

# Long-term effectiveness of sowing high and low diversity seed mixtures to enhance plant community development on ex-arable fields

Lepš, Jan<sup>1,2\*</sup>; Doležal, Jiri<sup>1,3</sup>; Bezemer, T. Martijn<sup>4,5,6</sup>; Brown, Valerie K.<sup>7</sup>; Hedlund, Katarina<sup>8</sup>; Igual Arroyo, Mariano<sup>9</sup>; Jörgensen, Helene Bracht<sup>8</sup>; Lawson, Clare S.<sup>7</sup>; Mortimer, Simon R.<sup>7</sup>; Peix Geldart, Alvaro<sup>9</sup>; Rodríguez Barrueco, Claudino<sup>9</sup>; Santa Regina, Ignacio<sup>9</sup>; Šmilauer, Petr<sup>1</sup> & van der Putten, Wim H.<sup>4,5</sup>

<sup>1</sup>Faculty of Biological Sciences, University of South Bohemia, Branišovská 31, CZ-37005 České Budějovice, Czech Republic;

<sup>2</sup>Institute of Entomology, Czech Academy of Sciences, Branišovská 31, CZ-37005 České Budějovice, Czech Republic;

<sup>3</sup>Institute of Botany, Section of Plant Ecology, Czech Academy of Sciences, Dukelská 135, CZ-379 82 Třeboň, Czech Republic;

<sup>4</sup>Netherlands Institute of Ecology, P.O. Box 40, 6666 ZG, Heteren, The Netherlands;

<sup>5</sup>Laboratory of Nematology, Wageningen University and Research Centre, P.O. Box 8123, 6700 ES Wageningen, The Netherlands;

<sup>6</sup>Laboratory of Entomology, Wageningen University and Research Centre, P.O. Box 8031, 6700 EH Wageningen, The Netherlands;

<sup>7</sup>Centre for Agri-Environmental Research (CAER), University of Reading, P.O.Box 237, Reading RG6 6AR, UK;

<sup>8</sup>Department of Ecology, Lund University, SE-223 62 Lund, Sweden; <sup>9</sup>Instituto de Recursos Naturales y Agrobiología CSIC, ES-37071 Salamanca, Spain; \*Corresponding author; E-mail [suspa@bf.jcu.cz](mailto:suspa@bf.jcu.cz); URL <http://botanika.bf.jcu.cz/suspa/>

## Abstract

**Questions:** How is succession on ex-arable land affected by sowing high and low diversity mixtures of grassland species as compared to natural succession? How long do effects persist?

**Location:** Experimental plots installed in the Czech Republic, The Netherlands, Spain, Sweden and the United Kingdom.

**Methods:** The experiment was established on ex-arable land, with five blocks, each containing three 10 m × 10 m experimental plots: natural colonization, a low- (four species) and high-diversity (15 species) seed mixture. Species composition and biomass was followed for eight years.

**Results:** The sown plants considerably affected the whole successional pathway and the effects persisted during the whole eight year period. Whilst the proportion of sown species (characterized by their cover) increased during the study period, the number of sown species started to decrease from the third season onwards. Sowing caused suppression of natural colonizing species, and the sown plots had more biomass. These effects were on average larger in the high diversity mixtures. However, the low diversity replicate sown with the mixture that produced the largest biomass or largest suppression of natural colonizers fell within the range recorded at the five replicates of the high diversity plots. The natural colonization plots usually had the highest total species richness and lowest productivity at the end of the observation period.

**Conclusions:** The effect of sowing demonstrated dispersal limitation as a factor controlling the rate of early secondary succession. Diversity was important primarily for its ‘insurance effect’: the high diversity mixtures were always able to compensate for the failure of some species.

**Keywords:** Diversity-function relationship; Grassland restoration; Old-field succession; Sampling effect.

**Abbreviations:** ED = Euclidian distance; HD = High diversity; LD = Low diversity; NC = Natural colonization.

## Introduction

Recent agricultural overproduction and price developments on the world market have resulted in the withdrawal of many arable fields from production in many industrialized countries. Abandoned fields then undergo ‘old field succession’. This process has been frequently studied, becoming a model for community ecological studies (e.g. Rejmánek 1990). Since the dynamics of secondary succession are much faster than those of primary succession, it has enabled experimental testing of various theories on dispersal, colonization and establishment (e.g. Armesto & Pickett 1986). Therefore, studying old field succession has both fundamental and applied value (Prach 2003). However, the predictability of old field succession and of the effects of various management strategies still needs improvement. Recent works suggest that changes in species trait composition during succession can be better predicted than species composition itself (Prach et al. 1997; Fukami et al. 2005).

Site managers attempt to direct the successional pathway towards stages of plant succession that conform to their reference or target community (Pakeman et al. 2002). For various reasons, meadows or pastures, i.e. grasslands kept open by human intervention are often considered more desirable than woodlands. As grasslands in Europe are often a source of biodiversity, their great species richness is one of the goals of restoration projects. Various incentive schemes, directives and rules for subsidies are used throughout Europe to encourage farmers to promote restoration of species-rich grasslands. These measures vary from agri-environment schemes,

where farmers get paid for ‘producing biodiversity’ to ecosystem restoration through land abandonment, where farmers are bought out to re-create former natural areas. The aim of most restoration projects is not to enhance diversity *per se*, but rather the presence and abundance of species that are characteristic of reference or target community. There can be two (not mutually exclusive) goals when sowing grassland species into abandoned fields – firstly to suppress potentially noxious arable weeds (Lawson et al. 2004) and, second to increase the diversity of grassland species that are specific to the reference or target systems.

It is commonly known for long time that secondary succession is affected by the initial presence of propagules (Egler 1954). Consequently, addition of propagules by sowing is an important management tool to affect the course of succession. When grasslands are created by sowing species-rich mixtures, it is important these grasslands maintain their high diversity – if not, sowing low diversity mixtures would be in most cases less expensive (see discussion of cost-effectiveness in Pakeman et al. 2002).

Here we describe how on abandoned arable land, sowing high (15 species) or low (four species) diverse seed mixtures influenced natural plant succession for a period of eight years. After sowing, the plots were not weeded but only mown once a year. As local abiotic and biotic conditions will be important in determining the impact of sowing on vegetation development, we replicated the experiment in five different environments by establishing the experiment in Northern, Southern, Atlantic and Continental countries in Europe.

A number of rather short-term experiments (including reports on the first three years of our experiment, van der Putten et al. 2000; Lepš et al. 2001; Hedlund et al. 2003) have indicated that, on average, species-rich mixtures are more productive and also more effective in suppression of weed species and preventing further colonization than species-poor mixtures (e.g. van der Putten et al. 2000; Naeem et al. 2000; Bullock et al. 2001), with some low diversity mixtures being as productive and as effective as the high diversity mixtures; those successful low diversity mixtures are usually composed of species dominating the high diversity mixtures (Lepš et al. 2001). Many experiments studying the effects of sowing species mixtures on vegetation development have been carried out for a limited period time, and some were accompanied by weeding (e.g. Hector et al. 1999). Whilst weeding is a useful tool to keep restricted sets of species in experimental plots, it is obviously not a feasible type of management practice and to allow weeds to become established after some years of hand-weeding completely misses out the effects pioneer plant species on plant community development after land abandonment.

It is also difficult to extrapolate the long-term effects of sowing from short-term experiments. For example, the chalk-grassland experiment of Pakeman et al. (2002) indicates that the system changes even 25 years after establishment. Moreover, some short-term ‘biodiversity’ effects disappeared after cessation of weeding (Phisterer et al. 2004). Therefore, we analysed and present effects of the sowing treatments and report on eight years of community development.

We address four questions: 1. How successful is the establishment of sown grassland species in ex-arable land and how long do the sown species persist at the site; 2. How does sowing of low- and high-diversity seed mixtures affect the naturally-colonizing plants; 3. How does sowing affect total species richness of the communities in a long-term; 4. How does sowing low- and high-diversity seed mixtures affect above-ground biomass production?

As species diversity has an important spatial component (e.g. Lepš & Štursa 1989), we analysed changes both at a small subplot scale (1 m<sup>2</sup>) and at a whole-plot scale (100 m<sup>2</sup>).

## Methods

### *Design of the experiment*

The experiment was established in five European countries: Czech Republic, CZ; The Netherlands, NL; Spain, ES; Sweden, SE; United Kingdom, UK). The basic site description is in Table 1, details are found in van der Putten et al. (2000) and Hedlund et al. (2003). At each site, the experiment was set-up with five randomized complete blocks. Each block consisted of three treatments, applied in 10 m × 10 m plots: NC: natural colonization (no species sown), LD: low diversity species mixture sown, HD: high diversity species mixture sown. The plots were separated by 2 m wide border rows. The HD plots were sown with a mixture of 15 species (Table 2): five grasses, five legumes, five non-legume forbs (referred to henceforth as ‘forbs’), and the composition was the same in all five blocks within a site.

In all countries species were selected that naturally occur in the grassland systems under investigation. In designing the experiment, we aimed at covering a wide range of environmental condition, with an experimental design as similar as possible among the five countries. Therefore a number of species were sown at all sites; two grasses (*Festuca rubra* and *Phleum pratense*), two legumes (*Lotus corniculatus* and *Trifolium pratense*) and one forb (*Plantago lanceolata*). The LD plots were composed of a split-set of the species sown in the HD plots: two grasses, one legume and one forb, so that each

**Table 1.** Characteristics of experimental sites in five European countries.

Country	Czech Republic	The Netherlands	Sweden	Spain	United Kingdom
Abbreviation	CZ	NL	SE	SE	UK
Site	Benesov	Mossel	Trolleholm	Muñovela	Bradenham
Co-ordinates	49°20' N, 15°00' E	52°04' N, 13°15' E	55°45' N, 13°15' E	40°54' N, 5°45' W	51°40' N, 0°48' W
Altitude (m a.s.l.)	659	30	85	840	140
Mean temperature (°C)	6.4	9.6	7.5	10.8	9.6
Warmest month (°C)	July (16.4)	July (17.0)	July (17.1)	August (29.9)	July (16.5)
Coldest month (°C)	January (-2.7)	January (2.4)	January (-0.9)	January (0.8)	January (3.6)
Average rainfall (mm.a <sup>-1</sup> )	680	820	700	500	750

grass species used in the HD treatment was sown in two LD blocks, while each legume and forb from the HD treatment was sown in one of the blocks. Total number of seeds was the same in LD and HD plots: grasses 2500 seeds/m<sup>2</sup> (divided equally among two species in LD and among five species in HD), legumes and forbs 500 seeds/m<sup>2</sup> each (single species of each group in LD, divided equally among five species of each group in HD). In the experiment, the NC treatment simulated undirected succession. However, since plots with sown vegetation produced (sometimes large quantities of) seeds, the development of vegetation in NC plots, particularly in the latter years, has been partly influenced by the adjacent sown plots. Plots were mown annually, exceptionally (in CZ) twice a year. Time of mowing was adjusted to local conditions and practices. In CZ, it was necessary to start mowing relatively early (June), and, with exception of dry years, to repeat mowing in autumn, otherwise the biomass started to rot. Similarly, in Spain, the plots were mown in June. In the other countries, plots were mown at the end of the season (August to September in SE, September to October in NL and UK).

#### *Data recording and analyses*

Within each main plot (10 m × 10 m), the cover of all the species was visually estimated in 12 permanent subplots (1 m × 1 m). In some countries, the cover was estimated using a simplified scale, for these, the centres of the cover classes are then used in calculations. Characteristics derived from the compound sample, i.e. pooled for the 12 permanent quadrats are further called whole-plot characteristics, characteristics of the 1-m<sup>2</sup> plots are called subplot characteristics.

For each plot the following characteristics were recorded every year: Whole-plot species richness, and average richness per subplot. This number was then separated into number of sown species and natural colonizers (i.e. all the species found in the plot other than the 15 species introduced to the site). The 15 species introduced to the site were not included in the list of natural colonizers, even in plots where they were not sown.

This is because in plots in which the species was sown, we are not able to exclude the possibility that the species also invaded the plot from other sources. In the case of sown species in LD plots, we distinguished between total number of sown species present, and number of species sown in that particular LD plot. This is important, because some of the sown species invaded into plots in which they were not sown. Furthermore, to estimate the success of sowing we calculated the total cover of sown species as a proportion of total cover of all species combined. Biomass was estimated using 12 (exceptionally 6) 0.25 m × 0.25 m plots in each 10 m × 10 m plot. The above-ground biomass was harvested at peak standing biomass, oven-dried and weighed.

The data were analysed by repeated measures ANOVA. In the model, treatment (NC, LD, HD) was the fixed factor, country, and block nested within country were the random factors, and year the repeated measures factor. The univariate repeated measurement model was calculated using the Statistica 5.5, while graphs were created using Statistica 7. Error terms used in individual tests are shown in Table 3. Because only years 1996 to 1998, and 2002 and 2003 were complete for all the countries, we used only those for the ANOVA based on all sites. However, all the available years are presented in graphs for individual countries.

Although we present results for all countries, treatments and years and their full interactions, the ecologically most interesting tests are for the effect of treatment, and the interaction between treatment and time. Significant effects of country and its interactions would show differences among countries. Significant effects of treatment and treatment by time interactions would show that there is a consistent effect of treatment, either constant over years (treatment), or variable over the years (treatment \* year interaction). Because 'country' is a random factor (and accordingly, the corresponding interactions with country are used as error MS for treatment and its interactions), the effects can be generalized, also outside the five experimental plots, irrespective of whether the effect of country or its interactions is significant or not. To test the effect of sowing different

**Table 2.** The performance of individual species in the HD (high-diversity) plots in individual countries. F96 = frequency of the species in 1996, i.e. the percentage of subplots in HD, in which the species was found. MC = Maximum cover attained by the species, with serial number of the year, when the maximum cover was attained in parentheses. F03 = species frequency in 2003, i.e. at the end of the experiment. AC03 = mean (Average) cover of the species in 2003. Numbers in bold signify the 'best' LD (low-diversity) species combinations in 2003. Numbers in bold in the F03 column signify the LD combination attaining the highest total biomass, bold in the AC03 the combination with highest proportion of sown species. Nomenclature follows Tutin et al. (1964–1980).

Species	CZ			NL			SE			ES			UK		
	F96	MC	F03	AC03	F96	MC	F03	AC03	F96	MC	F03	AC03	F96	MC	F03
<b>Grasses</b>															
<i>Agrostis capillaris</i>	-	-	-	-	98.3*	6.2 (2)	21.7	<b>0.5</b>	0	3.7 (6)	3.3	0.05	-	-	-
<i>Anthoxanthum odoratum</i>	-	-	-	-	98.3*	6.8 (2)	26.7	0.5	0	0	0	0	-	-	-
<i>Bromus inermis</i>	-	-	-	-	-	-	-	-	53.3	24.2 (7)	100	<b>14</b>	-	-	-
<i>Cynosurus cristatus</i>	98.3	6.3 (2)	<b>23.3</b>	0.5	-	-	-	-	0	0.01 (3)	0	0	98.3*	1.1 (4)	20
<i>Festuca rubra</i>	100	5.4 (5)	56.7	<b>2.7</b>	98.3*	61.5 (5)	<b>100</b>	43.3	0	22.3 (8)	<b>95</b>	<b>22.3</b>	96.7	84.8 (7)	<b>100</b>
<i>Holcus lanatus</i>	98.3	8.3 (2)	31.6	0.9	-	-	-	-	-	-	-	-	26.7	2.8 (4)	56.7
<i>Phleum pratense</i>	78.3	5.3 (3)	26.7	0.7	98.3*	9.9 (2)	<b>58.3</b>	<b>1.6</b>	-	0.5 (6)	<b>3.3</b>	0.03	86.7	4.6 (3)	<b>41.7</b>
<i>Poa pratensis</i>	-	-	-	-	1.7	6.6 (5)	86.7	4.3	-	-	-	-	-	-	1.1
<i>Poa trivialis</i>	-	-	-	-	-	-	-	-	1.7	0.03 (2)	<b>0</b>	0	-	-	-
<i>Trisetum flavescens</i>	98.3	76 (4)	<b>100</b>	<b>52</b>	-	-	-	-	-	-	-	-	43.3	10.4 (3)	<b>5.1</b>
<b>Forbs</b>															
<i>Achillea millefolium</i>	-	-	-	-	-	-	-	-	1.7	1.8 (7)	16.6	1.6	-	-	-
<i>Campanula rotundifolia</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Centaurea jacea</i>	20	2.6 (6)	20	1.02	-	-	-	-	-	-	-	-	100	22.7 (8)	100
<i>Centaurea nigra</i>	-	-	-	-	-	-	-	-	-	-	-	-	100	15.9 (8)	100
<i>Gadium verum</i>	5	0.02 (8)	1.7	0.02	-	-	-	-	0	0.7 (8)	5	0.7	0	0	0
<i>Hypericum perforatum</i>	-	-	-	3.3*	4.2 (5)	50	3	-	-	-	-	-	-	-	-
<i>Hypochaeris radicata</i>	-	-	-	-	100	5.6 (1)	1.7	0.06	-	-	-	-	-	-	-
<i>Leontodon hispidus</i>	-	-	-	-	11.7	11.5 (8)	100	<b>11.5</b>	0	0.12 (6)	1.7	0.02	31.7	2.1 (4)	50
<i>Linaria vulgaris</i>	0	0	0	0	-	-	-	-	-	-	-	-	-	-	-
<i>Lychnis flos-cuculi</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Matricaria chamomilla</i>	-	-	-	-	-	-	-	-	3.3	0.1 (1)	0	0	-	-	-
<i>Plantago lanceolata</i>	96.7	7.7 (1)	35	1.4	100	20.6 (2)	<b>71.7</b>	2.4	98.3	29.6 (2)	16.6	0.4	100	28.2 (3)	<b>98.3</b>
<i>Prunella vulgaris</i>	93.3	5.2 (6)	<b>20</b>	0.4	-	-	-	-	1.7*	0.96 (7)	8.3	0.3	-	-	-
<i>Sanguisorba minor</i>	-	-	-	-	-	-	-	-	88.3	11.7 (7)	60	<b>8.4</b>	100	13.1 (2)	61.7
<i>Tanacetum vulgare</i>	-	-	-	-	1.7	15.4 (8)	53.3	15.4	-	-	-	-	-	-	-
<b>Legumes</b>															
<i>Anthyllus vulneraria</i>	-	-	-	-	-	-	-	-	96.7	2.3 (1)	3.3	0.03	100	11.1 (3)	20
<i>Lathyrus pratensis</i>	93.3	53 (8)	<b>100</b>	<b>53</b>	-	-	-	-	-	-	-	-	-	-	-
<i>Lous corniculatus</i>	100	27.6 (5)	100	19	95	67.1 (3)	<b>98.3</b>	39.3	100	26 (6)	<b>43.3</b>	3.8	100	32.2 (8)	<b>100</b>
<i>Medicago lupulina</i>	55	0.5 (1)	<b>0</b>	-	-	-	-	-	81.7	0.64 (1)	0	<b>0</b>	100	56.8 (2)	88.3
<i>Trifolium arvense</i>	-	-	-	-	96.7	12 (2)	3.3	<b>0.12</b>	-	-	-	-	-	-	-
<i>Trifolium dubium</i>	76.7	3.4 (6)	0	0	45	0.2 (1)	0	0	-	-	-	-	55	1.2 (2)	6.7
<i>Trifolium fragiferum</i>	-	-	-	-	-	-	-	-	38.3	0.9 (3)	6.6	0.2	-	-	-
<i>Trifolium pratense</i>	93.3	48.1 (2)	8.3	0.1	46.7	4.7 (2)	5	0.2	100	73.1 (2)	0	0	3.3	1 (8)	40
<i>Trifolium repens</i>	-	-	-	-	-	-	-	-	100	6.6 (1)	0	0	-	-	-
<i>Trifolium subterraneum</i>	-	-	-	-	-	-	-	-	80.0	3.2 (1)	<b>0</b>	0	-	-	-
<i>Vicia cracca</i>	-	-	-	-	98.3	41.3 (1)	15	0.57	-	-	-	-	-	-	-

**Table 3.** Results of Repeated measurement ANOVA (*P*-values) for number of sown species (Sown sp.), number of unsown, i.e. colonizing species (Colon.), total number of plant species (All sp.), proportion of the sown species calculated on the basis of cover (prop. sown), plot heterogeneity characterized by average of standardized Euclidean distances (ED) and aboveground biomass. Number of species applies for the pooled sample of 12 quadrats, 1m<sup>2</sup> each, in the whole-plots (W after the name) and in subplots (averaged over the 12 plots, S after the name). The ANOVA model included three main plot factors (Country, Treatment, and Block (B) nested in Country), Country and Block being the random factors, and the repeated measures factor, Year. Factors in the error term and presented as the first letter of the factor name. Five years, for which the complete data are available, are used in the analysis. The last row of each subtable presents the analysis of the final year (2003). Only the treatment effect is shown. Significant terms (i.e. *P* < 0.05) are shown in bold. **a.** Complete data set; **b.** Comparison of LD and HD plots only.

Effect	df Effect	Error	df Error	Sown sp. W	Sown sp. S	Colon. W	Colon. S	All sp. W	All sp. S	prop. sown	ED	Biomass
<b>a.</b>												
Country (C)	4	B	20	<b>0.0000</b>	<b>0.0000</b>	<b>0.0000</b>	<b>0.0002</b>	<b>0.0000</b>	<b>0.0000</b>	<b>0.0000</b>	<b>0.0000</b>	<b>0.0000</b>
Treatment (T)	2	C × T	8	<b>0.0002</b>	<b>0.0004</b>	<b>0.0016</b>	<b>0.0008</b>	0.1687	0.1105	<b>0.0000</b>	0.1218	<b>0.0144</b>
Year (Y)	4	C × Y	16	0.4050	0.9314	0.2776	<b>0.0134</b>	0.4003	<b>0.0152</b>	<b>0.0096</b>	0.6163	<b>0.0103</b>
C × T	8	B × T	40	<b>0.0000</b>	<b>0.0000</b>	<b>0.0024</b>	<b>0.0001</b>	<b>0.0058</b>	<b>0.0000</b>	<b>0.0003</b>	<b>0.0000</b>	<b>0.0001</b>
C × Y	16	B × Y	80	<b>0.0000</b>	<b>0.0000</b>	<b>0.0000</b>	<b>0.0000</b>	<b>0.0000</b>	<b>0.0000</b>	<b>0.0000</b>	<b>0.0000</b>	<b>0.0000</b>
T × Y	8	C × B × Y	32	<b>0.0000</b>	<b>0.0000</b>	<b>0.0002</b>	<b>0.0000</b>	<b>0.0000</b>	<b>0.0000</b>	<b>0.0000</b>	0.1035	<b>0.0036</b>
C × T × Y	32	B × T × Y	160	<b>0.0000</b>	<b>0.0000</b>	<b>0.0003</b>	<b>0.0000</b>	<b>0.0003</b>	<b>0.0000</b>	<b>0.0000</b>	<b>0.0044</b>	<b>0.0333</b>
Final Y: Treatment	2	C × T	8	<b>0.0155</b>	<b>0.0059</b>	<b>0.0284</b>	<b>0.0233</b>	0.1342	0.2828	<b>0.0005</b>	<b>0.0048</b>	<b>0.0145</b>
<b>b.</b>												
Country (C)	4	B	20	<b>0.0000</b>	<b>0.0000</b>	<b>0.0004</b>	<b>0.0001</b>	<b>0.0008</b>	<b>0.0000</b>	<b>0.0000</b>	<b>0.0000</b>	<b>0.0001</b>
Treatment (T)	1	C × T	4	<b>0.0047</b>	<b>0.0090</b>	0.0576	<b>0.0286</b>	0.1471	<b>0.0290</b>	<b>0.0224</b>	0.6392	0.1011
Year (Y)	4	C × Y	16	0.8657	0.1013	0.0841	<b>0.0015</b>	0.0894	<b>0.0009</b>	<b>0.0019</b>	0.4986	<b>0.0031</b>
C × T	4	B × T	20	<b>0.0001</b>	<b>0.0000</b>	0.1075	0.1500	0.3529	<b>0.0066</b>	0.1019	0.2998	<b>0.0083</b>
C × Y	16	B × Y	80	<b>0.0000</b>	<b>0.0000</b>	<b>0.0000</b>	<b>0.0000</b>	<b>0.0000</b>	<b>0.0000</b>	<b>0.0000</b>	<b>0.0000</b>	<b>0.0000</b>
T × Y	4	C × B × Y	16	<b>0.0002</b>	<b>0.0002</b>	<b>0.0087</b>	<b>0.0410</b>	<b>0.0011</b>	<b>0.0050</b>	0.4248	0.6806	<b>0.0277</b>
C × T × Y	16	B × T × Y	80	<b>0.0050</b>	<b>0.0000</b>	0.2755	0.1799	0.3757	<b>0.0001</b>	<b>0.0226</b>	0.0730	0.3420
Final Y: Treatment	1	C × T	4	<b>0.0059</b>	<b>0.0126</b>	0.1328	0.1108	0.5252	0.1361	<b>0.0048</b>	0.5011	<b>0.0470</b>

numbers of species (same number of seeds of the three functional groups, but differing in the diversity within the functional groups), an ANOVA comparing LD and HD plots was also carried out. Since in LD plots, sown species composition differed among the replicates, we also compared the performance of the ‘best’ LD plot (i.e. the plot showing maximum proportion of sown species, maximum suppression of natural colonizers, etc.) with the HD treatment. Therefore in all graphs for individual countries, the error bars show the range of values observed, i.e. minimum and maximum values of the five main replicate plots in each country. For subplots, data were first averaged within main plot, and the main plot average minimum and maximum displayed. As the country and country by treatment effects were mostly significant, we also calculated repeated measures ANOVA for each country separately. This enables the use of all the years where data were collected in individual countries, and also show the treatments effects in individual countries. Further, to analyse which effects persisted until the end of experiment, we analysed the final year (2003) separately. The main treatment effect in Repeated measures ANOVA characterizes the average over the whole period, the treatment effect in 2003 characterizes that specific year only.

The heterogeneity of the vegetation might be an important factor increasing the richness of vegetation at the scale of the whole-plot. We have characterized heterogeneity by taking the average of standardized Euclidean distance (chord distance) between all pairs of subplots within a whole-plot. Chord distance is classical ED calculated after standardization by sample norm,

$$\sqrt{\sum_i x_i^2} \quad (1)$$

i.e. after each value is divided by the norm of the vector characterizing the community where  $x_i$  is representation of  $i$ -th species in the sample (see Lepš & Šmilauer 2003).

## Results

The behaviour of individual species is best characterized by their performance in the HD plots (Table 2). In Table 2, we also indicate the ‘best’ LD plots, selected according to two criteria: either as the combination attaining the highest biomass, or the combination with the



highest proportion of sown species, both at the end of the experiment (2003). Surprisingly, the best combinations based on those two criteria differed between countries (although the differences were small). In three countries, the two LD combinations shared the dominant grass species (*Trisetum flavescens* in CZ, *Festuca rubra* in SE and UK). In NL, the most productive LD combination also contained *Festuca rubra* (which was the most successful grass there). In ES, the most successful grass was *Bromus inermis*, and was present in the LD combination with highest proportion of sown species. In ES, very few sown species persisted, and the biomass in the last year was formed mostly by natural colonizers. In three countries, NL, SE, and UK, the most productive combination was composed of *Festuca rubra*, *Phleum pratense*, *Lotus corniculatus*, and *Plantago lanceolata*; this combination was reasonably successful also in CZ. This combination, containing the species widespread over the Europe, was the only LD combination identical in all the sites. In CZ, the one strong dominant species (*Trisetum flavescens*) was sufficient to create a highly productive LD mixture, regardless of the other species of the combination not being very successful (*Medicago lupulina* became extinct, *Cynosurus cristatus* and *Prunella vulgaris* had negligible cover in the last year).

The behaviour of individual species differed considerably among individual countries, but even so, some common features were observed. With the exception of SE, grasses established well, and the sown grasses have determined the community matrix till the end of experiment – *Festuca rubra* dominated in NL, SE, and UK, *Trisetum flavescens* in CZ (but also persisted well in the UK, the only other country where it was sown). This species also spread very well to plots where it was not sown. *Phleum pratense*, the other grass species sown in all countries, did not become dominant in any of the countries. Of the non-legumes, *Plantago lanceolata* established well in all countries, and was also quite persistent. Both sown legumes common to all sites, *Lotus corniculatus* and *Trifolium pratense* established well (with exception of *T. pratense* in UK) but only *Lotus* persisted well throughout the whole experimental period. The tiny leguminous species, *Medicago lupulina* and *Trifolium dubium*, disappeared quickly with exception of the UK site.

The results of the repeated measures ANOVAs of community characteristics are shown in Table 3, results for individual countries are presented in the electronic App. 1. The second order interaction Country  $\times$  Treatment  $\times$  Year was highly significant for all variables studied, indicating that vegetation development in individual countries has highly specific features. However, there was also a repeatable pattern in the dynamic response to treatments common to all sites, indicated by the Treat-

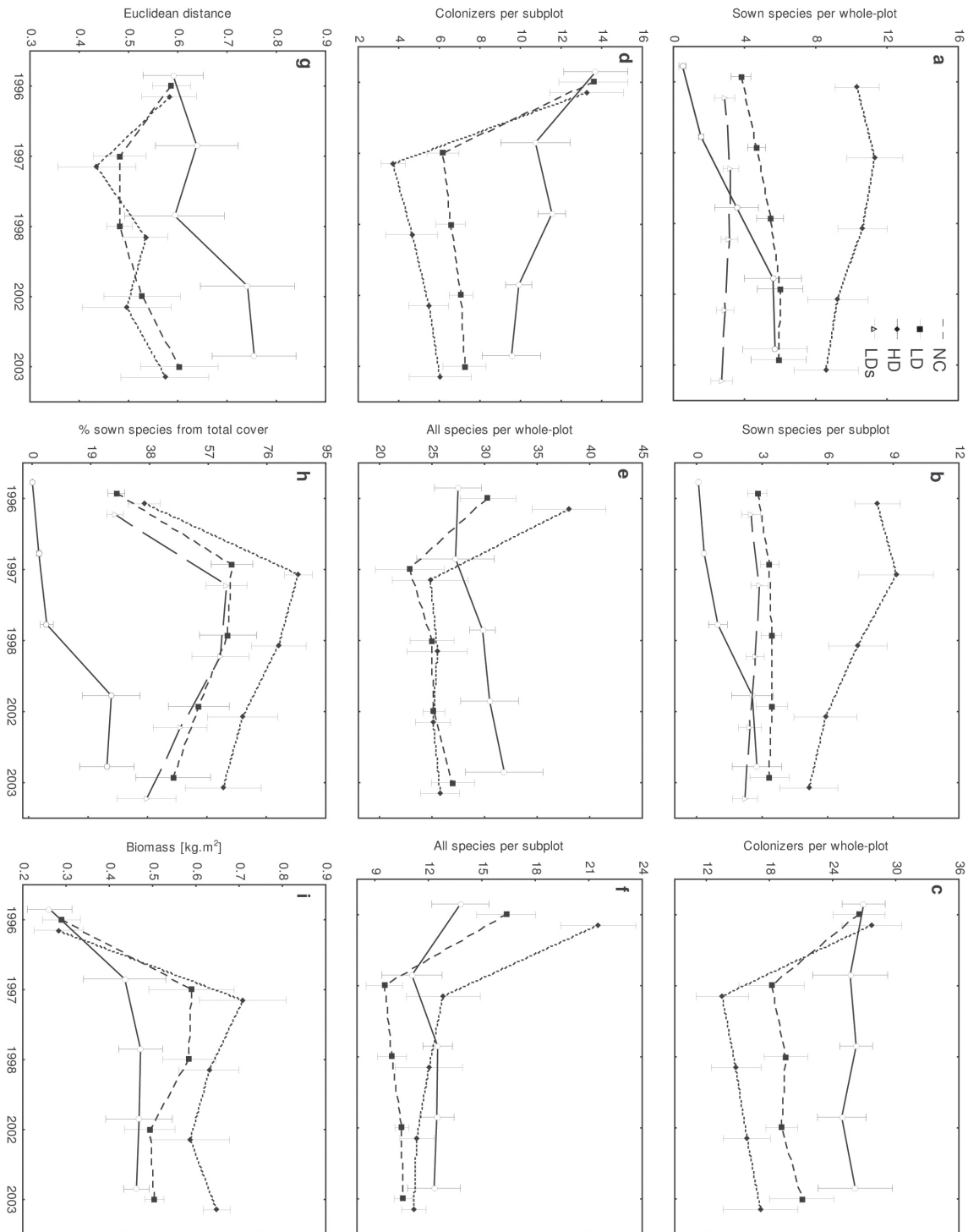
ment  $\times$  Year interaction, tested against the Country  $\times$  Treatment  $\times$  Year interaction, which was also highly significant (with exception of ED, i.e. heterogeneity). When comparing the LD and HD plots, the Treatment  $\times$  Year interaction was significant for most variables tested in the repeated measures ANOVA with the exception of heterogeneity, and proportion of sown species (Table 3b). However, in the latter case, the main treatment effect was significant, showing that there are consistent differences in the treatments over the whole experimental period. Also, the treatment effect in the last year was significant for most of the variables (with exception of total number of species, but including ED).

Most of the sown species established well at all locations but SE. In UK and NL, many species were recorded only from the second season onwards. In SE, less than half of the number of species that were sown established. At the other locations, during the first or second season, the number of sown species recorded in HD whole-plots was at least 11 but often close to 15 (Fig. 1a, Fig. 2). The number of sown species in subplots was also high (Fig. 1b, App. 1), showing that species established throughout the plots. At most locations, in HD plots the number of sown species decreased from the second season onwards. This decrease was more pronounced in subplots than in whole-plots. At three locations (CZ, NL, UK), even after 8 years, more than 70 percent of the sown species survived at the whole-plot level (Fig. 2). Species survived even better in the LD plots. During the studied period both the LD and NC plots were colonized step by step from adjacent HD and LD plots – consequently, the number of sown species in LD plots increased when all the sown species were taken into account – nevertheless, the survival of species sown in individual LD plot was generally good in CZ, NL, and UK. Despite the extinction of some sown species, and colonization from adjacent plots, the number of sown species was still highest in HD and lowest in NC at the end of experiment.

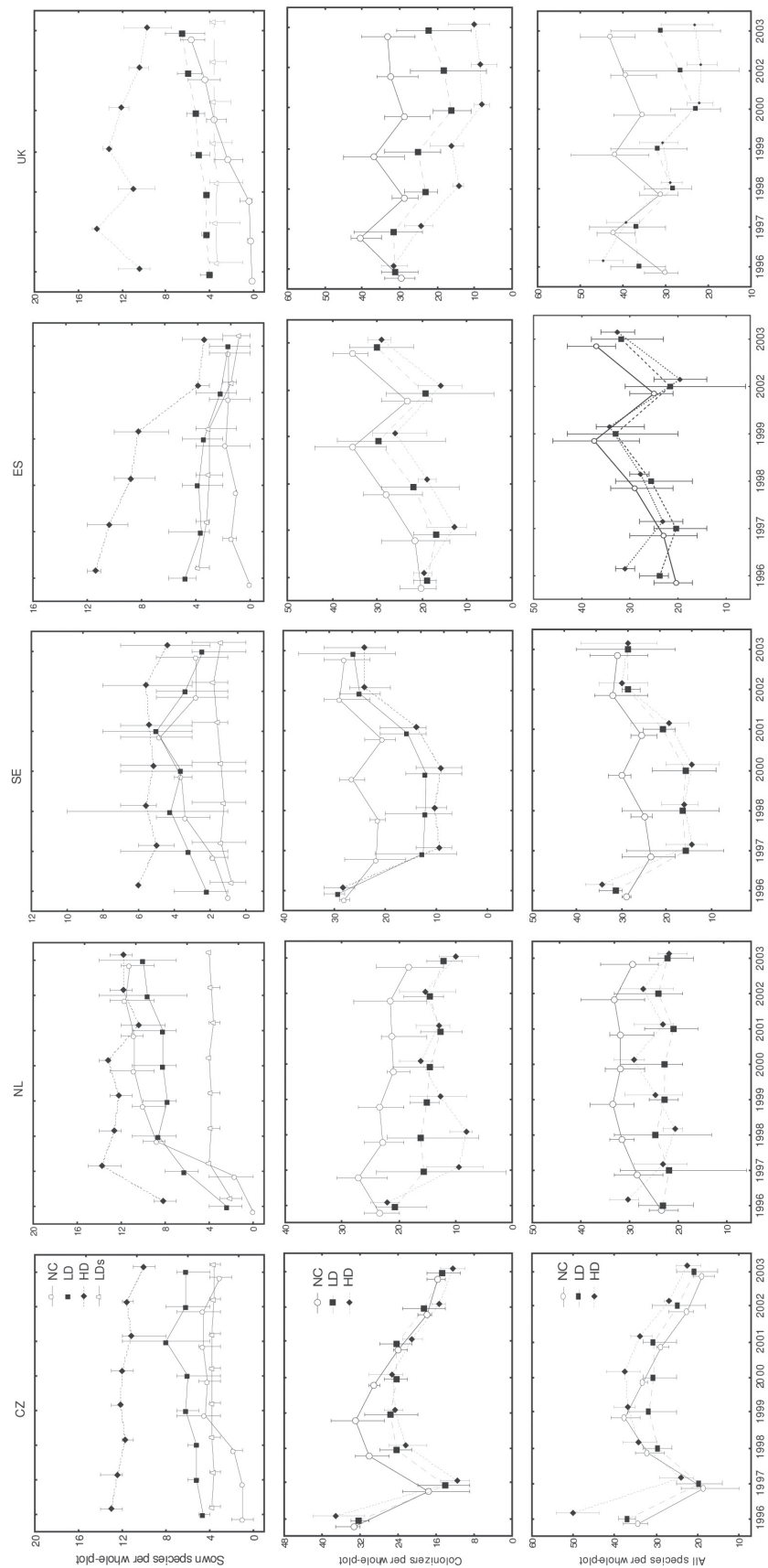
During the first season, sowing of species did not appear to affect the number of naturally colonizing plant species, neither at the scale of the whole-plot, or subplot (Fig. 1c, d, Fig. 2). The development of the number of natural colonizers then differed between locations (App. 1), however, there are some common features in all the countries.

Starting from the second (third in CZ) season, the number of colonizers started to differentiate according to the treatments, being on average lowest in the HD and highest in the NC plots. This effect was consistent and appeared in all countries (App. 2).

The differentiation was more pronounced at the scale of subplots rather than whole-plots, and was caused mainly by the decrease in the number of colonizing species in plots with sown species, which was most pro-

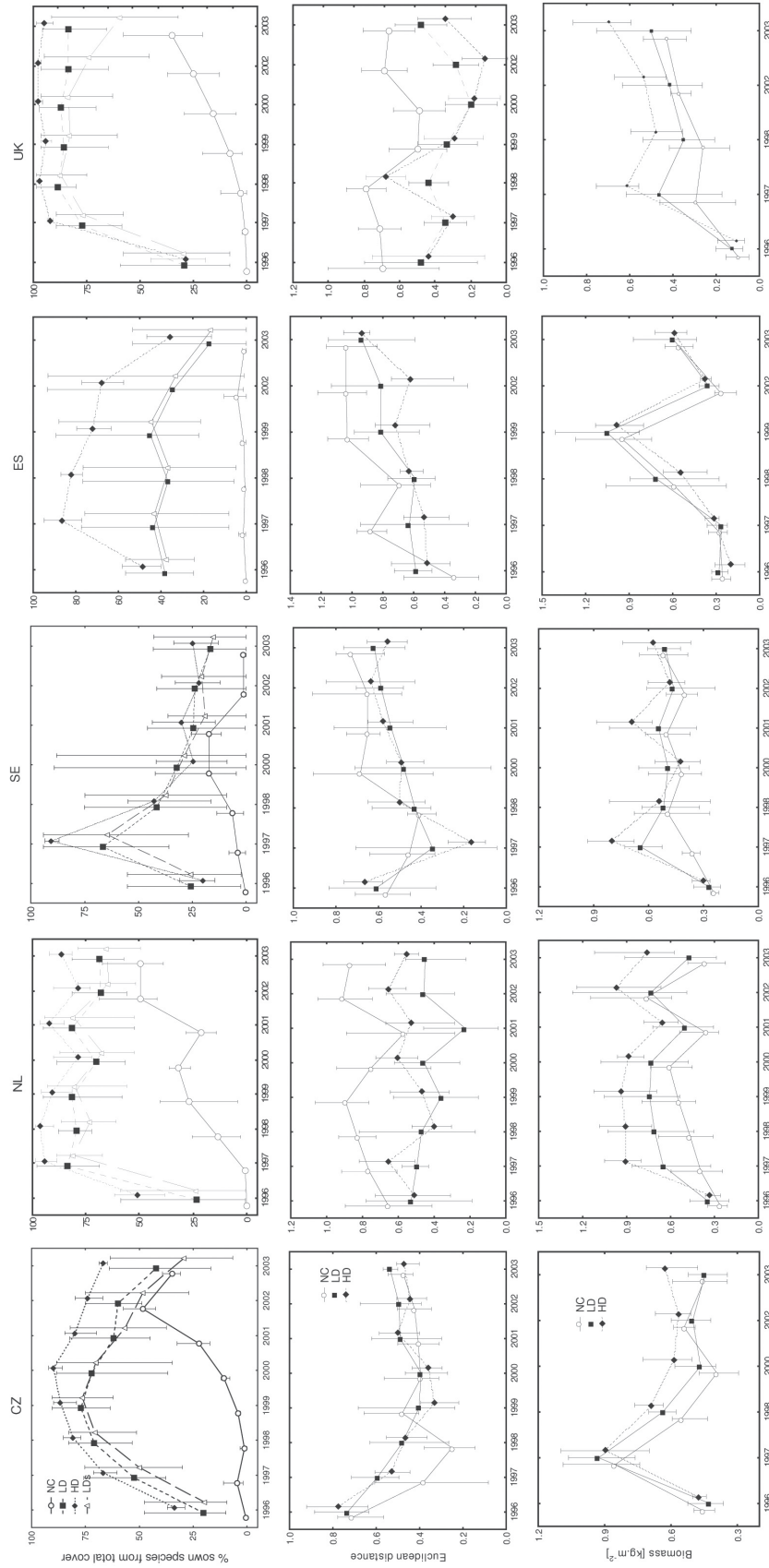


**Fig. 1.** Mean values of community characteristics averaged over the five countries for natural colonization (NC) and for sowing later-successional plant species in low-diversity (LD) and high-diversity (HD) treatments. LD plots are characterized by total number or proportions of all sown species (LD), and of species sown in respective plot (LDs) when applicable. The error bars are standard errors calculated from the variance among countries. The following characteristics are displayed: **a.** Number of sown species per whole-plot; **b.** Number of sown species per subplot; **c.** Number of colonizers per whole-plot; **d.** Number of colonizers per subplot; **e.** Number of all species per whole-plot; **f.** Number of all species per subplot; **g.** Heterogeneity characterized by mean standardized Euclidean distance among subplots; **h.** Proportion of sown species from total cover; **i.** Biomass. Results of repeated measure ANOVA are in Table 3.



**Fig. 2.** Changes in mean number of colonizers and total number of all species per whole-plot for natural colonization (NC) and for sowing later-successional plant species in low-diversity (LD) and high-diversity (HD) treatments. For sown species, LD plots are characterized by total number of all sown species (LD), and number of species sown in respective plot (LDs). Error bars extend between minimum and maximum value of the five blocks. Results of repeated measure ANOVA for individual countries are in App. 1.





**Fig. 3.** Changes in percentage of sown species from total cover, plot heterogeneity characterized by mean standardized Euclidean distance among subplots, and mean biomass for natural colonization (NC) and for sowing later-successional plant species in low-diversity (LD) and high-diversity (HD) treatments. For percentage of sown species, LD plots are characterized by proportion of all sown species (LD), and proportion of species sown in respective plot (LDs). Error bars extend between minimum and maximum value of the five blocks. Results of repeated measure ANOVA for individual countries are in App. 1.

nounced in HD plots. Nevertheless, the decrease in the number of colonizing species in the 'most suppressing LD mixture' was as great and sometimes even greater (ES) than in the average (and sometimes even than in the best) HD replicate. This corresponds well to the proportion of sown species in the mixture (Fig. 1h, Fig. 3), which is on average higher in HD than in LD (again, till the end of experiment), but the proportion of sown species in the best LD mixture is as high as the average HD mixture, and sometimes even higher than the best HD mixture. Also note that in all sites over all the years, the proportion of sown species in the best LD mixture is nearly the same for species sown in this mixture and all the sown species (Fig. 3). This shows that the successful LD species combinations do not leave space for further colonization. As shown in our previous paper (Lepš et al. 2001), during the first years, the successful LD mixture usually consisted of species becoming dominant in the HD mixtures; this continued till the end of period studied. In CZ, there was strong decrease in the number of colonizing species in the second year, in all the treatments. This was caused by a fast spread of *Trifolium repens*, a species that was not sown, but dominated all plots during the second year. The development of the number of colonizing species then became rather idiosyncratic with different responses at different locations (Fig. 2, App. 1). Even so, the number of colonizing species tends to be highest in the NC and lowest in the HD treatment, irrespective of whether sub- or whole-plots are considered and during most of the period studied. At the end of the experiment, the suppressing effect of sowing was still significant, but the difference between LD and HD was not.

The changes in the total number of species are obviously the combination of changes in richness of the two groups (sown and unsown species) and despite some common trends (Fig. 1e, f) differ considerably among locations (Fig. 2, App. 1). At the very beginning, in 1996, the number of colonizers was not affected by sowing and the total number of species (colonizers and sown species) was as a result of the sowing, highest in HD, intermediate in LD and lowest in NC. From the second season (1997) onwards, however, in all countries except CZ the sown communities started to decrease the number of non-sown species, so that as a result the NC plots contained the highest number of species (Fig. 1e, f). Sowing also appeared to homogenize the plant community, so that heterogeneity (measured by the Euclidean distance) was on average highest in NC plots (Fig. 1g). This effect was significant over all the countries in 2003 and in NL and UK over the whole period. At most locations, heterogeneity remained relatively constant, but it decreased slightly in CZ, and increased in SP. In general, the sown species were distributed more evenly over subplots. This can be

illustrated by comparison of Fig. 1e, f – the number of species at the end of the studied period is highest in NC on both spatial scales, however, the difference is much more pronounced at the large spatial scale.

Sowing did not affect productivity in the first year. However, during the following seasons, sown plots exhibited usually higher biomass than unsown plots (Fig. 1g). On average, the differences decrease slightly from the second season on, but remained significant until 2003. The development also differed between countries (Fig. 2). In CZ, productivity tended to decrease over time, probably as a consequence of nutrient depletion, whereas at the UK site, which is located on nutrient-poor calcareous soil, productivity steadily increased over time. Productivity was on average higher in HD than in LD plots, but within each country the highest LD replicate fell well into the HD range, in four out of five countries being higher than HD average. Interestingly, in CZ biomass was highest during the second season and in all treatments species richness was lowest during this season.

## Discussion

### *Behaviour of individual species*

Although the behaviour of individual species differed considerably among the countries, some common features emerged. With the exception of SE, most of the grasses established reasonably well (similar to the experiment of Pakeman et al. 2002). Of them, *Trisetum flavescens* was able to spread rapidly to unsown plots, both in our experiment (sown in CZ and UK) as well as in that of Pakeman et al. (2002). Again, in concordance with Pakeman et al. (2002), *Lotus corniculatus*, and *Plantago lanceolata*, established well, but the persistence varied considerably among countries for *P. lanceolata*. The success of other species varied considerably. The reasons for failure of a species to establish are often difficult to determine. For example, two species with rather poor establishment in the CZ site were *Centaurea jacea* and *Lychnis flos-cuculi*; while in experiments being established in other years at the same site, the two species became well established, even becoming dominant in the case of *Centaurea*. Similarly, *Centaurea nigra*, a closely related species, was highly successful at the UK site. The success of some species was thus highly habitat specific. Whereas *Galium verum* established well in chalk grassland habitats (both Pakeman et al. 2002, and our UK site), it generally failed at the other sites where it was sown (including the CZ site, where it grows naturally in the vicinity of the experimental field site). In line with other studies, our study shows that

the common and potentially dominant species can be used over a range of conditions. Nonetheless, detailed knowledge of local species behaviour is needed for species with high conservation value. Pakeman et al. (2002) also noted that differences in management such as grazing or mowing could be decisive for a species to fail or be successful in restoration attempts. Despite considerable variation in individual species' behaviour among sites, we were able to demonstrate some trends in the community characteristics, consistent among the sites.

#### *The effect of sowing and plant persistence on the course of succession*

Sowing considerably affected the course of succession and the effect of sowing persisted for the whole experimental period. These results support Egler's (1954) well established 'initial floristic composition' hypothesis. Therefore, sowing can be an important management option for vegetation restoration. However, while the identity of the species sown can strongly influence the species composition over a longer time course, the trait group composition of the plant species in both the sown and the unsown plots, that were analysed in the site in The Netherlands, converged over time (Fukami et al. 2005).

In our experiment, it was not feasible to prevent the spread of sown species into adjacent plots. In some countries plant species that were sown spread to adjacent plots, including the natural colonization plots, and sometimes dominated all the plots irrespective of sowing treatment. The increase in number of sown (i.e. grassland) species in NC and LD treatments, and resulting development toward dominance of these grassland species was higher than it would be in the absence of this effect.

We have observed large differences among individual sown species. Some of them were abundant in sown plots, but rarely or never spread to other plots. For example, at the CZ site, two sown species that successfully established and became abundant in plots where they were sown were *Trisetum flavescens* with very light seeds (0.18 mg – Grime et al. 1988) and *Lathyrus pratensis* with heavy seeds (12.85 mg). Whereas *Trisetum* subsequently colonized all plots in CZ (all LD replicates in which it was not sown and NC), *Lathyrus* was virtually absent from plots where it was not initially sown. This suggests that dispersal limitation is an important factor determining plant community composition in general (Poschlod et al. 1998; Ozinga et al. 2005), and it is thus important to consider dispersal abilities in grassland restoration (Hutchings & Booth 1996; Pywell et al. 2002; Donath et al. 2003). In general, plant traits are important determinants of species success in restoration (Pywell et al. 2003), and the ability to spread is amongst the most important ones. With exception of SE, 8 years

after initiating the experiment, the HD plots and the most successful LD mixtures were still dominated by the sown species. The dominance was highest in the UK, where the proportion of sown species was close to 90%, followed by NL, CZ and ES. At the whole-plot scale, more than half of the species survived in CZ, NL, and UK. This again shows that propagule addition can affect the successional trajectory for many years.

#### *Effect on natural colonizers*

As expected, sowing suppressed both the total cover, and the number of naturally-colonizing species. As a result, in most countries total number of species was highest in the natural colonization treatment. Is this desirable for restoration, as the community most successful in suppression of noxious weeds will most likely be the most successful in suppression of the desired colonizers? In most countries, the NC plots exhibited higher species richness than any of the sown plots at the end of the experiment. While NC plots were often colonized by sown species from adjacent plots, these species usually did not reach the level of dominance in plots in which they were sown, leaving space for other species to colonize. Sowing species mixtures is not a tool to maximize species richness, but a tool to introduce (and sometimes achieve high richness of) desired species. While some of the natural colonizers could also be classified as 'desirable' species, generally it is difficult to classify all the species into noxious, desirable, or indifferent. As the plots were mown, some annual arable weeds (e.g. *Veronica arvensis*, *Viola arvensis*) were able to germinate regularly from the permanent seed bank, and some even reached reproduction. The species that are considered noxious usually have some rather unique combination of traits making them 'pests'. For example, *Elymus repens*, abundant at several sites, is generally considered an undesirable weed. It is a perennial rhizomatous grass as are some desirable species such as *Avenula pubescens*, which are typically part of species-rich semi-natural grasslands. *Elymus repens*, however, has an excellent ability to regenerate from rhizomes. In the UK, where the unsown plots noticeably exhibited the highest species richness, there were many species of the semi-natural grasslands among the colonizers (e.g. *Centaurea erythraea*, *Clinopodium vulgare*, *Euphrasia nemorosa*, *Avenula pratensis*, *Avenula pubescens*, *Linum catharticum*, *Origanum vulgare*, *Picris hieracioides*, *Primula veris*).

The development of plant communities is often affected by the presence of strong dominants (e.g. Prach et al. 2001; Clarke et al. 2005) and is often highly idiosyncratic. Interestingly, at the UK site with the lowest productivity of all sites, no such dominant species ap-

peared. In CZ, starting from the fifth year, there was a continuous decrease in both the number and percent cover proportion of sown species in HD and LD treatments, which was partly caused by the spread of *Taraxacum officinale*, a species that was not sown and had less than 5% cover until the fourth year, but reaching 30-50% cover later on. Since the sown species were spreading reasonably well over short distances (within our experimental fields), high species richness in restoration sites might be achieved by sowing mixtures in strips, leaving enough free space between them for natural colonizers. However, this would decrease the suppression of noxious weeds which may invade the sown strips over time. Therefore, in places where a high incidence of noxious weeds is expected, sowing highly suppressive mixtures would be recommendable. At locations with invasion potential of the species from a target community nearby one should be aware that sowing mixtures could suppress desired natural colonization. It seems that highly suppressive mixtures were either the high diversity ones, or the ones composed of strong competitors. However, the presence of a strong competitor in a mixture not only suppresses the colonizers, but also leads to decreased survival of other sown species.

#### *Sowing with low vs. high diversity seed mixtures*

When comparing the success of sowing of HD and LD mixtures, measured either by proportion of sown species, by suppression of natural colonizers, or by the total biomass, on average, the HD mixtures were more successful. However, the most successful LD mixture was usually equally successful as the HD mixtures. This suggests that diversity was important primarily for its 'insurance effect' (Yachi & Loreau 1999). Whilst some of the LD mixtures failed to establish successfully, the HD mixtures were always able to compensate the failure of some species to establish. As we have already demonstrated for the first three years (Lepš et al. 2001), the LD plots containing the species that dominated in HD plots were the successful ones. Often, the presence of the dominant grass was sufficient for the LD mixture qualify as 'the best', even if the other species in the combination exhibited low cover, or even fail to survive. Consequently, we consider the better average performance of HD plots mainly a consequence of the sampling effect, i.e. presence of the productive and highly competitive species (Huston 1997). Similar patterns persisted for the whole experimental period.

#### *Effects on productivity*

Sowing increased the productivity, and, on average, the high diversity plots had the highest productivity of

all the treatments. This corresponds well with most published studies, executed on smaller spatial scales (Hector et al. 1999; van Ruijven & Berendse 2005; Lanta & Lepš 2006). However, with the exception of the first two years, the natural colonization treatment resulted in the most species rich community, and still exhibited relatively low productivity. So, in our experiment high productivity was connected with high diversity of sown (relatively productive grassland) species, not with the total diversity of the plant community, supporting the views that biodiversity-functioning relationships are highly species and context dependent (Wardle & Zackrisson 2005) and that the identity of (dominant) species is more important for ecosystem functioning (including the productivity) than the number of species *per se* (Lepš et al. 1982).

#### *Spatial scale and design of biodiversity experiments*

The design of biodiversity experiments, and in particular the selection of species combinations has been a topic of many controversies since Huston (1997). There is a profound trade-off between demands for correct and powerful analyses on the one hand and feasibility and realism of manipulations on the other. In short-term experiments carried out at limited spatial and temporal scales (e.g. Spačková & Lepš 2001), the number of replications is obviously less restricted than in long-term experiments, as presented here, that aim at realism and feasibility of treatments for restoration. In our experiment, the plot size of 10 m × 10 m, which is considerably larger than most other field experiments (e.g. Hector et al. 1999) was not sufficient to prevent the spread of species from the adjacent plots. Toward the end of the experiment, the species sown in LD and HD plots were common also in the natural colonization plots and consequently, the rate of development of plots with natural colonization toward a grassland community is overoptimistic. The number of possible combinations of low diversity mixtures that we employed in our experiment was rather limited. Therefore we are not able to statistically distinguish the effect of blocks caused by environmental factors (and consequently affecting all the treatments) and the effect of variation in the composition of the sown seed mixture. The large differences observed between minimum and maximum values for LD replicates suggest that the effect of species mixture was considerable. However, the fact that the best LD mixtures nearly always (i.e. in all the countries, for all the studied characteristics and over the whole experimental period) fell within the range of HD values is a striking feature of our experiment.

The larger plots are not only more realistic, but also enable the study of spatial heterogeneity and changes of species richness at two spatial scales. Sowing increased the number of species, but also led to homogenization.



Whereas the total number of species at the end of the experiment was highest in the natural colonization plots, this effect is more pronounced at the whole-plot scale. These results emphasize that we have to be extremely careful when extrapolating diversity features from small plot experiments to large restoration areas. In particular, the spatial extrapolations like the one suggested by Tilman (1999) estimating on the basis of species-area relationship how many species we need per hectare to achieve high productivity, might be misleading. In his extrapolation, Tilman (1999) expects constant  $z$  value (i.e. constant slope of the linear regression of  $\log(\text{Number of species})$  on  $\log(\text{Area})$ ), and local number of species determining the productivity. Our results suggest that the  $z$  value is variable and depends on the species life histories (natural colonization exhibited the greatest increase in the number of species from the subplot to the whole-plot level, but still had the lowest productivity), and that the productivity is mainly determined by the dominant species.

In conclusion, our study has demonstrated some common features of restoration by sowing late successional species. At the same time, the highly significant two-way Country  $\times$  Treatment and three-way Country  $\times$  Treatment  $\times$  Year interactions demonstrate that the effect and success of treatments was highly dependent on environmental conditions. This suggests that also the management recommendations for creation of grasslands on ex-arable land, for example through agri-environment schemes, should be highly country- and perhaps even region-specific taking local conditions into account.

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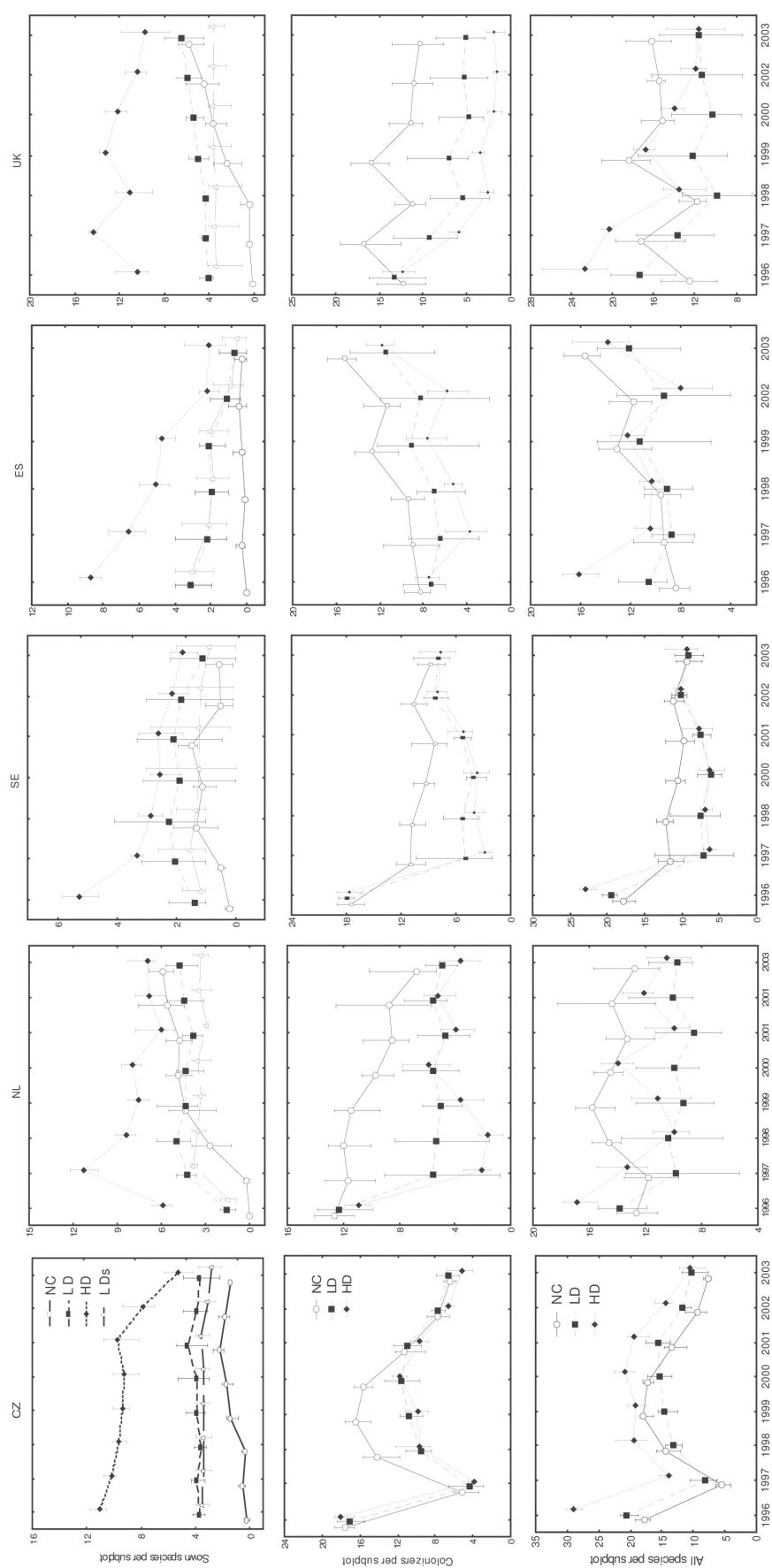
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**App. 1.** Results of Repeated measurement ANOVA (*P*-values) for individual countries. Only main effect of treatment (Tr) and its interaction with Year is shown. Results are presented for complete data sets, and for comparison of low-diversity and high-diversity plots (LD-HD instead of Tr). The number of species is considered on the whole-plot level (pooled species from all 12 quadrats, 1m<sup>2</sup> each) and on the subplot level (averaged number of species per 1m<sup>2</sup> quadrat. Significant terms (i.e. *P* < 0.05) are shown in bold.

Sown species		Whole plots			Subplots			
	Tr	LD-HD	Tr*Y	LD-HD*Y	Tr	LD-HD	Tr*Y	LD-HD*Y
CZ	0.0000	0.0002	0.0000	0.0008	0.0000	0.0000	0.0000	0.0000
NL	0.0001	0.0037	0.0000	0.0001	0.0000	0.0004	0.0000	0.0000
SE	0.0088	0.0432	0.1153	0.4159	0.0023	0.0646	0.0000	0.0000
ES	0.0000	0.0003	0.0000	0.0001	0.0000	0.0006	0.0000	0.0000
UK	0.0000	0.0001	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000
Colonizing species								
	Tr	LD-HD	Tr*Y	LD-HD*Y	Tr	LD-HD	Tr*Y	LD-HD*Y
CZ	0.0122	0.4112	0.0000	0.0058	0.0007	0.3622	0.0000	0.0484
NL	0.0001	0.2596	0.0001	0.0083	0.0000	0.0755	0.0000	0.0078
SE	0.0012	0.3667	0.0002	0.9926	0.0000	0.1529	0.0000	0.7613
ES	0.0584	0.3497	0.4331	0.7317	0.0096	0.1936	0.0219	0.2025
UK	0.0002	0.0238	0.0000	0.0085	0.0000	0.0465	0.0000	0.0164
All species								
	Tr	LD-HD	Tr*Y	LD-HD*Y	Tr	LD-HD	Tr*Y	LD-HD*Y
CZ	0.0037	0.0138	0.0000	0.0003	0.0000	0.0005	0.0000	0.0000
NL	0.0035	0.3463	0.0004	0.0585	0.0007	0.0317	0.0000	0.0345
SE	0.0149	1.0000	0.0031	0.9664	0.0050	0.4571	0.0000	0.1110
ES	0.5308	0.3674	0.0056	0.2357	0.2115	0.0528	0.0000	0.0022
UK	0.0298	0.7833	0.0000	0.0027	0.0185	0.0397	0.0000	0.0001
Proportion of sown species								
	Tr	LD-HD	Tr*Y	LD-HD*Y				
CZ	0.0000	0.0411	0.0000	0.5597				
NL	0.0000	0.0257	0.0000	0.1419				
SE	0.0004	0.5257	0.0001	0.4145				
ES	0.0004	0.0609	0.0000	0.0263				
UK	0.0000	0.0939	0.0000	0.5491				
Heterogeneity (ED)								
	Tr	LD-HD	Tr*Y	LD-HD*Y				
CZ	0.0502	0.3026	0.0073	0.8145				
NL	0.0011	0.0325	0.0007	0.0376				
SE	0.2215	0.9668	0.0736	0.3483				
ES	0.0771	0.2440	0.0044	0.7230				
UK	0.0000	0.4643	0.4945	0.1059				
Biomass								
	Tr	LD-HD	Tr*Y	LD-HD*Y				
CZ	0.0032	0.0333	0.0318	0.0272				
NL	0.0007	0.0315	0.0071	0.2331				
SE	0.0121	0.1922	0.0062	0.3743				
ES	0.2253	0.1619	0.5894	0.1453				
UK	0.0097	0.0774	0.1290	0.4064				



**App. 2.** Changes in mean number of colonizers and total number of all the species per subplot for natural colonization (NC) and for sowing later-successional plant species in low-diversity (LD) and high-diversity (HD) treatments. For sown species, LD plots are characterized by total number of all sown species (LD), and number of species sown in respective plot (LD). Error bars extend between minimum and maximum value of the five blocks. Results of repeated measure ANOVA for individual countries are in App. 1.