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2019. Trait-based approach confirms the importance of propagule limitation and assembly rules in old-field restoration

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Trait-based approach confirms the importance of propagule limitation and assembly rules in old-field restoration

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Authors' Contributions

KT, MH conceived and designed the field experiment; KSZ, MH collected field data; ACS, MH collected trait data and computed statistical analyses based on the scripts and advices provided by ZBD; MH wrote the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

Abstract

Community assembly theory is suggested as a guiding principle for ecological restoration to help understand the mechanisms that structure biological communities and identify where restoration interventions are needed. We studied three hypotheses related to propagule limitation, stress-dominance and limiting similarity concepts in community assembly in a restoration field experiment with a trait-based null model approach. The experiment aimed to assist the recovery of sand grassland on former arable land in the Kiskunság, Pannonian biogeographic region, Europe. Treatments included initial seeding of five grassland species, carbon amendment, low intensity mowing and combinations in 1 m by 1 m plots in three old-fields from 2003 to 2008. The distribution of ten individual plant traits was compared to the null model and the effect of time and treatments were tested with linear mixed effect models. Initial seeding had the most visible impact on species and trait composition confirming propagule limitation in grassland recovery. Reducing nutrient availability through carbon amendment strengthened trait convergence for length of flowering as expected based on the stress-dominance hypothesis. Mowing changed trait divergence to convergence for plant height with a strengthening impact with time, supporting our hypothesis of increasing dominance of limiting similarity with time. Our results support the idea that community assembly is simultaneously influenced by propagule limitation and multiple trait-based processes that act through different traits. The limited impact of manipulating environmental filtering and limiting similarity compared to seeding, however, supports the view that only targeting the dispersal and environmental filters in parallel would improve restoration outcome.

Key-words: carbon amendment, grassland restoration, limiting similarity, mowing, plant traits, seeding

Implication for Practice

Seeding of a limited number and amount of well-selected species can strongly enhance grassland restoration on old-fields both in terms of species and trait composition.

The dominant process in early succession is environmental filtering, so early restorative interventions should focus on this filter to accelerate the establishment of target ecosystems.

Carbon amendment can strengthen environmental filtering and help the establishment of species with stress-adapted traits.

Mowing strengthens environmental filtering in early succession and mitigates competitive exclusion later in succession.

As community assembly is simultaneously influenced by propagule limitation, environmental filtering and limiting similarity in old-field restoration, targeting the dispersal, abiotic and biotic filters in parallel would improve restoration outcome.

Introduction

Community assembly theory is suggested as a guiding principle for ecological restoration to help understand the mechanisms that structure biological communities and identify where restoration interventions are needed (Hobbs & Norton 2004, Temperton et al. 2004, Funk et al. 2008, Hulvey & Aigner 2014, Laughlin 2014). At the same time restoration projects and experiments provide opportunities to test assembly related theories by examining community responses to direct manipulations (Young et al. 2001).

According to the integrated conceptual framework of community assembly, stochastic processes dominate at the start of succession (e.g. due to chance dispersal) and deterministic processes (environment filtering and limiting similarity) will be significant later (Chang & HilleRisLambers 2016, Li et al. 2016). Many researches have shown that habitat restoration is strongly limited by early dispersal, which results from the depletion of the soil seed bank and dispersal limitation of target species in fragmented landscapes (e.g. Bakker et al 1996, Kiehl et al. 2010, Török et al. 2018a). In general, the soil seed bank of degraded sites (e.g. old-fields) mainly consists of undesired species adapted to disturbance by forming a persistence seed bank (Thompson et al. 1997, Halassy 2001, Kiss et al. 2016, Török et al. 2018b). Whereas spatial dispersal is more promising in Central and Eastern Europe where remnants of the natural vegetation are still present in the landscape (Halassy 2001, Ruprecht 2006, Csecserits et al. 2011, Albert et al. 2014, Prach et al. 2016, Valkó et al. 2016). However, the cover of specialist species in some cases remains very low (Molnár & Botta-Dukát 1998) and alien species can dominate old-fields (Csecserits et al. 2011). In case of propagule limitation seed introductions are needed (Kiehl et al. 2010) that can result in multiple development of restoration trajectories both at the species and the trait level (Fukami et al. 2005).

Once propagule limitation is overcome, species are further filtered by assembly processes. Two contrasting assembly processes are accepted as basic mechanisms that drive community structuring: environmental filtering and limiting similarity that are generally referred to as assembly rules (Weiher & Keddy 1995). The two processes are not exclusive, but multiple trait-based assembly processes can operate simultaneously that may change in their strength and importance with spatial (Díaz et al. 1998; de Bello et al. 2013), temporal (Douma et al. 2012), productivity or stress gradients (Lhotsky et al. 2016b). For the latter, the stress-dominance hypothesis predicts that abiotic constraints play a major role in harsh environments resulting in lower functional (“alpha”) diversity of traits useful in the adaptation of species to the given stress compared to random (Weiher & Keddy 1995; Coyle et al. 2014; Lhotsky et al. 2016b). In the absence of extreme stress, competition between species will result in higher functional (“alfa”) diversity of traits related to resource acquisition – in other words limiting similarity – that enables the coexistence of species (MacArthur & Levins 1967; Weiher & Keddy 1995; Lhotsky et al. 2016b). This tendency may be expected mostly at the finest spatial scales where species compete for the same local resources (de Bello et al. 2013) and in more or less homogenous environment (Botta-Dukát & Czúcz 2016). Similarly, the environmental filter dominates in early successional stages (Chang & HilleRisLambers 2016) when there is a plant colonization window due to the insaturation of the assembly (Bartha et al. 2003). Later in the course of succession, as the population sizes increase and the vegetation cover closes, the competition between species intensifies leading to the divergence of traits (Chang & HilleRisLambers 2016). Disturbance events (e.g. drought, fire, mowing) can control species with high competitive ability and create new colonization windows (Bartha et al. 2003), therefore resulting in an increased niche overlap and a decreased trait divergence (Grime 2006; Mason et al. 2011; de Bello et al. 2013).

Despite the recent shift towards adoption of assembly theory in restoration theory, only a limited number of studies test the relevance of propagule limitation and assembly rules jointly in restoration field experiments with a trait based null model approach. Examples include mostly microcosm or mesocosm (Grman & Suding 2010; Cleland et al. 2013; Yannelli et al. 2017) and garden experiments (Plückers et al. 2013) or comparison of previously restored sites (Pywell et al. 2003; Öster et al. 2009; Helsen et al. 2012; Hoelzle et al. 2012; Grman et al. 2013; Zirbel et al. 2017), but the number of real time-series in the field is limited (Sandel et al. 2011; Young et al. 2016; Torrez et al. 2017). There is a need for more in situ research to adequately quantify the importance of propagule limitation, environmental filtering and limiting similarity on long-term assembly and outcomes in natural systems (Götzenberger et al. 2012).

In the present paper we study propagule limitation, environmental filtering and limiting similarity in a microscale restoration field experiment (2003-2008). Treatments include the introduction of a low-diversity seed mixture, carbon amendment to lower soil available nitrogen and thus increase environmental stress and mowing to decrease competition (see also Halassy et al. 2016). We analyze traits separately and use the null model approach to reveal assembly rules, where we interpret negative effect sizes (functional diversity lower than expected by the randomization) as indication of environmental filtering, while positive effect sizes (functional diversity higher than expected by the randomization) as indication of competitive exclusion. We hypothesize that old-field restoration is both determined by propagule limitation and assembly rules (environmental filtering and competitive exclusion). The latter are dominantly trait-driven processes with changes from stress limitation dominating on the short-term to limiting similarity dominating on the longer term in succession (Cramer et al. 2008, Chang & HilleRisLambers 2016). Based on this, we tested the following hypotheses: i. seeding of a limited number of target species accelerates secondary succession and results in a divergence of seeded vs. non-seeded vegetation (propagule limitation); ii. reducing nutrient availability via carbon amendment results in increasing stress and thus lower functional diversity of traits compared to non-amended plots (stress-dominance hypothesis); iii. mowing counteracts the impact of interspecific competition for light (limiting similarity hypothesis) and decreases functional diversity of traits compared to unmown plots.

Materials and methods

Study area and experimental design

The study was conducted in the Kiskun LTER Fülöpháza Site (N 46°890 E 19°440), Hungary, Europe. The target of restoration efforts was the drought limited sand grassland (Festucetum vaginatae community, Fekete et al. 1995) that belongs to Natura 2000 priority habitat 6260* Pannonic sand steppes. The mean annual precipitation is 550 mm with frequent occurrence of long and severe droughts (Kovács-Láng et al. 2008). The maximum living biomass is estimated 65-179 g/m^2 (Ónodi et al. 2017) and the target community type is at the low productivity end of the local environmental gradient (Lhotsky et al. 2016b), due to its location mainly on dune tops and the poor water retention capacity of calcareous coarse sandy soils. The present landscape is the result of strong human impact (mainly arable cultivation and forest plantation) of recent centuries (Biró et al. 2013). From the 1980s abandonment of arable land is also widespread, especially in low productivity areas, and this provides potential for the regeneration and restoration of grasslands (Csecserits et al. 2011).

Three abandoned arable fields were selected for the experiment with similar climate, soil characteristics and earlier management (Halassy et al. 2016). Although the time of abandonment was different for the three sites (2002, 1999 and 1987), this had negligible impact on our treatments. Three types of treatments were applied in a full factorial design: 1) Seeding of five target species in 2002 after ploughing: *Festuca vaginata* (1.55 g/m^2), *Stipa borysthenica* (1.05 g/m^2), *Koeleria glauca* (1.00 g/m^2), plus two forb species (*Dianthus serotinus* and *Euphorbia segueriana* 0.20 g/m^2 together*,* nomenclature follows Király 2009). The species chosen for seeding are characteristic species of the target community – *F. vaginata* and *S. borysthenica* being dominant grasses, *K. glauca* a sub-ordinate grass, *E.*

segueriana a frequent subordinate forb and *D. serotinus* a rare forb that can become dominant locally –, but no prior selection was made to represent characteristic traits of the target community. 2) Carbon amendment with a dosage of 45 g sucrose/m^2 based on earlier experimental results (Török et al. 2000) was applied every three weeks in the vegetation period from April till October (2003-2008). Carbon amendment lowered soil available nitrogen (Halassy et al. 2016) that supposedly increased abiotic stress. 3) Mowing with hay removal was applied once a year in September to control interspecific competition for light (2003-2008). Treatments were applied in 1 m^2 plots in full factorial design in eight replicates for each treatment type, their combinations and for no seeding, no carbon, no mowing control at each of the three old-fields. Vegetation development was assessed based on the visual estimation of vascular species cover twice per year (in late May and early September 2003-2008). Cover data were pooled based on the yearly maximum per species. The study area and experimental design are described in details in Halassy et al. (2016).

Data on functional traits

We selected vegetative whole-plant and leaf traits (sensu Cornelissen et al. 2003) and reproductive traits that were accessible and relevant for restoration aims (cf. Laughlin 2014): life form, plant height (minimum and maximum), leaf size, specific leaf area (SLA), leaf dry matter content (LDMC), flowering (onset and length), seed mass and seed bank type. A short description of functional traits used in the analysis is given in Table 1. Data was obtained from local or Central European databases (LEDA – Kleyer et al. 2008; HUSEED – Peti et al. 2017) and literature sources (Csontos 2001; Halassy 2004; Király 2009; Lhotsky et al. 2016a). Where multiple trait data were available, the order of preference was local, national, and then regional data. Three woody species were excluded from the trait analysis since only seedlings occurred in the experimental sites, while databases usually contain traits for adult trees and shrubs. We compared the traits between seeded and non-seeded species using Chi square test in R version 3.3.1. (R Development Core Team, 2016).

Data analyses

Two separate principal coordinates analysis (PCoA, also referred to as metric multidimensional scaling of a data matrix, Legendre & Legendre 1998) were performed using the Euclidean distance to analyze species and trait composition changes. Species not reaching a total cover of 0.5 % summing all treatments and years were excluded from the analyses, resulting in 88 of the total 102 species found. To generate trait composition, community weighted means (CWM) were calculated separately for each trait and plot. CWM was derived for each continuous trait as the average of trait values weighted by the proportional abundance of species with the respective trait value. In case of categorical traits, CWM was calculated for binary dummy variables resulting in the relative abundance of each category. Four binary dummy variables not occurring in at least 10% of all samples for all treatments and years considered were excluded. The resulting 19 CWMs were used similarly to species in the ordination. All plots for all treatments (8), replicates (8), sites (3) and years (6) were included in the PCoA resulting in 1152 samples. The centroids of the eight treatment types were calculated for each year to draw the trajectories depicting changes in species and trait composition in 2003-2008 along the 1st and 2nd axis in the ordination space.

We analyzed if the functional diversity of a given trait was different from random expectation for each trait separately. We used Rao's quadratic entropy as a measure of the functional diversity (Botta-Dukát 2005). The differences between species' trait values were calculated using Gower-distance (Legendre & Legendre, 1998). We applied between-plot randomization (sensu Botta-Dukát & Czúcz 2016) to create the null model, equivalent to randomly drawing species from the pool of observed species. The combination of Rao's Q statistic and between-plot randomization is suitable for detecting both trait convergence due to environmental filtering and trait divergence due to limiting similarity (Botta-Dukát & Czúcz 2016). Since distributions of test statistic in the random communities do not follow normal distribution, we used probit-transformed p-values as effect sizes (Botta-Dukát 2018). Higher functional diversity than expected by the null model (trait divergence) is interpreted as evidence of limiting similarity and lower functional diversity than expected by the null model (trait convergence) is interpreted as environmental filtering. Statistical analyses were performed in R version 3.3.1. (R Development Core Team, 2016), using ‘vegan’ (Oksanen et al. 2016) and ‘FD’ (Laliberté & Legendre 2010, Laliberté et al. 2010) add-on packages.

We used general linear mixed models to test the changes of effect sizes of each trait with time and due to restoration treatments. The models were run in SPSS 14.0 for Windows (Beaumont, 2012) and included seeding, mowing and carbon amendment treated as fixed factors each with two levels (0=no treatment, 1=treatment). Year was included as a repeated measure with six levels according to the duration of the experiment (2003–2008) and plots were used as subject variable nested within field. We selected the first order autoregressive structure with homogenous variances for covariance structure and treatment means were separated using Fisher’s protected least significant difference (Halassy et al. 2016).

Results

Changes in species and trait composition

Both species and trait composition were primarily determined by seeding according to the PCoA analyses. Plots receiving seeding (with or without additional treatments) were separated in the ordination space from those not receiving seeding based on species cover data from the second year of the experiment and continued to be different throughout the study (Fig. 1). Species composition changed primarily with time for seeded plots along the first axis, whereas non-seeded plots remained more or less unchanged. The changes were primarily due to the establishment and growth of the five seeded species that reached 60-100% cover (mainly *D. serotinus* up to 70% and grass species up to 20%) in seeded plots, and remained less than 20% in non-seeded plots by 2008 (Fig. S1).

When trait composition was considered, seeding resulted in a visibly different composition from the third year on compared to non-seeded plots (Fig. 2). The trait composition of seeded plots changed considerably with time, whereas that of non-seeded plots had a more or less circular trajectory. All seeded species were Hemicryptophyte with smaller leaf size, SLA, but higher LDMC values compared to non-seeded species, and they also tend to have shorter viability in the seed bank, although these differences were scarcely significant statistically (Table S1).

Changes of assembly rules with treatments

Seeding resulted in significantly different functional diversity compared to non-seeded plots for all traits (Table S2). Seeding increased functional diversity for five traits (Fig. S2a). SLA (year*seeding: df=458.737 F=19.403 $p<0.001$) and length of flowering (year*seeding: df=501.908 F=8.746 $p<0.001$) remained convergent despite of increased trait divergence due to seeding. LDMC (year*seeding: df=460.030 F=20.244 $p<0.001$) and seed bank (year*seeding: df=456.157 F=5.324 $p<0.001$) became divergent earlier compared to non-seeded plots. As for seed mass (year*seeding: df=419.419 F=14.155 $p<0.001$), spontaneous recovery resulted in random trait pattern, compared to trait divergence found as result of seeding (Fig. 3). In four cases seeding decreased functional diversity compared to non-seeded plots, changing random (start of flowering, year*seeding: df=396.796 F=3.637 p<0.01) or divergent (leaf size, year*seeding: df=500.927 F=12.857 p<0.001, minimum height, year*seeding: df=483.148 F=6.706 p<0.001 and maximum height, year*seeding: df=447.822 F=7.164 p<0.001) distribution to convergent with time (Fig. S2b).

Carbon amendment resulted in increased trait convergence only for length of flowering by 2007 (year*carbon: df=501.908 F=2.501 p<0.05, Table S2, Fig. 4). We also detected the opposite trend, an increased trait divergence due to carbon amendment for SLA (year*carbon: df=458.737 F=6.070 p<0.001), seed mass (carbon: df=259.635 F=8.106 p<0.01) and seed bank type (carbon: df=232.902 F=4.341 p<0.05) (Fig. S3).

Mowing decreased trait divergence for life form (mowing: df=223.341 F=9.079 p<0.01), minimum height (mowing*year: df=483.148 F=3.759 p<0.01) and leaf size (mowing*year: df=500.927 F=2.896 p<0.05) (Fig. S4a). As for maximum height (mowing*year: df=447.822 F=6.936 p<0.001) mowing changed the assembly rule from divergent to convergent from the third year on (Fig. 5). Mowing decreased trait convergence for SLA (mowing*year: df=458.737 F=3.511 p<0.01) in some years compared to unmown plots (Fig. S4b). The full result of all treatments and years are presented in Table S2 and Fig. S5.

Discussion

From the treatments applied, initial seeding of five grassland species had the most visible impact on both species and trait composition resulting in divergent successional trajectory compared to non-seeded plots, a sign for strong propagule limitation. Spontaneous succession is increasingly involved in grassland restoration and the topic is especially important in Central and Eastern Europe where large areas of marginal croplands are being abandoned

(Török et al. 2018b). Although spontaneous recovery was shown to be successful within a few decades in the region (Halassy 2001, Ruprecht 2006, Csecserits et al. 2011, Albert et al. 2014, Prach et al. 2016, Valkó et al. 2016), the quick start of restoration by sowing a number of selected target species can shorten this period (Kövendi-Jakó et al. 2019).

We did not find contrast in vegetation development at the different levels of organization (species and traits), as others (Fukami et al. 2005; Helsen et al. 2012) who reported the dominance of historical contingency at the species level and a clear deterministic model of assembly at the trait level. This can be partly due to the small scale of investigations (Li et al. 2016), and partly due to the fact that the strong environmental filtering of drought in the studied region resulted in a small potential species pool, but principally because introducing target species primarily determined trait composition. Initial seeding of five target species accelerated old-field succession and induced a successional trajectory different from spontaneous regeneration which remained in the state of high inter-annual variation of vegetation composition, a sign of still dominating stochastic immigration processes (Cramer et al. 2008, Chang & HilleRisLambers 2016, Li et al. 2016).

Our restoration target was a drought limited sand grassland (Fekete et al. 1995), which is at the lower extreme of the regional productivity gradient (Lhotsky et al. 2016b). As follows from the stress-dominance hypothesis (Weiher & Keddy 1995; Coyle et al. 2014; Lhotsky et al. 2016b), environmental filtering is expected to be the dominant assembly process in our experimental sites. The impact of environmental filtering proved to be stronger than limiting similarity for most of the traits in the first six years of the studied old-field succession. We found convergent trait patterns throughout our study for life form, SLA and length of flowering (generally perennial species with smaller SLA and shorter flowering period). Further convergence was found as a result of seeding for start of flowering, leaf size, minimum and maximum height (earlier flowering, smaller leafs and stature).

The only exception was seed mass, where random trait patterns changed to trait divergence with time and as a result of seeding. This was the only trait where we could confirm the shift in assembly rules with time (Cramer et al. 2008, Chang & HilleRisLambers 2016) within six years of vegetation development. Seed mass determines dispersal (space and time), colonization and establishment success (Westoby et al. 2002; Cornelissen et al. 2003, Díaz et al. 2016) and as such, can be highly variable within communities (Westoby et al. 2002). In stressed environments large seeds (such as the seeds of the seeded Stipa borysthenica) are advantageous because they confer greater seedling survival (Westoby et al. 2002), while smaller seeds (such as the seeds of the seeded *Festuca vaginata*) can support animal and wind dispersal or escape from stress (Lavorel & Garnier 2002).

We hypothesized that carbon amendment further increases environmental stress due to decreased nutrient availability that would lead to increased trait convergence. This hypothesis was supported for length of flowering, carbon amendment inducing shorter flowering. When considering traits separately, convergence due to stress is usually found in vegetative traits, e.g. tall plants with large, soft leaves are filtered out with low soil productivity (Grime 2006; Sandel et al. 2011; Lhotsky et al. 2016b; Zirbel et al. 2017). However, some regenerative traits are also known to respond to stress, e.g. large seeds (see above) or shorter flowering period helps to avoid drought (Wellstein et al. 2014), the latter also confirmed by our results. The lack of further convergence is probably due to the fact that nutrient shortage had a minor impact compared to the already stressed environment and resulting trait convergences.

We hypothesized that mowing would decrease trait divergence with a strengthening impact in time. We detected decreased trait divergence for life form, leaf size and maximum plant height (generally perennial, small leafed short plants) as a result of mowing, and in the latter case the impact strengthened with time. Maximum height is often related to competitive ability in capturing light (Douma et al. 2012), and therefore is expected to be the subject of niche partitioning (MacArthur & Levins 1967; Weiher & Keddy 1995; Lhotsky et al. 2016b). Mowing leads to trait convergence as it benefits short stature species, which are better able to either avoid or rapidly recover from destruction (Sandel et al. 2011).

Community assembly can provide a theoretical basis to understand the mechanisms that structure biological communities and help identify beneficial restoration interventions (Hobbs & Norton 2004; Temperton et al. 2004). Compared to species-based analysis, trait-based analysis is more likely to capture general assembly patterns, independent of site history or the taxonomic composition of the species pool, therefore confers greater predictability and more generalizable outcomes to other restoration sites (Weiher & Keddy 1995; Gross et al. 2009; Götzenberger et al. 2012). Unfortunately, local measurement of traits is very time consuming and maybe impossible during restoration interventions, therefore most restoration studies cannot take intraspecific trait variability into account, but accept 'a central assumption of plant comparative ecology', which implies that variation within species is smaller than the differences between species (Westoby et al. 2002). Our results based on trait data gathered from databases were strong enough to reveal environmental filtering and limiting similarity, and we argue that this approach can be transferred to other restoration cases to assess the importance of assembly processes.

Our results in old-field restoration support the idea that community assembly is simultaneously influenced by propagule limitation and multiple trait-based processes (environmental filtering and limiting similarity) acting through different traits (Spasojevic & Suding 2012; de Bello et al. 2013; Lhotsky et al. 2016b). From the treatments applied, early seeding of a limited number of target species had the most visible impact on species and trait composition that is in line with strong propagule limitation expected in old-field restoration (Bakker et al 1996, Török et al. 2018b). Our results support the view that seeding (or introduction of vegetative forms) are crucial to vegetation restoration (Kiehl et al. 2010, Merritt & Dixon 2011) as they speed up the recovery of degraded habitats (Kövendi-Jakó et al. 2019).

The manipulation of the environmental filter (both abiotic and biotic) is often of secondary importance in restoration compared to dispersal as in our case (Halassy et al. 2016). Reducing nutrient availability through carbon amendment strengthened trait convergence as expected based on the stress-dominance hypothesis (Weiher & Keddy 1995; Coyle et al. 2014; Lhotsky et al. 2016b) for one trait related to stress avoidance (length of flowering). Mowing was hypothesized to decrease trait divergence with a strengthening impact with time that was strongly supported for maximum plant height. Both methods are used in restoration to alter community composition and our results contribute to understand the basic mechanisms in the background. Their limited impact compared to seeding, however, supports the view that only targeting the dispersal and environmental filters in parallel would improve restoration outcome.

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Data accessibility

Data are available from ZENODO https://zenodo.org/record/21048 and https://zenodo.org/record/1284143

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Table 1. Short description of functional traits used in the analysis.

Functional trait	*Short description*	*Scale*	*Data completeness (%)*	*Min-max value*	*Type of trait*	*Data source*
life form	Raunkier's categories (Th, Th-TH, TH, TH-H, H, G)	nominal	100		vegetative whole trait	Király 2009
plant height	minimum individual height (m)	ratio	100	0.03-0.60	vegetative whole trait	Király 2009
	maximum individual height (m)	ratio	100	0.10-2.50	vegetative whole trait	Király 2009
leaf size	mean leaf area (mm^2)	ratio	95.9	3.90-31468	vegetative leaf trait	Kleyer et al. 2007, Lhotsky et al. 2016, own measurement
specific leaf area	mean SLA (mg/g)	ratio	95.9	5.03-41.83	vegetative leaf trait	Kleyer et al. 2007, Lhotsky et al. 2016, own measurement
leaf dry matter content	mean LDMC (mm^2/mg)	ratio	95.9	92.09-594.06	vegetative leaf trait	Kleyer et al. 2007, Lhotsky et al. 2016, own measurement
flowering	first month of flowering	ordinal	100	2-8	regenerative trait	Király 2009
	length (months)	ratio	100	1-7	regenerative trait	Király 2009
seed mass	mean seed weight (g/1000 seeds)	ratio	96.9	0.01-43.74	regenerative trait	Peti et al. 2017
seed bank	transient; short-term persistent; long-term persistent	nominal	80.6	1-3	regenerative trait	Kleyer et al. 2007, Csontos 2001., Halassy 2004

Figure 1. Temporal changes of species composition in 2003-2008 based on PCoA. Trajectories are based on the centroids of plots per treatment per year. CO- control, C – carbon amended, M – mown, MC – mown and carbon amended, S – seeded, SC – seeded and carbon amended, SM – seeded and mown, SMC – seeded, mown and carbon amended plots. Seeded plots are highlighted with solid lines and full symbols.

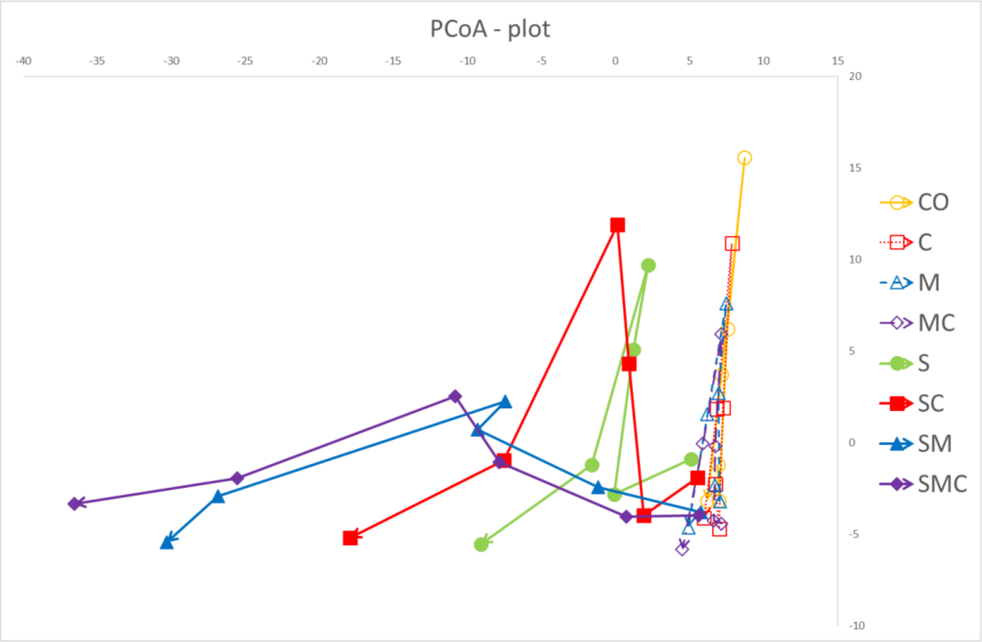
Figure 2. Temporal changes of trait composition (19 CWMs) in 2003-2008 based on PCoA. Trajectories are based on the centroids of plots per treatment per year. CO- control, C – carbon amended, M – mown, MC – mown and carbon amended, S – seeded, SC – seeded and carbon amended, SM – seeded and mown, SMC – seeded, mown and carbon amended plots. Seeded plots are highlighted with solid lines and full symbols.

Figure 3. Increased trait divergence with time for seed mass as a result of seeding. Positive values indicate that coexisting species are different in terms of a given trait (‘divergence’) compared to the null model, and negative values indicate similarity between coexisting species (‘convergence’). 0=all non-seeded plots (CO, C, M, MC), 1=all seeded plots (S, SM, SC, SMC). Within year significant differences ($p<0.05$) are marked by asterisk.

Figure 4. Increased trait convergence with time for length of flowering as a result of carbon amendment. Positive values indicate that coexisting species are different in terms of a given trait (‘divergence’) compared to the null model, and negative values indicate similarity between coexisting species (‘convergence’). 0=all non-amended plots (CO, M, S, SM), 1=all carbon amended plots (C, MC, SC, SMC). Within year significant differences ($p<0.05$) are marked by asterisk.

Figure 5. Decreased trait divergence for maximum height as a result of mowing. Positive values indicate that coexisting species are different in terms of a given trait (‘divergence’) compared to the null model, and negative values indicate similarity between coexisting species (‘convergence’). 0=all unmown plots (CO, S, C, SC), 1=all mown plots (M, SM, MC, SMC). Within year significant differences ($p<0.05$) are marked by asterisk.

Figure 1.


PCoA - plot
-40
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-30
-25
-20
-15
-10
-5
0
5
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15
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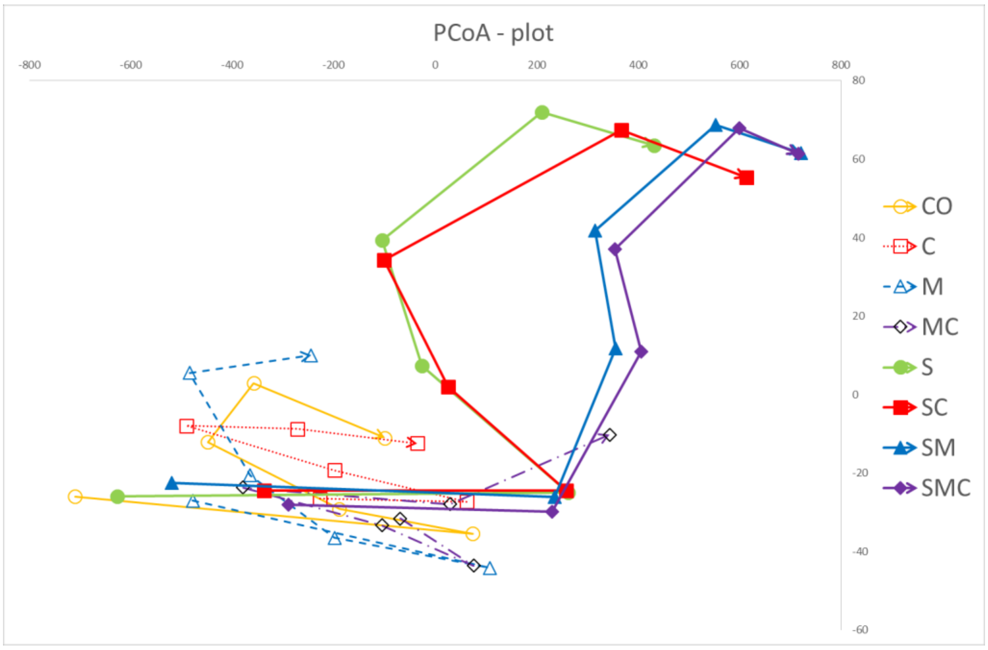
15
10
5
0
-5
-10
CO
C
M
MC
S
SC
SM
SMC

Figure 2.


PCoA - plot
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0
200
400
600
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CO
C
M
MC
S
SC
SM
SMC

Figure 3.

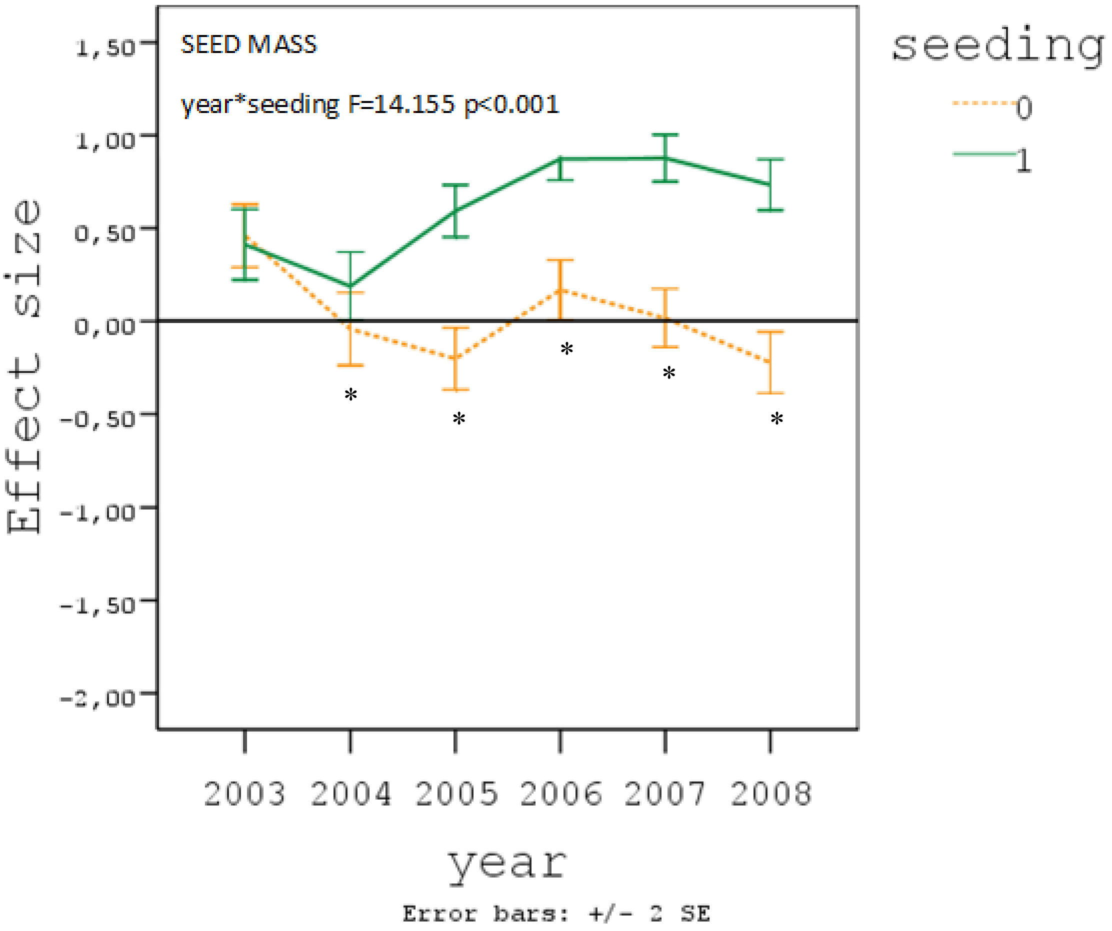

SEED MASS
year*seeding F=14.155 p<0.001
seeding
0
1
Effect size
1,50
1,00
0,50
0,00
-0,50
-1,00
-1,50
-2,00
*
*
*
*
*
2003
2004
2005
2006
2007
2008
year
Error bars: +/- 2 SE

Figure 4.

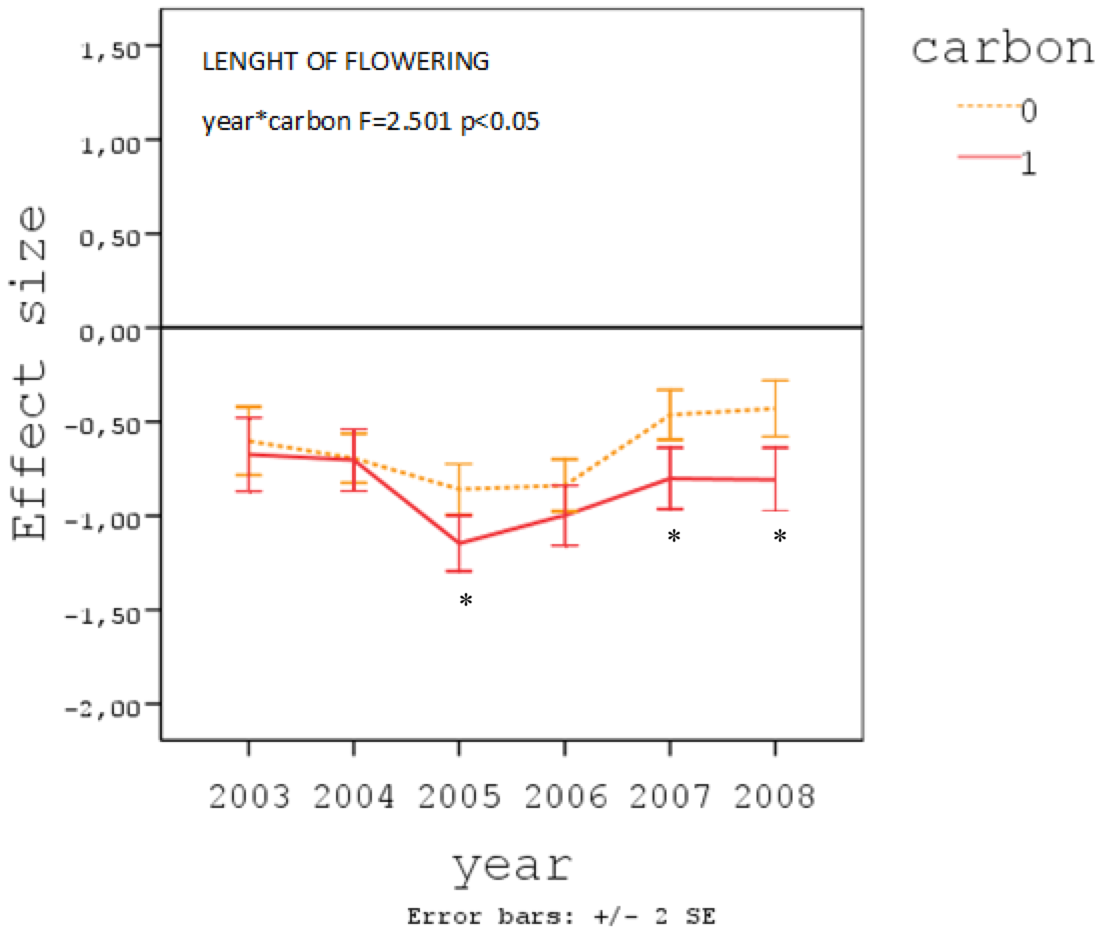
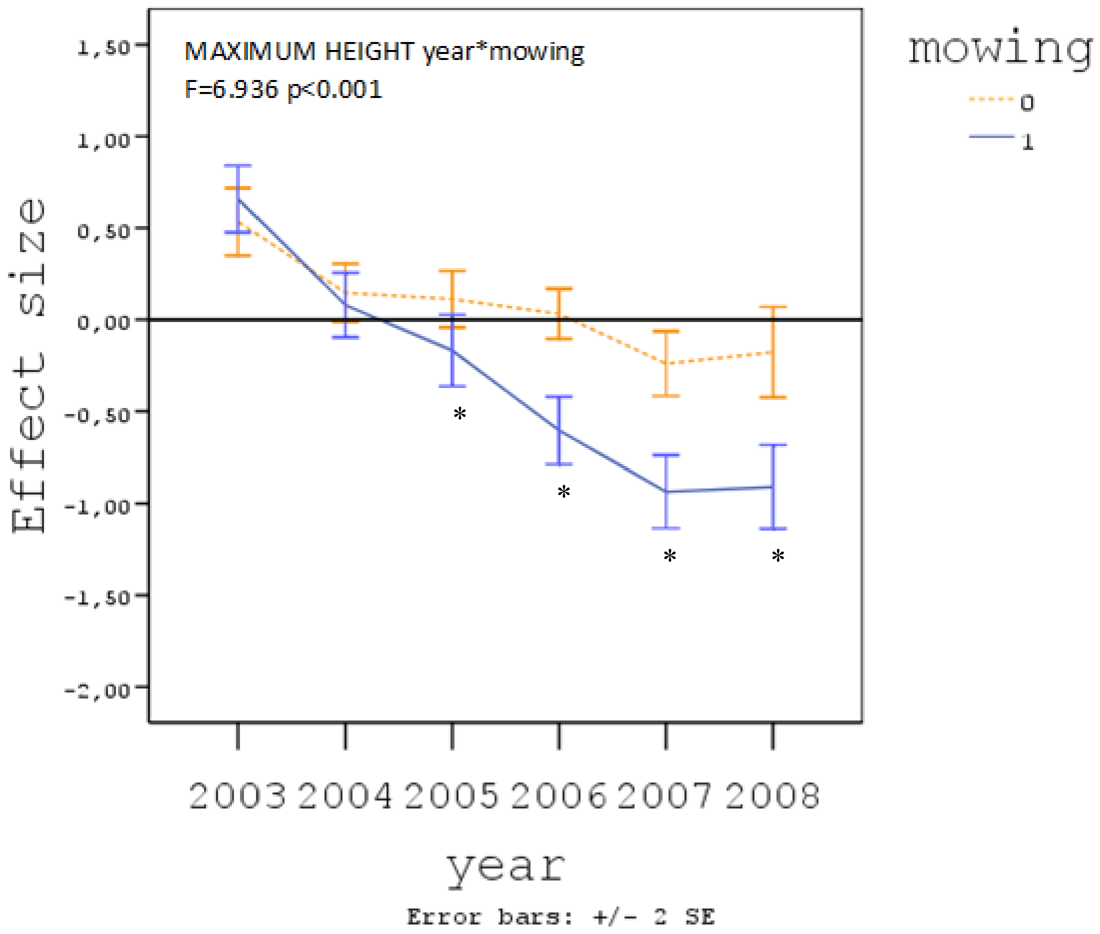

LENGHT OF FLOWERING
year*carbon F=2.501 p<0.05
carbon
0
1
Effect size
1,50
1,00
0,50
0,00
-0,50
-1,00
-1,50
-2,00
*
*
*
2003
2004
2005
2006
2007
2008
year
Error bars: +/- 2 SE

Figure 5.


MAXIMUM HEIGHT year*mowing
F=6.936 p<0.001
Effect size
1,50
1,00
0,50
0,00
-0,50
-1,00
-1,50
-2,00
*
*
*
*
2003
2004
2005
2006
2007
2008
year
Error bars: +/- 2 SE
mowing
0
1

Supporting information

Additional Supporting Information may be found in the online version of this article:

Table S1. Comparison of traits between seeded and non-seeded species.

Table S2. Summary of GLMM analyses.

Fig. S1. Changes in cover of seeded species.

Fig. S2. Changes of trait dispersion with seeding.

Fig. S3. Changes of trait dispersion with carbon amendment.

Fig. S4. Changes of trait dispersion with mowing.

Fig. S5. Changes of trait dispersion with time and all treatments.