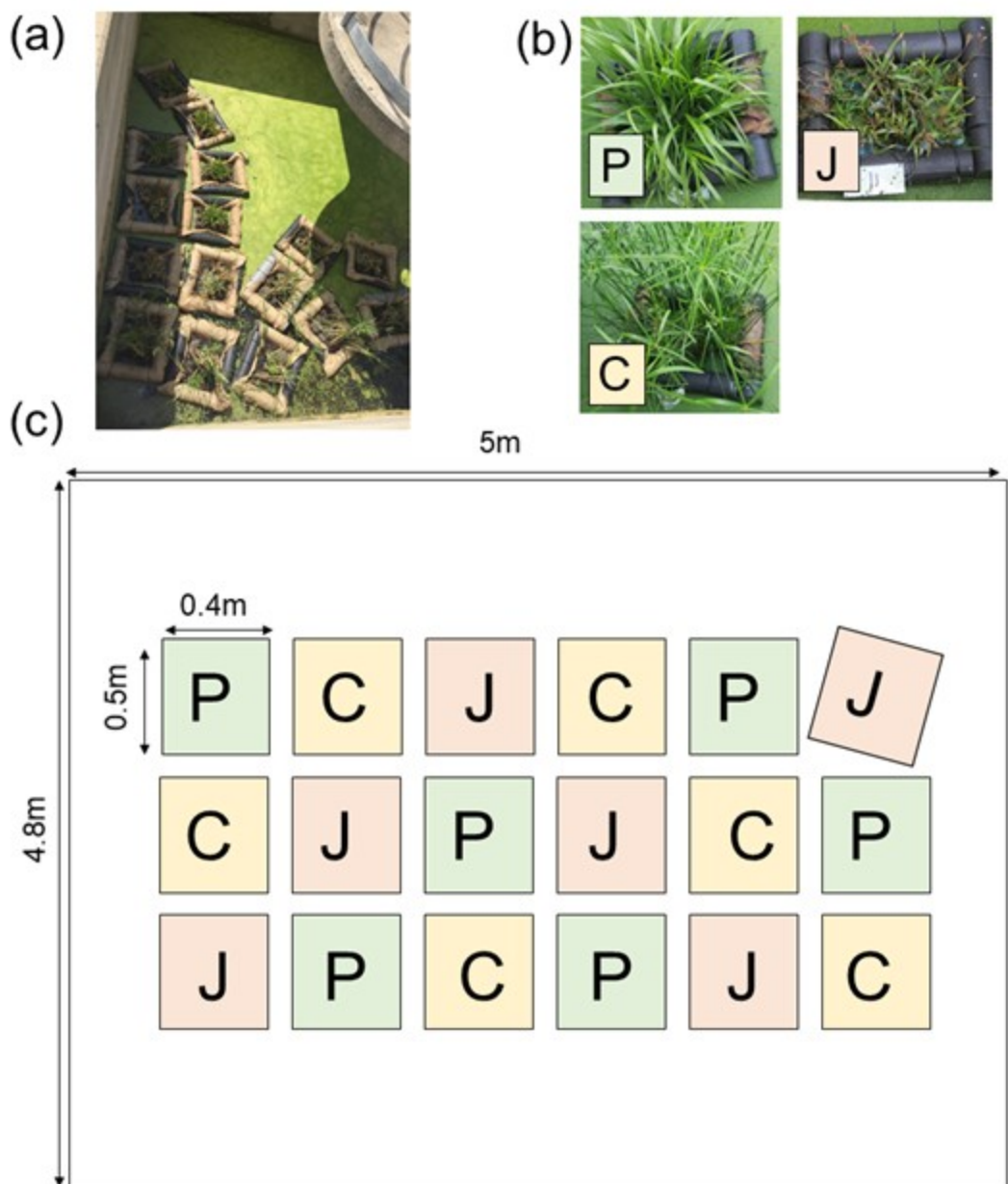


Figure 2

Design of the *in-situ* experiment in the treatment pond (a), showing the treatments: "P" is *Prionium serratum*, "J" is *Juncus lomatophyllus*, and "C" is *Cyperus textilis* (b) and the rough configuration (c) in Franschhoek, South Africa (n=18)

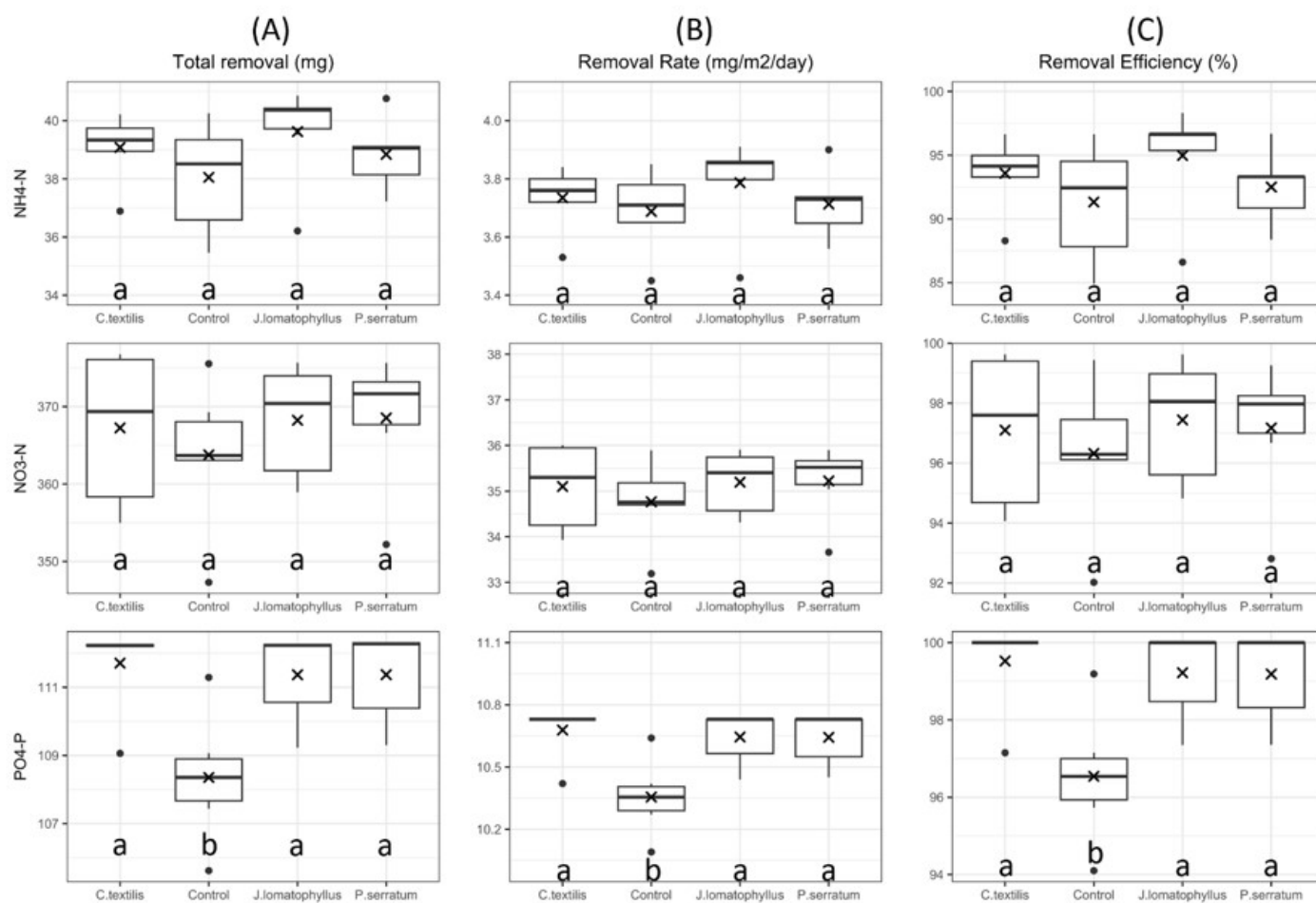


Figure 3

Box and whisker plots showing (A) Total removal (mg), (B) Removal rate ($\text{mg.m}^2.\text{d}^{-1}$), and (C) Removal efficiency (%) from water during the mesocosm experiment ($n=6$, $N=24$) under low nutrient conditions (1 month, from 27 January 2017 to 27 February 2017) for three endemic wetland species from South Africa. The solid black lines indicate median values, while the black crosses indicate the mean value per treatment. The solid black dots represent the outliers in the data. Letters denote significance differences at $p < 0.05$. Values can be found in in Table S7, Supplementary Material

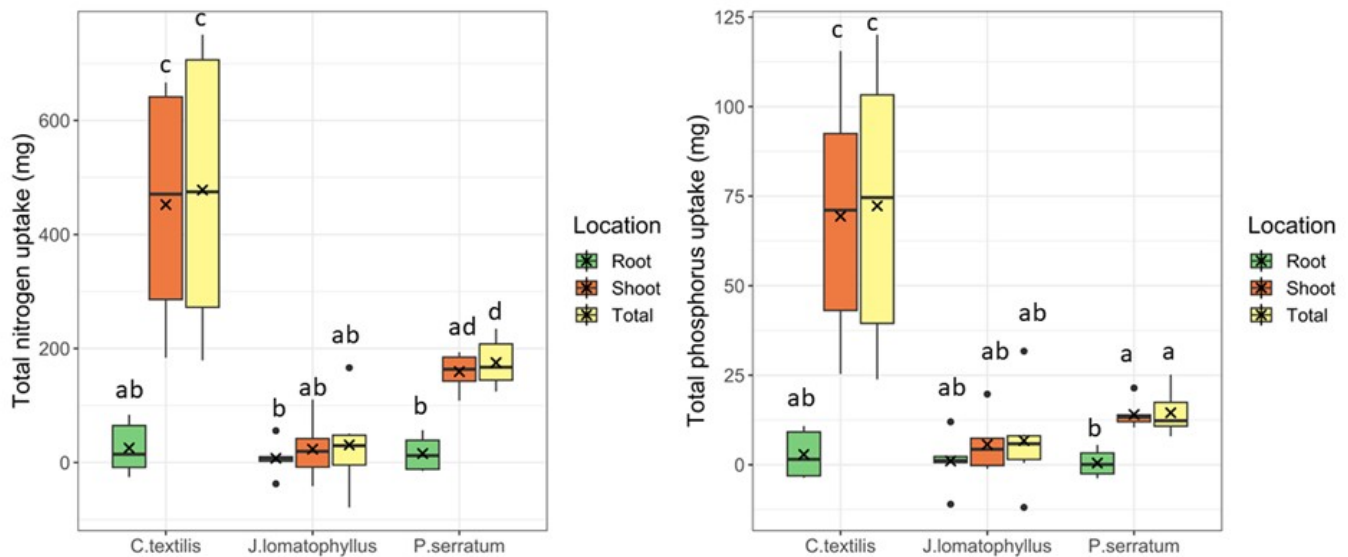


Figure 4

Mean monthly Total Nitrogen and Total Phosphorus plant uptake (mg) by *Cyperus textilis*, *Juncus lomatophyllus* and *Prionium serratum* for the *in-situ* experiment (3 months, from 12 March 2021 to 12 June 2021), South Africa. The solid black lines indicate median values, while the black crosses indicate the mean value per treatment. The solid black dots represent outliers. The boxes indicate the 25% and 75% percentiles. Letters denote significant differences between roots, shoots and total across species at $p < 0.05$. Values can be found in in Table S12, Supplementary Material

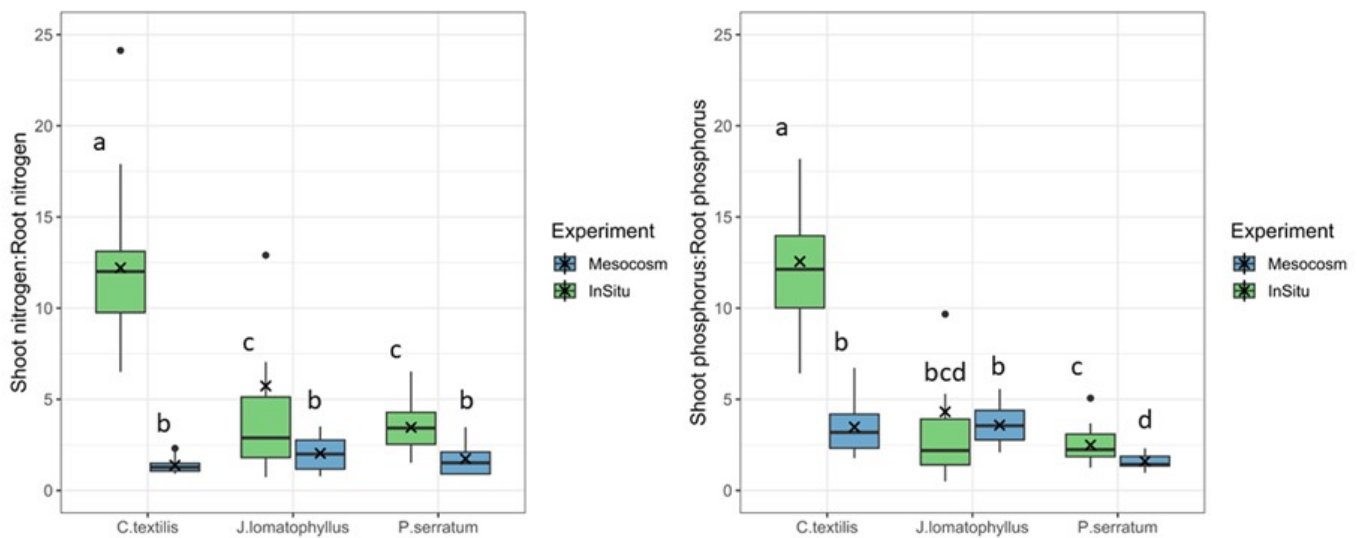


Figure 5

Ratios of dominant location of plant nutrient storage for both experiments – i.e. low (mesocosm experiment, 1 month in 2017) compared to high (*in-situ* experiment, 3 months in 2021) nutrient

conditions in South Africa. The solid black lines indicate median values, while the black crosses indicate the mean value per treatment. The solid black dots represent outliers. Letters denote significance at $p < 0.05$. Values can be found in Table S13, Supplementary Material

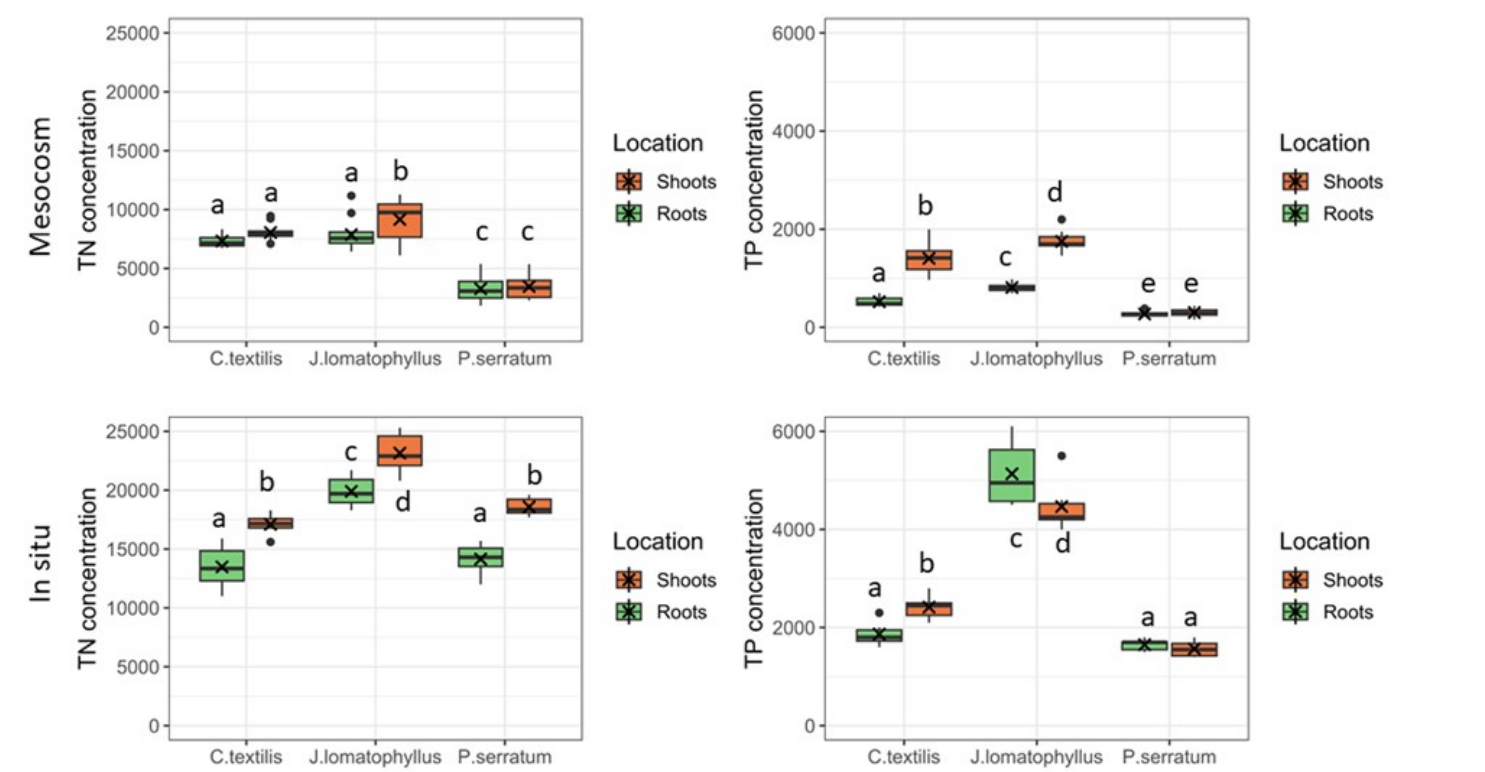


Figure 6

Graph showing difference in Total Nitrogen and Total Phosphorus concentrations between roots and shoots as well as between both experiments – i.e. low (mesocosm experiment, 1 month in 2017) compared to high (*in-situ* experiment, 3 months in 2021) nutrient conditions in South Africa. The solid black lines indicate median values, while the black crosses indicate the mean value per treatment. The solid black dots represent outliers. Letters denote significance at $p < 0.05$. Values can be found in Table S14, Supplementary Material

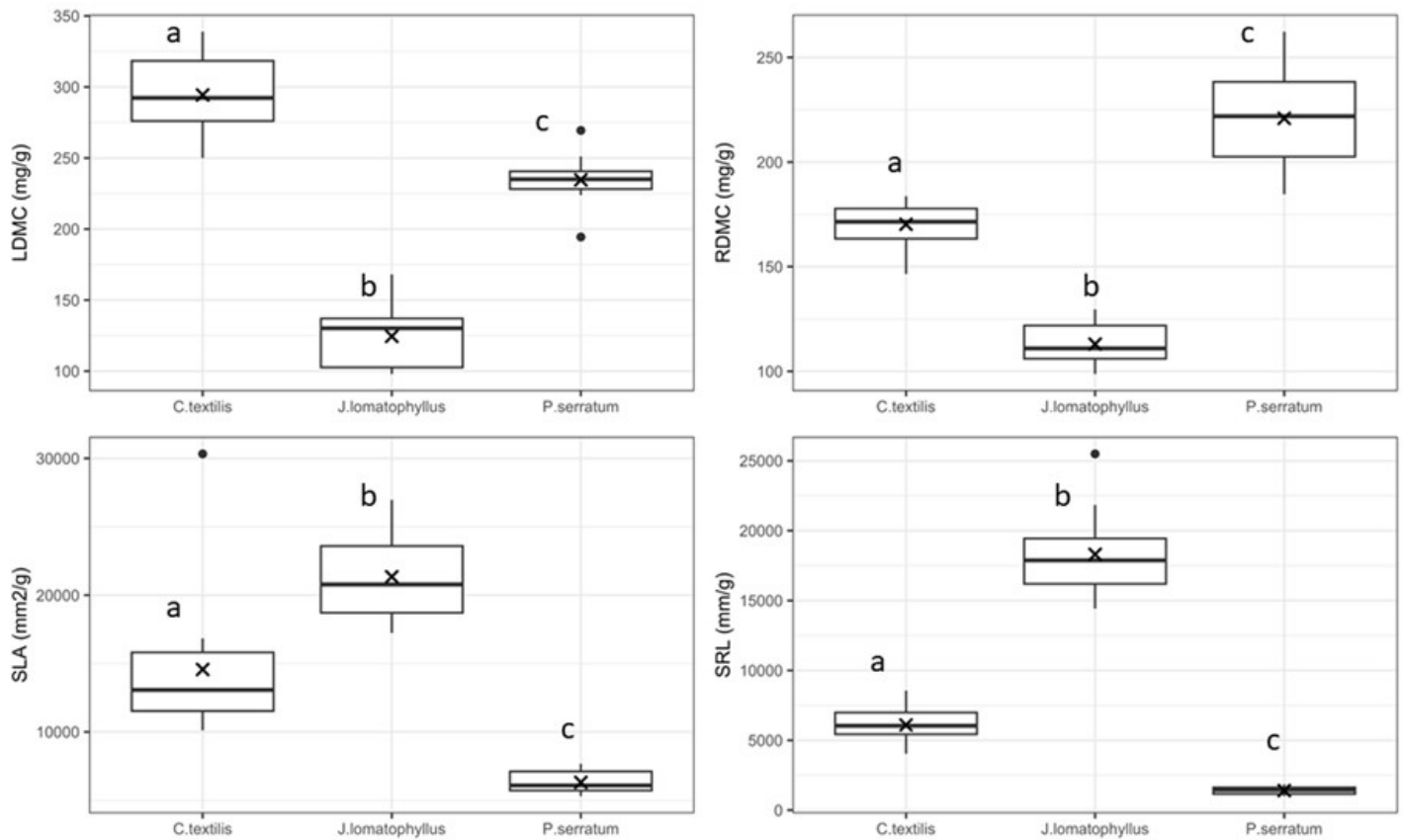


Figure 7

Indices derived from key plant functional traits for the three species measured within the one-month mesocosm experiment in the summer of 2017 in South Africa. LDMC = Leaf Dry Matter Content; RDMC = Root Dry Matter Content; SLA = Specific Leaf Area; and SRL = Specific Root Length. The solid black lines indicate median values, while the black crosses indicate the mean value per treatment. The solid black dots represent outliers. Letters denote significant differences at $p < 0.05$. Values for the other key plant function traits can be found in Table S16, Supplementary Material

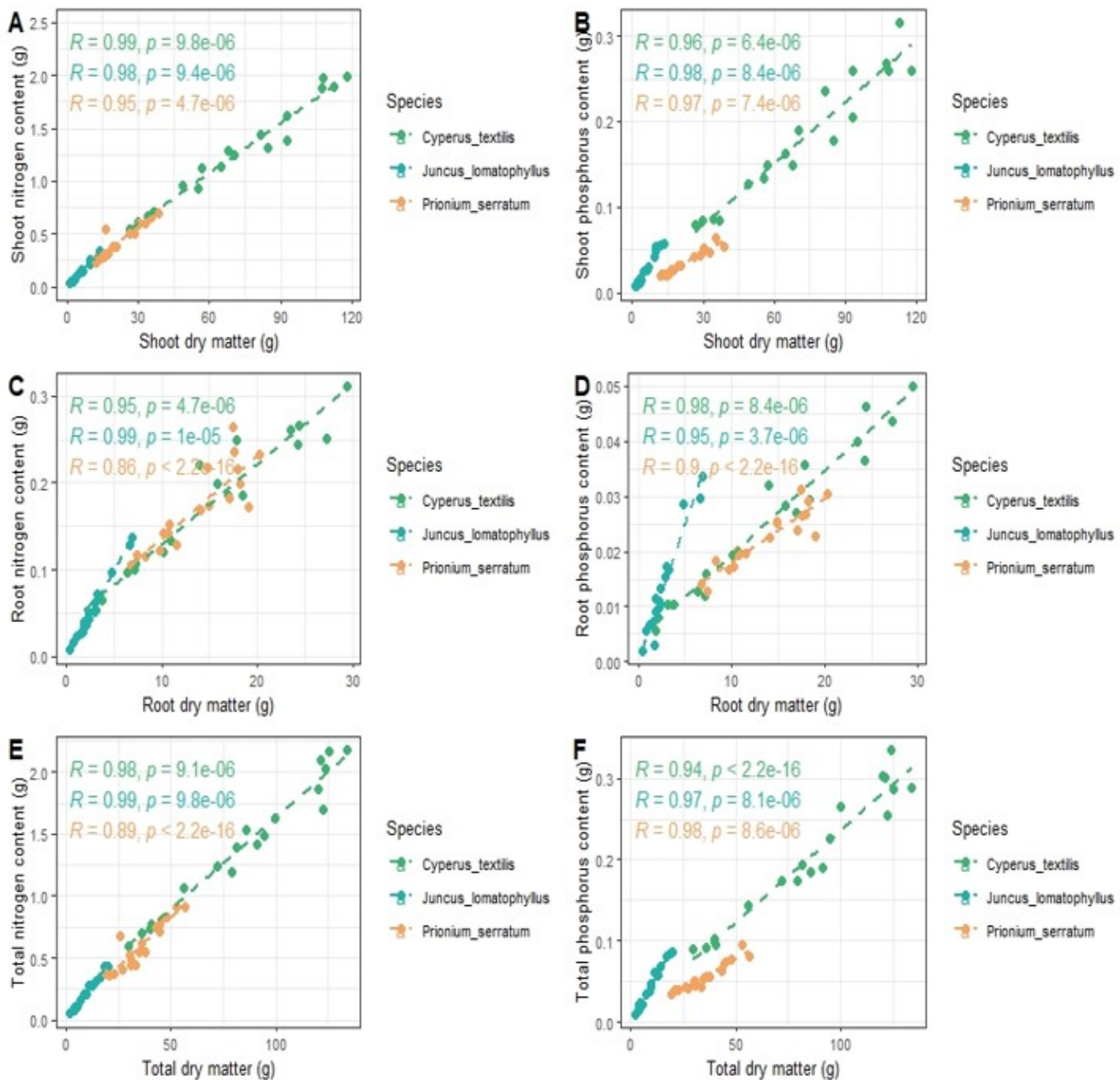


Figure 8

Relationship between plant growth (i.e. increase in root and shoot dry matter) and nutrient uptake for the three species measured for the three-month *in-situ* experiment (12 March 2021 to 12 June 2021) in South Africa. (A) Correlation between shoot dry matter (g) and shoot nitrogen content (g), (B) shoot dry matter (g) and shoot phosphorus content (g), (C) root dry matter (g) and root nitrogen content (g), (D) root dry matter (g) and root phosphorus content (g), (E) total dry matter (g) and total nitrogen content (g), (F) and total dry matter (g) and total phosphorus content (g). The dashed lines represent linear trendlines and the associated Spearman's correlation coefficient and p-value for each species is indicated in the relevant colour on each graph

Supplementary Files

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