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Seedling recruitment in mountain grassland restoration: effects of soil preparation and grazing

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Abstract

Questions: Seedling recruitment is a key step in any seed-based ecological restoration project. There is a controversial discussion i) whether soil preparation is required to reduce competition of pre-existing vegetation, or whether vegetation cover facilitate seedling recruitment, and ii) whether grazing should be excluded in initial stages of grassland restoration to protect seedlings, because grazing exclusion may also favour competitive ruderal species. We set up a combined soil preparation and grazing experiment to evaluate the effect of both factors on seedling recruitment of seeds transferred from a species-rich donor site.

Location: Upper Durance valley, Hautes-Alpes, France.

Methods: The experiment was set up using a full factorial split-plot design with five replicate sites. The treatments included soil preparation (harrowed or not) and grazing (excluded or not). Seeds were transferred using plant material brushed in a non-degraded reference grassland. The individuals of all occurring species were counted on the restoration sites, and the survival and reproduction of two focal species, *Bromopsis erecta* and *Rhinanthus alectorolophus*, were recorded for three months.

Results: Soil preparation by harrowing reduced the density of spontaneously emerging species and increased the seedling density of species transferred from the donor site. Grazing had only a weak negative effect on the recruitment of transferred species. The main effect of both treatments on seedling survival was not significant, but a significant interaction indicated that the grazing effect depended on soil preparation, with a negative effect of grazing only in non-harrowed plots.

Conclusions: The reduction of competition by soil preparation before seed addition of brush material had a positive effect on the seedling recruitment of transferred species and can thus

clearly be recommended in our study system. The weak negative effect of grazing may not always justify fencing costs.

KEYWORDS

Bromopsis erecta, brush harvesting, community ecology, ecological engineering, grassland restoration, grazing, harrowing, montane grasslands, *Rhinanthus alectorolophus*, seedbed preparation, seedling establishment, survival.

1 INTRODUCTION

2 Seedling recruitment is an important process driving the composition and diversity of plant
3 communities (Grubb, 1977; Zeiter *et al.*, 2006). Understanding the factors constraining seedling
4 recruitment is thus crucial to predict and improve restoration success. Dispersal (Münzbergová
5 and Herben, 2005; Frances *et al.*, 2010) and microsite limitation (Myers and Harms, 2009; Long
6 *et al.*, 2014) are known to be such constraining factors in re-colonisation processes. In grassland
7 restoration, the small soil seed bank of typical late-successional species may result in
8 insufficient seedling recruitment (Grman *et al.*, 2015; Buisson *et al.*, 2018). Poor seed dispersal,
9 as well as the lack of connectivity between source populations due to landscape fragmentation,
10 often limit the seed rain (Münzbergová and Herben, 2005; Bischoff *et al.*, 2009; Muller *et al.*,
11 2014) and successful restoration may require seed addition to overcome seed limitation (Öster
12 *et al.*, 2009; Török *et al.*, 2012; Valkó *et al.*, 2016). Thus, seed addition methods such as sowing
13 local seed mixtures, hay transfer or soil transfer, are increasingly used in grassland restoration
14 (Kiehl *et al.*, 2010; Scotton *et al.*, 2012; Kiss *et al.*, 2020).

15 Seed-addition experiments demonstrated that limited recruitment is the next major
16 constraint influencing plant establishment (Tilman, 1997; Turnbull *et al.*, 2000; Pywell *et al.*,
17 2002). Unfavourable microsite conditions may hamper germination and reduce seedling
18 survival often resulting in microsite limitation (Bissels, 2006). Soil disturbance, such as topsoil
19 removal or soil tillage, increases recruitment opportunities (Hölzel and Otte, 2003; Myers and
20 Harms, 2009; Long *et al.*, 2014; Klaus *et al.*, 2017). Soil preparation (also called “seedbed
21 preparation”; Shaw *et al.*, 2020) may promote the recruitment of plant species by (i) limiting
22 the competition of an already established vegetation, and by (ii) improving abiotic conditions
23 of seedling recruitment (Kiehl *et al.*, 2010). A low pre-existing vegetation cover may have a

1 facilitating effect on recruitment by protecting seedlings from high solar radiation and drought
2 stress (Callaway and Walker, 1997). However, if pre-existing vegetation is dense, the
3 competitive effect on introduced species may prevail because already established species limit
4 available space and resources (Kupferschmid *et al.*, 2000; Poschlod and Biewer, 2005).
5 Preparing the soil may favour seedling recruitment of new species by opening the vegetation
6 and destroying the root systems of competitive perennial species (Bischoff *et al.*, 2018). Soil
7 preparation further creates micro-reliefs improving seed and water retention as well as seed
8 adhesion (Chambers, 2000). However, soil disturbance increases nitrogen mineralisation
9 increasing plant available nitrogen in the soil (eutrophication) and may negatively affect the soil
10 fauna and soil structure potentially favouring erosion (Di and Cameron 2002; Holland 2004).

11 Human land management by grazing and mowing is a key factor regulating the
12 functioning and structure of semi-natural grasslands (Hejcman *et al.*, 2013). Several studies
13 confirmed the essential role of grazing in maintaining plant species richness of grassland
14 ecosystems (Dupré and Diekmann, 2001; Pykälä, 2003, Saatkamp *et al.*, 2018). However, in the
15 initial stages of grassland restoration, grazing may hamper seedling recruitment since seedlings
16 are particularly vulnerable to trampling and biomass removal by herbivory (Bakker, 2003;
17 Buisson *et al.*, 2015). Trampling may prevent species from germinating by crushing and
18 damaging seeds or coleoptiles (Rother *et al.*, 2013). Moreover, trampling on slopes may lead
19 to soil destabilization (Tasser *et al.*, 2003), increases soil erosion (Farrell and Fehmi, 2018) and
20 soil compaction (Allington and Valone, 2011). However, in mountain ecosystems, in which the
21 particular pedo-climatic conditions (soil erosion, shallow and stony soils) reduce seedling
22 recruitment, micro-reliefs created by hoof prints may increase seed trapping (Isselin-Nondedeu
23 *et al.*, 2006) and improve seed adhesion to the soil (Chambers, 2000). Accordingly, Eichberg
24 and Donath (2018) found an increased seedling recruitment in a trampling simulation

experiment on sandy soils also suffering from low stability and high run-off. Biomass removal by herbivory damages seedlings and often reduces initial seedling survival (Buisson *et al.*, 2015; Vidaller *et al.*, 2019b). However, Farrell and Fehmi (2018) only found a combined negative effect of trampling and herbivory whereas herbivory alone did not affect seedling establishment. Furthermore, Kladivová and Münzbergová (2016) even revealed a positive effect of grazing on both seedling recruitment and establishment but the grazing effect depended on habitat conditions at the specific microsite.

In our study, we aim to test the effect of soil preparation and grazing on seedling recruitment (combining germination and early survival) in a seed-addition experiment conducted in semi-natural mountain grasslands. Using brush material transferred from a species-rich donor grassland to restore degraded sites, we addressed the following questions: (1) Does soil preparation by harrowing before sowing improve seedling recruitment and survival of the transferred species? (2) What is the effect of early grazing on seedling recruitment and survival of the transferred species?

MATERIALS AND METHODS

Study area

The study area is the upper Durance valley ("Haute-Durance") in the Southern French Alps. The construction of a high-voltage transmission line led to a local destruction of species-rich grassland communities within a 1 km wide and 100 km long strip from L'Argentière-la-Bessée to La-Batie-Neuve (44°78'78"N, 6°59'41'E; 44°57'93"N, 6°20'77'E). The experimental sites representing a subsample of these grasslands are located between 1100 and 1300 meters above the sea level (upper montane altitudinal belt). The climate is temperate to sub-

Mediterranean depending on slope, exposition and elevation. At the closest meteorological station (Embrun), the annual precipitation is about 740 mm and the mean annual temperature 10.7°C. Considering the lower elevation of the station (880 m) the temperature is roughly 2°C lower resulting in an annual mean of 8.7°C at our experimental sites. The bedrock is predominantly calcareous with some quaternary glacial deposits. Non-degraded grasslands of the study area belong to Natura 2000 priority habitats: "Semi-natural dry grasslands of Festuco-Brometalia and scrubland facies on calcareous substrates" (N6210*, EU directive habitat 92/43/EEC; Calaciura and Spinelli, 2008) that are traditionally managed either by mowing, cattle and sheep grazing. On transitory access tracks and construction platforms, the vegetation was removed, and the soil compacted to facilitate the transport of heavy equipment. To recreate former soil conditions after electricity line construction the soil was de-compacted by deep tillage between May and July 2018.

Donor site and seed harvest

The donor site to collect brush material is located within the study area at Freissinières at an elevation of 1100 m (44°73'61"N; 6°56'72"E). The soil and climatic conditions of the donor site corresponded to the average of the five experimental sites. The seed material was harvested in July 2018 using a brush harvester mounted on a quad and equipped with an integrated vacuum system. On a surface of 4000 m², we obtained 2.6 kg of brush material. The brush material comprised seeds (60% of total mass) and vegetative parts (40%). Seed counts in ten subsamples of 0.5 g revealed an average density of 887 seeds/g of brush material. A single harvest was considered as sufficient since the short summer season in the study area has resulted in a concentration of the seed production period for most species.

1 **Experimental set-up and design**

2 The experiment was set up in October 2018 using a full factorial split-plot design replicated on
3 five sites (blocks) to test the effect of previous soil preparation, grazing and its interaction on
4 seedling recruitment (see Durbecq *et al.*, 2020, for the position of experimental sites). Each of
5 the five sites comprised eight plots of 16 m² each (4 m × 4 m) resulting in a total of 40 plots.
6 Four plots representing a half-block were exposed to extensive grazing and the remaining four
7 were fenced preventing grazing (whole-plot factor). The sowing and soil preparation
8 treatments (split-plot factors) were randomly assigned within half-blocks. The distance
9 between plots was 1 m except for the Embrun site where this distance was reduced to 50 cm
10 due to limited space.

11 The full design also comprised unsown control plots randomly assigned to half-blocks.
12 The unsown plots were used to evaluate which plant species of the donor grassland already
13 occurred at the restoration sites before the brush material was transferred (soil seed bank,
14 seed rain). Average vegetation cover of these unsown controls was 28% compared with 90% at
15 the donor site. Species occurring in the control plots of a given site were considered as
16 “spontaneously emerging” for this site. Only plant species of the donor site not occurring in the
17 control plots were considered as “transferred” (Appendix S1). The method may underestimate
18 the density of transferred species since species occurring in unsown control plots may still have
19 been transferred to the sown plots.

20 Sites already covered with spontaneous vegetation were mown prior to experimental
21 set-up. Harrowing was selected as a soil preparation method using a rotary harrow adjusted to
22 a working depth of 8 cm. In October 2018, just after harrowing, the brushed material was
23 spread at a density of 104 g/plot corresponding to a seed density of 3.75 g/m² and
24 approximatively 5765 seeds/m². The chosen seed density was based on recommendations of

a previous research project in the French Alps (Koch *et al.*, 2015). In agreement with the traditional local grazing regime, the unfenced experimental plots were grazed twice a year: in late October just after experimental set up, and in early June. Two sites were grazed by cattle, three sites by sheep, corresponding to the average proportion of both grazing types in the study region. The sites were neither fertilised nor irrigated.

Data collection

Vegetation relevés at the donor site and germination tests in the greenhouse

Prior to seed harvest, the cover of all occurring vascular plant species was estimated in five representative 3 m × 3 m plots as the vertical projection cover of above-ground vegetation in late June 2018. We used Tison *et al.* (2014) for plant species identification and as reference for plant species names. At the harvest in July 2018, the average phenological stage of all species was recorded in order to evaluate seed availability. Eighty percent of the species recorded in June were found to have seeds in July.

In order to obtain information on the germination potential of seeds contained in the brush material and to evaluate germination in the field compared with germination potential, we set up a germination test in November 2018. Subsamples of the brush material were transferred to five trays (0.03 m²) filled with standard peat substrate and placed in an unheated greenhouse. Each tray received 0.54 g of brush material corresponding to 18 g/m² and a density of approximately 18 000 seeds per m². The trays were regularly watered, and germination was recorded until April 2019. Greenhouse germination of the harvested brush material was recorded once a week for four months and seedlings were removed after identification.

Seedling survey and vegetation relevés at restoration sites

To quantify seedling recruitment of both transferred species and spontaneously emerging species, plant numbers were counted in three quadrats of 40 cm × 40 cm placed along the diagonal of each plot. Since we were not able to distinguish seedlings and plants re-sprouting from below-ground organs, the seedling counts may include young ramets of clonally growing plants. In order to identify spontaneously emerging species, we conducted at the same time vegetation relevés on the unsown control plots (9 m²).

Seedling survival of focal species

Seedlings of two typical species of the donor site, *Bromopsis erecta* (Huds.) Fourr. (former name: *Bromus erectus*) and *Rhinanthus alectorolophus* (Scop.) Pollich were monitored over 11 weeks in order to assess seedling survival. *B. erecta* is the characteristic species of semi-dry grasslands (Mesobromion type *sensu* Ellenberg, 1996) occupying a large range in Europe. It is further one of the typical species of the habitat type (N6210*, EU directive habitat 92/43/EEC; Calaciura and Spinelli, 2008). *B. erecta* is a perennial grass growing on calcareous and often nutrient-poor soils. *R. alectorolophus* is a summer annual, hemi-parasitic species growing in semi-dry to mesophilic calcareous grasslands. As *B. erecta*, it is among the characteristic species of the N6210*-habitat type. *R. alectorolophus* has a wide range of hosts that includes grasses and legumes (Sandner and Matthies, 2018). The species is used as a tool in restoration of species-rich grasslands as it reduces competition by grasses and may thus have a positive effect on plant diversity (Bullock and Pywell, 2005; Heer *et al.*, 2018). In all sown plots (four plots per site), ten randomly chosen seedlings of both species were tagged in May 2019 using coloured rings and poles (Appendix S2). A total of 400 seedlings were tagged over the five sites. The position of each individual was mapped, and each seedling was numbered in order to facilitate localization and recognition. Seedling survival and reproduction of these two focal species were recorded in late July 2019.

1

2 **Data analysis**

3 We analysed (i) seedling numbers and species richness of the total plant community (“all
4 species” = spontaneously emerging species + transferred species), and of transferred species
5 only, as well as (ii) the survival of the two focal species, *Bromopsis erecta* and *Rhinanthus*
6 *alectorolophus*.

7 Generalized linear mixed models were applied to assess the effect of soil preparation
8 (harrowing: yes/no) and of grazing (yes/no) on seedling recruitment and the survival of *B.*
9 *erecta* and *R. alectorolophus*. The full model included the two factors and the grazing-by-soil
10 preparation interaction as fixed effects and site as a random effect. In order to include the split-
11 plot design of the experiment, a grazing-by-site interaction (random) was fitted to the model
12 to test the whole-plot factor (grazing). Harrowing (split-plot factor) and the site-by-harrowing
13 interaction were tested against the model residuals. Abundance data were right-skewed but
14 models with Poisson error distribution and log-link function showed overdispersion. Thus
15 negative binomial error distribution and log-link function were finally used to test total
16 abundance and abundance of transferred species. Species richness was tested using Gaussian
17 error distribution and identity link. Both models additionally included quadrat within plots as a
18 random factor. Survival was analysed at plot level and did not include quadrat. A binomial error
19 distribution was fitted using a logit-link function.

20 Non-Metric Multidimensional Scaling (NMDS) based on Bray–Curtis similarity was
21 applied to compare plant species composition in different treatments (Borcard *et al.*, 2011). A
22 permutation multivariate analysis of variance (PERMANOVA, Anderson 2001) with 9999
23 permutations was used to analyse whether the community composition was significantly

different between the grazing and soil preparation treatments (R package “vegan”, Oksanen *et al.*, 2019). All analyses were performed using R 3.5.3 (R Core Team, 2019).

RESULTS

Seedling emergence

A total of 21 plant species germinated in the greenhouse corresponding to 37.5% of the total species number observed at the donor site. At the restoration sites, 29 donor site species (51.8%) were found. Taking into account the difference in the amount of seed material used in the greenhouse test and at the restoration sites, the final germination was 40 times higher in the greenhouse (7.31 seeds/g) than in the field (0.17 seeds/g; Appendices S3). In particular, *Plantago media* L., *Festuca marginata* (Hack.) K.Richt. and *Schedonorus pratensis* (Huds.) P.Beauv. showed a much higher greenhouse germination whereas the difference between greenhouse and field was much smaller in *B. erecta* (4 times higher in the greenhouse). *R. alectorolophus* did not germinate in the greenhouse.

Seedling recruitment

Soil preparation by harrowing reduced overall seedling recruitment including that of spontaneously emerging species. Grazing had a marginally significant negative effect on seedling recruitment (Table 1, Fig. 1a). The opposite effect of harrowing was observed for transferred plant species. Twice as many seedlings of transferred species were found in the harrowed than in the non-harrowed plots (Fig. 1b). Grazing resulted in a 50% reduction of the seedling density of transferred species, but due to the high between-site variation the grazing effect was only marginally significant. The grazing-by-soil preparation interaction was not significant for both response variables.

Table 1. Effect of harrowing and grazing on number of seedlings per m², species richness and the survival of two focal species. Results on seedling number and richness are presented separately for the whole plant community and the subsample of transferred species. H x G = grazing-by-harrowing interaction. Results of GLMM with Chi-Square (χ^2) and significance levels: . P < 0.1, * P < 0.05, ** P < 0.01, *** P < 0.001, NS not significant.

	<i>df</i>	Seedling number		Species richness		Survival	
		All species	Transferred species	All species	Transferred species	<i>Bromopsis erecta</i>	<i>Rhinanthus alectorolophus</i>
Harrowing	1	7.097 **	6.435 *	3.476 .	6.308 *	2.484 NS	0.741 NS
Grazing	1	3.726 .	3.440 .	2.661 NS	2.804 .	1.799 NS	2.585 NS
H x G	1	0.560 NS	0.011 NS	0.014 NS	1.095 NS	5.089 *	4.851 *

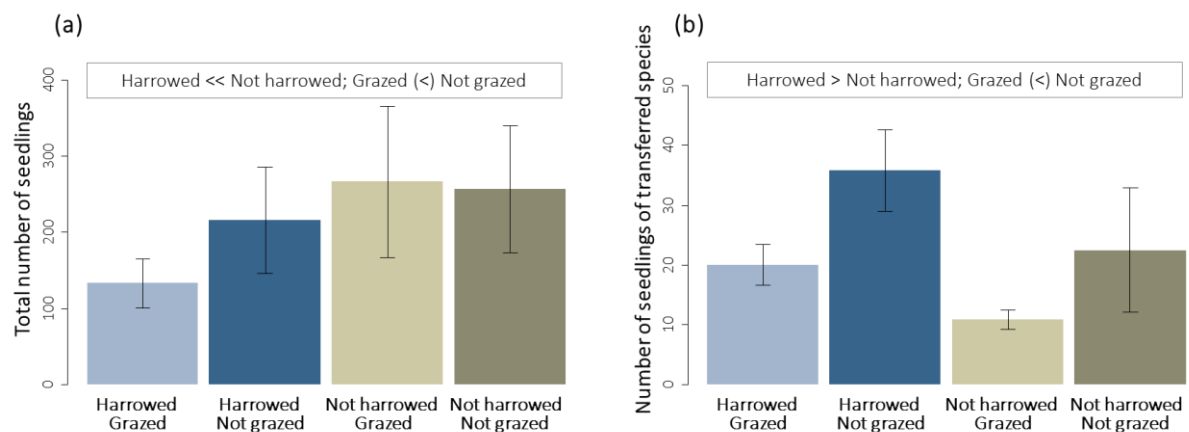


Fig. 1. Number of seedlings per m² of (a) all species, and of (b) transferred species only, in the four restoration treatments. The direction of main effects is indicated using "<<" (P<0.01), "<" (P<0.05), "<)" (P<0.1; marginally significant).

Plant species richness

The treatment effects on overall species richness were not (grazing) or marginally (increase when harrowed) significant (Table 1, Fig. 2a). The species richness of transferred species was 30% higher in the harrowed compared to the non-harrowed plots (Fig. 2b). Similarly to the abundance of transferred species, the richness of transferred species was reduced by grazing but the effect was only marginally significant. No significant grazing-by-soil preparation interaction was observed.

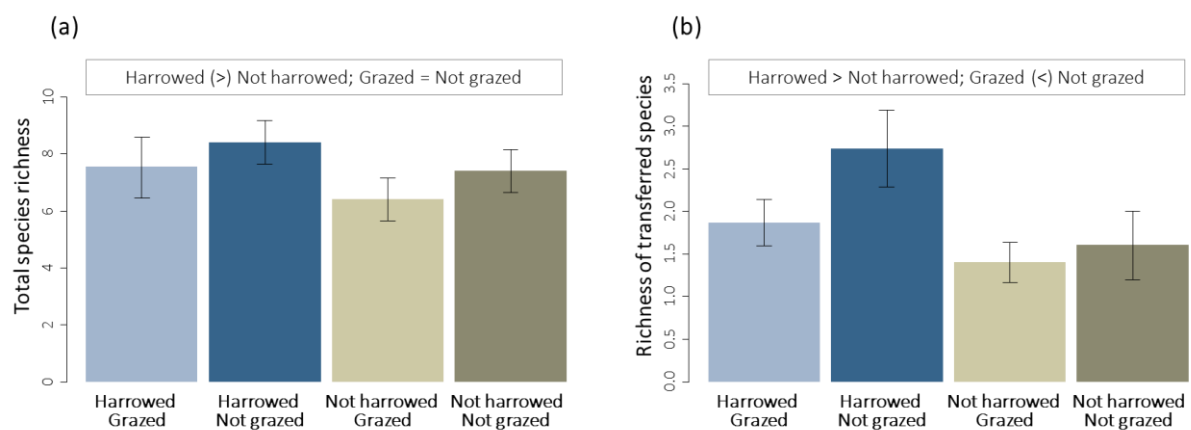


Fig. 2. (a) Species richness of seedlings of all species and of **(b)** transferred species in the four restoration treatments. The direction of main effects is indicated “<” (P<0.05), “(<)” (P<0.1; marginally significant) and “=” (not significantly different).

Plant species composition

The species composition was not clearly separated between treatments resulting in a large overlap of NMDS polygons (Fig. 3). Neither harrowing (P = 0.198), nor grazing (P = 0.392) nor

the grazing-by-soil preparation interaction ($P = 0.461$) were significant. The plots without soil preparation nor grazing showed higher variation in species composition resulting in a larger polygon. Independent of treatment, semi-dry grassland species such as *B. erecta*, *Saponaria ocymoides* L. and *Eryngium campestre* L. occurred in the upper part of the ordination plot, and ruderal species such as *Capsella bursa-pastoris* (L.) Medik., *Atriplex patula* L., *Anisantha sterilis* (L.) Nevski (former name: *Bromus sterilis*), *Veronica polita* Fr. and *Polygonum aviculare* L. in the lower right part.

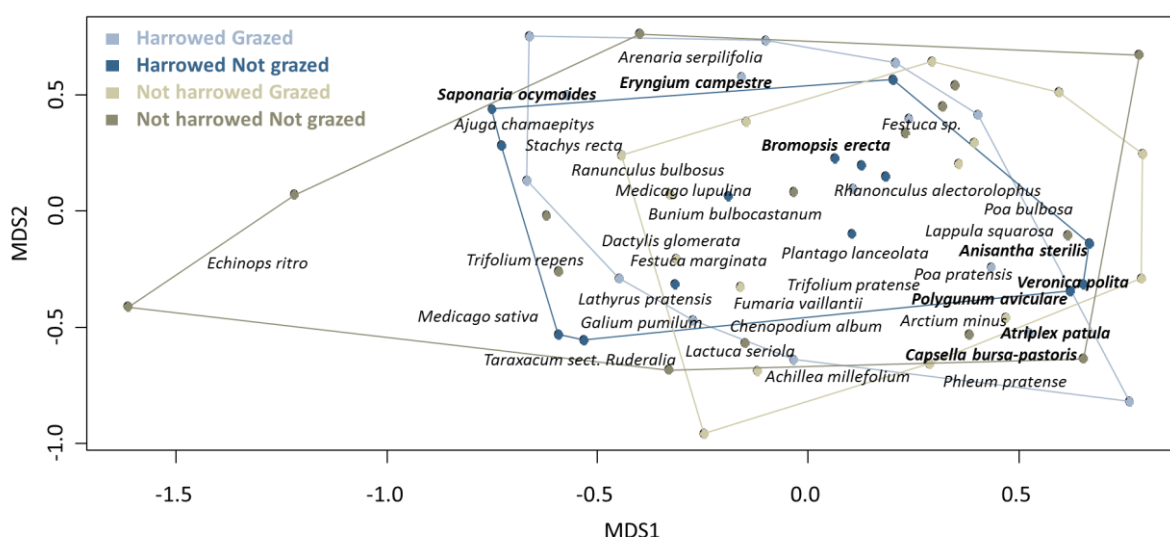


Fig. 3. Plant species composition in the four restoration treatments using NMDS. Polygons indicate the position of the outmost plots in each treatment (NMDS stress: 0.190).

Seedling survival of two focal species

Seedling survival of *B. erecta* was high in all treatments ($\geq 75\%$, Fig. 4a). The main effects of grazing and soil preparation were not significant, but a significant grazing-by-soil preparation

interaction indicated that the grazing effect depended on soil preparation (Table 1). Grazing reduced seedling survival only in non-harrowed plots. With few exceptions, *B. erecta* did not reach the reproductive stage in the first year. The survival rate of *R. alectorolophus* was low (<45%) compared to that of *B. erecta* (Fig. 4b). Similarly to *B. erecta*, no significant main effect was found for *R. alectorolophus* but a significant grazing-by-soil preparation interaction explained by a negative grazing effect in non-harrowed plots (Fig. 4b). Most surviving *R. alectorolophus* individuals of the un-grazed, non-harrowed plots were flowering in July, with a significant negative effect of grazing ($\chi^2 = 7.348$, $df = 1$, $P = 0.007$; Appendix S3). Few individuals reached the reproductive stage in the three other treatments types. Similarly to survival, the significant grazing-by-soil preparation interaction ($\chi^2 = 4.100$, $df = 1$, $P = 0.043$) was explained by a negative grazing effect in non-harrowed plots.

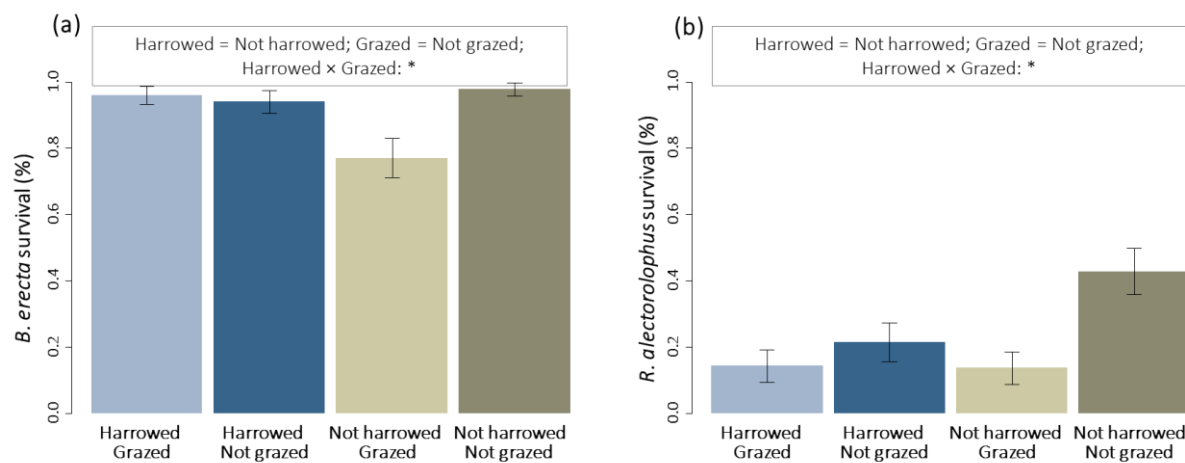


Fig. 4. Final seedling survival of (a) *Bromopsis erecta* and (b) *Rhinanthus alectorolophus*. The direction of main effects is indicated using "<" ($P < 0.05$) and "=" (not significantly different). Significant grazing-by-soil preparation interactions are presented using "*" ($P < 0.05$).

1 DISCUSSION

2 The low field emergence compared with greenhouse germination showed that seedling
3 recruitment may be a bottleneck in ecological restoration of grasslands (Öster *et al.*, 2009). In
4 our study on mountain grasslands, soil preparation largely increased the recruitment of
5 transferred species, whereas competition by spontaneously emerging ruderal species was
6 reduced. Grazing had only a weak negative effect on seedling density. While neither harrowing
7 nor grazing had an effect on focal species survival when applied alone, a significant interaction
8 effect indicated that the grazing effect depended on soil preparation or vice versa. The negative
9 grazing effect was generally stronger in non-harrowed plots. Despite significant grazing and
10 harrowing effects on seedling recruitment and early survival, none of the treatments influenced
11 the first-year plant community composition.

12 Several studies have demonstrated that soil preparation prior to seed addition increases
13 germination success and emergence of added seeds because it limits competition of pre-
14 existing vegetation (Edwards *et al.*, 2007; Bischoff *et al.*, 2018, Harvolk-Schöning *et al.*, 2020).
15 However, pre-existing vegetation or a mulch layer may also facilitate seedling recruitment
16 (Donath *et al.*, 2007; Scotton *et al.*, 2012). In particular in dry and open habitats, the absence
17 of vegetation cover increases drought stress reducing germination and seedling survival
18 (Callaway and Walker, 1997; Eckstein and Donath, 2005). Our restoration sites in the Upper
19 Durance valley clearly benefitted from previous harrowing indicating that the reduction of
20 competition and improved conditions for germination (Chambers, 2000) prevailed over the
21 reduction of facilitation effects. Similarly, Schmiede *et al.* (2012) and Harvolk-Schöning *et al.*
22 (2020) found that the cover of target species was higher following soil disturbance and seed
23 introduction in a floodplain grassland. To our knowledge, our study is the first showing a

1 simultaneous negative effect of soil preparation on spontaneously emerging species and a
2 positive effect on transferred species. This effect was observed shortly after construction work
3 leading to vegetation degradation and may be even stronger at restoration sites with higher
4 spontaneous vegetation cover (here only 28%). The reduced competition by already
5 established species created favourable microsite conditions for the recruitment of transferred
6 species. Soil tillage particularly reduced the abundance of ruderal, competitive species (*sensu*
7 Grime, 1988), such as *Anisantha sterilis* (L.) Nevski, *Capsella bursa-pastoris* (L.) Medik., *Atriplex*
8 *patula* L., and *Polygonum aviculare* L. Without soil disturbance, such competitive early
9 successional species hamper the establishment of the transferred late-successional species
10 (Donath *et al.*, 2007; Jaunatre *et al.*, 2014). Regardless of competition, harrowing has also been
11 shown to create favourable micro-reliefs improving the adhesion of the transferred seeds to
12 the soil and thus favouring recruitment and establishment (Isselin-Nondedeu *et al.*, 2006).
13 Chambers *et al.* (2000) showed that seed movements and seedling establishment clearly
14 depended on soil surface structure with holes limiting seed removal and increasing
15 germination. The quantity of removed seeds also depends on the number of transferred seeds
16 and their morphology (size, weight, shape). A better understanding of interactions between soil
17 preparation, seed adhesion to the soil and seed morphology may help to increase the
18 recruitment of transferred species and thus improve the efficiency of restoration measures
19 (Jakobsson and Eriksson, 2000).

20 Contrary to the soil treatment, the effect of grazing was not selective between
21 transferred species representing the target species of restoration, and non-target species.
22 Grazing had a small but generally negative effect on seedling recruitment. The negative grazing
23 effect was smaller than in other studies of the same geographical region but under
24 Mediterranean climate (Buisson *et al.*, 2015; Vidaller *et al.*, 2019b) in which the transferred

1 species clearly established better when grazing was excluded. Grazing animals negatively affect
2 seedling recruitment by trampling and later on by biomass removal. Trampling by livestock does
3 not only damage seedlings but also strongly affects the soil by erosion (Farrell and Fehmi, 2018)
4 or destabilization (Tasser *et al.*, 2003), particularly in mountain grassland slopes. On the other
5 hand, trampling may have a positive effect on seedling recruitment of transferred species, since
6 pre-existing vegetation is damaged by bruising, crushing, plant displacement or burial in mud
7 (Bilotta *et al.*, 2007), thus limiting competition with non-target species. Similar to soil
8 preparation, trampling may also create holes reducing seed removal of transferred species
9 (Isselin-Nondedeu *et al.*, 2006). Soil preparation and grazing may thus have a common positive
10 effect: the creation of microsites favourable to recruitment by limiting competition, increasing
11 seed adhesion and water retention (Isselin-Nondedeu *et al.*, 2006; Chambers, 2000). The
12 outcome of the trade-off between microsite creation and seedling damage depends on the
13 productivity of the sites and the timing of grazing. Biomass removal by grazing or mowing is
14 particularly required on highly productive sites where competitive grasses often hamper
15 seedling recruitment and establishment (Schmiede *et al.*, 2012; Kladvová and Münzbergová,
16 2016). Nutrient-poor sites rather need protection of seedlings against grazing than biomass
17 removal since competition is much lower (Kirmer *et al.*, 2012; Scotton *et al.*, 2012; Vidaller *et*
18 *al.*, 2019a, 2019b). The marginally significant negative grazing effect in our study may thus be
19 explained by the intermediate productivity level of our restoration sites. Post-pasture sowing
20 has been cited as a method to optimise timing, because it allows seedlings to develop before
21 the next grazing, reducing trampling and herbivory at the most vulnerable stage (Eichberg and
22 Donath, 2018). Our study sites were first grazed before seedling emergence, 2-3 weeks after
23 brush material transfer, and the following grazing period occurred seven months later. Thus,
24 the first grazing probably had a positive effect in reducing competition by pre-existing

1 vegetation, but the second grazing period may have negatively affected young seedlings that
2 predominantly emerged in spring, finally resulting in a slightly negative effect of grazing. The
3 vegetation period usually starts after snow melt in March resulting in a low probability to attain
4 maturity before the June grazing period. The timing of grazing needs to be taken into account
5 to limit the damage on seedlings, particularly if grazing exclusion is not possible. Livestock type
6 may influence grazing effects (Tóth *et al.*, 2016). In our study, we focused on an overall grazing
7 effect including sheep and cattle, and the grazer species effect was pooled with experimental
8 site. It may be interesting for future studies to distinguish effects of cattle and sheep grazing.

9 The analysis of seedling survival of two focal species confirmed these conclusions. The
10 grazing effect was small and limited to plots without soil preparation. The vegetation of these
11 non-harrowed plots was denser and thus more attractive for grazers explaining the higher
12 grazing effect. Grazing livestock prefers dense vegetation with higher food resource over sparse
13 vegetation resulting in a higher biomass removal (Török *et al.*, 2014; Meuret and Provenza
14 2015). *B. erecta* showed a generally high survival rate after three months suggesting that the
15 species, characteristic of semi-dry soils and semi-natural sites, is very well adapted to the pedo-
16 climatic conditions of our study zone and resistant to grazing (Calaciura and Spinelli, 2008).
17 *Rhinanthus alectorolophus* showed, however, a much lower survival since the abundance of
18 potential host plants was low during the first year of restoration. The species is a hemi-parasite
19 and its performance depends on the availability and performance of host plants (Matthies and
20 Egli, 1999; Bullock *et al.*, 2003). It cannot establish under low vegetation cover and its
21 population may go locally extinct as it is an annual species whose year-to-year survival relies on
22 the production of seeds and seedling recruitment (Coulson *et al.*, 2001; Bullock *et al.*, 2003).
23 Whereas the grazing effect on the survival of *R. alectorolophus* was not significant, the effect on

flower production was negative. The result suggests that the species may survive grazing, but grazing damage prevent species from flowering.

The differential effects of soil preparation and grazing on spontaneous and transferred species did not result in a change in first-year plant species composition. The abundance of spontaneously emerging species was between six and ten times higher than that of transferred species, explaining that the observed significant soil treatment effect on transferred species was not yet visible at community level. The soil of disturbed sites generally contains high numbers of seeds of annuals and/or ruderal species (Bischoff, 2002; Donath *et al.*, 2007). These species often dominate the vegetation of early successional stages before being replaced by typical grassland species that are usually perennials developing later in the succession (Kiehl *et al.*, 2010; Valkó *et al.*, 2016; Kiss *et al.*, 2020). The first year after the restoration of Eastern European grasslands, Lengyel *et al.* (2012) observed that annual weeds were dominant, but the cover of those weeds decreased dramatically after the third year due to the growth of perennial grasses. The authors observed an increase in the cover of target species from the first to the fourth year of restoration. A similar low initial restoration treatment effect due to initial low establishment of transferred target species and a subsequent high long-term restoration success (eight years) is known for riparian mesophilous grasslands (Auestad *et al.*, 2016; Bischoff *et al.*, 2018). Monitoring the plant community over several years will be necessary in order to evaluate whether the observed initial differences in seedling recruitment and early survival significantly influence plant species composition and restoration success in the long run.

CONCLUSION

Most species of the donor site were found at the restoration sites indicating that the applied brush harvesting technique was appropriate and allowed testing soil preparation and grazing effects. Similarly to studies in mesophilous grasslands (Edwards *et al.*, 2007, Bischoff *et al.*, 2018), soil preparation had a clearly positive effect on the seedling recruitment. The reduction in overall seedling density dominated by spontaneously emerging species demonstrated that the reduction in competition was the principal mechanism explaining the positive effect of previous soil tillage by harrowing on the recruitment of transferred species (Edwards *et al.*, 2007; Schmiede *et al.*, 2012). We thus recommend soil preparation before seed-based ecological restoration even in semi-dry grasslands to improve seedling recruitment of transferred target species. Our study did not confirm a strong negative effect of grazing on seedling recruitment found by several other authors (Scotton *et al.*, 2012; Vidaller *et al.*, 2019b). The marginally significant negative effect of extensive grazing may be tolerated if farmers are reluctant in putting up fences as in our study area. Further research is needed on the timing of sowing relative to grazing periods, in order to benefit from positive grazing effects by reduction of competition while avoiding seedling damage.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at
the end of the article.

Appendix S1. Photos of the two focal species.

Appendix S2. Seedling emergence *ex-situ* and *in-situ*.

Appendix S3. Reproductive individuals of *Rhinanthus alectorolophus*.