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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.

30
31
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 Vécirin & 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 (Vécirin & 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 μm) 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 μm). 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-m² 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-m²

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_{norm}) 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_{norm} 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_{norm}) 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_{norm} 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_{norm} 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 ± 0.6 species / sample)
232 than that collected in early spring (5.4 ± 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 ± 8.3 seeds / sample)
234 than that collected in early spring (8.7 ± 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_{norm}; $F = 14.35$; $p < 0.001$) 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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405 **References**

406 Bakker JP, Bakker ES, Rosén E, Verweij GL, Bekker RM (1996) Soil seed bank composition
407 along a gradient from dry alvar grassland to Juniperus shrubland. *Journal of Vegetation*
408 *Science* 7:165–176

409 Bossuyt B, Honnay O (2008) Can the seed bank be used for ecological restoration? An
410 overview of seed bank characteristics in European communities. *Journal of Vegetation*
411 *Science* 19:875–884

412 Braun-Blanquet J, Roussine N, Nègre R (1952) *Les groupements végétaux de la France*
413 *méditerranéenne*. CNRS. Paris, France

414 Buisson E, Alvarado ST, Le Stradic S, Morellato LPC (2017) Plant phenological research
415 enhances ecological restoration. *Restoration Ecology* 25:164–171

416 Buisson E, Dutoit T (2006) Creation of the natural reserve of La Crau: Implications for the
417 creation and management of protected areas. *Journal of Environmental Management*
418 80:318–326

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

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

425 Bulot A, Potard K, Bureau F, Bérard A, Dutoit T (2017) Ecological restoration by soil
426 transfer: impacts on restored soil profiles and topsoil functions. *Restoration Ecology*
427 25:354–366

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

431 Coiffait-Gombault C, Buisson E, Dutoit T (2011) Hay transfer promotes establishment of
432 Mediterranean steppe vegetation on soil disturbed by pipeline construction. *Restoration*
433 *Ecology* 19:214–222

434 Cramer V, Hobbs R, Standish R (2008) What’s new about old fields? Land abandonment and
435 ecosystem assembly. *Trends in Ecology & Evolution* 23:104–112

436 De Villiers AJ, Van Rooyen MW, Theron GK, Cowling RM (2004) The restoration of
437 Strandveld and Succulent Karoo degraded by mining: an enumeration of topsoil seed
438 banks. *South African Journal of Botany* 70:717–725

439 Dutoit T, Jaunatre R, Buisson E (2013) Mediterranean steppe restoration in France. In:
440 Ecological Restoration, Second Edition: Principles, Values, and Structure of an
441 Emerging Profession. Clewell AF, Aronson J (eds) p. 60

442 Espigares T, Peco B (1993) Mediterranean pasture dynamics: the role of germination. *Journal*
443 *of Vegetation Science* 4:189–194

444 Espigares T, Peco B (1995) Mediterranean annual pasture dynamics: impact of autumn
445 drought. *Journal of Ecology* 83:135–142

446 Fowler WM, Fontaine JB, Enright NJ, Veber WP (2015) Evaluating restoration potential of
447 transferred topsoil. *Applied Vegetation Science* 18:379–390

448 Good JEG, Wallace HL, Stevens PA, Radford GL (1999) Translocation of herb-rich
449 grassland from a site in Wales prior to opencast coal extraction. *Restoration Ecology*
450 7:336–347

451 Grillas P, Gauthier P, Yavercovski N, Perennou C (2004) Les mares temporaires
452 méditerranéennes: enjeux de conservation, fonctionnement et gestion vol1. *Station*
453 *biologique de la Tour du Valat, Arles*

454 Grman E, Bassett T, Zirbel CR, Brudvig LA (2015) Dispersal and establishment filters
455 influence the assembly of restored prairie plant communities. *Restoration Ecology*
456 23:892–899

457 Halassy M, Singh AN, Szabó R, Szili-Kovács T, Szitár K, Török K (2016) The application of
458 a filter-based assembly model to develop best practices for Pannonian sand grassland
459 restoration. *Journal of Applied Ecology* 53:765–773

460 Hautier Y, Niklaus PA, Hector A (2009) Competition for light causes plant biodiversity loss
461 after eutrophication. *Science* 324:636–638

462 Hosogi D, Kameyama A (2004) Timing for the collection of topsoil from a deciduous forest
463 for use as planting material in suburban Tokyo, Japan. *Ecological Engineering* 23:371–
464 386

465 Jaunatre R, Buisson E, Muller I, Morlon H, Mesléard F, Dutoit T (2013) New synthetic
466 indicators to assess community resilience and restoration success. *Ecological Indicators*
467 29:468–477

468 Jaunatre R, Buisson E, Coiffait-Gombault C, Bulot A, Dutoit T (2014b) Restoring species-
469 rich Mediterranean dry grassland in France using different species-transfer methods. In:
470 *Guidelines for Native Seed Production and Grassland Restoration*. Kiehl K, Kirmer A,
471 Shaw N (eds), Cambridge, UK pp. 182–197.

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

475 Kalamees R, Zobel M (2002) The role of the seed bank in gap regeneration in a calcareous
476 grassland community. *Ecology* 83:1017-1025

477 Kalamees R, Püssa K, Zobel K, Zobel M (2012) Restoration potential of the persistent soil
478 seed bank in successional calcareous (alvar) grasslands in Estonia. *Applied Vegetation*
479 *Science* 15:208–218

480 Kardol P, Bezemer TM, Van Der Putten WH (2009) Soil organism and plant introductions in
481 restoration of species-rich grassland communities. *Restoration Ecology* 17:258–269

482 Kiehl K, Kirmer A, Donath TW, Rasran L, Hölzel N (2010) Species introduction in
483 restoration projects – Evaluation of different techniques for the establishment of semi-
484 natural grasslands in Central and Northwestern Europe. *Basic and Applied Ecology*
485 11:285–299

486 Koch JM (2007) Alcoa’s mining and restoration process in South Western Australia.
487 *Restoration Ecology* 15:S11–S16

488 Legendre P, Gallagher ED (2001) Ecologically meaningful transformations for ordination of
489 species data. *Oecologia* 129:271–280

490 Moreira B, Tormo J, Pausas JG (2012) To resprout or not to resprout: factors driving
491 intraspecific variability in resprouting. *Oikos* 121:1577–1584

492 Ne’eman G, Goubitz S (2000) Phenology of east-Mediterranean vegetation. In: *Life and*
493 *environment in the Mediterranean*, WIT Press. Trabaud, L., pp 155–202

494 Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, Minchin PR, et al (2017) Package
495 ‘vegan’.

496 R Core Team (2016) *R: A Language and Environment for Statistical Computing*. R
497 Foundation for Statistical Computing, Vienna, Austria

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

501 Saatkamp A, Affre L, Dutoit T, Poschlod P (2009) The seed bank longevity index revisited:
502 limited reliability evident from a burial experiment and database analyses. *Annals of*
503 *Botany* 104:715–724

- 504 Smits NAC, Willems JH, Bobbink R (2008) Long-term after-effects of fertilisation on the
505 restoration of calcareous grasslands. *Applied Vegetation Science* 11:279–286
- 506 Ter Heerdt GNJ, Verweij GL, Bekker RM, Bakker JP (1996) An improved method for seed-
507 bank analysis: seedling emergence after removing the soil by sieving. *Functional*
508 *Ecology* 10:144–151
- 509 Török P, Vida E, Deák B, Lengyel S, Tóthmérész B (2011) Grassland restoration on former
510 croplands in Europe: an assessment of applicability of techniques and costs.
511 *Biodiversity and Conservation* 20:2311–2332
- 512 Van Assche JA, Debucquoy KLA, Rommens WAF (2003) Seasonal cycles in the
513 germination capacity of buried seeds of some Leguminosae (Fabaceae). *New*
514 *Phytologist* 158:315–323
- 515 Vécrin MP, Muller S (2003) Top-soil translocation as a technique in the re-creation of
516 species-rich meadows. *Applied Vegetation Science* 6:271–278
- 517 Volaire F, Norton MR, Lelièvre F (2009) Summer drought survival strategies and
518 sustainability of perennial temperate forage grasses in Mediterranean areas. *Crop*
519 *Science* 49:2386
- 520 Wubs ERJ, Putten WH van der, Bosch M, Bezemer TM (2016) Soil inoculation steers
521 restoration of terrestrial ecosystems. *Nature Plants* 2:16107
- 522

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

	reference vegetation	seed bank and season	topsoil transfer monitoring	objectives
sites	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
monitoring	6 × 4m ² 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 ² plots	Aut. 1:1 - FO: transfer from Ménudelle 1:1 (3 × 0.1 ha) in autumn: three 4-m ² 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 ² plots	
			Spring 1:3 - FO: transfer from Massilia 1:3 (1 × 1 ha) in spring: three 4-m ² plots	
Spring 1:1 - PL (1 × 5.5 ha) in spring: nine 4-m ² plots				
	vegetation sampling, BB index (total = 18 plots)	germination identification and count, vegetation sampling (presence / absence)	vegetation sampling, BB index	
references	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_{norm}, 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_{norm}, 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.

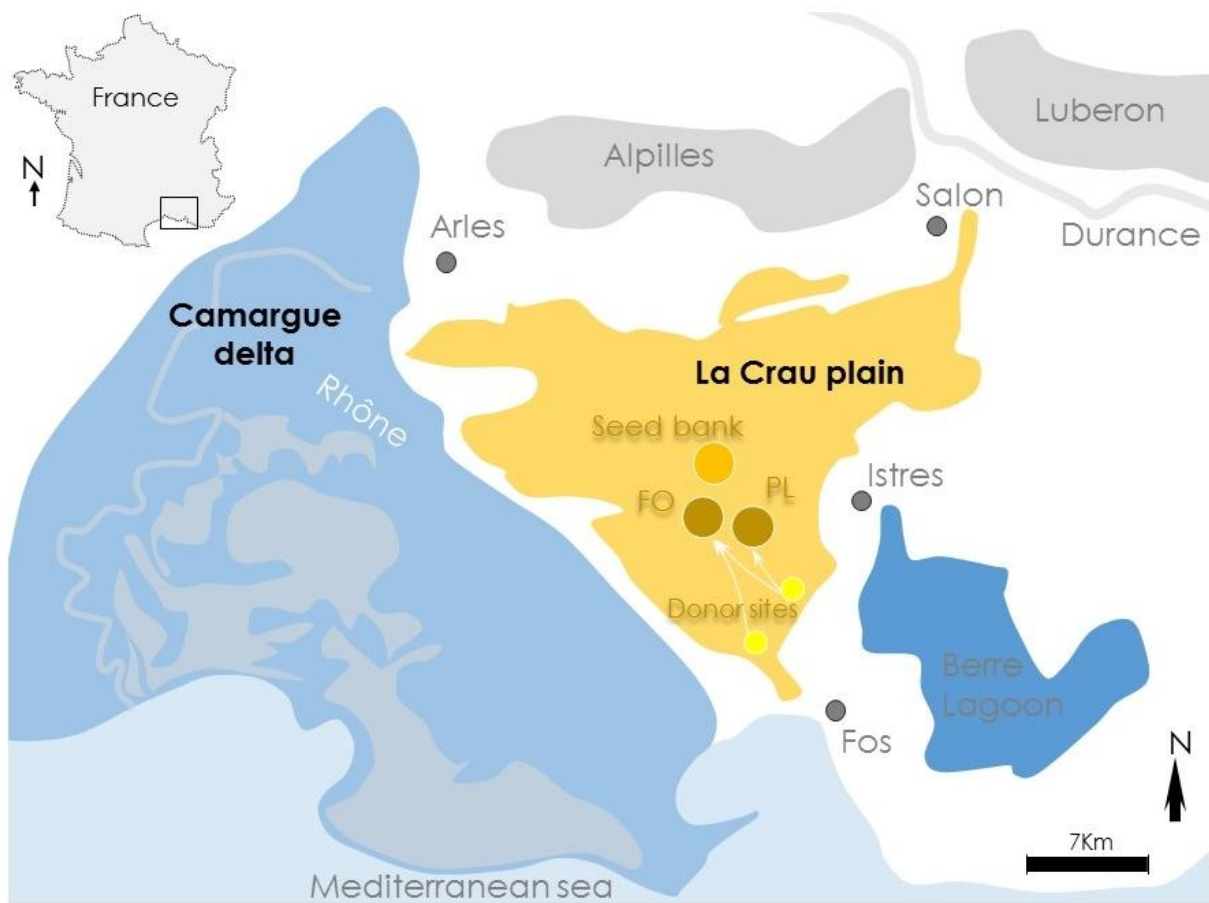


Figure 1. Map of the donor and restored (receptor) sites and as well as of the seed bank study site. FO = former orchard and PL = pipeline leak site where dry grassland topsoil transfers were implemented. See Table 1 for details.

Figure 2.

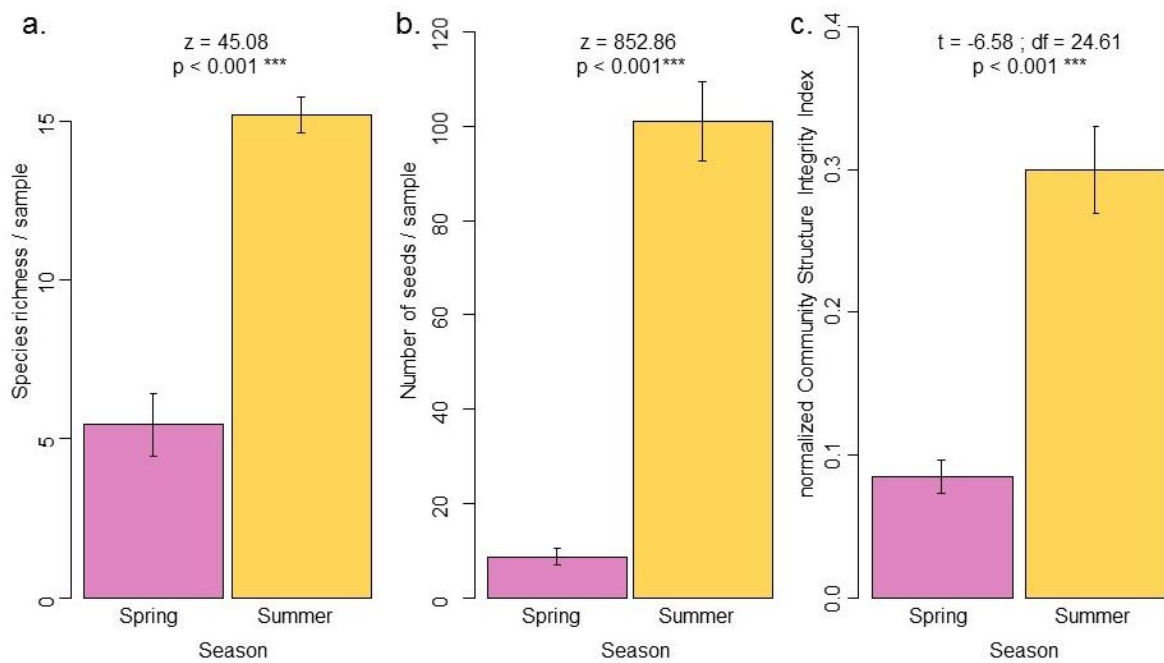


Figure 2. a. Species richness, b. seed density and c. normalized Community structure Integrity Index of the soil seed bank of Peau de Meau, Mediterranean dry grassland (reference grassland), at two seasons (spring: early spring in pink and summer: end of summer in yellow). Error bars represent \pm SE.

Figure 3.

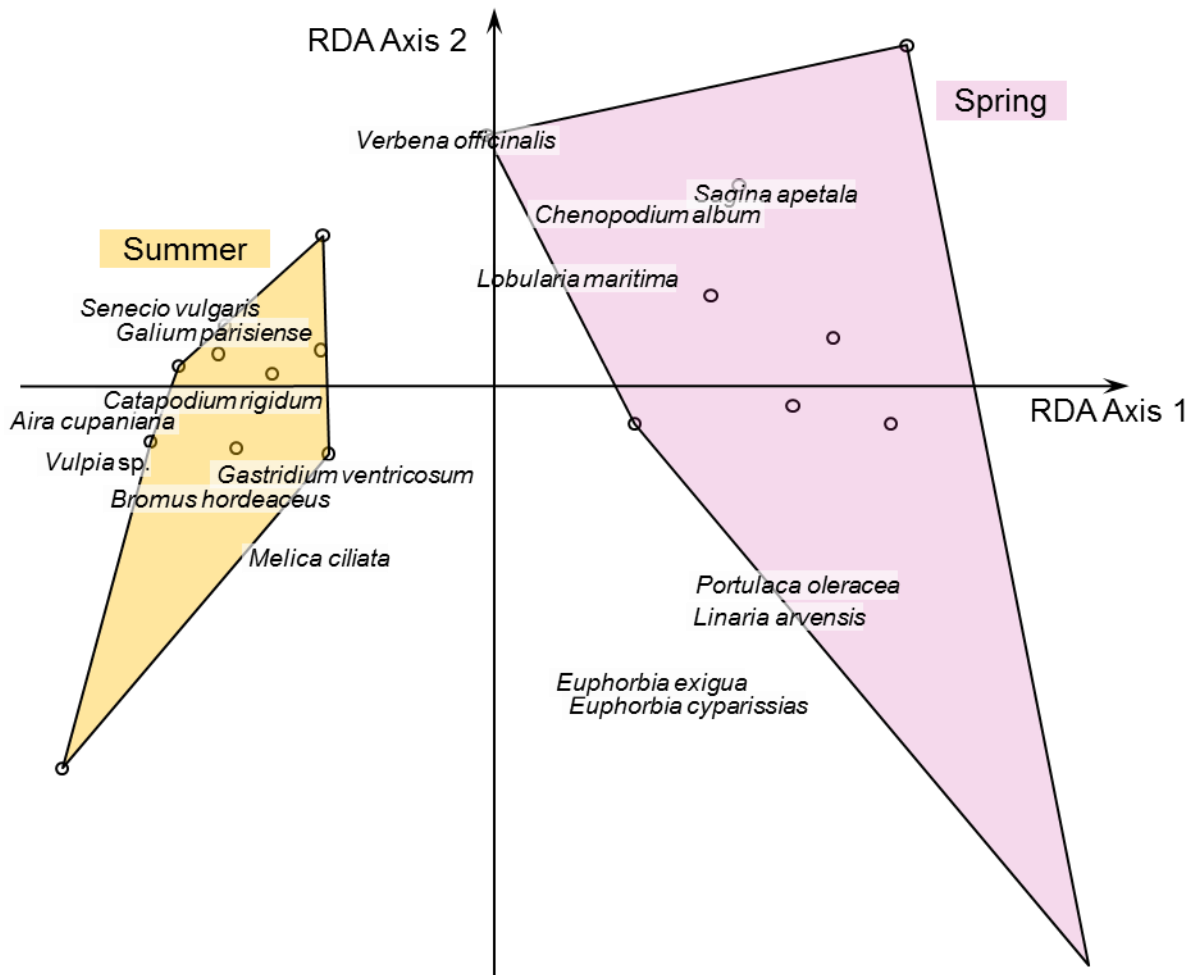


Figure 3. Soil seed bank ordination of samples from Peau de Meau, Mediterranean dry grassland (reference grassland) gathered at two seasons (spring: early spring in pink and summer: end of summer in yellow) based on RDA of plant community composition. For clarity, only the 16 most correlated species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

Figure 4.

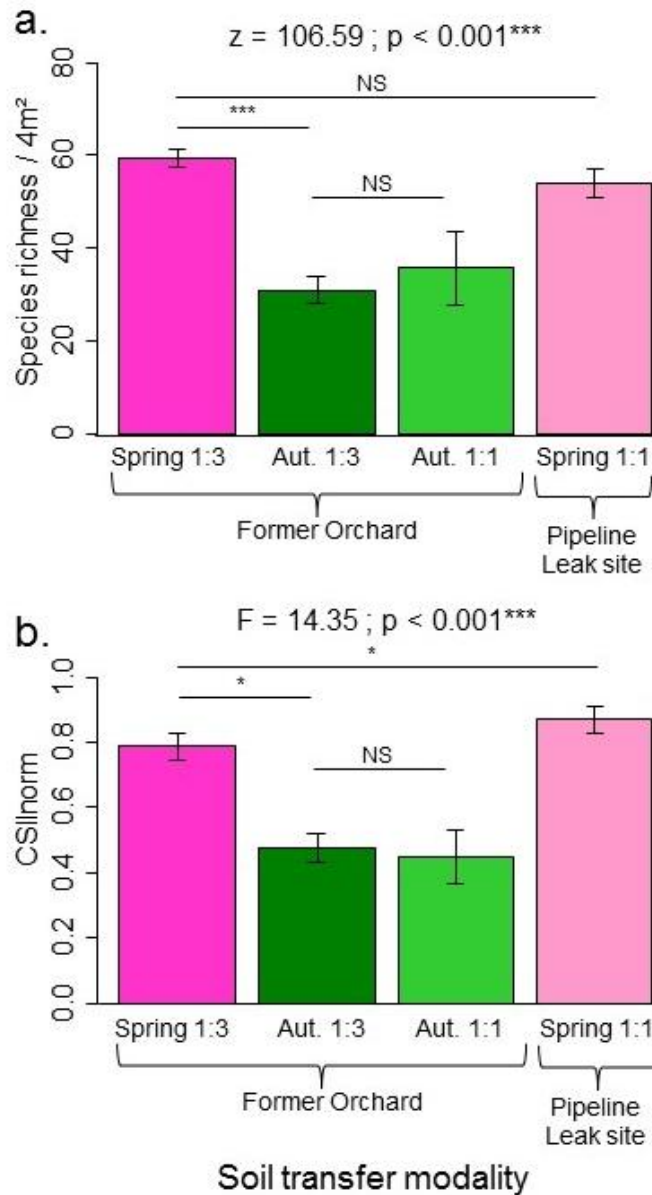


Figure 4. a. Species richness of the areas restored using topsoil transfers from Mediterranean dry grassland and; b. CSInorm, normalized Community Structure Integrity Index with reference grassland vegetation monitoring as reference compared with restored communities. Soil gathering seasons (spring in 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.

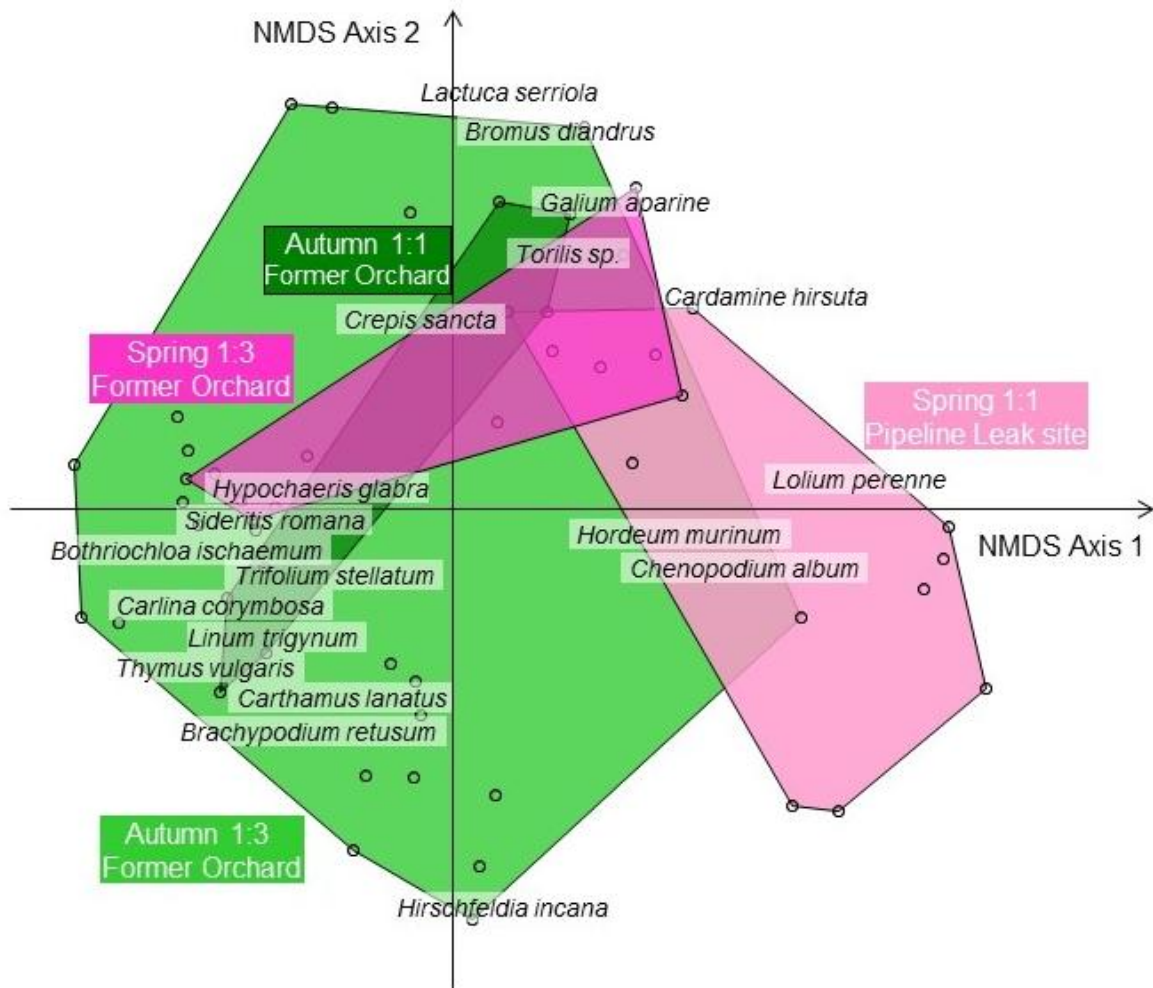


Figure 5. Plant community ordination one year after different topsoil transfers from Mediterranean dry grassland for different soil gathering seasons (spring in pink; autumn in green), transfer ratios (1:3 light colors; 1:1 dark colors) and receptor sites (Former Orchard; Pipeline Leak site) based on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

Figure 6.

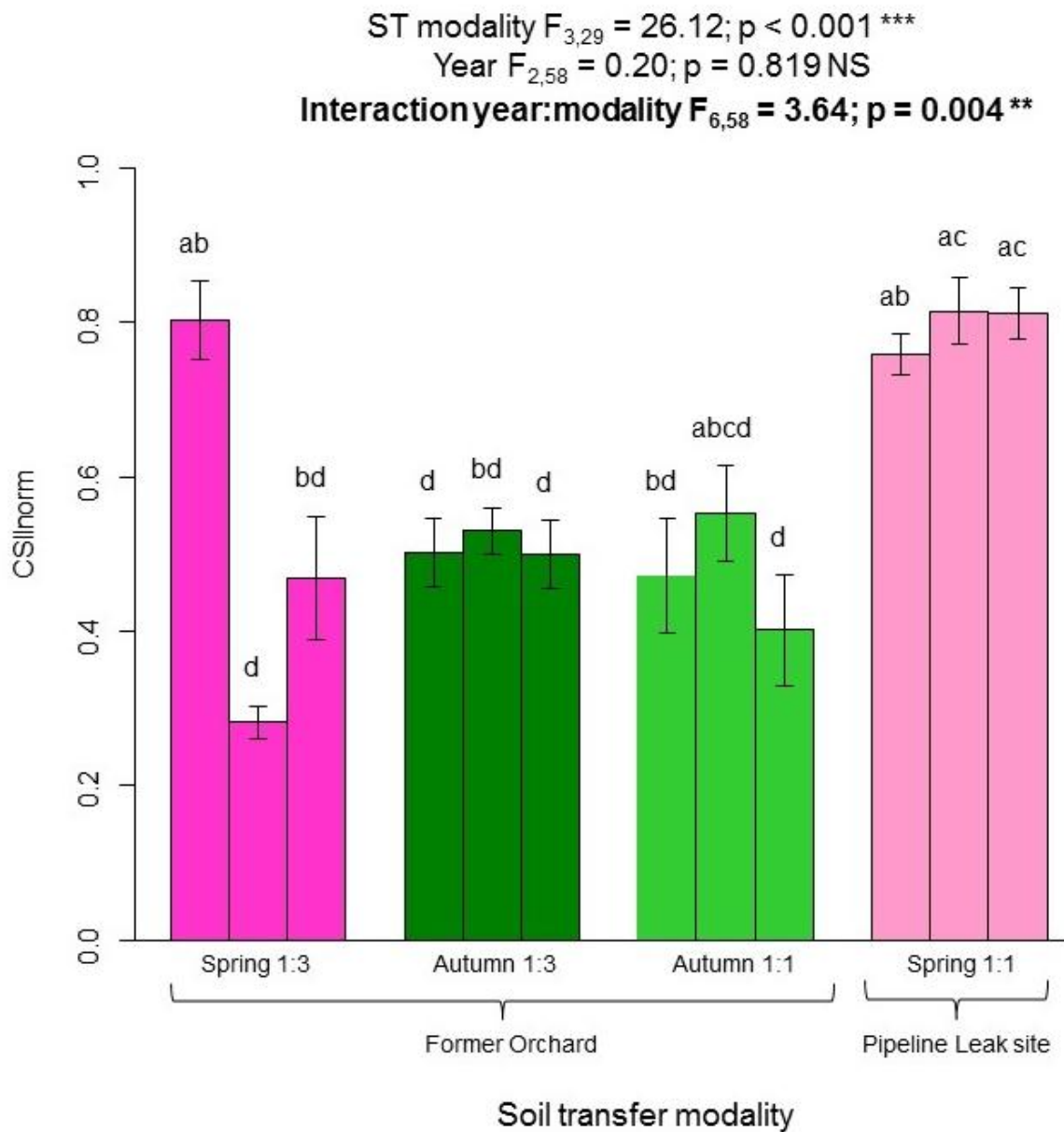


Figure 6. CSInorm, normalized Community Structure Integrity Index with reference grassland vegetation monitoring as reference compared with communities restored using topsoil transfers from Mediterranean dry grassland and interactions with years (1 to 3 years after transfers, from dark to light shades). 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; $p > 0.05$).