University of South Bohemia České Budějovice

Faculty of Science

The role of natural disturbances in a pasture

Master's thesis

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Annotation:

Seedling survival of species *Trifolium pratense* and *Achillea millefolium* was observed in microsites, such as molehills, anthills, wild boar disturbances, and artificial gaps at a pasture. In this thesis, we observed the influence of vegetation, litter, mosses, stones, bare soil cover, moisture, and temperature on seedling survival. The species composition of the seed bank and standing vegetation in different microsites was compared. The species composition of the microsites was also compared to the surrounding vegetation.

Poděkování

Moc děkuji své školitelce Marušce za pomoc v terénu, podnětné připomínky při psaní, a především za ochotnou pomoc, kdykoliv bylo potřeba.

Děkuji Šuspovi za komentáře a statistické připomínky.

Děkuji Markét, Alešovi, Peťovi, Helče a dalším za pomoc v terénu.

Děkuji svým blízkým za podporu a mým spolubydlícím za ochotu se mnou trávit dlouhé večery u psaní.

Obsah

1 Introduction

1.1 Grasslands and disturbances

Grasslands can be frequently found throughout Central Europe (Ellenberg, 1988). Human activity has played a significant role in shaping most grasslands, which are often regarded to be secondary vegetation that has supplanted the indigenous forest (Hejcman et al., 2013). These habitats typically featured a high species richness, which may be a result of previous mowing and grazing management (Chytrý et al., 2015) as well as specific environmental factors like soil moisture and pH (Merunková & Chytrý, 2012).

High biodiversity in meadows and pastures is at risk due to management changes such as intensive fertilization or abandonment of mowing and grazing (Valkó et al., 2018). Fertilization increases the biomass productivity of grasslands but at the cost of reducing their biodiversity (Lepš, 2004). Complete abandonment of management reduces the number of species and causes dominance of fewer species than before abandonment (Tasser & Tappeiner, 2002). In general, strong competition frequently shapes plant communities (Goldberg & Barton, 1992). Mowing reduces aboveground biomass thus plant species of smaller stature, which are considered to be weaker competitors, could persist (Zobel, 1992). The study by Mudrák et al. (2014) demonstrated that high productivity of a meadow significantly affects the establishment of *Rhinanthus* spp. seedlings. The solution is a properly timed mowing to reduce competition from surrounding vegetation and to remove litter Mowing positively affects species diversity but often results in a homogenous species composition (Lepš, 2014). Conversely, grazing can cause a more heterogeneous disturbance (Catorci et al., 2011). Thus, grazing is another management type preserving species diversity, dependently on intensity and frequency (Austrheim & Eriksson, 2001; Lanta et al., 2009). Additionally, as the grassland productivity increases, the effect of grazing also becomes more apparent (Herrero-Juregui & Oesterheld, 2018).

Besides large-scale disturbances (e.g. mowing and grazing (Catorci et al., 2011), grasslands are characterized by the frequent occurrence of small-scale disturbances that are considered an important part of the dynamics of species composition (Kohler et al., 2007;

Lavorel et al., 1994). Typical examples of these are wild boar disturbances, molehills, livestock trampling, or anthills. The consequence of small-scale disturbances is usually the local total removal of vegetation while after disturbance by mowing the majority of plants survived (Schippers & Joenje, 2002). One of the roles of small-scale disturbances is to open the space in the dense vegetation of grassland thus bringing opportunity for new species to establish (Kohler et al., 2007). In many studies (Bullock et al., 1995; Kiss et al., 2021; Kotorová & Lepš, 1999; Vandvik, 2004; Vítová et al., 2017) is widely used the term 'gap'. According to Bullock (2000) is defined as temporal, small-scale, and competitor-free space in vegetation. The term 'gap dynamics' is frequently used to describe the process of gap formation which is mostly associated with forest canopy (Diets & Clark, 2008; McCarthy, 2001; Yamamoto, 2000). This process is as important for grasslands but less commonly studied (Coffin & Lauenroth, 1990; Vandvik, 2004; Williams, 1992).

1.2 Plant regeneration in grassland

1.2.1 Gap colonization

Dispersal or habitat limitation affects species composition at small scales (Vítová & Lepš, 2011). Three resident and three non-resident species' seedling recruitment was observed in the mentioned study. The results showed that two non-residents proved capable of developing a viable population that might be limited by dispersal in the community. Also, Ehrlén & Eriksson (2000) claimed that the availability of seeds limits the target species within local populations, while seed dispersal to suitable habitats limits them on a larger scale. On the other hand, habitat limitation can result from the unsuitable abiotic conditions in a microsite around the mother plant on a local scale. However, these abiotic factors may fluctuate over time, which can impact the suitability of the microsite for certain species. Within the regional scale, habitat limitation is caused by the occupation of suitable microsites (Münzbergová & Herben, 2005) .

A new gap represents a microsite that is completely or barely uninhabited and present a chance for recruitment from seeds (Kalamees & Zobel, 2002; Vandvik, 2004) and may also play a crucial role in the coexistence of species and the maintenance of species diversity (Bullock et al., 1995; Grubb, 1977). Grubb (1977) defined the term 'regeneration niche' which

could be summarized as a set of conditions for a high probability of success in the replacement of an individual by a new individual. Bullock (2000) then mentioned the related term 'gap colonization ability', which is defined as a species' potential to spread seeds through space or time and its potential to recruit new plants into a gap compared to intact vegetation. Clonal growth plays an important role during the colonization of gaps (Vítová et al., 2017). Grime (2006) defined five different regenerative strategies which may be used by species, one species can use multiple (Fig. 1). These strategies reflect the different ways in which plants may respond to disturbances. (Kalamees & Zobel, 2002) studied the effect of the seed bank, short-distance dispersal, and clonal growth in gap regeneration. They found that 36 % of individuals germinated from the seed bank, 46 % of individuals came by seed rain and only 18 % of individuals arrived by clonal growth in the gaps. They also noticed that seed bank is probably promoted in larger gaps while clonal growth is more common in small gaps (Vandvik, 2004). Also important is the timing of disturbance, which can increase species diversity by postponing or eliminating competitive exclusion (Crawley, 2004).
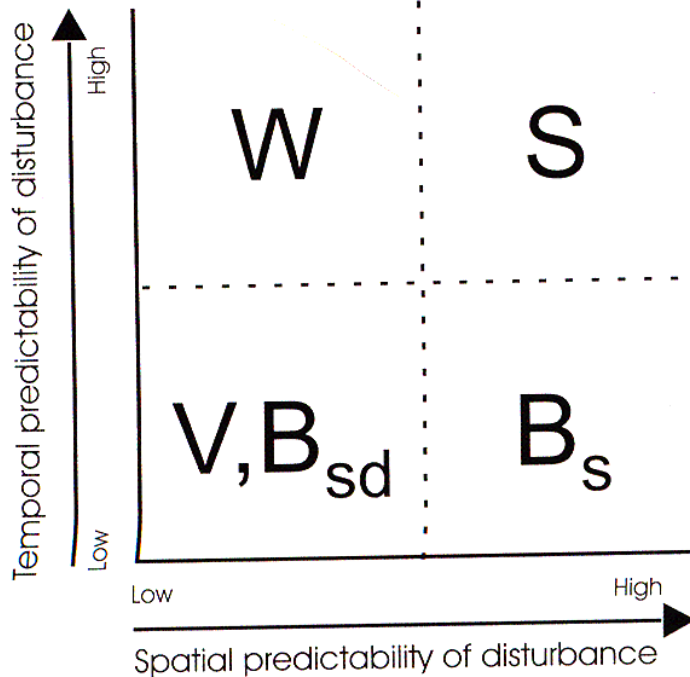
Fig. 1 Different regeneration strategies reply to disturbances that are predictable in both time and space. W – widely-dispersed seeds; V – vegetative expansion, B_{sd} – persistent juveniles; S – seasonal regeneration; B_s –persistent seed bank. Taken from Grime (2006).

In general, germination from seeds in intact vegetation in grasslands is not a very frequent event (Bullock, 2000; Kalamees & Zobel, 2002; Pywell et al., 2003). The study by Vítová et al. (2017) demonstrated through a manipulative experiment that the regeneration strategies in gaps change between four different functional groups (dicots, *Juncaceae*, *Poaceae*, *Cyperaceae*). The findings showed that *Poaceae* decreased the number of seedlings when the seed bank was eliminated, indicating that they primarily rely on seed rain as their source of seeds. In contrast, vegetative reproduction is typical for dicots in intact vegetation. Pywell et al. (2003) mentioned the traits that were positively connected to colonization ability, including highly viable seeds, autumn germination, and rapid rate of seedling growth. As a result, the key purpose is to establish quickly and outcompete other present seedlings during the process of colonization.

1.2.2 Abiotic and biotic conditions of gaps

Environmental conditions in forest canopy gaps were the subject of many studies (De Freitas & Enright, 1995; He et al., 2012; Latif & Blackburn, 2010) Also, the microclimate of grasslands was studied in a response to the different intensities of grazing as a type of disturbance (Vaieretti et al., 2018). Microclimate in gaps was investigated less frequently even though gaps in vegetation are microsites that may have distinct abiotic and biotic conditions compared to the surrounding intact vegetation. Gaps offer space with less intense competition, which is advantageous, especially for seedlings (Bullock, 2000). However, in the case of *Lysimachia vulgaris*, *Prunella vulgaris*, and *Plantago lanceolata*, the presence of vegetation was observed to enhance the chances of survival for their seedlings compared to artificial gaps in very early stages, because vegetation prevented the desiccation of soil surface (Kelemen et al., 2015). Also, the microclimate, particularly in the bigger gaps, may be harsh and unsuitable for seedling recruitment (Williams, 1992). In the gaps, wind and sunlight exposure are increased, and temperature and moisture levels are more variable (Vandvik, 2004). Morgan (1997), for example, assessed the establishment of the species *Rutidosis leptorrhynchoides* in gaps of different sizes. The results showed that the amount of light varies noticeably between the different sizes of gaps, with the level of light being lower in the areas with intact vegetation. The study also found that bigger gaps ($1m^2$) had moisture levels that were clearly lower.

The study by Kotorová & Lepš (1999) examined the impact of the moss layer on the recruitment of seedlings. The results indicated that moss removal with mowing has a positive effect on seedling recruitment as a result of reduced competitive pressure. However, mosses can strongly decrease the temperature and increase soil moisture in their surroundings (Li et al., 2022) According to Haugland & Froud-Williams (1999), *Lolium perenne* and *Trifolium pratense* seedling growth are significantly impacted by low soil moisture levels, which reduces their ability to compete. The study Špačková et al. (1998) demonstrated also positive response to moss removal for majority of species, however, for species *Myosotis nemorosa* and *Cardamine pratensis* found negative effect, likely as a result of decreased moisture or seed mortality. Litter can play a role in optimizing microclimate, providing shading to regulate the

light, and acting as a protective layer to moderate winter temperatures. However, litter also acts as a mechanical barrier for seedlings (Loydi et al., 2013).

1.3 Natural disturbances

Previously mentioned studies (Bullock, 2000; Morgan, 1997; Vandvik, 2004; Vítová et al., 2017; Williams, 1992) emphasized artificial gaps created by humans with the aim to resemble natural 'gap dynamic'. Experimentally created artificial gaps were created to mimic the role of natural disturbances in grasslands, but still this similarity should not be taken as granted. Moreover, these natural disturbances could be in temperate regions created for example by wild boars, moles, rodents, livestock, or ants. The differences in origin can lead to variations in environmental conditions, seedling recruitment, and species composition (Horčičková et al., 2019; Kohler et al., 2007; Konečná et al., 2021; Kovář et al., 2013; Parish & Turkington, 1990; Sebastià & Puig, 2008; Seifan et al., 2010).

One of the factors that distinguish natural disturbances is the soil nutrient content. The study by Canals & Sebastià (2000) compared the amount of nitrogen in molehills and surrounding vegetation. The findings indicate that molehills have a higher inorganic nitrogen content. The same pattern was observed in the study (Bueno et al., 2013) where the nitrogen mineralization rate was accelerated by the bioturbation of wild boars. Also, anthills differ from intact vegetation in terms of soil nutrients (nitrogen, phosphorus) and temperature (Konečná et al., 2021).

Natural disturbances could also contribute to seedling recruitment. Studies (Konečná et al., 2021; Parish & Turkington, 1990; Sondej & Kwiatkowska-Falińska, 2017) found similar results to a higher number of seedlings appearing in natural disturbances compared to intact vegetation. The activity of wild board disturbances negatively influences seed bank by decreasing its species richness (Bueno et al., 2011; Yusefi et al., 2022). Bueno et al. (2013); Kohler et al. (2007) and Hořčičková et al. (2019) also stressed the differences in species composition of the natural disturbances and surrounding vegetation.

In my thesis, I focused on the role of naturally occurring disturbances in grassland – anthills, molehills, and wild boar disturbances and also their comparison with artificial gaps

and intact vegetation. Seedling survival (species *Trifolium pratense, Achillea millefolium*), environmental conditions, and species composition were observed across these microsites. Considering the above, recent literature includes studies that usually use design comparing artificial gaps (Bullock, 2000; Morgan, 1997; Vandvik, 2004; Vítová et al., 2017; Williams, 1992) or certain natural disturbance type (Bueno et al., 2011; Sebastià & Puig, 2008; Seifan et al., 2012) with intact vegetation. The study by Hořčičková et al. (2019) showed that the composition of the vegetation differs not only between disturbances and intact vegetation, but also between artificial gaps and wild boar disturbances because of the timing of the disturbance origin, as the artificial gaps were created in one day, but the natural disturbances occurred at different times. All mentioned studies stressed out heterogeneity within individual natural disturbances. For instance, Parish and Turkington (1990) conducted a study at three localities over three years to examine the impact of moles and dung pats on species composition. The results indicated that species composition varied not only between microhabitats but also between years, indicating that disturbance development may be dynamic in both time and space. Nevertheless, the co-occurence of several types of disturbances is relatively usual. However, the variability across different types of disturbances is poorly studied (but see e.g., Milton et al. 1997). Animals regularly cause small-scale disturbances which are heterogeneous in space and time (Welander, 2000). Thus, there are many microsites of different sizes, depths, and successional stages which serve as hotspots with different competition pressure and environmental conditions (Canals & Sebastià, 2000; Parish & Turkington, 1990; Seifan et al., 2010, 2012) The thesis' major goal is to attempt to disentangle potential variability in species composition, seedling survival, and environmental conditions across different types of disturbances in grassland.

2 Objectives

1. Determine how environmental conditions affect seedling dynamics of two artificially sown species across different microsites.

2. Compare the species composition of the seed bank and standing vegetation across different microsites.

3. Compare the species composition of different microsites with the surrounding vegetation.

3 Materials and methods

3.1 Study site

The experiment site was situated in a mesophilic pasture close to the village Uhřice, near the town Vlachovo Březí, located in the region of South Bohemia (49° 4' 40" N, 13° 55' 19" W). The average annual temperatures in the range of years 2019 to 2021, the years of the experiment, were recorded as 9.1°C, 10.0 °C, and 8.6 °C. The mean annual precipitation for these years was 1.2 mm respectively per 24-hour period. The values are based on data gathered from a nearby CHI (Czech hydrometeorological institute) meteorological station in Husinec, located approximately 5 km away. The experimental plots were situated in a small pasture shaded by a bordering sparse shrub belt to the south and a forest to the northeast. A frequent occurrence of small-scale disturbances (molehills, anthills, and wild boar disturbances) is typical for the site. The pasture had been grazed by cows before the experiment and manually mown (microsites were omitted) each August during the experiment.

The vegetation at the locality is species rich (the average number of species is 38 in the 1 m^2 with the microsite in the centre). The vegetation on the site was classified as *Violion caninae* association, with prevalent species such as *Agrostis capillaris*[1], *Arrhenatherum elatius,* and *Anthoxanthum odoratum*. Several representatives of the *Carex* genus were also presented, including *Carex caryophyllea*, *Carex muricata*, and *Carex pallescens*. Diagnostic dicots for the *Violion caninae* association, such as *Carlina acaulis*, *Dianthus deltoides*,

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[1] The nomenclature of plant species was standardized according to the Key to the Flora of the Czech Republic (Kaplan et al., 2019)

Thymus pulegioides, and *Viola canina* were present at the site, as well as other species, such as *Lotus corniculatus*, *Plantago lanceolata*, and *Helianthemum grandiflorum*.

3.2 Experimental design

The experiment consists of three parts: a seedling survival experiment, a seed bank analysis, and a study of the species composition of small-scale microsites.

3.2.1 Seedling survival experiment

A seed sowing experiment of three resident species, *Achillea milefolium*, *Trifolium pratense*, and *Carlina acaulis*, was performed to compare their germination and seedling survival in different types of microsites in a pasture. The seeds were obtained from the commercial company Planta naturalis. The target species were chosen based on a germination test accomplished in a growing chamber. From a total of 11 tested species, the selected species had the highest germination rates. On April 9, 2019, seeds were sown into five types of microsites in the pasture, naturally occurring – molehills, anthills, and wild boar disturbances. Molehills and wild boar disturbances were created in the previous season, while anthills were older based on their size. Additionally, seeds were sown in artificially created gaps, which were made by removing the plant material from a turf, and then refilling the cleared space with a combination of soil taken from the surrounding area and the remaining soil from the turf. The last type of microsite was undisturbed vegetation that served as a control to monitor germination and seedling survival in a stronger competitive environment than in microsites.

Ten replications were selected for each type of microsite distributed as evenly as possible across the grassland. Then, a 15 × 15 cm area was marked in the center of each microsite, and a homogenous mixture of seeds of the three selected species was sown, with a count of one hundred seeds per species. To control the natural germination of selected resident species in the location, microsites without artificial sowing (three repetitions of each microsite type) were also marked. The experimental areas were observed approximately every three weeks from early April to early October 2019, 2020, and 2021 without re-sowing the

seeds. A total of twenty-five seedling censuses were taken from the experimental plot. The number of selected species' seedlings and the cover of vegetation, mosses, litter, bare soil, and stones in the marked areas were estimated.

Twenty-two TMS-4 dataloggers were also evenly placed in the plots. Dataloggers measured surface temperature every 15 minutes and humidity simultaneously. Dataloggers were not presented in all plots but were distributed evenly within each disturbance type with the following counts – intact vegetation five, anthills five, wild boar disturbances four, artificial gaps four, and molehills three (one did not preserve).

3.2.2 Species composition of the seed bank

The seed bank species composition was determined to assess variation among microsites and evaluate differences between vegetation and the composition of the seed bank. The viable seed bank composition was determined using the methods according to Knevel et al. (2003). Soil samples were collected on March 29, 2020, using a soil corer with a diameter of 5 cm, and were taken to a depth of 13 cm in each plot. The samples remained at ambient temperature until the next day. On March 30, 2020, the soil from each sample was mixed with a small amount of water and placed on sterile sand. The experiment was conducted in a glasshouse for approximately five months until September 16, 2020. Seedlings were identified into species and counted almost every week. After counting, the seedlings were removed to prevent competition. Boxes containing a mixture of soil and sand were watered every second day to maintain proper moisture levels.

3.2.3 Species composition of standing vegetation

Visual estimation of species cover (in %) was used to compare the species composition of different microsites and their surrounding vegetation. Every year at the beginning of July, phytosociological relevés of vegetation in each plot and its surrounding area were made (i.e. excluding the central part). Two relevés were conducted within a 1 × 1 m square. The first relevé was made on the microsite located at the center of the square, followed by a relevé of the surrounding area. The same process was applied for intact vegetation where the marked area of vegetation was the center of relevé.

3.3 Data analyses

Data were analyzed in R software (R Core Team, 2021) and multivariate analyses were performed using Canoco 5 software (ter Braak and Šmilauer 2012). Tests were performed at the 5% significance level. The significance of the Redundancy analysis and Redundancy analysis partial (hereinafter referred to as RDA and RDA-partial) was tested using the Monte Carlo permutation test with 999 permutations, and the explanatory power of the environmental variables was quantified using the adjusted R-squared value. In each case, the abundance of species was centered and standardized by sample norm. In this way, the RDA methods reflect the differences in relative species composition, not in the total abundance or cover.

3.3.1 Seedling survival experiment

Seedling survival of three target species was tested in my bachelor for two growing seasons (2019 – 2020) thesis (Vacková, 2021). To assess the change in the number of seedlings over three growing seasons (2019 – 2021) and the effect of microsites, a repeated measures ANOVA with fixed effects factors of microsite, time, and ID of the plot (1 – 50) as a nested random effect for both species was used. The number of seedlings was log-transformed using the formula $Y'=\log_{10}(Y+1)$. In this analysis, the main effect of the microsite refers to the effect of the microsite averaged over all the observation times, and the interaction to the differences in the dynamics.

3.3.1.1 Seedling response to vegetation, litter, and mosses

We used a generalized linear mixed-effects modeling (GLMM) approach to analyze the counts of seedlings. Due to the larger number of seedlings, only the first season of 2019 was analyzed (observation from following years have steadily very low number of seedlings of both species; Fig. 12, 13). The fixed effects included in the model are a categorical variable microsite and three continuous variables cover of vegetation, litter, mosses, stones, and bare soil which were log-transformed using the formula $Y'=\log_{10}(Y+0.1)$. The grouping factors ID of plot and time were included as random factors to control for the repeated measurements (using *glmer* from R package *lme4*, Bates et al., 2015 and *car* Fox & Weisberg, 2019). The

Poisson distribution with a log link function was used to model the counts. The ANOVA (Type III Sum of squares decomposition) was used to assess the significance of the fixed effects in the model. In this case, the individual effects are in fact averaged over all the observations, which would be problematic in the model with all the (necessarily correlated) effects and their interactions with time included.

3.3.2 Species composition of a seed bank and standing vegetation of microsites

A series of RDA were used to compare the species composition of the seed bank and standing vegetation across different microsites. Explanatory variables were a *type* (species composition of seed bank or standing vegetation) and *microsite* (molehills, anthills, wild boar disturbances, artificial gaps, and intact vegetation), and the explained variable was species abundance. The species composition of the seed bank was quantified as the number of seedlings, while the species composition of the vegetation was quantified as the percentage cover. Then, the species data were in both cases log-transformed by the formula $Y'=\log(Y+1)$. Note that with the standardization by sample norm, the data reflect the proportions of species – thus, this is probably the best way how to compare the species composition of seedbank and vegetation, which are necessarily characterized in different units, nevertheless, both are the compositional data.

To test the significance of the pure effect of interaction between the microsite and type of species composition (seed bank or vegetation) used the RDA-partial with an ID of pair (1-50) and main effects as covariates (type, microsite). Nevertheless, in this case, the ordination diagram is not informative. Thus, to visualize the differences in species composition among microsites, we used the RDA with the *microsite × type* interaction as the only explanatory variable.

An RDA-partial was also used for testing differences in the species composition between seed bank and vegetation with type as an explanatory variable and the ID of pair serving as a covariate.

The difference in species composition of seed bank across different microsites was performed by the RDA with explanatory variable *type*. Similarly, the RDA was performed to test potential contrasts in the species composition of vegetation across different microsites. A number of seedlings was log-transformed using the formula $Y'=\log_{10}(Y+0.1)$.

A series of RDA were used to compare the species composition of the different microsites and their surrounding vegetation. The analysis considered the explanatory variables of relevé *position* (centre or surrounding) and *microsite* (including molehills, anthills, wild boar disturbances, artificial gaps, and intact vegetation), while the explained variable was the abundance of species, which was measured as a percentage cover. The species data was log-transformed by the formula $Y'=\log(Y+0.1)$.

To test the pure effect of interaction between the microsite and the position of relevé (centre or surrounding) was used the RDA-partial with an ID of pair (1-50) and main effects as covariates (*position*, *microsite*). To visualize the differences of microsites in species composition (and also among *position* of species composition), we used the RDA with the microsite × position interaction as the only explanatory variable.

The RDA-partial was also used for testing differences in the species composition of vegetation between microsites and their respective surroundings with *position* as an explanatory variable and the ID of pair as a covariate.

4 Results

4.1 Seedling survival experiment

The results of the ANOVA for repeated measures showed that during the three growing seasons, the type of microsite had a significant effect on the number of seedlings for both selected species. Also, there was a significant interaction between microsite and time (Table 1 and 2).

Table 1 The results of repeated measurements ANOVA for species *Achillea millefolium*.

	DF effect	DF error	SS effect	MS effect	F	p-level
microsite	4	45	45.46	11.37	8.28	**p<0.001**
time	16	720	47.87	2.99	44.20	**p<0.001**
microsite*time	64	720	19.91	0.31	4.60	**p<0.001**

Table 2 The results of repeated measurements ANOVA for species *Trifolium pratense*.

	DF effect	DF error	SS effect	MS effect	F	p-level
microsite	4	45	21.56	5.39	3.62	**p=0.012**
time	16	720	131.33	8.21	115.88	**p<0.001**
microsite*time	64	720	13.41	0.21	2.96	**p<0.001**

4.1.1 Seedling response to vegetation, litter, and mosses

The GLMM showed significant differences in the number of seedlings of species *Achillea millefolium* among microsites (χ^2 = 51.258, DF= 4, p<0.001) and also showed a significant effect of the cover of vegetation, litter, stones, and mosses. The effect of bare soil was not significant (Table 3).

Table 3 The results of the GLMM for seedlings of species *Achillea millefolium.* Variables in bold signify the significant predictors.

	χ^2	DF	p-value
microsites	53.94	4	**p<0.001**
vegetation	230.07	1	**p<0.001**
litter	5.47	1	**p = 0.016**
cover of stones	12.01	1	**p<0.001**
mosses	6.04	1	**p=0.014**
bare soil	0.17	1	p=0.680

The GLMM showed significant differences in the number of seedlings of species *Trifolium pratense* among microsites and also showed a significant effect of vegetation litter cover of stone and bare soil ($\chi^2 = 6.307$, DF =1, p = 0.012). The effect of mosses was not significant ($\chi^2 = 0.6160$, DF =1, p=0.432; Table 4).

Table 4 The results of the GLMM for seedlings of species *Trifolium pretense.* Variables in bold signify the significant predictors.

	χ^2	DF	p-value
microsites	20.43	4	**p<0.001**
vegetation	132.04	1	**p<0.001**
litter	7.32	1	**p = 0.007**
cover of stones	9.48	1	**p=0.002**
mosses	0.62	1	p=0.432
bare soil	6.30	1	**p=0.012**

To visualize the seedling dynamic during the growing season (2019) was selected species *Trifolium pratense* because of a higher number of seedlings. Also, the trend of litter and vegetation (as significant effects for both species) was displayed. It is evidence that anthills are in a more advanced successional stage. The cover of vegetation and litter is higher from the beginning, similar to intact vegetation. While molehills and wild boar disturbances started with exposed ground similar to artificial gaps. The rapid growth of vegetation is commonly observed in artificial gaps, wild boar disturbances, and molehills. The cover of litter does not show signs of any discernible trend, we only see slight differences between microsites. The seedlings started to die off first in the anthills, where their decline was the fastest. The highest increase in seedling numbers was observed in the wild boar disturbances, followed by molehills and artificial gaps during May in the first year (Fig. 2). During the following two seasons, the number of seedlings in all microsites was very low, ranging in the units of individuals.
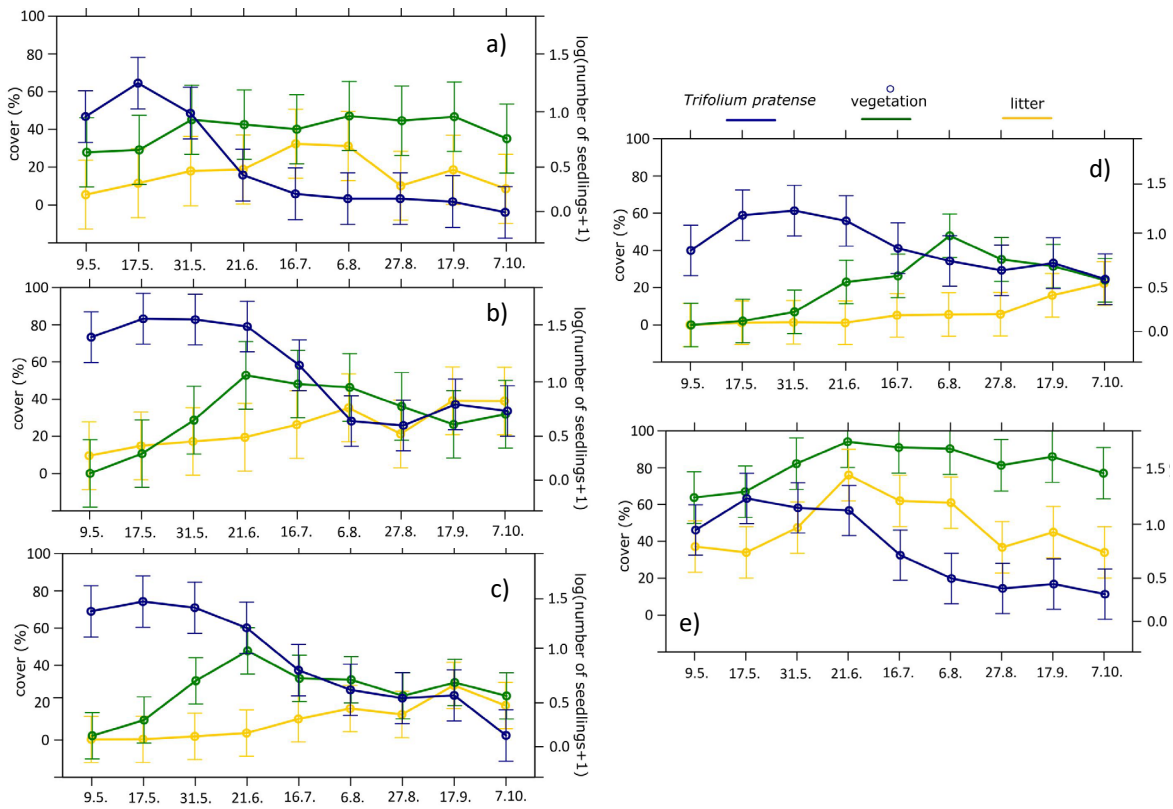
Fig. 2 Temporal dynamic of seedlings (*Trifolium pretense*), litter and vegetation in different microsites within one growing season a) anthills; b) wild boar disturbances; c) artificial gaps; d) molehills; e) intact vegetation. Error bars represent confidence intervals.

4.1.2 Seedling response to moisture and temperature

Moisture varies between disturbances, being highest in intact vegetation. Of the disturbances, it is the highest in wild boar disturbance and the lowest in anthills (Fig. 3).
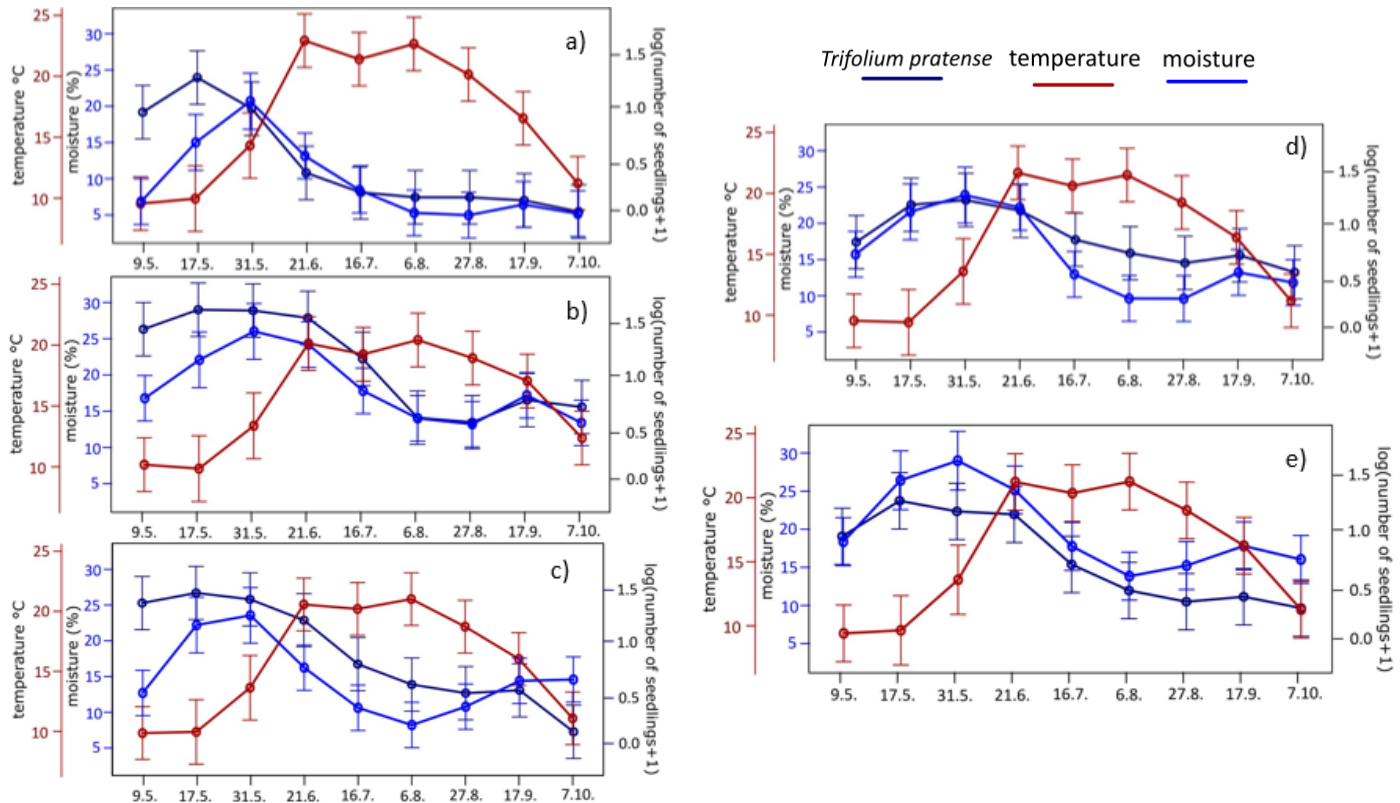

Fig. 3 Temporal dynamic of moisture and temperature (weekly averages before each census) in different microsites within one growing season a) anthills; b) wild boar disturbances; c) artificial gaps; d) molehills; e) intact vegetation. Error bars represent confidence intervals.

4.2 Species composition of the seed bank

A total of 36 species were recorded in the seed bank. In the vegetation, there were more than twice as many, specifically 66 species. During the experiment, 1800 seedlings germinated. The dominant species in the seed bank were *Agrostis capillaris*, *Juncus tenuis*, *Juncus effusus*, and *Oxalis acetosella*. In vegetation dominated species *Anthoxanthum odoratum*, *Arrhenatherum elatius*. The result of the RDA-partial with explanatory variable *microsite × type* and main effects (*microsite* and *type*) and ID of pair serving as covariates showed that the interaction is significant (pseudo – F= 1.5, p=0.002). The analysis was used only for testing the interaction. To visualize the results, an ordination diagram was generated showing the species composition and the interaction between microsite and type. The greatest variation in species composition is explained by the first axis, which is primarily associated with the effect of type. Additionally, the composition of the seed bank appears to be relatively consistent across microsites, while the vegetation composition shows greater variability (Fig. 4).
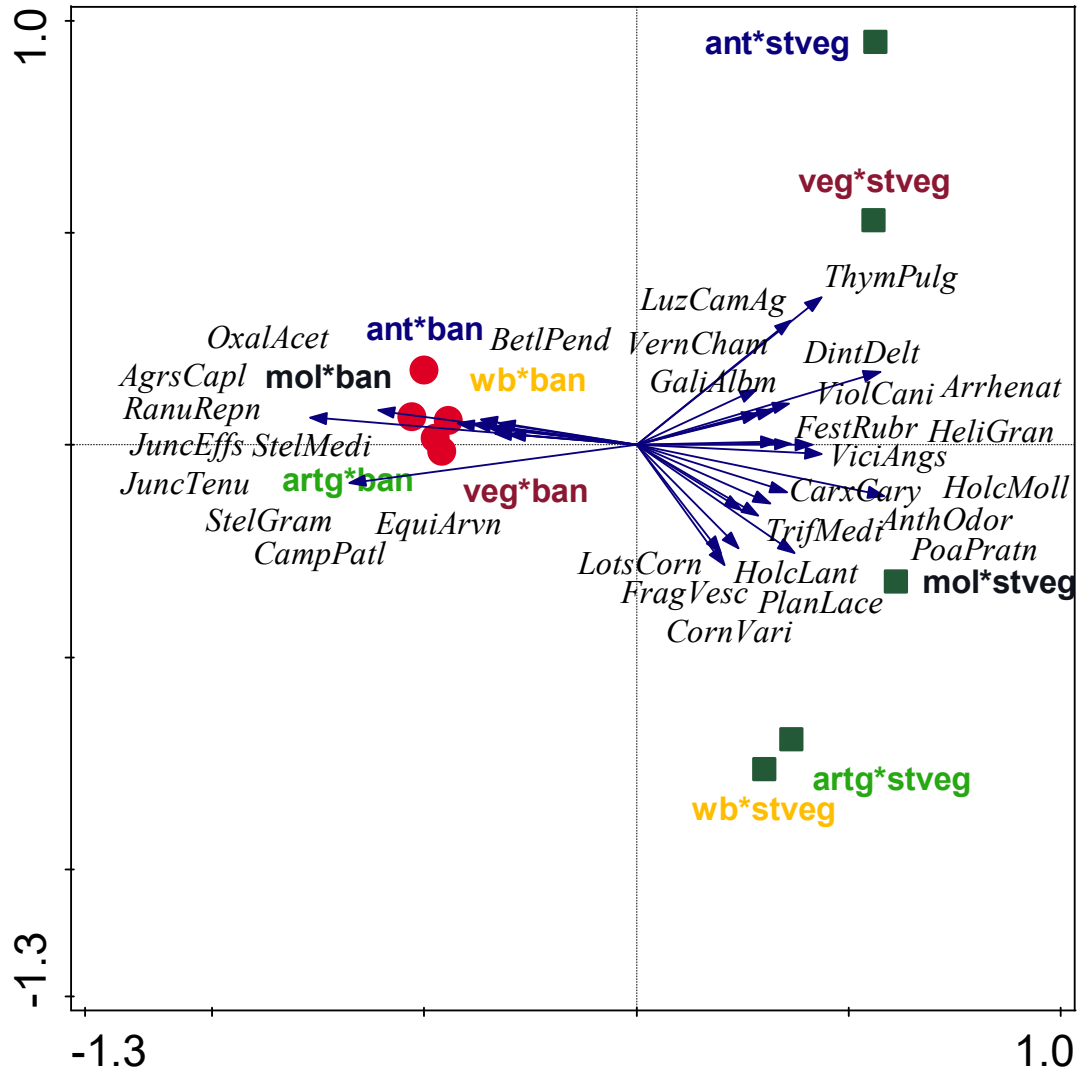
Fig. 4 The RDA diagram displays interaction *type* (stveg – standing vegetation; ban – seed bank; red circles) × *microsite* (ant – anthill; veg – intact vegetation; artg – artificial gap; wb – wild boar disturbances; mol – molehill; green squares) as the only explanatory variable. The adjusted explained variation is 21.92 %. The first axes explained 20.88 % of the total variation and the second axes only 2.42 %. Species abbreviations in Table 5.

Results from the partial RDA (with ID of pair as a covariate) indicated a significant effect of *type* (pseudo – F=25.8, p=0.001). Some species, such as *Agrostis capillaris*, *Campanula patula*, *Ranunculus repens*, *Juncus tenuis*, and *Juncus effusus*, exhibit greater

proportion in the seed bank than in the vegetation. In contrast, species such as *Poa pratensis*, *Festuca rubra*, and *Plantago lanceolata* are typical representatives of the vegetation (Fig. 5).
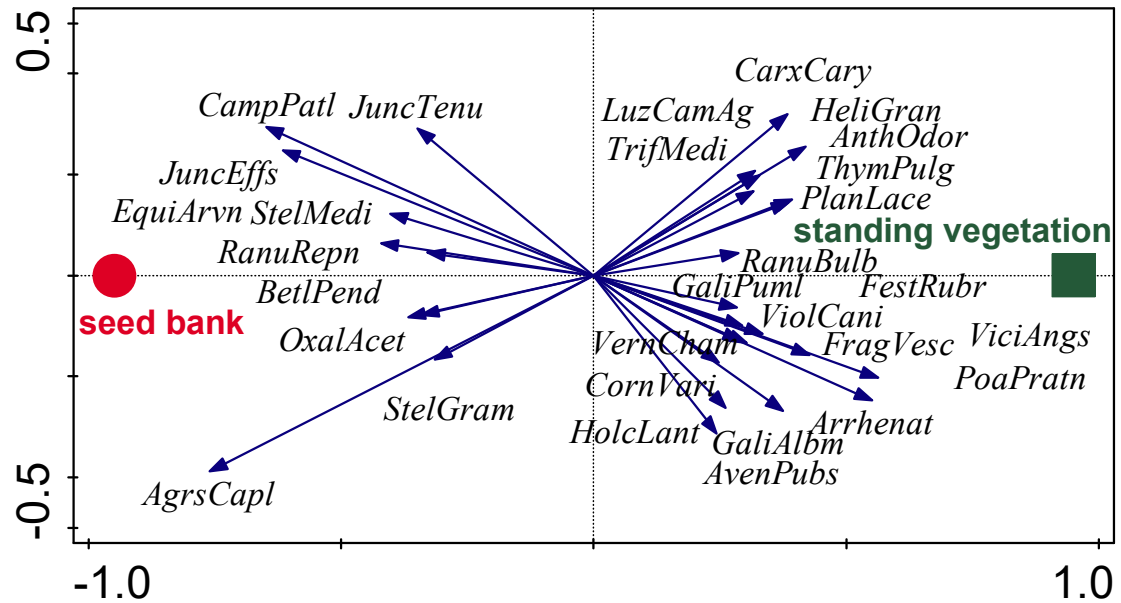

Fig. 5 The RDA-partial diagram displays differences in species composition explained by *type* (seed bank or standing vegetation). The adjusted explained variation is 33.13 %. The first axes explained 34.46 % of the total variation and the second axes only 8.25 %. Species abbreviations in Table 5.

The results of the RDA analysis of species composition in the seed bank, where the microsite was the explanatory variable, showed a non-significant effect (pseudo – F= 1.0, p=0.498).
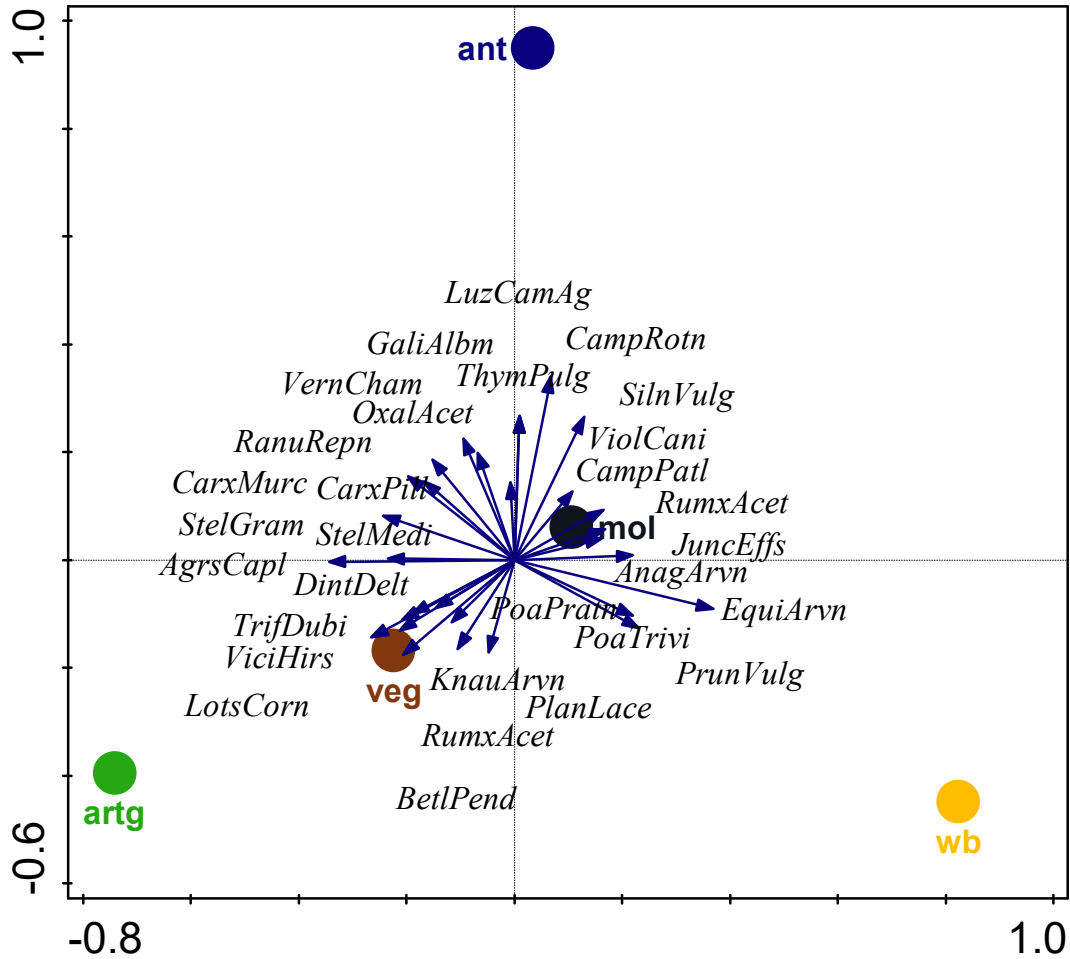
Fig. 6 The RDA diagram displays species composition of seed bank across microsites (ant – anthill, veg – intact vegetation, artg – artificial gap, wb – wild boar disturbances, mol – molehill). The differences are not significant, the diagram is shown only to display trends. Species abbreviations in Table 5.

The results of the RDA showed significant effect of microsite on species composition of the vegetation. (pseudo – F= 1.6, p=0.001). The species composition of the vegetation exhibits greater variability across microsites than that of the seed bank (Fig. 6, Fig. 7). The most different is the disturbances from wild boar and the artificial gaps. Species composition of anthills and molehills seem to be homogeneous (Fig. 7). Typical representatives of wild boar disturbances are species as *Sedum acre*, *Securigera varia* and *Brachypodium pinnatum*.
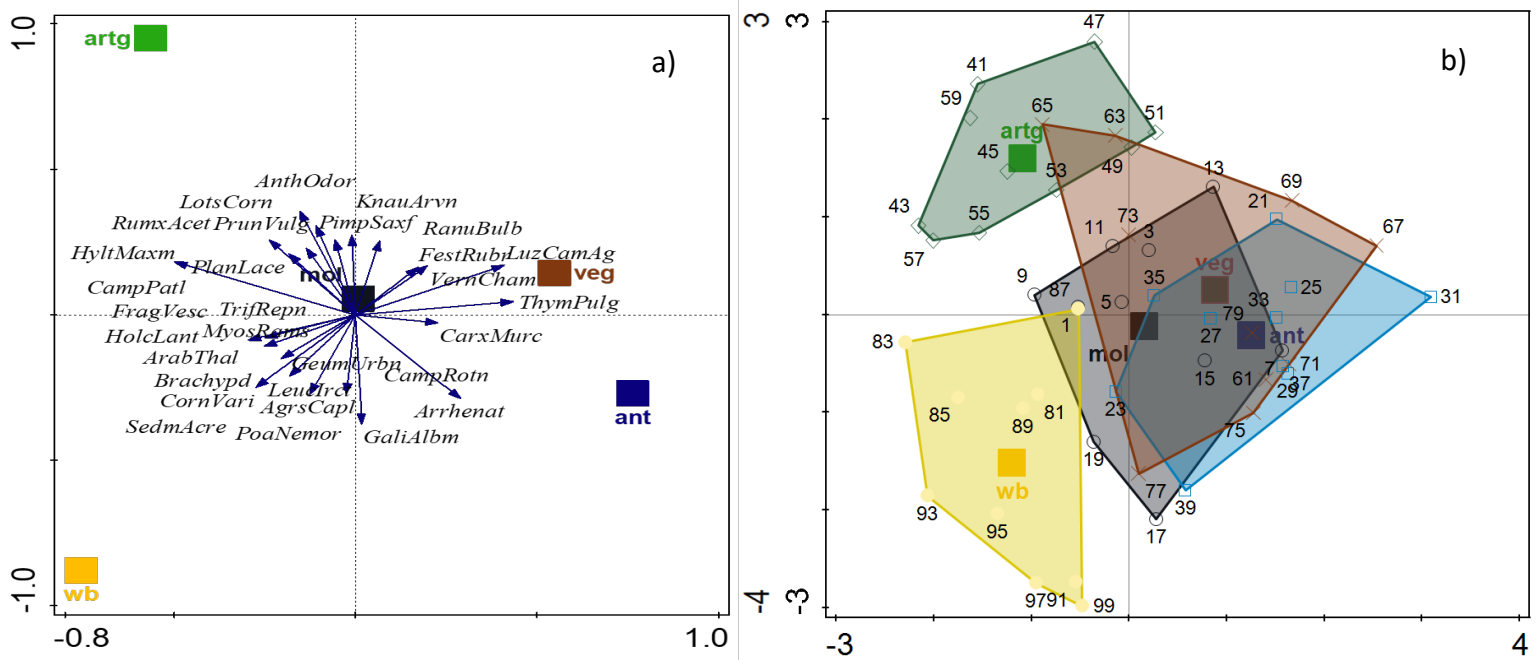
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Fig. 7 The differences in species composition of vegetation across microsites displayed by a) The RDA diagram and b) classified sample diagram. The adjusted explained variation is 4.80 %. The first axes explained 5.36 % of the total variation and the second axes only 3.50 %. *Microsite* categories are coded as ant – anthill; veg – intact vegetation; artg – artificial gap; wb – wild boar disturbances; mol – molehill. Species abbreviations in Table 5.

4.3 Species composition of vegetation

The outcome of the RDA-partial, which included the explanatory variable the interaction between *microsite × position* and the main effects of *microsite* and position, as well as the ID of the pairs acting as covariates, revealed that the interaction between *microsite* and *position* was found to be significant (pseudo – F=1.6, p=0.001). The analysis was used only for testing the interaction. To visualize the results, an ordination diagram was generated showing the species composition and the interaction between *microsite* and *position* (Fig. 8).

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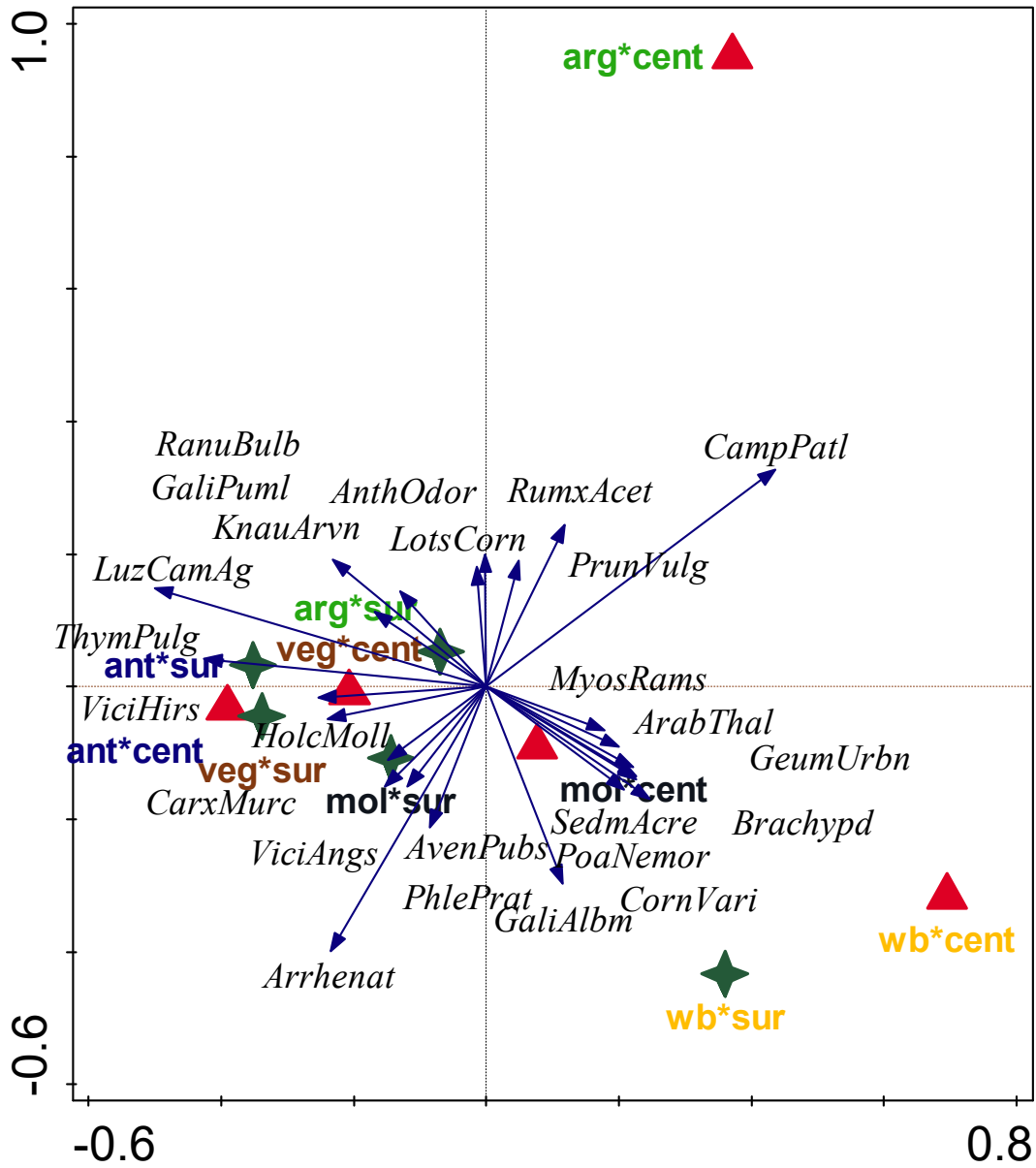
Fig. 8 The RDA diagram displays the interaction *microsite × position* as the only explanatory variable. The adjusted explained variation is 3.87 %. The first axes explained 3.89 % of the total variation and the second axes only 3.29 %. *position* (cent – center – red triangles; sur – surrounding vegetation – green stars) × *microsite* (ant – anthill; veg – intact vegetation, artg – artificial gap; wb – wild boar disturbances; mol – molehill). Species abbreviations in Table 5.

It is evident that the vegetation is more diversified within the microsite (center position). The intact vegetation (control in the center position) and its surrounding vegetation are (expectedly) very similar (Fig. 8). The artificial gaps located in the upper right corner of the plot are characterized by the species *Campanula patula*, *Rumex acetosella*, and *Lotus corniculatus*. Wild boar disturbances are probably less influenced by microsite because of their distinct position in the ordination space.

Results from the partial RDA (with ID of pair as a covariate) indicated a significant effect of *position* (pseudo – F=4.1, p=0.001). While species such as *Campanula patula* and *Stellaria graminea* have higher cover in microsites, the majority of species are typically associated with surrounding (Fig. 9).
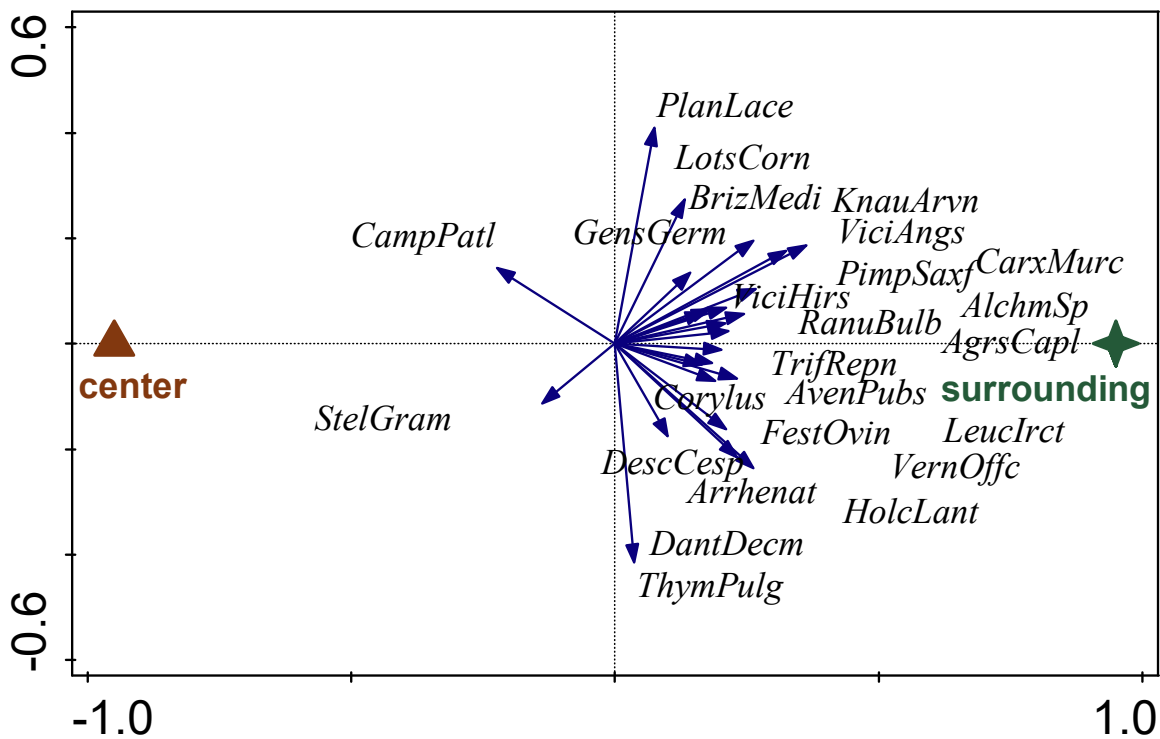
Fig. 9 The RDA-partial diagram displays differences in species composition explained by position (center or surrounding). The adjusted explained variation is 5.28 %. The first axes explained 2.14 % of the total variation and the second axes only 2.7 %. Species abbreviations in Table 5.

The results of the RDA analysis of species composition in the surrounding vegetation of the microsite showed a nonsignificant effect (pseudo – F= 1.0, p=0.465) which confirms the validity of the experimental design (Fig. 10). The species composition of vegetation in individual microsites is displayed in Fig. 7.

Fig. 10 The RDA diagram displays the species composition of surrounding vegetation across microsites which did not significantly differ. Species abbreviations in Table 5.

5 Discussion

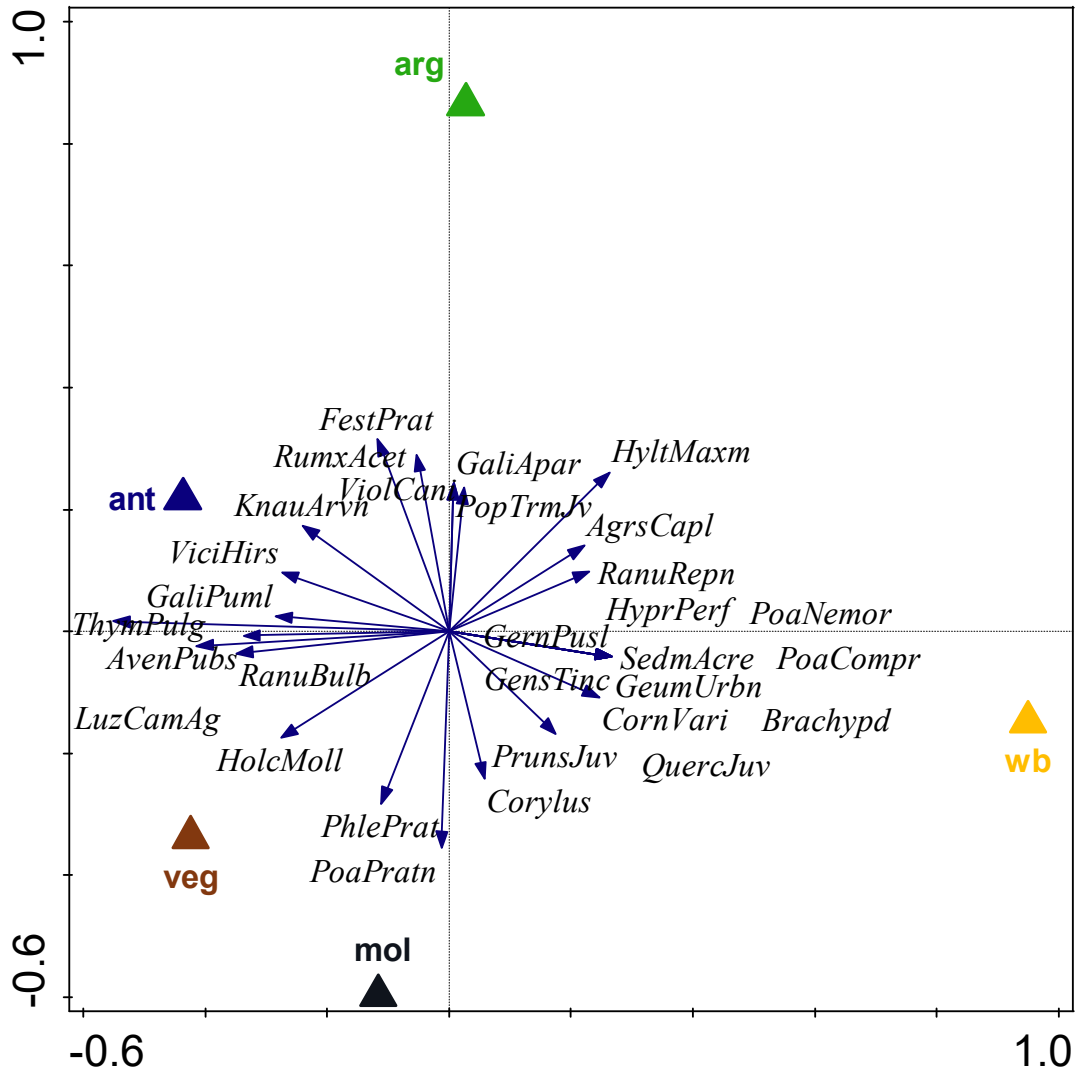Seedling survival after seed addition of three target species (*Trifolium pretense, Achillea millefolium,* and *Carlina acaulis*) across different types of microsites was studied in my bachelor thesis (Vacková, 2021). The results showed that the seedling survival during two growing seasons varied between microsites and the three species differed from each other. Additionally, microsites differed in nutrients (phosphorus, nitrogen, and carbon), pH, and size.

My master's thesis concentrated on the microclimate of microsites as well as the impact of vegetation, mosses, and litter cover. The results reveal a significant effect of litter and vegetation on the number of seedlings of *Trifolium pratense* and *Achillea millefolium* during one growing season. Furthermore, we also focused on the species composition of microsites and their seed bank. The results proved that species composition of extant vegetation varies across different microsites, but the composition of the seed bank did not differ across microsites. Also, the number of species is higher in standing vegetation than in seed bank. Furthermore, we also estimated the cover of vegetation in the surrounding area of each microsite. The result showed that species lists in the surrounding area and microsites overlap a lot, however, the proportion of species is significantly different between microsites and their surroundings. The main point of my thesis is to show the heterogeneity in seedling survival and species composition of different small microsites in grassland which has been poorly studied.

5.1 Seedling survival experiment

5.1.1 Seedling response to litter, vegetation, and mosses

In general, the temporal dynamics of seedlings across the growing season showed the peak of the number of seedlings in the middle of May in all microsites. A slight increase was

seen in autumn which corresponds to the observation of Sakanoue (2002) in a mown meadow where *Trifolium pratense* had an even higher number of seedlings in autumn than in spring. On the other hand, the highest peak of vegetation cover across all microsites was later in the middle of June when is an evident tendency to slight seedling mortality. When the soil is bare, it provides an opportunity for plants to colonize, and grow and leads to a quicker recovery of vegetation in those sites (Rusch & Fernández-Palacios, 1995). In this thesis, in microsites where there is intense biomass removal such as wild boar disturbances, artificial gaps, and molehills, there is often a faster colonization compared to intact vegetation and anthills. Vandvik (2004) suggested that species exhibit different responses to colonization dynamics across three successional stages of gaps. These stages are likely related to the age of gaps and may be characterized by different competition regimes or environmental conditions. In this thesis, the number of seedlings across all microsites started to stabilize at very low numbers at the beginning of August. An exception is anthills which were at the time of experiment start in an advanced stage of succession, i.e. already overgrown with vegetation and litter was present (Fig. 2a). The number of seedlings in anthills was at the beginning similar to that in intact vegetation but was then followed by a rapid decline. The reason is probably because of the strong intensity of disturbance at my locality caused by the constant bioturbation activity of ants. King (1977) also observed that seedlings establishment was slow and only persistent species survived in active anthills.

Mosses are often considered one of the first colonizers of bare soil or disturbed areas (Gloaguen, 1990; Porley, 1999). In this thesis, the moss cover was relatively low (median 1 %, maximum 30%) and its effect was not significant for species *Trifolium pratense*, thus, mosses could play a role as competitors in the first year which may change with the gap succession or between target species. Even though the mosses had such low abundance, they had a significant effect on the survival of *Achillea millefolium* seedlings. The study by Nystuen et al. (2014) concentrated on seedling recruitment affected by rodent disturbance and the cover of mosses in the Alps. One of the findings of the study was that mosses have no effect on seedling recruitment which corresponds with our results. On the other hand, mosses have sometimes been proven to facilitate by optimizing microclimate by preventing extreme temperature fluctuations and maintaining appropriate moisture (Ingerpuu et al., 2005). In contrast, the study

(Kotorová & Lepš, 1999) revealed the negative impact of the moss layer on the recruitment of seedlings. The results indicated that moss removal has a positive effect on seedling recruitment as a result of reduced competitive pressure. Note that the last study showing the negative effect is a manipulative experiment. It is possible that masses and seedlings are supported by some third factor (seedlings and mosses might be positively affected by e.g., moisture), which mitigates the negative effect of mosses on seedling recruitment in observational studies.

In this thesis, the results show that the seedling dynamic of the species *Trifolium pratense* and *Achillea millefolium* is affected by litter. These results partly correspond to study by Špačková et al. (1998) which demonstrated a positive response of seedling survival to moss and litter removal. On the other hand, litter can sometimes help seedlings by maintaining a suitable microclimate, shading against intense light, or releasing necessary nutrients during decomposition (Loydi et al., 2013). The results of the study Špačková et al. (1998) indicated that plots with only litter removal had fewer seedlings compared to plots with both moss and litter removal. Donath & Eckstein (2010) showed that the amount of litter also matters, as a denser layer leads to a more intense selection of seeds that are able to germinate, thereby significantly reducing diversity. Jensen & Gutekunst, (2003) and Jeschke & Kiehl, (2008) stressed that especially for germination and early seedlings, litter is considered to be a non-negligible barrier.

In my study, the number of seedlings was found to be significantly influenced by the presence of vegetation. Many studies (Barrett & Silander, 1992; Bullock, 2000; Gillet, 2008; Kohler et al., 2007; Parish & Turkington, 1990; Vandvik, 2004) revealed that in microsites where the vegetation cover was low or fully removed (natural disturbances and artificial gaps) the number of seedlings was. Out results showed that minimum of seedlings was observed in intact vegetation simultaneously with the highest cover of vegetation and litter (Fig. 2e). This probably indicates that seedlings were suppressed by dense vegetation.

In my thesis, microsites such as wild boar disturbances, artificial gaps, and molehills were at the beginning covered with almost no vegetation or litter (Fig. 2b, c, d). On the other hand, intact vegetation and anthills were already in an advanced stage of succession, with high vegetation cover and a layer of litter present. It is also apparent that in these two microsites,

there was not as fast colonization of vegetation and litter cover during the season as in uninhabited microsites (wild boar disturbances, molehills, and artificial gaps). This fact contributes to the heterogeneity of the environment, as both litter and vegetation can change over time while simultaneously affecting the microclimate in the microsite.

5.1.2 Seedling response to moisture and temperature

In my bachelor thesis (Vacková, 2021) soil moisture was significantly different across microsites. The major difference showed anthills as the driest microsites. The study by Dean et al. (1997) also demonstrated that anthills had lower moisture levels than the surrounding vegetation. In Vacková (2021), molehills, artificial gaps, intact vegetation, and wild boar disturbances did not demonstrate major differences in soil moisture. It is important to emphasize that the results were from a single soil sampling in March. On the other hand, dataloggers that were used in this thesis can record slight fluctuation in a microclimate which could strongly affects seed germination (Isselstein et al., 2002), thus we were able to observe greater differences in moisture between microsites (Fig. 11). According to Haugland & Froud-Williams (1999), *Lolium perenne* and *Trifolium pratense* seedling growth are significantly impacted by low soil moisture levels, which reduces their ability to withstand competition. Early seedling growth has frequently been considered to be the most vulnerable stage of a plant's life cycle (Isselstein et al., 2002; Morgan, 1997; Ryser, 1993). Moreover, seedlings have elevated mortality compared to adult plants throughout the growing season (Sarukhan & Harper, 1973). Besides that, seedlings are quite susceptible to various microclimate changes (Hofmann & Isselstein, 2004; Morgan, 1997; Stanik, 2021). Our results in this thesis revealed that the highest soil moisture was in intact vegetation (Fig. 3). This agrees with other studies (Dean et al., 1997; Schiffers et al., 2010; Williams, 1992; Zhou et al., 2017) where the soil moisture usually decreased in the gaps or natural disturbances compared to intact vegetation. The mentioned studies attributed the lower moisture in natural disturbances to the absence of vegetation which increased the temperature. Even though moisture was higher in intact vegetation than in other microsites, the number of seedlings was slightly lower than in wild boar disturbances, molehills, and artificial gaps (Fig. 2). The possible explanation is that the strong competition of vegetation outweighed the suitable conditions. On the other hand, in harsh conditions neighboring plants

can promote seedling survival and protect them from extreme conditions (Ryser, 1993). Also, the studies (Morgan, 1997; Williams, 1992; Zhou et al., 2017) stressed that the suitable microclimate is dependent on gap size, since in bigger gaps the microclimate is harsher, which negatively affects seedlings survival. From the perspective of grassland, which are considered to be a strong competitive environment, neighboring plants are probably more harmful than beneficial to seedlings. Nevertheless, (Kelemen et al., 2015) show that at the very early stages of recruitment, seedlings can be protected by vegetation (apparently due to preventing desiccation), but later on they are suppressed by competition of the extant vegetation.

Microtopography can influence microclimate and soil conditions. Harper et al. (1965) studied the effect of microtopography on seed germination. Slight depressions in the soil surface provide a space with enough soil moisture to protect the seeds from desiccation. In this thesis, slightly higher moisture than in molehills, artificial gaps, and anthills was indicated in wild boar disturbances. Wild boar disturbances vary in size, shape, and rooting intensity, which affects the properties of the soil (Bueno et al., 2013; Vacková, 2021). Wild boar disturbances are the only ones with a hollow shape, so moisture can be held there better which could positively impact the seedlings. It is also noticeable that the autumn increase in moisture is copied by the increased number of seedlings in wild boar disturbances. Molehills and anthills have different shapes as small hills. Anthills were found to be the driest microsites, which is consistent with studies by (Dean et al., 1997; Ehrle et al., 2017). Nevertheless, Konečná et. al 2021 found that the center of anthills did not differ in moisture from controls, but only the margins of the anthills. Thus, it also corresponds to our results because the dataloggers were situated in the margins of anthills, however, the seeds were sown into the center. Molehills and artificial gaps performed lower moisture levels than wild boar disturbances. When comparing artificial gaps to molehills, there was a more evident decrease in moisture in the artificial gaps compared to the molehills (Fig. 3c, d). Also, the rate of decrease in moisture levels was much higher in the artificial gaps than in the molehills. Williams (1992) studied the temperature and moisture levels in gaps of varying sizes. The results showed that gaps with a diameter of at least 30 cm exhibited extreme temperature values and had low moisture levels.

The temperature also affects seed germination and survival (Covell et al., 1986; Del Monte & Tarquis, 1997; Kolb & Robberecht, 1996). In this thesis, our results do not show much variation in temperature across different microsites. However, the highest temperature during the year typically occurred between June and August. In June, during the peak of the vegetation season, the average temperature in anthills can reach a value of around 25 °C. According to studies by Frouz (2000) and Konečná et al. (2021), the temperature on anthills tends to increase during the summer, although this can vary depending on the position of the anthill (Frouz, 2000). Molehills tend to exhibit slightly higher temperatures than artificial gaps and wild boar disturbances, which may be due to their shape. Exposure of bare soil to sunlight and air after vegetation removal can cause the soil to warm up, leading to elevated temperatures (Canals & Sebastià, 2000). The study by Williams (1992) conducted in Mountain plateau in Australia found that temperatures in artificial gaps reached almost 80°C. In my experiment, the results indicated that the temperature in artificial gaps was similar to the temperature in intact vegetation, which was on average per week around 21°C and with maximum value 47°C in intact vegetation and 46°C in artificial gaps.

5.2 Species composition of the seed bank and standing vegetation

In many studies, the seed bank is mentioned as an important source for maintaining of species diversity (Bakker et al., 1996; Erschbamer et al., 2001; Hopfensperger, 2007; Kalamees & Zobel, 2002; Vítová et al., 2017). It serves as a source of seed availability, which is particularly important in grasslands where the number of seeds can be limited. The natural disturbances are space in vegetation available for the establishment of new species (Kalamees & Zobel, 2002; King, 2007). Our results showed that the species composition of seed bank of different microsites does not differ. Even though that some studies (Bueno et al., 2011; Yusefi et al., 2022) demonstrated that seed banks could be negatively influenced by the bioturbation of animals as wild boar disturbances. According to Gómez-García et al. (1999), animal disturbances can have a positive impact on seed germination by exposing seeds to light and moisture, thereby creating opportunities for other plant species to grow. However, mentioned

studies (Bueno et al., 2011; Yusefi et al., 2022) have reported that wild boar disturbances can result in a lower abundance of seeds and species richness in the seed bank when compared to the surrounding vegetation. Indeed, there are cases where animal activity can support the seed bank. Schütz et al. (2008) studied the composition of the seed bank in anthills and in vegetation. Their results found that the species composition did not differ, but the number of seeds was many times greater in the seed bank of anthills than in vegetation. Another example is a study Koontz & Simpson (2010) showed a higher species richness of seed bank of kangaroo rat mounds compared to the surrounding soil. Kangaroo rats primarily consume seeds and accumulate them in their mounds when not eaten, which can contribute to local diversity and mounds provide microsites for seed germination and establishment.

The species composition of the seed bank and standing vegetation differed significantly. The number of species present in the standing vegetation was higher (66 species; Table 6) than in the seed bank across microsites (36 species; Table 6) which corresponds well to Eriksson & Eriksson (1977). Additionally, the standing vegetation contained a higher proportion of unique species (51.3 %) that were not found in the seed bank, while 35.5 % of species were present in both the seed bank and standing vegetation, and 13.2 % were only found in the seed bank. Kalamees & Zobel (2002) conducted a manipulative experiment to study species regeneration in gaps. They found that the majority of species in the gaps germinated from the seeds, with 36 % of species germinating from the seed bank and 46 % from seed rain, and only 18 % from clonal growth. Typical representatives of the seed bank were the species *Juncus tenuis*, *Juncus effusus*, *Agrostis capillaris* (it is typically abundant in both the seed bank and vegetation, Thompson, 1986). Besides grasses *Oxalis acetosella*, *Stellaria graminea*, and *Equisetum arvense* were frequent (Fig. 5). Species from genus *Juncus* often have a relatively large seed bank, with small seeds that can remain dormant in the soil for long periods of time (ref.). It is often the case for *Juncus* spp. to be absent from the standing vegetation in a locality, while still persisting in the soil seed bank. This may be due to the fact that seeds of *Juncus* are easily dispersed by wind, water, or animals to new locations (Thompson & Grime, 1979). Hopfensperger (2007) suggests that in grassland, the composition of the standing vegetation and the seed bank are typically similar, or do not differ significantly. This means that the species that are present in the aboveground vegetation are also likely to be represented in the seed bank.

However, according to Caballero et al. (2008), there could be differences in the persistence of perennial plants which mainly depends on the established plants' ability to survive over time, with the seed bank playing a minor role. On the other hand, for annual plants, their persistence relies on the seed bank to survive and propagate from one growing season to the next. This thesis indicated that the species composition of the standing vegetation in microsites differs from that of the seed bank. It is likely that the composition of microsites did not originate from a seed bank, but rather from seed rain or vegetative growth.

5.3 Species composition of microsites and their surroundings

There was a significant difference in the species composition between the microsites and their surrounding vegetation (Fig. 8). Regarding the number of species, there were 17 more species found in the surrounding vegetation compared to the microsites (Table 7). The cover proportions, especially of the species *Campanula patula* and *Stellaria graminea*, differed between the microsites and the surrounding vegetation. However, the results indicated that the surrounding vegetation around microsites did not differ significantly from each other i.e. the fact, that controls were in direct vicinity around microsites had not influenced surrounding vegetation enough to be distinct. In agreement with this, the species composition of gaps corresponds to the composition of their surrounding areas, but the proportions of species may differ between gaps and their surrounding areas because gaps provide more variable environmental conditions (Bullock, 2000). Several studies (Bueno et al., 2009; Kohler et al., 2007; Konečná et al., 2021; Seifan et al., 2010) compared a specific type of disturbances with surrounding vegetation and they revealed differences in species composition. For instance, the study by Seifan et al. (2010) observed species composition on molehills and their surrounding vegetation across a productivity gradient. In the more productive localities, there were mainly fewer shade-tolerant species present on the molehills.

Differences in species composition in disturbances are usually attributed to decreased competition pressure or different soil conditions. In Vacková (2021), we also focused on soil nutrients (phosphorus, nitrogen, and carbon), anthills exhibited notable differences in soil properties compared to other microsites. Specifically, anthills were found to have high concentrations of phosphorus and nitrogen, which is in line with the findings of e.g. Lenoir et

al. (2001), but contradicts those of Dean et al. (1997) Additionally, anthills had a high content of organic carbon and the highest pH among all the microsites examined. According to my bachelor thesis, there is no significant difference in terms of phosphorus and carbon content between molehills and both intact vegetation and wild boar disturbances. However, molehills were found to have a significantly higher nitrogen content than intact vegetation and other disturbances. These findings are in line with the study by Canals and Sebastià (2000), which suggests that the higher inorganic nitrogen content in molehills is due to the intensive nitrification process occurring in aerated soil resulting from the bioturbation by moles. The microsites also varied in size among different types. Molehills were the smallest with the least variation, similar to artificial gaps (which were aimed to constant size of 500 cm^2). Wild boar disturbances were the most variable in size, but also the largest. Individual microsites were significantly different from each other in terms of species composition (Fig. 7). The distinctive species composition was found for the wild boar disturbances and the artificial gaps. The previously mentioned differences in soil conditions, sizes, as well as temperature and moisture, can influence the composition of the plant community. There are only few studies that compare multiple disturbances with each other (Kohler et al., 2007; Parish & Turkington, 1990), although it is common for many types of disturbances to coexist within one locality. Milton et al. (1997) conducted a study in which they compared vole lawns with wild boar disturbances and found significant differences. The species composition of wild boar disturbances consisted mainly of therophytes and weeds, whereas in vole mounds the dominant species were those dispersed by wind and animals. In this thesis, typical representatives of plant species of wild boar disturbances and their surroundings were grasses *Brachypodium pinnatum*, *Poa nemorossa*, *Phleum pratense*, further dicots *Coronila varia*, and *Sedum acre*. Anthills were mostly dominated by *Thymus pulegioides* which were also dominated in their surroundings. Due to the proximity of different disturbances, species can potentially 'spill over' and create patches of specific species, ultimately increasing heterogeneity (see Milton, 1997).

Creating artificial gaps can be a useful tool for restoring species richness in grasslands, according to a study by Kiss et al. (2021). However, gaps can also serve as a holding area for invasive species, as noted by DiVittorio et al. (2007). Despite this, gaps can provide a refuge for seedlings in grasslands and contribute to maintaining species diversity by increasing local

heterogeneity. The variation in both temporal dynamics (as described by Vandvik in 2004, with different successional stages of disturbances offering varying conditions) and spatial dynamics (as observed by Seifan in 2010 and Milton in 1997) contributes to the overall species richness in the area.

6 Conclusion

The temporal dynamics of both artificially sown species (*Trifolium pratense* and *Achillea millefolium*) differed significantly between microsite types during the three growing seasons. There was a large drop in numbers of seedling in the second year for both species. The effect of litter, mosses, vegetation, and stones on the number of *Trifolium pratense* seedlings (during the first growing season) was also significant, but the effect of mosses was not. On the other hand, the effect of mosses was significant for species *Achillea millefolium.* Soil moisture was slightly higher in wild board disturbances than in other microsites. The temperature was the lowest in wild boar disturbances and the highest in anthills. The different microclimate in the microsites is probably caused by their different microtopography (shape and/or size).

Standing vegetation and seed bank had significantly different species compositions across microsites, with standing vegetation having a higher number of species than seed bank. While standing vegetation demonstrated differences between microsite types there was no difference in the species composition of the seed bank. The species composition of microsite vegetation differs significantly from surrounding vegetation, which had a greater number of species (17 more). Surrounding vegetation was not significantly different between microsites, confirming the validity of the experimental design. Microsites differ in terms of shape, size, and microclimate, which probably accounts for differences in species composition of standing vegetation and seedling survival. Small-scale disturbances increase temporal and spatial heterogeneity and thus contribute to species co-existence in grasslands.

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8 Appendix

Table 5 List of species and their abbreviation used in diagrams.

|					
Agrostis capillaris	*AgrsCapl*	*Fragaria vesca*	*FragVesc*	*Plantago lanceolata*	*PlanLace*
Alchemilla sp.	*AlchemSp*	*Galium album*	*GaliAlbm*	*Poa nemoralis*	*PoaNemor*
Anagallis arvensis	*AnagArvn*	*Galium aparine*	*GaliApar*	*Poa pratensis*	*PoaPratn*
Anthoxanthum odoratum	*AnthOdor*	*Galium pumilum*	*GaliPuml*	*Poa trivialis*	*PoaTrivi*
Anthriscus sylvestris	*AnthSylv*	*Genista germanica*	*GeniGerm*	*Potentilla heptaphylla*	*PotenHepta*
Arrhenatherum elatius	*Arrhenat*	*Genista tinctoria*	*GeniTinc*	*Prunella vulgaris*	*PrunVulg*
Helictotrichon pubescens	*AvenPubs*	*Geum urbanum*	*GeumUrbn*	*Prunus* sp. juv.	*PrunsuJuv*
Betula pendula	*BetlPend*	*Helianthemum grandiflorum*	*HeliGran*	*Quercus* robur juv.	*QuercJuv*
Brachypodium pinnatum	*Brachypd*	*Pilosella officinarum*	*HierPilo*	*Ranunculus acris*	*RanuAcri*
Briza media	*BrizMedi*	*Holcus lanatus*	*HolcLant*	*Ranunculus bulbosus*	*RanuBulb*
Campanula patula	*CampPatl*	*Holcus mollis*	*HolcMoll*	*Ranunculus repens*	*RanuRepe*
Campanula persicifolia	*CampPers*	*Hylotelephium maximum*	*HyltMaxm*	*Rumex acetosa*	*RumxAcet*
Campanula rotundifolia	*CampRotu*	*Hypericum perforatum*	*HyprPerf*	*Rumex acetosella*	*RumeAcel*
Carex caryophyllea	*CarxCary*	*Hypochaeris radicata*	*HypoRadi*	*Sedum acre*	*SedmAcre*
Carex muricata	*CarxMurc*	*Juncus effusus*	*JuncEffs*	*Silene nutans*	*SilnNusa*
Carex pallescens	*CarxPall*	*Juncus tenuis*	*JuncTenu*	*Silene vulgaris*	*SilnVulg*
Carex pilulifera	*CarxPill*	*Knautia arvensis*	*KnauArvn*	*Stellaria graminea*	*StelGram*
Centaurea jacea	*Centaurea*	*Lathyrus pratensis*	*LathPrat*	*Stellaria media*	*StelMedi*
Securigera varia	*CotnVari*	*Scorzoneroides autumnalis*	*LeonAutu*	*Taraxacum* sect. *Taraxacum*	*TaraxcSp*
Corylus avellana juv.	*Corylus*	*Leontodon hispidus*	*LeonHisp*	*Thymus pulegioides*	*ThymPulg*
Crataegus sp. juv.	*CratJuv*	*Leucanthemum ircutianum*	*LeucIrct*	*Trifolium campestre*	*TrifCamp*
Dactylis glomerata	*DactGlom*	*Linum catharticum*	*LinumSp*	*Trifolium dubium*	*TrifDubi*
Danthonia decumbens	*DantDecm*	*Lotus corniculatus*	*LotsCorn*	*Trifolium medium*	*TrifMedi*
Deschampsia cespitosa	*DescCesp*	*Luzula campestris* agg.	*LuzCamAg*	*Trifolium repens*	*TrifRepn*
Dianthus deltoides	*DintDelt*	*Melampyrum pratense*	*MelaPrat*	*Veronica arvensis*	*VeroArve*
Elymus repens	*ElymRepe*	*Myosotis ramosissima*	*MyosRams*	*Veronica chamaedrys*	*VeroCham*
Equisetum arvense	*EquiArvn*	*Oxalis acetosella*	*OxalAcet*	*Veronica officinalis*	*VeroOffi*
Festuca ovina	*FestOvin*	*Phleum pratense*	*PhlePrat*	*Vicia angustifolia*	*ViciAngs*
Festuca pratensis	*FestPrat*	*Pimpinella saxifraga*	*PimpSaxf*	*Vicia hirsuta*	*ViciHirs*
Festuca rubra	*FestRubr*	*Pinus sylvestris* juv.	*PinusJuv*	*Viola canina*	*ViolCani*
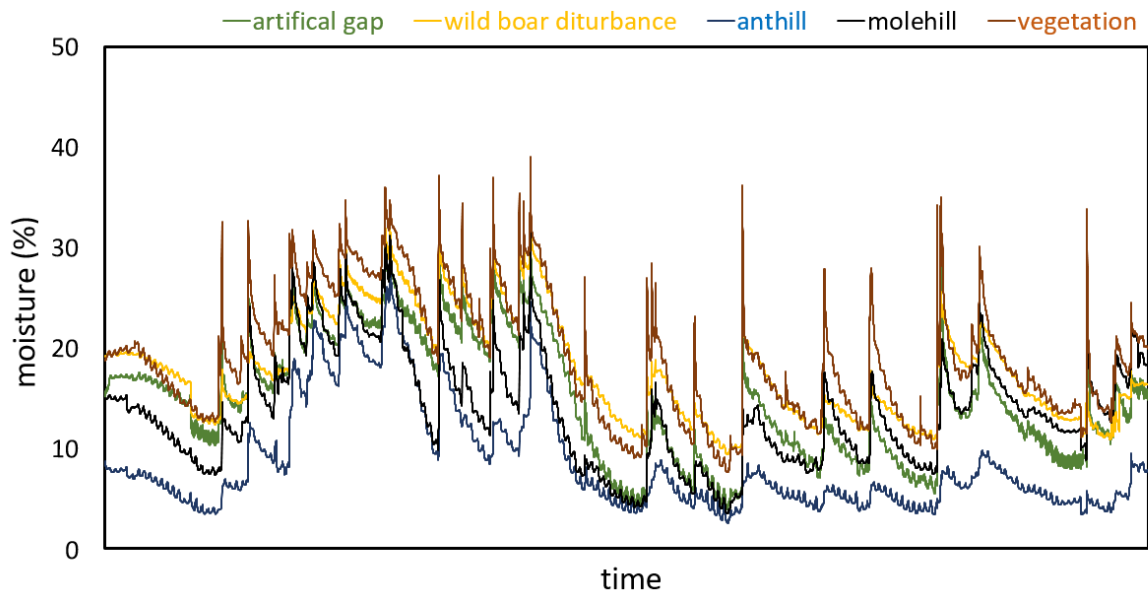
Fig. 11 Temporal dynamic of moisture in different microsites within the first growing season (April to early October; measurement each 15 minutes).

Fig. 12 Temporal dynamic of species *Achillea millefolium* in second and third year of experiment.
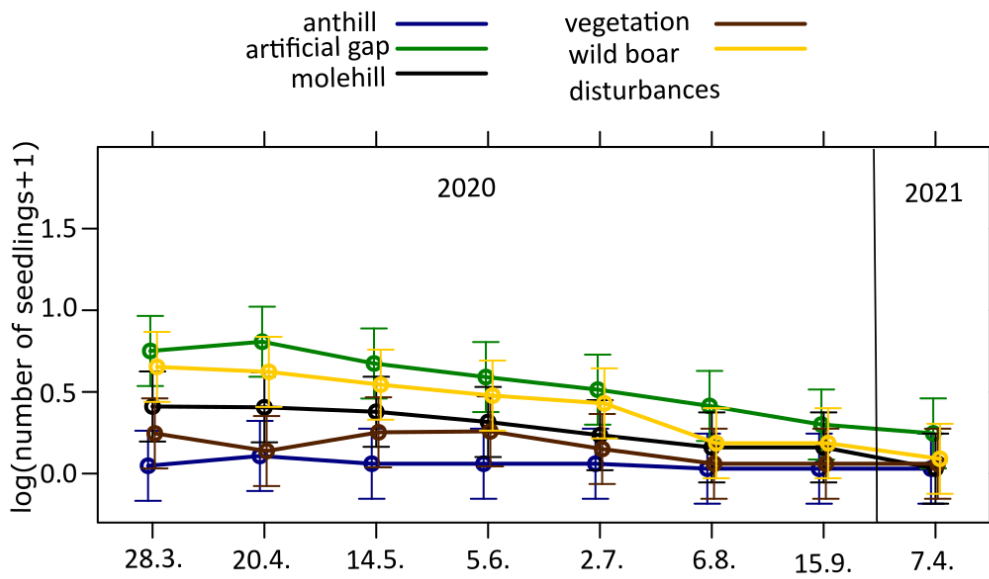

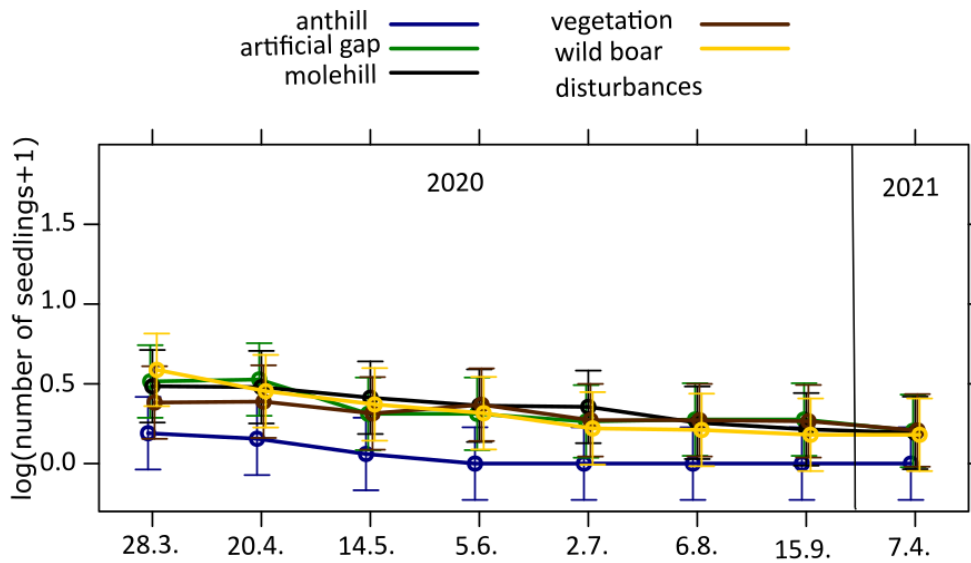Fig. 13 Temporal dynamic of species Trifolium pratense in second and third year of experiment.

Table 6 List of species in standing vegetation and seed bank.

overlapping species (35.5 %)	standing vegetation (51.3 %)	seed bank (13.2 %)
Agrostis capillaris	Anthoxanthum odoratum	Juncus effusus
Alchemilla sp.	Anthriscus sylvestris	Juncus tenuis
Campanula patula	Arabidopsis thaliana	Equisetum arvense
Campanula rotundifolia	Arrhenatherum elatius	Anagallis arvensis
Carex caryophyllea	Helictotrichon pubescens	Betula pendula
Carex muricata	Brachypodium pinnatum	Carex pilulifera
Galium album	Briza media	Oxalis acetosella
Helianthemum grandiflorum	Centaurea jacea	Ranunculus repens
Holcus lanatus	Securigera varia	Stellaria media
Holcus mollis	Crataegus sp. juv.	Poa trivialis
Knautia arvensis	Dactylis glomerata	
Lotus corniculatus	Danthonia decumbens	
Luzula campestris agg.	Elymus repens	
Poa pratensis	Festuca ovina	
Prunella vulgaris	Festuca pratensis	
Rumex acetosa	Festuca rubra	
Rumex acetosella	Fragaria vesca	
Silene vulgaris	Galium pumilum	
Stellaria graminea	Geum urbanum	
Thymus pulegioides	Pilosella officinarum	
Trifolium dubium	Hylotelephium maximum	
Trifolium repens	Scorzoneroides autumnalis	
Veronica chamaedrys	Leontodon hispidus	
Vicia hirsuta	Leucanthemum ircutianum	
Viola canina	Myosotis ramosissima	
Dianthus deltoides	Phleum pratense	
Plantago laceolata	Pimpinella saxifraga	
	Poa nemoralis	
	Potentilla heptaphylla	
	Prunus sp. juv.	
	Ranunculus acris	
	Ranunculus bulbosus	
	Sedum acre	
	Silene nutans	
	Taraxacum sect. Taraxacum	
	Trifolium medium	
	Veronica arvensis	
	Veronica officinalis	
	Vicia angustifolia	

Table 7 List of species in surrounding vegetation and vegetation of microsites.

surrounding vegetation (20.5 %)	overlapping species (79.5 %)	
Campanula persicifolia	*Agrostis capillaris*	*Scorzoneroides autumnalis*
Carex pallescens	*Alchemilla* sp.	*Leontodon hispidus*
Corylus avellana juv.	*Anthoxanthum odoratum*	*Leucanthemum ircutianum*
Deschampsia cespitosa	*Anthriscus sylvestris*	*Lotus corniculatus*
Galium aparine	*Arabidopsis thaliana*	*Luzula campestris* agg.
Genista germanica	*Arrhenatherum elatius*	*Myosotis ramosissima*
Genista tinctoria	*Helictotrichon pubescens*	*Phleum pratense*
Hypericum perforatum	*Brachypodium pinnatum*	*Pimpinella saxifraga*
Hypochaeris radicata	*Briza media*	*Plantago lanceolata*
Lathyrus pratensis	*Campanula patula*	*Poa nemoralis*
Linum catharticum	*Campanula rotundifolia*	*Poa pratensis*
Melampyrum pratense	*Carex caryophyllea*	*Potentilla heptaphylla*
Pinus sylvestris juv.	*Carex muricata*	*Prunella vulgaris*
Poa trivialis	*Centaurea jacea*	*Prunus* sp. juv.
Quercus robur juv.	*Securigera varia*	*Ranunculus acris*
Ranunculus repens	*Crataegus* sp.	*Ranunculus bulbosus*
Trifolium campestre	*Dactylis glomerata*	*Rumex acetosa*
	Danthonia decumbens	*Rumex acetosella*
	Dianthus deltoides	*Sedum acre*
	Elymus repens	*Silene nutans*
	Festuca ovina	*Silene vulgaris*
	Festuca pratensis	*Stellaria graminea*
	Festuca rubra	*Taraxacum* sect. *Taraxacum*
	Fragaria vesca	*Thymus pulegioides*
	Galium album	*Trifolium dubium*
	Galium pumilum	*Trifolium medium*
	Geum urbanum	*Trifolium repens*
	Helianthemum grandiflorum	*Veronica arvensis*
	Pilosella officinarum	*Veronica chamaedrys*
	Holcus lanatus	*Veronica officinalis*
	Holcus mollis	*Vicia angustifolia*
	Hylotelephium maximum	*Vicia hirsuta*
	Knautia arvensis	*Viola canina*