frontiers | Frontiers in Ecology and Evolution

TYPE Original Research
PUBLISHED 03 March 2023
DOI 10.3389/fevo.2023.1139053

OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to
Ecophysiology,
a section of the journal
Frontiers in Ecology and Evolution

RECEIVED 06 January 2023
ACCEPTED 15 February 2023
PUBLISHED 03 March 2023

CITATION
Luo Y, Chen Q, Liu F and Dai C (2023) Both species richness and growth forms affect nutrient removal in constructed wetlands: A mesocosm experiment.
Front. Ecol. Evol. 11:1139053.
doi: 10.3389/fevo.2023.1139053

Both species richness and growth forms affect nutrient removal in constructed wetlands: A mesocosm experiment

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Introduction: Plant richness is thought to improve the function of constructed wetlands (CWs), but most CWs are planted with monocultures, with only a few employed polycultures, which have drawn contradictory conclusions. We suppose functional diversity is the key to better performance of plant communities and hypothesize that CWs planted with diverse growth forms are superior in plant growth and nutrient removal.

Methods: In this study, six emergent plant species categorized into slender type (*Schoenoplectus tabernaemontani, Typha orientalis*), fan type (*Iris sibirica, Acorus calamus*) and large type (*Canna indica* and *Thalia dealbata*) were planted in monocultures, combinations (two species of the same growth form) and mixed polycultures (six species of three growth forms). We then compared how plant growth and nutrient uptake differed among treatments.

Results: It showed that the polyculture considerably increased the removal of total nitrogen (TN) and total phosphorus (TP), but the combination did not outperform monoculture. High consistency in the patterns between underground biomass and total biomass indicated that plant roots were essential for nutrient consumption. Compared with slender and fan plants, the large plants had a greater biomass increase in polycultures, which greatly accelerated the absorption and assimilation of TN and TP.

Conclusion: Our study indicated that plant community with various growth forms reduced the intensity of interspecific competition, increased the functional diversity, and greatly enhanced the ability of pollutant removal. Our results also provide some suggestions for plant selection and combination designs in CWs.

KEYWORDS
growth type, nitrogen, phosphorus, plant richness, sewage treatment, urban wetlands

1. Introduction

Urban wastewater has become a global concern due to the rapid progress of urbanization. A significant amount of untreated domestic and industrial wastewater loaded with high levels of nitrogen (N) and phosphorus (P) has been discharged into grounds, rivers, and lakes, gravely harming urban ecosystems with water bloom as one of the most prominent consequences (Villar-Navarro et al., 2018; Russo et al., 2019; Wurtsbaugh et al., 2019). Constructed wetlands (CWs) have been widely used because they are economic and high-efficient compared with traditional approaches. Planted CWs are more efficient than unplanted CWs in wastewater treatment (Paranychianakis et al., 2016; Zhu et al., 2018) because plants play an important role in assimilating nutrition such as N and P and help with nutrient retention (Geng et al., 2017). In addition, plant roots create a natural biofilm filtering system, which not only provides suitable habitats for microorganisms, but also exhibits large surface areas to reduce flow velocity and promote pollutant sedimentation (Carballeira et al., 2016; Sandoval et al., 2019).

Plant selection is a key when constructing CWs, because there may be species-specific effects on biomass accumulation, contaminant removal, and ornamental value (Long et al., 2016; Monokrousos et al., 2020). The most commonly used plants in CWs are *Phragmites australis*, *Typha latifolia*, *Canna indica*, and *Cyperus papyrus*, owing to their strong adaptability and high pollutant removal potential (Wu et al., 2019; Marín-Muñiz et al., 2020). Another component influencing the rate of pollutant removal is interspecific interaction when more than one species is grown in CWs, the outcome of which is typically dependent on tolerance and competitiveness. For instance, in a polyculture-CW, *Agapanthus africanus* had fewer new shoots and shorter stems than others and completely vanished from the community after 1 year of operation, possibly due to its weak competitive ability and slow growth (Calheiros et al., 2015). The competitiveness of *Pistia stratiotes* was found to be lower than that of *Phragmites karka* and *T. latifolia*, which resulted in a lower growth rate (Kumar et al., 2022). In comparison to a CW with *Oenanthe hookeri* and *Reineckia carnea*, the removal efficiency of P in a CW with *O. hookeri* and *Rumex japonicas* was much higher (Geng et al., 2017). All the above studies reveal that both species composition and interaction are crucial to the functioning of CWs.

It is generally considered that higher plant diversity enhances ecosystem resilience, stability, interference resistance, and pollutant removal rate (Hautier et al., 2018; Zhang et al., 2021). It then can be extrapolated that CWs in polycultures should outperform those in monocultures. The main reasoning is that the complementarity of different plant species across time, space, and function can make the CWs more resistant to environmental changes for a longer period of time (Zhang et al., 2010; Kumar et al., 2021). Additionally, diverse root morphology and the vertical distribution and layering in polyculture CWs increased the contact area with pollutants (Liang et al., 2011; Marchand et al., 2014), aided the absorption and storage of nutrients, as well as speeded up the decomposition of organic matters associated with elevated root exudates and microbial communities (Arslan et al., 2017; Hussain et al., 2018). However, many studies found that polyculture CWs were not different from or superior to monocultures in nutrient removal. When Rodriguez and Brisson (2016) compared the removal rates of N and P in the monoculture and polyculture of *P. australis* and *Phalaris arundinacea*, the monoculture of *P. australis* enjoyed a comparable or higher removal rate than polyculture. Furthermore, there was no significant difference in N removal between monoculture and polyculture composed of *C. papyrus* and *Zantedeschia aethiopica* (Leiva et al., 2018). The lack of difference was also found for the monoculture of *C. indica* and polyculture with *C. flabelliformis*, *P. australis*, *Pennisetum purpureum*, and *Hymenocallis littoralis* (Liang et al., 2011). It is worth mentioning that in the studies above, although more than one species was planted, they did not differ much in individual sizes or shapes. Such a mixture of similar species may not increase the functional richness of CWs, rendering intense interspecific competition and reduced growth in both parties (Samal et al., 2017; Leiva et al., 2018). Functional diversity is a biodiversity indicator to explore the connection between biodiversity and ecosystem function. It can reflect the functional differences of communities and be evaluated based on morphology, physiology, and anatomy (Steudel et al., 2016). Moreover, it is the fundamental driver of how biodiversity affects ecosystem function (Petchey and Gaston, 2002; Poos et al., 2009). Therefore, when building CWs, polycultures with functional diversity should be considered, which might minimize the degree of niche overlap, increase complementarity, and improve nutrient uptake.

Plant growth form, an ecological classification based on the morphology, appearance, and structure of plants (Wang et al., 2020), provides an informative indicator for evaluating functional diversity. It is a comprehensive reflection of the growth conditions and genetic background of plants, and to a certain extent, it reflects the strategies of adaptation in different ecological niches (Antos et al., 2021). Diversified growth forms in a plant community usually result in positive feedbacks (Dell'Osbel et al., 2020; Kumar et al., 2021). Indeed, differences in nutrient demands can elevate complementarity (Ma et al., 2017; Ediviani et al., 2018). Diverse growth rhythms could also extend the period for nutrient uptake across seasons with efficient nutrient retention (Manolaki et al., 2020). To date, most planted CWs are monoculture or with a low plant richness, and only a handful of researches employed polyculture, which has drawn contradictory conclusions (Aguinaga et al., 2018; Fahim et al., 2021; Carrillo et al., 2022). Very few studies have considered polyculture with various growth forms, in order to increase the functional diversity of plants and the nutrition turnover rate of CWs (Calheiros et al., 2015).

In this study, we are interested in how growth forms affect species interactions, which in turn influence biomass accumulation and nutrition removal. We selected six commonly-used species in CWs and categorized them into three growth forms: the slender, fan, and large types (Figure 1A). Growing them with different combination designs in CWs, we test three hypotheses. First, polycultures with various growth forms might be the most efficient in removing N and P from water. Because when the six species are mixed, the complementarity of interspecific relationships and the diversity of functional groups will promote plant growth and hence a greater nutrient removal rate (Zhang et al., 2010; Kumar et al., 2021). Second, an increase in plant richness with the same growth form should have little impact on water purification. In such a plant mixture, although species richness is increased, the functional diversity stays the same. We expect to see intense interspecific competition between plants of the same growth type (Samal et al., 2017; Leiva et al., 2018). Thus, there should be no evident increase in biomass accumulation or nutrient removal compared to monoculture. Third, plants of the large shape may present a higher ability in nutrient removal, because large-sized plants are usually with substantial biomass, complex subterranean systems, fast growth, rapid community establishment, and good tolerance (Licata et al., 2019 ; Kumar et al., 2022), all contributing to higher nutrient absorption rates. Therefore, they might be particularly useful in CWs given appropriate densities and accompanying species.

2. Materials and methods

2.1. Constructed wetland

The experiment was carried out in the greenhouse at the Wuchang campus of Hubei University (Wuhan, China) near

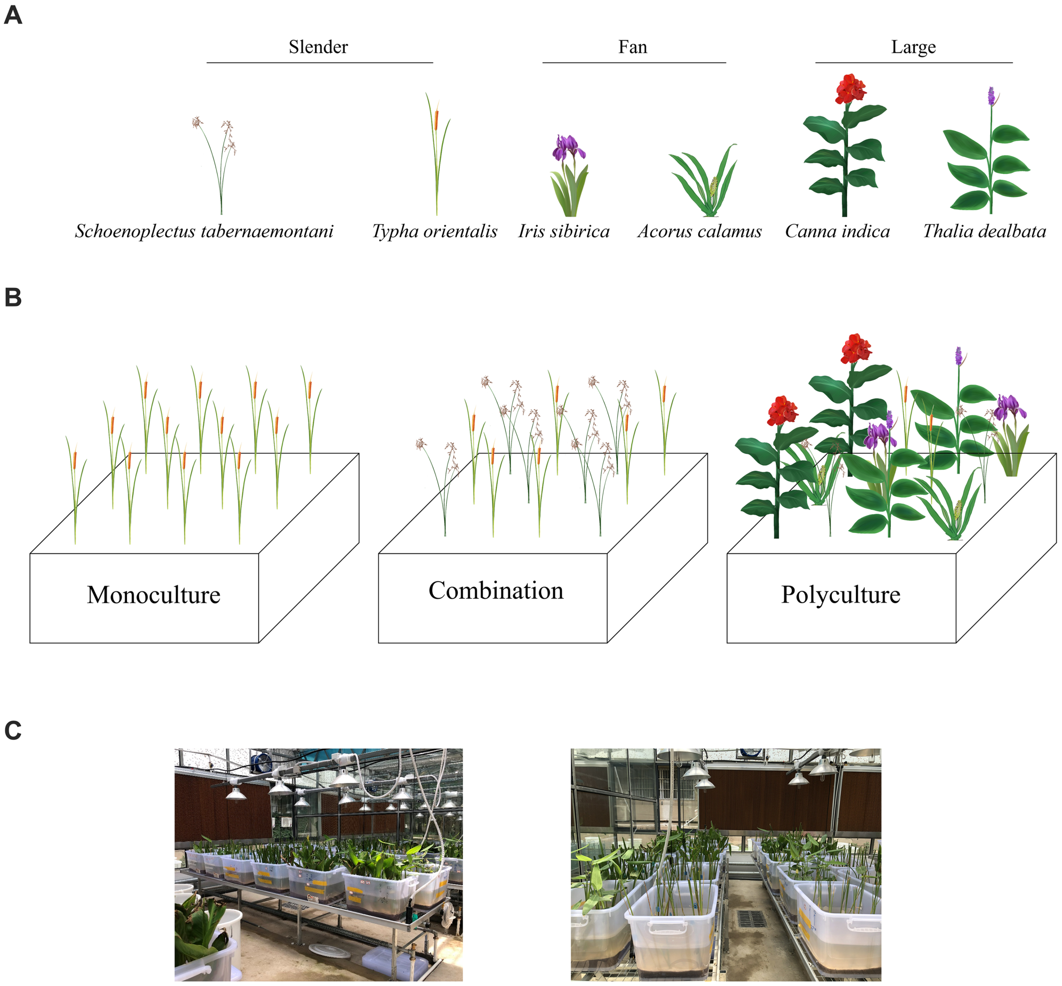

FIGURE 1
Plant species selection and the experimental setting: **(A)** drawings of six species and their growth form categories; **(B)** the layout of three treatments in culturing tanks (the location of different plant species in combination and polyculture was randomized); **(C)** growing condition in the greenhouse. Please note that all species have reproductive structures drawn for the ease of recognition, which did not represent the developmental state of individuals during culturing. See texts for detailed information.

School of Resources and Environmental Science (30°34′46′′N, 114°19′40′′E) in the year 2021. The room used in the greenhouse is 50 m^2 in size and contains four $1 \times 4\,m^2$ beds. Three overhead sodium lamps were used as supplementary lighting for each bed. Wet curtains and ventilating fans worked together to keep the moisture and temperature appropriate for plant growth. On average, the temperature was around 30°C during the day (8:00–20:00), and 25°C at night (20:00–8:00), whereas the humidity was about 50%. In this study, surface-flow CWs were built in plastic tanks of $63 \times 45 \times 39\,cm^3$ (length × width × depth). Sand was added to the bottom to a depth of 8 cm for plant anchorage. Before usage, all sand was washed 5–6 times with tap water until the solution turned clear to avoid any impurities or soluble minerals from influencing the results. To completely eliminate the remained forms of N and P, the sand was immersed in 0.2 mol/l diluted hydrochloric acids for 1–2 days and then successively rinsed with tap water and distilled water.

2.2. Plant selection

In order to choose plant species to construct experimental wetlands, we came up with three criteria that plant species should be: (1) commonly used in CWs and easily acquired; (2) emergent with ornamental values; and (3) of different growth forms. Thus, six common aquatic species were chosen with three growth forms, namely, the slender type (*Schoenoplectus tabernaemontani*, *Typha orientalis*), the fan type (*Iris sibirica*, *Acorus calamus*), and the large type (*Canna indica* and *Thalia dealbata*) (Figure 1A). Plants of slender growth form grow longitudinally with long and slender leaves. Two species of the fan form share fan-shaped leaf arrangements, usually with a flat and short appearance, whereas the large type is of big size, with dense leaves and massive rhizomes, and mature plants can reach 1.5–2.0 m tall. We bought seedlings of the six species from the same nursery and trimmed all into a certain height (slender: 45 cm; fan: 45 cm; large: 50 cm). Before planting, we made sure no extra minerals from leaves or roots were brought into culturing tanks by washing the whole plants 2–3 times with tap and distilled water. We also measured the initial fresh weight of each seedling.

2.3. Experimental design

Three treatments—monoculture, combination, and polyculture—representing one species, two species of the same growth form, and six species of three growth forms—were set up in this experiment (Figure 1B). After careful pilot trials, it was decided that 12 seedlings to be planted (3 × 4) in each culturing tank, with approximately a planting density of 44 plants/m^2. Because the main purpose of our study was to discover the effect of plant richness (with niches overlapping or diverse growth forms), we made sure that each treatment had the same number of repeating tanks ($n = 18$). For the polyculture treatment, each tank had all six species mixed and two seedling of each species were planted with randomized locations. In the monoculture, each species was repeated for three culturing tanks, together accounting for 18 replications. In the combination, each growth type (slender, fan, large) composed of two species was repeated for six tanks, and six seedlings of each species were planted per tank. In such a manner, not only did the monoculture and combination have the same number of growth types represented at tank level, but also the number of seedlings per plant species was balanced ($n = 36$) across three treatments. All 54 culturing tanks were evenly distributed among four beds in the greenhouse (Figure 1C). To plant all seedlings, each tank was filled with 30 L of 10% Hoagland nutrient solution (HB8870-1, Qingdao Haibo Biotechnology Co., Ltd.) at the initial stage. In order to replace dead plants and maintain an equal number of each species throughout the experiment, several extra plants of each species were simultaneously grown alongside. Seedlings were planted on July 6 and harvested on September 13, 2021. As a portion of plants showed tissue degradation after 2 months of cultivation, which might affect the effectiveness of nutrient removal if left untreated, we decided to end the experiment then.

In order to simulate the eutrophic state of urban sewage, additional N and P in the form of ammonium chloride (NH_4Cl) and potassium dihydrogen phosphate (KH_2PO_4) were added several times during plant growth (Figure 2). Our plan was to add 3 liters of nutrient solution with 25 mg/L N and 3 mg/L P (that is, 75 mg of N and 9 mg of P) every 5 days from July 25 (about 20 days after all seedlings were planted). Following each addition, the water volume in each tank was refilled with distilled water to 30 L. There were some modifications according to the growth condition and nutrient dynamics in culturing tanks throughout the course of the experiment (see Figure 2).

2.4. Data acquisition

To record the dynamics of nutrients with the growth of plants in the CWs, we kept measuring of N and P concentrations from the initial phase (several hours after plants were settled) to the end of the experiment. Each measurement was taken after the addition of N and P when the tanks were metered to a constant volume of 30 L. To collect water samples, we drew 20 mL of water from 5 sites within each tank, namely four corners and the center, and mixed them together for measurement. Total nitrogen (TN) and total phosphorus (TP) concentrations were measured by alkaline potassium persulfate digestion UV spectrophotometry method and ammonium molybdate spectrophotometry method, respectively. Total removal (TR) of N and P were calculated as follows:

$$TR_{(N,P)} = C1_{(N,P)} * V + A_{(N,P)} - C2_{(N,P)} * V$$

where C1 is the initial concentration; A is the total amount added; C2 is the final concentration; V is the total volume of culturing solution (=30 L).

In addition to the concentration of N and P, we also measured water dissolved oxygen (DO) concentration and pH in all tanks with a portable water quality analyzer (HQ40d, HACH). Ten measurements were taken once every 5 days across the experimental period. Each measurement was conducted between 9 and 11 a.m. and the probes were placed at the center of culturing tanks, roughly 5 cm below water surface. The probes were washed and dried between measurements.

At harvest, all plant individuals were cleaned and air-dried, after which final fresh weights were measured. Each plant was then cut into two parts: above- and below-ground. The dry biomass of above-ground, below-ground, and total were weighed and calculated after they had been placed in the drying oven for 48 h at 70°C. The fresh weight increase (FWI) was calculated by comparing final and initial states. During the process of the experiment, several plants died out of no clear reasons. In order to keep all culturing tanks at constant density, we replaced the dead plants with alive ones of similar sizes right away. However, strict controls over plant weight were not possible, which is why some FWIs turned to be negative.

2.5. Statistical analyses

All analyses were done using R version 4.0.2 (R Core Team, 2020) and packages including car (Fox and Weisberg, 2019), psych (Revelle, 2018), lmerTest (Kuznetsova et al., 2017), and emmeans (Lenth, 2022).

For plant biomass variables, each plant individual was an experimental unit. We initially added factors of "planting treatments" (monoculture, combination, and polyculture), "growth forms" (slender, fan, and large types), and "species nested within growth forms" in linear models. However, as the interactions between treatment and growth form, and between treatment and species turned out to be significant, we tested the effects of treatment in three hierarchical levels. First, we employed linear mixed models to test whether biomass was different among treatments by controlling species as a random effect to account for different growing patterns among species. Second, data were separated into three growth forms and the same model was used as above. Third, data were separated into six plant species and treatment effects were tested with ANOVA.

For N and P removal, each culturing tank was an experimental unit. One-way ANOVAs were used to test the effects of planting treatment on the total removal. Furthermore, to simultaneously test the effects of treatments and growth forms, only monoculture and combination tanks were included (polyculture tanks had diverse growth forms and thus were not applicable). Models were initially added with the interaction between treatments and growth forms, which showed a lack of significance and thus were removed. Hence, both variables were tested with two-way ANOVAs.

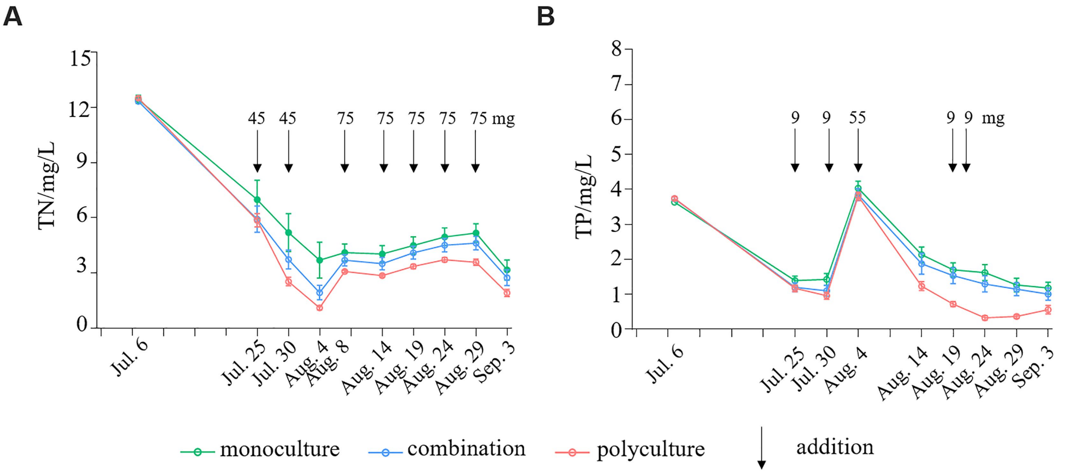

FIGURE 2
Concentration changes of total nitrogen [TN, **(A)**] and total phosphorus [TP, **(B)**] in culturing tanks during the whole experimental period under different treatments.

For water pH and dissolved oxygen (DO), ten measurements of each culturing tank were taken evenly spaced throughout the growing period, across which 5–7 times of nutrition addition were also conducted to disrupt the physicochemical property of the water. Thus, each measurement was regarded as relatively independent, and each measuring time was a block. Linear mixed models were used to test the difference in pH and DO among treatments. To further examine the effects of growth forms, only tanks of monoculture and combination were considered. Since the treatment factor might persist in the sub-dataset, its interaction with growth forms was tested for pH, which was not significant and removed. But for DO, the interaction term was significant, indicating altered response patterns of three growth forms between monoculture and combination. We, therefore, separated the dataset by treatment and looked into the effect of growth form individually. Results showed that in both treatments, the DO of those tanks with large growth forms was significantly lower than others. The only difference was that the degree of decrease appeared stronger in the combination treatment. Given the exact same trend and for a concise result presentation, we employed the same linear mixed model for both pH and DO, where "growth form" and "treatment" were fixed factors and "measuring time" a random factor.

In all ANOVAs, we have made sure the homogeneity of variances and normality of errors were met (pH was log-transformed). Only a few violations of homoscedasticity were detected, and data transformation did not make it better. But given significant differences among treatments and quite similar sample sizes, we considered the results trustable. Least-squares means and standard errors were reported for variables analyzed with mixed models using the package emmeans, otherwise, parametric means were reported. Significance in mixed models was evaluated by Type III Satterthwaite's method using the package lmerTest. *Post-hoc* comparisons were tested with Tukey adjustment.

3. Results

3.1. Plant biomass

Total dry biomass (TDB) showed significant differences among treatments ($F_{2,\,610} = 5.40$, $p = 0.005$), growth forms ($F_{2,\,610} = 360.15$, $p < 0.0001$) and species ($F_{3,\,610} = 96.17$, $p < 0.0001$). Compared with monoculture and combination, TDB of polyculture was significantly higher (Table 1; Figure 3A). The changes in TDB for each growth form under three treatments varied as well (Table 1; Figure 3B). While the TDB of polyculture was significantly lower than that of monoculture and combination in the case of slender plants, there was no significant difference among the three treatments in the fan-shaped group. Yet, the TDB of large plants in polyculture was significantly higher than in monoculture and combination. Regardless of the treatments, the TDB of the large type was always higher than that of slender and fan (Figure 3B). The TDB of each species also responded differently to treatments (Table 1; Figure 3C). For *S. tabernaemontani*, plants grown in polyculture had significantly lower TDB than those in combination. Compared to monoculture, the TDB of *T. orientalis* and *I. sibirica* were significantly reduced in polyculture. Plants of *A. calamus* exhibited no significant difference in TDB among three treatments. The TDB of *C. indica* and *T. dealbata* in polyculture was significantly higher than in monoculture and combination. Both of large growth form, *C. indica* had a higher TDB than *T. dealbata*.

When TDB was divided into aboveground (ADB) and belowground (BDB) components, the variations in dry biomass among treatments, growth forms, and species were also evident (Figures 3D–I). By comparing them with the patterns of TDB, it demonstrated great consistency in treatment effect on BDB and TDB. First, among the treatments, polyculture had a stronger impact on plants' dry biomass. Second, among different growth forms, the large type typically had a higher degree of increase in biomass. This was also true when examined at species level. Thus, the change in

TABLE 1 Treatment effects (monoculture, combination, and polyculture) on plant biomass variables in linear (mixed) models.

Response Variable	Whole model	Growth form			Species					
		Slender	Fan	Large	*Schoenoplectus tabernaemontani*	*Typha orientalis*	*Iris sibirica*	*Acorus calamus*	*Canna indica*	*Thalia dealbata*
Total dry biomass	$F_{2,610} = 5.40$, $p = 0.005$	$F_{2,191} = 5.48$, $p = 0.005$	$F_{2,211} = 0.19$, $p = 0.827$	$F_{2,204} = 11.46$, $p < 0.0001$	$F_{2,104} = 3.84$, $p = 0.025$	$F_{2,85} = 5.14$, $p = 0.008$	$F_{2,105} = 3.45$, $p = 0.035$	$F_{2,104} = 0.39$, $p = 0.678$	$F_{2,98} = 2.93$, $p = 0.058$	$F_{2,104} = 10.93$, $p < 0.0001$
Belowground dry biomass	$F_{2,610} = 13.05$, $p < 0.0001$	$F_{2,191} = 2.38$, $p = 0.096$	$F_{2,211} = 0.80$, $p = 0.452$	$F_{2,204} = 20.34$, $p < 0.0001$	$F_{2,104} = 2.08$, $p = 0.130$	$F_{2,85} = 1.28$, $p = 0.284$	$F_{2,105} = 0.49$, $p = 0.613$	$F_{2,104} = 0.40$, $p = 0.671$	$F_{2,98} = 7.46$, $p = 0.001$	$F_{2,104} = 13.85$, $p < 0.0001$
Aboveground dry biomass	$F_{2,610} = 1.54$, $p = 0.215$	$F_{2,191} = 11.88$, $p < 0.0001$	$F_{2,211} = 0.13$, $p = 0.881$	$F_{2,204} = 5.29$, $p < 0.006$	$F_{2,104} = 9.79$, $p = 0.0001$	$F_{2,85} = 6.451$, $p = 0.002$	$F_{2,105} = 11.88$, $p < 0.0001$	$F_{2,104} = 0.78$, $p = 0.460$	$F_{2,98} = 0.76$, $p = 0.472$	$F_{2,104} = 0.78$, $p = 0.001$
Fresh weight increase	$F_{2,617} = 10.41$, $p < 0.0001$	$F_{2,194.2} = 17.91$, $p < 0.0001$	$F_{2,212} = 6.40$, $p = 0.002$	$F_{2,207} = 12.67$, $p < 0.0001$	$F_{2,105} = 20.09$, $p < 0.0001$	$F_{2,87} = 11.82$, $p < 0.0001$	$F_{2,105} = 14.70$, $p < 0.0001$	$F_{2,105} = 24.96$, $p < 0.0001$	$F_{2,100} = 5.41$, $p = 0.009$	$F_{2,105} = 17.55$, $p < 0.0001$

plants' TDB could be largely attributed to the processes happening belowground.

The patterns in the fresh weight increase (FWI) across treatments, growth forms, and species share three important similarities with TDB. First, when compared to monoculture and combination, the increase in polyculture was significantly higher (Table 1; Figure 3J). Second, the degree of increase differed among growth types, where large plants enjoyed greater increase than slender and fan ones (Table 1; Figure 3K). And third, for individual species, polyculture significantly outperformed monoculture and combination in *C. indica* and *T. dealbata* (Table 1; Figure 3L). The figure also showed that *C. indica* had the strongest response among all species. As to the minute bars of fresh weight in slender and fan plants, it suggested greater water content in large plants, which might facilitate their nutrient metabolism.

3.2. Nitrogen and phosphorus removal

The concentrations of TN and TP in the culturing tanks started to differ among the three treatments after around 20 days of growth, and the differences persisted till the end of the experiment (Figure 2). The polyculture tanks had the lowest TN and TP concentrations, followed by the combination, and the monoculture with the greatest. The concentrations of TN and TP experienced the steepest drop over the first 20–25 days of the experiment, which should be caused by the rapid initial growth of plants. The concentration of TN rose in three intervals in all three treatments, probably owing to higher dosages of N were added and the plants' absorption became slower (Figure 2). The concentration of TP reached a peak on Aug 4th due to a high dosage of addition. Nevertheless, plants were able to consume most of the added P and brought the concentration to a low level at the end of the experiment (Figure 2). The concentration of TN considerably dropped at the end of the experiment, possibly because large-typed plants entered the reproductive period and required more nutrition.

The TN and TP removal in culturing water varied significantly among the three treatments. Compared to monoculture and combination, polyculture considerably increased the removal of TN ($F_{2,\ 51} = 3.17$, $p = 0.05$; Figure 4A) and TP ($F_{2,\ 51} = 5.16$, $p = 0.009$; Figure 4B). When polyculture was removed, both factors of growth forms and treatments were examined simultaneously in the remaining tanks. The effects of treatment were consistent with previous results, that is, monoculture and combination did not differ in TN ($F_{1,\ 32} = 0.34$, $p = 0.562$) and TP removal ($F_{1,\ 32} = 2.58$, $p = 0.118$). However, different growth forms varied remarkably in TN ($F_{2,32} = 17.78$, $p < 0.0001$) and TP removal ($F_{1,\ 32} = 19.28$, $p < 0.0001$), with a similar pattern that the large plants were of greater nutrition absorption (Figures 4C,D).

3.3. Water pH and dissolved oxygen

The concentration of DO had no significant difference among monoculture, combination, and polyculture (Table 2). However, there were notable differences across three growth types, with the large type having a much lower concentration of DO than slender and fan (Table 2). The pH of culturing water varied significantly depending on the treatment and growth type. The water was acidic in all three treatments, and the acidity rose with the number of species (Table 2).

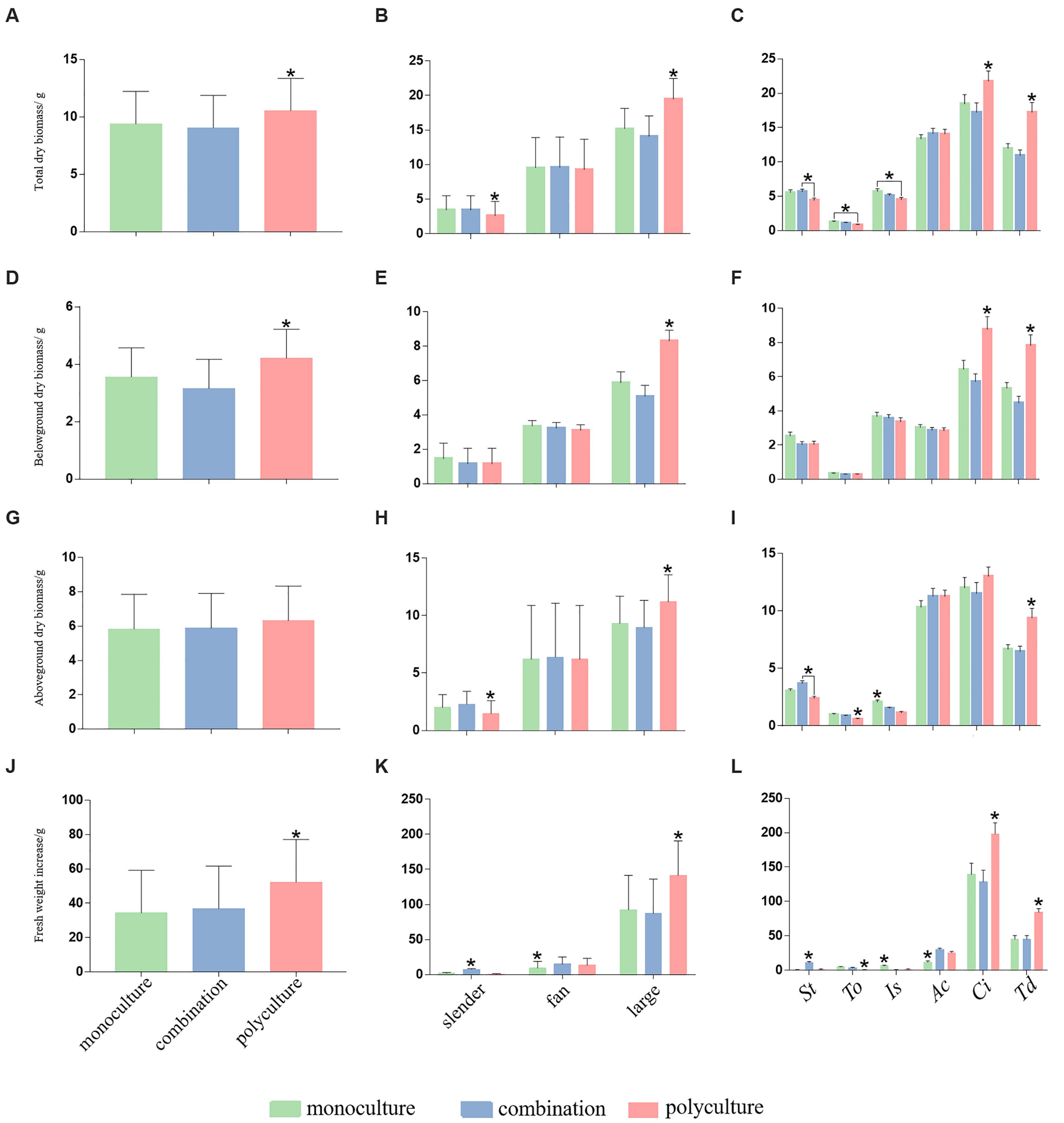

FIGURE 3
Differences in total dry biomass **(A-C)**, belowground dry biomass **(D-F)**, aboveground dry biomass **(G-I)**, and fresh weight increase **(J-L)**, among monoculture (green), combination (blue), and polyculture (rose) treatments. *Means significant differences with the other two treatments, means significant differences between two treatments, and unlabeled bars mean lack of significant differences among treatments.

The water was also acidic for all three growth types, with large type having the strongest acidity followed by fan, and slender the weakest (Table 2).

4. Discussion

Results showed that CWs planted with three different growth forms were the most efficient in consuming dissolved N and P, which is consistent with our hypothesis that combining plants with various growth forms in a polyculture is the most efficient in removing N and P from water. Given that the substrates, nutrient solution, temperature, light, and growing density of all culturing tanks were the same, the most possible reason is the higher species richness and functional diversity in polycultures with various growth forms (Cardinale, 2011; Huang et al., 2020). Correspondingly, both total dry biomass and fresh weight increase of plants in polyculture was also significantly higher than that of monoculture and combination, suggesting that plant growth effectively contributes to the removal of nutrients. Especially for *C. indica* and *T. dealbata* in polyculture, both had their fresh weight increased about 1.5 to 2.0 times than in monoculture, indicating promoted growth in response to niche differentiation and reduced competition. However, such positive effects were not universal for all species. For slender-formed *S. tabernaemontani* and *T. orientalis*, and *I. sibirica*, their growth, especially aboveground weight, was somewhat reduced. This may be due to strong shading effects (or allelopathy, see below) from large plants, which were not present in their monocultures and combinations. But the strength of the reduction in biomass was trivial compared to the strength of the increase in large plants (Figure 2), which had little impact on the overarching pattern.

Studies suggest that the removal of contaminants was strongly tied to belowground processes (Ge et al., 2011; Schultz et al., 2012; Wang et al., 2013). Hence, polyculture may have also elevated the activity of rhizospheric microorganisms through diverse structures and density of roots (Carballeira et al., 2016; Limpert et al., 2020). Indeed, we found greater belowground biomass in polycultures (Figure 2), which implies that belowground roots may have played an essential role. The roots of six planted species are of various structures. Specifically, the four species of slender and fan types grow rhizomes, in which *S. tabernaemontani* sends out shallow runners with dense root hairs; *T. orientalis* has horizontal fleshy rhizomes; both *A. calamus and I. sibirica* develop deeper rhizomes and dense fibrous roots. In contrast, *C. indica* and *T. dealbata* have tuberous rootstocks, on which extensive adventitious roots grow. It was quite evident and statistically significant that the roots of *C. indica* and *T. dealbata* in polyculture had higher biomass (1.5–1.7 times) than in monoculture and combination, which indicated greater ability in water purification. Increased root activity in polyculture and large plants could also explain the change in dissolved oxygen and pH in the culturing tanks, because rhizosphere should have consumed more oxygen and secreted more organic acids during decomposition (Tanner et al., 2005; Paranychianakis et al., 2016; Zhao et al., 2016). Other researchers have pointed out the crosstalk among distinct belowground microbial communities associated with different plants (Fan et al., 2018), and positive feedback between below and above grounds (Arslan et al., 2017), identifying alternative mechanisms in how plant diversity promotes purification functioning of CWs. It might be the case in our study, but more investigations need to be carried out to interpret the role of microorganisms.

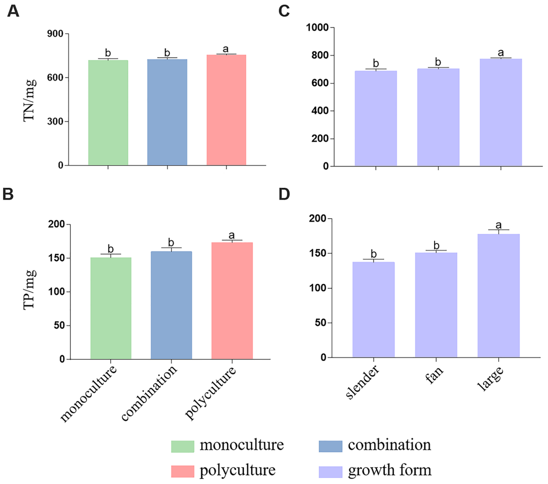

FIGURE 4
Differences of total nitrogen removal [TN, **(A,C)**] and total phosphorus removal [TP, **(B,D)**] among three treatments and different growth forms. Different letters above bars represent significant differences.

When two species of the same growth form were planted together, we did not find improved growth or nutrient removal. This agrees with our hypothesis that an increase in plant richness with the same growth form had little impact on water purification, further suggesting that it is the functional diversity, not the pure number of plant species that could facilitate the performance of plant communities (Geng et al., 2017). The key probably lies in antagonistic interspecific interactions when two species share a high level of similarities (Ellawala Kankanamge and Kodithuwakku, 2017). Hence, we also expected to see strong competition or even competitive exclusion in the combination treatment (Adler et al., 2018; Geng et al., 2019). Despite that none of the experimental combinations resulted in one species replacing the other, the results clearly suggested competitive relationships in slender and fan combinations, where *S. tabernaemontani* showed high competitiveness and increased growth in combination than monoculture, while plants of *I. sibirica* were of weaker ability than *A. calamus* and suppressed. It has been reported that the rhizome extract of *T. dealbata* had allelopathic effects on others in seed germination, seedling growth metabolism, and root activity (Miao et al., 2012). In our experiment, the water in some tanks became black after growing *C. indica* and *T. dealbata* together. The mortality rate of *C. indica* was unusually high, with one tank reaching 50% within a week. Such mortality, however, was not observed in the *C. indica* monocultures, demonstrating that cannas were probably inhibited by the allelopathy of *T. dealbata*. It should be noted that mortality was not included in our results because dead plants, whenever discovered, were replaced with healthy ones, in order to maintain the same plant density of all treatments. The allelochemicals of *T. dealbata* should also be present in polycultures (despite of lower

TABLE 2 The concentration of dissolved oxygen (mg/L) and pH in culturing tanks of different treatments and growth forms.

Response variable	Treatment/growth form			Comparison
Dissolved oxygen	Monoculture	Combination	Polyculture	$F_{2,527} = 2.28, p = 0.10$
	8.36 ± 0.36	7.94 ± 0.36	7.72 ± 0.36	
	Slender	Fan	Large	$F_{2,348} = 130.53, P < 0.0001$
	9.62 ± 0.35a	9.53 ± 0.35a	5.30 ± 0.35b	
pH	Monoculture	Combination	Polyculture	$F_{2,527} = 16.87, P < 0.0001$
	5.51 ± 0.12a	5.10 ± 0.10b	4.78 ± 0.08c	
	Slender	Fan	Large	$F_{2,348} = 67.72, P < 0.0001$
	6.26 ± 0.17a	5.10 ± 0.09b	4.54 ± 0.07c	

concentrations), which partly explains the suppressed growth of *S. tabernaemontani*, *T. orientalis*, and *I. sibirica*. Nonetheless, the performance of *A. calamus* and *C. indica* in polycultures were not negatively affected, perhaps suggesting their higher levels of tolerance.

Large-shaped plants, *C. indica* and *T. dealbata*, not only had higher rates of TN and TP removal than slender and fan types (Figure 4), but also seemed to contribute disproportionately to the biomass increase in polycultures (Figure 2). This echoes studies that found plants with extensive growth of roots and leaves speeded up nutrition absorption more than medium or small-sized plants (Liu et al., 2020; Teubner et al., 2022), which points out that it is essential to have species of large sizes considered and selected in CWs and sewage treatment (Levi et al., 2015). During our experiment, only the individuals of *C. indica* and *T. dealbata* entered the flowering stage, and reproduction might have elevated the uptake of various nutrients as well. Besides, large plants are also considered beneficial in suppressing sediment resuspension and keeping the aquatic environment warm by reducing wind speed in nature (Rehman et al., 2017), thus facilitating the water purification function of CWs. Nevertheless, large-sized plants are certainly not a panacea for all conditions. Studies have indicated that the removal rate of P by slender *Schoenoplectus* nearly doubled that of large-shaped *Phragmites*, especially in winter (López et al., 2016; Carrillo et al., 2022). *C. indica* was better than *P. purpureum* at TN removal in summer but the opposite was true in winter (Yang et al., 2007). Evidently, seasonality needs to be taken into consideration. While large plants mainly grow in summer, others may be more important in nutrient uptake across other seasons. In addition, plant density might play a role in the better growth of large plants in polycultures (Webb et al., 2013), because the density of large plants was essentially reduced when co-grown with slender- and fan-shaped plants of much lower biomass. However, the improved function of polycultures was more likely a collective effect of three growth forms rather than by the large form itself because each polyculture tank only had 2 large plants, compared with 12 in those monoculture and combination tanks.

Iris sibirica and *T. orientalis*, both commonly used in CWs, have been reported to greatly increase contaminant removal in wastewater (Ma et al., 2017; Zhu et al., 2017). However, the growth of the two plant species was poor in our study. We observed a higher mortality rate of *T. orientalis* in polyculture (16.67%) than that in monoculture (5.56%), suggesting that the growth of *Typha* was not promoted by niche differentiation, but instead inhibited, probably by the allelopathic effect of *T. dealbata*. Tillers of *T. orientalis* were not observed until after half a month of culturing. The slow developmental rate of this species might also result in shading by others when grown in a dense and diverse community. The poor growth of *I. sibirica* was likely ascribed to high temperatures during the experimental period. It has been reported that *I. sibirica* had the highest nutrient uptake rate in winter and was considered the most effective overwintering plant (Gao et al., 2014; Ma et al., 2017). We may observe better growth of *I. sibirica* if the trial duration was extended. In the future, it is worthwhile to conduct plant cultivation for longer durations, as it may reveal species interaction and nutrient dynamics under a more realistic scenario.

In conclusion, species richness and growth form did influence CW efficiency. Our results highlight that polyculture with various growth forms is the most efficient in pollutant removal. Meanwhile, various phenology and diverse ornamental value of different species add to the diversity of sceneries in urban wetlands (Rodriguez and Brisson, 2016; Leiva et al., 2018). It is noteworthy that the setting of our experiment was inside a greenhouse, which largely excluded natural pathogens and insect herbivores. If grown in natural habitats, species functional diversity in polyculture might provide additional benefits in diverse interspecific relationships, promoting more stable and sustainable ecosystem functions (Steudel et al., 2016).

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

YL: data analysis, visualization, and writing – original draft. QC: investigation. FL: conceptualization and methodology. CD: conceptualization, data analysis, writing – original draft, and supervision. All authors contributed to the article and approved the submitted version.

Funding

This study was funded by the Natural Science Foundation of Hubei Province of China grant 2019CFA066 (CD) and Hubei Engineering Research Center for Protection and Utilization of Special Biological Resources in the Hanjiang River Basin grant 2021-09 (CD).

Acknowledgments

We thank ZQ Li, LF Yang and JM Su at Hubei University for sharing lab equipment. We are grateful to all colleagues of Lab 302 at School of Resources and Environmental Science, Hubei University for experimental help. They are XT Xie, HQ Tang, ZY Xu, QT Peng, H Yang, HZ Mao, A Hu, BB Huo, SS Cui, C Liu, WH Yang, X Chen, YL Wu, CY Chen, JQ Zhang, and J Zhu.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher’s note

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