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Seed bank and seedling recruitment of endangered *Tephroseris longifolia* subsp. *moravica* (Asteraceae)

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Abstract Knowledge of seed-bank dynamics and seedling recruitment is crucial for effective in situ conservation of endangered species. Herein, we studied *Tephroseris longifolia* subsp. *moravica* in five of nine existing population sites to determine its spatio-temporal regeneration patterns. Our main aims were: (i) to confirm the existence of a soil seed bank and to determine its type and density, (ii) to assess the rate of natural seedling recruitment and the factors affecting seed germination and seedling survival, and (iii) to assess the potential of artificial disturbance regimes to enhance natural seedling recruitment. We used a series of experiments based on long-term seed burial, seedling emergence, germination and seedling establishment in permanent plots and artificial disturbance regimes. We found that this taxon forms a short-lived persistent seed bank and its seed germinability decreases over time. The average germination in situ was 3.8 %. While the moss and herb layer cover supported seedling survival, tree-litter cover negatively affected in situ germination. Turf removal had the strongest positive effect on germination percentage in our three tested in situ treatments, followed by litter removal and no management procedures. Seedling mortality was very high (60–100 % of seedlings died), with no difference determined between the treatments. Our results suggest that seedling recruitment from seed banks and artificial disturbance regimes might be an extremely beneficial conservation protocol to enhance small populations of this critically endangered taxon.

Keywords conservation · disturbance · germination · persistent seed bank · seedling emergence · seedling survival

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Introduction

Soil seed banks affect plant community colonization, succession and structure (Cabin and Marshall 2000; Saatkamp et al. 2014), but they are also clearly relevant for the long-term survival of populations of individual species (Baskin and Baskin 2014). They constitute a source of propagules for recruitment following disturbance (Levin 1990; Chambers 1995; Williams et al. 2005) and can substitute for seed production, which has failed over long periods (Cabin and Marshall 2000). Seed-bank recruitment is most important in plants with a short life cycle (Kalisz and McPeek 1992; Philippi 1993; Pake and Venable 1996) and plants occupying temporally heterogeneous and unpredictable environments (Volis et al. 2002). Delayed germination through persistence in the seed bank is an adaptive strategy buffering the detrimental effects of temporally varying environments (Cohen 1966; Venable and

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Lawlor 1980; Klinkhamer et al. 1987). In long-lived perennials the existence of persistent seed banks is usually associated with infrequent but major disturbances such as fires or floodings (Thompson 2000).

Recruitment from seed banks is especially important for rare and endangered species with restricted or changing population size. In addition to buffering, soil seedbanks help maintain plant genetic diversity at the population level and reduce the vulnerability of populations to local extinction (Hill and Vander Kloet 2005). However, many rare and most-endangered species lack persistent soil seed banks (Saatkamp et al. 2014), and their restoration is only rarely sufficiently effective (Poschlod 1996; Zehm et al. 2008). Nevertheless, persistence of seeds in the soil is an important species trait, since it indicates a spatiotemporal strategy a given species explored in its recent evolutionary history (Saatkamp et al. 2014).

Species' composition and abundance in soil seed banks are not directly translated into adult plant communities through germination and seedling recruitment (Saatkamp et al. 2014). Although many rare species germinate well in natural populations, seedling survival in their natural habitats is often extremely low and cannot compensate for mortality of senile individuals or sufficiently contribute to population maintenance and growth. Seedling recruitment success is often dependent on their size, microclimatic conditions and the availability of suitable microsites such as vegetation gaps (Milberg 1993; Kalamees and Zobel 2002). Another important conditions for successful seedling recruitment are timing of disturbances or gap creation (Lavorel et al. 1994; Pakeman et al. 2005) and seed or seedling predation (Forget et al. 2005).

We studied soil seed bank and seedling recruitment in the rare narrow endemic *Tephroseris longifolia* (Jacq.) Griseb. & Schenk subsp. *moravica* Holub, known only from nine sites in Slovakia and the Czech Republic (Janišová et al. 2012a,b). This species is legally protected in both countries and is treated as an endangered taxon of national (Eliáš et al. 2015; Grulich 2012) and European importance (NATURA 2000, Directive 92/43/EEC Anex II, Bilz et al. 2011). It is particularly vulnerable because of its preference for secondary, anthropogenic habitats currently threatened by land use changes, including intensification or abandonment (Batoušek and Grulich 1989; Janišová et al. 2005). The taxon populations are therefore under long-term monitoring, and various aspects of their ecology, coenology and demography have been studied to determine optimal habitat conservation management (Kochjarová 1998; Chmelová 2007; Gbelcová 2010; Janišová et al. 2005, 2012a,b; Hegedüšová et al. 2013).

We conducted a series of experiments to determine *T. longifolia* subsp. *moravica* spatial-temporal regeneration patterns and to provide basic know-how for the in-situ conservation efforts: (1) We used seed burial for different duration periods and subsequent germinability testing as the most reliable and accurate method of studying soil seed bank persistence (Telewski and Zeevart 2002; Saatkamp et al. 2009). (2) We studied seedling emergence in soil from its natural sites to confirm the existence of seed banks in natural conditions. (3) We followed the fate of seeds sown on permanent plots in five natural populations between summer 2009 and spring 2014. The permanent plots were located along transects covering the large heterogeneity of ecotone environmental condition in forest-grassland transitions. (4) We simulated conservation management treatments in a manipulated field experiment to test the positive influence of artificial disturbance on seedling recruitment.

The main aims of this article are (i) to confirm the existence of a soil seed bank in natural populations of *T. longifolia* subsp. *moravica*; (ii) determine its type (transient vs persistent and short-persistent vs long-persistent) and also its density; (iii) assess the rate of natural seedling recruitment and quantify the effects of vegetation structure and soil disturbance on seed germination and seedling survival; and (iv) analyse artificial disturbance potential to enhance taxon seedling recruitment in its natural sites.

Materials and methods

Taxon

Tephroseris longifolia subsp. *moravica* (2n = 48, hexaploid with basic chromosome number x = 8; Kochjarová 1997; Olšavská et al. 2015) is a long-lived perennial plant. It is an allogamous taxon without apomictic reproduction (Janišová et al. 2012b). In natural populations it flowers in May, with seeds ripening in June (Janišová et al. 2012b). Its natural occurrence is restricted to small areas with specific, mainly transitional, ecotone or grassland habitat (Janišová et al. 2012a; Hegedüšová et al. 2013). The annual fluctuation in number of flowering individuals is very high (Table 1). Flowering between 2005 and 2014 varied from 11 to 248 plants in the smallest population studied (Omšenie), and 74 to 2,117 in the largest (Radobica; Table 1). While the output of well-developed seeds per flowering shoot was estimated at 592 and averaged at 1,125 seeds per flowering plant (Janišová et al. 2012b), seed output can be significantly reduced by the *Phycitodes albatella* (family Pyralidae) granivorous butterfly identified at all population sites studied (Janišová et al. 2012b).

Seedling emergence

We collected soil samples at Radobica to prove seed bank presence in this taxon's natural site conditions. Here, the 0–4 cm and 5–8 cm layers were sampled because depth distribution is a reliable indicator of seed longevity (Thompson et al. 1997; Bekker et al. 1998). Three sets of soil samples were collected at each depth in 2011; one on 24th of March and two on 18th of May, and these were randomly distributed over 2 m^2 of vegetation which had contained a high density of flowering taxon plants the previous growing season. Each set consisted of 10 soil samples in metal cylinders with 100 cm^3 volume, and these were combined and spread on a sterilized substrate in 5 plastic containers, regularly watered and subsequently inspected for seedlings for 16 months; May 2011 to August 2012. Protection against herbivores was ensured by placing the containers in a wooden frame with a dense net cover, and these were embedded in an experimental garden lawn in Banská Bystrica in central Slovakia (48°45′09″ N, 19°0923′29″ E, 390 m a.s.l.). This simulated natural microclimatic conditions for seed germination, and container position within the frame was regularly changed to prevent different light and moisture availability. The first *T. longifolia* subsp. *moravica* seedlings appeared 12 days after embedding and the last seedling almost ten months later. Well-established seedlings were transplanted into garden-soil pots and cultivated in open-air conditions until identity confirmation.

Seed burial

To estimate the longevity of *T. longifolia* subsp. *moravica* soil seed bank, we used a burial experiment with burial periods between 1 and 5 years. Burial experiments for periods of different duration is advantageous as it does not only clearly differentiate between the transient and short-persistent seed banks (Thompson et al. 1997; Price et al. 2010), but also enables to quantify the temporal decline in seed germinability during the burial (Telewski and Zeevart 2002; Saatkamp et al. 2009). The seeds were collected on the 23rd of June 2008 from Radobica and Čavoj natural populations and dried and stored at 20.5°C room temperature. After excluding damaged and poorly developed seeds without embryo in November 2008, we placed 50 well-developed seeds in 20 nylon bags and buried 10 bags at each of the original Radobica and Čavoj sites. Two bags were excavated from each site in the 2009–2013 March–April spring seasons, and thx`x`e seeds were tested for ability to germinate. Seeds were regularly watered on Petri dishes and maintained in the laboratory until all germinated or decayed. Differences in final germination percentages (square-root transformed data) and the average starting day of germination were tested by general linear models with two predictors (Anonymus 1996): (1) time as a continuous predictor expressed as the number of months after seed collection and (2) Čavoj or Radobica population site as a categorical predictor. For each population, dry-stored seed percentage germination prior to seed burial was the baseline used to estimate buried seed decline in germination over time (details in Janišová et al. 2012a).

In situ germination

Freshly collected seeds of *T. longifolia* subsp. *moravica* were sown in the 2009 and 2011 summers in the five origin population sites: Čavoj, Radobica, Lysá, Omšenie and Stráž (Table 1). A total of 25 transects were established (4–6 transects at each population site). These consisted of five 25 × 25 cm plots separated by a 0.5 m-wide buffer zone and placed in various habitat conditions typical for local populations, thus ensuring microsite heterogeneity from open grasslands to shaded forest margins with exposed soil surface. The 2009 transects had three capitula with well-developed seeds spread in each plot, thus according to our previous study (Janišová et al. 2012b) number of well-developed seeds spread in each plot was estimated as 204 (with 68 seeds in each of 3 capitula with minimum 9, maximum 123 and $SD = 22$). The 2011 transects comprised fifty well-developed seeds sown in each plot. All plots were revisited several times during the following 5 years. Surveys of plots established in June 2009 were

Table 1 Population sites of *Tephroseris longifolia* subsp. *moravica*, their location and main attributes.

Population site	Mountain range	Longitude	Latitude	Altitude [m a.s.l.]	Area [m^2]	Current management	Number of flowering individuals (average and range for 2004–2014)	Number of flowering individuals per m^2
Lysá	**Biele Karpaty**	18°08′41″	49°04′17″	740–780	12,000	Long time without management or irregularly grazed	325 (10–724)	0.027
Čavoj	**Strážovské vrchy**	18°29′26″	48°52′57″	560–585	20,000	Mostly abandoned, partly cleared and mown	545 (94–1,585)	0.025
Omšenie	**Strážovské vrchy**	18°14′36″	48°54′52″	570–670	11,000	Regularly mown, partly without management	84 (11–248)	0.008
Radobica	**Tríbeč**	18°29′55″	48°34′27″	480–560	15,000	Mostly mown, partly without management	803 (74–2,117)	0.049
Stráž	**Vtáčnik**	18°32′40″	48°32′54″	770–780	5,000	Abandoned for decades and covered by large shrubs	99 (3–387)	0.020

performed 10–13 months (May 2010–August 2010), 22 months (May 2011), 34 months (June 2012), 45 months (May 2013) and 57 months (May 2014) after sowing. The surveys of plots established in June–July 2011 were carried out after 5 weeks (August 2011), 11 months (June 2012), 23 months (May 2013) and 35 months (May 2014). Old and newly germinated seedlings were distinguished at each recording, based on plot position. Same-size adjacent plots were inspected at each recording as controls, indicating seedling recruitment from seed bank and fresh seed dispersal. Analysis comprised cumulative numbers of seedlings recorded in the control plots subtracted from those in the sown plots. Percentage germination was calculated for each plot and expressed as a proportion of the highest 2009 and 2011 plot values, so that data from the two transect sets could be analysed simultaneously. Following square-root transformation, these values were used to test differences in germination at the sites by ANOVA (Anonymus 1996). The percentage of survived seedlings was calculated for each survey period, and average survival was calculated over the entire observation period. Differences in percentage survival during the first year and the average survival in all transition periods were tested by ANOVA (Anonymus 1996).

Vegetation and disturbance effects

We measured selected environmental variables to assess the effect of vegetation structure and disturbances on germination and seedling survival in all plots. Measurements were taken only once, in 2012, because there were no appreciable changes during the 2009–2014 experimental period. The following six variables were determined in each plot: herb and moss layer percentage cover, cover of bare soil, herb litter, tree litter, and disturbed surfaces. Simple regression models were calculated for germination percentage and seedling survival as dependent variables, and six plot characteristics comprised continuous predictors. We then calculated the percentage variance in dependent variables explained by each model in R, and Bayesian information criterion (BIC) selected the more appropriate linear or quadratic model.

In situ management experiment

We established a field manipulative experiment at the Radobica site and tested the effects of conservation management and artificial disturbance on *T. longifolia* subsp. *moravica* germination and seedling establishment. Sixteen plots were established on the 18th of September 2012, each 20 × 20 cm surrounded by a 0.8 m-wide buffer zone. Four control plots without seed addition and the three 'no management, litter removal and turf removal' treatments were organized in four replicates in a Latin square design. Fifty well-developed seeds collected in June 2012 were sown in a regular grid pattern in each treatment plot, and four sets of 25 seeds were simultaneously sown to dishes with 5 cm-deep sterilized substrate in the experimental garden at Banská Bystrica for comparison. These were regularly watered and surveyed, and all seeds and seedlings were surveyed after 5 weeks (23rd October 2012), 8 months (13th May 2013), 13 months (23rd October 2013) and 20 months (19th May 2014). Old and newly-germinated seedlings were distinguished at each recording, based on their plot coordinates. We found no seeds germinated in the control plots, so the number of seedlings in the treatment plots was used without adjustment. Differences in percentage germination between the three treatments were evaluated by ANOVA (Anonymus 1996) after square-root transformation, and seedling mortality differences were tested separately for each of the three transition periods. This was also performed by ANOVA with the number of seedlings at the beginning of each period set as a covariate.

Results

Seedling emergence

The first *T. longifolia* subsp. *moravica* seedlings in all cultivated soil samples appeared within two weeks. All except one seedling appeared five months after soil exposure and the last seedling germinated in spring 2012 after ten months soil exposure. In samples from the upper soil layer (0–4 cm below the soil surface), the numbers of seeds germinated were 6, 0 and 1 for the three sets of soil samples (the March and the two May samples), respectively. Accordingly, the mean number of germinable seeds calculated per 1 m^2 was 93 (range 40–240). In samples from the lower soil layer (5–8 cm below the soil surface), the number of seeds germinated were 1, 2 and 2 for the three sets of soil samples (the March and the two May samples), respectively. Accordingly, the mean number of germinable seeds per 1 m^2 was 67 (range 40–80).

Seed burial

Germination percentage of *T. longifolia* subsp. *moravica* was affected by time where the percentage gradually declined with duration of seed burial (GLM, $F = 12.83$, $P = 0.002$) but remained unaffected by population site (GLM, $F = 3.42$, $P = 0.082$, Fig. 1, Appendix 1). Seed ability to germinate decreased to approximately half the seed-burial reference value after two winters, and to 14 % of the reference value after five winters (Fig. 1; Janišová et al. 2012b). Average starting day of germination was unaffected by either time (GLM, $F = 1.63$, $P = 0.223$) or population site (GLM, $F = 3.857$, $P = 0.070$).

In situ germination

The in situ germination percentage was very low, at 3.8 % average of developed seeds sown, and no difference in germination percentage was noted in the five population sites (ANOVA, $P = 0.227$). An average 35.8 % seedlings survived the first year after sowing with 30.9 % average survival for all transition periods (Appendix 2). In addition, the population sites differed neither in survival during the first year (ANOVA, $P = 0.434$) nor in average survival for all transition periods (ANOVA, $P = 0.195$).

Vegetation and disturbance effects

While germination percentage was negatively affected by percentage tree-litter cover, initial year survival was supported by intermediate moss layer cover and high herb layer cover. The average survival was also positively affected by herb-litter cover, but negatively influenced by tree-litter cover, cover of bare soil and disturbed surfaces (Table 2, Appendix 3). Overall, 52 % of seedlings which germinated within the first five weeks following the 2011 sowing did not survive the May 2013 survey 10 months after their germination. In addition, 43 % of seedlings died between the 10th and 22th month following germination, in June 2012 and May 2013, and only 5 % of seedlings survived until

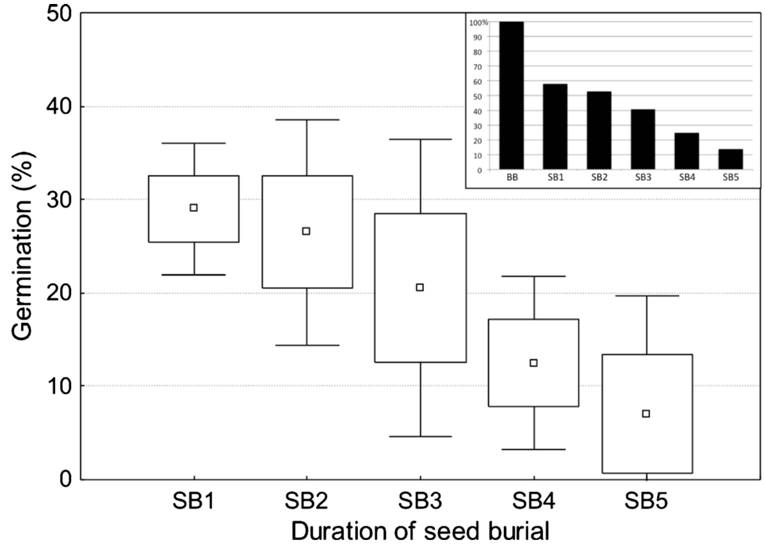

Fig. 1 Germination of *T. longifolia* subsp. *moravica* seeds collected and buried in 2008 and excavated in the five subsequent years: SB1 – 2009, SB2 – 2010, SB3 – 2011, SB4 – 2012, SB5 – 2013. The upper-right-corner chart shows decline in buried seed germination percentages, where germination of seeds occurring before burial is 100 %. (BB, data according to Janišová et al. 2012b). Middle point: mean, box: mean ± *SE*, whisker: mean ± *SD*.

the last May 2014 survey, 35 months after germination (Appendix 2).

In situ management experiment

Repeated-measures ANOVA analysis of data from plots with different treatments (Fig. 2) determined highly significant differences between the treatments ($P < 10^{-5}$ for both treatment and time effects, interaction between treatment and time was not significant, $P = 0.62$). While no seedlings germinated in control plots, the highest germination in treatment plots was observed in those with removed grassland turf (34.5 ± 10.5 %), followed by plots with removed herb litter (18.5 ± 8.2 %) and unmanaged plots (14 ± 4.3 %; Fig. 2). The cumulative percentage germination of 68 ± 14.2 % in cultivated plots was twice as high as in the most successful in situ treatment. Germination commenced and peaked by September/October 2012 in all treatments, within the first five weeks after sowing (Fig. 3). Out of the 134 germinated seedlings in this experiment, 81 % germinated 5 weeks after sowing (by the 23rd of October 2012), 14 % germinated between the 6th week (23 October 2012) and the 8th month (May 2013), 4 %

Table 2 Summary of simple regression models for in situ germination and seedling survival of *T. longifolia* subsp. *moravica* as dependent variables and plot characteristics as predictors. Linear and quadratic relationships were compared and the model with lower BIC is presented by arrows ↑ or ↓ for linear relationships and ∩ for a hump-back relationship. Percentage variance of dependent variable explained by the model is shown in parentheses. Scatter plots are shown in Appendix 4. Significant ($P < 0.05$) and marginally significant ($P \approx 0.05$) relationships are shown; n.s. – non–significant.

	Germination	1st-year survival	Average survival
Herb layer [%]	n.s.	↑ $P = 0.026$ (9.3 %)	↑ $P = 0.001$ (13.8 %)
Moss layer [%]	n.s.	∩ $P = 0.005$ (19.3 %)	∩ $P < 0.001$ (27.8 %)
Bare soil [%]	n.s.	n.s.	↓ $P = 0.053$ (5.1 %)
Herb litter [%]	n.s.	n.s.	↑ $P = 0.017$ (7.7 %)
Tree litter [%]	∩ $P = 0.029$ (5.7 %)	n.s.	↓ $P = 0.050$ (5.2 %)
Disturbed surface [%]	n.s.	n.s.	↓ $P = 0.054$ (5.1 %)

between the 8th (May 2013) and 13th months (October 2013), and 1 % between the 13th (October 2013) and 20th months (May 2014) (Appendix 4). We also determined that while germination did not continue in cultivated and litter-removed plots after May 2013, a limited number of seedlings appeared in non-managed plots and those with turf removal (Fig. 3). Seedling mortality was very high, where 60–100 % of seedlings died, and this result did not differ significantly between treatments in any of the three transition periods (ANOVA, $P = 0.767$, $P = 0.302$ and $P = 519$; Appendix 4).

Discussion

Seed bank in *T. longifolia* subsp. *moravica*

A persistent seed bank for *T. longifolia* subsp. *moravica* is newly described herein. Most previous researchers assumed absence of a seed bank in this taxon because of the sudden decline in dry-stored seed germination in the first year of seed ripening (Bábková-Hrochová 2004; Gbelcová 2006; Chmelová 2007). Chmelová (2007) also recorded reduced germination percentage to a mean value of 17 % 24 months after seed ripening, as the endosperm dried out rapidly in warm, dry conditions and the seed gradually died. However, we discovered that *T. longifolia* subsp. *moravica* seeds buried in the upper soil layer can survive for more than five years in their natural habitats. Coincidentally, our seedling emergence experiment proved that germinable *T. longifolia* subsp. *moravica* seeds are present in natural habitat soils one year after their dispersal, not only near the soil surface but also at 5 to 8 cm depth, which is comparable to the depth of our burial experiment. After disturbances of the upper soil layer, which are very frequent in the studied population sites, the naturally buried seeds may reach the soil surface with suitable light conditions and germinate.

The density of viable seeds usually decreases with depth in non-tilled soils (Thompson et al. 1997) and samples are therefore taken from the soil surface to approximately 10 cm depth in most seed bank studies. As expected, the density of viable seeds in our experiment was higher in soil samples from the upper soil layer than those at the lower depth. While this is proven in our burial results, with some temporal decrease in *T. longifolia* subsp. *moravica* seed germinability in the seed bank, seeds at greater depths were expected to be older with less ability to germinate; however, our discovery of germinable seeds in the deeper soil layer supports evidence of a persistent seed bank in this taxon.

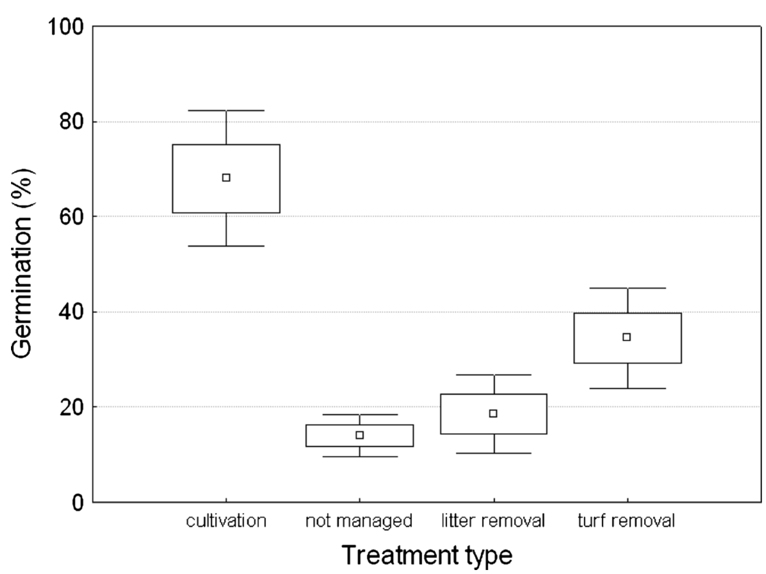

Fig. 2 Germination in cultivation (ex situ) and experimental plots (in situ) under three management treatments. Cumulative percentage germination is shown in all 20 months after seed sowing. Germination was twice as high in cultivation as in the most successful in situ treatment. In the treatment plots, the highest germination was recorded in plots with removed grassland turf. Middle point: mean, box: mean ± *SE*, whisker: mean ± *SD*.

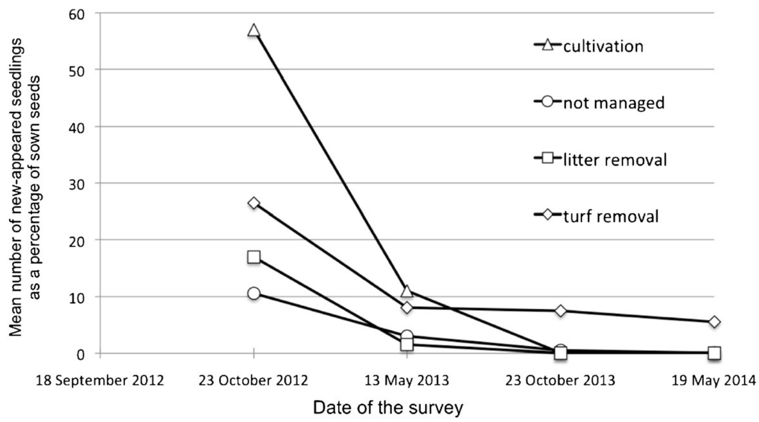

Fig. 3 Management treatment effects on seed germination dynamics. Germination in cultivated and all treatment plots peaked in the first five weeks after sowing and then decreased, remaining active mainly in plots with turf removal**.**

Our burial experiment indicated that seeds can survive in the seed bank no longer than 5–6 years, thus we classify the taxon seed bank short-lived. However, conditions in burial experiments are artificial compared to those encountered by seeds in natural populations and it is clear that seeds do not germinate in the dark. Bakker et al. (1996) warn that results from artificial burial experiments should be interpreted with caution, as they bypass the crucial role of natural burial mechanisms and are therefore prone to serious overestimation of seed longevity; and results from long-term burial experiments are also frequently close to the upper limit of longevity recorded for many species. Moreover, although artificially buried seeds are protected from the attention of potential predators including birds, mice, and beetles, they are also prevented from potential positive effects of soil organisms such as stimulation of germination by earthworm ingestion (van Tooren and During 1988; Thompson et al. 1994). While the seedling emergence method we used indicates a readily germinable seed bank, it does not provide reliable seed-bank assessment unless the soil sample is maintained for extended periods of time at simulated habitat conditions (Thompson and Grime 1979). This method can also dramatically underestimate the density of the seed bank due to errors associated with seed dormancy and specific environmental requirements for germination (Brown 1992; Bernhardt et al. 2008). All the above suggest that combined methods of determining seed bank characteristics and density are optimal to eliminate inadequacies in individually applied methods.

Soil seed bank in closely related taxa

As regards closely related *Tephroseris* species, a seed bank was confirmed in *Tephroseris helenitis* (L.) B. Nord. based on seedling emergence in a soil sample from natural vegetation (LEDA database), but we have no information on its longevity or density. Thompson et al. (1997) report that *Tephroseris palustris* (L.) Rchb. seeds survive in the soil for more than one year but less than five years; their maximum longevity, however, remains unknown (LEDA database; Staniforth et al. 1998). Widén (1987) inferred that *Tephroseris integrifolia* (L.) Holub species does not possess a seed-bank and most of its seeds germinate during the first year after dispersal, specifically in late summer or autumn. Meindl and Poschlod (2007) and Meindl (2011) record that *T. integrifolia* subsp. *vindelicorum* Krach has a transient seed bank. Most of its seeds germinate during the first autumn and, due to a missing seed dormancy and germination even in darkness, they are not able to form a persistent soil seed bank.

Seed bank role in *T. longifolia* subsp. *moravica* population dynamics

The seed-bank contribution to the persistence of *T. longifolia* subsp. *moravica* populations can be highly important during periods with weather conditions favourable to germination but with insufficient freshly-ripened seeds either due to low proportions of flowering individuals in the particular growing season or due to high seed predation (Janišová et al.

2012b). Theoretically, seed-bank seeds can rescue populations from extinction if the climate and habitat conditions are suitable for germination. Hence, Alexander and Schrag (2003) and Bucharová et al. (2012) registered seed bank importance in re-establishing plant populations and Dostálek and Münzbergová (2013) determined that seed bank recruitment is especially important for small populations endangered by low genetic diversity. However, other studies suggest that seed banks alone are insufficient to re-establish a species that has disappeared from its natural population site (Thompson et al. 1997; Baskin and Baskin 2014; Handlová and Münzbergová 2006).

In situ germination and vegetation and disturbance effects

Our results highlight that *T. longifolia* subsp. *moravica* seedling recruitment is very low in natural populations and that it is dependent on the availability of suitable germination microsites, including grassland patches without a close herb layer and disturbed areas with an open soil surface. These are most frequent in shaded tree and shrub understoreys, while thick tree-litter layers suppress germination. Seedling establishment and survival is subject to factors other than emergence; the positive effects of increased moss and herb cover and negative effects of bare soil highlight that this taxon's seedlings are susceptible to desiccation and that they thrive best in microsites protected from strong temperature and moisture fluctuations. In this context, herb litter may also have positive effect on seedling survival, although the general mechanisms of germination prevention by litter are well understood (Facelli and Pickett 1991). A positive effect of the moss layer on germination was reported also for *Ligularia sibirica* (Asteraceae) by Heinken-Šmídová and Münzbergová (2012) and for *Succisella inflexa* (Dipsacaceae) by Overbeck et al. (2003) whereas other studies indicated an inhibition of seedling establishment and growth by the moss layer (e.g. Kotorová and Lepš 1999; Rydin and Jeglum 2006). However, the later studies deal with wet habitats (wet meadows and peatlands), where the effect of the moss layer differs from effects in drier habitats exposed to dessication.

Management and conservation implications

Knowledge of soil seed bank dynamics and processes affecting seedling recruitment is crucial for efficient conservation, reintroduction of plants at risk of extinction and especially critical in restoring both population numbers and genetic variability (Allen 1994; Hurka 1994; Holsinger 1995; Cabin and Marshall 2000; Cochrane et al. 2007). *Tephroseris longifolia* subsp. *moravica* is a critically endangered plant species because of the restricted number and size of its populations. Our results suggest that low seedling recruitment in situ is responsible for decreased population size, especially in less abundant populations with limited seed output. In these populations, seedling recruitment from seed banks may be important during climatically suitable periods. Although seed germinability decreases rapidly over time, seed burial maintains seed longevity for at least several years. Moreover, germination time is shorter in buried seeds than in those dry-stored (cf. Janišová et al. 2012b), thus suggesting that buried seeds exposed to light can germinate almost immediately. Seed bank recruitment in low flowering years can be supported by artificial disturbances in early spring or autumn when sufficient moisture is ensured for seedling emergence and establishment. The conservation management including reducing vegetation cover by raking, small-scale exposure of the topsoil layer and tree-litter removal should be applied during a period of seed dispersal especially in high flowering years. Our results may be applied also in sites with extinct populations. Continued conservation management for several years after taxon extinction could increase the likelihood of its replenishment from a seed bank. The explicit role of seed banks in *T. longifolia* subsp. *moravica* population dynamics remains unclarified, and only a detailed long-term demographic study of marked individuals can elucidate this and related uncertainties.

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Appendix 1

Percentage germination and average starting day of germination of *T. longifolia* subsp. *moravica* seeds collected in June 2008 from two population sites, Čavoj (C) and Radobica (R).

Year of germination test	2009	2010	2011	2012	2013
Treatment	**Seed burial**				
Germination [%]	34 R	38 R	26 R	24 R	26 R
22 R	10 R	38 R	16 R	0 R	
36 C	26 C	18 C	4 C	2 C	
24 C	32 C	0 C	6 C	0 C	
Average starting day of germination	8.8 R	4.4 R	5.8 R	5.8 R	8.5 R
8.8 R	5.4 R	4.5 R	5.8 R	4.0 C	
6.2 C	4.9 C	4.7 C	5.0 C		
5.9 C	5.3 C		5.0 C		

Appendix 2

Numbers of Seedlings and % Seedling Survival in Transect Plots Sown in 2011 (A) and 2009 (B). Population Sites: O – Omšenie, C – Čavoj, R – Radobica, S – Stráž, L – Lysá

A – Explanation of columns: 1 – Transect_plot; 2 – Number of seeds sown in O (4 July 2011), C (29 June 2011), R (26 June 2011), S (26 June 2011), L (4 July 2011); 3 – Number of seedlings on the 1st survey in O (11 August 2011), C (11 August 2011), R (2 August 2011), S (2 August 2011), L (11 August 2011); 4 – Number of seedlings on the 2nd survey in O (7 June 2012), C (5 June 2012), R (4 June 2012), S (6 June 2012), L (8 June 2012), old/new-recorded; 5 – Number of seedlings on the 3rd survey in O (15 May 2013), C (16 May 2013), R (13 May 2013), S (14 May 2013), L (17 May 2013), old/new-recorded; 6 – Number of seedlings on the 4th survey in O (22 May 2014), C (23 May 2014), R (19 May 2014), S (20 May 2014), L (21 May 2014), old-/new-recorded; 7 – Cumulative number of seedlings; 8 – Number of seeds sown in the control plot; 9–12 – Number of seedlings in the control plots on the 1st, 2nd, 3rd and 4th surveys, old/new-recorded; 13 – Corrected cumulative number of seedlings (number of seedlings in the control plot subtracted); 14 – % germination expressed as a proportion from the highest value in 2011; 15 – % seedling survival between the 1st and 2nd surveys, 16 – % seedling survival between the 2nd and 3rd surveys; 17 – % seedling survival between the 3rd and 4th surveys; 18 – % cover of herb layer; 19 – % cover of moss layer; 20 – % cover of bare soil; 21 – % cover of herb litter; 22 – % cover of tree litter; 23 – % cover of disturbed surface.

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
O1_1	50	0	0/0	0/0	0/0	0	0	0	0	0	0	0	0.000				28	0	1	5	75	0
O1_2	50	8	1/0	0/0	0/0	8	0	0	0	0	0	8	0.333	13	0		55	0	0	15	60	0
O1_3	50	5	3/0	0/0	0/0	5	0	0	0	0	0	5	0.208	60	0		45	0	1	25	60	0
O1_4	50	1	1/2	0/0	0/0	3	0	0	0	0	0	3	0.125	100	0		90	0	0	40	40	0
O1_5	50	0	0/1	0/0	0/0	1	0	0	0	0	0	1	0.042		0		45	0	0	25	65	0
O2_1	50	0	0/0	0/0	0/0	0	0	0	0	0	0	0	0.000				20	0	2	25	60	0
O2_2	50	0	0/0	0/0	0/0	0	0	0	0	0	0	0	0.000				70	0	0	10	60	0
O2_3	50	0	0/0	0/0	0/0	0	0	0	0	0	0	0	0.000				75	0	0	7	60	0

(continued)

O2_4	50	7	0/0	0/1	0/0	8	0	0	0	0	0	8	0.333	0		0	40	0	0	60	60	0
O2_5	50	4	3/0	0/0	0/0	4	0	0	0	0	0	4	0.167	75	0		60	0	0	15	50	0
O3_1	50	0	0/0	0/0	0/0	0	0	0	0	0	0	0	0.000				42	0	0	8	75	0
O3_2	50	0	0/0	0/2	0/0	2	0	0	0	0	0	2	0.083			0	65	0	0	20	75	0
O3_3	50	3	0/2	2/0	0/0	5	0	0	0/1	0	0	4	0.167	0	100		25	0	1	5	85	0
O3_4	50	3	1/4	0/0	0/0	7	0	0	0	0	0	7	0.292	33	0		6	0	10	0	85	0
O3_5	50	0	0/0	0/0	0/0	0	0	0	0	0	0	0	0.000				4	0	1	0	97	0
C1_1	50	0	0/2	1/1	0/0	3	0	0	0	0	0	3	0.125		50	0	25	0	85	1	10	0
C1_2	50	0	0/2	0/1	0/0	3	0	0	0/1	0	0	2	0.083		0	0	20	5	10	0	70	0
C1_3	50	0	0/0	0/0	0/0	0	0	0	0	0	0	0	0.000				70	25	50	1	22	0
C1_4	50	0	0/0	0/0	0/0	0	0	1	1/0	0	0	-1	0.000				20	8	70	1	12	0
C1_5	50	2	2/0	0/0	0/0	2	0	0	0	0	0	2	0.083		100	0	21	2	65	0	15	0
C2_1	50	3	1/0	0/0	0/0	3	0	0	0	0	0	3	0.125	33	0		70	25	0	5	2	0
C2_2	50	1	0/0	0/0	0/0	1	0	0	0	0	0	1	0.042	0			25	60	0	8	5	0
C2_3	50	2	0/1	1/0	1/0	3	0	0	0	0	0	3	0.125	0	100	100	35	40	0	25	5	0
C2_4	50	0	0/0	0/0	0/0	0	0	0	0	0	0	0	0.000				55	30	2	25	2	2
C2_5	50	0	0/0	0/0	0/0	0	0	0	0	0	0	0	0.000				55	40	0	30	10	0
C3_1	50	0	0/0	0/0	0/0	0	0	0	0	0	0	0	0.000				45	2	3	35	3	2
C3_2	50	3	3/0	2/0	0/0	3	0	0	0	0	0	3	0.125	100	67	0	95	5	2	20	0	2
C3_3	50	0	0/0	0/4	4/1	5	0	0	0	0	0	5	0.208			100	65	5	3	20	0	5
C3_4	50	12	12/6	15/0	11/0	18	0	0	0/1	0	0	17	0.708	100	83	73	35	25	2	35	2	0
C3_5	50	3	1/0	1/0	1/0	3	0	0	0	0	0	3	0.125	33	100	100	80	1	1	5	0	0
L1_1	50	0	0/0	0/0	0/0	0	0	0	0	0	0	0	0.000				60	0	10	0	60	0
L1_2	50	19	8/0	0/2	1/0	21	0	0	0	0/1	0	20	0.833	42	0	50	30	10	45	10	10	15
L1_3	50	8	1/0	0/0	0/0	8	0	0	0	0	0	8	0.333	13	0		70	0	30	1	30	0
L1_4	50	1	0/0	0/0	0/0	1	0	0	0	0/1	1/0	0	0.000	0			85	1	20	10	50	0
L1_5	50	0	0/1	1/0	1/0	1	0	0	0	0	0	1	0.042		100	100	75	15	10	5	75	0
L2_1	50	2	0/0	0/0	0/0	2	0	0	0/1	0	0	1	0.042	0			65	0	0	25	60	0
L2_2	50	4	2/0	0/0	0/0	4	0	0	0/1	0	0	3	0.125	50	0		30	0	3	15	75	0
L2_3	50	2	1/0	0/0	0/0	2	0	0	0	0	0	2	0.083	50	0		50	0	30	15	55	10
L2_4	50	4	4/5	1/0	1/0	9	0	0	0	0	0	9	0.375	100	11	100	75	0	25	35	35	5
L2_5	50	2	0/1	0/0	0/0	3	0	0	0	0	0	3	0.125	0	0		65	0	5	45	50	5
L3_1	50	23	16/1	2/0	0/0	24	0	0	0	0	0	24	1.000	70	12	0	45	1	0	50	4	0
L3_2	50	4	0/0	0/0	0/0	4	0	0	0	0	0	4	0.167	0			30	1	30	15	2	30
L3_3	50	1	1/0	0/0	0/0	1	0	0	0	0	0	1	0.042	100	0		75	1	0	65	10	0
L3_4	50	7	2/0	1/0	0/0	7	0	0	0	0	0	7	0.292	29	50	0	30	1	25	30	1	30
L3_5	50	7	5/0	2/0	1/0	7	0	0	0	0	0	7	0.292	71	40	50	35	0	1	60	10	0
S1_1	50	3	0/0	0/0	0/0	3	0	0	0	0	0	3	0.125	0			30	0	15	2	60	3
S1_2	50	2	2/0	0/0	0/2	4	0	0	0	0	0	4	0.167	100	0		15	5	5	1	50	5
S1_3	50	2	0/4	0/3	0/0	6	0	0	0	0	0	6	0.250	0	0	0	6	5	40	1	35	5
S1_4	50	0	0/1	0/0	0/0	1	0	0	0	0/1	0	0	0.000		0		5	0	55	3	40	40
S1_5	50	1	0/0	0/0	0/0	1	0	0	0	0	0	1	0.042	0			35	0	0	2	90	0
S2_1	50	0	0/0	0/0	0/0	0	0	0	0	0	0	0	0.000				35	0	3	1	85	0
S2_2	50	0	0/0	0/0	0/0	0	0	0	0	0	0	0	0.000				25	0	20	2	60	20
S2_3	50	3	2/0	0/0	0/0	3	0	0	0	0	0	3	0.125	67	0		10	0	1	1	90	0
S2_4	50	5	2/0	0/0	0/0	5	0	0	0	0	0	5	0.208	40	0		12	2	7	3	65	0
S2_5	50	1	0/0	0/0	0/0	1	0	0	0	0	0	1	0.042	0			60	0	0	3	50	0
S3_1	50	0	0/0	0/0	0/0	0	0	0	0	0	0	0	0.000				35	0	1	30	75	3
S3_2	50	2	0/0	0/0	0/0	2	0	0	0	0	0	2	0.083	0			60	0	0	20	60	0
S3_3	50	0	0/0	0/0	0/0	0	0	0	0	0	0	0	0.000				65	0	0	15	30	0
S3_4	50	0	0/0	0/0	0/0	0	0	0	0	0	0	0	0.000				40	1	0	10	65	1
S3_5	50	2	1/0	0/0	0/0	2	0	0	0	0	0	2	0.083	50	0		45	0	0	15	80	0
S4_1	50	0	0/0	0/0	0/0	0	0	0	0	0	0	0	0.000				7	0	20	5	70	30
S4_2	50	0	0/1	1/1	0/0	2	0	0	0	0	0	2	0.083	100	0		10	0	2	1	75	3
S4_3	50	1	0/0	0/0	0/0	1	0	0	0	0	0	1	0.042	0			12	7	4	20	75	5
S4_4	50	1	0/0	0/0	0/0	1	0	0	0/1	0	0	0	0.000	0			25	0	0	10	80	0
S4_5	50	1	0/0	0/0	0/0	1	0	0	0	0	0	1	0.042	0			8	15	0	8	80	5
R1_1	50	1	0/0	0/0	0/0	1	0	0	0	0	0	1	0.042	0			2	0	0	0	100	0
R1_2	50	0	0/0	0/0	0/0	0	0	0	0	0	0	0	0.000				4	0	1	0	99	0

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R1_3	50	1	1/0	1/0	0/0	1	0	0/3	0	0	0/1	-3	0.000	100	100	0	65	15	0	5	80	0
R1_4	50	5	4/0	3/0	2/0	5	0	0	0	0	0	5	0.208	80	75	67	50	30	0	20	70	0
R1_5	50	5	4/0	0/0	0/0	5	0	0	0	0	0	5	0.208	80	0		30	45	0	10	30	0
R2_1	50	0	0/1	0/3	0/0	4	0	0	0	0	0	4	0.167		0	0	5	0	1	0	100	0
R2_2	50	1	0/0	0/0	0/0	1	0	0	0	0	0	1	0.042	0			30	0	2	0	98	0
R2_3	50	0	0/0	0/0	0/0	0	0	0	0	0	0	0	0.000				60	0	3	2	75	0
R2_4	50	0	0/2	0/0	0/0	2	0	0	0/1	0	0	1	0.042		0		60	0	4	3	80	0
R2_5	50	0	0/0	0/0	0/0	0	0	0	0	0	0	0	0.000				55	0	7	50	50	0

B – Explanation of columns: 1 – Transect_plot; 2 – Number of seeds sown (calculated as average number of well-developed seeds in three capitula, see Janišová et al. 2012b for details) in O (26 June 2009), C (25 June 2009), R (25 June 2009), S (25 June 2009), L (26 June 2009); 3 – Number of seedlings on the 1st survey in O (13 May 2010), C (11 May 2010), R (21 August 2010), S (21 August 2010); 4 – Number of seedlings on the 2nd survey in O (25 May 2011), C (20 May 2011), R (18 May 2011), S (18 May 2011), L (27 May 2011), old/new-recorded; 5 – Number of seedlings on the 3rd survey in O (7 June 2012), C (5 June 2012), R (4 June 2012), S (6 June 2012), L (8 June 2012), old/new-recorded; 6 – Number of seedlings on the 4th survey in O (15 May 2013), C (16 May 2013), R (13 May 2013), S (14 May 2013), L (17 May 2013), old/new-recorded; 7 – Number of seedlings on the 5th survey in O (22 May 2014), C (23 May 2014), R (19 May 2014), S (20 May 2014), L (21 May 2014), old/new-recorded; 8 – Cumulative number of seedlings; 9 – Number of seeds sown in the control plot; 10-14 – Number of seedlings in the control plots on the 1st, 2nd, 3rd, 4th and 5th surveys, old-/new-recorded; 15 – Corrected cumulative number of seedlings (number of seedlings in the control plot subtracted); 16 – % germination expressed as a proportion from the highest value in 2009; 17 – % seedling survival between the 1st and 2nd surveys, 18 – % seedling survival between the 2nd and 3rd surveys; 19 – % seedling survival between the 3rd and 4th surveys; 20 – % seedling survival between the 4th and 5th surveys; 21 – % cover of herb layer; 22 – % cover of moss layer; 23 – % cover of bare soil; 24 – % cover of herb litter; 25 – % cover of tree litter; 26 – % cover of disturbed surface.

1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**
L4_1	204	8	NA	7/0	5/0	5/0	8	0	NA	0	0	0	0	8	0.727			71	100	95	15	0	60	3	0
L4_2	204	3	NA	0/0	0/0	0/0	0	0	NA	0	0	0	0	0	0.000					50	30	0	60	20	0
L4_3	204	0	NA	0/0	0/0	0/0	0	0	NA	0	0	0	0	0	0.000					55	40	2	50	10	0
L4_4	204	0	NA	0/0	0/0	0/0	0	0	NA	0	0	0	0	0	0.000					85	25	0	50	1	20
L4_5	204	0	NA	0/0	0/0	0/0	0	0	NA	0	0	0	0	0	0.000					80	1	0	90	1	0
L5_1	204	NA	NA	0/0	0/0	0/0	0	0	NA	NA	0	0	0	0	0.000					55	25	0	25	55	0
L5_2	204	NA	NA	0/0	0/0	0/0	0	0	NA	NA	1	0	0	0	0.000					40	20	0	50	55	0
L5_3	204	NA	NA	0/0	0/0	0/0	0	0	NA	NA	0	0	0	0	0.000					32	10	3	25	50	5
L5_4	204	NA	NA	1	0/0	0/0	1	0	NA	NA	0	0	0	1	0.091			0		45	1	5	30	45	0
L5_5	204	NA	NA	0/0	0/0	0/0	0	0	NA	NA	0	0	0	0	0.000					55	0	0	10	90	0
O4_1	204	0	0/2	0/0	0/0	0/0	0	0	0	0	0	0	0	0	0.000		0			80	0	1	1	1	0
O4_2	204	25	8/0	0/1	0/1	0/0	10	0	0	0/1	1/0	0	0	9	0.818	32	0	0	0	22	0	75	2	15	75
O4_3	204	0	0/0	0/0	0/0	0/0	0	0	0	0	0	0	0	0	0.000					50	0	6	15	80	5
O4_4	204	3	3/0	0/0	0/0	0/0	3	0	0	0	0	0	0	3	0.273	100	0			70	0	0	15	65	0
O4_5	204	0	0/0	0/0	0/0	0/0	0	0	0	0	0	0	0	0	0.000					75	40	0	35	40	0
O5_1	204	0	0/0	0/0	0/0	0/0	0	0	0	0	0	0	0	0	0.000					45	0	0	1	95	0
O5_2	204	0	0/0	0/0	0/0	0/0	0	0	0	0	0	0	0	0	0.000					30	0	1	90	0	0

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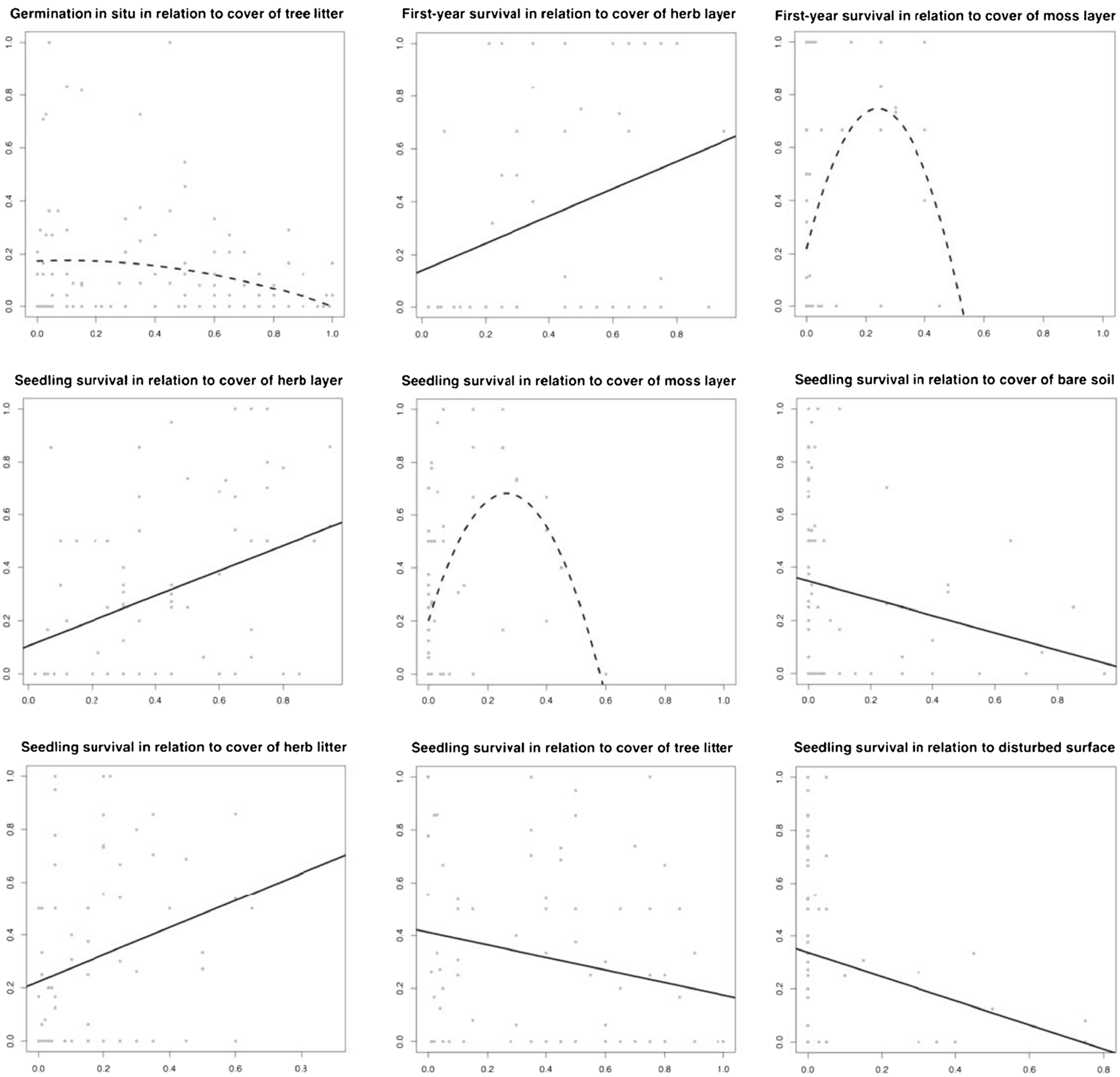
O5_3	204	0	0/0	0/0	0/0	0/0	0	0	0	0	0	0	0	0	0.000					25	0	1	2	80	0
O5_4	204	0	0/0	0/0	0/0	0/0	0	0	0	0	0	0	0	0	0.000					65	0	0	1	48	0
O5_5	204	3	2/0	0/0	0/0	0/0	3	0	0	0	0	0	0	3	0.273	67	0			45	0	1	50	40	0
S5_1	204	6	4/0	4/0	3/0	3/0	6	0	0	0	0	0	0	6	0.545	67	100	75	100	7	25	0	20	50	0
S5_2	204	0	0/1	1/0	0/0	0/0	1	0	0	0	0	0	0	1	0.091		100	0		75	2	2	40	15	0
S5_3	204	5	5/0	5/0	5/0	4/0	5	0	0	0	0	0	0	5	0.455	100	100	100	80	45	3	1	5	50	0
S5_4	204	1	1/0	1/0	1/0	1/0	1	0	0	0	0	0	0	1	0.091	100	100	100	100	70	25	0	22	35	0
S5_5	204	0	0/0	0/0	0/0	0/0	0	0	0	0	0	0	0	0	0.000					40	0	2	20	25	0
S6_1	204	7	7/1	2/0	1/0	1/0	7	0	0	0/3	0	0	0	4	0.364	100	25	50	100	60	3	0	45	45	0
S6_2	204	1	0/1	0/0	0/0	0/0	1	0	0	0	0	0	0	1	0.091	0	0			25	4	3	35	28	0
S6_3	204	8	8/0	7/0	4/0	3/0	8	0	0	0	0	0	0	8	0.727	100	88	57	75	75	1	0	30	35	0
S6_4	204	3	2/0	2/0	1/0	0/0	3	0	0	0	0	0	0	3	0.273	67	100	50	0	65	40	0	25	40	0
S6_5	204	15	11/1	9/0	4/0	4/0	15	0	0	0	0	0	0/4	11	1.000	73	75	44	100	62	30	0	20	45	0
R3_1	204	0	0/0	0/0	0/0	0/0	0	0	0	0	0	0	0	0	0.000					70	0	25	25	15	25
R3_2	204	1	0/0	0/0	0/0	0/0	1	0	0	0	0	0	0	1	0.091	0				30	0	70	4	12	35
R3_3	204	0	0/0	0/0	0/0	0/0	0	0	0	0	0	0	0	0	0.000					35	0	35	5	25	30
R3_4	204	4	0/0	0/0	0/0	0/0	4	0	0	0	0	0	0	4	0.364	0				2	0	95	1	7	75
R3_5	204	0	0/0	0/0	0/0	0/0	0	0	0	0	0	0	0	0	0.000					32	0	90	0	4	80
R4_1	204	0	0/0	0/0	0/0	0/0	0	0	0	0	0	0	0	0	0.000					10	100	0	1	5	0
R4_2	204	0	0/0	0/0	0/0	0/0	0	0	0	0	0	0	0	0	0.000					5	97	0	1	15	0
R4_3	204	0	0/0	0/0	0/0	0/0	0	0	0	0	0	0	0	0	0.000					20	75	0	2	30	0
R4_4	204	0	0/0	0/0	0/0	0/0	0	0	0	0	0	0	0	0	0.000					30	20	0	4	90	0
R4_5	204	0	0/0	0/0	0/0	0/0	0	0	0	0	0	0	0	0	0.000					50	0	0	10	85	0
C4_1	204	0	0/0	0/0	0/0	0/0	0	0	0	0	0	0	0	0	0.000					75	20	1	60	3	0
C4_2	204	0	0/0	0/0	0/0	0/0	0	0	0	0	0	0	0	0	0.000					40	10	0	40	10	0
C4_3	204	0	0/0	0/0	0/0	0/0	0	0	0	0	0	0	0	0	0.000					65	20	0	35	5	0
C4_4	204	0	0/0	0/0	0/0	0/0	0	0	0	0	0	0	0	0	0.000					85	10	0	5	50	0
C4_5	204	0	0/0	0/0	0/0	0/0	0	0	0	0	0	0	0	0	0.000					35	40	0	2	65	0
C5_1	204	0	0/0	0/0	0/0	0/0	0	0	0	0	0/1	1/0	0	-1	0.000					40	0	22	20	5	2
C5_2	204	0	0/0	0/4	1/1	0/0	5	0	1	0	0	0	0	4	0.364			25	0	30	0	40	5	4	50
C5_3	204	0	0/0	0/0	0/0	0/0	0	0	0	0	0	0	0/1	-1	0.000					25	2	10	55	5	20
C5_4	204	3	2/0	0/0	0/0	0/0	3	0	0	0	0	0	0	3	0.273	67	0			30	12	45	1	3	45
C5_5	204	5	2/0	0/0	0/0	0/0	5	0	0	0	2/0	0	0	3	0.273	40	0			35	40	0	4	5	0

Appendix 3

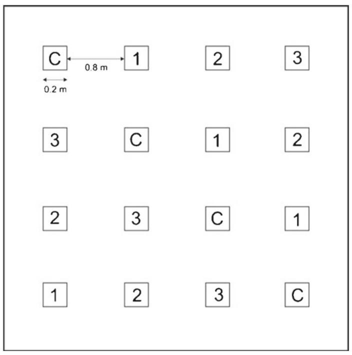
Scatter plots and regression trends expressing relationships between in situ germination and seedling survival of *T. longifolia* subsp. *moravica* as dependent variables and plot characteristics as predictors. See Table 1 for details.



Appendix 4

a – Scheme of the management experiment and position of the treatment plots: 1 – not managed, 2 – litter removal, 3 – turf removal, C – non-managed control plot without seed addition. In the control plots, no seedlings were recorded during the experiment (18 September 2012-19 May 2014). **b** – Number of seedlings in the cultivation ex situ and the treatment plots in situ. Differences in cumulative germination [%] between the treatments in situ tested by ANOVA: F = 16.44, $d.f.$ = 3, P < 0.001. Differences in '% survival between the surveys' between the treatments in situ tested by ANOVA: survey 1 (23 October 2012) – survey 2 (13 May 2013): ANOVA, F = 2.26, $d.f.$ = 3, P=134; survey 2 (13 May 2013) – survey 3 (23 October 2013): ANOVA, F = 1.47, $d.f.$ = 3, P = 0.277.

a



b

Treatment _replicate	**Date of survey**					
	18 Sept 2012 [number of seeds sown]	**23 Oct 2012**	**13 May 2013**	**23 Oct 2013**	**19 May 2014**	**Cumulative number of seedlings**
Cumulative number of seedlings						
cultivation ex situ_1	25	22	22	22	22	22
cultivation ex situ_2	25	10	14	14	14	14
cultivation ex situ_3	25	14	15	15	15	15
cultivation ex situ_4	25	11	17	17	17	17

Number of seedlings (old/new-recorded)						
not-maneged_1	50	3	0/3	0/1	0/0	7
not managed_2	50	10	1/0	0/0	0/0	10
not managed_3	50	4	0/1	0/0	0/0	5
not managed_4	50	4	2/2	0/0	0/0	6
litter removal_1	50	3	1/1	0/0	0/0	4
litter removal_2	50	10	0/0	0/0	0/0	10
litter removal_3	50	9	2/0	0/0	0/0	9
litter removal_4	50	12	4/2	1/0	0/0	14
turf removal_1	50	6	0/4	0/0	0/0	10
turf removal_2	50	12	1/4	0/5	2/1	22
turf removal_3	50	20	4/0	0/0	0/0	20
turf removal_4	50	15	1/2	0/0	0/0	17
Total number of seedlings survived in treatments						
not managed			3	0	0	
litter removal			7	1	0	
turf removal			6	0	2	
Total number of new-recorded seedlings in treatments						
not managed		21	6	1	0	28
litter removal		34	3	0	0	37
turf removal		53	10	5	1	69

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