



1	Nitrogen and Warming Control the Vegetation in Inner Mongolia Tourist Area
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3	Qiong Sun <sup>(1)</sup> , Xiaobing Hu <sup>(2)</sup> , Chi Zhang <sup>(1,3)</sup>
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5	(1) Tourism Institute of Beijing Union University, Beijing, 100101, China
6	(2) School of Engineering, University of Warwick, Coventry, CV4 7AL, United Kingdom.
7	(3) Centre for Creative Computing, Bath Spa University, Corsham SN13 0BZ, UK
8	
9	ABSTRACT
10	The global warming and atmospheric nitrogen deposition problem has become more and more serious
11	under the influence of human activities, and it has become one of the hot issues in this field, which will
12	have far-reaching impact on all kinds of vegetation, thus the functioning of the ecosystem will be
13	changed, which will be reflected in climate warming process. Inner Mongolia Autonomous Region is
14	mainly composed of desert grasslands, so the development and protection of vegetation has
15	considerable significance on the region. However, in the current environment of global warming, few
16	studies have been carried out on desert grassland plants. In this paper, an in-depth study was carried
17	out on the impact of warming and nitrogen addition on soil temperature, vegetation reproductive
18	phenology and vegetation community seed rain under natural conditions during five-year period from
19	2011 to 2015. During the experimental period, we found that soil temperature and soil moisture
20	decreased with the increase of soil layer, and warming obviously shortened the time of budding,
21	flowering and fruiting of vegetation. However, no significant effect was found on nitrogen addition.
22	Meanwhile, the impact of interaction effect of warming and nitrogen addition on seed rain was not
23	obvious, but the year difference of all relevant indicators was significant.

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25 Key words: Inner Mongolia; desert grassland; warming; nitrogen addition; vegetation

#### 26 INTRODUCTION

27 Since 2000, with the increase in population, rapid economic development and increasing human 28 activities, there appear global environmental changes, including global warming, carbon dioxide 29 concentration increase, global nitrogen deposition (Zaldívar & Sanz, 2014; Oliva et al., 2014), which to 30 some extent, affected the original ecosystem of various species (Buendia et al., 2015; Liu et al., 2014). 31 The United Nations Intergovernmental Panel on Climate Change summarized on its fourth work report in 32 2007 that in the past few decades, earth's surface temperature increased by about 0.6 degrees Celsius. 33 Meanwhile, it was estimated that average global temperature would increase by a minimum of 1.8 34 degrees Celsius in the next 2100 years. With the harsh temperature changes, nitrogen settlement of the 35 Earth's surface is also facing a grim situation, which increased by at least 1 time. Moreover, estimated 36 based on the model of Elisabeth, in the next few decades, with the increase of various nitrogen 37 complexes with different activities, nitrogen settlement amount will continue to increase (Lin et al., 2011). 38 The rising temperature and nitrogen settlement has great impact on the growth of plants. It will change 39 the relationship between the various species, and thus affect the vegetation. Apart from the impact on





40 community, reproductive phenology and seeds of vegetation, it will also cause indirect effects on plants 41 through changing soil temperature, soil moisture and spring snowmelt time. There are many scholars 42 who had studied in these areas, and these to some extent appeal to human to get the warning that we should protect the environment. For example, Xiaocheng Wen and Guangxin Lu put forward that 43 44 warming and nitrogen addition and interaction effect of the two changed the height of alpine grassland 45 plant communities and the fresh weight of aboveground biomass (Wen & Lu, 2015). Hongyu Guo, Wei 46 Wang and Guangxin Lu (2015) studied on how plant growth was influenced by warming and nitrogen 47 precipitation increase. While Jianan Cui, Yuexi Chen and Hui Sun (2015) studied on how warming and 48 nitrogen addition affected the mineral elements release of sequoia fresh litter. As one of the necessary 49 nutrients for the growth of plants and a kind of important fertilizer in the earth, nitrogen has drawn 50 extensive attention from experts. Abbasi et al. performed a study to determine the nitrogen-release 51 potential of residues added to soil and indicated that nitrogen concentration was an indicator for the 52 mineralization of organic residues (Abbasi et al., 2015). Yu and Jia observed the increase of soil organic 53 carbon (SOC) and total nitrogen (TN) in the soil and revealed that root biomass could increase SOC and 54 TN (Yu & Jia, 2014). Scharenbroch et al. proved that nitrogen availability of soil could promote the 55 growth of trees (Scharenbroch et al., 2013). 56 This paper carries out an in-depth analysis on three aspects of soil temperature, vegetation reproductive

56 This paper carries out an in-depth analysis on three aspects of soil temperature, vegetation reproductive 57 phenology and vegetation seed rain based on the results obtained from the experiments during 2011 -58 2015. It analyzes what impact will be brought by man-made warming and nitrogen addition to plants 59 under natural conditions, so as to estimate the crisis which Inner Mongolia grassland ecosystem may 50 face when there is natural environmental warming and increase of amount of nitrogen precipitation. Also, 51 it can be taken as a warning to call upon humans that we should protect the environment.

### 62 MATERIALS AND METHODS

In this experiment, we selected a base located in south central part of Inner Mongolia Ordos Grassland, with a total area of 33,000 acres and a large part of the base are natural grasslands (Johansson et al., 2008). Then we chose an area of desert grasslands and farming-grazing zone as the base of this experiment since this region is relatively local with regional characteristics. The desert grasslands in that region belong to the grassland vegetation subtype with strongest xerophytism among temperate grassland vegetation (Urano et al., 2000), so it is a very suitable base for this experiment.

We carried out a five-year experiment on Inner Mongolia Ordos Grassland from March 2011 to March 2015. Firstly, a warming control device was set up for the quadrat. In the five consecutive years, we conducted a soil warming and nitrogen addition process. Take the soil with 10 cm, 20 cm and 30 cm surface layer as samples and make comparison. Meanwhile, record the relevant data on vegetation reproductive phenology changes during 2011- 2013. Also, analyze the seeds obtained by the 64 seed rain collectors in the five years, and the following can be concluded based on the analysis on its temperature and humidity.

According to the test project, a control test on simulated warming and nitrogen addition under wild natural conditions was carried out. In this test, warming was taken as a simulated global climate change factor and nitrogen addition was taken as a man-made interference factor of global nitrogen settlement. The design was divided into four kinds of treatment experiments which were warming treatment (W), nitrogen fertilizer treatment (F), warming and nitrogen fertilizer treatment (WF) and control treatment (





81 without warming or nitrogen fertilizer treatment, C) respectively. Each treatment was repeated six times 82 and 24 treatment plots were included. The designed area of each plot was 8 m<sup>2</sup> (4mx2 m) and the total 83 area of the test was 192 m<sup>2</sup>. All treatment groups in the plot were equally distributed in order to avoid the 84 influence of direction and orientation on test results. Infrared heating method was adopted for simulated 85 warming in the test. The infrared radiator, with a length 1.5 m and a width of 18 cm, was hung 2.75 m 86 high above the ground through a steel pipe with a diameter of 4.6 cm. The maximum power of the 87 infrared radiator was 2250 watt and the gear was adjusted to 8-speed when used. In order to avoid test 88 errors caused by shade effect of infrared radiator as well as block effect of infrared ray on rainfall, false 89 radiators of the same shape and size were installed at the same position of the same height as the real 90 ones for treatment groups which didn't require warming. Ammonium nitrate, whose chemical name is 91 NH<sub>4</sub>NO<sub>3</sub>, was selected as the nitrogen fertilizer, with a nitrogen content of 33.7%. The application time of 92 nitrogen fertilizer was during the end of June and beginning of July when the rainy season approaches 93 annually.

94 The test project began from March 2011 and the infrared heating test never stopped or was interrupted 95 for even a single day all year round from the beginning to the duration of this test.

### 96 RESULTS

97 When only soil warming was conducted, temperature increased respectively by 1.29 °C, 0.83 °C, 0.80 °C and 0.71 °C; when only nitrogen addition was conducted, the temperature of the surface layer 99 decreased by 0.18 °C, soil temperature decreased by 0.05 °C, 0.03 °C and 0.01 °C respectively at 10 100 cm, 20 cm and 30 cm layer. However, when both warming and nitrogen addition were conducted, soil 101 temperature of each layer increased respectively by 1.11 °C, 0.78 °C, 0.77 °C and 0.70 °C.

Through repeated variance measurement, it is found that all of the four dominant species were affected by warming and nitrogen addition in the three growing seasons during 2011-2013 in the experiment. And for the time of duration of budding, flowering, fructification and reproductive growth of vegetation, the shortening of average time is more obvious under the warming situation (figure 1); while the shortening of average time under nitrogen addition situation is not obvious (figure 1). When under both warming and nitrogen addition situation, the shortening of average time is less obvious than that under mere warming situation (figure 1).

109 Through the observation on data results of the impact of warming and nitrogen addition and the 110 interaction of the two on budding time of various species during the three growing seasons from 2011 to 111 2013, it is found that impact of warming varies on different vegetation species, i.e. there exists the 112 specificity; while for the same vegetation, there presents the difference on impact of warming in different 113 years. Seen from table 1, the budding time of breviflora griseb, convolvulus ammannii and bassia 114 prostrate was shortened significantly due to warming process in 2011. While in 2012, the budding time of 115 breviflora griseb and allium tenuissimum was shortened significantly under warming treatment condition. 116 And in 2013, the budding time of allium tenuissimum and bassia prostrate was shortened significantly 117 due to warming treatment. 118 At the same time, we also analyzed the experimental data of flowering time of each species in each

growing season under both warming and nitrogen addition treatment during 2011-2013. The results show that the response of flowering time of each species to warming is similar with that of budding time;





121 there is also specificity in response of each species to warming. For example, in 2011, the flowering time 122 of stipa breviflora and bassia prostrate was significantly reduced under the influence of warming, but no 123 obvious effect was found on the flowering time of convolvulus ammannii and allium tenuissimum. In 124 2012, only the flowering time of allium tenuissimum was shortened obviously under the influence of 125 warming. While according to the data of 2013, warming has no significant effect on the flowering time of 126 all the species (table 2). Therefore, interaction effect of nitrogen addition and warming has little impact 127 on the flowering time of various species. 128 From the analysis of data results of fructification time of various species under both warming and

129 nitrogen addition treatment in the three growing seasons during 2011-2013, each species' response to 130 warming has the specificity, that is to say, in the same year, the results affected by warming have 131 different performance in different species. Similarly, for the vegetation itself, its response to warming 132 varies from year to year. In 2011, under the man-made warming treatment, the fructification time of stipa 133 breviflora, convolvulus ammannii and bassia prostrate was significantly shortened. In 2012, there was 134 significant shortening in fructification time of allium tenuissimum due to warming treatment; while seen 135 from table 3, fructification time of stipa breviflora was significantly shortened only by warming in 2013. 136 The response of each species to nitrogen addition conforms to the same law. There is no significant 137 effect on each species in each year.

138 From the analysis of data results of duration of reproductive growth of various species under both 139 man-made war ming and nitrogen addition treatment in the three growing seasons during 2011-2013, we 140 find that no matter what year it is, there is no significant effect of warming and nitrogen addition on 141 reproductive growth duration of convolvulus ammannii and allium tenuissimum. However, there is a year 142 difference on the effect of warming on the reproductive growth duration of stipa breviflora and bassia 143 prostrate. As can be seen from the table, in 2011 and 2012, the reproductive growth duration of stipa 144 breviflora was significantly shortened under the influence of warming; while in 2012, no significant effect 145 was found on the reproductive growth duration of stipa breviflora and bassia prostrate under the 146 influence of warming; in 2013, a significant shortening of the reproductive growth duration of bassia 147 prostrate was found, as seen in table 4.

148 During the five year period of growing seasons from 2011 to 2015, the 72 seed rain collectors on the 149 base collected altogether 147,757.19 seeds. The average density of the seed rain is 1229.6 ± 145.8 150 grains (Mean±SE), and all the seeds belong to 6 families, 8 genera and 8 species. In table 5, there is 151 detailed record of the seed rain information, including stipa breviflora, cleistogenes songorica and 152 wheatgrass, which are perennial bunch grass belonging to grass family. And stipa breviflora and 153 cleistogenes songorica are the vegetation which contribute most to seed rain with rate of contribution of 154 22.37 ± 2% and 13.11 ± 1% respectively. There are 2 families, 2 genuses and 2 species of perennial 155 herbs which are called allium tenuissimum and convolvulus ammannii. Among all the observed species, 156 allium tenuissimum (its contribution rate is 37.92 ± 2%) makes the greatest contribution to the quantity of 157 seeds rain, and the contribution rate of subshrub bassia prostrate is 14.86 ± 3%. As for annual herbs and 158 biennial herbs, their contribution rates are lower compared with those of the former ones and appear 159 randomly in different years.

Figure 2~6 show the impact of warming and nitrogen addition on seed rain density of cleistogenes
 songorica, allium tenuissimum, bassia prostrate, stipa breviflora and plant community.





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# 163 DISCUSSION

164 Under the circumstances of global warming, in order to study the functional mechanism between 165 ecosystem and climate warming, various climate warming simulation tests were adopted, including 166 resistance heating, far-infrared irradiation, top-open and top-close field greenhouse (Choi et al., 2012), 167 intercross transplantation etc. In this study, warming through infrared radiation was carried out under wild 168 natural conditions. Through the test, we found that soil temperature increased while soil humidity decreased after warming, suggesting that the warming test had a warming effect. However, the warming 169 170 effect and amplitude of this study is lower than that of the top-open warming method, which is mainly due 171 to the difference of warming methods. Far infrared warming method can keep the climatic factors of 172 sample plot such as natural air, wind speed, rainfall, temperature and humidity basically the same as the 173 natural climate conditions of the desert steppe, which minimizes the interference of human factors 174 caused by the warming method based on short-term control of air flow (Murakami et al., 2000), thus can 175 truly and objectively reflect and simulate the influence of climate warming on desert steppe.

176 Soil respiration (Nordgren et al., 2001) is a very complex soil ecological process. To some extent, 177 temperature is considered as the most important factor which influences total soil respiration rate. 178 However, in this study, no significant difference was found on soil respiration rate between warming 179 group and control group, no matter on seasonal change or on daily variation. For small area of sample 180 plot, the response of soil respiration to soil temperature changes showed great uncertainty, which may 181 be related to the spatial difference of water conditions, soil nutrient (Drechsel et al., 2001), biomass of 182 plants and microorganisms, etc, in addition to the short experimental time and low increasing extent of 183 temperature. Soil respiration is a very complicated biogeochemical process and not only the above 184 factors can affect directly or indirectly the generation and emissions of carbon dioxide in soil but also the 185 status and role of these factors will have corresponding changes with the change of temperature. Under 186 certain conditions, they may have modification, correction, and even cover-up effect on the effect of 187 temperature.

188 The far infrared warming test caused changes in dominant species and composition of the community 189 instead of changes of plant community. This may be because that far infrared warming effect, to some 190 extent, meets the demand for heat of the plant and changes the microclimate environment of plant 191 community and therefore influences the growth and development of plant to a certain degree (Hu et al., 192 2015). Besides, warming has also changed the content of moisture in soil and influenced moisture 193 absorbing of plant. The growth and biomass of the plant are also affected to varying degrees. The results 194 showed that, for any kind of plant community under the environment of global warming, there are always 195 some species whose response to temperature rises are more sensitive, so as to destroy the interspecific 196 competition and cause changes in community dominant species and composition.

According to some experts, soil moisture decrease caused by warming can prevent the growth of superficial roots, increase root mortality and enhance plant respiration, thus lower the net primary productivity of plants. Warming changed the distribution of the underground biomass (Singh et al., 2014). Compared with the control sample plot, underground biomass decreased in 0 ~ 10 cm soil layer of the warming sample plot while that in 10 ~ 20 cm soil layer increased slightly and the underground biomass





202 increased significantly in 20 ~ 30 cm soil layer, which is also related to soil moisture decrease caused by

203 warming. Since warming reduces soil moisture, water becomes the most key factor limiting plant growth.

204 In order to better adapt to the environment, plant roots extend to a deeper level for water, thus the 205 underground biomass transfers to deep soil.

Nitrogen is one of the most needed nutrients (Parras-Alcántara et al., 2013) for plant growth in terrestrial ecosystem. The addition of nitrogen increases soil mineral nutrients and improves carbon nitrogen ratio in soil. Moreover, it can improve microbial activity and increase supply of soil respiration, so as to promote the soil respiration. The result showed that soil respiration rate of nitrogen fertilizer sample plot was higher than that of the control sample plot. However, statistics showed that the difference was not significant, indicating no effect of nitrogen in soil respiration.

Though the addition of nitrogen changed the height, density, coverage and frequency as well as important value of some species of plants, it increased the above-ground biomass of plant species and plant functional groups, suggesting that the increase of above-ground biomass is not related with the addition of nitrogen, instead, it may be related to water conditions, soil environment and affinity interaction of the environment of the year.

217 Seed rain density of the four main species which make major contribution to plant community was 218 analyzed. From the perspective the plant community level, we conclude that the seed rain density will not 219 be significantly affected due to the warming and nitrogen addition process. However, there is significant 220 difference in years, which is mainly reflected in 2012, 2013 and 2015. From the perspective of species 221 level, these plants were not significantly affected (expect bassia prostrate), but difference between years 222 of the four species are significant. For different species, their response to years is different from each 223 other. The largest seed rain of stipa breviflora occurred in 2014 and 2015 while that of cleistogenes 224 songorica occurred in 2011, 2013 and 2015. The largest seed rain of allium tenuissimum occurred during 225 2011-2013 while that of bassia prostrate occurred in 2012, 2013 and 2015. Take the year as the statistic 226 unit and analyze the impact of interaction effect of warming and nitrogen addition on seed rain density 227 from 2011 to 2015 (figure 2). Under the condition of warming and nitrogen addition, the seed rain density 228 of cleistogenes songorica and plant community was not affected. But a significant influence can be found 229 on allium tenuissimum, bassia prostrate and stipa breviflora in some years. Besides, significant effect 230 can also be found on the seed rain of allium tenuissimum and bassia prostrate. In addition, when 231 comparing the impact of warming and nitrogen addition on seed rain of stipa breviflora respectively, a 232 significant difference is found.

233 Through the comparison on seed rain of plant community and the fastigium species number of 234 aboveground vegetation and the analysis of the similarity index applying three-factor analysis of 235 variance, the results show that aboveground vegetation species number decreased significantly under 236 the warming condition. However, from the perspective of seed rain species number and shared species 237 number, there is no significant impact. Still there are significant differences between years (Stott et al., 238 2014). The maximum and minimum species number of aboveground vegetation was found in 2012 and 239 2014 and there is significant difference between them. Besides, there is no significant difference in the 240 similarity index of Sorense and Jaccard under both warming and nitrogen addition conditions.

241 CONCLUSION





- 242 This study takes the stipa breviflora desert grassland in Siziwang Banner, Inner Mongolia as the object 243 of study, carried out a five-year research by applying the method of man-made warming and artificial 244 simulation of atmospheric nitrogen settlement, combined with field survey and ecosystem control test. 245 And an in-depth understanding was obtained through the aspect of soil temperature and humidity, 246 reproductive phenology of vegetation, as well as seed rain. However, because desert steppe ecosystem 247 response to climate change involves many aspects and is a process of multi-channel interaction, there 248 exist some flaws in the results of the research, and there are some factors of uncertainty and ambiguity 249 as well in the study, which still needs further improvement in future researches.
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average time of duration of budding, flowering, fructification and reproductive growth of four dominantspecies in the three growing seasons during 2011-2013





316 Fig. 2 Impact of warming and nitrogen addition on seed rain density of cleistogenes songorica









318 Fig. 3 Impact of warming and nitrogen addition on seed rain density of allium tenuissimum



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Fig. 4 Impact of warming and nitrogen addition on seed rain density of bassia prostrate





323 Fig. 5 Impact of warming and nitrogen addition on seed rain density of stipa breviflora









325 Fig. 6 Impact of warming and nitrogen addition on seed rain density of plant community

327 Table 1 Two-factor variance analysis results of budding time of the four main species under nitrogen

328	addtion,	warming treatment	and the intereaction	effect of the two in	growing seasons f	rom 2011 to 2013
	,	0			0 0	

Year	Source of variation	plant species			
		Sb	Ca	At	Кр
	Warming	0.005	0.031	0.148	<0.001
2011	Nitrogen	0.159	0.588	0.262	0.459
	W*N	0.309	0.194	0.062	0.153
	W	0.008	0.231	0.004	0.166
2012	N	0.666	0.546	0.474	0.959
	W*N	0.619	0.816	0.125	0.815
	W	0.210	0.132	0.035	<0.001
2013	N	0.704	0.731	0.120	0.431
	W*N	0.634	0.977	0.680	0.537





- 340 Table 2 Two-factor variance analysis results of flowering time of four main species under interaction
- 341 effect of nitrogen addition and warming in three growing seasons during 2011-2013

year	Source of variation	plant species			
		Sb	Са	At	Кр
	Warming	0.007	0.054	0.121	<0.001
2010	Nitrogen	0.148	0.783	0.411	Kp           <0.001
	W*N	0.950	0.204	0.091	0.118
	W	0.213	0.289	0.003	0.116
Sb           2010         Warming         0.007           2010         Nitrogen         0.148           W*N         0.950           2011         W         0.213           2011         N         0.471           W*N         0.192           W         0.089           2012         N         0.845           W*N         0.630	N	0.471	0.229	0.512	0.485
	0.192	0.575	0.191	0.761	
	W	0.089	0.123	0.081	0.059
2012	N	0.845	0.286	0.671	0.163
	W*N	0.630	0.436	0.829	0.487

343	Table 3	Two-factor	variance	analysis	results	of	fructification	time	of	four	main	species	under	the

3//	intoraction offect of warming	and nitrogon addition in t	broo growing soasons during 2011 to 2013
544	Interaction enect of warming	j anu muoyen auullon mu	lifee growing seasons during 2011 to 2013

Year	Source of	Plant species					
	variation	Sb	Ca	At	Кр		
	Warming	0.015	0.023	0.101	<0.001		
2011	Nitrogen	0.122	0.653	0.734	0.091		
	W*N	0.343	0.262	0.172	0.112		
	W	0.983	0.556	0.012	0.240		
2012	Ν	0.389	0.156	0.550	0.117		
	W*N	0.083	0.923	0.520	0.013		
	W	0.015	0.811	0.089	0.632		
2013	Ν	0.623	0.980	0.158	0.801		
	W*N	0.711	0.836	0.739	0.881		





- 357 Table 4 Two-factor variance analysis results of reproductive growth duration of four main species under
- 358 the interaction effect of warming and nitrogen addition in three growing seasons during 2011-2013

year	Source of	Plant species					
	variation	Sb	Са	At	Кр		
	Warming	0.003	0.405	0.139	0.736		
2011	Nitrogen	0.374	0.385	0.189	0.965		
	W*N	0.054	0.959	0.871	0.545		
	W	0.009	0.269	0.359	0.291		
2012	N	0.989	0.112	0.605	0.831		
	W*N	0.379	0.602	0.125	0.511		
	W	0.105	0.037	0.653	<0.001		
2013	N	0.022	0.146	0.027	0.931		
	W*N	0.604	0.545	0.222	0.139		

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361 Table 5 The basic information of plant species and seed rain percentage

Species name	Latin name	Life form	Seed percentage (%)
Cleistogenes songorica	Cleistogenes songorica	Perennial hydrophobic cluster grass	13.11±1
allium tenuissimum	Allium tenuissimum	perennial herb	37.92±2
Bassia prostrata	Kochia prostrata	subshrub	14.86±3
Stipa breviflora	Stipabreiflora	Perennial bunch grass	22.37±2
Neopallasia pectinata	Neopallasiapectinata	Annual and biennial	2.11±1
Agropyron cristatum	Agropyron cristatum	Perennial clustered	0.98±0.5
Common Russianthistle	Salsola collina	Annual herb	7.96±0.3
Convolvulus ammannii	Convoivuius ammannii	perennial herb	1.12±0.1

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