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CHANGES IN THE SPECIES DIVERSITY OF GRASSLAND COMMUNITIES DURING SECONDARY SUCCESSION

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Abstract

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Treatments of N, P and K fertilization levels were studied over the course of 18 years (1992–2009) of secondary succession in a semi-cultural permanent meadow community of *Sanquisorba Festucetum comutatae* on a mesophytic site in a fodder production area. Results of the evaluation of species diversity levels show that on average only 23.7 species (max. 34 species) contribute to actual fodder production, i.e. only 44% of species ascertained. A high level of N fertilization – 180 kg.ha^{-1} year^{-1} – significantly lowered the number of species (average – 19.9) in comparison to the variant without NPK (25.3 species), as well as in comparison to PK (26.3) and 90 kg N^{-1} year^{-1} + PK (23.4). Grasses make up on average 43.0% of the total number of species, legumes 5.9% and other herbs 51.1%. In the course of secondary succession, the number of species averaged across all treatments significantly increased, with 25.6 species in the 3rd post-stabilization phase (years 13–18) and 20.8 species in the 1st phase of succession (years 1–6). From % dominance point of view (expressed as the percentage weight of different species groups relative to total weight), there was a significant decrease in % dominance of grass components from 60.8% (1st phase) to 47.8% (3rd phase) and an increase in % dominance of other herbs from 35.3% (1st phase) to 46.8% (3rd phase). Across all successional phases, intensive N + PK fertilization significantly increased % dominance of grass components from 41.1%, and 39.8% for the variants without N fertilization, to 59.2% for N 90 and 69.7% for N 180 kg.ha^{-1} year^{-1} variant.

Key words: dominance; fertilization; grasses; legumes; herbs; permanent meadow

Introduction

The evaluation of the influence of four nutrition levels on species diversity (α-diversity) and its changes in individual phases of secondary succession of semi-natural *Sanquisorba-Festucetum comutatae* meadow communities has been the subject of a long-term, 18-year experiment. The qualitative aspect of the sward is expressed as % dominance (weight percentage in fodder) of grasses, legumes and other herbs in fodder produced in a dry state.

The species abundance of the meadow community was and still is the subject of research. With the formula p = f.(s.) (p /sward/ = f.(s.) /functions of the site/) Klecka and Fabian (1934) emphasize that any change in the soil environment results in species composition changes. Opitz von Boberfeld's data (1994) is proof of differences in competition of grasses and clovers on arable and meadow soils. Zelena (1993) and Mrkvicka and Vesela (2001) indicate lowered species diversity with the application of N+PK fertilizer exceeding the optimal level. Jancovic et al. (1999) indicate number of species decreased from 36 to 24–16 when fertilized with 60 to 240 kg N.ha^{-1} year^{-1} and were simplified to 3–4 main productive species. The dominant influence of N-nutrition is documented also, while observing significant differences in forage DM. Volume has increased from 4.8 t.ha^{-1} without N fertilization to 9,83 t.ha^{-1} with 180 kg N.ha^{-1} a year (Hrabe and Knot, 2011). According results in Park Grass Silwerton (2006) has biomass (productivity) negative effect upon species richness.

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Also, according Tilman (1987), addition of nitrogen significantly influences an increase of biomass and plant hight, which leads to decrease in species diversity. According to the multivariate model of species density variation of Crawley et al. (2005), 50 kg $N.ha^{-1}$ $year^{-1}$ added as fertilizer reduces species number by 6.5 species, compared to the N free plots. Velich (1986) emphasizes that the number of significant species with coverage above 1.0% is in negative linear correlation to the amount of nitrogen. Krajcovic et al. (1968) clarify the term of classic primary success of grassland swards (without anthropogenic effects) and regressive secondary succession, i.e. our swards in question.

Sward resistance and resilience values, which are described by Tilman and Downing (1994), can be determined from the alpha diversity, i.e. the number of species within an area, according to Britanak et al. (2007). As Novak (2009) describes, *Carex* spp*., Juncus* spp. and other "ruderal" herbs expand in grassland swards under mountainous conditions, on acidic soils and with a low level of exploitation. The qualitative aspects of the sward can be characterized in the % of grass, legumes components and other herbs dominance. According to Potsch (1999) the optimal ratio is 60–70% of grasses, 15–20% of clovers and 20–30% of other herbs. The possibility of increasing species diversity and reaching the optimal component ratio with no-till sowing was the subject of research by Buchgraber and Gindl (2004). Oerlemans (2006) studied methodological aspects of species diversity quantification by analyzing Shannon-Index and Simpson-Index and a number of scientific communications. Referring to Peet (1974), she claims that the number of species can contribute to the comparison of different communities only if the species play an important role in production.

The aim of this work is to evaluate the impact of long-term application of mineral fertilizers in the context of climate change on the proportion of grasses, legumes and herbs in semi-natural grassland.

Materials and Methods

The experiment site is located in a protected landscape area in the Bohemia-Moravian Uplands (Czech Republic). It is a fodder producing area (formerly classified as sub-mountainous). The elevation of the experimental area is 650 m. The average yearly temperature is 6.3°C (during the growing season 12.4°C); the yearly precipitation amount is 786 mm (465 mm during the growing season). The land is slightly sloped (3°) with southeast exposition. Soil type – stagnosol, acidic Pleosticene gneiss. Soil type – sandy loam soil, slightly gravelly and with a boggy upper sod layer, pH 4.45, water regime – mesohygrophytic, nutrition regime – mesoligotrophic.

Sanquisorba-Festucetum comutatae swards were mowed once a year up to 1992; in 1992 the experiment was started and they were mowed three times a year (always mowed at the start of June, August and October).

Fertilizing variants**:** H_0 – without N, P, K fertilization; H_1 – fertilized with P_{30}+K_{60} $kg.ha^{-1}$; H_2 – N_{90}+P_{30}+K_{60} $kg.ha^{-1}$; H_3 - N_{180}+P_{30}+K_{60} $kg.ha^{-1}$. The N supply is always divided by 1/3 for each mowing.

Arrangement of the experiment – classic small parcels (15.0 m^2 parcels) with random parcel arrangement and replicated 4 times. In the first harvest a fodder specimen was taken from a stable area of 1.00 m^2 from two of the four replicates. An analysis of each species was conducted (the number of species when fresh) and after being dried, mass was determined.

Dominance, as a ration of agribotanical groups in forage production was calculated in %. In the paper the % dominance of individual agribotanical groups (grasses, legumes and other herbs (including *Juncus* and *Carex*) is assessed.

The results were statistically analysed by repeated-measures Anova and followed by Tukey's test with p value 0.05 ($P \leq 0.05$), operated by Statistika 8.0. program version.

The main goal of the paper is to evaluate the long-term influence of various fertilization levels on changes in species abundance and trends in these changes in the initial phase of secondary succession (years 1–6), the stabilization phase (years 7–12) and the post-stabilization phase (years 13–18).

Results

Analytical data for individual years (Table 1) show the maximum total number of species 34, in the grass group 15, legumes group 4 and other herbs 17. According to synthetic data (Table 1) the average species number in harvested fodder is 23.7, of which 43.0% comprise grass species, 5.9% legumes species and 51.1% other herb species. At the time the experiments started Zelena (1993) found in total 57 species and on average per experimental parcel (replication) 34 species, of which 7 were important cultural grasses, 4 legumes and 6 other herbs, including *Juncus* and *Carex.*

The influence of NPK nutrition on the number of species

In total average (Table 2) the highest number of species was found for the P+K fertilization variant, 26.3. A reduction in the number of species for the unfertilized variant (rel. – 3.8%) and for the N_{90}+PK variant (– 11.0%) is not significant. On the other hand the H_3 N_{180}+PK variant showed 19.9 species, which is significantly lower, i.e. rel. – 24.3% on contrary to the variant with P + K fertilization and also

Table 1
The number of grass, legumes and other herb species in semi-natural grassland swards. Kameničky II., 1st cut, 1992–2009

Year of harvest	Variation of fertilization																			
	Ho (without NPK)				H_1 (P+K)				H_2 (N90+PK)				H_3 (N180+PK)				average var. Ho - H3			
	G[1]	L[2]	O[3]	∑	G	L	O	∑	G	L	O	∑	G	L	O	∑	G	L	O	∑
1992	7	1	10	18	8	1	9	18	9	0	7	16	9	0	7	16	8	1	8	17
1993	10	2	11	23	11	1	10	22	10	0	8	18	10	0	7	17	10	1	9	20
1994	12	2	11	25	11	2	15	28	11	1	11	23	11	0	9	20	11	1	12	24
1995	10	3	11	24	11	3	11	25	9	0	11	20	9	0	7	16	10	2	10	21
1996	10	1	9	20	7	3	12	22	10	1	9	20	6	0	5	11	8	1	9	18
1997	10	4	10	24	12	4	13	29	13	1	14	28	7	0	9	16	11	2	12	24
1998	10	2	11	23	10	2	14	26	12	0	10	22	8	0	8	16	10	1	11	22
1999	9	1	13	23	9	2	17	28	12	0	12	24	8	0	13	21	10	1	14	24
2000	11	2	12	25	11	3	13	27	13	1	13	27	10	0	12	22	11	2	13	25
2001	10	3	15	28	11	1	14	26	12	1	16	29	10	0	12	22	11	1	14	26
2002	12	1	15	28	15	2	17	34	13	1	13	27	12	1	14	27	13	1	15	29
2003	10	1	12	23	11	2	11	24	12	1	10	23	8	1	11	20	10	1	11	23
2004	13	3	15	31	9	3	11	23	10	0	13	23	9	1	10	20	10	2	12	24
2005	11	2	16	29	10	3	13	26	9	2	9	20	7	1	12	20	9	2	13	24
2006	10	2	16	28	10	3	15	28	8	1	15	24	7	1	16	24	9	2	16	26
2007	11	2	17	30	12	3	18	33	11	2	13	26	10	1	16	27	11	2	16	29
2008	14	2	15	31	11	4	14	29	12	2	14	28	10	1	13	24	12	2	14	28
2009	10	2	11	23	10	2	13	25	10	2	12	24	8	1	10	19	10	2	12	23
average 92-09	10.5	2.0	12.8	25.3	10.5	2.4	13.3	26.2	10.9	0.9	11.7	23.4	8.8	0.4	10.6	19.9	10.2	1.4	12.1	23.7

[1] Grasses; [2] Legumes; [3] Other herbs

Table 2
The average number of species in semi-natural grassland swards per individual phases of succession and in relation to fertilization level. Kameničky II., 1st cut, 1992–2009

Phases of succesion	Agribotanical groups	Variation of fertilization											
		Ho (without NPK)			H_1 (P+K)			H_2 (N90+PK)			H_3 (N180+PK)		
		number of species	%	% D	number of species	%	% D	number of species	%	% D	number of species	%	% D
I. 1992 - 1997	grasses	9.8	44.0	47.8	10.0	41.7	46.7	10.3	49.6	69.3	8.7	54.2	79.3
	legumes	2.2	9.7	3.9	2.3	9.7	11.6	0.5	2.4	0.1	0.0	0.0	0.0
	other herbs	10.3	46.3	48.3	11.7	48.6	41.7	10.0	48.0	30.6	7.3	45.8	20.7
	total	22.3	100		24.0	100		20.8	100		16.0	100	
II. 1998-2003	grasses	10.3	41.3	37	11.2	40.6	35.8	12.3	48.7	55.6	9.3	43.8	66.2
	legumes	1.7	6.7	2.5	2.0	7.3	7.4	0.7	2.6	0.3	0.3	1.6	0.0
	other herbs	13.0	52.0	60.5	14.3	52.1	56.8	12.3	48.7	44.1	11.7	54.7	33.8
	total	25.0	100		27.5	100		25.3	100		21.3	100	
III. 2004-2009	grasses	11.5	40.1	38.4	10.3	37.8	36.8	10.0	41.4	52.6	8.5	38.1	63.7
	legumes	2.2	7.6	2.5	3.0	11.0	13.8	1.5	6.2	4.5	1.0	4.5	0.5
	other herbs	15.0	52.3	59.1	14.0	51.2	49.4	12.7	52.4	42.9	12.8	57.5	35.8
	total	28.7	100		27.3	100		24.2	100		22.3	100	
average 1992-2009	grasses	10.6	41.7	41.1	10.5	40.0	39.8	10.9	46.4	59.2	8.8	44.4	69.7
	legumes	2.0	7.9	2.9	2.4	9.3	10.9	0.9	3.8	1.6	0.4	2.2	0.2
	other herbs	12.8	50.4	56	13.3	50.7	49.3	11.7	49.8	39.2	10.6	53.4	30.1
	total	25.3	100		26.3	100		23.4	100		19.9	100	

in comparison to variants without fertilization and with the application of 90 kg N.ha^{-1}. Intensive N application significantly reduces the number of legumes species and particularly the number of herb species from 12.8 – 13.3 for the unfertilized variant to 11.7 – 10.6 for variants fertilized with N. Their relative percentage of the structure of individual components is however increased by N fertilization to just above the 50% level.

The number of species in relation to succession

As far as the dynamics of succession changes (Table 3) is concerned the total average number of species has increased for all fertilization variants from 20.8 in the initial phase (years 1–6) to 24.8 in the stabilization phase (years 7–12) and to 25.6 in the post-stabilization phase (years 13–18), i.e. significant increases in comparison to the initial phase of succession. Correspondingly to the relationship to fertilization level a conclusive trend is indicated for the unfertilized variant and for variant H_3, i.e. with the application of 180 kg of N+PK. ha^{-1}. At the same time structural changes have occurred, i.e. a reduction in the percentage of grass species from the total number, from 46.6% in the initial phase to 39.5% in the post-stabilization phase, and a conclusive increase in the percentage of other herbs from 47.7% to 53.1%. The percentage of legumes elements, despite relative stability on average in total and even with the gradual increase of the percentage in the 3rd period of succession is significantly lower than with N+PK fertilization (range 3.8–2.2%) in comparison with the variant without NPK fertilization and with PK fertilization 7.9–9.3%.

Structural sward changes expressed as ratio of individual agribotanical groups in forage production (% dominance)

The number of species in grassland communities has a significant relationship to yield and ecological stability according to Britanak (2008); Tilman and Downing (1984); Dle Silvertown et al. (2006). The qualitative assessment of the sward composition however can be better expressed as a percentage of individual species or sward components, determined by the projective dominance method. The data we present (Tables 4 and 5) expresses the portion of grasses, legumes and other herbs in % of fodder mass (Figures 1, 2, 3). From this perspective N fertilization significantly increases – in contrast to species number trends – on average in total % dominance of grass components to 59.2–69.7% in comparison to 41.1% and 39.8% for variants without N fertilization and is at the expense of other herbs and legumes components. Dominance of legumes components (D = 10.9%) was significantly higher for the PK fertilization variant; for other variants it was under < 3.0%.

From the perspective of change dynamics (succession) of % dominance of individual sward components, in the post-

Table 3
The influence of fertilization on change in the structure of individual grassland community components over the course of succession. Kameničky II., 1st cut, 1992–2009

Phases of succession	Year	Variation of fertilization											
		Ho (without NPK)				H_1 (P+K)				H_2 (N90+PK)			
		G[1]	L[2]	O[3]	∑	G	L	O	∑	G	L	O	∑
initial	average 92-97	9.8a[4]	2.2a	10.3a	22.3a	10.0a	2.3a	11.7a	24.0a	10.3a	0.5a	10.0a	20.8a
stabilization	average 98-03	10.3a	1.7a	13.0b	25.0ab	11.2a	2.0a	14.3a	27.5a	12.3b	0.7ab	12.3a	25.3a
post-stabilization	average 04-09	11.5a	2.2a	15.0b	28.7b	10.3a	3.0a	14.0a	27.3a	10.0a	1.5b	12.7a	24.2a
Phases of succession	Year	H_2 (N90+PK)				H_3 (N180+PK)				average var. Ho - H_3			
		G	L	O	∑	G	L	O	∑	G	L	O	∑
initial	average 92-97	10.3a	0.5a	10.0a	20.8a	8.7a	0.0a	7.3a	16.0a	9.7a	1.3a	9.8a	20.8a
stabilization	average 98-03	12.3b	0.7ab	12.3a	25.3a	9.3a	0.3a	11.7b	21.3b	10.8a	1.2a	12.8b	24.8ab
post-stabilization	average 04-09	10.0a	1.5b	12.7a	24.2a	8.5a	1.0b	12.8b	22.3b	10.1a	1.9b	13.6b	25.6b

[1] Grasses.
[2] Legumes.
[3] Other herbs.
[4] Means followed by the same letter within a column are not significantly different (Tukey test) for $P \leq 0.05$.

Table 4
Dominance (% weight) of grasses, legumes and other herbs in semi-natural grassland swards in relation to fertilization level. Kameničky II., 1st cut, 1992–2009

Variation of fertilization	Dominance (in %) of vegetation segments		
	grasses	legumes	other herbs
without N, P, K	41.1a[1]	2.9a	56.0a
P_{30}+K_{60} kg.ha^{-1}	39.8a	10.9b	49.3a
N_{90}+P_{30}+K_{60} kg.ha^{-1}	59.2b	1.6a	39.2b
N_{180}+P_{30}+K_{60} kg.ha^{-1}	69.7c	0.2a	30.1c

[1] Means followed by the same letter within a column are not significantly different (Tukey test) for $P \leq 0.05$

Table 5
Changes in grass, legumes and other herbs dominance during succession semi-natural grassland. Kameničky; 1st cut, 1992–2009

Phases of succession	Dominance (in %) of vegetation segments		
	grasses	legumes	other herbs
initial (1st–6th year)	60.8a[1]	3.9a	35.3a
stabilization (7th–12th year)	48.6b	2.6a	48.8b
post-stabilization (13th–18th year)	47.8b	5.4a	46.8b

[1] Means followed by the same letter within a column are not significantly different (Tukey test) for $P \leq 0.05$

stabilization phase on average in total there was a significant decrease in the dominance of grass components (Figure 1) in production from 60.8% (1st phase) to 47.8% (3rd phase) and an increase in the dominance of herb components from 35.3% to 46.8% (Figure 3). Dominance of legumes components over the course of succession grew (average 1.9) and is significantly higher in the 3rd phase.

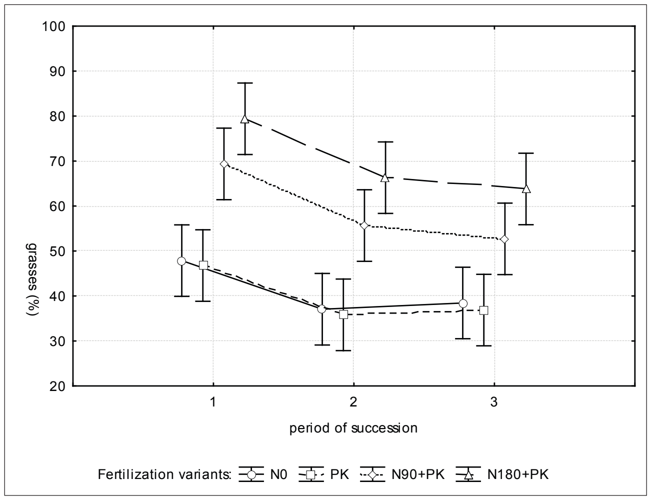

Fig. 1. Change dynamics in the % dominance of grass components in semi-natural grassland swards in relation to fertilization level (mean ± standard error). Kameničky II., 1st cut, 1992–2009

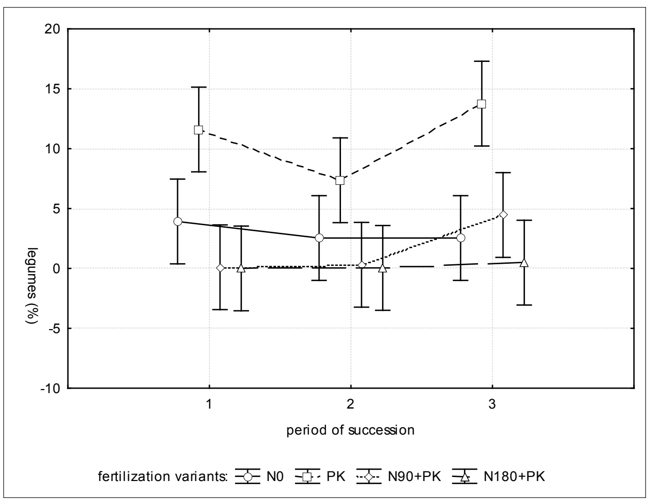

Fig. 2. Change dynamics in the % dominance of leguminous species in semi-natural grassland swards in relation to fertilization level (mean ± standard error). Kameničky II., 1st cut, 1992–2009

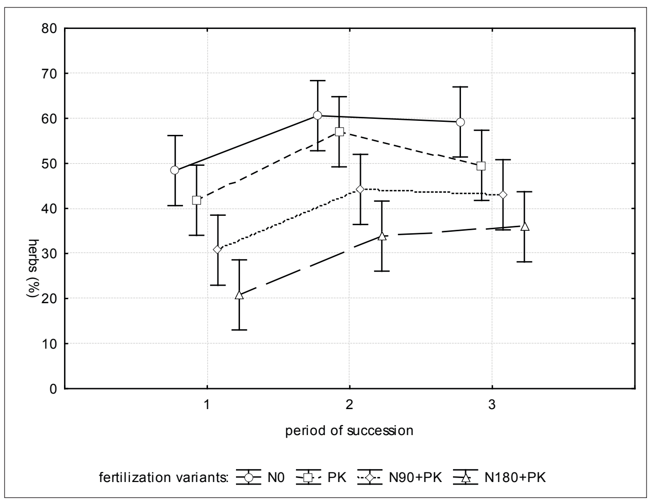

Fig. 3. Change dynamics in the % dominance of other herbs in semi-natural grassland swards in relation to fertilization level (mean ± standard error). Kameničky II., 1st cut, 1992–2009

Discussion

As Klecka and Fabian (1934) already indicated in the past, a significant decrease in species diversity of permanent grasslands with changes of abiotic factors, i.e. in this case fertilization levels. Ration 180 kg N+PK per 1 ha is according to economic and energetic analyses of production characteristics (Velich, 1986) ineffective for sites in question and leads to a reduction in species composition of swards. The results presented by Zelena (1993) and Jancovic et al (1999) confirm this. Odstrcilova et al (2007) and Ziliotto et al (2002) also confirm this trend, although they emphasize the necessity to assess species diversity and the influence of nutrition levels in relation to harvest numbers. Our results also confirm that there is a conclusive degree of species diversity change with distance from the optimal level (note fertilization), i.e. higher for the variant without NPK fertilization and lower for the variant with 180 kg N+PK.ha^{-1}. This conclusion is also valid for assessing species diversity of secondary succession over time. Increasing of plant species number in the post stabilization phase of grassland development occurred in all agri-botanical groups, it was observed being faster in groups of legumes and herbs. A ratio of grasses in the botanical biodiversity thereby decreased although the number of grass species increased.

These trends for extensively utilized (fertilized) swards manifest themselves in higher species distribution of stress selected species (= stress bearing), e.g. *Nardus stricta*, *Festuca ovina*, but also *Carex sp.* etc.

The reason for gradually increased number of species on the most intensively fertilised plots in post stabilized phase might be limited persistence of the most productive grass species or by imbalance of main nutrients as is mentioned by Hejcman et al. (2007). At the used fertilizing rate (N 180 + P30 + 60 kg ha^{-1}) occurs this imbalance between the nutrient demand and supply (Whitehead, 2008).

The assessment of % dominance (hereinafter % D) of individual species, or sward components, expressed in % (mass) in the harvested fodder is of greater significance to fodder quality in comparison to the species diversity of the grassland sward. The results confirm the findings of several scientific communications (Jancovic et al*.,* 1999; Velich, 1986; Nawrath et al., 2013, etc.) on increasing the grass mass portion and decreasing the portion of other herbs and the practical absence of legumes components with higher levels of N fertilization. However from the temporal perspective during the course of succession for on average all fertilization variants there was a conclusive decrease in % dominance of grass components (Figure 1). With decreasing component of grass species on total species number is decreasing the dominance of grass species, which confirms their positive correlation relationship (r = 0.72). Together the % of herbs is increasing. This "herb phenomenon", that is especially clear in extensively fertilized grassland swards (Isselstein, 1994), is the result of the ability of this group to react to changes in the abiotic environment, for example a change in the morphological structure of the root system and with it increased competition with grass species.

Achieving the optimal component ratio in semi-natural grassland swards from the perspective of cattle nutrition, especially increasing the percentage of vetch components, as presented by Potsch et al. (1999), with differing fertilization levels is difficult. Attempts to increase species diversity and also dominance of quality species with no-till sowing (Komarek et al., 2007; Buchgraber and Gindl, 2004) were not successful on our experimental environment (Fiala et al., 2002). Surprisingly high species diversity under these conditions indicates that the sward is restored (newly sowed) (Hrabe et al., 2002).

Conclusion

Treatments of N, P and K fertilization levels were studied over the course of 18 years (1992–2009) of secondary succession. On average the highest number of species was found for the P + K fertilization variant (26.3). The same variant showed the highest proportion of lugumes. On the other hand the N180 + PK variant showed only 19.9 species, which is significantly lower ($P < 0.05$). The results indicate that while the frequency of nitrogen doses is increasing, the number of species is decreasing. The dynamics of succession changes has increased for all fertilization variants on average from 20.8 species in the initial phase (years 1–6) to 25.6 in the post-stabilization phase (years 13–18). This increase was statistically significant ($P < 0.05$). At the same time structural changes have occurred, i.e. a reduction in the percentage of grass species from the total number and a conclusive increase in the percentage of other herbs. Across all successional phases, also intensive N + PK fertilization significantly increased percentage weight of grass components. The assessment of dominance individual species, or sward components, in the harvested fodder over time is of greater significance to fodder quality in comparison to the species diversity of the grassland sward.

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