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Effects of grazing intensity on seed size, germination and fungal colonization of *Lespedeza davurica* in a semi-arid grassland of northwest China

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ABSTRACT

Lespedeza davurica, a major semi-shrub leguminous plant of the vast semi-arid grasslands in northwest China, has decreased greatly in abundance due to overgrazing. Seed characteristics and fungal pathogens affect seedling recruitment from soil seed banks. However, little is known about how grazing affects seed characteristics and fungal colonization on seeds. Seeds of *L. davurica* were obtained both from plants and from soil from plots where four intensities of grazing (0, 2.7, 5.3 and 8.7 sheep/ha) had been applied for 13 years. The morphological and germination characteristics and colonization of seed by fungi were examined. Seed size showed a hump-shaped trend with increasing grazing intensity. With freshly harvested seed, the intensity of 8.7 sheep/ha significantly enhanced germination and extended the time over which germination occurred. The occurrence of pathogenic *Fusarium* species on seeds decreased with increasing grazing intensity. Our study strongly suggests that the intensity of grazing can alter seed morphological and germination characteristics, as well as the colonization of fungi on seeds. Additional studies involving more members of the plant community are needed to determine whether the intensity of grazing results in a compensatory mechanism that serves to enhance the possibility of plant recruitment from seeds under field conditions.

1. Introduction

The grasslands in northwest China occupy vast semi-arid and arid areas and are sensitive to climate change and grazing disturbance (Christensen et al., 2004). To date, approximately 97% of the grassland has degraded to various extents and more than one-third of the grassland is severely degraded due to many years of overgrazing, nearby deforestation leading to erosion, and other human activities (Zhou et al., 2010). Intense grazing results in a dramatic decline in the number of some preferred plant species (Li et al., 2002), and leads to an increase in bare land, resulting in soil loss from wind and water, which intensifies the risk of desertification (Nash et al., 2004; Zhao et al., 2005). *Lespedeza davurica* is a common woody semi-shrub legume in the grassland. This species is semi-prostrate in growth and the taproot of one-year old seedlings can reach a depth of 1 m. It is a highly preferred species for grazing animals in the area (Cheng et al., 2011; Zhao et al., 2010). It also plays a key role in reducing soil loss because of its highly developed root systems (Cheng et al., 2011). According to our long-term field observation, the abundance of *L. davurica* has declined greatly due to many years of intense grazing. Restoration of *L. davurica* in degenerated grasslands would be highly beneficial for the grassland ecosystem and also for local farmers.

The soil seed bank plays a key role in the regeneration of degraded grasslands (Baskin and Baskin, 1998; Thompson, 1987). However, many abiotic and biotic factors cause seedlings to die, resulting in the failure of seedling establishment (Baskin and Baskin, 1998). Seed characteristics, including seed size and dormancy, are important factors affecting seedling establishment (Tripathi and Khan, 1990). In addition, fungal pathogens can cause pre- and post-emergence death and hence limit seedling establishment (Gallery et al., 2007; Packer and Clay, 2000; Schafer and Kotanen, 2004). For example, in semi-arid regions of western

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North America, establishment failure of the winter annual grass *Bromus tectorum* in thousands of hectares was considered to be caused by soil-borne fungal pathogens (Baughman and Meyer, 2013). Although heavy grazing has been reported to cause a great reduction in the densities of soil seed banks (Kinloch and Friedel, 2005; Sternberg et al., 2009), little is known about how grazing alters seed characteristics and the colonization of fungal pathogens on seeds and hence influences seedling establishment.

In the present study, we explored how the intensity of grazing affects seed size, germination characteristics and colonization of fungal pathogens on seeds of *L. davurica*. Within an established grazing trial located in a semi-arid grassland of northwest China, seeds were collected from mature plants and also from the soil seed bank within plots that had been subjected to four different intensities of grazing over a 13-year period. These seeds were used to test three hypotheses. The first hypothesis was that size of seed of *L. davurica* will increase with increasing grazing intensity. This is based on two previous findings. The first study involving the forage legume *Desmodium paniculatum* (panicled leaf ticktrefoil) revealed that the size of seed was increased by defoliation when grown at high temperatures (Wulff, 1986). The second reason for expecting seed size to increase with grazing intensity was that the addition of mineral fertilizer resulted in an increased size of seed for *Asclepias syriaca* (common milkweed) (Willson and Price, 1980). In our study area, the content of soil mineral N has been shown to increase with grazing intensity (Liu et al., 2011). Our second hypothesis was that germination of seed of *L. davurica* will increase with the increased size of seed. This is because previous studies have indicated that large seeds usually have better germination characteristics than light seeds, especially in dry soils (Leishman and Westoby, 1994; Tripathi and Khan, 1990). The third hypothesis was that colonization by fungal pathogens on seeds will increase with grazing intensity. Fungal pathogens are one of the important factors leading to species rarity (Klironomos, 2002; Mangan et al., 2010). In the long-term grazing trial being used in our study, we have observed that the abundance of *L. davurica* has decreased greatly with increased grazing intensity (Chen et al., 2017), which could potentially be associated with increased fungal activity. By testing these hypotheses, we intend to further our mechanistic understanding of how the intensity of grazing affects seed characteristics and provide a theoretical foundation for the restoration of *L. davurica* in northwest China.

2. Materials and methods

2.1. Study site

The study site is located in Huan County in the Northern Loess Plateau of China (37.12°N, 106.82°E, 1650 m in elevation). This area has a typical semi-arid monsoon climate. The mean annual temperature is about 7.1 °C and average annual rainfall is approximately 360 mm, more than 80% of which occurs from July to September. Vegetation in the area is dominated by the forb *Artemisia capillaris* Thunberg (Asteraceae), semi-shrub *Lespedeza davurica* (Laxm.) Schindl (Fabaceae), and bunch grass *Stipa bungeana* Trin. (Poaceae). The vegetation of the grassland starts to re-green in late April to early May, and starts to wither in late October.

In 2001, 12 adjacent experimental plots within the semi-arid grassland with a visually similar botanical composition, slope, and aspect were selected and fenced to establish a grazing trial. Four grazing intensities were established as follows: 0, 4, 8, and 13 lambs were rotationally grazed in three replicated 0.5 ha plots of each treatment, which equated to stocking rates of 0, 2.7, 5.3 and 8.7 sheep/ha, respectively. All plots were arranged in a completely randomized design. Lambs of the 'Tan' breed weighing approximately 20 kg were purchased from local farmers in the spring of each year. Each plot is rotationally grazed three times per year (10 days each time with a rotation interval of 30 days) from early June to early September. Prior to the onset of our experiment, the four grazing intensities were maintained for 13 years from 2001 to 2014.

2.2. Study species and seed collection

Lespedeza davurica starts to flower in August with the seeds becoming mature and starting to disperse in late September. Each pod produces a single seed, approximately 1.9–2.2 mm in length and 1.2–1.5 mm in width. Seeds of *L. davurica* exhibit physical dormancy when they ripen. As with many other legumes, dormancy breakage can occur under natural conditions and also by physically damaging the seed coat allowing ready penetration of water (Baskin and Baskin, 2004).

Seed recovery from the soil seed bank took place in the middle of September 2014 when germination of most seeds of *L. davurica* present in the soil had ceased and new seeds had not been produced yet to replenish the seed bank. In each plot, approximately 80 kg soil samples were collected by taking 48 sub-samples (10 cm wide × 10 cm long × 10 cm deep) about 10 m apart along a W-shaped transect. No sampling was undertaken within 2 m of the plot fences to avoid edge effects. Subsamples from each plot were bulked into a composite sample, resulting in three replicates for each grazing intensity, matching with the field grazing design. Soil samples from each plot were stored in Nylon bags and immediately taken to the laboratory, where they were squashed and passed through two sieves, a 5 mm mesh size that allowed the passage of seeds of *L. davurica* but not of plant roots and other larger residues, and a 0.5-mm mesh size that allowed the passage of small soil particles but not *L. davurica* seeds. Seeds of *L. davurica* retained within the 0.5-mm mesh were picked out using sterilized tweezers. In October, mature seeds of *L. davurica* were harvested by hand from approximately 100 plants in each plot. The seeds collected from the same plot were combined. Seeds that were recovered from the soil or harvested from the plants were air-dried for a month, carefully cleaned by hand and then stored at 4 °C in sealed containers until required.

2.3. Measurement of seed size and germination

Seed samples from the same plot were well mixed. Seed pericarps were removed before the measurement of seed morphological characteristics. A hundred seeds were randomly selected from each of three replicates per grazing intensity, and were weighed individually on a five decimal place balance, followed by measuring the length and width of seed each using vernier calipers. Fifty seeds per replicate were then placed on the surface of a 5-mm deep layer of autoclaved-sterilized moist soil (50 g) in a Petri dish (diameter 20 cm) to determine germination. All Petri dishes were incubated at 20 °C and injected daily with 5 ml sterilized water to keep the soil moist. The number of germinated seeds was recorded daily for a month until no new seedlings developed. Seeds were considered to have germinated when the radicle was visible. After the germination trial, seeds that did not germinate were picked out, washed in tap water to remove soil, and their viability was assessed by germinating on moistened paper in Petri dishes following the seed coat being cut using a scalpel. All of the non-germinated seeds recovered from soil, and half of the non-germinated seeds from plants (approximately 20 seeds per replicate) were selected randomly and used for testing seed viability.