Significance of legumes for the distribution of plant species in grassland ecosystems at different altitudes in the Alps

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Abstract

The composition of plant species in grassland communities is determined in part by negative and positive interactions among neighbouring plants; such interactions could be dispersal or – in the case of legumes – symbiotic N_2 fixation or resource acquisition. We hypothesised that small scale distribution of plant species may be in part affected by (i) the plant's narrow dispersal in the close neighbourhood, (ii) by the N input into the soil through symbiotic N_2 fixation by legumes, and (iii) by small scale heterogeneity of N availability in the soil. To address these hypotheses, in four species-rich semi-natural and natural grasslands along an altitudinal gradient from 900 to 2100 m a.s.l., the association of species that grew within a radius of 5 cm of selected plant species (central plant, CP) was studied. The distribution of the plant species within the plant community was not random and the species composition that grew within a radius of 5 cm of a CP varied with altitude. The strength of the association between a CP and its neighbouring species decreased with increasing altitude. The 5 cm radius was most dominated by individuals of the same species as the CP, suggesting that narrow dispersal (either through seeds or through vegetative propagates) may be most important for the species composition within the sward. $\delta^{15}N$ values of non leguminous species growing near legumes compared to non leguminous species that grew far from legumes indicate that N from symbiotic N_2 fixation may be another factor influencing the association of plant species. Effects of small scale heterogeneity in apparent N availability in the soil were detected to be the least important for the species composition.

Introduction

In natural environments, the distribution of living organisms is usually patchy, arranged along gradients or in combination of both. The species composition of plant communities is determined in part by negative and positive interactions among neighbouring plants. Differences in the plant's demand for resources, in the pattern of growth, propagation and the way in which species modify the environment for other plant species may all contribute to the spatial pattering in a plant community (Callaway 1995). On the other hand small scale differences or gradients in the physical environment may lead to associations of plant species which all grow well under particular

micro-environmental conditions. Turkington and Harper (1979a; 1979b) and Turkington et al. (1985) showed many interspecific, though mainly negative associations in a small area. Few studies have revealed that slight differences in the frequency of species or genotypes of plants may be related to differences in the physical environment (Turkington et al. 1979a) or in the plant species that grow nearby (Turkington 1989; Lüscher et al. 1992) or to a combination of both (Turkington 1989). In alpine regions, a considerable variability in the environmental conditions over a small area is assumed. Moreover, specific climatic and edaphic conditions require particular strategies of plants for growth. Thus plant-plant and plant-environment interactions may be important for the structure of permanent grassland communities such as alpine grasslands (Kikvidze 1993; Bertness and Callaway 1994; Callaway 1995; Hacker and Gaines 1997).

Insufficient supply of N substantially limits plant growth in many terrestrial ecosystems (Vitousek and Howarth 1991). Thus, the role of symbiotic N_2 fixation in structuring plant communities and its effect on the ecosystem may be considerable. Symbiotic N_2 fixation is an important component of the N balance of various legume species in the Alps, which acquire 59 to 90% of N from symbiotic N_2 fixation up to the upper limit at which they can grow (Jacot et al. 2000a). The amount of N that is apparently transferred from legumes to non leguminous species may be as much as 80 kg N ha^{-1} a^{-1} in low land grassland ecosystems (Boller and Nösberger 1988; Hogh-Jensen and Schjoerring 1994; Seresinhe et al. 1994; Zanetti et al. 1997); a beneficial effect of leguminous plants on the growth of the non leguminous companion plants was clearly shown by Hebeisen et al. (1997) under low-land conditions. Many studies have shown the importance of plants that symbiotically fix N_2 in primary successional series (Lawrence et al. 1967; Morris and Wood 1989; Chapin et al. 1994). Moreover, Thomas and Bowman (1998) found an apparent effect of legumes on the pattern of plant species in permanent alpine grassland; both facilitative and inhibitive effects of *Trifolium* were found.

Most work on possible positive and negative interactions between neighbouring plants was based on the statistical association between pairs of species (e.g., Kershaw 1958, 1959; Turkington

and Harper 1979a; 1979b). These patterns of association can be described either by an analysis of covariance or by correlation coefficients. In this study, we developed a 'plant-centred' sampling method to study all the neighbouring plant species within 5 cm of different plant species as central plant (CP). Analysing these neighbourhoods by means of the logistic regression technique enabled us to include all the neighbouring plant species in the same analyses as compared to the analysis of pairs of plant species only. The frequency of plant species near legume CPs was compared with that of plant species around non leguminous CPs. With the selection of non leguminous CPs with a high and a low N indicator value respectively we aimed to cover a larger range of small scale variability of the physical environment within each site.

The goal of this work was to determine whether the frequency of species is related to the plant species that grow nearby or to the physical environment or to a combination of both. Effects of the CPs on the species frequency in their neighbourhood were expected to be related to (i) narrow seed or vegetative dispersal (the same species as the CP would be dominant in the near neighbourhood) or (ii) increased N availability in the close neighbourhood of legumes. [15]N natural abundance was measured to provide information about apparent transfer of N between legumes and non leguminous species in their close neighbourhood. (iii) Association of plants which all grow well under particular physical environmental conditions would be visible in a low and high N indicator value of the neighbouring plant of the respective N indicator CPs.

Materials and methods

Sites

The experiments were conducted on a southern slope in the upper Rhine valley, between Sumvitg and Trun (approximately 45 km to the south-west of Chur in the eastern Alps of Switzerland; 46°45′, 8°57′ E) at four altitudes (900, 1380, 1900 and 2100 m a.s.l). All the studies were made on natural permanent grassland, where Gneiss (consisting of granite) dominates. The sites were described in detail by Jacot et al. (2000a).

Before the investigation began, the sites at 900 and 1380 m a.s.l. were mown in the traditional way in the summer and grazed late in the autumn. The higher sites were grazed only during the summer.

Coexistence studies

To assess the spatial pattern within a plant community, the plant species in the nearest neighbourhood (< 5 cm) around selected individuals were recorded in 1996 and 1997. Except at 2100 m a.s.l., recording began two weeks after the first mowing at each altitude: end of June at 900 m a.s.l., beginning of July at 1380 m a.s.l., beginning of August at 1900 m a.s.l. and mid-August at 2100 m a.s.l. (before mowing). At each altitude one study area (100 m^2) was selected and was divided into 50 subplots, 2 m^2 each. In each of 40 subplots chosen from the 50 subplots, two legume species (*Trifolium pratense/nivale*, *Lotus corniculatus/alpinus*), one non leguminous species indicating N-rich conditions (Landolt 1977) and one non leguminous species indicating N-poor conditions were chosen as central plants (CP). At 2100 m a.s.l., no non leguminous species indicating N rich conditions were chosen due to low abundance of high N indicators. Instead, two non leguminous species indicating N-poor conditions were selected as CPs. In each of the 2 m^2 subplots, one plant of each of the four different species (CPs) near to the centre of the subplot was chosen. All plant species within a radius of 5 cm of the CP were recorded separately in eight equal sectors. If a plant species was found in several sectors it was recorded once in each sector. Thus, one species may be recorded from 0 (did not occur) to 8 times (was found in each sector) in the neighbourhood of each individual CP. At each of the four sites, the neighbourhood of a total of 320 individual CPs (2 years × 4 CPs × 40 replicates) was examined.

^{15}N natural abundance

At all altitudes, three randomly selected non leguminous species from positions immediately neighbouring to legumes and in an area far from legumes (0.25 m^2) and legumes themselves were sampled (see also Table 3). All the dried (65 °C for 48 h) plant material was ground to very fine powder in sequence using a Cyclotec 1093 sample mill (Tecator, Höganäs, Sweden) and a ball mill (type MM2; Retsch, Arlesheim, Switzerland). After drying again (35°C for 24 h), the samples (2 mg) were weighed into tin caps (0.04 ml, Lüdi, Flawil, Switzerland) and analysed for ^{15}N concentration using a continuous-flow mass spectrometer (Europa Scientific, Cambridge, UK) at the Stable Laboratory Facility of the University of Saskatchewan, Saskatoon, Canada. The precision of the natural abundance mass spectrometer was ± 0.3 delta per mil.

When determining small differences in the ^{15}N concentration, data are usually recorded as δ^{15}N (‰) (Shearer and Kohl 1986), which is defined as follows:

$$\delta^{15}\text{N}(‰)$$
$$= \left(\frac{\text{atom\%}^{15}\text{N(sample)} - \text{atom\%}^{15}\text{N(standard)}}{\text{atom\%}^{15}\text{N(standard)}} \right)$$
$$* 1000,$$

(The standard is atmospheric N$_2$)

Soil nitrogen (N) and temperature (T) indicator values

The Swiss flora has been classified empirically according to various environmental parameters (e.g., soil N status, temperature, soil pH, soil water content); five categories reflect a mixture of physiological characteristic and frequency of occurrence under particular conditions (Landolt 1977). This classification may help to classify growing conditions of a plant community. Low N indicator values indicate that these plants occur mainly at nutrient-poor sites while high values indicate main occurrence at very rich sites. Low T indicator values indicate that these plants occur mainly under relatively low temperatures while high values indicate main occurrence at warm climatic conditions.

At all four sites, N and T indicator values (Landolt 1977) were recorded weighted for all species growing within a 5 cm radius of each CP (see Table 2).

Data analyses

The association of the neighbouring species and a particular CP was analysed with logistic regression

(LOGISTIC procedure in SAS; SAS Institute, Cary, NC, USA). Four different regressions were performed per altitude, each for one CP. For a particular regression, the value of the target CP was defined as 1 at recordings for that CP, while it was 0 for the recordings of the three remaining CPs. The present–absent data of the neighbouring species were used as explanatory variables. This procedure enabled to calculate a probability of a CP being present at the presence of a set of species in the close environment.

In a first analysis (model A), only the same species as the four CPs were used as explanatory variables resulting in a first set of 16 regressions (4 CPs × 4 altitudes). In a further analysis (model B), we aimed to assess a characteristic set from all the neighbour species which could predict the occurrence of the CPs. Thus, additional species were included as explanatory variables by a stepwise procedure. In these regressions, only neighbour species which reached a significance level of $p \leq 0.10$ were included in the final model. The exception was the same species as the four CPs, which remained always in the model. In all the regressions, species with less than ten records per site (altitude) were not included in the analysis.

[15]N natural abundance data were analysed with ANOVA applying the GLM procedure of SAS. The nitrogen and temperature indicator values (Landolt 1977) of the neighbourhoods of the CPs were tested with the Kruskal–Wallis test for non-parametric multiple comparisons.

Results

Coexistence studies

A total of 97 plant species were recorded over the two years (Table 1). The species were not randomly distributed. The four species chosen as central plants (CP) were usually associated with a characteristic set of plant species that influenced the presence of the CP positively or negatively (Table 2a–d).

For example, at 900 m a.s.l. the probability of *Trifolium pratense* being the CP was increased by the presence of *Trifolium pratense* itself, *Arrhenaterum elatius*, *Lolium perenne*, *Salvia pratensis*, and *Trisetum flavescens*, (higher probability of these species compared to the intercept; Table 2a,

Table 1. Plant species at the experimental sites at four altitudes between 900 and 2100 m a.s.l.

Species	Altitude (m a.s.l.)			
	900	1380	1900	2100
Agropyron repens	X	X	.	.
Agrostis tenuis	X	X	X	X
Anthoxantum alpinum	.	.	.	X
Anthoxantum odoratum	X	X	X	.
Arrhenaterum elatius	X	X	.	.
Avena pubescens	.	.	.	X
Cynosurus cristatus	X	X	X	.
Dactylis glomerata	X	X	.	.
Deschampsia flexuosa	.	.	.	X
Festuca pratensis	X	X	.	.
Festuca rubra	X	X	X	X
Holcus lanatus	X	X	.	.
Lolium perenne	X	X	X	.
Nardus stricta	.	.	X	X
Phleum alpinum	.	.	X	X
Poa alpina	.	.	X	X
Poa annua	X	X	X	.
Poa pratensis	X	X	.	.
Poa supina	.	.	.	X
Trisetum flavescens	.	X	.	.
Carex sp	X	X	X	X
Luzula sudetica	.	.	X	.
Lotus alpinus	.	.	.	X
Lotus corniculatus	X	X	X	.
Trifolium alpinum	.	.	X	X
Trifolium badium	.	.	.	X
Trifolium medium	.	X	.	.
Trifolium nivale	.	.	.	X
Trifolium pratense	X	X	X	.
Trifolium repens	X	X	X	.
Trifolium thalii	.	.	X	.
Vicia sativa	X	X	.	.
Achillea millefolium	X	X	X	.
Aegopodium podagraria	.	X	.	.
Ajuga genevensis	X	.	.	.
Ajuga pyramidalis	.	X	X	.
Alchemilla vulgaris	.	X	X	.
Arabis hirsuta	X	.	.	.
Arnica montana	.	.	X	X
Bellis perennis	.	X	.	.
Calluna vulgaris	.	.	.	X
Campanula barbata	.	.	X	X
Campanula rotundifolia	X	X	X	.
Carum carvi	.	.	.	X
Cerastium caespitosum	X	X	X	X
Chrysanthemum leucanthemum	X	X	.	.
Crepis aurea	.	.	.	X
Crepis biennis	.	X	.	.
Echium vulgare	X	.	.	.
Euphrasia minima	.	.	X	X
Galium anisophyllum	.	.	X	X
Galium mollugo	X	X	.	.
Gentiana kochiana	.	.	X	X
Geranium silvaticum	.	.	.	X

Table 1. Continued.

Species	900	1380	1900	2100
Altitude (m a.s.l.)				
Geum montanum	.	.	X	X
Gnaphallium silvaticum	.	.	X	X
Heracleum sphondylium	.	X	.	.
Hieracium alpinum	.	.	.	X
Hieracium pilosella	.	.	X	X
Homogyne alpina	.	.	.	X
Hypericum maculatum	.	.	X	.
Hypochoeris radicata	X	X	.	.
Knautia arvensis	X	X	.	.
Leontodon helveticus	.	.	X	X
Leontodon hispidus	X	X	.	.
Myosotis alpestris	.	.	.	X
Myosotis silvatica	X	X	.	.
Phyteuma betonicifolia	X	X	X	X
Plantago lanceolata	X	X	X	.
Potentilla aurea	.	.	X	X
Potentilla erecta	.	.	X	X
Ranunculus alpestris	.	.	X	.
Ranunculus bulbosus	X	.	.	.
Ranunculus friesianus	.	X	.	.
Rhinantus alectorolophus	X	X	.	.
Rumex acetosa	X	X	X	X
Rumex acetosella	.	X	.	.
Rumex obtusifolius	.	.	.	X
Salvia pratensis	X	X	.	.
Satureja vulgaris	X	X	.	.
Sempervirum montanum	.	.	.	X
Silene alba	X	X	.	.
Silene nutans	.	.	X	.
Silene vulgaris	X	X	.	X
Soldanella pusilla	.	.	.	X
Solidago alpestris	.	.	.	X
Taraxacum officinale	X	X	.	.
Thymus humifusus	.	.	X	.
Thymus polytrichus	.	.	.	X
Thymus pulegioides	X	X	.	.
Vaccinium myrtillus	.	.	X	X
Vaccinium uliginosum	.	.	.	X
Vaccinium vitis	.	.	.	X
Veronica chamaedris	X	X	X	.
Veronica officinalis	.	.	X	.
Viola tricolor	X	X	.	.
Botrychium lunaria	.	.	.	X

Plant nomenclature according to Hess et al. (1984) (⊡ grami-noids, ▣ legumes, □ non-legume dicots).

Model B). In contrast, the presence of *Lotus corniculatus*, *Dactylis glomerata*, *Silene vulgaris*, *Festuca rubra*, and *Trifolium repens* decreased the probability of *Trifolium pratense* being the CP (lower probability of these species compared to the intercept; Table 2a, Model B).

Patterns of associations of CPs with neighbour species varied with altitude. For example, at 900 m a.s.l. the CP *Lotus corniculatus* was positively associated with the presence of *Lotus corniculatus*, *Agrostis tenuis*, *Arrhenaterum elatius*, and *Festuca rubra* in the neighbourhood, while it was negatively associated with *Trifolium pratense* and *Silene vulgaris*. In comparison, at 1900 m a.s.l. the CP *L. corniculatus* was positively associated with *Lotus corniculatus*, *Potentilla erecta*, *Agrostis tenuis*, *Leontodon helveticus*, and *Nardus stricta*, while it was negatively associated with the presence of *Trifolium pratense*.

Only a few associations were found along a wider altitudinal gradient: *Agrostis tenuis* was one of the characteristic species in the neighbourhood of *Lotus corniculatus* at three altitudes (from 900 to 1900 m a.s.l.), and *Lolium perenne* was one of the characteristic species associated with *Trifolium pratense* at 900 and 1380 m a.s.l.

As evident from the generally decreasing R^2 values with increasing altitude, the strength of the association between a CP and neighbouring plants decreased with increasing altitude (Table 2a–d). Moreover, the number of plants that predicted (positively or negatively) the presence of the CPs clearly decreased with altitude.

In many cases, a CP was best characterised by the neighbourhood of individuals of the same species (see diagonal of the associations for the four CPs; e.g. in Table 2a, Model A and B). The CP *Trifolium pratense/nivale* was positively associated with other individuals of *Trifolium pratense/nivale* even at all four sites (from 900 to 2100 m a.s.l.). Similarly, the CP *Lotus corniculatus* was positively associated with other individuals of *Lotus corniculatus* at three sites (from 900 to 1900 m a.s.l.). This relationship is also revealed when the models A (only the same predictor as the four CPs) are compared with models B (all species potentially included as predictors). Though in most cases the models B explained significantly more variance than the reduced models A (likelihood ratio test), there was often only a minor increase in the R^2 value in model B compared to model A.

^{15}N natural abundance

$\delta^{15}N$ of the legumes was always close to 0 (Table 3). At the four sites between 900 and

Table 2. Backtransformed probabilities of logistic regression for the prediction of the central plant (CP) through the neighbouring plants (radius of 5 cm) at the altitude of **900 m a.s.l.** (900 m).

Central plant	*Trifolium pratense*	*Lotus corniculatus*	*Dactylis glomerata*	*Silene vulgaris*	
N indicator value[a]	3	3	4	2	*n*
Model A, Intercept	0.298****	0.169****	0.270****	0.320***	
Trifolium pratense	**0.675******	0.048**	0.147**	0.161**	135
Lotus corniculatus	0.021**	**0.540******	0.099***	0.202**	166
Dactylis glomerata	0.200**	0.149 ns	**0.417******	0.196**	220
Silene vulgaris	0.053*	0.013****	0.058†	**0.852******	77
Model B, Intercept	0.322*	0.074****	0.274****	0.428 ns	
Trifolium pratense	**0.697******	0.019**	0.150**	0.208*	135
Lotus corniculatus	0.012**	**0.340******	0.100***	0.296*	166
Dactylis glomerata	0.200***	0.065 ns	**0.422******	0.275**	220
Silene vulgaris	0.071*	0.005****	0.048*	**0.973******	77
Agrostis tenuis	–	0.096*	–	–	306
Arrhenaterum elatius	0.454*	0.147***	–	0.164***	135
Festuca rubra	0.097*	0.152*	0.392†	0.228†	58
Leontodon hispidus	–	–	–	0.328*	140
Lolium perenne	0.433*	–	–	0.329*	143
Salvia pratensis	0.453†	–	–	–	53
Trifolium repens	0.220***	-	–	0.475†	388
Trisetum flavescens	0.399†	–	–	–	128
p-value (Model A)	****	****	****	****	
R^2 (Model A)	51.8	54.9	36.2	42.6	
p-value (Model B)	****	****	****	****	
R^2 (Model B)	61.2	61.1	37.2	53.1	
Likelihood ratio test (Model A versus B)	****	****	†	****	

ns: $p > 0.1$; † $p \leq 0.1$; *$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$; ****$p \leq 0.0001$; – : term was not included in the final model.
[a]Landolt (1977).
In model A, only the same species as the four CPs were evaluated as predictor species (neighbour species). In model B, additional neighbour species with distinct effects ($p < 0.1$) were selected by a stepwise procedure. The backtransformed coefficients are the probability of presence of the CP when the predictor species is present. A probability higher than the intercept means a positive association between neighbour and CP; a probability lower than the intercept indicates a negative association. 'n' indicates total records of each species of a maximum of 2560 (2 years × 4 CPs × 8 sectors × 40 reps.). Bold are the same neighbour species as the CP.

2100 m a.s.l., the $\delta^{15}N$ values of the non leguminous species differed, depending on whether they grew in the immediate neighbourhood of a legume (near legumes) or in an area far from legumes (Table 3). With the exception of the site at 1380 m a.s.l., non leguminous plant species always had a $\delta^{15}N$ value closer to zero near a leguminous plant species than in an area far from legumes.

N and T indicator values

At 900 and 1900 m a.s.l. the N indicator value (Landolt 1977) of all associated species differed significantly among the central plants (Table 4a). The values were highest close to the legumes and the N indicator at 900 m a.s.l.

The T indicator values decreased from 900 to 2100 m a.s.l. (Table 4b). At 2100 m a.s.l., the T values of the plants around *Trifolium nivale* were lower than the T values around the other CPs.

Discussion

The frequency of some of the species varied according to the neighbouring plants (Table 2), indicating that there may be positive and/or negative interactions among plants. Our data indicate that narrow-range dispersal and soil N, the latter presumably in part influenced by symbiotic N_2 fixation in legumes, may affect the pattern of species in grassland ecosystems from 900 to 2100 m a.s.l. However, one has to bear in mind that our data

Table 3. Backtransformed probabilities of logistic regression for the prediction of the central plant (CP) through the neighbouring plants (radius of 5 cm) at the altitude of **1380 m a.s.l.** (1380 m).

Central plant	Trifolium pratense	Lotus corniculatus	Dactylis glomerata	Silene vulgaris	
N indicator value[a]	3	3	4	2	*n*
Model A, Intercept	0.244****	0.154****	0.332***	0.316****	
Trifolium pratense	**0.529****	0.076*	0.221*	0.131*	92
Lotus corniculatus	0.165 ns	**0.331****	0.246†	0.124*	84
Dactylis glomerata	0.215 ns	0.188**	**0.322** ns	0.281*	526
Silene vulgaris	0.124†	0.147 ns	0.178*	**0.551****	70
Model B, Intercept	0.154****	0.082****	0.487 ns	0.372*	
Trifolium pratense	**0.426****	0.034*	0.345*	0.160**	92
Lotus corniculatus	0.100*	**0.230****	0.403 ns	0.152*	84
Dactylis glomerata	0.152 ns	0.105***	**0.476** ns	0.333*	526
Silene vulgaris	0.080†	0.066 ns	0.293*	**0.617****	70
Achillea millefolium	–	–	–	0.310*	325
Agrostis tenuis	–	0.098**	0.451*	–	958
Cynosurus cristatus	–	0.035†	0.615*	–	58
Leontodon hispidus	–	–	0.390†	–	141
Lolium perenne	0.180†	–	0.354†	–	105
Plantago lanceolata	0.197*	0.058*	–	–	226
Poa pratensis	0.201*	–	0.376†	–	96
Trifolium repens	–	0.092†	–	–	482
Trisetum flavescens	–	0.097†	–	–	204
p-value (Model A)	****	****	***	****	
R^2 (Model A)	26.1	23.1	8.2	21.3	
p-value (Model B)	****	****	****	****	
R^2 (Model B)	29.9	34.2	19.4	23.1	
Likelihood ratio test (Model A versus B)	*	****	****	*	

ns: $p > 0.1$; † $p \leq 0.1$; *$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$; ****$p \leq 0.0001$; – : term was not included in the final model.
[a]Landolt (1977).
In model A, only the same species as the four CPs were evaluated as predictor species (neighbour species). In model B, additional neighbour species with distinct effects ($p < 0.1$) were selected by a stepwise procedure. Further details see Table 2a.

cannot fully explain how plant species influence each others growth in grassland ecosystems.

Narrow dispersal as one possible determinant of plant community structure

Neighbouring plants of the same species as the CP often grow close to the CP (Table 2). Many of these plants were seedlings, indicating either that seed dispersal was limited in distance or that conditions were favourable there for plant establishment, or both. Most of the CPs have large seeds (e.g., *Trifolium pratense* and *Lotus corniculatus*), which indicates limited distance in seed dispersal; thus, species patterning may also be affected by seed dispersal. This interpretation is supported by the finding that the patchiness of seed banks corresponds to the patchiness of surface plants (Cabin and Marshall 2000).

Beside seed dispersal, vegetative propagation could also contribute to this phenomenon as many of the CPs form short rhizomes. As a result, such offsprings dominate the community around their putative mother plants.

Symbiotic N_2 fixation of leguminous plants as a possible determining factor of species composition

In many other studies, co-adaptation resulted in a close relationship between legumes and certain grass species in permanent temperate grassland communities (Turkington and Harper 1979a, 1979b; Turkington 1989; Lüscher and Jacquard 1991; Lüscher et al. 1992). In this study, legumes as CPs seem to have a determining effect on the surrounding species (Table 2). As a mechanistic explanation, the most important positive interaction between a legume and its neighbours may be

Table 4. Backtransformed probabilities of logistic regression for the prediction of the central plant (CP) through the neighbouring plants (radius of 5 cm) at the altitude of **1900 m a.s.l.** (1900 m).

Central plant	Trifolium pratense	Lotus corniculatus	Phleum alpinum	Potentilla erecta	
N indicator value[a]	3	3	4	2	*n*
Model A, Intercept	0.169****	0.244****	0.403 ns	0.335**	
Trifolium pratense	**0.311****	0.202*	0.270***	0.202**	250
Lotus corniculatus	0.166 ns	**0.289***	0.374 ns	0.263*	141
Phleum alpinum	0.161 ns	0.232 ns	**0.418** ns	0.353 ns	636
Potentilla erecta	0.162 ns	0.265†	0.400 ns	**0.306**†	515
Model B, Intercept	0.280**	0.107****	0.427 ns	0.409 ns	
Trifolium pratense	**0.462****	0.089†	0.287***	0.255**	250
Lotus corniculatus	0.279 ns	**0.130***	0.410 ns	0.323*	141
Phleum alpinum	0.263 ns	0.105 ns	**0.446** ns	0.426 ns	636
Potentilla erecta	0.269 ns	0.122*	0.428 ns	**0.379**†	515
Agrostis tenuis	0.231*	0.130*	–	–	318
Festuca rubra	–	–	–	0.379†	557
Leontodon helveticus	–	0.178**	–	0.257†	53
Nardus stricta	0.259*	0.118**	0.406*	–	1244
Trifolium repens	–	–	0.462†	–	354
p-value (Model A)	****	**	****	****	
R^2 (Model A)	33.9	7.1	15.1	14.3	
p-value (Model B)	****	****	****	****	
R^2 (Model B)	37.4	16.8	18.8	18.0	
Likelihood ratio test (Model A versus B)	**	****	*	*	

ns: $p > 0.1$; † $p \leq 0.1$; *$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$; ****$p \leq 0.0001$; – : term was not included in the final model.
[a]Landolt (1977).
In model A, only the same species as the four CPs were evaluated as predictor species (neighbour species). In model B, additional neighbour species with distinct effects ($p < 0.1$) were selected by a stepwise procedure. Further details see Table 2a.

the transfer of symbiotically fixed N (Hartwig 1998). A previous study showed that symbiotic N_2 fixation is important for the N balance of the various legume species in the study area up to the highest altitudes of legume growth (Jacot et al. 2000a). A species that is efficient in fixing N_2 usually has a $\delta^{15}N$ close to 0 (Table 3) because, per definition, the fixed N derived from the atmosphere has a $\delta^{15}N$ value of 0. When a non leguminous species profits from the N that originated from the legume, its $\delta^{15}N$ value would, thus, become diluted and expected to be close to zero. The observed decrease in the $\delta^{15}N$ values of non leguminous species that grow near to legumes compared to non leguminous species growing far from legumes suggests an apparent transfer (mineralised legume N) of symbiotically fixed N (Table 3). For example, *Agrostis tenuis* at 900 m a.s.l., which was strongly associated with *Lotus corniculatus* (Table 2), had a $\delta^{15}N$ value of 2.10 when it grew near leguminous plants and a value of 3.59 in an area far from legumes, indicating that *A. tenuis* was influenced by symbiotic N that was fixed originally by the leguminous plant. This suggests that a relatively N-rich environment, as created by symbiotic N_2 fixation in the neighbourhood of *L. corniculatus*, may give *A. tenuis* a competitive advantage. Although this result suggests that there may be a transfer of N, the $\delta^{15}N$ values were too low to quantify. $\delta^{15}N$ values around 6.0 are necessary since the accuracy of the method decreases when the $\delta^{15}N$ values are low (Domenach and Corman 1984; Ledgard and Peoples 1988; Peoples et al. 1989; Jacot et al. 2000a).

The concentration of N in the legumes in this study was consistently higher than in non leguminous species (Jacot et al. 2000b), presumably leading to a higher N concentration in the litter. The importance of the quality of litter (decomposition) was demonstrated for grass species by Wedin and Tilman (1990); the dramatic divergence of net mineralization rates of N in initially identical soil under five perennial grass species showed a potential for strong interactions among the species composition and N cycling in the ecosystem. Likewise, Thomas and Bowman (1998) showed that extractable soil mineral N was significantly higher in legume patches than in patches

Table 5. Backtransformed probabilities of logistic regression for the prediction of the central plant (CP) through the neighbouring plants (radius of 5 cm) at the altitude of **2100 m a.s.l.** (2100 m).

Central plant	Trifolium nivale	Lotus alpinus	Leontodon helveticus	Potentilla erecta	
N indicator value[a]	3	3	2	2	n
Model A, Intercept	0.195****	0.257**	0.288**	0.279**	
Trifolium nivale	**0.332****	0.127*	0.270 ns	0.189*	101
Lotus alpinus	0.194 ns	**0.282** ns	0.270 ns	0.273 ns	180
Leontodon helveticus	0.184 ns	0.246 ns	**0.303** ns	0.285 ns	238
Potentilla erecta	0.184 ns	0.271 ns	0.272 ns	**0.284** ns	329
Model B, Intercept	0.170****	0.257**	0.225***	0.363 ns	
Trifolium nivale	**0.303****	0.127*	0.201 ns	0.265†	101
Lotus alpinus	0.169 ns	**0.282** ns	0.210 ns	0.346 ns	180
Leontodon helveticus	0.165 ns	0.246 ns	**0.241** ns	0.397 ns	238
Potentilla erecta	0.157 ns	0.271 ns	0.214 ns	**0.362** ns	329
Alchemilla vulgaris	–	–	0.281†	–	53
Campanula rotundifolia	–	–	–	0.231†	63
Geum montanum	–	–	0.301†	0.221*	62
Soldanella pusilla	–	–	–	0.053*	43
Thymus polytrichus	0.296*	–	–	–	25
Vaccinium myrtillus	–	–	–	0.457*	86
p-value (Model A)	****	**	ns	ns	
R^2 (Model A)	24.6	13.4	1.9	6.7	
p-value (Model B)	****	**	ns	****	
R^2 (Model B)	29.2	13.4	7.7	27.4	
Likelihood ratio test (Model A versus B)	*	–	*	****	

ns: $p > 0.1$; † $p \leq 0.1$; *$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$; ****$p \leq 0.0001$; – : term was not included in the final model.
[a]Landolt (1977).
In model A, only the same species as the four CPs were evaluated as predictor species (neighbour species). In model B, additional neighbour species with distinct effects ($p < 0.1$) were selected by a stepwise procedure. Further details see Table 2a.

Table 6. δ^{15}N of legumes and plant species near legumes (*Trifolium pratense/nivale* and *Lotus corniculatus/alpinus*) or in an area far from legumes at four altitudes in 1998 ($n = 12$).

Altitude (m a.s.l.)	Plant species	Near legumes	Far from legumes	SE	p
		δ^{15}N			
900	Legumes	− 0.18		0.17	
	Agrostis tenuis	2.10	3.59	0.16	***
	Trisetum flavescens	2.00	2.29	0.28	ns
	Leontodon hispidus	3.88	4.07	0.22	ns
1380	Legumes	1.5		0.66	
	Festuca rubra	4.48	2.85	0.46	*
	Trisetum flavescens	1.63	0.78	0.24	ns
	Leontodon hispidus	4.59	2.95	0.41	*
1900	Legumes	− 0.59		0.16	
	Festuca rubra	− 0.65	1.87	0.28	***
	Nardus stricta	0.42	2.19	0.58	*
	Potentilla aurea	− 0.26	0.09	0.30	ns
2100	Legumes	− 0.20		0.16	
	Festuca rubra	0.71	− 2.24	0.46**	
	Leontodon helveticus	− 1.82	− 3.38	0.20***	
	Potentilla aurea	− 2.78	− 3.90	0.29**	

ns: $p > 0.1$; † $p \leq 0.1$; *$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$; ****$p \leq 0.0001$.

of soil without legumes. In this respect, the high rate of symbiotic N_2 fixation of legumes measured at these sites (Jacot et al. 2000a) would result in greater amounts of mineral N being left for the associated non leguminous plants. The finding that the average N indicator values of plant species in

Table 7. Medians of nitrogen (N) and temperature (*T*) indicator value (Landolt 1977) of all plant species (Table 1) close (*r* = 5 cm) to different central plant species (CP) (two legumes, a high N indicator and a low N indicator) at four altitudes in 1996 and 1997.

Central plant	*Trifolium pratense/nivale*	*Lotus corniculatus/ alpinus*	high N indicator	low N indicator		
(a) Altitude (m a.s.l.)	N indicator value				*p* (CP)	n
900	3.20[a]	3.17[a]	3.26[a]	2.83[b]	****	77
1380	3.25	3.31	3.30	3.25	ns	77
1900	2.77[a]	2.55[b]	2.68[ac]	2.52[bc]	***	80
2100	2.34	2.27	Nm	2.24	ns	40
(b) Altitude (m a.s.l.)	*T* indicator value				*p* (CP)	n
900	3.25[ab]	3.18[b]	3.33[a]	3.17[b]	****	77
1380	3.00	3.05	3.03	3.00	ns	77
1900	2.23	2.24	2.11	2.17	ns	80
2100	1.73[b]	1.85[a]	Nm	2.00[a]	***	40

nm: not measured; ns: $p > 0.05$; $p \leq 0.1$; *$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$; ****$p \leq 0.0001$.

the vicinity of legumes as CPs were generally higher than around the low N indicator CPs (Table 4a), is consistent with this interpretation.

The fact that some $\delta^{15}N$ values of non leguminous species were well above zero when growing near to legumes, e.g., at 1380 m a.s.l., indicates that spatial N uptake may also influence the $\delta^{15}N$ values in the vicinity of legumes & non leguminous species. Moreover, the effects of management such as animal grazing or spreading of manure (e.g. from concentrate fed animals) may affect the $\delta^{15}N$ in such a way it can no longer be used to determine symbiotic N_2 fixation or apparent N transfer.

Another indication for the benefit of legume-originated N for non leguminous plants could be a comparison of N concentrations between those growing in the neighbourhood of legumes with those growing in the neighbourhood of non leguminous plants. However, one has to be aware that available soil N affects also the development of plants which itself interferes with their N concentration in tissue. Indeed, our results show no clear pattern (data not shown); in tendency, non leguminous plants growing in the neighbourhood of legumes have even a lower N concentration compared to those growing in the neighbourhood of non leguminous plants.

Other biotic or abiotic mechanisms determining species composition in the near neighbourhood of CPs

In addition to the beneficial effect of N, dissimilarity in size, growth habit, plant spacing or physiological and morphological adjustments (Soussana and Lafarga 1998) as well as a temporal partitioning of the root and shoot zone may also contribute to the co-existence of certain plant species (Gimingham 1978). We found that species that indicate smaller amounts of soil N (e.g., *Festuca rubra*) coexist with species that indicate N-rich conditions (e.g., *Dactylis glomerata*); similarly, tall species (e.g., *Silene vulgaris*) often grow close to short species (e.g., *Trifolium repens*) (Table 2). At the highest altitude, the vicinity of *Potentilla erecta* was dominated to a great extent by *Vaccinium myrtillus* (Table 2). This indicates that certain herbaceous species that grow near to shrubby species may have a protective benefit from the latter. Such 'complementary strategies' may enable species to coexist and may in part be responsible for the stability of certain aggregations of species.

Beside positive plant-plant interactions, competition may also contribute to species composition. At 900 m a.s.l., e.g., *Silene vulgaris* did not usually grow close to *Trifolium pratense*, *Lotus corniculatus* and *Dactylis glomerata* (Table 2), indicating competition for below-ground resources and/or above-ground resources (light).

Legumes may not only influence neighbouring plant species through N availability; Thomas and Bowman (1998) showed that extractable P was lower in the soil of legume patches than in the soil where other plant species grew. This may be the result of a greater uptake of P by legumes relative to non leguminous plants. The fact that *Agrostis tenuis* for example was not associated *with Trifolium pratense* at 900 m. a.s.l. – but was associated with *Lotus corniculatus* – (Table 2) clearly indicates that symbiotically fixed N is not the only

determinant of the pattern of plants with respect to the neighbourhood of legumes.

Spatial heterogeneity in species composition may also be the result of microtopographic diversity (e.g., soil depth, texture and roughness of the surface) or microclimatic conditions (Fransen et al. 2001). The influence of such factors may increase at higher altitudes where soil is shallower, thus resulting in a rougher surface and many niches with relatively deep and very shallow soil patches (Franz 1987). This is also evident from the fact, that with increasing altitude, the occurrence of legumes becomes increasingly patchy (Jacot et al. 2000a, b). The effect of abiotic factors may be one of the reasons why, for example, the vicinity of *Trifolium pratense* was not characterised by the same set of species along the altitudinal gradient (Table 2). Different environmental conditions lead to site-specific plant species, e.g., with a different growth form and different competitive behaviour.

Soil nutrient heterogeneity may likewise contribute to species composition. This is evident from our data, which demonstrate that N availability was apparently highest close to the N indicator CP at 900 m a.s.l., whereas apparent N availability was highest close to *Trifolium pratense/nivale* at 1900 and 2100 m a.s.l. (Table 4a). This soil N heterogeneity may be the result of differences in the soil environment (soil depth, nutrient input, rate of leaching) and of the species which regulates soil mineral N.

In particular, local deposits of dung and urine at higher altitudes may create pockets of high available N in the soil. For example, we observed that *Phleum alpinum* grows more vigorous close to deposits of dung at 2300 m a.s.l. This indicates that this species, which requires large amounts of N, is mainly competitive at high altitudes due to input of N through dung and, thus, contributes to species composition.

The distinct T values of the CPs (Table 4b) indicate that small-scale differences in temperature conditions may exist in a sward leading to respective patterning of plant species.

The strength of the association between a CP and its neighbouring species was lower at higher altitudes (Table 2); moreover, the number of plants associated significantly with certain CPs decreased as well. This may indicate that biotic effects – mainly decisive for the composition of plant species at lower sites – become increasingly overridden by abiotic factors, such as microclimatic (e.g. (Table 4b) and/or edaphic conditions, at higher altitudes.

Conclusion

Mechanisms, such as narrow-range seed and/or vegetative propagation, may affect plant species patterning. Moreover, this study shows that, through symbiotic N_2 fixation, legumes may affect the patterning of plant species in grassland ecosystems by providing extra nitrogen to their near surrounding. However, other biotic or abiotic factors may be decisive for the composition of plant species.

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