Effect of water exchange rate on interspecies competition between submerged macrophytes: functional trait hierarchy drives competition

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Abstract

Background and aims The “competition-trait similarity” and “competition-trait hierarchy” hypotheses can be used to predict competitive outcomes between terrestrial plant species in response to environmental variation. However, their validity in aquatic plants remains poorly understood, particularly in terms of variation in the water exchange rate (WER).

Methods We compared competitive outcomes and variation in functional traits in two pairs of submerged macrophytes (Vallisneria natans vs. Myriophyllum aquaticum and V. natans vs. Myriophyllum spicatum) at three levels of WER using the replacement series method.

Results We found that the relative competitive ability of V. natans consistently decreased with increasing WER and decreasing the planting proportion of V. natans. Under these conditions, V. natans experienced reduced plant nutrient content and the inhibition of dissolved carbon dioxide absorption, which eventually shifted this species from a stronger to weaker competitor. With increasing WER and decreasing the planting proportion of V. natans, the below:above-ground biomass ratio and root diameter increased, while the root length and specific root length decreased, for V. natans. In contrast, all of these traits exhibited the opposite patterns for the co-cultured Myriophyllum species. In addition, the branch number per plant mass increased in V. natans but exhibited no change in co-cultured Myriophyllum species under the same conditions.

Conclusions Our results indicate that WER affected the outcome of interspecies competition between submerged macrophyte species. Moreover, the relative competitive ability of each species within a pair was more strongly linked to species competition-trait hierarchy than to competition-trait similarity.

Keywords Hydrological variation · Plant competition · Plant morphology · Stoichiometric characteristics · Trait hierarchy · Trait similarity

Introduction

Understanding how resource competition influences the relative abundance of plants is among the oldest pursuits in ecological research (Dong et al. 2020). The minimum resource requirement and maximum growth rate theories are two early conceptual frameworks that provide mechanistic insights into species’
competitive interactions. The minimum resource requirement theory recognizes that species with lower demand will become dominant if all species use the same limiting resource (Tilman 1980), whereas the maximum growth rate theory states that species with higher growth rates (or greater ability to capture environmental nutrients) will displace the others (Grime 1977). Subsequently, researchers have used quantitative methods to verify the validity of these two theories. Functional traits (morphological and physiological characteristics) determine the adaptive specialization, fitness, and persistence of species within a habitat (Kraft et al. 2015). Therefore, quantifying interspecific differences in functional traits is a promising approach for testing competition hypotheses in plants under different environmental conditions (Coomes and Grubb 2003).

With the development of trait-based approaches, several trait-based theories relating resource competition and relative abundance of plants have been derived; they include the “competition-trait similarity” (CTS; Cahill et al. 2008) and “competition-trait hierarchy” (CTH; Kunstler et al. 2012; Kraft et al. 2015) hypotheses. The CTS hypothesis assumes that competition is more intense among species with similar trait values because functional similarity is often associated with similar resource use. In contrast, the CTH hypothesis states that functional dissimilarity (differences in traits directly associated with plant competitive ability and fitness) should favor particular competitors over others, i.e., competitive intensity is strong between species with different trait values. Although these contrasting hypotheses have been explored repeatedly in terrestrial plants under various environmental stresses, their applicability in aquatic macrophytes has yet not been investigated (Goldberg and Novoplansky 1997), particularly with respect to hydrological variation.

Hydrological variation caused by global climate change and the development of hydrological engineering works is a major threat to aquatic organisms, often leading to a pervasive loss of biodiversity and productivity declines (Good et al. 2009; Tisseuil et al. 2012). Hydrological variation is particularly challenging for aquatic macrophytes because they cannot escape unfavorable environments (Pires et al. 2016). Among various hydrological elements, the exchange rate of water (WER) between a lake and its surrounding waters and among different areas within a lake, directly influences various physical and chemical properties of the water column and sediments, and thus greatly impacts the growth and functional traits of aquatic macrophytes (Yuan et al. 2018; Reitsema et al. 2020). In general, rapid water exchange increases hydrodynamic forces and decreases water transparency, reducing the rooting and regeneration capacity of aquatic macrophytes, or even causing mechanical damage to plant organs (Zhu et al. 2012; Zhang et al. 2014). Conversely, slow water exchange reduces sediment nutrient release; moreover, it also prevents the replenishment of oxygen and carbon dioxide from incoming water, and inhibits the transfer of these two gases at the air–water interface due to weakened flow turbulence (Jirka et al. 2010; Pan et al. 2019).

Such changes will further alter root and leaf traits that are associated strongly with nutrient, as well as oxygen and carbon dioxide, absorption (Pan et al. 2012; Hussner et al. 2016; Hessen et al. 2017). More importantly, species-specific responses of these root and leaf traits to the WER are predictable owing to differences in species tolerance thresholds. In a previous study (Yuan et al. 2018), we found that WER increases stimulated root growth for the separately cultured submerged macrophytes Hydrilla verticillata and Myriophyllum aquaticum due to increasing oxygen availability. Changes in root growth involved increased root length, specific root length (SRL), and below-ground to above-ground (B:A) biomass ratio. However, these adjustments were generally greater in M. aquaticum than in H. verticillata, resulting in a more dramatic increase of plant biomass in M. aquaticum by enhancing the ability of roots to absorb nutrients. Similar links between hierarchical traits and plant fitness to hydrological variation have been observed in various aquatic macrophytes (Pan et al. 2012; Gustafsson and Norkko 2019).

These phenomena indicate that when these species coexist under hydrological variation conditions (including the WER) and compete for limited sediment resources, the trait hierarchy may favor some competitors over others. It seems that CTH may play a more important role than CTS in explaining
the outcome of interspecies competition for aquatic macrophytes. Understanding how functional traits vary with the WER is of primary importance for verification of the validity of the CTS and CTH hypotheses, and the prediction of future changes in aquatic plant communities (Wang et al. 2010).

This study was conducted to compare variation in functional traits and relative competitive ability in two coexisting pairs of submerged macrophytes—*Vallisneria natans* vs. *M. aquaticum*, and *V. natans* vs. *Myriophyllum spicatum*—under the stress of WER variation. *V. natans* and *M. spicatum* are common vegetation components that can coexist in freshwater lakes throughout the world (Cao et al. 2011). *M. aquaticum* is an invasive species native to South America that has spread throughout many countries, including the lakes of Yunnan Province, China (You et al. 2013). The invasion of *M. aquaticum* and displacement of indigenous species (including *V. natans*) are crucial ecological issues of increasing significance (Xie et al. 2013). The following research questions will be addressed: (1) to investigate whether WER could induce interspecific differences in functional traits and thus affect interspecies competition; (2) to verify the validity of the CTS and CTH hypotheses in predicting the outcome of interspecies competition between these co-cultured submerged species. We hypothesized that relative competitive ability in the submerged macrophyte pairs would be positively related to the hierarchical difference in their functional traits.

**Materials and methods**

**Experimental material**

Three species of aquatic macrophytes were used in this experiment: *V. natans*, *M. aquaticum*, and *M. spicatum*. *V. natans* is ubiquitous in aquatic ecosystems, regardless of hydrological conditions (Zhu et al. 2018a). Both *Myriophyllum* species can become dominant species only in areas with strong hydrodynamic forces. For example, *M. aquaticum* tends to distribute in lakeshore zones strongly affected by waves, and *M. spicatum* is more frequently found in inlet and outlet channels, and regions with strong wind and fast WER (Wersal & Madsen, 2011; Zhu et al., 2018b).

All samples of the three aquatic macrophytes were collected in April 2018 from Shaping Bay (25° 56′ N, 100° 6′ E), east of Erhai Lake, Yunnan Province, China. Lake sediment (containing 0.16 g kg⁻¹ total N and 1.69 g kg⁻¹ total P) was collected simultaneously in these sites as experimental sediment. All ramets collected were planted in plastic buckets (67 cm × 45 cm × 55 cm; filled with 10 cm lake sediment and 20 cm tap water) in a greenhouse at Yunnan University for pre-culturing, where they sprouted new ramets.

**Experimental design**

We conducted two laboratory experiments (a schematic of the experimental device is presented in Fig. 1) to examine the effects of varying WER.
variation on environmental conditions in the absence of plants (experiment 1), and plant functional dissimilarity and relative competitive ability in two coexisting pairs of submerged macrophytes: \textit{V. natans} vs. \textit{M. aquaticum} and \textit{V. natans} vs. \textit{M. spicatum} (experiment 2), respectively. Three water exchange rates, i.e., static water (0% replacement of the whole water body per day), medium WER (20%), and high WER (40%), were applied in both experiments. This range of WER variation is reasonable for natural ecosystems (including Erhai Lake) according to our previous studies (e.g., Yuan et al. 2018). Two replacement series (of \textit{V. natans} vs. \textit{M. aquaticum} and \textit{V. natans} vs. \textit{M. spicatum}) were performed as replicates of the competition system in experiment 2. In addition, five combination modes (ratio of \textit{V. natans} numbers to those of corresponding \textit{Myriophyllum} species of 8:0; 6:2; 4:4; 2:6, and 0:8) were used in experiment 2 for each replacement series according to the standard replacement method used in previous studies (e.g., Connolly 1986; Gibson et al. 1999).

Three treatments were contained in experiment 1, with each treatment had three replicates (using three plastic buckets of 100 cm × 60 cm × 50 cm, approximately 300 L). While, a total of 30 treatments (three WERs × two replacement series × five combination modes) were implemented in experiment 2, each treatment with five replicated; the replicate were plastic pots (20-cm in height and 35-cm in diameter). In total, 150 pots were transferred to 30 plastic buckets before the experiment. Ten buckets were exposed to each of the three WER levels, and each bucket contained five pots of different combination modes belonging to the same replacement series. Collectively, 600 \textit{V. natans} ramets and 300 of ramets each \textit{Myriophyllum} species of a similar size were used in the experiments, with mean (± SD) lengths \((n > 150)\) of 5.36 ± 0.43, 7.23 ± 0.41, and 7.21 ± 0.47 cm, and mean (± SD) fresh weight \((n > 150)\) of 0.58 ± 0.10, 0.54 ± 0.10, and 0.55 ± 0.11 g for \textit{V. natans}, \textit{M. aquaticum} and \textit{M. spicatum}, respectively. The bottom of each pot was encosed with nylon net and filled with 10 cm of the same sediment used for plant incubation.

The experiments were conducted in the same greenhouse starting on June 1, 2018. Tap water was first aerated with sterile filtered air (Sartorius, Midisart 2000) in an aeration tank to homogenize the dissolved oxygen (DO) and dissolved carbon dioxide (DCD) concentrations, and then supplied to each WER treatment as needed throughout the experiment. An additional three-day experiment was conducted before the start of the main experiment to investigate indicator (DO and DCD) differences between incoming water and bucket water in the absence of ramets (see Table S1).

Experiment in the absence of ramets

This experiment was designed to accurately estimate the effects of the WER on water properties without the interference of submerged ramets. The DO and DCD concentrations of the system water were measured on days 1, 3, 5, 7, 9, 11, 13, and 15 at a depth of 45 cm (10 cm above the pot bottom) during the daylight (12:00–14:00) and night (22:00–24:00). The DO and DCD concentrations were determined using a DO meter (HQ40d, Hach, Loveland, USA) and a DCD meter (AMT-SC200, Shenzhen Yunchuan Wulian Technologies Co. Ltd, Shenzhen, China), respectively.

Experiment in the presence of ramets

This main experiment was designed to explore the effects of the WER on functional traits and interspecies competition between co-cultured submerged macrophytes during a period of 50 days. At the end of the experiment, we first measured plant height as the distance between the ground surface and leaf tip. Subsequently, these plants were carefully removed from the sediment to maintain intact root systems and rinsed with tap water. The branch number of newly developing ramets was recorded for each plant. Only \textit{V. natans} plants produced new ramets throughout this experiment. Plants were divided into above-ground (leaves for \textit{V. natans}, and leaves and stems for the two \textit{Myriophyllum} species) and below-ground parts (roots, stolons and rhizomes for all three species), and then the fresh biomass of each part was weighed using an electronic analytical balance (AX224ZH/E, Ohaus, Florham Park, NJ, USA; sensitivity 0.0001 g).

Fresh roots of the stock plant (excluding the roots of newly developing ramets) were used for root morphological analyses. At least three representative fully grown adventitious roots (growing from rhizomes and below the sediment surface) were selected from each ramet, and the mean root length (hereafter referred to as root length) and diameter were measured using
a Vernier caliper and a microscope equipped with an ocular micrometer (Olympus BX51; Olympus, Japan), respectively. The total root length and fresh weight of the three adventitious roots (cut from lateral roots) were recorded for each plant, and SRL was defined as total root length divided by fresh root mass. The root length, diameter and SRL of each plant species were first averaged first for each pot, and then for each treatment.

The remaining tissues of each plant were dried at 85 °C for 48 h and weighed to determine the wet to dry conversion factor. Then the dry weights were used to calculate the B:A biomass ratio, biomass accumulation, and branch number per plant mass. Biomass accumulation was calculated as the sum of the above-ground and below-ground tissue biomasses. The branch number per plant mass was calculated as the branch number divided by the total biomass of the stock plant (excluding the biomass of newly developing ramets). Biomass accumulation and branch number per plant mass values were averaged first for each pot, and then for each treatment.

Finally, the plant parts from each treatment were ground into powder and mixed together to measure the stoichiometric properties of plant carbon (C), nitrogen (N), and phosphorus (P). Samples were digested with H$_2$SO$_4$-H$_2$O$_2$ and evaluated for P and N contents per plant using colorimetric analyses (Xie et al. 2005). Organic C, which usually occupies the vast majority of total C in plants, was measured using the K$_2$Cr$_2$O$_7$ oxidation procedure (Lu 2000). The C:N, C:P, and N:P ratios of each plant were calculated from the total P, total N, and organic C results obtained.

Five parameters were chosen for the analysis of functional traits between co-cultured submerged macrophytes: root length, SRL, root diameter, the B:A biomass ratio, and the branch number per plant mass. The root length and SRL are the most functionally important traits by which plants absorb sediment nutrients (Johns et al. 2014). The root diameter affects oxygen transfer from plant shoots to roots through the formation of root aerenchyma (Pan et al. 2014). Biomass allocation between below-ground and above-ground parts reflects the trade-off between the maximization of sediment nutrient uptake and acclimation to DO and DCD deficiencies (Pan et al. 2019). Finally, the partitioning of available resources between growth and reproduction determines the balance between acclimation to specific environmental stress or escape from these adverse conditions (Nord and Lynch 2008).

Statistical analyses

In the experiment performed without ramets, we used one-way repeated-measures analysis of variance (ANOVA) followed by a Bonferroni post-hoc test to determine the effects of the WER on waterbody variables (DCD and DO concentrations in the bucket water) for each observation period. In the experiment performed with ramets, we used three competition indices to investigate the strength of competition between co-cultured species in each replacement series: relative yield (RY), total relative yield (RY$_T$), and aggressivity (A).

RY for *V. natans* (RY$_V$) and *Myriophyllum* species (RY$_M$) were calculated from the dry weight of each sample for different treatments using the following equations (Bi and Turvey 1994; Burnett and Mealar 2008):

\[
RY_V = P_V \times \frac{V_{\text{min}}}{V_{\text{mon}}} \\
RY_M = P_M \times \frac{M_{\text{min}}}{M_{\text{mon}}}
\]

where $P_V$ and $P_M$ are the proportions of *V. natans* and *Myriophyllum* species in the mixture for each replacement series. $V_{\text{mix}}$ and $V_{\text{mon}}$ are the mean biomass accumulation of *V. natans* in mixture and monoculture, respectively. $M_{\text{mix}}$ and $M_{\text{mon}}$ are the mean biomass of the corresponding *Myriophyllum* species in mixture and monoculture, respectively.

RY$_T$ can be used to predict species interaction between co-cultured species, which was calculated using the following equation (Bi and Turvey 1994):

\[
RY_T = RY_V + RY_M
\]
compete for different environmental resources) (Bi and Turvey 1994).

A (aggressivity) is a useful indicator of the relative competitive ability of two co-cultured plant species (Willey 1979). The A value for *V. natans* (A\textsubscript{V}) was calculated using the following equation (Dhima et al. 2007):

\[
A_V = 0.5 \left( \frac{V_{\text{mix}}}{V_{\text{mon}}} - \frac{M_{\text{mix}}}{M_{\text{mon}}} \right)
\]  

(4)

A\textsubscript{V} = 0 indicates that the competitive ability of *V. natans* is equal to those of *Myriophyllum* species; A\textsubscript{V} < 0 indicates that the competitive ability of *V. natans* is lesser than that of *Myriophyllum* species; and A\textsubscript{V} > 0 indicates that the competitive ability of *V. natans* is greater than that of *Myriophyllum* species.

Subsequently, multiple comparisons using Tukey test was performed to determine the differences among different treatments for plant growth (biomass and height), functional traits, stoichiometric properties (plant C, N, and P contents, and the ratios of C:N, C:P, and N:P ratios), and competition indices for each species at a given competition system. Two-way and three-way ANOVAs were performed to determine the effects of the WER, combination mode, and/or plant species on plant biomass, height, functional traits, stoichiometric properties, and competition indices. These analyses were followed by the Tukey test.

Additionally, linear regression analyses were conducted to determine the relationships among variation in the system DCD content, plant growth, functional traits, stoichiometric properties, and competition indices. All data were tested for normality and variance homogeneity before analyses. Data on the SRL, B:A biomass ratio, and plant height were log\textsubscript{10}-transformed to meet the assumptions of homogeneity and normality of variance prior to one-way ANOVA. All statistical analyses were performed using the SPSS 25.0 software (SPSS Inc., Chicago, IL, USA).

**Results**

Environmental variables in the absence of ramets

The DCD concentration was significantly lower, and the DO concentration was higher in incoming water than in the experimental waterbodies (*P* < 0.05; Table S1). As a result, the DO concentration significantly increased (*P* < 0.001; Fig. 2a, b; Table S2), whereas the DCD concentration sharply decreased, as the WER increased throughout the entire experiment during both the day and night (*P* < 0.001; Fig. 2c, d; Table S2). Throughout the experimental period, the DCD concentration ranged from 356 to 488 ppm, and the DO concentration was always exceeded 6.0 mg L\textsuperscript{-1}.

**Fig. 2** Variations (means ± SD, n = 3) in dissolved oxygen (DO) concentration (a and b) and dissolved carbon dioxide (DCD) concentration (c and d) of the system water in both daytime and nighttime in treatments with different water exchange rates throughout the 15-days-long experiment without ramets. Different letters indicate significant differences among treatments through one-way RM-ANOVA at the 0.05 significance level.
The plant biomass and height were significantly affected by the WER and plant species \((P < 0.01)\), regardless of the competition system (Tables 1 and 2). In addition, the WER had opposite effects on the biomass and height of \(V. natans\) and the \(Myriophyllum\) species. Specifically, increases in WER frequency led to decreases in the biomass and height of \(V. natans\), but increases in the biomass and height of \(Myriophyllum\) species, in both competition systems \((P < 0.001; \text{Fig. 3, Tables S3 and S4})\). For example, at the end of the experiment, the biomass of \(V. natans\) co-occurring with \(M. aquaticum\) was 31.62%, 39.11%, 37.16%, and 34.81% lesser (8:0, 6:2, 4:4, and 2:6 combination modes, respectively) in the high WER treatments than in the control treatments (static waterbodies). Likewise, the biomass of \(V. natans\) was 32.95%, 30.48%, 25.61%, and 26.51% lesser. In contrast, the biomass of \(M. aquaticum\) was 58.80%, 33.17%, 20.19%, and 30.59% (6:2, 4:4, 2:6, and 0:8 combination modes, respectively) greater in the high WER treatments than in the control treatments, and the biomass of \(M. spicatum\) was 77.34%, 84.74%, 66.12%, and 68.02% greater under the same conditions.

Moreover, the combination mode had significant effects on these two variables, which led to decreases below: above-ground biomass ratio (B:A biomass ratio), root length, specific root length (SRL), root diameter, plant C, plant N and plant P in \(V. natans—M. aquaticum\) system

### Table 1

| Species (S; df = 1) | WER (W; df = 2) | CM (C; df = 4) | \(S \times W\) | \(S \times C\) | W \(\times C\) | \(S \times W \times C\) |
|---------------------|-----------------|----------------|----------------|---------------|--------------|-------------------|
| Biomass (g dry wt plant\(^{-1}\)) | 241.070*** | 26.467*** | 0.861 | 205.408*** | 72.893*** | 1.181 | 0.610 |
| Plant height (cm) | 12.780** | 55.483*** | 2.432 | 335.826*** | 96.011*** | 1.437 | 2.250* |
| B:A biomass ratio | 31.078*** | 17.266*** | 0.880 | 490.162*** | 289.472*** | 1.622 | 2.156 |
| Root length (cm) | 51.877*** | 47.269*** | 2.293 | 318.072*** | 135.139 | 12.530*** | 1.355 |
| SRL (m g\(^{-1}\) fresh wt) | 94.079*** | 5.728** | 6.418** | 53.537*** | 142.444*** | 0.653 | 2.725* |
| Root diameter (um) | 0.797 | 5.509** | 3.411* | 111.219*** | 36.347*** | 0.904 | 4.584** |
| C (mg g\(^{-1}\)) | 2.189 | 1.523 | 0.894 | 13.430*** | 4.427** | 1.803 | 1.002 |
| N (mg g\(^{-1}\)) | 2.387 | 67.068*** | 0.497 | 1.598 | 9.170*** | 0.551 | 0.356 |
| P (mg g\(^{-1}\)) | 2.544 | 0.707 | 2.033 | 35.738*** | 78.497*** | 0.843 | 0.460 |

*\(P < 0.05; \; **P < 0.01; \; ***P < 0.001*

### Table 2

| Species (S; df = 1) | WER (W; df = 2) | CM (C; df = 4) | \(S \times W\) | \(S \times C\) | W \(\times C\) | \(S \times W \times C\) |
|---------------------|-----------------|----------------|----------------|---------------|--------------|-------------------|
| Biomass (g dry wt plant\(^{-1}\)) | 51.131*** | 15.517*** | 2.535 | 236.563*** | 136.130*** | 2.852* | 0.713 |
| Plant height (cm) | 5380.696*** | 73.580*** | 2.063 | 370.947*** | 23.383*** | 1.771 | 0.905 |
| B:A biomass ratio | 34.163*** | 5.492** | 2.063 | 17.042*** | 30.664*** | 0.562 | 1.404 |
| Root length (cm) | 573.931*** | 11.349*** | 1.095 | 699.913*** | 115.012*** | 1.504 | 7.301*** |
| SRL (m g\(^{-1}\) fresh wt) | 2068.652*** | 47.554*** | 1.074 | 173.030*** | 312.123*** | 2.057 | 0.971 |
| Root diameter (um) | 1099.865*** | 1.340 | 0.622 | 126.118*** | 99.856*** | 5.461*** | 1.553 |
| C (mg g\(^{-1}\)) | 813.920*** | 4.630* | 0.560 | 62.87*** | 59.93*** | 1.120 | 0.370 |
| N (mg g\(^{-1}\)) | 11.395** | 133.838*** | 0.131 | 2.817 | 1.718 | 1.228 | 1.537 |
| P (mg g\(^{-1}\)) | 86.772*** | 1.370 | 0.323 | 41.623*** | 20.213*** | 1.418 | 1.041 |

*\(P < 0.05; \; **P < 0.01; \; ***P < 0.001*

Plant biomass and height

The plant biomass and height were significantly affected by the WER and plant species \((P < 0.01)\), regardless of the competition system (Tables 1 and 2). In addition, the WER had opposite effects on the biomass and height of \(V. natans\) and the \(Myriophyllum\) species. Specifically, increases in WER frequency led to decreases in the biomass and height of \(V. natans\), but increases in the biomass and height of \(Myriophyllum\) species, in both competition systems \((P < 0.001; \text{Fig. 3, Tables S3 and S4})\). For example, at the end of the experiment, the biomass of \(V. natans\) co-occurring with \(M. aquaticum\) was 31.62%, 39.11%, 37.16%, and 34.81% lesser (8:0, 6:2, 4:4, and 2:6 combination modes, respectively) in the high WER treatments than in the control treatments (static waterbodies). Likewise, the biomass of \(V. natans\) co-occurring with \(M. aquaticum\) was 32.95%, 30.48%, 25.61%, and 26.51% lesser. In contrast, the biomass of \(M. aquaticum\) was 58.80%, 33.17%, 20.19%, and 30.59% (6:2, 4:4, 2:6, and 0:8 combination modes, respectively) greater in the high WER treatments than in the control treatments, and the biomass of \(M. spicatum\) was 77.34%, 84.74%, 66.12%, and 68.02% greater under the same conditions.

Moreover, the combination mode had significant effects on these two variables, which led to decreases below: above-ground biomass ratio (B:A biomass ratio), root length, specific root length (SRL), root diameter, plant C, plant N and plant P in \(V. natans—M. aquaticum\) system
in species biomass and height as the species planting proportion decreased, regardless of the species and competition system ($P<0.01$; Tables S3 and S4). Collectively, as the WER increased and the planting proportion of $V. natans$ decreased, the relative competitive ability of $V. natans$ gradually decreased. In addition, the biomass and height correlated positively with the DCD concentration for $V. natans$, but negatively with the DCD concentration for the $Myriophyllum$ species. This association was particularly strong for $V. natans$, regardless of the competition system ($R^2>0.33$; $P \leq 0.05$; Figures S1 and S2).

Plant functional traits

The WER significantly affected the B:A biomass ratio, root length, and SRL of both co-cultured species ($F>5.4$; $P<0.01$; Tables 1 and 2), and the branch number per plant mass of $V. natans$ ($P<0.001$; Tables S3 and S4), regardless of the competition system. The WER affected the root diameter only in the $V. natans$—$M. aquaticum$ system ($P<0.01$; Table 1).

In general, each functional trait exhibited opposite responses to the WER between co-cultured plant species. For $V. natans$, the B:A biomass ratio (Fig. 4a, c), branch number per plant mass (Fig. 4e, f), and root diameter (Fig. 5i, k) increased, while the root length (Fig. 5a, c) and SRL (Fig. 5e, g) decreased with increasing WER, regardless of the competition system ($P<0.001$; Tables S3 and S4). The opposite effects were observed for the B:A biomass ratio, root length, SRL, and root diameter in the two co-cultured $Myriophyllum$ species, with strong responses observed in the $V. natans$—$M. aquaticum$ system ($P<0.001$; Table S3). Neither $Myriophyllum$ species allocated biomass to new ramet production (i.e., branch formation) throughout the study period. With the exception of the branch number per plant mass, the effects of the combination mode on these parameters showed similar patterns for each co-cultured species, such as decreased root length and SRL and increased B:A biomass ratio and root diameter, as the species planting proportion decreased ($P<0.001$; Tables S3 and S4). Collectively, as the WER increased and the planting proportion of $V. natans$ decreased, the functional traits of co-cultured species generally showed opposite patterns, regardless of the competition system.

In addition, the B:A biomass ratio and root diameter were generally associated negatively with the

| Water exchange rate | Vallisneria natans | Myriophyllum aquaticum | Vallisneria natans | Myriophyllum spicatum |
|---------------------|-------------------|------------------------|-------------------|---------------------|
|                     | Biomass (g dry wt plant⁻¹) |                       | Biomass (g dry wt plant⁻¹) |                       |
| 0%                  | a                  | b                      | a                  | b                    |
| 20%                 | c                  | d                      | c                  | d                    |
| 40%                 | e                  | f                      | e                  | f                    |
| 60%                 | g                  | h                      | g                  | h                    |
| 80%                 | i                  | j                      | i                  | j                    |

Fig. 3 Variations (mean ± SD, n = 5) in plant biomass (a–d) and plant height (e–h) between co-cultured species (in Vallisneria natans—Myriophyllum aquaticum system, and $V. natans$—$Myriophyllum$ spicatum system) under three levels of water exchange rates and five combination modes (as the $V. natans$: $Myriophyllum$ species density ratio of 8:0, 6:2, 4:4, 2:6, and 0:8). Different letters indicate significant differences among treatments. Multiple comparisons of means were performed using the Tukey test at the 0.05 significance level.
DCD concentration for *V. natans*, but positively with the DCD concentration for the *Myriophyllum* species. This association was particularly strong for *V. natans* in the *V. natans*-*M. aquaticum* system during both the day and night ($R^2 > 0.53$; $P < 0.01$; Figures S3 and S4). In contrast, the root length and SRL were generally correlated positively with the DCD concentration for *V. natans*, but negatively with the DCD concentration for *Myriophyllum* species, regardless of the competition system and observation period (Figures S3 and S4). For example, the daytime DCD concentration was related significantly to root length in both competition systems, and to the SRL for *M. spicatum* in the *V. natans*-*M. spicatum* system ($R^2 > 0.43$; $P < 0.05$; Figure S3). In addition, four parameters (excluding the branch number per plant mass) were associated significantly with biomass, regardless of the species and competition system ($R^2 > 0.48$; $P < 0.05$; Figure S5).

Plant nutrients

The plant N content was significantly affected by WER in both competition systems ($P < 0.01$; Tables 1 and 2), whereas the effects of WER on P content were dependent on species, with a significant plant species × WER interaction ($P < 0.001$; Tables 1 and 2). The plant C content was significantly affected by WER, plant species, and their interaction in the *V. natans*-*M. spicatum* system ($P < 0.05$; Table 2), but was only affected by the interactive effects between WER and plant species, and between combination mode and plant species in the *V. natans*-*M. aquaticum* system ($P < 0.01$; Table 1).
Concentrations of both C and P gradually decreased in *V. natans*, but increased in co-cultured *Myriophyllum* species as WER increased (*P* < 0.05; Fig. 6; Tables S5 and S6). The plant N concentration significantly increased as WER increased in both co-cultured species, regardless of the competition system (*P* < 0.001; Tables S5 and S6). As a result, the C:N ratio decreased, whereas the N:P ratio increased with increasing WER, regardless of the species and competition system (*P* < 0.01; Table S6). N:P ratios were far below 14 throughout the experiment, regardless of the species and competition system.

The combination mode generally has similar effects on the nutrient status of co-cultured species. In the *V. natans—M. aquaticum* system, decreases in species planting proportions led to significant decreases in N and P concentrations (*P* < 0.05; Table S5) but did not significantly affect C concentration (*P* > 0.05). In the *V. natans—M. spicatum* system, the C and P concentrations were significantly affected by the combination mode, which decreased sharply as the species planting proportion decreased (*P* < 0.01; Table S6).

These results suggest that as WER increases and the planting proportion of *V. natans* decreases, *V. natans* will gradually be limited by nutrients, and the opposite effect would occur in co-cultured *Myriophyllum* species. Accordingly, the relationships of the N concentration to plant biomass and functional traits showed completely opposite patterns among co-cultured species. Specifically, the N concentration was associated negatively with the plant height, SRL, and root length and positively with the B:A biomass ratio and root diameter in *V. natans*, regardless of the competition system; the opposite pattern was observed...
in co-cultured *Myriophyllum* species, in which the N concentration was related significantly to the plant height and SRL ($R^2 > 0.41$; $P < 0.05$; Figure S8).

**Interspecies interaction**

The RY (relative yield) differed significantly between competition systems ($P < 0.01$; Table S7), and the effects of WER on RY$_V$ and RY$_T$ were dependent on the competition system, with significant competition system × WER interactions ($P < 0.01$; Table S7). The $A_V$ (aggressivity of *V. natans*) was affected by the combination mode ($P < 0.001$) and its interaction with the competition system ($P < 0.05$).

In the *V. natans*—*M. aquaticum* system, the RY$_V$ and $A_V$ significantly decreased as the planting proportion of *V. natans* decreased and the WER increased throughout the experiment ($P < 0.05$; Tables 3 and S8), with a significant combination mode × WER interaction ($P < 0.05$; Tables 3 and 5). Notably, in the 6:2 combination mode, the decreasing trends of these two variables became significant as WER increased ($P < 0.05$; Tables 3 and 5). In the *V. natans*—*M. spicatum* system, the RY$_V$ and RY$_T$ were affected significantly by the WER and combination mode ($P < 0.05$; Table S8), which generally increased as WER increased throughout the entire experiment (Tables 3 and 4). Finally, as WER increased and the planting proportion of *V. natans* decreased, $A_V$ gradually decreased, from positive values in the 6:2 combination mode to negative values in the 2:6 combination mode. These results suggest that the relative competitive ability of *V. natans* gradually decreased as WER increased and planting proportion decreased, eventually changing from a stronger to weaker competitor. Moreover, throughout the experiment, the RY$_T$ was always less than 1, regardless of the competition system (Table 4).

Linear regression analyses were conducted to determine the relationships of the competition rates and five combination modes (as the *V. natans*: *Myriophyllum* species density ratio of 8:0, 6:2, 4:4, 2:6, and 0:8). Different letters indicate significant differences among treatments. Multiple comparisons of means were performed using the Tukey test at the 0.05 significance level.
indices to the functional traits and stoichiometric characteristics of the plants. Lower $A_v$ values were generally associated positively with lower values for root length ($R^2 = 0.3065$ and $P < 0.001$ in the $V. natans$—$M. aquaticum$ system, but $R^2 = 0.0846$ and $P = 0.053$ in the $V. natans$—$M. spicatum$ system), SRL ($R^2 > 0.27$; $P < 0.001$), plant C content ($R^2 = 0.0839$ and $P = 0.054$ in the $V. natans$—$M. aquaticum$ system), and plant P content ($R^2 = 0.5081$ and $P < 0.001$ in the $V. natans$—$M. spicatum$ system). By contrast, $A_v$ values were associated negatively with the branch number per plant mass ($R^2 > 0.24$; $P \leq 0.001$), root diameter ($R^2 > 0.15$; $P < 0.01$), and B:A biomass ratio ($R^2 > 0.16$; $P < 0.01$; Figures S10—S13). Clearly, almost all functional traits (except root length in the $V. natans$—$M. spicatum$ system) contributed to changes in competition between co-cultured plant species, regardless of the competition system.

**Discussion**

Despite accumulating evidence of interspecific differences in functional traits in aquatic plants subjected to WER (Yuan et al. 2018; Pan et al. 2019), it remains unknown whether such differences can consequently alter the outcome of interspecies competition. Here, we find that these functional traits contribute to changes in relative yield (RY) and total relative yield (RYT) values among different treatments (three water exchange rate × three competition modes) for two competition systems ($Vallisneria natans$ vs. $Myriophyllum aquaticum$ and $V. natans$ vs. $M. spicatum$). Different letters indicate significant differences among treatments.

### Table 3

Multiple comparisons (using the Tukey test) for relative yield (RY) values of each species among different treatments (three water exchange rate × three competition modes) at a given competition system. Different letters indicate significant differences among treatments.

| Species WER | Density ratio of *Vallisneria natans*: *Myriophyllum* species |
|-------------|---------------------------------------------------------------|
|             | 6:2 | 4:4 | 2:6 |
| *V. natans*—*M. aquaticum* system |
| $V. natans$ 0 | 0.68 ± 0.03a | 0.42 ± 0.02c | 0.19 ± 0.01d |
| 20%          | 0.62 ± 0.02b | 0.38 ± 0.04c | 0.19 ± 0.02d |
| 40%          | 0.61 ± 0.01b | 0.39 ± 0.01c | 0.18 ± 0.0d |
| *M. aquaticum* 0 | 0.15 ± 0.04c | 0.41 ± 0.09b | 0.72 ± 0.05a |
| 20%          | 0.18 ± 0.02c | 0.4 ± 0.06b  | 0.67 ± 0.04a |
| 40%          | 0.18 ± 0.01c | 0.4 ± 0.02b  | 0.66 ± 0.04a |
| *V. natans*—*M. spicatum* system |
| $V. natans$ 0 | 0.63 ± 0.03a | 0.35 ± 0.05b | 0.15 ± 0.01c |
| 20%          | 0.63 ± 0.05a | 0.37 ± 0.03b | 0.17 ± 0.01c |
| 40%          | 0.66 ± 0.06a | 0.39 ± 0.02b | 0.17 ± 0.02c |
| *M. spicatum* 0 | 0.15 ± 0.02c | 0.34 ± 0.06b | 0.65 ± 0.04a |
| 20%          | 0.16 ± 0.03c | 0.4 ± 0.04b  | 0.7 ± 0.04a  |
| 40%          | 0.16 ± 0.02c | 0.37 ± 0.04b | 0.64 ± 0.03a |

### Table 4

Multiple comparisons (using the Tukey test) for total relative yield (RYT) values among different treatments (three water exchange rate × three competition modes) for two competition systems ($Vallisneria natans$ vs. $Myriophyllum aquaticum$ and $V. natans$ vs. $Myriophyllum spicatum$). Different letters indicate significant differences among treatments.

| WER | Density ratio of *Vallisneria natans*: *Myriophyllum* species |
|-----|---------------------------------------------------------------|
|     | 6:2 | 4:4 | 2:6 |
| *V. natans*—*M. aquaticum* system |
| 0  | 0.83 ± 0.08ab | 0.83 ± 0.11ab | 0.91 ± 0.06a |
| 20% | 0.80 ± 0.02ab | 0.78 ± 0.07b  | 0.86 ± 0.05ab |
| 40% | 0.79 ± 0.02ab | 0.81 ± 0.03ab | 0.84 ± 0.04ab |
| *V. natans*—*M. spicatum* system |
| 0  | 0.78 ± 0.03ab | 0.69 ± 0.08c  | 0.80 ± 0.04ab |
| 20% | 0.79 ± 0.08abc| 0.76 ± 0.05abc| 0.87 ± 0.04a |
| 40% | 0.81 ± 0.06ab | 0.76 ± 0.04bc | 0.80 ± 0.04ab |
we examined the potential effects of WER on interactions between coexisting submerged macrophytes, and the links between relative competitive ability and functional traits in these species. Our results suggest that the WER is an important factor driving plant competition, and that shifts in relative competitive ability in response to the WER and combination mode are attributable directly to consistently increasing the differences in functional traits between co-cultured species. Thus, our results show more support for the CTH hypothesis than the CTS hypothesis. To the best of our knowledge, this is the first study to validate the CTH hypothesis by evaluating interspecies competition among aquatic macrophytes.

Environmental variation in response to WER

In the experiment without added ramets, DO concentration was consistently higher than 6.0 mg L\(^{-1}\) and within the range of those observed in natural environments (Zhu et al. 2017; Tong et al. 2019; Ishikawa et al. 2019). The DCD concentration consistently decreased as the WER increased throughout the entire experiment (Fig. 2) and was always less than 490 ppm (minimum: 361 ppm), lower than the values reported in other indoor and field studies involving *V. natans* (Cao and Ruan 2015). DCD is among the principle sources of C for the photosynthesis of submerged macrophytes (Dülger et al. 2017). Thus, the DCD concentration may be the main factor limiting normal growth of submerged macrophytes of our experimental system (Fig. 2), as revealed by its significant linear relationships with biomass accumulation and plant height, regardless of the species and competition system (Figures S1 and S2).

In aquatic ecosystems, the effects of WER on DCD availability are mainly determined by the diffusion rate of CO\(_2\) across the air–water interface, and its concentration difference between inflow and outflow water (Dülger et al. 2017; Yuan et al. 2018; Pan et al. 2019). Given that the DCD concentration was obviously lower in incoming water than in bucket water (Table S1), the decrease in DCD concentration can be simply attributed to increasing WER, i.e., DCD released by the microbial decomposition of sediment organic matter was carried away by outlet water.

Responses of plant functional traits to WER

Optimal partitioning theory states that plants usually allocate biomass to the organ that acquires the most limiting resource to enhance fitness (Bloom et al. 1985). Thus, in our experimental system, DCD should be the factor limiting normal growth of submerged macrophytes. Leaf growth can promote the absorption of DCD, and the allocation of more biomass to stems benefits plant growth near or above the water surface where they can acquire more DCD (Murillo et al. 2019; Li et al. 2020). Thus, as the WER increased, more biomass was allocated to above-ground tissues (leaves and stems) in the two *Myriophyllum* species (Fig. 4). In contrast, more biomass was allocated to below-ground tissues and the production of new ramets for co-cocultured *V. natans*, regardless of the competition system. The production of new ramets allows plants to escape unfavorable environments (Wolfer and Straile 2004; Nord and Lynch 2008), but may be disadvantageous for the fitness of the stock plant, including the *in-situ* acquisition of DCD. In the present study, the effects of interspecific competition
were greater than those of intraspecific competition, as the RY_T was always less than 1. Thus, the two functional dissimilarities may have contributed to changes in relative competitive ability between species pairs, i.e., consistently decreasing the competitive ability of *V. natans*. This speculation was further demonstrated by the significant negative relationships of the two traits to A_v values, regardless of the competition system (Figures S10 and 11).

Completely opposite response patterns were observed between co-cultured species for root functional traits after exposure to WER variation. For the *Myriophyllum* species, the allocation of more biomass to above-ground tissues and new ramets inhibits the root growth of the stock plant, which is the main factor influencing the absorption of environmental nutrients (Xie et al. 2007). Under such conditions, the regulation of root morphology allows plants to efficiently increase nutrient absorption ability by developing longer and/or thinner roots (see Xie et al. 2007; Pan et al. 2012, 2014). Consistent with this pattern, roots were longer and thinner and SRL was higher in higher-WER than in lower-WER treatments for the two *Myriophyllum* species, regardless of the planting proportion of either species (Fig. 5). Through these adjustments, the nutrient absorption ability of the *Myriophyllum* species was not inhibited by reduced allocation of biomass to roots, as revealed by gradually increased concentrations of plant N and P as WER increased (Fig. 6). The growth and development of submerged macrophytes is directly dependent on the rapid accumulation of elements, particularly N and P, which are essential for the formation of various fundamental metabolites (Van de Waal et al. 2010). These results indicate that *Myriophyllum* species adapted well to WER variation through root functional trait plasticity. In contrast, roots of *V. natans* became shorter and thicker, and the SRL generally decreased with increasing WER. These responses will undoubtedly inhibit the absorption of sediment nutrients. As predicted, P concentration of *V. natans* exhibited a decreasing trend with increasing WER, further demonstrating the important role of root functional traits in determining nutrient absorption ability (Fig. 6). These trait divergences can further affect species interactions (Callaway et al. 2003; Coomes and Grubb 2003; Fort et al. 2014), particularly interspecies competition, when interspecific differences are large (Pan et al. 2019). Consistently, A_v values were associated positively with the root length and SRL, but negatively with the root diameter in this study (Figure S11).

In addition, by using the replacement series method, we clearly showed that combination mode also induced trait divergence. As the planting proportion of *V. natans* decreased, the B:A biomass ratio and root diameter increased, while the root length and SRL decreased in *V. natans*; whereas the opposite trends were observed in co-cultured *Myriophyllum* species, regardless of the competition system. Furthermore, the branch number per plant mass increased significantly in *V. natans*, but did not differ significantly in the *Myriophyllum* species under the same conditions. Clearly, both WER variation and combination mode induced species-specific changes in functional values between co-cultured submerged macrophytes. Previous studies have demonstrated that *Myriophyllum* species are usually weaker competitors than other submerged macrophytes in stable waterbodies (Wang et al. 2008; Miler et al. 2012). Thus, *V. natans* was a stronger competitor than *Myriophyllum* species in static waterbodies due to generally longer root length and SRL, as well as lower below-ground biomass ratio and root diameter, which allowed it to acquire DCD and nutrients more efficiently than the *Myriophyllum* species. However, the relative competitive ability of *Myriophyllum* species consistently increased with increasing WER and decreasing the planting proportion of *V. natans*, which would eventually transform it into a stronger competitor than *V. natans* (Tables 3, 4, 5). Given that the functional dissimilarity of the two coexisting pairs increased consistently during shifts in relative competitive ability, we conclude that CTH played a more important role than CTS in determining the relative abundance of the plants.

**Conclusion**

The results of this study provide insight into the mechanism by which WER affects interspecific interaction between submerged macrophytes. Variation in relative competitive ability between co-cultured species was strongly associated with hierarchical differences in plant functional traits associated with DCD and nutrient acquisition, providing more support for the CTH hypothesis than the CTS hypothesis. Understanding interactions
between competing individuals under WER variation is important for explaining plant distribution patterns and predicting further changes in communities of submerged macrophytes. For example, the present results will contribute to understanding the potential mechanisms driving *Myriophyllum* species distribution patterns in natural aquatic ecosystems, such as in tunnels and lakeshores, which are usually characterized by strong water exchange. In addition, *M. aquaticum*, a common indigenous species in South America, is now widespread in many countries, where it is considered a troublesome invasive (Lastrucci et al. 2017). In Yunnan Plateau lakes, invasive *M. aquaticum* successfully outcompetes native macrophytes (You et al. 2013). Our results provide valuable information for our understanding of the establishment of *M. aquaticum* elsewhere in the world. In particular, WER increases appear to favor this invasive species due to its stronger ability to tolerate high degrees of WER variation and nutrient deficiency compared with that of other submerged macrophytes (e.g., *V. natans* in the present study).

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**Author contributions** YP: designed the experiments, Writing—original draft, Formal analysis. DY: Writing and methodology. YG: Methodology. CD: Methodology.

**Declarations**

**Conflict of interest** The authors declare no competing financial interests.

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