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Original Research

Effect of Fertilizer Additions on Plant Communities and Soil Properties in a Temperate Grassland Steppe

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Abstract

Temperate grassland steppe is an important natural ecosystem in the world, which is being degraded under the combined pressure of climate change and human over-utilization. Our objective was to determine the effect of inorganic and organic fertilizer addition and determine the appropriate fertilizer resource and additional rate for temperate grassland restoration. The treatments consisted of an inorganic and organic fertilizer, each with three additional rates. The effect of fertilizer addition on aboveground biomass, community structure, soil properties, and N balance were examined using a 2-year *in situ* controlled trial at Guyuan Grassland Station, Hebei Province, China. The results showed that inorganic fertilizer treatments increased the aboveground biomass, and decreased diversity and evenness of the plant community. Organic fertilizer treatments increased soil total carbon and the carbon-to-nitrogen ratio, but did not significantly change the aboveground biomass and soil available nutrition during the 2-year experiment. The N quantity of plant harvest output is approximate to that of fertilizer addition input in inorganic fertilizer treatment with 75 kg hm^{-2} urea and 45 kg hm^{-2} diammonium phosphate. The study demonstrated that using fertilizer addition to restore temperature grassland steppe requires a comprehensive evaluation of the diverse services functions of the present and future.

Keywords: fertilizer, aboveground biomass, community structure, soil properties, temperate grassland steppe

Introduction

Temperate grassland steppe is an important natural ecosystem in the world, providing a diverse range of plant and animal species and ecosystem services, including wildlife habitat, forage resource for livestock, and carbon sequestration [1]. *Leymus chinensis* (Trin.) Tzvel is a native, perennial, rhizomatous grass. Grasslands dominated by *L. chinensis* are the dominant vegetation type in eastern Eurasian temperate grasslands. This grassland type occupies an area of 3×10^5 hm^2 in Inner Mongolia. It is typically utilized as hay and supplied

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to animals in the winter, and it also plays a key role in environmental security to the nation [2-3]. However, *L. chinensis* steppe has been degrading under the combined pressure of climate change and human over-utilization and has been subjected to unsustainable use and conflicting interests since the 1980s [4-7]. Today, the planet is warming under atmospheric CO_2 concentration increases [8-9], resulting in abnormal hydrothermal distribution and in erratic precipitation in arid and semiarid grassland ecosystems [10]. This represents a major challenge to temperate grassland, and encourages researchers to conduct studies relating to the successful restoration of ecological environments.

Infertile soil is one of the major factors restricting temperate grassland steppe sustainable development caused by over-utilization and the lack of an effective nutritional feedback approach. Limiting nutrients will change some key ecosystem processes and services, including the nutritional cycle, carbon sequestration, and changes to plant and soil communities [11-14]. An increasing number of studies have focused on the effect of nutrition addition in grassland ecosystems [15-18]. The fact that fertilizer addition can directly increase productivity has long been recognized [19-20]. A temperate grassland ecosystem is unlike a crop field, and in addition to the productivity function to the livelihood of herds, ecosystem services are also important services for human life. There is currently little research on both grassland productivity and ecosystem services. Most of the current research has demonstrated that fertilizer addition can alter soil properties, soil nitrogen cycling, and the stocks of soil carbon [21-23], which can result in a decline in species and functional group diversity [24-26]. In addition, aboveground biomass (AGB) changes are accompanied by changes in soil microbial processes [27-31], which is closely related to soil fertility. However, fertilizer addition can also cause environmental problems such as nutrient leaching, groundwater contamination, eutrophication, and soil acidification [17]. Although fertilization is a regular practice on crop fields, there has not been effective guidance on its appropriate use in temperate grassland due to a lack of reliable experimental data to support it.

Some previous studies have identified the effect of N addition on grassland, especially on the effect of simulate atmospheric N deposition [32-37]. However, little research has demonstrated the effect of fertilizer addition, especially the effect when N and P were added simultaneously, on both productivity and ecosystem services. Therefore, our objective was to demonstrate the effect of fertilizer addition on plant community and soil properties, search the effective methods to promote vegetation growth and soil fertility, and realize sustainable development in a temperate grassland steppe.

Experimental

Site Description

The study was conducted from 2014 to 2015 at Guyuan Station (41.77°N, 115.67°E, 1,400 m a.s.l.), located at the southern edge of the Inner Mongolian Plateau in Hebei Province, northern China. This region

Table 1. Dominant plant species, life forms, photosynthetic pathways, and life history traits.

Species	Life form	Photosynthetic pathway	Life history traits
Leymus chinensis	Grass	C3	Perennial
Cleistogenes squarrosa	Grass	C4	Perennial
Artemisia frigida	Forb	C3	Perennial
Stipa krylovii	Grass	C3	Perennial
Agropyron cristatum	Grass	C3	Perennial
Heteropappus altaicus	Forb	C3	Perennial
Iris ensata	Forb	C3	Perennial
Phlomis tuberosa	Forb	C3	Perennial
Ixeris sonchifolia	Forb	C3	Perennial
Koeleria cristata	grass	C3	Perennial
Medicago sativa	Legume	C3	Perennial
Melissitus ruthenica	Legume	C3	Perennial
Potentilla anserine	Forb	C3	Perennial
Chenopodium glaucum	Forb	C3	Annual
Saussurea amara	Forb	C3	Perennial
Thalictrum petaloideum	Forb	C3	Perennial

has a semiarid continental climate with mean annual precipitation of 400 mm, primarily distributed between May and September. The mean annual temperature was 2.5ºC, the mean minimum temperature in the coldest month (January) was -17.0ºC, and the mean maximum temperature in the hottest month (July) was 18.9ºC (1995-2013). The growing season is approximately 100 days, from May to September. The major soil type is sandy loam dark chestnut soil (Calcic-orthic Aridisol in the U.S. classification system) [38], and is slightly alkaline (pH 7.6). The experiment was conducted within a fenced, permanent 10-ha research paddock of a typical temperate grassland dominated by *Leymus chinensis* (Trin.) Tzvelev. Additional species included *Stipa krylovii* Roshev. (a tall perennial bunchgrass), *Cleistogenes squarrosa* (Trin.) Keng (a short perennial bunchgrass), and *Artemisia frigida* Willd (a short shrub) (Table 1). The nomenclature used is according to the international plant names index (IPNI 2017) [39].

Experimental Design

The treatments consisted of two different types of fertilizer, inorganic and organic, each with three additional rates. This factorial experiment was arranged in a randomized complete block design and replicated three times. The plot size was 3×5 m (15 m^2) with 100 cm spacing between plots. Urea (46% N) and diammonium phosphate (DAP, 18% N and 46% P_2O_5) was used as the source of N and P for inorganic fertilizer treatment. Organic fertilizer came from the Xin Xing manure company of Hebei Province, China (3% N, 1.5% P_2O_5, 45% organic matter; Table 2). All the fertilizer addition was broadcast in the spring annually during 2014 to 2015.

Data Collection

Precipitation and air temperature were recorded at the Guyuan Ranch meteorological station located 30 km from the experimental site.

The AGB and plant community characteristics were measured at the peak of AGB on 13 August 2014 and 18 August 2015. All species were harvested with scissors within a 1×1 m quadrat in each plot, oven-dried to a constant mass at 70°C for 48 h, weighed, and ground for N concentration. Total N in plants was measured by elemental analyzer (vario ELIII, Elementar, Germany). A 0.5×0.5 m quadrat with 100 sub-grids (0.05×0.05 m) was used to measure the coverage of each species, with three replicates per plot. We determined the relative coverage of each (percentage cover). The individual numbers of each species and the plant community in the frame were counted. We determined the Shannon-Wiener index (*H'*), species richness (S), and Pielou evenness index (*J'*) for each plot. The Shannon-Wiener index (*H'*) calculated as $H' = -\sum pi \ln (pi)$, where *pi* is the proportional number of species *i*, and S is the species richness in the community. The Pielou evenness index was calculated as $J' = H'/\ln(S)$.

Three soil cores (5 cm diameter and 10 cm depth) were collected from each plot on 13 August 2014 and 18 August 2015. Each fresh soil core was sieved through 2 mm mesh and separated into two parts. One was used for soil moisture (oven dried at 105ºC for 48 h) and soil pH analysis. The other was used to estimate soil available N (AN, the sum of extractable soil ammonium (NH_4^+-N) and nitrate (NO_3^--N) concentrations), soil available P (AP), soil total C (STC), soil total N (TN), and soil C/N ratio. Soil pH value was determined in a 1:2.5 soil-water suspension at 25°C using a pH meter (FE20 from FiveEasy, Mettler, Toledo). NH_4^+-N and NO_3^--N were extracted by 2 M KCl and determined with a flow injection autoanalyzer (FIAstar 5000 Analyzer, Foss Tecator, Denmark). Extractable NH_4^+-N and NO_3^--N concentrations were converted to dry mass basis using soil moisture data. Soil available P was extracted by 0.5 M $NaHCO_3$ solutions and determined with a continuous flow analyzer (Seal AutoAnalyzer 3, Seal, Germany), and STC and TN were measured by an elemental analyzer (vario ELIII, Elementar, Germany).

The N balance is N addition minus plant N output by harvest on each treatment in both 2014 and 2015.

Data Analysis

We examined the effects of fertilizer using the fertilizer addition treatment as sources of variance using the analysis of variance (ANOVA) model of SPSS

Table 2. Details of the fertilizer treatments in the *Leymus chinensis* steppe experiment.

Treatment	Detail	Total nutrient addition (kg hm^{-2} y^{-1})		
		N	P_2O_5	Organic matter
CK	No fertilizer applied	–	–	–
C1	75 kg hm^{-2} urea, 45 kg hm^{-2} DAP	42.6	20.7	–
C2	150 g hm^{-2} urea, 90 kg hm^{-2} DAP	85.2	41.4	–
C3	225 g hm^{-2} urea, 135 kg hm^{-2} DAP	127.8	62.1	–
M1	1,400 kg organic fertilizer	42	21	630
M2	2,800 kg organic fertilizer	84	42	1,260
M3	4,200 kg organic fertilizer	126	63	1,890

software (version 19.0, SPSS Inc., Chicago, Illinois, USA, 2004). One-way ANOVA with least significant difference (LSD) multiple range tests was used to test the statistical significance in the mean values of the treatments. Statistical significances of all tests were set at $p<0.05$. Results from the statistical analyses of the data were graphed using Microsoft Excel software (version 2010, Microsoft, Seattle, Washington, USA, 2010).

Results

Precipitation and Air Temperature

Precipitation and air temperatures varied over the 2 years of the study. April mean temperature was 8.2ºC in 2014 and only 5.7ºC in 2015. November precipitation was 43.3 mm in 2015 and only 1.3 mm in 2014 (Fig. 1). During the growing season from May through August, the amounts and distributions of precipitation and air temperatures were similar between the 2 years. The average air temperatures of growing season were 16.1 and 15.6ºC in 2014 and 2015, respectively, and precipitation was 250 and 228 mm in 2014 and 2015, respectively. Compared with the 1995-2013 average, both 2014 and 2015 had a higher monthly temperature in January and lower monthly precipitation in August (Fig. 1).

Effect of Fertilization on Aboveground Biomass

The AGB was 2.47 and 2.51 Mg hm^{-2} in C2 and C3 in 2014, respectively, both of which were significantly greater than that of M1, M2, and M3 (1.52, 1.75, and 1.84 Mg hm^{-2}; $p<0.05$). There were no significant differences among CK and organic fertilizer treatment (M1, M2, and M3) for AGB in 2014 (Fig. 2a). In 2015, AGB was 2.46, 2.96, and 2.71 Mg hm^{-2} in C1, C2, and C3, greater than that of M1, M2, and M3 (1.36, 1.50, and

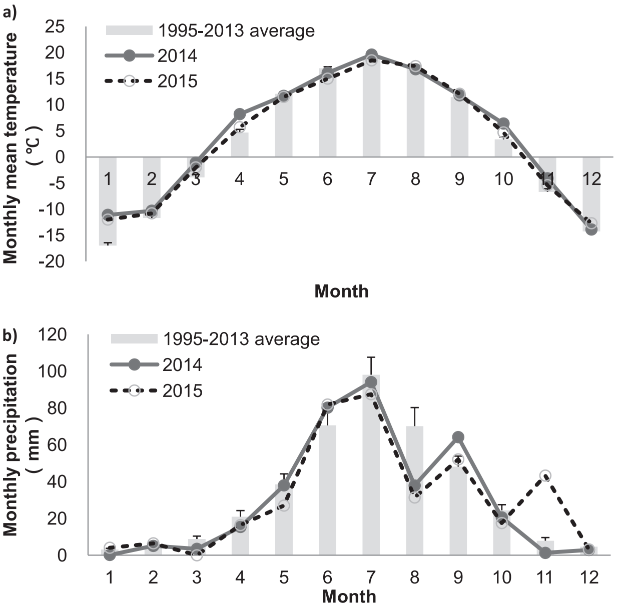

Fig 1. Monthly average temperature a) and total monthly precipitation b) in the experimental field 2014-2015; grey bars indicate the regional monthly averages (mean+SE) 1995-2013.

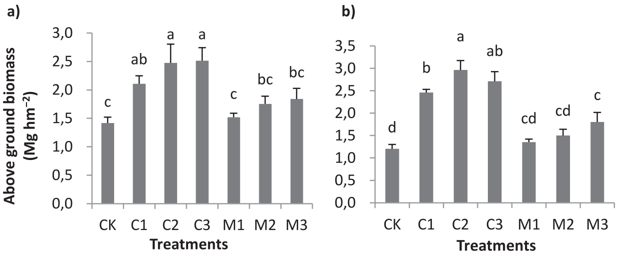

Fig. 2. Effect of fertilizer addition on aboveground biomass; for each panel, bars not labeled with the same letter indicate significantly different values at $p<0.05$ (based on LSD tests): a) effect of fertilizer addition in 2014 and b) in 2015.

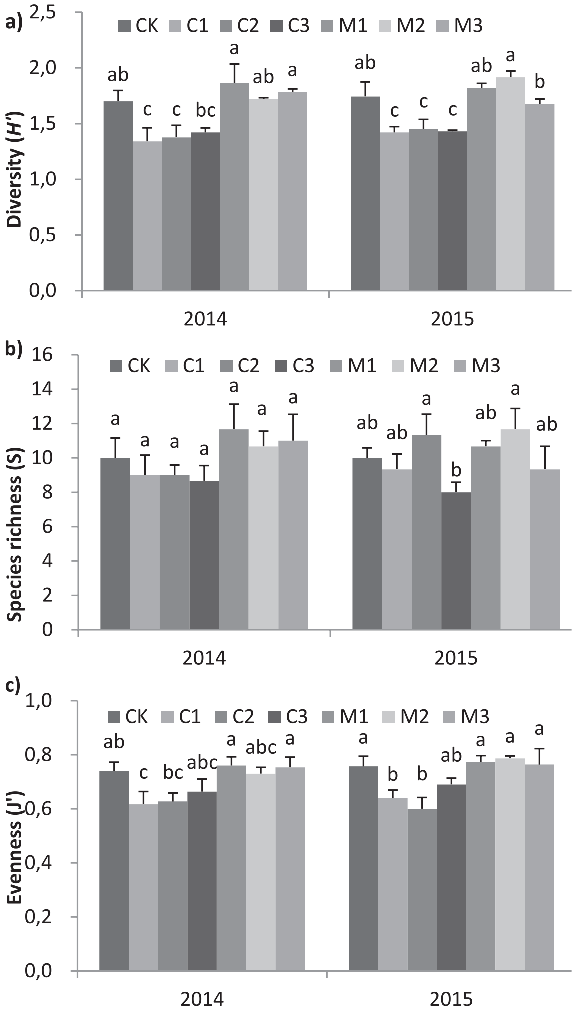

Fig. 3. Effect of fertilizer addition on Shannon-Wiener index, species richness, and Pielou evenness index; for each panel, bars not labeled with the same letter indicate significantly different values at $p<0.05$ (based on LSD tests): a) effect of fertilizer addition on *H'*, b) on S, and c) on *J'*.

1.80 Mg hm^{-2}; $p<0.05$). There were no significant differences among CK and organic fertilizer treatments (M1 and M2) for AGB in 2015 (Fig. 2b).

Effect of Fertilization on the Community Structure

The Shannon-Wiener index (*H'*) was 1.86, 1.72, and 1.78 in M1, M2, and M3 in 2014, respectively, all of which were greater than that of C1, C2, and C3 (1.34, 1.38, and 1.42; $p<0.05$). There were no significant differences among CK and organic fertilizer treatments (M1, M2, and M3) for *H'* in 2014. In 2015, *H'* was 1.82, 1.92, and 1.68 in M1, M2, and M3, respectively, significantly greater than those of C1, C2, and C3 (1.42, 1.45, and 1.43; $p<0.05$). There were no significant differences among CK and organic fertilizer treatments (M1 and M2) for *H'* in 2015 (Fig. 3a).

There were no significant differences among treatments on species richness (S) in 2014. In 2015, S was 8.0 in C3, lower than that of C2 and M2 (11.33 and 11.67; $p<0.05$), and there were no significant differences among the other treatments (Fig. 3b).

The Pielou evenness index (*J'*) was 0.62 and 0.63 in C1 and C2 in 2014, respectively, and both were lower than that of M1 and M3 (0.76 and 0.75; $p<0.05$). There were no significant differences among CK, organic fertilizer treatment (M1, M2, and M3) and C3 for *J'* in 2014. In 2015, *J'* was 0.64 and 0.60 in C1 and C2, both significantly lower than that of M1, M2, and M3 (0.77, 0.79 and 0.76; $p<0.05$). There were no significant differences among CK, organic fertilizer treatments (M1, M2, and M3), and C3 for *J'* in 2015 (Fig. 3c).

Effect of Fertilization on Soil Nutrition

Soil AN was 54.82, 63.53, and 66.94 mg kg^{-1} in C1, C2, and C3, respectively, greater than that of M1, M2, M3, and CK (38.39, 41.27, 38.38, and 37.71 mg kg^{-1}; $p<0.05$) in 2015. There were no significant differences among CK and organic fertilizer treatment (M1, M2, and M3) for AN (Fig. 4a).

Soil AP was 8.13, 6.60, and 6.77 mg kg^{-1} in C1, C2, and C3, respectively, significantly greater than that of M1, M2, and CK (4.66, 4.73 and 3.99 mg kg^{-1}; $p<0.05$). There were no significant differences among organic fertilizer treatments (M1, M2, and M3) for AP (Fig. 4b).

STC was 38.62, 36.02, and 48.67 g kg^{-1} in M1, M2, and M3, respectively, significantly greater than that of C1, C2, C3, and CK (26.39, 29.20, 23.36, and 27.20 g kg^{-1}; $p<0.05$) (Fig. 4c).

Soil C/N ratios were 14.84, 14.28, and 17.88 in M1, M2, and M3, respectively, significantly greater than those of C1, C2, C3, and CK (10.28,11.24, 9.07, and 10.37; $p<0.05$) (Fig. 5d). There were no significant differences among CK and inorganic fertilizer treatments (C1, C2, and C3; Fig. 4d), and no significant differences among all the treatments on soil TN (Fig. 4e) and pH (Fig. 4f) in 2015.

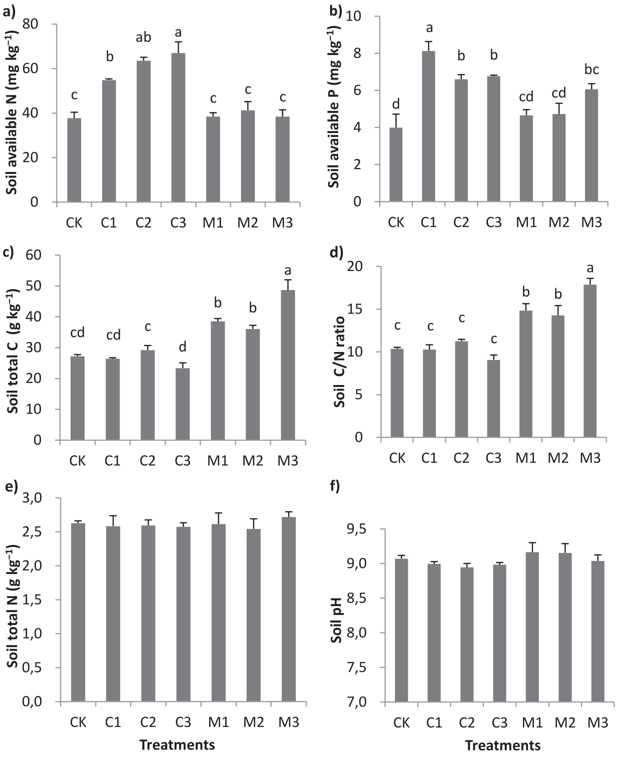

Fig. 4. Effect of fertilizer addition on soil available N, soil available P, soil total C, soil C/N ratio, soil total nitrogen, and pH; for each panel, bars not labeled with the same letter indicate significantly different values at $p<0.05$ (based on LSD tests): a) effect of fertilizer addition on AN, b) on AP, c) on STC, d) on C/N, e) on TN, and f) on pH.

Plant N Concentration

Plant N concentration (PNC) was 2.40% of C3 in 2014, significantly greater than that of organic fertilizer treatments M1, M2, and M3 (1.55, 1.49, and 1.36%, respectively; $p < 0.05$). There were no significant differences among CK and organic fertilizer treatments (M1, M2, and M3) for PNC in 2014 (Fig. 5a). In 2015, PNC was 2.52% in C3, greater than that of M1, M2, and M3 (1.63, 1.54, and 1.35%, respectively; $p<0.05$). As in 2014, there were no significant differences among CK

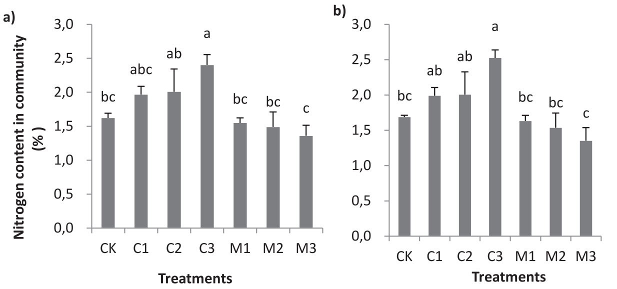

Fig. 5. Effect of fertilizer addition on nitrogen content in community; for each panel, bars not labeled with the same letter indicate significantly different values at $p<0.05$ (based on LSD tests): a) effect of fertilizer addition in 2014 and b) in 2015.

Table 3. N balance of each of fertilizer treatment during 2014-2015. For each column, value (mean±SE) not labeled with the same letter indicates significantly different values at $p<0.05$ (based on LSD tests).

Treatment	N addition (hm^{-2} y^{-1})	Harvest N output (kg hm^{-2})		N Balance (kg hm^{-2})
		2014	2015	
CK	0	23.14±3.12c	20.23±2.31c	-43.37
C1	42.6	41.09±1.87b	48.64±0.63b	-4.54
C2	85.2	46.58±0.82b	58.42±9.14ab	65.41
C3	127.8	59.60±3.55a	68.41±6.96a	127.59
M1	42	23.67±2.75c	22.19±2.14c	38.15
M2	84	25.76±3.76c	22.52±2.82c	119.72
M3	126	24.13±3.47c	23.77±1.46c	204.10

and organic fertilizer treatment (M1, M2, and M3) for PNC in 2015 (Fig. 5b).

N Balance

Harvest N output was 59.60 kg hm^{-2} of C3 in 2014, greater than that of C1 and C2 (41.09 and 46.58 kg hm^{-2}, respectively; $p<0.05$), and greater than that of CK and organic fertilizer treatments ($p<0.05$). The same findings were shown for 2015. The highest value of N balance was 204.10 kg hm^{-2} in M3, the lowest value of N balance was -43.37 kg hm^{-2} in CK during 2014 to 2015 (Table 3). N balance was -4.54 kg hm^{-2} of C1 treatment, of which N input is approximately that of harvest N output.

Discussion

Effect of Fertilizer Addition on AGB and Community Structure

Our results clearly show that fertilizer addition has led to marked differences on AGB and community structure between treatment plots in this temperature grassland (Fig 2-3). Soil AN and AP of CK was 37.71 mg kg^{-1} and 3.99 mg kg^{-1}, respectively, which demonstrates that the original soil is highly infertile. Therefore, the addition of inorganic fertilizer had a marked influence on biomass, which is in accordance with previous research [19-20]. The dominant species is *L. chinensis,* which is a poaceae plant that is very sensitive to N addition. However, the addition of more inorganic fertilizer did not enhance AGB, and there was no significant difference between inorganic fertilizer treatments in 2014. Compared with the C2 treatment, AGB declined in the C3 treatment in 2015. Previous studies have shown that N limitation is tightly coupled with water availability in semiarid grassland ecosystems [10, 25, 32]. The AGB responds more to added N in wet years than in dry years [17, 33-34]. The precipitation was 250.2 mm and 227.8 mm in 2014 and 2015, respectively. Lack of precipitation limited the fertilizer effect on biomass. Moreover, more inorganic fertilizer will cause physiological drought, which limits plant growth. Compared with inorganic fertilizer, organic fertilizer showed no marked influence on biomass; since the nutrition concentration was very low, soil AN and AP cannot increase over such a short period (Fig. 4).

Previous studies have shown that fertilizer addition also results in a declined in plant species diversity [23-24, 26]. In our study, there was no significant difference in the species number in 2014, and only maximum fertilizer C3 showed a significant difference in 2015. The Shannon-Wiener index decreased under inorganic fertilizer treatments, since the fertilizer increased poaceae species growth, producing an overshadowing effect on aboveground, repressing the growth of other species [40-41]. If fertilizer addition is used over the long term, it is likely to cause species losses from the community. The Pielou evenness index decreased with the Shannon-Wiener index since the species numbers did not significantly change over the 2-year experiment, except for the inorganic fertilizer treatment C3 in 2015 (Fig. 3).

Effect of Fertilizer Addition on Soil Properties

Fertilizer addition changed soil properties. Although the inorganic fertilizer can increase soil AN and AP quickly, but not significantly change STC and soil C/N ratio, organic fertilizer increases STC and C/N ratio but not soil AN and AP (Figs 4a-d). This is because inorganic fertilizer has a higher nutrition concentration and the nutrition in the inorganic form, which is easily taken into solution in the soil and absorbed by plants. The organic fertilizer provides nutrients in organic form, most of which must be transformed into an inorganic form before plant absorption and utilization. Moreover, organic fertilizer contains a large amount of organic matter, resulting in soil carbon content and C/N ratio increase which can promote soil microbial processes and soil enzyme activity [27-31], enhancing soil fertility. Both inorganic fertilizer and organic fertilizer cannot significantly change the soil total N over the course of 2 years (Fig. 4e). In our study, we also found that soil pH of inorganic fertilizer treatment showed a declining tendency compared with that of organic fertilizer (Fig. 4f), which supports some previous research [17, 25], organic fertilizer can increase the soil buffering capacity to resist soil acidification due to the presence of bivalent cations (Ca^{2+} and Mg^{2+} in manure), and/or the oxidation of organic anions that will consume H^+ ions during manure decomposition [42].

Effect of Fertilizer Addition on N Balance

Maintenance of the N balance is very important in ecosystem sustainable management. Although a lack of soil N nutrition will limit plant productivity, adding it could threaten the natural environment, causing problems such as nutrient leaching, groundwater contamination, and eutrophication [17, 43-45]. The CK treatment carried out 43.37 N kg hm^{-2} from the soil by harvest removal, which resulted in AN decrease and limited plant growth. The C1 treatment carried out 89.73 N kg hm^{-2}, the N balance was -4.54 N kg hm^{-2}, which also increased soil AN reach to 54.82 mg kg^{-1}. The inorganic treatments C2 and C3 released 65.41 and 127.59 N kg hm^{-2} to the environment, respectively, which represents a potential environmental risk. In addition, the C2 and C3 treatments did not significantly increase the AGB in our study. Although organic treatment releases more N to the soil, it also provides plentiful organic matter to the soil, which increases the STC and C/N ratio, benefiting soil fertility.

Our research findings therefore present a choice. Inorganic fertilizer may be applied at 42.6 N kg hm^{-2} to temperate grassland when local people need to increase the AGB in order to supply more hay feed to the animals in the winter and guarantee the livelihood of herds. However, we do not recommend this fertilizer approach continuously, as it will decrease community diversity and evenness. In addition, N limitation is tightly coupled with water availability in semiarid grassland ecosystems and precipitation distribution is erratic; therefore, inorganic fertilizer addition may represent a considerable environmental risk.

Conclusions

Inorganic fertilizer addition increased AGB, but decreased diversity and evenness of the temperate grassland community. Organic fertilizer addition increased STC and C/N ratio, but had little influence on AGB and soil available nutrition over the 2 years of our experiment. Our research demonstrated that using fertilizer addition to restore temperature grassland steppe need to comprehensively evaluate the diverse services functions.

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