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Biotic resistance to plant invasion in grassland: Does seed predation increase with resident plant diversity?

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Abstract

Seed predation impacts heavily on plant populations and community composition in grasslands. In particular, generalist seed predators may contribute to biotic resistance, i.e. the ability of resident species in a community to reduce the success of non-indigenous plant invaders. However, little is known of predators' preferences for seeds of indigenous or non-indigenous plant species or how seed predation varies across communities. We hypothesize that seed predation does not differ between indigenous and non-indigenous plant species and that seed predation is positively related to plant species diversity in the resident community. The seed removal of 36 indigenous and non-indigenous grassland species in seven extensively or intensively managed hay meadows across Switzerland covering a species-richness gradient of 18–50 plant species per unit area (c. $2\,m^2$) was studied. In mid-summer 2011, c. 24,000 seeds were exposed to predators in Petri dishes filled with sterilized soil, and the proportions of seeds removed were determined after three days' exposure. These proportions varied among species (9.2–62.5%) and hay meadows (17.8–48.6%). Seed removal was not related to seed size. Moreover, it did not differ between indigenous and non-indigenous species, suggesting that mainly generalist seed predators were active. However, seed predation was positively related to plant species richness across a gradient in the range of 18–38 species per unit area, representing common hay meadows in Switzerland. Our results suggest that generalist post-dispersal seed predation contributes to biotic resistance and may act as a filter to plant invasion by reducing the propagule pressure of non-local plant species.

Zusammenfassung

Samenprädatoren haben einen starken Einfluss auf Pflanzenpopulationen und die Artenzusammensetzung von Graslandökosystemen. Die Generalisten unter den Samenprädatoren können zur biotischen Resistenz beitragen, das heißt zur Fähigkeit der einheimischen Arten einer Gemeinschaft, den Invasionserfolg nicht-einheimischer Pflanzenarten zu vermindern. Es ist jedoch nicht bekannt, ob einheimische oder nicht-einheimische Pflanzenarten von Samenprädatoren bevorzugt werden, und wie stark die Samenprädation zwischen verschiedenen Pflanzengesellschaften variiert. Wir vermuten, dass Samen von einheimischen und nicht-einheimischen Pflanzenarten gleich häufig gefressen werden und dass die Samenprädation mit

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zunehmender Artenvielfalt einer Pflanzengesellschaft zunimmt. In sieben extensiv oder intensiv bewirtschafteten Heuwiesen der Schweiz untersuchten wir die Samenprädation von 36 einheimischen und nicht-einheimischen Graslandarten über einen Gradienten von 18–50 Pflanzenarten je Flächeneinheit (ca. 2 m^2). Im Hochsommer 2011 wurden ca. 24 000 Samen auf sterile Erde in Petrischalen in die verschiedenen Heuwiesen ausgebracht. Nach drei Tagen wurde der Anteil der fehlenden Samen bestimmt. Dieser Anteil variierte zwischen den einzelnen Arten (9.2–62.5%) und Heuwiesen (17.8–48.6%). Es konnte jedoch kein Zusammenhang zwischen Verlust und Größe der Samen festgestellt werden. Der Samenverlust unterschied sich nicht zwischen einheimischen und nicht-einheimischen Pflanzenarten, was darauf hinweist, dass hauptsächlich Generalisten unter den Samenprädatoren aktiv waren. Hingegen konnte über einen für Schweizer Heuwiesen repräsentativen Bereich von 18–38 Pflanzenarten je Flächeneinheit ein positiver Zusammenhang zwischen der Samenprädation und der Pflanzenartenvielfalt festgestellt werden. Unsere Ergebnisse lassen vermuten, dass Generalisten unter den Samenprädatoren zur biotischen Resistenz beitragen und als Filter gegen Pflanzeninvasion wirken, indem sie den Diasporendruck von nicht-einheimischen Pflanzenarten vermindern.

Keywords: Biotic filter; Community invasibility; Native non-native comparison; Propagule pressure; Seed availability; Seed provenance; Seed removal; Seed size; Semi-natural grassland; Species richness

Introduction

The number of non-indigenous plant species has increased in Europe (Hulme, Pysek, Nentwig, & Vilà 2009) and indeed around the world as a consequence of expanding transport and commerce. To be successful in a new habitat, non-indigenous species have to pass through various stages: being transported from the native range, colonizing the new habitat, surviving and reproducing. However, most non-indigenous species fail to establish or spread (Williamson & Fitter 1996). The failure of non-indigenous species in a new habitat may be explained by the biotic-resistance hypothesis, which states that resident species in a community reduce the success of exotic invasion (Levine, Adler, & Yelenik 2004). Biotic filters potentially impeding plant invasion include herbivores, pathogens and competition from indigenous species. While biotic resistance due to competition has often been studied, biotic resistance due to herbivores, including seed predators, has received less attention (Levine et al. 2004).

Propagule pressure, i.e. the number of propagules arriving at a site, has a well-documented influence on the success of species invasion (Lockwood, Cassey, & Blackburn 2005; Simberloff 2009). Therefore, the reduction of propagules of non-indigenous plant species by seed predators could be a potentially effective filter to invasion. In grasslands, and also in most other habitats, seed predators such as invertebrates and rodents have an important impact on the post-dispersal phase of seeds and thus affect plant population dynamics and community composition (Hulme 1993, 1996; Crawley 2000). Biotic resistance to plant invasion by means of seed predation would require that seed predators are generalists, i.e. they predate both indigenous and non-indigenous species. This requirement may not be fulfilled if non-indigenous species are released from seed predation as suggested by the enemy-release hypothesis, which states that invader species are successful in a new range either because they have lost some of the specialist enemies from their home range or suffer less from generalist enemies in the new range (Keane & Crawley 2002). A second prerequisite for biotic resistance via seed predation is that resident communities do not show predator satiation, i.e. the supply of seeds does not outweigh the predator's ability to consume them. Predator satiation is a common explanation for mast seeding of woody species in forests (Janzen 1971; Kelly 1994; Kelly & Sork 2002). Hay meadows, however, are often seed limited (Zeiter, Stampfli, & Newbery 2006; Stein, Auge, Fischer, Weisser, & Prati 2008) and therefore probably only rarely predator satiated. So far, very few studies, mainly performed with few plant species, have compared the effects of seed predation between indigenous and non-indigenous plant species and they have found mixed results (Blaney & Kotanen 2001; Shahid, Garneau, & McCay 2009; Pearson, Callaway, & Maron 2011).

Invasion resistance has long been assumed to increase with resident plant diversity (Elton 1958). More recent studies have supported this idea (Fargione & Tilman 2005; Scherber et al. 2010; Cardinale et al. 2012), and competition from resident plants has often served as an initial explanation for biotic resistance. Yet, herbivory, including seed predation, may act as a parallel process because the abundance and species richness of herbivores is often positively related with plant species richness (Knops et al. 1999; Haddad et al. 2009; Scherber et al. 2010), and this higher abundance and diversity of herbivores might result in more extensive damage. So far, the relationship between seed predation and plant species richness has only been examined by a single study (Pufal & Klein 2013), which found no relationship probably because the seeds of only three plant species were examined. Studies which have examined leaf damage found mixed results (Mulder, Koricheva, Huss-Danell, Högberg, & Joshi 1999; Scherber et al. 2006; Fischer, Weyand, Rudmann-Maurer, & Stöcklin 2012).

We performed a seed-removal study across a gradient of plant species richness in Swiss hay meadows with seeds of 36 indigenous and non-indigenous grassland species in order to explore the potential of seed predation as a filter to plant invasion according to the biotic resistance hypothesis

(Levine et al. 2004). Our research questions were: (1) Does seed removal differ between indigenous and non-indigenous plant species? (2) Does seed removal increase with plant species richness? If the seeds of non-indigenous species were removed, we would conclude that seed predation contributes to biotic resistance. However, if seeds of non-indigenous species were not removed, the alternative conclusion that seed predation does not contribute to biotic resistance would only hold if seeds of indigenous species still were removed, providing evidence for the existence and action of seed predators. Seed predation could be said to contribute to increased biotic resistance with increasing resident plant diversity if a positive relationship between seed removal and resident plant species richness is combined with the removal of non-indigenous seeds.

The effect of seed size on seed predation was also explored because seed size can play a role in the selection of seeds by seed predators (Reader 1993; Honek, Martinkova, Saska, & Pekar 2007) and because smaller seeds may better escape from predation than larger seeds as they are more easily incorporated into the soil (Thompson 1987; Van Tooren 1988; Chambers, MacMahon, & Haefner 1991).

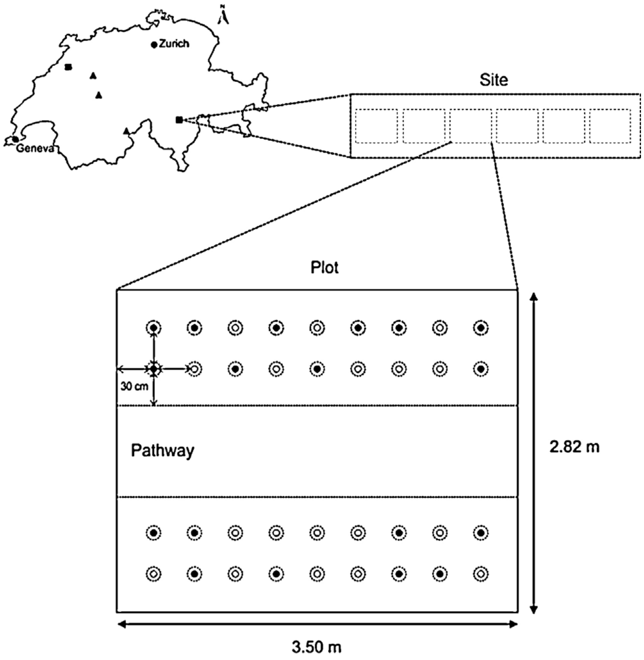

Fig. 1. Study design at seven sites in Switzerland, triangles representing one and rectangles two sites. Each site with six plots consisting of 36 Petri dishes, each Petri dish containing 16 seeds of one species. Petri dishes with 18 indigenous (open circles) and 18 non-indigenous (closed circles) species and plastic covers (crushed circles) in randomly assigned positions.

Materials and methods

Study sites

The field study was conducted in seven hay meadows in four regions across Switzerland: Jura, Plateau, Central Alps and Southern Alps (Fig. 1, Table 1). The sites represent intensively managed *Arrhenatherion*-type and extensively managed *Mesobromion*-type grassland (sensu Ellenberg 1996) with annual productivities in the range of 153–1107 gm^{-2} (Table 1). The intensively managed grasslands were fertilized with manure or slurry and mown up to three times yearly, often followed by autumn grazing, while the extensively managed grasslands were not fertilized and mown once or twice a year (Table 1). Whereas six sites span a common range of average plant species richness (24.8–32.5 per c. 2 m^2, Table 1) representative of the majority of hay meadows in Switzerland (Dietl & Lehmann 2006), average species richness is exceptionally high (46.7 per c. 2 m^2, Table 1) at the Negrentino site (Stampfli 1992, Zeiter & Stampfli 2012).

Investigated species

The seed removal of 36 temperate-grassland species, 18 of Swiss (CH) and 18 of North American (US) provenance (see Appendix A: Table 1), were recorded. The non-indigenous species used represent a random selection of the species which might arrive in a new habitat; none of the 18 species was resident at the study sites. Species which had not previously shown invasive behaviour in Europe were selected in order to reduce the risk of environmental hazards. Species names follow Lauber, Wagner, & Gygax (2012) for the Swiss species and USDA-NRCS (2013) for the North American species. From each provenance, nine species typically occurring in mesic nutrient-rich grassland and nine typically occurring in dry nutrient-poor grassland were selected to balance unknown ecological characteristics which may be linked with the habitat preference of these grassland species. The sets of species were taxonomically balanced between Swiss and North American species by selecting species from seven families including eight grasses, four legumes and 24 other forbs. The two North American species of the genus *Penstemon* were classified as *Plantaginaceae* according to Stevens (2001). The seeds were purchased in June 2010 from three companies (UFA Samen, Winterthur, Switzerland; Western Native Seed, Colorado, USA; Jellito, Schwarmstedt, Germany) and hand cleaned. A germinability test under controlled conditions in a growth chamber revealed no difference in seed viability between the seeds of indigenous and non-indigenous species (data not shown). The seeds were stored in cool and dry conditions until the start of the seed predation study in July 2011. Mean seed mass was determined by weighing eight sets of 100 seeds and these data showed a 52-fold range (0.13–6.80 mg; see Appendix A: Table 1). Seeds of indigenous and non-indigenous species did not differ in seed mass (t-test; $t_{34} = 0.36$; $p = 0.72$; log-transformed data).

Table 1. Geographic location, land-use characteristics and period of seed exposure in summer 2011 at study sites: regions (Reg), Jura (J), Plateau (P), Central Alps (A_C), Southern Alps (A_S); latitude and longitude (LL); elevation (E); exposure (Ex); inclination of slope (I); species richness (SR, mean ± SE, $n = 6$); land-use type (T), extensive (e), intensive (i); annual productivity measured in 2010 (AP); recent history of fertilization (1990–2009) (F), annual manure of goat (m_g), annual slurry (s), biennial slurry 1991–2000 (s_b), no fertilization (–); cutting frequency (C), additional autumn grazing (+).

Site	Geographic location					SR	Land use				Period of seed exposure
	Reg	LL, °N, °E	E m a.s.l.	Ex	I°		T	AP $gm^{-2}\,yr^{-1}$	F	C yr^{-1}	
Monthey	J	47°4′, 7°3′	650	SE	10	32.5 ± 1.5	e	512	–	1+	18–21 July
Combazin	J	47°3′, 7°3′	670	SSE	<5	28.7 ± 1.5	i	535	s_b	1+	18–21 July
Zollikofen	P	46°59′, 7°27′	555	ESE	5	24.8 ± 0.7	i	754	–	3	5–8 July
Thun	P	46°44′, 7°35′	570	–	–	25.5 ± 1.5	e	153	–	1	30 July–2 August
Bister-Breite	A_C	46°21′, 8°4′	1000	NW	2	28.7 ± 1.1	i	550	m_g	2+	13–16 September
Negrentino	A_S	46°27′, 8°55′	820	S	8	46.7 ± 1.1	e	414	–	2	28 June–1 July
Casserio	A_S	46°26′, 8°56′	770	NE	5	26.3 ± 0.8	i	1107	s	3+	28 June–1 July

Seed exposure

After haymaking, six plots of 2.82 × 3.50 m were established at each site with a minimum distance of at least 1 m between the plots. Within each plot, the 36 species were randomly allocated to 36 positions at regular distances of 35.5 cm along rows separated by a central pathway of 80 cm (Fig. 1). Seeds were exposed to predators in groups of 16 seeds per species and plot between late June and mid-September 2011 (Table 1). To facilitate seed retrieval after exposure, seeds were placed on sterilized soil (Spiel- und Rasensand, Ricoter, Aarberg, Switzerland) in Petri dishes of 5.5 cm in diameter and 1.2 cm in height. A soil core of the same dimensions was removed with a sharpened tube at each position, the Petri dish was placed level with the soil surface, the seeds were dropped from a bag containing 16 seeds through a funnel, and the Petri dish was covered with a white plastic plate of 12 cm in diameter fixed above ground with two nails of 7 cm in length to prevent seed loss by raindrops. The Petri dish covers did not prevent small rodents or insects from accessing the seeds but probably restricted access by birds, which are mainly generalist seed predators (Crawley 2000). The product of seven sites, six plots, two provenances, 18 species and 16 seeds resulted in a total of 24,192 exposed seeds. After three days' exposure, the Petri dishes were collected, placed in zip-lock bags and stored in cool and dry conditions. During exposure, a total of nine Petri dishes were lost across all sites and species due to animal activity. After separating the seeds from the soil by using a sieve with a mesh size of 1 mm, the remaining seeds were counted with the naked eye. Seeds of small-seeded species (*Erigeron speciosus*, US; *Agastache foeniculum*, US; *A. urticifolia*, US; *Holcus lanatus*, CH) were counted using a binocular microscope with 16-fold magnification (Wild Heerbrugg AG, Switzerland). Two other species, *Drymocallis fissa* (US) and *Penstemon digitalis* (US), turned out not to be reliably distinguishable from soil particles. The analysis was therefore restricted to 34 species, i.e. we compared 18 indigenous and 16 non-indigenous species. The number of seeds removed was calculated as the difference between 16 (the number of exposed seeds) and the number of seeds retrieved after three days' exposure.

We acknowledge that our approach to measuring seed predation may not have correctly estimated absolute seed predation. Firstly, the temporal activity of predators and their foraging decisions may change over time (Hulme 1994). Secondly, placing the Petri dishes caused small-scale disturbance and this may distract seed predators (Mittelbach & Gross 1984). And thirdly, the artificial conditions in the Petri dishes did not exactly match the soil conditions in the field, which may affect the incorporation of seeds in the soil, and thus their potential escape from predators. However, as our approach was identical at all sites and for both provenances, we assume that this affected neither the indigenous versus non-indigenous comparison nor the relationship between seed predation and plant species diversity.

Vegetation records

The frequency of vascular plant species rooting in the subplot was recorded in twelve subplots (18 × 36 cm^2) nested within unit areas of 1.36 × 1.48 m^2 (see Zeiter, Preukschas, & Stampfli 2013) located at a distance of 1–2 m, or 4 m (Negrentino), from the seed-exposure plots. Plant species were sampled outside the plots used for seed exposure to avoid further disturbance by trampling. Sampling areas and seed-exposure plots were still close to each other, and they did not noticeably differ in community composition, small-scale disturbance or management. Species richness, calculated as the cumulative number of species in twelve subplots, and the Shannon index [$H' = -\Sigma\,(p_i \ln p_i)$], combining information about species richness and frequency, were used as diversity measures at the plot scale. Within the unit areas used for species frequency recording, the abundance of seeds in the topsoil after the first harvest was also measured at all sites (see Zeiter et al. 2013). Seed density of the resident species in the topsoil was not related to plant species richness ($r^2 = 0.02$, $p = 0.34$, $n = 7$).

Statistical analysis

All statistical analyses were performed with the R statistical package (version 2.15.2; R Development Core Team 2012). We fitted and evaluated generalized linear mixed models (GLMMs) with binomial error distributions, using a logit-link function, and with Laplace approximation (lme4 package; Bates, Maechler, & Bolker 2010) for maximum likelihood estimation of the parameters (Bolker et al. 2009). The response variable was the proportion of seeds removed from the 16 seeds exposed. The models included provenance (indigenous versus non-indigenous) as a categorical fixed factor, diversity as a continuous fixed factor, and the interaction between diversity and provenance. Polynomial models were tested using linear and quadratic terms for the fixed factor diversity. Seed mass was included as a covariate. Random effects were fitted for species, site and plot nested within site. For each species, random linear and quadratic terms were also fitted for diversity.

Full models were simplified by removing non-significant terms to obtain minimum adequate models. The significance of fixed factors was first assessed by removing these factors from the model and comparing models with likelihood ratio tests; the significance was then confirmed using Wald *z* statistics. Separate models were fitted for species richness and Shannon index as continuous fixed factors because the two diversity measures were positively correlated with each other ($r = 0.983$, $n = 7$ sites). Akaike's information criterion (AIC) was used to compare the goodness of fit of models with species richness and Shannon index.

Results

30.5% of all seeds (i.e., 6917 out of 22,704 seeds) were removed from the Petri dishes during a three-day period. Across all sites, the proportion of removed seeds varied between species (see Appendix: Table 1) from (mean ± SE, $n = 7$) 9.2 ± 5.2% (*Astragalus canadense*, US) to 62.4 ± 10.2% (*Centaurea scabiosa*, CH). Across all species, the proportion of removed seeds varied between sites, from (mean ± SE, $n = 34$) 17.8 ± 3.6% (Casserio, Southern Alps) to 48.6 ± 6.0% (Combazin, Jura).

Across all sites, we found a hump-shaped relationship with a significant linear and quadratic effect of resident species richness on the proportion of removed seeds (Table 2, Fig. 2). This pattern was strongly influenced by seed removal at Negrentino, the site with extraordinarily high plant species richness compared to the other six sites. When we excluded Negrentino from the data set and re-ran the analysis (Table 3), we found a significantly positive relationship between resident species richness and the proportion of removed seeds across the six sites of lower species richness ($\chi^2 = 4.769$, df = 1, $p = 0.029$). Analogous analyses with Shannon diversity (H') as explanatory variable yielded very similar results to those with species richness (see Appendix A: Tables 2 and 3). Also the model fits for H' and species diversity were very similar both in the analysis across all sites (species richness AIC 9242 vs. H' AIC 9249) and in the analysis across six sites (species richness AIC 8350 vs. H' AIC 8353).

Table 2. Minimum generalized linear mixed model (GLMM) statistical table of seed predation of 34 species during three days of exposure in six plots at each of seven sites in hay meadows (minimum model not retaining seed provenance and seed mass; significant effects in bold type).

Fixed factors	Estimates ± SE	*z*	*p*
Intercept	−6.527 ± 1.860	−3.509	**0.000**
Resident species richness (linear term)	0.335 ± 0.115	2.924	**0.003**
Resident species richness (quadratic term)	−0.005 ± 0.002	−2.789	**0.005**

Random factors	Std. deviation
Site	0.441
Plot	0.476
Species, intercept	4.547
Species, resident richness linear term	0.275
Species, resident richness quadratic term	0.004

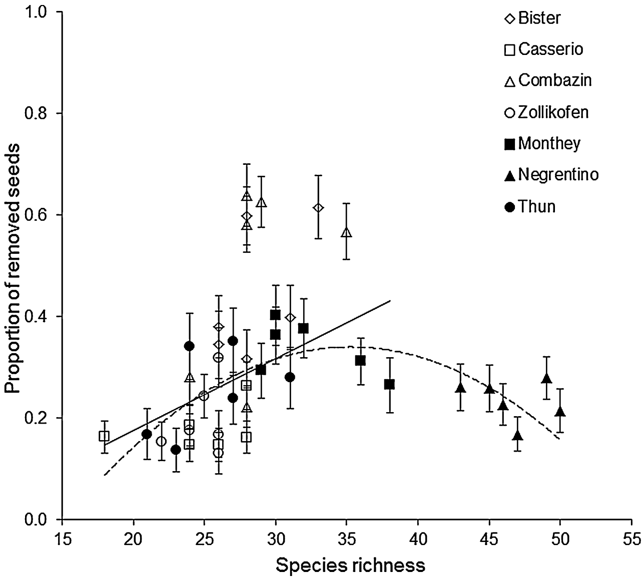

Fig. 2. Relationship between local plant species richness and proportion of seeds removed during three days of exposure, across all species (means ± SE, $n = 34$), across seven sites (dashed curve) and after exclusion of the most species-rich site (solid line). Each symbol represents one plot.

Table 3. Minimum generalized linear mixed model (GLMM) statistical table of seed predation of 34 species during three days of exposure in six plots at each of six sites in hay meadows (most species-rich site excluded; minimum model not retaining seed provenance and seed mass; significant effects in bold type).

Fixed factors	Estimates ± SE	z	p
Intercept	−2.861 ± 0.870	−3.290	**0.001**
Resident species richness	0.069 ± 0.031	2.276	**0.023**
Random factors		**Std. deviation**	
Site		0.485	
Plot		0.519	
Species, intercept		2.037	
Species, resident richness linear term		0.072	

Seed provenance and seed mass were not retained as significant effects in minimum adequate models (Tables 2 and 3, see Appendix A: Tables 2 and 3) of seed predation. Thus, the proportion of removed seeds did not differ between non-indigenous and indigenous species, and it was also independent of seed mass (Fig. 3).

Discussion

Seed-provenance effect on post-dispersal seed predation

Among seeds of the 34 indigenous and non-indigenous grassland species analysed we found no evidence that non-indigenous seeds benefitted from reduced seed predation in hay meadows. This is in contrast to the assumption of the enemy release hypothesis that generalist enemies should have a greater impact on indigenous than on non-indigenous species (Keane & Crawley 2002). Similar seed removal of indigenous and non-indigenous species was also found in an experiment performed with 43 old-field plant species in North America (Blaney & Kotanen 2001). These authors showed that both vertebrates and insects contributed to the pattern and vertebrates were more important than insects as post-dispersal seed predators. At our grassland sites, various seed-predator groups were present, including mice, voles, birds, ants, slugs and snails (J. Preukschas, personal observation) but we have no information on their relative contribution to seed predation. The presence of various seed predators, potentially contributing to a more equal removal of seeds of different size, might also explain why we found no relationship between seed removal and seed mass. Previous studies found that seed size can affect the preferences of seed predators (Reader 1993). Vertebrate predators such as rodents prefer large-seeded species (Blaney & Kotanen 2001; Pearson et al. 2011), and the relationship between seed predation and seed size depends on body size in invertebrates (e.g. *Carabids*, Honek et al. 2007). Thus, the lack of a relationship between seed size and predation may reflect the activity of different predator groups with different seed-size preferences in our grasslands. Alternatively, the absence of a relationship between seed size and predation may also result from a stronger impact on seed predation of other seed attributes such as content of nutrients or chemical defence compounds. Shahid et al. (2009) found that seeds of three non-native shrub species were less predated by rodents than seeds of two native species, likely because two of the three non-native species were toxic plants. In our study, however, chemical defence compounds likely played a minor role as all species except for *Astragalus canadensis* (US) and *Stipa viridula* (US) were non-toxic based on information from the species' home ranges (Roth, Daunderer, & Kormann 2008; Burrows & Tyrl 2013).

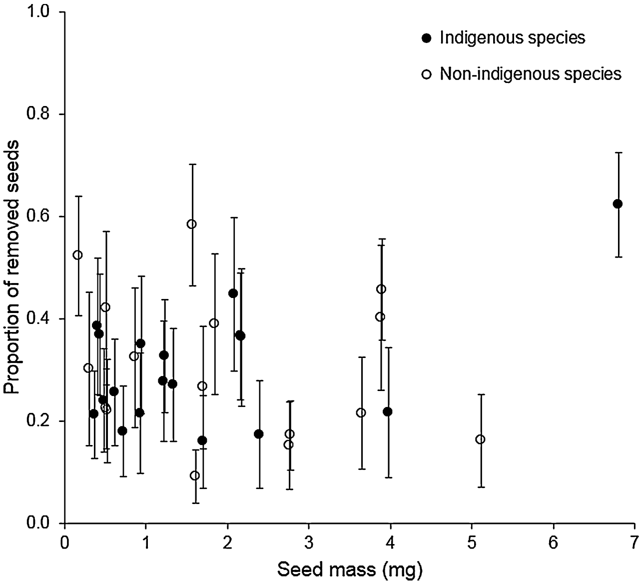

Fig. 3. Proportion of seeds removed during three days of exposure, of species across seven sites in relation to seed mass. Means (±SE) of indigenous ($n = 18$) and non-indigenous ($n = 16$) species.

Similar seed-predation of indigenous and non-indigenous species by generalist post-dispersal seed predators was also recently shown for seed predators in grassland of western Montana by Pearson et al. (2011), who also concluded that generalist seed predators may act as a filter to plant invasion by reducing propagule pressure of non-indigenous species. By corroborating the generalist behaviour of post-dispersal seed predators, our study extends the validity of the conclusion of Pearson et al. (2011) to temperate European grassland.

We cannot fully exclude that a certain enemy-release effect on pre-dispersal seed predation could favour the non-indigenous species of our study in a later phase of establishment in Swiss hay meadows because quite different predator groups are involved in pre- and post-dispersal seed predation. While post-dispersal seed predators tend to be relatively large, mobile, generalist herbivores, such as rodents and granivorous birds, pre-dispersal seed predators tend to be small, sedentary, specialist feeders belonging to the insect orders of Diptera, Lepidoptera, Coleoptera, and Hymenoptera (Crawley 2000). Thus, despite undergoing similar post-dispersal predation as indigenous species, the spread of non-indigenous species which might colonize grasslands may still be enhanced by the release from pre-dispersal seed predators (e.g. see Castells et al. 2013).

Relationship between seed predation and plant species diversity

We found a positive relationship between plant species richness and seed predation across the six sites representing common Swiss hay meadows in terms of species richness. Similar relationships between plant species richness and plant damage were found due to leaf herbivory (Mulder et al. 1999; Scherber et al. 2006), probably because herbivores increase in abundance and species richness with increasing plant species richness (Scherber et al. 2010).

A positive diversity–predation relationship, combined with the removal of seeds of non-indigenous species, implies that plant invasions may decrease as the species diversity of resident plants increases because seed predators reduce propagules of invaders. This is consistent with the biotic-resistance hypothesis (Levine et al. 2004) and implies that plant invasions may not only decrease with increasing species diversity of resident plants because of competition (Elton 1958; Fargione & Tilman 2005) but also because of seed predation. However, in contrast to experimental studies performed in artificially assembled communities, our evidence of biotic resistance in semi-natural hay meadows is correlative, which means that diversity effects cannot be separated from the effects of environmental factors.

Across the full diversity gradient in our study, the relationship between species richness and seed predation was not linear but hump-shaped due to relatively low seed predation at one site (Negrentino) with extraordinarily high species richness. Evidence from artificially assembled communities would rather support the idea that the effect of biodiversity on any single process is nonlinear and saturating (Cardinale et al. 2012). The hump-shaped curve found in this study suggests that seed predation might be reduced when plant species richness is exceptionally high but this evidence is only based on a single site. More sites should be investigated to clarify the relationship between seed predation and plant species richness and the potential mechanism which might reduce seed predation in highly diverse plant communities.

Limitations of the seed-exposure approach

Our suggestion that seed predation can contribute to biotic resistance to plant invasion by reducing the propagule pressure of non-indigenous plants requires that the seeds removed are killed by predators. However, exposing seeds and measuring seeds removed may overestimate seed predation because seed predation cannot be separated from seed dispersal by animals and from seed dislocation by abiotic factors such as wind and rain (Vander Wall, Kuhn & Beck 2005). In a study performed in temperate grassland, Pufal and Klein (2013) found low rates of removed seeds which were not predated. The majority of seed dislocation was due to rain splash. We can exclude rain splash as a source of seed loss in our study. Seeds removed by ants tend to be consumed unless they are equipped with elaiosomes which promote dispersal. According to a recent global survey on seed dispersal by ants (Lengyel, Gove, Latimer, Majer, & Dunn 2010), only two (*Centaurea nigrescens*, *Centaurea scabiosa*) of the 34 species analysed belong to genera with seeds known to have elaiosomes. We therefore assume that seeds removed by ants were mainly predated. As animals dispersing seeds also predate seeds (Chambers & MacMahon 1994; Hulme 2002), we assume that the indigenous versus non-indigenous comparison between seeds removed, and the relationship between plant species diversity and seeds removed correctly reflect attractiveness to seed predators.

Conclusion

In our study, seed removal did not differ between indigenous and non-indigenous plant species and it was positively related to plant species richness across a gradient of plant species richness covering a common range. This suggests that generalist post-dispersal seed predation contributes to biotic resistance and acts as a filter to plant invasion by reducing the propagule pressure of non-indigenous plant species in most of the semi-natural grasslands present today. This filter may be less effective in exceptionally species-rich semi-natural grasslands. Future studies should combine seed predation with biotic and abiotic factors which influence the establishment of non-native species. This will help to assess the relative importance of post-dispersal seed predation as a filter to plant invasion.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.baae.2014.01.004.

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