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# **Assembly rules in plant communities**

Ph.D. Thesis

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## ■ Annotation

Understanding the mechanisms of species co-occurrence in plant communities and determining the most important drivers of community assembly is one of the central questions in community ecology. Problematics of assembly rules is relatively difficult and most of the studies are based on null models, simulations, or observational methods rather than on experimental approach. This thesis focused on an experimental approach which can clarify many ecological mechanisms contributing to answer many questions related to assembly rules concept. Research in this thesis was focused both on stochastic and deterministic processes influencing the species community composition. Adding seeds or transplants of different species (including also the non-resident species) into community and also into plots with restricted competition and monitoring their establishment and survival for several years, the research highlighted the important role of priority effects on species community composition, the importance of the biotic filter as one of the main drivers in composition of meadow species and suggested that species composition is necessary to compare with the appropriately defined species pool. Research in this thesis also compared different estimation methods of species pool assessment with experimentally determined species pool trying to find the most appropriate possibility of the estimation of species pool.

## ■ Declaration

I hereby declare that I am the author of this dissertation and that I have used only those sources and literature detailed in the list of references.

České Budějovice, 25.11.2021

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Eva Švamberková

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## ■ List of papers and author's contribution

The thesis is based on the following papers (listed chronologically):

I. Švamberková, E., Vítová, A., & Lepš, J., (2017). The role of biotic interactions in plant community assembly: What is the community species pool? *Acta Oecologica*, 85, 150-156 (IF = 1.674). <https://doi.org/10.1016/j.actao.2017.10.011>

*EŠ participated in establishment of the study conception and designed the experiment together with JL and AV. EŠ established the experiment together with AV. EŠ was responsible for data collection in the field and data analysis in Experiment 2. EŠ was drafting a manuscript with the support of AV and JL. Her contribution was 45%.*

II. Švamberková, E., Doležal, J., & Lepš, J., (2019). The legacy of initial sowing after 20 years of ex-arable land colonisation. *Oecologia*, 190, 459-469 (IF = 3.225). <https://doi.org/10.1007/s00442-019-04415-y>

*EŠ partially participated in the data collection in the field. EŠ was responsible for data analysis with support of JL. EŠ was drafting a manuscript. Her contribution to the data collection (1996 – 2016) was correspondingly small, her contribution to the data analysis and writing was 65%.*

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IV. Švamberková, E., & Lepš, J. Methods of species pool determination as predictors of survival in seeding and transplanting experiments. (manuscript).

*EŠ established the study conception and designed the experiment together with JL. EŠ established the experiment and collected data. EŠ was responsible for data analysis with support of JL. EŠ was drafting a manuscript. Her contribution was 75%.*

#### ■ Co-author agreement

Jan Lepš, the supervisor of this Ph.D. thesis and co-author of all papers, fully acknowledges the stated contribution of Eva Švamberková to these manuscripts.

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Jan Lepš

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## **Chapter 1**

### **General introduction**



## **Concept of assembly rules**

Understanding the mechanisms of species co-occurrence in plant communities and determining the most important drivers of community assembly is one of the central questions in community ecology. In the history of research of this problem, there were two main opinions. The one idea was that species co-occur according to specific rules which determine the species community composition formation and stress the holistic nature of the communities (Clements 1916, Phillips 1931). Contrary, the second point of view promoted that composition of species in communities is based in individualistic species reactions to environmental gradients (Gleason 1926) and stress the randomness of community composition formation (Hubell 2001). Although many contemporary researchers accept that both deterministic and stochastic processes can influence the species community composition (Ulrich 2004, Kembel 2009, Gornish et al. 2019), random events are not often considered as important as assembly rules.

The concept of “assembly rules” was first used by Diamond (1975) who studied fruit-eating birds in New Guinea archipelago. He found that bird species do not co-occur randomly, but their co-occurrence is constrained by many interspecific interactions, especially by competition. Although this first concept of assembly rules was associated only with biotic limitations, other researchers were starting to develop this approach in wider interpretation. Weiher and Keddy (1999) defined assembly rules as a set of any ecological constraints which limit the species sorting from the regional species pool determining thus a local community composition. The basic ecological limitations deciding the coexistence of species in communities are biotic interactions (Grime 2006), abiotic environment (Lambers et al. 2012, Schamp et al. 2016) and dispersal ability (Houseman & Gross 2006). The concept of assembly rules is based on assumption that species living in ecologically similar habitats must differ in their niches

to coexist. Nevertheless, Hubbell (2001) referred that all species are ecologically similar (i.e., they can have some differences, but these do not influence the diversity) and the processes, which influence the community assembly, are generally random events (he included among them speciation, extinction, and dispersal). However, it depends on what is considered as random events. For example, dispersal abilities and reproductive traits can differentiate species in their fitness and thus, even though they can be very unpredictable, it does not mean that they are totally random (Clark 2009, Lowe & McPeck 2014).

### **Priority effects in a concept of assembly rules**

Species co-occurrence is affected by historical patterns such as speciation and migration on a geographical scale (i.e., phylogeographic assembly rules), by species dispersal abilities on a regional scale (i.e., dispersal assembly rules) and by both the abiotic environment and biotic interactions on a local scale (i.e., abiotic and biotic assembly rules). On the other hand, there is also the effect of random events which should be taken into consideration during studying processes forming a species community composition and which can definitely supplement the concept of assembly rules. Nevertheless, these random events are contemporary very often studied separately and are mostly considered exclusive of assembly rules (Cottenie 2005). One of the factors which can influence the species community composition very importantly but is not usually studied as a part of the assembly rules concept is “priority effect”.

Priority effect is a phenomenon of sequential arrivals of species from a regional species pool to a local community (Drake 1991, Facelli & Facelli 1993). The migration of new species in a site was considered as a decisive factor for forming communities and development of vegetation ages ago. For example, Clements (1916) suggested in his relay floristic theory that species of earlier successional stages enable an arrival of later successional species by ameliorating environmental

conditions (e.g., by forming soil layer needed for latter species). Nevertheless, this theory was responded by Egler (1954) in his initial floristic composition model which was developed specifically for the succession on abandoned old fields. It suggests that the vegetation development depends on the fact which species reached the target habitat at the beginning of the succession. Thus, after agricultural land abandonment, species of both early and late successional stages are present, although each is in different life span, for example while early successional stage species dominate at the first years, the species of later successional stages could be either very small or even dormant in this time (Breugel et al. 2007). However, in the literature, there are many ambiguities in the Eger's initial composition model and thus it is not always interpreted coincidentally (Wilson et al. 1992).

Regardless of fact whether really all species are present at the site just at the beginning of the succession or some species come later, it is proven that species reaching the habitat first have a significant effect on the later arriving species (Körner et al. 2008, Plückers 2013, Fraser et al. 2015, Popp et al. 2017). And this effect is what we can call priority effect. This species ordering and timing is related with the historical contingency of community assembly which is highlighted by many studies (Chase 2003, Leanne & Wilsey 2012; Fukami & Nakajima 2011, Fukami, 2015). Connell and Slatyer (1977) proposed the possible influence of early arriving species on later ones specifying facilitation, inhibition, and tolerance model. This classical model of succession then was the inspiration for studies really considering the arrival order of species as one of the assembly rules forming species communities (Fukami et al. 2005, Moore & Franklin 2012, Zuo et al. 2016). Fukami (2015) then developed a theory describing the functioning of priority effect based on niches overlap as niche pre-emption and niche modification. Niche pre-emption is considered if species requirements are highly overlapping. Then species arriving first reduces the colonisation of later arriving species by decreasing the

availability of resources which are the same for both (Gause 1934, MacArthur & Levins 1967), i.e., only species within niches are affected. On the other hand, in the case of niche modification, priority effect influences primarily species across niches because early arriving species alter the types of niches present in local communities and subsequently also the identity of later colonising species (Jones et al. 1994). Ordinarily it is expected that more successful colonist can be outcompeted by better competitor irrespective of priority effect, mostly because the trade-off between colonization ability and competitive strength (Tilman 1994). Nevertheless, if early arriving colonist manages to adapt to novel conditions, the assembly history and thus ordering of species arrival will become more important and this colonist will take the advantages even over the relatively better but later arriving competitor (Urban & de Meester 2009, Leanne & Wilsey 2012). It means that community assembly is historically conditioned and thus is influenced by priority effects (Carlstrom et al. 2019). The colonization ability of individual species is partially predictable based on functional traits. Nevertheless, which species will arrive first, and actual order of arrivals is affected by many factors, including the composition of surrounding communities (Vellend 2010). Moreover, for species establishment, it is not important just that its propagules arrive to the site, but also the number of propagules, factor dependent on the landscape context and rather difficult to predict (Lepš 2013). This is why priority effect is often referred to as a random effect (Lowe & McPeck 2014). Thus, random effects (e.g., priority effects) play their important role especially at the beginning of the structuring of the community but the patterns of these stochastic events can persist and influence the community development long time turning into a non-random development to a particular community (Ulrich 2004, Gornish et al. 2019). We focused on the importance of the priority and founder effect for community development in Chapter 2.



## **Species pool theory in a concept of assembly rules**

The idea of constraints selecting species from a regional to a local species pool became very popular in assembly rules problematic (de Bello et al. 2012, Lambers et al. 2012). It was identified with the concept of hierarchical filters acting at the finer and finer spatial scales which determine the rules of species community composition (Götzenberger et al. 2012, Biswas et al. 2016). At the broadest scale, species are filtered by a phylogeographic assembly which is speciation, extinction, and migration of species (Zobel 1997, Belyea & Lancaster 1999, Swenson 2011, Lambers et al. 2012, Götzenberger et al. 2012). It forms different regional species pools (Pärtel et al. 1996) from which species are selected by dispersal assembly which could be influenced by landscape arrangement and the distance among suitable habitats (Houseman & Gross 2006, Helm et al. 2014). The results of the effect of dispersal assembly are local species pools from which species are filtered out by biotic and abiotic assembly (Zobel 1997, Götzenberger et al. 2012) and form the actual species pool. The approach of hierarchical filtering definitely interconnected the concept of assembly rules with the species pool hypothesis.

The species pool hypothesis was first presented by Taylor et al. (1990) who used this theory to explain a local diversity of communities based on differences of habitats of various historical and regional areas. Nevertheless, the term of species pool was already used in the theory of island biogeography (MacArthur & Wilson 1963) as a set of species which are able to reach an investigated island and subsequently colonise it. Graves & Gotelli (1983) considered the definition of species pool as species present in a biogeographical area insufficient and they suggested to define a species pool of every bird community of the investigated island separately (i.e., local species pool). Eriksson (1993) highlighted the importance to study on which distance species potentially occupying particular community are able to disperse because the probability, that species would pass through the

community filter increases with the number of propagules entering into community which is related to the number of propagules produced by surrounding communities (Vellend 2010, Lepš 2013). The absence of particular species in a community thus can be a consequence of the inability of potentially colonising species to produce a sufficient number of seeds able to pass through the community filter (Vítová & Lepš 2011). Consequently, the species pool definition was developed from species present in the community to species potentially able to occur in a particular habitat (Houseman & Gross 2011, Cornell & Harrison 2014, Moor et al. 2015, Karger et al. 2016). De Bello et al. (2012) suggested functional species pool defined as a set of species functional traits and thus species pool could be assessed for each habitat independently including all potentially co-existing species driven by abiotic processes and dispersal filter. Lessard et al. (2012) also highlighted the need to define species pool independently for each community and in addition, they pointed out to studies which have ignored species that could potentially disperse into the target communities although they are not present there.

With development of such definition of species pool, researchers started to be interested more and more in species absent from a focal community even though these species have a potential to establish there (i.e., they are present in the regional species pool), and the environmental requirements of a particular habitat are appropriate (i.e., they should be able to pass through the environmental filter). These species were termed as “dark diversity” (Pärtel et al. 2011). Pärtel (2014) supplemented the problematic of dark diversity by species which are present in the community but were not noticed because they are for example very rare or in dormant stages (i.e., “hidden diversity”) and other absent species which in contrast to species from the dark diversity completely differed by their ecological requirements from the investigated habitat. Many studies have attempted to estimate the size and composition of dark diversity because it is not possible to observe

it directly (Lewis et al. 2016, de Bello et al. 2016, Moeslund et al. 2017, Brown et al. 2019, Carmona & Pärtel 2020). Nevertheless, all these methods are based on the observational data only which reflect the effect of both the abiotic and biotic filter on the level of environmental filtering (Cadotte & Tucker 2017). In theory, environmental filter should exclude only species which are not able to survive under specific environmental conditions of the given site (i.e., species affected by abiotic filter) and not also species which are able to withstand these abiotic conditions but are absent from a community because of failure in competition with other species present in the given site (i.e., species affected by biotic filter; Butaye et al. 2001). Without experimental approach and with observational data only, it is very difficult or even impossible to separate the effect of the abiotic and biotic filter and thus absence of many species can be wrongfully attributed to the abiotic filter. We focused on the experimental assessment of species pool and the determination of the species affiliation to the suitable habitat in Chapter 3 (and subsequently together with the other questions also in Chapter 4 and 5). Although that the research of the dark diversity has contributed significantly to the development of species pool perception, the concept of species pool hypothesis is not up to now definitely settled and there are many methods of species pool determination which can influence the set of species belonging to species pool and thus also the results of our research. In Chapter 5, we compared four different methods of species pool assessment as possible predictors of the real species survival tested experimentally to suggest which of these methods is the most appropriate to determine species pool.

## Species pool assessment

There are three main groups how to determine species pool. The first group (1) is based on the similarity of the composition of target community with ecological preferences of the focal species. Within this group, three approaches are used. The first one (1a.) is based on the phytosociological knowledge of local experts establishing the extensive lists of species and habitat types (Sádlo et al. 2007, Jiménez-Alfaro et al. 2018). The second approach (1b.) is based on ecological preferences of individual species, known as indicator values, determined for specific areas (e.g., Ellenberg et al. 1991 for western part of central Europe, Zarzycki et al. 2002 for Poland, Hill et al. 2004 for British Islands, Pignatti et al. 2005 or Guarino et al. 2012 for Italy, Landolt et al. 2010 for Switzerland and Alps, Didukh 2011 for Ukraine, Chytrý et al. 2018 for the Czech Republic). The third approach (1c.) is based on co-occurrence patterns usually derived from large phytosociological databases. One of the most widely used co-occurrence methods is Beals index (Beals 1984, Ewald 2002, Münzbergová & Herben 2004, Botta-Dukát 2012, Lewis et al. 2016). Beals index is the expression of probability (values from zero to one) that a species is able to live in a particular community without the condition that it has to occur there in a reality (Münzbergová & Herben 2004). The calculation is based on comparison of co-occurrence of examined species with other species in the focal community in a reference database (containing many phytosociological relevés). Thus, we use information about multivariate structure of the real data which are based on neither any environmental gradient nor any other classification and get a species pool with values of species occurrence probability (Ewald 2002, Botta-Dukát 2012). Unfortunately, Beals index is not optimal for rare species because it positively depends on species frequency in a region and thus rare species tend to get values very close to 0 regardless to their real suitability to the target habitat (Lewis et al. 2016, Carmona & Pärtel 2020). This problem could be

solved by special corrections (Münzbergová & Herben 2004, Real et al. 2006) or we can use other, quite novel, probabilistic models based also on species co-occurrence but for which the problem of species frequency seems not to be so serious. One is based on an unconstrained ordination using species and plot position along multivariate axes (UNO; Brown et al. 2019). Second uses the randomly expected co-occurrence given by the mean value of the hypergeometric distribution to compare it with the observed co-occurrence of each pair of species (Carmona & Pärtel 2020).

The second group (2) of methods of species pool assessment is based on species functional traits (Sonnier et al. 2010, de Bello et al. 2012, Moor et al. 2015). Functional traits are species characteristics which should be possible to measure on an individual, not only estimated from the species ecological behavior as it is done for ecological characteristics (1 b.) (Westoby 1998). Functional traits are not dependent on the species distribution in landscape; thus, we are losing the generality at the cost of increasing explaining power (Cornelissen et al. 2003). However, also functional traits, same as all methods from the group 1, are based on realized niches of species (Violle & Jiang 2009).

All the above-mentioned methods of species pool assessment (both group 1 and 2) reflect both the abiotic and biotic factors acting in the communities because they are based on realized niches of species. It means that in this case, only species able to survive in the community and thus also withstand the competition with surrounding vegetation are a part of this species pool. However, it could cause us many problems, especially if we compare the actual community composition with this species pool in the case of differentiation of the effect of the biotic and abiotic filter. For this purpose, we need to separate the effect of the biotic filter from the complete community filtering (Butaye et al. 2001) and look for community composition which also includes species not able to withstand the competitive pressure in this

community. In this case, only possibility to find out the affiliation of investigated species in a tested species pool is experimental assessment (3), third group how to determine species pool. Although it takes a lot of time and efforts, the advantage of this approach is that we can experimentally extend a tested species pool by seed introduction or transplanting experiment (i.e., to exclude dispersal limitation of species added as seeds or transplants) and then observe the effect of the community filter which includes both the abiotic environment and the biotic interactions (Turnbull et al., 2000; Zobel & Kalamees, 2005). If we, in addition, experimentally remove the biotic filter (especially competition), we can compare the effect of the abiotic filter working in an artificial competition-free gaps with the effect of the biotic filter acting in the intact vegetation (HilleRisLambers et al. 2012). This question is treated mainly in Chapter 4 (but also in Chapter 3 and 5).

### **Abiotic and biotic filter in community assembly**

Ecological theory of species coexistence predicts how species should be functionally convergent or divergent to co-occur (Grime 2006, de Bello et al. 2013). While the abiotic environmental filter should select species with similar ecological adaptations (Zobel et al. 1998, Cornwell & Ackerly 2009), biotic filter can select either functionally different species, i.e. limiting similarity because of niche differentiation (Siefertt 2012, de Bello et al. 2012, Carboni et al. 2014) or functionally similar species because of competitive exclusion of species which do not have sufficiently competitive traits (Chesson 2000, de Bello et al. 2012, Schamp et al. 2016). Nevertheless, the abiotic and biotic filters are the essential factors driving the species community composition from the regional to local scales (de Bello 2012). Although the abiotic environmental filter and niche differentiation are often referred as distinct processes, they very often operate together and form the community composition (Breitschwerdt et al. 2015, Lhotsky et al. 2016). However, their relative importance is

very often unknown and difficult to disentangle (Araújo & Rozenfeld 2014, Kraft et al. 2015, Cadotte & Tucker 2017, Münkemüller et al. 2020). Although the pattern of trait convergence / divergence is frequently used to infer the relative importance of biotic and abiotic filters in the community, this approach is not straightforward and has many problems (Cadotte & Tucker 2017). It is based on null models which were defined by Gotelli and Graves (1996) as models generating patterns based on randomization of real ecological data when an observed assemblage is compared with the randomly simulated one. If an observed assemblage differs from the random one, we can conclude that there is an ecological mechanism influencing a target community. Nevertheless, the settlement of the criteria for determining the appropriate mechanisms could be very difficult (Fox 1999, de Bello 2012, Götzenberger et al. 2012, Zhang 2020) especially if we want to infer the relative importance of the biotic and abiotic filters in the community. For this task we need to separate the effect of biotic filter from the environmental filtering which is actually possible only by experimental approach and not on basis of null models with combination of observational data only. We focused on the problematic of the importance of the effect of the biotic and abiotic filter on the community assembly especially in Chapter 4 (but partially, this problem is discussed also in Chapter 3 and 5).

Competition which can cause complete exclusion of some species from the community (Palmer 1994) is considered as one of the most important biotic interactions shaping plant community composition (Butaye et al. 2001; Pärtel et al. 2013; Wellstein et al. 2014). Although the competition is considered one of the most important processes in assembly rules, it is not definitely determined if there is a higher effect of the above- or below-ground competition on community composition. Zobel (1992) supposed that the main factor decreasing the species richness of the local species pool is asymmetric competition. The above-ground competition for light which is really a

very asymmetric type of competition has thus studied very often (ter Heerdt et al. 1991, Zobel et al. 1996). On the other hand, the below-ground competition primarily for water and nutrients (Denslow et al. 1991) which is much less asymmetric can influence much more individuals occurring in a community and thus its effect can be much higher than the effect of the above-ground competition (Wilson 1988, Seager et al. 1992). Nevertheless, it is very difficult to separate these effects with experimental manipulations, and thus if at all, the below-ground competition is mainly studied together with the above-ground competition (McPhee & Aarssen 2001). Although it is not easy, it is important and very useful to attempt to study the importance of the effect of below-ground competition against the effect of above-ground competition. We attempted to reveal the importance of the below-ground competition in Chapter 5.

### **The aims of thesis**

In this thesis, I aimed to answer the questions related to which factors can influence a plant community assembly and what are the main drivers of plant species community composition. To answer these questions, I concentrated on an experimental approach which was compared with other possibilities of species pool determination and methods usually employed to reveal the effect of different factors on plant species community composition.

Firstly, in Chapter 2, we focused on analysing of 20 years long experiment sowing high- and low-diversity seed mixtures with the main aim to answer if founder effect of the initial species composition and the priority effects of the early arriving species are important determinants of plant species community composition and how long can last their legacy. In Chapter 3, we conducted a seed introduction experiment on an oligotrophic wet meadow with quite wide variety of species, especially non-resident species typical for different ecological conditions than a target locality, with the aim to reveal if these species



could be able to establish there if competition was removed. With this answer, it was possible to deliberate how should be determined a species pool which is employed to a comparison of realized vegetation composition for the purpose of determination of the effect of biotic filtering. Nevertheless, for disentangling the relative importance of the effect of the biotic and abiotic filtering on the plant species community composition, the multisite experiments with variation of abiotic conditions are needed. Thus, we conducted two other seed / transplant addition experiments, one in three different meadows with distinct plant species composition (Chapter 4) and second in four localities on a moisture and productivity gradient (Chapter 5). Removing the competition, we compared the importance of the biotic and abiotic effects on plant species establishment in different life stages of species with the aim to compare the importance of regeneration and realized niches for community filtering. We then compared the results from our experimental approach with other methods determining the species affiliation to plant species community, specifically with Beals index in Chapter 4, and with Ellenberg indicator values, functional traits, Beals index and UNO in Chapter 5 where one of the main aims was to find the best predictor of the real species survival from different estimation methods of species pool assessment. In Chapter 5, we also aimed to compare the importance of below- and above-ground competition on transplant survival.

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## **Chapter 2**

### **The legacy of initial sowing after 20 years of ex-arable land colonisation.**

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# The legacy of initial sowing after 20 years of ex-arable land colonisation

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## Abstract

Priority effects provide an advantage to early establishing species, and are thought to significantly affect the course of succession. We conducted a 20-year long experiment sowing high- and low-diversity mixtures in an ex-arable field. We ask how long the effect of sowing persists and which sown species affect the course of succession. The experiment was established in the Czech Republic in five replicate blocks, each containing three random 10 × 10 m plots with three treatments: natural colonisation, sowing low- and high-diversity seed mixtures. The species cover was annually estimated in 12 permanent 1m<sup>2</sup> quadrates within each plot. To identify the effects of sowing, we used an innovative method analysing the data separately for each year using Redundancy analysis (RDA) with identity of sown species as explanatory variables. In the first year, the effect of sowing was small; the peak of explained variability occurred between third and fifth year. The legacy of sowing was detectable in the natural colonisers for 18 years and in the sown species for the whole 20-year period. For some species the difference between the plots where they were and were not sown remained significant for the whole 20- year period (e.g. *Lathyrus pratensis*) although the plots were adjacent and the area was mown with the same machine. Other ones (e.g. *Trisetum flavescens*)

colonised all the plots evenly. The long-lasting effect of the initial sowing confirms contingency of successional pathway on the propagule pressure in the time of start of succession due to the priority effects.

### **Keywords**

Founder effect, Initial composition, Long-term experiment, Old-field succession, Priority effect.

## Introduction

Secondary succession on abandoned land has long been a favoured model for ecological studies (Rejmánek and van Katwyk 2004). Vegetation development in ex-arable fields is characterised by a high species turnover and tends to lead to grasslands or forests depending on environmental conditions and management (Hansson and Fogelfors 1998; Harmer et al. 2001). However, the development could be limited by lack of available diaspores.

Secondary succession is significantly affected by propagules initially present and by propagule pressure from the surrounding area (Lanta and Lepš 2009). Reaching the target habitat is the first requirement for the occurrence of a species there (Schamp et al. 2016). The process depends on the number of propagules in the source habitat, on the distance between the source and target habitat and also on the presence of appropriate seed dispersers (Vellend 2010). Many grassland species are not capable to disperse over long distances (Kiehl et al. 2010) and thus dispersal limitation is an important obstacle in grassland restoration. Adding diaspores to the target habitat can therefore accelerate the process (Prach et al. 2015).

Clements (1916) suggested that species of earlier successional stages pave the way for late successional species. Nevertheless, this relay floristic theory was challenged by Egler's (1954) initial floristic composition model suggesting that vegetation development depends on the species which reached the target habitat first. Although there are many ambiguities in interpreting Egler's initial composition model (Wilson et al. 1992), the question how much the initial species composition affects the course of succession is important for both ecological theory and practical restoration.

It has been suggested that species reaching a habitat first have a significant effect on species later arriving (Körner et al. 2008; Plückers et al. 2013), a phenomenon known as the priority effect (Drake 1991; Facelli and Facelli 1993), causing the historical contingency of

community assembly (Chase 2003; Martin and Wilsey 2012; Fukami 2015). However, the impact of the priority effect can persist a rather long time, apparently longer than contemporary studies have demonstrated (Fukami et al. 2005; Martin and Wilsey 2012; Fry et al. 2017). Urban and de Meester (2009) suggested that if the early arriving species has enough time to adapt to local conditions, the priority effect is stronger because this better adapted competitor will exclude the later arriving colonist.

Both the effect of initial species composition and sequence of species arrival can be examined by seed addition experiments (Körner et al. 2008; Plückers et al. 2013). Whereas the short-term effects of sowing are obvious and expected, there is a very limited number of studies providing long-term perspectives concerning the effect of sowing (nine years in Bezemer and van der Putten 2007; 25 years in Pakeman et al. 2002). These long-term effects are of basic practical importance because the aim of restoration projects is rather maintenance of than temporal change in species composition and diversity. Studying the effect of initial vegetation composition and assembly history thus help both in habitat restoration (Kiehl et al. 2010; Fry et al. 2017) and invasive species prevention (Lang et al. 2017).

We used data series from a Biodiversity – Ecosystem function experiment established in 1996, originally investigating the effect of sowing high- and low-diversity seed mixtures as compared to natural colonisation on ecosystem functioning. The continuous 20-year data series enabled us to quantify the legacy of initial sowing. Specifically, we asked: (1) For how long can we detect the differences in cover of individual species between plots where the species were and were not sown? (2) Do particular sown species affect the composition of natural colonisers, and if so, how long does the effect persist? (3) For how long can we detect the effect of sowing on the total species composition (of both sown species and colonisers)? (4) Do traits of sown species predict species success? Finally, based on a single-time sampling 20



years after the start of the experiment, we checked whether there was any effect of particular species on soil characteristics.

### **Material and methods**

Originally, the same experiments were established in five different European countries as part of the CLUE project (Changing Land Usage: Enhancement of biodiversity and ecosystem development) with the aim to investigate the diversity effect on ecosystem functioning. The basic results are presented in van der Putten et al. (2000), Lepš et al. (2001), Hedlund et al. (2003) and Lepš et al. (2007). In this paper, we present the vegetation development at the Czech Republic site over the first 20 years.

### **Study site**

The experiment was established in Benešov, Czech Republic (N 49°19.9', E 15° 0.3', 659 m a.s.l.) in spring 1996 on an ex-arable field where the last crop, barley *Hordeum vulgare*, was cultivated in 1995. It had been alternated with potatoes *Solanum tuberosum* and pea *Pisum sativum* in the past. The experimental site is surrounded by arable land and recently established species-poor grasslands. The mean annual temperature of this site is 6.4 °C and the average rainfall amounts to 680 mm per year. Both the warmest and the wettest month is July (mean temperature 16.4 °C and average precipitation 78 mm). The coldest month is January (-2.7 °C) and the driest one is February (36 mm). The bedrock is paragneiss, which is covered with brown loamy soil. Starting the experiment, the content of organic matter in soil was 4.88%, the total N in 100 g of soil was 1538 mg, available P in 100 g of soil was 24.3 mg and pH was 5.88 (van der Putten et al. 2000).

## Design of the experiment and data recording

The experiment was carried out in five randomised blocks, each containing three  $10 \times 10$  m plots with the following treatments: 1) natural colonisation without any sowing (NC), 2) low-diversity seed mixture with four species sown (LD), and 3) high-diversity seed mixture with 15 species sown (HD). The plots were located in a continuous grid with 2 m wide aisles between the plots. The area was mown annually, twice a year, in June and October (in dry summer years the autumn mowing was omitted), with the same mowing machine used over the whole experimental site (enabling seed dispersal among individual plots in the experiment). Regular mowing was introduced to direct the secondary succession toward an extensively managed meadow. Sown species were selected respecting their participation in typical extensively managed meadows in the area. The selection was further restricted by the condition of the multisite CLUE experiment that two grasses, two legumes and one other forb should be common to all five countries (van der Putten et al. 2000).

For the HD treatment, five grasses (*Cynosurus cristatus*, *Festuca rubra*, *Holcus lanatus*, *Phleum pratense*, *Trisetum flavescens*), five legumes (*Lathyrus pratensis*, *Lotus corniculatus*, *Medicago lupulina*, *Trifolium dubium*, *Trifolium pratense*) and five other forbs (*Centaurea jacea*, *Galium verum*, *Lychnis flos-cuculi*, *Plantago lanceolata*, *Prunella vulgaris*) were sown in each plot. The density of each sown species in HD plots was 500 seeds / m<sup>2</sup> for grasses and 100 seeds / m<sup>2</sup> for both legumes and other forbs. For the LD treatment, four species were sown (a subset of the 15 species sown in HD): two grasses in a density of 1250 seeds / m<sup>2</sup> for each species, the one legume and the other forb both in densities of 500 seeds / m<sup>2</sup>, resulting in total densities of 2500 sown grass seeds / m<sup>2</sup> and 500 other species seeds / m<sup>2</sup>. Species composition of the LD plots differed between blocks (Table 1), enabling an assessment of the effect of particular sown species on the course of succession (Huston 1997). NC plots served as controls left to

be colonised naturally (with time increasingly also from the sown plots of the experiment).

In each 10 × 10 m plot, cover of each species was visually estimated in 12 permanent 1 × 1 m subplots every year from 1996 to 2016 at the peak of the vegetation season (i.e. in June). Although dozens of experts participated in the vegetation sampling, JL was present in all the years assuring consistency of estimates. In 2007 and 2008, only even-numbered subplots were sampled (providing thus only 6 subplots per plot). Data from 2006 are unavailable. The plant nomenclature is according to Kubát et al. (2002).

Table 1: Species combinations sown in low diversity seed mixture treatments. Five combinations of species (LD1 - LD5) were sown in the experiment.

Treatment	Sown species
LD1	<i>Phleum pratense</i> , <i>Holcus lanatus</i> , <i>Trifolium pratense</i> , <i>Centaurea jacea</i>
LD2	<i>Cynosurus cristatus</i> , <i>Trisetum flavescens</i> , <i>Medicago lupulina</i> , <i>Prunella vulgaris</i>
LD3	<i>Trisetum flavescens</i> , <i>Festuca rubra</i> , <i>Lathyrus pratensis</i> , <i>Lychnis flos-cuculi</i>
LD4	<i>Holcus lantus</i> , <i>Cynosurus cristatus</i> , <i>Trifolium dubium</i> , <i>Galium verum</i>
LD5	<i>Festuca rubra</i> , <i>Phleum pratense</i> , <i>Lotus corniculatus</i> , <i>Plantago lanceolata</i>

### Soil samples

Soil was sampled in late June 2016. In each plot, a topsoil core (0–10 cm depth) was collected using a split-tube sampler (5 cm diameter). The soil samples were oven-dried at 100 °C, ground to fine powder and sieved to a fraction of < 2 mm after removal of the roots. Ammonia, nitrate and total nitrogen were determined colorimetrically after Kjeldahl mineralisation using an automatic FIAstar 5010 Analyzer (Tecator) at the Analytical laboratory of the Institute of Botany, Czech

Republic. Phosphorous was determined colorimetrically after digestion in  $\text{HClO}_4$  using a SHIMADZU UV - 1650PC spectrophotometer. Also other physico-chemical parameters were measured: pH, water content, organic matter content (OM) and texture (fraction of particles  $> 0.5$  mm in diameter).

### **Data analysis**

To answer the questions how long the effect of sowing and initial composition can persist and which species affect the course of succession the most, we used RDA (Redundancy analysis, in CANOCO 5, ter Braak and Šmilauer 2012) with centring and no standardisation by species nor by samples (i.e. RDA on a covariance matrix). RDA was carried out for each sampled year separately. In the version with a single predictor and single response variable, the RDA is equivalent to simple regression. We used this analysis to test whether the sowing effect still persists for individual species, i.e. whether the individual species cover is still higher in plots where it was sown (use of RDA in CANOCO enabled to perform a permutation test reflecting the hierarchical design of the experiment, in the same manner as for the analyses of species composition, see below). Species sown in individual plots were used as explanatory variables – the values were 0 if the species was not sown in the plot, 1 if the species was sown at low density, i.e. in the HD plots, and 2 at high density, i.e. in LD plots. The values 1 and 2 were selected arbitrarily (the sowing densities in LD were 2.5 times higher for grasses and 5 times higher for legumes and other forbs, but the establishment did definitely not proportionally increase in the LD plots). Species composition (cover estimates) of 1) only unsown, 2) only sown and 3) all species (i.e. both sown and unsown species recorded in sampling) were the response variables in the RDA analyses. To find out which sown species were still detectable in the species composition, we applied forward selection (which thus selected the species of which the effect is still detectable). Nevertheless, the maximum number of selected species was a priori six

because of seven different sown species combinations (i.e. NC, HD and LD1–LD5). Thus, after selecting six species, no explainable variability remained, and thus no more species could be selected. However, the selected species were the most influential ones. A problem arose with pairs of species (legume and other forb), which were always sown together (and thus perfectly collinear): *Trifolium pratense* and *Centaurea jacea*, *Medicago lupulina* and *Prunella vulgaris*, *Lathyrus pratensis* and *Lychnis flos-cuculi*, *Trifolium dubium* and *Galium verum*, *Lotus corniculatus* and *Plantago lanceolata*. These species thus always have the same predictive power in the forward selection. In these cases, the species with higher average cover in all plots in individual years was selected. In addition, the changes of the average cover of individual sown species in HD, LD (taking into consideration only plots where individual species were sown) and NC over the 20 years of experiments are displayed to show the dependence of their dynamics in the sowing.

For all analyses, the significance was determined by a permutation test with a hierarchical design of permutations reflecting the individual quadrates being split-plots in the main  $10 \times 10$  m whole-plot. We permuted only the whole-plots (because these  $10 \times 10$  m plots are our independent observations), see Šmilauer and Lepš (2014, p. 79) for details of hierarchical permutations. In forward selection, the species were selected if  $p < 0.05$ . We did not use any adjustment for multiple tests. We used this approach to test the hypotheses we are interested in. The global null hypothesis (i.e. that the sowing had no effect over the whole course of succession) is patently unrealistic: in the first year(s) after sowing, the species established mainly in the plots where they were sown. Similarly, the differences between sown and unsown plots changed with time (so there must be significant interactions of sowing with time), as the secondary colonization appeared mainly in the unsown plots. On the contrary, it is clear that the sown species will spread also to the unsown plots and not all the

sown species will survive for the whole 20 years period. We were thus specifically interested for each individual year effect of sowing of which species is still detectable, and we designed our analyses to answer this question. All the p-values thus report the comparison-wise Type I error rates. To exclude the possibility that the significant results are a consequence of Type I error, we complemented these analyses with a global test for the whole period. We have used Principal Response Curves analysis (Šmilauer and Lepš 2014, p. 167, which is a multivariate counterpart of Repeated measurements analysis) followed by year by year analyses, both testing the differences between NC and HD plots (because these are the two “homogeneous treatments”, LD affects species composition according to the species sown in individual blocks). As these analyses can only demonstrate that the sowing affects the species composition but not which species (our main task), we present these analyses in the Online Supplementary Material 1 only.

We characterised the strength of the relationship by the explained variability, with both all the explanatory variables (i.e. by the complete sowing design) and the selected variables (sown species), and then by the number of species selected as influential. Theoretically, if a species has a significant effect on the composition of either sown or unsown species, it must have some effect on the total species composition as well. Nevertheless, by pooling the two groups of species, we changed the power of the test, by which different species might appear as influential. Consequently, we present the results of all three analyses.

Further, we attempted to predict the success of individual sown species by means of their traits. For this purpose, we calculated several characteristics of success for each sown species. First of all, these were frequency (proportion of 1 m<sup>2</sup> subplots where the species was present) and average cover. These two characteristics were calculated for plots where the species was sown and for all the plots, using data of the tenth and the twentieth year after the start of the experiment. They show the overall success of the species. Finally, we calculated the distinction

score for each species, i.e. the number of times that the species was selected as a significant predictor – indicating the differences between plots where the species was and was not sown. Then, we tested whether these values can be predicted using four sown species traits, canopy height determining competitive ability, seed mass related to reproductive ability, specific leaf area (SLA) associated with competition, and plant growth strategy (all three from the LEDA database, Kleyer et al. 2008), and additionally lateral spread (with exclusion of freely dispersible organs) determining asexual reproductive ability from the CLO-PLA 3.3 database (Klimešová et al. 2017) (Online Supplementary Material 2).

Soil characteristics were tested by forward selection analysis using the densities of sown species as explanatory variables and measured soil characteristics as response variables.

## Results

### General dynamics of unsown and sown species

All the plots underwent a typical secondary succession on abandoned fields, with the first year being dominated by typical arable weeds (e.g. *Elymus repens*) and by *Trifolium repens*, spreading vigorously through the whole locality, with many annuals (e.g. *Veronica arvensis*, *V. persicifolia*, *Cerastium fontanum*, *Capsella bursa pastoris*). The sown species established already in the first year, but their proportion was low, and they mostly started to be a significant part of the community from the second year on. During the early phases, the *Taraxacum officinalis* (sect. *ruderalia*) established and kept relatively high cover during the whole 20 years. Then, the plot was completely dominated by perennials, including some typical meadow grasses (*Arrhenatherum elatius*, *Dactylis glomerata*) that were not sown and established spontaneously. The PRC analysis shows significant differences between the NC and HD plots (Online Supplementary Material FigA 1 and FigB 1). The year by year analysis shows that for all the species,

the differences were significant throughout the experiment whereas the effect of sowing on the unsown species was detectable only till 2013 (Online Supplementary Material FigC 1). Despite these differences, the dynamics of natural colonizers was similar in all the plot types (Online Supplementary Material 5). The dynamics of sown species differed considerably among individual species (Fig. 1 and Online Supplementary Material FigA 4), and at least some effects of the initial sowing persisted in the plant community composition for the entire period of the experiment, i.e. for 20 years.

### **Differences in sown species cover between sown and unsown plots**

The cover values of *Lathyrus pratensis*, *Lotus corniculatus*, and *Centaurea jacea* were significantly affected by their sowing during the entire experiment (Table 2). These species remained in plots where they were sown and did not disperse very much elsewhere (Fig. 1 and Online Supplementary Material FigA 4). Four other species (*Prunella vulgaris*, *Cynosurus cristatus*, *Plantago lanceolata* and *Phleum pratense*) showed a detectably increased cover in plots where they were sown over 17 or more years out of 20 (Table 2). The cover of *Trisetum flavescens* differed between sown and unsown plots only in the first seven years, with the cover for the LD plots and HD plots being nearly the same. Afterwards, *Trisetum flavescens* dispersed also into NC plots (nearly exponential increase till the seventh year) and differences in cover between sown and unsown plots disappeared (Fig. 1a). *Lathyrus pratensis* dominated the plots where it was sown (Online Supplementary Material 6) but nearly did not disperse into unsown plots during the whole period – its cover was higher in LD than in HD plots for first 10 years, in the second half of the experiment the differences due to the sowing density disappeared (Fig. 1b). *Holcus lanatus* achieved high cover in LD plots during the first seven years, whereas in HD plots its cover remained low and the species subsequently decreased and remained low in all the plots for several years. Nevertheless, from 2012, it started unexpectedly increase in all



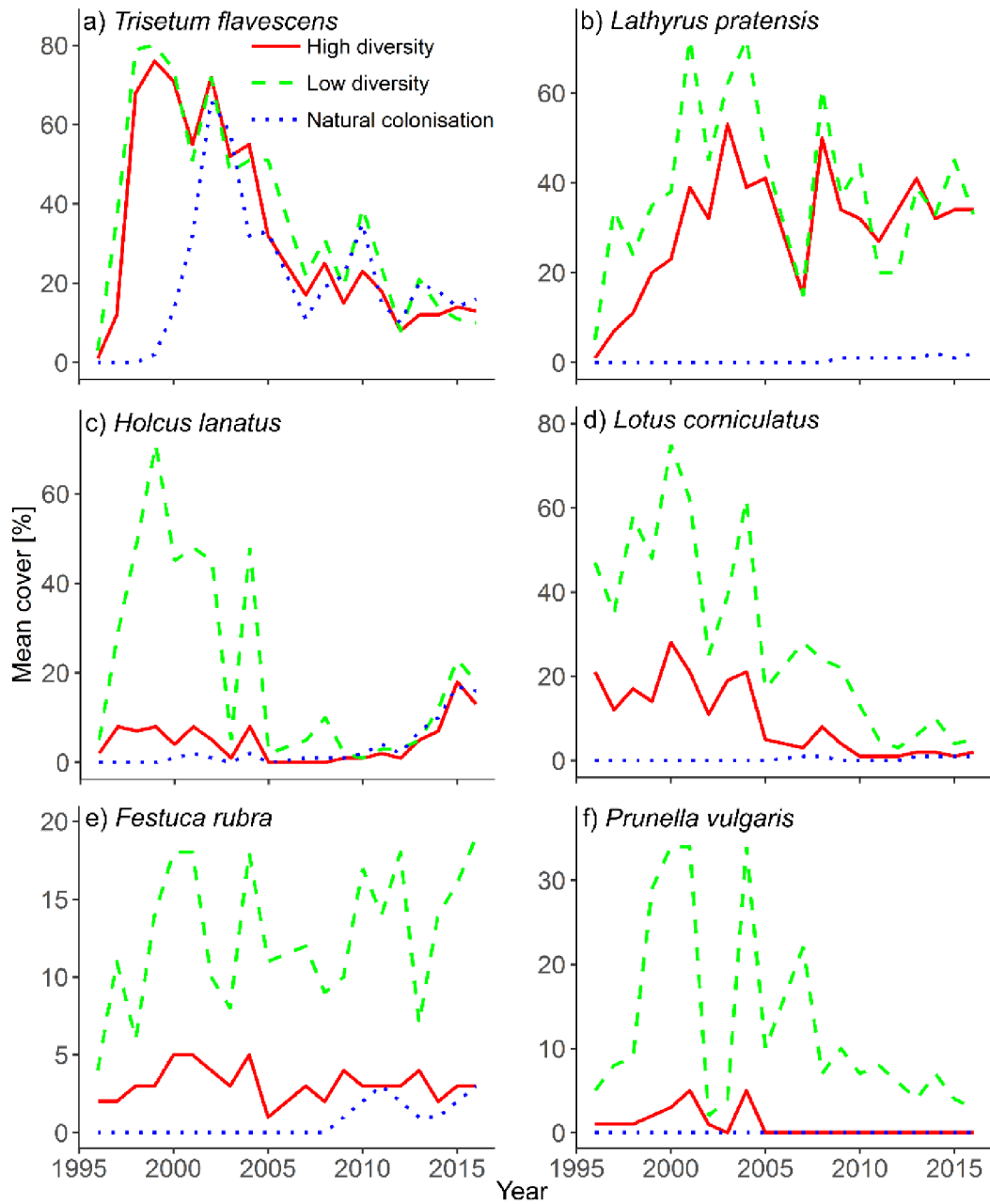


Fig. 1: Average cover of six sown species between 1996 and 2016 for high and low diversity seed mixtures plots where species were sown and natural colonisation plots. Other nine sown species are represented in Online Supplementary Material FigA 4. Y-axes scale differs for each panel. Color version of the figure is available online.

Table 2: Number of years when a sown species had a significant influence on themselves in regression of cover of each sown species and its densities in each year individually (SP) and on the remaining species after forward selection in the Redundancy analysis (RDA) in each year using cover of only unsown, only sown and all species as response variables. Detailed results for individual years are represented in Online Supplementary Material 3.

Species	Years			
	SP	Unsown	Sown	All
<i>Centaurea jacea</i>	20	0	2	1
<i>Cynosurus cristatus</i>	17	1	5	6
<i>Festuca rubra</i>	14	4	8	7
<i>Galium verum</i>	9	0	0	0
<i>Holcus lanatus</i>	12	7	9	9
<i>Lathyrus pratensis</i>	20	9	20	19
<i>Lotus corniculatus</i>	20	0	11	10
<i>Lychnis flos-cuculi</i>	1	0	0	0
<i>Medicago lupulina</i>	4	0	0	0
<i>Phleum pratense</i>	18	3	5	3
<i>Plantago lanceolata</i>	17	0	7	4
<i>Prunella vulgaris</i>	19	0	10	9
<i>Trifolium dubium</i>	8	0	2	5
<i>Trifolium pratense</i>	7	5	4	3
<i>Trisetum flavescens</i>	8	5	12	9

sown and unsown plots (Fig. 1c). Dispersion of *Lotus corniculatus* to unsown plots was rather low and its cover was higher in LD than HD plots, but the species tended to disappear completely toward the end of experiment (Fig. 1d). *Festuca rubra* established well at the beginning, and its cover roughly corresponded to sowing densities, i.e. was higher in LD plots. It was absent from the NC plots for more than ten years, and started appear to grow there only after 2008 (Fig. 1e). *Prunella vulgaris* established well and achieved high cover in LD plots only. In HD plots, it appeared with low cover in the first years and then

disappeared. It never dispersed into NC plots (Fig. 1f). The trends in other sown species were less conspicuous and are thus shown in Online Supplementary Material FigA 4. Nevertheless, we can say that there are no general patterns of sown species behaviour during the 20 years, and the dynamics of each species is idiosyncratic, but in some species, the legacy of sowing is detectable also after 20 years.

### **The effect of sowing on the community composition**

Accordingly, the community composition analysed by RDA detected significant sowing effects on the community composition. Sown species increased their effect on both unsown and sown species composition during the early years, with the maximum of explained variation in the fifth year for unsown species (Fig. 2a) and in the third year for sown species (Fig. 2b). The explained variation in the unsown species cover was considerably lower than that in the sown species (compare Figs. 2a and 2b). Also, the effects of sowing on the unsown species were detectable until 2013, i.e. for 18 years (Fig. 2a), while significant differences were observed for some sown species for the entire period of our experiment (Fig. 2b). In 2013, the composition of unsown species was significantly influenced by only one species, *Lathyrus pratensis* (pseudo-F = 17.3,  $p = 0.015$ ) but since 2014 no effects have been detected (Online Supplementary Material 3). On the other hand, the composition of sown species was significantly influenced during all 20 years, even in 2016 (i.e. the last year of our experiment), by two species: *Lathyrus pratensis* (pseudo-F = 41.9,  $p = 0.015$ ) and *Festuca rubra* (pseudo-F = 18.7,  $p = 0.03$ ) (Online Supplementary Material 3).

The influence of sown species on the total species composition (i.e. considering both sown and unsown species cover as response) initially increased to the maximum of explained variation in the fifth year of the experiment and subsequently decreased (Fig. 2c), remaining significant until the end of the experiment (Online Supplementary Material 3).

Species with the most significant effect on the composition of remaining species was *Lathyrus pratensis* if considering the cover of unsown species only (9 times chosen by forward selection), sown species only (20 times selected) and all species (19 times selected) (Table 2). This species was dominant in plots where it was sown and did not disperse very much into other plots (Fig. 1b, Online Supplementary Material 6). The least successful species *Galium verum*, *Lychnis flos-cuculi* and *Medicago lupulina* did not have any significant effect on the composition of the remaining species (Table 2) because their establishment was poor (Online Supplementary Material FigA 4).

Even the species, that maintained differences between plots where they were and were not sown, differed considerably in their effect on other species. While sowing of *Lathyrus pratensis* significantly affected sown species during the whole time of our experiment and unsown species for nine years, sowing of *Lotus corniculatus* and *Prunella vulgaris* had a significant influence on sown species only (which however includes also the effect on its own population, Table 2). While *Lotus corniculatus* was quite frequent in the first half of our experiment, it later started to decrease (Fig. 1d) and thus also its influence on the remaining vegetation declined (Online Supplementary Material 3). The cover of *Phleum pratense*, *Cynosurus cristatus* and *Plantago lanceolata* differed considerably in the plots where they were sown but just had a weak or no effect on the remaining species (Online Supplementary Material 3). These species did not dominate the vegetation and dispersed slightly, especially *Phleum pratense* (Online Supplementary Material FigAa 4). Although *Festuca*

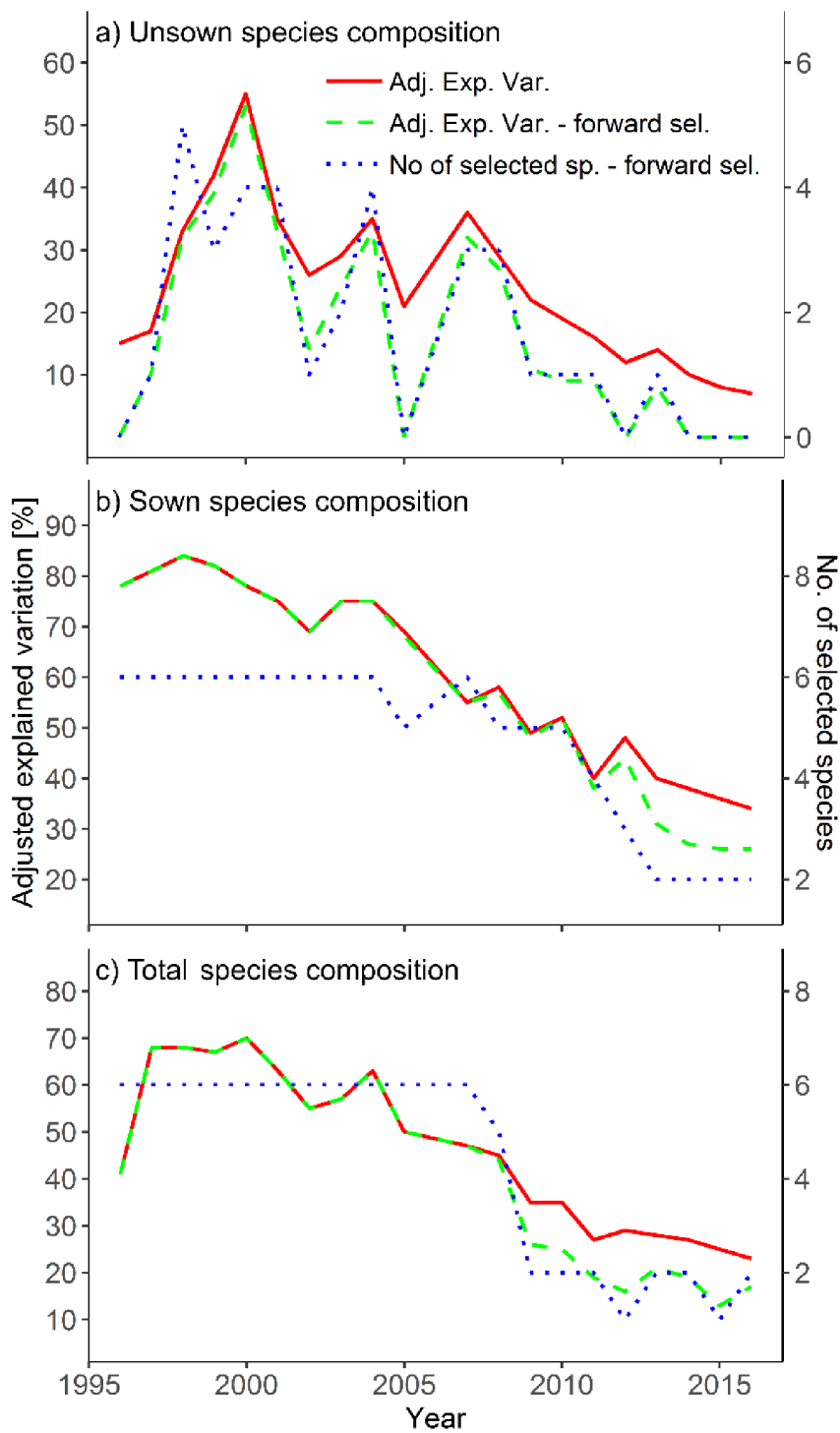


Fig. 2: Results of Redundancy Analysis (RDA) of a) unsown species composition, b) sown species composition and c) total species composition (i.e. both sown and unsown species) between 1996 and 2016. The adjusted explained variation (Adj. Exp. Var.) explained by the complete sowing design and by the species selected in forward selection and the number of selected species are shown. Color version of the figure is available online.

*rubra* was not dominant in character and only had a weak ability to disperse to plots where it was not sown (Fig. 1e), it had a significant effect on the remaining species at least in some years (Table 2). *Trifolium pratense* and *Trifolium dubium* were not dominant in the vegetation (with the exception of *Trifolium pratense* in 1997) and did not disperse very much into unsown plots (Online Supplementary Material FigA 4). *Holcus lanatus* and *Trisetum flavescens* could be called dominants at least for the first half of the time of our experiment, and *Trisetum flavescens* also dispersed very well to all the plots of our experiment (Fig. 1).

### **Species traits and soil characteristics**

Neither the frequency nor the average cover or the difference between sown and unsown plots of sown species (Table 2) correlates with any examined functional species traits (Online Supplementary Material 2).

No sown species had a significant effect on soil chemistry. The only trend we noticed was a very weak effect of *Lathyrus pratensis* on the nitrogen content of the soil. The effect was significant if only nitrogen (i.e. total nitrogen, N-NH<sub>4</sub> and N-NO<sub>3</sub>) was considered as a response variable (pseudo-F = 9.2, p = 0.042).

### **Discussion**

We found that the sown species composition affected the course of succession in the ex-arable field during the entire 20-year period. The only seed mixture sowing experiment of comparable length is Pakeman's grassland experiment, suggesting that initially sown species can influence the course of succession even 25 years after their establishment (Pakeman et al. 2002), other experiments provide evidence of the effect after eight (Fry et al. 2017), nine (Fukami et al. 2005) or 10 years (Mitchley et al. 2012). Nevertheless, most experiments with sowing seed mixtures are relatively short-term investigating the effect of sown mixtures on community assembly for two (Valkó et al. 2016), three (Sengl et al. 2017), four (Plückers et al.

2013) or five years (Martin and Wilsey 2012). As we have continuous time series, we were able to show that the identity of “influential” species, but also how the strength of the effect changes (mostly decreases) with time; it is thus very difficult to infer the long-term effects of initial species composition from short-term studies.

Our experiment was originally established with two aims (van der Putten et al. 2000): the first one was testing hypotheses about the relationship between diversity and productivity, typical of Biodiversity – Ecosystem function experiments. The other aim of the original experiment was to demonstrate the possibility of restoring meadows with higher species richness than intensively managed grasslands. Consequently, the sown species are typical meadow species, differing in their life history from typical natural colonisers starting spontaneous succession, mostly arable weeds.

In the first year of our experiment, the effect of sown species on the remaining vegetation was negligible, since fast-growing weeds mostly from the seed bank (Dutoit et al. 2003) dominated the vegetation in all the treatments. From the second year, we recorded a sharp increase in the effect of sown species on the remaining vegetation with the maximum effect between the second and fourth year. The replacement of fast-growing ruderal annuals by competitively strong perennial plants is typical of old-field succession (Hansson and Fogelfors 1998; Prévosto et al. 2011), even though the detailed mechanisms of competitive superiority might differ (Fry et al. 2017). We accelerated the process by adding diaspores of competitively strong meadow species, mostly perennials.

Our results support Egler’s initial floristic composition (Egler 1954) in the sense that species added to the community at the start of the succession determined significantly its course. Nevertheless, some species, namely *Holcus lanatus* and *Trisetum flavescens* achieved a high cover in plots where they were not sown after the period of their highest cover in sown plots (Fig. 1). Provided there is a sufficient

diaspore influx (here from the neighbouring sown plots), at least some species established and reached a high cover in the communities where they were initially absent, even though other species had already established there (as predicted by the model of Fibich et al. 2018).

Although all the plots were placed adjacent to each other, with two-metre wide aisles, and the area was mown with the same machinery, significant differences for some species between plots where they were and were not sown remained for 20 years. The differences need not necessarily be caused by low dispersal ability only, but also by the priority effects causing the inability of new-coming species to establish from seeds in competition of mature individuals of species established earlier, i.e. due to absence of their regeneration niche (Grubb 1977; Švamberková et al. 2017). While the effect of sown species on unsown species was noticeable until 2013 in our experiment, their effect on only sown species was apparent in all the years. This means that the time during which sown species are able to suppress the establishment of non-target species is shorter than their persistence at the site. From restoration point of view, the unwanted species often include early successional species, mostly arable weeds. Creating a priority effect of target species could be very effective in preventing their invasion (Fry et al. 2017).

There are different opinions of the importance of sowing in grassland restoration. For example Prach et al. (2015) suggested that spontaneous establishment is the best option in grassland habitat restoration. On the other hand, they also admitted that sowing of regional species mixtures accelerates restoration especially in the first years. Our results suggest that for some grassland species, a distance of twelve metres (difference in the centres of neighbouring plots) is sufficient to keep the difference between sown and unsown plots for twenty years. Our experiment was not established to test the persistence of the effect of sowing particular species, and thus its design is not ideal for this purpose. However, we are not aware of any



20-year long series with a more appropriate design. We used in all the analyses as explanatory variables the composition of the sown species (i.e. the manipulated variable), so that the statistically proved effects of sowing signify causality. Still, we should be aware of the limitations posed mainly by the collinearity in sown species as explanatory variables. The least affected is the persistence of the differences between plots where individual species were and were not sown. The only possible spurious effects can be found in LD plots, where individual species were mixed with other species and thus might have been under varying competition pressure. Because the natural colonisers were at the start more abundant than the sown species, the effect of other sown species on the initial establishment is probably small. The collinearity problem is slightly more serious for the effect of particular sown species on natural colonisers. The effects of pairs of legume and forb species, which were always sown together, are principally indistinguishable – we thus used the rule that the more abundant species is the affecting one. However, even in these pairs, the results are quite reliable – species from three pairs, *Prunella vulgaris* and *Medicago lupulina*, *Trifolium dubium* and *Galium vernum*, and *Lotus corniculatus* and *Plantago lanceolata*, were never selected as affecting the unsown species. From the other two pairs, *Trifolium pratense* was, unlike *Centaurea jacea*, able to achieve dominance (although only in the first half of the experiment). Of the last pair, *Lathyrus pratensis* reached dominance in plots where it was sown, and did not spread much outside these plots, and clearly affected natural colonisers (Online Supplementary Material 6), whereas the establishment of *Lychnis flos-cuculi* was very poor, never reaching a level of abundance at which it would be able to affect other species. The effects on sown species are most problematic, because the identity of each sown species determined from the very beginning which other species were sown. Nevertheless, even if the selection of influential species suffers from the collinearity problems, the fact that we have

found significant results even 20 years after sowing unequivocally demonstrates a long-lasting legacy.

For being detected as influential on remaining vegetation, sown species have to be dominant in a community and have to remain in the sown plot and not disperse very much. This was typically the case of *Lathyrus pratensis*. On the contrary, *Trisetum flavescens* had a very high cover during the entire experiment but had no statistical impact on the remaining vegetation, especially in the second half of the experiment, because it dispersed almost everywhere. This different dispersal character could probably have been caused by a difference in seed weight (Online Supplementary Material 2); *Lathyrus pratensis* has heavy seeds, giving the lighter seeds of *Trisetum flavescens* higher dispersal ability (Cornelissen et al. 2003).

None of the investigated functional traits explained differences between sown species in their establishment success or in their effect on the remaining species composition. This means that it is not easy to determine a universal species trait combination defining which species will either travel over the entire locality or stay in a small patch because this species trait combination seems to be rather idiosyncratic. Also, each of the sown species exhibited different pattern of dynamics during the 20 years of the experiment. *Lathyrus pratensis* kept the difference between plots where it was sown (regardless whether it was sown in high density in LD or low density in HD, Online Supplementary Material 6), *Festuca rubra* became important toward the end of experiment only in LD plots, and just as accessory species in both NC and HD and *Trisetum flavescens*, showing the most pronounced differences between sown and unsown plots at the beginning in relatively short time equalized its cover in all the plot types. This clearly shows that the priority effects are important for some species, whereas they do not play any role for the others.

The long persisting influence of *Lathyrus pratensis* on remaining vegetation could also be explained by its N<sub>2</sub>-fixing ability, which

exempts it from competition for resources with other species (Drouin et al. 1996; Manninen et al. 2010). Although the effect of *Lathyrus pratensis* on soil nitrogen content was rather weak, there is a trend of increased nitrogen content in plots where the species was sown (and where it still attains higher cover). Part of the legacy of the initial sowing could thus have been caused by the effect of *Lathyrus pratensis* on the soil composition.

Our long-term field study has shown that the founder effect of the initial species composition and the priority effect of early arriving species are important determinants of the course of secondary succession, because the species community assembly was found to be affected still 20 years after sowing. The effect of sowing target species in grassland restoration persists long enough to be considered a useful tool in landscape management. Our data suggest that more important than the number of sown species is their identity; some species were successful and influential, whereas others were not. The experience of local managers is indispensable in similar situation (Jongepierová et al. 2007) because it is very difficult to find a general rule predicting just from traits which of the sown species will be influential and will persist for a long time. Sowing many species is a type of insurance increasing the probability that at least some of the target (i.e. sown) species become a part of the restored community.

## Acknowledgements

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## **Supplementary material**

The online version of this article (<https://doi.org/10.1007/s00442-019-04415-y>) contains supplementary material, which is available to authorized users.



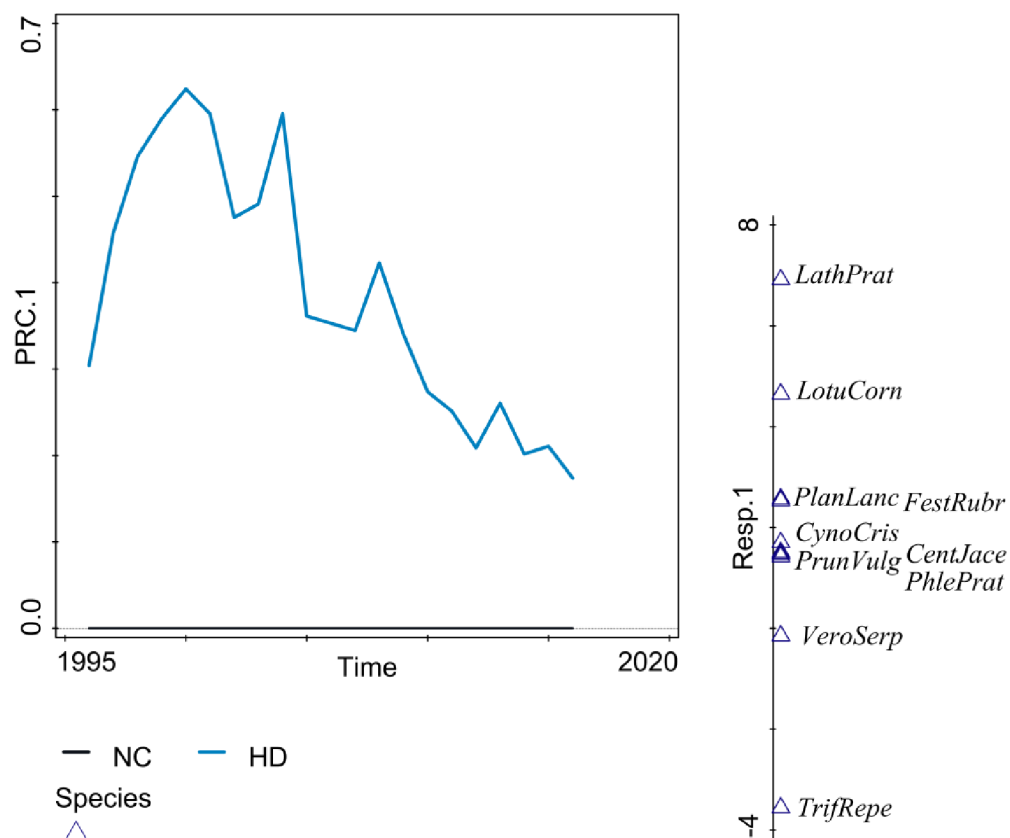
## Online Supplementary Material 1

### *Differences between plots under natural colonization (NC) and sown by high diversity mixture (HD)*

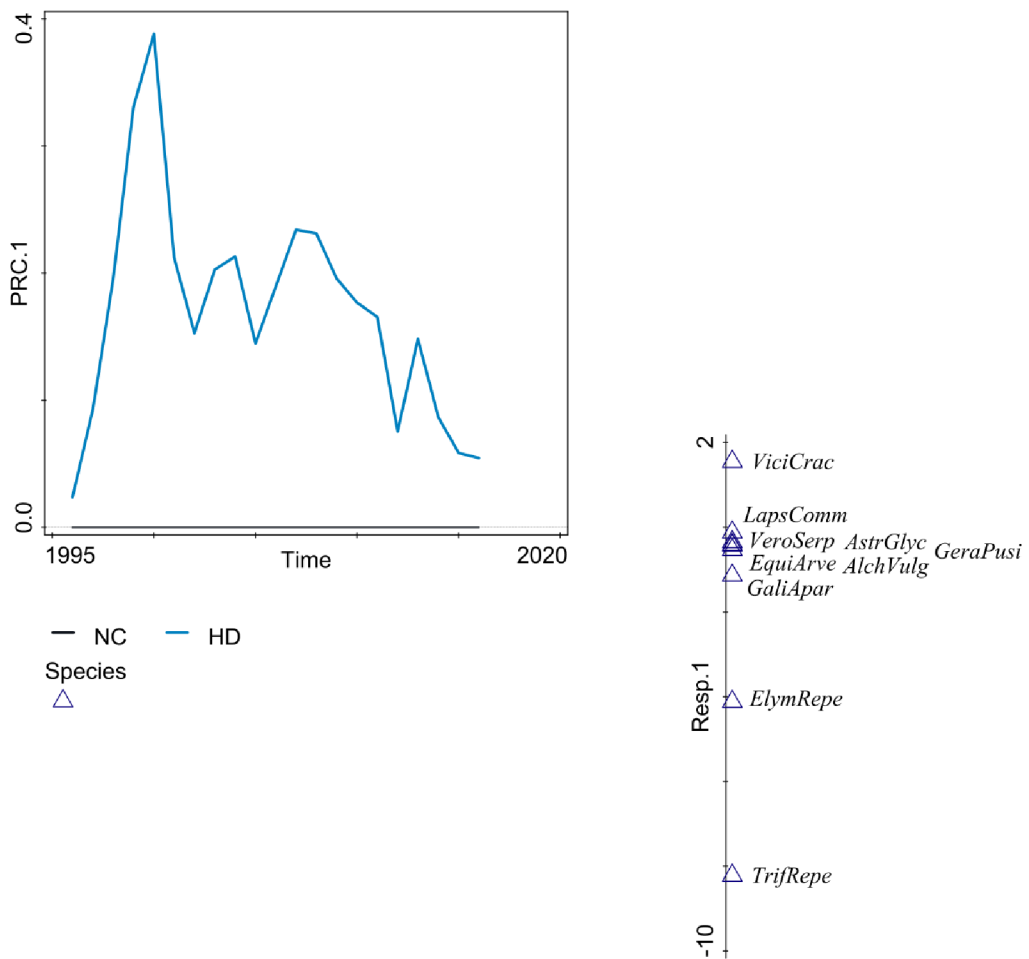
The principal response curves were highly significant for both all the species (FigA 1) and also for the unsown species (FigB 1).

For the analysis of all the species, not surprisingly, most of the species are the sown ones, and all of them are positively related to the sowing (i.e. to the HD treatment), with the best fitting species being *Lathyrus pratensis* (FigA 1). For the unsown species, the effect is less pronounced, with vast majority of the best fitting species being negatively correlated to the sowing. The most pronounced negative response was found in *Trifolium repens* and *Elymus repens*, which were two species, vigorously colonizing the NC plots (FigB 1).

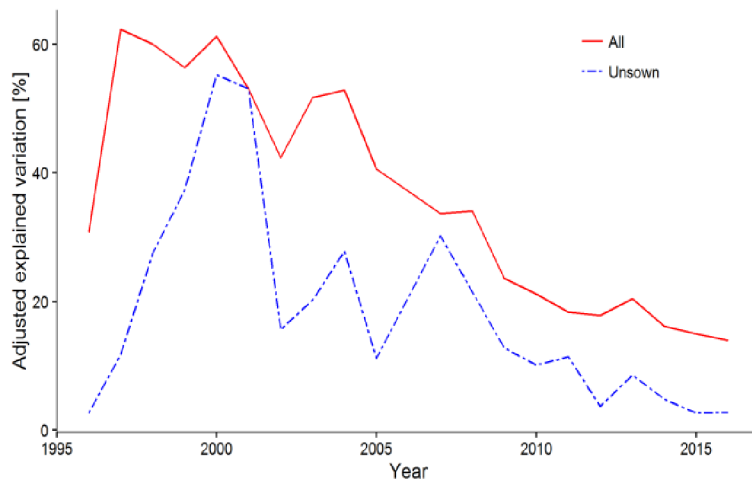
The year by year analysis (FigC 1, analogical to the analyses in the main text of the paper) demonstrated that the difference between NC and HD plots was always more pronounced when all the species were considered, and this was significant throughout the 20 years of experiment, with maximum effect between the second and the fifth year. The effect on the unsown species was less pronounced, delayed (maximum in the fifth year) and was significant only from 1997 to 2013 (without significant effect in 2012). In the last 3 years, the effect was negligible and not significant.



FigA 1: Principle Response Curves for the difference between NC and HD plots of all species (both sown and unsown species). The global test is significant (pseudo-F = 6.8,  $p = 0.004$ ). The vertical axis shows the best fitting species. *CentJace* – *Centauera jacea*, *CynoCris* – *Cynosurus cristatus*, *FestRubr* – *Festuca rubra*, *LathPrat* – *Lathyrus pratensis*, *LotuCorn* – *Lotus corniculatus*, *PhlePrat* – *Phleum pratense*, *PlanLanc* – *Plantago lanceolata*, *PrunVulg* – *Prunella vulgaris*, *TrifRepe* – *Trifolium repens*, *VeroSerp* – *Veronica serpyllifolia*.



FigB 1: Principle response curves for the difference between NC and HD plots of unsown species only. The global test is significant (pseudo-F = 2.4,  $p = 0.002$ ). The vertical axis shows the best fitting species. *AlchVulg* – *Alchemilla vulgaris*, *AstrGlyc* – *Astragalus glycyphyllos*, *ElymRepe* – *Elymus repens*, *EquiArve* – *Equisetum arvense*, *GaliApar* – *Galium aparine*, *LapsComm* – *Lapsana communis*, *TrifRepe* – *Trifolium repens*, *VeroSerp* – *Veronica serpyllifolia*, *ViciCrac* – *Vicia cracca*.



FigC 1: Results of RDA of total species composition (All) and unsown species composition (Unsown) in NC and HD plots in particular years.

## Online Supplementary Material 2

TableA 2: Examined plant functional traits of sown species. CentJace – *Centaurea jacea*, CynoCris – *Cynosurus cristatus*, FestRubr – *Festuca rubra*, GaliVeru – *Galium verum*, HolcLana – *Holcus lanatus*, LathPrat – *Lathyrus pratensis*, LotuCorn – *Lotus corniculatus*, LychFlos – *Lychnis flos-cuculi*, MediLupu – *Medicago lupulina*, PhlePrat – *Phleum pratense*, PlanLanc – *Plantago lanceolata*, PrunVulg – *Prunella vulgaris*, TrifDubi – *Trifolium dubium*, TrifPrat – *Trifolium pratense*, TrisFlav – *Trisetum flavescens*.

	Canopy height [m]	Seed mass [mg]	Lateral spread [m]	SLA [mm <sup>2</sup> /mg]
<i>CentJace</i>	0.85	1.99	0.04	16.41
<i>CynoCris</i>	0.55	0.55	0.01	23.92
<i>FestRubr</i>	0.48	0.91	0.07	19.36
<i>GaliVeru</i>	0.38	0.51	0.13	20.79
<i>HolcLana</i>	0.33	0.41	0.05	34.04
<i>LathPrat</i>	0.57	12.52	0.21	25.42
<i>LotuCorn</i>	0.43	1.40	0.00	23.65
<i>LychFlos</i>	0.41	0.20	0.00	24.22
<i>MediLupu</i>	0.25	1.78	0.00	27.24
<i>PhlePrat</i>	0.37	0.59	0.01	25.08
<i>PlanLanc</i>	0.16	1.62	0.00	18.52
<i>PrunVulg</i>	0.12	0.69	0.13	29.55
<i>TrifDubi</i>	0.23	0.47	0.00	25.96
<i>TrifPrat</i>	0.28	1.58	0.00	23.37
<i>TrisFlav</i>	0.55	0.30	0.07	20.73

### Online Supplementary Material 3

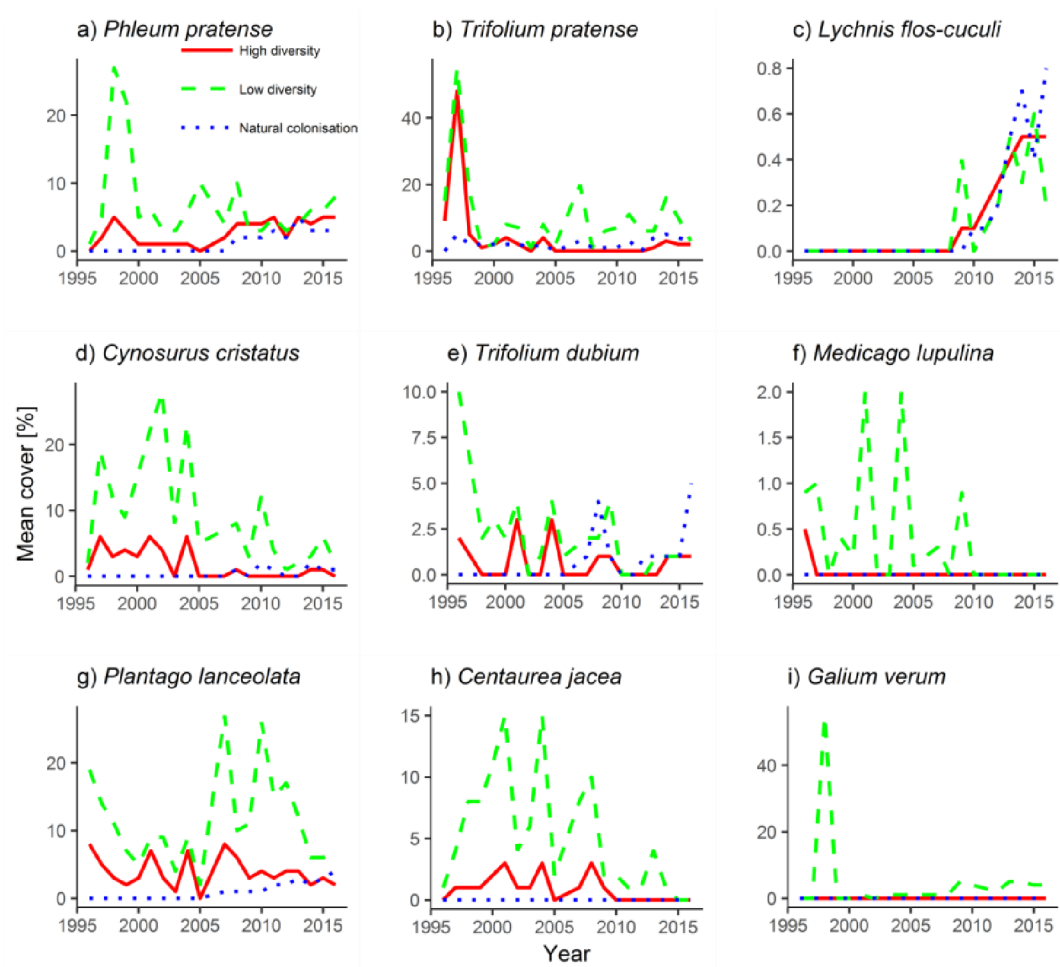
TableA 3: Results of regression of cover of each sown species and its densities in each year individually (SP). Sown species chosen after forward selection in the RDA analysis in each year using cover of only unsown (U), only sown (S) and all species (A) as response variables. \*  $0.01 < p < 0.05 >$ , \*\*  $p \leq 0.01$ . Total selection – number of times a species had a significant influence on the remaining species or themselves. *CentJace* – *Centaurea jacea*, *CynoCris* – *Cynosurus cristatus*, *FestRubr* – *Festuca rubra*, *GaliVeru* – *Galium verum*, *HolcLana* – *Holcus lanatus*, *LathPrat* – *Lathyrus pratensis*, *LotuCorn* – *Lotus corniculatus*, *LychFlos* – *Lychnis flos-cuculi*, *MediLupu* – *Medicago lupulina*, *PhlePrat* – *Phleum pratense*, *PlanLanc* – *Plantago lanceolata*, *PrunVulg* – *Prunella vulgaris*, *TrifDubu* – *Trifolium dubium*, *TrifPrat* – *Trifolium pratense*, *TrisFlav* – *Trisetum flavescens*.

Species / Year	1996			1997			1998			1999			2000			2001			2002			2003			2004			2005								
	SP	U	S	A	SP	U	S	A	SP	U	S	A	SP	U	S	A	SP	U	S	A	SP	U	S	A	SP	U	S	A	SP	U	S	A				
<i>CentJace</i>	**				**				**				**				**				**				**				**				**			
<i>CynoCris</i>	**	**			**	**			**	**			**	**			**	**			**	**			**	**			**	**			**	**		
<i>FestRubr</i>	**				**	*			**	**			**	**			**	**			**	**			**	**			**	**			**	**		
<i>GaliVeru</i>													*												*											
<i>HolcLana</i>	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**
<i>LathPrat</i>	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**
<i>LotuCorn</i>	**	*	*	*	**	*	*	*	**	*	*	*	**	*	*	*	**	*	*	*	**	*	*	*	**	*	*	*	**	*	*	*	**	*	*	*
<i>LychFlos</i>																																				
<i>MedLupu</i>	**			*				*				*				*				*				*				*				*				
<i>PhlePrat</i>	**	**			**	**			**	**			**	**			**	**			**	**			**	**			**	**			**	**		
<i>PlanLanc</i>	**	**			**	**			**	**			**	**			**	**			**	**			**	**			**	**			**	**		
<i>PrunVulg</i>	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**
<i>TrifDubu</i>	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**
<i>TrifPrat</i>	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**
<i>TrisFlav</i>	**	**	*	*	**	**	*	*	**	**	*	*	**	**	*	*	**	**	*	*	**	**	*	*	**	**	*	*	**	**	*	*	**	**	*	*

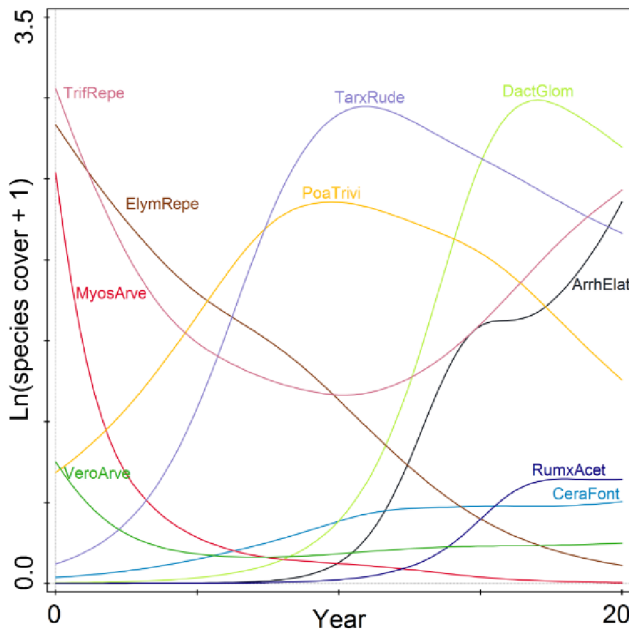
Species / Year	2007			2008			2009			2010			2011			2012			2013			2014			2015			2016			Total selection													
	SP	U	S	A	SP	U	S	A	SP	U	S	A	SP	U	S	A	SP	U	S	A	SP	U	S	A	SP	U	S	A	SP	U	S	A	SP	U	S	A								
<i>CentJace</i>	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	20	0	2	1				
<i>CynoCris</i>	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	17	1	5	6				
<i>FestRubr</i>	**	*	*	*	**	*	*	*	**	*	*	*	**	*	*	*	**	*	*	*	**	*	*	*	**	*	*	*	**	*	*	*	**	*	*	*	**	*	*	*	14	4	8	7
<i>GaliVeru</i>	**	*	*	*	**	*	*	*	**	*	*	*	**	*	*	*	**	*	*	*	**	*	*	*	**	*	*	*	**	*	*	*	**	*	*	*	**	*	*	*	9	0	0	0
<i>HolcLana</i>	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	**	**	**	**	12	7	9	9
<i>LathPrat</i>	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	20	9	20	19
<i>LotuCorn</i>	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	20	0	11	10
<i>LychFlos</i>				*				*				*				*				*				*				*				*				*	1	0	0	0				
<i>MedLupu</i>				*				*				*				*				*				*				*				*				*	4	0	0	0				
<i>PhlePrat</i>	**	**	*	*	**	**	*	*	**	**	*	*	**	**	*	*	**	**	*	*	**	**	*	*	**	**	*	*	**	**	*	*	**	**	*	*	**	**	*	*	18	3	5	3
<i>PlanLanc</i>	**	*	*	*	**	*	*	*	**	*	*	*	**	*	*	*	**	*	*	*	**	*	*	*	**	*	*	*	**	*	*	*	**	*	*	*	**	**	**	**	17	0	7	4
<i>PrunVulg</i>	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	19	0	10	9
<i>TrifDubu</i>				*				*				*				*				*				*				*				*				*	**	**	**	**	8	0	2	5
<i>TrifPrat</i>				*	**	*	*	**	**	*	*	**	**	*	*	**	**	*	*	**	**	*	*	**	**	*	*	**	**	*	*	**	**	*	*	**	**	**	**	**	7	5	4	3
<i>TrisFlav</i>	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	**	**	**	**	8	5	12	9

## Online Supplementary Material 4



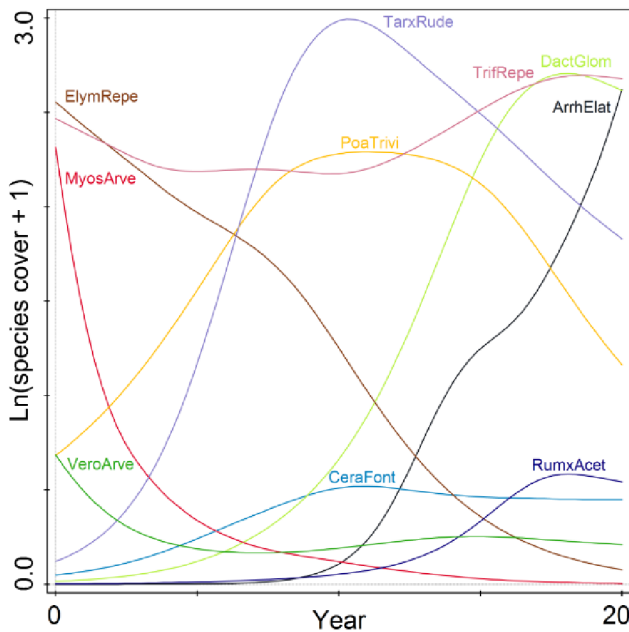
FigA 4: Average cover of nine sown species (which are not represented in Fig. 1) between 1996 and 2016 for high and low diversity seed mixtures plots where species were sown and natural colonisation plots. Y-axes scale differs for each panel.

## Online Supplementary Material 5



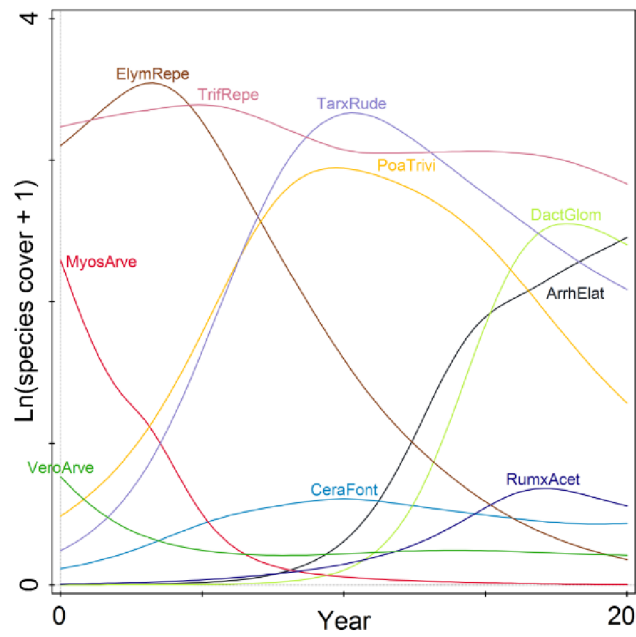
FigA 5: Response curves of ten unsown species covers in HD plots with the highest frequencies, fitted using generalized additive models. *ArrhElat* – *Arrhenatherum elatius*, *CeraFont* – *Cerastium fontanum*, *DactGlom* – *Dactylis glomerata*, *ElymRepe* – *Elymus repens*, *MyosArve* – *Myosotis arvensis*, *PoaTrivi* – *Poa trivialis*, *RumxAcet* – *Rumex acetosa*, *TarxRude* –

*Taraxacum sect. ruderalia*, *TrifRepe* – *Trifolium repens*, *VeroArve* – *Veronica arvensis*.



FigB 5: Response curves of ten unsown species covers in LD plots with the highest frequencies, fitted using generalized additive models. *ArrhElat* – *Arrhenatherum elatius*, *CeraFont* – *Cerastium fontanum*, *DactGlom* – *Dactylis glomerata*, *ElymRepe* – *Elymus repens*, *MyosArve* – *Myosotis arvensis*, *PoaTrivi* – *Poa trivialis*, *RumxAcet* – *Rumex acetosa*, *TarxRude* –

*Taraxacum sect. ruderalia*, *TrifRepe* – *Trifolium repens*, *VeroArve* – *Veronica arvensis*.



FigC 5: Response curves of ten unsown species covers in NC plots with the highest frequencies, fitted using generalized additive models. *ArrhElat* – *Arrhenatherum elatius*, *CeraFont* – *Cerastium fontanum*, *DactGlom* – *Dactylis glomerata*, *ElymRepe* – *Elymus repens*, *MyosArve* – *Myosotis arvensis*, *PoaTrivi* – *Poa trivialis*, *RumxAcet* – *Rumex acetosa*, *TarxRude* –

*Taraxacum sect. ruderalia*, *TrifRepe* – *Trifolium repens*, *VeroArve* – *Veronica arvensis*.



### Online Supplementary Material 6

Illustrative pictures of selected plots in 2004. These pictures show that all the HD plots were dominated by *Lathyrus pratensis* (FigA 6), similarly as the LD plot in block 3 (FigB 6), whereas the species was generally missing in all the other plots (FigC 6 and FigD 6).



FigA 6: Dominance of *Lathyrus pratensis* in high diversity plot (HD) in 2004.



FigB 6: Dominance of *Lathyrus pratensis* in low diversity plot (LD3) in 2004. Sown species were *Trisetum flavescens*, *Festuca rubra*, *Lathyrus pratensis*, *Lychnis flos-cuculi*.



FigC 6: Vegetation in low diversity plot (LD1) in 2004. Sown species were *Phleum pratense*, *Holcus lanatus*, *Trifolium pratense*, *Centaurea jacea*.



FigD 6: Vegetation in natural colonisation plot (NC) in 2004.

## **Chapter 3**

### **The role of biotic interactions in plant community assembly: What is the community species pool?**

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# The role of biotic interactions in plant community assembly: What is the community species pool?

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## Abstract

Differences in plant species composition between a community and its species pool are considered to reflect the effect of community filters. If we define the species pool as a set of species able to reach a site and form a viable population in a given abiotic environment (i.e. to pass the dispersal and abiotic filter), the difference in species composition should correspond to the effect of biotic interactions. However, most of the operational definitions of the species pool are based on co-occurrence patterns and thus also reflect the effect of biotic relationships, including definitions based on functional plant traits, Ellenberg indicator values or Beals index. We conducted two seed introduction experiments in an oligotrophic wet meadow with the aim of demonstrating that many species excluded, according to the above definitions, from a species pool are in fact able to establish there successfully if competition is removed. In sowing experiments, we studied the establishment and survival of species after the removal of competition (i.e. in artificial gaps) and in intact vegetation. We also investigated inter-annual variability of seed germination and seedling establishment and competitive exclusion of sown species. The investigated species also included those from very different habitats (i.e. species with very low corresponding Beals index or Ellenberg indicator values that were different from the target community weighted mean). Many of these species were able to grow in the focal

wet meadow if competition was removed, but they did not establish and survive in the intact community. These species are thus not limited by abiotic conditions, but by the biotic filter. We also recorded a great inter-annual variability in seed germination and seedling establishment. Competitive exclusion of species with different ecological requirements could be quite fast (one and half seasons) in some species, but some non-resident species were able to survive several seasons; the resident species were able to persist in competition. Comparison of realized vegetation composition with the corresponding species pool greatly underestimates the potential impact of the biotic filter if the delimitation of the species pool is based on the realized niches of species and co-occurrence patterns.

### **Keywords**

Abiotic filter, Biotic filter, Competitive exclusion, Disturbance, Sowing experiment, Species pool.

## 1. Introduction

Differences in species composition between a community and its local species pool are considered to reflect the effect of community filters (Lambers et al., 2012). However, there are many types of species pool and various methods of how to determine their species composition. The regional species pool includes all species present in a region (Belyea & Lancaster, 1999). It is mainly determined by the area where the species evolved and by their ability to migrate to a specific locality (Lambers et al., 2012; Lepš, 2013; Pärtel et al., 1996; Swenson, 2011; Zobel, 1997). The local species pool consists of those species from the regional species pool which are able to pass through the dispersal filter. Finally, species present in a community (“actual species pool”) are those species which passed through the community filter, including the abiotic filter (i.e. are able to tolerate abiotic conditions of the locality) and also the biotic filter (i.e. are able to withstand the biotic interactions in the community) (Götzenberger et al., 2012; Houseman & Gross, 2006; Lambers et al., 2012). Nevertheless, the basic concepts and definitions of the actual species pool are not consistent: some of the definitions filter out the species that are not able to withstand competition from other species, while others do not. Butaye et al. (2001) defined the local species pool as a set of species able to pass through dispersal and abiotic filters, whereas according to definition by Zobel (1997), species must be also able to tolerate local biotic interactions. This difference is often not explicitly considered in the methods of species pool determination. However, if we expect that the difference between the local species pool and actual species composition reflects the effect of the biotic filter, we would argue that the definition of the species pool should include all the species that are able to grow in given abiotic conditions, and should not be affected by their ability to pass the biotic filter.

Most of the operational definitions of the species pool are based on co-occurrence patterns (e.g. Sádlo et al., 2007) and thus also reflect

the effect of biotic relationships, including definitions based on functional plant traits (de Bello et al., 2012), Ellenberg indicator values (Zobel, 1997; Zobel et al., 1998) or Beals index (Münzbergová & Herben, 2004). But there are apparently many species able to reach the site and grow in the specific abiotic conditions, i.e. they are limited neither by dispersion nor by abiotic factors, but they are not able to grow in the community because of competition with other species (Butaye et al., 2001; Vítová & Lepš, 2011). If species pool is defined as a set of species able to reach a site and form a viable population in a given abiotic environment, it is not possible to determine this species pool using any methods based on co-occurrence patterns in real vegetation. Then, the only possibility is to use a seed introduction experiments in combination with experimental competition removal. In these experiments, seeds of various species are introduced to the community so that we can exclude any dispersal limitation, and by comparison of competition removal and control plots we can test directly the effect of the biotic filter, whereas the competition removal plots demonstrate the effect of abiotic conditions. Quite a few sowing experiments have been carried out, simply sowing the potential colonizers into intact vegetation with the aim of demonstrating dispersal limitation (Turnbull et al., 2000; Zobel & Kalamees, 2005).

Seedling establishment itself is a quite improbable event. The propagule pressure and ability of seedlings to withstand competition plays a crucial role (Cornelissen et al., 2003; Houseman & Gross, 2006). Relatively high numbers of propagules from potential colonizing species are needed because the probability of establishment from seeds is usually low, not only for colonizers, but also for the resident species (Clark et al., 2007; Vítová & Lepš, 2011). Sensitivity of seedling establishment to abiotic conditions and especially to competition is species specific (Kotorová & Lepš, 1999) and much higher than the sensitivity of already established plants. Further, only a small amount of seedlings from a large number of seeds can establish



and reach the reproductive stage (Lepš, 2013; Zobel, 1997). Seeds of many species germinate and survive in the community for several years in the stage of seedlings but they never establish a viable population (Vítová & Lepš, 2011). Successful seedling establishment is often dependent on disturbances (Kotorová & Lepš, 1999; Lepš, 2013).

Local, often small scale disturbances can temporarily remove the competition and enable local seedling establishment, e.g. in gaps (Grubb, 1977). However, the gaps typically do not persist for very long, and the newly established individuals might soon face competition, which might lead to competitive exclusion, but for already established plants, this competitive exclusion can take a rather long time (Adler et al., 2010; Lepš, 2014).

We conducted two seed introduction experiments in an oligotrophic wet meadow with the aim of comparing establishment success within intact vegetation and gaps (i.e. with competition removed) for species widely varying in the degree of their membership in the community species pool as predicted by the Beals index and Ellenberg indicator values. The investigated species also included those from different habitats, i.e. species with a low corresponding Beals index or with Ellenberg indicator values different from the target community weighted mean. As this is a single site experiment, it is impossible to test directly for the effect of abiotic conditions. However, we predict that many species from different habitats (as indicated by Beals index and Ellenberg values) will be able to grow in the site only in the absence of competition, and this would be strong indication of the effect of biotic interactions on species habitat preferences. To account for seasonal and inter-annual variability, the seeds were sown in various years and seasons. As we expected that these species will eventually be competitively excluded, we also aimed to determine the time needed for their competitive exclusion after gap overgrowth by extant vegetation. To account for possible methodological differences

in species demands for germination, we compared seed germination in laboratory conditions and in the field.

## 2. Materials and methods

### 2.1. Study site

The experiment was carried out in an oligotrophic wet meadow Ohrazení, 10 km southeast of České Budějovice, Czech Republic (48°57'N, 14°35'E, 510 m a.s.l.). This locality has been used for experimental studies for more than a decade (see, e.g. Lepš, 1999; 2014 for detailed characteristics of a site); the meteorological characteristics during the years of experiments are in Table 1. The species rich wet meadow can be characterized as *Molinion*. The dominant species *Molinia caerulea* forms the community together with other grasses (e.g. *Festuca rubra*, *Holcus lanatus*, *Briza media*), sedges (e.g. *Carex panicea*, *C. hartmanii*, *C. pallescens*), and many forbs (e.g. *Lysimachia vulgaris*, *Potentilla erecta*, *Ranunculus* spp.). The meadow is surrounded by forest and from the southeast side the meadow borders with a field. The experimental plots were established in a part of the meadow that could not be affected by possible nutrient runoff from the field and with homogeneous light conditions (possibly very limited shading from the forest edge).

Table 1: Mean annual temperature (derived using the altitude correction from values of the meteorological station in České Budějovice, 10 km from Ohrazení) and total annual precipitation (from the meteorological station in Ledenice, 3 km from Ohrazení) in Ohrazení from 2010 to 2014 (Czech Hydrometeorological Institute České Budějovice). Mean monthly temperature and total monthly precipitation are noted in appendices (Table A.1).

	2010	2011	2012	2013	2014
Mean annual temperature [°C]	7.5	7.1	6.2	7.7	9.0
Total annual precipitation [mm]	743.2	581.9	761.9	739	694.2

## 2.2. Seed introduction experiments

Species with various habitat preferences were introduced to the community of the wet meadow, both in artificial gaps and intact vegetation, in order to test their ability to establish and survive. Artificial gaps, 30 x 30 cm in size, were created by digging (to depth of 15 cm) and refilling with the soil from the target locality to remove the competition with surrounding vegetation. Seeds of each species were added to its own gap and control plot (intact vegetation, where the effect of competition was maintained) of the same size, to the central part of 20 x 20 cm. In both experiments, we used seeds from a commercial supplier (Planta Naturalis, Markvartice, Czech Republic). Seed germination and seedling survival was followed during several seasons. Gaps were not weeded, so that for species that established in gaps, we were able to observe their possible competitive exclusion. The experimental plots were (similarly to the whole meadow) mown regularly twice a year in the second half of June and October.

First, we established the experiment with 12 species sown into the plots in three replications each. Experimental plots were arranged in a regular grid, with 30 cm distance among the plots, with gap and control of each replicate of each species located next to each other. Individual replicates were established 1 m from each other. All sown species were absent from the locality and thus not a part of the local species list except *Plantago lanceolata* and *Succisa pratensis* which are residents there (*Experiment 1*; see Table A.3 for list of sown species). 200 seeds of one species per plot were sown in spring and autumn 2010 to account for the possible effect of season. Their recruitment was observed from 2010 to 2014.

To investigate a larger range of species, another experiment of similar design was established with 60 species (*Experiment 2*; see Table A.3 for list of sown species) sown into the plots in November 2011 in two replications distant 30 m one from the other. Experimental plots were arranged in a regular grid of six rows, distant 1 m from each

other. In each row, ten gaps and ten control plots were established in 30 cm distance between two neighbouring gaps and between gap and appropriate control plot. Species were randomly sown, each in one gap and their associated control plot. According to classical methods of species pool determination (de Bello et al., 2012; Münzbergová & Herben, 2004; Sádlo et al., 2007; Zobel, 1997), all these sown species were not a part of the species pool of the locality. In each plot, 200 seeds were sown for species with seed weight around 1 mg. For the lighter seeds, the densities were increased, and for heavier seeds decreased to account for the expected dependence of establishment on seed weight (nevertheless, the germination and establishment success was always related to seeding densities). The successful establishment was expressed as the number of survivors out of the number of sown seeds. The numbers of recruited individuals were monitored from 2012 to 2014. In 2013, we estimated the percentage cover of re-colonizing species in gaps.

### **2.3. Germination test**

For both experiments, germination tests were carried out to compare the ability of seeds to germinate in laboratory conditions with that of natural conditions. In *Experiment 1*, seeds were stored at -14 °C for three weeks, after that their germinability was observed in standardized climatic conditions (i.e. 19 °C, mode day/night). In *Experiment 2*, we decided to test additional methods of seed storage to find out which led to the best species germinability in the field. Thus, the germinability of seeds stored dry in -14 °C as in *Experiment 1* and in +4 °C was tested in standardized climatic conditions; germinability of seeds without any cold storage was tested at the room temperature (about +20 °C).

### **2.4. Data analysis**

The data on seedling establishment and survival were analyzed with respect to the repeated measure character of the data, i.e. with a mixed effect model with species and gap/control plot as the between subject

factor, and the date as a within-subject factor, with species and replicate identity being the random factor. Prior to the analysis, numbers of seedlings were logarithmic transformed  $x' = \log_{10}(x + 1)$  for *Experiment 1*, where  $x$  is the number of surviving individuals and  $x' = \log_{10}(x*200 + 1)$  for *Experiment 2*, where  $x$  is a number of surviving individuals per sown seed, i.e. number of individuals/number of seeds sown. Thus,  $x*200$  estimates the expected number of seedlings if 200 seeds would be sown for each species. Simple linear regression and correlation were used to characterize the relationships between pairs of variables using Statistica 12 (StatSoft, 2013).

Ellenberg indicator values (Ellenberg et al., 1991) for soil humidity, light availability and soil productivity for each sown species were obtained from the database JUICE of version 7.0 (Tichý, 2002). These were used to compute the absolute values of the difference between the community weighted mean (CWM) and Ellenberg indicator values of sown species (the higher the difference, the less suitable the habitat was for a species). Community weighted means of the indicator values were calculated for the two relevés of the target community, with weights being the species relative cover (in percentage without any transformation).

The phytosociological suitability was further characterized by Beals index (Beals, 1984; Münzbergová & Herben, 2004), calculated as  $P_{ij} = (1/S_i) \sum_k N_{jk} / N_k$  where  $P_{ij}$  is a probability to find species  $j$  at habitat  $i$ ,  $S_i$  is the number of species at habitat  $i$ ,  $N_{jk}$  is the number of joint occurrences of species  $j$  and  $k$  and  $N_k$  is number of occurrences of species  $k$  in the reference database. This index thus estimates the probability of occurrence of a species on the basis of co-occurrence patterns in a large collection of samples. Beals index of sown species was calculated for the two phytosociological relevés of the experimental plots on the basis of an external database of relevés of Czech National Phytosociological Database (all 55163 relevés from this database in total were used without any sorting, Chytrý &

Rafajová, 2003) using the program R of version 3.1.1 (R Development Core Team, 2014). For this calculation, the package “vegan” and function “beals” of “type” = 2 (i.e. abundances were used to compute weighted averages of conditioned probabilities) were used. The average value of the two phytosociological relevés was used.

### **3. Results**

#### **3.1. Seed introduction experiments**

In both experiments, the gaps were overgrown rather quickly (mainly by vegetative spreading) by surrounding vegetation, so that they can be considered a competition free space just during the first season. At the end of the second season, the cover of the vegetation (other than the sown species) was about 70 – 80 %, and reached to completely closed canopy at subsequent seasons, which decreased the difference between gaps and control plots.

In both experiments, all the analyses of seedling establishment and survival in control plots and gaps demonstrated that all the tested terms were significant. All the main effects were highly significant ( $p \ll 0.01$  in all the cases) – i.e. species differed among themselves in establishment success, gaps had on average more established individuals and dates differed in the number of established individuals, simply because the seedling died out. Also, all of the first order interactions were significant – there were differences between species in their reaction to gaps (gap/control plot \* species interaction), and significant interactions with the date showed that the dynamics was different both among the species and according to gaps. As a matter of fact, the decrease was faster in gaps – this was caused mainly by an increase in competition intensity within gaps; whereas in the control plots, the competition was constant, in gaps, due to their overgrowing, the intensity of competition increased. Looking at individual dates, seedling emergence of individual species (species taken as a random factor) significantly differed between gaps and control plots: in

*Experiment 1*  $F_{1,22} = 6.403$ ,  $p = 0.0294$  for spring sowing in August 2010 (second census after sowing, when the numbers of seedlings reached their maxima);  $F_{1,22} = 10.818$ ,  $p = 0.0203$  for autumn sowing in the first spring census in May 2011; in *Experiment 2*  $F_{1,59} = 66.590$ ,  $p < 0.0001$  in the first spring census in May 2012. In *Experiment 1*, these differences remained significant to May 2011 for spring sowing and May 2012 for autumn sowing. The numbers of surviving seedlings remained, in both sowing dates, higher in gaps during the whole period; nevertheless, the differences were non-significant toward the end of the monitoring of both spring and autumn sowing, particularly because different species responded to gaps in rather different ways (highly significant species \* gap/control plots interaction:  $F_{11,22} = 2.705$ ,  $p = 0.0226$  for spring sowing,  $F_{11,22} = 22.090$ ,  $p < 0.0001$  for autumn sowing, both in August 2014); note that the species \* gap/control plot interaction Mean Square is in the denominator of the F-test. Seedling numbers in *Experiment 2* became insignificant by the end of the first monitoring season ( $F_{1,59} = 11.986$ ,  $p = 0.0824$  in September 2012).

In *Experiment 1*, recruit establishment temporal dynamics differed significantly between gaps and control plots (gap/control plot \* time interaction,  $F_{9,91.96} = 2.849$ ,  $p = 0.0053$  for spring sowing,  $F_{6,66.00} = 7.707$ ,  $p < 0.0001$  for autumn sowing). At the beginning, recruit numbers in gaps were significantly higher compared to control plots, before they started to die off up until the end of our observation when the difference in recruit numbers in gaps did not differ from those in control plots (Figs. 1 and 2, Table A.2). As expected, both resident species (*Plantago lanceolata* and *Succisa pratensis*) survived very well in gaps as well as in control plots (see Table A.2). Surprisingly, the next species with the highest survival rates were non-resident *Sanguisorba minor* (spring sowing), *Hypericum perforatum*, *Galium verum* and *Geranium pratense* (autumn sowing) which survived to the end of our monitoring (i.e. for five seasons for spring sowing and four seasons for autumn sowing), especially in gaps (see Table A.2). The

weedy species *Aphanes arvensis* was the only one which reached a reproductive stage, although only in gaps and in the first year (tens of reproductive individuals, see Table A.2).

We did not record any large difference in the recruitment of seeds sown in spring and autumn, particularly in comparison with differences between seeds sown into gaps vs. control plots. Whether sown in spring or autumn, emergence of all seedlings was greatest in the first half of the year. We observed differences in the course of emergence only in a few species. For three of these species the behaviour was similar in both gaps and control plots: *Aphanes arvensis* emerged better from spring sowing, on the contrary, species *Geranium pratense* and *Trifolium hybridum* emerged better if they were sown in autumn. For *Sanguisorba minor*, we found that seeds sown into control plots in spring emerged better than those from autumn sowing. Nevertheless, at the end of the experiment the numbers of survivors were comparable (see Table A.2). The most obvious difference was found in species survival in control plots where seedlings from spring sowing died faster than those sown in autumn (particularly in *Galium verum* and *Scabiosa ochroleuca*).

In *Experiment 2*, 47 species germinated from 60 sown species in the locality. Nearly all species emerged better in gaps than in control plots (Fig. 3). *Potentilla palustris* was the only species that initially emerged better in control plots but subsequently the number of seedlings in gaps became higher. Species germinated until June 2012 (i.e. the first post-sowing season), from that time we noticed a sharp decline in recruit number in both gaps and control plots. After two years, all the sown species were absent from the control plots, while in gaps some individuals still survived – *Dianthus deltoides*, *Geum urbanum*, *Hypericum hirsutum*, *Lythrum salicaria*, *Potentilla recta*. However, no species reached a reproductive state.



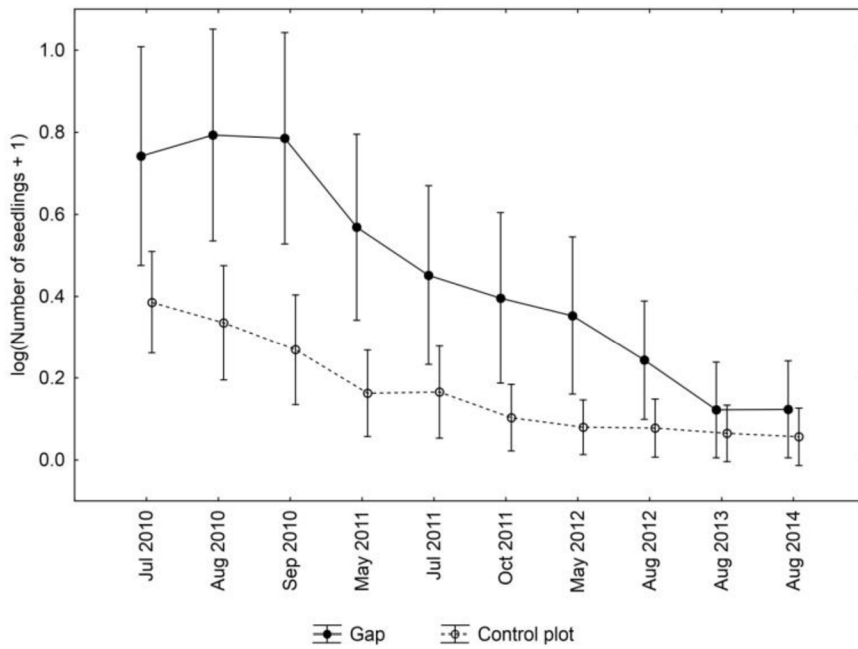


Fig. 1: Mean numbers of survivor individuals ( $\log(x+1)$  transformed) in gaps and control plots sown in spring 2010 during the five-year monitoring period of *Experiment 1* (pooled over all sown species and replicates). Error bars are 95% confidence intervals.

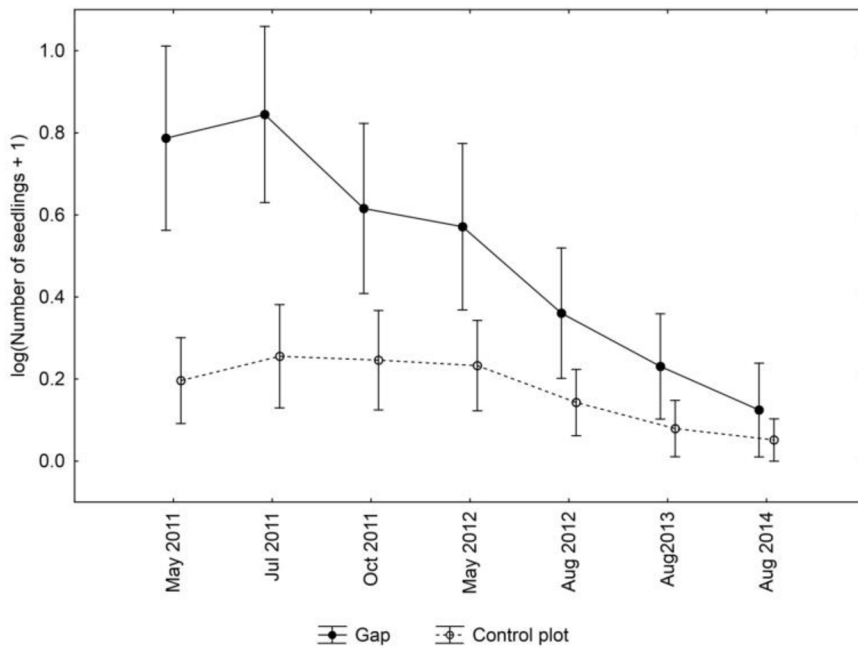


Fig. 2: Mean numbers of survivor individuals ( $\log(x+1)$  transformed) in gaps and control plots sown in autumn 2010 during the four-year monitoring period of *Experiment 1* (pooled over all sown species and replicates). Error bars are 95% confidence intervals.

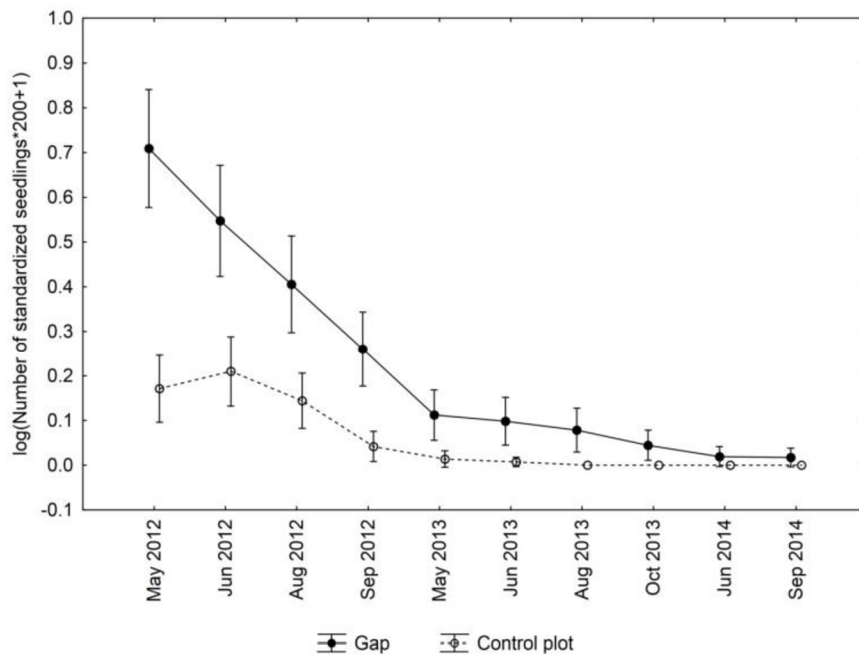


Fig. 3: Mean number of survivor individuals standardized by the number of sown seeds in gaps and control plots ( $\log(x*200+1)$  transformed) during the three-year monitoring period of *Experiment 2* (pooled over all sown species and replicates). Error bars are 95% confidence intervals.

There were six species common to both experiments which allowed a comparison of their inter-annual variability in their emergence and survival (*Aphanes arvensis*, *Euphorbia cyparissias*, *Papaver rhoeas*, *Salvia pratensis*, *Sanguisorba minor* and *Scabiosa ochroleuca*). We compared the two autumn sowings, however, because of slightly different methodology and also timing of individual censuses in the two experiments, we decided not to statistically compare the two experiments, but the differences in emergence and survival patterns are rather conspicuous. Five out of the six species emerged and survived significantly better in *Experiment 1* (sown in autumn 2010) than in *Experiment 2* (autumn 2011). The only species which established better in *Experiment 2* was *Papaver rhoeas* which did not emerge in *Experiment 1* at all. Seeds sown in *Experiment 2* emerged better in gaps but none of the seedlings reached the reproductive stage. In *Experiment 1*, the weedy species *Aphanes arvensis* established only in gaps, and was the only species that reached

the reproductive stage (tens of individuals). In contrast, only one seedling of this species was recorded in gaps in the *Experiment 2*. The greatest seedling emergence and survival was recorded for *Sanguisorba minor* in *Experiment 1*. In *Experiment 2*, just several seeds emerged but only one seedling survived in gaps until the second season. A similar course of survival was recorded also for seedlings of *Scabiosa ochroleuca* in *Experiment 1*, both in gaps and control plots. In *Experiment 2*, the seedlings survived only in the first season and only in gaps, in control plots they did not emerge at all. *Euphorbia cyparissias* and *Salvia pratensis* emerged very little and only in gaps and survived only one or two seasons in *Experiment 1*. Seeds from *Experiment 2* did not emerge at all.

The range of Beals index values in *Experiment 1* was from 0.0004 (*Aphanes arvensis*) to 0.2320 (resident *Plantago lanceolata*) and in *Experiment 2*, the values were rather low, from 0.00001 (*Hyoscyamus niger*) to 0.2210 (*Caltha palustris*) (see Table A.3). Interestingly, the species with the lowest Beals index value in the first experiment was the only species which reached the reproductive stage. In *Experiment 1*, the success of seedling survival (species survival to the end of the experiment) both in gaps and control plots was positively correlated with Beals index, but the relation was driven just by the two resident species with high Beals index, no relationship was found in *Experiment 2* (where, however, all the Beals index values were extremely low). In *Experiment 1*, the relationship with the Beals index became significant for spring sowing into both gaps and control plots in May 2012, for autumn sowing into gaps in August 2012, into control plots in August 2013. For spring sowing, the value of the correlation coefficient was more or less increasing in gaps as well as control plots throughout the experiment, although the value for control plots was higher than for gaps (Fig. 4). By contrast, the value of the correlation coefficient for control plots in autumn sowing was decreasing till May

2012 after which it started to increase again, with its value being lower than it was for gaps (Fig. 5).

Further, in both experiments, the success of seedling recruitment was independent of Ellenberg indicator values. The seedlings of many species with low Beals index and/or high difference between CWM and Ellenberg indicator values (see Table A.3) survived in the locality for several seasons and were usually found in gaps (*Galium verum*, *Geranium pratense*, *Hypericum perforatum* and *Sanguisorba minor* in *Experiment 1*, *Dianthus carthusianorum*, *Dianthus deltoides*, *Geum urbanum*, *Hypericum hirsutum*, *Lythrum salicaria* and *Potentilla recta* in *Experiment 2*). By this time, the gaps were completely overgrown by the vegetation, suggesting that for established individuals, competitive exclusion might take quite some time. On the other hand, the only species able to survive in gaps as well as in control plots were those with high Beals index (i.e. resident species *Plantago lanceolata* and *Succisa pratensis*).

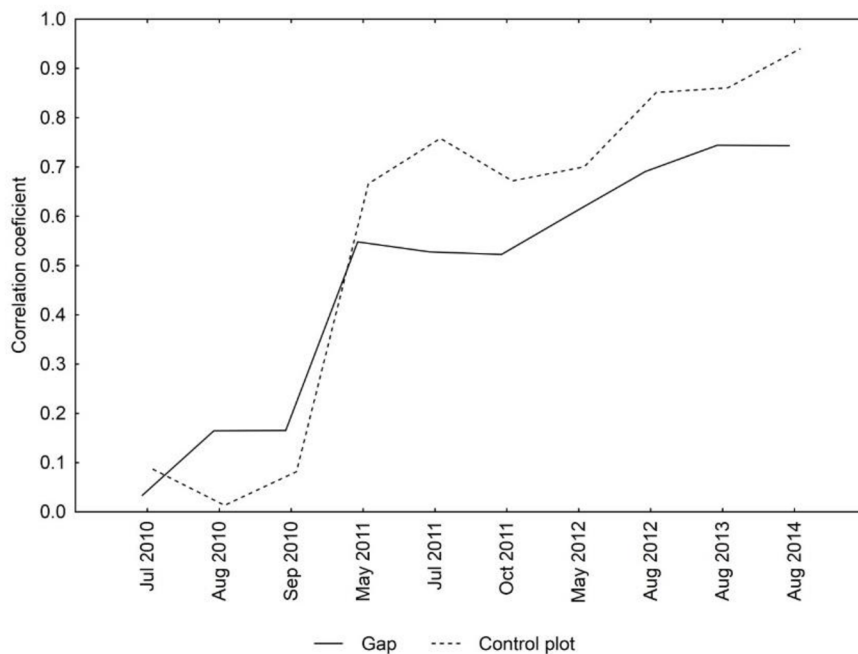


Fig. 4: The changes of the correlation coefficient between the number of seedlings and Beals index of species for spring sowing into gaps and control plots during five-year monitoring period in *Experiment 1*.

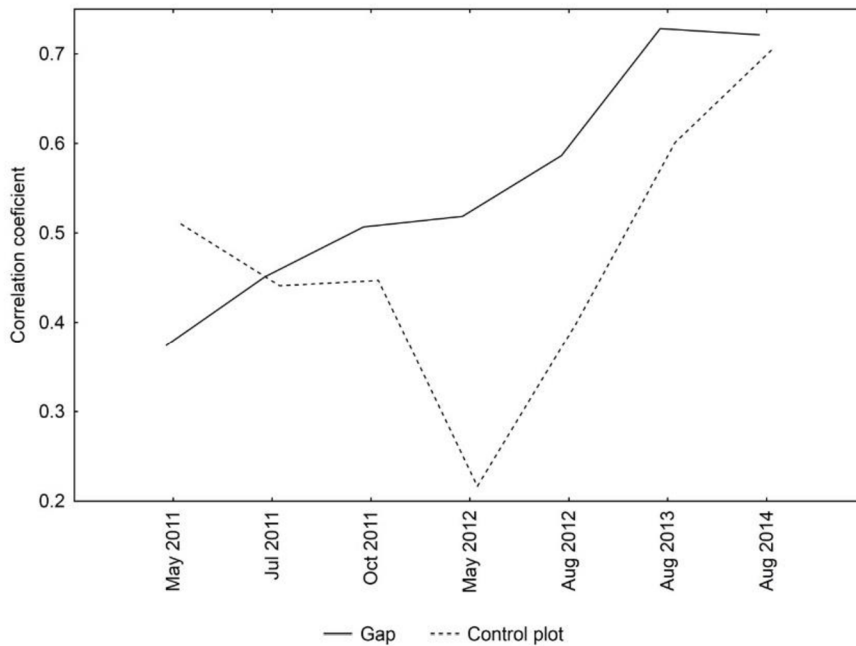


Fig. 5: The changes of correlation coefficient between number of seedlings and Beals index of species for autumn sowing into gaps and control plots during four-year monitoring period in *Experiment 1*.

### 3.2. Comparison of species germinability in the field relative to the germination test

In *Experiment 1*, seed germinability in standardized climatic conditions corresponded best to germinability of seeds sown into gaps in field plots in spring ( $r = 0.8773$ ,  $N = 12$ ,  $p < 0.001$ ), the correlation with emergence in control plots is also high ( $r = 0.6432$ ,  $N = 12$ ,  $p = 0.024$ ). In *Experiment 2*, three types of seed storage were used in the germination test. The germination of seeds stored in  $-14\text{ }^{\circ}\text{C}$  was the best predictor of field germination both in gaps ( $r = 0.3311$ ,  $N = 60$ ,  $p = 0.010$ ) and in control plots ( $r = 0.3606$ ,  $N = 60$ ,  $p = 0.005$ ). There were several species that did not germinate at all or very little in the germination test, but they germinated quite well in the field (e.g. *Lathyrus niger*, *Lycopsis arvensis*, *Myrrhis odorata*).

## 4. Discussion

In the first year of both experiments, the majority of the species took advantage of gaps for seed germination and seedling establishment regardless of their Beals index values. However, with gap vegetative re-colonization from the surrounding area, the number of recruits decreased quite quickly. After which, just species resident to the locality (and accordingly those which have high Beals index value) and only a few species with different habitat requirements (and thus with low Beals index values) were able to survive in overgrown gaps. The relatively long survival of species with low Beals index demonstrates that if a species is able to establish, its competitive exclusion could take quite some time. The increase of correlation between surviving individuals and species Beals index clearly shows that competitive exclusion is the most important determinant of species presence at the locality. Most species would be able to survive at the target locality if competition were to be removed, thus their occurrence is limited particularly by the biotic filter.

Gap re-colonization is a rapid process starting immediately after disturbance and returning to the pre-disturbance state after just a few years (Hölzel, 2005; Vítová et al., 2016). In our case, the canopy gap in both experiments was almost closed by the end of the second season, primarily through vegetative spread from the surrounding area, the exception being *Juncus* species which regenerated from the seed bank.

The ability of a species to establish in a target community was tested for species with very different habitat requirements. We introduced most species (basically all with exception of the two resident species) with low Beals index and pronounced differences in Ellenberg indicator values. This is in sharp contrast to most seed introduction studies, where species with habitat requirements corresponding to the locality (i.e. high Beals index, similar Ellenberg indicator values) were used (Turnbull et al., 2000; Zobel & Kalamees, 2005). As expected, resident species survived best. Turnbull et al.

(2000) assumed that resident species are limited neither by abiotic nor by microclimate conditions, whereas for non-resident species, the probability of this type of limitation increases and consequently their probability of survival is expected to lower. In our single site experiment, we were not able to test directly for possible effect of variation in abiotic conditions. Nevertheless, the ability of some of the non-resident species with very different habitat preferences (as, e.g. *Sanguisorba minor*) to establish in absence of competition is a clear indication, that (at least for these species) the biotic limitation is decisive.

Most of the non-resident species with very different habitat preferences were able to emerge and survive in plots for a certain time period in both experiments, even though only some of them established there successfully. In the first season, most species took advantage of gaps and we recorded the highest numbers of seedlings. Gaps acted here as safe sites for species emergence where aboveground as well as belowground competition was removed. In general, disturbance could be a decisive factor for successful establishment of species, both residents as well as non-residents (Grubb, 1977; Kotorová & Lepš, 1999; Morgan, 1997; Tofts & Silvertown, 2002). With proceeding gap re-colonization, environmental conditions of gaps started to change, as well as demands of newly established seedlings (Fibich et al., 2013; Isselstein et al., 2002; Puerta-Pinero et al., 2013). Thus, we recorded a high germination from seeds for most sown species, but also high seedling mortality during the first year of observation, indicating high sensitivity of species in the seedling stage (Kotorová & Lepš, 1999; Moore & Elmendorf, 2006). Only annual weedy species *Aphanes arvensis* in *Experiment 1* reached the reproductive stage, but as expected, species did not establish a viable population because of competition with species re-colonizing the gaps in the following season. Despite increasing competition, several non-resident species persisted in gaps till the following seasons and were

able to avoid, or at least postpone, their competitive exclusion. The most successful non-resident species was *Sanguisorba minor* (Beals index value 0.0147), which survived in several individuals till the end of the observation, i.e. till the fourth season after sowing, demonstrating that competitive exclusion can be rather slow for perennial plants that have become established. This suggests that the biotic filter plays a more important role than abiotic conditions for the establishment of these species, and thus might be the main factor preventing the “strangers” from establishing in the community, and to lesser extent accounting for low survival of those species. These non-resident species surviving till the end of both experiments would probably be able to reach the reproductive stage if the gaps were weeded permanently (as suggested by our experience from another experiment, unpublished data by Švamberská).

Unlike in the pot experiments in ideal conditions, development to reproductive age is much slower in field conditions. For example, Frei et al. (2012) estimated that the mean reproduction age of sown species *Campanula thyrsoides* could be 10 years. Similarly, our experience with *Plantago lanceolata* showed, that the species was able to produce strong fertile individuals within two months under ideal conditions in a pot experiment (e.g. in Stachová et al., 2013); in a field experiment, only a fraction of individuals reached maturity within a season, but only in gaps and when transplanted to the field as ca 2 cm tall seedlings (Kelemen et al., 2015), but no individual reached maturity in four years when sown in the present experiment. This suggests that the early establishment phase is the most critical one. In our experiment generally, only resident species were able to establish and survive in intact vegetation. Apparently this is due to their adaptation to local conditions and thus higher persistence in interspecific competition in the community in comparison with non-resident species (Ehrlén et al., 2006).



Comparison of the two experiments suggests large inter-annual variability in seed germination and seedling establishment. Seed germination is strongly influenced by environmental conditions and this effect is species specific (Pitt & Heady, 1978). In our case, the weather was considerably colder throughout the entire season of 2012 than in 2011 with particularly warm spring; precipitation differed from month to month, however their total sum in spring 2011 was higher than in spring 2012 (see Table A.1). These differences could provide worse conditions for species emergence in *Experiment 2*.

Similarly to Kotorová & Lepš (1999), we found that the best predictor of seed germination in the field was laboratory germination of seeds stored in -14 °C. Nevertheless, there were several species germinating well in the field but not in laboratory, probably because of our inability to provide species specific requirements for its germination in the laboratory (Hobbs & Mooney, 1991; Kotorová & Lepš, 1999). This suggests a requirement for additional germination tests to enable better interpretation of seed addition experiments. If seeds emerged well in a germination test but not in a field experiment, we can conclude that species is habitat limited. But if seeds did not emerge in both the germination test and the field, we should be cautious when interpreting the results.

Once established in gaps, seedlings of many species with low Beals index survived well for two or three years at the locality. In contrast, species with high Beals index, mostly resident ones, survived well to the end of the observation period, both in gaps but also in intact vegetation. Similarly, many species differing in CWM and Ellenberg indicator values survived well in gaps but not in control plots. These species with low Beals index and/or with high differences between CWM and Ellenberg indicator values were able to grow in the given abiotic conditions if the competition was eliminated. In most cases, nobody would consider these species to be a part of the local species pool (de Bello et al., 2012; Sádlo et al., 2007; Zobel, 1997). However,

if we use the definition of species pool referring to Butaye et al. (2001), i.e. set of species which are able to pass the abiotic filter (regardless of the possible effect of biotic filter), then at least all the species surviving in gaps to the end of our experiments should be considered as a part of local species pool (and perhaps also the species which were able to establish, but were subsequently outcompeted by the vegetatively spreading surrounding vegetation). Competition obviously plays a crucial role in species composition of the community and is the main force generating the biotic filter (Butaye et al., 2001; Pärtel et al., 2013; Wellstein et al., 2014). Zobel (1992) assumed that the main force decreasing species richness on the level of local species pool is asymmetric competition. Nevertheless, if competition is considered to be a part of the community filter, then all of the species in the community species pool should be those able to withstand the competition. It should be noted that all of the methods of determination of the species pool based on the co-occurrence patterns (e.g. based on the Beals index), or on realized species distribution (Ellenberg indicator values) characterize, in fact, the species realized niche, so species preferences after accounting for the effect of competition. Thus, they correspond to the species pool according to Zobel (1997), and not to Butaye et al. (2001). The same is true also for the expert estimates of species pool based on field experience (Sádlo et al., 2007).

In seed introduction experiments, the long term monitoring of seedling survival is necessary (Ehrlén et al., 2006; Houseman & Gross, 2006; Münzbergová & Herben, 2004; Zobel et al., 1998). Many species can survive in a seedling stage for several years and after that they can or cannot establish a viable population (Vítová & Lepš, 2011; Zobel & Kalamees, 2005). In our case, if seedlings in both experiments were monitored only for one season, results would be interpreted in a largely different way – seedlings survived both in gaps and control plots, so species are probably only dispersal limited. In contrast, after five and four years of monitoring in *Experiment 1* and three years of

monitoring in *Experiment 2* we can conclude that species are limited more by the biotic filter than dispersal.

## **5. Conclusion**

All the species had a preference for establishing in sites without vegetation (i.e. gaps) where the competition is lower. Relatively small gaps (30 x 30 cm) were re-covered by surrounding vegetation after just two years from their creation. However, it seems that one and half years might be a sufficient time for the establishment of resident species, which are then able to withstand the competition of surrounding vegetation, whereas the species with different ecological requirements are often subsequently outcompeted. Moreover, we recorded large inter-annual variability in seed germination and seedling establishment. The traditional ways of determining species pool using the Beals index and Ellenberg indicator values correspond well to Zobel's (1997) definition, i.e. definition based on species realized niches, and thus exclude species potentially sensitive to competitive exclusion. Accordingly, only the comparison between actual species composition and species pool according to Butaye et al. (2001), i.e. species pool determined independently of biotic interaction, is able to reflect the effect of the biotic filter. Comparisons with the species pool delineated on the basis of co-occurrence patterns greatly underestimated the importance of the biotic filter (i.e. of the competition) if the aim of this comparison is determination of the effect of biotic filtering. However, for disentangling the relative importance of biotic and abiotic limitation, the multisite experiments with variation of abiotic conditions are needed.

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### **Supplementary material**

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.actao.2017.10.011>.

## Appendices

Table A.1: Mean monthly temperature (based on altitude and measurements from the meteorological station in České Budějovice, 10 km from Ohrazení) and total monthly precipitation (from the meteorological station in Ledenice, 3 km from Ohrazení) in Ohrazení from 2010 to 2014 (Czech Hydrometeorological Institute České Budějovice).

	Year/Month	1	2	3	4	5	6	7	8	9	10	11	12
Temperature [°C]	2010	-4.1	-1.4	2.8	8.1	12.2	16.7	20.0	17.2	11.3	6.1	4.9	-3.9
	2011	-0.7	-1.6	3.5	10.4	13.3	16.8	16.4	18.2	14.4	7.3	2.1	2.5
	2012	-0.6	-7.7	1.6	5.2	11.7	14.9	16.2	15.1	10.2	5.8	3.1	-1.5
	2013	-1.4	-1.7	-0.4	7.9	11.6	15.5	18.3	17.4	12.1	8.4	3.8	0.6
	2014	0.4	1.4	5.4	9.1	11.5	16.0	18.5	15.3	13.5	10.0	5.5	1.8
Precipitation [mm]	2010	57.3	23.1	31.6	52.1	99.8	90.2	98.5	142.2	66.0	16.4	33.9	32.1
	2011	37.6	9.6	44.7	28.4	86.1	46.6	135.7	47.5	63.7	59.2	1.8	18.1
	2012	52.9	22.8	9.3	47.9	46.6	140.3	152.0	114.4	57.1	42.4	24.1	52.1
	2013	95.7	42.1	29.7	10.9	102.8	221.4	40.5	74.4	43.6	42.9	24.2	10.8
	2014	37.1	8.6	31.1	41.6	136.8	34.2	126.2	99.2	84.7	58.4	15.3	21.0



Table A.2: Temporal changes in the total numbers of seedlings established in gaps and control plots in *Experiment 1*. Recruits from seeds sown in autumn 2010 were not recorded before May 2011 and their numbers are listed as the second number (recruit number of spring/autumn sowing). AphaArve – *Aphanes arvensis*, EuphCypa – *Euphorbia cyparissias*, GaliVeru – *Galium verum*, GeraPrat – *Geranium pratense*, HypePerf – *Hypericum perforatum*, PapaRhoe – *Papaver rhoeas*, PlanLanc – *Plantago lanceolata*, SalvPrat – *Salvia pratensis*, SangMino – *Sanguisorba minor*, ScabOchr – *Scabiosa ochroleuca*, SuccPrat – *Succisa pratensis*, TrifHybr – *Trifolium hybridum*.

Plot	Species	2010		2011			2012		2013	2014	
		Jul	Aug	Sep	May	Jul	Oct	May	Aug	Aug	Aug
Gap	AphaArve	337	276	259	11/11	0/6	0/1	0/0	0/0	0/0	0/0
	EuphCypa	0	1	1	1/7	0/1	0/1	0/1	0/0	0/0	0/0
	GaliVeru	23	23	32	9/9	7/62	4/37	3/33	1/7	0/3	0/1
	GeraPrat	3	6	4	3/36	3/38	1/16	1/12	1/2	0/1	0/1
	HypePerf	26	38	42	35/94	30/108	23/70	17/63	6/29	0/7	0/1
	PapaRhoe	1	0	0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
	PlanLanc	207	253	231	216/152	160/150	137/119	124/113	59/59	46/57	48/44
	SalvPrat	1	1	1	1/1	1/2	0/2	0/0	0/0	0/0	0/0
	SangMino	281	262	235	132/103	113/90	95/74	59/68	18/24	1/4	1/0
	ScabOchr	3	5	7	0/14	0/14	0/7	0/4	0/2	0/1	0/0
	SuccPrat	4	7	9	6/2	9/5	5/8	4/6	4/3	2/2	2/0
	TrifHybr	7	7	4	18/122	0/75	0/0	0/2	0/0	0/0	0/0
	Control	AphaArve	8	2	2	0/0	0/0	0/0	0/0	0/0	0/0
EuphCypa		4	3	1	0/0	0/0	0/0	0/0	0/0	0/0	0/0
GaliVeru		10	8	6	0/1	0/1	0/2	0/2	0/1	0/0	0/0
GeraPrat		4	4	4	2/8	2/14	1/16	0/17	0/8	0/5	0/1
HypePerf		0	0	0	0/2	0/0	0/0	0/0	0/0	0/0	0/0
PapaRhoe		0	0	0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
PlanLanc		1	13	9	23/17	36/24	13/22	8/12	8/9	10/9	9/7
SalvPrat		3	0	0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
SangMino		42	61	60	7/8	6/10	5/11	3/11	3/4	1/0	0/0
ScabOchr		2	2	0	0/3	0/10	0/3	0/2	0/2	0/0	0/0
SuccPrat		12	7	1	1/0	4/0	1/0	1/0	4/0	3/0	5/0
TrifHybr		8	3	3	7/3	2/3	2/3	2/4	0/0	0/0	0/0

Table A.3: List of species used in *Experiment 1* and *Experiment 2* and their characteristics.

Species	Beals index	CWM - Ellenberg indicator value for light availability	CWM - Ellenberg indicator value for soil humidity	CWM - Ellenberg indicator value for soil productivity	Experiment
<i>Acinos arvensis</i>	0.0004	2.28	4.76	2.73	2
<i>Adonis aestivalis</i>	0.0000	0.72	3.76	0.73	2
<i>Alyssum alissoides</i>	0.0001	2.28	3.76	2.73	2
<i>Aphanes arvensis</i>	0.0004	0.72	0.76	1.27	1, 2
<i>Bupleurum falcatum</i>	0.0050	0.72	3.76	0.73	2
<i>Caltha palustris</i>	0.2210	0.28	2.24	2.27	2
<i>Cirsium acaule</i>	0.0017	2.28	3.76	1.73	2
<i>Cirsium canum</i>	0.0502	1.28	1.24	1.27	2
<i>Clinopodium vulgare</i>	0.0299	0.28	2.76	0.73	2
<i>Cyperus fuscus</i>	0.0006	2.28	0.24	0.27	2
<i>Dianthus carthusianorum</i>	0.0071	1.28	3.76	1.73	2
<i>Dianthus deltoides</i>	0.0170	1.28	3.76	1.73	2
<i>Eleocharis palustris</i>	0.0166	1.28	3.24	0.73	2
<i>Eryngium campestre</i>	0.0013	2.28	3.76	0.73	2
<i>Euphorbia cyparissias</i>	0.0315	1.28	3.76	0.73	1, 2
<i>Euphorbia esula</i>	0.0028	1.28	2.76	1.27	2
<i>Falcaria vulgaris</i>	0.0005	0.28	3.76	1.73	2
<i>Galeopsis angustifolia</i>	0.0000	1.28	4.76	0.27	2
<i>Galium verum</i>	0.0757	0.28	2.76	0.73	1
<i>Geranium pratense</i>	0.0336	1.28	1.76	3.27	1
<i>Geum rivale</i>	0.0489	0.72	1.24	0.27	2

Species	Beals index	CWM - Ellenberg indicator value for light availability	CWM - Ellenberg indicator value for soil humidity	CWM - Ellenberg indicator value for soil productivity	Experiment
<i>Geum urbanum</i>	0.0134	2.72	1.76	3.27	2
<i>Gypsophila muralis</i>	0.0003	1.28	1.24	0.73	2
<i>Hyoscyamus niger</i>	0.0000	1.28	2.76	5.27	2
<i>Hypericum hirsutum</i>	0.0010	0.28	1.76	3.27	2
<i>Hypericum perforatum</i>	0.0379	0.28	2.76	0.27	1
<i>Hypericum tetrapterum</i>	0.0109	0.28	1.24	1.27	2
<i>Inula britannica</i>	0.0016	1.28	0.24	1.27	2
<i>Inula ensifolia</i>	0.0017	2.28	4.76	1.73	2
<i>Juncus inflexus</i>	0.0094	1.28	0.24	0.27	2
<i>Lathyrus niger</i>	0.0038	1.72	3.76	0.73	2
<i>Linaria vulgaris</i>	0.0050	1.28	2.76	1.27	2
<i>Lycopsis arvensis</i>	0.0002	0.28	2.76	0.27	2
<i>Lycopus europaeus</i>	0.0610	0.28	2.24	3.27	2
<i>Lychnis viscaria</i>	0.0070	0.28	3.76	1.73	2
<i>Lythrum salicaria</i>	0.0912	0.28	1.24	-	2
<i>Medicago falcata</i>	0.0073	1.28	3.76	0.73	2
<i>Melilotus officinalis</i>	0.0009	1.28	3.76	0.73	2
<i>Mentha aquatica</i>	0.0174	0.28	2.24	1.27	2
<i>Meum athamanticum</i>	0.0100	1.28	1.76	0.73	2
<i>Myosurus minimus</i>	0.0001	1.28	0.24	1.27	2
<i>Myrrhis odorata</i>	0.0003	0.28	1.76	3.27	2
<i>Origanum vulgare</i>	0.0024	0.28	3.76	0.73	2
<i>Papaver rhoeas</i>	0.0005	0.72	1.76	2.27	1, 2
<i>Plantago lanceolata</i>	0.2320	0.72	-	-	1

Species	Beals index	CWM - Ellenberg indicator value for light availability	CWM - Ellenberg indicator value for soil humidity	CWM - Ellenberg indicator value for soil productivity	Experiment
<i>Potentilla palustris</i>	0.0851	1.28	2.24	1.73	2
<i>Potentilla recta</i>	0.0005	2.28	3.76	1.73	2
<i>Potentilla supina</i>	0.0007	0.28	1.24	3.27	2
<i>Prunella grandiflora</i>	0.0050	0.28	3.76	0.73	2
<i>Ranunculus arvensis</i>	0.0001	0.72	2.76	-	2
<i>Ranunculus sceleratus</i>	0.0031	0.72	2.24	5.27	2
<i>Rumex acetosella</i>	0.0189	1.28	3.76	1.73	2
<i>Salvia pratensis</i>	0.0258	1.28	3.76	0.27	1, 2
<i>Sanquisorba minor</i>	0.0147	0.28	3.76	1.73	1, 2
<i>Scabiosa ochroleuca</i>	0.0089	1.28	3.76	1.73	1, 2
<i>Scutellaria galericulata</i>	0.0511	0.28	2.24	2.27	2
<i>Seseli osseum</i>	0.0002	1.28	4.76	2.73	2
<i>Schoenoplectus lacustris</i>	0.0005	1.28	4.24	2.27	2
<i>Silene noctiflora</i>	0.0003	0.28	3.76	1.27	2
<i>Solanum dulcamara</i>	0.0097	0.28	1.24	4.27	2
<i>Stachys palustris</i>	0.0047	0.28	0.24	2.27	2
<i>Succisa pratensis</i>	0.1940	0.28	0.24	1.73	1
<i>Thalictrum minus</i> ssp. <i>minus</i>	0.0124	0.72	3.76	0.73	2
<i>Trifolium hybridum</i>	0.0568	0.28	0.76	1.27	1
<i>Trifolium montanum</i>	0.0206	1.28	3.76	1.73	2
<i>Veronica anagalis-aquatica</i>	0.0018	0.28	2.24	2.27	2

## **Chapter 4**

### **Experimental assessment of biotic and abiotic filters driving community composition**

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# Experimental assessment of biotic and abiotic filters driving community composition

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## Abstract

Species occurrence in a site can be limited by both the abiotic environment and biotic interactions. These two factors operate in concert, but their relative importance is often unclear. By experimentally introducing seeds or plants into competition-free gaps or into the intact vegetation, we can disentangle the biotic and abiotic effects on plant establishment.

We established a seed sowing/transplant experiment in three different meadows. Species were introduced, as seeds and pre-grown transplants, into competition-free gaps and the intact vegetation. They included 12 resident plants from the locality and 18 species typical for different habitats. Last two years, gaps were overgrown with vegetation from surrounding plants and we observed the competitive exclusion of our focal plants. We compared plant survival with the expected occurrence in target locality (Beals index).

Many of the species with habitat preferences different from our localities were able to successfully establish from seeds and grow in the focal habitat if competition was removed. They included species typical for much drier conditions. These species were thus not limited by the abiotic conditions, but by competition. Pre-grown transplants were less sensitive to competition, when compared to seedlings germinated from seeds. Beals index significantly predicted both

species success in gaps and the ability to withstand competition. Survival in a community is dependent on the adaptation to both the abiotic environment and biotic interactions. Statistically significant correlation coefficients of the ratio of seedling survival in vegetation and gaps with Beals index suggest the importance of biotic interactions as a determinant of plant community composition.

To disentangle the importance of abiotic and biotic effect on plant establishment, it is important to distinguish between species pool as a set of species typically found in given community type (determined by Beals index) and a set of species for which the abiotic conditions are suitable.

### **Keywords**

Abiotic filter, Beals index, Biotic filter, Competitive exclusion, Sowing and Transplanting experiment, Species pool.



## 1 Introduction

Each plant community is formed by a subset of the species pool, i.e. a subset of all species available to colonize a given site (Cornell & Harrison, 2014). The basic question is then which mechanisms decide which species from the species pool will finally form the community.

Dispersal limitation is an important factor for species occurring in the region. For example, the successful establishment of a single individual often requires the arrival of hundreds or thousands of seeds (Vítová & Lepš, 2011). Interestingly, low favourability of a particular habitat can be overcome by massive numbers of propagules (Fibich et al., 2018). Nevertheless, the main processes limiting species occurrence in a local scale are abiotic environment and biotic interactions (HilleRisLambers et al., 2012). Abiotic environment is influenced by many factors such as temperature and precipitations, availability of nutrients and other resources which plants need for their survival. Biotic interactions include the relationships among living organisms in a community. Although other biotic interactions (e.g. mycorrhiza, facilitation, pollination, herbivory) play an important role in plant communities, competition is considered a significant factor that limits co-occurrence among species (Grubb, 1977, Palmer, 1994, Wellstein et al., 2014, Li et al., 2018). Furthermore, studies typically use competition as biotic filter in community assembly studies (HilleRisLambers et al., 2012).

In local communities, abiotic environment and biotic interactions operate simultaneously, but their relative importance in structuring local community composition is often unknown and difficult to disentangle on the basis of observational data only (Araújo & Rozenfeld 2014, Kraft et al., 2015, Cadotte & Tucker, 2017). Although many studies based on observational data use the concept of environmental filtering as the effect of abiotic environment only, they in fact reflect environmental filtering which include not only the species ability to survive under specific environmental condition of the

given site but also withstand under the competition of other species present in a given site (Cadotte & Tucker, 2017). By this approach, the effect of biotic interactions on local community structuring could be significantly underestimated. Very probably, only experimental approach manipulating biotic interactions in species communities can reliably distinguish the effect of abiotic environment and biotic interactions (Kraft et al., 2015). Nevertheless, some studies (e.g. D'Amen et al., 2018) claim that the analysis based on combination of observational data and null models is able to separate the effect of biotic filter from the environmental filtering.

Sowing and transplant experiments are excellent approaches to disentangle the effects of various “filters” on community composition (Turnbull et al., 2000, Zobel & Kalamees, 2005, Švamberková et al., 2017). Excluding dispersal limitation, failure to establish after sowing or transplanting can be attributed to habitat limitation. There are many examples of species that are able to grow in given abiotic conditions, but are excluded by the biotic filter. These species are present within a regional species pool, but are representative for very different habitats. In order to examine the ability of these species to withstand the abiotic conditions of a given habitat, seed/transplant introduction experiments, where biotic filters (especially competition) are experimentally removed, are required (Cornell & Harrison, 2014, Švamberková et al., 2017). Species that successfully establish in competition-free experimental plots should be considered a part of the species pool defined as species able to pass only through abiotic filters (Butaye et al., 2001) while they cannot be a part of usually used species pool defined as species able to pass through the both abiotic and biotic filters (Zobel, 1997). Comparing plant performance across artificial competition-free gaps and intact vegetation (where the biotic and abiotic filters work in concert) can separate the importance of biotic and abiotic effects on plant establishment (HilleRisLambers et al., 2012, Kraft et al., 2015).

Many species require some type of gap (i.e. plot with reduced competition) in natural settings (Puerta-Piñero et al., 2013). In nature, gaps are the result of various disturbances, which create competition-free microhabitats and enable species to germinate and subsequently establish. When studying species establishment in seed/transplant introduction experiments, competition can be artificially excluded (or substantially reduced) using experimentally generated gaps (Vítová et al., 2017, Kotorová & Lepš, 1999, Tofts & Silvertown, 2002, Lemke et al., 2015). In gaps, competition for light, nutrients, and water is reduced (Frei et al., 2012, Lemke et al., 2015). On the other hand, species present in gaps are more exposed to extreme environmental conditions, such as desiccation (Kotorová & Lepš, 1999, Vítová & Lepš, 2011). Seedlings growing in gaps are also more apparent to herbivores than seedlings occurring within intact vegetation (Gustafsson et al., 2002, Lemke et al., 2015). Both gap size and the time of their formation play a crucial role in the establishment of new seedling species, affecting which species is first to colonize this gap. Even so, the establishment of seedlings in a community is unlikely and seedling survival does not always assure the long-term persistence of the species (Zobel, 1997, Gustafsson et al., 2002, Vítová & Lepš, 2011).

Most species are filtered out of a community during the germination phase and subsequent establishment of individuals (Kotorová & Lepš, 1999). The importance of factors (both abiotic and biotic) affecting species survival in a community can differ in different life stages of plants because their regeneration and realised niches are often quite distinct (Grubb, 1977). One of the primary reasons for the absence of some species in a community is their inability to establish in the presence of competition from other species. Although biotic interactions affect plants in later stages of their lifespan, the effect is not as strong as in their early phases of seedling development because older individuals are more biotic resistant than small seedlings (Tofts

& Silvertown, 2002, Bennett et al. 2016). It suggests that competitive exclusion of well-established individuals in a community may be rather slow (Adler et al., 2010). The studying of different life stages is thus necessary to get a complete insight into local processes influencing a whole life cycle of species.

When comparing the effect of abiotic and biotic filter on species composition of a local community, we need to define a local species pool, ideally as the ability of a given species to establish based on the abiotic environment alone without the effect of competition filter (Butaye et al., 2001, Švamberková et al., 2017). There are various methods to help determine the species pool: Ellenberg indicator values (Pärtel et al. 1996, Zobel, 1997, Zobel et al., 1998), functional traits (Sonnier et al., 2010, de Bello et al., 2012, Moor et al., 2015), phytosociological knowledge from local experts (Sádlo et al., 2007), Beals index (Ewald, 2002, Münzbergová & Herben, 2004, Botta-Dukát, 2012) or ordination methods (Brown et al., 2019). Nevertheless, with exception of experimental approach, all other methods of species pool determination reflect the influence of the both biotic and abiotic filters. Nevertheless, because experimental approach is very time consuming, Beals index can be quite invaluable approach to species pool assessment. While most of the above mentioned approaches for determination of species pool size depend on either expert's phytosociological experience or models corresponding with environmental gradients, methods related to Beals index employ information based on multivariate structure of real data. It compares species co-occurrence of examined species with other species of the appropriate habitat from a database of many phytosociological relevés (Chytrý & Rafajová, 2003), reflecting thus concerted effect of biotic and abiotic filters. Although Beals index is, in fact, also one of phytosociological methods, neither any classification nor any environmental gradients determined in advance are employed. It

transforms a species pool definition from a strictly determined set of species into species occurrence probability (Botta-Dukát 2012).

We conducted a seed/transplant introduction experiment across three different meadow habitats (Appendix 1). Species, both resident in the locality and typical for different habitats (not expected to be part of the species pool), were introduced as either seeds or pre-grown transplants into either competition-free gaps or the intact vegetation. Subsequently, we computed the expected occurrence of species from our experiment on target habitats using Beals index derived from the species co-occurrence pattern in the National phytosociological database (Chytrý & Rafajová, 2003) and compared these results with the real plant survival from our experiment. During the last two years of the experiment, surrounding vegetation was left to overgrow into gaps and we observed the competitive exclusion of our focal plants.

Our study aimed to: 1) compare the species pool determined by seed/transplant introduction experiment with the species pool delimited using Beals index; 2) disentangle the importance of the biotic and abiotic effects on plant establishment via the removal of competition; and 3) compare the survival of target species in different life stages (i.e. sown as seeds and planted as pre-grown transplants) and their competitive exclusion.

We expect that: 1) some species determined by Beals index as improbable to occur in target habitats will be able to establish experimentally in competition-free gaps. 2) Both abiotic and biotic effects will influence the species establishment but competition will be the most important determinant. We suggest that if survival is affected by both intrinsic characteristics of individual species and their interaction with the environment, the more an environment discriminates among species, correlations of species successes across ecologically different habitats should be weaker. In this way, we can identify, whether the discrimination among species is more pronounced in gaps (suggesting mainly effect of abiotic environment),

or in controls (discrimination by the whole habitat including competition by extant vegetation). 3) Competitive exclusion will be more important for seedlings growing from seeds in the field than for pre-grown transplants.

## 2 Materials and Methods

### 2.1 Study site

The experiment was conducted in the north-eastern region of Czech Republic, in a species rich locality named Strašovský rybník (50°6'N, 15°31'E, 217 m a.s.l.). The study site contained a pond, surrounded by a mosaic of wet meadows and fens. A littoral zone of the pond, with stands of *Phragmites australis*, accounted for the largest area. These reed beds are bordered by stands of tall sedges; with the remaining part of the locality being composed of *Molinion* and *Arrhenatherion* meadows with small patches of alluvial meadows and calcareous fens. The climatic conditions during the years of our experiment are provided in Table S1.

Our experiment was carried out in locations (at least 200 m distant from each other), which were referred to according to their two main dominant plants: 1) „*Carex acuta-Carex panicea*” (50°6'0.8"N, 15°31'0.5"E), 2) „*Deschampsia caespitosa-Carex tomentosa*” (50°5'59.4"N, 15°31'11.3"E), and 3) „*Sesleria uliginosa-Briza media*” (50°5'57.6"N, 15°31'14.4"E) habitats respectively. Moisture regime of all three habitats was dynamic in time (Fig. S1, Table S4) and contained distinct species compositions (Fig. S4). They differed in overall productivity (Tables S2 and S4) and several soil characteristics (Table S3). Between 2013 (i.e. the first year of our experiment) and 2016, all three habitats, as well as our experimental plots, were mowed regularly twice a year at the end of June and in mid-October; with the exception in 2015 when only one mowing event occurred due to an abnormally dry summer. Since 2017, the study locations, including our plots, were mowed only once a year.

## 2.2 Seed introduction experiment

To assess species establishment and survival in the presence and absence of competition, we introduced seeds and pre-grown young individuals (transplants) of both resident and non-resident plant species to our three habitats (Appendix 1). We selected species with good germination rate (knowledge from previous studies, e.g. Švamberková et al., 2017) from species typical for the region of our target locality. A species residence was determined for individual habitats based on whether a species was present in at least one of the five phytosociological relevés (5x5m) of given habitat type recorded in June 2014 (i.e. “habitat residency”, Table S5). We also used an additional classification, where any species present in at least one habitat type (according to phytosociological relevés from June 2014) or found within the study site during the nature conservation-screening inventory by Jan Horník et al. (unpublished data) were considered residents for the entire locality (i.e. “whole locality residence”, Table S5). Non-resident species include species typical for both drier and wetter conditions than target locality. Nevertheless, all the non-resident species can be considered part of the regional species pool, because they are found in close surrounding (see maps of species distribution at [www.pladias.cz/en/](http://www.pladias.cz/en/), accessed on May 8, 2019) and their propagules are thus able to reach the target locality. Seeds and transplants were placed into either control plots, with the intact vegetation, or artificially created gaps.

We created 30 artificial gaps (40 x 40 cm) in two replications in each habitat type, each by digging a hole 20 cm deep, and refilling with soil from the target habitat. To prevent competition from surrounding vegetation, gaps were weeded regularly two times a year (in spring and autumn) until 2016 when gaps were weeded once during spring for the last time. In 2017 and 2018 we observed the potential competitive exclusion of established individuals in gaps from the neighbouring vegetation. Control plots of the same size were established without any

manipulation of extant vegetation. Seeds from 30 species, 12 residents and 18 non-residents, (Table S5) were sowed to the centre of 20 x 20 cm plots within gap and control treatments in spring 2013. We used seeds from a commercial supplier (Planta Naturalis, Markvartice, Czech Republic). Each species was sowed separately in its own plot. Within a plot, 200 seeds of species, which had a seed weight of one seed 1 mg or more, were sown for each plant species. We sowed more than 200 seeds for plant species with seeds lighter than 1 mg because small seeds are expected to have reduced probability of establishment (Cornelissen et al., 2003). We used an *ad hoc* formula to increase the amount of seeds lighter than 1mg:  $x = 200(1 - \log m)$ , where  $x$  was a weight of seeds required for sowing and  $m$  a weight of one seed in mg. This process helped provide enough individuals for the assessment of mortality. The success of seedling establishment and survival was subsequently expressed as the number of survivors out of the number of the sown seeds. The proportion of seedling recruitment and survival was monitored from 2013 to 2018 several times per year.

### **2.3 Transplant experiment**

Transplants of the same species used in the seed introduction experiment (Table S6) were pre-grown in jiffy peat pots in a growth chamber (12 h light and 12 h darkness, 19°C) during 50 days. These transplants were planted within a 10-cm wide border region of the same gap and control plots as those used for the seed introduction experiment. We completely excluded six species from the transplant experiment (i.e. from all habitat types) and four others only from *Carex acuta-Carex panicea* habitat and from one replication of *Deschampsia cespitosa-Carex tomentosa* habitat because their pre-growth was unsuccessful (Table S6). In all other cases, three transplants of each species were planted and their initial height and number of leaves were measured (Table S6). All transplants were planted in target habitats at the end of May 2013, with the exception of the *Carex acuta-Carex panicea* habitat, where they were transplanted in the second half of



June because of an unexpected flood. Transplant survival was monitored from 2013 to 2018 several times per year and subsequently compared with success of seedlings in the seed introduction experiment.

## 2.4 Data analysis

We used the ratio of the living individuals, to the number of seeds sown/planted transplants as our measurement of success for individual species. This measurement was characterized for each sampling date and combination of habitat and treatment (i.e. gap/control). Each value is represented as the average of two replications. For convenience, we use the term *survival* throughout the text, but acknowledge that it is the outcome of germination (in case of sown seeds) and establishment success and survival.

Seedling and transplant survival were analysed using a repeated measures (split-plot) ANOVA in Statistica 13 (StatSoft, 2015), where time and treatment were modelled as within subject effects and species residence as a between subject effect. This analysis was carried out for each habitat separately. In a subsequent analysis, habitat type, time, and treatment were modelled as within subject effects and species residence as a between subject effect. Species identity was not included in these analyses. Prior to both analyses, survival of seedlings and transplants were arcsine transformed to help meet assumptions for ANOVA.

For each sown species and habitat, we calculated a Beals index (Beals, 1984) as an average of the conditional probability of a focal species occurrence, provided the presence of the other species in the target habitat relevé (five 5x5m relevés per habitats were recorded in June 2014):  $P_{ij} = \frac{1}{S_i} \sum_{k \neq j} \frac{N_{jk}}{N_k}$  where  $P_{ij}$  is the estimate of probability to find species  $j$  in habitat  $i$  (i.e. the Beals index),  $S_i$  is the number of species in a relevé characterized by habitat  $i$  (minus 1 if species  $j$  is present),  $N_{jk}$  is the number of joint occurrences of species  $j$  and  $k$ , and

$N_k$  is number of occurrences of species  $k$  in the reference database, where  $k$  is index of species in the relevé (Münzbergová & Herben, 2004). The Czech National Phytosociological Database (Chytrý & Rafajová, 2003) in stratified form to reduce oversampling of some areas (Těšitel et al., 2015) was used as the reference database. After the subsampling, the reference database contained 31,512 relevés. We used the weighted form of the Beals index, i.e. the function “beals” of “type” = 2 (abundances were used to compute weighted averages of conditioned probabilities instead of the plain average used in the above formula) in the R-package “vegan” (Oksanen et al., 2019). The index was calculated for each relevé separately and the average value across the five phytosociological relevés per each habitat was subsequently used. Beals index can be thus considered a measure of favourability of habitat for a given species.

For each combination of observation time, habitat, and species, we calculated average survival (from two replications) in gaps and controls, and the ratio of average control/gap survival. This ratio provided an estimate of competitive reduction, where a value of 1 denotes no effect of competition and 0 signifies the strongest effect of competition. In cases where survival in gaps was zero, the effect of competition could not be estimated and thus was not considered in subsequent analyses performed in Statistica 13 (StatSoft, 2015). We tested for significant correlations between Beals index and species survival across the different treatments to examine whether we can predict habitat favourability for a species. We also calculated the correlation of species survival always between two different habitats (each habitat taken in pair with each other habitat), for gaps and controls separately to identify, whether the differentiation in species survival between two habitats is determined mainly by abiotic environment or biotic interactions. Higher correlation coefficients for species survival in gaps than for control plots mean the more important

discrimination of species between these two habitats by the biotic interactions than by abiotic environment.

### 3 Results

#### 3.1 Seed germination and survival of seedlings in contrast to transplants

Most of the 30 sown species succeeded in germination in target habitats (the highest germination success averaged over the three habitats was 42% in a gap for *Plantago lanceolata*, and 12% in intact vegetation for *Nardus stricta*, median was 5.43% in gap and 0.62 in intact vegetation). Only two of the 18 non-resident species (*Bistorta major* and *Viola hirta*) did not successfully germinate in any habitat type. *Lathyrus vernus* was unable to germinate in the *Carex acuta-Carex panicea* habitat, but it was able to germinate in the other two habitats, but only in gaps. *Bupleurum falcatum* successfully germinated in *Carex acuta-Carex panicea* and *Deschampsia caespitosa-Carex tomentosa* habitat gaps, but it was unable to germinate in *Sesleria uliginosa-Briza media* habitat. All 12 sown resident species germinated in all habitat types.

Both resident and non-resident sown species achieved higher rates of germination and survival in gaps compared to intact vegetation in all habitat types (Tables 1 and 2, Fig. 1). Similar to sown species, transplants generally survived better in gaps than intact vegetation, but only in *Carex acuta-Carex panicea* and *Deschampsia caespitosa-Carex tomentosa* habitats (Table 2, Fig. 2). In the *Carex acuta-Carex panicea* habitat, gaps were initially stressful for transplants: their survival in the first year was higher in intact vegetation compared to gaps (Fig. 2a)). We did not observe a significant difference between transplant survival in gaps and vegetation in the *Sesleria uliginosa-Briza media* habitat (Table 2, Fig. 2c)) which also displayed the lowest mean dry biomass values (Table S2). Thus, the habitat with the lowest difference between species survival in gaps and intact vegetation

(Table 2) was also associated with the lowest mean biomass (Table S2).

Table 1: Repeated Measures ANOVA of seedling/transplant survival of resident and non-resident species (“whole locality residence”) in gaps and control plots (Treatment) during the experiment for all habitat types (taken in one analysis together). Statistically significant ( $p < 0.05$ ) results are in bold.

	Seedlings			Transplants		
	Degree of freedom	F	p	Degree of freedom	F	p
Residence	<b>1,28</b>	<b>16.8</b>	<b>&lt;0.001</b>	<b>1,18</b>	<b>135.737</b>	<b>&lt;0.001</b>
Habitat	2,56	0.588	0.559	<b>2,36</b>	<b>8.892</b>	<b>0.001</b>
Habitat*Residence	2,56	0.988	0.379	2,36	0.97	0.389
Time	<b>11 308</b>	<b>42.914</b>	<b>&lt;0.001</b>	<b>10 180</b>	<b>136.713</b>	<b>&lt;0.001</b>
Time*Residence	<b>11 308</b>	<b>5.293</b>	<b>&lt;0.001</b>	10 180	0.699	0.725
Treatment	<b>1,28</b>	<b>67.06</b>	<b>&lt;0.001</b>	<b>1,18</b>	<b>8.845</b>	<b>0.008</b>
Treatment*Residence	<b>1,28</b>	<b>11.525</b>	<b>0.002</b>	1,18	0.012	0.913
Habitat*Time	<b>22 616</b>	<b>3.794</b>	<b>&lt;0.001</b>	<b>20 360</b>	<b>3.149</b>	<b>&lt;0.001</b>
Habitat*Time*Residence	22 616	0.92	0.568	20 360	0.912	0.572
Habitat*Treatment	2,56	2.582	0.085	<b>2,36</b>	<b>3.303</b>	<b>0.048</b>
Habitat*Treatment*Residence	2,56	0.533	0.59	2,36	0.176	0.839
Time*Treatment	<b>11 308</b>	<b>35.411</b>	<b>&lt;0.001</b>	<b>10 180</b>	<b>3.497</b>	<b>&lt;0.001</b>
Time*Treatment*Residence	<b>11 308</b>	<b>2.715</b>	<b>0.002</b>	10 180	0.767	0.66
Habitat*Time*Treatment	<b>22 616</b>	<b>2.528</b>	<b>&lt;0.001</b>	<b>20 360</b>	<b>1.783</b>	<b>0.021</b>
Habitat*Time*Treatment*Residence	22 616	1.528	0.58	20 360	0.789	0.728

The effect of competition differed among habitats in time both for sown species and transplants (Table 1). In cases when residency was defined across the “whole locality” (i.e. “whole locality residence”), resident species survived significantly better than non-residents, both in gaps and vegetation across all habitats (Tables 1 and 2). When residency was defined within a habitat (i.e. “habitat residency”) resident sown species achieved higher rates of survival than non-residents, but this effect was only significant in the *Sesleria uliginosa-Briza media* habitat (Table S7). Contrary, “habitat residency” influenced the survival of transplants neither in gaps nor intact vegetation across any habitat type (Table S10).

Table 2: Repeated Measures ANOVA of seedling/transplant survival of resident and non-resident species ("whole locality residence") in gaps and control plots (Treatment) during the experiment in different habitat types (separate analysis for each habitat type). Statistically significant results ( $p < 0.05$ ) are in bold.

		Residence	Time	Time* Residence	Treatment	Treatment*Residence	Time* Treatment	Time* Treatment* Residence	
Seedlings	Degree of freedom	1,28	11 308	11 308	1,28	1,28	11 308	11 308	
	<i>Carex acuta-Carex panicea</i> habitat	F	<b>17.707</b>	<b>33.83</b>	<b>4.288</b>	<b>56.99</b>	<b>10.241</b>	<b>18.064</b>	<b>2.112</b>
		p	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>0.003</b>	<b>&lt;0.001</b>	<b>0.019</b>
	<i>Deschampsia caespitosa-Carex tomentosa</i> habitat	F	<b>8.901</b>	<b>26.24</b>	<b>3.249</b>	<b>51.89</b>	<b>9.041</b>	<b>31.918</b>	<b>3.633</b>
		p	<b>0.006</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>0.006</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>
	<i>Sesleria uliginosa-Briza media</i> habitat	F	<b>18.046</b>	<b>41.51</b>	<b>5.282</b>	<b>62.021</b>	<b>10.006</b>	<b>20.258</b>	1.245
		p	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>0.004</b>	<b>&lt;0.001</b>	0.256
	<i>Carex acuta-Carex panicea</i> habitat	Degree of freedom	<b>1,18</b>	<b>10 180</b>	10 180	<b>1,18</b>	1,18	<b>10 180</b>	10 180
		F	<b>8.241</b>	<b>94.08</b>	0.664	<b>6.202</b>	0.059	<b>4.102</b>	0.748
		p	<b>0.01</b>	<b>&lt;0.001</b>	0.756	<b>0.023</b>	0.811	<b>&lt;0.001</b>	0.679
Transplants	<i>Deschampsia caespitosa-Carex tomentosa</i> habitat	Degree of freedom	<b>1,22</b>	<b>10 220</b>	<b>10 220</b>	<b>1,22</b>	1,22	10 220	10 220
		F	<b>7.448</b>	<b>47.5</b>	<b>1.922</b>	<b>12.028</b>	0.048	1.595	0.241
		p	<b>0.012</b>	<b>&lt;0.001</b>	<b>0.043</b>	<b>0.002</b>	0.828	0.109	0.992
	<i>Sesleria uliginosa-Briza media</i> habitat	Degree of freedom	<b>1,22</b>	<b>10 220</b>	10 220	1,22	1,22	10 220	10 220
		F	<b>7.766</b>	<b>89.7</b>	0.759	1.803	0.227	1.594	1.819
	p	<b>0.011</b>	<b>&lt;0.001</b>	0.669	0.193	0.638	0.11	0.059	

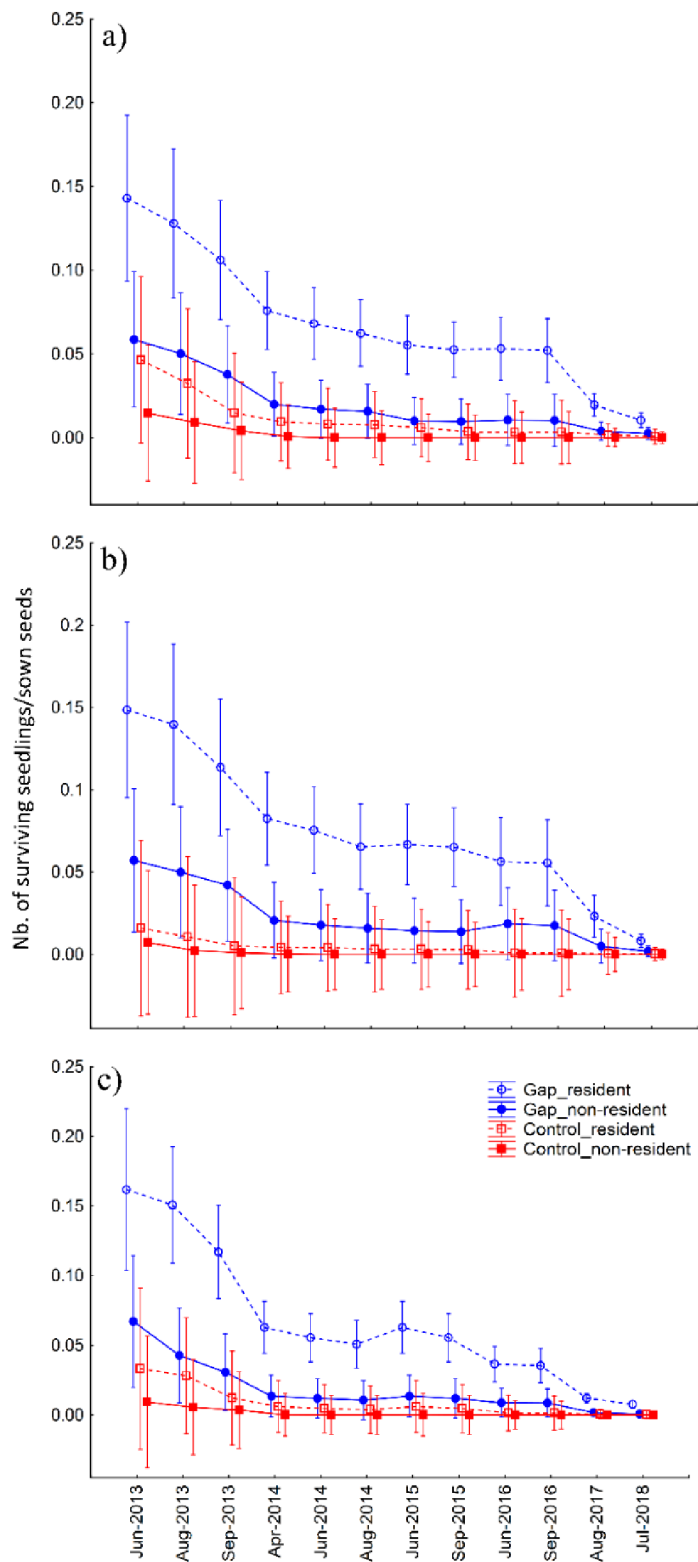


Fig. 1: Average survival of resident and non-resident seedlings in gaps and intact vegetation (Control) during the experiment within each habitat: a) *Carex acuta-Carex panicea*, b) *Deschmpsia caespitosa-Carex tomentosa*, c) *Sesleria uliginosa-Briza media* habitat. Error bars represent 95% confidence intervals.

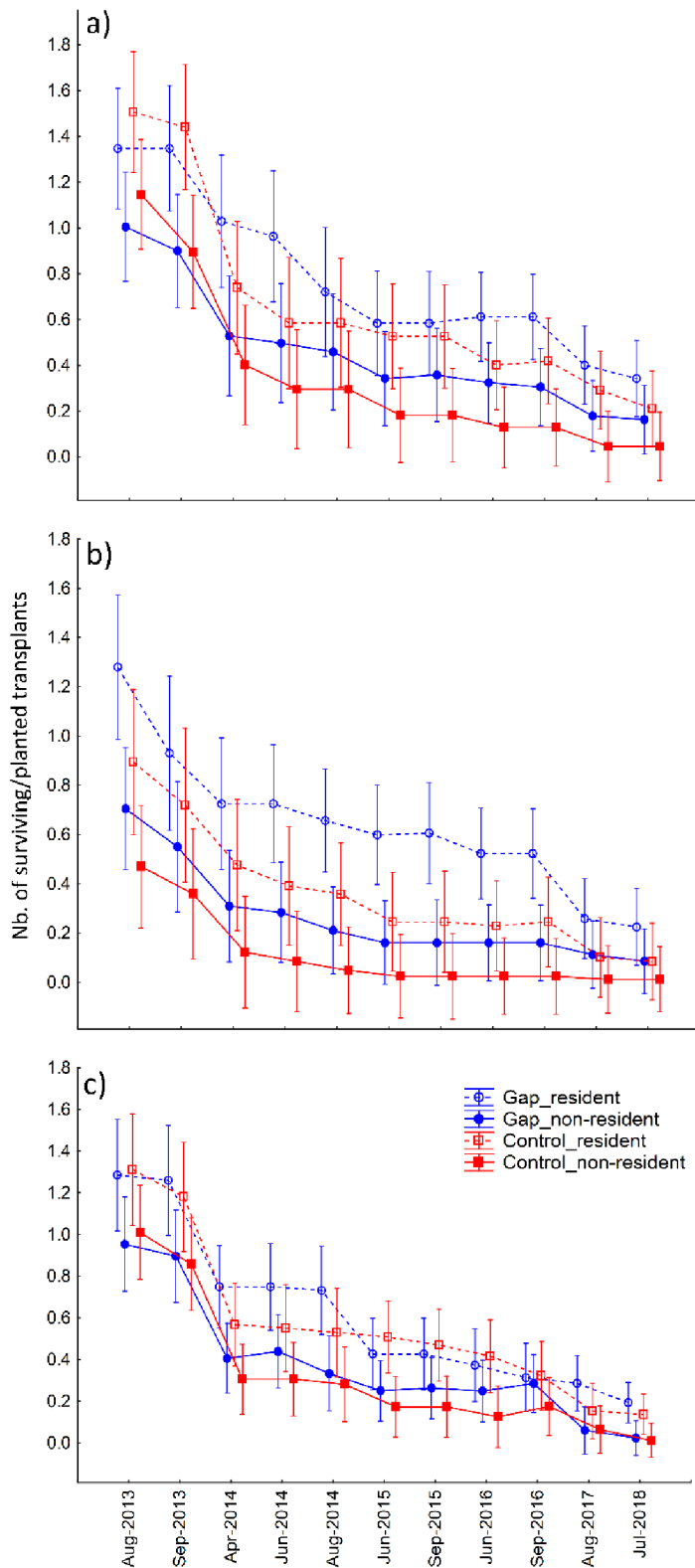


Fig. 2: Average survival of resident and non-resident transplants in gaps and intact vegetation (Control) during the experiment within each habitat: a) *Carex acuta-Carex panicea*, b) *Deschmipsia caespitosa-Carex tomentosa*, c) *Sesleria uliginosa-Briza media* habitat. Error bars represent 95% confidence intervals.

If competition was removed, many non-resident species were able to establish from seeds and grow in the focal habitat (Fig. 3a, b)). They included species typical for much drier conditions (i.e. *Carlina acaulis*, *Geranium sanguineum*, *Nardus stricta*, *Origanum vulgare*, *Sanguisorba minor*, *Thymus pulegioides*, *Trifolium montanum*), and forest species (*Hypericum hirsutum*, *Lathyrus vernus*). In the case of seed sowing experiment, none of these species survived within the intact vegetation. On the other hand, there were species, both resident and non-resident, which were unable to establish in intact vegetation as seeds in the seed introduction experiment, but were able to survive as transplants: *Carlina acaulis*, *Filipendula ulmaria*, *F. vulgaris*, *Geranium pratense*, *G. sanguineum*, *Hypericum hirsutum*, *Nardus stricta* and *Sanguisorba officinalis*.

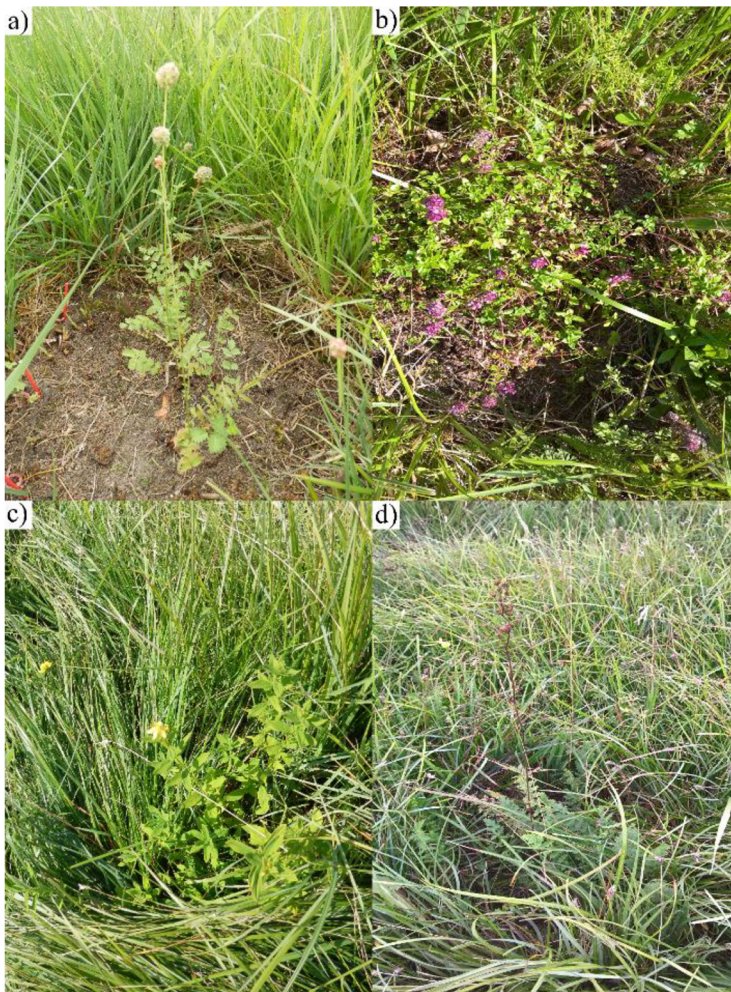


Fig. 3: Examples of non-resident species well prospering in competition-free gaps in 2015 (a) *Sanguisorba minor*, b) *Thymus pulegioides*) and in non-weeded gaps in 2018 (c) *Hypericum hirsutum*, d) *Filipendula vulgaris*).



During the last two years (i.e. 2017 and 2018), when gaps were no longer controlled for weeds, the differences of survival of species in gaps and vegetation began to diminish, especially in the case of seed sowing experiment (Fig.1). Nevertheless, many non-resident species that became established in gaps were able to survive also in overgrown gaps. The most successful non-resident species, which survived until the summer 2018 (Fig.3c, d), were *Geranium sanguineum*, *Hypericum hirsutum*, *Nardus stricta*, *Origanum vulgare*, *Sanguisorba minor*, *Thymus pulegioides* and *Trifolium montanum*. Several (e.g., *Hypericum hirsutum*, *Sanguisorba minor*, *Thymus pulegioides*) were even flowering in 2018. This suggests that once a species has established, its rapid competitive exclusion is difficult and unlikely.

### **3.2 Seedling/transplant survival compared with species respective Beals index values and among different habitat types**

Beals index (range for our species was from 0.346 to 0.001, Table S5) was a significant predictor for seedling survival in gaps and control plots (Table S8). Seedlings of species with high Beals index (i.e. species more probable to occur in the target habitat) survived better in both gaps and intact vegetation, than species with a low Beals index (i.e. species more improbable to occur in the target habitat). Nevertheless, there were many species with low Beals index (range from 0.007 to 0.08, Table S5), and thus improbable to occur in the target habitat, which survived if competition was removed but not under competition (e.g. *Carlina aculis*, *Geranium sanguineum*, *Hypericum hirsutum*, *Lathyrus vernus*, *Nardus stricta*, *Origanum vulgare*, *Sanguisorba minor*, *Thymus pulegioides*, *Trifolium montanum*). In the case of transplants, there were also significant correlations of survival with Beals index but not so often, and what is more, there was practically no significant correlation in the *Sesleria uliginosa-Briza media* habitat (compare Table S8 and S11).

Correlation coefficients between seedling survival and Beals index were generally higher in intact vegetation than in gaps (Fig. 4).

For transplants, the trend was similar but weaker, especially in the case of *Sesleria uliginosa-Briza media* habitat where correlation coefficients were higher for intact vegetation only during 2015 and 2016. During other time points, correlations were even lower for intact vegetation than for gaps (Fig. 5). Also, correlations between the ratio of survival in vegetation and in gaps and Beals index were significant and positive in the case of seedlings (Table S8). On the other hand, for transplants, they were significant only in *Carex acuta-Carex panicea* habitat in 2015 and 2016 (Table S11). Correlations between both seedling and transplant survival in non-weeded gaps (last weeded in spring 2016) and Beals index were not significant. Similarly, correlations between the ratio of surviving both seedlings and transplants in vegetation and gaps and Beals index started to weaken once weeding stopped (Tables S8 and S11).

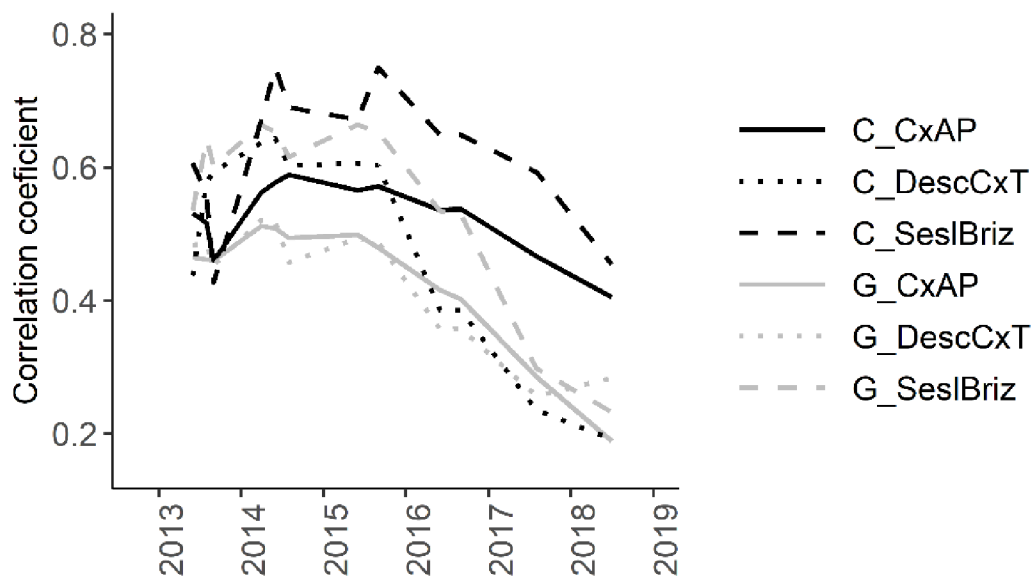


Fig. 4: Values of Pearson's correlation coefficients between seedling survival and Beals index across years and different habitat types (*CxAP* = *Carex acuta-Carex panicea*, *DescCxT* = *Deschampsia caespitosa-Carex tomentosa*, *SeslBriz* = *Sesleria uliginosa-Briza media* habitat, C = control plots – black line, G = gap – grey line).

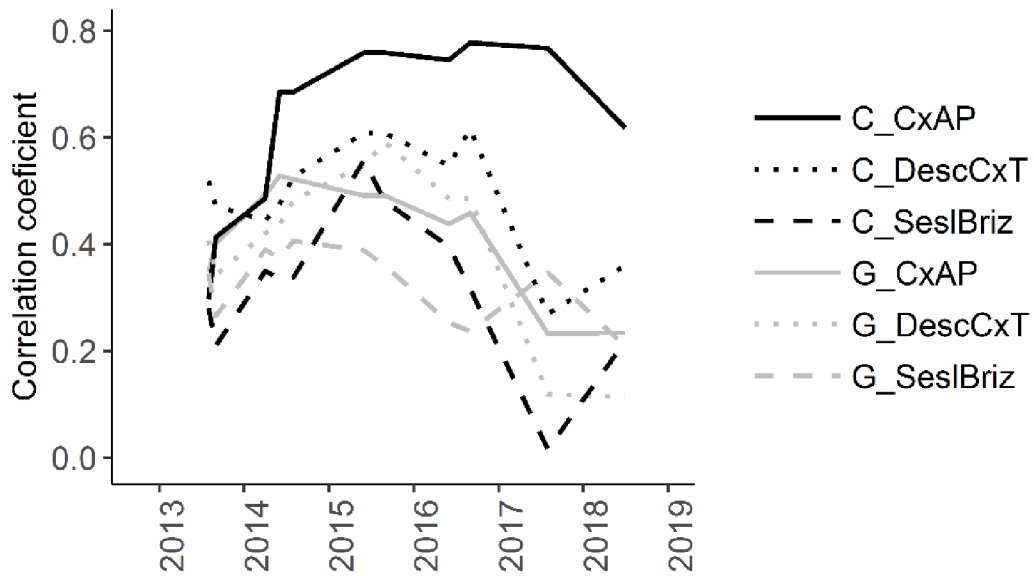


Fig. 5: Values of Pearson's correlation coefficients between transplant survival and Beals index across years and different habitat types (*CxAP* = *Carex acuta-Carex panicea*, *DescCxT* = *Deschampsia caespitosa-Carex tomentosa*, *SeslBriz* = *Sesleria uliginosa-Briza media* habitat, C = control plots – black line, G = gap – grey line).

Correlations of seedling survival across different habitat types (always taken in pairs) were significant, with the exception of intact vegetation between *Carex acuta-Carex panicea* and *Sesleria uliginosa-Briza media* habitats in 2017 and the seedling survival in vegetation between *Deschampsia caespitosa-Carex tomentosa* and *Sesleria uliginosa-Briza media* habitats during the last three years (i.e. when gaps were no longer weeded) (Table S9). Correlation coefficients were higher for seedling survival in gaps when compared to control plots, especially in the case of paired *Carex acuta-Carex panicea* and *Deschampsia caespitosa-Carex tomentosa* habitats (Fig. 6), thus these two habitats differed more by the biotic interactions than by abiotic environment.

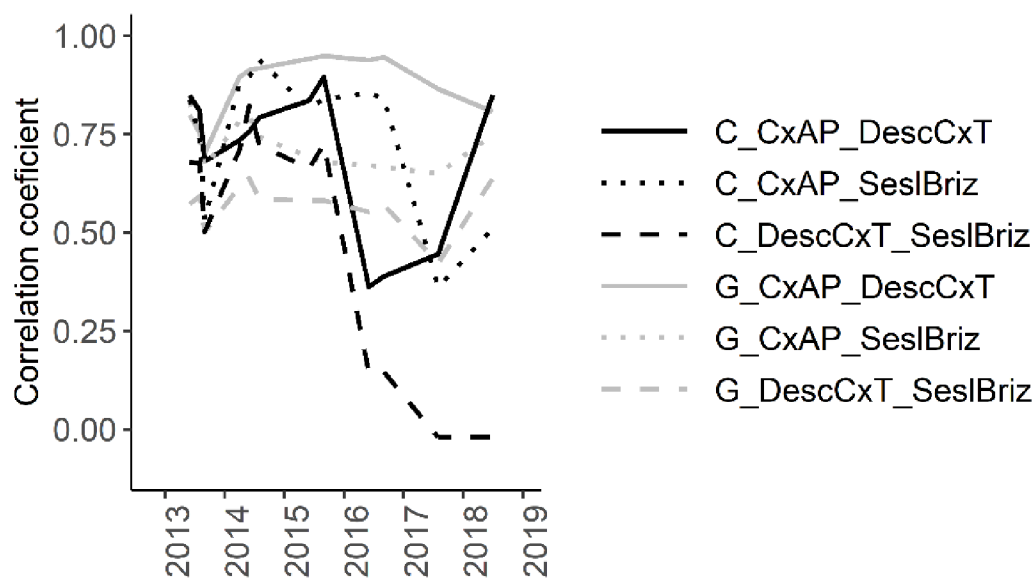


Fig. 6: Values of Pearson's correlation coefficients between seedling survival in different habitat types (in pairs): *CxAP* = *Carex acuta*-*Carex panicea*, *DescCxT* = *Deschampsia caespitosa*-*Carex tomentosa*, *SeslBriz* = *Sesleria uliginosa*-*Briza media* habitat, C = control plots – black line, G = gap – grey line.

## 4 Discussion

### 4.1 Seed germination and survival of seedlings in contrast to transplants

Across all habitat types, sown species, both resident and non-resident, germinated and subsequently survived better in gaps than in intact vegetation. This result corresponds to many other studies where most species persisted significantly better in plots without competition (Kotorová & Lepš, 1999, Tofts & Silvertown, 2002, Švamberková et al., 2017). Zobel et al. (1998) suggested that one of the most important factors affecting species survival is the surrounding vegetation. Frei et al. (2012) highlighted the positive effect that disturbances have on the establishment of *Campanula thyrsoides* seedlings, which responded positively to cutting the surrounding vegetation and disturbing the turf. Also in our experiment, many non-resident species with habitat preferences different from our habitats were able to establish from

seeds and grow when competition was removed (similarly as in Toft & Silvertown, 2002), but not in the intact community.

Also transplants survived better in gaps than in intact vegetation. However, the difference between transplant survival in gaps and intact vegetation was smaller than when seeds were introduced. In the *Sesleria uliginosa-Briza media* habitat, there were no differences between gaps and intact vegetation in the case of transplants in contrast to sown species. Aboveground biomass was there the lowest of the three habitats (Table S2), and thus we can expect least amount of competition for light. While also this small competition was crucial for seedlings growing from seeds in the field, it was not so important problem for transplants, which are generally more resistant than seedlings (Bennett et al., 2016). There were many species that were unable to establish from seeds in intact vegetation, but survived as transplants. The biotic filter had thus a more pronounced effect on establishment from seeds, than on transplant establishment (even though they were still young individuals). In concordance with Kotorová & Lepš (1999) it seems that very early phases of seedling establishment are the most sensitive stages of many plant species and their suppression is an important filtering mechanism in the community.

Species survival was dependent on the regular weeding within gaps because both artificially created gaps and other types of naturally disturbed plots tend to become overgrown with surrounding vegetation (Puerta-Piñero et al., 2013). Accordingly, during the last two years of our experiment (i.e. 2017 and 2018) when weeding ceased, the differences between gaps and vegetation started to decrease. Nevertheless, many non-resident species with habitat preferences different from our habitats (i.e. also species with very low Beals index and thus species very improbable to occur in target habitats), successfully established in gaps and survived also after weeding ceased and even reached their reproductive stage; confirming that competitive

exclusion can be a slow process (Adler et al., 2013). However, once weeding was stopped, plant mortality increased considerably, especially for seedlings. This supports the results in Gustafsson et al. (2002), which suggest that initial seedling establishment does not guarantee long-term species survival and it is important to monitor the complete vegetation cycle of target species because sudden changes can occur in late stages of seedling establishment (Münzbergová & Herben, 2004). Also, other studies (Ehrlén et al., 2006, Houseman & Gross, 2006, Frei et al., 2012, Pärtel et al., 2013) highlight the importance of long-term monitoring in seed addition experiments because it is possible that seeds of many species germinate and survive as seedlings for several years, but never establish a viable population (Vítová & Lepš, 2011).

#### **4.2 Seedling/transplant survival compared with their respective Beals index values and among different habitat types**

While the effect of species residence is a rather crude binary variable (resident/non-resident), the Beals index is based on individual species performance within an extensive set of phytosociological records from the whole region of the Czech Republic. This metric is able to distinguish between resident species regularly found within a given vegetation type and non-resident species found in similar and dissimilar habitats. In all habitat types and during the entirety of the experiment, seedling survival was positively correlated with Beals index in gaps and intact vegetation. This suggests that species are adapted to both the abiotic (correlation of survival in gaps with Beals index) and biotic conditions (correlation of survival in intact vegetation with Beals index) of particular habitats (HilleRisLambers et al., 2012). Positive correlations of species survival with Beals index was also reported by Mudrák et al. (2014), which sowed *Rhinanthus* species into a wide range of habitat types and by Milden et al. (2006) for *Succisa pratensis*. On the other hand, Münzbergová & Plačková (2010) and Frei et al. (2012) did not observe a positive relationship between Beals

index and seedling survival of sown species. For transplants, the correlation of survival with Beals index was weaker than for seedlings. This again confirms that transplants are less sensitive to competition than seedlings. This supports previous observation that the primary reason for the absence of some species in a community is their inability to establish as seedlings from seeds (Vítová & Lepš, 2011, Tofts & Silvertown, 2002).

Higher correlation coefficients between Beals index and survival in intact vegetation compared to gaps and the positive correlations between the ratio of seedling survival in intact vegetation and gaps suggest that competition was the most important determinant of species community composition. These dependences were generally similar also for transplants although they were rather weak. Higher correlation coefficients of survival across habitats in gaps compared to control plots (especially in case of pair *Carex acuta-Carex panicea* and *Deschampsia caespitosa-Carex tomentosa* habitats) also revealed that differences in species survival within these two habitats are caused more by biotic interactions than by environmental conditions (i.e. the competition is more discriminating among species than the effect of the abiotic environment). Bar-Massada (2015) suggested that biotic interactions are the most important drivers of species co-occurrence, although their effect could be influenced by environmental heterogeneity. Many other studies highlight the importance of biotic interactions in determining species community composition and the necessity to incorporate them into models (Myers & Harms, 2011, Boulangeat et al., 2012, Wisz et al., 2013, Pollock et al., 2014, Morales-Castilla et al., 2015). Conversely, D'Amen et al. (2018) suggested that environmental filtering and dispersal limitation are more important drivers of species co-occurrence than biotic interactions, but this conclusion was based on the analyses of observational data and the use of null models. In our view, without direct experimental manipulation of biotic interactions, it is difficult to

distinguish the direct effect of environment from environmentally modified biotic interactions (Cadotte & Tucker, 2017).

## **5 Conclusions**

Many non-resident species very improbable to occur in the target habitats (i.e. with low Beals index) were able to perform well in competition-free gaps, but were unable to survive in intact vegetation. These species were thus not limited by the abiotic conditions, but by competition with neighbouring plants. Although the appropriate abiotic conditions are important for seedling survival, our experiment suggests that biotic interactions are likely the most important determinants of plant species community composition and operate mainly through prevention of establishment of the “unsuitable” species. Although Beals index is a good predictor of species survival in plant communities, we should be careful to use it as species pool determinant, especially in disentangling the effect of abiotic and biotic filter on species community composition. If we define the community species pool as a set of species able to survive and reproduce in given abiotic environment (Butaye et al., 2001), the set of species will be much wider than predicted by Beals index (and generally any comparative method) because we extend the species pool about species otherwise excluded by biotic filter. Comparative methods generally exclude species which are not able to withstand the competition from species pool. If we compare the actual community composition with this species pool with the aim to disentangle the importance of biotic and abiotic factors, we would underestimate the effect of competition because species affected by competition are already excluded from this species pool.



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## Data Accessibility Statement

Data associated with this study are available from the Dryad Repository (doi:10.5061/dryad.fqz612jq9).

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## **Supplementary material**

Additional supporting information may be found online in the Supporting Information section.

## Appendix 1 – Study site characteristics

### Meteorological data

Table S1: Mean annual temperature and total annual precipitation in Strašovský rybník from 2013 to 2018 (based on the measurements of the Czech Hydrometeorological Institute in a meteorological station in Mokošín, 12 km from Strašovský rybník).

	2013	2014	2015	2016	2017	2018
Mean annual temperature [°C]	9.5	11.1	11.1	10.3	10.1	11.5
Total annual precipitation [mm]	559.8	548	451.1	394.1	594.5	375.1

### Measurement of moisture

From July 2013 to October 2016, we measured the volumetric soil moisture both in gaps and in the intact vegetation using TOMST dataloggers TMS3 in 15 minutes period. Then we calibrated data using TMS3Calibr (TOMST 2013) and counted mean daily soil volumetric moisture (Fig. S1).

### Species composition data

In June 2014, five phytosociological relevés (3x3m) on each habitat type were conducted. We recorded the cover of present species in each relevé. Species nomenclature refers to Kubát et al. (2002).

To show different species composition of each study habitat type, we conducted a Redundancy analysis (RDA) in CANOCO 5 (ter Braak and Šmilauer 2012) with centring and no standardisation neither by specie nor by samples (Fig. S4). Species composition (cover estimates) was used as response variables and different habitat types (*Carex acuta-Carex panicea*, *Deschampsia cespitosa-Carex tomtosa* and *Sesleria uliginosa-Briza media* habitat) as explanatory variables.

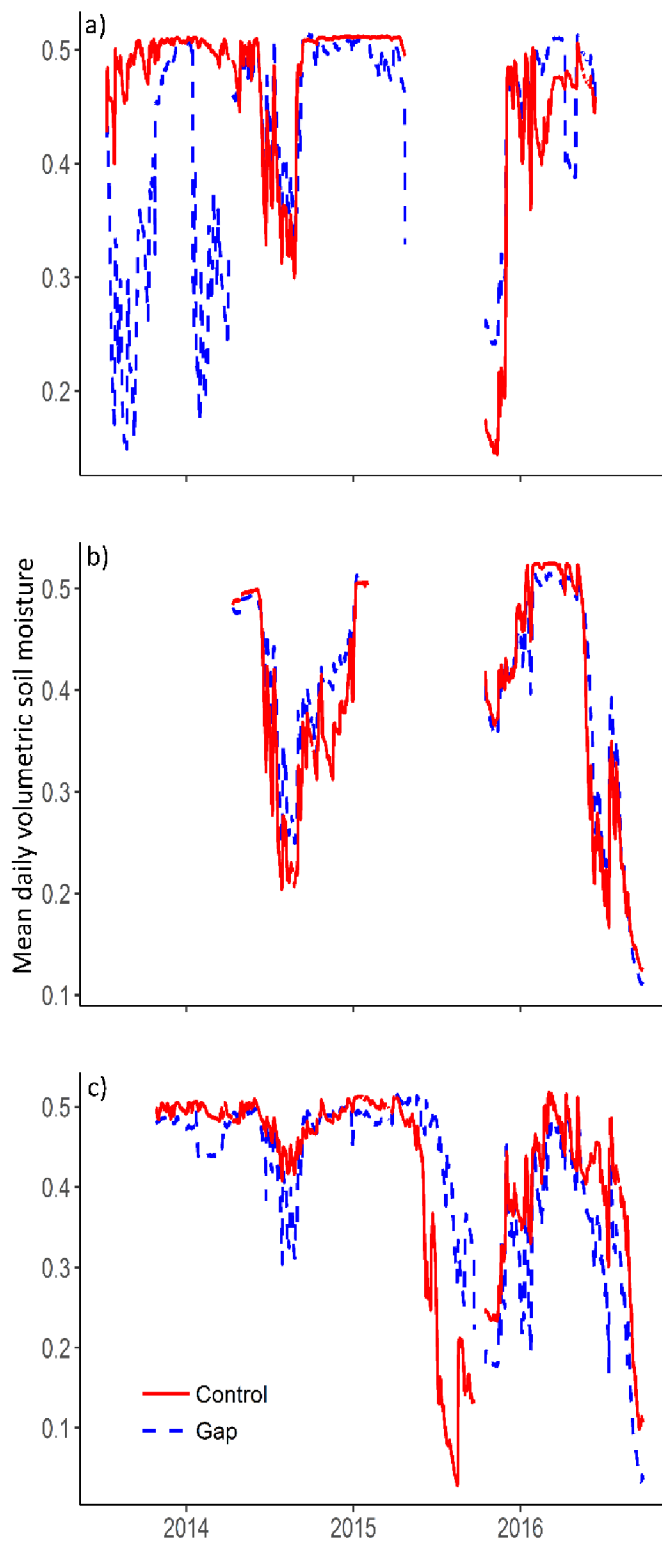


Fig. S1: Mean daily volumetric soil moisture in gaps and control plots in a) *Carex acuta-Carex panicea*, b) *Deschampsia cespitosa-Carex tomentosa*, c) *Sesleria uliginosa-Briza media* habitat.

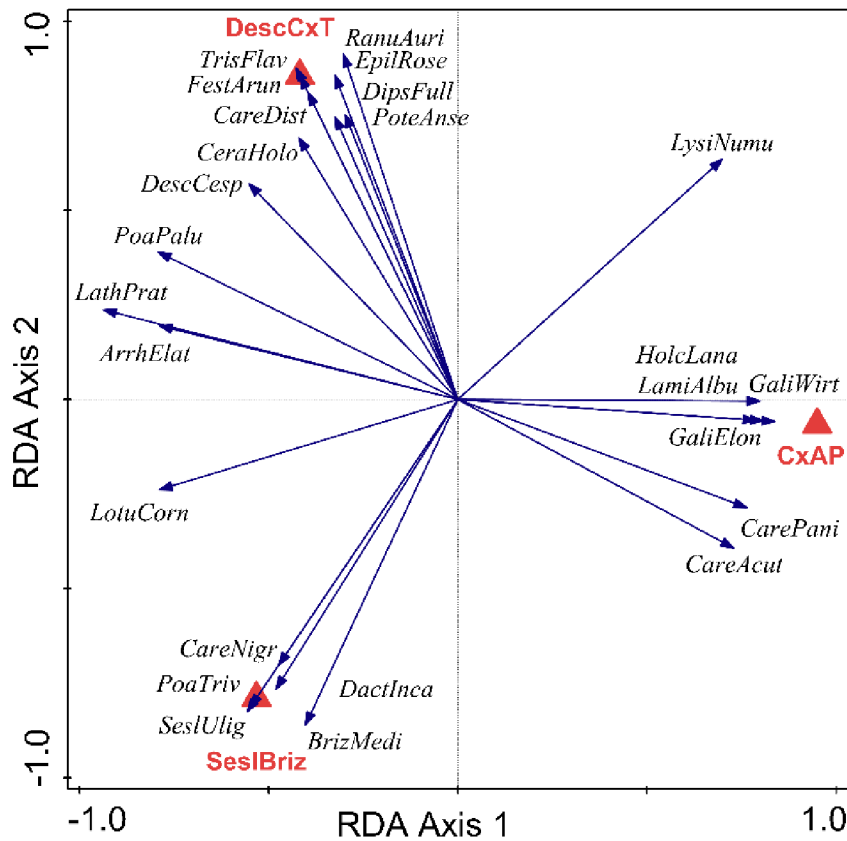


Fig. S2: RDA of species composition on different habitat types (CxAP = *Carex acuta*-*Carex panicea*, DescCxT = *Deschampsia cespitosa*-*Carex tomentosa*, SeslBriz = *Sesleria uliginosa*-*Briza media* habitat). Adjusted explained variation = 50.72%, pseudo-F = 8.2,  $p = 0.002$ . Red triangles mark different habitat types and blue arrows individual species. ArrhElat – *Arrhenatherum elatius*, BrizMedi – *Briza media*, CareAcut – *Carex acuta*, CareDist – *Carex disticha*, CareNigr – *Carex nigra*, CarePani – *Carex panicea*, CeraHolo – *Cerastium holosteoides*, DactInca – *Dactylorhiza incarnata*, DescCesp – *Deschampsia cespitosa*, DipsFull – *Dipsacus fullosa*, EpilRose – *Epilobium roseum*, FestArun – *Festuca arundinacea*, GaliElon – *Galium elongatum*, GaliWirt - *Galium wirtgenii*, HolcLana – *Holcus lanatus*, LamiAlbu – *Lamium album*, LathPrat – *Lathyrus pratensis*, LotuCorn – *Lotus corniculatus*, LysiNumu – *Lysimachia nummularia*, PoaPalu – *Poa palustris*, PoaTriv – *Poa trivialis*, PoteAnse – *Potentilla anserina*, RanuAuri – *Ranunculus auricomus*, SesiUlig – *Sesleria uliginosa*, TrisFlav – *Trisetum flavescens*.



### Biomass samples

In June 2019, we cut the biomass from five 0.5 x 0.5 m plots in each experimental habitat type. The biomass from each plot was dried at 110°C until its mass was considered to become constant. Then we weighed the dry biomass of each sample. Subsequently, we calculated the mean biomass weight from five samples for each habitat type.

Differences in biomass among the three habitat types were tested using one-way ANOVA with post-hoc comparisons using Tukey HSD method in Statistica 13 (StatSoft, 2015). Homogeneity of variances was tested using Bartlett test.

There were significant differences in biomass among different habitat types ( $F_{2,12} = 11.458$ ,  $p = 0.002$ ) and post-hoc tests have demonstrated significant difference between biomass on *Carex acuta-Carex panicea* and *Sesleria uliginosa-Briza media* habitat type ( $p = 0.023$ ) and on *Deschampsia cespitosa-Carex tomntosa* and *Sesleria uliginosa-Briza media* habitat type ( $p = 0.002$ ). The lower mean dry mass of biomass was recorded on *Sesleria uliginosa-Briza media* habitat type (Table S2). There was no significant difference between the biomass on *Carex acuta-Carex panicea* and *Deschampsia cespitosa-Carex tomntosa* habitat ( $p = 0.284$ , Table S2).

Table S2: Mean dry weight of biomass from different habitat types and their standard deviation. Letters *a* and *b* illustrate the differences in biomass among different habitat types in post-hoc test of one-way ANOVA.

Habitat type	Mean dry mass of biomass [g/0.25m <sup>2</sup> ]	Standard deviation [g/0.25m <sup>2</sup> ]
<i>Carex acuta-Carex panicea</i>	90.14 <i>a</i>	6.06
<i>Deschampsia cespitosa-Carex tomntosa</i>	98.87 <i>a</i>	11.35
<i>Sesleria uliginosa-Briza media</i>	73.158 <i>b</i>	3.68

### Soil samples

In August 2014, we took five soil samples (5cm of diameter, 20 cm deep) from six plots (0.5x0.5m) from each habitat type. Five samples from each plot were mixed together and subsequently laboratory analysed.

Differences in soil characteristics among the three habitat types were tested by the same analysis as in case of biomass. P-PO<sub>4</sub> and granularity 50-100% values were log-transformed to achieve, resp. to improve homogeneity of variances required by the F-test in one-way ANOVA.

There were significant differences in all soil characteristics among different habitat types with exception of the soil granularity 10-50 and 50-100 µm (Table S3). Post-hoc tests have demonstrated some significant differences between some soil characteristics on different habitat types (Table S3).

### Ellenberg indicator values

Community weighted mean (CWM) for each habitat type was calculated according to Garnier et al. (2004) using species cover from five phytosociological relevés for each habitat type from June 2014 weighted by Ellenberg indicator values for light, moisture and nutrients. Differences in CWM among the three habitat types were tested by the same analysis as in case of biomass and soil characteristics. CWM of Ellenberg indicator values for moisture was log-transformed to improve homogeneity of variances required by the F-test in one-way ANOVA.

There were significant differences in CWM in case of all Ellenberg indicator values among different habitat types (Table S4). Post-hoc tests have demonstrated some significant differences in CWM of some Ellenberg indicator values on different habitat types (Table S4).

Table S3: Soil sample characteristics (mean values from 6 plots for each habitat type) in different habitat types and results of their ANOVA. Letters *a*, *b* and *c* illustrate the differences in soil characteristics among different habitat types in post-hoc test of one-way ANOVA.

	<i>Carex acuta-Carex panicea</i> habitat	<i>Deschampsia cespitosa-Carex tomtosa</i> habitat	<i>Sesleria uliginosa-Briza media</i> habitat	F <sub>2,15</sub>	p
pH H <sub>2</sub> O	7.61 <i>a</i>	7.76 <i>a</i>	7.99 <i>b</i>	15.89	< <b>0.001</b>
pH KCl	7.17 <i>a</i>	7.32 <i>b</i>	7.55 <i>c</i>	28.3	< <b>0.001</b>
conductivity [μS/cm]	758.00 <i>b</i>	224.50 <i>a</i>	299.50 <i>a</i>	147.63	< <b>0.001</b>
organic contain (loss by annealing) [%]	25.31 <i>b</i>	18.79 <i>a</i>	18.91 <i>a</i>	17.02	< <b>0.001</b>
dry matter [%]	93.41 <i>b</i>	96.04 <i>a</i>	96.30 <i>a</i>	46.5	< <b>0.001</b>
N-NH <sub>4</sub> [mg/kg]	10.355 <i>b</i>	2.583 <i>a</i>	17.010 <i>c</i>	56.08	< <b>0.001</b>
P-PO <sub>4</sub> [mg/kg]	10.799 <i>a</i>	19.815 <i>b</i>	8.573 <i>a</i>	10.27	<b>0.002</b>
P [mg/kg]	545.943 <i>a</i>	572.311 <i>a</i>	363.287 <i>b</i>	17.8	< <b>0.001</b>
N [%]	1.20 <i>a</i>	1.03 <i>b</i>	1.22 <i>a</i>	17.81	< <b>0.001</b>
C [%]	12.58 <i>b</i>	11.37 <i>a</i>	14.73 <i>c</i>	53.81	< <b>0.001</b>
K [mg/kg]	245.700 <i>b</i>	313.083 <i>c</i>	107.290 <i>a</i>	56.68	< <b>0.001</b>
granularity [μm]					
0-2 [%]	16.51 <i>b</i>	13.90 <i>ab</i>	10.75 <i>a</i>	8.38	<b>0.004</b>
2-10 [%]	35.03 <i>a</i>	32.81 <i>a</i>	26.50 <i>b</i>	6.84	<b>0.008</b>
10-50 [%]	21.31 <i>a</i>	14.35 <i>a</i>	17.82 <i>a</i>	1.77	0.205
50-100 [%]	0.32 <i>a</i>	2.76 <i>a</i>	2.93 <i>a</i>	2.4	0.125
100-2000 [%]	26.83 <i>b</i>	36.68 <i>a</i>	42.14 <i>a</i>	12	<b>0.001</b>

Table S4: Community weighted mean (CWM) of Ellenberg indicator values (for light, moisture and nutrients) for different habitat type (mean from five phytocenological relevés) and results of their ANOVA. Letters *a* and *b* illustrate the differences in CWM among different habitat types in post-hoc test of one-way ANOVA.

	<i>Carex acuta-Carex panicea habitat</i>	<i>Deschampsia a cespitosa- Carex tomntosa habitat</i>	<i>Sesleria uliginosa -Briza media habitat</i>	F <sub>2,12</sub>	p
CWM_Light	6.68 <i>a</i>	6.71 <i>a</i>	7.27 <i>b</i>	25.68	< <b>0.001</b>
CWM_Moisture	6.92 <i>b</i>	6.31 <i>a</i>	6.67 <i>ab</i>	9.22	<b>0.004</b>
CWM_Nutrients	4.47 <i>a</i>	4.72 <i>a</i>	3.37 <i>b</i>	76.38	< <b>0.001</b>

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## Appendix 2 – Additional tables and figures

Table S5: Beals index and residence of species sown in seed introduction experiment.

Species	Beals index			Residence			Whole locality
	<i>Carex acuta-panicea</i> habitat	<i>Deschampsia cespitosa-Carex tomntosa</i> habitat	<i>Sesleria uliginosa-Briza media</i> habitat	<i>Carex acuta-panicea</i> habitat	<i>Deschampsia cespitosa-Carex tomntosa</i> habitat	<i>Sesleria uliginosa-Briza media</i> habitat	
<i>Anthoxanthum odoratum</i>	0.326	0.313	0.346	resident	non-resident	resident	resident
<i>Aphanes arvensis</i>	0.001	0.003	0.001	non-resident	non-resident	non-resident	non-resident
<i>Arabis glabra</i>	0.002	0.002	0.002	non-resident	non-resident	non-resident	non-resident
<i>Bistorta major</i>	0.116	0.121	0.105	non-resident	non-resident	non-resident	non-resident
<i>Bupleurum falcatum</i>	0.029	0.030	0.031	non-resident	non-resident	non-resident	non-resident
<i>Campanula persicifolia</i>	0.016	0.019	0.020	non-resident	non-resident	non-resident	non-resident
<i>Carlina acaulis</i>	0.050	0.059	0.070	non-resident	non-resident	non-resident	non-resident
<i>Cirsium acaule</i>	0.015	0.016	0.032	non-resident	non-resident	non-resident	non-resident
<i>Filipendula ulmaria</i>	0.221	0.190	0.213	non-resident	non-resident	non-resident	resident
<i>Filipendula vulgaris</i>	0.040	0.042	0.063	non-resident	non-resident	non-resident	resident
<i>Galium boreale</i>	0.080	0.086	0.131	resident	resident	resident	resident
<i>Geranium pratense</i>	0.069	0.085	0.063	non-resident	non-resident	non-resident	resident
<i>Geranium sanguineum</i>	0.007	0.007	0.007	non-resident	non-resident	non-resident	non-resident
<i>Hypericum hirsutum</i>	0.003	0.003	0.002	non-resident	non-resident	non-resident	non-resident
<i>Lathyrus vernus</i>	0.012	0.014	0.011	non-resident	non-resident	non-resident	non-resident
<i>Lotus corniculatus</i>	0.165	0.192	0.254	resident	resident	resident	resident
Species	Beals index			Residence			

	<i>Carex acuta-panicea</i> habitat	<i>Deschampsia cespitosa-Carex tomntosa</i> habitat	<i>Sesleria uliginosa-Briza media</i> habitat	<i>Carex acuta-panicea</i> habitat	<i>Deschampsia cespitosa-Carex tomntosa</i> habitat	<i>Sesleria uliginosa-Briza media</i> habitat	Whole locality
<i>Lycopus europeus</i>	0.065	0.043	0.037	non-resident	non-resident	non-resident	resident
<i>Lychnis flos-cuculi</i>	0.308	0.281	0.280	resident	resident	resident	resident
<i>Malva neglecta</i>	0.001	0.002	0.001	non-resident	non-resident	non-resident	non-resident
<i>Melica nutans</i>	0.017	0.020	0.018	non-resident	non-resident	non-resident	non-resident
<i>Nardus stricta</i>	0.098	0.086	0.105	non-resident	non-resident	non-resident	non-resident
<i>Origanum vulgare</i>	0.016	0.018	0.018	non-resident	non-resident	non-resident	non-resident
<i>Plantago lanceolata</i>	0.276	0.326	0.325	non-resident	non-resident	resident	resident
<i>Prunella vulgaris</i>	0.176	0.171	0.215	non-resident	non-resident	resident	resident
<i>Sanguisorba minor</i>	0.059	0.067	0.074	non-resident	non-resident	non-resident	non-resident
<i>Sanguisorba officinalis</i>	0.301	0.292	0.337	resident	resident	resident	resident
<i>Scutellaria galericulata</i>	0.040	0.024	0.027	non-resident	non-resident	non-resident	resident
<i>Thymus pulegioides</i>	0.069	0.083	0.103	non-resident	non-resident	non-resident	non-resident
<i>Trifolium montanum</i>	0.030	0.033	0.047	non-resident	non-resident	non-resident	non-resident
<i>Viola hirta</i>	0.049	0.051	0.072	non-resident	non-resident	non-resident	non-resident

Table S6: Transplants planted in different habitat types (in two replications) and their initial characteristics in time of planting. Transplant identity characterises the numeric mark of planted transplant (1, 2, 3) and treatment (g = gap, c = control plot) where it was planted. Transplants excluded from the experiment because their pre-growth was unsuccessful are marked as “-“.

	Tra nsp lant ide ntit y	<i>Carex acuta- Carex panicea 1</i>		<i>Carex acuta- Carex panicea 2</i>		<i>Descham psia cespitosa- Carex tomntosa 1</i>		<i>Descham psia cespitosa- Carex tomntosa 2</i>		<i>Sesleria uliginosa- Briza media 1</i>		<i>Sesleria uliginosa- Briza media 2</i>	
		Hei ght [c m]	Nb . of lea ves	Hei ght [c m]	Nb . of lea ves	Hei ght [c m]	Nb . of lea ves	Hei ght [c m]	Nb . of lea ves	Hei ght [c m]	Nb . of lea ves	Hei ght [c m]	Nb . of lea ves
<i>Anthoxant hum odoratum</i>	1g	4.6	1	7.7	2	3.7	3	4.7	2	4	3	3.5	3
	2g	3.5	2	4.4	3	6.5	2	8.9	2	3.1	3	8.8	5
	3g	2.1	2	3.6	2	8	4	9.1	3	4.3	3	4.7	2
	1c	3.2	2	9.2	4	5.1	3	10. 7	2	3.2	2	3	3
	2c	3.7	4	5	2	4.8	5	5.1	2	2.6	2	9.1	5
	3c	3.7	2	5.6	5	6.5	3	5	2	4.8	3	6.6	3
<i>Aphanes arvensis</i>	1g	1	4	2	4	1	4	1.1	7	1.7	7	1.2	5
	2g	1	3	2.2	5	1.3	5	0.5	4	1.5	7	1	4
	3g	1.1	4	1.1	6	1.5	6	1.3	5	1.5	8	1.3	5
	1c	1	3	0.9	3	1.2	7	0.6	3	1.4	7	1	4
	2c	0.8	4	1.1	5	1.5	7	1.2	6	1.6	6	1.5	6
	3c	1.1	6	0.7	4	1	6	1.1	6	1.6	6	1.6	4
<i>Arabis glabra</i>	1g	1	4	0.6	4	0.5	4	0.2	3	0.8	4	1	3
	2g	0.7	6	0.7	6	1	5	0.4	4	0.6	2	0.5	3
	3g	0.7	4	0.7	4	0.7	5	0.7	4	0.9	3	0.8	4
	1c	0.7	3	0.9	4	0.6	4	0.5	3	0.7	3	0.6	3
	2c	0.8	4	0.7	6	0.3	6	0.4	4	0.6	2	0.5	5
3c	0.7	4	1.2	6	0.5	5	0.3	6	0.7	3	0.5	4	
<i>Bistorta major</i>	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Bupleuru m falcatum</i>	-	-	-	-	-	-	-	-	-	-	-	-	-

	Tra nsp lant ide ntity	<i>Carex acuta- Carex panicea 1</i>		<i>Carex acuta- Carex panicea 2</i>		<i>Descham psia cespitosa- Carex tomntosa 1</i>		<i>Descham psia cespitosa- Carex tomntosa 2</i>		<i>Sesleria uliginosa- Briza media 1</i>		<i>Sesleria uliginosa- Briza media 2</i>	
		Hei ght [c m]	Nb . of lea ves	Hei ght [c m]	Nb . of lea ves	Hei ght [c m]	Nb . of lea ves	Hei ght [c m]	Nb . of lea ves	Hei ght [c m]	Nb . of lea ves	Hei ght [c m]	Nb . of lea ves
<i>Campanula persicifolia</i>	1g	1.1	2	0.2	2	0.7	2	0.5	2	0.8	1	0.6	2
	2g	1.2	2	0.6	2	0.5	2	0.2	2	0.5	2	0.4	2
	3g	1.3	2	0.5	2	0.4	3	0.6	2	0.6	2	0.5	3
	1c	1.5	3	0.9	2	0.5	2	0.3	2	0.7	2	0.5	2
	2c	1.7	3	0.2	1	0.5	2	0.3	4	0.3	2	0.6	2
	3c	1	2	0.3	2	0.3	2	0.2	2	0.8	2	0.5	4
<i>Carlina acaulis</i>	1g	1.9	4	3.8	4	1.8	4	2.1	4	3	4	3	3
	2g	3.4	4	2.5	4	2.5	4	1.9	4	3.5	4	1.7	3
	3g	2.9	4	2.7	4	2	3	3.3	4	3.5	3	1.5	4
	1c	2.6	4	3.6	4	3.5	4	2.3	4	2.5	3	3.1	4
	2c	2.1	4	2.6	4	3.5	4	1.7	3	3.2	4	3.2	4
	3c	2	4	3.4	4	2.5	4	2.4	4	2.7	3	2	4
<i>Cirsium acaule</i>	1g	-	-	-	-	1.5	2	-	-	1.8	1	0.5	0
	2g	-	-	-	-	2.3	2	-	-	2	2	1.9	2
	3g	-	-	-	-	-	-	-	-	1.1	1	1.4	2
	1c	-	-	-	-	2	2	-	-	1.5	2	2.1	4
	2c	-	-	-	-	1.7	2	-	-	2.4	2	3.1	3
	3c	-	-	-	-	-	-	-	-	2.5	4	2.3	2
<i>Filipendula ulmaria</i>	1g	-	-	-	-	1	2	-	-	0.7	2	0.9	2
	2g	-	-	-	-	0.8	2	-	-	1	2	0.7	2
	3g	-	-	-	-	0.7	2	-	-	0.9	2	1	2
	1c	-	-	-	-	0.9	2	-	-	1.2	3	1	2
	2c	-	-	-	-	0.6	3	-	-	0.6	2	0.8	2
	3c	-	-	-	-	1.1	3	-	-	0.6	1	0.8	2



	Tra nsp lant ide ntit y	<i>Carex acuta- Carex panicea 1</i>		<i>Carex acuta- Carex panicea 2</i>		<i>Descham psia cespitosa- Carex tommtosa 1</i>		<i>Descham psia cespitosa- Carex tommtosa 2</i>		<i>Sesleria uliginosa- Briza media 1</i>		<i>Sesleria uliginosa- Briza media 2</i>	
		Hei ght [c m]	Nb . of lea ves	Hei ght [c m]	Nb . of lea ves	Hei ght [c m]	Nb . of lea ves	Hei ght [c m]	Nb . of lea ves	Hei ght [c m]	Nb . of lea ves	Hei ght [c m]	Nb . of lea ves
<i>Filipendula vulgaris</i>	1g	1	4	0.9	3	1	4	1	3	0.7	3	1.1	4
	2g	1.1	3	0.9	2	1.3	4	1.6	5	0.7	3	0.8	3
	3g	1	3	0.6	3	0.7	3	1	3	1	3	1	3
	1c	0.8	3	1	4	1.2	4	0.8	3	1	3	1.2	2
	2c	1.3	4	1	3	1.8	4	0.9	3	0.8	2	1.5	3
	3c	1.2	3	0.7	3	2	4	1.3	3	0.4	4	1	3
<i>Galium boreale</i>	1g	1.2	8	0.7	4	1.5	12	1.5	4	1.5	6	2	19
	2g	2.2	8	2.1	5	1.7	9	1.5	8	1	10	2.5	11
	3g	1.5	6	1.4	7	2.3	12	1.2	6	1.5	13	2.3	6
	1c	0.9	6	2.5	8	1.7	7	1.8	9	1.9	14	1.7	8
	2c	1.3	8	1.1	8	2.5	10	1	6	1.5	8	2.5	13
	3c	1.9	8	0.8	4	2.5	7	2.3	8	0.5	4	1	5
<i>Geranium pratense</i>	1g	8.1	1	8	1	8	1	5.7	3	9	1	11	1
	2g	8.7	1	7.6	0	4.5	1	7.6	2	8.5	1	11.5	1
	3g	11.1	2	5.1	1	5.5	1	7.4	1	6.8	1	10.5	1
	1c	6	2	8.5	1	6	1	14.6	1	8	1	12.5	1
	2c	7.3	1	6	1	8.5	1	5.5	1	6	1	10.5	2
	3c	6.8	1	5.6	1	8	1	3.4	1	7.5	1	8	1
<i>Geranium sanguineum</i>	1g	5	2	6.3	1	7	2	3.3	2	4	2	2.5	1
	2g	4.3	2	6.1	2	6	1	5.8	1	6	1	3.5	1
	3g	5.5	2	8	1	5	2	4	2	7	1	4.5	1
	1c	6.5	1	7.2	2	6.7	2	4.2	1	5.5	2	5.5	2
	2c	4.9	1	6.3	1	5	2	4.1	1	5.7	2	6	2
	3c	3.7	1	4.8	1	3.5	2	3.5	1	4.5	2	6.5	1

	Tra nsp lant ide ntit y	<i>Carex acuta- Carex panicea 1</i>		<i>Carex acuta- Carex panicea 2</i>		<i>Descham psia cespitosa- Carex tommtosa 1</i>		<i>Descham psia cespitosa- Carex tommtosa 2</i>		<i>Sesleria uliginosa- Briza media 1</i>		<i>Sesleria uliginosa- Briza media 2</i>	
		Hei ght [c m]	Nb . of lea ves	Hei ght [c m]	Nb . of lea ves	Hei ght [c m]	Nb . of lea ves	Hei ght [c m]	Nb . of lea ves	Hei ght [c m]	Nb . of lea ves	Hei ght [c m]	Nb . of lea ves
<i>Hypericum hirsutum</i>	1g	0.6	4	0.6	4	1	8	0.2	4	0.1	4	0.2	4
	2g	0.7	4	0.2	2	0.2	2	0.3	4	0.2	6	0.5	4
	3g	0.6	4	0.6	4	1	6	0.4	4	0.1	4	0.5	4
	1c	0.6	2	0.7	4	0.7	6	0.5	6	0.1	2	1	6
	2c	0.6	4	0.6	4	0.5	6	0.4	6	0.1	4	1	6
	3c	0.6	4	0.7	2	1	4	0.2	4	0.1	4	0.5	4
<i>Lathyrus vernus</i>	1g	4.2	2	1.2	1	2.2	2	3.5	2	2	1	5.5	2
	2g	1.9	1	2	1	2.8	2	2.8	2	3	1	3.5	4
	3g	3.1	2	2.6	1	2	1	2.7	2	4.1	1	5.5	2
	1c	6	2	2	2	2.1	1	5.6	2	2	2	3.5	2
	2c	3	2	2.9	1	8	3	3.8	2	3.7	2	3.5	2
	3c	3.7	2	3.4	1	3	3	5.5	2	2.2	1	6	2
<i>Lotus corniculat us</i>	1g	14	18	2.9	2	3.5	7	7.5	10	2.8	2	7.5	6
	2g	1.9	11	2.6	4	6	7	3.2	5	1.5	2	8.5	5
	3g	3.1	5	2.3	4	4.5	5	4.7	5	1.8	3	8	5
	1c	6	3	2.5	4	8	12	3.2	4	2.1	4	7.5	7
	2c	3	7	2.1	3	6	8	6.7	11	3.5	3	14	12
	3c	3.7	3	3.4	7	10. 2	8	2.5	7	2.7	4	12. 5	14
<i>Lycopus europeus</i>	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Lychnis flos- cuculi</i>	1g	0.8	6	0.6	4	1	4	0.5	4	0.5	4	1	6
	2g	0.7	6	0.6	4	1	6	0.6	4	0.5	4	1	6
	3g	0.7	4	0.7	4	1	7	0.6	6	0.4	5	0.7	4
	1c	0.8	4	0.8	4	1	6	0.5	4	0.3	6	1	4
	2c	0.6	6	0.6	4	1.3	8	0.3	4	0.2	4	1	6
	3c	0.6	4	0.8	6	1	5	0.6	6	0.5	4	0.5	4

	Tra nsp lant ide ntit y	<i>Carex acuta- Carex panicea 1</i>		<i>Carex acuta- Carex panicea 2</i>		<i>Descham psia cespitosa- Carex tomntosa 1</i>		<i>Descham psia cespitosa- Carex tomntosa 2</i>		<i>Sesleria uliginosa- Briza media 1</i>		<i>Sesleria uliginosa- Briza media 2</i>	
		Hei ght [c m]	Nb . of lea ves	Hei ght [c m]	Nb . of lea ves	Hei ght [c m]	Nb . of lea ves	Hei ght [c m]	Nb . of lea ves	Hei ght [c m]	Nb . of lea ves	Hei ght [c m]	Nb . of lea ves
<i>Malva neglecta</i>	1g	-	-	-	-	1.5	1	-	-	1.4	1	0.5	1
	2g	-	-	-	-	1	1	-	-	0.9	0	0.5	1
	3g	-	-	-	-	-	-	-	-	1	1	0.6	2
	1c	-	-	-	-	1	2	-	-	1.5	1	0.5	1
	2c	-	-	-	-	0.7	1	-	-	0.8	2	1.3	1
<i>Melica nutans</i>	3c	-	-	-	-	-	-	-	-	1	0	1	2
	-	-	-	-	-	-	-	-	-	-	-	-	-
	1g	3	4	3.3	4	2.3	5	5	2	2.8	4	2.2	3
<i>Nardus stricta</i>	2g	2.6	2	3.1	3	4.5	4	3.1	3	3.6	5	4.2	2
	3g	3.5	4	3.1	3	3.5	4	9	3	3.2	3	3.3	2
	1c	3.2	3	2.7	3	3	5	2.9	3	2	4	3.2	4
	2c	3.3	4	4.7	3	3	3	10. 7	3	2.6	4	2	2
	3c	2.4	4	3	3	2.8	4	20. 3	3	3	4	2.6	4
<i>Origanum vulgare</i>	1g	0.6	4	0.7	4	1.5	4	0.3	4	0.3	2	0.5	6
	2g	0.8	4	0.8	4	1	3	0.4	6	0.2	4	0.7	4
	3g	0.9	4	0.6	4	0.8	4	0.2	4	0.5	4	0.4	4
	1c	0.6	4	0.8	6	0.3	4	0.4	4	0.3	6	0.4	4
	2c	0.6	4	0.6	2	0.6	4	0.7	6	0.3	4	0.5	4
<i>Plantago lanceolat a</i>	3c	0.6	2	0.6	2	1	4	0.6	4	0.5	5	0.8	4
	1g	4.4	2	3.4	2	5.9	1	4	3	2.5	2	8.2	4
	2g	5.2	1	4.3	1	5	2	4.1	1	5.6	1	8.5	3
	3g	5.4	2	3.1	1	8.5	2	5.9	2	3.5	2	11	4
	1c	7.6	2	4.4	1	4.5	1	2.6	2	5.7	2	7.5	4
	2c	4.4	1	6.3	2	6.5	1	5.9	2	3.7	1	7.8	4
3c	5	2	3.9	1	8.5	2	4	1	5	2	2.5	3	

	Tra nsp lant ide ntit y	<i>Carex acuta- Carex panicea 1</i>		<i>Carex acuta- Carex panicea 2</i>		<i>Descham psia cespitosa- Carex tomntosa 1</i>		<i>Descham psia cespitosa- Carex tomntosa 2</i>		<i>Sesleria uliginosa- Briza media 1</i>		<i>Sesleria uliginosa- Briza media 2</i>	
		Hei ght [c m]	Nb . of lea ves	Hei ght [c m]	Nb . of lea ves	Hei ght [c m]	Nb . of lea ves	Hei ght [c m]	Nb . of lea ves	Hei ght [c m]	Nb . of lea ves	Hei ght [c m]	Nb . of lea ves
<i>Prunella vulgaris</i>	1g	0.8	4	1.2	4	1.5	6	0.2	4	1	4	0.5	6
	2g	1.1	4	1.3	4	1	4	0.3	4	0.7	4	1	6
	3g	0.7	4	1.7	6	0.5	4	0.9	4	0.3	4	0.9	8
	1c	0.6	4	1.3	4	0.5	4	0.4	4	0.6	4	1	4
	2c	0.7	4	1.2	4	0.7	6	0.5	4	0.5	2	1.3	6
	3c	1.2	4	1.3	4	0.8	6	0.8	4	0.9	4	1	4
<i>Sanguisor ba minor</i>	1g	11. 2	2	7.7	3	6.5	3	4.7	5	8.5	3	7	3
	2g	10. 5	3	8.4	4	7.8	4	7.9	4	6.1	2	9	2
	3g	12. 4	2	7.7	4	6.5	3	8.8	4	6.3	3	4.9	2
	1c	9.4	3	11. 1	4	8.5	5	8.8	3	12. 5	2	4.8	5
	2c	9.3	2	6.2	3	9.5	3	5.6	3	8.5	4	8.7	2
	3c	6	3	6.6	3	5.5	2	5.4	3	7	2	9.3	3
<i>Sanguisor ba officinalis</i>	1g	6.6	2	5.4	2	4.8	2	4.1	2	5	2	3.5	2
	2g	3.4	1	4.8	1	3	1	3.2	2	8	2	6	1
	3g	4.6	2	4.9	1	2.5	2	4.2	2	7	2	3.7	1
	1c	6.9	2	5.2	2	4.5	2	3.8	1	5.5	3	6.1	3
	2c	7.4	2	2.9	2	3.7	2	2.5	2	6	2	4	2
3c	4.6	2	4.4	1	3.5	2	5.1	2	6.5	2	5.5	2	
<i>Scutellari a galericula ta</i>	-	-	-	-	-	-	-	-	-	-	-	-	-

	Tra nsp lant ide ntit y	<i>Carex acuta- Carex panicea 1</i>		<i>Carex acuta- Carex panicea 2</i>		<i>Descham psia cespitosa- Carex tommtosa 1</i>		<i>Descham psia cespitosa- Carex tommtosa 2</i>		<i>Sesleria uliginosa- Briza media 1</i>		<i>Sesleria uliginosa- Briza media 2</i>	
		Hei ght [c m]	Nb . of lea ves	Hei ght [c m]	Nb . of lea ves	Hei ght [c m]	Nb . of lea ves	Hei ght [c m]	Nb . of lea ves	Hei ght [c m]	Nb . of lea ves	Hei ght [c m]	Nb . of lea ves
<i>Thymus pulegioid es</i>	1g	-	-	-	-	1.5	6	-	-	0.5	4	0.7	6
	2g	-	-	-	-	1.8	4	-	-	0.3	2	1.5	3
	3g	-	-	-	-	0.3	0	-	-	1.3	4	0.4	2
	1c	-	-	-	-	1.3	6	-	-	2.5	6	2	10
	2c	-	-	-	-	0.5	4	-	-	0.9	6	1.1	6
<i>Trifolium montanu m</i>	3c	-	-	-	-	0.7	8	-	-	1	10	1.8	10
	1g	0.6	2	0.2	3	0.8	1	0.6	2	0.5	3	1.1	3
	2g	0.7	3	0.7	2	1	3	0.3	4	0.8	1	1	2
	3g	0.7	1	0.3	2	1.8	2	0.6	3	1.2	3	1.6	3
	1c	1	3	0.5	3	1.3	1	0.3	2	1	2	0.6	2
<i>Viola hirta</i>	2c	0.9	2	0.6	3	1.8	6	0.5	3	1	3	1.1	2
	3c	0.8	2	0.2	1	1	2	1.5	2	0.8	2	0.9	2
		-	-	-	-	-	-	-	-	-	-	-	-

Table S7: Repeated Measurement ANOVA of seedling survival of resident and non-resident species (“habitat residence”) in gaps and control plots (Treatment) during the experiment in different habitat types (separate analysis for each habitat type). Statistically significant results are in bold.

	Degree of freedom	<i>Carex acuta-Carex panicea</i> habitat		<i>Deschampsia caespitosa-Carex tomentosa</i> habitat		<i>Sesleria uliginosa-Briza media</i> habitat	
		F	p	F	p	F	p
Residence	1,28	2.927	0.098	1.834	0.186	<b>23.968</b>	<b>&lt;0.001</b>
Time	11,308	<b>28.358</b>	<b>&lt;0.001</b>	<b>21.891</b>	<b>&lt;0.001</b>	<b>58.011</b>	<b>&lt;0.001</b>
Time*Residence	11,308	<b>3.607</b>	<b>&lt;0.001</b>	<b>3.551</b>	<b>&lt;0.001</b>	<b>12.996</b>	<b>&lt;0.001</b>
Treatment	1,28	<b>21.396</b>	<b>&lt;0.001</b>	<b>23.158</b>	<b>&lt;0.001</b>	<b>54.808</b>	<b>&lt;0.001</b>
Treatment*Residence	1,28	0.035	0.852	1.059	0.312	<b>6.955</b>	<b>0.013</b>
Time*Treatment	11,308	<b>9.456</b>	<b>&lt;0.001</b>	<b>26.476</b>	<b>&lt;0.001</b>	<b>24.18</b>	<b>&lt;0.001</b>
Time*Treatment*Residence	11,308	0.283	0.989	<b>4.115</b>	<b>&lt;0.001</b>	<b>4.003</b>	<b>&lt;0.001</b>

Table S8: Correlations between seedling survival and Beals index in time on different habitat types for species from seed introduction experiment. Gap – seedling survival in gaps, control – seedling survival in the intact vegetation, control/gap – the ratio of seedling survival in the intact vegetation and in gaps. Statistically significant results are in bold.

		<i>Carex acuta-Carex panicea</i> habitat			<i>Deschampsia caespitosa-Carex tomentosa</i> habitat			<i>Sesleria uliginosa-Briza media</i> habitat		
		r	N	p	r	N	p	r	N	p
Jun-13	gap	<b>0.465</b>	<b>30</b>	<b>0.010</b>	<b>0.483</b>	<b>30</b>	<b>0.007</b>	<b>0.535</b>	<b>30</b>	<b>0.002</b>
	control	<b>0.531</b>	<b>30</b>	<b>0.003</b>	<b>0.437</b>	<b>30</b>	<b>0.016</b>	<b>0.606</b>	<b>30</b>	<b>0.000</b>
	control/gap	<b>0.629</b>	<b>27</b>	<b>0.000</b>	0.139	27	0.488	0.270	27	0.173
Aug-13	gap	<b>0.462</b>	<b>30</b>	<b>0.010</b>	<b>0.485</b>	<b>30</b>	<b>0.007</b>	<b>0.641</b>	<b>30</b>	<b>0.000</b>
	control	<b>0.516</b>	<b>30</b>	<b>0.004</b>	<b>0.578</b>	<b>30</b>	<b>0.001</b>	<b>0.560</b>	<b>30</b>	<b>0.001</b>
	control/gap	<b>0.645</b>	<b>27</b>	<b>0.000</b>	0.330	28	0.086	0.006	27	0.976
Sep-13	gap	<b>0.460</b>	<b>30</b>	<b>0.011</b>	<b>0.453</b>	<b>30</b>	<b>0.012</b>	<b>0.602</b>	<b>30</b>	<b>0.000</b>
	control	<b>0.460</b>	<b>30</b>	<b>0.011</b>	<b>0.593</b>	<b>30</b>	<b>0.001</b>	<b>0.427</b>	<b>30</b>	<b>0.019</b>
	control/gap	<b>0.451</b>	<b>25</b>	<b>0.023</b>	0.315	27	0.109	-0.131	26	0.525
Apr-14	gap	<b>0.512</b>	<b>30</b>	<b>0.004</b>	<b>0.521</b>	<b>30</b>	<b>0.003</b>	<b>0.664</b>	<b>30</b>	<b>0.000</b>
	control	<b>0.562</b>	<b>30</b>	<b>0.001</b>	<b>0.637</b>	<b>30</b>	<b>0.000</b>	<b>0.672</b>	<b>30</b>	<b>0.000</b>
	control/gap	0.353	22	0.107	<b>0.588</b>	<b>24</b>	<b>0.003</b>	-0.134	24	0.533

		<i>Carex acuta-Carex panicea</i> habitat			<i>Deschampsia cespitosa-Carex tomntosa</i> habitat			<i>Sesleria uliginosa-Briza media</i> habitat		
		r	N	p	r	N	p	r	N	p
Jun-14	gap	<b>0.509</b>	<b>30</b>	<b>0.004</b>	<b>0.519</b>	<b>30</b>	<b>0.003</b>	<b>0.653</b>	<b>30</b>	<b>0.000</b>
	control	<b>0.577</b>	<b>30</b>	<b>0.001</b>	<b>0.644</b>	<b>30</b>	<b>0.000</b>	<b>0.750</b>	<b>30</b>	<b>0.000</b>
	control/gap	<b>0.481</b>	<b>22</b>	<b>0.023</b>	<b>0.570</b>	<b>24</b>	<b>0.004</b>	0.320	24	0.128
Aug-14	gap	<b>0.494</b>	<b>30</b>	<b>0.006</b>	<b>0.457</b>	<b>30</b>	<b>0.011</b>	<b>0.616</b>	<b>30</b>	<b>0.000</b>
	control	<b>0.589</b>	<b>30</b>	<b>0.001</b>	<b>0.603</b>	<b>30</b>	<b>0.000</b>	<b>0.690</b>	<b>30</b>	<b>0.000</b>
	control/gap	<b>0.531</b>	<b>22</b>	<b>0.011</b>	<b>0.598</b>	<b>21</b>	<b>0.004</b>	<b>0.449</b>	<b>23</b>	<b>0.032</b>
Jun-15	gap	<b>0.499</b>	<b>30</b>	<b>0.005</b>	<b>0.493</b>	<b>30</b>	<b>0.006</b>	<b>0.664</b>	<b>30</b>	<b>0.000</b>
	control	<b>0.566</b>	<b>30</b>	<b>0.001</b>	<b>0.606</b>	<b>30</b>	<b>0.000</b>	<b>0.672</b>	<b>30</b>	<b>0.000</b>
	control/gap	<b>0.485</b>	<b>21</b>	<b>0.026</b>	<b>0.593</b>	<b>21</b>	<b>0.005</b>	<b>0.453</b>	<b>22</b>	<b>0.034</b>
Sep-15	gap	<b>0.479</b>	<b>30</b>	<b>0.007</b>	<b>0.488</b>	<b>30</b>	<b>0.006</b>	<b>0.653</b>	<b>30</b>	<b>0.000</b>
	control	<b>0.572</b>	<b>30</b>	<b>0.001</b>	<b>0.603</b>	<b>30</b>	<b>0.000</b>	<b>0.750</b>	<b>30</b>	<b>0.000</b>
	control/gap	<b>0.447</b>	<b>21</b>	<b>0.042</b>	<b>0.595</b>	<b>21</b>	<b>0.004</b>	<b>0.496</b>	<b>22</b>	<b>0.019</b>
Jun-16	gap	<b>0.416</b>	<b>30</b>	<b>0.022</b>	0.357	30	0.053	<b>0.534</b>	<b>30</b>	<b>0.002</b>
	control	<b>0.536</b>	<b>30</b>	<b>0.002</b>	<b>0.385</b>	<b>30</b>	<b>0.036</b>	<b>0.649</b>	<b>30</b>	<b>0.000</b>
	control/gap	<b>0.468</b>	<b>21</b>	<b>0.032</b>	0.369	20	0.109	<b>0.445</b>	<b>21</b>	<b>0.043</b>
Sep-16	gap	<b>0.402</b>	<b>30</b>	<b>0.027</b>	0.358	30	0.052	<b>0.530</b>	<b>30</b>	<b>0.003</b>
	control	<b>0.538</b>	<b>30</b>	<b>0.002</b>	<b>0.385</b>	<b>30</b>	<b>0.036</b>	<b>0.649</b>	<b>30</b>	<b>0.000</b>
	control/gap	<b>0.486</b>	<b>21</b>	<b>0.025</b>	0.369	20	0.110	<b>0.458</b>	<b>21</b>	<b>0.037</b>
Aug-17	gap	0.285	30	0.126	0.257	30	0.171	0.298	30	0.110
	control	<b>0.466</b>	<b>30</b>	<b>0.009</b>	0.235	30	0.210	<b>0.593</b>	<b>30</b>	<b>0.001</b>
	control/gap	0.439	16	0.089	0.523	14	0.055	<b>0.667</b>	<b>13</b>	<b>0.013</b>
Jul-18	gap	0.189	30	0.318	0.283	30	0.129	0.232	30	0.217
	control	<b>0.405</b>	<b>30</b>	<b>0.026</b>	0.192	30	0.310	<b>0.455</b>	<b>30</b>	<b>0.012</b>
	control/gap	0.507	15	0.054	0.535	11	0.090	0.517	10	0.126

Table S9: Correlations between seedling survival in time on different habitat types (in pairs) for species from seed introduction experiment. Gap – seedling survival in gaps, control – seedling survival in the intact vegetation, control/gap – the ratio of seedling survival in the intact vegetation and in gaps. Statistically significant results are in bold.

		<i>Carex acuta-Carex panicea / Deschampsia cespitosa-Carex tomtosa</i> habitat			<i>Carex acuta-Carex panicea / Sesleria uliginosa-Briza media</i> habitat			<i>Deschampsia cespitosa-Carex tomtosa / Sesleria uliginosa-Briza media</i> habitat		
		r	N	p	r	N	p	r	N	p
Jun-13	gap	<b>0.799</b>	<b>30</b>	<b>0.000</b>	<b>0.832</b>	<b>30</b>	<b>0.000</b>	<b>0.572</b>	<b>30</b>	<b>0.001</b>
	control	<b>0.849</b>	<b>30</b>	<b>0.000</b>	<b>0.838</b>	<b>30</b>	<b>0.000</b>	<b>0.679</b>	<b>30</b>	<b>0.000</b>
	control/gap	<b>0.531</b>	<b>26</b>	<b>0.005</b>	<b>0.492</b>	<b>26</b>	<b>0.011</b>	0.132	27	0.511
Aug-13	gap	<b>0.754</b>	<b>30</b>	<b>0.000</b>	<b>0.737</b>	<b>30</b>	<b>0.000</b>	<b>0.592</b>	<b>30</b>	<b>0.001</b>
	control	<b>0.810</b>	<b>30</b>	<b>0.000</b>	<b>0.739</b>	<b>30</b>	<b>0.000</b>	<b>0.676</b>	<b>30</b>	<b>0.000</b>
	control/gap	<b>0.588</b>	<b>27</b>	<b>0.001</b>	0.159	26	0.439	-0.002	27	0.992
Sep-13	gap	<b>0.701</b>	<b>30</b>	<b>0.000</b>	<b>0.668</b>	<b>30</b>	<b>0.000</b>	<b>0.500</b>	<b>30</b>	<b>0.005</b>
	control	<b>0.681</b>	<b>30</b>	<b>0.000</b>	<b>0.533</b>	<b>30</b>	<b>0.002</b>	<b>0.502</b>	<b>30</b>	<b>0.005</b>
	control/gap	<b>0.574</b>	<b>24</b>	<b>0.003</b>	0.081	23	0.713	-0.152	25	0.470
Apr-14	gap	<b>0.895</b>	<b>30</b>	<b>0.000</b>	<b>0.782</b>	<b>30</b>	<b>0.000</b>	<b>0.618</b>	<b>30</b>	<b>0.000</b>
	control	<b>0.735</b>	<b>30</b>	<b>0.000</b>	<b>0.880</b>	<b>30</b>	<b>0.000</b>	<b>0.709</b>	<b>30</b>	<b>0.000</b>
	control/gap	0.376	21	0.093	0.018	21	0.937	-0.041	22	0.856
Jun-14	gap	<b>0.913</b>	<b>30</b>	<b>0.000</b>	<b>0.796</b>	<b>30</b>	<b>0.000</b>	<b>0.637</b>	<b>30</b>	<b>0.000</b>
	control	<b>0.757</b>	<b>30</b>	<b>0.000</b>	<b>0.897</b>	<b>30</b>	<b>0.000</b>	<b>0.822</b>	<b>30</b>	<b>0.000</b>
	control/gap	<b>0.437</b>	<b>21</b>	<b>0.047</b>	<b>0.579</b>	<b>21</b>	<b>0.006</b>	<b>0.520</b>	<b>22</b>	<b>0.013</b>
Aug-14	gap	<b>0.918</b>	<b>30</b>	<b>0.000</b>	<b>0.743</b>	<b>30</b>	<b>0.000</b>	<b>0.587</b>	<b>30</b>	<b>0.001</b>
	control	<b>0.792</b>	<b>30</b>	<b>0.000</b>	<b>0.939</b>	<b>30</b>	<b>0.000</b>	<b>0.723</b>	<b>30</b>	<b>0.000</b>
	control/gap	0.372	20	0.106	<b>0.893</b>	<b>20</b>	<b>0.000</b>	0.082	20	0.731
Jun-15	gap	<b>0.942</b>	<b>30</b>	<b>0.000</b>	<b>0.692</b>	<b>30</b>	<b>0.000</b>	<b>0.581</b>	<b>30</b>	<b>0.001</b>
	control	<b>0.835</b>	<b>30</b>	<b>0.000</b>	<b>0.816</b>	<b>30</b>	<b>0.000</b>	<b>0.664</b>	<b>30</b>	<b>0.000</b>
	control/gap	<b>0.530</b>	<b>20</b>	<b>0.016</b>	<b>0.768</b>	<b>20</b>	<b>0.000</b>	0.247	20	0.294
Sep-15	gap	<b>0.948</b>	<b>30</b>	<b>0.000</b>	<b>0.677</b>	<b>30</b>	<b>0.000</b>	<b>0.581</b>	<b>30</b>	<b>0.001</b>
	control	<b>0.895</b>	<b>30</b>	<b>0.000</b>	<b>0.840</b>	<b>30</b>	<b>0.000</b>	<b>0.725</b>	<b>30</b>	<b>0.000</b>
	control/gap	<b>0.605</b>	<b>20</b>	<b>0.005</b>	<b>0.870</b>	<b>20</b>	<b>0.000</b>	<b>0.528</b>	<b>20</b>	<b>0.017</b>
Jun-16	gap	<b>0.938</b>	<b>30</b>	<b>0.000</b>	<b>0.671</b>	<b>30</b>	<b>0.000</b>	<b>0.553</b>	<b>30</b>	<b>0.002</b>
	control	<b>0.362</b>	<b>30</b>	<b>0.050</b>	<b>0.854</b>	<b>30</b>	<b>0.000</b>	0.145	30	0.444
	control/gap	0.345	20	0.137	<b>0.877</b>	<b>19</b>	<b>0.000</b>	0.227	18	0.365



		<i>Carex acuta-Carex panicea / Deschampsia caespitosa-Carex tomtosa</i> habitat			<i>Carex acuta-Carex panicea / Sesleria uliginosa-Briza media</i> habitat			<i>Deschampsia caespitosa-Carex tomtosa / Sesleria uliginosa-Briza media</i> habitat		
		r	N	p	r	N	p	r	N	p
Sep-16	gap	<b>0.946</b>	<b>30</b>	<b>0.000</b>	<b>0.665</b>	<b>30</b>	<b>0.000</b>	<b>0.570</b>	<b>30</b>	<b>0.001</b>
	control	<b>0.388</b>	<b>30</b>	<b>0.034</b>	<b>0.841</b>	<b>30</b>	<b>0.000</b>	0.145	30	0.444
	control/gap	0.341	20	0.141	<b>0.874</b>	<b>19</b>	<b>0.000</b>	0.229	18	0.362
Aug-17	gap	<b>0.865</b>	<b>30</b>	<b>0.000</b>	<b>0.650</b>	<b>30</b>	<b>0.000</b>	<b>0.423</b>	<b>30</b>	<b>0.020</b>
	control	<b>0.445</b>	<b>30</b>	<b>0.014</b>	0.361	30	0.050	-0.021	30	0.914
	control/gap	0.238	13	0.434	<b>0.617</b>	<b>12</b>	<b>0.033</b>	<b>0.852</b>	<b>10</b>	<b>0.002</b>
Jul-18	gap	<b>0.807</b>	<b>30</b>	<b>0.000</b>	<b>0.747</b>	<b>30</b>	<b>0.000</b>	<b>0.638</b>	<b>30</b>	<b>0.000</b>
	control	<b>0.850</b>	<b>30</b>	<b>0.000</b>	<b>0.510</b>	<b>30</b>	<b>0.004</b>	-0.019	30	0.923
	control/gap	0.055	10	0.880	0.522	10	0.122	<b>0.826</b>	<b>8</b>	<b>0.011</b>

Table S10: Repeated Measurement ANOVA of transplant survival of resident and non-resident species (“habitat residence”) in gaps and control plots (Treatment) during the experiment in different habitat types (separate analysis for each habitat type). Statistically significant results are in bold.

	<i>Carex acuta-Carex panicea</i> habitat			<i>Deschampsia caespitosa-Carex tomentosa</i> habitat			<i>Sesleria uliginosa-Briza media</i> habitat		
	Degree of freedom	F	p	Degree of freedom	F	p	Degree of freedom	F	p
Residence	1,18	2.54	0.128	1,22	0.33	0.572	1,22	0.78	0.386
Time	<b>10,180</b>	<b>82.38</b>	<b>&lt;0.001</b>	<b>10,220</b>	<b>31.5</b>	<b>&lt;0.001</b>	<b>10,220</b>	<b>76.33</b>	<b>&lt;0.001</b>
Time*	<b>10,180</b>	<b>2.01</b>	<b>0.034</b>	10,220	1.18	0.303	10,220	0.41	0.939
Residence									
Treatment	1,18	3.01	0.1	<b>1,22</b>	<b>6.98</b>	<b>0.015</b>	1,22	1.08	0.309
Treatment									
*	1,18	1.06	0.316	1,22	0.01	0.941	1,22	0.44	0.514
Residence									
Time*	<b>10,180</b>	<b>2.16</b>	<b>0.022</b>	<b>10,220</b>	<b>2.56</b>	<b>0.006</b>	10,220	1.43	0.168
Treatment									
Time*									
Treatment	10,180	1.81	0.062	10,220	1.29	0.237	10,220	1.76	0.069
*									
Residence									

Table S11: Correlations between transplant survival and Beals index in time on different habitat types for species from transplanting experiment. Gap – transplant survival in gaps, control – transplant survival in the intact vegetation, control/gap – the ratio of transplant survival in the intact vegetation and in gaps. Statistically significant results are in bold.

		<i>Carex acuta-Carex panicea</i> habitat			<i>Deschampsia cespitosa-Carex tomentosa</i> habitat			<i>Sesleria uliginosa-Briza media</i> habitat		
		r	N	p	r	N	p	r	N	p
Aug-13	gap	0.348	20	0.133	<b>0.407</b>	<b>24</b>	<b>0.048</b>	0.346	24	0.098
	control	0.297	20	0.203	<b>0.519</b>	<b>24</b>	<b>0.009</b>	0.280	24	0.185
	control/gap	-0.160	20	0.499	-0.127	22	0.573	-0.081	24	0.708
Sep-13	gap	0.402	20	0.079	0.342	24	0.102	0.265	24	0.211
	control	0.413	20	0.070	<b>0.472</b>	<b>23</b>	<b>0.023</b>	0.210	24	0.325
	control/gap	-0.227	20	0.336	-0.122	19	0.618	0.000	24	0.998
Apr-14	gap	<b>0.492</b>	<b>20</b>	<b>0.028</b>	<b>0.421</b>	<b>24</b>	<b>0.041</b>	0.390	24	0.060
	control	<b>0.486</b>	<b>20</b>	<b>0.030</b>	<b>0.445</b>	<b>24</b>	<b>0.029</b>	0.350	24	0.094
	control/gap	0.349	17	0.169	0.155	16	0.566	0.169	22	0.453
Jun-14	gap	<b>0.528</b>	<b>20</b>	<b>0.017</b>	<b>0.436</b>	<b>24</b>	<b>0.033</b>	0.373	24	0.072
	control	<b>0.686</b>	<b>20</b>	<b>0.001</b>	<b>0.474</b>	<b>24</b>	<b>0.019</b>	0.337	24	0.108
	control/gap	0.401	17	0.111	0.306	16	0.249	0.179	22	0.426
Aug-14	gap	<b>0.522</b>	<b>20</b>	<b>0.018</b>	<b>0.482</b>	<b>24</b>	<b>0.017</b>	<b>0.407</b>	<b>24</b>	<b>0.049</b>
	control	<b>0.686</b>	<b>20</b>	<b>0.001</b>	<b>0.526</b>	<b>24</b>	<b>0.008</b>	0.338	24	0.106
	control/gap	0.366	16	0.163	0.387	16	0.139	0.216	21	0.347
Jun-15	gap	<b>0.492</b>	<b>20</b>	<b>0.028</b>	<b>0.549</b>	<b>24</b>	<b>0.005</b>	0.389	24	0.061
	control	<b>0.759</b>	<b>20</b>	<b>0.000</b>	<b>0.608</b>	<b>24</b>	<b>0.002</b>	<b>0.558</b>	<b>24</b>	<b>0.005</b>
	control/gap	<b>0.722</b>	<b>16</b>	<b>0.002</b>	0.397	15	0.143	0.334	17	0.190
Sep-15	gap	<b>0.491</b>	<b>20</b>	<b>0.028</b>	<b>0.591</b>	<b>24</b>	<b>0.002</b>	0.359	24	0.085
	control	<b>0.759</b>	<b>20</b>	<b>0.000</b>	<b>0.608</b>	<b>24</b>	<b>0.002</b>	<b>0.480</b>	<b>24</b>	<b>0.018</b>
	control/gap	<b>0.734</b>	<b>17</b>	<b>0.001</b>	0.307	15	0.266	0.330	17	0.196
Jun-16	gap	0.438	20	0.053	<b>0.486</b>	<b>24</b>	<b>0.016</b>	0.253	24	0.233
	control	<b>0.746</b>	<b>20</b>	<b>0.000</b>	<b>0.548</b>	<b>24</b>	<b>0.006</b>	0.397	24	0.055
	control/gap	<b>0.706</b>	<b>16</b>	<b>0.002</b>	0.418	15	0.121	0.205	16	0.447
Sep-16	gap	<b>0.459</b>	<b>20</b>	<b>0.042</b>	<b>0.486</b>	<b>24</b>	<b>0.016</b>	0.237	24	0.264
	control	<b>0.778</b>	<b>20</b>	<b>0.000</b>	<b>0.616</b>	<b>24</b>	<b>0.001</b>	0.316	24	0.132
	control/gap	<b>0.763</b>	<b>16</b>	<b>0.001</b>	0.487	15	0.065	0.012	18	0.962

		<i>Carex acuta-Carex panicea</i> habitat			<i>Deschampsia cespitosa-Carex tomentosa</i> habitat			<i>Sesleria uliginosa-Briza media</i> habitat		
		r	N	p	r	N	p	r	N	p
Aug-17	gap	0.233	20	0.323	0.119	24	0.581	0.347	24	0.097
	control	<b>0.768</b>	<b>20</b>	<b>0.000</b>	0.270	24	0.203	0.016	24	0.941
	control/gap	0.447	11	0.168	0.461	10	0.180	-0.343	9	0.367
Jul-18	gap	0.234	20	0.321	0.114	24	0.596	0.208	24	0.330
	control	<b>0.618</b>	<b>20</b>	<b>0.004</b>	0.360	24	0.084	0.218	24	0.305
	control/gap	0.514	11	0.106	0.694	8	0.056	-0.139	6	0.793



## **Chapter 5**

### **Methods of species pool determination as predictors of survival in seeding and transplanting experiments**

Švamberková & Lepš (manuscript).



# Methods of species pool determination as predictors of survival in seeding and transplanting experiments

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## Abstract

1. Species composition is constrained by the ability of species to reach the site, establish, and survive there. The establishment and survival of species is constrained by both abiotic conditions and biotic interactions. These two operate in local scales together and it is very challenging to distinguish their effects. For this case, it is very important to define well the species pool with which the community composition is compared.

2. We can determine the species pool experimentally or use some of the estimation methods. But which of these methods is the closest to the reality? We compared four estimation methods of species pool assessment with the real survival of species from seed/transplant addition experiment.

3. We added both resident and non-resident species into plots with and without competition in four localities. We tested the ability of species to survive in competition-free gaps (abiotic conditions) and in the intact vegetation (complete community filter). We tested the recruitment of species from seeds and their establishment and survival of species from pre-grown transplants in these treatments. We tested, which of methods of species pool assessment can predict species performance in individual treatments and which part of the

environmental filter is the main driver of species community composition.

4. Methods of species pool assessment based on species co-occurrence patterns (Beals index and UNO) were the best predictors of the performance of species in the intact vegetation. These methods were less successful for prediction of species performance in competition free environment where the predictions based on some functional traits were a slightly more successful although that it was quite variable for different traits. Methods based on co-occurrence patterns were the most effective for prediction of seedling establishment, while seed germination and transplant survival were not predictable so well.

5. The biotic filter had the principal role for our species community composition, especially for the establishment process of seedlings. The role of biotic and abiotic filter is very difficult to distinguish without experimental approach and it is important to remember that the ratio of their importance is changing during the plant ontogeny.

### **Keywords**

Abiotic filter, Biotic filter, Co-occurrence, Community composition, Species pool, Seed addition experiment, Establishment, Transplants.



## Introduction

Determinants of composition of ecological communities have been in the focus of researchers for several decades (Götzenberger et al., 2012; Czortek et al., 2021). Species composition is constrained by a species ability to reach the site (i.e., dispersal limitation, Houseman & Gross, 2006), and its ability to establish and survive there. The latter is constrained by both abiotic conditions and biotic interactions (Cadotte & Tucker, 2017; Bruelheide et al., 2018; Belinchón et al., 2020). These two operate in local scales in concert and it is very challenging to distinguish their effects (Adler et al., 2013; Kraft et al., 2015; Švamberková and Lepš, 2020).

Regional species pools are determined by phylogeographic aspects such as speciation, extinction, and migration of species (Zobel, 1997; Swenson, 2011; Götzenberger et al., 2012), but the dispersal limitation might still prevent some species from the regional pool to reach a specific site (Pärtel et al., 1996; Schamp et al., 2016). Species which reached the target habitat are then sorted according to their ability to withstand the abiotic conditions of the site and the biotic interactions ongoing in the community (Cadotte & Tucker 2017; Bruelheide et al., 2018; Belinchón et al., 2020). Whereas the competition is probably the most limiting biotic factor for plants (Grubb, 1977; Wellstein et al., 2014; Li et al., 2018), the interactions with other trophic levels might be also limiting (herbivores, pathogens; Dobson & Crawley, 1994), but in some instances might have even a positive effect (e.g., mycorrhizal fungi, van der Heijden & Horton, 2009). These constraints are often described as series of filters: dispersal filter, community filter composed of abiotic and biotic parts (Butaye et al., 2021; Zobel, 1997).

Are we capable to predict, which species will be able to pass individual filters and become part of the actual community? Can the trait or known ecological preferences of individual species help to predict it? These questions are often solved by comparing the species

pool with the actual species composition (de Bello et al., 2012; Czortek et al., 2021), or with the ability of species to establish when sown (Zobel & Kalamees, 2005; Švamberková et al., 2017; Švamberková & Lepš, 2020). The problem is which of the species pools to use. Comparison with all the species able to reach the site (local species pool according to Zobel, 1997) will show the effect of complete community filter (biotic and abiotic factors in concert). Comparisons with pool of all environmentally suitable species (local species pool according to Butaye et al. (2001)) should show directly the effect of biotic filter (the species not able to withstand the abiotic conditions are by definition absent from the pool) and comparison with pool of species suitable to withstand the complete community filter (actual species pool according to Zobel (1997)) should show mainly an effect of some stochastic factors, because by definition, all the species should be able to pass the filter. Unless we add the known number of propagules (sufficiently exceeding the natural seed rain; Vítová & Lepš, 2011), we can hardly exclude the dispersal limitation. In the sowing experiments, we can directly study the effect of the community filter, and by manipulating of the target vegetation, we can form microhabitats affected (mainly) by abiotic filter (removing competition by forming artificial gaps; Švamberková et al., 2017; Švamberková & Lepš, 2020), even though removing competition does not remove other biotic factors.

To establish a viable population, enough seeds must germinate, establish, and finally develop into mature, reproductive individuals. This might take several years, and usually very small fraction of seeds reach the reproductive stage (Turnbull et al., 2000; Klimeš, 2005; Vítová & Lepš, 2011). It is thus desirable to follow the results of sowing for several years, and to estimate the chances of young individuals to reach maturity, to complement the sowing experiments with pre-grown transplants (Švamberková & Lepš, 2020).

Attempts to identify the set of species suitable for a habitat are very old – as a matter of fact, the knowledge of species habitat preferences and identification of habitat specific sets of appropriate species was the basis of classical Braun-Blanquet phytosociology (van der Maarel, 1975). Today, these attempts continue, and are often formulated as methods of the species pool determination. The first group (1) of these methods is based on the similarity of the composition of target community with ecological preferences of the focal species. Within this group, three approaches are used. The first one (1a.) is based on the phytosociological knowledge of local experts which establish the extensive lists of species and habitat types (Sádlo et al., 2007; Jiménez-Alfaro et al., 2018). Nevertheless, this approach depends on researchers with good experience from the study areas (Eriksson, 1993; Losvik, 2007), and is difficult to reproduce.

The second approach (1b.) is based on known ecological preferences of individual species on selected environmental gradients considered important, frequently published as indicator values (e.g., Ellenberg et al., 1991). The community weighted mean (CWM) of the indicator values of the target community should be indicative of the environment and thus the closer is species to CWM, the more should be the environment favourable for it (Pärtel et al., 1996; Zobel, 1997; Zobel et al., 1998). Original Ellenberg indicator values (EIV; Ellenberg et al., 1991) were published for taxa of western part of central Europe. The species preferences might differ among areas, and similar system of indicator values were published for other areas (e.g., Landolt et al., 2010 for Switzerland and Alps; Didukh, 2011 for Ukraine; Chytrý et al., 2018 for Czech Republic). The indicator values are based on the knowledge of local experts, and on the selection of gradients considered important.

The third approach (1c.) is based on co-occurrence patterns which are usually derived from large phytosociological databases. If a focal species frequently co-exists with the species of the target

community, it should belong to the community species pool (Lessard et al., 2016; Real et al., 2017; Belinchón et al., 2020). Typically, Beals index (BI; Beals, 1984; Ewald, 2002; Lewis et al., 2016) evaluates the probability of co-occurrence of the focal species with species in the target community based on the frequency of their real co-occurrence in phytosociological relevés in large phytosociological databases (Münzbergová & Herben, 2004). The multivariate structure of compositional data is used without any prior classification of community types, selection of environmental gradients, and is based on the data in databases, not on knowledge of experts. This is also the case for recently suggested method based on an unconstrained ordination analysis (UNO), which assesses the habitat favorability for a species based on relative position of a focal species and target community sample scores in an unconstrained ordination, based on the reference database (Brown et al., 2019).

The second group (2) of methods of predicting species pool is based on species functional traits (Sonnier et al., 2010; Moor et al., 2015). According to the functional species pool concept (de Bello et al., 2012), species with similar functional traits as the target community (typically characterized by CWM) should belong to the species pool. Although that the methodology of this concept might seem like EIV approach (1b.), there is a very important difference. While EIV are based on subjective knowledge of wide scale ecological preferences of individual species (based on wide co-occurrence patterns), the method using functional traits (2) is based just on direct measurements of the species traits. They are not thus dependent on the species distribution in landscape and their results are reproducible (Westoby, 1998; Pérez-Harguindeguy et al., 2016). For the EIV, the assumption is that the closer is species indicator value to CWM, the higher chance that species will be successful there. Whereas the same might be assumed also for traits, it is not unrealistic to expect that some traits will support

help species to thrive in the community, regardless of the CWM (e.g., the bigger seeds, the better is the chance to establish successfully).

All the methods of the group 1 clearly rely on the co-occurrence patterns and generally on the realized niches of species, so they should correspond to the actual species pool according to Zobel (1997). Species traits reflect the species ability to cope with both, biotic and abiotic conditions, and thus the methods of group 2 should be also related to actual species pool, i.e., to the realized niches of species (Violle & Jiang, 2009; Adler et al., 2013; Belinchón et al., 2020). However, because some of the functional traits might be more important for coping with the abiotic environment and some with the competition, various functional traits might be useful for predicting either species success in the community or in the competition free species in given abiotic environment.

Species belonging to the actual species pool, if not limited by dispersal, should be able to establish in a community and form there a viable population. Methods of species pool determination should be thus suitable also for predicting the success of species in sowing/transplanting experiments. We conducted a seed/transplant addition experiment adding both resident and non-resident species into plots (and thus removing the possible dispersal limitation) with and without competition in four localities. We tested the performance of species in competition free gaps, i.e., the ability of species to survive in given abiotic conditions, and the performance of species in vegetation, i.e., the ability of species to survive in given both abiotic and biotic conditions. We also assumed that a ratio of species performance in gaps and in vegetation should reflect the effect of biotic filter itself. We tested the performance of species in these treatments for two phases of species life cycle – 1) recruitment of species from seeds and 2) establishment of species from pre-grown transplants. We then tested, which of methods of species pool assessment can predict species performance in individual treatment

types. Some of the species pool assessment methods use some threshold for decision whether the species belongs to the pool. We are convinced that species pool is a fuzzy, rather than a crisp set (i.e., each species should have some quantitative indicator, rather than to either be or not to be a pool member), and thus we directly used the quantitative indicators as predictors.

We asked: (a) Which of the methods of species pool determination can predict species success when introduced by sowing or as transplant? Are the methods more successful in predicting the performance in the intact vegetation or in the competition free space? (b) Are the methods more effective when predicting success of sowing or the survival of transplants? The answers to the above questions enable to compare the importance of biotic and abiotic parts of the community filter and answer the question (c) about the importance of filtering on the establishment process and on the adult survival.

## Materials and methods

### Study site

The seed/transplant addition experiment was conducted in four different oligotrophic species rich meadows in South Bohemia in the Czech Republic near České Budějovice. These four localities formed a moisture gradient. The wettest locality Ohrazení (48°57'N, 14°35'E, 510 m a.s.l.) is a wet meadow characterized as *Molinion*. Vrcov (48°55'N, 14°39'E, 510 m a.s.l.) is a mezic meadow characterized as *Alopecurion* with some elements of *Molinion*. Zvíkov (48°59'N, 14°36'E, 500 m a.s.l.) is a mezic meadow characterized as *Arrhenatherion* (association *Poo-Trisetetum*). The driest locality Závraty (48°56'N, 14°23'E, 460 m a.s.l.) is a relatively dry grassland with the lowest productivity (Table S1) characterised as *Arrhenatherion* (association *Ranunculo bulbosi-Arrhenatheretum elatioris*). All these localities were extensively managed with a single mowing term at the end of June with exception of Vrcov where the

meadow was mown twice a year (June and September). The localities are close to each other (maximum distance is 20 km) and experience very similar climatic conditions (Table S2).

### **Species**

We selected 30 meadow plant species (their Ellenberg indicator values for light varied from 5 to 8, for moisture from 3 to 10 and for nutrients from 2 to 7), both residents typical for target localities and non-residents typical for different habitats (Table S3). Species residence was determined for each locality individually: species were considered resident if present in at least one of the five phytosociological relevés (5x5m) recorded in each locality in June 2016. All non-resident species can be a part of the regional species pool (i.e., should be able to disperse to the target localities) – all are present in the area (within radius of 30 km from the localities) according to the species distribution database ([www.pladias.cz](http://www.pladias.cz), access 13<sup>th</sup> of November 2020).

### **Seed introduction experiment**

Seeds were sown into both the artificially created gaps (i.e., plots without competition from surrounding vegetation) and the intact vegetation (i.e., control plots with competition from surrounding vegetation) at the beginning of April 2016 in the four localities. Gaps (20x20cm) were created artificially by digging a hole 20 cm deep. This hole was lined by geotextile to prevent the growing of roots of surrounding vegetation on a one hand and to ensure the permeability for both water and microbiota on the other hand. Gaps were refilled with the soil from the target locality without any rest of roots or rhizomes mixed with sand in the ratio of 3:1. Gaps were regularly weeded two times per year from non-target species which reached gaps from seeds or exceeded the geotextile vegetatively from the above. In each locality, two blocks, each containing 30 gaps and 30 control plots, were established. 200 seeds were sown evenly into whole area of each plot, each species separately into its own plot. The germination of seeds

and survival of seedlings was monitored from April 2016 to September 2019.

### **Transplant introduction experiment**

Seeds germinated in a growth chamber (12 h light and 12 h darkness, 19°C) and subsequently, seedlings were replanted into greenhouse. Each individual was planted into its own pot (7x7x6.5cm) both with and without geotextile. As substrate, we used the soil from the target localities (to assure the natural composition of microbiota) mixed with sand in a ratio of 3:1 (to balance the level of nutrients increased in soil after its moving from the field). Pre-grown transplants were planted in the four target localities in middle June 2016. Two pre-grown transplants of each species were planted (i.e., one transplant without and one with geotextile around it to identify the effect of geotextile on species survival) randomly in each treatment type. The distance among individuals in each treatment plot was 20 cm. In each locality, design of the experiment was arranged in four randomly replicated blocs with three treatments in each bloc: 1) gaps without any competition (i.e., exclusion of both below- and aboveground competition), 2) clipped plots (i.e., exclusion of only aboveground competition) and 3) control plots with the intact vegetation (i.e., no exclusion of competition). Gaps (40x600cm) were created and weeded similarly as in the case of seed introduction experiment. In the case of clipping treatment, the vegetation of 20 cm around each transplant was clipped by scissors regularly one time per three weeks. The survival of transplants was monitored from June 2016 to April 2019.

### **Data analysis**

To assess, how much a species “belongs to the community”, we used four different methods of species pool assessment (Table S3). These indicators were subsequently correlated with measures of species performance in the field experiment. The methods used were: Ellenberg indicator values (EIV; absolute value of difference from the



CWM, assumed to be negative predictor), species functional traits, Beals index (BI) and unconstrained ordination (UNO). In Supplementary materials (Fig. S1), we also presented some results with EIV without CWM and with the difference of functional trait values from the CWM. We used EIV for moisture, light and nutrients which were taken from a list of these values for the Czech Republic (Chytrý et al., 2018). Then, we used five species functional traits. Four traits were exported from databases: canopy height determining competitive ability of species, specific leaf area (SLA) and leaf dry-matter content (LDMC) associated with leaf economic spectrum and thus trade of between resource acquisition and conservation (all three traits taken from the LEDA database; Kleyer et al., 2008), and additionally lateral spread (with exclusion of freely dispersible organs) determining ability of clonal spread from the CLO-PLA 3.3 database (Klimešová et al., 2017). Fifth used species functional trait was seed mass which is related to reproductive ability. Average mass of one seed was derived from the real mass of 50 seeds (the seeds that were finally sown in the experiment).

We used two methods of species pool determination based on species co-occurrence patterns – Beals index (BI) and unconstrained ordination (UNO) which both were calculated individually for each sown species and experimental locality. For their calculation, we used phytosociological relevés (five 5x5m relevés per locality recorded in June 2016) and the Czech National Phytosociological Database (Chytrý & Rafajová, 2013) in stratified form (31 512 relevés) to reduce oversampling of some areas (Těšitel et al., 2015) as the reference database. For calculating BI, we used R-package “vegan” (Oksanen et al., 2019) of function “beals” of “type” = 2 (abundances used to compute weighted averages of conditioned probabilities). UNO was calculated using R function “dark.pred.ca (Brown et al., 2019) of “method = minpred” (using abundance data based on species cover). We calculated both BI and UNO for each relevé separately and used

the average value across the five relevés per each locality for subsequent analyses.

We compared each method of species pool assessment with survival of seedlings germinating from sown seeds in field (seedlings) and survival of pre-grown transplants (transplants) across the different treatments by Pearson's correlation (if two variables are highly correlated, one of them can be a good predictor of the other, Lepš & Šmilauer, 2020). We used the average survival (from two and four replication for seedlings and transplants respectively) for each combination of observation time, locality, and species in each treatment type. We also correlated the ratios of seedling or transplant survival in different treatments ("Clipped/Gap" representing the effect of belowground competition, "Control/Clipped" representing the effect of aboveground competition and "Control/Gap" representing the effect of full competition) with different methods of species pool determination. In these ratios a value 1 denotes no effect of competition and 0 the strongest effect of competition.

To compare the predictive power of different methods of species pool assessment, we analysed the relationship between absolute values of obtained correlation coefficients (through all experimental time and localities) and different methods of species pool determination, treatments and their interaction using general linear models (GLM) in programme R. Because some of the correlations between traits and survival were negative, we used for all the methods the absolute values of  $r$ , to have comparable procedure for all the methods. To analyse the changes of predictive power of different methods of species pool assessment in time, we calculated repeated measurement analysis of variance of absolute value of correlation coefficients between seedling/transplant survival and different methods of species pool assessment in different treatments from four studied localities. We used Linear Mixed Effects Models with locality as a factor with random effect in R-package "nlme" (Pinheiro et al., 2021).

Nevertheless, to enable ecological interpretation of the results, we presented in the pictures the real value of correlation coefficients, particularly to show which values of which traits increase the survival in individual cases. For the values of correlation coefficients, we show in the figures the limits for significance ( $p < 0.05$  in a two-tailed test) for single correlation coefficient for given number of species (30 in our case). It can be interpreted that if the average exceeds this limit, correlation coefficient for given treatment is mostly significant.

Finally, we choose one method of species pool determination which had generally the best predictive power for the real seedlings/transplant survival (i.e., BI) to compare in detail its results with the seedling/transplant survival. In the case of seedlings, we used the values of correlation coefficients of seedling survival with BI to determine their differences between treatments, in time, among localities and in the interactions between treatment and time and between locality and treatment in GLM in programme R. The same analysis was used for transplants, but we included there also the effect of geotextile (Textile) and the interaction between treatment and Textile and among treatment, Textile, and locality.

## **Results**

### **Predictive power of different methods of species pool assessment**

Both for seedlings and transplants, on average, the methods differed in their efficiency, but the differences between methods depended considerably on the treatments (Method\*Treatment interaction, Table 1). Methods based on the co-occurrence patterns (BI and UNO) correlated with the survival of both seedlings and transplants in controls the best (the performance of those two was very similar, with BI being usually slightly better), and similarly were superior for the effects of competition, particularly for the Control/Gap ratio (Fig. 1). Their performance for predicting survival in gaps was much worse, particularly for seedlings. There, the seed mass was the best predictor

for seedling survival (seedlings with heavy seeds survived better, Fig. 1a) and height for transplants (potentially tall species survived worse, Fig. 1b). When predicting ratios for transplants, BI and UNO were best or second best for ratios that included the belowground competition (Control/Gap and Clipped/Gap) but below average for the one reflecting only aboveground competition (Control/Clipped) (Fig. 1b).

Table 1: Results of analysis of variance of absolute value of correlation coefficient between seedling/transplant survival and different methods of species pool determination in different treatments (data for each measuring time and each locality taken together). General linear model with Gaussian distribution and identity link canonical function. Statistically significant results ( $p < 0.05$ ) are in bold.

	Seedlings			Transplants		
	Df	F	p	Df	F	p
Method	<b>9,710</b>	<b>3.6</b>	<b>&lt; 0.001</b>	<b>9,1670</b>	<b>87.73</b>	<b>&lt; 0.001</b>
Treatment	1,709	0.85	0.356	2,1668	2.22	0.109
Method*Treatment	<b>9,700</b>	<b>11.69</b>	<b>&lt; 0.001</b>	<b>18,1650</b>	<b>3.98</b>	<b>&lt; 0.001</b>

Table 2: Results of repeated measurement analysis of variance of absolute value of correlation coefficient between seedling/transplant survival and different methods of species pool determination in different treatments from four localities. Linear Mixed Effects Models with locality as a random factor. Statistically significant results ( $p < 0.05$ ) are in bold.

	Seedlings			Transplants		
	Df	F	p	Df	F	p
Method	<b>9,537</b>	<b>3.86</b>	<b>&lt; 0.001</b>	<b>9,1467</b>	<b>91.3</b>	<b>&lt; 0.001</b>
Treatment	1,537	0.91	0.339	2,1467	2.31	0.1
Time	8,537	0.37	0.937	6,1467	0.55	0.771
Method*Treatment	<b>9,537</b>	<b>12.52</b>	<b>&lt; 0.001</b>	<b>18,1467</b>	<b>4.15</b>	<b>&lt; 0.001</b>
Method*Time	72,537	0.8	0.885	<b>54,1467</b>	<b>2.75</b>	<b>&lt; 0.001</b>
Treatment*Time	8,537	0.57	0.801	12,1467	0.61	0.833
Method*Treatment*Time	72,537	0.61	0.995	108,1467	0.6	1

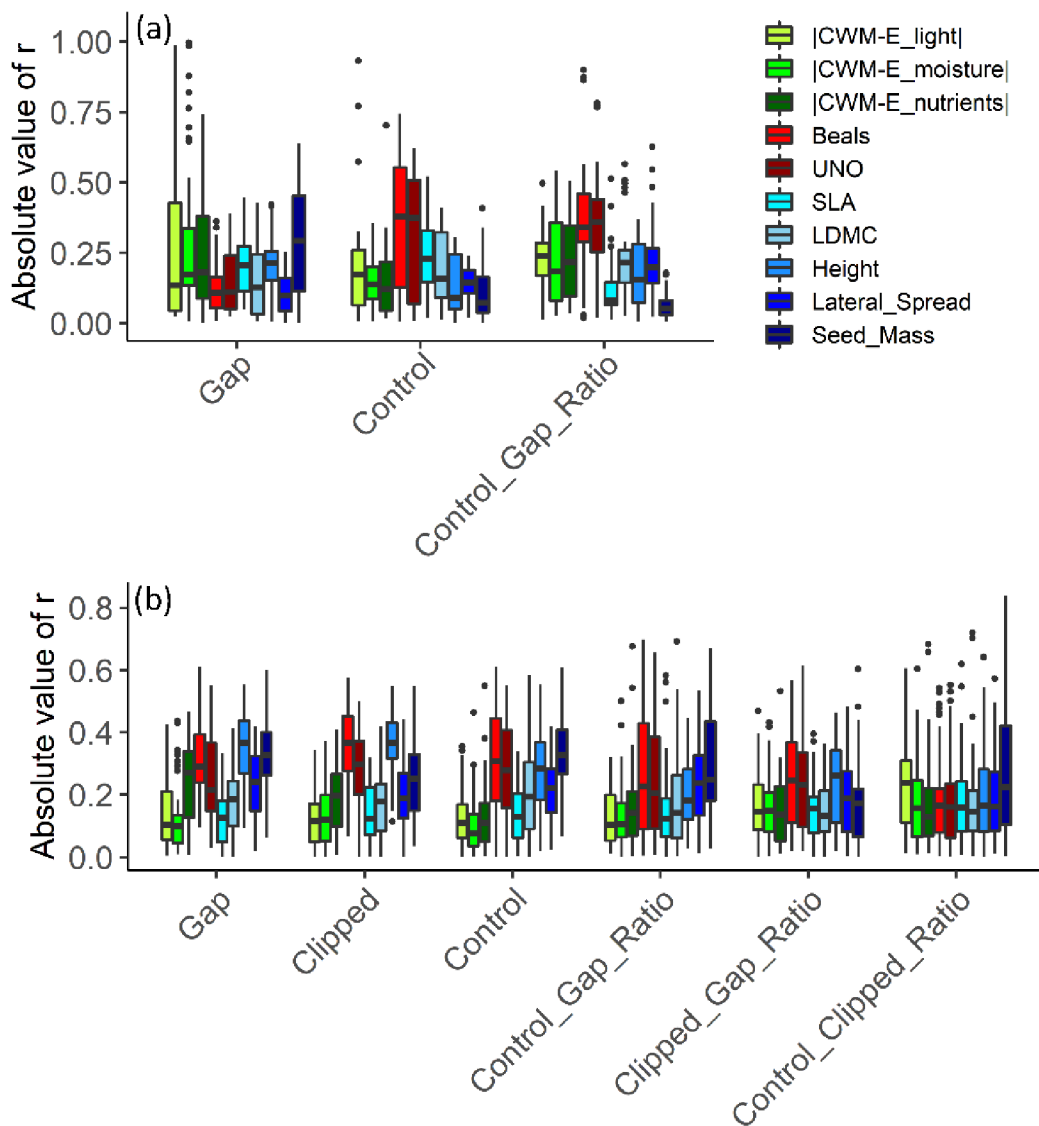


Fig. 1: The absolute values of Pearson's correlation coefficient  $r$  between real seedling (a)/transplant (b) survival from experimental approach and values of different methods of specie pool determination (absolute value of difference of Ellenberg indicator values for light, moisture, and nutrients from the CWM; functional traits – SLA, LDMC, canopy height, lateral spread, and seed mass; and Beals index and UNO based on species co-occurrence) in different treatments (gaps, clipped plots, control plots) and their ratios.

## Changes in time

There was no common temporal pattern or trend in the performance of all the individual methods for neither seedlings nor transplants (Table 2). Nevertheless, relative performance of individual methods differed in time for transplants (significant Method\*Time), but not treatments for neither seedlings nor transplants (Table 2). In the case of seedlings in gaps, BI and UNO were surpassed by some EIV and functional traits in each time (Fig. 2a), nevertheless, the average values of correlation coefficients indicated that the correlations were mostly not significant. For seedlings in control plots, it lasted two years to stabilise the predictive power of individual methods. Nevertheless, from the third year, BI and UNO were the best predictors of seedling survival, and their predictive power has had an increasing tendency indicating mostly significant relationships (Fig. 2b). In the case of the ratio of seedling survival in control plots and gaps, BI and UNO were the real top among all other methods during all the times of the experiment, and unlike the other methods, had increasing tendency (Fig. 2c).

In the case of transplants, height led in the predictive power in gaps (with negative sign, i.e., the taller species survived worse) and BI was the second on the top in gaps (Fig. 2d). BI and UNO held the top with height in clipped plots (with the increasing importance of seed mass and lateral spread from third year, Fig. 2e) and with both height and seed mass (which increased so steeply in the third year that it exceeded all other methods) in control plots (Fig. 2f). However, the values of  $r$  indicate that in most cases, the relationships were not significant.

Correlations of EIV with seedling survival in gaps was surprisingly mostly positive (the more different from CWM, the better seedling survival, Fig. 2a) while in control plots and for the ratio of control plots and gaps, they were more negative (as expected, close to CWM means better survival, Fig. 2b, c). In the case of transplants, EIV for light and moisture correlated with transplant survival mostly

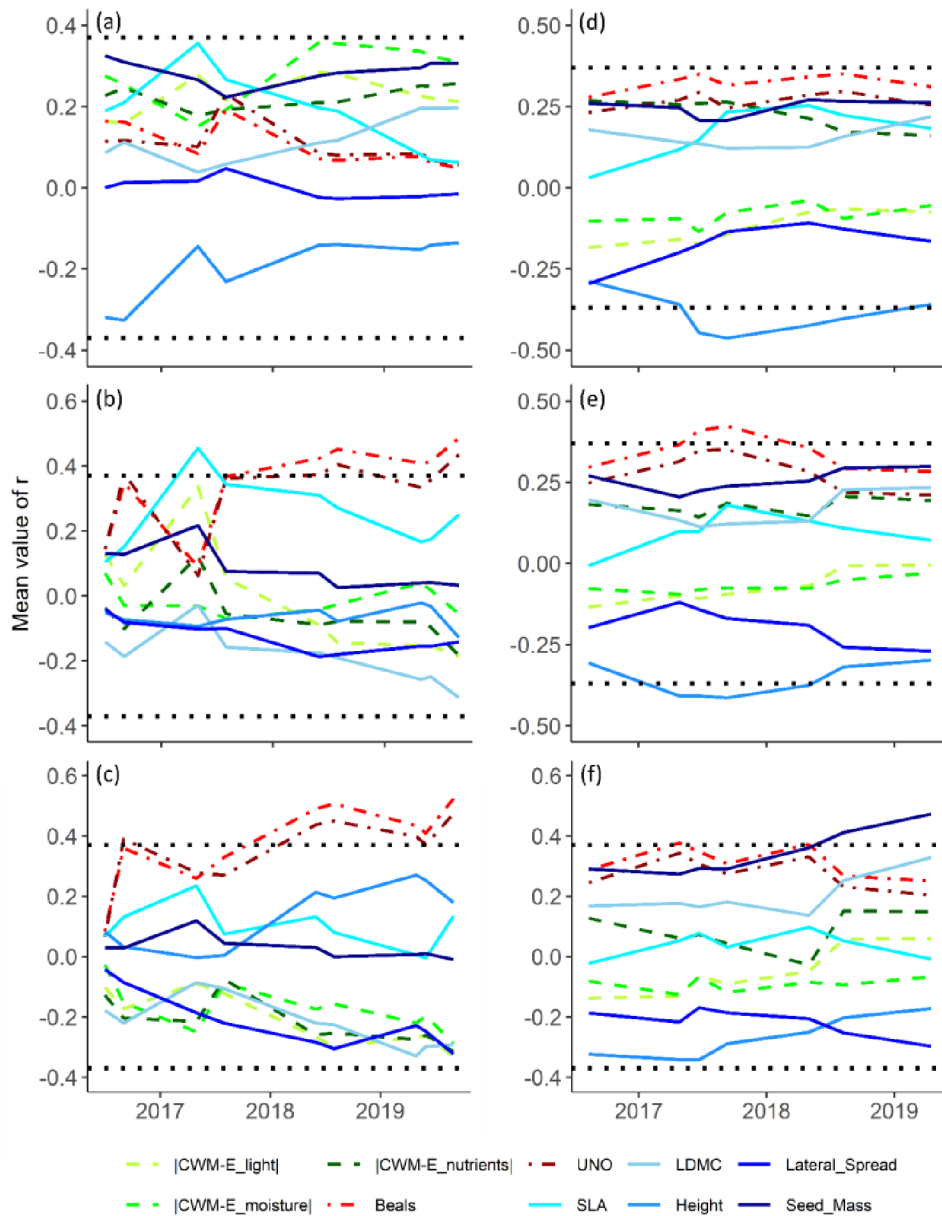


Fig. 2: The average values of Pearson's correlation coefficient  $r$  between real seedling/transplant survival from experimental approach and values of different methods of specie pool determination (difference of Ellenberg indicator values for light, moisture, and nutrients from the CWM; functional traits – SLA, LDMC, canopy height, lateral spread, and seed mass; and Beals index and UNO based on species co-occurrence) in different measuring times of the experiment for different treatments: (a) Seedlings in gaps, b) Seedlings in control plots, c) Seedlings for the ratio of control plots and gaps, d) Transplants in gaps, e) Transplants in clipped plots, and e) Transplants in control plots). Horizontal black dotted lines represent the level of significance of correlation coefficients if  $N = 30$  (sometimes less in the case of ratio (c)) and  $p < 0.05$ .

negatively while for nutrients positively in all treatment types (Fig. 2d, e, f). Nevertheless, these correlations were under the level of significance. Correlations of BI and UNO with both seedlings and transplants survival were mostly positive in all treatment types and their ratios (Fig. 2). While correlation of SLA and seed mass with seedling survival was mostly positive in all treatments, LDMC, lateral spread and height (except for Control/Gap ratio) correlated with seedling survival mostly negatively (Fig. 2). In the case of transplants, SLA, LDMC and seed mass correlated predominantly positively while height and lateral spread mostly negatively in all treatment types (Fig. 2). However, with few exceptions, correlations with functional traits were under the level of significance.

### **Performance of Beals index (BI) in different localities and treatments**

Seedling survival correlated significantly positively with BI in control plots (with exception of Zvíkov), and the correlations generally increased with time (Fig. 3a). On the contrary, the correlations with survival in gaps were weak, non-significant, and did not show any temporal trend. The BI also correlated tightly (and with increasing tendency over time) with the ratio of survival in control to gap. On the other hand, for transplant survival, there were significant correlations with BI predominantly for gaps and clipped plots in Ohrazení and Vrcov, for control and clipped plots in Zvíkov and for all plot types in Závraty (Fig. 3c).

Although correlation coefficients of seedling survival with BI were higher for control plots than for gaps (Fig. 3a), there was no significant difference among treatments in the case of transplants (Fig. 3c, Table 3). Treatments differed among localities in the case of both seedlings and transplants (Treatment\*Locality in Table 3). In the case of seedlings, there was significantly higher difference between gaps and control plots in Ohrazení ( $F_{1,16} = 22.74$ ,  $p = 0.001$ ) and Vrcov ( $F_{1,16} = 16.51$ ,  $p = 0.004$ ), localities with the highest biomass (Table S1), than in Zvíkov ( $F_{1,16} = 7.86$ ,  $p = 0.023$ ) and Závraty ( $F_{1,16} = 9.38$ ,  $p = 0.016$ ),



where also the biomass was lower (Table S1). In the case of transplant survival, the effect of the biotic filter was not so important with exception of Zvíkov, where correlation coefficients were higher for control plots with competition than for both gaps without competition and clipped plots with only below-ground competition (Fig. 3c).

Correlation coefficients of ratio of seedling survival in control plots and gaps with BI increased in time ( $F_{8,27} = 2.61$ ,  $p = 0.033$ , Fig. 3 b) but not differed significantly among localities ( $F_{3,24} = 2.32$ ,  $p = 0.101$ , Fig. 3 b). In the case of transplants, three ratios (“Clipped /Gap”, “Control/Clipped” and “Control/Gap”) differed among localities, but they did not differ in time (Table 3). Nevertheless, there was a predominating trend that the most important was the full or/and belowground competition while the effect of aboveground competition was not so important.

Table 3: Results of analysis of variance of correlation coefficients of seedling/transplant/transplant ratio survival with Beals index. General linear model with Gaussian distribution and identity link canonical function. Statistically significant results ( $p < 0.05$ ) are in bold.

	Seedlings			Transplants			Transplant ratio		
	df	F	p	df	F	p	df	F	p
Treatment	<b>1,70</b>	<b>55.69</b>	<b>&lt; 0.001</b>	2,165	2.16	0.12	<b>2,165</b>	<b>15.28</b>	<b>&lt; 0.001</b>
Locality	<b>3,59</b>	<b>13.19</b>	<b>&lt; 0.001</b>	<b>3,156</b>	<b>8.01</b>	<b>&lt; 0.001</b>	<b>3,156</b>	<b>26.60</b>	<b>&lt; 0.001</b>
Time	8,62	1.66	0.134	<b>6,159</b>	<b>5.12</b>	<b>&lt; 0.001</b>	<b>6,159</b>	<b>2.61</b>	<b>0.021</b>
Textile	-	-	-	<b>1,155</b>	<b>9.91</b>	<b>0.002</b>	<b>1,155</b>	<b>34.80</b>	<b>&lt; 0.001</b>
Treatment *Locality	<b>3,48</b>	<b>3.66</b>	<b>0.019</b>	<b>6,137</b>	<b>28.88</b>	<b>&lt; 0.001</b>	<b>6,137</b>	<b>8.79</b>	<b>&lt; 0.001</b>
Treatment *Time	<b>8,51</b>	<b>2.87</b>	<b>0.011</b>	12,143	1.72	0.07	12,143	0.67	0.778
Treatment *Textile	-	-	-	<b>2,135</b>	<b>31.41</b>	<b>&lt; 0.001</b>	<b>2,135</b>	<b>17.77</b>	<b>&lt; 0.001</b>
Treatment *Textile *Locality	-	-	-	<b>6,126</b>	<b>2.67</b>	<b>0.018</b>	<b>6,126</b>	<b>2.85</b>	<b>0.012</b>

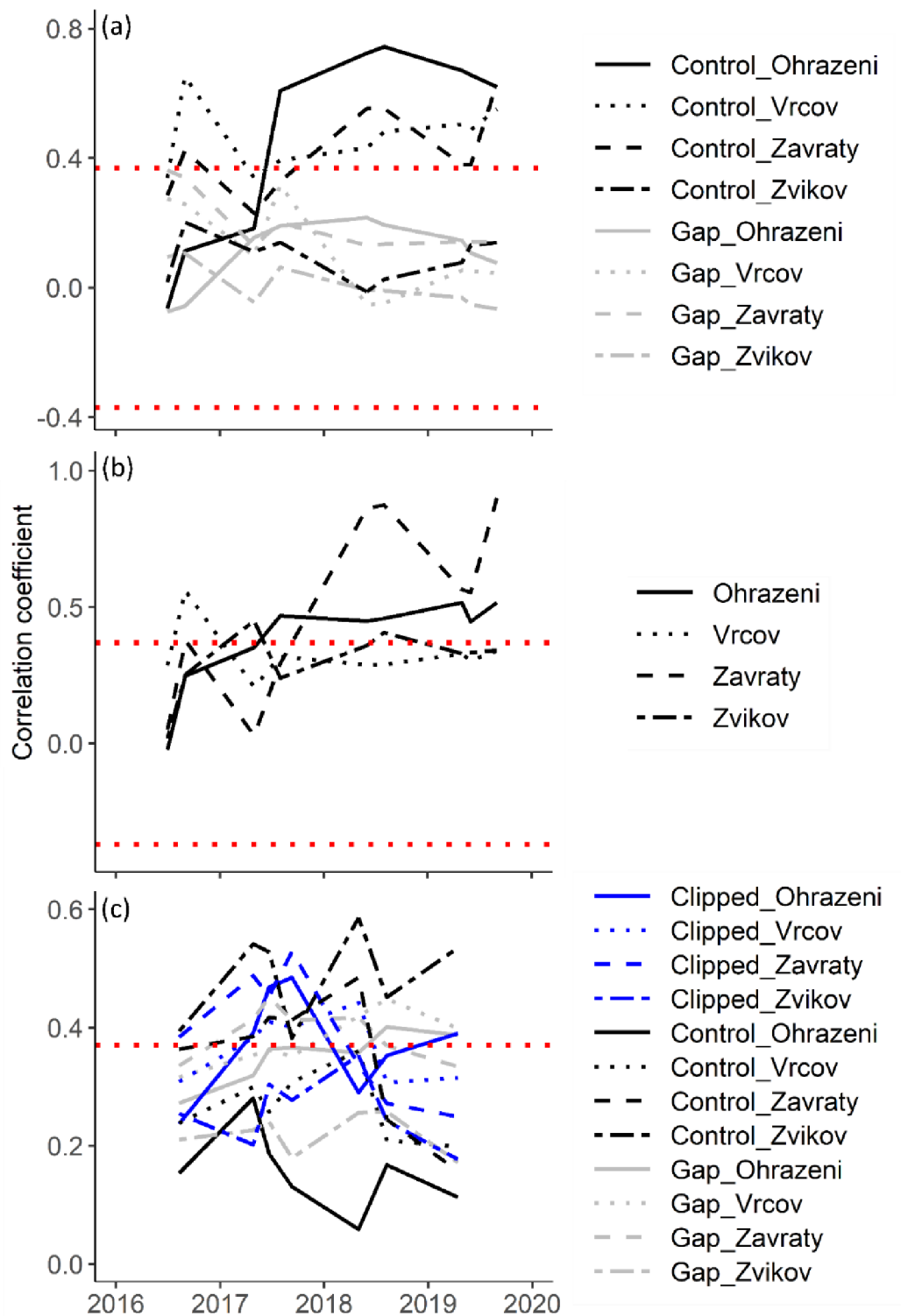


Fig. 3: Correlation coefficients between Beals index and seedling survival in gaps and control plots (a) and for the ratio between control plots and gaps (b), and transplant survival (c). Horizontal red dotted lines represent the level of significance of correlation coefficients if  $N=30$  (sometimes less in the case of ratio (b)) and  $p < 0.05$ .

## **Discussion**

None of the species pool determination methods was superior for all the treatments and for all plant developmental stages. The species performance was best predictable in controls (or control/gap ratios), and in this case, the methods based on direct use of vegetation databases (BI and UNO) were superior. This demonstrates the importance of the biotic filter for the formation of vegetation patterns. The performance of seedlings was better predictable than of transplants, suggesting the importance of filtering during the establishment from seeds. In competition free gaps, the methods based on vegetation databases were often outperformed by species traits.

### **Predictive power of different methods**

All the tested methods had some limitation and none of them can predict the species performance perfectly: these methods can only estimate the favorability of the target habitat for individual species (Ronk et al., 2016; Carmona & Pärtel, 2020). Thus, if we are not able to test the composition of local species pool experimentally, it is necessary to choose the best from estimation methods because some of them do it at least better, some of them worse (Belinchón et al., 2020). The reliability of individual methods differed in dependence on treatment type and the species life stage.

In competition free gaps, which reflected the effect of abiotic filter only and thus the local species pool according to Butaye et al. (2001), the prediction of species performance by whichever tested methods was not very successful. It is because all used methods are related to realized niches of species (Violle & Jiang, 2009; Adler et al., 2013; Belinchón et al., 2020) and thus correspond with actual species pool (Zobel, 1997), i.e., include species able to pass through the complete community filter. Many of studies use expression “environmental filtering” which should be associated to the effect of only abiotic filter (Kraft et al., 2015), but in fact, they used it for community filtering which combine both the abiotic and biotic filter

(Ronk et al., 2016; Belinchón et al., 2020). It is very difficult and may be even impossible to separate the effect of the abiotic environment from the biotic interactions only from observational data without any experimental approach (Adler et al., 2013; Švamberková & Lepš, 2020). Thus, none of the methods tested was able to predict species survival in competition free gaps well because species, which are not present in the locality because of competition, are excluded a priori from the actual species pool and thus never can become a part of the local species pool, although they might be able to survive the abiotic conditions but without competition (Kraft et al., 2015; Cadotte & Tucker, 2017).

In control plots with the intact vegetation, which reflect the effect of both the abiotic and biotic filter together (i.e., community filter), the prediction of species success in seed addition experiment was more successful. Here, BI and UNO were unequivocally better than other methods, and also reflected well the Control/Gap ratio, i.e., the effect of biotic filter. Unlike EIV, they do not have any a priori assumption about which gradient is the decisive one, and they compare the real community composition with a large amount of empirical data from the large phytosociological databases (Münzbergová & Herben, 2004). Contrary, EIV reflect relatively narrow amplitudes of individual species specific to the area where they were determined (Zobel et al., 1998; Ewald, 2002; Lewis et al., 2016). In the case of functional traits, although that they are not dependent on the species distribution in landscape, there is very questionable which trait in which case to choose, if chose only one trait or rather use the combination of more traits and which ones (Bruehlheide et al., 2018). Moreover, also the intraspecific variability in functional traits can play an important role (Albert, 2015; Lisner et al., 2021).

The effectiveness of the methods was also influenced by the life stage of species. None of the methods (with exception of SLA in the second spring in controls) was statistically significant predictor of

performance in the first two springs of seed addition experiment – this is probably because these were affected more by seed germination than by the survival. Some species, including non-residents, did not germinate the first year directly after sowing, but as late as the second spring and subsequently, these species died out. Consequently, the numbers of seedlings being still alive fluctuated erratically. The establishment of seedlings in the community (i.e., the seedling performance after the second spring) was well predicted by BI index and UNO. Nevertheless, BI and UNO were not so good predictors for species performance in transplant experiment (i.e., for the survival after establishment), and were surpassed by some functional traits, at least during some periods. The negative correlation of high with transplant performance showed that smaller species prospered better in gaps and clipped plots than taller plants. The height is very often associated with competitive ability for light (Pérez-Harguindeguy et al., 2016) and taller plants are supposed to be more able to withstand the competition (Grime, 2002). This ability provides very limited advantage in microhabitats with no or only belowground competition. On the other hand, due to various trade-offs (e.g., tall plants usually mature later, invest more in the stem than in the leaves), being potentially tall might hinder fast establishment. Height is also associated with abiotic characteristics such as for example soil depth or water availability (Harzé et al., 2016). Both these factors could be a little bit constrained in gaps which were lined in 20 cm depth by geotextile and generally gaps are more prone to dry out (Kotorová & Lepš, 1999; Vítová & Lepš, 2011). On the other hand, height was negatively correlated with BI (Fig. S2), thus, it is possible that it can also influence our results. The positive correlation of seed mass with transplant performance was quite surprising. It could be the case of syndrome traits, when tested trait is associated with other traits which were not tested (Körner, 2018) and thus it seems, that seed mass had the influence on the transplant survival but in fact, it could be the effect

of another trait. From traits we tested, seed mass correlated significantly only with lateral spread ( $r = -0.423$ ,  $N = 30$ ,  $p = 0.02$ , Fig. S2). Nevertheless, for example Herben et al. (2016) did not find any correlation between seed traits and traits associated with clonality. Thus, it is possible that there should be other traits which we not tested, and which are correlated with seed mass and cause its correlation with transplant performance in control plots.

While BI and UNO were very successful in predicting the seedling performance in Control/Gap ratio representing the effect of biotic filter, in the case of transplants, BI and UNO were best or second best for ratios that included the belowground competition (Control/Gap and Clipped/Gap) but below average for the one reflection only aboveground competition (Control/Clipped). This suggests that belowground competition was more important for our species community composition than aboveground competition.

### **Performance of Beals index (BI) in different localities and treatments**

The importance of the effect of biotic filter on the community composition was confirmed also in detailed analysis of species performance based on BI (which was chosen as a best predictor of real species performance), where seedling survival was correlated better in the case of control plots than in the case of gaps. Nevertheless, there were still large differences among localities. Ohrazení and Vrcov, localities with the highest biomass, exhibited higher difference between gaps and control than Zvíkov and Závraty, localities with lower biomass. It means that the effect of the biotic filter on seedling performance was the most important in wet (and thus more productive) localities, suggesting that the productivity can be considered an indicator of intensity of the biotic filter (Rajaniemi, 2003). Moreover, the effect of biotic filter increased in time in the case of seedlings. The biotic filter (mainly competition) is essential for species establishment in early phases of plant life (Švamberková & Lepš, 2020) and it is a principal factor influencing the meadow species community

composition (Boulangeat et al., 2012; Wisz et al., 2013; Bar-Massada, 2015).

The effect of biotic filter on transplants was smaller and the values of correlation coefficients for control plots did not differ very much or were even smaller than for gaps or clipped plots. There were also differences among localities, but the effect of biotic filter did not change in time. It suggests that the biotic filter is important especially for recruitment of small seedlings, but its importance decreases for more established individuals where the effect of the abiotic filter can increase. Higher sensitivity to competition of plants in early phases of seedling establishment than of mature and established plants was suggested also by other studies (Kotorová & Lepš, 1999; Bennett et al., 2016; Švamberková & Lepš, 2020). Nevertheless, from the results for transplant ratio, it was visible at least the trend that the most important from the biotic filter was the effect of full or/and belowground competition while the effect of aboveground competition was not so important.

## **Conclusion**

Methods of species pool determination based on species co-occurrence patterns (1c.) were good predictors of species performance in the intact vegetation (i.e., for species affected by the complete community filter) but not in competition free environment (i.e., for species affected only by the abiotic filter). In this case, some functional traits (2) were more successful as predictors. Methods based on co-occurrence patterns (1c.) were the most effective for prediction of seedling establishment, while seed germination itself and transplant survival was not predictable so easy. The biotic filter plays most important role in species community composition, especially on the establishment process of seedlings. For established plants, the effect of the abiotic filter increased on the expense of decreased and of the biotic filter.

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## Supplementary material

### Biomass samples

In June 2019, we cut the biomass from five 0.5 x 0.5 m plots in each locality. The biomass from each plot was dried at 80°C until its mass was considered to become constant. Then we weighed the dry biomass of each sample. Subsequently, we calculated the mean biomass weight from five samples for each locality.

Differences in biomass among the four localities were tested using one-way ANOVA with post-hoc comparisons using Tukey test from R-package “multcomp” (Hothorn et al. 2008) of function “glht”. Homogeneity of variances was tested using Bartlett test.

There were significant differences in biomass among different habitat types ( $F_{3,16} = 22.74$ ,  $p < 0.001$ ) and post-hoc tests have demonstrated significant difference between biomass in Ohrazení and Závraty ( $p < 0.001$ ), Ohrazení and Zvíkov ( $p = 0.003$ ), Závraty and Vrcov ( $p < 0.001$ ), Závraty and Zvíkov ( $p = 0.011$ ). The lower mean dry mass of biomass was recorded in Závraty (Table S1). The biomass in Vrcov did not differ from biomass neither in Ohrazení nor in Zvíkov ( $p = 0.14$ , resp.  $p = 0.214$ , Table S1).

Table S1: Mean dry weight of biomass from different localities and their standard deviation. Letters *a*, *b*, *c*, and *d* illustrate the differences in biomass among different localities in post-hoc test of one-way ANOVA.

Locality	Mean dry mass of biomass [g/0.5 m <sup>2</sup> ]	Standard deviation [g/0.5 m <sup>2</sup> ]
Ohrazení	113.33 <i>ab</i>	8.78
Vrcov	93.29 <i>abc</i>	17.18
Zvíkov	75.47 <i>c</i>	13.79
Závraty	43.97 <i>d</i>	5.07

Table S2: Mean annual temperature and total annual precipitation in studied localities from 2016 to 2019 (based on the measurements of the Czech Hydrometeorological Institute).

Locality	Year	Mean annual temperature [°C]	Total annual precipitation [mm]
Ohrazení	2016	8.3	600.9
	2017	8.4	660.1
	2018	9.1	578.2
	2019	9.4	657.5
Vrcov	2016	8.3	600.9
	2017	8.4	660.1
	2018	9.1	578.2
	2019	9.5	657.5
Zvíkov	2016	8.4	637.2
	2017	8.5	646.1
	2018	9.2	634.2
	2019	9.6	648.7
Závraty	2016	8.4	642.8
	2017	8.6	530.3
	2018	9.3	562.5
	2019	9.9	656.2

Table S3: Species used in seed/transplant addition experiment and their Ellenberg indicator values for light, moisture and nutrients, functional traits SLA, LDMC, Seed Mass, Canopy Height, and Lateral Spread (3 categories: 1 for < 0.01 m/year, 2 for 0.01 – 0.25 m/year, and 3 for > 0.25 m/year), and Beals index, unconstrained ordination (UNO), and residence of sown species (1 for resident species, 0 for non-resident species) specified for each study locality.

Species	Ellenberg Light	Ellenberg Moisture	Ellenberg Nutrients	SLA [mm <sup>2</sup> /mg]	LDMC [mg/g]	Seed Mass [mg]	Canopy Height [m]	Lateral Spread	Beals Ohrazeni	Beals Vrcov	Beals Zvikov	Beals Zavraty	UNO Ohrazeni	UNO Vrcov	UNO Zvikov	UNO Zavraty	Residence Ohrazeni	Residence Vrcov	Residence Zvikov	Residence Zavraty
<i>Anthoxanthum odoratum</i>	7	5	4	35.16	258.75	0.66	0.165	1	0.35	0.3	0.3	0.3	73.5	97.5	94.7	98.7	1	1	1	1
<i>Betonica officinalis</i>	6	4	4	22.3	215.7	1.52	0.375	1	0.08	0.0	0.0	0.0	21.1	23.7	21.9	9.40	1	1	1	0
<i>Bromus erectus</i>	8	3	4	16.47	339.13	4.67	0.5	1	0.04	0.0	0.0	0.0	0.61	5.76	8.19	4.54	0	0	0	0
<i>Campanula persicifolia</i>	5	4	5	29	174.43	0.01	0.4	1	0.03	0.0	0.0	0.0	0.00	0.14	4.80	0.82	0	0	0	0
<i>Clinopodium vulgare</i>	6	4	4	23.6	258.45	0.75	0.3	2	0.03	0.0	0.0	0.0	1.92	1.33	10.4	3.96	0	0	1	0
<i>Dianthus deltoides</i>	8	4	3	16.2	262.18	0.2	0.2	2	0.02	0.0	0.0	0.0	3.37	5.50	10.5	28.8	0	0	1	1
<i>Eleocharis palustris</i>	8	1	6	10.31	217.76	0.72	0.525	3	0.00	0.0	0.0	0.0	0.00	0.00	0.00	0.00	0	0	0	0
<i>Filipendula vulgaris</i>	7	4	3	17.43	295	1.16	0.325	1	0.05	0.0	0.0	0.0	4.64	8.07	8.08	3.30	0	0	0	0
<i>Galium boreale</i>	6	6	4	21.78	209.97	0.77	0.317	2	0.07	0.0	0.0	0.0	25.3	30.8	24.0	11.7	1	1	1	0
<i>Geum rivale</i>	6	8	5	22.3	237.83	1.17	0.3	2	0.04	0.0	0.0	0.0	1.87	2.58	0.42	0.00	0	0	0	0
<i>Geum urbanum</i>	5	5	7	37.96	302.42	0.67	0.3	1	0.04	0.0	0.0	0.0	0.02	0.00	0.27	0.00	0	0	0	0
<i>Hypericum hirsutum</i>	7	5	6	27.2	275.5	0.04	0.575	2	0.00	0.0	0.0	0.0	0.00	0.04	0.66	0.07	0	0	0	0
<i>Juncus inflexus</i>	7	8	5	3.65	322.41	0.19	0.45	1	0.01	0.0	0.0	0.0	0.00	0.00	1.03	0.00	0	0	0	0
<i>Linaria vulgaris</i>	8	4	5	20.03	189.25	0.11	0.417	3	0.01	0.0	0.0	0.0	0.14	0.89	2.71	3.23	0	0	0	0
<i>Lotus corniculatus</i>	7	4	4	23.67	186.186	1.22	0.429	1	0.19	0.2	0.2	0.2	12.5	43.7	56.1	78.1	0	1	1	1
<i>Lychnis flos-cuculi</i>	7	7	4	24.3	140.140	0.22	0.413	2	0.26	0.2	0.2	0.1	62.0	77.4	61.0	40.9	1	1	1	0
<i>Medicago falcata</i>	8	3	3	22.08	218.67	1.24	0.35	1	0.03	0.0	0.0	0.0	0.11	0.00	0.80	0.00	0	1	1	1
<i>Melilotus officinalis</i>	8	4	5	14.8	237.67	2.16	1.075	1	0.00	0.0	0.0	0.0	0.07	0.81	0.00	0.00	0	0	0	0



Species	Ellenberg Light	Ellenberg Moisture	Ellenberg Nutrients	SLA [mm <sup>2</sup> /mg]	LDMC [mg/g]	Seed Mass [mg]	Canopy Height [m]	Lateral Spread	Beals Ohrazeni	Beals Vrcov	Beals Zvikov	Beals Zavraty	UNO Ohrazeni	UNO Vrcov	UNO Zvikov	UNO Zavraty	Residence Ohrazeni	Residence Vrcov	Residence Zvikov	Residence Zavraty
<i>Nardus stricta</i>	8	6	2	10.	416	0.	0.1	1	0.14	0.1	0.0	0.0	47.7	25.1	23.6	23.0	1	0	1	0
<i>Origanum vulgare</i>	7	3	3	18.	233	0.	0.4	2	0.01	0.0	0.0	0.0	0.21	0.00	3.13	0.41	0	0	0	0
<i>Plantago lanceolata</i>	7	5	5	19.	166	1.	0.1	1	0.31	0.4	0.4	0.4	32.7	94.2	106.	131.	1	1	1	1
<i>Potentilla supina</i>	7	7	7	12.	317	0.	0.2	1	0.00	0.0	0.0	0.0	0.24	1.41	1.03	0.28	0	0	0	0
<i>Prunella vulgaris</i>	7	5	5	30.	162	0.	0.1	2	0.16	0.1	0.1	0.1	22.0	43.3	43.1	40.5	0	1	1	1
<i>Rumex acetosella</i>	8	3	2	25.	113	0.	0.1	3	0.04	0.0	0.0	0.0	2.09	1.28	4.32	29.8	0	0	0	1
<i>Salvia pratensis</i>	7	3	4	25.	171	1.	0.4	1	0.05	0.0	0.0	0.1	0.23	3.37	5.42	4.32	0	0	0	0
<i>Sonchisorba minor</i>	7	3	3	20.	311	2.	0.2	1	0.06	0.0	0.0	0.1	0.00	0.00	2.82	13.3	0	0	0	0
<i>Sonchisorba officinalis</i>	7	7	5	20.	199	1.	0.4	1	0.25	0.2	0.2	0.1	61.8	82.7	71.5	42.6	1	1	1	0
<i>Scabiosa ochroleuca</i>	8	3	3	11.	248	1.	0.2	1	0.03	0.0	0.0	0.0	0.26	0.00	0.07	2.06	0	0	0	0
<i>Thymus pulegioides</i>	7	4	3	25.	251	0.	0.1	2	0.08	0.1	0.1	0.1	6.08	8.10	19.2	62.3	0	0	0	0
<i>Trifolium montanum</i>	7	3	3	19.	260	0.	0.2	1	0.04	0.0	0.0	0.0	0.50	1.52	3.47	7.65	0	0	0	0

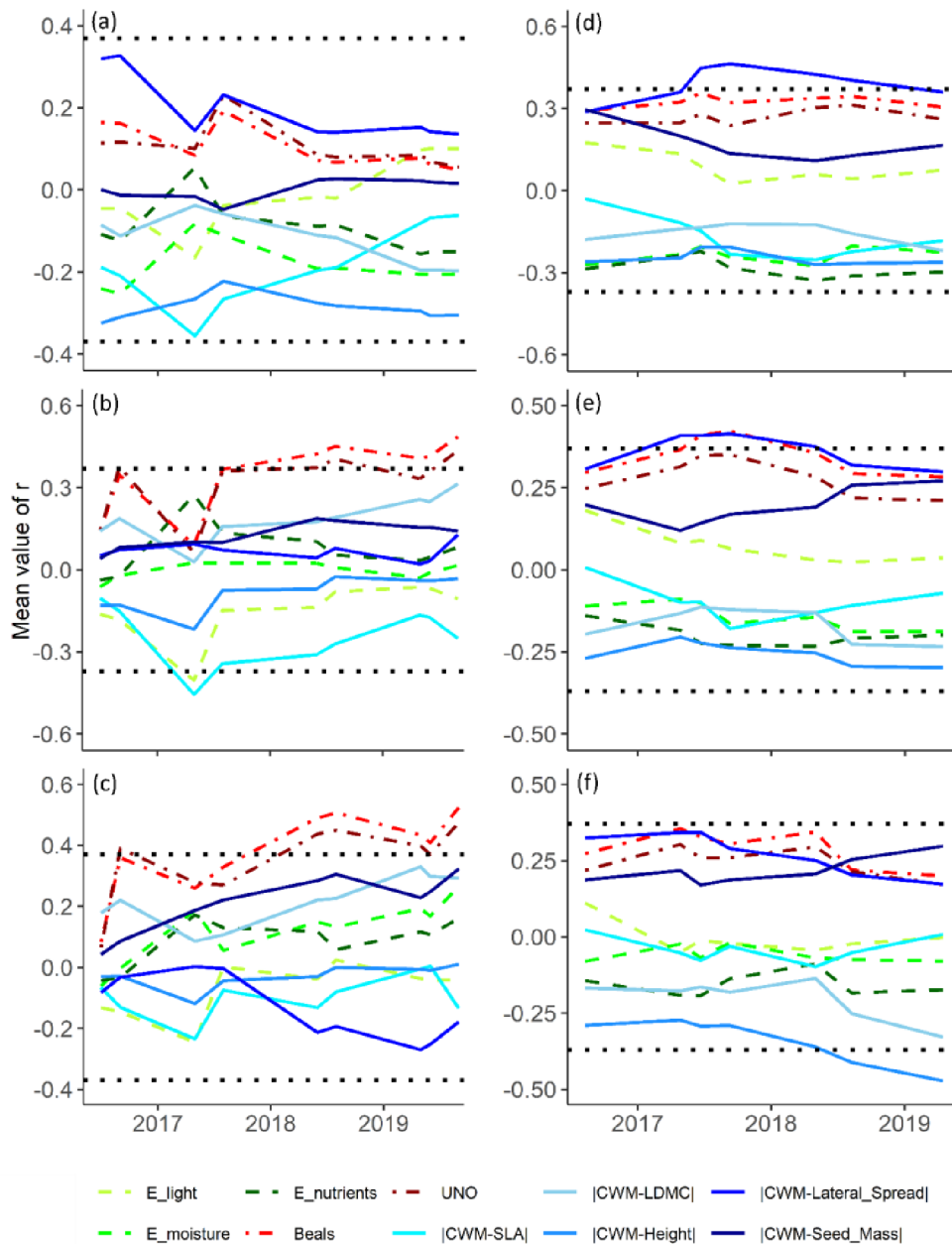


Fig. S1: The average values of Pearson's correlation coefficient  $r$  between real seedling /transplant survival from experimental approach and values of different methods of specie pool determination (Ellenberg indicator values for light, moisture, and nutrients; absolute value of difference of functional traits – SLA, LDMC, canopy height, lateral spread, and seed mass from the CWM; and Beals index and UNO based on species co-occurrence) in different measuring times of the experiment for different treatments: (a) Seedlings in gaps, b) Seedlings in control plots, c) Seedlings for the ratio of control plots and gaps, d) Transplants in gaps, e) Transplants in clipped plots, and e) Transplants in control plots). Horizontal black dotted lines represent the level of significance of correlation coefficients if  $N = 30$  (sometimes less in the case of ratio (c)) and  $p < 0.05$ .

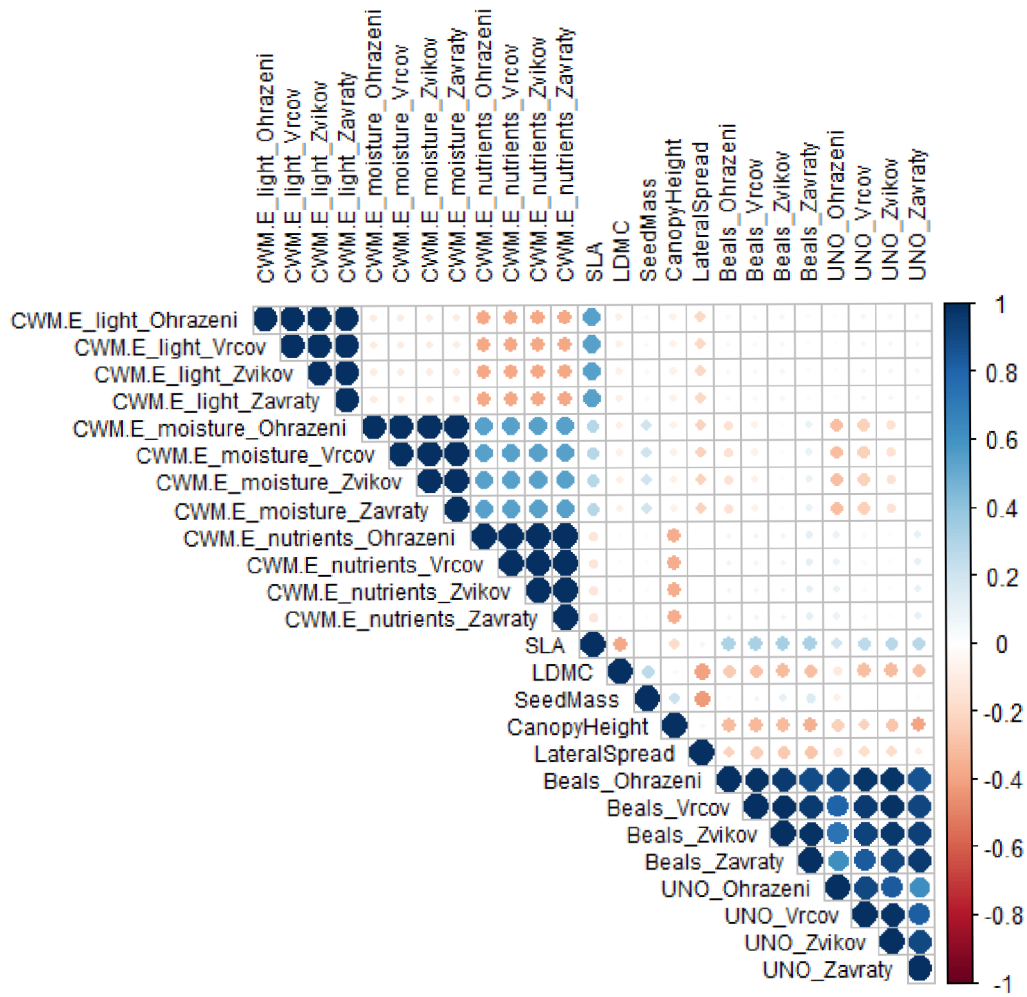


Fig. S2: Pearson's correlation matrix of different methods of specie pool determination.



## **Chapter 6**

### **General conclusions**



## **General conclusions**

The main aim of my thesis was to answer the questions related to assembly rules in plant communities – i.e., which factors can influence, and which species form a plant community assembly and what are the main drivers of plant species community composition. Problematic of assembly rules is relatively difficult and most of studies are based on null models, simulations, or other observational methods rather than on experimental approach (Lambers et al. 2012, Lessard et al. 2012, Cornell & Harrison 2014). In my thesis, I focused on an experimental approach only which can really clarify many ecological mechanisms contributing to answer many questions related to assembly rules concept. For example, without experiments, it is not possible to properly differentiate the importance of the abiotic and biotic effect on species community composition (Chapter 4 and 5) and we cannot distinguish the historical contingency of community assembly (Chapter 2). Also only with experimental approach, we can reliably determine habitat suitability of target species (Chapter 3 and 4). The importance of field experiments was highlighted already for example by Connell (1983) but because experimental approach is quite demanding and time consuming, data modelling and observations became faster and often easier way to get at least acceptable information. Nevertheless, the advantages of using permanent plots, especially with manipulation of the target community and with long-term perspective, are huge and enable to answer many ecological questions (de Bello et al. 2020).

In my research, I found out that both stochastic (Chapter 2) and deterministic (Chapter 3, 4 and 5) processes influence the species community composition. In Chapter 2, we found that founder effect of the initial species composition and the priority effects of the early arriving species are important determinants of plant species community composition. Species added to the community at the start of the succession significantly determined its course. The legacy of

species influenced by these effects, which are commonly considered as stochastic processes of historical contingency, was detectable also after 20 years from sowing these species. Nevertheless, these effects were quit species specific, and they were disappearing with time. For some species, the difference between the plots where they were and were not sown remained significant for the whole 20-year period (e.g., *Lathyrus pratensis*), other ones (e.g., *Trisetum flavescens*) colonised all the plots evenly. After 20 years there was only one species influencing the course of succession. Although that priority effects which are commonly suggested as stochastic processes (because they are very hard to determine and thus thought as random although that their patterns are not random) play very important role in the plant species community formation, the within community processes (i.e., abiotic and biotic filtering) seem to be still a little bit more important. Thus, in Chapter 3, 4 and 5, I have focused on the community filtering.

Although that the survival of species in a community is affected by both parts of the community filtering (i.e., abiotic and biotic filter), the biotic filter showed to be after all more important driver of species community composition than abiotic filter in our studied meadow localities (Chapter 3, 4 and 5). Many non-resident species, with very different habitat preferences than the target locality, were able to survive under specific abiotic conditions of the site but they cannot withstand the competitive pressure of surrounding vegetation (Chapter 3 and 4). Thus, these species were not limited by the abiotic filter but by biotic interactions, specifically by competition; we also found out that belowground competition can play even more important role than aboveground competition (Chapter 5). Nevertheless, these species are not commonly considered as a part of the species pool which is determined by different estimation methods such as Ellenberg indicator values, functional traits or methods based on co-occurrence patterns (e.g., Beals index, UNO) because these methods are not able to differ the effect of the abiotic and biotic filtering (Chapter 5). To



disentangle the importance of the abiotic and biotic filtering on the species community composition is very hard and without experimental approach even impossible (Chapter 4 and 5). Nevertheless, it is very important to precisely define species pool which is compared with realized vegetation composition and if we want to study the biotic interactions occurring in a community, it is necessary to use species pool determined independently of biotic interactions (i.e., species pool according to Butaye et al. 2001; Chapter 4).

However, because species pool assessment based on the experimental approach is quite difficult and time consuming and it is never possible to check up all potential species, we were searching for the most appropriate estimation methods of species pool assessment in Chapter 5. All tested methods had some limitation and no of them can predict the species performance perfectly. Nevertheless, the most suitable were methods based on co-occurrence patterns (i.e., Beals index and UNO) but only for the case of plots, which reflect the effect of both the abiotic and biotic filter together (i.e., community filter). It is because all used methods were related to realized niches of species and thus corresponded with actual species pool (Zobel 1997) including species passed through the complete community filter. Thus, although those methods based on co-occurrence patterns were quite good predictors of the real species community composition (Chapter 4 and 5), it is important to pay attention to use these methods as species pool determinants if we compare the effect of biotic and abiotic filters because these methods greatly underestimate the effect of competition and thus biotic filtering. What is more, the effectiveness of tested methods of species pool assessment was also influenced by the life stage of species. Methods based on co-occurrence patterns (i.e., Beals index and UNO) were the most effective for prediction of seedling establishment, while seed germination and transplant survival was not predictable so easy (Chapter 5).

Especially in Chapter 4 and 5, we compared the importance of regeneration and realized niches for community filtering. The biotic filter played really an important role especially on the establishment process of seedlings, for the recruitment of small seedlings, the competition of surrounding species was a radical limitation. In the case of survival of adult transplants, the importance of biotic filter for well-established individuals was decreasing and the effect of the abiotic filter sometimes started being increasing. Differences between regeneration and realized niches were also visible during studying the competitive exclusion of species from the community (Chapter 3 and 4). Well-established species were able to survive overgrowing by surrounding vegetation while for seedlings, the competitive pressure was limiting and caused their increasing mortality. Nevertheless, the competitive exclusion can be very slow process and it is very important to study really all life cycle of species because the conclusions can significantly differ among individual periods of species life. Suppression of seedling establishment turned out to be very important filtering mechanism in a community.

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## **Chapter 7**

### **Curriculum vitae**



## **Curriculum vitae**

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### **Publications not included in the thesis**

Szefer, P., Carmona, C. P., Chmel, K., Konečná, M., Libra, M., Molem, K., Novotný V., Segar S. T., **Švamberková E.**, Topliceanu S-T., & Lepš, J. (2017). Determinants of litter decomposition rates in a tropical forest: functional traits, phylogeny and ecological succession. *Oikos*, 126, 1101-1111. <https://doi.org/10.1111/oik.03670>

Godó, L., Béla, T., Valkó, O., Tóth, K., Kiss, R., Radócz, S., Kelemen, A., Torok, P., **Švamberková, E.**, & Deák, B. (2018). Ecosystem engineering by foxes is mediated by the landscape context - A case study from steppic burial mounds. *Ecology and Evolution*, 8, 7044-7054. <https://doi.org/10.1002/ece3.4224>

Kiss, R., Deák, B., Tóthmérész, B., Migléc, T., Tóth, K., Török, P., Lukács, K., Godó, L., Körmöczi, Z., Radócz, S., Kelemen, A., Sonkoly, J., Kirmer, A., Tischew, S., **Švamberková, E.**, & Valkó, O. (2020). Establishment gaps in species-poor grasslands: artificial biodiversity hotspots to support the colonization of target species. *Restoration Ecology*, 29, e13135. <https://doi.org/10.1111/rec.13135>

### **Citations of all my publications**

Total number of citations 47, h = 4 (Scopus 24 November 2021).



## **Conference presentations**

18 – 20 October 2013:

Česká společnost pro ekologii [Czech Society for Ecology], Brno, Czech Republic.

Poster: Role biotických vztahů pro složení rostlinného společenstva: Co je zásobník druhů společenstva? [The role of biotic interactions in plant community assembly: What is the community species pool?]

9 – 12 December 2014:

British Ecological Society and Société Française d'Écologie Annual meeting, Lille, France.

Poster: The role of biotic interactions in plant community assembly: What is the community species pool?

19 – 20 July 2015:

58th Annual Symposium of the International Association for Vegetation Science, Brno, Czech Republic.

Talk: Experimental assessment of the role of biotic interactions in delimitation of the community species pool.

23 – 25 October 2015:

Česká společnost pro ekologii [Czech Society for Ecology], České Budějovice, Czech Republic.

Poster: Experimentální vyhodnocení role biotických interakcí při stanovování zásobníku druhů společenstev. [Evaluation of the role of biotic interactions in determination of the community species pool using seed addition experiment.]

13 – 16 December 2015:

British Ecological Society Annual meeting, Edinburgh, Scotland.

Poster: Evaluation of the role of biotic interactions in determination of the community species pool using seed addition experiment.

5 – 7 May 2016:

29th Conference on Plant Population Biology 2016, Třeboň, Czech Republic.

Talk: Experimental assessment of the role of biotic interactions in delimitation of the community species pool.

11 – 15 December 2017:

British Ecological Society Annual meeting, Ghent, Belgium.

Poster: The legacy of sowing after 20 years long colonisation of ex-arable land.

3 – 5 May 2018:

31st Conference on Plant Population Biology, Innsbruck, Austria.

Poster: The legacy of sowing after 20 years long colonisation of ex-arable land.

14 – 18 July 2019

62nd Annual Symposium of the International Association for Vegetation Science, Bremen, Germany.

Talk: Experimental assessment of biotic interactions driving species co-occurrence in community composition.

### **Internship**

22 May – 5 August 2017:

University of Debrecen, Department of Terrestrial Ecology, Hungary, workgroup led by Orsolya Valkó, DSc.

### **Teaching**

Field course I. (introductory excursion for bachelor students, CZ, 2017 – 2018).

Ecology – Dendrochronology and Analysis of the Forest Structure (tutorials, CZ, 2017 – 2019).

Biological Laboratory Technique (tutorials, CZ, 2017 – 2021).

Basic Course of Botany, Phycology and Mycology (botanical tutorials, CZ, 2016 – 2021).

### **Thesis supervision**

Kraus, J. (2021). Klíčivost semen, metody stanovení a její ovlivnění faktory prostředí. [Seed germination, methods of determination and its influence by environmental factors. Bc. Thesis, in Czech] – 56 pp., Faculty of Science, University of South Bohemia, České Budějovice, Czech Republic.

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