5. Stoichiometry and functional traits of reed (*Phragmites australis*)

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5.1 Introduction

Functional traits and life histories of plant species as well as the structure and function of ecosystems are of general and fundamental interest in ecology (Kerkhoff et al. 2006; McGill et al. 2006; Pérez-Harguindeguy et al. 2013). Plants grow in varying environments and there is a challenge for these organisms to adapt to extreme habitats such as very wet environments (e.g. floodplains, lakes and mires) or extremely dry ones (e.g. deserts and semi-deserts). There are many studies on functional traits available on terrestrial plants (Reich & Oleksyn 2004; Han et al. 2011), but only a very few on aquatic ones. Hydrophytes, for example, grow in the water where a limitation of oxygen availability is given. Thus, they require the development of certain tissue characteristics in order to combat this limitation.

Leaf and root traits are important indicators for plant strategies as they have an associated functional purpose (Eissenstat & Yanai 1997). The root traits are closely associated with the nutrient availability of the environment and the plant growth rates (Grime 1977). Plants with high growth rates usually have a high specific root length (SRL), low root diameter, low tissue density, high plasticity and are more adapted to nutrient rich environments. On the

Part of
Cirella, G. T. & Zerbe, S. (Eds.). (2014). *Sustainable water management and wetland restoration strategies in Northern China*. bu,press. https://doi.org/10.13124/9788860461094

contrary, species with a low growth rate are found on nutrient poor sites and have a high root:shoot ratio (RS) (Chapin et al. 2002). Leaves with a low specific leaf area (SLA) have a high dry matter content (DMC), high longevity, high nutrient-use efficiency and better defence against herbivores and physical hazards (Baruch 2011). While leaves with a high SLA have a high photosynthesis and growth rate, correspondingly they have low longevity and less developed defence systems (Baruch 2011; Shi et al. 2010). Factors such as top-soil freezing in winter and nutrient limitations determine the relationships among leaf and root biomass and nutrient content (Craine et al. 2005). Leaves and roots might also be functionally associated with other organs, for example, plant stems (Freschet et al. 2010).

In our investigation with regard to stoichiometry and functional traits, we choose reed (*Phragmites australis*) as a focal species. Reed can cope with a broad range of ground water levels, nutrient supplies, soil and water salinity and land-use (Zerbe & Thevs 2011; Taisan 2009). Reed not only has important ecosystem functions, but provides also a broad range of ecosystem services, such as building material, fodder for grazing, energy source and water purification (Köbbing et al. 2013; Patuzzi et al. 2013a). We examined the stoichiometry, functional traits and community structure of common reed in two settlements of northern China, differentiating between terrestrial and aquatic habitats. Thus, this research also contributes to the knowledge of nutrient content of water and sediment in both Wuliangsuhai Lake and the wetlands of Zhangye. The potential of N and P removal by reed with regard to wetland restoration is discussed. The findings of this study could have implications for the management of reed-dominated wetland ecosystems by understanding trade-offs of plant functional traits in changing climatic conditions. Climate change will become increasingly an issue especially in high latitude areas, like in northern China, where there has been a strong warming effect in recent years (Piao et al. 2010). Water plants are more sensitive to the impacts of climatic change than terrestrial plants. Recent research stresses a significant loss of species and alteration of community structure of wetlands in the last four decades (Fang et al. 2006). To better understand this impact, differences in the responses of functional traits of water and terrestrial plants to environmental change are important for predictive and scenario-based action. *P. australis* could be a model plant to exemplify wetland ecosystem changes in a dynamic global climate.

5.2 Materials and methods

5.2.1 Sampling and lab analysis

Within the Wuliangsuhai Lake area and the wetlands of Zhangye, we sampled reed in August 2011 when it was at its peak biomass. We laid out 45 plots in the water, covering a wide range of water pollution and salinisation levels (Figure 1), and 12 plots in terrestrial habitats. The plots had an area of 0.5 × 0.5 m^2. Mean plant height (m) and basal diameter (cm) related to the sediment surface were measured and the stem numbers were counted. Two to five reed individuals (ramets) were sampled on each plot. Roots and rhizomes were sampled 30 cm below the sediment surface. We divided each individual into five parts (organs), i.e. flowers, leaves, stems, roots and rhizomes. The five organs were weighed on site, directly after sampling.

Additionally, water and sediment were sampled at root depths near the experimentation areas at the same time as sample collection. Using this method, for the comparison of nutrients in reed organs, we evaluated differing developmental conditions by sampling ten plots with five aquatic and five terrestrial habitats in November 2011 (Li et al. 2014a).

In the laboratory, we determined the DMC, total carbon (mg C g^{-1}), total nitrogen (mg N g^{-1}) and total phosphorous (mg P g^{-1}) contents of reed flowers, leaves, stems, roots and rhizomes. All samples were oven dried at 60 °C for 72 h for subsequent nutrient content analyses. C and N were measured using an elemental analyser (2400 II CHN Elemental Analyzer, Perkin-Elmer, USA) with a combustion temperature of 950 °C and a reduction temperature of 640 °C. P was measured following the molybdate stannous chloride method (He et al. 2008). A total ranging from 10–30 leaves and 0.5 g wet root (1–5 replicates) were scanned with a Canon scanner (4400F), per sample. Then, SLA, SRL, mean root diameter (R^{diam}) and mean root area of unit mass (R^{area}) were determined with WinFOLIA and WinRHIZO (Régent, Quebec, Canada).

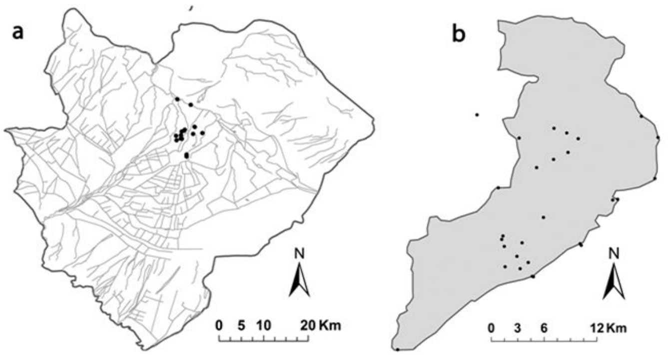

Figure 1 – Situated within northern China, locations of the reed sampling plots within the [a] wetlands in Zhangye (Zhang et al. 2005) and [b] Wuliangsuhai Lake (USGS 2011) (Li et al. 2014b).

5.2.2 Statistical data analysis

We compared the functional traits of *P. australis* from both study sites and found no significant differences. Hence, we pooled together the data from the two study sites for further analyses. One-way ANOVA and Bonferroni *post hoc* tests were carried out to assess the differences in N and P contents within and across reed organs. Standardised major axis (SMA) slopes with 95 % confidence intervals were used to examine N and P relationships within reed organs (Wright et al. 2004). Ordinary least square (OLS) regressions were performed to test the relationships of plant nutrient contents and water and sediment characteristics. ANCOVA was used to test the differences between regression slopes (Townend 2002). Since the sampling sites of the terrestrial and aquatic habitat were in close vicinity, we expected no other variations in regard to environmental conditions. The paired t-tests assess the differences in the traits of *P. australis* as well as characteristic differences between the two habitats were performed. One-sided paired t-tests were used for DMC, SLA, SRL, R^{area} and R^{diam}. DMC and R^{diam} were expected to be higher in the terrestrial reed and SLA, SRL and R^{area} higher in aquatic. Two-sided paired t-tests were used to test for differences in biomass allocation in the five *P. australis* organs and RS. In this case, the proportion of root to the total biomass was also defined as Root Ratio (RR). SMA analysis was used to detect relationships between biomass allocations in different organs. Slopes of SMA were compared between the two reed ecotypes. We considered more the trait differences of *P. australis* due to the two types of habitats (mostly moisture differences) and ignored the differences of other environmental variables, for example, soil and sediment nutrient contents. For further information on the methodological procedure, see (Li et al. 2014a, 2014b). The t-tests were used for the comparison of reed N and P content between two seasons.

5.3 Findings: N and P content

5.3.1 N and P content of reed

The N and P content of reed was on average 14.1 and 0.95 mg g^{-1}, respectively. The average N:P ratio was about 16, with 13 for the belowground and 17 for aboveground part. It differed significantly for the N and P contents in the five organs of reed. The N and P contents (mg g^{-1}) and the N:P ratio were 22.9, 2.05, and 12 in the flowers, 31.4, 1.33 and 24 in the leaves, 6.4, 0.65 and 10 in the stems, 16.1, 1.03 and 17 in the roots, and 7.2, 0.66 and 10 in the rhizomes, respectively (Figure 2). The N content was highest in the leaves while the P content was largest in the flowers compared to the other reed organs ($P < 0.05$).

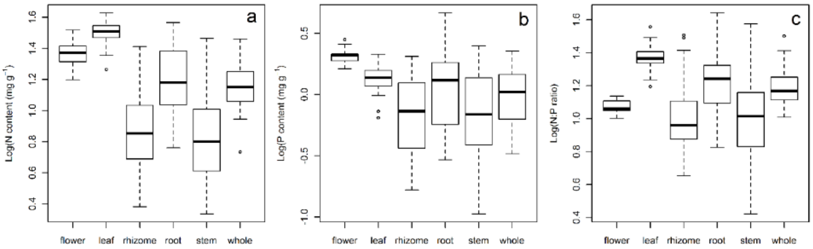

Figure 2 – Boxplot of N and P content and N:P ratio in five organs of reed with data $\log_{10}$-transformed. The lines in the middle of the boxes indicate median values, the upper and lower ranges of the boxes show the third and the first quartiles whereas upper and lower lines out of the boxes indicate the maximum and minimum values, extreme values are shown as dots (Li et al. 2014b).

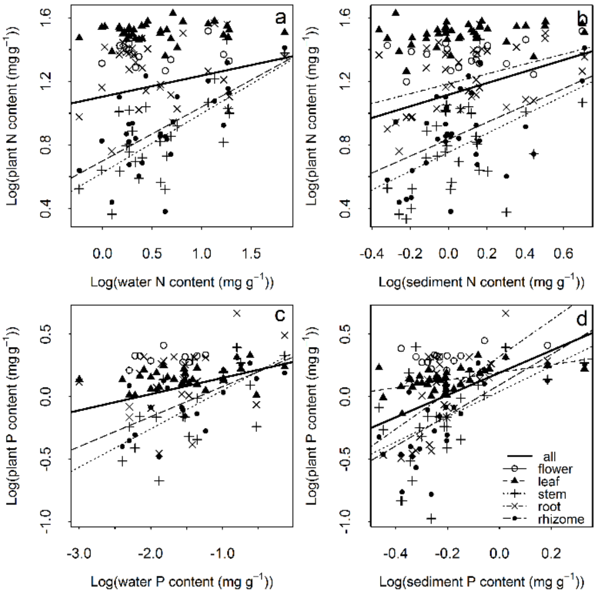

Figure 3 – The influence of water and sediment N and P availability on the N and P content of reed with data $\log_{10}$- transformed and with regression line shown when the slope is significant ($P < 0.05$) and not shown when the slope is not significant ($P > 0.05$) (Li et al. 2014b).

5.3.2 N and P stoichiometry of reed along environmental gradients

At the whole plant level, plant N and P contents were significantly and positively correlated with water and sediment N and P contents ($r^2 = 0.38$ and 0.18 for reed-water and reed-sediment N, respectively; $r^2 = 0.62$ and 0.43 for reed-water and reed-sediment P, respectively; all $P < 0.05$, Figure 3). At the organ level, stem and rhizome N contents increased significantly with the water and sediment N contents ($P < 0.05$, Figure 3). Flower and leaf N contents did not exhibit significant changes with the water or sediment N content ($P > 0.05$).

Stem and rhizome P contents increased significantly with the water and sediment P contents ($P < 0.05$). Root and leaf P contents increased significantly with the sediment P content ($P < 0.05$), with root P increasing fastest (slope = 1.45, $r^2 = 0.39$, $P < 0.05$) and leaf P slowest (slope = 0.31, r^2 =

0.26, $P < 0.05$). Flower P content did not exhibit significant changes with water or sediment P contents ($P > 0.05$).

5.3.3 The influence of nutrients on reed biomass allocation and community structures

Reed community structure is influenced by the environment and plant nutrient contents. Reed stem density was negatively correlated with reed P content and sediment P availability (r^2 = 0.54, 0.17, $P < 0.05$, Figure 4). Reed growth height and basal diameter were negatively correlated with stem density (r^2 = 0.48, 0.67, $P < 0.05$, Figure 4).

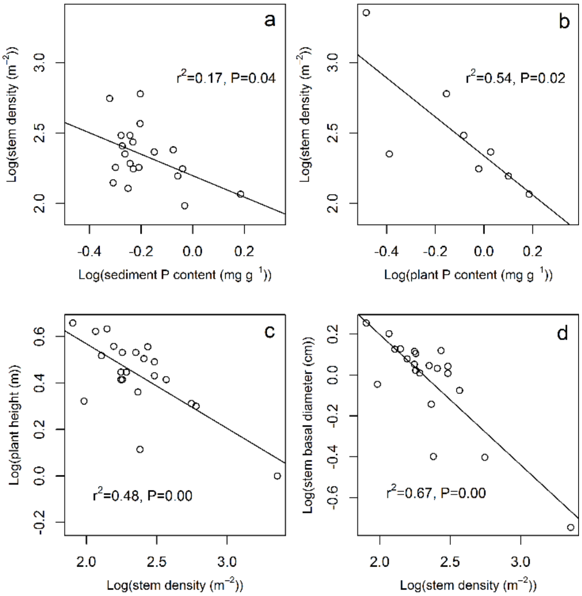

Figure 4 – The relationships of reed stem density with sediment per plant P content and reed growth height and basal diameter with stem density (with data $\log_{10}$- transformed) (Li et al. 2014b).

5.3.4 The functional trait and biomass allocation comparison of reeds in aquatic and terrestrial habitats

The mean SLA was 14.9 and 11.7 $m^2\ kg^{-1}$ and the mean SRL for aquatic and terrestrial reeds were 94.9 and 40.4 $m\ g^{-1}$, respectively. The SLA and SRL were both higher for aquatic than for terrestrial reeds ($P < 0.05$, Figure 5a, b). The mean root area of unit mass (R^{area}) was greater but the mean root diameter (R^{diam}) was smaller for aquatic than for terrestrial reeds ($R^{area} = 0.09$ and 0.05 $m^2\ g^{-1}$; and $R^{diam} = 0.32$ and 0.42 mm for aquatic and terrestrial reeds, respectively, $P < 0.05$, Figure 5c, d). A further illustrative comparison of reed growing in terrestrial and aquatic habitats (i.e. habitat, leaf and root) can be found in Li et al. (2014a).

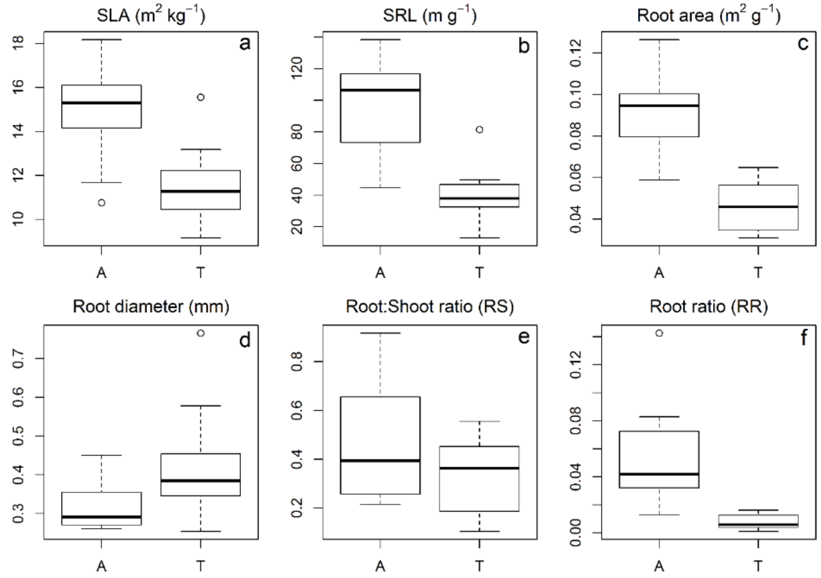

Figure 5 – The functional traits and biomass allocation of aquatic (A) and terrestrial (T) reeds with [a] = specific leaf area (SLA), [b] = specific root length (SRL), [c] = mean root area of unit mass (R^{area}), [d] = mean root diameter (R^{diam}), [e] = Root:Shoot ratio (RS) and [f] = root ratio (RR) (Li et al. 2014a).

The biomass proportions of the aboveground organs flower, leaf, and stem as well as belowground rhizome did not significantly differ for the aquatic and terrestrial plants (70 % and 76 % for aboveground biomass proportions, 3.9 % and 8.9 % for flower, 29.5 % and 36.2 % for leaf, 39.5 % and 32.3 % for stem, 21.4 % and 21.9 % for rhizome, for aquatic and terrestrial reeds, respectively, all $P > 0.05$, Table 1). The mean RS did not differ significantly between aquatic and terrestrial reeds (0.45 and 0.32, $P > 0.05$, as shown in both Table 1 and Figure 5e). However, the biomass proportion of root was significantly higher for aquatic than for terrestrial reeds (5.5 % and 0.7 %, respectively, $P < 0.05$, see Table 1 and the RR in Figure 5f).

Table 1 – The biomass allocation in reed of the aquatic and terrestrial ecotypes. Leaf:Stem indicates the biomass ratio of leaf and stem, analogously for the Root:Rhizome, Root:Leaf and Stem:Rhizome. The different superscripts indicate significant differences in the means (paired t-test, $P < 0.05$), SD = standard deviation. * The proportion of root biomass is also defined as root ratio (RR). (Li et al. 2014a).

	Aquatic form		Terrestrial form	
	Mean	SD	Mean	SD
Flower	3.9^a	2.9	8.9^a	5.9
Leaf	29.5^a	9.5	36.2^a	8.1
Stem	39.5^a	9.6	3.5	7.0
Root*	5.5^a	3.5	0.7^b	0.6
Rhizome	21.4^a	7.0	21.9^a	8.0
RS	0.45^a	0.24	0.33^a	0.15
Leaf:Stem	0.83^a	0.47	1.28^a	0.79
Root:Rhizome	0.26^a	0.13	0.04^b	0.02
Root:Leaf	0.22^a	0.18	0.02^b	0.02
Stem:Rhizome	2.09^a	0.95	1.84^a	1.22

Aquatic and terrestrial reeds, established in contrasting environments, have similar biomass allocation patterns but distinct leaf and root functional traits, which suggests different resource acquisition strategies. Aquatic reed grows faster with high SLA and SRL and is more receptive to the environment, while terrestrial reed is able to resist more to adverse environment and it is less responsive to the environment.

5.4 Comparative analysis of reed N and P content between summer and winter

The nutrient contents in reed organs were different between the two seasons studied (Photograph 6 and 7; Table 2). The N contents of reed's aboveground part (including leaf, stem and flower) in winter were lower than the contents in summer when reed has its peak biomass levels ($P < 0.05$). We did not find significant differences for the belowground elements (i.e. root and rhizome ($P > 0.05$)). The P contents of reed in leaf and stem in winter were lower than the contents in summer ($P < 0.05$). The P contents of reed in flower, root and rhizome were not significantly different ($P > 0.05$). The N:P ratio in stem and flower of reed was significantly different in the two seasons ($P < 0.05$). The results showed the movement of nutrient contents from leaf and stem to other organs from summer to winter.

With 10 samples (five for each of the two seasons studied), we compared the N and P contents from the winter for aquatic and terrestrial ecotypes reeds and found no significant differences in each organ. Future research and more sampling are needed to verify this result.

Table 2 – Comparison of N and P content (mg g^{-1}) of five organs of reed (aquatic form) in northern China in summer and winter. The different superscripts indicate significant differences in the means (t-test, $P < 0.05$) (Li et al. 2014a, 2014b).

	Summer N	Winter N	Summer P	Winter P	Summer N:P	Winter N:P
Leaf	31.37^{a}	8.01^{b}	1.33^{A}	0.34^{B}	$23.5^{\wedge}$	$23.4^{\wedge}$
Stem	6.37^{a}	1.91^{b}	0.65^{A}	0.09^{B}	$10.0^{\wedge}$	$22.0^{\vee}$
Flower	22.94^{a}	13.46^{b}	2.05^{A}	2.38^{A}	$11.7^{\wedge}$	$5.7^{\vee}$
Rhizome	7.18^{a}	6.38^{a}	0.66^{A}	0.8^{A}	$10.4^{\wedge}$	$8.0^{\wedge}$
Root	16.13^{a}	19.66^{a}	1.03^{A}	1.82^{A}	$17.2^{\wedge}$	$10.8^{\wedge}$

5.5 Conclusion

This research comprehensively sampled reed plants growing in different water bodies and on land in two seasons of northern China. It analysed the N and P content and biomass allocation patterns in each organ. The the N and P content of reed organs is closely related but the correlations may slightly alter with a change in environment-nutrient availability. In particular, N and P content of reed in leaf and flower is higher and more flexible, while it is lower and relatively stable in the stem. Significant increase of N and P content for aboveground parts of reed were found with an increase of sediment N and P availability, but any such corresponding increase of aboveground biomass ratio was not significant (Li et al. 2014b). Additionally, plant nutrient stoichiometry and nutrient availability are important drivers of these stand structures. The stem density of reed decreased, while stem height and basal diameter increased with the increase in nutrient availability. These results showed that with the eutrophication of wetlands and lakes, reed plants could absorb more N and P, and any extra N and P will result in more leaf and flowering of plants.

Moreover, N and P content in stem and leaf of reed decreased significantly during the winter season. This shows that, to restore a wetland ecosystem, harvesting reed in summer, when reed has its peak biomass, is not only removing more biomass but also more N and P. Limited by the sample size, we did not find significant changes of N and P content in reed belowground part for summer or winter.

Reed grows quite different in various environments, most notably is its functional trait differences in aquatic and terrestrial habitats. We found that both SLA and SRL, characteristics of leaf and root, respectively, are significantly different for reed in the two habitats even when the two grow about 10 meters in distance from each other. Being different, with the results of functional traits, we did not find very different biomass allocation patterns of reed between the two ecotypes. We preliminary concluded that this species adapted to a changing environment probably by way of eutrophication, climatic pressures and salinisation – mostly due to changes of functional traits rather than biomass allocation patterns. The scope of this research could be interlaced with climate change modelling or for the evaluation of N and P removal via reed harvesting in different eutrophication statuses for planning and restoring wetlands.

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