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Above-ground biomass distribution among species during early old-field succession

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Abstract. The interdependence of species richness and plant biomass has widely been accepted as a general biodiversity rule. However, there is no information about how relationships are established during colonization and how total biomass is distributed among plants. The main objective of this study was to determine the role of several factors which we have hypothetized as affecting biomass distribution among species in an early old-field community. To test this hypothesis, we conducted an experiment in a deeply ploughed *Agropyro-Rumicion crispi* community in the Basque Country (Spain). Light, water and soil nitrogen content were factorially manipulated resulting in eight experimental treatments. We also examined several site features, which could potentially influence final biomass distribution: seed bank composition, soil physico-chemical heterogeneity and cover and density of the dominant plant species in the community. A partition hypothesis testing approach using Redundancy Analysis was conducted to determine the fraction of biomass distribution variability assigned to each treatment and site feature. The most important species, in terms of biomass, were *Agropyron repens*, *Sinapis arvensis*, *Arrhenatherum bulbosum* and *Picris echioides*. As a general conclusion, biomass distribution among species during early secondary succession primarily depends on light availability and nitrogen supply. Several soil variables, such as magnesium, calcium and clay contents, also explain a relevant fraction of the biomass distribution among plant species. On the contrary, we found no effect of seed bank composition on biomass distribution. Finally, the total species number and cover of dominant species such as *Sinapis arvensis*, may determine final biomass distribution.

Keywords: Environmental constraint; Fertility; Hypothesis testing; Redundancy Analysis.

Abbreviations: DCA = Detrended Correspondence Analysis; GLM = General Linear Model; MANOVA = Multiple Analysis of Variance; PAR = Photosynthetically Active Radiation; RDA = Redundancy Analysis; TVE = Total Variation Explained; uSD = units of Standard Deviation.

Nomenclature: Castroviejo et al. (1986-2002); for species not included in this work: Tutin et al. (1964-1980).

Introduction

Many studies have explored the relationships between plant biomass and species richness in terrestrial plant communities (e.g. Al-Mufti et al. 1977; Austin 1987; Shipley et al. 1991; Tilman & Pacala 1993; Gough et al. 1994). Different levels of productivity, density dependence and impact of shading have been discussed as important factors (e.g. Grace et al. 1993; Wilson & Tilman 1991), either in descriptions of natural productivity gradients (Wilson & Tilman 1993; Gough et al. 1994; Tilman 1993) or through experiments (Shipley & Keddy 1994). Results of these studies have led us to infer the relevance of competition (Grime 1979), together with disturbance (Huston 1979), spatial and temporal soil heterogeneity (Tilman 1982; Tilman & Pacala 1993) and factors operating during colonization processes (Tilman 1993; Tilman & Pacala 1993; Silvertown et al. 1994) in regulating the mutual relationship between species richness and plant biomass.

Many studies have explored the way in which biomass is allocated among plant organs but few studies deal with the way in which biomass is distributed among species at the community level. The main aims of this study are to understand (1) the processes that affect biomass accumulation among species; (2) the role of colonization. During colonization, recruitment after severe perturbations is directly conditioned by availability of propagules (Lavorel et al. 1991; Fenner 1995). Emerging seedlings usually face complex competitive scenarios in which mortality is usually density-dependent and shaped by the establishment of competitive hierarchies (Keddy & Shipley 1989). Seedling survival at this stage is also affected by weather (Escudero et al. 1999), soil characteristics (García-Fayos et al. 2000) and the presence of herbivores and pathogens (Schupp & Fuentes 1995). Obviously, these environmental constraints affect each life stage, but also species composition and the distribution of biomass among species (Tilman 1993). Three questions need to be answered: 1. What are the

factors controlling the relationships between the two biodiversity attributes? 2. How is the interdependence established? 3. How is biomass distributed among species?

We hypothesized that community-level relationships between these attributes established during early old-field succession depends on soil resource levels (Gough et al. 1994) and heterogeneity (Rubio & Escudero 2000), along with biotic constraints such as seed bank composition and seed density. To test this hypothesis, an experimental study was conducted in a deeply ploughed field to examine the effect of plant resources such as light, water and nitrogen availability on species composition and biomass distribution among species. Also, seed bank characteristics, soil heterogeneity and the resulting plant community structure, were studied.

Material and Methods

Study site

This study was carried out in the Arkaute Agricultural Experimental Station, 3 km east of Vitoria-Gasteiz (Basque Country), N Spain (42°51' N; 2°37' W; 510 m a.s.l.). Climate is classified as temperate submediterranean and subhumid (Rivas-Martínez & Loidi 1999), with mean annual rainfall of 843 mm and mean temperature of 11.7 °C, ranging from 1.3° C in the coldest month to 26.1 °C in the warmest. Precipitation is highly unpredictable in time and quantity and is characterized by marked summer droughts. The study year (1999) had more rainfall than usual. The study site is an old field, abandoned 5 yr ago, which covers ca. 3 ha on level land and is grazed by sheep. The plant community was assigned to the alliance *Agropyro-Rumicion crispi*, which is widely distributed in the area. Main species were *Agropyron repens*, *Arrhenatherum bulbosum*, *Rumex crispus*, *Geranium dissectum* and *Picris echioides*. Soils were classified as vertisols with some hydromorphy.

Experimental design

In February 1999, the soil was ploughed to a depth of 30 cm and hand-raked to smooth the surface and remove any remaining clumps of vegetation. Then, 40 plots (4.75 m × 1.75 m) were regularly distributed with a 1.75-m wide buffer. Levels of light (shaded: S^+; not shaded: S^o), water (watered: W^+; not watered: W^o) and nitrogen availability (nitrogen addition: N^+; no nitrogen addition: N^o) were factorially manipulated (1 *df* per treatment) resulting in eight treatments. We used a randomized design, with five replicates per treatment.

On N^+ plots 6 $g.m^{-2}.yr^{-1}$ N was added by manually scattering urea in granular form from the walkways in the last week of March. On S^+ plots a wooden frame was installed, covered with a neutral shading cloth 1.5 m above the soil. This device filtered 80% of daylight photosynthetically active radiation (PAR). On W^+ plots we added 8.65 $l.m^{-2}$ fortnightly during June and July (five irrigations). During this period water demands reach a maximum and the summer drought usually begins, so it is the most critical time for plant development. Total increase of water supply above mean rainfall during this period was 21.5%.

Soil and seed bank sampling

Soil sampling was carried out in March immediately after the ploughing treatment. Three soil cores, 11 cm diameter and 3 cm deep, were collected per plot, thoroughly mixed and then separated into two fractions. The soil analysis fraction was air-dried at room temperature for a month, sieved in the laboratory and analysed for fine earth (< 2 mm), fine-gravel (2-20 mm) and gravel (> 20 mm) percentages, pH in H_2O (1:2.5), organic matter content, total N, P, Ca, Mg and cation exchange capacity. The other soil fraction, 140.45 cm^3, was spread in 10 cm × 10 cm × 1 cm pots mixed with vermiculite (1:1) and placed in a glasshouse. Pots were regularly watered and the seedlings identified, counted and removed throughout a year.

Plant sampling

During the second week of July 1999, a 0.25-m^2 quadrat was delimited in the centre of each plot and harvested by clipping plants at ground level. We considered above-ground tissues to be in the quadrat if they were connected to a tiller that was rooted in the quadrat. In the laboratory, plant samples were identified to species, separated and dried at 70 °C for 4 d and subsequently weighed. A 'biomass matrix' of 40 plots × 60 species above-ground biomass values was constructed.

Plant species were classified into two groups according to their establishment type (field observations): plants mainly originated from vegetative organs and mainly established from seeds. Seeder plants were also classified into two types in relation to their dispersal strategy: anemochorous and non-anemochorous plants.

Plant densities and cover were determined in a quadrat adjacent to that for estimating biomass (0.25 m^2). We counted the number of tillers of each species and estimated percentage cover for those species with at least 1.5% of the total cover in each plot.

Numerical analyses

Most data were heteroscedastic and did not meet the normality test of Kolmogorov-Smirnov; they were log-transformed. Differences among treatments in species richness, total biomass and other attributes, e.g. grass and forb biomass, biomass related to establishment type (vegetative or seeder), and to dispersal (anemochores or not), were tested by three-way ANOVA models in which the three-way interaction was the error term. We used type III sums of squares to avoid difficulties due to unbalanced data. Bonferroni adjustments were performed to control for the significance levels after multiple comparisons. We also conducted non-parametric tests in cases where normality assumptions were not met.

The biomass matrix was subjected to constrained ordination for hypothesis testing (ter Braak & Prentice 1988; Legendre & Anderson 1999; Rubio & Escudero 2000). The major advantages of this technique over classical multivariate analysis of variance are that it is distribution-free and has no restrictive upper limit to the number of species included (Verdonschot & ter Braak 1994). Statistic modelling by constrained ordination performs better than General linear model (GLM) approaches, which work better for single-species models (Guisan et al. 1999). Our null hypothesis (H_0) was that the influence of environmental variables on the biomass matrix was not significantly different from random. With this in mind, Detrended Correspondence Analysis (DCA) was also conducted – detrending by segments and non-linear rescaling of the axes, which has the property that the extracted axes are scaled in units of average standard deviation (Gauch 1982). As the length of the extracted gradients was relatively short (Standard Deviation units; uSD = 2.08), we followed Legendre & Anderson (1999) and conducted Redundancy Analysis (RDA) which assumes linear relationships (ter Braak & Šmilauer 1997). We tested for collinearity among variables in each constraining matrix by calculating variation inflation factors (VIFs) for each variable. None of the variables were eliminated because their VIF values were below 10 (Chatterjee & Price 1991), but the $S^+N^+W^+$ variable in the treatment matrix was not considered.

Six matrices were built to constrain the biomass matrix:

1. *Treatment matrix*; six vectors: three treatment factors, previously transformed into dummy variables, and three dummy variables for the coincidence by pairs of treatments (N^+S^+, W^+S^+, N^+W^+);
2. *Soil data matrix*; 12 vectors: soil texture (gravel, fine gravel, sand, clay), organic matter, pH, total N, C:N ratio, P, Ca, Mg, cation exchange capacity;
3. *Seed bank matrix*; 22 species with numbers of individuals germinated in the glasshouse per experimental plot;
4. *Species richness matrix*; one vector: number of species in each plot;
5. *Species cover matrix*; 10 species, those that made up at least 1.5% of the cover;
6. Species density matrix; 11 (most frequent) species (App. 1).

Total Variation Explained (TVE) by each data set was calculated as the sum of all canonical extracted axes using each of these matrices as the constraining data matrix (Bocard et al. 1992). A Monte Carlo permutation test was performed to determine the accuracy of the relationship (1000 randomizations) between the two data sets. The sum of all canonical eigenvalues or trace was used to build the *F*-ratio statistic (ter Braak 1990; Legendre & Anderson 1999). Only when $p < 0.05$ (adjusted for multiple comparisons by the Holm's method; Legendre & Legendre 1998) the relationship between the two data sets was considered significant. If the RDA model was significant, a forward stepwise procedure was carried out to select a reduced model including only significant variables. We incorporated explanatory variables one at a time and step by step in the order of their decreasing eigenvalues after partialling out the variation accounted for the already included variables. The process stopped when the new variable was not significant ($p > 0.05$). Improvement of the reduced model with each new selected variable was determined by a Monte Carlo permutation test with 1000 randomizations.

A variance partitioning with RDA was performed to evaluate the relative importance of these constraining matrices after adjusting the variability of other data sets which were considered as covariables (Bocard et al. 1992; Bocard & Legendre 1994; Legendre & Legendre 1998). This procedure has been called partial RDA because it determines the variation explained by the explanatory variables after removing the variation accounted for the covariable data set. All the partial models were also tested for significance using a Monte Carlo permutation approach. The treatment matrix was the explanatory data set whereas the other five data sets (Cover, Density, Soil, Seed bank, Species number) were considered as covariable matrices. A similar approach was conducted by only considering the reduced RDA models and the significant variables. The fraction of variability from the biomass data set was assigned to each of the considered matrices. These analyses were conducted with CANOCO (ter Braak & Šmilauer 1997).

Results

Descriptive results

The total number of weed species was 60. *Agropyron repens* accounted for 33.3% of the total biomass, *Sinapis arvensis* for 15.1%. Species richness per plot was similar to the pre-disturbed *Agropyro-Rumicion* ($U = 63.0$, $p = 0.163$) and the only experimental treatment that had a significant effect on species richness was the

shading treatment ($p < 0.008$, Table 1). Richness decreased from 20 species per non-shaded quadrat to 16.5 species per shaded quadrat (Fig. 1a). The number of forb species (mean = 14.5) did not show this trend (Kruskal-Wallis including only main effects: $\chi^2 = 3.53$; $p = 0.063$; $df = 3$).

Total above-ground biomass per plot ranged from 275 g.m^{-2} to 538 g.m^{-2} and differences among treatments were highly significant. Specifically, the main factors affecting this community trait were shade and N-addition (Table 1). Although irrigation did not have a clear effect, the interaction with shading resulted in higher total biomass values in shaded and irrigated plots (Fig. 1b). Total biomass reached a maximum value of 538 g.m^{-2} in treatments combining N and no shading (N^+S^o) and a minimum of 275 g.m^{-2} in the opposite combination (N^oS^+) (Fig. 1b). Grass biomass only increased significantly with N-addition (Table 1) and forb biomass had a large decrease under shaded conditions (Fig. 2) (Kruskal-Wallis, $\chi^2 = 10.71$; $df = 3$; $p = 0.001$).

Species with vegetative regeneration hardly increased biomass after N-addition, whereas those regenerating from seed produced much less under the shade treatment (Table 1 and Fig. 1c). As a result, species with vegetative reproduction became dominant under shaded conditions, and those with seed reproduction took advantage in full light. Nevertheless, shade treatments did not affect species richness (Fig. 1d) (Kruskal-Wallis, $\chi^2 = 2.49$; $df = 3$; $p = 0.114$; mean = 15.0). Biomass of anemochorous plants decreased significantly in shaded conditions, while non-anemochorous plants were affected to the same extent as total biomass by shade and nitrogen addition (Table 1 and Fig. 1e). To evaluate if these differences were a consequence of depletion in seed rain due to the shading mesh, we established additional control quadrats (25 cm × 25 cm) within the 40 experimental plots. In these control plots, soil to 20 cm in depth was removed, sterilized in the laboratory and relocated. Thus, emergence would only be a consequence of seed rain. We found no significant difference between shaded and non-shaded treatments in the total number of seedlings germinated until July ($U = 177.5$; $p = 0.55$).

Variance partitioning results

We excluded 19 of the 60 species occurring in less than three plots to avoid potential biases; herewith only 12% of the total biomass was excluded.

The first DCA axis was relatively short; thus, we conducted RDA ordinations. In order to determine the fraction of the Total Variation of the biomass matrix explained by each data set, we conducted six RDAs (Table 2). The treatment matrix accounted for a high and significant fraction (43.6%) of variation in the biomass matrix. The soil data matrix and cover species matrix significantly explained 41.2% and 37.7% of variance, respectively. Conversely, densities and seed bank matrices explained no significant fraction of variation. Finally, the one-vector number-of-species matrix significantly accounted for a relevant fraction of variance (18.2%). To evaluate the existence of any underlying spatial trend that could generate spatial autocorrelation among our plots, we performed an RDA including northing and easting components in the constraining matrix (Dutilleul 1993). TVE was below 2% and not significant ($p = 0.55$).

To determine the relevant variables of each of the explanatory data sets, we conducted several stepwise forward selection analyses. When the constraining matrix was the treatment matrix, only four of the six variables were selected: S^+ (21% of the total variation explained), N^+ (8%), N^+S^+ (6%) and N^+W^+ (5%). In the soil matrix only three variables significantly entered the reduced model: Clay fraction (8%), Mg-content (6%) and Ca-content (7%). In the cover matrix only *Sinapis arvensis* (11%) was selected. The resulting reduced models show relatively small drops in TVE.

We performed partial RDAs in which the variation attributable to soil, cover and total species number data sets – acting as covariable data sets – was partialled out before adjusting the remaining information (Table 3). Treatment and soil data sets together explained 75.4% of the total biomass variation with a small overlap of 9.4% (Fig. 3). Treatment and cover sets accounted for 64.4% of the total variation, but overlapped 16.9%. Species number and treatment sets accounted together for 55.7% of total variance.

Table 1. Three-way-fixed-effects ANOVA. N^+ = Nitrogen; S^+ = Shading; W^+ = water. The *F* statistic is presented. Significance levels are: n.s. = Not significant; * = $p < 0.008$ (Bonferroni adjustment for $p = 0.05$); ** = $p < 0.001$.

	N^+	S^+	N^+S^+	S^+W^+	W^+	N^+W	$N^+W^+S^+$
Species number	n.s.	8.1*	n.s.	n.s.	n.s.	n.s.	n.s.
Total biomass	12.13**	60.76**	n.s.	17.25**	n.s.	n.s.	n.s.
Vegetative plant biomass	7.90*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Non-vegetative plant biomass	n.s.	85.38**	n.s.	n.s.	n.s.	n.s.	n.s.
Anemochorous biomass	n.s.	31.79**	n.s.	n.s.	n.s.	n.s.	n.s.
Non-anemochorous biomass	12.69**	27.44**	7.95*	11.82*	n.s.	n.s.	n.s.
Grass biomass	8.18*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

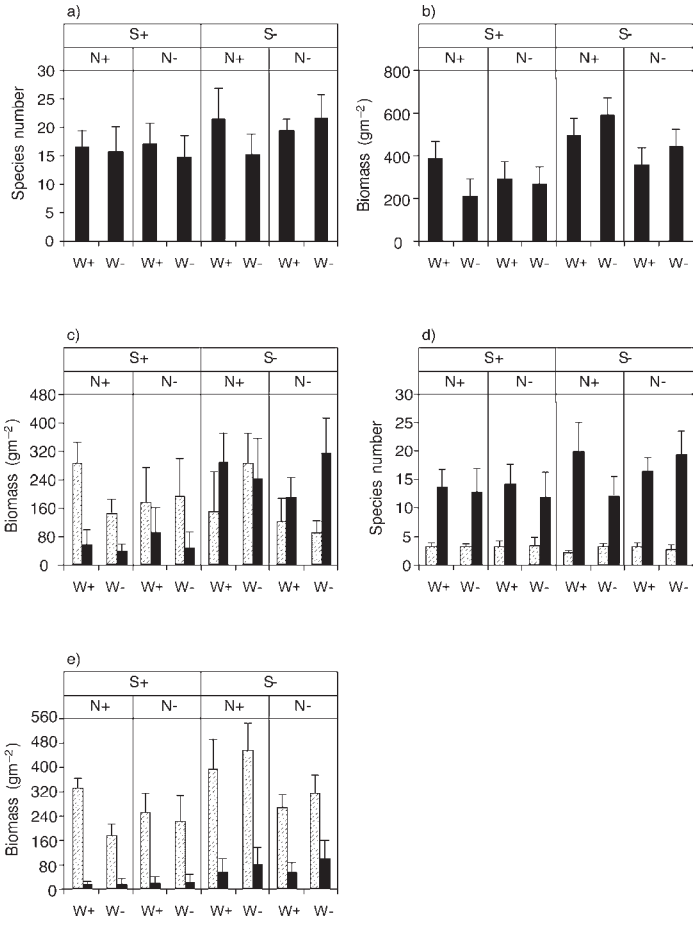

Fig. 1. Mean values and standard deviation of biomass and species number per plot in the eight treatments. S^+ = Shade; S^- = no-shade; N^+ = Nitrogen addition; N^- = No addition of nitrogen; W^+ = Water irrigation; W^- = no irrigation. **a.** Species number per quadrat **b.** Total biomass **c.** Vegetative plant biomass (striped bars) and biomass of plants establishing from seed (black bars). **d.** Vegetative species number (striped bars) and species number of plants establishing from seed (black bars) **e.** Non-anemochorous (striped bars) and anemochorous (black bars) plant biomass.

We also performed partial RDA analyses including as covariables only those that had previously entered reduced models (see Tables 3 and 5). The treatment matrix together with the reduced soil matrix still explained 60.4% of biomass variation (overlap < 1%). Treatments significantly explained a high fraction of variation after fitting *Sinapis arvensis* cover and species number (34.8% and 34.4%, respectively).

The biplot of the RDA model with the four selected treatment variables (App. 2, Fig. 4a) showed that S^+ and N^+S^+ are the major environmental factors on axis 1 and N^+ and N^+W^+ are correlated with axis 2. Biomass of some weed species was clearly related to the shade centroid*: Agropyron repens*, *Convolvulus arvensis* and *Lolium perenne*. *Anagallis arvensis*, *Medicago lupulina* and others were negatively correlated with shade. Nitrogen treatment (N^+) induced an increase in the biomass of *Sinapis arvensis*, *Arrhenatherum bulbosum* and *Rumex crispus*. The RDA biplot using the three selected soil variables (App. 2, Fig. 4b) showed that the first axis was

Table 2. RDA models using the biomass data set as the main matrix and other environmental data sets as constraining matrices (treatment, densities, covers, species number, soil and soil seed bank). *Constraining matrix* indicates data set used to build models. $\lambda 1$, $\lambda 2$, $\lambda 3$ are the eigenvalues of the corresponding extracted axes. Σcons is the sum of all constrained axes, i.e. the variation explained by the constraining data set. Total variation in linear methods as in RDA sums up to 1. The Total Variation Explained (*TVE*) corresponds with the sum of all constrained axis eigenvalues (Σcons). *F* is the *F*-ratio statistic, which is computed using the trace or sum of all eigenvalues, and *p* is significance of the model after Holm's method ($P < 0.05$) (with 1000 randomisations). Note that $\lambda 2$ of the last model (sp.-num.) is not constrained

Constraining matrix	$\lambda 1$	$\lambda 2$	$\lambda 3$	Σcons	TVE	Monte Carlo test	
						F	*p*
Treatment (6 variables)	0.241	0.137	0.038	0.436	43.6	4.246	**
Soil (12 variables)	0.192	0.092	0.078	0.412	41.2	1.576	*
Covers (10 variables)	0.193	0.097	0.042	0.377	37.7	1.757	*
Densities (11 variables)	0.177	0.07	0.05	0.348	34.8	1.356	n.s.
Seed bank (22 variables)	0.380	0.204	0.100	0.804	80.4	1.615	n.s.
Species number (1 variable)	0.182	0.309	0.236	0.182	18.2	8.48	**

Table 3. Partial RDA models using the treatment data set as the constraining matrix and subsequent data sets as covariable matrices. Rows 1-3 correspond to models in which all variables and covariables are considered, rows 4-6 correspond to models including significantly selected variables and covariables. *Constraining matrix* indicates the data set used to build models. *Covariables* indicates the covariables data sets for partial RDA models. *λ1, λ2, λ3* are the eigenvalues of the corresponding extracted axes. *Scons* is the sum of all constrained axes. The sum of all eigenvalues after fitting covariables is in brackets (Ico). In partial RDAs, TVE represents the proportion between the sum of all constrained eigenvalues after fitting covariables and the sum of all unconstrained eigenvalues after fitting covariables. *F* = F-ratio statistic computed using the trace or sum of all eigenvalues as reference; *p* = level of significance of the model (1000 randomizations). Number of variables of the main matrix (va) and number of covariables of the corresponding matrix (co) are shown.

Constraining matrix	Covariables	λ1	λ2	λ3	Scons (Ico)	TVE	Monte Carlo test	
							F	*p*
TREAT. (6va)	soil (12co)	0.231	0.077	0.018	0.342 (0.588)	58.16	4.846	0.002
TREAT. (6va)	species no. (1co)	0.215	0.114	0.026	0.375 (0.82)	45.61	4.519	0.002
TREAT. (6va)	cover (10co)	0.122	0.110	0.018	0.267 (0.62)	43.06	2.887	0.003
TREAT. (4va)	soil (3co)	0.238	0.121	0.031	0.394 (0.79)	49.87	7.942	0.001
TREAT. (4va)	species no. (1co)	0.207	0.112	0.02	0.344 (0.818)	42.05	11.528	0.001
TREAT. (4va)	cover (1co)	0.184	0.128	0.033	0.348 (0.89)	39.1	5.467	0.001

correlated with clay content, whereas the second was correlated with the content of magnesium. The species related to the clay factor were *Agropyron repens* and *R. crispus* while magnesium had a positive effect on the biomass of *Cirsium arvense* and *A. bulbosum*.

Discussion

Species richness

We found no differences in species richness in any of the productivity scenarios despite higher biomass values on fertilized plots. The only significant differences in species richness were detected under shaded conditions. In this sense our results do not support any of the two postulated and complementary models to explain the relationship between species richness and productivity: the unimodal model (Tilman 1982; Tilman & Pacala 1993; Gough et al. 1994) and the monotonic model (Abrams 1995). Furthermore, above-ground biomass has been reported as an efficient species-richness predictor in several experiments (Wisheu & Keddy 1989; Wheeler & Shaw 1991). Our results, however, are in accordance with those of García et al. (1993), Tilman (1993) and Gough et al. (1994) in which no relationships were found between biomass and species richness. These studies were also carried out in early succession stages.

We detected a drop in species richness under shad-

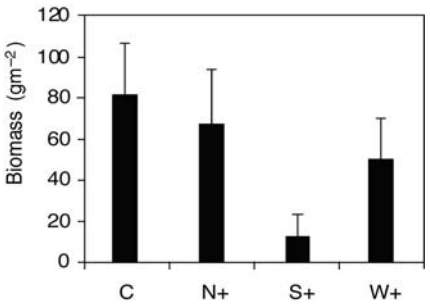

Fig. 2. Mean values and standard deviation of forb biomass in four treatments (C = control; N^+ = Nitrogen addition; W^+ = Water addition; S^+ =Shading).

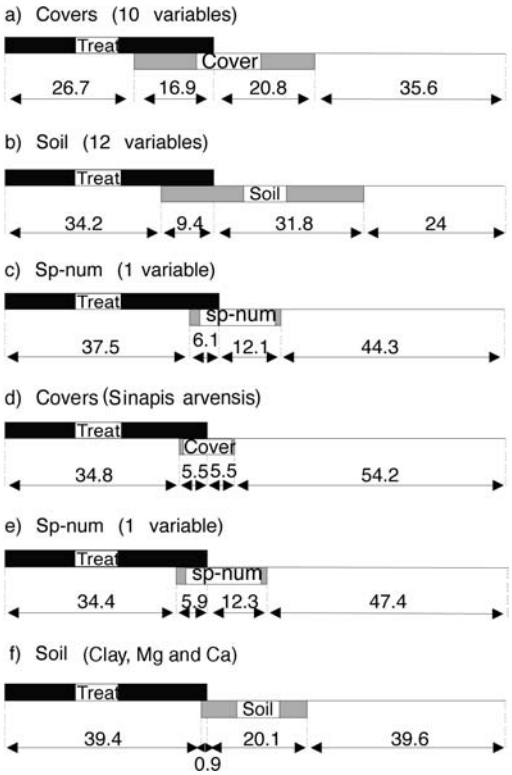

Fig. 3. Percentage of variation in plant biomass explained by each of the data matrices (treatments, soil characteristics, cover and species number) and the overlapping of the variation explained by the treatment matrix with each of the other constraining matrices in full (**a**, **b** and **c**) and reduced (**d**, **e** and **f**) RDA models.

ing. The number of species establishing from seeds was significantly lower in shaded plots (Fig. 1d). Goldberg & Miller (1990) observed that light reduction increased mortality rates of slow growing and shade-intolerant species, as well as seedlings, and consequently it also increased local extinction rates and decreased diversity in a 1-yr old-field. Experiments carried out with litter as the shading agent also generated a decrease of species richness (Foster & Gross 1998).

Biomass distribution among species: general remarks

We have focused our study on biomass distribution among species because it is considered an indicator of both productivity (Pickett & Bazzaz 1976) and plant reproductive success (Mimbé & Lafarge 1995). The extent to which a plant species affects the ecosystem functioning is predictable from its contribution to the total biomass of the plant community (Grime 1998).

Many studies support the hypothesis that either light availability (Foster & Gross 1998; Huante et al. 1998), or nutrient (Tilman 1987) and water supply (Lane 1995) control species abundance throughout succession. Competitive hierarchies are common in plant communities, and there is evidence that above-ground competitive success is controlled by the ability to gain height and to produce biomass (Dietz et al. 1998). Our results seem to support these general statements.

Nutrient availability in biomass distribution

Conspicuous increments of annual forb biomass in early succession have previously been found after fertilization treatments (Carson & Barrett 1988; Huberty et al. 1998) and nutrient pulses after intense perturbations have been proposed as mechanisms to allow annuals to persist in recently abandoned old-fields. However, we have not detected a significant increase in total biomass of plants reproducing from seeds. In contrast, plants with vegetative growth showed a significant increase in their biomass when nitrogen was added. This effect may be explained by the fact that the original community was dominated by grasses with a remarkable ability to develop vegetative organs (*Agropyron repens*, *Arrhenatherum bulbosum*). Thus, after ploughing, broken pieces of rhizomes and bulbs should have been able to take advantage of nutrient addition, due to their higher relative growth rate (Grime et al. 1988) and generally faster nutrient uptake rates (Heil & Bruggink 1987) and consequently enhanced biomass production. Subsequent asymmetric competitive interaction may have prevented the expected increase in biomass of annuals.

Despite this marked effect on biomass, we detected no significant effect of fertilization on species richness (Table 1). In a number of short-term studies fertilization had no consistent effect on diversity (Carson & Pickett 1990; Goldberg & Miller 1990); in others, it led to higher richness (Pringle 1990) or caused an increase followed by a decrease (Carson & Barrett 1988). Long-term studies, in contrast, revealed a reduction in diversity when nitrogen was added (Tilman 1987, 1993; Foster & Gross 1998) or showed no effects (Huberty et al. 1998). Two mechanisms to explain a drop in species diversity with nutrient addition have been suggested: competitive exclusion (Sardans 1997) and a change in the number of simultaneously limiting resources (Braakhekke & Hooftman 1999). Most likely, both explanations are feasible but their importance may depend on the initial fertility levels and the nature of the available propagules. In our experiment the initial soil nitrogen availability was high (C:N = ca. 7.2). Thus, the initial floristic composition was well suited to be competitive in nutrient-rich environments. This would explain why the effect of nitrogen addition was not as dramatic as in non-fertile soils (Huberty et al. 1998).

In early secondary succession, fertilization has been observed to favour plants with a short life span, high seed production and fast growth (Tilman 1988). In this study, some species which fit well with these characteristics, such as *Sinapis arvensis*, *Papaver rhoeas* or *Aphanes arvensis,* performed better on fertilized plots. There is also a strong tendency of *Agropyron repens* to develop better in fertilized plots. Some studies have indicated that *Agropyron repens* is favoured by N-addition and it is considered an early successional species on nutrient rich soils (Tilman 1987, 1990). *Arrhenatherum bulbosum* also had a clear tendency to perform better on enriched plots.

Light availability in biomass distribution

Shade accounts for more than 21% of the variability of the partition biomass data matrix. Biomass contribution of some grass species such as *Agropyron repens* and *Lolium perenne* increased in shaded plots, whereas biomass of some annual forbs such as *Kickxia spuria* and *Anagallis arvensis* increased in non-shade plots.

The effect of shading treatment on total biomass was highly significant ($p < 0.001$) and it is in line with a number of previous studies (Parker & Muller 1982; Sparks & Oechel 1993). In contrast, Shaver et al. (1998) detected no effect of shade on total biomass. This contradiction may be explained by the fact that Shaver et al. (1998) studied tundra communities which are usually limited primarily by nutrients and not by light. Our study area was a nutrient-rich old-field where nutrient limitation may be secondary to light limitation if shading is applied. In fact, theories proposed to explain the

relationship between diversity and biomass production consider light competition to be one of the main factors responsible for a diversity decrease in high productivity environments (Abrams 1995; Foster & Gross 1998). The biomass of plants established from seeds significantly decreased under shaded conditions (Table 1 and Fig. 1c). Similar results were obtained by Tilman (1987) and Huberty et al. (1998). These results may be explained as a consequence of the lower growth rate of annual seedlings under shaded conditions compared with that of perennials which usually have a higher capability to use reserves.

Water availability in biomass distribution

Surprisingly, irrigation had no significant effect on total biomass or biomass distribution (Table 1) despite water being widely considered the most relevant determinant of plant growth in a mediterranean climate (Thomas & Davis 1989; Davis & Midgley 1990). Our study area is near the border between the Mediterranean and Eurosiberian regions and has a precipitation well above mean Mediterranean (843 $mm.yr^{-1}$). A second relevant element is related to the fact that the *Agropyro-Rumicion crispi* grasslands usually exist in soils with some hydromorphy. These two features probably explain the lack of plant response in biomass terms to experimentally enhanced water supply. The very wet study year may also be at least partially responsible for this unexpected result.

Effects of plant cover and soil factors on biomass distribution

The cover matrix explained 37.7% of the total variance. Only 16.9% of the variability is explained jointly by cover and treatment matrices. Plants growing in open areas tend to maintain a constant cover/biomass ratio due to modular growth and lateral expansion (Röttgermann et al. 2000). This growth strategy leads to the maintenance of a high and relatively stable light harvesting efficiency on a per biomass basis. At this point, our results seem to support the findings of Röttgermann et al. (2000). However, the RDA forward selection procedure carried out with the cover matrix, showed that only the cover of *Sinapis arvensis* had a significant effect on species biomass distribution. The constrained model including only this variable (*Sinapis arvensis* cover) explained 11% of variance and was highly significant. These results agree with the Mass Ratio Hypothesis (Grime 1998) which predicts that a particular species may influence the community properties depending on its relative contribution to the total biomass. As suggested by Grime & Hunt (1975) and Wilson & Tilman (1991), the development of individual plants in a community may be limited by the growth of the dominant species. Thus, *Sinapis arvensis* negatively affected the development of other species. *S. arvensis* performed better in plots with high levels of nitrogen (see Paolini et al. 1999).

Soil characteristics significantly explained 41.2% of the biomass distribution. Soil and treatment matrices jointly accounted for 75.4% of the total variation. Only three variables were selected in the reduced model: clay, Mg- and Ca- content. The main expected effect of these factors is the enhancement of water retention by the soil (Jenny 1980) and decrease of P-availability, but it is uncertain how important these effects are for plant development under different intensities of competition.

Finally, the lack of a significant effect of the seed bank must be a consequence of the low seed densities on the soil surface due to the deep ploughing (3.5 seeds.100 cm^{-3}).

Concluding remarks

Biomass and species richness did not show a significant relationship in this early successional community, as found in other studies (García et al. 1993; Tilman 1993; Gough et al. 1994). Biomass distribution among species may be for the major part explained by nutrient, water and light availability. Fertilization treatments had no effect on species richness, possibly due to the initial high fertility levels of our experimental field.

Reduction of species richness in shaded plots can be related to higher mortality rates of shade-intolerant species or shade-sensitive forb seedlings (Goldberg & Miller 1990; Foster & Gross 1998). Grasses increased their contribution in shaded plots, while annual forb biomass increased in non-shaded plots.

Fertilization did favour a short life span and fast growing species. We found no increase in biomass for species that establish from seed, but we did for species with vegetative reproduction. Nutrient enrichment may favour vegetative fragments that remained in the soil after ploughing the pre-disturbance community. The Mass Ratio Hypothesis was confirmed in that species with a high biomass contribution, such as *Sinapis arvensis*, partly determined the biomass distribution among species.

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