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Effects of Experimental Sowing on Agroforestry Ecosystem Primary Production during Recovery from Agricultural Abandonment in a Semi-Arid Region of Central Western Spain

Jimmy-Edgar Alvarez-Diaz, María del Carmen Santa-Regina and Ignacio Santa-Regina

Abstract

We present a study of plant species diversity manipulation on abandoned arable fields and show that most diversity effects can be explained by a successional shift from annuals to perennial plant species. We tested the hypothesis that plant mixtures consisted of mid-successional plant species that were expected to occur on the site following secondary succession, and an increase in the initial plant species diversity at the beginning of secondary succession improves the amount of biomass produced. The main aim was to compare the aboveground biomass for the whole plant community and for different functional groups, using 7 years of field data at abandoned arable land in a semi-arid region of Central Western Spain. Significant differences were established for the treatment-year interaction, analysing the perennial-annual species ratio (P:A) in the HD and LD of sowing treatments. The differences were established at the start of the experiment, when the sown species were more effective in the HD-sowing treatment. There was a negative relationship among the productivity and mean richness of the natural colonization (NC) and the analysed sowing treatments (HD and LD). The coefficient of determination of this relationship was significant ($R^2 = 0.307$, $F(1, 13) = 5.75, P = 0.032$).

Keywords: aboveground biomass, biodiversity, land-use change, secondary succession, plant species richness, plant functional groups
1. Introduction

In some parts of the world, such as in North America and Europe [1], there is an opposing trend, towards set-aside policies and the abandonment of agricultural land. Land abandonment provides opportunities to restore ecosystem properties such as biodiversity and biogeochemical cycles. However, the recovery of pre-agricultural soil conditions can be very slow (e.g. about 200 years for soil carbon and nitrogen [2]). The restoration of former species diversity is often constrained by abiotic and biotic conditions, such as eutrophization or seed-bank depletion [3]. In the mean time, and especially in the years just after abandonment, unmanaged land may favour nutrient leaching, constitute reservoirs of aggressive weeds damageable to adjacent fields and alter the aesthetics of the landscape.

Sampling effects may be the result of the enhanced chance of including species with a specific trait (e.g. high productivity) in species-rich compared to species-poor plant mixtures [4]. Sampling effects are demonstrated by varying the plant species composition of low-diversity (LD) treatments [5]. A positive impact of species diversity on, for example, primary productivity may be the result of resource-use complementarity allowing more diverse communities to utilize a larger proportion of the ecosystem resources [6]. In order to establish whether such overyielding occurs in mixtures of plant species, information on the productivity of the monocultures of all individual plant species needs to be included in the comparison [7]. Using the performance of individual plant species in high-diversity (HD) mixtures, their performance in low-diversity mixtures may be predicted, but this is not sufficient to separate the sampling effect and from that of resource-use complementarity [8].

In the debate on the relationship between biodiversity and ecosystem function relationships, the current focus on experimental procedures and the statistical interpretation of experimental data [9] means that many questions on the response of biodiversity to global changes are still wide open. One of the types of global change having the most immediate effects on biodiversity is that of land-use change [10, 11]. Agricultural expansion and intensification is a major component of such changes resulting in low-diversity ecosystems and large losses of carbon and nitrogen, affecting global carbon balance and fresh water quality, respectively [12, 13].

The effects of increasing plant diversity often saturate at rather low number of species (in average 90% of the known cases, the productivity of the most diverse treatment is reached with mixtures of five species [14]; note that these cases may be biased). Since by definition, functional differences are larger between functional groups than between species, functional group diversity has been found to have a larger impact on ecosystem processes than species diversity [9, 15, 16]. In these experiments, plant functional groups have been identified on the basis of species physiology (C3 vs. C4 species, N fixers vs. non-N fixers, woody vs. non-woody species) or life history (early- vs. late-season species, annuals vs. perennials). However, in removal studies, plant functional traits were found to have little impact on soil communities [17].

We tested the hypothesis that plant mixtures consisted of mid-successional plant species that were expected to occur on the site following secondary succession, and an increase in the
initial plant species diversity at the start of secondary succession improves the amount of biomass produced. The aim of this study was to compare the aboveground biomass for the entire plant community and for various functional groups, using 7 years of field data at abandoned arable land in a semi-arid region of Central Western Spain.

2. Materials and methods

2.1. Site description

The study area was located at 850 m a.s.l., 15 km to the west of Salamanca city, Spain (its coordinates: 40° 54′ 00″ N, 5° 45′ 30″ W), where a 1-ha experimental plot was chosen at the Muñovela experimental farm (C.S.I.C). The plot is edaphically homogeneous, with a dehesa-like woodland.

The climate of the zone features rainy winters and hot summers and may be classified as semi-arid Mediterranean (C\textsubscript{1}B′\textsubscript{1}S\textsubscript{2}b′4). Long-term mean rainfall and temperature are 500 mm and 12.3°C, respectively, with November being the rainiest month (99 mm) and July the driest (17 mm). January is normally the coldest month (0.8°C).

The tree covering comprises Quercus rotundifolia Lam, with a density of 98 trees ha\textsuperscript{−1}, a mean height of 5.9 m and a mean diameter of 29.1 cm. Chronologically, the area lies in the Mediterranean Region, Carpetano-Ibérico-Leonesa province, Salmantino sector and Genisto hystricis-Querceto-rotundifoliae sigmetum series. The estimated mean age of the trees was 150 years. Other characteristic species defining the series are Dorycnium pentaphyllum Scop., Thymus zygis L., T. Mastichina L. and Crataegus monogyna Jacq.

The soil is a chromic Luvisol [18], developed over red clays and Miocene conglomerates. Soil texture A/B is loam/clay. The slope of the plot is 2 %.

2.2. Experimental design

A field experiment was carried out on abandoned arable land with sown low- and high-diversity treatments and natural colonization (NC) following typical farming practice for the site. In April-May 1996, experimental plots were installed on former agricultural land that had been cropped with (crop rotation) monocultures until the end of 1995. The experiment was organized according to a block design with five replicate blocks. Within each block, four plots measuring 10 × 10 m were marked out and each of the three treatments was randomly assigned to one plot. All plots were separated by 2-m walkways. The three treatments (low-diversity-sown, high-diversity-sown and natural colonization) were randomly allocated to the plots in each block.

As the initial vegetation development at abandoned land is usually highly unstable and unpredictable, late-successional types of functional groups of plants were experimentally sown in both low- and high-diversity mixture. Based on the specific characteristics of plants,
the functional groups most widely recognized in tempered grassland communities and which were used in this study are (1) grasses, (2) legumes and (3) other forbs. The low- and high-diversity mixtures comprised the same amounts of seed (grasses: 2500 seeds m\(^{-2}\), legumes: 500 seeds m\(^{-2}\) and other forbs also 500 seeds m\(^{-2}\)). Fifteen species (five per functional group) were sown as the high-diversity-sown treatment. For the low-diversity-sown treatment, low-diversity seed mixtures (two grasses, one legume and one other forb species) were used as random choices from the total set of plants available for each replicate in order to take account of the sampling effects. The plant mixtures used consisted of species typical of later-successional stages (Table 1).

2.3. Measurements

Each year at peak-standing biomass (May/June), vegetation sampling biomass was clipped at 5 cm from the soil surface. In each replicate, a plot of 10 × 10 m and 12 subplots of 25 × 25 cm were harvested and the clipped material was sorted into litters and living parts (the standing

<table>
<thead>
<tr>
<th></th>
<th>LD1</th>
<th>LD2</th>
<th>LD3</th>
<th>LD4</th>
<th>LD5</th>
<th>HD</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Grasses</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bromus inermis Leyss.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>500</td>
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<tr>
<td>Festuca rubra L.</td>
<td></td>
<td></td>
<td></td>
<td>1250</td>
<td></td>
<td>500</td>
</tr>
<tr>
<td>Phleum pratense L.</td>
<td></td>
<td>1250</td>
<td></td>
<td></td>
<td></td>
<td>500</td>
</tr>
<tr>
<td>Poa pratensis L.</td>
<td></td>
<td></td>
<td>1250</td>
<td></td>
<td>1250</td>
<td>500</td>
</tr>
<tr>
<td>Poa trivialis L.</td>
<td>1250</td>
<td>1250</td>
<td>1250</td>
<td></td>
<td></td>
<td>500</td>
</tr>
<tr>
<td><strong>Legumes</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>100</td>
</tr>
<tr>
<td>Lotus corniculatus L.</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>100</td>
</tr>
<tr>
<td>Medicago lupulina L.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>100</td>
</tr>
<tr>
<td>Trifolium fragiferum L.</td>
<td></td>
<td></td>
<td></td>
<td>500</td>
<td></td>
<td>100</td>
</tr>
<tr>
<td>Trifolium pratense L.</td>
<td></td>
<td></td>
<td></td>
<td>500</td>
<td></td>
<td>100</td>
</tr>
<tr>
<td>Trifolium subterraneum L.</td>
<td></td>
<td></td>
<td></td>
<td>500</td>
<td></td>
<td>100</td>
</tr>
<tr>
<td><strong>Forbs</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>100</td>
</tr>
<tr>
<td>Achillea millefolium L.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>100</td>
</tr>
<tr>
<td>Galium verum L.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>100</td>
</tr>
<tr>
<td>Matricaria chamomilla L.</td>
<td></td>
<td></td>
<td></td>
<td>500</td>
<td></td>
<td>100</td>
</tr>
<tr>
<td>Plantago lanceolata L.</td>
<td></td>
<td></td>
<td></td>
<td>500</td>
<td></td>
<td>100</td>
</tr>
<tr>
<td>Sanguisorba minor Scop.</td>
<td></td>
<td></td>
<td></td>
<td>500</td>
<td></td>
<td>100</td>
</tr>
</tbody>
</table>

\( P < 0.05 \)

Table 1. Density of sown seeds (seeds.m\(^{-2}\)) in the five blocks of low-diversity treatments (LD1-LD5) and in the plots of high-diversity treatments (HD).
biomass). In addition, the standing biomass was sorted into grasses, legumes and other forbs. All standing biomass was dried to constant weight at 80°C and weighed. Each year, the clipping was done in different subplots. In addition, in 12 permanent subplots of 1 m² each, adjacent to the clipped subplots, the number of plant species was counted and grouped into two life history classes: annual and perennial species.

2.4. Statistical analyses

The effects of the treatment on aboveground biomass using analyses of variance (ANOVA) with treatments and years as factors were analysed. Also, mixed ANOVAs (treatment, year and interactions among them) were carried out to relate the dominance of total number of species, annuals and perennials and for each functional group, in the NC, LD and HD treatments. Additionally, regression analyses were performed correlating biomass to the perennial-annual plant species ratio (P:A) and the number of species (S). The relationship established between biomass and diversity (P:A species ratio and the total number of species S) was examined using a stepwise multiple regression analysis and an analysis of covariance (ANCOVA) to separate the possible influence of the different diversity parameters P:A and S on biomass production.

Analysis of correspondence (CA) on the matrix of frequency of dominant species in the natural colonization, low-diversity and high-diversity treatments was also performed. A step-by-step multiple regression analysis was conducted to test the variability in the biomass and to explain the model of biomass, total number of species and total cover.

3. Results

3.1. Aboveground biomass production

The aboveground biomass follows a similar pattern among natural colonization and sowing treatments (HD and LD); therefore, the treatment-year interaction was not significant (Table 2). However, the annual differences emerge when comparing HD and LD separately, with the year 1998 showing these differences. Generally, the aboveground biomass tends to reduce across years of succession, but with a significant increase in 1998 with respect to other years, being favoured by the precipitations in the previous years (Figure 1).

When focusing on the aboveground biomass as stratified to functional groups, grasses were the functional group which had the most biomass in each treatment, except the first years, when the forb group had the most amount of biomass. However, the great increase of biomass in 1998 was mainly due to the contribution of grasses, with their significant differences for the treatment-year interaction (Table 2). This was supported for the high relation between total biomass and grasses biomass ($R^2 = 0.72; F_{(1,103)} = 262.99; P < 0.000$). This group began to be the most important from 1998 and had an influence on the total functionality of the ecosystem. The relationship between forb biomass and total biomass although significant was smaller ($R^2 = 0.37; F_{(1,66)} = 40.12; P < 0.000$); this value shows that the forb species
were important through 1998, when they were substituted by better competitor grass species (Figure 2), grasses sowing in HD and grasses colonizing in LD and NC. The cover of sown species was defined by 60% of the total biomass in HD ($R^2 = 0.60; F_{(1, 33)} = 48.54; P < 0.000$); meanwhile, this only explains the 15% in LD ($R^2 = 0.15; F_{(1, 33)} = 5.62; P < 0.024$). Comparing the relationship between total cover (sown species and colonizing species) and the total biomass in each treatment separately, there were important annual variations, which only explained the 37% of the biomass variability in HD ($R^2 = 0.37; F_{(1, 33)} = 19.74; P < 0.000$) and the 48% in LD ($R^2 = 0.48; F_{(1, 33)} = 30.70; P < 0.000$).

The sowing effect did not affect the total biomass, neither the functional groups nor the agreement of block performance suppressing colonizing species. The best block was observed in LD (blocks 2 and 3) (Figure 3), which did not establish an obvious increase in the biomass of the dominant sown species (*Bromus inermis*). In the same way, there was no significant correlation between the total biomass and the total number of colonizing species ($R^2 = 0.017; F_{(1, 66)} = 1.15, P = 0.28$).

Table 2. Mixed analysis of variance of three factors (treatment, year and interactions among them).

<table>
<thead>
<tr>
<th>Variables</th>
<th>Treatment</th>
<th>Year</th>
<th>Tr*Y</th>
<th>HD-LD</th>
<th>Year</th>
<th>HD-LD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total biomass</td>
<td>0.268</td>
<td>0.000</td>
<td>0.344</td>
<td>0.197</td>
<td>0.000</td>
<td>0.012</td>
</tr>
<tr>
<td>Grass biomass</td>
<td>0.553</td>
<td>0.000</td>
<td>0.526</td>
<td>0.369</td>
<td>0.000</td>
<td>0.037</td>
</tr>
<tr>
<td>Forb biomass</td>
<td>0.998</td>
<td>0.000</td>
<td>0.019</td>
<td>0.992</td>
<td>0.000</td>
<td>0.059</td>
</tr>
<tr>
<td>Legume biomass</td>
<td>0.645</td>
<td>0.039</td>
<td>0.976</td>
<td>0.619</td>
<td>0.126</td>
<td>0.961</td>
</tr>
</tbody>
</table>

Figure 1. Aboveground biomass (g·m$^{-2}$) in the natural colonization (NC), low-diversity (LD) and high-diversity (HD) treatments for 7 years studies is compared.
3.2. Achieved number of plant species and perennial/annual ratio on biomass production (P:A)

In the HD and LD of sowing treatments, significant differences were established for the treatment-year interaction, analysing the perennial-annual species ratio (P:A) \((F = 3.78, 6 \text{ d.f.}, P > 0.009)\). The differences were established at the start of the experiment, when the sown species were more...
effective in the HD-sowing treatment (Figure 4). From 2002, when the cover and the number of sown species decrease, these differences disappear, whereas there was a relative importance of annual species in HD from 2002.

The annual species followed the same pattern in the two sowing treatments ($F = 1.75, 6$ d.f. $P = 0.15$), although there were annual differences at the beginning of the experiment (Figure 5).

![Figure 4. Perennial/annual species ratio in the natural colonization (NC), low-diversity (LD) and high-diversity (HD) treatments for the 7 studied years.](image)

![Figure 5. Annual colonizing number of species in the natural colonization (NC), low-diversity (LD) and high-diversity (HD) treatments for the 7 studied years.](image)
3.3. Dominance model of experimentally established community

The dominance of the species in the natural colonization, low-diversity and high-diversity treatments is indicated in Table 3 and Figure 6. Both sowing treatments (HD) and (LD) differed significantly from the natural colonization in terms of the total number of dominant species, except the dominance of annual species (Table 3). In 1999, there was a great increase in the number of annual dominant species, both in HD and in LD treatments, which prevent the significance of this interaction (Figure 6).

The dominance of the annual species followed the same pattern like that of the total dominance. In both variables, there were no significant differences between treatments or treatments-year interaction (Table 3). In the same way, the dominance of annual species explained the 74% of total dominance variability showed for the treatments during all sampling period ($R^2 = 0.74, F_{1,68} = 196.60, P < 0.000$), while the dominance of perennial species only explained the 48% ($R^2 = 0.48, F_{1,68} = 63.88, P < 0.000$) and showed significant differences among treatment-year; therefore, the sowing effect had no negative effect on beta-diversity, despite the fact that there were two sown species clearly dominant in both treatments (B. inermis and Sanguisorba minor). There was a positive relationship ($r = 0.83$) between the total dominance of the community of both treatments with the Shannon index, which shows that increasing this index will increase the beta-diversity ($R^2 = 0.69, F_{1,68} = 149.11, P < 0.000$), while there was a negative relationship with dominance indexes of Berger-Parker ($r = -0.66; R^2 = 0.44, F_{1,68} = 55.04, P < 0.000$) and Simpson ($r = -0.70; R^2 = 0.49, F_{1,68} = 65.99, P < 0.000$), indicating that increasing the dominant species will not decrease the diversity.

The sowing effect was a little relevant in implanting or suppressing the perennial-colonizer species. Nevertheless, during the sowing period where there was a greater number of sown species in HD treatment, the perennial-colonizer species had a lower success than in LD or NC (Figure 7). Although there were a few perennial-colonizer species in natural conditions

<table>
<thead>
<tr>
<th>Variables</th>
<th>Treatment</th>
<th>Year</th>
<th>Tr*Y</th>
<th>LD-HD</th>
<th>Y</th>
<th>LD-HD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total dominance</td>
<td>0.308</td>
<td>0.000</td>
<td>0.018</td>
<td>0.813</td>
<td>0.000</td>
<td>0.506</td>
</tr>
<tr>
<td>Annual species</td>
<td>0.053</td>
<td>0.000</td>
<td>0.204</td>
<td>0.185</td>
<td>0.000</td>
<td>0.526</td>
</tr>
<tr>
<td>dominance</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Perennial species</td>
<td>0.164</td>
<td>0.000</td>
<td>0.000</td>
<td>0.006</td>
<td>0.003</td>
<td>0.013</td>
</tr>
<tr>
<td>dominance</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grasses dominance</td>
<td>0.047</td>
<td>0.000</td>
<td>0.000</td>
<td>0.098</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>Forbs dominance</td>
<td>0.180</td>
<td>0.000</td>
<td>0.008</td>
<td>0.217</td>
<td>0.000</td>
<td>0.041</td>
</tr>
<tr>
<td>Legumes dominance</td>
<td>0.201</td>
<td>0.003</td>
<td>0.000</td>
<td>0.233</td>
<td>0.000</td>
<td>0.000</td>
</tr>
</tbody>
</table>

*P < 0.05.

Table 3. Mixed analysis of variance of three factors (treatment, year and interactions among them).
at the end of the experiment, all species belong to the forb group: *Carduus tenuiflorus* Curtis, *Chondrilla juncea* L., *Convolvulus arvensis* L. and *Echium plantagineum* L. (Figure 7).

The sowing effects on the dominance of colonizer species are indicated by a correspondence analysis (Figure 8). The first axis explains the 11% variability of species composition and there was a very high correlation ($r = 0.97$) among species and the explanatory variables of this composition. These values indicate that the treatment-year interaction may be explained for this horizontal axis with a high percentage, for the high number of species involved in this analysis.
Figure 7. Annual frequency of species appearance in the natural colonization (NC), low-diversity (LD) and high-diversity (HD) treatments. Only the species with dominance >3% are indicated.
3.4. Temporal change of the aboveground biomass

There is a negative relationship between the productivity and mean richness of the natural colonization and the analysed sowing treatments (HD and LD). The coefficient of determination of this relationship was significant ($R^2 = 0.307$, $F_{(1, 13)} = 5.75$, $P = 0.032$) (Figure 9). The species richness influenced with a very short percentage on the biomass production, with the species composition being more important, directly influencing certain functional groups. Thus, the biomass of forb group may be explained at about 55%, due to the total cover presented for all species in both sowing treatments ($R^2 = 0.43$, $F_{(1, 68)} = 52.11$, $P < 0.000$), but the biomass of the grasses was independent of this variable ($R^2 = 0.08$, $F_{(1, 68)} = 5.79$, $P = 0.019$).

In a step-by-step multiple regression analysis, these variables explain the 61% of the variability in the biomass of forb group (Table 4), and only two explain the model of biomass, total number of species and total cover. The contribution of species richness (value BETA) had a greater and negative effect on the biomass of forbs than on the total cover in natural conditions.

The biomass in HD treatment responded positively to the cover of sown species, while in LD treatment, this effect was null and clearly showed the effect of cover on all grouped species (Table 5).

Figure 8. Analysis of correspondence (CA) on the frequency matrix of the dominant species in the natural colonization (NC), low-diversity (LD) and high-diversity (HD) treatments.
Figure 9. Correlation among mean species richness and aboveground biomass from natural colonization (NC), Low-diversity sown (LD) and high-diversity sown (HD) for the 7 studied years.

\[ R = 0.78; R^2 = 0.61; R^2\text{-adjusted} = 0.59; F_{(2, 32)} = 25.03; P < 0.000 \]

**Table 4.** Multiple regression model of the aboveground biomass for the forbs group, explained for the defined variables from the number and coverage species shown in the natural colonization treatment.

<table>
<thead>
<tr>
<th>MODEL</th>
<th>(B)</th>
<th>BETA</th>
<th>(t)</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total number of species</td>
<td>-22.850</td>
<td>-0.735</td>
<td>-6.224</td>
<td>0.000</td>
</tr>
<tr>
<td>Total number of species</td>
<td>-21.593</td>
<td>-0.694</td>
<td>-6.218</td>
<td>0.000</td>
</tr>
<tr>
<td>Total cover</td>
<td>1.910</td>
<td>0.268</td>
<td>2.396</td>
<td>0.023</td>
</tr>
</tbody>
</table>

BETA: Partial coefficients of standardized regression; \(B\): regression coefficients not standardized; \(t\): to prove the null hypothesis \(B_i = 0\); \(P(\alpha = 0.05)\)

**Table 5.** Multiple regression model of the aboveground biomass for the forbs group, explained for the defined variables from the number and coverage of species shown in the low and high treatments.

<table>
<thead>
<tr>
<th>HD ((R = 0.81; R^2 = 0.65; R^2\text{-adjusted} = 0.63; F_{(2, 32)} = 29.49 P &lt; 0.000))</th>
<th>MODEL</th>
<th>(c)</th>
<th>BETA</th>
<th>(t)</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shown species coverage</td>
<td>6.350</td>
<td>0.772</td>
<td>6.967</td>
<td>0.000</td>
<td></td>
</tr>
<tr>
<td>Shown species coverage</td>
<td>5.128</td>
<td>0.623</td>
<td>4.993</td>
<td>0.000</td>
<td></td>
</tr>
<tr>
<td>Total coverage</td>
<td>3.185</td>
<td>0.274</td>
<td>2.195</td>
<td>0.036</td>
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</tbody>
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<thead>
<tr>
<th>LD ((R = 0.69; R^2 = 0.48; R^2\text{-adjusted} = 0.47; F_{(1, 33)} = 30.70 P &lt; 0.000))</th>
<th>MODEL</th>
<th>(c)</th>
<th>BETA</th>
<th>(t)</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total coverage</td>
<td>12.367</td>
<td>0.694</td>
<td>5.541</td>
<td>0.000</td>
<td></td>
</tr>
</tbody>
</table>

BETA: Partial coefficients of standardized regression; \(B\): regression coefficients not standardized; \(t\): to prove the null hypothesis \(B_i = 0\); \(P(\alpha = 0.05)\)
The high correlation among the sum of variance and the sum of covariance also indicated a stabilization effect in the temporal change where there was a greater amount of perennial species. In the HD treatment, when the sown species were more successful, the smaller fluctuations on the biomass change with the number of perennial species (Figure 10). There was a positive relationship between the total biomass and the sum of variance (Figure 11), indicating inter-annual changes inside and between treatments, due to fluctuations of dominant species.

Figure 10. Correlation among the perennial number of species and the biomass temporal change and the sum of variances and covariances, in the natural colonization (NC), low-diversity (LD) and high-diversity (HD) treatments for the 7 studied years.
4. Discussion

4.1. Aboveground biomass production

The highest plant biomass in 1998 was found in low-diversity plots. All treatments had a similar pattern, displaying an important increase of both the grass and secondarily the forb biomass. The treatment-year interaction was not significant. The differences were established when considering HD and LD treatments separately. The forb biomass increased only under diversity treatments (LD and HD). The effect of the dominant species development could explain both the greater plant biomass yield in LD plots, where the number of sown plant species was less in HD plots, while the highest grass biomass yield was in NC (some sown and natural grass species are strong competitors). Our aboveground biomass values were greater than those obtained by Puerto et al. [19] in nine different types of dehesa, whose values ranged between 150 and 200 g.m$^{-2}$, and similar to values found by Pecó et al. [20], 150–250 g.m$^{-2}$, in Mediterranean semi-arid environments.

The contribution of grasses is known to increase as a result of sowing and temporal development when managing grassland communities [21]. The influence of functional group traits on productivity has been reviewed by Díaz and Cabido [22] who found a high number of field studies rather to be correlated to functional group traits than to species diversity. When comparing the two sown treatments, the aboveground biomass was not affected by the number of sown species in each functional group, like in naturally colonized treatments where there are higher probabilities of one functional group missing or being present in a low proportion.

![Figure 11. Correlation among the perennial number of species and the biomass temporal change of biomass and the sum of variances and covariances, in the natural colonization (NC), low-diversity (LD) and high-diversity (HD) treatments for the 7 studied years.](http://dx.doi.org/10.5772/67665)
A greater productivity shown in both qualitative ways was that plots with initial diversity were intently increased by sowing different species. Plots where more species were sown are those that eventually had higher biomass; however, the higher productivity for 1998 was not accompanied by the higher diversity. In addition to inherent production capabilities of each species, the evenness of the distribution (the relative proportion of the component species) can bear on productivity [23], especially the relative contribution of dominants and codominants. So, where fast-growing herbs are able to exercise competitive dominance, it is unusual to find exceedingly low floristic diversity; the resulting community may be closer to monoculture [24].

There were no significant differences considering treatment-year interaction when comparing HD and LD separately for the total number of dominant species or for the annual total of dominant species. Therefore, a similar pattern of both treatments during part and all period of experimentation could be expected. In this way, at the beginning of the experiment in HD, the dominance of annual species decreases significantly, but these differences disappeared with time. This behaviour is in connection with the dominance of perennial species, being highest in HD treatment through the year 1999, that is, in the same period of time, the dominant increase of perennial species (sown and colonizers) has a negative influence on the dominance of annual species, but only in this treatment. Then, the dominance of perennial species fell over minimum in HD, allowing the equalization of the same value than in LD, while the number of annual dominant species increases again. Firstly, this effect was important during the first period of experimentation when the sown species were performing better as cover. From 2002, many species disappeared or decreased its cover and only a few species were seen.

The effectiveness of both treatments in deleting annual species was clear, since there was a negative relationship \((r = -0.64)\) between the cover of sown species and the number of annual dominant species \((R^2 = -0.41, F_{(1, 68)} = 47.68, P < 0.000)\). However, the elimination of annual dominant species was not involved, at the same time the sowing has provided the implantation of colonizer perennial species. At the end of the experimentation, the HD, LD and NC seemed to converge again and the sowing effect tends to disappear with time. The sowing effect was comparatively different in HD treatment, due to the combined action of the greater number of sown species in the initial stages of experiment. During all periods, the LD and NC had a similar pattern, although the influence of sown species in those LD blocks is clear, where the performance of species in determined mixtures was successful. Considering total plant species richness, the observed patterns were in disagreement with the hypothesis that increased productivity results from higher species diversity in grasslands [9].

4.2. Enhancement of productivity and resource capture along secondary succession

The increase in aboveground biomass with grassland succession is in agreement with earlier results for mesic environments [25]. This trend is assumed to be related to successional changes in the traits of the dominant plant species. A gradual increase in the contribution of grasses as a result of sowing and temporal development was established when biomass was sorted into grasses, legumes and non-legume forbs.

During the course of secondary succession, there was a change in the number of species as well in productivity. In general, plant diversity decreases while productivity increases [26].
These trends are found in several studies [27]. The manipulations of the succession assay include the introduction of later-successional plant species, mainly perennial species. As a consequence of this manipulation, the course of succession has been affected as a result of the change in the dominance of vegetation pattern. Leps et al. [8] from a study on the first 4 years of the succession concluded that the floristic composition changed more rapidly than the performance of the dominant species. The effect of the sown diversity treatments was the rearrangement of most seasonally variable community [28] of early-successional species by weed suppression to a community when grasses and perennials dominated. The effects of species richness may be only effective in narrow degrees of the functional composition of communities [29], due to combination of some factors such as the species identity, effects of species attributes [30] and in the lower degree, as the complementary of resources used [31].

The poor relationship found between the perennial and annual species ratio (P:A) and the aboveground biomass in HD treatment ($R^2 = 0.18$, $F_{(1, 33)} = 7.34$, $P = 0.011$) and in BD ($R^2 = 0.14$, $F_{(1, 33)} = 5.32$, $P = 0.027$) indicated the limited contribution of perennial species to the aboveground biomass in the sowing treatments and the poor relationship between species richness and productivity.

4.3. Temporal change of the aboveground biomass

The diversity has no positive effect on aboveground biomass in the three treatments: HD, LD and NC. The results of temporal change in the aboveground biomass may confirm the null answer of the aboveground biomass to changes of diversity in the sowing treatments. However, the results confirmed the negative effect of diversity change on system productivity. There are sampling effects that may partially explain the total biomass [4]. This effect predicts that the total biomass was determined by the increase of probability which comprise a particular species in a community. Species such as the perennial grass *Lolium rigidum* or the forbs *Matricaria inodora*, *Polygonum aviculare* or *Plantago coronopus*, which were dominant in natural conditions, were removed from the sown species, particularly in LD treatment.

In general, the sowing effect caused destabilization in the system, increasing the number of species that may coexist in the established communities, at the expense of the biomass, that is, a negative productivity-diversity relationship. The HD treatment where there was more effective sowing effect exhibited a lower variation among blocks than the remaining treatments. Analysing the biomass by functional groups, a negative relationship was found in NC which was also valid for the forb group.

There was no significant relationship found between the temporal change of the aboveground biomass and mean species richness in each plot-treatment relationship ($R^2 = 0.012$, $F_{(1, 13)} = 0.154$, $P < 0.701$). However, the temporal change of the aboveground biomass was positively related with the number of perennial present species (sown and natural colonizer) ($R^2 = 0.306$, $F_{(1, 13)} = 5.723$, $P = 0.033$). This may indicate that this effect was mainly due to the group of perennial sown species.

The sown species negatively influenced the cover of colonizer-dominant species in HD treatment, allowing a redistribution of colonizer species taking a greater degree of competition.
This reflected a lower variability of the temporal change of the aboveground biomass because there was no dominant species in natural conditions. The sowing effect did not increase the species richness, but the uniformity of abundant colonizer species was increased. There was a diversity effect on the variability of established communities depending on the variation of biomass among dominant species or another species.

4.4. Vegetation composition effects: a negative productivity-diversity relationship

The biomass pattern cannot be explained by the hypothesis that more diverse plant communities are more productive [32]. Indeed, clear relationships have been found for biomass with the relative abundance of perennial species; this is taken as the mean number of perennial species/mean number of annual species ratio.

From the analysis of data, there were no great differences regarding the number of perennial species along the years. These considerations recommended the use of an index relating both groups of species (P:A); it was once observed that the relative abundance of perennial number of species is what changes along the years, and in doing so, the vegetation pattern observed could be explained satisfactorily. In fact, our data suggest that there was a change in the dominance pattern after sowing, so a higher P:A and a general trend towards decreasing richness were reached from the sown treatments.

The functional groups differ in both phenology and rooting depth implying a complementary exploitation of habitat; competitive interactions in mixture may have a strong effect on total plant biomass. In fact, the sampling effect and resource complementary, either in time or in space, may operate simultaneously [15].

As the productivity-diversity relationship was negative, the biomass of forb group was also influenced negatively by the richness species in the plots, showing that the functional diversity also had an effect on productivity and the efficiency of capture resources [33].

Sowing mixtures of later-successional plant species may enhance the initial functional diversity of plant species by stimulating the establishment of mid-successional perennials. This may have undesirable side effects through the introduction of alien genotypes, but it enhances the exclusion of arable weeds [5], and high-diversity mixtures enhance the reliability of ecosystem functions [8]. Introduction of mid-successional plant species stimulates the restoring of ecosystem processes, such as efficient light capture (LAI) and light utilization (photosynthesis), and it affects aboveground insect assemblages [34]. However, the short-term effects of the experimental treatments on different trophic groups of nematodes [35] and on microbial activity [36] are limited. In fact, in the first 2 years, the effect of ending agricultural practices alone had much larger effects on the soil community than any of the experimental manipulations of the plant assemblages [34, 36].

5. Conclusions

The aboveground biomass follows a similar pattern among natural colonization and sowing treatments (HD and LD); therefore, the treatment-year interaction was not significant.
However, the annual differences appear when comparing HD and LD separately, with the year 1998 showing these differences. In general, the aboveground biomass tends to decrease across years of succession, but with a significant increase in 1998 with respect to other years, being favoured by the precipitations in the previous years.

The sowing effect did not affect the total biomass, neither the functional groups nor the agreement of block performance suppressing colonizing species. The best block was observed in LD (blocks 2 and 3), which did not establish an obvious increase in the biomass of the dominant sown species (*B. inermis*). In the same way, there was no significant correlation among the total biomass and the total number of colonizing species.

Significant differences were established for the treatment-year interaction, analysing the perennial-annual species ratio (P:A) in the HD and LD of sowing treatments. The differences were established at the beginning of the experiment, when the sown species were more effective in the HD-sowing treatment. From 2002, when the cover and the number of sown species decrease, these differences disappear, whereas there was a relative importance of annual species in HD from 2002.

There is a negative relationship between the productivity and mean richness of the natural colonization and the analysed sowing treatments (HD and LD). The coefficient of determination of this relationship was significant ($R^2 = 0.307$, $F_{(1,13)} = 5.75$, $P = 0.032$). The species richness influenced with a very short percentage on the biomass production, being more important the species composition influencing directly on certain functional groups. Thus, the biomass of forb group may be explained at about 55%, due to the total cover presented for all species in both sowing treatments ($R^2 = 0.43$, $F_{(1,46)} = 52.11$, $P < 0.000$), but the biomass of the grasses was independent of this variable ($R^2 = 0.08$, $F_{(1,46)} = 5.79$, $P = 0.019$).

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