



Enhancing rangeland sustainability by reseeding legumes in temperate steppe

Chuan Guo¹, Yue Pang¹, Meiqi Guo¹, Jiqiong Zhou², Hao Zhang³, Nan Liu¹, Yingjun Zhang¹, Gaowen Yang¹

¹College of Grassland Science and Technology, China Agricultural University, Beijing 100193, China; ²College of Grassland Science & Technology, Sichuan Agricultural University, Chengdu 611130, China; ³College of Animal Science & Technology, Henan University of Animal Husbandry and Economy, Zhengzhou 450044, China

Corresponding author: yanggw@cau.edu.cn; zhangyj@cau.edu.cn

Key words: legumes; reseeding; production; no-tillage; soil nitrogen; soil organic matter

Abstract

Legumes, acting as important fixers of nitrogen (N), are characterized by high forage quality. However, these key species are suffering from substantial declines in rangeland worldwide, especially in degraded ecosystems, with subsequent constraints on livestock performance. There is an urgent need to restore legumes to improve forage quality and quantity. We developed a cutting-edge reseeding technology, and a new re-seeding machine, to introduce legumes into degraded rangeland. Compared with traditional no-tillage reseeding, the new reseeding technology can dramatically improve germination rates of reseeded legumes by creating suitable micro-habitat for seed germination and seedling establishment, e.g., higher soil temperature and moisture. Legume-reseeded rangeland was more productive than non-reseeded rangeland, primarily due to enhanced ecological niche complementarity and compensatory growth through interspecific facilitation. Four years after legume reseeding, soil nitrogen and organic matter content were increased by more than 10%. Beneficial soil microbes, e.g., the abundance of arbuscular mycorrhizal fungi, was significantly increased by 31% after legume reseeding. In general, introducing legumes into degraded rangeland can improve forage production and quality, and simultaneously enhance rangeland sustainability by promoting soil health.

Introduction

Legumes are integral components of rangeland ecosystems, contributing significantly to soil fertility and enhancing the overall nutritional value of forages (Ganjurjav et al., 2024). However, rangeland degradation, driven by climatic change and anthropogenic disturbances, has led to a rapid decrease in diversity and abundance of legumes (Xu et al., 2020; Tognetti et al., 2021). This loss of legumes has profound ecological and economic consequences, for instance, reducing soil carbon sequestration and nitrogen fixation, and decreasing forage quality and quantity (Stagnari et al., 2017). Reintroducing legumes into degraded rangelands has been recognized as a critical strategy for restoration and renewal (Waddington, 1992; Mi et

al., 2024). Nevertheless, the successful establishment and persistence of legumes are often hindered by abiotic factors (e.g., drought; light competition) and biotic factors such as competition from native vegetation in rangeland. To facilitate seedling establishment and achieve long-term maintenance of legume proportion in rangeland, there is an urgent need to develop effective agronomic practices. Here we focus on the approach of reintroducing legumes into degraded steppes and then investigate the effect of legume reintroduction on forage production and soil nutrient content. This study aims to provide practical implications for rangeland restoration and ecosystem sustainability.

Methods

Study area and experimental design

The study site is located at a temperate steppe in Hulunbuir, northeastern Inner Mongolia, China (49 ° 20 ' ~ 49 ° 26 ' N, 119 ° 55 ' ~ 120 ° 9 ' E, altitude 628 ~ 649 m), with an annual average temperature of -3 ~ 0 °C, and an annual average precipitation of 350 ~ 410 mm. The precipitation is seasonal and mainly occurs from June to September. The study area is moderately degraded steppe. In June 2020, we reseeded yellow-flower alfalfa (*Medicago falcata*) into degraded rangeland using no tillage machine with inverted T-shaped or V-shaped slots, and the un-reseeded area was treated as non-reseeding (NR) treatment. We randomly selected ten 1 × 1 m sample plots for paired sampling, applying both non-reseeding (NR) and reseeding (R) treatments.

Sampling and measurements

After reseeding, we buried button-type temperature and humidity monitors in each slot, which can automatically record data every hour. These monitors recorded soil relative humidity data from July 19th to September 6th, 2020, and soil temperature data from June 17th to September 23rd, 2020. We randomly selected three 1-meter-long slots in each plot, and counted the number of alfalfa seedlings in each slot 7 days after reseeding to calculate germination rate. Aboveground living plants in each plot were harvested and sorted by species in middle August 2024 (peak growth period). All plant species were classified into three functional groups: legume, forb, and grass. We collected soil samples at the depths of 0-15 cm and measured total nitrogen and organic carbon content using an elemental analyser (Vario Macro, elemental, Germany) and a CN analyser (Elementar, Germany), and measured the abundance of arbuscular mycorrhizal fungi (AMF) using phospholipid fatty acid (PLFA) analysis.

Statistical analysis

When investigating the impact of inverted T- or V-shaped slot reseeding on soil temperature and humidity, we performed one-way ANOVA using the *aov* function in the *stats* package. If significant effects were found, we used the *agricolae* package for a Duncan test. We used the *t.test* function in the base *stats* package to detect the difference between non-reseeding (NR) and reseeding (R) treatments. The *ggplot2* package was used for data visualization. All statistics and data visualization were performed in R 4.3.3 (R Core Team, 2024).

Results

The inverted T-shaped slot increased soil temperature and humidity.

We developed a new re-seeding machine using inverted T-shaped slot to introduce legumes into degraded rangeland (Fig. 1). The inverted T-shaped slot significantly increased soil temperature and relative humidity in the ditch by 1.2°C and 60%, respectively. The increase in soil temperature and relative humidity provided a suitable microenvironment for seed germination (Fig. 2, Table 1), and therefore the inverted T-shaped slot increased seed germination rate by 120%.

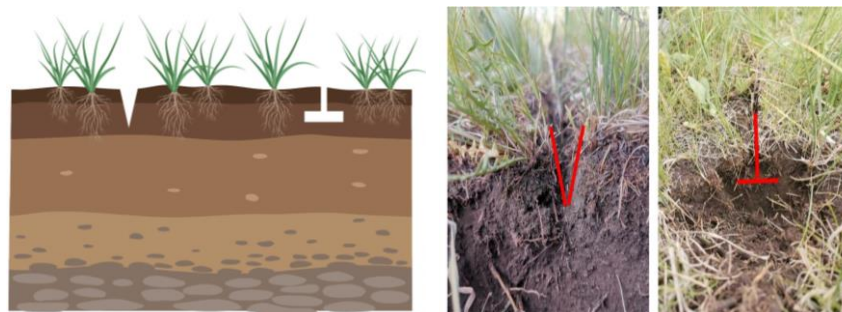


Fig. 1 The ditch created by the V-shaped slot or the inverted T-shaped slot.

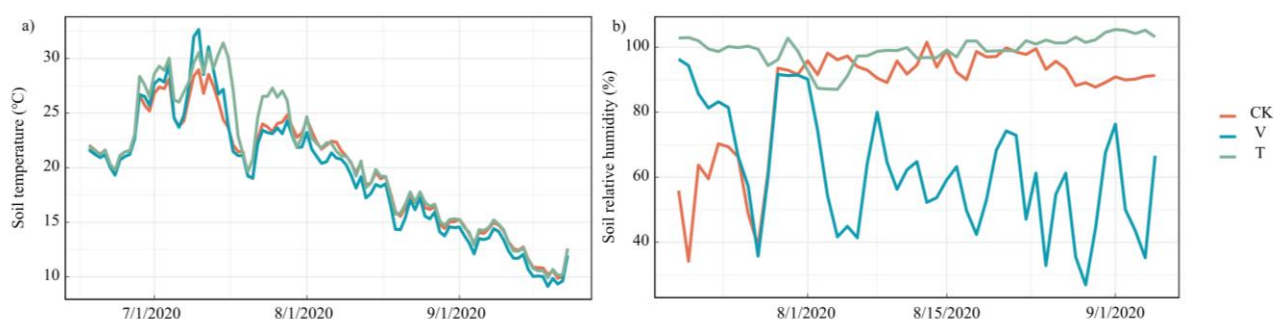


Fig. 2 Effects of the inverted T-shaped slot on soil temperature (a) and humidity (b). CK, control; V, V-shaped slot; T, inverted T-shaped slot.

Table 1 Effects of reseeding method on soil temperature and humidity and germination rate

Reseeding method	Soil relative humidity (%)	Soil temperature (°C)	Germination rate (%)
Control (CK)	86.31 ± 2.34 b	19.69 ± 0.51 b	-
V-shaped slot (V)	62.20 ± 2.59 c	19.32 ± 0.57 b	25.41 ± 7.93 b
Inverted T-shaped slot (T)	99.24 ± 0.62 a	20.42 ± 0.60 a	55.96 ± 11.53 a

Note: different lowercase letters following values (mean ± SE) represent significant ($P < 0.05$) differences, while identical lowercase letters indicate no significant differences among or between treatments.

Reseeding legumes increased soil nutrient content and AMF biomass.

Legume introduction increased soil total nitrogen content 14% and increased soil organic carbon content by 7.5% after four years of reseeding legumes (Fig. 3a, b). Reseeding legumes increased the abundance of AM fungi by 31% (Fig. 3c).

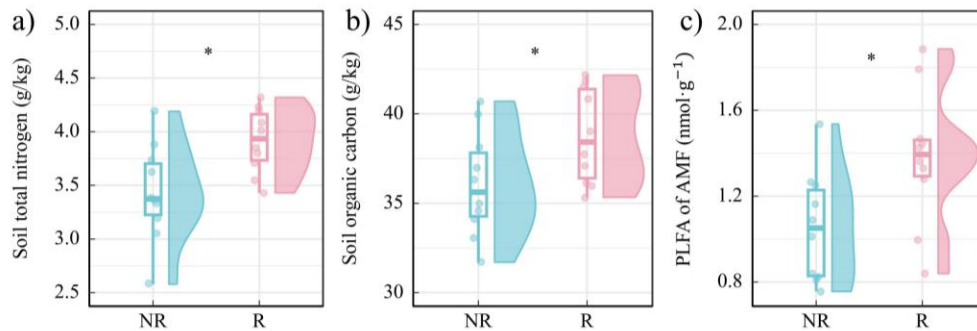


Fig. 3 Effects of reseeding legumes on soil nutrient content and AMF abundance. The difference between the reseeding (R) and non-reseeding (NR) treatments is indicated by asterisks ($P < 0.05$).

Reseeding legumes increased plant biomass and changed community composition.

Reseeding legumes significantly improved the productivity of degraded rangeland. For instance, total biomass production was increased by 148%, which mainly came from the increase in the biomass of legume functional group (Fig. 4a). After reseeding, the proportion of legume functional group significantly increased, while the proportion of grass and forb has significantly decreased (Fig. 4b).

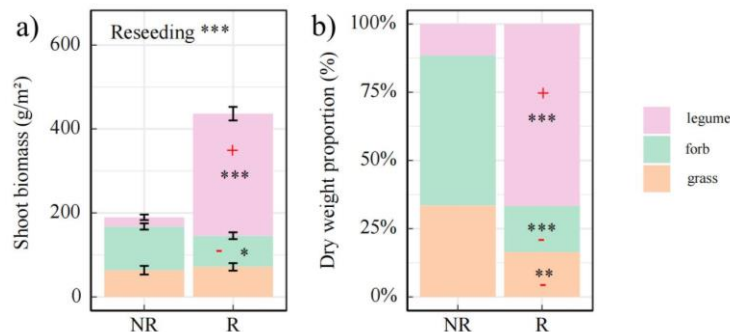


Fig. 4 Effects of reseeding legume on plant biomass and community composition. The difference between the reseeding (R) and non-reseeding (NR) treatments is indicated by asterisks (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$). The symbols ‘+’ and ‘-’ indicate a significant increase and decrease by reseeding, respectively.

Discussion

In summary, we have developed an effective solution for restoring temperate degraded steppes through reseeding legumes. The inverted T-shaped slot significantly improved the germination rates of legume seedlings by promoting soil temperature and humidity. The successful establishment of legumes can dramatically improve forage production and simultaneously enhance soil health indicated by an increase in soil fertility and the abundance of AMF. Our study shows that reseeding legumes in temperate steppe can promote forage production and enhance rangeland sustainability.

Reseeding via the inverted T-shaped slot serves as an effective practice for creating favourable microhabitats. Compared to the traditional V-shaped slot, the inverted T-shaped slot can create a ditch with smaller surface soil cracks and larger underground voids. These attributes not only help reduce soil water loss and maintain soil temperature, but also mitigate the underground competition of native species against the reseeded ones. The improvement of microhabitat could benefit seedling establishment, according to a

previous study (Lett and Dorrepaal, 2018). The successful introduction of legumes led to substantial increases in plant productivity, driven primarily by a high proportion of legumes in plant communities. One of our recent studies found that reseeded legumes can further improve the performance of neighbouring species (Guo et al., 2024). This is because the presence of legumes can enhance ecological niche complementarity and promote compensatory growth through interspecific facilitation (Guo et al., 2024). It is well known that legumes can gradually enrich soil nitrogen availability through biological nitrogen fixation. This will stimulate microbial processes that enhance soil organic carbon sequestration (Abalos et al., 2020; Gou et al., 2023; Hu et al., 2024). Our findings align with previous studies, showing significant increases in soil total nitrogen and carbon storage following legume introduction. Additionally, microbial restoration is a critical objective for degraded ecosystems. In our study, the abundance of AMF was increased dramatically by reseeded legumes. The increase in AMF abundance can improve multiple ecosystem functions, e.g., the formation of soil aggregates and plant growth, leading to positive feedback for restoration.

Our study has revealed the multifaceted benefits of legume restoration in improving ecosystem function within degraded rangelands. In the future, efforts should be directed towards harnessing the ecological advantages of legumes to restore degraded rangelands. Furthermore, it is essential to develop appropriate management strategies to maintain the proportion of legumes in plant communities, thereby ensuring ecosystem sustainability.

Acknowledgements

The study was funded by Special Funds of Inner Mongolia Autonomous Region for Transformation of Scientific and Technological Achievements (2022YFDZ0101) and the National Natural Science Foundation of China (32192462, 32271771).

References

- Abalos D, De Deyn GB, Philippot L, Oram NJ, Oudová B, Pantelis I, Clark C, Fiorini A, Bru D, Mariscal-Sancho I, van Groenigen JW, Cheng L (2020) Manipulating plant community composition to steer efficient N-cycling in intensively managed grasslands. *Journal of Applied Ecology* 58(1), 167-180.
- Ganjurjav H, Li M, Han L, Sha Y, Li Z, Han X, Ji G, Wu R, Ma Y, Hu G, Gornish E, Gao Q (2024) Reseeding increased plant biomass production and soil fertility, but not plant species diversity in degraded grasslands in China. *Journal of Environmental Management* 370, 122966.
- Gou X, Reich PB, Qiu L, Shao M, Wei G, Wang J, Wei X (2023) Leguminous plants significantly increase soil nitrogen cycling across global climates and ecosystem types. *Global Change Biology* 29(14), 4028-4043.
- Guo M, Guo T, Zhou J, Liang J, Yang G, Zhang Y (2024) Restored legume acts as a “nurse” to facilitate plant compensatory growth and biomass production in mown grasslands. *Agronomy for Sustainable Development* 44, 60.
- Hu Q, Zhang Y, Cao W, Yang Y, Hu Y, He T, Li Z, Wang P, Chen X, Chen J, Shi X (2024) Legume cover crops sequester more soil organic carbon than non-legume cover crops by stimulating microbial transformations. *Geoderma* 450, 117024.
- Lett S, Dorrepaal E (2018) Global drivers of tree seedling establishment at alpine treelines in a changing climate. *Functional Ecology* 32(7), 1666-1680.
- Mi W, Zheng H, Chi Y, Ren W, Zhang W, Zhang H, Liu Y, Yuan F (2024) Reseeding inhibits grassland vegetation degradation – Global evidence. *Agriculture, Ecosystems & Environment* 374, 109144.
- Stagnari F, Maggio A, Galieni A, Pisante M (2017) Multiple benefits of legumes for agriculture sustainability: an overview. *Chemical and Biological Technologies in Agriculture* 4, 2.
- R Core Team (2024) R: A language and environment for statistical computing (Version 4.3.3). R Foundation for Statistical Computing, Vienna, Austria. Available at <https://www.R-project.org/> [Accessed 03 Oct 2024].

- Tognetti PM, Prober SM, Báez S, Chaneton EJ, Firn J, Risch AC, Schuetz M, Simonsen AK, Yahdjian L, Borer ET, Seabloom EW, Arnillas CA, Bakker JD, Brown CS, Cadotte MW, Caldeira MC, Daleo P, Dwyer JM, Fay PA, Gherardi LA, Hagenah N, Hautier Y, Komatsu KJ, McCulley RL, Price JN, Standish RJ, Stevens CJ, Wragg PD, Sankaran M (2021) Negative effects of nitrogen override positive effects of phosphorus on grassland legumes worldwide. *Proceedings of the National Academy of Sciences* 118(28), e2023718118.
- Waddington J (1992) A comparison of drills for direct seeding alfalfa into established grasslands. *Journal of Range Management* 45, 483-487.
- Xu B, Hujiltu M, Baoyin T, Zhong Y, Bao Q, Zhou Y, Liu Z (2020) Rapid loss of leguminous species in the semi-arid grasslands of northern China under climate change and mowing from 1982 to 2011. *Journal of Arid Land* 12(5), 752-765.