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# **Response of Plant Community Carbon and Nitrogen Stoichiometry** to Experimental Warming on the Qinghai-Tibet Plateau

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**Abstract:** Low temperature is an important limiting factor for alpine ecosystems on the Tibetan Plateau. This study is based on data from on-site experimental warming platforms (open top chambers, OTC) at three elevations (4300 m, 4500 m, 4700 m) on the Qinghai-Tibet Plateau. The carbon and nitrogen stoichiometry characteristics of plant communities, both above-ground and below-ground, were observed in three alpine meadow ecosystems in August and September of 2011 and August of 2012. Experimental warming significantly increased above-ground nitrogen content by 21.4% in September 2011 at 4500 m, and reduced above-ground carbon content by 3.9% in August 2012 at 4300 m. Experimental warming significantly increased below-ground carbon content by 5.5% in August 2011 at 4500 m, and the below-ground ratio of carbon to nitrogen by 28.0% in September 2011 at 4300 m, but reduced below-ground nitrogen content by 15.7% in September 2011 at 4700 m, below-ground carbon content by 34.3% in August 2012 at 4700 m, and the below-ground ratio of carbon to nitrogen by 37.9% in August 2012 at 4700 m. Experimental warming had no significant effect on the characteristics of community carbon and nitrogen stoichiometry under other conditions. Therefore, experimental warming had inconsistent effects on the carbon and nitrogen and nitrogen stoichiometry of plant communities at different elevations and during different months. Soil ammonium nitrogen and nitrate nitrogen content were the main factors affecting plant community carbon and nitrogen stoichiometry.

Key words: Damxung County; alpine meadow; open top chambers; carbon and nitrogen stoichiometry

### 1 Introduction

Nitrogen is one of the most important limiting nutrient factors in terrestrial ecosystems and plays an important role in the carbon cycle and climate change (Nicolas and James, 2008). The carbon content of plant communities is closely related to photosynthesis. Increases in net  $CO_2$  absorption by plants slow down the impact greenhouse gases have on climate warming (Day et al., 2008). The carbon-nitrogen ratio (C: N) can reflect the relationship between carbon and nitrogen. The coupling relationship that exists between carbon and nitrogen affects plant growth. If an increase in carbon content is not accompanied by a change of nitrogen, the result may be nitrogen restrictions for plants (Luo et al., 2004). Moreover, C: N can directly affect the functions of terrestrial ecosystems (Xiong et al., 2015). Climate warming, which is an obvious feature of climate change, can change the growth rate and metabolism of plants (Fu et al., 2015a); such changes, in turn, affect the carbon and nitrogen metrology of plants. Therefore, it is very important to study the effect of climate warming on the carbon and nitrogen me-

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trology of plant communities.

Effects of climate warming on ecosystem carbon and nitrogen content and pools vary with ecosystem types (Dou et al., 2010; Mau et al., 2018). The fact that climate warming effects vary may be attributable to the following reasons. Firstly, species composition and diversity vary among ecosystems (Li et al., 2015). Secondly, the duration of warming can impact the effect the warming has on the growth of individual plants (Arft et al., 1999). Thirdly, ecosystems have growing seasons of different lengths. Warming leads to changes in plant phenology and helps to determine the length of time plants grow. (Rustad et al., 2001). The Qinghai-Tibet Plateau is the world's highest plateau and has unique alpine ecosystems that are more sensitive to climate change than ecosystems elsewhere. Therefore, the impact of warming on the carbon and nitrogen stoichiometry in alpine meadows on the Qinghai-Tibet Plateau may be different than that occurring in other areas due to the unique community composition and elevation.

There have been numerous studies of the effects of warming on carbon and nitrogen in alpine meadows. For example, Zhang found that soil microbial biomass (MBC) and soil microbial nitrogen (MBN) showed stronger positive responses to warming in colder environments, and that warming may have no significant impact on soil carbon and nitrogen pools (Zhang et al., 2015). Previous studies focused on the response of soil carbon and nitrogen to warming in alpine meadows, but there has been little research on the response of plant community carbon and nitrogen to the warming of alpine meadows (Fu et al., 2015b). In this study, the carbon and nitrogen content of plant communities were measured in alpine meadows around the grassland station of Damxung county, Lhasa city, Tibet Autonomous Region. The measurements took place in August and September 2011 and in August 2012. The research results revealed the impact of global climate change on the production processes and functions of grassland ecosystems.

#### 2 Materials and methods

#### 2.1 Study area

This study was conducted at the grassland station of Damxung County, Lhasa City, Tibet Autonomous Region (90°04′E, 30°30′N), located on the southern edge of Tanglha Mountain. The study area has a continental plateau monsoon climate, with stronger total radiation from the sun, lower temperatures, a larger daily range of temperatures, and a smaller annual range of temperatures than other plains. Precipitation is mainly concentrated in June-August. The main vegetation types are typical to alpine meadows, and the main soil types are alpine meadow soils. According to the observation data of Damxung county from 1963 to 2010, the average annual temperature is  $1.8^{\circ}$ C, the hottest month is July (average  $11.0^{\circ}$ C) and the coldest month is January (average  $-9.1^{\circ}$ C). Observed monthly temperature and precipitation are shown in Table 1.

Table 1Monthly temperature and precipitation in DamxungCounty

Observation	Air temperature (°C)			Precipitation (mm)			
	4300 m	4500 m	4700 m	4300 m	4500 m	4700 m	
2011-08	11.41	10.11	9.01	56.81	57.52	58.18	
2011-09	10.49	9.29	8.29	58.14	59.95	61.04	
2012-08	12.02	10.80	9.76	80.53	83.40	85.91	

# 2.2 Experiment design and determination of carbon and nitrogen contents in plant communities

In 2008, three fenced plots were established at three elevations (4300 m, 4500 m, 4700 m) along the base of Nyenchen Mountain. Four sets of paired open top chambers (OTC) and control quadrats were randomly set within the fenced plots.

In August and September 2011 and August 2012, at each elevation, three pairs of OTCs and control samples were randomly selected, and the ground tissues of the plants within an area of 0.5 m×0.5 m were clipped. Open top chambers (bottom diameter: 1.45 m; top diameter: 1.00 m; height: 0.40 m) were used to increase temperature. For each plot, a 3.7-cm soil auger was used to collect soil samples (0-20 cm). Experimental warming increased average temperatures of growing season soil by 1.13, 1.34, and 1.09°C, and air temperatures by 1.04, 1.41, and 1.01°C at the 4300 m, 4500 m and 4700 m elevations, respectively. At the same time, the soil humidity of 0.05, 0.04, and 0.05  $\text{m}^3 \cdot \text{m}^{-3}$  was significantly reduced (Fu et al., 2013). The visible roots were cleaned, and the above-ground parts of the plants were placed in an oven at 65°C for 48 hours to constant weight. Then the plant samples were pulverized in a pulverizing device and ground with a ball mill instrument for 30 seconds. After the samples were pulverized, above-ground and below-ground carbon and nitrogen contents were determined by an Elementar Variomax CN. Soil inorganic nitrogen (ammonium nitrogen and nitrate nitrogen) was determined by LACHAT Quickchem Automated lon Analyzer.

#### 2.3 Statistical analysis

Based on the analysis of repeated measures of variance, the effects of experimental warming in the observation months on carbon content, nitrogen content, and the C:N of above-ground and below-ground parts of seedlings were investigated. Based on the independent *t*-test, the effects of experimental warming on the carbon content, nitrogen content and C: N of the above-ground and belowground parts were investigated. Variation partitioning analyses (VPA) and regression analysis were used to investigate the causes of changes in plant community carbon and nitrogen. All statistical analyses were performed using R 3.5.2 software and SPSS 22. All figures were generated with Sigmaplot 12.5.

### 3 Results

# 3.1 The response of carbon and nitrogen metrology to warming

The analysis of repeated measures of variance showed that warming had no significant effects on above-ground carbon and nitrogen metrology (Table 2). The independent *t*-test analysis showed that warming significantly increased the above-ground nitrogen content by 21.4% (3.57 g kg<sup>-1</sup>) in September 2011 at 4500 m, but significantly decreased the above-ground carbon content by 3.9% (15.66 g kg<sup>-1</sup>) in August 2012 at 4300 m (Fig. 1). Analysis of repeated measures of variance showed that warming significantly reduced the below-ground carbon content by 15.75% (65.51 g kg<sup>-1</sup>) at 4700 m (Table 3).

The independent *t*-test analysis showed that warming significantly increased the below-ground carbon content by 5.5% (23.25 g kg<sup>-1</sup>) in August 2011 at 4500 m, and the below-ground C:N by 28.0% (8.30) in September 2011 at 4300 m. Warming significantly decreased below-ground nitrogen content by 15.7% (1.27 g kg<sup>-1</sup>) in September 2011 at 4700 m, below-ground carbon content by 34.3% (146.88 g kg<sup>-1</sup>) in August 2012 at 4700 m and the belowground C:N by 37.9% (26.00) in August 2012 at 4700 m (Fig. 2).

# 3.2 Relationship between carbon and nitrogen metrology and environmental factors

Warming increased the soil  $NH_4^+$ -N content in August 2012 at 4300 m, but decreased soil  $NO_3^-$ -N content in September 2011 at 4500 m and August 2012 at 4300 m (Fig. 3).

Table 2 Analysis of repeated measures of variance for the effects of experimental warming and the observation month were taken on carbon content, nitrogen content and the ratio of carbon to nitrogen for the above-ground parts of plant communities.

Elevation	Model	Nitrogen content		Carbon content		C/N	
		F	Р	F	Р	F	Р
4300 m	Warming(W)	0.96	0.382	3.41	0.139	3.84	0.122
	Month(M)	13.44	0.003	4.09	0.060	18.93	0.001
	W×M	0.78	0.492	0.25	0.788	0.40	0.682
4500 m	Warming(W)	0.47	0.532	0.01	0.948	1.25	0.327
	Month(M)	40.52	0.000	4.50	0.049	63.79	0.000
	W×M	2.34	0.159	1.26	0.336	7.71	0.014
4700 m	Warming(W)	0.34	0.593	0.02	0.906	1.61	0.273
	Month(M)	53.72	0.000	18.31	0.001	103.77	0.000
	W×M	5.17	0.036	0.23	0.800	6.29	0.023

Note: F: f-valued; P: significance probability.

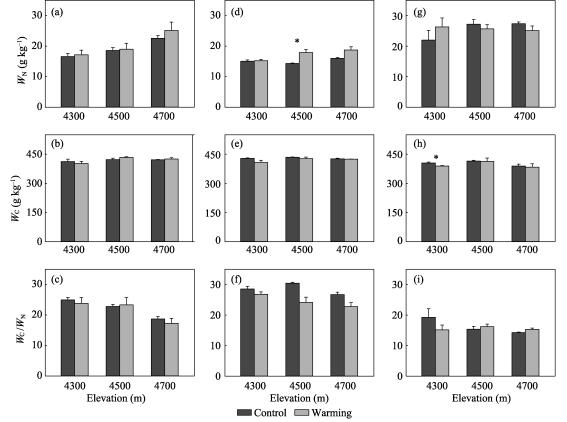


Fig. 1 Effects of experimental warming on the carbon content, nitrogen content and the ratio of carbon to nitrogen for the above-ground parts of plant communities in alpine meadows at elevations 4300 m, 4500 m and 4700 m in August 2011 (a, b, c), September 2011 (d, e, f) and August 2012 (g, h, i)

Note: Values with "\*" show significant differences in the carbon and nitrogen content after experimental warming at the 0.05 level.

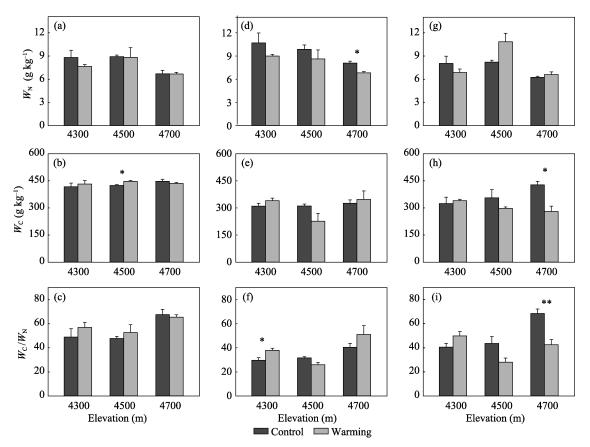


Fig. 2 Effects of experimental warming on the carbon content, nitrogen content and the ratio of carbon to nitrogen for the below-ground parts of plant communities in alpine meadows at elevations 4300 m, 4500 m and 4700 m in August 2011 (a, b, c), September 2011 (d, e, f) and August 2012 (g, h, i)

Note: Values with "\*" "\*\*" show significant differences in the carbon and nitrogen content after experimental warming at the 0.05 level and 0.01 level, respectively.

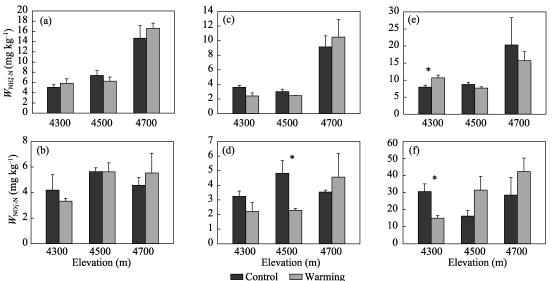


Fig. 3 Effects of experimental warming on soil NH<sub>4</sub><sup>+</sup>-N and NO<sub>3</sub><sup>-</sup>-N in alpine meadows at elevations 4300 m, 4500 m and 4700 m in August 2011 (a, b), September 2011 (c, d) and August 2012 (e, f) Note: Values with "\*" show significant differences in the NH<sub>4</sub><sup>+</sup>-N and NO<sub>3</sub><sup>-</sup>-N content after experimental warming at the 0.05 level .

We divided environmental factors into three categories: available nitrogen  $(NH_4^+-N, NO_3^--N)$ , precipitation and air

temperature. Using variation partitioning (VPA), we found that these three categories explained the change of carbon and nitrogen metrology by 34.0%, 7.1% and 3.7%, respectively (Fig. 4).

Table 3 Analysis of repeated measures of variance for the effects of experimental warming and the observation month were taken on carbon content, nitrogen content and the ratio of carbon to nitrogen for the belowground parts of plant communities

Elevation	Model	Nitrogen content		Carbon content		C/N	
		F	Р	F	Р	F	Р
4300 m	Warming	7.62	0.051	1.469	0.292	4.936	0.090
	Month	4.00	0.115	14.69	0.002	14.63	0.002
	Interaction	0.06	0.814	0.08	0.923	0.02	0.981
4500 m	Warming	1.10	0.354	2.24	0.209	6.05	0.070
	Month	0.22	0.808	28.87	0.000	11.76	0.004
	Interaction	1.99	0.199	3.16	0.097	2.57	0.137
4700 m	Warming	2.10	0.221	13.80	0.021	3.40	0.139
	Month	7.00	0.017	7.34	0.016	9.56	0.008
	Interaction	4.42	0.051	4.54	0.048	7.74	0.013

Note: F: f-valued; P: significance probability.

Regression analyses of  $NH_4^+N$  and  $NO_3^-N$  in the soil with above-ground and below-ground carbon-nitrogen indexes of plants were performed. We found that  $NH_4^+N$  and  $NO_3^-N$  had a logarithmic relationship for all above-ground and below-ground indicators, while  $NO_3^-N$  was only related

to the carbon-nitrogen indicators of the below-ground parts and showed a logarithmic relationship. All models were significant at the level of P = 0.1 (Fig. 5).

#### 4 Discussion

# 4.1 Characteristics of carbon and nitrogen metrology during different months

This research found that the effects of warming on the characteristics of carbon and nitrogen metrology were different in the three observation months. This was consistent with previous findings (Fu et al., 2019b; Zong and Shi, 2019). This may be attributable to the following reasons. Firstly,

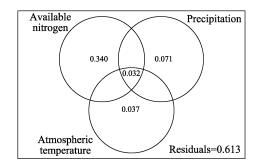


Fig. 4 Variation partitioning analyses (VPA) of the carbon content, nitrogen content and the ratio of carbon to nitrogen of plant communities in alpine meadows.

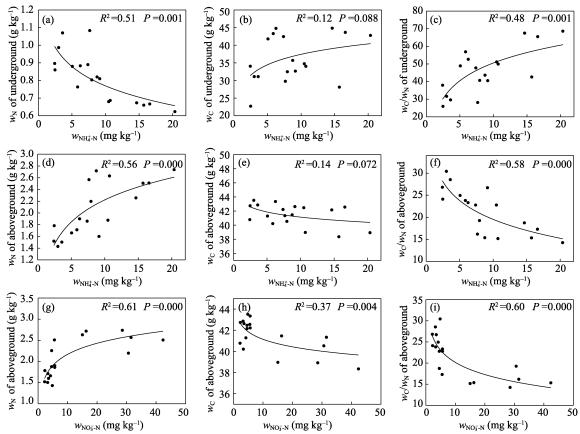


Fig. 5 Regression analyses of the carbon content, nitrogen content and the ratio of carbon to nitrogen of plant communities with  $NH_4^+$ -N and  $NO_3^-$ -N in the soil

the temperature on the Tibetan Plateau is lower than that in other areas at the same latitude, thus the growing season of plant communities is relatively short. In this study area, July and August are the most active period for plant growth; the withering period begins gradually in September (Shen et al., 2016). The physiology of plants are different between different month (Fu et al., 2019a). Compared with July and August, chlorophyll content, the activity of enzymes, and physiological functions all decrease, and this may in turn affect plant photosynthesis for carbon fixation and root absorption of nitrogen. Moreover, the available nitrogen content ( $NH_4^+$ -N and  $NO_3^-$ -N) of plants varied in the observation months (Fig. 3). The available nitrogen content can explain the 34% change of plant carbon and nitrogen (Fig. 4). Secondly, the temperature and precipitation conditions varied in the three observation months (Table 1), and these variations caused differences in soil temperature and soil humidity conditions. This in turn affected the absorption of soil inorganic nitrogen by root systems and the fixation of carbon by photosynthesis of vegetation redistribution (Kuchenbuch et al., 1986; Bouda and Saiers, 2017).

# 4.2 Carbon and nitrogen metrology characteristics at different elevation

This research found that the effects of warming on the characteristics of carbon and nitrogen metrology were different at the three elevations examined. This was consistent with previous findings (Zhang et al., 2015; Chang et al., 2016; Ma and Chang, 2019). This may be attributable to the following reasons. Firstly, there are differences between the three elevations in both mean annual and growing-season environmental humidity and temperature (Table 1, Fu et al., 2011). Soil temperature changes with elevation, and soil temperature affects the decomposition of litter in the soil, affecting in turn the carbon and nitrogen content of plant communities by influencing the supply of effective nutrients. Atmospheric temperature and humidity also affect evapotranspiration. Lower temperatures close the pores and affect the photosynthesis of plants, thus affecting the carbon content of plant communities (Pan et al., 2009). Secondly, soil organic matter and soil microbial community composition change with elevation. The available nitrogen and phosphorous in soils are dependent on soil microbes (Yu et al., 2019b), and this leads to differences in plant carbon and nitrogen contents at different elevations (Fig. 3). Thirdly, the species composition and diversity of plant communities varies at different elevations, and this may in turn lead to different carbon and nitrogen contents (Yu et al., 2019a).

## 4.3 The different effects on the characteristics of carbon and nitrogen metrology between above-ground and below-ground

This research found that the effects of warming on above-

ground and below-ground carbon and nitrogen content changes were not synchronized. This may be attributable to the following reason. First, above-ground plant carbon and nitrogen were closely related to ammonium and nitrate nitrogen, while the below-ground plant carbon and nitrogen content had nothing to do with nitrate nitrogen content (Fig. 5). Fast-acting nitrogen is the main influencing factor (Fig. 4) and may cause asynchronous changes (Frechilla et al., 2001). Second, illumination intensity was an important factor. Under conditions with higher radiation, plants distribute more carbon to the above-ground parts (stem, leaf) in order to obtain more light energy to promote plant growth and more carbon synthesis. This provides the plants with more abundant carbon to supply roots to absorb minerals in the soil (Edwards et al., 2004). By contrast, under conditions with lower radiation, most biomasses distribute more carbon to the stem, resulting in less carbon in the roots (Edwards et al., 2004). 75% of the nitrogen in plants is concentrated in the chloroplast and this is a key factor in the metabolism of photosynthesis (Takashima et al., 2010), so the intensity of photosynthesis also affects the distribution of nitrogen.

### 5 Conclusions

Overall, the effects of warming on the above-ground and below-ground carbon and nitrogen metrology of plant communities were inconsistent, and the responses to warming were not synchronized. Different observation months and different elevations changed the effect of warming on the carbon-nitrogen metrology of the communities. Environmental factors (temperature, precipitation and soil nitrogen) dominated by soil available nitrogen ( $NH_4^+$ -N and  $NO_3^-$ -N) were the main factors that affected the carbon-nitrogen metrology of plant communities in response to warming.

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## 模拟增温对青藏高原高寒草甸群落碳氮计量学的影响

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摘 要:低温是影响青藏高原生态系统的重要限制因子。本研究基于青藏高原三个海拔(4300 m、4500 m、4700 m)上的 模拟增温实验平台(开顶式增温箱, open top chambers, OTC),观测了2011年8-9月和2012年8月的高寒草甸生态系统的群落 地上和地下碳氮计量学特征。结果表明:模拟增温显著增加了21.4%的2011年9月4500m的群落地上氮含量,显著降低了3.9% 的2012年8月4300m的群落地上碳含量,而对其他情况下的群落碳氮计量学特征无显著影响;模拟增温显著增加了5.5%的2011 年8月4500m的群落地下碳含量,显著增加了28.0%的2011年9月4300m的群落地下碳氮比,显著降低了15.7%的2011年9 月4700m的群落地下氮含量,显著增加了28.0%的2011年9月4300m的群落地下碳含量,显著降低了15.7%的2011年9 月4700m的群落地下氮含量,显著降低了34.3%的2012年8月4700m的群落地下碳含量,显著降低了37.9%的2012年8月4700 m的群落地下碳氮比,而对其他情况下的群落碳氮计量学特征无显著影响。因此,模拟增温对不同海拔高度和不同月份的群落碳 氮计量学的影响不一致,土壤铵态氮与硝态氮含量是影响植物群落碳氮计量学的主要因子。

关键词:当雄县; 高寒草地; 开顶式增温箱; 碳氮计量学