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Growth characteristics and N accumulation of *Phragmites karka* in response to N addition: A candidate species for use in tropical riparian buffers

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ABSTRACT: High nutrient runoff from fertilizers and livestock practices can significantly degrade water quality and aquatic habitats. Riparian buffers act as a barrier zone, slowing down runoff and reducing the leaching of nutrients into aquatic ecosystems. Plants play an important role in nutrient removal via root uptake. But they may lose their effectiveness if they grow at very high levels of nutrients and pollutants. To find out how rising nitrogen levels affected the growth, biomass production, and N accumulation of the tropical reed (*Phragmites karka* (Retz.) Trin. ex Steud.), an experiment with nitrogen addition was conducted. Fifteen similarly-sized specimens were selected and grown in containers filled with 8.5 l of a standard growth medium. The N level was progressively increased by weekly additions of 1.4, 7, and 14 mg N l^{-1} (as NH_4NO_3) over 11 weeks. The results showed that N addition enhanced plant growth, total plant biomass, and N accumulation in leaves and roots. The plants showed a preference for NH_4^+ over NO_3^- as the N source. Under high N concentrations, the plants appeared to adjust their N demand by increasing tissue N accumulation, resulting in limited N removal capacities. Hence, our study demonstrates that *P. karka* positively responds to elevated N levels and can adapt to high N availability.

KEYWORDS: nature-based solutions, nitrogen removal, nutrient runoff, tropical reed, wetland vegetation

INTRODUCTION

Eutrophication is an environmental problem that is increasing along with the rapid growth of population and economic development. Agricultural non-point source pollution has been considered a primary factor accelerating the eutrophication process. For decades, rivers and lakes have experienced intensive discharge from agriculture and farmland, degrading water quality and consequently impairing aquatic ecosystems [1–3]. In Southeast Asia, cultivation and intensive use of fertilizers are major contributors to the loading of nutrients into water bodies. The nutrient leaching and runoff after rainfall and irrigation, particularly nitrogen, can cause changes in soil acidification, increase eutrophic levels, and degrade biodiversity [3]. Therefore, the effort to find a suitable method to reduce non-point source nitrogen in these areas has become of increasing interest. Traditional approaches to controlling non-point source nitrogen have focused on managing fertilization, reducing runoff emissions, and removing excess nitrogen by bioremediation [4, 5]. Freshwater riparian buffers, the interface between cultivated land and aquatic ecosystems, play an important role in reducing adverse impacts from upland runoff. They can slow down the water flow rate, promoting sedimentation and protecting riverbanks from erosion. Nutrients and pollutants are retained within the soil and reduced before being delivered directly into the water bodies. Previous studies found that riparian wetlands can reduce approximately 70–80% of nitrogen from agricultural runoff [6, 7]. Nitrogen retained in riparian soils can be removed by plant uptake and microbially mediated N transformation. Plants have contributed to N removal during periods of rapid growth, and N removal capacities rely on plant species and their performance in response to various environments. It has been reported that several wetland plant species have large effects on N removal in riparian wetlands, for example, *Carex* spp., *Phragmites australis* (Cav.) Trin. ex Steud., *Typha* spp. [8], *Phalaris arundinacea* L., *Salix* sp. [9], and *Zizanioides vetiveria* (L.) Nash ex Small [10].

Riparian buffers act as nutrient sinks, so the intensive drainage of N fertilizer can lead to nitrogen accumulation in the soil. Nitrogen enrichment can stimulate plant growth, particularly in invasive species. For example, a previous study found that high N availability increased the growth and biomass production of *P. australis*, in contrast to *Spartina pectinata* Bosc ex Link, which did not change growth under similar conditions. Opportunistic species with a high affinity for N can displace native species, thereby altering wetland plant communities [11]. However, there has been a decline in plant N uptake capacity in riparian zones throughout the last 20 years, which may be related to poor soil quality caused by overuse of N fertilizer [7, 12]. Another study found that an increase in nitrogen loading can cause a decrease in the N removal efficacy of switchgrass (*Panicum virgatum* L.

cv. Cave-n-Rock) buffer [13]. Thus, plants in riparian buffers must adapt to variations in N availability [14]. Consequently, their N removal effectiveness may be compromised under excessively high N levels. There is limited information on the response of wetland plant species to N enrichment in tropical riparian zones. Therefore, an improved understanding of plant traits and N acquisition that allows the plant to grow at higher N levels is needed.

Phragmites karka (Retz.) Trin. ex Steud, commonly known as tropical reed, is a species prevalent along riverbanks and in floodplains in tropical regions, including Thailand [15, 16]. *P. karka* has been recommended for preventing soil erosion [17, 18]. Little is known about the ecophysiology of this species, particularly its performance in high-N environments. Therefore, this study assesses the growth and biomass, root morphology, and N accumulation in the tissue of *P. karka* in response to gradual cumulative N addition. We hypothesize that (1) *P. karka* will show increased growth and biomass with rising N levels, but its growth will plateau at high N levels, and (2) the plants will adapt by modifying their root structure, for example by reducing root length and size. This adaptation should correlate with a decreased N demand, leading to reduced N uptake at high cumulative N levels. This study will provide knowledge on N nutrition of *P. karka* that can be used for predicting N removal efficiency in riparian buffers.

MATERIALS AND METHODS

Plant preparation and experimental set-up

Shoot stocks of *P. karka* were collected from canal banks adjacent to a road in the Mae Taeng District, Chiang Mai Province, Thailand. For propagation, approximately 300 mm of the basal stem from each shoot was selected. The leaves were removed, and the stems were then cut into 100 mm long segments, including a node. Each cut stem was placed in a sand-containing tray and watered every day. After approximately two weeks, new shoots and roots spouted from the nodes. Fifteen similarly-sized plants (4–6 g fresh mass, 160–230 mm tall) were selected for the experiment. Each new shoot was transplanted into a plastic pot ($\varnothing = 120$ mm × 200 mm height) containing gravel ($\varnothing \sim 20$ mm). Then, each pot was placed in a container built from polyvinyl chloride (PVC) pipes ($\varnothing = 150$ mm × 550 mm height) and filled with 8.5 l of a standard growth medium prepared according to Smart and Barko [19] supplemented with 0.1 mM KH_2PO_4 and 0.85 ml of a commercial plant micronutrients solution (Tropica, Denmark). Then, pH was adjusted to 6.5 using 1 M HCl and 1 M NaOH. Initially all plants were supplied with 1.4 mg N l^{-1} (as NH_4NO_3) for a week. Over an 11-week period, the nitrogen level was progressively increased with weekly additions of 1.4, 7, and 14 mg N l^{-1}, using NH_4NO_3 solutions, to create three treatments ($n = 5$). All plants were cultivated under greenhouse conditions at the Department of Biology, Faculty of Science, Chiang Mai University, Thailand. The light regime provided approximately 80% of full sunlight with a light:dark cycle of 10:14 h. Temperature was 26 ± 10 °C during the day and 14 ± 5 °C at night.

Medium nitrogen analysis

The concentrations of NH_4^+ and NO_3^- in the medium of each column were analyzed weekly before and after adding N to estimate the total N removal and medium N accumulation. A water sample (40 ml) was withdrawn from each container and then analysed for NH_4^+ and NO_3^-. The NH_4^+ and NO_3^- were measured using a modified salicylate method (Quikchem Method No. 10-107-06-3B; Lachat Instruments, Milwaukee, WI, USA) and a UV-method [20], respectively.

Growth study

On day 77, all plants were harvested, roots were removed from the gravel and cleaned with tap water. The number of shoots and their length were recorded. Subsequently, all plants were segregated into shoots and roots, then dried at 60 °C to constant weight using a hot-air oven. Some parts of the shoots were freeze-dried using a freeze-dryer (FreeZone 2.5 L Benchtop, Labconco, USA). The freeze-dried leaf materials were then used for chlorophyll analysis. The relative growth rates (RGRs) were calculated based on plant dry mass according to Evans [21].

Chlorophyll measurement

Chlorophyll *a* (Chl *a*) and Chlorophyll *b* (Chl *b*) were measured from homogenised leaf tissues. Eight milligrams of freeze-dried sampled were extracted with 96% ethanol (8 ml) and placed in the dark at room temperature. After 24 h, the absorbance of the extracts was determined at 648.6 nm and 664.2 nm using a UV-VIS Spectrophotometer (i3, China). Concentrations of Chl *a* and Chl *b* were calculated according to Lichtenthaler [22].

Inorganic nitrogen and C/N analysis

Inorganic nitrogen (NH_4^+ and NO_3^-) in dried leaf and root materials was analysed by hot water extraction. Five mg of homogenised subsamples were extracted with 15 ml of distilled water at 80 °C for 20 min. NH_4^+ and NO_3^- concentrations in the extracts were determined by using a modified salicylic method (Quikchem Method No. 10-107-06-3-B; Lachat Instruments) and UV-method [20], respectively.

Total carbon (C) and total nitrogen (N) in the plant tissue were analysed. Three milligrams of the fine-ground plant materials (leaves, roots) were placed into tin foil capsules. Then C and N were analysed by a CN analyser (Leco, CN828, Saint Joseph, Michigan, USA). The nitrogen accumulation in leaves and roots

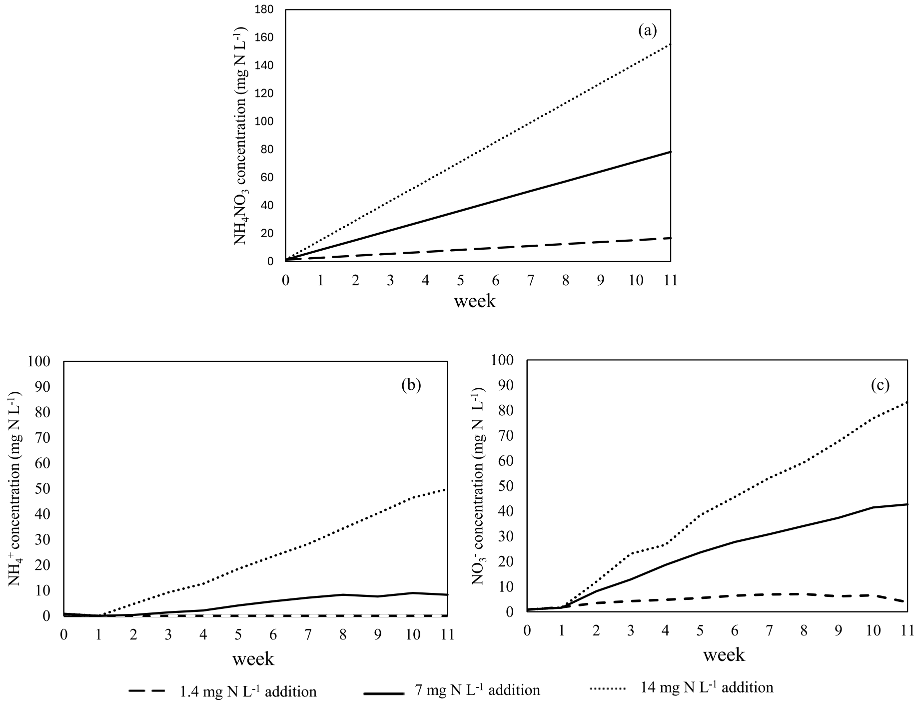

Fig. 1 Ideal accumulative nitrogen in growth medium (a), accumulative NH_4^+ (b) and NO_3^- (c) in the growth medium during the experimental period.

were calculated by multiplying their N content and dry biomass. The nitrogen use efficiency (NUE) was evaluated as the ratio between plant biomass production and the amount of N supply in the systems (mg plant DW mg^{-1} N supply).

Statistics

The statistics was performed using Past326b software [23]. All data were tested by one-way analysis of variance (ANOVA). The multiple comparisons between the groups at the 5% significance level were performed using Dunn's post hoc test.

RESULTS

Accumulative N in medium

Nitrogen accumulated in the growth medium over time, with higher levels of NO_3^- compared to NH_4^+ (Fig. 1). In the low-N addition (1.4 mg N l^{-1}) treatment, NO_3^- slightly increased after N was added, whereas NH_4^+ was completely removed. At the higher-N addition treatments, both NO_3^- and NH_4^+ accumulated in the medium. Treatments receiving N adding at 7 and 14 mg N l^{-1}, NO_3^- concentrations increased up to approximately 32 mg N l^{-1} (3 mM) and 84 mg N l^{-1} (6 mM), while NH_4^+ concentrations increased to approximately 8.4 mg N l^{-1} (0.6 mM) and 49 mg N l^{-1} (3.5 mM), respectively, after 11 weeks.

N removal

Over the experimental period, total inorganic N removal from the systems was significantly different among treatments (Fig. 4b). The N removal capacity was significantly increased with increasing medium N concentrations. However, the N removal capacity of the system receiving high N adding at 14 mg N l^{-1} significantly decreased by approximately 20% compared with the system obtained at 7 mg N l^{-1}.

Plant growth study

Overall, the growth of *P. karka* significantly increased with escalating levels of N addition. At low-N addition, the RGRs of the plants were significantly lower than that of the plants grown at higher-N addition (Fig. 2a). The plants with high growth rates also had greater biomass production, particularly the shoot biomass which were nearly 3 times higher than the plants grown at low-N addition (Fig. 2c). As the result, the high-N added plants had high shoot:root ratios (Fig. 2d). Likewise, total plant height significantly increased in the plants received N adding at 1.4 and 14 mg N l^{-1} (Fig. 2b). However, root length tended to decrease in the plants exposed to higher N levels (Fig. 4a).

Chlorophylls

Chl *a* concentration increased in the plants received high-N addition (Fig. 3a). Similarly, Chl *b* concentra-

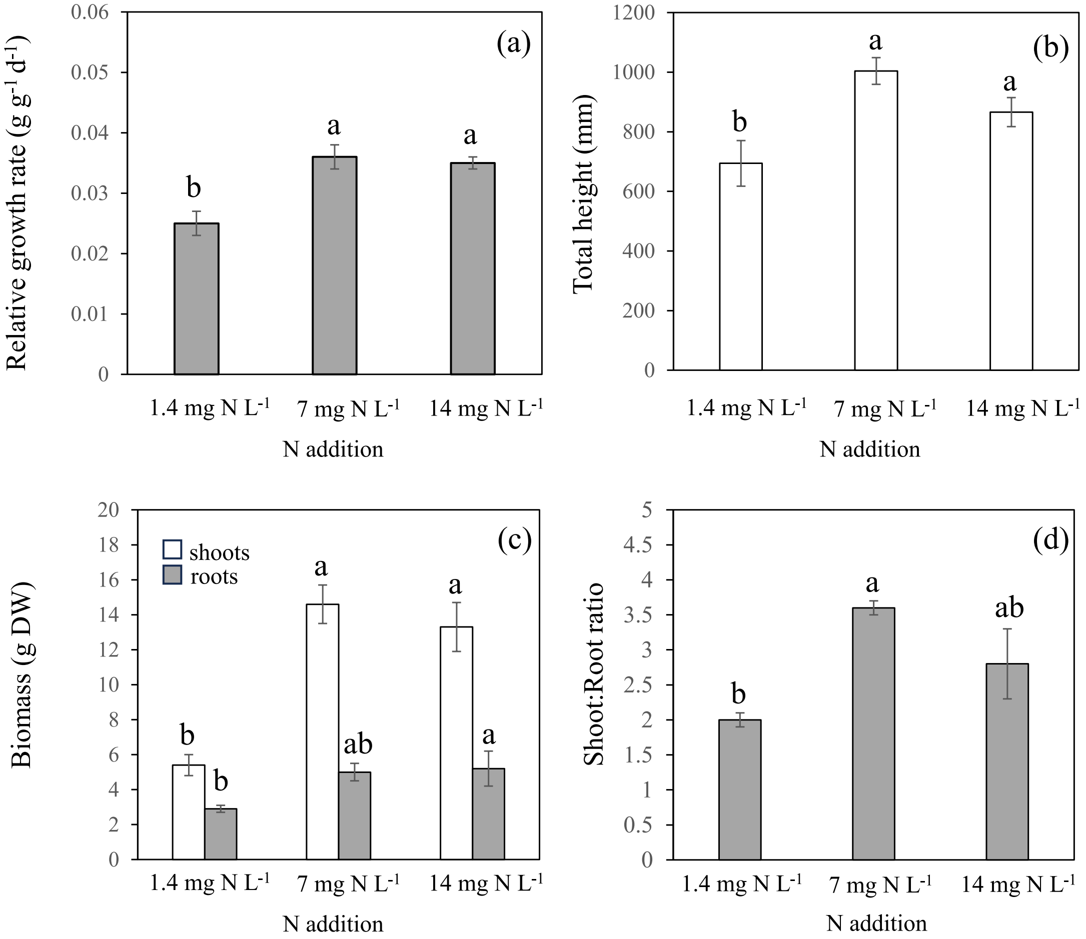

Fig. 2 Relative growth rate (a), total height (b), biomass (c) and shoot:root ratio (d) (mean ± SE) of *P. karka* grown at different N addition (1.4, 7, and 14 mg N l^{-1} as NH_4NO_3). Different letters above columns indicate significant differences between treatments.

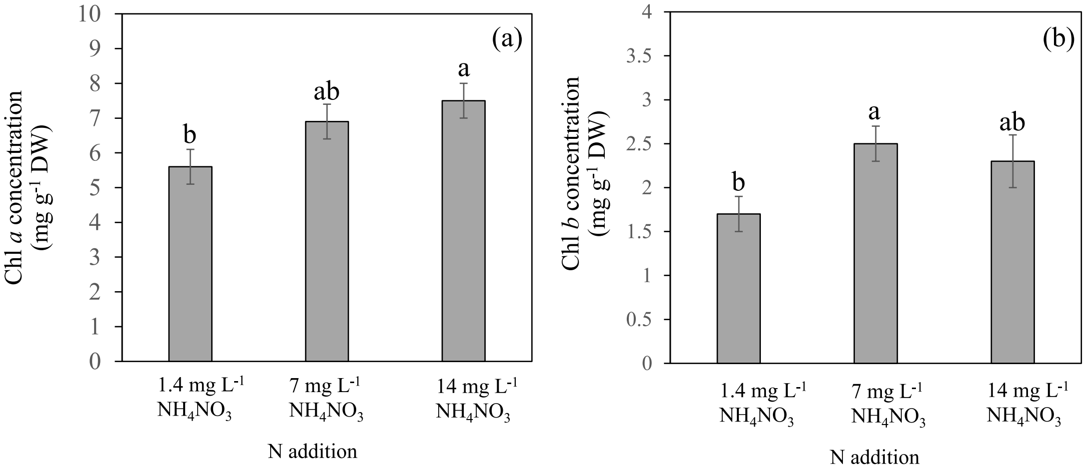

Fig. 3 Concentration of chlorophyll *a* (a) and chlorophyll *b* (b) (mean ± SE) of *P. karka* grown at different N addition (1.4, 7, and 14 mg N l^{-1} as NH_4NO_3). Different letters above columns indicate significant differences between treatments.

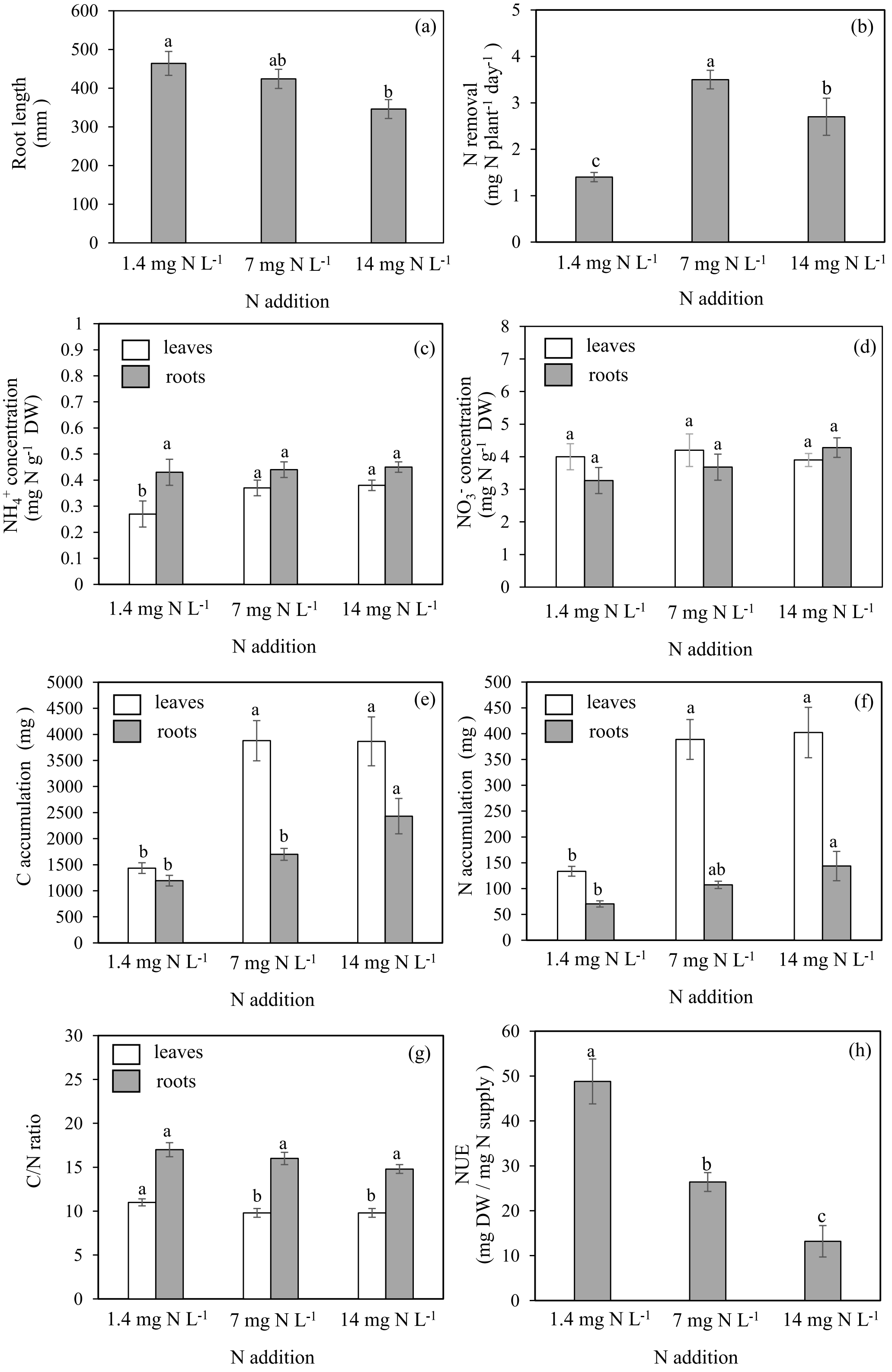

Fig. 4 Root length (a), N removal (b), NH_4^+ concentration (c), NO_3^- concentration (d), total C accumulation (e), total N accumulation (f), C/N ratio (g) in leaves and roots, and nitrogen use efficiency, NUE (h), (mean ± SE) of *P. karka* grown at different N addition (1.4, 7, and 14 mg N l^{-1} as NH_4NO_3). Different letters above columns indicate significant differences between treatments.

tion increased in the plants grown at the medium- and high-N addition treatments (Fig. 3b). According to the increase of Chl *a* concentration in the high-N addition, total chlorophylls concentration also increased.

N in the tissue and C/N ratio

There was high NO_3^- accumulation in both roots and leaves and no significant difference across treatments (Fig. 4d). By contrast, at high-N addition (14 mg N l^{-1}), NH_4^+ concentrations in leaves were significantly higher than in the plants grown in the 1.4 mg N l^{-1} addition treatments. However, NH_4^+ concentrations in roots were not significantly different among treatments (Fig. 4c). The total C and N accumulation in leaves and roots significantly increased with increasing N addition (Fig. 4e,f). Leaf C/N ratios significantly decreased in the plants received higher-N addition, but no significant difference in root C/N ratios (Fig. 4g). Also, the significantly decreased in the NUE was found when the plants received higher-N addition (Fig. 4h).

DISCUSSION

In this study, we observed that N concentrations in the growth medium increased with higher-N additions, leading to significant N accumulation, especially in the high-N addition treatments. Notably, NO_3^- accumulated more than NH_4^+, aligning with the established preference of most wetland plants for NH_4^+ due to its lower energy requirements for uptake [24]. In the low-N addition treatment, we found that NH_4^+ was completely absorbed by the plants, while NO_3^- accumulated in the growth medium. Similar results were found in the higher-N addition treatments showing that accumulated NO_3^- was much higher than NH_4^+. It appears that *P. karka* prefers NH_4^+ likely because of the lower energy costs associated with NH_4^+ uptake and assimilation. This preference is consistent with the natural growth environment of *P. karka*, typically along riverbanks or in flooded littoral zones where NH_4^+ is usually the dominant N-form. The initial response of the plants to NH_4^+ was positive, indicating a good performance with NH_4^+ supply. The study by Tylova-Munzarova et al [25] revealed that *P. australis* had high uptake capacity for NH_4^+ compared to NO_3^- even though this species can grow well in both NH_4^+ and NO_3^-. It was suggested that *P. australis* show growth plasticity with respect to N form and shared characteristics of wetlands species which grew in water-saturated soil where nitrification is restricted. As a result, NH_4^+ is typically preferred over NO_3^-. Our study also observed positive responses of *P. karka* to nitrogen addition. Specifically, plant growth and biomass production increased with increasing N. The relative growth rates of the plants grown at 7 and 14 mg N l^{-1} addition were about 44% higher compared to those grown in the 1.4 mg N l^{-1} addition, indicating that this species adapts well to high-N conditions. Similar results were found in *P. australis* which increased growth and could displace a native species like *S. pectinata* in N-rich environments [11]. Previous studies have documented that wetland plant species which are strong competitors, generally respond positively to N addition. The ability to take advantage from increased N can encourage them to colonize N-rich habitats [26]. However, it is important to note that excessive cumulative N, particularly NH_4^+, can adversely affect plant growth. In our study, when medium NH_4^+ concentrations reached 49 mg N l^{-1} (3.5 mM), a reduction in shoot growth of *P. karka* was observed along with a significant reduction in root length. Nevertheless, chlorophyll concentrations did not significantly decrease with increasing N addition, thus no symptoms of NH_4^+ toxicity such as chlorosis were observed. This response has also been found in other wetland plants species that tolerate high NH_4^+ concentrations [27, 28].

Riparian buffers typically receive pollutants from surface runoff. They can suffer from intensive anthropogenic disturbance, particularly agricultural activities [7]. The high nutrient loading often exacerbates the enrichment of nitrogen in soil, affecting plant growth and their ability to remove N in riparian buffers [13, 29]. A study by Zhang et al [30] showed that *Carex schmidtii* Meinsh. had reduced growth and biomass production after being supplied with high-N concentrations (10 mg N l^{-1}). The plants had small leaves and short roots compared with the control. The excess N inhibited plant growth and affected the plant morphology and function. In our study, the plants grown at low-N addition had low growth rates compared with the plants grown at higher-N addition. The growth of plants was triggered when NH_4NO_3 was applied at 7 mg N l^{-1} per week, and no change in growth was observed in the plants when receiving N at 14 mg N l^{-1}. This showed that increased nitrogen can enhance the growth capabilities of *P. karka*. However, the growth rates seem to be impacted when N supplies are overstretched. Down-regulation of N uptake, influenced by internal N demand, has been documented [31–33]. In this study, at high-N addition, the plants accumulated higher N concentrations, particularly in leaves, which may reduce N demand and consequently lower N removal capacity. This is consistent with observed changes in root morphology, where plants exposed to high-N addition exhibited reduced root length. Under such conditions, the incentive for root elongation is diminished, as N availability is no longer limiting. This morphological shift reduces root surface area available for nutrient uptake, therefore decreasing N uptake rates even when external N availability remains high [34]. As a result, leaf C/N ratios decreased, and NUE declined with long-term N addition. It indicates that *P. karka* can modulate N acquisition when faced with increased nitrogen. A similar result has been reported in *P. australis* growing with increasing N availability [35, 36]. Moreover, the study by Luo and Zhou [37] showed that six species of *Popularis* had high NUE when growing at low N supply, but the NUE was significantly reduced with increasing N availability. In this study changes in root traits were influenced more by genotype than N levels. Consequently, the plant's N nutritional status had a greater impact on the NUE than root morphological traits. Due to its high biomass production, high N accumulation, and tolerance to elevated N levels, *P. karka* appears to be a strong candidate species for nitrogen mitigation in riparian buffer zones. For comparison, other wetland species such as *P. australis* and *P. arundinacea* have also demonstrated effective N removal capacities in similar environments. For instance, *P. australis* has exhibited high N removal rates in constructed wetlands, ranging from 4.0 to 6.8 mg N plant^{-1} day^{-1} [38, 39]. While *P. arundinacea* has shown N removal of approximately 3.2 mg N plant^{-1} day^{-1} [38]. In this study, *P. karka* demonstrated a comparable N removal capacity of up to 3.5 mg N plant^{-1} day^{-1}. Although microbial diversity and activity related to N removal were not assessed in this study, rhizosphere microorganisms play a key role in N cycling, particularly through nitrification and denitrification processes that govern NO_3^- and NH_4^+ availability to plants. Therefore, shifts in soil microbial communities can alter N transformation and availability [40]. Moreover, N-fixing microorganisms, including cyanobacteria can also contribute to N-fixation that affects plant N uptake and ecosystem N availability [41]. These microbial activities collectively influence plant N uptake and overall removal efficiency in riparian systems [42]. Hence, future studies incorporating microbial dynamics will offer a more comprehensive understanding of *P. karka*'s potential in nitrogen mitigation.

CONCLUSION

Nitrogen availability has a significant influence on growth, biomass production, and N removal efficiency in *P. karka*. Overall, the species demonstrates better growth and biomass production with NH_4^+ over NO_3^- as its nitrogen source. Under high nitrogen conditions, *P. karka* accumulates considerable amounts of total N in its tissues, particularly in the leaves. However, this accumulation is accompanied by a decline in N removal efficiency. These findings indicate that *P. karka* adjusts its N acquisition strategies to support growth under N-enriched conditions. Nevertheless, the use of lab-scale mesocosm with a limited sample size does not fully replicate the complexity of natural riparian buffer systems, which include factors such as soil-microbe interactions, fluctuating water regimes, and variable nitrogen loading. Consequently, future field-based research in N-enriched environments is necessary to evaluate the true potential of *P. karka* for mitigation under realistic ecological conditions.

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