NUTRIENT RESPONSES OF CAREX SCHMIDTH TO CONSECUTIVE DROUGHT AND RE-FLOODING EPISODES IN SEMI-ARID WETLANDS: IMPLICATIONS FOR TUSSOCK RESTORATION

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Abstract. The regulation mechanism of wetland plants in response to consecutive drought (D) and reflooding (RT) events can be identified via nutrient responses. Nutrient responses of *Carex schmidtii* were tested using a simulation experiment with five treatments: D, wetting (W) and RT resulted in *C. schmidtii* being re-flooded after 30, 40 and 60 days of drought. D and RT treatments partly affected nutrient stoichiometry of *C. schmidtii*. Plant nutrient stoichiometry experienced a shift of adaption from drought to flooding and making part or full recoveries after RT with reference to D and W treatments. Plant P concentration had a negative relationship with N:K ratio. D and RT effects on plant trade-offs and scaling exponents of *C. schmidtii* were profound; the scaling exponent of P~N increased with initial re-flooding time, while that of K~N decreased. Although nutrient trade-offs in D gradually differed from W and RT over time, the nutrient flexibility of *C. schmidtii*; however, strong internal stability of stoichiometric relationships effectively relieved changes in plant nutrient stoichiometry. The findings aid our understanding of nutrient responses of *C. schmidtii* to D and RT.

Keywords: nutrient trade-offs, hydrological changes, Carex schmidtii tussocks, Momoge wetland, wetland restoration, semi-arid environments

Introduction

Nutrient homeostasis or flexibility focused on nutrient stoichiometry and stoichiometric relationships can reveal the internal mechanisms of wetland plants in response to environmental fluctuations (Elser et al., 2000; Güsewell et al., 2003; Sistla and Schimel, 2012; Julian et al., 2020). Nutrient homeostatic or flexibility are closely associated with plant growth strategy and adaption, by which plants regulate the underlying physiological processes and biochemical allocations in response to environmental fluctuations (Yu et al., 2015; Hu et al., 2018; Li et al., 2018). Nutrient trade-offs are the dynamic equilibrium of nutrients and their ratios in organisms, regulating their nutrient stoichiometry and stoichiometric relationship, thereby affecting nutrient homeostasis or flexibility (Sistla and Schimel, 2012; Corrales-Carvajal et al., 2016; Zhang et al., 2021). As nutrient trade-offs

are a response to the degree of coupling effects of multi nutrient stoichiometry and their environment on plant growth and eco-physiological processes (Wright et al., 2004; Yan et al., 2019), nutrient homeostasis or flexibility relying on nutrient trade-offs are therefore widely applied to solve related problems in ecological systems. However, whether nutrient homeostasis and flexibility together regulate plant growth and nutrient responses by nutrient trade-offs is currently unclear.

Nutrient stoichiometry and responses of wetland plants with respect to nutrient tradeoffs and homeostasis or flexibility are important factors regulating the adaptability of plants to hydrological fluctuation (Sistla and Schimel, 2012; Rong et al., 2015; Mariotte et al., 2017). Nitrogen (N), phosphorus (P) and potassium (K) are the abundant essential elements in higher plants; these nutrients are also common nutrients supporting plant growth, having a strong relationship to individual performance (Wright et al., 2011; Chiwa et al., 2019). N and P are also important sources for the synthesis of photosynthetic pigments, proteins and nuclei (Wan et al., 2020; Huang et a., 2021; Zhang et al., 2019a). K plays a vital role in the production, transportation and storage processes of carbohydrates, it promotes protein synthesis and activates certain enzymes or coenzymes (Chiwa et al., 2019; Gierth and Mäser, 2007; Liu et al., 2021). Additionally, ratios of N, P and K are proxies for stoichiometric relationships regulating plant nutrient acquisition, transportation and availability, as well as being used to indicate nutrient limitation of species and ecosystems (Güsewell et al., 2003; Pan et al., 2020; Zhang et al., 2021). Whether nutrients and their ratios vary within a certain range is closely related to nutrient homeostasis or flexibility (Sistla and Schimel, 2012; Julian et al., 2020). Previous studies indicated a general pattern of nutrient stoichiometry under multi environments, highlighting that nutrient homeostasis relieved environmental fluctuation effects on plants (Reich et al., 2010; Tian et al., 2018; Hu et al., 2021). Therefore, examining nutrient stoichiometry and responses is an effective method to help understand the adaption of plant functional trait adaptation under environmental fluctuations. However, the majority of studies have only focused on nutrient performance across species on global or zonal scales, few investigations have been undertaken on the nutrient response of wetland plants at a single species level.

In a wetland system, hydrological fluctuations with changes in flooding depth, frequency and duration are master factors controlling plant growth and ecophysiological processes (Casanova and Brock, 2000; Zhang et al., 2019a). Extreme hydrological fluctuations due to changes from consecutive drought episodes to flooding events have complex effects on plant growth and nutrient trade-offs. These effects influence community structure and vegetation patterns in wetlands around the world, especially in semi-arid zones (Li et al., 2017b; Wen et al., 2017; Zhang et al., 2019b). Although it has been previously shown that initial flooding results in a decrease of plant N, P and K concentrations in plant leaves, flooding events also result in an increase of nutrient concentration in roots (Chen et al., 2005). Concentration of N and P in plant shoots of submersed macrophytes significantly decreased along a water depth gradient, while the N:P ratio increased (Li et al., 2015). Chen et al. (2020) reported speciesspecific responses of leaf N and P stoichiometry to flooding duration at the intra-species level, other than at the inter-species level. Additionally, leaf N and P concentrations of wetland plants significantly increased with flooding duration, while their ratio decreased (Chen et al., 2020). Recently, Zhang et al. (2021) recorded hydrological fluctuations to have complex effects on the dynamic of nutrient response and stoichiometric relationships of wetland plants. However, the majority of studies focused on plant growth performance under hydrological fluctuations (Hamdan et al., 2010; Garssen et al., 2015; Yuan et al., 2019); few investigations have examined nutrient response and trade-offs of wetland plants to hydrological fluctuations, especially re-flooding conditions after consecutive drought episodes.

Carex schmidtii is a native tussock-forming species with hydrological fluctuation tolerance, contributing to the wide distribution of tussocks in riparian wetlands and mountainous marshes in north temperate climates (Qi et al., 2021a; Zhang et al., 2019a). *C. schmidtii* tussocks have an abundant root system, facilitated to absorb and conserve nutrients in biomass and the hummocks (Lawrence et al., 2013; Wang et al., 2019a). Due to climate changes and anthropogenic activities, *C. schmidtii* tussocks have suffered serious degradation and death over the last few decades (Pan et al., 2006; Qi et al., 2021b). The effects of hydrological gradient and fluctuations on plant growth and eco-physiological responses of *C. schmidtii* have been previous reported, indicating that this plant relies on final or dynamic performance (Yan et al., 2015; Zhang et al., 2019a, b). However, despite the importance of nutrient stoichiometry for the ecological adaptation of wetland species, few investigations have focused on nutrient responses of *C. schmidtii* to consecutive drought and re-flooding episodes from the perspective of nutrient trade-off and recovery.

As there is a substantial information gap relating to nutrient trade-offs regarding hydrological fluctuations and plant growth recovering in wetland ecosystems, we examine in detail the role of nutrient homeostasis or flexibility with respect to ecological responses (Julian et al., 2020; Chen et al., 2020; Zhang et al., 2021). Nutrient responses of C. schmidtii in a simulation experiment were tested under various hydrological treatments, including consecutive drought, wetting and re-flooding treatments. The aims of this study are: (1) to examine the effects of consecutive drought and re-flooding on nutrient stoichiometry of C. schmidtii at each growth stage;(2) to identify the responses of nutrient trade-offs and their scaling exponents to re-flooding; and (3) to evaluate the nutrient recovery effect of C. schmidtii. We hypothesized that (1) consecutive drought episodes and re-flooding significantly affected plant nutrient stoichiometry and their trade-offs, (2) and re-flooding effectively recovered plant nutrients in C. schmidtii. We also hypothesized that consecutive drought and re-flooding promoted nutrient flexibility of C. schmidtii, and nutrient homeostasis and flexibility together regulate plant nutrient responses. Findings from this investigation will aid in understanding the response of nutrient stoichiometry and trade-offs of C. schmidtii to consecutive drought and re-flooding treatments, providing information about nutrient recovery of C. schmidtii in semi-arid zones.

Materials and methods

Experimental process

In May 2018, hummocks of *C. schmidtii* tussocks and soil were collected from a riparian wetland situated along the Nenjiang River in Momoge Wetland Nature Reserve, Northeast China. Hummocks and soil were transported to a greenhouse (Northeast Institute of Geography and Agroecology, Chinese Academy of Sciences) within 12 h of collection. Hummocks without above-biomass were cut into uniform pieces (20 cm in height and 15 cm in diameter). The bottom 30 cm of each plastic container (cubes with a side length of 50 cm) was filled with mixed soil, in which hummocks were individually planted; the top half of the section was left uncovered.

The experimental culture and equipment used in this simulation can be found in Zhang et al. (2019b, 2020). Plant rhizomes were successfully sprouted from the hummocks and were cultivated for 18 days with a soil water content of $36.32 \pm 1.46\%$. Physicochemical properties of tap water and soil used in this experiment are the same as those highlighted in Zhang et al. (2019b). The soil has a total nitrogen content of $4.27 \pm 0.67 \text{ mg} \cdot \text{g}^{-1}$, total phosphorus content of $0.52 \pm 0.07 \text{ mg} \cdot \text{g}^{-1}$. During the experimental period, the air temperature and relative humidity in the greenhouse ranged from 25 to 38 °C and 36 to 58%, respectively.

In June 2018, 30 C. schmidtii tussocks with a similar plant size (a height of 52.98 ± 1.19 cm and biomass of 0.12 ± 0.02 g) were selected for our experiment. Five treatments were undertaken in this analysis: wet treatment (W), consecutive drought treatment (D) and reflooding treatments (RT: DF30, DF40 and DF60). Each treatment had six duplicates. A soil water content ranging from 37.11 to 41.62% was maintained in treatment W by replenishing the water every two or three days (keep the soil surface moist); treatment D naturally reduced the soil water content over time; and treatments RT resulted in C. schmidtii tussocks being re-flooded about 5 cm after 30 days (DF30), 40 days (DF40) and 60 days (DF60) of drought, respectively. The changes in soil water content under D and W treatment can be found in Zhang et al. (2019b). Plants were flooded after RT so as to simulate submergence events caused by irregular heavy rainfall in July and August. Notably, C. schmidtii tussocks naturally reduced the soil water content over time before reflooding, and then the treatments maintained a 5 cm flooding depth above the soil surface. Plants from the independent tussocks were then randomly analyzed for each treatment every ten days. The experiment lasted for 110 days (from June 1 to September 18, 2018), covering the entire growth stage of C. schmidtii (Zhang et al., 2019b).

Sampling and analysis

In order to evaluate the effects of consecutive drought episodes and re-flooding on nutrient stoichiometry and trade-offs of *C. schmidtii*, plants were randomly selected from each treatment at different growth stage (Day 0, 10, 20, 30, 40, 50, 60,70, 80, 90, 100, 110, respectively) and dried for 2 h at 120 °C before being dried at 65 °C until a constant weight was recorded, Samples were then ground into powder and sieved through a 0.25 mm sieve. Sulfuric acid ($\omega = 98\%$; $\rho = 1.84$) and hydrogen peroxide ($\omega = 30\%$; $\rho = 1.13$) were added to the plant samples before they were digested at 375 °C using a muffle furnace until the liquid was clear. Before analysis, the clear liquid was cooled to room temperature and made up to a volume of 500 mL. Plant N and P concentrations were determined using Kjeldahlanalysis and the Molybdenum-Antimony-Spectropho-Tometric Method with an automatic chemistry analyzer (Smartchem 300, Advanced Monolithic Systems, Graz, Italy), respectively. Plant K concentrations were determined by atomic emission spectrometer (ICPS-7500, Shimadzu, Japan). All N:P, N:K and K:P ratios for every treatment were calculated as mass ratios (Zhang et al., 2021).

Statistical analysis

Statistical analyses were performed using SPSS 20, Origin 9.2 and R v4.0.1 (R Development Core Team 2021). Plant N, P, K concentrations and their ratios in different growth stages were checked for normality and homogeneity before further analyses. To meet the assumptions of homoscedasticity, some variables were log-

transformed. One-way analysis of variance (ANOVA) was used to determine the effects of consecutive drought and re-flooding on plant nutrients and their ratios of *C. schmidtii*. Multiple comparisons of means were undertaken using Duncan's test at the 0.05 significance level. Correlations between plant nutrients and their ratios were performed using "PerformanceAnalytics" package (https://CRAN.R-project.org/package=PerformanceAnalytics) in R. Strong correlations between plant nutrients and their ratios were plant nutrients and their ratios were project.org/package=PerformanceAnalytics) in R. Strong correlations between plant nutrients and their ratios were plant nutrients and their ratios were expressed by the power function as *Equation 1:*

$$Lgi = \alpha \times Lgj + \beta$$
 (Eq.1)

where α and β indicate the slope (i.e. the scaling exponent) and 'elevation' or Y-intercept (i.e. normalization constant; Reich et al., 2010; Tian et al., 2018); *i* and *j* indicate N, P, K and their ratios of *C. schmidtii*, respectively.

Fittings were performed to describe plant nutrient trade-offs in each treatment based on data collected from the entire *C. schmidtii* growth stage (Day 0~110) and data collected after re-flooding treatments, respectively. Ordinary least square (OLS) regression was used to determine the *i~j* scaling relationship under different treatments using package "Imodel2" in R (https://CRAN.R-project.org/package=Imodel2). Principal component analysis (PCA) of plant nutrients and their ratios of *C. schmidtii* in different growth stages were performed using "FactomineR" (https://CRAN.R-project.org/package=FactoMineR) and "factoextra" (https://CRAN.R-project.org/package=factoextra) packages.

Results

Effects of consecutive drought and re-flooding events on nutrients and their ratios of C. schmidtii

Consecutive drought and re-flooding treatments had complex effects on N, P and K concentrations of C. schmidtii (Figs. 1 and A1). Fluctuation curves of plant N concentrations were observed in D and W treatments, with significant differences being identified on the Day 10 (Fig. 1 and Table 1). The highest plant N concentration was recorded in the W treatment recorded as 29.26% higher than the value in the D treatment. However, plant N concentrations in D and W treatments decreased to 13.68 mg·g⁻¹ and 13.91 mg·g⁻¹ by the Day 30, respectively. From Day 40 to Day 70, plant N concentrations in the W treatment increased with time; N concentration in the D treatment decreased from 17.29 mg·g⁻¹ to 15.43 mg·g⁻¹ in the same time period (Fig. A1). Compared to the D and W treatments, plant N concentrations recorded partial or full recoveries under re-flooding treatments (DF30, DF40 and DF60) due to a shift of adaptation from drought to flooding. Plant P concentrations in the D treatment increased from 0.73 mg·g⁻¹ to 1.04 mg·g⁻¹ before decreasing to 0.41 mg·g⁻¹ with time (*Fig. 1b*). Significant differences in plant P concentration were identified between D and W treatments on Day 20 (Fig. 1a). From Day 40 to Day 70, plant P concentrations in the W treatment were significantly higher than those in the D treatment. No significant differences in plant P concentrations were recorded among W and RT (DF30, DF40 and DF60) during the late growth stage. Plant K concentrations changes with time in the D treatment (Day 0 ~Day 70); across the entire growth stage, K concentrations recorded a decreasing tend in the W treatment. Although plant K concentration recorded a decrease at the early stage of re-flooding treatments, concentrations in DF40 and DF60 treatments recorded full recoveries compared to the W treatment.

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Figure 1. Effects of consecutive drought and re-flooding episodes on N concentrations (a), P concentrations (b), K concentrations (c), N:P ratios (d), N:K ratios (e) and K:P ratios (f) of Carex schmidtii. W is the wet treatment; D is the consecutive drought treatment; DF30, DF40 and DF60 refer to re-flooding after 30, 40 and 60 days of consecutive drought, respectively. Different letters stand for significant differences at the 0.05 significance level in treatments in the same growth stage and there are no significant differences among treatments without any letters

Nutrient	Day 0		Day 10		Day 20		Day 30		Day 40		Day 50	
	F	р	F	р	F	р	F	р	F	р	F	р
Ν	0.000	1.000	14.868	0.008	0.253	0.630	0.061	0.818	1.531	0.248	0.780	0.527
Р	0.005	0.948	0.328	0.588	6.090	0.043	0.111	0.756	2.895	0.086	0.960	0.443
Κ	0.000	0.993	3.159	0.126	0.051	0.828	7.172	0.055	6.537	0.009	0.940	0.452
N:P	0.136	0.731	2.304	0.180	10.282	0.015	0.011	0.920	11.026	0.001	0.931	0.456
N:K	0.008	0.933	0.011	0.921	1.850	0.216	44.143	0.003	5.881	0.013	0.640	0.604
K:P	0.025	0.881	3.697	0.103	10.128	0.015	0.684	0.455	29.907	0.000	1.883	0.186
Nutrient	Day 60		Day 70		Day 80		Day 90		Day 100		Day 110	
	F	р	F	р	F	р	F	р	F	р	F	р
Ν	3.337	0.077	1.291	0.315	5.549	0.023	3.767	0.059	0.921	0.473	0.505	0.687
Р	3.059	0.092	2.565	0.078	3.100	0.089	0.282	0.837	1.595	0.265	2.852	0.086
Κ	0.164	0.917	1.141	0.373	3.991	0.052	20.759	0.000	1.173	0.379	4.312	0.031
N:P	13.557	0.002	7.115	0.002	0.330	0.804	1.573	0.270	2.860	0.104	0.764	0.537
N:K	1.973	0.197	0.550	0.702	0.996	0.443	20.437	0.000	0.672	0.593	2.811	0.089
K:P	12.768	0.002	7.532	0.001	0.037	0.990	13.463	0.002	0.510	0.686	1.236	0.343

Table 1. One-way ANOVAs results (F and p values) for the effects of consecutive drought and re-flooding treatments on N, P, K concentrations and their ratios for Carex schmidtii at each growth stage

In treatment D, the N:P ratio decreased from 26.11 to 18.40 before increased to 40.73; in the W treatment, moderate fluctuations were recorded between 20.01 and 28.79 (*Figs. 1* and *A1*). Similar N:P recovery patterns were also recorded among DF30, DF40 and DF60 treatments. The N:P ratio decreased at the early stage of RT and then recovered to a higher value (>16) by the end of the experiment. The N:K ratio recorded a variation under D treatment, with values < 1.2 between Day 30 to Day 60 (except for

Day 50). N:K ratio, however, recorded a significant increase under W and RT treatments, especially during the late growth stage (*Fig. le*). Under treatment D, the K:P ratio decreased before increasing, under the W treatment it recorded an increase before decreasing in W treatment (*Fig. lf*). Significant differences in K:P ratios were identified on the Day 20, Day 40, Day 60~70 and Day 90. On the Day 20, the highest value of the K:P ratio was recorded in the W treatment, this being 1.71 times of the value in D treatment. However, the K:P ratio in the D treatment was significantly higher than W treatment between the Day 40 ~ Day 70. Re-flooding treatments effectively relieved the negative effects of the D treatment, recorded full recovery of the K:P ratio with reference to the W treatment.

Effects of consecutive drought and re-flooding on nutrient trade-offs of C. schmidtii

Positive relationships were identified among N, P and K concentrations of *C. schmidtii* using correlations analysis. Divergent pairwise correlations among the N:P, N:K and K:P ratios were recorded. The N:P ratio was positively related to N:K and K:P ratios for *C. schmidtii*, and the N:K ratio was negatively related to the K:P ratio. Plant P concentrations exhibited relatively moderate and negative relationships with the N:K ratio (*Fig. 2*).



Figure 2. Correlation analysis of N, P, K concentrations and their ratios of Carex schmidtii

Consecutive drought and re-flooding had profound effects on the trade-offs among N, P and K concentrations of *C. schmidtii* (*Fig. A2; Table 2*). The P~N scaling exponent in treatment D was 1.201, 21.68% higher than that recorded in the W treatment (0.987). The P~N scaling exponents in re-flooding treatments increased with the order of DF30

(0.464) < DF40 (0.684) < DF60 (0.999); the scaling exponent in treatment DF60 was much similar to that recorded in treatment W. K~N scaling exponents in D and W treatments were 0.733 and 1.708, respectively; scaling exponents in re-flooding treatments decreased with the order of DF30 (0.833) > DF40 (0.707) > DF60 (0.564). K~P scaling exponents in treatments D, DF60, DF40, DF30 and W were 0.136, 0.214, 0.347, 0.500 and 1.036, respectively. However, trade-offs between N:P ratio~K, N:K ratio~N and K:P ratio~N were not clear under consecutive drought and re-flooding treatments (*Table A1*). Trade-offs of K~P based on nutrient performance after reflooding were significant and their scaling exponent had the order of DF30 (0.540) < DF40 (0.606) < DF60 (0.981; *Table A2*). Based on data for the entire growth stage of *C. schmidtii*, these results were contrary to those for the K~P scaling exponent (*Table 2*).

Table 2. Summary of fitting results between N, P and K concentrations of Carex schmidtii under consecutive drought and re-flooding conditions. Fittings in D, W, DF30, DF40 and DF60 were based on nutrient data collected from the entire growth stage of C. schmidtii. α Scaling exponents, CI Confidence interval

Lg i~ Lg j	Treatments	Fittings	α (95%CI)	R ²	р
Lg P~ Lg N	W	Y = 0.987X - 1.350	0.987 (0.678~1.297)	0.484	< 0.001
	D	Y = 1.201X-1.655	1.201 (0.554~1.848)	0.324	< 0.001
	DF30	Y = 0.464X-0.668	0.464 (0.101~0.828)	0.133	< 0.05
	DF40	Y = 0.684X-947	0.684 (0.256~1.113)	0.199	< 0.01
	DF60	Y = 0.999X-1.368	0.999 (0.557~1.441)	0.331	< 0.001
Lg K~ Lg N	W	Y = 1.708X-1.088	1.708 (1.218~2.198)	0.529	< 0.001
	D	Y = 0.733X + 0.240	0.733 (0.440~1.026)	0.465	< 0.001
	DF30	Y = 0.833X + 0.016	0.833 (0.385~1.281)	0.246	< 0.001
	DF40	Y = 0.707X + 0.211	0.707 (0.282~1.132)	0.211	< 0.01
	DF60	Y = 0.564X + 0.400	0.564 (0.133~0.996)	0.142	< 0.05
Lg K~ Lg P	W	Y = 1.036X + 1.167	1.306 (0.644~1.429)	0.391	< 0.001
	D	Y = 0.136X + 1.158	0.136 (-0.046~0.319)	0.072	0.138
	DF30	Y = 0.500X + 1.067	0.500 (0.124~0.876)	0.143	< 0.05
	DF40	Y = 0.347X + 1.117	0.347 (0.056~0.640)	0.121	< 0.05
	DF60	Y = 0.214X + 1.124	0.214 (-0.046~0.474)	0.062	0.104

Nutrient recovery of C. schmidtii under consecutive drought and re-flooding conditions

PCA results of nutrients and their ratios revealed two PCs explaining 78.6% ~87.7% of the total variation in the seven growth stages (Day 0~110; *Figs. 3* and *A3*). Between Day 0~10, plant N, P, K concentrations and their ratios in D and W treatments were similar (*Fig. 3a*). Significant differences in nutrient balance between D and other treatments (W, DF30, DF40 and DF60) were identified along PC1 as they occupied unique positions in the four growth stages from Day 20~70. DF30, DF40 and DF60 treatments recorded similar nutrient balance results as that in treatment W in the entire re-flooding stage (Day 40~110). Additionally, DF30 recorded significant differences in

the nutrient balance along PC1 with DF40 between Day 80~90, and with DF60 from Day 100~110. The nutrient balance in DF60 was much more convergent than that in other treatments in the early re-flooding stage (Day 70). Additionally, PCA of nutrients and their ratios revealed two PCs explaining 77.1% and 77.8% of total variation based on the nutrient data collected after re-flooding (*Fig. A4 a* and *b*) and the entire growth stages (*Fig. A4 c* and *d*), respectively. Nutrient trade-offs in treatment D recorded significant differences along PC1 and PC2 due to their unique positions (*Fig. A4 b*). Interestingly, nutrient trade-offs developed to the negative direction along PC1 with the order of D, DF60, DF40, DF30 (*Fig. A4 d*).



Figure 3. The PCA plot showing the locations of nutrients in Carex schmidtii under consecutive drought and re-flooding conditions based on nutrient data collected from the entire growth stage. (a) Day 0~10, (b) Day 20~30, (c) Day 40, (d) Day 50~60, (e) Day 70, (f) Day 80~90 and (h) Day 100~110. Markers represent individual sample locations, while ellipses represent the 95% confidence zone for each treatment

The coupling relationship of biomass nutrients and their ratios in Carex schmidtii

The pathway analysis model explained 48.33%, 81.43% and 36.00% of plant N, P, K concentrations, respectively (*Fig. 4*). Due to the strong internal stability of plant nutrient stoichiometry, results indicated that consecutive drought and re-flooding conditions had no significant contribution and path flux to plant nutrient and their ratios. Treatment time had negative effects on plant K concentration, N:P ratio and K:P ratio. Plant P and K concentrations, N:P ratio and treatment time had positive effects on plant N concentration. Additionally, plant K concentration has a high contribution to plant P concentration.



Figure 4. Structural equation modeling (SEM) of treatment time, plant N, P, K concentrations and their ratios ($\chi^2 = 1.647$, Df = 5, p = 0.895, GFI = 0.996, RMSEA = 0). Red and blue solid arrows indicate significant positive and negative pathways, respectively. Numbers in the arrows indicate the path coefficients. Percentage values close to the variables refer to the proportion of explained variance (R^2) by the model. Day, treatment time; NP, N:P ratio; KP, K:P ratio

Discussion

Nutrient homeostasis or flexibility are recognized as the middle ground of ecological stoichiometry, affecting the stoichiometric relationships of terrestrial vegetation (Sistla et al., 2015; Gu et al., 2017; Mariotte et al., 2017). In previous studies, most plants displayed clear homeostatic behavior between the plant-soil system under conditions where nutrients were added (Wang et al., 2018; Yang et al., 2019). Additionally, the majority of previous studies focused on nutrient homeostasis of wetland plants regarding multi species on a global or zonal scale (Yu et al., 2015; Li et al., 2018; Hu et al., 2018). However, it is also controversial that nutrient homeostasis or flexibility regulate the ecological, physiological and biochemical processes of plants in response to environmental fluctuations (Sistla and Schimel, 2012; Julian et al., 2020). Therefore, debates regarding to nutrient homeostasis or flexibility have highlighted the significance of understanding nutrient responses of wetland plants to hydrological fluctuations. In semi-arid zones, the hydrological management using re-flooding to relieve drought is a common method to relieve the effects of consecutive drought episodes on plant growth performance (Li et al., 2017b; Zhang et al., 2019b; Qi et al., 2021a). Whether nutrient homeostasis or flexibility together regulate nutrient responses of C. schmidtii, and what is the degree of re-flooding treatments effect on C. schmidtii, are interesting and meaningful ecological questions.

Inconsistent with our hypothesis, consecutive drought and re-flooding treatments have complex effects on nutrient stoichiometry of *C. schmidtii* at each growth stage. Flooding condition promoted plant N concentration compared to consecutive drought treatment (Day10). This finding is consistent with that of Li et al. (2017a), who found that increasing soil water content promoted foliar nutrient concentrations of *Carex brevicuspis*. Additionally, short-term drought contributed to an increase in plant P concentrations and the decrease of N:P and K:P ratios (Day 20) by enhancing P-related physiological processes under an aerobic environment. However, a sharp decrease in nutrients occurred in D and W treatments on Day 30. This result may be related to 1) the dilution effects of nutrients due to the rapid growth of wetland plants, and 2) the protective strategy of plants by transferring nutrients to root systems in response to a

sharp increase temperature stress. Additionally, as our study paid close attention to the dynamic process of nutrient performance in wetland plants within a certain time period and on key hydrological transition nodes, and we found that re-flooding after varying degrees of drought resulted in complex wetland plant responses. The growth and nutrient responses of wetland plants are influenced by the transition order, duration and frequency of drought-flooding (Li et al., 2017a; Yuan et al., 2019; Lan et al., 2021). In this study, re-flooding after drought (one form of alternating flooding-drought conditions) contributed to an increase of plant P concentrations and a decrease in the N:P ratio compared to consecutive drought events. Zhang et al. (2019a) reported that plant N and P concentrations decreased under alternating flooding-drought conditions; the ratio of N:P increased compared to those under consecutive flooding treatments. Previous studies have also revealed that hydrological fluctuations have significant effects on the dynamic of plant N and K concentrations, and their ratios in C. schmidtii (Zhang et al., 2021). These results indicate that suitable water conditions are conducive to nutrient absorption and storage in wetland plants. Significant differences in some nutrient stoichiometry among the treatments recorded nutrient flexibility of wetland plants. However, no significant differences of plant nutrients were recorded despite hydrological changes, finding that strongly support the regulation of nutrient homeostasis in plant response processes (Sistla and Schimel, 2012; Gu and Grogan, 2017).

Alternating flooding-drought conditions is an effective water-saving method to recover plant growth and nutrient stoichiometry of wetland plants in a semi-arid zone (Li et al., 2017b; Yang et al., 2020). Hydrological management in this zone with respect to alternating flooding-drought conditions effectively restores typical wetland plants including *Phragmites australis* and *C. schmidtii* (Wen et al., 2017; Zhang et al., 2019a). However, only a few studies have reported nutrient responses and trade-offs of wetland plants to re-flooding despite the significance for understanding the recovery dynamic and mechanism of plants under alternating flooding-drought conditions. In this study, re-flooding effectively alleviated drought effects on plant nutrient stoichiometry, except for K concentrations. However, plant P and K concentrations and their ratio recorded full recoveries; plant N concentration, N:P and N:K ratios recovered to a certain ratio of that in treatment W. P is closely associated with nucleus and eco-physiological processes in plants (Ghimire et al., 2017). Consecutive drought events exerted negative effects on P absorption, transportation and conservation, limiting the synthesis of ATP and phospholipid, resulting in withering of plants and death (Elser et al., 2000; Mariotte et al., 2020). Additionally, a significant fluctuation of plant nutrient stoichiometry occurred during the early stage of re-flooding after drought, indicating that plants experienced a shift from drought-adaption to flooding-adaption (Zhang et al., 2019b). The recovery dynamic of nutrients and their ratios are also influenced by initial reflooding time, affecting drought duration and plant growth (Baldwin et al., 2006; Wang et al., 2015). Re-flooding in the early growth stage facilitated rapid and successful plant growth recovery (Zhang et al., 2019a, b). However, the recovery of plant nutrient concentrations increased with an increase in initial re-flooding time after Day 70. Although wetland plants have strong plastic deformation in the early growth stage (Bizet et al., 2015; Yao et al., 2021), re-flooding in the late growth stage can affect nutrient stoichiometry, increase plant vitality and extend the phenology period of plants (Feng et al., 2021; Zhang et al., 2021).

Nutrient responses to consecutive drought and re-flooding plays vital roles in the ecological adaption of wetland species. Taking into account the growth of *C. schmidtii* in this experiment (Zhang et al., 2019b), we can more intuitively understand the regulation of nutrients on plant ecological and physiological adaptation. Both D and RT had positive or negative interactive effects on the growth and photosynthesis of *C. schmidtii*. D treatment decreased plant height, specific leaf area, chlorophyll content in the middle growth stage. Although RT were found to successfully recovery plant mass, chlorophyll content and photosynthesis of *C. schmidtii*, flooding condition inhibited plant performance in the late growth stage (Zhang et al., 2019b). This is closely related to the recovery dynamic of plant nutrients. Nutrient responses to consecutive drought and re-flooding are synergistic with plant physiological and ecological traits.

Nutrient trade-off and stoichiometric relationships are important proxies for nutrient homeostasis and flexibility (Sistla and Schimel, 2012; Corrales-Carvajal et al., 2016; Jevasingh et al., 2017). Robust stoichiometric relationships of P~N and K~N under various hydrological conditions provided strong evidence for nutrient homeostasis (Tian et al., 2018; Zhang et al., 2021). Scaling exponents (SE) were undertaken on data collected during the entire growth stage of C. schmidtii, resulting in comparable SE results among the five treatments. SE of P~N increased with an increase of initial reflooding time, and SE of P~N in DF60 was almost the same as that in treatment W. This result indicated that the relationship of P~N in DF60 recovered in relation to that in treatment W. However, due to consecutive drought events promoting the reduction of N and P, maintaining their robust stoichiometric relationships, the highest SE of P~N were recorded in treatment D. SE of K~N decreased with an increase of initial re-flooding time, results that were similar to those for treatment D. Under hydrological fluctuations, the stoichiometric relationship of K~N changed, except for that under treatment W. With consideration of nutrient stoichiometric relationships of K~P, N:P ratio~K, N:K ratio~P and K:P ratio~N, nutrient flexibility was much more common, a finding that supported the responses of internal nutrient relationships to hydrological fluctuations. Additionally, based on the data collected after re-flooding, the majority of SE in RT were absent, a finding that indicates nutrient flexibility. PCA results indicated that nutrient trade-offs of RT were also similar to W, however, they gradually separated with those in treatment D (Figs. 3 and A4). Differences in area and position of confidence ellipses revealed nutrient flexibility, and the overlapping of confidence ellipses supported nutrient homeostasis despite hydrological fluctuations (Zhang et al., 2021). SEM analysis indicated a strong relationship between nutrients and their ratios, ignoring hydrological changes. Although consecutive drought and re-flooding conditions contributed to nutrient flexibility of C. schmidtii, strong internal stability of stoichiometric relationships effectively relieved changes in plant nutrient stoichiometry. Therefore, nutrient homeostasis and flexibility together regulate plant growth and nutrient responses.

In semi-arid zones, water scarcity limited plant growth and wetland development (Pan et al., 2006; Wang et al., 2019b). A key ecological question for wetland restoration and management in these areas is how to effectively recovery plant growth utilizing a water-saving methods (Li et al., 2017b; Zhang et al., 2019a, b; Yang et al., 2020). Although wetland restoration and conservation have been strengthened in this zone recently, hydrological management methods suitable for specific wetland species are still being explored (Qi et al., 2021a). Thus, comprehensive and deepening of wetland

restoration and protection for tussock wetlands require further development (Guo et al., 2016; Zhang et al., 2019a; Oi et al., 2021c). Recovery technologies based on techniques utilizing seedbanks, transplanting rhizomes, and creating microtopography have been conducted to restore tussocks in wetlands, especially in semi-arid zones (Wang et al., 2020; Qi et al., 2021c). Additionally, hydrological management and regulation has been applied to restore tussock wetlands (Zhang et al., 2019a, b). Although previous studies examining hydrological fluctuations paid close attention to plant growth and physiological characteristics (Yan et al., 2015; Guo et al., 2016; Zhang et al., 2019a), few studies have evaluated the effects of consecutive drought and re-flooding events on nutrient responses of C. schmidtii. In this study, DF30, DF40 and DF60 were effectively found to alleviate consecutive drought effects on plant nutrients; the final performance of C. schmidtii after re-flooding increased with an increase in initial re-flooding time. By considering results from previous investigations with the aim of recovering plant growth, we recommend flexible alternating flooding-drought conditions to restore C. schmidtii wetlands in semi-arid zones (Zhang et al., 2019a, b; Wang et al., 2019b; Qi et al., 2021c). Reasonable irrigation according to the available water can guarantee the survival and development of tussocks.

Conclusion

Consecutive drought and re-flooding treatments had complex effects on nutrient stoichiometry of C. schmidtii at each growth stage. With reference to drought (D) and wetting (W) treatments, plant nutrient stoichiometry recorded partial or full recoveries under re-flooding treatments (RT: DF30, DF40 and DF60) by experiencing a shift of adaption from drought to flooding. Positive relationships were identified among N, P and K concentrations of C. schmidtii, and plant P concentration exhibited a relatively moderate and negative relationship with the N:K ratio. Consecutive drought and re-flooding treatments profoundly affected the tradeoffs among N, P and K of C. schmidtii. Significant stoichiometric relationships of P~N and K~N were quantified using the empirical equation model, and the scaling exponent of P~N increased with the order of DF30 < DF40 < DF60, while that of K~N decreased. With an increase in treatment time, nutrient trade-offs in treatment D gradually differed from W and re-flooding treatments, however nutrient trade-offs of re-flooding treatments recovered in comparison to W. Although consecutive drought and re-flooding conditions contributed to nutrient flexibility of C. schmidtii to a certain degree, strong internal stability of nutrient stoichiometric relationships of C. schmidtii regulated nutrient trade-offs and effectively relieved changes in plant nutrient stoichiometry due to treatment time and hydrological conditions. Nutrient homeostasis and flexibility together regulate plant growth. Efficient evaluation of nutrient recovery aids our understanding of nutrient responses of C. schmidtii to consecutive drought and re-flooding treatments. Simultaneously, our findings provide valuable information for hydrological management and restoration of C. schmidtii tussocks in semi-arid zones.

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APPENDIX

Figure A1. Effects of consecutive drought and re-flooding episodes on N concentrations (a), P concentrations (b), K concentrations (c), N:P ratios (d), N:K ratios (e) and K:P ratios (f) of Carex schmidtii. Dotted lines show the changes in nutrients and their ratios of Carex schmidtii after re-flooding. W is the wet treatment; D is the consecutive drought treatment; DF30, DF40 and DF60 refer to re-flooding after 30, 40 and 60 days of consecutive drought, respectively



Figure A2. Relationships between N, P and K concentrations of Carex schmidtii under consecutive drought and re-flooding conditions. (a) $\ln P = \alpha \ln N + \beta$. (b) $\ln K = \alpha \ln N + \beta$. (c) $\ln K = \alpha \ln P + \beta$. α Scaling exponents, β elevation. Fittings in D, W, DF30, DF40 and DF60 were based on nutrient data collected from the entire growth stage of C. schmidtii. W is the wet treatment; D is the consecutive drought treatment; DF30, DF40 and DF60 refer to re-flooding after 30, 40 and 60 days of consecutive drought, respectively



Figure A3. PCA plot showing the contribution of nutrients and their ratios of Carex schmidtii under consecutive drought and re-flooding conditions based on nutrient data collected from each growth stage of C. schmidtii. Markers represent individual sample locations, while ellipses represent 95% confidence zone for each treatment



Figure A4. PCA plot showing the locations of nutrients and their ratios of Carex schmidtii under consecutive drought and re-flooding conditions (Day 0~110). Figure A4 a and b were based on nutrient data of C. schmidtii collected after re-flooding. Figure A4 c and d were based on nutrient data of C. schmidtii collected from the entire growth stage. Markers represent individual sample locations, while ellipses represent 95% confidence zone for each treatment

Table A1. Summary of fitting results between nutrients and their ratios of Carex schmidtii under consecutive drought and re-flooding conditions. Fittings in D, W, DF30, DF40 and DF60 were based on nutrient data collected from the entire growth stage of C. schmidtii. α Scaling exponents, CI Confidence interval

Lg Y~ Lg X	Treatments	Fittings	α (95%CI)	\mathbf{R}^2	р
	W	Y = 0.721X + 0.263	0.721 (0.209~1.234)	0.155	< 0.01
	D	Y = -0.469X + 1.895	-0.469 (-1.230~0.233)	0.050	0.218
Lg K:P ratio~ I g N	DF30	Y = 0.368X + 0.684	0.368 (-0.135~0.872)	0.048	0.147
Lg IV	DF40	Y = 0.022X + 1.158	0.022 (-0.524~0.569)	0.000	0.934
	DF60	Y = -0.435 X + 1.768	-0.435 (-1.040~0.170)	0.048	0.154
	W	Y = -0.546X + 0.139	-0.546 (-0.885~-0.207)	0.193	< 0.01
T) T T Z (D	Y = 0.133X + 0.111	0.133 (-0.005~0.271)	0.114	0.058
Lg N:K ratio~	DF30	Y = -0.213X + 0.160	-0.213 (-0.561~0.136)	0.034	0.225
Lgi	DF40	Y = -0.058X + 0.142	-0.058 (-0.340~0.335)	0.004	0.682
	DF60	Y = 0.117X + 0.154	0.117(-0.140~0.375)	0.020	0.363
	W	Y = -0.068X + 1.438	-0.068 (-0.198~0.062)	0.025	0.296
	D	Y = 0.108X + 1.288	0.108 (-0.497~0.713)	0.004	0.718
Lg N:P ratio~	DF30	Y = 0.009X + 1.300	0.009 (-0.229~0.247)	0.000	0.941
Lg K	DF40	Y = -0.048X + 1.386	-0.048 (-0.334~0.237)	0.003	0.735
	DF60	Y = -0.036X + 1.409	-0.036 (-0.332~0.259)	0.001	0.807

Table A2. Summary of fitting results between nutrients and their ratios of Carex schmidtii after re-flooding. Fittings in DF30, DF40 and DF60 were based on nutrient data collected after re-flooding. α Scaling exponents, CI Confidence interval

Lg Y~ Lg X	Treatments	Fittings	α (95%CI)	R ²	р
Lg P~ Lg N	DF30	Y = 0.283X-0.464	0.283 (-0.152~0.717)	0.060	0.194
	DF40	Y = 0.573X - 0.800	0.573 (-0.022~1.168)	0.160	0.058
	DF60	Y = 0.520X-0.742	0.520 (-0.217~1.257)	0.140	0.152
Lg K~ Lg N	DF30	Y = 0.644X + 0.195	0.644 (0.079~1.209)	0.163	< 0.05
	DF40	Y = 0.730X + 0.129	0.730 (0.122~1.338)	0.229	< 0.05
	DF60	Y = 0.616X + 0.255	0.616 (-0.428~1.659)	0.103	0.226
Lg K~ Lg P	DF30	Y = 0.540X + 1.024	0.540 (0.049~1.031)	0.153	< 0.05
	DF40	Y = 0.606X + 1.082	0.606 (0.209~1.004)	0.324	< 0.01
	DF60	Y = 0.981X + 1.114	0.981 (0.420~1.542)	0.501	< 0.01
I WD I	DF30	Y = 0.362X + 0.660	0.362 (-0.226~0.950)	0.054	0.218
Lg K:P ratio~	DF40	Y = 0.157X + 0.928	0.157 (-0.463~0.777)	0.013	0.604
Lg IV	DF60	Y = 0.095X + 0.996	0.095 (-0.681~0.871)	0.005	0.796
	DF30	Y = -0.329X + 0.180	-0.329 (-0.815~0.157)	0.064	0.176
Lg N:K ratio~	DF40	Y = -0.326X + 0.171	-0.326 (-0.734~0.080)	0.117	0.110
Lgi	DF60	Y = -0.711X + 0.164	-0.711 (-1.363~-0.058)	0.281	< 0.05
	DF30	Y = -0.031X + 1.338	-0.031 (-0.354~0.292)	0.001	0.845
Lg N:P ratio~ L g K	DF40	Y = -0.220X + 1.548	-0.220 (-0.618~0.178)	0.059	0.263
Lg K	DF60	Y = -0.344X + 1.697	-0.344 (-0.703~0.014)	0.232	0.058

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