BIODIVERSITY RESEARCH

Traits related to species persistence and dispersal explain changes in plant communities subjected to habitat loss

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ABSTRACT

Aim Habitat fragmentation is a major driver of biodiversity loss but it is insufficiently known how much its effects vary among species with different life-history traits; especially in plant communities, the understanding of the role of traits related to species persistence and dispersal in determining dynamics of species communities in fragmented landscapes is still limited. The primary aim of this study was to test how plant traits related to persistence and dispersal and their interactions modify plant species vulnerability to decreasing habitat area and increasing isolation.

Location Five regions distributed over four countries in Central and Northern Europe.

Methods Our dataset was composed of primary data from studies on the distribution of plant communities in 300 grassland fragments in five regions. The regional datasets were consolidated by standardizing nomenclature and species life-history traits and by recalculating standardized landscape measures from the original geographical data. We assessed the responses of plant species richness to habitat area, connectivity, plant life-history traits and their interactions using linear mixed models.

Results We found that the negative effect of habitat loss on plant species richness was pervasive across different regions, whereas the effect of habitat isolation on species richness was not evident. This area effect was, however, not equal for all the species, and life-history traits related to both species persistence and dispersal modified plant sensitivity to habitat loss, indicating that both landscape and local processes determined large-scale dynamics of plant communities. High competitive ability for light, annual life cycle and animal dispersal emerged as traits enabling species to cope with habitat loss.

Main conclusions In highly fragmented rural landscapes in NW Europe, mitigating the spatial isolation of remaining grasslands should be accompanied by restoration measures aimed at improving habitat quality for low competitors, abiotically dispersed and perennial, clonal species.

Keywords
Clonality, competition, connectivity, dispersal, fragmentation, life-history trait.

INTRODUCTION

Metapopulation ecology and the theory of island biogeography have provided analytical and conceptual frameworks to predict the effects of fragmentation on animal populations and communities. In the last decade, efforts have been made to adapt these frameworks to understand also how plants respond to habitat loss and isolation (Freckleton & Watkinson, 2002). For plants, it is expected that species' ability to both persist locally and disperse is critical in shaping communities

(Jakobsson & Eriksson, 2003). A general hypothesis is that both low ability to persist locally and low dispersal capacity are associated with higher species sensitivity to habitat fragmentation (Cadotte *et al.*, 2006). To what extent one of these two processes is dominant in determining plant species composition in fragmented landscapes is, however, still unclear.

One approach to clarify this is to explore species richness responses to habitat loss and isolation for groups of species with shared life-history traits, where the measured traits are clearly linked either to dispersal or to a key local process such as competition (Fréville *et al.*, 2007; Ozinga *et al.*, 2009). This community approach, which may provide more insights than analyses on species richness alone and aid in confronting theoretical predictions, has been successfully applied for several animal taxa in continental- or global-scale synthesizing studies (e.g. Öckinger *et al.*, 2010). Surprisingly, such large-scale trait-based analyses using primary data and assessing the effect of habitat loss and isolation on species richness are still lacking for plants.

Plants exhibit an array of adaptations and a great diversity of life-history traits, some of which are combined in complex trade-offs that mediate species' ability to persist and to colonize new patches (Tilman *et al.*, 1994). Asymmetric competition for light is recognized as a key mechanism that affects plant survival and recruitment where species with tall canopy height and large leaf area have higher probability of persistence than small, subordinate species (Grime, 2001). Among herbaceous species, clonal growth is a complementary strategy to persist locally that may lead to extensive time delays in extinction ('extinction debt') (Eriksson, 1996; Honnay & Bossuyt, 2005; Helm *et al.*, 2006; Lindborg, 2007; Kuussaari *et al.*, 2009). Many plant species can also disperse through time in a persistent seed bank by producing seeds that persist in the soil for many years (Thompson *et al.*, 1996). The soil seed bank may thus act as a buffer against the extinction of small and isolated plant populations that are typical for present-day highly fragmented agricultural landscapes (Piessens *et al.*, 2004).

Although dispersal has long been suggested as a key factor in plant metacommunity dynamics (MacArthur & Wilson, 1967; Levin *et al.*, 2003) only recently has this been tested in large-scale empirical studies (e.g. Damschen *et al.*, 2008; Ozinga *et al.*, 2009). Species with poorer dispersal capacity are expected to have lower opportunity for recolonization (Damschen *et al.*, 2008) and therefore to be more sensitive to habitat fragmentation (Collins *et al.*, 2009). Plant dispersal is, however, a very complex process controlled by several attributes such as diaspore size, number and morphology, dispersal syndrome or propagule type that, at least considered separately, might be difficult to directly link to dispersal distance. There are basically two options for plants to enhance dispersal: to favour various attributes on diaspores to increase dispersal distance, or simply to produce larger number of diaspores (Eriksson & Jakobsson, 1999). Concerning the former, recent advances in metapopulation theory have proposed that directional animal dispersal might increase species robustness to habitat loss (Johst *et al.*, 2002; Purves & Dushoff, 2005).

Although this has been empirically demonstrated for tree species (Montoya *et al.*, 2008; Sutton & Morgan, 2009), no general patterns have emerged yet.

The simultaneous analysis of traits related to species persistence and dispersal and their potential interaction remain largely overlooked for plants (but see Tremlová & Münzbergová, 2007; Fréville *et al.*, 2007; Knapp *et al.*, 2009). Hence, using a large consolidated dataset on the distribution of plant species in grassland fragments situated in five regions across NW Europe, we empirically tested how plant traits related either to species persistence or to dispersal and their interaction modify plant species vulnerability to decreasing habitat area and increasing isolation. We hypothesize (1) that life-history traits that enhance local persistence such as high competitive ability for light, clonality and a persistent seed bank lead to lower species vulnerability to habitat fragmentation (Cadotte *et al.*, 2006), and (2) that species traits enhancing colonization ability, such as directional animal dispersal (Purves & Dushoff, 2005) and production of large number of seeds (Eriksson & Jakobsson, 1999), provide further increased robustness to habitat fragmentation.

METHODS

Sampling and study regions

Our dataset was composed of primary data from studies on plant community distribution in grassland fragments in Central and Northern Europe (Fig. 1a; Table 1). First, the regional datasets were consolidated by standardizing plant species nomenclature and by recalculating standardized landscape measures from the original geographical data. Then, for each species, we compiled several life-history traits as explained below.

As extinction processes caused by habitat fragmentation often occur with a time delay (Kuussaari *et al.*, 2009), we included in this study only regions for which we assume that a high proportion of an extinction debt has been paid, that is, where previous studies testing for extinction debt in the regions included in this study demonstrated null or low extinction debt (see Adriaens *et al.*, 2006; Krauss *et al.*, 2010). This selection enabled us to reduce the potential bias resulting from extinction debt on interactions between species richness and current landscape configuration. A total of 300 semi-natural grassland patches in highly fragmented rural landscapes in five regions across four European countries were included (Table 1). In Finland and Denmark, the studied grassland patches were dry-mesic grasslands, both calcareous and siliceous, and in Germany and Belgium, they all belonged to the calcareous grassland type. All chosen semi-natural grassland patches occurred as discrete habitat patches within landscapes composed of either agricultural land or forest plantations. In the original studies, area and connectivity were kept independent (i.e. no correlation) by specifically planning a priori to achieve the required appropriate sampling scheme (Table 1).
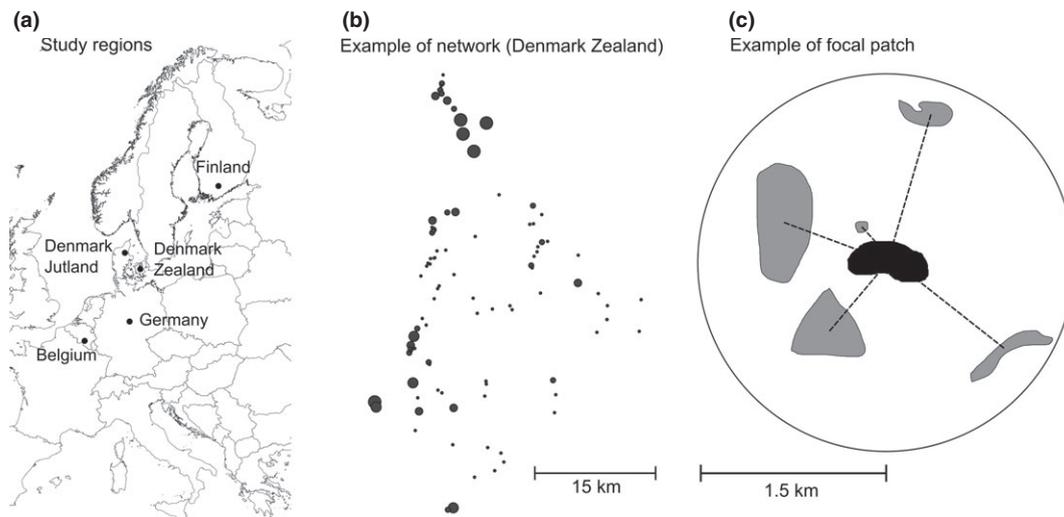
Figure 1 (a) Distribution of the study regions (networks) in which the 300 grassland patches were sampled, (b) example of one grassland network (each dot is a grassland patch; dot size is proportional to patch area) and (c) example of fragmented focal patch (the black patch indicates the focal one, while the grey patches are those included in the computation of connectivity measures).

Table 1 Descriptive statistics of specialist species richness, habitat area and connectivity (SI with $\alpha = 1$ and $b = 0.5$ see Moilanen & Hanski, 2006 for details) in 300 semi-natural patches in the five regions across NW Europe. The Pearson correlation between area and connectivity ($r_{Area, SI}$) and associated *P*-value are also reported.

	Species richness Mean ± SD	Area (ha) Mean ± SD (Min–Max)	$SI_{(\alpha = 1, b = 0.5)}$	$r_{Area, SI}$ (*P*-value)	References
Belgium (*n* = 63)	56 ± 14.8	1.06 ± 1.72 (0.01–8.46)	2.47 ± 1.81 (0–5.95)	0.101 (0.43)	Adriaens *et al.* (2006)
Denmark Jutland (*n* = 63)	40 ± 17.6	1.96 ± 7.44 (0.03–52.26)	1.34 ± 1.13 (0–4.38)	0.054 (0.67)	Bruun (2001)
Denmark Zealand (*n* = 97)	43 ± 17.9	1.18 ± 2.46 (0.03–12.92)	0.80 ± 0.86 (0–3.53)	0.054 (0.60)	Bruun (2000)
Finland (*n* = 46)	65 ± 6.9	1.07 ± 0.87 (0.21–3.94)	8.89 ± 4.33 (0–18.19)	0.278 (0.06)	Raatikainen *et al.* (2007)
Germany (*n* = 31)	64 ± 13.1	2.53 ± 2.84 (0.10–10.89)	1.88 ± 1.72 (0–7.48)	0.029 (0.88)	Krauss *et al.* (2004)

Plant species data

Vascular plant species occurrence was recorded in all the focal grassland patches while keeping sampling effort proportional to patch area and local habitat complexity. Although the sampling effort differed among regions, the consistent application of a proportional sampling made the regions comparable using a mixed model approach. More details about the sampling in each region can be found in the original publications (Table 1). Plant species were further classified for each region separately, as specialist grassland species (i.e. a species confined to, or at least strongly preferring the focal grassland type) or as generalist (a species that thrives in grasslands but is not dependent on the focal grassland type), with the help of field guides, available literature and local expert advice. We excluded woody species from the analyses due to their low occurrence, and we excluded generalist species due to their weaker dependence on semi-natural grasslands. While both arable land and forest can be considered a relatively permeable matrix for habitat generalists, they can

be considered an unsuitable habitat for the large majority of grassland specialists. Taxonomy was harmonized across all the datasets following BiolFlor (Kühn *et al.*, 2004).

Plant life-history traits

Our approach was to empirically test specific hypotheses derived from ecological theory using only traits that are clearly related to different species persistence and/or dispersal strategies. We did therefore not test all the potential life-history traits available from the literature, but examined instead the importance of canopy height, longevity (i.e. life span), seed bank persistence, seed dispersal agent and seed number, testing the expectations described below. All the traits (including seed number and plant height) were used as categorical factors to contrast the species richness response of groups of species with different strategies to persist or to disperse. The use of categories had the great advantage to create a factorial design where we could test interactions between traits, area and connectivity (refer to Data Analysis

section). Trait data were derived from the BiolFlor (Kühn *et al.*, 2004), LEDA (Kleyer *et al.*, 2008) and Seed Information Database (SID) databases (Royal Botanic Gardens Kew, 2008).

Canopy maximum height

We classified species into two groups according to their canopy maximum height (defined as the distance between the highest photosynthetic tissue and the base of the plant): small (< 50 cm, $n = 142$) vs. tall species (> 100 cm, $n = 77$). Species with intermediate height (50–100 cm, $n = 132$) were not included in the analyses. Canopy height was chosen because it is a central trait to carbon accumulation strategy and is a major determinant of a plant's ability to compete for light (Grime, 2001). We tested the expectation that species with higher competitive ability for light (tall species) will be less sensitive to habitat loss and isolation than less competitive species (small species) (Cadotte *et al.*, 2006). Moreover, tall species are also expected to have longer seed dispersal distances than short species, increasing their ability to colonize new patches (Thomson *et al.*, 2011).

Longevity

We considered two groups of species with contrasting longevity (i.e. life span) and growth form: annual and biennial species ($n = 85$) vs. perennial species that often exhibit clonal growth ($n = 159$). We included clonality in the longevity trait as clonal reproduction can be considered as an alternative life cycle loop that allows local persistence of a species without seed production (Honnay & Bossuyt, 2005). Perennial non-clonal species ($n = 76$) were not included in the analysis. The population dynamics of plant species with a short life cycle (annuals and biennials) is, compared with long-lived species, expected to be characterized by larger fluctuations in abundance and therefore more susceptible to habitat loss and isolation.

Seed bank persistence

Analogous to the spatial rescue effect of immigration, where recolonization from neighbouring patches prevents a species from going extinct, the existence of a persistent seed bank can lead to a temporal rescue effect, where the extinction of a plant species is prevented through survival in the seed bank of a patch (Piessens *et al.*, 2004). We classified the species into two seed bank groups: species with persistent (seeds persist in the soil for at least 5 years, $n = 103$) and species with transient seed bank (seeds persist in the soil for < 1 year, often much less, $n = 56$). Plant species with long-lived seeds are thereby expected to be less susceptible to local extinction, because the seed bank buffers populations against the detrimental consequences of abundance fluctuations caused by demographic and environmental stochasticities (MacDonald & Watkinson, 1981).

Seed dispersal agent

Recent theoretical (Johst *et al.*, 2002; Purves & Dushoff, 2005) and empirical studies (Montoya *et al.*, 2008; Sutton & Morgan, 2009) have proposed that directional animal dispersal might be a key trait increasing species robustness to habitat fragmentation. We classified the species into two dispersal groups: animal-dispersed species ($n = 36$) including epizoochory and endozoochory and abiotically dispersed species ($n = 136$) including explosive, wind and unspecialized species. Myrmecochorous species were excluded because of the low number of species having this dispersal agent.

Seed number

Irrespective of any dispersal attribute of diaspores, a larger reproductive output is likely to increase species chance of colonization (Eriksson & Jakobsson, 1999). We classified the species into two categories according to their mean number of seed produced per individual plant: low (no. seed< 1000, $n = 59$) and large seed number (no. seed > 10,000, $n = 107$). The intermediate class (1000 < no. seed<10,000, $n = 116$) was excluded from the analysis. Although there is a relatively high degree of uncertainty in estimating this trait because seed production can vary in response to environmental conditions and resource availability, intraspecific variation is likely to be small relative to the variation among our two extreme classes (Shipley & Dion, 1992).

Trait correlation analysis

We found a significant association between being an annual and biennial species and having a short canopy height (Fig. S1a in Supporting Information) and between having a persistent soil seed bank and a short canopy height (Fig. S1b). We found that producing large seed number was associated with having short canopy height (Fig. S1d), being annual and biennial (Fig. S1g), being abiotically dispersed (Fig. S1h) and having a persistent seed bank (Fig. S1i). All other pairs of traits were uncorrelated.

Habitat area and isolation

All patches of semi-natural grassland located within a radius of 1.5 km from the 300 focal patches ($n = 4161$) were mapped using digital topographic maps and aerial photographs (Fig. 1c). The maximum radius has been selected considering that previous studies testing the effect of habitat connectivity on species richness have used similar radii (e.g. Piessens *et al.*, 2004; Brückmann *et al.*, 2010). In all the regions, isolation of the surrounding grassland patch network was calculated for each of the 300 focal grassland patches using the three most used connectivity measures: nearest neighbour distance (d^{NN}), buffer connectivity measure (BUF) and incidence function model connectivity measure (SI) (Moilanen & Hanski, 2006).

The nearest neighbour measure is the smallest distance between the focal grassland patch *i* and any other grassland patch *j* and was computed as follows:

$$d_i^{NN} = \min_{j \neq i} d_{ij}$$

where d_{ij} is the distance between focal patch *i* and any other patch *j*, calculated between patch centres.

The buffer connectivity measure defines connectivity as the area of habitat of the patches *j* within a buffer (circle) around the focal patch:

$$BUF_i = \sum_{i \neq j} A_j \text{ for } d_{ij} < 1.5 \text{ km}$$

where A_j is the area of patch *j* and d_{ij} is distance between patches *i* and *j*.

Finally, the incidence function model connectivity measure was computed as follows:

$$SI_i = \sum_{i \neq j} e^{-\alpha d_{ij}} A_j^b \text{ for all } d_{ij} < 1.5 \text{ km}$$

where SI_i is connectivity of patch *i*, α is a coefficient of the negative exponential function that determines how the weight given to the surrounding patches decreases with distance, d_{ij} is distance between patches *i* and *j*, and A_j is area of patch *j* that is related to emigration by factor *b*. As plant dispersal over long distances is difficult to predict, different values of α (0.5, 1, 3 and 5), describing how fast the number of migrants declines with increasing distance, were used. Different values of α did not affect our connectivity measures significantly and only $\alpha = 1$ was used in further analyses. However, different values of the parameter *b*, which accounts for the tendency of per capita emigration to be greater from smaller habitat patches, affected the connectivity measures more strongly. To explore the potential effect of different *b*-values, we analysed SI values calculated with $b = 0.3$, 0.5 and 1, respectively.

Large habitat connectivity is expected to be associated with a larger regional species pool potentially arriving at the focal patch by dispersal. Whatever the local and regional processes that have produced the observed species richness patterns (e.g. mass effect, metapopulation dynamics, source-sink dynamics), a larger amount of potential suitable habitats in the surrounding landscape is expected to be positively associated with local species richness in the focal patch (Helm *et al.*, 2006; Lindborg, 2007; Brückmann *et al.*, 2010).

Data analysis

To assess the responses of plant species richness to habitat area, connectivity and plant life-history traits across the five regions, three broad analytical approaches using linear mixed models (LMMs) were adopted. We could use LMMs because model residuals approximated a normal distribution and exhibited homogeneity of variance. All the LMMs were estimated using the lme(nlme) function in R, version 2.12.1 (R Development Core Team, 2010) with the restricted maximum-likelihood (REML) estimation method.

First, we analysed overall effects of habitat area and connectivity on raw plant species richness irrespective of life-history traits. These models included habitat area, connectivity and their interaction as continuous fixed factors and region assigned as random factor (i.e. a random intercept model). As focal patch area was not correlated with our measures of connectivity (SI_i) within each of the five regions (Table 1), both habitat area and connectivity could be simultaneously included in the same model. Within this set-up, we tested the four measures of connectivity (d^{NN}, BUF, $SI_{(\alpha = 1, b = 1)}$, $SI_{(\alpha = 1, b = 0.5)}$ and $SI_{(\alpha = 1, b = 0.3)}$) in separate models and evaluated the consistency of the connectivity effect using different measures. In all further analyses, we used the connectivity measure that gave the strongest effect on species richness, that is, with the lowest AIC value.

Second, to test our main hypothesis that life-history traits modify the relationship between species richness and habitat area and connectivity, we examined the interactions between area or connectivity and life-history traits using sequential *F*-tests (Pinheiro & Bates, 2000). In all the mixed models, traits were entered as categorical fixed factors and area and connectivity as continuous fixed factors, while the number of species with each combination of traits, at each grassland patch, was the response variable; that is, there were four species richness values from each patch in this analysis. The random structure included patch within region, to account for the fact that species richness of the different combinations of traits was computed at the same patches. A major advantage of this statistical approach was that any collinearity between traits, area and connectivity was avoided, allowing tests of interactions and main effects within a factorial design (see also Bommarco *et al.*, 2010; Öckinger *et al.*, 2010). Species numbers were standardized (mean = 0, SD = 1) within each combination of traits and within each region in all models where we tested for trait effects, to make model coefficients comparable. The strength of this standardization was that the average species richness of the different trait categories was kept constant, thereby removing potential biases when comparing slopes of categories with very different number of species.

In a first set of models, we included two traits at a time. For each pair of traits (e.g. trait A and trait B), the following model was built:

Species richness~ *Fixed effects*: all main effects + Area × Trait A + Area × Trait B + Connectivity × Trait A + Connectivity × Trait B + Area × Trait A × Trait B + Connectivity × Trait A × Trait B, *Random effects*: patch within region.

In the models described above, species richness was computed for each combination of traits such that all combinations of traits were present in each grassland patch. Species for which data were not available on the two traits included in each model were excluded from the species richness computation. Starting from the full models described above, we simplified each model with a manual backward model simplification ($P > 0.05$) testing the interactions using sequential *F*-tests. The interesting terms in these models are the 3-way interactions

between Trait A, Trait B and area or connectivity. A significant interaction would imply that the slope of the species–area or species–connectivity relationship was different between groups of species belonging to different combination of the two traits (e.g. annual and short canopy vs. annual and tall canopy vs. perennial and short canopy vs. perennial and tall canopy).

Third, if no 3-way interactions involving two traits were significant, we stopped the model simplification and we analysed each trait separately building one model with the following structure (e.g. Trait A):

Species richness~ *Fixed effects*: all main effects + Area × Trait A + Connectivity × Trait A, *Random effects*: patch within region.

In these five models, the number of species within each trait category was recomputed to include the species that were initially omitted from the models testing two traits (because they lacked information on both traits). Starting from the full models described above, we simplified each model with a manual backward selection ($P > 0.05$) testing the interactions using sequential F-tests. The interesting terms in these models are the interactions between Trait A and area or connectivity. A significant interaction would imply that the slope of the species–area or species–connectivity relationship was different between groups of species belonging to different trait categories (e.g. annual vs. perennial; short vs. tall canopy).

RESULTS

From the 300 grassland patches across the five regions, a total of 353 herbaceous grassland species were recorded. Mean species richness per patch was 51 (SD = 18.4). Considering overall species richness, there was a strong positive effect of patch area, whereas connectivity and the interaction between area and connectivity were not significant (Table 2). In the four models, the slope of the species–area relationship varied between 0.37 and 0.38. Different connectivity measures showed consistent positive but non-significant effects. Compared with the other connectivity measures, the model including SI with $\alpha = 1$ and $b = 0.5$ had the lowest AIC value. We therefore used this measure in the following models testing trait effects.

The mixed models where we simultaneously tested all possible combinations of two traits did not show any significant 3-way interactions with either area or connectivity, that is, the effect of one trait did not modify the effect of any other trait.

We therefore analysed single traits with recomputed species richness within separate trait categories. The five models, testing the single life-history traits separately, showed that canopy maximum height, longevity and seed dispersal agent modified the response of species richness to habitat patch area, that is, significant interaction between area and the trait (Table 3). No interaction between traits and habitat connectivity was found. We found an interaction between habitat area and canopy height, where the species–area relationship was flatter for tall (> 1.0 m) than for short (< 0.5 m) species (slope difference = 0.097, SE = 0.041, $P = 0.019$) (Fig. 2a). Except

Table 2 Results of the linear mixed models testing the effect on overall plant species richness of area, different measures of connectivity and their interaction. The models included region as random factor. d^{NN} indicates nearest neighbour distance, BUF the buffer connectivity measure and SI the Hanski's connectivity index (for details, see Moilanen & Hanski, 2006). All connectivity measures were computed within a 1.5 km radius around the focal patch. Area, d^{NN}, BUF and SI were log-transformed in all models. Non-significant interactions were removed with a backward elimination procedure ($P > 0.05$).

	d.f.	Coefficient	SE	t	P	AIC
(a)						
Intercept	293	0.2910	0.1230	2.36	0.0187	772.8
Area	293	0.3802	0.0348	10.91	< 0.001	
d^{NN}	293	−0.1545	0.1581	−0.98	0.329	
$d^{NN} \times$ Area	–	–	–	–	–	
(b)						
Intercept	293	0.1239	0.1304	0.95	0.342	773.3
Area	293	0.3713	0.0349	10.62	< 0.001	
BUF	293	0.0695	0.0530	1.31	0.191	
BUF × Area	–	–	–	–	–	
(c)						
Intercept	293	0.1651	0.1241	1.33	0.185	773.6
Area	293	0.3729	0.0350	10.66	< 0.001	
$SI_{(\alpha = 1, b = 1)}$	293	0.0626	0.0717	0.87	0.383	
$SI_{(\alpha = 1, b = 1)} \times$ Area	–	–	–	–	–	
(d)						
Intercept	293	0.0914	0.1546	0.59	0.555	772.4
Area	293	0.3776	0.0348	10.85	< 0.001	
$SI_{(\alpha = 1, b = 0.5)}$	293	0.1236	0.0888	1.39	0.165	
$SI_{(\alpha = 1, b = 0.5)} \times$ Area	–	–	–	–	–	
(e)						
Intercept	293	0.0974	0.16018	0.61	0.544	774.9
Area	293	0.3801	0.0348	10.93	< 0.001	
$SI_{(\alpha = 1, b = 0.3)}$	293	0.1090	0.0867	1.26	0.209	
$SI_{(\alpha = 1, b = 0.3)} \times$ Area	–	–	–	–	–	

for Finland, the explained variation of the species–area relationship was larger for short than for tall species (Fig. 3a). We found a significant interaction between habitat area and longevity, where the slope of the species–area relationship was steeper for perennial species with clonal growth than for annual species (slope difference = 0.083, SE = 0.036, $P = 0.024$) (Fig. 2b). Except for Germany, in all regions, we consistently found that the explained variation by the species–area relationship was larger for perennial clonal than for annual species (Fig. 3b). Concerning seed dispersal agent, the slope of the relationship between species richness and area was steeper for abiotically dispersed species than for species dispersed by animals (slope difference = 0.054, SE = 0.026, $P = 0.024$) (Fig. 2c). In all regions, the species–area relationship was tighter for abiotically dispersed species than for species dispersed by animals (Fig. 3c).

Table 3 Results of the linear mixed models testing effects on species richness of habitat area, connectivity (SI with α = 1 and *b* = 0.5, see Moilanen & Hanski, 2006), single trait and their interactions as fixed effects and patch within region as random effect. Sequential *F*-tests are used to test the fixed effects (Pinheiro & Bates, 2000). Species richness was standardized (mean = 0, SD = 1) within each region and level of the trait analysed. Area and SI were log-transformed in all the models. Non-significant interactions were removed with a backward elimination procedure (*P* > 0.05).

Variables	d.f.	F	P*
(a)			
Canopy max height	1, 298	0	1.000
Area	1, 293	113.68	< 0.001
$SI_{(\alpha = 1, \ b = 0.5)}$	1, 293	0.19	0.662
Area × Canopy max height	1, 298	5.60	0.019
$SI_{(\alpha = 1, \ b = 0.5)}$ × Canopy max height	–	–	–
(b)			
Longevity	1, 298	0	1.000
Area	1, 293	123.02	< 0.001
$SI_{(\alpha = 1, \ b = 0.5)}$	1, 293	0.06	0.850
Area × Longevity	1, 298	5.13	0.024
$SI_{(\alpha = 1, \ b = 0.5)}$ × Longevity	–	–	–
(c)			
Soil seed bank	1, 299	0	1.000
Area	1, 293	96.05	< 0.001
$SI_{(\alpha = 1, \ b = 0.5)}$	1, 293	0.114	0.736
Area × Soil seed bank	–	–	–
$SI_{(\alpha = 1, \ b = 0.5)}$ × Soil seed bank	–	–	–
(d)			
Dispersal agent	1, 298	0	1.000
Area	1, 293	111.51	< 0.001
$SI_{(\alpha = 1, \ b = 0.5)}$	1, 293	0.04	0.843
Area × Dispersal agent	1, 298	5.17	0.024
$SI_{(\alpha = 1, \ b = 0.5)}$ × Dispersal agent	–	–	–
(e)			
Seed number	1, 299	0	1.000
Area	1, 293	102.84	< 0.001
$SI_{(\alpha = 1, \ b = 0.5)}$	1, 293	0.40	0.529
Area × Seed number	–	–	–
$SI_{(\alpha = 1, \ b = 0.5)}$ × Seed number	–	–	–

*P-value of main effect of the each trait is 1 because we standardized species richness (mean = 0, SD = 1), that is, standardized mean species richness did not differ between the trait categories (Fig. 2).

DISCUSSION

Metapopulation theory suggests that the distribution of a species in a fragmented landscape is the result of the outcome of extinction and colonization processes (Eriksson, 1996). Under these assumptions, populations should be more likely to persist in larger habitat patches and in highly connected rather than in isolated patches leading to positive species–area and species–connectivity relationships (MacArthur & Wilson, 1967). Our results indicated that the negative effect of habitat loss on plant species richness was pervasive across different regions, whereas the effect of habitat isolation was not evident. This area effect was, however, not equal for all the species, and life-history traits related to both persistence and dispersal processes modified plant sensitivity to habitat loss.

Although plant dispersal from source populations is likely to be related to the interpatch distance and the likelihood of colonization is therefore expected to decline as habitat patches become more isolated (e.g. Helm *et al.*, 2006; Lindborg, 2007; Brückmann *et al.*, 2010), we found that the degree of isolation did not explain plant species richness (see also Bruun, 2000; Dupré & Ehrlén, 2002; Stiles & Scheiner, 2010). We acknowledge that our test of connectivity effects may have several potential problems. First, testing such effects on species richness may mask important differences among species in their likelihood of showing a relationship between occupancy and habitat connectivity. Although we restricted our analyses to grassland specialists, the joint examination of a large number of species (*n* = 353) might have veiled a true connectivity effect as more ubiquitous species have lower probability to demonstrate sensitivity to reduced connectivity in their occupancy among patches (Hanski & Pöyry, 2007). Therefore, our community approach cannot rule out a connectivity effect on single species occupancy. Second, isolation acting on dispersal limitation can also be scale-dependent (Burns, 2005). Our connectivity measure was computed up to a maximum distance of 1.5 km, and it is possible that processes working at larger spatial scales remain undetected (e.g. Bruun, 2000). Third, a contrasting, but also possible, explanation is that the high level of fragmentation in several regions has lead to a general dispersal limitation among communities. A majority of grassland species might, in other words, be equally and highly dispersal limited irrespective of their traits (Pardini *et al.*, 2010), that is, in several regions even relatively large values of connectivity indicated situations of dispersal limitation. Finally, our measures of isolation do not include information on the presence and absence of the individual species in the neighbouring habitats. While such information was not possible to obtain for our large study area, this approach might have revealed stronger effects of isolation (e.g. Kirmer *et al.*, 2008).

Our results on the effects of life-history traits suggest that both species' ability to persist and disperse modify species vulnerability to habitat loss but not to habitat isolation. We found a clear trait effect on both the slope and strength (measured as proportion of the explained variation) of the species–area relationship, that is, steeper relationships were also tighter. Confirming recent perspectives in ecological theory, the directions of the observed responses suggest that both stochastic regional processes related to dispersal and local processes contribute in determining large-scale spatial dynamics of plants (see also Freckleton & Watkinson, 2002; Römermann *et al.*, 2008).
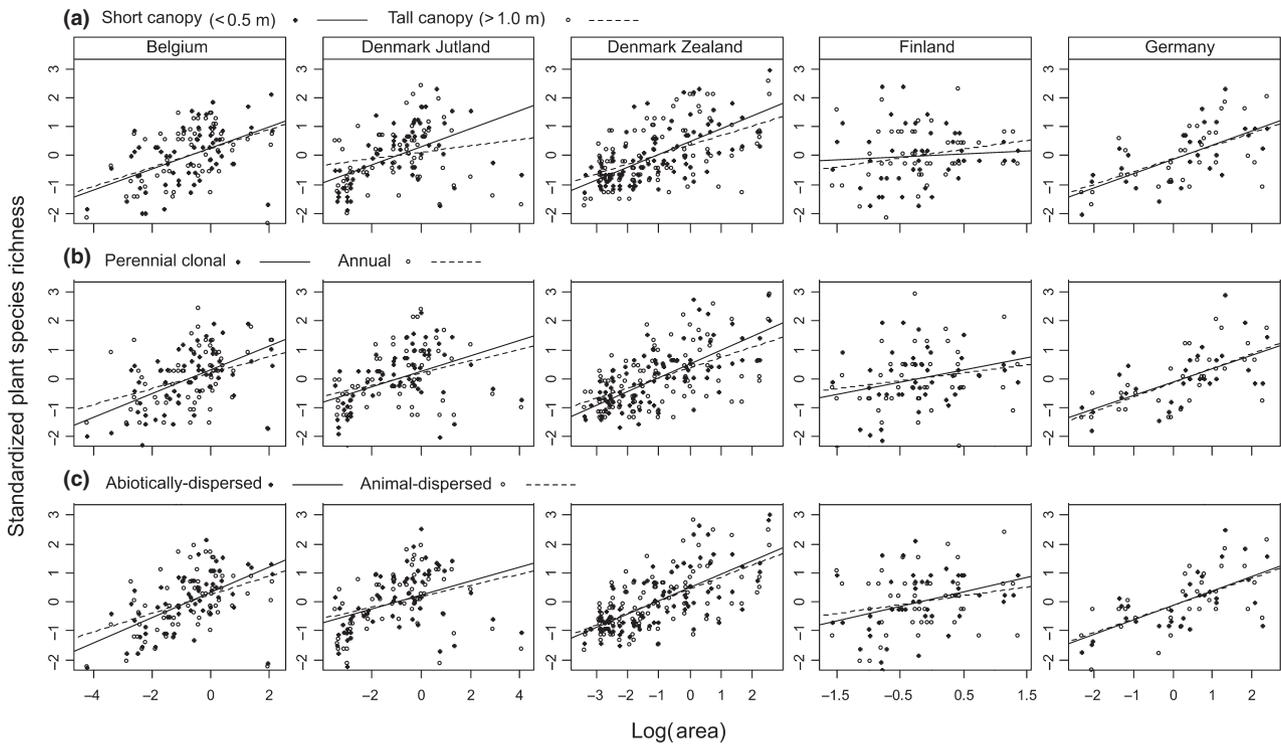
Figure 2 Species–area relationship separately for (a) canopy maximum height (< 0.5 m vs. > 1.0 m), (b) longevity (annual and biennial vs. perennial with clonal growth) and (c) seed dispersal agent (abiotic vs. animal dispersal) in the five regions across NW Europe. Species numbers were standardized (mean = 0, SD = 1) within each class of traits and within each region.
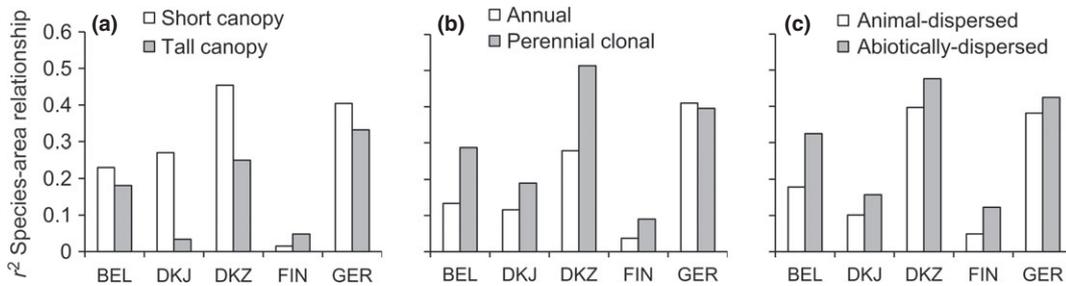

Figure 3 Explained variation (r^2) of the species–area relationship in the five regions (BEL: Belgium, DKJ: Denmark Jutland; DKZ: Denmark Zealand; FIN: Finland, and GER: Germany) separately for (a) canopy maximum height (< 0.5 m vs. > 1.0 m), (b) longevity (annual and biennial vs. perennial with clonal growth) and (c) seed dispersal agent (animal vs. abiotic dispersal).

In line with our initial expectations, we found an interaction between habitat area and canopy height, suggesting that small species tend to be more negatively affected by fragmentation than tall species (see also Sutton & Morgan, 2009). Because several factors other than fragmentation may influence plant populations in fragmented grasslands, notably eutrophication through atmospheric nitrogen deposition (Dupré *et al.*, 2010), plant competitive ability could play an important role in maintaining plant populations. An interaction between habitat area and competitive ability fits well with the expectation that small habitat patches are more prone to eutrophication from the intensive agricultural matrix than larger patches (Kiviniemi & Eriksson, 2002) posing stronger pressure to low competitive

species. Moreover, tall species are also expected to have more effective seed dispersal than short species, increasing their ability to colonize new patches and to persist in fragmented landscapes (Thomson *et al.*, 2011).

Contrary to our expectation that perenniality and clonality would increase species persistence and therefore species robustness to fragmentation (Lindborg, 2007), we found that annual species were less affected by habitat loss than perennial clonal species. Compared with perennial clonal species, annual species invest more in seed dispersal and often in dispersal through time by the establishment of a persistent seed bank (Collins *et al.*, 2009). Annual plants have also been shown to be more robust against several human disturbances that may have

further influenced plant species in addition to habitat fragmentation (Adriaens *et al.*, 2006). Therefore, clonal species may have more limited capacity to respond rapidly to changing environmental conditions (Dupré & Ehrlén, 2002; Buckley & Freckleton, 2010; Lindborg *et al.*, 2012).

Although plant dispersal has long been suggested as a key factor in population dynamics and species distribution in fragmented habitats (MacArthur & Wilson, 1967; Levin *et al.*, 2003), its role has only recently been empirically tested in large-scale experiments (Damschen *et al.*, 2008) or continental empirical studies (Ozinga *et al.*, 2009). We found that animal-dispersed species were less impacted by habitat loss than abiotically dispersed species, while no effect of seed number was ascertained. Compared with abiotic agents, which mostly exhibit random dispersal, animal agents preferentially deliver seeds towards suitable habitats (Johst *et al.*, 2002; Purves & Dushoff, 2005). This directional dispersal is expected to enhance the colonization of small habitat patches compared with abiotically dispersed species. This relationship has also been found in several regional empirical studies (Montoya *et al.*, 2008; Damschen *et al.*, 2008; but see Dupré & Ehrlén, 2002).

Conclusions

Our trait-based approach, where we simultaneously considered intrinsic species vulnerabilities, extrinsic pressures and their interaction, indicates that regional dynamics of plants in fragmented grasslands can be understood as a combination of both dispersal and local processes. The consistency of the observed patterns across different biogeographic regions allows for generalizations regarding the life-history traits that enhance plant species robustness to grassland habitat loss: a high competitive ability for light (i.e. tall canopy height), an annual life cycle and animal-mediated dispersal. These trait effects and the pervasive effect of habitat area rather than isolation indicate that, in highly fragmented semi-natural grasslands, changes in landscape configuration after habitat loss are probably not the only driver of plant extinctions and that local processes controlling plant recruitment and local coexistence should probably play a further important role (Bruun, 2000; Dupré & Ehrlén, 2002). This finding has important consequences for developing future conservation strategies to mitigate the effect of habitat fragmentation. Our results indicate that, in highly fragmented rural landscapes, mitigating the spatial isolation of remaining grasslands should be accompanied by restoration measures aimed at improving habitat quality for small, low competitors and perennial, clonal species.

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REFERENCES

Adriaens, D., Honnay, O. & Hermy, M. (2006) No evidence of a plant extinction debt in highly fragmented calcareous grasslands in Belgium. *Biological Conservation*, **133**, 212–224.

Bommarco, R., Biesmeijer, J.C., Meyer, B., Potts, S.G., Pöyry, J., Roberts, S.P.M., Steffan-Dewenter, I. & Öckinger, E. (2010) Dispersal capacity and diet breadth modify the response of wild bees to habitat loss. *Proceedings of the Royal Society B: Biological Sciences*, **277**, 2075–2082.

Brückmann, S.V., Krauss, J. & Steffan-Dewenter, I. (2010) Butterfly and plant specialists suffer from reduced connectivity in fragmented landscapes. *Journal of Applied Ecology*, **47**, 799–809.

Bruun, H.H. (2000) Patterns of species richness in dry grassland patches in an agricultural landscape. *Ecography*, **23**, 641–650.

Bruun, H.H. (2001) Determinants of species richness in patches of grassland and heathland in Himmerland (Denmark). *Nordic Journal of Botany*, **21**, 607–614.

Buckley, H.L. & Freckleton, R.P. (2010) Understanding the role of species dynamics in abundance–occupancy relationships. *Journal of Ecology*, **98**, 645–658.

Burns, K.C. (2005) A multi-scale approach for dispersal filters in an island plant community. *Ecography*, **28**, 552–560.

Cadotte, M.W., Mai, D.V., Jantz, S., Collins, M.D., Keele, M. & Drake, J.A. (2006) On testing the competition-colonization trade-off in a multispecies assemblage. *The American Naturalist*, **168**, 704–709.

Collins, C.D., Holt, R.D. & Foster, B.L. (2009) Patch size effects on plant species decline in an experimentally fragmented landscape. *Ecology*, **90**, 2577–2588.

Damschen, E.I., Brudvig, L.A., Haddad, N.M., Levey, D.J., Orrock, J.L. & Tewksbury, J.J. (2008) The movement ecology and dynamics of plant communities in fragmented landscapes. *Proceedings of the National Academy of Sciences USA*, **105**, 19078–19083.

Dupré, C. & Ehrlén, J. (2002) Habitat configuration, species traits and plant distributions. *Journal of Ecology*, **90**, 796–805.

Dupré, C., Stevens, C.J., Ranke, T., Bleeker, A., Peppler-Lisbach, C., Gowing, D.J.G., David, J., Dise, N.B., Dorland, E.,

Bobbink, R. & Diekmann, M. (2010) Changes in species richness and composition in European acidic grasslands over the past 70 years: the contribution of cumulative atmospheric nitrogen deposition. *Global Change Biology*, 16, 344–357.

Eriksson, O. (1996) Regional dynamics of plants: a review of evidence for remnant, source–sink and metapopulations. *Oikos*, 77, 248–258.

Eriksson, O. & Jakobsson, A. (1999) Recruitment tradeoffs and the evolution of dispersal mechanisms in plants. *Evolutionary Ecology*, 13, 411–423.

Freckleton, R.P. & Watkinson, A.R. (2002) Large-scale spatial dynamics of plants: metapopulations, regional ensembles and patchy populations. *Journal of Ecology*, 90, 419–434.

Fréville, H., McConway, K., Dodd, M. & Silvertown, J. (2007) Prediction of extinction in plants: interaction of extrinsic threats and life history traits. *Ecology*, 88, 2662–2672.

Grime, J.P. (2001) *Plant strategies, vegetation processes, and ecosystem properties*. Wiley, New York.

Hanski, I. & Pöyry, J. (2007) Insect populations in fragmented habitats. *Insect conservation biology* (ed. by A.J.A. Stewart, T.R. New and O.T. Lewis), pp. 175–202. Proceedings of the Royal Entomological Society's 23rd Symposium, CABI, Wallingford.

Helm, A., Hanski, I. & Pärtel, M. (2006) Slow response of plant species richness to habitat loss and fragmentation. *Ecology Letters*, 9, 72–77.

Honnay, O. & Bossuyt, B. (2005) Prolonged clonal growth: escape route or route to extinction? *Oikos*, 108, 427–432.

Jakobsson, A. & Eriksson, O. (2003) Trade-offs between dispersal and competitive ability: a comparative study of wind-dispersed Asteraceae forbs. *Evolutionary Ecology*, 17, 233–246.

Johst, K., Brandl, R. & Eber, S. (2002) Metapopulation persistence in dynamic landscapes: the role of dispersal distance. *Oikos*, 98, 263–270.

Kirmer, A., Tischew, S., Ozinga, W.A., von Lampe, M., Baasch, A. & van Groenendael, J.M. (2008) Importance of regional species pools and functional traits in colonization processes: predicting re-colonization after large-scale destruction of ecosystems. *Journal of Applied Ecology*, 45, 1523–1530.

Kiviniemi, K. & Eriksson, O. (2002) Size-related deterioration of semi-natural grassland fragments in Sweden. *Diversity and Distributions*, 8, 21–29.

Kleyer, M., Bekker, R.M., Knevel, I.C. *et al.* (2008) The LEDA Traitbase: a database of life-history traits of Northwest European flora. *Journal of Ecology*, 96, 1266–1274.

Knapp, S., Kühn, I., Bakker, J., Kleyer, M., Klotz, S., Ozinga, W., Poschlod, P., Thompson, K., Thuiller, W. & Römermann, C. (2009) How species traits and affinity to urban land use control large-scale species frequency. *Diversity and Distributions*, 15, 533–546.

Krauss, J., Klein, A.-M., Steffan-Dewenter, I. & Tscharntke, T. (2004) Effects of habitat area, isolation, and landscape diversity on plant species richness of calcareous grasslands. *Biodiversity and Conservation*, 13, 1427–1439.

Krauss, J., Bommarco, R., Guardiola, M., Heikkinen, R.K., Helm, A., Kuussaari, M., Lindborg, R., Öckinger, E., Pärtel, M., Pino, J., Pöyry, J., Raatikainen, K.M., Sang, A., Stefanescu, C., Teder, T., Zobel, M. & Steffan-Dewenter, I. (2010) Habitat fragmentation causes immediate and time-delayed biodiversity loss at different trophic levels. *Ecology Letters*, 13, 597–605.

Kühn, I., Durka, W. & Klotz, S. (2004) BiolFlor - a new plant-trait database as a tool for plant invasion ecology. *Diversity and Distributions*, 10, 363–365.

Kuussaari, M., Bommarco, R., Heikkinen, R., Helm, A., Krauss, J., Lindborg, R., Öckinger, E., Pärtel, M., Pino, J., Rodà, F., Stefanescu, C., Teder, T., Zobel, M. & Steffan-Dewenter, I. (2009) Extinction debt: a challenge for biodiversity conservation. *Trends in Ecology and Evolution*, 26, 564–571.

Levin, S.A., Muller-Landau, H.C., Nathan, R. & Chave, J. (2003) Ecology and evolution of seed dispersal: a theoretical perspective. *Annual Review of Ecology, Evolution and Systematics*, 34, 575–604.

Lindborg, R. (2007) Evaluating the distribution of plant life history traits in relation to current and historical landscape configurations. *Journal of Ecology*, 95, 555–564.

Lindborg, R., Helm, A., Bommarco, R., Heikkinen, R.K., Kühn, I., Pykälä, J. & Pärtel, M. (2012) Effect of habitat area and isolation on plant trait distribution in European forests and grasslands. *Ecography*, doi: 10.1111/j.1600-0587.2011.07286.x.

MacArthur, R.H. & Wilson, E.O. (1967) *The theory of island biogeography*. Princeton University Press, Princeton, NJ.

MacDonald, N. & Watkinson, A.R. (1981) Models of an annual plant population with a seed bank. *Journal of Theoretical Biology*, 93, 643–653.

Moilanen, A. & Hanski, I. (2006) Connectivity and metapopulation dynamics in highly fragmented landscapes. *Connectivity conservation* (ed. by K.R. Crooks and M. Sanjayan), pp. 44–71. Cambridge University Press, Cambridge.

Montoya, D., Zavala, M.A., Rodríguez, M.A. & Purves, D.W. (2008) Animal versus wind dispersal and the robustness of tree species to deforestation. *Science*, 320, 1502–1504.

Öckinger, E., Schweiger, O., Crist, T.O., Debinski, D.M., Krauss, J., Kuussaari, M., Petersen, J.D., Pöyry, J., Settele, J., Summerville, K.S. & Bommarco, R. (2010) Life-history traits predict species responses to habitat area and isolation – a cross-continental synthesis. *Ecology Letters*, 13, 969–979.

Ozinga, W.A., Römermann, C., Bekker, R.M., Prinzing, A., Tamis, W.L.M., Schaminée, J.H.J., Hennekens, S.M., Thompson, K., Poschlod, P., Kleyer, M., Bakker, J.P. & van Groenendael, J.M. (2009) Dispersal failure contributes to plant losses in NW Europe. *Ecology Letters*, 12, 66–74.

Pardini, R., Bueno, Ad.A., Gardner, T.A., Prado, P.I. & Metzger, J.P. (2010) Beyond the fragmentation threshold hypothesis: regime shifts in biodiversity across fragmented landscapes. *PLoS ONE*, 5, e13666.

Piessens, K., Honnay, O., Nackaerts, K. & Hermy, M. (2004) Plant species richness and composition of heathland relics in

northern Belgium: evidence for a rescue effect? *Journal of Biogeography*, **31**, 1683–1692.

Pinheiro, J. & Bates, D. (2000) *Mixed effects models in S and S-Plus*. Springer Verlag, New York.

Purves, D.W. & Dushoff, J. (2005) Directed seed dispersal and metapopulation response to habitat loss and disturbance: application to *Eichhornia paniculata*. *Journal of Ecology*, **93**, 658–669.

R Development Core Team (2010) *R: a language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0. Available at: http://www.R-project.org/.

Raatikainen, K.M., Heikkinen, R.K. & Pykälä, J. (2007) Impact of within-site and between-site factors on vegetation of boreal semi-natural grasslands. *Plant Ecology*, **189**, 155–173.

Römermann, C., Tackenberg, O., Jackel, A.-K. & Poschlod, P. (2008) Eutrophication and fragmentation are related to species' rate of decline but not to species rarity: results from a functional approach. *Biodiversity and Conservation*, **17**, 591–604.

Royal Botanic Gardens Kew (2008) Seed Information Database (SID). Available at: http://data.kew.org/sid/.

Shipley, B. & Dion, J. (1992) The allometry of seed production in herbaceous angiosperms. *The American Naturalist*, **139**, 467–483.

Stiles, A. & Scheiner, S.M. (2010) A multi-scale analysis of fragmentation effects on remnant plant species richness in Phoenix, Arizona. *Journal of Biogeography*, **37**, 1721–1729.

Sutton, F.M. & Morgan, J.W. (2009) Functional traits and prior abundance explain native plant extirpation in a fragmented woodland landscape. *Journal of Ecology*, **97**, 718–727.

Thompson, K., Bakker, J. & Bekker, R. (1996) *The soil seed banks of North West Europe: methodology, density and longevity*. Cambridge University Press, Cambridge.

Thomson, F.J., Moles, A.T., Auld, T.D. & Kingsford, R.T. (2011) Seed dispersal distance is more strongly correlated with plant height than with seed mass. *Journal of Ecology*, **99**, 1299–1307.

Tilman, D., May, R.M., Lehman, C.L. & Nowak, M.A. (1994) Habitat destruction and the extinction debt. *Nature*, **371**, 65–66.

Tremlová, K. & Münzbergová, Z. (2007) Importance of species traits for species distribution in fragmented landscapes. *Ecology*, **88**, 965–977.

SUPPORTING INFORMATION

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

Figure S1 Association between the five life-history traits included in the study.

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