Acidity and availability of aluminum, iron and manganese as factors affecting germination in European acidic dry and alkaline xerothermic grasslands

Mateusz Wala[1,*], Jeremi Kołodziejek[1], Tomasz Wilk[2,3]

[1]University of Lodz, Faculty of Biology and Environmental Protection, Department of Geobotany and Plant Ecology; Banacha 12/16, 90-237 Łódź, Poland

[2]Przedsiębiorstwo Produkcyjno-Consultingowe ADOB Sp. z o.o. Sp. jawna; Kołodzieja 11, 61-070 Poznań, Poland

[3]Faculty of Chemistry, Adam Mickiewicz University in Poznań; Uniwersytetu Poznańskiego 8, 61-614 Poznań, Poland

Corresponding Author:

Mateusz Wala[1]

Banacha 12/16, 90-237 Łódź, Łódź Voivodeship, Poland

Email address: mateusz.wala@biol.uni.lodz.pl

Abstract

Germination ecology of 10 species from acidic dry grasslands and 10 species from alkaline xerothermic grasslands was studied. The seeds were subjected to different pH, iron (Fe), manganese (Mn) and aluminum (Al) treatments under controlled conditions. Effects of ionic (chlorides) and chelated forms (HBED chelates) of Fe and Mn were also compared. Final germination percentage (FGP) and index of germination velocity (IGV) were calculated. The results indicate that pH and extremely high availability of Al are the major edaphic filters

29   regulating germination-based revegetation, while availability of Fe and Mn is of the secondary

30   importance. Both chelates and ionic forms of Fe and Mn exerted similar effects on the ability of

31   seeds to complete germination. It suggests that both chelates are not hazardous for early

32   ontogenetic stages of plants. Neither group has group-specific adaptations pertaining to

33   germination characteristics in the context of the studied chemical stimuli, which indicates a

34   diversity of germination strategies and individual species-specific reactions to the tested factors.

Introduction

37   Germination is a process which is controlled by environmental conditions (Koornneef, Bentsink

38   & Hilhorst, 2002). Light, temperature and availability of water are known to be crucial factors

39   affecting completion of germination of seeds (Gibson, 2009; Graeber et al., 2012). However,

40   there are also many secondary (but important) abiotic factors affecting germination, such as

41   availability and type of nitrogen-containing compounds and other macronutrients, acidity (pH),

42   availability of micronutrients and nonessential trace/ballast elements as well as the amount and

43   type of pollutants (Graeber et al., 2012; Kranner & Colville, 2012). Therefore, completion of

44   germination that is observed in the field depends on a multifactorial interaction between

45   environmental determinants. These stimuli directly affect metabolism of seeds and/or they are

46   recognized and translated into a signal affecting hormonal equilibrium that prevents or triggers

47   germination (Nonogaki, 2017; Duermeyer et al., 2018).

48   Grasslands are plant communities characterized with high solar irradiance and light

49   heterogeneity (Bakker, Blair & Knapp, 2003). Light that reaches the soil surface of 'vegetation

50   gaps', where completion of germination is most favored, is not strongly filtered due to the lack of

51   dense canopy. Thus, it has an optimal spectrum that supports the germination process of

52   photoblastic-positive plant species (Deregibus et al., 1985). Temperature of soil surface, where

53   seeds of photoblastic-positive plant species complete germination, depends partially on physical-

54   chemical properties of the soil (Bothe, 2015). Although non-calcareous sandy soils and

55   calcareous soils can reach respectively 70 °C and 60 °C on a sunny day in summer (Jentsch &

56   Beyschlag, 2003; Bothe, 2015), the temperatures of soil surface and diurnal amplitude of soil

57   temperature in spring and autumn (when seeds of many herbs complete germination; Czarnecka,

58   2004) are lesser (Burmeier et al., 2010). This creates a safe environment in terms of major

59   factors controlling the germination process. Soil solution, which makes imbibition of seeds

60 possible, contains a wide spectrum of chemical compounds (Marques et al., 1996). Many of them

61 interfere with the germination process, enhancing (e.g., the appropriate form and dose of

62 nitrogen; Duermeyer et al., 2018) or halting it (e.g. due to toxicity of ions or due to supraoptimal

63 osmoticum; Kranner & Colville, 2012).

64 Dry acidic grasslands from *Koelerio-Corynephoretea* Klika in Klika et V. Novák 1941

65 class and xerothermic alkaline grasslands from *Festuco-Brometea* Br.-Bl. et R.Tx. 1943 class are

66 the priority types of habitats in Europe (*6120 and *6210, respectively; Council Directive,

67 1992). Plants from those habitats share two common traits, namely, tolerance to heat stress and

68 high irradiance (Leuchner & Ellenberg, 2017). However, there are several iconic characteristics

69 of each type of soil that differ them (Lee, 1999; Bothe, 2015). Soils of dry acidic grasslands

70 (mostly Podzols) are known to be remarkably acidic and to contain high concentrations of

71 available Al, Mn and Fe due to low pH (Abedi, Bartelheimer & Poschlod, 2013; Strawn, Bohn

72 & O'Connor, 2020). Depending on many soil-forming factors, concentration of Ca in non-

73 calcareous sandy soils vary, but it is established that typical inland sandy soils (not affected by

74 marine or alluvial deposition) are poor in this element (Török et al., 2009). Soils on which

75 alkaline xerothermic grasslands develop (mostly Rendzinas) are completely opposite – they are

76 rich in Ca and are characterized with low acidity due to composition of bedrock (in most cases

77 bedrock is rich in $CaCO_3$; sometimes it originates from $CaSO_4$ – then they are susceptible to

78 acidification). Furthermore, due to high pH, several elements, e.g., Fe, Mn and Al are

79 precipitated in insoluble forms (Bothe, 2015; Strawn, Bohn & O'Connor, 2020). Considering two

80 major characteristics of alkaline and acidic soils, namely availability of Ca and soil pH, plants on

81 calcareous soils are recognized as calcicoles (sometimes referred to as basophiles or calciphiles),

82 whereas those on silicate soils are calcifuges (or calciphobes/acidophiles, considering their

83 reaction to Ca and pH; Bothe, 2015). Interestingly, the abovementioned factors (pH and

84 availability of Ca, Fe, Mn and Al) were only rarely studied in the past as the factors affecting

85 germination process in those types of grasslands.

86 It is known that different forms of mineral nutrients and ballast elements are not equally

87 available for plants. For example, availability of Al is driven by soil pH, as soil solutions of

88 acidic soils are remarkably rich in this element, while in alkaline soils its availability is definitely

89 lesser (Abedi, Bartelheimer & Poschlod, 2013; Bothe, 2015). In the case of Fe and Mn, the most

90 common species available for plants are their free ions (strictly limited source) and complexed

91   forms (Bothe, 2015; Shao et al., 2017). Relatively much is known of Fe and Mn nutrition and

92   effects of availability of these two elements on vegetative growth of higher plants (Andresen,

93   Peiter & Küpper, 2018). Chelated Fe can be readily acquired and utilized (Strategy II plants) or

94   reduced and then acquired by plants (Strategy I plants; Grillet & Schmidt, 2017). Furthermore,

95   Fe and Mn are subjected to constant ligand (chelator) exchange (including competition of these

96   metals for a given chelator), which probably shifts their availability in soil environment (Yehuda

97   et al., 1996; Duckworth, Bargar & Sposito, 2009). The phenomenon of metal chelation (by

98   biological as well as synthetic chelators, e.g., N,N'-di(2-hydroxybenzyl)ethylenediamine-N,N'-

99   diacetic acid; HBED) underlies research on soil chemistry and nutrition of plants (where chelates

100   can be used as a tool; Strawn, Bohn & O'Connor, 2020; Wala et al., 2020), but it was also found

101   to be a remedy for losses of productivity in agriculture (Venturas et al., 2014). Even apart from

102   the scientific and economical significance of this problem, there are some other practical issues

103   associated with the influence of ionic and chelated forms of Fe and Mn on plants. Although the

104   usage of metal chelates in agriculture and horticulture has gained popularity in recent years, not

105   much is known about their environmental effects and safety, including their influence on the

106   early ontogenetic stages of plants. According to the available data, HBED chelates were not

107   studied in this context. Furthermore, Mn-HBED was never investigated in the context of plant

108   physiology, thus nothing is known about its mode of action and safety.

109   Although functional ecology of seeds, including effects of habitat sieves (e.g., edaphic

110   factors) has recently gained more attention in plant ecology (Poschlod, 2020), there is only

111   fragmental information about germination patterns that can be observed in temperate grasslands.

112   For example, germination-related requirements pertaining to pH of calcicole and calcifuge plant

113   species from temperate grasslands are hardly known. Furthermore, although influence of metals

114   on the completion of germination was solicitously studied and reviewed, information pertaining

115   to the role of Al as well as Fe and Mn (both in ionic and complexed forms) in this process is very

116   scarce (Kranner & Colville, 2012). So far, some studies addressed only the problem of influence

117   of Al toxicity on the ability of seeds to complete germination in some wild-living plant species

118   (Abedi, Bartelheimer & Poschlod, 2013). Therefore, we performed the experiments to shed light

119   on less studied aspects of germination ecology of the selected plant species from contrasting

120   European grasslands (Table 1). The following hypotheses were addressed: 1) calcicole plant

121   species (in terms of their centers of abundance) prefer alkaline pH while calcifuge plant species

122   prefer acidic pH for completion of germination; 2) availability of iron (Fe-HBED and FeCl$_3$) and

123   manganese (Mn-HBED and MnCl$_2$) impacts germination-related characteristics, and 3)

124   increasing concentration of AlCl$_3$ affects germination, whereby acidophilous species are more

125   resistant than basophilous ones. The following questions were addressed: 1) do pH and

126   availability of Fe, Mn and Al influence the ability of seeds to complete germination? 2) do

127   HBED-chelated Fe and Mn affect germination to a greater extent than their chlorides? 3) is Al a

128   real threat to germination-based revegetation?

129

Materials & Methods

131   **Criteria of species selection and collection of seeds.** The plant species used in this study were

132   selected based on their habitat preferences (Table 1). In total, 20 species were selected (10 from

133   acidic dry grasslands and 10 from alkaline xerothermic grasslands). Their occurrence on the

134   respective sites in central Europe was also taken into consideration (Czyżewska, 1992; Mucina &

135   Kolbek, 1993; Matuszkiewicz, 2001; Kącki, Czarniecka & Swacha, 2013, as well as Illustrierte

136   Flora von Mitteleuropa; Hegi, 1931). According to the available literature (Ellenberg, 1991), all

137   the selected species are light-loving plants that can be found in warm and relatively dry sites

138   characterized with low nitrogen soil status (Table 1). Furthermore, the selected plant species

139   belong to different plant families. With such a wide selection of species, we intended to examine

140   the most common as well as most valuable plants that can be found in the studied communities.

141   The propagules (fruits – achenes for the studies species from Asteraceae and Rosaceae,

142   nutlets for the studied species from Boraginaceae and Lamiaceae – and seeds for rest of the

143   studied species; therein after referred as seeds) were manually collected from plants cultivated in

144   the Didactic-Experimental Garden of Faculty of Biology and Environmental Protection,

145   University of Lodz (51°78'N, 19°48'E; 223 m a.s.l.), at full maturity stage in summer, 2019

146   (exact timing depended on the species). The seeds were gathered from at least 20 healthy and

147   representative plant individuals growing in homogenous and optimal conditions (matching

148   individual requirements for growth and reproduction of each species). The seeds were stored in

149   paper bags in the laboratory for 14 days (at a relative humidity of 30%). Then they were

150   inspected (all malformed, discolored and damaged seeds were removed) and weighed (four lots

151   of 100 randomly selected seeds). Dimensions of the seeds were taken from the literature

152 (Bojñnanský & Fargašová, 2007). Then, the seeds were stratified in a refrigerator (5 °C) for 16

153 weeks.

154

155 **General germination procedures.** The ability of seeds to complete germination was tested on

156 glass Petri dishes (ø = 5 cm). The dishes were lined with two pieces of filter paper (Whatman no.

157 1; GE Healthcare, Chicago, IL, United States). The seeds were mixed before the tests in order to

158 fulfil the randomization requirement. On each dish, 25 seeds were placed and moistened with 1.5

159 cm^3 of the tested solutions.. Subsequently, the dishes were sealed with parafilm to maintain

160 homogenous moisture and humidity and placed in a germination cabinet. All experiments were

161 conducted under identical conditions of fully-controlled thermo-photoperiod (16 h of light phase

162 at 25 °C and 8 h of dark phase at 15 °C; light was supplied with white fluorescent lamps, PAR =

163 40 $\mu mol\ m^{-2}\ s^{-1}$, measured with FluorPen; PSI, Drasov, Czech Republic). Those photo-thermal

164 conditions were selected due to the fact that many of the species we studied complete

165 germination in spring (Czarnecka, 2004), when air temperature is relatively high in central

166 Europe. This thermoperiod was previously used also in other multispecies investigations

167 focusing on a similar topic (Tudela-Isanta et al., 2018) and it is within optimal temperature range

168 allowing completion of germination of many species from the temperate zone, including those

169 from temperate grasslands (Baskin & Baskin, 1998; Dürr et al., 2015; Ladouceur et al., 2019).

170 Furthermore, based on the data pertaining to the seed weight of the species we studied (in most

171 cases the seeds weighted < 1.5 mg; Table 1), they are likely to be photoblastic positive or at least

172 photoblastic neutral (Jankowska-Blaszczuk & Daws, 2007), which was confirmed by preliminary

173 tests showing that all the species we studied were able to complete germination under the

174 described conditions. All tested solutions were prepared using distilled water (conductivity <

175 0.08 $\mu S\ cm^{-1}$). The pH values of each solution (Supplemental Table 1) were measured with

176 digital pH-meter (CP-401; Elmetron, Zabrze, Poland). Completion of germination was counted

177 daily for 21 d. During the experiments, the seeds were monitored for any signs of their

178 unviability (seed softness and brownish embryo color), however no such situation was observed.

179

180 **Experiment 1: Effects of pH.** Phosphate buffer (KH_2PO_4:Na_2HPO_4; 0.066 mol · dm^{-3}) was

181 used in this study as a medium ensuring the desired pH. It was the buffer of choice due to its

182 relatively wide range of available pH values. Moreover, it is not toxic to cells and its acidity does

183   not change substantially with change of temperature. Furthermore, each used phosphate species

184   co-occurs in soil with a given soil pH, i.e., $H_2PO_4^-$ occurs predominantly in acidic soils, while

185   HPO_4^{2-} in alkaline ones (Strawn, Bohn & O'Connor, 2020), thus it reflects the available forms of

186   P in the studied grasslands (Leuchner & Ellenberg, 2017). The effect of pH on germination was

187   studied within the pH range of 5.0–8.0 in increments of 1.0 pH unit (similarly to setup used

188   elsewhere; Tudela-Isanta et al., 2018). The buffers were prepared using analytical grade salts.

189   The experiment was run according to the setup described in 'General Germination Procedures'.

190

191   **Experiment 2: Effects of ionic and complexed iron.** Fe-HBED chelate (*N,N*'-di(2-

192   hydroxybenzyl)ethylenediamine-*N,N*'-diacetic acid iron(III) sodium salt; 7% Fe; synthesized

193   according to European Patent Application EP20461587.6 by PPC ADOB, Poland, and

194   authenticated using LC-MS/MS, FTIR and UV-VIS analyses; Supplemental Figure 1) was used

195   as a complexed form of Fe (chelation rate 1:1 with favored Fe(III) oxidation state). This agent is

196   characterized by high stability under a wide range of conditions, including pH, thus it can be

197   used as a tool simulating natural metal chelation under high soil pH. Anhydrous $FeCl_3$ of

198   analytical quality was used as the ionic form of Fe^{3+}. The effect of Fe was evaluated using

199   isomolar concentrations of both compounds (0, 5 and 25 $\mu mol \cdot dm^{-3}$). This range of

200   concentrations was the same as that used in our previous studies evaluating physiological

201   response of plants at vegetative stage (Wala et al., 2020). These concentrations were also within

202   the range maintaining optimal plant growth under hydroponic conditions (Venturas et al., 2014;

203   Kasozi et al., 2019) and are higher than the concentration of available Fe in a soil solution of

204   Rendzinas (picomolar/nanomolar values; Bothe, 2015). The experiment was run according to the

205   setup described in 'General Germination Procedures'.

206

207   **Experiment 3: Effects of ionic and complexed manganese.** The complexed form of Mn

208   (chelation rate 1:1 with favored Mn(III) but possible Mn(II) oxidation state; Pinto et al., 2019)

209   was supplied as Mn-HBED (*N,N*'-di(2-hydroxybenzyl)ethylenediamine-*N,N*'-diacetic acid

210   manganese (II) sodium salt; 7% Mn; synthesized by PPC ADOB, Poland). HBED was selected

211   as Mn-complexing agent in order to reduce bias resulting from comparison of different chelators

212   (in this case Fe-HBED vs Mn-HBED). Furthermore, high stability of HBED under alkaline

213 conditions simulates chelation process in the studied type of community (alkaline xerothermic

214 grassland).

215   Mn-HBED was synthesized as following: a 250 cm^3 flask with a magnetic stirrer, a pH-

216 meter and a reflux condenser was charged with 100 g of water and 23.96 g (0.05 mol) of *N,N'*-

217 di(2-hydroxybenzyl)ethylenediamine-*N,N'*-diacetic acid (HBED) monohydrochloride (purity

218 88.6%). Next, 1 mol · dm^{-3} solution of sodium hydroxide (NaOH) was added to the slurry until

219 pH 7 was reached. After complete homogenization, 10.39 g (0.0525 mol) of MnCl$_2$ · 4H$_2$O

220 (purity 99%) in 30 ml of water were added in portions. During the addition of MnCl$_2$ · 4H$_2$O, pH

221 was maintained in range of 6–8 using 1 mol · dm^{-3} solution of NaOH. After the complete

222 addition of MnCl$_2$ · 4H$_2$O, mixture was stirred for 1 hour at room temperature. Then, the mixture

223 was alkalized to pH 11 using a 1 mol · dm^{-3} solution of NaOH. The solution was left without

224 stirring for 24 hours in darkness to allow excess Mn (to precipitate as oxide) then filtered through

225 a cellulose filter and the solvent was removed *in vacuo*. Finally, 23.50 g of *N,N'*-di(2-

226 hydroxybenzyl)ethylenediamine-*N,N'*-diacetic acid, manganese(II) sodium salt, were obtained.

227 The product assay was 7.33% of manganese according to EN 16963:2018-03. Mn-HBED was

228 authenticated using LC-MS/MS, FTIR and UV-VIS analyses (Supplemental Figure 1).

229   The ionic form of Mn was supplied with analytical grade MnCl$_2$ · 4H$_2$O. The effect of Mn

230 was evaluated using concentrations equal to those used in Experiment 2 (0, 5 and 25 μmol ·

231 dm^{-3}). These concentrations were used in order to 1) compare the effects of Fe and Mn and 2)

232 simulate Mn-over-supplemented environment, more Mn-rich than a soil solution (where average

233 availability of Mn ranges c.a. 0.1–10 μmol · dm^{-3} soil solution and in near-alkaline and acidic

234 soils is close to 0.4 and 16.0 μmol · dm^{-3} soil solution, respectively; Marschner, 1988; Shao et

235 al., 2017) and artificial media optimal for soil-less plant cultivation (e.g. Hoagland solution,

236 where Mn concentration in full strength medium is equal to 9 μmol · dm^{-3}; Asher & Edwards,

237 1983). The experiment was run according to the setup described in 'General Germination

238 Procedures'.

239

240 **Experiment 4: Effects of aluminum.** The test was conducted according to the available data

241 reporting a nonlinear relation between exchangeable Al and the pH of soil (Abedi, Bartelheimer

242 & Poschlod, 2013). Analytical grade AlCl$_3$ · 6H$_2$O was used as source of ionic Al (Abedi,

243 Bartelheimer & Poschlod, 2013). The effect of Al on seed germination was evaluated using 0.00,

244   0.01, 0.10, 1.00 and 10.00 mmol · dm^{-3} solutions, which reflects the availability gradient of this

245   element from alkaline to acidic soils (Abedi, Bartelheimer & Poschlod, 2013). Tested solutions

246   were prepared using the serial dilution method. The experiment was run according to the setup

247   described in 'General Germination Procedures'.

248

249   **Calculation of germination-related parameters.** To determine reactions of the species we

250   studied, the final germination percentage (FGP; ranging 0–100%) and index of germination

251   velocity (IGV, known as modified Timson's index, ranging 0–100; Khan & Ungar, 1997) were

252   calculated. The greater values of FGP and IGV, the greater ability to complete germination or the

253   more rapid germination, respectively. The IGV was calculated with the germinationmetrics

254   package v. 0.1.3 (Aravind et al., 2020) run in R software (v. 3.5.2, 64 bit version; R Core Team,

255   2018) using preprogrammed equations of the package.

256

257   **Statistical analysis.** All experiments were replicated four times (n = 4). Normality of the data

258   was analyzed with the Kolmogorov–Smirnov's test and homogeneity of variances was analyzed

259   with the Brown–Forsythe's test. To detect differences between treatments within a given species,

260   one-way ANOVA followed by the Bonferroni post-hoc test was mounted (differences were

261   accepted as statistically significant at p < 0.05). To inspect effects of the studied factors, two-way

262   ANOVA (Experiment 2 and Experiment 3) and three-way ANOVA (comparison between

263   Experiment 2 and Experiment 3) were used. Differences between effects of isomolar solutions of

264   Fe and Mn (Experiment 2 and Experiment 3, respectively) were detected with two-way ANOVA

265   followed by the Bonferroni post-hoc test (differences were accepted as statistically significant at

266   p < 0.05). Correlation between seed weight and germination reaction was calculated with

267   Spearman's sum rank test. Analysis was conducted for all tested species (n = 20) as well as for

268   separated groups of acidophilous (n = 10) and basophilous (n = 10) species. For each FGP value,

269   the data were normalized to FGP values recorded at pH = 7 (Experiment 1) or control (null

270   concentration) conditions (Experiments 2–4). Correlation was considered as statistically

271   significant at p < 0.05. All statistical analyses were performed with Statistica™ v. 13.3 (Tibco

272   Software Inc.; Palo Alto, CA, USA).

273   To find out if there are similarities between the studied species (segregation of species on

274   the basis of germination strategies), FGP-based hierarchical cluster analysis (HCA) was

275   performed, merging data from Experiments 1–4. For each species, in order to reduce bias from

276   the species-specific reaction (namely due to differences in completion of germination under

277   control conditions), the data were normalized by calculation of ratio of FGP value at given

278   treatment and FGP value recorded at pH = 7 (Experiment 1; due to neutral acidity and the most

279   proportional KH_2PO_4: Na_2HPO_4 ratio) or control (null concentration) conditions (Experiments 2–

280   4) as it was done for calculation of correlations. Phenograms were constructed using Ward's

281   method of row clustering and Manhattan distance and the tightest clusters were presented first.

282   HCA analysis was performed using ClustVis online tool (Metsalu & Vilo, 2015) with mean

283   values from normalization, as proposed recently (Chen et al., 2020).

284

Results

286   **Experiment 1: Effects of pH.** FGP values did not depend on pH of the buffered solutions in Cst

287   and Dde (Figure 1A), whilst in the other studied species they were differentially regulated.

288   Acidophilous species showed preference to acidic conditions (Amo, Hpi, Hpe, Hra, Tse and Vth)

289   and neutral conditions (Evu) or the preference was not clear (Rac; Figure 1A). On the other hand,

290   basophiles showed 4 general types of reactions: 1) preference for near-neutral pH (Csc, Gcr,

291   Vte), 2) good completion of germination at acidic and neutral pH with strongly marked

292   inhibition of completion of germination at alkaline pH (Aam, Bof, Pme, Pre, Pgr and Vte), 3)

293   relatively wide tolerance of the tested pH values of the buffered solutions with marked

294   acidophilism (Dca) and 4) strong preference to neutral and alkaline conditions with marked

295   decrease in FGP under acidic conditions (Sge) (Figure 1A). Interestingly, Tse showed marked

296   decrease in FGP at optimal pH value, when compared to null concentrations in Experiments 2–4

297   (Figure 1).

298   IGV values were nearly proportional to corresponding FGP values with two exceptions. In

299   the case of Aam and Gcr completion of germination was slow (Figure 1B). Germination speed in

300   Gcr did not dependent on the buffer composition, as this species completed germination with

301   similar velocity in Experiments 2–4, whilst in Aam this was probably caused by the compounds

302   present in the buffered solutions (KH_2PO_4 and Na_2HPO_4) or their concentration (when compared

303   to IGV values from Experiments 2–4).

304

305   **Experiment 2: Effects of ionic and complexed iron.** The tested Fe-containing compounds

306   affected completion of germination of the studied species except that of Bof, Csc, Cst and Tse

307   (Figure 2A). Considering the $FeCl_3$-treated seeds, reduction of completion of germination was

308   observed both after the treatment with 5 μmol · dm^{-3} (Gcr, Hpi and Pgr) and with 25 μmol ·

309   dm^{-3} (Aam, Dca, Dde, Gcr, Hpi, Hra, Pgr, Sge, Vth and Vte; Figure 2A). Similar results were

310   observed for Fe-HBED-treated ones, as the solution of 5 μmol · dm^{-3} negatively affected

311   completion of germination in Dca, Dde, Evu, Hpi and Hra, whilst that of 25 μmol · dm^{-3} reduced

312   completion of germination in Dca, Dde, Evu, Hpi, Hpe, Hra, Pme, Pre, Sge and Vth (Figure 2A).

313   Both $FeCl_3$ (5 and 25 μmol · dm^{-3}) and Fe-HBED (5 μmol · dm^{-3}) positively affected completion

314   of germination in Amo (Figure 2A).

315   Effects of the tested conditions on FGP and IGV values were similar (Figure 2B). With

316   exception for Gcr which germinated slowly but very successfully (Figure 2A).

317   Two-way ANOVA showed that the type of Fe-bearing compound influenced FGP values

318   in 9 species (Amo, Aam, Evu, Gcr, Hpi, Hra, Pre, Pgr and Rac), whilst the dose of tested

319   compound affected 8 species (Amo, Aam, Csc, Dde, Hpe, Hra, Sge and Vth; Supplemental Table

320   2). Interaction between the type and the dose of the tested compounds affected significantly 4

321   species in total (Amo, Dde, Hpe and Hra; Supplemental Table 2). IGV values were significantly

322   affected by Fe type in 11 species (Amo, Aam, Cst, Dca, Evu, Gcr, Hpi, Hpe, Hra, Pre, Pgr and

323   Rac) and by Fe dose in 12 species (Amo, Aam, Csc, Dca, Dde, Hpe, Hra, Pme, Rac, Sge, Vth

324   and Vte; Supplemental Table 2). Interaction between these factors affected IGV values in four

325   species (Amo, Dde, Hpe and Hra; Supplemental Table 2).

326

327   **Experiment 3: Effects of ionic and complexed manganese.** Mn treatments had no effects on

328   FGP values in Cst, Dca, Gcr, Hpi, Pre, Tse, Vth and Vte (Figure 3A). On the other hand, $MnCl_2$

329   solution of 5 μmol · dm^{-3} had negative effect on the ability of seeds to complete germination in

330   Aam, Bof, Csc, Hpe and Pme (Figure 3A). Decreases in FGP values were observed in Amo,

331   Aam, Bof, Csc, Hpe, Hra, Pme, Rac and Sge due to application of 25 μmol · dm^{-3} $MnCl_2$

332   solutions (Figure 3A). Negative effects of Mn-HBED were also observed in the species treated

333   with 5 μmol · dm^{-3} (Aam, Csc, Dde, Hpe and Pme) as well as with 25 μmol · dm^{-3} solutions

334   (Aam, Csc, Dde, Evu, Hpe, Hra, Pme and Pgr; Figure 3A).

335   The changes of IGV values were similar to those in FGP (Figure 3B). Similarly as it was

336   shown in Experiment 2, Gcr was proved to be an exceptionally slow germinator (Figure 3B).

337   Two-way ANOVA showed that the type of Mn-containing agent affected 5 species (Aam,

338   Bof, Dde, Pme and Rac) and Mn dose affected significantly 4 species (Amo, Csc, Hra and Rac;

339   Supplemental Table 2). Only one species was affected significantly by the interaction between

340   the tested factors (Rac; Supplemental Table 2). Similar effects were observed for IGV values

341   (where Aam, Bof, Dde, Pme and Rac were affected by Mn type and Amo, Csc, Hra and Rac

342   were affected by Mn dose; Supplemental Table 2), but in the case of interaction between Mn

343   type and dose, two species were affected significantly (Gcr and Rac; Supplemental Table 2).

344

345   **Comparison of iron and manganese as stimuli for germination.** Five species (Cst, Pre, Sge

346   Tse and Vth) had similar tolerance to both metals (Supplemental Table 3). Seven species

347   completed germination better in Fe than in Mn solutions (Amo, Aam, Csc and Rac in the case of

348   chlorides; Aam, Csc, Gcr and Hpe of HBED-chelated forms) while the others quite the opposite

349   (e.g., Dca for 25 μmol $\cdot$ dm^{-3} of chloride; Dde, Hra and Vte for 25 μmol $\cdot$ dm^{-3} of chlorides; Pgr

350   for both doses of chlorides; Hpi and Rac for both concentrations of HBED-chelated forms;

351   Supplemental Table 3).

352   Three-way ANOVA showed that the element (Fe/Mn) affected FGP values in 11 species

353   (Amo, Aam, Bof, Csc, Dca, Dde, Evu, Hpi, Hra and Pgr), the type of metal bearing compounds

354   influenced FGP values significantly in 11 species (Amo, Aam, Bof, Csc, Dde, Evu, Gcr, Hpi,

355   Hra, Pme and Pre) and the dose affected FGP values in 9 species (Amo, Aam, Csc, Hpe, Hra,

356   Rac, Sge and Vth; Supplemental Table 4). Interactions between two factors affected 5–11

357   species and interaction between all three factors affected only 5 species (Amo, Dde, Hpe, Hra

358   and Rac; Supplemental Table 4). Very similar effects were observed for IGV values

359   (Supplemental Table 4).

360

361   **Experiment 4: Effects of aluminum.** Six species (Amo, Cst, Dca, Rac, Tse and Vth) completed

362   germination well in all tested concentrations of Al, as their FGP values did not differ

363   significantly (Figure 4A). The solutions ≥ 0.01 mmol $\cdot$ dm^{-3} inhibited completion of germination

364   in Pgr and Sge, ≥ 0.1 mmol $\cdot$ dm^{-3} and greater solutions inhibited completion of germination in

365   Aam, Dde, Hpi and Pme, ≥ 1 mmol $\cdot$ dm^{-3} and greater solutions reduced FGP values in Bof, Hpe

366   and Pre and 10 mmol · dm^{-3} solutions reduced FGP values in Gcr, Hra and Vte (Figure 4A).

367   Interestingly, Al-stimulated completion of germination was observed in Hpe (0.01 mmol · dm^{-3})

368   and in Csc (10 mmol · dm^{-3}; Figure 4A). The observed patterns of FGP responses were similar

369   to those of IGV (Figure 4B).

370

371   **Classification of germination requirements and relations of seed traits to germination.** Seed

372   weight was negatively correlated with FGP values only when the seeds were subjected to 5 μmol

373   · dm^{-3} MnCl$_2$ (R = -0.447; p = 0.048; Supplemental Table 5). IGV values were also negatively

374   correlated with weight only when the seeds were subjected to 0.01 mmol · dm^{-3} AlCl$_3$ (R = -

375   0.503; p = 0.024 for all the studied species and R = -0.648; p = 0.043 for acidophiles;

376   Supplemental Table 5). All other correlations were insignificant (p > 0.05; Supplemental Table

377   5).

378   HCA analysis showed that acidity and extremely high concentration of Al (10.00 mmol ·

379   dm^{-3}) were major factors allowing clustering of the studied species, whilst the other studied

380   treatments had only minor contribution to species segregation (Figure 5). According to the HCA

381   analysis, five major groups of species were distinguished: 1) species with preference for sub-

382   neutral acidity (pH = 6) that are able to tolerate high doses of Al (Csc, Evu, Gcr and Vth); 2)

383   species with marked inhibition of germination completion at pH = 8 and reduced ability to

384   complete germination in the presence of Al (Sge, Bof, Pre and Pgr); 3) species with rather wide

385   tolerance to acidity and not strongly affected by Al (Dde and Hra); 4) species with marked

386   preference for acidic conditions and no obvious reaction to Al (Amo, Tse, Hpe, Pme and Vte);

387   and 5) species that prefer acidic conditions (with marked susceptibility to alkalization) and no

388   reaction to Al (Rac, Aam, Cst, Dca and Hpi; Figure 5). The estimated soil preference and seed

389   weight were not good predictors of completion of germination under the tested conditions, as

390   clustering did not distinguished sharp and homogenous groups (Figure 5). On the other hand,

391   three first clusters were relatively well segregated considering R EIVs, but the remaining two

392   clusters did not show any logical pattern (Figure 5).

393

Discussion

395   It is commonly accepted that grasslands established on alkaline calcareous soils are composed of

396   calcicole/calciphile/basophile plant species, while those established on acidic sandy soils are

397 composed of calcifuges/calciphobes/acidophiles (Bothe, 2015). However, this concept was only

398 occasionally tested to show its viability (Bothe, 2015), especially considering germination

399 requirements. Although drastic changes of soil chemistry (e.g., pH – Wagner et al., 2017 – and

400 availability of nutrients, most remarkably Fe, Mn and Al) in Rendzinas and Podzols are not very

401 likely, slightly varying physical-chemical properties of soil (including its patchy structure; Cain

402 et al., 1999) create a mosaic of microhabitats which contributes to plant diversity (Leuchner &

403 Ellenberg, 2017). It suggests that some species within a given habitat type can be more/less

404 calciphilic (or acidophilic) than others or show an undifferentiated reaction to soil pH and

405 availability of the studied metals (in terms of germination requirements). The presented work is

406 in line with this conception. It must be however noted that further validation of the presented

407 findings on annual dicotyledonous plants as well as grasses is needed in order to fully describe

408 the studied plant communities.

409   The recorded data clearly demonstrated that ability of the studied species to complete

410 germination was pH-dependent (hypothesis 1). Even the species considered as undeniable

411 acidophiles and basophiles (considering their centers of abundance) are able to complete

412 germination under the conditions which are not their typical ones (e.g., Aam, Bof, Csc, Cst, Dca,

413 Dde, Evu, Pre, Pgr, Rac, Vth and Vte). Furthermore, for some species that can be found both in

414 xerothermic and dry grasslands (Cst, Dca, Hpe, and Evu) the amplitude of soil pH allowing them

415 completion of germination is relatively wide. However, it should be interpreted with caution

416 because mismatch between the conditions allowing completion of germination and those making

417 vegetative growth optimal is known, which was shown previously, e.g., for soil moisture

418 (Wilman et al., 2014). Overall, it is worth noting that both alkaline xerothermic and acidic dry

419 grasslands are composed of 1) species with pH requirements for completion of germination

420 exactly matching pH of the substratum on which they can be found, 2) pH-undifferentiated

421 species (wide theoretical regeneration niche) and 3) species characterized with marked pH

422 preferences however having also partial ability to complete germination under non-optimal

423 conditions. It is known that there is diversity of germination strategies within flora of alkaline

424 and acidic grasslands, including such adaptations as reaction to nitrogen (Wala, Kołodziejek &

425 Patykowski, 2021), ability to withstand thermal aging (Tausch et al., 2019) and complete

426 germination under a given thermoperiod (Tudela-Isanta et al., 2017). Although previous studies

427 suggested that alpine calcicoles and calcifuges possessed group-specific adaptations allowing

428 completion of germination under acidity matching their centers of abundance (Tudela-Isanta et
429 al., 2018), a homogeneous reaction within a group is probably not as plausible in lowland
430 temperate grasslands (Margreiter et al., 2020). Our study is in agreement with the conception of
431 heterogeneity of germination strategies within calcicole/calcifuge groups, as it clearly shows that
432 a potential germination-based regeneration niche of several species (e.g., Aam, Cst, Dde, Pgr,
433 Rac) is wide and does not match their realized niches and centers of abundance. These species
434 cannot be usually found under non-typical soil conditions, which probably results from weak
435 competitiveness at the vegetative stage. For example, Rac is known to be a pH-indifferent
436 germinator (Yazdi et al., 2013), but is not able to win competition with basophilic species during
437 establishment on calcareous soils, also due to susceptibility to Fe-dependent limitations
438 (Hutchinson, 1967; Leuchner & Ellenberg, 2017). On the other hand, some calcicole species
439 (occurring naturally on alkaline soils, e.g. Rendzinas) showed exceptional tolerance to low pH at
440 the germination stage (e.g., Aam and Pgr) but they were not able to develop correctly under high
441 soil acidity of Podzols (Wala et al., 2020).

442   It must be noted that the composition of buffer solution used for studies on pH optimum
443 for completion of germination can affect the results (Ma et al., 2015). Due to the fact that we
444 used in this study isomolar buffers containing only phosphates, the results can be the effect of
445 any of the three traits of the used buffers, namely differing acidity (pH) or phosphate ratio
446 (HPO_4^{2-}: $H_2PO_4^-$) or ratio of Na^+: K^+. Thus, species limited by the highest pH values used in this
447 study (calcicole Aam, Amo, Bof, Pre, Pgr and Pme and calcifuge Hpi) may also additionally
448 encounter adverse effects of HPO_4^{2-} and/or Na^+. As both phosphate forms can be utilized by
449 plants; Schachtman, Reid & Ayling, 1998), Na-caused toxicity is more probable explanation.
450 This element (whose availability can be increased due to agronomical load; Núez-Delgado,
451 López-Periago & Díaz-Fierros Viqueira, 2002) can disturb the germination-based revegetation of
452 the studied types of grasslands because it is relocated with percolate during soil surface runoff
453 across slopes (Schulze et al., 2019) from arable fields to lower areas. Sodic-saline conditions
454 were repeatedly shown to deteriorate edaphic conditions, ability of seeds to complete
455 germination, plant performance (Läuchli & Grattan, 2012) and regeneration sites (Ma et al.,
456 2015). Therefore, it can be treated as another threat for the species we studied. Additionally, it is
457 worth noting that alkaline soils, on which xerothermic grasslands are settled in Central Europe,
458 are rather non-sodic (Tóth, Montanarella & Rusco, 2008). It partially explains why the studied

459   xerothermic calcicoles are probably not adapted to elevated Na concentrations and can suffer

460   from adverse effects of this element. It provokes further studies concerning the effects of sodic-

461   alkaline environment on the ability of seeds to complete germination.

462   Influence of Fe on the ability of seeds to complete germination was only occasionally

463   studied (El Rasafi et al., 2016; Reis et al., 2018). For example, 20 μmol $\cdot$ dm^{-3} solution of FeCl$_3$

464   was shown to have no significant effect on the ability to complete germination of crop plants

465   such as *Triticum aestivum* L. and *Phaseolus vulgaris* L. (El Rasafi et al., 2016) and similar

466   reactions were observed for Bof, Csc, Cst and Tse investigated in this study. On the other hand, a

467   reduction of FGP due to application of ionic Fe (given as FeSO$_4$) was observed in *T. aestivum*

468   treated with solutions of c.a. 90–143 μmol Fe^{2+} $\cdot$ dm^{-3} (Reis et al., 2018). Interestingly, there are

469   also some available data suggesting that completion of germination in rice (*Oryza stativa* L.)

470   could be accelerated due to application of FeSO$_4$ (c.a. 6.6 mmol Fe^{2+} $\cdot$ dm^{-3}; Wang et al., 2020).

471   In the presented study, detrimental changes pertaining to germination parameters due to

472   application of FeCl$_3$ could be the result of Fe itself or Cl$^-$ load (15/75 μmol Cl$^-$ $\cdot$ dm^{-3}).

473   However, it is worth noting that due to low concentrations of the tested solutions, effects of

474   osmotic pressure are not high enough to affect germination at imbibition phase, which can be

475   extrapolated from numerous studies showing effects of polyethylene glycol-simulated

476   osmoticum on the ability of seeds to complete germination (Chamorro & Moreno, 2019; Yi et

477   al., 2019). This strongly suggests that several studied species (Aam, Dca, Dde, Gcr, Hpi, Hra,

478   Pgr, Vth and Vte) are susceptible to overdose of Fe^{3+}, as considerable concentrations of Cl$^-$ (up

479   to 30 mmol $\cdot$ dm^{-3}) are known to have no or marginal influence on germination process (Abedi,

480   Bartelheimer & Poschlod, 2013). It can be hypothesized that reduced completion of germination

481   due to supraoptimal doses of Fe can be the result of Fe-dependent disturbances in a cell cycle

482   (Reis et al., 2018) resulting from oxidative stress caused by photo-Fenton and Fenton-like

483   reactions catalyzed by Fe (Rincón & Pulgarin, 2006; Ahile et al., 2021) and/or non-oxidative

484   ion-specific toxicity. In nature, release of Fe species from Fe-bearing minerals is to a great extent

485   controlled by soil pH (Strawn, Bohn & O'Connor, 2020). In fact, concentration of Fe^{3+} released

486   from Fe oxides under alkaline conditions (pH = 8.5) is very low and becomes significant only at

487   remarkably acidic conditions (Robin et al., 2008). It partially explains why some undeniable

488   calcicoles (Aam, Dca, Gcr, Pgr, Sge, and Vte) are negatively influenced by ionic Fe. However,

489   some studied calcifuges (Cst, Dde, Hpi, Hra and Vth) also seem to be prone to Fe-dependent

490   limitations at the germination stage (hypothesis 2). It can be proposed that Fe soil status is an

491   additional but minor ecological sieve filtering establishment of new individuals in the studied

492   communities. However, the way in which Fe becomes a potential limiting factor in each of the

493   studied types of communities may vary. For example, in the case of dry acidic grasslands,

494   availability of iron can be shaped by succession (Sparrius, Sevink & Kooijman, 2012). Another

495   way to increase availability of Fe on soil surface is severe soil disturbance (caused by

496   anthropogenic and natural activities) and eventually displacement of Fe-rich hardpan, which is

497   then solubilized. A different mode of action is more plausible in alkaline xerothermic grasslands,

498   where Fe chelation due to biological activity at the local scale is probably the major factor

499   influencing its availability (Singer, Schwertmann, & Friedl; 1998 Gries & Runge, 1992; Ferreira

500   et al., 2019). Although not much is known about the quantitative siderophoral status of

501   Rendzinas, studies on Podzols showed that this kind of soil contains nanomolar concentrations of

502   a given siderophore/chelator (a dozen or so nanomoles on average; Winkelmann, 2007; Ahmed

503   & Holmström, 2015). Thus, it is very likely that the total pool of Fe chelators (and thus chelated

504   Fe as there is a linkage between concentration of chelators and chelated Fe; Liu & Millero, 1999)

505   is at the micromolar level in both soils (Robin et al., 2008), but their biological recovery from the

506   alkaline soil may be even harder than from the acidic one in some cases due to lime-dependent

507   sequestration (Boiteau et al., 2020). In the context of the presented study, such chelator-based Fe

508   dissolution in soil may have some impact on germination-based revegetation, affecting the

509   studied plants in a species-specific manner.

510   The role of ionic and complexed forms of Mn on geochemical processes and life

511   (including plant ontogenesis, e.g., a germination process) still needs elucidation (Duckworth,

512   Bargar & Sposito, 2009). Up to date, it has been demonstrated that Mn (solutions of c.a. 5–91

513   μmol $Mn^{2+} \cdot dm^{-3}$ given as $MnSO_4$) did not significantly affect FGP of lettuce (*Lactuca sativa*

514   L.) seeds sown on Hoagland medium (Liu, Zhang, Lal, 2016). On the other hand, the completion

515   of germination of *Nicotiana tabacum* L. seeds was significantly reduced up to 75% after

516   application of 2–20 mmol $Mn^{2+} \cdot dm^{-3}$ (given as $MnSO_4$) on Murashige and Skoog medium

517   (Santandrea, Schiff & Bennici, 1997). Reduction of FGP due to increasing availability of Mn

518   seems to be species-specific, as plants can differ in their reaction to Mn concentration at the

519   germination stage (e.g., *Arabidopis thaliana* L. and *Arabis paniculata* Franch. subjected to 1 and

520   10 mmol $Mn^{2+} \cdot dm^{-3}$ given as $MnCl_2$; Tang, Tao & Li, 2021). On the basis of the presented

521 study it can be stated that Mn species-specifically influenced the ability of seeds to complete

522 germination (hypothesis 2). As eight studied species did not suffer from the negative effects of

523 Mn (Csc, Dca, Gcr, Hpi, Pre, Tse, Vth and Vte) and there was no sharply pronounced pattern of

524 reaction to the tested stimuli within each ecological group, it can be proposed that the reaction to

525 Mn availability is individual for each species, which further implies that microhabitats (in terms

526 of Mn availability gradient or hotspots) can play a role in establishment of the studied plants. It is

527 however worth noting that oxidative state of Mn also should not be underestimated, as chemical

528 structure HBED is believed to favor Mn(III) state (similarly to other Mn chelators (Duckworth,

529 Bargar & Sposito, 2009) due to two phenolato donors of this chelator (Pinto et al., 2019) and/or

530 oxidation under oxygen environment (Wahsner et al., 2019), although occurrence of Mn(II) state

531 is also possible. From an environmental point of view, it is believed that complexed forms of Mn

532 prevail in soil solution at neutral pH (> 90% of Mn pool), while under acidic conditions, free

533 Mn^{2+} is probably the predominant form of this element (> 70% of Mn pool; Ritchie, 1989).

534 Slightly reduced FGP and IGV values in basophilic species (Aam, Bof, Csc, Pme, Pgr and Sge)

535 treated with Mn-HBED as well as in acidophilic ones (Amo, Evu, Hpe, Hra and Rac) treated

536 with $MnCl_2$ suggest that the predominant form of Mn in each type of grasslands plays a role in

537 assembly of plant community.

538   Interplay between endogenous Fe and Mn is known to influence the seed germination

539 process (Eroglu et al., 2017). The presented study showed that exogenous Fe and Mn also

540 affected the ability of seeds to complete germination. Additionally, considering the possible

541 application of our results, it seems that the chelated forms of the studied metals (Fe-HBED and

542 Mn-HBED) are not more detrimental to the germination process in the studied plants when

543 compared to their respective ionic forms (isomolar solutions). This indicates that reasonable

544 usage of HBED-chelated Fe and Mn should not pose a risk to terrestrial vegetation, which was

545 suggested for other Fe chelates (European Food Safety Authority, 2013).

546   Limitations caused by increased Al availability were widely shown to occur in many

547 terrestrial plant species (Singh, 2017). Inhibition of root elongation caused by destabilization of

548 cell homeostasis is the most recognized sign of Al toxicity at the early stages of plant growth

549 (Alves Silva et al., 2014). However, relatively little is known about the direct influence of Al on

550 the ability of seeds to complete germination. Up to date, the most detailed study on this topic has

551 been conducted on plants from dry temperate grasslands that can be found along an acidity

552   gradient (Abedi, Bartelheimer & Poschlod, 2013). Among the 15 investigated species, a little

553   more than half of them were resistant to Al toxicity at the germination stage (which was also

554   shown for Amo, Cst, Dca, Rac, Tse and Vth investigated in this study), but for the other near half

555   Al was toxic when the seeds were treated with its concentrations exceeding 1–2 mmol · dm^{-3}

556   (Abedi, Bartelheimer & Poschlod, 2013). Reduction of completion of germination (by c.a. 25%)

557   due to increased availability of Al^{3+} (320 μmol · dm^{-3}) was also observed in *Ricinus communis*

558   L. (Alves Silva et al., 2014) as well as in *Eugenia dysenterica* DC. (200–800 μmol Al^{3+} · dm^{-3};

559   reduction by 30%; Rodrigues et al., 2019). On the other hand, *L. sativa* is known to complete

560   germination in the presence of Al within the range of c.a. 2–800 μmol Al^{3+} · dm^{-3}, but

561   concentrations higher that 2 μmol Al^{3+} · dm^{-3} slow down this process (Silva & Matos, 2016).

562   Decelerated germination was also observed in *E. dysenterica* subjected to 600–800 μmol Al^{3+} ·

563   dm^{-3} (Rodrigues et al., 2019). Similar mode of action was also noted in this study, but the

564   thresholds for the observed effect were species-specific. For example, undeniable basophilic

565   species (Aam, Pgr and Sge) together with Dde (acidophilic species) were susceptible to Al

566   toxicity even at its low concentrations, whereas the reduction of completion of germination for

567   some other species was observed in 1 mmol Al^{3+} · dm^{-3} (Bof, Hpi, Pme and Pre) and in 10 mmol

568   Al^{3+} · dm^{-3} (Evu, Gcr, Hra and Vte). It suggests that Al can be a barrier preventing establishment

569   of basophiles on non-alkaline soils, whilst for acidophiles it probably contributes to the

570   formation of a mosaic structure of vegetation. It must be however noted that slight acidification

571   events in alkaline xerothermic grasslands probably do not lead to strong Al-driven shifts in

572   germination-dependent revegetation, as basophilic species are able to complete germination

573   efficiently under low Al concentrations. Furthermore, the basophiles seemed not to be more

574   susceptible to Al toxicity than the tested acidophiles (hypothesis 3). It is known that the

575   acquisition of Al is connected with generation of reactive oxygen species leading to oxidative

576   stress and cellular damages (Šimonovičová et al., 2004; Bothe, 2015). It can be hypothesized that

577   these processes occurs also in a growing embryo when seeds encounter high amounts of freely

578   available Al during imbibition. However, there are also some suggestions (Silva & Matos, 2016)

579   that morpho-anatomical and chemical traits of a seed coat contribute to Al resistance at the

580   germination stage, probably because of limited penetration of this element due to chemical

581   constituents of this structure (most notably phenolic compounds that are able to complex Al^{3+}

582   into insoluble forms; Moïse et al., 2005; Zhang et al., 2016). Alternatively, germinating seeds of

583   tolerant species are able to withstand Al-caused toxicity on account of efficient stress coping

584   mechanisms that reduce ion-specific toxicity and oxidative stress (resulting in fast compensation

585   of damages). As many species in our investigation completed germination under high

586   concentrations of Al, their tolerance buffer for completion of germination is wider than it could

587   be predicted from their centers of abundance (Abedi, Bartelheimer & Poschlod, 2013).

588   However, further studies are needed to determine if the tolerance level at the germination stage

589   matches that at further ontogenetic stages, up to maturity (including reproduction). It can be

590   predicted that if the tolerance buffer to Al toxicity during germination of a given species is

591   greater than those during further developmental stages (e.g., growth of seedlings), depletion of

592   seed banks is very plausible, which was proposed for other environmental stimuli, e.g., nitrogen

593   (Bird & Choi, 2017).

594   Interestingly, some species may even experience positive effects of increased availability

595   of Al, e.g., remarkably acidophilic grass, *Corynephorus canescens* L. (most markedly at 0.01

596   mmol $Al^{3+} \cdot dm^{-3}$, but possibly up to 2 mmol $Al^{3+} \cdot dm^{-3}$; Abedi, Bartelheimer & Poschlod,

597   2013). Similar slight stimulation was observed in Hpe and Csc (0.01 and 10 mmol $Al^{3+} \cdot dm^{-3}$,

598   respectively) investigated in this study. Enhanced completion of germination may be in this case

599   the result of an increased pro-oxidative state due to Al-dependent stimuli (Šimonovičová et al.,

600   2004), as reactive oxygen species (most notably hydrogen peroxide) are known to trigger the

601   release from dormancy and accelerate remobilization of resources and endosperm weakening

602   (Wojtyla et al., 2016). It can be proposed that such stimulation plays a role in detection of

603   optimal conditions for growth and development in some Al-tolerant specialists (in this case Hpe

604   and Csc).

605   Finally, the presented study showed that flora from both acidic dry and alkaline

606   xerothermic grasslands was diverse in its germination strategies. Hierarchical clustering analysis

607   indicated that there was no homogenous reaction to the tested conditions within each group.

608   Furthermore, seed size and estimated preference for soil acidity should not be used as common

609   delimiters allowing sharp segregation of germination strategies of acidophilic and basophilic

610   plants (in terms of influence of edaphic conditions, e.g., pH, Al, Fe and Mn). Moreover, seed

611   weight did not correlate with the ability of seeds to complete germination under increased

612   availability of the tested metals or changing acidity (with sparse exceptions). This suggests the

613   existence of some more complicated species-specific structural and/or physiological adaptations

614 in seeds that are probably not yet well recognized to be the traits associated with tolerance to

615 chemical stimuli.

616

Conclusion

618 Overall, the presented study showed a diversity of germination strategies in plants from acidic

619 dry as well as alkaline xerothermic grasslands. Tolerances to pH and availability of Fe, Mn and

620 Al are other traits contributing to floristic diversity of a given plant community due to the

621 formation of microhabitats. This seems to promote mosaic structure of the studied plant

622 communities, however in our opinion pH and extremely high availability of Al are major factors,

623 while availability of Fe and Mn is probably of secondary importance. The studied edaphic

624 factors seem to filter plant establishment on remarkably acidic and alkaline soils and might be

625 among the driving forces of evolution of plant germination strategies. As specialization of

626 germination requirements in the studied species was found, changes in soil pH and availability of

627 Al, Fe and Mn should be taken into consideration in further studies on biology of dry acidic, and

628 xerothermic alkaline grasslands. It can be expected that spatial and temporal changes in local

629 vegetation have an impact on germination-based revegetation in the studied types of

630 communities. Thus, we believe that ecology of acidic and alkaline grasslands should be co-

631 investigated with soil chemistry. Such studies cannot only deepen understanding of species

632 diversity and natural processes in these kinds of communities (e.g., succession), but also can

633 improve management, protection and ecological restoration of grasslands in a worldwide scale.

634 Furthermore, both Fe-HBED and Mn-HBED exerted similar effects on the ability of seeds to

635 complete germination when compared to ionic forms of these metals. This suggests that both

636 chelates are not detrimental to early ontogenetic stages of plants when they are reasonably used.

637

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644

References

1. Abedi M, Bartelheimer M, Poschlod P, 2013. Aluminium toxic effects on seedling root survival affect plant composition along soil reaction gradients - a case study in dry sandy grasslands. *Journal of Vegetation Science* 24:1074–1085. DOI: 10.1111/jvs.12016.

2. Ahile UJ, Wuana RA, Itodo AU, Sha'Ato R, Malvestiti JA, Dantas RF. 2021. Are iron chelates suitable to perform photo-Fenton at neutral pH for secondary effluent treatment? *Journal of Environmental Management* 278:111566. DOI: 10.1016/j.jenvman.2020.111566.

3. Ahmed E, Holmström SJM. 2015. Siderophore production by microorganisms isolated from a Podzol soil profile. *Geomicrobiology Journal* 32:397–411. DOI: 10.1080/01490451.2014.925011.

4. Alves Silva GE, Toledo Ramos F, de Faria AP, Costa França MG. 2014. Seeds' physicochemical traits and mucilage protection against aluminum effect during germination and root elongation as important factors in a biofuel seed crop (*Ricinus communis*). *Environmental Science and Pollution Research* 21:11572–11579. DOI: 10.1007/s11356-014-3147-6.

5. Andresen E, Peiter E, Küpper H. 2018. Trace metal metabolism in plants. *Journal of Experimental Botany* 69:909–954. DOI: 10.1093/jxb/erx465.

6. Aravind J, Vimala Devi S, Radhamani J, Jacob SR, Kalyani S. 2020. Germinationmetrics: Seed germination indices and curve fitting. R package version 0.1.3. https://cran.r-project.org/web/packages/germinationmetrics/vignettes/Introduction.pdf.

7. Asher CJ, Edwards DG. 1983. Modern solution culture techniques. In: Läuchli A, Bieleski RL, eds. *Inorganic Plant Nutrition. Encyclopedia of Plant Physiology, Vol. 15*. Berlin: Springer, 147–180. DOI: 10.1007/978-3-642-68885-0_4.

8. Bakker C, Blair JM, Knapp AK. 2003. Does resource availability, resource heterogeneity or species turnover mediate changes in plant species richness in grazed grasslands? *Oecologia* 137:385–391. DOI: 10.1007/s00442-003-1360-y.

9. Baskin C, Baskin JM. 1988. Germination ecophysiology of herbaceous plant species in a temperate region. *American Journal of Botany* 75:286–305. DOI: 10.1002/j.1537-2197.1988.tb13441.x.

675 10. Bird EJ, Choi YD. 2017. Response of native plants to elevated soil nitrogen in the sand
676   dunes of Lake Michigan, USA. *Biological Conservation* 212:398–405. DOI:
677   10.1016/j.biocon.2016.12.001.

678 11. Boiteau RM, Kukkadapu R, Cliff JB, Smallwood CR, Kovarik L, Wirth MG, Engelhard
679   MH, Varga T, Dohnalkova A, Perea DE, Wietsma T, Moran JJ, Hofmockel KS. 2020.
680   Calcareous organic matter coatings sequester siderophores in alkaline soils. *Science of the*
681   *Total Environment* 724:138250. DOI: 10.1016/j.scitotenv.2020.138250.

682 12. Bojñnanský V, Fargašová A. 2007. *Atlas of seeds and fruits of Central and East-*
683   *European flora: the Carpathian Mountains region*. Dordrecht: Springer. DOI:
684   10.1007/978-1-4020-5362-7.

685 13. Bothe H. 2015. The lime-silicate question. *Soil Biology and Biochemistry* 89:172–183.
686   DOI: 10.1016/j.soilbio.2015.07.004.

687 14. Burmeier S, Donath TW, Otte A, Eckstein RL. 2010. Rapid burial has differential effects
688   on germination and emergence of small- and large-seeded herbaceous plant species. *Seed*
689   *Science Research* 20:189–200. DOI: 10.1017/S0960258510000127.

690 15. Cain ML, Subler S, Evans JP, Fortin MJ. 1999. Sampling spatial and temporal variation
691   in soil nitrogen availability. *Oecologia* 118:397–404. DOI: 10.1007/s004420050741.

692 16. Chamorro D, Moreno JM. 2019. Effects of water stress and smoke on germination of
693   Mediterranean shrubs with hard or soft coat seeds. *Plant Ecology* 220:511–521. DOI:
694   10.1007/s11258-019-00931-2.

695 17. Chen Y, Hu G, Rodriguez C, Liu M, Binder BM, Chervin C. 2020. Roles of SlETR7, a
696   newly discovered ethylene receptor, in tomato plant and fruit development. *Horticulture*
697   *Research* **7**:17. DOI: 10.1038/s41438-020-0239-y.

698 18. Council Directive. 1992. Council Directive 92/43/EEC of 21 May 1992 on the
699   Conservation of Natural Habitats and of Wild Fauna and Flora. *Official Journal of the*
700   *European Communities* L206 (22.7.92.): 7–50.

701 19. Czarnecka J. 2004. Seed longevity and recruitment of seedlings in xerothermic grassland.
702   *Polish Journal of Ecology* 52:505–521.

703 20. Czyżewska M. 1992. Syntaksonomia śródlądowych, pionierskich muraw napiaskowych.
704   (Syntaxonomy of inland and pioneer psammophilous grasslands). *Monographiae*
705   *Botanicae* 74:1–174. DOI: 10.5586/mb.1992.001.

706   21. Deregibus VA, Sanchez RA, Casal JJ, Trlica MJ. 1985. Tillering responses to enrichment
707   of red light beneath the canopy in a humid natural grassland. *Journal of Applied Ecology*
708   22:199–206. DOI: 10.2307/2403337.

709   22. Duckworth OW, Bargar JR, Sposito G. 2009. Coupled biogeochemical cycling of iron
710   and manganese as mediated by microbial siderophores. *Biometals* 22:605–613. DOI:
711   10.1007/s10534-009-9220-9.

712   23. Duermeyer L, Khodapanahi E, Yan D, Krapp A. 2018. Regulation of seed dormancy and
713   germination by nitrate. *Seed Science Research* 28:1–8. DOI:
714   10.1017/s096025851800020x.

715   24. Dürr C, Dickie JB, Yang X, Pritchard HW. 2015. Ranges of critical temperature and
716   water potential values for the germination of species worldwide: Contribution to a seed
717   trait database. *Agricultural and Forest Meteorology* 200:222–232. DOI:
718   10.1016/j.agrformet.2014.09.024.

719   25. El Rasafi T, Nouri M, Bouda S, Haddioui A. 2016. The effect of Cd, Zn and Fe on seed
720   germination and early seedling growth of wheat and bean. *Ekológia (Bratislava)* 35:213–
721   223. DOI: 10.1515/eko-2016-0017.

722   26. Ellenberg H. 1991. Indicator values of vascular plants in central Europe 1. Indicator
723   values of vascular plants not including *Rubus*. *Scripta Geobotanica* 18:9–166.

724   27. Eroglu S, Giehl RFH, Meier B, Takahashi M, Terada Y, Ignatyev K, Andresen E, Küpper
725   H, Peiter E, von Wirén N. 2017. Metal Tolerance Protein 8 mediates manganese
726   homeostasis and iron reallocation during seed development and germination. *Plant
727   Physiology* 174:1633–1647. DOI: 10.1104/pp.16.01646.

728   28. European Food Safety Authority. 2013. Scientific opinion on the safety and efficacy of
729   iron compounds (E1) as feed additives for all species: iron chelate of amino acids,
730   hydrate, based on a dossier submitted by Zinpro Animal Nutrition Inc. *EFSA Journal*
731   11:3287. DOI: 10.2903/j.efsa.2013.3287.

732   29. Ferreira CMH, Vilas-Boas Â, Sousa CA, Soares HMVM, Soares EV. 2019. Comparison
733   of five bacterial strains producing siderophores with ability to chelate iron under alkaline
734   conditions. *AMB Express* 9:78. DOI: 10.1186/s13568-019-0796-3.

735   30. Gibson DJ. 2009. *Grasses and Grassland Ecology*. Oxford: Oxford University Press.
736   DOI: 10.1093/aob/mcp219.

737   31. Graeber K, Nakabayashi K, Miatton E, Leubner-Metzger G, Soppe WJJ. 2012. Molecular
738   mechanisms of seed dormancy. *Plant, Cell and Environment* 35:1769–1786. DOI:
739   10.1111/j.1365-3040.2012.02542.x.

740   32. Gries D, Runge M. 1992. The ecological significance of iron mobilization in wild
741   grasses. *Journal of Plant Nutrition* 15:37–41. DOI: 10.1080/01904169209364434.

742   33. Grillet L, Schmidt W. 2017. The multiple facets of root iron reduction. *Journal of
743   Experimental Botany* 68:5021–5027. DOI: 10.1093/jxb/erx320.

744   34. Hegi G. 1931. *Illustrierte Flora von Mittel-Europa, Band VII: Gesamtregister*. München:
745   JF Lehmanns Verlag.

746   35. Hutchinson TC. 1967. Lime-chlorosis as a factor in seedling establishment on calcareous
747   soil. *New Phytologist* 66:697–705. DOI: 10.1111/j.1469-8137.1967.tb05440.x.

748   36. Jankowska-Blaszczuk M, Daws MI. 2007. Impact of red: far red ratios on germination of
749   temperate forest herbs in relation to shade tolerance, seed mass and persistence in the
750   soil. *Functional Ecology* 21:1055–1062. DOI: 10.1111/j.1365-2435.2007.01328.x.

751   37. Jentsch A, Beyschlag W. 2003. Vegetation ecology of dry acidic grasslands in the
752   lowland area. *Flora* 198:3–25. DOI: 10.1078/0367-2530-00071.

753   38. Kącki Z, Czarniecka M, Swacha G. 2013. Statistical determination of diagnostic, constant
754   and dominant species of the higher vegetation units of Poland. *Monographiae Botanicae*
755   103:1–267. DOI: 10.5586/mb.2013.001.

756   39. Kasozi N, Tandlich R, Fick M, Kaiser H, Wilhelmi B. 2019. Iron supplementation and
757   management in aquaponic systems: A review. *Aquaculture Reports* 15:100221. DOI:
758   10.1016/j.aqrep.2019.100221.

759   40. Khan MA, Ungar IA. 1997. Effects of thermoperiod on recovery of seed germination of
760   halophytes from saline conditions. *American Journal of Botany* 84:279–283. DOI:
761   10.2307/2446089.

762   41. Koornneef M., Bentsink L. & Hilhorst H. Seed dormancy and germination. *Curr. Opin.
763   Plant Biol.* **5**, 33–36. DOI: 10.1016/S1369-5266(01)00219-9 (2002).

764   42. Kranner I, Colville L. 2011. Metals and seeds: Biochemical and molecular implications
765   and their significance for seed germination. *Environmental and Experimental Botany*
766   72:93–105. DOI: 10.1016/j.envexpbot.2010.05.005.

767   43. Ladouceur E, Bonomi C, Bruelheide H, Klimešová J, Burrascano S, Poschlod P, Tudela-
768   Isanta M, Iannetta P, Mondoni A, Amiaud B, Cerabolini BEL, Cornelissen JHC, Craine J,
769   Louault F, Minden V, Öllerer K, Onipchenko V, Soudzilovskaia N, Jimémez-Alfaro B.
770   2019. The functional trait spectrum of European temperate grasslands. *Journal of*
771   *Vegetation Science* 30:777–788. DOI: 10.1111/jvs.12784.

772   44. Läuchli A, Grattan SR. 2012. Plant responses to saline sodic conditions. In: Wallendar
773   WW, Tanji KK, eds. *Agricultural salinity assessment and management. Second edition.*
774   Reston: ASCE, 169–205. DOI: 10.1061/9780784411698.ch06.

775   45. Lee JA. 1999. The calcicole—calcifuge problem revisited. *Advances in Botanical*
776   *Research* 29:1–30. DOI: 10.1016/S0065-2296(08)60306-7.

777   46. Leuchner C, Ellenberg H. 2017. *Ecology of Central European non-forest vegetation:*
778   *coastal to alpine, natural to man-made habitats. Vegetation ecology of Central Europe,*
779   *volume II.* Cham: Springer International Publishing AG. DOI: 10.1007/978-3-319-43048-
780   5.

781   47. Liu R, Zhang H, Lal R. 2016. Effects of stabilized nanoparticles of copper, zinc,
782   manganese, and iron oxides in low concentrations on lettuce (*Lactuca sativa*) seed
783   germination: Nanotoxicants or nanonutrients? *Water, Air, and Soil Pollution.* 227:42.
784   DOI: 10.1007/s11270-015-2738-2.

785   48. Liu X, Millero FJ. 1999. The solubility of iron hydroxide in sodium chloride solutions.
786   *Geochimica et Cosmochimica Acta* 63:3487–3497. DOI: 10.1016/S0016-7037(99)00270-
787   7.

788   49. Ma H, Yang H, Lü X, Pan Y, Wu H, Liang Z, Ooi MKJ. 2015. Does high pH give a
789   reliable assessment of the effect of alkaline soil on seed germination? A case study with
790   *Leymus chinensis* (Poaceae). *Plant and Soil* 394:35–43. DOI: 10.1007/s11104-015-2487-
791   4.

792   50. Margreiter V, Pagitz K, Berg C, Schwager P, Erschbamer B. 2020. Pros and cons of
793   using a standard protocol to test germination of alpine species. *Plant Ecology* 221:1045–
794   1067. DOI: 10.1007/s11258-020-01061-w.

795   51. Marques R, Ranger J, Gelhaye D, Pollier B, Ponette Q, Gœdert Q. 1996. Comparison of
796   chemical composition of soil by zero-tension plate lysimeters with those lysimeters in a

797   forest soil. *European Journal of Soil Science* **47**, 407–417. DOI: 10.1111/j.1365-
798   2389.1996.tb01414.x.

799 52. Marschner H. 1988. Mechanisms of manganese acquisition by roots from soils. In:
800   Graham RD, Hannam RJ, Uren NC, eds. *Manganese in soils and plants. Developments in*
801   *plant and soil sciences, vol 33*. Dordrecht: Kluwer Academic Publishers, 191–204. DOI:
802   10.1007/978-94-009-2817-6_14.

803 53. Matuszkiewicz W. 2001. *Przewodnik do oznaczania zbiorowisk roślinnych Polski*.
804   Warszawa: Wydawnictwo Naukowe PWN.

805 54. Metsalu T, Vilo J. 2015. ClustVis: A web tool for visualizing clustering of multivariate
806   data using Principal Component Analysis and heatmap. *Nucleic Acids Research*
807   43:W566–W570. DOI: 10.1093/nar/gkv468.

808 55. Moïse JA, Han S, Gudynaitę-Savitch L, Johnson DA, Miki BLA. 2005. Seed coats:
809   Structure, developent, composition, and biotechnology. *In Vitro Cellular and*
810   *Developmental Biology – Plant* 41:620–644. DOI: 10.1079/IVP2005686.

811 56. Mucina L, Kolbek J. 1993. *Festuco-Brometea*. In: Mucina L, Grabherr G, Ellmauer T,
812   eds. *Die Pflanzengesellschaften Österreichs. Teil I*. Jena: Gustav Fischer Verlag, 420–
813   492.

814 57. Nonogaki H. 2017. Seed biology updates – highlights and new discoveries in seed
815   dormancy and germination. *Frontiers in Plant Science* 8:524. DOI:
816   10.3389/fpls.2017.00524.

817 58. Núez-Delgado A, López-Periago E, Díaz-Fierros Viqueira F. 2002. Chloride, sodium,
818   potassium and faecal bacteria levels in surface runoff and subsurface percolates from
819   grassland plots amended with cattle slurry. *Bioresource Technology* 82:261–271. DOI:
820   10.1016/S0960-8524(01)00183-3.

821 59. Pinto SM, Tomé V, Calvete MJF, Castro MMCA, Tóth É, Geraldes CFGC. 2019. Metal-
822   based redox-responsive MRI contrast agents. *Coordination Chemistry Reviews* 390:1–31.
823   DOI: 10.1016/j.ccr.2019.03.014.

824 60. Poschlod P. 2020. Let us have a functional view of seeds! A special Issue on "Functional
825   Seed Ecology". *Seed Science Research* 30:235–237. DOI: 10.1017/S0960258521000027.

826 61. R Core Team. 2018. R: A language and environment for statistical computing. *R*
827   *Foundation for Statistical Computing, Vienna, Austria.* https://www.R-project.org.

828 62. Reis S, Pavia I, Carvalho A, Moutinho-Pereira J, Correia C, Lima-Brito J. 2018. Seed
829   priming with iron and zinc in bread wheat: effects in germination, mitosis and grain yield.
830   *Protoplasma* 255:1179–1194. DOI: 10.1007/s00709-018-1222-4.

831 63. Rincón A-G, Pulgarin C. 2006. Comparative evaluation of Fe^{3+} and TiO_2 photoassisted
832   processes in solar photocatalytic disinfection of water. *Applied Catalysis B:*
833   *Environmental* 63:222–231. DOI: 10.1016/j.apcatb.2005.10.009.

834 64. Ritchie GSP. 1989. The chemical behaviour of aluminium, hydrogen and manganese in
835   acid soils. In: Robson AD, ed. *Soil acidity and plant growth*. Marrickville: Academic
836   Press Australia, 1–60. DOI: 10.1016/B978-0-12-590655-5.X5001-4.

837 65. Robin A, Vansuyt G, Hinsinger P, Meyer JM, Briat JF, Lemanceau P. 2008. Iron
838   dynamics in the rhizosphere. Consequences for plant health and nutrition. *Advances in*
839   *Agronomy* 99:183–225. DOI: 10.1016/S0065-2113(08)00404-5.

840 66. Rodrigues AA, Filho SCV, Müller C, Rodrigues DA, Sales JF, Zuchi J, Costa AC,
841   Rodrigues CL, da Silva AA, Barbosa DP. 2019. Tolerance of *Eugenia dysenterica* to
842   aluminum: Germination and plant growth. *Plants* 8:317. DOI: 10.3390/plants8090317.

843 67. Santandrea G, Schiff S, Bennici A. 1997. Manganese toxicity to different growth
844   processes in vitro in *Nicotiana*. *Plant Cell, Tissue and Organ Culture* 50:125–129. DOI:
845   10.1023/A:1005917114146.

846 68. Schachtman DP, Reid RJ, Ayling SM. 1998. Phosphorus uptake by plants: From soil to
847   cell. *Plant Physiology* 116:447–453. DOI: 10.1104/pp.116.2.447.

848 69. Schulze E-D, Beck E, Buchmann N, Clemens S, Müller-Hohenstein K, Scherer-Lorenzen
849   M. 2019. *Plant ecology. Second edition*. Heidelberg: Springer-Verlag GmbH Germany.
850   DOI: 10.1007/978-3-662-56233-8.

851 70. Shao JF, Yamaji N, Shen RF, Ma JF. 2017. The key to Mn homeostasis in plants:
852   Regulation of Mn transporters. *Trends Plant Science* 22:215–224. DOI:
853   10.1016/j.tplants.2016.12.005.

854 71. Silva P, Matos M. 2016. Assesment of the impact of aluminum on germination, early
855   growth and free proline content in *Lactuca sativa* L. *Ecotoxicology and Environmental*
856   *Safety* 131:151–156. DOI: 10.1016/j.ecoenv.2016.05.014.

857   72. Šimonovičová M, Huttová J, Mistrík I, Široká B, Tamás L. 2004. Root growth inhibition
858   by aluminum is probably caused by cell death due to peroxidase-mediated hydrogen
859   peroxide production. *Protoplasma* 224:91–98. DOI: 10.1007/s00709-004-0054-6.

860   73. Singer A, Schwertmann U, Friedl J. 1998. Iron oxide mineralogy of Terre Rosse and
861   Rendzinas in relation to their moisture and temperature regimes. *European Journal of*
862   *Soil Science* 49:386–395. DOI: 10.1046/j.1365-2389.1998.4930385.x.

863   74. Singh S. 2017. Toxicity of aluminium on various levels of plant cells and organism: A
864   review. *Environmental and Experimental Botany* 137:177–193. DOI:
865   10.1016/j.envexpbot.2017.01.005.

866   75. Sparrius LB, Sevink J, Kooijman AM. 2012. Effects of nitrogen deposition on soil and
867   vegetation in primary succession stages in inland drift sands. *Plant and Soil* 353:261–
868   272. DOI: 10.1007/s11104-011-1029-y.

869   76. Strawn DG, Bohn HL, O'Connor GA. 2020. *Soil chemistry. Fifth edition.* Oxford: Wiley
870   Blackwell.

871   77. Tang T, Tao F, Li W. 2021. Characterisation of manganese toxicity tolerance in *Arabis*
872   *paniculata*. *Plant Diversity* 43:163–172. DOI: 10.1016/j.pld.2020.07.002.

873   78. Tausch S, Leipold M, Reisch C, Poschlod P. 2019. Dormancy and endosperm presence
874   influence the ex situ conservation potential in central European calcareous grassland
875   plants. *AoB Plants* 11:plz035. DOI: 10.1093/aobpla/plz035.

876   79. Török P, Matus G, Papp M, Tóthmérész B. 2009. Seed bank and vegetation development
877   of sandy grasslands after goose breeding. *Folia Geobotanica* 44:31–46. DOI:
878   10.1007/s12224-009-9027-z.

879   80. Tóth G, Montanarella L, Rusco E. 2008. *Threats to soil quality in Europe. JRC scientific*
880   *and technical reports. EUR 23438 EN.* Luxembourg: European Commission, Office for
881   Official Publications of the European Communities.

882   81. Tudela-Isanta M, Fernández-Pascual E, Wijayasinghe M, Orsenigo S, Rossi G, Pritchard
883   HW, Mondoni A. 2017. Habitat-related seed germination traits in alpine habitats.
884   *Ecology and Evolution* 8:150–161. DOI: 10.1002/ece3.3539.

885   82. Tudela-Isanta M, Ladouceur E, Wijayasinghe M, Pritchard HW, Mondoni A. 2018. The
886   seed germination niche limits the distribution of some plant species in calcareous or
887   siliceous alpine bedrocks. *Alpine Botany* 128:83–95. DOI: 10.1007/s00035-018-0199-0.

83. Venturas M, Fernández V, Nadal P, Guzmán P, Lucena JJ, Gil L. 2014. Root iron uptake efficiency of *Ulmus laevis* and *U. minor* and their distribution in soils of the Iberian Peninsula. *Frontiers in Plant Science* 5:104. DOI: 10.1016/10.3389/fpls.2014.00104.

84. Wagner V, Chytrý M, Zelený D, von Wehrden H, Brinkert A, Danihelka J, Hölzel N, Jansen F, Kamp J, Lustyk P, Merunková K, Palpurina S, Preislerová Z, Wesche K. 2017. Regional differences in soil pH niche among dry grassland plants in Eurasia. *Oikos* 126:660–670. DOI: 10.1111/oik.03369.

85. Wahsner J, Gale EM, Rodríguez-Rodríguez A, Caravan P. 2019. Chemistry of MRI contrast agents: Current challenges and new frontiers. *Chemical Reviews* 119:957–1057. DOI: 10.1021/acs.chemrev.8b00363.

86. Wala M, Kołodziejek J, Patykowski J. 2021. Nitrogen signals and their ecological significance for seed germination of ten psammophilous plant species from European dry acidic grasslands. *PLoS ONE* 16:e0244737. DOI: 10.1371/journal.pone.0244737.

87. Wala M, Kołodziejek J, Mazur J, Patykowski J. 2020. Differences in iron acquisition strategies of calcicole plant species from xerothermic grasslands. *Geoderma* 377:114572. DOI: 10.1016/j.geoderma.2020.114572.

88. Wang L, Liu B, Wang Y, Qin Y, Zhou Y, Qian H. 2020. Influence and interaction of iron and lead on seed germination in upland rice. *Plant and Soil* 455:187–202. DOI: 10.1007/s11104-020-04680-4.

89. Wilman V, Campbell EE, Potts AJ, Cowling RM. 2014. A mismatch between germination requirements and environmental conditions: Niche conservatism in xeric subtropical thicket canopy species? *South African Journal of Botany* 92:1–6. DOI: 10.1016/j.sajb.2013.12.007.

90. Winkelmann G. 2007. Ecology of siderophores with special reference to the fungi. *Biometals* 20:379–392. DOI: 10.1007/s10534-006-9076-1.

91. Wojtyla Ł, Lechowska K, Kubala S, Garnczarska M. 2016. Different modes of hydrogen peroxide action during seed germination. *Frontiers in Plant Science* 7:66. DOI: 10.3389/fpls.2016.00066.

92. Yazdi SAF, Rezvani M, Mohassel MHR, Ghanizadeh H. 2013. Factors affecting seed germination and seedling emergence of sheep sorrel (*Rumex acetosella*). *Romanian Agricultural Research* 30:373–380.

919   93. Yehuda Z, Shenker M, Romheld V, Marschner H, Hadar Y, Chen Y, 1996. The role of
920   ligand exchange in the uptake of iron from microbial siderophores by gramineous plants.
921   *Plant Physiology* 112:1273–1280. DOI: 10.1104/pp.112.3.1273.

922   94. Yi F, Wang Z, Baskin CC, Baskin JM, Ye R, Sun H, Zhang Y, Ye X, Liu G, Yang X,
923   Huang Z. 2019. Seed germination responses to seasonal temperature and drought stress
924   are species-specific but not related to seed size in a desert steppe: Implications for effect
925   of climate change on community structure. *Ecology and Evolution* 9:2149–2159. DOI:
926   10.1002/ece3.4909.

927   95. Zhang L, Liu R, Gung BW, Tindall S, Gonzalez JM, Halvorson JJ, Hagerman AE. 2016.
928   Polyphenol-aluminum complex formation: Implications for aluminum tolerance in plants.
929   *Journal of Agricultural and Food Chemistry* 64:3025–3033. DOI:
930   10.1021/acs.jafc.6b00331.

List of the studied plant species, their growth forms, seed characteristics, habitat preferences and ecological indicator values describing their realized centers of abundance.

Nomenclature of studied species follows the Plant List (http://www.theplantlist.org); [a]growth form of a given species, where: B – biennial herb, P – perennial herb, S – subshrub/semishrub; [b]seed size follows the literature (Bojñnanský & Fargašová, 2007); [c]seed weight was measured in this study (determined by weighing 100 air-dried seeds; mean ± SD, n = 4); [d]soil preference of the studied species estimated prior the experimental phase (basing on criteria presented in the article), where: A – acidophilous species from dry acidic grasslands, B – basophilic species from xerothermic alkaline grasslands; [e]ordinal scale (1–9) of Ellenberg's Indicator Values (Ellenberg, 1991), where: L – light requirements ranging from 7 to 9, where 7 indicates well-lit/slightly-shaded conditions (c.a. 30% of relative illumination) and 9 indicates full light conditions (>50% of relative illumination); T – temperature requirements ranging from 5 to 7, where 5 indicates species preferring moderately cool to warm conditions (characteristic of montane and submontane conditions, mostly southern Fennoscandia) and 7 indicates species preferring warm conditions (characteristic of North European Plain); K – continentiality requirements ranging 2 to 5, where 2 indicates atlantic conditions and 5 indicates subatlantic to subcontinental conditions; F – soil moisture requirements ranging from 2 to 5, where 2 indicates dry soils and 5 indicates moist soils; R – soil pH requirements ranging from 2 to 9, where 2 indicates extremely acidic to acidic soils and 9 indicates extremely alkaline soils originating from limestones; N – soil nutrient requirements ranging from 1 to 7, where 1 indicates extremely infertile soils and 7 indicates fertile soils; 0 – indifferent behaviour, wide amplitude or unequal behaviour in different areas; * – uncertain and not fully described behaviour.

1 **Table 1. List of the studied plant species, their growth forms, seed characteristics, habitat**
2 **preferences and ecological indicator values describing their realized centers of abundance.**

Species	Abbreviation	Family	Growth Form[a]	Seed size [mm][b]	Seed weight [mg][c]	Preference to soil pH[d]	Ellenberg's Indicator Value[e] L	T	K	F	R	N
Alyssum montanum L.	Amo	Brassicaceae	P	1.5-1.9 x 1.1-1.3	0.369 ± 0.002	A	9	6	4	2	7	1
Aster amellus L.	Aam	Asteraceae	P	3.4-3.8 x 1.5-1.8	1.010 ± 0.029	B	8	6	6	4	9	3
Betonica officinalis L.	Bof	Lamiaceae	P	2.7-3.1 x 1.3-1.5	1.147 ± 0.035	B	7	6	5	*	0	3
Centaurea scabiosa L.	Csc	Asteraceae	P	4.5-5.0 x 2.0-2.2	4.815 ± 0.211	B	7	0	3	3	8	4
Centaurea stoebe Tausch	Cst	Asteraceae	B	2.5-3.0 x 1.2-1.4	2.146 ± 0.033	A	8	7	5	2	8	3
Dianthus carthusianorum L.	Dca	Caryophyllaceae	P	2.0-2.4 x 1.5-1.8	0.283 ± 0.008	A	8	5	4	3	7	2
Dianthus deltoides L.	Dde	Caryophyllaceae	P	1.1-1.6 x 0.7- 1.0	0.106 ± 0.010	A	8	5	4	3	3	2
Echium vulgare L.	Evu	Boraginaceae	B/P	2.4-2.8 x 1.5-1.8	2.242 ± 0.048	A	9	6	3	4	8	4
Gentiana cruciata L.	Gcr	Gentianaceae	P	1.1-1.3 x 0.5-0.6	0.090 ± 0.003	B	7	6	4	3	8	3
Hieracium pilosella L.	Hpi	Asteraceae	P	2.1-2.3 x 0.5-0.5	0.147 ± 0.002	A	7	0	3	4	0	2
Hypericum perforatum L.	Hpe	Hypericaceae	P	1.0-1.2 x 0.5-0.6	0.076 ± 0.002	A	7	6	5	4	6	3
Hypochaeris radicata L.	Hra	Asteraceae	P	5.0-10.0 x 0.5-0.6	0.962 ± 0.025	A	8	5	3	5	4	3
Plantago media L.	Pme	Plantaginaceae	P	1.7-2.1 x 1.0-1.2	0.249 ± 0.007	B	7	0	7	4	7	3
Potentilla recta L.	Pre	Rosaceae	P	1.3-1.7 x 1.9-2.1	0.385 ± 0.018	B	9	7	5	3	5	2
Prunella grandiflora (L.) Scholler	Pgr	Lamiaceae	P	1.7-1.9 x 1.5-1.7	1.217 ± 0.031	B	7	0	5	3	8	3
Rumex acetosella L.	Rac	Polygonaceae	P	1.4-1.8 x 1.1-1.3	0.200 ± 0.013	A	8	5	3	4	2	2
Stachys germanica L.	Sge	Lamiaceae	B	1.9-2.2 x 1.4-1.6	1.392 ± 0.065	B	7	7	4	3	8	5
Thymus serpyllum L.	Tse	Lamiaceae	S	0.6-0.8 x 0.5-0.7	0.143 ± 0.004	A	7	6	5	2	5	1
Verbascum thapsus L.	Vth	Scrophulariaceae	B	0.8-1.0 x 0.5-0.6	0.131 ± 0.004	A	8	0	3	4	7	7
Veronica teucrium L.	Vte	Plantaginaceae	P	1.3-1.6 x 1.1-1.2	0.320 ± 0.022	B	7	6	5	3	8	2

4 Nomenclature of studied species follows the Plant List (http://www.theplantlist.org); [a]growth form of a
5 given species, where: B – biennial herb, P – perennial herb, S – subshrub/semishrub; [b]seed size follows
6 the literature (Bojñnanský & Fargašová, 2007); [c]seed weight was measured in this study (determined by
7 weighing 100 air-dried seeds; mean ± SD, n = 4); [d]soil preference of the studied species estimated prior
8 the experimental phase (basing on criteria presented in the article), where: A – acidophilous species
9 from dry acidic grasslands, B – basophilic species from xerothermic alkaline grasslands; [e]ordinal scale (1–
10 9) of Ellenberg's Indicator Values (Ellenberg, 1991), where: L – light requirements ranging from 7 to 9,
11 where 7 indicates well-lit/slightly-shaded conditions (c.a. 30% of relative illumination) and 9 indicates
12 full light conditions (>50% of relative illumination); T – temperature requirements ranging from 5 to 7,
13 where 5 indicates species preferring moderately cool to warm conditions (characteristic of montane and
14 submontane conditions, mostly southern Fennoscandia) and 7 indicates species preferring warm
15 conditions (characteristic of North European Plain); K – continentiality requirements ranging 2 to 5, where
16 2 indicates atlantic conditions and 5 indicates subatlantic to subcontinental conditions; F – soil moisture
17 requirements ranging from 2 to 5, where 2 indicates dry soils and 5 indicates moist soils; R – soil pH
18 requirements ranging from 2 to 9, where 2 indicates extremely acidic to acidic soils and 9 indicates

19   extremely alkaline soils originating from limestones; N – soil nutrient requirements ranging from 1 to 7,

20   where 1 indicates extremely infertile soils and 7 indicates fertile soils; 0 – indifferent behaviour, wide

21   amplitude or unequal behaviour in different areas; * – uncertain and not fully described behaviour.

Figure 1

Effect of pH on the final germination percentage (FGP; A) and index of germination velocity (IGV; B) of the tested species from acidic dry and alkaline xerothermic grasslands.

Values are the mean ± SD (n = 4). Different letters indicate significant differences between groups (ANOVA with Bonferroni post-hoc test, $p < 0.05$).
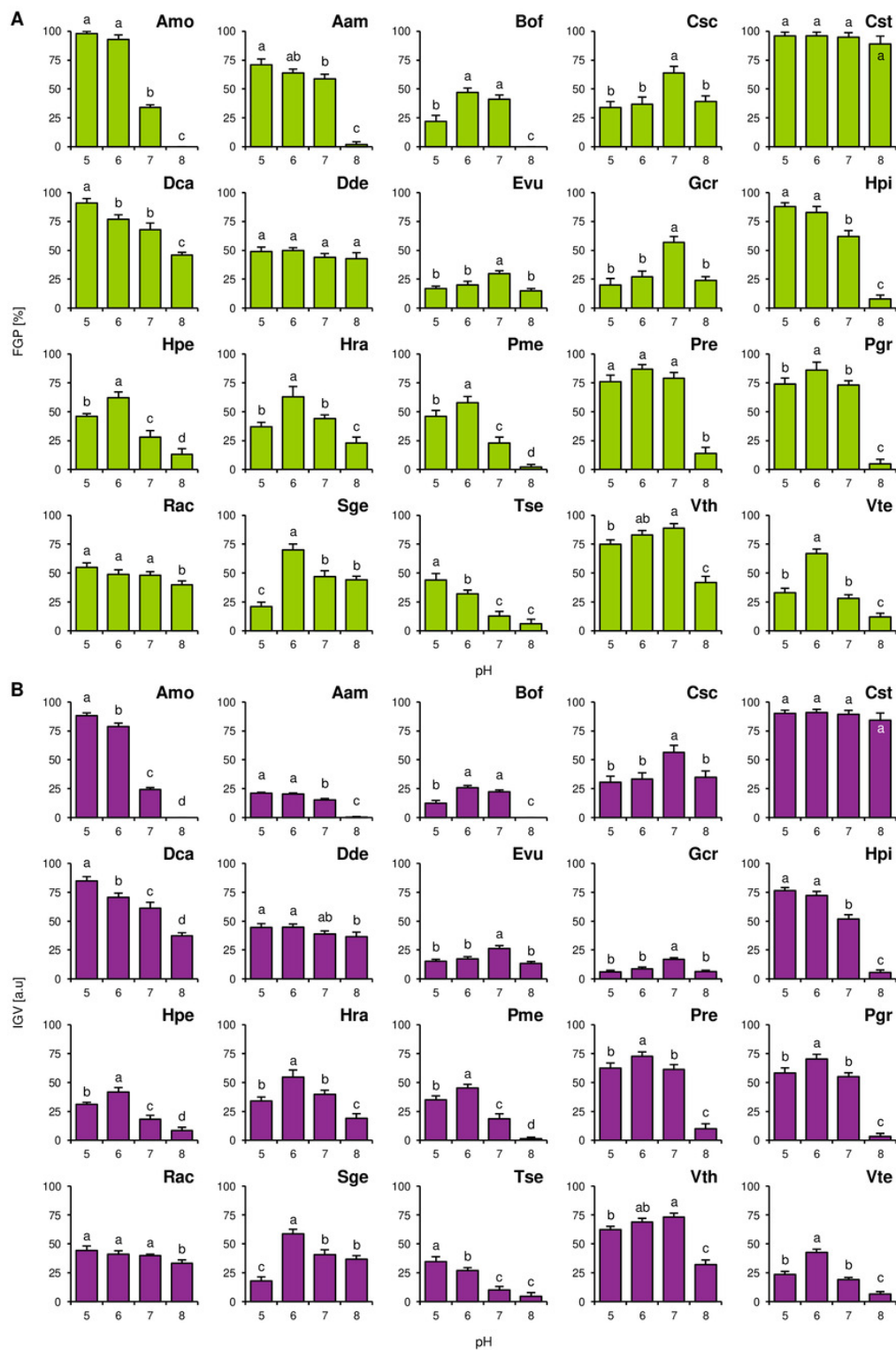
Figure 2

Effect of ionic and chelated iron (FeCl$_3$ and Fe-HBED) on the final germination percentage (FGP; A) and the index of germination velocity (IGV; B) of the tested species from acidic dry and alkaline xerothermic grasslands.

Values are the mean ± SD (n = 4). Different letters indicate significant differences between groups (ANOVA with Bonferroni post-hoc test, $p < 0.05$).
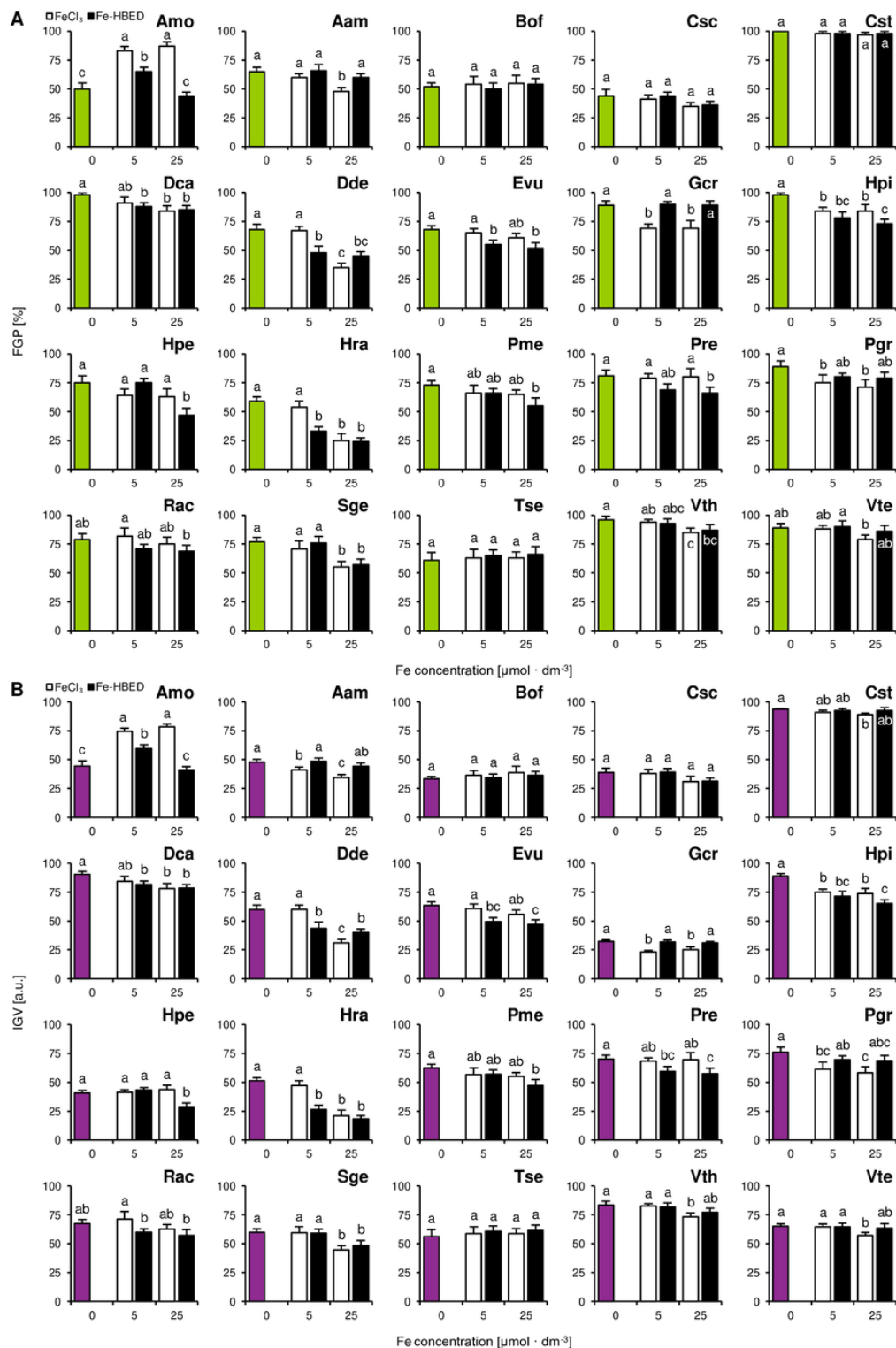
Figure 3

Effect of ionic and chelated manganese (MnCl$_2$ and Mn-HBED) on the final germination percentage (FGP; A) and index of germination velocity (IGV; B) of the tested species from acidic dry and alkaline xerothermic grasslands.

Values are the mean ± SD (n = 4). Different letters indicate significant differences between groups (ANOVA with Bonferroni post-hoc test, $p < 0.05$).
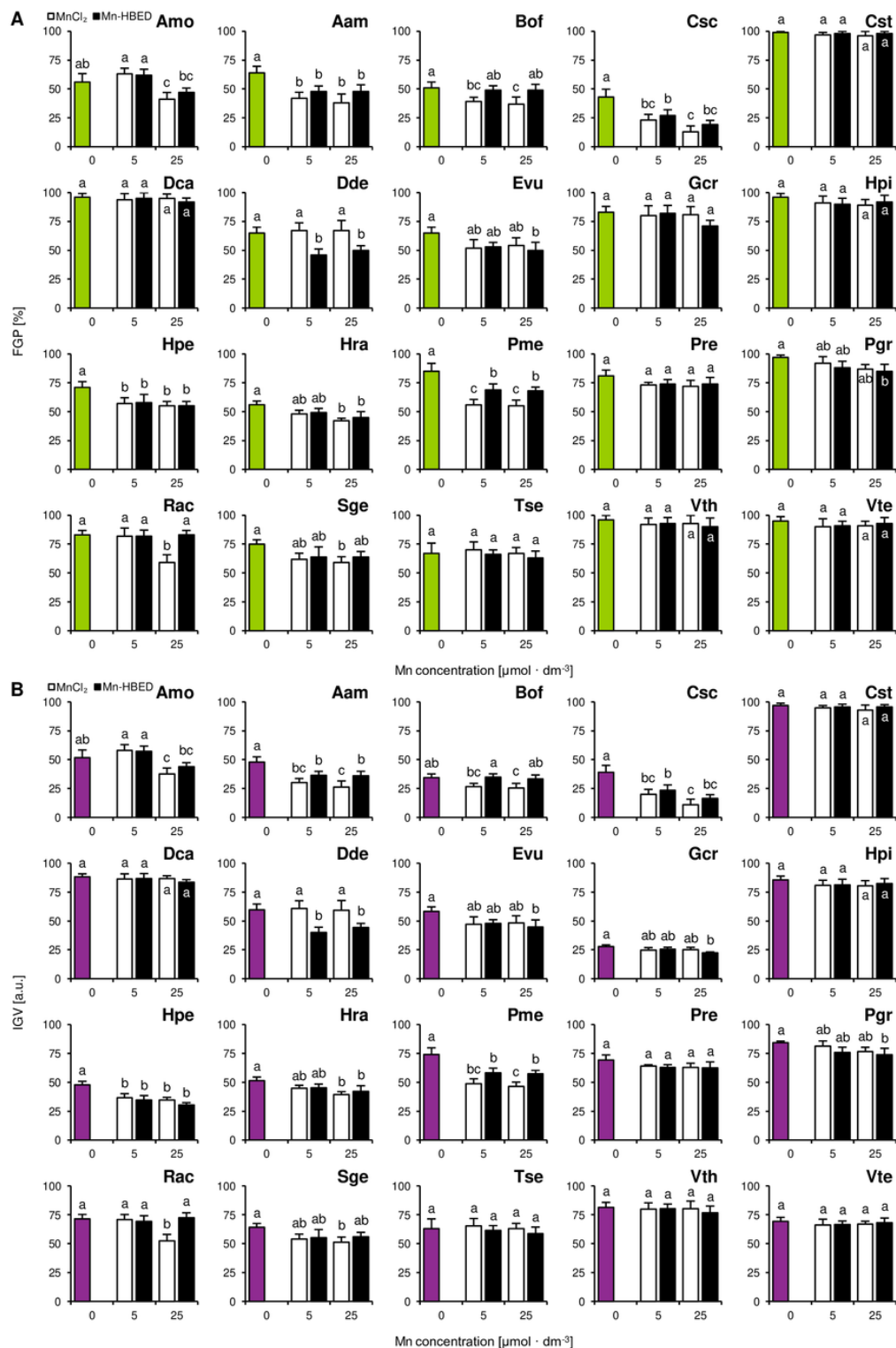
Figure 4

Effect of aluminum ($AlCl_3$) on the final germination percentage (FGP; A) and index of germination velocity (IGV; B) of the tested species from acidic dry and alkaline xerothermic grasslands.

Values are the mean ± SD (n = 4). Different letters indicate significant differences between groups (ANOVA with Bonferroni post-hoc test, $p < 0.05$).
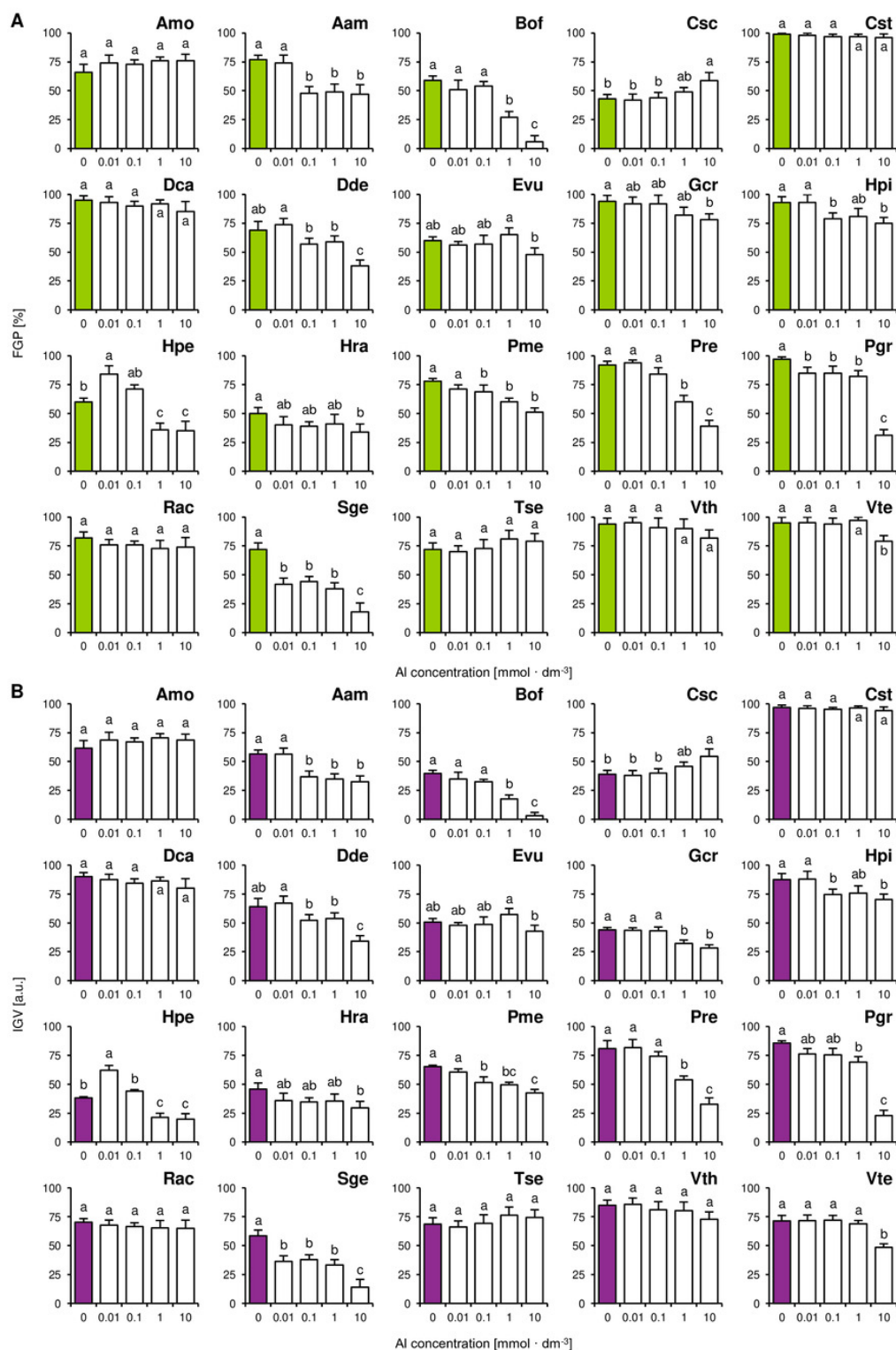
Figure 5

Hierarchical clustering analysis (HCA) of germination reactions (FGP) of the studied species to the tested conditions.

Analysis was conducted using data from Experiments 1–4. For each species, the data were normalized to FGP values recorded at pH = 7 (Experiment 1) or control (null concentration) conditions (Experiments 2–4). Three levels of annotations represents data presented in Table 1 (R Ellenberg's Indicator Value, estimated soil preference and seed weight). Green and red colors in scale bar represents stimulation and inhibition of FGP, respectively. Phenograms were constructed using Ward's method of row clustering and Manhattan distance.