



HAL
open science

Identifying reference communities in ecological restoration: the use of environmental conditions driving vegetation composition

Aure Durbecq, Renaud Jaunatre, Elise Buisson, Alexandre Cluchier, Armin Bischoff

► To cite this version:

Aure Durbecq, Renaud Jaunatre, Elise Buisson, Alexandre Cluchier, Armin Bischoff. Identifying reference communities in ecological restoration: the use of environmental conditions driving vegetation composition. *Restoration Ecology*, 2020, 28 (6), pp.1445-1453. 10.1111/rec.13232 . hal-02964222

HAL Id: hal-02964222

<https://hal.science/hal-02964222v1>

Submitted on 30 Oct 2020

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

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

1 **Identifying reference communities in ecological restoration: the use of environmental**
2 **conditions driving vegetation composition**

3 **Running head:** Identification of references in ecological restoration

4

5 *Aure Durbecq^{1,2}, Renaud Jaunatre³, Elise Buisson¹, Alexandre Cluchier², Armin Bischoff¹

6

7 ¹Mediterranean Institute of Biodiversity and Ecology (IMBE), Avignon University, CNRS,
8 IRD, Aix Marseille University, IUT, Campus Jean Henri Favre, 337 Chemin des Meinajaries,
9 84140 Avignon, France.

10 ²Engineering consulting ECO-MED, Tour Méditerranée, 65 Avenue Jules Cantini, 13006
11 Marseille, France

12 ³Univ. Grenoble Alpes, INRAE, LESSEM, 2 rue de la Papeterie-BP 76, 38402 St-Martin-
13 d'Hères, France

14 *corresponding author – aure.durbecq@gmail.com

15

16 **Author contributions:** AD, AB, RJ, EB conceived the project; AD, AB, RJ, EB conducted the
17 field surveys; AD conducted the literature review and organized the data; RJ, AD wrote the
18 personalized R functions; AD, RJ, AB analyzed results; AD wrote the first draft; AD, AB, RJ,
19 EB revised and edited the manuscript.

20

21

22

23 **Abstract**

24 In restoration ecology, the reference ecosystem represents a key concept which is well defined
25 from a theoretical point of view. In practice, however, selecting reference systems, such as
26 reference plant communities, often lacks clear methodology. In order to facilitate this selection,
27 we provide a framework based on ecological theory and more precisely on relationships
28 between vegetation and environmental factors to identify reference plant communities. The four
29 major steps are: 1) the delimitation of a geographical zone in which habitat types similar to
30 restoration sites occur; 2) the identification of environmental factors structuring non-degraded
31 plant communities within this geographical zone; 3) the comparison of the environmental
32 factors between non-degraded and degraded sites; 4) the selection of the non-degraded sites
33 most similar to restoration sites in terms of environmental factors to use them as references. We
34 concept-proved our approach by identifying reference communities using environmental factor
35 combinations for five mountain grassland sites degraded by the construction of a high-voltage
36 line. In a multivariate analysis of eighteen non-degraded sites, we identified six major
37 environmental factors explaining plant species compositions. A second multivariate analysis
38 including degraded sites provided environmental distances of the eighteen non-degraded to
39 each of the degraded sites. The results demonstrated that the environmentally most similar sites
40 were not necessarily the geographically closest ones. In conclusion, the analysis of regional
41 plant-environment interactions provides an important tool to identify reference communities or
42 source sites for seed transfer if not available adjacent to degraded habitats.

43

44 **Keywords:** abiotic factors, grassland restoration, plant succession, degradation, target
45 reference

46

47 **Implications for Practice**

48 - A good knowledge of the relationships between structuring environmental factors and plant
49 community composition allows the identification of reference communities based on the
50 measurement of these factors at restoration sites.

51 - The selected environmental factors need to be robust and should not be too much affected by
52 ecosystem degradation prior to restoration.

53 - The study of several potential reference communities makes it possible to select the best
54 reference(s) for each restoration site in terms of such environmental factors.

55 - In grassland ecological restoration, the identification of reference communities using
56 combinations of factors of restoration sites allows a more efficient sourcing of donor sites in
57 particular if close-by reference communities are not available.

58

59

60

61

62

63

64

65

66

67

68 **Introduction**

69 The definition of reference systems is a crucial step in ecological restoration as it allows
70 defining restoration targets and evaluating restoration success (Hobbs & Norton 1996; Giardina
71 et al. 2007; McDonald et al. 2016). The reference paradigm represents a cornerstone concept
72 which is well defined from a theoretical point of view (Aronson et al. 1993, 2016; SER Primer
73 2004). However, in practice, the specific selection of reference ecosystems or communities
74 often lacks clear scientific criteria, or this step is not sufficiently taken into account in
75 restoration planning. A review of 301 articles focusing on terrestrial ecological restoration
76 projects showed that less than 75% of the studies used a reference, 62% used “negative
77 reference(s)” representing the pre-restoration degraded state, and only 60% used “positive
78 target reference(s)” defined by the pre-degradation system, with only 22% presenting both.
79 Both negative and positive references may be used together to evaluate restoration success: i) a
80 negative one to evaluate the success of an active restoration compared with the degraded system
81 , and ii) a positive one to assess success by comparing the restored and the reference
82 communities (Benayas et al. 2009; Wortley et al. 2013). In many studies, the ecosystem prior
83 to its degradation is considered as a (positive) reference (Aronson et al. 1995; SER Primer
84 2004). However, ecosystems may already be degraded prior to the latest disturbance that is
85 considered as starting point of restoration, and/or the information on the state of the ecosystem
86 before degradation might not be available. This is a very common situation in European
87 grassland restoration where ecosystems suffer from land abandonment or intensification
88 (Bischoff 2002; Baasch et al. 2016).

89 An important objective of restoration is to accelerate or jumpstart succession by facilitating
90 late-successional species (Palmer et al. 1997). Late-successional stages or even endpoints of
91 succession are determined by abiotic conditions although historical contingency may influence
92 final community composition (Fukami et al. 2005; Young et al. 2005). While it is useful to

93 consider endpoints (attractors) of succession, earlier successional stages and potential variation
94 of endpoints also need to be taken into account (Prach et al. 2016). The succession towards
95 natural vegetation representing such endpoints is often prevented by human land management.
96 A constant and/or predictable disturbance regime, such as grazing by livestock of semi-natural
97 grasslands, may result in stable intermediate states of plant succession (or “alternate steady
98 states” or metastable states; Clewell & Aronson 2013). These intermediate states can thus be
99 considered as quasi-stable representing an equilibrium with environmental conditions and land
100 management (Young et al. 2001; Bouzillé 2007).

101 The use of several positive references may be a solution to take into account such intermediate
102 states and is increasingly common in ecological restoration (Ruiz-Jaen & Aide 2005; Suganuma
103 & Durigan 2015; McDonald et al. 2016). The definition of various reference systems resulting
104 from different land management types (Le Floc'h & Aronson 1995; Cortina et al. 2006) would
105 provide a range of restored ecosystem trajectories. Furthermore, considering the potential
106 variation in successional endpoints allows integrating ecosystem dynamics and natural
107 variability (Asbjornsen et al. 2005; Sluis et al. 2018; Erskine et al. 2019).

108 In cases of high spatial heterogeneity such as varying slope and aspect, small-scale differences
109 may compromise the preference of adjacent plant communities and populations usually
110 recommended for active ecological restoration (Kiehl et al. 2010, Vander Mijnsbrugge et al
111 2010; Scotton et al. 2011). Additionally, restoration sites may sometimes be surrounded by
112 other more or less degraded ecosystems. In such cases, references need to be found at higher
113 distances requiring a clear concept and methodology to identify the most appropriate sites.

114 In our study, we provide a framework to define positive reference communities by using
115 environmental factors that drive plant species composition. This method is particularly useful
116 in the following cases: i) information on plant species composition prior to latest degradation
117 is not available; ii) the sites were already (at least partially) degraded before the latest

118 disturbance for which restoration is required and several environmental factors structuring plant
119 communities remain within the natural range after a particular ecosystem degradation; iii) if
120 due to economic or legal constraints, only one propagule source site can be selected for several
121 restoration sites.

122 We first present a framework to select reference communities by using environmental factors
123 that determine plant species composition. To concept-prove our framework, we applied the
124 approach to a mountain grassland project involving the restoration of temporary access tracks
125 required to build a new high-voltage electricity line. Habitat conditions at the sites change at
126 relatively small scales thus complicating the search for appropriate reference communities.
127 Such extensively grazed and/or mowed grassland ecosystems represent a particular case of
128 succession in which regular management truncates succession by preventing the development
129 of woody vegetation (Young et al. 2001). Most European grasslands are thus considered as such
130 alternative quasi-stable states since they are maintained by regular grazing and mowing. Their
131 species composition is not only dependent on climate and soil conditions but also on
132 management type (Isselstein et al. 2005; Härdtle et al. 2006). However, management regimes
133 are similar in our study region allowing an analysis of environmental factors driving plant
134 community composition. Due to the scope of the construction work, the different non-degraded
135 grasslands selected as potential references should thus make it possible to obtain a wide range
136 of quasi-stable states representative of the different restoration sites.

137

138

139 ***Framework to identify reference communities by combinations of environmental factors***

140 We suggest a 4-step methodological framework to select references by analyzing the species
141 composition of non-degraded systems and measuring the environmental factors structuring
142 them (Fig. 1): 1) delimit a geographical zone in which habitat types similar to restoration sites
143 occur; 2) identify environmental factors structuring non-degraded plant communities within
144 this geographical zone and measure them in non-degraded and degraded sites; 3) evaluate
145 environmental distances between degraded sites and non-degraded sites; 4) identify the non-
146 degraded sites most similar to restoration sites in terms of environmental factors. First, a rough
147 estimation of habitat type based on existing habitat or biogeographical maps is required to
148 delimit the geographical zone in which the reference communities may be found (Alard 2002).
149 Existing pedoclimatic or historical vegetation data may increase the precision of habitat type
150 information. Secondly, within the delimited geographical zone, major environmental factors
151 that typically drive species composition in the study area are measured on non-degraded sites.
152 Factors that are significantly modified by the habitat degradation cannot be taken into account.
153 At the same time, the relationship between these environmental factors and plant species
154 composition is analyzed. The number of analyzed sites needs to be representative of the
155 variation of the geographical zone and sufficient to identify the best subset of environmental
156 factors using multivariate statistics. Once identified, the major environmental drivers of non-
157 degraded sites need to be measured on sites to be restored. The non-degraded sites presenting
158 the most similar values compared with restoration sites can therefore be used as reference sites.
159 This method allows identifying the best corresponding plant communities for each restoration
160 site within the study zone. For each restoration site, one or more reference sites that correspond
161 best in terms of structuring environmental factors and thus of potential plant community
162 composition will be chosen.

163

164 ***Proof of concept***

165 We tested our approach to identify reference communities using combinations of environmental
166 factors in mountain grasslands degraded by the construction of a high-voltage electricity line.
167 The construction work included the creation of access tracks and working platforms on 13 ha
168 of mountain grasslands. The grasslands were separated in hundreds of patches separated by
169 woodlands. Due to small-scale changes in abiotic conditions and degradation of adjacent sites
170 by land abandonment, arable use or afforestation, the closest sites were often inappropriate
171 references. The identification of reference communities was thus a priority action to define
172 restoration targets but also to find appropriate grassland sources for hay transfer used as a
173 restoration technique (Kiehl et al. 2010). Following the four suggested steps we asked: (1) What
174 are the environmental factors explaining best the species composition of non-degraded
175 mountain grassland sites? (2) What are the non-degraded sites most similar to the degraded sites
176 in terms of these environmental factor combinations? (3) Are the environmentally most similar
177 sites also the geographically closest ones?

178

179 **Material and methods**

180 ***Restoration sites and delimitation of geographical zone***

181 The study area is the upper Durance valley (“Haute-Durance”) in the Southern French Alps
182 from L'Argentière-la-Bessée to La-Batie-Neuve (44°78'78"N, 6°59'41'E; 44°57'93"N,
183 6°20'77'E, Fig.2). The study sites are located 100 to 600 m above the valley floor on slopes at
184 an elevation of 1000 to 1400 m (upper montane altitudinal belt). The climate is temperate to
185 sub-Mediterranean depending on elevation and exposition. The Pelvoux and Mont Viso
186 mountains provide a rain shadow, reducing precipitation to 740 mm per year, with a mean
187 annual temperature of 10.7°C (based on data from 1991 to 2010, meteorological station of
188 Embrun). The bedrock is predominantly calcareous, but many sites are covered by quaternary

189 glacial deposits. The degraded areas were characterized by dry to mesophilic grasslands. Most
190 of these grasslands are Natura 2000 priority habitats: “Semi-natural dry grasslands of *Festuco-*
191 *Brometalia* and scrubland facies on calcareous substrates” (N6210, EU directive habitat
192 92/43/EEC). The construction work affected soil structure and soil organic matter composition
193 by stripping soil, compacting and decompacting. A total of 18 species-rich non-degraded
194 grassland sites as well as five restoration sites were selected as study sites in the Haute-Durance
195 valley (Fig. 2). Selection criteria were traditional management by grazing (sheep, cattle) and
196 mowing, geographical representation of the study area and representative microclimatic
197 conditions (semi-dry to mesophilic) in avoiding driest and most humid sites. We further
198 excluded restoration sites with particularly strong soil modification by gravel addition.

199

200 ***Data collection***

201 To link species composition to environmental conditions, vegetation cover of all vascular plant
202 species was evaluated between 21 June and 5 July 2018 in 3 randomly placed quadrats (2m x
203 2m) within each of the 18 non-degraded grassland sites resulting in a total of 54 quadrats. We
204 measured abiotic factors that are usually not or little influenced by soil disturbance involved in
205 access track or working platform creation. These factors included soil variables such as
206 phosphorus, potassium and carbonate content, pH value and moisture but also
207 geomorphological variables influencing climatic conditions such as slope, aspect and elevation.
208 Three soil samples per site were thus taken to a depth of 20 cm (diameter 5 cm). They were
209 dried and sieved using a 2 mm mesh size. Plant available phosphorus (P_2O_5) was determined
210 using the Olsen Sterling method (1954), exchangeable potassium ions (K_2O) was determined
211 according to Thomas (1982) and Ciesielski et al. (1997). Soil moisture was measured in the
212 field using Theta Probe ML3 sensors. The soil pH was measured in a 1:5 water solution. The
213 carbonate content was estimated using a 3-level scale of effervescence with 1 M HCl (non-

214 effervescent; slightly effervescent; strongly effervescent). In mountain ecosystems, plant
215 species composition and richness are strongly influenced by slope and aspect driving solar
216 radiation (Pykälä et al. 2005). We estimated the annual direct incident solar radiation (ASR)
217 using the method of McCune & Keon (2002). The ASR takes into account that southwest facing
218 slopes show a higher radiation than southeast-facing slopes in folding the aspect (Supplement
219 S1). This folded aspect is combined with slope and latitude to calculate the ASR.

220

221 *Statistical analysis*

222 *Factor selection: test of variance inflation to avoid collinearity and correlation matrix*

223 In order to reduce collinearity within the measured environmental variables we tested Variance
224 Inflation Factors (VIF; Zuur et al. 2010). Highly correlated variables (VIF>2) were sequentially
225 removed (R package “vegan”, Oksanen et al. 2019). We used the pairwise correlation analysis
226 (Pearson correlation coefficient) to crosscheck VIF removal procedure. Since slope and aspect
227 are included in the ASR, they were not considered. Carbonate content (correlated to pH) was
228 removed according to VIF analysis whereas elevation, ASR, soil moisture, soil pH, P₂O₅ and
229 K₂O were kept and used in subsequent analyses in order to test the response of plant species
230 composition to environmental factors of non-degraded sites.

231 *Analysis of factors driving species composition*

232 We analyzed the plant species composition of the non-degraded sites using redundancy analysis
233 (RDA) to model the relationship between species composition and environmental variables (R
234 package “vegan”, Oksanen et al. 2019). Species cover was first Hellinger-transformed since
235 this transformation provides a better resolution in linear ordination techniques than chi-square
236 distance (Legendre & Gallagher 2001). We removed rare species (occurring in less than eight
237 plots) from the analysis in order to improve the detection of relationships between community
238 composition and abiotic factors. Removing rare species reduces bias due to stochastic sampling

239 effects since rare species occurrence is often a poor predictor of environmental conditions
240 (McCune and Grace 2002). The significance of the six selected variables (ASR, elevation, soil
241 moisture, soil pH, P₂O₅, K₂O) was tested using the “envfit” function with N = 999 permutations
242 (R package “vegan”; Oksanen et al. 2019) to determine relationships with plant community
243 composition.

244 *Environmental distances between non-degraded and restoration sites*

245 The environmental distance (= dissimilarity) between each restoration and non-degraded sites
246 was assessed by calculating the Euclidean distance using the “vegdist” function (R package
247 “vegan”; Oksanen et al. 2019). Non-metric multidimensional scaling (NMDS) based on
248 Euclidean distances of environmental factors was used to illustrate the most similar reference
249 for each restoration site (R code: <https://github.com/RenaudJau/ChooseRef>). In order to
250 compare the distribution of plant communities according to environmental factors values and
251 to geographical zone, a second NMDS was performed on plant species composition of non-
252 degraded sites. Sites were grouped by each environmental factor (high, medium, low factor
253 values) and major geographical zones (south; central; north) in different ordination plots. All
254 analyses were performed using R 3.5.3 (R Core Team 2019).

255 **Results**

256 *Environmental drivers of plant species composition*

257 One-hundred and eighty plant species belonging to 40 families were identified across the 18
258 study grasslands. The RDA model relating plant species composition and environmental
259 variables at non-degraded sites explained 44.8% of the total variance. The first two constrained
260 axes accounted for 20.2% and 11.4% of total variance and were mainly determined by P₂O₅,
261 soil pH, soil moisture and solar radiation (Fig. 3). The environmental variables retained by the
262 VIF analysis did not show strong correlations confirming that applying it efficiently removed
263 correlated environmental variables (Table S1). Four of the six selected variables (ASR: $p =$

264 0.004, P₂O₅: $p = 0.025$, soil moisture: $p = 0.023$, pH: $p = 0.026$) had a significant influence on
265 plant species composition whereas the influence of elevation ($p = 0.201$) and K₂O ($p = 0.830$)
266 was not significant (Table S2). Sites located on the right side of Fig. 3 were associated with low
267 ASR, relatively low (neutral) pH and high soil moisture, as well as high P₂O₅. They were well
268 represented by mesophilic species, such as *Dactylis glomerata*, *Galium verum*, *Poa pratensis*,
269 *Plantago lanceolata* and *Lathyrus pratensis*. In contrast, plants associated with higher ASR and
270 pH included *Bromopsis erecta*, *Brachypodium rupestre*, *Pilosella officinarum*, *Lotus*
271 *corniculatus* and *Teucrium chamaedrys*. Sites with higher abundance of *Astragalus danicus*,
272 *Pilosella officinarum*, *Lotus corniculatus* and *Teucrium chamaedrys* were negatively
273 correlated with elevation and soil moisture, whereas sites with higher abundance of *Salvia*
274 *pratensis* and *Dactylis glomerata* were positively correlated with both environmental variables.

275

276 ***Identification of reference sites by comparing similarity in environmental factors***

277 The five restoration sites revealed different combinations of environmental factors resulting in
278 different environmental distances with non-degraded sites (Fig. 4). Restoration sites 1R and 3R
279 showed a low environmental distance to the non-degraded site 21 indicating high similarity in
280 measured abiotic factors. However, the second environmentally closest non-degraded sites
281 were different (1R: site 22, 3R: site 18). The three sites closest to 2R in terms of environmental
282 variables were 6, followed by 4 and 5. The most similar sites to 11R were 12, 10 and 16, and
283 the most similar to 20R were 9, 17 and 14 (Fig. 4.a, Table 1). If technical and/or logistic
284 constraints require the choice of one reference community as a source site for plant material for
285 all five degraded sites, the non-degraded site 9 would represent the best reference (Table 1),
286 which has the lowest mean distance to five restored sites (Fig. 4.b).

287 The geographical zonation of plant species composition hardly corresponded to environmental
288 factor values. NMDS according to geographical position separated the southernmost zone from
289 the northernmost zone whereas the central zone was not well separated from the latter one (Fig.
290 S1). The Northern zone was characterized by low levels of P_2O_5 and soil moisture, low solar
291 radiation (ASR) and neutral pH. The Central zone showed highest soil moisture and P_2O_5 ,
292 medium ASR and the most acidic pH. The Southern zone was represented by high solar
293 radiation and a basic pH. However, there were many exceptions to these general trends and
294 environmental zonation was very different from geographical zonation in P_2O_5 and soil
295 moisture. The lack of correspondence between geographical and environmental zonation
296 explains that the environmentally most similar non-degraded site was not necessarily the
297 geographically closest one.

298

299 **Discussion**

300 Our results indicate that among the seven tested environmental factors , ASR, soil moisture,
301 P_2O_5 and soil pH best explain the distribution of plant communities in our 18 species-rich
302 mountain grasslands and can thus be used as indicators for reference communities in this
303 particular case.

304 The simultaneous analysis of environmental factors such as pedoclimatic variables, and
305 vegetation, allows identifying major ecological processes that govern the studied system, since
306 they are considered as major drivers of plant community composition (Box 1981). Elevation
307 known has an important driver of mountain grassland composition did not significantly
308 influence plant species compositionsince limited to a relatively small elevational gradient of
309 400 m. ASR included slope and aspect that are known to determine plant species composition
310 in mountain grasslands (Srinivasan et al. 2005). As many other plant communities, grasslands
311 are controlled by edaphic factors, such as water content, organic matter and nutrient

312 concentration (Sebastiá 2004; Klimek et al. 2007). Several studies have emphasized the relation
313 between pH and phosphorus. Phosphorus availability decreases with increase in pH because at
314 alkaline pH, phosphate ions react with calcium or magnesium (Cerozi & Fitzsimmons 2016; Li
315 et al. 2019). This is consistent with our results that show a negative correlation between soil pH
316 and exchangeable phosphorus (P_2O_5).

317 The lack of methods for reference community choice has often been cited as a shortcoming in
318 restoration ecology (Halle & Fattorini 2004) although some recent studies have developed a
319 more explicit methodology. Suganuma & Durigan (2015) selected nine references divided into
320 three categories of riparian forest according to their ecological integrity (3 references in old
321 growth forest, 3 in degraded forest and 3 in secondary forest). Erskine et al. (2019) combined
322 vegetation surveys and mapping data to select five reference sites for the ecological restoration
323 of uranium mines, while McManamay et al. (2018) suggest a spatial framework in six steps to
324 identify reference systems for stream restoration. All these studies used multiple references that
325 were selected according to relationships between environmental factors and vegetation patterns.
326 They included floristic surveys (contemporary data) and the search of historical data. These
327 studies suggest extending the surveys beyond the close vicinity of restoration sites and using
328 statistical tools to identify and to rank structuring variables in order to select appropriate
329 reference communities. In our framework, we filled this gap in providing a method to identify
330 reference plant communities based on statistical analyses of specific relationships between
331 environmental factors and plant community composition.

332 Since soil conditions are among the most important factors driving plant species composition,
333 a careful variable choice is needed to exclude factors strongly influenced by previous ecosystem
334 degradation. Soil disturbance, such as tillage or soil removal, may lead to a drastic reduction in
335 organic matter and total nitrogen storage, and can strongly affect their distribution in the soil
336 profile (Hölzel & Otte 2003; Dolan et al. 2006; Du et al. 2010). Intermediate arable use, as the

337 most common degradation of European grasslands, increases soil nutrient content, in particular
338 plant available potassium and phosphorous (Bischoff et al. 2009). In such a case of
339 eutrophication, the depletion of fertilizer residues is crucial to obtain abiotic conditions that are
340 appropriate for the establishment of plant species adapted to the pre-degradation state (Tallowin
341 et al. 1998; Bischoff et al. 2009). Thus, our method of reference community identification is
342 difficult to apply to ecological restoration of fertilized soils. However, if habitat degradation is
343 limited to mechanical soil disturbance, many soil variables typically driving plant species
344 composition remain unchanged. In our approach, we avoided the use of the soil parameters
345 cited above (total and mineral nitrogen, organic matter) that are known to be influenced by
346 mechanical soil disturbance. Thus, we are convinced that our selected abiotic factors are
347 appropriate for the search of best matching reference sites. Such data collected *in situ*
348 (contemporary data) may be complemented by historical data (wildfire records, land use
349 changes) that may still affect plant species composition (Asbjornsen et al. 2005; Urgenson et
350 al. 2018).

351 Our method is applicable to ecological restoration beyond grasslands if the specific
352 environmental factors structuring the plant communities are correctly identified. For example,
353 in ecological restoration of lagoon ecosystems corresponding to ancient salt marshes (EU
354 habitat code 1150), environmental factors structuring plant communities are water level,
355 duration of flooding and degree of salinity that may also be used to identify reference
356 communities (Bouzillé 2007).

357 In restoration practice, adjacent sites of very similar environmental conditions are not always
358 available. Either the degradation includes the whole habitat with completely different
359 conditions outside the degraded zone (e.g. degraded grasslands within woodlands), adjacent
360 sites are not accessible (or not available as source sites for seed transfer) or abiotic conditions
361 change at very small scales. In such cases, our approach allows the identification of

362 geographically more distant reference or source sites that match best with environmental
363 conditions of restoration sites. In this case, the selection of multiple potential references is
364 therefore necessary to select the one(s) that will be closest to the restored sites in terms of
365 environmental variables driving plant community composition. The concept of multiple
366 references is a helpful tool in restoration practice allowing a more flexible choice depending on
367 availability (identification of source sites) or on different priorities for environmental factor
368 match. Additionally, even if the restoration sites are close, the best potential reference sites are
369 not necessarily the same, depending on the environmental factors that can vary on a relatively
370 small scale. Finally, in grassland restoration practice, the identification of multiple references
371 allows to combine different source sites if non-degraded sites do not comprise the entire species
372 pool (area not sufficient, environmental conditions do not fully correspond). Although not
373 directly tested in our proof-of-concept, the method also allows the integration of land
374 management variation resulting in different quasi-stable states of plant succession.

375 To conclude, our approach to use the close relationships between environmental conditions and
376 plant community composition is a straightforward method to identify reference communities
377 when environmental structuring factors are not affected by degradation. It can be easily applied
378 to restoration practice but requires a good knowledge of plant ecology, in particular on plant-
379 environment interactions, environmental filters and local species pools (Lortie et al. 2004;
380 Cristofoli & Mahy 2010). A better understanding of plant-environment interactions and of
381 potential effects of degradations on physical and chemical soil conditions would be required to
382 improve the method.

383

384 **Acknowledgments**

385 We thank Alice Dupré-la-Tour (INRAE) and Sophie Duhautois for the restoration project
386 implementation and management, Daniel Pavon (IMBE) for his valuable help in plant species

387 identification and Marie Pisson (ECO-MED) for her technical assistance in GIS work. We also
388 thank Thierry Dutoit, François Mesléard, Emmanuel Corcket, Thomas Spiegelberger,
389 Stéphanie Huc, Christel Vidaller and Tiago Toma for their helpful advice on design and
390 analyses. Financial support was provided by RTE (Electricity Transmission Network), the
391 environmental consultancy ECO-MED and ANRT (National Agency of Research and
392 Technology via a grant CIFRE). We also thank the anonymous reviewer and the associate editor
393 Karel Prach for helpful suggestions on the manuscript.

394

395 **LITERATURE CITED**

396 Alard D, Poudevigne I (2002) Biodiversity in changing landscapes: from species or patch
397 assemblages to system organisation. In: Leuven RSEW, Poudevigne I, Teeuw RM (eds),
398 Application of Geographic Information Systems and Remote Sensing in River Studies,
399 R.S.E.W. Leuven, I. Poudevigne, R.M. Teeuw. Backhuys Publishers, Leiden, The
400 Netherlands, pp 9–24

401 Aronson J, Clewell A, Moreno-Mateos D (2016) Ecological restoration and ecological
402 engineering: Complementary or indivisible? *Ecological Engineering* 91:392–395

403 Aronson J, Floret C, Floc'h EL, Ovalle C, Pontanier R (1993) Restoration and Rehabilitation
404 of Degraded Ecosystems in Arid and Semi-Arid Lands. I. A View from the South.
405 *Restoration Ecology* 1:8–17

406 Aronson J, Floret C, Le Floc'h E, Ovalle C, Pontanier R (1995) Restauration et réhabilitation
407 des écosystèmes dégradés en zones arides et semi-arides : le vocabulaire et les concepts.
408 In: *L'homme peut-il refaire ce qu'il a défait ?* J. Libbey Eurotext, Montrouge, pp 11–29

409 Asbjornsen H, Brudvig LA, Mabry CM, Evans CW, Karnitz HM (2005) Defining Reference
410 Information for Restoring Ecologically Rare Tallgrass Oak Savannas in the Midwestern
411 United States. *J* for 103:345–350

412 Baasch A, Engst K, Schmiede R, May K, Tischew S (2016) Enhancing success in grassland
413 restoration by adding regionally propagated target species. *Ecological Engineering*
414 94:583–591

415 Benayas JMR, Newton AC, Diaz A, Bullock JM (2009) Enhancement of Biodiversity and
416 Ecosystem Services by Ecological Restoration: A Meta-Analysis. *Science* 325:1121–
417 1124 .

418 Bischoff A (2002) Dispersal and establishment of floodplain grassland species as limiting
419 factors in restoration. *Biological Conservation* 104:25–33

420 Bischoff A, Warthemann G, Klotz S (2009) Succession of floodplain grasslands following
421 reduction in land use intensity: the importance of environmental conditions,
422 management and dispersal. *Journal of Applied Ecology* 46:241–249

423 Box EO (1981) *Macroclimate and plant forms: an introduction to predictive modelling in*
424 *phytogeography*. Kluwer, The Hague, NL

425 Bouzillé JB (2007) *Gestion des habitats naturels et biodiversité. Concept, méthodes et*
426 *démarches*. Lavoisier, Paris, France 331

427 Cerozi B da S, Fitzsimmons K (2016) The effect of pH on phosphorus availability and
428 speciation in an aquaponics nutrient solution. *Bioresource Technology* 219:778–781

429 Ciesielski H, Sterckeman T (1997) A comparison between three methods for the determination
430 of cation exchange capacity and exchangeable cations in soils. *Agronomie* 17:9–15

- 431 Clewell AF, Aronson J (2013) *Ecological Restoration, Second Edition: Principles, Values, and*
432 *Structure of an Emerging Profession*. Island Press
- 433 Cortina J, Maestre FT, Vallejo R, Baeza MJ, Valdecantos A, Perez-Devesa M (2006)
434 *Ecosystem structure, function, and restoration success: Are they related?* *Journal for*
435 *Nature Conservation* 14:152–160
- 436 Cristofoli S, Mahy G (2010) *Restauration écologique: contexte, contraintes et indicateurs de*
437 *suivi*. *Biotechnologie, Agronomie, Société et Environnement* 14:203–211
- 438 Dolan MS, Clapp CE, Allmaras RR, Baker JM, Molina JAE (2006) *Soil organic carbon and*
439 *nitrogen in a Minnesota soil as related to tillage, residue and nitrogen management*. *Soil*
440 *and Tillage Research* 89:221–231
- 441 Du Z, Ren T, Hu C (2010) *Tillage and Residue Removal Effects on Soil Carbon and Nitrogen*
442 *Storage in the North China Plain*. *Soil Science Society of America Journal* 74:196–202
- 443 Erskine PD, Bartolo R, McKenna P, Humphrey C (2019) *Using reference sites to guide*
444 *ecological engineering and restoration of an internationally significant uranium mine in*
445 *the Northern Territory, Australia*. *Ecological Engineering* 129:61–70
- 446 Fukami T, Bezemer M, Mortimer S, Vander Putten, WH (2005) *Species divergence and trait*
447 *convergence in experimental plant communities*. *Ecology Letters*, 8:1283-1290.
- 448 Giardina CP, Litton CM, Thaxton JM, Cordell S, Hadway LJ, Sandquist DR (2007) *Science*
449 *Driven Restoration: A Candle in a Demon Haunted World—Response to Cabin (2007)*.
450 *Restoration Ecology* 15:171–176

- 451 Halle S, Fattorini M (2004) Advances in restoration ecology: insights from aquatic and
452 terrestrial ecosystems. In: Temperton VM et al. Assembly rules and Restoration
453 Ecology., Island Press. Whashington, pp 10–33
- 454 Härdtle W, Redecker B, Assmann T, Meyer H (2006) Vegetation responses to environmental
455 conditions in floodplain grasslands: Prerequisites for preserving plant species diversity.
456 Basic and Applied Ecology 7:280–288
- 457 Hobbs RJ, Norton DA (1996) Towards a Conceptual Framework for Restoration Ecology.
458 Restoration Ecology 4:93–110
- 459 Hölzel N, Otte A (2003) Restoration of a species-rich flood meadow by topsoil removal and
460 diaspore transfer with plant material. Applied Vegetation Science 6:131–140
- 461 Isselstein J, Jeangros B, Pavlu V (2005) Agronomic aspects of biodiversity targeted
462 management of temperate grasslands in Europe – A review. Agronomy Research 3:139–
463 151
- 464 Kiehl K, Kirmer A, Donath TW, Rasran L, Hölzel N (2010) Species introduction in restoration
465 projects – Evaluation of different techniques for the establishment of semi-natural
466 grasslands in Central and Northwestern Europe. Basic and Applied Ecology 11:285–
467 299
- 468 Klimek S, Richter gen. Kemmermann A, Hofmann M, Isselstein J (2007) Plant species richness
469 and composition in managed grasslands: The relative importance of field management
470 and environmental factors. Biological Conservation 134:559–570
- 471 Le Floch E, Aronson J (1995) Écologie de la restauration. Définition de quelques concepts de
472 base. Natures Sciences Sociétés, Hors série. 3:s29–s35

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

475 Li F-R, Liu L-L, Liu J-L, Yang K (2019) Abiotic and biotic controls on dynamics of labile
476 phosphorus fractions in calcareous soils under agricultural cultivation. *Science of The*
477 *Total Environment* 681:163–174

478 Lortie CJ, Brooker RW, Choler P, Kikvidze Z, Michalet R, Pugnaire FI, Callaway RM (2004)
479 Rethinking plant community theory. *Oikos* 107:433–438

480 McCune B, Grace JB (2002) *Analysis of ecological communities*, MjM Software Design,
481 Gleneden Beach, Oregon.

482 McCune B, Keon D (2002) Equations for potential annual direct incident radiation and heat
483 load. *Journal of Vegetation Science* 13:603–606

484 McDonald T, Gann GD, Jonson J, Dixon KW (2016) *International standarts for the practice of*
485 *ecological restoration – includind principles and key concepts (first edition)*, Society for
486 *Ecological Restoration (SER)*, Washington, D.C

487 McManamay RA, Smith JG, Jett RT, Mathews TJ, Peterson MJ (2018) Identifying non-
488 reference sites to guide stream restoration and long-term monitoring. *Science of The*
489 *Total Environment* 621:1208–1223

490 Oksanen J, Blanchet F, Friendly M, Kindt R, Legendre P, McGlinn Dol et al. (2019) *vegan:*
491 *Community Ecology Package*. R package version 2.5-4

492 Olsen SR (1954) *Estimation of Available Phosphorus in Soils by Extraction with Sodium*
493 *Bicarbonate*. U.S. Department of Agriculture

494 Palmer MA, Ambrose RF, Poff NL (1997) Ecological Theory and Community Restoration
495 Ecology. *Restor Ecology* 5:291–300

496 Prach K, Tichý L, Lencová K, Adámek M, Koutecký T, Sádlo J et al. (2016) Does succession
497 run towards potential natural vegetation? An analysis across seres. *Journal of*
498 *Vegetation Science* 27:515–523

499 Pykälä J, Luoto M, Heikkinen RK, Kontula T (2005) Plant species richness and persistence of
500 rare plants in abandoned semi-natural grasslands in northern Europe. *Basic and Applied*
501 *Ecology* 6:25–33

502 R Core Team (2019) R: A language and environment for statistical computing. R Foundation
503 for Statistical Computing, Vienna, Austria. URL <https://www.R-project.org/>

504 Ruiz-Jaen MC, Aide TM (2005) Restoration Success: How Is It Being Measured? *Restoration*
505 *Ecology* 569–577

506 Sebastiá M-T (2004) Role of topography and soils in grassland structuring at the landscape and
507 community scales. *Basic and Applied Ecology* 5:331–346

508 SER Primer: Society for Ecological Restoration International (2004) Science & Policy Working
509 Group. The SER International Primer on Ecological Restoration. www.ser.org &
510 Tucson: Society for Ecological Restoration International.

511 Scotton M, Kirmer A, Krautzer B (2011) Practical handbook for seed harvest and ecological
512 restoration of species-rich grasslands. Practical handbook for seed harvest and
513 ecological restoration of species-rich grasslands

- 514 Sluis WJ, Bowles M, Jones M (2018) Multiscale metrics differentiate among tallgrass prairie
515 restorations and remnant ecosystems along a restorative continuum. *Restoration*
516 *Ecology* 26:466–475
- 517 Srinivasan MP, Bhatia S, Shenoy K (2005) Vegetation-environment relationships in a South
518 Asian tropical montane grassland ecosystem: restoration implications. *Tropical Ecology*
519 56:201–221
- 520 Suganuma MS, Durigan G (2015) Indicators of restoration success in riparian tropical forests
521 using multiple reference ecosystems. *Restoration Ecology* 23:238–251
- 522 Tallowin, JRB, Kirkham FW, Smith REN, Mount-ford JO (1998) Residual effects of
523 phosphorous fertilisation on the restoration of floristic diversity to wet hay meadows.
524 In: Joyce CB and Wade PM (eds) *European Lowland Wet Grasslands: Biodiversity,*
525 *Management and Restoration*, John Wiley & Sons, Ltd., Chichester, United Kingdom,
526 pp. 249-263
- 527 Thomas GW (1982) Exchangeable Cations. *Methods of Soil Analysis Part 2 Chemical and*
528 *Microbiological Properties agronomyonogra:159–165*
- 529 Urgenson LS, Nelson CR, Haugo RD, Halpern CB, Bakker JD, Ryan CM et al. (2018) Social
530 perspectives on the use of reference conditions in restoration of fire-adapted forest
531 landscapes. *Restoration Ecology* 26:987–996
- 532 Vander Mijnsbrugge K, Bischoff A, Smith B (2010) A question of origin: Where and how to
533 collect seed for ecological restoration. *Basic and Applied Ecology* 11:300–311

534 Wortley L, Hero J-M, Howes M (2013) Evaluating Ecological Restoration Success: A Review
535 of the Literature: Trends and Gaps in Empirical Evaluations. *Restoration Ecology*
536 21:537–543

537 Young TP, Petersen D A, Clary JJ (2005) The ecology of restoration: historical links, emerging
538 issues and unexplored realms. *Ecology Letters* 8:662–673

539 Young TP, Chase JM, Huddleston RT (2001) Community Succession and Assembly
540 Comparing, Contrasting and Combining Paradigms in the Context of Ecological
541 Restoration. *Ecological Rest* 19:5–18

542 Zuur AF, Ieno EN, Elphick CS (2010) A protocol for data exploration to avoid common
543 statistical problems. *Methods in Ecology and Evolution* 1:3–14

544

545

546

547

548

549

550

551 **Table 1.** Similarities between restoration sites (1R, 2R, 3R, 11R, 20R) and the non-degraded
552 sites (from site 4 to 24, without R) of the study zone (similarity decreasing from left to right)
553 using the Euclidean distance for environmental variables shown in Fig. 3. Here the colors show
554 examples for choosing only one reference for (a) one site (example of restoration site 2R: \triangle);
555 (b) 2 sites (example of 1R and 3R: \circ); (c) all the restoration sites (1R, 2R, 3R, 11R and 20R:
556 \hexagon).

Restoration sites	Reference sites																	
	Higher similarity \longrightarrow Lower similarity																	
1R	\circ 16	22	\hexagon 9	23	15	19	4	17	18	13	6	5	8	16	10	7	12	14
2R	\triangle 6	4	5	15	\hexagon 9	16	16	8	18	7	19	13	23	10	22	12	17	14
3R	\circ 16	18	15	6	19	\hexagon 9	4	22	8	23	5	13	16	17	7	10	12	14
11R	12	10	16	13	\hexagon 9	14	7	5	8	15	17	19	18	22	4	6	16	23
20R	\hexagon 9	17	14	19	10	22	16	12	7	13	16	18	4	15	8	6	5	23

557

558

559

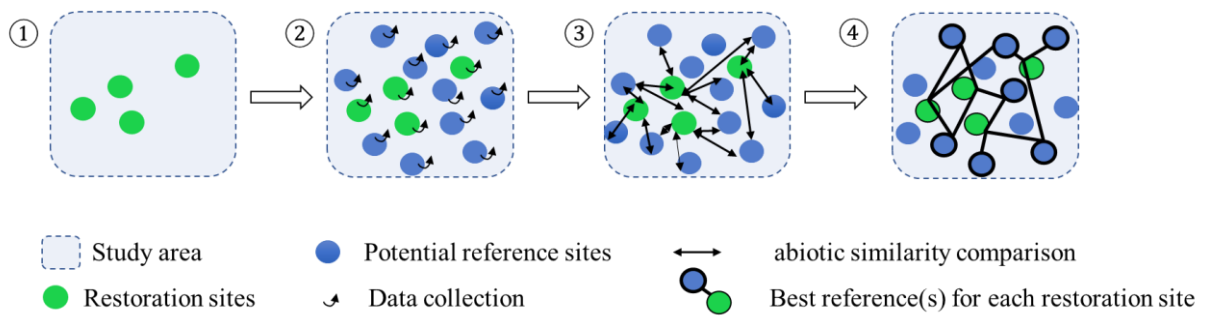
560

561

562

563

564



565

566

567 **Fig. 1.** The four major steps of the proposed method to select references. ① Delimit an extend
568 study area around restoration sites; ② Identify environmental factors structuring plant
569 communities (data collection and driver analysis); ③ Evaluate environmental distances
570 between restoration and potential reference sites (dissimilarity coefficient); ④ Select the most
571 similar references to restoration sites (dissimilarity coefficient).

572

573

574

575

576

577

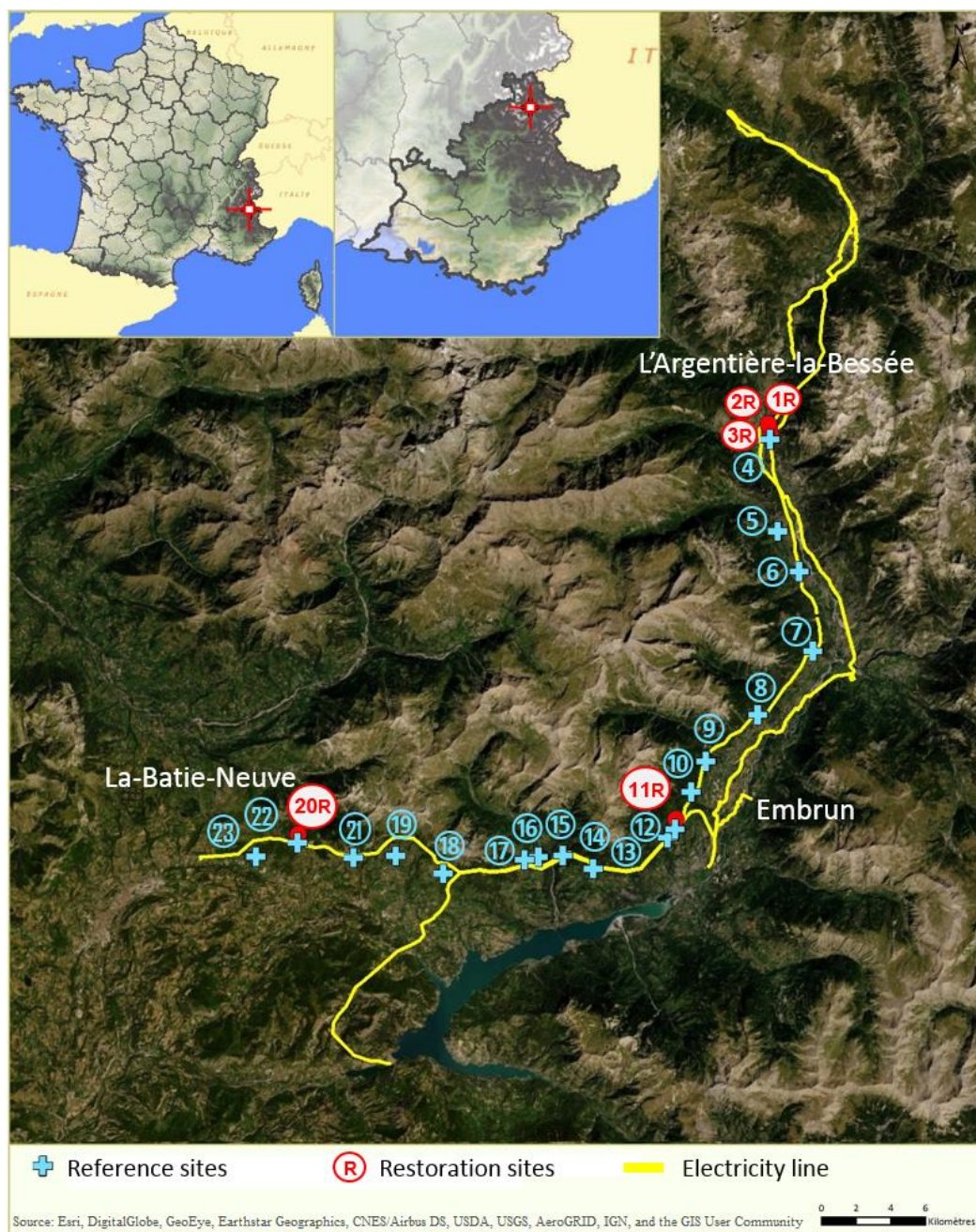
578

579

580

581

582



583

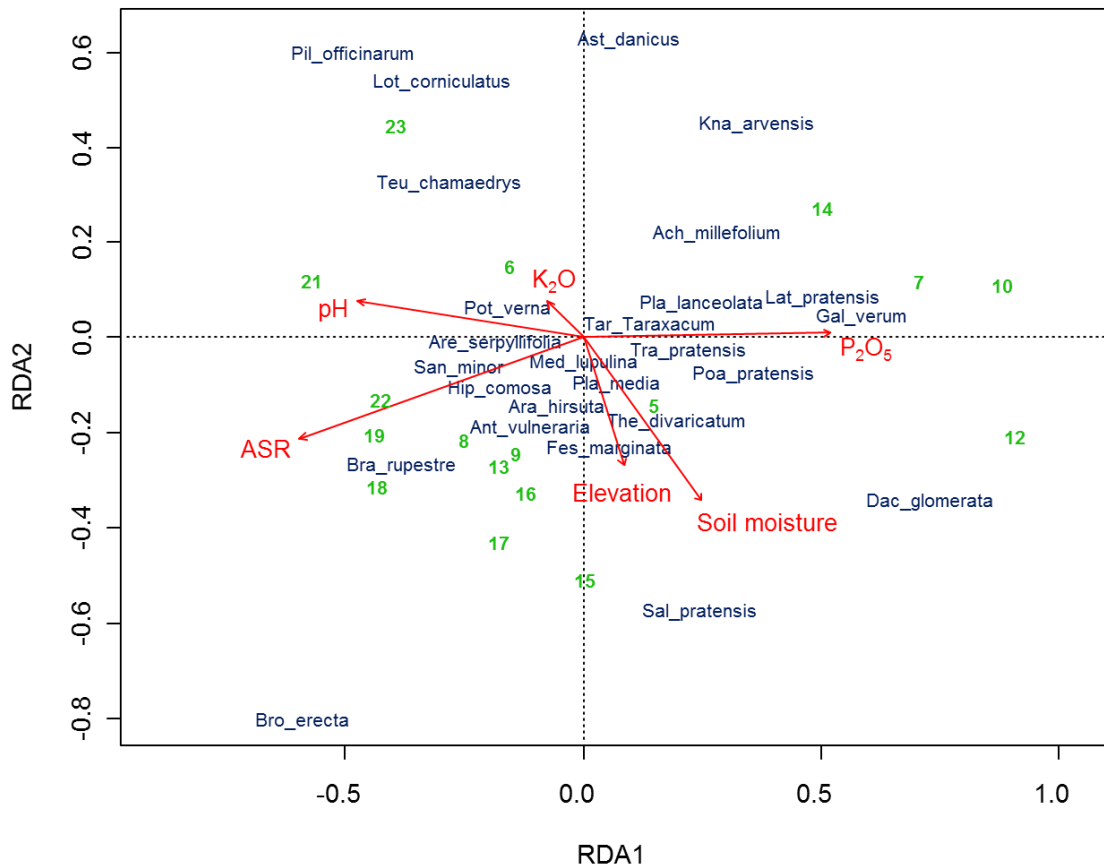
584

585 **Fig. 2.** Location of the study area with restoration sites (1R, 2R, 3R, 11R and 20R, in red) and
586 reference sites (numbered from north to south, without R, in blue) along the construction of a
587 new electricity line.

588

589

590



591

592 **Fig. 3.** RDA biplot using Hellinger-transformed plant species cover (species names in blue) and
593 reference sites (green) constrained by environmental variables (red arrows). Environmental
594 variables: ASR = Annual Solar Radiation; P₂O₅ = plant available phosphorous; soil moisture;
595 soil pH; elevation and K₂O = plant available potassium. Full species names: Table S3.

596

597

598

