



**THE EFFECT OF FERTILIZATION,
MOWING AND ADDITIONAL
ILLUMINATION ON THE STRUCTURE
OF A SPECIES-RICH GRASSLAND
COMMUNITY**

LIINA EEK-PIIRSOO

DISSERTATIONES BIOLOGICAE UNIVERSITATIS TARTUENSIS

73

**THE EFFECT OF FERTILIZATION,
MOWING AND ADDITIONAL ILLUMINATION
ON THE STRUCTURE
OF A SPECIES-RICH GRASSLAND
COMMUNITY**

LIINA EEK-PIIRSOO



TARTU UNIVERSITY
PRESS

Chair of Plant Ecology, Department of Botany and Ecology, University of Tartu, Estonia

The dissertation is accepted for the commencement of the degree of *Doctor philosophiae* in plant ecology at University of Tartu on June 3, 2002 by the Council of the Faculty of Biology and Geography of the University of Tartu

Opponent: Ph.D. Graciela Rush, Norwegian Institute for Nature Research (NINA), Trondheim, Norway.

Commencement: Room No 218, Lai 40, Tartu on June 3, 2002, at 10.15.

The publication of this dissertation is granted by the Department of Botany and Ecology, University of Tartu

CONTENTS

LIST OF ORIGINAL PUBLICATIONS.....	6
INTRODUCTION	7
MATERIAL AND METHODS	11
Study site	11
Experimental design	11
Sampling methods	13
Data analysis	14
<i>Estimation of diversity</i>	14
<i>Number of contacts in a point quadrat</i>	14
<i>Degree of biomass aggregation</i>	14
<i>The variance ratio statistic</i>	14
<i>Standardized species richness</i>	15
<i>Leaf orientation index</i>	15
<i>Direct radiation index</i>	15
RESULTS	16
Species richness and diversity	16
Canopy density	17
Degree of biomass aggregation	18
The variance ratio statistic	18
Standardized species richness	18
The orientation of plant aerial parts in the canopy	19
Response of species with different growth form and light requirements	19
DISCUSSION	20
REFERENCES	24
ACKNOWLEDGEMENTS	27
VÄETAMISE, NIITMISE JA LISAVALGUSTAMISE MÕJU LIIGIRIKKA NIIDUKOOSLUSE STRUKTUURILE. Summary in Estonian	28
PUBLICATIONS	31

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on three papers, which are referred to in the text by the relevant Roman numeral.

- I Eek, L. and Zobel, K. 1997 Effects of additional illumination and fertilization on seasonal changes in fine-scale grassland community structure. *Journal of Vegetation Science* 8: 225–234.
- II Eek, L. and Zobel, K. 2001. Structure and diversity of a species-rich grassland community, treated with additional illumination, fertilization and mowing. *Ecography* 24: 157–164.
- III Zobel, K. and Eek, L. Seasonal direct light availability affects mean leaf orientation in a herbaceous multi-species canopy (manuscript, submitted to *Plant Biology*)

Published papers are reproduced with due permission from the publishers.

INTRODUCTION

The role of competition in structuring plant communities has been a matter of discussion for several decades already. Although the importance of competition has been generally accepted, the exact role of competition and the precise mechanisms by which it operates are still unclear. While everyone would agree that competition is likely to be an important determinant of community structure in at least some cases, there is considerable debate as to the general importance of competition in this respect. Though a large number of works reporting the importance of competition can be found in literature (Connell 1983, Schoener 1983), it is unclear, in many cases, how much the presented evidence in fact tells about the actual long-term and large-scale role of competition in community processes.

As competition is, by definition, negative interaction between organisms, caused by their need for a common resource, it is most logical that the change of its relative importance along resource gradients has been one of the much discussed topics in ecology. Frequently, this discussion has been integrated with another important question — why do certain spatial and temporal patterns of species diversity exist in the natural world (Rosenzweig 2000)? Mostly, the debate has been concentrated on the relative importance of competitive exclusion of species in diversity maintenance. Competitive exclusion is, by definition, (local) extinction of a species due to interspecific competition. The principle of competitive exclusion, so-called Gause's principle, originally stated that species cannot coexist as long as they are limited by the same resource (Gause 1934). Later, the principle has been re-formulated several times, and nowadays the most widely used formulation states that long-term co-existence is impossible as long as species occupy the same (multidimensional) niche (Vandermeer 1972).

Most of the current approaches agree that competitive exclusion of species may occur, at least in some stages of plant community dynamics, but there is no agreement on the relative role of above-ground and below-ground interactions in competitive exclusion processes (Wilson 1988, Aerts *et al.* 1991, Wilson and Tilman 1993, Belcher *et al.* 1995, Cahill 1999). Competition for light has generally been considered more crucial a determinant in competitive exclusion processes (Grime 1979, Tilman 1990, Bengtsson *et al.* 1994, Nagashima *et al.* 1995) mainly because it tends to be strongly asymmetric (Weiner 1990). On the other hand, in competition experiments the effect of root competition usually turns out to be clearly stronger (review by Wilson 1988). This leads to one of the main questions underlying this thesis: what is the relative importance of root and shoot competition in the processes which determine plant community structure and diversity?

In fact, below and above-ground components have seldom been separated in competitive interactions (Cahill 1999). Studies where such a separation has been made report different results. Some argue that the overall intensity of competition is greater with increased productivity (Grime 1973, 1979, Keddy 1989, Wilson and Tilman 1993). Others claim that the overall strength of competition should not change with increased soil resources (Newman 1973, Tilman 1988, Wilson and Tilman 1991, 1995), but, at the same time, the relative importance of shoot competition should increase with fertility (Wilson and Tilman 1991, 1995).

I have admitted above that the pair of dependences — below and above ground resource balance vs. root and shoot competition balance \Rightarrow root and shoot competition balance vs. diversity maintenance — has been widely discussed. It is much more difficult to find case studies where aspects of natural multi-species plant community structure other than diversity have been observed, as responding to changing resource levels and competitive pressure. At the same time, light competition intensity is surely dependant on the way in which the existing plant material is positioned in the vegetation (leaf orientation, number of layers in the foliage, horizontal pattern of shoots, *etc.*). Though these aspects have been quite thoroughly investigated in agricultural crops and wooded plants, there is very little reliable data for herbaceous natural or semi-natural communities.

The body of theoretical problems, considered above, is closely related to one of the crucial topics in the planning of management and protection measures for temperate semi-natural plant communities — maintenance of species diversity in traditionally grazed or mown grasslands. There has been a drastic decrease in the area of traditionally managed grasslands in the Baltic Sea region in the last decades, resulting in a fall of diversity readings (see Kukk and Kull 1997 for review). Mostly, cessation of the long-term management regime has meant two things — neglect of the grassland (cessation of grazing or mowing) or fertilization (mainly addition of nitrogen) for better productivity. In both cases the resulting decrease in species diversity has been explained through competitive exclusion of species due to intensified interspecific competition (Gurevich and Unnasch 1989, Kull and Zobel 1991, Huber 1994). Thus clarification of the above-mentioned problems in ecological theory would surely be of some use for planning optimal protection and restoration strategy in semi-natural grasslands.

One of the key problems in community ecology is experimental design. As far as competition studies are concerned, most of the knowledge has been gathered from pot, garden or greenhouse experiments (Connell 1983, Schoener 1983). Manipulation of natural stands has also been popular, especially by altering soil nutrient availability and plant shoot density. The experimental alteration of light availability, if applied, has meant either shading (reduction of incoming light) or physical manipulations with plant shoots in order to avoid contact between them (Connell 1983, Schoener 1983). Addition of light resource is a treatment that has never been tested as a possible way of altering

light climate in natural vegetation and thus releasing the pressure of light competition in the community. One of the novelties in this thesis is the application of an original experimental manipulation of incoming light availability — the placement of South-facing mirrors (building a 'second sun').

The main aim of the experiment providing data for this thesis, was to study the relative importance of above- and below-ground competitive processes in determining small-scale canopy structure and diversity pattern in a herbaceous community. Given that possible short-term changes in species diversity are most easily detectable in communities with high species diversity, we chose a species-rich semi-natural grassland site (annually mown Laelatu wooded meadow in western Estonia) as the target community. Our main interest was to find out what the relative role of root and shoot competition in diversity maintenance is. Initially we assumed that intensified light competition is the main mechanism responsible for the decrease in diversity in the parts of the grassland where mowing had been discontinued or where nutrients had been added (Kull and Zobel 1991). Thus, we hypothesized that additional illumination of the canopy could mitigate the effect of fertilization and cessation of mowing.

We concentrated on the following main working hypotheses:

1. *Intensified interspecific competition for light, and hence competitive exclusion of species, is the main reason behind the decrease of diversity in those semi-natural plant communities where the long-term disturbance regime has been discontinued, or where fertilizer treatment has been applied.* More specifically:
 - a. In the studied annually mown grassland, light normally does not limit above-ground productivity and shoot competition is not the major determinant of small-scale diversity pattern and plant canopy structure.
 - b. In the case of nutrient addition, and/or cessation of annual mowing, light may become limiting for canopy productivity, and competition for light may become an important determinant of diversity and community structure.
 - c. Additional light resource, if provided, would mitigate the effect of fertilizer and cessation of mowing, by making competition for light less intense.

Additionally, in the course of our work, several new problems came up which could be formulated as separate working hypotheses:

2. Due to the obvious positive dependence between small-scale richness and density of plant ramets, richness per unit coverage could be a diversity measure more sensitive to experimental treatments than richness per unit area.
3. Mean leaf orientation is regressed to the amount of direct radiation received in the growing season. The abundance of direct sunlight causes exposition of

leaf surfaces predominantly towards the North, the deficit of direct sunlight towards the South.

4. Plants with different basic growth form (graminoid vs. forbs) and plants with different light requirement (light-demanding vs. shade-tolerant) differ in the plasticity of response to seasonal direct light availability.

MATERIAL AND METHODS

Study site

The study site is situated in a calcicolous species-rich wooded meadow in Laelatu, Western Estonia, on the eastern coast of the Baltic Sea (58°35'N, 23°34'E).

The plant community at the site has been assigned to the *Sesleria coerulea* — *Filipendula hexapetala* association (Krall and Pork 1970). The majority of species (98%) in the herbaceous layer are perennials, 71% of the species are forbs and 29% are graminoids (III). There is a scattered tree layer in the meadow with *Quercus robur*, *Fraxinus excelsior*, *Betula spp.* and *Populus tremula* as the most abundant species. Average tree canopy coverage is *ca* 20%.

The Laelatu meadow has been more or less regularly annually mown in early July for more than 200 years. Currently only about 20 ha of the whole 153 ha area is being mown every year. The herbaceous community in the meadow is extremely species-rich on the small scale — as many as 25 vascular plant species have been recorded in a 10 cm × 10 cm plot, 42 species in a 20 cm × 20 cm plot (Kull and Zobel 1991), and 68 species in a 1 m² plot (Kull and Zobel 1995). In 2001 even 76 vascular plant species were counted in one square meter (T. Kukk *et al.*, unpublished data). It is important to note that the high species richness figures are characteristic only to the part of the meadow which has been permanently mown for centuries. In the remaining parts, which have probably been left unmanaged for certain periods of time, the small-scale diversity is considerably lower — *ca* 35 species per square meter as an average.

Data on some relevant climatic parameters (number of sunshine hours per month, mean monthly air temperature, monthly precipitation) were acquired from the observatory of the Estonian Institute of Meteorology and Hydrology, situated 3 km from our experimental site (Table 1 in II). According to their data the total number of sunshine hours over three months (May to July) varied seasonally between 826 and 1081, being only 745 in the darkest and cloudiest year (1996). In this year the mean monthly temperature was also only 12.6°C compared to 13.7...14.5°C in the remaining years. The first year of the experiment (1994) was the driest; the total precipitation during the three summer months was only 94.0 mm, compared to 174.6...219.5 mm in the remaining years.

Experimental design

The field experiment was established in late May/early June 1994 and lasted five seasons, until 1998 when half of the experimental plots (those that were not mown) were destroyed by taking root biomass samples for another data-set. In mown plots, species richness was estimated also in 1999.

Three different experimental treatments were applied to the herbaceous vegetation:

- 1) additional illumination with the help of vertical south-facing glass mirrors;
- 2) fertilization with NPK and microelements;
- 3) mowing.

The experiment had a randomized factorial design. Each of the treatments had two levels (treated or not, + or -), and all of the eight possible (2×2×2) treatment variants had three replicates; thus we had 24 permanent plots altogether. Given that the long-term management regime in the grassland has only been annual mowing for hay (without fertilizer addition), the control plots were those that were non-fertilized, not additionally illuminated and annually mown.

The size of experimental permanent plots where changes in vegetation structure were recorded each summer was 40×40 cm. Actually, quadrats of 1m² (with the sample 40×40 cm area in the center) were experimentally treated, in order to avoid edge effects.

The plots were additionally illuminated with the help of glass mirrors measuring 212 cm (horizontal) × 124 cm (vertical), their lower edge being 20 cm from ground level. The mirrors faced south and reflected sunlight to the plots for ca 6 hours per day during the growing season. The mirrors increased the amount of photosynthetically active radiation (PAR) and also temperature (measured with linear light meter Licor LI-250 and Quantum Sensor Licor LI-191SA and thermometer, respectively; see Table 2 in II). Mirrors increased the amount of PAR above the canopy by ca 65%. In the lowest canopy strata (5 cm from ground) the amount of PAR increased approximately twofold.

The change of temperature was the highest in intermediate canopy layers (Table 2 in II).

The plots were fertilized twice a year — in early May and in late September. The annual amounts and element composition of the fertilizer are shown in table 1.

Table 1. The chemical composition of the mixture used in the fertilizer treatment and the annual amounts of nutrient addition

Target nutrient element	The chemical compound used	Annual addition of the target element (g m ⁻² yr ⁻¹)
N	NH ₄ NO ₃	10
P	P ₂ O ₅	4.2
K	KNO ₃	4.2
B	Na ₂ B ₄ O ₇	0.12
Mo	(NH ₄) ₂ MoO ₂₄	0.005
Mg	MgSO ₄ × 7 H ₂ O	2.5
Fe	FeSO ₄ × 7 H ₂ O	0.2
Mn	MnSO ₄ × 5H ₂ O	0.5
Zn	ZnSO ₄ × 5H ₂ O	0.5
Cu	CuSO ₄ × H ₂ O	1.2

The plots were mown once a year, in mid-July.

Sampling methods

During first three years of the experiment (1994...1996) the plots were examined twice a year — in the end of June to early July and early September. Last two years (1997...1998) the plots were studied only once a year, in July.

We studied plant canopy structure using the point quadrat method. Instead of the usual metal pins we used a laser beam with a diameter of 1 mm (see Fig. 1 in I for the equipment) to mark the quadrats. During the first year we positioned the point quadrats at three different angles — 60° (the angle approximating the mean angle of incoming direct light around noon), 90° (vertical) and 120° (inclination of the point quadrat towards the north; see Fig. 2 in I). Since 1995 we continued to make investigations at two angles instead of three — 60° and 120°. Studying canopy from different angles enabled us to roughly assess the mean orientation of leaves along the south-north direction (Fig. 3 in I; III).

We made observations at 50 different randomly chosen points at each angle in every plot in a sampling season — during the first year at 150 points (3 angles × 50 points) and in the remaining years at 100 points (2 angles × 50 points). The coordinates of the points were determined beforehand, using a random number generator, to an accuracy of 1 mm. In each point quadrat the number of contacts of living plant biomass with the point quadrat beam were recorded.

In 1994, in the first sampling session (summer), in eight plots — one from each treatment variant — we identified the plant species for every contact in the point quadrats. In the second sampling session (autumn), four plots were studied in this way (annually mown plots were excluded). In 1995 and 1996 the autumn session was omitted.

Complete floristic inventory was performed after point quadrat survey. We also estimated visually the percentage coverage for each species.

To study separately functionally different species we classified them: a) according to growth form — graminoids (*Cyperaceae* and *Poaceae*) vs. forbs (the remaining vascular plant species); b) according to the Ellenberg ecological indicator value for light (Ellenberg *et al.* 1991). A species was considered light demanding if its indicator value for light exceeded 6 (not included).

Availability of direct light in sample plots was estimated from hemispherical photographs made in 1999. The direct site factor *Tdir* (proportion of direct radiation under tree canopy relative to that above the tree canopy; Machado and Reich 1999) was estimated as an integral for the period of one month (30 days before maximum sun angle till maximum sun angle), using cosine correction (III).

Data analysis

Estimation of diversity

In addition to counting species, we estimated species diversity in plots using indices of the Hill's family N_1 and N_2 (exponent of Shannon's index and inverse of Simpson's index, respectively) and evenness measures derived from these two — N_1/N_0 ($E_{1,0}$) and N_2/N_0 ($E_{2,0}$) where N_0 is species richness (Routledge 1979). The dynamics of species richness (N_0) and diversity (N_1 , N_2 , $E_{1,0}$, $E_{2,0}$) in experimental plots were analysed using ANOVA and Repeated Measures ANOVA. Cumulative species richness was calculated as the sum of all species that had occurred in the particular plot throughout the observation years 1994...1999 (number of species in the first year plus number of new species during next years; van der Maarel and Sykes 1993). For this period of time there was species composition data available only for permanently mown plots. For all plots the last observation year was 1998.

The effect of experimental treatments and time on cumulative species richness was analyzed using Repeated Measures ANOVA.

Number of contacts in a point quadrat

The effect of experimental treatments and time on the mean number of contacts per point quadrat (from now number of contacts) was analyzed using Repeated Measures ANOVA (see I and II for details).

Degree of biomass aggregation

The degree of biomass aggregation in a plot was calculated as the ratio of observed and expected (in the case of random spatial distribution of biomass) variance of the number of contacts in a plot (see I for details). The effects of experimental treatments, time and angle of observation on the degree of biomass aggregation were analyzed using Repeated Measures ANOVA.

The variance ratio statistic

The relative spatial variability of species richness at a point was estimated by calculating the variance ratio statistic (Palmer 1987, Wilson *et al.* 1987, Zobel and Zobel 1988) — observed variance of the number of species in a point quadrat divided by the expectancy from a null model assuming random and independent distribution of species inside a permanent plot (see I for details of the computation technique).

Standardized species richness

Standardized species richness (richness per unit coverage) was calculated as species richness in a plot divided by total plant coverage (sum of coverages of all species) in this plot. The relationship between species richness and total coverage was analysed using linear regression analysis. The same analysis was used for studying the relationship between annual changes of species richness and total coverage. The effect of experimental treatments and time on standardized species richness was analysed using Repeated Measures ANOVA.

Leaf orientation index

Mean plant aerial part azimuth orientation was represented in a single index *LOI* – leaf orientation index. The statistic was calculated as follows:

$$LOI_{ij} = \ln (N(60)_{ij} / N(120)_{ij}) \quad (1)$$

$N(\alpha)_{ij}$ — total number of contacts with vascular plants registered in the 50 point quadrats positioned at angle α , in i -th plot, j -th year. The values $LOI_{ij} > 0$ denote the exposition of leaf surface predominantly towards the South.

Direct radiation index

We calculated direct radiation index (*DRI*) to estimate the relative amount of direct light in a plot in a particular year. The statistic was calculated as follows:

$$DRI_{ij} = S_j * Tdir_i * M \quad (2)$$

DRI_{ij} — direct radiation index for i -th plot in j -th year;

S_j — number of sunshine hours in May and June in j -th year;

$Tdir_i$ — direct site factor in i -th plot;

M — mirror efficiency coefficient, i.e. estimate of the overall multiplicative effect of additional illumination (in plots without mirrors $M = 1$). The estimate of M was achieved statistically (see III).

All statistical procedures were performed using Statistica 6.0 software.

RESULTS

Species richness and diversity

Altogether 119 species of vascular plants were recorded in the permanent plots during 5 years of the experiment. 87 vascular plant species were recorded in those eight plots where we specified every species in point quadrats during the first three years of the experiment (III).

The dynamics of species diversity in the 24 permanent plots is shown in Table 2. Unfortunately, the experiment did not yield any significant responses of small-scale diversity in the experimentally manipulated plots. None of the experimental treatments caused clear changes in species number, nor were the interactive effects of treatments significant. This was true regardless of the particular diversity measure used as a dependent variable in the ANOVA model (richness, change of richness when compared to initial situation, N_1 , N_2 , evenness of abundance).

Table 2. Species richness in permanent plots (40x40 cm) with different treatment variants in different years (1994–1998). Not fertilized, not additionally illuminated and annually mown plots served as control plots.

Fertilization	Mirror	Mowing	1994	1995	1996	1997	1998
–	–	–	37	31	34	32	33
–	–	–	27	28	30	27	27
–	–	–	24	24	23	19	21
–	–	+	46	33	39	34	35
–	–	+	29	30	31	30	29
–	–	+	37	35	38	35	34
–	+	–	37	38	37	36	36
–	+	–	27	30	28	26	28
–	+	–	44	36	33	29	34
–	+	+	23	25	21	17	21
–	+	+	45	30	31	32	31
–	+	+	24	21	22	19	20
+	–	–	26	35	29	28	30
+	–	–	22	25	25	20	19
+	–	–	43	30	36	34	31
+	–	+	30	29	28	30	30
+	–	+	41	32	35	30	36
+	–	+	29	25	30	30	28
+	+	–	30	30	29	19	24
+	+	–	37	34	36	32	31
+	+	–	44	37	38	34	33
+	+	+	28	25	26	22	22
+	+	+	46	34	40	37	38
+	+	+	34	33	34	31	35

Somewhat surprisingly, considering that no significant annual changes of diversity were detected, the effect of experimental treatments appeared when cumulative species richness in a plot was used as the dependent variable. This was true, though, only for permanently mown plots, observed also in 1999. For all the 24 plots (last observation year 1998) there were no significant effects of treatments on cumulative richness. The highest cumulative species richness in the mown series was found in those plots where additional illumination and fertilization had been applied simultaneously (interaction fertilization*illumination significant at $p = 0.0194$, Fig. 1). At the same time the main effects of fertilization and additional illumination were not significant, although both treatments led to a certain decrease in mean cumulative richness, when applied alone (Fig. 1).

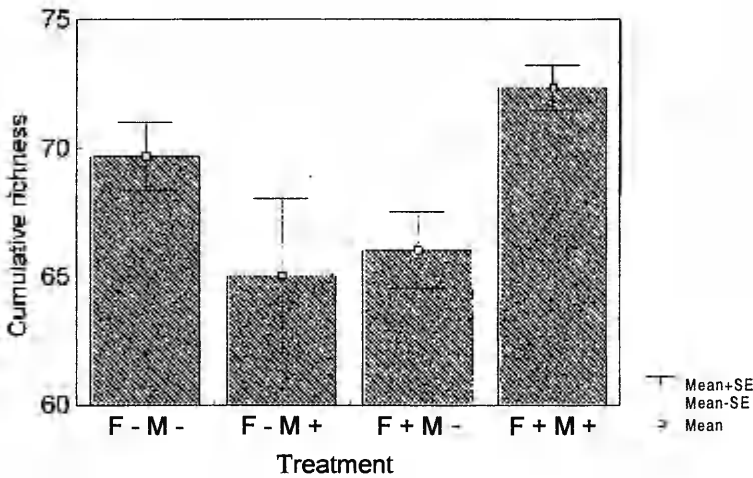


Figure 1. The effect of fertilization (F) and additional illumination (M) on cumulative richness in permanent plots (1994...1999). The interaction $F*M$ is significant at $p = 0.0194$. Bars indicate standard errors.

Canopy density

The grand mean number of contacts per point quadrat beam in a plot over the five years of the experiment was 5.29 (min 2.18, max 11.8, SD 1.68; III).

Canopy density increased significantly during the growing season in all plots (impact of time significant at $p < 0.0001$; Fig. 4 in I). Logically, the increase was the greatest in fertilized plots (impact of fertilizer significant at $p = 0.0437$).

Fertilization led to an increase in canopy density in the long term also (Table 3 in II, effect of fertilization $p < 0.0001$). However, the highest number of con-

tacts per point quadrat beam was in plots with the highest resource levels – in fertilized plots with mirrors (Fig 1 in II).

Degree of biomass aggregation

The observations in the first year of the experiment showed that fertilization leads to a significantly more aggregated spatial distribution of biomass in the canopy (Fig 5 in I; time*fertilization interaction significant at $p=0.0353$). Mirrors, in turn, decreased this effect significantly ($p=0.0189$), so that in additionally illuminated plus fertilized conditions the canopy was even more homogeneous than in control plots.

The variance ratio statistic

There was demonstrated to be a variance deficit (observed variance of richness smaller than expected) in all the analyzed 36 data-sets (representing treatment combination, time and angle — eight plots and three angles in the first sampling date, four plots and three angles in the second sampling date). Generally, expected variance could be predicted well from the observed one (Fig. 6 in I, note that the names of the X and Y axes have been confused in the graph — X axis actually measures expected variance and *vice versa*). Also, variance ratio statistic was in good correlation with the degree of biomass aggregation. Great variance deficit predicts a highly aggregated spatial distribution of shoot biomass (Fig. 7 in I).

During the first season, fertilization as well as light addition, when applied alone, caused a considerable decrease of the variance ratio (Fig. 8 in I). When these treatments were applied simultaneously, no detectable change in variance ratio could be observed.

Standardized species richness

Species richness was significantly and positively related to total plant coverage in a plot (Fig. 3 in II, $p=0.0017$). Also, annual change of species richness in a plot could be predicted from the annual change in total plant coverage (Fig. 4 in II, $p=0.0001$). Decrease of coverage generally meant a decrease in richness as well, and *vice versa*. Only during the first year of the experiment was the annual change in species richness unrelated to annual change in coverage.

As the relationship between species richness and total plant coverage in a plot could with great probability shade the possible relationships between species richness and experimental treatments, we invented another weighed richness measure — species richness per coverage, i.e. standardized species richness (Sst).

The only factors that affected standardized species richness significantly were time and fertilization (Table 4 in II). Standardized species richness proved to be significantly smaller in fertilized plots ($p < 0.0001$).

The orientation of plant aerial parts in the canopy

The positioning of point quadrats at different angles in a South-North direction made it possible to study changes in the azimuth orientation and inclination of plant leaves in the canopy (Fig. 3 in I).

In the first year of the experiment the number of contacts observed at different angles were quite similar, i.e. leaves did not show any preference of orientation. The exception was again these illuminated and fertilized plots where the number of contacts was the smallest at 60° (Fig 4 in I) — leaf surface was exposed predominantly towards the North.

When observations over the five years of the experiment were pooled, it appeared that there were consistently more contacts recorded at 120° , except in the darkest and coolest year (1996) when leaves were exposed predominantly towards the sun in all plots, regardless of treatment (Fig 2 in II).

We got the same result when studying the relationship between leaf orientation index (LOI) and direct radiation index (DRI). There was a significant positive relationship between these characteristics ($p = 0.0021$, Fig. 2 in III). In conditions when there was enough sunlight the surface of leaves was orientated towards the North and in years with less light, towards the sun.

Response of species with different growth form and light requirements

Not all plants responded similarly to the change in light conditions by adjusting the mean leaf azimuth angle of their aerial parts. Mostly, light-demanding (Fig. 4 in III) and graminoid (Fig. 3 in III) species were responsible for such an effect: 41% of the variation in leaf orientation (LOI) was explainable through direct light availability (DRI) for light-demanding species and 26% for graminoid species. Shade-tolerant species (Ellenberg's indicator value for light below seven) and forbs did not show any sign of plastic reaction to changes in direct light availability (III).

DISCUSSION

Unfortunately, the results of the experiment did not provide firm evidence in support of our main working hypothesis (*Hypothesis 1, 1a, 1b, 1c*, see introduction): *intensified interspecific competition for light, and hence competitive exclusion of species, is the main reason behind the decrease in diversity in those semi-natural plant communities where the long-term disturbance regime has been discontinued, or where fertilizer treatments have been applied*. The main reason for that was the failure of the fertilizer treatment to suppress richness in permanent plots. Thus, *Hypothesis 1c — additional illumination, if provided, would mitigate the effect of fertilizer ...* — turned out to be impossible to test. Species diversity *per se*, regardless of how it was estimated (richness, N_1 , N_2 , evenness), did not respond significantly to any of the experimental manipulations. This was an unexpected result, given that a sharp decrease in species richness due to fertilization and cessation of mowing had been recorded in the same meadow earlier (Kull and Zobel 1991), and a similar effect has been shown in several previous experiments (see Gurevich and Unnasch 1989 for review; Gough *et al.* 1994). Most probably, we failed to affect richness in permanent plots because of the quite small areas manipulated with experimental treatments (only 1 m²). The invasion of species from surrounding areas through lateral vegetative spread and propagule input (mass effect) might have been intense enough to compensate the probable competitive extinction of certain species, since, in Laelatu meadow, vegetative regeneration is prevailing and regeneration from seeds is obviously not so important (Kalamees and Zobel 2002).

However, we managed to find some indirect evidence in favour of *Hypothesis 1*. When the series of permanently mown plots was observed, cumulative species richness was the highest in the plots with the highest resource levels — in fertilized plots with mirrors. There, additional illumination obviously mitigated the intensified light competition expected to occur when plots are only fertilized and no additional light is provided. Unfortunately, we could not detect a significant decrease in cumulative species richness due to fertilization, though visually there seems to be some negative effect (Fig. 1).

The testing of *Hypothesis 2* (see introduction) — due to the obvious positive dependence between small-scale richness and density of plant ramets, richness per unit coverage could be a diversity measure more sensitive to experimental treatments than richness per unit area — revealed a way of detecting the effect of fertilizer treatment on species richness. The first step in testing this hypothesis was to check whether the assumption on the richness — canopy density relationship holds. It appeared that species richness and total coverage of plants showed a significant positive correlation over all treatment variants, over all years of the experiment. Also, year-to-year changes in plot richness could be predicted from year-to-year changes in total coverage. The first year of the ex-

periment was the exception here — change of richness 1994...1995 was not correlated with change of total coverage 1994...1995. This possibly reflects the consequences of the severe drought during the first year of the experiment (1994, Table 1 in II) — in the following year (1995) richness in a plot evidently depended mostly on the drought-tolerance of the particular species inhabiting the plot in 1994. Positive correlation between species richness and plant density per constant area has been shown before on a broad gradient of community productivity and small-scale diversity (Zobel and Liira 1997) and in artificial communities with experimentally manipulated plant density (Goldberg and Estabrook 1998) but not between small-scale richness and canopy coverage inside a compact plant community.

Though fertilized plots generally carried a denser canopy, the mentioned relationship did not hold in fertilized plots, which were significantly more species-rich. The overall variability of richness, as well as canopy coverage, were great enough to shade the possible effects of experimental treatments on the richness — coverage relationship. Our results somewhat contradict several previous experiments where fertilization has led to decreasing species density with increasing canopy density (e.g. Pasternak-Kusmierska 1992, Gough and Grace 1998, but see Huberty *et al.* 1998).

The overall positive relationship between plot richness and canopy coverage encouraged the use of standardized richness (S_{st}) for detecting the effects of experimental treatments on diversity. Unfortunately, we failed to find evidence on competition for light being the key factor in diversity formation. Although nutrient addition led to a decrease in richness per unit coverage, there was no indication of additional illumination mitigating this effect. Neither did cessation of mowing affect S_{st} significantly.

The results of studying the spatial structure of the canopy by means of point quadrat analysis generally supported the assumption made in *Hypothesis 1a*: *in the studied annually mown grassland light normally does not limit above-ground productivity and shoot competition is not the major determinant of small-scale diversity pattern and plant canopy structure*. Light does not seem to be the limiting resource under normal conditions — additional illumination, when applied as a single treatment, caused no significant increase in canopy density, measured as number of vascular plant contacts per point quadrat beam (Fig. 1 in II). Neither did additional illumination have apparent harmful effect on productivity. The considerable rise in radiation intensity and the notable rise in temperature in the case of direct sunshine (by more than 60% and by 10...20%, respectively, see Table 2 in II) has not led to a significantly sparser canopy in additionally illuminated plots. As expected, fertilization led to a significant increase in canopy density (Fig. 1 in II).

Also, there was evidence, supporting the Hypotheses 1b, 1c, stating that light would become limiting after nutrient addition, and that additional illumination would mitigate this effect, respectively. Fertilization in fact led to denser and also more aggregated canopy, but the densest and most homogeneous canopies

were found in fertilized plots with mirrors. Additional illumination most probably mitigated the intensified light competition that occurred after fertilization and prevented the uneven development of canopy-forming species in these plots so that the changes in biomass aggregation were even smaller than in control plots or in plots with only mirrors (Fig. 5 in I).

The results of computing the variance ratio statistic were somewhat unexpected. The good predictability of the null model expectation from observed spatial variability of richness in a point quadrat, and the rather close relationship between the degree of small-scale biomass aggregation and variance ratio (Fig. 7 in I) makes it dubious whether such a statistic can be used as an independent and biologically meaningful characteristic of community organization. Variance ratio appears to follow mainly the spatial variability of the amount of plant material in a quadrat (the latter is well correlated with the expected variance of point quadrat richness) and says little about the competitive pressure or possible niche limitation (I, Palmer and van der Maarel 1995, van der Maarel *et al.* 1995).

Our *third working hypothesis* was concerned with the ability of grassland plants to respond to changes in light availability by adjusting the mean inclination of leaves: *mean leaf orientation is regressed to the amount of direct radiation received in the growing season. The abundance of direct sunlight causes exposition of leaf surfaces predominantly towards the North, the deficit of direct sunlight, towards the South.*

The results of our study demonstrate that the relationship between mean leaf orientation and the amount of direct radiation received during the growing season can be quantified (Fig. 1 in III). Though the predictive power of the regression model remains very low there is evidence supporting our hypothesis.

There was strong indication that the general orientation of leaves of herbaceous species depends on the climatic conditions prevailing during the growing season. In more or less sunny 'ordinary' years (in our experiment 1994, 1995, 1997, 1998, Table 1 in II) the orientation of leaves was similar to what has been reported in earlier studies (e.g. Cooper *et al.* 1970) — leaves were predominantly inclined towards the sun, exposing their surfaces towards the North. In the exceptionally cool and cloudy year (1996, Table 1 in II) the situation was strikingly different, independent of the experimental treatment (Fig. 2 in II) — then the prevailing inclination of leaves was towards the North, i.e. leaf surfaces were mostly exposed to the South. Plants apparently showed considerable plasticity by orientating their photosynthetic surface towards the direction of the sun to absorb more PAR.

The more detailed study of leaf North-South orientation, with species identified for each contact in the point quadrat analysis, revealed the reason why the overall regression of *LOI* on *DRI* was so weak as to describe only less than seven percent of the observed variation of the dependent variable (Fig. 1 in III). It appeared that *only certain functional groups of species are responsible for the plasticity of mean leaf orientation in the canopy (our fourth hypotheses).* In

light-demanding species with Ellenberg indicator value for light seven or higher (73% of all species recorded) the seasonal amount of direct PAR was a good predictor of mean leaf orientation, describing 41% of total variation in *LOI* (Fig. 3 in III). The azimuth angle of shade species was not related to the seasonally received amount of direct radiation. Evidently, the ability to escape too intense direct radiation, and thus avoid photoinhibition, is an important trait of those species which are adapted to grow in full light. In shade species the danger of experiencing excessive direct radiation is too small in the long run to adopt an ability of adjusting the canopy to seasonal light climate.

Another functional group of species that demonstrated clear response in leaf orientation were species with graminoid growth form (grasses and sedges in the Laelatu meadow, 29% of the recorded species; Fig. 4 in III). The mean azimuth angle of the remaining species (forbs) appeared to be insensitive to the seasonal PAR availability. Most probably, such difference should be explained through the basic differences in how leaves are produced and maintained in forbs *vs.* graminoids. In the latter the vegetative leaves are often obligatory asymmetrical in respect to the vertical axis of the ramet while forbs can be obligatory symmetrical. Thus, providing that most of the graminoid ramets are vegetative (and this is the case in the Laelatu meadow), a certain azimuth angle of inclination may easily prevail, if there is a signal in the environment that alters the azimuth direction of leaf emergence in the early development phase of the leaves (see Myers *et al.* 1997 for comparison).

REFERENCES

- Aerts, R., Boot, R. G. A. and van der Aart, P. J. M. 1991. The relation between above- and belowground biomass allocation patterns and competitive ability. *Oecologia* 87: 551–559.
- Belcher, J. W., Keddy, P. A. and Twolan-Strutt, L. 1995. Root and shoot competition along soil depth gradient. *Journal of Ecology* 83: 673–682.
- Bengtsson, J., Fagerström, T. and Rydin, H. 1994. Competition and coexistence in plant communities. *Trends in Ecology and Evolution* 9: 246–250.
- Cahill, J. F. 1999. Fertilization effects on interactions between above- and belowground competition in an old field. *Ecology* 80: 466–480.
- Connell, J. H. 1983. On the prevalence and relative importance of interspecific competition: evidence from field experiments. *American Naturalist* 122: 661–696.
- Cooper, J. P., Rhodes, J. and Sheehy, J. E. 1970. Canopy structure, light interception and potential production in forage grasses. — Aberystwyth Univ. Coll. Wales. Welsh Plant Breeding Sta. Rep., pp. 57–69.
- Ellenberg, H., Weber, H. E., Düll, R., Wirth, V., Werner, W., Paulissen, D. 1991. Zieglerwerte von Pflanzen in Mitteleuropa. *Scripta Geobotanica* 18: 1–248.
- Gause, F. G. 1934. The struggle for existence. Williams and Wilkins, Baltimore, Md.
- Goldberg, D. E. and Estabrook, G. F. 1998. Separating the effects of number of individuals sampled and competition on species diversity: an experimental and analytic approach. *Journal of Ecology* 86: 983–988.
- Gough, L., Grace, J. B. and Taylor, K. L. 1994. The relationship between species richness and community biomass: the importance of environmental variables. *Oikos* 70: 271–279.
- Gough, L. and Grace, J. B. 1998. Herbivore effects on plant density at varying productivity levels. *Ecology* 79: 1586–1594.
- Grime, J. P. 1973. Competitive exclusion in herbaceous vegetation. *Nature* 242: 344–347.
- Grime, J. P. 1979. *Plant Strategies and Vegetation Processes*. Wiley, Chichester.
- Gurevich, J. and Unnasch, R. 1989. Experimental removal of a dominant species at two levels of soil fertility. *Canadian Journal of Botany* 67: 3470–3477.
- Huber, R. 1994. Changes in plant species richness in a calcareous grassland following changes in environmental conditions. *Folia Geobotanica et Phytotaxonomica* 29: 469–482.
- Huberty, L. E., Gross, K. L. and Miller, C. J. 1998. Effects of nitrogen addition on successional dynamics and species diversity in Michigan old fields. *Journal of Ecology* 86: 794–803.
- Kalamees, R. and Zobel, M. 2002. The role of the seed bank in gap regeneration in a calcareous grassland community. *Ecology* (in press)
- Keddy, P. A. 1989. *Competition*. Chapman and Hall, New York.
- Krall, H. and Pork, K. 1970. Laelatu puisniit. Kumari, E. (ed.) *Lääne-Eesti rannikualade loodus*. pp. 14–27. Valgus, Tallinn.
- Kukk, T. and Kull, K. 1997. Puisniidud. *Estonia Maritima* (Publication of the West-Estonian Archipelago Biosphere Reserve) 2.

- Kull, K. and Zobel, K. 1995. Kas on veel ruumi? Ei, kõik on täis. *Eesti Loodus* 48: 33–35.
- Kull, K. and Zobel, M. 1991. High species richness in an Estonian wooded meadow. *Journal of Vegetation Science* 2: 711–714.
- Liira, J. and Zobel, K. 2000. Vertical structure of a species-rich grassland canopy, treated with additional illumination, fertilization and mowing. *Plant Ecology* 146: 185–195.
- Machado, J.-L. and Reich, P. B. 1999. Evaluation of several measures of canopy openness as predictors of photosynthetic photon flux density in deeply shaded conifer-dominated forest understory. *Canadian Journal of Forest Research* 29: 1438–1444.
- Myers, D. A., Jordan, D. N. and Vogelmann, T. C. 1997. Inclination of sun and shade leaves influences chloroplast light harvesting and utilization. *Physiologia Plantarum* 99: 395–404.
- Nagashima, H., Terashima, I. and Katoh, S. 1995. Effects of plant density on frequency distributions of plant height in *Chenopodium album* stands: Analyses based on continuous monitoring of height-growth of individual plants. *Annals of Botany* 75: 173–180.
- Newman, E. I. 1973. Competition and diversity in herbaceous vegetation. *Nature* 244: 310–311.
- Palmer, M. W. 1987. Variability in species richness with Minnesota oldfields: a use of variance test. *Vegetatio* 70: 61–64.
- Palmer, M. W. and van der Maarel, E. 1995. Variance in species richness, species association, and niche limitation. *Oikos* 73: 203–213.
- Pasternak-Kusmierska, D. 1992. Density and species structure of sward of an intensively fertilized meadow after reduction and discontinuance of fertilization. *Ekol. Pol.* 40: 47–61.
- Rosenzweig, M. L. 2000. Species diversity in space and time. Cambridge, University Press, United Kingdom.
- Routledge, D. R. 1979. Diversity indices: which ones are admissible? *Journal of Theoretical Biology* 76: 503–515.
- Schoener, T. W. 1983. Field experiments on interspecific competition. *American Naturalist* 122: 240–285.
- Tilman, D. 1988. Plant strategies and the dynamics and structure of plant communities. Princeton University Press, Princeton, New Jersey, USA.
- Tilman, D. 1990. Constraints and tradeoffs: toward a predictive theory of competition and succession. *Oikos* 58: 3–15.
- van der Maarel, E. and Sykes, M. T. 1993. Small-scale plant species turnover in a limestone grassland: the carousel model and some comments on the niche concept. *Journal of Vegetation Science* 4: 179–188.
- van der Maarel, E., Noest, V. and Palmer, M.W. 1995. Variation on species richness on small grassland quadrats: niche structure or small-scale plant mobility? *Journal of Vegetation Science* 6: 741–752.
- Vandermeer, J. H. 1972. Niche theory. *Annual Review of Ecology and Systematics* 3: 107–132.
- Weiner, J. 1990. Asymmetric competition in plant populations. *Trends in Ecology and Evolution* 5: 360–364.
- Wilson, J. B. 1988. The effect of initial advantage on the course of plant competition. *Oikos* 51: 19–24.

- Wilson, J. B., Gitay, H. and Agnew, A. 1987. Does niche limitation exist? *Funct. Ecol.* 1: 391–397.
- Wilson, S. D. and Tilman, D. 1991. Components of plant competition along an experimental gradient of nitrogen availability. *Ecology* 74: 1050–1065.
- Wilson, S. D. and Tilman, D. 1993. Plant competition and resource availability in response to disturbance and fertilization. *Ecology* 74: 599–611.
- Wilson, S. D. and Tilman, D. 1995. Competitive responses of eight old-field plant species in four environments. *Ecology* 76: 1169–1181.
- Zobel, K. and Liira, J. 1997. A scale-independent approach to the richness vs biomass relationship in ground-layer plant communities. *Oikos* 80: 325–332.
- Zobel, K. and Zobel, M. 1988. A new null hypothesis for measuring the degree of plant community organization. *Vegetatio* 75: 17–25.

ACKNOWLEDGEMENTS

I am most thankful to my supervisor Dr. Kristjan Zobel for his unbelievable patience in working with me through all these years and guiding me towards the truth in plant ecology.

I would like to thank my colleagues who helped me in my tiresome field-works and made long working days more cheerful, especially Rein Kalamees, Meeli Mesipuu, Pille Urbas, Jaan Liira, Mare Toom and, of course, my husband Marko Piirsoo.

My special thanks to Jaan Liira for his valuable statistical help and help in graphical questions.

I thank Ilmar Part for revising English.

My studies were supported financially by Tartu University and the Estonian Science Foundation (ESF grant No 261 and 3887).

VÄETAMISE, NIITMISE JA LISAVALGUSTAMISE MÕJU LIIGIRIKKA NIIDUKOOSLUSE STRUKTUURILE

Kokkuvõte

Liikidevahelise konkurentsi rolli taimekoosluse struktuuri kujunemisel, püsimisel ja muutumisel on taimeökoloogias palju uuritud. Saati pole senini ühest arusaamist, mil määral, kuidas ja miks konkurents koosluse protsesse mõjutab.

Konkurentsi võib taimedel jaotada maa-aluseks ja maapealseks. Maapealset konkurentsi ehk valguskonkurentsi peetakse üldiselt olulisemaks koosluse elu mõjutajaks, sest, olles tugevalt ebasümmeetriline, viib see kergesti liikide konkurentsele väljatõrjumisele. Samas osutub eksperimentaalselt mõõdetud juurkonkurents tihtipeale tugevamaks kui valguskonkurents. Niisiis jääb ebaselgeks, milline on kahe konkurentsitüübi suhteline roll taimekoosluse struktuuri ja mitmekesisuse määramisel ning sellesisuline uudne teave peaks taimeökoloogias üldist huvi pakkuma.

Käesolevas töös eeldasime, et intensiivistunud liikidevaheline valguskonkurents on peamine liigilise mitmekesisuse vähenemise põhjus nendes poollooduslikes kooslustes, kus on lakanud pikaajalise häirimise (niitmine, karjatamine) režiim või kus on kasutatud väetisi. Peamine tööhüpotees oli, et *meie uuritavas taimekoosluses (väetamata ja iga aasta niidetav Laelatu puisniit Lääne-Eestis) valgus ei ole limiteerivaks faktoriks, aga muutub limiteerivaks pärast toitainete lisamist või niitmise katkestamist. Sel juhul peaks väetamise ja/või niitmise lakkamise mõju olema neutraliseeritav lisavalgustamisega lõunasuunaliste peeglite abil.*

Püüdsime leida vastust ka küsimusele, kas keskmine lehe orientatsioon rohusu võras sõltub kasvuperioodi jooksul saadud otsese kiirguse hulgast. Eeldasime, et otsese päikesekiirguse küllus põhjustab lehepinna pöördumist enamasti põhja poole, otsese kiirguse defitsiit aga lõuna poole. Samuti pakkus huvi, kas paindlikkus valgustatusele reageerimisel on erinev eri kasvuvormiga (kõrrelised vs. rohunid) ja erineva valgusnõudlusega taimedel (valgusnõudlikud vs. varjualuvad).

Töös on kirjeldatud viieaastase (1994...1998) eksperimendi tulemusi. Uurisime liigirikka niidukoosluse võra struktuuri ja liigilise mitmekesisuse muutusi. Eksperimendis muutsime taimede maapealset ja maa-alust konkurentsi väetamise, niitmise ja lisavalgustamisega. Lisavalgust andsime vertikaalsete peeglite abil, mis peegeldasid päikesevalgust prooviruudule ca 6 tundi päevas kasvuperioodi vältel. Niitsime kord aastas juuli keskel ja väetasime kaks korda aastas, mai algul ja septembri lõpus.

Prooviruutude suurus oli 40×40 cm. Töötlesime 1 m² suurust ala prooviruudu ümber. Igal töötlusel oli kaks taset (töödeldud või töötlemata), kõiki ka-

heksta (2×2×2) võimalikku töötluste variante oli kolmes korduses, seega oli prooviruute kokku 24.

Rohustu võra struktuuri uurisime nn. nõelaproovi meetodil, kasutades laserikiirt nõela asemel. Vaatlusi tegime kahe erineva nurga — 60° ja 120° alt lõuna–põhja teljel (vastavalt keskmine päikesenurk sel aastaajal ja peegelduva päikese nurk). Vaatlusi tegime kummagi nurga alt 50, juhuslike koordinaatidega punktides. Igal ruudul määrasime ka kõik seal kasvavad taimeliigid.

Liigirikkus prooviruutudel paraku ei muutunud oluliselt tänu eksperimentaalsetele töötlustele. Põhjus on ilmselt see, et prooviruudud olid väikesed (40×40 cm) ja ümbritsevalt, töötlemata alalt levisid taimed vegetatiivselt ka prooviruutudesse, kompenseerides seega oodatud liigirikkuse vähenemist pärast väetamist ja niitmise lakkamist.

Kumulatiivne liigirikkus (liikide arv esimesel vaatlusaastal, millele on liidetud lisandunud liikide arv järgnevatel aastatel) oli suurim ruutudel, kus oli enim ressursse taimede kasvuks — väetatud peeglitega ruutudel. Tundub tõenäoline, et nendel ruutudel lisavalguse andmine leevendas valguskonkurentsi, mis muutus tugevamaks pärast väetamist nendel ruutudel. Samuti oli taimede võra kõige tihedam ja ühtlasema jaotusega nendel ruutudel, kus oli nii väetatud kui ka lisavalgust antud. Nende ruutude võra oli ühtlasem isegi kui kontrollruutude (niidetud väetamata ja peeglita ruudud) oma.

Taimede üldkatvus prooviruudul oli ainus parameeter, mis oli oluliselt seotud väikeseskaalalise liigirikkusega, olenemata eksperimentaalsest tööstusest või aastast.

Liigirikkus ühikulise katvuse kohta (nn. standardiseeritud liigirikkus) vähenes oluliselt pärast väetamist. Taimede kogukatvuse aastane muutus oli hea aastase ruudu liigirikkuse muutuse ennustaja, välja arvatud erandlikult kuival perioodil (1994...1995).

Keskmine lehtede orientatsioon rohusus sõltus otsese pealelangeva kiirguse hulgast kasvuperioodil, seda eriti valgusnõudlike ja graminooidsete liikide puhul. Tavaliselt (neljal aastal viiest) ei olnud valgus vaadeldud niidurohusus taimede jaoks limiteeriv ja taimed pöörasid suurema osa oma lehepinnast päikesest eemale. Erandlikult pilvisel aastal (1996) oli aga taimede lehepind pööratud valdavalt päikese poole.

PUBLICATIONS

Eek, L. and Zobel, K. 1997
Effects of additional illumination and
fertilization on seasonal changes in fine-scale
grassland community structure.
Journal of Vegetation Science 8: 225–234.

Effects of additional illumination and fertilization on seasonal changes in fine-scale grassland community structure

Eek, Liina¹ & Zobel, Kristjan^{1*}

¹Department of Botany and Ecology, Tartu University, EE-2400 Tartu, Estonia;

*Corresponding author: Fax +372 34 35440; E-mail kzobel@ut.ee

Abstract. Fine-scale structure of a species-rich grassland was examined for seasonal changes caused by manipulated changes in the availability of above and below-ground resources (additional illumination with the help of mirrors and fertilization) in a field experiment. If changes induced by fertilization – which are expected to lead to a reduction in small-scale diversity – are due to intensified light competition, they should be compensated for by additional light input.

Permanent plots of 40 cm × 40 cm were sampled by the point quadrat method at three angles (60°, 90° and 120° from the horizontal North-South direction), using a laser beam to position the quadrats, in early July and early September. The applied treatments did not cause apparent changes in plant leaf orientation. The degree of spatial aggregation of biomass increased seasonally in fertilized, non-illuminated plots: greater productivity at a constant light supply led to a faster growth rate of potentially dominant species, as compared to the subordinate ones. Additional illumination mitigated this effect of fertilization, indicating that the observed changes in biomass aggregation were due to increased light competition.

There was a considerable seasonal decrease of variance ratio (ratio of observed variance of richness at a point and variance expected at random) in fertilized only and in illuminated only plots. In fertilized plots this was due to the positive relationship between biomass aggregation and expected variance of richness. Biomass constancy occurs to be inversely related to deficit in variance of richness. In illuminated plots, in contrast, only the observed variance of richness decreased seasonally, indicating a more uniform use of space by different species. Evidently, a deficit in variance of richness can be caused by drastically different processes, showing that the variance ratio statistic may not have a significant explanatory value in fine-scale community studies.

Keywords: Field experiment; Grassland community; Root competition; Shoot competition; Species richness.

Nomenclature: Kuusk (1972).

Introduction

Ecologists generally agree that interspecific competition is a force which may affect the structure and dynamics of plant communities. However, there is little agreement on how competition intensity, and the relative importance of competitive interactions for community development, is related to the availability of essential resources. Competition intensity has been considered (1) to be unrelated to habitat productivity (S.D. Wilson & Shay 1990; Taylor et al. 1990; S.D. Wilson & Tilman 1991, 1993; Belcher et al. 1995), or (2) to increase with habitat productivity (Grime 1979; Gurevitch 1986; J.B. Wilson 1988; Reader & Best 1989). Logically, there are also different views on the role of competition as a determinant of community structure and diversity in relation to habitat productivity (Grime 1979; Tilman 1990; Tilman & Pacala 1993; Bengtsson et al. 1994). Much of the controversy is probably due to the fact that below and above-ground competition have rarely been separated in ecological experiments. Studies where such a separation has been made usually report that root competition is more important (J.B. Wilson 1988; Aerts et al. 1991; Belcher et al. 1995) and is negatively related (S.D. Wilson & Tilman 1993) or unrelated (Belcher et al. 1995) to soil fertility.

On the other hand, most of the approaches agree that shoot competition intensity is positively related to productivity (though it has seldom been measured separately; Grime 1979; J.B. Wilson 1988; S.D. Wilson & Tilman 1993), and that competition for light may be a major determinant of community change or community stability (Grime 1979; Tilman 1990; Bengtsson et al. 1994). The relative importance of the two types of competition for community dynamics still remains unclear, even if we assume that (1) nutrient competition and light competition are uncorrelated or negatively correlated, (2) nutrient competition is unrelated or negatively related to productivity and (3) light competition is positively related to productivity, and then look at the latest results of root/shoot competition experiments. Root and shoot competition may differ greatly in their

symmetry (Weiner 1990) so it may be that nutrient competition, which usually appears to be more intense in the experiments, is less important as a determinant of community structure and diversity than previously assumed.

Though diversity may, in some cases, promote productivity (Kareiva 1994) the common understanding is that diversity tends – at least in medium to fertile conditions – to decrease with productivity (Grime 1979; Moore & Keddy 1989; Gough et al. 1994). For example, fertilization of species-rich grasslands, with constant disturbance rates, usually results in a decline of diversity (Bobbink & Willems 1987; Kull & Zobel 1991; Willems et al. 1993; van der Maarel & Sykes 1993; Tilman & Pacala 1993). If we agree that such a decline is caused by competitive exclusion of certain species, for instance because of intensified competition (a view supported by many authors; cf. Zobel 1992; Bengtsson et al. 1994), one may pose the question: is it root competition, shoot competition, or both, that cause the change? This study attempts to seek evidence to clarify this question, while analysing the short-time effect of grassland fertilization.

So far, in all field experiments where competition for light has been manipulated, it has been done either by physically preventing contact among the leaves of target and non-target plants (and thus at the same time introducing disturbance as an additional manipulation), or by reducing resource availability by shading (see Aarssen & Epp 1990 for a review). None of the field experiments have included an increase of resource availability for shoots, by adding light (a procedure comparable to fertilization in the case of below-ground resources). The experiment that provided data for this study applies (in a factorial design) two treatments to increase resource level both below- and above-ground. The broad objective of the experiment is to learn whether the expected fertilization-induced change of community structure and the decrease of species diversity in a species-rich grassland can be compensated for by additionally illuminating the canopy with the help of mirrors. If the changes caused by increased productivity are due to intensified shoot competition illumination should (at least partly) compensate for the effect of fertilizer.

We present the results of studying early changes in grassland canopy fine-scale structure induced by fertilizing and additionally illuminating a species-rich calcareous wooded meadow community in western Estonia.

Methods

Study site

The experiment was carried out in the Laelatu calcicolous wooded meadow in western Estonia, on the eastern coast of the Baltic Sea (58° 35' N, 23° 34' E).

The plant community at the site has been assigned to the *Sesleria coerulea-Filipendula hexapetala* association (Kral & Pork 1970). About 20 ha of the 153-ha meadow has been permanently mown for hay for more than 200 yr. It has a scattered tree layer with *Quercus robur*, *Fraxinus excelsior*, *Betula* spp. and *Populus tremula* as the most abundant species. The grassland community is extremely rich in vascular plant species at a small scale – the maximum number of species found was 25 in a 10 cm × 10 cm plot, 42 in a 20 cm × 20 cm plot (Kull & Zobel 1991) and 68 in a 1-m² plot (Kull & Zobel 1995). High species richness is characteristic only of this part of the meadow which has been mown permanently and not been fertilized. Fertilization is known to cause a rapid and severe decline of small-scale richness in the community (Kull & Zobel 1991).

Experimental design

The experiment is based on a randomized factorial design. Three treatments, applied to 40 cm × 40 cm permanent plots, are combined: (1) additional illumination with the help of vertical glass mirrors, (2) fertilization with NPK and microelements and (3) mowing. Each of the treatments has two levels (– +), and all the eight (2 × 2 × 2) possible treatment variants are in three replicates. Thus, 2 × 2 × 2 × 3 = 24 plots are studied altogether. In this paper only data from the 12 not-mown plots are used.

The plots are fertilized with N (10 gm⁻² yr⁻¹), P (4.2 g m⁻² yr⁻¹), K (4.2 g m⁻² yr⁻¹) and microelements twice a year, in late May and late September. An area of 1 m² is fertilized, with the permanent plot situated in the center of the area, in order to avoid edge effects.

Vertical glass mirrors (width 212 cm, height 124 cm, lower edge of the mirror being 20 cm from the ground) are used to increase the influx of light in illuminated plots. The mirrors face south and reflect sunlight to the 40 cm × 40 cm permanent plots for ca. 6 hr a day, increasing the illumination of the plots nearly twofold. Even under overcast conditions the light intensity increased by ca. 20–30% (measured with a linear light meter).

The treatment of additional illumination has the side additional effect of increasing temperature in the plant canopy, especially in bright sunlight. Measurements showed a temperature increase of 10–22% in the

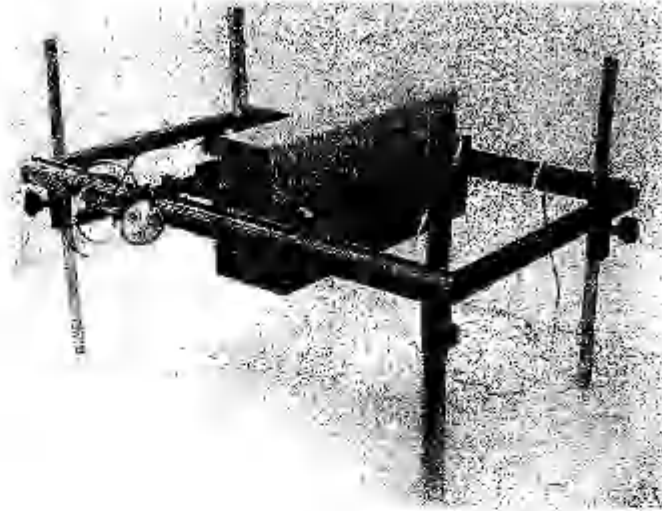


Fig. 1. The point quadrat sampling equipment is mounted on a frame alongside the permanent plot. A laser on a trolley moves along one axis. An extendable arm attached to the trolley and carrying a small rounded mirror, serves as the second axis. The coordinates of the point quadrat are read from the position of the laser trolley and the length of the mirror arm.

upper layer of the canopy (measured in direct sunlight), the relative rise of temperature depending on wind speed. However, the possible effect of the 'warmer' microclimate in illuminated plots will not be considered separately in this study. The main aim of the experiment was to see whether additional illumination could slow down the processes initiated by fertilization, i.e. processes leading to a decrease of diversity. If the increased temperature is harmful to some plant species

(and they suffer or disappear from the plot) the power of refuting the null hypothesis of identical richness dynamics in fertilized and fertilized + illuminated plots will simply be decreased. It is most improbable that the rise of temperature would promote the establishment of new species in the plots with mirrors, so this effect can only be an additional noise factor, but is not likely to lead to false rejections of the null hypothesis.

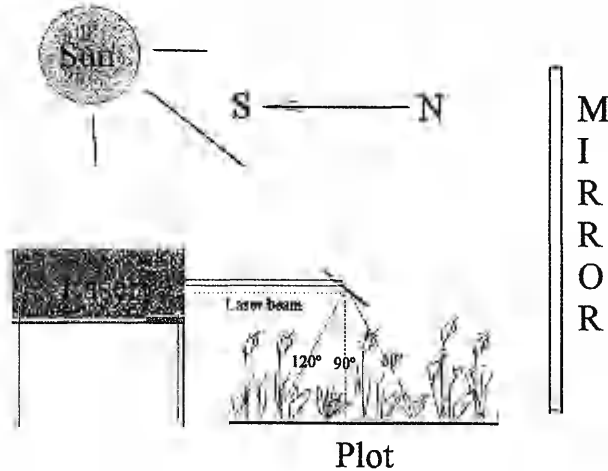


Fig. 2. The use of a mirror for additional illumination, and a laser beam as point quadrat pin at three different angles to examine a 40 cm x 40 cm permanent plot.

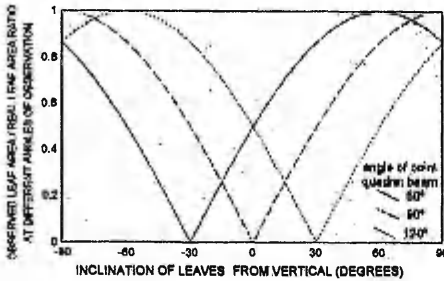


Fig. 3. Graph illustrating the way cover estimates depend on plant leaf inclination in the case of the three angles of positioning the point quadrat beam. The Y-axis measures percent leaf area visible from a certain angle of observation; the X-axis measures inclination of plant leaves from vertical (minus values indicate inclination towards the sun). The three curves are calculated as

$$Y = |\sin(X + (90^\circ - \alpha))|, \quad (1)$$

where α is angle of the point quadrat beam (60° , 90° and 120° , respectively).

Sampling methods

The plots were examined twice a year (early July and early September). In this study data from the first season of the experiment (1994) are used. For technical reasons it was not possible to examine the plots before fertilization and establishment of the mirrors, the first sampling was performed 40 days after the first fertilization, and immediately after placing the mirrors.

We did not expect significant effects of experimental treatments on species richness during the first sampling season, so a complete floristic inventory of the plots was performed only at the first sampling date (July).

Species composition and plant canopy structure were studied using the point quadrat method. We used a laser beam with a diameter of 1 mm instead of the usual metal pins to mark the point quadrats. The sampling laser is shown in Fig. 1. The beam was randomly positioned in the canopy at 50 points in each plot (coordinates of each point being selected randomly beforehand with a precision of 1 mm), at three different angles – 60° , 90° and 120° (Fig. 2). So, in total 150 point quadrats were investigated in each plot, on one sampling date. The positioning of the point quadrat beam at different angles makes it possible to learn about the mean orientation (inclination) of plant leaves. If the mean number of touches is recorded at three different angles, the approximate mean orientation of leaves can be assessed graphically (Fig. 3).

In all investigated points the coordinates of the point

quadrat and the number of touches of the beam by vascular plant species and litter (if positioned above green biomass at the particular angle), were recorded. In eight plots at the first sampling date (representing the eight possible treatment variants), and in the four non-known plots at the second sampling date, we identified plant species for each touch of green biomass by the point quadrat beam, and recorded the vertical sequence of the species.

Statistical methods

The effect of fertilization, illumination and time on the mean number of touches (of green biomass by the laser beam in a plot; from now on simply number of touches), was analysed using Repeated Measures ANOVA (within subjects design). The angle of the laser beam was included as an additional independent variable in the analysis.

The degree of biomass aggregation in a plot was computed as the ratio of observed variance of the number of touches in a plot to the variance expected in the case of a random spatial distribution of biomass. The expected variance was estimated by the mean of the observed numbers of touches in a plot (in the case of randomly distributed biomass the number of touches should be Poisson-distributed with the variance equal to the mean).

The effects of experimental treatments, time and angle on the degree of biomass aggregation were also analysed using Repeated Measures ANOVA.

Computation of the variance ratio statistic

We were interested whether the variability of species richness at a point differs from what could be expected from the null hypothesis of random distribution of species (variance deficit or excess; Palmer 1987; J.B. Wilson et al. 1987; Zobel & Zobel 1988). The ratio of observed and expected variances (variance ratio) served as the statistic of variance deficit or excess.

We estimated the expected variance of the number of species at a point using a slightly more complicated randomization procedure than usual. First, to retain the observed spatial distribution of biomass in the null model, numbers of touches were determined beforehand in hypothetical point quadrats by selecting randomly – with replacement from the pool of the 50 observed numbers of touches in the particular plot – at the particular angle and at the particular sampling date. Then, a species was assigned for each touch in each point quadrat. This was done by selecting randomly with replacement from the pool of all the registered touches in the studied 50 point quadrats. The number of species in each point quadrat

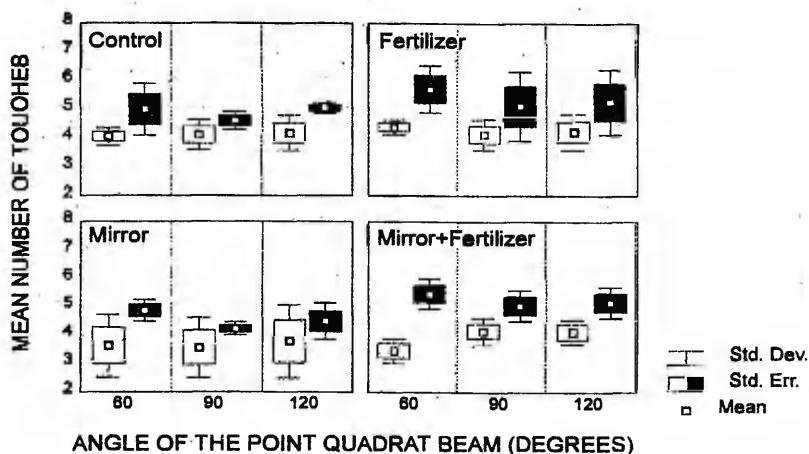


Fig. 4. Mean numbers of touches of green biomass and litter, recorded in a point quadrat at different angles, treatments and sampling time. Each pair of box-whisker plots represents the mean number of touches in July (open plots) and September (shaded plots), respectively. The main effects of fertilization ($p = 0.0437$), angle ($p = 0.0013$) and sampling time ($p < 0.0001$) are significant.

was then counted and the expected variance calculated.

We added an additional random procedure to avoid bias in estimating the expected variance of richness at a certain point. In the case of counting species, touched by a point quadrat beam (pin), it is important to realize that richness can be severely overestimated in the null model by simply randomly selecting touches from the entire pool. For several species the probability that its particular touch by the beam is accompanied by a touch of the same species (itself), can be much larger than the probability of being accompanied by a touch of some other species – i.e. it is quite usual that a beam touches the same individual several times. If this is not considered in building the null model, it is likely that the expected mean number of species at a certain point would be much greater than observed in the randomized data, and hence the variance of richness, as proportional to the mean, would be systematically larger as well.

To overcome this problem we estimated for all target species the empirical probability of: (1) being accompanied on a beam by a touch of a non-target species, and (2) the probability of being accompanied on a beam by a touch of the same species (itself). We used the whole dataset to estimate these probabilities. The probabilities were used in the randomization procedure only if they had been estimated on the basis of more than 10 observations (otherwise the 0.5/0.5 ratio was used).

In the randomization procedure the selected touch was approved only if a simultaneously generated ran-

dom number (evenly distributed between 0 and 1) was smaller than a probability value, calculated according to the list of species, already selected for this hypothetical point quadrat (while selecting the first touch for a hypothetical quadrat, the whole procedure was naturally omitted). The already selected touches in the point quadrat could be (1) of the same species or (2) of some other species, than the touch under selection. As each species had empirical probabilities of (1) co-occurring with itself and (2) co-occurring with some other species, calculated earlier, the probability for the selection procedure was evaluated as the mean probability of co-occurrence, over all the touches already selected for the particular hypothetical quadrat. In the case of disapproval of the selection (see above), the procedure was repeated until approval (note that the total number of touches in each hypothetical point quadrat was determined beforehand, by selecting it randomly from the pool of observed numbers of touches).

For a total of 36 datasets, representing treatment combination, time and angle – eight plots and three angles in the first sampling date, four plots and three angles in the second sampling date – we estimated the expected variance and calculated the variance ratio 1000 times. In the case of more than 97.5 % of the trials providing a variance ratio smaller or greater than unity, we concluded that variance deficit/excess was significant (two-sided test, $p = 0.05$).

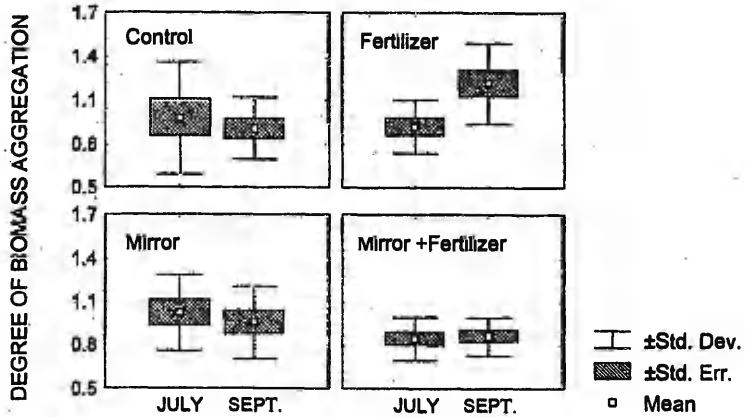


Fig. 5. Change of the degree of spatial aggregation of biomass (ratio of observed and expected variance of the number of touches in a point quadrat) with time in plots with different treatment. Main effect of sampling time is significant ($p < 0.0001$), so are the interactions fertilizer \times time ($p = 0.0353$) and fertilizer \times mirror ($p = 0.0189$).

Results

The mean number of touches per point quadrat increased significantly ($p < 0.0001$) during the season in all plots (Fig. 4). As expected, fertilization also led to a significant increase of biomass density ($p = 0.0437$). The numbers of touches were significantly different when counted at different angles (60° , 90° and 120°). Fig. 4 shows that there were systematically fewer touches at 90° , compared to 60° and 120° (situation in fertilized + illuminated plots in July is the exception here). Partly,

this result is trivial, because the distance that a beam at angles 60° and 120° has to penetrate in the canopy is $1.155 (= 1/\cos 30^\circ)$ times longer than a vertical beam. When this coefficient is applied to the results, it appears that biomass density was equal at all angles in July, in all plots except fertilized + illuminated ones. Such equality indicates that leaves did not have certain inclined orientation. Equal estimates of coverage at all three angles is not possible in the case of an inclination of leaves, it can be nearly equal when leaves are horizontal (Fig. 3). Consequently, in July leaves were either horizontal or

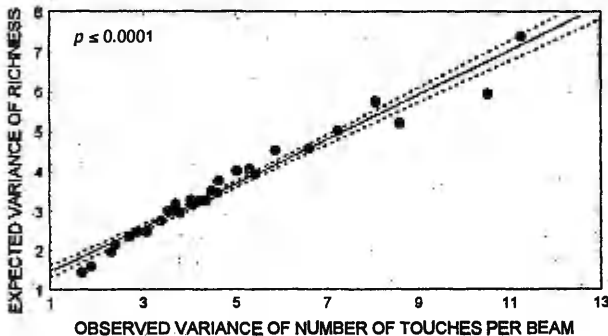


Fig. 6. Expected variance of richness at a point (predicted from the null model stating the independent distribution of species), plotted against the observed variance of touches recorded in a point quadrat.

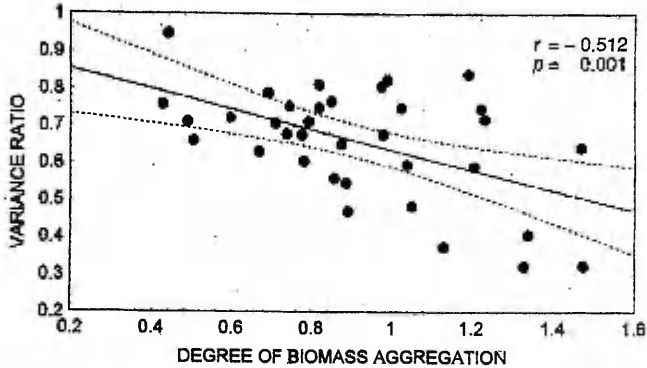


Fig. 7. Relationship between the variance ratio (observed/expected variance of richness at a point) and the degree of biomass aggregation (observed/expected variance of number of touches at a point).

strongly curved (a round object would have identical projections on surfaces with any orientation). In fertilized plots with mirrors the density of biomass was the lowest, when observed at 60° (angle of the sun), at the first sampling date. It means that leaves tended to be inclined towards the sun (Fig. 3). By September the biomass density in all investigated plots was greater at 60° and 120°, compared to 90°. This shows that most of the leaves tended to be more or less vertically orientated. It is obvious from Fig. 3 that if point quadrats, positioned at angles 60° and 120°, provide systematically larger numbers of touches than vertically positioned point quadrats, it can only indicate the mean inclination of leaves from vertical being less than 15° towards the North or South (of course, the latter will be true only if the inevitably greater number of touches in an inclined point quadrat is taken into account).

There were only minor (non-significant) seasonal changes in the degree of biomass aggregation in control plots and in plots with only additional illumination (Fig. 5). Fertilization, if applied alone, increased significantly the degree of biomass aggregation (fertilization × time interaction term significant at $p = 0.0353$, fertilization × mirror interaction term significant at $p = 0.0189$). In fertilized plots without mirrors the rather homogeneous spatial distribution of green biomass, observed in the beginning of the experiment, had developed into a pattern, consisting of spots with high biomass density (thickets) and spots with little biomass (openings). When illumination and fertilization are combined, seasonal increases in biomass aggregation can no longer be seen. Additional illumination has compensated for the effect of fertilizer — the changes were even smaller than in control plots or in plots with mirrors only (Fig. 5).

The results of computing the variance ratio statistic for the 36 datasets (see methods) are challenging to interpret. First, all the estimates of the variance ratio indicated variance deficit. The expected variance of richness was always greater than the observed variance. Second, it appeared that the expected variance of richness at a point was almost exactly predictable from the variance of the number of touches (Fig. 6). It means that in the null model the number of species expected in a point quadrat mostly depended on the density of biomass at this point. Consequently, there should also exist a strong correlation between the degree of biomass aggregation and the variance ratio (first of these two statistics includes variance of the number of touches at a point, second includes expected variance of richness). There was a strong relationship between these variables (Fig. 7).

The above-mentioned relationships help to interpret the results concerning the seasonal change of the variance ratio, as related to the applied experimental treatments (Fig. 8). Fertilization, as well as illumination, if applied alone, caused a considerable decrease in variance ratio. In fertilized only plots this must be because of the increase of biomass aggregation (see Figs. 5 and 6). At the same time, in illuminated only plots, where biomass aggregation did not change seasonally (Fig. 5) the decrease of variance ratio should be due to a smaller observed variance of richness. When the treatments were applied simultaneously, the seasonal change of variance ratio was negligible and comparable to that in control plots. It means that neither the observed variance of richness, nor the expected variance of richness (biomass aggregation, see Figs. 5 and 6), changed in time.

Owing to the uncertain explanatory value of the variance ratio statistic, neither the p -values for the variance ratio, nor the results of applying ANOVA to the variance ratio, are referred to in this paper.

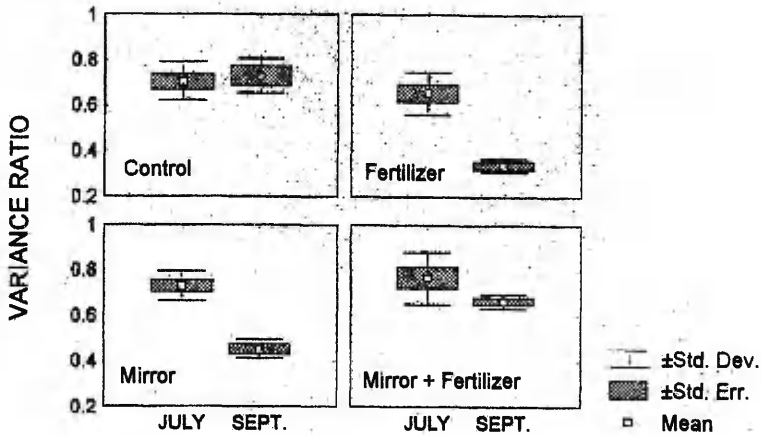


Fig. 8. Change in variance ratio (observed/expected variance of richness at a point) with time in plots with different treatments.

Discussion

The results of studying the orientation of plant leaves in experimental plots were unexpected. Generally, there was no evidence of any orientation being preferred by the leaves, except that the nearly vertical position prevailed at the second sampling date (Figs. 3 and 4). There was an indication of leaves being inclined towards the sun in fertilized plots with mirrors at the first sampling date. As the mirrors had just been established at this time, this could be an early reaction of plants to the 'second sun'. However, in September there was no evidence for an effect of additional illumination on plant leaf orientation. Assuming that there could be some light deficit in the lower canopy layers of fertilized non-illuminated plots by September, one may conclude that either (1) the morphological flexibility of grassland plants is too small for quickly orientating their leaves for the best capture of light, or (2) orientation of leaves for catching direct sunlight is not an effective strategy for plants in the lower canopy layer.

One of the main interests in this study was the detection of changes in plant canopy structure, induced by fertilization, and indicating processes which in the long run would cause a decrease in diversity. If competition for light is responsible for diversity decrease, it would be logical to expect an increased spatial aggregation of biomass as an early reaction to fertilization. The reason why shoot competition may easily lead to

competitive exclusion of species lies in its asymmetric nature – a small initial advantage allows a stem to capture a disproportionately great amount of resources and *vice versa* (Weiner 1990; Bengtsson et al. 1994). Thus, species capable of rapid resource uptake and having a higher growth potential, would be expected to rapidly improve their position in the canopy, while subordinate species are even more suppressed (shaded). Results from this experiment support this view. In fertilized plots there was a significant increase of biomass aggregation (Fig. 5). However, additional illumination completely eliminated this process, so the more aggregated spatial distribution of biomass must have been a result of competition for light. Increased light resource has prevented the uneven development of canopy-forming species in fertilized plots. This result is encouraging, considering the long-term objective of the experiment.

The early changes of plant canopy structure with above- and below-ground resource addition support the view that one of the main forces which lead to a reduced number of species, as compared to the entire set of potential inhabitants (species pool; Taylor et al. 1990; Pärtel et al. 1996) is competition for light (Zobel 1992; Bengtsson et al. 1994).

The variance ratio statistic (Palmer 1987; J.B. Wilson et al. 1987) was calculated for data sets as a potential indicator of seasonal changes in the spatial organization of multi-species canopy. Variance deficit, as compared to null model prediction, has been considered evidence of

negative associations among species (Zobel & Zobel 1988; Palmer 1987), nucleation (Zobel et al. 1993) or niche limitation (saturation of community by species; J.B. Wilson et al. 1987; J.B. Wilson & Sykes 1988; Watkins & Wilson 1992; Zobel et al. 1993). As pointed out by Palmer & van der Maarel (1995; van der Maarel et al. 1995) and J.B. Wilson (1995) there can also be other reasons for variance deficit, e.g. limitation to the number of plant individuals that can coexist in a quadrat. To overcome this problem we designed a null model where the distribution of the number of plant modules in point quadrats was the same as in the empirical data. We also corrected the null model to cope with a certain kind of spatial dependence arising from morphological features of species (one individual may be recorded several times in a point quadrat). However, our results showed that strong variance deficit could be a direct reflection of an increased degree of biomass spatial aggregation, caused by a variable ability of resource capture by species. The null model prediction of variance in richness depended strongly and positively on relative variance of the number of plant modules in a quadrat (Fig. 6), making the observed decrease of variance ratio in fertilized non-illuminated quadrats (Fig. 8) merely an indication of the uneven growth of species during the season. J.B. Wilson & Gitay (1995) have sought evidence for competitive interactions in a low variance of total quadrat biomass. Our results showed, in contrast, that intensified shoot competition and deficit in variance of richness, could be explained by an increased variance of total quadrat biomass (degree of biomass aggregation).

The seasonal decrease in variance ratio in illuminated, non-fertilized plots cannot be explained by the increased biomass aggregation (Figs. 5 and 8). Richness has become less variable in space, with variance of the number of plant modules at a point still being close to random expectation. It is very difficult to interpret such a phenomenon in the framework of the existing theoretical approaches: how could niche limitation be caused by increased light resource? More likely, the variance deficit here is an indication of a more uniform use of space in the canopy – the abundance of light may have reduced the importance of local shading and made it easier for species with different morphology to coexist at a point. In those illuminated plots where nutrients were added and the canopy was more dense, the status quo, observed in control plots, has been retained (Figs. 5 and 8).

Acknowledgements. This work was supported by grant No. 261 from the Estonian Science Foundation. We thank Michael W. Palmer and an anonymous referee for helpful comments on the manuscript.

References

- Aarssen, L.W. & Epp, G.A. 1990. Neighbour manipulations in natural vegetation: a review. *J. Veg. Sci.* 1: 13-30.
- Aerts, R., Boot, R.G.A. & van der Aart, P.J.M. 1991. The relation between above- and belowground biomass allocation patterns and competitive ability. *Oecologia (Berl.)* 87: 551-559.
- Belcher, J.W., Keddy, P.A. & Twolan-Strutt, L. 1995. Root and shoot competition along soil depth gradient. *J. Ecol.* 83: 673-682.
- Bengtsson, J., Fagerström, T. & Rydin, H. 1994. Competition and coexistence in plant communities. *Trends Ecol. Evol.* 9: 246-250.
- Bobbink, R. & Willems, J. 1987. Increasing dominance of *Brachypodium pinnatum* (L.) Beauv. in chalk grassland: a threat to a species-rich ecosystem. *Biol. Conserv.* 40: 301-314.
- Gough, L., Grace, J.B. & Taylor, K.L. 1994. The relationship between species richness and community biomass: the importance of environmental variables. *Oikos* 70: 271-279.
- Grime, J.P. 1979. *Plant strategies and vegetation processes*. Wiley, Chichester.
- Gurevich, J. 1986. Competition and the local distribution of the grass *Stipa neomexicana*. *Ecology* 67: 46-57.
- Kareiva, P. 1994. Diversity begets productivity. *Nature* 368: 686.
- Krall, H. & Pork, K. 1970. Laelatu puisniit. In: Kumari, E. (ed.) *Lääne-Eesti rannikualade loodus*, pp. 14-27. Valgus, Tallinn.
- Kull, K. & Zobel, K. 1995. Kas on veel ruumi? Ei, kõik on täis. *Eesti Loodus* 48: 33-35.
- Kull, K. & Zobel, M. 1991. High species richness in an Estonian wooded meadow. *J. Veg. Sci.* 2: 711-714.
- Kuusik, V. 1972. *Taimede välimääräraja*. Valgus, Tallinn.
- Moore, D.R.J. & Keddy, P.A. 1989. The relationship between species richness and standing crop in wetlands: the importance of scale. *Vegetatio* 79: 99-106.
- Palmer, M.W. 1987. Variability in species richness with Minnesota oldfields: a use of variance test. *Vegetatio* 70: 61-64.
- Palmer, M.W. & van der Maarel, E. 1995. Variance in species richness, species association, and niche limitation. *Oikos* 73: 203-213.
- Pärtel, M., Zobel, M., Zobel, K. & van der Maarel, E. 1996. The species pool and its relation to species richness: evidence from Estonian plant communities. *Oikos* 75: 111-117.
- Reader, R.J. & Best, B.J. 1989. Variation in competition along an environmental gradient: *Hieracium floribundum* in an abandoned pasture. *J. Ecol.* 77: 673-684.
- Taylor, D.R., Aarssen, L.W. & Loehle, C. 1990. On the relationship between r/K selection and environmental carrying capacity: a new habitat template for plant life history strategies. *Oikos* 58: 239-250.
- Tilman, D. 1990. Constraints and tradeoffs: toward a predictive theory of competition and succession. *Oikos* 58: 3-15.
- Tilman, D. & Pacala, S. 1993. The maintenance of species richness in plant communities. In: Ricklefs, R.E. & Schlüter, D. (eds.) *Species diversity in ecological communities*, pp. 13-25. University of Chicago Press, Chicago, IL.

- van der Maarel, E. & Sykes, M. 1993. Small-scale spatial dynamics of plant species in a grassland community over six years. *J. Veg. Sci.* 4: 179-188.
- van der Maarel, E., Noest, V. & Palmer, M.W. 1995. Variation in species richness on small grassland quadrats: niche structure or small-scale plant mobility? *J. Veg. Sci.* 6: 741-752.
- Watkins, A.J. & Wilson, J.B. 1992. Fine-scale community structure of lawns. *J. Ecol.* 80: 15-24.
- Weiner, J. 1990. Asymmetric competition in plant populations. *Trends Ecol. Evol.* 5: 360-364.
- Willems, J.H., Peet, R.K. & Bik, L. 1993. Changes in chalk-grassland structure and species richness resulting from selective nutrient addition. *J. Veg. Sci.* 4: 203-212.
- Wilson, J.B. 1988. Shoot competition and root competition. *J. Appl. Ecol.* 25: 279-296.
- Wilson, J.B. 1995. Variance in species richness, niche limitation, and vindication of patch models. *Oikos* 73: 277-279.
- Wilson, J.B. & Gitay, H. 1995. Limitations to species coexistence: evidence for competition from field observations, using a patch model. *J. Veg. Sci.* 6: 369-376.
- Wilson, J.B. & Sykes, M. 1988. Some tests for niche limitation by examination of species diversity in the Dunedin area, New Zealand. *N. Z. J. Bot.* 26: 237-244.
- Wilson, J.B., Gitay, H. & Agnew, A. 1987. Does niche limitation exist? *Funct. Ecol.* 1: 391-397.
- Wilson, S.D. & Shay, J.M. 1990. Competition, fire and nutrients in a mixed-grass prairie. *Ecology* 71: 1959-1967.
- Wilson, S.D. & Tilman, D. 1991. Components of plant competition along a productivity gradient. *Ecology* 72: 1050-1065.
- Wilson, S.D. & Tilman, D. 1993. Plant competition and resource availability in response to disturbance and fertilization. *Ecology* 74: 599-611.
- Zobel, K. & Zobel, M. 1988. A new null hypothesis for measuring the degree of plant community organization. *Vegetatio* 75: 17-25.
- Zobel, K., Zobel, M. & Peet, R.K. 1993. Change in pattern diversity during secondary succession in Estonian forests. *J. Veg. Sci.* 4: 489-498.
- Zobel, M. 1992. Plant species coexistence - the role of historical, evolutionary and ecological factors. *Oikos* 65: 314-320.

Received 16 November 1995;

Revision received 24 April 1996;

Accepted 11 May 1996.

EEK, L. and ZOBEL, K. 2001.
Structure and diversity of a species-rich grassland community,
treated with additional illumination, fertilization and mowing.
Ecography 24: 157–164.

Structure and diversity of a species-rich grassland community, treated with additional illumination, fertilization and mowing

Liina Eek and Kristjan Zobel

Eek, L. and Zobel, K. 2001. Structure and diversity of a species-rich grassland community, treated with additional illumination, fertilization and mowing. – *Ecography* 24: 157–164.

We examined the changes of species-rich herbaceous community canopy structure and species diversity in a five-year field experiment. Above- and below-ground resource addition (fertilization and additional illumination with the help of mirrors) and cessation of annual mowing were applied as experimental treatments in 24 permanent plots of 40 × 40 cm. Canopy structure was studied by point quadrat sampling using laser beam to randomly position point quadrats at two different angles of observation – 60° and 120° from horizontal, north-south direction. We hypothesized that the studied plant community is not light-limited under the normal conditions (non-fertilized and annually mown) but would become light-limited after nutrient addition and/or cessation of mowing. In this case the effect of fertilization and cessation of mowing could be mitigated by introducing additional light resource (placing south-facing mirrors).

Species richness in plots was not significantly altered by experimental treatments, most probably because of a strong input of species from surrounding areas by the means of lateral vegetative spread, compensating for the expected decrease of diversity with fertilization and cessation of mowing. Surprisingly, plant total coverage in a plot was the only variable significantly explaining plot richness, independent of experimental treatments and the year of observation. We therefore studied also species richness per unit coverage (standardized richness) which appeared to be significantly reduced by fertilization. Annual change of plant total coverage of a plot was good predictor of annual change of plot richness, except for the exceptionally dry period (1994–1995). There was no indication of light being limiting for the plants in control plots, but light obviously became limiting after nutrient addition – in fertilized plots additional illumination lead to a notably denser canopy. Generally, the average exposition of plant leaves surface was towards the north, independent of experimental treatments. However, in the exceptionally cloudy and cool year 1996 the situation was radically different – leaves were exposed predominantly towards the south, indicating that multi-species canopy can plastically react to long-term changes in light availability.

L. Eek and K. Zobel (correspondence: kzobel@ut.ee), Dept of Botany, Tartu Univ., 40 Lai St., EE-51005 Tartu, Estonia.

It is widely acknowledged that light is a major determinant of plant community structure (Wilson 1988, Tilman 1990, 1993, Bengtsson et al. 1994, Grace and Pugsek 1997, Kleijn and Van der Voort 1997) and the intensity of light competition is positively related to productivity (Wilson 1988, Wilson and Tilman 1993). However, it is not clear, what exactly is the relative

importance of light (vs nutrient) competition in community change.

Light competition has been considered important even in low swards (Mitchley and Willems 1995), occurring immediately after plants are high enough to shade each other. Evidently, the better is nutrient availability, the faster and higher the plants will grow and the less

Accepted 7 July 2000

Copyright © ECOGRAPHY 2001.

ISSN 0906-7590

Printed in Ireland – all rights reserved

equally will light be distributed in the canopy (Willems 1983, Hirose and Werger 1995). Apparent from many experiments, fertilization tends to lead to a decrease in species richness (Gurevich and Unnasch 1989, Kull and Zobel 1991, Tilman 1993, Tilman and Pácala 1993, Willems et al. 1993) as inferior competitors are likely to be excluded from such communities due to intensified competition, especially asymmetric light competition.

If light competition is equalized and insufficiency of light in lower levels of canopy is mitigated, then inferior light competitors will not be outcompeted (Piper 1995). This is obvious in several biomass removal experiments where plant individuals have been kept in the same size by physically preventing species from having opportunity to dominate (Gibson et al. 1987, Kull and Zobel 1991, Jonsson 1995, Mitchley and Willems 1995, Ryser et al. 1995, Güsewell et al. 1998, Proulx and Mazumder 1998). Moderate biomass removal, especially in fertile sites, has another important effect on plant canopy structure, connected to the removal of dead parts of plants. Low litter biomass has been considered to be a factor favouring higher species richness (Day et al. 1988, Tilman 1993, Foster and Gross 1998) at least partly due to resulting better light conditions, although other reasons have also been suggested (e.g. litter removal has been shown to enhance seed germination, Tilman 1993). Beside biomass removal (e.g. grazing or mowing) and decreased nutrient availability the only way of improving light availability in the canopy is additional illumination. The latter is also the only method of adding the light resource to lower canopy layers without physically disturbing the community (Eek and Zobel 1997).

An important feature affecting the light climate in a canopy is foliage inclination. Inclination of plant leaves has been shown to change in response to several environmental variables (light availability, water and temperature regime, etc.), in order to optimize the effectiveness of resource capture and avoid photoinhibition (Ehleringer and Forseth 1989, Tappeiner and Cernusca 1989). In case of complete light interception the mean inclination of grass leaves is reported to vary mainly between 60°–90°, south-north direction (i.e. leaf surfaces tend to be exposed towards the north, Cooper et al. 1970). However, there are practically no information available on how plastically does the mean inclination of plant leaves in a multi-species grassland canopy respond to certain changes in light availability (e.g. seasonal, year-to-year, successional, experimentally induced changes).

In 1994 we established a field experiment in a species-rich wooded meadow to study the relative importance of above- and below-ground competitive processes in determining small-scale canopy structure (incl. mean leaf inclination) and diversity pattern. Initially we assumed that intensified competition for light is the main mechanism responsible for the decrease in diversity in

these parts of the grassland where mowing had been ceased or where nutrients had been added (Kull and Zobel 1991). Thus, we hypothesized that additional illumination of the canopy could be applied to mitigate the effect of fertilization and cessation of mowing (Eek and Zobel 1997, Liira and Zobel 2000, Urbas and Zobel 2000). In this paper we shall present the results from this experiment obtained after manipulating vegetation in permanent plots with additional illumination (with the help of mirrors), fertilization and cessation of annual mowing for four years. We shall mainly concentrate on seeking evidence for the following working hypotheses: 1) in the studied annually mown grassland light normally does not limit above-ground productivity and shoot competition is not an important force in small-scale diversity pattern formation; 2) in the case of nutrient addition and/or cessation of annual mowing light may become limiting for canopy productivity and competition for light an important determinant of diversity; 3) additional light resource, if provided, would mitigate the effects of fertilizer and cessation of mowing by making competition for light less intense; 4) given species richness per unit area is known to depend on plant ramet density (Zobel and Liira 1997), and given plots manipulated with fertilization are expected to develop denser vegetation (more ramets and higher coverage), richness per unit coverage could be a diversity measure more sensitive to experimental manipulations than richness per unit area. 5) Mean foliage inclination, depending on radiation intensity and other various environmental factors (Ehleringer and Forseth 1989, Tappeiner and Cernusca 1989) might be used as complex characteristic of light conditions and microclimate in the grassland canopy (as manipulated by experimental treatments and subject to year-to-year climatic variation).

Methods

Study site

The experimental site is situated in the Laelatu wooded meadow on the eastern coast of Baltic sea, in western Estonia.

The plant community of the meadow has been assigned to the *Sesleria coerulea* – *Filipendula hexapetala* association (Krall and Pork 1970). There is a scattered tree layer with *Quercus robur*, *Fraxinus excelsior*, *Betula* spp. and *Populus tremula* as the most abundant species, average coverage of tree canopy being ca 20%. It has been permanently cut for hay for > 200 yr. The total area of the meadow is 153 ha, but only ca 20 ha has been annually mown.

This ecosystem is extremely rich in vascular plant species at a small scale – the maximum number of species found is 25 in a 10 × 10 cm plot, 42 in a 20 × 20

Table 1. The number of sunshine hours per month (measured with heliotrope), mean monthly air temperature (°C) and monthly precipitation (mm) during the growing season in the five years of experiment (measured by Estonian Inst. of Meteorology and Hydrology in Virtsu observatory, 3 km from experimental site). Data on the exceptionally dark, cool and dry years are in boldface.

The number of sunshine hours per month				
	May	June	July	Total
1994	339	303	439	1081
1995	252	315	372	939
1996	206	270	269	745
1997	284	295	364	943
1998	307	241	278	826

Mean monthly air temperature (°C)				
	May	June	July	Mean
1994	9.0	12.5	19.5	13.7
1995	9.8	16.9	16.6	14.4
1996	9.1	13.3	15.4	12.6
1997	8.4	16.2	18.9	14.5
1998	10.5	14.7	16.0	13.7

Monthly precipitation (mm)				
	May	June	July	Total
1994	29.0	62.0	3.0	94.0
1995	57.3	87.3	59.9	204.5
1996	59.2	28.6	86.8	174.6
1997	56.2	93.1	70.2	219.5
1998	38.4	78.2	60.6	177.2

cm plot (Kull and Zobel 1991) and 68 in a 1 m² plot (Kull and Zobel 1995). The high species richness is found only in the annually mown part of the meadow. In the case of cessation of mowing taller and faster growing species would overtop lower and slower-growing ones, and as a result weaker light competitors would be outcompeted and species richness would decrease sharply (Kull and Zobel 1991, Jonsson 1995, Ryser et al. 1995).

Data on some climate characteristics at the experimental site (number of sunshine hours per month, mean monthly air temperature, monthly precipitation), in the growing seasons of 1994 by 1998, are presented in Table 1.

Experimental design

The experiment had a randomized factorial design. Three treatments applied to 40 × 40 cm permanent plots were combined: 1) additional illumination with the help of vertical glass mirrors, 2) fertilization with NPK and microelements and 3) mowing. Each treatment had two levels (- +), and all the eight (2 × 2 × 2) possible treatment variants were in three replicates. Thus, (2 × 2 × 2 × 3) 24 plots were studied altogether.

The plots were fertilized with N (10 g m⁻² yr⁻¹), P (4.2 g m⁻² yr⁻¹), K (4.2 g m⁻² yr⁻¹) and microelements twice a year, in early May and in late September. The plots were mown in mid-July. Fertilization and cessation of mowing were applied to an area of 1 m², with permanent plot situated in the center of the area, in order to avoid edge effects.

Vertical glass mirrors (width 212 cm, height 124 cm, lower edge of the mirror being 20 cm from the ground) were used to increase the amount of photosynthetically active radiation (PAR). The mirrors faced south and reflected sunlight to the permanent plots for ca 6 h a day. The intensity of PAR was measured during sunny days at ca 12 AM–1 PM in 3–6 July 1998 using linear light meter Licor LI-250 and Quantum Sensor Licor LI-191SA (light sensitive area 100 × 1 cm), temperature was measured at midday in 3 July. Light interception in the grassland canopy was measured at different heights with 5 cm intervals up to 35 cm from the ground level. At each level measurements were repeated twice. Temperature inside the canopy was also measured at the same height intervals, but in four replicates. In Table 2 data on fertilized and mown plots from 3 different canopy levels is presented to demonstrate that the applied additional illumination increased light intensity considerably even under a dense and comparatively high canopy (measurements were done just before annual mowing). One can see that the mirrors increased the level of illumination in lower canopy layers approximately twofold.

The temperature gradient was not that sharp. The highest temperature was observed in the intermediate canopy layers in plots with mirrors. Direct radiation appears not to be the most important determinant of temperature regime in the canopy.

Table 2. Average values of relative PAR intensity and temperature (°C) in illuminated and non-illuminated plots. Values of light are expressed relative to those measured above the canopies of non-illuminated plots (= 1.0, boldface).

Height of measurement (cm, from ground level)	Without mirror		With mirror	
	Light	Temperature (°C)	Light	Temperature (°C)
5	0.1	26.4	0.22	29.7
15	0.45	26.8	0.67	32.1
35	1.0	24.1	1.65	28.4

Sampling methods

The plots were examined in the end of June and beginning of July during 5 yr (1994–1998).

The point quadrat method was applied to examine canopy spatial structure. This method is known as effective, speedy and non-destructive way to study plant canopy (Jonasson 1983, Floyd and Anderson 1987, Mitchley and Willems 1995). A laser beam with the diameter of 1 mm was used to mark the point quadrats. The beam was positioned in the canopy randomly at 50 points in each plot at two different angles – 60° and 120° from horizontal, south-north direction – in order to be able to roughly assess the mean orientation of leaves along south-north direction (see Fig. 3 in Eek and Zobel 1997). Thus, altogether 100 point quadrats were investigated in each 40 × 40 cm plot. At this the angle of the point quadrat beam 60° meant inclination towards the south (the angle approximating the mean angle of incoming direct PAR around noon) and the angle 120° meant inclination of the point quadrat beam towards the north. Prior to observations the coordinates of the point quadrats were determined using random number generator, with the precision of 1 mm (for more details see Eek and Zobel 1997).

In each investigated point the coordinates of the point quadrat and the number of touches per beam by vascular plant species and litter (if positioned above green biomass), were recorded.

Additionally, in all plots all vascular plant species were recorded and percent coverage estimated visually. The total coverage was calculated as the sum over all vascular plant species.

Statistical methods

The dynamics of plant canopy spatial structure was analysed using Repeated Measures ANOVA (ANOVAR). The number of touches of vascular plants per point quadrat beam (hereafter number of touches) was regarded as dependent variable. Experimental treatments (fertilization, illumination and mowing – hereafter FERT, ILLUM and MOWING), angle of point quadrat beam (60° and 120°, ANGLE) and time (1994–98, TIME) served as fixed independent variables. The random factor PLOT (denoting the three replicate permanent plots) was included as additional independent variable, nested within the three experimental treatments.

The relationship between species richness in a plot (S) and total plant coverage (C, calculated as the sum of visually estimated coverage over all species) as well as the relationship between annual changes of these variables was analyzed using linear regression analysis.

Given species richness in a plot was significantly positively related to total plant coverage in the same

Table 3. The effect of fertilization (FERT), additional illumination (ILLUM), mowing (MOWING), angle of point quadrat beam (60° and 120°, ANGLE) and time (1994–1998, TIME) on the number of touches of vascular plants per point quadrat; analysed with repeated measures ANOVA. Experimental plot (PLOT) was included in the model as an additional random independent variable, nested within the three experimental treatments. None of the interactions among three or more factors were significant and these are not shown. * = $p < 0.05$.

Factor	DF	F-value	p-value
PLOT	16,2352	13.3180	0.0000*
ILLUM	1,16	0.1721	0.6838
FERT	1,16	221.3583	0.0000*
MOWING	1,16	0.0005	0.9820
ANGLE	1,16	4.6674	0.0463*
TIME	4,64	29.5606	0.0000*
ILLUM × FERT	1,16	8.5783	0.0098*
ILLUM × MOWING	1,16	0.1213	0.7321
FERT × MOWING	1,16	4.6705	0.0452*
ILLUM × ANGLE	1,16	0.9516	0.3438
PLOT × ANGLE	16,2352	19.6479	0.0000*
FERT × ANGLE	1,16	1.3509	0.2622
MOWING × ANGLE	1,16	0.5753	0.4592
PLOT × TIME	64,9408	6.4166	0.0000*
ILLUM × TIME	4,64	4.5073	0.0029*
FERT × TIME	4,64	16.1021	0.0000*
MOWING × TIME	4,64	2.6975	0.0383*
ANGLE × TIME	4,64	10.7860	0.0000*

plot, and given the latter could possibly shade the presumable relationships among species richness and experimental treatments, it occurred reasonable to measure richness also per unit coverage. Therefore, standardized richness was estimated for each plot: $S_{st} = S/C$.

ANOVAR was used to study the effect of experimental treatments (FERT, ILLUM and MOWING) and TIME on standardized species richness. All statistical procedures were performed using STATISTICA 6.0 software.

Results

Number of touches

The highest number of touches of vascular plants per point quadrat beam was observed in plots with the highest resource levels – in fertilized plots with mirrors. As expected, fertilization increased significantly the number of touches whenever applied (main effect of FERT significant at $p < 0.0001$, Table 3). Additional illumination alone had no effect (main effect of ILLUM non-significant at $p = 0.6838$, Table 3), but in fertilized plots it obviously smoothed the presumably intensified light competition by providing more light to lower canopy levels (interaction ILLUM × FERT significant at $p = 0.0098$, Fig. 1, Table 3). Somewhat surprisingly, mowing did not affect the number of touches per point quadrat beam (main effect of MOWING non-significant at $p = 0.9820$, Table 3).

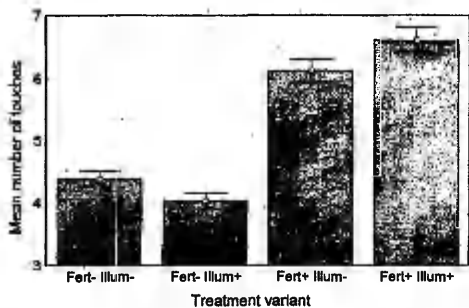


Fig. 1. Mean number of touches per point quadrat beam (and standard errors) in plots with different fertilization and illumination treatments (Fert – fertilization, illum – additional illumination). The interaction between illumination and fertilization is significant at $p = 0.0129$.

There was an overall significant effect of sampling year on canopy density (main effect of TIME significant at $p < 0.0001$, Table 3), indicating that the years of experiment differed greatly in above-ground productivity. Also, the relative effect of fertilization on canopy density changed in time (interaction FERT \times TIME significant at $p < 0.0001$, Table 3).

Comparing number of touches at different angles (60° and 120°) it was obvious that leaves generally tended to be exposed towards the North (i.e. there were more touches at 120° ; main effect of ANGLE significant at $p = 0.0463$, Table 3). However, in 1996 a steep decrease appeared in the number of touches at 120° . In this particular summer the highest number of touches was found at 60° – i.e. leaves were predominantly exposed to the sun (interaction ANGLE \times TIME significant at $p < 0.0001$, Fig. 2, Table 3). This phenomenon was obvious in all plots, independent of

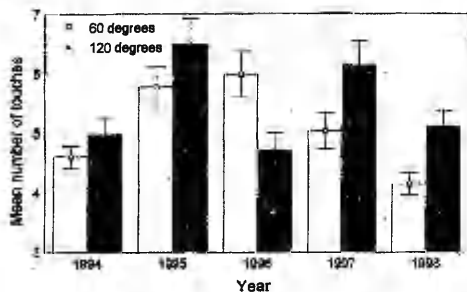


Fig. 2. Mean number of touches per point quadrat beam (and standard errors) at different angles of observation in different years. The angles 60° and 120° denote inclination from horizontal of the point quadrat beam, in a N-S direction. 60° means inclination of point quadrat beam towards the sun; 120° , towards the north. The interaction between angle of observation and time (year of observation) is significant at $p < 0.0001$.

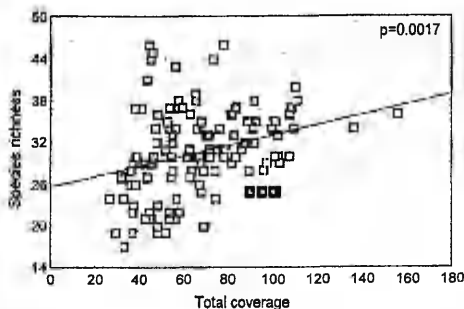


Fig. 3. Relationship between species richness in a plot and total plant coverage in the same plot. Data from 5 yr of observation are pooled.

treatments. It can be explained with the fact that the year 1996 was extremely cool and cloudy when compared to the remaining years (Table 1). Most probably plants did not get enough light and leaves had orientated to direction of sun (60°) to absorb more PAR.

Species richness

There was a significant and positive relationship between species richness in plots and total coverage of plants (Fig. 3, $p = 0.0017$).

Also, annual changes of species richness in a plot were significantly and positively related to annual changes in plant total coverage in the same plot (Fig. 4, $p = 0.0001$). The first year of the experiment was the exception here – change in species richness 1994–1995 was unrelated to change in coverage.

Standardized species richness (S_{st}) was significantly smaller in fertilized plots (Table 4, FERT significant at $p < 0.0001$).

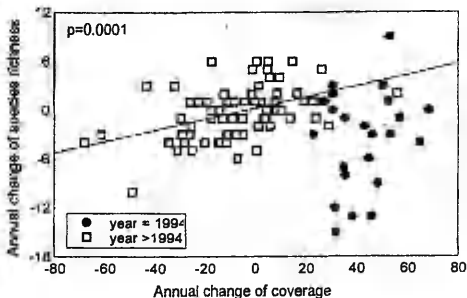


Fig. 4. Annual change of species richness in a permanent plot plotted against annual change of total plant coverage in the same plot. Changes from the exceptionally dry period (1994–1995) are marked with filled symbols. Data from the remaining 4 yr of the experiment is pooled (open symbols). Regression line is drawn for the last 4 yr.

Table 4. The effects of fertilization (FERT), additional illumination (ILLUM), mowing (MOWING) and time (1994–1998, TIME) on standardized species richness (species richness in experimental plot per unit coverage of vascular plants in that plot), analysed with repeated measures ANOVA. None of the interactions among three or more factors were significant and these are not shown. * = $p < 0.05$.

Factor	DF	F-value	p-value
FERT	1,16	39.42971	0.0000*
ILLUM	1,16	1.20913	0.2878
MOWING	1,16	0.01627	0.9001
TIME	4,64	78.00124	0.0000*
FERT × ILLUM	1,16	1.07900	0.3144
FERT × MOWING	1,16	2.50998	0.1327
ILLUM × MOWING	1,16	2.81918	0.1126
FERT × TIME	4,64	1.73915	0.1523
ILLUM × TIME	4,64	0.77913	0.5429
MOWING × TIME	4,64	2.19136	0.0799

Discussion

The results of studying vascular plant touches per point quadrat beam and inclination of leaves generally supported our working hypothesis (1). Light does not seem to be the limiting resource under normal conditions – additional illumination, when applied as the single treatment, caused no significant increase in canopy density, measured as number of vascular plant touches per point quadrat beam. One has to note here that neither did additional illumination have apparent harmful effect on productivity. The considerable rise of radiation intensity and the notable rise of temperature in the case of direct sunshine (by >60% and by 10–20%, respectively, see Table 2) has not led to a significantly sparser canopy in additionally illuminated plots. As expected, fertilization led to a significant increase in canopy density.

Also, there was evidence, supporting the second and third working hypotheses (2, 3), stating that light would become limiting after nutrient addition, and that additional illumination would mitigate this effect, respectively. There was a clear indication that plants were not able to make entire use of the extra nutrients in these plots which were not additionally illuminated – the densest canopies were found in fertilized plots with mirrors. Assuming that light being the limiting resource also means intensified competition for light, one could speculate that additional illumination most probably mitigated this effect of nutrient addition as well. Thus, given light competition has been responsible for the decrease of diversity, observed earlier in the fertilized parts of the Laelatu meadow, we have a basis for expecting a slower decline of diversity in fertilized + illuminated plots, when compared to only fertilized ones.

Surprisingly, we could not detect any change in species richness due to experimental treatments, though a sharp decrease in species richness due to fertilization and cessation of mowing had been recorded in the same

meadow earlier (Kull and Zobel 1991). Most probably we failed to affect richness in permanent plots because of the quite small areas manipulated with experimental treatments (only 1 m²). The invasion of species from surrounding areas through lateral vegetative spread and propagule input (mass effect) might have been as intense as to compensate the probable competitive extinction of certain species. Mostly, ingrowing rhizomes and stolons should be considered responsible for the mass effect here – in Laelatu meadow vegetative regeneration is prevailing and regeneration from seeds is obviously not so important (Kalamees and Zobel unpubl.).

It is quite logical to assume that if competition for light is not determining small-scale diversity pattern (light is not limiting the productivity of species) richness in a plot will depend positively on number of ramets inhabiting the plot (Oksanen 1996, Zobel and Liira 1997) and, consequently, on canopy density in the plot. Thus, assuming also that in early phases of the experiment nutrient addition could cause the formation of a denser canopy without light starting to limit productivity, we hypothesized that richness per unit coverage would be a more sensitive measure of richness than just the number of species in a plot (4). The first step in testing this hypothesis was to check whether the assumption on richness–canopy density relationship holds. To some surprise, especially given we still expected a decline of diversity with increasing plant coverage in fertilized and/or non-mown plots, species richness and total coverage of plants occurred to exhibit a significant positive correlation over all treatment variants, over all years of the experiment. Also, year-to-year changes in plot richness could be predicted from year-to-year changes in total coverage (significant positive correlation). The first year of experiment was the exception here – change of richness 1994–1995 was not correlated with change of total coverage 1994–1995. This possibly reflects the consequences of the severe drought during the first year of the experiment (1994, Table 1) – in the following year (1995) richness in a plot evidently depended mostly on the drought-tolerance of the particular species inhabiting the plot in 1994. Positive correlation between species richness and plant density per constant area has been shown before on a broad gradient of community productivity and small-scale diversity (Zobel and Liira 1997) and in artificial communities with experimentally manipulated plant density (Goldberg and Estabrook 1998). However, as far as we know, no such relationship has been detected inside a more or less ecologically homogenous (semi-) natural grassland community with nutrients and light manipulated, i.e. there is no data available in the literature demonstrating small-scale richness positively depending on canopy density in the particular microsite. It is important to note here that though fertilized plots generally carried a denser canopy, the mentioned relationship did not yield in fertilized plots

being significantly more species-rich. The overall variability of richness as well as canopy coverage were as great as to shade the possible effects of experimental treatments on the richness-coverage relationship. Our results somewhat contradict several previous experiments where fertilization has led to decreasing species density with increasing canopy density (e.g. Pasternak-Kusmierska 1992, Gough and Grace 1998, but see Huberty et al. 1998).

The overall positive relationship between plot richness and canopy coverage encouraged to use standardized richness S_{st} for detecting the effects of experimental treatments on diversity. Unfortunately, we failed to achieve evidence on competition for light being the key factor in diversity formation. Although nutrient addition led to a decrease in richness per unit coverage, there was no indication of additional illumination mitigating this effect. Neither did cessation of mowing affect S_{st} significantly.

Our fifth working hypothesis (5) was concerned with the ability of grassland plants to respond to changes in light availability by adjusting the mean inclination of leaves. We could not detect any changes in mean leaf inclination as a response to experimental treatments. However, there was strong indication that the general orientation of leaves of herbaceous species depends on the climatic conditions prevailing during the growing season. In more or less sunny "ordinary" years (in our experiment 1994, 1995, 1997, 1998, Table 1) the orientation of leaves was similar to what has been reported in earlier studies (e.g. Cooper et al. 1970) – leaves were predominantly inclined towards the sun, exposing their surfaces towards the north. In the exceptionally cool and cloudy year (1996, Table 1) the situation was strikingly different, independent of the experimental treatment (Fig. 2) – then the prevailing inclination of leaves was towards the north, i.e. leaf surfaces were mostly exposed to the south. Plants apparently showed considerable plasticity by orientating their photosynthetic surface to the direction of sun to absorb more PAR. To our knowledge this is the first clear evidence showing that mean leaf inclination in a multi-species herbaceous community can respond plastically to year-to-year changes in light availability during the growing season.

Acknowledgements – This study was supported by grant 3887 from the Estonian Science Foundation.

References

- Bengtsson, J., Fagerström, T. and Rydin, H. 1994. Competition and coexistence in plant communities. – *Trends Ecol. Evol.* 9: 246–250.
- Cooper, J. P., Rhodes, J. and Sheehy, J. E. 1970. Canopy structure, light interception and potential production in forage grasses. – *Aberystwyth Univ. Coll. Wales, Welsh Plant Breeding Stn Rep.*, pp. 57–69.
- Day, R. T. et al. 1988. Fertility and disturbance gradients: a summary model for riverine marsh vegetation. – *Ecology* 69: 1044–1054.
- Eek, L. and Zobel, K. 1997. Effects of additional illumination and fertilization on seasonal changes in fine-scale community structure. – *J. Veg. Sci.* 8: 225–234.
- Ehleringer, J. R. and Forseth, I. N. 1989. Diurnal leaf movements and productivity in canopies. – In: Russell, G., Marshall, B. and Jarvis, P. (eds), *Plant canopies: their growth, form and function*. Cambridge Univ. Press, pp. 129–142.
- Floyd, D. A. and Anderson, J. E. 1987. A comparison of three methods for estimating plant cover. – *J. Ecol.* 75: 221–228.
- Foster, B. L. and Gross, K. L. 1998. Species richness in a successional grassland: effects of nitrogen enrichment and plant litter. – *Ecology* 79: 2593–2602.
- Gibson, C. W. D. et al. 1987. Spring grazing by sheep: effects of seasonal changes during early old field succession. – *Vegetatio* 70: 33–43.
- Goldberg, D. E. and Estabrook, G. F. 1998. Separating the effects of number of individuals sampled and competition on species diversity: an experimental and analytic approach. – *J. Ecol.* 86: 983–988.
- Gough, L. and Grace, J. B. 1998. Herbivore effects on plant density at varying productivity levels. – *Ecology* 79: 1586–1594.
- Grace, J. B. and Pugsek, B. H. 1997. A structural equation model of plant species richness and its application to coastal wetland. – *Am. Nat.* 149: 436–460.
- Gurevich, J. and Unnasch, R. 1989. Experimental removal of a dominant species at two levels of soil fertility. – *Can. J. Bot.* 67: 3470–3477.
- Güsewell, S., Buttler, A. and Klotzli, F. 1998. Short-term and long-term effects of mowing on the vegetation of two calcareous fens. – *J. Veg. Sci.* 9: 861–872.
- Hirose, T. and Werger, M. J. A. 1995. Canopy structure and photon flux partitioning among species in a herbaceous plant community. – *Ecology* 76: 466–474.
- Huberty, L. E., Gross, K. L. and Miller, C. J. 1998. Effects of nitrogen addition on successional dynamics and species diversity in Michigan old fields. – *J. Ecol.* 86: 794–803.
- Jonasson, S. 1983. The point intercept method for non-destructive estimation of biomass. – *Phytocoenologica* 11: 385–388.
- Jonsson, L. 1995. Effects of restoration on wooded meadows in southeastern Sweden. – *Lund Univ., Sweden*.
- Kleijn, D. and Van der Voort, L. A. C. 1997. Conservation headlands for rare arable weeds: the effects of fertilizer application and light penetration on plant growth. – *Biol. Conserv.* 81: 57–67.
- Krall, H. and Pork, K. 1970. Laelatu puisniit. – In: Kumari, E. (ed.), *Lääne-Eesti rannikualade loodus*. Valgus, Tallinn, in Estonian.
- Kull, K. and Zobel, M. 1991. High species richness in an Estonian wooded meadow. – *J. Veg. Sci.* 2: 711–714.
- Kull, K. and Zobel, K. 1995. Kas on veel ruumi? Ei, kõik on täis. – *Eesti Loodus* 48: 33–35, in Estonian.
- Liira, J. and Zobel, K. 2000. Vertical structure of a species-rich grassland canopy, treated with additional illumination, fertilization and mowing. – *Plant Ecol.* 146: 185–195.
- Mitchley, J. and Willems, J. H. 1995. Vertical canopy structure of Dutch chalk grasslands in relation to their management. – *Vegetatio* 117: 17–27.
- Oksanen, J. 1996. Is the humped relationship between species richness and biomass an artefact due to plot size? – *J. Ecol.* 84: 293–295.
- Pasternak-Kusmierska, D. 1992. Density and species structure of sward of an intensively fertilized meadow after reduction and discontinuance of fertilization. – *Ekol. Polsk.* 40: 47–61.
- Piper, J. K. 1995. Composition of prairie plant communities on productive versus unproductive sites in wet and dry years. – *Can. J. Bot.* 73: 1635–1644.

- Proulx, M. and Mazumder, A. 1998. Reversal of grazing impact on plant species richness in nutrient-poor vs nutrient-rich ecosystems. - *Ecology* 79: 2581-2592.
- Ryser, P., Langenauer, R. and Gigon, A. 1995. Species richness and vegetation structure in a limestone grassland after 15 years management with six biomass removal regimes. - *Fol. Geobot. Phytotax.* 30: 157-167.
- Tappeiner, U. and Cernusca, A. 1989. Canopy structure and light climate of different alpine plant communities; analysis by means of a model. - *Theor. Appl. Climatol.* 40: 81-92.
- Tilman, D. 1990. Constraints and tradeoffs: toward a predictive theory of competition and succession. - *Oikos* 58: 3-15.
- Tilman, D. 1993. Species richness of experimental productivity gradients: how important is colonization limitation? - *Ecology* 74: 2179-2191.
- Tilman, D. and Pacala, S. 1993. The maintenance of species richness in plant communities. - In: Ricklefs, R. E. and Schluter, D. (eds), *Species diversity in ecological communities*. Univ. of Chicago Press, pp. 13-25.
- Urbas, P. and Zobel, K. 2000. Adaptive and inevitable morphological plasticity of three herbaceous species in a multi-species community: field experiment with manipulated nutrients and light. - *Acta Oecol.* 21: 139-147.
- Willems, J. H. 1983. Species composition and above ground phytomass in chalk grassland with different management. - *Vegetatio* 52: 171-180.
- Willems, J. H., Peet, R. K. and Bik, L. 1993. Changes in chalk-grassland structure and species richness resulting from selective nutrient additions. - *J. Veg. Sci.* 4: 203-212.
- Wilson, J. B. 1988. The effect of initial advantage on the course of plant competition. - *Oikos* 51: 19-24.
- Wilson, S. D. and Tilman, D. 1993. Plant competition and resource availability in response to disturbance and fertilization. - *Ecology* 74: 599-611.
- Zobel, K. and Liira, J. 1997. A scale-independent approach to the richness vs biomass relationship in ground-layer plant communities. - *Oikos* 80: 325-332.

Zobel, K. and Eek, L. 2002.
Seasonal direct light availability affects mean leaf orientation
in a herbaceous multi-species Canopy.
(manuscript, submitted to Plant Biology)

SEASONAL DIRECT LIGHT AVAILABILITY AFFECTS MEAN LEAF ORIENTATION IN A HERBACEOUS MULTI-SPECIES CANOPY

Kristjan Zobel¹ and Liina Eek¹

¹Department of Botany and Ecology, Tartu University, 40 Lai St.,
EE-51005 Tartu, Estonia; E-mail: kzobel@ut.ee.

Abstract

Relationship between leaf orientation in natural or semi-natural herbaceous communities and the local abundance of direct light has rarely been investigated. We present evidence from a permanent plot experiment that seasonal direct light availability in 40×40 cm plots significantly affects mean leaf orientation in a semi-natural wooded meadow. In sunny years and in well-illuminated conditions the leaf surface is exposed predominantly to the North, and *vice versa*. Two groups of species are responsible for such a response: of the variation in leaf orientation 41% is explainable through direct light availability for light-demanding species, and 26% for graminoid species. In shade-tolerant species and in forbs there is no sign of a regression between leaf orientation and direct light abundance. Ability to plastically react to variation in seasonal and local direct light availability appears to be a trait under considerable selective pressure only in light-demanding plants, and more characteristic to species with graminoid growth form.

Introduction

Mean foliage inclination and mean leaf azimuth orientation in herbaceous multi-species stands has never been shown to demonstrate a clear response pattern to abiotic environmental variables. Moreover, it is not clear whether such foliage characteristics have any ecologically interpretative value at all. The existing knowledge on plant leaf inclination and orientation mainly comes from experiments with monocultures or mixtures of arable crops, or studies of tree foliage. Regarding the present state of the arts, it is difficult, if not impossible, to extrapolate this knowledge on natural or semi-natural herbaceous plant communities.

There is a lot of empirical evidence, supported by rather convincing theoretical considerations, that spherical (or ellipsoidal) distribution of leaf angle (Baldocchi *et al.* 1985, Campbell 1986, Goudriaan 1988, Wang and Jarvis 1988, Utsugi 1999) and random distribution of orientation for compass direction (McMillen and McClendon 1979) are reasonable guesses for the majority of plant assemblages. However, it has been claimed that these assumptions may be unlikely to occur in practice (Anderson 1966). Several studies show that foliage can be non-randomly distributed with respect to azimuth angle (Scott and Wells 1969, Caldwell *et al.* 1986) as well as inclination angle (Caldwell *et al.* 1986, Herbert and Nilson 1991, Gibson *et al.* 1992). At least it has been clearly shown that certain species can plastically respond to the orientation of diffuse

and/or direct radiation, in order to maximize water-use efficiency and avoid photoinhibition (Berg and Heuchelin 1990, Ryel and Beyschlag 1995, Hikosaka and Hirose 1997).

Besides light availability several other environmental variables have been documented to influence leaf inclination and orientation — water and nutrient availability, temperature regime, etc. (Tappeiner and Cernusca 1989, Ehleringer and Forseth 1989). Several stress factors (e.g. water stress) have been shown to result in steeper midday leaf angles (paraheliotropism; Kao and Tsai 1998, Herbert 1991).

While studying mean azimuth angle of plant aerial parts in a long-term experiment in a species-rich herbaceous community we found an indication that it could be related to the amount of direct radiation received in the particular growing season (Eek and Zobel 2000). There was an indication that in sunny years most of the leaves tended to be exposed towards the North and *vice versa*. There are studies which describe similar phenomenon in herbaceous monocultures (e.g. Scott and Wells 1969) or single-species experiments (Myers *et al.* 1997) but these are restricted to one vegetation period and do not reveal year-to-year variation pattern in leaf orientation.

In this study we investigated to which extent mean leaf azimuth angle in a grassland canopy could be predicted from seasonal direct light availability. Given that the data were collected from a long-term permanent plot experiment where light availability had been manipulated with the help South-facing mirrors (providing a 'second sun' during *ca.* six hours around midday; Eek and Zobel 1997, 2000, Liira and Zobel 2000, Urbas and Zobel 2000), we were also interested in the possible effect of such experimental treatment to leaf azimuth angle. More specifically, we wanted to test the following working hypothesis:

H₁ — Mean leaf orientation in a grassland canopy is regressed to the amount of direct radiation received in the growing season. The abundance of direct sunlight causes exposition of leaf surface predominantly towards the North, the deficit of direct sunlight towards the South.

Additionally, we interested to learn whether plants with different basic growth forms (graminoids vs. forbs) and plants with different light requirements (light-demanding vs. shade-tolerant) differ in their plasticity of response to seasonal direct light availability.

Material and methods

The experiment was carried out in a mesic semi-natural species-rich calcicolous wooded meadow in Laelatu, western Estonia, on the eastern coast of the Baltic Sea (58°35' N, 23°34' E). The grassland has been annually cut for hay in early July for more than 200 years. The majority of species in the herbaceous layer (98%) are perennials, 71% of the species are forbs (incl. tree seedlings) and 29% are graminoids. Among species with known synecological light requirement (106 species out of 120), most of the graminoids (86%), as well as forbs (69%) are light-demanding (Ellenberg's indicator value for light exceeding 6). Of the light-demanding species 25% are graminoids and 75% were forbs (Table 1).

Table 1. The counts of light-demanding and shade tolerant graminoid and forb species, recorded in the permanent plots. A species was considered light-demanding when its Ellenberg's indicator value for light (Ellenberg et al. 1991) exceeded 6 (9 showing maximal light demand). The tendency of graminoids to be more light-demanding than forbs is not significant ($P = 0.1051$)

	Graminoids	Forbs
Light-demanding	19	58
Shade-tolerant	3	26

The vertical structure of the herbaceous canopy in the Laelatu meadow has been described in detail by Liira and Zobel 2000. Generally, grasses dominate in the uppermost canopy layers, grand mean of the height of plant organs being *ca* 18 cm. Mean height of sedges and forbes is significantly smaller (grand mean of the height of plant organs *ca* 13 cm). There are no obvious layers in the canopy, biomass decreases monotonically with increasing height (Liira *et al.* 2002). Mean leaf area index (LAI) of the canopy is *ca* 2.2 (min 1.4, max 2.9; L. Laanisto, unpublished data).

In 1994 24 permanent plots of 40×40 cm were marked. In half of the plots light availability was manipulated by placing south-facing vertical glass mirrors (width 212 cm, height 124 cm, lower edge of the mirror being 20 cm from the ground). The mirrors reflected additional sunlight onto the permanent plots for *ca* 6 hours a day.

The plots were examined annually in the end of June/beginning of July during 5 years (1994–1998). A laser beam with the diameter of 1 mm was used to mark 100 randomly placed point quadrats in each plot at two angles — 60° and 120° from horizontal, South-North direction. The angle 60° meant inclination of the point quadrat towards the South. In each investigated point the number of contacts with green plant material were recorded.

During three years (1994–1996) we additionally studied eight permanent plots (four plots with mirrors and four without) for plant species composition in the point quadrats.

Data on the number of May, June and July sunshine hours, mean monthly air temperature and monthly precipitation (Table 2) for the period 1994–1998 were acquired from the Virtsu Observatory, situated 3 km from experimental site.

Table 2. The number of sunshine hours, mean monthly air temperature and monthly precipitation during the growing season (May, June, July) in the five years of experiment (measured by Estonian Institute of Meteorology and Hydrology in Virtsu observatory, 3 km from experimental site).

Observation year	Number of sunshine hours	Mean monthly air temperature (°C)	Total precipitation (mm)
1994	1081	13.7	94
1995	939	14.4	205
1996	745	12.6	175
1997	943	14.5	220
1998	826	13.7	177

Availability of direct light in sample plots was estimated from hemispherical photographs made in 1999. The direct site factor $Tdir$ (proportion of direct radiation under tree canopy relative to that above tree canopy; Machado and Reich 1999) was estimated as integral for the period of one month (30 days before maximum sun angle till maximum sun angle), using cosine correction.

Species were roughly classified according to Ellenberg's ecological indicator values for light (Ellenberg *et al.* 1991) and according to their growth form. A species was considered light-demanding if its indicator value for light exceeded 6 (not included). According to growth form species were classified into two groups — graminoids (*Cyperaceae* and *Poaceae*) and forbs (the remaining vascular plant species).

We used point quadrat data to estimate average plant aerial part azimuth orientation in a single index. The leaf orientation index (LOI) was calculated as:

$$LOI_{ij} = \ln(N(60)_{ij} / N(120)_{ij}) \quad (1)$$

Here: $N(\alpha)_{ij}$ — total number of contacts with vascular plants registered in the 50 point quadrats positioned at angle α , in i -th plot, j -th year. The values $LOI_{ij} > 0$ denote the exposition of leaf surface predominantly towards the South.

For each sampling season and each permanent plot direct radiation index was calculated as follows:

$$DRI_{ij} = S_j * Tdir_i * M \quad (2)$$

Here: DRI_{ij} — direct radiation index for i -th plot in j -th year; S_j — number of sunshine hours in May and June in j -th year; $Tdir_i$ — direct site factor in i -th plot; M — mirror efficiency coefficient, i.e. the estimate of the overall multiplicative effect of additional illumination (in plots without mirrors $M = 1$). The estimate of M was achieved statistically. For this the coefficient M (mirror efficiency coefficient) in Eq. (2) was given values: $M = 1, 1.05, 1.1, 1.15$, etc. and each time the strength of the regressive relationship $LOI = f(DRI)$ was estimated for the two data subsets which showed strong regression between LOI and DRI — light demanding and graminoid plants. Strength of regression was measured using adjusted determination coefficient (R^2). The value of M , providing maximum strength of the relationship, was considered the best estimate. Note that the direction of the additional direct radiation (North-South) should be disregarded in estimating M because the main signal for adjusting leaf azimuth angle is still South-North orientated.

The dependence between plant growth form (graminoids vs. forbs) and light requirement was studied using Pearson chi-square test for 2×2 contingency table.

Results

Over the five years of the experiment the grand mean number of contacts recorded per one point quadrat pin in a sample plot was 5.29 (min. = 2.18, max. = 11.8, SD = 1.68). Altogether, 87 vascular plant species were recorded in those eight permanent plots which were studied for species composition in the point quadrats during the first three years of the experiment. Altogether, 120 species were recorded in the permanent plots.

The analysis of dependence between plant growth form and light requirement (Table 1) using chi-square test showed that the null hypothesis (light requirement is independent of growth form) could not be refuted ($P = 0.1051$). Though there is a tendency towards graminoids being more light-demanding, this cannot be proved statistically.

The results of estimating the value of the mirror efficiency coefficient M in equation (2), using iteration, are shown in Fig. 1. The strength of the regression of leaf orientation index (LOI) on direct radiation index (DRI) was in both cases maximal at $M \approx 0.15$. Thus, the overall effect of additional illumination on plant performance, as observed in the response of plant leaf orientation, is comparable to one that would be expected after a 15% increase of the natural direct PAR level.

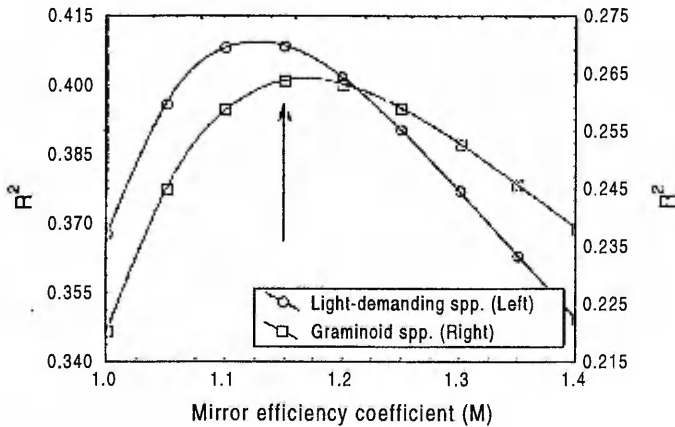


Figure 1. The results of estimating the value of mirror efficiency coefficient M in Eq. (2) by iteration for two data subsets — light-demanding species (A) and graminoid species (B). The strength of regression between leaf orientation index (LOI , Eq. (1)) and direct radiation index (DRI , Eq. (2)) is measured using adjusted determination coefficient (R^2) for different M -values. In both cases the value $M \approx 0.15$ yields a maximum of R^2 and is considered the best estimate for the mirror efficiency coefficient.

The overall relationship between plant leaf orientation (LOI) and seasonal abundance of direct PAR (DRI) was significant at $P = 0.0021$ (Fig. 2). There is an indication that in years with abundant direct sunlight most of the leaf surface tends to be exposed towards the North and in cloudy seasons towards the South.

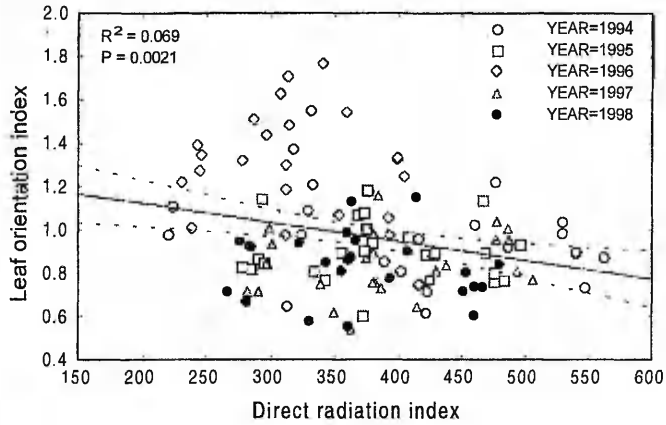


Figure 2. The regression of leaf orientation index (*LOI*) on direct radiation index (*DRI*). Data from 24 permanent plots over the five years of observation are pooled. *DRI* is calculated using the estimate of $M = 0.15$ (Eq. (2), see Fig. 1).

The more detailed study of the relationship between *LOI* and *DRI* in eight permanent plots revealed that functionally different plant species responded differently to the availability of direct PAR. Species with Ellenberg's indicator value for light being equal to seven or higher responded significantly ($P = 0.0005$) to changes in seasonal direct radiation level by adjusting the mean azimuth angle of their aerial parts (Fig. 3). As much as 41% of the variation in *LOI* over three years could be explained through *DRI* in light-demanding species. In species with Ellenberg's indicator value for light below seven there was no significant relationship between *LOI* and *DRI*.

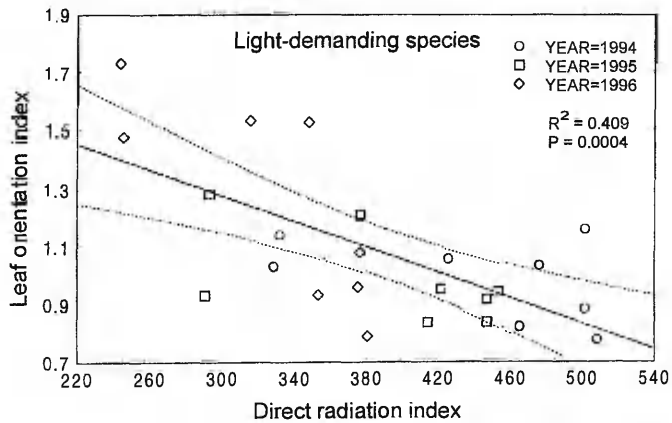


Figure 3. The regression of leaf orientation index (*LOI*) on direct radiation index (*DRI*) for light-demanding species (Ellenberg's indicator value for light exceeding 6). Data from 8 permanent plots over the three years of observation are pooled. *DRI* is calculated using the estimate of $M = 0.15$ (Eq. (2), see Fig. 1).

When species with different growth form (graminoids vs forbs) were studied separately, a significant relationship between *LOI* and *DRI* could be detected only in graminoids ($P = 0.0060$, Fig. 4). More than 25% of the variation in mean leaf azimuth angle of grasses and sedges was attributable to variation in direct PAR level. In forbs no significant relationship was found between *LOI* and *DRI*.

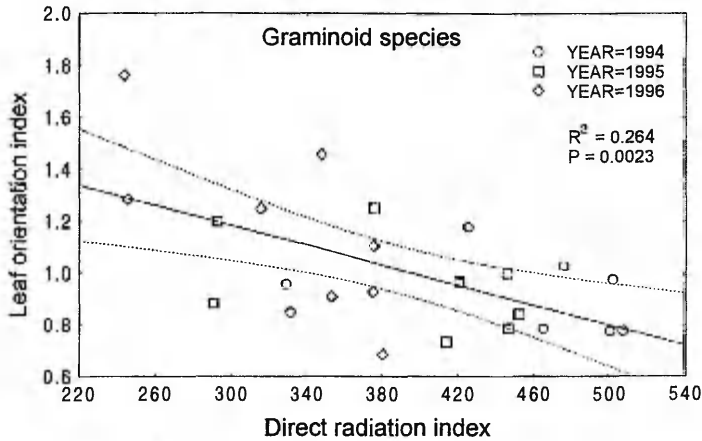


Figure 4. The regression of leaf orientation index (*LOI*) on direct radiation index (*DRI*) for graminoid species (*Poaceae* and *Cyperaceae*). Data from 8 permanent plots over the three years of observation are pooled. *DRI* is calculated using the estimate of $M = 0.15$ (Eq. (2), see Fig. 1).

There was no significant difference between the mean orientation of graminoid and forb leaves, nor between the mean orientation of the leaves of light-demanding and shade tolerant plants.

Discussion

There are virtually no data in the literature on plant leaf angle and orientation that would make it possible to predict with any certainty the mean azimuth angle of leaves in a multi-species herbaceous community. Neither can one find evidence showing that herbaceous plants in natural stands can plastically respond to variation in seasonal availability of PAR by adjusting their architecture to an excess or deficit of the light resource. The results of this study demonstrate that the relationship between mean leaf orientation and the amount of direct radiation received during the growing season can be quantified (Fig. 2). One can also see in the graph that in the cloudy and cool year of 1996 (Table 2) mean *LOI* is considerably higher (leaves more South-exposed) than in the remaining, sunny and warm years. Though the predictive power of the regression model remains very low there is evidence supporting our main hypothesis.

Leaf inclination and orientation have frequently been shown to depend also on other environmental factors than light, especially water availability (Ehleringer and Forseth 1989, Tappeiner and Cernusca 1989, Herbert 1991, Kao and Tsai 1998). The graph in Fig. 2 shows that changes in LOI can hardly be explained through possible water stress in the Laelatu meadow. In the exceptionally dry year of 1994 (Table 2) mean LOI is similar to that in the sunny but considerably rainier years of 1995, 1997 and 1998.

The more detailed study of leaf North-South orientation, with species identified for each contact in the point quadrat analysis, revealed the reason why the overall regression of LOI on DRI was relatively weak ($R^2 < 0.07$; Fig. 2). It appeared that only certain functional groups of species are responsible for the plasticity of mean leaf orientation in the canopy. In light-demanding species with Ellenberg's indicator value for light seven or higher (73% of recorded species) the seasonal amount of direct PAR was a good predictor of mean leaf orientation, describing 42% of total variation in LOI (Fig. 3). The azimuth angle of shade species was not related to the seasonally received amount of direct radiation. Evidently, the ability to escape too intense direct radiation, and thus avoid photoinhibition, is an important trait of those species which are adapted to grow in full light. In shade species the danger of experiencing excessive direct radiation for a long time is too small in the long run to adopt an ability of adjusting the canopy to seasonal light climate.

Another functional group of species that demonstrated clear response in leaf orientation were species with graminoid growth form (grasses and sedges in the Laelatu meadow, 29% of the recorded species; Fig. 4). The mean azimuth angle of the remaining species (forbs) appeared to be insensitive to the seasonal PAR availability. One must admit here that the results depicted in Fig. 3 and 4 are probably not entirely independent. Table 1 shows that only three graminoid species are classified as shade-tolerant ones. Thus, the significant relationship between LOI and DRI in graminoids could principally result from the fact that graminoids are largely a subset of light-demanding species. Though the relatively higher light-requirement of graminoid species could not be statistically proved ($P = 0.1051$), the question remains.

Assuming that the relationship in Fig. 4 still reflects reality, and graminoids, as a whole, can more plastically re-arrange their morphology according to seasonal light climate than forbs, this phenomenon needs an (somewhat speculative) interpretation. Most likely, it should be explained through the basic differences in how leaves are produced and maintained in forbs vs graminoids. In the latter the vegetative leaves are often obligatory asymmetrical with respect to the vertical axis of the ramet while forbs can be obligatory symmetrical. Thus, provided that most of the graminoid ramets are vegetative (and this is the case in the Laelatu meadow), certain azimuth angle of inclination may easily prevail if there is a signal in the environment that alters the azimuth direction of leaf emergence in the early development phase of the leaves (see Myers *et al.* 1997 for comparison).

In conclusion, our results clearly indicate that mean leaf orientation in a multi-species herbaceous canopy is a function of the amount of incoming direct radiation during the growing season, and can be predicted with some certainty from the latter, especially for light-demanding species and for graminoid species.

Acknowledgements

This work was supported by ESF grant 3887. We thank Olevi Kull and two anonymous referees for valuable comments on the manuscript and Ilmar Part for revising the English.

References

- Anderson, M.C. 1966. Stand structure and light penetration. II. A theoretical analysis. *Journal of Applied Ecology* 3, 41–54.
- Baldocchi, D.D., Hutchinson, B.A., Matt, D.R. and McMillen, R.T. 1985. Canopy radiative transfer models for spherical and known leaf inclination angle distributions: A test in an oak-hickory. *Journal of Applied Ecology* 22, 539–555.
- Berg, V.S. and Heuchelin, S. 1990. Leaf orientation of soybean seedlings. 1. Effect of water potential and photosynthetic photon flux density on paraheliotropism. *Crop Science* 30, 631–638.
- Caldwell, M.M., Meister, H.-P., Tenhunen, J.D. and Lange, O.L. 1986. Canopy structure, light microclimate and leaf gas exchange of *Quercus coccifera* L. in a Portuguese macchia: measurements in different canopy layers and simulations with a canopy model. *Trees* 1, 25–41.
- Campbell, G.S. 1986. Extinction coefficients for radiation in plant canopies calculated using an ellipsoidal inclination angle distribution. *Agricultural and Forest Meteorology* 36, 317–321.
- Eek, L. and Zobel, K. 1997. Effects of additional illumination and fertilization on seasonal changes in fine-scale community structure. *Journal of Vegetation Science* 8, 225–234.
- Eek, L. & Zobel, K. 2000. Structure and diversity of a species-rich grassland community, treated with additional illumination, fertilization and mowing. *Ecography* 24, 157–164.
- Ellenberg, H., Weber, H.E., Düll, R., Wirth, V., Werner, W., Paulissen, D. 1991. *Ziigerwerte von Pflanzen in Mitteleuropa*. *Scripta Geobotanica* 18, 1–248.
- Gibson, D., Casal, J.J. and Deregibus, V.A. 1992. The effects of plant density on shoot and leaf lamina angles in *Lolium multiflorum* and *Paspalum dilatatum*. *Annals of Botany* 70, 69–73.
- Goudriaan, J. 1988. The bare bones of leaf-angle distribution in radiation models for canopy photosynthesis and energy exchange. *Agricultural and Forest Meteorology* 43, 155–169.
- Herbert, T. J. 1991. Variation in interception of the direct solar beam by top canopy layers. *Ecology* 72, 17–22.
- Herbert, T.J. and Nilson, T. 1991. A model of variance of photosynthesis between leaves and maximization of whole plant photosynthesis. *Photosynthetica* 25, 597–606.
- Hikosaka, K. and Hirose, T. 1997. Leaf angle as a strategy for light competition: optimal and evolutionary stable light extinction coefficient within a leaf canopy. *Écoscience* 4, 501–507.
- Kao, W.Y. and Tsai, T.T. 1998. Tropic leaf movements, photosynthetic gas exchange, leaf $\delta^{13}\text{C}$ and chlorophyll a fluorescence of three soybean species in response to water availability. *Plant, Cell and Environment* 21, 1055–1062.
- Liira, J. and Zobel, K. 2000. Vertical structure of a species-rich grassland canopy, treated with additional illumination, fertilization and mowing. *Plant Ecology* 146, 185–195.
- Liira, J. Zobel, K., Mägi, R. and Molenberghs, G. 2002. Vertical structure of herbaceous canopies: the importance of plant growth-form and species-specific traits. *Plant Ecology* (in press).
- Machado, J.-L. and Reich, P.B. 1999. Evaluation of several measures of canopy openness as predictors of photosynthetic photon flux density in deeply shaded conifer-dominated forest understory. *Canadian Journal of Forest Research* 29, 1438–1444.
- McMillen, G.G. and McClendon, J.H. 1979. Leaf angle: an adaptive feature of sun and shade leaves. *Botanical Gazette* 140, 437–442.

- Myers, D.A., Jordan, D.N. and Vogelmann, T.C. 1997 Inclination of sun and shade leaves influences chloroplast light harvesting and utilization. *Physiologia Plantarum* 99, 395–404.
- Ryel, R.J. and Beyschlag, W. 1995. Benefits associated with steep foliage orientation in two tussock grasses of the American Intermountain West. A look at water-use-efficiency and photo-inhibition. *Flora* 190, 251–260.
- Scott, D. and Wells, J.S. 1969. Leaf orientation in barley, lupin, and lucerne stands. *New Zealand Journal of Botany* 7, 372–388.
- Urbas, P. and Zobel, K. 2000. Adaptive and inevitable morphological plasticity of three herbaceous species in a multi-species community: Field experiment with manipulated nutrients and light. *Acta Oecologica* 21, 139–147.
- Utsugi, H. 1999. Angle distribution of foliage in individual *Chamaecyparis obtusa* canopies and effect of angle on diffuse light penetration. *Trees — Structure and Function*, 14, 1–9.
- Wang, Y.P. and Jarvis, P.G. 1988. Mean leaf angles for the ellipsoidal inclination angle distribution. *Agricultural and Forest Meteorology* 43, 319–321.

CURRICULUM VITAE

Liina Eek-Piirsoo

Nationality: Estonian

Time and place of birth: 9 February 1972 in Tallinn, Estonia

Marital status: Married, husband Marko Piirsoo

Current employment / organization: Estonian Ministry of the Environment,
Department of Nature Protection

Address home: Suvila 8-2, Tallinn 10913

Telephone number: + 37 26 576 652

e-mail: leek@ut.ee

Education

Magister scientiarum (MSc) in biology (plant ecology)

28 August 1997 at Tartu University (supervisor K. Zobel, PhD) master thesis
"The effect of fertilization, additional illumination and mowing on the fine-
scale structure of the grassland canopy"

Language skills: Estonian — mother tongue, English — good, Russian and
German — satisfactory, Spanish — poor

Former employment

1995–1997 Institute of Botany and Ecology of Tartu University

1996 May – November Zürich ETH Geobotanical Institute

1997 August – September Switzerland Zürich ETH Geobotanical Institute
(Eidgenössische Technische Hochschule Geobotanisches Institut) visiting
student

Since November 1997 Ministry of the Environment

Membership of societies and organisations

Chairperson of the Advisory Committee for Genetic Modifications — 2000

Member of Novel Food Committee — 2000

Member of Commission of Academy of Science of International Biological
Diversity Observation Year — 2001

Member of Committee on Agricultural Genetic Resources — 2001

Publications

- Eek, L. and Zobel, K. 1997 *Effects of additional illumination and fertilization on seasonal changes in fine-scale grassland community structure*. *Journal of Vegetation Science* 8: 225–234.
- Ryser, P. and Eek, L. 2000. *Consequences of phenotypic plasticity in leaf and root traits for acquisition of multiple limiting resources*. *American Journal of Botany* 87 (3): 402–411.
- Eek, L. and Zobel, K. 2001. *Structure and diversity of a species-rich grassland community, treated with additional illumination, fertilization and mowing*. *Ecography* 24: 157–164.
- Zobel, K. and Eek, L. *Seasonal direct light availability affects mean leaf orientation in a herbaceous multi-species canopy* (manuscript, submitted to *Plant Biology*)

CURRICULUM VITAE

Liina Eek-Piirsoo

Sünniaeg: 9. veebruar 1972 Tallinn
Kodakondsus: Eesti
Perekonnaseis: abielus, abikaasa Marko Piirsoo
Elukoht: Suvila 8–2, Tallinn 10913, tel. 6 57 66 52
e-post: leek@ut.ee
Praegune töökoht: Keskkonnaministeeriumi looduskaitse osakond

Haridus

1979–1987 Tallinna 34. 8-klassiline Kool
1987–1991 Tallinna 3. Keskkool
1991–2002 Tartu Ülikool
Teaduskraad: *Magister scientiarum* taimeökoloogias
28. augustil 1997 Tartu Ülikoolis dr. K. Zobeli juhendamisel magistritöö “The effect of fertilization, additional illumination and mowing on the fine-scale structure of the grassland canopy”
Keelteoskus: eesti keel (emakeel), inglise keel (hea), vene ja saksa keel (rahuldav), hispaania keel (halb).

Teenistuskäik

1995–1997 tähtjaliste lepingite alusel TÜ botaanika ja ökoloogia instituudis;
1996 mai – november Šveitsis Zürichi Kõrgema Tehnikaülikooli geobotaanika instituudi (Eidgenössische Technische Hochschule Geobotanisches Institut) külalisüliõpilane;
1997 august – september samas;
1999 august samas;
novembrist 1997 Keskkonnaministeeriumis.

Teadusorganisatsiooniline ja -administratiivne tegevus

2000 geenitehnoloogiakomisjoni esimees
2000 uuendtoidukomisjoni liige
2001 Eesti Teaduste Akadeemia rahvusvahelise bioloogilise mitmekesisuse aasta Eesti komisjoni liige
2001 Põllumajanduskultuuride Geneetiliste Ressursside Nõukogu liige

Publikatsioonid

Eek, L. and Zobel, K. 1997 *Effects of additional illumination and fertilization on seasonal changes in fine-scale grassland community structure*. Journal of Vegetation Science 8: 225–234.

Ryser, P. and Eek, L. 2000. *Consequences of phenotypic plasticity in leaf and root traits for acquisition of multiple limiting resources*. American Journal of Botany 87 (3): 402–411.

Eek, L. and Zobel, K. 2001. *Structure and diversity of a species-rich grassland community, treated with additional illumination, fertilization and mowing*. Ecography 24: 157–164.

Zobel, K. and Eek, L. *Seasonal direct light availability affects mean leaf orientation in a herbaceous multi-species canopy* (käsikiri saadetud Plant Biology'sse)

DISSERTATIONES BIOLOGICAE UNIVERSITATIS TARTUENSIS

1. **Toivo Maimets**. Studies of human oncoprotein p53. Tartu, 1991, 96 p.
2. **Enn K. Seppet**. Thyroid state control over energy metabolism, ion transport and contractile functions in rat heart. Tartu, 1991, 135 p.
3. **Kristjan Zobel**. Epifüütsete makrosamblike väärtus õhu saastuse indikaatoritena Hamar-Dobani boreaalsetes mägimetsades. Tartu, 1992, 131 lk.
4. **Andres Mäe**. Conjugal mobilization of catabolic plasmids by transposable elements in helper plasmids. Tartu, 1992, 91 p.
5. **Maia Kivisaar**. Studies on phenol degradation genes of *Pseudomonas* sp. strain EST 1001. Tartu, 1992, 61 p.
6. **Allan Nurk**. Nucleotide sequences of phenol degradative genes from *Pseudomonas* sp. strain EST 1001 and their transcriptional activation in *Pseudomonas putida*. Tartu, 1992, 72 p.
7. **Ülo Tamm**. The genus *Populus* L. in Estonia: variation of the species biology and introduction. Tartu, 1993, 91 p.
8. **Jaanus Remme**. Studies on the peptidyltransferase centre of the *E.coli* ribosome. Tartu, 1993, 68 p.
9. **Ülo Langel**. Galanin and galanin antagonists. Tartu, 1993, 97 p.
10. **Arvo Käär**. The development of an automatic online dynamic fluorescence-based pH-dependent fiber optic penicillin flowthrough biosensor for the control of the benzylpenicillin hydrolysis. Tartu, 1993, 117 p.
11. **Lilian Järvekülg**. Antigenic analysis and development of sensitive immunoassay for potato viruses. Tartu, 1993, 147 p.
12. **Jaak Palumets**. Analysis of phytomass partition in Norway spruce. Tartu, 1993, 47 p.
13. **Arne Sellin**. Variation in hydraulic architecture of *Picea abies* (L.) Karst. trees grown under different environmental conditions. Tartu, 1994, 119 p.
13. **Mati Reeben**. Regulation of light neurofilament gene expression. Tartu, 1994, 108 p.
14. **Urmas Tartes**. Respiration rhythms in insects. Tartu, 1995, 109 p.
15. **Ülo Puurand**. The complete nucleotide sequence and infections *in vitro* transcripts from cloned cDNA of a potato A potyvirus. Tartu, 1995, 96 p.
16. **Peeter Hõrak**. Pathways of selection in avian reproduction: a functional framework and its application in the population study of the great tit (*Parus major*). Tartu, 1995, 118 p.
17. **Erkki Truve**. Studies on specific and broad spectrum virus resistance in transgenic plants. Tartu, 1996, 158 p.
18. **Illar Pata**. Cloning and characterization of human and mouse ribosomal protein S6-encoding genes. Tartu, 1996, 60 p.

19. **Ülo Niinemets.** Importance of structural features of leaves and canopy in determining species shade-tolerance in temperature deciduous woody taxa. Tartu, 1996, 150 p.
20. **Ants Kurg.** Bovine leukemia virus: molecular studies on the packaging region and DNA diagnostics in cattle. Tartu, 1996, 104 p.
21. **Ene Ustav.** E2 as the modulator of the BPV1 DNA replication. Tartu, 1996, 100 p.
22. **Aksel Soosaar.** Role of helix-loop-helix and nuclear hormone receptor transcription factors in neurogenesis. Tartu, 1996, 109 p.
23. **Maido Remm.** Human papillomavirus type 18: replication, transformation and gene expression. Tartu, 1997, 117 p.
24. **Tiiu Kull.** Population dynamics in *Cypridium calceolus* L. Tartu, 1997, 124 p.
25. **Kalle Olli.** Evolutionary life-strategies of autotrophic planktonic microorganisms in the Baltic Sea. Tartu, 1997, 180 p.
26. **Meelis Pärtel.** Species diversity and community dynamics in calcareous grassland communities in Western Estonia. Tartu, 1997, 124 p.
27. **Malle Leht.** The Genus *Potentilla* L. in Estonia, Latvia and Lithuania: distribution, morphology and taxonomy. Tartu, 1997, 186 p.
28. **Tanel Tenson.** Ribosomes, peptides and antibiotic resistance. Tartu, 1997, 80 p.
29. **Arvo Tuvikene.** Assessment of inland water pollution using biomarker responses in fish *in vivo* and *in vitro*. Tartu, 1997, 160 p.
30. **Urmas Saarma.** Tuning ribosomal elongation cycle by mutagenesis of 23S rRNA. Tartu, 1997, 134 p.
31. **Henn Ojaveer.** Composition and dynamics of fish stocks in the gulf of Riga ecosystem. Tartu, 1997, 138 p.
32. **Lembi Lõugas.** Post-glacial development of vertebrate fauna in Estonian water bodies. Tartu, 1997, 138 p.
33. **Margus Pooga.** Cell penetrating peptide, transportan, and its predecessors, galanin-based chimeric peptides. Tartu, 1998, 110 p.
34. **Andres Saag.** Evolutionary relationships in some cetrarioid genera (Lichenized Ascomycota). Tartu, 1998, 196 p.
35. **Aivar Liiv.** Ribosomal large subunit assembly *in vivo*. Tartu, 1998, 158 p.
36. **Tatjana Oja.** Isoenzyme diversity and phylogenetic affinities among the eurasian annual bromes (*Bromus* L., Poaceae). Tartu, 1998, 92 p.
37. **Mari Moora.** The influence of arbuscular mycorrhizal (AM) symbiosis on the competition and coexistence of calcareous crassland plant species. Tartu, 1998, 78 p.
38. **Olavi Kurina.** Fungus gnats in Estonia (*Diptera: Bolitophilidae, Keroplatidae, Macroceridae, Ditomyiidae, Diadocidiidae, Mycetophilidae*). Tartu, 1998, 200 p.
39. **Andrus Tasa.** Biological leaching of shales: black shale and oil shale. Tartu, 1998, 98 p.

40. **Arnold Kristjuhan.** Studies on transcriptional activator properties of tumor suppressor protein p53. Tartu, 1998, 86 p.
41. **Sulev Ingerpuu.** Characterization of some human myeloid cell surface and nuclear differentiation antigens. Tartu, 1998, 163 p.
42. **Veljo Kisand.** Responses of planktonic bacteria to the abiotic and biotic factors in the shallow lake Võrtsjärv. Tartu, 1998, 118 p.
43. **Kadri Põldmaa.** Studies in the systematics of hypomyces and allied genera (Hypocreales, Ascomycota). Tartu, 1998, 178 p.
44. **Markus Vetemaa.** Reproduction parameters of fish as indicators in environmental monitoring. Tartu, 1998, 117 p.
45. **Heli Talvik.** Prepatent periods and species composition of different *Oesophagostomum* spp. populations in Estonia and Denmark. Tartu, 1998, 104 p.
46. **Katrin Heinsoo.** Cuticular and stomatal antechamber conductance to water vapour diffusion in *Picea abies* (L.) karst. Tartu, 1999, 133 p.
47. **Tarmo Annilo.** Studies on mammalian ribosomal protein S7. Tartu, 1998, 77 p.
48. **Indrek Ots.** Health state indices of reproducing great tits (*Parus major*): sources of variation and connections with life-history traits. Tartu, 1999, 117 p.
49. **Juan Jose Cantero.** Plant community diversity and habitat relationships in central Argentina grasslands. Tartu, 1999, 161 p.
50. **Rein Kalamees.** Seed bank, seed rain and community regeneration in Estonian calcareous grasslands. Tartu, 1999, 107 p.
51. **Sulev Kõks.** Cholecystokinin (CCK) — induced anxiety in rats: influence of environmental stimuli and involvement of endopioid mechanisms and erotonin. Tartu, 1999, 123 p.
52. **Ebe Sild.** Impact of increasing concentrations of O₃ and CO₂ on wheat, clover and pasture. Tartu, 1999, 123 p.
53. **Ljudmilla Timofejeva.** Electron microscopical analysis of the synaptosomal complex formation in cereals. Tartu, 1999, 99 p.
54. **Andres Valkna.** Interactions of galanin receptor with ligands and G-proteins: studies with synthetic peptides. Tartu, 1999, 103 p.
55. **Taavi Virro.** Life cycles of planktonic rotifers in lake Peipsi. Tartu, 1999, 101 p.
56. **Ana Rebane.** Mammalian ribosomal protein S3a genes and intron-encoded small nucleolar RNAs U73 and U82. Tartu, 1999, 85 p.
57. **Tiina Tamm.** Cocksfoot mottle virus: the genome organisation and translational strategies. Tartu, 2000, 101 p.
58. **Reet Kurg.** Structure-function relationship of the bovine papilloma virus E2 protein. Tartu, 2000, 89 p.
59. **Toomas Kivisild.** The origins of Southern and Western Eurasian populations: an mtDNA study. Tartu, 2000, 121 p.

60. **Niilo Kaldalu.** Studies of the TOL plasmid transcription factor XylS. Tartu 2000. 88 p.
61. **Dina Lepik.** Modulation of viral DNA replication by tumor suppressor protein p53. Tartu 2000. 106 p.
62. **Kai Vellak.** Influence of different factors on the diversity of the bryophyte vegetation in forest and wooded meadow communities. Tartu 2000. 122 p.
63. **Jonne Kotta.** Impact of eutrophication and biological invasions on the structure and functions of benthic macrofauna. Tartu 2000. 160 p.
64. **Georg Martin.** Phytobenthic communities of the Gulf of Riga and the inner sea the West-Estonian archipelago. Tartu, 2000. 139 p.
65. **Silvia Sepp.** Morphological and genetical variation of *Alchemilla L.* in Estonia. Tartu, 2000. 124 p.
66. **Jaan Liira.** On the determinants of structure and diversity in herbaceous plant communities. Tartu, 2000. 96 p.
67. **Priit Zingel.** The role of planktonic ciliates in lake ecosystems. Tartu 2001. 111 p.
68. **Tiit Teder.** Direct and indirect effects in Host-parasitoid interactions: ecological and evolutionary consequences. Tartu 2001. 122 p.
69. **Hannes Kollist.** Leaf apoplastic ascorbate as ozone scavenger and its transport across the plasma membrane. Tartu 2001. 80 p.
70. **Reet Marits.** Role of two-component regulator system PehR-PehS and extracellular protease PrtW in virulence of *Erwinia Carotovora* subsp. *Carotovora*. Tartu 2001. 112 p.
71. **Vallo Tilgar.** Effect of calcium supplementation on reproductive performance of the pied flycatcher *Ficedula hypoleuca* and the great tit *Parus major*, breeding in Northern temperate forests. Tartu, 2002. 126 p.
72. **Rita Hõrak.** Regulation of transposition of transposon Tn4652 in *Pseudomonas putida*. Tartu, 2002. 108 p.



ISSN 1024-6479
ISBN 9985-56-648-3