Oecologia (2011) 166:1–10
DOI 10.1007/s00442-010-1902-z

PHYSIOLOGICAL ECOLOGY - ORIGINAL PAPER

Stoichiometric homeostasis of vascular plants in the Inner Mongolia grassland

Qiang Yu · James J. Elser · Nianpeng He · Honghui Wu · Quansheng Chen · Guangming Zhang · Xingguo Han

Received: 23 April 2010 / Accepted: 28 December 2010 / Published online: 11 January 2011

Abstract Stoichiometric homeostasis, the degree to which an organism maintains its C:N:P ratios around a given species- or stage-specific value despite variation in the relative availabilities of elements in its resource supplies, is a key parameter in ecological stoichiometry. However, its regulation and role in affecting organismal and ecosystem processes is still poorly understood in vascular plants. We performed a sand culture experiment and a field nitrogen (N) and phosphorus (P) addition experiment to evaluate the strength of N, P and N:P homeostasis in higher plants in the Inner Mongolia grassland. Our results showed that homeostatic regulation coefficients (H) of vascular plants ranged from 1.93 to 14.5. H varied according to plant species, aboveground and belowground compartments, plant developmental stage, and overall plant nutrient content and N:P ratio. H for belowground and for foliage were inversely related, while H increased with plant developmental stage. H for N (H_N) was consistently greater than H for P (H_P) while H for N:P ($H_{N:P}$) was consistently greater than H_N and H_P. Furthermore, species with greater N and P contents and lower N:P were less homeostatic, suggesting that more homeostatic plants are more conservative nutrient users. The results demonstrate that H of plants encompasses a considerable range but is stronger than that of algae and fungi and weaker than that of animals. This is the first comprehensive evaluation of factors influencing stoichiometric homeostasis in vascular plants.

Keywords Ecological stoichiometry · N:P ratio · Developmental stages · Steppe · Plant functional groups

Communicated by Hermann Heilmeier.

Q. Yu · N. He · H. Wu · Q. Chen · G. Zhang · X. Han (✉)
State Key Laboratory of Vegetation and Environmental Change, Institute of Botany, Chinese Academy of Sciences, Beijing 100093, China
e-mail: xghan@ibcas.ac.cn

Q. Yu · H. Wu · X. Han
Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang 110016, China

J. J. Elser
School of Life Sciences, Arizona State University, Tempe, AZ 85287, USA

Introduction

The expanding sophistication of techniques in biology and ecology at all levels of biological organization has resulted in an increasingly fragmented knowledge base, making it challenging to integrate research across different scales and organisms (Vogel 1998; Elser et al. 2000b). However, entities at all levels of organization from molecules to biosphere are composed of various chemical elements in diverse ratios. Ecological stoichiometry, the study of the balance of multiple chemical elements in ecological interactions and processes (Elser et al. 2000b; Sterner and Elser 2002), makes it possible to connect studies of various levels of organization, diverse organisms, and different habitats (Elser et al. 2000b; Elser and Hamilton 2007). Stoichiometric theory is becoming a powerful framework for ecology and biology (Elser et al. 2000a; Sterner and Elser 2002; Karimi and Folt 2006), especially for research on the cycling of chemical elements and trophic transfer (Vanni 2002; Hessen et al. 2004).

Stoichiometric homeostasis is a central concept in ecological stoichiometry. Homeostasis represents the ability of organisms to maintain constant conditions in the body despite fluctuations in the environment (Kooijman 1995).

In the theory of ecological stoichiometry, the ability of an organism to maintain a given elemental composition despite variation in the elemental composition of its resource supplies is called "stoichiometric homeostasis" (Reiners 1986; Sterner and Elser 2002). Knowledge of the degree of stoichiometric homeostasis in plants is important because it underpins our interpretation of observed variation in plant C:N:P ratios in nature (Sterner and Elser 2002). For example, are variations in biomass C:N:P ratios a reflection of local physiological adaptation or of species replacements (Elser et al. 2010)? Stoichiometric homeostasis also affects the assumptions of models of ecological dynamics under stoichiometric constraints, which generally assume weak stoichiometric homeostasis in phototrophs and strong homeostasis in heterotrophs (Sterner and Elser 2002). Stoichiometric homeostatic regulation reflects underlying physiological and biochemical allocations as organisms respond to their surrounding environments (Hessen et al. 2004), and thus the degree of homeostasis may be highly relevant to fitness and to a species' ecological strategy (Frost et al. 2005; Jeyasingh et al. 2009). Indeed, our previous work has shown that species with strong homeostasis are dominant and stable in the community, while ecosystems dominated by homeostatic taxa are productive and stable (Yu et al. 2010), implying that stoichiometric homeostasis is a powerful and predictive mechanism underpinning important ecological phenomena.

Despite the importance of stoichiometric homeostasis, quantitative data are still relatively rare. While various studies have examined changes in elemental composition in response to nutrient supply across a wide range of taxa, including bacteria (Goldman et al. 1987; Makino et al. 2003), fungi (Levi and Cowling 1969), zooplankton (Andersen and Hessen 1991), algae (Rhee 1978), and vascular plants (Güsewell and Bollens 2003; Güsewell 2004), only a few studies have provided sufficient information to rigorously quantify the degree of stoichiometric homeostasis (Güsewell 2004; DeMott and Pape 2005; Karimi and Folt 2006; Jeyasingh et al. 2009).

The strength of stoichiometric homeostasis is readily quantified by the regulation coefficient H, calculated by $y = cx^{1/H}$ (Sterner and Elser 2002) where y is the elemental ratio of the organism, x is the elemental ratio in its nutrient supply (diet or external environment) and c is a constant. Higher values of H indicate greater stoichiometric homeostasis. Stoichiometric models have often assumed strict homeostasis for animals (H approaching infinity; Sterner and Elser 2002). However, previous stoichiometric homeostasis studies focused on zooplankton have shown that there exists considerable variation in H (H for P ranged from 4 to 40, DeMott and Pape 2005; H for P:C ranged from 16 to 161, Jeyasingh et al. 2009), indicating that the assumption of strict homeostasis in animals needs to be relaxed. As mentioned previously, ecological stoichiometry has generally adopted an assumption of weak stoichiometric homeostasis in vascular plants (Sterner and Elser 2002; Güsewell 2004), based primarily on observations of unicellular algae such as the green alga *Scenedesmus* (Rhee 1978). While work on freshwater invertebrates has shown that H differs across various chemical elements (Karimi and Folt 2006; e.g., H for N, C, P were 34.1, 13.8, and 7.7, respectively), data are particularly lacking for higher plants (Persson et al. 2010), despite the fact that vascular plants form the basis of nearly all terrestrial food webs. Thus, the validity of a weak homeostasis assumption is uncertain and considerably more data are needed for a clearer view (Persson et al. 2010; Elser et al. 2010).

Based on previous studies, we expected that (1) H of plants varies considerably, but is stronger than in algae and fungi and weaker than in animals, and (2) that H varies across different plant species, for aboveground versus belowground biomass, across developmental stages, and with overall plant nutrient content and N:P ratio. To test these expectations for stoichiometric homeostasis in higher plants, we conducted a sand culture experiment and a field N and P addition experiment in Inner Mongolia, China. Three grassland plant species were planted in sand with various nitrogen (N) and phosphorus (P) solutions and sampled at different developmental stages to evaluate how H might vary across various species, aboveground and belowground compartments, developmental stages, and elements. Furthermore, the consequences of variation in H across species and elements were tested in a field N and P addition experiment. This comparative approach provides a first step towards synthetic understanding of stoichiometric homeostasis in plants and its ecological consequences.

Materials and methods

Sand culture and field sampling

The sand culture experiment was conducted at the Inner Mongolia Grassland Ecosystem Research Station (IMGERS) in 2006. Three species, *Leymus chinensis* (a perennial rhizome grass, C-3), *Cleistogenes squarrosa* (a perennial bunchgrass, C-4) and *Chenopodium glaucum* (an annual forb, C-3), respectively, representing the dominant, subdominant, and the annual minor species, were planted from seeds in pots filled with sand and watered with nutrient solutions. Sand between 0.2 and 2.0 mm was screened with mesh and, to minimize the soil nutrient content, washed five times using tap water prior to filling plastic pots (30 cm diameter, 35 cm height). For each plant

species, there were 6 N level treatments (2, 4, 8, 16, 24, 32 mmol N L^{-1}, added as NH_4NO_3) and 6 P level treatments (0.15, 0.3, 0.6, 1.2, 2.4, 4.8 mmol P L^{-1}, added as KH_2PO_4), respectively. Each level had three replicates, with three pots randomly allocated to a replicate and a total of 36 pots (3 replicates × 3 pots × 4 harvests) for each species. The intermediate concentrations of N and P are generally considered to be optimal for plant growth and development (Hoagland and Arnon 1950). With the exceptions of N and P, the macroelement composition of the solution followed a formula developed by Hoagland and Arnon (1950) and the microelement composition was based on Jensen and Collins (1985). Each experimental pot received the same amount of macro- and micronutrients except for N and P. Each pot had four drainage holes and received 250 mL solutions every day to maintain a relatively constant macro- and micronutrient concentration. To avoid ionic toxicity, the pots were washed with 500 mL water twice every 10 days immediately followed by 250 mL nutrient solutions. All pots were covered when it rained and, if rainwater entered pots, additional 250 mL nutrient solutions were added. There were 10–30 individuals in each pot depending on the plant size. The density was controlled to ensure that the plant individuals did not shade each other. The aboveground and belowground biomass of 30 plants (within 3 pots) of each plant species for each treatment replicate were harvested at 15-day intervals from 25 June to 25 August 2006. Healthy, fully expanded leaves and roots were oven-dried at 60°C, powdered, and screened with 0.1-mm mesh for chemical analysis (total N and total P).

The field N and P addition experiments were conducted in a *Leymus chinensis* grassland that had been fenced since 1999 to prevent grazing by large animals. The mean annual temperature in the study area is 0.3°C with mean monthly temperatures ranging from −21.6°C in January to 19.0°C in July. The annual precipitation was 304 mm in 2006 and 240 mm in 2007, lower than the mean annual precipitation (346 mm). Both N and P addition series had seven treatment levels and six replicates for each level: control, 0, 5.6, 11.2, 22.4, 39.2, 56 g N m^{-2} (added as urea) and control, 0, 1.55, 3.1, 6.2, 9.3, 12.4 g P m^{-2} (as KH_2PO_4) respectively. Except for control, each plot in the N addition experiment also received 1.55 g P m^{-2}, or 2.8 g N m^{-2} in the P addition experiment. The effect of K was controlled by adding appropriate levels of KCl so that all plots received similar levels of K. The fertilizer was thoroughly mixed with sand and then applied to the plot surfaces in May 2006 and 2007, respectively. The aboveground biomass was sampled by clipping all plants at ground level within a 1 m × 1 m quadrat on 20 July 2006 and 2007. All living vascular plants were sorted to species, oven-dried at 60°C and weighed. Fifty fully grown and healthy leaves for each different species (12 species total) were selected, ground, and mixed evenly for analysis. Soil was randomly sampled with three replicates at the depth of 0–10 cm within quadrats, and the three replicates were mixed to produce one sample.

The 12 species were divided into four plant functional groups on the basis of life forms according to Bai et al.'s method (2004). Perennial rhizome grass (PR): *Leymus chinensis*; perennial bunchgrasses (PB): *Stipa grandis*, *Agropyron cristatum*, *Cleistogenes squarrosa, Achnatherum sibiricum, Koeleria cristata*; perennial forbs (PF): *Poa sphondylodes*, *Carex korshinskyi*, *Allium ramosum*, *Allium tenuissimum*; annuals and biennials (AB): *Axyris amarantoides*, *Chenopodium glaucum*.

Element analysis

Total N concentrations (% of dry mass) were analyzed using the micro-Kjeldahl method (Bremner 1996) while total P concentrations (% of dry mass) were measured by the ammonium molybdate method after persulfate oxidation (Kuo 1996).

For soil inorganic N measurement, dried and pre-weighed soil samples were first extracted with 2 mol L^{-1} KCl. The concentrations of inorganic N (NH_4^+-N and NO_3^--N) in the filtrates were determined using a flow injection autoanalyzer (FIAstar 5000 Analyzer; Foss Tecator, Denmark, Wang et al. 2006). To measure available P, air-dried and pre-weighed soil was extracted with 0.5 mol L^{-1} $NaHCO_3$ (Schoenau and Huang 1991) and P concentration in the extract was determined by the ammonium molybdate method (Kuo 1996). The concentrations of soil inorganic N and available P were calculated based on dry soil weight. Soil water content was determined gravimetrically by oven-drying subsamples at 105°C for 24 h.

Data analysis and statistics

To estimate the strength of plant homeostasis of a given measure of plant nutrient content, we used regression analysis to fit the data to the homeostatic model equation (Sterner and Elser 2002): $y = cx^{1/H}$, where y is the N or P concentration or N:P ratio of plants, x is N or P concentration or N:P ratio in the environment. For the sand culture experiment, x is the solution concentration or its N:P ratio. For the field N addition experiment, x is the soil inorganic N concentration (NH_4^+-N + NO_3^--N) and N:P ratio, and for the P addition experiment, x is the 0.5 mol L^{-1} $NaHCO_3$ extractable P concentration and N:P ratio. In the equation, c is a constant. The values of H and c were obtained when we analyzed the relationship between y and

x using regression analysis. A high value of H indicates strong stoichiometric homeostatic regulation.

Variation of H across species, developmental stage, and element in the sand culture experiment was analyzed by repeated measurements ANOVA, while variation of H across years, species, and elements in the field experiment was analyzed by three-way ANOVA. Linear regression was used to assess the relationships between H and mean element concentrations and N:P in biomass across all treatments. All statistical analyses were performed using SAS (version 9.0; SAS Institute, Cary, NC, USA).

Results

Patterns of tissue N and P concentrations in response to N and P concentrations in solution in the sand culture experiment

Both N and P concentrations in the plant tissue increased with increasing N and P addition rates in the solutions. The nature of the response pattern was similar for all species: tissue nutrient content showed a sharp increase at lower N and P levels and then leveled off. This pattern was well-captured by the homeostasis model: $y = cx^{1/H}$. On average, the homeostasis model could explain 87 ± 6% (mean ± SD) of variation in biomass elemental composition. H varied from 1.93 to 14.49 (Fig. 1), with an average of 5.60 ± 2.61.

Aboveground H of *Leymus chinensis* was consistently the highest among the three species (Fig. 1). H_N varied from 5.88 (N concentration varying from 3.21 to 5.39%) to 8.80 (N concentration varying from 2.29 to 3.57%), and from 6.73 to 12.16 for H for N:P in the N addition experiment [$H_{N:P(+N)}$]. H_P varied from 3.37 (P concentration 0.28–0.82%) to 6.77 (P concentration 0.14–0.27%), and from 4.49 to 9.46 for H for N:P in the P addition experiment ($H_{N:P(+P)}$). However, H values for belowground tissues of *Leymus chinensis* were consistently the lowest among the three species (Fig. 1). H_N varied from 2.95 (N concentration varying from 0.63 to 1.74%) to 3.64 (N concentration varying from 0.63 to 1.44%), and from 5.11 to 7.99 for $H_{N:P(+N)}$. H_P varied from 1.67 (P concentration 0.07–0.65%) to 2.56 (P concentration 0.08–0.37%), and from 2.19 to 2.77 for H for N:P in the P addition experiment.

Aboveground H of *Chenopodium glaucum* was consistently the lowest among the three species (Fig. 1). However, values of H for belowground of *Chenopodium glaucum* were consistently the greatest among the three species. For *Cleistogenes squarrosa*, both H values of foliage and belowground were intermediate for the three species (Fig. 1).

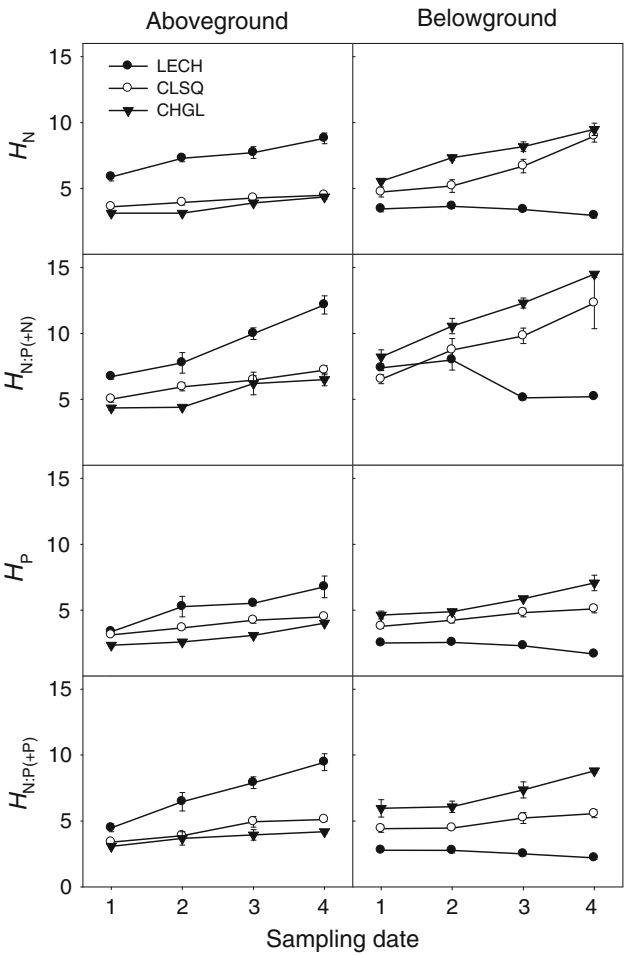

Fig. 1 Changes in H for aboveground (*left*) and belowground (*right*) tissue with plant developmental stages for three grassland species in the sand culture experiment. H_N and $H_{N:P(+N)}$ reflect changes in tissue N and N:P when N was added, and H_P and $H_{N:P(+P)}$ reflect changes of tissue P and N:P when P was added. Sampling dates 1, 2, 3, and 4 represent 10 and 25 July, and 10 and 25 August, respectively. *LECH Leymus chinensis*, *CLSQ Cleistogenes squarrosa*, *CHGL Chenopodium glaucum*. *Error bars* SEM

Patterns of tissue N and P concentrations in response to N and P contents in the soil in field N and P addition experiment

Changes of tissue N and P concentrations in response to soil N and P contents (soil inorganic N varied from 8.2 to 68 and 8.8 to 103 mg kg^{-1} in 2006 and 2007, respectively, and available P varied from 2.1 to 22.7 and 2.2 to 23.5 mg kg^{-1}) in the field N and P addition experiments were similar to those in the sand culture experiment. On average, the homeostasis model explained 80 ± 17% (mean ± SD) of the variation in plant elemental composition for the three plant species (77 ± 13% for the 12 species; Yu et al. 2010). H ranged from 2.60 to 9.45 (Fig. 2) with an average of 5.42 ± 2.44.

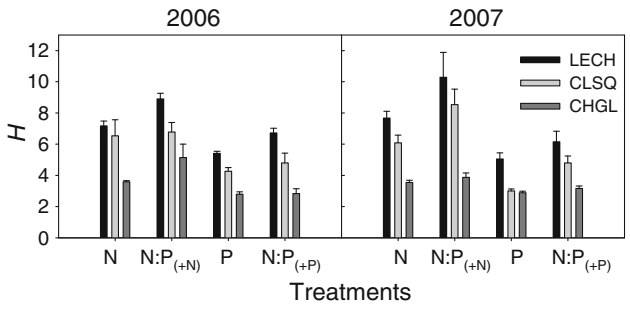

Fig. 2 Inter-annual differences in *H* for aboveground tissues of three species in field N and P addition experiment. CLSQ, *Leymus chinensis*; *Cleistogenes squarrosa*; CHGL, *Chenopodium glaucum*. *Error bars* SEM

Aboveground *H* values of *Leymus chinensis* were the highest among the 12 species in the field experiment. H_N, $H_{N:P(+N)}$, H_P, and $H_{N:P(+P)}$ in 2007 were 7.68 (N varying from 2.01 to 2.91%), 10.29, 5.6 (P varying from 0.10 to 0.18%), and 6.15, respectively (Fig. 2). *H* values of *Chenopodium glaucum* were the lowest among the 12 species. H_N, $H_{N:P(+N)}$, H_P and $H_{N:P(+P)}$ in 2007 were 3.54 (N varying from 2.31 to 4.67%), 3.87, 2.88 (P varying from 0.20 to 0.48%), and 3.15, respectively. There were no significant differences between the results from 2006 and 2007. *H* values for the other species were within the range of *Leymus chinensis* and *Chenopodium glaucum*. The order of *H* values for plant functional groups was PR > PB > PF > AB (Fig. 4). Within a given functional group, *H* values of species were similar.

Variation of *H* with developmental stage

All *H* parameters (including H_N, $H_{N:P(+N)}$, H_P, $H_{N:P(+P)}$) for foliage in the sand culture experiment increased significantly with sampling date (Fig. 1; Table 1; $P < 0.001$). This pattern was consistent for the three species. However, the magnitude of change in *H* differed among species. For example, *Leymus chinensis* had the greatest change while *Cleistogenes squarrosa* and *Chenopodium glaucum* changed more slowly.

Temporal changes in *H* for belowground differed from those for aboveground biomass (Fig. 1), increasing significantly with sampling date for *Cleistogenes squarrosa* and *Chenopodium glaucum* ($P < 0.001$) while remaining stable for *Leymus chinensis* ($P > 0.05$). However, overall, developmental stage had a significant effect on *H* ($P < 0.001$; Table 1), while there were no significant differences in *H* values between July 25 and August 10 ($P > 0.05$).

Variation of *H* among tissues within species

Overall, *H* differed significantly according to plant species ($P < 0.001$; Table 1). Foliage and belowground of the same species appear to have different degrees of stoichiometric homeostasis. In the sand culture experiment, aboveground *H* values for *Leymus chinensis* were higher than root *H* but aboveground *H* values were much lower for *Cleistogenes squarrosa* and *Chenopodium glaucum* (Fig. 1). Interestingly, *H* appeared to have inverse relationships for foliage and belowground: the species with the highest aboveground *H* (*Leymus chinensis*) tended to have the lowest belowground *H* ($P < 0.05$, Table 1), while the species with lowest aboveground *H* (*Chenopodium glaucum*) had the highest belowground *H* ($P < 0.05$).

For the field N and P addition experiments, there are foliage data only. Both in 2006 and 2007, *H* for the three species had the same rank as the sand culture experiment; that is, *Leymus chinensis* > *Cleistogenes squarrosa* > *Chenopodium glaucum* ($P < 0.05$; Fig. 2; Table 2). Furthermore, the values of *H* for each species were similar between the two experiments. Overall, species had significant effect on *H* ($P < 0.001$) in the field experiment and there were no significant differences in *H* values estimated for data from 2006 and 2007 (Table 2).

Variation of *H* for different chemical elements

Values of *H* also varied with regard to which aspect of elemental composition was considered. In general, the sand culture experiment results showed that both foliage and belowground had higher H_N than H_P ($P < 0.05$; Fig. 1; Table 1). Furthermore, $H_{N:P(+N)}$ was consistently higher than H_N ($P < 0.05$), while $H_{N:P(+P)}$ was greater than H_P ($P < 0.05$).

Similarly, results from the field experiments showed overall that *H* differed according to which elemental parameter was measured ($P < 0.001$; Table 2). More specifically, H_N was higher than H_P ($P < 0.05$; Fig. 2; Table 2). $H_{N:P}$ was also consistently higher than corresponding H_N and H_P ($P < 0.05$).

Relationship between *H* and N, P concentrations and N:P

In the sand culture experiment, both aboveground mean N concentration ($P = 0.099$; Fig. 3) and P concentration ($P < 0.001$) were negatively related to their corresponding aboveground *H*, while aboveground N:P was positively related with $H_{N:P}$ ($P < 0.001$). For belowground, both N and P concentrations were negatively related with corresponding *H* ($P < 0.001$); however, no relationship was found between $H_{N:P}$ and N:P ($P = 0.17$). For the field experiment, mean concentrations of N and P were negatively associated with H_N and H_P while N:P was positively related with $H_{N:P}$ for 2006 and 2007 combined ($P < 0.001$; Fig. 4).

Table 1 Repeated measurements ANOVA results for the effects of species, sampling time, and elements on *H* in the sand culture experiment

H for aboveground				*H* for belowground			
Species		Date		Species		Date	
LECH	7.38 ± 2.60 a	Jul 10	4.04 ± 1.28 d	LECH	3.65 ± 1.84 c	Jul 10	4.99 ± 1.80 c
CLSQ	4.61 ± 1.15 b	Jul 25	4.89 ± 1.94 c	CLSQ	6.29 ± 2.54 b	Jul 25	5.70 ± 2.49 b
CHGL	3.96 ± 1.29 c	Aug 10	5.87 ± 2.54 b	CHGL	7.92 ± 2.73 a	Aug 10	6.13 ± 2.92 b
		Aug 25	6.46 ± 2.61 a			Aug 25	6.98 ± 3.97 a
Elements				Elements			
N	5.04 ± 1.95 b			N	5.80 ± 2.28 b		
$N:P_{(+N)}$	7.10 ± 2.71 a			$N:P_{(+N)}$	9.05 ± 3.04 a		
P	4.04 ± 1.36 c			P	4.12 ± 1.60 c		
$N:P_{(+P)}$	5.08 ± 2.03 b			$N:P_{(+P)}$	4.84 ± 2.06 b		
Species	$F = 428.592$, $df = 2$, $P < 0.001$			Species	$F = 455.142$, $df = 2$, $P < 0.001$		
Date	$F = 87,94$, $df = 3$, $P < 0.001$			Date	$F = 37.271$, $df = 3$, $P < 0.001$		
Elements	$F = 150.353$, $df = 3$, $P < 0.001$			Elements	$F = 348.189$, $df = 3$, $P < 0.001$		
Species × date	$F = 10.759$, $df = 6$, $P < 0.001$			Species × date	$F = 25.362$, $df = 6$, $P < 0.001$		
Species × elements	$F = 7.958$, $df = 6$, $P < 0.001$			Species × elements	$F = 3.665$, $df = 6$, $P = 0.010$		
Date × elements	$F = 2.501$, $df = 9$, $P = 0.015$			Date × elements	$F = 3.118$, $df = 9$, $P = 0.003$		
Species × date × elements	$F = 0.89$, $df = 18$, $P = 0.592$			Species × date × elements	$F = 2.763$, $df = 18$, $P = 0.001$		

Different lowercase letters represent statistically significant differences between treatments at $P < 0.05$. Values of *H* are expressed by mean ± SD. The degrees of freedom (*df*) for the numerator, which come from each effect, are given in the table. The denominator *df* is 96, which comes from within variance

LECH Leymus chinensis, *CLSQ Cleistogenes squarrosa*, *CHGL Chenopodium glaucum*

Discussion

Ecological stoichiometry has often assumed a strict homeostasis for animals and a flexible stoichiometry for autotrophs (Sterner and Elser 2002). However, emerging data indicate that the situation is more complicated (Persson et al. 2010). Our findings indicate that vascular plant taxa common in the Inner Mongolia grassland present a considerable range in the strength of their stoichiometric homeostasis and do not generally conform to a concept of close tracking of environmental nutrient supply originally emphasized in stoichiometric theory. While our *H* values for plants were generally lower than those of freshwater invertebrates reported by Karimi and Folt (2006), especially for N ($H = 34.1$), and were also lower than zooplankton for P and P:C (H_P 9–40, except *Daphnia magna* and *D. pulex*, DeMott and Pape 2005; $H_{P:C}$ 16–162, Jeyasingh et al. 2009), our data indicate that some plant species can be quite homeostatic (*Leymus chinensis* in the sand culture experiment, $H_P = 6.61$), and even stronger than some zooplankton, such as *Daphnia magna* and *D. pulex* ($H_P = 4$–6; DeMott and Pape 2005). Suggestions of strong stoichiometric homeostasis were also found from field studies of wetland plants and aquatic macrophytes (Demars and Edwards, 2007). For aboveground N:P ratio, Güsewell (2004) reported *H* values for herbaceous plants ranging from 1.7 to 4.6 (recalculated from Shaver and Melillo 1984; Ryser and Lambers 1995; Güsewell and Bollens 2003; Güsewell 2005). These are somewhat lower than those observed in the present study for aboveground biomass of Inner Mongolia plants, which ranged from 3.08 to 14.49. Makino et al. (2003) proposed that heterotrophic bacteria are more like animals than plants in terms of C:N:P homeostasis based on data from several experiments (Tezuka 1990; Nakano 1994; Chrzanowski and Kyle 1996). Their studies showed that *H* values of bacteria for C:P and N:P are similar, ranging from 1.1 to ∞. However, a majority of the studies for different bacteria strains had $H < 6$, while the remainder had *H* values equivalent to ∞. So, bacterial homeostasis also appears to vary considerately among species and studies. Overall, it appears that stoichiometric homeostasis in plants is stronger than in algae or fungi (Rhee 1978; Sterner and Elser, 2002) but is weaker than in animals (DeMott and Pape 2005; Karimi and Folt 2006; Jeyasingh et al. 2009), perhaps because vascular plants are more developmentally complex than algae and fungi but less so than animals. Differences in the mechanisms of nutrient absorption and storage may also play a role (Sterner and Elser 2002). However, there is a large range of variation within each group that requires further investigation.

While many factors can influence *H* (discussed below), the results obtained from the sand culture experiment and

Table 2 Three-way ANOVA results for the effects of years, species, and elements on H in the field experiments

Years		Species		Elements	
2006	5.41 ± 2.14 ns	LECH	7.17 ± 2.26 a	N	5.77 ± 2.03 b
2007	5.42 ± 2.72 ns	CLSQ	5.60 ± 2.19 b	$N:P_{(+N)}$	7.25 ± 3.04 a
		CHGL	3.48 ± 1.09 c	P	3.90 ± 1.87 d
				$N:P_{(+P)}$	4.74 ± 1.77 c

Years	$F = 0.002$, $df = 1$, $P = 0.969$
Species	$F = 82.973$, $df = 2$, $P < 0.001$
Elements	$F = 37.795$, $df = 3$, $P < 0.001$
Years × species	$F = 0.336$, $df = 2$, $P = 0.716$
Years × elements	$F = 0.975$, $df = 3$, $P = 0.407$
Species × elements	$F = 2.477$, $df = 6$, $P = 0.027$
Years × species × elements	$F = 1.712$, $df = 6$, $P = 0.124$

Different lowercase letters represent statistically significant differencse between treatments at $P < 0.05$. Values of H are expressed by mean ± SD. The degrees of freedom (df) for the numerator are given in the table. The denominator df is 120

ns No significant difference, *LECH Leymus chinensis*, *CLSQ Cleistogenes squarrosa*, *CHGL Chenopodium glaucum*

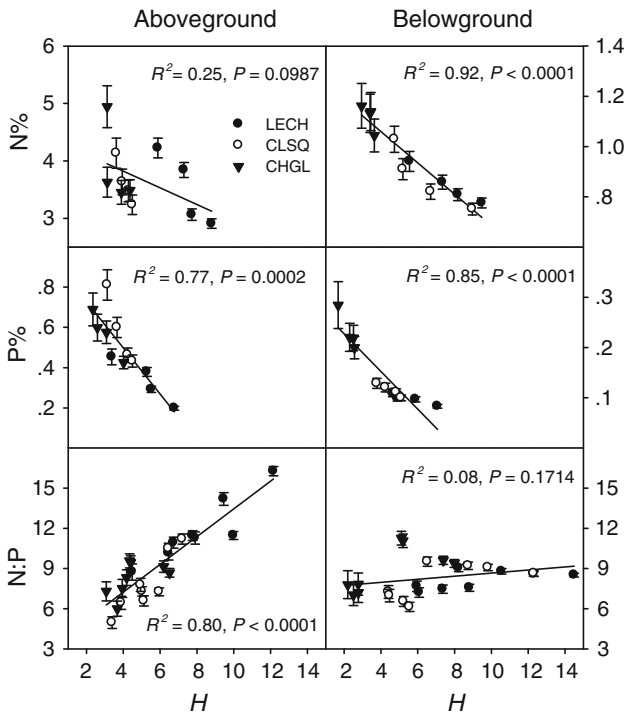

Fig. 3 Relationships between N%, P% and N:P and corresponding H in aboveground (*left*) and belowground (*right*) tissues across the four developmental stages and three species in the sand culture experiment. The N%, P% and N:P values reflect the mean values of the tissues across all the treatments for a given developmental stage (sampling date) and species. *Error bars* SEM

field N and P addition experiments are consistent and comparable. Rank values of H values for the study species from highest to lowest for both experiments were in the order of *Leymus chinensis* > *Cleistogenes squarrosa* > *Chenopodium glaucum* in both studies, suggesting that values of H obtained from different experiments can be reasonably compared and that H was not strongly influenced by possible difficulties arising from differences in N and P actually available in soil versus analytical estimates of soil N and P. Furthermore, there was no significant difference between the results from 2006 and 2007 ($P = 0.97$) in the field N and P addition experiments even though precipitation differed considerably between the two years (304 and 240 mm, respectively). While it would be interesting to assess H in years of drought compared to years of high rainfall, the consistency of these results suggests that our estimation of H is appropriate and that H may reasonably be considered as a trait of particular species.

Our results show that H generally increased with plant developmental stage (excluding belowground of *Leymus chinensis*); that is, older plants in more advanced developmental stages were more tightly constrained in tissue elemental concentration than less advanced plants. This may be because older plants have more developed modulation systems than young plants or that young plants contain more functional materials than old plants, which makes younger plants respond more quickly and more strongly to different nutrient regimes than older plants.

Interestingly, different parts of the same plant appear to exhibit different degrees of stoichiometric homeostasis, with an apparently inverse relationship for aboveground and belowground biomass. While this pattern might reflect different phenological timing between aboveground and belowground growth, it may also reflect a fundamental tradeoff in nutrient investment and allocation associated with ecological success in this low-fertility grassland. For example, *Leymus chinensis*, the dominant plant species in the grassland, had the highest H for aboveground biomass

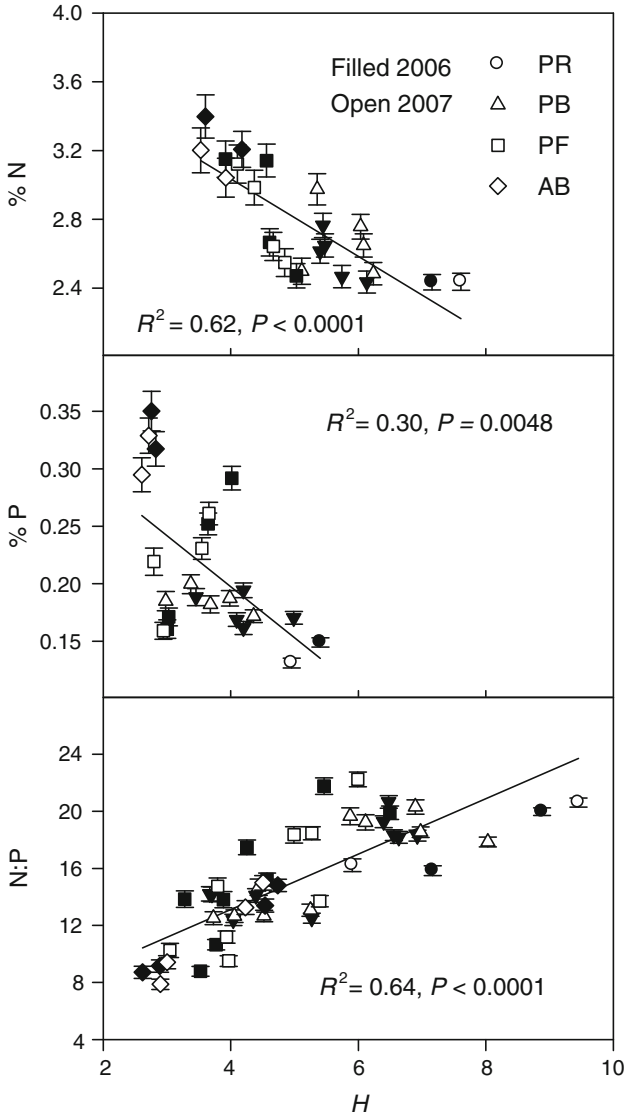

Fig. 4 Relationships between foliage N%, P% and N:P and corresponding H in the field experiment. The N%, P% and N:P values reflect the mean values of the tissues among all the treatments for a given species (*one point* represents a species). Plant functional groups: perennial rhizome grass (*PR*), perennial bunchgrasses (*PB*), perennial forbs (*PF*), annuals and biennials (*AB*). *Error bars* SEM

but the lowest H for belowground biomass, likely because this species has adapted to the infertile environment by adopting a storage strategy in belowground for potentially limiting nutrients, corresponding well with other plants dominating infertile sites (Chapin 1980). Thus, high aboveground H species keep their foliar elemental composition stable by modulation of belowground composition, which is an appropriate adaptation to the steppe environment. While more data on aboveground and belowground H are needed for a wider range of species, this belowground versus aboveground relationship in nutrient regulation may help to explain why high H species dominate in the Inner Mongolia grassland and exhibit stable dynamics and why ecosystems dominated by high H species have higher and more stable productivity (Yu et al. 2010).

H also varied with regard to which aspect of elemental composition was considered. H_N was constantly higher than H_P, a result that may simply reflect the fact that there is a greater ability to vary the relative abundance of elements, such as P, that is less abundant overall in tissue. This result also corresponds well with the finding that zooplankton strongly regulate macronutrients but weakly regulate essential micronutrients and do not regulate non-essential metals (Karimi and Folt 2006). We also found that $H_{N:P}$ was consistently higher than corresponding H_N and H_P. This is in line with a general trend that increases in N concentration are usually accompanied by increases in P simply because of coupling of major elements in various biochemical and cellular constituents, making the ratio of these two elements relatively constant compared to the potentially decoupled changes in the surrounding environment (Sterner and Elser 2002).

Higher aboveground tissue nutrient concentrations were associated with reduced values of H and higher N:P ratios coincided with higher H (Figs. 3 and 4). This suggests that plants with stronger stoichiometric homeostasis are more conservative nutrient users (lower nutrient content and higher N:P) and that plants with more flexible nutrient stoichiometry are more lavish nutrient users. Strong stoichiometric homeostasis and conservative nutrient use may be critical for species in arid and infertile environments, such as typical grassland, and thus may be important ecophysiological mechanisms underpinning their biomass production and stability (Yu et al. 2010).

Our findings demonstrate comprehensively for the first time how H values vary across plant species, plant developmental stages, plant aboveground and belowground compartments, and according to which set of elements was considered. It is essential to account for these factors when characterizing plant elemental composition and its regulation. In addition, previous studies have shown that the degree of homeostatic regulation depends on overall nutrient supply and on light intensity (Güsewell 2004). Limpens and Berendse (2003) found that the strength of homeostasis may change as a long-term adaptation to site fertility. Furthermore, data from algae suggest that even growth rate itself could be associated with stoichiometric homeostasis (Elrifi and Turpin 1985; Shafik et al. 1997; Persson et al. 2010), as the relatively constrained biochemical allocations required under fast growth might constrain biomass N:P within a narrow range under situations that permit high growth rates. Similar H values of species within a given functional group and significant differences of H among functional groups suggest that life

form and resource use strategy significantly influence stoichiometric homeostasis. It seems that many biotic and abiotic factors can affect stoichiometric homeostasis of vascular plants. More systematic research is needed to determine what factors can impact *H* and how *H* relates to other aspects of plant ecological strategy.

Acknowledgments We are grateful to the Inner Mongolia Grassland Ecosystem Research Station (IMGERS) for providing the experimental sites and elemental analysis. This work was supported by the Key Project of National Natural Science Foundation of China (NSFC, 30830026), the Innovative Research Group Project of NSFC (30821062), and the State Key Basic Research Development Program (2007CB106801). J. Elser acknowledges support from the National Science Foundation (DEB-0618193). We would like to thank R.W. Sterner, H. Heilmeier and two anonymous reviewers for their comments that greatly improved earlier versions of this manuscript. The main participants for the field experiment included Lishi Zhou, Fumei Lv, Hao Yang, Yanshu Liu, Deliang Kong, Xiaotao Lv, Qiang Li, Jianjun Chen, and Fuli Zhang; they are gratefully acknowledged.

References

Andersen T, Hessen DO (1991) Carbon, nitrogen, and phosphorus content of freshwater zooplankton. Limnol Oceanogr 36: 807–814

Bai Y, Han X, Wu J, Chen Z, Li L (2004) Ecosystem stability and compensatory effects in the inner Mongolia grassland. Nature 431:181–184

Bremner JM (1996) Nitrogen: total. In: Sparks DL et al (eds) Methods of soil analysis. Part 3. Chemical methods. Soil Science Society of America and American Society of Agronomy, Madison, pp 1085–1123

Chapin FS (1980) Nutrient allocation and responses to defoliation in tundra plants. Arct Alp Res 12:553–563

Chrzanowski TH, Kyle M (1996) Ratios of carbon, nitrogen and phosphorus in *Pseudomonas fluorescens* as a model for bacterial element ratios and nutrient regeneration. Aquat Microb Ecol 10:115–122

Demars BOL, Edwards AC (2007) Tissue nutrient concentrations in freshwater aquatic macrophytes: high inter-taxon differences and low phenotypic response to nutrient supply. Freshw Biol 52:2073–2086

DeMott WR, Pape BJ (2005) Stoichiometry in an ecological context: testing for links between *Daphnia* P-content, growth rate and habitat preference. Oecologia 142:20–27

Elrifi IR, Turpin DH (1985) Steady-state luxury consumption and the concept of optimum nutrient ratios: a study with phosphate and nitrate limited *Selenastrum minutum* (Chlorophyta). J Phycol 21:592–602

Elser JJ, Hamilton A (2007) Stoichiometry and the new biology—the future is now. Plos Biol 5:1403–1405

Elser JJ et al (2000a) Nutritional constraints in terrestrial and freshwater food webs. Nature 408:578–580

Elser JJ et al (2000b) Biological stoichiometry from genes to ecosystems. Ecol Lett 3:540–550

Elser JJ, Fagan WF, Kerkhoff AJ, Swenson NG, Enquist BJ (2010) Biological stoichiometry of plant production: metabolism, scaling and ecological response to global change. New Phytol 186:593–608

Frost PC, Evans-White MA, Finkel ZV, Jensen TC, Matzek V (2005) Are you what you eat? Physiological constraints on organismal stoichiometry in an elementally imbalanced world. Oikos 109:18–28

Goldman JC, Caron DA, Dennett MR (1987) Regulation of gross growth efficiency and ammonium regeneration in bacteria by substrate C:N ratio. Limnol Oceanogr 32:1239–1252

Güsewell S (2004) N:P ratios in terrestrial plants: variation and functional significance. New Phytol 164:243–266

Güsewell S (2005) Responses of wetland graminoids to the relative supply of nitrogen and phosphorus. Plant Ecol 176:35–55

Güsewell S, Bollens U (2003) Composition of plant species mixtures grown at various N:P ratios and levels of nutrient supply. Basic Appl Ecol 4:453–466

Hessen DO, Ågren GI, Anderson TR, Elser JJ, de Ruiter PC (2004) Carbon sequestration in ecosystems: the role of stoichiometry. Ecology 85:1179–1192

Hoagland R, Arnon DI (1950) The water culture method for growing plants without soil. Circular 347, California Agricultural Experiment Station, College of Agriculture. University of California, Berkeley

Jensen MH, Collins WL (1985) Hydroponic vegetable production. Hortic Rev 7:483–558

Jeyasingh PD, Weider LJ, Sterner RW (2009) Genetically-based trade-offs in response to stoichiometric food quality influence competition in a keystone aquatic herbivore. Ecol Lett 12: 1229–1237

Karimi R, Folt CL (2006) Beyond macronutrients: element variability and multielement stoichiometry in freshwater invertebrates. Ecol Lett 9:1273–1283

Kooijman S (1995) The stoichiometry of animal energetics. J Theor Biol 177:139–149

Kuo S (1996) Phosphorus. In: Sparks DL et al (eds) Methods of soil analysis. Part 3. Chemical methods. Soil Science Society of America and American Society of Agronomy, Madison, pp 869–920

Levi MP, Cowling EB (1969) Role of nitrogen in wood deterioration VII. Physiological adaptation of wood-destroying and other fungi to substrates deficient in nitrogen. Phytopathology 59:460–468

Limpens J, Berendse F (2003) Growth reduction of *Sphagnum magellanicum* subjected to high nitrogen deposition: the role of amino acid nitrogen concentration. Oecologia 135:339–345

Makino W, Cotner JB, Sterner RW, Elser JJ (2003) Are bacteria more like plants or animals? Growth rate and resource dependence of bacterial C:N:P stoichiometry. Funct Ecol 17:121–130

Nakano S (1994) Carbon:nitrogen:phosphorus ratios and nutrient regeneration of a heterotrophic flagellate fed on bacteria with different elemental ratios. Arch Hydrobiol 129:257–271

Persson J, Fink P, Goto A, Hood JM, Jonas J, Kato S (2010) To be or not to be what you eat: regulation of stoichiometric homeostasis among autotrophs and heterotrophs. Oikos doi:10.1111/j.1600-0706.2010.18545.x

Reiners WA (1986) Complementary models for ecosystems. Am Nat 127:59–73

Rhee GY (1978) Effects of N:P atomic ratios and nitrate limitation on algal growth, cell composition and nitrate uptake. Limnol Oceanogr 23:10–25

Ryser P, Lambers H (1995) Root and leaf attributes accounting for the performance of fast- and slow-growing grasses at different nutrient supply. Plant Soil 170:251–265

Schoenau JJ, Huang WZ (1991) Anion-exchange membrane, water, and sodium bicarbonate extractions as soil tests for phosphorus. Commun Soil Sci Plan 22:465–492

Shafik HM, Herodek S, Presing M, Voros L, Balogh KV (1997) Growth of *Cyclotella meneghiniana* Kutz. II. Growth and cell composition under different growth rates with different limiting nutrient. Ann Limnol Int J Limnol 33:223–233

Shaver GR, Melillo JM (1984) Nutrient budgets of marsh plants: efficiency concepts and relation to availability. Ecology 65: 1491–1510

Sterner RW, Elser JJ (2002) Ecological stoichiometry: the biology of elements from molecules to the biosphere. Princeton University Press, Princeton

Tezuka Y (1990) Bacterial regeneration of ammonium and phosphate as affected by the carbon:nitrogen:phosphorus ratio of organic substrates. Microb Ecol 19:227–238

Vanni MJ (2002) Nutrient cycling by animals in freshwater ecosystems. Annu Rev Ecol Evol Syst 33:341–370

Vogel S (1998) Academically correct biological science. Am Sci 86:504–506

Wang C, Wan S, Xing X, Zhang L, Han X (2006) Temperature and soil moisture interactively affected soil net N mineralization in temperate grassland in Northern China. Soil Biol Biochem 38:1101–1110

Yu Q et al (2010) Linking stoichiometric homeostasis with ecosystem structure, functioning, and stability. Ecol Lett 13:1390–1399