

1 **Long-term grazing exclusion effects populations genetics and functional traits of**
2 ***Artemisia frigida* in Mongolia**

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14 **Abstract**

15 Mongolian steppes cover the largest still intact parts of the Palearctic steppe biome. They have
16 experienced a long history of nomadic pastoralism, and grazing has a significant impact on steppe
17 vegetation, soils and ecosystem functioning. These effects vary depending on the interplay of local
18 climatic conditions, yet relationships between grazing and plant population genetics have been
19 scarcely studied, and thus remain poorly understood. We investigated influence of grazing on
20 population genetics and functional traits of the most prominent steppe species, *Artemisia frigida*.
21 We sampled a total of 187 individuals from eight locations comparing fenced and unfenced sites
22 along the Trans-Mongolian Railway and Hustai National Park, and analyzed them using ten
23 microsatellite loci.

24 Grazing had a significant positive impact on the genetic diversity of *A. frigida*, but no effect
25 on population genetic differentiation. In contrast, climatic and soil variables strongly influenced
26 population genetic structure, and higher amounts of soil nutrients increased *A. frigida*'s genetic
27 diversity. Grazing significantly influenced plant functional traits, i.e. plant height, and specific leaf
28 area, yet no relationship was found between genetic diversity and plant functional traits. Overall,
29 given that fenced *A. frigida* populations were genetically less diverse, a moderate level of grazing
30 is beneficial for this species and hence may stabilize ecosystem functioning of steppes.

32 Keywords: Long-term grazing, Mongolia, *Artemisia frigida*, population genetics, functional traits

33

1. Introduction

Steppe ecosystems of Eurasia have been heavily influenced by humans, primarily by agriculture, livestock herding, and human settlement. Steppes have undergone significant transformations, and large areas of steppes in Central and Eastern Europe have been turned into cultivated landscapes. In China, 90 % of the grasslands are considered degraded due to overgrazing and plowing, and steppe vegetation in Russia is also severely fragmented today (Smelansky and Tishkov, 2012; Hurka et al., 2019). In contrast, Mongolia's steppe ecosystems are still remarkably intact due to the country's sparse human population and a continued tradition of nomadic pastoralism. However, as of 2016, 58 % of the Mongolian rangeland area is estimated to be degraded due to strong increases in livestock numbers over the last three decades (Densambuu et al., 2018).

Reviews on the impact of livestock grazing on dryland vegetation have found significant reductions in vegetation cover and species richness, especially in habitats with harsh climate and low productivity (Jones, 2000; Wang and Wesche, 2016; Herrero-Jáuregui and Oesterheld, 2018; Munkhzul et al., 2021). Long-term grazing could also cause severe damages on soil structure, and decreases of soil nutrient as well as water contents, which result in increased vulnerability to soil erosion and grassland degradation (Zhang et al., 2017; Jamsranjav et al., 2018; Hao and He, 2019). Moreover, it is well documented that grazing has a significant effect on plant functional traits related to relative growth rate and photosynthetic capacity, e.g., above-ground biomass, plant height, leaf size, leaf dry mass and specific leaf area (SLA; Diaz et al., 2007). This reflects the fundamental trade-off between growth and defense against herbivores (Díaz et al., 2001; Klimesova et al., 2008; Borchardt et al., 2013; Hui and Guoqi, 2014; Lang et al., 2020). Plant functional traits are usually highly plastic, allowing a genotype to adjust to a range of abiotic and biotic stressors (Reich et al., 2003). Under extreme climates, grazing effects on plant functional traits may be overruled by climatic controls (Wang and Wesche, 2016). However, it still is poorly understood if intraspecific variation in functional traits is under either genetic or environmentally controls (Scheepens et al., 2010; Karbstein et al., 2020); and different results were obtained depending on the traits and species studied (Caruso et al., 2020).

Grazing can also affect population genetic diversity and structure. Effects on plant genetic diversity have, however, not been extensively studied, particularly so in drylands (Greenville et al., 2017). Plant genetic diversity can respond negatively or positively to grazing, and can be subject to both direct and indirect grazing effects. Grazing can directly reduce the effective population size and restrict gene flow when whole plants and/or their reproductive parts are consumed (Leimu et al., 2006; Souto and Tadey, 2019). It can have an indirect negative effect on the frequency of certain genotypes due to the selection of grazing-tolerant phenotypes (Brougham and Harris, 1967; McKinney and Fowler, 1991). Herbivory can also result in higher mutation rates due to direct damage, and thus grazing could have a positive impact on genetic variation (Marcotrigiano, 2000). Grazers disperse seeds via endozoochory or epizoochory, facilitating gene flow across long distances (Bläß et al., 2010; García-Fernández et al., 2019). Finally, grazing disturbance can provide safe sites for germination due to gaps in vegetation (Oesterheld and Sala, 1990; Frank, 2005).

74 Previous studies revealed that the of grazing on population genetics varies with its intensity
75 (Peng et al., 2015; Pelliza et al., 2020) and this can be strongly modified by environmental
76 conditions, i.e. climatic and soil factors (Lohmann et al., 2012; Oyundelger et al., 2020, 2021b).
77 Increased grazing has been demonstrated to reduce genetic diversity in common grassland species,
78 like *Stipa* spp. and *Kobresia pygmaea* (C.B. CLARKE) C.B. CLARKE, while moderate grazing has
79 been proposed as a superior strategy (compared to fencing) for grassland management in China
80 (Shan et al., 2006; Liu et al., 2009; Peng et al., 2015). Several studies have assessed the effect of
81 climatic factors on population genetics. In particular, precipitation is an important factor shaping
82 population genetic structures (Zhang et al., 2018; Jiang et al., 2019; Oyundelger et al., 2021b), and
83 grazing effects were less pronounced in drier regions (Jamnadass et al., 2006). Only few studies
84 reported a relationship between soil nutrient availability and genetic variation in plant populations.
85 Abraham et al., (2018) found that soil moisture and soil nitrogen content were positively correlated
86 with genetic diversity of *Thymus sibthorpii* BENTH., while a higher phosphorous content increased
87 population genetic variation of *Carex nigra* (L.) REICHARD in alpine fens, and of *Artemisia frigida*
88 in drylands (Reisch et al., 2020; Oyundelger et al., 2021b).

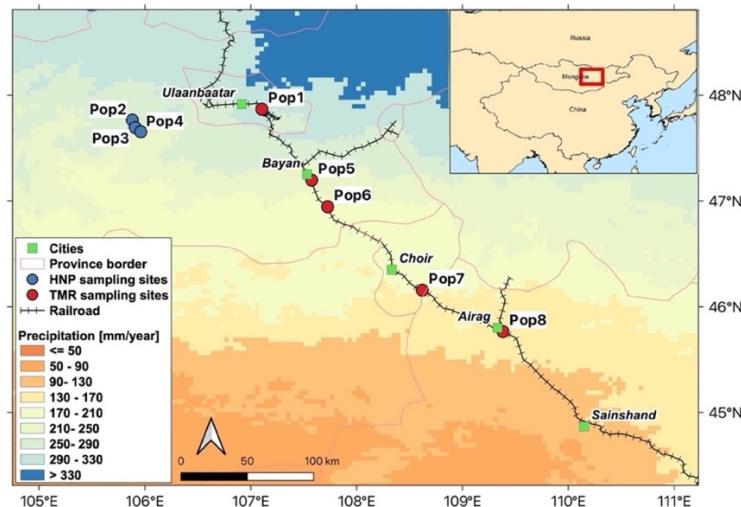
89 Changes in genetical structures associated with grazing have been investigated primarily with
90 neutral molecular markers (Smith et al., 2009; Faville et al., 2020). These allow for a general
91 description of grazing-related genetic consequences, although they are not directly related to
92 fitness-related traits such as grazing resistance (Matлага and Karoly, 2004). Hence, to investigate
93 long-term grazing effect (hereinafter referred to as grazing) on genetic diversity and structure, we
94 employed neutral markers, namely microsatellites (SSRs), in *Artemisia frigida* as one of the most
95 widely distributed rangeland species in Mongolian grasslands.

96 Assessing the impact of grazing in pastoral grazing systems is challenging in the absence of
97 control sites, given that all natural rangelands have typically been utilized for many generations.
98 Yet, in Mongolia, such reference sites could be found along the Trans-Mongolian Railway (TMR),
99 where fences were built and maintained since 1955 resulting in over 60 years of grazing exclusion
100 (Gansukh et al., 2018). The fenced railway line effectively works as a large-scale transect across
101 different vegetation zones of Mongolia. Comprehensive vegetation studies along the TMR have
102 been conducted by Bazha et al., (2012), who found that grazed sites were dominated by (dwarf)
103 shrubs, wih notably *A. frigida* becoming dominant in mountain, meadow, typical and dry-steppes
104 under overgrazing. *Artemisia frigida* can tolerate harsh climatic conditions and mechanical
105 disturbance (Bai et al., 1995; Li et al., 2002), and thus, is one of the first perennials to be established
106 at moderately disturbed sites (Sarvis, 1941). Against the backdrop of an apparent positive impact
107 of grazing on *A. frigida*, we studied its genetic and functional diversity under grazing exclusion.
108 Specifically, we aimed to answer the following questions: i). How and to what extent does grazing
109 affect the genetic diversity and structure of *A. frigida*? ii). In contrast to grazing, how do climatic,
110 soil, and vegetation conditions influence genetic diversity and differentiation of *A. frigida*
111 populations? iii). How do functional traits respond to grazing and are those traits related to genetic
112 variation?

113 2. Materials and methods

114 2.1. Study area

115 The study was conducted in the central and southern parts of Mongolia, between Ulaanbaatar
116 (47°52'N, 107°06'E) and Sainshand (45°26' N, 109°39' E; Fig. 1). From north to south, different
117 types of steppe vegetation occurred: mountain steppe, tall grass steppe, typical steppe, and desert
118 steppes (Pfeiffer et al., 2019). Mongolia is characterized by a continental dry climate, and our
119 study sites along the NW-SE stretch over a mean annual precipitation (MAP) range between 160
120 and 300 mm, while mean annual temperature (MAT) lies between -0.8 and 3.5°C, and altitude is
121 on average 1300 m a.s.l (Table 1). Along this aridity gradient, productivity and biomass decrease,
122 while the share of xerophytic species increases (e.g., shrubs and dwarf semi-shrubs increase from
123 ca. 5 % in the meadow steppe to 80 % in the desert steppe; Bazha et al., 2012)). Regular burning
124 of the vegetation has taken place inside the fences, while grazing by large mammals has been
125 almost completely restricted since building of the fences (Fig. 2a, b). In order to increase the
126 sample size of our study, three large mammal exclosures established in 2003 (100 m² each; Fig.
127 2c, d) in Hustai National Park (HNP) have been added as control sites. These sites undergo regular
128 observations and livestock grazing is allowed only in extremely harsh winters (Tserendulam et al.,
129 2018).



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131 Figure 1. Location of sampled populations of *A. frigida* in Hustai National Park (HNP) and along the Trans-
132 Mongolian Railway (TMR). Precipitation data were derived from Hijmans et al. (2005).

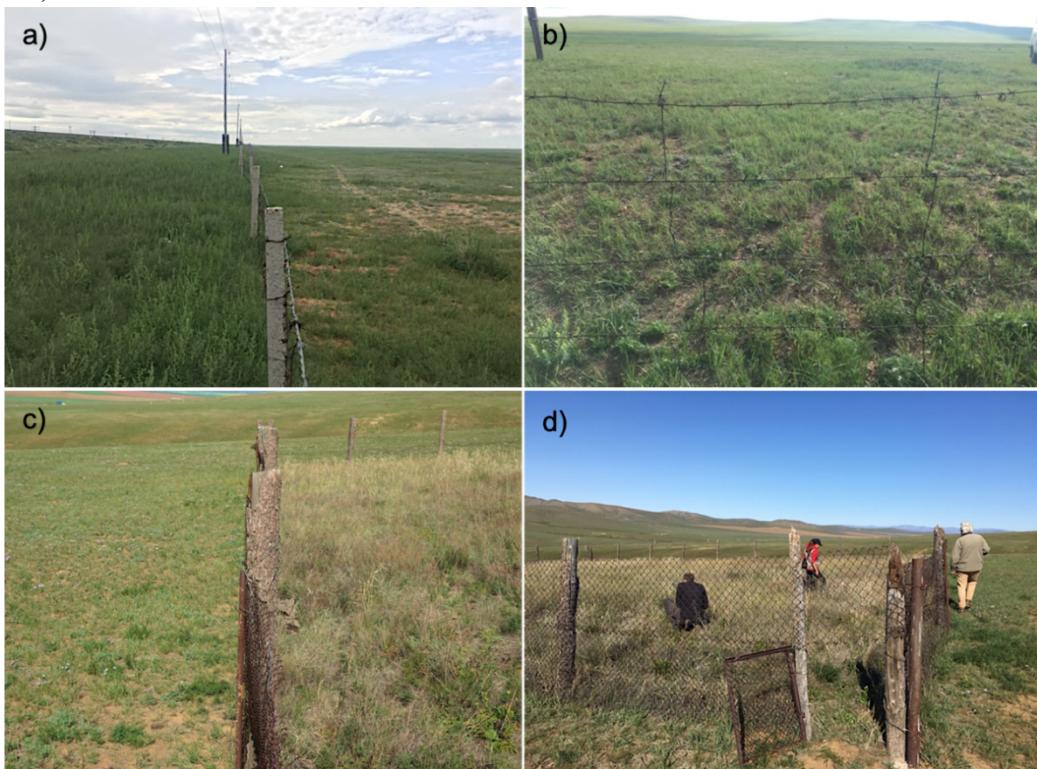
133 2.2. Studied species

134 We selected *Artemisia frigida* WILLD. (Asteraceae) because it is one of the most grazing tolerant
135 key dryland species and it is highly consumed by livestock throughout the year, particularly
136 preferred by sheep, goat and camel (Damiran, 2005). The species occurs not only widely in
137 Mongolia, but is distributed across Central and Middle Asia and North America (Oyundelger et
138 al., 2021a). *Artemisia frigida* is a perennial subshrub of 0.2 – 0.6 m height bearing deeply
139 pinnatisect leaves covered with dense silky pubescence. Flowers are arranged in panicles of

140 yellowish capitula, each of which produces around a thousand seeds that contribute to maintaining
141 a persistent seed bank in the soil (Harvey, 1981). Diploid ($2n = 2x = 18$) and tetraploid cytotypes
142 ($2n = 4x = 36$) are reported (Garcia et al., 2004; Pellicer et al., 2010; Wan et al., 2011; Korobkov
143 et al., 2014).

144 2.3. Collection and measurement

145 We sampled five populations along the TMR transect and three in the HNP. At each population,
146 we compared 10×10 m plots inside and outside of the fences and thus grazed and ungrazed sub-
147 populations. Distance between grazed and ungrazed sites was a minimum of 20 meters. At each
148 sub-population, 15 individuals of *A. frigida* were sampled. Fresh leaf material was collected for a
149 total of 187 individuals in summer of 2019 (Table 1). In addition, three functional traits were
150 measured on the same individuals: height of inflorescence (if plants were flowering), height of
151 vegetative parts, and specific leaf area (SLA). Height of inflorescence was determined as height
152 from the ground surface to the tip of the highest inflorescence of that plant, and height of vegetative
153 parts as height of a random branch. For leaf area measurements, two fresh leaves from one
154 individual were taken (30 leaves per site) and scanned with a Conrad P-573 handheld document
155 scanner in the field. Scanned pictures were analyzed with ImageJ (Abràmoff et al., 2004) to
156 determine leaf area. In the laboratory, leaves were air-dried and weight was measured with Mettler
157 Toledo XP6, and the SLA was calculated by dividing leaf area by dry mass (Perez-Harguindeguy
158 et al., 2013).



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160 Figure 2. Differences in vegetation structure (a, c) between grazed and ungrazed sites with different fence
161 structures (b, d) along the Trans-Mongolian Railway and in the Hustai National Park, respectively. Picture
162 'a' is taken along the TMR close to the Choir city.

163 2.4. Vegetation samples and soil analyses

164 Species composition (SC; cover of each species within a plot), species richness (SR) and total
165 vegetation cover (VC, %) of vascular plants were recorded within a 10 m × 10 m plot to reveal the
166 grazing effect on local vegetation. A sample of top soil (0.05 m depth) was obtained from each
167 site including fine plant roots and the humic layer. Soil samples were first separated from litter and
168 debris, and further measurements were taken in the laboratory after sifting through a 2 mm sieve
169 and incubating at 75 °C for 18 h. To assess the amount of plant available nutrients (P, Mg, Ca, K
170 and Al), we prepared soil extractions following the Olsen method (Sims, 2000). Extracts were
171 measured by spectrometry (ICP-OES, Institute of Soil Science, Hannover University). Soil pH was
172 measured by a pH meter (Metler Toledo; water: soil volume = 5:1). Total N and C concentrations
173 of the soil were measured through dry combustion in a CN analyzer (Vario Pyro Cube; Elementar,
174 Langenselbold, DE). Carbonate content was first assessed with a quick test using 10% HCl, and
175 samples showing a reaction were further analyzed using a calcimeter following Scheibler's method
176 (ON L 1084-99, 1999). Soil C was then corrected for inorganic carbon. Rest water was measured
177 after drying of samples at 105 °C for 24h, and was used to calibrate nutrient contents to g dry soil.

178 2.5. Climate data extraction

179 The meteorological data for 39 years (mean annual temperature (MAT), mean annual precipitation
180 (MAP), coldest quarter temperature and precipitation (December-February; CQTemp and
181 CQPrec), and the warmest quarter temperature and precipitation (June-August; WQTemp and
182 WQPrec) between 1980 – 2018) were retrieved for each locality from the high resolution
183 CHELSA_V2 dataset, which has the advantage of capturing inter-annual precipitation variation
184 (Karger et al., 2017). The coefficient of variation of annual precipitation (cvP) was estimated based
185 on the retrieved MAP data and was also used as a predictor.

186 2.6. Molecular analyses

187 Genomic DNA of 187 individuals was isolated from silica gel dried leaf material of *A. frigida*
188 using the ATMAB protocol (Doyle and Doyle, 1987), with some modifications (Ziegenhagen,
189 1990). We had developed species-specific SSR markers which were proven to be polymorphic and
190 informative in a former study (see Oyundelger et al., 2021b for marker details). Amplifications for
191 a total of ten SSR markers were performed in a volume of 12.5 µl, and customized PCR reaction
192 mixtures and cycling programs were used (see list of the markers and PCR details from Table A.1).
193 Fragment sizes were determined by the central laboratory of the Senckenberg Biodiversity and
194 Climate Research Center (SBiK-F, Germany) using an ABI3730 sequencer (Life Technology)
195 with the LIZ600 size standard. The program Geneious® 10.2.6 (<https://www.geneious.com>) was
196 used to score allele sizes. Individuals exhibited a maximum of four microsatellite alleles per locus,
197 which was consistent with the hypothesized tetraploidy as described in Oyundelger et al. (2021b).

198 2.7. Statistical analyses

2.7.1. Analysis of genetic diversity and population structure

To estimate genetic diversity between and within populations, we used two different programs, which allow handling of microsatellite data for polyploid species: GenoDive v. 3.04 (Meirmans 2020) and the R-package Polysat v. 1.7 (Clark and Jasieniuk, 2011) in R v.4.0.3 (R Core Team, 2020). Measurements of genetic diversity comprised: allele diversity, effective number of alleles, percentage of polymorphic loci, observed heterozygosity, expected heterozygosity, and inbreeding coefficient, which were all estimated with GenoDive. The Bruvo distances were estimated in the R-package Polysat ; they are specifically designed to estimate distances for microsatellite data with higher and mixed ploidy, while accounting for mutational processes (Bruvo et al., 2004). We calculated the mean Bruvo distance among individuals for any given population (hereafter ‘Bruvo index’; see detailed from Oyundelger et al., (2021b)) using the R-package vegan (Oksanen et al., 2007) and used this as surrogate for genetic diversity. Bruvo index and measures of genetic diversity metrics were highly correlated ($r > 0.7^{***}$; Table A.2). Therefore, we opted to use the Bruvo index as a representative of genetic diversity throughout.

2.7.2. Effect of grazing on genetic diversity and structure in contrast to the local environment

Linear Mixed Models (LMMs) implemented in R-package lme4 (Bates et al., 2007) were fitted to assess the effects of grazing pressure and the local environment on genetic diversity. The log-transformed Bruvo distance was used as response variable. Due to multicollinearity of the environmental variables (Table A.3), only selected predictors for climate, soil and community composition were used: MAP, Ca, SR, “fence” was included as a factor. All predictors were scaled to zero mean-unit-variance prior to modeling to make estimates comparable, and population was included as a random factor. The analysis involved stepwise reduction of the full model by progressively removing the least significant factor until the null model. We compared subsequently reduced models in an ANOVA framework, and retained the final model with significant predictors.

We performed a Principal Coordinate Analysis (PCoA) using population level mean Bruvo distances using the R-package vegan (Oksanen et al., 2007). Environmental and vegetation variables were fitted *post hoc* on that ordination using vegan (Oksanen et al., 2007), and visualized using ggplot2 (Wickham 2011), to reveal environmental variables that are significantly associated with population genetic structure, in particular with fenced and unfenced sites. Environmental variables covered geography (latitude, longitude, and altitude), climate (MAT, WQTemp, CQTemp, MAP, WQPrec, CQPrec and cvP), soil nutrient contents (P, Mg, Ca, K, Al, C, N and pH), and vegetation variables (species richness and vegetation cover). Data on geographic, main climatic and vegetation variables are given in Table 1. Further data on climate, analyzed soil nutrient values and plant species composition of each site (each species' cover within a plot) are provided in Table A.4 & Table A.5.

235 2.7.3. *Effect of grazing on functional traits, and its relationship to the genetic diversity*

236 To investigate grazing effects on plant functional traits, we fitted LMMs using mean and
237 coefficient of variation of the height of inflorescence, vegetative height, and SLA. In all models,
238 population was included as a random factor. In addition to the environmental variables mentioned
239 above, the Bruvo index was included as a predictor. Height of vegetative parts and inflorescence
240 had normal distributions, and were not log-transformed, while SLA and cover of *A. frigida* were
241 log-transformed to achieve normality. The model simplification procedure was conducted as
242 described above.

243 2.7.4. *Relationship between genetic and spatial distances*

244 To assess the overall relationship between genetic and spatial distances, Mantel tests between
245 genetic distance (population level mean Bruvo) and geographic distances (Euclidean distances)
246 were computed with 10,000 randomizations using the R-package vegan (Oksanen et al., 2007).
247 Further Mantel tests were conducted between genetic distances and a) climatic differences
248 (Euclidean distance of standardized climatic variables described in 2.5); b) distance of soil
249 indicator variables (Euclidean distance of standardized variables described in 2.4); and c)
250 differences in plant community composition (Bray-Curtis distance based on log-transformed
251 species' cover).

252 Table 1. Characteristics of the study sites (locality and climate) and respective genetic diversity parameters.
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Locality, province	Pop code	Fence	N	E	Altitude, m	MAT, C°	MAP, mm	cvP	SR	VC, %	N	PIC	AD	EfN	H _O	H _E	G _{IS}	Bruvo index
Ulaanbataar, Tuv	Pop1	outside	47.8675	107.1051	1358	-0.65	237.9	28.2	27	75	14	0.78	11.8	6.07	0.62	0.78	0.20	0.62
		inside	47.8678	107.1053	1357	-0.65	237.9	28.2	20	85	8	0.72	7.7	5.62	0.69	0.79	0.12	0.60
Hustai NP, Tuv	Pop2	outside	47.7555	105.8915	1404	-0.31	276.0	29.5	22	40	11	0.66	7.2	3.85	0.44	0.73	0.39	0.56
		inside	47.7648	105.8818	1352	-0.05	278.3	29.5	35	95	11	0.74	8.9	4.66	0.57	0.75	0.24	0.57
Hustai NP, Tuv	Pop3	outside	47.6915	105.9301	1325	-0.05	283.9	29.1	24	76	12	0.71	7.2	3.54	0.53	0.72	0.26	0.54
		inside	47.6944	105.9093	1358	-0.79	304.6	29.1	27	90	12	0.73	8.3	4.58	0.55	0.77	0.29	0.59
Hustai NP, Tuv	Pop4	outside	47.6659	105.9682	1325	0.38	252.8	29.1	25	61	10	0.76	9.9	5.02	0.64	0.78	0.18	0.60
		inside	47.6540	105.9619	1232	0.80	251.0	29.0	22	84	12	0.66	8	3.34	0.52	0.66	0.21	0.51
Bayan, Tuv	Pop5	outside	47.1964	107.5764	1388	0.30	225.6	26.8	25	56	11	0.58	5.8	2.87	0.44	0.70	0.37	0.43
		inside	47.1961	107.5760	1388	0.30	225.6	26.8	21	69	7	0.46	3.7	2.35	0.36	0.68	0.48	0.36
Bayan, Tuv	Pop6	outside	46.9449	107.7278	1324	0.82	204.2	28.4	19	63	10	0.72	7.7	4.31	0.58	0.76	0.24	0.59
		inside	46.9448	107.7270	1331	0.82	204.2	28.4	12	80	9	0.57	5.7	3.36	0.37	0.69	0.47	0.51
Choir, Dundgovi	Pop7	outside	46.1549	108.6206	1221	2.38	179.6	34.1	25	70	15	0.71	9.8	4.75	0.63	0.73	0.15	0.50
		inside	46.1554	108.6208	1222	2.29	180.8	34.0	23	95	15	0.74	10	5.36	0.61	0.78	0.22	0.54
Airag, Dundgovi	Pop8	outside	45.7644	109.3826	1032	3.48	161.2	33.3	23	76	15	0.74	10.3	5.08	0.60	0.77	0.22	0.56
		inside	45.7648	109.3827	1041	3.48	161.2	33.3	22	70	15	0.70	8.9	4.77	0.59	0.73	0.19	0.54
Mean					1291	0.78	229	29.8	23	74	12	0.69	8.18	4.34	0.55	0.74	0.26	0.54

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255 N – latitude, E – longitude, MAT – mean annual temperature, MAP – mean annual precipitation, SR – plant species richness, VC, % – vegetation
256 cover, N – number of samples, PIC – polymorphic information content, AD – allele diversity, EfN – number of effective alleles, H_O – observed
257 heterozygosity, H_E – expected heterozygosity and G_{IS} – inbreeding coefficient.

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3. Results

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3.1. Effect of grazing on genetic diversity and structure, in contrast to the local environment

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Genetic diversity of *A. frigida* along the TMR was significantly affected by grazing, which had a significant positive effect on genetic diversity of *A. frigida*. Soil calcium content had a positive impact on genetic diversity, although both effects were modest. In contrast, climatic variables showed no significant effect on *A. frigida* genetic diversity (Table 2; Fig. 3).

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Table 2. Summary of Linear Mixed Models assessing the effects of grazing and environmental variables on genetic diversity of the populations of *A. frigida*.

	Estimate	Std. Error	p value	
(Intercept)	-0.29	0.02	<0.001	***
Grazing	0.04	0.01	0.02	*
Soil calcium	0.03	0.01	0.02	*

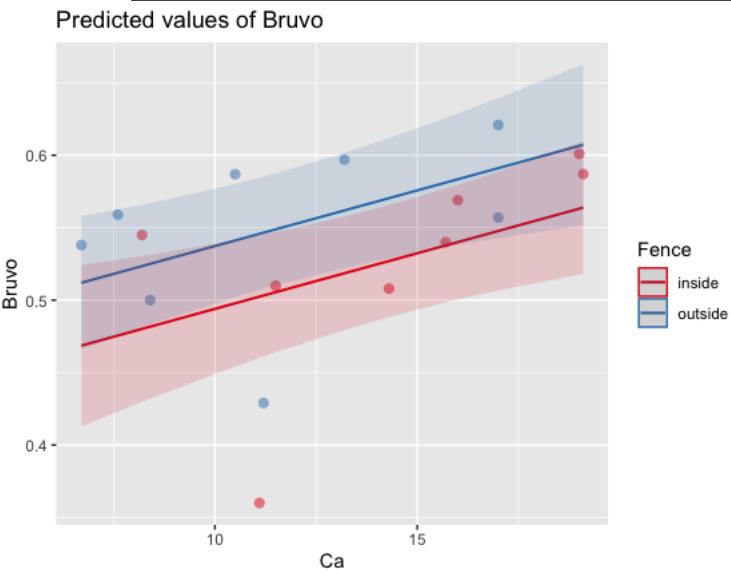
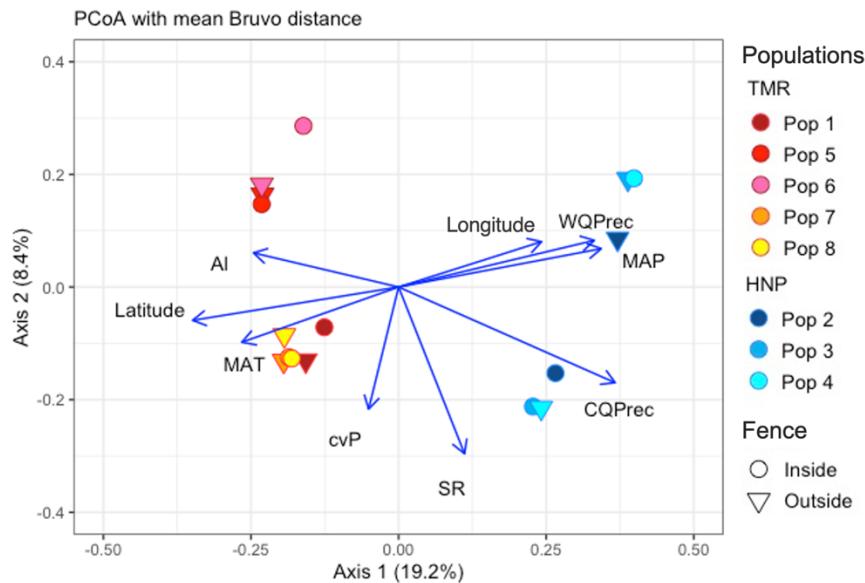
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Figure 3. Predicted effects of soil calcium amount (Ca) and grazing based on the best-fitting linear mixed models (LMMs; Table 2). Shaded areas indicate marginal effects.

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We found no main effect of grazing on genetic distance between populations (Fig. 4). In the PCoA ordination, populations were segregated along the x-axis, representing three populations from HNP (Pop2 – 4) located on the right side, and populations along the TMR on the left side. Environmental variables and plant species richness showed a more substantial relationship with the genetic distance than grazing according to *post hoc* fitted predictor variables (see Fig. 4).



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Figure 4. Principle Coordinate Analysis (PCoA) based on mean Bruvo distance between populations obtained from ten SSR markers for fenced (circles) and unfenced (triangles) sub-populations of *A. frigida*. Environmental predictors were fitted *post hoc* on the ordination plot (only those that passed $p < 0.05$ according to a test with 1,000 permutations are shown). MAP – mean annual precipitation, MAT – mean annual temperature, WQPrec – warmest quarter precipitation, CQPrec – coldest quarter precipitation, cvP – coefficient of variation of annual precipitation, Al – soil aluminum content, and SR – plant species richness, TMR – Trans-Mongolian Railway, HNP – Hustai National Park.

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We assessed whether effect of grazing on genetic differentiation interacts with climatic variation by calculating mean pair-wise Bruvo distance between pairs of fenced and unfenced sites at a given location. Grazing effect tended to be smaller under higher cvP ($r = -0.67$, $p = 0.007$).

The Mantel tests indicated that genetic distance (Bruvo index) was positively correlated with all environmental distances, i.e., geographic distance ($r^2 = 0.12$, $p < 0.001$), climatic differences ($r^2 = 0.19$, $p < 0.001$), difference in plant species composition ($r^2 = 0.13$, $p < 0.001$), and distance in soil nutrient content ($r^2 = 0.08$, $p = 0.001$). In terms of effect size, the correlations were, however, modest.

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3.2. Effect of grazing on plant functional traits, and its relationship to genetic diversity

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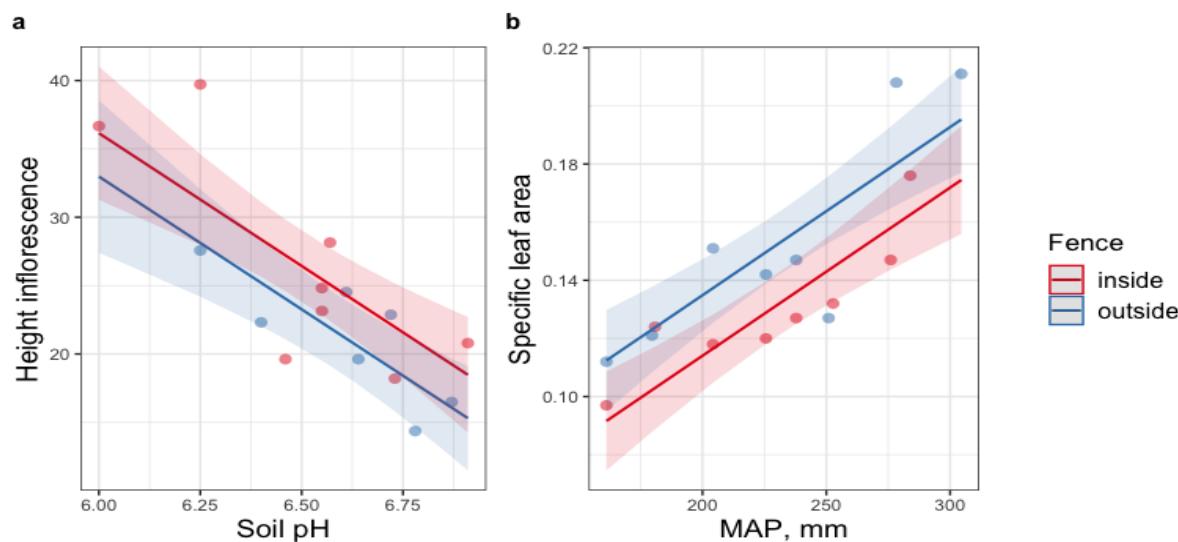
We found that grazing had a significant impact on all functional traits measured, albeit with varying responses. Grazing reduced both height of inflorescence and vegetative parts, but increased SLA of *A. frigida*. Furthermore, a higher MAP resulted in a significant increase in SLA, whereas higher soil pH resulted in lower inflorescence height (Table 3; Fig. 5). We observed no relationship between genetic diversity and functional traits: neither with respect to trait variance (coefficient of variation of the respective variables), nor regarding mean values (mean height of the vegetative and inflorescence, as well as of the SLA).

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Table 3. Summary of Linear Mixed Models assessing the effects of grazing and environmental variables on the functional traits of *A. frigida* populations.

Functional traits	Predictor	Estimate	Std. Error	Pr(> t)	
Height inflorescence	(Intercept)	23.72	1.51	<0.001	***
	Grazing	-2.75	0.93	0.024	*
	Soil pH	-8.19	1.52	<0.001	***
Height vegetative parts	(Intercept)	11.51	0.70	<0.001	***
	Grazing	-3.00	0.98	0.008	**
	MAP	0.08	0.01	<0.001	***
Specific leaf area	(Intercept)	-0.89	0.01	<0.001	***
	Grazing	0.06	0.02	0.010	*
	MAP	0.08	0.01	<0.001	***

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Figure 5. Predicted effects of a) soil pH values on plant inflorescence height and b) MAP on specific leaf area of *A. frigida* between grazed and ungrazed sites based on the best-fitting Linear Mixed Models (LMMs; Table 3). Shaded areas indicate marginal effects of the model.

311

4. Discussion

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4.1. Effect of grazing on genetic diversity and structure, in contrast to the local environment

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4.1.1. Population genetic diversity

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Long-term grazing disturbance in dryland populations can lead to a loss of genetic diversity across generations (Pelliza et al., 2020), while intermediate levels of grazing can facilitate gene flow between populations (Peng et al., 2015). We found that the fences along the TMR and HNP had a negative effect on *A. frigida*'s genetic diversity (Table 2), demonstrating that grazing promotes genetic diversity.

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Large herbivores are important seed dispersal vectors among different pasturelands (Plue et al., 2019). In particular, herbivores play an important role in Mongolian steppes, where livestock

322 are herded in order to seek for fresh pastures with frequent movements during the year. Therefore,
323 livestock movement across long distances can promote genetic exchange between populations,
324 given that small diaspores like those of *A. frigida* have high adhesion potential and can be
325 transported in animal fur for up to 15 km per day (Bläß et al., 2010). Furthermore, free soil patches
326 are formed for seed germination in the vegetation as a result of browsing and grazing (safe sites;
327 Frank, 2005) for seed germination in the vegetation, which would otherwise have a lesser chance
328 of germination, especially in conditions of long-term vegetation succession, such as, inside fences.
329

330 Our previous study conducted in rangelands of Mongolia, examining the effect of different
331 grazing intensity levels on *A. frigida* (Oyundelger et al., 2021b) revealed no substantial grazing
332 effect on the genetic diversity. There, we compared different levels of grazing intensity from
333 modest to high, but lacked control sites without any grazing. Another study on *A. frigida* conducted
334 in Inner Mongolia, China, found a detrimental grazing effect, with genetic heterozygosity
335 decreasing as grazing intensity increasing from enclosure to heavy grazing (Wang et al., 2004),
336 but with high standard deviations of the genetic diversity indices. Mean values of the genetic
337 diversity is decreased as grazing increased according to Wang et al., (2004), however, standard
338 deviations are often as high as the mean values, making differentiation among different grazing
339 intensity levels questionable.

340 We also found that higher soil calcium amount promotes genetic diversity in *A. frigida*.
341 Soil calcium content was higher inside the fences, possibly due to the construction work of the
342 railway, where different calcium-based stabilizers (e.g., calcium carbonate and calcium chloride)
343 are often used for soil stabilization (Afrin, 2017). Calcium is required for plant cell elongation in
344 both shoots and roots (Hepler, 2005), and Feagley and Fenn (1998) found that calcium increases
345 ammonium, potassium and phosphorus absorption, promotes photosynthesis, and increases plant
346 size. Furthermore, it has been shown that soil carbon and phosphorous have significant positive
347 effects on the establishment of seedlings and plant growth of *Artemisia* species (Yang et al., 2015).
348 our former study (Oyundelger et al., 2021b) found that higher levels of soil phosphorus promoted
349 the genetic diversity of *A. frigida*, through facilitating successful seed germination enhancing
350 reproductive success, resulting in more effective sexual reproduction and a consequent increase in
351 genetic diversity. Regrettably, there is a general lack of studies investigating the relationship
352 between soil nutrient content and plant genetic diversity, so it remains speculative if observed
353 effects of Ca and P are direct or just due to covariance with other potential beneficial soil factors.
354

354 4.1.2. Population genetic differentiation

355 *Artemisia frigida* is a wind-pollinated, wide-spread perennial species, and a prolific seed producer
356 (Harvey, 1981). Its life form, geographical distribution, and reproduction system have direct
357 implications for genetic differentiation among populations. Former studies have demonstrated that
358 populations of *A. frigida* sustain adequate gene flow, and therefore, levels of genetic differentiation
359 is low in drylands of China and Mongolia ($D_{ST} = 0.04$; $D_{ST} = 0.05$; $F_{ST} = 0.32$; $G_{ST} = 0.034$; Wang
360 et al., 2004; Liu et al., 2010, 2012; Oyundelger et al., 2021b, respectively). In our study, we found
361 moderate genetic differentiation among populations at $G_{ST} = 0.07$. This is, however twice as high

362 as compared to our former study on different grazing intensity levels conducted in Mongolia. In
363 the present study, the higher genetic differentiation among populations was possibly caused by
364 clear differentiation between regions, i.e. populations of HNP and along the TMR, as illustrated in
365 the PCoA (Fig. 4). This pattern was previously observed in our study as well, where HNP
366 populations differed genetically from other populations, confirming an earlier hypothesis about
367 high diaspora input, i.e. new genotypes transported by moving herds in the national park (see
368 details from Oyundelger et al., 2021b). However, we did not find an overall grazing effect on
369 genetic differentiation between grazed and ungrazed sites in both HNP and TMR (mean Bruvo
370 distance inside fence = 0.61, and outside fence = 0.62), as fences permit diaspora and pollen
371 transport through wind (Fig. 2 b, d), and thus genetic exchange. In addition, under drier condition,
372 vegetation is less dense even inside the fences, may limiting differentiation between grazed and
373 ungrazed sites.

374 In comparison to the grazing effect, environmental variables and plant species richness
375 showed a more substantial relationship with genetic distance. Specifically, the amount of total
376 precipitation, the variability of precipitation, annual temperature and geographical locations
377 showed significant relationships with genetic structure (Fig. 4). This is consistent with our former
378 study (Oyundelger et al., 2021b), where precipitation variability and habitat types were the main
379 drivers of population genetic differentiation in Mongolian drylands. Furthermore, we found a
380 correlation between the coefficient of variation of annual precipitation (cvP) and grazing effect,
381 with stronger grazing impacts on genetic distance in moister habitats with lower cvP ($r = -0.67$,
382 $p = 0.007$). The northern part of Mongolia with forest steppes and mountain- and meadow-steppe
383 habitats receive higher amounts of precipitation, and in moister habitats grazing has a stronger
384 impact due to the direct effect of high stocking rates according to the equilibrium system dynamics
385 (Ahlborn et al., 2020).

386

387 4.2. Effect of grazing on plant functional traits, and its relationship to genetic diversity
388 We found that grazing had a significant impact on all functional traits measured, which could be
389 interpreted as adaptation to grazing. Specifically, the heights of both plant inflorescence and
390 vegetative parts were smaller, but SLA was larger at grazed sites. This is consistent with the
391 general strategy of grazing resistant species displaying both avoidance traits (high SLA; Díaz et
392 al., 2001) Smaller plant height of *A. frigida* under disturbance was also observed by Li., et al
393 (2002). These authors simulated a grazing effect by cutting individuals during their reproductive
394 stage, and reported that severe cutting reduced the height of re-growth and the number of branches,
395 resulting in miniaturized plants. Increased SLA in grazing resistant species under intensified
396 grazing is known from previous studies (Díaz et al., 2001; Cingolani et al., 2005; Lang et al.,
397 2020); higher SLA should improve the photosynthetic ability and compensate for biomass loss via
398 higher growth rates in response to long-term grazing. Moreover, Lang et al., (2018) found that
399 SLA of *A. frigida* increased with higher water availability, which is concordant with our result of
400 a significant positive correlation between SLA and MAP (Table 3). However, we did not find

401 interactions between climate and grazing on the difference of SLA between populations. Such
402 interactions were found by Lang et al., (2020) in *A. frigida*, yet with weak correlation ($R = 0.04^*$).

403 We observed no relationship between genetic diversity and mean values of functional traits
404 of *A. frigida*, nor with trait variance. This reflects that many traits exhibit great plasticity in
405 response to local environmental conditions (Stinchcombe et al., 2012; Mason et al., 2020). Few
406 studies have examined the genetic basis of functional trait variation and its heritability. Geber and
407 Griffen (2003) reviewed the relationship between selection of traits and heritability, finding that
408 the traits under strong selection have the lowest heritability due to a demand for adaptability to
409 high environmental variance. Recently, Ahrens et al., (2020) confirmed that heritability is not
410 consistent among traits, and different levels of variability can be observed in the traits depending
411 on local adaptation to selection pressure. Specifically, SLA in a tree species (*Corymbia calophylla*
412 (LINDL.) K.D. HILL & L.A.S. JOHNSON) was found to be barely heritable confirming that SLA is
413 highly plastic (Shipley, 2000). In addition, Scheepens et al., (2010) found a vary plastic response
414 of SLA in the widespread alpine plant *Campanula thrysoides* L. as well, but with some evidence
415 of genetic constraints on SLA variance.

416 Conclusion

417 Grazing of large herbivores is an integral aspect of Mongolian steppe ecosystems, soil function,
418 and nomadic livelihoods. Our study is one of the few studies, where exclosures were used to
419 investigate grazing effect on population genetic structure of dominant steppe species. We found
420 that fences along the TMR and in the HNP had negative effects on the genetic diversity of
421 *A. frigida*, while soil nutrient contents might promote seedling establishment, thus increasing
422 genetic diversity. In contrast to climate and soil characteristics, grazing showed no significant
423 influence on population genetic structure. Moreover, grazing had a significant impact on all the
424 functional traits measured, in particular plant height decreased and SLA increased as a likely
425 adaptation against grazing. Yet, we found not relationships between genetic differentiation among
426 sub-population and functional traits, pointing to high phenotypic plasticity. Overall, a certain level
427 of grazing may be even beneficial for species genetic diversity and hence functioning of the
428 grassland.

429 Still, relationships between dryland plant genetic diversity and environmental variables,
430 specifically with soil factors remain not fully understood. This aspect should be considered in
431 future studies.

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