

# Mountain grassland restoration using hay and brush material transfer combined with temporary wheat cover

Aure Durbecq, Léo Rocher, Renaud Jaunatre, Alice Dupré La Tour, Elise

Buisson, Armin Bischoff

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1	Mountain grassland	restoration using	g hay and br	ush material t	transfer combined	l with temporary
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- 2 wheat cover
- \*Aure Durbecq<sup>1,2</sup>, Léo Rocher<sup>1</sup>, Renaud Jaunatre<sup>3</sup>, Alice Dupré la Tour<sup>3</sup>, Elise Buisson<sup>1</sup>, Armin
  Bischoff<sup>1</sup>
- 5
- 6 <sup>1</sup>Mediterranean Institute of Biodiversity and Ecology (IMBE), Avignon University, CNRS, IRD, Aix
- 7 Marseille University, Avignon, FR
- 8 <sup>2</sup> Environmental consultancy ECO-MED, Marseille, FR
- 9 <sup>3</sup> Univ. Grenoble Alpes, INRAE, LESSEM, F-38402 St-Martin-d'Hères, FR

- 11 Correspondence: Aure Durbecq, IMBE, Avignon University, CNRS, IRD, Aix Marseille University,
- 12 IUT, Avignon, FR.
- 13 Email: aure.durbecq@gmail.com
- 14
- 15 Short title: Restoring mountain grasslands by propagule transfer
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## 21 Abstract

22 Mountain grassland restoration success may be hampered by limited seed dispersal and poor soil seed banks of many grassland species. These constraints can be overcome by actively 23 introducing propagules from nearby non-degraded communities. We tested different 24 25 restoration techniques in order to understand the mechanisms favouring target species 26 seedling recruitment and establishment. In five degraded mountain grasslands, we analysed (i) 27 the effect of two techniques increasingly used in ecological restoration to overcome low seed dispersal: transfer of brush-harvested seed material and hay transfer, and (ii) the potentially 28 facilitative effect of a temporary plant cover (common wheat) on the recruitment of transferred 29 brush-harvested propagules. We found that both propagule transfer techniques were 30 successful in establishing plant species of the donor community with an increase of plant 31 32 species richness, cover and abundance of transferred species. Hay transfer was more efficient in transferring species of the donor grassland than brush-harvested material transfer. Brush-33 harvested material transfer only increased abundance and cover of donor grassland species 34 when sown together with wheat. The results indicated that hay mulch favoured seedling 35 36 recruitment of target species, and that propagule transfer without hay mulch needs to be 37 compensated by additional temporary plant cover in order to create favourable conditions for seedling recruitment. A comparison with best reference communities for each restoration 38 39 grassland confirmed that hay transfer and brush material transfer with wheat sowing were successful in driving plant community composition towards the desired reference state. In 40 conclusion, restoration of mountain grasslands with shallow and stony soils clearly benefits 41 42 from a facilitative effect of dead (hay) or living (wheat) vegetation cover.

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45	Keywords
46	Semi-natural montane grasslands, Seedling recruitment, Nurse crop, Erosion control, Mulch
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# 64 1. Introduction

Grassland ecosystems represent 25% of terrestrial biomes (<u>Blair et al., 2014</u>; <u>Wilsey, 2020</u>) and
are essential habitats for the conservation of biodiversity and associated ecosystem services
(<u>Stoate et al., 2009</u>; <u>Amiaud & Carrère, 2012</u>; <u>Valkó et al., 2016</u>). Since the 1980ies, the
conservation of species-rich semi-natural grasslands has become a major concern in Europe.
Most European semi-natural grasslands were integrated in the habitat management Natura
2000 network in order to stop habitat degradation and to re-establish grassland species
diversity using ecological restoration approaches (<u>Wilsey, 2020</u>).

72 Successful grassland restoration may be primarily hampered by limited dispersal capacity and/or poor soil seed banks of many grassland species, and by a grazing management 73 favouring clonal reproduction over sexual reproduction (Halassy et al., 2016; Török et al., 2018). 74 75 These constraints are well documented and can be overcome by actively introducing 76 propagules (Kiehl et al., 2010; Scotton et al., 2012; Hölzel et al., 2012). The use of native plant species of local origin is recommended to assure adaption to local environmental conditions 77 and to maintain genetic diversity (Vander Mijnsbrugge et al., 2010; Bucharova et al., 2019). 78 Consequently, transferring local seeds from nearby non-degraded communities is increasingly 79 used in ecological restoration (Scotton, 2019). Diverse techniques are currently applied 80 81 depending on specific environmental conditions at restoration and donor sites (Kiehl et al., 82 2010; Hedberg & Kotowski, 2010). The increasing number of studies illustrates the scientific interest in comparing restoration effectiveness of various seed harvesting and transfer 83 techniques (Scotton & Ševčíková, 2017), such as hay transfer vs. sowing of seed mixtures 84 (Auestad et al., 2015; Kövendi-Jakó et al., 2019), brush material vs. green hay transfer (Albert 85

86 et al., 2019), or sowing combined with various additional treatments (Török et al. 2012; Pawluśkiewicz et al., 2019; Havrilla et al., 2020). 87

In our study, we compared the restoration effectiveness of two mechanical harvesting 88 techniques increasingly used in mountain grassland restoration: the transfer of brush-89 harvested seed material and the transfer of dry hay both collected from the same donor site 90 91 located close to the degraded grasslands. The brush harvester strips and aspirates the plant 92 material without cutting (Scotton et al., 2012). The harvested brush material comprises seeds 93 but also vegetative parts of fruits and, to a lesser degree, other vegetative material. Lowgrowing and less abundant species may be under-represented in this material (Edwards et al., 94 2007; Scotton et al., 2009; Wagner et al., 2020), but brush harvesting allows concentrating 95 seeds thus reducing humidity and facilitating storage. It also reduces the mulch layer that may 96 97 hamper germination (Mollard et al., 2014). Dry hay transfer involves cutting of plant material close to the soil surface, drying it on the field before storage and spreading it on the restoration 98 site. The higher vegetative biomass compared with brush material may hamper germination 99 100 but protects seedlings against high solar radiation, drought and soil erosion (Eckstein & Donath, 2005; Graiss & Krautzer, 2011; Havrilla et al., 2020).

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102 As seedling recruitment and establishment are important constraints in semi-natural grassland restoration (Öster et al., 2009), we also tested whether simultaneous transfer of 103 104 brush material and sowing of an annual plant species may compensate for the absence of a 105 protecting mulch layer. Such annuals provide temporary living cover that may facilitate seedling 106 recruitment and establishment (Padilla et al., 2006; Wright et al., 2014). Similarly to hay mulch, 107 a living cover may, however, increase root and light competition (Donath et al., 2006). We 108 tested a mix of two lowland varieties of common wheat Triticum aestivum as a facilitative 109 species. These varieties were expected to disappear within a couple of years since climatic conditions of our mountain study sites limit their seed production and self-sowing. *Triticum*cover may reduce soil erosion, an important criterion for the functionality of restoration
measures. Such soil stabilisation and erosion control are particularly important in mountain
grasslands on slopes facing a high risk of soil loss and propagule run-off (<u>Huc et al., 2018;</u>
Scotton, 2019).

115 In order to understand the underlying mechanisms of seedling recruitment and 116 establishment, we analysed first-year seedling abundance and second-year cover, respectively. 117 To determine which technique is the most appropriate in mountain grassland restoration, we addressed the following questions: (1) Which method of plant material transfer is most 118 119 successful in establishing species transferred from the donor community? (2) Does sowing of 120 wheat improve the seedling establishment of brush material? (3) Do transfer techniques and 121 wheat sowing increase the similarity between restored and reference communities? (4) What is the influence of these treatments on soil erosion? 122

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#### 125 2. Material and methods

126 *2.1. Study area* 

The study area was the upper Durance valley in the Southern French Alps. The five selected restoration sites were degraded by the construction of a high-voltage transmission line involving the creation of access tracks and working platforms. The construction work locally destroyed the vegetation and affected the soil structure. The study zone extends over about 100 km, from L'Argentière-la-Bessée in the north to La-Bâtie-Neuve in the south (44°78'78''N, 6°59''41'E; 44°57'93''N, 6°20''77'E). The study sites are located on slopes above the Durance valley, at an elevation of 1060 m to 1320 m above sea level. They are characterised by a

134 subcontinental climate, with an average annual temperature of 8.7°C and an annual rainfall of 740 mm (Embrun meteorological station, annual temperature corrected for an altitudinal 135 difference of roughly 2°C). Dry to mesophilic grasslands occur at sites managed by grazing or 136 mowing. Most of these surrounding non-degraded grasslands are priority habitats of Natura 137 2000: "Semi-natural dry grasslands of Festuco-Brometalia and scrubland facies on limestone" 138 139 (N6210, European habitat directive 92/43/EEC; Calaciura & Spinelli, 2008). The five restoration sites used for the experiments were former access tracks and working platforms, and 140 141 restoration goal was the re-establishment of typical Festuco-Brometalia grasslands. Traditional 142 management by grazing was re-introduced.

143

#### 144 2.2. Donor and reference sites

145 One typical non-degraded Mesobromion grassland of the study area was chosen as donor site 146 for harvesting plant material. The site was located in Freissinières (44°73'61"N; 6°56'72"E) at 147 an elevation of 1100 m and a 3° south-west-facing slope. Two harvest techniques were applied, brush harvesting and traditional haymaking. The brush material was harvested on 13 July 2018 148 using a brush harvester mounted on a quad and equipped with an integrated vacuum system. 149 On a surface of 4000 m<sup>2</sup>, 2.6 kg of brush material was obtained. The harvested material 150 151 comprised seeds (60 % of total mass) and vegetative parts (40 %). Seeds were counted in 10 152 subsamples of 0.5 g harvested material revealing an average density of 887 seeds/g. Hay was 153 cut at 15 July 2018 on an additional area of 800 m<sup>2</sup> at the same donor site using a rotary mower. 154 Harvest conditions were dry and warm without rainfall during the previous days. The hay was dried for two days on the harvested grassland and was baled in five bales of 7.5 kg ( $45 \times 30 \times$ 155 75 cm) for a total of 37.5 kg, with approximately 1 % of seeds in the total raw material. Brush 156

157 material and hay were kept for three months at room temperature under dry and dark158 conditions.

159 Although the donor site reflected the medium environmental conditions of the five restoration sites we identified "best references" for each site to evaluate restoration success. 160 Since best reference sites are not always the closest ones (Durbecq et al., 2020), we surveyed 161 162 eighteen non-degraded grasslands along the new electricity line. These potential references included the donor site and were located at the same altitudinal belt as restoration sites (1000 163 - 1400 m). Soil and microclimatic conditions measured at restoration sites and potential 164 reference communities were used to identify the best references of each restoration site 165 according to Durbecq et al., (2020, methodology). 166

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#### 168 2.3. Experimental design

At the five restoration sites, we repeated the following five treatments in 4 m × 4 m plots: control without hay or brush material transfer ('Control'), hay transfer ('Hay'), brush material transfer ('Seed'), wheat sowing ('Triticum') and both brush material and wheat seed addition together ('Triticum + Seed'). The restoration sites that were already colonized by spontaneous vegetation (mainly *Chenopodium album* L. and *Atriplex patula* L.) were mown before the setup of the experiments and they were then extensively grazed in spring and autumn. The position of the five treatments was randomised within sites.

In October 2018, 6.5 g/m<sup>2</sup> of harvested brush material was spread in the brush material
treatments, corresponding to 3.75 g/m<sup>2</sup> of seed and 5765 seeds/m<sup>2</sup>. The chosen seed density
is a little higher than recommended for lowland grasslands and lower than usually sown in
alpine grasslands (<u>Kiehl et al., 2010; Scotton et al., 2012; Koch et al., 2015</u>). The brush material

180 was pressed to the ground using a lawn roller. In the hay transfer treatment, a bale of hay of 7.5 kg was spread (0.5 kg/m<sup>2</sup>) on each plot. The amount of hay was adjusted to an estimated 181 seed content of 1% (Scotton et al., 2012) corresponding to roughly 5 g/m<sup>2</sup> of seeds. The hay 182 was pressed to the ground by the wheels of tractor in order to maintain hay on the ground. The 183 184 sown wheat treatment was a mixture of two local lowland winter wheat (*Triticum aestivum*) 185 varieties, "Meunier d'Apt" and "Saissette de Provence". These early lowland varieties are not 186 well adapted to high altitude resulting in low seed production and a rapid decline during 187 mountain grassland succession. The seed density was adjusted to 9 g/m<sup>2</sup>. Like for the brush material transfer, a lawn roller was used to improve seed adhesion to the ground. Controls 188 were not sown but they were also rolled. 189

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#### 191 *2.4. Data collection*

#### **192** *2.4.1. Vegetation surveys in donor and reference grasslands*

193 In the donor grassland, vegetation surveys were conducted on 29 June 2018, and the 194 occurrence of mature seeds was evaluated for each plant species at the harvest date. Surveys were used to establish a list of 37 potentially transferred species (hereafter: transferred 195 species). The vegetation surveys of the eighteen potential reference sites were conducted 196 197 between 21 June and 5 July 2018 by visual estimation of plant cover for each species in quadrats of 2 m  $\times$  2 m. In the donor grassland, five quadrats were sampled, and three in each of the 198 199 other potential reference grasslands. Additionally, we monitored seed maturity in each plant 200 species at the day of harvest.

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202 2.4.2. Germination test in a greenhouse

In November 2018, brush material and hay were spread in an unheated greenhouse to evaluate
and compare the germination potential of seeds in brush material and hay. Harvested brush
material and dry hay were sown to ten trays (0.03 m<sup>2</sup>) filled with standard potting soil substrate
(mixture of 1/3 vermiculite and 2/3 of sterile peat). Five trays received 0.54 g of brush material
corresponding to 18g/m<sup>2</sup> of seeds, and five trays received 12.5 g of hay corresponding to 4
g/m<sup>2</sup> of seed material according to an estimated seed content of 1%. The trays were regularly
watered, and germinations identified and removed until April 2019.

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#### 211 2.4.3. Seedling abundance and vegetation surveys in grasslands under restoration

212 To measure seedling abundance, plant number was counted in three quadrats of  $40 \times$ 40 cm placed along the diagonal of each plot in June 2019, at the five restoration sites. Since it 213 214 was not possible to distinguish seedlings and plants re-sprouting from belowground organs, the 215 seedling counts may include stems or ramets of vegetative regeneration. We considered all 216 reproducing plant species of the donor site as transferred although some of them also occurred in the control plots of sites under restoration, indicating their presence in the soil seed bank or 217 218 seed rain. In June 2020, the cover of plant species was measured following the same protocol as in the potential reference grasslands: in each plot, one quadrat of 2 m × 2 m was sampled. 219

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#### 221 2.4.4. Erosion monitoring

To evaluate the effect of the different treatments on soil erosion, we measured erosion according to Feret & Sarrailh (2005) at four restoration sites (at one site, the permanent points were accidentally removed in autumn 2019 not allowing any analysis). The device comprised a

225 1.5 m long aluminium bar and two 0.5 m long iron poles fixed in the ground of each plot. These 226 iron poles were the permanent points of the measurements. They were adjusted to provide a 227 horizontal support for the aluminium bar before measurements. Vertical holes were drilled in the centre of the aluminium bar every 10 cm resulting in a total of 13 regularly spaced holes 228 (Fig. S1). To take the erosion measurements, a graduated rod was introduced in each of the 229 230 holes. The scale of the graduated rods allowed a direct measurement of the space between the 231 aluminium bar and the ground. An increase in space between measurements indicates erosion 232 whereas a decrease suggests an accumulation of soil. Measurements were taken in June 2019, October 2019, June 2020 and September 2020, in order to assess the effect of restoration 233 234 treatments on soil erosion.

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#### 236 2.5. Data analysis

237 We used plant abundance, cover and richness of the entire plant community and of 238 transferred species to run statistical tests and to calculate restoration indices. Major soil and microclimatic variables of restoration sites and potential reference sites were fitted to 239 240 multivariate models (NMDS) to identify the best references of each restoration site. The identification was based on dissimilarity coefficients using Euclidean distances and is detailed 241 242 in Durbecq et al., (2020). We compared restoration sites and these best reference sites using 243 the restoration indices CSII<sub>norm</sub> (normalized Community Structure Integrity Index) and HAI 244 (Higher Abundance Index). The CSII allows measuring which proportion of the species 245 abundance of the reference communities is represented in the restored communities, and the HAI evaluates the proportions of the species abundance in the restored communities that are 246 higher than in the reference communities (Jaunatre et al., 2013). 247

248 We ran linear and generalized mixed models with transfer treatments as fixed effect 249 and site as random effect to analyse response variables (including restoration indices) to the 250 restoration treatments. In the analyses of plant abundance, guadrat within treatment was additionally included as a random effect. Plant cover, species richness and restoration indices 251 were normally distributed and thus analysed using a Gaussian distribution with identity link. 252 253 Plant abundance was fitted using a Poisson distribution with log-link function. In the case of a significant treatment effect, pairwise comparisons were used to compare differences between 254 255 transfer techniques (glht function in multcomp package; Hothorn et al., 2008).

A NMDS (Non-Metric Multidimensional Scaling) based on Bray–Curtis similarity was applied to compare plant species composition of the five restoration sites and their best reference sites. A Permutational Multivariate Analysis of Variance (PERMANOVA) was used to analyse whether the community composition was significantly different between treatments (R package "vegan"). All analyses were performed using R 3.5.3 (<u>R Core Team 2019</u>).

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263 **3. Results** 

The greenhouse tests revealed a mean density of 197.4 viable seeds/g in the brush harvest material and 2.4 viable seeds/g in the hay. This corresponds to a comparable density of 1283 viable seeds per m<sup>2</sup> and 1200 viable seeds per m<sup>2</sup> transferred with brush material and hay to the restoration sites, respectively.

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269 3.1. Effects of transfer techniques and wheat sowing on target species establishment

Seedling emergence of transferred species was only 50/m<sup>2</sup> (without wheat) to 65/m<sup>2</sup> (with 270 wheat) in the brush material transfer and 200/m<sup>2</sup> in the hay transfer corresponding to 4 %, 5 271 %, and 17 % of the number of transferred viable seeds, respectively. Transferred species 272 abundance was significantly different between the restoration treatments ( $\chi^2$  = 51.85, df = 4, P 273 < 0.001; Fig. 1). We found the clearly highest density of transferred species in the 'Hay' 274 275 treatment although seed density was similar to brush material according to germination tests. The 'Seed' treatment involving brush material transfer without wheat addition was neither 276 significantly different to the control nor to the 'Triticum' treatment. Transferred seedling 277 abundance in the combined 'Triticum + Seed' treatment was significantly higher than in the 278 control but lower than in the 'Hay' treatment. 279



Fig. 1. Effect of seed transfer techniques on the abundance of transferred species estimated in quadrats of 40  $\times$  40 cm in 2019. Error bars represent ±SE and different letters indicate significant differences between treatments (P < 0.05).

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The treatments also had a significant effect on the second-year cover of transferred species ( $\chi^2 = 16.28$ , df = 4, P = 0.002; Fig 2.a). However, the strongly positive effect of the 'Hay' treatment vanished and the difference to the control was not significant any more. Transferred species cover was significantly higher in the combined 'Triticum + Seed' treatment than in the 'Seed' and 'Triticum' treatments whereas all other treatments were not significantly different.

Similarly to transferred species cover (Fig. 2.a), richness of transferred species varied 290 significantly between treatments ( $\chi^2$  = 19.65, df = 4, P < 0.001; Fig. 2.b). The 'Seed' treatment 291 was again only significantly different from the control if combined with wheat sowing ('Triticum 292 + Seed'). Contrary to transferred species cover but in line with first-year abundance, species 293 richness was significantly higher in the 'Hay' treatment than in the control. The 'Hay' and the 294 combined 'Triticum + Seed' treatments comprised on average seven more transferred species 295 296 (± 3 species) than the control. The 'Seed' treatment without wheat sowing was not significantly 297 different from the control or the 'Triticum' treatment. Similar treatment effects were visible for the total species richness (Fig. S2). However, only the difference between the combined 298 299 'Triticum + Seed' treatment and the control was significant.



Fig. 2. Effect of seed transfer techniques on (a) transferred species cover, and (b) transferred
 species richness, both based on 2020 cover values and estimated in quadrats of 2 × 2 m. Error
 bars represent ±SE and different letters indicate significant differences between treatments (P
 < 0.05).</li>

# 307 3.2. Comparison with best references of each restoration site

308 The plant species composition of the restoration sites was very different from that of reference sites in all treatments (Fig. 3). Restoration and reference sites were particularly 309 310 separated on the first axis. This axis represented a disturbance gradient with annual and ruderal species occurring on the left together with restoration sites (Arctium minus (Hill) Bernh., 311 Polygonum aviculare L., Anisantha sterilis (L.) Nevski, etc.) and perennial grassland species on 312 the right together with reference sites (Stipa pennata L., Helianthemum nummularium (L.) Mill., 313 Pilosella officinarum F.W.Schultz & Sch.Bip., etc.) . The differences between reference sites 314 reflect the heterogeneity of plant communities according to different environmental conditions 315 that are independent of degradation. They were particularly separated on the second NMDS 316

317 axis representing a productivity gradient from stony, xeric sites in the lower part of the biplot to more humid, mesophilic sites in the upper part. There was a large overlap between the five 318 319 treatments within restoration sites, but the treatment effect was still significant (F = 1.432, df = 4, P = 0.012). As expected, the unsown control showed the greatest distance to the 320 references. The 'Hay' and 'Triticum + Seed' treatments were closest to the reference sites, 321 322 followed by the 'Seed' treatment without wheat. However, the latter treatment was not much closer to the reference communities than wheat sowing alone. The 'Triticum' treatment 323 324 showed the highest variation between sites resulting in a large NMDS polygon.



Fig. 3. NMDS of plant species composition in the five transfer technique treatments compared
to plant species composition in the best reference for each restoration site previously identified
in Durbecq et al., (2020). Polygons indicate the position of the outmost plots in each treatment
(stress = 0.20). Different symbols indicate the five different restoration sites and corresponding

references: • and • for sites corresponding to reference n°16 (same reference for the two
restoration sites), ■ to reference n°12, \* to reference n°6, and ▲ to reference n°9.

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The CSII<sub>norm</sub> calculated for each restoration site compared with their respective best reference showed a significant difference between treatments ( $\chi^2 = 18.02$ , df = 4, P < 0.01; Fig. 4.a and 5). CSII<sub>norm</sub> was significantly higher in the combined 'Triticum + Seed' treatment than in the control, the 'Triticum' and the 'Seed' treatments. The 'Hay' treatment was not significantly different from other treatments.

The HAI was also significantly different between treatments ( $\chi^2 = 11.44$ , df = 4, P < 0.05, Fig. 4.b and 5). The lowest HAI was found in the 'Seed' treatment being significantly lower than the control and the 'Triticum' treatment, indicating significantly lower non-target abundances in the 'Seed' treatment. Differences between other treatments were not significant.



Fig. 4. (a) CSII<sub>norm</sub> and (b) HAI, comparing the plant communities of transfer technique
treatments of each restoration site with their respective best reference site. Error bars

347 represent ±SE and different letters indicate significant differences between treatments (P <</li>
348 0.05).

350	The significant differences in $CSII_{norm}$ were explained by a higher number of target
351	species in the 'Hay' and in the combined 'Triticum + Seed' treatments compared to the other
352	treatments. Target species were those occurring in the best references such as Plantago media
353	L., Festuca cinerea Vill., Teucrium chamaedrys L., Poa pratensis L., Hippocrepis comosa L., Lotus
354	corniculatus L., Silene vulgaris (Moench) Garcke, Eryngium campestre L., Poterium sanguisorba
355	L. and Trifolium pratense L. (Fig. 5). In the 'Hay' and the 'Triticum + Seed' treatments, the cover
356	of several target species was even higher than in the references, (e.g. Poterium sanguisorba L.,
357	Medicago lupulina L., Trifolium repens L., Trifolium pratense L., Convolvulus arvensis L.).
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370 Fig. 5. Mean target plant cover of abundant species (> 9 samples, 41 of 259 species in total) in restoration treatments and the best reference community. Different colours represent the 371 372 mean cover proportion in references (*black*), the mean cover proportion in communities under restoration up to the mean cover in the reference communities (green), and the mean cover 373 proportion exceeding that of the reference communities (orange). Asterisks indicate 374 375 transferred species. For readability, the x-axis is limited to 10 % cover (only Bromopsis erecta (Huds.) Fourr., Schedonorus arundinaceus (Schreb.) Dumort. and Galium molugo agg. 376 exceeded). 377

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# 380 *3.3. Ecosystem function: erosion control*

Erosion monitoring did not show significant treatment effects at the end of the observation period. Lowest erosion was observed in the 'Hay' treatment but due to high between-site variation the difference to other treatments was not significant (Fig. 6). Intermediate measurements demonstrated a high temporal variability of erosion and of treatment effects on erosion (Fig. S3).



Fig.6. Effect of seed transfer techniques on soil erosion. Error bars represent ±SE. Positive
values show higher erosion, thus a lesser amount of material on the ground 14 months after
the beginning of the experiment.

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#### 393 4. Discussion

Both techniques, brush-harvested propagule transfer and hay transfer, allowed the 394 introduction of high numbers of viable seeds. However, first-year seedling recruitment was 395 396 much higher in hay transfer than in brush material transfer. Additional wheat sowing had a 397 particularly positive effect on seedling establishment from brush material resulting in a second-398 year cover of transferred species similar to that of hay. Brush material transfer alone did neither increase transferred species cover nor plant species richness. According to transferred species 399 abundance and cover, hay transfer and brush material transfer combined with wheat sowing 400 were also most successful in restoring the plant species composition of the reference. Soil 401 erosion was not affected by any restoration treatments. 402

### 404 4.1. Effect of seed-addition techniques on transferred species recruitment

Brush harvesting has many technical advantages compared to hay cutting allowing selection 405 406 and cleaning of seeds, drying and particularly easy storage (lower volume than hay) under optimum temperature and humidity (Edwards et al., 2007; Vitis et al., 2020; Frischie et al., 407 2020). Contrary to hay cutting, it is possible to brush-harvest several times a season thus 408 409 maximizing the number of transferred species (Edwards et al., 2007; Scotton & Ševčíková, 410 2017). The possibility to use grasslands for hay making after brush harvesting is another advantage reducing losses for livestock farmers. However, several studies obtained a low 411 efficiency in plant establishment from brush material compared to hay transfer (Edwards et al., 412 413 2007; Sengl et al., 2017; Albert et al., 2019). Albert et al., (2019) explained the lower seed 414 number per harvested area by the higher working height of brushes compared to mowing 415 machinery missing the seeds of low-growing species.

In our study, the brush material contained as many viable seeds as the hay suggesting that 416 the low recruitment compared to hay transfer was due to lower germination and seedling 417 418 survival. The mulch layer resulting from hay transfer may improve seedling recruitment of 419 transferred species through an increase in soil moisture retention (Donath et al., 2007; Mollard 420 et al., 2014; Havrilla et al., 2020). Soil moisture retention and temperature buffering are 421 important factors influencing germination in our study region characterized by shallow soils, 422 spring frosts and high solar radiation. Furthermore, hay mulch protects seeds from predation, 423 and animal trampling (Scotton et al., 2012; Vanderburg et al., 2020) and limits competition of ruderal species in early stages of grassland restoration (Kiehl & Wagner, 2006). Additionally, a 424 hay layer may reduce surface runoff (Graiss & Krautzer, 2011) and improve seed adherence to 425

426 the soil (Chambers, 2000; Havrilla et al., 2020). High recruitment in the hay transfer treatment suggests that such positive effects prevailed in our study over negative effects of a mulch layer 427 on transferred species establishment, such as light limitation (Eckstein & Donath, 2005). Such 428 mulch effects are highly species-specific (Sonkoly et al., 2020), and in our study the mulch layer 429 had most likely an indirect positive effect on target species by having a direct negative effect 430 431 on ruderal species. Furthermore, a mulch layer may stronger limit light availability of seeds in the soil (spontaneous, mostly ruderal species) than of seeds of transferred species that remain 432 433 at the soil surface or even in upper mulch layers.

Effects of temporary wheat cover on seedling recruitment may be similar to hay mulch 434 435 effects, such as protection against drought and solar radiation (Wright et al., 2014). Additionally, belowground effects may facilitate seedling recruitment and establishment since 436 437 wheat roots stabilize the soil and foster the recolonization of the soil fauna (Faivre 2000). In modifying soil structure and composition (Pohl et al., 2009; Liu et al., 2018), wheat can be 438 considered as a nurse crop (Padilla et al., 2006). Many high-altitude restoration studies aimed 439 440 at improving recruitment conditions and reducing soil erosion using nurse species or wheat straw addition to stabilize the soil (Graiss & Krautzer, 2011; Kavian et al., 2018; Scotton, 2019). 441 442 However, propagule run-off, germination and early survival are less likely affected by wheat sowing than by hay mulch since wheat cover develops later. In our study, this was probably the 443 reason for an absence of a wheat sowing effect on first-year abundance of species transferred 444 with brush material whereas the effect on second-year cover was strongly positive. Like hay 445 transfer, facilitative effects of wheat sowing prevailed over negative effects of competition for 446 447 light and water observed in other studies on additional sowing (Donath et al., 2006; Wright et 448 al., 2014).

449 Soil preparation before sowing has often been recommended to improve seedling recruitment in sowing of brush-harvested seeds or seed mixtures (Kiehl et al., 2010; Edwards 450 et al., 2007; Klaus et al., 2017; Shaw et al., 2020). Similarly to hay mulch, soil preparation 451 increases seed and water retention as well as seed adhesion (Chambers, 2000) and may thus 452 compensate for the absence of a hay mulch layer limiting seed run-off. In our study area, a 453 454 previous experiment testing soil preparation effects on seedling recruitment showed a strong increase in first-year abundance of transferred species (Durbecq et al., 2021). Thus, the plots 455 456 that received brush material in our study may particularly benefit from previous soil tillage and a combination with additional wheat sowing may be the optimum strategy for seedling 457 recruitment and establishment. 458

459

## 460 4.2. Successful restoration of reference communities and erosion protection

461 Seed addition using brush material and hay of the donor site was successful in increasing the 462 similarity to best references at plant community level. As in the analysis of cover and number of species transferred from the donor site, best restoration results were obtained for hay 463 transfer and the combination of brush material with wheat sowing, showing highest similarity 464 to references in plant species composition and highest target abundance (higher CSIInorm = 465 466 abundance of species occurring in the reference). This finding confirmed that a dead (hay) or 467 living (wheat) "nurse cover" improves seedling establishment of target (reference 468 communities) and transferred (donor community) species (Graiss & Krautzer, 2011; Kavian et 469 al., 2018; Scotton, 2019). The brush material transfer without wheat sowing did not increase 470 similarity to references nor target abundance. However, HAI (proportion of non-target abundances) of this treatment was lower compared to the control whereas the HAI of brush 471

472 material with wheat and hay transfer were not significantly different from the control. These
473 results indicated that hay mulch and wheat cover did not only provide better conditions for
474 target species but also for non-target species recruitment.

475 In mountain and alpine grasslands with more or less steep slopes, soil stabilization processes are slow, which increases soil erosion (Wiesmair et al., 2017). Erosion control is thus 476 essential to restore these ecosystems (Pohl et al., 2009; Scotton, 2019; Löbmann et al., 2020). 477 478 Without plant cover, erosion, depletion of organic matter and gully formation may compromise restoration approaches (Dupin et al., 2019). Several authors have shown a negative linear 479 relationship between vegetation cover and soil runoff (Peratoner, 2003; Liu et al., 2018; Gu et 480 al., 2020). Hence, immediate revegetation by locally adapted propagules is widely 481 recommended (Scotton et al., 2009; Wiesmair et al., 2017; Dupin et al., 2019). Using well-482 483 adapted temporary nurse crop may have a stronger positive effect on soil erosion control than 484 dead plant material, such as hay or straw, since developing roots allow a belowground stabilisation of the soil (Pohl et al., 2009). According to Liu et al., (2018) and Gu et al. (2020), an 485 486 average total vegetation cover of 60 % observed in our study may reduce soil erosion by up to 80 % compared to bare soil. However, our restoration treatments did not sufficiently influence 487 488 total plant cover compared to control and thus the treatment effect on soil erosion was not significant. Run-off at several places may have been compensated by sediment accumulation 489 in others without changing the overall erosion measurement. To analyse erosion and sediment 490 accumulation within plots in detail combined sediment-runoff collection systems may be used 491 with water collection and storage tanks being placed at the down slope side (Parsons et al., 492 493 <u>2006; Phan Ha et al., 2012</u>).

Contrary to our results, Kavian et al. (2018) found a long-term reduction in soil runoff using
wheat residual as agriculture mulch. Our restoration sites were not characterized by steep
slopes, thus limiting soil run-off and the probability to find differences between treatments.
Additionally, spontaneous vegetation emerging from the soil seed bank dominated in the
beginning limiting the treatment effect.

499

500 5. Conclusions

501 All applied transfer techniques were successful in establishing plant species of the donor 502 community. However, the transfer of brush harvested seed material was only efficient together 503 with wheat sowing. Our results demonstrated that restoration of grasslands of the mountain altitudinal belt with shallow and stony soils clearly benefit from dead (hay) or living (wheat) 504 vegetation cover providing a nurse effect. Facilitation effects prevail over competition and/or 505 506 reduction in resources such as light (Eckstein & Donath, 2005; Padilla et al., 2006; Wright et al., 507 2014). Hay transfer was the best method to increase germination and seedling recruitment but the difference to brush material transfer with wheat sowing vanished in the second year 508 509 indicating higher seedling mortality in the hay mulch (Eckstein & Donath, 2005). The lower 510 seedling recruitment in brush material transfer may be compensated by harrowing the soil 511 before brush material transfer to improve recruitment conditions (Kiehl et al., 2010; Klaus et 512 al., 2017; Durbecq et al., 2021). Propagule transfer did not improve the soil erosion control. 513 Short-lived species spontaneously emerging from the soil seed bank dominated in the 514 beginning reducing the magnitude of treatment effects. Long-term monitoring is required to 515 evaluate the replacement of short-lived ruderals by the transferred perennial species often 516 observed in restoration studies (Albert et al., 2019; Kiss et al., 2020; Shaw et al., 2020).

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533	Appendix 1. Supplementary material
534	
535	ORCID
536	<i>Aure Durbecg</i> https://orcid.org/0000-0002-9201-1916

- 537 *Léo Rocher* <u>https://orcid.org/0000-0003-0122-1588</u>
- 538 *Renaud Jaunatre* <u>https://orcid.org/0000-0001-6970-8304</u>
- 539 *Alice Dupré la Tour* <u>http://orcid.org/0000-0001-8713-6514</u>
- 540 *Elise Buisson* <u>https://orcid.org/0000-0002-3640-8134</u>
- 541 *Armin Bischoff* <u>https://orcid.org/0000-0003-2865-8720</u>

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