Functional traits of seeds dispersed through endozoochory by native forest ungulates

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Abstract

Aims: Zoochory is a prominent dispersal process in a wide range of plant species. However, the extent to which the trait composition of dispersed seed assemblages differs from the regional assemblage remains untested, as does the extent to which sympatric native herbivores disperse traits non-randomly.

Location: Lorris and Montargis forests, central France.

Methods: We compared the taxonomic and functional composition of seed assemblages dispersed by three wild ungulates to the characteristics of the regional flora in an agro-forested landscape. We collected roe deer (*Capreolus capreolus*), red deer (*Cervus elaphus*) and wild boar (*Sus scrofa*) faeces in two forests, and subjected the samples to germination under controlled conditions. We first examined how different vectors influence the composition of the dispersed plant assemblages by comparing abundance and species richness of the seedlings emerging from the faeces of the three ungulates. We then compared the functional characteristics of the dispersed pool with those of the regional flora in a multivariate functional space built from 20 relevant plant traits.

Results: A total of 754 seedlings and 46 plant species germinated from 300 faeces samples, with higher plant species richness and abundance for red deer faeces. All three ungulates widely use forest habitat, but the proportion of non-forest plants was higher in red deer and wild boar faeces than in the regional species pool. Traits such as seed shape, seed size or seed bank longevity affected dispersal probability, but their effects were overshadowed by habitat effects.

Conclusions: Endozoochory acts as an indirect functional filter, filtering species according to the vectors' feeding habitat. This could affect the composition of forest plant communities by allowing plants from open habitats to colonize forested areas.

Introduction

Seed dispersal is a critical process in plant population spatial dynamics (Cain et al. 2000). Exchanging individuals among populations allows genetic flow and/or colonization of new suitable areas, lower intraspecific competition and pathogen prevalence, and may rescue populations from local extinction (Howe & Smallwood 1982; Cain et al. 2000; García et al. 2007). By spreading individuals, dispersal influences the spatial patterns of plant diversity but also has an impact on species assembly processes, and through these processes affects community composition patterns (Cousens et al. 2008). Animals are common vectors of plant dispersal (Vittoz & Engler 2007). Many, but not all animal-dispersed plants have morphological traits assumed to result from adaptations to epizoochory, including hooked or bristly seeds, or to endozoochory, with fleshy fruits or a resistant seed coat, for instance (Howe & Smallwood 1982; Pakeman et al. 2002; Cousens et al. 2008). In endozoochorous dispersal, because many herbaceous seeds, particularly smaller ones, without such morphological adaptations can be accidentally ingested in considerable quantities by large herbivores as they consume plant foliage ('foliage is the fruit'; Janzen 1984), adaptations which improve seed resistance to digestion condition their dispersal effectiveness (Pakeman et al. 2002). Seeds able to germinate after defecation by herbivores have been shown to share common characteristics, including small size, round shape and low mass (Pakeman et al. 2002; Couvreur et al. 2005; Mouissie et al. 2005a).

Thompson et al. (1993) demonstrated that these seeds also have persistent seed banks, which could explain their higher survival rates.

Studies on a variety of domestic and wild herbivores have established the wide variety of plant species dispersed through endozoochory (Pakeman et al. 2002; Mouissie et al. 2005a,b; Eycott et al. 2007; Jaroszewicz et al. 2013). Additionally, herbivores trigger 'directed dispersal', as they transfer plant species between similar habitats corresponding to their feeding grounds (Wenny 2001; D'hondt et al. 2012; Rico et al. 2014). Since their home ranges are larger than those of smaller mammals and domesticated species limited by human confinement, wild ungulates potentially disperse seeds over long distances and across varied habitats in mosaic landscapes. Thus, large wild ungulates may not only link distant plant populations, but also promote colonization of new areas; these ungulates are therefore suitable as models to assess the effects of zoochory on plant distribution. Furthermore, as plant dispersal probability differs depending on seed traits, wild ungulates could filter plant communities according to their dietary preferences and patterns of habitat use within their home ranges.

Most existing studies on the community-level consequences of endozoochory have not compared the dispersed assemblage to the composition of the regional flora (Cosyns et al. 2005; Mouissie et al. 2005a,b; Jaroszewicz et al. 2013), even if the pool of dispersed species is necessarily a sub-sample of the regional pool. In situations where animals feed randomly, one could assume the commonest plant species in the regional pool would be consumed and dispersed more frequently by animal vectors. On the other hand, if animals express dietary preferences or/and feed in specific habitats, they should not consume plants at random, and dispersed plant species would not necessarily be the commonest in the regional pool. Yet, all consumed seeds do not have the capacity to survive ingestion and germinate after defecation. Hence, the dispersed pool would reflect both the vectors' dietary and/or habitat preferences, as well as seed germination ability. Wild ungulates usually disperse small and round seeds, which have no particular morphological adaptations (i.e. pulp or appendages; Pakeman et al. 2002; Heinken et al. 2002). Yet, the extent to which the prominence of seed morphological traits reflects their abundance in the regional assemblage remains untested, as does whether or not herbivores disperse traits randomly. Furthermore, while recent studies have suggested that combinations of traits explain seed survival after defecation (Couvreur et al. 2005; D'hondt & Hoffmann 2011), the studies that compare dispersed and non-dispersed species consider each plant attribute separately. In a lowland coniferous forest mosaic Eycott et al. (2007) observed a lower proportion of seeds without physical dispersal adaptations (awns, burrs,

fleshy fruit, nuts, wings or plumes) in faecal material than in their study area (25 vs 39%). In contrast, Heinken et al. (2002), who studied zoochory by roe deer, wild boar, hare and marten in a deciduous forest, found that the proportion of light seeds without morphological adaptations to zoochory was higher in wild mammal faeces than in the regional flora. Non-forest plant species were also significantly more dispersed.

In this study, we evaluated whether forest ungulates act as a biotic filter for plants dispersed by endozoochory. In two forests surrounded by an agro-forested mosaic landscape in central France, we sampled faeces from three wild ungulates: roe deer (*Capreolus capreolus*), a herbivorous ruminant browser; red deer (*Cervus elaphus*), an intermediate herbivorous ruminant mixed feeder; and wild boar (*Sus scrofa*), an omnivorous-frugivorous hindgut fermenter (Hofmann 1989; Clauss et al. 2008). We first compared the abundance and species richness of the seedlings dispersed by the three ungulates to assess their relative contribution to the composition of the dispersed plant assemblages. We then compared the assemblages of plant species dispersed by each of the three animal species to the regional pool of plant species in a multivariate functional space built from ecological traits known to affect dispersal propensity. We specifically tested the following hypotheses: (1) due to their differing feeding strategies, the three wild ungulates should disperse different seed quantities and plant species richness. We hypothesized that red deer, as a mixed feeder with a wider feeding regime, would disperse more seeds and species than the more selective roe deer (Eycott et al. 2007; Jaroszewicz et al. 2013). We also hypothesized that wild boar would disperse fewer seeds and plant species than the two ruminant species (Heinken et al. 2002; Schmidt et al. 2004; Jaroszewicz et al. 2013). (2) Species abundance in the faeces should reflect the animals' dietary preferences rather than the plant species abundance in the study area. (3) Plant ecological traits relevant to dispersal should distinguish dispersed species from the species in the regional assemblages. We assumed that the dispersed pool of species would be biased towards small and round seeds, and that these species would also have a persistent seed bank. We also expected that the two herbivores would preferentially disperse herbaceous species, with shrubs in addition for roe deer, while the frugivorous wild boar should preferentially disperse seeds with pulp.

Methods

Study area

We conducted our study in two forests in central France, the Lorris (14 500 ha) and Montargis (4100 ha) forests, separated from each other by approximately 25 km of

agricultural mosaic composed of crops, pastures, villages and small woods (Fig. 1a). These two forests are managed through rotational clear-felling. They are dominated by *Quercus petraea* (45 and 50% cover in Lorris and Montargis, respectively) and *Pinus sylvestris* (39 and 20% cover, respectively); they each include approximately 5% open habitats. They differ in soil and vegetation composition. Lorris is dominated by conifers trees (*Pinus sylvestris*, *Pinus nigra*), while Montargis is mainly deciduous (*Quercus petraea*, *Fagus sylvatica*, *Carpinus betulus*). Lorris includes a number of ponds of different sizes, and the soil is more acidic (pH < 4.5) than in Montargis. Roe deer and wild boar are present in both forests (respectively, approximately five and ten animals killed per km² in 2009–2010 in Lorris, five and two in Montargis; unpubl data from the Loiret hunting federation), while red deer are present only in Lorris (about two animals killed per km² during the same hunting season, just prior to faeces collection).

Faeces collection and treatment

We collected faeces from the three ungulate species in the two forests from May 2010 to Dec 2012. Each faeces sample corresponded to one defecation event. We collected faeces roughly every fortnight in 84 strip transects of 2 × 100 m (44 in Lorris, 40 in Montargis). We placed the transects in order to stratify the search effort and reflect the diversity of stand age (young, intermediate or old), structure (regular, coppice) and composition (*Quercus* spp., *Pinus* spp.) of both forests (Fig. 1b). We only collected fresh faeces and removed the lowermost layer in order to avoid contamination by seeds from the soil seed
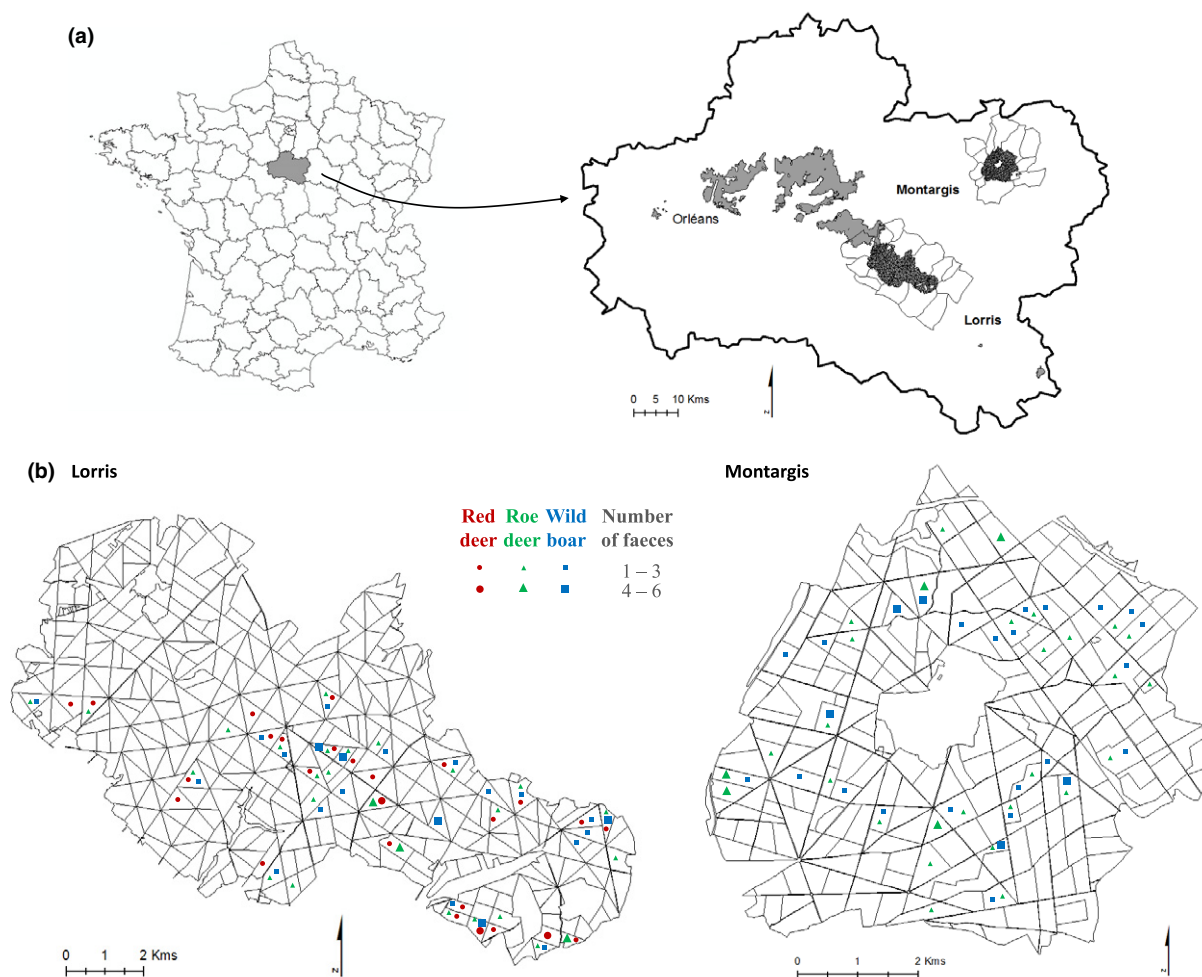

Fig. 1. (**a**) Map of the regional district where the two study areas are located, showing the Lorris and Montargis forests and the surrounding municipalities considered for the regional plant species pool (14 for Lorris, 13 for Montargis), and (**b**) location and number of faeces samples collected per forest and animal species.

bank. We recorded the collection date of each faeces sample, dried the samples at 20–25 °C for 2 d, then weighed and stored them at 5 °C until the end of field collection. We obtained a total of 844 faeces samples. However, for some month–stand pairs, we found no faeces. Furthermore, wild boar faeces were often found on the same transects from month to month, usually in the younger stands. We decided to select an equal number of faeces per animal species, per month and per forest; in order to cater for the available space available in our greenhouse. We finally used 300 faeces samples: 180 from Lorris and 120 from Montargis; 60 for each animal species (five samples × 12 mo). These 300 faeces were randomly selected so as to reflect the higher diversity of month–stand pairs. In order to sort the seeds contained in the faeces, we washed each sample through 2-mm and 200-μm sieves to remove large components (fibre fragments) and to retain the smallest seeds expected (*Juncus* species, according to local vegetation surveys). We then spread the content of the 200-μm sieve onto trays containing a 3–5-mm thick layer of sterilized potting compost, as recommended for seed bank analyses (Ter Heerdt et al. 1996). We kept the sample trays in a greenhouse under moist conditions and let temperatures in the greenhouse vary with outside temperature. We only heated the greenhouse at night in winter to avoid negative temperatures, maintaining 5 °C. Mean temperatures were 15 °C in winter and 25 °C in summer, 35 °C were reached during two very warm weeks. In order to control for seed rain contamination in the greenhouse, we also placed ten control trays containing pure sterilized potting compost with no sieved faecal content near the sample trays.

Estimation of viable seed content

Over 1 yr, we regularly recorded all seedlings from the trays as soon as they were large enough to be identified, and then removed them in order to prevent competition. After each identification session, we stirred the tray compost. We identified seedlings at the species level whenever possible, unfortunately 4% died before any identification was possible; 36% could only be identified to genus level, 32% of which (i.e. 12% of the overall seedling pool) were *Juncus* spp. that died before they grew enough to enable species level identification. We excluded the six species that also emerged in the controls and were considered to be contaminations: *Conyza canadensis*, *Epilobium tetragonum*, *Populus alba*, *Salix* spp., *Senecio vulgaris* and *Oxalis* spp.

Regional flora

We defined the regional flora as the flora hosted within all the surrounding municipal territories if they included at least a part of the study forest, and their neighboring non-forest habitats, up to 2 km from the nearest forest edge (Fig. 1a). We compiled separate regional species pools for the two forests from municipality-level plant species inventories (data available online, from the Bassin Parisien National Botanical Conservatory: http://cbnbp.mnhn.fr/cbnbp/, accessed Jun 2014). We computed species occurrence frequency separately for each forest as the relative number of municipalities in which a species was present. We only considered spermatophytes, herbaceous and woody species.

All plant species recorded in the faeces samples were listed in the regional flora. Local plant diversity was higher in Lorris (818 species, for a corresponding surface area of 39 941 ha) than in Montargis (672 species, for 22 929 ha).

Trait matrix

We compiled 20 plant attributes known to be associated with endozoochorous dispersal (Table 1) from the LEDA Traitbase (Kleyer et al. 2008), the Ecoflora database (Fitter & Peat 1994) and the Baseflor (Julve 1998). We also retrieved the seed bank longevity index (SLI) from Thompson et al. (1997). We calculated seed shape (variance in dimensions: Vs) following Bekker et al. (1998): $Vs = \sum(x_i - \bar{x})^2/3$, with $x_1 = $ length/length, $x_2 = $ width/length and $x_3 = $ height/length. Seed coat hardness and leaf nutrient content, usually associated with seed resistance to digestion and plant foliage attractiveness (Janzen 1984; D'hondt & Hoffmann 2011), were available for <11% of the species present in the regional flora; we therefore did not include these traits in our study. In total, 19% of the traits were missing some form of data (when considering each species–trait pair), so we completed the data through supplementary searches in different sources of grey literature. Since multivariate analyses (see below) do not tolerate missing data, we filled the remaining 7% of the missing data with the mean value (for continuous traits) or the modal value (for categorical traits).

Data analyses

We tested for differences in seedling abundance and species richness among the animal species, while accounting for possible phenological and forest-related variations in the seed pools. Due to the high level of zero inflation in our response variables (seedling abundance and plant species richness), we built hurdle models (Potts & Elith 2006) with animal species, sampling month and forest as covariates on the truncated Poisson component and no covariate on the binomial component. We included the log-transformed weight of each faeces sample as an offset to ensure

Table 1. Attributes and ecological traits used in our analyses.

Attribute or Trait	Expected Effect on Endozoochory	Categories or Span (Unit)	Source
Plant Traits			
Plant Class	Browser ruminants preferentially feed on dicots, grazers on monocots (Hofmann 1989)	Monocot, dicot	Baseflor
Plant Habitat Preference	Plants and their vectors must be in contact	Forest, peri-forest, non-forest, hygrophile*	Baseflor
Life Form	A proxy for plant phenology and seed accessibility for animals	Chamaephyte, geophyte, helophyte, hemicryptophyte, hydrophyte, phanerophyte, therophyte	Ecoflora, Baseflor
Life Span		Bi-annual, vernal, aestival, deciduous, evergreen, shrub, sub-shrub, bulb, rhizome, tuber, stolon, erect, rosette, tuft	Baseflor
L Ellenberg Indicator Value	Light requirement; a proxy for habitat openness	2 (shade)–9 (light)	Ecoflora, Baseflor, Tela Botanica[1]
N Ellenberg Indicator Value	High nutrient plants are more attractive for herbivores (Janzen 1984)	1 (oligotrophy)–9 (eutrophy)	Ecoflora, Baseflor, Tela Botanica
Seed Production	If higher, enhances contact probability with an animal	1–10, 10–100, 100–1000, 1000–10.000, >10.000 (seed number·plant^{-1})	LEDA, Ecoflora
Seed Release Height	Gives an idea of seed accessibility for animals	0.003–55 m	LEDA
Seed Traits			
No Appendages	Well dispersed by endozoochory (Janzen 1984)	Yes/No	LEDA, Ecoflora, Digital Seed Atlas of the Netherlands[2] (Cappers et al. 2006)
Elongated Appendages	Favours epizoochory	Yes/No	
Hooks	Favours epizoochory	Yes/No	
Flat Appendages	Favours anemochory	Yes/No	
Balloon Structures	Favours hydrochory	Yes/No	
Pulp	Favours endozoochory by frugivores	Yes/No	
Elaiosomes	Favours myrmecochory	Yes/No	
Mucilage	Sticky substance which favours epizoochory	Yes/No	
Length	Small seeds germinate better (Pakeman et al. 2002)	0.29–58.75 mm	LEDA
Mass	Light seeds germinate better (Couvreur et al. 2005)	0.01–4753 mg	LEDA
Shape (Vs)	Round seeds germinate better (Janzen 1984)	0.0 (spherical)–0.2 (disc- or needle-shaped)	Bekker et al. (1998)
Seed Bank Longevity Index (SLI)	Seeds from persistent seed bank germinate better (Pakeman et al. 2002)	0 to 1	Thompson et al. (1997)

Categorical traits: Plant class, plant habitat preference (*with a separate category for hygrophilous species, which mostly depend on highly specific microclimatic and local edaphic conditions), plant life form, life span and seed production. Continuous traits: L and N indicator values, seed length, mass, shape, release height and seed bank longevity index. Binary traits: Seed morphology (presence of appendages, elongated or flat appendages, hooks, balloon structures, pulp, elaiosomes and mucilage).

[1]http://www.tela-botanica.org/site:accueil; accessed: Jun 2014.

[2]http://seeds.eldoc.ub.rug.nl/?pLanguage=en; accessed date: Jun 2014.

Table 2. Summary of the dispersed species assemblage.

Plant Species	Red Deer	Wild Boar	Roe Deer	Total
Sample Size	60	120 (60 + 60)	120 (60 + 60)	300
Total Number of Seedlings Dispersed	416	276	62	754
Total Number of Species Dispersed	34	24	10	46
Chao2 Estimator of Total Number of Species Dispersed (± SD)	63 ± 14	40 ± 11	13 ± 25	72 ± 22
Total Number of Genera Dispersed	25	20	9	34
Total Number of Families Dispersed	16	14	7	19
Top Five Plant Species, in Terms of Frequency in Faeces:	*Juncus* spp. *Calluna vulgaris* *Plantago major* *Poa annua* *Agrostis capillaris*	*Juncus* spp./*Juncus effusus* *Digitaria sanguinalis* *Chenopodium album* *Urtica dioica*	*Calluna vulgaris* *Juncus* spp. *Digitaria sanguinalis* *Urtica dioica/Kickxia elatine/Agrostis capillaris*	
Seedling Abundance/Faeces (± SD)	7.05 ± 20.12	2.34 ± 11.82	0.51 ± 1.92	
Specific Richness/Faeces (± SD)	1.56 ± 1.78	0.66 ± 1.04	0.25 ± 0.50	
Mean Weight of Faeces (± SD)	21.7 ± 10.6 g	22.9 ± 18.2 g	7.4 ± 4.4 g	
Mean Seedling Density·g^{-1} (± SD)	0.30 ± 0.60	0.13 ± 0.62	0.09 ± 0.24	
Mean Species Density·g^{-1} (± SD)	0.08 ± 0.09	0.05 ± 0.09	0.06 ± 0.15	

that sample size-related variations in the response variables were accounted for. We used similar hurdle models to check whether faeces collection date influenced species richness. We also built species accumulation curves based on the Chao2 estimator to check whether the composition of our seed samples reflected the actual species richness found in the two forests (Chao 1987).

We then compared the taxonomic and functional composition of the dispersed species pool to that of the regional flora. First, we used Spearman's rank correlations to test whether species frequency of occurrence in our faeces samples was correlated to species frequency of occurrence in the regional flora. For this test, we used all plant species from the regional pool, including the plant species absent from the faeces (frequency of occurrence in faeces = 0). Then, to test for differences between the functional composition of the dispersed species pool and that of the regional flora, we used a Hill and Smith multivariate analysis, equivalent to principal components analysis, which allows categorical and continuous data to be used simultaneously (Hill & Smith 1976). The multivariate functional space was built from 20 plant traits relevant for endozoochorous seed dispersal (see Table 1). We computed separate multivariate spaces for both forests since we could not assume that both regional plant assemblages were similar. We used species frequency of occurrence in the regional flora as the row weight, and retained the first two components of the analyses, which accounted for 19.6% and 19.4% of the total variance for Lorris and Montargis, respectively. We drew ellipses around the centroids of each dispersed pool (by roe deer, red deer and wild boar), with points weighted by the species frequency of occurrence in the faeces (for each

forest–animal species pair), and with axes representing 1.5× the SD of the species' coordinates on each principal component. We then tested for differences between the functional characteristics of the pool transported by each animal species (roe deer, red deer or wild boar) and the regional pool. To do this, we used a MANOVA to compare the coordinates of the plant species of each of the three dispersed pools with the coordinates of all species from the regional pool. All statistical analyses were performed using R 3.1 software (R Foundation for Statistical Computing, Vienna, AT) and the pscl, fossil and ade4 libraries (Dray & Dufour 2007; Zeileis et al. 2008; Vavrek 2011).

Results

Qualitative results

A total of 754 seedlings of 46 plant species germinated from the 300 collected faeces samples, after exclusion of contaminations and unidentified seedlings (Appendix S1). Two taxa dominated the dispersed pool in terms of seedling number: *Juncus* spp. (260 seedlings) and *Portulaca oleracea* (160 seedlings). We observed *Juncus* spp. in 12.7% of all the faeces, while *P. oleracea* was only recorded in 1%. 48% of the species only occurred in a single faeces sample, and 32% of the germinating species produced only one seedling, while no seeds germinated in 63% of the faeces.

Red deer dispersed more plant species and more seedlings than either wild boar or roe deer (Table 2). Red deer, wild boar and roe deer, respectively, dispersed 17, ten and one plant species exclusively, while five species were shared by these three ungulates: *Agrostis capillaris*, *Calluna vulgaris*, *Juncus* spp., *Luzula* spp. and

P. oleracea (Appendix S1). Four of these species had a frequency of occurrence >50% in both forests, with *P. oleracea* at 36%.

Seedling abundance and plant species richness

Red deer dispersed more seeds than either roe deer (difference between roe and red deer = −0.49 ± 0.17 (±SD), $z = -2.89$, $P < 0.01$) or wild boar (difference between red deer and wild boar = −0.53 ± 0.10, $z = -5.56$, $P < 0.01$). The deviance explained was 27.8%. Wild boar faeces also exhibited lower species richness than red deer faeces (difference between red deer and wild boar = −0.82 ± 0.24, $z = -3.42$, $P < 0.001$). Richness did not differ significantly between roe and red deer (−0.66 ± 0.52, $z = -1.26$, $P = 0.21$); however, this might be due to the low amount of non-null data combined with the high variability in species richness dispersed by roe deer. Our model explained 17.8% of the deviance in species richness. As a consequence of plant phenology, both seedling abundance and species richness were correlated with time. Seedling abundance was highest from Jul to Dec ($P = 0.03$ in Jul, $P < 0.001$ from Aug to Dec), while species richness was highest in Jul ($P = 0.04$), and tended to be higher between Sept and Oct ($P = 0.08$ and 0.06 respectively; Appendix S2). There was no correlation between species frequency of occurrence in faeces and in the regional flora ($\rho = 0.05$ and 0.02 for Lorris and Montargis, respectively). Cumulative species richness curves (Fig. 2) showed that a number of infrequently dispersed species may not have

been recorded in our study, but did not suggest that this incomplete sampling affected the results of our analyses (see also Table 2). The estimated species richness showed that wild boar dispersed a higher number of species than did roe deer.

Functional traits

The functional patterns described were consistent from one forest to the other. The first axis of the Hill and Smith multivariate analysis segregated species according to their habitat preference, from forest species (phanerophytes with fruits, high seed release height and large heavy seeds) to non-forest species (heliophilous species with persistent seed banks; Figs 3 and 4). The second axis differentiated species according to their seed morphology – with perennial monocots and species bearing few seeds with morphological adaptations (elongated or flat appendages, hooks, balloon structures) towards negative values; and shrubs and annual species with round seeds without appendages towards positive values (Figs 3 and 4, and see coordinates of all traits in Appendix S3). The MANOVA revealed that the pools dispersed by red deer and wild boar differed from the regional pool on the first axis (Lorris: $P = 0.01$ for red deer, $P = 0.03$ for wild boar; Montargis: $P = 0.02$ for wild boar), but not on the second axis (Lorris: $P = 0.5$ for red deer, $P = 0.6$ for wild boar; Montargis: $P = 0.5$ for wild boar). The pool dispersed by roe deer did not differ from the regional pool (Lorris: $P = 0.2$ for axis 1 and 2; Montargis: $P = 0.4$
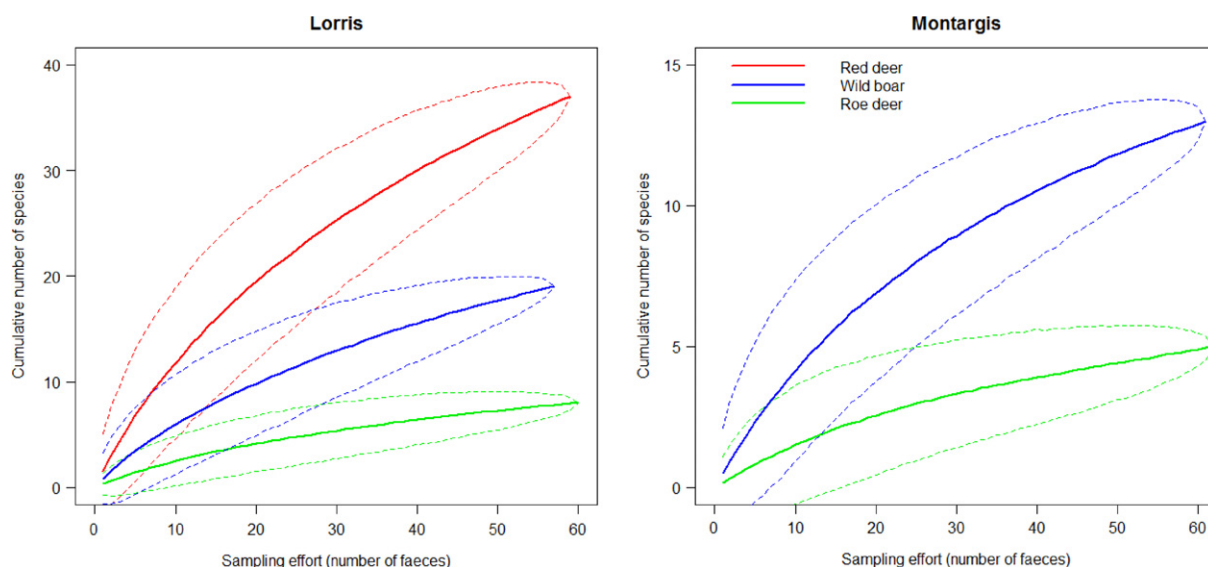

Fig. 2. Cumulative number of plant species dispersed by each animal species, with increasing cumulative faeces weight (red deer: 1302 g, roe deer: 444 g, wild boar: 1374 g) in Lorris (left) and Montargis (right). Solid lines correspond to Chao2 estimations of species richness, and dotted lines are the 95% confidence intervals.
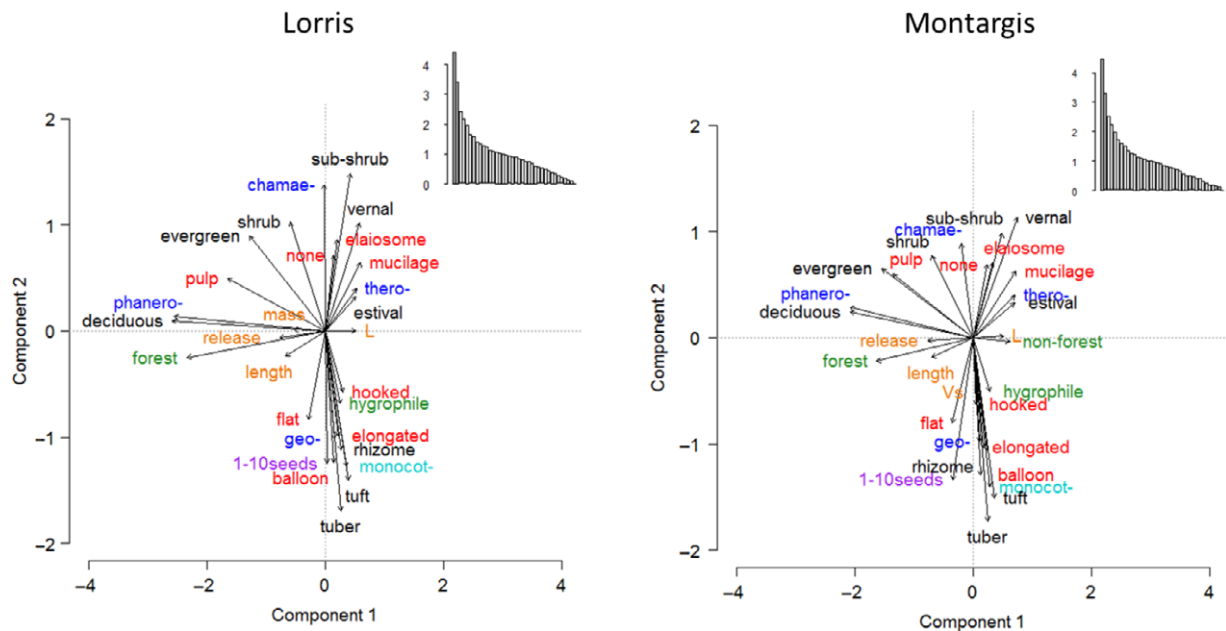
Fig. 3. Correlations between 20 plant attributes (see Table 1 for description) along the two-first components of the Hill and Smith analyses in Lorris (left) and Montargis (right). The insets show the variance explained by each component. For better readability, we only present plant attributes with coordinates >0.5.
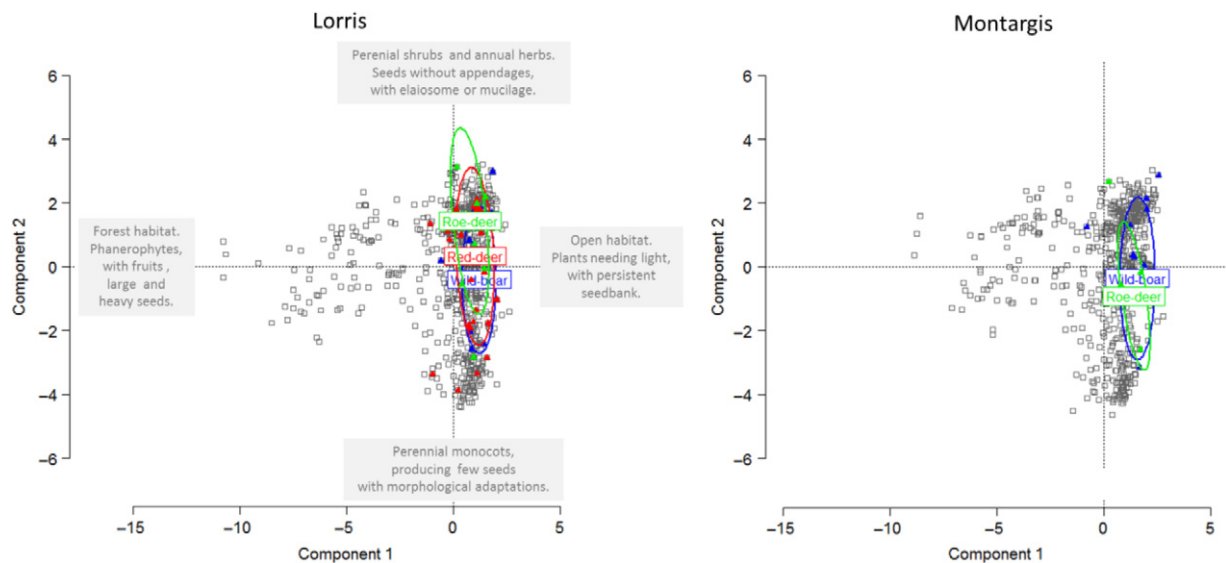

Fig. 4. Distribution of the plant species in the regional vegetation along the two-first components of the Hill and Smith analyses built on 20 plant attributes (see Fig. 3). The centroid of the regional pool is located at the intersection of the two axes. Ellipses are centred on the centroids of the plant species dispersed by each of the animal species: red deer, roe deer and wild boar in Lorris (left), roe deer and wild boar in Montargis (right). The axes of the ellipses represent 1.5× the SD of the species' coordinates on each component. Grey boxes recall the functional complexes of plant attributes, applicable both to Lorris and Montargis.

for axis 1, $P = 0.8$ for axis 2). Indeed, while the regional flora was spread along the two components of the Hill and Smith, the dispersed assemblage was dominated by non-forest species (right of the first axis) and covered a wide range of morphological characteristics along the second component (Fig. 4).

Discussion

Our results show that species richness and abundance of seeds dispersed through endozoochory vary among the three common ungulate vectors studied, and differ in both taxonomic and functional composition from that of the regional flora. Ungulate-mediated dispersal could therefore act as a filter of plant assemblages and thus affect regional and local floristic patterns. In particular, our results suggest that ungulates could favour the intrusion of non-forest plants into forested areas.

Seedling abundance and species richness

Differences among the three ungulates in terms of dispersed seeds and species are consistent with their feeding preferences. As we hypothesized, more seedlings and species germinated from red deer than from roe deer faeces. Our results largely demonstrate that as a large-bodied mixed feeder, red deer consume a wider range of plant species than the smaller-bodied and more selective roe deer (Hofmann 1989). The roe deer is known to feed preferentially on leaves and buds rather than seeds and fruits (Schmidt et al. 2004); this may explain why it dispersed so few species in our study. We found that wild boar dispersed larger quantities of seeds with higher species richness than did roe deer, suggesting that the wild boar diet in our study area involves high seed intake, and probably only small amounts of rhizomes, roots or insects (Heinken et al. 2002).

Taking mean faeces weight and daily defecation rates into account (M. Picard, unpubl data), wild boar dispersed more species per day than did roe deer (three species vs two species), while red deer dispersed the most (eight species·d^{-1}). Our results therefore suggest that wild boar could have a larger role in seed dispersal than was previously thought (Schmidt et al. 2004; Jaroszewicz et al. 2013), and that red deer provides the highest contribution in terms of dispersed seeds and species. Depending on the relative abundance of the three vector species, plants that correspond to the feeding preferences of the most abundant vector should be dispersed in higher numbers.

Surprisingly, we did not find seedlings from plants known to be consumed by forest herbivores (*Molinia caerulea*, *Holcus lanatus*, *Dactylis glomerata*, *Glechoma hederacea* and *Betula pendula*) in our faeces samples, even though these plants were among the most abundant in our regional pool. In addition, apple tree seeds (*Malus sylvestris*) and those from cultivated plants, including maize and wheat (*Zea mays*, *Triticum aestivum*), were observed in wild boar faeces during sieving, but never germinated. These absences, and the lack of germination in 63% of the faeces, could be because the larger seeds were damaged during the sieving process or because our experimental design did not fulfill the germination conditions for some species (breaking of dormancy; Cosyns & Hoffmann 2005). The latter explanation appears especially relevant for fleshy-fruited species. Indeed, in all the faeces samples from the three ungulates, we recorded only two species with fleshy berries (*Rubus fruticosus* and *Solanum nigrum*). Fleshy-fruited shrubs often bear seeds with physiological or morphological dormancy (Heinken et al. 2002); such species may therefore have failed to germinate under our experimental conditions. The small amount of fleshy-fruited plants usually found in herbivore faeces also suggests that these species better survive endozoochorous dispersal by specialist frugivores like birds or mustelids than by large ungulates (Heinken et al. 2002; Couvreur et al. 2005). The costs associated with endozoochory by large ungulates may be too high for plants bearing fleshy fruits, because their typically large seeds are damaged during chewing and their transit through the ungulate digestive tract, partly due to a longer retention time than in birds or mustelids.

Species occurrence frequency in faeces and in the regional flora

The plant species most frequently dispersed by the three animal species were present in the regional assemblage at an occurrence frequency >50% (except for two species dispersed only by roe deer: *Digitaria sanguinalis* and *Kickxia elatine*). Abundant plant species in feeding habitats have a higher probability of being consumed and, through a sampling effect, of being effectively dispersed. Yet, species occurrence frequencies in faeces and in the study areas were not correlated, showing that many regionally abundant species are not dispersed by wild ungulates, which is consistent with feeding preferences. All the plant species dispersed in our study had previously been found to be dispersed by roe deer, red deer, wild boar, fallow deer or white-tailed deer (except *Kickxia elatine*) in a primeval forest (Jaroszewicz et al. 2013), a coniferous forest mosaic (Eycott et al. 2007), a Mediterranean dehesa (Malo & Suárez 1995) and in both European and North American agro-forested landscapes (Heinken et al. 2002; Myers et al. 2004; Schmidt et al. 2004; Von Oheimb et al. 2005). The dispersed plants in our two study areas were similar to the species found in the above-mentioned studies, irrespective of the species pool. This confirms that the composition of assemblages dispersed through endozoochory is driven by vector feeding preferences rather than by plant species abundance, even when the vectors are relatively generalist feeders, as for the three ungulates we studied. Furthermore, a large proportion of the dispersed species, including regionally common ones, emerged from one faeces sample

only, which seems to suggest that vector diet and feeding behaviour – not plant occurrence – drive dispersal probability. To conclude, wild ungulates could have a filtering effect on the composition of dispersed plant assemblages through their feeding preferences, irrespective of the composition of the regional species pool.

Habitat and traits of the dispersed species

The pool of dispersed species was a non-random sample of the regional flora and was dominated by non-forest species. This may reflect wild ungulate preference for feeding in open areas, at least for red deer and wild boar (Hemami et al. 2005). This could also mean that seeds from forest species (often trees and shrubs) are less gut transit-resistant than open field species. Indeed, Heinken et al. (2002) suggested that tree seeds are too large to survive the chewing process and gut transit, and are produced in numbers that are too low for a high ingestion probability. Since roe deer are known to feed often on shrubs, this could explain why we found only a few seedlings germinated from their faeces, and why the functional pool dispersed by roe deer differed from those of the other two species. Furthermore, in our functional trait space, the dispersed assemblages corresponded to small light seeds with a high seed bank longevity index. As suggested by Thompson et al. (1993), survival in the seed bank probably requires similar adaptations to those needed to survive transit through the digestive tract. However, the presence of appendages, elaiosomes or mucilage was similar in the regional and dispersed species pools, suggesting that these morphological structures only slightly affect the probability of being internally dispersed via ungulates. Couvreur et al. (2005) also found that endozoochory was associated with a broad range of plant functional characteristics in donkeys, suggesting that low functional differences from the regional species pools may be a general characteristic of dispersed seed assemblages. Among traits that might distinguish dispersed from non-dispersed plants, plant edibility and attractiveness or nutrient content could have a significant influence (Janzen 1984), but these traits will need to be more exhaustively quantified in trait databases to assess their contribution to the composition of endozoochory-dispersed species assemblages.

Implications for endozoochory

Although it is thought that zoochory is the main mode of dispersal in forested areas (Heinken et al. 2002), forest species are rarely dispersed by ungulates (Schmidt et al. 2004; Von Oheimb et al. 2005), probably because other vectors such as birds or mustelids predominate (Heinken et al.

2002; Couvreur et al. 2005). The scarcity of forest species in ungulate-dispersed seed assemblages could also contribute to the limited colonization capacities of many ancient woodland species (Hermy et al. 1999; Panter & Dolman 2012). As herbaceous non-forest species dominate the dispersed species pool, and most wild ungulates forage in open habitat and sleep, ruminate or shelter in the forest (Kuijper et al. 2009), seeds are more likely to be dispersed from open areas to forested areas, thus entering the forest soil seed bank (Van Calster et al. 2008). The dispersed plants in our study had a high seed bank longevity index and may be able to germinate when suitable conditions arise, even a long time after defecation. They may thus potentially colonize intra-forest gaps, like forest road edges or tree-fall gaps (Naaf & Wulf 2007).

Conclusion

We showed that the composition of assemblages dispersed through endozoochory is driven by vector feeding habitat preferences more than by seed traits. We therefore suspect that the composition of the dispersed species pool is mainly affected by the most abundant vectors. Endozoochorous dispersal may act as an indirect ecological filter, not associated with plant niche as in a classic ecological filter (Keddy 1992), but rather with a plant–animal interaction. Our results stress the need to better estimate the effect of plant–animal relationships on patterns of plant community assemblage in heterogeneous landscapes.

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References

Bekker, R.M., Bakker, J.P., Grandin, U., Kalamees, R., Milberg, P., Poschlod, P., Thompson, K. & Willems, J.H. 1998. Seed size, shape and vertical distribution in the soil: indicators of seed longevity. *Functional Ecology* 12: 834–842.

Cain, M.L., Milligan, B.G. & Strand, A.E. 2000. Long-distance seed dispersal in plant populations. *American Journal of Botany* 87: 1217–1227.

Cappers, R.T.J., Bekker, R.M. & Jans, J.E.A. 2006. *Digital seed atlas of the Netherlands*. Barkhuis, Eelde, NL.

Chao, A. 1987. Estimating the population size for capture-recapture data with unequal catchability. *Biometrics* 43: 783–791.

Clauss, M., Nijboer, J., Loermans, J.H.M., Roth, T., Van der Kuilen, J. & Beynen, A.C. 2008. Comparative digestion studies in wild suids at Rotterdam Zoo. *Zoo Biology* 27: 305–319.

Cosyns, E. & Hoffmann, M. 2005. Horse dung germinable seed content in relation to plant species abundance, diet composition and seed characteristics. *Basic and Applied Ecology* 6: 11–24.

Cosyns, E., Claerbout, S., Lamoot, I. & Hoffmann, M. 2005. Endozoochorous seed dispersal by cattle and horse in a spatially heterogeneous landscape. *Plant Ecology* 178: 149–162.

Cousens, R., Dytham, C. & Law, R. 2008. *Dispersal in plants: a population perspective*. Oxford University Press, Oxford, UK.

Couvreur, M., Cosyns, E., Hermy, M. & Hoffmann, M. 2005. Complementarity of epi- and endozoochory of plant seeds by free ranging donkeys. *Ecography* 28: 37–48.

D'hondt, B. & Hoffmann, M. 2011. A reassessment of the role of simple seed traits in mortality following herbivore ingestion. *Plant Biology* 13(Suppl 1): 118–124.

D'hondt, B., D'hondt, S., Bonte, D., Brys, R. & Hoffmann, M. 2012. A data-driven simulation of endozoochory by ungulates illustrates directed dispersal. *Ecological Modelling* 230: 114–122.

Dray, S. & Dufour, A. 2007. The ade4 package: implementing the duality diagram for ecologists. *Journal of Statistical Software* 22: 1–20.

Eycott, A.E., Watkinson, A.R., Hemami, M.-R. & Dolman, P.M. 2007. The dispersal of vascular plants in a forest mosaic by a guild of mammalian herbivores. *Oecologia* 154: 107–118.

Fitter, A.H. & Peat, H.J. 1994. The ecological flora database. *Journal of Ecology* 82: 415–425.

García, C., Jordano, P. & Godoy, J.A. 2007. Contemporary pollen and seed dispersal in a *Prunus mahaleb* population: patterns in distance and direction. *Molecular Ecology* 16: 1947–1955.

Heinken, T., Hanspach, H., Raudnitschka, D. & Schaumann, F. 2002. Dispersal of vascular plants by four species of wild mammals in a deciduous forest in NE Germany. *Phytocoenologia* 32: 627–643.

Hemami, M.-R., Watkinson, A.R. & Dolman, P.M. 2005. Population densities and habitat associations of introduced muntjac *Muntiacus reevesi* and native roe deer *Capreolus capreolus* in a lowland pine forest. *Forest Ecology and Management* 215: 224–238.

Hermy, M., Honnay, O., Firbank, L., Grashof-Bokdam, C. & Lawesson, J.E. 1999. An ecological comparison between ancient and other forest plant species of Europe, and the implications for forest conservation. *Biological Conservation* 91: 9–22.

Hill, M.O. & Smith, A.J.E. 1976. Principal component analysis of taxonomic data with multi-state discrete characters. *Taxon* 25: 249–255.

Hofmann, R.R. 1989. Evolutionary steps of ecophysiological adaptation and diversification of ruminants: a comparative view of their digestive system. *Oecologia* 78: 443–457.

Howe, H. & Smallwood, J. 1982. Ecology of seed dispersal. *Annual Review of Ecology and Systematics* 13: 201–228.

Janzen, D.H. 1984. Dispersal of small seeds by big herbivores: foliage is the fruit. *The American Naturalist* 123: 338–353.

Jaroszewicz, B., Pirożnikow, E. & Sondej, I. 2013. Endozoochory by the guild of ungulates in Europe's primeval forest. *Forest Ecology and Management* 305: 21–28.

Julve, Ph. 1998. Baseflor. Index botanique, écologique et chronologique de la flore de France. http://perso.wanadoo.fr/philippe.julve/catminat.htm (accessed January 2013)

Keddy, P.A. 1992. Assembly and response rules: two goals for predictive community ecology. *Journal of Vegetation Science* 3: 157–164.

Kleyer, M., Bekker, R.M., Knevel, I.C., Bakker, J.P., Thompson, K., Sonnenschein, M., Poschlod, P., Van Groenendael, J.M., Klimeš, L., (. . .) & Peco, B. 2008. The LEDA Traitbase: a database of life-history traits of the Northwest European flora. *Journal of Ecology* 96: 1266–1274.

Kuijper, D.P.J., Cromsigt, J.P.G.M., Churski, M., Adam, B., Jędrzejewska, B. & Jędrzejewski, W. 2009. Do ungulates preferentially feed in forest gaps in European temperate forest? *Forest Ecology and Management* 258: 1528–1535.

Malo, J.E. & Suárez, F. 1995. Herbivorous mammals as seed dispersers in a Mediterranean dehesa. *Oecologia* 104: 246–255.

Mouissie, A.M., Van Der Veen, C.E.J., Veen, G.F. (ciska) & Van Diggelen, R. 2005a. Ecological correlates of seed survival after ingestion by Fallow Deer. *Functional Ecology* 19: 284–290.

Mouissie, A.M., Vos, P., Verhagen, H.M.C. & Bakker, J.P. 2005b. Endozoochory by free-ranging, large herbivores: ecological correlates and perspectives for restoration. *Basic and Applied Ecology* 6: 547–558.

Myers, J.A., Vellend, M., Gardescu, S. & Marks, P.L. 2004. Seed dispersal by white-tailed deer: implications for long-distance dispersal, invasion, and migration of plants in eastern North America. *Oecologia* 139: 35–44.

Naaf, T. & Wulf, M. 2007. Effects of gap size, light and herbivory on the herb layer vegetation in European beech forest gaps. *Forest Ecology and Management* 244: 141–149.

Pakeman, R.J., Digneffe, G. & Small, J.L. 2002. Ecological correlates of endozoochory by herbivores. *Functional Ecology* 16: 296–304.

Panter, C.J. & Dolman, P.M. 2012. Mammalian herbivores as potential seed dispersal vectors in ancient woodland fragments. *Wildlife Biology* 18: 292–303.

Potts, J.M. & Elith, J. 2006. Comparing species abundance models. *Ecological Modelling* 199: 153–163.

Rico, Y., Holderegger, R., Boehmer, H.J. & Wagner, H.H. 2014. Directed dispersal by rotational shepherding supports landscape genetic connectivity in a calcareous grassland plant. *Molecular Ecology* 23: 832–842.

Schmidt, M., Sommer, K., Kriebitzsch, W.-U., Ellenberg, H. & von Oheimb, G. 2004. Dispersal of vascular plants by game in northern Germany. Part I: Roe deer (*Capreolus capreolus*) and wild boar (*Sus scrofa*). *European Journal of Forest Research* 123: 167–176.

Ter Heerdt, G.N.J., Verweij, G.L., Bekker, R.M. & Bakker, J.P. 1996. An improved method for seed-bank analysis: seedling emergence after removing the soil by sieving. *Functional Ecology* 10: 144–151.

Thompson, K., Band, S.R. & Hodgson, J.G. 1993. Seed size and shape predict persistence in soil. *Functional Ecology* 7: 236.

Thompson, K., Bakker, J.P. & Bekker, R.M. 1997. *The soil seed banks of North West Europe: methodology, density and longevity.* Cambridge University Press, Cambridge, UK.

Van Calster, H., Chevalier, R., van Wyngene, B., Archaux, F., Verheyen, K. & Hermy, M. 2008. Long-term seed bank dynamics in a temperate forest under conversion from coppice-with-standards to high forest management. *Applied Vegetation Science* 11: 251–260.

Vavrek, M.J. 2011. fossil: Palaeoecological and palaeogeographical analysis tools. *Palaeontologia Electronica* 14: 1T.

Vittoz, P. & Engler, R. 2007. Seed dispersal distances: a typology based on dispersal modes and plant traits. *Botanica Helvetica* 117: 109–124.

Von Oheimb, G., Schmidt, M., Kriebitzsch, W.-U. & Ellenberg, H. 2005. Dispersal of vascular plants by game in northern Germany. Part II: red deer (*Cervus elaphus*). *European Journal of Forest Research* 124: 55–65.

Wenny, D.G. 2001. Advantages of seed dispersal: a re-evaluation of directed dispersal. *Evolutionary Ecology Research* 3: 51–74.

Zeileis, A., Kleiber, C. & Jackman, S. 2008. Regression models for count data in R. *Journal of Statistical Software* 27: 1–25.

Supporting Information

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

Appendix S1. List of plant species that germinated from the faeces samples.

Appendix S2. Monthly variability and difference between the two forests for seedling abundance and species richness.

Appendix S3. Coordinates of plant attributes on the first two components of the Hill and Smith analyses.