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DIRECTING SUCCESSION: EXPERIMENTAL SOWING AND TRANSFER OF VEGETATION INTO AN ABANDONED FIELD

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Content

Abstract

A recently abandoned field located in south Czech Republic was used to determine how the level of enrichment of the soil with diaspores of plants typical for species rich meadows can affect the plant community development directed intentionally with regular mowing to the species rich meadow.

Two types of diaspore enrichment were used at the experimental 2x2 m plots (each type with three levels, yelding nine combinations) arranged in five completely randomised blocks: seed mixtures of plants characteristic of species rich meadows (high diversity mixture of 15 sp., low diversity mixtures of 4 sp. and unsown control) and blocks of vegetation transferred from a species rich meadow to the plots (blocks transferred in spring, autumn and control). In addition, soil from the source meadow was broadcasted on all plots with meadow blocks to test, if both plant diaspores and soil (micro)organisms present at the meadow could positively affect development of the plant community. Vegetation cover in plots and meadow blocks has been estimated annually since the start of experiment in 1996.

Analyses of data revealed that there is a significant effect of sown species on absolute species representation of natural colonisers and this effect is more intensive in plots sown with high diversity mixture. There are significant changes in species composition inside transferred meadow blocks. Broadcasting soil did not show any significant effect on species composition.

Introduction

Emerging from many data collected around the world is the conclusion that people, where they congregated, were a strong force in vegetation change during several last millennia. Thus landscapes, at least in forested areas, were changed from nearly closed forests to a mosaic of woodland, meadow and field as well as urban patches (Hadač 1982). People drove certain species to near extinction while at the same time facilitating extensions of other species (Delcourt 1987) and whole plant communities, because new niches and new ways of species migration were created or lost. Some man-made communities (like heathlands, pastures, wooded, hay or wet meadows) which have been developing under traditional land use techniques during many centuries and which often contained a lot of unique species, are nowadays endangered due to changes in their management.

Intensifying human impact on (semi)natural plant as well as animal communities is associated with overall decrease of their species diversity to such an extent, that the key issue of nature conservation is to preserve and restore biodiversity. It is supposed that many ecosystem features like stability, productivity, respiration, decomposition, nutrient and water retention are in many cases diversity-dependent. Impacts of biodiversity on population dynamics and ecosystem functioning have long been debated, however with many theoretical explorations but few field studies (Tilman et al. 1994, 1996, 1997, Naeem et al. 1994, Hooper & Vitousek 1997, Scherer-Lorenzen 1998, Symstad et al. 1998).

Here I provide evidence from direct experimental manipulation of diversity using meadow plant communities differing in the number of species which were artificially established onto a recently abandoned field. Two kinds of diaspore enrichment were used: seed mixtures of meadow species and blocks of vegetation transferred from a species rich meadow ('stepping stones'). The aims of the experiment are both theoretical and applied: (1) To study the effects of community complexity as well as manipulation of community development by increasing plant species diversity. (2) To examine if, and how, a stepping stone approach may be used to enhance the colonization of the bare soil of abandoned land by addition of plant diaspores and soil (micro)organisms of expected/desired successional stages. This study is a part of a larger European project CLUE (Changing Land Usage, Enhancement of biodiversity and ecosystem development), where the effects of community complexity (of both vegetation and soil (micro)organisms) on ecosystem processes and vegetation dynamics on former agricultural, set-aside, land are studied. There are five participating countries: CZ, SE, UK, NL, SP. Details of the whole CLUE project are available on our homepage: `http://www.nioo.knaw.nl/cto/clue/clue.htm.`

In several respects, grassland communities are particularly suitable for ecological experimentation: they react soon after the start of experiments and can be monitored easily. Finally, recent changes in grassland use (abandonment, overfertilisation) have resulted in the endanger of such communities (Krahulec 1995) so that data on their ecology are important for successful restoration. There are a number of studies on meadow restoration in terms of reestablishment of traditional land-use techniques to restitute the former richness. In some cases, the revegetation of intensively disturbed soils such as that created by mining and construction activities (Luken 1990) or abandoning of arable soil (McDonald 1993, Jongepierová 1995) has been tested too.

Material and Methods

Nomenclature: Rothmaler (1978).

Study Site

The experimental field was located near the village Benešov nad Lipou, South Bohemia, Southwest margin of the Bohemian-Moravian Upland, Czech Republic, at an altitude of 665 m a.s.l. The locality has a moderately humid climate typical of the highlands (mean annual temperature 6.4 °C, mean annual precipitation 677 mm). The soil is a brown earth on ground rock biotitic gneiss. The field was withdrawn from agricultural use after the last crop harvest (barley) in autumn 1995, the experiment was set up in spring 1996.

Design of the Experiment

In the 2 x 2 m plots with 2-m walkways two types of diaspore enrichment were used (each with three levels, yielding nine combinations) in five completely randomised blocks. Treatments were seed mixtures of plants characteristic of species-rich meadows (high diversity mixture of 15 species, low diversity mixture of four species and unsown control) and blocks of vegetation ('stepping stones') transferred from a species-rich meadow to the plots (stepping stones transferred in spring, autumn and control). The plant community development is directed with regular mowing to the species rich meadow. For more details, see Fig. 1. and 2.

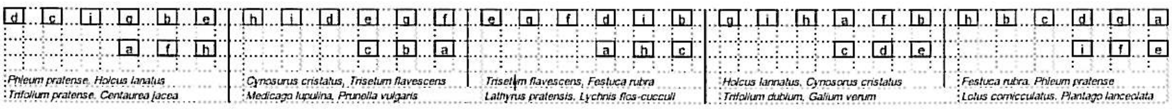

Fig. 1. Layout of the five randomised blocks. Combinations of treatments: a = natural colonization (NC); b = low diversity treatment (LD); c = high diversity treatment (HD); d = NC + spring stepping stones; e = LD + spring stepping stones; f = HD + spring stepping stones; g = NC + autumn stepping stones; h = LD + autumn stepping stones; I = HD + autumn stepping stones. Names of species used as the LD assemblages are at the bottom of each block.

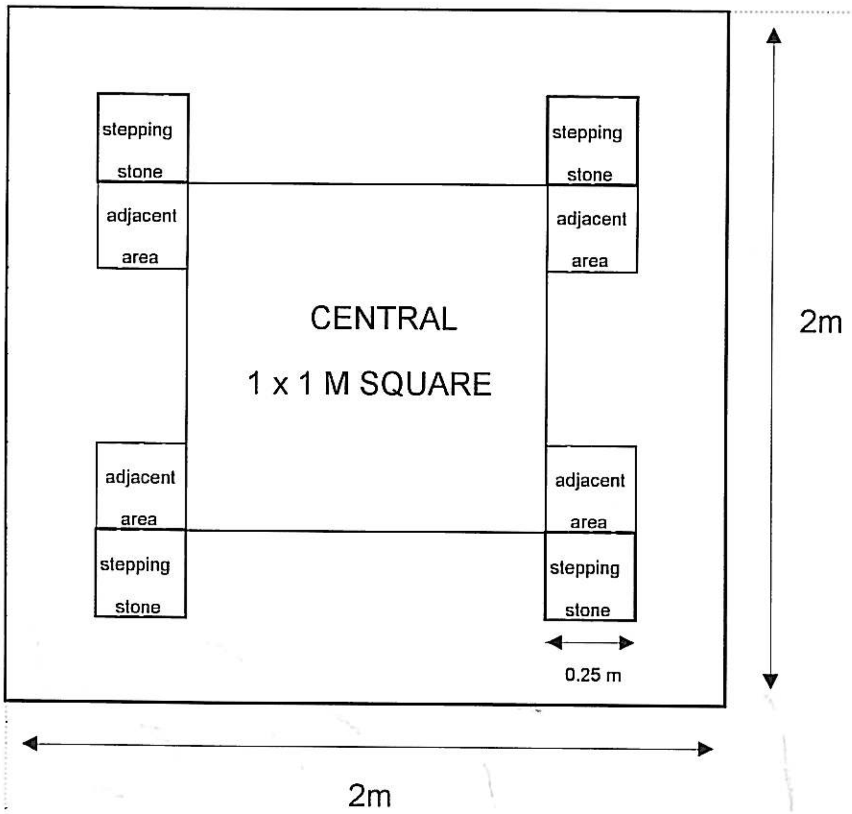

Fig. 2. Detail of the 2 x 2 m plot with four installed stepping stones. Vegetation cover was estimated separately in all central 1 x 1 m squares, stepping stones and its adjacent areas.

The relevés of adjacent areas were taken to record spreading of stepping stone vegetation and, if needed, to record other possible impacts of the stepping stone (i.e. soil (micro)organisms) onto its surroundings.

Seed mixtures

Some main factors were taken into account before the selection of species suitable for seeding: (1) relatively wide ecological valence with an optimum in *Arrhenatherion* and *Cynosurion* plant associations, (2) presence in a local flora pool of native grasslands in vicinity of the study site, (3) keep a balanced ratio of plant functional groups (4) availability of sufficient amount of seeds, and (5) endeavour to keep at least similar composition of a core species according to the differences in ecological conditions of the other participating countries (SE, UK, NL, SP).

Also another problem has emerged concerning the supply of sufficient seed mixtures as well as preservation of genetic diversity respecting a certain region. Seeds used in this experiment were got from several Czech companies (Planta Naturalis in Markvartice u Sobotky and others), because it was the only possible way of obtaining such a number of meadow species, which are not currently the component of commercially distributed seed mixtures. The distance of seed source (less than 200 km in our case) could be discussed/doubted, nevertheless there exists a wide range of views about. For example, several seed mixtures of species coming from different ecological conditions and different regions in England (Jongepierová 1995) were tested. One may nowadays buy a number of diverse commercial seed mixtures in specialised stores. The Czech company Planta Naturalis offers five such mixtures rich in species from diverse meadow communities (for example the 'Česká květnice' mixture consists of 65 species).

Based on specific characteristics of plants, the functional groups were assembled as grasses, legumes and other forbs. Fifteen species (five per functional group) were sown as a high diversity treatment (HD) and four species (two grasses, one legume and one forb) were sown as a low diversity treatments (LD). In both the low and high diversity mixtures we used a constant seed density per plot, with each species sown in proportion (grasses: 2500 seeds/m2, legumes: 500 seeds/m2 and other forbs also 500 seeds/m2 yielding together about 4.35 g/m2). Different species combination in LD treatment was used in each block. This is an important detail, because if we want to compare low and high diversity treatments, we shall not always sow the same four species in each of the five complete blocks. In such a case, we would test not the difference between low and high diversity but the difference between high diversity and four particular species. Sown species together with their germination in the field are summarized in Table 1.

Before sowing the field in spring 1996, soil samples were collected to determine the field seed bank. The amount of available nitrogen was determined in the second year. Detailed results are summarized in a bachelor dissertation of my colleague Hejcman (1997), here I quote only the most frequent species found in the seed bank: *Fallopia convonvulus, Myosotis arvensis, Plantago major, Poa annua, Spergula arvensis, Veronica arvensis, Veronica persica*. Mean number of all seedlings per 2 litres of topsoil was 77.

Sown species	germination on field
Grasses	
Holcus lanatus	good
Festuca rubra	good
Phleum pratense	reasonable
Cynosurus cristatus	good
Trisetum flavescens	good
Legumes	
Lotus corniculatus	good
Trifolium dubium	good
Lathyrus pratensis	reasonable
Medicago lupulina	reasonable
Trifolium pratense	reasonable
Forbs	
Plantago lanceolata	good
Galium verum	bad
Lychnis flos cuculi	disaster
Prunella vulgaris	good
Centaurea jacea	bad

Tab. 1. List of sown species. The greenhouse test of seed viability was also done: altogether 14 species germinated well except Lychnis flos-cuculi (did not germinate at all).

Tab. 2. List of species from the source meadow (estimates of mean percentage cover).

Poa pratensis + trivialis	33
Alopecurus pratensis	33
Alchemilla monticola	30
Dactylis glomerata	13
Phleum pratense	8
Taraxacum sp.	7
Ranunculus acer	5
Agrostis tenuis	4
Veronica serpyllifolia	4
Trisetum flavescens	4
Ranunculus auricomus	4
Achillea millefolium	4
Agropyron repens	4
Cerastium holosteoides	3
Festuca pratensis	2
Anthoxanthum odoratum	2
Rumex acetosella	1
Veronica arvensis	1
Plantago major	+
Stellaria graminea	+
Cardamine pratensis	+
Holcus mollis	+
Holcus lanatus	+
Vicia cracca	+
Juncus effusus	r
Poa annua	r

Stepping stones

The first half of stepping stones was transplanted in April 1996 (short time after seeds were dispersed) and the second half in October 1996. As a source, a relatively species rich meadow (cca 10 species per one stepping stone on the average) characterised by the species list in Tab. 2 was chosen. Size of each the stepping stone was 25 x 25 cm and cca 20 cm in depth, four stepping stones were transplanted per one 2 x 2 m plot (Fig. 2.).

In addition in April 1996, the top soil from the source meadow was broadcasted on all 2 x 2 m plots assigned for spring and autumn stepping stones. Soil was taken after removal of a thin sod layer (2-3 cm), homogenized (visible plant material was got out of the way) and disperzed as 10 litres per plot. To determine which plant diaspores were present, 10 litres of homogenized soil were collected. Sterile sand was placed on the bottom of each of 10 trays and 1 litre soil subsamples were placed over the sand in 1 cm thick layer. One part of the trays was watered immediately whilst the other part was first kept dry for ten days and watered again. The trays were placed in a greenhouse and regularly watered, emerged seedlings were identified, counted and removed at regular intervals during the first four months. After this period, when no more seedlings emerged, the soil was stirred and watered again to encourage germination of persisting seeds.

Vegetation Sampling

Percentage species cover was estimated in three successive years (1996-1998). Sampling was done in mid summer according to the phenological aspects of particular year. Three data sets were collected (see Fig. 2.): (1) central $1m^2$ square of each 2 x 2 m plot; (2) vegetation of transplanted stepping stones; (3) vegetation of 25 x 25 cm area adjacent to each the stepping stone.

Data Analysis

The data on changes in species composition were evaluated using multivariate ordination analysis of the new version of CANOCO - Canoco for Windows package (ter Braak & Šmilauer 1998).

I used the following nominal explanatory variables (with classes in brackets): sowing (HD, LD, NC) and stepping stones (spring, autumn, no stepping stones transplanted). These factors are coded as series of dummy variables (Jongman et al. 1987). Factor time is coded as a sole quantitative variable. This type of coding was chosen after a special Detrended Correspondence Analysis (DCA). In this analysis the factor time was coded as a series of three dummy variables and I depicted centroids of its interactions with HD, LD, and NC treatments to ensure, whether all the treatments 'move' linearly according to the level of the time. The response of particular treatments was confirmed to be linear.

After the second preliminary DCA analysis on all three data sets yielded axes of length 4.36 SD, the question emerged, whether unimodal or linear response model would best fit. Only if the gradient length is reduced to less than about 3 SD, the approximations involved in weighted averaging (i.e. method used in calculations of DCA and CCA) become worse (ter Braak & Prentice1988), so that in our case the unimodal response model (CCA) would seem to be adequate. On the other hand, DCA analyses of separate data sets yielded axes of lower lengths what implies that in those cases, a linear method could/should be used. Finally it was switched to the RDA as majority of explaining variables were nominal ones and also results of all analyses of separate data sets would be more comparable and easily be interpreted.

All data sets are in the form of repeated measurements, and the analyses have to reflect this fact (including the permutation scheme in the Monte Carlo permutation test). In all the analyses, the sown species were made passive. This means, that one is interested in the response of natural colonizers. (If the sown species were not made passive, the significant response to sowing might be caused solely by the fact that the species that were sown were present just in the plots where they were sown.)

The data were analyzed by several analyses (i.e. several combinations of environmental variables and covariables) to answer the particular questions. The questions, together with corresponding design of analysis and results of the Monte Carlo permutation test are in Tab. 5. & 6. Similarly as in the univariate repeated measures, the interaction term of treatment with time corresponds to differential development. If the interaction is zero, then all the differences from the beginning are conserved over the time; this means that the successional trajectories are parallel. So the difference in development means non-parallel successional trajectory. In practice this usually means, that the plots under differing treatments are becoming more dissimilar with time.

I used two types of analyses: standardized and unstandardized. Standardization in this paper means standardization by sample norm. In this way one is able to distinguish differences in the absolute representation of species (in our case, species cover) and relative representation (proportion of cover of the species within a community). This distinction is important because the total cover of natural colonizers differs between treatments. Consequently, a significant result from nonstandardized analysis shows that there are some differences between treatments; the species respond to the treatment, but it is not clear, if the species differ in their response (the proportion of species need not be changed). If the standardized analysis shows some significant differences, one can conclude that the species respond, and that not all the species respond in the same way, and, consequently, the proportion of species differ. The first test is more powerful, however, the significant result of the second test with standardized data is ecologically interesting. The results of CANOCO analyses were displayed as the ordination diagrams using program CanoDraw 3.1. (CanoDraw is now bundled with Canoco for Windows).

Results

Meadow Seed Bank Determination

Emergence of seedlings from the seed bank was observed in 21 species, of which 8 were perennials. The similarity according to Sörensen´s index, between the actual meadow vegetation (Tab. 2.) and the soil seed bank (Tab. 3.) is 43 %. The results of drying versus non-drying of the seed bank samples show that more seeds germinated (only) after drying.

Tab. 3. Number of viable seeds per 10 litres of the meadow top soil. Perennials are indicated with an asterisk.

	droughted	non droughted
*Agrostis tennuis**	4	-
*Alopecurus pratensis**	-	6
*Cardamine pratensis**	2	-
*Cerastium holosteoides**	16	6
Chenopodium album	2	2
Chenopodium polyspermum	2	-
Fallopia convonvulus	2	-
Galeopsis tetrahit	6	4
Galinsoga ciliata	4	-
Gnaphalium uliginosum	8	-
*Holcus mollis**	-	12
Juncus bufonius	62	2
*Juncus effusus**	84	8
*Plantago major**	4	4
Poa annua	14	16
Polygonum hydropiper	4	2
Sagina procumbens	60	44
Spergula arvensis	-	2
*Taraxacum officinale**	8	6
Veronica persica	2	-
*Veronica serpyllifolia**	6	-

Two-way analysis of variance

Most sown species have become established, except *Lychnis flos-cuculi*, *Medicago lupulina*, and *Galium verum* (Tab. 1. & 4.). The highest number of natural colonizers was observed in the first year regardless of the treatment, nevertheless conspicuous differences were found among plots with high diversity (HD), low diversity (LD), and natural colonization (NC) treatments in suppression of their density. Total percentage cover of natural colonizers was negatively correlated with the diversity treatment and this relation has became more intensive in the second and third year. After the three years of experiment, the number of species sown in the LD-treated plots has persisted, whereas the HD plots have lost a part of their initial diversity (Fig. 3. & 4.). The HD, LD, and NC plots were compared regardless of the stepping stone treatment, because only few stepping stone species have spread through the whole plots so far. The effect of stepping stone treatment is involved in the CANOCO analyses.

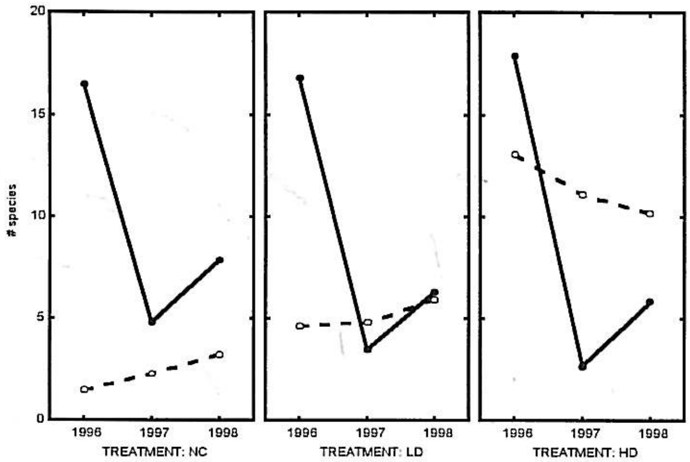

Fig. 3. Changes in a mean number of natural colonizers (solid line) and sown species (broken line). Treatments on the x-axis are as in the text. Significant main effects and interactions from Two-way ANOVA (data on natural colonizers): year (p << 0.0001); treatment x year (p = 0.02). The effect of diversity treatment on the number of species of natural colonizers was not significant (p = 0.18).

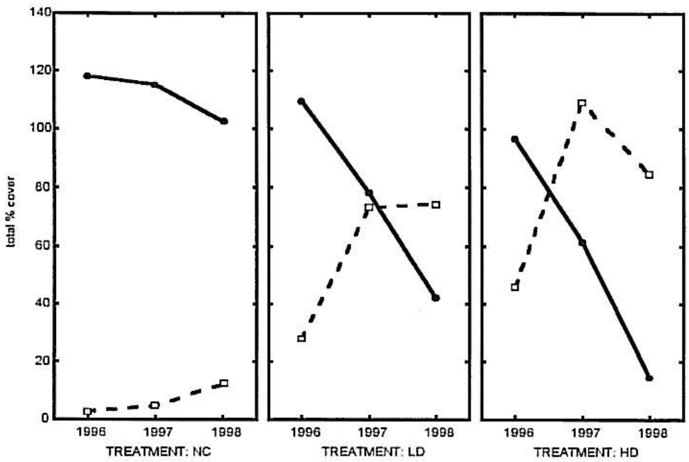

Fig. 4. Changes in a mean total percentage cover. Species were divided into two groups: natural colonizers (solid line) and sown species (broken line). Treatments on the x-axis are as in the text. Significant main effects and interactions from Two-way ANOVA (data on natural colonizers): year (p << 0.0001); treatment (p << 0.0001); treatment x year (p << 0.0001).

Tab. 4. Changes in cover of the most important species in particular treatments in three successive years. All the sown species are listed, weeds only with cover ≥ 7 in at least one category. Estimated as percentage cover, r = cover lower than 1%.

year	1996			1997			1998		
treatment	HD	LD	NC	HD	LD	NC	HD	LD	NC
Lotus corniculatus	18	53	9	11	48	0	6	39	0
Trisetum flavescens	1	2	0	29	37	0	54	54	1
Trifolium pratense	10	19	2	34	48	3	6	27	3
Plantago lanceolata	7	22	3	9	44	0	3	20	0
Holcus lanatus	2	3	1	6	12	0	4	25	0
Lathyrus pratensis	1	8	0	3	23	0	2	15	0
Cynosurus cristatus	0	1	0	11	23	0	3	10	0
Festuca rubra	1	4	1	4	11	0	2	9	0
Phleum pratense	0	0	0	1	1	0	2	17	0
Prunella vulgaris	2	7	0	1	4	0	1	2	0
Trifolium dubium	1	3	0	r	4	0	r	2	0
Centaurea jacea	r	1	0	0	r	0	0	1	0
Galium verum	r	r	0	0	r	0	0	0	0
Lychnis flos-cuculi	0	0	0	0	0	0	0	0	0
Medicago lupulina	r	r	0	0	r	0	0	0	0
Trifolium repens	35	41	38	49	62	92	5	13	34
Agropyron repens	10	10	9	12	14	17	4	18	43
Fallopia convonvulus	10	16	16	r	r	r	r	r	r
Myosotis arvensis	5	7	7	r	r	r	3	4	9
Stellaria media	7	11	13	r	r	r	r	1	1
Viola arvensis	6	7	8	r	r	r	r	2	3

Redundancy analysis

Following variables were used to characterize the results of particular analyses (with abbreviations in brackets): species - environment correlation on the first canonical axis (% expl.); the sum of all constrained eigenvalues - i.e. the ratio of variability explained by all the explanatory variables used in the test (Σ all constr.); corresponding probability value obtained by Monte Carlo permutation test (P) - i.e. the type one error probability in testing the hypothesis that the effect of all explanatory variables is zero. Results are summarized in Tab. 5. The sown species, although passive in the analyses, are displayed in ordination diagrams.

Tab. 5. Analyses of samples from the central 1x1 metre squares. Explanatory variables are environmental variables in CANOCO terminology; factors, whose effect was excluded from the analysis are listed as covariables. If the standardisation by samples was applied, the numeral of particular analysis is signed with „s". Names of variables in following tables: T = time; S = sowing (HD, LD, NC); StSt = stepping stone treatment; S/A = season of stepping stone removal (spring/autumn); B = block. The interactions of couples of the treatments are indicated with an asterisk.

	Question	Answer	Explanat. variables	Covariables	% expl.	Σ all constr.	P
1	Is the absolute representation of species related to explaining variables?	YES	T, S, StSt, T*S, T*StSt	B	28.4	0.35	0.002
1s	Is the relative representation of species related to explaining variables?	YES	T, S, StSt, T*S, T*StSt	B	26.7	0.34	0.006
2	Is there a common successional trend in absolute species representation?	YES	T	B, S, StSt	29.3	0.26	0.01

Tab. 5.

	Question	Answer	Explanat. variables	Covariables	% expl.	Σ all constr.	P
2s	Is there a common successional trend in relative species representation?	YES	T	B, S, StSt	27.6	0.25	0.01
3	Is there any effect of sown species on absolute species representation and/or their development?	YES	S, S*T	B, StSt,T	8.6	0.07	0.002
3s	Is there any effect of sown species on relative species representation and/or their development?	YES	S, S*T	B, StSt,T	7.8	0.07	0.002
4	Is there any large-scale effect of stepping-stone treatment on absolute species representation and/or their development?	YES	StSt, StSt*T	B, S, T	1.5	0.02	0.04
5	Is there any small-scale effect of stepping-stone treatment on absolute species representation and/or their development?#	YES	C, AB, T*C, T*AB	B, S, T, S/A	3.6	0.03	0.002

Comparison of samples recorded from the central 1 x 1 m squares and from the areas adjacent to the stepping stones.

The first analysis revealed, that the constrained canonical axes explained about 35% of the data variability (Σ all constr. = 0.35). This is the total part of community variability, which one can ascribe to environmental variables, because in this case all available variables were used as explanatory ones (except blocks, of course). The first axis was determined mainly by the factor time, whereas the differentiation along the other axes was mainly due to the type of sowing (HD, LD, NC). Stepping stone treatment had the weakest explanatory power. To single out the effects of the particular treatments from each other, separate tests were calculated: the greatest explanatory power (26 % of total data variability, i.e. about 70 % of constrained variability calculated in analysis 1) could one ascribe to the factor time (analyses 2 & 2s). Species with a high score on the first axis, i.e., those with increasing density, were few: *Poa trivialis* (spreading from the stepping stones and road verges closed to the field), *Trisetum flavescens* (sown species which became a dominant wherever was sown) and *Taraxacum sp.* Low scores on the first axis were found with a number of annual weed species (Fig. 5.). Consequently, the ordination diagram shows a negative correlation between their density and density of clonally spreading *Trifolium repens* (the most frequent natural colonizer). The density of *Agropyron repens* (the second most frequent natural colonizer) did not show any common trend in time, and hence it was not depicted in the ordination space.

The second question is whether there is any effect of sown species on the composition of natural colonizers. Analysis 3 (without standardisation by sample norm) yielded, that only 7 % of total variability, i.e. about 20 % of constrained variability may be ascribed to the effect of sown species. As shown in ordination diagram (Fig. 6.), sown species were partly able to suppers the most problematic and still persisting weeds *Agropyron repens* and *Trifolium repens*. Standardized analysis revealed similar results. The hypothesis that the application of stepping stones will enhance the succession was tested by analyses 4 & 5. This environmental variable explained only 2 % of total variability, i.e. 6 % of variability constrained. The most successful colonizers originating from the stepping stones were *Poa* *trivialis*, *Achillea millefolium*, *Cerastium holosteoides*, *Stellaria graminea*, and *Agrostis tenuis*.

Data on vegetation changes of stepping stones were tested separately (Tab. 6.).

Tab. 6. Stepping stone samples analyses. S/A = season of stepping stone transplantation. See for further explanation Tab. 5.

	Question	Answer	Explanat. variables	Covari-ables	% expl.	Σ all constr.	P
6	Did the season of the stepping stone transplantation and/or the kind of the treatment (sowing) have any effect on the composition of the stepping stone vegetation? Is there any effect of time?	YES	S/A, S, T	B	8.2	0.20	0.01
7	Is there a common successional trend in absolute species representation inside stepping stones?	YES	T	B, S, S/A	8.3	0.08	0.008
7s	Is there a common successional trend in relative species representation inside stepping stones?	YES	T	B, S, S/A	8.1	0.08	0.008
8	Differ the stepping stones in accord with the season of transplantation?	YES	S/A, S/A*T	B, S, T	3.5	0.05	0.002
9	Differ the stepping stones in accord with the kind of the treatment?	Weak support	S, S*T	B, S/A, T	1.3	0.02	0.08

Similarly as in the case of data from central 1m^2 squares, I first used all environmental variables as explanatory ones to obtain the total part of explainable community variability and then I calculated separate tests to single out the effects of the particular treatments from each other. In the analysis 6 with all environmental variables the first ordination axis was determined mainly by the factor time, whereas the differentiation along the second axis was mainly due to the season when the stepping stones were transplanted (environmental variables explained together only 20 % of total community variability). The comparison of sums of all constrained eigenvalues from the particular tests revealed, that the most powerful explanatory variable was the factor time (about 10 % of total variability), season of transplantation explained approximately 5 % and there was not found statistically significant difference among stepping stones transplanted into different diversity treatments.

As shown in the ordination diagram (Fig.7.), *Trifolium repens*, *Agropyron repens*, *Myosotis arvensis*, *Viola arvensis* (natural colonizers), *Trisetum flavescens*, and *Lathyrus pratensis* (sown species) have a high scores on the first axis, i.e. their cover has increased inside stepping stones. On the contrary, *Veronica serpyllifolia*, *Alopecurus pratensis* and *Ranunculus acris* (stepping-stone species) became less frequent. *Anthoxanthum odoratum* (stepping-stone species) has a high score on both the axes, because it thrived well mainly inside stepping stones transplanted in the autumn. Cover of *Cerastium holosteoides* has decreased mainly in spring stepping stones, *Cardamine pratensis* has slightly increased inside autumn stepping stones.

The whole CLUE project also focused on the changes in dry matter production and here I would like state a part of the results (Tab. 7.).

Tab. 7. Dry matter yield in ton ha-1 yr-1. Data originate from corresponding experimental design (Šmilauer & Lepš, personal communication). Dynamics of dry matter yield was studied separately at HD, LD, and NC treated plots. I also mention yields from one treatment, which was not involved in my experimental design: the continual crop rotation (CCR). It can give a good imagine about how different the treatments are in nutrient utilization. CCR was not withdrawn from the agricultural use and the crop is harvested. The values for 1998 were not processed yet but they are expected to be markedly lower than those from 1997.

treatment	HD	LD	NC	CCR
1996	4.80	4.24	4.56	10.60 (oat)
1997	10.08	9.16	7.92	13.40 (barley)

Discussion

In summary it can be stated that sowing of the meadow plants was successful: most species became established and have created community altogether closed to the desired hay meadow. The levels of dry matter yield in 1997 were as high as those obtained from high productive species-poor meadows, which were intensively fertilized (Oomes 1990, 1992). Oomes observed significant decrease in dry matter production, especially during the first three to five years (depending upon the soil type) after fertilization had been stopped and regular mowing began. Similar changes are also expected in our experiment.

Results of CANOCO analyses show that there are significant differences among the three diversity treatments. Sown species were able to successfully compete with natural weed colonizers and their effect was more intense in the high diversity treatment. The higher competitive ability of species richer community can be ascribed primarily to following reasons:

First, the more diverse ecosystems are more likely to contain some species which thrive well at particular environmental conditions (Tilman & Downing 1994, Naeem et al. 1994, Symstad et al. 1998). Presence of such keystone species may have great effect on ecosystem functioning and in some cases may be more important than the species number per se (Symstad et al. 1998). Our data imply, that the difference between HD and LD treatments is partly caused by absence of such well thriving species in part of five alternative species assemblages used in LD treatments.

Second, plant productivity and resource utilization are greater at higher diversity (Tilman et al. 1996, Naeem et al. 1994, Symstad 1998, Scherer-Lorenzen 1998) so that more intensive competition for resources like nutrients or light may occur. Under such conditions plants with pure ruderal strategy (annual field weeds in our case) may be strongly outcompeted by C-S-R strategists (late-successional meadow species). For the same reason weaker ability of sown species to compete with *Agropyron repens* and *Trifolium repens* - plants having C-S-R to C-R strategy (Grime 1988) was observed. Such species (ruderal-perennials sensu Grime 1979) are most abundant in circumstances in which the impact of disturbance is less intensive, such as those which occur during the second and third year after colonization of bare soil in abandoned arable fields. Both *A. repens* and *T. repens* are clonally spreading perennials.

Agropyron repens has an extensive system of rhizomes which allowed the plant to cover large area. *Agropyron* was only rarely recorded from the seed bank but it regenerated from numerous rhizome fragments (Hejcman 1997). Experiences of many researcherchs (for example Schmidt & Brübach (1993), Grime (1979)) confirm that it is species which did not change either the spatial pattern or the mean annual cover for a long time: it is the most persistent natural colonizer in the site. The mean cover did not change at all, hence it is not depicted in ordination diagram of analysis testing the common successional trend in the field (Fig.5.). On the other hand, the impact of LD and HD treatments is obvious: *Agropyron* became least abundant in HD treatment while it is a dominant plant in NC treatment (Fig.6.).

Trifolium repens is a creeping stoloniferous legume, which regenerates almost exclusively by rooted stolons in closed communities. Despite only a few seedlings were recorded from the seed bank (Hejcman 1997), this species reached almost 40 % cover in the field in the first year (similar results experienced McDonald (1993)). The 'creeping' strategy enabled the colonization of stepping stones also (Fig.7.). *Trifolium* is intolerant to shade (Grime 1988), and hence it has been partly suppressed in the tall vegetation of HD as well as LD-treated plots (Fig.6.). The highest cover was observed in all plots in the second year (Tab. 4). Consequently, if some species has an optimum in second year, it is impossible to reveal this behaviour using RDA because that method is based on *linear* species response. This is a price one has to pay while focusing on certain model of species response: a part of change in the field community remains hidden anyway. For similar reason neither RDA nor CCA are able to test bimodal responses.

With regard to the results of analysis on the common successional trend and known cover of particular species it is assumed that some species performed this way: densities of *Myosotis arvensis* and partly *Viola arvensis* and *Stellaria media* were negatively correlated with *Trifolium repens* (Fig.5.) and, in the second year (when the *Trifolium* density culminated) these plants were rarer than the next year. Nevertheless these densities were ten times lower than in the first year, that is why CANOCO calculated negative scores on the first axis correlated with time (Fig.5.). The other natural colonizers depicted in the diagram (*Fallopia convonvulus*, *Veronica persica*, *V. arvensis*, *Poa annua*, *Polygonum persicaria*, *P. aviculare*, *P. hydropiper*, *Galeopsis tetrahit*, *Lamium purpureum*, *Gnaphalium uliginosum*, and *Sagina procumbens*) show predominantly strong decrease which is independent upon *Trifolium* densities. These results imply that the first group can partly endure the competition with present perennials whilst the remaining annuals are weak competitors specialised on the colonization of bare soil. There are more mechanisms playing a part in successional changes of weed communities.

First, the regeneration from seeds is essential for all annual weeds and therefore one can not wonder that there are many strategies. Germination often depends upon soil moisture, temperature as well as upon the amount and quality of light: number of plants germinate only when exposed to the daylight. *Veronica persica* and *V. arvensis* increase germination rate after dry storage; *Polygonum sp.*, *Galeopsis tetrahit*, *Fallopia convonvulus*, and perennial weed *Plantago major* often need chilling period to break the dormancy of seeds; *Polygonum persicaria* and *Myosotis arvensis* also more germinate after dry storage (Grime 1988).

Second, if sprouted, the saplings and mature plants also have particular preferences and demands. There is evidence that soil moisture, light penetration through the canopy, and the nutrient availability are in some cases diversity dependent (Tilman et al. 1996, Tilman et al. 1997, Naeem et al. 1994, Scherer-Lorenzen 1998) and thus the composition of plant community may be indirectly affected by species diversity.

It is probable that main plant characteristics allowed better performance of following annuals (fore a nice review, see Hejcman 1997):

Myosotis arvensis is a semi-rosette winter-annual with height to 30 cm, which enables it to compete with *Trifolium repens* for light. Germination of seeds is usually delayed until autumn which may also be advantageous, because *Myosotis* does not sprout till the meadow is mown.. According to Grime (1988) *M.a.* has strategy intermediate between ruderal and stress-tolerant.

Stellaria media is a short-lived winter or summer annual. It probably persists due to the ability to have two peaks in spring and autumn, when the vegetation cover is not too dense.

With regard to the performance of sown species, the absolute dominance of *Trisetum flavescens* (about 54% in HD as well as LD treatment) can be hardly explained with an easy theory because there are more alternative explanations. *Trisetum flavescens* has early seed set and almost intermediate germination has been documented in sites subject to summer drought. This may be expected to confer an advantage in hayfields. (Grime 1988). *T.f.* appears to show only modest specialization towards any ecological factor or turf structure, and Grime concludes that the species is a 'congenital subordinate', i.e. never more than a minor component of grassland communities which is in contrast to our observations. One can argue that *T.f.* is a quite frequent component of mesic upland meadows in Czech but unfortunately it is often caused by resowing those sites with mixtures containing cultivars of *T.f.* For the same reason it is still not clear whether the clone used in our experiment originated from an unaffected source.

The other sown grasses ranged from two (*Festuca rubra*, *Phleum pratense*, *Cynosurus cristatus*) to four (*Holcus lanatus*) percent in HD-treatment.

Lychnis flos-cuculi, *Medicago lupulina*, and *Galium verum* did not survived in any experimental design (*L.f.* did not germinate at all and only few seedlings of *M.l.* and *G.v.* were found in the first season). *M.l.* is annual to short-lived perennial which is usually absent from vegetation, dominated by robust perennial species and associated with relatively unproductive habitats (Grime 1988). Both the nutrient status and competition intensity reached obviously high levels to maintain this species in the field. *Trifolium dubium* with similar life form (winter-annual) and nutrient demands was also close to extinction.

The low densities of *Centaurea jacea* (one percent in LD only) and *Galium verum* (disappeared) are probably due to the weak germination in field. I have evidence from another similar experiment closed to our field, where both *Centaurea* and *Galium* were sown and performed very good. The other sown species are present in amounts close to values expected for comparable meadow communities.

From the botanical point of view, the transplantation of stepping stones can be considered 'medium successful'. Although almost all species survived the transplantation and performed well, the stepping stones had only a slight impact on the surrounding vegetation. They significantly contributed to the spreading of *Poa trivialis*. It is a plant having a high potential for the colonization of such recently disturbed habitats which are dominated by larger perennials and has often been observed in disturbed meadows (Grime 1979, 1988, McDonald 1993). Other stepping-stone species have spread only occasionally while the pool of species invading stepping stones were considerably larger: first colonizer was *Trifolium repens*. *Trisetum flavescens* (sown species) was able to invade only marginal parts of stepping stones. *Lathyrus pratensis* (sown species) invaded whole area of many stepping stones successfully due to the vegetative spreading by rhizome growth (see Fig. 8. for more comments). Since insignificant differences between the spring and autumn stepping stones were observed it could therefore be concluded that both ways of transplantation are possible.

Broadcasting soil on the stepping stone plots did not show any significant effect on species composition. The positive result can be hardly expected with regard to known composition of source-meadow seedbank. It consisted mainly of annuals (Tab.3.) which could not contribute to the meadow development. There is evidence that meadow species tend to have transient seed bank while most dicotyledonous weed species may remain viable in the soil for many years (McDonald 1993), thus the seed bank is often found to represent an earlier stage in vegetation succession.

Comparison of the stepping stone transplantation versus simple sowing revealed in our case a clear answer: the stepping stone approach can be recommended only as a 'last chance' practice if all alternatives to preserve some extremely rare habitat failed and there was possibility to remove the vegetation to a place close by. I have met with only one well documented restoration experiment (set up in Germany) so far: 0.4 ha of grassland vegetation together with a 0.5 m layer of soil was removed and monitored for the whole area over a period of five years (Bruelheide 1998). The new meadow was completely recovered and almost all species performed well.

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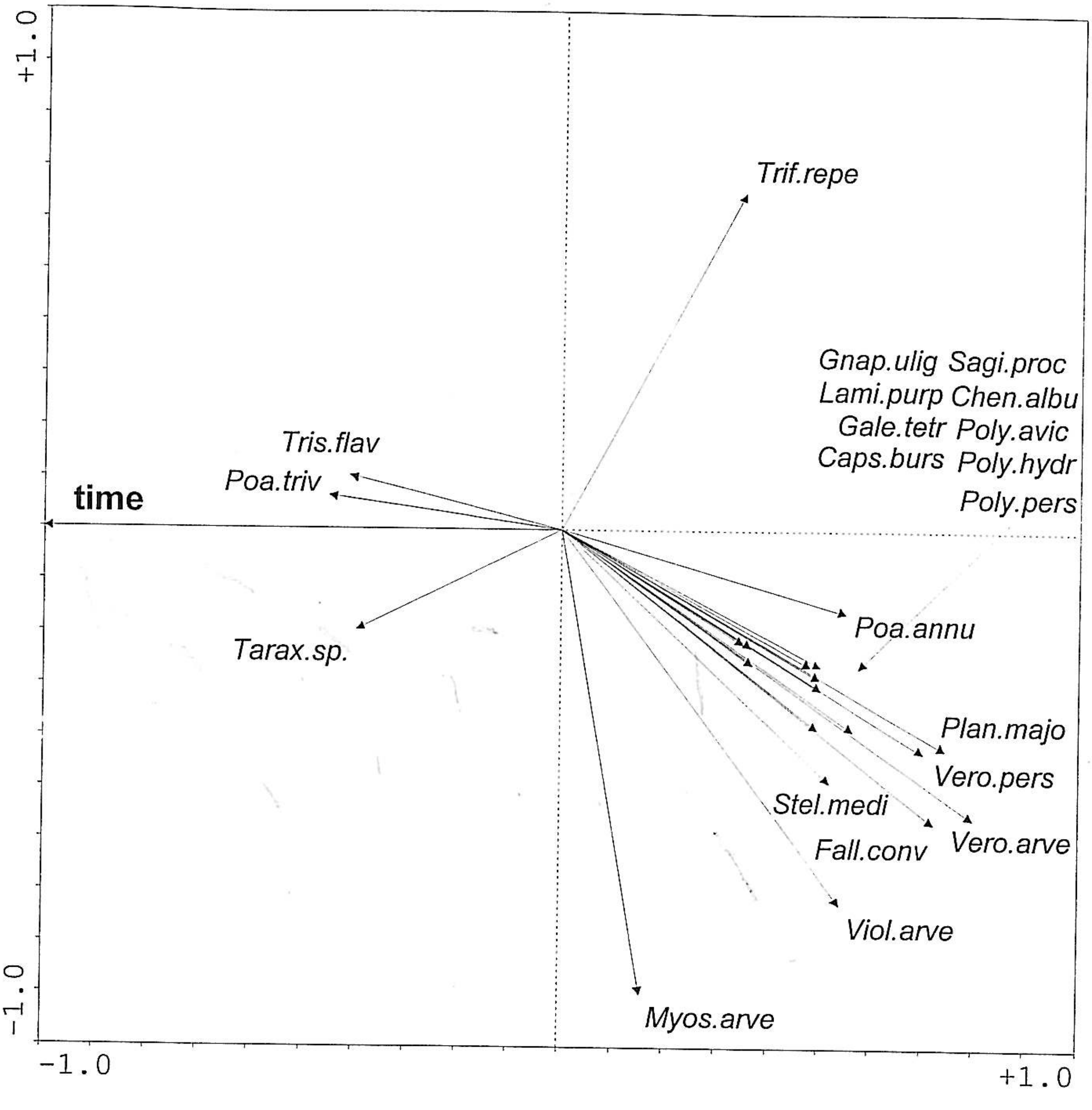

Fig. 5. Ordination diagram showing the trends in relative species representation in the field (analysis 2.). The cover of natural colonizers markedly decreased except *Poa trivialis*, *Taraxacum sp.* (natural colonizers) and *Trisetum flavescens* (sown species). Abbreviations of species are in Tab. 8. The diagram explains 35 % of the variance in the fitted species data. For clarity, only species with the highest correlation with the ordination axes were displayed.

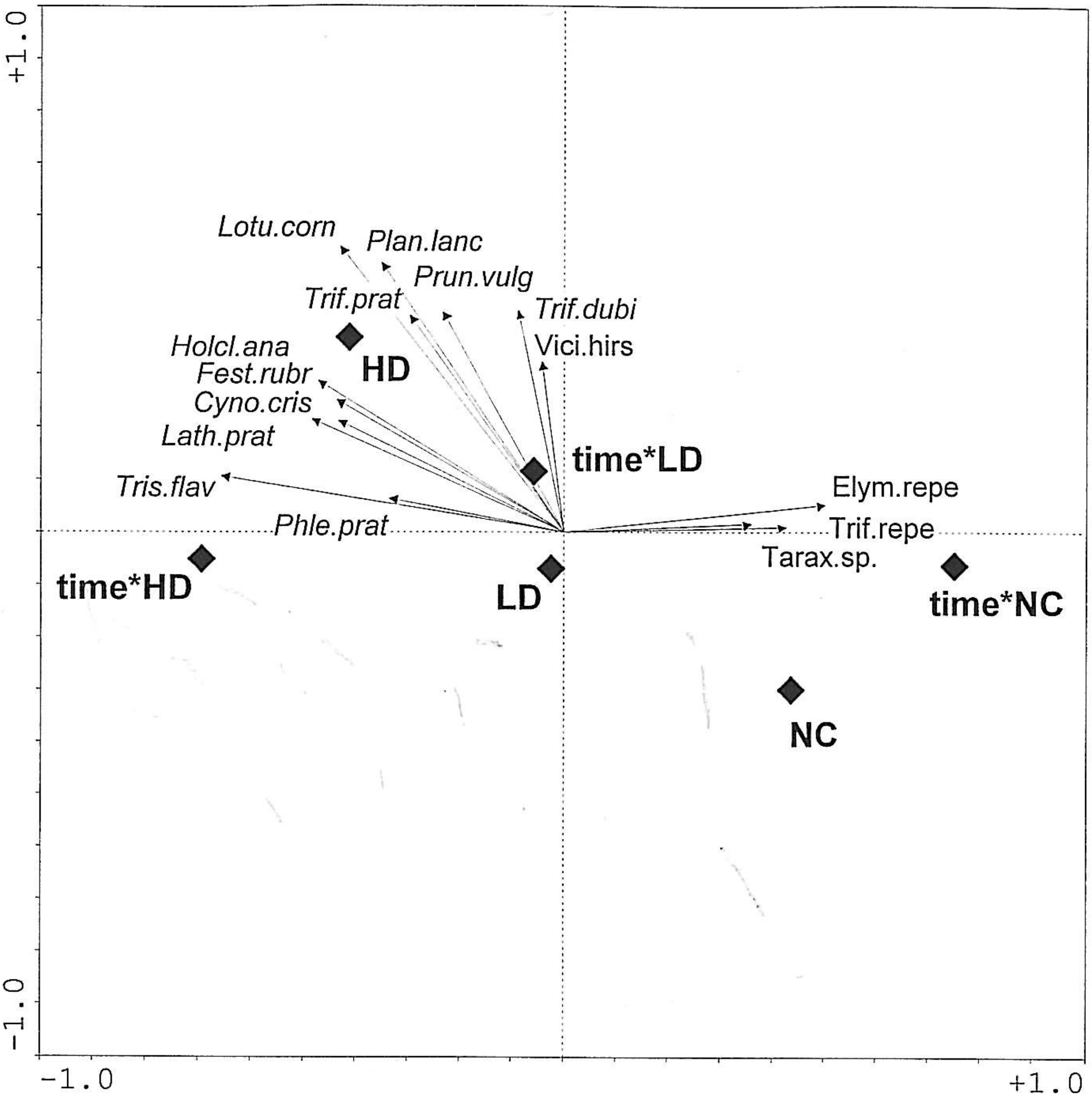

Fig. 6. Ordination diagram (analysis 3) showing the effect of sown species on the cover of *Agropyron repens*, *Trifolium repens*, and *Taraxacum sp*. Sown species are written in italics, abbreviations of species names are in Tab. 8. HD = high diversity treatment, LD = low diversity treatment, NC = natural colonization treatment. Interactions of particular treatments with time are indicated with an asterisk. The diagram explains 7 % of the variance in the fitted species data. For clarity, only species with the highest correlation with the ordination axes were displayed.

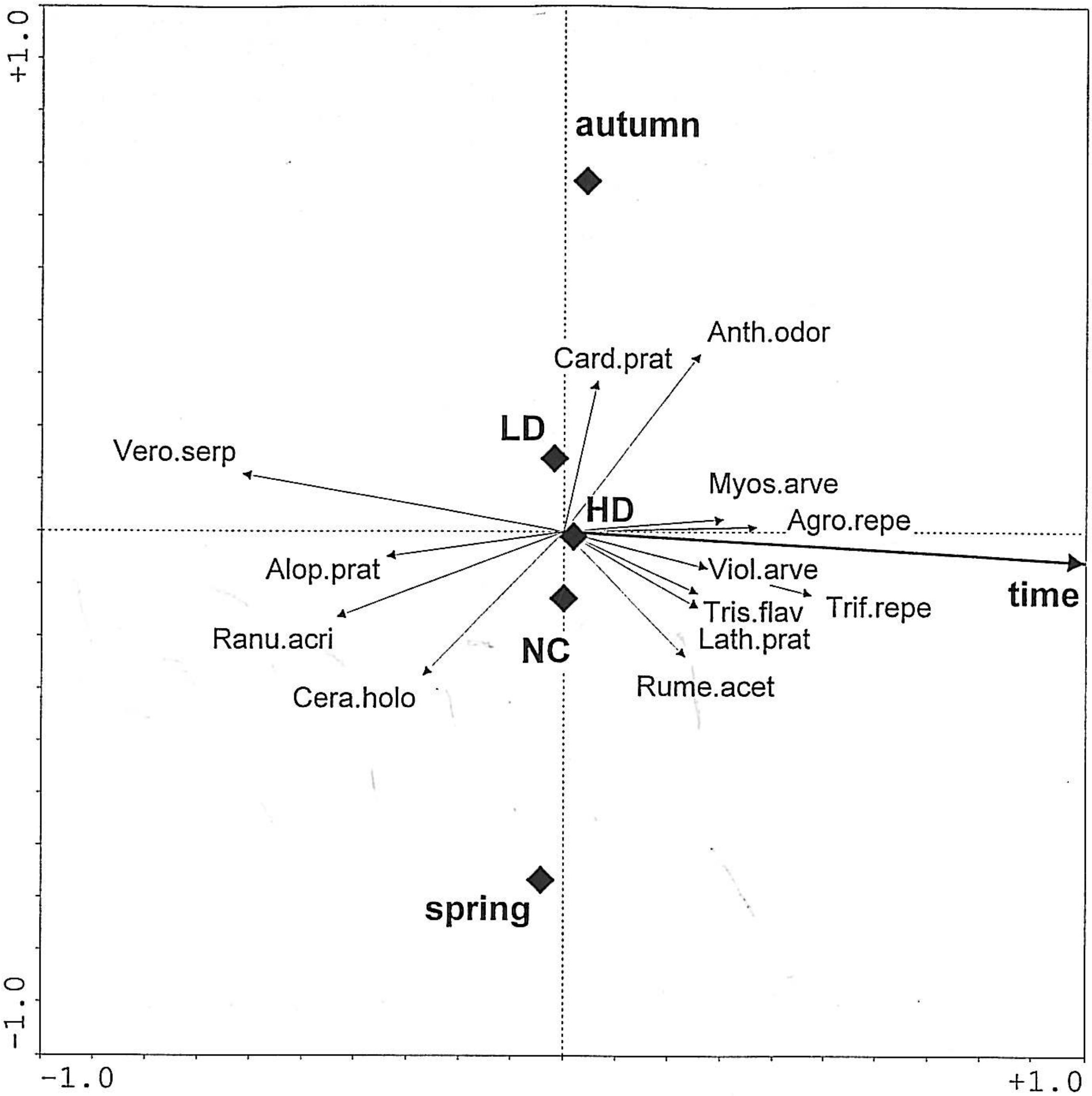

Fig. 7. Ordination diagram showing the changes of plant community inside stepping stones (analysis 6.). The density of *Veronica serpyllifolia* decreased probably due to the more intensive competition for light. *Anthoxanthum odoratum* preferred the autumn transplantation. This species has a second flush of shoot growth in autumn (Grime 1988). Thus, *A. o.* might regenerate immediately after the autumn removal. *Trisetum flavescens* and *Lathyrus pratensis* are the only sown species which were able expand inside stepping stones. Abbreviations of species names are in Tab. 8.; HD = high diversity treatment; LD = low diversity treatment; NC = natural colonization treatment; autumn/spring = season of stepping-stone transplantation. The diagram explains 20 % of the variance in the fitted species data. For clarity, only species with the highest correlation with the ordination axes were displayed.

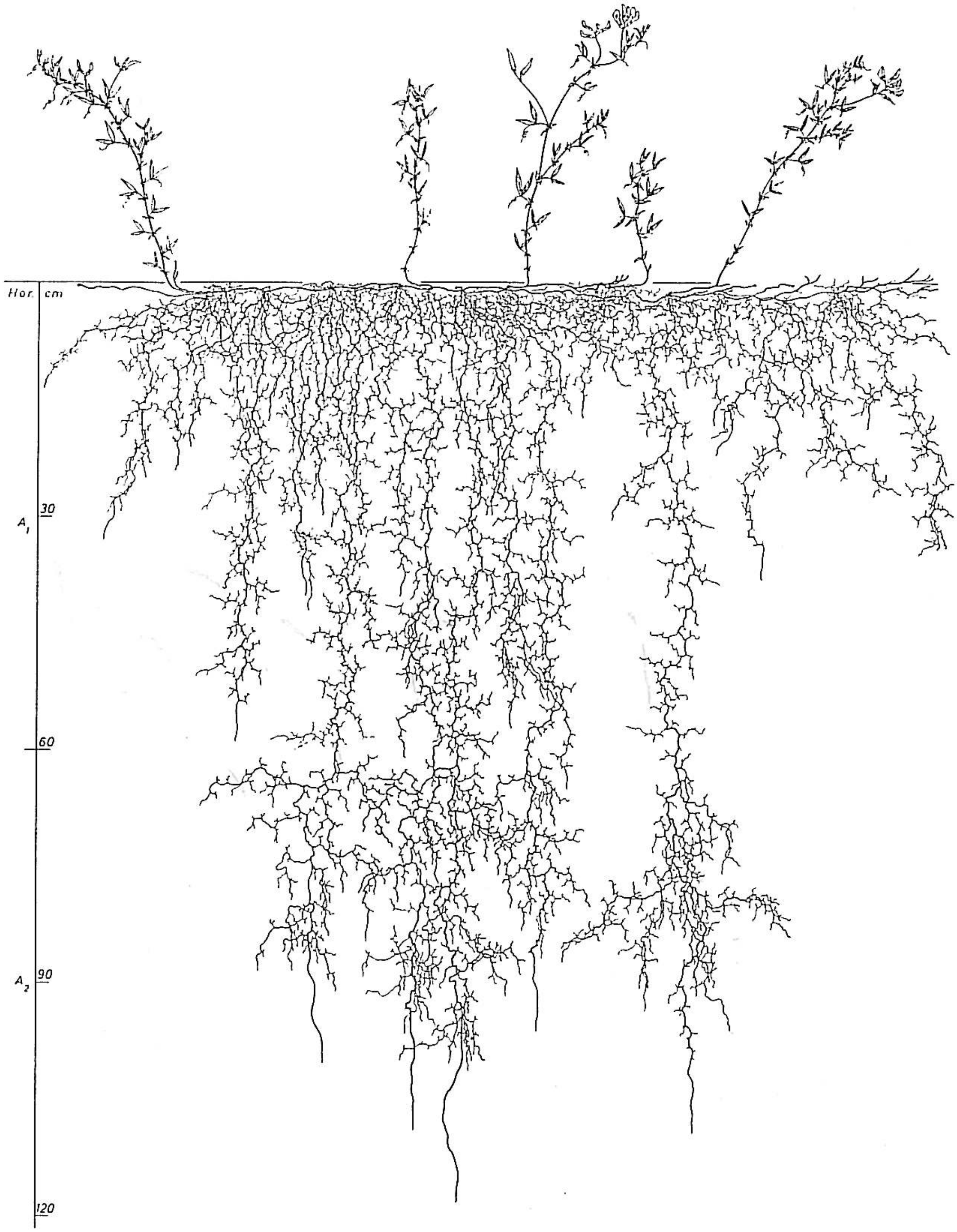

Fig. 8. *Lathyrus pratensis* has the capacity for lateral spread by means of rhizome growth. The picture clearly documents the strategy and gives also good idea about how *Lathyrus* could invade the stepping stones (picture originates from the root atlas of L. Kutchera (1995)).

Tab. 8. List of abbreviations used in ordination diagrams.

abbreviation	correct name	abbreviation	correct name
Agro.repe	*Agropyron repens*	Poa.triv	*Poa trivialis*
Alop.prat	*Alopecurus pratensis*	Poly.avic	*Polygonum aviculare*
Anth.odor	*Anthoxanthum odoratum*	Poly.hydr	*Polygonum hydropiper*
Caps.burs	*Capsella bursa-pastoris*	Poly.pers	*Polygonum persicaria*
Card.prat	*Cardamine pratensis*	Prun.vulg	*Prunella vulgaris*
Cera.holo	*Cerastium holosteoides*	Ranu.acri	*Ranunculus acris*
Cyno.cris	*Cynosurus cristatus*	Rume.acet	*Rumex acetosa*
Fall.conv	*Fallopia convonvulus*	Sagi.proc	*Sagina procumbens*
Fest.rubr	*Festuca rubra*	Stel.medi	*Stellaria media*
Gale.tetr	*Galeopsis tetrahit*	Tarax.sp	*Taraxacum sp.*
Gnap.ulig	*Gnaphalium uliginosum*	Trif.dubi	*Trifolium dubium*
Holc.lana	*Holcus lanatus*	Trif.prat	*Trifolium pratense*
Chen.albu	*Chenopodium album*	Trif.repe	*Trifolium repens*
Lami.purp	*Lamium purpureum*	Tris.flav	*Trisetum flavescens*
Lath.prat	*Lathyrus pratensis*	Vero.arve	*Veronica arvensis*
Lotu.corn	*Lotus corniculatus*	Vero.pers	*Veronica persica*
Myos.arve	*Myosotis arvensis*	Vero.serp	*Veronica serpyllifolia*
Plan.lanc	*Plantago lanceolata*	Vici.hirs	*Vicia hirsuta*
Plan.majo	*Plantago major*	Viol.arve	*Viola arvensis*
Poa.annu	*Poa annua*		

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⇧ Autumn stepping stone immediately after its transplantation. Treatment: low diversity.

⇦ Spring stepping stone immediately after its transplantation. Treatment: natural colonisation.

The 'high diversity' plot in the second year.