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## Species transfer via topsoil translocation: lessons from two large Mediterranean restoration projects

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1 **Species transfer via topsoil translocation: lessons from two large Mediterranean**  
2 **restoration projects**

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7 **Running head: Mediterranean grassland topsoil translocation**

8  
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23  
24  
25 **Author contributions**

26 EB identified the article content, wrote the first version of the article and managed the  
27 manuscript preparation; all coauthors collected the data; RJ analyzed the data, produced all  
28 figures and participated in writing the discussion; all coauthors edited the various versions of  
29 the manuscript.

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32

33 **Abstract**

34 Restoration success of species-rich grasslands is often limited by low seed dispersal. To  
35 reintroduce target species of local provenances, bulk topsoil transfer is performed by  
36 excavating soil and vegetation from donor sites and spreading these on receptor sites. Our  
37 first objective was to determine the most advantageous season for transferring topsoil in  
38 Mediterranean grasslands. The second objective was to assess which treatment - combination  
39 of season (spring or autumn) and transfer ratio (1:1 or 1:3) - performed best in restoring a  
40 Mediterranean grassland through bulk topsoil transfer after two large restoration projects.  
41 Just-in-time soil transfer (i.e. with no stockpiling) was implemented 1) on a former 3-ha  
42 orchard where topsoil was spread after removing trees and levelling soil, and 2) after a 5-ha  
43 oil pipeline leak where polluted soil was removed prior to treatment (soil horizons were  
44 reconstituted). A seed bank study showed that the summer seed bank contained higher seed  
45 densities, species richness and similarity to the reference site than the spring seed bank.  
46 Spring transfers gave better results than autumn transfers in terms of species richness and  
47 composition similarity with the reference site, while transfer ratios gave similar results. Long-  
48 term success was not driven by season or transfer ratio but by the underlying seed bank at  
49 receptor sites: the former orchard's weed-containing seed bank hampered topsoil transfer  
50 success. This study also suggests that restoration success cannot be deduced from seed bank  
51 studies alone, as species establishment is highly dependent on differences in growing  
52 conditions (including competition at receptor sites).

53

54 **Keywords:** bulk topsoil transfer; sod dumping; soil inoculation; seed bank; steppe

55

56

57 **Implications for practice**

- 58 • Mediterranean grassland summer seed bank (vs. spring seed bank) contains higher  
59 seed densities and species richness, indicating that summer is better than spring for  
60 transferring seed banks of Mediterranean grasslands; using spring topsoil transfer (vs.  
61 autumn transfer) to restore Mediterranean grasslands gives better results in terms of  
62 species richness and composition similarity with the reference site (summer transfer could  
63 not be tested here);
- 64 • Topsoil transfer success is not greatly impacted by transfer ratio: the tested ratios 1:1  
65 and 1:3 yield identical species richness and composition similarity with the reference  
66 Mediterranean grassland;
- 67 • The underlying seed bank at the receptor sites is an important factor in long-term  
68 topsoil transfer success to restore Mediterranean grasslands.

69  
70  
71 **Introduction**

72 The restoration of species-rich grasslands is often limited by low seed dispersal (Buisson et  
73 al. 2006; Grman et al. 2015; Halassy et al. 2016), both in space and time (e.g. Römermann et  
74 al. 2005). Successful methods of reintroducing target species of local provenances commonly  
75 include sowing seed mixtures and transferring seed-containing hay or soil (Kiehl et al. 2010).  
76 Soil transfer can be accomplished through i) turf transfer, also called community  
77 translocation, or ii) bulk topsoil transfer, also called sod dumping (Bullock 1998; Kiehl et al.  
78 2010; Jaunatre 2014a; Bulot et al. 2014, 2017). Soil transfer means that not only seeds are  
79 transferred, but also other propagules such as rhizomes, small shallow-rooted plants, and  
80 micro-organisms that may play an important role in the restoration of the plant community  
81 (Török et al. 2011; Wubs et al. 2016). Of the two techniques, bulk topsoil transfer is the  
82 cheapest (Good et al. 1999), especially if soil is spread at a ratio lower than 1:1, i.e. spread on  
83 an area larger than where it was gathered (e.g. ratio of 1:2, Good et al. 1999; ratio of 1:3,  
84 Jaunatre et al. 2014a).

85  
86 While both soil transfer techniques are based on extracting soil from donor sites  
87 scheduled to be destroyed and thus are not sustainable, they are fundamentally different. Turf  
88 transfer consists in cutting and transferring turfs and mainly relies on the survival and  
89 colonization potential of the plants contained in the turfs (Bullock 1998; Kardol et al. 2009;

90 Vécrin & Muller 2003). Bulk topsoil transfer, the type explored in this study, consists in  
91 excavating soil and vegetation from donor sites and spreading these on receptor sites, often at  
92 a ratio of 1:1 (see Kiehl et al. 2010 for review); it relies mainly on the availability of viable  
93 soil-stored seeds and other propagules.

94

95 Prior to large-scale bulk topsoil transfer, seed bank studies are often carried out at the  
96 donor sites. First, restorationists need to evaluate the potential of implementing such a  
97 technique: topsoil transfer is worth implementing only if the topsoil contains seeds of target  
98 species, particularly in regions where seeds are not available commercially (Fowler et al.  
99 2015). Using soil seed banks as a source of material for restoration of various plant  
100 communities (forests, marshes, grasslands, heathlands) has been widely explored *via* seed  
101 bank studies (e.g. the review by Bossuyt & Honnay 2008 of 102 studies and the case study by  
102 Kalamees et al. 2012). With the exception of some grasslands, heathlands and early  
103 successional communities, these 103 seed bank studies concluded that restoration cannot rely  
104 solely on seed banks. However, bulk topsoil transfer is still used in many countries and for  
105 the restoration of various types of ecosystems, such as roadsides, meadows, grasslands,  
106 forests, etc., with relative success (Kiehl et al. 2010; Fowler et al. 2015). While this  
107 discrepancy between the findings of seed bank studies and the continuing practice of bulk  
108 topsoil transfer (Fowler et al. 2015) remains insufficiently studied, both bulk topsoil transfer  
109 and seed bank studies continue to be performed.

110 The second reason for performing seed bank studies prior to large-scale bulk topsoil  
111 transfer is to determine the most advantageous time for transfer. Ideally, transfer should be  
112 carried out at a season when a maximum proportion of the target species are found as seeds in  
113 the soil (De Villiers et al. 2004; Hosogi & Kameyama 2004), typically at the end of the  
114 vegetation period and, in the Mediterranean region, before the onset of the autumn rainy  
115 season (Grillas et al. 2004; De Villiers et al. 2004). This is particularly important when  
116 aiming to restore plant communities containing many species with a transient seed bank  
117 (Buisson et al. 2006). The third objective of prior seed bank studies is to permit subsequent  
118 evaluation of restoration success. After topsoil transfer, monitoring is carried out to document  
119 vegetation establishment on the receptor sites; the composition of the vegetation is often  
120 compared with the seed bank and vegetation of the donor sites to evaluate restoration success  
121 (Vécrin & Muller 2003).

122

123 The objectives of this study are 1) using a seed bank study, to assess whether spring or  
124 summer is more advantageous for transferring topsoil to restore Mediterranean grasslands  
125 and 2) using a topsoil transfer experiment, to assess the best combination of season (spring or  
126 autumn) and bulk topsoil transfer ratio (1:1 or 1:3 hereafter termed topsoil transfer ratio) to  
127 restore a Mediterranean grassland plant community. The best transfer time should be  
128 summer, when topsoil contains both species that form a more persistent seed bank and  
129 species that form only a transient seed bank; however, this season could not be tested mainly  
130 due to constraints inherent to worksites. Soil transfers were implemented in autumn and  
131 spring. Our hypotheses are that i) the best transfer time between spring and autumn is spring,  
132 when topsoil contains the few species fruiting in late autumn, ii) the best topsoil transfer  
133 treatment is at a ratio of 1:1 (delivering a higher number of seeds per area unit).

134

## 135 **Methods**

### 136 Study sites

137 The study sites are situated in south-eastern France, in a pseudo-steppe located in the plain of  
138 La Crau which has recently undergone several large restoration projects, three of which  
139 included soil transfer. La Crau is the ancient delta of the Durance River, whose dry grassland  
140 vegetation once covered 60 000 ha. Dominated by perennial species and also including many  
141 annual species, this vegetation has been shaped by a dry and windy Mediterranean climate,  
142 particular soil conditions (i.e. shallow, relatively poor Haplic Cambisol (Calcaric) soil with  
143 siliceous stones, covering about 50% of the ground) and many centuries of itinerant sheep  
144 grazing (Buisson & Dutoit 2006; Figure S1). This now fragmented grassland vegetation, of  
145 which only 11 500 ha remain, serves as a reference ecosystem and donor sites for soil  
146 transfers (Jaunatre et al. 2014b). The first restoration site is an area of 357 ha managed from  
147 1987 to 2006 as an orchard, and rehabilitated in 2008 as part of the first mitigation banking  
148 experiment launched by the French government (Dutoit et al. 2013). Concurrently with this  
149 rehabilitation project, restoration trials were carried out on this orchard: direct topsoil transfer  
150 from La Crau dry Mediterranean grassland (with no stockpiling) was implemented on a few  
151 hectares in spring and autumn 2009 at ratios of 1:1 or 1:3 (partly described in Jaunatre et al.  
152 2014a; Figure S1). Less than one kilometer away from this former orchard (Figure 1), the  
153 grassland soil and vegetation of a 5.5 ha grassland area were completely destroyed by an oil  
154 spill due to an underground pipeline leak in 2009 (second site hereafter referred to as pipeline  
155 leak site). Restoration consisted in excavating the polluted soil down to the bedrock (40 cm)

156 followed by direct topsoil transfer from La Crau dry Mediterranean grassland, reconstituting  
157 soil horizons in spring 2011 (Bulot et al. 2017; Figure S1).

158

#### 159 Seed bank study

160 To identify the season when the topsoil seed bank of the reference site has the highest seed  
161 content, ten replicate 2.5 L soil samples were taken at the site of Peau de Meau, in the center  
162 of the plain of La Crau, in April and August 2001 (Table 1; Römermann et al. 2005). These  
163 20 samples were left to germinate following the protocol described hereafter. Each soil  
164 sample taken in the field consisted of 10 pooled subsamples, as recommended by Bakker et  
165 al. (1996) taken between 0 and 20 cm deep using a 4-cm diameter soil corer. The seedling  
166 emergence method (Ter Heerdt et al. 1996) was selected to qualify and quantify germinable  
167 seeds in the soil seed banks. Soil samples were concentrated by washing them with water on  
168 two different sieves (2 mm and 200  $\mu\text{m}$ ) to reduce bulk and clay. The concentrated soil was  
169 spread in a thin layer (0.5 cm) on plastic trays filled with 2 cm vermiculite topped with  
170 medical compresses (mesh size about 100  $\mu\text{m}$ ). The trays were covered with fine gauze to  
171 prevent contamination. All trays were watered frequently from below. Emerged seedlings  
172 were identified, counted and removed weekly. Unknown seedlings were grown for  
173 identification. After three months of cultivation (greenhouses), the samples were allowed to  
174 dry out. Following six weeks of cold stratification (5°C), the samples were cultivated for a  
175 further three months. Vegetation monitoring was carried out in ten replicate 4-m<sup>2</sup> quadrats in  
176 May 2001 (Table 1): we recorded the presence of each species.

177

#### 178 Topsoil transfers and vegetation sampling

179 Direct topsoil transfer of the 20-cm upper soil layer was implemented on the former orchard  
180 in mid-March 2009 at a ratio of 1:3 on 1 ha (Table 1 and 2), and in late October 2009 both at  
181 a ratio of 1:3 on 3 ha (Jaunatre et al. 2014a) and at a ratio of 1:1 on 0.3 ha (Jaunatre et al.  
182 2014a). Direct topsoil transfer was also implemented on the pipeline leak site in spring 2011  
183 at a ratio of 1:1 on 5.5 ha (Table 1 and 2; Bulot et al. 2017). At the former orchard, topsoil  
184 was spread over the orchard soil (Jaunatre et al. 2014a). At the pipeline leak site, all soil  
185 horizons were reconstituted: i) altered bedrock (35–40 cm deep), ii) subsoil (20–35 cm deep)  
186 and iii) topsoil (0–20 cm deep) (Bulot et al. 2017, 2014). Vegetation monitoring was carried  
187 out in May 2010, May 2011 and May 2012 on the former orchard and in May 2012, May  
188 2013 and May 2014 on the pipeline leak site. At each site, a minimum of three replicate 4-m<sup>2</sup>

189 quadrats were sampled (Table 1): we recorded the cover of each species and the total percent  
190 cover of vegetation using a modified Braun-Blanquet scale (Braun-Blanquet et al. 1952): 0.5  
191 for species covering less than 1% of the quadrat, 1 for those covering between 1% and 5%, 2  
192 for those covering between 5% and 25%, 3 for those covering between 25% and 50%, 4 for  
193 those covering between 50% and 75% and 5 for species covering more than 75% of the  
194 quadrat.

195

196

#### 197 Data analysis

198 To identify the best time to transfer topsoil to the former orchard and pipeline leak sites, we  
199 compared spring and summer seed bank characteristics. Differences in composition between  
200 seed banks were assessed using i) species richness and seed density per sample and ii) a  
201 redundancy analysis (RDA; matrix: 39 samples  $\times$  50 species) based on abundance data from  
202 the seed bank, after a Hellinger transformation (Legendre & Gallagher 2001). Differences in  
203 composition between seed banks with above-ground vegetation were assessed using  
204 normalized Community Structure Integrity Indices (CSII<sub>norm</sub>) calculated on plant species  
205 presence / absence data (Jaunatre et al. 2013). To compare means between seasons, GLMs  
206 using a Poisson distribution were performed on species richness and seed abundance data as  
207 they are count data, and a t-test was performed on CSII<sub>norm</sub> as they met parametric  
208 conditions.

209 To compare topsoil transfers implemented to restore Mediterranean dry grassland at the  
210 former orchard and pipeline leak sites, and to test the effects of season and transfer ratio  
211 (treatments = ratio-season combination) on success, we compared plant species richness and  
212 composition between the restored sites. Here again, differences in composition were assessed  
213 using i) a normalized Community Structure Integrity Index (CSII<sub>norm</sub>) calculated on plant  
214 species presence / absence data and ii) a non-metric multidimensional scaling (NMDS;  
215 matrix: 33 quadrats  $\times$  99 species) based on Bray–Curtis distance calculated from presence /  
216 absence data. As species richness data is count data, they were analyzed with a Generalized  
217 Linear Model (GLM) procedure using a Poisson distribution to compare means between  
218 restored sites. CSII<sub>norm</sub> met parametric conditions; we therefore performed an ANOVA.  
219 Three pairwise comparisons, with Benjamini-Hochberg adjustment, were carried out: 1) to  
220 compare dates of transfer at the 1:3 ratio on the former orchard, 2) to compare autumn  
221 transfer ratios on the former orchard and 3) to compare the spring 1:3 transfer on the former



222 orchard and the spring 1:1 transfer on the pipeline leak site. To determine the combined effect  
223 of year (1, 2 and 3 years after transfer), and treatments (ratio-season combination) on  
224 CSII<sub>norm</sub> we performed two-way ANOVA on repeated measures, followed by Tukey  
225 honestly significant difference post-hoc tests.

226 All analyses were performed with R software 3.3.2 (R Core Team 2016) and its ‘vegan’  
227 package (Oksanen et al. 2017).

228

## 229 **Results**

### 230 **Seed bank**

231 The soil seed bank collected in summer contained more species ( $15.2 \pm 0.6$  species / sample)  
232 than that collected in early spring ( $5.4 \pm 1.0$  species / sample) ( $z = 45.08$ ,  $p < 0.001$ ; Figure 2a).

233 The seed bank collected in summer also contained more seeds ( $101.1 \pm 8.3$  seeds / sample)  
234 than that collected in early spring ( $8.7 \pm 1.8$  seeds / sample) ( $z = -852.86$ ,  $p < 0.001$ ; Figure 2b).

235 Although they shared many species, the two seed bank communities were separated along the  
236 RDA first axis (Figure 3). Spring samples were differentiated from summer samples by the  
237 presence of *Sagina apetala*, absent from the summer seed bank. The summer seed bank  
238 samples were characterized by species like *Gastridium ventricosum*, *Vulpia* sp., *Aira*  
239 *cupaniana*, *Catapodium rigidum*, *Galium parisiense*, *Bromus hordeaceus* and *Melica ciliata*  
240 which were not, or only rarely, found in the spring seed bank. The samples collected in spring  
241 were more dispersed on the RDA ordination than those collected in summer. Most of the  
242 species found in the spring and summer seed bank were species found in the reference  
243 grassland (thus target species), although similarity was below 0.4 for both seasons (Figure 3;  
244  $t = -6.58$ ;  $p < 0.001$ ). Out of the 1089 germinations, we identified 50 species (14 in spring and  
245 38 in summer), only 5 of which were non-target species. *Senecio vulgaris* (49 germinations),  
246 which mainly germinated outside the summer seed bank, is naturally and sporadically found  
247 in La Crau grasslands. The other four species, *Verbena officinalis* (65 germinations, 13 in  
248 spring and 52 in summer), *Kickxia elatine* (3 germinations, 1 in spring and 2 in summer)  
249 *Chenopodium album* (2 germinations, 1 in spring and 1 in summer) and *Portulaca oleracea*  
250 (2 germinations in spring) were not found in the above-ground grassland vegetation; they are  
251 arable weeds dispersed from formerly irrigated fields found around the study site  
252 (Römermann et al. 2005; Buisson et al. 2006).

253

### 254 **Topsoil transfers**

255 The year following topsoil transfer, species richness ( $z = 106.59$ ;  $p < 0.001$ ) and similarity to  
256 the reference site (CSII<sub>norm</sub>;  $F = 14.35$ ;  $p < 0.001$ ) significantly differed between treatments  
257 (ratio-season combination): both these variables were higher for spring soil transfer than for  
258 autumn soil transfer on the former orchard (see results of post-hoc tests, Figure 4).  
259 Moreover, for the spring transfers, similarity to the reference site was slightly significantly  
260 higher with the 1:1 transfer ratio on the pipeline leak site than with the 1:3 transfer ratio on  
261 the former orchard (Figure 4). Transfer ratios did not affect species richness and similarity to  
262 the reference site for the former orchard. In spring, similarity was higher for the pipeline leak  
263 site transfer at a ratio of 1:1 than for the former orchard transfer at a ratio of 1:3 (Figure 4).  
264 Overall, the plant compositions of the different topsoil transfers were rather similar, with  
265 many species characteristic of the reference grassland such as *Carlina corymbosa*, *Thymus*  
266 *vulgaris* or *Trifolium stellatum* (Figure 5). However the topsoil transferred onto the pipeline  
267 leak site had a slightly different plant composition characterized by ruderal species like  
268 *Cardamine hirsuta* or *Lolium perenne*. The topsoil transferred onto the former orchard was  
269 characterized by ruderal species like *Bromus diandrus*, *Chenopodium album*, *Hirschfeldia*  
270 *incana* and *Lactuca serriola* (Figure 5).

271 From the second year after transfers, only the spring pipeline leak site transfer at a  
272 ratio of 1:1 retained high similarity to the reference site, above 80%. For all transfers  
273 implemented at the former orchard site, regardless of transfer season or ratio, similarity  
274 dropped to below 60% (Figure 6).

275 **Discussion**

276 Topsoil transfer timing

277 The La Crau Mediterranean dry grassland has a mainly transient seed bank (Buisson et al.  
278 2006). Since most of the plants are annuals and spend the harsh season (summer) as seeds in  
279 the seed bank, the most advantageous season for topsoil transfer should clearly be summer.  
280 Moreover, seeds can be expected to germinate in autumn: in a Mediterranean climate, not  
281 only is germination triggered by temperature and light, but it also occurs only after a  
282 threshold amount of precipitation (Ne'eman & Goubitz 2000). Differences cannot simply be  
283 explained by dormancy cycles, as germination of annual Mediterranean species depends on  
284 the timing and amount of autumn precipitation (Espigares & Peco 1993, 1995). The results of  
285 our seed bank study support our first hypothesis, showing that species richness and seed  
286 density and similarity of species composition between seed bank and above-ground  
287 vegetation of the reference site are much higher in the summer than in the spring seed bank.  
288 The latter is more dispersed on the community ordination because only a few seeds  
289 germinated in each spring sample (Römermann et al. 2005), giving each species a higher  
290 weight than the species composing the more abundant summer seed bank community.

291 While summer appears to be the most appropriate season for transferring the seed  
292 bank, restoration work cannot always be implemented when it would be ideal in terms of  
293 resilience of the restored plant community. This is mainly due to constraints inherent to  
294 worksites and because restoration planning usually takes into account not only the phenology  
295 of plant communities but also that of endangered plant species and of animals, such as  
296 nesting birds (Buisson et al. 2017). Thus, topsoil transfer was never implemented in summer  
297 to restore our Mediterranean dry grassland.

298

299 Season for Mediterranean dry grassland topsoil transfer

300 Topsoil transfer was not carried out in summer on the Mediterranean grassland due to site  
301 preparation delays (soil levelling on the former orchard and polluted soil excavation on the  
302 pipeline leak site; see Jaunatre et al. 2014a; Bulot et al. 2014; 2017 for details). As many  
303 annual species germinate with the first autumn rains, it was difficult to hypothesize that either  
304 autumn or spring would be the best transfer season. In the first year, spring topsoil transfer  
305 led to high species richness on the restoration site and high similarity with the reference site  
306 (ca. 80%) compared to autumn transfer (slightly over 40%). This may be partly due to the  
307 fact that a few species, such as *Bellis sylvestris*, *Lobularia maritima*, flower and produce

308 seeds mainly in autumn and are thus added to the pool of potentially germinable species in  
309 spring. In this study, these two species germinated with both transfers but their frequency of  
310 occurrence was higher on the restoration sites where spring topsoil transfers were performed.  
311 We observed annual Fabaceae common in the reference grassland, such as *Medicago minima*,  
312 *Medicago monspeliaca*, *Medicago truncatula*, *Trifolium cherleri*, *Trifolium scabrum* and  
313 *Trifolium stellatum*, more frequently with the spring than with the autumn transfers. Van  
314 Assche et al. (2003) showed that some species congeneric to those cited here show increased  
315 germination efficiency with cold stratification. They would therefore germinate better after  
316 winter, explaining the results from spring transfers in our study. Surprisingly, numerous  
317 annual species known through seedling emergence studies to have a transient seed bank and  
318 accordingly not found in our spring seed bank study, were found under spring soil transfer.  
319 This may partly be due to the fact that species with low seed production and species whose  
320 germination requirements are narrow are less likely to be detected by seed bank studies  
321 (Saatkamp et al. 2009). Moreover, species whose seeds massively germinate with autumn  
322 rain are hard to detect in spring. It can thus be expected that a wider range of species will  
323 establish from spring topsoil transfer than predicted from seed bank studies (Kalamees &  
324 Zobel 2002). Here, the restoration following polluted soil excavation on the pipeline leak site,  
325 which involved a spring transfer of 41 000 tons of soil, gave better results than predicted *via*  
326 seed bank studies on a few kilos of soil (Bulot et al. 2014).

327

328 Perennial species of this Mediterranean dry grassland do produce seeds that can  
329 germinate in autumn in the field and form a seed bank (e.g. *Dactylis glomerata*; 64  
330 germinations in the summer seed bank vs. 14 in the spring seed bank / 1089 germinations).  
331 Perennial plants also make it through summer drought thanks to the survival of the organs  
332 (meristematic tissues at the bases of leaves, roots, underground storage organs) from which  
333 they regrow once water is available (Volaire et al. 2009; Moreira et al. 2012). They store  
334 carbohydrates during the growing season, before the onset of drought, which allows them to  
335 maintain respiration demands during summer and to initiate resprouting (Volaire et al. 2009;  
336 Moreira et al. 2012). Thus, topsoil transfer in autumn may not have given them enough time  
337 to replenish their reserves to be resilient to a second stressful situation, such as translocation:  
338 *Brachypodium retusum* established better in spring than in autumn, as did *Cynodon dactylon*  
339 and *Bothriochloa ischaemum*, though to a lesser extent. An attempt to translocate perennial  
340 species in summer would show whether experiencing two simultaneous disturbances (drought

341 and translocation), rather than one occurring after the other, provides a better chance of  
342 survival.

343

344 Differences between restored grassland sites

345 Topsoil transfer on the pipeline leak site yielded few ruderal species, and even those were  
346 ruderal species also found on the reference grassland, such as *Cardamine hirsuta* or *Lolium*  
347 *perenne*. Contrastingly, topsoil transfer on the former orchard led to communities dominated  
348 by ruderal species like *Bromus diandrus*, *Chenopodium album* and *Hirschfeldia incana*,  
349 absent from the reference grassland but common on the rehabilitated areas of the former  
350 orchard where topsoil transfer was not performed (Jaunatre et al 2014a). This major  
351 difference between the two restored sites is due to a difference in restoration process: on the  
352 pipeline leak site, the soil horizons were entirely reconstituted, while on the former orchard,  
353 topsoil was spread on top of orchard soil containing its own seed bank of weeds (Jaunatre et  
354 al 2014a). It is likely that the different restoration processes also largely explain the  
355 differences between transfer ratios in spring: the 1:1 ratio soil transfer implemented on the  
356 pipeline leak site produced results more similar to the reference one year after transfer than  
357 the 1:3 ratio soil transfer implemented on the former orchard.

358 Autumn soil transfer ratios on the former orchard did not affect species richness and  
359 similarity to the reference one year after transfer. The representation on the ordination of the  
360 autumn 1:3 transfer ratio on the former orchard is more dispersed, most probably due to the  
361 greater sampling effort.

362 In subsequent years, 80% similarity to the reference site was maintained with the  
363 spring 1:1 ratio topsoil transfer on the pipeline leak site, whereas the spring 1:3 ratio topsoil  
364 transfer on the former orchard led to similarity falling to below 50%. There are two possible  
365 explanations. First, the 1:3 ratio does not transfer enough seeds for the community to be  
366 maintained long-term. However, this is contradicted by the fact that similarity with the  
367 reference is identical with both ratios for the autumn transfers. The second likely explanation  
368 is the differences between sites and restoration processes. The seed bank and soil nutrient  
369 content are affected by cultivation legacy, and there can be long-term effects on plant  
370 communities (Cramer et al. 2008; Smits et al. 2008). On the former orchard, weed seed load  
371 in the seed bank and nutrients in the soil are therefore greater, resulting in high average  
372 vegetation height and cover (Jaunatre et al 2014a). This may have had a negative effect on

373 the germination and growth of less competitive light-demanding grassland target species  
374 (Hautier et al. 2009).

375

376 Seed bank studies help acquire a better understanding of ecosystem functioning and  
377 thus help restorationists plan better transfer timing. However, they do not provide a clear  
378 prediction of restoration success, since they largely underestimate the true seed density in the  
379 seed bank due to detection probabilities, dormancy and germination niches (De Villiers et al.  
380 2004; Fowler et al. 2005). The outcome for bulk soil transfers in Mediterranean ecosystem  
381 restoration depends on several factors: transferred soil depth, whether the soil is stockpiled  
382 and whether soil horizons are reconstituted (Koch 2007; Coiffait-Gombault et al. 2011; Bulot  
383 et al. 2014), the proportion of annual species in the donor site seed bank, and the fertility and  
384 seed bank of the receptor site (Jaunatre et al 2014a; Fowler et al. 2015). Restoration success  
385 is also highly dependent on appropriate scheduling of the various tasks, although in practice  
386 timing is also governed by worksite imperatives. More importantly, restoration implies taking  
387 into account a set of environmental parameters not at play in seed bank studies. The patterns  
388 of some of these parameters can partly be foreseen on worksites (e.g. sites scheduled for  
389 restoration may be colonized by arable weeds germinating from the seed bank dating back to  
390 former agricultural activities; Buisson et al. 2006; Jaunatre et al. 2014a). However, this does  
391 not necessarily mean that they can be dealt with in advance. Ecological restoration will  
392 therefore partly remain a trial and error process and restored sites will require subsequent  
393 adaptive management.

394

395

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403

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523 Table 1. Dry grassland seed bank study and topsoil transfer details, showing protocols as well  
 524 as subsequent vegetation monitoring. FO stands for former orchard and PL for  
 525 pipeline leak site.  
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 527

	<b>reference vegetation</b>	<b>seed bank and season</b>	<b>topsoil transfer monitoring</b>	<b>objectives</b>
<b>sites</b>	3 sites of dry grassland	1 site of dry grassland (Peau de Meau; 43°34'01.85"N, 4°50'07.12"E)	2 sites (FO, PL), see Table 1 for details	to determine the best time to transfer topsoil in Mediterranean grasslands
<b>monitoring</b>	6 × 4m <sup>2</sup> plots / site	Seed bank: 10 2.5-L samples (pooled subsamples) taken between 0 and 20 cm deep, 2 seasons (April, August) Vegetation: 10 × 4m <sup>2</sup> plots	Aut. 1:1 - FO: transfer from Ménudelle 1:1 (3 × 0.1 ha) in autumn: three 4-m <sup>2</sup> plots	to determine the best topsoil transfer treatment (ratio-season combination)
			Aut. 1:3 - FO: transfer from Ménudelle 1:3 (3 × 1 ha) in autumn: eighteen 4-m <sup>2</sup> plots	
			Spring 1:3 - FO: transfer from Massilia 1:3 (1 × 1 ha) in spring: three 4-m <sup>2</sup> plots	
Spring 1:1 - PL (1 × 5.5 ha) in spring: nine 4-m <sup>2</sup> plots				
	vegetation sampling, BB index (total = 18 plots)	germination identification and count, vegetation sampling (presence / absence)	vegetation sampling, BB index	
<b>references</b>	Jaunatre et al. 2014a	Römermann et al. 2005 and unpublished	Jaunatre et al. 2014a,b; Bulot et al. 2017 and unpublished	

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Table 2. Topsoil transfer data used to determine the best topsoil transfer treatment (ratio-season combination). Coordinates of and distance between donor and restored (receptor) sites. Area covered (ha), ratio and season are indicated for each transfer.

donor sites	coordinates	restored (receptor) sites	coordinates	distance (km) between donor and restored sites	Surface area (ha) covered by topsoil transfer	ratio	season	month
Massilia	43°29'03.61"N , 4°53'16.56"E	Former orchard	43°31'07.38"N, 4°51'35.05"E	3.5	1 × 1 ha	1:3	spring	March 2009
Ménudelle	43°30'30.22"N , 4°54'03.14"E				3 × 1 ha	1:3	autum n	Oct. 2009
					3 × 0.1 ha	1:1	autum n	Oct. 2009
		Pipeline leak site	43°31'36.77"N, 4°53'04.50"E	2.5	1 × 5.5 ha	1:1	spring	March- April 2011

Figure 1. Map of the donor and restored (receptor) sites and as well as of the seed bank study site. FO = former orchard and PL = pipeline leak site where dry grassland topsoil transfers were implemented. See Table 1 for details.

Figure 2. a. Species richness, b. seed density density and c. normalized Community structure Integrity Index of the soil seed bank of Peau de Meau, Mediterranean dry grassland (reference grassland), at two seasons (spring: early spring and summer: end of summer).

Figure 3. Soil seed bank ordination of samples from Peau de Meau, Mediterranean dry grassland (reference grassland) gathered at two seasons (spring: early spring and summer: end of summer) based on RDA of plant community composition. For clarity, only the 16 most correlated species are shown (out of 38).

Figure 4. a. Species richness of the areas restored using topsoil transfers from Mediterranean dry grassland and; b. CSII<sub>norm</sub>, normalized Community Structure Integrity Index with reference grassland vegetation monitoring as reference compared with restored communities. Soil gathering season (spring in pink; Aut.: autumn in green), transfer ratio (1:3 light colors; 1:1 dark colors) and receptor sites (Former Orchard; Pipeline Leak site). Error bars represent  $\pm$ SE, horizontal bars represent pairwise comparisons (NS:  $p > 0.05$ ; \*:  $p < 0.05$ ; \*\*\*:  $p < 0.001$ ).

Figure 5. Plant community ordination one year after different topsoil transfers from Mediterranean dry grassland for different soil gathering seasons (spring in pink; autumn in green), transfer ratios (1:3 light colors; 1:1 dark colors) and receptor sites (Former Orchard; Pipeline Leak site) based on NMDS of plant community composition, final stress=0.16. For clarity, only the 19 most correlated species are shown (out of 99).

Figure 6. CSII<sub>norm</sub>, normalized Community Structure Integrity Index with reference grassland vegetation monitoring as reference compared with communities restored using topsoil transfers from Mediterranean dry grassland and interactions with years (1 to 3 years after transfers, from dark to light shades). Soil gathering seasons

(spring in pink; autumn in green), transfer ratios (1:3 light colors; 1:1 dark colors) and receptor sites (Former Orchard; Pipeline Leak site). Error bars represent  $\pm$ SE, bars having a common letter are not significantly different (Tukey honest significant differences post-hoc tests after ANOVA on repeated measures;  $p > 0.05$ ).

Figure 1.

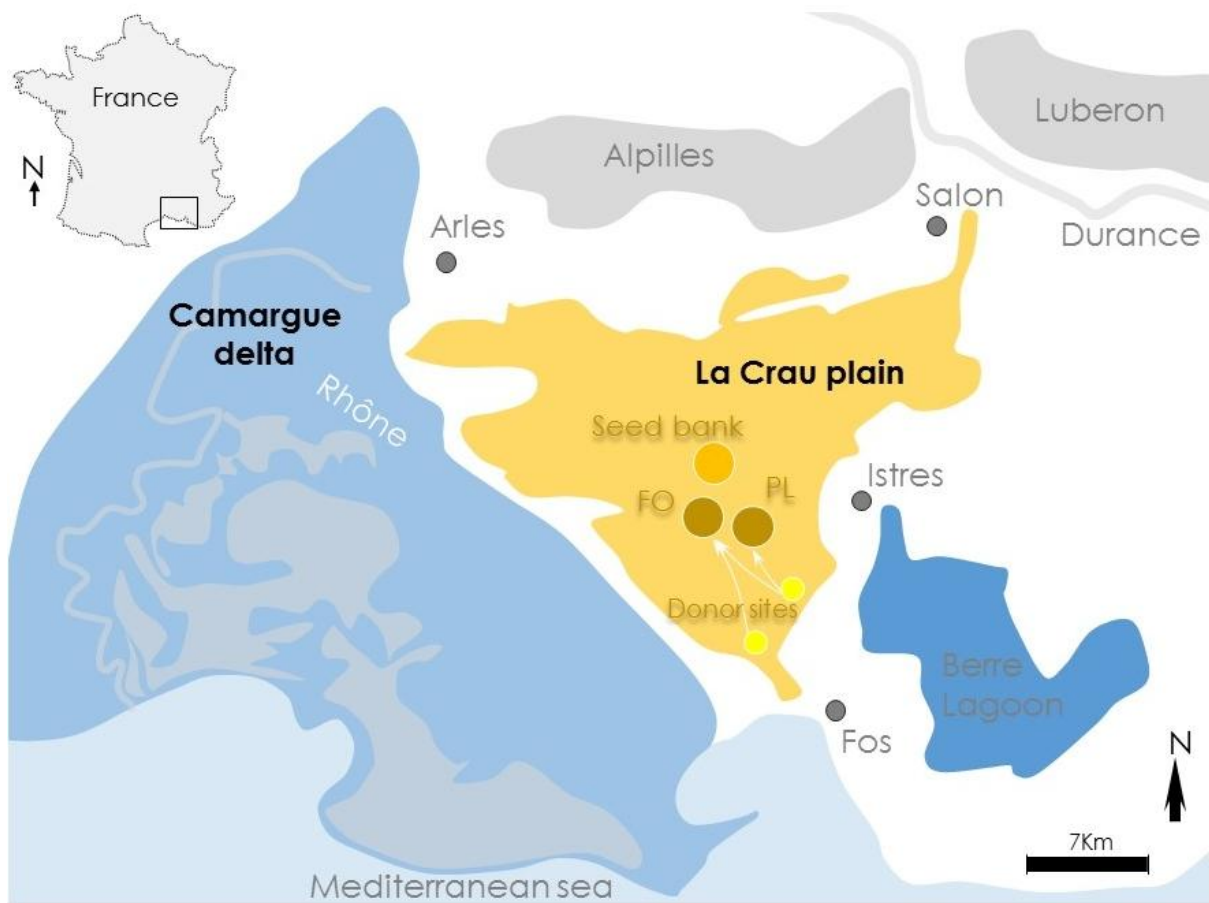


Figure 1. Map of the donor and restored (receptor) sites and as well as of the seed bank study site. FO = former orchard and PL = pipeline leak site where dry grassland topsoil transfers were implemented. See Table 1 for details.

Figure 2.

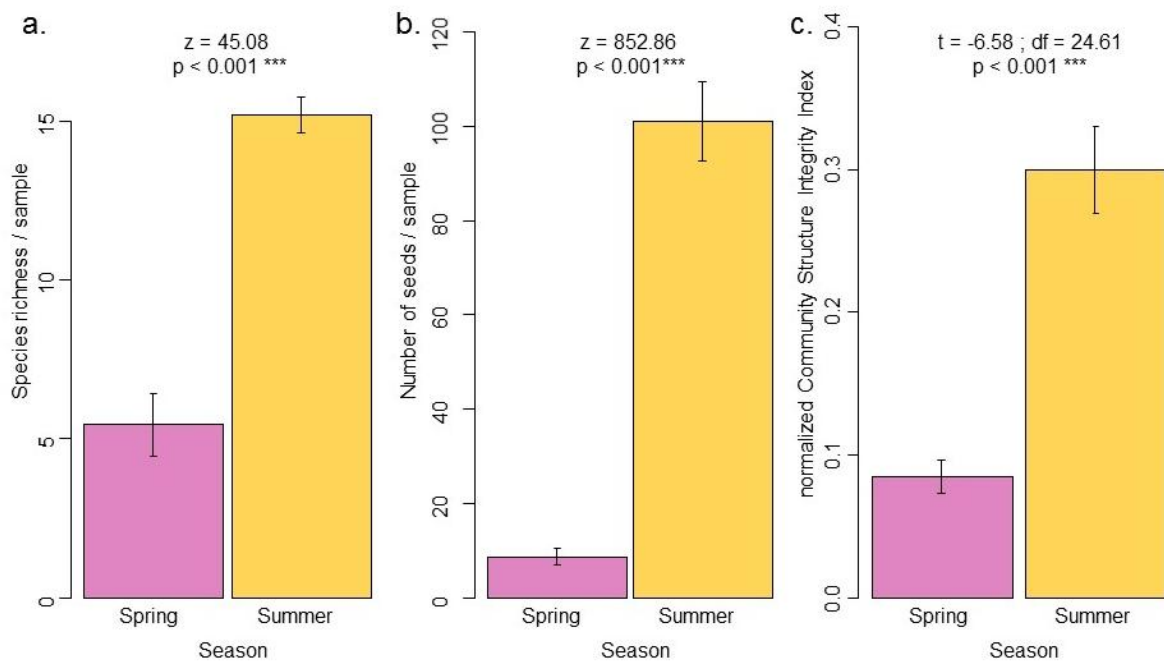


Figure 2. a. Species richness, b. seed density and c. normalized Community structure Integrity Index of the soil seed bank of Peau de Meau, Mediterranean dry grassland (reference grassland), at two seasons (spring: early spring in pink and summer: end of summer in yellow). Error bars represent  $\pm$ SE.



Figure 3.

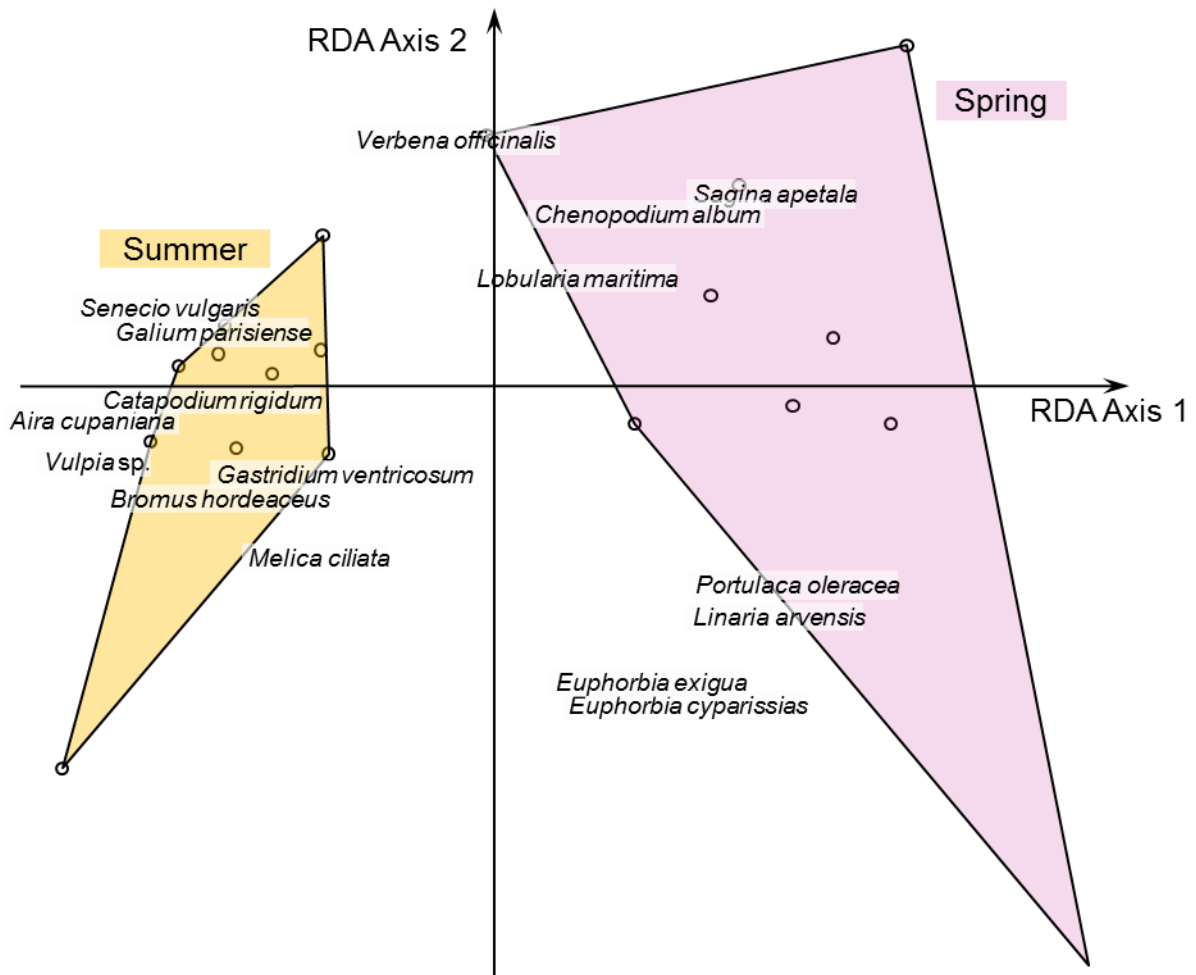


Figure 3. Soil seed bank ordination of samples from Peau de Meau, Mediterranean dry grassland (reference grassland) gathered at two seasons (spring: early spring in pink and summer: end of summer in yellow) based on RDA of plant community composition. For clarity, only the 16 most correlated species are shown (out of 38).

Figure 4.

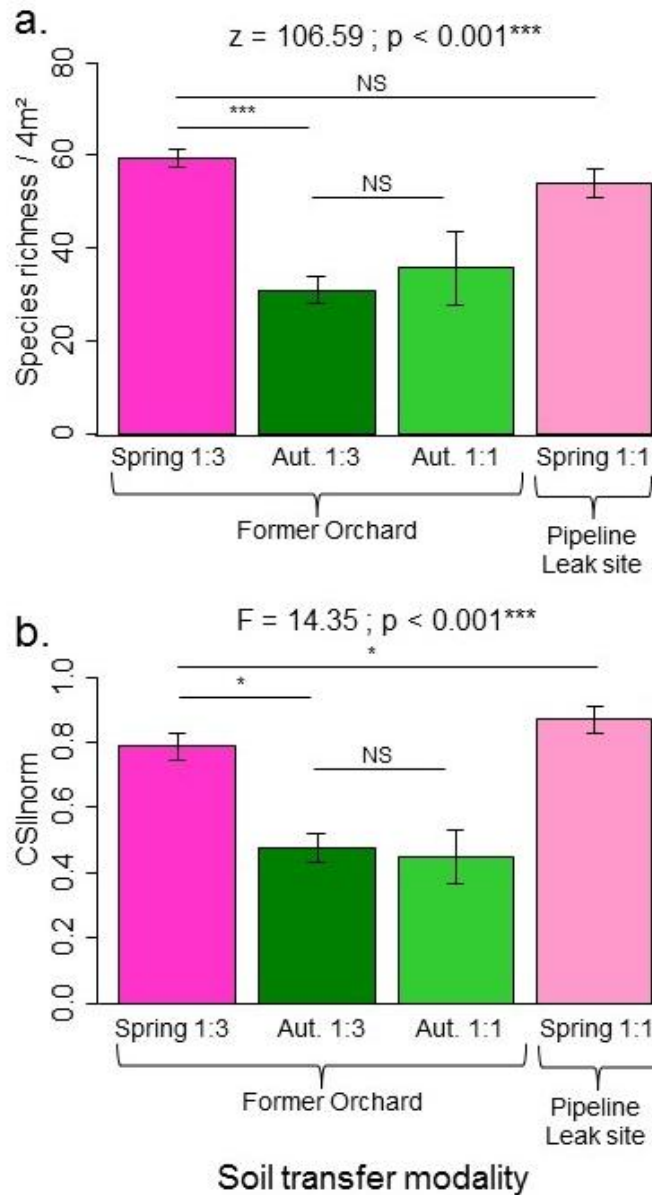


Figure 4. a. Species richness of the areas restored using topsoil transfers from Mediterranean dry grassland and; b. CSInorm, normalized Community Structure Integrity Index with reference grassland vegetation monitoring as reference compared with restored communities. Soil gathering seasons (spring in pink; Aut.: autumn in green), transfer ratios (1:3 light colors; 1:1 dark colors) and receptor sites (Former Orchard; Pipeline Leak site). Error bars represent  $\pm$ SE, horizontal bars represent pairwise comparisons (NS:  $p > 0.05$ ; \*:  $p < 0.05$ ; \*\*\*:  $p < 0.001$ ).

Figure 5.

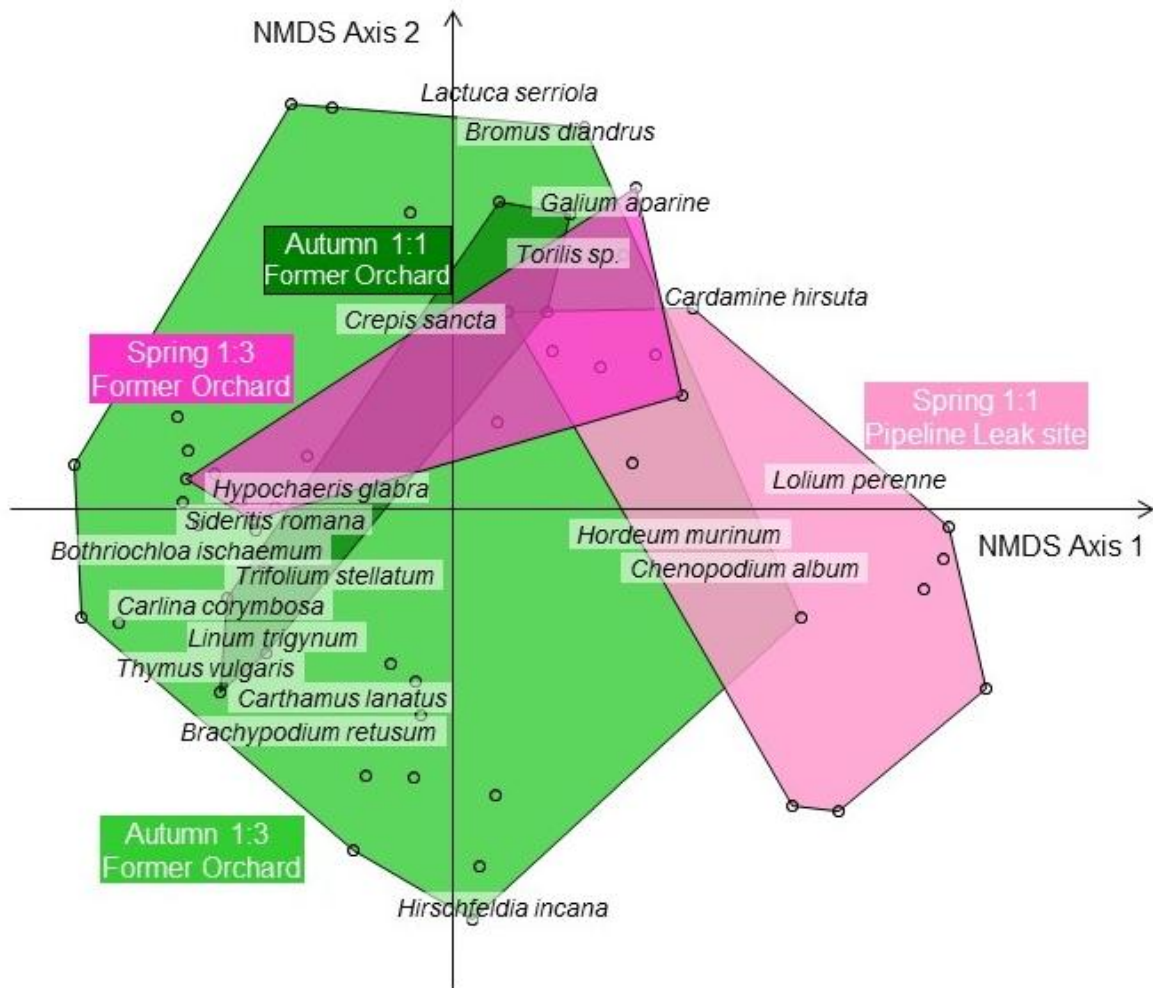


Figure 5. Plant community ordination one year after different topsoil transfers from Mediterranean dry grassland for different soil gathering seasons (spring in pink; autumn in green), transfer ratios (1:3 light colors; 1:1 dark colors) and receptor sites (Former Orchard; Pipeline Leak site) based on NMDS of plant community composition, final stress=0.16. For clarity, only the 19 most correlated species are shown (out of 99).

Figure 6.

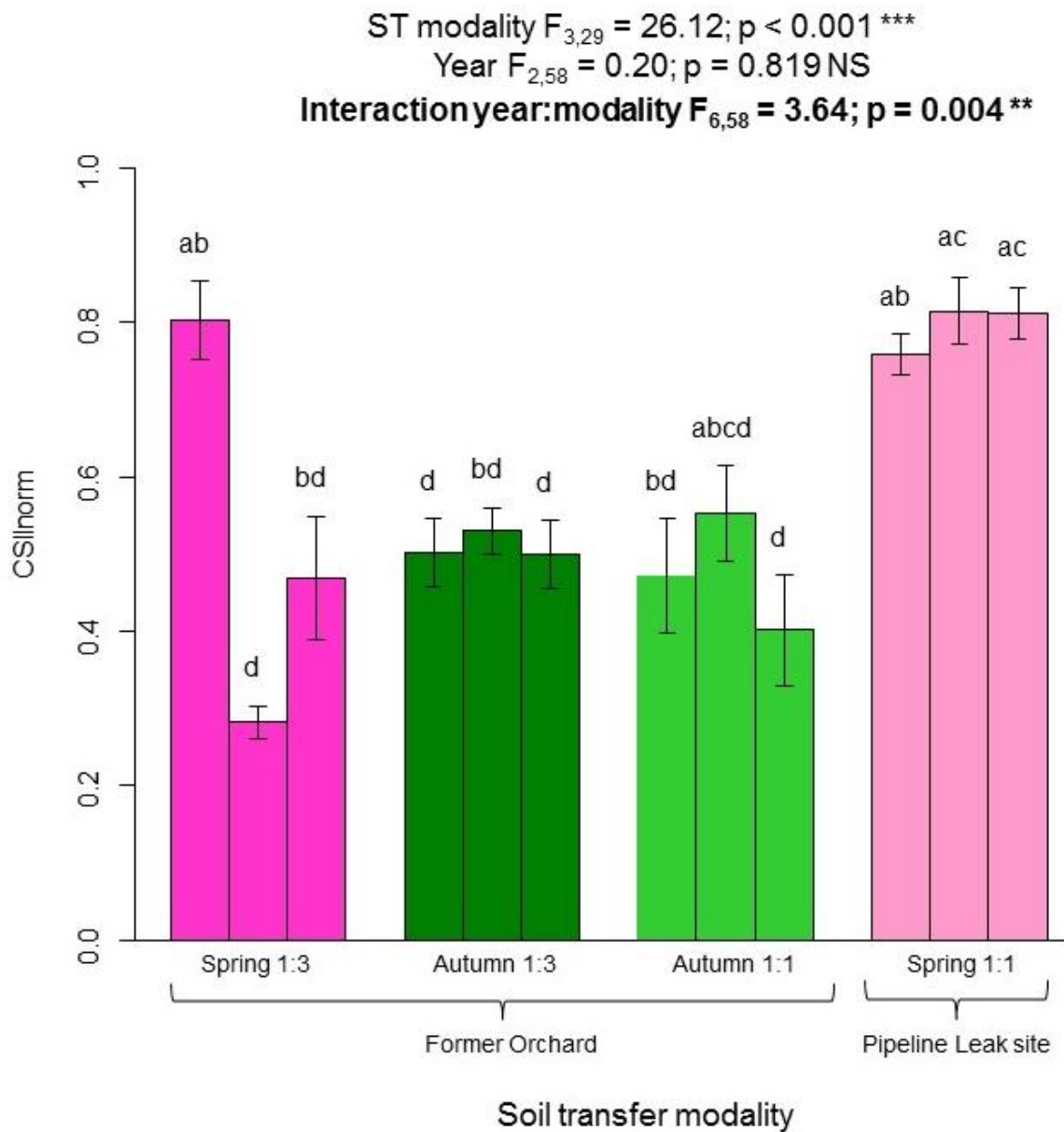


Figure 6. CSInorm, normalized Community Structure Integrity Index with reference grassland vegetation monitoring as reference compared with communities restored using topsoil transfers from Mediterranean dry grassland and interactions with years (1 to 3 years after transfers, from dark to light shades). Soil gathering seasons (spring in pink; autumn in green), transfer ratios (1:3 light colors; 1:1 dark colors) and receptor sites (Former Orchard; Pipeline Leak site). Error bars represent  $\pm$ SE, bars having a common letter are not significantly different (Tukey honest significant differences post-hoc tests after ANOVA on repeated measures;  $p > 0.05$ ).