Leaf phenotypic plasticity and integration balance plant adaptation to water table decline: a mesocosm experiment

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Abstract

Functional trait-based approaches have been widely used to explore the relationship between plants and their surroundings. Yet, whether phenotypic plasticity and phenotypic integration are differently functional coordination to enhance plant adaptation to declining water levels is still lacking in empirical knowledge. We conducted a mesocosm experiment in an alpine wetland with two dominant plants, *Carex muliensis* (hygrophytes) and *Pedicularis longiflora* var. *tubiformis* (mesophytes), exposed to four water table gradients (WT10, WT0, WT-20 and WT-50, representing the water table at 10 cm, 0 cm, -20 cm and -50 cm from the surface). We measured leaf traits related to resource use strategies, and the relationship between leaf phenotypic plasticity and integration. We found that hygrophytes shifted their leaf traits towards resource-conserving strategies, such as increasing leaf thickness and decreasing leaf area and specific leaf area, under water table decline. In contrast, mesophytes shifted their leaf traits towards resource-acquisition strategies, enhancing their competitiveness and fitness at low water levels. We also found a negative correlation between leaf phenotypic plasticity and integration in both plant species, suggesting a trade-off between them. which was attributed to the fact that wetland plants may prioritize traits that reduce water loss (e.g. larger leaf thickness), resulting in lower integration with other traits (photosynthetic and nutrient use related traits). We conclude that, water table decline alters plant leaf resource use strategies and that the balance between leaf phenotypic plasticity and integration contributes to plant adaptation to water table decline. This study improves our understanding of the role of leaf phenotypic plasticity and integration in plant adaptation in the context of declining water levels in wetlands will help predict the future structure and composition of alpine wetland ecosystems.

Introduction

Global wetland area has shrunk by a third in the past 50 years due to climate change and artificial drainage, with an accelerating trend since 2000 (Ramsar Convention on Wetlands., 2018). The Zoige alpine wetland, the world's largest, is no exception: its water table has dropped by 15 cm in the past 30 years (Chen et al., 2014; Xiang et al., 2009; Dong et al., 2010), exceeding the critical threshold for ecological resilience (Yang et al., 2014). Excessively low water levels can trigger wetland degradation, organic carbon decomposition and biodiversity loss, although lower water levels can boost wetland plant growth and livestock productivity (Potvin et al., 2015; Yang et al., 2017; Miller et al., 2015). These effects may alter the structure, function and biogeochemical cycles of alpine wetland ecosystems and exacerbate the greenhouse effect (Dong et al., 2020; Cui et al., 2013). Wetland plants are key components of alpine wetlands, so understanding how they adapt and cope with water table decline is crucial for predicting the future of these ecosystems (Cao et al., 2017). hygrophytes and mesophytes are two functional types with different water requirements: hygrophytes depend more on water availability and are more sensitive to water stress than mesophytes (Markesteijn et al., 2009; Riva et al., 2017). Thereby, understanding the morpho-physiological strategies of both types of plants under water level gradients is crucial for understanding the stability and dynamics of alpine wetlands ecosystems under water level decline (Wang et al., 2017; Aatin et al., 2015).

Leaf functional traits reflect how plants optimize resource use in response to environmental changes, by adjusting their morphology and physiology to cope with stress and enhance resource uptake (Yin et al., 2018; Reich. 2014; Donovan et al., 2011). According to leaf economics spectrum theory, plants display a coordinated set of trait combinations along a one-dimensional axis (Violle et al., 2007; Wright et al., 2004). At one end of the spectrum are plants with high nutrient (e.g., N, P) content, high photosynthetic capacity and short lifespan, representing a rapid resource acquisition strategy (Osnas et al., 2013; Zhang et al., 2021). This strategy is linked to low environmental stress levels, enabling fast growth and nutrient turnover in non-stressed ecosystems, while increasing carbon sequestration (Mason et al., 2016; Reich et al., 2003). At the other end, in resource-limited and stressful environments, plants tend to adopt a conservative strategy of lowering photosynthetic functionality and leaf nutrient content, and investing more in leaf tissue structure (Wright et al., 2006). Within this framework, water level decline reduces soil water availability, and may trigger a trait trade-off transition between hygrophytes and mesophytes (Krab et al., 2013).

Phenotypic plasticity and phenotypic integration, defined as the ability of plants to change their phenotype in different environments and the coordinated relationship between different functional traits, respectively (Pigliucci., 2003; Lin et al., 2020; Turcotte et al., 2016). The former is seen as a mechanism by which plants adapt to environmental change, while the latter can either act as an internal constraint on phenotypic plasticity or coordinate with phenotypic plasticity to facilitate plant adaptation to the environment (Stotz et al., 2021; Shi et al., 2023). Currently, ecologists are trying to understand the complex trade-offs among adaptive species and the potential effects of environmental heterogeneity on them (Matías et al., 2018; Wainwright et al., 2019). A general theory addressing plant-environment relationships is the integration of functional traits, phenotypic plasticity and phenotypic integration, rather than relying on any single component (Levine., 2016; Matesanz et al., 2023). However, how species modulate their trade-off strategies in response to changes in their plasticity and integration abilities in different environments, especially under declining water levels, is poorly understood (Dawson et al., 2017). Based on current knowledge of the functional traits of hygrophytes and mesophytes leaves, we hypothesize that: (i) declining water levels alter the strategies of alpine wetland plants in terms of investment, nutrient and water resource use; (ii) and there is a trade-off between leaf phenotypic plasticity and phenotypic integration that facilitates adaptation of wetland plants to water table decline.

In this study, we tested the above two hypotheses by conducting a two-year mesocosm experiment to analyse key leaf functional traits of tow dominant plants (*Carex muliensis* and *Pedicularis longiflora* var. *tubiformis*) in alpine wetlands. Specifically, we investigated the response of 12 leaf functional traits to a water table gradient and explored the variation in resource trade-off strategies based on leaf economic spectrum under different water table gradients. We then identified differences in phenotypic plasticity and integration of leaf traits in plants differing between the two water-demanding strategies, and assessed constraints on leaf phenotypic plasticity expression through overall leaf integration levels. Our results reveal the trait trade-off strategies of alpine wetland plants under water stress gradients and enhance our understanding of the mechanisms underlying their adaptation to declining water levels.

Materials And Methods

2.1. Study site and mesocosm collection

This study was conducted at the Zoige Alpine Wetland Ecological Station (32°58' N latitude, 102°37' E longitude, 3458 m a.s.l.) in the eastern Tibetan Plateau, as illustrated in Fig. 1a and c. The region experiences a continental plateau cold-temperate monsoon climate, characterized by a lack of clear seasonal boundaries, with short spring and autumn periods and a long winter. The average annual precipitation is 860.8 mm, with 80% of the precipitation occurring between May and August, and the average annual evaporation is 1,250 mm, mainly concentrated in the summer months. The average annual temperature is 2.9℃, with a minimum of -22.8℃ in January and a maximum of 24.6℃ in August (Wu et al., 2014). The wetland is dominated by *Carex muliensis* (over 90% vegetation cover) and dotted with *Juncus allioides*, *Pedicularis longiflora* var. *Tubiformis*, and *Juncus leucanthus* (Hu et al., 2016). Based on the results of previous studies (Han et al., 2012), it has been shown that *Carex muliensis* is adapted to high moisture habitats, whereas *Pedicularis longiflora* var. *Tubiformis* is adapted to relatively low moisture habitats, and the importance value of *Pedicularis longiflora* var. *Tubiformis* is overwhelmingly superior to that of *Carex muliensis* in low moisture habitats. Therefore, we refer to *Carex muliensis* as hygrophytes and to *Pedicularis longiflora* var. *Tubiformis* as mesophytes.

A representative alpine wetland area (Fig. 1a, SS) located approximately 30 km from the Zoige Alpine Wetland Ecological Station was selected for mesocosm material collection. In October 2019, 32 bottomless metal tanks (60 cm length × 60 cm width × 100 cm height) made of uniform material were inserted into the wetland soil. The tanks were dug out and seamlessly welded when the soil froze in early December and transported to the rain shelter at the ecological station (Fig. 1b, c). The rain shelter, constructed using light-permeable plastic sheets, effectively eliminated rainwater interference with the experiment and minimized the disruption of physiological processes such as plant photosynthesis. The outer layer of the tanks was covered with polystyrene plastic foam to prevent heat exchange between the interior and exterior of the tanks. The water level inside the tanks was maintained at the same level as the in situ wetland until the onset of the plant-growing season in April 2020.

2.2. Experimental design

The collected mesocosm material was stratified into four water levels: WT10, WT0, WT-20, and WT-50, representing the water table at 10 cm, 0 cm, -20 cm and -50 cm from the surface). WT10 simulated the maximum water depth during the wet season in the alpine wetland, while WT-50 simulated the lowest groundwater depth during the dry season. Alpine wetland herbaceous plant roots are predominantly found within 20 cm below the soil surface, thus WT-20 represents the critical depth at which plant roots

access water. WT0 represents the optimal water level for plant growth, where plants are free from flooding or drought stress. Eight replicates were used for each water level, but it was deemed more prudent to avoid further replication after sampling, as it could negatively impact the long-term maintenance of the experiment. Therefore, the 32 collected tanks were evenly divided into two parts, with one part utilized as experimental material and sampled regularly, while the other part was maintained solely by adding water, without any sampling treatment. The tanks were connected to an automatic water refill system for precise control of the water level. A liquid level transmitter (CYW11, Xingyi, China) was installed inside the Plexiglas tube at the periphery of the box to monitor the water level inside the box, and a solenoid valve (SMCVDW22JA, SMC, Japan) was activated to replenish water when the water level fell below a set value. The water level was maintained throughout the growing season (June to October), and replenishment ceased at the end of the growing season to prevent any potential freeze damage to the system.

2.3. Functional traits measurement

Based on standardized protocols, 12 functional traits were measured in this study for five dominant wetland species. In order to fully assess the response of wetland plants to declining water levels, the functional traits were categorized into morphological, photosynthetic, and stoichiometric traits (Pérez-Harguindeguy et al., 2013; Liu et al., 2021).

Photosynthetic traits were assessed in situ in the field, including Fv/Fm (maximum photosynthetic quantum yield from PSII reaction centers), ETRmax (maximum electron transfer rate), qP (photochemical quenching coefficient), and NPQ (non-photochemical quenching coefficient). Specifically, five individuals of each species were randomly selected for four replicates (n = 4) in each of the four water level treatments. From these individuals three healthy, intact leaves were randomly selected for measurement. A portable photosynthesizer (GFS-3000, Walz, Germany) was selected to continuously monitor the fluorescence parameters of the leaves from 9:00-11:00 in clear weather by clamping the leaf midrib without altering the natural growth angle of the leaf. Light intensity, relative air humidity, CO_2 concentration and temperature were controlled at 1200 $\mu mol \cdot m^{-2} \cdot s^{-1}$, 50%, 390 $\mu mol \cdot mol^{-1}$ and 12°C, respectively, to exclude environmental perturbations to the experiment (Woitke et al., 2004; Maxwell et al., 2000).

Morphological traits, including leaf lengh (LL, cm), leaf area (LA, cm^2), leaf thickness (LT, mm), leaf dry matter content (LDMC, $g \cdot g^{-1}$), and specific leaf area (SLA, $cm^2 \cdot g^{-1}$). After photosynthetic trait measurements plant leaves were collected, carefully wrapped in wet paper and placed in a bubble box filled with ice packs for transport back to the laboratory for further functional trait measurements. leaf lengh was measured directly using a straightedge, and leaf thickness with a vernier caliper (0.01 mm). The plant leaves were scanned using Canon Scan LiDE210 software, and the resulting images were analyzed using Image J (Version 1.51, NIH, United States) to calculate the leaf area. After scanning, the

fresh weight of the leaves (FLFW, g) was measured using an electronic balance (10,000 ppm). The leaves were then dried in an oven at 80°C for 48 h until a constant weight was achieved, and the dry weight of the leaves (LDW, g) was measured. Finally, the specific leaf area and dry matter content were calculated.

The main stoichiometric traits of the plants were: total nitrogen (TN, $g \cdot kg^{-1}$), total phosphorus (TP, $g \cdot kg^{-1}$), and total nitrogen/phosphorus ratio (TN/TP). Plant leaves were dried and ground into powder (< 0.25 mm) using a ball mill (JX-2GL, Jingxin, China). Plant total nitrogen and phosphorus were determined using a fully automated continuous flow analyzer (AA3, SEAL, Germany) and the total nitrogen/phosphorus ratio was calculated.

2.4. Statistical analysis

Leaf phenotypic integration was calculated as the number of target traits significantly correlated with other traits. Phenotypic plasticity was calculated with reference to the formulae of Shi et al. (2023) and Godoy et al. (2012) as follows:

$$\text{Plasticity} = \frac{\text{Mean (Water table)} - \text{Mean (CK)}}{\text{Max [Mean (Water table), Mean (CK)]}} \qquad (1)$$

Equation (1), Mean (Water table) is the mean of leaf traits under the corresponding water table treatment and Mean (CK) is the mean of the control, i.e. the trait value at WT10 water table, indicating the undegraded water table of the wetland. Max [Mean (Water table), Mean (CK)] is the maximum value of the corresponding leaf trait between the two treatments.

All statistical analyses were performed using the R software (version 3.3.3; R Development Core Team, Vienna, Austria). To satisfy the normality and homoscedasticity tests of the data in the analysis of variance (ANOVA), the variable data were $\lg_{(X+1)}$ transformed, with X being the original value of the functional trait. A two-way ANOVA was conducted to assess the effects of species, water level treatments and their interactions on leaf functional traits, using the *agricolae* package in R (de Mendiburu., 2017). Principal component analysis (PCA) was performed with the R 'prcomp' function to determine the pattern of variation between leaf functional traits and resource acquisition strategies under different water level treatments. One-way ANOVA with post-hoc Tukey HSD (honestly significant difference) analyses were used to compare differences between plant leaf traits at different water level gradients and differences in PC scores. Student's *t*-test was used to compare differences in phenotypic plasticity and integration between the two plant leaf. Finally, we again used the *agricolae* package to determine the correlation

between phenotypic plasticity and integrative of the leaves of the two plants. The results of all the above data analyses were presented visually via Origin 2023 (OriginLab, MA, USA).

Results

3.1. Leaf functional trait responses

Both species and water level had significant effects on functional traits of leaves (Table 1 and Fig. 2). Specifically, species had a significant effect on leaf morphology and photosynthetic traits ($p < 0.05$), except for NPQ, but not on stoichiometric traits ($p > 0.05$). Notably, there was a significant interaction between species and water level on leaf morphology, photosynthetic and stoichiometric traits ($p < 0.05$), with the exception of TP, and the interaction was independent of species type (Table 1).

Overall, Leaf traits of *Carex muliensis* and *Pedicularis longiflora* var. *tubiformis* responded differently to water stress (Fig. 2). Specifically, as water levels decreased, *Carex muliensis* showed reductions LL, LA, LDMC, SLA, Fv/Fm, ETRmax, qP, and N content (Figs. 2a, b, d, e, f, g, h, j), and increases LT and NPQ (Figs. 2c, i), and had non-linear effects on other stoichiometric traits except N content (Fig. 2k, l). In contrast, *Pedicularis longiflora* var. *tubiformis* exhibited increases in LL, LA, LDMC, SLA, Fv/Fm, ETRmax, qP and N/P (Figs. 2a, b, d, e, f, g, h, l), and decreases in NPQ and P content. The effect of water stress on LL was not statistically significant for *Pedicularis longiflora* var. *tubiformis* (Fig. 2c).

Table 1

Two-way ANOVA for species and water level on functional traits of leaves. Bold indicates *F*-values indicating significant effects at the $p < 0.05$ level.

Trait types	Species ($df = 1$)		Water level gradients ($df = 3$)		Species × Water level gradients ($df = 3$)	
	F	P	F	P	F	P
LL	365.495	**0.000**	0.666	0.579	6.01	**0.002**
LA	22.903	**0.000**	7.816	**0.000**	35.899	**0.000**
LT	47.078	**0.000**	3.732	**0.021**	7.549	**0.001**
LDMC	14.249	**0.001**	1.204	0.324	11.187	**0.000**
SLA	103.552	**0.000**	9.019	**0.000**	155.830	**0.000**
Fv/Fm	6.672	**0.020**	2.203	0.127	13.550	**0.000**
ETRmax	13.094	**0.002**	0.122	0.946	7.819	**0.002**
qP	35.537	**0.000**	0.583	0.635	5.245	**0.010**
NPQ	0.925	0.350	6.189	**0.005**	16.13	**0.000**
TN	0.874	0.357	3.028	**0.044**	37.710	**0.000**
TP	1.602	0.215	0.414	0.744	1.021	0.396
N:P	2.310	0.138	0.934	0.436	6.071	**0.002**

3.2. Trait correlation

Figure 3 demonstrates the correlations between leaf traits of *Carex muliensis* and *Pedicularis longiflora* var. *tubiformis*. In this case, SLA in *Carex muliensis* showed a strong positive correlation ($p < 0.01$) with photosynthetic traits (i.e., Fv/Fm, ETRmax, and qP), and a moderate negative correlation ($p < 0.05$) with NPQ. Furthermore, LT exhibited a positive correlation ($p < 0.05$) with SLA, Fv/Fm, ETRmax, TN, and TP, and strongly negatively correlated ($p < 0.01$) with NPQ. For *Pedicularis longiflora* var. *tubiformis*, TN was moderately positively correlated with Fv/Fm, ETRmax and qP in photosynthetic traits ($p < 0.05$) and strongly negatively correlated with NPQ ($p < 0.01$). Overall, LT and NPQ for both species were negatively correlated with the remaining morphological, photosynthetic and stoichiometric traits (Fig. 3).

3.3. Leaf resource utilization strategies compared

The first two principal components (PC1 and PC2) captured 74% and 72% of the variation in 12 leaf functional traits for *Carex muliensis* and *Pedicularis longiflora* var. *tubiformis*, respectively (Fig. 4a, b). Traits related to resource acquisition (LL, LA, LDMC, SLA, Fv/Fm, ETRmax, qP and TN content) loaded positively on PC1, while traits reflecting resource conservation (LT, NPQ) loaded negatively (Table 2 and

Fig. 4a, b). Except for LT in *Pedicularis longiflora* var. *tubiformis*, all traits contributed significantly to either PC1 or PC2 in both species (Table 2).

Table 2

Differences in leaf functional trait PC and PC2 scores. Significant correlations are represented with bold font and asterisks. *$p < 0.05$, **$p < 0.01$.

Trait types	*Carex muliensis*		*Pedicularis longiflora* var. *tubiformis*	
	PC1	PC2	PC1	PC2
LL	**0.637****	0.104	**0.778****	0.128
LA	**0.797****	0.114	**0.930****	0.032
LT	**-0.774****	0.067	-0.322	-0.071
LDMC	**0.738****	-0.181	**0.712****	-0.211
SLA	**0.885****	-0.102	**0.939****	-0.022
Fv/Fm	**0.829****	-0.235	**0.943****	0.08
ETRmax	**0.946****	0.083	**0.849****	0.325
qP	**0.830****	0.228	**0.777****	0.204
NPQ	**-0.719****	0.429	**-0.830****	-0.253
TN	**0.933****	0.019	**0.793****	-0.072
TP	0.084	**-0.966****	-0.212	**0.910****
N:P	**0.506***	**0.826****	**0.714****	**-0.633****

Water level significantly affected the scores of both *Carex muliensis* and *Pedicularis longiflora* var. *tubiformis* on PC1 and PC2 ($p < 0.05$). *Carex muliensis* scores on PC1 decreased with lower water levels ($p < 0.01$), while *Pedicularis longiflora* var. *tubiformis* scores on PC1 increased. PC2 scores did not differ between the two species (Fig. 4c, d).

3.4. The relationship between phenotypic plasticity and integration of leaf traits

The plasticity of leaf traits varied considerably between the two plants (Fig. 5a, b). For *Carex muliensis*, LA, N:P and LDMC showed the highest mean plasticity, while for *Pedicularis longiflora* var. *tubiformis*, LA, LL and Fv/Fm did. Morphological (LL, LA and SLA) and photosynthetic (Fv/Fm and ETRmax) traits were most responsive to the WT10 gradient in *Pedicularis longiflora* var. *tubiformis*, whereas stoichiometric (TP and N:P) traits were most responsive to the WT0 gradient (Fig. 5b). In *Carex muliensis*, stoichiometric (TP and N:P) and photosynthetic (ETRmax and qP) traits exhibited the highest plasticity at WT-50 (Fig. 5a).

Phenotypic plasticity and integration of leaf traits did not differ significantly between *Carex muliensis* and *Pedicularis longiflora* var. *tubiformis*. However, as water levels decreased, *Pedicularis longiflora* var. *tubiformis* showed higher plasticity and lower integration than *Carex muliensis* (Fig. 6a, b). Both plants exhibited a significant negative correlation between plasticity and integration, with more plastic traits being less integrated with others (Fig. 6c, d).

Discussion

4.1. A range of tradeoffs among leaf functional traits in the context of declining water levels

As two co-dominant plants in alpine wetlands, the morphological and physiological traits of *Carex muliensis* (hygrophytes) and *Pedicularis longiflora* var. *tubiformis* (mesophytes) are essential for predicting the species' multivariate trait and plasticity strategies in the context of declining water tables (Bai et al., 2013; Wu et al., 2017). We found that both plants adapted significantly to the environmental changes induced by lower water levels, but with different trait strategies. Declining water levels increased morphological traits such as LL and LA in *Pedicularis longiflora* var. *tubiformis* (except for LT), but decreased them in *Carex muliensis* (except for LT). These changes are in line with previous wetland studies that showed lower water levels enhance the competitive advantage of mesophytes leaves but reduce that of hygrophytes (Bubier et al., 2003; Laine et al., 2011; Laiho et al., 2003). As key economic traits of leaves, Leaf morphological traits such as LL, LA, SLA and LDMC are closely related to plant light capture and carbon acquisition capacity (Wright et al., 2001; Kattge et al., 2011; Garnier et al., 2012). In our study, traits such as LL and SLA increased in *Pedicularis longiflora* var. *tubiformis* but decreased in *Carex muliensis* with lower water levels, suggesting that lower water levels promote mesophytes growth by improving photosynthesis and resource capture capacity (Hanke et al., 2015; Luo et al., 2016). Moreover, we observed that lower water levels increased LT in hygrophytes and decreased LT in mesophytes, which is consistent with other microcosm experiments (Wolfe et al., 2006; Weltzin et al., 2000). Lower water levels cause drought stress in hygrophytes with high water demand, which may increase leaf thickness to reduce the distance and resistance of intracellular water diffusion to the outside of the cell (Li et al., 2018; Lei et al., 2018). Alternatively, increased leaf thickness in hygrophytes may reduce light flux and water consumption by physiological processes, such as photosynthesis and

respiration, compared to mesophytes, thus facilitating hygrophytes adaptation to low water table habitats (Gonzalez-Paleo et al., 2018).

Chlorophyll fluorescence parameters can more accurately reflect the processes of light energy absorption, transfer and dissipation in plants (Lin et al., 1992). We found that lower water table increased ETRmax and qP in *Pedicularis longiflora var. tubiformis*, possibly because it reduced anoxic metabolic pathways such as respiration and ethanol fermentation and used the saved energy for photosynthesis (Chen et al., 2016). Meanwhile, NPQ in *Pedicularis longiflora var. tubiformis* decreased significantly with lower water levels, suggesting that it enhanced photosynthetic products by reducing non-photochemical quenching and improving light energy utilization (Osmond et al., 2000; Shi et al., 2014). In contrast, Fv/Fm, ETRmax and qP decreased, while NPQ increased in *Carex muliensis* with lower water levels. This indicates that the photosynthetic electron transport pathway of *Carex muliensis* was inhibited, and that under drought stress, *Carex muliensis* dissipated excess light energy by increasing non-photochemical quenching, thereby avoiding photoinhibition (Zhang et al., 2005; Ma et al., 2020; Maxwell et al., 2020; Goss et al., 2015; Bai et al., 2011). Considering our results and those of previous studies, we suggest that the photosynthetic trait trade-off strategy of mesophytes is superior to that of hygrophytes in alpine wetlands with declining water levels.

Stoichiometric traits are related to nutrient storage and reflect the physiological and biochemical responses of the organism to the environment (He et al., 2010; Sardans et al., 2012; Güsewell et al., 2004). For instance, nitrogen content indicates the photosynthetic capacity of the plant, phosphorus content is associated with leaf longevity, and the N:P ratio reflects the degree of nitrogen or phosphorus limitation (Santiago et al., 2004; Takahashi et al., 2008). In this study, both *Carex muliensis* and *Pedicularis longiflora* var. *tubiformis* had N:P ratios below 14, suggesting that plant growth in alpine wetlands was strongly N-limited rather than phosphorus-limited (Du et al., 2020). This result is consistent with long-term experimental observations, which indicate that N or P limitation may depend on the amount and form of elements in the soil (Vitousek et al., 1997). Prolonged inundation of alpine wetlands reduces the rate of N mineralisation in soils, decreasing N availability, while phosphorus is often present as water-soluble phosphate in water and easily leached for plant uptake (Fonseca et al., 2000). We also found that although both plants had imbalances in N and P content, they showed different patterns of change in N and P content under flooded (WT10) or wet (WT0) water levels and under dry (WT-20 and WT-50) water levels. *Carex muliensis* had higher N:P ratios than *Pedicularis longiflora* var. *tubiformis* at WT10 and WT0 water levels, but lower at WT-20 and WT-50 water levels, indicating that hygrophytes had more flexible stoichiometric stability than mesophytes under flooded or wet water levels, but the opposite strategy under drought stress (Xiong et al., 2022; Yu et al., 2019; Guo et al., 2021). Thus, mesophytes adapted better to the stresses associated with lower water levels in alpine wetlands by regulating the stoichiometric element ratios in vivo compared to hygrophytes.

As expected, Our results support hypothesis (i) that lower water levels affect functional traits in wetland plant leaves, leading to changes in the intrinsic trait trade-off strategies of plants. Specifically, morphological traits such as LA, photosynthetic traits such as Fv/Fm and stoichiometric traits such as N

content decreased in hygrophytes but increased in mesophytes with lower water levels. This suggests that hygrophytes and mesophytes adapted their functional traits and trade-off strategies to the lower water table in the alpine wetland (Kurokawa et al., 2010; Diaz et al., 2016; Niinemets et al., 2001; Liu et al., 2015). This is in line with previous findings that plant growth under stressful environments tends to favour resource-conserving strategies. When wetland water level declines, hygrophytes experience drought stress and reduce their water dependence by limiting cell division and growth rates (Ma et al., 2019; Khanday et al., 2017; Liu et al., 2018; Wang et al., 2015). In contrast, a lower water table improves soil aeration and microbial activity, which enhances the availability of soil soluble nutrients. This allows mesophytes to increase their resource input to photosynthetic and respiratory functions in a high nutrient and suitable water environment, enabling them to improve their suitability for low water table habitats (Baird et al., 2013, Macrae et al., 2013). These results confirm that hygrophytes and mesophytes adapt to lower water levels by adopting different trait trade-off strategies to meet their ecological needs. That is, as the wetland water table declines, hygrophytes shift towards a slow return on investment strategy (conservative strategy), while mesophytes shift towards a fast return on investment strategy (acquisition strategy).

4.2. Heterogeneity in leaf phenotypic plasticity and integration in the context of declining water table

Phenotypic plasticity is considered to be the ability of plants to change their phenotype in a heterogeneous environment (Nicotra et al., 2010). We found that phenotypic plasticity of morphological, physiological and stoichiometric traits of hygrophytes and mesophytes differed significantly among water levels. This agrees with northern peatlands findings that lower water levels induce changes in plant trait plasticity, increasing the competitive advantage of mesophytes and decreasing the adaptive capacity of hygrophytes (Guo et al., 2018; Duan et al., 2019). Our data suggest that stoichiometric traits (TP and N:P) and photosynthetic traits (ETRmax and qP) showed the highest plasticity at WT-50 in *Carex muliensis*, while morphological traits (LL, LA and SLA) and photosynthetic traits (Fv/Fm and ETRmax) showed the highest plasticity at WT10 in *Pedicularis longiflora* var. *tubiformis*. These suggest that phenotypic plasticity typically occurs in stressful environments, with inundation (WT10) enhancing the adaptability of hygrophytes, and drought (WT-50) improving the water access optimization of mesophytes through altered plasticity (Craine et al., 2001; Robroek et al., 2017). Moreover, the opposite patterns of phenotypic plasticity responses of hygrophytes and mesophytes under lower water levels suggest that the two types of plants have different adaptive strategies to water stress, and differ in their sensitivity and stability to water level changes (Tucić et al., 2018). Taken together, we suggest that hygrophytes and mesophytes have two distinct patterns of phenotypic plasticity. Compared to mesophytes, hygrophytes increase their resilience to wetland water table declines by improving more efficient water retention traits (e.g. increasing LT) and reducing phenotypic plasticity such as photosynthesis.

Phenotypic plasticity and integration reflect the flexibility and coordination of plant responses to heterogeneous environments, respectively, and both can facilitate plant adaptation to environmental change, improve their competitiveness and fitness, and ultimately influence their ecological distribution and evolutionary direction (Weiner et al., 2004; Van Kleunen M et al., 2005). We observed a negative correlation between phenotypic plasticity and integration in both hygrophytes and mesophytes in the context of declining water levels, which supports our second hypothesis (ii) that the trade-off between phenotypic plasticity and integration in plant leaves favours improved plant fitness under lower water levels. Plasticity enables plants to adapt to their environment in the short term, while integration ensures adaptation and survival advantage in the long term evolution of plants (Liu et al., 2022; Gao et al., 2008). The negative correlation between leaf phenotypic plasticity and integration suggests that plant leaves may invest more in traits that reduce water loss (e.g. larger LT), resulting in less integration with other traits (photosynthetic, nutrient use related traits) (Wang et al., 2017). Moreover, phenotypic plasticity was lower and integration and trade-offs were higher in *Carex muliensis* than in *Pedicularis longiflora* var. *Tubiformis* (Figs. 3 and 6a, b). This suggests that hygrophytes are more sensitive to water status and require a stronger trade-off between phenotypic plasticity and integration. Mesophytes, on the other hand, are more tolerant of water stress, and they can regulate phenotypic plasticity and integration more flexibly (Matesanz et al., 2021; Luizzi et al., 2021; Li et al., 2016). Therefore, we suggest that hygrophytes and mesophytes have distinct adaptation strategies to water stress. That is, under lower water levels, hygrophytes prefer conservative and efficient trait combinations, while mesophytes prefer flexible and diverse trade-off strategies.

Conclusions

The present study provides an integrated perspective of how plants express their leaf phenotypic plasticity and integration to adapt to wetland water table decline. We show that lower water levels decrease morphological (e.g. LT, SLA), photosynthetic (e.g. Fv/Fm, ETRmax) and stoichiometric traits (e.g. N, P content) in hygrophytes, driving hygrophytes leaves towards resource-conserving strategies. In contrast, the corresponding traits for mesophytes increase with lower water levels, enabling mesophytes to use resources better and improve plant competitiveness under low water conditions. Moreover, leaf phenotypic plasticity and integration in hygrophytes and mesophytes were negatively correlated, indicating a trade-off between leaf phenotypic plasticity and integration, and that phenotypic integration remains an internal constraint on phenotypic plasticity. Considering our results, we conclude that lower water levels induce a rapid response in plant leaf functional traits, and that the trade-off between leaf phenotypic plasticity and integration facilitates plant adaptation to lower wetland water levels. The relationship between phenotypic plasticity and integration needs further exploration in future wetland studies to assess the link between trait-environment adaptation.

Declarations

CRediT authorship contribution statement

Jun Yang: Sampling and measurement, Data analysis, Writing-original draft. **Yongheng Gao:** Conceptualization, Methodology, Supervision, Writing-Review and Editing. **Chuan Zhao:** Funding acquisition, Supervision, Methodology, Writing-Reviewing and editing. **Huai Chen:** Conceptualization, Methodology, Supervision, Writing-reviewing and editing, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Figures

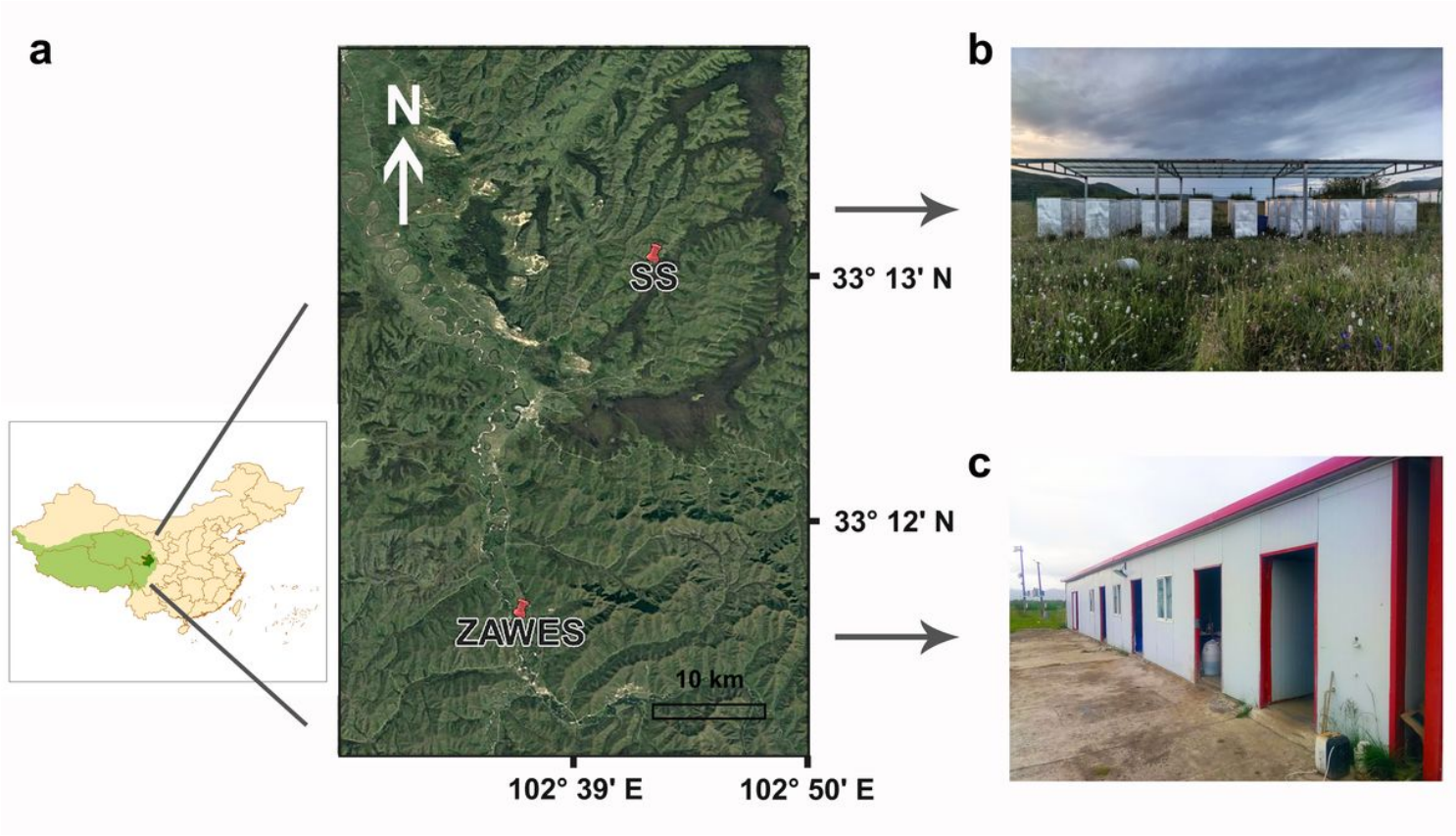

Figure 1

Location of the study area in China's Zoige Plateau (**a**); SS: sample site; Mesocosm experiment (**b**); ZAWES: Zoige Alpine Wetland Ecological Station (**c**).

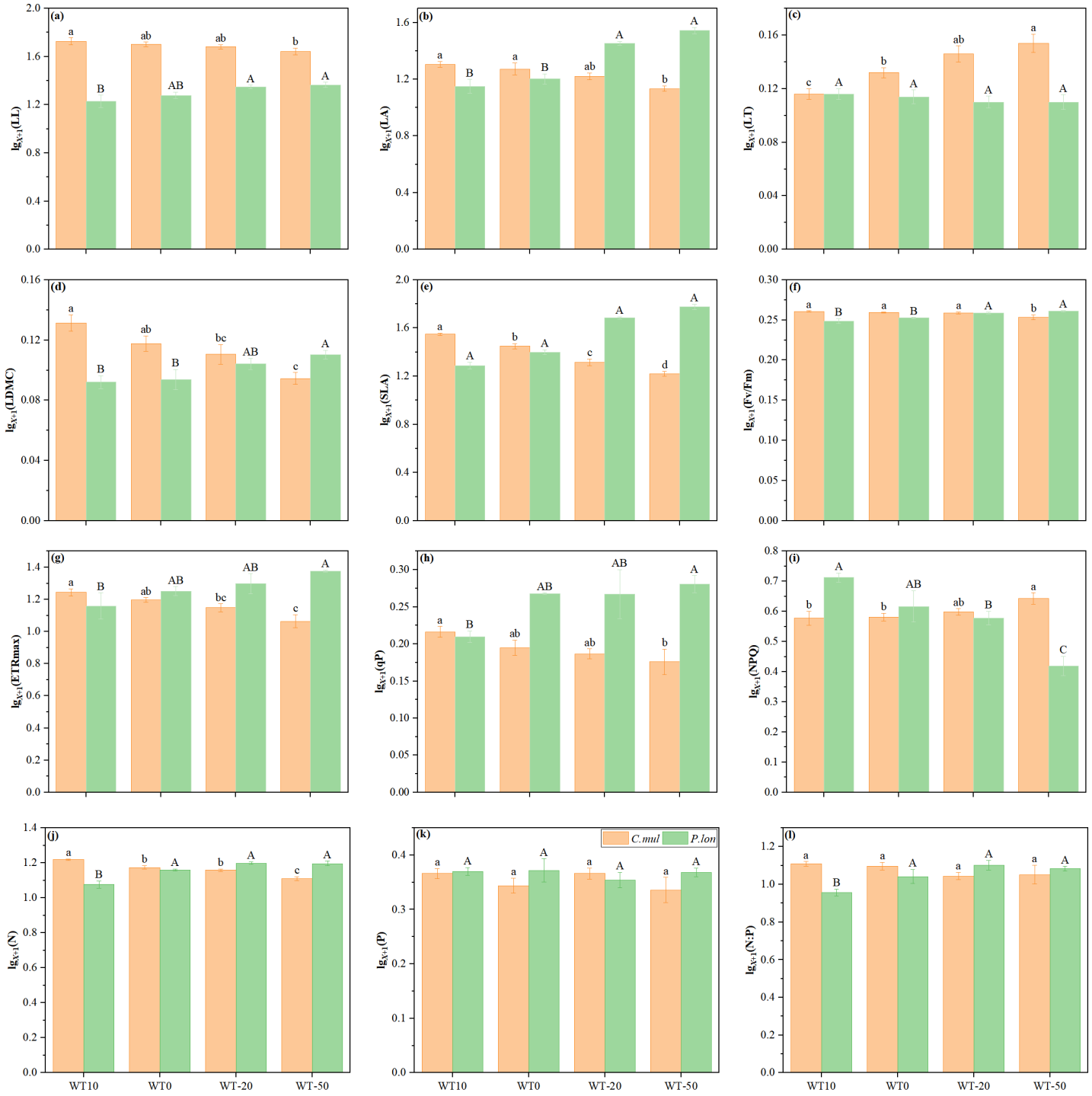

Figure 2

Difference of plant functional traits under water level gradient. LL: leaf lenght (a); LA: leaf area (b); LT: leaf thickness (c); LDMC: leaf dry matter content (d); SLA: specific leaf area (e); Fv/Fm: maximum photosynthetic quantum yield from PSII reaction centers (f); ETRmax: maximum electron transport rate (g); qP: photochemical quenching coefficient (h); NPQ: non-photochemical quenching coefficient (i); TN: total nitrogen (j); TP: total phosphorus (k); TN/TP: ratio of total nitrogen and phosphorus (l). *C. mul*: *Carex muliensis*; *P. lon*: *Pedicularis longiflora* var. *tubiformis.* Different upper and lower case letters on the

columns indicate significant differences ($p < 0.05$) between treatments at different water levels for the same species.

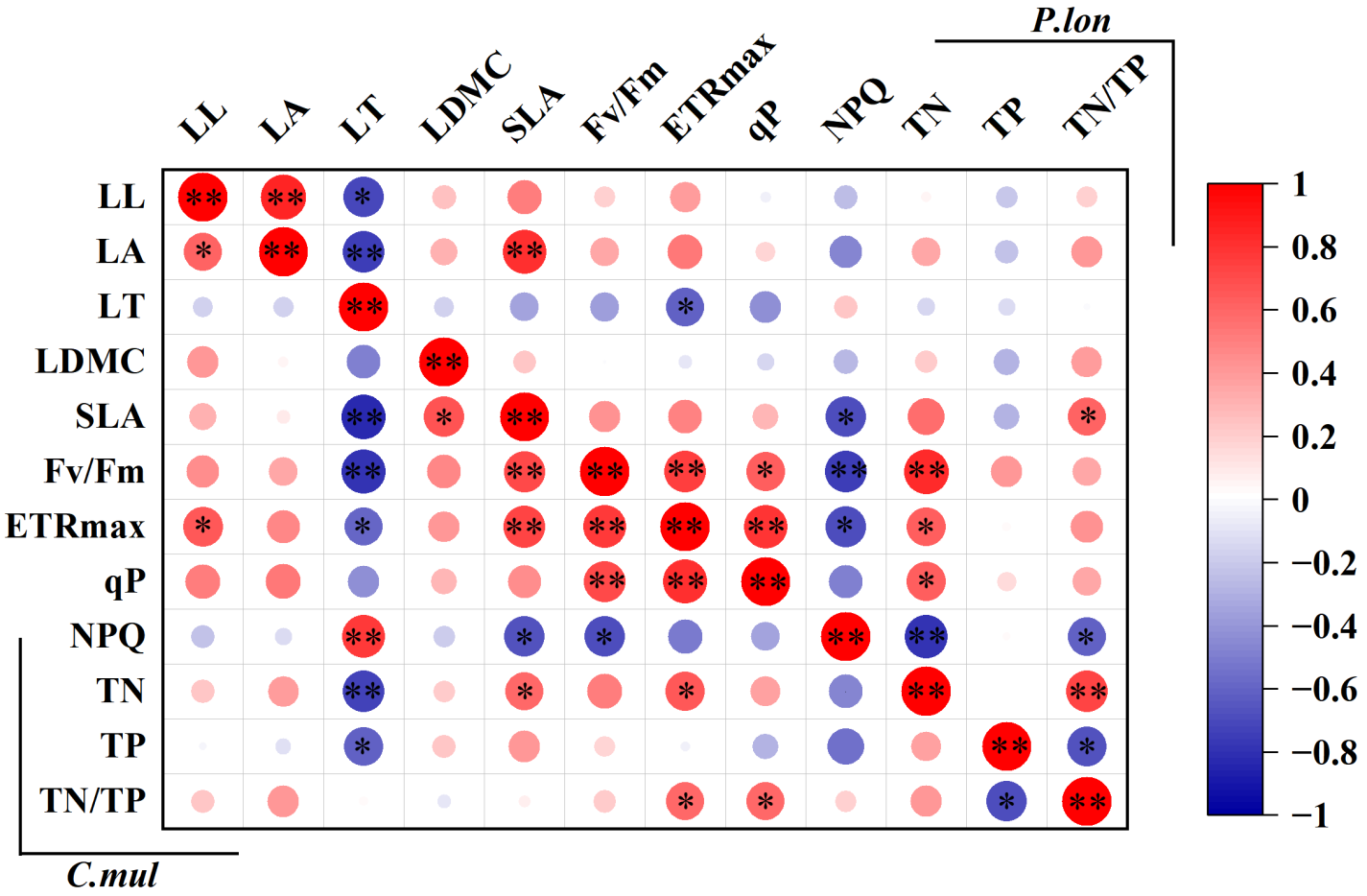

Figure 3

Correlation between traits of hygrophytes and mesophytes. *C. mul*: *Carex muliensis*; *P. lon*: *Pedicularis longiflora* var. *tubiformis.* The color of the box indicates the correlation coefficient, red indicates positive correlation, and blue indicates negative correlation, the darker the color, the larger the absolute value of the correlation coefficient. *$p < 0.05$, **$p < 0.01$.

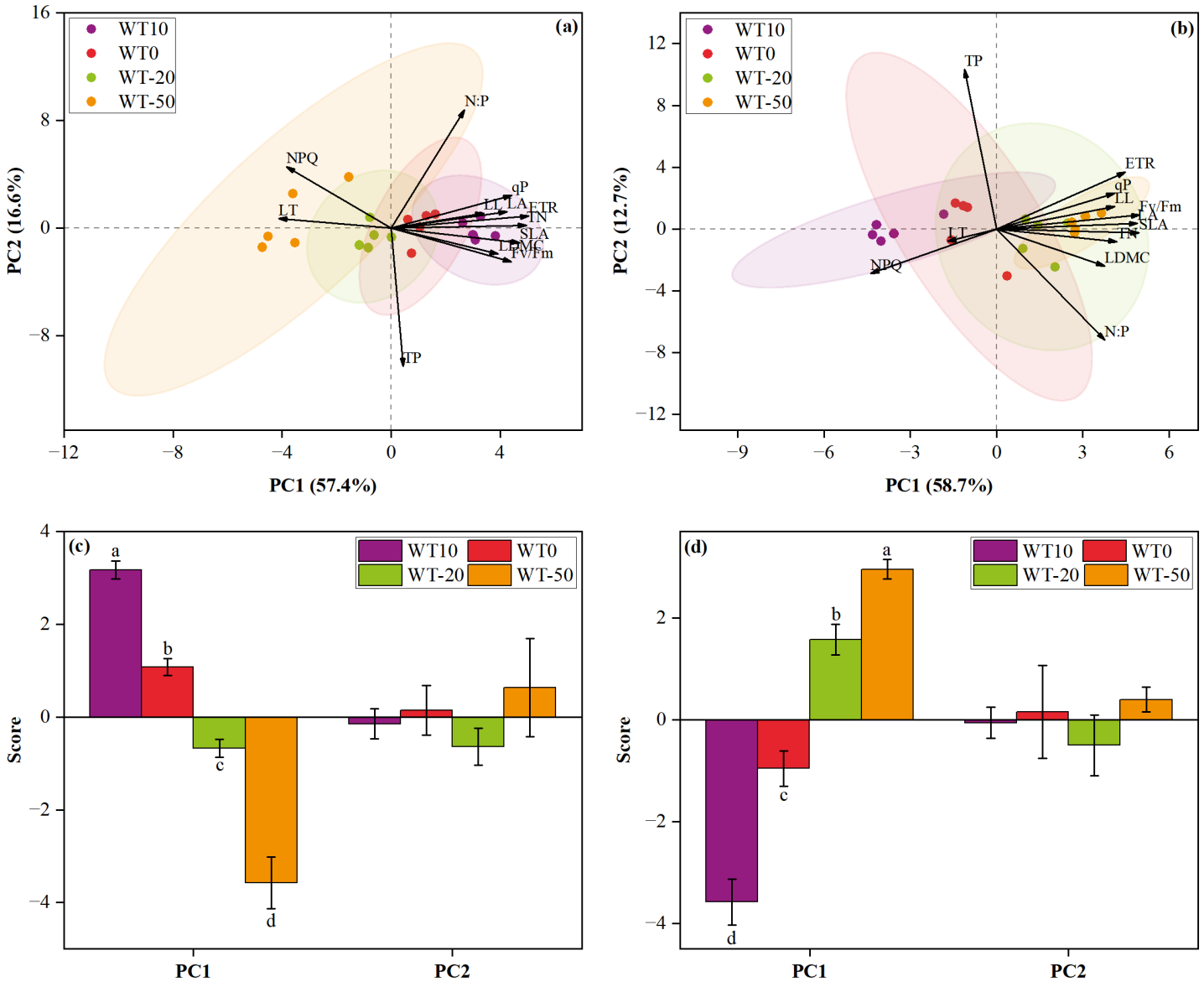

Figure 4

Principal component analysis (PCA) and scores on the first two PC axes for *Carex muliensis* (a, c) and *Pedicularis longiflora* var. *tubiformis* (b, d) under different water level treatments.

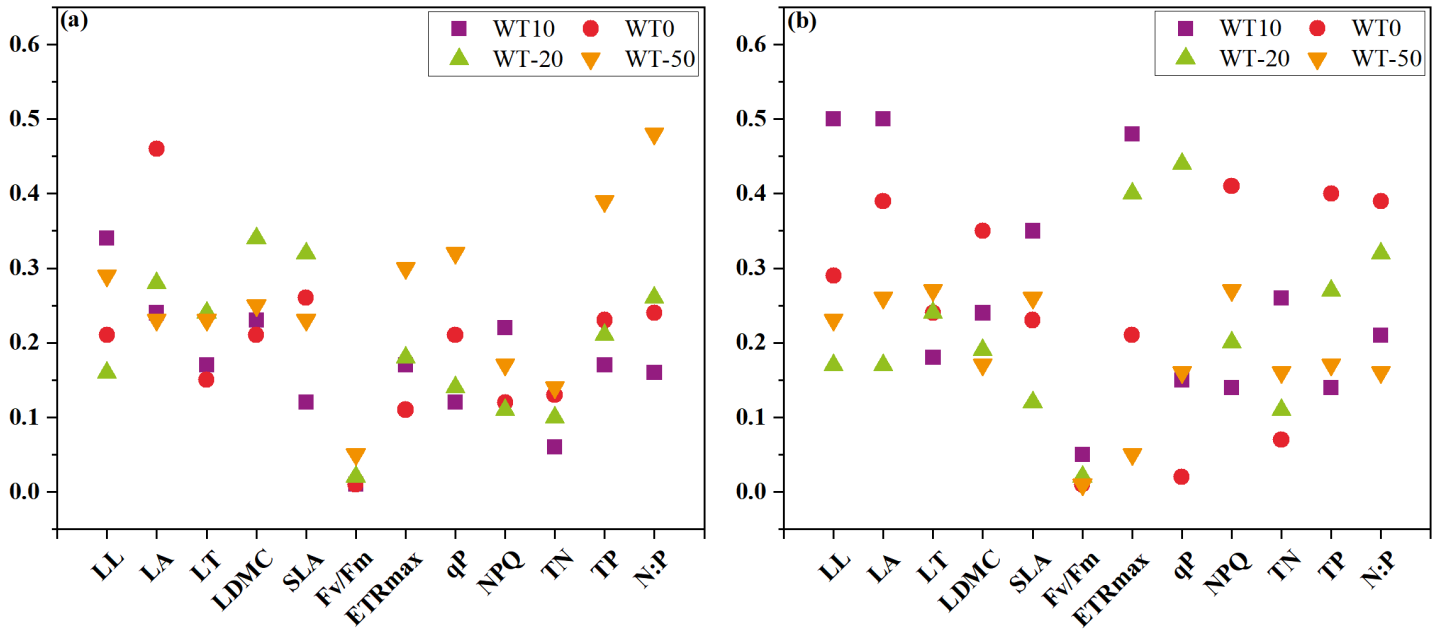

Figure 5

Leaf phenotypic plasticity of *Carex muliensis* (a) and *Pedicularis longiflora* var. *tubiformis* (b) under different water level treatments.

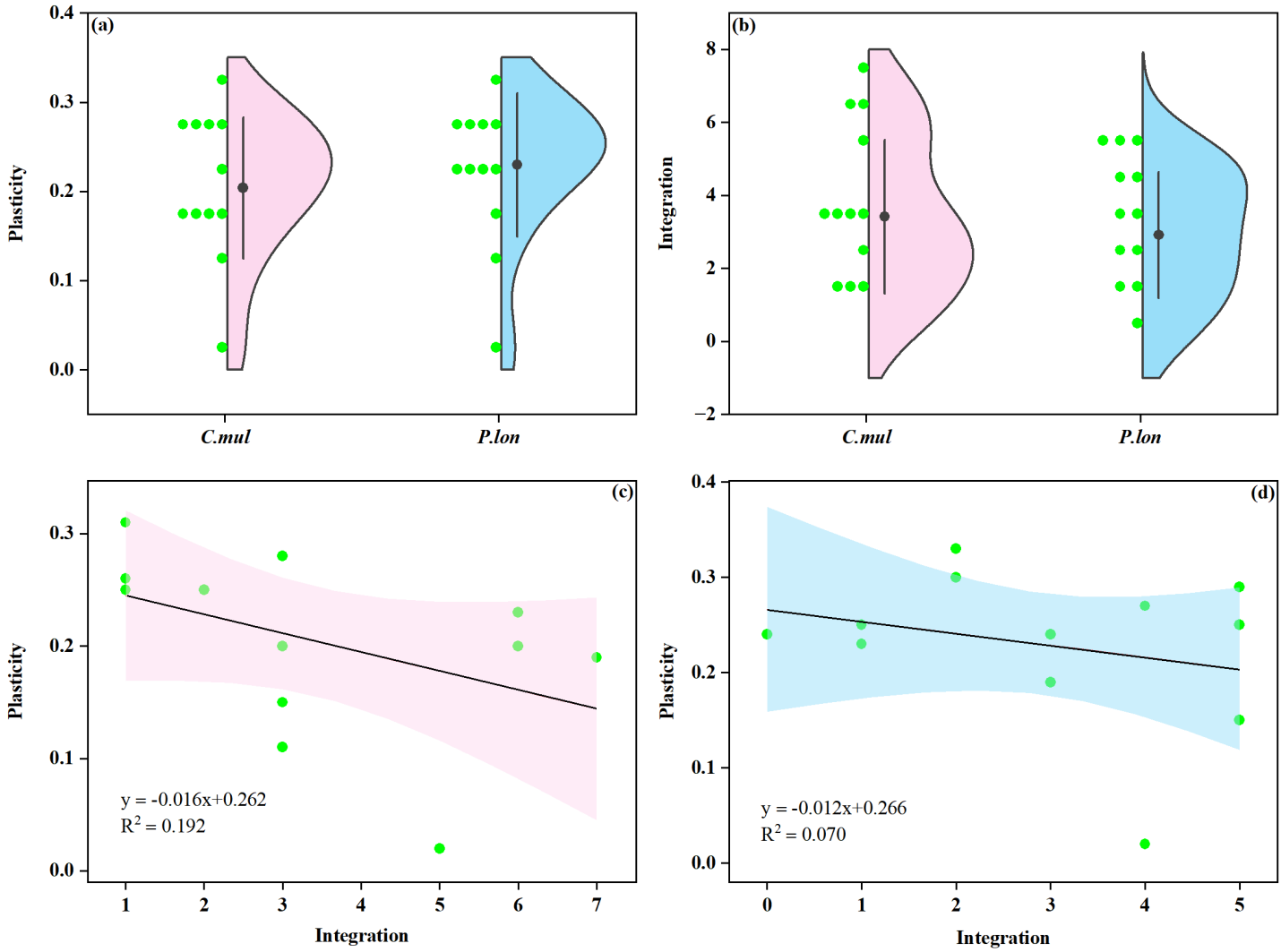

Figure 6

Relationship between phenotypic plasticity and integration in leaves of *Carex muliensis* (a) and *Pedicularis longiflora* var. *Tubiformis* (b). Each point corresponds to a leaf trait (n = 12). The shaded area indicates the 95% confidence interval of the fit test.