



Plant assemblages respond sensitively to aluminium solubility in acid soils

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Abstract: Aluminium as a growth limiting factor has been recognized for many years. At high concentrations, aluminium (Al) ions reduce nutrient availability in soils, harm plant cells and thus inhibit plant growth. In addition, Al concentration may be a major factor filtering species composition on acid soils in favour of Al-resistant plants. In this study we analyse species responses and turnover along soil pH and Al gradients and we attempt to interpret the results with respect to the recognised aluminium solubility patterns. Plant community and soil data collected from mesophilous and acidophilous submontane broad-leaved forests of Western Slovakia were used for this purpose. Topsoil horizons were analysed for soil reaction (pH), organic carbon and extractable total aluminium. Species responses to the Al measurements were analysed and tested using CCA and the Huisman-Olff-Fresco (HOF) model. We calculated species turnover by accumulating the first derivatives of all HOF response curves, and interpreted them with respect to the Al solubility pattern observed in the soil dataset. We also performed a bioindication experiment to test how a species assemblage indicates the aluminium gradient. In total, 81% of species shows a significant response to the soil Al gradient. We identified that a rapid retreat of many species and, in consequence, high compositional turnover (ecotone) corresponded with a discontinuity in Al solubility observed at 130 mg Al kg⁻¹ (pH 3.8). Here, the exchangeable Al became increasingly under-saturated with respect to the equilibrium attained at higher pH. This discontinuity was also visible in the bioindication experiment, where the prediction algorithm operated better at the acidic end of the gradient. The results indicate that the studied plant assemblages respond sensitively to soil Al solubility. Changes in aluminium solubility in soils correspond with ecotone between adjacent types of vegetation.

Plant nomenclature: Marhold and Hindák (1998).

Abbreviation: HOF–Huisman-Olff-Fresco model.

Introduction

Plant assemblages respond sensitively to changing soil acidity (Ellenberg et al. 1992, Schaffers and Sýkora 2000, Wamelink et al. 2005). Soil reaction alone (pH) and various related soil properties, comprising available calcium and aluminium (Al), carbonates, base saturation or nitrates have been used to explain such responses in plant community data (e.g., Schaffers and Sýkora 2000, Pepler-Lisbach and Kleyer 2009). Among different soil properties, high Al concentration has been recognized as a relevant factor driving plant growth and species transitions along the soil pH gradient (e.g., Abedi et al. 2013, Pepler-Lisbach and Kleyer 2009). In neutral soils, aluminium occurs predominantly in an undissolved form and does not affect plants in any significant way. However, it is increasingly solubilised when soils turn more acidic and aqueous Al³⁺ then becomes a crucial growth-limiting factor for plants (Foy 1992, Poschenrieder et al. 2008). Besides toxicity

of monomeric aluminium, it reduces phosphorus, molybdenum and sulphur availability, and by occupying a major share of ion-exchange sites aluminium becomes a driving competitor for other cation nutrients, including calcium and magnesium (e.g., McLean 1976, Boudot et al. 1994). Therefore, soil aluminium has repeatedly been used to explain vegetational patterns on acidic soils in numerous studies (e.g., Neave et al. 1995, Abedi et al. 2013). However, so far, a little attention has been paid to the aluminium solubility, which represents a major forcing mechanism for Al availability in acidic soils (Ulrich 1983, Wesselink et al. 1996).

In fact, bioavailability of Al may vary considerably depending on the solubility of Al solids present in soils. Recent studies have indicated that a pH decrease of one unit may result in Al dissolution varying by almost three orders of magnitude, dependent on which Al-solids are present (Mulder and Stein 1994, Wesselink et al. 1996, Dlapa 2002), thus resulting in different growing conditions for plants. The main solubility differences that apply for mineral soils in Slovakia are

demonstrated in the solubility diagram (Fig. 1) constructed from experimental data published by Dlapa (2002) and thermodynamical data from Lindsay (2001). In general, organic solids (SOM, dashed lines) were identified to equilibrate lower aluminium concentrations at low pH and demonstrated less intensive, though highly variable, aluminium release along the pH gradient compared to the mineral Al hydroxides, such as gibbsite for instance (solid lines in Fig. 1). In addition, Wesselink et al. (1996) have shown that the SOM-binding mechanisms prevail in extremely acid and carbon-rich mineral soil horizons, while mineral solids take control in less acidic soils. We therefore expect that Al concentration does not demonstrate a simple, monotonous response to changing soil reaction, but shows discontinuities depending on which mechanisms take control over Al release. We hypothesize that these discontinuities may interpret a part of vegetational pattern along the soil acidity gradient since they represent transition points between different edaphic conditions. Foremost, the discontinuities are believed to correspond with ecotones between plant assemblages.

Species response and turnover modelling were used to analyse the vegetational transitions along the aluminium gradient in this study. Various aspects of these approaches have exhaustively been discussed by Austin et al. (1994), Huisman et al. (1993), Peppler-Lisbach and Kleyer (2009) and Walker et al. (2003). From the variety of methods, the Huisman-Olff-Fresco model (HOF, Huisman et al. 1993) was identified by Lawesson and Oksanen (2002) as the most superior tool to model species responses. This fits species data using sets of the following five increasingly complex and theoretically sound response models, in a forward selection: type I – a flat linear relationship, type II – a monotone relationship, type III – a plateau-like relationship, type IV – a symmetric Gaussian relationship and type V – a skewed unimodal relationship. In addition, the HOF models can also be used for evaluating species turnover (Peppler-Lisbach and Kleyer 2009, Peper et

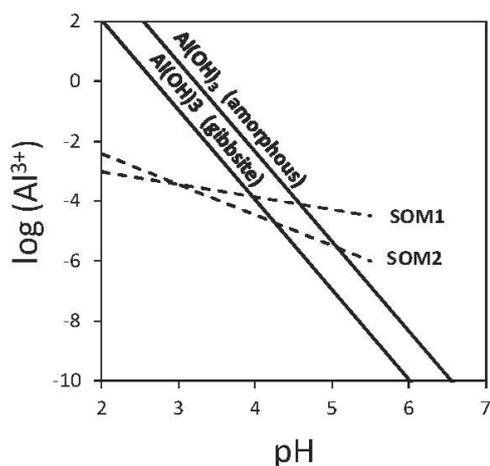


Figure 1. Solubility diagram showing logarithmic relationships between Al^{3+} and H^+ ions activities in soil solution as equilibrated by soil organic matter (SOM) and mineral Al-hydroxide (amorphous and crystalline gibbsite); Al-hydroxides were recalculated from Lindsay (2001), SOM lines show two different equilibriums from Dlapa (2002).

al. 2011), which quantifies an instantaneous rate of change in species composition at any point along the gradient. The species turnover can therefore identify ecotones where a distinct change in species composition occurs as a result of aluminium discontinuities. The use of species responses to rate the vegetational transitions has thoroughly been discussed and various measures have been suggested, including the absolute values of the derivatives of species response curves (Oksanen and Tonteri 1995), the arctangent-transformed absolute values of the curve derivatives (Peppler-Lisbach and Kleyer 2009), or the two values above relativised for species richness (Oksanen and Tonteri 1995). Walker et al. (2003) provided an alternative approach using ordination scores to identify the ecotone locations and widths.

In this study we analyse species responses and turnover along pH and aluminium gradients and we attempt to interpret the results with respect to recognised aluminium solubility patterns in soils. Plant assemblage and field measurement data collected from mesophilous and acidophilous submontane broad-leaved forests of Western Slovakia were used for this purpose. We answer the following questions: (1) How do individual plant species respond to the soil pH and aluminium gradient? (2) How does species compositional turnover change along the pH and aluminium gradient and how do these changes relate to aluminium solubility? (3) How does discontinuity in Al solubility correspond with the observed vegetational transitions?

Material and methods

Vegetation and soil data

The plant community data were collected in Western Slovakia at N 48°51' to 48°10' and E 17°01' to 18°12'. The dataset includes 142 phytocoenological relevés from submontane forests which belong to the following four alliances: *Carpinion betuli* Issler 1931 (88 relevés), *Quercion petraeae* Zólyomi et Jakucs in Jakucs 1960 (17 relevés), *Genisto germanicae-Quercion* Neuhäusl et Neuhäuslová-Novotná 1967 (30 relevés), and *Pino-Quercion* Medwecka-Kornaš in Medwecka-Kornaš et al. 1959 (7 relevés). All relevés were sampled on siliceous bedrock, mostly of weathered granite, phyllite, gneiss, quartzite, and decalcified loess and eolian sands in few cases. All forest stands were sampled in 400 m² quadrates using the standard field methods of the Zürich-Montpellier geobotanical school and the original Braun-Blanquet (1964) scale.

Topsoil mineral A horizons were sampled in the corners of a 5 m × 5 m square in the centre of each relevé quadrate. Samples from the four corners were mixed together, providing one average sample. These samples were air-dried, homogenized and sieved through a 2 mm mesh. The aluminium was studied after the soils were equilibrated under a constant 1 hour shaking with 0.01 M CaCl_2 solution (soil:solution = 1:5). Soil acidity $\text{pH}(\text{CaCl}_2)$ was measured by WTW pH-meter immediately after shaking. The supernatants were analysed for total extractable aluminium (Al_{ex}) using the alumi-

Table 1. Descriptive statistics of the soil data. Number of soil samples is 142.

	Transf.	Mean	Median	Min.	Max.	s.d.	Skew.	Kurt.	W	P
pH(CaCl ₂)	no	4.06	4.04	3.23	5.03	0.34	0.026	-0.723	0.985	0.143
pH(H ₂ O)	no	4.85	4.84	3.98	5.92	0.40	0.195	-0.271	0.989	0.398
Al _{ex} (mg kg ⁻¹)	no	75.90	43.91	0.60	457.45	82.71	1.718	3.689	0.808	< 0.001
	log	1.58	1.64	-0.22	2.66	0.57	-0.278	-0.902	0.965	0.002
C _{org} (%)	no	2.84	2.68	0.76	6.24	0.93	1.059	1.876	0.938	< 0.001

s.d. – standard deviation, Skew. – skewness, Kurt. – kurtosis, W – Shapiro-Wilk's W test, P – probability.

non method according to Pauwels et al. (1992). Soil reaction in water suspension (pH(H₂O), soil:water = 1:2.5) was measured by the WTW pH-meter, and organic carbon (C_{org}) by the Walkley-Black method (USDA-NRCS-NSSC 1996).

The analyzed soils mainly comprised: Leptic, Haplic and Stagnic Cambisols, Haplic Stagnosols (Albic, Dystric), Albic Luvisols (Epidystric) and Brunic Arenosols (Dystric) – nomenclature by FAO-ISRIC-ISSS (2006). The studied soils were moderately to strongly acidic with pH(H₂O) varying between 3.9 and 5.9 and pH(CaCl₂) between 3.2 and 5. The pH values measured in CaCl₂ extracts were in the order of 0.1 to 1.7 unit lower (0.8 on average) than those measured in water, with the Pearson's correlation $r = 0.74$ ($P < 0.001$) between the two values. Both pH data were normally distributed as indicated by the Shapiro-Wilk's W test in Table 1. Soil organic carbon (C_{org}) varied between 0.8 and 6.2%, which is a common range for the studied soil and vegetation types in Western Slovakia. The CaCl₂-extractable Al contents (Al_{ex}) ranged between 1 and 457 mg kg⁻¹. As expected, the Al_{ex} values clearly demonstrate a left-centred distribution which shifted close to normal when values were logarithmically transformed. Therefore, the logarithms of Al_{ex} were further utilized as Al gradient in this study.

Data analysis

The Canonical Correspondence Analysis (CCA) and the Monte Carlo permutation test of the species presence/absence data (excluding seedlings) were calculated in CANOCO software (ter Braak and Šmilauer 2002) to test whether the plant community data corresponded significantly with the measured aluminium. Species response curves were calculated from the presence/absence community data (understorey species) and the log-transformed Al_{ex} measurements using the HOF model (Huisman et al. 1993). The HOF curves were expressed as probabilities of species occurrence (P_s) along the Al gradient. Only species with a minimum relevé frequency of 10 were included. The HOF model was run on R software (R development team 2013) using the *gravy* package (Oksanen and Minchin 2002a,b). The best model was selected according to Bayesian Information Criterion at $P=0.05$. In addition to model curve parameters, we also estimated species "optima" (X_s) and "tolerances" (T_s) at the Al gradient. The X_s value, which is assumed to be a local expectancy value rather than a true species optimum, was calculated as the value at the Al gradient where the area under the HOF curve attained half its maximum (Balkovič et al. 2010). The T_s value was calculated

as the Al interval width, where the area under the curve contains 80% probability (Gégout and Pierrat 1998).

The compositional turnover was calculated from the species curves of type II to V (87 species in total) as suggested by Pepler-Lisbach and Kleyer (2009). Species with flat response (type I) were considered indifferent and were omitted from the analyses. The cumulative turnover rates (TR_{tot}) were calculated using the arctangent transformation given by:

$$TR_{tot} = \sum_{s=1}^m \arctan\left(\frac{P'_s}{|P_s|}\right) \quad (1)$$

where P'_s is the first derivative of the response function of species "s" at a given log-Al, and "m" is the total number of modelled species. The arctangent transformation was used to avoid single or few species with very steep slopes dominating the turnover rates (Pepler-Lisbach and Kleyer 2009). The TR_{tot} rates were also calculated separately for positive and negative turnovers. A positive turnover rate is caused by species with an ascending response curve at a given log-Al, whereas negative turnover rate is caused by species with decreasing response. Furthermore, the TR_{tot} curve was used to identify ecotones. The midpoint of an ecotone was identified as the position of maximum turnover rate along the gradient (Walker et al. 2003). In addition, species richness (P_{tot}) was calculated by the summation of all P_s of II to V species over the Al gradient (Pepler-Lisbach and Kleyer 2009).

According to the community-unit concept (Whittaker 1975), species with similar responses to major environmental gradients tend occur under similar conditions. We therefore performed a bioindication experiment to test whether the soil Al gradient significantly corresponds with species assemblages at particular sites. In this bioindication, the HOF curves of species present in a phytocoenological relevé were added together to give the cumulative probability curve (P_r) for the relevé as given below:

$$P_r = \sum_{s=1}^k P_s \quad (2)$$

where P_s is the HOF probability curve of species "s", and "k" is the number of species in relevé "r". These cumulative curves were then adjusted by subtracting their baselines. Subsequently, relevé optima (X_r) were calculated as values at the Al gradient where the area under the P_r curves attains half its maximum. A sample calculation result is demonstrated in Fig. 7a. The piecewise regression model was used

to test whether the X_i values corresponded significantly with the measured Al values. For all piecewise regressions in this study, the breakpoints were estimated iteratively to minimize overall sum of squares as described by Ryan and Porth (2007). The goodness of fit was assessed using the coefficient of determination (R^2). A “leave-one-out” approach was used for bioindication. This is an intensive computing method, involving a loop function over the data deleting a relevé from the HOF analysis when the bioindication is calculated for that particular relevé.

Results

Soil aluminium gradient

The Al_{ex} values increased exponentially with decreasing pH(CaCl₂) as an equilibrium was reached: an increase from Al_{ex} of 1 mg kg⁻¹ at pH 5.0 to more than 457 mg kg⁻¹ at pH 3.2 was observed. This shows that aluminium is released from the reactive Al solids as soil acidity increases. The logarithms of Al_{ex} demonstrate a tight linear relationship with pH values (Fig. 2a), highlighting a discontinuity in the trend slope at the acidic end of the pH range. This relationship can be satisfactorily described by a piecewise linear regression with a breakpoint calculated at pH 3.8, which approximately equals an Al_{ex} of 130 mg kg⁻¹ ($R^2 = 0.99$, $P < 0.001$). It is expected from the regression that two different mechanisms control Al solubility in the studied topsoils: one at pH above 3.8, and the second below this pH value. At pH > 3.8, the regression slope equals 1.8 (in absolute value), indicating an almost two-fold increase in log- Al_{ex} with a pH decrease of one unit. However, the Al_{ex} values become under-saturated with respect to the first mechanism when pH falls below 3.8. The slope of the

regression line decreases from 1.8 to 1.0, thus signifying only a one-fold increase in log- Al_{ex} with a unitary pH decrease. The same discontinuity is also visible in Fig. 2b where Al_{ex} became increasingly undersaturated at pH less than 3.8 with respect to the equilibrium at higher pH (vertical line), as indicated by arrow. The discontinuity at pH 3.8, which equals a pH of about 4.2 when measured in water (based on our measurements, but data not shown here), coincides with the threshold for the aluminium buffer range reported by Ulrich (1983).

The soil organic carbon (C_{org}) does not demonstrate any significant correlation to the logarithms of Al_{ex} (Pearson's $r = 0.10$, $P > 0.05$) and it alone cannot directly be used to explain the solubility pattern above. As indicated by numerous studies (e.g., Berggren and Mulder 1995, Wesselink et al. 1996, Dlapa 2002), Al-saturation of the organic matter would be needed to quantify the real stoichiometry of how SOM contributes to the pattern. Nevertheless, it is obvious that horizons with pH below 3.8 contain more organic carbon than horizons where pH > 3.8 (Fig. 2c; t-test, $t = 2.55$, $P = 0.012$). We may therefore hypothesize that more organic carbon and, in consequence, lower Al-saturation resulted in more conservative solubility at pH below 3.8.

Species responses to soil aluminium gradient

The CCA analysis (Fig. 3) and the Monte Carlo permutation test indicate that the plant community data relate significantly to the log(Al_{ex}) contents: $F = 3.76$, $P < 0.001$, while HOF models provide the possibility of analyzing these relationships in more detail. In total, 87 of the 108 species (81%) demonstrate a significant response to the Al gradient, whereas the remaining 21 species show no response at all

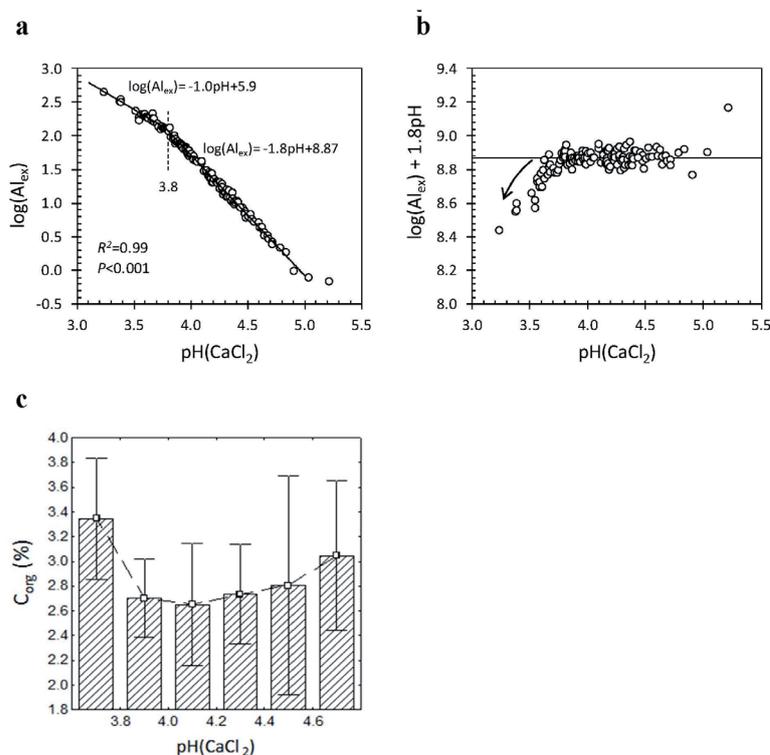


Figure 2. Soil aluminium, pH and organic carbon gradient; **a**) piecewise linear regression between log(Al_{ex}) and pH(CaCl₂) with breakpoint at pH 3.8, **b**) scatter of the measured Al_{ex} values along the regression line calculated for pH > 3.8, **c**) soil organic carbon (C_{org}) distribution along the soil pH gradient (column: mean, whiskers: 95% confidence interval).

(type I model), and these were therefore considered indifferent in this study.

The most common responses are type II or III models, covering monotonically increasing or decreasing curves, which were fitted to 45 species. The species of type II and III respond positively or negatively, but their “true” niches may remain hidden beyond the sampled Al gradient. In summary, 21 of the 45 species respond positively to high aluminium contents (increasing models). Many of these are true acidophilous plants, such as *Genista pilosa*, *Avenella flexuosa* or *Luzula luzuloides*, where X_s values range between 21 and 255 mg Al_{ex} kg⁻¹ and tolerances (T_s) between 330 and 455 mg Al_{ex} kg⁻¹. On the other hand, 24 of the 45 species show a monoton-

ically decreasing response with their optima on Al-poor soils. The X_s values are ranging between 6 and 12 mg Al_{ex} kg⁻¹ and tolerances between 8 and 71 mg Al_{ex} kg⁻¹ in this case. Species types IV and V, fitted with unimodal models, demonstrate a distinct optimum at the Al gradient. A symmetric Gaussian (type IV) response fitted 27 species and a skewed unimodal (type V) response fitted 15 species. The species of type IV model have optima at moderate aluminium contents, with the X_s and T_s values ranging between 10 and 89 mg Al_{ex} kg⁻¹ and 49 and 386 mg Al_{ex} kg⁻¹, respectively. Finally, type V species show an abrupt disappearance at high or low Al contents, with the X_s values here between 11 and 42 mg Al_{ex} kg⁻¹ and the tolerance T_s values between 57 and 451 mg Al_{ex}

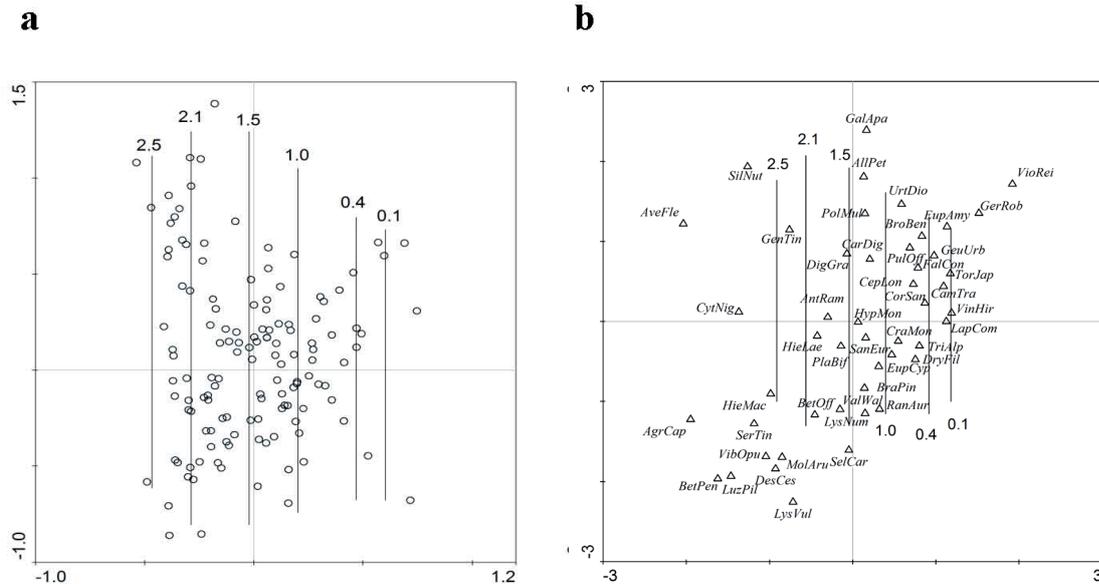


Figure 3. CCA ordination diagram with **a**) phytoecological relevés, and **b**) the most important species calculated of the species presence/absence data and the total extractable aluminium (log-transformed); axis 1 eigenvalue = 0.068, eigenvalue Y = 0.144, total eigenvalue = 1.758, canonical axis 1 (log Al_{ex}): F-ratio = 5.27, $P=0.001$ estimated by the Monte-Carlo permutation test; vertical lines demonstrate surface of log-transformed aluminium calculated using the Generalized Linear Model in CANOCO; for species labels refer to Appendix.

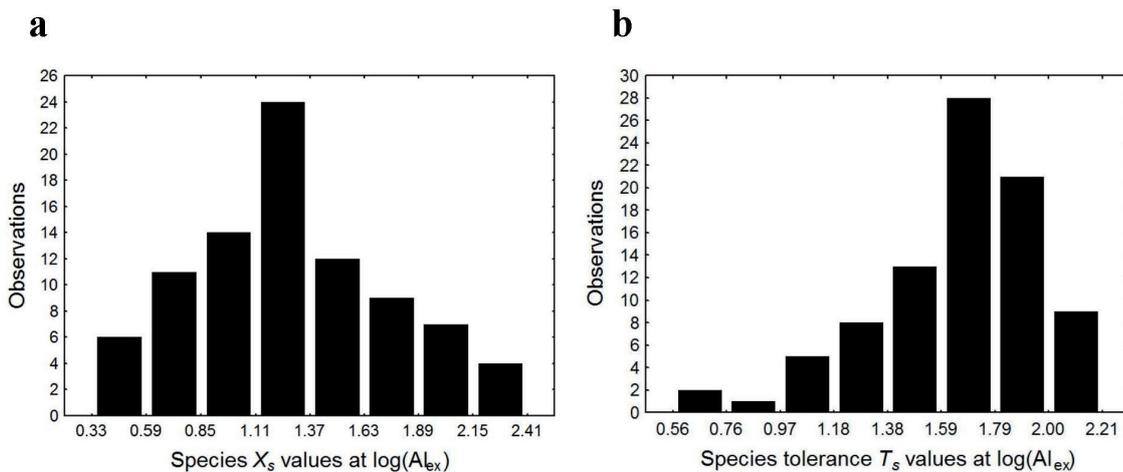


Figure 4. Frequency histogram of **a**) species optima (X_s , Shapiro-Wilk's $W = 0.980$, $P = 0.198$), and **b**) tolerances (T_s , $W = 0.947$, $P = 0.002$) along the logarithms of Al_{ex}; with the exclusion of indifferent species of type I model.

kg⁻¹. The X_s values of the entire species pool with indifferent species excluded from the analysis demonstrate a normal distribution (Fig. 4a) with a mode of approximately 17 mg Al_{ex} kg⁻¹. The species tolerances provide a slightly right-centred histogram, with a mode value of approximately 50 mg Al_{ex} kg⁻¹ (Fig. 4b). The tolerances, however, vary strongly among HOF-model types, as depicted in the entire list of HOF model results in Appendix.

Compositional turnover rate along the soil aluminium gradient

Compositional turnover rates (TR_{tot}) demonstrate apparent peaks along the studied Al gradient (Fig. 5a, solid line). This curve displays a maximum at approximately 140 mg Al_{ex} kg⁻¹, which corresponds to pH of 3.8, and another peak around 3 mg Al_{ex} kg⁻¹ (ca. pH 4.8). The contribution of HOF models II-V to the total species turnover rates is demonstrated in Fig. 5b. The peak at 140 mg Al_{ex} kg⁻¹ is mainly due to turnover rates of species with decreasing responses at this point (i.e. negative turnover rate). This is particularly noted in types IV and V unimodal response models. A positive turnover (i.e. increasing response) of acidophilous species with type II models contributes less to this 140 mg Al_{ex} kg⁻¹ peak. On the other hand, the peak at 3 mg Al_{ex} kg⁻¹ is largely created by the high positive turnover rates of types IV and V species which exhibit an ascending unimodal response at this point of the underlying Al gradient. The peaks determine midpoints of ecotones at the aluminium gradient (arrows in Fig. 5a). The maximum species diversity (P_{tot} , dot-dashed line in Fig. 5a) occurs in the middle of the Al gradient at 18 mg Al_{ex} kg⁻¹ (ca. pH 4.2). As reported by Peppler-Lisbach and Kleyer (2009), the maximum diversity occurs at the gradient value where positive and negative turnover rates cross (Fig. 5a, dashed lines).

The species turnover peak at 140 mg Al_{ex} kg⁻¹ (ecotone 1) equates with the discontinuity in Al solubility observed at pH 3.8 (~4.2 measured in water). This is approximately the point where Al solubility takes control over proton buffer-

ing in acid soils (Ulrich 1983), which is connected with an increase in toxic aqueous Al and only a minimum alleviating effect of exchangeable base cations. These specific soil conditions result in a rapid retreat of many common species (e.g., *Veronica chamaedrys*, *Anthericum ramosum*, *Dactylis polygama* or *Mycelis muralis*, see negative turnover rates in Appendix). The emergence of acidophilous species, particularly those with monotonically increasing type II curves, contributes to a lesser extent to the overall species turnover at this point in the gradient. The species turnover peak at Al of 3 mg kg⁻¹ (ecotone 2) corresponds to a pH of ca. 4.8 (pH 5.5 measured in water) where Al occurs in an undissolved form. It is obvious that aluminium concentrations are not high enough to limit plant growth here and, therefore, aluminium is not directly responsible for this ecotone. This peak is related to the rapid appearance of many species, both acidophilous and acid-sensitive (e.g., *Campanula persicifolia*, *Astragalus glycyphyllos*, *Brachypodium sylvaticum*, *Luzula luzuloides* or *Melampyrum pratense*; see positive turnover rates in Appendix). The mean Ellenberg indicator values (EIV) for nitrogen plotted against exchangeable aluminium in Fig. 6 suggest that increased nutrient availability at log-Al_{ex} less than 0.4 may be responsible for this peak. A relationship between the above ecotone centres and inter-relevé and inter-species variability is presented using the CCA results in Fig. 3 (notice line 2.1 and 0.4 for ecotone 1 and 2, respectively). It is worth noting that the highest species diversity (P_{tot}) was observed at moderate values of the Al gradient, where acidophilous species and species less tolerant to soil acidity both occur at low turnovers.

Species assemblage responses to soil aluminium gradient

We expect aluminium to be a factor affecting species composition at a particular site. Hence the species composition should correspond with the soil Al content at each site. This expectation was tested through an iterative bioindication where the cumulative probability curve (P_r) and its X_r value

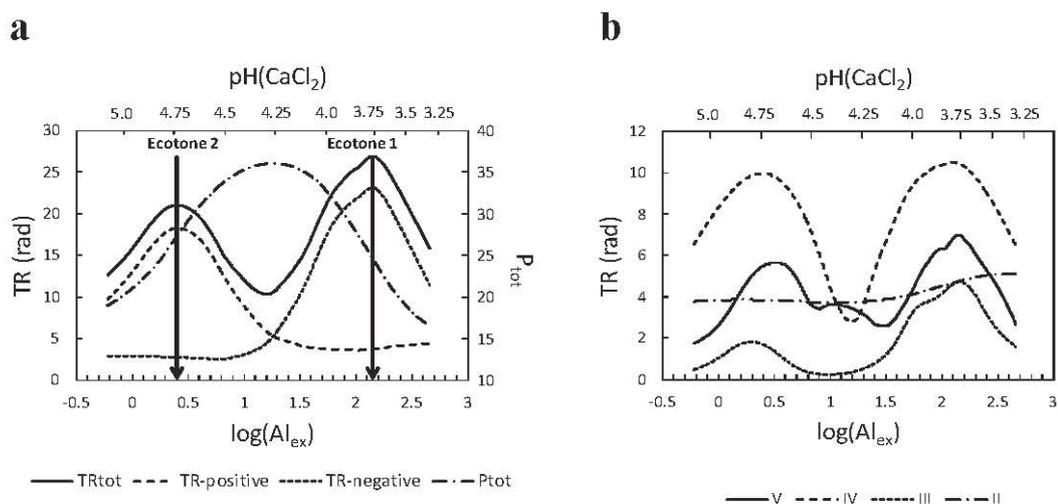


Figure 5. Species turnover rates; **a)** cumulative species turnover rate (TR) in radians and species richness (P_{tot}); **b)** cumulative turnover rate for species with the HOF response types II to V; the arctangent transformation was used for Y-axis to avoid species with very steep slopes.

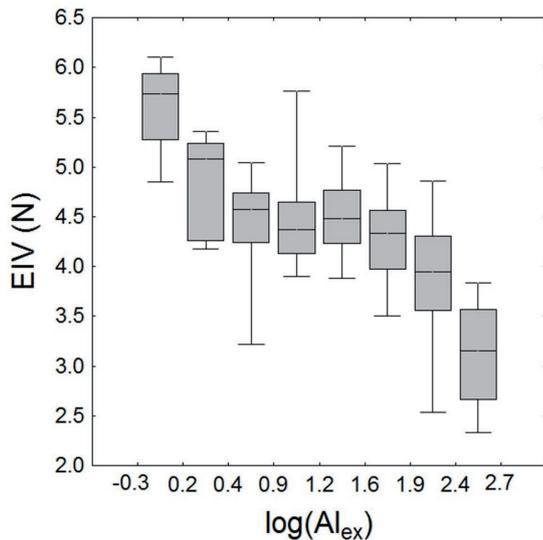


Figure 6. Mean Ellenberg's indicator values (EIV) for nitrogen plotted against log-transformed extractable aluminium (Al_{ex}); line: median, boxes: 25th and 75th percentile, whiskers: 5th and 95th percentile.

were calculated for each relevé from HOF models of plants present in the relevé understorey (see Fig. 7a for a sample analysis). The bioindication resulted in X_r values which demonstrate a curvilinear relationship with $\log(Al_{ex})$. The piecewise linear regression in Fig. 7b accounted for 48% of the variance ($P < 0.001$), showing the inflection point at ca. 70 mg Al_{ex} kg^{-1} (equal to pH 3.9). This inflexion point is close to the pH 3.8 discontinuity observed at the above described Al solubility gradient. The regression-segment slope is steeper for sites with $Al_{ex} > 70$ mg kg^{-1} , indicating a stronger relationship at the acidic end of the gradient. It is evident that the X_r values have a narrower range of 7 to 60 mg Al_{ex} kg^{-1} compared to the measured 1 to 457 mg kg^{-1} Al_{ex} range. This is due to the well recognized shrinking effect in the bioindication procedure which shifts the estimations toward the overall average (Balkovič et al. 2010, Wamelink et al. 2011). This could be removed by a deshinking procedure (e.g., ter Braak and van Dam 1989, Birks et al. 1990), however, it is not necessary in this case since we focus on the trend, rather than on the calibration alone.

Discussion

Aluminium as a growth limiting factor has been recognized for many years (Miyake 1916). At high concentrations, Al ions reduce nutrient availability in soils, harm plant cells and inhibit plant growth (Poschenrieder et al. 2008). High Al resistance is therefore an important trait of plant species occupying acidic soils. Although aluminium impacts on wild plants received considerable less attention than crop plants, there are numerous studies identifying aluminium as a major factor filtering species composition in favour of Al-resistant plants (Abedi et al. 2013 and literature cited therein). In this study we show that besides Al concentration, aluminium

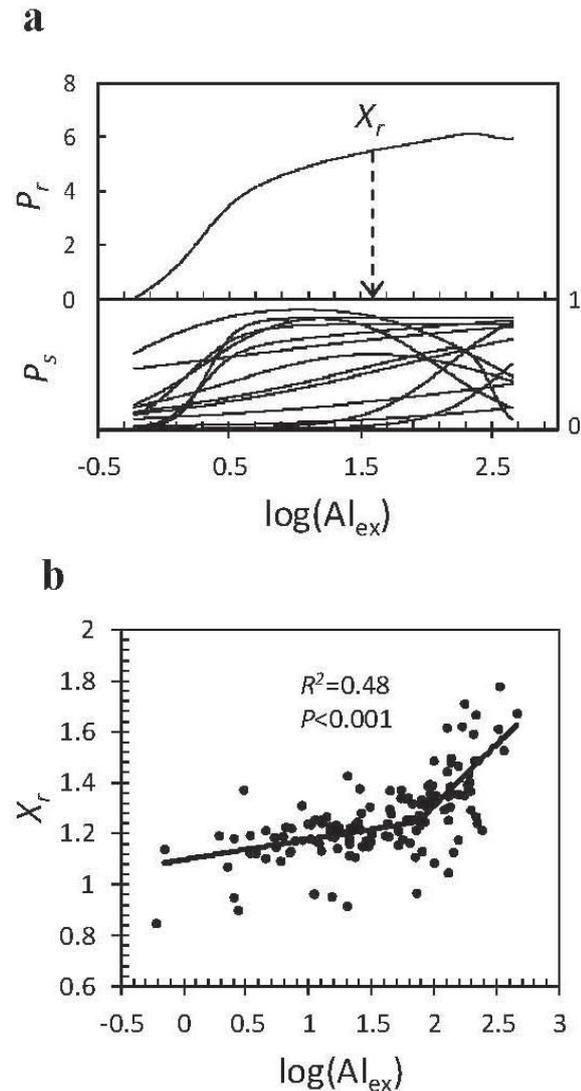


Figure 7. Bioindication calculation; a) HOF models of species along the soil Al gradient for a sample relevé (Nr. 102) and adjusted cumulative probability curve of the relevé; the arrow points at the Al_{ex} content where the area under the adjusted cumulative probability curve reaches half its maximum (X_r); P_s – probabilities of species occurrence from the HOF model, P_r – cumulative probability curve for the sample relevé; b) X_r values of relevés ($N = 142$) plotted against logarithms of $CaCl_2$ -extractable total Al and the piecewise linear regression: $X_r = 0.08\log(Al_{ex}) + 1.10$ for $\log(Al_{ex}) \leq 1.87$, and $X_r = 0.48\log(Al_{ex}) + 0.35$ for $\log(Al_{ex}) > 1.87$, $R^2 = 0.48$, $P < 0.001$.

solubility can interpret some of the vegetational transitions. We know from literature that Al concentration in soils corresponds with complex mechanisms responsible for Al solubility, and that these mechanisms vary under different soil environments (Berggren and Mulder 1995, Wesselink et al. 1996, Dlapa 2002). Consequently, Al concentrations demonstrate discontinuities as different mechanisms take control over its solubility across different soils, thus forcing distinct edaphic conditions for vegetation. In this study we identified that a rapid retreat of many species and, in consequence, high

compositional turnover (ecotone) in mesophilous and acidophilous submontane forests corresponded with a discontinuity in Al solubility observed at $140 \text{ mg Al}_{\text{ex}} \text{ kg}^{-1}$ (pH 3.8). Here, the extractable Al became increasingly under-saturated with respect to the equilibrium attained at higher pH (Fig. 2b). Aluminium concentration as high as 140 mg kg^{-1} is expected to have adverse effects on many species. Although there is only little information on critical concentrations for wild plants (Abedi et al. 2013), 5 mg kg^{-1} of Al extracted by CaCl_2 is commonly used as an indicator of aluminium stress for Al-tolerant crop plants (e.g., Edmeades et al. 1983). Abedi et al. (2013) showed that such Al concentration ($\sim 1.1 \text{ mM}$) significantly reduced the root growth of numerous grassland species. Due to extreme complexity of aluminium chemistry in soils (Lindsay 2001, Poschenrieder et al. 2008), we did not investigate the soil mechanisms responsible for this discontinuity. Nevertheless, we hypothesize that aluminium was controlled by less saturated SOM at $\text{pH} < 3.8$ as extensively documented in the literature (e.g., Mulder and Stein 1994, Berggren and Mulder 1995, Wesselink et al. 1996, Kanianska and Dlapa 2000). Here-in, we focused solely on the solubility pattern alone and its relationships to vegetational transitions. Future work should expand on the underlying soil mechanisms and closer understanding of soil-vegetational connections.

Some methodological considerations on the analytical Al values used in this study need to be discussed. Quite a few soil Al indices have been used in plant and vegetational studies, including monomeric Al, exchangeable Al, Al/Ca ratio and Al toxicity index (e.g., Garuer and Horst 1991, Boudot et al. 1994, Cronan and Grigal 1995, Pepler-Lisbach and Kleyer 2009). Although phytotoxic Al^{3+} would be more appropriate for our study than the others, it implies demanding and costly analytical procedures and it is therefore suboptimal here. The total Al extracted from soil samples by 0.01 M CaCl_2 is commonly used instead of Al^{3+} since it closely correlates with phytotoxic Al (Boudot et al. 1994). Due to its simplicity, this method was recommended as a routine extraction procedure for diagnosing Al toxicity in soils by Conyers et al. (1991) and was therefore used in this study. However, the total extractable Al does not sufficiently comply with the real soil equilibria and a detailed Al speciation would be needed to conclude more on the solubility mechanisms (Lindsay 2001).

Most of uncertainty in our vegetation analysis originates from the HOF model use. In general, HOF is presumed to perform optimally here because it enables us to address both symmetric and skewed responses. With skewed responses, HOF provides the possibility of reflecting an exponentially-growing limitation toward the extreme part of the Al gradient (cf. Austin 1990). Furthermore, we do not expect any bimodality in species responses biasing the modelled HOF curves, because the gradient in question is quite short. However, the following limitations toward the HOF model should be considered: (i) gradient truncation and (ii) representativeness of the dataset. Firstly, the completeness of the Al gradient may be questioned since extremely acid podzols were not included. Therefore, it could be expected that some portion of a true species response remains hidden beyond the limits of

the sampled Al gradient, resulting in HOF model truncation. However, this limitation can hardly be avoided since podzols in Slovakia occur in distinctly different conditions, as observed in high mountains, or they contain only secondary vegetation in submontane regions. Both these situations imply a distinct species pool and composition. Secondly, the Al gradient is not fully representative, because a non-stratified sampling was used due to data availability restrictions, and autocorrelation was not considered in this study. The data set comprises only few samples with lower Al concentration and, with such a low number, the HOF model may be influenced at the low end of the Al gradient. The measurements of low Al concentrations were also burdened by higher analytical error. In addition to the above-mentioned potential pitfalls emanating from HOF, the species turnover rates can deviate as a result of different species richness along the gradient (Pepler-Lisbach and Kleyer 2009). We applied the adjustment for species richness as suggested by the above authors, and found no significant difference between the two turnover rates (not shown here). Pepler-Lisbach and Kleyer (2009) reported consistent findings and similar total turnover rates with a maximum at pH 3.7 from semi-natural deciduous forests in NW Germany. The authors explain the peak at pH 3.7 with an exponential increase in exchangeable Al. Here we show that there are discontinuities in the exponential Al increase which corresponded with the observed species turnover rates.

A bioindication simulation was used to reveal how plant assemblages correspond with the measured Al at particular sites by analysis of the response curves of the species present. It is evident from Fig. 7b that the bioindication algorithm does not operate with the same effectiveness over the entire gradient. A rapid increase in aluminium concentration at the acidic end of the gradient influences the relevé composition to a higher extent, since it quickly promotes acidophilous species and suppresses species less tolerant to soil acidity. This simulation also provides validation of the HOF analysis, wherein the presented bioindication accounted for 48% of the total variance. Due to data availability, we used the “leave-one-out” approach rather than an independent dataset to circumvent tautological data use in this study.

The following additional methodology considerations are important. Firstly, we carefully distinguished between organic O and mineral A horizons when sampling the soils. All our conclusions apply to mineral A horizons. A substantially lower increase, or even a decrease, in extractable Al with increasing soil acidity could be expected when both A and O horizons were mixed, or when O horizons were used alone. Secondly, a tight interaction between soil acidity, aluminium and moisture can be expected in our semi-hydromorphic and hydromorphic soils (e.g., stagnosols). Pakeman et al. (2008) showed that a combination of soil reaction and moisture can affect species responses. And finally, this ‘relevé’ versus ‘mixed-soil’ concept does not reflect the intra-relevé variability in soil aluminium and plant population data, which may vary significantly throughout the relevé area.

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Supplementary material

Appendix. Complete list of HOF-based species indicators (X_s , T_s) at the 0.01 M CaCl₂-extractable Al gradient, and turnover rates (TR) at Al_{ex} of 3 and 140 mg kg⁻¹. The file may be downloaded from the web site of the publisher at www.akademai.com.