Climatic variability and other site factor influences on natural regeneration of *Pinus pinaster* Ait. in Mediterranean forests

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ABSTRACT

Background: How environmental factors affect forest regeneration is relevant for systems that depend partially or fully on natural regeneration.

Objective*:* *P. pinaster* post-disturbance regeneration and its relationship to environmental factors was studied in five *P. pinaster* forest populations of central Spain. We expected that 1) different harvesting methods or wildfire would promote natural regeneration in all populations, but with local and regional variations; 2) alternatively, different site-dependent stand factors would affect natural regeneration, although generalized climate effects would be seen. Analysis of variance and multivariate analysis were used to test differences, to classify ecological variations and to search for the most important factors affecting regeneration.

Results: The results suggest that the recovery of *P. pinaster* forest in burnt stands, and stand replacement in harvested stands can be achieved soon after disturbance if climatic conditions and other local-site factors (e.g. soil and overstorey structure in harvested stands, cone bank in burnt stands) make the stand suitable for natural regeneration. Heterogeneous regeneration can be expected in all cases. The time of precipitation strongly influenced seedling density and successive regeneration development stages. Edaphic properties combined with water availability from precipitation can seriously limit the natural establishment of *P. pinaster* in xeric systems or during years of intense drought. Although many factors contribute to high variability natural regeneration was very effective (successful) in *P. pinaster* forests, which contributes to the generalization that natural regeneration is a viable forestry option in many forest types.

Keywords: Fire/ Harvesting/ Precipitation/ Regeneration development stages/ RDA/ Site factors/ Variation partitioning.

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1 INTRODUCTION

An evaluation of natural regeneration, from inner-stand complexities to landscape scale, is necessary for understanding secondary succession of forest species (see Puettmann and Ammer, 2007). Research in this area can be used for sustainable and multiple-objective stand structure design. For most forest management purposes, establishment is the first and most critical process following regeneration harvest or stand-replacing disturbance. Establishment determines future stand structures, habitat conditions and silvicultural options (Keyes and Maguire, 2005). A better understanding of regeneration processes is equally important for management where regeneration is undesirable, for example where the species is not autochthonous or there is excessive natural regeneration. On the other hand, how environmental factors affect forest regeneration is relevant for systems that depend partially or fully on natural regeneration. The natural regeneration of woody plants is a complex process driven by different factors and shaped by the ecological and demographic characteristics of the species, disturbances and stochastic events (Paluch, 2005). Regeneration involves many stages in the life cycle of plants, and the highest mortality occurs in the early seedling stages. Transition from seedlings to later life stages and the success of each stage depend on site factors, which can be considered as all physical and biotic factors that define the habitat (Matney and Hodges, 1991), including their interactions and disturbances that may alter that habitat. They include climatic, physiographic and soil factors as well as biotic factors, which involve interactions with associated plants, animals and microorganisms above and below ground (Barnes et al., 1998).

Pinus pinaster Ait. is a species widely distributed over the Mediterranean landscape, forming different ecotypes or populations that are adapted to regional edaphic and climatic factors; and where forest management is carried out by the same method (Gil et al., 1990; Alía et al., 1996). Outside its natural range, this species is considered one of the most invasive plants in the world (Lowe et al., 2000). In Mediterranean *P. pinaster* forests both even-aged (seed-tree or shelterwood systems) and uneven-aged (selection cuttings) silvicultural selection systems are applied homogeneously within populations for ecological, economical or social reasons (Rodríguez et al., 2008). However, stand conditions and suitability for natural regeneration may differ within populations even if the same silvicultural system is applied, resulting in heterogeneous natural establishment that includes null, deficient or excessive regeneration (Rodríguez et al., 2008; Rodríguez-García et al., 2010; Ruano et al., 2009). Post-harvest natural regeneration of this species is generally considered easy, although the effects of forest management are poorly understood, and there are several examples of poor regeneration success (González-Alday et al., 2008; Rodríguez et al., 2008). In Mediterranean forests, *P. pinaster* is among the conifers most frequently subjected to forest fires (Calvo et al., 2008). Several studies have indicated that *P. pinaster* responds to fire through rapid seed dispersal and high post-fire seedling density, which can vary depending on the stand conditions (Calvo et al., 2003; Gallegos et al., 2003), the level of serotiny among populations (Tapias et al., 2004), and the severity of the fire (Vega et al., 2008).

However, information about the overall relationship between *P. pinaster* natural regeneration (regardless of disturbance type) and site factors is scarce. This study offers an evaluation of *P. pinaster* natural regeneration and the main environmental factors that shape it, in different scenarios. The main objectives were to examine the suitability for natural regeneration and classify regeneration density and development stages (seedling, sapling, recruited trees) after harvesting and wildfire, the most common disturbances to *P. pinaster* in Mediterranean forest communities, and to understand the relationship between post-disturbance regeneration and environmental factors. We expected that 1) harvesting with different methods or wildfire would promote natural regeneration in all populations, with variations due to local and regional environmental factors; 2) alternatively, different site-dependent stand factors would affect natural regeneration, although generalized climate effects would be seen. Fulfillment of these objectives should lead to an improved understanding of the ecology of the natural regeneration of *Pinus pinaster* and how environmental factors interact with and affect different disturbances in Mediterranean forests.

2 MATERIAL AND METHODS

2.1 Study sites

The study was conducted in fourteen naturally regenerated *P. pinaster* stands located along the Central and Meridional Iberian Ranges of Spain (Fig. 1), which were representative forests of different *P. pinaster* populations and silviculture systems (Tab. 1). Stand selection was restricted to *P. pinaster*-dominated forests with an average area of 30 ha, which had been disturbed within a 10-year period prior to sampling (Tab.1) and where the stand regenerated naturally, with no subsequent management or natural disturbance.

The stand area requirement was difficult to satisfy in many cases, leading to different numbers of sampled plots per stand. Stands from the Meseta Castellana (MC, referred to as the Castilian Plateau in other studies) and Guadarrama (GD) were harvested by the seed-tree selection and shelterwood methods, respectively. Often, shelterwood method application is reduced to two partial cuttings instead of the three classic interventions described in the text-books (Nyland 2002). Seed-tree and shelterwood selection methods were based on tree vigour, dominance and phenotype. Stands from Albarracín (AB) and Maestrazgo (MG) were harvested by the cutting selection method. GH stands were located in burnt forests, where deliberate fires have become more frequent in the last few decades. Felled trees in all harvested stands were trailed on the ground and removed from the stands, leaving harvest debris. No soil treatments were carried out in any of the stands. In the GH population, wood debris had been removed from Stands 10 and 11, but was still present in Stand 9 at the time of sampling.

The density of final shelter trees varied between 25 and 50 trees ha^{-1} in stands submitted to the seed-tree method, between 50 and 150 trees ha^{-1} in stands submitted to the shelterwood method, and between 200-300 trees ha^{-1} in stands submitted to the cutting selection method. Shelter trees in burnt stands varied between 0 and 15 trees ha^{-1}. The study stands share a Mediterranean-type climate with hot dry summers, cold winters and precipitation mainly in autumn and spring. GH stands presented mainly Cambisol-type soils, MC stands presented Arenosol-type soils, AB and MG stands presented Cambisols changing to Luvisols in humid sites and GD stands presented siliceous Entisol-type soil. Shrub and herbaceous heterogeneity as well as different Mediterranean woody species were found (e.g. *Quercus pyrenaica* Willd. in mesic sites, *Q. ilex* L. and *Pinus pinea* L. in xeric sites).

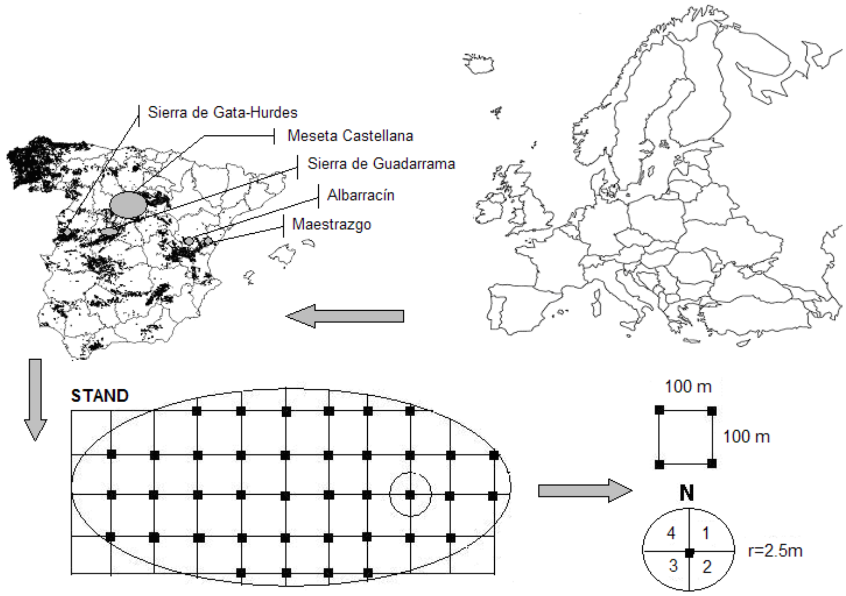

Figure 1. Populations included in the study and layout of the sampling design.

Table 1. Main characteristics of the stands studied in five Mediterranean *Pinus pinaster* populations of central Spain.

D	Po	Stand	Stand location	N	ST	SA	BA ($m^2 ha^{-1}$)	DSS (m)	Alt (m.a.s.l.)	P (mm)	T (ºC)	WSL
STr	MC	S1	41º15'N-4º45'W	22	6	2007	8.80±2.9	20.70±32.8	750±14.8	440	13.8	41º17'N-4º41'W
		S2	41º30'N-4º30'W	23	4	2005	9.93±3.7	9.89±9.0	788±12.4	493	12.1	41º19'N-4º18'W
		S3	41º30'N-4º30'W	16	3	2005	6.52±5.7	10.00±17.9	795±39.3	493	12.1	41º19'N-4º18'W
SW	GD	S7	40º33'N-4º20'W	13	2	2007	12.88±3.1	4.61±3.1	1261±11.3	739	10.6	40º35'N-4º03'W
		S8	40º33'N-4º20'W	14	10	2007	12.32±2.7	3.96±3.1	1097±12.3	739	10.6	40º35'N-4º03'W
CS	AB	S14	40º20'N-1º20'W	9	3	2007	31.61±11.6	56.91±51.1	1375±32.7	355	11	40º25'N-1º26'W
		S15	40º20'N-1º20'W	5	4	2007	29.20±5.5	3.15±1.5	1321±15.6	355	11	40º25'N-1º26'W
		S16	40º20'N-1º20'W	6	4	2007	15.50±6.4	4.08±2.6	1259±10.5	355	11	40º25'N-1º26'W
		S17	40º20'N-1º20'W	6	3	2007	11.92±6.6	4.76±2.8	1209±15.2	355	11	40º25'N-1º26'W
	MG	S12	40º24'N-0º45'W	29	7	2006	23.07±6.9	2.95±1.3	1115±32.9	404	12.3	40º14'N-0º44'W
		S13	40º24'N-0º45'W	29	4	2006	24.12±4.4	2.63±1.1	1101±32.9	404	12.3	40º14'N-0º44'W
WF	GH	S9	40º30'N-6º30'W	26	5	2006	0.58±2.2	27.90±25.0	351±24.9	672	15.4	40º04'N-6º39'W
		S10	40º30'N-6º30'W	25	7	2006	0.38±1.5	58.14±42.9	607±61.5	1050	15.4	40º18'N-6º20'W
		S11	40º30'N-6º30'W	30	6	2006	0.00±0.0	90.68±28.4	804±89.7	1050	15.4	40º18'N-6º20'W

D, disturbance; STr, seed-tree selection method; SW, shelterwood method; CS, cutting selection method; WF, wildfire; Po, population; MC, Meseta Castellana; GD, Guadarrama; GH, Sierra de Gata-Las Hurdes; MG, Maestrazgo; AB, Albarracín; N, number of sampled plots per stand; ST, years since disturbance, i.e., the number of years between the disturbance and the sampling year (SA); BA, basal area of the residual trees; DSS, distance to the seed-source; Alt, meters above sea level; P, average annual precipitation; T, average annual temperature. WSL, weather station location. Values shown correspond to the mean of the variable± SE).

2.2 Sampling and measurements

Sampling within the stands was conducted systematically using a 100 x 100 m random start grid and the *stocked quadrant* method (see Rodríguez-García et al., 2010 for further explanations). Circular plots with a 2.5 m radius were established at the grid intersections (nodes) and then divided into four quadrants (Fig. 1). With this radius, each quadrant has an area of 4.9 m^2, which corresponds to the space available for one hypothetical tree in a regular distribution pattern with a density close to 2000 trees ha^{-1}: the minimum density required for natural regeneration to be considered successful (Matney and Hodges, 1991). A stem count of seedlings with a maximum diameter of 7.5 cm at breast height was taken within the plots. Height (cm), basal diameter (mm) at ground level, vigour state (undamaged or damaged by climatic and other conditions such as desiccation, grazing or pathogens) and seedling social position (dominant or suppressed) with respect to others of the same species or nearby shrubs were recorded for each seedling. A seedling was considered viable when it was rated as both undamaged and dominant. While we cannot assume that seedlings classified as non-viable will not eventually grow to be canopy trees, their apparently low fitness for growth or survival implies a likelihood that these seedlings would either not survive or would be eliminated in future stand thinnings.

Seedling age was estimated visually on site by counting the number of branches. Recently germinated seedlings (from autumn or spring of the sampling year) were classified as 0 years old. Mean, modal, median and maximum seedling ages were calculated in each plot in order to identify the main post-disturbance establishment years. The median age was used for all analyses because two or more modal years of establishment were observed, although the main modal age and median age per plot coincided in 82.6% of the plots. Of the descriptive statistics, the median represented the central value of the main years of establishment much better than the mean and the modal (Härdle and Simar, 2007). Precipitation and temperature variables were used to characterize the local climate of the stands. Mean annual precipitation (mm) and monthly minimum and maximum temperatures (ºC) were calculated with series of data from 1960 (Stand 1), 1975 (Stands 2 and 3), 1973 (Stands 7 and 8), 1991 (Stands 12, 13, 14-17), and 1999 (Stands 9-11) to the sampling year (Tab.1). Weather stations were located at a similar altitude to the stands. Precipitation in each season (autumn, spring, summer and winter) of the median year of establishment per plot and the year preceding it was taken into account. Considering the median seedling age per plot we obtained several central years of establishment per stand and the values of precipitation which characterized the different seasons of those years. But, since only in 82.6% of the plots coincided these years with the years of highest frequency of establishment (modal year of establishment), the term precipitation in the median year of establishment and precipitation in the year prior to the median year of establishment should be nuanced and be though as precipitation in different seasons of two consecutive central years within an establishment period (between the first and the last event). Total density (TD) and viable density (VD) of seedlings ha^{-1} were calculated for each plot without considering age groups. Ten categories for evaluating the regeneration development stages were established according to the average density of viable seedlings per plot (none, scarce, desirable and excessive regeneration) and the average seedling height (0-30 cm; 31-130 cm, and >130 cm) per plot (Tab. 2). The presence or absence of the regeneration stage was codified as a dummy variable (1/0) in each plot per stand. We consider a satisfactory (successful) natural regeneration density to be around 2000 viable seedlings per hectare (Matney and Hodges, 1991). Lower density might be considered insufficient and therefore unsuccessful, while establishment above 5000 seedlings per hectare may result in pre-commercial thinning.

Table 2. Regeneration development stages established for describing *P. pinaster* natural regeneration according to density of viable seedlings and average regeneration height.

Regeneration development stage				
VD (N ha^{-1})	Abundance	Plant height (cm)	Life stage	Category
0	None		No regeneration	V0
1-2000	Scarce	0-30	Seedlings	V1
		31-130	Saplings	V2
		>130	Recruited trees	V3
2001-5000	Desirable	0-30	Seedlings	V4
		31-130	Saplings	V5
		>130	Recruited trees	V6
>5000	Excessive	0-30	Seedlings	V7
		31-130	Saplings	V8
		>130	Recruited trees	V9

VD, viable seedling density

Different variables were recorded within the plots (Tab. 3). Site factors studied in relation to understory vegetation included the percentage cover of moss, herbaceous grasses, and total shrubs per plot, as well as the percentage cover of harvest debris and litter. All coverage percentages were measured visually to the nearest 5%. The height of shrubs and herbaceous grasses (measured from their geometrical center) and of harvest debris, was measured with a tape to the nearest 0.5 cm in each plot. A soil sample was obtained from 20 cm below the soil surface (González-Martínez et al., 2001) in a random sub-sample of 10% of the plots in each stand. Local soil maps consulted before selecting the sampling plots confirmed that the soils were homogenous within each stand. Soil samples were analysed to determine the percentage of sand, silt and clay, the percentage of carbonate and organic matter (carbon method), and the concentration of phosphorus (Olsen method). Concentrations of potassium, calcium, magnesium and sodium were determined after extraction using 1N ammonium acetate; pH (1: 2.5 suspension) and electrical conductivity were also determined. Laboratory soil analyses were carried out following standard procedures for agricultural soil research (MAPA, 1994).

2.3 Statistical analysis

Silvicultural selection systems are applied homogeneously within populations due to ecological, economical or social reasons. Each disturbance type was represented by a different *P. pinaster* geographical area or population, with the exception of two populations for the cutting selection method. Differences in TD and VD between disturbance types (with data gathered from AB and MG in the cutting selection treatment) were explored using multiple analysis of variance. But, given the relative importance of the temporal component imposed by differences in the number of years after disturbance and the sampling year among the populations, most of the analyses were performed separately for each population in order to better see compositional differences among ecological territories. However, treating each population separately limited the environmental factors affecting regeneration to a specific disturbance type. Plots were the main experimental unit, but results and discussion are provided at different levels of analysis (plot, stand, disturbance type and population) to better describe the regeneration patterns observed.

2.3.1 Stand suitability and post-disturbance regeneration

A multifactor analysis of variance (GLM) with a nested design was used to check significant differences in seedling density (TD and VD) among stands and disturbance types. The model included stands nested within the disturbance, so the disturbance mean square was then tested over the stand mean square. TD and VD were subjected to a natural logarithm transformation. Tukey's test was used for all pairwise comparisons of least-squares means to detect differences between treatments. The analysis was performed with the Statistica 6.0 statistical package.

Table 3. Description of the environmental variables measured within the plots and used as environmental factors in the variation partitioning and the CVA analysis.

Variable abbreviation	Variable name and units
Shrh	Average height of shrubs (cm)
Shrcov	Shrub cover (%)
Grassh	Average height of herbaceous grass (cm)
Grasscov	Herbaceous grass cover (%)
Mosscov	Moss cover (%)
Litter	Litter cover (%)
Loggh	Logging debris height (cm)
Loggcov	Logging debris cover (%)
Stones	Soil surface stone cover(%)
Coarse	Soil coarse elements content (%)
Sand	Soil sand content (%)
OM	Organic matter concentration (%)
P	Phosphorous concentration (ppm)
K	Potassium concentration (ppm)
Ca	Calcium concentration (meq/100g)
Mg	Magnesium concentration (meq/100g)
EC	Electrical conductivity (dSm/m)
pH	Soil pH
DSS	Distance to the nearest seed source (m)
BA	Residual trees basal area (m^2/ha)
Treeh	Residual tree height (m)
Altitude	Elevation above sea level (m)
Pwin0	Winter precipitation for the median establishment year (mm)
PwinA	Winter precipitation for the year prior to the median year (mm)
Psp0	Spring precipitation for the median establishment year (mm)
PspA	Spring precipitation for the year prior to the median year (mm)
Psum0	Summer precipitation for the median establishment year (mm)
PsumA	Summer precipitation for the year prior to the median year (mm)
Paut0	Autumn precipitation for the median establishment year (mm)
PautA	Autumn precipitation for the year prior to the median year (mm)

2.3.2 RDAs and breakdown of ecological variation

Since the stands had been harvested or burned, and also sampled in different years, the relative importance of the number of years after disturbance (ST), and sampling year (SA) on the regeneration density variation (TD and VD were used as response variables throughout the whole procedure) was calculated for each disturbance, but separately for AB and MG populations. A Detrended Correspondence Analysis (DCA) with TD and VD as response variables revealed a linear environmental response (length of the axes <2 SD each), so the data were submitted to a Redundancy Analysis (RDA), (Ter Braak 1995). RDA is a direct gradient analysis technique, or the canonical form of PCA, which can be employed to break down ecological variation. In each population, full and partial redundancy analyses (RDAs) were then used to separate the pure ST and SA effects from the environmental or site factors (E) (see Tab. 3) by means of a variation partitioning method (Borcard et al. 1992; Qinghong and Brakenhielm 1995; Park 2001).

Note that stands were sampled in the same year within GD, AB, MG and GH populations, so SA was not considered in the variation partitioning method. First, full RDAs were used to ascertain the total explained variance (TEV) by the combined set of E and ST. Then, a partial RDA was compiled from four RDA runs, where either E or ST was used as the explanatory variable and the other as the covariable, obtaining the unique contribution of each matrix (E and ST) together with its joint effect. The joint effect represents the combined two and/or three way covariance between particular combinations of single and/or paired variable matrices (Borcard et al. 1992; Qhinghon and Brakenhielm 1995). The procedure for MC included SA (see Appendix). First, a full RDA was used to find out the total variance explained by the set of E, ST and SA together. Then, a partial RDA was run three times using TD and VD as response variables throughout the whole procedure. E, ST and SA were each used as the explanatory variable with the other two as covariables. For each combination, four runs of RDA were obtained for variance partitioning; which rendered the unique contribution of each matrix and the two and three-way covariance (joint effects) between all three matrices (Borcard et al. 1992; Qhinghon and Brakenhielm 1995). The significance of the total canonical variation in each partial RDA was tested with 999 Monte-Carlo permutations of the reduced model in the CANOCO 4.5 package. Species data (TD and VD) were submitted to logarithmic transformation.

2.3.3 Regeneration development stages, site factors and discriminant analysis

The ten categories established for describing the natural regeneration development stages (Tab. 2) were used as response variables in canonical variates analysis (CVA) or Fisher's linear discriminant analysis, to see which linear combinations of environmental variables (E, see Tab. 3) discriminated best between groups (of plots) in each population. The presence or absence of the regeneration stage was codified as a dummy variable (1/0) in each plot per population (Ter Braak and Smilauer, 2002). ST and SA effects were controlled by specifying them as covariables in the partial CVAs. Partial CVA is also known as one-way Multivariate Analysis of Covariance (MANOCO); it tests for additional discrimination between clusters, beyond the discrimination obtainable with the covariables (Ter Braak and Smilauer, 2002). Then, a forward stepwise method was used to select environmental variables, with independent variables added in decreasing order of the total explained variance. The relationship of each independent variable to the regeneration variables was tested against a null hypothesis of random association using a permutation test (Monte Carlo), with 999 unrestricted permutations under a full model (p<0.05). Eigenvalues in the CVA (h) were derived from the eigenvalues in the CCA (k) as h=k/(1-k), for plotting selected variables in a biplot diagram. The variance was readjusted using the RDA and the specifications suggested by Leps and Smilauer (2003).

3 RESULTS

3.1 Stand suitability and disturbance type

P. pinaster natural establishment was confirmed and considered successful in all stands evaluated except in Stand 1, 9 and 17 (viable density under 2000 viable seedlings ha^{-1}). The total seedling density differed among all disturbances (F=3.27; p<0.001), except between shelterwood and wildfire disturbances (Fig. 2.1). Total seedling density differed among stands within the cutting selection disturbance (F=3.04; p=0.001). The highest total density was observed in Stand 8 (shelterwood in GD), while the lowest in Stand 17 (cutting selection in AB). Viable seedling density differed among all disturbances (F=9.57; p<0.001), except between wildfire and cutting selection disturbances (Fig. 2.2). Viable seedling density varied significantly among stands within the fire disturbance (F=3.27; p<0.001). The highest viable density was observed in Stands 8 (shelterwood in GD) and 10 (fire in GH), and the lowest viable density in Stand 17 (cutting selection in AB).

3.2 Relative importance of the number of years after disturbance, sampling year, and measured environmental factors

The environmental factors (E) measured had a significant effect on natural regeneration and explained much of the post-disturbance total density and viable seedling density (Fig. 3). Temporal differences in the number of years after disturbance and the sampling year exerted small to moderate influence, but had no significant influence on regeneration density variation. Around half of the variation in regeneration density distribution in the stands submitted to the shelterwood method was explained by the covariance between environmental factors and the number of years after the disturbance (E+ST). This covariance was lower in the other populations (Fig. 3). The amount of unexplained variance (U) differed, and was higher in burnt stands, followed by the cutting selection method in MG and the seed-tree method in MC.

3.3 Regeneration development stages and mixed patches of different densities

The regeneration development stages varied depending on the stands with mixed patches of different densities that were found in all populations (Tab. 4). Young seedling stages (V1, V4 and V7) were the most frequently observed. Excessive regeneration density in the sapling stage was observed 6-7 years after fire in Stands 10 and 11, and 10 years after harvesting with the shelterwood method in Stand 8. However, scarce regeneration, with 53.8 % of the plots lacking regeneration, was observed in Stand S7, which was harvested using the shelterwood method two years prior to sampling. Similarly, 70% of the plots in Stand 9 (burnt five years prior to sampling) lacked post-fire regeneration, alongside mixed plots with patches of scarce sapling density. Desirable and even excessive seedling-stage regeneration was found 3-4 years after cutting selection in AB stands. In contrast, the MC stands submitted to the seed-tree method and the MG stands that underwent cutting selection presented a relatively high percentage of plots with null or scarce regeneration, mixed with a lower percentage of patches with desirable and even excessive seedling density.

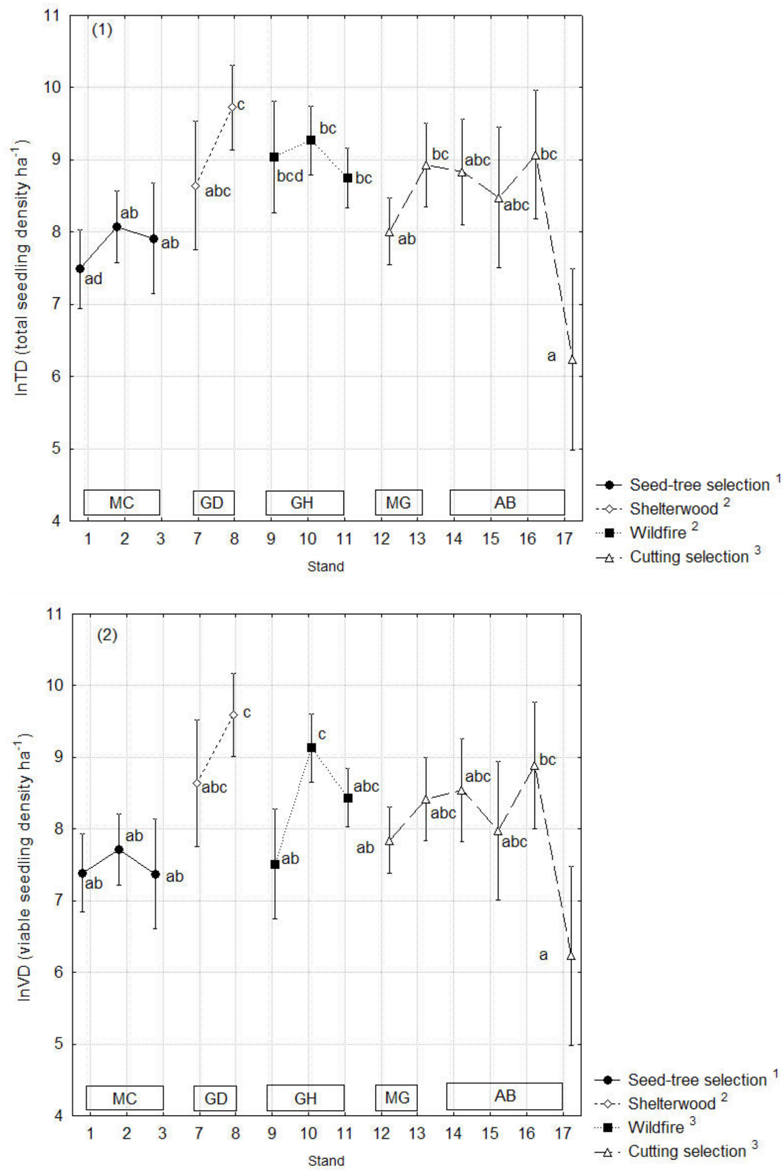

Figure 2. Natural logarithm of total seedling density (1) and viable density (2) of *P. pinaster* in different populations: GH, Sierra de Gata-Las Hurdes; GD, Guadarrama; MC, Meseta Castellana; AB, Albarracín; MG, Maestrazgo. Vertical bars denote a 0.95 confidence interval. *Letters* and *numbers* show significant differences (p<0.001) between stands and disturbance types, respectively.

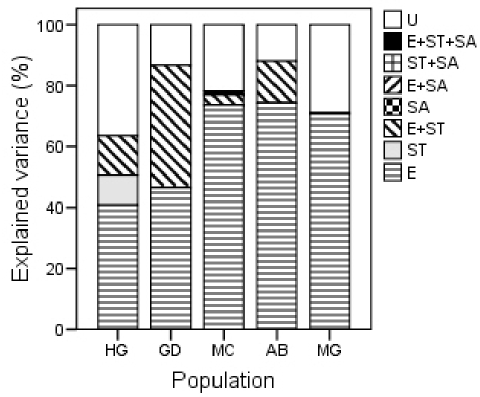

Figure 3. Total explained variance of *P. pinaster* regeneration density (TD and VD), accounted for by environmental factors (E), the number of years since disturbance (ST), sampling year (SA), and double and triple covariance between the different matrices of variables used in the ecological variation breakdown, and unexplained (U).

3.4 Environmental factors affecting natural regeneration development stages

All biplots of the CVA analysis are shown in Fig. 4. The influence of the different environmental factors varied with the disturbance and the population, although the effect of precipitation was significant in all of them. Only significant ($p<0.05$) variables are shown. Plots lacking regeneration (V0) appear separately from those with regeneration in all biplots. In stands submitted to the seed-tree method (Fig. 4a), the significant variables ($p<0.05$) related to the first CVA axis were autumn and spring precipitation in the year prior to the median year of establishment and spring precipitation in the median year of establishment. This suggests a drought gradient that increases as precipitation in the main seasons for rainfall in the Mediterranean climate becomes scarce. The significant variables related to the second CVA axis were summer precipitation in the year prior to the median year of establishment, winter precipitation in the median year of establishment and the percentage of sand content in the soil, which may be related to soil texture and water retention capacity. This suggest a second drought gradient that increases as precipitation in the not-typical seasons for rainfall in the Mediterranean climate becomes scarce.

Table 4. Percentage of plots per stand and population with different regeneration development stages according to seedling density and height. The category names are defined in Table 2. MV indicates mean value per population.

Po	Stand	V0	MV0	V1	MV1	V2	MV2	V3	MV3	V4	MV4	V5	MV5	V6	MV6	V7	MV7	V8	MV8	V9	MV9
MC	S1	27.3	30.5	40.9	23.1	4.5	15.5	0	0	9.1	11.7	0	1.4	0	1.4	18.2	10.4	0	2.2	0	3.7
	S2	17.4		21.7		8.7		0		26.1		4.3		4.3		13		0		4.3	
	S3	46.7		6.7		33.3		0		0		0		0		0		6.7		6.7	
GD	S7	70	35	0	0	0	0	0	0	0	0	0	5	0	0	30		0		0	0
	S8	0		0		0		0		0		10		0		20	25	70	35	0	0
AB	S14	0	12.5	11.1	15.3	0	4.2	0	0	22.2	25.6	0	0	0	0	55.6	35.6	11.1	7	0	0
	S15	0		0		0		0		80		0		0		20		0		0	
	S16	0		16.7		0		0		0		0		0		66.7		16.7		0	
	S17	50		33.3		16.7		0		0		0		0		0		0		0	
MG	S12	24.1	37.9	31	20.7	0	0	0	0	27.6	19	0	0	0	0	10.3	19	3.4	1.7	0	0
	S13	51.7		10.3		0		0		10.3		0		0		27.6		0		0	
GH	S9	69.2	29.63	0	6.17	19.2	11.1	0	0	0	4.94	7.7	11.1	0	0	3.84	9.88	0	27.6	0	0
	S10	16		8		0		0		4		12		0		8		52		0	
	S11	6.67		10		13.3		0		10		13.3		0		16.67		30.		0	

Po, population; MC, Meseta Castellana; GD, Guadarrama; AB, Albarracín; MG, Maestrazgo; GH, Sierra de Gata-Las Hurdes

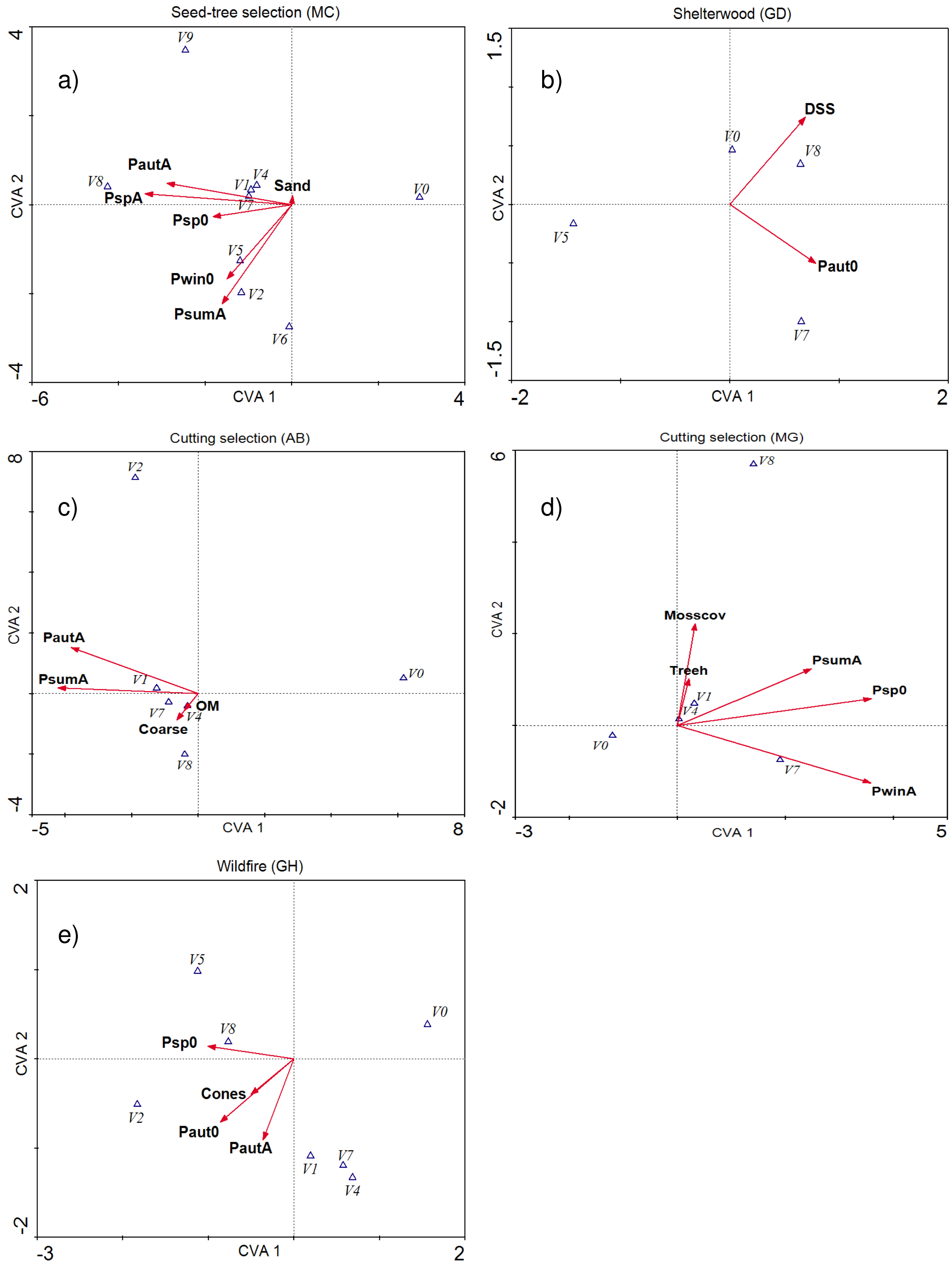

Figure 4. Species-environment biplots of CVA axes 1 and 2 in five *Pinus pinaster* populations submitted to different disturbances. Only significant variables (p<0.05) are shown (see Tab. 2 and Tab. 3). MC, Meseta Castellana; GD, Guadarrama; AB, Albarracín; MG, Maestrazgo GH, Sierra de Gata-Las Hurdes.

In stands submitted to the shelterwood method (Fig. 4b), the gradient represented along the first CVA axis may be related to water availability during autumn, which could increase the density of new seedlings. The second axis suggests an environmental gradient related to overstory structure, light conditions or some other interaction with adult trees. This could regulate natural establishment in a system with excessive density of established saplings. Significant explanatory variables in stands submitted to cutting selection in the AB population (Fig. 4c) were precipitation events in summer and autumn, the percentage of coarse particles in the soil, and soil organic matter content (which is related to water retention capacity). This suggests a link between the regeneration development stage, a drought gradient (first axis) and soil water availability (second axis). In MG stands (Fig. 4d), the first axis might suggest a seed-bed-conditions gradient during seed germination and initial seedling growth, while the second axis may suggest an environmental gradient related to overstorey structure, light, water or other physical factors affecting seed-bed conditions. Finally, in the CVA analysis of post-fire regeneration in the GH stands (Fig. 4e), the first CVA axis of this population is suggestive of a limitation gradient of seed availability after fire and water availability in the spring. The second axis could be interpreted as a water availability gradient during autumn.

4 DISCUSSION

This study has identified the driving factors for natural regeneration of *P. pinaster* in five populations submitted to different disturbances in Mediterranean forests. The results suggest that the recovery of *P. pinaster* forest in burnt stands, and stand replacement in harvested stands may be achieved soon after disturbance if climatic conditions and other local-site factors make the stand suitable for natural regeneration (Rodríguez-García et al., 2010). Natural regeneration of *P. pinaster* was confirmed for all disturbances and was successful in almost all stands per population, with significant differences in regeneration density among stands and disturbance types. Regeneration was very heterogeneous, with patches of desirable to excessive density for different life stages mixed with patches of scarce to null regeneration. This heterogeneity may be the effect of microsites within the stand that provide suitable microclimatic conditions for early establishment (Holmgren et al., 1997).

Regardless of this variability, our study clearly shows that natural regeneration can be a viable forestry option in many forest types and can be very effective in *P. pinaster* forests. All studied silvicultural systems have great potential for successful forest regeneration. Natural regeneration method has several disadvantages such as the dependence on adequate seed crops, little control over spacing and initial stocking, or the production of irregular stands not well suited for mechanical harvesting or other stand treatments (Barnett and Baker, 1991). However, it has other advantages such as the conservation of genetic resources and therefore local adaptations, continuous supply of seeds, little risk of seedling loss by insects and disease problems, low establishment cost, little soil disturbance and relatively little labor and heavy equipment required (Barnett and Baker, 1991).

The results also show that the amount and season of precipitation at the regional level are significantly related to post-disturbance regeneration. Many studies have shown similar climatic influences on various stages of the regeneration process of *P. sylvestris* (see Tegelmark 1998) and *P. ponderosa* (League and Veblen 2006). *P. pinaster* is an estenoic species with a narrow range of optimal precipitation conditions (Gandullo and Sánchez Palomares, 1994), and has been classified as a drought-avoiding species with sensitive stomata (Picon et al., 1996). Once germination occurs, regeneration would persist in situations where a combination of precipitation-substrate-relief and/or other factors promotes enough average annual water availability to support tree recruitment (Gil et al., 1990). This indicates that *P. pinaster* natural regeneration may be temporally limited in intense drought conditions or xeric environments. It may also indicate that precipitation in autumn and spring, the main seasons for rainfall in the Mediterranean climate, are the main factors triggering natural establishment. However, precipitation during winter and summer may also positively affect forest regeneration. Ruano et al. (2009), worked in the same area (MC) using different approaches, and reported that summer rainfall had a significant influence on germination, early development, and total and viable seedling density of *P. pinaster*.

Soil textural properties appeared to be important in MC stands submitted to the seed-tree method and in MG stands submitted to cutting selection. These stands presented a high percentage of plots with scarce regeneration. The significant effects of sand content and coarse particles in the soil may indicate that soil texture and water holding capacity are key structural factors in determining natural tree establishment, since they modify the depth and availability of pulse-delivered rainfall (Noy-Meir, 1973). Percentage of organic matter in the soil was significantly related to natural regeneration in the AB stands. Organic matter has a profound effect on a wide array of physical, chemical and biological soil properties, and its contribution to aggregate formation influences the amount of soil water available to plants (Barnes et al., 1998). Moss cover and adult tree-height were significantly related to natural regeneration in MG stands. The variation in regeneration among these stands may be due to differences in the physical characteristics of the seedbeds, water supply, mineral nutrients, light or temperature (Kozlowski, 2002). Previous studies on *P. pinaster* natural regeneration and environmental factors found that moss cover and logging debris cover reduced the probability of obtaining at least 2000 viable and dominant seedlings per hectare (Rodríguez-García et al., 2007). This suggests that the effects of environmental factors on natural regeneration may vary from mesic to xeric sites, or according to a water availability gradient within a particular forest stand.

Establishment in burnt stands was confirmed; indicating that these forests are prone to recover after fire if cone availability is high and when autumn and spring precipitation are abundant. Residual basal area was almost null in these stands (Tab. 1). The significance of the number of cones on the soil surface, together with the relatively short distance to the seed source, makes it likely that natural establishment after fire depended mainly on seed dispersal and the cone bank. This relationship was observed by Vega et al. (2008) for *P. pinaster* and other pine species such as *P. banksiana* (de Groot et al., 2004); where the cone bank and initial seed rain were the variables most closely related to initial seedling density. Overstorey structure was relatively important in MG stands submitted to cutting selection and GD shelterwood stands compared to other stand types. The significant relationship between natural regeneration and adult tree height in the first case, and the distance to the nearest seed source in the second case, may indicate that other non-measured factors could be more important. Such factors may involve light in the understory or interaction with shrubs or adult trees (Rodríguez-García et al., 2010). Although not significant, the large temporally conditioned variation attributed to environmental variables (E+ST) in GD stands submitted to the shelterwood method may come from different seedling responses to environment conditioned by temporal factors (resource availability, interaction with other organisms, appropriate microsites for establishment, etc.) that affect their net growth and survival. The relatively large degree of unexplained variation found in some populations (MC, MG, GH) may be an indication that fine-scale disturbance, fluctuations or stress, which weaken vegetation-environment relationships (Okland and Eilersten, 1994), are the more important structuring factors in those stands. This variance is independent of the measured environmental variables (Borcard et al., 1992) and points to more complex succession dynamics. Such dynamics can occur in post-fire new forests, or where management is guiding forests to uneven-aged systems (cutting selection) or in forests where other stages of regeneration may be limited or controlled by different factors, such as canopy openness or light in the MC stands (Ruano et al., 2009).

5 CONCLUSIONS

There is a clear need for greater understanding regarding environmental influences on the natural forest regeneration and early establishment dynamics of post-fire and post-harvest established seedlings in Mediterranean forest communities. The results of this study suggest that the recovery of *P. pinaster* forest in burnt stands, and stand replacement in harvested stands may be achieved soon after disturbance if climatic conditions and other site-specific factors make the stand suitable for natural regeneration. Heterogeneous regeneration can be expected in all cases. Knowledge of the site conditions is necessary for planning regeneration treatments and optimizing soil water status and seedbed conditions to meet the requirements of the species or populations. Climatic conditions strongly influenced seedling density and successive regeneration development stages. Foresters might be encouraged to carry out regeneration harvesting in autumn of years with abundant precipitation. Edaphic properties, especially soil texture combined with water availability from precipitation can seriously limit the natural establishment of *P. pinaster* in xeric systems or during years of intense drought. Additionally, environmental influences may change from xeric to mesic sites. Although further research is needed to clarify these issues, and many factors contribute to high variability, our study clearly shows that natural regeneration is often very effective in *P. pinaster* forests, which contributes to the generalization that natural regeneration is a viable forestry option in many forest types.

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7.1 Appendix 1.

Table 1A. Variation partitioning procedure by partial RDAs for natural regeneration of P. pinaster in the Meseta Castellana (MC) population. TD and VD have been used as response variables for the entire procedure.

	Population			
TEV %	MC	Env. Var.	Covariable	Eig.
		(E) + (ST) + (SA)	None	0.782
RDAs 1	Env. Var.	Covariable		
Run				Eig.
1	E	ST + SA		0.736
2	ST + SA	E		0
3	ST + SA	None		0.046
4	E	None		0.782
Joint effect	E ←→ ST + SA=	0.782- 0.736=	0.046- 0=	0.046
RDAs 2	Env. Var.	Covariable		
Run				Eig.
1	ST	E + SA		0
2	E + SA	ST		0.736
3	E + SA	None		0.782
4	ST	None		0.046
Joint effect	ST ←→ E + SA =	0.046-0 =	0.782-0.736 =	0.046
RDAs 3	Env. Var.	Covariable		
Run				Eig.
1	SA	E + ST		0
2	E + ST	SA		0.770
3	E + ST	None		0.782
4	SA	None		0.012
Joint effect	SA ←→ E + ST=	0.012-0 =	0. 782-0.770 =	0.028

Env. Var., environmental variables; Eig., eigenvalue

From Table 1A and Figure 1A we have:

[E+ST] + [E+SA] + [E+SA+ST]= 0.046

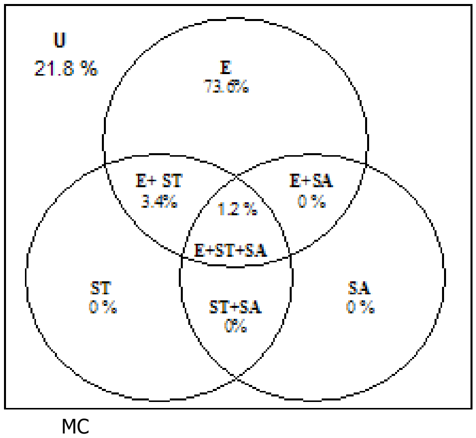
[E+ST] + [ST+SA] + [E+SA+ST]= 0.046 (1) and

[E+SA] + [ST+SA] + [E+SA+ST]= 0.028

[E] + [ST] + [E+ST]= 0.736+0+ [E+ST]= 0.782-0.012

[E] + [SA] + [E+SA]= 0.736+0+ [E+SA]= 0.782-0.046 (2)

[ST] + [SA] + [ST+SA]= 0+0+[E+SA]= 0.782-0.046



Population

Figure 1A. Variation partitioning procedure by partial RDAs for natural regeneration of *P. pinaster* in the Meseta Castellana (MC) population

By solving equations in 1 and 2, we get

[E] + [ST]= 0.034

[E] + [SA]= 0 and

[ST] + [SA]= 0

Where E is the interaction between environmental factors, ST is the years since disturbance and SA is the sampling year:

[E+SA+ST]= 0.012

Finally, the total variation was broken down into:

Common variation [E+SA+ST]

Unique variation [E] + [ST] + [SA]

Total explained variance= 0.782

Partial common variation [E+ST] + [E+SA] + [ST+SA]