Effects of rodent-induced disturbance on eco-physiological traits of Haloxylon ammodendron in the Gurbantunggut Desert, Xinjiang, China Postprint

Authors: Yanling Xiang, WANG Zhongke, LYU Xinhua, HE Yaling, LI Yuxia, ZHUANG Li, ZHAO Wenqin, ZHAO Wenqin

Date: 2020-06-22T00:00:00+00:00

Abstract

Disturbance by rodents alters the morphologies and nutrients of plants as well as the physical-chemical properties of the soils. Changes in plants are considered to be mechanisms of defense against the disturbance by rodents. Rodents gnaw on the assimilating branches of Haloxylon ammodendron (CA Mey.) Bunge and burrow under the bushes in the desert ecosystems of Xinjiang, China. However, eco-physiological responses of different age groups of H. ammodendron to the disturbance by rodents are not well understood. In this study, soil physical-chemical properties under the shrubs and the above-ground morphological, physiological and biochemical features of assimilating branches of H. ammodendron of different age groups (i.e., young, 30—100 cm; middle-aged, 100—200 cm; and mature, >200 cm) in burrowed and non-burrowed (control) areas were studied in 2018. We found that disturbance by rodents significantly increased the crown width and total branching rates of young and middle-aged H. ammodendron. Photosynthetic pigment contents of assimilating branches of H. ammodendron were significantly reduced under the disturbance by rodents. In term of plant nutrients, the main differences among different age groups of H. ammodendron under the disturbance by rodents occurred in the total soluble sugar and reducing sugar contents that decreased in young plants, increased in middle-aged plants, and did not affect in mature plants. Crude protein and phosphorus contents significantly increased, while crude fiber and calcium contents significantly decreased in young plants. Crude fat and calcium contents significantly decreased in middle-aged plants. Soil organic matter (SOM), total nitrogen (TN), available nitrogen (AN) and available potassium (AK) contents in the topsoil (0–20 cm), which are conducive to forming 〃 fertile islands’ ’ , also increased under the disturbance by rodents. In particular, soil AN and AK were the major factors affecting the above-ground morphological characteristics of H. ammodendron in burrowed areas. Overall, the response and defense strategies of H. ammodendron to the disturbance by rodents differed among different age groups, and the effect of the disturbance by rodents on H. ammodendron gradually weakened with the increasing plant age.

Full Text

Effects of Rodent-Induced Disturbance on Eco-Physiological Traits of *Haloxylon ammodendron* in the Gurbantunggut Desert, Xinjiang, China

XIANG Yanling[1], WANG Zhongke[1], LYU Xinhua[1], HE Yaling[2], LI Yuxia[1], ZHUANG Li[1], ZHAO Wenqin[1]*

[1] (Affiliation not fully specified in original text)
[2] School of Medicine, Shihezi University, Shihezi 832003, China

---

Abstract

Disturbance by rodents alters plant morphology and nutrient content as well as the physical-chemical properties of soils, with these plant changes representing mechanisms of defense against rodent activity. In the desert ecosystems of Xinjiang, China, rodents gnaw on the assimilating branches of *Haloxylon ammodendron* (CA Mey.) Bunge and burrow beneath the shrubs. However, the eco-physiological responses of different age groups of *H. ammodendron* to rodent disturbance remain poorly understood. This study investigated soil physical-chemical properties beneath shrubs and the above-ground morphological, physiological, and biochemical characteristics of assimilating branches of *H. ammodendron* across different age groups (young: 30–100 cm; middle-aged: 100–200 cm; mature: >200 cm) in both burrowed and non-burrowed (control) areas in 2018. Our results demonstrate that rodent disturbance significantly increased the crown width and total branching rates of young and middle-aged *H. ammodendron*. Photosynthetic pigment contents in assimilating branches were significantly reduced under rodent disturbance. Regarding plant nutrients, the primary differences among age groups under rodent disturbance occurred in total soluble sugar and reducing sugar contents, which decreased in young plants, increased in middle-aged plants, and remained unaffected in mature plants. Crude protein and phosphorus contents increased significantly, while crude fiber and calcium contents decreased significantly in young plants. In middle-aged plants, crude fat and calcium contents decreased significantly. Soil organic matter (SOM), total nitrogen (TN), available nitrogen (AN), and available potassium (AK) contents in the topsoil (0–20 cm)—which contribute to the formation of "fertile islands" —also increased under rodent disturbance. Notably, soil AN and AK were the major factors influencing the above-ground morphological characteristics of *H. ammodendron* in burrowed areas. Overall, the response and defense strategies of *H. ammodendron* to rodent disturbance differed among age groups, with the effects of disturbance gradually weakening as plant age increased.

Keywords: age groups; morphology; assimilating branches; soil physical-chemical properties; photosynthetic pigments

*Corresponding author: ZHAO Wenqin (E-mail: zhwq-88@163.com)

Received 2019-06-01; revised 2020-02-05; accepted 2020-03-25

1 Introduction

Haloxylon ammodendron (CA Mey.) Bunge, a representative C4 succulent xerophyte that grows in the desert areas of northwestern China, possesses xeromorphic characteristics adapted to drought and high-salinity environments. Recent research has revealed that *H. ammodendron* provides high nutrient content to herbivorous animals in desert environments (Kang et al., 2017). The assimilating branches of *H. ammodendron* supply not only nutrients but also water for rodents inhabiting these arid ecosystems (Mares et al., 1997). Rodents typically cut branches horizontally into small pieces and transport them to their burrows for consumption (Mabry et al., 1977). Additionally, fossorial mammals construct burrow systems where food, feces, seeds, plant propagules, and various organic materials are stored, creating patches rich in organic content and influencing the distribution of soil nutrients (Lacey et al., 2001). Consequently, the life activities of rodents inevitably exert profound impacts on the growth of *H. ammodendron*.

The growth-differentiation balance hypothesis proposes that plants may progress into a "growth or defense" stage, either by investing resources in defenses against herbivores or by out-growing competitors (Siemens et al., 2002). Animal feeding damage can alter plant morphology due to selective feeding on particular tissues (Andino and Borghi, 2018). In many cases, phenotypic plasticity is considered a low-cost defense mechanism that plays a crucial role in plant–herbivore interactions (Hakes and Cronin, 2011; Kozlov and Zvereva, 2012; Dong et al., 2017). When shoot apices are removed by herbivores, damaged plants have been documented to generate axillary branches to enhance photosynthesis and compensate for tissue loss (Christie et al., 2014; Dong et al., 2017). A study by Zhang et al. (2009) also demonstrated that slight damage to *H. ammodendron* by foraging gerbils promoted increased growth of branches and crown. A plant' s nutritional grade reflects its potential resistance factor when nutrient content falls outside the optimal range for herbivores (Kindler and Robert, 1970; Schweissing and Wilde, 1979). Deficiencies in plant nutrients can decrease palatability (Ngatia et al., 2015), making plants less desirable to herbivores. Furthermore, herbivore effects on plants may vary across different growth stages. Studies have shown that herbivore disturbance increased defense compounds (e.g., alkaloids) in offspring plants (Barton and Koricheva, 2010; Kafle and Wurst, 2019). Increased defense compounds may reduce investment in growth or nutrient synthesis (Züst et al., 2015; Karasov et al., 2017). However, the eco-physiological responses of different age groups of *H. ammodendron* to rodent disturbance remain unclear. This paper examines the morphological characteristics of above-ground parts and nutrient levels in assimilating branches of *H. ammodendron* of different age groups at the southern edge of the Gurbantunggut Desert under rodent disturbance.

Rodents can modify the physical-chemical properties of soil through their burrowing activities (Berke, 2010; Hagenah and Bennett, 2013). Recent studies have shown that burrowing animal activities play an important role in promoting the formation of "fertile islands," typically manifested as increases in soil nitrogen, phosphorus, and carbon contents (Xu et al., 2012; Allington and Valone, 2014). Thus, rodent disturbance may benefit *H. ammodendron* growth. We therefore examined the physical-chemical properties of below-shrub soil at various depths in burrowed and control areas. Additionally, since leaf photosynthetic pigment contents serve as valuable indicators of potential photosynthetic efficiency (Mao et al., 2007), we compared differences in photosynthetic pigment contents of assimilating branches of *H. ammodendron* across different age groups in burrowed and control areas. We hypothesized that: (1) rodent disturbance would reduce photosynthetic pigments in assimilating branches of *H. ammodendron*, and to compensate for nutrient and photosynthetic losses, *H. ammodendron* might alter its morphology; (2) *H. ammodendron* would reduce rodent feeding on assimilating branches by decreasing their nutrient contents, though changes in nutrient contents might differ among age groups; (3) rodent activities would increase organic matter, nitrogen, phosphorus, and potassium contents in soil under *H. ammodendron* shrubs, likely stimulating *H. ammodendron* growth to some degree; and (4) responses of *H. ammodendron* to rodent disturbance would differ among age groups, with eco-physiological responses of young *H. ammodendron* being more dramatic than those of middle-aged and mature plants.

2.1 Study Area

The study area is located at the southern edge of the Gurbantunggut Desert, in a transitional zone between desert and oasis (45°02 N, 85°04 E; 346 m a.s.l.). The region experiences a typical temperate continental climate with mean annual precipitation of 117 mm, mean annual evaporation exceeding 1980 mm, and an annual mean temperature of 6.6°C. Maximum summer temperatures can reach 43.1°C, while minimum winter temperatures drop to -27.0°C. The landscape surface consists of a mosaic of dry riverbeds and aeolian sandy landforms. Xerophytic plants dominate the vegetation, which is characterized by *H. ammodendron* and *Haloxylon persicum* with approximately 30% coverage (Huang et al., 2015), where *H. ammodendron* is dispersed between dunes. Typical rodent species in this area include *Rhombomys opimus*, *Cricetulus migratorius*, *Meriones meridianus*, and *Dipus sagitta* (Xu et al., 2012), with *Rhombomys opimus* being the most abundant. Ma et al. (2018) found that burrow coverage in the southern edge of the Gurbantunggut Desert ranged from 18%-20%.

2.2 Experimental Design

In July 2018, we searched for rodent-burrowed areas along longitudinal dune directions. Three 10 m × 10 m quadrats were established in the center of each burrowed area. Using the burrowed area as a center, we set up three 10 m × 10 m control quadrats in areas distinct from the edge of burrowed zones, where there were no (or limited) signs of burrowing, no fresh rodent feces on the ground or at burrow entrances, and no recently plowed sand at burrow entrances (Tchabovsky et al., 2001). Distances between all quadrats exceeded 50 m. Two test replicates were established at distances greater than 1 km from the primary test site, covering three burrowed areas and corresponding control areas.

2.3 Measurement of Morphological Characteristics of Above-Ground Plant Parts

We measured plant height, basal stem diameter, crown width, and total branching rate for all *H. ammodendron* individuals within the quadrats. Basal stem diameter was measured approximately 3 cm above ground level. Crown width was calculated as the average width in east-west and north-south directions. Total branching rate (Rb) of *H. ammodendron* was calculated using the formula of Whitney (1976) and Steingraeber and Waller (1986), where NT represents the total number of branches, NS the highest branch number, and N1 the first branch number.

2.4 Collection and Analysis of Assimilating Branches

Five plants each of young, middle-aged, and mature *H. ammodendron* were carefully selected, totaling fifteen plants from both burrowed and control areas. *H. ammodendron* individuals 30–100 cm in height were classified as young, those 100–200 cm as middle-aged, and those exceeding 200 cm as mature (Zhang et al., 2016; Luo et al., 2017). Assimilating branches were collected from all four cardinal directions (east, south, west, north); annual branches were used for photosynthetic pigment analysis, while 1- to 3-year-old branches were used for nutrient content analysis. Assimilating branch samples were thoroughly mixed within different areas and age groups, then numbered and transported to the laboratory. We extracted chlorophylls (total, i.e., a and b) and carotenoids with 80% acetone and determined their concentrations spectrophotometrically according to Lichtenthaler (1987). Assimilating branch samples were oven-dried at 65°C to constant weight, then ground using a grinder. After grinding, samples were passed through 40-mesh sieves for nutrient content measurement. Total soluble sugar and reducing sugar were quantified according to Roe (1955) and Nelson (1944) using glucose as the standard. Crude protein content was measured by the Kjeldahl method with a conversion coefficient of 6.25. Crude fat content was determined via diethyl-ether extraction using the Soxhlet method. Crude fiber content was measured by the weight method (Somogyi, 1952). Phosphorus and calcium contents were measured using atomic absorption spectrometry (AAS, Type 932GBC, Scientific Equipment Pty Ltd., Australia).

2.5 Soil Collection and Analyses

We selected areas beneath the canopy of fifteen plants each of young, middle-aged, and mature *H. ammodendron* in both burrowed and control areas. For each selected area, soil samples were collected at four compass directions and three depths (0–20, 20–40, and 40–60 cm) at approximately 20 cm from the shrub base, then mixed to form a single composite sample. Soil samples were sealed, labeled, and transported to the laboratory for analysis. All soil samples were air-dried, ground, and passed through a 0.2-mm sieve for analysis. Soil pH (1:5 soil:water mix) was measured using a pH meter (PHS-25 digital explicit, Shanghai Yi Dian Appliance Co., Ltd., China). The following soil properties were measured using methods described by Bao (1999): soil organic matter content (SOM, KCr_2O_7 method), total nitrogen (TN, $HClO_4$-H_2SO_4 digestion method), total phosphorus (TP, Mo-Sb colorimetric method), total potassium (TK, atomic absorption spectrometry), available nitrogen (AN, $CaCl_2$ extracts analyzed with a flow analyzer), available phosphorus (AP, $NaHCO_3$ extracts analyzed with the Mo-Sb colorimetric method), and available potassium (AK, NH_4OAc extracts analyzed by atomic absorption spectrometry).

2.6 Data Analyses

SPSS 19.0 and Origin 9.5 software were used for data analysis and plotting. Data in figures are presented as means ± SE. Above-ground morphology of *H. ammodendron*, physiological characteristics of assimilating branches, and soil physical-chemical properties in burrowed and control areas were compared using independent-sample t-tests ($P < 0.05$). Photosynthetic pigment concentrations in assimilating branches of different ages and physical-chemical properties of soil at different depths were compared using one-way ANOVA with LSD multiple comparisons. Pearson' s correlation analysis was used to examine relationships among above-ground morphological characteristics. Canoco 5.0 redundancy analysis (RDA) was employed to investigate associations between above-ground morphological characteristics and topsoil (0–20 cm) physical-chemical properties in burrowed and control areas ($P < 0.05$). Prior to analysis, above-ground morphological characteristics and soil physical-chemical properties were log-transformed ($\lg(x+1)$). Detrended correspondence analysis (DCA) was used to analyze above-ground morphological characteristics (Lepš and Šmilauer, 2003). Results showed that extended gradients at all axes in burrowed and control areas were 0.325 and 0.328, respectively, indicating suitability for linear redundancy analysis as these values were less than 3.000. All expansion factors of soil physical-chemical properties were less than 10.000, permitting RDA analysis (Braak and Prentice, 1988). Monte Carlo permutation tests were used to progressively select significant soil physical-chemical properties.

3.1 Effects of Rodent Disturbance on Above-Ground Morphological Characteristics of *H. ammodendron*

Rodent disturbance reduced the mean height of *H. ammodendron*, though differences were not significant among age groups ($P < 0.05$; Fig. 1a [Figure 1: see original paper]). The effect of rodent disturbance on above-ground morphological characteristics was most pronounced in young *H. ammodendron.* Basal diameter and crown width of young plants in burrowed areas were significantly larger than those in control areas ($P < 0.05$), increasing by 38.92% and 41.43%, respectively (Fig. 1b). Rodent disturbance significantly increased the crown width of middle-aged *H. ammodendron* by 21.17% ($P < 0.05$; Fig. 1c). Total branching rates of young, middle-aged, and mature *H. ammodendron* increased significantly by 20.88%, 38.65%, and 48.19%, respectively ($P < 0.05$; Fig. 1d) under rodent disturbance.

Plant height showed a strong positive correlation with basal stem diameter, though this relationship was more significant in control areas than in burrowed areas ($P < 0.01$; Fig. 1e). A strong positive correlation was observed between height and crown width in control areas ($P < 0.01$; Fig. 1f), while a low-level positive correlation was found in burrowed areas ($P < 0.05$; Fig. 1f), suggesting that rodent disturbance affected crown width more than basal stem diameter.

Fig. 1 Difference in above-ground morphological characteristics (a–d) of *H. ammodendron* with different ages (heights) in burrowed and control areas. Boxes represent interquartile range (containing 50% of values), lines across boxes represent medians, whiskers indicate highest and lowest values, and circles indicate outliers. * and ** indicate significant differences between burrowed and control areas at $P < 0.05$ and $P < 0.01$ levels, respectively. (e) Correlation between height and basal stem diameter of *H. ammodendron* in burrowed (R^2) and control (${R_0}^2$) areas; and (f) correlation between height and crown width of *H. ammodendron* in burrowed (R^2) and control (${R_0}^2$) areas.

3.2 Effects of Rodent Disturbance on Physiological Characteristics of Assimilating Branches

3.2.1 Nutrient Content in Assimilating Branches

Rodent disturbance effects on nutrient content in assimilating branches of *H. ammodendron* differed among age groups (Fig. 2 [Figure 2: see original paper]). Disturbance significantly reduced most nutrient contents—including total soluble sugar, reducing sugar, crude fiber, and calcium—in young plants, which decreased by 15.21%, 25.78%, 15.74%, and 17.40%, respectively. However, crude protein and phosphorus contents were 18.04% and 15.62% higher in burrowed areas than in control areas (Figs. 2a, c, d, e, and f). In contrast, total soluble sugar and reducing sugar contents in assimilating branches of middle-aged *H. ammodendron* in burrowed areas were 29.44% and 27.05% greater than those in control areas, whereas crude fat and calcium contents were 16.36% and 33.01% lower in burrowed areas (Figs. 2b, c, e, and f). For mature *H. ammodendron*, rodent disturbance only reduced phosphorus content in assimilating branches ($P < 0.05$; Fig. 2). Overall, nutrient content changes in assimilating branches were lowest in mature *H. ammodendron* compared to young and middle-aged plants.

Fig. 2 Difference in nutrient content of assimilating branches of *H. ammodendron* in burrowed and control areas. Bars represent standard error. * and ** indicate significant differences between burrowed and control areas at $P < 0.05$ and $P < 0.01$ levels, respectively.

3.2.2 Photosynthetic Pigment Content in Assimilating Branches

Rodent disturbance significantly affected photosynthetic pigment content in assimilating branches of *H. ammodendron*. Photosynthetic pigment content in young plants decreased significantly ($P < 0.05$; Fig. 3 [Figure 3: see original paper]), while in middle-aged plants, photosynthetic pigment content also decreased significantly, though carotenoid content showed no significant effect ($P < 0.01$; Fig. 3a). Chlorophyll a, total chlorophyll (chlorophyll a and b), and carotenoid contents in assimilating branches of mature *H. ammodendron* decreased significantly in burrowed areas ($P < 0.05$; Fig. 3).

Fig. 3 Comparison of photosynthetic pigment content of *H. ammodendron* in burrowed and control areas. Different lowercase letters represent significant differences among different age groups (heights) of *H. ammodendron* at $P < 0.05$ level. * and ** indicate significant differences between burrowed and control areas at $P < 0.05$ and $P < 0.01$ levels, respectively.

3.3 Soil Physical-Chemical Properties

Soil depth had no significant effect on SOM, TP, AP, TK, and AK contents in either burrowed or control areas ($P > 0.05$; Fig. 4 [Figure 4: see original paper]). Soil TN content was highest in surface soil (0–20 cm) and decreased with depth by 55.56% and 25.00% in burrowed and control areas, respectively, whereas soil water content increased with depth by 75.60% and 67.75% in burrowed and control areas, respectively ($P < 0.05$; Fig. 4). Rodent disturbance enhanced the "fertile island" effect of *H. ammodendron*, primarily manifested as increased soil AN content at 0–40 cm depth, particularly in topsoil (0–20 cm). SOM, TN, AN, and AP contents increased significantly by 20.09%, 55.57%, 45.06%, and 8.94%, respectively, at 0–20 cm depth in burrowed areas compared to control areas ($P < 0.05$; Fig. 4). AN and AP contents and soil pH at 20–40 cm depth were significantly higher in burrowed areas than in control areas ($P < 0.05$; Fig. 4), having increased by 40.39%, 22.71%, and 1.57%, respectively. However, soil pH at 40–60 cm depth in burrowed areas was significantly lower (2.12%) than in control areas ($P < 0.01$; Fig. 4). Additionally, rodent disturbance significantly reduced surface soil (0–20 cm) water content by 35.56% ($P < 0.05$; Fig. 4).

Fig. 4 Difference in soil physical-chemical properties of *H. ammodendron* in burrowed and control areas. Different lowercase letters indicate significant differences among soil depths at $P < 0.05$ level. Boxes represent interquartile range (containing 50% of values), lines across boxes represent medians, whiskers indicate highest and lowest values, and circles indicate outliers. * and ** indicate significant differences between burrowed and control areas at $P < 0.05$ and $P < 0.01$ levels, respectively. SOM, soil organic matter; TN, total nitrogen; TP, total phosphorus; TK, total potassium; AN, available nitrogen; AP, available phosphorus; AK, available potassium.

3.4 Correlation Between Soil Physical-Chemical Properties and Above-Ground Morphological Characteristics

RDA was performed to evaluate associations between topsoil (0–20 cm) physical-chemical properties and above-ground morphology of *H. ammodendron* (Fig. 5 [Figure 5: see original paper]; Table 1). No significant differences in soil water content were observed among deep soil samples (data not shown), so soil water content was excluded from RDA analysis. The first two axes explained 70.2% of morphological variation in burrowed areas (Fig. 5a) and 63.8% in control areas (Fig. 5b). AN ($P = 0.002$) and AK ($P = 0.002$) significantly influenced above-ground morphology of *H. ammodendron* in burrowed areas, with influence order AN > AK (Fig. 5a). In control areas, AN ($P = 0.02$), AK ($P = 0.004$), TN ($P = 0.012$), and AP ($P = 0.008$) notably affected above-ground morphology, with influence order AN > AK > TN > AP (Fig. 5b). These results indicate that AN and AK in topsoil are the primary factors affecting above-ground morphology of *H. ammodendron* regardless of rodent disturbance.

Fig. 5 Correlation between physical-chemical properties of topsoil and above-ground morphology of *H. ammodendron* as determined by redundancy analysis (RDA). (a) Burrowed areas; and (b) control areas. •, young *H. ammodendron*; , middle-aged *H. ammodendron*; , mature *H. ammodendron*; SOM, soil organic matter; TN, total nitrogen; TP, total phosphorus; TK, total potassium; AN, available nitrogen; AP, available phosphorus; AK, available potassium.

Table 1 Forward selection of soil variables during redundancy analysis

Sample plot	Soil variable	Eigenvalues	Explained variance (%)
Burrowed area	AN	(value not specified)	(value not specified)
Burrowed area	AK	(value not specified)	(value not specified)
Control area	AN	(value not specified)	(value not specified)
Control area	AK	(value not specified)	(value not specified)
Control area	TN	(value not specified)	(value not specified)
Control area	AP	(value not specified)	(value not specified)

Note: AN, available nitrogen; AK, available potassium; TP, total phosphorus; TK, total potassium; SOM, soil organic matter; AP, available phosphorus; TN, total nitrogen.

4.1 Soil Physical-Chemical Properties Under *H. ammodendron* Shrubs

Rodent activity significantly increased SOM, TN, AN, and AP contents in soil (Fig. 4), primarily due to burrow systems constructed beneath *H. ammodendron* shrubs that accumulate plant residues, feces, and urine, thereby strengthening the "fertile island" effect. Differences were most pronounced in topsoil (0–20 cm) (Fig. 4) due to the range of rodent activity. Studies have shown that gerbils, the most abundant rodent species in this area, are mainly active at 20–30 cm soil depth (Jiang et al., 2007; Xu et al., 2012). In addition to underground activities, frequent surface activities of rodents also increase nutrient contents in topsoil. Increased soil AN and AP contents at 20–40 cm depth in burrowed areas may be related to microbial activity. Research has demonstrated that increased organic matter and nitrogen contents enhance microbial biomass and diversity, improving mineral bioavailability (Ewing et al., 2007; Jiang et al., 2007). Furthermore, rodent activities likely affect root exudate release from *H. ammodendron*, thereby influencing soil physical-chemical properties and microbial biomass and diversity, with this interaction potentially being more pronounced in rhizosphere soil. However, this speculation requires further investigation.

As a typical salt-tolerant plant, *H. ammodendron* creates high soil salt content beneath its canopy through water absorption and salt transport by the root system (Munns and Tester, 2008; He et al., 2018), a phenomenon known as the "salt island" effect (Li et al., 2011). Our study found that rodent interference significantly increased soil pH at 20–40 cm depth (Fig. 4), suggesting that rodent activities may impact the "salt island" effect under *H. ammodendron*. Root damage from rodent activities may weaken the plant' s ability to absorb and transport salt. Since our study did not measure soil salt content, further research is needed. Additionally, because rodent activities vary seasonally (Liu et al., 2012), physical-chemical changes in burrow area soils will vary depending on sampling timing. For example, during food storage seasons, the number of foraging holes increases substantially, affecting soil physical-chemical properties (Whitford and Kay, 1999). Studies examining seasonal changes in soil properties due to rodent disturbance are needed to address this question.

4.2 Above-Ground Morphological Characteristics of *H. ammodendron*

We found that AN and AK were positively correlated with height, basal diameter, and crown width of *H. ammodendron* and were the main determinants affecting growth in rodent-disturbed areas (Fig. 5a). This may be because nutrient return to soil is slow in desert ecosystems (Barthelemy et al., 2018), and nitrogen and potassium sources in rodent feces can be readily absorbed by vascular plants to stimulate growth (Sitters et al., 2017). However, rodent disturbance had a minor inhibitory effect on plant height, though the difference was not statistically significant (Fig. 1a), indicating that herbivores have a small inhibitory effect on *H. ammodendron* growth. Young *H. ammodendron* in burrowed areas had thicker stems than those in control areas (Fig. 1b), likely related to subsequent increases in soil nutrients, though rodent disturbance only affected basal diameter in young plants. Additionally, young *H. ammodendron* disturbed by rodents exhibited greater crown widths and total branching rates than undisturbed plants (Figs. 1c and d), probably related to increased soil nutrient content and availability. Increased branching rate may also be triggered by amputation, which tends to increase crown width. Amputation generally leads to greater branch strength induced by axillary meristems released from apical dominance (Shimizu-Sato et al., 2009). Furthermore, the effect of rodent disturbance on total branching rate was much greater in middle-aged and mature *H. ammodendron* than in young plants (Fig. 1d), possibly because rodents favor larger plants when constructing burrows and remain near burrows most of the time, moving only short distances (Falkenberg and Clarke, 1998; Rogovin et al., 2003). Thus, larger *H. ammodendron* display a greater branching response to damage. Increased crown width and branching help expand leaf area, allowing plants to absorb more sunlight, CO_2, and O_2, reflecting the morphological flexibility of *H. ammodendron* in response to rodent disturbance.

4.3 Photosynthetic Pigment and Nutrient Contents of Assimilating Branches of *H. ammodendron*

Rodent disturbance significantly decreased photosynthetic pigment content in annual assimilating branches of *H. ammodendron*, except for carotenoid content in middle-aged plants (Fig. 3). Jasmonic acid (JA) or methyl jasmonate (JA-Me) promotes chlorophyll degradation in leaves (Jung, 2004; Li et al., 2016), and both mechanical injury and herbivore attack can lead to rapid accumulation of JA or JA-Me in plants (Campos-Vargas and Saltveit, 2010; Nabity et al., 2013). We therefore speculate that chlorophyll reduction triggered by rodent disturbance may be associated with JA-induced chlorophyll degradation after branch feeding. Mechanical damage to chloroplasts and reactive oxygen species-mediated lipid peroxidation of chlorophyll pigments may also contribute to chlorophyll content reduction (Sıvritepe et al., 2009), though this phenomenon requires further investigation.

Compared with control areas, no significant correlations were observed between photosynthetic pigment content and total soluble sugar or reducing sugar in assimilating branches in burrowed areas (Fig. S1), indicating that decreased photosynthetic pigment content is not the primary reason for changes in soluble sugar and reducing sugar contents. In our study, rodent disturbance significantly increased total soluble sugar and reducing sugar contents while significantly decreasing crude fat content in assimilating branches of middle-aged *H. ammodendron* (Figs. 2b, e, and f), possibly related to mechanical damage of photosynthetic tissues by herbivores altering carbohydrate status and distribution in plants (Koch, 1996). In burrowed areas, total soluble sugar and reducing sugar in assimilating branches may primarily function as osmoregulation substances for middle-aged *H. ammodendron*. In contrast, total soluble sugar and reducing sugar did not increase but instead decreased significantly in young *H. ammodendron* after rodent disturbance (Figs. 2e and f), possibly because young trees lack well-developed root and stem food reserves present in mature plants (Barton, 2016). Therefore, after browsing, young *H. ammodendron* reduced nutrient contents such as total soluble sugar, reducing sugar, crude fiber, and calcium in assimilating branches (Fig. 2) to discourage rodent feeding. According to optimal allocation theory, highly defensive plants produce chemical substances to prevent animal feeding, which may decrease nutrient availability for growth and development (Züst et al., 2015; Karasov et al., 2017). However, our study focused only on nutrient levels; changes in chemical defense substances require further investigation.

Modification of nutrient contents in assimilating branches of *H. ammodendron* due to rodent disturbance was only significant in young plants. Ulappa et al. (2014) reported that browsed plants with long-term occupancy were more likely to have greater crude protein content. Our study found a significant positive correlation between crude protein and soil N (Table S1), though rodent disturbance only significantly increased crude protein content in young *H. ammodendron* (Fig. 2a). Nutrient contents in assimilating branches of mature *H. ammodendron* were virtually unaffected except for phosphorus (Fig. 2), findings comparable to those reported by O' Reilly-Wapstra et al. (2005) and Sotelo et al. (2014). The age at which plants are fed upon also affects optimal defense response (Karasov et al., 2017), and mature plants of the same species may have stronger defensive capabilities than young specimens. Thus, the degree of rodent disturbance observed in burrowed areas of this study was insufficient to significantly impact nutrient composition of assimilating branches in mature *H. ammodendron*.

It should be noted that the amount of *H. ammodendron* feeding by rodents varies with season and burrow density (Liu et al., 2012; Xu et al., 2012). *H. ammodendron* was much more abundant than any other shrub species and became the main food source for rodents in the study area during autumn when most herbaceous plants withered (Liu et al., 2012). Our experiment was conducted in summer when burrow coverage was 18%-20% in this area (Ma et al., 2018). However, as disturbance degree changes, eco-physiological responses in *H. ammodendron* may fluctuate. Our results provide a preliminary examination of some eco-physiological responses of *H. ammodendron* to rodent disturbance at the southern edge of the Gurbantunggut Desert.

5 Conclusions

Rodent feeding decreased photosynthetic pigment contents of *H. ammodendron* but increased branch volume and crown size to compensate for losses of plant tissue, nutrients, and photosynthetic machinery. In response to losses from rodent activity, young *H. ammodendron* tended to reduce nutrient content in assimilating branches to discourage herbivore feeding. The effect of increased soil nutrients on young *H. ammodendron* was more dramatic than on middle-aged and mature plants. Middle-aged *H. ammodendron* tended to invest more energy in defense, increasing chemical defense substances such as total soluble sugar and reducing sugar while decreasing nutrient content in assimilating branches. The response of mature *H. ammodendron* to rodents was the least pronounced among age groups. Overall, *H. ammodendron* exhibits varying degrees of tolerance to rodent disturbance across age groups, with defense systems strengthening as plants grow and the effects of rodent disturbance gradually decreasing with age.

Acknowledgements

This study was funded by the National Natural Science Foundation of China (41561010, 31560177) and the Key Laboratory of Xinjiang Phytomedicine Resource and Utilization and Academic Leader of "3152" in Shihezi University, China. We are grateful for assistance from Ms. JIANG Fengjiao, Mr. GE Jinxiong, and Mr. LUO Yunfeng.

References

Allington G R H, Valone T J. 2014. Islands of fertility: A byproduct of grazing? *Ecosystems*, 17(1): 127-141.

Andino N, Borghi C E. 2018. Occurrence of ctenomys mendocinus in a high-altitude cold desert: Effect on density, biomass, and fitness of sagebrush plants. *Arctic, Antarctic, and Alpine Research*, 49(1): 53-60.

Bao S D. 1999. *Methods of Agrochemical Soil Analysis*. Beijing: China Agriculture Press, 25-35. (in Chinese)

Barthelemy H, Stark S, Michelsen A, et al. 2018. Urine is an important nitrogen source for plants irrespective of vegetation composition in an Arctic tundra: Insights from a ^{15}N-enriched urea tracer experiment. *Journal of Ecology*, 106(1): 367-378.

Barton K E, Koricheva J. 2010. The ontogeny of plant defense and herbivory: Characterizing general patterns using meta-analysis. *American Naturalist*, 175(4): 481-493.

Barton K E. 2016. Low tolerance to simulated herbivory in Hawaiian seedlings despite induced changes in photosynthesis and biomass allocation. *Annals of*

Botany, 117(6): 1053-1062.

Berke S K. 2010. Functional groups of ecosystem engineers: A proposed classification with comments on current issues. *Integrative & Comparative Biology*, 50(2): 147-157.

Braak C J F T, Prentice I C. 2004. A theory of gradient analysis. *Advances in Ecological Research*, 34: 23-28.

Campos-Vargas R, Saltveit M E. 2010. Involvement of putative chemical wound signals in the induction of phenolic metabolism in wounded lettuce. *Physiologia Plantarum*, 114(1): 73-84.

Christie K S, Ruess R W, Lindberg M S, et al. 2014. Herbivores influence the growth, reproduction, and morphology of a widespread arctic willow. *PloS ONE*, 9(7): e101716.

Dong B C, Fu T, Luo F L, et al. 2017. Herbivory-induced maternal effects on growth and defense traits in the clonal species *Alternanthera philoxeroides*. *Science of the Total Environment*, 605-606: 114-123.

Ewing S A, Southard R J, Macalady J L, et al. 2007. Soil microbial fingerprints, carbon, and nitrogen in a Mojave Desert creosote-bush ecosystem. *Soil Science Society of America Journal*, 71(2): 469-475.

Falkenberg J C, Clarke J A. 1998. Microhabitat use of deer mice: Effects of interspecific interaction risks. *Journal of Mammalogy*, 79(2): 558-565.

Hagenah N, Bennett N C. 2013. Mole rats act as ecosystem engineers within a biodiversity hotspot, the Cape Fynbos. *Journal of Zoology*, 289(1): 19-26.

Hakes A S, Cronin J T. 2011. Resistance and tolerance to herbivory in *Solidago altissima* (Asteraceae): Genetic variability, costs, and selection for multiple traits. *American Journal of Botany*, 98(9): 1446-1455.

He A L, Niu S Q, Zhao Q, et al. 2018. Induced salt tolerance of perennial ryegrass by a novel bacterium strain from the rhizosphere of a desert shrub *Haloxylon ammodendron*. *International Journal of Molecular Sciences*, 19(2): 469.

Huang G, Cao Y F, Wang B, et al. 2015. Effects of nitrogen addition on soil microbes and their implications for soil C emission in the Gurbantunggut Desert, center of the Eurasian Continent. *Science of the Total Environment*, 515-516: 215-224.

Jiang H P, Wu N, Yang W K. 2007. Effects of *Rhombomys opimus* on microbial quantity, soil moisture content, and soil nutrient content in a desert. *Arid Zone Research*, 24(2): 187-192. (in Chinese)

Jung S. 2004. Effect of chlorophyll reduction in *Arabidopsis thaliana* by methyl jasmonate or norflurazon on antioxidant systems. *Plant Physiology & Biochemistry* (Paris), 42(3): 225-231.

Kafle D, Wurst S. 2019. Legacy effects of herbivory enhance performance and resistance of progeny plants. *Journal of Ecology*, 107(1): 58-68.

Kang J J, Zhao W, Ying Z, et al. 2017. Calcium chloride improves photosynthesis and water status in the C4 succulent xerophyte *Haloxylon ammodendron* under water deficit. *Plant Growth Regulation*, 82(3): 467-478.

Karasov T, Chae E, Herman J, et al. 2017. Mechanisms to mitigate the tradeoff between growth and defense. *Plant and Cell*, 29(4): 666-680.

Kindler D S, Robert S. 1970. Nutrients and the reaction of two alfalfa clones to the spotted alfalfa aphid. *Journal of Economic Entomology*, 63(3): 938-940.

Koch K E. 1996. Carbohydrate-modulated gene expression in plants. *Annual Review of Plant Physiology and Plant Molecular Biology*, 47(1): 509-540.

Kozlov M V, Zvereva E L. 2012. Delayed local responses of downy birch to damage by leafminers and leafrollers. *Oikos*, 121(3): (page numbers not specified in original)

Lacey E A, Patton J L, Cameron G N, et al. 2001. *Community Ecology of Subterranean Rodents*. Chicago: University of Chicago Press, 75-76.

Lepš J, Šmilauer P. 2003. *Multivariate Analysis of Ecological Data Using CANOCO*. Cambridge: Cambridge University Press.

Li C J, Li Y, Ma J. 2011. Spatial heterogeneity of soil chemical properties at fine scales induced by *Haloxylon ammodendron* (Chenopodiaceae) plants in a sandy desert. *Ecological Research*, 26(2): 385-394.

Li L, Li H X, Zeng H L, et al. 2016. Exogenous jasmonic acid and cytokinin antagonistically regulate rice flag leaf senescence by mediating chlorophyll degradation, membrane deterioration, and senescence-associated genes expression. *Journal of Plant Growth Regulation*, 35(2): 366-376.

Lichtenthaler H K. 1987. Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. *Methods in Enzymology*, 148: 350-382.

Liu W, Xu W X, Yang W K, et al. 2012. Food habits of the great gerbil (*Rhombomys opimus*) in the Southern Gurbantunggut Desert, Xinjiang, China. *Pakistan Journal of Zoology*, 44(4): 931-936.

Luo Q, Chen Q, Ning H, et al. 2017. Chronosequence-based population structure and natural regeneration of *Haloxylon ammodendron* plantation in the southern edge of the Gurbantunggut Desert, Northwestern China. *Russian Journal of Ecology*, 48(4): 364-371.

Ma T, Zheng J H, Wen A M, et al. 2018. Group coverage of burrow entrances and distribution characteristics of desert forest-dwelling *Rhombomys opimus* based on unmanned aerial vehicle (UAV) low-altitude remote sensing: A case study at the southern margin of the Gurbantunggut Desert in Xinjiang. *Acta Ecologica Sinica*, 38(3): 953-963. (in Chinese)

Mabry T J, Hunziker J H, Difeo D R J. 1977. Creosote bush: Biology and chemistry of *Larrea* in new world deserts. *Desert Plants*, 31(5): 399.

Mao L Z, Lu H F, Wang Q, et al. 2007. Comparative photosynthesis characteristics of *Calycanthus chinensis* and *Chimonanthus praecox*. *Photosynthetica*, 45(4): 601-605.

Mares M A, Ojeda R A, Borghi C E, et al. 1997. How desert rodents overcome halophytic plant defenses. *Bioscience*, 47(10): (page numbers not specified in original)

Munns R, Tester M. 2008. Mechanisms of salinity tolerance. *Annual Review of Plant Biology*, 59(1): 651-681.

Nabity P D, Zavala J A, DeLucia E H. 2013. Herbivore induction of jasmonic acid and chemical defences reduce photosynthesis in *Nicotiana attenuata*. *Journal of Experimental Botany*, 64(2): 685-694.

Nelson N. 1944. A photometric adaptation of the Somogyi method for determination of glucose. *Journal of Biological Chemistry*, 153: 375-380.

Ngatia L W, Turner B L, Njoka J T, et al. 2015. The effects of herbivory and nutrients on plant biomass and carbon storage in vertisols of an East African savanna. *Agriculture, Ecosystems & Environment*, 208: 55-63.

O' Reilly-Wapstra J M, Potts B M, McArthur C, et al. 2005. Inheritance of resistance to mammalian herbivores and of plant defensive chemistry in a *Eucalyptus* species. *Journal of Chemical Ecology*, 31(3): 519-537.

Roe J H. 1955. The determination of sugar in blood and spinal fluid with anthrone reagent. *Journal of Biological Chemistry*, 212(1): 335-343.

Rogovin K, Randall J A, Kolosova I, et al. 2003. Social correlates of stress in adult males of the great gerbil, *Rhombomys opimus*, in years of high and low population densities. *Hormones & Behavior*, 43(1): 132-139.

Schweissing F C, Wilde G. 1979. Temperature and plant nutrient effects on resistance of seedling sorghum to the greenbug. *Journal of Economic Entomology*, 72(1): 20-23.

Shimizu-Sato S, Tanaka M, Mori H. 2009. Auxin-cytokinin interactions in the control of shoot branching. *Plant Molecular Biology*, 69(4): 429-435.

Siemens D H, Garner S H, Mitchell-Olds T, et al. 2002. Cost of defense in the context of plant competition: *Brassica rapa* may grow and defend. *Ecology*, 83(2): 505-517.

Sitters J, te Beest M, Cherif M, et al. 2017. Interactive effects between reindeer and habitat fertility drive soil nutrient availabilities in arctic tundra. *Ecosystems*, 20(7): 1266-1277.

Sİvritepe N, Kumral N A, Erturk U, et al. 2009. Responses of grapevines to two-spotted spider mite mediated biotic stress. *Journal of Biological Sciences*, 9(4): 311-318.

Somogyi M. 1952. Note on sugar determination. *Journal of Biological Chemistry*, 195(1): 19-23.

Sotelo P, Pérez E, Najar-Rodriguez A, et al. 2014. *Brassica* plant responses to mild herbivore stress elicited by two specialist insects from different feeding guilds. *Journal of Chemical Ecology*, 40(2): 136-149.

Steingraeber D A, Waller D M. 1986. Non-stationarity of tree branching patterns and bifurcation ratios. *Series B, Biological Sciences*, 228(1251): 187-194.

Tchabovsky A V, Krasnov B, Khokhlova I S, et al. 2001. The effect of vegetation cover on vigilance and foraging tactics in the fat sand rat *Psammomys obesus*. *Journal of Ethology*, 19(2): 105-113.

Ulappa A C, Kelsey R G, Frye G G, et al. 2014. Plant protein and secondary metabolites influence diet selection in a mammalian specialist herbivore. *Journal of Mammalogy*, 95(4): 834-842.

Whitford W G, Kay F R. 1999. Biopedturbation by mammals in deserts: A review. *Journal of Arid Environments*, 41(2): (page numbers not specified in original)

Whitney G G. 1976. The bifurcation ratio as an indicator of adaptive strategy in woody plant species. *Bulletin of the Torrey Botanical Club*, 103(2): 67-72.

Xu M H, Liu T, Jiang L. 2012. Study on the harm characteristics of rodents to *Haloxylon ammodendron* and its control for ecological threshold value in the south of Gurbantonggut Desert. *Journal of Arid Land Resources and Environment*, 26(6): 126-133. (in Chinese)

Xu W X, Liu W, Yang W K, et al. 2012. *Rhombomys opimus* contribution to the “fertile island” effect of tamarisk mounds in Junggar Basin. *Ecological Research*, 27(4): 775-781.

Zhang K, Su Y Z, Liu T N, et al. 2016. Leaf C:N:P stoichiometrical and morphological traits of *Haloxylon ammodendron* over plantation age sequences in an oasis-desert ecotone in North China. *Ecological Research*, 31(3): 449-457.

Zhang S L, Kou M J, Bing J C, et al. 2001. Investigation of forest rodents fauna in Gansu province. *Forest Pest and Disease*, 6: 26-28. (in Chinese)

Zhang S L, Chen Y W, Ma J M, et al. 2009. Effect of the damage and feeding of *Rhombimys opimus* on the growth of *Haloxylon ammodendron*. *Forest Pest & Disease*, 28(1): 7-9. (in Chinese)

Züst T, Rasmann S, Agrawal A A. 2015. Growth-defense tradeoffs for two major anti-herbivore traits of the common milkweed *Asclepias syriaca*. *Oikos*, 124(10): 1404-1415.

Appendix

Fig. S1 Pearson' s correlation coefficients among physiological indices of assimilating branches of *H. ammodendron* in burrowed and control areas. * and ** indicate significant differences between indices at $P < 0.05$ and $P < 0.01$ levels, respectively. TSS, total soluble sugar; RS, reducing sugar; CF, crude fiber; EE, crude fat; CP, crude protein; P, phosphorus; Ca, calcium; Cha, chlorophyll a; Chb, chlorophyll b; Car, carotenoid; Total Chl, total chlorophyll.

Table S1 Correlation of nutrients between topsoil and assimilating branches of *H. ammodendron* in burrowed areas

Nutrient	Correlation coefficient
TSS	-0.82**
RS	0.62*
CF	-0.74*
EE	0.70*
CP	-0.79*
P	0.75*
Ca	0.78*
Cha	-0.88**
Chb	0.71*
Car	0.78*
Total Chl	-0.82**

Note: TSS, total soluble sugar; RS, reducing sugar; CF, crude fiber; EE, crude fat; CP, crude protein; P, phosphorus; Ca, calcium; SOM, soil organic matter; TN, total nitrogen; TP, total phosphorus; TK, total potassium; AN, available nitrogen; AP, available phosphorus; AK, available potassium. * and ** indicate significant correlations at $P < 0.05$ and $P < 0.01$ levels, respectively.

Note: Figure translations are in progress. See original paper for figures.

Source: ChinaXiv —Machine translation. Verify with original.