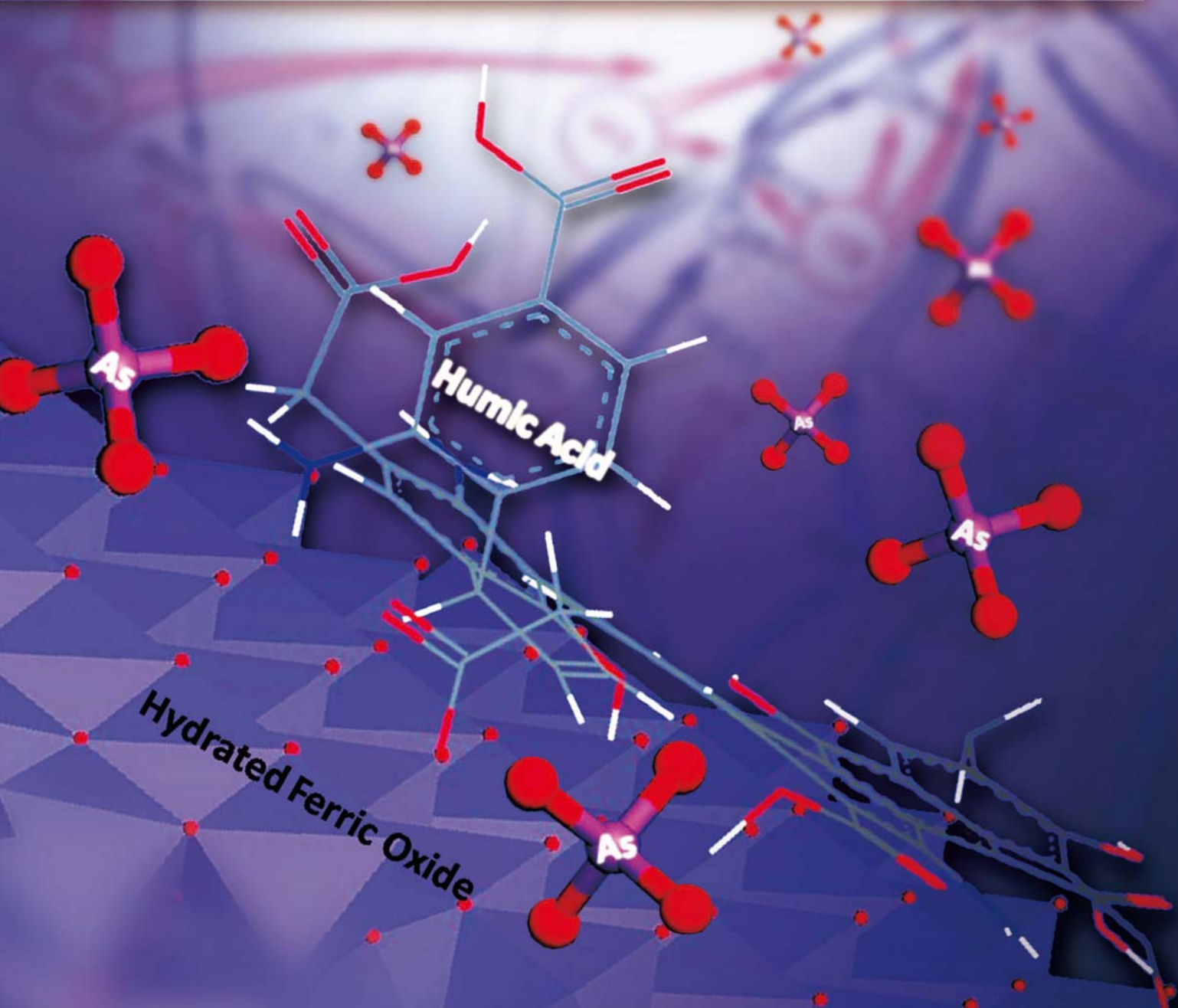


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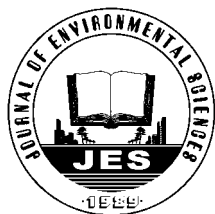
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Remediation of nutrient-rich waters using the terrestrial plant, *Pandanus amaryllifolius* Roxb.

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ABSTRACT

Effective control of eutrophication is generally established through the reduction of nutrient loading into waterways and water bodies. An economically viable and ecologically sustainable approach to nutrient pollution control could involve the integration of retention ponds, wetlands and greenways into water management systems. Plants not only play an invaluable role in the assimilation and removal of nutrients, but they also support fauna richness and can be aesthetically pleasing. *Pandanus amaryllifolius*, a tropical terrestrial plant, was found to establish well in hydrophytic conditions and was highly effective in remediating high nutrient levels in an aquatic environment showing 100% removal of NO_3^- -N up to 200 mg/L in 14 days. Phosphate uptake by the plant was less efficient with 64% of the PO_4^{3-} -P removed at the maximum concentration of 100 mg/L at the end of 6 weeks. With its high NO_3^- -N and PO_4^{3-} -P removal efficiency, *P. amaryllifolius* depleted the nutrient-rich media and markedly contained the natural colonization of algae. The impediment of algal growth led to improvements in the water quality with significant decreases in turbidity, pH and electrical conductivity. In addition, the plants did not show stress symptoms when grown in high nutrient levels as shown by the changes in their biomass, total soluble proteins and chlorophyll accumulation as well as photochemical efficiency. Thus, *P. amaryllifolius* is a potential candidate for the mitigation of nutrient pollution in phytoremediation systems in the tropics as the plant requires low maintenance, is tolerant to the natural variability of weather conditions and fluctuating hydro-periods, and exhibit good nutrient removal capabilities.

Introduction

Eutrophication caused by excess nutrient loads is one of the common problems in surface waters globally. Excessive nutrients, primarily nitrogen (N) and phosphorus (P), arise from rapidly expanding urban development as well as intensifying agricultural and industrial activities. Restriction of nutrient loading is the essential key to eutrophication control. Phytoremediation presents a cost effective and ecologically sustainable way of reducing high nutrient inputs to water bodies by treating point and non-

point source pollution. Removal of N and P using aquatic macrophytes in constructed wetlands, and storm- or wastewater retention ponds has been shown to successfully remediate eutrophic waters (Vymazal, 2007; Hu et al., 2008; Lu et al., 2010). Floating aquatic plants, in particular, have been reported to achieve very high nutrient removal efficiencies due to their high nutrient uptake capacity, fast growth rates and high biomass productivity (Reddy and DeBusk, 1987). In a study on the improvement of water quality of dairy manure wastewater using three floating aquatic macrophytes, *Eichhornia crassipes*, *Pistia stratiotes* and *Hydrocotyle umbellata*, *E. crassipes* has been shown to reduce total nitrogen (TN) by 91.7%, ammonium by 99.6% and total phosphorus (TP) by 95.8%

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(Sooknah and Wilkie, 2004). Significant removal of nitrate from sewage water by *Azolla filiculoides* has also been reported with removal rates up to 100% (Forni et al., 2001). Phytoremediation using emergent macrophytes has also become a prevalent treatment process. Experimental wetland beds planted with *Phragmites australis* have been shown to remove more than 96% of TN and TP from simulated plant nursery runoff (Huett et al., 2005). Similar studies on *Carex lacustris*, *Phalaris arundinacea*, *Scirpus validus* and *Typha latifolia* also indicated that these wetland plant species were effective in reducing TN and TP concentrations (Fraser et al., 2004). Hunter et al. (2001) reported that *S. validus* exhibited removal efficiency of up to 80% for N and 55% for P in a wetland microcosm. Although they play an active role in nutrient recycling in freshwater ecosystems, several macrophytes, such as water hyacinth, water fern and common reed, are notoriously invasive and their capacity for rapid colonization presents a major drawback and limits their use in actual field conditions (Hershner and Havens, 2008).

Vascular plants, both herbaceous and woody, that are widely cultivated for horticultural purposes in the tropics, could be a good depository from which to discover new candidate plant species with phytoremediative capabilities (Tan and Yeo, 2009). In our laboratory, a variety of herbaceous plants was investigated for their tolerance to partial submergence based on their partiality to moist-loving conditions. *Pandanus amaryllifolius* was one of the terrestrial plant species found to adapt well to hydrophytic conditions. *P. amaryllifolius*, commonly known as fragrant Pandan, is distributed in tropical Southeast Asia, where its leaves are widely used in cooking for its fragrance as well as a natural food dye. The ability of *P. amaryllifolius* to grow emersed allowed for its deployment in a hydroponics experimental system. Hence, the primary objective of the present study was to evaluate the potential of *P. amaryllifolius* for nutrient removal. Batch experiments were carried out to determine the effectiveness of *P. amaryllifolius* in removing nutrients, specifically nitrate and phosphate. In addition, the performance and productivity of the plant grown under eutrophic conditions as well as its ability to improve water quality were investigated.

1 Material and methods

1.1 Plant materials and culture conditions

P. amaryllifolius Roxb. were propagated from shoot cuttings collected from the teaching garden of the Department of Biological Sciences, National University of Singapore. The cuttings were allowed to root in water before they were individually planted in perforated plastic pots filled with lightweight expanded clay aggregates held in place by a top layer of glass pebbles. All plants were grown in

100 times diluted Hoagland's solution (1/100 HS) in the roof-top green-house of the Department, and subjected to natural diurnal variation in temperatures (34 ± 2)°C in the day and (24 ± 2)°C in the night). Light intensity varied within 60–800 $\mu\text{mol}/(\text{m}^2\text{-sec})$.

1.2 Experimental design

The experiments were each replicated 4 times. In each replicate, 4 established plants were kept partially submerged in a plastic tank (30 cm \times 25 cm \times 18 cm) containing 5 L of 1/100 HS modified to eliminate nitrate (NO_3^- -N) or phosphate (PO_4^{3-} -P) (1/100 HS^{-N/P}), depending on the treatment. To study the nutrient removal efficiency of the plants, the growth medium was supplemented either with 0, 5, 50, 100, 200 mg/L NO_3^- -N (KNO_3) for nitrate treatment or 0, 5, 20, 50, 100 mg/L PO_4^{3-} -P (KH_2PO_4) for phosphate treatment. The medium was maintained without aeration and 1/100 HS^{-N/P} was added weekly to offset evaporative losses and to ensure that the roots remained submerged in water.

1.3 Chemical analysis

For NO_3^- -N and PO_4^{3-} -P determinations, water samples were collected and filtered through 0.2 μm syringe filters before analysis by ion chromatography using a Dionex ICS-1000 ion chromatograph with a Dionex AS12A column (Sunnyvale, California). Total Kjeldahl Nitrogen (TKN) in the plant samples was determined using the micro-Kjeldahl method (Eastin, 1978). Dried plant samples were digested in 4 mL concentrated H_2SO_4 and 1 tablet Kjeltab (1.5 g K_2SO_4 , 1.5 g Se) for 2 hr at 350°C and the TKN content determined using the Tecator Kjeltac Auto 1030 Analyzer (Sweden). For analysis of TP, dried plant materials were first digested using the wet ashing method (Parkinson and Allen, 1975). TP content was determined spectrophotometrically using the Phosphomolybdenum Blue assay (Murphy and Riley, 1962).

1.4 Biomass determination

The collected plant samples were washed thoroughly with deionized water and blotted dry. The fresh weight (fw) of the plants was recorded before they were divided into shoot and root portions. These were dried separately at 70°C for two weeks before determining the dry weight (dw).

1.5 Total soluble protein determination

The total soluble protein (TSP) concentration was determined according to Bradford (1976) using the Bio-Rad Protein Assay Kit (Bio-Rad, Hercules, USA) with bovine serum albumin as calibration standard. The concentration of TSP was expressed as mg proteins per g fw.

1.6 Chlorophyll determination

Chlorophylls were extracted by grinding 0.03 g of fresh

leaf samples in 5 mL of absolute acetone and absorbances were determined at 645 and 663 nm. Concentrations of chlorophylls were calculated according to Arnon (1949), and are expressed as mg chlorophylls per g fw.

1.7 Chlorophyll fluorescence

Chlorophyll fluorescence emission from the upper surface of attached leaves was determined using a versatile pulse amplitude fluorometer (FMS II, Hansatech Instruments Ltd., Norfolk, UK). Each leaf sample was dark-adapted for 30 min before analysis. Minimal fluorescence level, F_0 , was determined at a light level lower than $0.01 \mu\text{mol}/(\text{m}^2\text{-sec})$. The maximal fluorescence yield of the dark-adapted leaf, F_m , was determined by applying a $10,000 \mu\text{mol}/(\text{m}^2\text{-sec})$ saturating light pulse. The maximal fluorescence yield of the light-adapted leaf, F_m' , was then determined by the application of a saturating light pulse of $10,000 \mu\text{mol}/(\text{m}^2\text{-sec})$ at 1 sec duration, at intervals of 60 sec for 30 min. The values of F_v/F_m , F_v'/F_m' , Φ_{PSII} , q_p and NPQ were recorded using the Modfluor software V. 2.00 (Hansatech Instruments Ltd., Norfolk, UK).

1.8 Chlorophyll *a* water analysis

Chlorophyll *a* in the algae was determined by spectrophotometry according to Radojevic and Bashkin (2006) with modifications. Water sample was first filtered through a glass fibre filter paper and the filter macerated in 10 mL of 80% methanol. The suspension was incubated in a water bath at $65\text{--}80^\circ\text{C}$ for 5 min, cooled and the extract clarified by centrifugation. Absorbances of the chlorophyll *a* extract were determined at 663, 665 and 750 nm before and after acidification with 0.1 mL of 0.1 mol/L HCl. Calculation for the concentration of chlorophyll *a* was corrected for

turbidity as well as the presence of pheophytin *a*.

1.9 Water quality analysis

The pH of water was determined using the YSI 556 Multi-Probe System (YSI Environmental, Ohio, USA). Turbidity was determined using the Thermo Orion Turbidimeter (Thermo Electron Corporation, Ohio, USA) and expressed in Nephelometric Turbidity Units (NTU).

2 Results

2.1 Nutrient removal efficiency

P. amaryllifolius was highly efficient in NO_3^- -N uptake, completely removing up to 100 mg/L NO_3^- -N for 3 days after the start of the experiment (Fig. 1a). Nitrate at 200 mg/L was totally removed by the plants after 14 days. However, only 20% of the NO_3^- -N was removed in the control set-ups without plants after 28 days. Phosphate removal by *P. amaryllifolius* was less efficient; PO_4^{3-} -P at concentrations up to 20 mg/L was completely removed only after 35 days of experiment (Fig. 1b). At higher concentrations, only 80% and 64% of 50 and 100 mg/L PO_4^{3-} -P, respectively, were removed at the end of 42 days.

All the experimental set-ups were exposed to natural diurnal variation in light and air temperature in the roof-top green-house and growth of algae in the nutrient-rich media usually set in 21 days after the start of the experiment. In control set-ups without plants, the nutrient removal was attributed to the algal growth, which could completely remove PO_4^{3-} -P up to 20 mg/L by the end of 42 days (Fig. 1b).

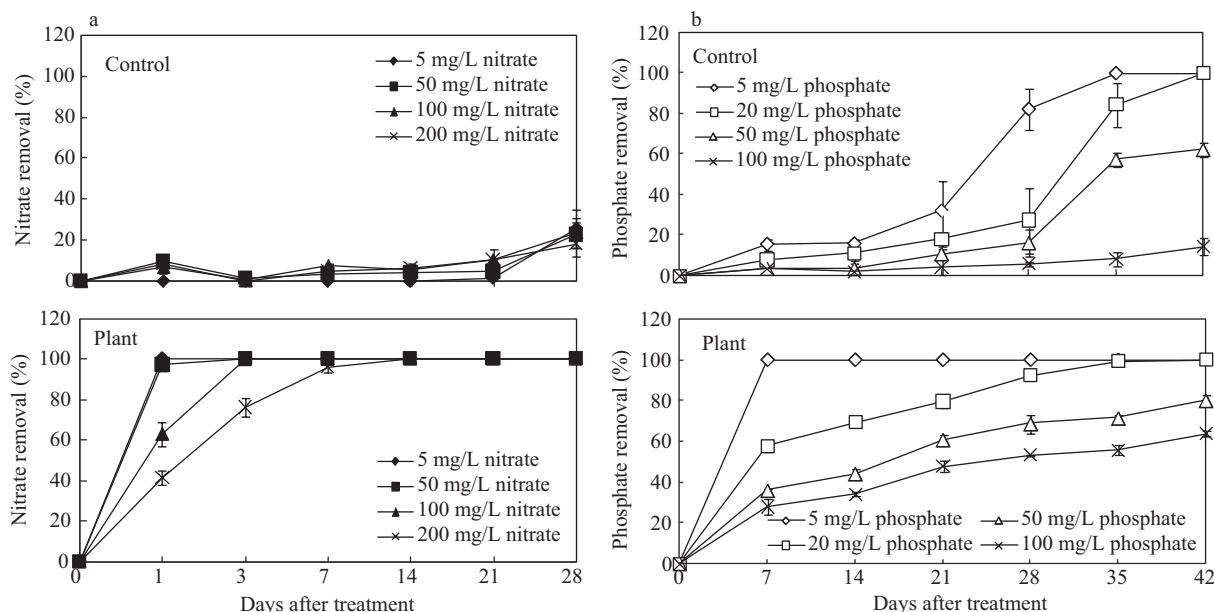


Fig. 1 Removal of nitrate (a) and phosphate (b) by *P. amaryllifolius*. Values represent the mean \pm SE of four replicates.

2.2 Plant responses to increased nutrient concentrations

2.2.1 Plant growth

P. amaryllifolius showed an increase in plant biomass when grown in increasing NO_3^- -N concentrations (Fig. 2a). This gain in biomass was largely due to increased foliage growth. Phosphate, on the other hand, did not significantly affect plant biomass as compared to NO_3^- -N (Fig. 2b). The plants under both NO_3^- -N and PO_4^{3-} -P treatments did not exhibit nutrient toxicity symptoms during the period of study. Exposure to high levels of NO_3^- -N even promoted new shoot formation. In preliminary experiments, *P. amaryllifolius* was found to be tolerant to very high levels of NO_3^- -N or PO_4^{3-} -P up to 1000 mg/L, although the plants exhibited visible signs of stress such as leaf edge burn (data not shown).

2.2.2 TKN, TP, TSP and chlorophyll content

The TKN and TP contents in the tissues of *P. amaryllifolius* increased with the increase in external nutrient concentration. Plants exposed to 50 mg/L NO_3^- -N or higher resulted in greater N bioaccumulation and the increase was primarily due to increased N accumulation in the shoots (Fig. 3a). Similarly, the plant TP content increased when treated with 5 mg/L PO_4^{3-} -P or higher (Fig. 3b). However, unlike NO_3^- -N accumulation, PO_4^{3-} -P accumulation in the roots was higher than that in the shoots.

Exposure of plants to increasing concentrations of NO_3^- -N resulted in increased TSP accumulation in the leaves and roots (Fig. 3a). At 200 mg/L NO_3^- -N, TSP accumulation in

the leaves increased by more than 2 folds after 28 days of treatment. Chlorophyll content in the leaves also increased with exposure to increasing NO_3^- -N concentrations largely as a result of chlorophyll *a* accumulation. The total chlorophyll concentration increased by 61% at 200 mg/L NO_3^- -N compared to the control treatment (0 mg/L NO_3^- -N). A slight decrease in TSP levels was observed in the leaves and roots exposed to PO_4^{3-} -P and chlorophyll concentration remained almost constant in response to PO_4^{3-} -P treatment (Fig. 3b).

2.2.3 Chlorophyll fluorescence

Changes in chlorophyll *a* fluorescence parameters were determined to evaluate the photochemical responses of *P. amaryllifolius* to high nutrient levels. Photosynthetic efficiency, indicated by F_v/F_m of dark-adapted leaves, showed that the plants were healthy when exposed to NO_3^- -N or PO_4^{3-} -P treatments; F_v/F_m values ranged from 0.80 to 0.85 (Fig. 4). Plants treated with increasing concentration of NO_3^- -N showed a corresponding increase in photochemical activity in the leaves. Increases in the photochemical efficiency of open PSII reaction centres (F_v'/F_m') resulted in significant increases in Φ_{PSII} , the quantum efficiency of PSII, starting 7 days after NO_3^- -N treatment (Fig. 4a). After 21 days, the Φ_{PSII} of plants treated with 100 and 200 mg/L NO_3^- -N increased by more than 2 folds that of the control. Increases in q_p , which indicates the proportion of open PSII centres, were also observed in plants subjected to increasing external NO_3^- -N concentrations (Fig. 4a). As a result, with increasing NO_3^- -N concentration, the non-photochemical quenching

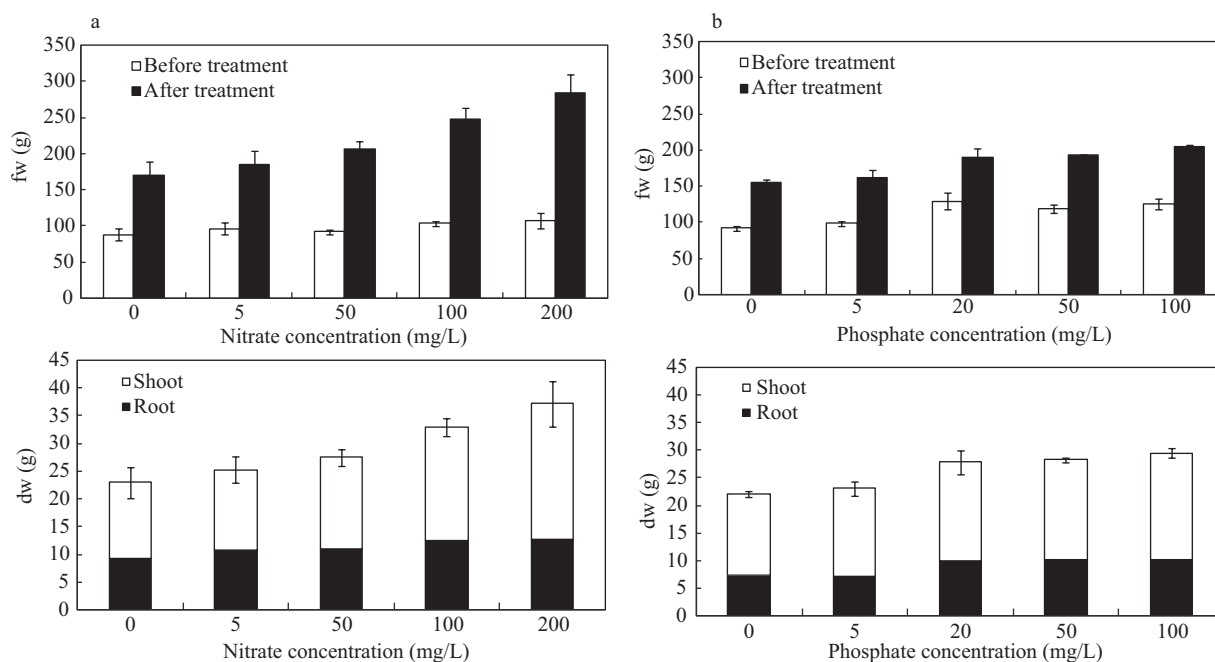


Fig. 2 Effects of nitrate (a) and phosphate (b) on biomass accumulation (top panel) and partitioning (bottom panel) in *P. amaryllifolius*. The plants were grown in aqueous media containing (a) 0, 5, 50, 100 and 200 mg/L nitrate for 28 days, or (b) 0, 5, 20, 50 and 100 mg/L phosphate for 42 days. Values represent the mean \pm SE of four replicates.

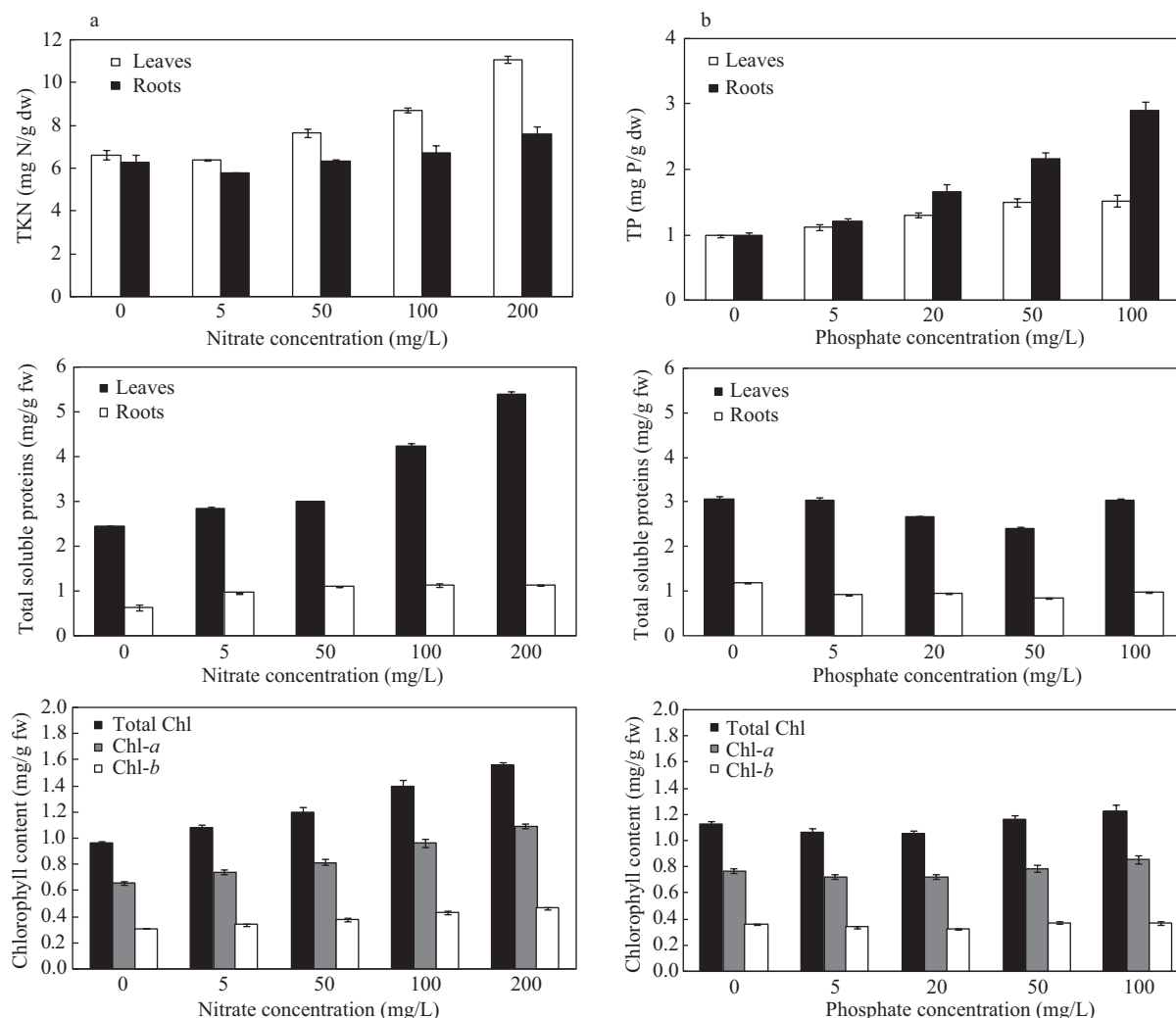


Fig. 3 Effects of nitrate (a) and phosphate (b) on total kjeldahl nitrogen (TKN), total phosphate (TP), total soluble protein and chlorophyll content in *P. amaryllifolius*. Treatment notations are the same as in Fig. 2. Values represent the mean \pm SE of four replicates.

(NPQ) decreased, indicating the reduction of thermal energy dissipation at PSII. No obvious changes in Fv'/Fm' , Φ_{PSII} , q_p and NPQ were detected in the plants treated with PO_4^{3-} -P (Fig. 4b).

2.3 Plant effects on water quality

Chlorophyll *a*, a principal algal pigment, was determined as an algal biomass indicator in the media. Increasing concentrations of NO_3^- -N resulted in high levels of algal bloom in the control setups, whereas nutrient depletion due to the high NO_3^- -N removal efficiency of *P. amaryllifolius* severely reduced the algal growth in the plant setups (Fig. 5a). Algal growth, in the presence of increasing PO_4^{3-} -P concentrations, was also significantly impeded in the plant setups, compared to the control (Fig. 5b). However, this reduction was less severe compared to that observed in the NO_3^- -N treatment, as there were still residual levels of PO_4^{3-} -P in the media that promoted algae proliferation (Fig. 1b).

Improvements in water quality were observed as a result of the mitigation of high nutrient levels by *P. amaryllifolius*. Turbidity was significantly higher in the control setups than in the plant setups (Fig. 6). In both NO_3^- -N and PO_4^{3-} -P treatments, the drastic increase in turbidity in the control setups from day 21 to day 28 of the study was attributable to the proliferation of algae in the growth media. The EC and pH of the growth media increased in the control setups but decreased in the plant setups (Fig. 7). As EC provides a measure of the amount of dissolved salts in solution, the removal of ions from the media by plant uptake led to a significant reduction in EC in the plant setups, most notably by 70% and 56% in the highest NO_3^- -N and PO_4^{3-} -P treatments, respectively, by the end of the study. The slight but steady increase in EC in the control setups could be due to the weekly supplement of 1/100 $HS^{-N/P}$ that was added to offset evaporative losses of the media during the period of study. In the presence of plants, water pH was maintained at a stable range of pH 6.5 to 7.0, irrespective of the nutrient treatment. On the other

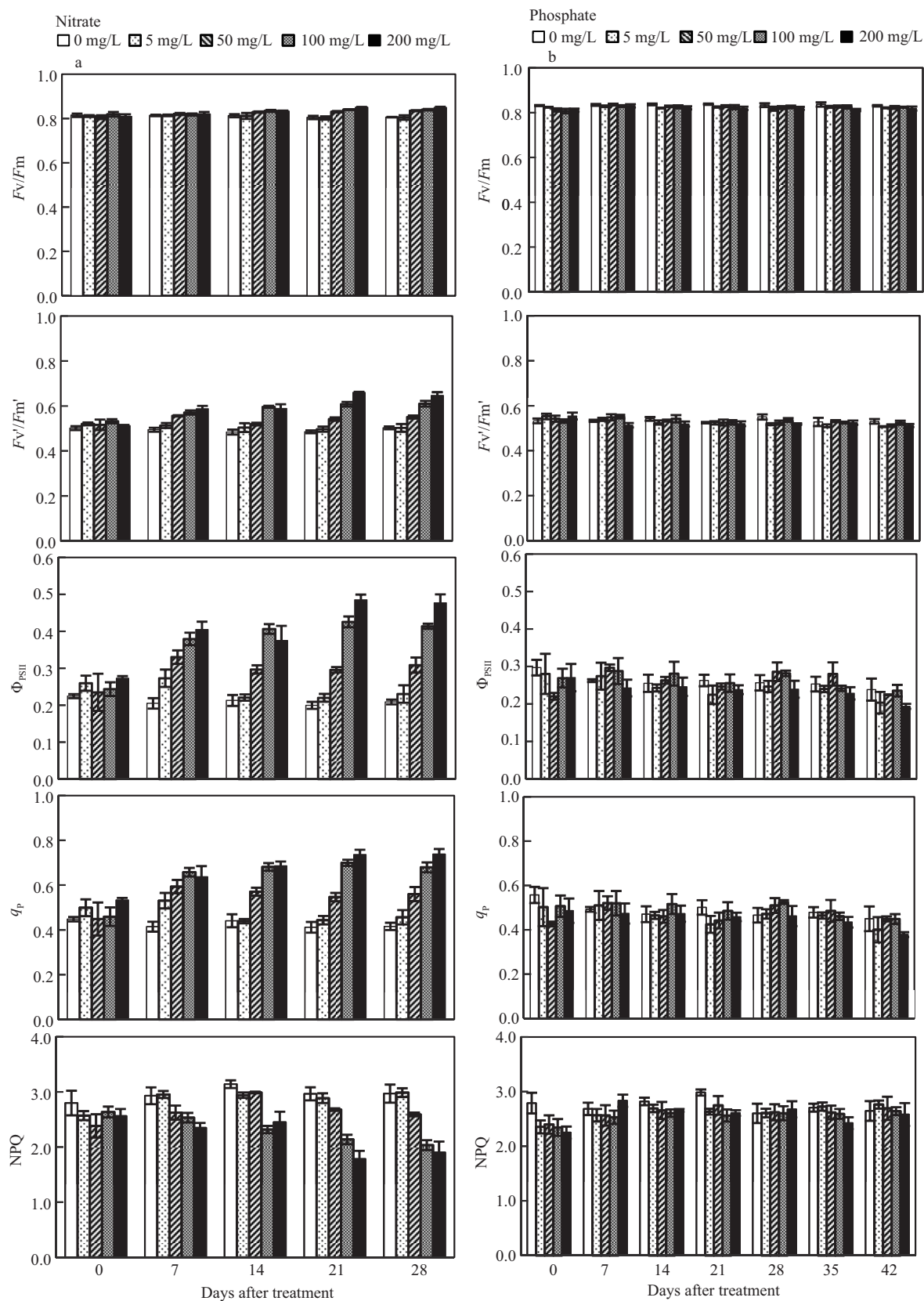


Fig. 4 Effects of nitrate (a) and phosphate (b) on chlorophyll fluorescence parameters of *P. amaryllifolius*. Treatment notations are the same as in **Fig. 2**. Values represent the mean \pm SE of four replicates. F_v'/F_m' : photochemical efficiency of open PSII reaction centres in light-adapted state, Φ_{PSII} : quantum efficiency of PSII in light-adapted state, q_p : photochemical quenching, NPQ: non-photochemical quenching.

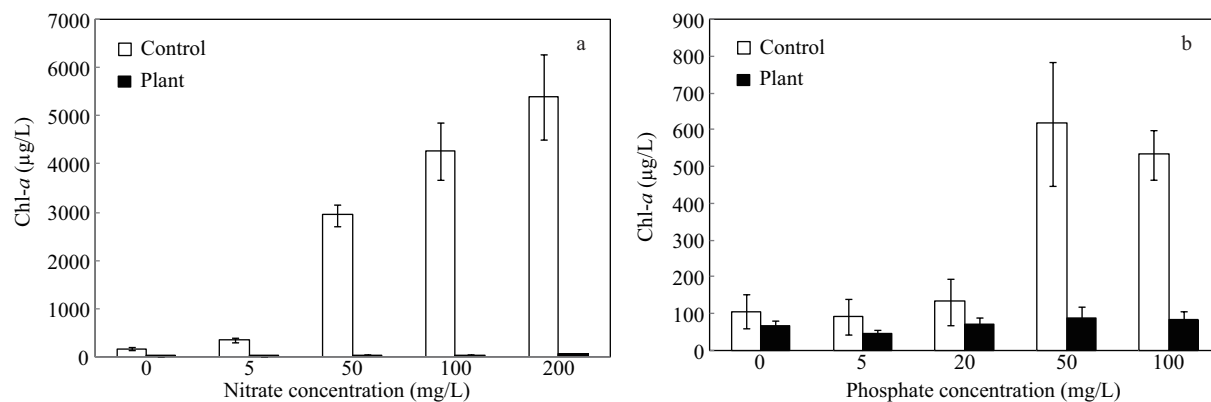


Fig. 5 Algal growth in the nutrient-rich media. Chlorophyll *a* (Chl-*a*) concentration in growth media containing nitrate (a) and phosphate (b). Treatment notations are the same as in Fig. 2. Experimental controls without plants were carried out with the same nutrient concentrations. Values represent the mean \pm SE of four replicates.

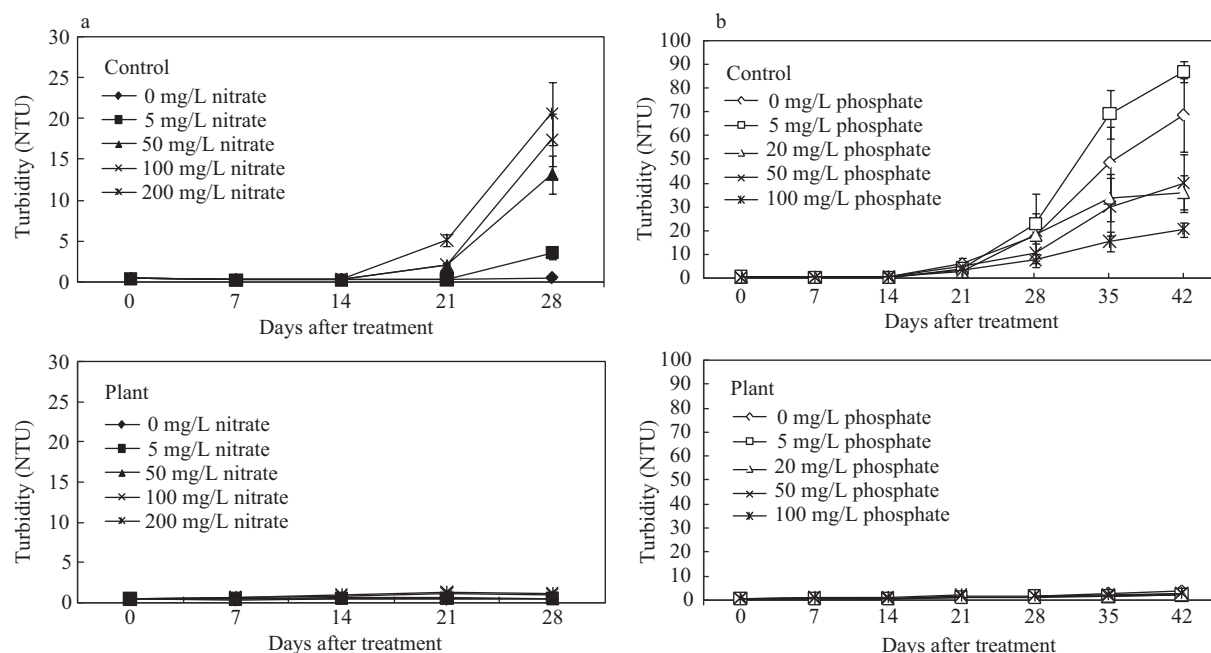


Fig. 6 Effects of nitrate (a) and phosphate (b) on turbidity of growth media. Treatment notations are the same as in Fig. 2. Experimental controls without plants were carried out with the same nutrient concentrations. Values represent the mean \pm SE of four replicates.

hand, the pH values in the control setups increased sharply after 14 days, coinciding with the rise in algal growth in the media.

3 Discussion

Inorganic N and P are the primary causes of eutrophication in surface waters. In an urban environment, significant amounts of N and P enter surface waters from non-point sources such as construction sites and runoff from landscape fertilizers. Current approaches to control non-point nutrient pollution include the creation of wetlands, retention ponds, riparian zones and greenways (Carpenter et al., 1998). The deployment of plants enhances nutri-

ent removal through nutrient uptake, filtration of organic and inorganic particulates, and formation of rhizospheric bacteria associations (Srivastava et al., 2008). Vegetated buffer strips in riparian zones, for example, have been shown to remove about 38%–63% of the incoming nitrate load from agricultural lands to streams (Hefting et al., 2006). The key to effective plant-assisted remediation of nutrient pollution is the establishment and sustainability of the desired vegetation communities. Plants have been reported to exhibit species-specific efficiency for heavy metal uptake as well as nutrient removal (Maschinski et al., 1999, Lin et al., 2002). The potential removal of N and P by plants depends on their growth rate and the N and P contents in the tissues. Thus, the desirable traits of a plant used for nutrient assimilation and removal should include

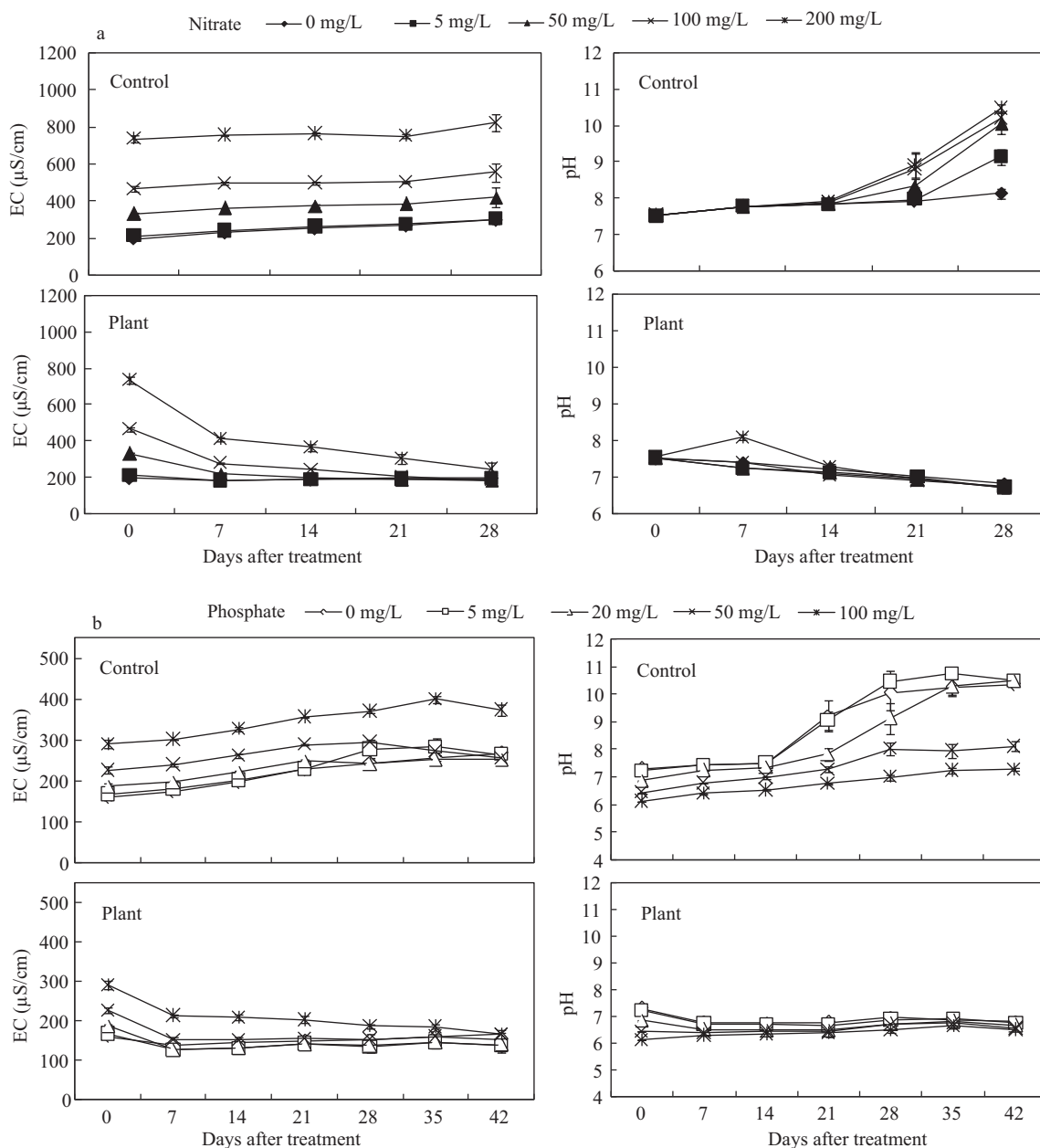


Fig. 7 Effects of nitrate (a) and phosphate (b) on electrical conductivity (left panel) or pH (right panel) of the growth media. Treatment notations are the same as in **Fig. 2**. Experimental controls without plants were carried out with the same nutrient concentrations. Values represent the mean \pm SE of four replicates.

rapid growth, high capacity for biomass accumulation and high tissue nutrient content (Paranychanakis et al., 2006). Hence, for many such applications, invasive plants with their high nutrient uptake capacity, fast growth rates and high biomass productivity were used in preference to native plants.

In this study, *P. amaryllifolius*, a tropical terrestrial plant, was found to be highly efficient in removing nutrients, particularly NO_3^- -N, from an aquatic environment. Up to 200 mg/L NO_3^- -N was shown to be completely removed after 14 days. The nutrient removal efficiency was significantly higher than that reported for *A. filiculoides*, which

showed 88% removal in water contaminated with 30 mg/L NO_3^- -N after 12 days (Forni et al., 2001). The NO_3^- -N removal capacity of *P. amaryllifolius* (100%) was also better than that of riparian plants *Phragmites japonica* and *Salix gracilistyla*, which were reported to remove 20%–50% of NO_3^- -N at concentrations higher than 100 mg/L (Shin et al., 2004). While *P. amaryllifolius* was more efficient in NO_3^- -N removal, its PO_4^{3-} -P removal efficiency (100%–64% of 5–100 mg/L PO_4^{3-} -P) was, nonetheless, better than that of *P. japonica* and *S. gracilistyla*, which were shown to remove less than 40% of the influent PO_4^{3-} -P concentration (25–30 mg/L) (Shin et al., 2004).

A comparative study on *P. stratiotes* (water lettuce), *Spirodela polyrhiza* (duckweed) and algal-based treatment of wastewater showed that macrophytes were more efficient in N and P removal than algal ponds (Awuah et al., 2004). In particular, TP removals were 33% and 9% for water lettuce and duckweed systems, while a TP increase of 19% was observed in the algal ponds. In the present study, however, algae attributed to significant removal of $\text{PO}_4^{3-}\text{-P}$ from the control setups most noticeably after day 21. Nonetheless, at high concentrations of $\text{PO}_4^{3-}\text{-P}$ (100 mg/L), the removal efficiency of *P. amaryllifolius* (64%) was considerably higher than that of algae (15%).

Of all the essential mineral nutrients, plants require N in the largest amounts and N availability is often the major limiting factor for plant growth. However, as with all nutrients, excessive levels can be detrimental to the normal growth and development of plants. A high N concentration of 112 mg/L was shown to affect the mortality of *T. latifolia* (Fraser et al., 2004). On contrary, *P. amaryllifolius* was not only tolerant to high nutrient conditions but it also grew better and accumulated greater biomass, particularly in the leaves, with increased $\text{NO}_3^- \text{-N}$ exposure. The positive correlation between improved removal efficiencies of N and biomass production in plants has also been reported in a previous study on wastewater treatment (Tzanakakis et al., 2009). In a study on the biomass allocation of plants in response to various abiotic factors, nutrient supply was found to have the strongest effect on allocation (Poorter and Nagel, 2000). When exposed to a high nutrient environment, plants usually partition more biomass to the above ground part to acquire more carbon resource (Xie et al., 2004). In *P. amaryllifolius*, increases in $\text{NO}_3^- \text{-N}$ availability also resulted in greater fractional investments of biomass in foliage compatible to observations by Xie et al. (2004). High $\text{NO}_3^- \text{-N}$ levels not only increased foliage growth in *P. amaryllifolius* but also promoted lateral shoot development suggesting enhanced plant vigour.

Plants that accumulated greater nutrient content in tissues when exposed to higher nutrient loading have been recommended for use in waste effluent treatment due to their high nutrient recovery (Zheng et al., 2008; Tzanakakis et al., 2009). In *P. amaryllifolius*, increased nutrient availability resulted in greater N and P accumulation in the plant tissues suggesting a capacity for greater assimilation of N and P under conditions of abundant supply, thus making it an attractive plant for use in phytoremediation. As N is a macronutrient essential for the production of proteins, nucleic acids and chlorophylls, the increase in tissue N content led to concomitant increases in TSP and chlorophyll concentrations in the leaves of *P. amaryllifolius*. Consequently, with the increased availability of N resources, the photosynthetic efficiency and activity increased resulting in higher plant growth. The Fv/Fm ratios of the plants under $\text{NO}_3^- \text{-N}$ and $\text{PO}_4^{3-}\text{-P}$ treatments were typical of those of healthy plants (0.75–

0.85), indicating that they were not stressed from the excess nutrient feed (Maxwell and Johnson, 2000). Moreover, plants exposed to increasing levels of $\text{NO}_3^- \text{-N}$ showed higher values of Fv'/Fm' and q_p , suggesting an increased capacity of the plants to harvest and transduce light energy. As the quantum yield of electron transfer through PSII (Φ_{PSII}) is the product of the efficiency of open PSII reaction centres (Fv'/Fm') and the photochemical quenching (q_p) (Genty et al., 1989), increases in Fv'/Fm' , and q_p concomitantly resulted in significant increases in Φ_{PSII} . Thus, the results indicated that *P. amaryllifolius* plants were not only tolerant to high $\text{NO}_3^- \text{-N}$ levels (up to 200 mg/L), but showed increased photosynthetic light reactions and carbon assimilation, resulting in increased plant growth under eutrophic conditions. In contrast, exposure of *P. amaryllifolius* plants to increasing $\text{PO}_4^{3-}\text{-P}$ concentrations did not affect Fv/Fm , Fv'/Fm' , q_p and Φ_{PSII} values, suggesting that the efficiency of PSII photochemistry was dependent on plant N, but not on P, nutrition.

The most recognizable manifestation of nutrient enrichment in water is algal bloom, which leads to the impairment of water quality and its appearance. In this study, exogenous supply of $\text{NO}_3^- \text{-N}$ and $\text{PO}_4^{3-}\text{-P}$ promoted abundant algal growth in the control setups without plants. The increase in algal growth had a deleterious effect on water quality, resulting in significant increases in turbidity and pH. The increase in alkalinity was likely the consequence of the consumption of carbonate during algal photosynthesis (Sooknah and Wilkie, 2004). Efficient removal of $\text{NO}_3^- \text{-N}$ and $\text{PO}_4^{3-}\text{-P}$ by *P. amaryllifolius* effectively impeded algal proliferation, thereby drastically lowering water turbidity and maintaining the pH. The rapid uptake of $\text{NO}_3^- \text{-N}$ by the plant led to nutrient-deficient conditions that almost completely inhibited algal growth. Reduction of $\text{PO}_4^{3-}\text{-P}$ levels by *P. amaryllifolius*, although less efficient as compared to $\text{NO}_3^- \text{-N}$, also reduced algal growth by up to 86%, resulting in significantly lower water turbidity (68%–90%). Similarly, the growth of *P. amaryllifolius* plants also led to a 55%–70% reduction in water EC as N- and P-containing inorganic ions were removed from the waters via plant uptake. The improvements in water quality due to the remediation capability of *P. amaryllifolius* were comparable, if not better, to those reported in studies using the water fern and water lettuce (Forni et al., 2001; Lu et al., 2010).

It has been widely reported that the utilization of aquatic macrophytes, riparian plants and even ornamental hydrophytes to remove nutrients from urban wastewater offers a safe and effective alternative to conventional cleanup techniques (Abe and Ozaki, 1998; Shin et al., 2004; Zhang et al., 2007). The removal and disposal of biomass with the accumulated N and P appear to be an efficient technique to control freshwater eutrophication, as it terminates the nutrient cycles in aquatic systems (Vy-mazal, 2007). In this study, *P. amaryllifolius*, a terrestrial

plant species, was not only able to adapt to hydrophytic conditions but also exhibited very active NO_3^- -N and PO_4^{3-} -P uptake from eutrophic water. Its nutrient removal efficiency was shown to be significantly better than that observed for aquatic macrophytes, such as the *P. australis* and *Pennisetum purpureum* (Lin et al., 2002). Such high nutrient removal efficiency, in addition to the ability to accommodate land-water ecotones, makes the plant desirable for use on river banks for the natural attenuation of streams or fresh water bodies. The amphibious characteristic of *P. amaryllifolius* is particularly advantageous in the tropics as the plant can be highly adaptive to extreme fluctuations in water levels that can occur as a result of long dry periods, seasonal monsoons and flash floods.

It is important to note, however, that the uptake of nutrients by plants has been shown to be dependent on the hydraulic retention time with removal efficiencies improving with longer retention times (Hunter et al., 2001; Shin et al., 2004). In addition, interactions between N and other mineral nutrients, mainly P and K, are important because a deficiency in one may restrict the uptake of another, thereby affecting nutrient cycling and crop yield (Reid and Hayes, 2003). Hence, the remediative capability of *P. amaryllifolius* in mesocosms under variable flow conditions, as well as the effects of N-P limitations on nutrient mitigation and the influence of microbiota on nutrient uptake and fixation by the plant, should be investigated in future studies.

4 Conclusions

Successful management and control of eutrophication are based primarily on the restriction of nutrient inputs into the water body. Plants are not only beneficial for the assimilation and removal of excess nutrients, but they are also aesthetically pleasing and help to create an environment conducive for wildlife. In this study, *P. amaryllifolius*, a tropical terrestrial plant, was shown to adapt well to hydrophytic conditions and was highly effective in mediating high nutrient levels in an aquatic environment. The plants did not exhibit stress responses when grown in eutrophic conditions for up to 42 days, indicating that this species was well-adapted for growth in nutrient-rich habitats. The high NO_3^- -N and PO_4^{3-} -P removal efficiency of *P. amaryllifolius* resulted in rapid depletion of nutrients, particularly NO_3^- -N, which led to a significant reduction in the natural colonization of algae. The resulting impediment of algal growth had positive implications on the water quality. Thus, *P. amaryllifolius* exhibited good remediation potential for nutrient control and could be highly suited for use in freshwater systems to mitigate nutrient pollution from sources such as stormwater runoff, dairy farms and aquaculture.

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