CrossMark

Responses of Non-structural Carbohydrates and C:N:P Stoichiometry of *Bothriochloa ischaemum* to Nitrogen Addition on the Loess Plateau, China

Ze-Min Ai[1,2] · Sha Xue[1,3] · Guo-Liang Wang[1,3] · Guo-Bin Liu[1,3]

Abstract *Bothriochloa ischaemum*: (L.) Keng is an excellent native pasture and forage species on the Loess Plateau, China, which is low in soil nitrogen. Little is known about the effects of N addition on biomass, concentrations of non-structural carbohydrates (NSCs), and the carbon:nitrogen:phosphorus (C:N:P) stoichiometry of *B. ischaemum* in this area. We examined these responses in both plant tissues and soil to N additions of 0 (N0), 2.5 (N1), 5.0 (N2), and 10.0 (N3) g N m^{-2} year^{-1}. Biomass was maximal with N additions between 2.5 and 5.0 g N m^{-2} year^{-1}. The increase in biomass was more rapid above- than belowground. N addition had no significant effect on the C:N:P ratios in the plant tissues, except for the aboveground biomass N:P ratio which increased significantly. The response to N addition of aboveground biomass and the N:P ratio suggests that N is not likely the limiting nutrient for the growth of *B. ischaemum* when biomass N:P ratios are between 25.5 and 27.1. In contrast to the plant tissues, the soil C:N and C:P ratios, but not the N:P ratio, decreased significantly with increasing N addition, indicating that N addition may help improve soil quality in this area. Both above- and belowground NSC concentrations varied significantly with N addition, with maxima of 102.4 g kg^{-1} in N1 and 49.6 g kg^{-1} in N2. The application of N could be a management option for improving ecosystem biomass production and NSC concentrations in the grasses on the Loess Plateau.

Keywords Plant · Biomass · Allocation · Concentration · Management · Fertiliser

Introduction

Carbohydrates are an important currency of energy for plant growth and metabolism (Mooney 1972), and the storage and distribution of carbohydrates in growth and survival have become foci of research (Dietze and others 2014). When the production of photosynthates exceeds the growth demand for carbon (C) compounds, carbohydrates will be stored in plant tissues and used later for growth and reproduction (Chapin and others 1990; Mooney 1972). Non-structural carbohydrates (NSCs) are the forms temporarily stored by plants (Kozlowski 1992) and are important substrates for growth and metabolism (Li and others 2002). The amount of stored NSCs generally reflects a plant's C supply (Chapin and others 1990) and the balance between C acquisition and expenditure (Poorter and Kitajima 2007). NSCs consequently play a central role in the response of plants to environmental stress (Chapin and others 1990; Kozlowski 1992). Stored NSCs have thus been proposed as a key determinant of drought resistance in plants (Rosas and others 2013).

The roles of NSCs in physiological, ecological, and evolutionary plant responses to biotic and abiotic stimuli have been challenging subjects in plant science (Zhang and others 2013). Soluble sugars and starch are commonly studied

✉ Guo-Bin Liu
  gbliu@ms.iswc.ac.cn

1 State Key Laboratory of Soil Erosion and Dryland Farming on the Loess Plateau, Institute of Soil and Water Conservation, Chinese Academy of Sciences and Ministry of Water Resources, Xinong Road 26#, Yangling 712100, Shaanxi, People's Republic of China

2 University of Chinese Academy of Sciences, Beijing 100101, People's Republic of China

3 Institute of Soil and Water Conservation, Northwest A & F University, Yangling 712100, Shaanxi, People's Republic of China

NSCs (Dietze and others 2014). Soluble sugars are products of photosynthesis and are used to meet current plant requirements and osmotic regulation, and starch is the main form for storage and is used to meet future plant needs (Dietze and others 2014; von Arx and others 2012). Evidence suggests that plants use sugars and starch to support reproductive activity (Chapin and others 1990), but some of the synthesised carbohydrates can transiently be stored as starch, and soluble sugars may serve as osmolytes during water stress (Kozlowski 1992).

Ecological stoichiometry, the balance of multiple elements required for metabolic processes and interactions, provides new approaches for studying ecosystem processes at various levels, from foliar physiology to ecosystem productivity (Hessen and others 2004). Environmental and organismic carbon:nitrogen:phosphorus (C:N:P) stoichiometries represent the relationships between organisms and ecosystem structure and function (Elser and others 2000a). The study of ecological stoichiometry has recently focused on documenting the C:N:P stoichiometry of plants and its driving factors (Güsewell 2004). The C:N:P ratio of plants can be associated with important ecological processes such as litter decomposition (Güsewell and Gessner 2009), N_2 fixation (Sañudo-Wilhelmy and others 2001), plant–herbivore–predator relationships (Ngai and Jefferies 2004), ecosystem-specific composition and diversity (Olde Venterink and others 2003), and the ability of organisms to adapt to environmental stresses (Woods and others 2003). Our knowledge of stoichiometric flexibility is thus crucial for understanding C–N–P interactions, ecosystem dynamics, and future biogeochemical cycles under changing terrestrial ecosystems (Sistla and Schimel 2012).

N is one of the most commonly limiting nutrients in terrestrial plants (Vitousek and Howarth 1991) and is critical for the production of biomass and typically the most limiting factor to plant productivity (Lemus and others 2008). Increased N input generally has a large potential to affect photosynthetic rates (Granath and others 2009), growth (Xia and Wan 2008), and ultimately higher primary productivity (Bai and others 2010) in most ecosystems. Previous studies have demonstrated that experimental nutrient addition is a classic approach to identifying the nutrients that limit plant growth and the relative degrees of nutrient limitation of a study area (Vitousek and Howarth 1991). Experimental N addition is thus widely used as a strategy of agricultural management to alleviate ecosystem N limitation, which will potentially influence the stoichiometric composition of plants (Esmeijer-Liu and others 2009) and increase growth and productivity (Bai and others 2010). Much of our present understanding of the N limitation of plant biomass production in grassland ecosystems has been determined from experiments of N addition (LeBauer and Treseder 2008). Nutrient limitations simultaneously drive

stoichiometric and metabolic constraints on carbohydrate use (Chapin and others 1990; Millard and others 2007). The storage of carbohydrates is also mediated by nutrient availability, with a larger allocation to storage when growth is limited by nutrients (Knox and Clarke 2005). Whether this response is simply stoichiometric, however, is unclear; an active stress response would also promote carbohydrate storage over growth (Kobe and others 2010).

The deposition of atmospheric N has had a large impact on the grassland ecosystem of the Loess Plateau in China (Liu and others 2011). The current rate of N deposition in this area is 2.2 g N m^{-2} year^{-1} (Wei and others 2010) and will likely increase in the future (Galloway and others 2004). Soil fertility, especially N levels, however, is low (Zhu and others 1983). N may thus be one of the most limiting nutrient elements in this area (Vitousek and Howarth 1991; Yang and others 2011a). *Bothriochloa ischaemum* (L.) Keng, a C_4 perennial grass distributed mainly in warm temperate zones, propagates rapidly, is drought tolerant, and has a strong ability to regenerate. *B. ischaemum* is an excellent native pasture and forage species on the Loess Plateau due to its ability to adapt to the local conditions and to the palatability and forage quality of its leaves (Xu and others 2011). Little is known, however, about the effect of N addition on biomass, NSC concentrations, and on *B. ischaemum* tissues and soil C:N:P stoichiometry of in the hilly-gully region of the plateau.

We experimentally added N at the rates of 0, 2.5, 5.0, and 10.0 g N m^{-2} year^{-1} in soil tanks to test the effects of N addition on the biomass and NSC concentrations of *B. ischaemum* and on the plant and soil C:N:P stoichiometries. We hypothesised that N addition would (1) significantly increase both above- and belowground plant biomass, but a greater increase in aboveground biomass would result in a decreased ratio of below- to aboveground biomass, (2) result in lower C:N ratios in both plant biomass and soil and higher N:P ratios in each, and (3) have a negative impact on plant NSC concentrations along the N addition gradient and significantly reduce the ratio of soluble sugar to starch concentrations.

Materials and Methods

Plant Materials

Seeds of *B. ischaemum* were acquired in autumn 2012 from the experimental fields at the Ansai Research Station (ARS) of the Chinese Academy of Sciences (36°51′30″N, 109°19′23″E, 1068–1309 m a.s.l.), which is in the centre of the semiarid, hilly-gully region of the Loess Plateau. The seeds were dried in direct sunlight and then stored in sealed plastic bags at a laboratory in Yangling, Shaanxi Province,

under natural dry conditions. The rates of seed germination on moist filter paper in Petri dishes at 25 °C were greater than 90% before the experiment (Xu and others 2011).

Growth Conditions

The experiment was conducted at the State Key Laboratory of Soil Erosion and Dryland Farming on the Loess Plateau in Yangling (34°12′N, 108°7′E, 530 m a.s.l.). The mean annual temperature is 12.9 °C, and the mean annual precipitation is 637.6 mm. The loessial soil used in our experiment was obtained from the upper 20 cm of an arable field at ARS. The soil had a bulk density of 1.2 g cm^{-3}, organic matter concentration of 1.3 g kg^{-1}, and total N and P concentrations of 0.19 and 0.27 g kg^{-1}, respectively. The soil was air-dried and passed through a 2-mm mesh. Soil gravimetric water contents at field capacity and wilting point were 20.0 and 4.0%, respectively.

Experimental Design

The experiment began in June 2013. Soil was added to soil tanks (200.0×100.0×50.0 cm in length×width×height) in 10-cm layers to a depth of 40 cm (Wu and others 2008). The soil was well watered before sowing to ensure seedling establishment. Seeds of *B. ischaemum* were sown into holes on slopes with gradients of 15° (Chen and others 2007) at a density of 10×10 cm. The soil moisture content was maintained at greater than 80% of field capacity during seedling establishment. Excess grass plants were manually removed to restrict plants to one per hole of the same size. Three replicates of four treatments received additional N in the form of urea at the rates of 0 (N0), 2.5 (N1), 5.0 (N2), and 10.0 (N3) g N m^{-2} year^{-1}. The N was applied in August 2013, and May, June, July, and August of 2014, as a solution of urea in 1 l of deionised water (the amount of N in a year was one time application in August 2013, and equivalent to the amount of N in a year divided into four applications in 2014; N0 received the same volume of water).

Sampling

All aboveground plant tissues were harvested at the end of August 2014 at the soil surface using scissors. Belowground tissues and soil samples (0–20 cm soil layer, with a soil-core sampler 2.5 cm in diameter) were collected from six 20 × 20 cm quadrats in each soil tank after clipping the aboveground tissues, then the belowground tissues and soil samples were combined to form one sample for each soil tank, respectively. The belowground plant samples were washed gently with water over a 60-mesh screen until the roots were separated from the soil. All above- and belowground plant samples were dried at 65 °C to constant weight, weighed, ground through a 1-mm sieve, and stored for chemical analysis. Each soil sample was air-dried after removing gravel, and coarse fragments were homogenised and sieved to 0.25 mm prior to analysis. The total C, N, and P concentrations in both plants and soils were then analysed.

The plant samples for NSC analysis were immediately frozen in liquid N, heated in a microwave oven (40 s at 600 W), dried to a constant weight at 65 °C (Li and others 2008), and then stored until chemical analysis. The concentrations of the soluble sugars and starch were determined using an anthrone reagent.

Chemical Analyses: Soluble Sugars

Approximately 0.1 g of dried sample was put into 10-ml centrifuge tubes, and 5 ml of 80% ethanol was added. The centrifuge tubes were incubated in a water bath at 80 °C for 30 min and then centrifuged at 5000×g for 5 min. The supernatant was removed, the residue was extracted two more times as above, and the residue was retained for starch analysis. The supernatants were analysed for soluble sugars using the anthrone method (Seifter and others 1950). The anthrone reagent (2 g anthrone in 100 ml 95% H_2SO_4) was added to the supernatants and incubated in a boiling water bath for 15 min. After cooling, the soluble sugar concentration was determined spectrophotometrically (Shimadzu, Japan) at 620 nm. The sugar concentration was determined by comparison with glucose standards on a dry matter basis.

Chemical Analyses: Starch

The residues were resuspended in 2 ml of distilled water, and the mixtures were incubated in a water bath at 100 °C for 15 min to evaporate the ethanol. The samples were cooled to room temperature, 2 ml of 9.2 mol l^{-1} $HClO_4$ was added, and then 4 ml of distilled water was added 15 min later. The samples were centrifuged at 5000×g for 5 min and extracted once more as above. The supernatants were analysed for starch spectrophotometrically at 620 nm. The starch concentration was determined by comparison with glucose standards using a conversion factor of 0.9 (Osaki and others 1991) on a dry matter basis.

Chemical Analyses: Total C, N, and P Concentrations

Plant total C and soil organic matter concentrations were determined by the H_2SO_4–$K_2Cr_2O_7$ oxidation method. Plant and soil total N concentrations were measured using the Kjeldahl method (Bremner and Mulvaney 1982). Plant total P concentration was measured by persulfate oxidation followed by colorimetric analysis, and soil total P

concentration was determined colorimetrically after digestion with H_2SO_4 and $HClO_4$ (Schade and others 2003).

Data Analysis

C:N:P ratios in plant biomass and soil were expressed as molar ratios. NSC concentrations were calculated as the sum of the starch and total soluble sugars for each sample (Li and others 2002). The data from all treatments were analysed by a one-way analysis of variance (ANOVA) followed by a least significant difference test. A correlation analysis was based on Pearson's correlation coefficients ($P < 0.05$). All ANOVA and correlation analyses were tested for significance at $P < 0.05$ using SPSS 18.0 (SPSS Inc., Chicago, USA) and structural equation models were analysed using AMOS package (expansion pack of SPSS), and graphs were plotted using SigmaPlot 12.5.

Results

Above- and Belowground Biomasses, Nutrient Concentrations, and Stoichiometries

The above- and belowground and total biomasses of *B. ischaemum* responded significantly to N additions. The biomasses all significantly increased with increasing N additions and reached the maximum value at N2, when compared with N0, and N2 biomasses were significantly increased by 240, 146, and 209%, respectively. The ratio of below- to aboveground biomass in the N1 treatment was similar to that of N0, and later again in the N2 and N3 treatments the ratios were significantly lower with increasing N additions, because the increase of aboveground biomass was more rapid than that of belowground biomass (Fig. 1).

There were no significant differences in biomass C, N, and P concentrations among treatments. Similarly, biomass C:N and C:P ratios did not respond to N additions (Fig. 2a–e). The N:P ratio of aboveground biomass, but not belowground, responded significantly to N additions and averaged 24.4 over all treatments. The aboveground biomass N:P ratio increased with increasing N addition, but only in the N3 treatment the biomass N:P ratio was significantly larger than that in the control, by 22% (Fig. 2f).

Soil Nutrient Concentrations and Stoichiometries

Soil C concentration increased significantly at the lowest N addition but decreased significantly at the higher treatment levels, soil N and P concentrations significantly increased, whereas C:N and C:P ratios significantly decreased with increasing N additions (Fig. 3a, b).
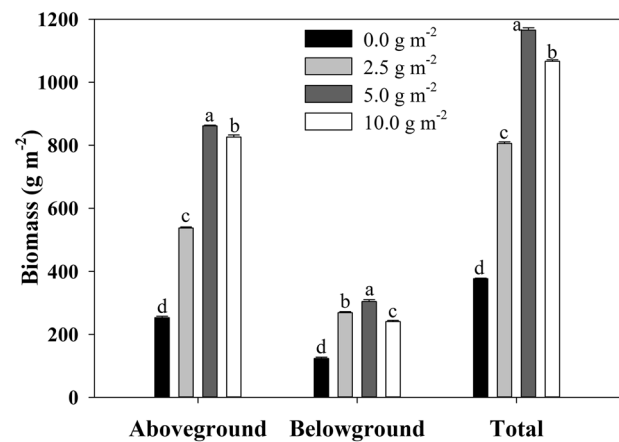

Fig. 1 Effects of N addition on plant biomass of *B. ischaemum*. *Error bars* are SE ($n = 3$). *Different letters* above bars indicate significant difference at $P = 0.05$

Above- and Belowground NSC Concentrations

The soluble sugar, starch, and NSC concentrations of belowground tissues and starch and NSC concentrations of aboveground tissues varied significantly with N additions across treatments, but the aboveground soluble sugar concentrations did not. An overall trend indicated that the starch and NSC concentrations above- and belowground and the belowground soluble sugar concentrations increased significantly at the lowest N addition but decreased significantly at the higher treatment levels (Fig. 4a–c). The NSC concentrations aboveground reached the maxima of 102.4 g kg^{-1} in N1, when it was 49.6 g kg^{-1} in N2 belowground (Fig. 4c). The ratio of aboveground soluble sugar to starch concentrations did not change significantly with increasing amounts of added N (Fig. 4a, b). But the belowground ratio of soluble sugar to starch concentrations was significantly increased at the lowest N addition but decreased significantly at the higher treatment levels, because the increase in concentrations was more rapid for belowground soluble sugars than for starch at low amounts of added N, but the increase was slower at higher amounts of added N (Fig. 4a, b).

Discussion

Effects of N Addition on Plant Biomass and C, N, and P Concentrations

As we hypothesised, N addition had significantly increased the total biomass of *B. ischaemum*. The response of biomass to N addition was significantly greater in above- (111.9–240.1%) than belowground (94.8–146.2%) tissues,
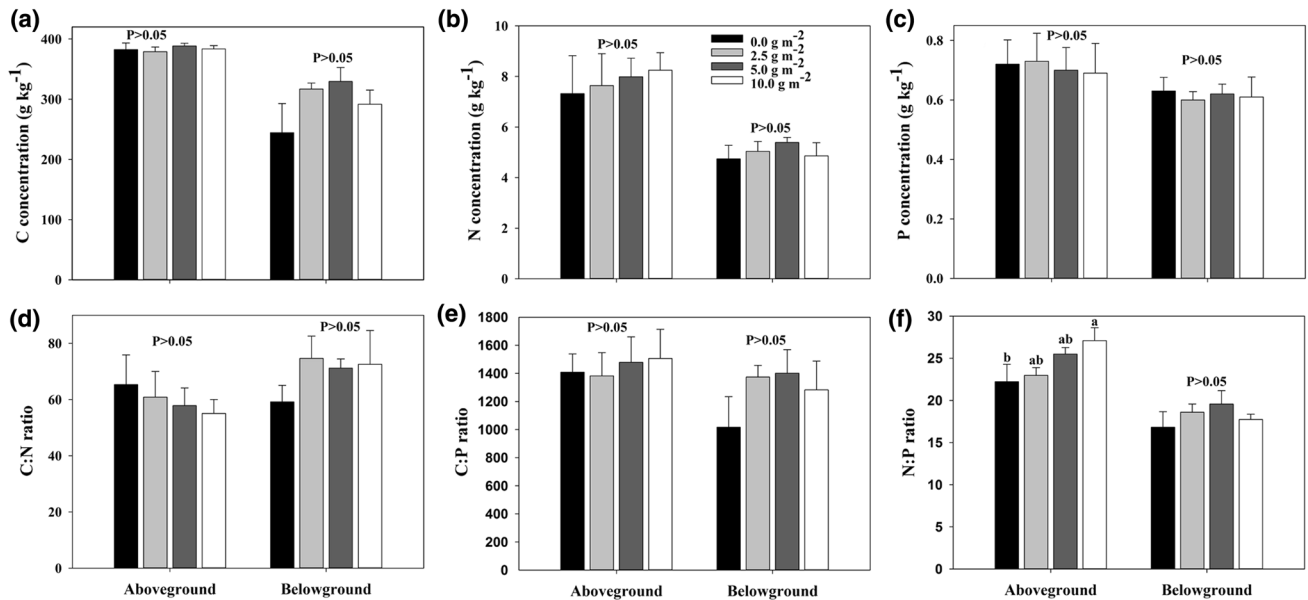
Fig. 2 Effects of N addition on plant biomass C, N, and P concentrations and their stoichiometric ratios of C:N, C:P, and N:P of *B. ischaemum*. *Error bars* are SE (*n*=3). *Different letters* above bars indicate significant difference at P=0.05

Fig. 3 Effects of N addition on soil C, N, and P concentrations and their stoichiometric ratios of C:N, C:P, and N:P of *B. ischaemum*. *Error bars* are SE (*n*=3). *Different letters* above bars indicate significant difference at *P*=0.05
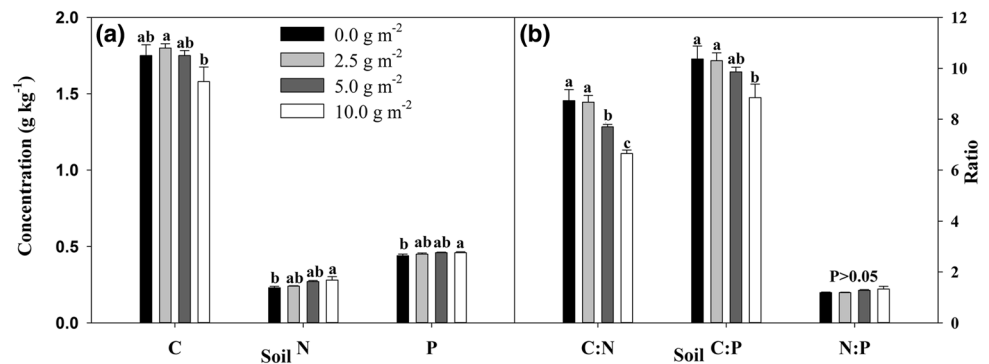
so the ratio of below- to aboveground biomass decreased significantly with increasing N addition (Fig. 1). This result was consistent with that of a previous study (Li and others 2011) and our hypothesis. The application of fertiliser replenishes soil nutrients, so fertilised plants are not subject to nutrient stress, but plants can be subject to light stress. Plants may enhance the ability to absorb light by increasing the distribution of photosynthetic products to the crown, thereby reducing light stress, so that plants could grow faster.

The significant increase in *B. ischaemum* biomass with N addition demonstrated that N was a limiting factor to growth on the Loess Plateau in China. This phenomenon was also demonstrated by a meta-analysis (Xia and Wan 2008) and previous studies by grassland studies in other regions (Bai and others 2010; Clark and Tilman 2008; Han and others 2014) The total biomass of *B. ischaemum* in

N3 was significantly lower than that in N2. The addition of large amounts of N may produce an imbalance between plant and soil nutrient elements (Lieb and others 2011) and may acidify the soil and remove basic ions (Wei and others 2013). These effects would be detrimental to plants. High amounts of added N may also lead to limitations of other elements (for example, P), which are also not conducive to plant growth (Zhang and others 2013). The allocation of resources to belowground biomass increases with N deficiency, consistent with our finding that the ratio of below- to aboveground biomass decreased significantly with increasing amounts of added N.

Effects of N Addition on Plant Biomass C:N:P Ratios

N addition in *B. ischaemum* led to a lower aboveground biomass C:N ratio in our study, consistent with
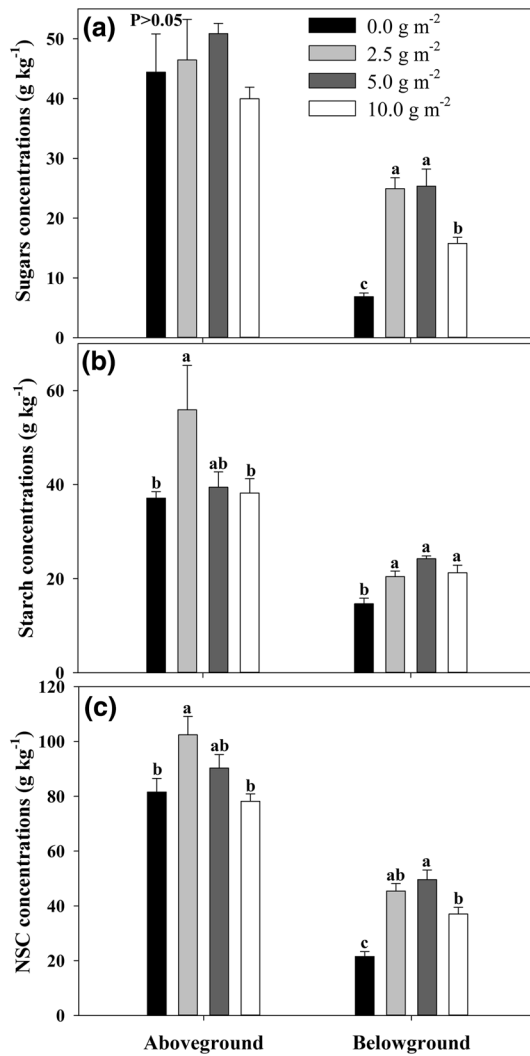
Fig. 4 Effects of N addition on plant soluble sugar, starch, and NSC concentrations of *B. ischaemum*. *Error bars* are SE (*n* = 3). *Different letters* above bars indicate significant difference at *P* = 0.05

previous studies (Yang and others 2011b), which support our hypothesis that N addition would result in lower aboveground biomass C:N ratio.

The plant biomass N:P ratio has been suggested as a simple index of the limitation of N and P (Güsewell 2004). The relationships between biomass and the N:P ratio are dependent on plant species or region (Güsewell 2004). In our study, the aboveground biomass was positively correlated (*R* = 0.631, *P* < 0.05) with the aboveground biomass N:P ratio. The aboveground biomass N:P ratios were all above 22, with an average of 24.4 ± 2.9 (Fig. 2f), lower than the mean ratio of 33.3 on the Loess Plateau in China (Zheng and Shangguan 2007), and a little more than the mean ratio of 22.8 in the terrestrial plant species (Elser and others 2000b), all of which were much higher than the ratios of 5–9 in vegetation in general (Güsewell 2004). The

aboveground biomass N:P ratio in our study increased significantly with N addition, in accordance with our hypothesis, and similar to the ratios observed by other studies in Chinese grasslands (Han and others 2014; Lü and Han 2010). Our results were insufficient to propose a new use of plant biomass N:P ratios to identify nutrient limitation in the grasslands of the Loess Plateau. The applicability of the value of N:P stoichiometry for predictions of nutrient limitation or as a management tool for planning fertilisation, however, remains debatable.

Effects of N Addition on Soil C:N:P Ratios

The soil C:N (6.7–8.7), C:P (8.9–10.4), and N:P (1.2–1.3) ratios were lower than the previously reported (Liu and others 2013) ratios of 6.9–14.9, 12.1–59.7, and 1.5–4.2, respectively, and of 10.7, 38, and 3.6, respectively, in China (Tian and others 2010).

The C:N ratio decreased significantly with increasing amounts of added N, which was consistent with our hypothesis and results, suggesting that N deposition may not greatly contribute to additional C sequestration because of the lower N use ratio (Yang and others 2011b). Previous studies have indicated that a decrease in the soil C:N ratio may be conducive to microbial decomposition (Norby 1998).

Soil C:P ratios are often used as indicators of the decomposition and accumulation of organic matter, and the enrichment and the effective amount of P can be used as an index of soil fertility within a certain range and can also affect the accumulation and distribution of nutrients in plants (Méndez and Karlsson 2005). The soil C:P ratio in our study decreased significantly with increasing amounts of added N when the P concentration significantly increased, indicating that N addition would increase the availability of soil P (Perring and others 2008). The N:P ratio did not change significantly in our study, inconsistent with our hypothesis.

Effect of N Addition on Plant NSC Concentrations

We hypothesised that N addition would significantly influence plant NSC concentrations, which was well supported by our findings. We found a significant increase in NSC concentrations in *B. ischaemum* at the lowest level of N additions (2.5 g m^{-2}) but at higher levels of N additions NSC concentrations were indistinguishable from those in the control treatment. This finding was consistent with previous findings that low amounts of added N would contribute to the accumulation of soluble carbohydrates, thereby increasing their concentration, but high amounts of added N could significantly reduce concentrations (White 1973). The pattern of increased NSC concentrations only in the

lowest N addition treatment was found in both the above- and belowground biomass, and in belowground biomass the NSC concentrations increased with all N additions to statistically the same amount. Structural equation modelling showed that N addition had a significant impact on soil P concentration ($P = 0.013$), while soil P concentration had the significant positive effect on both the belowground biomass starch ($P = 0.008$) and NSC concentrations ($P = 0.046$), so N addition had the strong positive effect on the belowground biomass NSC concentrations (Fig. 5). The appropriate amount of N application would likely improve growth, which would increase photosynthetic activity and theoretically increase carbohydrate reserves (White 1973). High N addition would stimulate the synthesis of amino acids and amide compounds to the detriment of carbohydrate reserves when N was excessive (White 1973).

Higher amounts of NSC concentrations thus accumulate to balance the osmotic pressure of cells (Millard and others 2007) and can be used by plants to cope with stress. Stored C would be used under conditions of C deficits until reserves are exhausted (Poorter and Kitajima 2007). Low levels of N additions could help perennial plants such as *B. ischaemum* to maintain a sufficiently high ratio of soluble sugar to starch concentrations in the belowground to overwinter successfully (White 1973) and increase its drought resistance (Li and others 2013). The result was consistent with our hypothesis that N addition would significantly reduce the ratio of soluble sugar to starch concentrations along the N addition gradient. Adding the proper amount of N would thus favour the accumulation of NSCs and improve the resistance of *B. ischaemum* to stresses on the Loess Plateau. These pools of NSCs would increase the

plant's potential resistance to drought stress (von Arx and others 2012).

Conclusions

This study demonstrated that N addition has significant effects on the biomass, NSC concentrations, and aboveground N:P ratio in *B. ischaemum* in the grassland of the Loess Plateau in China. Our observations indicated that the grassland was N-limited and improved our understanding of nutrient limitation in a semiarid grassland ecosystem. These findings will help us understand the effects of different management strategies on nutrient cycling in this ecosystem. Our results for the responses of biomass and NSC concentrations suggest that the application of N could be a management option for improving ecosystem biomass production, C:N:P stoichiometry, and NSC concentrations of the grass on the plateau. The amount of fertilisation, however, should be considered with caution due to the possible negative effects, such as loss of species diversity or large changes in species composition (Yang and others 2011a), or a decline in soil quality (Li and others 2010). Further study is required to look into the possibility that other nutrients are also limiting the productivity.

Fig. 5 Results of structural equation models of N addition on plant NSC concentrations. The model fits the data well: $x^2 = 3.461$, $P = 0.177$. Numbers at *arrows* are standardised path coefficients (equivalent to correlation coefficients). *Circles* indicate error terms (e1–e3). Percentages close to endogenous variables indicate the variance explained by the model

References

Bai YF, Wu J, Clark CM, Naeem S, Pan Q, Huang J, Zhang L, Han X (2010) Tradeoffs and thresholds in the effects of nitrogen addition on biodiversity and ecosystem functioning: evidence from inner Mongolia Grasslands. Glob Chang Biol 16:358–372

Bremner J, Mulvaney C (1982) Nitrogen—total. Methods of soil analysis Part 2 Chemical and microbiological properties. American Society of Agronomy, Madison, pp. 595–624

Chapin FS, Schulze E-D, Mooney HA (1990) The ecology and economics of storage in plants. Annu Rev Ecol Syst 21:423–447

Chen LD, Gong J, Fu BJ, Huang ZL, Huang YL, Gui LD (2007) Effect of land use conversion on soil organic carbon sequestration in the loess hilly area, loess plateau of China. Ecol Res 22:641–648

Clark CM, Tilman D (2008) Loss of plant species after chronic low-level nitrogen deposition to prairie grasslands. Nature 451:712–715

Dietze MC, Sala A, Carbone MS, Czimczik CI, Mantooth JA, Richardson AD, Vargas R (2014) Nonstructural carbon in woody plants. Annu Rev Plant Biol 65:667–687

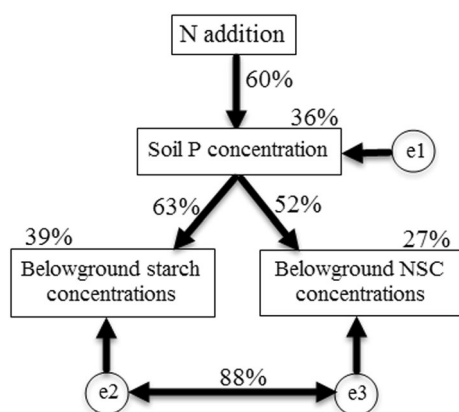Elser J, Sterner RW, Gorokhova E, Fagan WF, Markow TA, Cotner JB, Harrison JF, Hobbie SE, Odell GM, Weider LW (2000a)

Biological stoichiometry from genes to ecosystems. Ecol Lett 3:540–550

Elser JJ, Fagan WF, Denno RF, Dobberfuhl DR, Folarin A, Huberty A, Interlandi S, Kilham SS, McCauley E, Schulz KL, Siemann EH (2000b) Nutritional constraints in terrestrial and freshwater food webs. Nature 408:578–580

Esmeijer-Liu A, Aerts R, Kürschner W, Bobbink R, Lotter A, Verhoeven J (2009) Nitrogen enrichment lowers Betula pendula green and yellow leaf stoichiometry irrespective of effects of elevated carbon dioxide. Plant Soil 316:311–322

Galloway JN, Dentener FJ, Capone DG, Boyer EW, Howarth RW, Seitzinger SP, Asner GP, Cleveland CC, Green PA, Holland EA, Karl DM (2004) Nitrogen cycles: past, present, and future. Biogeochemistry 70:153–226

Granath G, Strengbom J, Breeuwer A, Heijmans MM, Berendse F, Rydin H (2009) Photosynthetic performance in Sphagnum transplanted along a latitudinal nitrogen deposition gradient. Oecologia 159:705–715

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

Güsewell S, Gessner MO (2009) N: P ratios influence litter decomposition and colonization by fungi and bacteria in microcosms. Funct Ecol 23:211–219

Han X, Sistla SA, Zhang YH, Lü XT, Han XG (2014) Hierarchical responses of plant stoichiometry to nitrogen deposition and mowing in a temperate steppe. Plant Soil 382:175–187

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

Knox K, Clarke P (2005) Nutrient availability induces contrasting allocation and starch formation in resprouting and obligate seeding shrubs. Funct Ecol 19:690–698

Kobe RK, Iyer M, Walters MB (2010) Optimal partitioning theory revisited: nonstructural carbohydrates dominate root mass responses to nitrogen. Ecology 91:166–179

Kozlowski T (1992) Carbohydrate sources and sinks in woody plants. Bot Rev 58:107–222

LeBauer DS, Treseder KK (2008) Nitrogen limitation of net primary productivity in terrestrial ecosystems is globally distributed. Ecology 89:371–379

Lemus R, Parrish DJ, Abaye O (2008) Nitrogen-use dynamics in switchgrass grown for biomass. Bioenerg Res 1:153–162

Li M, Hoch G, Körner C (2002) Source/sink removal affects mobile carbohydrates in Pinus cembra at the Swiss treeline. Trees 16:331–337

Li M-H, Xiao WF, Wang SG, Cheng GW, Cherubini P, Cai XH, Liu XL, Wang XD, Zhu WZ (2008) Mobile carbohydrates in Himalayan treeline trees I. Evidence for carbon gain limitation but not for growth limitation. Tree Physiol 28:1287–1296

Li XY, Han SJ, Guo ZL, Shao DK, Xin LH (2010) Changes in soil microbial biomass carbon and enzyme activities under elevated CO_2 affect fine root decomposition processes in a Mongolian oak ecosystem. Soil Biol Biochem 42:1101–1107

Li LJ, Zeng DH, Yu ZY, Fan ZP, Mao R, Peri PL (2011) Foliar N/P ratio and nutrient limitation to vegetation growth on Keerqin sandy grassland of North-east China. Grass and Forage. Science 66:237–242

Li MH, Cherubini P, Dobbertin M, Arend M, Xiao WF, Rigling A (2013) Responses of leaf nitrogen and mobile carbohydrates in different Quercus species/provenances to moderate climate changes. Plant Biol 15:177–184

Lieb AM, Darrouzet-Nardi A, Bowman WD (2011) Nitrogen deposition decreases acid buffering capacity of alpine soils in the southern Rocky Mountains. Geoderma 164:220–224

Liu XJ, Duan L, Mo J, Du E, Shen J, Lu X, Zhang Y, Zhou X, He C, Zhang F (2011) Nitrogen deposition and its ecological impact in China: an overview. Environ Pollut 159:2251–2264

Liu JX, Huang WJ, Zhou GY, Zhang DQ, Liu SZ, Li YY (2013) Nitrogen to phosphorus ratios of tree species in response to elevated carbon dioxide and nitrogen addition in subtropical forests. Global Chang Biol 19:208–216

Lü XT, Han XG (2010) Nutrient resorption responses to water and nitrogen amendment in semi-arid grassland of Inner Mongolia, China. Plant Soil 327:481–491

Méndez M, Karlsson PS (2005) Nutrient stoichiometry in Pinguicula vulgaris: nutrient availability, plant size, and reproductive status. Ecology 86:982–991

Millard P, Sommerkorn M, Grelet GA (2007) Environmental change and carbon limitation in trees: a biochemical, ecophysiological and ecosystem appraisal. New Phytol 175:11–28

Mooney H (1972) The carbon balance of plants. Annu Rev Ecol Syst 3:315–346

Ngai JT, Jefferies RL (2004) Nutrient limitation of plant growth and forage quality in Arctic coastal marshes. J Ecol 92:1001–1010

Norby RJ (1998) Nitrogen deposition: a component of global change analyses. New Phytol 139:189–200

Olde Venterink H, Wassen M, Verkroost A, De Ruiter P (2003) Species richness-productivity patterns differ between N-, P-, and K-limited wetlands. Ecology 84:2191–2199

Osaki M, Shinano T, Tadano T (1991) Redistribution of carbon and nitrogen compounds from the shoot to the harvesting organs during maturation in field crops. Soil Science and Plant. Nutrition 37:117–128

Perring MP, Hedin LO, Levin SA, McGroddy M, de Mazancourt C (2008) Increased plant growth from nitrogen addition should conserve phosphorus in terrestrial ecosystems. Proc Natl Acad Sci 105:1971–1976

Poorter L, Kitajima K (2007) Carbohydrate storage and light requirements of tropical moist and dry forest tree species. Ecology 88:1000–1011

Rosas T, Galiano L, Ogaya R, Peñuelas J, Martínez-Vilalta J (2013) Dynamics of non-structural carbohydrates in three Mediterranean woody species following long-term experimental drought. Front Plant Sci 4:1–16

Sañudo-Wilhelmy SA, Kustka AB, Gobler CJ, Hutchins DA, Yang M, Lwiza K, Burns J, Capone DG, Raven JA, Carpenter EJ (2001) Phosphorus limitation of nitrogen fixation by Trichodesmium in the central Atlantic Ocean. Nature 411:66–69

Schade JD, Kyle M, Hobbie S, Fagan W, Elser J (2003) Stoichiometric tracking of soil nutrients by a desert insect herbivore. Ecol Lett 6:96–101

Seifter S, Dayton S, Novic B, Muntwyler E (1950) The estimation of glycogen with the anthrone reagent. Arch Biochem 25:191–200

Sistla SA, Schimel JP (2012) Stoichiometric flexibility as a regulator of carbon and nutrient cycling in terrestrial ecosystems under change. New Phytol 196:68–78

Tian HQ, Chen GS, Zhang C, Melillo JM, Hall CA (2010) Pattern and variation of C: N: P ratios in China's soils: a synthesis of observational data. Biogeochemistry 98:139–151

Vitousek PM, Howarth RW (1991) Nitrogen limitation on land and in the sea: how can it occur? Biogeochemistry 13:87–115

von Arx G, Archer SR, Hughes MK (2012) Long-term functional plasticity in plant hydraulic architecture in response to supplemental moisture. Ann Bot (Lond) 109:1091–1100

Wei Y, Tong YA, Qiao L, Liu XJ, Duan M, Li J (2010) Preliminary estimate of the atmospheric nitrogen deposition in different ecological regions of Shaanxi Province. J Agro-Environ Sci 29:795–800

Wei CZ, Yu Q, Bai E, Lü X, Li Q, Xia J, Kardol P, Liang W, Wang Z, Han X (2013) Nitrogen deposition weakens plant–microbe

interactions in grassland ecosystems. Glob Chang Biol 19:3688–3697

White LM (1973) Carbohydrate reserves of grasses: a review. J Range Manag 26:13–18

Woods H, Makino W, Cotner J, Hobbie S, Harrison J, Acharya K, Elser J (2003) Temperature and the chemical composition of poikilothermic organisms. Funct Ecol 17:237–245

Wu CH, Chen YM, Wang GL, Lu JL (2008) Vertical distribution characters of typical communities root system in relation to environmental factors in the Hilly-gully region of Loess Plateau, China. Chin Sci Soil Water Conserv 6:65–70

Xia JY, Wan SQ (2008) Global response patterns of terrestrial plant species to nitrogen addition. New Phytol 179:428–439

Xu BC, Xu WZ, Huang J, Shan L, Li FM (2011) Biomass production and relative competitiveness of a C_3 legume and a C_4 grass co-dominant in the semiarid Loess Plateau of China. Plant soil 347:25–39

Yang HJ, Li Y, Wu MY, Zhang Z, Li LH, Wan SQ (2011a) Plant community responses to nitrogen addition and increased precipitation: the importance of water availability and species traits. Glob Chang Biol 17:2936–2944

Yang YH, Luo YQ, Lu M, Schädel C, Han WX (2011b) Terrestrial C:N stoichiometry in response to elevated CO_2 and N addition: a synthesis of two meta-analyses. Plant Soil 343:393–400

Zhang NY, Guo R, Song P, Guo JX, Gao YZ (2013) Effects of warming and nitrogen deposition on the coupling mechanism between soil nitrogen and phosphorus in Songnen Meadow Steppe, northeastern China. Soil Biol Biochem 65:96–104

Zheng S, Shangguan Z (2007) Spatial patterns of leaf nutrient traits of the plants in the Loess Plateau of China. Trees 21:357–370

Zhu XM, Li YS, Peng XL, Zhang SG (1983) Soils of the loess region in China. Geoderma 29:237–255