

# Endozoochorous dispersal by herbivores and omnivores is mediated by germination conditions

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## Research article

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# Abstract

**Background:** Vertebrate-mediated seed dispersal is probably the main long distance dispersal mode. Through endozoochory, large mammals act as mobile links between habitats within and among forest patches. Along with other factors, their feeding regimes do affect their contribution as dispersal vectors. We conducted a cross-species comparative experiment involving two herbivores, red deer and roe deer; and two opportunistic omnivores, wild boar and brown bear, all occurring in the forest and steppe-forest ecotone habitats of the south-eastern Caspian region. We compared their role as endozoochorous seed dispersal agents by monitoring seedling emergence in their dungs under greenhouse and natural conditions.

**Results:** In total, 3078 seedlings, corresponding to 136 plant taxa sprouted from 445 paired dung sub-samples, under greenhouse and natural conditions. Only 336 seedlings, corresponding to 36 plant taxa, emerged under natural conditions, among which five taxa did not appear under greenhouse conditions. Graminoids and forbs composed 91% of the seedlings in the greenhouse whereas shrubs were more abundant under natural conditions, representing 55% of the emerged seedlings. Under greenhouse conditions, first red deer and then wild boar dispersed more species than the other two mammals, while under natural conditions brown bear was the most effective vector. We observed remarkably higher species richness and seedling abundance per dung sub-sample under buffered greenhouse conditions than we did under natural conditions.

**Conclusions:** The four sympatric mammals studied provided different seed dispersal services, both in terms of seedling abundance and species richness and may therefore be regarded as complementary. Our results highlight a positive bias when only considering germination under buffered greenhouse conditions. This must be taken into account when planning management options to benefit plant biodiversity based on the dispersal services concluded from greenhouse experiments.

## Background

Seed dispersal is an important process connecting the end of the adult plant's reproductive cycle to the **establishment** of its **offspring** (1). It is divided into three main phases: emigration, when the seed leaves the adult plant; transfer, or the distance covered between the adult plant and the seed landing site; and finally, immigration, when the released seed germinates and the new individual starts to grow. Seed dispersal also determines plant community dynamics and influences potential recruitment rates, recolonization, gene flow and consequently, genetic diversity (2). Seed dispersal can also enable plant migration in response to environmental changes (3), accompany *plant community responses* to habitat fragmentation (4, 5), contribute to the soil seed bank (6), but also spread invasive exotic species (7).

Large herbivores are one of the most important drivers of vegetation dynamics in grazed ecosystems (8). Through endozoochory, they act as long-distance plant dispersal agents, between habitats within and among forest patches (9, 10). In comparison with smaller herbivores, large herbivores consume more

seeds, cause less damage to the seed during the chewing and ruminating processes (11) and disperse seeds over longer distances within their larger home ranges (12). Herbivore traits such as body size, feeding regime and digestive physiology (i.e. ruminant or not) and spatio-temporal habitat preferences may affect the efficiency of endozoochorous dispersal (7, 13-15). However, few studies have simultaneously assessed the role of endozoochorous dispersal by sympatric herbivores and omnivores in local plant communities (7, 15, 16).

In addition to animal functional traits, plant phenology also matters. Seasonal variations in seed availability affect the number of species and seeds dispersed by the vectors (17). Seed availability for herbaceous species peaks during spring and summer, while seeds from fleshy-fruited shrub species are more common in summer and early autumn.

Each step in the seed dispersal cycle is crucial (1) and this is also true for the establishment of seedlings emerging from faeces. Though several methods have been used to assess the composition, density and viability of seeds in dung content, most studies have investigated germination success under controlled greenhouse conditions or in standardised laboratory environments with a regular water supply and a relatively constant temperature (18, 19). Such approaches actually indicate potential germination success rather than effective seed dispersal (20-22). We therefore launched our study to test the effect of specific germination conditions on the outcome of endozoochorous plant dispersal.

We used a cross-species comparative experiment involving the four most common wild mammals occupying the forest and prairie-forest ecotone of the south-eastern Caspian region. There are two herbivores: an intermediate mixed feeder, the red deer (*Cervus elaphus maral*), and a browser, the roe deer (*Capreolus capreolus*) (23); and two rather opportunistic omnivores (i.e. which make use of all available resources, including e.g. fruits, insects and earthworms) (24, 25): the wild boar (*Sus scrofa*) and the brown bear (*Ursus arctos*).

We compared the plants germinating from their faeces by habitat and by season to investigate the different vectors' contribution to the pool of plants dispersed. We shaped the following hypotheses:

1. According to body size, feeding regime and digestive strategy (ruminant or not), each dispersal agent would disperse different sets of plants. For instance, red deer should disperse more plants than the more selective roe deer, and omnivores like brown bear should disperse more fleshy-fruited plants than do herbivores.
2. The diversity of the plant species dispersed by the studied dispersal agents would vary spatially and temporally across the study area according to their habitat preferences and various periods of seed release. Seed dispersal by omnivores should be at its highest in late summer/early autumn when fleshy fruits are abundant, whereas herbivores should disperse more seeds (compared to omnivores) in spring/early summer, when herbaceous plants predominate.
3. Since the plants dispersed should differ with dispersal vectors and since each plant requires specific germination conditions, we also expected differences between greenhouse and natural conditions to

vary for each vector studied. According to previous studies, we also expected lower germination under natural conditions than under buffered greenhouse conditions.

## Results

### Seedling emergence under greenhouse conditions

After the exclusion of two unidentified individuals, seedlings representing 129 plant species and 29 families germinated from the 445 dung sub-samples collected. More than 88% of the sub-samples contained germinated seeds (97% for red deer, 91% for brown bear, 89% for wild boar and 88% for roe deer). Most plant species belonged to the *Poaceae* (n = 29), *Brassicaceae* (n = 12) or *Asteraceae* (n = 11) family. Twenty percent of the families were represented by a single species. *Urtica dioica* (623 seedlings) and *Cyperus fuscus* (383 seedlings) were the most abundant seedlings. *Portulaca oleracea* and *C. fuscus* were the most frequent species, respectively occurring in 24% and 23.5% of the dung sub-samples. Thirty-seven percent of the species occurred in only one dung sample, 65% of the species emerged less than five times, and 27% of the germinating species produced a single seedling. The dispersed species included 81 herbaceous species, 33 graminoids, 13 woody species (shrubs, subshrubs or trees) and one non-spiny cushion form plant. Forty-seven percent of the species were annuals.

A greater number of seedlings generally meant a greater number of species (Spearman's  $r_s = 0.76$ ;  $P < 0.0001$ ), though some wild boar samples were dominated by a single species (e.g. *U. dioica*). Certain plant species were dispersed by a single animal vector: 40 by red deer only, 29 by wild boar only, ten by brown bear only and six by roe deer only (Additional file 1, Fig. 1a). Red deer dispersed the greatest number of plant species (Fig 1a; Fig 2; Table 4).

### Seedling emergence under natural conditions

By comparison, under natural conditions, fewer sub-samples provided seedlings (55% for brown bear, 30% for wild boar, 26% for red deer, and 20% for roe deer). No seeds germinated from 67% of the dung sub-samples. After the exclusion of five contaminant species, 334 seedlings of 36 plant species from 16 families germinated from the 445 paired dung sub-samples. Most species belonged to the *Poaceae* (n = 8), *Rosaceae* (n = 5) or *Polygonaceae* (n = 5; Table 4) family. Fleshy-fruited shrubs and trees (*Berberis* sp. with 67 seedlings, 4%; and *Cerasus* sp. with 46 seedlings, 3%) were the most frequently dispersed plant species (Additional file 1).

Germinating plants include 20 herbaceous species, nine shrubs and seven graminoids; 37% of the species were annuals. Some plants were dispersed by a single vector (6 for red deer only; 5 for wild boar only; 4 for brown bear only and 1 for roe deer only) (Table 4; Fig. 1b).

**Fig. 1** Number of species dispersed by one to four dispersal vectors in the study area (both habitats combined), a: under greenhouse conditions; and b: under natural conditions.

**Fig. 2** Species accumulation curves for the plants dispersed by the different animal vectors (total dung mass for red deer: 3640 g; wild boar: 2980 g; brown bear: 1280 g; roe deer: 285.5 g) (a) for herbivores - top panel and (b) omnivores - bottom panel; under greenhouse (G) and natural (N) conditions, based on the Chao 2 estimator with 95% confidence intervals.

### **Seedling abundance and species richness**

Based on the GLMM results for species richness, the best model included the intercept and Animal species (Additional file 2). For seedling abundance, the best model included the intercept, Animal species, Season and the interaction Animal × Season (Additional file 3).

Variability of seedling abundance per gram of faeces was low for all vectors, whether herbivores or omnivores, and whatever the season.

Seedling abundance per gram of faeces was higher for roe deer than for omnivores in the spring. It was lower for red deer in the summer compared to wild boar and roe deer. Finally, we detected no differences in the fall (Fig 3a, Table 1).

Species richness per faeces was higher for red deer than for roe deer; furthermore, it was similar for red deer, wild boar and brown bear (Fig 3b, Table 2).

Habitat type (forest versus prairie-forest ecotone) had no effect on seedling abundance per gram of faeces or species richness per faeces, whatever the dispersal vector (Additional file 2, Additional file 3).

**Fig. 3** Plots of least square mean (with 95% confidence intervals) for (A) seedling abundance per gram of faeces vs. animal×season interaction, and (B) species richness per faeces vs. animal vector. Means sharing a letter are not significantly different (Turkey's post hoc tests). The results were back-transformed to the original scale by taking antilogarithms of the least square means (LSM) and the 95% confidence intervals (CI).

Table 1 Differences between dispersal vectors in terms of seedling abundance per gr of faeces in each season. Significant differences are in bold.

Table 2 Differences between dispersal vectors in terms of species richness per faeces. Significant differences are in bold.

### **Natural versus greenhouse conditions**

Of the 136 plant taxa which emerged from the 445 paired dung sub-samples under both greenhouse and natural conditions, 131 taxa appeared under greenhouse conditions versus only 36 taxa under natural conditions, five of which only germinated under natural conditions.

Based on the GLMM results for seedling abundance and species richness, the best model included the intercept, Animal species, Germination conditions and the interaction Animal species × Germination

conditions. Seedling abundance and species richness per faeces sample were significantly higher under greenhouse than natural conditions ( $P \leq 0.001$ ).

We defined three groups of plants. The first group comprises species that germinated solely under greenhouse conditions. This group includes 62 herbs, 26 graminoids, six shrubs, one sub-shrub and one cushion plant (Additional file 1). The second group includes the five taxa (*Aegilops cylindrical*, *Cornus sanguinea*, *Calamintha nepeta*, *Rosa canina* and *Silene* sp.) that germinated only under natural conditions, though in low numbers. Finally, the third group includes the 31 plants that germinated under both natural and greenhouse conditions, though species generally ( $n=21$ ) emerged in lower abundance under natural conditions. However, the following eight taxa (*Cerasus* sp., *Polygonum minus*, *Parietaria officinalis*, *Poa masenderana*, *Poa nemoralis*, *Poa pratensis*, *Rumex* sp. and *Sorbus torminalis*) germinated more successfully under natural conditions.

Seedling abundance of the third plant group was significantly higher under greenhouse conditions for roe deer ( $U = 7$ ,  $P = 0.046$ ) and wild boar ( $U = 34$ ,  $P = 0.005$ ), but not for red deer ( $U = 103.5$ ,  $P = 0.184$ ) or brown bear ( $U = 45.5$ ,  $P = 0.382$ ).

Dispersed plant species composition differed when animal and germination conditions were taken into account in the Canonical Correspondence Analysis (CCA) ( $F_{540, 7} = 2.534$ ,  $P = 0.001$ ; Fig. 4). Pairwise comparisons indicated strong differences in the composition of the dispersed species for each combination of animal and germination condition, except between red deer and roe deer (Table 3). Comparisons among animals also showed that red deer, roe deer and wild boar had greater differences with brown bear in terms of dispersed plant composition than they did with one another (Table 3). Comparisons among animals showed richer composition under greenhouse than under natural conditions for roe deer ( $R = 0.23$ ;  $P = 0.001$ ), red deer ( $R = 0.14$ ;  $P = 0.001$ ) and wild boar ( $R = 0.15$ ;  $P = 0.001$ ), but not for brown bear ( $R = 0.02$ ;  $P = 0.091$ ).

**Fig. 4** Biplots showing the results of the canonical correspondence ordination analysis. The first plot (a) shows the position of each combined factor (animal plus germination condition, e.g. WBG = Wild Boar under Greenhouse conditions) on the first two CCA axes of the dispersed plant species. The second plot (b) shows the position of the same factors on the same axes of the dispersed species' growth form (Graminoid, Herb, Shrub and Tree). Animal vectors: RO = Roe deer, RE = Red deer, BB = Brown bear, WB = Wild boar; and germination condition: G = Greenhouse conditions, N = Natural conditions. The plant species scientific names are written in shorthand form: the first two letters of the genus and the first two letters of the species (Additional file 4).

Table 3 ANOSIM results (r statistic) of pairwise tests for differences between animals using abundance data under greenhouse conditions (a) and natural conditions (b).

## Discussion

The four animal vectors studied dispersed a large set of the plants available in the Golestan National Park (GNP) through endozoochory. Several of the plant species that germinated frequently in our dung sub-samples or produced abundant seedlings (*U. dioica*, *P. oleracea*, *C. fuscus*, *C. album*, *P. lapathifolium*, *B. virgatum*, *Berberis* sp. and *S. media*) had been highlighted in previous studies (13, 15, 26, 27). However, most of the species germinating in our study emerged only once as a single seedling. This can be explained by accidental seed intake (7), forage contamination by surrounding seeds (28), low local abundance of the plant during the sampling time (29) or rare feeding bouts.

Under greenhouse conditions, we found a higher proportion of dung sub-samples with emerging seedlings than previously reported for all our animal vectors (5, 15, 30). Nearly all the red deer dung sub-samples contained viable seeds.

### **Seedlings abundance and species richness**

The differences among vectors in the seed content dispersed can be attributed to the animals' dietary preferences (mixed feeders vs. *browsers*; (15, 31) and herbivores versus omnivores), digestive physiology (ruminant or not; (32)), body size, habitat preferences and ranging behaviour. These factors may also cause animal vectors to deposit the seeds in different habitats (7, 17).

Consistent with previous studies (7, 30), we found that *grazers* (i.e. red deer) dispersed the highest number of species, followed by *browsers* and then *omnivores*. We observed significant differences between the two deer species. The red deer has a wider diet than the roe deer and occupies a much larger home range (33); these characteristics increase the animal's encounter rate with numerous and diverse plant species and increases the likelihood of their consumption (13). Apart from this difference between herbivores, however - and contrary to our expectations - the number of species dispersed did not differ among the other vectors, seasons, or between habitats. This can be attributed to the wide diversity of habitats and associated plants encountered in the GNP (34), making complementary food sources available in different seasons and across habitats. The similarity between omnivorous and herbivorous vectors in terms of number of species dispersed may also be due to an exceptionally high number of plants dispersed by the two omnivores in our study area, suggesting that they may have shifted their diet towards *plant* resources from animal sources (15, 31, 35). Anthropogenic factors may also explain the absence of difference in species richness among our animal vectors. In the GNP, there has been a dramatic decline in large herbivores (16) mainly due to livestock grazing, habitat loss and intensive poaching (36). The small remaining deer populations are now limited to the less disturbed sites, where, consequently, herbivory pressure has been increasing. In contrast, wild boar numbers have been increasing at the same time across a wide variety of habitats due to religious restrictions on eating wild boar meat (37), thus increasing the animal's encounter rate with a wider range of plants. This corroborates a previous study (31) reporting more species dispersed by wild boar than by roe deer.

Even though the GNP hosts very rich flora, some studies have reported higher numbers of plant species dispersed by vectors similar to brown bear (35); by red and roe deer (7, 13), and by wild boar (15). The preparation of the sub-samples in these studies could explain these results, as some plants have specific

germination requirements other than cold stratification (e.g. warm stratification and exposure to smoke). A long germination period may have also increased the number of germinated seedlings and species (7) *under both germination conditions*. Finally, the difference in altitude (1616 m for the greenhouse and 450 m for the natural conditions) probably affected the germination pattern.

As hypothesised, we showed that the abundance of emerging seedlings was positively correlated with season, peaking in summer or autumn. In general, seed density in dung depends on the feeding regime of the vectors and follows the seasonal pattern of plant seed-shedding (17).

Again, and as predicted, the herbivores in our study were more effective dispersal vectors than the omnivores in spring, when herbaceous plants are more frequent and abundant. Shrubs were mostly present in the omnivorous dung samples, collected in summer and autumn, when palatable fleshy fruits are available (35, 38).

In agreement with previous studies, roe deer dispersed more seeds per gram of faeces than wild boar (7, 27). There is no comparable study dealing with roe deer and brown bear. In summer, even red deer dispersed fewer viable seeds per gram of dung than did roe deer; this could be due to the seasonal summer reduction in herbaceous plant cover, which constitutes the main food resource for red deer.

As a consequence of their feeding regime and body size (39), we also expected red deer to disperse more seeds than wild boar. However, the opportunistic feeding regime of wild boar considerably increased the number of seeds they dispersed (more than half of which emerged in summer). Indeed, in summer, wild boar dispersed more seeds than did red deer. Generally, the phenology of seedling emergence varied among animal species. Roe deer dung showed maximum seed density in spring, coinciding with the dispersal of vernal herbs such as *Sonchus oleraceus* (75% of the seedlings emerged from roe deer dung in spring). The number of seedlings in red deer samples peaked in autumn, with the dispersal of *Polygonum lapathifolium* (accounting for one third of the seedlings that emerged from red deer dung in autumn). Finally, the highest seed density in brown bear dung samples was in summer, which coincided with the peak seeding period for fleshy-fruited shrubs and trees.

## Species composition

Both the functional traits of the animal vectors and plant phenology were reflected in the changes occurring in animal diet and habitat use across seasons, which in turn affected the composition of the plants dispersed. The largest dissimilarities among the sets of dispersed plant species were between brown bear and the three other vectors.

Most of the plant species dispersed in this study produced small seeds with no particular morphological structure or specific adaptations for endozoochory, with the exception of the seeds dispersed by the brown bear. This supports the “foliage is the fruit” hypothesis (28), which states that the edible vegetative parts of the plants act as the ecological ‘fruit’, or attractant reward. Brown bears preferentially selected fleshy fruits from shrubs and trees (*Berberis* sp., *Crataegus* sp. and *Cerasus* sp.), even though seeds from

dry fruits were also dispersed by brown bear in lower densities than for fleshy fruits, and especially during spring. Indeed, though fleshy fruits from shrubs are an important source of energy during fall hyperphagia (40); in spring, when those fruits are scarce, bears mainly rely on herbaceous plants and other vegetative parts (as well as animal carcasses) (35, 38).

Our results also reveal the potential of large native herbivores and omnivores, especially wild boar, to disperse ruderal, early-successional species, though only two of them (*Portulaca oleracea* and *Cyperus fuscus*) were dispersed in large quantities. A larger proportion of these species germinated from the wild boar dung collected during the dry season, when the animal spends more time feeding along roadsides. Wild boar can disperse some cultivated plants, like *C. vulgaris* and *S. lycopersicum*, into natural areas when they take advantage of the food left by humans along roads or when they roam in agricultural areas (41). Therefore overabundant wild boar populations may facilitate the spread of ruderal, early-successional or exotic plant species from such habitats, and consequently lead to mixed effects on species diversity (42). On the other hand, decreasing deer populations translate into both reduced browsing pressure and reduced dispersal service with unpredictable outcomes for plant community composition. Therefore, conservation efforts should focus on the protection of animal populations which provide seed dispersal services to desired local plants (43).

### **Natural versus greenhouse germination conditions**

As predicted, more plant species germinated under greenhouse than natural conditions, though the difference was low for brown bear. The greatest difference was observed for wild boar dung sub-samples, and may be linked to the large number of *U. dioica* seedlings, not observed under natural conditions.

Different reasons can account for the lower seed germination rates found under natural conditions. First, artificial cold stratification was not applied to the dung sub-samples placed under natural conditions where they depended on natural cold stratification. Most species must undergo a cold period to break seed dormancy and facilitate germination (44). The climatic conditions during our experiment probably did not provide the necessary conditions to break dormancy. Germination requirements are species-dependent. For example, *Cornus sanguinea* seeds did not germinate in the greenhouse; they require a warm phase to degrade their stony endocarp (45) followed by chilling at 3°C for 8-12 weeks to break seed dormancy (46). In other cases, *R. canina* for instance, 11 weeks of warm stratification followed by cold stratification appears to be the most appropriate treatment (47). The regular water supply and relatively constant temperature under greenhouse conditions are likely to positively affect germination results if most of the plants require such conditions. *U. dioica*, for instance, has been frequently observed in dung (27, 48); however, successful emergence under natural conditions did not occur in our study, probably because this plant prefers moist or damp soil (49). Natural climatic fluctuations should facilitate germination for seeds with particular requirements (22); we can therefore suppose that most of the plants dispersed in our study had quite similar needs. Finally, we did not check the seed content of the two sub-samples before submitting them to contrasting conditions (22). This means that rare species, present as a single seed in the original dung sample would have germinated either in the greenhouse or under

natural conditions, or not germinated at all, and this would lead to artificial heterogeneity between the sub-samples (22).

## Conclusions

Our results reveal that endozoochorous dispersal assessed under greenhouse conditions likely overestimates establishment rate under natural conditions. Factors affecting germination success under natural conditions include the environmental filter (local abiotic conditions, dung decay speed) and biotic interactions among plants (competition, facilitation) or with other organisms (seed predation, herbivory, secondary seed dispersal) (50). Nevertheless, the seeds that did not emerge from the dung under natural conditions over the course of this study may remain in the seed bank until abiotic edaphic conditions become suitable for germination. Extending studies similar to ours over the long term to cover the different climatic conditions in different years may provide more comprehensive results. In addition, to understand the ecological relevance of endozoochory, we need to mimic dung deposition in realistic conditions (i.e. compare germination in dung samples placed in different micro-habitats).

As revealed in our study, large mammals are important vectors for seed dispersal, and their loss or population reduction in natural ecosystems may have cascading effects on other taxa. We show that the four sympatric vectors we studied provide different, complementary seed dispersal services in terms of seasonality, seedlings and species number. It is also likely that dispersal distances covered by each vector also differ (14) in relation to both home range size and path tortuosity.

## Methods

### Study area

Golestan National Park (GNP) is situated in the north-eastern part of Iran (37°16'43"N 55°43'25"E-37°31'35"N 56°17'48"E) and is among the oldest and most diverse protected areas in the Middle East. It covers around 920 km<sup>2</sup> of eastern Iranian Caspian forests with altitudes ranging from 450 to 2411 m above sea level (Fig. 5).

The average annual temperature varies from +11.8 °C to +18.8 °C. The climate is seasonal, marked by cold winters (January, mean temperature -0.8°C) and warm summers (July, mean temperature 23.3°C). Summers with high temperatures in the dry regions can cause extremely hot, dry conditions in the east, south and northeast and a humid climate in the western part of the region (34). Yearly precipitation ranges from 150 mm in the south-eastern part of the park to more than 1000 mm in the more central areas. The area receives 32.3%, 25.6%, 11.8%, and 30.3% of its annual rainfall during winter, spring, summer and fall, respectively.

**Fig. 5** Location of Golestan National Park, highlighting the Hyrcanian forests (in green) in the western half and the surrounding steppes towards the east, north and south. Transitional vegetation zones occur in between and at high altitudes.

The GNP lies across the Euro-Siberian and Irano-Turanian phytogeographical regions (Hyrcanian and Khorassan–Kopet–Dagh provinces, respectively). The park contains a wide range of flora and fauna, which are unique in many aspects. It encompasses diverse vegetation entities including Hyrcanian mesophytic forests, shrublands, scrublands (occasionally mixed with C4-composed grasslands), *Juniperus* sp., woodlands, mountain steppes and meadows, *Artemisia* sp. steppes, and communities composed of halophilous plants (34). We divided these vegetation entities into two major habitat types where the target animal vectors are known to be present: Hyrcanian closed forests (hereafter, forests) and transitional scrub and Juniper woodlands (hereafter, prairie-forest ecotone). We therefore located our study plots within these two major habitat types, replicated twice; resulting in four sampling sites.

At the time of our study, there were about 257 (95% CI: 91-423) red deer (51), 150 roe deer, 6,000 (95% CI: 3,050-9,906) wild boars (52), and 60 brown bears in the park (Golestan Provincial Department of Environment, 2016).

In our study area, the roe deer typically prefer a closed-forest habitat, which overlaps only slightly with the habitats favoured by the two omnivorous species. Red deer partly share the closed-forest and the prairie-forest ecotones with the other three species. The wild boar inhabit a wide range of habitats and brown bears usually prefer mountainous forested sites with high densities of fleshy-fruited shrubs and trees.

Home range (HR) sizes have not been evaluated in GNP for the four target animal species, however, other studies can provide information on the gradient of HR size among species (e.g. 17 ha, 81 ha, 283 ha and 5000 ha, respectively for roe deer, red deer, wild boar and brown bear (14, 53)).

### **Dung collection and treatment**

Dung samples were collected monthly from mid-May to November 2016 (spanning the seeding period) along random transects in the two habitat types. We could not find any faecal samples for brown bear or roe deer during certain months; therefore, samples were allocated to the following three seasons (spring, summer and autumn) to obtain at least two samples for each season-animal pair. We restricted dung collection to intact, fresh wet samples to limit post-dispersal modifications (7). We prevented contamination from seeds sticking to the surface of the samples by removing the lowermost layer of the collected dung (15). A small number of wild boar dung samples had been hollowed out by coprophagous beetles (5%) and were therefore discarded. The collected samples were air dried in paper bags for 10 days and weighed to the nearest 0.01 g. For red deer, wild boar and brown bear, we extracted two 20-g paired sub-samples from each faecal sample to investigate seedling emergence and plant establishment under greenhouse versus natural conditions. Because samples were lighter for roe deer (average weight of  $5.67 \pm 2.21$  g; Table 4) than for the other three species, each individual roe deer dung sample was divided into two equally-sized sub-samples.

Table 4 Summary of the dispersed species assemblages by animal vector. G = greenhouse conditions; N = natural conditions. Sample size was similar for greenhouse and natural conditions.

## **Germination experiments**

Both the greenhouse and the natural experiments had a randomised block design with seven blocks (corresponding to sampling month) and four treatments (corresponding to animal vector). Over a 15-month period, we recorded the germinated seedling species and then removed them. To obtain seedling species richness and abundance for each sampling season (spring, summer, autumn), we pooled the monthly data from May-June, July-August and October-November for each site and each animal vector.

### ***Greenhouse germination conditions***

The samples were stored at 3-5°C until field collections were completed (15), then each sample was carefully crushed to break apart the pellets. Each crushed sample was mixed with a similar volume of soil and sand and poured into pots (diameter 20 cm, depth 25 cm), making a layer approximately 1-2-cm thick. We then filled the pots with a 1:2:1 mixture (sand: soil: peat moss), which had previously been sterilised in an autoclave at 120 °C for 45 minutes (54).

The samples were then allowed to grow under natural daylight with daytime temperatures of around 25 °C in a greenhouse located at the Isfahan University of Technology (1616 m above sea level). The *average minimum temperature* was 18 °C. The samples were monitored every two days to maintain humidity. To prevent competition, we identified, counted, and removed the emerging seedlings as soon as possible. When no new seedlings emerged, the soil in each pot was thoroughly mixed and the experiment was continued for two more months to enable more deeply buried seeds to germinate (55). To control for possible seed bank or seed rain contamination in the greenhouse, 30 control pots (without faecal samples) containing a similar substrate were placed among the pots with dung samples and were maintained under the same conditions.

Seedlings were identified at species level whenever possible. Overall, 5.3% of the species could only be identified to the family level (seven *Poaceae* taxa) and 10% only to the genus level (13 taxa). Two seedlings died before they had grown sufficiently to enable identification. We did not observe any contaminating seedlings in the control pots.

### ***Natural germination conditions***

To examine germination success under natural conditions, a 10×20 m enclosure was established (located in the Tangrah region: 37°23'53.7"N latitude, 55°47'54.4"E longitude, 450 m above sea level) and the experiment was carried out within the fenced area to prevent disturbance from grazing animals. To prevent any seeds in the soil seed bank from contaminating the experimental soil, we inverted the soil by bringing a layer of soil from a depth of more than 35 cm up to the surface of the experimental site (21). Planting pots were filled with this deep soil and placed on the surface. The faecal samples were carefully crushed to break apart the pellets and were placed directly into each planting pot. To allow natural soil

moisture into the planting pots and to improve rainwater drainage, the bottoms of the pots were removed. The faecal samples were not subjected to artificial cold treatment but were exposed to natural temperatures. Average annual rainfall was about 580 mm during the germination period. In order to control for air-borne seed input and soil seed bank content, seven control pots with soil only and no dung were positioned among the pots with dung samples, organized by month. Temperature and light were not controlled and no irrigation was applied during the experiment. The samples were completely exposed to natural climatic conditions. Emerged seedlings were identified to the species level whenever possible (11% could only be identified to the genus level). In the control pots, five species (*Hesperis hyrcana*, *Lamium album*, *Torilis japonica*, *Nonea lutea*, and *Veronica persica*) were recorded. These five species occurred more often in the control pots than in the non-control pots and were therefore eliminated from further analyses.

## **Data analysis**

We built species accumulation curves with a Chao 2 estimator to assess how well we sampled the expected species richness (56). These species accumulation curves helped us compare the different animal vectors under both greenhouse and natural conditions.

### ***Greenhouse data analysis***

We used generalized linear mixed models (GLMM) to compare seedling abundance and the number of species among dispersal vectors, season and habitat type (dung sample as the statistical unit). Negative binomial and Poisson regression models were respectively assigned for seedling abundance and species richness (count response variables) including additional over-dispersion in the model. Animal species (4 species), sampling season (spring, summer and autumn) and habitat (forest and prairie-forest ecotones) were fixed factors, and site within habitat was a random effect. The log-transformed weight of each dung sample was taken as an offset to account for differing sample weights.

We used the *lsmeans* package to obtain the predicted values for each combination of factors. We then performed a Tukey post-hoc test for pairwise comparisons.

First, we fitted the full model to include all the main factors and Animal × Site and Animal × Season interactions (Additional file 5 & 6). The final model was obtained by backward stepwise selection. Best model selection was based on the lowest Akaike Information Criterion value (AIC).

### ***Data analysis for natural versus greenhouse conditions***

We used Poisson regression models to compare seedling abundance and species richness among animal species and between germination conditions (greenhouse vs. natural). First, we fitted the full model to

include all the main factors and Animal species × Germination conditions interaction, with dung sample as the statistical unit. Best model selection was based on the lowest Akaike Information Criterion value (AIC). The lsmeans package and Tukey post-hoc test were used to obtain the predicted values for each combination of factors and for pairwise comparisons, respectively.

Pairwise comparisons between greenhouse and natural conditions for seedling abundance of common plant species were made with the nonparametric Mann-Whitney U test.

We used canonical correspondence analysis (CCA) to compare the composition of germinating plants (square root of seedling abundance for each plant species) among the animal vectors and between germination conditions. Due to the high number of plant species, plotting priority was given to most abundant plant species in the dung samples, following Hill's N2 diversity index. We used Monte-Carlo permutation tests ( $n = 999$  permutations) to test the significance ( $P < 0.05$ ) of the variables and the axes of the CCA. We compared differing plant species composition among animal vectors, and between germination conditions by an analysis of similarities (ANOSIM), with a Bray-Curtis similarity index ranging from zero (complete species overlap) to one (no species in common). This index excludes double-zero comparisons and does not weight rare or abundant species (57).

We performed all statistical analyses with the R 3.6.2. software (R Foundation for Statistical Computing, Vienna, AT) in the vegan (58), vennDiagram (59), lme4 (60), lsmeans (61) and MuMIn (62) libraries.

## Additional Files

**Additional file 1.** Alphabetical list of the plant species germinated from the faecal samples of the four different animal vectors. Fruit type (FT): Fleshy (+) or dry fruit (-), Life cycle (LF): A=Annual, B=Biennial, P=Perennial. Group: D=Dicotyledon, M=Monocotyledon. Degree of rarity in GNP (38): END=endangered, VUL=vulnerable, RAR=rare, NOT=non-threatened, UND=undetermined, UNK=unknown status. G=greenhouse conditions, N=natural conditions.

**Additional file 2.** Results from the best model selected by the Akaike Information Criterion for species richness per faeces

**Additional file 3.** Results from the best model selected by the Akaike Information Criterion for seedling abundance per gram of faeces

**Additional file 4.** The full scientific names of the plant species that are mentioned in the CCA plot

**Additional file 5.** Estimated means  $\pm$  SE obtained from generalized linear mixed effects models with a Poisson distribution with species richness per faeces as the response variable; animal, season and site, animal-site and animal-season interactions as fixed effects, and sample plot repetitions within each site as a random effect. One level of each factor (i.e. brown bear, ecotone, fall) is constrained in the "intercept" of the model.

**Additional file 6.** Estimated means  $\pm$  SE obtained from generalized linear mixed effects models with a negative binomial distribution with seedling abundance per gram of faeces as the response variable; animal, season and site, animal-season and animal-site interactions as fixed effects; and sample plot repetitions within each site as a random effect. One level of each factor (i.e. brown bear, ecotone, fall) is constrained in the "intercept" of the model.

## Declarations

### Abbreviations

GNP: Golestan National Park; GLMM: Generalised linear mixed model; CCA: Canonical correspondence analysis; Chao2: Chao's estimator of species richness.

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### Authors' contributions

SK: Investigation, Methodology, Analysis, Writing- Original draft preparation

MRH: Conceptualisation, Methodology, Writing- Reviewing and Editing, Supervision

MTE: Data Curation, Methodology, Supervision

CB: Resources, Methodology, Writing-Reviewing and Editing

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### Availability of data and materials

All data generated or analysed during this study accompanying with R scripts have been submitted to <https://github.com/KARIMI66/Endozochorous-seed-dispersal-data?files=1>.

### Ethics approval and consent to participate

Not applicable. There was no requirement to seek ethical approval to carry out the work described above.

### Consent for publication

Not applicable.

## Competing interests

The authors declare that they have no competing interests.

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## Tables

Table 1 Differences between dispersal vectors in terms of seedling abundance per gr of faeces in each season. Significant differences are in bold.

	Contrast	Ratio	SE	Z.ratio	P.value
Spring	Brown bear-Wild boar	0.542	0.257	-1.290	0.980
Summer		0.742	0.263	-0.840	0.999
Fall		1.006	0.247	0.028	1
Spring	Brown bear- Roe deer	0.118	0.066	-3.799	<b>0.008</b>
Summer		0.695	0.286	-0.833	0.999
Fall		0.712	0.246	-0.979	0.998
Spring	Brown bear- Red deer	0.343	0.153	-2.390	0.412
Summer		2.046	0.723	2.025	0.676
Fall		0.868	0.211	-0.582	1
Spring	Red deer- Roe deer	0.346	0.147	-2.495	0.340
Summer		0.340	0.102	-3.586	<b>0.017</b>
Fall		0.821	0.248	-0.651	1
Spring	Red deer-Wild boar	0.632	0.175	-1.649	0.890
Summer		2.757	0.491	5.694	<b>&lt;0.0001</b>
Fall		0.862	0.173	-0.739	0.999
Spring	Wild boar- Roe deer	0.219	0.099	-3.343	<b>0.039</b>
Summer		0.936	0.283	-0.217	1.000
Fall		0.707	0.221	-1.107	0.994

Table 2 Differences between dispersal vectors in terms of species richness per faeces. Significant differences are in bold.

	Contrast	Ratio	SE	Z.ratio	P.value
Brown Bear	Red deer	0.864	0.098	-1.285	0.572
	Roe deer	1.237	0.193	1.357	0.526
	Wild boar	0.882	0.102	-1.085	0.698
Red deer	Roe deer	1.431	0.189	2.704	<b>0.034</b>
	Wild boar	0.980	0.076	-0.262	0.993
Roe deer	Wild boar	1.402	0.189	2.495	0.060

Table 3 ANOSIM results (r statistic) of pairwise tests for differences between animals using abundance data under greenhouse conditions (a) and natural conditions (b).

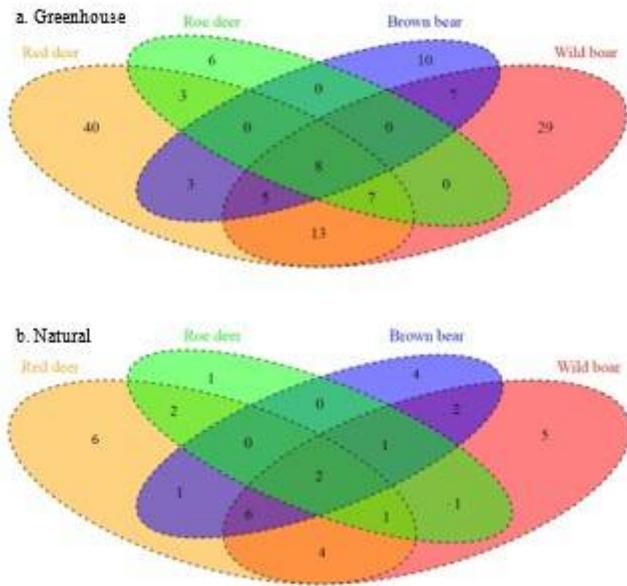
	Red deer	Roe deer	Brown bear
(a) Greenhouse conditions			
Roe deer	0.03		
Brown bear	0.11***	0.18***	
Wild boar	0.03**	0.09***	0.12***
(B) Natural conditions			
Roe deer	0.01		
Brown bear	0.10***	0.22***	
Wild boar	0.01*	0.05**	0.07***

\*Significant differences: 0.001 '\*\*\*' 0.01 '\*\*' 0.05 '\*'

Table 4 Summary of the dispersed species assemblages by animal vector. G = greenhouse conditions; N = natural conditions. Sample size was similar for greenhouse and natural conditions.

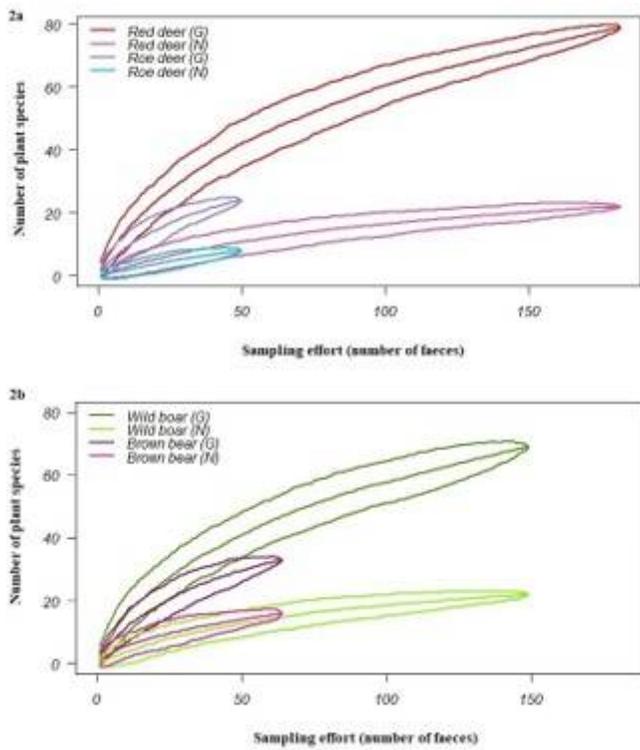
	Brown bear		Wild boar		Roe deer		Red deer	
	G	N	G	N	G	N	G	N
Sample size (spring, summer, autumn)	64 (10, 11, 43)		149 (25,73,51)		50 (9, 20, 21)		182 (46, 71, 65)	
Mean weight of faeces ( $\pm$ SD)	202.81 $\pm$ 128.55		87.79 $\pm$ 49.85		5.67 $\pm$ 2.21		60.84 $\pm$ 19.09	
Total number of seedlings dispersed	268	153	1301	78	120	13	1053	90
Total number of species dispersed	33	16	69	22	23	8	79	22
Chao2 estimator of species dispersed ( $\pm$ SD)	43.7 $\pm$ 7.5	26.5 $\pm$ 10.1	107.0 $\pm$ 19.1	46.8 $\pm$ 24.0	28.6 $\pm$ 4.9	12 $\pm$ 4.7	115.1 $\pm$ 16.7	26.5 $\pm$ 4.2
Total number of genera dispersed	29	14	55	19	23	7	59	18
Total number of families dispersed	19	7	25	11	15	6	23	10
Top six plant taxa, in terms of total seedling abundance in faeces	<i>Berberis</i> sp.	<i>Berberis</i> sp.	<i>Urtica dioica</i>	<i>Cerasus</i> sp.	<i>Portulaca oleracea</i>	<i>Convolvulus arvensis</i>	<i>Blitum virgatum</i>	<i>Polygonum lapathifolium</i>
	<i>Rubus</i> sp.	<i>Cerasus</i> sp.	<i>Cyperus fuscus</i>	<i>Sorbus torminalis</i>	<i>Sonchus oleraceus</i>	<i>Stellaria media</i>	<i>Polygonum lapathifolium</i>	<i>Poa masenderana</i>
	<i>Crataegus</i> sp.	<i>Rubus</i> sp.	<i>Phleum paniculatum</i>	<i>Veronica beccabunga</i>	<i>Convolvulus arvensis</i>	<i>Poa nemoralis</i>	<i>Cyperus fuscus</i>	<i>Berberis</i> sp.
	<i>Cerasus</i> sp.	<i>Sorbus torminalis</i>	<i>Aegilops tauschii</i>	<i>Poa masenderana</i>	<i>Echinochloa crus-galli</i>	<i>Cornus sanguinea</i>	<i>Portulaca oleracea</i>	<i>Stellaria media</i>
	<i>Lonicera</i> sp.	<i>Rhamnus pallasii</i>	<i>Amaranthus blitoides</i>	<i>Rumex sanguineus</i>	<i>Apetiolata</i>	<i>Melilotus albus</i>	<i>Urtica dioica</i>	<i>Dysphania botrys</i>
	<i>Rhamnus pallasii</i>	<i>Prunus divaricata</i>	<i>Blitum virgatum</i>	<i>Berberis</i> sp.	<i>Phleum paniculatum</i>	<i>Poa masenderana</i>	<i>Amaranthus blitoides</i>	<i>Clinopodium umbrosum</i>

## Figures



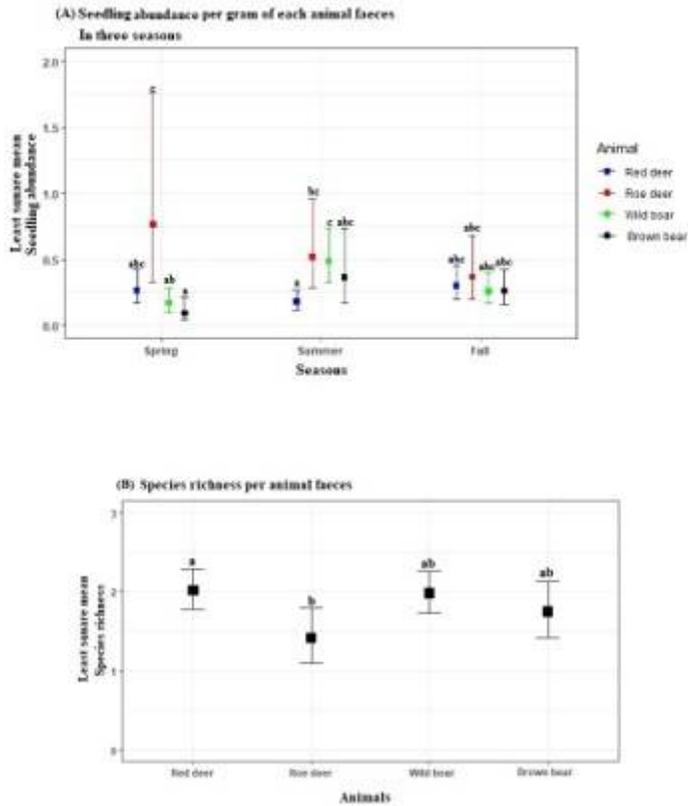
**Figure 1**

Number of species dispersed by one to four dispersal vectors in the study area (both habitats combined), a: under greenhouse conditions; and b: under natural conditions.



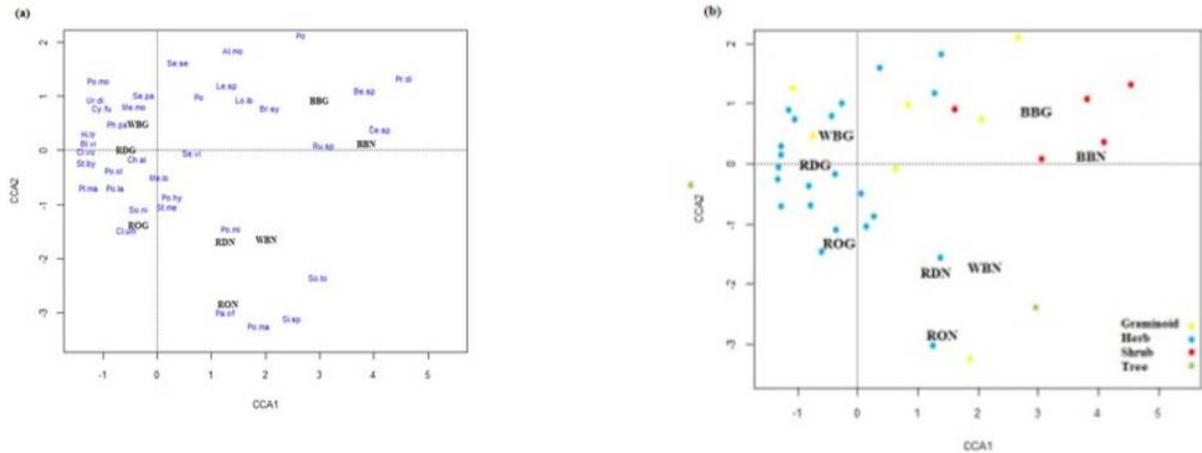
**Figure 2**

Species accumulation curves for the plants dispersed by the different animal vectors (total dung mass for red deer: 3640 g; wild boar: 2980 g; brown bear: 1280 g; roe deer: 285.5 g) (a) for herbivores - top panel and (b) omnivores - bottom panel; under greenhouse (G) and natural (N) conditions, based on the Chao 2 estimator with 95% confidence intervals.



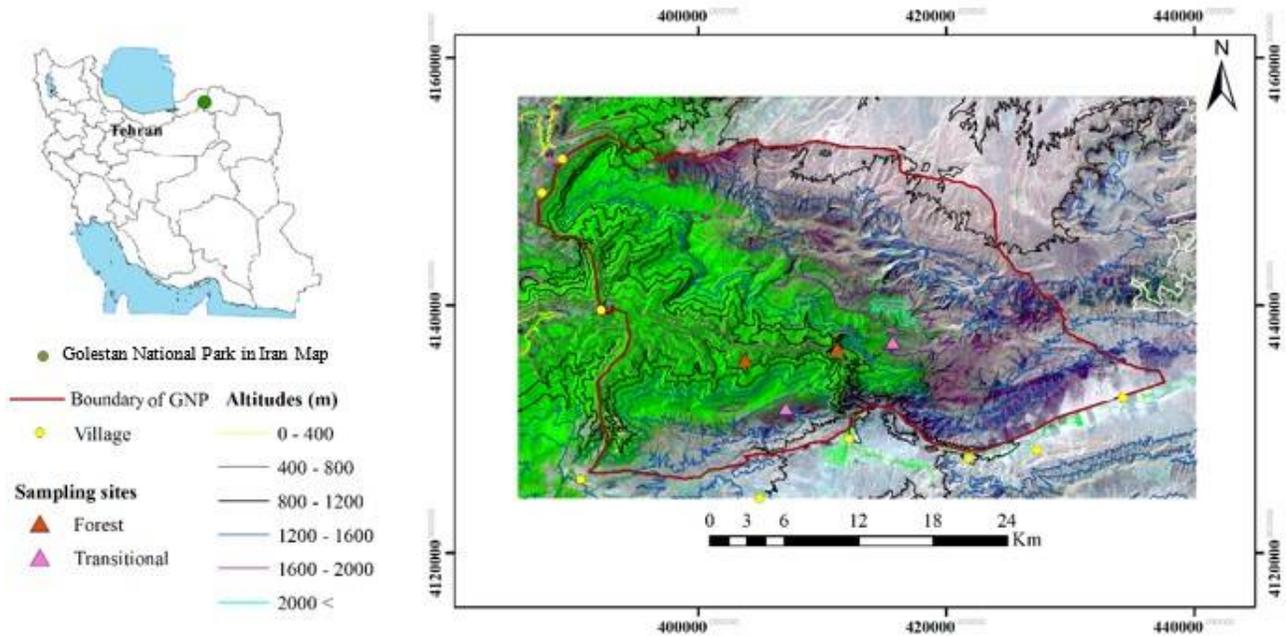
**Figure 3**

Plots of least square mean (with 95% confidence intervals) for (A) seedling abundance per gram of faeces vs. animal×season interaction, and (B) species richness per faeces vs. animal vector. Means sharing a letter are not significantly different (Turkey's post hoc tests). The results were back-transformed to the original scale by taking antilogarithms of the least square means (LSM) and the 95% confidence intervals (CI).



**Figure 4**

Biplots showing the results of the canonical correspondence ordination analysis. The first plot (a) shows the position of each combined factor (animal plus germination condition, e.g. WBG = Wild Boar under Greenhouse conditions) on the first two CCA axes of the dispersed plant species. The second plot (b) shows the position of the same factors on the same axes of the dispersed species' growth form (Graminoid, Herb, Shrub and Tree). Animal vectors: RO = Roe deer, RE = Red deer, BB = Brown bear, WB = Wild boar; and germination condition: G = Greenhouse conditions, N = Natural conditions. The plant species scientific names are written in shorthand form: the first two letters of the genus and the first two letters of the species (Additional file 6).



**Figure 5**

Location of Golestan National Park, highlighting the Hyrcanian forests (in green) in the western half and the surrounding steppes towards the east, north and south. Transitional vegetation zones occur in between and at high altitudes.

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [DataRcodes.rar](#)
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