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Short-term acute nitrogen deposition alters the interaction between Korean pine seeds and food hoarding rodents

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ABSTRACT

Although the environmental impacts of nitrogen (N) deposition have been well studied, how nitrogen deposition affects the interaction between seeds and food hoarding animals has never been investigated. In this study, N was added twice as NH_4NO_3 to Korean pines *Pinus koraiensis* in order to explore the impacts of N deposition on seed traits and food-hoarding behaviors of small rodents in the northeastern deciduous forests in China. Pine cones were collected to measure seed traits and to test food-hoarding behaviors of small rodents both in the field and in semi-natural enclosures. Our results showed that short-term N addition significantly enhanced the ratio of seed meat to seed coats and the concentrations of crude protein. Moreover, seed volatile compounds were significantly altered by N addition, resulting in lower emission of α-pinene but higher emissions of D-limonene. Consequently, nitrogen addition increased seed scatter-hoarding by both *Tamias sibiricus* and *Apodemus peninsulae* in enclosures. Field experiments showed the consistent results that small rodents preferred to scatter-hoard seeds with N addition over the control. To our knowledge, this is the first study showing that nitrogen deposition shapes the interaction between seeds and food-hoarding animals through altering the physical and chemical traits of seeds.

1. Introduction

Seed dispersal mediated by animals has been recognized as playing an important role in plant regeneration and community structure (Vander Wall, 1990; Hirsch et al., 2012; Jansen et al., 2014; Steele et al., 2015). Scatter-hoarding animals (e.g., rodents and birds) have been regarded as key vectors in seed dispersal for many tree species in various forest ecosystems (Yi et al., 2012; Steele et al., 2014; H. Zhang et al., 2015). Whether a seed can be successfully transported far away from the parent trees and produces a seedling is often determined by various seed traits (Lai et al., 2014; Wang et al., 2014; Garzon-Lopez et al., 2015). An increasing literature has shown that the nutrition status of seeds is decisive in affecting seed dispersal patterns and final seed fates (Yang et al., 2012; Z. Zhang et al., 2015). Large seeds with high nutrition are more likely to be dispersed and hoarded by animals than those with low nutrition (Zhang and Zhang, 2008; Xiao et al., 2013, 2015), while small seeds or seeds with low nutrition tend to be consumed rather than dispersed away from the seed source (Wang et al., 2012; Rusch et al., 2013). In addition, seeds with high concentrations of protein and fat are more likely to be scatter-hoarded by food-hoarding animals (Wang and Chen, 2012). Recently, several studies provided new insight into the role of seed volatile compounds in seed dispersal and seed relocation by small rodents (Hollander et al., 2012; Paulsen et al., 2013, 2014; Yi et al., 2016). Apart from nutritional status, anti-nutritional properties, such as physical and chemical defense traits (e.g., seed coat thickness, and tannins) have been well documented to influence seed dispersal (Yi and Zhang, 2008; Zhang and Zhang, 2008; Rusch et al., 2013). Therefore, these observations indicate that seed dispersal patterns and plant regeneration can be greatly affected by the physical and chemical traits of seeds (Z. Zhang et al., 2015).

Use of fossil fuels and fertilizers has increased worldwide, resulting in anthropogenic emissions of reactive nitrogen of NO_x and NH_x (Zhang et al., 2012; Fowler et al., 2013; Huang et al., 2015). These emissions have led to increasing atmospheric deposition of fixed nitrogen, both as nitrate and ammonium through wet deposition or as nitrogen dioxide, nitric acid and ammonia via dry deposition (Liu et al., 2013; Zhu et al., 2015). This unintended nitrogen addition has shown significant effects on plant species composition, diversity, and ecosystem functioning (Vitousek et al., 1997; Sala et al., 2000). Forest ecosystems in the industrial-

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ized regions also have experienced greater rates of nitrogen deposition in recent decades (Bahr et al., 2013; Talhelm et al., 2013; Dirnböck et al., 2014; Guerrieri et al., 2015). Previous studies in agricultural ecosystems provided solid evidence that the composition of proteins and amino acids in crop seeds exhibits high plasticity in response to artificial nitrogen addition (Kirkman et al., 1982; Nakasathien et al., 1999; Tabe et al., 2002). Although the environmental impacts of high rates of nitrogen addition have been well investigated (Wedin and Tilman, 1996; Gough et al., 2000; Suding et al., 2005), little is known whether or how increases in N availability to the forest ecosystems accompanying increased N deposition might influence seed traits (e.g., seed nutrition and/or volatile emission) of tree species bearing large seeds. If we accept that seed physical and chemical traits of forest trees can be greatly altered by N addition, changes in seed dispersal patterns mediated by food-hoarding animals should be expected because seed traits play important role in determining seed dispersal. However, we still have no knowledge on the interaction between seeds and food-hoarding animals in response to nitrogen deposition.

In the present study, nitrogen was added twice in the form of NH_4NO_3 to Korean pine (*Pinus koraiensis*) trees in an artificial forest in northeastern China. Our aim was to explore the impacts of N deposition on seed physical and chemical traits as well as food-hoarding behaviors of small rodents. Because plant growth and reproduction in the temperate forests are often limited by N availability (Schleppi et al., 2012), we expect that artificial nitrogen deposition will increase the contents of seed nutrients (e.g., proteins or fat). If this is true, seed volatile compounds are expected to change under nitrogen addition as odors in plants often mirror their nutritional value (Goff and Klee, 2006). As a consequence, variations in seed dispersal patterns by small rodents should be witnessed due to the changes in seed traits under nitrogen deposition.

2. Materials and methods

2.1. Study site

Our experiments were conducted in the Qingyuan Forest Ecosystem Research Station of Chinese Ecosystem Research Network (CERN) in the eastern Liaoning Province, Northeast China (41°51′9.94″N, 124°56′11.22″E, elevation 600–800 m). The climate of the region is a continental monsoon type with a humid and rainy summer and a cold and snowy winter. Mean annual air temperature varies between 3.9 °C and 5.4 °C with the minimum of −37.6 °C in January and the maximum of 36.5 °C in July. The mean annual precipitation ranges between 700 mm and 850 mm, 80% of which falls from June to August. The frost-free period lasts for 130 days on average, with an early frost in October and late frost in April (Zhu et al., 2007).

2.2. Nitrogen addition

In May 2015, we selected 20 Korean pine trees (DBH ≈ 20 cm) bearing several immature pine cones 20 m apart from each in a 2 ha artificial forest. They were randomly assigned into two groups each consisting of 10 trees. Zhu et al. (2015) estimated annual total dissolved nitrogen deposition as 32.49 kg N ha^{-1} $year^{-1}$ in Shenyang, around 140 km far from our study area. If we assume that the contribution of dry deposition was approximately 40% in China (Zhu et al., 2015), the total N deposition in our study area would be 50–60 kg N ha^{-1} $year^{-1}$. According to the previous study (Sun et al., 2015) and given the trends in nitrogen deposition in the study area, 2.5 g N m^{-2} yr^{-1} of NH_4NO_3 was added twice in 20 l of solution to each of the 10 Korean pine trees of the first group in early June and July during the rainy season in the study area. An area of 12.56 m^2 around each tree trunk (radius = 2 m) was selected for nitrogen addition based on the underground root distribution of these pine trees. At the same time, 20 l of water free of NH_4NO_3 was added to each of the other 10 fruiting Korean pine trees in the same way.

2.3. Seed physical traits

In September 2015, when the seeds of the Korean pine were mature, pine cones from each of the 20 trees were artificially collected. Seeds in each cone were artificially picked out and dried under ambient conditions for 10 days. Then, 300 seeds were randomly selected from the control and N-added trees, respectively. These 300 seeds were then divided into 15 groups each containing 20 seeds and dried at 70 °C for 48 h. Each seed was carefully cracked and then the kernels as well as seed coats of each group were weighed, respectively. The proportion of aborted seeds was recorded for each group to determine if nitrogen deposition affects seed abortion rate. The ratio of seed meat to seed coats of each group was calculated as: $R = \frac{\text{Mass of kernels (g)}}{\text{Mass of coats (g)}}$ for the control and N treatment groups, respectively. However, the aborted seeds were discarded for calculating the ratios.

2.4. Seed nutrition traits

After the physical traits were recorded, the seed meat from the control and N addition seeds were collected. Seed meat was then sent to the Beijing Physical–Chemical Analysis Center (Beijing) for analyses of crude protein, crude fat and crude starch according to the methods described previously (Yi and Zhang, 2008; Zhang and Zhang, 2008).

2.5. Seed odor emissions

Twenty seeds were randomly selected from the N addition and control groups. For each group, the 20 seeds were divided into 4 sub-samples, each containing five seeds. Then, the five seeds of *P. koraiensis* of each sub-sample were initially sealed in 10-ml gas chromatography (GC) headspace vials (Daobang Technology, Nanjing, China) and stored at room temperature for 24 h before headspace analyses. Odorous compound analyses were performed using Agilent 7697A headspace autosampler + Agilent 7890-5975 GC–MS (Agilent Technologies, Inc., USA). The odorous compounds were separated using GC on a HP-17MS column (30 m length, 0.32 mm internal diameter, 0.25 mm film thickness; Agilent Technologies, Inc., USA) running a temperature program (15 min hold at 40 °C, 1 °C per min to 250 °C, and 10 min hold; helium carrier gas at constant flow rate of 1 ml per min). The volatile compounds were identified from the NIST (National Institute of Standards and Technology, Gaithersburg, MD, USA) mass spectral database. The abundance of each volatile compound detected in the headspace was expressed as a percentage of total peak area.

2.6. Capture of small rodents

We used SJL601 steel-framed live-traps (9 cm × 10 cm × 25 cm, manufactured by Sichuan Shujile Company, Sichuan, China) to trap small rodents for our enclosure experiments. The traps were baited with peanuts and carrots and then placed in forests at 5-m intervals along four transects at 0800 h. All traps were pre-baited for 1 day and protected from predators by wrapping with steel mesh. Traps were checked twice daily for 6 consecutive days. The captured focal animals were immediately transferred to the animal housing room and caged individually (30 cm × 40 cm × 50 cm) at