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Restoration of European habitats in mainland Portugal, using commercial seed mixtures. Considerations for its management and conservation

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ABSTRACT
Permanent mountain pastures include meadows and other perennial pastures of high ecological, economic, cultural and scenic value. Increasing desertification limits the maintenance and conservation of its biodiversity and the associated landscape mosaic. A restoration experiment in permanent high altitude grasslands in Beira Alta (Centre East (CE) mainland Portugal) was made, by sowing adequate cultivars of existing grass and legume species. The main objectives addressed were: (1) comparison of floristic composition between reference communities included in the previous habitats and the improved communities; (2) evaluation of the success of sowing adequate cultivars of autochthonous species; (3) evaluation of the establishment of target species in terms of the maintenance of floristic composition of reference. The experiment was carried out in 2014 on nine farms situated in Beira Alta (Guarda District) and the phytosociological method was applied in the floristic surveys. The sown species with highest percentage of soil cover were *Trifolium subterraneum*, *Lolium multiflorum*, *Ornithopus sativus* and *Trifolium vesiculosum*. In the priority habitat 6220 it was observed a re-establishment of many species in their original composition and a high cover of several cultivars of *Trifolium subterraneum*. These results highlight the importance of using cultivars of autochthonous species in the improvement of altitude pastures.

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Floristic composition; field experiment; grasslands; productivity; re-establishment

1. Introduction

Natural and semi-natural Mediterranean pastures are important resources in traditional land-use systems integrating the most protected ecosystems in Europe (EC 2007). They have important functions such as the prevention of soil erosion, reducing the risk of fire and carbon sequestration. Generally, they are grazed mainly by cattle and sheep; some of them are cut and improved with the aim of increasing their nutritional value. They are recognised as key habitats for maintaining biodiversity in agricultural landscapes (Pykälä 2000). They are dependent on traditional land use systems (Gustavsson et al. 2007) and their ecological and socio-economic multi-functionality are generally recognised (Zimkova et al. 2007). However, their diversity has been declining due to changes in management. It is known that the changes in biodiversity registered in recent decades come in response to strong changes induced by human action (Vitousek 1994).

In mountain areas of CE mainland Portugal these habitats have high environmental, ecological and landscape value. Generally, they are subjected to grazing by cows or sheep and some of them are cut in the summer.

Due to their importance in biodiversity conservation and economic sustainability, it is necessary to re-establish their diversity, also improving nutritional value. Studies and experiments in grassland restoration have been performed in many parts of Europe (e.g. Benayas et al. 2009; Török et al. 2010; Prach et al. 2014). Ecological restoration has been widely used to reverse environmental degradation and its benefits in provision of biodiversity and ecosystems services are known (Benayas et al. 2009).

The low levels of productivity and feed quality obtained in semi-natural grasslands in mountain areas of CE mainland Portugal led to a set of experimental restorations and improvements through sowing adequate cultivars of existing grass and legume species in order to improve their yield and quality. These experiments were carried out in 2013–2014 on nine farms situated in Beira Alta (Guarda District), including some communities which corresponded with the priority habitat 6220 (Pseudo-steppe with grasses and annual of the *Thero-Brachypodietea*) or habitat 6510 (Low hay meadows; Council Directive 92/43/EEC 1992). Seed mixtures of cultivars of autochthonous species were sown, composed mainly by legume species and grasses, with the expectation of increased productivity in line with other studies (e.g. Schipanski and Drinkwater 2012; Teixeira et al. 2015).

In order to better understand ecological key processes in grazing systems and assessing the success of restoration and management, the following questions were addressed: (1) How do floristic composition and ecological variables differ between reference communities included in the previous habitats and the

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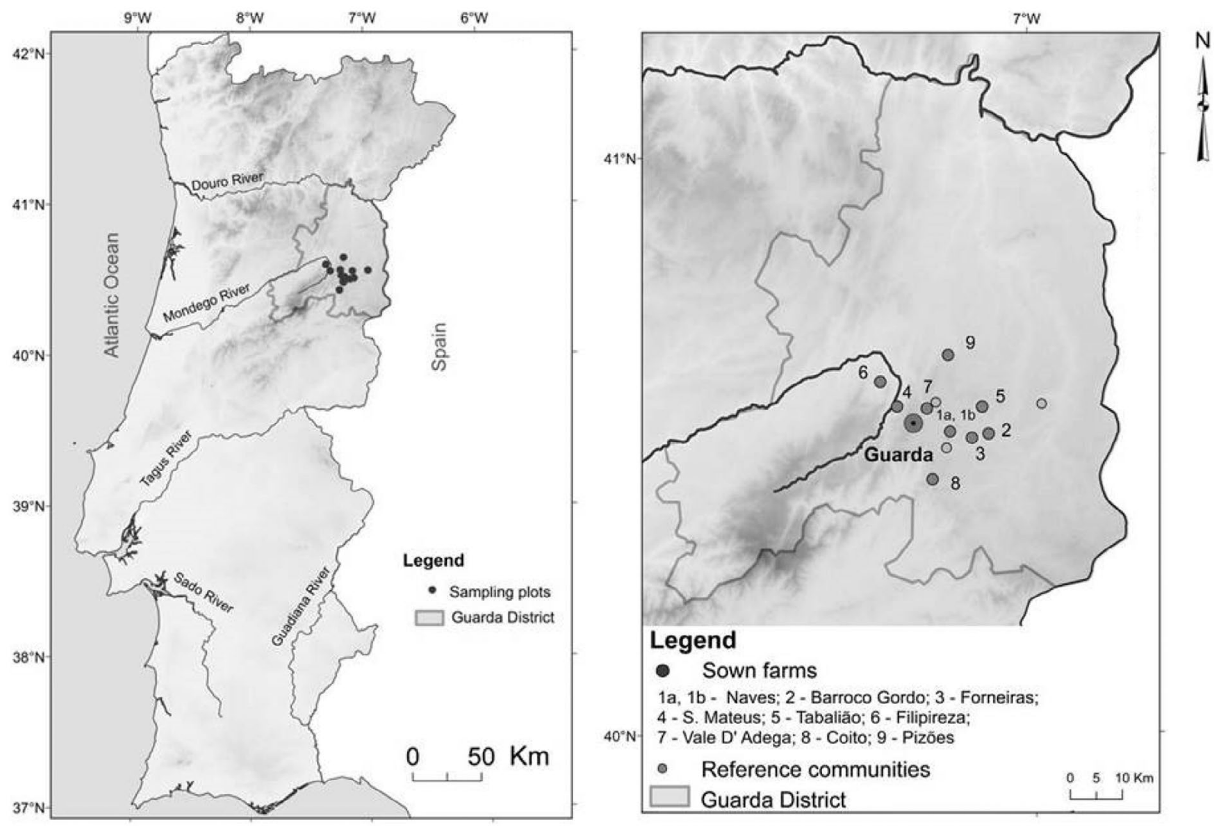

Figure 1. Study area and location of the sown farms and reference sites.

improved communities? (2) How do restored grasslands differ in the success of sowings? (3) How do restored grasslands differ in the maintenance of floristic composition of autochthonous target species?

2. Methodology

2.1. Study area

The study area (Figure 1) covered the entire Guarda District in CE mainland Portugal in natural permanent mountain grasslands (500 to 850 m a.s.l.).

According to the biogeographic typology of Rivas-Martínez et al. (2014), the study area is included in the Eurosiberian Region, Atlantic-Central European Subregion, European Atlantic Province, Cantabrian Atlantic Province, Atlantic Orolusitanian Subprovince, in which the Guardan District of Montemuro and Estrela Sierran Sector is included.

The study area covers mainly mesomediterranean and supramediterranean territories with sub-humid upper and lower ombrotypes. The identification of the communities was based mainly on the publications of Meireles (2010) and Ribeiro (2013).

2.2. Sampling design and data collection

During 2013, nine farms were sown with seed mixtures of cultivars of autochthonous species (Figure 1). The soil was previously fertilised in order to correct pH and balance nutrients. From late winter (April) to summer (July) of 2014, 55 floristic surveys were made (Figure 1) on reference sites and on the nine farms sown in order to evaluate the success of the sowings. The phytosociological method of Braun-Blanquet (1979), modified by Géhu and Rivas-Martínez (1981) was applied. The plot size of each relevé was 16 m^2. Each taxon's percentage cover was recorded to replace the scale of Braun-Blanquet (1964) since this is not appropriate for many multivariate analyses (Podani 2006).

The geographic position of each sample unit was obtained with GPS and digitalised through a geographic information system (GIS) ArcGis (version 9.0; ESRI).

Explanatory variables were recorded for each meadow or grassland, including both qualitative and quantitative environmental variables and management variables. The environmental factors included: (a) pedological factors that include the percentage of stone, soil texture and parameters obtained by standard soil analyses such as the determination of pH (H_2O), potassium and phosphorus by the Egnér-Riehm (Riehm 1958) method and (b) orographic factors that include aspect.

Management influence was evaluated by using: (a) grazing intensity; (b) type of livestock (cow and sheep).

The sown mixtures in each farm are shown in Table 1.

2.3. Nomenclature

Botanical nomenclature follows Aedo and Herrero (2005), Benedí et al. (2009), Castroviejo et al. (1986–1990, 1993a, 1993b, 1997a, 1997b, 2008), Devesa et al. (2007), Franco (1984), Franco and Rocha Afonso (1994, 1998), Morales et al. (2010), Muñoz Garmendia and Navarro (1998), Nieto Feliner et al. (2003), Paiva et al. (2001), Talavera et al. (1999, 2000, 2010, 2012), Franco

Table 1. Seed mixtures of cultivars autochthonous species. M1 (in Naves and Tabalião farms), M2 (in Pizões farm), M3 (in S. Mateus farm), M4 (Forneiras farm), M5 (in Filipireza farm), M6 (in Barroco Gordo, Vale D'Adega and Coito farms).

Mixture		M1				M2		M3		M4		M5		M6					
		NAVES		TABALIÃO		PIZÕES		S. MATEUS		FORNEIRAS		FILIPIREZA		BARROCO GORDO		VALE D'ADEGA		COITO	
Species	Cultivar	kg	%	kg	%	kg	%	kg	%	kg	%	kg	%	kg	%	kg	%	kg	%
	'Seaton Park'	45	12	36	12	66	8			30	12	33	12	78	12	15	12	78	12
	'Woogenelup'									30	12	22	8	52	8	10	8	52	8
	'Clare'							18	12.5										
Trifolium subterraneum	'Dalkeith'					33	4												
	'Campeda'	30	8	24	8	99	12			20	8			78	12	10	8	52	8
	'Gosse'	45	12	36	12	66	8	12	8,3	30	12	44	16	78	12	15	12	78	12
	'Antas'	45	12	36	12	33	4	18	12.5			22	8			5	4	26	4
Trifolium resupinatum	'Hykon'					66	8												
Ornithopus sativus	'Emena'	60	16	48	16	132	16	18	12.5	30	12	33	12	78	12	15	12	78	12
Medicago sativa	'Paraggio'							12	8.3										
Trifolium resupinatum	'Nitro-plus'					33	4					22	8						
Trifolium incarnatum	'Contea'	15	4	12	4	33	4			20	8			52	8	5	4	26	4
Trifolium michelianum	'Bolta'	15	4	12	4	33	4	6	4.2			11	4	26	4				
	'Paradana'									20	8					10	8	52	8
Trifolium vesiculosum	'Zulu II'	30	8	24	8	66	8	12	8.3	20	8	33	12	78	12	15	12	78	12
Lolium rigidum	'Wimmera'							18	12.5										
Lolium multiflorum	'Trinova'	90	24	72	24	165	20	30	20.8										
	'Rapido'									50	20	55	20	130	20	25	20	130	20
TOTAL MIXTURE (kg)		375	100	300	100	825	100	144	100	250	100	144	100	650	100	125	100	650	100
kg/ha		25		25		25		24		25		24		25		25		25	

(1971) for the genus *Tuberaria* and *Geraniaceae* and *Linaceae* families, Oliveira (2005) for the genus *Arrhenatherum*, Vázquez and Barkworth (2004) for the genus *Celtica*. The bioclimatological, biogeographical and syntaxonomical typology was checked according to Rivas-Martínez (2007), Costa et al. (2012) and Rivas-Martínez et al. (2014).

2.4. Data analysis

The data-set includes 55 relevés and 164 plant species and was submitted to a Canonical Correspondence Analysis (CCA). A previous Detrended Correspondence Analysis (DCA) was made in order to confirm a unimodal response of species to ecological variables. The relationships between a species occurrence and the main explanatory variables were assessed through CCA performed using the CANOCO 4.5 software (ter Braak and Smilauer 2002). For improving normality all data were log-transformed in line with Lepš and Šmilauer (2003). Detection of collinearity between explanatory variables was made with a forward selection (Borcard et al. 1992; Heikkinen et al. 2004), performed by the Monte Carlo permutation test (999 permutations) which allowed the exclusion of variables that did not contribute significantly ($p < 0.05$) to the ordination model. Only ordination axes with an eigenvalue greater than 0.3 were considered to be ecologically acceptable (ter Braak 1987). Furthermore, variance inflation factors for the explanatory variables were examined to detect collinearity.

The Monte Carlo permutation test (999 permutations) was also used to calculate statistical differences of the germination of cultivars autochthonous species composition between the nine farms and of the floristic composition (native species) between farms and reference communities.

Fidelity was calculated through the *phi* (ϕ) coefficient (Sokal and Rohlf 1995; Chytrý et al. 2002) based on cover data and Fisher's exact test was applied to test statistical significance (Chytrý et al. 2002; Tichý and Chytrý 2006) using the Juice 7.0.67 software (Tichý 2002). Only fidelity values of species with significance ($p < 0.05$; $p < 0.01$) are displayed and all groups were standardised to equal size.

3. Results

3.1. Relationship between species composition and ecological variables between the improved and reference communities

The floristic composition of the nine sown farms and three reference communities were analysed. The three reference communities (Ref. com.) have the following classification: Ref. com 1 – community dominated by *Vulpia bromoides* (*Leontodonto longirostris-Vulpietum bromoidis*); Ref. com. 2 – community dominated by *Festuca ampla* and *Festuca rothmaleri*; Ref. com. 3 – community dominated by *Arrhenatherum elatius* subsp. *bulbosum*.

Some of the surveyed communities have correspondence to habitats of Directive 92/43/EEC. In Naves farm (farm 1) the community *Trifolio subterranei-Poetum bulbosae* was identified, which has correspondence with priority habitat 6220, subtype 2 (Mediterranean perennial swards of *Poa bulbosa*) (ICNF 2006) and Ref. com. 2 has correspondence to subtype 4 of the same priority habitat. The surveyed Ref. com. 3 has correspondence to the habitat 6510 (Lowland hay meadows). Due to the absence of soil data for this community, it was excluded from CCA analysis.

In Naves farm (farm 1), we distinguish two parcels: one that is improved (farm 1a) and another with the same community type included in the referred priority habitat, but not improved and subjected to the same grazing intensity and management (farm 1b).

The Monte Carlo permutation test ($p < 0.05$) allowed the identification of the key variables for the ordination model: pH, potassium, organic matter and grazing by sheep. The two first axes of the CCA accounted for 63.1% of the species-environmental variance. The CCA ordination diagram (Figure 2) shows the relation of the floristic patterns with the selected key variables. The eigenvalues were 0.424 for axis 1 and 0.308 for axis 2, which indicates a reasonable relationship between species and the selected key variables. Axis 1 separates the plots in function of organic matter and potassium concentration, which are higher in farm 1b (Naves). Axis 2 separates mainly the reference communities (not mobilised, not sown, not fertilised) of the sown communities.

3.2. Assessing the success of sowings

The cultivar species with best establishment and highest percentage cover (Table 2) were: *Trifolium subterraneum* (Farm 1a); *Lolium multiflorum* (Farms 2, 3, 7 and 8); *Ornithopus sativus* (Farms 2, 7 and 8); *Trifolium vesiculosum* (Farms 4 and 7).

The total cover (total sum of individual species cover, which can be greater than 100%) of cultivar species was higher in Farm 2 (Barroco Gordo), farm 4 (S. Mateus), farm 7 (Vale d'Adega) and farm 8 (Coito) (Table 2).

The total number of cultivar species was higher in farm 1a (Naves), farm 5 (Tabalião), farm 6 (Filipireza), farm 7 (Vale d'Adega), farm 8 (Coito) and farm 9 (Pizões) (Table 2).

In Table 3 the results are presented of the Monte Carlo Permutation tests used to evaluate the statistical significance of the composition of cultivar of autochthonous species germinated between the nine farms. Results of these tests revealed highly significant differences ($p < 0.01$ and $p < 0.05$) between the nine farms.

Analysing the results shown in Table 3, it is clear that farm 1 (Naves) differs significantly from all the others. In fact, farm 1 (Naves) is the only farm which is grazed by sheep and the only one where the predominant communities surveyed belong to the Mediterranean perennial sward of *Trifolio subterranei-Poetum bulbosae*, which has correspondence to subtype 2 of priority habitat 6220 of the Directive 92/43/EEC. Comparing otherwise, the results presented in the synoptic table (Table 5) and Table 2 highlight the frequency and high cover of the several sown cultivars (Table 1) of *Trifolium subterraneum* in farm 1 (Naves, Table 1). Regarding these results, although the seed mixture applied was similar in the different farms, it was clear that in farm 1 (Naves) the establishment of the cultivars of *Trifolium subterraneum* was more successful.

Farm 2 (Rochoso) and farm 3 (Forneiras) differ significantly from farm 4 (S. Mateus) and from farm 6 (Filipireza) (Table 3). On the other hand, Table 2 shows that in farm 2 (Rochoso) the dominant species were *Lolium multiflorum* and *Ornithopus sativus*, which were almost absent in farm 4 (S. Mateus) and farm 6 (Filipireza).

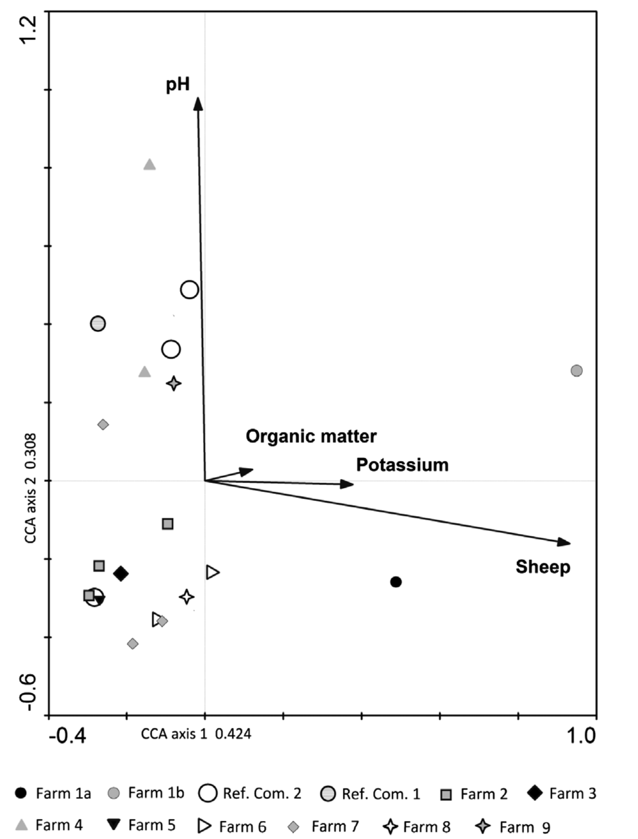

Figure 2. Canonical correspondence analysis (CCA). Biplot with samples and explanatory ecological variables selected using Monte Carlo permutation test ($p < 0.05$).

Farm 4 (S. Mateus) differs significantly from all the others (Table 3), verifying not only an absence of *Lolium multiflorum* and *Ornithopus sativus* but also a high cover of *Trifolium vesiculosum* and *Trifolium michelianum* (Table 2).

Farm 6 (Filipireza) differs significantly from farm 8 (Coito) (Table 3) and in the table it is shown that the dominant species in farm 8 (Coito) were *Lolium multiflorum* and *Ornithopus sativus*, which were almost absent in farm 6 (Filipireza). Farm 8 (Coito) also differs significantly from farm 9 (Pizões) (Table 3) in which *Ornithopus sativus* has the highest cover (Table 2).

3.3. Maintenance of floristic composition of autochthonous target species of the reference communities in the improved communities

Table 4 presents the results of the Monte Carlo Permutation tests used to evaluate the statistical significance of the floristic composition of native species of the improved communities and the reference communities. Results of these tests revealed significant differences ($p < 0.001$ and $p < 0.05$) between reference communities and the improved communities.

Results presented in Table 4 show that improved communities of farm 1 (Naves) differ significantly from all the reference communities. In this farm, it was surveyed a parcel that was not improved, which was subjected to the same management and belonged to the same community type of the improved community before its improvement (Farm 1b). A significant difference was found between the Mediterranean perennial swards

Table 2. Mean cover (higher values in bold), total cover and total number of plant species in each farm. 1 – Farm 1a (Naves); 2 – Farm 2 (Barroco Gordo); 3 – Farm 3 (Forneiras); 4 – Farm 4 (S. Mateus); 5 – Farm 5 (Tabalião); 6 – Farm 6 (Filipireza); 7 – Farm 7 (Vale d'Adega); 8 – Farm 8 (Coito); 9 – Farm 9 (Pizões).

	Farms								
Plant species	1	2	3	4	5	6	7	8	9
Trifolium subterraneum	**53**	0	0	0	4	11	7	10	1
Ornithospus sativus* subsp. *sativus	1	**35**	20	1	28	3	**47**	30	**59**
Trifolium incarnatum	2	6	5	0	5	5	17	4	9
Trifolium michelianum	3	2	13	6	19	7	8	5	1
Trifolium vesiculosum	1	8	11	**32**	7	8	**26**	18	14
Lolium multiflorum	3	**60**	**50**	30	20	18	**47**	**50**	11
Trifolium resupinatum	0	0	0	0	0	18	1	0	2
Medicago sativa	0	0	0	1	0	0	0	0	0
Total cover of cultivar species (%)	63	**112**	98	**112**	83	69	**151**	**117**	97
Total number of cultivar species	6	5	5	4	6	7	7	6	7

Note: Significant differences are marked in bold.

of *Trifolio subterranei-Poetum bulbosae* (which corresponds to subtype 2 of priority habitat 6220 of the Directive 92/43/EEC) that was improved from the same community type not improved. In synoptic table (Table 5), it is possible to better understand this floristic difference. Although *Poa bulbosa* is dominant in both, the *Trifolium subterraneum* cover of Farm 1a is related to the cultivar sown while in farm 1b *Trifolium subterraneum* belongs to native species and/or other cultivar sown in 1985. *Vulpia geniculata* (nitrophilous species) has a high cover in non-improved farm 1b and it is absent in the improved communities of farm 1a.

Ref. com. 1 relates to an annual successional reference community and the results presented in Table 4 show that five farms have no significant differences in their native species composition, which means that many of the species of the Ref. com. 1 are present in the improved communities. However, in relation to Ref. com. 2 and 3 which relate to perennial successional herbaceous communities dominated by perennial grasses such as *Festuca ampla*, *Festuca rothmaleri* or *Arrhenatherum elatius* subsp. *bulbosum* (see also synoptic Table 5), almost no significant differences were found, which means that herbaceous perennial

Table 4. Monte Carlo Permutation Test results for the improved and reference communities.

Communities	Ref. com. 1	Ref. com. 2	Ref. com. 3	Farm 1b
Farm 1a	**0.016***	**0.002****	**0.002****	**0.01***
Farm 1b	**0.02***	**0.001****	**0.041***	–
Farm 2	**0.006****	**0.001****	**0.001****	–
Farm 3	0.06	**0.001****	**0.001****	–
Farm 4	**0.046***	**0.001****	**0.001****	–
Farm 5	**0.046***	**0.001****	**0.001****	–
Farm 6	0.17	**0.01***	0.075	–
Farm 7	0.09	**0.001****	**0.001****	–
Farm 8	0.101	**0.002****	0.074	–
Farm 9	0.102	**0.001****	**0.041***	–

Note: Significant differences are marked in bold.
$^{**}p < 0.01$; $^{*}p < 0.05$

species characteristics of these reference communities do not yet have an ecological niche in these restored or improved communities. In fact, synoptic Table 5 shows that annual species of *Tuberarietea guttatae* class are present and frequent in all the considered groups, while perennial species of *Stipo giganteae-Agrostietea castellanae* and *Molinio-Arrhenatheretea* are frequent only in reference communities 3 and 4 and they are almost absent in sown communities one year after the sowing.

4. Discussion

The present study highlights the importance of improvement of productivity of grasslands ensuring socio-economic and environmental benefits, also regarding nature conservation, and promoting biodiversity conservation. Thus, it assists the compatibilisation of biodiversity conservation and agricultural practices such as livestock grazing.

Experiments conducted in CE mainland Portugal demonstrated that application of the legume *T. subterraneum* recovers the similar abundance of native *T. subterraneum* in native communities. In addition, in this subtype habitat, the experiment confirms the spontaneous establishment of target species of adjacent communities of the same habitat or present in the soil seed-bank. Relevés of not sown farm 1b (Naves) had high values of potassium and organic matter, probably related with the long history of grazing and presence of sheep. A correlation was found between high values of potassium and grazing by sheep, which seems to be in concordance with results obtained by Catani (1956) in which he found high values of potassium in sheep excrement. This particular outcome suggests the need of a few years of monitoring of the evolution of the effects of livestock on the nutrient balance of the soil, namely organic

Table 3. Monte Carlo Permutation Test results for the improved communities between the nine farms.

Communities	Farm 1a	Farm 2	Farm 3	Farm 4	Farm 5	Farm 6	Farm 7	Farm 8	Farm 9
Farm 1a		**0.002****	**0.01***	**0.002****	**0.006****	**0.022***	**0.018***	**0.018***	**0.018***
Farm 2			0.449	**0.001****	0.211	**0.026***	0.1	0.08	0.16
Farm 3				**0.016***	0.469	**0.026***	0.1	0.084	0.164
Farm 4					**0.001****	**0.02***	**0.001****	**0.001****	**0.001****
Farm 5						0.132	0.788	0.295	0.132
Farm 6							0.13	**0.03***	0.134
Farm 7								0.431	0.099
Farm 8									**0.036***
Farm 9									

Note: Significant differences are marked in bold.
$^{*}p < 0.05$; $^{**}p < 0.01$.

Table 5. Synoptic table of the communities in the nine farms and reference communities. Diagnostic species with $\phi \geq 0.5$ and significant fidelity are marked.

Group number	1	2	3	4	5	6
Number of relevés	6	4	2	5	2	26
Cultivar species						
Trifolium subterraneum	V	.	.	.	.	III
Trifolium michelianum	IV	.	.	.	.	IV
Trifolium incarnatum	III	.	.	.	.	IV
Trifolium vesiculosum	II	.	.	.	.	V **
Lolium multiflorum	III	.	.	.	.	V **
Ornithopus sativus subsp. *sativus*	II	.	.	.	.	V
Trifolium resupinatum	.	.	.	.	.	I
Medicago sativa	.	.	.	.	.	I
Native species						
Characteristics of sintaxonomic units of *Poetea bulbosae*						
Parentucelia latifolia	V **	.	.	.	.	.
Trifolium glomeratum	II	.	V	.	.	I
Poa bulbosa	V	V	.	.	III	II
Trifolium subterraneum	I	V **	V	.	.	I
Erodium botrys	II	II	III	.	.	I
Characteristics of sintaxonomic units of *Tuberarietea guttatae* (annual species)						
Vulpia bromoides	V	II	V	IV	V	IV
Leontodon taraxacoides subsp. *longirostris*	III	V	V	II	V	I
Ornithopus compressus	I	II	V	I	V	I
Trifolium striatum subsp. *striatum*	I	.	V **	.	.	I
Ornithopus pinnatus	.	.	III	.	.	I
Trifolium arvense	.	.	III	.	III	I
Hypochaeris glabra	III	.	III	.	.	II
Hymenocarpus lotoides	I	II	V	.	.	II
Tuberaria guttata	.	.	III	.	.	I
Tolpis barbata	.	.	V	.	.	II
Lathyrus angulatus	.	.	.	I	III	.
Characteristics of sintaxonomic units of *Stipo gigantea-Agrostietea castellanae* and *Nardetea*						
Festuca ampla subsp. *ampla*	.	II	.	V **	.	.
Festuca rothmaleri	.	.	.	III **	.	.
Carex leporina	.	II	.	III **	.	.
Agrostis castellana	.	.	.	II	V	II
Characteristics of sintaxonomic units of *Molinio-Arrhenatheretea*						
Poa trivialis subsp. *trivialis*	.	.	.	III **	.	.
Poa pratensis	.	.	.	III **	.	.
Carum verticillatum	.	.	.	IV **	.	I
Trifolium repens var. *repens*	I	.	.	III	.	I
Trifolium pratense subsp. *pratense*	.	.	.	III **	.	.
Trifolium resupinatum	I	.	V	I	.	I
Plantago lanceolata	I	.	V	I	V	I
Trifolium dubium	III	III	.	IV	III	.
Hypochaeris radicata	.	.	.	II	V	II
Arrhenatherum elatius subsp. *bulbosum*	.	.	.	.	V	I
Carex divisa	.	II	.	.	III	.
Crepis capillaris	.	.	.	II	.	III
Holcus lanatus	.	II	.	I	.	.
Other species with frequency higher than 40% in two groups						
Chamaemelum mixtum	IV	III	V	I	.	V
Avena barbata	.	.	III	II	III	III
Plantago coronopus	V **	IV	III	.	.	II
Vulpia myuros	III	.	V	.	.	II
Lupinus gredensis	.	II	.	.	V	II
Bromus diandrus	.	.	V	I	.	II
Chondrilla juncea	.	.	.	.	III	II
Cerastium glomeratum	V **	.	.	II	.	I
Plantago lagopus	III	III	.	IV	.	I
Diplotaxis catholica	.	III	.	.	.	I
Hordeum murinum subsp. *leporinum*	I	II	III	.	.	I
Raphanus raphanistrum	.	.	III	.	III	I
Bromus tectorum	.	II	.	.	.	I
Bromus rigidus	.	II	.	.	.	I
Anthoxanthum aristatum	.	II	.	III	.	I

(*Continued*)

Table 5. (*Continued*).

Notes: Frequency classes: V: 80–100% of the relevés, IV: 60–80%, III: 40–60%, II: 20–40%, I: 0–20%. 1 – Farm 1a (Naves); 2 – Farm 1b (Naves); 3 – Ref. Com. 1 (of *Vupia bromoides*); 4 – Ref. Com. 2 (of *Festuca ampla* and *Festuca rothmaleri*); 5 – Ref. Com. 3 (of *Arrhenatherum elatius* subps. *bulbosum*); 6 – Farms 2–9.
$^{*}p < 0.05$; $^{**}p < 0.01$.

matter and potassium. Unexplained variance was moderate; however, in ordination models it is common to find moderate amounts of unexplained variance (Økland1999), possibly related to unmeasured non-spatial explanatory variables.

In the improved grasslands, the presence of target annual species of the annual reference community was common. However, the presence of target species of perennial reference communities was less frequent. The establishment of all target species of the reference communities is not to be expected as also observed in other similar studies, e.g. Lencová and Prach (2011), Pärtel et al. (1998), Prach et al. (2014) and Török et al. (2010). But those target species certainly have high chance of becoming established considering the role of dispersal (Matsamura and Takeda 2010). The establishment of cultivar species in farm 1a seems to follow the original composition of these community types replacing the nitrophilous species niche that occurs as a consequence of many years of grazing.

The study made by Prach et al. (2014) demonstrates that the establishment of target species in grasslands restored using commercial seed mixtures is slow. Nevertheless, the application of seed material from commercial origin is one of the main approaches in ecological restorations (Scotton et al. 2012) and in this study the applied restoration responds to the need of the farmers in terms of increasing grassland productivity. In this way, socio-economic benefits and also environmental benefits (e.g. soil carbon sequestration or soil erosion prevention) are ensured.

According to Janišová et al. (2011) and Madruga-Andreu et al. (2011), sowing commercial seed mixtures is not the best solution in terms of nature conservation measures, namely in the re-establishment of floristic composition of native communities included in habitats of Directive 92/43/EEC. Other solutions based on regional seed mixtures will certainly be a better choice for recreating the composition of original native species (Prach et al. 2014).

However, in this experimental study, the seed-mixture sown in communities of the priority habitat 6220 resulted in legume species (cultivars) and, at the same time, in the maintenance of high cover of *Poa bulbosa* and other target species of this habitat type, highlighting that sustainability of increased productivity is possible while maintaining biodiversity. Therefore, a monitoring programme is necessary to evaluate the evolution of the improved communities in terms of permanence of sown legume species and in terms of re-establishment of target species in the improved grasslands in CE mainland Portugal and it is also important to expand this experiment to restoring grasslands with regional seed mixtures.

5. Conclusions

Mediterranean perennial swards (priority habitat 6220) restored with a commercial seed mixture presented a high colonisation by *Trifolium subterraneum* cultivars and maintain a high percentage of target species after the first year, characteristic of the native community (*Trifolio subterranei-Poetum bulbosae*), highlighting that sustainability of increased productivity is possible while maintaining biodiversity. However, it is important to monitor the establishment of the target species in the improved grasslands in CE mainland Portugal and to expand this experiment to restoring grasslands with regional seed mixtures.

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