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## Long-term effects of topsoil transfer assessed thirty years after rehabilitation of dry alluvial quarries in Southeastern France

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1 **Long-term effects of topsoil transfer assessed thirty years after**  
2 **rehabilitation of dry alluvial quarries in Southeastern France**

3  
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19 **Abstract**

20  
21 Of the many techniques tested to date to rehabilitate degraded ecosystems, topsoil transfer  
22 appears to offer the best results. However, this method is recent, and results achieved in  
23 the short term (months to years) may not provide a sound indication of long-term  
24 vegetation reestablishment. In the plain of La Crau in southeastern France, many dry  
25 alluvial quarries were exploited in the 1970s -1980s and are nearly all now abandoned.  
26 Various topsoil transfers were performed when operations ceased, for various  
27 rehabilitation purposes (aesthetic, security, agricultural, etc.) and now provide the  
28 opportunity to test their efficacy in the 30-year long-term. We used an ecological analysis  
29 of plant communities and soil carried out in 2015 to compare the reference ecosystem  
30 (the steppe of La Crau, positive control) with un-rehabilitated quarry pits (negative  
31 control) and with four different rehabilitation treatments: (1) soil transfer (40 cm) with no  
32 contact with the water table; (2) soil transfer (40 cm) in contact with the water table; (3)  
33 more than 40 cm of soil transfer and (4) anthropogenic material deposits (spoils) unrelated  
34 to the characteristics of the reference soil. Our results show that the treatment most  
35 favorable to restoration of the species richness, diversity and composition of the steppe  
36 vegetation is transferring soil with fine particles (clay, silt) (treatment 1), at least 40 cm  
37 thick and without contact with groundwater. However, even after thirty years, and the re-  
38 establishment of traditional sheep grazing, full restoration of the reference steppe is far  
39 from being achieved. The other treatments lead to the emergence of hybrid or novel  
40 ecosystems, such as temporary ponds, riparian woodlands or matorrals with new variables  
41 and common variables to the historical ecosystem. Additional techniques to enhance the  
42 effectiveness of topsoil transfer are discussed.

43  
44 **Keywords:** alpha diversity, gamma diversity, quarry rehabilitation, Mediterranean  
45 ecosystem, soil analyses, steppe plant community.

## 46 1. Introduction

47 The constant acceleration of urbanization and industrialization worldwide, with  
48 the ensuing demand for mineral resources, means that newly-created quarries have largely  
49 destroyed many natural ecosystems (Wang et al., 2011). Quarrying results in significant  
50 visual and ecological impacts (Simón-Torres et al., 2014), not all of which have been  
51 identified yet (El-TaHER et al., 2016). Quarrying causes drastic alterations. It destroys flora  
52 and fauna, thereby reducing biodiversity and disrupting fundamental ecological  
53 relationships. Moreover, it extensively damages soil by modifying the original site  
54 topography and depleting and altering soil microbial communities (Corbett et al., 1996;  
55 Pinto et al., 2001; Milgrom, 2008; Mouflis et al., 2008; Simón-Torres et al., 2014). Other  
56 impacts identified at regional level include nuisance to local residents, with dust, noise  
57 pollution and ground vibrations when topsoil is dug up with heavy machinery (Mohamed  
58 and Mohamed, 2013; Dontala et al., 2015). Quarrying can also cause chemical  
59 contamination of groundwater by increasingly hazardous materials (Misra, 2013; Dontala  
60 et al., 2015).

61  
62 Today, many countries have legislation requiring mining and quarrying  
63 companies to implement ecological restoration after closure (DITR, 2005; UNEP et al.,  
64 2005; European Parliament, 2014). Ecological restoration *sensu lato* is the process of  
65 assisting the recovery of an ecosystem that has been degraded, damaged, or destroyed  
66 (SER, Society for Ecological Restoration International, Science & Policy Working  
67 Group, 2004). The goals of restoration *sensu stricto* include the re-establishment of the  
68 pre-existing biotic integrity in terms of species composition and community structure.  
69 Many quarries thus implement rehabilitation or reclamation actions of benefit to  
70 biodiversity (Damigos and Kaliampakos, 2003; Carrick and Krüger, 2007). Rehabilitation  
71 seeks to repair one or more ecosystem attributes, processes, or services. Reclamation, on  
72 the other hand, includes land stabilization, public safety guarantees, aesthetic  
73 improvement and usually a return of the ecosystem considered useful in the regional  
74 context (Society for Ecological Restoration International, Science & Policy Working  
75 Group, 2004).

76  
77 Numerous experiments conducted worldwide testify to the many quarry  
78 rehabilitation techniques in use, in particular for vegetation. For example, shrub species  
79 were planted in dolomite rock quarries in China (Wang et al., 2011) or in limestone  
80 quarries in Portugal (Oliveira et al., 2011) to rehabilitate areas formerly degraded by an  
81 increased bare ground soil surface sensitive to water or wind erosion. This involved  
82 adding an improved marl substrate layer on top of the bare rock (Oliveira et al., 2011).  
83 Other techniques using substitute substrates with fertilizers, water-holding polymers, geo-  
84 textiles and mycorrhizal inoculations were used to enhance vegetation growth after  
85 marble mining in Spain. However, long-term monitoring is rarely implemented, and those  
86 results available are not encouraging (Oliveira et al., 2011). Yet this rather limited effect  
87 of rehabilitation contrasts with the sometimes high diversity found in some abandoned  
88 quarries (Remacle, 2009; Chapel, 2011; Prach et al., 2013; Pitz et al., 2014). This paradox  
89 suggests a need to determine the most beneficial measures for the rehabilitation of  
90 singular flora and vegetation in disturbed areas, and for research specifically aimed at  
91 those inhabiting quarry habitats (Oliveira et al., 2011; Ballesteros et al., 2014).

93 One of the most frequently used techniques is to cover the post-quarrying exposed  
94 geological substrate, either with a layer of topsoil removed pre-quarrying and conserved  
95 in stockpiles during quarrying (topsoil transfer) (Simón-Torres et al., 2014), or with an  
96 artificially created soil (artificial soils) (Frouz et al., 2008; Weber et al., 2015). Topsoil  
97 transfer consists in removing the uppermost centimeters of topsoil from a donor site of  
98 ecological interest. This topsoil can be the pre-existing soil from the site itself, or can  
99 come from another site already programmed for destruction (Bulot et al., 2016). The soil  
100 is then re-spread on the sites to be restored (Ghose, 2001; Sheoran et al., 2010). It can  
101 consist in a direct topsoil transfer from another site that begins to be exploited. In such  
102 case, there is no storage of topsoil and therefore a better chance of rehabilitation  
103 successful (Rivera et al., 2014). In other cases, topsoil is stored for the duration of mining  
104 exploitation, the quality of soil and seed bank are then altered and this reduces the chances  
105 of rehabilitation success (Ghose, 2001; Strohmayr, 1999). Topsoil transfer can be used  
106 to save ecological features and their associated ecosystem services from donor sites (Box,  
107 2003). It has been evaluated as the best rehabilitation method to compensate for the many  
108 projects necessarily involving destruction to make way for consented or permitted  
109 development (Box, 2014). However, success monitoring rarely exceeds the first few years  
110 (Koch, 2007; Oliveira et al., 2011; Muller et al., 2013; Bulot et al., 2014; Jaunatre et al.,  
111 2014a; Bulot et al., 2016). Most restoration involving soil transfer has been recent and/or  
112 has not been examined scientifically (Fowler et al., 2015). The long-term effects (over  
113 several decades) of topsoil transfer on target species survival are therefore not known.  
114 Moreover, results obtained in the first months or years after implementation do not  
115 necessarily provide a good indication of longer-term responses (Cooke and Johnson,  
116 2002; D'Antonio and Meyerson, 2002; Herrick et al., 2006; Oliveira et al., 2011;  
117 Gaucherand et al., in press). Studies assessing medium- and long-term results are,  
118 therefore, essential for a fuller evaluation of these techniques.

119 In the plain of La Crau in southeastern France, many dry alluvial quarries were  
120 exploited in the 1970s for the creation of the Fos-sur-Mer port zone. These activities  
121 resulted in the destruction of nearly 300 hectares of the unique La Crau steppe ecosystem  
122 (Buisson and Dutoit, 2006). When quarrying ceased fifteen years later, the companies  
123 were not required by law to rehabilitate the environment after mining. Various  
124 rehabilitation trials, mainly using different soil transfer techniques, were thus  
125 implemented, mainly with aesthetic aims (i.e. creating a flat area like the steppe  
126 landscape) or security objectives (i.e. to avoid potholes).

127 Monitoring the soil and vegetation of these quarry pits today therefore provides  
128 the opportunity for 30-year long-term feedback on these techniques (i.e. involving  
129 different soil sources, composition, thickness and with or without contact with the  
130 groundwater table). Plant communities and soil physico-chemical parameters in  
131 rehabilitated pits were compared to a positive control, the steppe, generally considered as  
132 the reference ecosystem (Dutoit et al., 2013; Hobbs et al., 2013) and to a negative control  
133 without any soil transfer treatment. The soil of this surrounding steppe was the one that  
134 existed before it was destroyed by quarrying. To evaluate the biological importance of  
135 the newly created habitats, we worked at different spatial scales. We took into account  
136 both local (alpha) and regional biodiversity (gamma diversity), as well as the originality  
137 of the plant communities in relation to a landscape repository.

## 139 2. Materials and methods

### 140 2.1. Study site

141 Our study was carried out in the plain of La Crau, located in southeastern  
142 Mediterranean France (Bouches-du-Rhône) (Fig. 1A). Characterized by a unique natural  
143 habitat, the plain was classified in 1990 as a Special Protection Area under Natura 2000,  
144 called *Steppe of La Crau*, the last remaining French Mediterranean steppe (Buisson and  
145 Dutoit, 2006). Its Mediterranean substeppic grassland plant community features the  
146 phytosociological association *Asphodeletum fistulosii* (Molinier and Tallon, 1950)  
147 dominated by species such as *Brachypodium retusum* (Pers.) P. Beauv, *Thymus vulgaris*  
148 L. and *Asphodelus ayardii* Jahand and Marie. This plant community is unique in terms of  
149 species richness, composition and diversity (Cherel, 1988; Badan et al., 1995; Henry,  
150 2009). There are on average 30 to 40 plant species per square meter, 50% of which are  
151 annuals (Römermann et al., 2005). This plant community may result from a regressive or  
152 allogenic plant succession of thousands of years under the combined effects of i) the dry  
153 and windy Mediterranean climate, ii) specific soil conditions: the 40 cm deep soil is  
154 composed of 50% siliceous stones and lies on a calcareous conglomerate which cannot  
155 be penetrated by plant roots (Molliex et al., 2013) and iii) a recurring disturbance regime  
156 constituted by itinerant sheep grazing since the end of the Neolithic period (Badan et al.,  
157 1995; Lebaudy, 2004; Henry et al., 2010; Tatin et al., 2013).

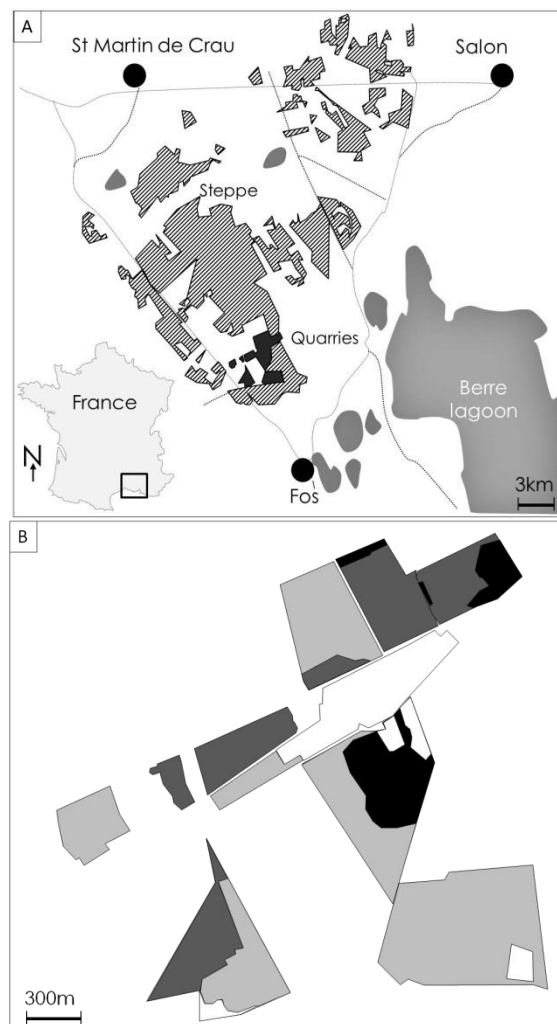
158  
159 Our study site was located in the south of the plain of La Crau (43°31'36.77''N,  
160 4°53'04.50''E). Present on the site are 296 ha of open quarries once exploited for road  
161 ballast or materials for the construction of docks in the Fos-sur-Mer port zone (Fig. 1).  
162 All these quarries were abandoned in the 1980s, as seen from old aerial photographs (1938  
163 - 2015) (Fig. 2). A shallow Haplic Cambisol soil WRB (IUSS Working Group WRB,  
164 2006) 40 cm deep lying above the geological conglomerate (composed of quartz pebbles  
165 in a calcium carbonate matrix forming a limestone 1 to 5 m thick), hereafter named  
166 topsoil, was removed from these quarries and exported, sold or stored. This made way for  
167 quarrying the underlying geological substrate (a 10 to 50 m thick mixture of Quaternary  
168 pebbles and sands deposited in a former large alluvial fan during the Riss-Wurm glacial  
169 period, (Molliex et al., 2013)) below the conglomerate.

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171 In most quarry pits (44% of the study site area), no material was replaced after  
172 quarrying, leaving exposed the un-disturbed geological material, or the surface of the  
173 water table when quarrying went more than nine meters deep (average water table height).  
174 We therefore considered this situation a negative control with no attempt at rehabilitating  
175 the soil or the vegetation for ecological or other reasons (Table 1). However, in some  
176 cases, quarry pits were partly filled with anthropogenic spoil materials combining various  
177 proportions of gravels, clays and sands, conglomerate or concrete blocks, etc. (11% of  
178 the study site area). This situation was considered as a rehabilitation treatment, since these  
179 new artificial soils provided conditions favorable to new primary plant successions, even  
180 though nothing was done to drive these successions towards the plant composition and  
181 dynamics of the reference steppe plant community. Contrastingly, at the initiative of  
182 certain quarry operators, other quarry pits had stored topsoil re-spread on top of the spoil  
183 materials (45 % of the study site area) to various depths, including a depth similar to the  
184 reference steppe (40 cm) and a greater depth (more than 40 cm of topsoil added).

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186 Differing quarry operating conditions, followed by differing rehabilitation  
187 treatments in the eighties, provided us with a great diversity of artificial  
188 geomorphological and pedological situations whose impacts on the present vegetation  
189 could be compared. We compared this vegetation both to the steppe vegetation (positive  
190 control) generally considered as the reference ecosystem in the previous restoration  
191 projects realized in this area (Dutoit et al., 2013), and to quarry pits with no rehabilitation  
192 treatments (negative control) (Table 1). In total, four situations were identified along a  
193 rehabilitation gradient: (1) soil transfer (40 cm) with no contact with the water table, (2)  
194 soil transfer (40 cm) in contact with the water table, (3) more than 40 cm of soil transfer  
195 and (4) anthropogenic deposits (spoils) (Table 1). These four situations were identified  
196 by the use of ancient aerial photographs (Fig. 2), soil profiles and interviews of quarry  
197 operators.  
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**Fig. 1.** Location of study site and experimental design. (A) Location of the plain of La Crau area in France and location of the remnant patches of steppe (striped grey) and quarries (black); (B) experimental design of the different rehabilitation treatments realized in the 1980s: 40 cm soil transfer (dark grey); > 40 cm soil transfer (black); anthropogenic deposits (white) and no rehabilitation (light grey).



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**Fig. 2.** Quarry expansion in the seventies and eighties visible from old aerial photographs (1968 – 1974 – 1984 – 2011 -IGN). The extension of intensive orchard areas since 1984 can also be observed between the quarries.

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**Table 1.** Various rehabilitation treatments and controls used in this study and analyses performed on each treatment

Treatments		Sample number for soil analyses	Transect number for vegetation sampling
Positive control	Reference Steppe	5	6
Topsoil transfer	40 cm soil transfer with no contact with the water table	4	4
	40 cm soil transfer in contact with the water table	5	3
	> 40 cm soil transfer	5	3
Anthropogenic soil	Anthropogenic deposits (spoils)	5	5
Negative control	No rehabilitation: absence of deposits or soil spreading in the quarried geological substratum	5	4

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## 2.2. Vegetation analyses

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Vegetation sampling using 4 m<sup>2</sup> quadrats was carried out in May 2015. For each of the six treatments, depending on available area, 3 to 6 100-meter transects were replicated, each composed of five pseudo-replicated quadrats (Fig. 1; Table 1). Quadrats were pseudo-replicated on the same site along each transect, so as to reveal any heterogeneity in the spatial distribution of plant communities for the same treatment. The number of replicated transects differed according to type of treatment in the quarries sampled. On each transect, quadrats were spaced more than 10 m apart, to prevent spatial autocorrelation between quadrats (Buisson et al., 2006). The abundance of each plant species was visually estimated, in percent cover (Gillet, 2000).

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## 2.3. Soil analyses

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Soil analyses were carried out on twenty-nine 200g soil samples taken from abandoned quarries in 2015 (Table 1). Samples were extracted from the first ten centimeters of the upper soil/material layer in February 2015. Each sample was dried and sieved with a 2 mm mesh sieve. Chemical content (organic C (Allison, 1965), total N (Bremner, 1996), C:N, CEC Metson (Metson, 1956; Ciesielski et al., 1997), K, P<sub>2</sub>O<sub>5</sub> (Olsen, 1954), CaCO<sub>3</sub>, pH (Sparks et al., 1996; Thomas, 1996)) and fine particle sizes (<2 mm) (percentages of 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) (Gee and Bauder, 1986) were measured without decarbonization by the soil analysis laboratory at INRA (*Institut National de la Recherche Agronomique*) in Arras.



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#### 240 2.4. Data analysis

241 To test the effects of treatments on physico-chemical parameters, soil  
242 characteristics were ordinated using principal component analysis (PCA) (6 treatments ×  
243 29 soil samples), a method appropriate for continuous variables (Borcard et al., 2011).

244 To test the effects of the different treatments on species richness, plant community  
245 composition and structure, these variables were compared between treatments.  
246 Community structure was measured using different indices (alpha diversity, gamma  
247 diversity, CSII<sub>norm</sub> and HAI index). Alpha diversity, which is the number of species  
248 present in a plant community, was calculated for each 4 m<sup>2</sup> quadrat. Gamma diversity,  
249 which is the total number of species in a landscape, was calculated as the cumulated  
250 species-richness of the five quadrats for each transect in the different quarries and the  
251 reference steppe. As the number of transects used in the quarries (N = 19) is higher than  
252 in the steppe (N = 6), six quarry transects were randomly selected and their cumulated  
253 species-richness was calculated. This operation was repeated 100 times. Mean cumulated  
254 species richness determined quarry gamma diversity. Two indices were used to assess the  
255 success of plant community rehabilitation by topsoil transfer: the CSII<sub>norm</sub> index  
256 (normalized Community Structure Integrity Index) measuring the proportion of the  
257 abundance of the species of the reference community represented in the community of  
258 the restored community, and the HAI index (Higher Abundance Index) measuring the  
259 proportion of the abundance of species in the restored community which is higher than in  
260 the reference community (Jaunatre et al., 2013). When parametric conditions were met,  
261 we used ANOVA tests followed by post-hoc Tukey HSD tests if a significant difference  
262 was detected. When parametric conditions were not met, we used non-parametric  
263 Kruskal-Wallis tests followed by pairwise Wilcoxon comparisons with a *p-value*  
264 adjustment according to Bonferroni if a significant difference was detected (Bonferroni,  
265 1936).

266 Vegetation composition and abundance were ordinated using non-metric  
267 multidimensional scaling (NMDS) (6 treatments × 125 quadrats × 241 plant species)  
268 based on Bray-Curtis dissimilarity (Borcard et al., 2011). We were thus able to visualize  
269 the vegetation structure of each treatment and to compare them.

270 All the analyses were conducted using R 2.13.0 (R Development Core Team,  
271 2011), multivariate analyses with the “ade4” (Chessel et al., 2004; Dray and Dufour,  
272 2007; Dray et al., 2007) and “vegan” (Oksanen et al., 2015) packages.

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### 275 3. Results

#### 276 3.1. Effect of topsoil transfer on soil physico-chemical parameters

277 The different rehabilitation treatments showed significant differences in soil  
278 physico-chemical properties (Table 2, Fig. 3). The PCA ordination based on soil  
279 properties significantly discriminated the different treatments. Axis 1 (45.3% of inertia)  
280 separated topsoil (steppe and soil) transfer from the negative control with no spoil  
281 deposits or soil spreading. This separation is explained partly by the quantities of clay,  
282 which were significantly higher in the steppe and soil transfers. Conversely, in the  
283 absence of deposits or soil spreading, the substrate was characterized by significantly  
284 higher amounts of coarse sand, higher C:N ratios and higher pH.

285 Axis 2 (25.8% of inertia) clearly separated the soils of the reference steppe and of  
286 the soil transfer treatments from those of the negative control and spoil deposits. The soils

287 of topsoil transfer treatments tended to be closer to that of the reference steppe, especially  
 288 those which were not in contact with the water table.

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290 **Table 2**

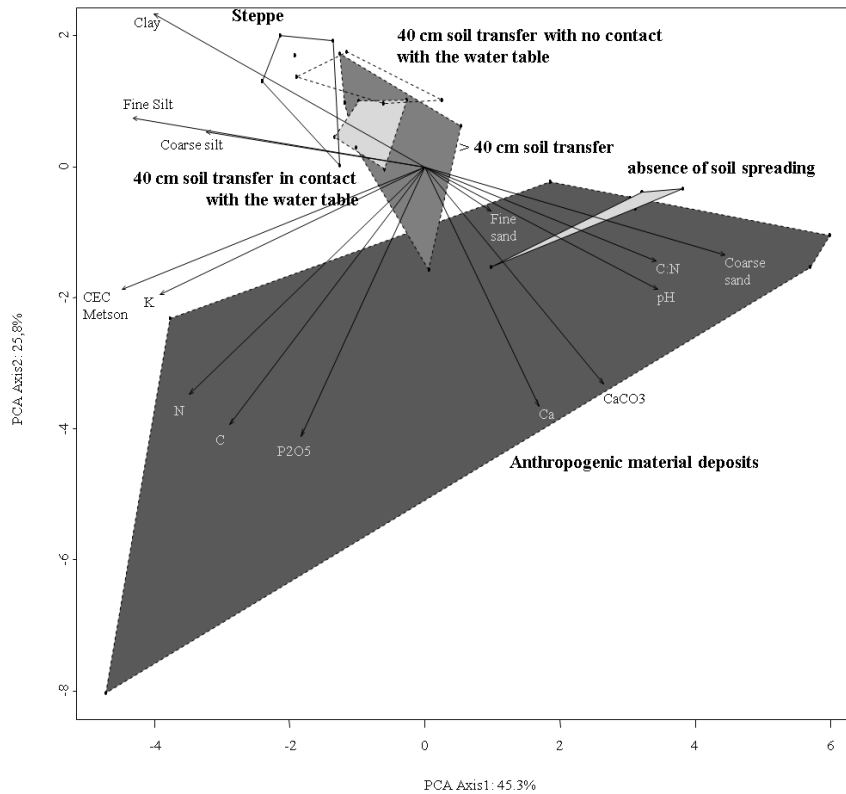
291 Soil analyses for each rehabilitation treatment realized in May 2015. The given values are means  $\pm$  standard  
 292 errors. The F value and p value are from ANOVA and the X<sup>2</sup> value is from Kruskal-Wallis tests on the  
 293 effects of treatments on each variable. Two values in the same row with a different letter are significantly  
 294 different according to Tukey post-hoc tests or Mann–Whitney–Wilcoxon tests (Bonferroni, 1936).

	Statistic parameter	p-value	Reference steppe (Positive control)	Topsoil transfer (40 cm) with no contact with the water table	Topsoil transfer (40 cm) in contact with the water table	Topsoil transfer (More than 40 cm)	Anthropogenic material deposits	No rehabilitation Absence of soil spreading/material deposits (Negative control)
Clay (g kg <sup>-1</sup> )	X <sup>2</sup> =19.38, Df=6	0.004**	231.2±6.6 b	199.5±33.9 b	223.4±4 b	204±17.5 b	109.8±28.1 a	98.8±8.9 a
Fine silt (g kg <sup>-1</sup> )	F <sub>6,27</sub> = 1.7	0.16	210.8±5.5	173.5±15.3	177.4±2.7	176.8±6.5	150.8±46.3	131.6±13
Coarse silt (g kg <sup>-1</sup> )	F <sub>6,27</sub> = 0.56	0.76	143.0±3.7	116.5±11.6	128.4±2.6	123.6±5.9	123.8±66.1	81.2±6.9
Fine sand (g kg <sup>-1</sup> )	F <sub>6,27</sub> = 0.5	0.801	202.8±5.1	239.5±34.9	218.0±6.2	205.8±5.3	224.0±47.1	234.6±16.1
Coarse sand (g kg <sup>-1</sup> )	F <sub>6,27</sub> = 2.91	0.026*	212.2±13.6 a	271.0±26.9 ab	252.8±3.4 ab	289.8±29.1 ab	391.6±118.7 ab	453.8±35.5 b
Total carbon (g kg <sup>-1</sup> )	F <sub>6,27</sub> = 1.1	0.385	17.4±1.4	12.4±2.1	15.4±1.9	15.9±1.6	31.7±15.6	11.4±3.2
Total nitrogen (g kg <sup>-1</sup> )	F <sub>6,27</sub> = 0.72	0.637	1.6±0.1	1.1±0.2	1.4±0.1	1.5±0.2	2.2±1.3	0.8±0.3
C:N	F <sub>6,27</sub> = 2.72	0.034*	10.7±0.3 a	11.8±1.8 ab	10.7±0.5 ab	10.9±0.6 ab	22.1±6.2 b	15.1±0.9 ab
pH	F <sub>6,27</sub> = 4.31	0.004**	7.6±0.3 a	8.2±0.2 ab	8.3±0.1 ab	8.2±0.1 ab	8.7±0.2 b	8.6±0.1 b
CaCO <sub>3</sub> (g kg <sup>-1</sup> )	X <sup>2</sup> = 19.38, Df=6	0.004***	5.8±4.8 a	70.8±62.3 a	20.8±5.7 a	58±46.1 a	216.6±20.4 b	223.4±19.1 b
P <sub>2</sub> O <sub>5</sub> (g kg <sup>-1</sup> )	X <sup>2</sup> = 10.59, Df=6	0.102	0.184±0.173	0.009±0.001	0.014±0.003	0.030±0.0162	0.016±0.010	0.026±0.008
CEC Metson (cmol + kg <sup>-1</sup> )	F <sub>6,27</sub> = 1.37	0.261	9.1±0.4	7.1±1.3	8.4±0.2	7.8±0.4	8.7±4	3.4±0.6
Ca (g kg <sup>-1</sup> )	F <sub>6,27</sub> = 2.89	0.026*	2.8±1 a	4.1±1.1 ab	4.9±0.6 ab	4.3±1 ab	6.7±0.4 b	6.4±0.1 b
K (g kg <sup>-1</sup> )	F <sub>6,27</sub> = 1.32	0.282	0.1±0	0.1±0	0.2±0	0.1±0	0.1±0.1	0.1±0

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**Fig. 3.** PCA ordination based on 29 soil sample analyses. Samples from each rehabilitation treatment and control are identified by dashed lines: (1) soil transfer (40 cm) with no contact with the water table in white (2) soil transfer (40 cm) in contact with the water table in light grey (3) more than 40 cm of soil transfer in medium grey and (4) anthropogenic material deposits in dark grey. Both samples from controls are identified by solid lines: Steppe in white, No rehabilitation in grey. Arrows represent soil variables (chemical content (organic C, total N, C:N, CEC Metson, K, P<sub>2</sub>O<sub>5</sub>, CaCO<sub>3</sub>, pH) and fine particle sizes (<2 mm) (percentages of 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))

### 3.2. Effect of topsoil transfer on plant communities

#### 3.2.1. Alpha and gamma diversity

Vegetation inventories allowed us to identify 21 plant taxa of conservation interest out of the 241 species recorded in total (nearly 10%): 5 with high conservation priority and 16 with moderate conservation priority on the red list of the Provence-Alpes-Côte-d’Azur region (Table 3). 224 species were inventoried in the quarries, including 20 regional red list species and 131 in the reference steppe, including 11 regional red list species (Table 3). Only one plant species of conservation interest was identified in the steppe: *Phlomis lychnitis* L., against 11 in quarries. Four species were recorded in anthropogenic material deposits alone (*Bupleurum semicompositum* L., *Limonium cuspidatum* (Delort) Erben, *Limonium echioides* (L.) Mill., *Velezia rigida* L.) and one in the negative control (*Lythrum hyssopifolia* L.) (Table 3). All plant species of conservation interest identified in the topsoil transfer areas were also identified in the reference steppe vegetation, except for *Campanula erinus* L. and *Paronychia capitata* (L.) Lam. (Table 3).

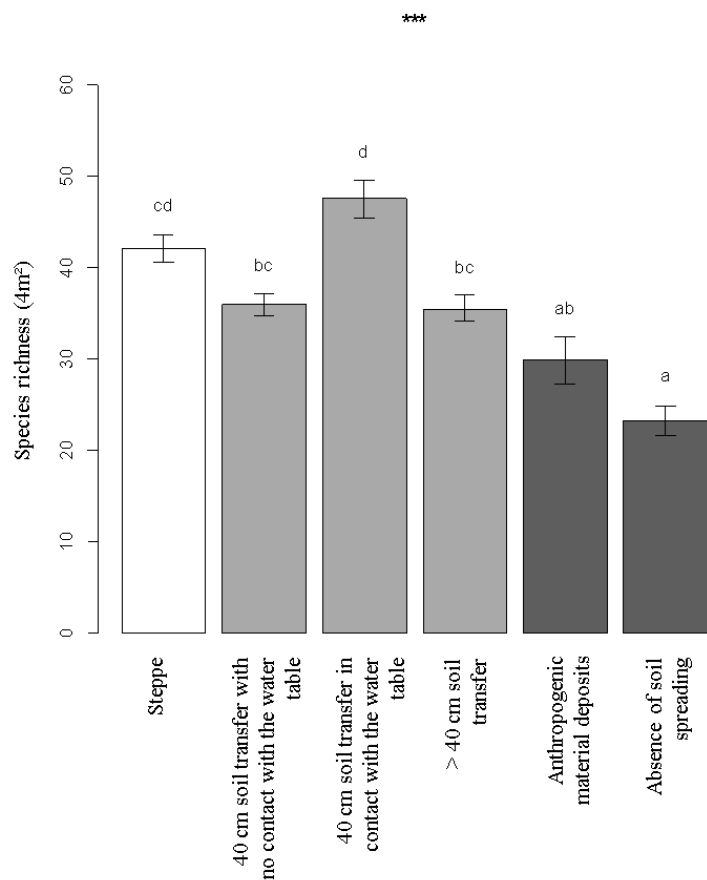
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**Table 3**  
Species of conservation interest identified for each treatment in May 2015. The species' regional conservation status was determined from the regional red list of the Provence-Alpes-Côte-d'Azur region (Noble et al., 2015)

Species	Regional conservation status	Reference steppe (Positive control)	Topsoil transfer (40 cm) with no contact with the water table	Topsoil transfer (40 cm) in contact with the water table	Topsoil transfer (More than 40 cm)	Anthropogenic material deposits	No rehabilitation Absence of soil spreading /material deposits (Negative control)
<i>Bupleurum semicompositum</i> L.	Moderate					x	
<i>Campanula erinus</i> L.	Moderate		x	x		x	x
<i>Centaurea melitensis</i> L.	Moderate	x		x		x	
<i>Gastroidium ventricosum</i> (Gouan) Schinz & Thell.	Moderate	x		x	x		
<i>Limonium cuspidatum</i> (Delort) Erben	Strong					x	
<i>Limonium echioideis</i> (L.) Mill.	Moderate					x	
<i>Linaria arvensis</i> (L.) Desf.	Moderate	x	x	x		x	
<i>Lythrum hyssopifolia</i> L.	Moderate						x
<i>Melilotus indicus</i> (L.) All.	Moderate					x	
<i>Parapholis incurva</i> (L.) C.E.Hubb.	Moderate	x		x	x	x	x
<i>Paronychia capitata</i> (L.) Lam.	Moderate		x			x	
<i>Phlomis lychnitis</i> L.	Moderate	x					
<i>Polygala monspeliaca</i> L.	Moderate	x			x		x
<i>Polypogon maritimus</i> Willd.	Moderate					x	x
<i>Psilurus incurvus</i> (Gouan) Schinz & Thell.	Moderate	x	x	x	x	x	x
<i>Ruta Montana</i> (L.) L.	Moderate	x	x				
<i>Taeniatherum caput-medusae</i> (L.) Nevski	Strong	x	x	x	x	x	x
<i>Trifolium glomeratum</i> L.	Strong	x	x	x		x	
<i>Trifolium subterraneum</i> L.	Moderate	x			x		
<i>Valerianella microcarpa</i> Loisel.	Strong				x	x	
<i>Velezia rigida</i> L.	Strong					x	

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At the regional scale (gamma diversity), the mean species richness of the plant communities of the different rehabilitation treatments ( $156.78 \pm 0.77$ ) was higher than the mean species richness of the reference steppe vegetation ( $130 \pm 0$ ) ( $F_{1, 198} = 1219$ ,  $p < 0.001$ ). However at the scale of the plant community, the species richness (alpha diversity) of the different topsoil transfer treatments (contact with water table ( $47.6 \pm 2.02$ ); no contact with water table ( $36 \pm 1.20$ ); more than 40 cm soil transfer ( $35.55 \pm 1.46$ )) showed no significant difference from the vegetation of the reference steppe ( $42.2 \pm 1.51$ ) (Fig. 4). In contrast, the average species-richness on anthropogenic material deposits ( $29.88 \pm 2.66$  species) and on the negative control ( $23.25 \pm 1.64$ ) was significantly lower than in the steppe ( $F_{5, 119} = 18.98$ ,  $p < 0.001$ ) (Fig. 4).



342  
 343 **Fig. 4.** Means and standard errors of species richness (4m<sup>2</sup>) for each treatment and the two controls: steppe  
 344 (white), topsoil transfer (light grey), anthropogenic material deposits and absence of soil spreading (dark  
 345 grey). Within a treatment, bars sharing common letters are not significantly different according to Tukey  
 346 post-hoc test.

347  
 348

### 349 3.2.2. *Effects of topsoil transfer on plant community composition*

350 The NMDS ordination showed a gradient in plant community composition, from the  
 351 steppe (positive control) to the no-rehabilitation negative control, and clearly  
 352 discriminated plant communities (NMDS-Stress= 0.20) (Fig. 5).

353 In the NMDS axis1-axis2 plane, the reference steppe was characterized by its most  
 354 representative species, such as perennial species (*Eryngium campestre* L., *Stipa capillata*  
 355 L., *Phlomis lychnitis* L., etc.), especially *Brachypodium retusum* (Pers.) P. Beauv., usually  
 356 dominant in the steppe plant community. It was also characterized by annual species such  
 357 as *Aegilops ovata* L., *Clypeola jonthlaspi* L., *Cynosurus echinatus* L., *Hedypnois cretica*  
 358 Willd., *Reseda phyteuma* L., *Stipa capillata* L., *Trifolium arvense* L., which were  
 359 recorded only in the steppe.

360 The reference steppe community was close to the two topsoil transfer treatments with  
 361 no contact with the water table ("> 40 cm topsoil transfer" and "40 cm topsoil transfer  
 362 with no contact with the water table") (Fig. 5). These two plant communities overlapped  
 363 and were characterized mainly by grasses and thistles, such as *Avena barbata* Brot., and  
 364 *Galactites tomentosus* Moench., as well as *Euphorbia cyparissias* L., *Marrubium vulgare*  
 365 L., *Medicago truncatula* Gaertn., etc.. This was confirmed by the CSII<sub>norm</sub>, which showed  
 366 that only 18 to 22% of species identified in topsoil transfer plant communities were also

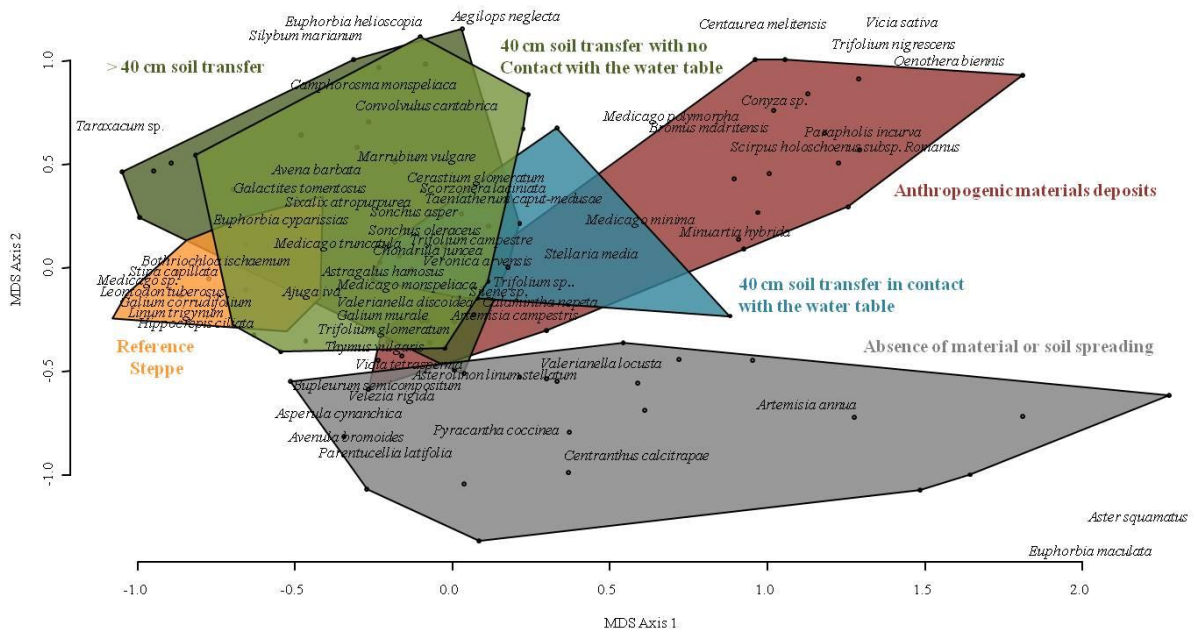
367 present in the steppe community with the same composition, abundance and richness (Fig.  
368 6A) even thirty years after these rehabilitation treatments were applied.

369 The topsoil transfer in contact with the water table showed a different plant  
370 community composition from that of the reference steppe. It contained *Anagallis arvensis*  
371 L., *Catapodium rigidum* (L.) C.E.Hubbs., *Hypericum perforatum* L., *Stellaria media* (L.)  
372 Vill., *Veronica arvensis* L., etc. (Fig. 5), pioneer species common to anthropogenic and  
373 wetland margin environments.

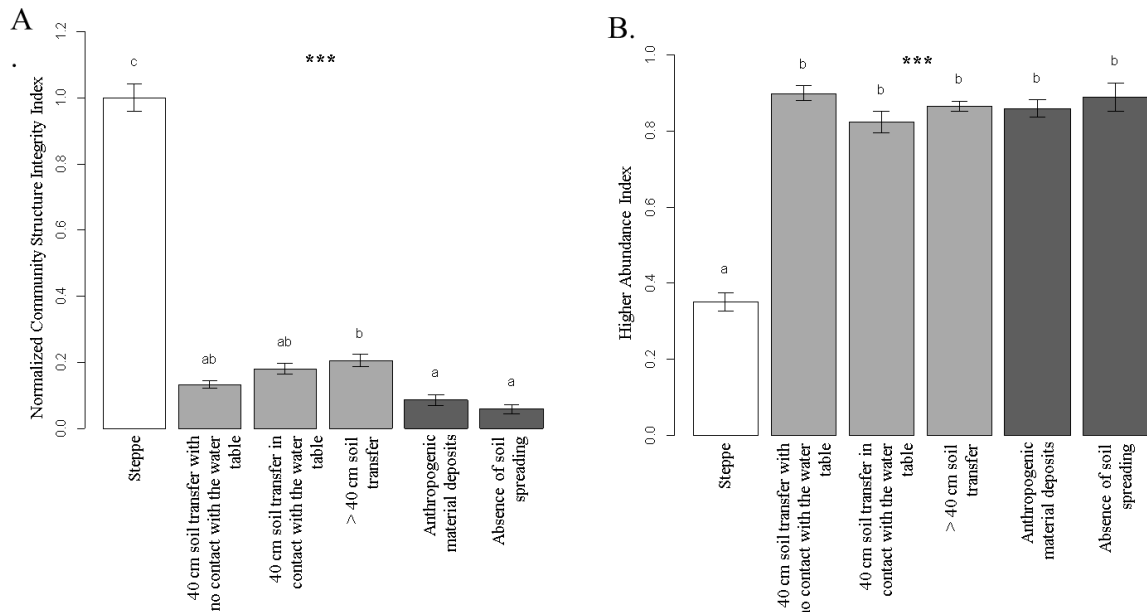
374 In the NMDS axis1-axis2 plane, the rehabilitated areas (the topsoil transfer and the  
375 anthropogenic material deposits treatments) and the reference steppe were also clearly  
376 separated from the no-rehabilitation ("negative control") treatment in terms of plant  
377 community composition (Fig. 5). The no-rehabilitation control was characterized by  
378 pioneer annual target species also occurring in the reference steppe, such as *Centranthus*  
379 *calcitrapae* (L.) Dufur., *Valerianella locusta* (L.) Laterr., *Velezia rigida* L., and other non-  
380 target species such as *Artemisia annua* L., *Pyracantha coccinea* M. Roem. (Fig. 5). These  
381 last two species can be considered potential invasive species. Due to the presence of the  
382 water table, this area contained temporary wetland species such as *Lythrum hyssopifolia*  
383 L., a species of conservation interest.

384  
385 The plant communities identified in quarries were far from similar to those of the  
386 reference steppe ( $F_{5, 119} = 227.20$ ,  $p < 0.001$ ) (Fig. 6A) in terms of composition, richness  
387 and diversity. Not only did they not include all reference steppe species, but they included  
388 other species not present in the steppe community (*Anagallis foemina* Mill., *Anagallis*  
389 *arvensis* L., *Cynodon dactylon* (L.) Pers., *Dittrichia viscosa* (L.) Greuter, *Medicago*  
390 *orbicularis* (L.) Bartal., *Rumex pulcher* L., etc.). The CSII<sub>norm</sub> index showed a significant  
391 difference between treatments (Fig. 6A). The anthropogenic material deposits treatment  
392 and the no-rehabilitation treatment showed a significantly lower CSII<sub>norm</sub> index than the  
393 reference steppe (Fig. 6A). The HAI index was relatively high both in the different  
394 rehabilitated areas and in the no-rehabilitation treatment, and significantly different from  
395 that of the reference steppe ( $F_{5, 119} = 88.66$ ,  $p < 0.001$ ) (Fig. 6B).

396



399  
400 **Fig. 5.** NMDS ordination based on 125 vegetation samples (4m<sup>2</sup>) and 175 species present in at least 3  
401 samples. For clarity, only the 63 most correlated species are shown. Samples from each rehabilitation  
402 treatment are identified: (1) soil transfer (40 cm) with no contact with the water table in light green (2) soil  
403 transfer (40 cm) in contact with the water table in blue (3) more than 40 cm of soil transfer in dark green  
404 and (4) anthropogenic material deposits in red. Both samples from control are identified: steppe in yellow,  
405 absence of material or soil spreading in grey.  
406



407  
408 **Fig. 6.** Means and standard errors of normalized Community Structure Integrity Index (A) and higher  
409 Abundance Index (B) for each treatment: steppe (white), topsoil transfer (light grey), anthropogenic  
410 material deposits and absence of soil spreading (dark grey). Within a treatment, bars sharing common letters  
411 are not significantly different according to Tukey post-hoc test.  
412

#### 413 4. Discussion

414 Topsoil transfer has been recognized as the best method for restoring soil and  
415 vegetation when this resource has been conserved or is available elsewhere (Box 2014,  
416 Bulot et al., 2014, in press). To date, however, the restoration success of this technique  
417 has mainly been assessed over the short term (a few years after treatment). Assessing  
418 restoration over the long term (several decades), our results show that topsoil transfer is  
419 still the best method, especially when initial soil characteristics (thickness, absence of  
420 contact with the water table) are respected. Nevertheless, in our case, soil transfer still  
421 does not compensate for the destruction of the pre-existing ecosystem: the steppe soil and  
422 plant community have not yet fully recovered even thirty years after its application. These  
423 results confirm those of Holl and Cairns, Mulligan et al., Nichols (Holl and Cairns, 1994;  
424 Mulligan et al., 2006; Nichols, 2006), who found that plant community composition was  
425 never completely restored after rehabilitation, in quarries or elsewhere. However, it is  
426 generally acknowledged that natural successions towards steppe occur on quarries after  
427 topsoil applications and re-vegetation is positive, although species composition still  
428 differs from that of the reference steppe (Martínez-Ruiz and Fernández-Santos, 2005).

429  
430 The negative control, where there was no rehabilitation, no transfer of materials  
431 or soil, is the worst situation compared to the undisturbed reference steppe in terms of  
432 total number of plant species and total number of plant species of conservation interest.  
433 In 30 years, only a fraction of the steppe vegetation has recovered. This is due to the depth  
434 of quarrying, which involves potential contact with the water table. New primary plant  
435 succession is therefore blocked by irregular changes in ground water table levels,  
436 including by the extreme conditions that develop between dry and flood periods (Prach  
437 et al., 2014; Balázs A. Lukács et al., 2015; Deák et al., 2015; Masson et al., 2015). The  
438 lack of rehabilitation, together with the new abiotic conditions created by quarrying, thus  
439 generated “novel ecosystems” (Hobbs et al., 2009). The long-term dynamics of these new  
440 ecosystems are unknown, since we have no experience of similar habitat conditions, and  
441 because they are also impacted by global change (climate and land-uses) (Doley and  
442 Audet, 2013; Hobbs et al., 2013). However, we do know that these environments will not  
443 spontaneously evolve towards the reference steppe in the long-term, barring any major  
444 ecological change (significant and permanent reduction in the level of the water table).  
445 Usually, for these radically disturbed mining sites, it is not practicable to aim for the  
446 restoration of historical ecosystems. However, hybrid or novel ecosystems, which consist  
447 of new combinations of physical and biological components with new variables and  
448 variables common to historical ecosystems, could provide acceptable levels of stability  
449 and functionality (Doley and Audet, 2013). For example, vegetation may include both  
450 native species and non-native species.

451  
452 Deposition of various types of anthropogenic materials after the end of quarrying  
453 created great heterogeneity in terms of physico-chemical soil parameters, but less  
454 diversity and species-richness in terms of vegetation. However, these materials provide  
455 some very specific habitats for certain species of high conservation value, which have  
456 particular ecological requirements linked to their narrow ecological niche. At some places  
457 on our site, depositing sandy salty soils excavated closer to the Mediterranean coast has  
458 provided a habitat for *Limonium cuspidatum* (Delort) Erben and *Limonium echioides* (L.)  
459 Mill., two halophytic species of conservation interest at the regional scale. However,  
460 deposition of anthropogenic materials is certainly not the best method to restore plant



461 communities similar to the reference steppe in the plain of La Crau. A better method  
462 might be steppe geomorphological rehabilitation by filling the bottom of quarry pits, thus  
463 isolating the upper transferred soil layers from the water table (Meredith, 2007; Frouz et  
464 al., 2008; Mchergui et al., 2014). Nevertheless, our results show that spoils must be  
465 covered by at least 40 cm of topsoil from the reference steppe.

466  
467 Thus, topsoil transfer appears to be the most favorable treatment for rehabilitation  
468 of the reference sub-steppe plant community, in terms of physico-chemical properties and  
469 plant community composition. Our long-term results on soil transfer restoration confirm  
470 those obtained over the short term by Bulot et al. (2014) and Jaunatre et al. (Jaunatre et  
471 al., 2014a, 2014b) for the same steppe area. Our study shows that these reconstituted soils  
472 present the most favorable abiotic conditions for restoring the reference steppe,  
473 particularly because they entail no significant differences in fine particle size content from  
474 the soil of the reference steppe (Buisson et al., 2006; Bulot et al., 2016; Römermann et  
475 al., 2005). However, the plant communities identified in these treatments cannot yet be  
476 considered similar to those of the reference steppe vegetation, as measured by the various  
477 diversity indices. Soil alone does not account for the diversity of vegetation encountered  
478 here after different types of soil transfer. Other factors are involved, such as the plant  
479 composition of the different surrounding patches, i.e. steppe, abandoned quarries, fallow  
480 land, orchards, etc. (Pärtel et al., 1998; Zobel et al., 1998), the reproduction capacity of  
481 their communities, the presence of dispersal vectors (Bakker et al., 1996; Ozinga et al.,  
482 2004) and between-species competition or facilitation (Maestre et al., 2009; Teixeira et  
483 al., 2016). The topsoils transferred most probably had a very poor seed bank, having been  
484 conserved in large piles for several years, and the soil of the reference steppe is known to  
485 have a very poor permanent seed bank (Römermann et al., 2005; Buisson et al., 2006;  
486 Saatkamp et al., 2009; Rivera et al., 2012; Bordez, 2015). Therefore there would be very  
487 little similarity between the topsoil transferred and the reference steppe seed banks. Soil  
488 transfers in contact with the water table are ruled out as a restoration treatment aiming to  
489 restore the vegetation from the reference steppe (Mediterranean xeric grasslands), since  
490 they favor species adapted to moist soils, such as *Scirpus holoschoenus* subsp. *romanus*  
491 (L.) auct., *Aster squamatus* (Sperng.) Hieron, etc.. The solution would be to reconstitute  
492 the conglomerate bedrock occurring in the reference steppe, or to deposit spoil materials  
493 covered by topsoil as described above, which would prevent plant roots from reaching  
494 the water table.

495 Compared to techniques that only impact species dispersion, such as hay transfer  
496 (Hölzel and Otte, 2003; Coiffait-Gombault et al., 2011a, 2011b; Török et al., 2012) or  
497 seeding (Oliveira et al., 2014; Gilardelli et al., 2016), topsoil transfer offers the best  
498 results, both by dispersing target species and by at least partially recreating soil abiotic  
499 conditions (Box, 2003; Jaunatre et al., 2014b). However, real success is only possible if  
500 certain criteria are met. The topsoil's particle size fraction needs to be adequate and it  
501 should not contain blocks of conglomerate that will raise soil CaCO<sub>3</sub> content. This could  
502 negatively impact some slightly acidophilus species which characterize the steppe  
503 vegetation, especially "tonsures" (vegetation patches composed of very small vegetation,  
504 dominated by bryophytes, lichens and vascular plants that are more acidophilus and  
505 xerophilous than other steppe species (Coiffait-Gombault, 2011)).

506 Confirming findings by Simón-Torrez et al. (Simón-Torres et al., 2014), our study  
507 demonstrates that in the long term, the best way to restore the reference steppe vegetation is  
508 by using the most similar soil, which has conserved its fine particle size fraction, with a thickness

509 of at least 30 to 40 cm and without contact with the water table (Redente et al., 1997; Holmes,  
510 2001; Bowen et al., 2005). Schladweiler et al. (Schladweiler et al., 2005) found that 30 cm of  
511 topsoil produced the greatest diversity; while they found that more than 50 cm ensured the  
512 greatest biomass, this was not our goal here. Despite topsoil transfer, 30 years was not  
513 sufficient to reach the reference state, even though the traditional sheep-grazing regime  
514 was re-established after quarry abandonment. Other factors may have been involved in  
515 changing the plant succession. At soil level, fine elements such as clays could have been  
516 lost by leaching during soil transport and / or the storage phase (Weng et al., 2004; Zeng  
517 et al., 2011). This would have modified soil physico-chemical properties and  
518 consequently the plant communities that developed there (Citeau, 2008; Amir et al.,  
519 2014). On our soil transfer treatments, species richness was similar to the steppe, but the  
520 most dominant species of the steppe was still absent (*Brachypodium retusum*).  
521 Conversely, species not present in the steppe were able to develop. In all cases, our  
522 monitoring should be pursued on the long-term. There is clearly room for improvement  
523 in the methods used for future restoration of quarries still in operation. Topsoil transfer  
524 in turfs could be one answer (Good et al., 1999; Vécrin and Muller, 2003; Bulot et al.,  
525 2014), as this method allows the conservation of the soil structure (Bulot et al., 2014).  
526 The transfer could also be direct, without storage, thereby limiting degradation of the soil  
527 and of the seed bank (Granstrom, 1987; Jones and Esler, 2004; Fenner and Thompson,  
528 2005; Hall et al., 2010; Golos and Dixon, 2014). This would also ensure that plant species  
529 remain established on the turfs, preventing undesired species implantation. Although  
530 direct topsoil transfer is advocated, it is often not feasible because soil in place in the  
531 steppe may not be available when required for transfer (Bulot et al., 2014). When only  
532 ordinary soil transfer is possible, storage time must be as short as possible: numerous  
533 studies have shown the negative impact of soil storage (Strohmayr, 1999; Stahl et al.,  
534 2002; Boyer et al., 2011; Park et al., 2011; Golos and Dixon, 2014; Bordez, 2015).  
535 Storage can lead to negative impacts on the soil seed bank. Direct topsoil transfer results  
536 in loss of less than 50% of the soil seed bank, whereas stockpiling causes loss of 80-90%  
537 (Koch et al., 1996). In addition, stored soil piles form a barrier and can trap many non-  
538 target anemochorous species (Bordez, 2015). Storage can also cause rotting of organic  
539 matter when piles are too high (Stahl et al., 2002). It is therefore recommended that soil  
540 be stored on low elevation piles (Bordez, 2015). Moreover, while soil transfer may go  
541 some way toward restoring the reference ecosystem, the trajectories of rehabilitated  
542 ecosystems are not those of the reference steppe ecosystem. Additional techniques should  
543 be implemented to enhance restoration, such as seeding target species or removing  
544 undesirable species (Bordez, 2015). For example, native species seeding favors the  
545 development of native species to the detriment of exotic or ruderal species (Norman et  
546 al., 2006).

547

548 In conclusion, topsoil transfer methods can be enhanced by various techniques  
549 offering prospects of improved restoration. This should encourage current quarrying  
550 operations to implement these findings when planning for rehabilitation.

551

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560

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