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RESEARCH PROJECT

An experimental protocol to assess the effects of plant diversity on ecosystem functioning utilized in a European research network

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Summary

1 This paper describes the methods of an experiment which provides a direct test of the relationship between biodiversity and ecosystem functioning.

2 Contrary to earlier biodiversity studies, here true replication of diversity levels is achieved. The experimental design and supplementary treatments designed to test invasibility and effects of disturbance on biodiversity are described.

3 A long-term monitoring program to evaluate ecosystem responses, incorporating eco-physiological, demographic and agricultural methods, is outlined.

4 We discuss the utility, advantages and shortcomings of the experiments, both in theoretical and applied contexts.

Keywords: agriculture, biodiversity, climate change, experimental design, methodology, stability

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Introduction

Estimates of world-wide species extinctions currently attain the unprecedented rate of about 150 species per day (Reid & Miller 1989), leading to a continuous decline of biodiversity. But to what extent the stability of ecosystems may depend on their biological diversity is largely unknown. Recent research suggests that the loss of biodiversity may impair the functioning of ecosystems. This provides an additional argument for the fundamental need to protect biodiversity (Lawton 1994; Naeem *et al.* 1994, 1996; Schulze & Mooney 1994; Tilman *et al.* 1996) and raises questions, such as: Does high diversity increase ecosystem resilience, stress tolerance and invasion resistance? Do different species, ecotypes or genotypes show differential responses to declining diversity? How are ecosystem processes and the population dynamics of target species influenced by biodiversity and how is this effect modified by environmental heterogeneity? What is the impact of various functional groups and their presence on ecosystem functioning?

Since 1996 an international consortium of researchers (BIODEPTH, Biodiversity and Ecological Processes in Terrestrial Herbaceous Ecosystems: Experimental Manipulations of Plant Communities, EC-Framework IV) has been addressing the above questions. At eight sites across Europe (Germany, Great Britain, Greece, Ireland, Portugal, Sweden, Switzerland), grassland communities with a range of plant-species diversities were created by either eliminating the natural vegetation or by ploughing arable fields, and sowing mixtures of randomly chosen species from the local species pool. The different diversities which were established on the sites varied from monocultures to a maximum diversity, the later representing the average species richness per m^2, characteristic of the surrounding grassland. Within these differing communities functional properties of terrestrial ecosystems, e.g. nutrient and carbon dynamics, succession and primary production, are measured.

Since herbaceous communities are widespread throughout Europe, they permit a comparative approach and were therefore chosen as experimental targets. Grasslands comprise diverse ecosystems, spanning intensively-managed species-poor pastures (e.g. *Trifolium-Lolium* mixtures) to extensive-utilized pastures and meadows with high biodiversity (e.g. calcareous grasslands, fens). The latter have shown a significant loss of plant species, due to climatic or management change (Fischer & Stöcklin 1997). Yet it is unknown to which extent these reductions of diversity will impair ecosystem processes and functions.

This description subsequently focuses on the Swiss experiment. We will describe the approach and the experimental design.

Experimental site

The experimental site at Lupsingen (47°27' N, 7°41' E; altitude 439 m a.s.l.) lies in the Swiss Jura mountains. The mean temperatures in January and July are 0.7 °C and 18.3 °C, respectively (mean annual temperature: 9.0 °C, 1986–1995), and the average rainfall amounts to 1046 ± 38 mm $year^{-1}$, based on data from the nearest meteorological station (*c.* 15 km to the east). The calcareous loamy soil overlies Jurassic bedrock, with a roughly neutral pH (6–7) in the upper soil horizon. The field site is a former arable field, previous crops were maize (1993) and rape seed (1994). After the harvest of the last crop the soil was ploughed and left bare over the winter months. In early spring 1995 the experimental area was harrowed twice within three weeks before the experimental plots were established.

Plant species selection

Selection of suitable plant species

A pool of species suitable for the experiment was selected from semi-natural species-rich grassland communities (belonging to the Arrhenatherion alliance, Ellenberg 1988) typical for this kind of habitat in Switzerland. Phytosociological (Ellenberg 1988) and agronomic data (Lehmann *et al.* 1992) were used for the selection of species. The ultimate set of 48 plant species used in the experiment was derived from the larger species pool by pragmatic choices such as availability of seeds and plant competitiveness (see also Table 3).

Allocation of species to diversity treatments

To assess the impact of species diversity on the ecosystem function random allocation of the species to diversity treatments is important (Givnish 1994). This requirement was

Table 1. *Levels of species diversity created in the experimental grassland plots of the Swiss BIODEPTH experiment. Included are the levels of replication and the occurrence of three "functional groups"* (G, *grasses;* H, *herbs;* L, *legumes)*

Diversity level (no. of species)	Replicates per block (diversity level)	No. of functional groups per plot	
1	10	1	(G, H, L)
2	7	1,2	(G, GH, GL)
4	8	1,2,3	(G, GH, GL, GHL)
8	5	1,2,3	(G, GH, GL, GHL)
32	2	3	(GHL)

followed within the following constraints: Each diversity level contained species belonging to three "functional groups" (*G,* grasses; *H,* non-legume herbs; *L,* legumes), and each mixture with two or more species had to contain at least 50% grasses (except for the maximum diversity treatment). In mixtures with the three functional groups the latter two had to occur in equal proportions. The species combinations within the diversity levels were randomly selected from two different subsets of species. "Set 1" contained typical species of commercial seed-mixtures for Arrhenatherion grasslands in Switzerland (Lehmann *et al.* 1992). They were included in every diversity level. "Set 2" contained species only included in diversity levels with 4, 8 or 32 species. For these diversity levels sampling with replacement of species from "set 1" was used to enhance the probability of obtaining realistic species mixtures.

Experimental design

PLOT LAYOUT

The maximum-diversity plots in the experiment contained 32 species, representing the species richness of surrounding semi-natural grassland communities in the Swiss Jura range. A range of monoculture plots was also established. Additionally, we chose three levels of intermediate species diversity, producing a short geometric series (Table 1).

Each diversity treatment had a different species composition, for example, there were monocultures of ten different species or eight-species mixtures of five different, partly overlapping species combinations. The reason for this "within-treatment variation" of species was to avoid the confounding of diversity. If the individual species and not the treatment is replicated (i.e. low diversity), results may depend on the individual traits of a particular species, more than on the diversity level *per se.* Such confounding was not avoided in previous biodiversity experiments (cf. Naeem *et al.* 1994; Leadley & Körner 1996; Schmid *et al.* 1996). In the present experiment the individual species combinations were randomly allocated within each of two blocks, yielding two replicates for each mixture. Fewer individual species combinations were used at the higher diversities because variability in ecosystem processes was expected to be lower among compositions with higher, compared to those with lower diversities.

The number of functional groups contained in the experimental communities increased with the number of species (Table 2); however, care was taken to produce as little confounding as possible among the two factors. An overview of the different species mixtures is given in Table 3.

Table 2. *Number of different species combinations per diversity level, arranged according to functional groups contained in the experimental grassland communities. Column sums equal the number of replicates per diversity level (see also Table 1)*

Functional groups	Diversity level (geometric series of species richness)				
	1	2	4	8	32
Grasses	6	3	2	1	0
Legumes	2	0	0	0	0
Herbs	2	0	0	0	0
Grasses + legumes		2	2	1	0
Grasses + herbs		2	2	1	0
Grasses + legumes + herbs			2	2	2
Number of replicates	10	7	8	5	2

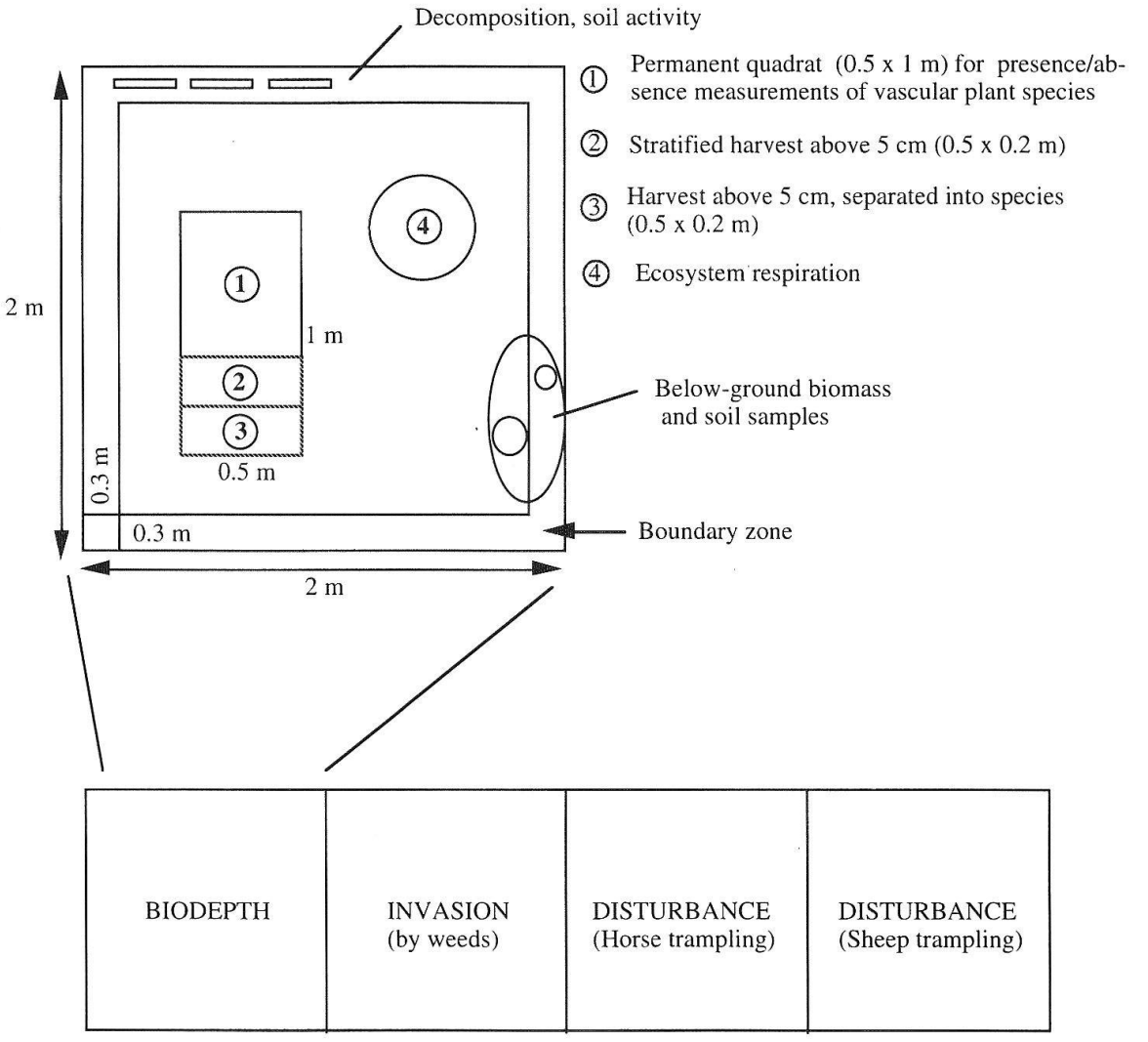

Fig. 1. *Experimental design within a given plot and zones utilized for various measurements. Assignment of various treatments to subplots was random.*

Table 3. *List of plant species utilized in the five diversity levels (1–32 spp.), arranged according to functional groups. Numbers and letters identify individual species mixtures (i.e. the number refers to the diversity level, while the letter refers to the species combination). Nomenclature follows Binz & Heitz (1990)*

1 species	2 species	4 species	8 species	32 species	
1a *Dactylis glomerata*	2a *Dactylis glomerata*	3a *Festuca pratensis*	4a *Anthoxanthum odoratum*	5a *Agropyron repens*	5b *Agrostis tenuis*
	Lolium perenne	*Holcus lanatus*	*Arrhenatherum elatius*	*Agrostis stolonifera*	*Anthoxanthum odoratum*
1b *Lolium perenne*		*Poa pratensis*	*Cynosurus cristatus*	*Alopecurus pratensis*	*Arrhenatherum elatius*
	2b *Arrhenatherum elatius*	*Trisetum flavescens*	*Dactylis glomerata*	*Arrhenatherum elatius*	*Dactylis glomerata*
1c *Poa pratensis*	*Festuca pratensis*		*Festuca pratensis*	*Cynosurus cristatus*	*Festuca ovina*
		3b *Cynosurus cristatus*	*Holcus lanatus*	*Dactylis glomerata*	*Festuca pratensis*
1d *Arrhenatherum elatius*	2c *Poa pratensis*	*Dactylis glomerata*	*Poa pratensis*	*Festuca rubra*	*Holcus lanatus*
	Trisetum flavescens	*Festuca pratensis*	*Trisetum flavescens*	*Lolium perenne*	*Lolium perenne*
1e *Trisetum flavescens*		*Lolium perenne*		*Poa pratensis*	*Phleum pratense*
	2d *Lolium perenne*		4b *Alopecurus pratensis*	*Trisetum flavescens*	*Poa pratensis*
1f *Festuca pratensis*	*Trifolium repens*	3c *Arrhenatherum elatius*	*Anthoxanthum odoratum*		
		Festuca rubra	*Cynosurus cristatus*	*Anthyllis vulneraria*	*Lathyrus pratensis*
1g *Trifolium pratense*	2e *Arrhenatherum elatius*	*Trisetum flavescens*	*Festuca rubra*	*Lathyrus pratensis*	*Lotus corniculatus*
	Trifolium pratense	*Trifolium repens*	*Lolium perenne*	*Lotus corniculatus*	*Medicago sativa*
1h *Trifolium repens*			*Poa pratensis*	*Onobrychis viciifolia.*	*Trifolium pratense*
	2f *Festuca pratensis*	3d *Holcus lanatus*	*Lathyrus pratensis*	*Trifolium pratense*	*Trifolium repens*
1i *Plantago lanceolata*	*Plantago lanceolata*	*Lolium perenne*	*Trifolium pratense*	*Trifolium repens*	*Vicia cracca*
		Poa pratensis			
1j *Taraxacum officinale*	2g *Poa pratensis*	*Trifolium pratense*	4c *Alopecurus pratensis*	*Achillea millefolium*	*Achillea millefolium*
	Taraxacum officinale		*Arrhenatherum elatius*	*Ajuga reptans*	*Ajuga reptans*
		3e *Dactylis glomerata*	*Dactylis glomerata*	*Anthriscus sylvestris*	*Campanula patula*
		Festuca rubra	*Festuca pratensis*	*Bellis perennis*	*Centaurea jacea*
		Trisetum flavescens	*Holcus lanatus*	*Centaurea scabiosa*	*Daucus carota*
		Ranunculus acris	*Trisetum flavescens*	*Crepis biennis*	*Geranium pratense*
			Plantago lanceolata	*Daucus carota*	*Heracleum sphondylium*
		3f *Arrhenatherum elatius*	*Ranunculus acris*	*Galium verum*	*Knautia arvensis*
		Cynosurus cristatus		*Knautia arvensis*	*Leucanthemum vulgare*
		Poa pratensis	4d *Arrhenatherum elatius*	*Plantago lanceolata*	*Pimpinella major*
		Achillea millefolium	*Dactylis glomerata*	*Ranunculus acris*	*Plantago lanceolata*
			Festuca rubra	*Salvia pratensis*	*Potentilla erecta*
		3g *Arrhenatherum elatius*	*Lolium perenne*	*Sanguisorba officinalis*	*Prunella vulgaris*
		Lolium perenne	*Lotus corniculatus*	*Scabiosa columbaria*	*Ranunculus acris*
		Lathyrus pratensis	*Trifolium repens*	*Silene vulgaris*	*Sanguisorba officinalis*
		Taraxacum officinale	*Achillea millefolium*	*Taraxacum officinale*	*Taraxacum officinale*
			Daucus carota		
		3h *Dactylis glomerata*			
		Festuca pratensis	4e *Festuca pratensis*		
		Lotus corniculatus	*Lolium perenne*		
		Plantago lanceolata	*Poa pratensis*		
			Trisetum flavescens		
			Trifolium pratense		
			Trifolium repens		
			Knautia arvensis		
			Taraxacum officinale		

WITHIN-PLOT TREATMENTS

Each plot measuring 8 m x 2 m was subdivided into four 2 m x 2 m subplots (Fig. 1). Each subplot was randomly assigned to one of four treatments: (1) undisturbed control, (2) invasion, (3) sheep disturbance, and (4) horse disturbance.

(1) Undisturbed subplots were used for measurements under the standard BIODEPTH protocol (see below), i.e. no manipulations were made apart from biomass removal (mowing) and soil coring (root biomass, soil nutrient status). They served as control in relation to the following treatments.

(2) Invasion subplots were established to test the effects of diversity on the establishment and spread of weed species. A number of experiments using a clonal perennial species (*Ranunculus repens*), annual and perennial weeds, and an annual root hemiparasite (*Rhinanthus alectorolophus*) were initiated in spring 1997.

(3, 4) Disturbance subplots were used to simulate the effects of livestock trampling and how it relates to plant diversity. The two treatments differed in the spatial scale of the trampling events mimicking the effects of sheep (treatment 3) and horses (treatment 4). A researcher equipped with ballast, yielding a total weight of *c.* 120 kg, and wearing artificial "hooves"-boots trampled the subplots twice per year during moist soil conditions following the mid- and late season biomass harvests. Roughly 15% of the total subplot area was trampled in both (3) and (4) with a force applied of about 1.9 kg cm^{-2}.

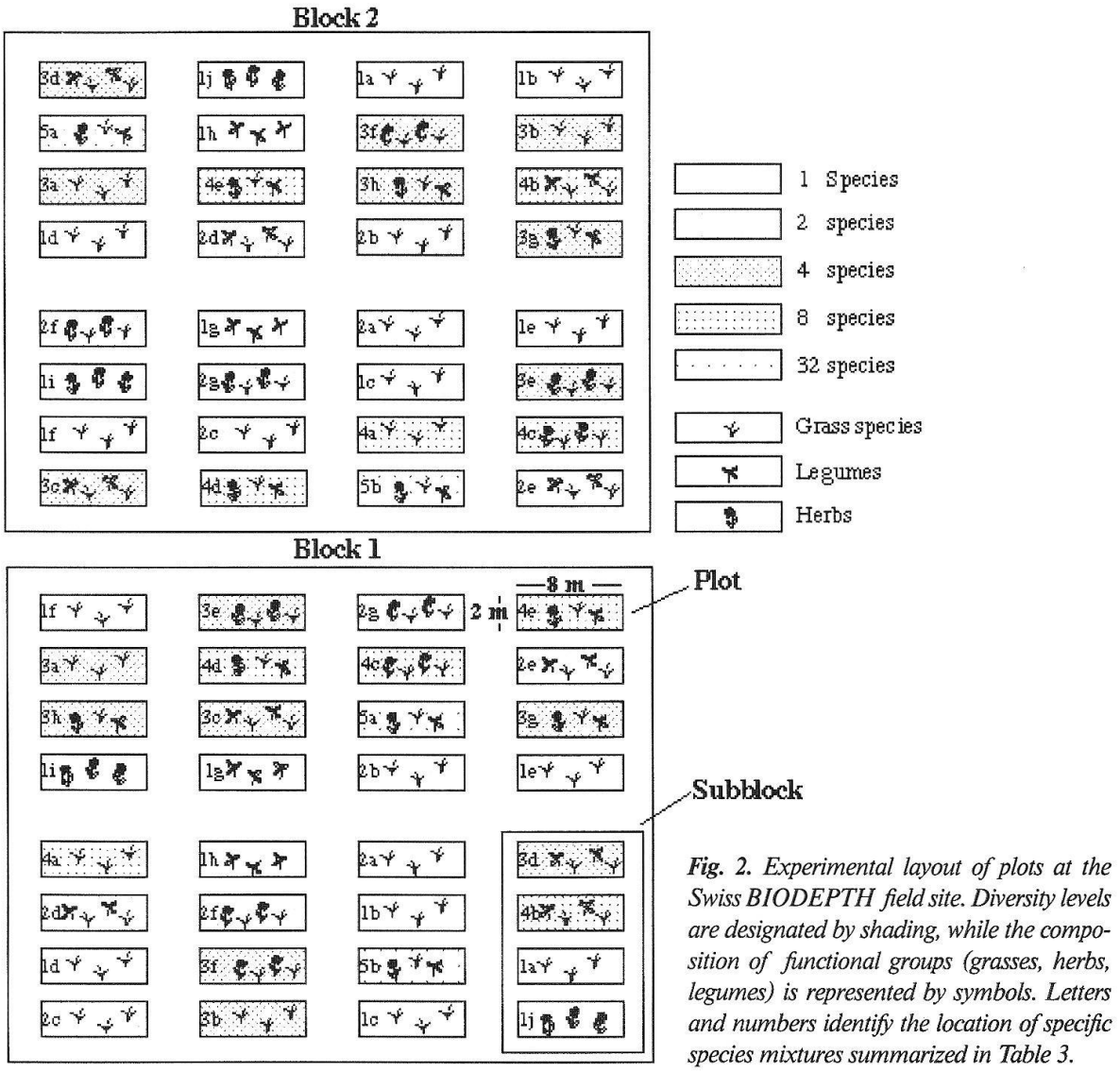

Fig. 2. Experimental layout of plots at the Swiss BIODEPTH field site. Diversity levels are designated by shading, while the composition of functional groups (grasses, herbs, legumes) is represented by symbols. Letters and numbers identify the location of specific species mixtures summarized in Table 3.

Experimental site

SITE PREPARATION

In late April 1995 two replicate blocks of 40 m x 30 m were set up at the experimental site. Within each block 32 plots of 2 m x 8 m (divided into four quadrats) were arranged into eight sub-blocks (Fig. 2). Within sub-blocks the width of the paths was 1 m and among sub-blocks and between blocks it was 3 m. All plots were separated from adjacent plots by paths sown with *Lolium perenne* in May 1995. We used *Lolium* to maintain vegetative cover between plots to avoid "island" and edge effects between plots with respect to ecosystem processes (rooting, nutrient dynamics). In addition, since clonal growth is negligible in this species, the likelihood of *Lolium* invasions into plots is minimal.

SOWING OF PLOTS

Seeds were obtained from commercial suppliers; the list of seed companies can be given on request. We attempted to select the nearest local variety, however in a few cases seeds from more distant sources had to be used. Prior to the sowing, germination tests were carried out in pots filled with soil and kept outside (three 10 cm x 10 cm pots per species, seeded with 10 seeds per pot) to test the seed viability (first column in Table 4). We tried to achieve a constant number of 2000 viable seeds m^{-2} divided equally among the species sown in each plot. Thus the amount of seeds taken for each species was roughly adjusted according to the observed germination rate. From 1–3 May 1995 the species mixtures were sown by hand after each plot had been weeded and raked. The soil surface of all plots was slightly compacted (rolled) after the sowing, to prevent desiccation and mortality of germinating seeds.

SITE MAINTENANCE

For the first two years after the sowing, all plants that were not sown were weeded in all plots in order to maintain diversity levels and to permit the establishment of the sown mixtures. The immigration of aggressive clonal weeds into the plots from outside was prevented by spot-treatment with a contact herbicide (glyphosate [N-(Phosphonomethyl)-glycine], Roundup®). the abundance of voles was controlled by frequent trapping and occasional fumigation with carbon monoxide.

The experimental protocol

PRELIMINARY MEASUREMENTS

Site characteristics

In each plot one pooled soil sample was collected 1995 by taking five soil cores (4 cm diameter, 10 cm depth) prior to the sowing on 1–3 May. The soil pH of the fresh soil samples from each plot yielded a range of 6.4 to 7.6 (0.01-M $CaCl_2$; pH meter 761, Knick, Berlin, Germany); no obvious gradient across the field site was conceivable. The soil nutrient status was analyzed by standard methods (Anonymous 1995). Measurements of NO_3^- were carried out on fresh material, whereas total N, PO_4^{3-}, K^+, Mg^{2+} and C-anorganic were analyzed on dried soil samples (40 °C, 48 h). The total carbon and nitrogen contents of soil samples were determined with a CHNS-analyzer (LECO-932, St. Joseph, Michigan, USA). The analytical results are summarized in Table 5. Unfortunately, comparative values from converted pastures are scarce. Data from a grassland site that was established on an arable field in Northern Germany more than two decades ago (Manzke 1995), suggest that C and N-pools at Lupsingen are higher and may decline in the future.

Table 4. Germination of species under controlled conditions and seedling establishment in the experimental plots, arranged according to functional groups (grasses, legumes, herbs). The amount of seeds taken for each species was roughly adjusted according to the observed germination rate

	Germination (%)	Surviving plants after 75 days in various diversity levels (m^{-2})				
		1 species	2 species	4 species	8 species	32 species
Agropyron repens	13	–	–	–	–	12
Agrostis stolonifera	37	–	–	–	–	2
Agrostis tenuis	53	–	–	–	–	10
Alopecurus pratensis	23	–	–	–	17	2
Anthoxanthum odoratum	53	–	–	–	31	6
Arrhenatherum elatius	7	520	265	133	77	37
Cynosurus cristatus	16	–	–	50	25	8
Dactylis glomerata	10	420	340	133	51	9
Festuca ovina	–	–	–	–	–	28
Festuca pratensis	16	450	200	163	55	32
Festuca rubra	13	–	–	120	60	20
Holcus lanatus	30	–	–	50	33	–
Lolium perenne	30	800	470	150	180	55
Phleum pratense	30	–	–	–	–	14
Poa pratensis	6	210	110	43	21	4
Trisetum flavescens	7	220	110	70	31	6
Anthyllis vulneraria	23	–	–	–	–	2
Lathyrus pratensis	10	–	–	90	60	13
Lotus corniculatus	33	–	–	260	100	50
Medicago sativa	53	–	–	–	–	42
Onobrychis viciifolia	–	–	–	–	–	4
Trifolium pratense	43	210	410	130	108	30
Trifolium repens	87	620	340	160	140	22
Vicia cracca	–	–	–	–	–	–
Achillea millefolium	83	–	–	84	40	15
Ajuga reptans	–	–	–	–	–	1
Anthriscus sylvestris	3	–	–	–	–	–
Bellis perennis	97	–	–	–	–	18
Campanula patula	43	–	–	–	–	4
Centaurea jacea	33	–	–	–	–	22
Centaurea scabiosa	3	–	–	–	–	18
Crepis biennis	13	–	–	–	–	20
Daucus carota	27	–	–	–	70	29
Galium verum	–	–	–	–	–	32
Geranium pratense	3	–	–	–	–	8
Heracleum sphondylium	–	–	–	–	–	–
Knautia arvensis	3	–	–	–	54	20
Leucanthemum vulgare	73	–	–	–	–	16
Pimpinella major	7	–	–	–	–	–
Plantago lanceolata	7	300	390	160	150	27
Potentilla erecta	–	–	–	–	–	–
Prunella vulgaris	7	–	–	–	–	26
Ranunculus acris	–	–	–	36	24	2
Salvia pratensis	10	–	–	–	–	24
Sanguisorba officinalis	–	–	–	–	–	8
Scabiosa columbaria	3	–	–	–	–	10
Silene vulgaris	10	–	–	–	–	20
Taraxacum officinale	30	270	200	120	70	20
Mean ± SE		402±249	554±365	445±169	517±242	545±108

Table 5. *Soil nutrient status at the beginning of the experiment (site 1), and from a grassland established on an arable field 20 years ago (site 2, Manzke 1995). Sample size for site 1 (the Lupsingen experiment) is 64, i.e. one sample per plot (means ± 1SE)*

Site	pH	Total C	Anorganic C	Total N	Soluble NO_3^-	Soluble PO_4^{3-}	K^+	Mg^{2+}
		(%)	(%)	(%)	(mg kg^{-1})	(mg kg^{-1})	(mg kg^{-1})	(mg kg^{-1})
1	7.2±0.1	3.74±0.04	0.18±0.03	0.38±0.00	81±1	1.61±0.05	5.83±0.24	14.0±0.5
2	–	2.41	0.54	0.17	–	–	–	–

Plant and community characteristics

The frequency of sown seedlings and weed species as well as the total vegetation cover and the cover of weed species were recorded in a permanent square of 0.5 m x 0.5 m in mid July 1995. A second estimate of the species composition and the total vegetation cover was carried out at the beginning of October 1995. The effective densities observed after 75 days are summarized in Table 4. Although the variability within treatment levels was high, the seedling density in plots ranged from 400–500 seedlings m^{-2} independent of diversity level. Furthermore, mean plant cover exceeded 80% by late summer 1995, in contrast to Tilman *et al.* (1996) who obtained cover values of merely 30–60%.

Mean stand height was estimated twice in 1995, in mid July and late September. Aboveground biomass was harvested at the end of July and at the end of September (to a height of 15 cm) for measuring community and species dry mass. The total nitrogen content of a dried subsample of each plot was analyzed with the CHNS-analyzer. After the biomass harvests in various subplots (see below), all plots were mown to a height of 15 cm at the end of July 1995, and the biomass was removed. Since autumn 1995 the plots have been mown to 5 cm in June and September. The plot and subplot boundaries, i.e. access paths maintained with *Lolium,* were mown more frequently to prevent the grass from flowering.

LONG-TERM MEASUREMENTS

Climatic and environmental measurements

The following climatic measurements were made with an automatic weather-station (WS01, Delta-T Devices, Burwell, UK): rainfall, soil temperature (5 cm and 10 cm depth), air temperature (0 cm, 5 cm and 2 m height), and relative humidity (2 m). Solar radiation was measured with a dome solarimeter at 2 m (GS1 pyranometer; Delta-T Devices). Hourly means of 1-minute measurement intervals were stored. Weekly means for all climatic data were calculated from daily means based on daily maxima and minima.

Plant diversity, phenology and vegetation structure

Twice a year, in early and mid season, the plant-species richness was recorded prior to mowing in the 2 m x 2 m subplot used for the core measurements (undisturbed subplot). At the same time presence and absence of plant species was recorded in 50 cells, each 10 cm x 10 cm, within a permanent area of 0.5 m x 1 m located at random in the central quadrat of 1.7 m x 1.7 m of each BIODEPTH subplot (see Fig. 1). Flowering phenology was censused by monthly visual surveys (starting in mid April) according to Mueller-Dombois & Ellenberg (1974).

An estimate of plant architecture in the different communities was obtained by measuring the canopy height with a sward stick and

by a stratified harvest in June (20 cm x 50 cm area), during which we differentiated three strata (5–15 cm, 15–50 cm, >50 cm; cf. Monsi & Saeki 1953). The vertical structure of the canopies was also recorded semi-quantitatively by taking a photograph of each canopy profile prior to the destructive harvest. Concurrently, measurements of light extinction in different height intervals of the canopies were performed with a ceptometer (Delta-T Devices). The leaf area index *(LAI)* of each plot was determined with an electronic fisheye sensor (LAI 2000, LI-COR, Lincoln, Nebraska, USA), the incident and transmitted *PAR* was measured with a Delta-T sunscan device (Delta-T Devices) at two heights: above the canopy and at ground level; the measurements were taken before the harvest in June and September.

Productivity and carbon fluxes

In a sampling area of 20 cm x 50 cm standing crop was harvested twice, down to a height of 5 cm at the end of June and at the beginning of September prior to each mowing. The harvested biomass was dried (80 °C, 24 h) and weighed. Community root biomass was measured at the same time as above-ground biomass in autumn. Two soil cores were taken at random positions within each undisturbed subplot, avoiding the centre which contained the permanent area (see Fig. 1). The 4-cm diameter, 20-cm deep cores were divided into four 5-cm deep strata and roots were extracted by washing and sieving (2 mm mesh size).

The soil CO_2 efflux was measured immediately before the first harvest with an infrared gas analyzer and a respiration cuvette of 10 cm diameter (EGM1, PP-Systems, Stotfold Hitchin Herts, GB). Respiration measurements of the whole ecosystem during nighttime were initiated before the second harvest. We used a closed system (short-term enrichment technique) with a chamber of 35 cm diameter and 90 cm height and an infrared gas analyzer (Li 6200, LI-COR, Lincoln, Nebraska, USA).

The decomposition rates were measured in each plot by inserting cotton strips vertically into the soil to a depth of 10 cm. The decomposition of the strip was assessed as the loss of dry mass over a period of time (May–July 1996 and onwards). As an estimate of long-term decomposition we used the loss of dry mass of buried wooden sticks (birch sticks) over 1.5 years (Naeem *et al.* 1994).

Water relations

An infrared thermometer (KT19.82, Heitronics, Wiesbaden, Germany) was used to measure the canopy surface temperature, as an indirect measure of moisture availability and evapo-transpiration according to the Bowen ratio concept (Jones 1992). This measurement was made once before the first yearly harvest, from 1997 onwards simultaneously with the soil moisture measurements of the upper layer (0–6 cm depth). The soil moisture was determined in each plot at the beginning of dry periods in June and July by time-domain reflectometry *(TDR)* using a portable Delta-T theta probe (Delta-T Devices).

Nutrient fluxes

Soil cores were taken prior to each autumn harvest to assess pH in calcium chloride, available P, total C and total N (2-cm diameter, 20-cm depth). From 1997 onwards, samples were also obtained below a depth of 20 cm. Nitrogen and phosphorus contained in the plant biomass were determined from a subsample of each biomass harvest to estimate nutrient retention in plant biomass.

Soil activity

To assess the feeding activity of the soil fauna a "bait lamina test" was carried out (Thörne 1990). The bait sticks were placed vertically in the soil down to a depth of 10 cm for 10 days in May 1996, sampling will continue in the following years. As another indicator of soil activity we measured the above-ground cast production of earthworms per ground area in spring and autumn 1996.

Insect diversity

Beginning in mid summer 1996 the diversity and abundance of major insect groups were determined with sweep net and suction sampler counts in undisturbed plots. Preliminary results indicated no direct correlation between insect and plant diversity, but herbivore diversity tends to be lowest in monocultures (J. Koritcheva, pers. comm.).

"Biosensor" experiment

This experiment is not integrated in the experimental design (cf. Fig. 2), it deals with another aspect of diversity, namely intraspecific variation. Three perennial species which occurred nearly at all eight sites of the BIODEPTH program were selected as model systems: *Dactylis glomerata* (grass), *Trifolium pratense* (legume), and *Plantago lanceolata* (herb). Seeds from each species were collected during the summer of 1996. At each site seeds from the local population and the other seven European sites were used for a reciprocal transplant experiment. The species will be planted in a grid in cleared, supplemental plots located adjacent to the experimental plots. Comparisons of the performance of plants from the same population at the different sites provide a measure of plasticity of ecotypes in various environments, thus providing information about the specific adaptations of ecotypes to their native habitats.

In a related experiment by A. Schreiber (Basel), ten different genotypes of *Trifolium repens* were planted into diversity plots and their performance was monitored. In addition, Ch. Binder (Basel) examined the survival, growth and biomass yields of six phytometer species planted into diversity plots. The performance was quite variable among species and genotypes, again supporting the idea that biodiversity experiments should include a number of species mixtures per diversity level to account for individualistic responses.

The general utility of this experimental protocol

The purpose of this protocol is twofold. We see it (1) as a guideline for designing and executing experiments on plant biodiversity, and (2) as a baseline to evaluate the re-establishment of species-rich grasslands. As mentioned previously, earlier experiments that examined the role of biodiversity in maintaining ecosystem functions suffered from methodological flaws (Givnish 1994; Naeem *et al.* 1994; Leadley & Körner 1996; Schmid *et al.* 1996). In this BIODEPTH experiment we undertook great efforts to avoid the confounding of diversity levels by species, and also paid attention to the fact that the diversity of functional groups increases with increasing biodiversity.

With respect to the agricultural policy (re-establishment of diverse grasslands) three questions needed to be addressed. Although it is deemed desirable to extensify grassland use and to re-establish species-rich grasslands, the best method to achieve this goal is scarcely known. Our project and another project (e.g. *Artenreiche Wiesen als ökologische Ausgleichsflächen,* BUWAL) address this issue

at present, and will provide long-term data on establishment success, persistence and the maintenance of biodiversity in re-established grasslands. Our protocol also describes effective monitoring tools. The second question, with respect to the agricultural policy, concerns the desirable level of biodiversity. It is not clear, neither from the theory nor from existing data (Lawton 1994), how much plant diversity is necessary to maintain sustainable (levels of) species diversity and to maintain various ecosystem processes (e.g. biomass yield, nutrient retention). The experiment described above should provide a quantitative basis leading to concise management objectives. The third question is related to the European agricultural policy that enforces restrictions on the free use of seed varieties in managed grassland (e.g. discourages use of locally-adapted varieties), and thus is likely to reduce genetic diversity within species across EU-countries. Results from the "biosensor" experiment will demonstrate the extent of ecotypic variation in three common grassland species.

Preliminary results and outlook

The described experiment will continue until 1999, to give us the opportunity to study established diversity plots for another three seasons. Although plant species diversities could be successfully established within a short period of 3–6 months at our site, leading to a monotonic increase in aboveground biomass with increasing diversity, other processes such as the establishment of full cover and maximum belowground biomass have not attained equilibrium. In calcareous grassland plots established from seedlings, root biomass did not reach values of undisturbed calcareous grassland within the third year of the experiment (P. Leadley, pers. comm.). Furthermore, attainment of steady-states of microbial biomass and soil nutrient dynamics require even longer time spans. However, the grasslands re-created in the course of the BIODEPTH-experiment more closely correspond to sown agricultural grasslands on fertile soil, thereby differing from nutrient-poor calcareous grassland not only in nutrient status, but presumably also in the rate of reaching steady-state conditions. Furthermore, it is important to study the effects of biodiversity on ecosystem functions not only at equilibrium, but also during the establishment phase.

Continued monitoring of insect diversity will yield information on colonization rates by herbivores. We suspect that this process did not yet attain equilibrium during summer 1996, the second year of the experiment. Establishment and effects of introduced weeds on diversity are studied in invasion subplots. Aside from these experimental invasions, we are still faced with continued and unwanted germination of weeds from the seed bank and wind dispersal, particularly in low diversity plots. Weed removal is necessary to maintain experimentally established diversity levels until equilibrium conditions are reached.

Despite these problems mentioned above our experimental design is likely to yield comprehensive data about the interrelationships between grassland biodiversity and ecosystem functions and processes, community invasibility and long-term maintenance of species richness, that will allow us to test several hypotheses (cf. Lawton 1994; Johnson *et al.* 1996). Combined with data from the other European members of the BIODEPTH consortium, we will be able to substantiate our local results and conclusions, and to extend them on a European scale.

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