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Effect of N, P and K fertilization on the species succession of an established grass sward during a decade

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Summary: Effects of different soil N, P and K supply levels and their combinations were examined on the species composition of a grass sward between 2006 and 2015 in a field experiment. The grass was established in autumn of 2000 with seed mixture of eight grass species. The calcareous chernozem loamy soil of the growing site contained around 3% humus, 3–5% $CaCO_3$, 20–22% clay in the ploughed layer and was originally moderately supplied with available N and K, and relatively poorly supplied with P and Zn. The trial included 4N×4P×4K=64 treatments in 2 replications, giving a total of 128 plots. The cover of grass species was surveyed every year at the end of May before the first cut. Perennial ryegrass *(Lolium perenne)*, timothy (*Phleum pratense*), meadow fescue (*Festuca pratensis*) and red fescue (*Festuca rubra*) disappeared from the experiment in the first few years. Crested wheatgrass (*Agropyron cristatum*) expanded through the years and required higher N and P supply to thrive. Smooth brome grass (*Bromus inermis*) was not a sown species, but established and expanded through the years. It benefited from N and K fertilization up to the highest supply, whereas a moderate P supply level was already satisfactory. Tall fescue (*Festuca arundinacea*) was the dominant species in the first years, but was continuously supressed. It required moderate N fertilization, since higher doses reduced the cover below the level of N control. P control treatment was the most favourable, whereas K had a slightly positive effect. The cover of cocksfoot (*Dactylis glomerata*) also showed a decreasing trend. Moderate N supply was the most effective, whereas P and K had only a slight effect. Reed canarygrass (*Phalaris arundinacea*) could not really spread, but remained present sporadically during the studied period. It preferred fertilized plots over control plots. Cover of other plant species, primarily herbs, increased continuously from 2% to 30–37% through the years mainly on control, especially on N control plots. The species composition was considerably modified by N, P and K supply of the soil.

Introduction

Different effects of fertilization on grasslands has been studied for a long time. Lawes et al. (1882) made comprehensive observations about the effect of

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fertilization on the species composition of grass in the Rothamsted experiment. Properly and scientifically established plant nutrition creates the possibility for forage yield increment of grasses. The extent of fertilizer effects may widely vary depending on many factors, e.g. the composition of fertilizer, soil characteristics, climatic conditions and species composition of the grass. Fertilization have different effects on each component of the lawn, i.e. on grasses, on leguminous plants and on other herbaceous plants. It can promote or maintain some species, but suppress others (Berendse et al. 1992, Jacobsen et al. 1996). However, in spite of changes at species level, the functional groups of the lawn may remain unchanged, thus its functions, i.e. carbon uptake can be constant (Czóbel et al. 2013).

The nutrient-status of the soil influences biomass production. Optimum nutrient supply creates condition to reach higher production, but species richness can be reduced at higher nutrient levels, or at least the species composition of a grassland can be modified (Janssens et al. 1998, Isbell et al. 2013). In the French Alps at 2000 m altitude, 15-years residual effect of high dose liming as well as P and K fertilization had significantly changed the species composition, but not species richness or yield. However, some typical plant species were replaced by more common species (Spielberger et al. 2010).

Among management practices, increasing nutrient supply due to fertilization has the greatest effect on species composition by promoting some species and suppressing others. On heavy clay soil near Wageningen, the Netherlands, on a nutrient-poor field the common grass species *Holcus lanatus*, *Anthoxanthum odoratum*, *Festuca rubra* and *Agrostis* spp. were replaced by *Alopecurus pratensis*, *Dactylis glomerata*, *Lolium perenne* and *Poa trivialis* on NPK treated hay plots within ten years (Elberse et al. 1983).

N and P fertilization can increase water-use efficiency of grasses, thus fertilization might help to mitigate adverse effects of climate change (Brueck 2008, Erickson and Kenworthy 2011). Considering the long-term effects, probably fertilizers can modify the composition of the grasslands in the most effective way and with the smallest investment. McLeod (1965) examined the effect of different N and K fertilizer doses on the species composition of a mixed leguminous and grass sward in a pot experiment. The ratio of the yield of the species alone and in mixture was considered as the "competition index". In Hungary, Szemán (2009) found that fertilizer application had no negative effect on the species number of grass species, but overall it reduced the diversity of the flora, whereas farmyard manure improved it on a rangeland.

Effect of fertilization on grass yield and element composition of this long-term grass experiment was published earlier. In the first year of the experiment, NPK fertilization increased the yearly air-dry hay yield from 3 to 13 t/ha mostly due to nitrogen and phosphorus fertilization. The element concentration of the grass alternated in a wide range due to the synergisms and antagonisms caused by the element content of the fertilizers, as well as the dilution effect as a result of yield increase (Ragályi and Kádár 2006). The trends of the effect of fertilization on grass yield was similar in the subsequent years, affected also by the amount and distribution of precipitation (Kádár et al. 2014, Ragályi et al. 2014). Szemán et al. (2010) found that NxP supply had a major influence on the coverage of the dominant grass species in this experiment between 2007 and 2009. Yearly results of the experiment between 2001 and 2012 was published by Kádár (2013a).

The aim of this present study was to determine the effect of different N, P and K nutrient supply levels of soil on the species composition and succession dynamics of a grass sward established with seed mixture of eight grass species.

Materials and methods

The grass experiment was set up in 2000 at Nagyhörcsök Research Station of the Institute for Soil Science and Agricultural Chemistry, Centre for Agricultural Research, Hungarian Academy of Sciences which is located near to Sárbogárd, Hungary (N 46° 51’ 56.84”; E 18° 31’ 10.17”; alt. 140 m a.s.l.). The calcareous chernozem soil of the site contained originally about 3% humus, 3–5% $CaCO_3$, 20–22% clay, 60–80 mg kg^{-1} AL (ammonuim-lactate soluble)-P_2O_5, 140–160 AL-K_2O mg kg^{-1}, 150–180 mg kg^{-1} KCl-soluble Mg in the ploughed layer according to Egner et al. (1960). The soil was originally moderately well supplied with available K, Mg, Mn, Cu and poor in P and Zn according to the Hungarian advisory system (Buzás et al. 1979). The area was drought sensitive with the groundwater table at a depth of 13–15 m and had an average yearly precipitation of 537 mm (1967–2015).

Plots were set up with the total combinations of four different N, P and K levels. The 0, 100, 200, 300 kg ha^{-1} N doses were applied yearly divided into two halves, one was applied in autumn and the other in spring. P and K fertilizations were performed with 0, 500, 1000, 1500 kg P_2O_5 and K_2O load in 1999 autumn. The applied fertilizers were Ca-ammonium nitrate, superphosphate and potassium chloride. The trial included 4N×4P×4K= 64 treatments in 2 replications, giving a total of 128 plots, sized 6 m by 6 m each, arranged in full factorial design within four stripes (96 m long each) divided by unsurveyed and unfertilized homogenous buffer stripes. Soil analyses were made in autumn 2010. The main goal of the experiment was not only to study the effect of different applied fertilizer doses, but rather to create different N, P and K nutrient supply levels in the soil. The experimental variants represent low, moderate, high and very high supply levels and all of their combinations. Treatments and the P and K contents of the ploughed layer of the soil are shown in Table 1.

Table 1. Fertilizer treatments (a–c) and their effects on the AL-soluble P and K contents (d–e) in the ploughed layer of the soil in 2010.
1. táblázat. Műtrágya kezelések (a–c) és hatásuk a talaj szántott rétegének AL-oldható P és K elemkészletére (d–e) 2010-ben. (1) kijuttatott műtrágya vagy talaj analízis; (2) kísérleti kezelés vagy műtrágyázás dózisa.

Fertilization and soil analysis (1)		Treatments or fertilization levels (2) 0	1	2	3
(a) N kg ha^{-1} yr^{-1}		0	100	200	300
(b) P_2O_5 kg ha^{-1}	In 1999	0	500	1000	1500
(c) K_2O kg ha^{-1}	In 1999	0	500	1000	1500
(d) AL*-P_2O_5 mg kg^{-1}	In 2010	82	201	374	601
(e) AL*-K_2O mg kg^{-1}	In 2010	131	174	240	301

*Ammoniumlactate soluble

Prior to the grass experiment, between 1973 and 2000, on these quadrats the same treatments were applied to study the effects on yearly varying crops (Kádár 2000, 2013b; Kádár and Földesi 2001). The long-term grass experiment was initiated by sowing a mixture of 8 grass species, on 20th September 2000. The relatively large number of components was intended to give adequate coverage and to indicate the species which are suitable for growing in this area. The dose of the applied grass seed mixture was 60 kg ha^{-1}. Based on the kernel weight of the sown grasses, the grass species rate was also calculated. Seed weight differences is often considered when multi-species experiments are established (Németh et al. 2017). The species composition of the sown grass, the seed application doses, seed weight rates and grass species rate is shown in Table 2.

Species composition was surveyed in every year by visual estimation of percentage cover values of the species on each plot at the end of May, before the first harvest. In each quadrat, aboveground vegetation was harvested yearly in late May or early June from 2001 to 2015. Hay was harvested once in dry years and twice in wet years.

Estimation of the average cover of species concerning the whole experiment was made from the first year. However, deeper plot by plot survey was carried out yearly from 2006. Thus, in this paper the dataset of a decade from 2006 until 2015 is presented.

Precipitation was measured by Hellmann rain gauge at the experimental station 400 m away from the quadrats. The solid snow, sleet, freezing rain and hail were measured after melting. Two years were relatively wet (2010, 2014), two years were near to average (2007, 2013), while in the remaining six years the yearly precipitation remained below the 537 mm average of the measured rainfall on the experimental site in the last 55 years.

Factorial ANOVA statistical analyses were carried out using Statistica 13 software (Dell Inc. 2015) with Tukey's HSD posthoc test ($p < 0.05$).

Results

Species in the whole experiment

Figure 1 gives an overview of the changes in average cover values of the sown grass species, the colonizer smooth brome, as well as all other plant species between 2006 and 2015 as an average of all the treated plots of the experiment.

The composition of the grass has been considerably changed throughout the years. Despite of their relatively high seeding rate, *Lolium perenne* disappeared from the whole experiment by 2006, and the same happened to *Phleum pratense*, *Festuca pratensis* and *Festuca rubra* by 2008 (Table 2). Thus, the effect of fertilization on these species are not evaluated in this paper.

Agropyron cristatum definitely expanded through the years almost constantly. Compared to its 6% seeding rate, its cover increased by 67% by the year 2006. By 2015 it covered over four times more area compared to its sown seed ratio. According to the trends (Fig. 1), it became the dominant species from the year 2012 taking the place of *Festuca arundinacea*.

The colonizer *Bromus inermis* was not a sown species, but became established and expanded through the years. In 2006 its cover was 10%, and in the following years this value varied in the range of 12–15%. *Festuca arundinacea* was sown with 12% seed rate. It spread and thrived especially between 2006 and 2008 reaching 35–32% cover, but was gradually supressed to 5% by the year 2015. Seeding rate of *Dactylis glomerata* was 9%, which remained stable until 2006 when

Table 2. Seed mixture of the eight grass species sown in autumn 2000.
2. táblázat. A 2000 őszén elvetett nyolcfajos fűmagkeverék összetétele. (1) a keveréket alkotó fajok; (2) vetett magmennyiség; (3) százalékos részesedés magtömeg alapján; (4) fűfajok százalékos részesedése.

Components (1)	Sown seed kg ha^{-1} (2)	Seed weight rate % (3)	Grass species rate % (4)
Meadow fescue (*Festuca pratensis*)	15.0	25	18
Tall fescue (*Festuca arundinacea*)	12.6	21	12
Perennial ryegrass (*Lolium perenne*)	12.6	21	13
Crested wheatgrass (*Agropyron cristatum*)	5.4	9	6
Red fescue (*Festuca rubra*)	3.6	6	8
Timothy (*Phleum pratense*)	3.6	6	19
Reed canarygrass (*Phalaris arundinacea*)	3.6	6	15
Cocksfoot (*Dactylis glomerata*)	3.6	6	9
Total	60	100	100

its cover was 8%, but by 2015 it decreased to 1.5%. *Phalaris arundinacea* could not really establish, but remained present in traces during the studied period.

Cover of other plant species increased from 2% to 30–37% due to the thinning and aging of sown grass species. The major species of this category concerning the whole experiment are: chickweed (*Cerastium* sp.), hawksbeard (*Crepis rhoeadifolia*), black medick (*Medicago lupulina*), field bindweed (*Convolvulus arvensis*) and drooping brome (*Bromus tectorum*). Total plant cover fluctuated between 64 and 85%.

Effect of precipitation on sown species

Effect of monthly and seasonal sums of precipitation prior to botanical survey on the cover of each grass species was evaluated, but no significant correlations were found in the period between 2006 and 2015. A relatively higher, about 0.4 Pearson correlation coefficient was found between *Bromus inermis* and sum of precipitation from March till May, whereas about –0.4 was found between the cover of *Festuca arundinacea* and sum of precipitation in March and April. This might indicate that these two species might be more sensitive to the amount of precipitation in the spring months, *Bromus inermis* might prefer more rainfall, whereas *Festuca arundinacea* can take more advantage form drier spring months, but this trend could not be validated.

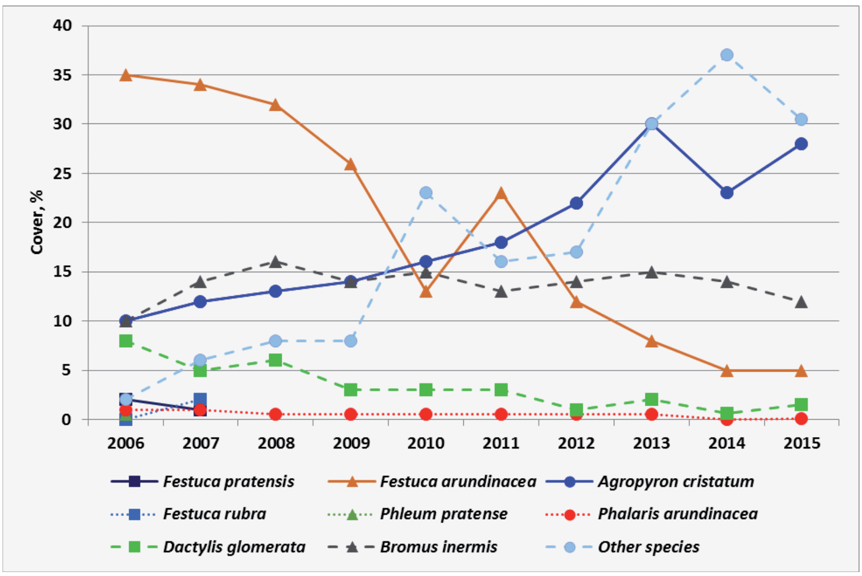

Fig. 1. Average cover percentages of the sown grass species, the *Bromus inermis* and all other spontaneously established species between 2006 and 2015 at Nagyhörcsök, Hungary.
1. ábra. A vetett fűfajok, a *Bromus inermis* és az egyéb betelepült fajok átlagos borításának alakulása 2006 és 2015 között a nagyhörcsöki tartamkísérletben.

Effect of fertilization on the sown species

Generally, the upward tendency of *Agropyron cristatum* was mainly due to N fertilization (Fig. 2). Already the N1 treatment, i.e. 100 kg ha^{-1} year^{-1} fertilization rate resulted in significantly larger cover from the year 2010, but in fact 200–300 kg N fertilization could provide a clear competitive advantage. There was no justifiable difference between the latter two doses. As a result of increasing P doses, the cover also increased significantly to the highest P3 supply level. The P0 level was significantly exceeded by even the P1 level, which was further enhanced by the increasing supply. K showed no meaningful effect, a slight downward trend could be observed. Similarly to *Agropyron cristatum*, *Bromus inermis* also benefited from fertilization, which helped its competition against other species. Within the over-

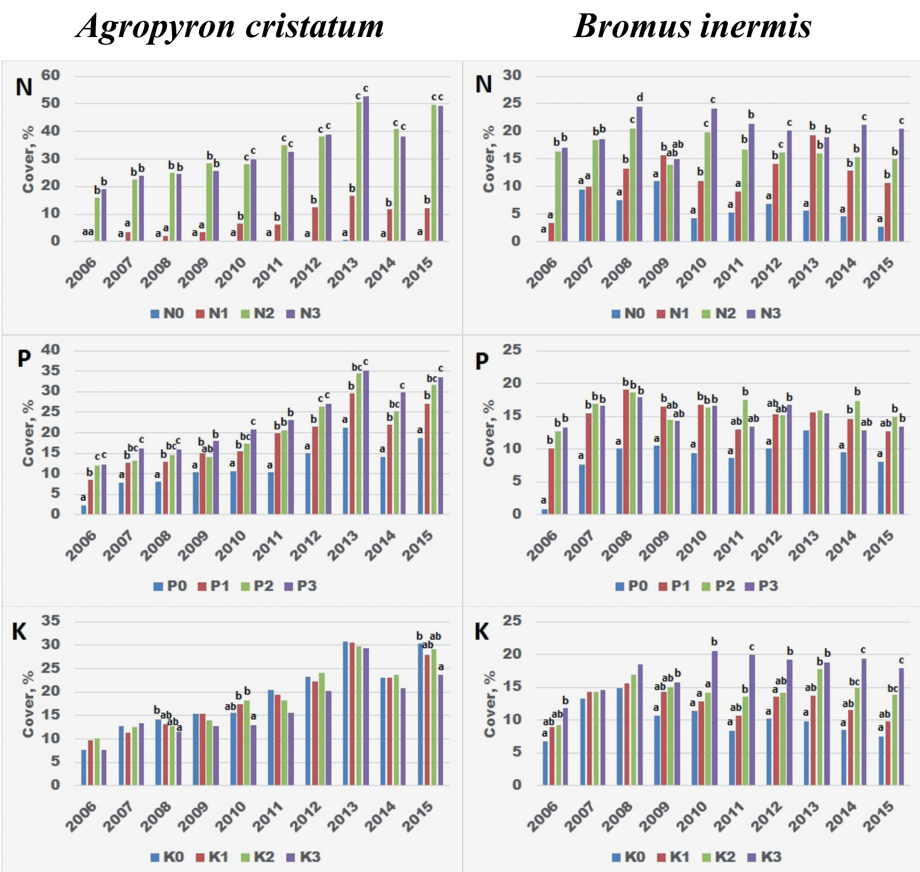

Fig. 2. Effect of increasing N, P and K supply levels on the yearly cover of *Agropyron cristatum* and *Bromus inermis*. Significant differences ($p < 0.05$, Tukey's HSD) are indicated by lower case letters between treatments within each year. The absence of letters indicates no significant difference.
2. ábra. Növekvő N, P és K ellátottság hatása az *Agropyron cristatum* és a *Bromus inermis* évenkénti borítottságára. Az egyes éveken belül kialakult szignifikáns eltéréseket ($p < 0.05$, Tukey's HSD) az eltérő betűk jelzik. Betűk hiányában a különbségek statisztikailag nem igazolhatók.

all experiment, its cover was relatively stable, only slightly fluctuating. N2 and N3 treatments resulted in significantly greater coverage than N0 treatment, but also N1 treatment had the same effect in most of the years. Unlike *Agropyron cristatum*, *Bromus inermis* was able to utilize even the highest N3 dose since in some years the cover of this treatment was veritable higher compared to that of N2 treatment. Increasing P supply also had a beneficial effect on the cover, but above P1 supply level only moderated effects were observed. K also had a significant effect on cover, moreover, the highest K supply resulted the highest cover every year.

Festuca arundinacea showed a downward trend during the studied years. Contrary to the two species discussed above, it had much moderate fertilizer demand. Though N1 treatment significantly increased its cover in almost all of the years, the higher doses reduced the cover below the level of N0 (Fig. 3). In the case of phosphorus, P0 treatment was the most favourable, whereas K had

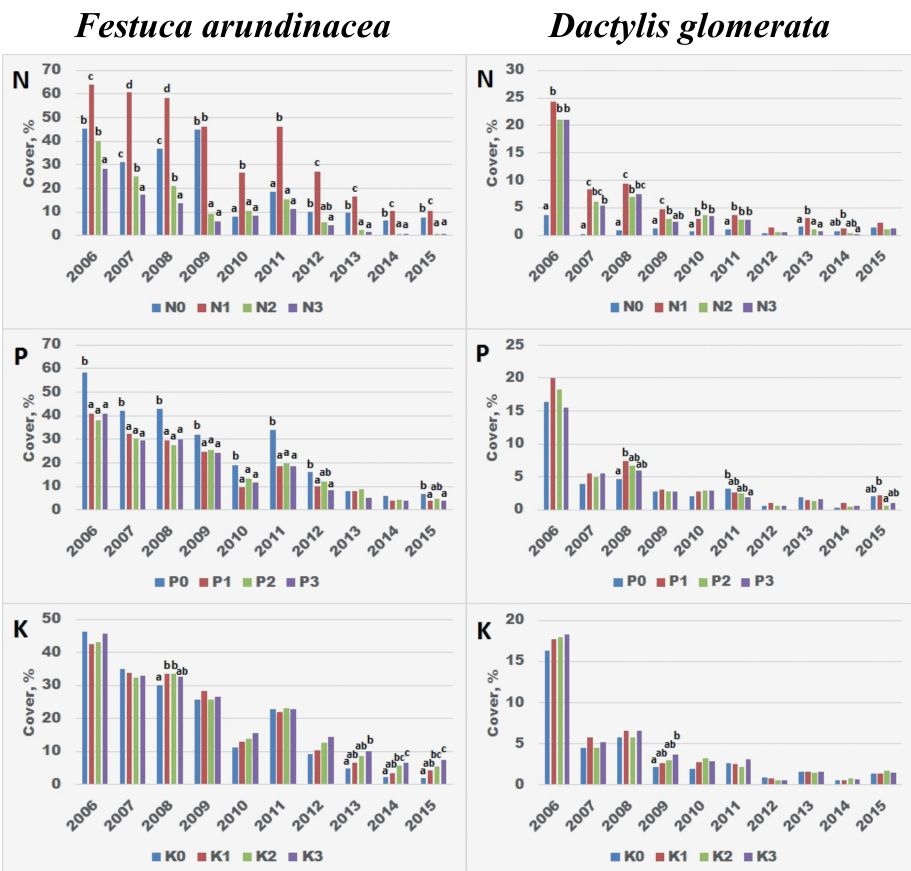

Fig. 3. Effect of increasing N, P and K supply levels on the yearly cover of *Festuca arundinacea* and *Dactylis glomerata*. Explanation of the results of statistical tests are indicated in Figure 2.
3. ábra. Növekvő N, P és K ellátottság hatása a *Festuca arundinacea* és a *Dactylis glomerata* évenkénti borítottságára. A statisztikai eredmények magyarázata megegyezik a 2. ábránál leírtakkal.

a fluctuating but overall slightly positive effect. The cover of *Dactylis glomerata* was primarily characterized by a year-to-year decline. Especially in the first few studied years, N has clearly increased cover, and N1 was the most effective. Both P and K had only a slight effect. P1 level, as well as K2-K3 levels seemed to be optimal, but the differences were not considerable and rarely significant. *Phalaris arundinacea* remained only in traces, but according to the surveys, it reached the highest cover on N2-N3, P1-P3 and K1-K3 treated plots, depending on the year, and disappeared from control plots (data not shown).

The cover of all other species was growing over the years. However, Figure 4 shows clearly that especially on N fertilized plots the presence and cover of this predominantly dicotyledonous group decreased significantly. This phenomenon can be observed primarily in year 2010 and later when the cover reached a remarkably higher value. The suppressive effect of P and K fertilization was lower, but it was

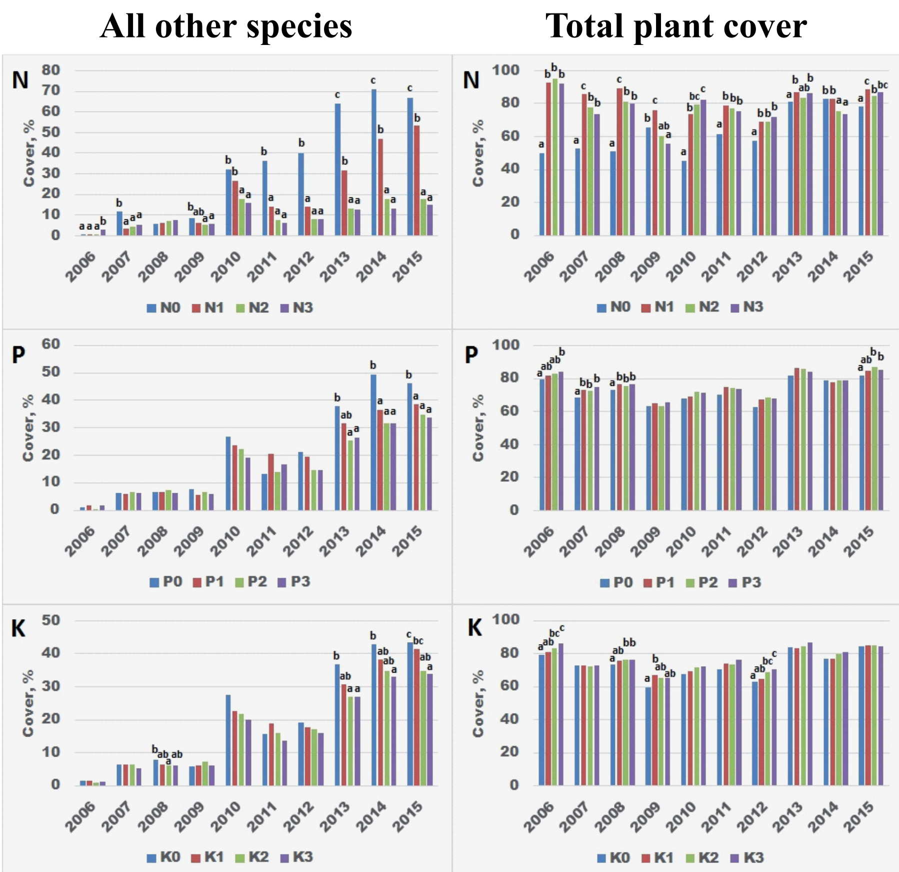

Fig. 4. Effect of increasing N, P and K supply levels on the yearly cover of all other species and total plant cover. Explanation of the results of statistical tests are indicated in Figure 2.

4. ábra. Növekvő N, P és K ellátottság hatása az egyéb növényfajok és az összes növényi borítás évenkénti értékeire. A statisztikai eredmények magyarázata megegyezik a 2. ábránál leírtakkal.

significant between 2013 and 2015. Total plant cover has not changed remarkably over the years. In this case also N has a decisive role, for the majority of the years the cover of N-treated plots was significantly higher than N0 control. P and K treatments slightly, in most cases non-significantly increased the total plant cover.

Discussion

When evaluating data, it is important to note that the results of this experiment do not necessarily show the absolute nutritional requirements of the species but rather their competitiveness with other specified species in the case of different nutrient supplies within the present experimental and natural conditions. The main reason for the disappearance of *Lolium perenne*, *Phleum pratense*, *Festuca pratensis* and *Festuca rubra* by 2008 might be their lower drought tolerance compared to the other survivor species. Lawes et al. (1882) also found that *Festuca pratensis* diminished very fast, after a few years, from the Rothamsted experiment since it has less sturdy habit, less branched root and did not resist drought so well. Generally, water demand of the decreaser and the increaser group of grass species is contrasting. Decreasers had a WB ≥ 5, while increasers (*Agropyron cristatum* and *Bromus inermis*) had WB ≤ 4 values on an expert knowledge based empirical scale ranging from 1 to 12 (Horváth et al. 1995). Water shortage, due to a low-lying groundwater table, can be one of the overall directing forces in succession at this area.

As the results showed, both *Agropyron cristatum* and *Bromus inermis* required higher doses of N, P and in the latter case K in order to successfully compete with other species. *Dactylis glomerata* benefited from N fertilization, while *Festuca arundinacea* required only moderate N supply, but liked control plots as well. According to Ellenberg and Leuschner (2010), *Bromus inermis* and *Festuca arundinacea* prefer habitats with moderate N or nutrient supply (5 on a scale of 10), whereas *Dactylis glomerata* and *Agropyron* species rather occur on better supplied sites (6 on a scale of 10). Hungarian classification scales placed *A. cristatum* among the least N demanding species (Horváth et al. 1995), which contradicts the strong vigour and cover increment due to N fertilization in our experiment.

Falkengren-Grerup (1998) reported a twofold biomass increase of *Dactylis glomerata* treated with 250 µM nitrogen (in a 7.5 × 7.5 × 5.0 cm pot) versus 50 µM, however 1250 µM treatment did not result in further increase or decrease compared to 250 µM. This is consistent with our results, i.e. *D. glomerata* benefits form even moderate N supply, but it is not depressed by higher rates of N fertilizer. In the Rothamsted experiment, *D. glomerata* thrived on plots with complex high dose fertilization (Lawes et al. 1882).

In a field experiment, existing bermuda grass (*Cynodon dactylon*) sharply decreased in favour of overseeded tall fescue (*Festuca arundinacea*) due to yearly

270 kg ha^{-1} N fertilization, especially on unharvested, but also on hayed plots during a seven years observation period (Franzluebbers et al. 2013).

All other species, predominantly dicotyledonous, were primarily found in control, especially on N control plots, while they were most remarkably retrieved from plots N2 and N3, i.e. due to 200 and 300 kg ha^{-1} year^{-1} N fertilization. It is likely that grass species are better able to utilize N, so the cover of all other species was indirectly disadvantaged by fertilization (Czóbel et al. 2013). Based on 13 monocotyledonous and 15 dicotyledonous species, Falkengren-Grerup (1998) also found that N primarily increases the biomass of monocots compared to dicotyledons. In the Park Grass Experiments at Rothamsted, Williams (1978) reported decreasing biomass of grasses parallel with increasing contribution of other species on unfertilized plots, which are also in accordance with our results.

The positive effect of N on total plant cover was particularly evident in earlier years, from 2006 to 2012. However, during the years from 2012 to 2015, these differences were considerably mitigated. One of the reasons for this may be that the heavily fertilized lawn often became patchy, i.e. it did not produce a continuous cover, but rather tussocks. The other reason might be the increasing cover in control plots by plant species that could well tolerate nutritional deficiencies. In years with different weather conditions, the increased number and the more varied ecological tolerance of the not-sown species could be responsible for maintaining the homogeneity of total plant cover.

Tilman and Wedin (1991) tested the effect of different seed ratios and N doses on pairwise competition of grass species in pot experiments. *Agrostis scabra* was seeded together with either *Agropyron repens* or *Schizachyrium scoparium* or *Andropogon gerardi* with 20–80%, 80–20% and 50–50% initial seed abundance. *Agrostis* was competitively suppressed by *Schizachyrium* and *Andropogon* on low N soils, and by all the other three species on high N soils. Generally, different N doses had significant effect, however, different seed ratios had little or no effect on the biomass of each species in pairwise comparison three years after sowing. This finding is also consistent with our results, and clarifies that in the long run species composition of established grasslands are primarily determined by the environmental conditions rather than the ratio of the sown seeds.

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N, P és K műtrágyázás hatása telepített gyep fajainak szukcessziójára tíz év során

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Különböző N, P és K ellátottsági szintek, és azok kombinációi hatását vizsgáltuk egy mesterséges gyep fajösszetételére 2006 és 2015 között szabadföldi kísérletben. A gyepet 2000 őszén telepítettük nyolc komponensből álló fűmag ke-

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verékkel. A kísérleti terület mészlepedékes csernozjom talaja a szántott rétegben mintegy 3% humuszt, 3–5% $CaCO_3$-ot és 20–22% agyagot tartalmazott. A kezeletlen talaj N és K elemekben közepesen, P és Zn elemekben gyengén ellátottnak minősült. A kísérlet 4N×4P×4K = 64 kezelést tartalmazott 2 ismétléssel, így összesen 128 parcellát foglalt magában. A gyepfajok százalékos borítását évente felvételeztük május végén, az első kaszálás előtt.

Az angolperje (*Lolium perenne*), a réti komócsin (*Phleum pratense*), a réti csenkesz (*Festuca pratensis*) és a vörös csenkesz (*Festuca rubra*) már a kísérlet első éveiben eltűnt a területről. A taréjos búzafű (*Agropyron cristatum*) az évek során növelte a borítását és meghálálta a nagyobb N és P ellátottságot. Az árva rozsnokot (*Bromus inermis*) nem vetettük, ám spontán megtelepedett a területen, és folyamatosan terjeszkedett. A N és K adagok a legmagasabb szintig növelték a borítását, míg a P esetében mérsékeltebb volt az igénye. A kísérlet első éveiben a nádképű csenkesz (*Festuca arundinacea*) volt a legmeghatározóbb faj, de borítása folyamatosan csökkent. Mérsékelt N műtrágyázást igényelt, a nagyobb adagok már a kontroll szintje alá csökkentették a tömegességét. Foszforból a kontroll volt a legkedvezőbb, míg a K trágyázás enyhe pozitív hatással volt rá. A csomós ebír (*Dactylis glomerata*) borítása csökkenő tendenciát mutatott az évek során. Mérsékelt N ellátottság bizonyult számára a legkedvezőbbnek, míg a különböző P és K ellátottságoknak nem volt számottevő hatásuk. A zöld pántlikafű (*Phalaris arundinacea*) nem tudott elterjedni, de szórványosan megtalálható volt végig a vizsgált időszak alatt. A kezelt parcellákon fordult elő, míg a kontroll parcellákon nem volt megtalálható. Az egyéb növényfajok, többnyire kétszikűek, borítása elsősorban a kontroll, azon belül is főleg a N kontroll parcellákon folyamatosan nőtt az évek során 2%-ról 30–37%-ra. A gyep fajösszetételét jelentősen befolyásolta a talaj N, P és K ellátottsága.