Central European Journal of **Biology**

Effect of experimental top soil removal on vegetation of Pannonian salt steppes

Research Article

Zuzana Melečková^{1,*}, Dobromil Galvánek¹, Daniel Dítě¹, Pavol Eliáš Jr.²

¹Institute of Botany, Slovak Academy of Sciences, 845 23 Bratislava, Slovakia

²Department of Botany, Slovak University of Agriculture, 949 76 Nitra. Slovakia

Received 17 March 2013; Accepted 24 June 2013

Abstract: Inland saline habitats of the Pannonian Lowland exhibit a specific variety of grasslands determined by a soil salinity gradient. Changes in the hydrological regime and absence of management have resulted in heavy degradation of the vegetation. The impact of topsoil removal on salt steppes was tested by a 3-year small-scale manipulated experiment in SW Slovakia (Kamenínske Slanisko Nature Reserve). Topsoil was removed in three contrasting types of vegetation with different soil salinities, *i.e.* in different stages of habitat degradation. Data were analysed by multi-way ANOVA and by multivariate methods. Species richness decreased and the proportion of halophytes increased significantly in the two types with the highest soil salinity; however, the total number of halophytes was not influenced by soil removal. The treatment caused inhibition of secondary succession on the plots with the highest salinity. The effect of the soil removal was only short-term in the vegetation with moderate salinity and on heavily degraded and desalinized types it even stimulated further recruitment of ruderal species. Topsoil removal has only limited potential for the restoration of Pannonian salt steppes. It should be applied only in slightly degraded vegetation, where salt accumulation is still present and target species propagules are available.

Keywords: Inland halophytic vegetation • Degradation • Ecological restoration • Slovakia

© Versita Sp. z o.o.

1. Introduction

Grasslands of high nature value in Europe are extremely threatened by recent changes in land use as a consequence of changes in the agricultural sector [1]. An active ecological approach of restoration is required, based on scientific knowledge and taking into account economic and social constraints [2,3]. Among the grasslands requiring restoration are those of saline habitats. They belong to the most ancient lowland vegetation types of Central Europe, dating back to the middle Würm period (33,000 yr BP) [4]. The largest area of the analogous habitat type in Eurasia is known to be within the steppe zone of the Southern Ural region [5].

Pannonian saline habitats reach their northern distribution limit in Slovakia [6,7]. The vegetation in the periphery of its natural range is particularly threatened. For instance, about 8,300 ha of Pannonian

saline vegetation existed in the south-western part of Slovakia up to the middle of the 20th century [8], but after reclamation and land use changes their total area has been reduced to around 500 ha and is limited to 20 scattered locations [9].

The patrimonial value of these habitats has been recognized in Western Europe [10,11] and also in Central Europe, where the local and regional studies [12-18] document devastation of the Pannonian saline vegetation. The specific problems of the habitat degradation and fragmentation arise from drainage, river flow regulation and eutrophication, *i.e.* decrease in salinity and increase in nutrients that cause expansion of weedy species and decline of halophytes. This progressive succession has a negative impact on the survival of halophytic plant communities. Effective maintenance can be achieved by supporting the opposite, retrogressive succession [19].



Several large-scale restoration projects have been implemented on salt marshes of Europe so far, including application of grazing by different herbivores [20,21], de-embankment or natural periodic flooding [22,23] and sowing of low-diversity seed mixtures and hay transfer [24,25]. They usually lead to a successful establishment of targeted halophytic species. Excavation of the soil surface [26] may also be a feasible restoration method for saline habitats.

Top soil removal is a form of active management which favours retrogressive succession. It is primarily intended to decrease the available nutrients and weed propagules in the upper soil layers, permitting successful establishment of the target species [27]. Top soil removal stimulates seedling emergence and it may reduce competition for light [28]. A lower level of competition in the community is crucial, since halophytes have less competitive ability as compared to glycophytes [29] and the pioneer vegetation of small alkali depressions is strongly dependent on areas with bare soil and extreme salinity [30]. However, responses of different species are extremely dependent upon species-specific traits [31].

Top soil removal has been carried out in different non-forest habitat types in Western Europe where the reduction of nutrient levels is also necessary, such as fens [32], heathlands [33-35] and dry grasslands [36]. To date, however, studies on the effectiveness of top soil removal in saline habitats are lacking, despite the fact that it can be applied on a more local scale than other restoration methods (*e.g.* manipulations of hydrological regime). Such enormous intervention may affect large areas [23,37] and cannot be easily implemented in the intensively farmed landscapes of Central Europe.

Succession of halophytic vegetation is generally fast [38]. Our experiment was focused on the development of initial stages of the vegetation in response to soil removal. We investigated whether top soil removal may create suitable micro-sites for the establishment of low competition halophytic species in relation to soil salinity. The following questions were asked: 1) How does top soil removal affect the small-scale species richness of salt steppes? 2) What is the impact of top soil removal on species composition of saline grasslands with different levels of soil-salinity (different stages of degradation)? 3) Do halophytes profit more from top soil removal than glycophytes?

2. Experimental Procedures

2.1 Study area

The experiment was carried out in the Kamenínske Slanisko Nature Reserve in SW Slovakia, 12 km north of the town of Štúrovo in the alluvium of the Hron river (47°52'38"N, 18°38'44"E, 109 m a.s.l.). The mean annual temperature is about 10.3°C, averaging 20°C in July and -1.5°C in January. The mean annual rainfall is 550 mm [39], but in exceptional years, as in 2010 when extremely high rainfall was recorded in all of Central Europe, 967 mm was recorded in the closest weather station at Čata, approx. 8 km from the site (unpublished data of the Slovak Hydrometeorological Institute, Bratislava).

The reserve contains the last remnants of formerly widely distributed salt steppes and meadows of SW Slovakia which now survive on just 150.7 ha. It was recognized as the floristically richest saline habitat in Slovakia until the 1980s [40], with different patches of halophytic vegetation such as large salt flats covered with the most salt demanding plant community *Camphorosmetum annuae* and alkali shortgrass steppes with *Artemisio-Festucetum pseudovinae* creating smaller mosaics with wet salt meadows like *Scorzonero parviflorae-Juncetum gerardii*. The micro-topography was typically developed, which had an effect on the diversity of the vegetation mosaic, responding to even the slightest changes in the soil parameters [12,41].

However, from the 1950s changing agricultural practices have strongly influenced the hydrological regime in the reserve *i.e.* salt accumulation caused by highly-mineralized groundwater. In addition, regular grazing was excluded from the reserve in the 1970s; in recent times, only occasional low intensity sheep grazing has taken place. As a consequence, a drastic reduction in the area of the habitat and in the floristic composition has occurred [42-44].

2.2 Experimental design and data sampling

The present vegetation of the locality is a fragmented patchy mosaic. Consequently, it was decided to use a stratified random experimental design (random distribution within three vegetation types).

The design was strongly influenced by the limited expanse of the existing relictual halophytic communities, since the area of the target association *Camphorosmetum annuae* was less than 45 m² [43]. Therefore, we were able to place only relatively small experimental plots, because a larger plot area would have greatly increased the heterogeneity of the plots. In spite of this fact, there was no evidence of an edge effect in the plots (halophytes were distributed in all cells, not only in the central ones) and ruderal species were found not only in peripheral positions. Twelve 2x1 meter plots were established in three different vegetation types (four replicates per type) interspersed in the locality. The types corresponded on the salinity gradient with

the degradation stages of the halophytic vegetation. Their names are abbreviated on the basis of the most characteristic species of the vegetation (Table 1). *Artemisia santonicum*-type ('ARSA') denotes stands the optimal stage dominated by obligate halophytes, *Festuca pseudovina*-type ('FEPS') represents moderately degraded vegetation with mesic species and *Arrhenatherum elatius*-type ('AREL') includes plots occupied by generalists and ruderal species.

The top soil removal was performed on half of each plot (1x1 m) by manually removing vegetation cover with soil in 2008; the second half of the plot was not removed (controls). The depth of removal varied according the depth of the vegetation root zone, ranging from 5 cm for the stands of ARSA to 20 cm for stands of AREL. Vegetation recordings were carried out from 2008 (the baseline record before application of the treatment) until 2010 in mid-September. The presence or absence of vascular plants was recorded in a 0.5×0.5 m wooden frame divided by wires into 25 cells of 0.1×0.1 m. The position of the frame was fixed in the central part of each plot to insure consistent sampling.

Soil samples were taken once (in 2009) during the experiment from each of the 12 plots from a depth of 15 cm, each with a volume of 300 g and the following properties were analyzed: soil reaction (pH/CaCl₂); exchangeable Na⁺; amount of total salts (NaCl and Na₂SO₄) and total nitrogen (N_{TOT}). Exchangeable Na⁺ was evaluated in an acidified soil extract of barium chloride buffer (pH 8.1) and triethanolamine by flame atomic absorption spectrometry (AAS-F) at a wavelength of the individual cation [45]. Salt content was determined by water extraction followed by evaporation of the water extract and drying the residue at a temperature of 105°C. Soil reaction was measured potentiometrically in a saturation extract obtained from saturated soil paste

according to international standards [46]. Total nitrogen was determined by a patented method of dynamic combustion in an oxygen atmosphere: the soil sample is dispensed into the combustion tube. The result of combustion is a mixture of gases, nitrogen oxides, CO₂, H₂O, SO₂ and the oxygen excess. The mixture of gases is passed through the reduction catalyst, which eliminates redundant oxygen and reduces the nitrogen oxides to nitrogen. This mixture is split on thin-layer chromatography column and detected using a thermal conductivity detector. The purpose of the soil sampling was to describe soil chemistry conditions in all three studied vegetation types, especially in relation to salt, exchangeable sodium and nutrient content (Table 1). Soil samples were not taken in subsequent years in order to minimize the effects of soil disturbance on vegetation development of the experimental plots.

2.3 Data analysis

Data on species richness, number of halophytes (obligatory halophytes according to [6]) and percentage of halophytes from all recorded species were subjected to multi way hierarchical analysis of variance (ANOVA) using the average per plot of 10x10 cm. The following transformations were used: $y'=\log y$ (species richness), $y'=\log(10^*y+1)$ (number of halophytes), and $y'=\arcsin\sqrt{y}$ (ratio of the number of halophytes to species richness). Transformations were applied to remove the heterogeneity of variances and transform data into a multiplicative scale which is of higher ecological interest in our analysis. In addition, the problems with zero values in our data sets were settled, as were issues concerning percentage values close to 0 or 1 [47,48].

Three between-subject (whole plot) factors were tested, two with fixed effect (REMOVAL, VEGTYPE) and one random factor (PLOT). Factor REMOVAL

Acronym of the characteristic species in the vegetation type	ARSA	FEPS	AREL	
Syntaxonomical classification of the vegetation	Puccinellion limosae	Festucion pseudovinae	Successional stage of Arrhenatherion elatioris	
Characteristic and dominant species	Artemisia santonicum subsp. patens Camphorosma annua Puccinellia distans agg. Plantago maritima	Plantago maritima Limonium gmelinii Achillea millefolium Galium verum	Arrhenaterum elatius Cirsium arvense Dipsacus fullonum Jacea pannonica	
Total salts in the soil (NaCl and Na_2SO_4 . in %)	0.5173 (SD=0.0314)	0.313 (SD=0.093)	0.1095 (SD=0.0193)	
Soil reaction (pH/CaCl ₂)	9.2925 (SD=0.1489)	8.2825 (SD=0.3043)	7.805 (SD=0.1248)	
Exch. Na+ (cmol+/kg)	12.16 (SD=2.3698)	3.096 (SD=2.1061)	0.2203 (SD=0.1192)	
Nutrients N _{TOT} (%)	0.0745 (SD=0.007)	0.1323 (SD=0.035)	0.2175 (SD=0.0928)	



indicates application of the experimental treatment, factor VEGTYPE describes three different types of vegetation (*Artemisia santonicum*-type, *Festuca pseudovina*-type and *Arrhenatherum elatius*-type) and the random factor PLOT identifies plots. This factor is nested in factor VEGTYPE. One repeated measurement (within-subject factor) TIME was tested, describing the temporal variability of our data (3 subsequent years, one recording per year). Calculations were performed using the software Statistica 5.5.

Data on species composition from all experimental plots were analyzed by detrended correspondence analysis (DCA) [48] to discover the main environmental gradients determining the vegetation pattern on the experimental plots. To analyze the trajectories of vegetation development with different salt content and treatment applied, the three different types of plots were used as supplementary environmental variables. The results of the first-step DCA detected a strong unimodal structure of the vegetation data, with a gradient length of 5.413 SD.

Because the temporal development on the plots was of our highest interest and we used before-after control impact (BACI) design [49], we examined the interaction of factors REMOVAL*TIME to test the effect of top soil removal on species composition. Factor REMOVAL was considered as a dummy variable with values 0 and 1, factor TIME was a consecutive TIME from the beginning of the experiment (values 0, 1, 2).

Three separate direct gradient analyses were performed, one for each studied vegetation type, using only the dataset from a particular vegetation type. Due to a shorter gradient (2.1 SD units), a linear response of species to treatment was expected. Therefore, redundancy analysis (RDA) was the most appropriate method for these data [48].

The frequency data concerning the occurrence of species were standardized by norm because the relative

proportion of particular species was of the highest interest in our case. Monte Carlo permutation tests (499 permutations) were performed (permutations respecting split-plot design and repeated measurements). Factor TIME was used as a covariate to filter any possible temporal trend of vegetation.

The gradient analyses were calculated using the software package CANOCO for Windows 4.5 [50]. The nomenclature of taxa follows Marhold and Hindák [51] and the names of syntaxa are by Molnár and Borhidi [52].

3. Results

3.1 Changes of the species richness

Three variables and one interaction of variables (PLOT, REMOVAL, TIME and REMOVAL*TIME) were considered as significant by ANOVA. The interaction REMOVAL*TIME (P<0.001) confirms the fact that removal of top soil on the experimental plots had a highly significant impact on the studied vegetation (Table 2). The average species richness decreased in all three types to approx. 75% of the previous value (Figure 1) in the year after the removal; after another year it began to increase everywhere.

The highest species richness (5 species per 0.01 m²) was recorded in *Festuca pseudovina*-type, where the fastest recovery of species richness was observed two years after the removal. The values of species richness in this type were nearly the same two years after the treatment as at the beginning of the observation. The recovery of species richness in *Artemisia santonicum*-type and *Arrhenatherum elatius*-type was slower, otherwise all three types had more or less the same pattern of species richness changes after the intervention: a decrease in the year after the removal, followed by an increase (insignificant interaction REMOVAL* VEGTYPE *TIME).

	dF effect	dF error	Species richness		Number of halophytes		Halophytes/ all species	
			F	Р	F	Р	F	Р
removal	1	9	23.803	< 0.001	2.638	0.139	24.899	< 0.001
VEGTYPE	2	9	10.049	0.005	243.033	< 0.001	52.172	< 0.001
time	9	18	22.103	< 0.001	1.556	0.238	10.246	< 0.001
removal* VEGTYPE	2	9	1.977	0.194	2.620	0.127	4.985	0.035
removal* time	2	18	13.270	<0.001	0.300	0.745	20.788	< 0.001
removal* VEGTYPE *time	4	18	1.597	0.218	0.817	0.531	5.782	0.004

Table 2. Results of multi-way ANOVA for the effects plot, removal, VEGTYPE and time. Only fixed repeated measurement factors are displayed.





Figure 1. Changes in species composition after top soil removal in the Kamenínske Slanisko Nature Reserve. Species richness, number of halophytes and proportion of halophytes to total species richness were calculated by ANOVA, data transformed (species richness y' = log y, number of halophytes y' = log (10*y+1) and ratio of the number of halophytes to species richness y' = arcsin √ y). ARSA*R: plots of *Artemisia santonicum*-type, removed vegetation cover; ARSA*U unremoved control; FEPS*R: *Festuca pseudovina*-type removed; FEPS*U: *Festuca pseudovina*-type unremoved control; AREL*R: *Arrhenatherum elatius*-type removed; AREL*U: *Arrhenatherum elatius*-type unremoved control.

3.2 Changes of the number of halophytes

The removal had no impact on total number of halophytes in our plots (insignificant interactions REMOVAL*TIME, REMOVAL*VEGTYPE *TIME) (Table 2). The only significant variable is VEGTYPE (P<0.001), because there are significant differences in the presence of halophytes among all three types (Figure 1).

3.3 Changes in the proportion of halophytes to total species richness

Although no significant interaction was found in total number of halophytes, the situation is different when we take into account the proportion of halophytes in total species richness (Table 2). Both interactions (REMOVAL*TIME and REMOVAL*VEGTYPE*TIME) were significant (P<0.001and P=0.004 respectively). The proportion of halophytes increased in *Artemisia santonicum*-type and in *Festuca pseudovina*-type, but two years after the treatment it remained higher only in the *A. santonicum*-type. The least pronounced increase in halophyte proportion was observed in *Arrhenatherum elatius*-type (Figure 1).

3.4 Changes of species composition after top soil removal

Results of the indirect gradient analysis (DCA) show the main ecological gradients which determine the vegetation on the experimental plots. The main gradient represents the salinity gradient, from stands with high salinity level on the left, represented by specialists (*Camphorosma annua*, *Puccinellia distans*, *Tripolium pannonicum*, *A. santonicum*) to the stands on the right where generalists dominate (*e.g. Cirsium arvense*, *Carduus acanthoides*, *Carex hirta*) (Figure 2). The three types follow this main gradient with *Artemisia santonicum*-type on the left position, *Festuca pseudovina*-type in the middle and *Arrhenatherum elatius*-type on the right (Figure 3). The y-axis represents the moisture gradient with some hygrophilous taxa on the top (*Juncus compressus*, *Inula britannica*) and with taxa of dry grasslands in the bottom part of the graph (e.g. Jacea pannonica, Festuca rupicola).

Artemisia santonicum and F. pseudovina-plots with removed top soil moved towards a higher salinity level (Figure 3). In F. pseudovina plots this trend is observed only in the first year; subsequently, the development trajectory of these stands reverts to lower salinity. Plots of A. elatius show the opposite tendency, moving



Figure 2. DCA ordination diagram of species recorded between 2008 and 2010 in the Kamenínske Slanisko Nature Reserve. Matching codes of the species used in the ordinations: Achmill - Achilliea millefolium L., allivine - Allium vineale L., anagarve - Anagallis arvensis L., arrhelat - Arrhenatherum elatius (L.) P. Beauv. ex J. Presl et C. Presl, artesant - Artemisia santonicum subsp. patens (Neilr.) K. M. Perss., atripatu - Atriplex patula L., betupend - Betula pendula Roth., bromhord - Bromus hordeaceus L., bromjapo - B. japonicus Thunb., bupltenu - Bupleurum tenuissimum L., calaepig - Calamagrostis epigejos (L.) Roth., campannu - Camphorosma annua Pall., cardacan - Carduus acanthoides L., carehirt - Carex hirta L., caremela - C. melanostachya M. Bieb. ex Willd., ceraholo - C. holosteoides Fr., cerapumi - Cerastium pumilum Curt., cirsarve - Cirsium arvense (L.) Scop., cratmono - Crataegus monogyna Jacq., conycana - Conyza canadensis (L.) Cronquist, dactglom - Dactylis glomerata L., dauccaro - Daucus carota L., desccesp - Deschampsia cespitosa (L.) P. Beauv., dipsfull – Dipsacus fullonum L., elytrepe – Elytrigia repens (L.) Desv., epiltetr – Epilobium tetragonum L., festarun – Festuca arundinacea Schreb., festpseu – F. pseudovina Hack. ex Wiesb., festrupi – F. rupicola Heuff., galapunc – Galatella punctata (Waldst. et Kit.) Nees, galiapar - Galium aparine L., galimoll - G. mollugo L., galiveru - G. verum L., gleched - Glechoma hederacea L., inulbrit - Inula britanica L., jacepann - Jacea pannonica (Heuff.) Soják, juncarti - Juncus articulatus L., junccomp - J. compressus Jacq., lactserr - Lactuca serriola L., lathprat - Lathyrus pratensis L., limogmel - L. gmelinii (Willd) Kuntze, lotucorn - Lotus corniculatus L., lotutenu – L. tenuis Waldst. et Kit. ex Willd., medilupu – Medicago lupulina L., odonvulg – Odontites vulgaris Moench, picrhier – Picris hieracioides L., planlanc - Plantago lanceolata L., planmajo - P. major L., planmari - P. maritima L., poa_angu - Poa angustifolia L., podocanu – Podospermum canum C. A. Mey., polygavic – Polygonum aviculare agg. L., puccdist – Puccinellia distans agg. (Jacq.) Parl., ranurepe - Ranunculus repens L., ranusard - R. sardous Crantz, rumecris - Rumex crispus L., senejaco - Senecio jacobaea L., soncarv - Sonchus arvensis L., sympoffi - Symphytum officinale L., trifcamp - Trifolium campestre Schreb., trifrepe - T. repens L., trippann - Tripolium pannonicum (Jacq.) Dobrocz., verbblat - Verbascum blattaria L., veroarve - Veronica arvensis L., verocham - V. chamaedrys L., vicitetr - Vicia tetrasperma (L.) Schreb.



Figure 3. DCA scatter plot ordination diagram, using the three types of plots as nominal supplementary variables, showing trends in the experimental plots in three types of vegetation in the Kamenínske Slanisko Nature Reserve from 2008 to 2010. 08 – year 1, 09 – year 2, 10 – year 3. For plots, see the legend under Figure 1.

towards a higher occurrence of ruderal species in the first year after the removal, but in the following year a slight shift back to the left can be observed. Undisturbed plots in all three types show a small movement towards less optimal stages of the salinity level (Figure 3).

The fact that top soil removal had a significant impact (P=0.002) on the vegetation of *A. santonicum* and *F. pseudovina*-types was also confirmed by the three partial RDA biplots. Interaction REMOVAL*TIME explained a rather high percentage of total variance in these two vegetation types (26.8% for *A. santonicum* and 25% for *F. pseudovina*-type). Less significant (P=0.06) changes were observed in *A. elatius*-type where the interaction explained only a small portion of the variability in our data (8.6%).

The RDA diagrams of *A. santonicum* and *F. pseudovina* plots (Figures 4a, b) demonstrate an increasing abundance of perennial halophilous species (*Plantago maritima* and *Limonium gmelinii*) during the experiment. Annual target halophyte *C. annua* appeared after the first year of the soil removal only on the plots of *A. santonicum*-type, where it was growing before the treatment and it was establishing and persisting gradually in the following years. The top soil removal reduced the abundance of species more typical of mesic grasslands, such as *J. pannonica*, *D. glomerata*, and *A. elatius*. Other salt-tolerant species and glycophytes responded with considerable expansion of the opened canopy, for example *Daucus carota*, *I. britannica* and

C. arvense. On the plots of *A. elatius*, after removal there was an increased abundance of ruderal taxa, like *Conyza canadensis* and *P. major*, and hygrophytes such as *Juncus articulatus* (Figure 4c).

4. Discussion

4.1 The effect of top soil removal on smallscale species richness of saline vegetation and its impact on species composition with different levels of soil-salinity (different stages of degradation)

Top soil removal is a strong disturbance, as the whole vegetation cover is removed and a new succession series is opened. Therefore, subsequent significant decline of species richness a year after removal in all three studied vegetation types is not surprising. Two years after the removal, species richness development starts to differentiate among the three vegetation types. The only type in which the previous species richness is nearly recovered two years after removal is the Festuca pseudovina-type; the two other types continue to display guite low species richness after this time. This development is probably caused by very strong environmental filters which determine vegetation changes after the removal. High salt content is probably such a filter in the Artemisia santonicum-type. Halophytes are the only species able to tolerate such



Figure 4. RDA ordination diagram representing species response to the interaction of topsoil removal and time (Rem*time) in stands of ARSA (a), FEPS (b) and AREL (c) in the Kamenínske Slanisko Reserve. Species are displayed by 3% of fit range at ARSA and 10% of fit range at FEPS and AREL. The matching codes of species occurring in the plots are in the legend of Figure 2.

extreme conditions and have a competitive advantage compared to glycophytes [29]. This can explain the low species richness as only halophytes managed to establish.

Although the total number of halophytes was not changed, the proportion of halophytes to overall species richness changed significantly after the treatment. Soil removal brought about different effects in each vegetation type. Pronounced increase of halophyte proportion was recorded in *A. santonicum*-type because most of the glycophytes present in the vegetation were not able to recover after soil removal. No increase was found in *F. pseudovina*-type. The reason might be the insufficient salt content for halophytes which favors glycophytes and salt-tolerant generalists.

Changes of species composition along the salinity gradient were of the highest interest in the experiment. The results of indirect and direct gradient analyses (DCA, RDA) confirmed the important role of soil salinity.

Soil removal had a positive effect (delaying secondary succession) only on the optimal stage of the saline vegetation, the *A. santonicum*-type. In moderately degraded *F. pseudovina*-type, it has only a short-term positive effect without the persistence of halophytes, due to increased competition of glycophytes. On degraded vegetation (*Arrhenatherum elatius*-type) the soil removal even stimulated further recruitment of ruderal species without the establishment of halophytes.

4.2 Different responses of halophytes to top soil removal

The effect of soil removal was not equal for all halophytic species present in the experiment. *Camphorosma annua*, the indicator of the highly sodic saline habitats of the ponto-pannonian floristic area [53] established only on the removed plots of *Artemisia santonicum* vegetation type. The reason of the slow recovery process can be the short-term viability of its seeds, not present in the seed bank, but further research is needed because no exact data are known. Our hypothesis is supported by the fact that seed longevity of the related species *C. lesingii* is only 8-10 months [54] and seed germinantion of therophytes can be markedly reduced by high soil salinities [55].

Perennial halophytes like *P. maritima* and *L. gmelinii* had a positive response to top soil removal in the plots with higher salinity (*A. santonicum* and *F. pseudovina*-type), due to reduced competition caused by soil removal. They are deep-rooting species [56] which are able to grow even on degraded, formerly saline soils. In addition, those species are rhizomatous, and their underground parts might have remained in place after the treatment. *P. maritima* may regenerate from the shoots quite quickly and thus colonize bare soil [57]. On the other hand, it is a weak competitor, typical of early succession stages [58,59].

4.3 Temporal changes of the vegetation influenced by the precipitation

Strong trend of movement towards the wetter edge of the y-axis gradient was detected by DCA in our study. It may be related to the extreme precipitation, which occurred in the year 2010 (highest annual rainfall ever recorded in this region).

The most degraded *A. elatius* plots have the lowest relative position in microrelief (removal was the deepest here) and therefore rainwater fills them easily. This may result in seedlings of ruderal species having

high mortality rates because they are not able to grow when flooded by water. On the other hand, extreme precipitation may also impact halophytic vegetation. Saline grasslands are dependent on groundwater with a high salt content but the amount of rain water also plays an important role [26,60]. The leaching of salts from the top soil is most common in the rainy seasons [61], during local flooding of depressions after short-term heavy rainfall. Soil moisture is also a very important ecological factor influencing halophytic vegetation [62]. If the groundwater table is far below the top soil, then rainfall is the only relevant water source; but being rather neutral, it does not contain the soluble minerals which cause salt accumulation.

4.4 Recommendations for restoration and conclusions

The salinity gradient is crucial for the differentiation of halophytic vegetation in the Pannonian [41,52] and in other Central European saline habitats [13]. It plays a limiting role for the successful application of top soil removal as a restoration measure for saline habitats on large scale. If the abiotic conditions have changed to the extent that soil removal itself is not able to restore the natural movement of salinization, it may be combined with hydrological restoration [63].

However, in highly fragmented vegetation, as our study site, rehabilitating substrates by hydrological restoration is not feasible. Soil removal itself is not the ultimate condition for successful restoration on habitats with reduced salt accumulation. It should be used in combination with other methods. In order to reduce the pressure of glycophytes, and to induce disturbances promoting a regeneration niche [64] for the establishment of halophytic species, grazing might be the most appropriate management on the degraded inland salt steppes [65] as large herbivores are effective propagule dispersers of target species, even on the desalinized soils or isolated systems [20,66]. Moreover, the intensive trampling results in soil compaction and prevents salt leaching [21]. We consider implementation of grazing as a mitigation measure on the fragmented and eutrophicated habitats to be an urgent priority.

Acknowledgements

The authors are grateful to Róbert Šuvada (Slovak Karst National Park Administration) and Pavol Polák for their help during the data sampling, Richard Hrivnák (Institute of Botany, Bratislava) and other reviewers for critical comments on the previous versions of the manuscript. The study was financed by projects

VEGA No. 2/0030/09, No. 2/0003/11 and by the grant SK0115 through the European Economic Area Financial

References

- Küster H., Keenleyside C., The origin and use of agricultural grasslands in Europe, In: P. Veen P., Jefferson R., de Smidt J., van der Straaten J. (Eds.), Grasslands in Europe of high nature value, KNNV Publishing, Zeist, 2009
- [2] Kiehl K., Plant species introduction in ecological restoration: Possibilities and limitations. Basic Appl. Ecol., 2010, 11, 281-284
- [3] Miller J.R., Hobbs R.J., Habitat restoration do we know what we're doing?, Restorat. Ecol., 2007, 15, 382-390
- [4] Sümegi P., Molnár A. Szilágyi G., Alkalinization in the Hortobágy [Szikesedés a Hortobágyon], Term. világa, 2000, 131, 213-216, (in Hungarian)
- [5] Karpov D.N., Yuritsyna N.A., Saline soils vegetations of the Southern Ural and adjacent regions, In: Golub V. B., Saksonov S. V. (Eds.), Togliatti, 2006 (in Russian)
- [6] Krist V., Halophytic vegetation of southwestern Slovakia and the northern part of the Lesser Plain [Halofytní vegetace jihozápadního Slovenska a severní části Malé uherské nížiny], Práce Moravské Přírodovědecké Společnosti 12, Brno, 1940 (in Czech)
- [7] Vicherek J., Vegetace ČSSR, Ser. A 5, The plant communities of halophytes and Subhalophytenvegetation Czechoslovakia [Die Pflanzengesellschaften der Halophyten- und Subhalophytenvegetation der Tschechoslowakei], Academia, Praha, 1973 (in German)
- [8] Osvačilová V., Svobodová Z., Floristical and phytocoenological survey of the Nitra district (tematical map) [Floristicko-fytocenologický prieskum Nitrianskeho kraja], VŠP, Nitra, 1961 (in Slovak)
- [9] Sádovský M., Eliáš jun. P., Dítě D., Distibution of halophytic communities in southwestern Slovakia: History and present [Historické a súčasné rozšírenie slaniskových spoločenstiev na juhozápadnom Slovensku], Bull. Slov. Bot. Spoločn. Bratislava, 2004, Supplement, 10, 127-129, (in Slovak)
- [10] Bouzillé J.B., Tournade F., Blockage of vegetational succession over several centuries in wetlands of Western France, C.R. Acad. Sc., Paris Sciences et Vie, 1994, 317, 571-574, (in French)
- [11] Bakker J.P., Esselink P., Dijkema K.S., van Duin W.E., de Jong D.J., Restoration of salt marshes in the Netherlands, Hydrologia, 2002, 478, 29-51

Mechanism and the Norwegian Financial Mechanism and from the state budget of the Slovak Republic.

- [12] Borhidi A., Kevey B., Lendvai G., Plant Communities Of Hungary, Akadémiai Kiadó, Budapest, 2013
- [13] Piernik A., Inland halophilous vegetation as indicator of soil salinity, Basic Appl. Ecol., 2003, 4, 525-536
- [14] Schmidt D., Alkali vegetation fragments in the surroundings of Győr [A Győr környéki szikesek növényzete], Flora Pannonica, 2007, 5, 95-104 (in Hungarian)
- [15] Šumberová K., Novák J., Sádlo J., Saline grasslands
 [Slaniskové trávníky (Festuco-Puccinellietea)], In: Chytrý M. (Ed.), Vegetation of the Czech republic 1
 [Vegetace České Republiky 1], Praha, Academia, 2007
- [16] Dajic-Stevanovic Z., Pecinar I., Kresovic M., Vrbnicanin S., Tomovic Lj., Biodiversity, utilization and management of grasslands of salt affected soils in Serbia, Community Ecol., 2008, 9 (Suppl 1), 107-114
- [17] Šefferová Stanová V., Janák M., Ripka J., Management models for habitats in Natura 2000 Sites. 1530 *Pannonic salt steppes and salt marshes, European Commission, 2008, http://ec.europa. eu/environment/nature/natura2000/management/ habitats/pdf/1530_Pannonic_salt_steppes.pdf
- [18] Szabados K., Bošnjak T., Tucakov M., Kicošev V., The importance of the hydrological network of Vojvodina for biodiversity conservation [Značaj hidrološke mreže Vojvodine za očuvanje biološke raznovrsnosti], Savetovanje, Melioracije 11, Novi Sad, Temat. zb. radova, 2009, 207-214 (in Serbian)
- [19] Bakker J.P., Ruyter J.C., Effects of five years of grazing on a salt-marsh vegetation. A study with sequential mapping, Vegetatio, 1981, 44, 81-100
- [20] Loucougaray G., Bonis A., Bouzillé J. B., Effects of grazing by horses and/or cattle on the diversity of coastal grasslands in western France, Biol. Conserv., 2004, 116, 59-71
- [21] Bonis A., Bouzillé J. B., Amiaud B., Loucougaray G., Plant community patterns in old embanked grasslands and the survival of halophytic flora, Flora, 2005, 200, 74-87
- [22] Wolters M., Garbutt A., Bakker J. P., Salt-marsh restoration: evaluating the success of deembankments in north-west Europe, Biol. Conserv., 2005, 123, 249-268

- [23] Bernhardt K. G. Koch M., Restoration of a salt marsh system: temporal change of plant species diversity and composition, Basic Appl. Ecol., 2003, 4, 441-451
- [24] Lengyel S, Varga K, Kosztyi B, Lontay L, Déri E, Török P, et al., Grassland restoration to conserve landscape-level biodiversity: a synthesis of early results from a large-scale project, Appl. Veg. Sci., 2012, 15, 264-276
- [25] Török P., Miglécz T., Valkó O., Kelemen A., Tóth K., Lengyel S., et al., Fast restoration of grassland vegetation by a combination of seed mixture sowing and low-diversity hay transfer, Ecol. Eng., 2012, 44, 133-138
- [26] Lindig-Cisneros R., Zedler J.B., Halophyte recruitment in a salt marsh restoration site. Estuaries, 2002, 25, 1174-1183
- [27] Török P., Vida E., Deák B., Lengyel S., Tóthmérész B., Grassland restoration on former croplands in Europe: an assessment of applicability of techniques and costs, Biodivers. Conserv., 2011, 20, 2311-2332
- [28] Weijtmans K., Jongejans E., van Ruijven J., Sod cutting and soil biota effects on seedling performance, Acta Oecol., 2009, 35, 651-656
- [29] Engels J.G., Rink F., Jensen K., Stress tolerance and biotic interactions determine plant zonation patterns in estuarine marshes during seedling emergence and early establishment, J. Ecol., 2001, 99, 277-287
- [30] Molnár Z., Biró M., Bölöni J., Horváth F., Distribution of the (semi-)natural habitats in Hungary I. Marshes and grasslands, Acta Bot. Hung., 2008, 50, 59-106
- [31] Lenssen J.P.M., van de Steeg H.M., de Kroon H., Does disturbance favour weak competitors?, Mechanisms of changing plant abundance after flooding, J. Veg. Sci., 2004, 15, 305-314
- [32] Klimkowska A., Dzierza P., Brzezińska K., Kotowski W., Medrzycki P., Can we balance the high costs of nature restoration with the method of topsoil removal?, Case study from Poland, J. Nat. Cons., 2010, 18, 202-205
- [33] Verhagen R., Klooker J., Bakker J.P., van Diggelen R., Restoration success of low-production plant communities on former agricultural soils after topsoil removal, App. Veg. Sci., 2001, 4, 75-82
- [34] Allison M., Ausden M., Successful use of topsoil removal and soil amelioration to create heathland vegetation, Biol. Conserv., 2004, 120, 221-228
- [35] Diaz A., Green I., Tibbett M., Re-creation of heathland on improved pasture using top soil removal and sulphur amendments: Edaphic drivers

and impacts on ericoid mycorrhizas, Biol. Conserv., 2008, 141, 1628-1635

- [36] Kiehl K., Pfadenhauer J., Establishment and persistence of target species in newly created calcareous grasslands on former arable fields, Plant Ecol., 2007, 189, 31-48
- [37] Wolters M., Garbutt A., Bekker R.M., Bakker J.P., Carey P.D., Restoration of salt-marsh vegetation in relation to site suitability, species pool and dispersal traits, J. App. Ecol., 2008, 45, 904-912
- [38] Zhao B., Yan Y., Guo H.Q., He M.M., Gu Y.J., Li B., Monitoring rapid vegetation succession in estuarine wetland using time series MODIS-based indicators: An application in the Yangtze River Delta area, Ecol. indicators, 2009, 9(2), 346-356
- [39] Miklós L., Hrnčiarová T., (Eds.), Atlas of the Slovak republic, 1. edition [Atlas krajiny Slovenskej republiky, 1. vydanie] MŽP SR Bratislava, SAŽP Banská Bystrica, 2002 (in Slovak)
- [40] Svobodová Z., Řehořek V., The recent stage of flora and vegetation of Kamenínske slanisko Natural Reserve and problem of its protection [Súčasný stav flóry a vegetácie Štátnej prírodnej rezervácie Kamenínske slanisko a problematika jeho ochrany], Sprav. Obl. Podun. Múzea – prír. vedy, 1985, 5, 67-74 (in Slovak)
- [41] Illyés E., Botta-Dukát Z., Molnár Zs., Patch and landscape factors affecting the naturalness based quality of three model grassland habitats in Hungary, Acta Bot. Hung., 2008, 50 (Suppl), 179-197
- [42] Fehér S., Origin and development of the salt steppes and marshes in SW Slovakia, Flora Pannon., 2007, 5, 67-93
- [43] Dítě D., Eliáš jun. P., Sádovský M., Camphorosmetum annuae Rapaics ex Soó 1933
 vanishing plant community of saline habitats in Slovakia, Thaiszia, 2008, 18, 9-20
- [44] Eliáš jun. P., Fehér S., Dítě D., Šuvada R., Current occurrence of Hog's Fennel (Peucedanum officinale) in Slovakia [Recentný výskyt smldníka lekárskeho (Peucedanum officinale) na Slovensku], Bull. Slov. Bot. Spoločn, Bratislava, 2010, 32/1, 29-35 (in Slovak)
- [45] Richards L.A., Diagnosis and Improvement of Saline and Alkali Soils, Salinity Laboratory Staff, Washington, 1954
- [46] STN ISO 13536, Soil quality. Determination of cationic exchange capacity and content of exchangeable cations using barium chloride buffer pH 8.1, SÚTN, Bratislava, 2001 (in Slovak).
- [47] Underwood A. J., Experiments in ecology, Cambridge University Press, Cambridge, 1997

- [48] Lepš J., Šmilauer P., Multivariate Analysis of Ecological Data using CANOCO. Cambridge University Press, Cambridge, 2003
- [49] Stewart-Oaten A., On rejection rates of paired intervention analysis: comment, Ecology, 2003, 84, 2795-2799
- [50] ter Braak C.J.F., Šmilauer P., CANOCO reference manual and CanoDraw for Windows user's guide. Software for Canonical Community Ordination (version 4.5), Wageningen and České Budějovice, Biometris, 2002
- [51] Marhold K. (Ed.), Ferns and higher plants, In: Marhold K., Hindák F. (Eds.), Checklist of Non-Vascular and Vascular Plants of Slovakia [Zoznam nižších a vyšších rastlín Slovenska], Veda, Bratislava, 1997
- [52] Molnár Z., Borhidi A., Hungarian alkali vegetation: Origins, landscape history, syntaxonomy, conservation, Phytocoenologia, 2003, 33, 377-408
- [53] Knežević A., Andelić M., Merkulov L.J., Ekomorphological characteristics of Camphorosma annua Pall. (Chenopodiaceae) [Eko-morfološke karakteristike vrste Camphorosma annua Pall.], Zb. radova PMF, Novi Sad, 1992, 22, 31-38 (in Serbian)
- [54] Gintzburger G., Toderich K.N., Mardonov B.K., Mahmudov M.M., Rangelands of the Arid and Semiarid Zones in Uzbekistan, CIRAD, Montpellier, 2003
- [55] Ungar I. A., Influence of salinity on seed germination in succulent halophytes, Ecology, 1962, 43, 763-764
- [56] Muraközy E., Nagy Z., Duhazé C., Bouchereau A., Tuba Z., Seasonal changes in the levels of compatible osmolytes in three halophytic species of inland saline vegetation in Hungary, J. Plant Physiol., 2003, 160, 395-401

- [57] Makowczyńska J., Andrzejewska-Golec E., Micropropagation of Plantago maritima L. - a vanishing species in Poland, Acta Soc. Bot. Pol., 2009, 78, 13-18
- [58] Dormann C.F., van der Wal R., Bakker J.P., Competition and herbivory during salt marsh succession: the importance of forb growth strategy, J. Ecol., 2000, 88, 571-583
- [59] Dormann C.F., Bakker J.P., The impact of herbivory and competition on flowering and survival during saltmarsh succession, Plant Biol., 2000, 2, 68-76
- [60] Kovács D., Tóth T., Marth P., Soil salinity between 1992 and 2000 in Hungary, Agrokém. Talajtan, 2006, 55(1), 89-98
- [61] Armstrong A.S.B., Rycroft D.W., Tanton T.W., Seasonal movement of salts in naturally structured saline-sodic clay soils, Agr. Water Manage., 1996, 32, 15-27
- [62] Tóth T., Medium-term vegetation dynamics and their association with edaphic conditions in two Hungarian saline grassland communities, Grassland Sci., 2010, 56, 13-18
- [63] Beyen, W., Meire P., Ecohydrology of saline grasslands: Consequences for their restoration, App. Veg. Sci., 2003, 6, 153-160
- [64] Grubb P. J., Maintenance of species-richness in plant communities – importance of regeneration niche, Biol. Reviews, 1977, 52, 107-145
- [65] Bakker J.P., Bravo L.G., Mouissie A.M., Dispersal by cattle of salt-marsh and dune species into saltmarsh and dune communities, Plant Ecol., 2008, 197, 43-54
- [66] Auffret A.G., Schmucki R., Reimark J., Cousins S.A.O., Animal movement provides useful functional connectivity for plants in fragmented systems, J. Veg. Sci., 2012, 23, 970 -977