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Distribution and utilization of nitrogen on moderately and heavily grazed temperate desert steppe using the ^{15}N tracing technique

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ABSTRACT

Due to overgrazing, grasslands in northern China have been severely degraded since 1980s. Grassland biomass and plant populations decreased dramatically. Application of fertilizer is a common technique for pasture management in many countries, however, it has not been widely used on China grasslands. Nitrogen (N) is an important productivity driver in grassland ecosystems, but its transportation, allocation and utilization in temperate desert steppe remained unclear. In this study, a sequential nitrogen addition experiment was conducted on a farm in southwestern Jingtai County, Gansu Province, in the transition zone between Loess Plateau and Tengger Desert. Two different grassland plots with moderate grazing (MG) and heavy grazing (HG) were fenced in 2008. N (NH_4NO_3) was applied at the rate of: 12.25 g N·m^{-2}, 24.5 g N·m^{-2}, 36.75 g N·m^{-2} and 49 g N·m^{-2}, respectively. To determine the amount and distribution of nitrogen applied to the grassland, the ^{15}N tracer technique was adopted. The results demonstrated that the recovery rates of ^{15}N ranged from 3.8% to 11.7% in the plants. The nitrogen recovery amount from both shoots and roots increased significantly ($P < 0.05$), while the recovery rate decreased gradually with increasing nitrogen application. The ^{15}N recovery from shoots under HG was much less than from MG. In one growing season, only 18.2%–8.0% of labeled ^{15}N was derived from fertilizer, so soil nitrogen is still the main source absorbed and utilized by plants. As much as 50.4%–84.4% labeled ^{15}N remained in soil, which was evenly distributed throughout 0–20 cm soil layer, and significantly increased with nitrogen application rates. The nitrogen remained in the top soil (0–10 cm) pool was much less than that of 10–20 cm layer ($P < 0.05$). The nitrogen loss amounts and rates were significantly different among N treatments, which indicated that there was an increasing trend in N loss with increasing N application rates. Loss/recovery analysis showed that 12.25 g N·m^{-2} was the recommended application rate. This work is expected to provide the lowest risk/return ratio and produce both economic and environmental benefits for the degraded grassland in Gansu and analogus regions in China.

1. Introduction

Nitrogen is one of the most important nutrients and an essential component of many important biomolecules (Camargo and Alonso, 2006). The deficiency of nitrogen is a key factor restricting the improvement in recovering grassland productivity and quality (Pan et al., 2005; Cheng et al., 1997; Chen et al., 1985), while reasonable application can not only significantly improve the density, height, aboveground biomass, below ground biomass and total biomass (Pan et al., 2005), but also increase soil nitrogen absorption and utilization rate (Zhou et al., 2010; Xie et al., 2010; Liu et al., 2010). However, human activities, such as excessive fertilizer application, and fossil fuel consumption have greatly accelerated and reinforced the natural nutrient cycles in soil, water and atmosphere (Bu et al., 2011; Payne et al., 2012). Nitrogen loss in some rangelands will impose an ecological threat to environmental security, while excessive nitrogen application also can reduce plant leaf quality and nitrogen utilization rates (Feng et al., 2005). Therefore, it is important to study the fate of nitrogen fertilizer transportation, application, and distribution. To date, many studies have focused on nitrogen leaching, accumulation and balance of cultivated lands, wetlands, forests and waters (Krug and Winstanley, 2002; Prasad et al., 2004; Sawaittayothin and Polprasert, 2007; Ross et al., 2008; Schröder et al., 2003). Some researches were also conducted in grassland using the ^{15}N isotope tracer. Clark (1977) found the

Abbreviations: N, nitrogen; MG, moderate grazing; HG, heavy grazing; OM, organic matter; N_T, total nitrogen; N_A, available nitrogen; GI, grazing intensity

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added nitrogen was mainly absorbed by plants and entered the plant nitrogen pool in grassland ecosystem. However, some of results on shortgrass steppe didn't support this conclusion (Pan et al., 2004). Chen et al. (1985) reported that adding nitrogen had a significant effect on *Leymus chinensis* productivity in rainy season, but the nitrogen fate was not studied. As an effective nitrogen research method, the ^{15}N tracer technique can be used to study the rate of utilization, accumulation, allocation, and transportation of different nitrogen resources and has become one of several important methods and tools available (Wang and Zhang, 2002; Hauck and Bremner, 1976). Therefore, the objectives of the research were to: 1) determine the allocation and fate of nitrogen fertilizer in both plant and soil using ^{15}N tracing technique; 2) analyze nitrogen movement and transformation in different plant parts; 3) determine the most economical fertilizer rate for temperate desert steppe; and 4) provide sound scientific recommendations for nitrogen fertilization management on temperate desert steppe.

2. Materials and methods

2.1. Experimental site

The experiment was conducted on a farm (103°31′E, 37°06′N) in southwestern Jingtai County (2400 m above sea level), Gansu Province, in the transition zone between Loess Plateau and Tengger Desert. The region had a temperate arid continental climate with relatively warm summers with long daylight (annual sunshine hours = 2658 h) and cold winters (annual mean temperature = 8.3 °C). The average annual rainfall is 185 mm with most of the rainfall events occurring between July and September. The annual mean evaporation is 3081 mm, 16 times of the rainfall.

The major types of grasslands in the region were temperate desert steppe and alpine shrub meadow with the former being accounted for 78.5% of the total grassland area. The dominant species were xeric plants and mesoxerophytes such as Stipa (*Stipa capillata Linn.*), crested wheatgrass (*Agropyron cristatum (Linn.) Gaertn*), Kobresia humilis (*Kobresia humilis*), Kobresia capillifolia (*Kobresia capillifolia*), Medicago ruthenica (*Pocockia Ruthenia (L.) Boiss),* Herba Oxytropis falcatae (*Oxytropis aciphylla Ledeb.*), Carex (*Carex liparocarpos Gaudin*), Rhizoma polygoni vivipari (*Polygonum viviparum*), *Leontopodium leontopodioides*, Herba potentillae chinensis (*Potentilla spp*), and Nutans Elymus (*Elymus nutans Griseb.*). The soil was diluvial grey and brown desert soil. Details of soil information are given in Table 1. The main livestock were sheep (*Ovis aries*), goats (*Capra hircus*) and camels (*Camelus*).

Four farmlets of 100–134 ha each in size had the same soil type (Grey-brown desert soil) and similar pasture conditions (pasture species, density and groundcover) prior to the commencement of a major development program (Jiao et al., 2016). In 2008, four grazing intensities were imposed in each farmlet. They were: (1) light grazing (LG) with 0.45 sheep unit/ha; (2) moderate grazing (MG), 0.75 sheep unit/ha; (3) heavy grazing (HG), 1.50 sheep unit/ha, and (4) no grazing (NG). These treatments were maintained consistently from 2008 to 2013. Our previous research showed that light and moderate grazing improved grassland quality and fertility, while no and heavy grazing had a negative impact on temperate desert steppe (Jiao et al., 2016). So in this study only two grazing areas were selected to carry on nitrogen application test. One was MG (0.75 sheep unit/ha) that had improved grassland quality, and the other was HG (1.5 sheep unit/ha) that had a severe detrimental effect on the grassland. Details of the two farmlets including size, vegetation, animals used for grazing and their grazing intensities are given in Table 2.

Table 2
Information for two fertilization sites of moderate and heavy grazing. GI- grazing intensity; MG – moderate grazing; HG – heavy grazing.

Area	GI (sheep unit/ ha)	Size	Vegetation
MG	0.75	134	*Stipa breviflora* + *Cleistogenes mutica*
HG	1.50	100	*Stipa* glareosa + *Allium L.* + *Leontopodium leontopodioides*

2.2. Experimental design

The ammonia nitrate fertilizer used in the experiment was a double-labeled, $^{15}NH_4{}^{15}NO_3$, with an atom percent excess of 10.3%. The nitrogen was provided by Shanghai Research Institute of Chemical Industry, Ministry of Chemical Industry (Shanghai).

A completely random sequential design experiment was conducted at the MG and HG sites, which were fenced off in 2008. Twelve 1 m × 1 m plots were randomly selected at each site, with approximately 20 m intervals. A 20 cm height × 9.5 cm radius soil core was removed from the center of each plot and carefully put into a pot (20 cm height × 10 cm radius). Then the pot was placed back into the original soil core pit. Ammonium nitrate fertilizer (NH_4NO_3) was applied on April 1st, 2013, at the rate of 12.25 (35 g $^{15}NH_4{}^{15}NO_3$ m^{-2}), 24.5 (70 g $^{15}NH_4{}^{15}NO_3$ m^{-2}), 36.75 (105 g $^{15}NH_4{}^{15}NO_3$ m^{-2}) and 49 g N·m^{-2}(140 g $^{15}NH_4{}^{15}NO_3$ m^{-2}), respectively. Each treatment was replicated 3 times at each site. The labeled ^{15}N solution was prepared by weighing 20 g of $^{15}NH_4{}^{15}NO_{3,}$ dissolved in 2000 ml distilled water, evenly mixed and added to 4 pots at the rate of 100, 200, 300, and 400 ml, respectively. To maintain equal volume, 300, 200, 100 and 0 ml of distilled water were applied to each pot, respectively. Then each plot was fenced off using wire mesh (1.5 m height) to keep wild animals from entering.

2.3. Sample collection

Forage and soil samples inside and outside all pots in the two experimental areas were collected on August 30th, 2013. All litter, forage stems and leaves above ground, and all underground roots and soil samples of 200 g each at 0–10 cm, and 10–20 cm were collected and weighed for each pot. Plant samples were all cut to ground level, air dried, mixed with all litter, and weighed for biomass calculation. All samples were crushed and passed through a 1 mm sieve (TTF-100) before chemical analysis. Roots and soil samples from different layers underwent the same methods after removing sand and gravels.

2.4. Samples analyses

Total nitrogen (N_T) in plant and soil samples was determined by the semi-micro Kjeldahl procedure (Bao, 2000). The abundance of ^{15}N was determined using Gas Isotope Mass Spectrometer (MAT-271, Germany) and analyses were conducted by Chemical Engineering Research Institute of Shanghai Chemical Department. Based on sample ^{15}N abundance, the recovery amount and recovery rate for labeled ^{15}N by plant, the retention amount and retention rate in different soil layers and the

Table 1
Physical and chemical characteristics of the tested soils for grazing intensity of moderate and heavy grazing. GI – grazing intensity; MG – moderate grazing; HG – heavy grazing.

GI	Soil depth (cm)	OM (%)	pH	Bulk density (g/cm^3)	N_T (%)	N_A (mg/kg)	Soil texture
MG	0 – 30	3.60	8.64	1.00	0.17	111.70	Diluvial gray and brown desert soil
HG	0 – 30	2.63	8.94	1.17	0.13	81.69	Diluvial gray and brown desert soil