



**HAL**  
open science

## Using various artificial soil mixtures to restore dry grasslands in quarries

Julie Chenot-lescure, Renaud Jaunatre, Elise Buisson, Hervé Ramone, Thierry Dutoit

► **To cite this version:**

Julie Chenot-lescure, Renaud Jaunatre, Elise Buisson, Hervé Ramone, Thierry Dutoit. Using various artificial soil mixtures to restore dry grasslands in quarries. *Restoration Ecology*, 2022, 30 (7), pp.1-46. 10.1111/rec.13620 . hal-03495884

**HAL Id: hal-03495884**

**<https://hal.science/hal-03495884>**

Submitted on 26 Jan 2022

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

# Using various artificial soil mixtures to restore dry grasslands in quarries

## Running head: Substrates for grassland restoration in quarries

Julie Chenot-Lescure<sup>1,2,3\*</sup>, Renaud Jaunatre<sup>2</sup>, Elise Buisson<sup>3</sup>, Hervé Ramone<sup>3</sup>, Thierry Dutoit<sup>3</sup>

1. Société des Carrières de la Ménudelle, 13. BP 80011, 13551 Saint-Martin-de-Crau, France

2. Univ. Grenoble Alpes, INRAE, UR LESSEM, 2 rue de la Papeterie-BP 76, 38402 St-Martin-d'Hères, France

3. Institut Méditerranéen de Biodiversité et d'Ecologie (IMBE), Avignon Université, UMR CNRS IRD Aix Marseille Université, IUT site Agroparc, BP 61207, 84911 Avignon cedex 09, France

\*author corresponding: [elise.buisson@univ-avignon.fr](mailto:elise.buisson@univ-avignon.fr)

### Author contributions

JCL, EB, TD, RJ conceived and designed the research; JCL, EB, HR, TD conducted the vegetation survey; JCL, EB, RJ analyzed the data; JCL, EB wrote the manuscript; EB, RJ, TD corrected the manuscript.

### Abstract

Quarry restoration is difficult to implement due to the scarcity of the original soil. The restoration of Mediterranean dry grasslands to mitigate similar ecosystem/habitat loss is thus still a developing process. So far, very few studies have created artificial substrates to address the lack of soil. In the *La Crau* plain (southeastern France), quarries were established in an ancient Mediterranean steppe-like dry grassland. Once the remaining grassland zones were protected in 2001, quarries were extended over former intensive orchards established on the grassland in the 1990s before it was protected. Now, restoration has to be done without the unaltered protected grassland soil but with the soil from orchards, which contains fertilizers spread during the orchard exploitation. To recreate a more suitable substrate, the orchard topsoil was mixed with poor substrate materials directly extracted from the quarry (0-30 mm pebbles with sandy matrix). Different substrate mixtures were tested with or without sowing the dominant species of the grassland, a perennial grass: *Brachypodium retusum* (and an annual grass *Brachypodium hybridum*). After five years of monitoring, our results show that raw substrate materials (100%) limit the establishment of all species, and that, at the opposite, orchard topsoil (100%) favors a

35 significantly higher species richness of target and non-target species. The recommended substrate  
36 mixture to reach a compromise between high target cover and low non-target species cover is  
37 composed of 50% raw quarry material and 50% arable soil. Sowing *Brachypodium* reduces target  
38 species richness establishment but also limits non-target species richness and cover.

39  
40 **Keywords:** Grassland, Raw substrate materials, Mediterranean pseudo-steppe, Artificial soil  
41 mixture, Soil [restoration](#), Thero-Brachypodietae.

42  
43 **Implications for practice:**

- 44 - Soil resources may be scarce for ecological restoration therefore creating substrates based  
45 on soil mixed with raw quarry material is an opportunity to increase [available substrate](#)  
46 [volumes](#)
- 47 - Substrate composed of 100% arable soil maximized both target and non-target species  
48 establishment
- 49 - Substrate mixtures composed of 50% arable soil and 50% raw quarry material are a good  
50 compromise to create more substrate than there is soil, favor target species and lower  
51 non-target species establishment
- 52 - Sowing *Brachypodium* lowers non-target species richness but also target species cover;  
53 it creates a physiognomy close to that of the reference grassland.

54  
55 **Introduction**

56 Ecological restoration in former quarries is often difficult because of the definitive loss of the  
57 original soil resource (Castillejo & Castelló 2010; Jarvis & Walton 2012). Its replacement by low  
58 quality substrates (e.g. rubble sometimes mixed with inert waste: low nutrient and organic  
59 contents, low mycorrhize, poor soil structure, low water storage, etc.) is often insufficient [for](#)  
60 [successful revegetation](#) because it limits the establishment and growth of plants, which are also  
61 exposed to recurrent drought stress in the Mediterranean region (Oliveira et al. 2011). To date,  
62 very few studies have focused on the creation *in integrum* of a soil in ecological restoration  
63 projects, while this is known to be one of the key drivers of restoration success (Mchergui et al.  
64 2014; Muñoz-Rojas 2018).

65 When quarrying is performed, soils can be discarded or stockpiled, altering their quality  
66 (Stahl et al. 2002). If quarrying occurs in pristine / undamaged ecosystems, [immediately](#)  
67 transferring the soil to a restoration site (just-in-time soil transfer) is a good way to restore high  
68 species-diversity grasslands in Southeastern Mediterranean France (Jaunatre et al. 2014; Bulot et

69 al. 2017; Buisson et al. 2018). However, this technique is hardly applied because it is costly and  
70 need to be performed concomitantly with undesirable soil extraction on undamaged well  
71 preserved ecosystems. It is thus limited to cases where a reference site is planned to be destroyed  
72 (Bullock 1998).. Other sources of soils, such as from abandoned agricultural areas may also be  
73 available for restoration. However, fertilizers spread in the course of intensive exploitation  
74 (nitrogen, phosphorus and potassium) may have considerably increased the nutrient contents of  
75 these soils (Römermann et al. 2005; Wang et al. 2017; Helm et al. 2019). The use of the arable  
76 layer (i.e. topsoil) resulting from abandoned agricultural areas might therefore favor the  
77 establishment of undesirable species from the seedbank (Buisson et al. 2018), including ruderal  
78 and exotic invasive species, to the detriment of target stress-tolerant plant species (Castillejo &  
79 Castelló 2010; Ballesteros et al. 2012; Jaunatre et al. 2014). Thus, the use of such arable soil may  
80 not enable the restoration of nutrient poor grassland vegetation with high species-diversity, thus  
81 impeding the restoration of such ecosystems.

82 The selection of the materials at the outset of a project determines the success of the  
83 restoration process (Bradshaw 2000), and is particularly decisive for the recovery of the target  
84 vegetation associated with specific substrates (Ballesteros et al. 2014, 2012; Whiting et al. 2004).  
85 In order to recreate an adequate substrate starting with arable soil as a base, mixing it with natural  
86 materials (for example, originating from the geological substrate), not impacted by the chemical  
87 inputs of former agricultural practices, should allow approaching the oligotrophic characteristics  
88 of the soils of the reference communities.

89 The first aim of the present experimental setup was to test different mixtures of substrates  
90 in the context of a Mediterranean dry grassland (*La Crau*, Southern France) restoration. This will  
91 allow us to select the best substrate for initiating the successional trajectory of the vegetation  
92 towards the reference dry grassland (*Asphodeletum fistulosi* – Molinier & Tallon 1950). During  
93 the restoration process, species may not recolonize owing to a lack of seed dispersal. In that case,  
94 the recolonization process may be accelerated by sowing target species, or by sowing so-called  
95 'nurse' species to encourage the establishment of other species (Padilla & Pugnaire 2006; Ren et  
96 al. 2008; López & Cavallero 2017). The second aim of this experimental setup is thus to test  
97 whether *Brachypodium retusum* (Poaceae) can act as a nurse species for grassland restoration in  
98 quarries. It is a perennial rhizomatous grass species which represents the most abundant species  
99 in biomass and cover in many north-western Mediterranean grasslands (Molinier & Tallon 1950;  
100 Luis et al. 2004; Buisson et al. 2021). It is common within its natural range (Contu 2013), where  
101 it plays an important role particularly in post-wildfire recovery (Caturla et al. 2000). *B. retusum*  
102 is one of the first species to reproduce after a fire (Vidaller et al. 2019), and to rapidly recolonize

103 the environment. It plays a key role in protecting the soil against erosion, favoring the  
104 establishment of target shrub species and preventing the establishment of undesirable species  
105 (Caturla et al. 2000; Larchevêque et al. 2010; Raventós et al. 2012). Its rather tall and dense  
106 physiognomy can be expected to enable the capture of windborne seeds (Bullock & Moy 2004;  
107 Giladi et al. 2013) and the protection of seedlings from grazing. Consequently, *B. retusum* would  
108 be a good candidate to have an indirect positive impact on the establishment and survival of target  
109 species (defined here as the species found in the reference grassland).

110 The experimental setup was designed to test the effect of (1) the physical-chemical  
111 composition of the substrate, (2) *B. retusum* sowing and (3) their interaction, on the natural  
112 colonization of the regional species pool. We tested the following hypotheses: (1) The substrate  
113 mixtures, constituted exclusively of materials extracted from the quarry (pebbles of 0-30 mm  
114 with sandy matrix hereafter raw quarry material), are poor in nutrients and organic matter, and  
115 will limit the establishment of all species, i.e. undesirable ruderal species and target species; (2)  
116 Spontaneous colonization enables plant communities to tend towards the reference grassland, as  
117 long as i) they are not in competition with dense patches of ruderal species (like on the substrate  
118 mixtures with the highest proportion of arable soil), or ii) the abiotic conditions are not too harsh  
119 (like on the substrate mixtures with raw quarry material); and (3) Sowing *Brachypodium retusum*  
120 enables the rapid establishment of this species, and potentially facilitates the trapping of  
121 windborne seeds from the reference grassland. Sown plots will then have higher cover of *B.*  
122 *retusum*, increased richness of target species and greater similarity to the reference grassland.

123

## 124 **Methods**

### 125 *Study area*

126 Our study was carried out on a quarry in the *La Crau* plain, located in southeastern Mediterranean  
127 France (Bouches-du-Rhône). An area of the gravel quarry “*La Ménéudelle*” was dedicated to this  
128 study. It was in operation from 2008 to 2011, impermeabilized in 2012 (using deposits of  
129 decantation sludge – clays and silt - presenting a strong water retention capability) and filled up  
130 in 2012-2013 (with inert materials that are non-fermentable, non-polluting and not likely to alter  
131 the physical and chemical properties of the soil down to 3.50m depth (BO-MEDDTL 2011;  
132 Figure 1). After compacting and levelling of the surface of the landfill, the site was then at an  
133 elevation of 40 cm below the original surface of the reference grassland and ready to receive the  
134 experimental setup.

135

### 136 *The different substrate mixtures*

137 Two types of substrates were used in the experiment for the different mixtures. 1) Arable soil  
138 from an orchard abandoned in 2015, composed of the topsoil (organo-mineral layer, 0-20 cm)  
139 and the subsoil (mineral layer, 20-40 cm) of cultivated Haplic Cambisol soil mixed together  
140 (Chenot et al. 2018). It was directly collected at the donor site at the same time as the different  
141 treatments were constituted to avoid any storage period of the soil and any enrichment in  
142 undesirable non-target species during storage (in particular windborne Asteraceae species)  
143 (Bordez 2015). 2) The raw quarry material was composed of sand and of alluvial gravel, sifted  
144 so as to keep only elements between 0 and 30 mm, also stored for less than 24h as in order to  
145 avoid enrichment with seeds of non-target species. Overall, five substrate mixtures with different  
146 proportions of each substrate were tested : 100% raw quarry material, 75% raw quarry material  
147 with 25% arable soil, 50% raw quarry material with 50% arable soil, 25% raw quarry material  
148 with 75% arable soil, and 100% arable soil (Table 1).

149 The various mixtures made it possible to obtain significant differences in substrate  
150 mixture physical-chemical properties (Supplement S1). The mixtures were spread in spring 2016  
151 in the form of 30-40 cm thick 5m × 5m plots (similar thickness of soil to the reference ecosystem)  
152 using back hoes and hand tools. During the mixing of materials using backhoes, the soil was  
153 stirred, mixed and thus aired to ensure the growth of the plants (Jarvis & Walton 2012). Overall,  
154 50 plots were installed at the site in February 2016 (Supplement S2).

155

### 156 *Sowing the dominant species*

157 Two sowing treatments were tested: (1) Each substrate mixture without sowing of any species  
158 (natural colonization); (2) Each substrate mixture with sowing of *Brachypodium* spp..  
159 Commercially available non-certified seeds of non-local origin (Spain) were sown (germination  
160 rate: 73%) in March 2016 over 625m<sup>2</sup> of experimental plots (25m<sup>2</sup> × 25 plots), with a ratio of  
161 10g of seeds/m<sup>2</sup> (3000 seeds/m<sup>2</sup>). While we purchased *Brachypodium retusum* seeds only, our  
162 seed mix turned out to be only 32% *B. retusum* and 68% *Brachypodium hybridum*, the latter  
163 being an annual *Brachypodium* which seems to have been confused during seed collection by the  
164 seed company. However this species is also present in the reference grassland with a lower plant  
165 cover (Pavon & Pires 2020). Throughout the remainder of this article, only the term  
166 *Brachypodium* was used for the sown seed mix. The experimental setup thus includes five  
167 replicates of each treatment (5 substrate mixtures × 2 sowing treatments × 5 replicates).

168

### 169 *Experimental implementation*

170 The different treatments were spread randomly over the site (Supplement S2). No material was  
171 spread in the paths between the plots. Once a year, paths were mowed using a [grass trimmer](#) to  
172 limit their colonization by plants and avoid their seeding, which might have influenced the  
173 establishment of species on the plots by edge effects. As the reference grassland is grazed  
174 (Saatkamp et al. 2010; Saatkamp et al. 2018), grazing by an itinerant flock of sheep, connecting  
175 the reference grassland and all the plots, was reintroduced after the experiment was set up (for a  
176 total of 3 days a year between February and May). Finally, a drainage outlet was dug at the end  
177 of the experimental plot to avoid the stagnation of water during rainy episodes.

178

### 179 *Annual vegetation surveys*

180 Monitoring of the vegetation was carried out every year from 2016 to 2018 and in 2020 to assess  
181 plant recolonization dynamics and to compare the different treatments. For that purpose,  
182 permanent 4m<sup>2</sup> quadrats situated at the center of each 25m<sup>2</sup> plot (to avoid any edge effect) were  
183 monitored in May 2016, May 2017, May 2018 and May 2020. The abundance of each plant  
184 species was estimated on the basis of percent cover (Gillet 2000) and total plant cover was also  
185 estimated. The vegetation developing on the quadrats was compared with the reference  
186 ecosystem after a survey of randomly placed 4m<sup>2</sup> quadrats on the reference grassland: ten  
187 quadrats in 2018 and five quadrats in 2020. Reference plant communities contained  $52.2 \pm 2.2$   
188 species per 4m<sup>2</sup>, and 109 species overall in these 15 quadrats.

189

### 190 *Data analyses*

191 All the data analyses were performed using the packages *vegan* (Oksanen et al. 2015), *car* (Fox  
192 et al. 2020), *multcomp* (Hothorn et al. 2020), *emmeans* (Lenth et al. 2020) and *lme4* (Bates et al.  
193 2016) in R 4.0.3. (R Core Team 2020).

194 We evaluated the effects of substrate mixtures, sowing and time on species composition,  
195 richness and cover of plant communities. We also evaluated the effects of substrate mixtures and  
196 sowing five years after the experiment was set up, in 2020. This was done on species  
197 composition, richness and cover of plant communities, as well as on *Brachypodium retusum* and  
198 *Brachypodium hybridum* covers. In 2020, we also assessed the effects of treatments on plant  
199 species richness and plant cover for target and non-target species separately (target species being  
200 the species found on the reference grassland plots in 2020). For species composition, we used the  
201 data of all species but *Brachypodium* spp. as the aim was to assess the effect on the plant  
202 community independently of *Brachypodium* cover. As the sum of species cover usually largely  
203 exceed total species cover, we therefore used as cover without *Brachypodium* the product of the

204 sum of species cover excluding *Brachypodium* spp. cover by the estimated total plant cover,  
205 divided by the sum of all species cover, hereafter called plant cover without *Brachypodium*.

206 Two indices were used to assess the state of the plant communities compared to the  
207 reference ecosystem. 1) The CSII<sub>norm</sub> Index (normalized Community Structure Integrity Index)  
208 measuring the proportion of the abundance of species of the reference community represented in  
209 the restored community (it shows the percentage of similarity in terms of composition and  
210 abundance of the communities studied compared to the reference community). 2) The HAI Index  
211 (Higher Abundance Index) measuring the proportion of the abundance of species in the restored  
212 community which is higher than in the reference community (it shows what is 'in excess', in terms  
213 of species and abundance in the studied communities studied compared to the reference  
214 community) (Jaunatre et al. 2013).

215 In order to characterize the composition of the plant communities sampled on the different  
216 plots, two Non-Metric Multidimensional Scaling (NMDS) based on Bray–Curtis similarity were  
217 performed (Borcard et al. 2011). One NMDS was run on all years (200 quadrats (5 mixtures × 2  
218 sowing treatments × 5 replicates × 4 years (2016, 2017, 2018, 2020)) × 155 species) and one in  
219 2020 (55 quadrats (5 mixtures × 2 treatments × 5 replicates + 5 quadrats in the reference  
220 grassland) × 135 species).

221 To test the effects of the substrate mixtures × *Brachypodium* sowing × time on plant  
222 species richness, plant cover, CSII<sub>norm</sub> and HAI, one Generalized Linear Mixed Model (GLMM)  
223 was performed on each of these variables. For each, we used substrate mixtures, *Brachypodium*  
224 sowing and years as fixed factors and quadrats as a random factor to take repeated measures into  
225 account. They were followed by pairwise contrast comparisons with a Tukey adjustment when  
226 significant.

227 To test for possible effects of the substrate mixtures × *Brachypodium* sowing on plant  
228 species richness, plant cover CSII<sub>norm</sub> and HAI in 2020, one Generalized Linear Model GLM was  
229 performed on each of these variables. They were followed by pairwise contrast comparisons with  
230 a Tukey adjustment when significant. All models were fitted with a poisson error distribution for  
231 plant species richness and a gamma error distribution with log link for the other variables.

232

233

## 234 **Results**

235 *Effects of substrate mixtures, Brachypodium sowing and time on plant communities*



236 All plant communities changed over time (Supplement S3). They all started in 2016 with arable  
237 weeds, such as *Diploaxis tenuifolia* (L.) DC., *Polygonum aviculare* L., *Solanum nigrum* L. and  
238 *Veronica persica* Poir.. With time, they gained target species of the reference grassland, such as  
239 *Avena barbata* Pott ex Link, *Catapodium rigidum* (L.) C.E.Hubb., *Hordeum murinum* L.,  
240 *Medicago truncatula* Gaertn., *Rostraria cristata* (L.) Tzvelev, *Trifolium campestre* Schreb.,  
241 *Trifolium glomeratum* L. and *Trifolium scabrum* L..

242 Overall, species richness increased with time on all plots. It increased by 5 fold between  
243 2016 and 2017 and only by 10% from 2017 to 2020 (Supplement S3). Percent covers of unsown  
244 species increased by two fold from 2016 to 2018, then decreased by 10% in 2020 (Supplement  
245 S3). Substrate mixtures with 100% or 75% of raw quarry material had significantly lower species  
246 richness and lower percent cover of unsown species than other substrate mixtures on all years.  
247 Plots where *Brachypodium* was not sown had significantly higher species richness and higher  
248 percent cover of unsown species (Supplement S3).

249 CSInorm increased by ten fold between 2016 and 2017 (especially on unsown plots and  
250 on sown plots with the highest proportion of arable soil) and then remained relatively stable or  
251 underwent a 20% decrease by 2020 (Supplement S3). HAI on unsown plots were always high  
252 (close to 1). HAI on sown plots first increased by 3 fold and then decrease by the same order to  
253 go back below 0.6 in 2020 (Supplement S3).

254

#### 255 *Effects of substrate mixtures on Brachypodium retusum cover in 2020*

256 *Brachypodium retusum* had a significantly higher cover on sown plots ( $\chi^2=381.23$ ,  $df=1$   
257  $p<0.001$ \*\*\*; Figure 2). It established very well on all sown plots (found on 25 plots out of 25  
258 sown in 2020; average percent cover = 38.8%). It was found on two of the 25 unsown plots in  
259 2020, but with a significantly lower percent cover on average (0.04%). *Brachypodium hybridum*  
260 had a significantly higher cover only on plots sown on the substrate mixture with 100% arable  
261 soil ( $\chi^2=23.97$ ,  $df=4$ ,  $p<0.001$ ; Figure 2). Its percent cover (8.8%) was much lower than that of  
262 *Brachypodium retusum* (35%).

263

#### 264 *Effects of substrate mixtures and Brachypodium sowing on plant communities in 2020*

265 *Brachypodium* sowing and the substrate mixtures influenced plant community composition in  
266 2020 (Figure 3). Axis 1 of the NMDS on plant community data showed two major groups: the  
267 quadrats where *Brachypodium* was sown on the left and those where *Brachypodium* was not  
268 sown on the right. The sown quadrats were characterized by target species such as *Andryala*  
269 *integrifolia* L., *Lobularia maritima* (L.) Desv. and *Medicago monspeliaca* (L.) Trautv.. In

270 contrast, those without sowing of *Brachypodium* were characterized by *Dittrichia viscosa* (L.)  
271 Greuter, *Oloptum miliaceum* (L.) Röser & Hamasha and *Symphytotrichum squamatum* (Spreng.)  
272 G.L.Nesom. Axis 2 of the NMDS showed a slight gradient in the structure of the plant  
273 communities in relation to substrate mixtures, and the reference grassland (Figure 3): mixtures  
274 with higher proportions of arable soil sown with *Brachypodium* were slightly more similar to the  
275 reference grassland than mixtures with higher proportions of raw quarry material.

276 Substrate mixtures with a higher proportion of arable soil had a significantly higher  
277 species richness of both target and non-target species (respectively:  $\chi^2=37.94$ ,  $df=4$ ,  $p<0.001$  and  
278  $\chi^2=30.94$ ,  $df=4$ ,  $p<0.001$ , Figure 4). *Brachypodium* sowing did not influence target plant species  
279 richness ( $\chi^2=0.05$ ,  $df=1$ ,  $p=0.825$ ), and plots where *Brachypodium* was sown had lower richness  
280 of non-target species ( $\chi^2=13.10$ ,  $df=1$ ,  $p<0.001$ , Figure 4). Substrate mixtures with a higher  
281 proportion of arable soil also had a higher plant cover of target species, although this was  
282 significant only on plots sown with *Brachypodium* ( $\chi^2=38.57$ ,  $df=4$ ,  $p<0.001$ , Figure 5). The plant  
283 cover of non-target species was only significantly higher on unsown plots with a substrate  
284 mixture with 100% of arable soil compared with sown plots with the same substrate mixture  
285 ( $\chi^2=13.62$ ,  $df=4$ ,  $p=0.009$ , Figure 5).

286  
287 *Similarity of plant communities obtained in the different soil mixtures to those of the reference*  
288 *grassland*

289 In 2020, 60 species, out of the 85 species inventoried in the five reference grassland plots, were  
290 recorded on the experimental substrate mixtures (50 plots – total number of species in 2020 =  
291 136). Among these 60 species, we found *Anisantha madritensis* (L.) Nevski, *Anisantha rubens*  
292 (L.) Nevski, *Catapodium rigidum* (L.) C.E.Hubb., *Crepis foetida* L., *Galactites tomentosus*  
293 Moench, *Medicago monspeliaca* (L.) Trautv., *Rostraria cristata* (L.) Tzvelev, *Sonchus oleraceus*  
294 L., *Trifolium campestre* Schreb., *Trifolium scabrum* L. and *Vulpia ciliata* Dumort. (Supplement  
295 S4, S5). The plant communities developing on newly created substrates remained highly different  
296 from the reference grassland five years after implementation (the best CSII<sub>norm</sub> reached 18.15%  
297 for substrates composed of 100% arable soil;  $\chi^2=31.08$ ,  $df=4$ ,  $p<0.001$ ; Figure 6). The substrate  
298 mixtures with the poorest nutrient content (QM and QM\_AS), presented the plant communities  
299 that were farthest from the reference grassland in terms of composition and abundance, in  
300 contrast to those with a high proportion of arable soil, such as AS and AS\_QM (Figure 6). The  
301 CSII<sub>norm</sub> restoration index also showed that plots where *Brachypodium* was not sown were more  
302 similar to the grassland than where *Brachypodium* was sown ( $\chi^2=11.89$ ,  $df=1$ ,  $p<0.001$ ; Figure  
303 6).

304 Unsown plots contained species not found in the reference grassland nor on sown plots,  
305 such as *Artemisia verlotiorum* Lamotte, *Clinopodium nepeta* (L.) Kuntze and *Bituminaria*  
306 *bituminosa* (L.) C.H.Stirt.. Sown plots contained species not found in the reference grassland nor  
307 on unsown plots, such as *Hirschfeldia incana* (L.) Lagr.-Foss. and *Medicago orbicularis* (L.)  
308 Bartal. (Supplement S4, S5). Unsown plots contained 10 reference grassland species not found  
309 on sown plots, such as *Carduus nigrescens* Vill., *Eryngium campestre* L., *Linum strictum* L.,  
310 *Neatostema apulum* (L.) I.M.Johnst., *Sideritis romana* L. and *Urospermum dalechampii* (L.)  
311 Scop. ex F.W.Schmidt. Sown plots contained 6 reference grassland species not found on unsown  
312 plots, such as *Aira cupaniana* Guss., *Logfia gallica* (L.) Coss. & Germ., *Petrorhagia prolifera*  
313 (L.) P.W.Ball & Heywood and *Plantago berlardii* All. (Supplement S4, S5). Most plots also  
314 contained high abundances (>5%) of species that are common in the reference grasslands but  
315 usually recorded there with lower abundances (<0.5%) (*A. madritensis*, *R. cristata*, *H. murinum*,  
316 *A. rubens*, *T. campestre*, *A. barbata*, *M. truncatula*, *C. foetida*, *Geranium molle*, etc.). These three  
317 sets of species explain the relatively high HAI index (86.4%; Figure 6; Supplement S4, S5). The  
318 higher HAI restoration index on the unsown plots showed that sowing *Brachypodium* limited the  
319 establishment of non-target species, but this was significant only on substrate with 100% raw  
320 quarry material ( $\chi^2=11.89$ ,  $df=1$ ,  $p<0.001$ ; Figure 6). There was no significant differences of HAI  
321 between substrate mixtures ( $\chi^2=2.42$ ,  $df=4$ ,  $p=0.660$ ; Figure 6).

322

323

## 324 **Discussion**

325 Despite the fact that plant communities are still distant from the reference grassland after five  
326 years, the tested substrate mixtures allow the rapid establishment of a grassland-like vegetation  
327 with numerous Poaceae species. The total number of target species (defined here as the species  
328 found in the reference grassland) increased with the age of succession in the majority of substrate  
329 mixtures as already demonstrated in other restoration experiments undertaken in the *La Crau*  
330 grasslands (Coiffait-Gombault et al. 2012; Bulot et al. 2014) or in other dry grasslands (Zobel et  
331 al. 1996). The first years after restoration are often characterized by weedy ruderal species (Vida  
332 et al. 2010). Our results confirmed this pattern with a strong dominance of ruderal species, such  
333 as *Diplotaxis tenuifolia*, *Polygonum aviculare*, etc. which then become rare or are replaced by a  
334 mix of ruderal-competitive species *sensu* Grime (1977) and some target species all of which  
335 naturally colonize the site from the available regional pool of species. The most common  
336 reference grassland species found on the experimental plots after five years are i) wind-dispersed  
337 Asteraceae (*Crepis foetida*, *Carduus pycnocephalus*, *Galactites tomentosa*), ii) small Poaceae

338 (*Anisantha madritensis*, *Anisantha rubens*, *Catapodium rigidum*) which are common in the short-  
339 term seed bank and can easily be transported by wind over small distances as well as by sheep  
340 and ants, and iii) Fabaceae (*Trifolium campestre*, *Trifolium glomeratum*, *Trifolium scabrum*,  
341 *Medicago truncatula*) which are found in the short-term and long-term seed banks and are  
342 transported by ants (Buisson et al. 2006; De Almeida et al. 2020). This finding is similar to that  
343 of Alday et al. (2011), where species that exhibited zoochory and anemochory were most  
344 abundant in the early stages of post-mining succession. Monitoring over the longest term possible  
345 is recommended as recovery rates and patterns of change in the plant communities may be slow  
346 (Jones et al. 2018). Recovery is particularly slow in the *La Crau* grasslands (Coiffait-Gombault  
347 et al. 2012; Bulot et al. 2014). In the long term, it is expected that the plant communities on the  
348 different substrate mixtures will keep developing in different directions and will never achieve  
349 the entire restoration of the reference grassland because of highly different starting conditions  
350 (substrate mixtures) (Prach et al. 2014). Such results have been already demonstrated for *La Crau*  
351 grasslands with former cultivation (Jaunatre et al. 2016) and sheep paddocks (Saatkamp et al.  
352 2020) which still show different plant composition and diversity to the reference grassland  
353 several centuries or millennia after their abandonment (Römermann et al. 2005; Helm et al.  
354 2018).

355 While some target species colonized the **restored** areas, the high Higher Abundance  
356 Indices (HAI) show that some target species over develop (compared to their abundance on the  
357 reference grassland) and that non-target species are common. The latter may be due to the fact  
358 that the site is not directly surrounded by reference grassland areas, which impede possibilities  
359 of target species recolonization (Lanta et al. 2020). The neighboring areas are strongly impacted  
360 by quarry exploitation, former quarries, or former orchards where ruderal species predominate.  
361 Non-target species may thus easily find their way to the experimental plots, carried in the seed  
362 bank of the arable soil used for the experiment, by the wind or by itinerant grazing, sheep  
363 transporting indistinctively target and non-target species (Albert et al. 2015). The pool of plant  
364 species, that is, the source of seeds, of diaspores and the potential for colonization of species  
365 plays a major role in the restoration, by influencing the process of spontaneous succession  
366 (Řehounková & Prach 2008; Strykstra et al. 1998; Zobel et al. 1998). Therefore, the  
367 characteristics of the landscape should be properly taken into account for any restoration  
368 initiatives (Bakker et al. 1998; Prach et al. 2001; Son et al. 2020), and in our case, may have a  
369 higher success if adjacent to patches of the reference ecosystem (Buisson et al. 2006).

370

371 The substrates represent major variables determining the rhythm and direction of plant  
372 successions and should unavoidably be taken into account in restoration programs (Prach et al.  
373 2001). The substrate mixtures poor in nutrients, i.e. with a higher percentage of raw quarry  
374 material, may not be the best as they have lower target species plant covers and lower species  
375 richness of both target and non-target plant species than the richer soils (with a higher percentage  
376 of arable soil). Survival of target species is indeed severely impeded by harsh abiotic conditions  
377 (Pueyo et al. 2009). Moreover, as they generally have a lower plant cover, these substrate  
378 mixtures may lead to higher soil erosion, the region having very strong winds blowing 334  
379 days/year (Buisson & Dutoit 2004).

380 In the present study, fertilizers (N, P, K) applied during the phase of exploitation of the  
381 orchards (1983-2015) and thus found in the arable soil have certainly contributed to increasing  
382 plant cover on richer substrate mixtures, as it has already been demonstrated elsewhere (Norman  
383 et al. 2006). Higher plant species richness on nutrient-richer soils is likely due to the fact that  
384 arable soils contain a seed bank while raw material does not (Bulot et al. 2014). Substrate  
385 mixtures with 50% or less of arable soils have significantly less non-target species richness and  
386 substrate mixtures with 50% or more of arable soils have significantly more target species  
387 richness or cover. Therefore, in order to favor target species richness and cover and maintain  
388 non-target cover as low as possible, the best compromise would be the substrate mixture with  
389 50% arable soil and 50% raw quarry material. This substrate mixture also has a similarity to the  
390 reference grassland plant community which is amongst the highest.

391  
392 Sowing *Brachypodium* seeds allowed *Brachypodium retusum* to establish on all plots,  
393 although its cover was lower on the raw quarry material. In 2020, sown plots have a lower  
394 richness of non-target species, a lower cover of target species, and a lower similarity to the  
395 reference grassland (as well as a lower cover of non-target species on arable soils only). These  
396 results contrast with other studies where seeding increased the establishment of target species  
397 (Baasch et al. 2012; Norman et al. 2006). These experiments were however greatly different from  
398 this one: we sowed one dominant grassland species and they sowed species-rich mixtures or hay  
399 collected from a reference grassland. *B. retusum* therefore seems to compete with non-target  
400 species establishment and with target species growth: its clonal growth and ability to cover the  
401 ground, and its rhizome and root system make it a quite competitive species (Bonet 2004; Clary  
402 et al. 2004; Saatkamp et al. 2018). This seems to contradict Raventós et al. (2012) study which  
403 showed a facilitative effect of *B. retusum* during the establishment phase of target species after  
404 fire. However, that study was carried out on shrubby chamaephyte species (*Ulex parviflorus*

405 Pourr., *Cistus albidus* L., *Helianthemum marifolium* Mill. and *Ononis fruticosa* L.) and not on  
406 grassland plants as it is the case here. The reason for a greater competitive effect of *B. retusum*  
407 on target species than on non-target species cover most probably lays in the fact that target  
408 grassland species are stress tolerant and non-target species rather ruderal (e.g. *D. tenuifolia*) and  
409 competitive (e.g. *O. miliaceum*) species.

410       There was no significant effect of *B. retusum* on target species richness. We can therefore  
411 conclude that *B. retusum* impacts target species cover rather than target species establishment. In  
412 addition, these data suggest that *B. retusum* may not facilitate target species arrival by capturing  
413 windborne seeds as we initially hypothesized, although this could also be due to the fact that few  
414 target species seeds were wind-blown onto the experimental plots as there is no adjacent  
415 reference grassland nearby. While we show that *B. retusum* cover is much higher than *B.*  
416 *hybridum*, it is not possible to fully disentangle the specific effect of *B. retusum* from that of *B.*  
417 *hybridum*. However, so far, it is still not possible to obtain pure and certified seeds of *B. retusum*  
418 in Southern France. Future restoration projects in Southern France will therefore still use this  
419 mixture of *B. retusum* + *B. hybridum* from Spain, until a better option is available for purchase.

420  
421       While plots with a substrate composed of 100% of arable soil and not sown with  
422 *Brachypodium* had the highest richness of target species and highest similarity of the plant  
423 communities to the reference grassland community, they also had the highest cover of non-target  
424 species. Moreover, using a substrate composed of 100% arable soil may limit opportunities for  
425 restoration activities due to insufficient substrate being available to restore more surface area  
426 than the area from which it was excavated. In order to increase the volume of substrate produced  
427 to restore wider areas than solely that of abandoned orchards (but also quarries), arable soil  
428 should be mixed / diluted with raw quarry material. Therefore, the next best option would be the  
429 substrate mixture with 50% arable soil and 50% quarry material. This substrate mixture does not  
430 differ significantly from the 100% arable soil mixtures in similarity to the reference grassland  
431 community and target species richness and cover. It is then a good compromise for increasing  
432 target and decreasing non-target species richness and cover.

433       Not sowing *Brachypodium* allows better growth and thus higher cover of target species;  
434 but also leads to higher non-target species richness and cover and some over-abundance of target  
435 species (*A. madritensis*, *R. cristata*, *H. murinum*, *A. rubens*, *T. campestre*, *A. barbata*, *M.*  
436 *truncatula*, *C. foetida*, *Geranium molle*, etc.). However, sowing *Brachypodium*, while not  
437 preventing the colonization of non-target species, does reduce their cover and richness and  
438 creates a physiognomy similar to that of the reference grassland with *B. retusum* as a dominant

439 grass. *B. retusum* is known to be extremely slow at recolonizing areas with disturbed soils  
440 (Coiffait-Gombault et al. 2012). Hence, sowing *B. retusum* is a major step towards reintroducing  
441 it.

442 Further studies should look at enriching sown and unsown plots with a few target species  
443 to identify the potential key roles of *B. retusum* in interacting with grassland species  
444 establishment. A new experiment could be about sowing a more diverse seed mixture of target  
445 species (which is still a problem as most seeds of the target species are not available for purchase)  
446 and whether sowing of *B. retusum* and this seed mix should be simultaneous or sequential.  
447 Longer monitoring of these experimental plots may allow us to refine the best treatment to be  
448 applied, i.e. where non-target species decrease the most and where target species increase.

449

450

#### 451 **Acknowledgements**

452 This study was supported by Gagneraud construction funding. We thank Pierre Bourguet (*Société*  
453 *des Carrières de la Ménudelle*) for his collaboration. We thank Jean-Marc Arnal for the  
454 preparation and implementation of the plots, Florian and Pascal for substrate mixtures. We are  
455 also grateful to students, research assistants and field ecologists for help with fieldwork: Anne  
456 Aurière, Cannelle Moinardeau, Christel Vidaller, Chloé Malik and Daniel Pavon.

457

458

#### 459 **Literature Cited**

- 460 Albert A, Auffret AG, Cosyns E, Cousins SAO, D'hondt B, Eichberg C, Eycott AE, Heinken  
461 T, Hoffmann M, Jaroszewicz B, Malo JE, Mårell A, Mouissie M, Pakeman RJ, Picard  
462 M, Plue J, Poschlod P, Provoost S, Schulze KA, Baltzinger C (2015) Seed dispersal by  
463 ungulates as an ecological filter: a trait-based meta-analysis. *Oikos* 124:1109–1120
- 464 Alday JG, Pallavicini Y, Marrs RH, Martínez-Ruiz C (2011) Functional groups and dispersal  
465 strategies as guides for predicting vegetation dynamics on reclaimed mines. *Plant*  
466 *Ecology* 212:1759
- 467 Baasch A, Kirmer A, Tischew S (2012) Nine years of vegetation development in a postmining  
468 site: effects of spontaneous and assisted site recovery. *Journal of Applied Ecology*  
469 49:251–260
- 470 Bakker JP, van Andel J, van der Maarel E (1998) Plant Species Diversity and Restoration  
471 *Ecology: Introduction*. *Applied Vegetation Science* 1:5–8

472 Ballesteros M, Cañadas EM, Foronda A, Fernández-Ondoño E, Peñas J, Lorite J (2012)  
473 Vegetation recovery of gypsum quarries: short-term sowing response to different soil  
474 treatments. *Applied Vegetation Science* 15:187–197

475 Ballesteros M, Cañadas EM, Foronda A, Peñas J, Valle F, Lorite J (2014) Central role of  
476 bedding materials for gypsum-quarry restoration: An experimental planting of  
477 gypsophile species. *Ecological Engineering* 70:470–476

478 Bates D, Maechler M, Bolker B, Walker S, Christensen RHB, Singmann H, Dai B,  
479 Grothendieck G, Green P (2016) Package ‘lme4’: Linear Mixed-Effects Models using  
480 “Eigen” and S4. R package version 1.1-12 113. [https://CRAN.R-](https://CRAN.R-project.org/package=lme4)  
481 [project.org/package=lme4](https://CRAN.R-project.org/package=lme4)

482 BO-MEDDTL (2011) Circulaire du 22/08/11 relative à la définition des déchets inertes pour  
483 l’industrie des carrières au sens de l’arrêté du 22 septembre 1994 relatif aux  
484 exploitations de carrières et aux installations de premier traitement des matériaux de  
485 carrières.

486 Bonet A (2004) Secondary succession of semi-arid Mediterranean old-field in south-eastern  
487 Spain: insights for conservation and restoration of degraded lands. *Journal of Arid*  
488 *Environments* 56:213–233

489 Borcard D, Gillet F, Legendre P (2011) *Numerical Ecology with R*. Springer New York, New  
490 York, NY

491 Bordez L (2015) Stratégies de revégétalisation des maquis miniers nickélicifères de Nouvelle-  
492 Calédonie étude sur les potentiels biologiques des Topsoils en vue de leur utilisation  
493 pour la restauration écologique des milieux dégradés application au massif de Koniembo  
494 pour KNS. PhDthesis, Institut Agronomique Néo-calédonien, Nouvelle Calédonie

495 Bradshaw A (2000) The use of natural processes in reclamation — advantages and difficulties.  
496 *Landscape and Urban Planning* 51:89–100

497 Buisson E, Almeida TD, Durbecq A, Arruda AJ, Vidaller C, Alignan J-F, Toma TSP, Hess  
498 MCM, Pavon D, Isselin-Nondedeu F, Jaunatre R, Moinardeau C, Young TP, Mesléard  
499 F, Dutoit T, Blight O, Bischoff A (2021) Key issues in Northwestern Mediterranean dry  
500 grassland restoration. *Restoration Ecology* 29:e13258

501 Buisson E, Dutoit T (2004) Colonisation by native species of abandoned farmland adjacent to a  
502 remnant patch of Mediterranean steppe. *Plant Ecology* 174:371–384

503 Buisson E, Dutoit T, Torre F, Römermann C, Poschlod P (2006) The implications of seed rain  
504 and seed bank patterns for plant succession at the edges of abandoned fields in  
505 Mediterranean landscapes. *Agriculture, Ecosystems & Environment* 115:6–14



506 Buisson E, Jaunatre R, Römermann C, Bulot A, Dutoit T (2018) Species transfer via topsoil  
507 translocation: lessons from two large Mediterranean restoration projects. *Restoration*  
508 *Ecology* 26:S179–S188

509 Bullock JM (1998) Community translocation in Britain: Setting objectives and measuring  
510 consequences. *Biological Conservation* 84:199–214

511 Bullock JM, Moy IL (2004) Plants as seed traps: inter-specific interference with dispersal. *Acta*  
512 *Oecologica-International Journal of Ecology* 25:35–41

513 Bulot A, Potard K, Bureau F, Bérard A, Dutoit T (2017) Ecological restoration by soil transfer:  
514 impacts on restored soil profiles and topsoil functions. *Restoration Ecology* 25:354–366

515 Bulot A, Provost E, Dutoit T (2014) Comparison of different soil transfer strategies for  
516 restoring a Mediterranean steppe after a pipeline leak (La Crau plain, South-Eastern  
517 France). *Ecological Engineering* 71:690–702

518 Castillejo JM, Castelló R (2010) Influence of the application rate of an organic amendment  
519 (Municipal Solid Waste [MSW] Compost) on gypsum quarry rehabilitation in semiarid  
520 environments. *Arid Land Research and Management* 24:344–364

521 Caturla RN, Raventós J, Guàrdia R, Vallejo VR (2000) Early post-fire regeneration dynamics  
522 of *Brachypodium retusum* Pers. (Beauv.) in old fields of the Valencia region (eastern  
523 Spain). *Acta Oecologica* 21:1–12

524 Chenot J, Jaunatre R, Buisson E, Bureau F, Dutoit T (2018) Impact of quarry exploitation and  
525 disuse on pedogenesis. *CATENA* 160:354–365

526 Clary J, Savé R, Biel C, Herralde FD (2004) Water relations in competitive interactions of  
527 Mediterranean grasses and shrubs. *Annals of Applied Biology* 144:149–155

528 Coiffait-Gombault C, Buisson E, Dutoit T (2012) Are old Mediterranean grasslands resilient to  
529 human disturbances? *Acta Oecologica* 43:86–94

530 Contu S (2013) *Brachypodium retusum*. The IUCN Red List of Threatened Species 2013:  
531 e.T44393494A44505478.

532 De Almeida T, Blight O, Mesléard F, Bulot A, Provost E, Dutoit T (2020) Harvester ants as  
533 ecological engineers for Mediterranean grassland restoration: Impacts on soil and  
534 vegetation. *Biological Conservation* 245:108547

535 Fox J, Weisberg S, Price B, Adler D, Bates D, Baud-Bovy G, Bolker B, Ellison S, Firth D,  
536 Friendly M, Gorjanc G, Graves S, Heiberger R, Krivitsky P, Laboissiere R, Maechler  
537 M, Monette G, Murdoch D, Nilsson H, Ogle D, Ripley B, Venables W, Walker S,  
538 Winsemius D, Zeileis A, R-Core (2020) car: Companion to Applied Regression.  
539 <https://CRAN.R-project.org/package=car>

540 Giladi I, Segoli M, Ungar ED (2013) Shrubs and herbaceous seed flow in a semi-arid  
541 landscape: dual functioning of shrubs as trap and barrier. *Journal of Ecology* 101:97–  
542 106

543 Gillet F (2000) La phytosociologie synusiale intégrée. Guide méthodologique. Université de  
544 Neuchâtel, Laboratoire d'écologie végétale et de phytosociologie 68

545 Grime JP (1977) Evidence for the existence of three primary strategies in plants and its  
546 relevance to ecological and evolutionary theory. *Am Nat* 111:1169–1194

547 Helm J, Dutoit T, Saatkamp A, Bucher SF, Leiterer M, Römermann C (2019) Recovery of  
548 Mediterranean steppe vegetation after cultivation: Legacy effects on plant composition,  
549 soil properties and functional traits. *Applied Vegetation Science* 22:71–84

550 Hothorn T, Bretz F, Westfall P, Heiberger RM, Schuetzenmeister A, Scheibe S (2020)  
551 multcomp: Simultaneous Inference in General Parametric Models. [https://CRAN.R-](https://CRAN.R-project.org/package=multcomp)  
552 [project.org/package=multcomp](https://CRAN.R-project.org/package=multcomp)

553 Jarvis D, Walton G (2012) So you have no soil for quarry restoration? pp. 160-165. In: Hunger,  
554 E. and Walton, G. (Eds.) *Proceedings of the 16th Extractive Industry Geology*  
555 *Conference*, EIG Conferences Ltd. p. 194.

556 Jaunatre R, Buisson E, Muller I, Morlon H, Mesléard F, Dutoit T (2013) New synthetic  
557 indicators to assess community resilience and restoration success. *Ecological Indicators*  
558 29:468–477

559 Jaunatre R, Buisson E, Dutoit T (2014) Can ecological engineering restore Mediterranean  
560 rangeland after intensive cultivation? A large-scale experiment in southern France.  
561 *Ecological Engineering* 64:202–212

562 Jaunatre R, Fonvieille N, Spiegelberger T, Buisson E, Dutoit T (2016) Recovery of arbuscular  
563 mycorrhizal fungi root colonization after severe anthropogenic disturbance: four species  
564 assessed in old-growth Mediterranean grassland. *Folia Geobotanica* 1–14

565 Lanta V, Mudrak O, Liancourt P, Dvorský M, Bartoš M, Chlumská Z, Šebek P, Čížek L,  
566 Doležal J (2020) Restoring diversity of thermophilous oak forests: connectivity and  
567 proximity to existing habitats matter. *Biodiversity and Conservation* 29:3411–3427

568 Larchevêque M, Ballini C, Baldy V, Korboulewsky N, Ormeño E, Montès N (2010)  
569 Restoration of a Mediterranean postfire shrubland: Plant functional responses to organic  
570 soil amendment. *Restoration Ecology* 18:729–741

571 Lenth R, Singmann H, Love J, Buerkner P, Herve M (2020) emmeans: Estimated Marginal  
572 Means, aka Least-Squares Means. <https://CRAN.R-project.org/package=emmeans>

573 López DR, Cavallero L (2017) The role of nurse functional types in seedling recruitment  
574 dynamics of alternative states in rangelands. *Acta Oecologica* 79:70–80

575 Luis MD, Raventós J, Cortina J, González-Hidalgo JC, Sánchez JR (2004) Fire and torrential  
576 rainfall: effects on the perennial grass *Brachypodium retusum*. *Plant Ecology* 173:225–  
577 232

578 Mchergui C, Aubert M, Buatois B, Akpa-Vinceslas M, Langlois E, Bertolone C, Lafite R,  
579 Samson S, Bureau F (2014) Use of dredged sediments for soil creation in the Seine  
580 estuary (France): Importance of a soil functioning survey to assess the success of  
581 wetland restoration in floodplains. *Ecological Engineering* 71:628–638

582 Molinier R, Tallon G (1950) La végétation de la Crau (Basse-Provence). *Revue générale de*  
583 *Botanique* 1–111

584 Muñoz-Rojas M (2018) Soil quality indicators: critical tools in ecosystem restoration. *Current*  
585 *Opinion in Environmental Science & Health* 5:47–52

586 Norman MA, Koch JM, Grant CD, Morald TK, Ward SC (2006) Vegetation succession after  
587 bauxite mining in Western Australia. *Restoration Ecology* 14:278–288

588 Oksanen JF, Blanchet G, Kindt R, Legendre P, Minchin PR, O’Hara RB, Simpson GL,  
589 Solymos P, Henry M, Stevens H, Wagner H (2015) Package ‘vegan’: Community  
590 Ecology Package. R package version 2.3-2 285. [https://CRAN.R-](https://CRAN.R-project.org/package=vegan)  
591 [project.org/package=vegan](https://CRAN.R-project.org/package=vegan)

592 Oliveira G, Nunes A, Clemente A, Correia O (2011) Effect of substrate treatments on survival  
593 and growth of Mediterranean shrubs in a revegetated quarry: An eight-year study.  
594 *Ecological Engineering* 37:255–259

595 Padilla FM, Pugnaire FI (2006) The role of nurse plants in the restoration of degraded  
596 environments. *Frontiers in Ecology and the Environment* 4:196–202

597 Pavon D, Pires M (2020) *Flore des Bouches-du-Rhône*. Naturalia Publications. Turriers, France

598 Prach K, Pyšek P, Bastl M (2001) Spontaneous vegetation succession in human-disturbed  
599 habitats: A pattern across seres. *Applied Vegetation Science* 4:83–88

600 Prach K, Řehouňková K, Lencová K, Jírová A, Konvalinková P, Mudrák O, Študent V,  
601 Vaněček Z, Tichý L, Petřík P, Šmilauer P, Pyšek P (2014) Vegetation succession in  
602 restoration of disturbed sites in Central Europe: the direction of succession and species  
603 richness across 19 seres. *Applied Vegetation Science* 17:193–200

604 Pueyo Y, Alados CL, García-Ávila B, Kéfi S, Maestro M, Rietkerk M (2009) Comparing direct  
605 abiotic amelioration and facilitation as tools for restoration of semiarid grasslands.  
606 *Restoration Ecology* 17:908–916

607 R Core Team (2020) R: A language and environment for statistical computing. R Foundation  
608 for Statistical Computing, Vienna, Austria

609 Raventós J, Wiegand T, Maestre FT, de Luis M (2012) A resprouter herb reduces negative  
610 density-dependent effects among neighboring seeders after fire. *Acta Oecologica*  
611 38:17–23

612 Řehouňková K, Prach K (2008) Spontaneous vegetation succession in gravel–sand pits: a  
613 potential for restoration. *Restoration Ecology* 16:305–312

614 Ren H, Yang L, Liu N (2008) Nurse plant theory and its application in ecological restoration in  
615 lower subtropics of China. *Progress in Natural Science* 18:137–142

616 Römermann C, Dutoit T, Poschlod P, Buisson E (2005) Influence of former cultivation on the  
617 unique Mediterranean steppe of France and consequences for conservation  
618 management. *Biological Conservation* 121:21–33

619 Saatkamp A, Henry F, Dutoit T (2020) Romans Shape Today’s Vegetation and Soils: Two  
620 Millennia of Land-Use Legacy Dynamics in Mediterranean Grasslands. *Ecosystems*

621 Saatkamp A, Henry F, Dutoit T (2018) Vegetation and soil seed bank in a 23-year grazing  
622 exclusion chronosequence in a Mediterranean dry grassland. *Plant Biosystems*  
623 152:1020–1030

624 Saatkamp A, Römermann C, Dutoit T (2010) Plant functional traits show non-linear response  
625 to grazing. *Folia Geobotanica* 45:239–252.

626 Son D, Alday JG, Chu Y, Lee EJ, Park S, Lee H (2020) Plant species colonization in newly  
627 created road habitats of South Korea: Insights for more effective restoration. *Science of*  
628 *The Total Environment* 719:137476

629 Stahl PD, Perryman BL, Sharmasarkar S, Munn LC (2002) Topsoil stockpiling versus exposure  
630 to traffic: A case study on in situ uranium wellfields. *Restoration Ecology* 10:129–137

631 Strykstra RJ, Bekker RM, Bakker JP (1998) Assessment of dispersule availability: its practical  
632 use in restoration management. *Acta botanica neerlandica* 47:57–70

633 Vida E, Valkó O, Kelemen A, Török P, Deák B, Miglécz T, Lengyel Sz, Tóthmérész B (2010)  
634 Early vegetation development after grassland restoration by sowing low-diversity seed  
635 mixtures in former sunflower and cereal fields. *Acta Biologica Hungarica* 61:226–235

636 Vidaller C, Dutoit T, Ramone H, Bischoff A (2019) Fire increases the reproduction of the  
637 dominant grass *Brachypodium retusum* and Mediterranean steppe diversity in a  
638 combined burning and grazing experiment. *Applied Vegetation Science* 22:127–137






- 639 Wang C, Zheng M, Song W, Wen S, Wang B, Zhu C, Shen R (2017) Impact of 25 years of  
640 inorganic fertilization on diazotrophic abundance and community structure in an acidic  
641 soil in southern China. *Soil Biology and Biochemistry* 113:240–249
- 642 Whiting SN, Reeves RD, Richards D, Johnson MS, Cooke JA, Malaisse F, Paton A, Smith J a.  
643 C, Angle JS, Chaney RL, Ginocchio R, Jaffré T, Johns R, McIntyre T, Purvis OW, Salt  
644 DE, Schat H, Zhao FJ, Baker AJM (2004) Research Priorities for Conservation of  
645 Metallophyte Biodiversity and their Potential for Restoration and Site Remediation.  
646 *Restoration Ecology* 12:106–116
- 647 Zobel M, Suurkask M, Rosén E, Pärtel M (1996) The dynamics of species richness in an  
648 experimentally restored calcareous grassland. *Journal of Vegetation Science* 7:203–210
- 649 Zobel M, van der Maarel E, Dupré C (1998) Species pool: the concept, its determination and  
650 significance for community restoration. *Applied Vegetation Science* 1:55–66
- 651
- 652
- 653

654

**Table 1:** Composition of the different mixtures used for the experiment.

655

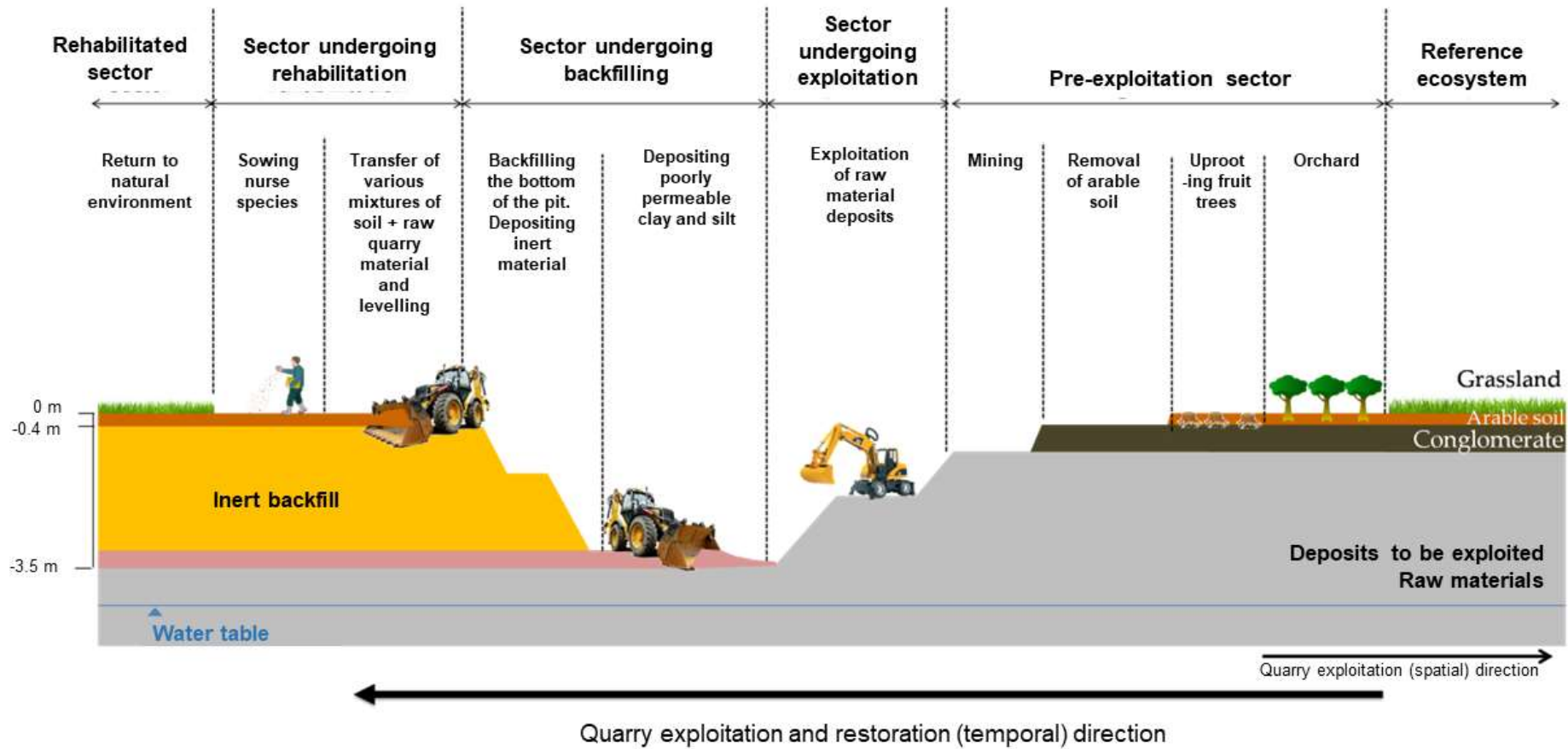
656

<b>Code for figures</b>	<b>raw Quarry Material (QM)</b>	<b>Arable Soil (AS)</b>	<b>Color code for figures</b>
<b>QM</b>	100%	0%	
<b>QM_AS</b>	75%	25%	
<b>HALF</b>	50%	50%	
<b>AS_QM</b>	25%	75%	
<b>AS</b>	0%	100%	

657

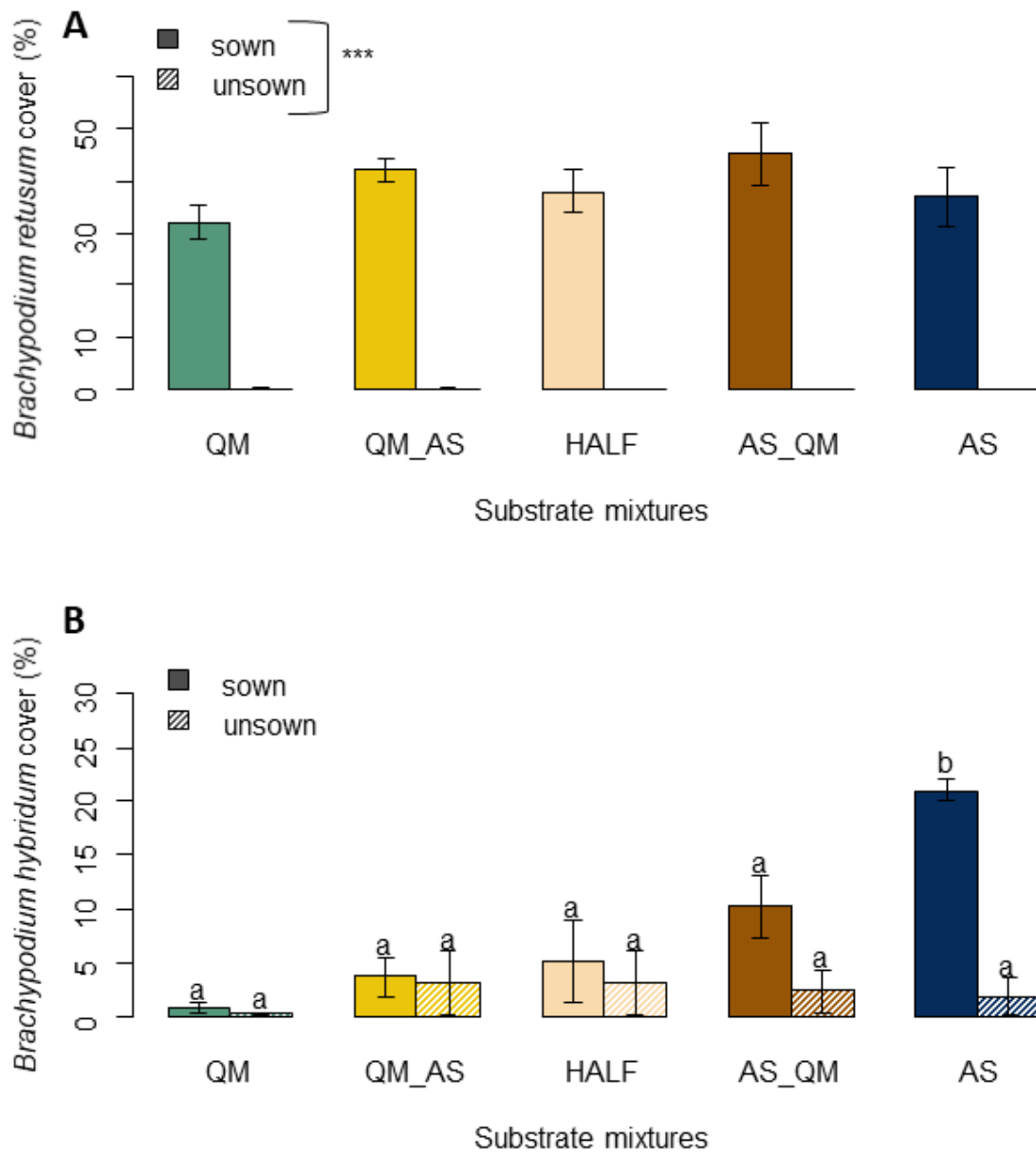
658

659  
660



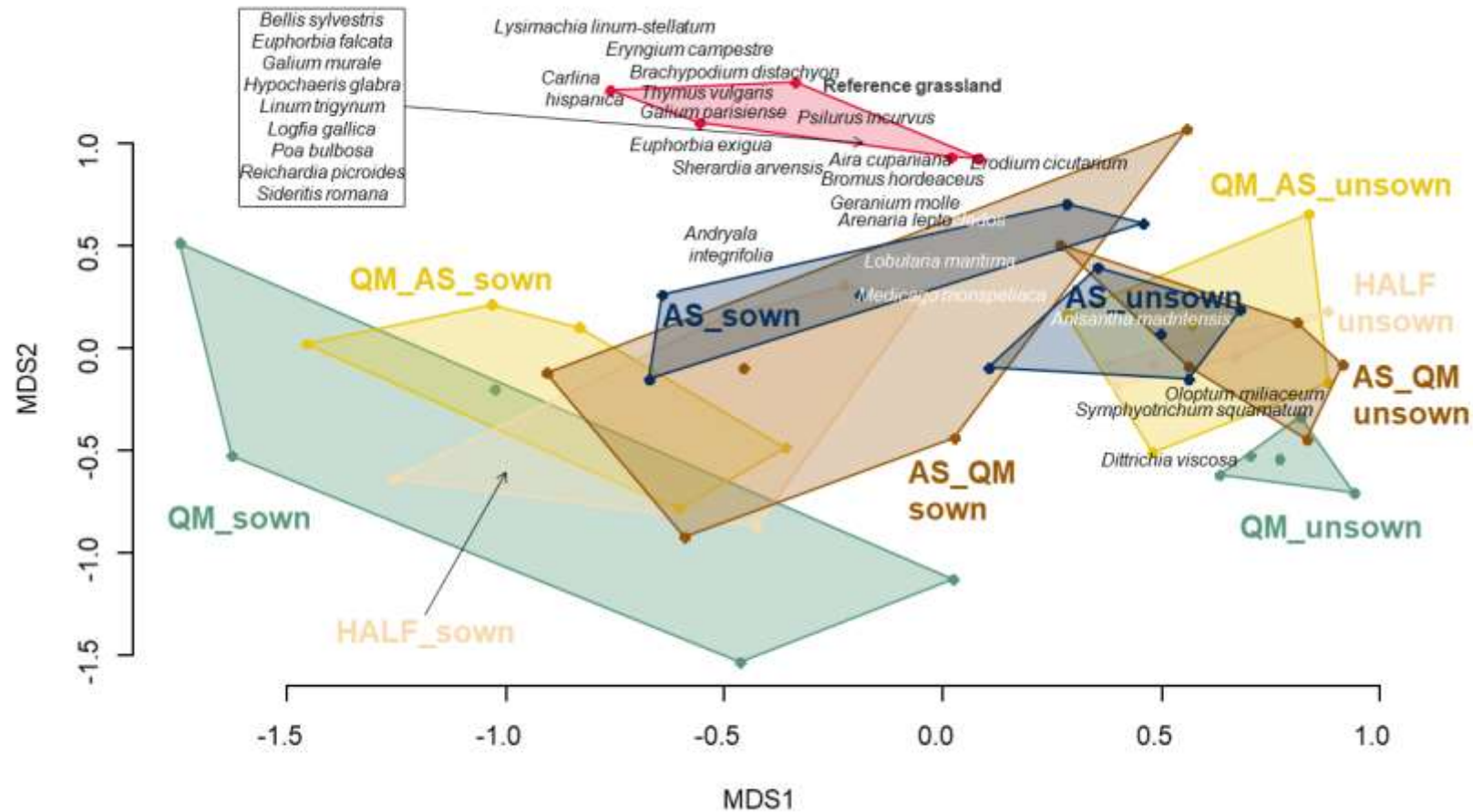
661  
662 Figure 1. Diagram of the exploitation and restoration of the Ménéduelle quarry in Southeastern France.

663  
664



666  
 667 Figure 2. *Brachypodium* cover on experimental plots in 2020 (mean cover  $\pm$  standard error). Plots  
 668 were sown (dark grey) or not (light grey) with *Brachypodium*. Substrate mixtures included QM  
 669 – Quarry raw Material or AS – Arable Soil (see Table 1 for mixture codes). A. *Brachypodium*  
 670 *retusum* (mean cover = 38.8%): Substrate mixtures  $\chi^2=4.98$ ,  $df=4$ ,  $p=0.290$  n.s.; *Brachypodium*  
 671 sowing  $\chi^2=381.23$ ,  $df=1$ ,  $p<0.001$ \*\*\* (Substrate mixtures  $\times$  *Brachypodium* sowing  $\chi^2=5.05$ ,  $df=4$ ,  
 672  $p=0.282$  n.s.). B. *Brachypodium hybridum* (mean cover = 8.2%): Substrate mixtures  $\times$   
 673 *Brachypodium* sowing  $\chi^2=23.97$ ,  $df=4$ ,  $p<0.001$ \*\*\* (Substrate mixtures  $\chi^2=25.07$ ,  $df=4$ ,  
 674  $p<0.001$ \*\*\*; *Brachypodium* sowing  $\chi^2=17.44$ ,  $df=1$ ,  $p<0.001$ \*\*\*).





676

677 Figure 3. Plant species composition in 2020 using NMDS. Polygons indicate of plots with the same treatment (NMDS stress: 0.21). Plots were  
 678 sown (sown) or not (unsown) with *Brachypodium*. Substrate mixtures included QM: 100% of raw Quarry Material; QM\_AS: 75% of raw Quarry  
 679 Material and 25% of Arable Soil; HALF: 50% of each; AS\_QM: 75% of Arable Soil and 25% of raw Quarry Material; AS: 100% of Arable Soil  
 680 (see Table 1 for mixture codes).

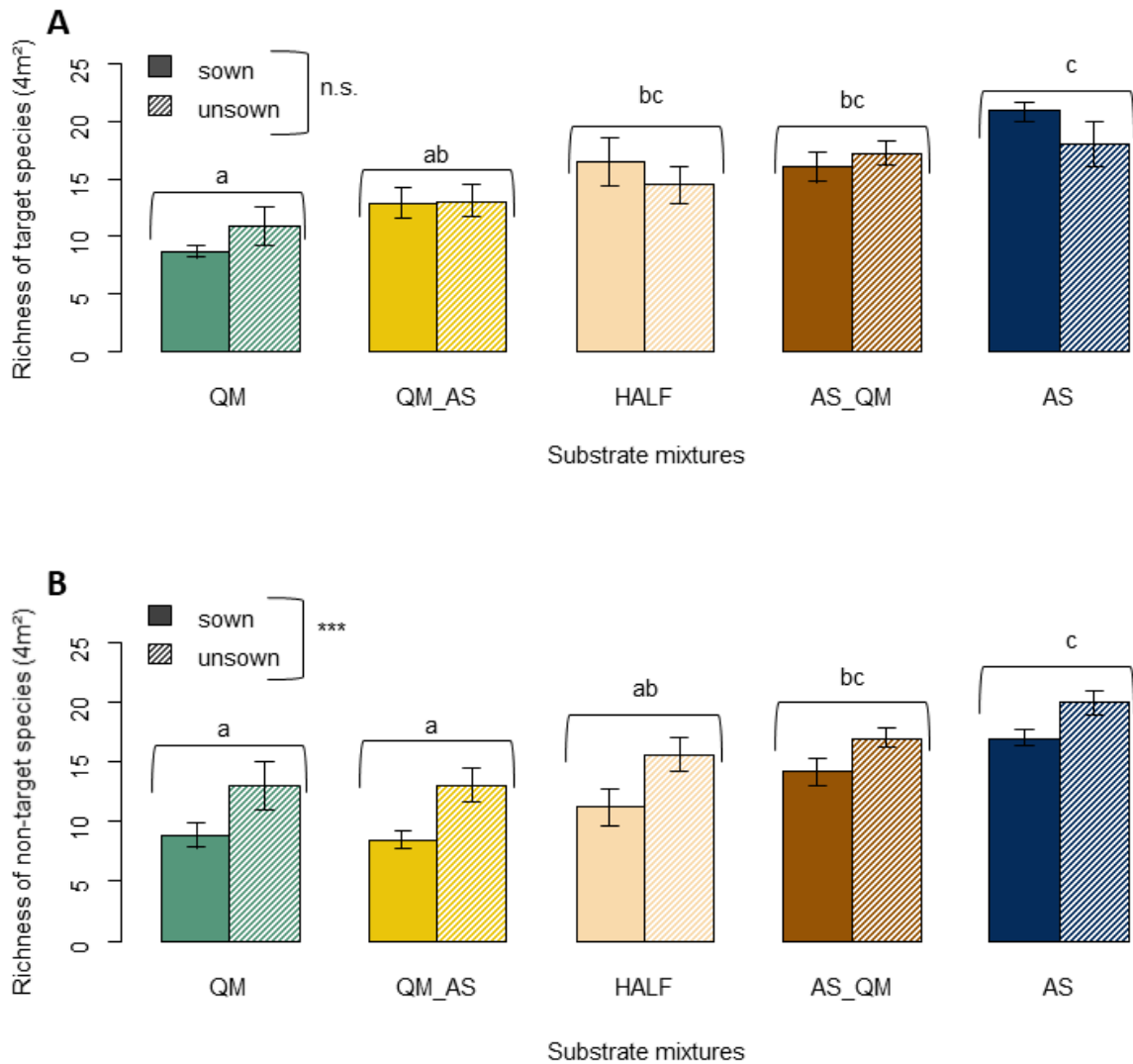


Figure 4. Mean species richness ( $\pm$  standard error) of A. target species and B. non-target species on experimental plots in 2020. Plots were sown (dark grey) or not (light grey) with *Brachypodium*. Substrate mixtures included QM – Quarry raw Material or AS – Arable Soil (see Table 1 for mixture codes). A. Substrate mixtures  $\chi^2=37.94$ ,  $df=4$ ,  $p<0.001^{***}$ ; *Brachypodium* sowing  $\chi^2=0.05$ ,  $df=1$ ,  $p=0.825$  n.s. (Substrate mixtures  $\times$  *Brachypodium* sowing  $\chi^2=3.09$ ,  $df=4$ ,  $p=0.543$  n.s.). B. Substrate mixtures  $\chi^2=30.94$ ,  $df=4$ ,  $p<0.001^{***}$ ; *Brachypodium* sowing  $\chi^2=13.10$ ,  $df=1$ ,  $p<0.001^{***}$  (Substrate mixtures  $\times$  *Brachypodium* sowing  $\chi^2=2.06$ ,  $df=4$ ,  $p=0.726$  n.s.).

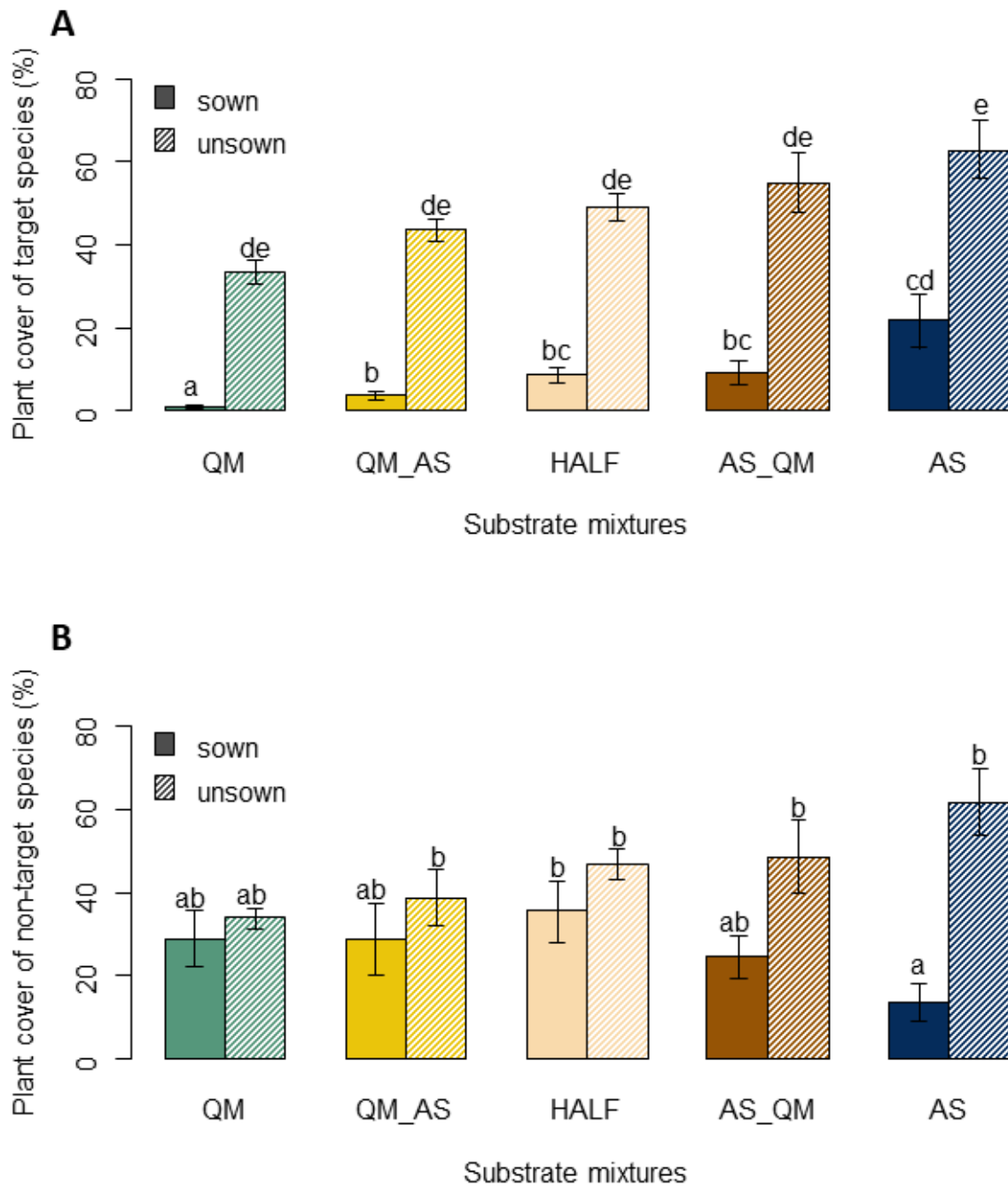


Figure 5. Mean plant cover ( $\pm$  standard error) (without *Brachypodium*) of A. target species and B. non-target species on experimental plots in 2020. Plots were sown (dark grey) or not (light grey) with *Brachypodium*. Substrate mixtures included QM – Quarry raw Material or AS – Arable Soil (see Table 1 for mixture codes). A. Substrate mixtures  $\times$  *Brachypodium* sowing  $\chi^2=38.57$ ,  $df=4$ ,  $p<0.001^{***}$  (Substrate mixtures  $\chi^2=61.00$ ,  $df=4$ ,  $p<0.001^{***}$ ; *Brachypodium* sowing  $\chi^2=174.19$ ,  $df=1$ ,  $p<0.001^{***}$ ). B. Substrate mixtures  $\times$  *Brachypodium* sowing  $\chi^2=13.62$ ,  $df=4$ ,  $p=0.009^{**}$  (Substrate mixtures  $\chi^2=2.01$ ,  $df=4$ ,  $p=0.735$  n.s.; *Brachypodium* sowing  $\chi^2=17.81$ ,  $df=1$ ,  $p<0.001^{***}$ ).

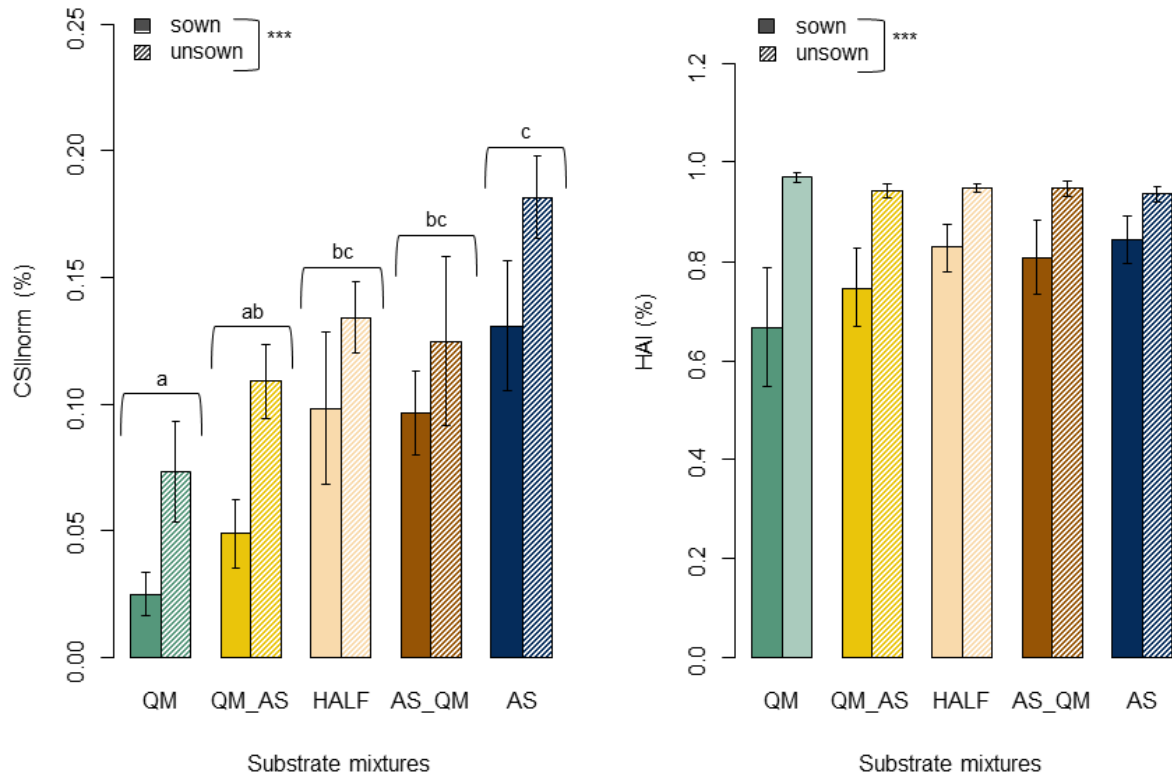


Figure 6. Mean CSII and HAI ( $\pm$  standard error) on experimental plots in 2020. A. CSII: Substrate mixtures  $\chi^2=31.08$ ,  $df=4$ ,  $p<0.001^{***}$ ; *Brachypodium* sowing  $\chi^2=11.89$ ,  $df=1$ ,  $p<0.001^{***}$  (Substrate mixtures  $\times$  *Brachypodium* sowing  $\chi^2=0.815$ ,  $df=4$ ,  $p=0.936$  n.s.). B. HAI: Substrate mixtures  $\chi^2=2.42$ ,  $df=4$ ,  $p=0.660$ ; *Brachypodium* sowing  $\chi^2=19.98$ ,  $df=1$ ,  $p<0.001^{***}$  (Substrate mixtures  $\times$  *Brachypodium* sowing  $\chi^2=4.14$ ,  $df=4$ ,  $p=0.387$  n.s.).

## **Supplement S1:** Physical-chemical properties of mixtures tested at the Ménudelle quarry in the Crau plain

### Soil parameter sampling

The physical and chemical analyses of the soil were performed twice: 1) in 2016 before sowing (35 samples, 6 replicates on each of the 5 substrate mixtures + 5 on the reference grassland) and 2) in 2018 (50 samples, 5 replicates on each of the 5 substrate mixtures \* sowing/no sowing). Each 200-g sample of soil was collected by extracting the first ten centimeters of the soil layer outside the vegetation survey quadrats. Each sample was dried and sieved with a 2 mm mesh sieve. The physical and chemical analyses were performed by the soil analysis laboratory at INRA (*Institut National de la Recherche Agronomique*) in Arras, France. The reference(s) for the method used for each parameter is detailed here: pH water ratio 1:5 (Thomas 1996), CaCO<sub>3</sub> using a Bernard calcimètre (Sparks et al. 1996), organic C (Allison 1965), total N by dry combustion (Bremner 1996; Dumas 1831), C:N ratio, Cation Exchange Capacity (CEC) and exchangeable K<sup>+</sup> by Metson (Metson 1956; Ciesielski et al. 1997), available P (Olsen 1954). Particle-size distribution was studied only in 2016 in 5 fractions following the Robinson method without prior decarbonisation (clay (<0.002 mm), fine silt (0.002-0.02 mm), coarse silt (0.2-2 mm), fine sand (0.05-0.2 mm) and coarse sand (0.2-2 mm)).

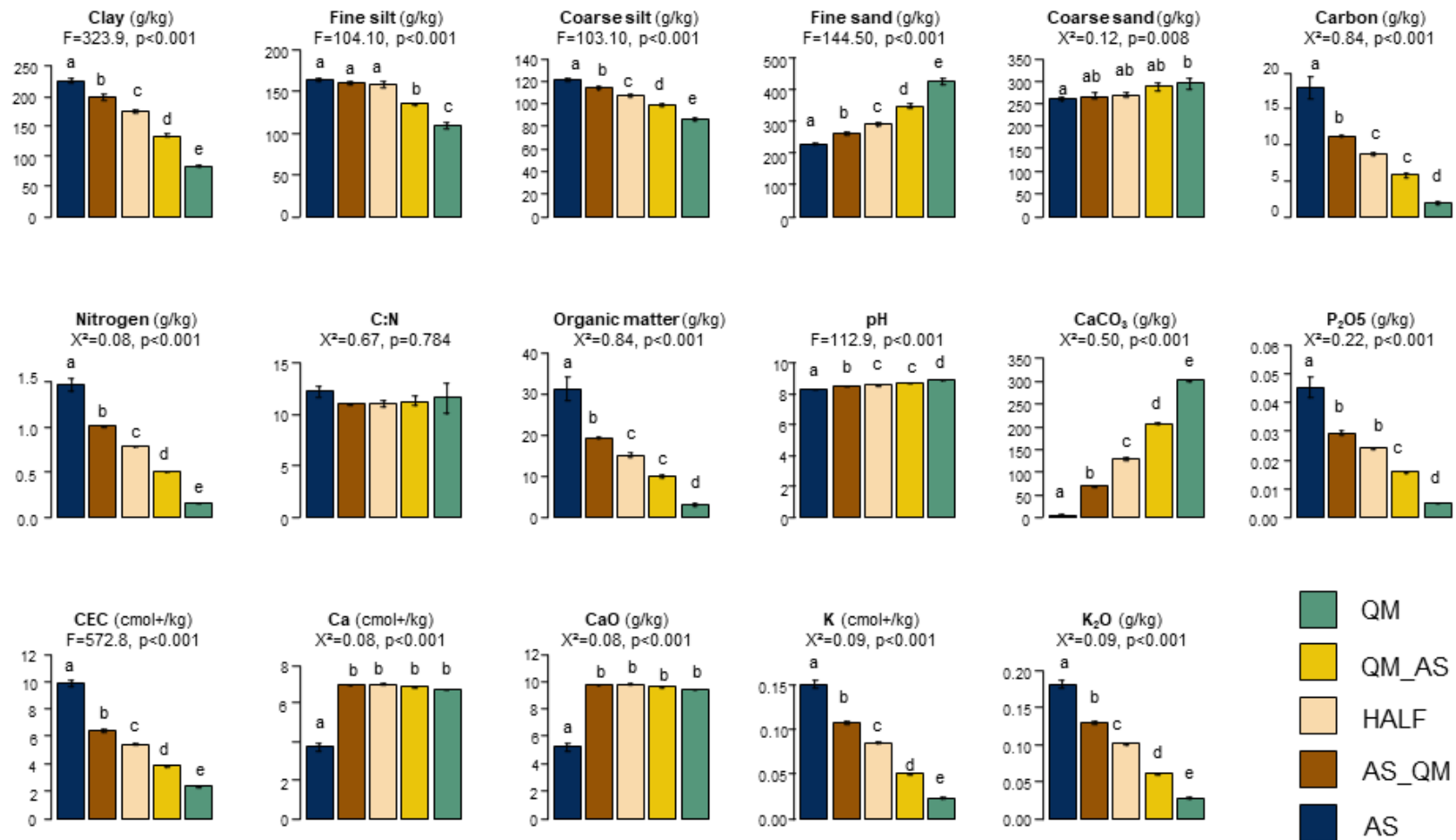
### Data analyses of physical-chemical properties of soils

In order to compare 2016 soil physical and chemical parameter values between substrate mixtures, univariate analyses were performed. When the parametric conditions were met, an ANOVA test, followed by a post-hoc Tukey Honestly Significant Difference test, were performed whenever significant differences were encountered (Stoll 2017). When the parametric conditions were not met, Generalized Linear Models (GLM) under gamma distribution were run. These analyses were followed by pairwise contrast comparisons with a Tukey adjustment. To study the changes in the chemical parameters over years (comparing the 2016 analyses with those of 2018), a principal component analysis (Dray et al. 2020) was run on the chemical data of both years, including that of the reference grassland, to show potentially differences between years and mixtures.

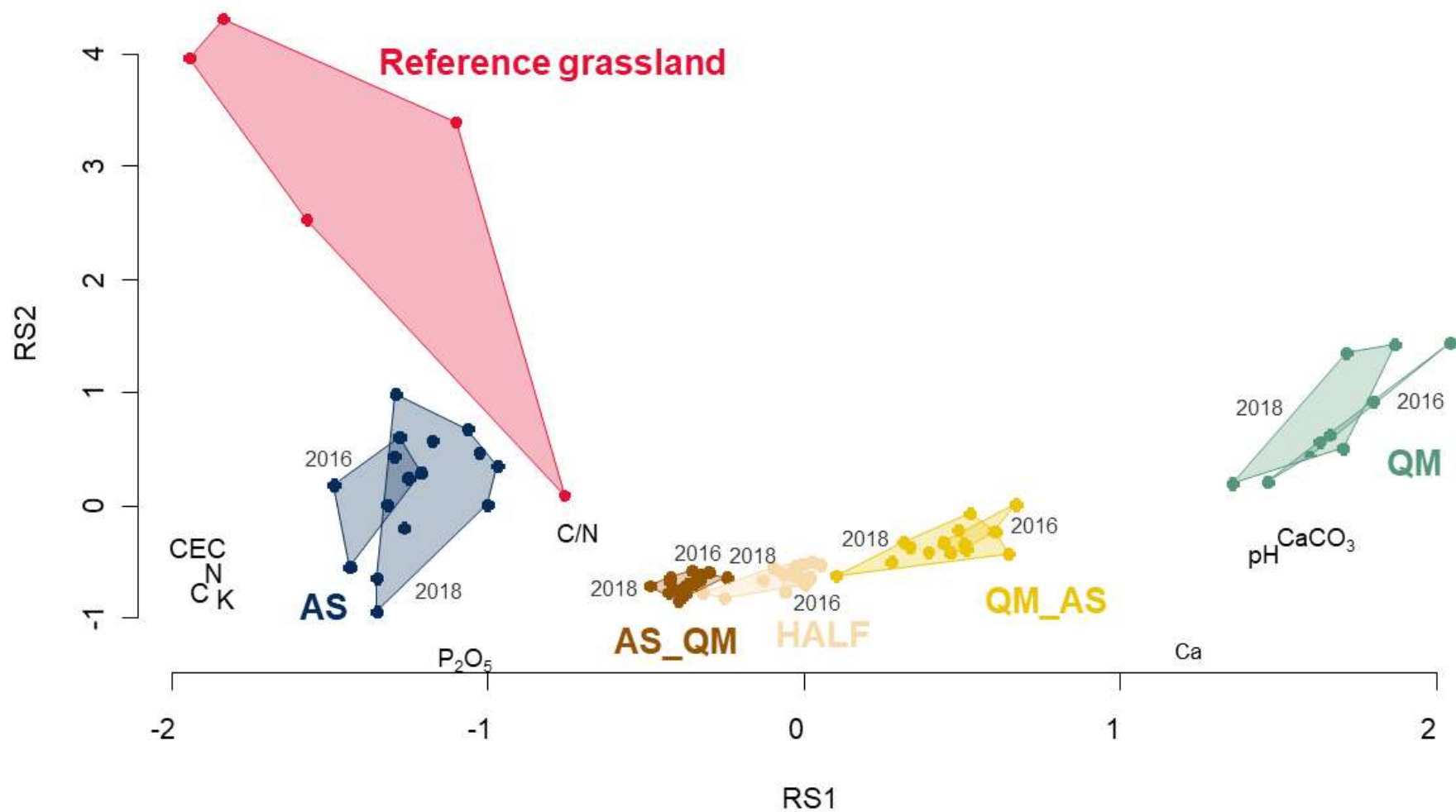
### Patterns of change in the physical and chemical parameters of the soils

The mixture of arable soil with raw quarry material reduced the fertility of the soil (Figure S1). The concentrations of nitrogen, phosphorus and potassium were reduced by a factor of five between the substrates composed entirely of arable soil (Figure S1) and those composed exclusively of raw quarry material. The proportion of sand (fine and coarse) was much greater in the raw quarry material. In contrast, clay was found in greater quantities in the mixtures with arable soil (Figure S1). The raw quarry material was characterized by higher CaCO<sub>3</sub> and Ca content, whereas the arable soil contained significantly more organic matter and organic carbon (Figure S1). Finally, a gradient in the Cationic Exchange Capacity was observed, with greater quantities for the arable soil compared to the raw quarry material (Figure S1).

The PCA (Figure S2) opposed the reference grassland from all experimental plots. Substrate mixtures with higher levels of arable soils had higher phosphorus, nitrogen, potassium and Cationic Exchange Capacity, while substrate mixtures with higher levels of raw quarry materials had higher CaCO<sub>3</sub> and Ca content and higher pH.



**Figure A.** The bars represent the mean  $\pm$  standard error (QM: 100% of raw Quarry Material; QM\_AS: 75% of raw Quarry Material and 25% of Arable Soil; HALF: 50% of each; AS\_QM: 75% of Arable Soil and 25% of raw Quarry Material; AS: 100% of Arable Soil). These analyses were run on 2016 data



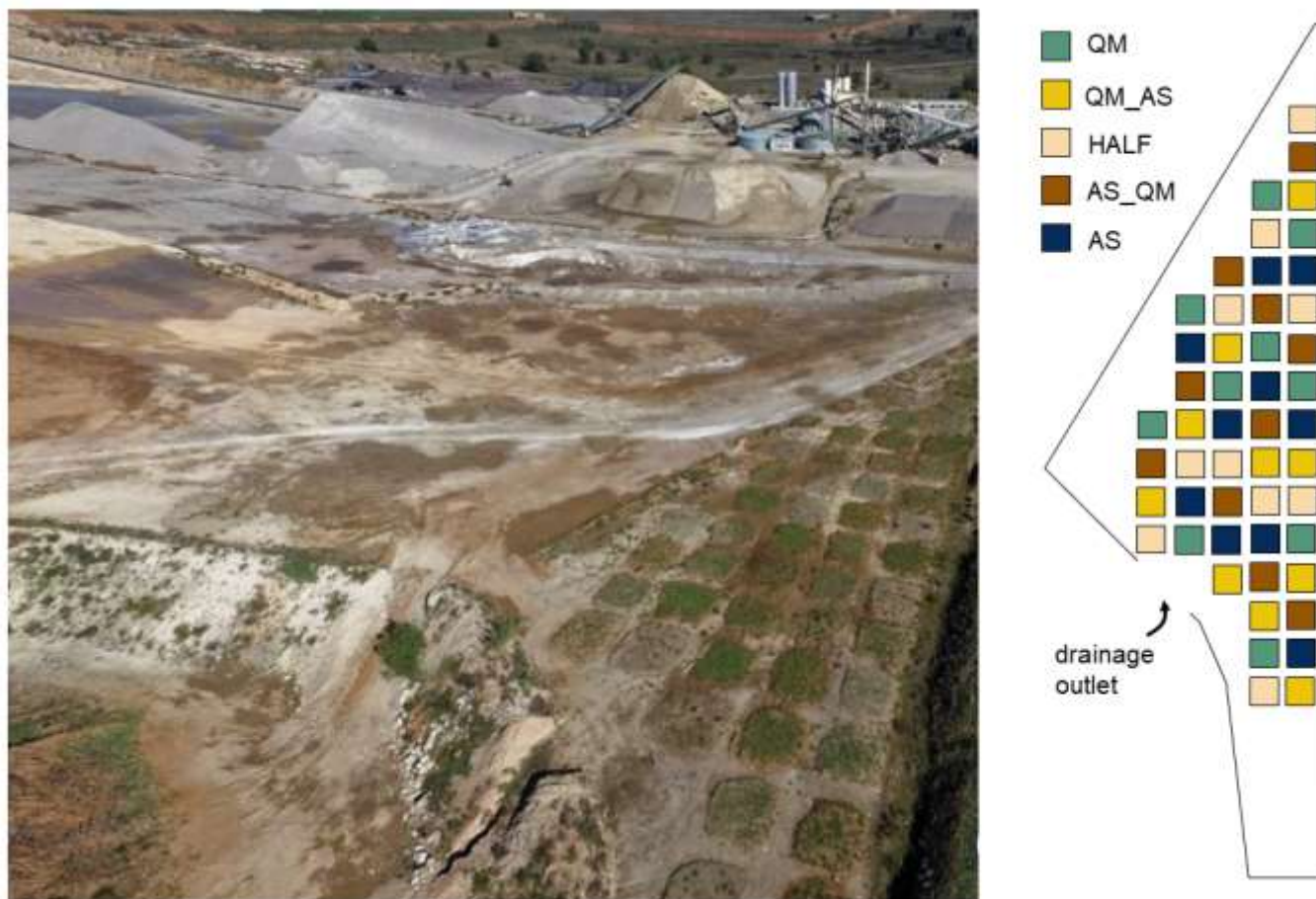
**Figure B.** Principal Component Analysis of chemical soil parameters on the reference grassland and on the experimental plots in 2016 and 2018. QM: 100% of raw Quarry Material; QM\_AS: 75% of raw Quarry Material and 25% of Arable Soil; HALF: 50% of each; AS\_QM: 75% of Arable Soil and 25% of raw Quarry Material; AS: 100% of Arable Soil



- Allison LE (1965) Organic carbon. In: Black, C.A., Evans, D.D., White, J.L., Enisminger L.E., & Clark, F.E., 1965. *Methods of Soil Analysis*. American Society of Agronomy, Madison, Wisconsin pp. 1372–1378.
- Bonferroni CE (1936) Teoria statistica delle classi e calcolo delle probabilità. *Publicazioni del R Istituto Superiore di Scienze Economiche e Commerciali di Firenze* 8:1–62
- Bremner JM (1996) Nitrogen-Total. In: Sparks, D.L., Page, A.L., Helmke, P.A., Loeppert, R.H., Soltanpour, P.N., Tabatabai, M.A., Johnston, C.T., & Sumner, M.E., 1996. *Methods of soil analysis. Part 3 – chemical methods*. Vol. 5 Soil Science Society of America Book Series pp. 1085–1121.
- Ciesielski H, Sterckeman T, Santerne M, Willery JP (1997) A comparison between three methods for the determination of cation exchange capacity and exchangeable cations in soils. *Agronomy for Sustainable Development* 17:9–15
- Coelho R, Infante P, Santos MN (2013) Application of Generalized Linear Models and Generalized Estimation Equations to model at-haulback mortality of blue sharks captured in a pelagic longline fishery in the Atlantic Ocean. *Fisheries Research* 145:66–75
- Dray S, Dufour A-B, Thioulouse J (2020) ade4: Analysis of Ecological Data: Exploratory and Euclidean Methods in Environmental Sciences. *Comprehensive R Archive Network (CRAN)*. <https://CRAN.R-project.org/package=ade4>
- Dumas J (1831) Procédés de l'analyse organique. *Annal. Chem. Phys.*
- McCullagh P (1984) Generalized linear models. *European Journal of Operational Research* 16:285–292
- Metson AJ (1956) *Methods of chemical analysis for soil survey samples*. New Zealand Department of Scientific and Industrial Research
- Olsen SR (Sterling R (1954) Estimation of available phosphorus in soils by extraction with sodium bicarbonate. Washington, D.C. : U.S. Dept. of Agriculture
- Sparks DL, Page AL, Helmke PA, Loeppert RH, Soltanpour P, Tabatabai MA, Johnston CT, Sumner ME (1996) *Methods of soil analysis. Part 3 - chemical methods*. 1390
- Stoll A (2017) Post Hoc Tests: Tukey Honestly Significant Difference Test. In: *The SAGE Encyclopedia of Communication Research Methods*. SAGE Publications, Inc, Thousand Oaks pp. 1306–1307.
- Thomas GW (1996) Soil pH and Soil Acidity. In: Sparks, D.L., Page, A.L., Helmke, P.A., Loeppert, R.H., Soltanpour, P.N., Tabatabai, M.A., Johnston, C.T., & Sumner, M.E., 1996. *Methods of Soil Analysis Part 3 - Chemical Methods*. Vol. 5 Soil Science Society of America Book Series pp. 475–490.
- Wilcoxon F (1945) Individual Comparisons by Ranking Methods. *Biometrics Bulletin* 1:80–83

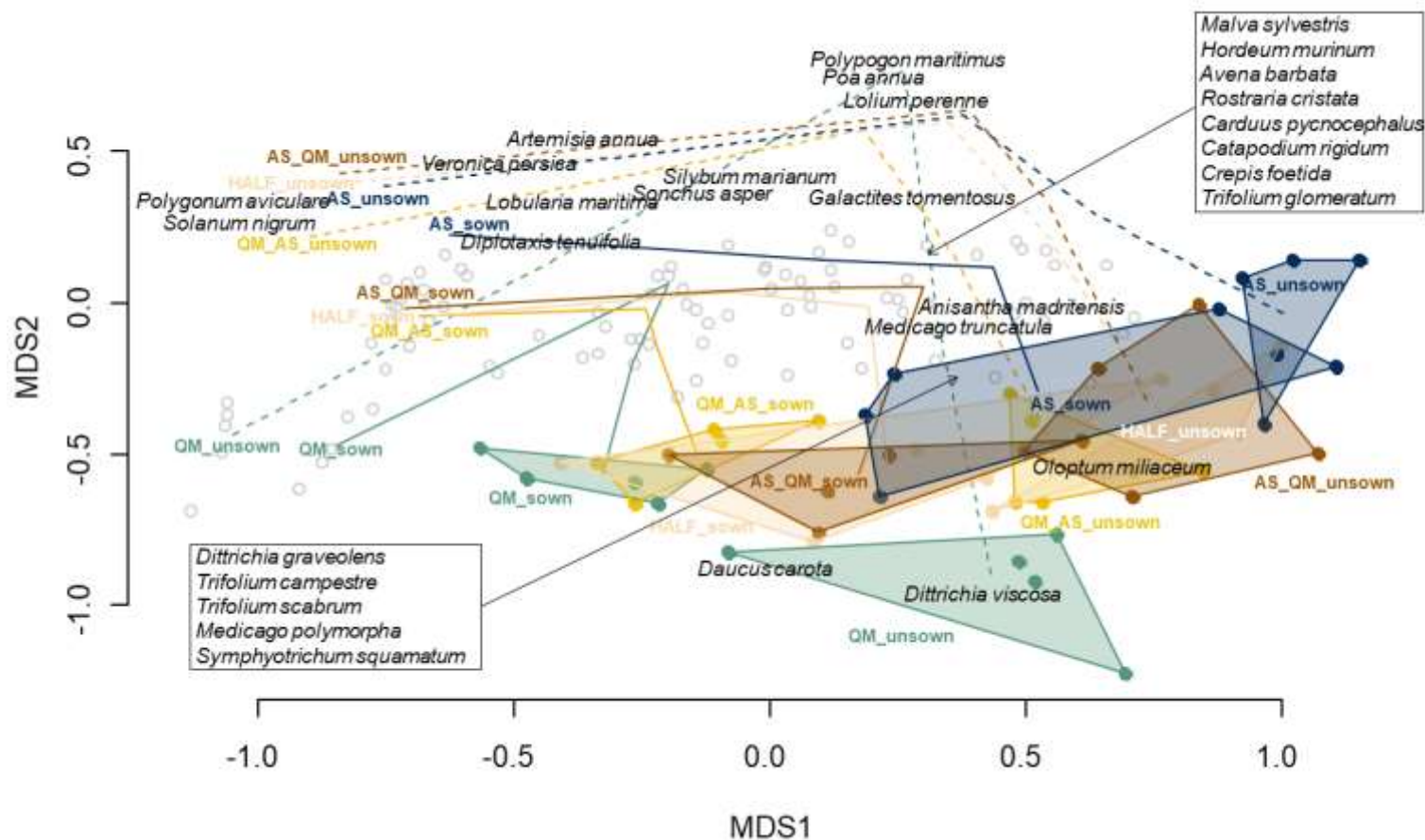


**Supplement S2.** Experimental plots set up in the Ménéudelle quarry in Southeastern France.

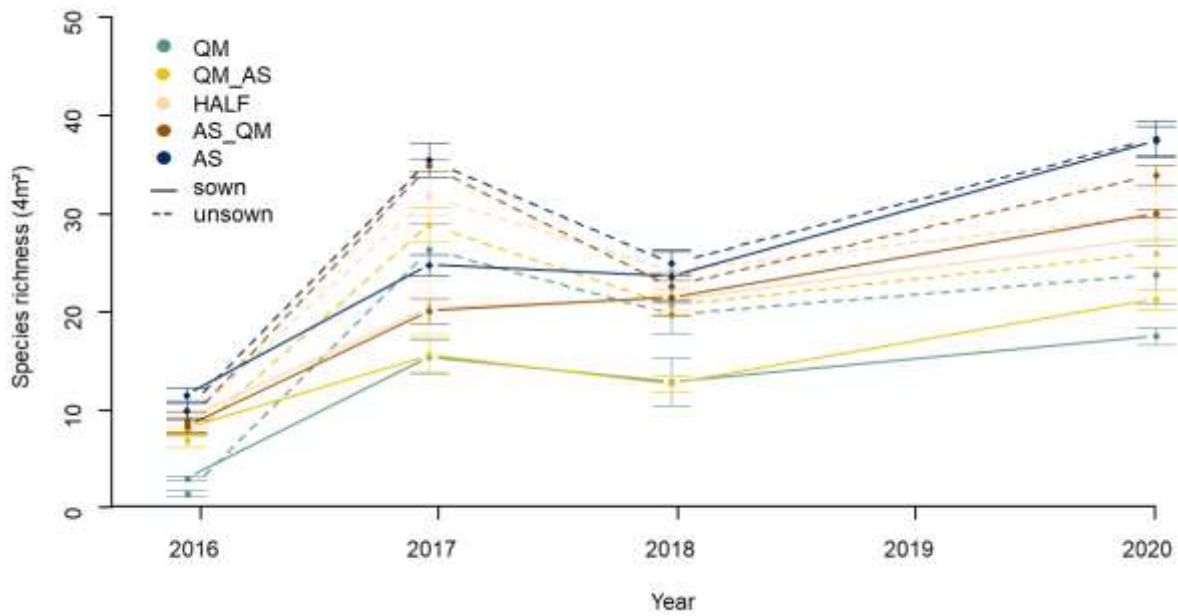


**Figure C.** Experimental plots set up in the Ménéudelle quarry in 2016 (50 plots  $\times$  5 mixtures (QM: 100% of raw Quarry Material; QM\_AS: 75% of raw Quarry Material and 25% of Arable Soil; HALF: 50% of each; AS\_QM: 75% of Arable Soil and 25% of raw Quarry Material; AS: 100% of Arable Soil)  $\times$  2 sowing treatments + a drainage outlet) Photography: SCLM

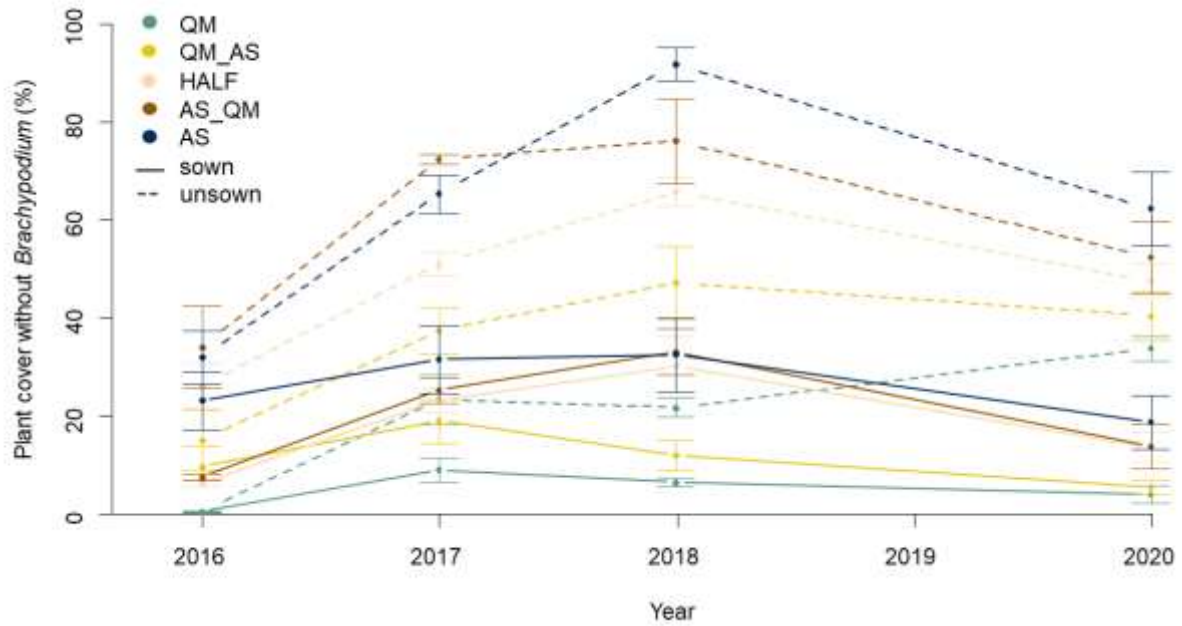
**Supplement S3:** Influence of substrate mixtures, *Brachypodium* sowing and time on plant communities



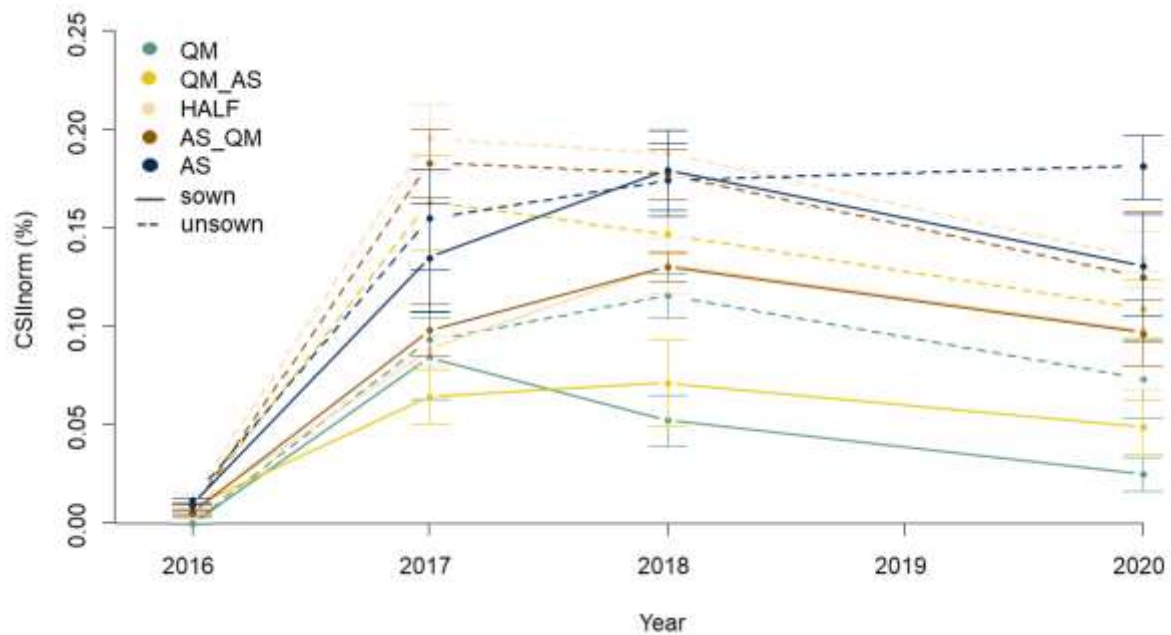
**Figure D.** Plant species composition using NMDS. Polygons indicate of plots with the same treatment in 2020 and lines the trajectory in 2016, 2017, 2018 and 2020 (NMDS stress: 0.21). Plots were sown (sown) or not (unsown) with *Brachypodium*. Substrate mixtures included QM: 100% of raw Quarry Material; QM\_AS: 75% of raw Quarry Material and 25% of Arable Soil; HALF: 50% of each; AS\_QM: 75% of Arable Soil and 25% of raw Quarry Material; AS: 100% of Arable Soil



**Figure E.** Mean species richness on experimental plots in 2016, 2017, 2018 and 2020. Plots were sown (solid lines) or not (dashed lines) with *Brachypodium*. Substrate mixtures included QM – raw Quarry Material or AS – Arable Soil (see Table 1 for mixture codes). Substrate mixtures  $\times$  *Brachypodium* sowing  $\chi^2=9.40$ ,  $p=0.052$ ; Substrate mixtures  $\times$  Years  $\chi^2=27.49$ ,  $p=0.007^{**}$ ; *Brachypodium* sowing  $\times$  Years  $\chi^2=34.75$ ,  $p<0.001^{***}$ . QM: 100% of raw Quarry Material; QM\_AS: 75% of raw Quarry Material and 25% of Arable Soil; HALF: 50% of each; AS\_QM: 75% of Arable Soil and 25% of raw Quarry Material; AS: 100% of Arable Soil

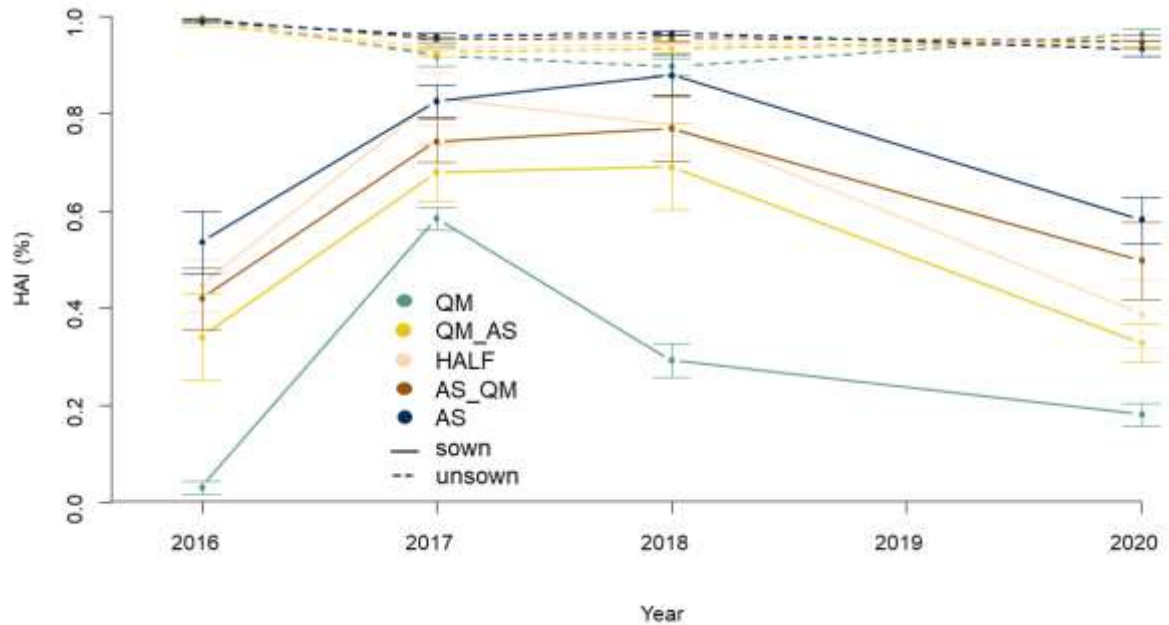


**Figure F.** Mean plant cover (without *Brachypodium*) on experimental plots in 2016, 2017, 2018 and 2020. Plots were sown (solid lines) or not (dashed lines) with *Brachypodium*. Substrate mixtures included QM – raw Quarry Material or AS – Arable Soil (see Table 1 for mixture codes). Substrate mixtures  $\times$  *Brachypodium* sowing  $\chi^2=152.13$ ,  $p<0.001^{***}$ ; Substrate mixtures  $\times$  Years  $\chi^2=49.96$ ,  $p<0.001^{***}$ ; *Brachypodium* sowing  $\times$  Years  $\chi^2=47.98$ ,  $p<0.001^{***}$ . QM: 100% raw quarry material; QM: 100% of raw Quarry Material; QM\_AS: 75% of raw Quarry Material and 25% of Arable Soil; HALF: 50% of each; AS\_QM: 75% of Arable Soil and 25% of raw Quarry Material; AS: 100% of Arable Soil



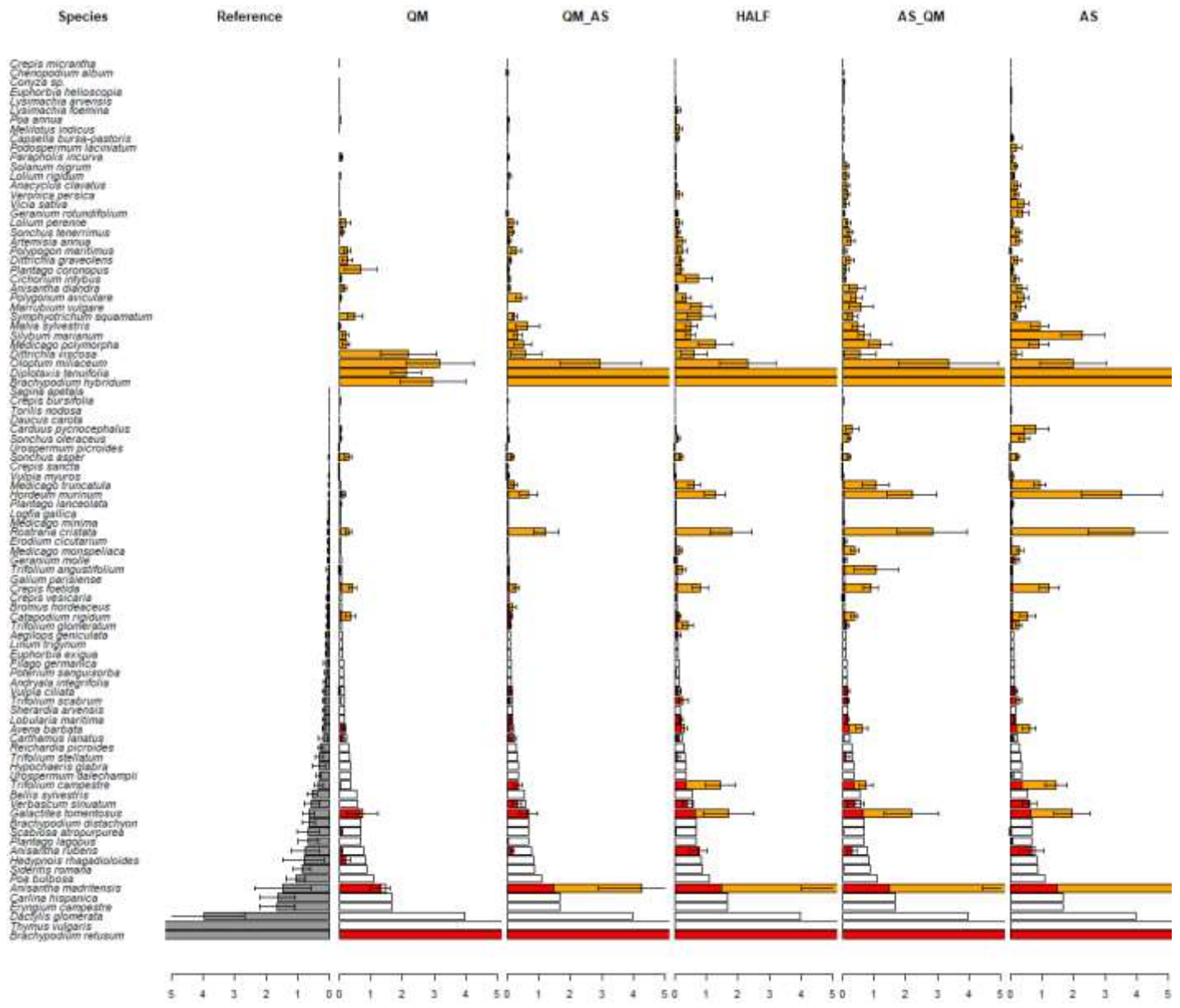
**Figure G.** Mean CSII on experimental plots in 2016, 2017, 2018 and 2020. Plots were sown (solid lines) or not (dashed lines) with *Brachypodium*. Substrate mixtures included QM – raw Quarry Material or AS – Arable Soil (see Table 1 for mixture codes). Substrate mixtures  $\times$  *Brachypodium* sowing  $\times$  Years  $\chi^2=26.24$ ,  $p=0.010^*$ . QM: 100% of raw Quarry Material; QM\_AS: 75% of raw Quarry Material and 25% of Arable Soil; HALF: 50% of each; AS\_QM: 75% of Arable Soil and 25% of raw Quarry Material; AS: 100% of Arable Soil





**Figure H.** Mean HAI on experimental plots in 2016, 2017, 2018 and 2020. Plots were sown (solid lines) or not (dashed lines) with *Brachypodium*. Substrate mixtures included QM – raw Quarry Material or AS – Arable Soil (see Table 1 for mixture codes). Substrate mixtures  $\times$  *Brachypodium* sowing  $\chi^2=152.13$ ,  $p<0.001^{***}$ ; Substrate mixtures  $\times$  *Brachypodium* sowing  $\times$  Years  $\chi^2=105.95$ ,  $p<0.001^{***}$ . QM: 100% raw quarry material; QM: 100% of raw Quarry Material; QM\_AS: 75% of raw Quarry Material and 25% of Arable Soil; HALF: 50% of each; AS\_QM: 75% of Arable Soil and 25% of raw Quarry Material; AS: 100% of Arable Soil

**Supplement S4:** Structures of plant communities recorded on the different substrate mixtures tested against the reference grassland plant community (grey: mean abundance of species recorded in the reference; white: abundance lacking in relation to the reference; orange: abundance of target species present in the community assessed; red: excess abundance of species present in the communities assessed and not in the reference). (QM: 100% of raw Quarry Material; QM\_AS: 75% of raw Quarry Material and 25% of Arable Soil; HALF: 50% of each; AS\_QM: 75% of Arable Soil and 25% of raw Quarry Material; AS: 100% of Arable Soil)





- 1 **Supplement S5:** Species recorded in 2020 on the different substrate mixtures for the two sowing treatments (with or without sowing of  
2 *Brachypodium*) and the reference grassland. QM: 100% of raw Quarry Material; QM\_AS: 75% of raw Quarry Material and 25% of Arable Soil;  
3 HALF: 50% of each; AS\_QM: 75% of Arable Soil and 25% of raw Quarry Material; AS: 100% of Arable Soil  
4

Genus	species	QM		QM_AS		HALF		AS_QM		AS		Grassland
		sown	unsown	sown	unsown	sown	unsown	sown	unsown	sown	unsown	
<i>Aegilops</i>	<i>geniculata</i>				x	x	x	x	x	x	x	x
<i>Aira</i>	<i>cupaniana</i>									x		x
<i>Ajuga</i>	<i>iva</i>					x						
<i>Anacyclus</i>	<i>clavatus</i>	x			x	x	x	x	x	x	x	
<i>Andryala</i>	<i>integrifolia</i>					x		x		x	x	x
<i>Anisantha</i>	<i>diandra</i>	x	x		x							
<i>Anisantha</i>	<i>madritensis</i>	x	x	x	x	x	x	x	x	x	x	x
<i>Anisantha</i>	<i>rubens</i>	x	x	x	x	x	x	x	x	x	x	x
<i>Arenaria</i>	<i>leptoclados</i>					x			x			x
<i>Artemisia</i>	<i>annua</i>						x	x	x	x	x	
<i>Artemisia</i>	<i>verlotiorum</i>						x				x	
<i>Asphodelus</i>	<i>ayardii</i>											x
<i>Avena</i>	<i>barbata</i>		x	x	x	x	x	x	x	x	x	x
<i>Bellis</i>	<i>sylvestris</i>											x
<i>Bituminaria</i>	<i>bituminosa</i>		x				x					
<i>Bothriochloa</i>	<i>ischaemum</i>											x
<i>Brachypodium</i>	<i>distachyon</i>											x
<i>Brachypodium</i>	<i>hybridum</i>	x	x	x	x	x	x	x	x	x	x	
<i>Brachypodium</i>	<i>retusum</i>	x	x	x	x	x		x		x		x
<i>Bromus</i>	<i>hordeaceus</i>	x			x	x	x			x	x	x
<i>Bupleurum</i>	<i>semicompositum</i>						x	x			x	
<i>Carduus</i>	<i>nigrescens</i>				x							x
<i>Carduus</i>	<i>pycnocephalus</i>	x			x	x	x	x	x	x	x	x
<i>Carduus</i>	<i>tenuiflorus</i>						x			x		

<i>Carlina</i>	<i>hispanica</i>												X
<i>Carlina</i>	<i>lanata</i>						X	X	X		X		X
<i>Carthamus</i>	<i>lanatus</i>		X	X	X		X	X	X	X	X		
<i>Catapodium</i>	<i>rigidum</i>	X	X	X	X	X	X	X	X	X	X	X	X
<i>Centaureum</i>	<i>erythraea</i>												X
<i>Centaurea</i>	<i>melitensis</i>												X
<i>Cerastium</i>	<i>glomeratum</i>	X									X		
<i>Cerastium</i>	<i>pumilum</i>		X				X		X				X
<i>Chondrilla</i>	<i>juncea</i>		X				X						
<i>Cichorium</i>	<i>intybus</i>					X					X		
<i>Clinopodium</i>	<i>nepeta</i>		X										
<i>Convolvulus</i>	<i>arvensis</i>						X	X	X	X			
<i>Convolvulus</i>	<i>cantabrica</i>			X	X				X	X	X		
<i>Conyza</i>	<i>sp.</i>		X		X			X	X	X	X		
<i>Crepis</i>	<i>bursifolia</i>		X	X	X			X	X	X			
<i>Crepis</i>	<i>foetida</i>	X	X	X	X	X	X	X	X	X	X	X	X
<i>Crepis</i>	<i>micrantha</i>		X	X		X	X	X		X			
<i>Crepis</i>	<i>sancta</i>			X	X	X		X	X	X	X	X	X
<i>Crepis</i>	<i>vesicaria</i>		X	X		X	X	X	X	X	X	X	X
<i>Cynodon</i>	<i>dactylon</i>	X				X				X	X		
<i>Cynosurus</i>	<i>echinatus</i>						X						X
<i>Dactylis</i>	<i>glomerata</i>				X	X	X		X				X
<i>Daucus</i>	<i>carota</i>	X	X	X	X	X	X	X	X	X	X		
<i>Diplotaxis</i>	<i>tenuifolia</i>	X	X	X	X	X	X		X	X	X		
<i>Dittrichia</i>	<i>graveolens</i>	X	X	X	X	X	X	X	X	X	X	X	
<i>Dittrichia</i>	<i>viscosa</i>	X	X	X	X	X	X	X	X	X	X	X	
<i>Echium</i>	<i>vulgare</i>		X		X		X		X		X		
<i>Erodium</i>	<i>cicutarium</i>							X	X		X	X	
<i>Erodium</i>	<i>malacoides</i>						X		X	X	X		

<i>Erodium</i>	<i>moschatum</i>	x		x		x			x			
<i>Eryngium</i>	<i>campestre</i>						x					x
<i>Euphorbia</i>	<i>cyparissias</i>					x						x
<i>Euphorbia</i>	<i>exigua</i>											x
<i>Euphorbia</i>	<i>falcata</i>											x
<i>Euphorbia</i>	<i>helioscopia</i>		x						x	x	x	
<i>Euphorbia</i>	<i>segetalis</i>								x			
<i>Euphorbia</i>	<i>serrata</i>		x									
<i>Filago</i>	<i>germanica</i>		x	x	x	x	x			x	x	x
<i>Filago</i>	<i>pygmaea</i>		x							x		x
<i>Filago</i>	<i>pyramidata</i>		x									
<i>Foeniculum</i>	<i>vulgare</i>		x		x		x	x				
<i>Galactites</i>	<i>tomentosus</i>	x	x	x	x	x	x	x	x	x	x	x
<i>Galium</i>	<i>murale</i>											x
<i>Galium</i>	<i>parisiense</i>	x			x				x			x
<i>Geranium</i>	<i>molle</i>			x	x	x	x			x	x	x
<i>Geranium</i>	<i>rotundifolium</i>	x	x			x	x		x	x	x	
<i>Hedypnois</i>	<i>rhagadioloides</i>		x					x		x		x
<i>Hippocrepis</i>	<i>ciliata</i>							x				
<i>Hirschfeldia</i>	<i>incana</i>					x		x		x		
<i>Hordeum</i>	<i>murinum</i>	x	x	x	x	x	x	x	x	x	x	
<i>Hypericum</i>	<i>perfoliatum</i>						x		x			
<i>Hypochaeris</i>	<i>glabra</i>					x			x	x	x	x
<i>Lactuca</i>	<i>serriola</i>											x
<i>Lagurus</i>	<i>ovatus</i>											x
<i>Lathyrus</i>	<i>cicera</i>									x		
<i>Leontodon</i>	<i>tuberosus</i>							x				
<i>Limonium</i>	<i>echioides</i>							x				
<i>Linaria</i>	<i>arvensis</i>											x

<i>Linum</i>	<i>strictum</i>									x		x	x
<i>Linum</i>	<i>trigynum</i>												x
<i>Lobularia</i>	<i>maritima</i>					x	x			x	x	x	x
<i>Logfia</i>	<i>gallica</i>										x		x
<i>Lolium</i>	<i>rigidum</i>	x	x	x	x	x	x	x	x	x	x	x	
<i>Lysimachia</i>	<i>arvensis</i>	x			x		x	x	x	x	x	x	
<i>Lysimachia</i>	<i>foemina</i>		x		x		x		x				
<i>Lysimachia</i>	<i>linum-stellatum</i>												x
<i>Malva</i>	<i>sylvestris</i>	x			x	x	x			x	x	x	
<i>Marrubium</i>	<i>vulgare</i>					x	x			x	x	x	
<i>Medicago</i>	<i>minima</i>	x		x		x		x			x	x	x
<i>Medicago</i>	<i>monspeliaca</i>	x	x	x	x	x	x	x	x	x	x	x	x
<i>Medicago</i>	<i>orbicularis</i>	x		x		x		x					
<i>Medicago</i>	<i>polymorpha</i>	x	x	x	x	x	x	x	x	x	x	x	
<i>Medicago</i>	<i>rigidula</i>	x			x	x	x	x				x	x
<i>Medicago</i>	<i>truncatula</i>	x		x	x	x	x	x	x	x	x	x	x
<i>Melilotus</i>	<i>indicus</i>	x		x	x	x		x	x	x			
<i>Misopates</i>	<i>orontium</i>			x							x		
<i>Neatostema</i>	<i>apulum</i>		x										x
<i>Oloptum</i>	<i>miliaceum</i>	x	x		x		x			x		x	
<i>Pallenis</i>	<i>spinosa</i>			x	x	x		x	x				
<i>Papaver</i>	<i>rhoeas</i>							x				x	
<i>Parapholis</i>	<i>incurva</i>	x	x	x	x	x	x	x	x	x		x	
<i>Petrorhagia</i>	<i>prolifera</i>								x				x
<i>Plantago</i>	<i>afra</i>												x
<i>Plantago</i>	<i>bellardii</i>					x							x
<i>Plantago</i>	<i>coronopus</i>	x	x		x	x	x	x	x	x	x	x	
<i>Plantago</i>	<i>lagopus</i>	x			x				x		x		x
<i>Plantago</i>	<i>lanceolata</i>	x	x			x						x	

<i>Poa</i>	<i>bulbosa</i>												X
<i>Podospermum</i>	<i>laciniatum</i>				X	X	X	X	X	X	X		
<i>Polygonum</i>	<i>aviculare</i>							X					
<i>Polypogon</i>	<i>maritimus</i>	X											
<i>Polycarpon</i>	<i>tetraphyllum</i>												X
<i>Poterium</i>	<i>sanguisorba</i>		X	X		X	X						X
<i>Prospero</i>	<i>autumnale</i>												X
<i>Psilurus</i>	<i>incurvus</i>												X
<i>Reichardia</i>	<i>picroides</i>												X
<i>Rostraria</i>	<i>cristata</i>	X	X	X	X	X	X	X	X	X	X	X	X
<i>Rumex</i>	<i>pulcher</i>								X				X
<i>Sagina</i>	<i>apetala</i>		X		X		X		X	X	X	X	X
<i>Scabiosa</i>	<i>atropurpurea</i>	X	X	X	X			X			X	X	X
<i>Scolymus</i>	<i>hispanicus</i>									X	X		
<i>Scorpiurus</i>	<i>muricatus</i>		X							X			
<i>Senecio</i>	<i>vulgaris</i>												X
<i>Sherardia</i>	<i>arvensis</i>							X	X	X	X		X
<i>Sideritis</i>	<i>romana</i>		X						X				X
<i>Silene</i>	<i>gallica</i>												X
<i>Silene</i>	<i>nocturna</i>			X			X	X	X	X			X
<i>Silybum</i>	<i>marianum</i>	X	X	X	X	X	X	X	X	X	X	X	
<i>Sonchus</i>	<i>oleraceus</i>				X				X				X
<i>Sonchus</i>	<i>asper</i>	X	X	X	X	X	X	X	X	X	X	X	X
<i>Sonchus</i>	<i>tenerrimus</i>					X		X		X	X		
<i>Stipa</i>	<i>capillata</i>												X
<i>Symphotrichum</i>	<i>squamatum</i>	X	X	X	X	X	X	X	X	X	X	X	
<i>Taeniatherum</i>	<i>caput-medusae</i>												X
<i>Thymus</i>	<i>vulgaris</i>												X
<i>Tolpis</i>	<i>umbellata</i>												X

<i>Torilis</i>	<i>nodosa</i>								x			
<i>Tragopogon</i>	<i>porrifolius</i>		x			x		x	x			
<i>Trifolium</i>	<i>angustifolium</i>	x	x	x	x	x	x	x	x	x	x	
<i>Trifolium</i>	<i>campestre</i>	x	x	x	x	x	x	x	x	x	x	x
<i>Trifolium</i>	<i>cherleri</i>							x				x
<i>Trifolium</i>	<i>glomeratum</i>			x	x	x	x	x	x	x	x	x
<i>Trifolium</i>	<i>scabrum</i>	x	x	x	x	x	x	x	x	x	x	x
<i>Trifolium</i>	<i>stellatum</i>					x		x	x			x
<i>Trifolium</i>	<i>suffocatum</i>						x				x	
<i>Trifolium</i>	<i>tomentosum</i>						x			x		
<i>Urospermum</i>	<i>dalechampii</i>		x									x
<i>Urospermum</i>	<i>picroides</i>		x		x	x	x	x	x	x	x	x
<i>Valantia</i>	<i>muralis</i>								x			
<i>Verbascum</i>	<i>sinuatum</i>		x	x	x		x		x	x	x	x
<i>Veronica</i>	<i>arvensis</i>							x		x	x	
<i>Veronica</i>	<i>persica</i>				x		x	x	x		x	
<i>Veronica</i>	<i>polita</i>										x	
<i>Vicia</i>	<i>lathyroides</i>									x		
<i>Vicia</i>	<i>sativa</i>						x	x		x	x	
<i>Vulpia</i>	<i>ciliata</i>	x	x	x	x	x	x	x	x	x	x	x
<i>Vulpia</i>	<i>myuros</i>	x	x	x	x	x	x		x	x	x	x

5

6