



## Historical grazing effects on kin recognition in clonal grass: Implications for soil nutrient management and grassland recovery

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### ABSTRACT

Grazing influences community stability and resilience by mediating plant-plant interactions. Kin recognition plays a crucial role in those interactions, however, the extent to which grazing-induced transgenerational effects modify kinship interaction under nutrient conditions remains poorly understood. We examined the responses of *Leymus secalinus* clonal offspring from different grazing contexts to kin and non-kin under varying soil nutrient conditions through greenhouse experiment and measured the growth of *L. secalinus* in grazed grassland. Our results showed that clonal offspring from non-grazing context exhibited facilitative interactions with neighbors and had increased in total biomass, tiller number and root traits when grown with kin than non-kin, demonstrating the presence of kin recognition in *L. secalinus*. Moreover, parental grazing context significantly affected kin recognition patterns. Specifically, clonal offspring from light grazing context significantly increased total biomass (38.6 %) when grown with non-kin, while those from heavy grazing context significantly increased total biomass (62.5 %) when grown with kin, potentially buffering grazing-induced plant productivity declines. External nutrients addition increased the tiller number of clonal offspring with grazing context when grown with non-kin. Field surveys confirmed *L. secalinus*' dominance, highlighting its adaptive advantage. Overall, our results demonstrate that historical grazing context and nutrient conditions together regulate kin recognition in clonal plants, influencing their competitive and cooperative strategies. These results extend the application of kin recognition theory to clonal plants and reveal the regulatory role of transgenerational effects and soil nutrient factor in plant adaptation to grazing, and offer a novel perspective on optimizing grassland recovery dynamics in grassland ecosystems.

### 1. Introduction

Herbivory grazing influences plant communities through the removing biomass and altering soil fertility, thereby affecting grassland recover (McSherry and Ritchie, 2013; Wang et al., 2016; Shu et al., 2024). Beyond immediate impacts, grazing has been shown to have long-lasting effects on plant growth through transgenerational effects (Li et al., 2022c; Ren et al., 2024), potentially regulating grassland ecosystem recovery processes. In grassland ecosystems, overgrazing is a major environmental stress factor and can reduce plant size with a dwarf individual phenotype through transgenerational effects which can be transmitted to vegetative progeny (Ren et al., 2018; Yin et al., 2023). Plant dwarfism could result in significant declines in the aboveground biomass and productivity in grassland, affecting the grassland ecosystem

resilience through a cascade of individual, population, and community reactions. However, most studies of transgenerational effects have focused on plant individual (Li et al., 2022c; Yin et al., 2023), with limited attention to their influence on plant-plant interaction.

In grassland, plant-plant interactions play a crucial role in shaping community assembly and maintaining community stability, driven by resource partitioning and niche divergence (Zhang et al., 2022a). Production benefits of grassland have been attributed to niche complementarity, which reduces resource competition among species and allows more resources to be allocated to growth. Overgrazing reduces grassland genotypic diversity and leads to population aggregation of genetically related neighbors (Wang et al., 2014; Wang et al., 2018). Such behavior often intensifies interspecies competition, causes resource waste and impacts grassland recovery under grazing pressure.

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Recent studies have revealed that plants exhibit a trade-off between competitive ability and stress tolerance, individuals with lower stress tolerance are less affected by competition but may rely more on facilitation. Therefore, transgenerational effects of grazing may enhance plant stress tolerance while influencing plant-plant interactions, thereby impacting population dynamics and community assembly.

Recent studies have demonstrated that plants can distinguish between kin and non-kin individuals and exhibit differential responses based on genetic relatedness, a phenomenon referred to as kin recognition (Zhang et al., 2016; Li et al., 2022b). The ability to detect relatedness would allow plants to cooperation among kin, such as optimized resource allocation and reduced competitive costs, which can enhance fitness. The role of kin recognition may be quite important for grassland resilience, where grazing often leads to individuals surrounded by genetically related neighbors (Wang et al., 2014; Wang et al., 2018), can reduce intraspecific competition and enhance cooperation, maximizing stand performance and increasing production. However, most studies of kin recognition have focused on contemporary environmental context, with much less attention paid to how historical context on kin recognition. Grazing-induced transgenerational effect can alter plant competitive ability, whether the kin recognition may potentially optimize interactions among plants affected by historical grazing context, thereby affecting grassland stability and productivity, remained poorly understood.

Long-term overgrazing can lead to soil nutrient availability reduction, impacting grassland vegetation recovery. The ability of kin recognition enhance cooperation among kin, maximizing allocate more resources to shoot biomass in nutrient-limited environments (Fan et al., 2022). While contemporary conditions directly shape plant responses, historical context of grazing may impose long-term evolutionary pressures that alter the capacity for kin recognition (Zhang and Tielbörger, 2019; Luo et al., 2022). Due to transgenerational effect, traits conferring grazing stress tolerance are usually associated with biological processes requiring extra cost, and the ability to compete for resources and maintain metabolic performance under stress cannot be maximized simultaneously (Munns and Tester, 2008). Therefore, there should be a trade-off between stress tolerance and competitive ability (Zhang and Tielbörger, 2019). Plants affected by grazed-induced transgenerational effect would likely to exhibit stronger kin cooperation under soil nutrient stress (Li et al., 2018). This enhanced cooperation among kin may involve reallocating resources to optimize growth and reproduction under stress, thereby improving overall fitness and population dynamics (Yang et al., 2018). Despite its ecological and evolutionary significance, how historical grazing effects interact with current soil nutrient availability to influence kin recognition patterns remains poorly understood.

In semi-arid grasslands, approximately 90 % of vegetation turnover relies on clonal reproduction. These plants rely on stolons or rhizomes to expand laterally, enabling them to exploit heterogeneous environments and persist under grazing pressure (Liu et al., 2016). Given its dominance and adaptability, clonal plant provides a unique model to investigate how kin recognition and transgenerational plasticity interact under grazing stress, and how these mechanisms influence grassland community dynamics. For clonal plant, the transgenerational effect can be passed on through vegetative propagation potentially creating persistent adaptation that enhances restoration success. Due to its stolon structure and the limited dispersal capabilities of these buds, clonal species often coexists with both kin and non-kin neighbors (Yang et al., 2018), and heavy grazing can increase the likelihood of interactions among related plants (Zhang et al., 2022b). Thus, the grazing-induced transgenerational effects and kin recognition in clonal plants may represent a potential mechanism influencing grassland recovery and population dynamics in grazing grassland.

To explore the potential mechanisms transgenerational effects and kin recognition under grazing environments, we conducted a greenhouse experiment using *Leymus secalinus* (Georgi) Tzvel., a widespread clonal grass species widely distributed across semi-arid grasslands of

northern China, specifically Inner Mongolia, Gansu, Shanxi and Qinghai (Ye et al., 2006; Sui et al., 2011), to examine how parental grazing experience influences offspring kin recognition under varying soil nutrient conditions. Then, we investigate the adaptive mechanisms of *L. secalinus* in grazed grasslands and verify their impact on population dynamics through field surveys. Specifically, we proposed two hypotheses closely related to grassland restoration practices: (i) Clonal offspring of *L. secalinus* from heavy grazing intensity exhibit enhanced kin cooperation as an adaptive response, which may explain its rapid dominance in degraded grassland recovery. (ii) Environmental resource conditions modulate the effect of grazing history on kin recognition, accounting for shifts in plant interaction patterns across restoration stages.

## 2. Materials and methods

### 2.1. Study site

Our grazing experiment was conducted at Anding district, Dingxi City, Gansu Province, China (latitude 34°26'–35°35'N and longitude 103°52'–105°13'E, 1450 m asl), characterized by a temperate continental monsoon climate. The mean annual precipitation in this region is 386 mm, and the mean annual temperature is 6.8°C.

The experimental site has a well-documented history of management. Sainfoin (*Onobrychis viciifolia* Scop., sowing quantity 112.5 kg ha<sup>-1</sup>) was sown in 2016. The site was subsequently managed under a mowing regime from 2017 to 2019, during which time *L. secalinus* gradually emerged naturally. By 2020, the vegetation had transitioned to a legume–grass mixed sward composed of sainfoin and *L. secalinus* with a yield ratio of 6:4 and a total forage yield of 2.4 t ha<sup>-1</sup>. Consequently, the sown pasture was converted from mowing to grazing in 2020. We did not focus on the effects of mowing on the sown pasture, but rather on the impacts of grazing after the grassland had succeeded to a legume–grass mixed pasture. To investigate the effects of grazing on the sown pasture, a grazing factor was applied in this study. The grazing experimental sites were built in 2020. The experimental sites include four levels of grazing intensity, i.e., non-grazing (0 sheep ha<sup>-1</sup>), light grazing (11 sheep ha<sup>-1</sup>), medium grazing (14.9 sheep ha<sup>-1</sup>) and heavy grazing (24 sheep ha<sup>-1</sup>). Each grazing intensity treatment was replicated across three blocks in a randomized block design. The blocks were separated by fencing to prevent movement of livestock between treatment areas. Grazing occurred annually for a 90-day period from June 1 to October 1, 2020.

### 2.2. Study material

During the grazing period, we observed that grazing significantly decreased shoot biomass, but increased the proportion of *L. secalinus* in grassland under light (65 %) and heavy grazing (78 %) intensity compared to non-grazing (Fig. S1). In addition, *L. secalinus* accounted for over 60 % of grassland, reaching 85 % under light grazing and 93 % under heavy grazing (Fig. S1). Such the proportion of shoot biomass and density of *L. secalinus* indicated that it was more dominant than sainfoin under light and heavy grazing stresses.

On October 16, 2020, clonal offspring of *L. secalinus* were collected from a grazing paddock in a steppe grassland. The clonal offspring of *L. secalinus* with three grazing intensities treatments, including non-grazed population (NG<sub>offspring</sub>), light grazing population (LG<sub>offspring</sub>) and heavy grazing population (HG<sub>offspring</sub>). Within each population, we randomly selected 12 soil blocks (25 × 25 × 35 cm) (at least 10 m apart) (Semchenko et al., 2017), resulting in a total of 36 soil blocks. Additionally, we selected rhizomes with no fewer than 4 buds as experimental materials in each soil blocks. Selected plant materials are taken back to the laboratory in an insulated box (4°C) to be used for a greenhouse experiment.

### 2.3. Kin recognition experiment

To explore the underlying mechanisms of parental grazing context and kin recognition on grassland recover after grazing, a greenhouse experiment was conducted at Lanzhou University (Lanzhou, China), beginning on October 17, 2020. Clonal offsprings of *L. secalinus* with three grazing contexts were used on this study. A total of 200 rhizome fragments (with one node, 2 cm in length) were placed into Petri dishes lined with moist tissue paper at 21–26 °C with a 14 h light /10 h dark cycle to promote root and shoot development. On November 1, 2020, 60 plantlets of similar size from each parental grazing context were transplanted into pots (17 cm in diameter and 15 cm deep) filled with a growth medium, consisting of a mixture of potting peat and sand (1:1 v/v, autoclave at 121°C for 20 min before use) with a pH of 6.0, available nitrogen of 0.83 g/kg, available phosphorus of 0.83 g/kg, and available potassium of 1.03 g/kg.

The experiment included 18 treatments, with six replicates, resulting in a total of 108 pots (three parental grazing contexts × three neighbor identities × two soil nutrient treatments) (Fig. 1). The experimental design was a completely randomized factorial design with balanced replication. Three different neighbor identities treatments were set as follows. Single treatment, plants were grown alone in the pots (A, Fig. 1). Kin treatment, two plants from the same rhizome were paired and planted in pots (AA, Fig. 1). Non-kin treatment, two plants from the different rhizome were paired and planted in pots (AB, Fig. 1). The same letter of plant indicated buds collected the same rhizome (Fig. 1). To explore the addition of external nutrients effects, half of pots received 300 ml of 100 % Hoagland solution as high nutrient treatments, while the other pots received the same volume of distilled water as low nutrient treatments (Fig. 1). Distilled water or Hoagland solution was

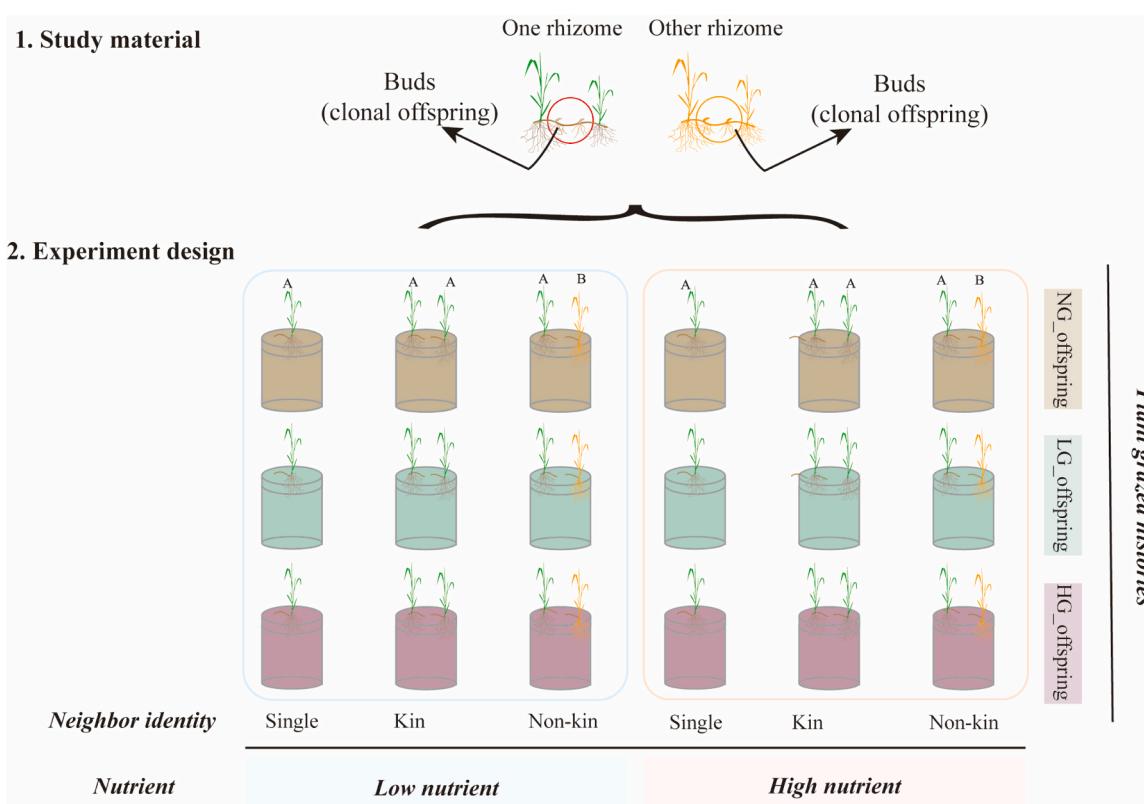
added weekly to each pot to replenish nutrients lost due to plant uptake. The pots were randomly assigned to the layout, which was randomized once a month within each block.

### 2.4. Field experiment

To further explore how effect of grazing history and kin recognition on potential impacts for *L. secalinus* under different grazing stresses and recovery in field. After 2020, we continued the grazing experiment, grazing occurred annually for a 90-day period from June 1 to October 1 for two consecutive years (2021–2022). The experiment spanned three grazing cycles. Following the three-year grazing period, all sheep were removed on October 1, 2022, and the grassland was enclosed to monitor recovery. For the current study, we analyzed vegetation data collected during the grazing period (2020–2022) and during the second year of recovery (2024). The experimental allows us to examine both the immediate effects of grazing and the initial recovery following cessation of grazing on *L. secalinus*.

### 2.5. Measured

The greenhouse experiment concluded on January 5, 2021. At the end of the experiment, the number of tillers for each plant in the experimental pots was recorded. The heights of clonal offspring were measured weekly using a straightedge, and growth rates were calculated based on these weekly height measurements and time intervals. After harvesting, the aboveground plant components were carefully separated into leaves and stems. For the belowground components, roots were meticulously washed to ensure the complete removal of soil particles without causing root damage. The cleaned roots were sealed in plastic



**Fig. 1.** Schematic fragment of the experimental control design. The cloned offspring collected from non-grazing (NG\_offspring), light grazing (LG\_offspring) and heavy grazing plots (HG\_offspring) are used as experimental materials for greenhouse experiments. We set up three neighbour identity treatment pots under two soil nutrient levels, including single pots (clonal offspring growth alone), kin pots (plants from the same rhizome), and non-kin pots (plants from the different rhizome). One or other rhizome mean two different rhizomes. The same colour of plant indicated buds from the same parental plant, whereas different colour of plant indicated buds from different parental plants.

bags for further analysis. In the laboratory, a root scanner (WinRHIZO, Regent Instruments, Canada) was used to measure root traits, including total root length and root area. Leaves, stems, and roots were dried in an oven at 70°C for 48 h until a constant weight was achieved. Above-ground biomass was determined as the combined dry weights of the leaf and stem components.

The plant community structure and biomass were investigated before (at May 28) and after (at July 16) the grazing treatment each year (from 2020 to 2022). It was also measured on July 16, 2024, to explore the recovery of the grassland. We randomly sampled fifteen 1 m × 1 m quadrats in each of the different grazing plots and recorded the species composition as well as the biomass and density by species. Each plot was spaced approximately 5 m apart. We then harvested the aboveground plants, placed them into paper bags according to species, and dried them in a 70°C oven for more than 48 h until a constant weight was achieved. Each dried plant was weighed to obtain the aboveground biomass of each species.

### 2.6. Relative interaction index analyse

To explore effect of different kinship neighbor on focal plant with different grazing intensities. The interaction between focal plant when grown with neighbor and when grown alone was quantified using the relative interaction index (RII) under the different treatments (Abd El-Gawad et al., 2017):

$$RII = \frac{B_{neighbor} - B_{single}}{B_{neighbor} + B_{single}} \quad (1)$$

where  $B_{neighbor}$  and  $B_{single}$  represent the total biomass of uninjured plant or injured plant in the presence and absence of neighbor, respectively. RII is bounded between -1 and 1, with positive values indicating net facilitative interactions, negative values indicating competition, and larger absolute values indicating stronger intensity of the interaction.

### 2.7. Effects of plant grazing history and neighbor identity

The effects of parental grazing context and neighbor identity on performance traits (i.e., biomass, tiller number, root traits) of focal plants were calculated by natural logarithm of response ratio ( $\ln RR$ ), representing the response of performance traits in grazing history or kin treatment compared with that in non-grazing history or non-kin treatment. The  $\ln RR$  for performance traits was calculated as (Yue et al., 2017; Ruan et al., 2024):

$$\ln RR_{GH} = \ln\left(\frac{X_{GZ}}{X_{NG}}\right) \quad (2)$$

$$\ln RR_{NI} = \ln\left(\frac{X_{kin}}{X_{non-kin}}\right) \quad (3)$$

where  $X_{GZ}$ ,  $X_{NG}$  represents when grown alone, the performance traits of focal plant with grazing history (i.e., light grazing history or heavy grazing history), non-grazing history, respectively.  $X_{kin}$ ,  $X_{non-kin}$  represents the performance traits of focal plant in kin and non-kin treatment, respectively.  $\ln RR_{GH} > 0$  represented focal plant with grazing history had larger performance traits than non-grazing history plant.  $\ln RR_{GH} < 0$  represented focal plant with grazing history had smaller performance traits than non-grazing history plant and  $\ln RR_{GH} = 0$  represented parental grazing context had no significantly affect on performance traits of focal plant. In addition,  $\ln RR_{NI} > 0$ ,  $< 0$ , or  $= 0$ , represents neighbor identity had a positive, negative, or no effect on the growth of clonal offspring, respectively.

### 2.8. Statistical analyses

A one-way ANOVA was used to examine the effects of grazing

intensity on biomass and density of species in grazing grassland. Moreover, a three-way ANOVA was used to examine the effects of parental grazing context, neighbor identity, and soil nutrient as well as their interactions, on biomass allocation, number of tillers, height, and root traits of *L. secalinus* clonal offspring. Statistical significance was set at  $p < 0.05$ . Significant differences of relative interaction index (RII), parental grazing context effect ( $\ln RR_{GH}$ ), and neighbor identity effect ( $\ln RR_{NI}$ ) were assessed by  $t$ -test. A result greater than 0 indicates a positive effect, while less than 0 indicates a negative effect, and equal to 0 signifies no effect. In addition, the correlation among kin responses ( $\ln RR_{NI}$ ) of plant traits was be analyzed, such as total biomass (TBM), aboveground biomass (ABM), belowground biomass (BBM), tiller number (TN), root length (RL), root area (RA), and specific root length (SRL). All analyses were performed with SPSS 25.0 for Windows (SPSS, Chicago, IL, USA), and figures were prepared using Origin 2021 (OriginLab Corp. USA).

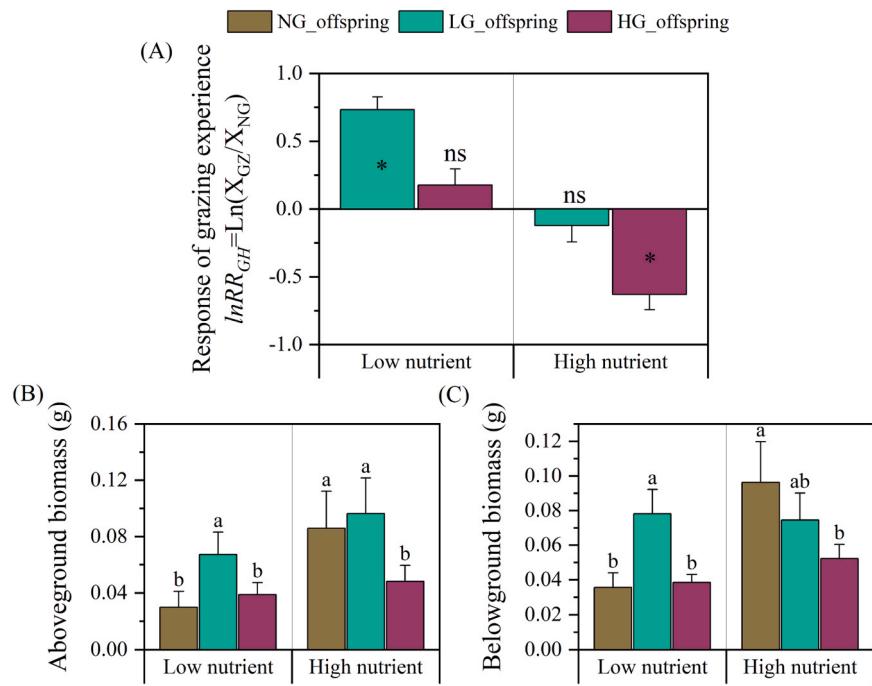
## 3. Results

### 3.1. Effect of parental grazing context and neighbor identity on performance of *L. secalinus* offspring

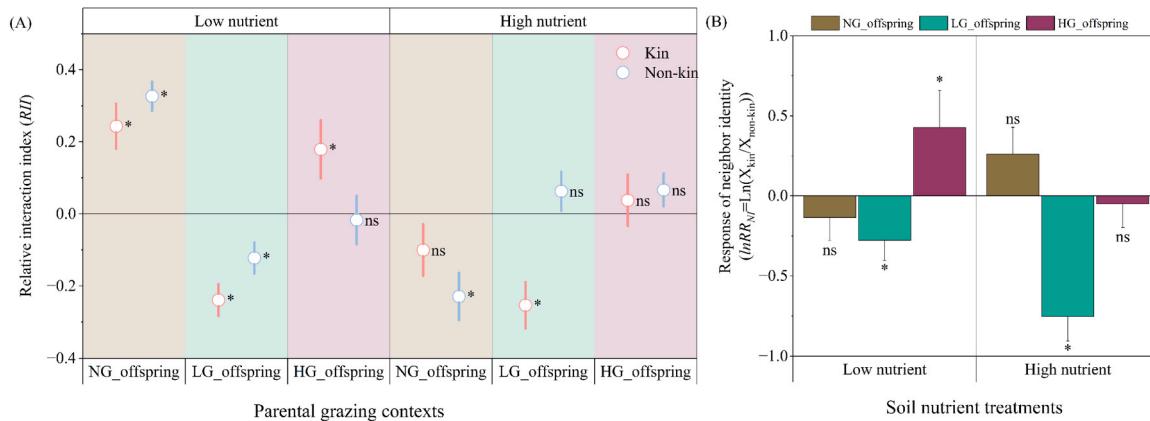
The parental grazing context significantly affected performance traits of *L. secalinus* offspring when grown alone (Fig. 2, Table S1). The offspring from the light grazing population (LG\_offspring) exhibited a significantly positive parental grazing experience response ( $\ln RR_{GH} > 0$ ) for total biomass and increased in above- (ABM, 123 %) and below-ground (BBM, 117 %) biomass, tiller number (TN, 96 %), and root traits ( $p < 0.05$ ) under low nutrient conditions ( $p < 0.05$ , Figs. 2B, 2C; Fig. S1), while those from heavy grazing population (HG\_offspring) showed neutral  $\ln RR_{GH}$  ( $p > 0.05$ ) and exhibited a trend of  $\ln RR_{GH} > 0$  (Fig. 2).

Interestingly, parental grazing context significantly affected kinship interaction when grown with different relative. The offspring from the non-grazing population (NG\_offspring) had a positive relative interaction index ( $RII=0.29$ , facilitate), suggesting that in non-grazing grasslands, kin recognition may facilitate population expansion and dominance. Presence of non-kin increased root traits and tiller number (Fig. 3; Fig. S2). However, LG offspring showed an opposite pattern ( $RII=-0.18$ , competition) when grown with neighbor ( $p < 0.05$ , Fig. 3), and presence of kin increased their root traits and tiller number. This competitive pattern may promote a more diverse community structure during the process of grassland restoration. In contrast, HG offspring displayed a more facilitate with kin ( $RII = 0.18$ ) compared to non-kin ( $RII = -0.02$ ), and presence of non-kin increased in biomass, tiller number, and root traits (Fig. 3; Fig. S2). Heavy grazing promotes cooperation between kin, helping to quickly establish a dominant monospecific population in grazing environments. Furthermore, LG\_offspring demonstrated lower growth rates (GR) when grown with kin compared to non-kin. The effect of kinship on the height of LG offspring gradually increased with growth time and nutrient addition enhanced this effect, while kinship had no effect on height of HG\_offspring (Fig. 4). The results indicated that parental grazing context and kin recognition mediated biomass and tiller number of *L. secalinus* offspring.

Moreover, soil nutrient availability can regulate the effect of parental grazing context on neighbor interaction (Fig. 3; Fig. S2). High nutrient shifted neighbor interaction among NG\_offspring from facilitate to competition (from  $RII = 0.29$  to  $RII = -0.165$ ;  $p < 0.05$ ), and presence of kin increased biomass, tiller number, and root traits (Fig. 3; Fig. S2). However, presence of non-kin biomass, tiller number, and root traits of LG\_offspring under high soil nutrient condition, whereas the neighbor identity did not differ significantly performance of HG\_offspring (Fig. 3). This result indicated that during the grassland restoration process, the nutrient supplementation strategy should be adjusted according to the specific grazing history.



**Fig. 2.** Effects of parental grazing context ( $\ln RR_{GH}$ ) on the biomass of *L. secalinus* offspring under different nutrient treatments when grown alone. NG\_offspring, LG\_offspring, and HG\_offspring represent the clonal offspring of *L. secalinus* collected from non-grazing, light grazing, and heavy grazing populations, respectively. The significance of each response was determined using a *t*-test (\*,  $p < 0.05$ ; ns,  $p > 0.05$ ). Letters in the figure indicate differences among grazing intensity treatments.



**Fig. 3.** Relative interaction index (A,  $RII$ ) and neighbor identity (B,  $\ln RR_{NI}$ ) of total biomass response of clonal offspring with different parental grazing contexts when grown with kin and non-kin neighbor. NG\_offspring, LG\_offspring, and HG\_offspring, clonal offspring of *L. secalinus* collected from non-grazing, light grazing, and heavy grazing population, respectively. The significance of each response was determined using a *t*-test (\*,  $p < 0.05$ ; ns,  $p > 0.05$ ).

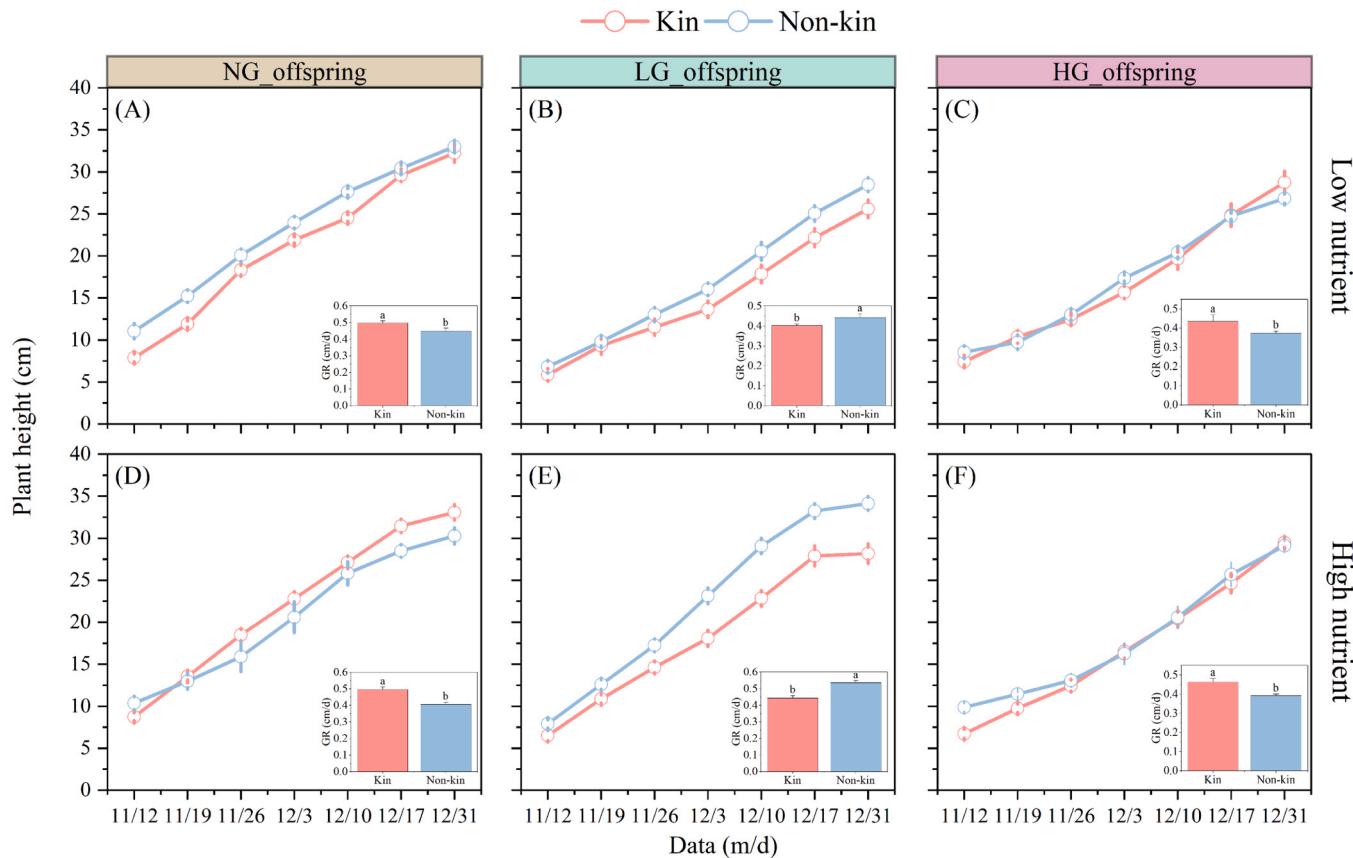
### 3.2. The impact of grazing intensity on biomass and density of the community and the proportion of *L. secalinus*

After two years of grazing (2021–2022) and two years (2023–2024) of grassland recovery, *L. secalinus* exhibits a greater degree of dominance within grassland. The biomass and density of *L. secalinus* accounted for over 50 % and 80 % of the shoot biomass, particularly under light and heavy grazing treatments. Compared to in 2021, the proportion of biomass and density of *L. secalinus* increased in stage of prior to light grazing (biomass, from 35 % to 72 %; density, from 95 % to 97 %) and during the grazing period (biomass, from 46 % to 73 %) under heavy treatment in 2022 (Fig. 5). The establishment of this dominance aligns with the adaptive strategies observed in the offspring with grazing context from the greenhouse experiment. It confirms the ecological significance of the kin recognition patterns and nutrient responses we

observed in actual grazing grassland. During the grassland recovery period following the cessation of grazing (2024), the species composition changed: sainfoin was no longer present, while Poa appeared in all treatments and *Stipa capillata* emerged specifically in the medium grazing treatment plots. *L. secalinus* continued to account for over 50 % of the population, particularly under light and heavy grazing treatments in the stage of recover (Fig. 5). In heavily grazed plots, *L. secalinus* established dominance more rapidly and maintained it throughout the recovery phase.

## 4. Discussion

The importance of parental grazing context and kin recognition, as well as the mechanisms in shaping plant adaptation strategies, has been a major focus of the science of plant adaption strategies and interactions



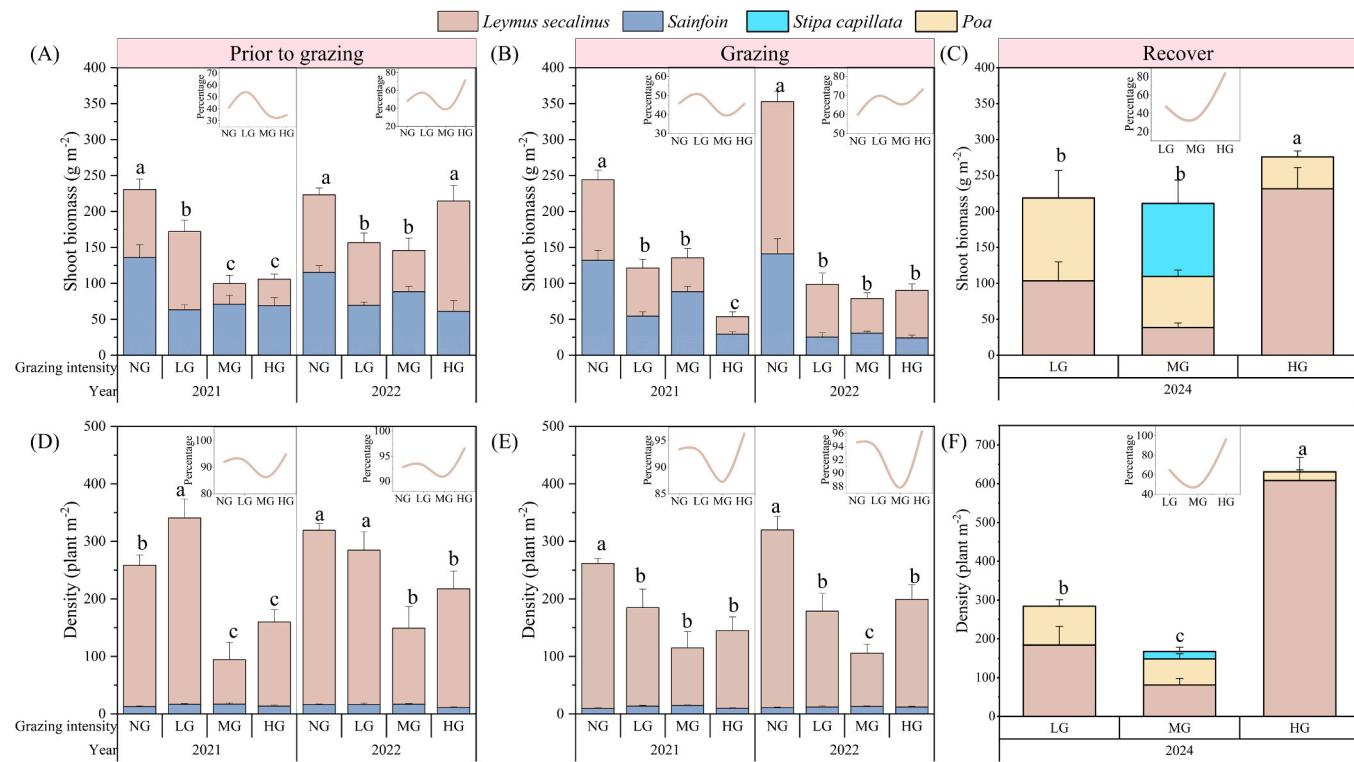
**Fig. 4.** Effects of parental grazing context and neighbor identity on plant height and growth rate (GR) of *L. secalinus* in each nutrient addition treatment. GR, growth rate, were calculated as (the height at December 31st - the height at November 12th) / the grown data. Different letters indicate significant difference ( $p < 0.05$ ).

in recent years (Yang et al., 2018; Li et al., 2022c). Transgenerational plasticity induced by grazing can enhance tiller production in clonal offspring, improving their adaptation to grazing pressure through epigenetic modifications that persist across generations (Li et al., 2022c). In overgrazing grassland, the increased density of clonal plants often results in spatial clustering of genetically related individuals, where kin recognition likely to become a potential crucial pathway influencing population structure and restoration dynamics. This recognition mechanism enables plants to optimize resource allocation and reduce competitive costs among closely related individuals, redirecting resources toward reproduction and survival, thereby enhancing overall fitness and population resilience during restoration processes (Biedrzycki and Bais, 2022). This study showed that clonal offspring with different grazing contexts had different kin recognition response, responding with altered biomass, root traits and tiller number, and the effect was mediated by soil nutrient availability. In addition, our field experiment found that *L. secalinus* gradually dominated the community under grazing stress, accounting for over 50 % of biomass and 80 % of density, particularly under light and heavy grazing treatments. Parental grazing context affected adaptive strategies of clonal offspring of *L. secalinus*. This dominance highlights adaptive mechanisms of the species not only reflects the species' adaptive mechanisms to grazing pressure but also offers important insights for restoring degraded grasslands. The complex interplay between parental grazing context and kin recognition might be adaptive mechanisms in *L. secalinus* contribute to community stability and restoration in grazed grasslands (Fig. 6).

#### 4.1. Transgenerational adaptation to grazing in clonal offspring

Parental grazing context shaped performance traits of clonal offspring through transgenerational effects, and enables offspring to

preadapt to environmental stress (Li et al., 2022c), creating a form of ecological memory to enhance system resilience. Grazing induced transgenerational effect tends to increase tiller number of clonal offspring to buffer grazing pressure. However, different adaptive strategies in clonal offspring depend on grazing intensity. Clonal offspring with light grazing context exhibited greater biomass, tiller number, and root traits compared to clonal offspring with heavy grazing context. Light grazing likely induces stress priming, enhancing resource allocation to growth and reproduction (Kleijn and Steinger, 2002), potentially accelerating the establishment phase of restoration. This positive response aligns with previous studies showing enhanced fitness in plants with moderate stress history (Zhou et al., 2022). The compensatory growth response observed may potentially enhance their ability to establish quickly and outcompete neighbors in relatively undisturbed environments, contributing to rapid vegetation cover development in heterogeneous grassland restoration sites. In contrast, clonal offspring with heavy grazing context exhibited the conservative strategy through reducing aboveground biomass while maintaining reproductive potential, reflects a resource allocation shift toward stress-tolerant traits, enabling survival under chronic grazing pressure (Zheng et al., 2015). These contrasting strategies between clonal offspring with different grazing contexts represent a fundamental trade-off between competitive ability and stress tolerance, consistent with Grime's competitive, stress-tolerant and ruderal theory (Graff and Aguiar, 2017; Zhang and Tielbörger, 2019). Light grazing appears to select for traits that enhance competitive performance in relatively favorable conditions, while heavy grazing selects for conservative resource allocation that prioritizes survival over growth under persistent disturbance (Puy et al., 2021). This adaptive strategy can be transmitted between generations through transgenerational effects, thereby promoting population development and enhancing grassland recovery after grazing.



**Fig. 5.** Effects of grazing intensity on the shoot biomass and density of species and percentage of *L. secalinus* (the broken line graph in the figure) in stage of prior to grazing (a, d, May 28, 2021–2022), grazing (b, e, July 15, 2021–2022), and recover (c, f, July 15, 2024). Prior to grazing, a vegetation survey of the grassland was conducted prior to the grazing. Grazing, vegetation surveys were conducted on the plants during the grazing period. Recover, a vegetation survey was conducted on the grazed grassland two years after grazing cessation in 2022. NG, non-grazing, LG, light grazing, MG, modest grazing, HG, heavy grazing. Letters in the figure indicate differences among grazing intensity treatments.

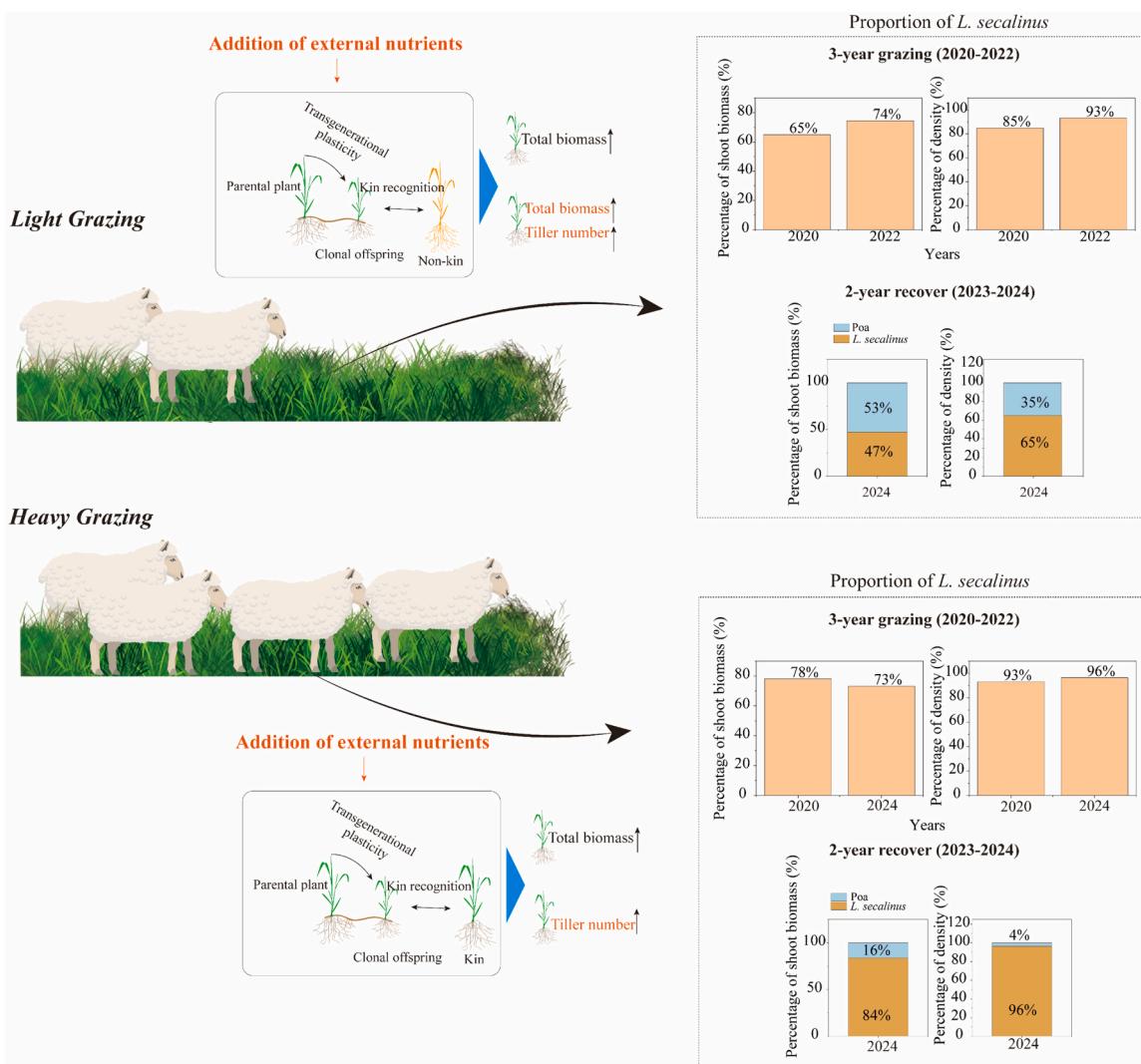
#### 4.2. Parental grazing context effects on kin recognition

We further found that historical grazing pressure significantly modifies kin recognition patterns in *L. secalinus*. While basic kin recognition mechanisms exist in non-grazed plants, our research reveals that grazing history fundamentally reshapes these patterns, creating distinct social interaction strategies that optimize survival under different disturbance regimes. The historical context of grazing fundamentally alters offspring responses to kin, creating distinct interaction patterns that optimize survival and growth (Luo et al., 2022). Our study supports previous findings, further demonstrating that parental grazing context modulated intraspecific interactions through kin recognition (Zhang et al., 2022b). In grazing ecosystems, plants must simultaneously manage both grazing-induced stress and competitive interactions with neighbors. Our findings suggest that the intensity of historical grazing pressure reconfigures these plant social behaviors in ways that reflect adaptive strategies. The intensity of parental grazing context influenced the competitive abilities of clonal offspring (Luo et al., 2022), thereby impacting their responses to kinship interactions (Zhang et al., 2022b). Clonal offspring with light grazing context exhibited greater biomass, tiller number, and root system when grown alone compared to non-grazing context under low nutrient condition. This pattern suggests that even moderate grazing can induce compensatory growth in plants and increased the growth and resource acquisition capacity of clonal offspring through transgenerational effects in resource-limited environments (Zhao et al., 2008; Yin et al., 2019). The enhanced root traits likely facilitate more effective exploration and uptake of soil nutrients and water (Li et al., 2018). However, this increase in competitive traits may also improve intraspecific competition among kin (Dudley et al., 2013; Zhang et al., 2022b), potentially reducing overall population growth rates in the initial phases of recovery. Resource allocation to competitive traits may lead to reduced biomass allocation (Rehling

et al., 2021), the trade-off (negative relative interaction index) could temporarily slow the development of dominant clonal populations, but may also create establishment opportunities for other species, thereby promoting community diversity during grassland restoration.

In contrast, clonal offspring with heavy grazing adopted a more conservative strategy focused on resource conservation through decreasing the above-ground expansion of clonal offspring to avoid competition for light resources when growing with kin (Martinez-Garcia and Rodriguez-Concepcion, 2023). This strategy could promote more stable, albeit slower, community development by minimizing competitive interactions among planted individuals. In addition, they enhanced nutrient uptake capacity by increasing individual biomass and altering root characteristics to support their growth in kin interactions. This stability suggests that offspring with heavy grazing context selects for traits that prioritize resilience over competitiveness through kin recognition, a strategy likely favored in environments with chronic disturbance (Li and Xu, 2018). This finding aligns with Zhang and Tielbörger (2019), who argued that intense grazing pressure promotes conservative growth strategies to ensure long-term survival. Stable biomass production across neighbor identities suggests that clonal plant promote kin responses via transgenerational effects, maintaining growth and aiding grassland recovery after heavy grazing.

Notably, Zhang et al. (2022b) found that kin interactions play a crucial role in determining the population dynamics during the early stage of *L. chinensis* growth through measuring plant size. Similarly, we also found the ramet-level responses to neighbor identity changed over time in both non-grazed and grazed groups. For a period of 2 months, plant size was measured seven times (using vertical height as a proxy), and the results revealed that the differences between the heights of clonal offspring with non-grazing grown with kin and non-kin gradually narrowed, this in line with the previous study (Fig. 5) (Zhang et al., 2022b). However, the differences between the heights of clonal



**Fig. 6.** Schematic diagram illustrates the potentially effect of grazing induced transgenerational plasticity and kin recognition in grassland recovery.

offspring with light grazing grown with kin and non-kin gradually increased while the plant height of clonal offspring with heavy grazing showed no significant difference throughout the entire period. This temporal divergence suggests that light grazing history may sensitize plants to prolonged kin competition, possibly through enhanced competitive trait expression. These findings align with the resource competition hypothesis (Li et al., 2022a), whereby initial growth advantages compound over time through positive feedback mechanisms, potentially accelerating dominance patterns in restored communities. In contrast, heavy grazing may select for stable growth strategies that prioritize predictable resource allocation over competitive responses that could promote more gradual and potentially more stable community development. Such temporal stability could represent an adaptive strategy in intensively grazed environments where consistent recovery capacity may be more important than competitive ability. These results suggest that parental grazing context regulated the stage of plant growth where kin recognition plays a role, offering a novel perspective on how their interplay affects grassland recovery over time.

#### 4.3. Nutrient availability modulates kin recognition effects

Our results demonstrate that soil nutrient availability acts as a critical environmental filter that modulates the expression of kin recognition patterns and their interaction with grazing history. High nutrient

prompted clonal offspring with light grazing context to allocate less resources to root system and tiller number compared to low nutrient condition. This reduction in resource allocation decreased competition for resources among kin but resulted in a decline in total biomass for clonal offspring with light grazing (Mazal et al., 2023), suggesting that nutrient addition might reduce initial productivity while potentially enhancing diversity. The reduced resource investment under high-nutrient conditions suggests a shift from competitive to avoidance strategies, which may promote species coexistence and maintain community diversity in nutrient-enriched grasslands (Wei et al., 2023). In contrast, clonal offspring with heavy grazing context exhibited neutralizing kin recognition effect. They maintained relatively stable biomass production regardless of nutrient availability, indicating a more conservative growth strategy that may reflect adaptation to chronic stress (Wooliver et al., 2016). Such neutralizing kinship interactions may promote genotypic diversity within populations, thereby improving intraspecific interactions and enhancing overall population productivity in heavily grazed grasslands. Therefore, our results suggested that the addition of external nutrients can influence how grazing history and kin recognition affect plants and promote grassland genotypic diversity, benefiting the post-grazing recovery growth and maintenance of population development.

#### 4.4. Grazing effects on community dominance of *L. secalinus*

The transgenerational effect enhances with increasing historical background duration. Our findings that *L. secalinus* gradually dominated the community under three-year grazing stress, accounting for over 50 % of biomass and 80 % of density represent a remarkably high level of dominance. Under light grazing, grasslands maintain higher genetic diversity, whereas heavy grazing reduces this diversity. Clonal offspring affected by grazing context increased tiller number and biomass through kin recognition that could enhance population establishment success. In addition, the observed community shifts, including the disappearance of sainfoin and emergence of Poa and *Stipa capillata* during the recovery phase, reflect typical patterns of grassland succession following disturbance (Bates and Davies, 2014). Notably, the persistence of *L. secalinus* as the dominant species even after two years of recovery suggests the presence of ecological memory, likely maintained through grazing legacy effects (Li et al., 2022c). These results highlight that clonal plants with appropriate grazing intensity play a significant role in maintaining grassland productivity and stability during restoration by occupying dominant positions in developing communities, potentially accelerating grassland recovery. This persistence suggests that clonal species based on grazing history could have lasting impacts on restoration outcomes well beyond the initial establishment phase.

Our findings have important implications for understanding grassland ecosystem restoration and management. The complex interactions between historical grazing, kin recognition, and soil nutrients suggest that grassland restoration strategies should consider both historical disturbance regimes and current environmental conditions. The distinct adaptive strategies we observed in offspring from different grazing histories indicate that plant populations may maintain a "memory" of past disturbance that influences their current interactions and performance (Latzel et al., 2023). This memory creates predictable patterns of plant behavior that enhance establishment success, accelerate recovery and increase resilience of restored systems to future disturbances. While our study provides restoration with novel insights into how grazing history shapes plant social behaviors that influence restoration outcomes, several limitations of our study that should be addressed in future research. First, our experimental grazing treatment was limited to a year, which may not fully capture the long-term transgenerational effects that develop over multiple grazing cycles. While short-term stress also can induce significant transgenerational responses in plants (Lampei, 2019; Puy et al., 2021), longer-term studies would strengthen our understanding of these mechanisms and their persistence across generations. Second, our experimental design assumed that plants from the same rhizome represent kin while those from different rhizomes (>10 m apart) represent non-kin, following established protocols in clonal plant studies (Li et al., 2018; Li et al., 2021; Zhang et al., 2022b). However, without genetic markers, we cannot definitively confirm the degree of genetic relatedness between plants. While we observed significant differential responses to these treatments, suggesting the presence of kin recognition mechanisms, future studies should incorporate molecular genetic analyses to provide more definitive confirmation of genotype identity and strengthen conclusions about kin recognition mechanisms. Furthermore, examining how these transgenerational effects and kin recognition mechanisms operate across multiple clonal species common in grassland recovery could reveal broader patterns in how grassland communities respond to and recover from grazing pressure, potentially allowing for more precise prediction of recovery and development of targeted management interventions.

#### 5. Conclusions

Our study provides novel insights for grassland recovery by revealing how historical grazing context shapes plant kin recognition and species interactions through transgenerational effects, mediated by soil nutrient conditions. We demonstrated that *L. secalinus* progressively dominates

grassland communities under grazing pressure, particularly with light and heavy grazing intensities, indicating its adaptive resilience to herbivory and potential value as a restoration species in grazing grassland. The transgenerational effects of grazing were evident in offspring performance, where light-grazed parental experience enhanced growth under nutrient-poor conditions, a likely adaptation to resource-limited environments typical of grazed systems. Additionally, we found that grazing history fundamentally alters plant-plant interactions through modified kin recognition patterns, with heavy-grazed offspring exhibiting cooperative strategies toward kin while light-grazed offspring displayed competitive responses. Nutrient availability further modulated these interaction patterns, highlighting the context-dependency of plant adaptive strategies. These findings significantly advance restoration ecology by revealing how clonal plants contribute to community stability and development in recovering grazed ecosystems through transgenerational plasticity and kinship-dependent interactions mechanisms that could be strategically manipulated to enhance restoration outcomes. By integrating considerations of historical grazing context, genetic relatedness of clonal plants, and their interactions with soil conditions, these natural adaptation mechanisms likely to accelerate recovery, enhance ecosystem function, and increase resilience to future disturbances. The differential responses to grazing intensity and neighbor identity highlight the sophisticated adaptive mechanisms employed by dominant grassland species to maintain fitness under variable disturbance regimes, providing valuable insights for sustainable grassland management practices that consider both historical grazing context and plant social dynamics.

#### CRediT authorship contribution statement

**Liping Mao:** Visualization, Methodology, Data curation. **Yiting He:** Validation, Data curation. **Zhen Zhang:** Writing – original draft, Formal analysis, Data curation, Conceptualization. **Zhixin Zhang:** Writing – review & editing, Supervision, Conceptualization. **Yuying Shen:** Writing – review & editing, Resources, Methodology, Funding acquisition.

#### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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#### Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.agee.2025.109860.

#### Data availability

Data will be made available on request.

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