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SUB SOIL P STATUS COULD EXPLAIN THE ABSENCE OF RESILIENCE IN PLANT SPECIES COMPOSITION OF SUBALPINE GRASSLAND 63 YEARS AFTER THE LAST FERTILIZER APPLICATION*

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A unique grassland fertilizer experiment (the 'Grass Garden') was discovered on a podzol in the Giant (Krkonoše) Mts. in 2006. Sixty three years after having been fertilized with wood ash and manure for over 200 years, a 5 ha plot was dominated by *Deschampsia cespitosa* in the centre of the plot and *Avenella flexuosa* on the edge, whereas *Nardus stricta* was dominant in adjacent land which has never been fertilized. To explain these differences, soil (to three depths) and biomass samples were collected from three quadrants in *Deschampsia*, *Avenella*, and *Nardus* areas. Although the total and Olsen P appeared unaffected by the former fertilizer application in the surface humus layer, both P forms (plus soil solution P) were higher in the Grass Garden than outside for the eluvial and illuvial layers. Concentration and storage of P in green and senescent biomass was the lowest in *Nardus* and the highest in *Deschampsia*.It is suggested that these differences in P status may be responsible for the different dominant species between the areas, since *Nardus* is known to be very efficient in P recycling and has high P use efficiency. Long-term after-effects of fertilizer application must be evaluated over the whole soil profile since nutrients can move into the subsoil where the plants are able to acquire them.

biomass N : P ratio**;** cation exchange capacity**;** long-term fertilizer experiment**;** *Nardus stricta***;** phosphorus and nitrogen use efficiency and nutrition indexes**;** podzol soil; residual after effect

Abbreviations: Ah = humus layer, AlS = aluminium saturation, Ave = *Avenella flexuosa,* Bhs = illuvial layer, BS = base saturation, Des = *Deschampsia cespitosa,* E = elluvial layer, ECEC = effective cation exchange capacity, GG = Grass Garden, Nar = *Nardus stricta,* NNI = nitrogen nutrition index, PNI = phosphorus nutrition index, NUE = nitrogen use efficiency, PUE = phosphorus use efficiency

INTRODUCTION

According to May's (1973) definition, resilience is the ability of an ecosystem to recover into its original state after termination of a perturbation. Recent studies suggest that resilience of plant species composition after termination of fertilizer application is inversely related to grassland productivity, because the residuals after effects of fertilizer application in highly productive lowland grasslands last for over 20 years (Olff, Bakker, 1991; Mountford et al., 1996; Clark et al., 2009; Hrevušová et al., 2009; Královec et al., 2009), while for several decades after Ca and P application in unproductive acid or calcareous grasslands (Hegg et al., 1992; Spiegelberger et al., 2006; Hejcman et al., 2007b; Smits et al., 2008; Klaudisová et al., 2009). Results from alpine grasslands show that the original plant species composition after short-term perturbation by fertilizer application can be achieved after six decades (Spiegelberger et al., 2006), but if the fertilizer application lasts for centuries, recovery of species composition may take much longer time, if indeed it occurs at all (Semelová et al., 2008).

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A unique piece of land called the Grass Garden (GG), fertilized with wood ash and manure for at least 200 years, was discovered in the Giant Mts. in 2006 (Semelová et al., 2008). The last fertilizer application was made in 1944. Sixty-two years later, *Nardus stricta* was still dominant in the unfertilized control, *Deschampsia cespitosa* in the central part of the GG, and *Avenella flexuosa* on the edge of the GG.

In the GG, the effect of the former fertilizer application on plant-available P in the humus (Ah) layer (extracted by Mehlich III reagent) was not significant, but the concentration of P in total plant biomass differed substantially (Semelová et al., 2008). This raised the question why the original plant species composition was not restored after more than six decades. Were there any differences in soil chemical properties and in P status in the soil profile between the *Nardus* control and the formerly fertilized areas dominated by *Deschampsia* and *Avenella*? Was there any movement of elements supplied by fertilizer due to leaching in the soil profile? To answer these questions, detailed analysis of soil properties was performed in the humus (Ah), eluvial (E), and illuvial (Bhs) layers of the podzol soil.

Further, concentrations of N and P, and the N : P ratio, in plant biomass can differ substantially because of nutritional status, inter-species differences, and developmental stage (Koerselman, Meuleman, 1996; Wassen et al., 2005; Pavlů et al., 2013). Generally, species with conservative growth strategy (S – stress tolerant *sensu* Grime et al., 1988) are well adapted on nutrient poor environments since they develop mechanisms for scarce nutrient acquisition or conservation, namely a decreased growth rate, low nutrient concentrations in plant tissues, modification of root growth (Hammond, White, 2008; White, Hammond, 2008), a long leaf life span (Simoes

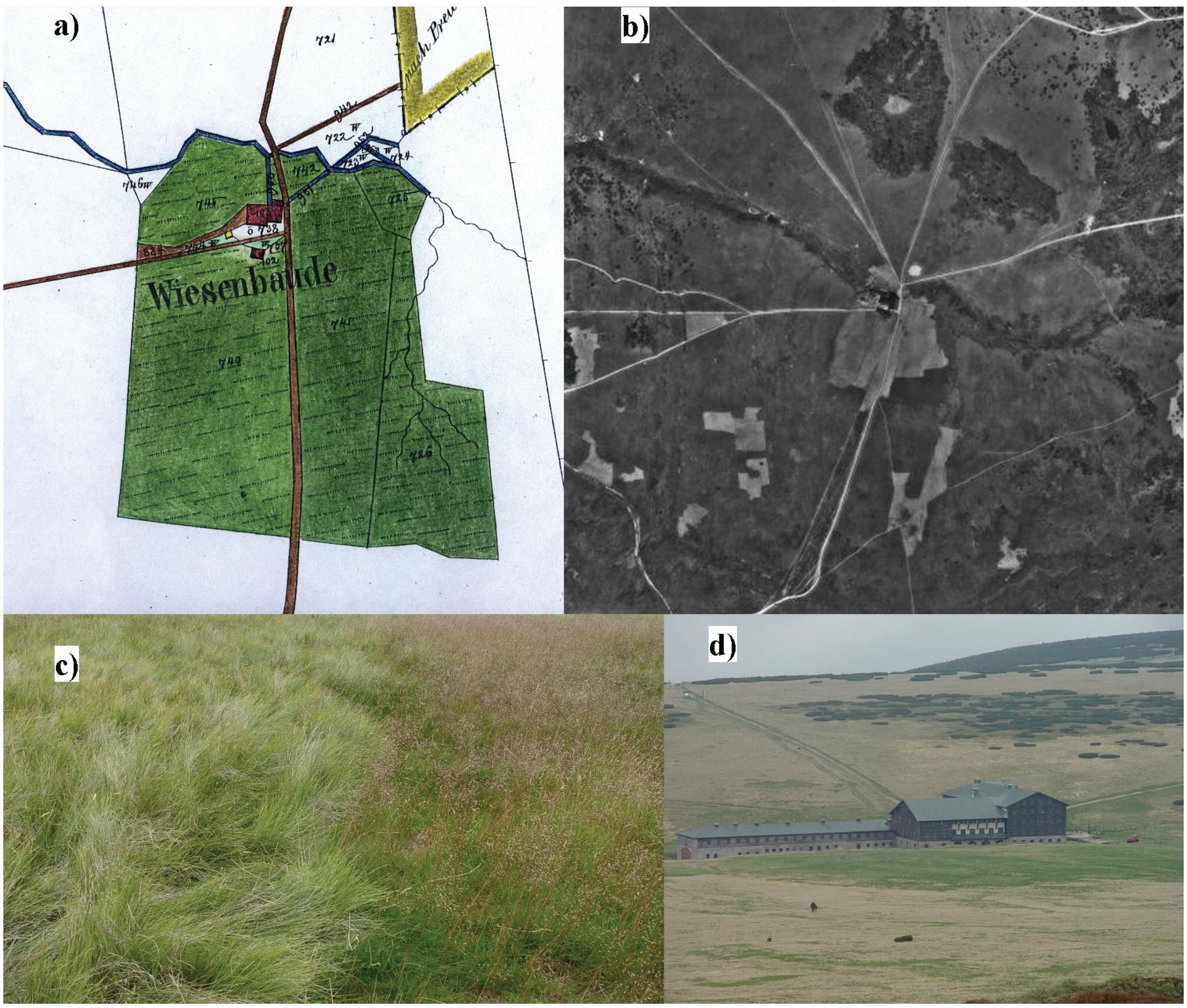

Fig. 1. (a) Map of Grass Garden (GG) near Meadow Chalet created in 1840–1841, (b) aerial photograph of GG taken by the Czechoslovak army in summer 1936 – freshly cut plots are light in colour; most of the GG area was freshly cut, (c) border between control (*Nardus stricta* dominated) and fertilized transition area (*Avenella flexuosa* dominated) was still well visible and accurate to a few decimeters 62 years after the last fertilizer application in summer 2006, (d) Meadow Chalet together with still well-visible GG (dark green colour in front of the building dominated by *Deschampsia cespitosa; Nardus* is yellow) in spring 2004. Photos: Michal Hejcman©

et al., 2008), and effective internal recycling of limiting nutrients, especially P (Vance et al., 2003; Güsewell, 2004).

Concentration of P and N : P ratio in green and senescent biomass of different species can be used as an 'indicator' of soil status and nitrogen and phosphorus use efficiency (NUE and PUE) which represents the ability to create from the same amount of N or P a different amount of biomass. The next aim of the paper was therefore to investigate differences in NUE and PUE among *Avenella*, *Deschampsia,* and *Nardus* by analyzing differences in N and P concentrations and N : P ratio in green and senescent biomass collected at the same time.

Alternative approach for investigation of nutritional status of different plants is the use of nitrogen (NNI) and phosphorus (PNI) nutrition indexes. Both indexes were developed by agronomists to predict effect of fertilizer application on biomass production in various crops (Duru, Ducrocq, 1997; Lemaire, 1997; Jouany et al., 2004; Ziadi et al., 2008). Advantage of this approach is that it takes into account the dilution effect, i.e. the decrease in nutrients concentrations during the accumulation of aboveground standing biomass.

Both indices, NNI and PNI, are obtained from the ratio, on a percent basis, of the actual (measured) nutrient content to the critical one which corresponds to the minimal concentration in sward needed to produce maximum dry matter, i.e. nutrient content allowing potential growth. For N, the critical curve given by Lemaire (1997) is the following:

$\%N_{critical} = 4.8\% (DM)^{-0.32}$

For P, the critical curve given by Duru, Ducrocq (1997) is the following:

$P\%_{critical} = 0.15 + 0.065\ N\%_{measured}$

The authors demonstrated that this relationship remains linear within the range of N and P concentrations observed in grasslands. NNI or PNI value of 100 indicates optimal N or P nutrition, while values below 60 indicate severe N and P deficiency limiting biomass production. It was proposed that NNI and PNI can be applied even in plant ecology as a tool characterizing NUE and PUE of different plant species. We suppose that NNI or NPI are inherently low for species well adapted to environments with low N or P availability. The last aim of the paper was therefore to test the suitability of NNI and PNI for investigation of nutrient use efficiency in investigated species.

MATERIAL AND METHODS

Study site

The Grass Garden is located on the top of the eastern part of the Giant Mts. in the borderland between the Czech Republic and Poland (latitude 50°44', longitude 15°42'). The study site lies above the upper tree limit at altitudes ranging from 1415 to 1430 m above sea level. The mean annual temperature is 2°C and the mean annual precipitation is 1380 mm (Vrbatova Bouda Meteorological Station). In the study area, highly acid podzol soils have developed on medium-grained porphyric granite.

The area of GG is approximately 5 ha and it had been fertilized by manure and wood ash for more than 200 years (Fig. 1). The GG and surrounding grassland was cut as intensively as possible because of a general shortage of forage in the Giant Mts. at that time. Grazing was restricted to the end of the growing season to give priority to hay production. The last fertilization and cutting were done in 1944. Mean annual doses of applied nutrients per ha were: 90–140 kg N, 250–350 kg K, 30–50 kg P, 300–450 kg Ca, 80–130 kg Mg (Semelová et al., 2008). The nomenclature of plant species follows Kubát et al. (2002).

Data collection

Three plant communities were distinguished: the control which had never been fertilized dominated by *Nardus* (Nar area), a transition zone on the edge of GG dominated by *Avenella* (Ave area), and the central part of GG, dominated by *Deschampsia* (Des area). In each area, the dominant species represented more than 80% of total biomass. Ave area probably received lower nutrient doses than Des area in the past. Three 1 m^2 sampling quadrates were established in each area, i.e. nine in total. Biomass and soil samples were collected on July 11^{th} and 12^{th}, 2007.

Soil sampling and analysis

Soil samples were collected within the profiles at 5 cm (Ah – humus layer), 10 cm (E –eluvial layer), and 30 cm (Bhs – illuvial layer) depths. Soil analysis was carried out by the Laboratoire d'Analyses des Sols of the National Institute for Agronomic Research (INRA, 62000 Arras, France) using French standards (Afnor, 1994). Conventional analyses were conducted on composite samples obtained by mixing an equal amount from the 3 replications for each horizon and area. Soil pH was measured in a water-soil suspension with a mass-to-volume ratio of 1 : 2.5 (NF ISO 10390). The organic C content was determined by oxidation with potassium dichromate and sulfuric acid (NF ISO 14235). Total organic nitrogen content was determined by the Kjeldahl method (NF ISO 11261) and the C : N ratio was calculated. The texture was determined by the pipette method of Robinson (NF X31-107). Effective Cation Exchange Capacity (ECEC) was determined with the cobaltihexamine method (Orsini, Rémy, 1976). The base saturation

Table 1. Soil texture and selected chemical properties under investigated areas in 0–5 cm (Ah), 5–10 cm (E), and 20–30 cm (Bhs) soil layers

Area	Layer	Clay (%)	Silt (%)	Sand (%)	C (%)	C : N	pH (H_2O)	ECEC ($cmol_c\ kg^{-1}$)	BS (%)	Ca ($cmol_c\ kg^{-1}$)	Mg ($cmol_c\ kg^{-1}$)	K ($cmol_c\ kg^{-1}$)	Al ($cmol_c\ kg^{-1}$)	AlS (%)
Des	Ah	23.2	42.9	33.9	20.7	14.7	3.9	16.4	56	7.14	1.43	0.50	4.0	25
Ave	Ah	32.2	48.9	18.9	27.5	15.9	4.2	10.7	36	1.49	1.16	1.03	5.1	47
Nar	Ah	29.9	47.7	22.4	29.3	15.4	4.4	11.9	40	2.16	1.18	1.29	5.0	42
Des	E	6.7	25.3	68	1.4	13.1	4.1	2.8	25	0.44	0.11	0.09	1.1	40
Ave	E	4.5	20.2	75.3	1.2	15.7	4.6	1.6	19	0.15	0.06	0.06	0.7	42
Nar	E	4.6	15	80.4	6.5	20.8	4.6	1.9	18	0.16	0.06	0.08	0.8	43
Des	Bhs	13.3	35.5	51.2	7.1	20	4.4	7.1	14	0.74	0.11	0.06	6.6	92
Ave	Bhs	10.4	30.1	59.5	5.8	23.3	4.5	6.1	7	0.26	0.06	0.05	6.3	104
Nar	Bhs	12	19.6	68.4	5.4	23.6	4.6	5.1	8	0.19	0.06	0.08	4.9	97

Areas abbreviations: Des = formerly fertilized dominated by *Deschampsia*, Ave = transition zone dominated by *Avenella,* Nar = unfertilized control dominated by *Nardus*

C = organic carbon content, C : N = carbon : nitrogen ratio in organic matter, ECEC = effective cation exchange capacity, BS = base saturation of ECEC, Ca, Mg, K, Al = concentration of exchangeable cations, AlS = aluminium saturation of ECEC

percentage (BS) and Al saturation percentage (AlS) were calculated according to B a i z e (2000).

Phosphorus status of the soils was measured on individual samples for each horizon and replication soils. Total soil P content was determined by ICP following calcination at 450°C and wet-digestion by HF and $HClO_4$ of soil (NF X 31-147). The plant available P was determined by the Olsen procedure (O l s e n et al., 1954) and the concentration of phosphate ions in the soil solution (Cp) was determined according to S t r o i a et al (2007).

Biomass collection and analysis

Total biomass samples were collected on each plot by cutting the sward within a 0.25 × 0.75 m quadrate with edging shears at a height of about 2 cm. Subsequently, an aliquot biomass was sorted manually to separate green and senescent biomasses for each sample. Collected samples were then oven-dried at 80°C for 48 h to determine the dry-matter yield and the relative contribution of green and senescent fractions to the total biomass. The bulk sample and fractions were ground to 0.5 mm and then analyzed for P content after wet digestion in H_2SO_4-H_2O_2 using the method of M u r p h y and R i l e y (1962). Total N concentration was determined with a CN gas analyzer (LECO Corporation, St Joseph, USA).

Calculation of N and P nutrition indexes

Dry matter yield and herbage N and P concentrations measured on the bulk green fraction were used to calculate the NNI and PNI indices with the following relationships (see D u r u , D u c r o c q , 1997 for detailed explanation of equations):

$$NNI = (\%N_{measured}/\%N_{critical}) \times 100;$$
$$\text{with } \%N_{critical} = 4.8\%\ (DM)^{-0.32} \tag{1}$$

$$PNI = (P\%_{measured}/P\%_{critical}) \times 100;$$
$$\text{with } P\%_{critical} = 0.15 + 0.065\ N\%_{measured} \tag{2}$$

Data analysis

All analysis was done with STATISTICA software (Version 12.0, 2013). One-Way ANOVA followed by comparison using Tukey's test was applied to identify significant differences among areas for soil chemical properties, biomass production, biomass mineral contents, and nutrition index data.

RESULTS

Soil analysis

Soil texture was similar for all areas in each layer and the main features of the soil were typical for podzols (Table 1). The soil was silty in Ah layer and sandy in E and Bhs layers. The gradient of soil chemical properties from Nar to Des areas was recorded. In Ah layer, the organic C content was highest in Ave and Nar and the lowest in Des area. In E layer, organic C content was substantially lower than in Ah layer: it was highest in Nar area layer. In Bhs layer, the content of organic C was similar in all areas. In Ah layer, there was no effect of area on the C : N ratio of the organic matter. In E and Bhs layers, there was an increase in C : N ratio from Des to Nar areas. The soil pH increased slightly with depth and was generally lowest in Des area, ranging 3.9–4.6 in all soils. ECEC was highest in Ah layer, intermediate in Bhs layer, and lowest in

E layer. In Ah layer, ECEC was highest in Des area. In all layers, percent base saturation (BS), especially by Ca and Mg, was highest in Des area. On the other hand, K and Al saturation (AlS) was highest in Ave and Nar areas.

Distribution of P over the soil profile is shown in Fig. 2. Total P concentration was highest in Ah layer followed by Bhs and much lower in E layer where P concentration was significantly higher in Des area. In the case of Olsen P concentration, the general pattern was similar to total P. No significant differences in either total or Olsen P among areas were recorded in Ah layer, whereas for layer E, there was a significantly higher Olsen P concentration in Des area and for Bhs layer the amount measured for Nar area was significantly lower. In the case of soil solution P concentration (Cp), a significantly higher value was measured for Nar area in comparison with Des area in Ah layer. In E layer, significantly higher Cp concentration was measured in Des area compared with Ave and Nar areas.

Biomass analysis

Effect of area on green biomass was not significant, although the highest amount of green biomass was recorded in Des followed by Ave area (Table 2). Weight of green biomass as a percentage of the total was highest in Ave area and lowest in Nar area.

In green and senescent biomass, concentrations of N and P were lowest and N : P ratio highest in Nar area (Table 2). The largest differences were recorded, especially for P concentrations, between Des and Nar areas. The amount of N and P fixed in green biomass differed substantially among areas; the amount of N was almost twice as high in Des as in Nar area and the amount of P was five times higher.

The majority of nutrients were stored in green biomass and only 23, 15, and 27% of N and 15, 12, and 17% of P was stored in senescent biomass in Des, Ave, and Nar areas, respectively.

A significant effect of area on NNI and PNI was recorded (Table 2). There were no significant differ-

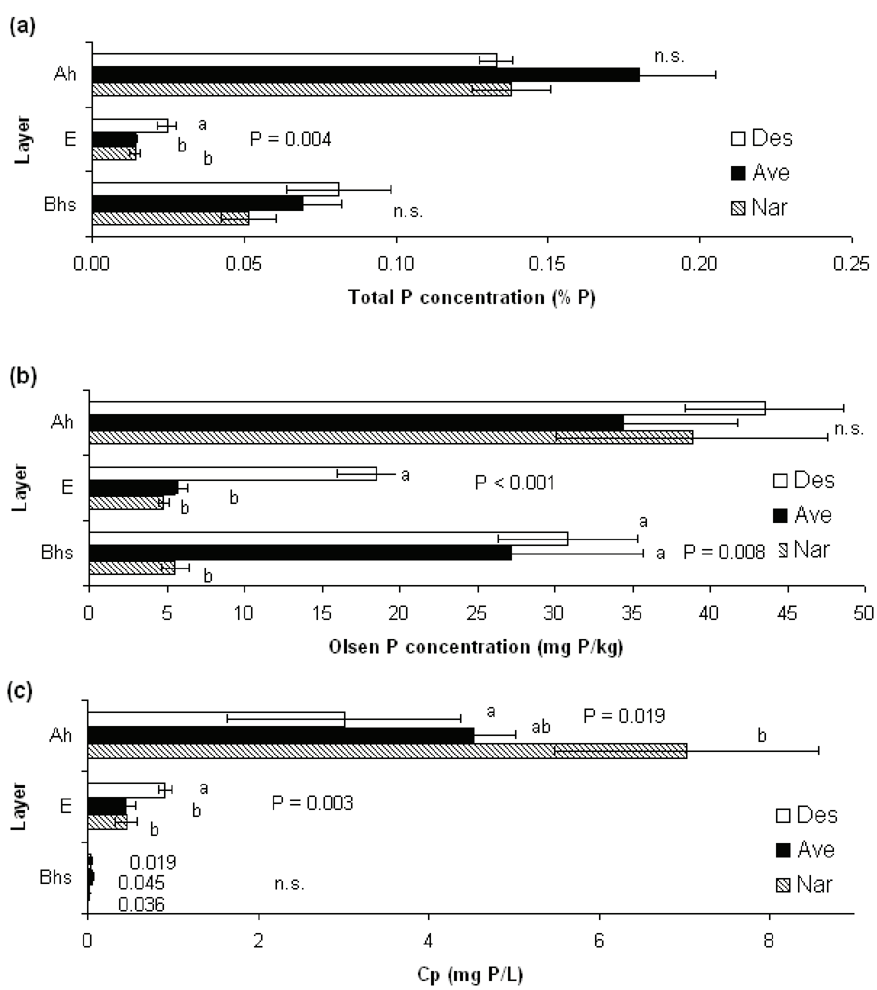

Fig. 2. (a) Concentration of total P, (b) Olsen P, and (c) Cp in 0–5 cm humus (Ah), 5–10 cm elluvial (E), and 20–30 cm illuvial (Bhs) soil layers; Areas abbreviations: Des = formerly fertilized dominated by *D. cespitosa*, Ave = transition zone dominated by *A. flexuosa*, Nar = unfertilized control dominated by *N. stricta*;P = probability value obtained by One Way ANOVA analysis, n.s. = non-significant result of ANOVA, using Tukey's post hoc test; areas with the same letter are not significantly different; error bars represent standard error

Table 2. Effect of areas on constituent characteristics (based on ANOVA results)

Tested variable	*P*-value	Des area	Ave area	Nar area
Green biomass				
% of green biomass from total	**0.009**	$66.8^{a} \pm 8.4$	$83.6^{b} \pm 2.4$	$63.8^{a} \pm 3.6$
Green biomass (t ha^{-1})	0.327	5.1 ± 2.0	4.0 ± 0.5	3.5 ± 0.3
N concentration in green biomass (%)	**< 0.001**	$2.9^{a} \pm 0.1$	$2.1^{b} \pm 0.1$	$2.2^{b} \pm 0.1$
P concentration in green biomass (%)	**0.001**	$0.34^{a} \pm 0.01$	$0.29^{a} \pm 0.07$	$0.10^{b} \pm 0.01$
N : P ratio	**< 0.001**	$8.5^{a} \pm 0.3$	$7.8^{a} \pm 2.0$	$22.9^{b} \pm 3.0$
Amount of N in green biomass (kg ha^{-1})	0.109	148.8 ± 64.1	85.7 ± 14.5	75.6 ± 2.7
Amount of P in green biomass (kg ha^{-1})	**0.034**	$17.5^{a} \pm 7.2$	$11.8^{b} \pm 4.4$	$3.3^{c} \pm 0.6$
N nutrition index	**0.013**	$101.1^{a} \pm 17.1$	$69.1^{b} \pm 6.4$	$67.4^{b} \pm 1.3$
P nutrition index	**0.002**	$101.2^{a} \pm 1.4$	$99.3^{a} \pm 5.1$	$33.0^{b} \pm 4.5$
Senescent biomass				
% of senescent biomass from total	**0.009**	$33.2^{a} \pm 8.4$	$16.4^{b} \pm 2.4$	$36.2^{a} \pm 3.6$
Senescent biomass (t ha^{-1})	**0.001**	$2.3^{a} \pm 0.02$	$0.8^{b} \pm 0.13$	$2.0^{a} \pm 0.41$
N concentration in senescent biomass (%)	**0.001**	$1.9^{a} \pm 0.07$	$2.0^{a} \pm 0.16$	$1.4^{b} \pm 0.04$
P concentration in senescent biomass (%)	**0.009**	$0.15^{a} \pm 0.01$	$0.21^{a} \pm 0.08$	$0.03^{b} \pm 0.001$
N : P ratio in senescent biomass	**< 0.001**	$12.7^{a} \pm 0.5$	$10.5^{a} \pm 3.0$	$41.3^{b} \pm 2.0$
Amount of N in senescent biomass (kg ha^{-1})	**< 0.001**	$44.7^{a} \pm 1.2$	$15.7^{b} \pm 1.4$	$27.6^{c} \pm 5.2$
Amount of P in senescent biomass (kg ha^{-1})	**< 0.001**	$3.2^{a} \pm 0.2$	$1.6^{b} \pm 0.6$	$0.7^{c} \pm 0.2$

Ave = *Avenella flexuosa,* Des = *Deschampsia cespitosa,* Nar = *Nardus stricta*

significant results are in bold; degrees of freedom were 2 in all analyses; ± values represent standard error; areas with the same letter were not significantly different

ences between NNI for Ave and Nar areas, but it was significantly higher in Des area. In the case of PNI, there were no significant differences between Des and Ave, and PNI was significantly lower in Nar area.

DISCUSSION

Soil analysis

In the humus layer, no differences in total P and Olsen (plant available) P were recorded. This is consistent with results of Semelová et al. (2008) who showed no significant difference in Mehlich III (plant available) P in upper Ah layers. Total P content was relatively high in all Ah layers as a consequence of organic matter accumulation (Stroia et al., 2007). Significantly increased soil solution P (Cp) in Nar area compared to Des area in Ah layer is surprising as no differences were detected using stronger Olsen or Mehlich III reagents. Cp concentration was positively correlated with the amount of organic matter in Ah layer. A large release of organic matter into extracted solution can lead to artifacts during phosphate ion titration in low pH media, leading to organic P mineralization and overestimation of the phosphate ions concentration in the solution measured (van Veldhoven, Mannaerts, 1987). On the other hand, the concentrations of total P, Olsen P, and soil solution P (Cp) were still significantly increased in E layer in Des area although the last fertilizer application was made 63 years prior to soil sampling. Increased concentration of Olsen P was still recorded in Bhs layer in both formerly fertilized Des and Ave areas compared with Nar area. These results indicate substantial movement of fertilizer P within the soil profile and probably explain still existing differences in P availability between formerly fertilized Ave and Des areas and control Nar area.

We believe that such a long-term positive residual effect of fertilizer application on P availability in the soil profile has never been published previously. In alpine conditions, higher P availability in a podzol Ah layer compared with a control was reported by Hejcman et al. (2007b) almost 40 years after the last P application; but in this work the subsoil E and Bhs layers were not investigated.

Measurement of the ECEC and exchangeable cations at soil pH revealed differences between the areas, since it allows determining all the exchangeable cations, including Al. High, regular, and long-lasting fertilizer application changed the soil reactivity on Des area: the ECEC increased as a consequence of applied wood ash in comparison with Ave and Nar areas. Similar results were reported by Yamoah et al. (1992) and by T a k a h a s h i et al. (2006) for soils amended with lime. Base saturation of the ECEC was increased by 50% and Al saturation decreased by 50% as expected from the large increase (50%) in ECEC for formerly fertilized soils. Exchangeable Ca was five and four times higher in Des area compared to Ave and Nar areas. Similar long-term residual effects of Ca application were reported by S p i e g e l b e r g e r et al. (2006).

The fact that availability of P and Ca was increased in E and Bhs layers of formerly fertilized areas indicates that the long-term residual effect of fertilizer application must be evaluated for all layers within the soil profile, not just the humus layer (Ah) as it has been frequently done (see N i i n e m e t s, K u l l, 2005; S p i e g e l b e r g e r et al., 2006; H r e v u š o v á et al., 2009; P a v l ů et al., 2012).

Biomass analysis

Mean amounts of green and senescent biomass were highest in Des, followed by Ave, and lowest in Nar area. Differences among areas were not significant because of high variability within the data especially in Des area.

The mean amount of N stored in green and senescent above-ground biomass was 1.9 times higher and the amount of P was 5 times higher in Des area compared with Nar area. Although all three areas differed in both N and P storage, the clearest differences were for P. This may indicate (1) a greater ability of *Avenella* and *Deschampsia* to acquire P from the soil profile or (2) a greater availability of P in Ave and Des areas. Based on results of soil P analyses and experiences of other authors (G ü s e w e l l et al., 2005; H e j c m a n et al., 2007a,b; C h y t r ý et al., 2009; K l a u d i s o v á et al., 2009), the second explanation seems to be more probable because *Nardus* generally dominates in environments with very low P availability.

Concentrations of N and P in green biomass of *Avenella* and *Deschampsia* were substantially higher than those reported by G r i m s h a w, A l l e n (1987) although the biomass samples were collected at the same time of the year. This is probably because they could have been collected at different developmental stages. For any given species, P and N concentrations in mature plants are lower than those measured in young biomass because of the dilution effect resulting from the increase in the proportion of stems, which have lower N and P concentrations than leaves during growth (D u r u, D u c r o c q, 1997). *Nardus* and *Avenella* N use efficiencies were similar, for the same level of biomass production the same N concentration was measured. N use efficiency in *Deschampsia* was substantially lower than in *Avenella* and *Nardus* since *Deschampsia* displayed higher N concentration in biomass under a high level of biomass production. These conclusions are well supported by the NNI observed for the different areas. N nutrition in the Des area appears to have been optimal since the NNI was about 100, whereas in Ave and Nar areas it was in the range of 65–70, indicating suboptimal N nutrition. Although NNI was developed by agronomists as a tool for assessing the effect of N supply on the dry matter yield and N limitation of different crops (L e m a i r e, 1997) and multi-species grasslands (G a r n i e r et al., 2007), it can be successfully used to characterize N use efficiency of different species as demonstrated for the first time in this study.

Phosphorus use efficiency was substantially lower for *Deschampsia* and *Avenella* than for *Nardus*, because P concentration was substantially higher in green biomass of *Deschampsia* and *Avenella* than in *Nardus*. This is also indicated by the N : P ratio which was highest in *Nardus* biomass. These conclusions are well supported by PNI values for different species. For *Deschampsia* and *Avenella,* PNI was close to 100 indicating optimal P nutrition, while in *Nardus*, although the value of PNI (33) was extremely low, indicating very high P limitation, the biomass production was not affected (J o u a n y et al., 2004). This indicates very high P use efficiency in *Nardus* compared to the other species. Like NNI, PNI can be successfully used for evaluation of P use efficiency for a given species. According to other authors (J o u a n y et al., 2004; G a r n i e r et al., 2007; H e j c m a n et al., 2010), such a low PNI has never been recorded in eutrophic and mesotrophic grasslands with species adapted to higher soil P availability.

Accumulation of senescent biomass was due to the absence of cutting in the locality (S e m e l o v á et al., 2008). N and P resorption efficiencies differed substantially for the investigated species. Based on differences in N and P concentrations between green and senescent biomass, which were smaller for N than for P, it was concluded that N resorption was generally lower than that of P in all three species. This agrees with results obtained from many other species (for review see G ü s e w e l l, 2004). P resorption was very intensive in *Nardus*, as the concentration of P in senescent biomass was as low as 0.03%. This highly effective P resorption in *Nardus* indicates effective internal cycling of P and the species' ability to cope with low soil P availability (G ü s e w e l l et al., 2005; K l a u d i s o v á et al., 2009).

Plant and soil interactions

It is highly probable that differences in P availability in E and Bhs layers can be at least partly responsible for the dominance of *Deschampsia* and *Avenella* in formerly fertilized areas and of *Nardus* in the unfertilized area because the investigated grasses can acquire scarce nutrients even from depths greater than 12 cm (P e c h á č k o v á et al., 2004). This indicates high competitiveness of *Nardus* in environments with very low P availability. Further, increased Ca availability in the fertilized areas can be partly responsible for differences in species composition among investigated areas as *Deschampsia* can grow in soils with higher Ca availability in comparison to *Avenella* and *Nardus*.

P status of *Deschampsia* and *Avenella* was the same, but N nutrition was different. Additionally, there was substantially lower Al mobility in the soil profile under *Deschampsia* compared to *Avenella* area. *Deschampsia* is probably more competitive than *Avenella* when Al toxicity level is lower.

CONCLUSION

As a consequence of former fertilizer application, the soil P status was similar for *Deschampsia* and *Avenella* areas, but different for unfertilized *Nardus* area. The differences were apparent from subsoil analysis indicating movement of fertilizer P within the profile. The soil effective cation exchange capacity, base cations (Ca especially), and exchangeable Al status were different for *Deschampsia* area in comparison with *Avenella* and *Nardus* areas.

Nardus, which is adapted to very low P availability, displayed very high P use efficiency as indicated by its low P concentration, low PNI, and high N : P ratio in green and senescent biomass compared to other species. N use efficiency was similar for *Avenella* and *Nardus,* but lowest for *Deschampsia* as indicated by NNI and N concentrations in biomass. The competitive ability of the three species was determined by P and N internal metabolism related to soil properties and probably also by Ca availability and Al toxicity.

It was concluded that long-term after-effects of fertilizer application must be evaluated over the whole soil profile since nutrients can move down by leaching and then be retained in the subsoil where the plants are able to acquire them. Like the N : P ratio, NNI and PNI can be effectively used to characterize N and P use efficiency in grassland species.

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