

Potential and realised endozoochorous dispersal by herbivores and omnivores in a Caspian forest steppe area

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Research article

Keywords: Different germination methods, Mammal functional groups, Potential endozoochory, Realised endozoochory

Posted Date: October 1st, 2019

DOI: <https://doi.org/10.21203/rs.2.15402/v1>

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Version of Record: A version of this preprint was published on August 31st, 2020. See the published version at <https://doi.org/10.1186/s12898-020-00317-3>.

Abstract

Background: Among other factors, feeding regimes of different mammals affect their roles as endozoochorous seed dispersal vectors. We thus conducted a cross-species comparative approach involving two herbivores, red and roe deer; and two omnivores and opportunistic frugivores, wild boar and brown bear, which occupy the forest and prairie-forest ecotone habitats of the south-eastern Caspian region. To compare their potential and realised endozoochorous seed dispersal, we used two different seed germination methods: controlled greenhouse and natural conditions. Results: Of the 136 plant taxa (3078 seedlings) recorded from the 445 paired dung sub-samples of both methods, only 36 taxa (336 seedlings) were recorded under natural conditions, among which five taxa showed no emergence in the greenhouse. Graminoids and forbs composed 91% of the seedlings in greenhouse but shrub species were more abundant under natural conditions (55% of emerged seedlings). Considering greenhouse condition, red deer and then wild boar dispersed more species than the other animals, while in natural condition brown bear was the most effective vector. We observed remarkably higher species richness and seedling abundance in the controlled greenhouse method than natural condition ($P \leq 0.001$). This difference may be related to dung sample preparation method or meteorological conditions in the field during our experiment. Conclusions: The studied sympatric vectors were shown to provide different seed dispersal services and may, therefore, be regarded as complementary. Our results provide evidence for the potential positive bias arising when only considering germination under controlled greenhouse conditions. Relativising the potential versus the realised endozoochorous dispersal of seeds by different mammals would help determine management options to maintain plant biodiversity. Keywords : Different germination methods, Mammal functional groups, Potential endozoochory, Realised endozoochory

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 also a major determinant of plant community dynamics influencing potential recruitment rates, recolonization, spread of invasive species, 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) and contribute to the build-up of seeds in the soil compartment.

As major long-distance plant dispersal agents (6), herbivores act as one of the most important drivers of vegetation dynamics in grazed ecosystems (7). Depending on animal body size, dietary and habitat preferences, and the animal's digestive physiology, herbivores functional groups play different roles in endozoochorous seed dispersal (8-10). However, few studies have evaluated the role of sympatric herbivores and omnivores in the endozoochorous seed dispersal of local plant communities (11-13).

The establishment phase of seedlings that have emerged from seeds dispersed through endozoochory is an important stage in the seed dispersal cycle. Though several methods have been developed to detect 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 (14, 15). Such results, at best, may merely indicate potential germination success of the dispersed seeds (16, 17). We therefore launched this study to specifically test the relative germination rates of internally dispersed seeds under natural and greenhouse conditions.

We propose herein a cross-species comparative approach involving the four most common wild herbivores and omnivores which occupy the forest and prairie-forest ecotone habitats of the south-eastern Caspian region: two herbivores - an intermediate mixed feeder, the red deer (*Cervus elaphus maral*) and a browser, the roe deer (*Capreolus capreolus*); and two omnivores and opportunistic frugivores - the wild boar (*Sus scrofa*) and the brown bear (*Ursus arctos*).

The plants germinating from the faecal samples of the four different animal vectors feeding in the same habitat and season were compared to investigate the contribution of each animal vector to the composition of the pool of plants dispersed in two different habitats (forest and prairie-forest ecotone).

We hypothesised that:

- 1) According to their varying body size, feeding regime and digestive strategy (ruminant versus non ruminant), the four dispersal agents would disperse different sets of seeds. Accordingly, red deer, as an intermediate mixed feeder, should disperse more diverse plants than the more selective roe deer, and frugivores like brown bear should disperse more fleshy-fruited plants than herbivores.
- 2) The composition of plant species dispersed by the studied dispersal agents would vary spatially and temporally across the study area. Seed dispersal by frugivores should be at its highest in late summer and autumn when shrubs are producing fleshy fruits. We expected lower seed dispersal by herbivores during the dry season (summer) due to lower herbaceous cover. Finally, we expected to find a larger proportion of invasive species in wild boar dung samples during the dry season, a period during which wild boar spend more time feeding along the roadsides in our study area.
- 3) Seed germination under both controlled and natural conditions would vary with dispersal agent, since each agent would disperse a specific set of seeds with specific germination conditions. We also expected lower germination under natural conditions than under greenhouse-buffered conditions, in agreement with previous studies.

Results

Seedling emergence under greenhouse conditions

Over 97% of red deer, 91% of brown bear, 89% of wild boar, and 88% of roe deer dung sub-samples contained germinating seeds; no seedlings emerged from 8% of the combined animal dung sub-samples. After the exclusion of the two unidentified seedlings, a total of 2,739 seedlings representing 129 plant species and 29 families germinated from the 445 dung samples collected. Most species belonged to *Poaceae* ($n = 29$), *Brassicaceae* ($n = 12$) and *Asteraceae* ($n = 11$). Twenty percent of the families were represented by a single species. Two species, *Urtica dioica* (623 seedlings) and *Cyperus fuscus* (383 seedlings), were the most abundant species in the dispersed pool. The most frequent species, *Portulaca oleracea* and *C. fuscus*, respectively occurred in 24% and 23.5% of all dung sub-samples. A large proportion of the species (37%) only occurred in a single dung sample and 65% of the species emerged less than five times. In addition, 27% of the germinating species produced only one seedling. The dispersed pool of species was composed of 81 herbaceous species, 33 graminoids, 13 shrubs, subshrubs or trees, and one non-spiny cushion form plant. Annuals and perennials comprised, respectively, 47% and 52% of the emerged seedlings.

A total of 919 seedlings, representing 26 species, belonged to native invaders (species that have become “invasive” in their own native range; (18)), five of which were solely dispersed by wild boar: *Amaranthus cruentus*, *Malva sylvestris*, *Plantago lanceolata*, *Rapistrum rugosum*, *Chenopodium vulvaria* (see Additional file 1).

Containing greater number of seedlings generally meant greater hosted species richness (Spearman's $r_s = 0.76$; $P < 0.0001$), though some wild boar samples were dominated by a large number of seedlings from a single species (i.e. *U. dioica*). Certain plant species were dispersed by only one animal vector; forty for red deer, twenty-nine for wild boar, ten for brown bear and six for roe deer. By comparison, eight species (*P. oleracea*, *Setaria viridis*, *Stellaria media*, *Phleum paniculatum*, *Convolvulus arvensis*, *Sisymbrium irio*, *Polygonum hyrcanicum* and *Chenopodium album*) were dispersed by all four animal vectors (Additional file 1, Fig. 1a). Red deer dispersed the greatest number of plant species (see also Table 1). The species accumulation curves (Fig. 2a) did not achieve a plateau, meaning that our sampling was insufficient to account for some rare plants. The overall observed species richness for the samples represented only 74% of the expected species richness, with percentages varying from 64% (wild boar) to 75% (brown bear, Table 1).

Seedling emergence under natural conditions

Under natural conditions, 55% of brown bear, 30% of wild boar, 26% of red deer, and 20% of roe deer dung sub-samples provided viable seeds. After the exclusion of the five contaminant species, 334 seedlings of 36 plant species and 16 families germinated from the 445 dung sub-samples. Most species belonged to the *Poaceae* ($n = 8$), *Rosaceae* ($n = 5$) and *Polygonaceae* ($n = 5$; Table 1). 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. *Poa masenderana* with 28 seedlings (3.5%) came third (Additional file 1).

No seeds germinated from 67% of the dung samples in natural conditions. Among the germinated seeds, 20 species were herbaceous, nine were shrubs and seven were graminoids. Annuals and perennials respectively constituted 37% and 63% of the germinated species. Some plants were dispersed by a single animal (6 for red deer; 5 for wild boar; 4 for brown bear and a single for roe deer). Two taxa, *Poa masenderana* and *Rumex* sp., were shared by all four animals (Fig. 1b).

The overall observed number of species corresponded to 81% of the expected species richness and varied among each animal species from 83% (red deer), 72% (roe deer), 60% (brown bear) to 47% for wild boar. Brown bear appeared to be the best dispersal agent under natural conditions (Fig. 2b).

Fig. 1 Number of plants dispersed by a single up to the 4 dispersal vectors in GNP (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 (dung mass for red deer: 3640 g, wild boar: 2980 g, brown bear: 1280 g, roe deer: 285.5 g) for herbivores on top (a) and omnivores at the bottom (b) in greenhouse (G) and natural (N) conditions, based on the Chao2 estimator with 95% confidence intervals.

Dispersed seed pool composition under greenhouse conditions

At the community level, plant sub-communities sharply differed by the species they were composed ($F_{436, 8} = 3.387$, $P = 0.001$) when animal, season and habitat were taken into account in the CCA (Fig. 3). Pairwise comparisons indicated strong differences ($P \leq 0.001$) in the composition of species among any season / animal / habitat combinations, except for the comparison between red deer and roe deer ($P = 0.07$). Autumn plant composition differed more from spring ($R = 0.15$; $P = 0.001$) than from summer ($R = 0.05$; $P = 0.001$) compositions. Comparisons among animals showed that red deer, roe deer and wild boar had greater differences with brown bear in terms of plant composition ($R = 0.12-0.18$; $P = 0.001$) than they did with one another ($R = 0.03-0.09$; $P = 0.001$). Of the dispersed plant species, 27% and 41% had been exclusively deposited in the prairie-forest ecotone and the forest habitat, respectively ($R = 0.03$; $P = 0.001$).

Fig. 3 Biplots showing the results of the canonical correspondence ordination analysis. The first plot (a) shows the position of each factor (season and habitat and animal vector) on the first two CCA axes of dispersed plant species and dung samples and the second (b) shows the position of each animal vector on the same axes in the dispersed growth form space. Animal vectors: RO = Roe deer, RE = Red deer, BB = Brown bear, WB = Wild boar in blue ellipses; Season: AU = Autumn, SU = Summer, SP = Spring in green ellipses; and habitat: F = Forest, E = prairie-forest Ecotone in red ellipses.

Seedling abundance and plant species richness per animal vector

Variability of seed abundance and species richness per gram of faeces were low for all vectors, either herbivores or omnivores. Roe deer dispersed more viable seeds per dung mass unit (spring mean \pm SD = 0.762 ± 0.325 , and summer mean = 0.517 ± 0.163) than any other mammals studied. Brown bear (0.090 ± 0.04) and wild boar (0.166 ± 0.046) dispersed the lowest amount of seeds per gram in spring. Seedling abundance (per gram of dung \pm SD) significantly differed between roe deer and brown bear (0.118 ± 0.066 , $z = -3.799$, $P = 0.008$) and, roe deer and wild boar (4.573 ± 2.080 , $z = 3.343$, $P = 0.039$) in spring. During the summer, wild boar dispersed the highest amount of viable seeds per gram of dung (mean \pm SD = 0.484 ± 0.102), while red deer dispersed the lowest amount (mean \pm SD = 0.175 ± 0.038). At the same time, there were less seeds dispersed by red deer than either wild boar (0.362 ± 0.064 (\pm SD), $z = -5.694$, $P = 0.0001$) or roe deer (0.339 ± 0.102 ; $z = -3.586$; $P = 0.017$). Roe deer faeces exhibited lower plant species richness per gram of faeces (mean \pm SD = 1.4 ± 0.178) than red deer (mean \pm SD = 2.02 ± 0.128 ; $z = 2.70$; $P = 0.028$). The habitat type did not have any significant relationship with seedling abundance and plant species richness per animal vector (all $P > 0.05$).

Natural versus greenhouse conditions

Of the 136 plant taxa recorded from the 445 paired dung sub-samples in both of the greenhouse and natural conditions, only 36 taxa were recorded under natural conditions, while 131 taxa were observed under greenhouse conditions. Seedling abundance and species richness were significantly higher under greenhouse than natural conditions ($P \leq 0.001$).

Differences in germination patterns between greenhouse and natural conditions concerned three groups of plants. In the first group, no seedlings emerged under natural conditions, suggesting that natural conditions were unsuitable for germination or establishment during our study period. Overall, 62 herbs, 26 graminoids, six shrubs, one sub-shrub and one cushion plant emerged only under greenhouse conditions. In this group, the most abundant species were *Urtica dioica*, *Cyperus fuscus*, *Blitum virgatum* and *Portulaca oleracea*. A second group of five taxa (*Aegilops cylindrical*, *Cornus sanguinea*, *Calamintha nepeta*, *Rosa canina* and *Silene* sp.) germinated only under natural conditions, though in low numbers. A third group of 31 plants germinated under both natural and greenhouse conditions. Most ($n = 21$) of these species emerged at a lower rate under natural than greenhouse conditions. However, eight taxa (*Cerasus* sp., *Polygonum minus*, *Parietaria officinalis*, *Poa masenderana*, *Poa nemoralis*, *Poa pratensis*, *Rumex* sp. and *Sorbus torminalis*) had higher emergence rates under natural conditions. *Polygonum convolvulus* and *Prunus divaricata* occurred similarly under natural and greenhouse conditions.

Germinated seedling abundance of third plant group significantly differed under greenhouse and natural conditions for roe deer (Mann-Whitney U test: $U = 7$, $P = 0.046$) and wild boar ($U = 34$, $P = 0.005$), but not for red deer ($U = 103.5$, $P = 0.184$) and brown bear ($U = 45.5$, $P = 0.382$). We observed a good match between growing conditions for the most abundant (i.e. *Berberis* sp., *Cerasus* sp. and *Rubus* sp.) and frequent (*Berberis* sp., *Cerasus* sp.) plant species germinated from brown bear dung sub-samples under both greenhouse and natural condition.

Discussion

The studied animal vectors dispersed a large set of plants were available in the GNP through endozoochory. Several plant species which frequently emerged in our dung sub-samples or produced abundant seedlings, such as *U. dioica*, *P. oleracea*, *C. fuscus*, *C. album*, *P. lapathifolium*, *B. virgatum*, *Berberis* sp. and *S. media*, have previously and regularly been mentioned (9, 13, 19, 20). Most of the germinated plant species emerged from a single dung sub-sample as a single seedling. This pattern could be attributed to four different causes: accidental seed intake (11), forage contamination by surrounding seeds (21), low abundance of the plant species in the local pool at the sampling time (22), and rare feeding bouts.

We found a higher proportion of dung sub-samples with emerging seedlings under greenhouse conditions than previously reported for all our animal vectors (5, 13, 23). Nearly all the red deer dung sub-samples contained viable seeds, corresponding to 60% of all emerged plant species, half of which was solely dispersed by red deer; a pattern previously observed (11). Roughly half of the total set of plants emerging from the dung sub-samples was dispersed by wild boar; with less than half of which was exclusively dispersed by this omnivore. Wild boar also dispersed the highest number of native invader plants.

Abundance of seedlings and species richness

The observed differences among animal vectors in plant species seed content of their faecal samples can be attributed to their dietary preferences (*grazers* vs. *mixed feeders* vs. *browsers*; (13, 24)), digestive physiology (ruminant or not; (25)), body size, habitat preferences and ranging behaviour. These factors may also lead to the deposition of seeds in different habitats (11, 26).

Consistent with previous studies (11, 23), we expected that *grazers* disperse the highest number of species, followed by *browsers* and *omnivores*. Indeed, red deer dung sub-samples had the highest number of species. We observed significant differences between the two deer species. The red deer is an intermediate mixed feeder while roe deer is a *selective browser* (27) which consumes small amounts of high-quality food like buds

and leaves more than fruits or seeds (20). The large-bodied red deer occupies much bigger home ranges than roe deer (28) and this increases chances to encounter and consume more plants (9) than roe deer (29). Other than this pattern between the two deer species, however - and contrary to our expectations - the species richness of the plants dispersed did not differ among the other animal vectors, seasons, and between habitats. This can be attributed to the wide diversity of habitats and associated plants encountered in GNP (30), which probably provided complementary food sources in different seasons and across habitats. The similarity between omnivorous and herbivorous vectors in terms of dispersed plant species richness may also be due to an exceptionally high number of plants dispersed by the two omnivores in our study area, suggesting that they have shifted their diet towards *plants* (high seed intake) to the detriment of *non-plant food resources* (13, 24, 31). Anthropogenic factors may have also been partly responsible for non-significant differences between animal vectors considering plant species richness. In GNP, there has been a dramatic decline in large herbivores mainly due to livestock grazing, habitat loss and intensive poaching (32). The small remaining deer populations are now limited to the less disturbed sites, where herbivory pressure has consequently been increasing. In contrast, because of religious restrictions on eating wild boar meat, their numbers have increased during the same period across a wide variety of GNP habitats. Consequently, wild boars encounter a wider range of plant species. This result is in line with findings of Heinken *et al.* (2002), who reported more species dispersed by wild boar than by roe deer.

Even though GNP hosts a very rich flora, some studies reported higher numbers of plant species dispersed by similar vectors (31) for brown bear; (9, 11) for red and roe deer, and (13) for wild boar). This could be related to insufficient spatial and temporal sampling as reflected by the species accumulation curves. Sample preparation method could also be involved, as some plants have specific germination requirements other than cold stratification (e.g. warm stratification and smoke exposure). Increasing the duration of the germination experiment would probably have increased the number of germinated seedlings and species (11). Then, the altitude difference (1616 and 450 m for the greenhouse and the natural conditions respectively) has probably affected the germination pattern.

As hypothesised, we showed that the abundance of emerging seedlings was positively correlated with season, increasing to a peak in summer or autumn. In general, seed density in the dung sub-samples depended on the feeding regime of the vectors and followed a seasonal pattern compatible with plant seed shedding periods (26).

As expected, the studied herbivores were more effective seed dispersal vectors than the omnivores in spring, when herbaceous plants dominate in terms of frequency and abundance. Shrubs were mostly present in omnivorous dung samples collected in summer and autumn, when palatable fleshy fruits are available (31, 33).

As we expected and in line with previous studies, roe deer were more effective dispersal vectors than either wild boar (11, 20) or brown bear in terms of dung seed density. Even red deer dispersed a lower mean number of viable seeds than roe deer in summer, despite their larger body size. It could be due to a reduction in the herbaceous plant cover in summer, which constitutes the main food of red deer. As a consequence of feeding regime and body size (34), we also expected red deer to disperse more seeds than wild boar. However, the opportunistic feeding regime of wild boars considerably increased the number of seeds dispersed by this species (more than half of which emerged in summer), as compared to red deer in summer. 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* (three quarters of the seedlings emerged from roe deer dung in spring). Red deer samples peaked in autumn, with the dispersal of *Polygonum lapathifolium* (one third of the seedlings emerged from red deer dung in autumn). Finally, the highest seed density in brown bear dung samples was in summer, which coincided with the maximum seeding period for fleshy-fruited shrubs and trees.

Species composition

Both the functional traits of the animal vectors and plant phenology reflected the changes occurring in their diet and habitat use across seasons, thereby affecting the composition of the plants dispersed. In terms of animal vector, the largest dissimilarities among the sets of dispersed plant species were between brown bear and the three other vectors, whatever their feeding regime. The differences in the seed content among herbivore and omnivore dung samples reached their peak in autumn. We also found significant differences in the composition of the seed sets between dungs deposited in the two different habitats, thus reflecting a habitat-dependent deposition pattern (35). This pattern can be partly influenced by feeding habitat preferences among animal vectors. Roe deer prefer closed-forest habitats with low visibility compare to open habitats, while other three species occur in both closed and open habitat considering food resources in different seasons.

Most of the plant species dispersed through endozoochory by the studied animal vectors 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 is in accordance with the "foliage is the fruit" hypothesis (21), where 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 in the *Berberis*, *Crataegus* and *Cerasus* genera. However, seeds from dry fruits were also present in brown bear faeces, though in lower densities than those from fleshy fruits, especially during spring. This result is in agreement with the brown bear's opportunistic feeding behaviour. Indeed, though fruits from shrubs represent an important source of energy for fat accumulation prior to hibernation (36), when nutritional fruits are rarely available, bears mainly rely on herbaceous species and other vegetative parts besides animal carcasses during spring (31, 33).

Our results also reveal the potential of large native herbivores and omnivores, especially wild boar, to disperse native invader species, mostly ruderal or early successional species. However, only two of these species (*Portulaca oleracea* and *Cyperus fuscus*) were dispersed in large quantities. As expected, a larger proportion of native invader species germinated from wild boar dung collected during the dry season, when it spends more time

feeding along roadsides. Some plants cultivated, like *C. vulgaris* and *S. lycopersicum*, are dispersed into natural areas by wild boar when they take benefit of the food left by humans along the roads or when wild boars roam in agricultural areas (37). Therefore overabundant wild boar populations may enhance invasion of native invader and exotic plant species from such habitats, and consequently lead to species diversity reduction (38). Reduced deer population and associated browsing pressure would also result in increased species diversity (39), but at the same time the reduction of seed vectors would negatively affect plant regeneration, and result in biodiversity decline (40). Therefore, conservation efforts should focus on the protection of animal population which provide seed dispersal services to plants (41).

Natural versus greenhouse germination conditions

More plant species germinated under greenhouse conditions; though the difference was low for brown bear. The greatest difference was observed between wild boar dung sub-samples. This may be due to the large number of *U. dioica* seedlings which was not observed under natural condition.

Three reasons can account for the lower seed germination rates found under natural conditions. First, cold stratification was not applied to the dung sub-samples placed under natural conditions to allow natural cold stratification. Most species have to undergo a cold period to break seed dormancy and facilitate germination (42). The climatic conditions in the field during our experiment might not have provided the necessary conditions to break dormancy of all seeds. It should also be noted that germination requirements are species-dependent. For example, *Cornus sanguinea* seeds, which did not germinate in the greenhouse, require a warm phase to degrade their stony endocarp (43) followed by chilling at 3°C for 8-12 weeks to break seed dormancy (44). In other cases, *R. canina* for instance, 11 weeks of warm stratification followed by cold stratification appears to be the most appropriate (45). Second, a regular water supply and relatively constant temperature, as in greenhouse conditions, is likely to positively affect germination results. *U. dioica*, for instance, has been frequently observed in dung (16, 20); however, successful emergence under natural conditions did not occur in our study, probably because the plant prefers moist or damp soil (46). However, climatic fluctuations under natural conditions seems to facilitate the germination process of seeds with particular requirements (17). Finally, we did not check the seed content of the two sub-samples before submitting them to contrasting conditions (17). This means that rare species with a single seed in the original dung sample would have either germinated in the greenhouse, or under natural conditions, or not germinated at all. According to Wessels and Schwabe (2008), splitting the original dung sample into two random sub-samples may lead to heterogeneous composition between sub-samples.

Conclusions

Our results reveal that potential endozoochorous dispersal, determined by germination success from dung sub-samples under greenhouse conditions, over-estimates likely establishment rates as compared to natural conditions. This difference shows the potential positive bias arising when only considering germination under controlled greenhouse conditions. Factors that affect germination success under natural conditions such as competition, seed predation, climate, decaying state of the dung and herbivory should also be taken into account (35). Nevertheless, seeds that did not emerge from dung under natural conditions over the course of this study may remain in seed bank until edaphic and climatic conditions become suitable for germination. Therefore, our study may only partially depict seed dispersal under natural conditions.

As we revealed in our study, large herbivores and omnivores are important vectors for seed dispersal, and their loss in natural ecosystems may lead to cascading effects on other taxa. The four studied sympatric vectors were shown to provide different seed dispersal services and may, therefore, be regarded as complementary. Understanding the potential of animal vectors would make it possible to determine management programs to maintain or enhance plant species diversity through long-distance seed dispersal.

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 of the Middle East. It covers around 920 km² of eastern Iranian Caspian forests with altitudes ranging from 450 to 2411 m above sea level (Fig. 4).

The climate is seasonal, marked by cold winters (January to March) and warm summers (July to September). The average annual precipitation is 400 mm and yearly precipitation ranges from 150 mm in the south-east part of the park to more than 1000 mm in more central areas. The most of the precipitation occurs from late autumn to early spring. Clearly the winter months with 32.3% of annual rainfall are the moistest months of the year. The area receives 25%, 11.8%, and 30.3% of annual rainfall during spring (April to June), summer, and fall (October to December), respectively. Minimum relative humidity of the region is 60% but increases up to 83% during summer. The average annual temperature varies from +11.8 °C to +18.8 °C. The summers with high temperature in the dry regions can cause really hot and dry conditions in the east, south and northeast and a humid climate in the west part of that region (30).

Fig. 4 Location of Golestan National Park, highlighting the Hyrcanian forests (in green) in the half western part and the surrounding steppes towards the east, north and south. Transitional vegetation zones occur in between and at high altitudes.

GNP lies across the Euro-Siberian and the Irano-Turanian phytogeographical regions (Hyrcanian and Khorassan–Kopet–Dagh provinces, respectively). GNP contains a wide range of flora and fauna, which are unique in many aspects; it is covered by diverse vegetation entities which include the

Hyrceanian mesophytic forests, shrublands, scrublands (mixed with C4-composed grasslands sometimes), woodlands of *Juniperus* sp., mountain steppes and meadows, *Artemisia* sp. steppes, and communities composed of halophilous plants (30). Mixed plant communities can be found in between these two phytogeographical regions. We incorporated these plant units into two major habitats types: Hyrceanian closed forests and transitional scrub and Juniper woodlands, where the studied animal vectors are known to be present. We therefore selected study plots within these two major vegetation types; each plot was replicated twice.

At the time of our study, there were about 257 (95% CI: 91-423) red deer (47), 150 roe deer, 6,000 (95% CI: 3,050-9,906) wild boars, and 60 brown bears in the park (Golestan Provincial Department of Environment, 2016).

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

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 sample of brown bear and 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 pairs. We restricted dung collection to intact, fresh and wet samples to limit interactions with the environment (11). We prevented contamination by seeds that stuck on the surface of the collected dung samples by removing the lowermost layer of dung samples (Picard et al., 2016). 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 both greenhouse and natural conditions. Because roe deer dung samples were lighter than those of the other three species (average weight of 5.67 ± 2.21 g; Table 1), each individual sample was divided into two equally-sized sub-samples.

Table 1 Summary of the dispersed species assemblages for all vectors combined and by vector. G = greenhouse conditions; N = natural conditions.

Germination experiments

Both the greenhouse and natural experiments had a randomised complete block design with 7 blocks (sampling month) and 4 treatments (animal vectors). Over a 15-month period, we recorded the germinated seedling species and subsequently removed them. To obtain seedling species richness and abundances in each sampling season (spring, summer, autumn), we pooled monthly data of 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 (Picard et al., 2016), and then separately crushed with care to break the pellets. They were mixed with a similar volume of soil and sand, then poured into pots (diameter 20 cm, depth 25 cm) making a layer of approximately 1-2-cm thick. We filled the pots with a 1:2:1 mixture (sand: soil: peat moss), previously sterilised in an autoclave at 120 °C for 45 minutes (10).

The samples were then allowed to grow under natural daylight with daytime temperatures of around 25 °C under greenhouse conditions located in the Isfahan university of technology (32°43'13.6"N latitude and 51°32'52.4"E longitude and at an altitude of 1616 m above sea level). The *average* minimum *temperature* fell to 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 months to enable the germination of more deeply buried seeds (48). To control for possible seed bank or seed rain contamination in the greenhouse, 30 control pots (without faecal samples) with a similar substrate were placed among pots with dung samples and kept equally.

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% 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 37°23'53.7"N latitude and 55°47'54.4"E longitude and at an altitude of 450 m above sea level) and the experiment was carried out within the fenced area to prevent disturbance by the grazing animals. We inverted the soil by bringing a deeper layer of soil (from a depth of more than 35 cm) up to the surface of the experimental site to prevent any seeds in the soil seed bank from contaminating the experimental soil (49). Planting pots were filled with the same deep soil and placed on this surface. The faecal samples were not subjected to artificial cold treatment. They were crushed carefully to break the pellets and were placed directly in each planting pot and were allowed to natural cold stratification. To allow the natural soil moisture into the planting pots and to improve rainwater drainage, the bottoms of the pots were removed. Average annual rainfall was about 580 mm during that period. In order to control for air-borne seed input and soil seed bank content, seven soil-only pots with no dung were similarly positioned for each month. Temperature and light

were not controlled and no irrigation was applied during the experiment. The samples were completely exposed to natural climatic conditions. Seedlings were identified to the species level whenever possible (11% could only be identified to 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 and were therefore discarded from further analyses.

Data analysis

We built species accumulation curves using Chao 2 estimator to estimate the expected species richness and check if our observed seed-dispersed species richness matched what should be expected in the study area (50).

Greenhouse data analysis

A generalised linear mixed model (GLMM) was used to compare the abundance of seedlings and the number of species between dung samples of the studied vectors, while accounting for potential phenological and habitat variations in the assemblage of dispersed seeds. Negative binomial regression and Poisson models were respectively assigned for seedling abundance and species richness (count response variables) based on over-dispersion in the model. Animal species, sampling season (spring, summer, and autumn) and habitat (forest, prairie-forest ecotone) were fixed factors, and habitat repetition was considered a random effect. The log-transformed weight of each sample was taken as an offset to account for differing sample weights.

The compositions of the germinated species (square root transformed) were compared to the main factors studied (animal vector, sampling season and habitat) through canonical correspondence analysis (CCA). We used Monte-Carlo permutation tests ($n = 999$ permutations) to test the significance ($P < 0.05$) of the forward selected variables and the axes of the CCA.

We compared differing plant species composition among animal species, sampling season, and habitat by analysing similarities (ANOSIM procedure), which provided an R statistic ranging from zero (complete species overlap) to one (no species in common). We also used Bray-Curtis similarity indices as they exclude double-zero comparisons and do not weight rare or abundant species (51).

Data analysis for natural versus greenhouse conditions

Poisson regression models were used to test abundance of seedlings and species richness between the planted dung samples of the animal vectors, while accounting for germination conditions (greenhouse vs. natural). Animal species and germination conditions were the fixed effects and dung sub-sample was the random effect. Pairwise comparisons between seedlings abundance of plant species under both conditions were made with the nonparametric Mann-Whitney U test.

We performed all statistical analyses with the R 3.1 software (R Foundation for Statistical Computing, Vienna, AT) using the *vegan* (52), *venndiagram* (53), *lme4* (54), *lsmmeans* (55) and *MuMIn* (56) libraries.

Abbreviations

GNP: Golestan National Park; GLMM: Generalised linear mixed model; CCA: Canonical correspondence analysis.

Declarations

Acknowledgements

We are grateful to Hossein Akhani and Atefeh Ghorbanalizadeh for their help in the field and assisting in identification of plants species. We also thank Esmail Ghadimi, Mojtaba Ghadimi and Javd Pourrezaei for assisting with data collection.

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

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Availability of data and materials

The datasets in this study are available from the corresponding author on reasonable request.

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 Summary of the dispersed species assemblages for all vectors combined and by vector. G = greenhouse conditions; N = natural conditions.

	Wild boar		Red deer		Roe deer		Brown bear		Total	
	N	G	N	G	N	G	N	G	N	G
Sample size (spring, summer, autumn)	149 (25,73,51)		182 (46, 71, 65)		50 (9, 20, 21)		64 (10, 11, 43)		445 (90, 175, 180)	
Mean weight of faeces (± SD)	87.79 ± 49.85		60.84±19.09		5.67±2.21		202.81±128.55		84.08±79.20	
number of seedlings dispersed	78	1301	90	1053	13	120	153	268	334	2742
number of species dispersed	22	69	22	79	7	24	16	33	36	131
Chao2 estimator of species dispersed (± SD)	46.8 ± 24.0	107.0 ± 19.1	26.5 ± 4.2	115.1 ± 16.7	9.6 ± 3.4	35.8 ± 9.5	26.5 ± 10.1	43.7 ± 7.5	44.3 ± 6.8	177 ± 17.3
number of genera dispersed	19	55	18	59	7	23	14	29	31	89
number of families dispersed	11	25	10	23	6	15	7	19	16	30
Top six plant taxa, in terms of total seedling abundance in faeces	<i>Cerasus sp</i>	<i>U.dioica</i>	<i>P.lapathifolium</i>	<i>B.virgatum</i>	<i>C.arvensis</i>	<i>P.oleracea</i>	<i>Berberis sp.</i>	<i>Berberis sp.</i>	<i>Berberis sp</i>	<i>U.dioica</i>
	<i>S.torminalis</i>	<i>C.fuscus</i>	<i>P.masenderana</i>	<i>P.lapathifolium</i>	<i>S.media</i>	<i>S.oleraceous</i>	<i>Cerasus sp.</i>	<i>Rubus sp.</i>	<i>Cerasus sp</i>	<i>C.fuscus</i>
	<i>V.beccabunga</i>	<i>P.paniculatum</i>	<i>Berberis sp</i>	<i>C.fuscus</i>	<i>P.nemoralis</i>	<i>C.arvensis</i>	<i>Rubus sp.</i>	<i>Crataegus sp.</i>	<i>P.masenderana</i>	<i>B.virgatum</i>
	<i>P.masenderana</i>	<i>A.tauschii</i>	<i>S.media</i>	<i>P.oleracea</i>	<i>C.sanguinea</i>	<i>E.crus-galli</i>	<i>S.torminalis</i>	<i>Cerasus sp.</i>	<i>S.torminalis</i>	<i>P.lapathifolium</i>
	<i>R.sanguineus</i>	<i>A.blitoides</i>	<i>D.botrys</i>	<i>U.dioica</i>	<i>M.albus</i>	<i>Apetirolata</i>	<i>R.pallasii</i>	<i>Lonicera</i>	<i>Rubus sp</i>	<i>P.oleracea</i>
	<i>Berberis sp</i>	<i>B.virgatum</i>	<i>C.umbrosum</i>	<i>A.blitoides</i>	<i>P.masenderana</i>	<i>P.paniculatum</i>	<i>Prunus</i>	<i>R.pallasii</i>	<i>P.lapathifolium</i>	<i>Berberis sp</i>

Figures

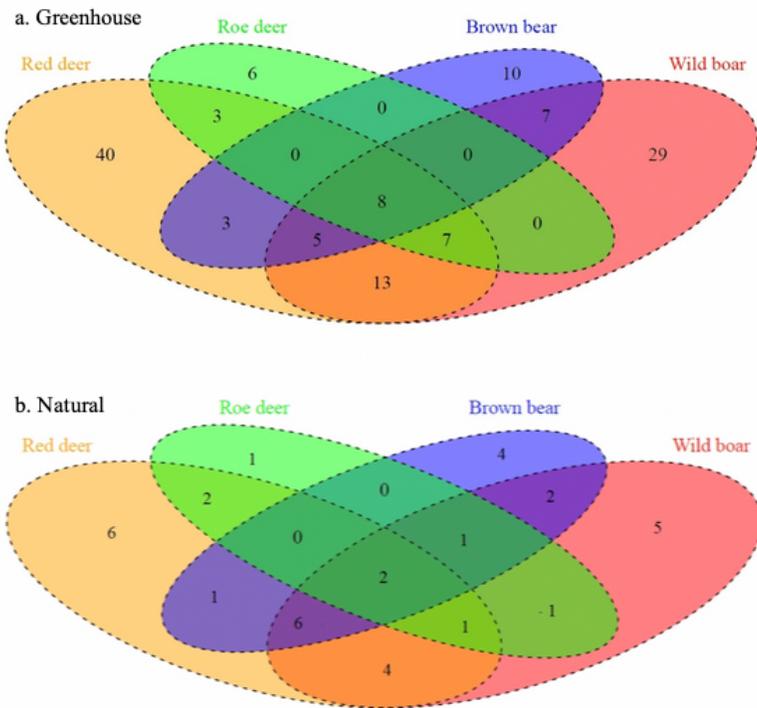


Figure 1

Number of plants dispersed by a single up to the 4 dispersal vectors in GNP (both habitats combined), a-under greenhouse conditions and b-under natural conditions.

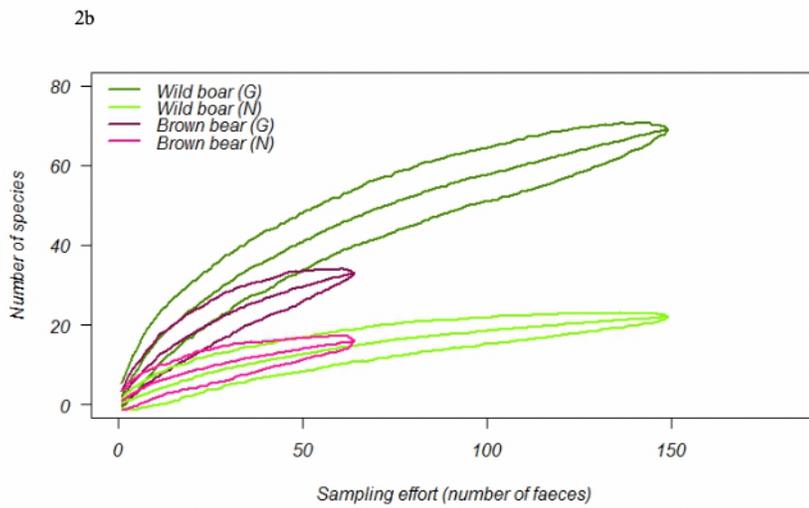
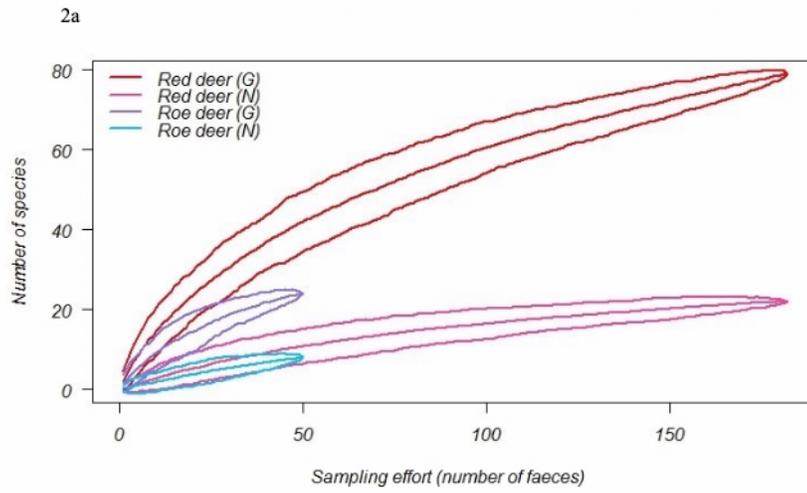


Figure 2

Species accumulation curves for the plants dispersed by the different animal vectors (dung mass for red deer: 3640 g, wild boar: 2980 g, brown bear: 1280 g, roe deer: 285.5 g) for herbivores on top (a) and omnivores at the bottom (b) in greenhouse (G) and natural (N) conditions, based on the Chao2 estimator with 95% confidence intervals.

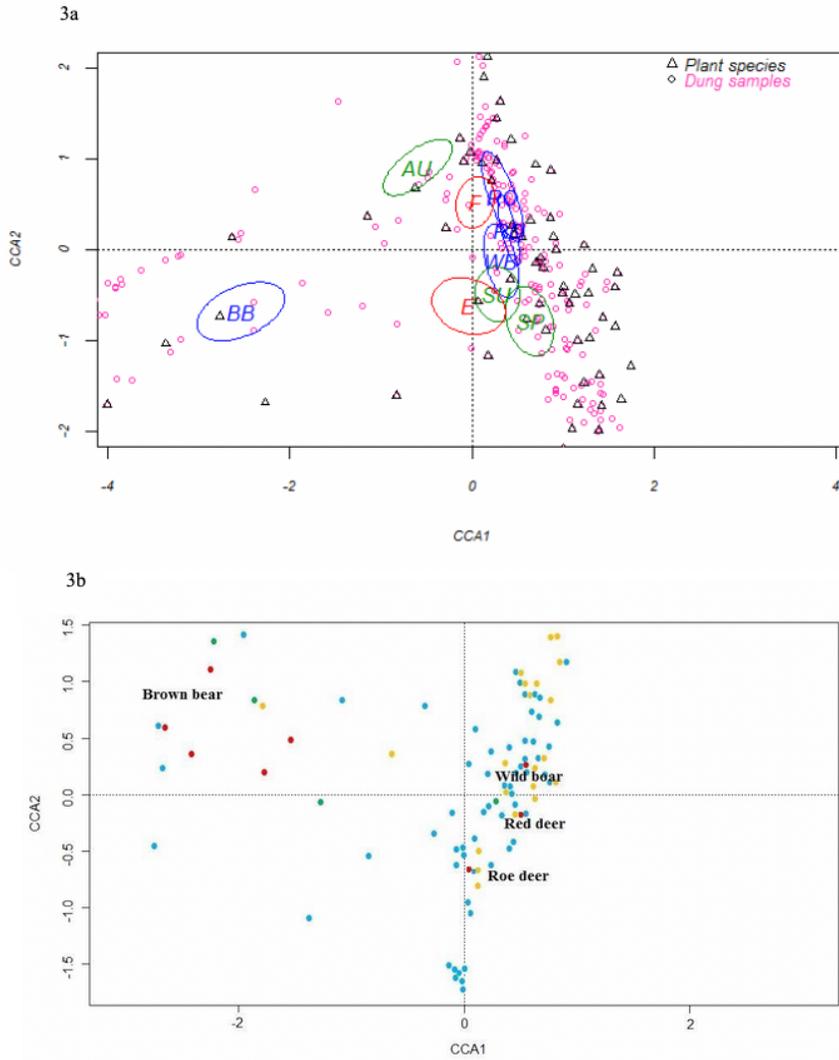


Figure 3
 Biplots showing the results of the canonical correspondence ordination analysis. The first plot (a) shows the position of each factor (season and habitat and animal vector) on the first two CCA axes of dispersed plant species and dung samples and the second (b) shows the position of each animal vector on the same axes in the dispersed growth form space. Animal vectors: RO = Roe deer, RE = Red deer, BB = Brown bear, WB = Wild boar in blue ellipses; Season: AU = Autumn, SU = Summer, SP = Spring in green ellipses; and habitat: F = Forest, E = prairie-forest Ecotone in red ellipses.

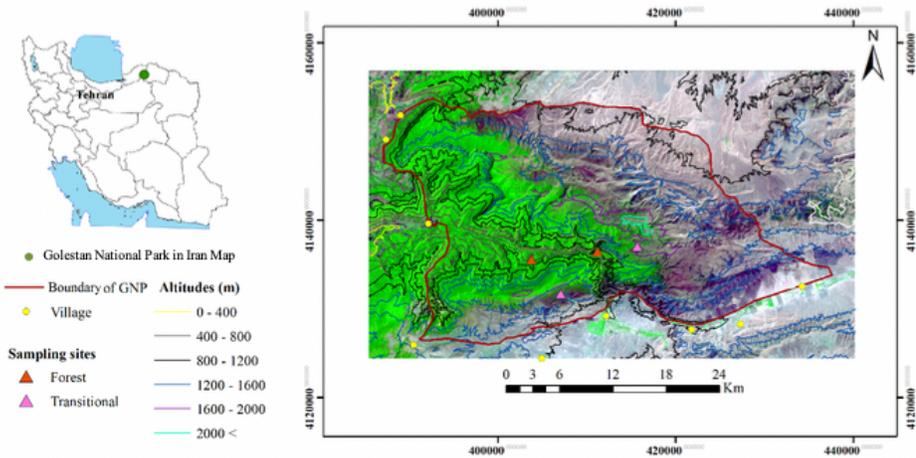


Figure 4
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Location of Golestan National Park, highlighting the Hyrcanian forests (in green) in the half western part and the surrounding steppes towards the east, north and south. Transitional vegetation zones occur in between and at high altitudes.

Supplementary Files

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