

FERTILIZER ADDITION INDUCES A CASCADE OF PLANT COMMUNITY RESPONSES IN A MEADOW

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Abstract. Fertilizer addition can improve soil nutrients in degraded grasslands, change plant community composition, and promote community productivity. An experiment was established in 2014 near Hulunbuir Grassland Ecosystem National Field Observation Station at Hulunbuir in Inner Mongolia, China, using L14 (3⁴) orthogonal test with 14 treatments totally, to compare the change of plant community productivity and plant diversity during three years of fertilization. We monitored the dynamic responses of aboveground biomass, plant species composition, species richness, Shannon diversity, Pielou's evenness after nitrogen (N), phosphorus (P), and potassium (K) addition once a year from 2014 to 2016. Compared to the control treatment of no additional fertilizer, we found that, (1) The fertilization combination of N (274 kg ha⁻¹), P (350 kg ha⁻¹), and K (57 kg ha⁻¹) had a greater effect on biomass after the third year of fertilizer addition; (2) Gramineae accounted for a major portion of biomass in 2016, whereas the proportion of other species declined or disappeared; and (3) the longer the fertilization treatments went on, the lower Shannon diversity and Pielou's evenness turned out to be. All these responses are strongly interrelated in a cascade of changes. The interrelationships between fertilizers and different plant communities attribute the change not only to climate shift but also to time.

Keywords: *nitrogen fertilizer, phosphorus fertilizer, potassium fertilizer, community biomass, species diversity*

Introduction

Arid and semi-arid lands cover nearly one-third of the terrestrial land surface and may be highly sensitive to anthropogenic nutrient inputs (Zhou et al., 2018). Nitrogen (N), phosphorus (P), and potassium (K) are macro nutrient elements constraining plant productivity in many grassland ecosystems. In recent decades, due to the high intensity of human activities, including the expansion of agriculture and fertilizer application, the content of N in the soil is changing on varying degrees (Yahdjian et al., 2011). N is

considered to be the second most limiting factor for plant growth in arid lands globally after water. Unlike most other nutrients, which generally are derived from the breakdown of rocks, nitrogen is derived from the atmosphere. Plants that form symbioses with nitrogen-fixing bacteria, which can acquire nitrogen from its vast atmospheric reservoir, are widespread in natural grasslands and well-managed pastures. The effects of N addition are altering plant community structure including plant productivity and biomass allocation (Gough et al., 2000; Plassmann et al., 2009; Han et al., 2011). Additionally, the nutrients such as phosphorus, potassium, and micronutrients constrain the activity of biological nitrogen fixers (Vitousek, 2015). Simultaneously, nitrogen-phosphorus co-limitation could reflect a nitrogen-induced increase in the production of nitrogen-rich phosphatase enzymes that render phosphorus more available (Pegtel et al., 1996; Houlton et al., 2008; Bai et al., 2017). The principle of grassland fertilization is not only based on the nutritional needs of the plant and the ability of providing nutrients for the soil, but should be also considered the effects and benefits of fertilization. Due to the diverse types of grassland soils in different regions, various types and concentrations of fertilizer will impact changes in grassland productivity differently. Therefore, the definition of fertilizer concentration levels are relative and there are no limits (Bai et al., 2010). Many studies focus on the effect of fertilization on biomass or nutrients of artificial grassland, but only few studies focus on the effect of biomass and species composition in natural grasslands (Blaxter et al., 2009; Xu et al., 2015). Both organic manure and chemical fertilizer applied on the soil of natural grassland can significantly increase grasses growth, but there is a difference in their effects on species composition (Eek et al., 2001; Jiang et al., 2013). A long-term location fertilization study in China indicated that a single application of nitrogen fertilizer on grassland could promote the development of grass, but was not favourable to the growth of legumes. Compared to nitrogen fertilizer, phosphorus fertilizer was more beneficial to the growth of legumes, and potassium fertilizer could promote the photosynthesis of plants and improve cold resistance and disease resistance of plants. Therefore, the balanced application of N, P, K fertilizer has positive effects on grassland yield and quality (Nie et al., 2012). Plant diversity plays an important role for grassland ecosystem functions and is a key for controlling productivity with changing climate (Hector et al., 1999; Zhang et al., 2018). When the diversity of plants is reduced, the buffering effect of the grassland will be reduced and therefore, the service function of the ecosystem is threatened. As a result, ecologists are continually concerned of the relationship between fertilization and plant diversity (Huberty et al., 1998; Richardson et al., 1999; Zeng et al., 2009). There may be positive, negative, or no significant correlations between fertilization and species richness, but thus far no definitive conclusion has been determined (Rajaniemi, 2002; Hejcman et al., 2007; Hautier et al., 2014; Leimer et al., 2015).

The Hulunbuir Meadow steppe is located in Northeast China. It is one of three world-famous grasslands, with an area of 1.08×10^4 km². The grassland ecosystem provides fodder for livestock by grazing or mowing. The plant productivity depends on climatic conditions such as temperature and precipitation. The decomposition rate of soil organic matter slowly is constrained mainly by low temperature, which leads to trapping of the nutrient in forms unavailable to the plant. One of the macro-nutrients N, P, and K is added to the soil in short-term, it could generally increase primary productivity and soil organic matter, but excessive fertilization may result in an imbalance of soil and plant nutrients, soil acidification, and toxic effects to plants. This leads to

uncertainty in the overall outcome for plant communities (Warman and Cooper, 2000; Pan et al., 2014). Therefore, the appropriate types and concentrations of fertilizers are the key factor for grassland improvement. Most studies on fertilizer for grassland pay more attention to comparing one or two types at different levels (Oomes, 1990). In order to analyze the effects of different fertilization regimes on the development of natural grassland ecosystems, we tested biomass, plant species composition, and plant diversity under various fertilizer treatments in the Hulunber *Leymus chinensis* Meadow steppe over a three years' period. This study was designed to address the following three questions: (i) How does short-term N, P, K fertilizer addition affect aboveground net primary productivity? (ii) Which is optimal option of fertilizer combination for improvement of grassland yield? (iii) How does species diversity change with optimal fertilization levels in different years?

Materials and methods

Study sites and experimental design

The fertilization experiment was conducted near Hulunbuir Grassland Ecosystem National Field Observation Station, China (49.2313N, 120.0247E). The station, which was built in 1997 by the Chinese Academy of Agricultural Sciences, is located in the core area of Hulunbuir Meadow steppe. The average altitude of this area is 650 m.a.s.l. The climate is continental semi-arid with a dry and cold winter and a rainy summer. The mean annual temperature is 0 °C. The annual precipitation is about 250-350 mm, of which about half falls between June and August. The frost-free period is 85 to 155 days. The type of soil is chestnut soil. The vegetation is dominated by *Leymus chinensis* (Trin.) Tzvel., *Vicia amoena* (Fisch.ex DC.), *Thalictrum squarrosus* (Steph.), *Pulsatilla turczaninowii* (Kryl. et Serg.), *Stipa baicalensis* (Roshev.), and *Cleistogenessquarrosa* (Trin.) Keng, and accompanied by *Carexduriuscula* (C. A. Mey.), *Allium bidentatum* (Allium L.), and *Artemisia tanacetifolia* (Linn.). Prior to 2014, the grassland was irregularly grazed by cattle and the site was mowed once a year in autumn.

In order to prevent grazing, the fertilization experiment was started at a permanent area within an enclosure in 2013, whereas the mowing regime also occurred within the enclosure. The plots had similar plant community composition and structure before fertilizing. Fourteen 6 × 10 m plots were randomly placed with an average separation distance of about 2 m in May 2014. An L14 (3⁴) orthogonal test was chosen to test their effects on plant community biomass, plant species composition, species richness, Shannon diversity, and Pielou's evenness. The orthogonal test is an efficient measurement to assay the comprehensive effect of multiple factors, finding the dominant factors and the best combination of levels for them with the least experimental trials, enhancing the reproducibility of the experimental results (Montgomery, 1991). The L14 (3⁴) orthogonal test for the experiment was to reduce workload, while it could arrange fourteen treatments with three factors and their four levels each, and test the interactions between factors if they exist. The local soil nutrient survey results in 2013, the total N content in 0-30 cm soil was 2.86 g·kg⁻¹, the total P was 0.49 g·kg⁻¹ and the total K was 22.96 g·kg⁻¹. Comprehensive consideration the soil survey result and local traditional fertilization, selecting fourteen plots (T1 to T14) with nitrogen fertilizer (N), phosphate fertilizer (P), and potassium fertilizer (K), and four fertilization concentration levels for each fertilizer (*Table 1*). The concentrations of four N treatments were 0, 91, 183 and 274 kg ha⁻¹ (hereafter denoted as N1, N2, N3 and N4, respectively), four P

treatments were 0, 175, 350, 525 kg ha⁻¹ (hereafter denoted as P1, P2, P3, and P4, respectively), and four K treatments were 0, 28, 57, 85 kg ha⁻¹ (hereafter denoted as K1, K2, K3, and K4, respectively), three replicates for each of the fourteen plots (hereafter denoted as T1-T14, respectively). The fertilizer was broadcast on the surface by hand once a year, and the date of fertilization was in early June every year.

Table 1. L14 (3⁴) orthogonal test for the combinational effect trial and fertilization amounts of Nitrogen (N), Phosphorous (P), and Potassium (K) applied

Plot number	Treatment	Fertilization amounts of N, P, K		
		CON ₂ H ₄ (kg ha ⁻¹)	CaP ₂ H ₄ O ₈ (kg ha ⁻¹)	K ₂ SO ₄ (kg ha ⁻¹)
T1	N0P0K0	0	0	0
T2	N0P2K2	0	350	57
T3	N1P2K2	91	350	57
T4	N2P0K2	183	0	57
T5	N2P1K2	183	175	57
T6	N2P2K2	183	350	57
T7	N2P3K2	183	525	57
T8	N2P2K0	183	350	0
T9	N2P2K1	183	350	28
T10	N2P2K3	183	350	85
T11	N3P2K2	274	350	57
T12	N1P1K2	91	175	57
T13	N1P2K1	91	350	28
T14	N2P1K1	183	175	28

The urea (N ≥ 46.4%), calcium superphosphate (P₂O₅ ≥ 16%), potassium sulfate (K₂O ≥ 51%) were applied for nitrogen fertilizer (N), phosphate fertilizer (P), and potassium fertilizer (K) respectively with artificial fertilization

Biomass sampling

Except for surface litter, the total biomass of all plants was harvested from 0.5 × 0.5 m quadrats in August of each year between 2014 and 2016 with three repetitions randomly selected in each plot (*Fig. 1*). Different species were separately cut to the ground level using scissors in each square area. All species were separately weighed after drying at 65 °C for 24 h in the oven. The plant community biomass was the total amount of all plants biomass in each plot (Sala et al., 1988).

Plant species composition and plant diversity

In each plot, we randomly selected three 0.5×0.5 m quadrats for the investigation of plant species composition and plant diversity. Every August was chosen to test because of the peak number of plant species biomass at this time. Plant species composition was calculated using the percentage of the species weight in the community. The richness was defined as the number of species per square area. The diversity index was calculated using the Shannon-Wiener diversity index (Shannon, 1949). Evenness of species was calculated using Pielou's evenness index (Pielou, 1966). During the three years, 62 plant species were identified within the study area, and divided into 6 plant

functional groups, including 9 Poaceae, 8 Fabaceae, 4 Ranunculaceae, 1 Cyperaceae, 11 Asteraceae, and 29 miscellaneous species or forbs (Table 2). We have selected cumulative important values $IV \geq 5$ to be classified as, Poaceae, Fabaceae, Ranunculaceae, Cyperaceae, Asteraceae; other important values (IV) < 5 were classified as miscellaneous species or forbs. The cumulative important values (IV) of different populations were calculated and classified according to the morphology of the different plants (Bu and Jiang, 2014).

The cumulative important values (IV):

$$IV = \frac{RD + RH + RM}{3} \quad (\text{Eq.1})$$

where: RD = relative density, RH = relative height, RM = relative dry mass.



Figure 1. The photo of experiment site and taking sample

Table 2. Classification of functional group

Plant functional groups	Plant species
Gramineae	<i>Leymus chinensis</i> <i>Stipa baicalensis</i> <i>Achnatherum sibiricum</i> <i>Calamagrostis epigeios</i> <i>Poa pratensis</i> <i>Koeleria cristata</i> <i>Cleistogenes squarrosa</i> <i>Bromus inermis</i> <i>Festuca ovina</i>
Leguminosae	<i>Astragalus adsurgens</i> <i>Vicia amoena</i> <i>Thermopsis lanceolata</i> <i>Astragalus melilotoides</i> <i>Astragalus hsinbaticus</i> <i>Oxytropis myriophylla</i> <i>Melissitus ruthenica</i> <i>Melilotus dentate</i>

Ranunculaceae	<i>Pulsatilla turczaninovii</i> <i>Thalictrum squarrosus</i> <i>Clematis hexapetala</i> <i>Caltha palustris</i>
Cyperaceae	<i>Carex duriuscula</i>
Asteraceae	<i>Artemisia dacunculus</i> <i>Artemisia tanacetifolia</i> <i>Scorzonera austriaca</i> <i>Serratula centauroides</i> <i>Heteropappus altaicus</i> <i>Artemisia annua</i> <i>Tanacetum parthenium</i> <i>Tripolium vulgare</i> <i>Lxerisson chifolia</i> <i>Artemisia frigida</i> <i>Saussurea amara</i>
Miscellaneous species or forbs	<i>Allium bidentatum</i> <i>Allium tenuissimum</i> <i>Lilium pumilum</i> <i>Veratrum nigrum</i> <i>Allium ledebourianum</i> <i>Allium prostratum</i> <i>Adenophora stenanthina</i> <i>Adenophora paniculata</i> <i>Viola vercumda</i> <i>Chenopodium glaucum</i> <i>Orobanche coerulescens</i> <i>Swertia pseudochinensis</i> <i>Gentianopsis barbata</i> <i>Gentiana squarrosa</i> <i>Galium verum</i> <i>Saposhnikovia divaricata</i> <i>Bupleurum chinense</i> <i>Dontostemon dentatus</i> <i>Lepidium apetalum</i> <i>Dianthus chinensis</i> <i>Linaria vulgaris</i> <i>Veronica longirolia</i> <i>Cymbariadahurica</i> <i>Iris ventricosa</i> <i>Potentilla acaulis</i> <i>Potentilla verticillaris</i> <i>Potentilla bifurca</i> <i>Potentilla nudicaulis</i> <i>Potentilla tanacetifolia</i>

Statistical analysis

We used one-way ANOVA to examine the main effects of fertilizer addition. Prior to analysis, we confirmed that our response variables met normality and equal variance assumptions. To test whether the impacts of fertilizer addition on community biomass, plant species composition, species richness, Shannon diversity, and Pielou's evenness differed across time, repeated analysis of variance (ANOVA) was performed with fertilizer addition as the between-subjects factors and years as the within-subject factor.

Based on the design and character of the orthogonal experiment, the effect of the nitrogen fertilizer gradient on biomass, species richness, Shannon diversity and Pielou's evenness were analyzed by using the same fertilizer gradient of phosphate fertilizer and potassium fertilizer. Nitrogen fertilizer was used as follows T1 (N0P0K0), T2 (N0P2K2), T3 (N1P2K2), T6 (N2P2K2), and T11 (N3P2K2); phosphate fertilizer was used as follows T1 (N0P0K0), T4 (N2P0K2), T5 (N2P1K2), T6 (N2P2K2), and T7 (N2P3K2); and potassium fertilizer was used as follows T1 (N0P0K0), T8 (N2P2K0), T9 (N2P2K1), T6 (N2P2K2), and T10 (N2P2K3). The average of no fertilizer (N0P0K0) and nitrogen-free fertilizer (N0P2K2) was used for the initial gradient of nitrogen fertilizer.

Data were subjected to analysis of variance (ANOVA) using SPSS 20.0 software (SPSS statistical package, Chicago, IL, United States). An alpha of 0.05 was used for statistical significance. If sources were significant, they then underwent an LSD test with significance again declared at the 0.05 level.

Results

Environmental conditions during the fertilization experiment

During the fertilization study, the average of growing-season monthly air temperature from May to August was similar in all three years, but there was a great difference in rainfall (Fig. 2). The average of growing-season rainfall in 2014 was 82.28 mm, 78% and 114% higher than that in 2015 and 2016 respectively. Especially in July, the rainfall in 2014 was four times more than that in 2015 and 2016, while June in 2014 was twice higher than that in 2015 and 2016 respectively.

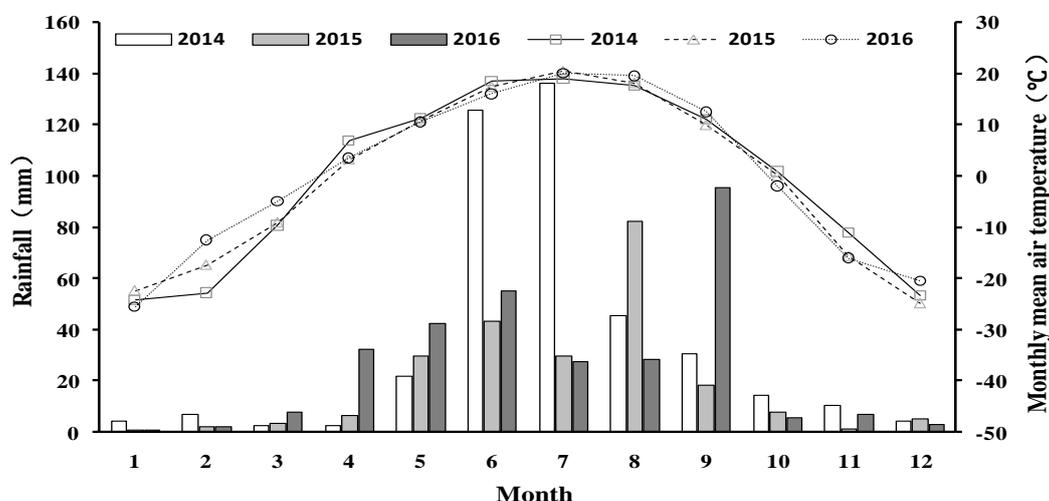


Figure 2. Monthly mean air temperature (lines) and rainfall (bars) received during the study period from 2014-2016 at the experiment site near Hulunbuir Grassland Ecosystem National Field Observation Station

Plant community biomass

The effect of years and fertilizer treatments strongly influenced the total biomass of the community from 2014 to 2016 (Fig. 3). A positive effect of fertilizer on biomass emerged from T10 in 2014, was significantly higher than T1 (control), T4, T7, T8, and T9 ($P < 0.05$). This effect was short-lived. Except for T2, other treatments were significantly higher than T1 in 2015 ($P < 0.05$). Interestingly, a cumulative effect of the community biomass was more significant in 2016, which was the third year of fertilizer. T11 was the highest biomass in 2016 and higher than others except for T9 ($P < 0.05$). From the year difference analysis, the same treatment from 2014 to 2016 was significantly different in T1, T10, T12, and T13. There was no obvious difference between T10 and T11 in 2014 and 2015, but T11 was significantly higher than T10 in 2016 ($P < 0.05$). It indicated that T11 was the better fertilizer effect during the three years. The analysis shows that all fertilizer treatments performed better than the control (T1), so it could be concluded that fertilization plays a positive role in grassland productivity.

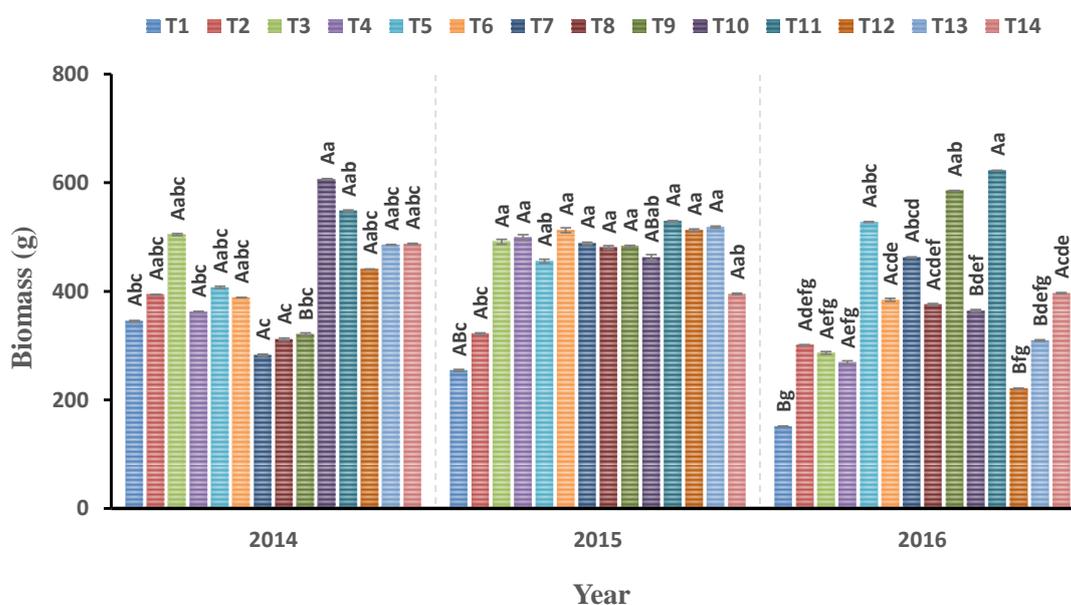


Figure 3. Effects of different fertilization treatments (Table 1) from 2014 to 2016 on total plant community biomass. The vertical bars represent the standard errors. Different upper-case letters indicate significant difference between years in the same treatment and lower-case letters indicate significant difference between treatments in the same year according to LSD test at $P < 0.05$

The mono-fertilizer effect was analyzed and the difference in partial treatments was significant (Fig. 4). In 2014, which was the first year of fertilization, there was only clear effect on K fertilizer at different levels. The community biomass increased with increasing the concentration of K when N and P fertilizer was the same fertilizer level. The trends of N, P, and K were similar in 2015. Except for N0 (P2K2), the biomass of all fertilizer treatments was significantly higher than the control (N0P0K0). Significant effects on biomass at the different level of N, P and K were also observed in 2016, which was the third year of fertilization. The biomass increased with N level increasing

and N3 the highest one ($P < 0.05$). P1 (N2K2) was the highest one, P1 (N2K2) and P3 (N2K2) were significantly higher than P0 (N2K2) and the control ($P < 0.05$). The low P concentration met the needs of plant growth for phosphorus. This phenomenon is more obvious in terms of potassium fertilizer. Low K concentration was significantly higher than other levels of K ($P < 0.05$).

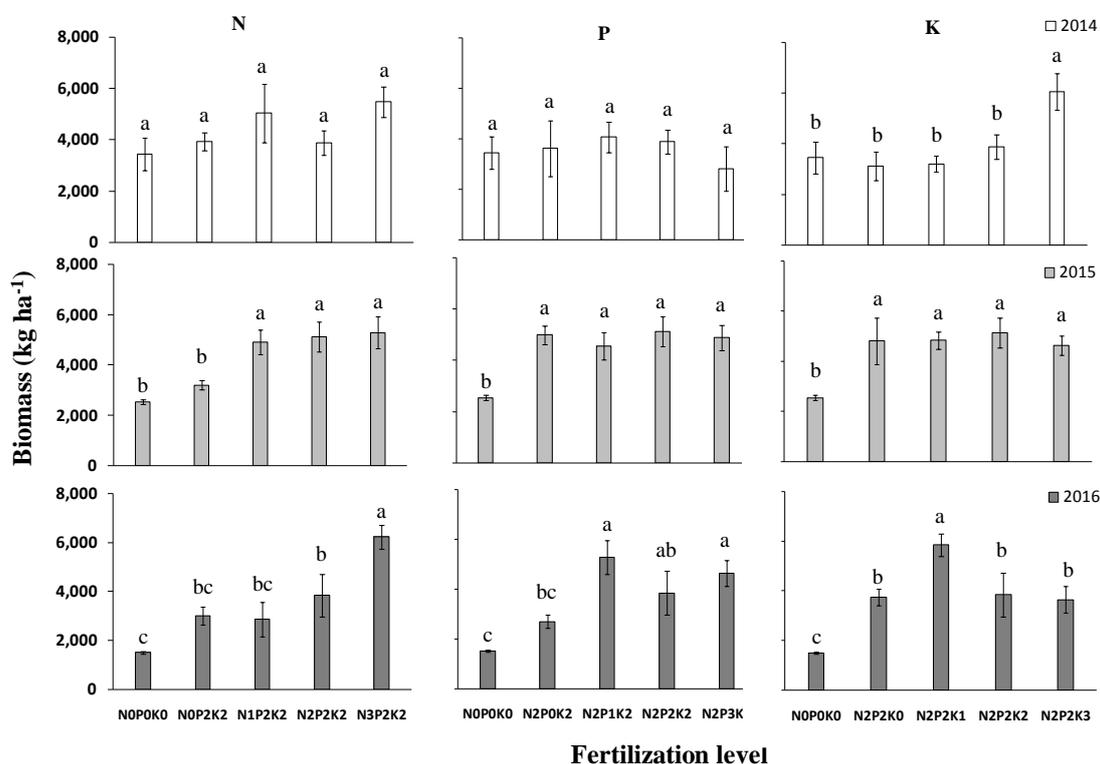


Figure 4. Total community biomass for different N, P, K levels at August fertilization in 2014, 2015, and 2016. NOP0K0 represents no fertilizer, N0 (P2K2), N1 (P2K2) and N3 (P2K2) represent that different concentration changes of nitrogen under the same phosphorus and potassium concentration. The same mean as phosphorus and potassium. The vertical bars represent the standard errors. Different letters indicate significant difference between treatments in the same year according LSD test at $P < 0.05$. NS represents no significant differences

Plant species composition

The relative biomass of the functional groups in different treatments exhibited a significant effect on Gramineae for the three years (Table 3). In 2014, the Gramineae achieved the highest percentage of biomass (Fig. 5). In 2015, the dominance of miscellaneous species or forbs was gradually promoted, and Gramineae was replaced as the second proportion of biomass and the proportion of other species also decreased to varying degrees. In 2016, the Gramineae achieved the highest percentage again and the proportion of Cyperaceae increased in different treatments compared to 2015. The proportion of miscellaneous species or forbs declined significantly in 2016, Leguminosae and Ranunculaceae even disappeared in most treatments (Table 3). Except for Gramineae and Cyperaceae, some species in all treatments disappeared and the highest biomass of Gramineae was still T11.

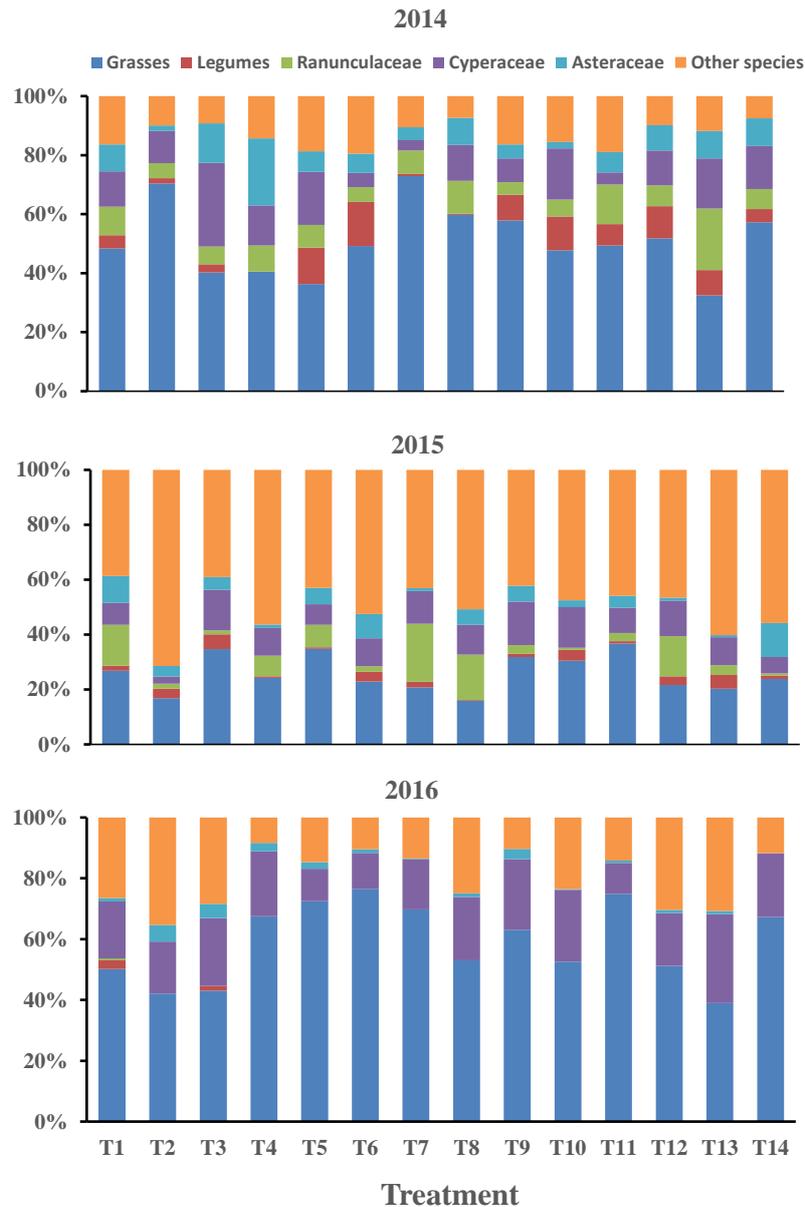


Figure 5. Treatment (Table 1) effects on aboveground biomass of functional groups of plants in August for 2014, 2015 and 2016

Table 3. Results (*P*-value) of repeated measures ANOVA for biomass of the functional groups within fertilization treatments

Biomass	Gramineae	Leguminosae	Ranunculaceae	Cyperaceae	Asteraceae	Miscellaneous species or forbs
Treatment (T)	< 0.001	0.495	0.894	0.692	0.958	0.991
Year (Y)	< 0.001	< 0.001	< 0.001	0.672	< 0.001	0.01
T* Y	0.001	0.784	0.99	0.504	0.839	0.998

Species richness, Shannon diversity and Pielou's evenness of plant community

Except for 2015, there were significant differences of species richness between 2014 and 2016, and T11 and T12 being the maximum in 2014 and 2016, respectively

(Fig. 6A). It was also observed that T11 in 2014 was significantly higher than in 2015 and 2016 ($P < 0.05$). In 2015, T12 was significantly higher than the other two years (Fig. 6a). From the single fertilizer and year effect analysis, the significant differences of N, P and K were observed (Fig. 7). In addition to the medium concentration of N (N2P2K2) and K (K2N2P2), other concentrations of N and K in 2014 were significantly higher than that in 2015 and 2016 ($P < 0.05$). The medium concentration of N, P, and K reached the maximum value in 2016. Similarly, a significant change in Shannon diversity during the three years was found ($P < 0.05$). The peak value of Shannon diversity was T13 in 2016 and no significant difference on T13 between 2014 and 2016 (Fig. 6B). The Shannon diversity of fourteen treatments in 2014 was no different or higher than that in 2015 and 2016 (Fig. 6b). This phenomenon was more obvious in the single fertilizer effect (Fig. 7). No matter which fertilizer and concentration, Shannon diversity for most treatments were higher in 2014 than that in 2015 and in 2016 ($P < 0.05$). From fertilizer concentration analysis, the control (N0P0K0) was always higher than other concentration of N, P and K during the three years. N0 (P2K2) was the lower one in 2014 and N2 (P2K2) became the lowest in 2016, while P3 (N2K2) in 2016 replaced P0 (N2K2) in 2014 to become the lowest ($P < 0.05$). For the concentration of K, the high concentration (K3N2P2) was always lowest during the three years ($P < 0.05$). In 2015 and 2016, the significant differences of Pielou's evenness between treatments appeared. T9 in 2015 and 2016 were higher than partial treatments and no significant change between the two years (Fig. 6C). For the trend of year effect analysis, Pielou's evenness was similar to Shannon diversity (Fig. 6c). No matter which fertilizer and concentration, Pielou's evenness in 2014 were no differences or significantly higher than that in 2015 and 2016 ($P < 0.05$). There was no difference in Pielou's evenness in different N concentration in 2014 and 2015, but the difference appeared in 2016 and N1 was the lowest. Pielou's evenness in different P concentration was similar to N, and the lowest value was P1 in 2015 and 2016. The commonality of different K concentrations within the three years was that high concentration (K3N2P2) was always the lowest value ($P < 0.05$).

Discussion

The plant community biomass response to fertilization

The plant community biomass has an obvious change during the three years, as well as the less biomass of some treatments in 2016 than the other two years when severe changed in rainfall. It suggests that effects of fertilizer on plant community biomass are mediated, at least in part, by changes in rainfall (Yu et al., 2015). Many previous studies observed that these divergent effects of fertilizer addition are mediated by many factors which vary in time and space, including but not limited to fertilizer rate, the traits of the species composing the community and soil moisture and climate (Yu et al., 2015; Brooks, 2003; Li et al., 2010). Although the biomass of some treatments in 2016 was lower, it could be seen that the difference in treatments in 2016 was more significant. T11 was the highest one in 2016 and it was still the best combination with the drought conditions between 2015 and 2016. Regardless of the amount of rainfall or scarcity between 2014 and 2016, the fertilizer effect continued to exist during these three years. The biomass of the fertilized plots was always higher than that of the non-fertilized one (T1). These could explain the addition of different concentrations of fertilizers applied that often led to different results in plant community biomass (Su et al., 2012; Zhou et

al., 2018). Until 2016, our results also revealed that high concentration of nitrogen fertilizer (N3P2K2) was optimum based on the maximum biomass resulting from nitrogen fertilizer level, the low concentration of phosphorus (P1N2K2) and potassium (K1N2P2) were also optimum after the three years fertilization. These results do indicate that the growth of plants in the grassland with additional fertilizer would be greater which agrees with previous research results (Bai et al., 2010). However, there is a threshold of grassland productivity response to fertilization (Bai et al., 2010). As the amount or the period of fertilizer has increased, the amount of biomass did not increase or even decrease until it plateaued.

Plant species composition response to fertilization

Fertilizer addition has commonly been observed to change plant species composition and response of plant community members may dictate different responses to fertilizer addition (Gough et al., 2000; Hautier et al., 2014; Su et al., 2012). Nutrient limitation is a widespread phenomenon in grassland ecosystems. The responses of plant community to fertilizer addition are also contingent upon environmental conditions, especially moisture (Yu et al., 2015; Brooks, 2003; Su et al., 2012; Yahdjian et al., 2011; Zhou et al., 2018). With increasing levels of fertilizer addition, it benefits the dominance of alien annual plants and possibly promotes the invasion of new species in semi-arid regions, at the expense of natives (Brooks, 2003). In our study, the Gramineae became the dominant proportion again in 2016 and some species disappeared. This finding was consistent with previous studies (Zhou et al., 2018). This might suggest that the current dominants possess traits that allow them to benefit from, or showing effects after a period of time, while rare species do not. These responses are probably explained by a combination of differing N, P and K acquisition strategies, fertilizer-use efficiency and maximum growth rates of these different functional types (Wamelink et al., 2009).

Species richness, Shannon diversity and Pielou's evenness of plant community response to fertilization

Diversity including species richness, Shannon diversity, Pielou's evenness and other dimensions commonly lead to enhanced productivity through the mechanism of complementarity (Hejerman et al., 2007; Yahdjian et al., 2011). Species losses and declines in species richness per unit area are commonly induced by fertilizer addition (Su et al., 2012; Clark et al., 2013). In our study, compared to the control, the richness of partial fertilizer treatments decreased significantly, but the highest value of richness appeared in T12 in 2016 (*Fig. 6a*). Regardless of N, P and K, the higher values were always in the fertilizer treatments in 2015 and 2016 (*Fig. 7*). However, there is a significant decline in the value of partial fertilization treatment compared to control. The reasons for the difference from other studies may be that P and K were applied together with N in this experiment but not as a sole treatment like in many other studies. Another reason was that our experiment was short-time and more time was required to fully demonstrate plant community effect. In contrast, the lowest value of Shannon diversity occurred in fertilized treatments, while the values with unfertilized treatment were always higher than some fertilized treatments. As the fertilizer year increased, the values of the same treatment in 2016 were all lower than that in 2014. This is a good illustration of the theory that the longer the fertilization treatments lasted, the lower Shannon diversity became. This phenomenon was also obvious in Pielou's evenness.

These results do correspond with those obtained in several published studies and document a decreased in species diversity with fertilizer effect (Bobbink and Willems, 1993; Kahmen et al., 2002; Moog et al., 2002).

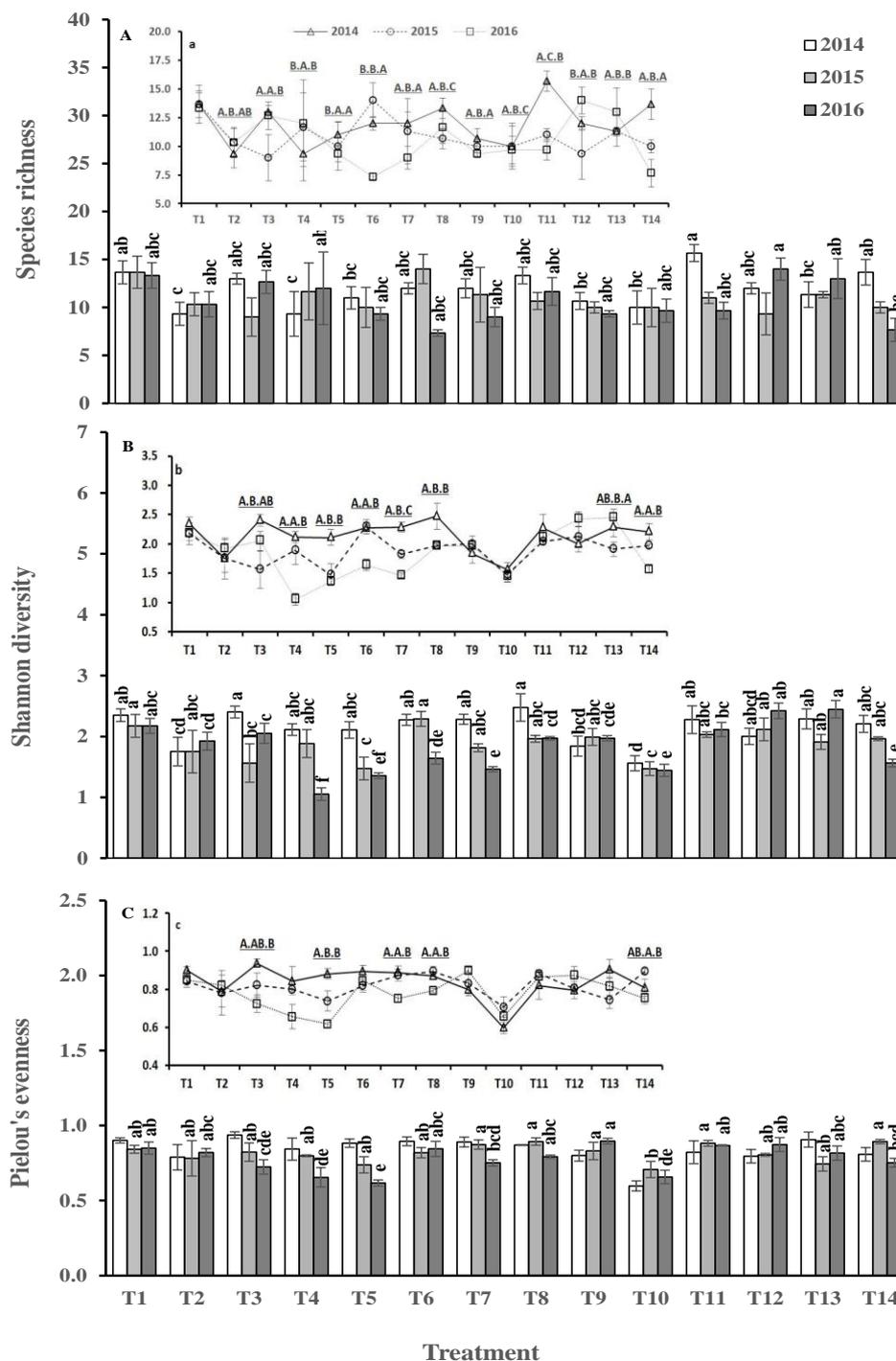


Figure 6. Treatment (Table 1) and year (2014, 2015 and 2016) effects on species richness, Shannon diversity and Pielou's evenness of plant community in August. Figure (A, B, C) represent treatment effects and different lower-case letters indicate significant difference in treatments (bars). Figure (a, b, c) represent year effects and different upper-case letters indicate significant difference in years (lines) according LSD test at $P < 0.05$. The vertical bars represent the standard errors

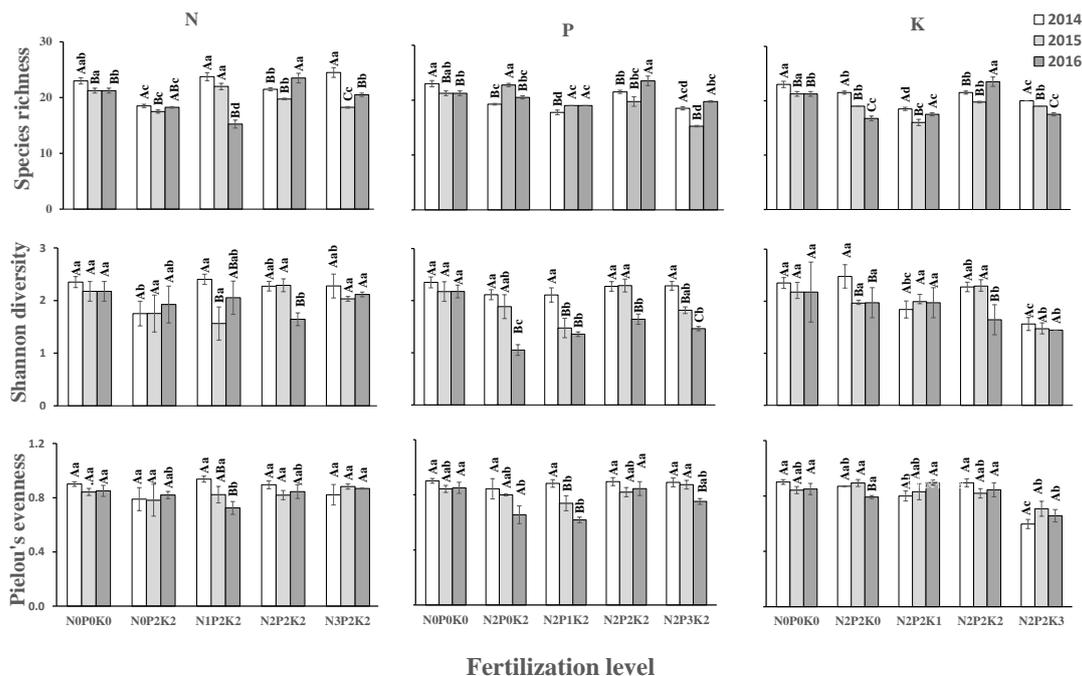


Figure 7. Species richness, Shannon diversity and Pielou's evenness for four different fertilization levels by year of August fertilization (2014, 2015, and 2016). N0P0K0 represents no fertilizer, N0 (P2K2), N1 (P2K2) and N3 (P2K2) represent that different concentration changes of nitrogen under the same phosphorus and potassium concentration. The same mean as phosphorus and potassium. The vertical bars represent the standard errors. Different letters indicate significant difference between treatments in the same year according LSD test at $P < 0.05$

Conclusion

Based on the result, we recommend the combination of N (274 kg ha^{-1}), P (350 kg ha^{-1}), and K (57 kg ha^{-1}), and annually to maximize plant community productivity from the semi-arid grassland in Hulunbuir Inner Mongolia, China. Gramineae accounted for the major proportion of biomass in the third year of fertilization, whereas the proportion of other species declined or disappeared. The longer the fertilization treatments went on, the lower Shannon diversity and Pielou's evenness turned out to be. All these responses are strongly interrelated in a cascade of changes. We further establish that effects on plant community biomass are additively influenced by the dose of fertilizer applied and duration of application. In our study, the effects of the year were consequently significant on many indicators. It could indicate that some other factor could have influence on the plant community indicators. Therefore, we conclude that water control will be carried out the original design, to simulate the impact of different precipitation on the experimental results of this study in the future.

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