Vertical distribution of soil seed bank and the ecological importance of deeply buried seeds in alkaline grasslands

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Background. Soil seed banks play a central role in vegetation dynamics and may be an important source of ecological restoration. However, the vast majority of seed bank studies examined only the uppermost soil layers (0-10cm); hence our knowledge on the depth distribution of seed bank and the ecological significance of deeply buried seeds is limited. The aim of our study was to examine the fine-scale vertical distribution of soil seed bank to a depth of 80cm, which is one of the largest studied depth gradients so far. Our model systems were alkaline grasslands in East-Hungary, characterised by harsh environmental conditions, due to Solonetz soil reference group with Vertic horizon. We asked the following questions: (1) How do the seedling density and species richness of soil seed bank change along a vertical gradient and to what depth can germinable seeds be detected? (2) What is the relationship between the depth distribution of the germinable seeds and the species traits? **Methods**. In each of the five study sites, four soil cores (4cm diameter) of 80cm depth were collected with an auger for soil seed bank analysis. Each sample was divided into sixteen 5-cm segments by depth (320segments in total). Samples were concentrated by washing over sieves and then germinated in an unheated greenhouse. Soil penetration resistance was measured in situ next to each core location (0–80cm depth, 1-cm resolution). We tested the number and species richness of seedlings observed in the soil segments (N=320), using negative binomial generalized linear regression models, in which sampling layer and penetration resistance were the predictor variables. We run the models for morphological groups (graminoids/forbs), ecological groups (grassland species/weeds) and life-form categories (short-lived/perennial). We also tested whether seed shape index, seed mass, water requirement or salt tolerance of the species influence the vertical distribution of their seed bank. **Results**. Germinable seed density

and species richness in the seed bank decreased with increasing soil depth and penetration resistance. However, we detected 9 germinable seeds of 6 species even in the deepest soil layer. Forbs, grassland species and short-lived species occurred in large abundance in deep layers, from where graminoids, weeds and perennial species were missing. Round-shaped seeds were more abundant in deeper soil layers compared to elongated ones, but seed mass and ecological indicator values did not influence the vertical seed bank distribution. Our research draws attention to the potential ecological importance of the deeply buried seeds that may be a source of recovery after severe disturbance. As Vertisols cover 335 million hectares worldwide, these findings can be relevant for many regions and ecosystems globally. We highlight the need for similar studies in other soil and habitat types to test whether the presence of deep buried seeds is specific to soils with Vertic characteristics.

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Abstract

Background. Soil seed banks play a central role in vegetation dynamics and may be an important source of ecological restoration. However, the vast majority of seed bank studies examined only the uppermost soil layers (0-10 cm); hence our knowledge on the depth distribution of seed bank and the ecological significance of deeply buried seeds is limited. The aim of our study was to examine the fine-scale vertical distribution of soil seed bank to a depth of 80 cm, which is one of the largest studied depth gradients so far. Our model systems were alkaline grasslands in East-Hungary, characterised by harsh environmental conditions, due to Solonetz soil reference group with Vertic horizon. We asked the following questions: (1) How do the seedling density and species richness of soil seed bank change along a vertical gradient and to what depth can germinable seeds be detected? (2) What is the relationship between the depth distribution of the germinable seeds and the species traits?

Methods. In each of the five study sites, four soil cores (4 cm diameter) of 80 cm depth were collected with an auger for soil seed bank analysis. Each sample was divided into sixteen 5-cm segments by depth (320 segments in total). Samples were concentrated by washing over sieves and then germinated in an unheated greenhouse. Soil penetration resistance was measured in situ

40   next to each core location (0–80 cm depth, 1-cm resolution). We tested the number and species
41   richness of seedlings observed in the soil segments (N = 320), using negative binomial
42   generalized linear regression models, in which sampling layer and penetration resistance were
43   the predictor variables. We run the models for morphological groups (graminoids/forbs),
44   ecological groups (grassland species/weeds) and life-form categories (short-lived/perennial). We
45   also tested whether seed shape index, seed mass, water requirement or salt tolerance of the
46   species influence the vertical distribution of their seed bank.
47   **Results**. Germinable seed density and species richness in the seed bank decreased with
48   increasing soil depth and penetration resistance. However, we detected 9 germinable seeds of 6
49   species even in the deepest soil layer. Forbs, grassland species and short-lived species occurred
50   in large abundance in deep layers, from where graminoids, weeds and perennial species were
51   missing. Round-shaped seeds were more abundant in deeper soil layers compared to elongated
52   ones, but seed mass and ecological indicator values did not influence the vertical seed bank
53   distribution. Our research draws attention to the potential ecological importance of the deeply
54   buried seeds that may be a source of recovery after severe disturbance. As Vertisols cover 335
55   million hectares worldwide, these findings can be relevant for many regions and ecosystems
56   globally. We highlight the need for similar studies in other soil and habitat types to test whether
57   the presence of deep buried seeds is specific to soils with Vertic characteristics.
58

59   # Introduction
60

61   Soil seed banks, including dormant and non-dormant seeds, present on the soil surface and in the
62   soil, play an important role in population dynamics and diversity maintenance in almost all
63   ecosystems throughout the world (Bakker et al., 1996; Douh et al., 2018; Sloey & Hester, 2019).
64   They serve as reservoirs for seeds that have already become independent of their mother plants
65   in terms of their metabolism and are able to germinate or acquire this ability in the future
66   (Csontos, 2001). Soil seed banks also play a considerable role in the long-term preservation of
67   genetic variation in plant populations and in shaping spatial and temporal distribution of species,
68   influencing the structure and composition of future plant communities (Bossuyt & Honnay,
69   2008; Münzbergová et al., 2018). In addition, they may have a crucial role in the spontaneous
70   recovery and restoration of various habitats (Ludewig et al., 2021; Sloey & Hester, 2019), as
71   they might contain the seeds of the target habitat even when its species have already disappeared
72   from aboveground vegetation (Hopfensperger, 2007; Shiferaw et al., 2018; Wang et al., 2020).
73   As seed banks usually include seeds from the earlier successional stages, they also function as a
74   so-called "successional memory", providing an insight into the processes of past vegetation
75   dynamics (Bakker et al., 1996; Bossuyt & Honnay, 2008; Ma et al., 2021). Recent studies also
76   emphasize their role when identifying and evaluating potential climate-change microrefugia
77   (Ashcroft et al., 2012).
78

79 Different vegetation types vary in their seed bank characteristics, such as seed density, seed bank
80 species richness and also in similarity level between the species composition of the seed bank
81 and vegetation (Bakker et al., 1996; Bossuyt & Honnay, 2008; Hopfensperger, 2007; Plue et al.,
82 2020; Wang et al., 2020). Species richness of the seed bank and the similarity between seed bank
83 and vegetation were found to be higher in grasslands than in forests (Plue et al., 2020). In
84 general, seed bank density in grasslands is higher than in forests, but lower compared to wetlands
85 (Bossuyt & Honnay, 2008; Hopfensperger, 2007). It is important to note that there may be major
86 differences in the seed densities of different grassland types. The diversity and density of a soil
87 seed bank is the highest in the most stressed grassland types, such as continental and seashore
88 salt-affected grasslands. In these types of grasslands, regeneration from the persistent soil seed
89 bank may play an essential role in maintaining the species richness of the aboveground
90 vegetation due to the unfavourable environmental/growing conditions (Bossuyt & Honnay, 2008;
91 Hopfensperger, 2007; Plue et al., 2020; Valkó et al., 2014).
92
93 Although several classification systems have been set up to classify seed banks into different
94 types, the system proposed by Thompson et al. (1997) became the most widely used one. It
95 defines three seed bank types (i.e., transient, short term persistent and long-term persistent) based
96 on the longevity of seeds in the seed bank. Plant species can be classified into certain types of
97 soil seed bank based on their relative frequency in aboveground vegetation and seed bank, and
98 on the relative prevalence of seeds in the upper and lower soil layers (Csontos, 2001). It is a
99 general trend that the more persistent the seeds of a given species, the higher its proportion found
100 in the deeper soil layers (Bekker et al., 1998).
101
102 A considerable proportion of the seed bank occurs in the layers close to the surface, mostly in the
103 top 5 cm, and its density and the number of germinable seeds decline with depth (Bekker et al.,
104 1998; Csontos, 2001; Shiferaw et al., 2018). The vertical distribution of soil seed bank is shaped
105 by several factors, such as soil type, seed size and seed shape based on the results of previous
106 studies focussing on surface layers of the seed bank. For instance, larger seeds are less likely to
107 enter the deeper soil layers by movement down along cracks or burial by soil-dwelling animals
108 than small ones (Bekker et al., 1998; Shiferaw et al., 2018; Thompson et al., 1994). Moreover,
109 small seeds tend to have greater longevity (Bu et al., 2016), therefore they have a higher chance
110 (i.e., longer time) to preserve their viability while being deeply buried (Shiferaw et al., 2018;
111 Thompson et al., 1993).
112
113 Soil seed bank studies may contribute to management activities that aim at maintaining the
114 species richness of the natural habitats. It is an especially important task, as the area of natural
115 habitats is in constant decline. However, our knowledge on the restoration potential of soil seed
116 bank in most ecosystems is incomplete. The reason for that is that vast majority of studies
117 examined only the uppermost soil layers, usually the upper 10 or 20 cm, as the seed bank
118 typology systems also consider these layers (Thompson et al., 1997). Hence, our knowledge on

119   the depth distribution of seed banks and the ecological significance of deeply buried seeds is
120   rather limited, nonetheless information on the soil seed bank persistence of characteristic species
121   may help to predict the effectiveness of habitat restoration, especially when it relies to
122   spontaneous vegetation processes (Ludewig et al., 2021; Poschlod et al., 1995; Saatkamp et al.,
123   2019). It would be crucial to know whether the deep soil layers can be suitable propagule sources
124   for the recovery of communities after degradation.
125
126   The aim of this study was to examine the soil seed bank to a depth which, to our best knowledge,
127   has not been done so far except for one study in moving sand dunes where soil layers are
128   constantly re-organized (Qian et al., 2016). We studied the fine-scale vertical depth distribution,
129   the species composition, and the seedling density of the soil seed bank to a depth of 80 cm. Our
130   model systems were inland alkaline grasslands which are priority habitats of the EU Natura 2000
131   network (Pannonic salt steppes and salt marshes, 1530). These habitats were developed under
132   continental climate and harsh environmental conditions. Their soil characteristics can be
133   specified by high electric conductivity, alkalinity and compactness and shrink-swell potential,
134   resulting in the development of seasonally opening and closing deep cracks, which is classified
135   as Vertic Solonetz according the WRB (IUSS WG WRB, 2015). Harsh environmental conditions
136   induce the formation of persistent seed banks and the high likeliness of crack-formation
137   increases the possibility of deep burial of seeds (Burmeier et al., 2010; Valkó et al., 2014). In
138   order to explore the potential of deeply buried seed bank in ecological processes and habitat
139   restoration we asked the following questions: (1) How do the seedling density and species
140   richness of soil seed banks change along a vertical gradient and to what depth can germinable
141   seeds be detected in alkaline grasslands? (2) What is the relationship between the depth
142   distribution of the germinable seeds and the species traits?
143

144   Materials & Methods

145
146   *Study area*
147   The study was conducted in five study sites located in the Hortobágy National Park, East-
148   Hungary. Hortobágy is the largest salt-affected inland grassland area in Europe. The landscape is
149   composed by a mosaic of various plant communities; it is characterized by alkaline grasslands
150   and marshes, loess grasslands with scattered arable fields, floodplains and wooded habitats
151   (Deák et al., 2014). The climate is temperate continental. The mean annual temperature is 9.5°C
152   and the average annual rainfall is 550 mm, with a maximum in June (80 mm). The average
153   temperature and average precipitation show a considerable fluctuation from year-to-year (Fick &
154   Hijmans, 2017).
155
156   The soil reference group of the study area is classified as Vertic Solonetz (IUSS WG WRB,
157   2015). Vertisols and Vertic horizons are heavy clayey soils with a minimum of 30% clay content
158   to a depth of 50 cm or more, characterised by clay minerals having high shrink-swell potential.

159 Despite their compactness, Vertic soil horizons show a notable temporal and spatial variability.
160 Because of the shrink-swell effect of the clay, dry seasons result in the formation of deep
161 desiccation cracks. Due to the shrinking and swelling behaviour, the upper 10–20 cm of these
162 soils has a mixed granular structure. Most of these types of soils are neutral or alkaline, because
163 of their calcareous smectic clay parent material. The average pH values of Vertisols range from
164 6.0 to 8.0, however higher pH values can occur as well. High electrical conductivity (EC) is a
165 typical feature of Solonetz, caused by the high base saturation and its sodium-dominated
166 composition, together with high exchangeable sodium percentage (ESP) (Coulombe et al., 1996;
167 IUSS WG WRB, 2015).

168

169 Sampling was carried out in natural stands of dry alkaline grasslands (association: Achilleo
170 setaceae-Festucetum pseudovinae). These short grasslands occur in dry areas and typical on
171 Vertic Solonetz soils (Deák et al., 2014). The dominant grass species is *Festuca pseudovina*,
172 typical forb species include *Achillea collina*, *A. setacea, Plantago lanceolata* and *Trifolium* spp.
173 (Deák et al., 2014).

174

175 ***Sampling design***
176 Samples were collected after snowmelt, during late February 2019. In each site, four soil cores
177 (4 cm diameter) of 80 cm depth were collected with a soil auger for soil seed bank sampling (a
178 total of 20 soil core samples). The samples were divided into sixteen 5-cm segments by depth: 0–
179 5 cm, 5–10 cm, 10–15 cm, 15–20 cm, 20–25 cm, 25–30 cm, 30–35 cm, 35–40 cm, 40–45 cm,
180 45–50 cm, 50–55 cm, 55–60 cm, 60–65 cm, 65–70 cm, 70–75 cm and 75–80 cm, and each
181 segment was placed separately in a plastic bag (320 soil segments in total). Samples were then
182 stored at 5°C for 3 weeks. Soil penetration resistance was measured *in situ* with Eijkelkamp
183 Penetrologger next to each core sampling location down to 80 cm depth with 1-cm resolution.
184 Permission for the research was granted by the Trans-Tisza Environmental, Nature Protection
185 and Water Inspectorate (approval no 6646/08/2014).

186

187 ***Seed bank analysis***
188 Soil seed banks were studied with the seedling emergence method. Samples were concentrated
189 by washing them through sieves according to the method of Ter Heerdt et al. (1996). Vegetative
190 parts and fine soil materials were removed using a 3-mm mesh sieve and then using a fine sieve
191 with a mesh width of 0.2 mm. As a result, sample volumes were reduced considerably. The
192 concentrated samples were spread out in a thin layer (3-4 mm) on trays filled with potting soil
193 topped with an approximately 8-cm layer of sterilized potting soil. Control trays were filled with
194 the same sterilized potting soil to filter out possible wind-borne and potting soil contaminants.
195 The trays were randomly placed in an unheated greenhouse, at the Botanical Garden of the
196 University of Debrecen, where germination was carried out from April to November 2019 under
197 natural light conditions and regular watering. Seedlings were counted regularly, identified when
198 possible then removed. Unidentifiable seedlings were transplanted into separate pots and grown

199 until identifiable. At the end of June no new seedlings emerged, so watering was stopped to
200 mimic arid summer conditions that are typical in the studied ecosystem. Dried sample layers
201 were carefully broken through and stirred, and then at the end of August watering was restarted,
202 which was continued until early November. Nomenclature of the species followed Király (2009).
203
204 ***Classification of species***
205 Plant species were categorized into two morphological groups (graminoids and forbs) and into
206 two ecological groups (grassland species and weeds), using the Hungarian Flora Database
207 (Horváth et al., 1995). Species were arranged into simplified life-form groups based on
208 Raunkiaer's life-form categories. These groups were short-lived species (Th, TH) and perennials
209 (H, G, Ch).
210
211 Seed length, seed width and seed thickness values were collected for each species from
212 Schermann (1967) to calculate seed shape index (Bekker et al., 1998). Seed shape was expressed
213 as the variance of three seed dimensions (length, width, and height), after standardizing all three
214 values, so that length was considered as 1 (dividing all three values by the length). As a result of
215 this, seed shape index was independent of seed size. The more round-shaped a seed was, the
216 closer the index value was to 0, while the more elongated was the seed, the closer the index value
217 was to 1. The average seed mass of each species was collected from the database of Török et al.
218 (2013). Ecological indicator scores for salt tolerance (SB), and relative water requirement (WB)
219 values (Borhidi 1995) – were assigned to the species. Based on these trait data, community
220 weighted means (CWM) of salt tolerance (SB), relative water requirement (WB), seed mass and
221 seed shape index were calculated by weighting with the number of the germinated seedlings in
222 each layer. The classification of each recorded species into morphological, ecological, and life-
223 form groups, as well as their SB, WB, seed mass and seed shape index are given in Appendix 1.
224
225 ***Statistical analysis***
226 For all data handling and analyses the R statistical software (ver. 4.0.1; R Core Team, 2021) was
227 used. We tested the total number of seedlings, and the total number of species observed in the
228 soil segments (N = 320), using negative binomial generalized linear regression models (GLMs;
229 utilizing the *glmmTMB* R-package: Brooks et al., 2017), in which sampling layer and penetration
230 resistance were predictor variables, with control for their interaction. Although these variables
231 show substantial correlation, re-scaling prior to model fitting (subtracting the mean from all
232 values and dividing by the standard variation), and controlling for their interaction mitigated the
233 adverse effect of their collinearity, as shown by the low variance inflation scores from the fitted
234 models (5> for all variables and models; tested with the "performance" R-package: Lüdecke et
235 al., 2021).
236
237 Similarly, we fitted negative binomial models on the number of seedlings and germinated species
238 separately for each level of our categorical variables, with sampling layer and penetration

239 resistance as predictors, with control for their interaction as well. To test the effect of sampling
240 layer and penetration resistance on community-weighted means (CWMs) we used log-linked
241 Gamma GLMs, with sampling layer and penetration resistance as predictor terms. Due to the
242 non-significant interactions, we left out interaction terms from the final models on CWMs. In
243 cases when a given variable had a minimum value of zero, we transformed it by incrementing by
244 one in order to be able to fit Gamma models.
245
246 In the preliminary analyses we fitted all models with sampling repetition nested in sampling site
247 used as random factor, but due to the low (close to zero) variances we excluded random effects
248 from the final models.
249

250 **Results**
251 During the study 402 seedlings germinated, which corresponds to an overall seedling density of
252 15,870 seedlings/m^2. A total of 51 vascular plant species (and one unidentifiable forb seedling)
253 were detected, of which 180 seedlings of 12 graminoid species and 222 seedlings of 39 forb
254 species were identified (Appendix 1). Short-lived species comprised 60% of the total species and
255 47% of the total number of germinated seeds. Grassland species were present in greater numbers
256 in the soil seed bank than weeds, both in terms of the number of species (37 *vs.* 14 species,
257 respectively) and the number of germinable seeds (374 *vs.* 27 seeds, respectively).
258
259 The most abundant species in the soil seed bank were *Juncus compressus* (37% of total
260 seedlings), *Trifolium angulatum* (12%), *Gypsophila muralis* (9%), *Inula britannica* (6%),
261 *Matricaria recutita* (4%), *Plantago lanceolata* (3.5%) and *Festuca pseudovina* (2.5%). These
262 seven species were accounted for about 75% of the total germinable seed number. Of the 7 most
263 numerous species in seed bank samples, 4 were short-lived and 3 were perennials.
264
265 The soil seed bank density and the species richness decreased significantly with soil depth
266 (GLMs, $p < 0.001$ in both cases; Table 1, Figure 1). The largest decline was shown between the
267 first (0–5 cm depth) and second layers (5-10 cm) of the soil. The upper 0–5 cm soil layer
268 contained 75% (302 seedlings) of all germinated seeds, whereas their proportion was 25% (100
269 seedlings) in the deeper layers (5–80 cm). In the uppermost soil layer (0–5 cm) 12 016
270 seedlings/m^2 seedling density was detected, while in the deepest layer (75–80 cm) this value was
271 358 seeds/m^2. There was only one soil layer, the 60–65 cm depth, from which no germination
272 has occurred. Although, there were 18 species that only germinated from the first soil layer (0–
273 5 cm depth), 34 species had seedlings in the deeper soil layers. The most frequent species from
274 these deeper layers (5–80 cm) were *Juncus compressus* (18 seedlings), *Inula britannica* (15
275 seedlings), *Gypsophila muralis* (8 seedlings), *Trifolium angulatum* (8 seedlings) and *Amaranthus
276 retroflexus* (5 seedlings). A total of 9 seedlings from 6 species emerged from the deepest layer
277 (75–80 cm depth), namely *Juncus compressus*, *Cerastium vulgare*, *Trifolium angulatum*,

278 *Trifolium repens*, *Vicia hirsuta* and a forb seedling that died before identification. Of these
279 species, 3 were short-lived and 2 were perennials.
280
281 General linear models (GLMs) showed that depth had a significant effect on the vertical
282 distribution of morphological (graminoids, forbs), ecological groups (grassland species, weeds)
283 and life-forms (perennials, short-lived species) (Table 1). In the zone between layers 9 to 13 (40–
284 65 cm depth), forbs, grassland species and short-lived species were dominant and occurred in
285 large abundance, while graminoids, weeds and perennial species were missing (Figure 2A-F).
286 The abundant species found in these layers were *Cerastium vulgare*, *Gypsophila muralis*, *Inula*
287 *britannica*, *Stenactis annua*, *Trifolium angulatum*, *T. retusum*, *Vicia angustifolia*.
288
289 Seeds in the deeper soil layers had significantly lower seed shape index compared to seeds in the
290 upper soil layers (GLM, p = 0.049), which means that round shaped seeds were more abundant
291 in the deeper layers than elongated ones. There was no difference in the depth distribution of the
292 seeds among species with different seed mass and ecological indicator values. GLMs showed no
293 effect of burial depth on the CWMs of seed weight, salt tolerance (SB) and relative water
294 requirement (WB) (Table 1).
295
296 Based on the average penetration resistance values of each soil segment, it can be determined
297 that soil penetration resistance increased significantly with depth (Figure 3). Under the 55–60 cm
298 depth, in the deeper soil layers a slight decrease was shown in the soil penetration resistance.
299 With increasing soil penetration resistance, the seedling number and species richness of all
300 studied morphological, ecological and life-form groups decreased significantly (Table 2).
301

302 **Discussion**
303
304 ***The vertical distribution of seed bank in alkaline grasslands***
305 We found that both the germinable seed density and species richness of the seed bank
306 significantly decreased with soil depth and that seedling density was the highest in the upper soil
307 layer (0–5 cm) in the studied ecosystem. These seed bank patterns are consistent with the results
308 of previous studies analysing the seed bank of different grassland (e.g., Ma et al., 2010; Niknam
309 et al., 2018), forest (e.g., Godefroid et al., 2006; Jaroszewicz, 2013) and wetland ecosystems
310 (e.g., de Souza et al., 2019). More importantly, despite the sharp decline below the first five
311 centimetres, deeper soil layers also contained germinable seeds (e.g., 1 154 seeds/m^2 in the 5-
312 10 cm soil layer) that were able to germinate (if seeds were brought to the surface or near the
313 surface). A novelty of our study is that we found that germinable seeds can be detected in soil
314 layers much deeper than previously noted in other studies.
315
316 Based on the reviewed literature and our current knowledge, we believe that this is the second
317 study in which germinable seeds from such a deep soil layer have been detected. The only study

318   that investigated similarly deep burial of seeds was performed by Qian et al. (2016), but they
319   studied the seed bank of mowing sand dunes – which are dynamic systems – where soil layers
320   are constantly re-organized. They found that wind activities and sand burial contribute to the
321   formation of the spatiotemporal pattern of the soil seed banks of active sand dunes. Due to the
322   displacement of the seeds in different directions over time, seed density in active sand dunes
323   showed a relatively constant vertical distribution across the studied sand profile. A significant
324   amount of germinable seeds were found in the deeper soil layers too, which could be a form of
325   adaptation of plant species to the effects of wind erosion on active sand dunes. In addition, their
326   study showed that deeply buried seeds are brought to or near to the surface by the wind, and thus
327   they play an important role in population regeneration and vegetation restoration in active sand
328   dunes.
329
330   The majority of seed bank studies carried out in grassland (Godefroid, et al. 2018; Jacquemyn et
331   al., 2011; Ma et al., 2010; Niknam et al., 2018) and forest ecosystems (Douh et al., 2018;
332   Godefroid et al., 2006; Jaroszewicz, 2013) usually investigated only the uppermost soil layers to
333   a depth of 20 cm. However, the vertical distribution of the seed bank in wetland ecosystems has
334   already been studied at greater depths (Burmeier et al., 2010; Dawson et al., 2019; Jauhiainen,
335   1998; McGraw, 1987; van der Valk & Davis, 1979; Wall & Stevens, 2015). According to the
336   reviewed literature, these greatest depths were 45 cm (McGraw, 1987) and 50 cm (Burmeier et
337   al., 2010; Jauhiainen, 1998), respectively. This also means that seed bank research has generally
338   neglected the deeply buried seeds. In the present study we found that deeper soil layers (down to
339   80 cm) may also play an important role in the preservation of at least some seeds of grassland
340   species. Our results showed that the penetration resistance of the soil significantly increased with
341   soil depth. This finding supports our result regarding the vertical distribution of soil seed bank;
342   that is, the more compact the soil was, the fewer seeds it contained.
343
344   ***Role of plant traits in vertical seed distribution***
345   Forb species were more abundant than graminoids in the seed bank, as has also been shown in
346   other studies (Douh et al., 2018; Jutila, 1996; Ma et al., 2010). Although, short-lived species
347   were dominant over perennials in the terms of species number, the seedling number of perennials
348   was higher, suggesting a higher seed bank density. Seeds of grassland species were represented
349   by a significantly higher density in the soil seed bank than weed species. Although forbs,
350   grassland species and short-lived species occurred in a relatively large abundance in the deeper
351   soil layers, graminoids, weeds and perennial species were scarce. A possible reason for this
352   pattern is that opportunistic germination strategy is vital in the case of short-lived plant species,
353   so they tend to have a greater longevity and might penetrate deeper than perennials (Jutila,
354   1996). The higher abundance of forbs compared to graminoids in the deep soil layers may be due
355   to the fact that many graminoids have elongated seeds, therefore they cannot penetrate as deeply
356   as rounded seeds (see also Bekker et al., 1998). Our findings that deeper soil layers were
357   characterised by seeds of grassland species rather than weeds imply that seeds of grassland

358   species have the potential to tolerate harsh environments for a longer time. The aboveground
359   vegetation in the studied habitat type is species-poor and dominated by one perennial grassland
360   specialist grass, *Festuca pseudovina*, with a percentage cover above 60% (see Deák et al., 2014;
361   Valkó et al., 2014, 2017). Despite its dominance in the aboveground vegetation, *F. pseudovina*
362   occurred with low density in the soil seed bank. The aboveground vegetation of alkaline
363   grasslands contains very few weed species in low cover, due to the high salt content of the soil
364   (Valkó et al., 2014) which was also reflected in the seed bank composition found in the current
365   study.
366
367   Our results suggest that there is no difference in the depth distribution of the seeds of species
368   with different seed mass and ecological indicator values. However, we found that seed shape was
369   an important factor in determining the vertical distribution of seeds. Seeds with low seed shape
370   index, i.e. round-shaped seeds, were dominant over elongated ones in deeper soil layers. This
371   result corresponds to the findings of Bekker et al. (1998) in relation to seed size, shape and
372   vertical distribution in the soil.
373

374   ***Implications for conservation and restoration***
375   The periodic drying and rewetting characteristic of soils with Vertic horizon can cause cracks
376   down to a depth of 1 m or more (Coulombe et al., 1996). This crack-formation may be a major
377   driver of the vertical distribution of the seed bank. This assumption is supported by the findings
378   of Burmeier et al. (2010) who studied the impacts of desiccation cracks on soil seed bank
379   formation in a flood-meadow ecosystem. They found that the distribution and composition of the
380   seed bank were different in samples taken along cracks and in samples taken beside them, as
381   these cracks acted as natural seed traps. Desiccation cracks also had an effect on the vertical
382   translocation of the seeds. The survival rate of these entrapped seeds increased with increasing
383   depth, which could imply that in combination with other abiotic factors desiccation cracks cause
384   a selection pressure on plant species to develop long-term persistent seed banks. Furthermore,
385   their study showed that desiccation cracks influenced the establishment of the seed bank, and
386   therefore, were an important driver of community assembly and dynamics in flood-meadows.
387

388   Our findings draw attention to the potential ecological importance of the deeply buried seeds in
389   Vertisols and other reference soil groups with Vertic horizons. Our results originate from a single
390   region and ecosystem type, and the number of samples was also limited by the field and
391   greenhouse capacities. Despite the limitations in the sampling strategy, our results show that
392   germinable seeds in Vertisols occur in deeper layers than previously thought. As Vertisols cover
393   335 million hectares worldwide (IUSS WG WRB, 2015) and further extended areas are covered
394   by soils with Vertic horizons, these findings may be relevant for many regions and ecosystems,
395   such as grasslands, savannas, shrublands and open forests, globally. Vertisols occur in the tropic
396   or semiarid regions in Australia, the Americas, India and Northeastern Africa (Coulombe et al.,
397   1996). To test the generality of our findings, studies are needed in other regions and ecosystems

398 with Vertisols on the vertical distribution of the soil seed bank. We highlight the need for similar
399 studies in other soil and habitat types to test whether the presence of deeply buried seeds is a
400 specific characteristic of Soils with Vertic horizons.
401
402 There are different views about the role of the persistent seed banks in the formation of species
403 richness in stressed communities, such as alkaline grasslands (Valkó et al., 2014). Some
404 researchers found that clonal spread has a greater role in stressed environment than sexual
405 reproduction, thus, persistent seed bank is less important for habitat restoration and maintaining
406 diversity (Chang et al., 2001). In contrast, others showed that despite the temporarily unsuitable
407 environmental conditions, species are able to germinate and establish from the persistent seed
408 bank; therefore, persistent seed banks may play an important role in the vegetation dynamics of
409 communities in stressful environments (Bossuyt & Honnay, 2008; Valkó et al., 2014). Our
410 results support the latter findings and imply that the persistent seed bank in the deeper soil layers
411 of alkaline grasslands can be a promising source for habitat restoration even many years after
412 degradation.
413
414 Our knowledge on the depth distribution of seed banks and the ecological significance of deeply
415 buried seeds is still limited. Our current study suggests that deep soil layers might contain more
416 germinable seeds than previously thought. Our results may 1) contribute to the better
417 understanding of the concept of seed bank persistence; 2) provide information about the past
418 vegetation of a given area, about the past land use practices and past abiotic and climatic
419 changes; 3) provide a better knowledge on the present conservation value of the studied area; 4)
420 contribute to the planning of conservation measures as well as habitat restoration design by
421 providing more detailed information on the seed bank dynamics of a given area.
422 Based on these results, the question arises whether these deeply buried seeds have a chance for
423 germinate. There are several natural and anthropogenic processes by which the deeply buried
424 seeds may return to the soil surface (Douh et al., 2018). For instance, the activity of the soil
425 fauna – especially the movements of soil-dwelling rodents – may enable buried seeds to return to
426 the surface (Reichman, 1979; Valkó et al., 2021). Soil layers are mixed during building and
427 construction (such as road and railway construction and mining) and agricultural activities (such
428 as ploughing) that can bring deeply buried seeds to the surface. These activities often occur in
429 the studied landscape, for instance via the establishment of drainage ditch systems, soil-filling of
430 channels and restoration of landscape scars (Deák et al., 2015; Valkó et al., 2017). It has been
431 observed in areas affected by such activities that salt-tolerant species (*Suaeda* spp., *Salsola soda*)
432 absent from the vegetation of the actual landscape, reappeared in the restored sites (Deák et al.,
433 2015; Valkó et al., 2017). These observations also suggest that through the mixing of soil layers,
434 deeply buried seeds may play an active role in vegetation dynamics. Besides the unintended
435 mixing of soil layers, restoration projects might actively activate deeply buried seeds by targeted
436 topsoil removal. Future studies need to clarify whether and how the deeply buried seeds can be
437 used in restoration projects.

438

Conclusions

440

441 In this study we found that in alkaline grasslands with Vertic soil horizon, the density and
442 species richness of soil seed bank decreases along a vertical gradient but germinable seeds can be
443 found also in deep soil layers down to 80 cm depth. This is the first study that found germinable
444 seeds in such deep soil layers in non-sandy soil. Our results suggest that it is important to also
445 consider the deep soil layers in seed bank studies and restoration ecological research as these soil
446 layers can also contain germinable seeds of the native species characteristic of the plant
447 community. Our results showed that round-shaped seeds can be found in deeper soil layers than
448 elongated ones, but seed mass, water requirement and salt tolerance of the plant species did not
449 affect the vertical distribution of the seed bank. Based on the results, the most important research
450 directions may be 1) analysing the vertical distribution of soil seed bank in other ecosystem and
451 soil types, 2) testing the role of natural and anthropogenic processes in mixing the soil layers and
452 bringing the deeply buried seeds closer to the soil surface and 3) to study the possibilities for
453 utilizing the deeply buried seeds of native species in ecological restoration projects.

454

Acknowledgements
456 The authors are grateful for Ferenc Báthori and Szilvia Radócz for their assistance during
457 fieldwork, and for Csaba Albert Tóth and István Kapocsi for discussions about the topic.

458

459

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Results of the generalized linear models (GLMs) about the effect of burial depth on the dependent variables.

Results of the generalized linear models (GLMs) about the effect of burial depth on the seedling number and species richness of the morphological, ecological and life-form groups, as well as on the CWMs of the studied traits. Significant effects are marked with boldface.

Dependent variable	Estimate	Standard error	p
No. seedlings	**-0.5043**	**0.1271**	**< 0.001**
Species richness	**-0.2505**	**0.0387**	**< 0.001**
Forbs, no. seedlings	**-0.4004**	**0.0785**	**< 0.001**
Forbs, species richness	**-0.2415**	**0.0416**	**< 0.001**
Graminoids, no. seedlings	-0.7413	1.1923	0.535
Graminoids, species richness	**-0.2758**	**0.0782**	**< 0.001**
Perennials, no. seedlings	-0.7351	0.9855	0.456
Perennials, species richness	**-0.3106**	**0.0772**	**< 0.001**
Short-lived spp., no. seedlings	**-0.3779**	**0.0749**	**< 0.001**
Short-lived spp., species richness	**-0.2284**	**0.0424**	**< 0.001**
Grassland spp., no. seedlings	**-0.5518**	**0.1917**	**0.004**
Grassland spp., species richness	**-0.2589**	**0.0456**	**< 0.001**
Weeds, no. seedlings	**-0.2434**	**0.0670**	**< 0.001**
Weeds, species richness	**-0.2513**	**0.0635**	**< 0.001**
CWM, seed mass	0.0055	0.0309	0.860
CWM, WB	-0.0084	0.0074	0.259
CWM, SB	-0.0150	0.0129	0.251
CWM, seed shape index	**-0.0037**	**0.0019**	**0.048**

Results of the generalized linear models (GLMs) about the effect of soil penetration resistance on thedependent variables.

Results of the generalized linear models (GLMs) about the effect of soil penetration resistance on the seedling number and species richness of the morphological, ecological and life-form groups, as well as on the CWMs of the studied traits. Significant effects are marked with boldface.

Dependent variable	Estimate	Standard error	p
No. seedlings	**-0.0056**	**0.0016**	**< 0.001**
Species richness	**-0.0036**	**0.0009**	**< 0.001**
Forbs, no. seedlings	**-0.0057**	**0.0015**	**< 0.001**
Forbs, species richness	**-0.0033**	**0.0010**	**< 0.001**
Graminoids, no. seedlings	**-0.0055**	**0.0024**	**0.023**
Graminoids, species richness	**-0.0044**	**0.0015**	**0.003**
Perennials, no. seedlings	**-0.0061**	**0.0022**	**0.007**
Perennials, species richness	**-0.0051**	**0.0014**	**< 0.001**
Short-lived spp., no. seedlings	**-0.0053**	**0.0014**	**< 0.001**
Short-lived spp., species richness	**-0.0030**	**0.0010**	**0.002**
Grassland spp., no. seedlings	**-0.0057**	**0.0017**	**< 0.001**
Grassland spp., species richness	**-0.0035**	**0.0010**	**< 0.001**
Weeds, no. seedlings	**-0.0053**	**0.0018**	**0.004**
Weeds, species richness	**--0.0054**	**0.0017**	**0.002**
CWM, seed mass	-0.0003	0.0008	0.754
CWM, WB	-0.0002	0.0002	0.452
CWM, SB	-0.0004	0.0003	0.196
CWM, seed shape index	**-0.0001**	**0.0001**	**0.037**

Figure 1

The effect of burial depth (layers 1-16) on the seedling number and species richness in the studied alkali grasslands.
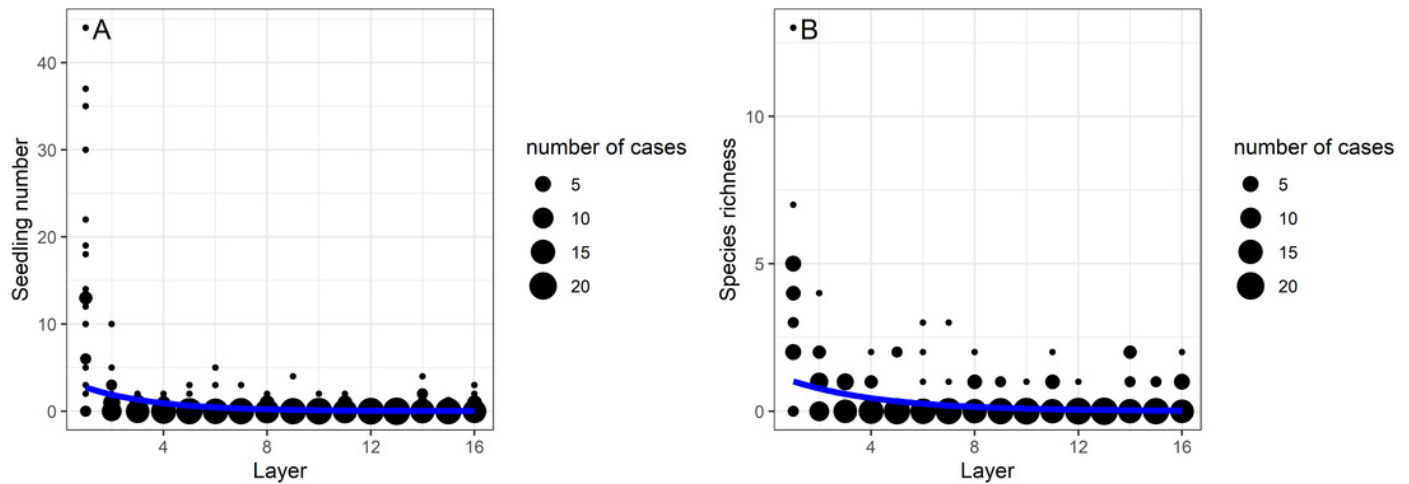
Figure 2

The effect of burial depth on the seedling number of the studied morphological (forbs, graminoids), life-form (short-lived species, perennials) and ecological groups (grassland species, weeds).

The effect of burial depth (layers 1-16) on the seedling number of the studied morphological (forbs, graminoids), life-form (short-lived species, perennials) and ecological groups (grassland species, weeds). Layers 9-13 are marked with red to highlight the contrasting distribution of the different species groups in this zone with high penetration resistance (see Figure 3).
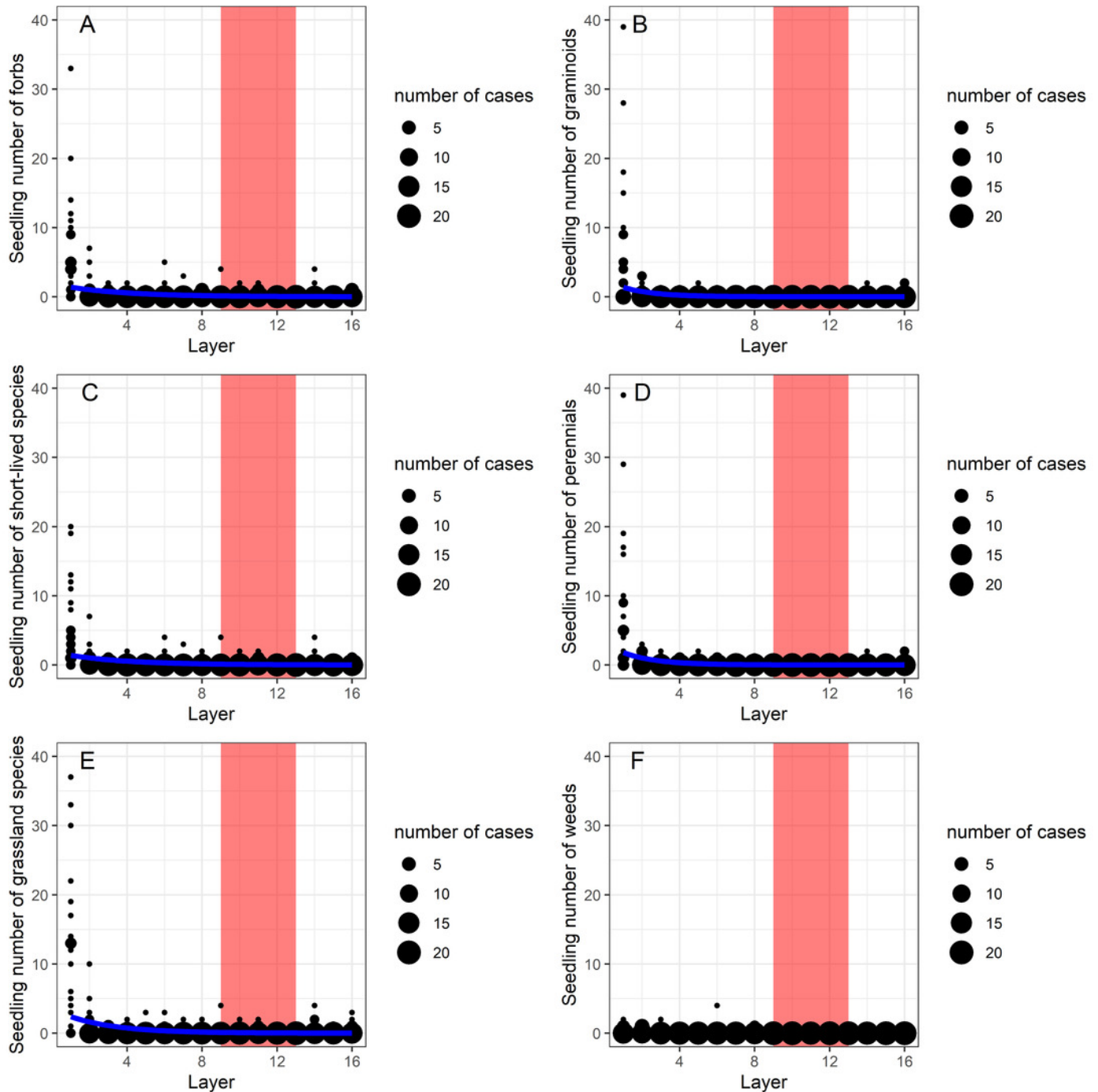
Figure 3

Penetration resistance curves (0-80 cm) in the studied Vertic Solonetz soils.