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## The effects of climate fluctuations and soil heterogeneity on the floristic composition of sown Mediterranean annual pastures

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### Abstract

Sown Biodiverse Permanent Pastures Rich in Legumes (SBPPRL) is a pasture system for Mediterranean climatic areas mixing a large number of pastures species and cultivars, with a predominance of *Trifolium subterraneum*. Since the nineties, it rapidly expanded in Portugal due to its high dry matter yields (DM) and C sequestration rates in soil organic matter. Although without clear experimental evidence, it is currently admitted that SBPPRL are able to adjust soil spatial heterogeneity and to reflect interannual climate fluctuations.

The study was realised on a SBPPRL sown in 2001 on a mixed farm located in “Serra da Estrela”, the main agricultural mountain system of Portugal. The effects of two explanatory variables – “Year-climate” (moderately dry in 2007-2008 and humid in 2009-2010) and “Slope” (three positions: hill shoulder, backslope and footslope) – on the floristic composition were explored. Three botanical levels of analysis were used: all species, sown species and *T. subterraneum* cultivar levels. The statistical significance and the influence of the two explanatory variables on the plant composition varied between these three levels. The distribution of *T. subterraneum* cultivars along the microtopographical gradient was congruent with their life cycle duration: short-cycle cultivars had higher relative cover in uphill plots while longer cycle cultivars tended to dominate down the slope. Diversifying the sown species/cultivar colonists’ pool in seed pasture mixtures appears to favour microtopographic gradients and interannual climatic fluctuation tracking. Sown species diversity and *T. subterraneum* cultivar diversity are complementary in this process; they promote, respectively, interannual climatic and microtopographic gradients fluctuation tracking.

Keywords: subterranean clover, pasture legumes, pasture ecology

### Introduction

In the seventies, the Portuguese agronomist David Crespo devised a new pasture system – the Sown Biodiverse Permanent Pastures Rich in Legumes (SBPPRL) – mixing a large number of improved pasture plants with a Mediterranean optimum. The SBPPRL can involve up to twenty different pasture plant species and cultivars, the majority of them self-regenerating winter annuals (only grasses are perennial), *Trifolium subterraneum* being the preponderant species.

FERTIPRADO (2011), the main pasture seed provider in Portugal, blends its SBPPRL mixtures according to three criteria: soil texture, soil pH and mean annual precipitation. In these mixtures, to a variable group of pasture species with different ecological requirements are added several cultivars of *T. subterraneum* covering a considerable life cycle length spectrum. *T. subterraneum* cultivar selection in agronomic practice depends on the date at which moisture stress can be expected to occur and the time of flowering required to allow the maturation of adequate seed before this occurs (SMETHAM, 2003). Pasture improvers and seed sellers currently determine these two factors by mean annual precipitation and the date of the cultivars’ first flowers. *T. subterraneum* is a cleistogamic species, its natural populations being constituted by pure lines (KATZNELSON, 1974). Consequently, morphologically consistent cultivars should be recognized several years after pasture sowing.

Several reasons explain SBPPRL rapid expansion in Portugal. The foremost cause is that they are more productive than semi-natural dry-pastures. In a five year study in Southern Portugal, CARNEIRO *et al.* (2005) found that SBPPRL had an average stocking rate 145% superior to contiguous semi-natural pastures (0.86 vrs. 0.35 LU.yr<sup>-1</sup>, p=0.03, paired t-test). In the DM yields summarised in table 2 for the year 2007-2008 in Quinta da França, Portugal, the SBPPRL total DM production was 347% higher than

its nearby semi-natural pasture (6.15 t DM.ha<sup>-1</sup> *vs.* 1.77 t DM.ha<sup>-1</sup>). All the available empirical evidences points to superior productive characteristics of SBPPRL when compared to semi-natural pastures, even under similar chemical fertilization schedules (TEIXEIRA, 2010). In Mediterranean Portugal areas, CRESPO (2011) reported on SBPPRL yields of 4 - 8 t DM.ha<sup>-1</sup>.yr<sup>-1</sup>, with more than 50% of legumes, and with production costs of about 15-20% of the compound feed costs. SBPPRL's have other advantages. In Mediterranean territories with large semi-natural hay-meadow areas (e.g. sub-humid to humid supramediterranean mountains), the SBPPRL DM production peak coincides with the period when hay-meadows are closed to herbivores to allow hay growth. So, the integration of hay-meadows and SBPPRL can optimize fodder production at the farm-level, and have a favourable impact on the conservation status of an important Natura 2000 habitat, fully dependent on agricultural management (HALAD *et al.*, 2011). In soils with a low soil organic matter (SOM) content, a general consequence of a millenary cereal and fallow rotation used in the Mediterranean basin, the SBPPRLs exhibited a remarkable carbon sequestration in SOM performance achieving 5 t.ha<sup>-1</sup>.year<sup>-1</sup> CO<sub>2</sub>e (TEIXEIRA *et al.*, 2011). The Portuguese government is now paying carbon sequestration on an area of up to 42'000 ha of SBPPRL, that could reach 0.91 x 10<sup>6</sup> t of CO<sub>2</sub>e from 2010 to 2012. Finally, it is also important to state the easy conversion of former agricultural soils to SBPPRL, their persistence in time (there are functional SBPPRL of more than 25 years old) and their simple and low cost maintenance.

The SBPPRL address a classical subject in community ecology: the effects of species diversity on ecosystem function. Pasture plants and communities have been a favourite experimental subject in these studies, with much scientific evidence produced (BALVANERA *et al.*, 2006). Empirical evidence accumulated during the past decades shows that pastures sown with species diverse seed mixtures are more productive (CLARK, 2001). Diverse pastures are also expected to be less permeable to spontaneous species of low palatability and feed value (FRANKOW-LINDBERG *et al.*, 2009), to track environmental heterogeneity (SANDERSON *et al.*, 2004) and to withstand extreme climate fluctuations (TILMAN & DOWNING, 1994). In spite of its obvious agronomic relevance, the majority of the published results on pasture diversity effects on ecosystem functions, were carried out on a small scale with strictly controlled experimental conditions (SYMSTAD *et al.*, 2003), involving a small number of species (SPEHN *et al.*, 2005), in homogeneous soil conditions (WACKER *et al.*, 2008) and during short time spans (CARDINALE *et al.*, 2007). Furthermore, these studies rarely brought the analysis to the cultivar level (intraspecific diversity).

At the microtopographical scale (local relief) slope influences key environmental factors important in plants - soil relationship (SWANSON *et al.*, 1988). GÓMEZ *et al.* (1978) proposed the slope system as the most appropriate model for the study of Mediterranean herbaceous communities subjected to grazing. Later authors have explored microtopographic gradient effects in herbaceous Mediterranean community attributes (e.g. species richness) on small spatial scales (e.g. OSSEM *et al.*, 2002; PECO *et al.*, 2006). Besides slope, the floristic structure of Mediterranean annual pastures is also remarkably sensitive to annual rainfall fluctuations, as ORTEGA & FERNÁNDEZ ALÉS (1987) or FIGUEROA & DAVID (1991) demonstrated in southern Spain.

It is expectable that species diversity in pastures will have a stronger effect on productivity in habitats with patchy resources that fluctuate over time. This putative positive effect is probably amplified in Mediterranean therophytic plant communities (and in sown SBPPRL) where plant recruitment restarts with the first rains each autumn. This fundamental question hasn't been properly addressed, and the published bibliography about it is scanty and not always straightforward (WACKER *et al.*, 2008).

Due to their species-genotypes diversity, SBPPRL are then expected to be able to track and tune to the spatial and temporal heterogeneity of Mediterranean soils. Three hypotheses will be tested in this paper. 1) Do SBPPRL track slope system microenvironments array? 2) Does the same happen with interannual climate fluctuations? 3) Do the SBPPRL responses to these microenvironments spatial arrays and climatic fluctuations occur at the sown species and *T. subterraneum* cultivars levels? If SBPPRL are an agronomic solution these hypotheses should be tested by observational studies under farm conditions at the plant community scale.

## Materials and methods

A stabilized SBPPRL was selected on a private farm – Quinta da França (Covilhã, Portugal), 40° 16' N 7° 30' W, ca. 425 m MSL – located on the lower east-facing slope of the highest continental Portugal mountain, the Serra da Estrela. This sward was sown in arable land in 2001 with a commercial SBPPRL. Since then it was intensely grazed with cattle and sheep and annually fertilized with 27 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>.

The studied SBPPRL is located on a gentle slope of a granite hill with 20 m elevation and 380 m length. Three clear microtopographic positions according to the simplified slope form model of RUHE & WALKER (1969, cit. BIRKLAND, 1999) were identified: hill shoulder, backslope and footslope. In 2007-2008 and 2009-2010, four sampling squares (stratified random sampling) were randomly located on each position. In the third week of May of 2008 and 2010, species cover was evaluated by use of the point-quadrat method, with a frame of 70x70 cm with 49 points. Plant species were identified to the species level or to the cultivar level in the case of *T. subterraneum*. Each of the quadrats was protected with an enclosure cage during the previous three weeks (the period of higher DM accumulation). DM yields were evaluated in 2007-2008 in enclosure cages that rotated around the quadrats. Soil samples were collected nearby the quadrats of 2009-2010.

The 2009 spring was exceptionally dry and the SBPPRL was impossible to sample. 2007-2008 and 2009-2010 agricultural years were, respectively, moderately dry (589 mm) and moderately wet (1023 mm) (annual rainfall mean of 781 mm). Temperature integrals of the two growing seasons were close to the mean.

In order to identify the *T. subterraneum* cultivars sown in Quinta da França, and to evaluate their life cycle length, a seed collection of the same *T. subterraneum* cultivars was sown in the autumn of 2007 in a greenhouse. To simulate herbivory, plants were manually clipped when reaching 5 cm. The collection was visited two times a week; the date of the first flowers was registered and used to order the cultivar's life cycle length. Of the five cultivars originally sown in Quinta da França it was possible to discriminate morphologically 'Campeda' + 'Woogenellup', 'Denmark', 'Gosse' and 'Losa'. 'Campeda' and 'Woogenellup' were difficult to separate. *T. subterraneum* 'Gosse' is a selection of subsp. *oxaloides* (= subsp. *yanninicum*); the remaining cultivars belong to subsp. *subterraneum*.

All the sampled *T. subterraneum* plants in Quinta da França were similar to one of the four sown cultivars or cultivar groups. Nevertheless, a biunivocal correspondence between them involves some uncertainty because the ingress of autochthonous lines with similar morphology to the sown cultivars in the SBPPRLs cannot be excluded. However, autochthonous *T. subterraneum* genotypes are probably less competitive in nutrient equilibrated soils than improved genotypes. On the other hand *T. subterraneum* is rather uncommon in the semi-natural pastures of Quinta da França, and appears to come from the sown populations.

The floristic data was explored at the community level with the multivariate ordination algorithms available in the CANOCO program (TER BRAAK & ŠMILAUER, 2002). The floristic data – number of pin contacts per species or *T. subterraneum* cultivars – was standardized by the norm to enhance the effect of species/cultivars relative cover on the ordinations. A Detrended Correspondence Analysis (DCA) showed that the species turnover was low and that linear ordination methods use is recommendable (PCA [principal component analysis] or RDA [redundancy analysis]) (LEPŠ & ŠMILAUER, 2003). Two explanatory variables were considered: "Year-climate" (dummy variable, expressed in the ordination diagrams – figs. 1 and 2 – with the 2009 and 2011 values) and "Slope" (semi-quantitative variable with three levels: hill shoulder, backslope and footslope; arrows in the diagrams point to uphill positions).

RDA models and Monte Carlo permutation tests were used to partition variation and to test the effects of each explanatory variable *per se*. "Year-climate" and "Slope" were used alternatively as explanatory variables or co-variables. They could be considered as crossed environmental factors and their effects on species variation partitioned with RDA models for two reasons: (1) each year, with the first autumn rains, there is a restart of the SBPPRL and of the semi-natural pastures flora; (2) the quadrats moved freely inside each slope position between years. A split-plot design with restricted random permutations within each year or each slope position ("design-based" random permutations) was adopted (for theory see LEPŠ & ŠMILAUER, 2003). This test is much more restrictive than free permutations. Point quadrat pin contacts were previously log transformed due to their huge variation between samples. Three botanical levels of analysis were explored in variation partition: all species level, sown species level, and *T. subterraneum* cultivar level. After confirming residuals normality and variance homogeneity (Shapiro-Wilk and Levene's tests, data not presented) one-way or two-way ANOVA were applied to soil fertility, DM production and *T. subterraneum* cultivars relative cover.

## Results and discussion

From the original sown mixture, five species were still detected in the studied SBPPRL: *Astragalus (Biserrula) pelecinus*, *Trifolium subterraneum*, *T. michelianum*, *T. resupinatum* and *Dactylis glomerata*.

The *T. subterraneum* cultivars sown in Quinta da França sward were distinguished based on the following characters: ‘Losa’ – hirsute twigs; ‘Denmark’ – glabrous petioles and stipules; ‘Gosse’ – sparsely villous petioles, glabrous stipules; ‘Campeda’ + ‘Woogenellup’ – villous petioles and stipules. The order of the timing of flowering was: ‘Losa’ (an early season cultivar); < ‘Goss’, ‘Campeda’ and ‘Woogenellup’ (middle season cultivars) with a few days of difference between them, almost two weeks after ‘Losa’; < ‘Denmark’ (late season cultivar), one week after middle season cultivars. To avoid flowering time redundancies and eventual identification errors, middle season cultivars were merged into one group. Soil fertility parameters and DM production are spatially variable across the studied SBPPRL (tables 1 and 2). P - trend along the slope system is difficult to interpret. SOM was smaller in the hill shoulder; although without a statistical confirmation, the same occurs with K<sub>2</sub>O.

Table 1. Soil fertility data from the studied SBPPRL (mean values, Quinta da França, Portugal). Analytical methods: soil organic matter – Walkley-Black; P<sub>2</sub>O<sub>5</sub> and K<sub>2</sub>O – Egner-Riehm. One-way ANOVA. Significant values (P < 0.05) are highlighted in bold. Different letters indicates significant differences between explanatory variables levels (P<0.05, Tukey’s HSD test).

	P <sub>2</sub> O <sub>5</sub> (mg.kg <sup>-1</sup> )	K <sub>2</sub> O (mg.kg <sup>-1</sup> )	Soil organic matter (%)	pH H <sub>2</sub> O
Hill shoulder	94	120	1.3 <b>b</b>	5.2 <b>a</b>
Backslope	115 <b>a</b>	128	3.6 <b>a</b>	4.9 <b>b</b>
Footslope	63 <b>b</b>	159	3.4 <b>a</b>	5.8
Mean	90	136	2.8	5.3
d.f.	2	2	2	2
p-value	0.039	0.179	<0.001	0.051

Table 2. Mean DM production (total and per fraction) in 2007-2008 along the slope gradient in the studied SBPPRL (Quinta da França, Portugal). One-way ANOVA.

	Legumes		Grasses		Other plants		Total (DM kg.ha <sup>-1</sup> )
	(DM kg.ha <sup>-1</sup> )	(%)	(DM kg.ha <sup>-1</sup> )	(%)	(DM kg.ha <sup>-1</sup> )	(%)	
Hill shoulder	2521	51.0%	928	18.8%	1490	30.2%	4939
Backslope	3358	51.0%	1344	20.4%	1883	28.6%	6585
Footslope	3215	46.4%	1640	23.7%	2074	29.9%	6929
d.f.	2		2		2		2
p-value	0.4		0.28		0.202		0.117

A PCA was performed with all sown and autochthonous species monitored in spring 2008 and 2010. The first three axes explained 55.3 % of species data variability; the eigenvalue of the fourth axis was already very low [ $\lambda_1=0.297$ ,  $\lambda_2=0.143$ ,  $\lambda_3=0.120$ ,  $\lambda_4=0.069$ ]. The first axis was mostly correlated with “Year-climate” (r= -0.95). Not surprisingly “Year-climate” significantly (p=0.02) explained 27.3% of the community variation. The PCA eigenvalues of axes 2 and 3 were alike ( $\lambda_2=0.143$ ,  $\lambda_3=0.120$ ). The second axis is difficult to interpret; probably reflects the effect of soil compaction – a soil physical parameter hard to evaluate in mineral Mediterranean soils – in community composition. The “Slope” variable was correlated with the third axis (r=-0.54), explaining 6.9% (p = 0.03) of the species variation partition. The marginal effects in a RDA with a forward selection of environmental variables confirmed that the species variability explained by the explanatory variables decreases in the order “Year-climate” > “Slope” and that soil fertility variables exerted a smaller effect on the flora structure of the sward (data not presented).

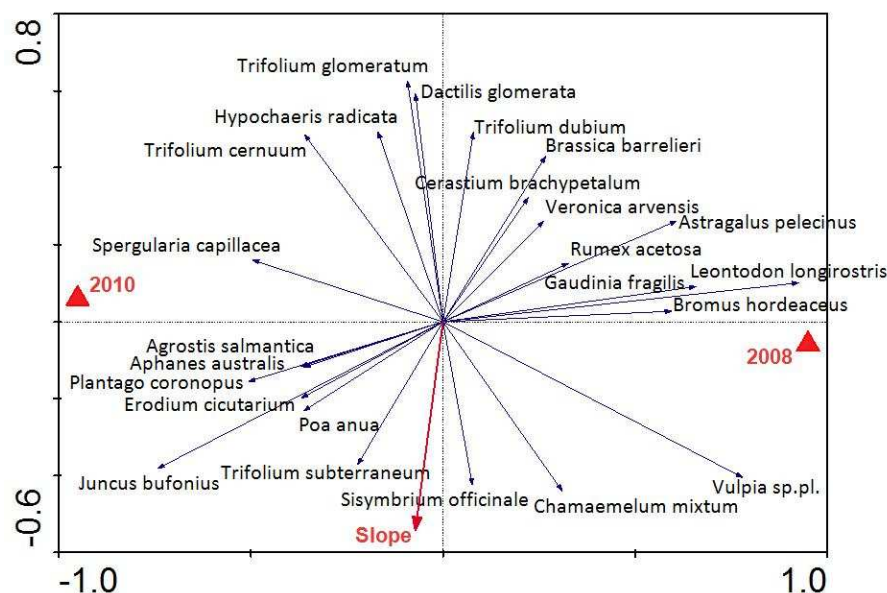


Figure 1. PCA triplot – 1<sup>st</sup> and 3<sup>rd</sup> axes. Data gathered in a SBPPRL of Quinta da França (Portugal). Variables are passively projected. In the figure are represented twenty one species with the highest fit with the 2<sup>nd</sup> and 3<sup>rd</sup> PCA axes.

Species of temporary wet soils like *Spergularia capillacea* (*Caryophyllaceae*), *Trifolium cernuum* (*Fabaceae*), *Juncus bufonius* (*Juncaceae*) and *Agrostis salmantica* (*Poaceae*) were promoted by wet condition in 2009-2010 (fig. 1). The opposite happened in 2007-2008, a dry year. Downslope positions favored perennial mesophyllous species (e.g. *Dactylis glomerata*, *Poaceae* and *Hypochaeris radicata*, *Asteraceae*) (fig. 1). Annual species gained dominance at the hill shoulder. *Trifolium subterraneum* relative cover – but not legume biomass (table 2) – was higher uphill because temporary wet soils species response in 2009-2010 was higher in downslope positions. The grass component in DM was also higher downslope than in the hill shoulder, at least in 2007-2008 (table 2). The short cycle and ubiquitous grasses of the genus *Vulpia* are abundant in all kinds of Mediterranean grazed pastures, and gain relative cover in dry years (fig. 1). Their short cycle releases them from the effects of dry springs, like in 2008. The same happens with other species like *Leontodon longirostris* (*Asteraceae*).

“Year-climate” retained a significant effect on botanical composition at the sown species level (table 3). Sown species relative cover fluctuated slightly between years, except for *Astragalus pelecinus*, which was higher in 2008 (data not presented). In fact the removal of *A. pelecinus* from the DCA model shattered the significance of the “Year-climate” variable. In the SBPPRL of Quinta da França aerial seeding clover species never surpassed a relative cover of 1% and the dominance of *T. subterraneum* in the legume fraction was absolute because grazing was intense and continuous throughout the year. SBPPRLs with higher covers of aerial seeding clovers probably respond more to annual rain fluctuations than its intensively grazed counterparts. In fact there was an abnormal increase in aerial seeding clovers cover in the wet spring of 2010, throughout moderately grazed SBPPRL in Portugal.

“Year-climate” didn’t had a significant effect in *T. subterraneum* cultivars relative cover at the community scale (table 3). Nevertheless an individualistic approach (table 4) showed that middle season cultivars retreated in the wet year. Apparently, the early season cultivar (‘Losa’) relative cover followed the same trend, and the long season cultivar (‘Denmark’) the inverse. Table 3 and fig. 2 reveal instead that “Slope” had a strong and highly significant effect in the spatial distribution of *T. subterraneum* cultivars at the community scale: uphill position were mainly colonized by the early season ‘Losa’; backslope is the preferred microhabitat of the middle season cultivar samples; and late season cv. Denmark largely surpassed the other cultivars in the footslope. Univariate statistics (table 4) confirm that *T. subterraneum* cultivars are spatially segregated according to the life cycle length, although this effect is only statistically significant with the short season ‘Losa’.

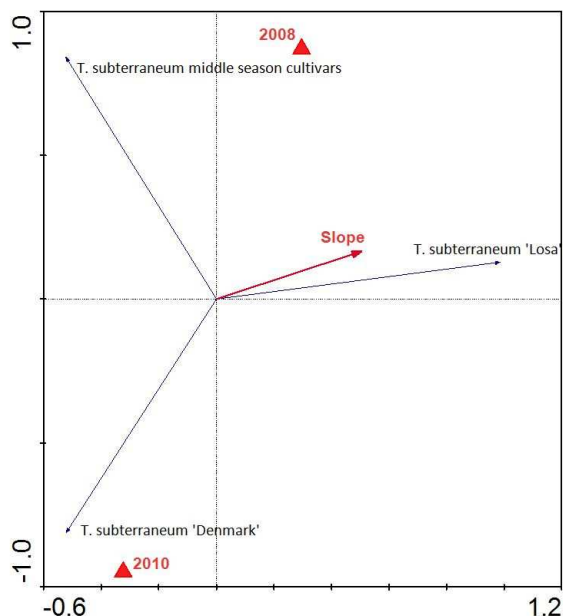


Figure 2. Biplot from a Principal Component Analysis – axes 1 and 2 – with the data gathered in the SBPPRL of Quinta da Frana (Portugal) for *T. subterraneum* cultivars. All variables passively projected.

Table 3. Variation partitioning of sown species or *T. subterraneum* cultivars composition. Significant values ( $P < 0.05$ ) are highlighted in bold.

Botanical level of analysis Explanatory variable	Explained variability (%)	Significance
Sown species level		
Year-climate	14.7	<b>0.05</b>
Slope	2.9	0.65
<i>T. subterraneum</i> cultivars level		
Year-climate	10.8	0.08
Slope	18.0	<b>0.004</b>

Table 4. *T. subterraneum* cultivars mean relative cover (%) variation between years and along the slope gradient in the SBPPRL of Quinta da Frana. Two-way ANOVA. Significant values ( $P < 0.05$ ) are highlighted in bold. Different letters indicate significant differences between slope positions in the same pasture type (Tukey's HSD test).

	'Losa' (early season cv.)	'Gosse', 'Campeda' and 'Woogenellup' (middle season cv.'s)	'Denmark' (late season cv.)
Year-climate			
2007-2008	10.4	17.0	20.2
2009-2010	7.5	7.1	27.6
d.f.	1	1	1
p-value	0.503	<b>0.015</b>	0.270
Slope			
Hill shoulder	21.3a	12.6	19.5
Backslope	3.1b	16.5	26.6
Footslope	2.4b	7.0	25.7
d.f.	2	2	2
p-value	<b>0.002</b>	0.125	0.627
Slope x Year-climate			
p-value	0.695	0.445	0.482



## Conclusions

The statistical significance and the influence of the explanatory variables “Year-climate” (a proxy interannual climatic fluctuations) and “Slope” (a proxy of soil resources and soil properties spatial variability) on the plant composition of the studied SBPPRL varied between the three botanical levels of analysis: all species level, sown species level, and *T. subterraneum* cultivar levels. “Year-climate” and “Slope” explained species variability reached a maximum, respectively, at the all species and *T. subterraneum* cultivar levels. The sown species pool in the studied SBPPRL was impoverished by intensive grazing, hampering a deep exploration of sown flora dynamics at the community scale. Anyway, “Year-climate” exercised a stronger and significant control in sown species assembling than “Slope”; the inverse occurred at the *T. subterraneum* cultivar level. *T. subterraneum* cultivars spatial segregation was related to their life cycle length: early season ‘Losa’ reached higher relative covers in uphill positions; middle season cultivars were more common in backslope positions; and the late season cv. Denmark surpassed the other cultivars on the footslope.

Diversifying the sown species/cultivar colonists’ pool in seed pasture mixtures appears to favour interannual climatic and microtopographic gradients fluctuation tracking. Sown species diversity and *T. subterraneum* cultivar diversity are complementary in this process; they promote, respectively, interannual climatic and microtopographic gradients fluctuation tracking. Our data is merely phenomenological: it corroborates the three hypotheses presented at the end of the introduction section but doesn’t prove them.

The gathered information is also too scanty to help a discussion about the underlying mechanisms of sown species and *T. subterraneum* cultivars spatial and temporal accommodation in the SBPPRL’s ecological gradients. However, it was found that the three slope positions were heterogeneous enough to permit the reseeding of different cycle length *T. subterraneum* cultivars. The reseeding probability and intensity certainly is not similar for each cultivar in each slope position, and culminates in a spatial distribution pattern of *T. subterraneum* cultivars that persists between years.

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