The role of the brown bear *Ursus arctos* as seed disperser: a case study with the bilberry *Vaccinium myrtillus*

Rola niedźwiedzia brunatnego *Ursus arctos* w rozprzestrzenianiu nasion: studium przypadku na przykładzie borówki czarnej *Vaccinium myrtillus*

PhD thesis
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To the memory of José Ignacio and Javier Rodríguez Val

Female brown bear with two cubs of the year feeding on bilberry fruits in Tatra National Park (July 2020)

"They thought they were burying you,

they did not know they were burying a seed"

Ernesto Cardenal, Nicaraguan priest, poet and politician

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CONTENTS

SUMMARY

Seed dispersal is an essential ecosystem service that, in temperate and boreal regions, is mainly provided by birds and mammals. Frugivores usually differ in their effectiveness as seed dispersers as a result of their different quantitative and qualitative contributions to seed dispersal. Long-distance seed dispersal events, mostly carried out by large frugivores, are key to guarantee gene flow among plant populations and the colonization of habitats, especially for clonal plants that rarely show seedling recruitment. The brown bear *Ursus arctos* is one of the most widely distributed terrestrial mammals and one of the few large-sized frugivorous species in non-tropical regions. In this PhD I evaluated the role of brown bears as seed dispersers, in particular of the bilberry *Vaccinium myrtillus*, a slow-growing clonal shrub common in Eurasian temperate and boreal regions and an important food resource for brown bears and many other animal species.

In Paper I I investigated the effectiveness of brown bears as seed dispersers worldwide. I analyzed their quantitative importance by extracting information about the species of fleshy fruits consumed and their importance in brown bears' diet from published data of 96 study areas. Brown bears consumed worldwide more than a hundred fleshy-fruited plant species whose fruits represented a quarter of the annual bears' diet. The bilberry was the second most commonly fruit eaten, only surpassed by the crowberry *Empetrum nigrum*, and it was consumed in most study areas located in Eurasian temperate and boreal regions. Ex-situ germination experiments with 11 species of fleshy fruits commonly eaten by brown bears (including the bilberry) were performed to check the quality of the seed dispersal service provided by the species. Ingestion by brown bears rarely damaged the seeds (99% of bilberry seeds were viable after bear ingestion), which usually germinated better than when embedded within the pulp. This study shows that, being one of the few megafaunal species inhabiting non-tropical areas, brown bears are pivotal seed dispersers across the entire range of the species.

In Paper II I analyzed the importance of fleshy fruits, and specifically of the bilberry, in the feeding ecology of brown bears inhabiting Tatra National Park (southern Poland). I assessed diet composition with DNA metabarcoding techniques in 246 bear scats collected between 2017 and 2019. Fleshy-fruited plant species were present in 56% of scats and across the whole bear activity period. The bilberry was the most common food (present in 42% of samples), followed by other fleshy-fruited plant species, the raspberry *Rubus idaeus* (20%). The bilberry was especially important during hyperphagia (from July to October), being detected in up to 73% of the scats in August. The prevalence of fleshy fruits in the diet of Tatra brown bears suggest that, even in areas highly humanized as Tatra National Park, bears may still provide essential ecosystem services such as seed dispersal.

In Paper III I analyzed the effectiveness of brown bears as bilberry dispersers in Tatra National Park in relation to the other frugivores inhabiting the area. To do that, in 2017 and 2018 I collected mammal scats and bird droppings containing bilberry seeds during transect inspections in coniferous forests and alpine meadows. Mammals were identified visually from their scats, and birds by DNA barcoding techniques. I counted the numbers of seeds in each faecal sample and conducted ex-situ germination experiments to address the quantity and quality of the bilberry dispersal services provided by each species. Among the three mammal and 13 bird species dispersing bilberry seeds in the study area, brown bears dispersed the vast majority of the seeds (more than 100,000 seeds per hectare and month). When quantitative and qualitative contributions were combined, brown bears were also among the most effective bilberry dispersers in the area, only surpassed by two species of thrushes. Additionally, brown bears complemented the dispersal activities provided by avian dispersers by defecating large amounts of bilberry seeds in alpine meadows and at the end of the fruiting season- areas and periods where seed dispersal by birds was less prevalent.

In Paper IV I investigated how important brown bears and other guilds of seed dispersers are for bilberry seedling recruitment in natural conditions. In 2018 and 2019 I marked 33 brown bear, 17 mesocarnivore (foxes *Vulpes vulpes* and martens *Martes sp.*) and 12 passerine faeces containing bilberry seeds in Tatra National Park. I revisited the locations of the faeces in 2019 and 2020 and counted the number of bilberry seedlings emerging. Bilberry germination was associated to all bear, 88% of mesocarnivore and 50% of bird faeces. The largest number of bilberry seedlings germinated from bear scats (154 seedlings/m^2); this was significantly higher than in scats of the other two guilds of dispersers and in control plots. Up to 16% of the seedlings germinated from bear scats survived at least one year after germination. Additionally, seedling density was significantly higher when associated to bear scats located in bear daybeds than in animal paths. Thus, brown bear resting behavior in daybeds, which usually involves the creation of small disturbances in the soil, enhanced bilberry germination. This study shows that bilberry seedling germination and survival is common when associated to faeces of frugivores, including brown bears, which implies that, contrary to previous suggestions, repeated bilberry seedling recruitment may occur in nature. Endozoochory, and especially specific behaviors of frugivores that create suitable conditions for bilberry germination, must not be neglected when analyzing reproductive strategies of plants, especially in the case of clonal species.

The results of this PhD demonstrate the importance of brown bears as bilberry seed dispersers and suggest that this may apply for all areas where both species coexist. Some features of brown bears such as the long distances they can travel and the disturbances they create in their resting sites, together with the large amounts of seeds they defecate in a single scat and the matching between their hyperphagia period and the bilberry fruiting season, are key to understand the singularity of the seed dispersal services provided by the species, especially considering that other large-sized frugivores are often missing in areas where brown bears are present. Proper management and conservation strategies are key to guarantee the essential ecosystem services brown bears still provide.

STRESZCZENIE

Rozsiewanie nasion jest podstawową usługą ekosystemową, którą w regionach o klimacie umiarkowanym i borealnym dostarczają głównie ptaki i ssaki. Zwierzęta owocożerne zwykle różnią się efektywnością, w wyniku ilościowego i jakościowego zróżnicowania ich udziału w rozprzestrzenianiu nasion. Zdarzenia rozsiewania nasion na duże odległości, realizowane głównie przez duże zwierzęta owocożerne, są kluczowe dla zagwarantowania przepływu genów pomiędzy populacjami roślin oraz kolonizacji siedlisk, szczególnie w przypadku roślin klonalnych, które rzadko wykazują rekrutację siewek. Niedźwiedź brunatny *Ursus arctos* jest jednym z najbardziej rozpowszechnionych ssaków lądowych i jednym z nielicznych dużych gatunków owocożernych w regionach nietropikalnych. W niniejszym doktoracie oceniałem rolę niedźwiedzi brunatnych jako rozsiewaczy nasion, zwłaszcza borówki czarnej *Vaccinium myrtillus*, która jest wolno rosnącym klonalnym krzewem, pospolitym w eurazjatyckich regionach umiarkowanych i borealnych, a także ważnym źródłem pokarmu dla niedźwiedzi brunatnych i wielu innych gatunków zwierząt.

W artykule nr I zbadałem efektywność niedźwiedzi brunatnych jako rozsiewaczy nasion w skali globalnej. Przeanalizowałem ich znaczenie ilościowe, pozyskując informacje o gatunkach owoców mięsistych i ich znaczeniu w diecie niedźwiedzi brunatnych z opublikowanych danych z 96 obszarów badawczych. Niedźwiedzie konsumowały ponad sto gatunków roślin o mięsistych owocach i owoce te stanowiły jedną czwartą ich rocznej diety. Borówka była drugim najczęściej spożywanym owocem, ustępując jedynie bażynie *Empetrum nigrum*, i była spożywana w większości obszarów badawczych położonych w eurazjatyckich regionach umiarkowanych i borealnych. Przeprowadzono doświadczenia kiełkowania *ex situ* z 11 gatunkami mięsistych owoców, powszechnie spożywanych przez niedźwiedzie brunatne (włączając w to borówkę czarną), aby sprawdzić jakość usługi rozsiewania nasion dostarczanej przez gatunek. Spożycie przez niedźwiedzia rzadko uszkadzało nasiona (99% nasion borówki zachowało żywotność), które zwykle kiełkowały lepiej niż gdy były osadzone w miąższu owocu. Badanie to pokazuje, że będąc jednym z nielicznych gatunków megafauny zamieszkujących obszary nietropikalne, niedźwiedzie brunatne są kluczowymi rozsiewaczami nasion w całym zasięgu gatunku.

W artykule nr II przeanalizowałem znaczenie owoców mięsistych, a szczególnie borówki czarnej, w ekologii żerowania niedźwiedzi brunatnych na terenie Tatrzańskiego Parku Narodowego. Stosując metody metabarkodingu DNA, określiłem skład diety na próbie 246 odchodów zebranych w latach 2017-2019. Owoce mięsiste występowały w 56% analizowanych prób i przez cały okres aktywności niedźwiedzi. Najczęściej obecna była borówka czarna (42% prób), a następnie malina *Rubus idaeus* (20% prób). Borówka czarna okazała się też szczególnie ważna podczas hiperfagii (od lipca do października) i była wykrywana nawet w 73% prób zebranych w sierpniu. Powszechne występowanie mięsistych owoców w diecie niedźwiedzi brunatnych sugeruje, że nawet

na obszarach tak silnie zhumanizowanych jak Tatrzański Park Narodowy, niedźwiedzie mogą nadal dostarczać podstawowe usługi ekosystemowe, takie jak rozsiewanie nasion.

W pracy nr III przeanalizowałem skuteczność niedźwiedzi brunatnych jako rozsiewaczy nasion borówki czarnej, w stosunku do innych owocożernych gatunków zwierząt występujących w Tatrzańskim Parku Narodowym. W tym celu, podczas badań wykonywanych w 2017 i 2018 roku na transektach wyznaczonych w lasach iglastych i na łąkach alpejskich, zbierałem odchody ssaków i ptaków zawierające nasiona borówki. Gatunki ssaków określiłem na podstawie wyglądu odchodów, a gatunki ptaków zostały oznaczone za pomocą metod metabarkodingu DNA. W każdej próbie kału policzyłem liczbę nasion i przeprowadziłem eksperymenty kiełkowania *ex situ*, aby zarówno ilościowo, jak i jakościowo określić usługi rozsiewania borówki czarnej świadczone przez każdy gatunek. Spośród trzech gatunków ssaków i 13 gatunków ptaków rozsiewających nasiona borówki na badanym terenie, niedźwiedzie brunatne rozsiały zdecydowaną większość (ponad 100 000 nasion na hektar miesięcznie). Przy łącznym potraktowaniu ilościowego i jakościowego wkładu w rozsiewanie, niedźwiedź brunatny okazał się również jednym z najskuteczniejszych rozsiewaczy nasion borówki czarnej na tym obszarze, ustępując jedynie dwóm gatunkom drozdów. Ponadto, niedźwiedzie brunatne uzupełniały usługi zapewniane przez ptasich rozsiewaczy poprzez wydalanie dużych ilości nasion borówki na łąkach alpejskich oraz pod koniec sezonu owocowania, czyli w obszarze i okresie, w którym rozsiewanie nasion przez ptaki jest mniej powszechne.

W artykule nr IV przeanalizowałem jaką rolę odgrywają niedźwiedzie brunatne i inne grupy rozsiewaczy nasion w rekrutacji siewek borówki czarnej w warunkach naturalnych. W 2018 i 2019 roku na terenie Tatrzańskiego Parku Narodowego oznaczyłem miejsca zdeponowania 33 odchodów niedźwiedzi brunatnych, 17 odchodów średnich drapieżników (lisów *Vulpes vulpes* i kun *Martes sp.*) oraz 12 odchodów ptaków wróblowych zawierających nasiona borówki. W 2019 i 2020 roku sprawdziłem oznaczone miejsca i policzyłem obecne siewki borówki. Kiełkowanie borówki zaobserwowałem na wszystkich odchodach niedźwiedzi, na 88% odchodów średnich drapieżników i na 50% ptasich odchodów. Najwięcej siewek borówki czarnej wykiełkowało z odchodów niedźwiedzia (154 siewki/m2), istotnie więcej niż w przypadku dwóch pozostałych grup rozsiewaczy nasion oraz na poletkach kontrolnych. Maksymalnie 16% siewek, które wykiełkowały z odchodów niedźwiedzi przetrwało co najmniej rok po wykiełkowaniu. Dodatkowo, zagęszczenie siewek było istotnie większe, gdy dotyczyło odchodów niedźwiedzi znajdującymi się w miejscach odpoczynku dziennego (barłogów), niż odchodów niedźwiedzi znajdujących się na ścieżkach przemieszczania się zwierząt. Tak więc, zachowanie niedźwiedzia brunatnego w miejscach dziennego odpoczynku, zwykle związane z powstawaniem niewielkich zaburzeń w glebie, wpływa korzystnie na kiełkowanie borówki czarnej. Badanie to pokazuje, że kiełkowanie nasion i przeżywalność siewek borówki czarnej w odchodach zwierząt owocożernych, w tym niedźwiedzi brunatnych, jest powszechnym zjawiskiem, co oznacza, że, wbrew wcześniejszym sugestiom, w przyrodzie może dochodzić do powtarzających się rekrutacji siewek borówki czarnej. Endozoochoria, w tym w szczególności specyficzne zachowania zwierząt owocożernych stwarzające dogodne warunki do kiełkowania borówki, nie mogą być lekceważone podczas analizowania strategii reprodukcyjnych roślin, zwłaszcza gatunków klonalnych.

Wyniki tego doktoratu pokazują, jak ważną rolę odgrywają niedźwiedzie brunatne w rozsiewaniu nasion borówki czarnej i sugerują, że rola ta może dotyczyć wszystkich obszarów, na których współistnieją oba gatunki. Niektóre aspekty biologii i ekologii gatunku, takie jak pokonywanie dużych odległości, dokonywanie przekształceń otoczenia w miejscach dziennego odpoczynku (barłogach), wraz z obecnością dużej ilości nasion w odchodach, które wydalane są podczas pojedynczego procesu defekacji oraz nakładanie się okresów występowania hiperfagii i sezonu owocowania borówki czarnej, stanowią klucz do zrozumienia wyjątkowości usług rozsiewania nasion zapewnianych przez niedźwiedzie, zwłaszcza biorąc pod uwagę, że na obszarach, na których występują, często brakuje innych dużych zwierząt owocożernych. Odpowiednie strategie zarządzania i ochrony mają kluczowe znaczenie dla zagwarantowania podstawowych usług ekosystemowych dostarczanych przez niedźwiedzie brunatne.

INTRODUCTION

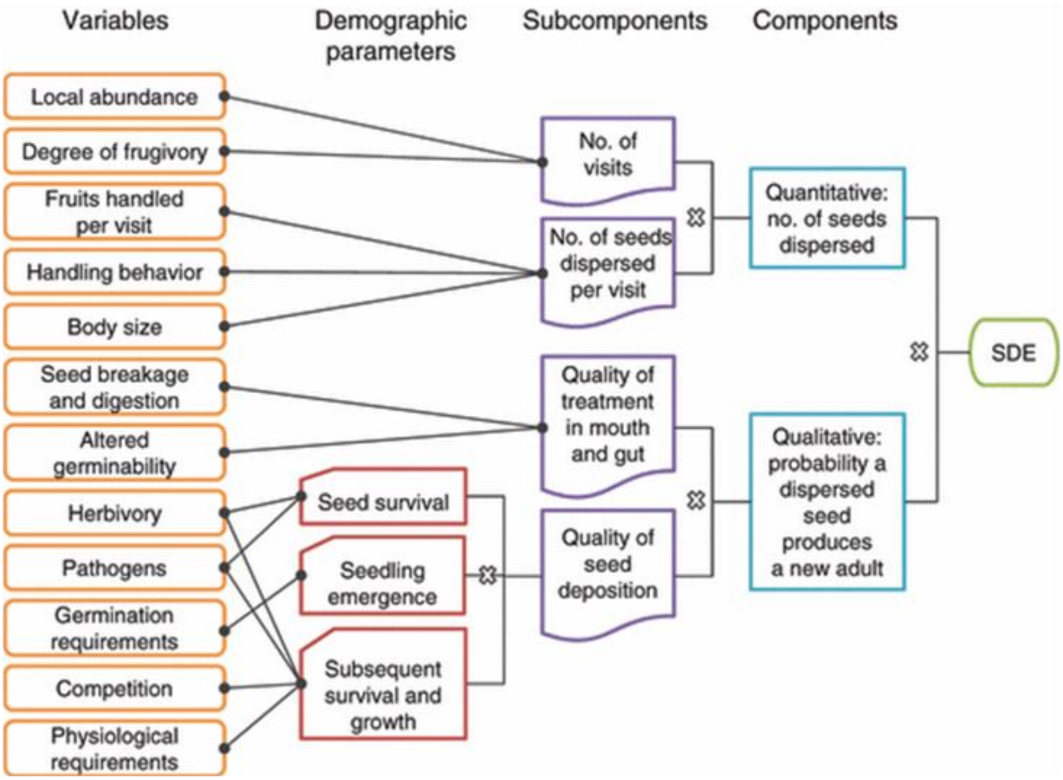
Female brown bear with two cubs feeding on bilberry fruits in the study area. Picture: Adam Wajrak

SEED DISPERSAL, FRUGIVORY AND ENDOZOOCHORY

Seed dispersal, i.e. the movement of seeds away from the parent plants, influences plant recruitment, colonization of new habitats and gene flow among plant populations. Consequently, it is a key stage in the regeneration process of plant populations (Jordano & Godoy, 2002; Bascompte & Jordano, 2007; Jordano et al., 2007; Traveset et al., 2014). The seed shadow, defined as the spatial pattern of seed distribution relative to parent trees, acts as the population recruitment surface and its shape depends on the dispersal strategy followed by each plant species (Janzen, 1970; Jordano & Godoy, 2002). Seeds can be dispersed by different means such as wind, gravity or water. However, zoochory or seed dispersal by animals represent the dispersal mean most frequently used by seed plants (Traveset et al., 2014). Animals can move seeds by ingestion and the subsequent regurgitation or defecation (endozoochory) or/and by the seeds being attached to the animal' fur, plumage or feet (epizoochory). Plants with seeds present different adaptations depending on their predominant seed dispersal mechanism. For instance, sugar-rich fruits are commonly produced by plant species dispersed by endozoochory (Herrera, 2002). Thus, frugivory and the subsequent endozoochory are processes that may have positive effects for both animals and plants (Janzen, 1984, Quintero et al., 2020).

The Seed Dispersal Effectiveness framework (SDE hereafter) was developed to standardize measurements of the contributions of dispersers belonging to different seed dispersal systems (Schupp, 1993). The total SDE of a disperser is usually understood as the number of new adults produced due to its dispersal activities, but other measurements such as the number of recruited seedlings have also been used (Schupp, 1993). Dispersers vary in their quantitative and qualitative contributions to SDE (Schupp, 1993; Schupp et al., 2010). The quantitative component relates to the number of seeds dispersed by a disperser agent and it depends on the number of visits made to a plant and the number of fruits and seeds consumed per visit. The qualitative component usually refers to the probability that a dispersed seed remains viable after animal ingestion (i.e. quality of mouth and gut treatment) multiplied by the probability that the seed will survive, germinate and produce a new adult in a given deposition site (Schupp, 1993; Schupp et al., 2010; Fig. 1a). SDE landscapes allow the location of different dispersers in a graph based on their respective SDEs and accounting for the different dispersal strategies followed by each disperser (i.e. their respective combinations of quantity and quality of seed dispersal, which results in a specific SDE, Fig. 1b).

a)


Variables
Demographic parameters
Subcomponents
Components
Local abundance
Degree of frugivory
Fruits handled per visit
Handling behavior
Body size
Seed breakage and digestion
Altered germinability
Herbivory
Pathogens
Germination requirements
Competition
Physiological requirements
Seed survival
Seedling emergence
Subsequent survival and growth
No. of visits
No. of seeds dispersed per visit
Quality of treatment in mouth and gut
Quality of seed deposition
Quantitative: no. of seeds dispersed
Qualitative: probability a dispersed seed produces a new adult
SDE

b)

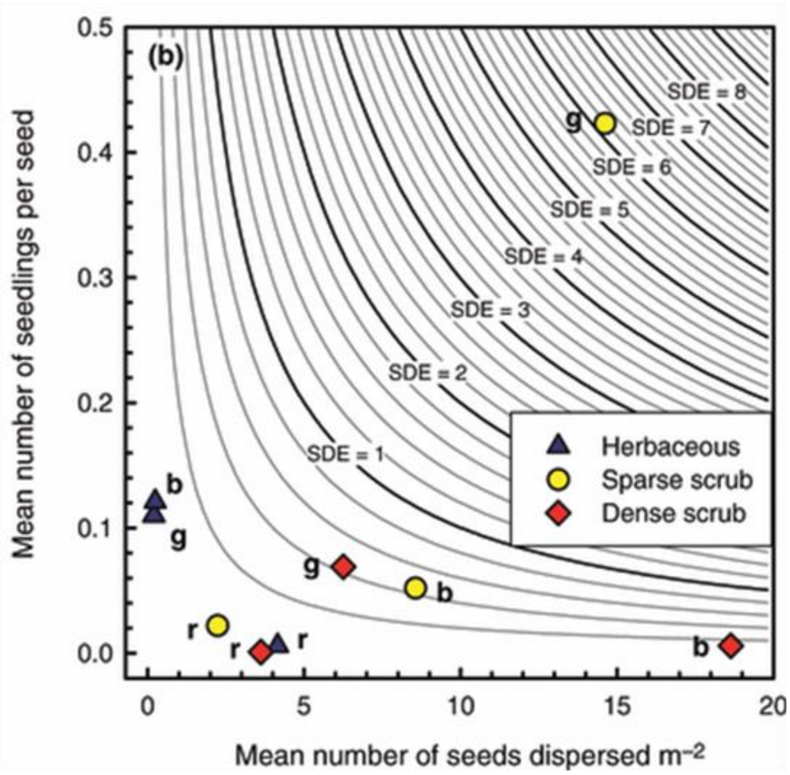

(b)
Mean number of seedlings per seed
Mean number of seeds dispersed m⁻²
0.0
0.1
0.2
0.3
0.4
0.5
0
5
10
15
20
SDE = 1
SDE = 2
SDE = 3
SDE = 4
SDE = 5
SDE = 6
SDE = 7
SDE = 8
▲ Herbaceous
○ Sparse scrub
◆ Dense scrub
b
g
r
g
b
r
g
r
r
b

Figure 1a) Flow chart representing the conditioning factors of seed dispersal effectiveness (SDE) in endozoochorous seed dispersal systems. The general framework for developing SDE studies is provided by the components and subcomponents. Demographic parameters refer to life stages that may determine the total quality component of a disperser. Measurable variables relevant for SDE studies are represented in the left part of the chart. Factors with multiplicative effects are interconnected with righ-angles lines and an "x" (e.g. the number of visits × the number of seeds dispersed per visit = the number of seeds dispersed). Variables affecting not multiplicatively to components or subcomponents are linked to them with straight lines and closed circles. Figure 1b) Tentative example of a well studied SDE landscape. The example, constructed based on data published in Calviño-Cancela & Martín-Herrero (2009), shows recruitment of *Corema album* in three adjacent habitats in northeastern Spain. Each geometric form (triangles, circles, diamonds) represents a different habitat, whereas each letter (r, b, g) represents a different dispersal agent. The quantitative component (x axis) is measured as the mean number of seeds dispersed per m^2 by each agent in a given habitat whereas the qualitative component (y axis) refers to the probability of seedling emergence per seed as result of the dispersal activities of each disperser in a given habitat. Both figures are extracted from Schupp et al., 2010.

Different dispersers agents may show different qualitative and quantitative contributions to SDE due to differences related to body size, population densities, dietary patterns, animal behavior and other related factors (Schupp, 1993; Schupp et al., 2010). Thus, differences in the quantity and/or quality of the SDE provided by different functional groups of dispersers is likely to be considerable and relevant. Birds and mammals are the most important seed dispersers, especially in temperate and boreal regions (Jordano, 2000; Albrecht et al., 2013; Tsuji et al., 2016). Even when fruit consumption by most avian and mammalian dispersers usually has either positive or neutral effects in seed germination (Traveset, 1998), the number of seeds, which affects seedling germination and survival, is usually much larger in mammal scats than in bird droppings. Large numbers of seeds in a single scat may attract seed predators and secondary dispersers (Enders & Vander Wall, 2012) and, after germination, intense competition is likely to occur among siblings (Lewis, 1987; Loiselle, 1990). On the other hand, more nutrients over longer periods are provided to seeds contained in large mammal scats than within small bird droppings. Additionally, large frugivores can disperse seeds from different species in a single scat whereas faeces of smaller frugivores, such as passerines, usually contain seeds of only one or two species (Traveset et al., 2007).

The shape of the seed shadow (the spatial distribution of seeds from the mother plant) determines the genetic structure of plant populations by influencing gene flow and recruitment (Jordano & Godoy, 2002). Based on differences in frugivores' foraging behavior, gut retention times and patterns of fruit selection, seeds located in different portions of a given seed shadow are most often dispersed by different species (Herrera, 1995; Jordano et al., 2007). As dispersal distance is directly constrained by body size (Santini et al., 2013), long distance dispersal events usually rely on a small subset of large species, even if smaller species are responsible for the majority of the total seed rain provided in the area. For instance, the vast majority of long-distance dispersal events in Mediterranean, temperate and boreal regions are carried out by mammals and by large birds (Jordano et al., 2007; Tsuji et al., 2016; Fig 2). Population declines of these key frugivorous species, including megafauna (i.e comparatively large animal species that have strong effects on ecosystems, present distinctive functional traits and habitat requirements and have escaped most-non anthropogenic predation when adults; Moleón et al., 2020), may seriously impair seed-mediated gene flow in fragmented landscapes by truncating the long-distance events and collapsing seed arrival to a restricted subset of available microsites (Jordano et al., 2007).

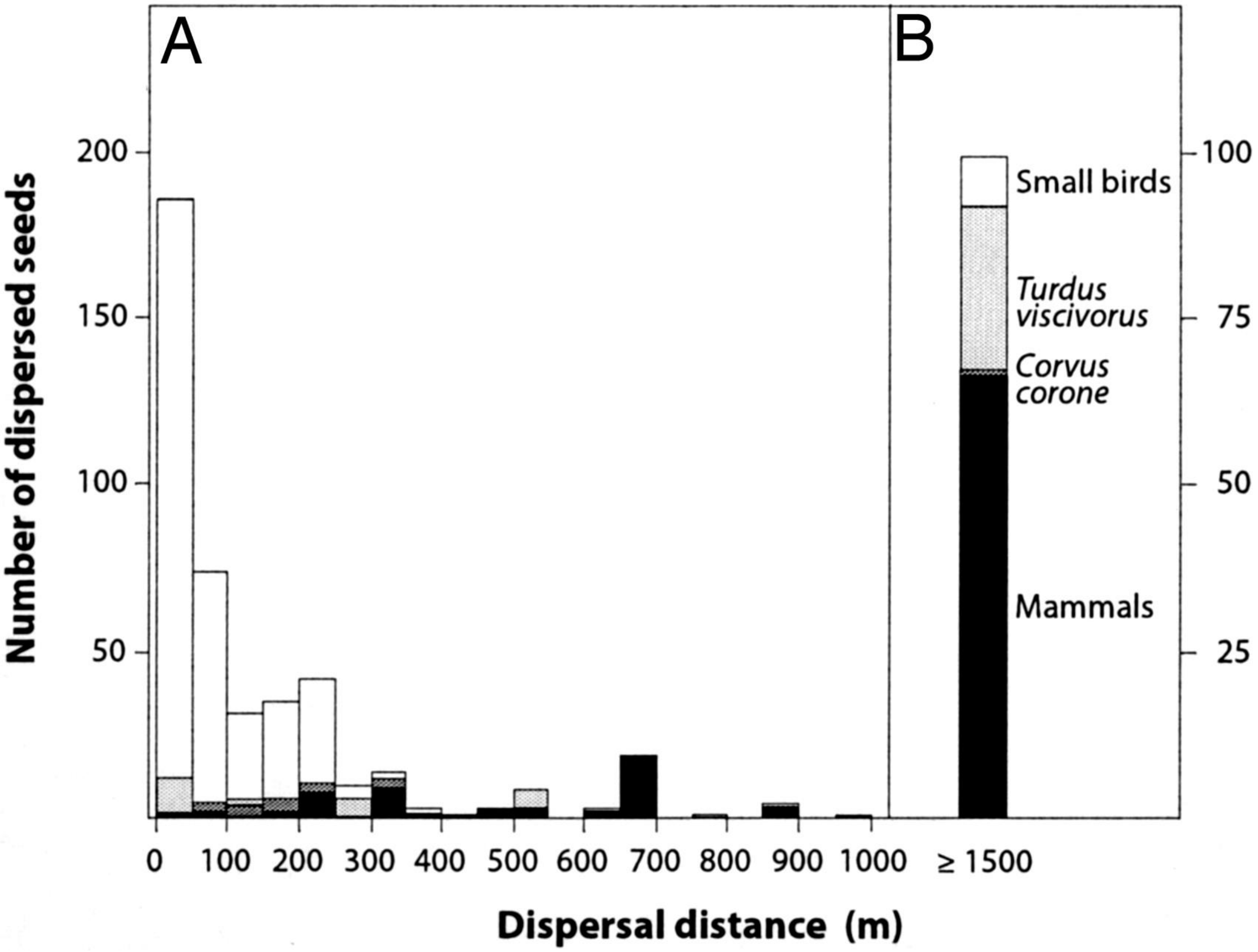


Fig. 2. Different disperser guilds contribute differently to each portion of the seed shadow. The example shows a) the number of seeds of *Prunus mahaleb* dispersed by mammals (black), carrion crows *Corvus corone* (dark grey), mistle thrushes *Turdus viscivorus* (light grey) and small birds (white) according to the distance to the parent tree in a Mediterranean forest of southern Spain and b) percentage of immigrant seeds (i.e. seeds becoming from other populations located further than 1,500 m away from the studied population) defecated by each disperser guild in the same study area. Mammals were responsible for most of the immigrant seeds, whereas small birds were responsible for the vast majority of the short-distance seed dispersal events. Extracted from Jordano et al., 2007.

Despite the essential role of large frugivores as seed dispersers, most studies about frugivory and seed dispersal have traditionally focused on birds and small- to medium-sized mammals (Herrera, 2002; Vidal et al., 2013). However, an increasing number of studies about seed dispersal by extant megafauna have been published, yet focused primarily in tropical areas (Vidal et al., 2013). Thus, further research about the contributions of large frugivores, and particularly megafauna, to seed dispersal is still required, especially in temperate and boreal regions.

STUDY SPECIES

- **The bilberry *Vaccinium myrtillus***

The bilberry (Ericaceae) is a deciduous, rhizomatous clonal shrub widely distributed in temperate and boreal regions of Eurasia, usually associated to acidic soils (Ritchie, 1956). It spreads mainly by clonal propagation, primarily after soil disturbances (Schimmel & Granström, 1996), with rhizomes usually located 15 to 20 cm deep into the soil (Ritchie, 1956). Leaves are elliptical, bright green and up to 3 cm long. Bilberry flowers are pink, bell-shaped and appear on one-year twigs, usually during spring, but a second less intense flowering season may happen during late summer (personal observation). Fruits are dark-blue to purple berries, with average diameter and weight of 0.5-1 cm and 0.25-0.5 g, respectively (Eriksson & Ehrlén, 1991; Ranwala & Naylor, 2004; Fig. 3). Each fruit contains several dozens of seeds (mean and maximum = 52 and 120 seeds respectively, personal observation). In temperate regions, bilberry fruiting season starts during mid-July at low elevations, with populations at elevations above 2,000 meters above sea level (m.a.s.l.) ripening as late as early September (personal observation).

Bilberry fruits are a key food for many animal species, including megafaunal frugivores like the brown bear *Ursus arctos*, mesocarnivores (e.g. red foxes *Vulpes vulpes* and martens *Martes spp*.), grouses (e.g. western capercaillie *Tetrao urogallus*, black grouse *Lyrurus tetrix*, hazel grouse *Tetrastes bonasia)* and passerines (Schaumann & Heinken, 2002; Honkavaara et al., 2007; Blanco-Fontao et al., 2010; Hertel et al., 2018). It is one of the economically most important wild berry species worldwide due to its large production of sugar-rich fruits and demand for human consumption (Nestby et al., 2011). Despite bilberry fruits being heavily consumed by frugivores, which additionally seldomly damage the seeds during ingestion (Honkavaara et al., 2007), seedling recruitment seems to be scarce within conspecific adult stands and appears usually restricted to open gaps with high moisture and organic soil content (Eriksson & Fröborg, 1996; Hegland et al., 2010). Due to this, certain substrates such as bare soil or decaying wood may facilitate recruitment at “windows of opportunity”, i.e. spatially or temporally unpredictable conditions in which seedling establishment is possible within stands of conspecific adults (Eriksson & Fröborg, 1996; Fig. 3). Some specific behaviors of bilberry consumers such as marking behavior in fallen trees or logs by mesocarnivores or defecation by brown bears next to their resting sites, where they usually create local disturbances in the ground by digging out the vegetation (Fig. 4), have been suggested to direct seed dispersal to specific deposition sites suitable for bilberry recruitment (Schaumann & Heinken, 2002; Steyaert et al., 2019).

Figure 3. Common appearance of bilberry fruits and leaves (top) and bilberry recruitment in the base of a cut Norway spruce *Picea abies*, illustrating an example of decaying wood as a suitable deposition site for bilberry recruitment (bottom). Pictures taken by Alberto García-Rodríguez and Nuria Selva.

- **The brown bear *Ursus arctos***

The brown bear (Order: Carnivora, Family: Ursidae) is one of the world's most widely distributed terrestrial mammals and the largest living terrestrial carnivore. The species was historically present in most of the Holarctic and still nowadays inhabits a broad variety of biomes, from tundra to deserts (Pasitschniak-Arts, 1993). Its heavy body mass and large habitat requirements, a distinctive reproductive biology when compared to other carnivores (e.g. late first reproduction in females and longer maternal care, Steyaert et al., 2012) and the lack of non-human natural predators when adults make the species a good representative of megafauna inhabiting northern latitudes. Brown bears' annual cycle may be divided into three different physiological states: (1) a winter dormancy period (lasting from three to seven months depending on the latitude), (2) a hypophagia period, when they emerge from winter dens in spring and start consuming small amounts of food until mid-summer, and (3) a hyperphagia period, characterized by a progressive increase of food intake that usually lasts until mid-autumn, when they enter again into the den for winter dormancy.

Food habits are crucial in brown bear ecology and behavior, especially during hyperphagia as they must gain fat reserves to meet the energetic requirements for hibernation during winter. Brown bears are omnivores and can adapt their dietary patterns to seasonal changes in food availability. Thus, their trophic niche is very flexible and strongly related to environmental conditions, with populations in warm and highly productive environments being almost completely herbivorous, whereas populations in cold, unproductive environments are more carnivorous (Bojarska & Selva, 2012). During hyperphagia, brown bears feed intensively on fleshy fruits, especially in those populations inhabiting boreal, temperate and Mediterranean areas (Bojarska & Selva, 2012). Resource availability, and more specifically bilberry abundance, is known to influence demographic parameters of brown bears (e.g. reproductive success, litter size, the age of first reproduction) and ecological features such as habitat selection or population density (Blanchard, 1987; Palomero et al., 1997; Welch et al., 1997; Hildebrand et al., 1999; McLoughlin et al., 2000; Hertel et al., 2016; Hertel et al., 2018). This indicates that the bilberry and other fleshy fruits are key food resources for the species. Besides the important effects that fleshy fruits have in brown bears' biology, brown bears may also benefit the fleshy-fruited plant species they consume as bear ingestion allows the germination of their seeds (Traveset & Willson, 1997; Willson & Gende, 2004; Steyaert et al., 2019). Additionally, a single brown bear scat can contain up to several thousand *Vaccinium* seeds that can be deposited over long distances (Willson & Gende, 2004; Lalleroni et al., 2017; Fig. 4). All this, together with the already mentioned bear resting behavior, suggests that the prevalence of fleshy fruits such as the bilberry in the diet of brown bears is not only essential for these animals but also for the population dynamics of the species they consume. However, our understanding of this mutualistic relationship is still poorly known, with the majority of the previous research being conducted, at least partially, in controlled environments. Thus, more field studies, such the ones I am presenting in this thesis, must be conducted in order to get a deeper knowledge of the real seed dispersal effectiveness of brown bears in natural conditions.

Figure 4. A female brown bear with two cubs of the year in a bilberry field (top), a brown bear daybed (bottom-left) and a brown bear scat containing bilberry leaves and seeds (bottom-right). Pictures taken by Alberto García-Rodríguez.

STUDY AREA

The fieldwork for this thesis was conducted in Tatra National Park (southern Poland), a 211 km^2 protected area located in the Tatra Mountains, a mountain range formed during the Alpine orogeny that belongs to the western part of the Carpathian Mountains and forms a natural border between Poland and Slovakia (Fig. 5). In the Polish side, mainly composed by valleys and north-facing slopes, the Tatra Mountains range from 774 to 2,499 m.a.s.l., and the entire area is under protection as national park. Snow cover lasts for about 100 days in lower parts and up to 290 days in the mountain tops. Mean annual temperatures are 5.4 and 2.4°C at the lowest elevations and timberline areas (1,550 m.a.s.l.), respectively. Precipitation increases with elevation and averages 1,100 mm for the lowest elevations and 1,700 mm for the timberline (Mirek & Piekos-Mirkowa, 1992).

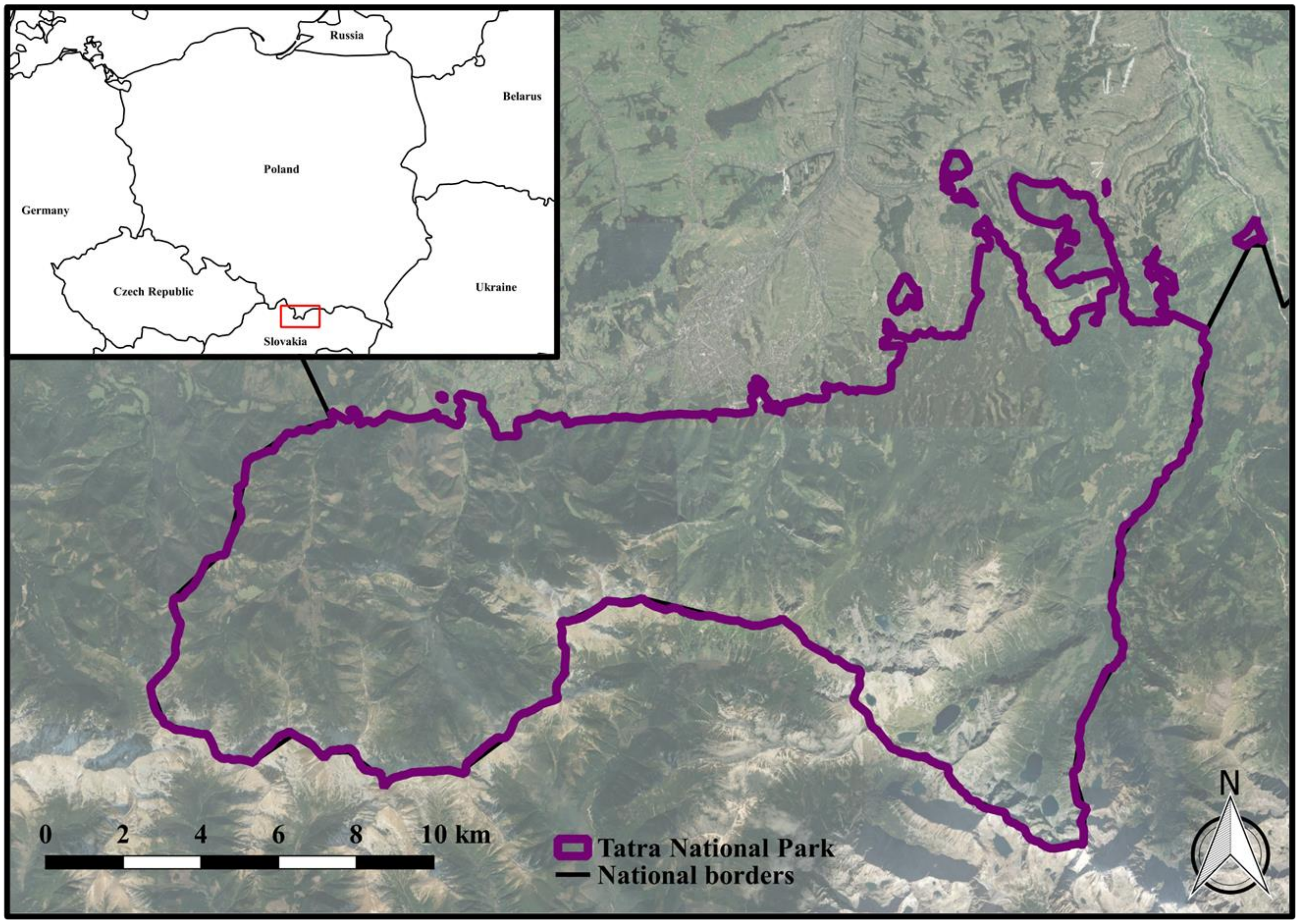


Figure 5. Map showing the location of the study area, the Tatra National Park, in Poland.

The entire surface of the Tatra Mountains lies in the temperate conifer forests terrestrial biome (Olson et al. 2001). Four different elevation zones can be found in the area: montane (700 – 1,550 m.a.s.l.), subalpine (1,550 – 1,800), alpine (1,800 – 2,300) and subnival zone (from 2,300 m a.s.l.; Mirek & Piekos-Mirkowa, 1992). Montane forests cover around 60% of the total surface of the study area and are dominated by Norway spruce *Picea abies*, partly introduced in the original habitat of silver fir *Abies alba* and European beech *Fagus sylvatica* during the 19th century (Matysek et al., 2019). Dwarf pines *Pinus mugo* are abundant in the subalpine zone. Montane, subalpine and alpine floors are dominated by bilberries (Fig. 6). Other shrub species such as the lingonberry *Vaccinium vitis-idaea*, the bog bilberry *V. uliginosum*, the mountain bilberry *V. gaultheriodes* and the red raspberry *Rubus* *idaeus* can be found at different elevations, but in smaller numbers (Mirek & Piekos-Mirkowa, 1992). More than 200 plant species and 90 endemisms have been described in the area. Acidic podzols upon granite bedrocks constitute the majority of the soils in the area (Mirek & Piekos-Mirkowa, 1992).

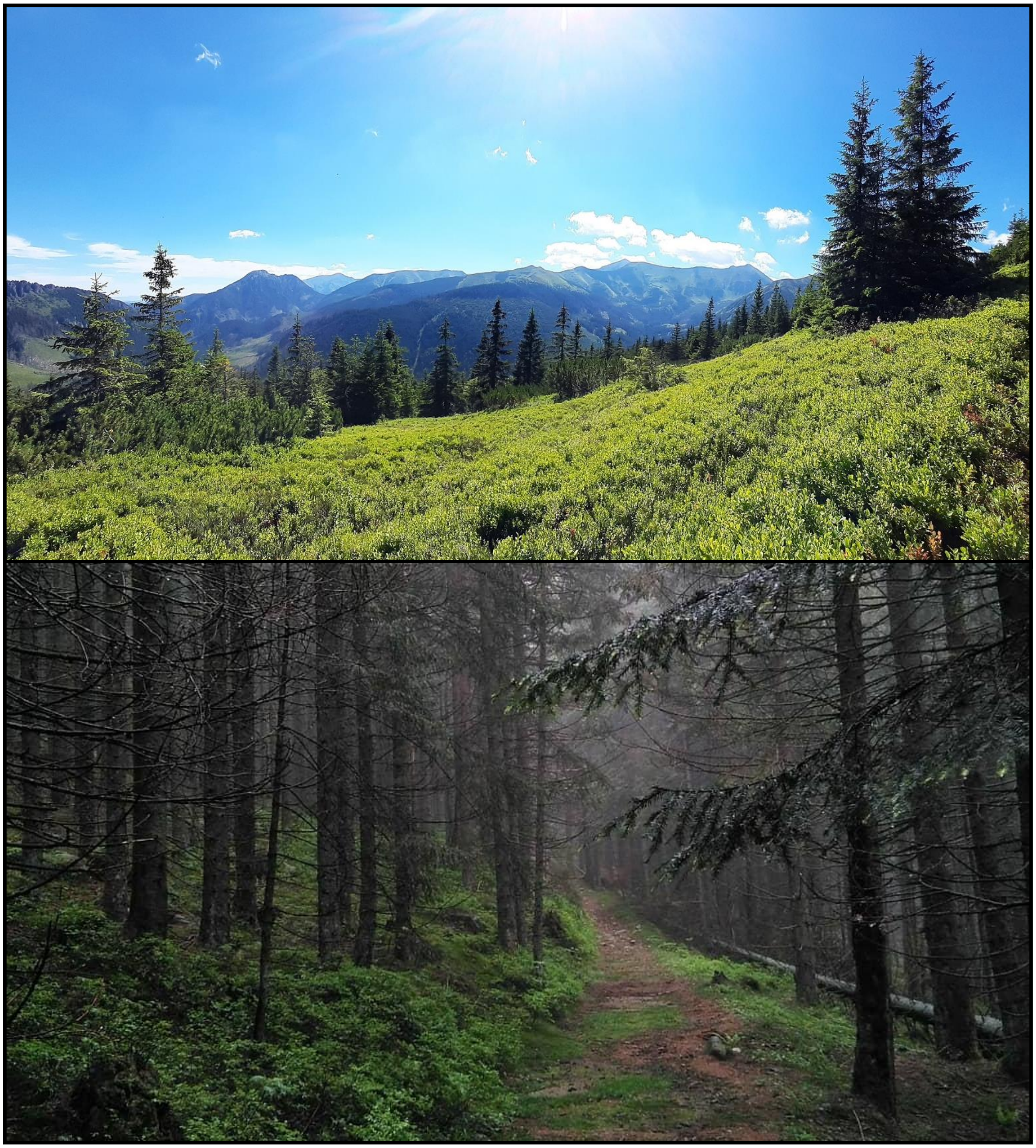


Figure 6. Landscape of the two elevation zones where most of the fieldwork was conducted. Top picture: alpine meadows predominate in the subalpine zone (1,550 – 1,800 m.a.s.l.). Bottom picture: Norway spruce forests dominate the landscape in the montane zone, below 1,550 m.a.s.l., which is the average elevation of the timberline. At ground level, bilberry shrubs dominate in both montane and subalpine elevation zones in the study area. Pictures taken by Alberto García-Rodríguez

The fauna of the Tatra Mountains is rich and comprise many species of both invertebrates and vertebrates, some of which are endemic. The region is inhabited by up to 40 mammal species, including the presence of ungulates, such as Tatra chamois *Rupicapra rupicapra tatrica*, red deer *Cervus elaphus* and roe deer *Capreolus capreolus*, marmots (*Marmota marmota*), red foxes, pine and stone martens *Martes martes* and *M. foina* and large carnivores such as brown bears, Eurasian lynx *Lynx lynx* and grey wolves *Canis lupus* (Fig. 7). The Tatra brown bear population segment is transboundary, with many animals moving regularly between Poland and Slovakia (Bartoń et al., 2019). The number of bears inhabiting Tatra National Park is estimated in 45-79 individuals, thus, the density is quite high (Konopiński et al., 2019). The area holds up to 200 different bird species, including some endangered ones, such as the three-toed woodpecker (*Picoides tridactylus*), the black grouse and the western capercaillie. Some of these avian species are important seed dispersers known to feed on bilberry fruits (Fig. 8). Many frugivorous animals present in the area, such as brown bears, red foxes, martens, grouses and thrushes, are known to be important seed dispersers in other study systems (Honkaavara et al., 2007; Blanco-Fontao et al., 2010; Albrecht et al., 2013; González-Varo et al., 2014; Lalleroni et al., 2017).

Figure 7. Up to 40 different mammal species are present in Tatra National Park. The Tatra chamois (top) is the symbol of the national park and can be found grazing the vegetation, usually in the subalpine and alpine zones. Marmots (bottom-left) and red deer (bottom-right) are also among the most emblematic mammal species found in the area. Pictures taken by Alberto García-Rodríguez.

Figure 8. Small to medium-sized frugivorous birds known to consume bilberry fruits such as ring ouzels *Turdus torquatus* (top-left), fieldfares *T. pilaris* (top-right), blackbirds *T. merula* (bottom-left) and Eurasian robins *Erithacus rubecula* (bottom-right) are present in the study area. Pictures taken by Alberto García-Rodríguez.

Tatra National Park is one of the most popular touristic destinations in Poland, currently visited by more than 3.5 million tourists annually, with an increasing trend in the recent years. The number of visitors peaks between July and mid-September (up to 40,000 people daily, https://tpn.pl/zwiedzaj/turystyka/statystyka), coinciding with the period where brown bears rely the most on bilberry fruits in the area. Tourists' presence is only allowed along touristic trails and in resting areas. However, the high abundance of these infrastructures yields a density of more than 1 km of public paths per km^2 within the protected area. Berry picking within the national park is only allowed under special permits (e.g. for scientific research), but illegal picking still happens both next to the public paths and in more restricted areas (personal observation). No artificial feeding of wildlife is conducted within the national park (Zwijacz-Kozica et al., 2017). However, artificial feeding sites for ungulates are found in the surroundings of the national park in small numbers in the Polish territory (personal observation), but in larger numbers in neighbouring areas in Slovakia (Rigg & Gorman, 2005).

OBJECTIVES OF THE THESIS

The main goal of this thesis was to assess the role that brown bears have as bilberry seed dispersers, addressing both the quantitative and qualitative components of the seed dispersal effectiveness provided by the species. To do that, I structured the research into four papers with their corresponding specific objectives.

Paper I

The main goal was to assess the role of brown bears as legitimate megafaunal seed disperser across their entire distribution range, considering both the quantitative and qualitative components of the seed dispersal effectiveness provided by the species. I specifically aimed to (1) identify all fleshy-fruited plant species eaten by brown bears worldwide, (2) evaluate the contribution of fleshy fruits in brown bear' diet across biomes, and (3) determine the effects of ingestion by brown bears on the proportion and speed of germination in 11 selected fleshy-fruited plant species commonly eaten by the species. I also explored the factors related to brown bears' biology and ecology that may influence their effectiveness as seed dispersers.

Paper II

Here I aimed to assess the relevance of the bilberry and other fleshy-fruited plant species in the diet of brown bears in Tatra National Park. Specific goals were to assess (1) the total diversity of food items consumed by brown bears, (2) the seasonal differences in their diet and (3) the effects that human disturbance may have in brown bears diet and, thus, in a relevant ecosystem service provided by brown bears - the dispersal of fleshy fruits.

Paper III

I determined the role of brown bears as bilberry dispersers in the Tatra Mountains in relation to the effectiveness of all bilberry dispersers in the study area. I specifically aimed to (1) estimate the quantitative, qualitative and combined -SDE- contribution of each bilberry disperser species to the total SDE landscape in Tatra National Park, (2) assess differences in the contributions of birds and mammals to the bilberry seed rain (i.e. number of bilberry seeds dispersed per hectare and month) according to the habitat (coniferous forests and alpine meadows) and the timing of seed dispersal (from July to October), (3) determine differences in the SDE between avian and mammalian dispersers in relation to body size and (4) check the SDE component -quantity or quality- that is a better surrogate of the total SDE of the different bilberry dispersers.

Paper IV

The main goal was to evaluate how important are brown bears and other seed disperser guilds (mesocarnivores, small to medium-sized passerines) for bilberry recruitment in natural ecosystems. Specific goals were to assess (1) the frequency and intensity of bilberry seedling germination from faeces of brown bears and other frugivores in the area, (2) the survival of bilberry seedlings emerged from brown bear faeces and (3) the differences in the bilberry germination in brown bear faeces located in animal paths and in the disturbances created at their resting sites.

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Female brown bear with two cubs feeding on bilberry fruits in the study area. Picture: Adam Wajrak

The role of the brown bear *Ursus arctos* as a legitimate megafaunal seed disperser

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ABSTRACT

Megafaunal frugivores can consume large amounts of fruits whose seeds may be dispersed over long distances, thus, affecting plant regeneration processes and ecosystem functioning. We investigated the role of brown bears (*Ursus arctos*) as legitimate megafaunal seed dispersers. We assessed the quantity component of seed dispersal by brown bears across its entire distribution based on information about both the relative frequency of occurrence and species composition of fleshy fruits in the diet of brown bears extracted from the literature. We assessed the quality component of seed dispersal based on germination experiments for 11 fleshy-fruited plant species common in temperate and boreal regions and frequently eaten by brown bears. Across its distribution, fleshy fruits, on average, represented 24% of the bear food items and 26% of the total volume consumed. Brown bears consumed seeds from at least 101 fleshy-fruited plant species belonging to 24 families and 42 genera, of which *Rubus* (Rosaceae) and *Vaccinium* (Ericaceae) were most commonly eaten. Brown bears inhabiting Mediterranean forests relied the most on fleshy fruits and consumed the largest number of species per study area. Seeds ingested by bears germinated at higher percentages than those from whole fruits, and at similar percentages than manually depulped seeds. We conclude that brown bears are legitimate seed dispersers as they consume large quantities of seeds that remain viable after gut passage. The decline of these megafaunal frugivores may compromise seed dispersal services and plant regeneration processes.

KEYWORDS

Ursus arctos, megafauna, seed dispersal effectiveness, long-distance seed dispersal, fleshy fruits, plant-animal mutualisms.

INTRODUCTION

Seed dispersal (i.e. the movement of seeds away from the parent plants) is essential for plant recruitment, colonization of habitats, gene flow among populations and plant community dynamics (Cain et al., 2000; Cousens et al., 2008). Across different biomes, a large proportion of vascular plant species depends on frugivorous animals for the dispersal of their seeds (Jordano, 2000). The spatial distribution of seeds dispersed by frugivores is strongly affected by animal species body size and mobility (Jordano et al., 2007; Bueno et al., 2013; Pérez-Méndez et al., 2016). Large frugivore species seem to be particularly important in connecting plant populations by increasing gene flow via dispersed seeds (Hamrick et al., 1993; Mueller et al., 2014; Pérez-Méndez et al., 2018).

Beyond differences in their mobility across landscapes, frugivores also vary in their seed dispersal effectiveness, which depends on the qualitative and quantitative contribution to seed dispersal services (Schupp, 1993; Schupp et al., 2010). The quantity component is defined as the number of seeds dispersed, which is determined by both the number of interactions between a disperser agent and a fruiting plant species and the number of seeds removed by a disperser per interaction. The quality component is traditionally defined as the probability that a dispersed seed will germinate, survive and grow to an adult plant, which is determined by the combined effects of fruit handling, gut passage treatment and seed deposition in suitable microhabitats (Schupp, 1993). Legitimate seed dispersers are usually defined as true mutualist agents that combine a high quality and quantity of seed dispersal and, thus, strongly impact regeneration processes and population dynamics of the dispersed plant species (Traveset & Richardson, 2014).

Traditionally, most studies about frugivory and seed dispersal have been focused on birds and small- to medium-sized mammals (Herrera, 2002; Vidal et al., 2013). In the last two decades, an increasing number of studies about seed dispersal by extant large frugivores from tropical areas have been published (Vidal et al., 2013). However, the information about the role of large frugivores as seed dispersers in temperate and northern regions is still limited. Megafaunal species are currently defined as comparatively large animal species that have strong effects on ecosystems, present distinctive functional traits and habitat requirements and have escaped most-non anthropogenic predation when adults (Moleón et al., 2020). Megafaunal frugivores are considered quantitatively and qualitatively pivotal seed dispersers, particularly because they can transport many seeds over long distances (Pires et al., 2018). The increased chance of long-distance dispersal events by these animals facilitates the colonization and re-colonization of new and former habitats, enhancing genetic diversity and reducing parent-sibling competition (Traveset & Richardson, 2014). In addition, megafaunal frugivores have the potential to consume many different fleshy-fruited plants, including species with both small and big seeds (Chen & Moles, 2015). The selective loss of large-bodied animals during the last centuries (Dirzo et al., 2014), particularly megafauna, can strongly impair the dispersal of large-seeded plant species, which may have serious consequences for their recruitment, population structure, genetic diversity and evolutionary trajectories (Galetti et al., 2013; Pérez-Méndez et al., 2016; Pérez-Méndez et al., 2018; Pires et al., 2018). Therefore, complete information about the role of extant megafauna for seed dispersal and plant regeneration processes is highly valuable.

The brown bear *Ursus arctos* (Order: Carnivora, Family: Ursidae) is one of the world's most widely distributed terrestrial mammals and the largest living terrestrial carnivore; it inhabits a broad variety of biomes, from tundra to deserts (Fig. 1). Its heavy body mass and large habitat requirements (Pasitschniak-Arts, 1993), together with a distinctive biology (e.g. late first reproduction in females and longer maternal care when compared to other carnivore species; Steyaert et al., 2012) and the lack of non-human natural predators at adult stages make the species a good representative of megafauna inhabiting northern latitudes. The brown bear is an omnivore with an important share of fleshy-fruited plants in its diet. Its trophic niche is very flexible and strongly related to environmental conditions, with populations in warm and highly productive environments being almost completely herbivorous, whereas populations in cold, unproductive environments are more carnivorous (Bojarska & Selva, 2012). Previous studies have shown that resource availability influences the age of first reproduction, litter size, population density, home range size and habitat selection in brown bear populations (Blanchard, 1987; Palomero et al., 1997; Welch et al., 1997; McLoughlin et al., 2000; Nomura & Higashi, 2000). Besides these more general effects of resource availability, the abundance of fruit resources is known to strongly affect female breeding success in some brown bear populations (Hertel et al., 2018). This indicates that fleshy fruits are a key food resource for the species. However, our understanding of the reciprocal effect of the brown bear on the dispersal and regeneration of its food plants remains incomplete; particularly the assessment of the seed dispersal service provided by the species.

Our main goal was to assess the role of the brown bear as a legitimate megafaunal seed disperser across its entire distribution range, addressing both the quantity and quality components of the seed dispersal effectiveness provided by the species. We specifically aimed to (1) identify all fleshy-fruited plant species eaten by brown bears worldwide, (2) evaluate the contribution of fleshy fruits in brown bear' diet across biomes, and (3) determine the effects of ingestion by bears on the proportion and speed of germination in selected fleshy-fruited plant species commonly eaten by brown bears. We also explored the factors related to brown bears' biology and ecology that may influence its seed dispersal effectiveness.

METHODS

- **Literature review – Species richness and quantitative importance of fleshy fruits in brown bear diet**

We compiled published information about brown bears' diet to analyse both the diversity and the quantitative importance of fleshy fruits in brown bear diet worldwide. We searched Google Scholar database for articles containing data on brown bear diet using the following keyword string: "(bear* or *ursus or *arctos) and (food* or habit* or forag* or diet* or faec* or scat* or stomach*)". The search yielded 13,900 hits for the period 1900-2016 and we screened the first 1,000 results. For those studies identified as relevant we also checked the reference lists for additional publications. If for a given population several studies had been published based on partly overlapping data, we only considered the latest study to avoid pseudo replicates in the database. We selected only studies covering the whole active period in brown bears and with a resolution of the food items good enough to distinguish fruits from other food items. In total, we selected for analyses 70 studies published between 1969 and 2016 that contained information about the diet of brown bears from 96 study areas covering the entire distribution of the species (Europe = 25, Asia = 30 and North America = 41 areas, Fig. 1, Appendix S6 and S7). In 69 out of these 70 publications (93 out of the total 96 study areas) bear scats were the only or the major source of information, whereas only one study used exclusively stomachs from killed animals. Thus, information was based on samples being already dispersed by brown bears or potentially dispersed in the case of killed animals. All the selected studies contained information from at least 15 brown bear diet samples. For 85 of the 96 areas fulfilling the above criteria, we extracted or calculated the relative frequency of occurrence of fleshy fruits as the number of occurrences of fleshy-fruited plant species divided by the total number of occurrences of all food items considered. Please note that it is different from the frequency of occurrence, where the number of occurrences is divided by the total number of samples. Whenever possible, we extracted the identity of the fleshy-fruited plants consumed at family, genus and species level. For each plant taxa, we also noted the number of times that it was recorded across study areas (e.g. 10 means that a taxon had been recorded in 10 different study areas). We extracted the latitude and longitude of the study areas and assigned each area to one of the following terrestrial biomes: (1) tundra, (2) boreal forests/taiga, (3) temperate coniferous forests, (4) temperate broadleaf and mixed forests, (5) montane grasslands and shrublands, (6) Mediterranean forest, woodlands and scrubs and (7) deserts and xeric shrublands (Olson et al., 2001). To assess whether the relative frequency of occurrence was a good indicator of the amount of fruits and seeds consumed by brown bears per study area we also extracted from the same articles the relative volume of fleshy fruits, defined as the average percentage of volume that fleshy fruits represented out of the total volume of a scat; this information was available in 46 out of 96 study areas.

- **Germination experiment - Quality of the seeds dispersed by brown bears**

We selected eleven fleshy-fruited plant species to investigate the effects of brown bear ingestion on the proportion and speed of germination. We chose species that are commonly eaten by brown bears in temperate and boreal forests, including our study area, the Carpathian Mountains, based on both literature review and previous field inspections, which also facilitated fruit collection to perform the germination experiments. The selected species represented a gradient of propagule size -from small to large seeds-, the number of seeds per fruit, and fruit type and size. We obtained data on the average seed weight from the Seed Information Database of the Royal Botanic Gardens - Kew (http://www.kew.org/data/sid/).

For each species, we collected ripe fruits from at least 20 individual plants in the Bieszczady Mountains (SE Poland), located in the North-Eastern part of the Carpathian Mountains, during August-October 2008. We mixed the fruits of each species and divided them into three different groups according to the following treatments: (1) whole fruit treatment: 100 entire fruits of each species planted, i.e. seeds with the pulp, (2) depulped seed treatment: 500 seeds manually extracted, i.e. seeds without pulp, and (3) bear treatment: 500 seeds recovered from fresh bear scats.

For the bear treatment, we fed three captive brown bears in the Warsaw Zoological Garden with ripe fruits of each of the eleven selected species. Bear feeding was conducted three times in total, once per month from August to October, depending on the ripening time of each species. We recovered bear scats up to 24 hours after feeding, kept them in plastic bags in a refrigerator for transport and processed them in the lab to extract the seeds. We mixed scats from each of the three trials to eliminate the potential effects of individual bears and washed them through a sieve (0.5 mm mesh size) with running water. Then, we sorted 500 intact seeds of each plant species for the bear treatment. We kept seeds for the depulped and bear treatment in a refrigerator at 6°C until all bear-treatment seeds from the eleven species were ready.

We sowed all seeds at the same time, between 13th and 18th October 2008. The whole fruits (with the pulp) were planted immediately after returning from the field in seedbeds (one fruit per pot), thus, before the feeding trials with captive bears. For the depulped and bear treatment, we planted one seed in each pot of the seedbed. We sowed seeds and fruits in potting soil (peat soil in the case of *Vaccinium myrtillus*) in open-air seedbeds at the Krakow Botanical Garden, therefore, in outdoor conditions. The seeds stayed outside, being covered by snow during winter. The seedbeds were distributed together on concrete ground and covered with a mesh lid to prevent seed predation by rodents and birds. We checked the seedbeds from early April to late June in 2009 and 2010 at intervals of three to seven days, until no further germination was observed. We watered seeds and seedlings regularly depending on weather conditions. We conducted visits also during winter to control the state of the seedbeds. We noted the date and the germination in each pot and seedbed during each inspection (17 and 18 inspections in 2009 and 2010, respectively). In total, we monitored the germination of 12,100 seeds from early April 2009 to late June 2010.

Additionally, we collected brown bear scats in the Bieszczady Mountains in 2008-2010 as part of a larger project. We selected a subsample of 100 scats containing seeds of fleshy fruits to estimate the amount of seeds dispersed per scat. The scats were soaked in water with detergent, washed through a sieve, dried and weighed. Each scat was divided in five parts, two of them were randomly chosen, weighed and examined to count the total number of seeds and the number of damaged seeds (broken or crashed). We estimated the total number of seeds and the fraction of seeds damaged for the whole weight of the scat. Additionally, in each of the 11 selected fleshy-fruited plant species, we counted the number of seeds in 30 randomly chosen fruits.

- **Data analyses**

We used generalized linear models to analyse the effects of the biome on the relative frequency of occurrence and on the number of species and genera of fleshy fruits consumed by brown bears. We fitted the model of the relative frequency of occurrence to a quasibinomial distribution and logit-link function and both models of the number of species and genera to a Poisson distribution and log-link function. We excluded deserts from the analyses because only one study area was located in this biome (Fig.1, Table 1).

We constructed a standardized study area × plant genus interaction matrix with the relative frequency of occurrence for each genus and included the study areas for which such data was available (66 out of 96 study areas). We performed a two-dimensional non-metric multidimensional scaling ordination to visualize differences and similarities in the contribution of the fleshy-fruited plant genera eaten by brown bears among the different biomes. We, then, performed a post-hoc permutational multivariate analysis of variance (permanova test) to check statistical differences in the contribution of the different fleshy-fruited plant genera among biomes.

We used data from the germination experiments to analyse the effects of bear ingestion on seed germination proportions and speed. Specifically, we calculated the mean germination times in 2009 and 2010 separately, and the proportion of seeds germinated at the end of the experiment for each combination of the eleven fleshy-fruited plant species and the three treatments. The mean germination time is often used as a proxy for the germination speed and it is measured as the weighted mean of the germination time (mean germination time = $\Sigma(n \times D)/\Sigma n$, where n is the number of seeds germinated on day D of the experiment; Nin et al., 2017). Based on evidence from the experiment, we considered April 1st and June 30th as the start and the end of the germination period in each year.

We performed generalized linear mixed effects models to test the effects of germination treatments on the proportion of seeds germinated at the end of the experiment (2010) and on the mean germination time during the first year (2009). We excluded *Viburnum opulus* and *Crataegus monogyna* from the analysis of mean germination time because the first species did not germinate during 2009 and the second germinated very little (Appendix S4 and S5); just some of the seeds germinated before the first inspection in 2010. We fitted the proportion of seeds germinated to a binomial distribution and logit-link function and the mean germination time to a Poisson distribution and log-link function. We included treatment (bear, depulped and whole fruit) as a fixed factor and plant species as a random intercept. We used Spearman correlation to analyse the relation between the weight of the seeds of each species and the proportion of intact seeds after brown bear ingestion.

We used the R statistical environment (version 3.4.0, R Development Core Team 2017) to perform all the statistical analyses. We used the R packages *lme4* (Bates et al., 2015) for the implementation of the generalized linear mixed effects models, *Vegan* (Oksanen et al., 2019) for the nonmetric multidimensional scaling ordination and the permanova test and *SeedCalc* (Silva & Medeiros, 2019) to calculate mean germination times.

RESULTS

- **Species richness and quantity of fleshy fruits consumed by brown bears**

We found that brown bears consumed fleshy fruits in the seven biomes where they were present. At least 101 fleshy-fruited plant species belonging to 42 genera and 24 families were eaten by brown bears across the 96 study areas (Table 1, Fig. 1, Appendix S1). Fruits from Rosaceae and Ericaceae families were the most frequently consumed, being recorded in 65% and 49% of the study areas, respectively. The genus most commonly consumed by bears was *Rubus*, appearing in 45% of the studied areas, followed by *Vaccinium* (42%) and *Prunus* (32%, Fig. 1). Regarding species, *Empetrum nigrum* (present in the diet in 21% of the study areas), *V. myrtillus* (20%), *V. vitis-idaea* (18%) and *Rubus idaeus* (17%) were the most common fleshy-fruited plants eaten by brown bears (Fig. 1). On average, brown bears consumed almost five fleshy-fruited plant species per study area, and fleshy fruits represented 24% of the consumed food items (relative frequency of occurrence, Table 1) and 26% of the total volume of all bear foods (n = 46 study areas). At the study area level, the percentage of volume of fleshy fruits in bear diet was highly correlated with the relative frequency of occurrence (Pearson's product-moment r = 0.82, n = 46 study areas).

The average number of fleshy-fruited taxa and the relative frequency of occurrence of fleshy fruits in brown bear's diet were the highest in Mediterranean areas, whereas intermediate values were observed in the diet of temperate and boreal regions (Table 1, Fig. 2, Appendix S2). The lowest numbers of fleshy-fruited plant taxa were consumed by brown bears in deserts and in the tundra, but also diet studies in these biomes were scarce (Table 1, Appendix S2). The ordination plot (Fig. 2) indicated that genera belonging to the Ericaceae family (e.g., *Vaccinium*, *Empetrum* and *Arctostaphylos*) were mainly associated with boreal biomes, i.e. tundra and taiga, whereas genera belonging to the Rosaceae (e.g. *Malus*, *Pyrus*, *Rosa*, *Rubus*) and Rhamnaceae (e.g. *Rhamnus* and *Frangula*) families were mainly associated with temperate biomes. Species from *Prunus*, *Viburnum* and *Cornus* genera were associated with both Mediterranean and temperate biomes. We found statistical differences in the relative frequency of occurrence of each fleshy-fruited plant genera eaten by brown bears among biomes (results from permanova test based on Bray-Curtis distances and 999 permutations: $R^2 = 0.28$; p-value = 0.001).

The mean number of seeds per brown bear scat found in the Bieszczady Mountains varied among fleshy-fruited plant species, ranging from 6,344 seeds in the case of *Rubus fruticosa* to only two seeds in the case of *Frangula alnus* (Table 2).

- **Quality of the seeds dispersed by brown bears**

After assessing the taxonomic composition and the frequency of occurrence of fleshy-fruits in brown bear diet across the 96 study areas and the number of seeds per scat found in the Bieszczady Mountains (quantity component), we assessed in detail the consequences of gut passage for the intactness and germination of seeds recovered from brown bear scats. Across the nine plant species recorded in bear scats in the Bieszczady Mountains, on average 88% of the seeds remained undamaged after bear ingestion (Table 2). In six out of nine species more than 93% of the seeds remained intact after bear ingestion, whereas in *Prunus avium* and *Malus sp.* the percentage was lower, with 62% and 49% of the seeds undamaged after gut passage, respectively (Table 2). We found that the seed weight was not related to the percentage of seeds undamaged after bear gut passage (Spearman's rank correlation: rho = -0.44, n = 9).

In general, seeds ingested by bears and manually depulped had higher percentages of germination than seeds sowed within the pulp (Table 2, Fig. 3, Appendix S3). Seeds ingested by brown bears germinated better than seeds manually depulped and whole fruits in seven out of the eleven fleshy-fruited plant species (Table 2). Germination percentages after bear ingestion were higher than 50% for seven species, whereas in the case of depulped and whole fruit treatments, six and one species exceeded this germination percentage, respectively (Table 2). Seeds from *V. myrtillus* and *F. alnus* germinated only during the first year, while *V. opulus* germinated exclusively in the second year (Appendix S4 and S5). We did not find any influence of the treatment on the mean germination times (Fig. 3, Appendix S3).

DISCUSSION

Our study provides a first comprehensive assessment of the role of brown bears as legitimate seed dispersers across their distribution range. We have shown that frugivory in the brown bear is not just a locally restricted phenomenon, but that fleshy fruits represent a major food resource for the species across its entire distribution. Across its geographic range the brown bear consumes fruits of more than 100 plant species belonging to 42 genera and 24 families, whose seeds remain mostly intact after bear ingestion and germinate better than when embedded within the pulp. The quantity and quality of seed dispersal services provided by brown bears highlight that this megafaunal species is a legitimate seed disperser and may have a substantial impact on plant regeneration services in all the biomes where the species is present. Therefore, brown bears must be recognised as one of the few extant and relevant legitimate megafaunal seed dispersers inhabiting non-tropical areas.

Our literature review revealed that frugivory by brown bears is prevalent across the species entire geographic range, and represents on average a quarter of the total volume and of the number of food items eaten during an entire year. As fleshy fruits are not available all year around in northern regions, these figures would probably be much higher if only the fruiting period would be considered. Previous studies analysing global dietary patterns of other frugivorous mammals sympatric with brown bears, such as red foxes *Vulpes vulpes* and pine martens *Martes martes*, has revealed that they also track fruit availability and change their dietary patterns along the year, feeding primarily on small vertebrates during winter, spring and early summer. Plants are less important in the diets of these mesocarnivores, being consumed mostly during late summer and autumn, and, on average, not exceeding 20% of the annual volume (Zalewski, 2005; Soe et al., 2017). Large herbivores sympatric with brown bears and considered as megafauna, such as the European bison *Bison bonasus* or the red deer *Cervus elaphus*, also disperse viable seeds from more than a hundred plant species, whereas others like the moose *Alces alces* are less efficient dispersers (Jaroszewicz et al., 2013; Lundgren et al., 2018). However, wild ungulates feed primarily on herbs and leaves, with fruits representing less than 5% of the total volume of their diet (Gebert & Verheyden-Tixier, 2008; Kowalczyk et al., 2019). Additionally, the germination success of seeds defecated by ungulates is usually below 10% (Cosyns et al., 2005). Therefore, in comparison with sympatric megafauna, the brown bear is among the most effective megafaunal seed dispersers in their distribution range, at least in areas where other ursid species are absent.

The importance of fleshy fruits in brown bear diet can be partly explained by the annual cycle of the brown bear. As a hibernator, the brown bear is adapted to seasonal climates with prolonged periods of energetic bottlenecks (Albrecht et al., 2017). Successful hibernation depends on the energy reserves at the onset of the denning period, which are crucial to survive the winter. To meet the energetic demands during hibernation, brown bears maximize their energy uptake during a period of hyperphagia, in which they feed intensively on fleshy fruits and mast to build up body fat before den entry (Welch et al., 1997; Hertel et al., 2018). Therefore, fleshy fruits represent a key food resource for the species, which can affect important aspects of brown bear biology such as habitat selection and breeding success (Nomura & Higashi, 2000; Hertel et al., 2016). Fleshy fruits contain an important proportion of hydrophobic lipids (Valido et al., 2011), used to gain body fat for hibernation. Importantly, the ripening time of most fleshy fruits coincides with the hyperphagic period of highly food demand by bears, when they spend most of their time foraging. In that period, brown bears can consume up to a third of their body weight of fleshy fruits per day (Welch et al., 1997).

Fleshy fruits are present in more than half of brown bears scats during late summer and early autumn and this applies to populations from different latitudes, including those from Northern Yukon, Hokkaido or the Pyrenees (McHutchon & Wellwood, 2003; Sato et al., 2005; Lalleroni et al., 2017). Other bear species such as the Asiatic black bear *U. thibetanus* and the American black bear *U. americanus* also feed intensively on fleshy fruits during the fruiting season and berries may represent more than the half of the total volume consumed (Baldwin and Bender, 2009; Koike, 2010). An increased frugivory during late summer and early autumn is a common phenomenon in other carnivore species inhabiting boreal and temperate regions such as red foxes or pine martens (Zalewski, 2005; Soe et al., 2017). However, these species feed primarily on vertebrates during mid-summer and the share of fleshy fruits in the diet of these species is much lower than in the brown bear during this period.

The most common genera eaten by brown bears across the species range are *Rubus*, *Empetrum* and *Vaccinium*. These genera typically form dense vegetation layers at ground level with exceptionally high local fruit abundances. Thus, these plant genera are attractive resources because, once a fruiting patch has been detected, brown bears can easily harvest large amounts of fruits growing close to the ground by ‘browsing’ the local vegetation (Welch et al., 1997). Fruits from four species of the Ericaceae familiy (*Empetrum nigrum, Vaccinium myrtillus, V. vitis-idaea* and *V. uliginosum*) are the most commonly eaten by brown bears in boreal regions. These few species dominate the ground layer in forests and meadows at northern latitudes, producing large amounts of nutritious and easily accessible fruits during late summer and autumn, when brown bears rely the most on fleshy fruits.

Up to our knowledge, comprehensive reviews on frugivory by given species at the global scale are scarce and the comparison between the number of fleshy-fruited plant species eaten by bears and other frugivores, including other ursids and megafauna species, is difficult to make, particularly in boreal and temperate regions. Megafaunal seed dispersers have been more studied in tropical areas, where more diverse fleshy fruits are highly available year-round. For instance, the Asian elephant *Elephas maximus* is known to disperse seeds of at least 122 species from 39 different families (Campos-Arceiz & Blake, 2011), a number that is somehow comparable to the diversity of fleshy fruits consumed by brown bears, moreover considering that they inhabit areas less rich in fleshy fruits than the tropics.

Brown bears are more frugivorous and consume the largest variety of fleshy-fruited plant species per study area in Mediterranean regions. These results support previous findings that brown bears inhabiting warmer and more productive areas rely more on fleshy fruits (Bojarska & Selva, 2012). As brown bears are omnivores that feed opportunistically on many food resources, their diet may reflect the local availability and diversity of fruiting plant species in a given study area rather than strong dietary preferences. This is supported by the fact that Mediterranean high mountains scrublands, which are the areas where brown bears are still present in the Mediterranean basin, show very high fruit densities, only comparable to the exceptionally high fruit availability in tropical forests (Jordano, 2000). Brown bears consume the lowest number of fleshy-fruited plant species in cold and less productive areas, which may be also explained by the lower availability of these food resources.

We showed that a single brown bear scat may contain up to several thousand seeds, which supports a previous study conducted in Alaska where brown bears faeces containing up to 7,000 seeds of *Vaccinium* and 2,000 of *Ribes* species were recovered from the field (Willson & Gende, 2004). This confirms that brown bears have the potential to disperse high quantities of seeds. However, the quantity component of the seed dispersal does not only depend on the number of seeds dispersed per scat but also on the abundance of the disperser (Schupp, 1993). All else being equal, local declines of brown bear populations due to harvesting and habitat loss can, therefore, be expected to reduce the quantity component of seed dispersal services provided by the species. Given that the effect of each individual brown bear is relatively large compared to the per individual effect of smaller-bodied seed dispersers (e.g., birds or small mammals), the potential effects of small population declines can translate into pronounced effects on seed dispersal processes and services. To our knowledge, there are no published studies yet about the relative contribution of brown bears to the total seed rain in comparison to other dispersers. However, preliminary results suggest that brown bears may disperse up to 90% of the total seeds mobilised by the whole frugivore community in European alpine forests (authors' unpublished data). Asiatic black bears have been shown to be major seed dispersers in relation to other species in mixed temperate forests in Japan (Naoe et al, 2016; Naoe et al., 2019). However, these are local studies and the relative importance of brown bears as seed dispersers may differ depending on the population densities of bears and other frugivores. In addition to the effects of local population declines, human activities can also compromise seed dispersal processes (McConkey & O'Farrill, 2016). Anthropogenic food resources (i.e. any food resource derived from human activities) and artificial feeding (i.e. intentional food provisioning to wildlife by humans) may jeopardise the seed dispersal services provided by brown bears by affecting habitat selection, movement behaviour and dietary preferences (Skuban et al., 2016; Štofík et al., 2016; Selva et al., 2017) as it has been already suggested for other carnivore species (López-Bao & González-Varo, 2011).

The germination experiments showed that brown bear ingestion improves the ability of seeds to germinate when compared to seeds germinating from whole fruits and that it does not reduce the ability of seeds to germinate in comparison to manually depulped fruits. These results are in line with previous works (Traveset & Willson, 1997; Nowak & Crone, 2012; Steyaert et al., 2019) and suggest that the passage of seeds through the bears' gut is beneficial for germination by removing the seeds from the pulp without harming the seeds (Samuels & Levey, 2005). Additionally, we first showed that bears rarely damaged the seeds of most fleshy fruits. Therefore, brown bears do not differ in the quality of their seed dispersal services from other major disperser guilds including birds, lizards, bats, mesocarnivores or elephants (Traveset & Willson, 1997; Traveset, 1998; Valido & Olesen, 2007; Campos-Arceiz & Blake, 2011). Thus, brown bears can be considered as legitimate seed dispersers also from a qualitative point of view. However, to fully understand the quality component of seed dispersal services provided by brown bears more information about the post-dispersal stages, such as the characteristics of the microhabitats in which brown bears defecate the seeds, is required. In this line, preliminary studies suggest that defecation of seeds in the surroundings of bears' resting sites might be beneficial for plant recruitment, especially in the case of clonal species (Steyaert et al., 2019). Seedling establishment is usually infrequent in these species and usually restricted to "windows of opportunity" (i.e. spatially or temporally unpredictable conditions in which seedling establishment is possible within stands of conspecific adults; Eriksson & Fröborg, 1996). Brown bears usually defecate next to their resting sites, where they dig and create local disturbances in the ground characterized by open top-soil with no or little vegetation (Steyaert et al., 2019). These small disturbances might be essential for seedling recruitment of clonal plants as they expose the defecated seeds to the perfect conditions for germination, representing a valid example of these windows of opportunity. In addition, processes such as secondary seed dispersal and seed or seedling predation might be relevant if secondary dispersers or predators are attracted to bear scats containing large amounts of seeds (Jansen et al., 2012; Koike et al., 2012). Secondary dispersal may enhance germination probabilities by decreasing seed densities and, thus, seed competition, in spots with high densities of seeds such as bear scats. Additionally, secondary seed dispersers usually dig the soil and create small disturbances to relocate the seeds, creating the perfect environment for seedling establishment (Koike et al., 2012). These aspects related to the quality of seed dispersal services provided by brown bears in comparison to other sympatric seed dispersers are relevant topics for future research.

The quality component of seed dispersal is not only related to fruit handling and gut treatment, but also to animal movement (Schupp, 1993; Schupp et al., 2010). Brown bear daily displacement in the Carpathian Mountains, taken as the straight-line distance between two most distant locations during a 24h period, is on average 2-4 km, but can reach up to 30 km (Bartoń et al., 2019). Seeds dispersed across such distances might facilitate the colonization of new areas, and might enhance genetic diversity via reduction of adult-sibling competition and via release from high pathogen pressure near adult plants (Willson & Traveset, 2000). However, to our knowledge, there is only one study discussing the potential seed dispersal kernel provided by brown bears. Lalleroni et al. (2017) found that brown bears in the Pyrenees move on average between 0.85 and 1.34 km every 6 hours, that corresponds to the median gut retention time for a berry-based diet (Elfström et al., 2013). Other bear species, such as the Asiatic black bear *Ursus thibetanus*, are known to move more than half of the seeds they consume over 500 meters and to disperse seeds up to 22 km away from the source (Koike et al., 2010). Based on the average daily displacement and gut retention times, brown bears would disperse seeds two-to-three times farther than other species considered long-distance dispersers, such as martens *Martes spp*. and the Japanese macaque *Macaca fuscata* (mean and maximum dispersal distances around 400-500 and 1,200 meters, respectively; Hickey et al., 1999; Terakawa et al., 2009; González-Varo et al., 2013; Tsuji et al., 2016). In temperate regions, red foxes may disperse seeds over similar average distances, but their maximum seed dispersal distances are still up to eight times smaller than the ones of bears (half of the seeds dispersed more than 1,000 meters away from the source and up to 2,846 meters for foxes; Hickey et al., 1999; Terakawa et al., 2009; Koike et al., 2010; González-Varo et al., 2013). The relationship between body size and dispersal distance has been proven in various taxa (Jordano et al., 2007; Santini et al., 2013). Brown bears may, thus, provide unique dispersal services by moving seeds over large distances, similar to recognised megafaunal long-distance seed dispersers inhabiting tropical areas such as African savanna elephants, which carried half of the seeds over 2.5 kilometres (Bunney et al., 2017).

We have shown that brown bears are legitimate seed dispersers across its range, providing a high quantity and quality dispersal services. We have also shown that brown bears consume large amounts of seeds from many different fleshy-fruited plant species, that the large majority of these seeds remains intact after gut passage, and that gut passage does not reduce and even enhances the ability of seeds to germinate. Given its large body size the brown bear has the potential to contribute substantially to long-distance seed dispersal. Many large bodied frugivores, including megafauna, have been extirpated since the 15th century and populations of the remaining species show 25% average decline in abundance (Dirzo et al., 2014). The loss of large-bodied frugivores frequently causes a reduction of seed dispersal distances, genetic diversity and effective population sizes of plants (Pérez-Méndez et al., 2016; Pires et al., 2018). Studies about megafaunal frugivores have often focused on their role as the exclusive dispersers of large seeds ("megafaunal fruit syndrome") and have been conducted mostly in tropical areas (Galetti et al., 2018). However, seed size is not expected to be a constraint of frugivore-mediated seed dispersal in Mediterranean, temperate and boreal regions, where fleshy fruits containing large seeds are missing. Consequently, we suggest that the uniqueness of megafaunal frugivores inhabiting these areas should be evaluated in terms of their significant contributions to the total seed rain and dispersal distances. We conclude that in Mediterranean, temperate and boreal biomes, where other megafauna species are unlikely to be effective seed dispersers, brown bears are an integral part of seed disperser communities. Our results suggest that brown bears are unique legitimate megafaunal seed dispersers that play an essential, though overlooked, role in plant regeneration processes and ecosystem functioning.

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AUTHORS´CONTRIBUTIONS

AGR, NS, AV and JA conceived the study, AGR wrote a first draft of the paper with significant contributions by NS and JA, JA compiled the data for the literature review, SS and NS were in charge of the germination experiments and contributed to field data collection, AGR analyzed the data; all authors provided comments that significantly improved the manuscript and approved the last version.

ADDITIONAL INFORMATION

Data availability. The datasets generated and analysed during the current study are available from the corresponding author upon reasonable request.

Competing interests: The authors declare that they have no competing interests.

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Figure 1. (a) Map showing the study areas (n=96, dots) from which data on brown bear diet were gathered. Brown bear distribution is shown in grey. The dot colors represent the biomes in which each study area is located. The percentages of study areas with presence of different families (b), genera (c) and species (d) of fleshy fruits are shown in the lower panels. Only the 20 most common taxa of each taxonomic rank are shown. Study areas are listed in Appendix S6 and the complete list of taxa recorded in the brown bear diet worldwide is presented in Appendix S1. The map in (a) was created with the R statistical environment (version 3.4.0; R Development Core Team, 2017), using the package *rworldmap* South, 2011). Historical and present brown bear distributions were extracted from the IUCN website (IUCN SSC Bear Specialist Group, 2017).

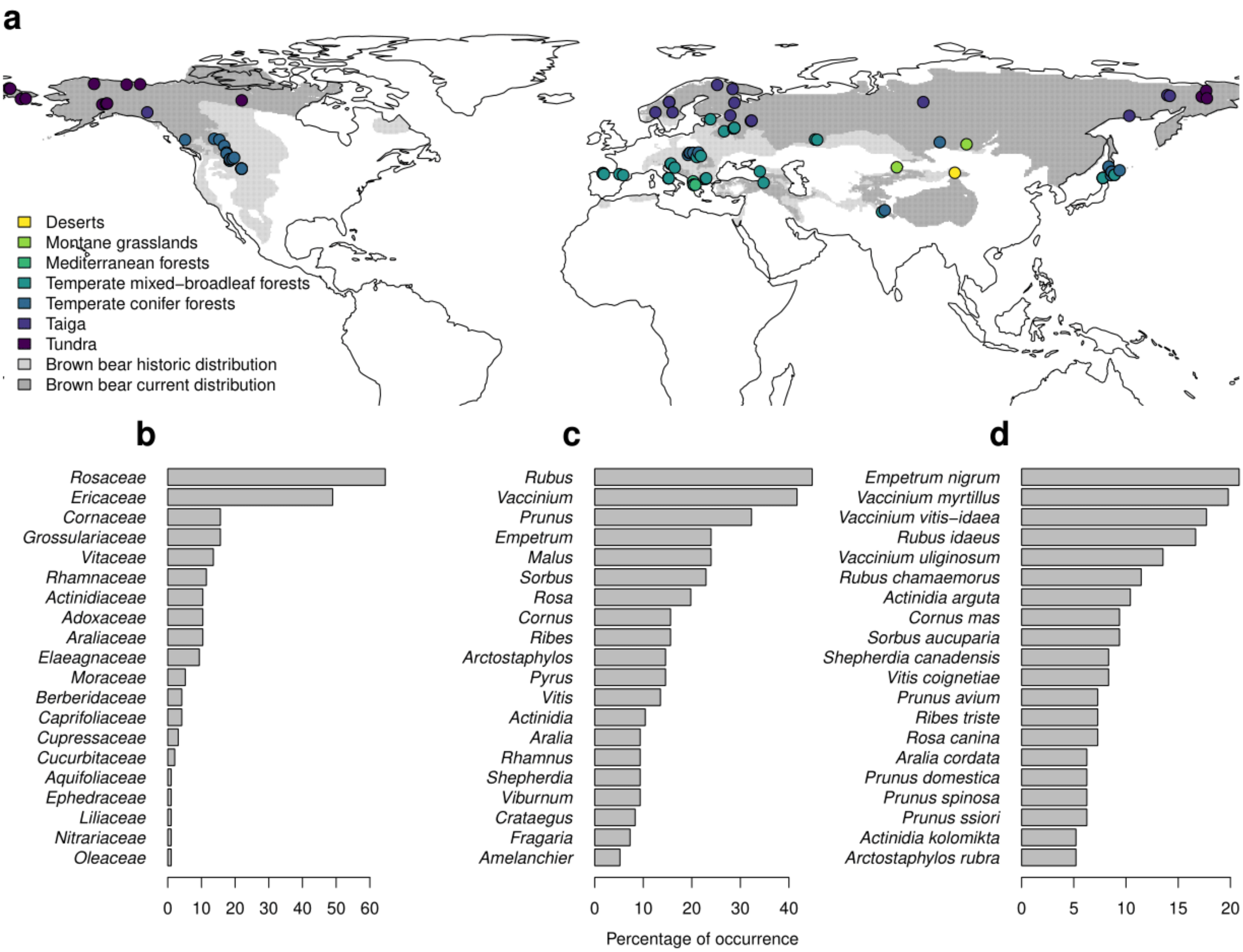

a
Deserts
Montane grasslands
Mediterranean forests
Temperate mixed–broadleaf forests
Temperate conifer forests
Taiga
Tundra
Brown bear historic distribution
Brown bear current distribution
b
Rosaceae
Ericaceae
Cornaceae
Grossulariaceae
Vitaceae
Rhamnaceae
Actinidiaceae
Adoxaceae
Araliaceae
Elaeagnaceae
Moraceae
Berberidaceae
Caprifoliaceae
Cupressaceae
Cucurbitaceae
Aquifoliaceae
Ephedraceae
Liliaceae
Nitrariaceae
Oleaceae
0 10 20 30 40 50 60
c
Rubus
Vaccinium
Prunus
Empetrum
Malus
Sorbus
Rosa
Cornus
Ribes
Arctostaphylos
Pyrus
Vitis
Actinidia
Aralia
Rhamnus
Shepherdia
Viburnum
Crataegus
Fragaria
Amelanchier
0 10 20 30 40
Percentage of occurrence
d
Empetrum nigrum
Vaccinium myrtillus
Vaccinium vitis–idaea
Rubus idaeus
Vaccinium uliginosum
Rubus chamaemorus
Actinidia arguta
Cornus mas
Sorbus aucuparia
Shepherdia canadensis
Vitis coignetiae
Prunus avium
Ribes triste
Rosa canina
Aralia cordata
Prunus domestica
Prunus spinosa
Prunus ssiori
Actinidia kolomikta
Arctostaphylos rubra
0 5 10 15 20

Figure 2. Nonmetric multidimensional scaling ordination plots illustrating differences in the contribution of fleshy-fruited plant genera consumed by brown bears across biomes. These differences are based on the relative frequency of occurrence of each genus in brown bear diet in 66 study areas. The location within the ordination plot of the study areas from which data were gathered (a) and the fleshy-fruited plant genera eaten by brown bears (b) are shown. *Shepherdia* and *Empetrum* are consumed by brown bears mostly in boreal biomes (i.e. tundra and taiga; left part in panel b), while *Vaccinium* and *Ribes* are eaten in both boreal and temperate regions (mid-left in panel b). The majority of the genera consumed by brown bears are found in temperate regions (mid part in panel b), with *Viburnum*, *Prunus* and *Cornus* being mostly eaten by brown bears inhabiting Mediterranean regions.

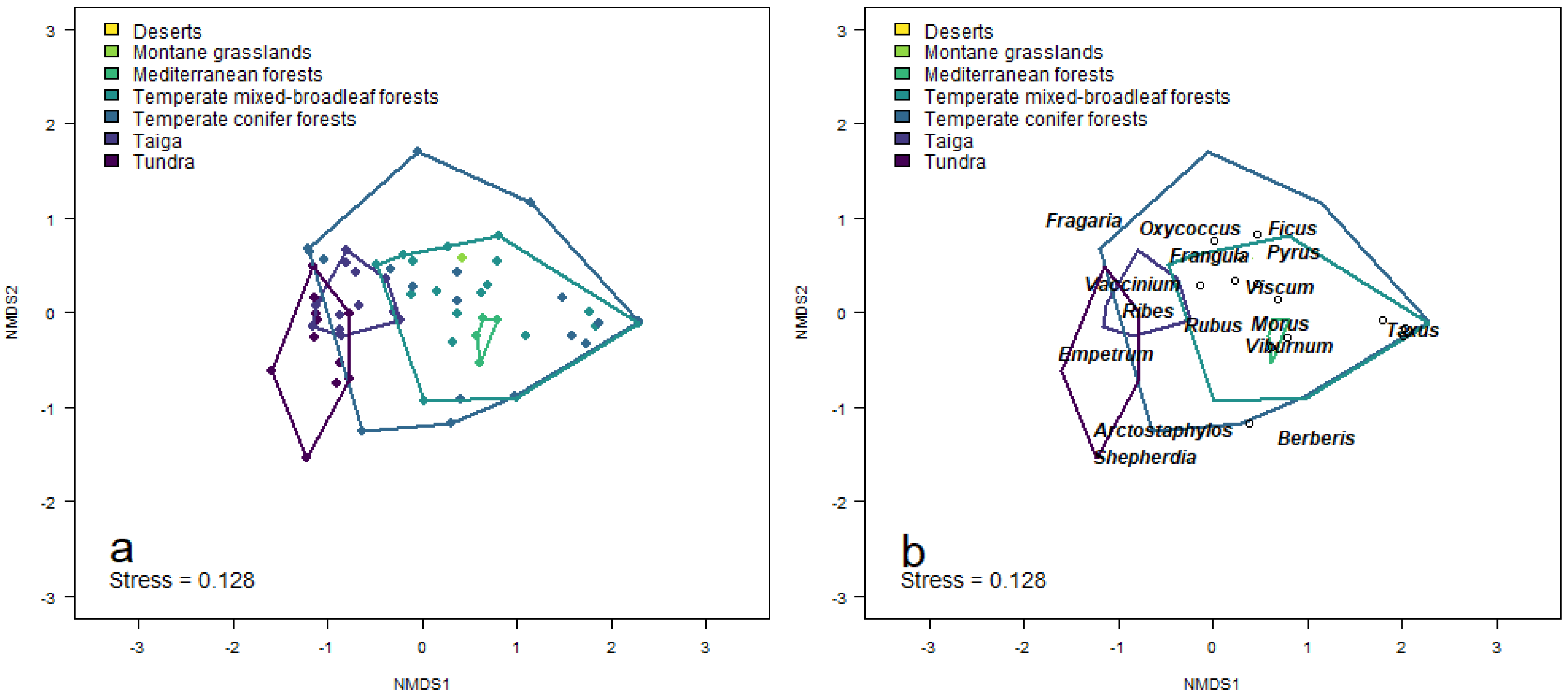

Deserts
Montane grasslands
Mediterranean forests
Temperate mixed-broadleaf forests
Temperate conifer forests
Taiga
Tundra
a
Stress = 0.128
NMDS1
NMDS2
-3
-2
-1
0
1
2
3
b
Stress = 0.128
Fragaria
Oxycoccus
Ficus
Frangula
Pyrus
Vaccinium
Viscum
Ribes
Rubus
Morus
Viburnum
Taxus
Empetrum
Arctostaphylos
Shepherdia
Berberis

Figure 3. (a) Mean proportion of seeds germinated at the end of the 2-year germination experiment and (b) mean germination times during the first year of germination (number of days elapsed since April 1st) for 11 fleshy-fruited plant species in relation to three germination treatments (Brown bear- seeds ingested by brown bears and recovered from the scats, Depulped- manually depulped fruits and, Whole fruits- seeds embedded within the whole fruit). Grey dots and black arrows represent the predicted mean values and the standard errors, respectively. Empty dots represent the actual observed values for each fleshy-fruited plant species sown. Fleshy-fruited plant species are listed in Table 2.

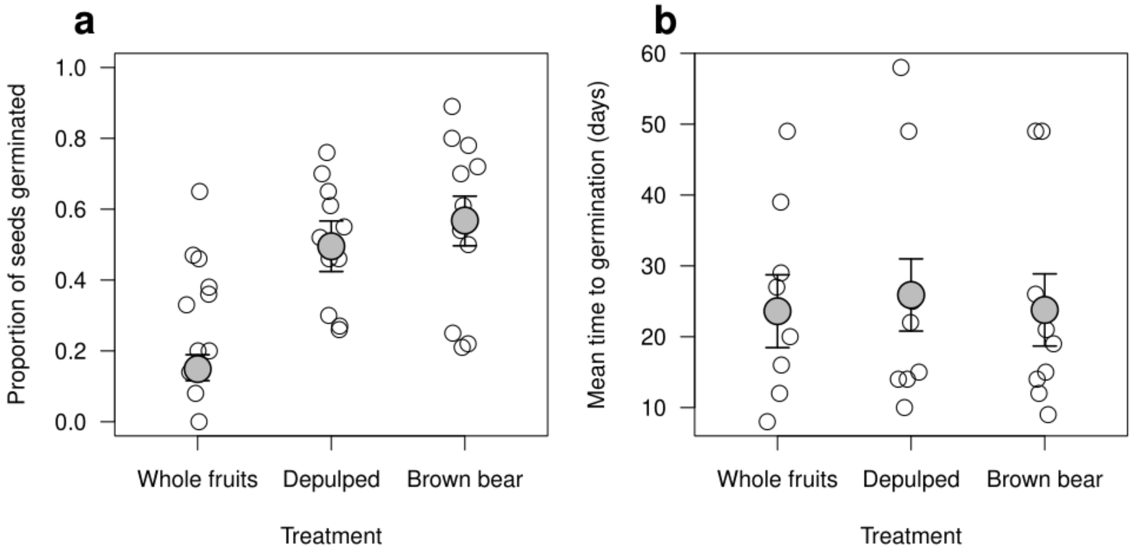


Table 1. Average values (mean ± SD) among study areas of the number of taxa (species and genera) and relative frequency of occurrence (number of occurrences of fleshy-fruited plant species divided by the total number of occurrences of all food items consumed) of fleshy fruits eaten by brown bears in each biome within brown bear distribution range and in the entire range. The total number of studies and samples examined is also indicated for each biome. The same letter (a, b, c) after the scores of the relative frequency of occurrence in different biomes indicate statistical differences between these biomes (e.g. letter "a" represents statistical differences in the frequency of occurrence of fleshy fruits in brown bears diet between boreal forests & taiga and temperate coniferous forests).

Biome	**No. species /study area (mean ± SD)**	**No. genera /study area (mean ± SD)**	**Relative frequency of occurrence (mean ± SD)**	**No. study areas**	**No. samples**
Tundra	3.43 ± 1.22	3.00 ± 1.11	0.25 ± 0.12	14	2,696
Boreal forests & taiga	4.87 ± 3.40	3.40 ± 2.06	0.27 ± 0.14^{a}	15	4,431
Temperate coniferous forests	4.40 ± 3.80	4.03 ± 3.47	0.16 ± 0.08^{abc}	30	17,272
Temperate mixed & broadleaf forests	5.53 ± 4.15	5.20 ± 4.04	0.26 ± 0.13^{b}	30	10,272
Montane grasslands & shrublands	5.00 ± 5.61	5.00 ± 5.66	0.24 ± 0.18	2	1,779
Mediterranean forests, woodlands and scrubs	7.25 ± 3.31	7.00 ± 3.37	0.45 ± 0.21^{c}	4	1,134
Deserts and xeric shrublands	1.00 ± NA	1.00 ± NA	NA	1	365
Average/Total	4.78 ± 3.63	4.26 ± 3.35	0.24 ± 0.14	96	37949

Table 2. Characterization of some of the quality and quantity components of brown bear seed dispersal for 11 fleshy-fruited plant species commonly eaten by brown bears in Eurasian temperate forests. The table shows the life form (tree, shrub or both), fruit type (berry, drupe, polydrupe or pome), number of seeds per fruit (average and range), average weight of 1,000 seeds (gr – information extracted from the Kew Royal Botanical Garden), average number of seeds found per bear scat (n =100 scats containing fleshy fruits) and the percentage of seeds that remain intact after bear ingestion. The results of the germination experiment are also shown and include the percentage of seeds germinated at the end of the two-year experiment and the mean germination time during the first year of germination (in number of days elapsed since April 1st) for the three treatments (Brown bear- seeds ingested by bears and recovered from the scats, Depulped- manually depulped fruits, and Whole fruits- seeds embedded within the whole fruit). * Asterisks mark species that only germinated during the second year of germination.

						Germination treatments					
						Brown bear		**Depulped**		**Whole fruits**	
Species	**Life form**	**Fruit type**	**No. seeds per fruit (range)**	**Weight 1000 seeds (gr)**	**No. seeds per scat (% intact seeds)**	**Seeds germinated (%)**	**Mean germination time (days)**	**Seeds germinated (%)**	**Mean germination time (days)**	**Seeds germinated (%)**	**Mean germination time (days)**
Rosa sp.	Shrub	Pome	25 (16-53)	16.0	4 (100%)	54.4	21.45	52.4	25.04	7.61	20.30
Frangula alnus	Tree/shrub	Drupe	2.5 (2-4)	20.6	2 (100%)	71.8	26.95	61.2	26.26	37.6	29.22
Vaccinium myrtillus	Shrub	Berry	52 (15-86)	0.3	2,190 (98.9%)	22.4	49.23	30.0	49.51	0.46	49.17
Rubus fruticosa	Shrub	Polydrupe	29 (7-44)	2.23	6,344 (98.7%)	60.8	49.88	45.6	58.21	20.3	39.42
Sambucus nigra	Tree/shrub	Drupe	3 (2-4)	12.0	136 (97.7%)	77.8	15.21	65.2	22.07	36.3	12.99
Sorbus aucuparia	Tree	Pome	2.2 (1-4)	7.0	242 (93.3%)	69.8	12.10	46.0	14.22	46.8	NA
Prunus spinosa	Shrub	Drupe	1 (1-1)	175	224 (88.7%)	79.8	19.81	76.0	15.70	46.0	27.50
Prunus avium	Tree	Drupe	1 (1-1)	183	875 (62.3%)	20.6	14.86	27.2	14.01	33.0	8.67
Malus sp.	Tree	Pome	4.2 (2-8)	12.4	74 (48.8%)	88.8	9.75	69.8	10.61	13.8	16.40
Viburnum opulus*	Tree/shrub	Drupe	1 (1-1)	33.3	-	49.6	-	55.4	-	65.0	-
Crataegus monogyna*	Tree	Pome	1 (1-1)	98.0	-	24.8	-	25.8	-	20.0	-

Supplementary material for:

The role of the brown bear *Ursus arctos* as a legitimate megafaunal seed disperser

Scientific Reports

Alberto García-Rodríguez, Jörg Albrecht, Sylwia Szczutkowska, Alfredo Valido, Nina Farwig and Nuria Selva

Appendix S1. List of fleshy-fruited plant taxa (families, genera and species) consumed by brown bears and number of study areas (n= 96) where each taxon was recorded as brown bear food.

Family	Study areas	Genus	Study areas	Species	Study areas
Rosaceae	62	*Rubus*	43	*Empetrum nigrum*	20
Ericaceae	47	*Vaccinium*	40	*Vaccinium myrtillus*	19
Cornaceae	15	*Prunus*	31	*Vaccinium vitis-idaea*	17
Grossulariaceae	15	*Empetrum*	23	*Rubus idaeus*	16
Vitaceae	13	*Malus*	23	*Vaccinium uliginosum*	13
Rhamnaceae	11	*Sorbus*	22	*Rubus chamaemorus*	11
Actinidiaceae	10	*Rosa*	19	*Actinidia arguta*	10
Adoxaceae	10	*Cornus*	15	*Cornus mas*	9
Araliaceae	10	*Ribes*	15	*Sorbus aucuparia*	9
Elaeagnaceae	9	*Arctostaphylos*	14	*Shepherdia canadensis*	8
Moraceae	5	*Pyrus*	14	*Vitis coignetiae*	8
Berberidaceae	4	*Vitis*	13	*Prunus avium*	7
Caprifoliaceae	4	*Actinidia*	10	*Ribes triste*	7
Cupressaceae	3	*Aralia*	9	*Rosa canina*	7
Cucurbitaceae	2	*Rhamnus*	9	*Aralia cordata*	6
Aquifoliaceae	1	*Shepherdia*	9	*Prunus domestica*	6
Ephedraceae	1	*Viburnum*	9	*Prunus spinosa*	6
Liliaceae	1	*Crataegus*	8	*Prunus ssiori*	6
Nitrariaceae	1	*Fragaria*	7	*Actinidia kolomikta*	5
Oleaceae	1	*Amelanchier*	5	*Arctostaphylos rubra*	5
Santalaceae	1	*Berberis*	4	*Arctostaphylos uva-ursi*	5
Solanaceae	1	*Lonicera*	4	*Malus sylvestris*	5
Taxaceae	1	*Ficus*	3	*Pyrus communis*	4
Thymelaeaceae	1	*Juniperus*	3	*Rhamnus alpina*	4
		Morus	3	*Ribes dikuscha*	4
		Arbutus	2	*Vaccinium oxycoccos*	4
		Frangula	2	*Vitis vinifera*	4
		Sambucus	2	*Actinidia polygama*	3
		Citrullus	1	*Amelanchier ovalis*	3
		Daphne	1	*Cornus sericea*	3
		Ephedra	1	*Crataegus monogyna*	3
		Ilex	1	*Ficus carica*	3
		Kalopanax	1	*Fragaria vesca*	3
		Nitraria	1	*Prunus cerasifera*	3
		Olea	1	*Sorbus aria*	3

Oplopanax	1	*Vaccinium scoparium*	3
Oxycoccus	1	*Viburnum opulus*	3
Solanum	1	*Amelanchier alnifolia*	2
Streptopus	1	*Arbutus unedo*	2
Taxus	1	*Arctostaphylos alpinus*	2
Ventia	1	*Berberis aristata*	2
Viscum	1	*Frangula alnus*	2
		Malus domestica	2
		Malus pumila	2
		Morus australis	2
		Prunus cornuta	2
		Prunus mahaleb	2
		Prunus padus	2
		Prunus persica	2
		Prunus salicina	2
		Prunus virginiana	2
		Rosa acicularis	2
		Rubus fruticosus	2
		Rubus sachalinensis	2
		Sambucus racemosa	2
		Sorbus commixta	2
		Sorbus domestica	2
		Vaccinium globulare	2
		Viburnum cotinifolium	2
		Viburnum furcatum	2
		Aralia elata	1
		Berberis repens	1
		Citrullus lanatus	1
		Cornus controversa	1
		Crataegus douglasii	1
		Fragaria virginiana	1
		Ilex aquifolium	1
		Juniperus communis	1
		Juniperus horizontalis	1
		Kalopanax septemlobus	1
		Lonicera involucrata	1
		Lonicera xylosteum	1
		Morus alba	1
		Olea europaea	1

Oplopanax horridus	1
Oxycoccus microcarpus	1
Prunus cerasus	1
Prunus cocomilia	1
Prunus sargentii	1
Rhamnus alnifolia	1
Rhamnus cathartica	1
Rhamnus virgata	1
Ribes bracteosum	1
Ribes montigenum	1
Ribes oxyacanthoides	1
Ribes uva-crispa	1
Rosa majalis	1
Rubus arcticus	1
Rubus caesius	1
Rubus spectabilis	1
Rubus ulmifolius	1
Shepherdia argentea	1
Solanum nigrum	1
Sorbus matsumurana	1
Sorbus sambucifolia	1
Streptopus lanceolatus	1
Taxus cuspidata	1
Vaccinium caespitosum	1
Vaccinium membranaceum	1
Viburnum edule	1
Viscum album	1

Appendix S2. Summary statistics (Estimate – Est., Standard Error – S.E., z value – z, t value – t, and p-value) for the three generalized linear models performed to test the effects of the biome on the number of taxa (genera and species) and the relative frequency of occurrence of fleshy fruits consumed by brown bears. The intercept corresponds to Montane grasslands & shrublands. Deserts are excluded due to small sample size (n = 1 study area). The list of study areas and their corresponding biomes are provided in Appendix S1.

	Number of genera				**Number of species**				**Relative frequency of occurrence**			
Biome	**Est.**	**S.E.**	**z**	**p-value**	**Est.**	**S.E.**	**z**	**p-value**	**Est.**	**S.E.**	**t**	**p-value**
Intercept	1.61	0.31	5.09	<0.001	1.61	0.31	5.09	<0.001	-1.15	0.47	-2.44	0.017
Tundra	-0.51	0.35	-1.45	0.147	-0.38	0.35	-1.08	0.278	0.08	0.51	0.15	0.877
Boreal forests & taiga	-0.38	0.34	-1.11	0.265	-0.03	0.34	-0.08	0.936	0.16	0.50	0.33	0.743
Temperate coniferous forests	-0.21	0.33	-0.65	0.514	-0.13	0.33	-0.39	0.697	-0.48	0.49	-0.97	0.335
Temperate mixed & broadleaf forests	0.04	0.33	0.12	0.904	0.10	0.32	0.31	0.756	0.09	0.49	0.20	0.841
Mediterranean forests, woodlands & scrubs	0.34	0.37	0.91	0.361	0.37	0.37	1.01	0.311	0.94	0.55	1.71	0.092

Appendix S3. Summary statistics (Estimate – Est., Standard Error – S.E., z value – z, and p-value) for the two generalized linear mixed models performed to test the effects of the germination treatment (seeds ingested by bears and recovered from the scats, manually depulped seeds, and seeds embedded within the whole fruit) on the proportion of seeds germinated at the end of the 2-year experiment and on the mean germination time during the first year of germination of eleven fleshy-fruited plant species commonly eaten by brown bears in Eurasian temperate regions. Fleshy-fruited plant species, final percentages of seeds germinated and mean germination times are given in Table 2. *Significant values are presented in bold.

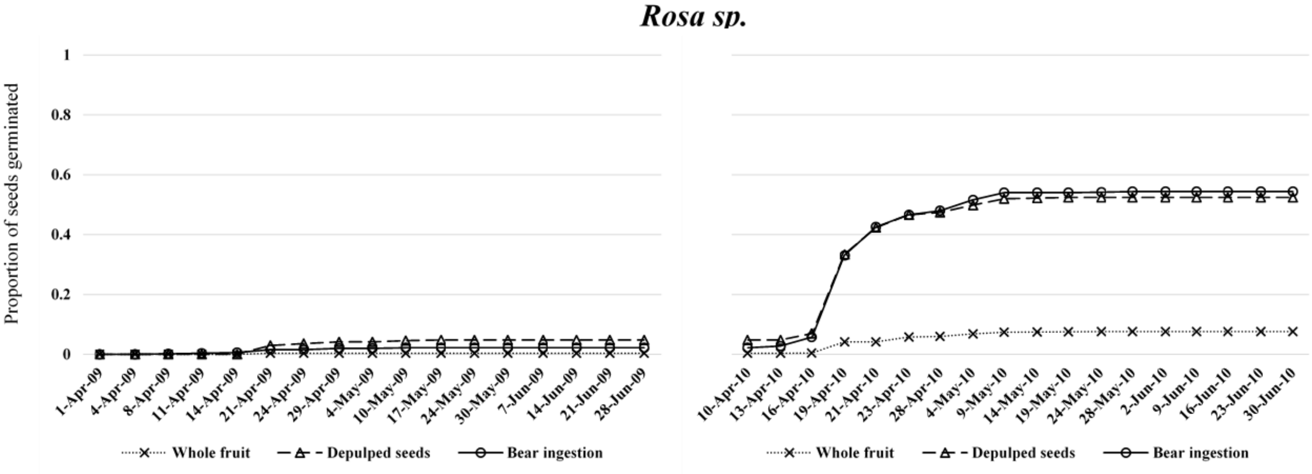
	Proportion of seeds germinated				**Mean germination time**			
	Est.	**S.E.**	**z**	**p-value**	**Est.**	**S.E.**	**z**	**p-value**
Fixed factors								
Intercept	-2.30	1.05	-2.19	**0.028***	3.03	0.19	16.10	**<0.001***
Bear	2.86	1.22	2.34	**0.019***	-0.01	0.10	-0.03	0.9
Depulped	2.48	1.21	2.05	**0.040***	0.05	0.10	0.54	0.591
Random factor								
Species	0.00	0.00			0.26	0.51		

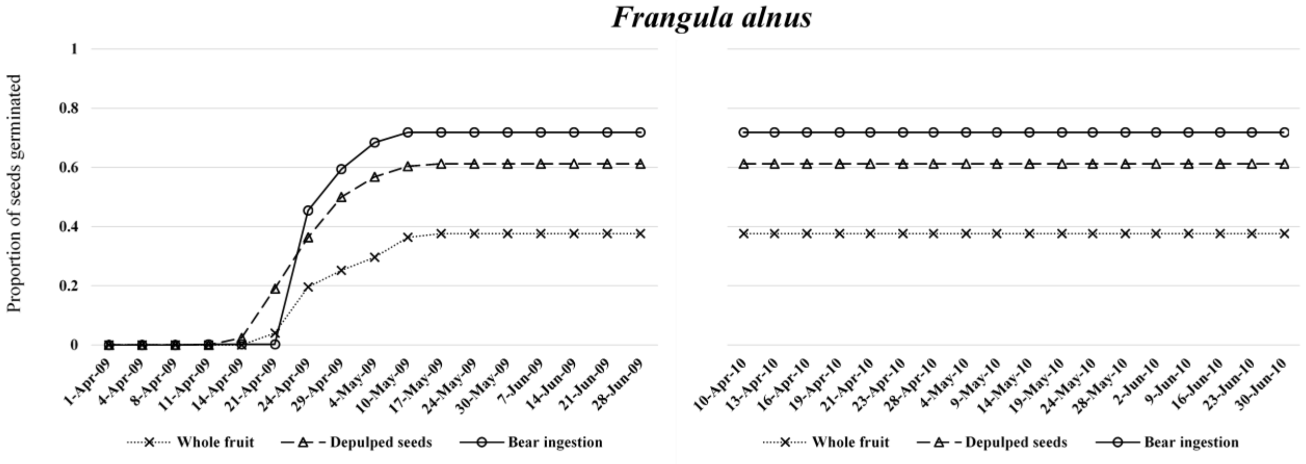
Appendix S4. Percentage of seeds germinated at the end of the 2-year experiment and mean germination times (number of days elapsed since April 1st) during the first year of germination for eleven fleshy-fruited plant species eaten by brown bears in Eurasian temperate regions and under three different germination treatments (seeds ingested by bears and recovered from the scats, manually depulped seeds, and seeds embedded within the whole fruit). The percentage of seeds germinated in 2010 is based on those seeds that did not germinate in 2009. NA values are present for those combinations of species and treatments for which mean germination times were not possible to calculate.

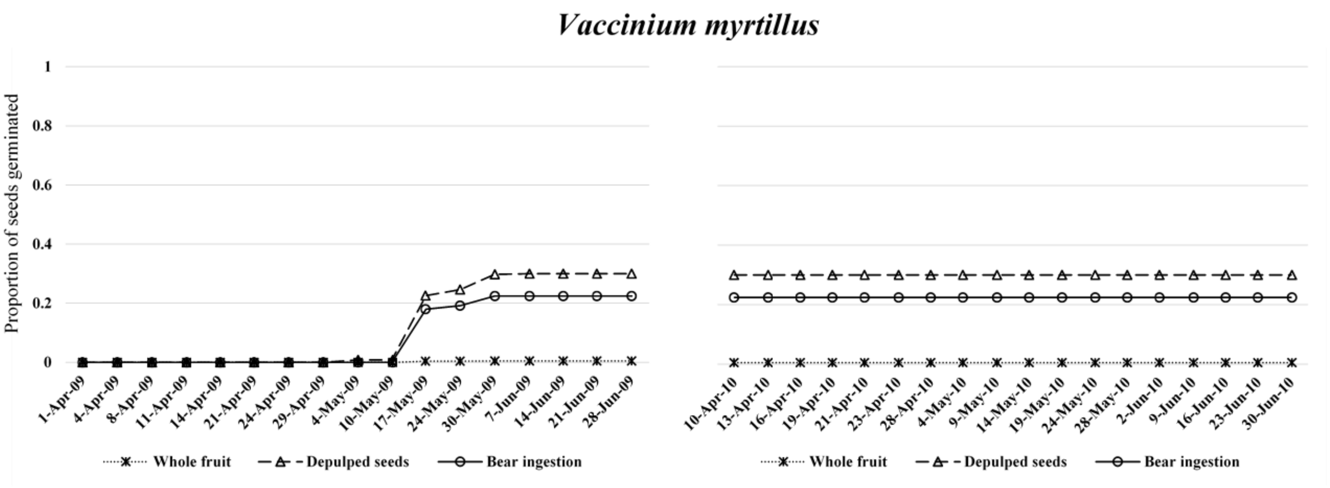
			2009		**2010**	
Species	**No. seeds per fruit**	**Treatment**	**Percentage germinated (%)**	**Mean germination time (days)**	**Percentage germinated (%)**	**Mean germination time (days)**
Rosa sp.	25	Whole	0.40	20.30	7.27	20.93
		Depulped	4.80	25.04	50.00	17.93
		Bear	2.20	21.45	53.37	19.05
Frangula alnus	2.5	Whole	37.60	29.22	0	NA
		Depulped	61.20	26.26	0	NA
		Bear	71.80	26.95	0	NA
Vaccinium myrtillus	52	Whole	0.46	49.17	0	NA
		Depulped	30.00	49.51	0	NA
		Bear	22.40	49.23	0	NA
Rubus fruticosa	29	Whole	7.83	39.42	13.40	19.42
		Depulped	18.40	58.21	33.33	19.29
		Bear	41.00	49.88	32.41	8.99
Sambucus nigra	3	Whole	35.67	12.99	1.03	1.38
		Depulped	61.20	22.07	10.20	2.51
		Bear	74.80	15.21	11.90	2.04
Sorbus aucuparia	2.2	Whole	0.91	NA	46.33	19.56
		Depulped	43.80	14.22	3.91	2.23
		Bear	64.60	12.10	14.69	2.76
Prunus spinosa	1	Whole	2.00	27.50	44.90	19.15
		Depulped	62.60	15.70	35.82	4.87
		Bear	68.00	19.81	36.87	4.18
Prunus avium	1	Whole	33.00	8.67	0	NA
		Depulped	27.20	14.01	0	NA
		Bear	16.20	14.86	5.25	4.59
Malus sylvestris	4.2	Whole	11.43	16.40	2.69	12.12
		Depulped	69.80	10.61	NA	NA
		Bear	88.20	9.75	5.01	1.11

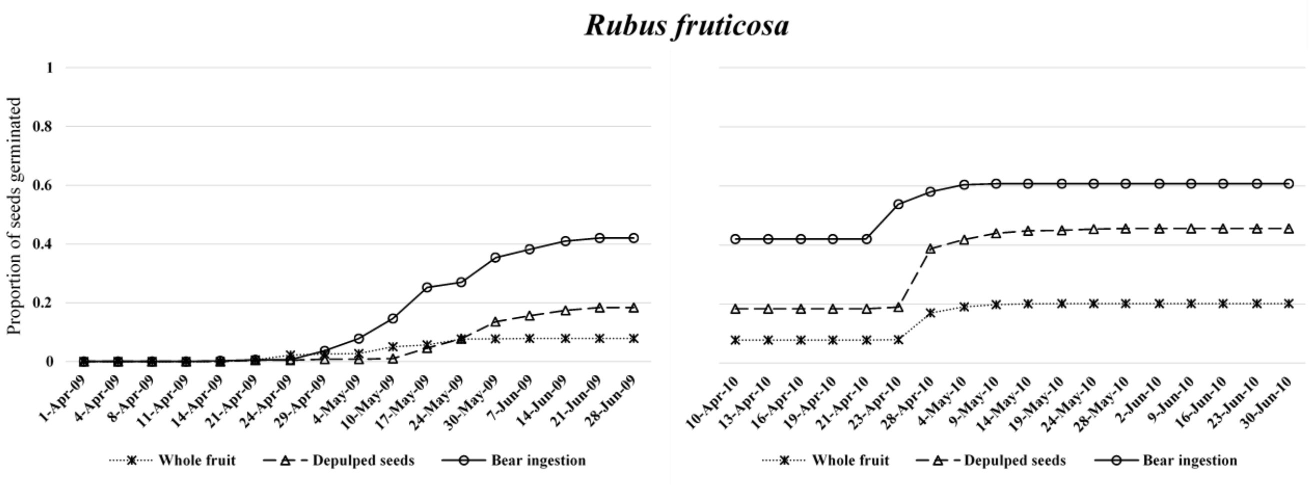
Viburnum opulus	Whole	0	NA	65.00	NA
	Depulped	0	NA	55.40	NA
	Bear	0	NA	49.60	NA
Crataegus monogyna	Whole	0	NA	20.00	12.90
	Depulped	2.00	NA	24.28	15.73
	Bear	0	NA	24.80	15.40

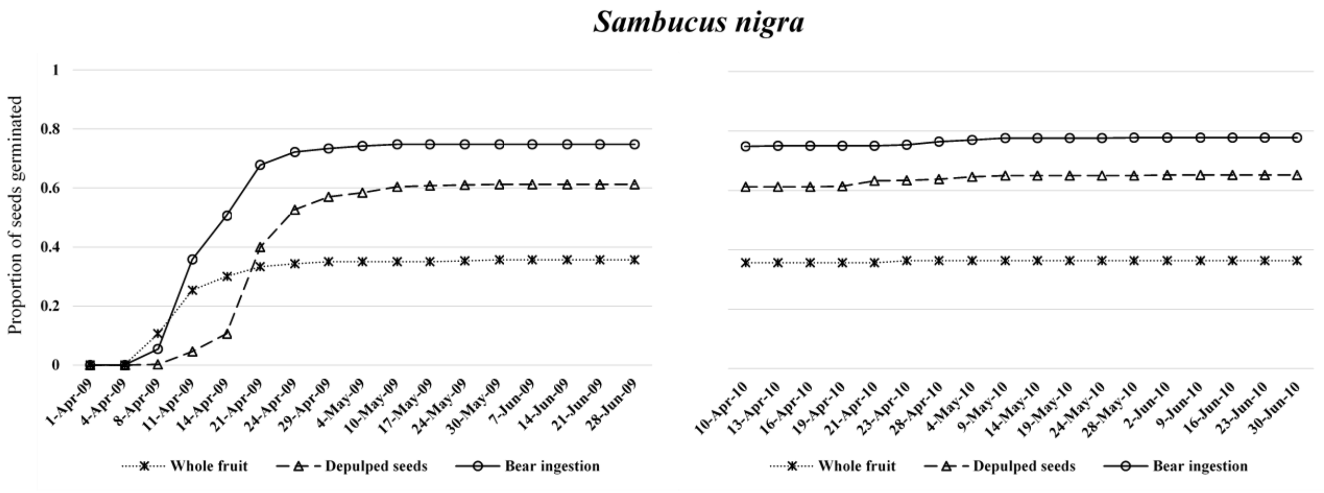
Appendix S5. Germination curves for eleven fleshy-fruited plant species eaten by brown bears in temperate regions in Eurasia and under three germination treatments: (1) seeds ingested by bears and recovered from the scats (empty dots), (2) manually depulped seeds (triangles) and (3) seeds embedded within the whole fruit (crosses) during 2009 (left pannel)- and 2010 (right pannel). Germination inspections started on April 1st and finished on June 30th each year.

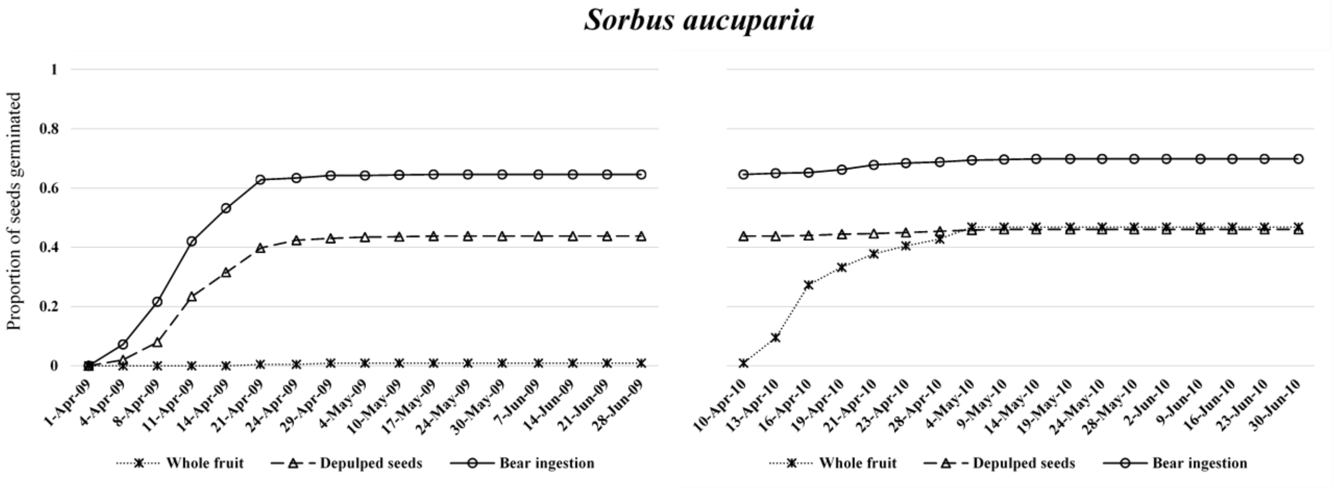

Rosa sp.


Frangula alnus


Vaccinium myrtillus


Rubus fruticosa


Sambucus nigra


Sorbus aucuparia
Proportion of seeds germinated
1
0.8
0.6
0.4
0.2
0
1-Apr-09
4-Apr-09
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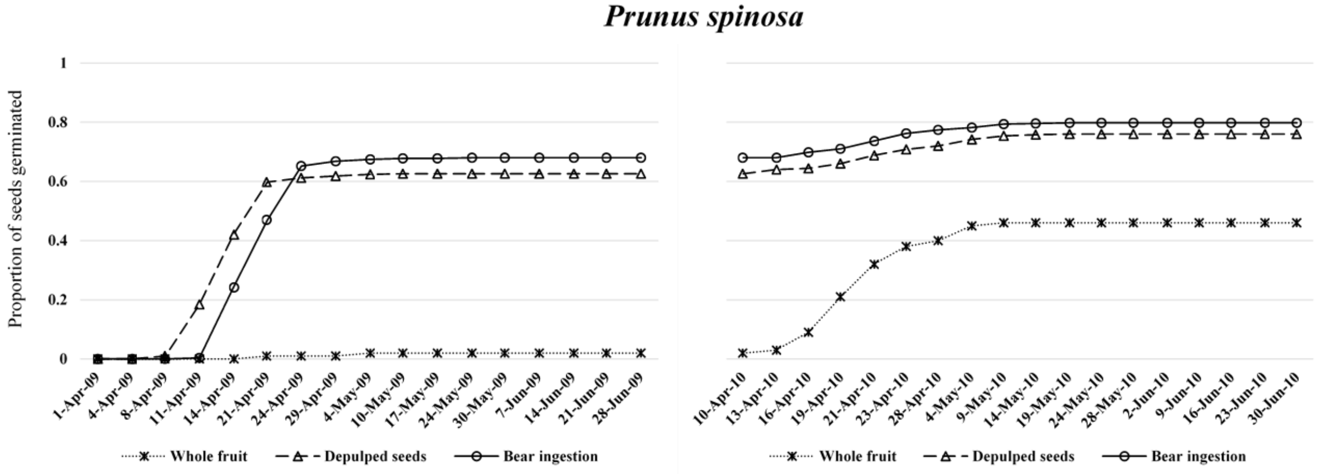
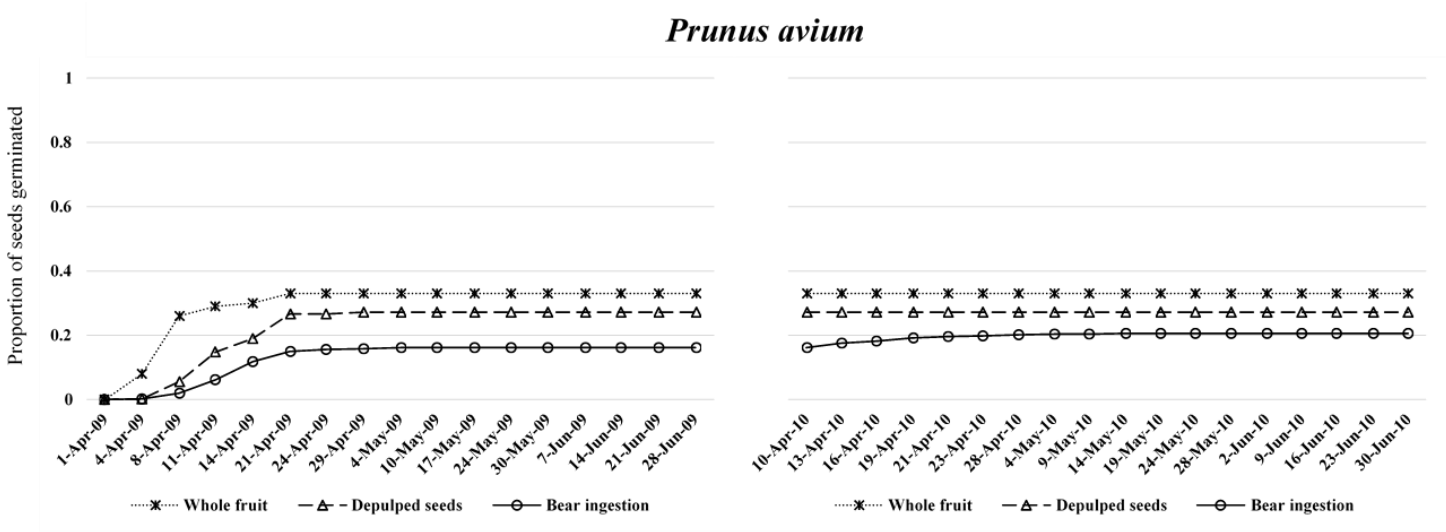
11-Apr-09
14-Apr-09
21-Apr-09
24-Apr-09
29-Apr-09
4-May-09
10-May-09
17-May-09
24-May-09
30-May-09
7-Jun-09
14-Jun-09
21-Jun-09
28-Jun-09
10-Apr-10
13-Apr-10
16-Apr-10
19-Apr-10
21-Apr-10
23-Apr-10
28-Apr-10
4-May-10
9-May-10
14-May-10
19-May-10
24-May-10
28-May-10
2-Jun-10
9-Jun-10
16-Jun-10
23-Jun-10
30-Jun-10
Whole fruit
Depulped seeds
Bear ingestion


Prunus spinosa
Proportion of seeds germinated
1
0.8
0.6
0.4
0.2
0
1-Apr-09
4-Apr-09
8-Apr-09
11-Apr-09
14-Apr-09
21-Apr-09
24-Apr-09
29-Apr-09
4-May-09
10-May-09
17-May-09
24-May-09
30-May-09
7-Jun-09
14-Jun-09
21-Jun-09
28-Jun-09
10-Apr-10
13-Apr-10
16-Apr-10
19-Apr-10
21-Apr-10
23-Apr-10
28-Apr-10
4-May-10
9-May-10
14-May-10
19-May-10
24-May-10
28-May-10
2-Jun-10
9-Jun-10
16-Jun-10
23-Jun-10
30-Jun-10
Whole fruit
Depulped seeds
Bear ingestion


Prunus avium
Proportion of seeds germinated
1
0.8
0.6
0.4
0.2
0
1-Apr-09
4-Apr-09
8-Apr-09
11-Apr-09
14-Apr-09
21-Apr-09
24-Apr-09
29-Apr-09
4-May-09
10-May-09
17-May-09
24-May-09
30-May-09
7-Jun-09
14-Jun-09
21-Jun-09
28-Jun-09
10-Apr-10
13-Apr-10
16-Apr-10
19-Apr-10
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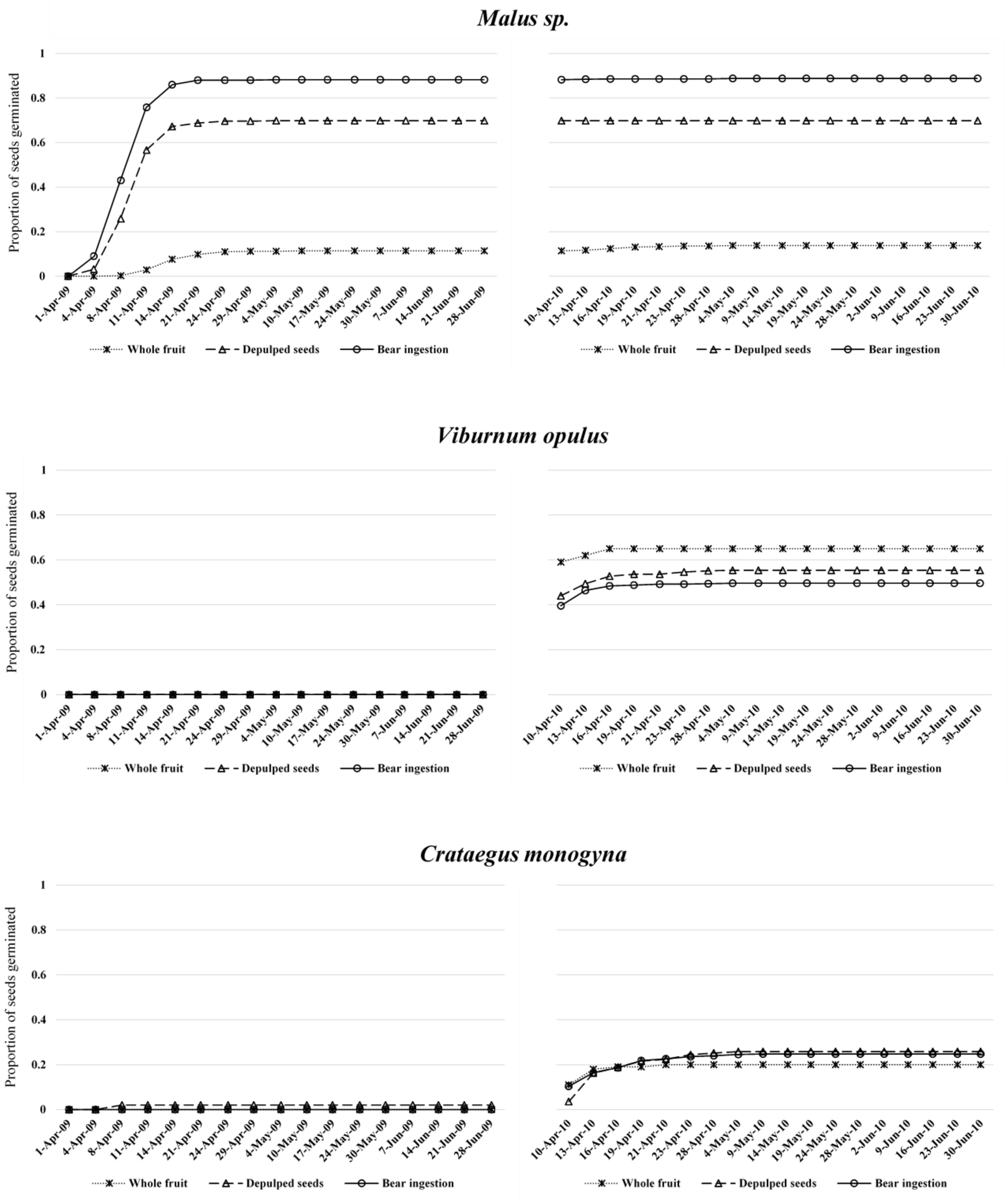
9-May-10
14-May-10
19-May-10
24-May-10
28-May-10
2-Jun-10
9-Jun-10
16-Jun-10
23-Jun-10
30-Jun-10
Whole fruit
Depulped seeds
Bear ingestion


Malus sp.
Viburnum opulus
Crataegus monogyna
Proportion of seeds germinated
0
0.2
0.4
0.6
0.8
1
1-Apr-09
4-Apr-09
8-Apr-09
11-Apr-09
14-Apr-09
21-Apr-09
24-Apr-09
29-Apr-09
4-May-09
10-May-09
17-May-09
24-May-09
30-May-09
7-Jun-09
14-Jun-09
21-Jun-09
28-Jun-09
10-Apr-10
13-Apr-10
16-Apr-10
19-Apr-10
21-Apr-10
23-Apr-10
28-Apr-10
4-May-10
9-May-10
14-May-10
19-May-10
24-May-10
28-May-10
2-Jun-10
9-Jun-10
16-Jun-10
23-Jun-10
30-Jun-10
Whole fruit
Depulped seeds
Bear ingestion

Appendix S6. ID, country, biome, latitude and longitude (in degrees) of the study areas (n = 96) from which data on brown bears diet were gathered. Number of species and genera, relative frequency of occurrence (rF) and relative volume (rV) of fleshy fruits in brown bear diet, number of samples analyzed and the reference from which data were collected are also provided for each study area.

ID	**Country**	**Biome**	**Latitude**	**Longitude**	**Species**	**Genera**	**rF**	**rV**	**No. samples**	**Reference**
1	Russia	Tundra	68.0	-178.3	4	3	0.27	NA	102	Chernjavskij & Krechmar (1993)
2	Russia	Tundra	67.9	-177.9	4	3	0.27	NA	102	Krechmar (1995)
3	Russia	Tundra	64.9	-174.8	3	2	0.14	NA	55	Chernjavskij & Krechmar (1993)
4	Russia	Tundra	65.0	-173.4	3	2	0.14	NA	55	Krechmar (1995)
5	USA	Tundra	69.3	-153.5	4	1	NA	NA	NA	Reynolds (1980)
6	USA	Tundra	63.3	-151.1	4	2	0.37	NA	810	Murie (1981)
7	USA	Tundra	63.5	-150.0	3	2	0.28	NA	196	Stelmock & Dean (1986)
8	USA	Tundra	63.6	-149.7	1	1	0.53	NA	406	Stelmock & Dean (1986)
9	USA	Tundra	69.1	-144.0	1	1	0.16	NA	113	Phillips (1987)
10	Canada	Tundra	69.2	-140.1	5	3	0.19	0.17	321	MacHutchon & Wellwood (2003)
11	Canada	Boreal forests & taiga	61.0	-138.0	2	2	0.27	NA	128	Pearson (1975)
12	Canada	Temperate coniferous forests	53.0	-127.0	10	8	NA	NA	NA	Hamilton & Bunnell (1987)
13	Canada	Temperate coniferous forests	53.3	-118.5	8	6	NA	NA	331	Cristescu et al. (2015)
14	Canada	Temperate coniferous forests	53.0	-117.0	1	1	NA	NA	665	Munro et al. (2006)
15	Canada	Temperate coniferous forests	51.2	-115.6	3	2	0.23	NA	381	Hamer & Herrero (1987)
16	USA	Temperate coniferous forests	49.3	-114.9	1	1	0.23	0.01	306	Mace & Jonkel (1986)
17	Canada	Temperate coniferous forests	49.0	-114.9	1	1	0.23	0.33	1100	McLellan & Hovey (1995)
18	USA	Temperate coniferous forests	47.0	-114.0	16	9	0.13	0.19	1094	Aune & Kasworm (1989)
19	USA	Temperate coniferous forests	47.0	-114.0	7	4	0.15	0.29	144	Aune (1985)
20	USA	Temperate coniferous forests	47.0	-114.0	1	1	0.15	0.21	1094	Aune (1994)
21	USA	Temperate coniferous forests	47.4	-113.9	1	1	0.18	NA	293	Mace & Jonkel (1986)
22	USA	Temperate coniferous forests	47.4	-113.9	1	1	NA	0.23	177	Servheen (1983)
23	USA	Temperate coniferous	47.4	-113.2	1	1	0.18	NA	140	Mace & Jonkel

		forests								(1986)
24	USA	Temperate coniferous forests	47.8	-112.7	1	1	0.19	0.01	417	Mace & Jonkel (1986)
25	USA	Temperate coniferous forests	44.6	-110.6	2	2	0.01	0.14	3028	Knight et al. (1982)
26	USA	Temperate coniferous forests	44.6	-110.6	1	1	NA	0.03	3938	Mattson et al. (1991)
27	USA	Temperate coniferous forests	44.6	-110.5	2	2	0.1	0.17	487	Craighead et al. (1995)
28	USA	Temperate coniferous forests	44.6	-110.5	3	3	0.03	0.03	615	Mealey (1980)
29	Canada	Tundra	64.5	-110.5	5	2	0.38	0.14	169	Gau et al. (2002)
30	Spain	Temperate broadleaf & mixed forests	43.3	-5.3	8	4	0.14	0.1	929	Clevenger et al. (1992)
31	Spain	Temperate broadleaf & mixed forests	43.3	-5.3	11	4	0.27	0.28	1500	Naves et al. (2006)
32	Spain	Temperate broadleaf & mixed forests	43.0	-5.0	10	3	0.19	NA	261	Braña et al. (1987)
33	France	Temperate broadleaf & mixed forests	43.0	-0.5	4	3	NA	0.4	252	Berducou et al. (1983)
34	France	Temperate broadleaf & mixed forests	42.7	0.6	6	4	0.07	NA	89	Lagalisse (2002)
35	Norway	Boreal forests & taiga	61.0	10.0	3	2	0.25	0.37	134	Elgmork & Kaasa (1992)
36	Italy	Temperate broadleaf & mixed forests	41.8	13.8	15	7	0.3	0.38	2359	Ciucci et al. (2014)
37	Italy	Temperate broadleaf & mixed forests	41.8	13.8	11	6	0.35	0.47	328	Di Domenico et al. (2012)
38	Italy	Temperate broadleaf & mixed forests	41.8	13.9	1	1	0.34	NA	232	Zunino & Herrero (1972)
39	Norway	Boreal forests & taiga	64.0	14.0	3	2	0.13	0.13	118	Dahle et al. (1998)
40	Sweden	Boreal forests & taiga	64.0	14.0	2	1	0.23	0.36	148	Dahle et al. (1998)
41	Slovenia	Temperate broadleaf & mixed forests	45.9	14.4	10	3	0.17	NA	363	Kavcic et al. (2015)
42	Slovenia	Temperate broadleaf & mixed forests	46.1	14.5	1	1	NA	0.13	200	Große et al. (2003)
43	Sweden	Boreal forests & taiga	61.0	15.0	3	1	0.32	0.53	539	Stenset et al. (2016)
44	Croatia	Temperate broadleaf & mixed forests	44.9	15.6	10	4	0.31	0.33	95	Cicnjak et al. (1987)
45	Slovakia	Temperate coniferous forests	48.6	19.5	7	3	0.19	0.14	243	Skuban et al. (2016)
46	Slovakia	Temperate coniferous forests	49.2	19.9	8	3	0.28	0.28	373	Rigg & Gorman (2005)
47	Poland	Temperate coniferous forests	49.2	20.1	6	3	0.31	NA	68	Jamnicky (1988)
48	Greece	Mediterranean forests, woodlands & scrubs	40.9	21.0	5	2	0.53	0.53	223	Mertzanis et al. (2000)

49	Poland	Temperate coniferous forests	49.2	21.0	2	1	0.08	0.08	46	Frackowiak & Gula (1992)
50	Greece	Mediterranean forests, woodlands & scrubs	39.9	21.1	5	3	0.54	NA	343	Mertzanis (1992)
51	Greece	Mediterranean forests, woodlands & scrubs	39.9	21.1	12	6	0.14	NA	360	Paralikidis et al. (2010)
52	Greece	Mediterranean forests, woodlands & scrubs	39.9	21.6	7	3	0.58	NA	208	Vlachos et al. (2000)
53	Slovakia	Temperate broadleaf & mixed forests	48.0	22.0	4	1	0.34	NA	215	Stofik et al. (2013)
54	Poland	Temperate coniferous forests	49.3	22.5	5	2	0.23	0.29	184	Frackowiak (1997)
55	Ukraine	Temperate broadleaf & mixed forests	48.3	23.2	1	1	0.3	NA	401	Vaisfeld & Chestin (1993)
56	Bulgaria	Temperate broadleaf & mixed forests	41.6	24.6	5	3	NA	0.42	128	Genov et al. (2008)
57	Bulgaria	Temperate broadleaf & mixed forests	41.8	24.8	9	3	0.51	0.52	148	Genov et al. (2010)
58	Estonia	Temperate broadleaf & mixed forests	59.0	26.0	4	2	0.23	0.35	142	Vulla et al. (2009)
59	Norway	Boreal forests & taiga	69.0	28.0	4	2	0.27	0.34	137	Persson et al. (2001)
60	Belarus	Temperate broadleaf & mixed forests	55.5	30.0	1	2	0.19	NA	732	Sidorovich (2006)
61	Russia	Boreal forests & taiga	60.1	31.8	7	2	0.08	NA	640	Novikov et al. (1969)
62	Russia	Boreal forests & taiga	67.8	32.5	1	1	0.64	NA	250	Semenov-Tian-Shanskii (1972)
63	Russia	Temperate broadleaf & mixed forests	56.3	32.7	12	4	0.59	0.6	474	Ogurtsov (2018)
64	Russia	Temperate broadleaf & mixed forests	56.5	33.0	1	1	0.05	NA	18	Pazhetnov (1990)
65	Russia	Temperate broadleaf & mixed forests	56.5	33.0	4	3	0.22	NA	200	Pazhetnov (1990)
66	Russia	Boreal forests & taiga	63.8	33.0	1	1	0.15	NA	114	Vaisfeld & Chestin (1993)
67	Russia	Boreal forests & taiga	58.5	37.8	7	2	0.35	NA	258	Kaleckaja (1973)
68	Russia	Boreal forests & taiga	58.6	38.0	7	2	0.15	NA	209	Razumovsky (1966)
69	Russia	Temperate broadleaf & mixed forests	43.8	40.4	1	1	0.32	NA	108	Vaisfeld & Chestin (1993)
70	Turkey	Temperate broadleaf & mixed forests	40.5	41.5	1	1	0.39	NA	72	Ambarl (2015)
71	Russia	Temperate broadleaf & mixed forests	53.1	56.5	1	1	0.28	NA	228	Sharafutdinov & Korotkov (1974)
72	Russia	Temperate broadleaf & mixed forests	53.0	57.1	1	1	0.08	NA	140	Vaisfeld & Chestin (1993)
73	India	Temperate broadleaf & mixed forests	32.0	76.0	5	4	0.11	NA	222	Rathore & Chauhan (2014)
74	India	Temperate coniferous	32.5	76.8	5	4	0.11	NA	222	Rathore (2008)

		forests								
75	Kazakhstan	Montane grasslands and shrublands	45.0	80.3	9	5	0.37	NA	884	Grachev & Fedosenko (1977)
76	Russia	Boreal forests & taiga	64.0	88.0	4	3	0.14	NA	39	Vaisfeld & Chestin (1993)
77	Russia	Temperate coniferous forests	52.3	92.8	1	1	0.23	NA	1279	Vaisfeld & Chestin (1993)
78	Mongolia	Deserts and xeric shrublands	43.4	97.2	1	1	NA	NA	365	Schaller et al. (1993)
79	Russia	Montane grasslands and shrublands	51.7	100.6	1	1	0.11	NA	895	Vaisfeld & Chestin (1993)
80	Japan	Temperate broadleaf & mixed forests	41.9	140.3	2	2	NA	0.17	18	Nomura & Higashi (2000)
81	Japan	Temperate broadleaf & mixed forests	41.9	140.3	7	6	0.45	0.59	44	Ohdachi & Aoi (1987)
82	Japan	Temperate broadleaf & mixed forests	41.9	140.3	10	7	0.16	0.14	223	Sato et al. (2005)
83	Japan	Temperate coniferous forests	45.0	142.0	9	6	0.13	0.16	90	Aoi (1985)
84	Japan	Temperate coniferous forests	45.2	142.0	4	3	0.14	0.31	73	Ohdachi & Aoi (1987)
85	Japan	Temperate coniferous forests	42.7	142.7	9	6	0.24	0.32	115	Sato et al. (2005)
86	Japan	Temperate coniferous forests	43.7	142.9	1	1	0.06	0.06	23	Ohdachi & Aoi (1987)
87	Japan	Temperate coniferous forests	43.7	142.9	7	6	0.13	0.12	218	Sato et al. (2005)
88	Japan	Temperate broadleaf & mixed forests	42.8	143.7	4	4	0.13	0.18	117	Sato et al. (2004)
89	Japan	Temperate broadleaf & mixed forests	42.8	143.7	6	4	0.23	0.19	34	Sato et al. (2004)
90	Japan	Temperate coniferous forests	44.1	145.2	8	5	0.14	NA	128	Ohdachi & Aoi (1987)
91	Russia	Boreal forests & taiga	60.0	148.0	11	3	0.47	NA	565	Chernjavskij & Petrichenko (1984)
92	Russia	Boreal forests & taiga	66.0	159.1	12	4	0.28	NA	1044	Chernjavskij & Petrichenko (1984)
93	Russia	Boreal forests & taiga	65.8	159.8	6	3	0.34	NA	108	Krechmar (1995)
94	Russia	Tundra	65.6	169.1	4	2	0.28	NA	107	Krechmar (1995)
95	Russia	Tundra	67.3	170.4	3	2	0.13	NA	148	Krechmar (1995)
96	Russia	Tundra	65.1	170.6	4	2	0.17	NA	112	Krechmar (1995)

Appendix S7. References (n=70) from which data on brown bear diet across its distribution range, including 96 study areas, were gathered. The ID of the study areas of each publication is also provided.

ID Study areas	**Reference**
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42	Große, C., Kaczensky, P., & Knauer, F. (2003). Ants: a food source sought by Slovenian brown bears (*Ursus arctos*)? *Canadian Journal of Zoology, 81*.
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Female brown bear with two cubs feeding on bilberry fruits in the study area. Picture: Adam Wajrak

The bear-berry connection: ecological and management implications of brown bears' food habits in a highly touristic protected area

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ABSTRACT

Diet studies are essential to understand animal ecology and ecosystem dynamics, especially in the case of large omnivores with a broad range of trophic interactions. These studies are particularly relevant in areas where human disturbance is intense and, thus, species dietary patterns might change and hinder the ecosystem services these species provide. We assessed the diet of brown bears *Ursus arctos* by DNA metabarcoding in Tatra National Park (southern Poland), a highly touristic protected area holding a high density of bears. Brown bears' diet showed a marked seasonality, a characteristic feature of brown bear populations relying on natural foods. Graminoids represented the main food during spring, while fleshy-fruited plants became more important from mid-summer. Fleshy-fruited plants were present in 56% of faeces and during the entire activity period of bears, revealing that fruits play a pivotal role in the feeding ecology of Tatra brown bears. Two berry species, *Vaccinium myrtillus* and *Rubus idaeus*, were the most frequently detected (in 42% and 20% of faeces, respectively). The large consumption of fleshy-fruited plants, and particularly of berries, suggests that, despite high levels of human disturbance in the area, brown bears still play a key role as seed dispersers. Management strategies focused on an effective waste management, control of berry picking, strict regulations about human activities in specific areas during sensitive periods in the feeding ecology of bears, and the lack of artificial food provisioning are crucial to ensure the seed dispersal processes and associated ecosystem services that bears and other frugivores provide.

KEYWORDS

Next-generation sequencing, DNA metabarcoding techniques, fleshy fruits, frugivory, *Vaccinium*, seed dispersal, *Ursus arctos*, diet.

INTRODUCTION

Human population is currently increasing in unprecedented numbers, with the environmental conditions worsening worldwide as a consequence of human-driven processes such as resource exploitation, pollution, climate change and their synergistic interactions (Brook et al., 2008). As a consequence, many wildlife species are forced to live in fragmented anthropogenic landscapes (Goudie, 2013). Human disturbance may act as a strong driver of biodiversity change that does not only compromise species persistence but also the ecological interactions in which they are involved and the related ecosystem services (Fontúrbel et al., 2015; Valiente-Banuet et al., 2015). For instance, habitat fragmentation and degradation have effects on animal-mediated seed dispersal, especially on the diversity of seed dispersing animals and the frequency of plant-seed disperser interactions (McConkey et al., 2012; Fontúrbel et al., 2015). However, we still know little about how other forms of human disturbance in natural areas such as mountain tourism, the provision of artificial food to wildlife or the extraction of natural foods by humans (e.g. berry picking) may hinder animal-mediated seed dispersal processes.

Understanding animal mediated ecosystem functions, such as seed dispersal by frugivores, requires a good knowledge of species diet, especially in those with a complex feeding ecology such as large carnivores with an omnivorous diet (Nawaz et al., 2019). Large carnivores have an important conservation value as they play key roles in ecosystems, thus, a better understanding of their feeding ecology is crucial to determine the effects they exert on other trophic levels (Monterroso et al., 2018; Ciucci et al., 2020). Accurate diet analyses are mandatory to enhance our understanding of ecosystem functioning and to implement effective conservation and management actions, particularly in highly humanized ecosystems. However, precise knowledge about the feeding ecology of omnivores and the ecological implications of consumer – resource interactions is still limited due to the difficulties in accurately determining the complex variety of food items consumed (De Barba et al., 2014). To solve this limitation, DNA metabarcoding approaches and high throughput sequencing (HTS hereafter) have been increasingly used as a tool to obtain more accurate identification of the food items consumed, even for food remains which are too small or too digested to be identified microscopically and from complex diet mixtures (Valentini et al., 2009; De Barba et al., 2014). By using universal primers, this approach allows the simultaneous identification of various DNA sequences of taxa present in dietary samples collected non-invasively in the field (e.g. faecal or regurgitate remains; Pompanon et al., 2012). Metabarcoding techniques have already been successfully applied to diet studies of different groups of mammals such as large carnivores, ungulates or rodents (Elfström et al., 2014; Fløjgaard et al., 2017; Lopes et al., 2020; Nørgaard et al., *in press*).

The brown bear *Ursus arctos* (Order: Carnivora, Family: Ursidae) is one of the world's most widely distributed terrestrial mammals and the largest living terrestrial carnivore. As other omnivore species, brown bears are flexible dietary generalists that rapidly adapt to environmental conditions and seasonal differences in food availability (Bojarska and Selva, 2012). During the hyperphagic season prior to hibernation, brown bears inhabiting boreal, temperate and Mediterranean areas feed intensively on fleshy fruits, whose seeds usually remain undamaged after bear ingestion and can be deposited over long distances. Due to this, bears' fleshy-fruit-based diet is important not only for bears but also for the seed dispersal and recruitment of the plant species they consume (García-Rodríguez et al., 2021). However, human activities, which are known to reduce natural resource use and alter spatiotemporal activity patterns in brown bears (Nevin and Gilbert, 2005a; Nevin and Gilbert, 2005b; Rode et al., 2007) are likely to have consequences for the seed dispersal services provided by brown bears. However, the impact of human activities on the diet of brown bears and their role as seed dispersers is still poorly understood. Here we analyzed the dietary patterns of brown bears inhabiting the Tatra National Park (southern Poland), a highly populated and touristic alpine area in the Carpathian Mountains holding a high density of bears, with a particular focus on their role as seed dispersers. Specifically, we aimed to answer the following questions: 1) Which is the dietary diversity of brown bears in Tatra National Park across the entire activity period of the species? 2) How important are fleshy fruits for the brown bears inhabiting the area? and 3) Can brown bears still provide essential seed dispersal services despite high levels of human disturbance? Finally, we discuss the management actions which should be implemented in order to preserve the ecosystem services provided by bears in highly disturbed areas.

MATERIAL AND METHODS

Study area

The study was conducted in Tatra National Park (Poland) and its vicinity, a 211 km^2 protected area located in the Tatra Mountains, a mountain range belonging to the western part of the Carpathian Mountains in the Poland - Slovakia borderline (Fig. S1). Tatra National Park ranges from 774 to 2,499 meters above sea level (m.a.s.l.) and lies entirely in the temperate conifer forests terrestrial biome (Olson et al. 2001). Montane forests (below 1,550 m.a.s.l.) occupy around 60% of the total surface of the area and are dominated by *Picea abies*, partly introduced in the original habitat of *Abies alba* and *Fagus sylvatica* during the 19th century. *Pinus mugo* are abundant in subalpine areas (1,550 – 1,800 m.a.s.l.). *Vaccinium myrtillus* dominates montane, subalpine and alpine floors (up to 2,300 m.a.s.l.). Other shrub species such as *V. vitis-idaea*, *V. gaultheriodes* and *Rubus idaeus* can be found at different elevations (Mirek and Piękoś-Mirkowa, 1992).

Tatra National Park is one of the most popular tourist destinations in Poland, visited currently by more than 3.5 million tourists annually. The number of visitors peaks in July and August, when up to 40,000 people may enter the park daily (https://tpn.pl/zwiedzaj/turystyka/statystyka). Visitors' presence, which from March to November is only allowed during daylight hours, is limited to a network of more than 270 km of touristic trails and associated resting areas, which yields a density of more than 1 km of public paths per km^2 within the protected area. Berry picking within the national park is only allowed under special permits (e.g. for scientific research), but illegal picking still happens, even on a large scale in some areas (AGR and TZK personal observations). No artificial feeding of wildlife is conducted within the national park (Zwijacz-Kozica et al., 2017). However, artificial feeding sites for ungulates are found in surrounding areas, although in small numbers in the Polish territory (AGR; personal observation), but in much larger numbers in neighbouring areas in Slovakia (Rigg and Gorman, 2005).

The Tatra brown bear population segment is transboundary, with some animals moving regularly between Poland and Slovakia, and it is estimated at 45-79 individuals for Tatra National Park (Konopiński et al., 2019, Bartoń et al., 2019), which suggests one of the highest bear densities in Europe. Brown bears in the area usually hibernate from November to March, have a low to normal feeding activity from den emergence and until the end of the mating season ("hypophagia" hereafter; April-June) and start feeding intensively from July until hibernation ("hyperphagia"; Bartoń et al., 2019; García-Rodríguez et al., 2020)). Bear sightings by tourists are frequent in the area and some animals can approach human settlements, especially during the autumn. However, bear attacks to humans are rare and damages to human properties (i.e. livestock, apiaries, agriculture, etc.) are uncommon when compared to other brown bear areas in Europe (Bautista et al., 2017; Bombieri et al., 2019).

- **Field collection of brown bear faeces**

We collected fresh brown bear faeces in Tatra National Park during a two-year period (from July 2017 to July 2019) and comprising the entire activity period of brown bears in the area (April to November). Based on our research interests, we considered three days as the maximum age for a fresh sample. We estimated the age of each sample based on its smell and shape, considering the weather conditions of the previous days. We followed three different approaches for collection of faeces: 1) during inspections of clusters of GPS-collared bears; 2) during inspections of nine transects (1.5 km length and 3 m width each) established in different areas of the national park and surveyed once a month from July to October in 2017 and 2018, and 3) during routine conservation and monitoring tasks conducted by Tatra National Park staff. In order to have a representative subsample of the food items contained in each brown bear faecal sample and to minimize external environmental contamination, we opened each sample and took two or three small pieces, depending on sample size, from different parts of the inside of the faeces, obtaining a total subsample of about 1 cm^3. For each sample, we noted the date of collection, the GPS coordinates (latitude and longitude) and the elevation (Table S1). We put each sample in a tube two thirds full with silica gel and stored it at room temperature until processing for DNA metabarcoding.

- **Diet composition identification**

DNA metabarcoding analysis for the identification of the food taxa contained in bear faeces was performed by amplifying and sequencing on a high-throughput sequencing platform short DNA fragments of plants, insects and invertebrates. We used three universal primer pairs to target each of these major groups of bear diet (one pair for each group; De Barba et al., 2014; Taberlet et al., 2018). DNA extraction, PCR amplification and sequencing, sequence data filtering and analysis as well as taxonomic identification were carried out based on published protocols (Taberlet et al., 2012; De Barba et al., 2014). Detailed information of all steps is described in Appendix A. Taxa identified as nonnative species in our study area and not considered to have been introduced by human activities were reassigned to a higher taxonomic level present in the study area. After sequence data filtering, we classified each identified taxon as (i) natural food item, if it naturally occurs in the study area, or as (ii) possibly human-related food item, if the item could be related to human activities (i.e. if natural counterparts also occurred in the area).

- **Data organization and statistical analysis**

Brown bear diet was assessed based on the taxonomically assigned DNA sequences obtained after analysis and filtering of the sequence data. Prior to statistical analysis, we classified all the food items detected in brown bear faeces into 10 different food categories nested in three major groups: vertebrates, insects and plants. Vertebrate taxa were subsequently classified as birds or mammals, whereas plants were divided into seven different categories: plant species producing fleshy fruits (e.g. berries, drupes), plant species producing hard mast (e.g. acorns, nuts), tree species with neither fleshy fruits nor hard mast (e.g. *Acer sp.*), plant species with edible roots (e.g. tubers), graminoids (i.e. herbaceous species with grass-like morphology), forbs (i.e. non-graminoid herbaceous species) and cryptogams (i.e. plant species reproducing by spores, without seeds).

We assigned each brown bear faecal sample to the month of collection and to the season of feeding activity (hypophagia or hyperphagia). We collected only three samples during early November (before November 11th) and one of them was discarded after sequence quality filtering. Thus, we included the other two samples in October for further analyses (Table S1). We extracted information about the number of unique DNA sequence reads produced and assigned them to each taxon in each faecal sample. Additionally, we calculated the frequency of occurrence of each taxon detected as the number of faeces in which a given food taxon is recorded divided by the total number of faeces. We constructed a brown bear faecal sample × food taxon matrix of presence/absence of each taxon in each faecal sample. We used this matrix to measure the diversity of Tatra brown bears' diet with two complementary indices: (1) the Hill series number 0 (i.e. the total number of taxa detected in each sample; "N_0" hereafter; Hill, 1973) and (2) the Gini-Simpson index ($\lambda = 1 - \sum p_i^2$; the probability that two items randomly taken from a faecal sample belong to different food categories; Simpson, 1949). We calculated these two indexes for each faecal sample separately.

We used univariate generalized linear models to analyze the effects of seasonality (separately for the feeding season – hypo and hyperphagia- and the month) on the scores of the two indexes calculated to measure trophic diversity (N_0 and Gini-Simpson index) and on the frequency of occurrence of each of the ten food categories considered. Models related to Gini-Simpson's index and to the frequency of occurrence of food categories were fitted with a binomial distribution, whereas the two models analyzing the influence of the phenology on N_0 index were fitted with a Poisson distribution. We used the *R* statistical environment (version 3.4.0, R Development Core Team 2017) to perform all statistical analyses. The map in Fig. S1 was built in *QGis* software (v2.14.22) whereas the rest of the figures was created using the R packages *ggplot2* (Wickham, 2011), *bipartite* (Dormann et al., 2008) and *VennDiagramm* (Chen and Boutros, 2011).

RESULTS

We collected a total of 253 brown bear faeces in the study area between July 2017 and July 2019. PCR products generated a total of 26,901,502 paired-end sequence reads. We discarded seven samples that yielded no sequence reads after sequence data analysis and filtering. Thus, we used 246 faeces for all further analyses (42 and 204 from hypo and hyperphagia, respectively; Table S1, Fig. S1).

- **Diet composition**

We detected a total of 285 different molecular operational taxonomic units -MOTUS- (see online supplementary information) corresponding to 173 different taxa identified in brown bear faeces. Among these, 25 taxa were identified as vertebrates (8 avian and 17 mammalian taxa), 48 were identified as insects and 100 taxa were identified as plants (7 of them were assigned to plant species producing fleshy fruits, 2 to plant species producing hard mast, 6 to tree species with neither fleshy fruits nor hard mast, 2 to plant species with edible roots, 14 to graminoids, 55 to forbs and 14 to cryptogams; Table S2). Taxonomic resolution varied among vertebrates, insects and plants, but in the three groups more than 50% of the taxa were assigned at least to genus level (Table S3). We could assign 46% of the detected insect taxa, 44% of vertebrate taxa and 12% of plant taxa to species level (Table S3).

The number of taxa detected per brown bear faecal sample ranged from one to 19 (mean±standard deviation = 6.37±3.47 taxa; Table S4). As much as 97% and 67% of the faeces contained more than one and five food items, respectively (Table S1). Forty six percent of the taxa were detected exclusively in one faecal sample and 58% of the taxa in less than 1% of the faeces (i.e. only in one or two faeces). Plant material was detected in 99% of the faeces (244 out of 246; Fig. 1). We found insect and vertebrate materials in 55% and 17% of the samples, respectively. Vertebrate, insect and plant material were present together in 10% of the faeces (Fig. 1).

Apiaceae and Ericaceae were the plant families most commonly detected, being present in 78% and 42% of the bear faeces. Psychodidae and Anisopodidae were the most frequent families among insects (found in 13% and 6% of samples, respectively), whereas Cervidae and Canidae were the mammalian families most often detected (7% and 3%, respectively; Fig. S2). Seven genera (six plants and one insect) were detected in more than 10% of the faeces. Among them, two fleshy-fruited plant genera, *Vaccinium* and *Rubus*, were the most frequently detected (in 42% and 20% of faeces, respectively), while *Psychomora* and *Cervus* were the most common insect and vertebrate genera (13% and 6%, respectively; Table 1). At species level, only four plants were detected in more than 10% of the samples. Two fleshy-fruited plant species were the most commonly found- *V. myrtillus* (present in 42% of faeces) and *R. idaeus* (20%), followed by the forb *Stellaria nemorum* (12%) and by the hard mast tree *Fagus sylvatica* (11%). The diptera *Sylvicola fenestralis* and the hymenoptera *Vespula vulgaris* were the insects most commonly found (6% and 5%, respectively) and *Cervus elaphus* the most common vertebrate (6%; Table 1).

Only 14 out of the 173 taxa identified could possibly derive from human activities, which means 8% of the taxa detected. Specifically, we detected seven vertebrate, one insect and six plant taxa with a possible anthropogenic origin (Table S2; Table S5). These food items were detected in 17 faeces (7% of the total). Among these taxa, only five vertebrates, mostly related to livestock activities, were found in more than one bear faeces. Additionally, in three cases, these taxa have a wild counterpart present in the study area and, thus, we could confirm the anthropogenic origin of only two of these five items (*Bos sp.* was found in five samples and *Meleagris gallipavo* in two; Table S2; Table S5).

- **Temporal patterns in brown bear diet**

We found both a seasonal and a monthly variation in the diet of Tatra brown bears, with the frequency of occurrence of four out of the ten different food categories considered being influenced by the feeding season (hypo and hyperphagia). Similarly, the month of collection also affected the frequency of occurrence of six out of these ten categories (Table S6). Thus, brown bear diet composition showed a strong variation in relation to both the feeding season and the month. When only the feeding season was considered (without taking into account months separately), we found that graminoids were more frequently consumed during hypophagia, whereas insects, cryptogams and fleshy-fruited plant species were more frequently consumed during hyperphagia (Fig. 2; Fig. S3, Table S6). When monthly variation in brown bear diet was analyzed, we additionally found that plant species producing hard mast were significantly more frequently consumed towards the end of the bears' activity period (September – October), whereas vertebrates were more common in bear diet just after den emergence (April) and just before hibernation (September – October; Fig. S3, Table S6).

- **Relevance of fleshy-fruited plant species in brown bear diet**

Taxa producing fleshy fruits were present in 56% of the faeces and during the entire activity period of brown bears, i.e. from April to November. We registered a peak during August (80%; Fig. 2; Fig. S3; Table 2). Native species *V. myrtillus* and *R. idaeus*, present in 42% and 20% of the samples respectively, were the fleshy-fruited plant taxa more frequently consumed. Moreover, *V. myrtillus* was found in faeces collected during the entire activity period of bears, whereas *R. idaeus* was detected in all months except in April (Fig. 2; Table 2). Apart from these two species, five more plant species producing fleshy fruits were consumed by brown bears (Table 2; Table S2). Three of them (*V. vitis-idaea*, *Prunus sp*. and *Ribes sp*.) are also native to the study area. *V. vitis-idaea* was detected in 3.6% of all faeces and was found in faeces collected at the end of the hyperphagia, coinciding with *V. vitis-idaea*' fruiting period (September and October), but also in faeces collected just after bear emergence from dens (April and May). *Prunus sp*. was detected in 3.2% of faeces and only in those collected from August to October. *Ribes sp.* was detected only in one faeces collected in July (0.4% of all samples). The other two taxa assigned as fleshy-fruited plant species (the family Actinidiaceae and *Capsicum sp*.) do not naturally occur in the area and, thus, their presence in brown bears' diet is most likely of human origin. These non-native fleshy-fruited taxa were found only in three bear faeces collected at the beginning of bears' activity period (i.e. April; Table 2; Table S6).

DISCUSSION

- **Ecological considerations of a berry-based diet in brown bears**

Here we have shown that fleshy fruits, and more specifically berries, are key food resources for brown bears inhabiting Tatra National Park. Two fleshy-fruited plant species, *V. myrtillus* and *R. idaeus*, were the most relevant food items for bears in the region, suggesting that, as it also happens in many brown bear populations worldwide, fleshy fruits are pivotal for the species, especially during hyperphagia, when they feed intensively in order to achieve the energetic demands needed to survive the winter (Welch et al., 1997; García-Rodríguez et al., 2021). *V. myrtillus* abundance is known to affect brown bears´ body size, movement and reproductive success and it is a key feature for habitat selection for Scandinavian brown bears (Hertel et al., 2016; Hertel et al., 2018).

Additionally, a recent work on the role of brown bears as seed dispersers across their distribution found that *V. myrtillus* and *R. idaeus* are among the fleshy-fruited plant species that are most commonly consumed by the brown bear across its geographic range (García-Rodríguez et al., 2021). Interestingly, we detected consumption of *V. myrtillus* and *V. vitis-idaea* during spring, whereas the fruiting season of these species starts during late summer in the study area. Some fruits, especially of *V. vitis-idaea*, may persist under the snow and they can be available for brown bears and other frugivores the next spring, once the snow has melted. However, for the case of *V. myrtillus*, whose fruits rarely persist in the shrub after heavy snowfalls (TZK personal observation), we believe that brown bears might have fed primarily on other plant parts such as twigs, young shots or flowers during spring, either intentionally or accidentally when eating other foods. This finding is supported by previous research conducted in Scandinavia where *V. vitis-idaea* fruits were found in up to 34% of brown bear faces collected during spring, whereas only 4% of these faeces contained *V. myrtillus* fruits (Stenset et al., 2016).

The large consumption of fleshy fruits, reaching up to 80% of their diet in some periods, suggests that brown bears may play an essential role as seed dispersers in Tatra National Park. In line with this, previous research found that brown bears are responsible for the vast majority of dispersal of *V. myrtillus* seedsin the area (García-Rodríguez et al., in preparation). Together with the huge amounts of berries they consume, other brown bear features are essential to understand the uniqueness of the seed dispersal services provided by the species. For instance, brown bear faeces can be deposited several kilometers away from the place where fruits were ingested and each of them may contain thousands of undamaged seeds that may germinate (Lalleroni et al., 2017; García-Rodríguez et al., 2021). These long-distance seed dispersal events are essential for gene flow and plant population dynamics (Nathan & Muller-Landau, 2000). Additionally, bears usually defecate next to their resting sites, where they dig out soil and create local disturbances on the ground that may enhance germination (Steyaert et al., 2019; García-Rodríguez and Selva, submitted). As seedling establishment is usually rare in clonal species such as *V. myrtillus,* especially within stands of conspecific adults (Eriksson and Fröborg, 1996), these small disturbances created by bears might facilitate seedling recruitment in these species by exposing the defecated seeds to suitable conditions for germination (Steyaert et al., 2019). Research conducted in the study area has found that bilberry germination was associated to all marked brown bear faeces, with up to the 16% of the seedlings surviving for at least one year (García-Rodríguez and Selva, submitted). All this combined, and especially considering that that long-distance seed dispersal by other large-bodied frugivores is often missing in brown bear areas, suggests that frugivory by brown bears might be essential for the regeneration of fleshy-fruited plants in temperate and boreal ecosystems.

Frugivory by brown bears may be crucial to support the adaptation of fleshy-fruited plant species to global warming, particularly in mountain regions, like the Tatra alpine ecosystem. Climate change is likely to affect the distribution, survivorship and productivity of essential natural foods of brown bears, including *V. myrtillus* and other fleshy-fruited plant species (Rodríguez et al., 2007; Roberts at al., 2014; Penteriani et al., 2019). As fruit maturation in most species occurs later at higher elevations and brown bears can track food availability (Rötzer and Chmielewski, 2001; Deacy et al., 2016), bears might be mobilizing seeds uphill while following the phenology of the fruits they rely on, facilitating the colonization of upper areas by the seeds they disperse (González-Varo et al., 2017). With average temperatures constantly increasing due to climate change and taking into account the negative relationship between temperature and elevation, this uphill movement might be especially important for plants that, as *Vaccinium* species, benefit from being covered by snow during winter. The uphill dispersal of seeds provided by other ursid species, the Asiatic black bear *U. thibetanus*, seems to support the adaptation of some fleshy-fruited plant species to global warming (Naoe et al., 2016).

- **Management considerations of a berry-based diet in brown bears**

Our findings suggest that brown bears can still play a crucial role in seed dispersal processes even with high levels of human disturbance when the management is appropriate. We believe that actions carried out by the National Park staff, such as the aversive conditioning to bears approaching human settlements, the proper waste management inside the Park and the strict regulations of berry picking by humans, together with the high productivity in terms of fruit production, are important factors explaining that bears still feed intensively on natural foods, and particularly on fleshy-fruits, in Tatra National Park, in spite of the large amount of visitors and high human disturbance. Additionally, tourists are neither allowed to leave the public paths nor to walk during the night within the national park from March to November, coinciding with the activity period of bears. Thus, human presence in the area is considered predictable both spatially and temporally. This predictability is known to reduce spatial displacement and minimize nutritional impacts in brown bears inhabiting areas disturbed by humans (Rode et al., 2007). For instance, total resource use by brown bears in Alaska declined when bears were exposed to 24-hour daily human activity, but not when human presence was limited to daylight, indicating that time regulations may be an effective management strategy to maintain the natural feeding ecology of the species (Rode et al., 2007). All this considered, we believe that the identification and protection of natural feeding areas important for bears should be a priority to make human presence more predictable in such areas. This information could be used to implement temporal and spatial restrictions to human activities such as hiking. This strategy has been already suggested and successfully implemented in other bear areas in Europe such as in the Cantabrian Mountains (Naves et al., 2006). Additionally, this may have positive effects not only for preserving the natural feeding ecology and the seed dispersal services provided by bears and other frugivores but also for minimizing the chances of human-bear encounters, thus, enhancing safety of both bears and humans.

Brown bears in highly disturbed areas can still have a natural diet with an insignificant contribution of human-related foods. Anthropogenic foods, which are known to disrupt temporal dietary patterns and lower the quality of bears' foods (Sergiel et al., 2020), are almost negligible in the diet of bears inhabiting Tatra National Park. This may be partially explained by the absence of artificial feeding practices and crops in the study area, in spite of these practices being common in the neighbouring areas of Slovakia. Our results are clearly in contrast with diet data from the Slovakian side of the Tatra Mountains, where crops, much more widespread there due to milder climatic conditions, represented 30% of the total volume of brown bears' diet and became the most abundant food during autumn (*Avena sativa*, *Zea mais* and *Triticum aestivum*; Rigg and Gorman., 2005), instead of fleshy fruits, as in our study. Artificial feeding practices are known to change dietary patterns in brown bears especially if provided in large amounts (Kavčič et al., 2015). For instance, bear diet in Tatra National Park had a significantly higher quality and a more seasonal pattern than the diet of bears inhabiting the Bieszczady Mountains, in the eastern part of the Polish Carpathians, where artificial food targeted to ungulates is provided and used year-round by brown bears (Sergiel et al., 2020). However, it is still unclear to what extent these artificial food subsidies can disrupt diet patterns of brown bears and compromise the seed dispersal services they provide.

Here we demonstrated that human presence *per se* does not necessarily compromise the feeding requirements of brown bears and that an effective and well-informed management is pivotal to guarantee a natural-based diet in brown bears and the subsequent ecosystem services they provide. However, some considerations should be still highlighted. First, this scenario may differ in areas where brown bears are hunted as human presence in such areas directly alters habitat selection and force bears to select less productive areas (Lodberg-Holm et al., 2019), which might compromise bears' nutritional requirements and the related ecosystem services. Second, little information is still available about how berry picking by humans might compromise feeding requirements of frugivore species and their subsequent seed dispersal services (Ticktin, 2004; McConkey et al., 2012). Berry picking is a common practice in many brown bear areas (Stryamets et al., 2012) and it might considerably reduce the amounts of fleshy fruits available for wildlife. For instance, berry picking by humans represent as much as 10%-15% of the total natural production of *V. myrtillus* and *V. vitis-idaea* in areas of Eastern Finland and Russian Karelia (Belonogova, 1988; Turtiainen et al., 2011). In addition, some countries are nowadays implementing development programs and public measures such as a tax-free income to encourage berry pickers (e.g. Finland; Saastomoiken, 1999). Therefore, there is a need to ensure that these practices do not disrupt seed dispersal processes carried out by bears and other frugivore species, especially considering that the effects of harvest on the productivity of fleshy-fruited plant species may take years to become apparent (McConkey et al., 2012), which might compromise population dynamics of both fleshy-fruited plants and their associated frugivores in the long term.

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AUTHORS' CONTRIBUTION

AGR, MDB and NS conceived the study and designed the methodology. PT designed the blocking primers to be used with Inse01 marker. AGR and TZK collected the samples in the field. MDB and DR prepared the samples and performed the extraction, amplification and sequencing of DNA material. CL performed bioinformatic analysis and preliminary data filtering, which was subsequently finished by AGR and TZK. AGR performed all statistical analyses and wrote a first draft of the manuscript with significant contributions of MDB and NS. All authors contributed critically to the draft and gave final approval for publication.

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Table 1. Diet composition of brown bears in the Tatra Mountains (Poland) in 2017-2019, indicating the most common species and genera identified in the faeces analyzed (n=246) by DNA metabarcoding techniques. The frequency of occurrence for each food taxon (proportion of faeces with a given taxon) is calculated for the entire activity period of brown bears (April-November), as well as for the hypophagia (April-June) and hyperphagia (July-November) seasons separately. Only taxa detected in more than 3% of the faeces are listed (see Table S2 for the full list).

			Frequency of occurrence		
	Family	**Category**	**Total**	**Hypophagia**	**Hyperphagia**
Species					
Vaccinium myrtillus	Ericaceae	Fleshy fruits	41.9%	9.3%	48.5%
Rubus idaeus	Rosaceae	Fleshy fruits	20.3%	23.3%	19.6%
Stellaria nemorum	Caryophyllaceae	Forbs	11.8%	16.3%	10.8%
Fagus sylvatica	Fagaceae	Hard mast	11.4%	0	13.7%
Sylvicola fenestralis	Anisopodidae	Insects	6.1%	0	7.4%
Cervus elaphus	Cervidae	Mammals	5.7%	0	6.9%
Vespula vulgaris	Vespidae	Insects	5.3%	0	6.4%
Vaccinium vitis-idaea	Ericaceae	Fleshy fruits	3.7%	4.7%	3.4%
Genera					
Vaccinium	Ericaceae	Fleshy fruits	42.3%	11.6%	48.5%
Rubus	Rosaceae	Fleshy fruits	20.3%	23.3%	19.6%
Luzula	Juncaceae	Graminoids	17.9%	51.2%	10.8%
Urtica	Urticaceae	Forbs	15.9%	25.6%	13.7%
Psychomora	Psychodidae	Insects	13.0%	2.3%	15.2%
Stellaria	Caryophyllaceae	Forbs	11.8%	16.3%	10.8%
Fagus	Fagaceae	Hard mast	11.4%	0	13.7%
Athyrium	Woodsiaceae	Cryptogams	8.1%	0	9.8%
Sylvicola	Anisopodidae	Insects	6.1%	0	7.4%
Cervus	Cervidae	Mammals	5.7%	0	6.9%
Vespula	Vespidae	Insects	5.3%	0	6.4%
Leontodon	Asteraceae	Forbs	4.9%	0	5.9%
Rhipidia	Limoniidae	Insects	4.5%	0	5.4%
Trifolium	Fabaceae	Forbs	4.1%	4.7%	3.9%
Dryopteris	Dryopteridaceae	Cryptogams	3.3%	0	3.9%
Prunus	Rosaceae	Fleshy fruits	3.3%	0	3.9%

Table 2. Frequency of occurrence (proportion of faeces with a given taxon) of fleshy-fruited plant taxa in brown bear faeces collected in the Tatra Mountains (Poland, n=246). Numbers are given for the entire activity period of brown bears and for each month separately. The number of faeces analyzed each month is provided in brackets. Two samples collected in November were assigned to October.

	Total (246)	**April (11)**	**May (12)**	**June (19)**	**July (36)**	**August (45)**	**September (32)**	**October (91)**
Frequency of occurrence								
Vaccinium myrtillus	41.9%	18.2%	8.3%	5.3%	38.9%	73.3%	56.3%	35.2%
Rubus idaeus	20.3%	0	16.7%	42.1%	27.8%	4.4%	15.6%	11.0%
Vaccinium vitis-idaea	3.7%	9.1%	8.3%	0	0	0	3.1%	6.6%
Prunus sp.	3.3%	0	0	0	0	4.4%	3.1%	4.4%
Capsicum sp.	0.8%	18.2%	0	0	0	0	0	0
Actinidiaceae	0.4%	9.1%	0	0	0	0	0	0
Ribes sp.	0.4%	0	0	0	2.8%	0	0	0
Total fleshy fruits	**56.1%**	**36.4%**	**33.3%**	**47.4%**	**58.3%**	**80.0%**	**65.6%**	**48.3%**

Figure 1. Venn diagrams showing the number and the percentage (in brackets) of brown bear faeces collected in the Tatra Mountains (Poland) containing food taxa belonging to each of the three major food groups (plants, vertebrates and insects) for the entire activity period of brown bears and for hypophagia (April – June) and hyperphagia (July – November), separately.

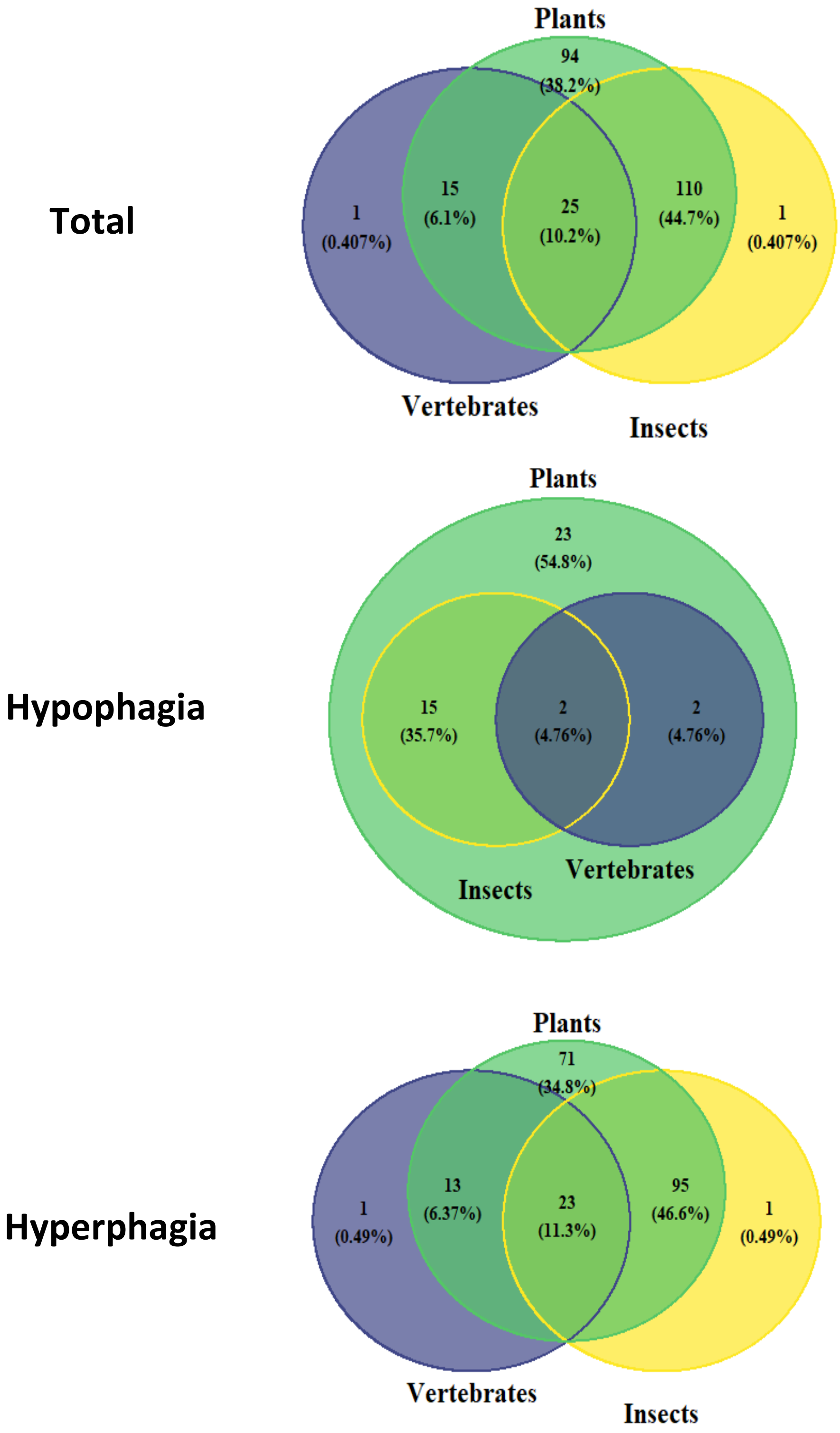
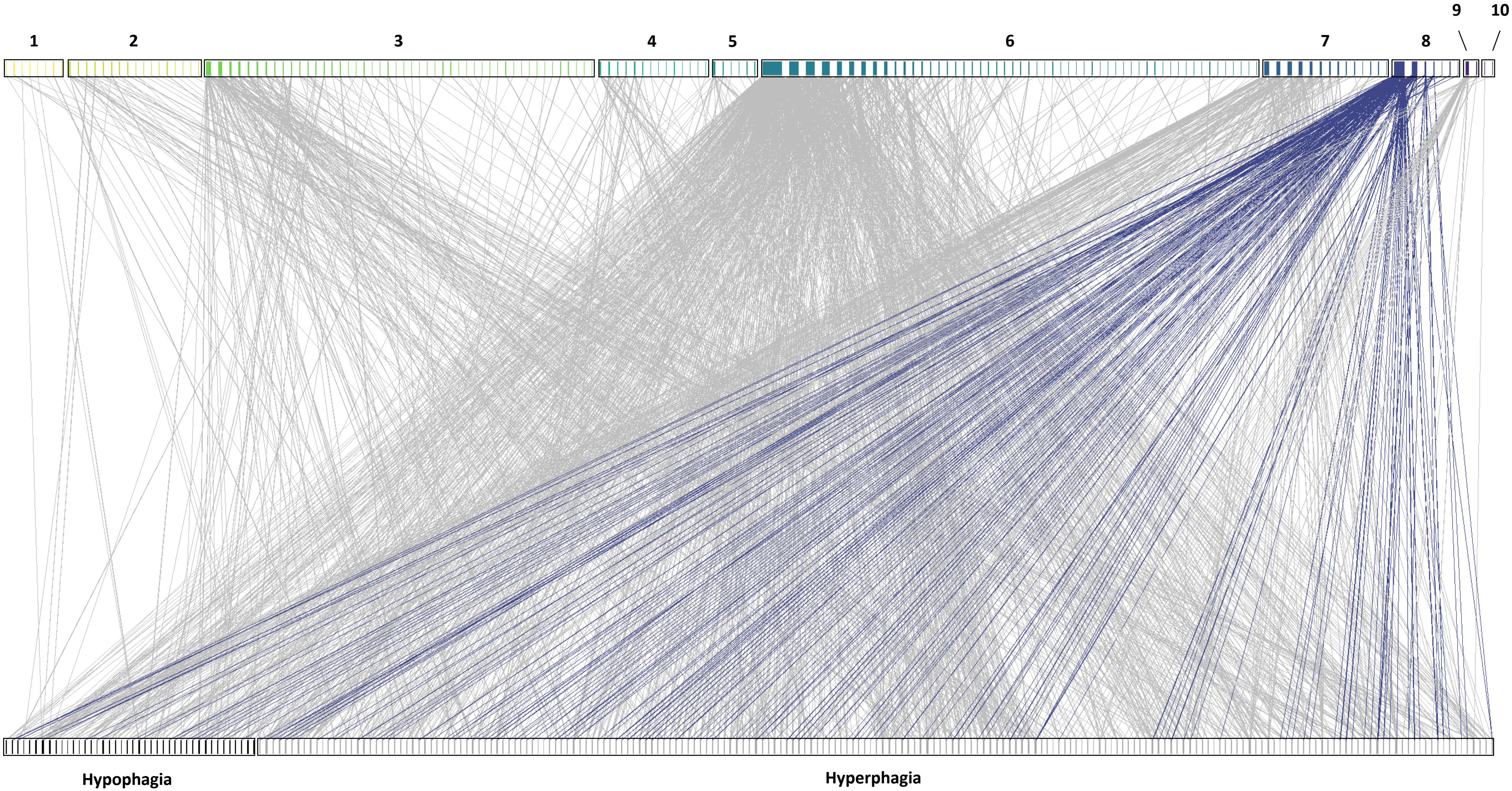


Figure 2. Bipartite graph linking each brown bear faecal sample and each food taxa identified in the diet of brown bears in the Tatra Mountains (Poland). Bottom bars represent individual brown bear faeces (n=246) and are ordered chronologically based on the dateof sample collection (both years merged); bear faeces collected during hypophagia (n=42) and hyperphagia (n=204) are grouped separately and represented in black and grey, respectively. Upper bars represent food taxa detected by DNA metabarcoding techniques (n = 173) in brown bear faeces and are organized by the 10 categories considered: 1) birds (n=8), 2) mammals (n=17), 3) insects (n=48), 4) cryptogams (n=14), 5) trees (tree species not producing neither fleshy fruits nor hard mast; n=6), 6) forbs (n=55), 7) graminoids (n=14), 8) fleshy fruits (plant species producing fleshy fruits; n=7), 9) hard mast (tree species producing hard mast; n=2) and 10) roots (plants with edible roots; n=2). All items belonging to the same food category are grouped and represented with a distinct color. Bottom bars width represents the total number of food taxa of each faecal sample, whereas upper bars width represents the number of faecal samples containing each taxon. Purple lines indicate links between brown bear faeces and fleshy-fruited plant species (category 8).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Hypophagia
Hyperphagia

Supplementary material for:

The bear-berry connection: ecological and management implications of brown bears' food habits in a highly touristic protected area

Submitted

Alberto García-Rodríguez, Nuria Selva, Tomasz Zwijacz-Kozica, Jörg Albrecht, Clement Lionnet, Delphine Rioux, Pierre Taberlet and Marta De Barba

Appendix A – DNA metabarcoding procedures for brown bear diet analysis

- **Laboratory methods**

DNA extraction from collected samples was performed following the protocol described in Taberlet et al. (2012), using approximately 1-2 g of dried faeces and 5-10 mL of phosphate buffer (Na2HPO4; 0.12 M; pH ~8) per sample, and recovering the DNA extracts in 100 µL. Faecal DNA extractions were carried out in a room dedicated to processing eDNA samples. Eleven extraction negative controls (containing only extraction reagents and no faecal material) were included to monitor for contamination and were analysed following the same protocol used for the faecal samples.

Diet analysis was performed by amplifying three universal markers, targeting short (<100 bp) and variable DNA fragments of components of the diet: for plants (i) the P6 loop of the chloroplast trnL (UAA) intron [GH in Taberlet et al. (2007) or Sper01 in Taberlet et al. (2018)]; for vertebrates (ii) marker V5 [V5 loop of the mitochondrial 12S gene in (Riaz et al., 2011) or Vert01 in Taberlet et al. (2018)]; and for insects (iii) the mitochondrial 16S gene [Inse01 in Taberlet et al. (2018)]. Blocking oligonucleotides were included in the amplification with the vertebrate and insect markers to minimize amplification of brown bear and human DNA (Table S7).

PCR amplifications were performed separately for each universal marker. For each marker, PCR contained 1x concentrated AmpliTaq Gold® Master Mix (Life Technologies, Carlsbad, CA, USA), 0.5 µM (GH) or 0.2 µM (V5 and Inse01) of each primer, 0.0032 mg bovine serum albumin (BSA, Roche Diagnostics, Basel, Switzerland) and 2 µL DNA extract, in 20 µL reaction volume. Amplifications with the vertebrate and insect markers also contained 2µM of the respective bear and human blocking oligonucleotides. Thermocycling conditions had an initial denaturation step of 10 minutes at 95°C, followed by 45 cycles of 30 seconds at 95°C, 30 seconds at 52°C (GH and Inse01) or 49°C (V5), 60 seconds at 72°C, and a final elongation of 7 minutes at 72°C. Following De Barba et al. (2014), we used PCR negative controls (n=7) and aliquots (n=7) of a positive control sample in the experiment to monitor the performance of the amplification and the sequencing, and to guide the selection of filtering parameters in the sequence analysis process. The positive control was made by mixing known quantities of the DNA extract of 10 species (Table S8). Four PCR replicates were performed for all samples. Primers used in each PCR were uniquely modified by the addition of molecular identifier tags on the 5' end, to allow the assignment of sequence reads to their source samples. Tags differed on both ends of a PCR product and were composed by eight nucleotides, containing at least five differences among them (Coissac, 2012). Empty PCR wells, corresponding to unused tag combinations, were included in the experiment to monitor potential tag-jumping events (Schnell et al., 2015).

PCR products of each marker were pooled together and purified using the MinElute PCR purification kit (QIAGEN GmbH). Libraries were prepared for each marker separately using the MetaFast protocol (http://www.fasteris.com/metafast), a PCR-free procedure enabling a significant reduction in bias associated with library preparation and sequencing, including the problem of tag-jumping (Schnell et al., 2015). The sequencing was carried out on Next-Seq Mid Output flow cell (2x150) (Illumina Inc., San Diego, CA, USA), following the manufacturer's instructions, using a commercial service (www.fasteris.com).

- **Sequence analysis and filtering protocol**

The sequence reads were first analysed using the OBITools package (Boyer et al., 2016). Forward and reverse reads corresponding to a single DNA molecule were assembled and primers and tags were identified. The amplified regions, excluding primers and tags, were kept for further analysis. A separate dataset was created for each marker and strictly identical sequences were clustered together, keeping the information about their distributions among samples. Sequences shorter than 10 (for plants), 50 (vertebrates), or 70 (insects) bp were excluded. Each sequence within a PCR product was classified into the categories of 'head' (the most common sequence within a group of sequences differing by a single indel/substitution), 'internal' (sequences less frequent within the group of related sequences; i.e. corresponding to amplification/sequencing errors) or 'singleton' (a sequence with no other variant differing by a single indel/substitution). A sequence reference database for the taxonomic identification of the sequences detected in the samples was built for each DNA metabarcoding marker. More specifically, we used EMBL nucleotide library (release 139.2) and *ecoPCR* program (Ficetola et al., 2010) to extract the relevant DNA region for plants (Viridiplantae), vertebrates (Vertebrata) and insects (Insecta) using GH, V5 and Inse01 markers, respectively. Taxon assignation was achieved by finding highly similar sequences to the query sequence in the reference databases, and assigning a unique taxon to each sequence.

Taxonomically assigned sequences of each marker were then further filtered using R v. 3.6.3 (R Core Team, 2018). Following De Barba et al. (2014), we used the positive and negative controls included in the experiment to set filtering parameters (i.e. for identification of erroneous sequences, sequences derived from contamination and samples more likely to produce unreliable results) and to evaluate the performance of the experiment. Sequences not identified as "head" in ≥3 replicates, or "singleton" in 4 replicates, or "head" in ≥2 and singleton ≥1 replicate, or "head" in ≥1 and singleton ≥3 replicates in a sample were considered erroneous and therefore deleted. We discarded sequences with a per-sample read frequency below a threshold specific for each marker and library. Each threshold was set separately based on the comparison of read counts of sequences of known taxa in the positive controls vs unexpected sequences (<0.01 for plants and vertebrates, <0.03 for insects). Sequences assigned to Ursidae, Hominidae, Heuteleostomi, and with identity <80% over the entire query sequence length with any reference sequence were removed. PCR sample replicates with read counts less then the median read count observed across the PCR negative controls were discarded. Only samples having at least 2 replicates after this step were retained. Within these samples, we kept sequences observed in >50% of sample replicates. We further discarded samples with total read count below the 1st quantile of read counts across all samples. Sample replicates were finally combined to obtain a consensus sequence profile for each sample by taking the sum of the sequence counts of the replicates. Sequence read counts were converted to frequencies for further analysis.

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Table S1. Month, day and year of collection of the brown bear faeces analyzed by DNA metabarcoding techniques to assess brown bear diet in the Tatra Mountains (Poland) (n=246). Latitude (north) and longitude (east) are given in degrees and elevation in meters above sea level. The number of food taxa detected is also provided for each faecal sample.

Faeces Id	Month	Day	Year	Latitude	Longitude	Elevation	Taxa
1	April	8	2019	49.2420	20.0754	1730	4
2	April	8	2019	49.2420	20.0753	1744	6
3	April	9	2019	49.2897	20.0673	960	5
4	April	11	2019	49.2769	19.9043	951	2
5	April	13	2019	49.2833	19.9525	892	4
6	April	13	2019	49.2783	19.9455	950	11
7	April	13	2019	49.2780	19.9455	959	19
8	April	20	2019	49.3341	20.0570	877	14
9	April	23	2019	49.2558	19.8954	1147	5
10	April	24	2018	49.2741	19.9341	970	1
11	April	26	2019	49.2697	19.9700	1282	2
12	May	2	2019	49.2426	19.8868	1677	7
13	May	4	2019	49.2083	19.7715	1652	1
14	May	5	2018	49.2538	19.9921	1258	8
15	May	9	2019	49.2712	19.9924	1130	5
16	May	10	2019	49.2503	19.9454	1535	2
17	May	19	2019	49.2634	19.8972	1146	10
18	May	19	2019	49.2634	19.8969	1150	10
19	May	21	2018	49.2691	19.8730	1040	3
20	May	27	2019	49.2296	20.0955	1128	2
21	May	28	2018	49.2457	20.0622	1656	8
22	May	31	2019	49.2090	19.7801	1769	3
23	May	31	2019	49.2072	19.7973	1608	8
24	June	4	2019	49.2167	20.0847	1284	4
25	June	5	2019	49.2188	20.0894	1243	8
26	June	5	2019	49.2190	20.0895	1245	4
27	June	5	2019	49.2173	20.0843	1295	6
28	June	7	2019	49.2123	20.0778	1356	6
29	June	7	2019	49.2126	20.0783	1353	4
30	June	9	2018	49.2720	19.8912	1093	5
31	June	9	2018	49.2720	19.8912	1093	4

32	June	13	2019	49.2584	19.9883	1182	7
33	June	13	2019	49.2519	19.9907	1415	10
34	June	13	2019	49.2520	19.9906	1403	11
35	June	17	2018	49.2450	19.8954	1381	4
36	June	17	2018	49.2448	19.8937	1499	7
37	June	17	2019	49.2348	19.8572	1127	6
38	June	20	2018	49.2676	19.9149	1144	4
39	June	20	2018	49.2676	19.9149	1144	5
40	June	25	2019	49.2457	19.9138	1730	6
41	June	25	2019	49.2337	19.8762	1314	8
42	June	27	2018	49.2635	19.9835	1090	2
43	July	2	2018	49.2203	19.8638	1249	7
44	July	2	2018	49.2203	19.8638	1249	18
45	July	2	2018	49.2191	19.8674	1297	8
46	July	2	2018	49.2210	19.8677	1255	10
47	July	3	2019	49.2180	20.0895	1229	10
48	July	4	2018	49.2295	19.7764	1427	7
49	July	4	2018	49.2295	19.7765	1417	8
50	July	7	2018	49.2594	19.8191	1042	6
51	July	8	2018	49.2394	19.8403	1581	10
52	July	8	2018	49.2394	19.8414	1543	6
53	July	8	2019	49.2775	19.9143	954	1
54	July	8	2019	49.2776	19.9136	955	4
55	July	8	2018	49.2315	19.7801	1308	6
56	July	8	2018	49.2378	19.7769	1406	3
57	July	11	2019	49.2855	19.8950	919	2
58	July	11	2019	49.2863	19.8943	925	5
59	July	12	2017	49.2582	19.9788	1238	8
60	July	13	2018	49.2609	19.8924	1157	8
61	July	13	2018	49.2609	19.8924	1157	8
62	July	13	2018	49.2633	19.8856	1245	5
63	July	16	2018	49.2453	19.9665	1372	8
64	July	16	2018	49.2453	19.9665	1372	9
65	July	17	2019	49.2468	19.9104	1684	6
66	July	17	2019	49.2471	19.9151	1557	4
67	July	17	2019	49.2237	19.8898	1384	5
68	July	18	2017	49.2346	19.8794	1398	8
69	July	18	2017	49.2916	20.1283	907	6
70	July	18	2019	49.2450	19.9649	1432	8

71	July	19	2019	49.2553	20.0140	1478	6
72	July	19	2019	49.2588	20.0129	1359	3
73	July	22	2017	49.2571	20.0559	1373	5
74	July	26	2019	49.2520	19.9705	1230	5
75	July	27	2018	49.2365	19.8155	1122	7
76	July	29	2017	49.2654	20.0241	1350	5
77	July	29	2017	49.2654	20.0241	1350	4
78	July	30	2017	49.2548	20.0212	1263	8
79	August	2	2017	49.2509	20.0084	1546	7
80	August	2	2017	49.2544	20.0129	1479	7
81	August	3	2018	49.2599	20.0149	1382	4
82	August	3	2018	NA	NA	1445	9
83	August	4	2018	49.2359	19.9710	1617	4
84	August	4	2018	49.2462	19.9689	1311	6
85	August	6	2018	49.2215	19.8670	1255	5
86	August	6	2018	49.2215	19.8670	1255	3
87	August	6	2018	49.2215	19.8670	1255	3
88	August	7	2018	49.2613	20.0026	1250	4
89	August	15	2017	49.2010	20.0391	1887	2
90	August	16	2017	49.2538	19.9671	1206	1
91	August	17	2017	49.2331	19.8503	1290	6
92	August	17	2017	49.2266	19.8563	1112	3
93	August	17	2017	49.2657	20.0281	1293	6
94	August	19	2017	49.2563	20.0148	1474	6
95	August	22	2017	49.5184	19.9989	744	13
96	August	22	2018	49.2622	20.0008	1249	5
97	August	22	2018	49.2603	20.0085	1282	5
98	August	22	2018	49.2602	20.0093	1278	5
99	August	22	2018	49.2592	20.0105	1327	6
100	August	22	2018	49.2592	20.0105	1327	4
101	August	22	2018	49.2592	20.0105	1327	8
102	August	22	2018	49.2589	20.0112	1323	5
103	August	22	2018	49.2530	20.0105	1519	4
104	August	22	2018	49.2538	20.0119	1503	4
105	August	23	2017	49.2897	20.0962	994	4
106	August	23	2018	49.2366	19.9710	1606	8
107	August	23	2018	49.2372	19.9704	1588	7
108	August	24	2017	49.2463	19.9691	1309	6
109	August	24	2017	49.2468	19.9701	1301	3

110	August	27	2018	49.2507	20.0081	1552	8
111	August	27	2018	49.2607	20.0165	1389	5
112	August	27	2018	49.2604	20.0159	1387	7
113	August	27	2018	49.2602	20.0094	1278	7
114	August	27	2018	49.2587	20.0115	1336	8
115	August	30	2017	49.2382	20.0341	1702	7
116	August	30	2018	49.2488	19.9360	1728	6
117	August	30	2018	49.2498	19.9392	1693	7
118	August	30	2018	49.2505	19.9404	1681	4
119	August	31	2017	49.2115	19.7972	1479	5
120	August	31	2018	49.2868	20.1055	1117	3
121	August	31	2018	49.2821	20.1066	1142	11
122	August	31	2018	49.2822	20.1081	1147	12
123	August	31	2018	49.2841	20.1108	1131	4
124	September	1	2018	49.2624	20.0213	1378	7
125	September	1	2018	49.2510	19.9542	1329	17
126	September	7	2017	49.2765	20.0039	1145	8
127	September	7	2017	49.2657	20.0280	1294	5
128	September	7	2017	49.2651	20.0262	1311	9
129	September	8	2017	49.2138	20.0800	1325	4
130	September	10	2017	49.2508	20.0082	1546	7
131	September	10	2017	49.2512	20.0089	1539	6
132	September	10	2017	49.2573	20.0154	1452	7
133	September	14	2017	49.2370	19.9707	1590	4
134	September	14	2017	49.2418	19.9695	1457	11
135	September	14	2017	49.2428	19.9698	1413	6
136	September	15	2017	49.2484	20.0030	1631	5
137	September	16	2018	49.2607	20.0177	1408	6
138	September	17	2018	49.2786	19.9594	957	3
139	September	18	2018	49.2766	19.9350	940	4
140	September	19	2018	49.2342	19.9723	1681	5
141	September	19	2018	49.2345	19.9701	1640	5
142	September	19	2018	49.2374	19.9701	1575	6
143	September	19	2018	49.2433	19.9700	1400	4
144	September	20	2018	49.2261	19.8581	1113	2
145	September	21	2018	49.2310	19.7753	1460	6
146	September	26	2018	49.2924	20.1284	906	15
147	September	26	2018	49.2978	20.1222	991	11
148	September	28	2018	49.2716	19.9007	1013	8

149	September	29	2018	49.3006	20.1226	959	7
150	September	29	2018	49.3006	20.1231	971	10
151	September	29	2018	49.3022	20.1291	879	4
152	September	29	2018	49.3081	20.1276	979	11
153	September	29	2018	49.3124	20.1292	957	16
154	September	30	2018	49.3109	20.1710	1023	8
155	September	30	2018	49.3044	20.1297	879	4
156	October	3	2018	49.2496	19.9534	1315	9
157	October	3	2018	49.2497	19.9531	1323	9
158	October	3	2018	49.2470	19.9506	1364	11
159	October	3	2018	49.2470	19.9506	1364	6
160	October	3	2018	49.2470	19.9506	1364	5
161	October	3	2018	49.2470	19.9506	1364	9
162	October	3	2018	49.2470	19.9506	1364	11
163	October	3	2018	49.2470	19.9506	1364	13
164	October	3	2018	49.2503	19.9506	1306	9
165	October	3	2018	49.2503	19.9506	1306	10
166	October	3	2018	49.2503	19.9506	1306	8
167	October	3	2018	49.2504	19.9514	1371	7
168	October	3	2018	49.2504	19.9503	1407	10
169	October	3	2018	49.2495	19.9501	1335	11
170	October	4	2017	49.2722	20.0349	1201	16
171	October	4	2018	49.2744	19.9389	989	3
172	October	4	2018	49.2744	19.9389	989	3
173	October	4	2018	49.2745	19.9382	1017	2
174	October	4	2018	49.2749	19.9372	1008	7
175	October	4	2018	49.2748	19.9371	1009	2
176	October	4	2018	49.2745	19.9366	1004	3
177	October	4	2018	49.2745	19.9366	1004	3
178	October	4	2018	49.2740	19.9353	994	2
179	October	4	2018	49.2740	19.9356	1006	4
180	October	4	2018	49.2739	19.9357	1009	1
181	October	4	2018	49.2738	19.9356	1014	10
182	October	4	2018	49.2737	19.9358	1018	3
183	October	4	2018	49.2735	19.9356	1026	2
184	October	4	2018	49.2726	19.9376	1057	5
185	October	4	2018	49.2737	19.9389	989	3
186	October	4	2018	49.2737	19.9389	989	3
187	October	4	2018	49.2737	19.9389	989	2

188	October	4	2018	49.2786	19.9410	914	4
189	October	4	2018	49.2749	19.9410	967	5
190	October	5	2018	49.2525	19.8771	1243	12
191	October	5	2018	49.2527	19.8769	1238	11
192	October	5	2018	49.2527	19.8769	1238	5
193	October	5	2018	49.2529	19.8755	1207	9
194	October	5	2018	49.2544	19.8745	1123	3
195	October	6	2017	49.2553	19.8953	1149	12
196	October	6	2017	49.3669	20.1438	817	12
197	October	7	2017	49.2542	19.9126	1222	7
198	October	7	2018	49.2988	19.8843	1113	6
199	October	7	2018	49.2985	19.8822	1107	3
200	October	7	2018	49.2790	19.9600	927	2
201	October	7	2018	49.2790	19.9596	926	4
202	October	7	2018	49.2790	19.9595	925	3
203	October	7	2018	49.2790	19.9595	925	6
204	October	7	2018	49.2783	19.9586	960	4
205	October	7	2018	49.2785	19.9578	936	5
206	October	7	2018	49.2790	19.9578	915	16
207	October	8	2017	49.2855	19.9751	900	4
208	October	8	2017	49.2855	19.9751	900	5
209	October	10	2017	49.3671	20.1369	794	10
210	October	10	2017	49.2578	20.0158	1455	4
211	October	10	2017	49.2526	20.0099	1524	1
212	October	10	2017	49.2515	20.0089	1539	8
213	October	10	2018	49.2332	19.9752	1749	7
214	October	10	2018	49.2332	19.9751	1744	2
215	October	10	2018	49.2371	19.9680	1525	6
216	October	11	2018	49.2303	19.7733	1521	7
217	October	11	2017	49.2518	19.9702	1226	3
218	October	12	2017	49.2942	20.0957	1049	13
219	October	12	2017	49.2189	20.0740	1559	3
220	October	12	2017	49.2123	20.0777	1357	5
221	October	12	2017	49.2324	19.9965	1625	5
222	October	12	2017	49.2319	19.9970	1637	6
223	October	12	2017	49.2312	19.9978	1647	3
224	October	12	2018	49.2588	19.9724	1137	6
225	October	12	2018	49.2557	19.9701	1182	8
226	October	12	2018	49.2485	19.9711	1274	15

227	October	12	2018	49.2470	19.9702	1294	14
228	October	12	2018	49.2467	19.9698	1316	5
229	October	12	2018	49.2436	19.9702	1419	7
230	October	12	2018	49.2433	19.9700	1415	15
231	October	12	2018	49.2868	19.9396	856	4
232	October	13	2017	49.2387	19.9690	1557	5
233	October	13	2017	49.2427	19.9694	1407	3
234	October	13	2017	49.2525	19.9696	1198	1
235	October	13	2017	49.2525	19.9696	1198	5
236	October	13	2018	49.2528	19.7904	1376	8
237	October	15	2017	49.2163	20.0669	1708	3
238	October	18	2017	49.2343	20.0715	1801	2
239	October	18	2018	49.2137	19.9995	1848	8
240	October	26	2017	49.3300	20.0788	880	6
241	October	29	2018	49.2678	19.9011	1042	1
242	October	29	2018	49.2778	19.9223	907	9
243	October	31	2018	49.3446	20.1433	789	12
244	October	31	2018	49.3376	20.1457	800	5
245	November	8	2018	49.2786	20.0085	1031	8
246	November	10	2018	49.2449	19.9188	1768	6

Table S2. List of the 173 taxa detected by DNA metabarcoding techniques in brown bear faeces collected in the Tatra Mountains (Poland). The rank (i.e. taxonomic resolution), group, category and frequency of occurrence are provided for each taxon. Family, genus and species also given whenever possible.

Group/Category Taxa ID	Taxa	Rank	Order	Family	Genus	Species	Frequency of occurrence
Vertebrates							
Birds							
Birds-4	Columbidae	Family	Columbiforme	Columbidae	NA	NA	0.41%
Birds-2	*Meleagris gallopavo*	Species	Galliformes	Phasianidae	*Meleagris*	*Meleagris gallopavo*	0.81%
Birds-3	Phasianinae	Subfamily	Galliformes	Phasianidae	NA	NA	0.81%
Birds-1	Passeriformes	Order	Passeriformes	NA	NA	NA	0.81%
Birds-6	*Prunella modularis*	Species	Passeriformes	Passeridae	*Prunella*	*Prunella modularis*	0.41%
Birds-5	Phylloscopidae	Family	Passeriformes	Phylloscopidae	NA	NA	0.41%
Birds-7	*Sylvia atricapilla*	Species	Passeriformes	Sylviidae	*Sylvia*	*Sylvia atricapilla*	0.41%
Birds-8	*Turdus philomelos*	Species	Passeriformes	Turdidae	*Turdus*	*Turdus philomelos*	0.41%
Mammals							
Mammals-4	*Bos*	Genus	Artiodactyla	Bovidae	*Bos*	NA	2.03%
Mammals-10	*Capra*	Genus	Artiodactyla	Bovidae	*Capra*	NA	0.41%
Mammals-16	Caprinae	Subfamily	Artiodactyla	Bovidae	NA	NA	0.41%
Mammals-1	*Cervus elaphus*	Species	Artiodactyla	Cervidae	*Cervus*	*Cervus elaphus*	5.69%
Mammals-2	*Capreolus capreolus*	Species	Artiodactyla	Cervidae	*Capreolus*	*Capreolus capreolus*	2.85%
Mammals-3	*Sus scrofa*	Species	Artiodactyla	Suidae	*Sus*	*Sus scrofa*	2.44%
Mammals-5	*Vulpes vulpes*	Species	Carnivora	Canidae	*Vulpes*	*Vulpes vulpes*	2.03%
Mammals-6	*Canis*	Genus	Carnivora	Canidae	*Canis*	NA	1.63%
Mammals-17	*Canis lupus familiaris*	Subspecies	Carnivora	Canidae	*Canis*	*Canis lupus*	0.41%
Mammals-11	*Martes*	Genus	Carnivora	Mustelidae	*Martes*	NA	0.41%
Mammals-14	*Mustela nivalis*	Species	Carnivora	Mustelidae	*Mustela*	*Mustela nivalis*	0.41%
Mammals-8	*Chionomys nivalis*	Species	Rodentia	Cricetidae	*Chionomys*	*Chionomys nivalis*	1.22%

Mammals-9	*Microtus*	Genus	Rodentia	Cricetidae	*Microtus*	NA	0.81%
Mammals-13	*Microtus subterraneus*	Species	Rodentia	Cricetidae	*Microtus*	*Microtus subterraneus*	0.41%
Mammals-15	*Sylvaemus group*	Sp. group	Rodentia	Muridae	*Apodemus*	NA	0.41%
Mammals-7	Boreoeutheria	No rank	NA	NA	NA	NA	1.22%
Mammals-12	Eutheria	No rank	NA	NA	NA	NA	0.41%

Insects

Insect-24	*Byturus*	Genus	Coleoptera	Byturidae	*Byturus*	NA	0.41%
Insect-25	*Carabus*	Genus	Coleoptera	Carabidae	*Carabus*	NA	0.41%
Insect-36	*Carabus violaceus*	Species	Coleoptera	Carabidae	*Carabus*	*Carabus violaceus*	0.41%
Insect-40	*Liparus coronatus*	Species	Coleoptera	Curculionidae	*Liparus*	*Liparus glabirostris*	0.41%
Insect-31	*Anoplotrupes stercorosus*	Species	Coleoptera	Geotrupidae	*Anoplotrupes*	*Anoplotrupes stercorosus*	0.41%
Insect-45	Cholevinae	Subfamily	Coleoptera	Leiodidae	NA	NA	0.41%
Insect-23	*Aphodius*	Genus	Coleoptera	Scarabaeidae	*Aphodius*	NA	0.41%
Insect-33	*Atheta aeneipennis*	Species	Coleoptera	Staphylinidae	*Atheta*	*Atheta aeneipennis*	0.41%
Insect-39	*Isotoma viridis*	Species	Collembola	Isotomidae	*Isotoma*	*Isotoma viridis*	0.41%
Insect-4	*Sylvicola fenestralis*	Species	Diptera	Anisopodidae	*Sylvicola*	*Sylvicola fenestralis*	6.10%
Insect-8	*Lasiomma seminitidum*	Species	Diptera	Anthomyiidae	*Lasiomma*	*Lasiomma seminitidum*	2.85%
Insect-7	*Bibio marci*	Species	Diptera	Bibionidae	*Bibio*	*Bibio marci*	2.85%
Insect-17	*Lucilia*	Genus	Diptera	Calliphoridae	*Lucilia*	NA	0.81%
Insect-34	*Calliphora vomitoria*	Species	Diptera	Calliphoridae	*Calliphora*	*Calliphora vomitoria*	0.41%
Insect-11	*Culicoides*	Genus	Diptera	Ceratopogonidae	*Culicoides*	NA	2.03%
Insect-27	*Drosophila*	Genus	Diptera	Drosophilidae	*Drosophila*	NA	0.41%
Insect-48	*Parascaptomyza*	Subgenus	Diptera	Drosophilidae	*Scaptomyza*	NA	0.41%
Insect-37	*Dryomyza anilis*	Species	Diptera	Dryomyzidae	*Dryomyza*	*Dryomyza anilis*	0.41%
Insect-14	*Euryomma*	Genus	Diptera	Fanniidae	*Euryomma*	NA	1.22%
Insect-6	*Rhipidia*	Genus	Diptera	Limoniidae	*Rhipidia*	NA	4.47%
Insect-16	*Huckettomyia*	Genus	Diptera	Muscidae	*Huckettomyia*	NA	0.81%
Insect-21	*Lophosceles cinereiventris*	Species	Diptera	Muscidae	*Lophosceles*	*Lophosceles cinereiventris*	0.81%
Insect-38	*Haematobosca stimulans*	Species	Diptera	Muscidae	*Haematobosca*	*Haematobosca stimulans*	0.41%

Insect-3	Diptera	Order	Diptera	NA	NA	NA	6.50%
Insect-20	Schizophora	No rank	Diptera	NA	NA	NA	0.81%
Insect-2	*Psychomora*	Genus	Diptera	Psychodidae	*Psychomora*	*Psychomora*	13.01%
Insect-12	*Scathophaga*	Genus	Diptera	Scathophagidae	*Scathophaga*	NA	2.03%
Insect-22	Scathophaginae	Subfamily	Diptera	Scathophagidae	NA	NA	0.81%
Insect-42	*Nemopoda nitidula*	Species	Diptera	Sepsidae	*Nemopoda*	*Nemopoda nitidula*	0.41%
Insect-44	*Sphaerocera curvipes*	Species	Diptera	Sphaeroceridae	*Sphaerocera*	*Sphaerocera curvipes*	0.41%
Insect-18	*Trichocera*	Genus	Diptera	Trichoceridae	*Trichocera*	NA	0.81%
Insect-10	*Cavariella*	Genus	Hemiptera	Aphididae	*Cavariella*	NA	2.44%
Insect-41	*Macrosiphum hellebori*	Species	Hemiptera	Aphididae	*Macrosiphum*	*Macrosiphum hellebori*	0.41%
Insect-43	*Philaenus spumarius*	Species	Hemiptera	Aphrophoridae	*Philaenus*	*Philaenus spumarius*	0.41%
Insect-28	*Stenodema*	Genus	Hemiptera	Miridae	*Stenodema*	NA	0.41%
Insect-13	*Trioza anthrisci*	Species	Hemiptera	Triozidae	*Trioza*	*Trioza anthrisci*	1.63%
Insect-32	*Apis mellifera*	Species	Hymenoptera	Apidae	*Apis*	*Apis mellifera*	0.41%
Insect-9	*Myrmica scabrinodis*	Species	Hymenoptera	Formicidae	*Myrmica*	*Myrmica scabrinodis*	2.85%
Insect-35	*Camponotus herculeanus*	Species	Hymenoptera	Formicidae	*Camponotus*	*Camponotus herculeanus*	0.41%
Insect-5	*Vespula vulgaris*	Species	Hymenoptera	Vespidae	*Vespula*	*Vespula vulgaris*	5.28%
Insect-26	*Dolichovespula*	Genus	Hymenoptera	Vespidae	*Dolichovespula*	NA	0.41%
Insect-47	Spilomelinae	Subfamily	Lepidoptera	Crambidae	NA	NA	0.41%
Insect-29	Ditrysia	No rank	Lepidoptera	NA	NA	NA	0.41%
Insect-1	Calyptratae	No rank	NA	NA	NA	NA	17.89%
Insect-15	Pancrustacea	No rank	NA	NA	NA	NA	1.22%
Insect-19	Neoptera	Infraclass	NA	NA	NA	NA	0.81%
Insect-30	Coleoptera	Order	NA	NA	NA	NA	0.41%
Insect-46	Gomphocerinae	Subfamily	Orthoptera	Acrididae	NA	NA	0.41%

Plants

Cryptogams

Cryp-8	Bartramiaceae	Family	Bartramiales	Bartramiaceae	NA	NA	0.41%
Cryp-12	Desmidiales	Order	Desmidiales	NA	NA	NA	0.41%

Cryp-7	*Dicranella heteromalla*	Species	Dicranales	Dicranaceae	*Dicranella*	*Dicranella heteromalla*	0.81%
Cryp-9	Dicranaceae	Family	Dicranales	Dicranaceae	NA	NA	0.41%
Cryp-14	*Hylocomium splendens*	Species	Hypnales	Hylocomiaceae	*Hylocomium*	*Hylocomium splendens*	0.41%
Cryp-3	Hypnales	Order	Hypnales	NA	NA	NA	3.25%
Cryp-11	Bryophytina	No rank	NA	NA	NA	NA	0.41%
Cryp-1	*Athyrium*	Genus	Polypodiales	Athyriaceae	*Athyrium*	NA	5.28%
Cryp-4	*Athyrium*	Genus	Polypodiales	Athyriaceae	*Athyrium*	NA	2.85%
Cryp-2	*Dryopteris*	Genus	Polypodiales	Dryopteridaceae	*Dryopteris*	NA	3.25%
Cryp-13	Polypodiales	Order	Polypodiales	NA	NA	NA	0.41%
Cryp-6	Polytrichaceae	Family	Polytrichales	Polytrichaceae	NA	NA	0.81%
Cryp-5	*Sphagnum*	Genus	Sphagnales	Sphagnaceae	*Sphagnum*	NA	2.85%
Cryp-10	*Tetraplodon*	Genus	Splachnales	Splachnaceae	*Tetraplodon*	NA	0.41%
Trees							
Trees-3	Betulaceae	Family	Fagales	Betulaceae	NA	NA	0.41%
Trees-2	Saliceae	Tribe	Malpighiales	Salicaceae	NA	NA	2.85%
Trees-1	Spermatophyta	No rank	NA	NA	NA	NA	6.50%
Trees-6	*Platanus*	Genus	Proteales	Platanaceae	Platanus	NA	0.41%
Trees-4	Ulmaceae	Family	Rosales	Ulmaceae	NA	NA	0.41%
Trees-5	*Acer*	Genus	Sapindales	Sapindaceae	Acer	NA	0.41%
Forbs							
Forbs-1	Apiaceae	Family	Apiales	Apiaceae	NA	NA	78.46%
Forbs-10	apioid superclade	No rank	Apiales	Apiaceae	NA	NA	6.10%
Forbs-13	Scandicinae	Subtribe	Apiales	Apiaceae	NA	NA	3.25%
Forbs-20	*Astrantia major*	Genus	Apiales	Apiaceae	*Astrantia*	*Astrantia major*	1.22%
Forbs-28	*Chaerophyllum*	Genus	Apiales	Apiaceae	*Chaerophyllum*	NA	0.81%
Forbs-49	Apioideae	Subfamily	Apiales	Apiaceae	NA	NA	0.41%
Forbs-6	Asteraceae	Family	Asterales	Asteraceae	NA	NA	17.48%
Forbs-11	*Leontodon*	Genus	Asterales	Asteraceae	*Leontodon*	NA	4.88%
Forbs-40	*Lactuca*	Genus	Asterales	Asteraceae	*Lactuca*	NA	0.41%
Forbs-50	Asteroideae	Subfamily	Asterales	Asteraceae	NA	NA	0.41%

Forbs-51	Cynoglossoideae	Subfamily	Boraginales	Boraginaceae	NA	NA	0.41%
Forbs-27	Brassicaceae	Family	Brassicales	Brassicaceae	NA	NA	0.81%
Forbs-9	*Stellaria nemorum*	Species	Caryophyllales	Caryophyllaceae	*Stellaria*	*Stellaria nemorum*	11.79%
Forbs-26	Alsineae	Tribe	Caryophyllales	Caryophyllaceae	NA	NA	1.22%
Forbs-36	*Cerastium*	Genus	Caryophyllales	Caryophyllaceae	*Cerastium*	NA	0.41%
Forbs-55	Sileneae	Tribe	Caryophyllales	Caryophyllaceae	NA	NA	0.41%
Forbs-2	Rumiceae	Tribe	Caryophyllales	Polygonaceae	NA	NA	36.18%
Forbs-25	Polygonoideae	Subfamily	Caryophyllales	Polygonaceae	NA	NA	1.22%
Forbs-33	Cucurbitaceae	Family	Cucurbitales	Cucurbitaceae	NA	NA	0.41%
Forbs-32	Caprifoliaceae	Family	Dipsacales	Caprifoliaceae	NA	NA	0.41%
Forbs-34	Primulaceae	Family	Ericales	Primulaceae	NA	NA	0.41%
Forbs-35	Theaceae	Family	Ericales	Theaceae	NA	NA	0.41%
Forbs-12	*Trifolium*	Genus	Fabales	Fabaceae	*Trifolium*	NA	4.07%
Forbs-23	*Vicia*	Genus	Fabales	Fabaceae	*Vicia*	NA	1.22%
Forbs-29	indigoferoid/millettioid clade	No rank	Fabales	Fabaceae	NA	NA	0.81%
Forbs-31	*Lathyrus pratensis*	Species	Fabales	Fabaceae	*Lathyrus*	*Lathyrus pratensis*	0.81%
Forbs-38	*Gentiana*	Genus	Gentianales	Gentianaceae	*Gentiana*	NA	0.41%
Forbs-19	*Galium*	Genus	Gentianales	Rubiaceae	*Galium*	NA	2.03%
Forbs-17	*Geranium*	Genus	Geraniales	Geraniaceae	*Geranium*	NA	2.44%
Forbs-54	Mentheae	Tribe	Lamiales	Lamiaceae	NA	NA	0.41%
Forbs-22	*Plantago*	Genus	Lamiales	Plantaginaceae	*Plantago*	NA	1.22%
Forbs-46	*Veronica*	Genus	Lamiales	Plantaginaceae	*Veronica*	NA	0.41%
Forbs-45	*Veratrum lobelianum*	Genus	Liliales	Melanthiaceae	*Veratrum*	*Veratrum lobelianum*	0.41%
Forbs-21	*Hypericum*	Genus	Malpighiales	Hypericaceae	*Hypericum*	NA	1.22%
Forbs-41	*Linum*	Genus	Malpighiales	Linaceae	*Linum*	NA	0.41%
Forbs-47	*Viola*	Genus	Malpighiales	Violaceae	*Viola*	NA	0.41%
Forbs-39	*Helianthemum nummularium*	Genus	Malvales	Cistaceae	*Helianthemum*	*Helianthemum nummularium*	0.41%
Forbs-53	Myrtoideae	Subfamily	Myrtales	Myrtaceae	NA	NA	0.41%
Forbs-15	*Chamaenerion*	Species	Myrtales	Onagraceae	*Chamaenerion*	*Chamaenerion*	2.85%

	angustifolium					*angustifolium*	
Forbs-37	*Epilobium*	Genus	Myrtales	Onagraceae	*Epilobium*	NA	0.41%
Forbs-53	Epilobieae	Tribe	Myrtales	Onagraceae	NA	NA	0.41%
Forbs-14	*Oxalis acetosella*	Genus	Myrtales	Oxalidaceae	*Oxalis*	*Oxalis acetosella*	2.85%
Forbs-3	Pentapetalae	No rank	NA	NA	NA	NA	33.33%
Forbs-4	Mesangiospermae	No rank	NA	NA	NA	NA	30.49%
Forbs-5	rosids	No rank	NA	NA	NA	NA	18.29%
Forbs-24	campanulids	No rank	NA	NA	NA	NA	1.22%
Forbs-48	fabids	No rank	NA	NA	NA	NA	0.41%
Forbs-42	*Piper*	Genus	Piperales	Piperaceae	*Piper*	NA	0.41%
Forbs-18	*Ranunculus*	Genus	Ranunculales	Ranunculaceae	*Ranunculus*	NA	2.44%
Forbs-8	Rosales	Order	Rosales	NA	NA	NA	13.01%
Forbs-16	Colurieae	Tribe	Rosales	Rosaceae	NA	NA	2.85%
Forbs-30	Rosoideae incertae sedis	No rank	Rosales	Rosaceae	NA	NA	0.81%
Forbs-43	*Potentilla*	Genus	Rosales	Rosaceae	*Potentilla*	NA	0.41%
Forbs-7	*Urtica*	Genus	Rosales	Urticaceae	*Urtica*	NA	15.85%
Forbs-44	*Saxifraga*	Genus	Saxifragales	Saxifragaceae	*Saxifraga*	NA	0.41%
<u>Graminoids</u>							
Gram-1	*Luzula*	Genus	Poales	Juncaceae	*Luzula*	NA	17.89%
Gram-13	*Oreojuncus*	Genus	Poales	Juncaceae	*Oreojuncus*	NA	0.41%
Gram-8	Poales	Order	Poales	NA	NA	NA	2.44%
Gram-2	Poeae Chloroplast Group 2 (Poeae type)	No rank	Poales	Poaceae	NA	NA	14.23%
Gram-3	Poeae	Tribe	Poales	Poaceae	NA	NA	13.82%
Gram-4	Poideae	Subfamily	Poales	Poaceae	NA	NA	12.60%
Gram-5	Dactylidinae	Subtribe	Poales	Poaceae	NA	NA	8.54%
Gram-6	Poeae Chloroplast Group 1 (Aveneae type)	No rank	Poales	Poaceae	NA	NA	7.32%
Gram-7	Poaceae	Family	Poales	Poaceae	NA	NA	3.66%
Gram-9	Agrostidinae	Subtribe	Poales	Poaceae	NA	NA	2.03%
Gram-10	*Cynosurus cristatus*	Species	Poales	Poaceae	*Cynosurus*	*Cynosurus cristatus*	1.22%

Gram-11	*Avena fatua*	Genus	Poales	Poaceae	*Avena*	*Avena fatua*	0.81%
Gram-12	*Holcus*	Genus	Poales	Poaceae	*Holcus*	NA	0.81%
Gram-14	BOP clade	No rank	Poales	Poaceae	NA	NA	0.41%
Fleshy fruits							
FF-7	Actinidiaceae	Family	Ericales	Actinidiaceae	NA	NA	0.41%
FF-1	*Vaccinium myrtillus*	Species	Ericales	Ericaceae	*Vaccinium*	*Vaccinium myrtillus*	41.87%
FF-3	*Vaccinium vitis-idaea*	Species	Ericales	Ericaceae	*Vaccinium*	*Vaccinium vitis-idaea*	3.66%
FF-2	*Rubus idaeus*	Species	Rosales	Rosaceae	*Rubus*	*Rubus idaeus*	20.33%
FF-4	*Prunus*	Genus	Rosales	Rosaceae	*Prunus*	NA	3.25%
FF-6	*Ribes*	Genus	Saxifragales	Grossulariaceae	*Ribes*	NA	0.41%
FF-5	*Capsicum*	Genus	Solanales	Solanaceae	*Capsicum*	NA	0.81%
Hard mast							
Mast-1	*Fagus sylvatica*	Species	Fagales	Fagaceae	*Fagus*	*Fagus sylvatica*	11.38%
Mast-2	*Abies alba*	Species	Pinales	Pinaceae	*Abies*	*Abies alba*	2.44%
Roots							
Roots-1	*Allium*	Genus	Asparagales	Amaryllidaceae	*Allium*	NA	0.81%
Roots-2	*Beta vulgaris*	Species	Caryophyllales	Chenopodiaceae	*Beta*	*Beta vulgaris*	0.41%

Table S3. Number of distinct molecular operational taxonomic units (MOTUS) and taxa identified by DNA metabarcoding techniques in brown bear faeces collected in the Tatra Mountains (Poland). The taxonomic resolution, expressed as percentage of taxa assigned to each taxonomic level for the three major food categories considered in this study (plants, vertebrates and insects), is also provided.

	Metabarcoder marker / Taxa groups		
	Plants	**Vertebrates**	**Insects**
Number of MOTUS	152	44	86
Number of taxa identified	100	25	48
Taxonomic level of taxa identified			
No rank	13.00%	8.00%	8.33%
Infraclass	NA	NA	2.08%
Subclass	NA	NA	NA
Class	NA	NA	NA
Order	5.00%	4.00%	4.17%
Family	14.00%	8.00%	NA
Subfamily	6.00%	8.00%	8.33%
Tribe	8.00%	NA	NA
Subtribe	3.00%	NA	NA
Genus	39.00%	20.00%	29.17%
Subgenus	NA	NA	2.08%
Species group	NA	4.00%	NA
Species	12.00%	44.00%	45.83%
Subspecies	NA	4.00%	NA

Table S4. Number of food taxa (mean, standard deviation – S.D., and maximum in a single faecal sample – Max.) belonging to each major group and category detected by DNA metabarcoding techniques in brown bear faeces collected in the Tatra Mountains (Poland). Numbers are given for the total activity period of the species in the study area as well as for the hypophagia (April to June) and hyperphagia (July to November) seasons separately. The number of faeces containing taxa belonging to each group and category are also provided for each period.

	Hypophagia				**Hyperphagia**				**Total**			
		Food taxa				**Food taxa**				**Food taxa**		
	Faeces	**Mean**	**S.D.**	**Max.**	**Faeces**	**Mean**	**S.D.**	**Max.**	**Faeces**	**Mean**	**S.D.**	**Max.**
Total	42	6.02	3.71	19	204	6.45	3.43	18	246	6.37	3.47	19
Vertebrates	4	0.14	0.47	2	39	0.30	0.83	7	43	0.27	0.78	7
Birds	2	0.07	0.34	2	8	0.04	0.19	1	10	0.04	0.23	2
Mammals	3	0.07	0.26	1	31	0.26	0.77	6	34	0.23	0.71	6
Insects	17	0.71	1.04	4	119	0.92	0.97	5	136	0.88	0.98	5
Plants	42	5.14	3.40	18	202	5.17	3.08	17	244	5.16	3.13	18
Cryptogams	0	NA	NA	NA	38	0.26	0.64	4	38	0.22	0.59	4
Trees	5	0.12	0.32	1	19	0.11	0.37	3	24	0.11	0.36	3
Forbs	38	2.97	2.20	10	183	3.14	2.21	11	221	3.11	2.21	11
Graminoids	34	1.48	1.35	5	76	0.74	1.21	7	110	0.86	1.26	7
Fleshy fruits	16	0.40	0.54	2	122	0.76	0.73	3	138	0.69	0.71	3
Hard mast	3	0.07	0.26	1	30	0.15	0.37	2	33	0.14	0.36	2
Roots	2	0.10	0.48	3	1	NA	NA	1	3	0.02	0.21	3

Table S5. Frequency of occurrence (proportion of faeces with a given taxon) of the food taxa possibly derived from human activities detected in brown bears faeces collected in the Tatra Mountains (Poland, n=246). An asterisk (*) indicates taxa whose wild counterparts are also present in the study area and, thus, the natural vs human origin of these food items in bear diet cannot be confirmed.

Taxa	Group	Category	Frequency of occurrence
*Sus scrofa**	Vertebrates	Mammals	2.44%
Bos	Vertebrates	Mammals	2.03%
*Canis**	Vertebrates	Mammals	1.63%
Meleagris gallipavo	Vertebrates	Birds	0.81%
Phasianinae*	Vertebrates	Birds	0.81%
Beta vulgaris	Plants	Roots	0.41%
Capsicum	Plants	Fleshy fruits	0.81%
Canis lupus familiaris	Vertebrates	Mammals	0.41%
*Apis mellifera**	Insects	Insects	0.41%
Lactuca	Plants	Forbs	0.41%
Cucurbitaceae	Plants	Forbs	0.41%
Actinidiaceae	Plants	Fleshy fruits	0.41%
Piper	Plants	Forbs	0.41%
Capra	Vertebrate	Mammals	0.41%

Table S6. Summary statistics (Chi-square -Chi^2-, degrees of freedom -df- and p-value) of the univariate generalized linear models (GLMs) performed to test the effects of the feeding season (hypophagia vs hyperphagia) and the month (from April to October) on the trophic diversity per faecal sample of brown bears, calculated as (1) number of taxa consumed -N_0- and (2) Gini-Simpson Index, and in the frequency of occurrence of each of the food categories considered in the study. Asterisks indicate p-values <0.05.

		Family GLM	**Feeding season**			**Months**		
			Chi^2	**df**	**p-value**	**Chi^2**	**df**	**p-value**
Diversity index								
N_0		Poisson	0.859	1	0.354	8.812	6	0.167
Simpson		Binomial	1.067	1	0.301	2.824	6	0.831
Frequency of occurrence each food category								
Birds	FO	Binomial	0.061	1	0.806	12.123	6	0.059
Mammals	FO	Binomial	2.176	1	0.140	19.632	6	0.003*
Insects	FO	Binomial	4.473	1	0.034*	28.482	6	<0.001*
Cryptogams	FO	Binomial	15.594	1	<0.001*	17.945	6	0.006*
Trees	FO	Binomial	0.253	1	0.615	8.654	6	0.194
Forbs	FO	Binomial	0.534	1	0.465	11.426	6	0.076
Graminoids	FO	Binomial	21.854	1	<0.001*	40.099	6	<0.001*
Fleshy fruits	FO	Binomial	6.631	1	0.012*	22.381	6	0.001*
Hard mast	FO	Binomial	1.958	1	0.162	37.756	6	<0.001*
Roots	FO	Binomial	3.691	1	0.055	10.962	6	0.089

Table S7. Mean scores (standard deviation) of trophic diversity indexes (mean number of taxa N_0 and Gini-Simpson index) performed to test the trophic diversity of brown bears in the Tatra Mountains (Poland) for the total activity period of Tatra brown bears and separately for each feeding season (hypophagia and hyperphagia) and month. Two samples collected in November were assigned to October.

Trophic diversity index	**Total**	**Hypophagia**				**Hyperphagia**				
			April	**May**	**June**		**July**	**August**	**September**	**October**
Mean number of taxa No	6.37 (3.47)	6.02 (3.71)	6.72 (5.88)	5.58 (3.29)	5.84 (2.27)	6.45 (3.43)	6.58 (2.95)	5.71 (2.46)	7.22 (3.70)	6.36 (3.81)
Gini-Simpson	0.61 (0.16)	0.67 (0.15)	0.70 (0.20)	0.65 (0.19)	0.68 (0.10)	0.59 (0.15)	0.66 (0.13)	0.60 (0.12)	0.62 (0.13)	0.55 (0.18)

Table S8. Blocking oligonucleotides developed to prevent amplification of brown bear and human DNA with the universal vertebrate marker (V5) and the insect marker (Inse01).

Universal marker	**Blocking primer name**	**Primer sequence 5'-3'**
V5	Ursus_V5_B2	CCACTATGCTTAGCCTTAAACATAAATAATTTATTAAAC-C3[1]
	HomoB	CTATGCTTAGCCCTAAACCTCAACAGTTAAATCAACAAAACTGCT-C3[1]
Inse01	Blk_Inse01_Ursus	TATGGAGCTTCAATTAATTAGCTCAA-C3[2]
	Blk_Inse01_Homo	TATGGAGCTTTAATTTATTAATGCAA-C3[2]

[1] De Barba, M., Miquel, C., Boyer, F., Mercier, C., Rioux, D., Coissac, E., Taberlet, P., 2014. DNA metabarcoding multiplexing and validation of data accuracy for diet assessment: application to omnivorous diet. Mol. Ecol. Resour. 14, 306-323.

[2] Developed for this study

Table S9. DNA concentration of taxa used to prepare the positive control DNA samples. Each DNA was diluted 1:100 in the positive control.

Species	**ng/µL**
Plants	
Nicotiana tabacum	1.69
Typha minima	4.98
Eritrichium nanum	2.62
Nothofagus pumilio	7.42
Insects	
Strobilomyia spp.	9.82
Parnassius apollo	2.88
Aedes Aegypti	1.66
Vertebrates	
Cephalophus monticola	7.94
Ovis aries	6.12
Capra hircus	1.14

Figure S1. Map showing the location of the brown bear faeces analyzed in the Tatra Mountains (Poland) between 2017 and 2019 for this study (n=246).

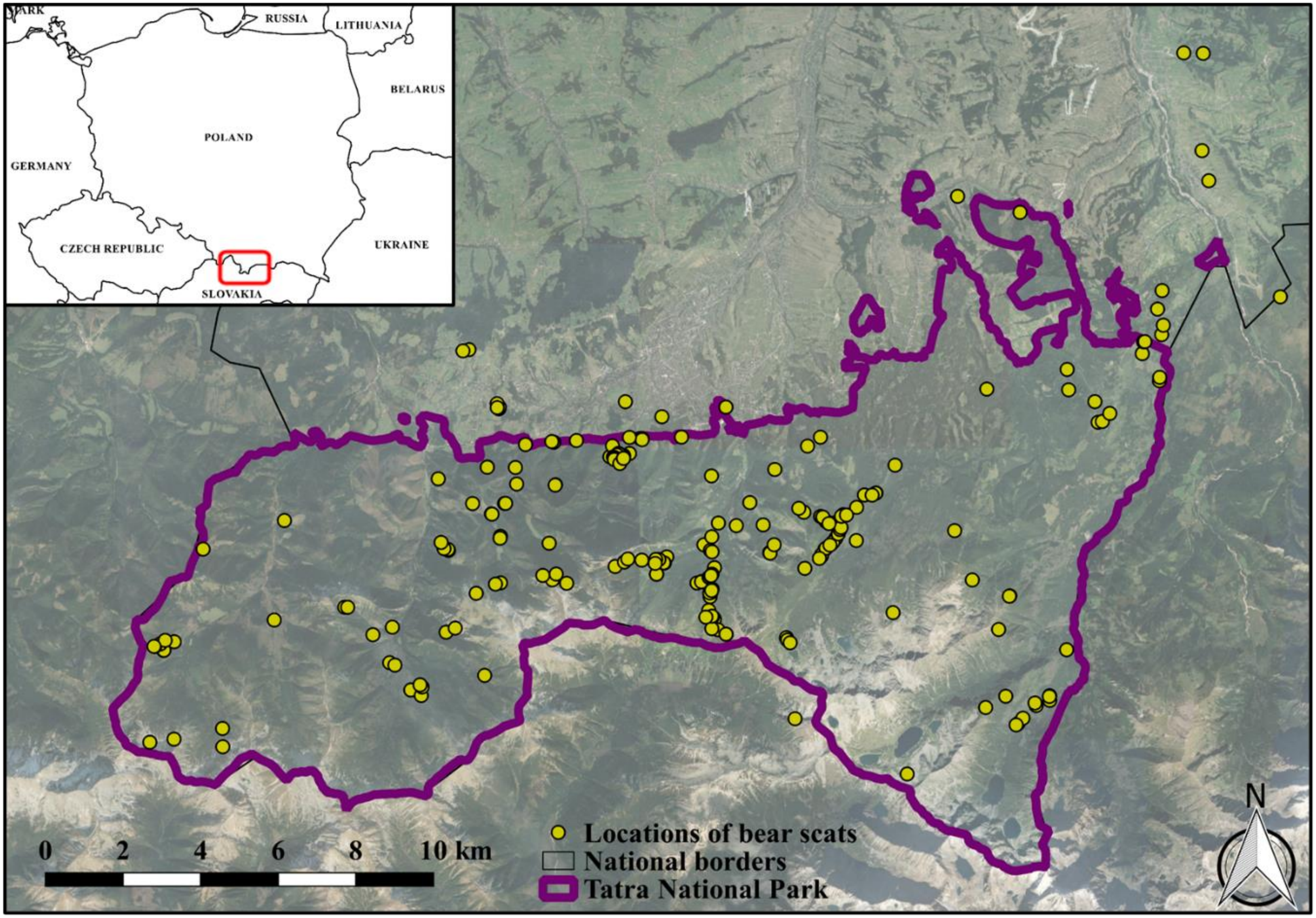


Figure S2. Frequency of occurrence (i.e. the number of faeces in which a specific taxon appears divided by the total number of faeces) of the families detected by DNA metabarcoding techniques in brown bear faeces collected in the Tatra Mountains (Poland). Vertebrate, insect and plant families are represented in blue, yellow and green, respectively.

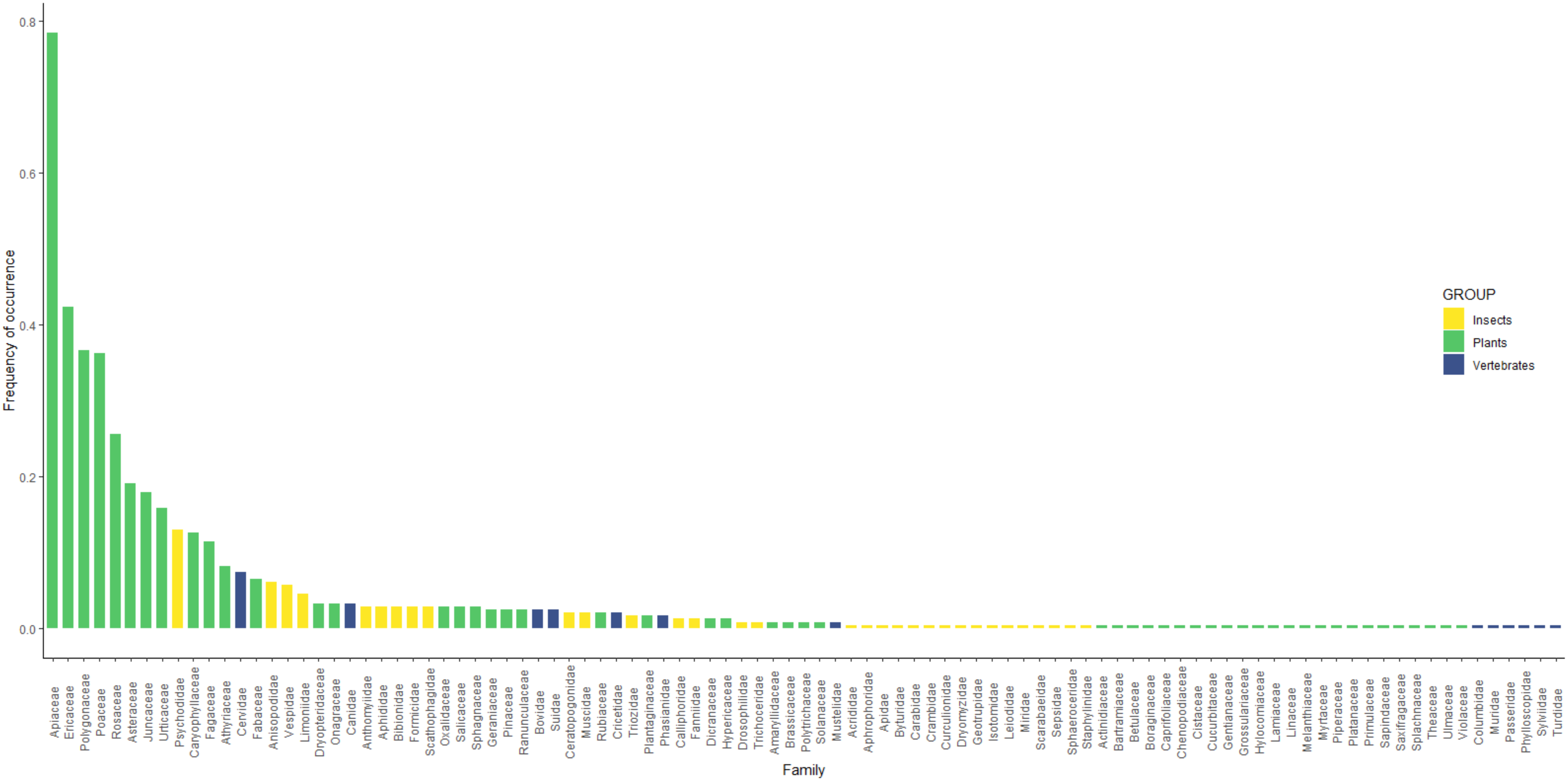
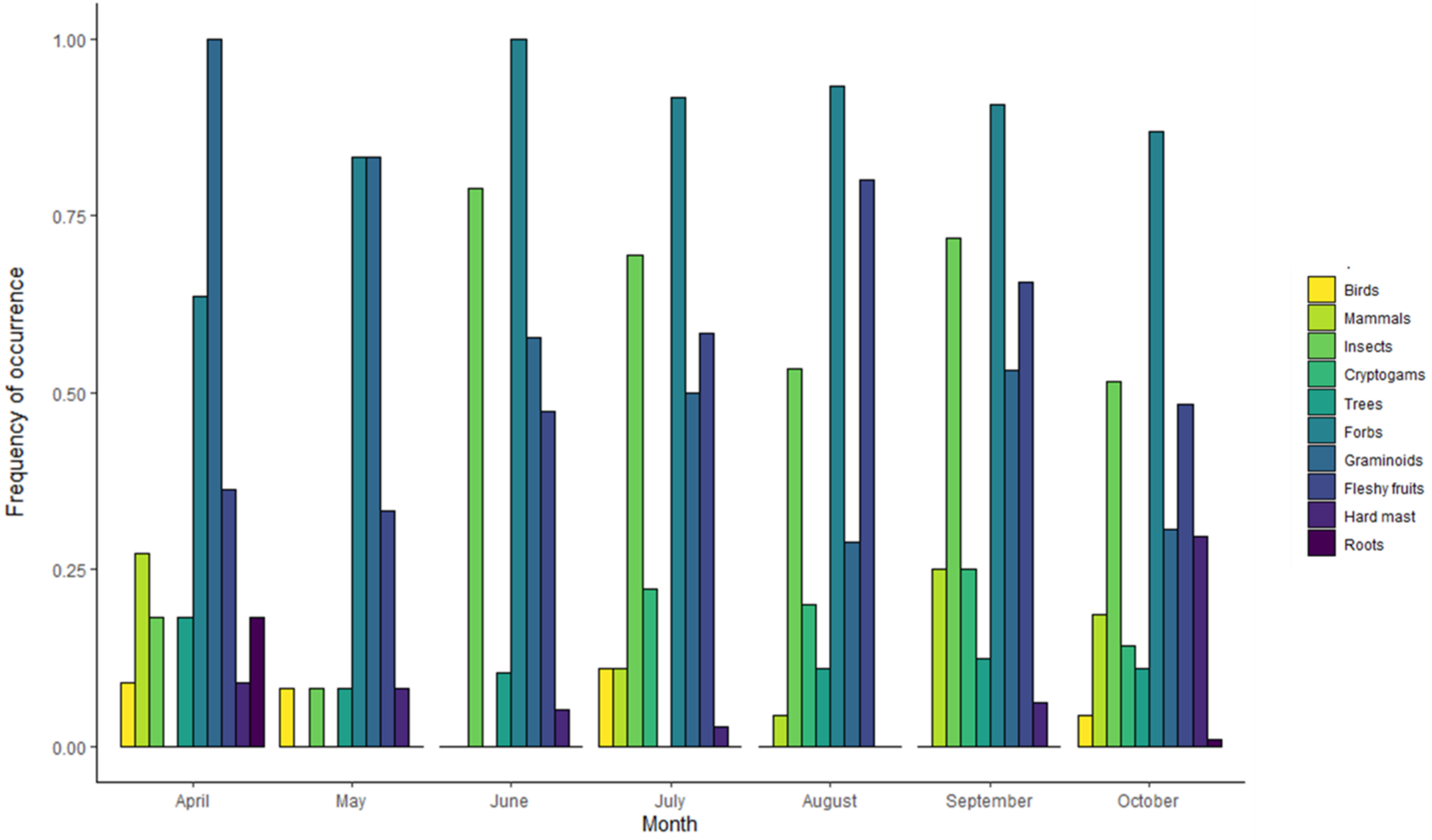


Figure S3. Monthly variation of the frequency of occurrence (proportion of faeces with a given food category) of the main food categories consumed by brown bears in the Tatra Mountains (Poland): 1) birds, 2) mammals, 3) insects, 4) cryptogams, 5) trees (tree species not producing neither fleshy fruits nor hard mast), 6) forbs, 7) graminoids, 8) fleshy fruits (plant species producing fleshy fruits), 9) hard mast (tree species producing hard mast) and 10) roots (plants with edible roots).



Female brown bear with two cubs feeding on bilberry fruits in the study area. Picture: Adam Wajrak

Functional complementarity of seed dispersal services provided by birds and mammals in an alpine ecosystem

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ABSTRACT

Frugivory and endozoochory are important ecosystem functions that in temperate and boreal regions are carried out mainly by birds and mammals, which usually differ in their effectiveness as seed dispersers as a consequence of their different quantitative and qualitative contributions to seed dispersal. However, there is still little information about how spatiotemporal differences in frugivory between these groups may provide complementarity to the seed dispersal services they provide. We investigated the complementarity of seed dispersal services provided by the community of bilberry *Vaccinium myrtillus* dispersers in an alpine ecosystem, the Tatra Mountains (southern Poland). We collected bird droppings and mammal scats containing bilberry seeds in two different habitats: coniferous forests and alpine meadows. Birds were identified by DNA barcoding techniques while mammals were visually identified in the field. We analyzed the influence of habitat, microhabitat and timing in the quantitative contributions of each species to the total seed rain and conducted ex-situ germination experiments to check the quality of the seed dispersal services they provide. At least thirteen bird and three mammal species dispersed bilberry seeds in the Tatra Mountains. Two species of thrushes -song thrushes *Turdus philomelos* and fieldfares *T. pilaris*-, brown bears *Ursus arctos* and red foxes *Vulpes vulpes* were the most effective bilberry dispersers. Quantitative and qualitative contributions to the seed dispersal differed between birds and mammals. Mammals, particularly brown bears, dispersed the majority of bilberry seeds in both habitats. Additionally, avian and mammalian dispersers differed in their contributions to the total seed rain according to the habitat and timing of seed dispersal. Birds dispersed most seeds at the middle of the fruiting season and within the forest, while seed dispersal by mammals peaked in upper meadows and at the end of the season. Seeds defecated by birds had higher chances to germinate as their droppings contained less seeds and were defecated in more suitable microhabitats. Our results demonstrate that, despite their different quantitative and qualitative contributions to seed dispersal, both birds and mammals may be effective bilberry dispersers. Complementary seed dispersal services guarantee the constant arrival of bilberry seeds to different microhabitats and along the entire fruiting season in the area, maximizing the chances of seedling germination.

KEYWORDS

Endozoochory, Seed Dispersal Effectiveness (SDE), Tatra Mountains, *Vacciniun myrtillus*, dispersers, DNA barcoding techniques, phenology, frugivory.

INTRODUCTION

Frugivory and the associated seed dispersal are essential ecological functions that benefit both plants and frugivores (Janzen, 1984; Quintero et al., 2020). From the plant perspective, seed dispersal by animals is known to influence other important processes such as plant recruitment, colonization of new habitats or gene flow among populations (Nathan & Muller-Landau, 2000; Cain et al., 2000; Wang & Smith 2002; Spiegel & Nathan, 2007). Most plant species, especially in temperate and boreal regions, are dispersed by birds and mammals (Jordano, 2000; Jordano et al., 2011; Albrecht et al., 2013). Frugivorous species within a community complement their seed dispersal services not only by specializing in different fleshy-fruited plant species (González-Varo et al., 2014; González-Castro et al., 2015), but also by contributing differently to seed arrival to both microhabitats and distances within the dispersal kernel (Jordano et al., 2007; McConkey & Brockelman, 2011; González-Varo et al., 2014; Rumeu et al., 2020). Additionally, sympatric frugivorous species may contribute differently to the seed rain across the fruiting season (González-Varo et al., 2019). All these factors can have direct consequences on the quantity and quality of the seed dispersal services provided by each species (Jordano et al., 2007; González-Varo et al., 2019). This complementarity in seed dispersal services may be essential for plant recruitment as the extinction or loss of major dispersers is difficult to compensate by the action of less important dispersers (González-Castro et al., 2015). However, we still know little about how the spatiotemporal complementarity in seed dispersal services influences seedling recruitment (González-Castro et al., 2015; González-Varo et al., 2019).

The seed dispersal effectiveness framework (SDE hereafter) was developed to standardize measurements of the contributions of dispersers belonging to different seed dispersal systems (Schupp, 1993). SDE is ideally measured as the number of new adults produced due to the dispersal activities of a disperser, but it can also be measured in terms of recruited seedlings (Schupp, 1993). Frugivores vary in their quantitative and qualitative contributions to SDE (Schupp, 1993; Schupp et al., 2010). The quantity component is usually measured as the total number of seeds dispersed by a frugivore and it depends on the number of visits made to a plant and the number of seeds taken per visit (Schupp, 1993; Schupp et al., 2010). The quality component is most often understood as the probability that a dispersed seed remains viable after animal ingestion (i.e. quality of mouth and gut treatment) multiplied by the probability that the seed will survive, germinate and produce a new adult (Schupp, 1993; Schupp et al., 2010). SDE landscapes allow the location of different dispersers in a graph based on their respective SDEs and accounting for the different dispersal strategies followed by different dispersers (i.e. their respective combinations of quantity and quality components of seed dispersal, which results in a specific SDE; Schupp, 1993, Schupp et al., 2010).

The quantity component has traditionally been considered as a better surrogate of the total effect of animal mutualists on plants than the quality component (Vázquez et al., 2005; but see also González-Castro et al., 2015). Therefore, many studies have focused on rates of fruit removal as a proxy for the effect of different frugivorous species on plant recruitment (e.g. García et al., 2001; Albrecht et al., 2012). However, fruit removal per se does not guarantee adult plant establishment (Wang & Smith, 2002), as factors such as seed and seedling competition, different characteristics of microhabitats (e.g. types of substrate, canopy cover) and the timing of the seed arrival influence seed viability and germination as well as seedling survivorship (Eriksson & Fröborg, 1996; González-Varo et al., 2014; González-Varo et al., 2019). Additionally, habitat preferences and animal movement, usually constrained by body size (Santini et al., 2013), are crucial to understand the fate of the dispersed seeds (Jordano et al., 2007; Rumeu et al., 2020) and determine the quality of the seed dispersal service provided by each species (Schupp, 1993).

Linking each seed dispersal event to a frugivorous species is essential to determine the role (e.g. redundancy or complementarity) of each frugivore in a particular disperser assemblage (Jordano et al., 2007; González-Varo et al., 2013). Classical methods of identification such as direct observations of seed removal, visual assignment of scats or faecal analyses of mist-netted birds have been usually used to identify the dispersal agents (Jordano, 1988; Jordano et al., 2007; González-Varo et al., 2013; Heleno et al., 2013). These techniques, however, have obvious limitations such as the difficulty of visually discriminating faecal remains belonging to related species or the impossibility of linking seed removal observations and mist-netted birds to seed deposition in particular microhabitats. Due to this, related species of frugivores have been traditionally joined into functional groups (González-Varo et al., 2014). To solve this problem, González-Varo et al. (2014) described DNA-barcoding protocols based on faecal remains, a non-invasive technique using DNA material for disperser species identification. As seeds are sampled after being dispersed, this technique links the frugivory process with patterns of seed deposition, capturing both quantity (number of seeds dispersed) and quality (microsite' quality for recruitment) components of SDE (González-Varo et al., 2014).

Here we analyzed the spatiotemporal complementarity of seed dispersal services provided by avian and mammalian frugivores inhabiting alpine regions. We studied the community of bilberry *Vaccinium myrtillus* dispersers in the Tatra Mountains (southern Poland) as a model system. We chose the bilberry because, despite their seeds being dispersed in important numbers by both birds and mammals every fruiting season, seedlings are rarely seen in nature and usually associated to disturbances in the soil. Thus, studying the differences in the dispersal strategies followed by the different bilberry consumers may be key to understand bilberry recruitment patterns in natural conditions. Specifically, we aimed to answer the following questions: (1) What is the quantitative, qualitative and combined -SDE- contribution of each bilberry disperser species to the total SDE landscape in the study area?; (2) Do birds and mammals differ in their contributions to the bilberry seed rain according to the habitat (i.e. coniferous forests, alpine meadows) and the timing (July, August, September, October) of seed dispersal?; (3) Does SDE differ between bird and mammal species and are these differences related to body size? and (4) Which component of the seed dispersal -quantity or quality- is a better surrogate of the total SDE of the different bilberry dispersers?

MATERIAL AND METHODS

- **Study species**

The bilberry (Ericaceae) is a clonal shrub widely distributed in temperate and boreal regions of Eurasia (Ritchie, 1956). It spreads mainly by rhizomes, primarily after disturbances (Schimmel & Granström, 1996). Fruits have average diameter and weight of 0.5-1 cm and 0.25-0.5 g, respectively (Eriksson & Ehrlén, 1991; Ranwala & Naylor, 2004). Each fruit contains several dozens of small seeds (up to 120 seeds of 0.4 mg each; Ranwala & Naylor, 2004; AGR personal observation). In temperate regions, bilberry fruiting season starts in mid-July at low elevations, with population at elevations above 2,000 meters above sea level (m.a.s.l.) ripening in early September (AGR personal observation). Fruit and seed production is abundant, especially during mast years (Selås, 2000), but recruitment through seedling establishment is rare and usually restricted to gaps exposing the soil surface (Eriksson & Froborg, 1996). The bilberry is a key food resource for many animal species, including large frugivores like the brown bear, mesocarnivores (e.g. red foxes and martens *Martes spp*.), grouses and passerines (Schaumann & Heinken, 2002; Honkavaara et al., 2007; Blanco-Fontao et al., 2010; Hertel et al., 2018; García-Rodríguez et al., 2021).

- **Study area**

The study was conducted in Tatra National Park (Poland) and its vicinity, a 211 km^2 protected area located in the Tatra Mountains, a mountain range belonging to the western part of the Carpathian Mountains that forms a natural border between Poland and Slovakia (Fig. S1). Tatra National Park ranges from 774 to 2,499 meters above sea level (m.a.s.l.) and four different elevation zones can be found in the area: montane (700 – 1,550 m.a.s.l.), subalpine (1,550 – 1,800), alpine (1,800 – 2,300) and subnival zones (from 2,300 m.a.s.l., Mirek & Piekos-Mirkowa, 1992). Montane forests occupy around 60% of the total surface of the study area and have been largely transformed and managed since the 19th century, when Norway spruce *Picea abies* was introduced as a plantation tree in the original habitat of silver fir *Abies alba* and European beech *Fagus sylvatica*. The subalpine zone is dominated by dwarf pines *Pinus mugo*. Montane, subalpine and alpine floors are dominated by bilberries. Other *Vaccinium* species such as the lingonberry *V. vitis-idaea*, the bog bilberry *V. uliginosum*, the mountain bilberry *V. gaultheriodes* can be found at different elevations, but in much smaller numbers (Mirek & Piekos-Mirkowa, 1992). Many frugivorous animals are present in the area, including species of conservation concern such as the brown bear, the western capercaillie *Tetrao urogallus* and the black grouse *Lyrurus tetrix*.

- **Quantity component of the SDE – Contributions of frugivores to the bilberry seed rain**

We defined the quantity component as the number of bilberry seeds dispersed per ha and month. To document the community of frugivores dispersing bilberry seeds in the study area we established six transects of approximately 1.5 km length and 3 m width each. These transects were set along an 800 m elevation gradient (1,125-1,925 m.a.s.l.) and encompassed the majority of the elevation range of the bilberry within the study area. We established all the transects in the same valley because of three reasons: 1) easy accessibility, 2) widely distributed bilberry populations and 3) abundant and diverse frugivorous community in the area (AGR personal information). Four transects were located within the montane zone (i.e. within coniferous forests) whereas the other two were in the subalpine and alpine zones (i.e. within alpine meadows; Fig. S1). We inspected each transect once per month during the bilberry fruiting season (from mid-July to mid-October) in 2017 and 2018, yielding a total of 48 transect inspections (6 transects × 4 sampling sessions × 2 years; Table S1). We recorded the total surface area covered in each transect inspection. During the inspections we collected all carnivore scats and bird droppings located within each transect. We did not collect either ungulate or rodent pellets because these animal groups are not considered efficient bilberry dispersers (Tolvanen et al., 1994; Welch et al., 2000; Steyaert et al., 2009). For each faecal sample, we recorded the elevation and type of microhabitat (i.e., bare soil, dead wood, vegetation or stone). A total of 1,812 faecal samples (1,656 bird droppings and 156 carnivore scats) were collected and stored in a freezer at -18ºC until sample processing and DNA extraction.

The identification of seed disperser species was done following two different approaches. Carnivore scats were identified to the species level in the field by trained people according to shape, size and smell, a procedure commonly used for the identification of mammal faecal remains (González-Varo et al., 2013). As both pine marten *Martes martes* and stone marten *M. foina* are present in the study area and their scats cannot be unambiguously distinguished visually, they were grouped as *Martes spp.* Eight mesocarnivore scats could not be linked to a species due to the washed and old condition of the samples. However, only three of these samples contained bilberry seeds and were subsequently kept for further analyses. Additionally, visual inspections in the field confirmed that none of the grey wolf *Canis lupus* and Eurasian lynx *Lynx lynx* scats found during transect inspections contained bilberry seeds and, thus, they were not collected. Carnivore scats (104 of the total 156 collected) were individually weighed, homogenized and mixed in the laboratory. We took three subsamples of each, weighed them and counted the number of bilberry seeds with the help of a 10× magnifying glass. We calculated the number of bilberry seeds per gram of fresh scat for each subsample and calculated the average number of seeds per gram of fresh scat across the three subsamples. For each scat separately, we calculated the total number of seeds by multiplying its average number of seeds per gram by its total fresh weight. We extrapolated the total number of seeds in the rest of the carnivore scats (n = 52 out of the total 156 collected) based on the species assigned, the fresh weight of the scat and the average number of seeds per gram of fresh scat for each species and month.

Avian dispersers were identified via DNA barcoding techniques in a laboratory specialized in low DNA concentration procedures. We selected a representative random sample of the collected droppings for genetic analyses ("processed samples" hereafter). The selection was done for each of the 48 transect inspections in the following way: a minimum of 50% of the samples were selected for those transect inspections with more than 50 bird droppings collected, a minimum of 75% of the samples were selected for those transect inspections with 21-50 samples collected and all the samples (100%) were selected for DNA analysis in the cases of transects inspections with 20 or less bird droppings collected. In total, we extracted and amplified DNA from 1,111 droppings out of total 1,656 bird droppings collected.

In the laboratory, we counted the total number of bilberry seeds in each dropping selected for barcoding before DNA extraction and assigned it to the matched species after DNA amplification and sequencing. We recovered DNA from these bird droppings via seed incubation in extraction buffer with rotation ("Method 3" of DNA recovery in González-Varo et al., 2014) and DNA extraction via a GuSCN/silica protocol for ancient DNA ("Protocol 1" for DNA extraction in González-Varo et al., 2014). We included negative controls in each extraction to check for contamination. We then amplified the extracted DNA using the COI-fsd-degF and COI-fsdR for degraded samples (González-Varo et al., 2017). For those samples without successful amplification we performed nested PCR with COI-fsd-degF / COI-fsdR (González-Varo et al., 2017) primer set on the amplicon of AWCintF2 / AWCintR4 (avian DNA barcodes, Lijtmaer et al., 2012). We increased primers and Taq DNA polymerase during PCR amplifications to overcome expected DNA amounts. We additionally increased bovine serum albumin (BSA) to counteract PCR inhibitors. PCRs were performed in a Biometra TOne Thermal Cycler. Each PCR started with an (1) initial 4 min of denaturation at 94°C, followed by (2) 42 cycles of 45s of denaturalization at 94°C + 45s of annealing at 54°C + 45s of extension at 72°C and (3) a final extension of 6 min at 72°C. Final products were sent for sequencing to LGC Genomics (www.lgcgenomics.com, Berlin – Germany) in 2018 and to Macrogen (www.macrogen.com, Amsterdam – Netherlands) in 2019. We edited the resulting sequences with CodonCode Aligner (Version 9.0.1, CodonCode Corporation) and linked each sequence (minimum 80 bp) to the different bird species using the "Barcode of Life Data" – BOLD (http://www.boldsystems.org; Ratnasingham & Hebert, 2007). BOLD accepts sequences from the 50 region of the COI gene and returns species-level identification whenever possible, assigning a percentage of similarity to matched sequences. We only assigned a disperser to samples for which a similarity of more than 99% between our sequence and the disperser species was found. More detailed information about primer designs and DNA recovery, extraction and amplification can be found in González-Varo et al. (2014; 2017).

As we did not extract DNA from all bird droppings, we extrapolated the results to assess the total contribution of each bird species to the bilberry seed rain. We linked each unprocessed dropping to a bird species based on the proportions of barcoded droppings belonging to each species. Once each sample was linked to a frugivore, we assigned different seed quantities to each unprocessed dropping based on the average number of bilberry seeds found per dropping for each species during barcoding. To account for differences in spatiotemporal patterns of seed dispersal among different frugivores we calculated the proportions of samples belonging to each species and the number of seeds per dropping for each combination of transect and sampling round separately. For each transect inspection, we calculated the seed rain provided by each frugivore as the total number of seeds dispersed by each species divided by the total surface covered in each inspection (i.e. number of bilberry seeds dispersed per ha and month). We assessed the sampling completeness of the disperser assemblage in each habitat type using rarefaction curves (Fig. S2).

- **Quality component of the SDE – Bilberry germination on different microhabitats**

We defined the quality component as the probability that a bilberry seed deposited by a specific frugivorous species will germinate. To study the effects of the microhabitat and seed density (i.e. the amount of seeds per scat/dropping) on the germination rates of bilberry seeds we established an ex-situ germination experiment. Ex-situ conditions allowed us to check the plots on a daily basis. We randomly collected 500 bilberry fruits on September 2017 from an area of around 100 m^2 in an alpine meadow with high bilberry productivity located in Tatra National Park (coordinates: 49.2533N, 20.0113E, elevation 1,515 m.a.s.l.) and kept them in the fridge at 4 Celsius degrees for two days before extracting the seeds. To separate the seeds from the pulp, we mixed all the berries and placed them into a bowl with a small amount of water. We gently smashed the berries with the help of a wooden mallet. After doing this, seeds moved to the bottom of the bowl, while pulp stayed on the surface. We carefully removed water and pulp with the help of a strainer. We repeated this step five times to separate all the seeds. We then placed the seeds on filter paper, dried them for 48 hours at room temperature and counted them with the help of 10× magnifying glass. To avoid damaging the seeds we took them by hand and put them in paper envelopes. Bilberry seeds were sown in 48 plastic pots (20×20×20 cm) at the botanical garden of the Centre for Research and Conservation of Mountain Plants of the Institute of Nature Conservation – Polish Academy of Sciences located in Zakopane, in the vicinity of the national park (around 1.3 km away from the national park border in straight line; coordinates: 49.2922N, 19.9759E, elevation: 915 m.a.s.l.). Pots were placed into three different blocks. Each block consisted of 16 pots, assigned to a two-factorial treatment of microhabitat and seed density. We simulated the four different microhabitat types on which we found the faecal samples during the transect inspections: bare soil, dead wood, stones and vegetation. Soil, dead wood, stones and vegetation were collected from the vicinity of the national park, in a place where bilberries naturally occur. The soil was placed directly into the pots without previous sterilization after being well mixed and homogenized. To simulate the effects of the microhabitat type we either covered the soil with (i) small pieces of dead wood of fallen coniferous trees (dead wood treatment), (ii) small stones (5-10 cm stones; stone treatment), (iii) mosses and alive herbaceous plants (vegetation treatment), or nothing (bare soil treatment). Four different amounts of bilberry seeds were sown in the pots: a control treatment with no seeds, as well as treatments with 10, 100 and 1000 seeds. The control with no seeds was used to check for the existence of a bilberry seed bank in the collected soil. The other three density treatments were selected to simulate the effects of seed numbers on the germination rates (Supp. Mat. Table S2). In total we sowed 13,320 bilberry seeds.

Coinciding with the germination season in the field, we inspected the pots once a day from May 20th to June 29th 2018 until the moment when no new seedlings appeared for three consecutive days (García-Rodríguez et al., 2021). Furthermore, we checked the pots once a month from July to October in 2018 and from May to October in 2019 to confirm that no more seeds germinated. Each day we checked for new seedlings and marked them with a wooden stick to avoid counting them again. If a seedling died, we removed the wooden stick. As bilberry seedlings are very small and grow slowly, we checked all the seedlings once a week to be sure that every marked seedling was a bilberry. Pots stayed outdoors and were covered by snow during winter and watered whenever the substrate was dry. To tried to mimic natural conditions the most and, thus, we did not prevent neither seed or seedling predation, nor competition with herbs and mosses growing in the pots.

- **Statistical analyses**

For statistical analyses we differentiated between the whole community of dispersers and the main functional groups: mammals were subdivided into brown bears and mesocarnivores (red foxes and martens) and birds were divided into small and large passerines and grouses. We distinguished between small and large passerines according to mean avian body masses. Based on existing differences in body masses between passerine families we included species with adult body masses heavier than 60g in the large passerine category (Corvidae and Turdidae) whereas lighter species were classified as small passerines (Sylvidae and Muscicapidae). Data about mean body mass of each disperser species was extracted from the Encyclopedia of Life, hosted by the National Museum of Natural History (https://eol.org/traitbank). We excluded grouses from the functional group analyses because of small sample size (n = 12 droppings).

We performed generalized linear mixed models with a negative binomial distribution and log link function to analyze the effects of habitat type, month and their interaction on the bilberry seed rain provided by the whole community of dispersers and by birds (small and large passerines separately). The models included habitat type (alpine meadow and coniferous forest), month (July, August, September and October) and their interaction as fixed factors. The transect was included as a random factor. We performed generalized linear models with a negative distribution and log link function to analyze the effects of habitat type, month and their interaction on the seed rain provided by mammals (mesocarnivores and brown bears separately).

We used a Chi^2 test to evaluate whether patterns of seed deposition across the microhabitat types differed between the four frugivorous functional groups (small passerines, large passerines, mesocarnivores and brown bears). To analyze the effects of seed density and microhabitat type on the germination rates of bilberry seeds we performed a generalized linear mixed effects model with a betabinomial distribution (to account for overdispersion) and logit link function based on the data from the germination experiment. The initial model included the number of seeds sown (10, 100 and 1000), the microhabitat (bare soil, vegetation, stones, dead wood) as well as their interaction as fixed factors and the block (n = 3) as a random factor. After inspection of the model, we removed the interaction term between the number of seeds sown and microhabitat type, because it was not significant. We used the estimated parameters of the most parsimonious model (i.e. the model with lowest AIC value) to predict the expected proportion of germinated seeds for each bird dropping and carnivore scat based on both the microhabitat in which the dropping or scat was found and the number of bilberry seeds contained in each dropping or scat. We calculated the individual SDE of each dropping/scat (i.e. the predicted number of seeds germinating from each faecal sample) by multiplying the number of seeds in each faecal sample by the estimated proportion of germinated seeds in the respective faecal sample. We then calculated the SDE of each disperser species by summing the individual SDE values of the samples assigned to each species.

We performed Kruskal-Wallis tests to test for differences in SDE values between bird and mammal species, for coniferous forests and alpine meadows separately. We used Spearman's rank correlations to test for a relationship between the SDE of each disperser species and its body mass. In addition, we performed Spearman's rank correlations to test for a relationship between the quantity and the quality components and to test for a relationship between each of these components with the total SDE of each disperser species in each habitat separately.

All the statistical analyses were done using the R statistical environment (version 3.4.0, R Development Core Team, 2017). We used the packages *effect.lndscp* (Jordano, 2017) to plot the effectiveness landscapes, *lme4* (Bates et al., 2015) for the implementation of the generalized linear mixed models and *Vegan* (Oksanen et al., 2007) for the implementation of the rarefaction curves. The map in Fig. S1 was performed in QGIS (version 2.14.22, 2018).

RESULTS

- **Species of bilberry dispersers and their seed rains**

In total, we collected 1,812 faecal samples during the transect inspections (1,656 bird droppings and 156 carnivore scats, Supp. Mat. Table S1). A total of 1,111 bird droppings were processed for barcoding, and 837 of these could be assigned to a bird species (75%). We found that at least 16 different frugivorous species dispersed bilberry seeds in the study area (Fig. 1, Table 1). Total bilberry seed rain in the area was 107,979 bilberry seeds per ha and month (115,597 and 92,744 seeds in forests and meadows, respectively). Brown bears provided the majority of the seed rain in both coniferous forests (94% of the total seed rain) and alpine meadows (93%), followed by mesocarnivores (3.6% and 6.0% of the seed rain in forests and meadows, respectively) and thrushes *Turdus spp*. (1.5% and 1.4% in forests and meadows, respectively; Table 1). We found that four bird species – the western capercaillie, the hazelgrouse *Tetrastes bonasia*, the Eurasian jay *Garrulus glandarius* and the Eurasian blackcap *Sylvia atricapilla* – dispersed bilberry seeds exclusively in coniferous forests, while one species – the winchat *Saxicola rubetra* – dispersed them exclusively in the alpine meadows (Fig. 1, Table 1). The remaining seed dispersers contributed to seed rain in both habitats. According to the rarefaction curves, we recorded 100% and 88% of the disperser species in coniferous forests and alpine meadows, respectively (Fig. S2). The mean altitudinal gradient of seed dispersal provided by the disperser species was 463 ± 244 m (mean ± SD; for species with $n > 20$ samples maximum elevation gradient = 776 m for fieldfares; minimum = 460 m for brown bears; Table S3).

The amounts of dispersed seeds differed between the two habitats across the bilberry season (Table 2, Fig. 2). Bilberry seed rain within the forest peaked in August and progressively decreased until October in all the disperser functional groups. On the other hand, bilberry seed arrival to the alpine meadows decreased from August to October in the case of birds, but progressively increased from July to October in the case of mammals (Table 2, Fig. 2).

- **Effect of seed density and microhabitat in bilberry germination**

Different functional groups of frugivores showed different preferences on microhabitats when dispersing the bilberry seeds ($Chi^2 = 163.93$, df = 12, p-value < 0.001, n = 991, Fig. S3). Both small and large passerines usually defecated on stones, while mesocarnivores did it across all microhabitats more randomly. Most brown bears scats were found on vegetation (Fig. S3). Seed quantity and, to a lesser extent, the microhabitat of the deposition site influenced seed germination rates. Bilberry germination rates significantly decreased with increasing seed densities (Fig. 3a, Table 3). Among microhabitats, the largest germination rates were found on dead wood, followed by bare soil, stones and vegetation (Fig. 3b, Table 3). Consequently, small passerines such as winchats *Saxicola rubetra*, Eurasian robins *Erithacus rubecula* or black redstarts *Phoenicurus ochruros* that dispersed only few seeds per dropping and defecated mainly on stones provided the highest quality of bilberry seed dispersal in terms of germination rates. In contrast, the quality of the seed dispersal provided by mammals was lower, because of the larger number of seeds per carnivore scat and because more scats were defecated on vegetated soil, less suitable microhabitat for bilberry seed germination (Fig. 1, Table 1, Fig. S3, Fig. S4).

- **SDE landscapes and functional groups**

Two species of thrushes – song thrushes *Turdus philomelos* and fieldfares *T. pilaris* – were the most effective bilberry dispersers in the study area (82 and 68 bilberry seeds potentially germinating from their droppings per ha and month, 23% and 19% of the total emerging seedlings in the study area), followed by brown bears (47 seeds/ha and month, 16%) and red foxes (37 seeds/ha and month, 12%; Table 1). In coniferous forests song thrushes were the most effective seed dispersers (101 seeds/ha and month, 27%), followed by fieldfares (71 seeds/ha and month, 19%), brown bears (47 seeds/ha and month, 15%) and robins *Erithacus rubecula* (37 seeds/ha and month, 12%). The predicted number of seedlings germinating from disperser faecal remains was 14% larger in forests than in alpine meadows (366 vs 318 seeds/ha and month; Table 1). Fieldfares (62 seeds/ha and month, 19%), brown bears (47 seeds/ha and month, 17%), mistle thrushes *T. viscivorus* (45 seeds/ha and month, 16%) and song thrushes (43 seeds/ha and month, 14%) were the most effective dispersers in alpine meadows (Fig. 1, Table 1).

We did not find differences in SDE values between birds and mammals neither in coniferous forests (Kruskal-Wallis Chi^2 = 1.69, df = 1, p-value = 0.19) nor in alpine meadows (Kruskal-Wallis Chi^2 = 1.44, df =1, p-value = 0.23). Accordingly, SDE was not related to disperser species body mass in forests (Spearman's rho = 0.13, p-value = 0.63). However, we found a positive relation between body size and SDE in alpine meadows (Spearman's rho = 0.63, p-value = 0.02).

- **Quantity and quality components as surrogates for the total SDE**

SDE was positively related to the quantity component in both coniferous forests and alpine meadows (forests: Spearman's rho = 0.90, p-value < 0.01; meadows: Spearman's rho = 0.82, p-value < 0.01) and negatively related with the quality component in alpine meadows (Spearman's rho = -0.67, p-value = 0.02). However, SDE was not related to the quality component in coniferous forests (Spearman's rho = -0.29, p-value = 0.30). We found a negative relation between the quantity and quality components in both habitats (forests: Spearman's rho = -0.63, p-value = 0.01; meadows: Spearman's rho = -0.90, p-value < 0.01).

DISCUSSION

Here we have demonstrated that birds and mammals show spatiotemporal complementarity in the bilberry seed dispersal services they provide in alpine ecosystems. Avian and mammalian contributions to the bilberry seed rain are spatially and temporally structured as their dispersal services peak in different habitats and at different times. Mammals, particularly brown bears, contribute the most to the bilberry seed rain in the study area, but seeds dispersed by birds have the highest chances to germinate, because birds consistently defecate in microhabitats that are more suitable for germination. The smaller quantities of bilberry seeds found in bird droppings minimize seed competition and increase the chances of germination. The uncoupled contribution of quantity and quality components to the total SDE landscapes implies that frugivorous species with different body sizes may have similar effectiveness as seed dispersers. Therefore, birds and mammals may be similarly essential for seed dispersal as species from both groups are effective bilberry dispersers, but they contribute differently to seed arrival in terms of habitat, microhabitat and timing. Our results also demonstrate that generalist species (i.e. species that occurred in both coniferous forests and alpine meadows) such as thrushes, brown bears and red foxes are the most effective dispersers in both habitat types, being responsible for the vast majority of the dispersal events occurring beyond the forest edge.

The seed rain provided by birds and mammals vary across the bilberry fruiting season. Avian seed dispersal peaked in August in both habitats, which may be explained by the annual cycle of the European migrant bird species present in the study area (e.g. robins and song thrushes), which usually move to their wintering grounds in September (for detailed information see Cramp & Perrins, 1998). These species are also the most relevant avian seed dispersers while wintering in Mediterranean regions (González-Varo et al., 2019). On the other hand, bilberry dispersal by mammals peaked at the end of the season (September – October) and took place primarily in alpine meadows. This suggests that, in alpine ecosystems, mammals closely follow the bilberry fruiting phenology along the elevation gradient, moving from the lower forests during mid-summer to the upper alpine meadows in early-autumn. Mesocarnivores and brown bears are flexible dietary generalists that are adapted to food seasonality, with fruit consumption being directly related to fruit availability (Herrera, 1989; Welch et al., 1997; García et al., 2001). In line with this, foxes and martens are known to change their dietary preferences from rodents and small birds to bilberry fruits throughout the summer in European boreal and temperate regions (Jędrzejewski et al., 1993; Schaumann & Heinken, 2002). Brown bears are quantitively important bilberry dispersers in other boreal and temperate populations (Hertel et al., 2018; Lalleroni et al., 2017; García-Rodríguez et al., submitted), especially before the hibernation, when they can consume up to a third of their body weight of fleshy fruits per day (Welch et al., 1997). The timing of frugivore-mediated seed dispersal is known to affect not only the quantitative contribution of each disperser to the total seed rain but also seed viability and germination (González-Varo et al., 2019). As bilberry seed viability is positively associated to snow cover and negatively affected by wet and warm conditions in the soil (Ranwala & Naylor, 2004; Nestby et al., 2011), one could expect that bilberry seeds survive and germinate better when dispersed at the end of the fruiting season, when mammals are providing the majority of the seed dispersal services in the study area. The effects of timing on the quality of seed dispersal in temperate and boreal regions is a relevant topic for future research.

Our results demonstrate that germination rates of bilberry seeds are in general very low, especially in scats containing large amounts of seeds and/or deposited on vegetation. Low germination rates of bilberry seeds, especially when deposited on vegetation, have been also reported in boreal coniferous forests in Scandinavia (Eriksson & Fröborg, 1996). All this supports the idea that recruitment of bilberry seeds is extremely rare in nature and constrained to “windows of opportunity”, i.e. spatially and temporally unpredictable conditions in which seedling recruitment is possible within established conspecific adults (Eriksson & Fröborg, 1996).

The results of the germination experiments also suggest that, qualitatively, birds are better bilberry dispersers than mammals. Previous studies have found that passerines, mesocarnivores and brown bears do not harm bilberry seeds and that, in some cases, they may enhance bilberry seed germination when compared to intact seeds manually extracted (Schaumann & Heinken, 2002; Honkavaara et al., 2007; Steyaert et al., 2019; García-Rodríguez et al., 2021). However, in all these experiments, bilberry seeds were sown individually or in similar densities after being recovered from faeces and, thus, possible effects of seed competition are omitted. Therefore, the huge differences in the number of seeds contained in bird droppings and carnivore scats, together with different preferences of deposition sites, may still explain why we found that birds are better bilberry seed dispersers qualitatively in our study area.

On the other hand, specific behaviors of different mammal species might enhance the qualitative contributions of these species to the bilberry seed dispersal. For instance, in order to delimit their territories, mesocarnivores usually defecate in visible spots such as fallen logs, disturbed vegetation or burnt areas, which are known to enhance germination of bilberry seeds (Schaumann & Heinken, 2002; Rost et al., 2012; Steyaert et al., 2018). Brown bears usually defecate next to their resting sites, where they often dig out the vegetation and create local disturbances that may facilitate bilberry germination (Steyaert et al., 2019; García-Rodríguez & Selva, submitted). Additionally, due to the much larger bilberry seed quantity per faecal remain, the probability of at least one seedling emerging from a brown bear scat is higher than for any other frugivore inhabiting the area. Therefore, the high seed densities in brown bear scats may act as an insurance that enhances the probability of seedling establishment. In plant species usually expanding by clonal propagation, such as the bilberry, this insurance of recruitment provided by bears and other large frugivores may be key for guaranteeing genetic diversity and gene flow within and among populations.

Our results suggest that, in alpine regions, the quantity of the seed dispersal service is a better indicator of the total effect of the dispersers on plant reproduction than the quality of the service they provide, which supports previous research (Vázquez et al., 2005). Given that differences in the qualitative contribution among bilberry dispersers are constrained by the general low bilberry germination rates (9.5 ± 15%), the quantitative contribution prevails as a better surrogate of the SDE in our study system. This may be explained by the extreme differences found in the quantitative contributions of the disperser species inhabiting the study area, with avian dispersers contributing much less than mammals to the total bilberry seed rain. In order to get a comprehensive picture of the disperser assemblage of the bilberry in our study area, we tried to deliberately allocate our sampling effort across the entire fruiting season of the bilberry in our study area. Yet this resulted on sampling intervals of three to four weeks between transect walks. Therefore, we cannot rule out that the lower contribution of birds to seed rain might in part be influenced by the shorter longevity of bird droppings than mammal scats under natural conditions. However, we are confident that the reported patterns are not unduly influenced by differences in the longevity of bird droppings and mammal scats. Moreover, brown bears have been recognized as the quantitatively most important seed dispersers also in other temperate and boreal regions. For instance, in a previous study conducted in southeast Alaska fruit removal by birds was around 15 and eight times smaller than by brown and black bears, respectively (Harrer & Levi, 2018). Thus, we believe that the primacy of bears as seed dispersers from a quantitative point of view might be a normal feature of areas holding healthy populations of these animals.

The spatiotemporal complementarity between birds and mammals in the total bilberry SDE landscape in the Tatra Mountains relates to differences in the quantity and timing of the seed dispersal services they provide, and in the seed arrival to different habitats and microhabitats. This complementarity may enhance gene flow among and within plant populations, recruitment probabilities and the colonization of new habitats (González-Castro et al., 2015). The important but different contributions of both birds and mammals to the total bilberry seed dispersal demonstrate the high complexity of the frugivore community in our study area. Functional differences in seed dispersal services, which provide robustness to the interactions between plants and dispersers (Bascompte & Jordano, 2007), has been reported for birds and mammals in Mediterranean montane forests and, more recently, in another temperate region in Europe, the Cantabrian Mountains (Jordano et al., 2007; Rumeu et al., 2020). As animal body size is directly related to dispersal distance (Santini et al., 2013), the contribution of both small and large-sized disperser agents to the bilberry dispersal in our study area guarantees that seeds reach different distances within the dispersal kernel (Jordano et al., 2007). Frugivorous mammals such as martens, foxes and brown bears are well-known long-distance seed dispersers that may move seeds more than one kilometer away from the parent plant (Otani, 2002; Jordano et al., 2007; Rost et al., 2012; Lalleroni et al., 2017). These long-distance dispersal events are crucial for gene flow, plant community dynamics and plant recruitment in new areas and young stands, which are the main contributions of sexual reproduction to the demography of clonal plants such as the bilberry (Cain et al., 2000, Cousens et al., 2008). Moreover, generalist species such as foxes and bears are crucial for transporting seeds above vegetation belts and play a key role for plants to migrate uphill in response to climate change (Nathan et al., 2008; González-Varo et al., 2017b; Naoe et al., 2016). Therefore, the extinction or severe decline of large-sized seed dispersers in temperate ecosystems would be difficult to compensate by birds as large frugivores provide most of the long-distance seed dispersal events (Jordano et al., 2007), especially taking into account that grouses, other important long-distance seed dispersers in boreal regions (Welch et al., 2000), seemed to be functionally extinct in these areas. Additionally, global warming is likely to affect fleshy-fruited plant species distribution, survivorship and productivity (Roberts et al., 2014; Penteriani et al., 2019) and may modify the phenology of some important aspects of the frugivores dispersing their seeds (e.g. bird migration, brown bear hibernation, etc.). These aspects relating fleshy-fruited plant species performance and disperser activity to climate change are relevant topics for future research.

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AUTHOR´S CONTRIBUTION

AGR, JA and NS conceived the study and designed methodology; AGR, AP and DF collected the data in the field; AGR prepared the samples and performed the extraction and amplification of DNA; AGR and JA analyzed the data; AGR wrote a first draft of the manuscript with significant contributions of JA and NS. All authors contributed critically to the drafts and gave final approval for publication.

DATA AVAILABILITY STATEMENT

The data used in this article are available in https://figshare.com/s/c48c49ca02515fee6f40

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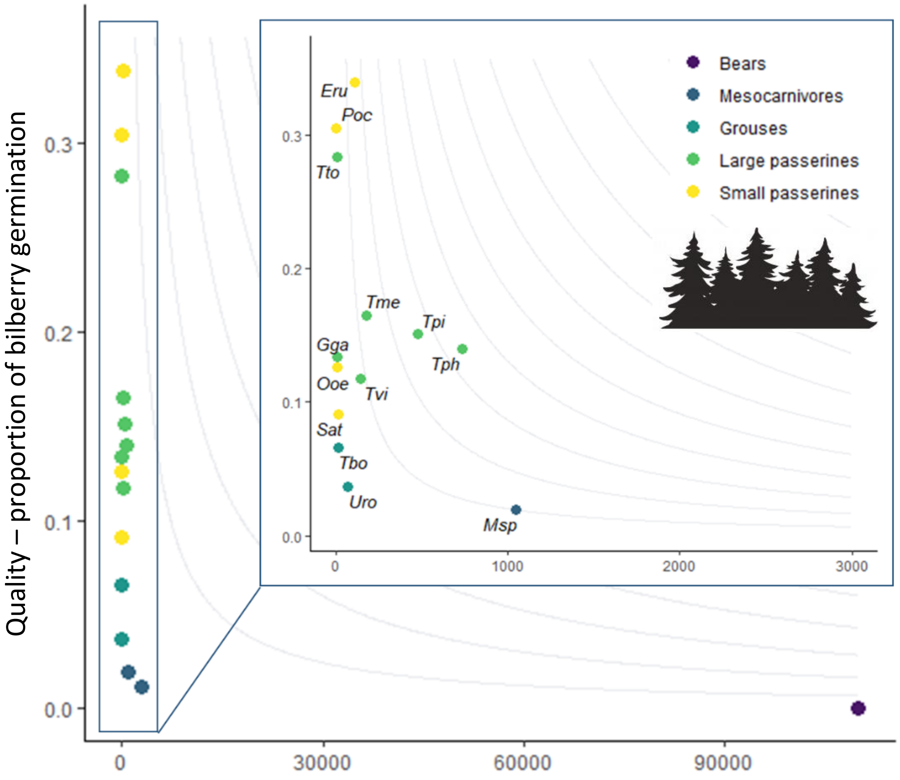
Figure 1. Seed dispersal effectiveness (SDE) landscapes of frugivorous species dispersing the bilberry coniferous forests (a) and alpine meadows (b) and in Tatra National Park, Poland. Each point corresponds to a species and its position relates to its effectiveness as bilberry seed disperser, which is the result of multiplying the quantity component (number of bilberry seeds that each species dispersed per ha and month – X axis) by its quality component (probability of germination of a bilberry seed dispersed by each species – Y axis). Each isocline represents a unique value of SDE (number of bilberry seedlings emerging per ha and month) and it can be the result of different combinations of values in quality and quantity components. The brown bear is represented with a purple point in the bottom-right corner of the figures. The embedded figures zoom in the left part of each SDE landscape to visualize differences in the quantity of bilberry seed dispersal provided by each of the bilberry disperser species. Acronyms represent the different disperser species: Poc – *Phoenicrurus ochruros*, Sru – *Saxicola rubetra*, Eru – *Erithacus rubecula*, Sat – *Sylvia atricapilla*, Ooe – *Oenanthe Oenanthe*, Tph – *Turdus philomelos*, Tme – *T. merula*, T.pi – *T. pilaris*, T.to – *T. torquatus*, Tvi – *T. viscivorus*, Gga – *G. glandarius*, Tbo – *Tetrastes bonasia*, Uro – *Tetrao urogallus*, Msp – *Martes sp*., Vvu – *Vulpes vulpes*. Values of quantity, quality and SDE for each species are provided in Table 1. Please, note that the X-axes are represented in different scales.

(a) **Coniferous forests**



(b) **Alpine meadows**

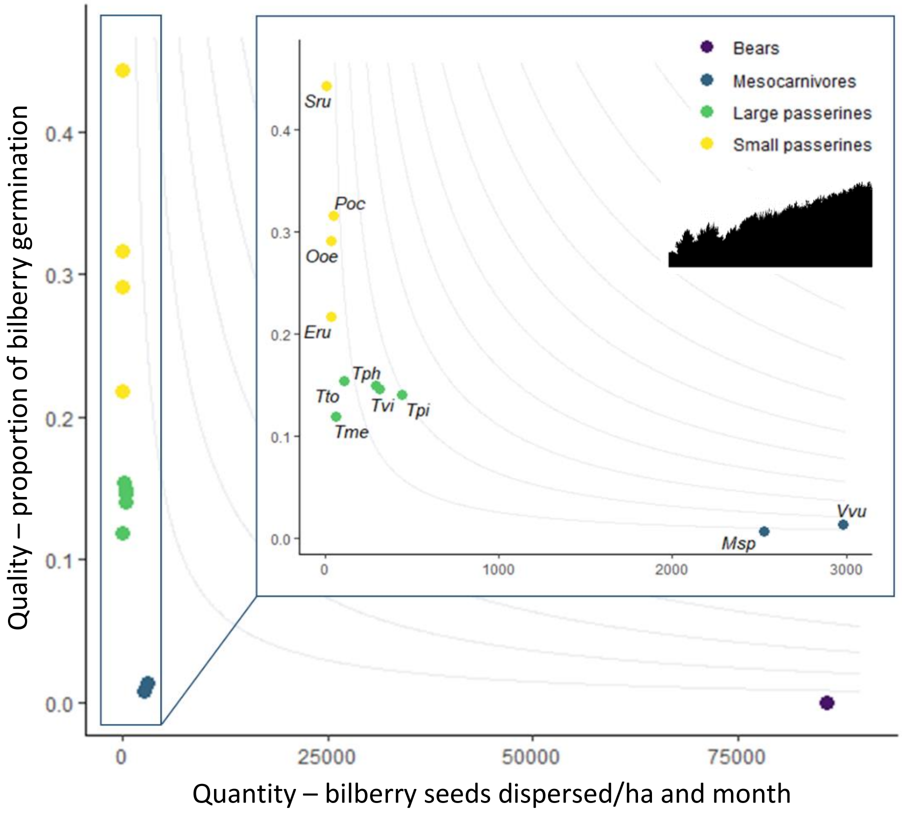


Figure 2. Boxplots representing monthly bilberry seed rains (number of bilberry seeds dispersed per ha and month; Y axes) provided by each functional group of seed dispersers (small passerines, large passerines, mesocarnivores and brown bears; X axes) in coniferous forests (left) and alpine meadows (right) in Tatra National Park, Poland. Please, note that the Y-axes are represented in different scales due to the very different seed rains provided by each functional group of seed dispersers.

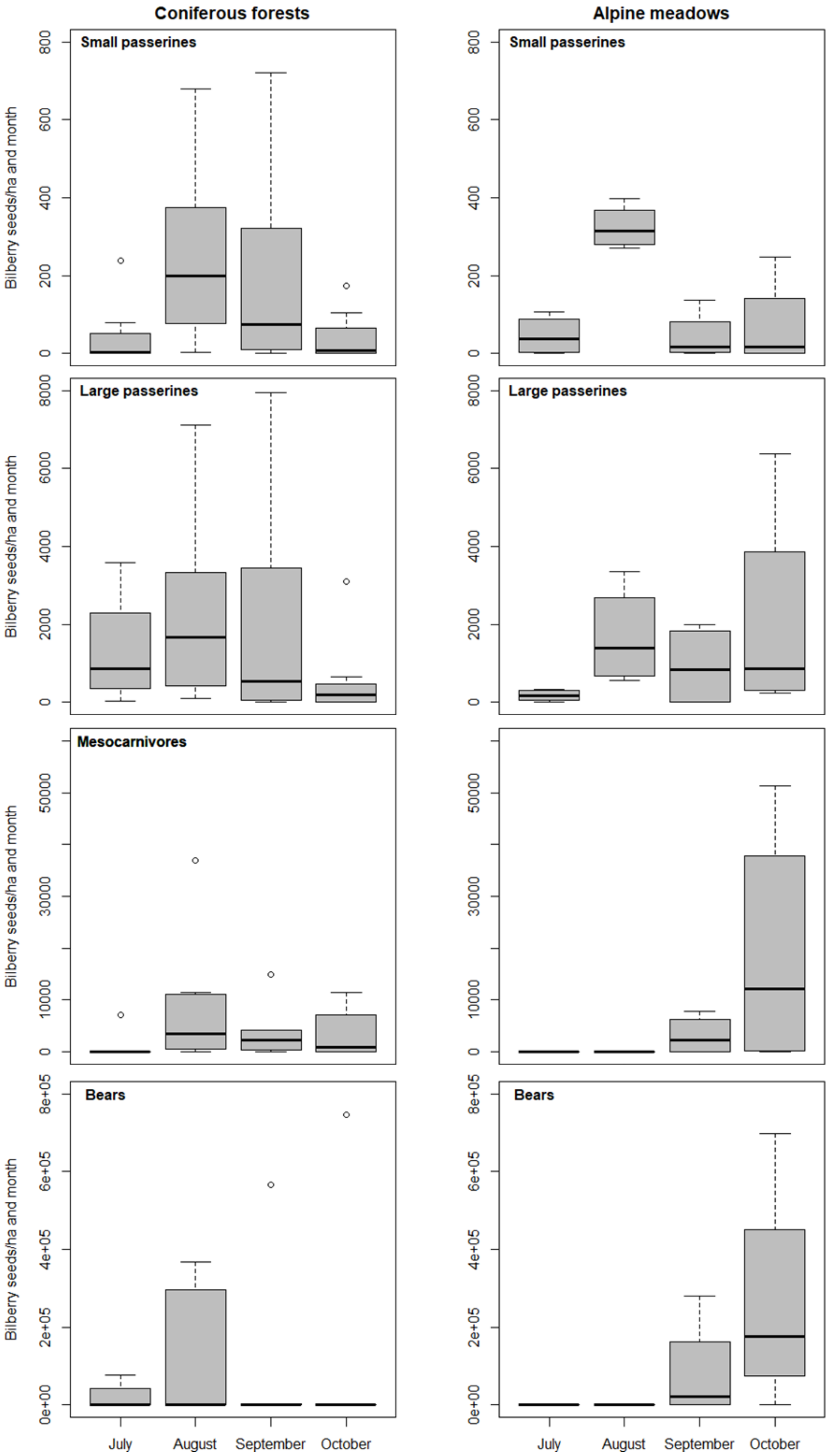

Coniferous forests
Alpine meadows
Small passerines
Large passerines
Mesocarnivores
Bears
Bilberry seeds/ha and month
0
200
400
600
800
2000
4000
6000
8000
10000
30000
50000
0e+00
2e+05
4e+05
6e+05
8e+05
July
August
September
October

Figure 3. Effects of seed density (number of bilberry seeds sown; left) and microhabitat (substrate of seed deposition; right) on the germination rates of bilberry seeds (Y axes). Please, note that Y-axes are represented in different scales.

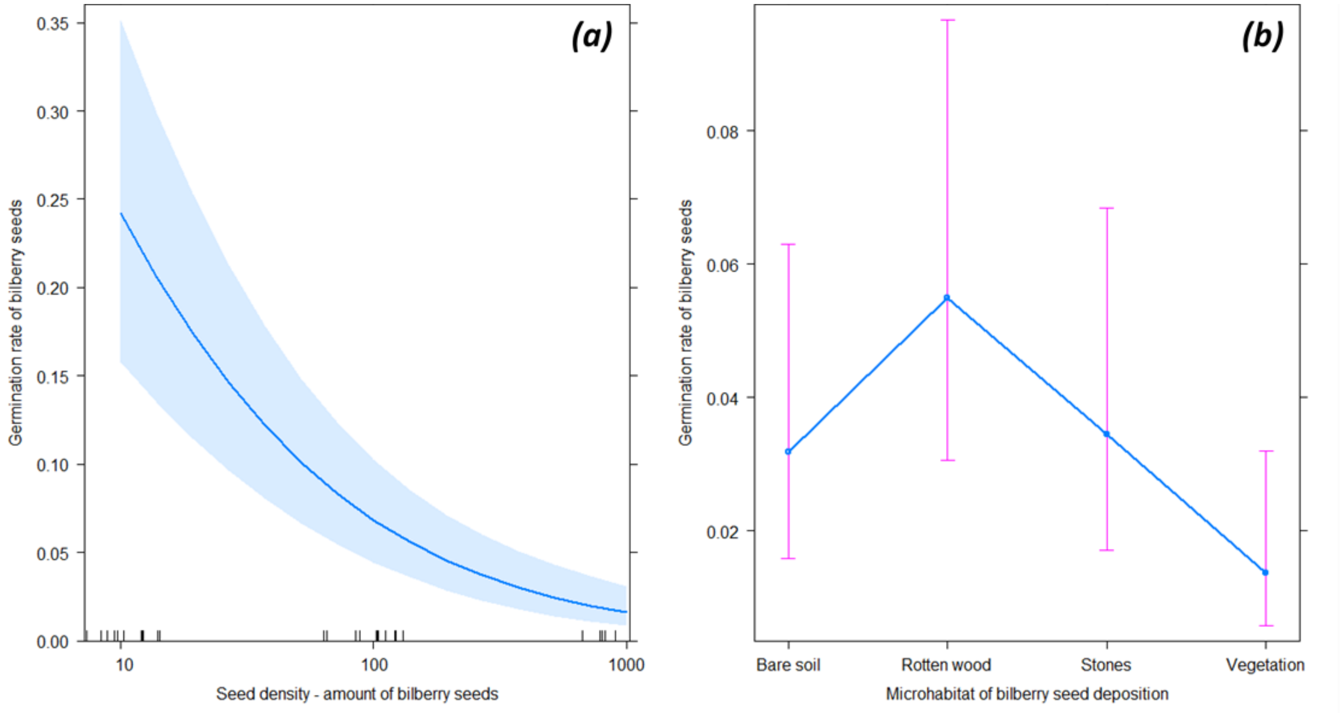


Table 1. Disperser species of bilberry seeds ranked according to their total SDE values (number of bilberry seedlings emerging per ha and month as the result of the dispersal activities of each species). The scores of the quantity (number of bilberry seeds that each species dispersed per ha and month) and quality (probability of germination of a bilberry seed dispersed by each species) components and the position in the ranking (in brackets) are also provided. SDE values and scores of quantity and quality components also provided for each of the two studied habitats separately (alpine meadows and coniferous forests).

			Total			**Coniferous forests**			**Alpine meadows**		
Species	**Group**	**Body mass (grams)**	**SDE**	**Quantity component**	**Quality component**	**SDE**	**Quantity component**	**Quality component**	**SDE**	**Quantity component**	**Quality component**
Turdus philomelos	Large passerines	70	82.52 (1)	584.46 (4)	0.14 (11)	101.90(1)	730.67 (4)	0.14 (10)	43.76 (4)	292.02 (5)	0.15 (9)
Turdus pilaris	Large passerines	100	68.30 (2)	462.76 (5)	0.15 (5)	71.43 (2)	473.86 (5)	0.15 (4)	62.05 (1)	440.55 (4)	0.14 (6)
Ursus arctos	Bears	100,000	47.44 (3)	101,808.67 (1)	<0.01 (16)	47.34 (3)	109,764.34 (1)	<0.01 (15)	47.66 (2)	85,897.35 (1)	<0.01 (12)
Vulpes vulpes	Mesocarnivores	5,000	37.04 (4)	3,021.50 (2)	0.01 (14)	34.43 (5)	3,039.03 (2)	0.01 (14)	42.24 (5)	2,986.43 (2)	0.01 (10)
Erithacus rubecula	Small passerines	16	27.42 (5)	84.91 (8)	0.32 (2)	37.65 (4)	111.13 (8)	0.34 (2)	6.97 (10)	32.45 (11)	0.21 (2)
Turdus viscivorus	Large passerines	114	26.40 (6)	199.80 (6)	0.13 (10)	16.90 (8)	144.16 (7)	0.12 (9)	45.38 (3)	311.08 (6)	0.15 (8)
Turdus merula	Large passerines	99	22.16 (7)	140.04 (7)	0.16 (7)	29.53 (6)	179.06 (6)	0.16 (6)	7.40 (11)	61.99 (8)	0.12 (5)
Martes sp.	Mesocarnivores	1,500	19.82 (8)	1,541.59 (3)	0.01 (15)	20.19 (7)	1,048.34 (3)	0.02 (13)	19.05 (6)	2,528.07 (3)	0.01 (11)
Phoenicurus ochruros	Small passerines	15	6.07 (9)	17.56 (11)	0.34 (1)	0.61 (14)	1.76 (15)	0.34 (1)	15.47 (8)	49.16 (9)	0.31 (1)
Turdus torquatus	Large passerines	102	6.02 (10)	37.53 (10)	0.16 (8)	0.85 (13)	3.20 (12)	0.26 (7)	16.37 (7)	106.21 (7)	0.15 (7)
Oenanthe oenanthe	Small passerines	23	4.10 (11)	16.39 (12)	0.25 (4)	0.88 (12)	6.82 (13)	0.13 (3)	10.50 (9)	35.52 (10)	0.30 (4)
Tetrao urogallus	Grouses	3,500	1.64 (12)	44.67 (9)	0.04 (13)	2.47 (9)	67.00 (9)	0.04 (12)	0	0	0
Sylvia atricapilla	Small passerines	18	0.67 (13)	7.27 (14)	0.09 (6)	1.01 (10)	10.90 (11)	0.09 (5)	0	0	0
Tetrastes bonasia	Grouses	430	0.62 (14)	9.44 (13)	0.07 (12)	0.93 (11)	14.16 (10)	0.07 (11)	0	0	0
Saxicola rubecula	Small passerines	16	0.44 (15)	1.07 (16)	0.41 (3)	0	0	0	1.33 (12)	3.22 (12)	0.41 (3)
Garrulus glandarius	Large passerines	170	0.27 (16)	1.94 (15)	0.14 (9)	0.40 (15)	2.91 (14)	0.14 (8)	0	0	0

Table 2. Summary statistics of the Generalized Linear Mixed Models -GLMM- and Generalized Linear Models -GLM- performed to test the effects of habitat type (alpine meadows and coniferous forests) and timing (month of the sampling session: July to October) on the bilberry seed rain provided by each functional group of seed dispersers (small passerines, large passerines, mesocarnivores and brown bears; more details in methods section). Asterisks show significant p-values < 0.05.

	Small passerines (GLMM)			**Large passerines (GLMM)**			**Mesocarnivores (GLM)**			**Brown bears (GLM)**		
Fixed factors	**Chi^2**	**Df**	**p-value**	**Chi^2**	**Df**	**p-value**	**Chi^2**	**Df**	**p-value**	**Chi^2**	**Df**	**p-value**
Intercept	13.15	1	<0.001*	296.70	1	<0.001*						
Habitat	1.62	1	0.20	6.70	1	0.009*	17.39	1	<0.001*	23.72	1	<0.001*
Timing	69.00	3	<0.001*	19.93	3	<0.001*	6.58	3	0.087	-40.44	3	1.00
Interaction	18.86	3	<0.001*	30.74	3	<0.001*	43.60	3	<0.001*	5.78	3	0.12
Random factors	**Var**	**S.D.**		**Var**	**S.D.**							
Site	1.36	1.17		0.39	0.62							

Table 3. Summary statistics of the Generalized Linear Mixed Model performed to test the effects of seed density (number of bilberry seeds sown: 10, 100, 1000) and microhabitat (substrate of seed deposition: bare soil, dead wood, stones and vegetation) in the germination rates of bilberry seeds. We suppressed the intercept by using means parameterization to show the mean germination probability for each microhabitat. Asterisks show significant p-values < 0.05

Fixed factors	Chi^2	Df	p-value
Seed density	53.73	1	<0.001*
Microhabitat	13.01	4	0.011
Random factors	**Var**	**S.D.**	
Block	0.54	0.23	

Supplementary material for:

Functional complementarity of seed dispersal services provided by birds and mammals in an alpine ecosystem

Alberto García-Rodríguez, Jörg Albrecht, Nina Farwig, Danuta Frydryszak, Aida Parres, Dana G. Schabo, Nuria Selva

Figure S1. Map showing the location of the transects for faecal sample collection in Tatra National Park, Poland. Transects 1-4 were established in coniferous forests and transects 5-6 in alpine meadows.

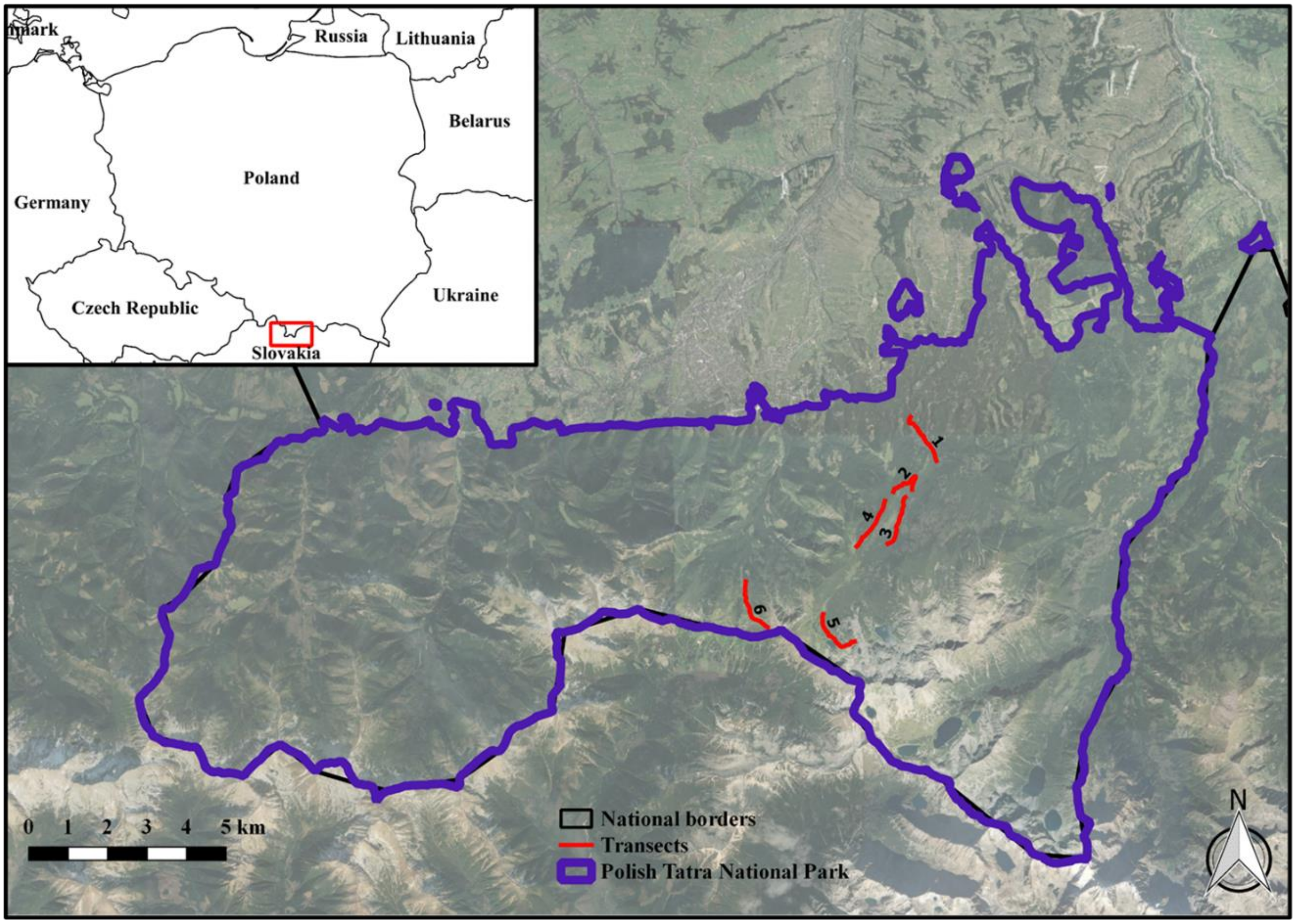


Figure S2. Rarefaction curves for the number of bilberry disperser species detected during the transect inspections in coniferous forests (dark green) and alpine meadows (light green) in Tatra National Park, Poland. The total number of species recorded, the potential number of species and the completeness (species recorded divided by the potential number of species) is provided for each habitat type.

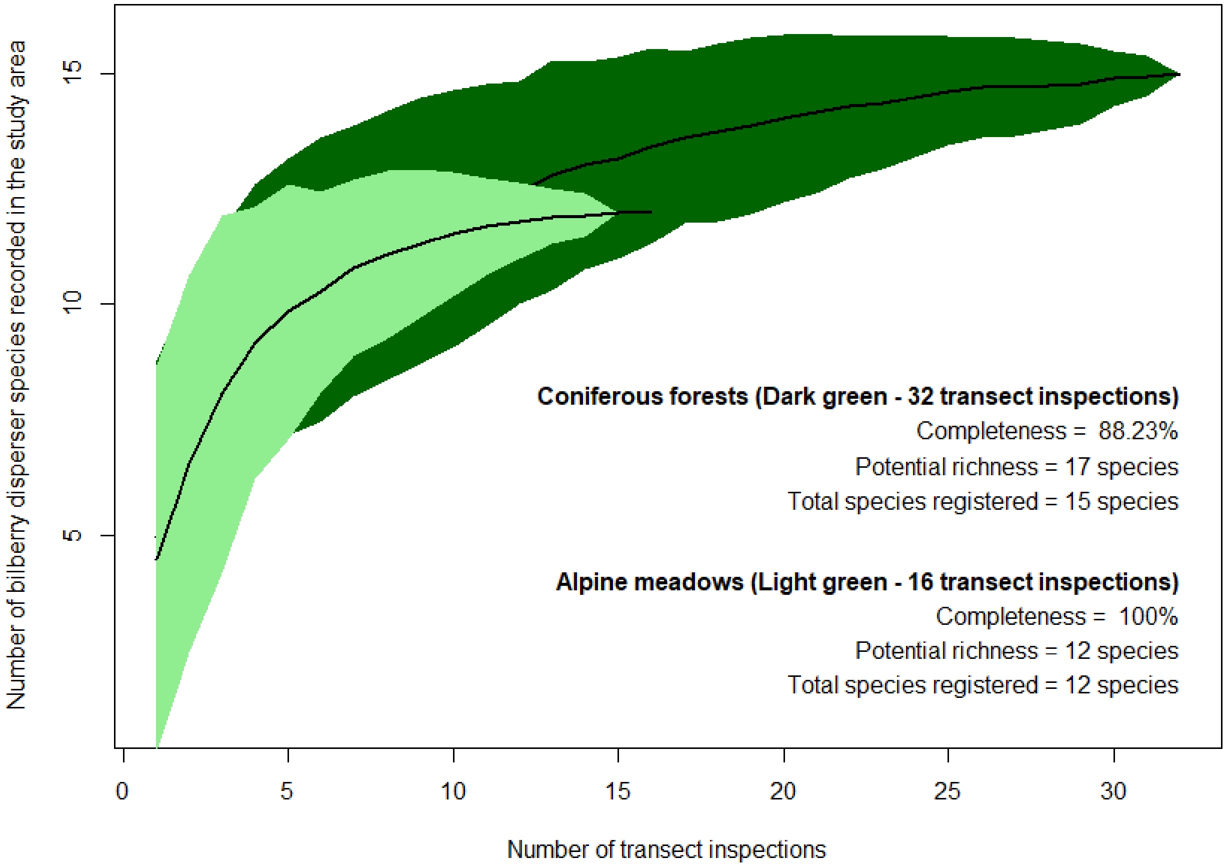


Figure S3. Proportion of faecal samples found at the four microhabitats for each functional group of bilberry dispersers (small passerines, large passerines, grouses, mesocarnivores and brown bears) in Tatra National Park, Poland. The number of faecal remains of each group provided in brackets.

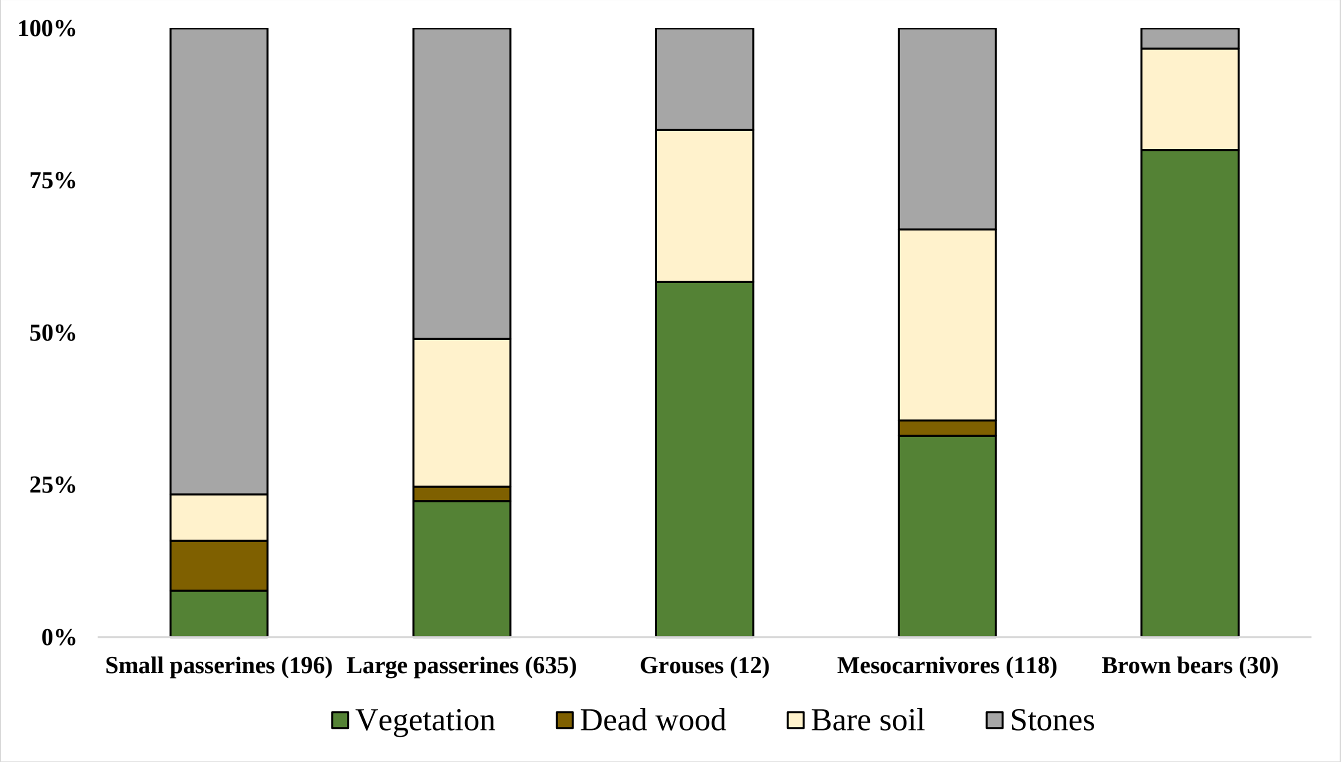


Figure S4. Seed dispersal effectiveness (SDE) landscape of frugivorous species dispersing the bilberry in Tatra National Park, Poland. Each point corresponds to a frugivorous species and its position relates to its effectiveness as bilberry seed disperser, which is the result of multiplying the quantity component (number of bilberry seeds that each species dispersed per ha and month – X axis) by its quality component (probability of germination of a bilberry seed dispersed by each of the species – Y axis). Each isocline represents a unique value of SDE (i.e. the number of bilberry seedlings emerging per ha and month) and it can be the result of different combinations of values in quality and quantity components. The brown bear is represented with a purple point in the bottom-right corner of the figure. The embedded figure zooms in the left part of the SDE landscape to visualize differences in the quantity of bilberry seed dispersal provided by each of the bilberry disperser species. Acronyms represent the different disperser species: Poc – *Phoenicrurus ochruros*, Sru – *Saxicola rubetra*, Eru – *Erithacus rubecula*, Sat – *Sylvia atricapilla*, Ooe – *Oenanthe Oenanthe*, Tph – *Turdus philomelos*, Tme – *T. merula*, T.pi – *T. pilaris*, T.to – *T. torquatus*, Tvi – *T. viscivorus*, Gga – *G. glandarius*, Tbo – *Tetrastes bonasia*, Uro – *Tetrao urogallus*, Msp – *Martes sp*., Vvu – *Vulpes vulpes*. Values of quantity, quality and SDE for each species are provided in Table 1.

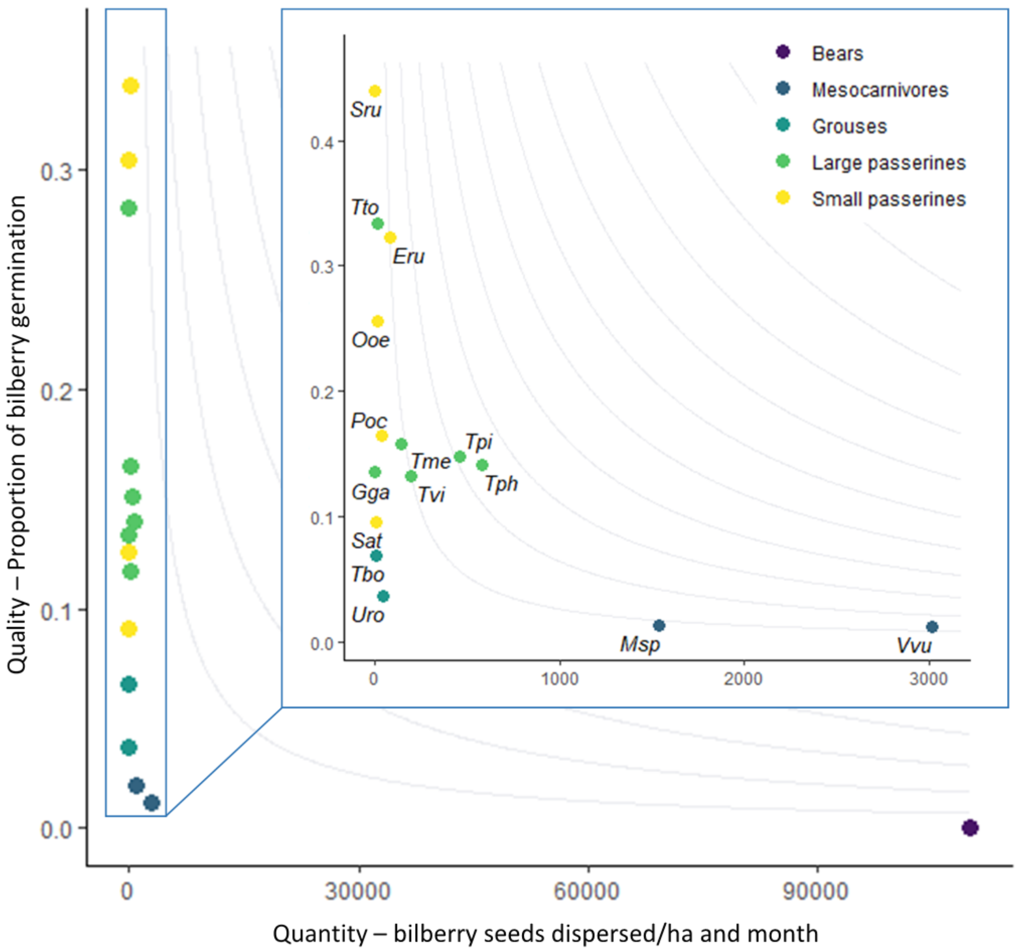


Figure S5. Altitudinal distribution (Y-axis: density; X-axis: elevation in meters above sea level -m.a.s.l.) of the bilberry dispersal service -based on the number of samples assigned to each species- provided by each frugivorous species dispersing the bilberry in Tatra National Park, Poland. For birds, distribution is based on the samples identified to species level by barcoding techniques.

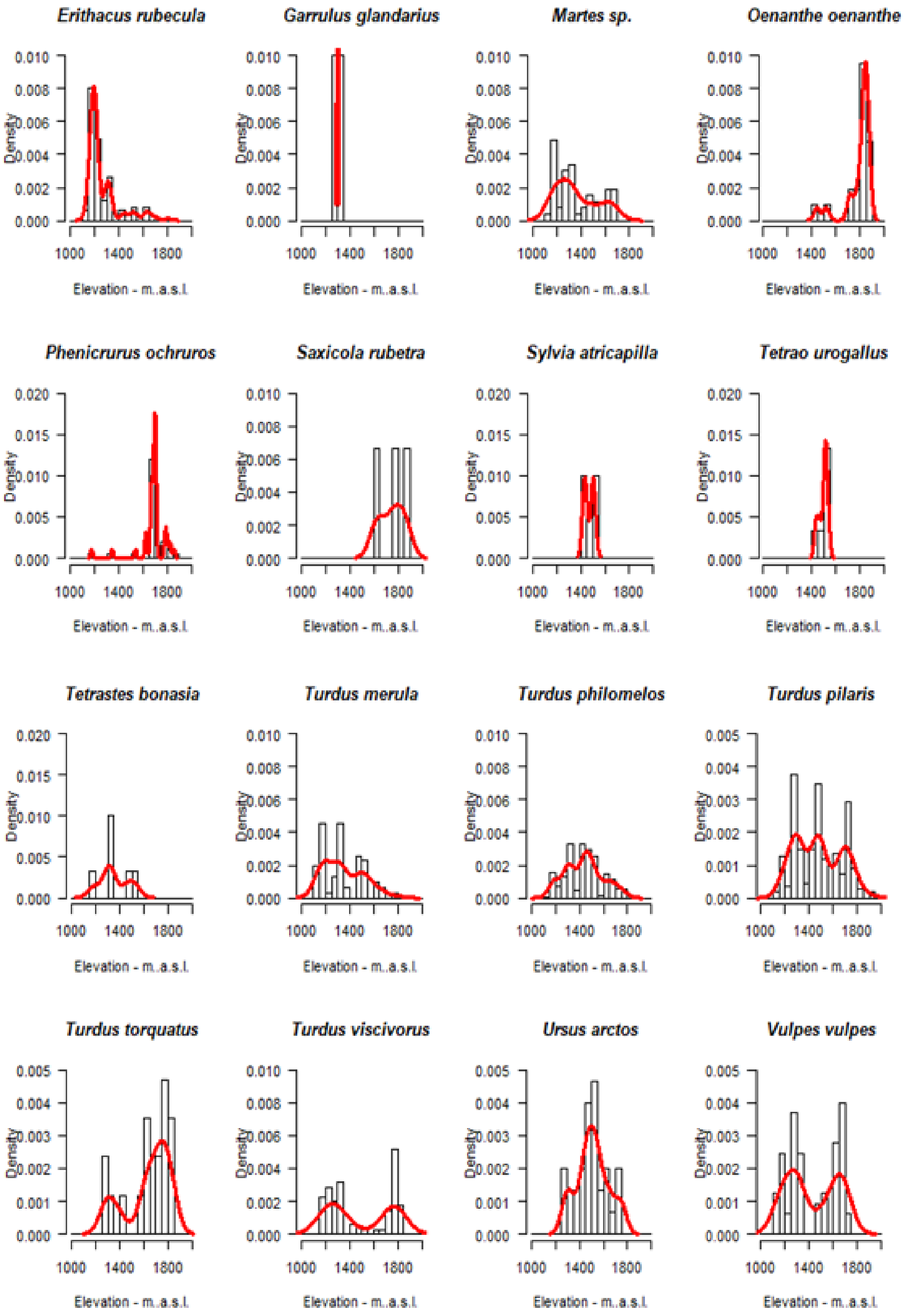


Table S1. Total number of samples collected and processed (i.e. samples whose seeds were counted and prepared for DNA extraction and amplification) for each transect inspection in 2017 and 2018. The percentage of samples processed among the total number collected in each transect inspection provided in brackets.

		2017		**2018**	
Transect	**Round**	**Samples collected**	**Samples processed (%)**	**Samples collected**	**Samples processed (%)**
1	July	39	30 (76.92%)	54	31 (57.41%)
1	August	66	34 (51.51%)	93	53 (56.99%)
1	September	85	51 (60%)	67	38 (56.72%)
1	October	36	31 (86.11%)	30	25 (83.33%)
2	July	30	22 (73.33%)	11	11 (100%)
2	August	46	34 (73.91%)	60	45 (75%)
2	September	53	32 (60.37%)	15	15 (100%)
2	October	34	26 (76.47%)	4	4 (100%)
3	July	12	12 (100%)	12	12 (100%)
3	August	18	18 (100%)	17	17 (100%)
3	September	35	28 (80%)	25	19 (76%)
3	October	22	21 (95.45%)	2	2 (100%)
4	July	59	31 (52.54%)	17	17 (100%)
4	August	85	50 (58.82%)	97	69 (71.13%)
4	September	95	51 (53.68%)	3	3 (100%)
4	October	75	43 (57.33%)	11	11 (100%)
5	July	11	11 (100%)	3	3 (100%)
5	August	28	23 (82.14%)	55	36 (65.45%)
5	September	37	32 (86.49%)	6	6 (100%)
5	October	53	34 (64.15%)	30	28 (93.33%)
6	July	38	33 (86.84%)	6	6 (100%)
6	August	46	38 (82.81%)	44	42 (95.45%)
6	September	38	31 (81.58%)	5	5 (100%)
6	October	98	51 (52.05%)	6	6 (100%)

Table S2. Number of samples processed (mammal scats visually identified and bird droppings from which we counted seeds prior DNA extraction), number of samples with bilberry seeds, number of bilberry seeds per scat/dropping (maximum, mean, standard deviation and median values), total number of seeds processed, total number of seeds dispersed (as a result of extrapolation, for more details please check methods section) by each frugivorous species dispersing the bilberry in Tatra National Park, Poland.

Species	No. samples processed	No. samples with seeds	Seeds per scat/dropping				Total processed seeds	Total samples with seeds	Total bilberry seeds
			Maximum	Mean	S.D.	Median			
Erithacus rubecula	130	129	56	5.45	6.60	4	709	232	1,242
Garrulus glandarius	2	2	44	23	NA	NA	46	2	46
Martes sp.	35	19	5,297	646	1,332	4	22,609	36	29,573
Oenanthe Oenanthe	21	21	34	8.38	8.23	7	176	32	278
Phoenicrurus ochruros	38	38	18	5.65	4.30	4	215	57	319
Saxicola rubetra	3	3	5	3.33	1.53	3	10	4	11
Sylvia atricapilla	2	2	84	48	NA	NA	86	3	134
Tetrao urogallus	6	6	651	122.69	258.88	19	736	6	736
Tetrastes bonasia	5	5	78	32.55	33.24	24	162	6	193
Turdus merula	62	62	126	20.12	20.51	15	1,248	106	2,144
Turdus philomelos	263	263	162	21.45	21.41	16	5,708	433	9,163
Turdus pilaris	216	215	115	21.62	19.75	17	4,735	358	7,655
Turdus torquatus	17	17	92	23.94	23.97	16	407	26	558
Turdus viscivorus	69	69	340	26.93	42.89	17.5	1,885	124	3,399
Ursus arctos	23	21	282,902	41,529	66,369	10,428	955,188	30	1.815,119
Vulpes vulpes	39	24	7,956	827	1,720	89	32,273	48	53,154

Table S3. Altitudinal distribution (minimum elevation, 1st quartile, median elevation, mean elevation, 3rd quartile, maximum elevation in meters above sea level -m.a.s.l.- and elevation gradient in meters) of the bilberry dispersal service provided by each frugivorous species -i.e. the number of samples assigned to each species by DNA barcoding or visual identification- dispersing the bilberry in Tatra National Park, Poland. The number of samples of each species used to check the altitudinal distribution also provided.

		Elevation						
Species	**Samples**	**Minimum (m.a.s.l.)**	**1st Quartile (m.a.s.l.)**	**Median (m.a.s.l.)**	**Mean (m.a.s.l.)**	**3rd Quartile (m.a.s.l.)**	**Maximum (m.a.s.l.)**	**Gradient (meters)**
Erithacus rubecula	129	1137	1187	1202	1264	1304	1808	671
Garrulus glandarius	2	1296	NA	NA	NA	NA	1304	8
Martes sp	19	1147	1198	1309	1364	1483	1699	552
Oenanthe oenanthe	21	1444	1781	1835	1792	1850	1871	427
Phoenicrurus ochruros	38	1175	1671	1698	1680	1700	1858	683
Saxicola rubetra	3	1626	NA	NA	NA	NA	1851	225
Sylvia atricapilla	2	1433	NA	NA	NA	NA	1503	70
Tetrao urogallus	6	1435	1477	1511	1495	1511	1534	99
Tetrastes bonasia	5	1181	1310	1314	1350	1420	1526	345
Turdus merula	62	1140	1199	1322	1350	1492	1788	648
Turdus philomelos	263	1140	1315	1444	1437	1538	1789	649
Turdus pilaris	215	1143	1298	1474	1476	1654	1919	776
Turdus torquatus	17	1284	1590	1704	1631	1754	1826	542
Turdus viscivorus	69	1158	1253	1342	1489	1763	1833	675
Ursus arctos	20	1289	1447	1511	1510	1582	1749	460
Vulpes vulpes	24	1140	1284	1322	1427	1635	1721	581

Female brown bear with two cubs feeding on bilberry fruits in the study area. Picture: Adam Wajrak

Constant gardeners: endozoochory promotes repeated seedling recruitment in the bilberry *Vaccinium myrtillus*

Submitted to *Biology Letters*

Alberto García-Rodríguez and Nuria Selva

ABSTRACT

The apparent rarity of bilberry seedlings within conspecific adults is an argument commonly used to propose the “recruitment at windows of opportunity” (RWO - spatially or temporally unpredictable conditions in which seedling recruitment may occur within conspecific adult stands) as the most plausible recruitment strategy for the species. However, this hypothesis does not account for the seed disperser community and has not been tested in natural conditions. We marked brown bear *Ursus arctos*, mesocarnivore and passerine faeces containing bilberry seeds in the Tatra Mountains (NW Carpathians, Poland) and followed the fate of the embedded seeds during two years. We detected bilberry germination associated to 100%, 87.5% and 50% of bear, mesocarnivore and passerine faeces, respectively, but also in 23.1% of the control plots located 30 m away from bear scats. In bear scats, studied in more detail, 15.7% of the seedlings survived at least one year and in 77.8% of the samples new seedlings germinated also in the second year. The largest numbers of seedlings were associated to bear scats (154.4±237.3 seedlings/m^2), particularly at resting sites. Our results demonstrate that repeated seedling recruitment, and not RWO, is the main bilberry recruitment strategy if frugivores are accounted for. Studies on reproductive strategies of clonal plants must not neglect the role of different seed disperser guilds.

KEYWORDS

Seed dispersal, clonal plants, recruitment at windows of opportunity, repeated seedling recruitment, germination, *Ursus arctos*, daybed.

INTRODUCTION

Seedling recruitment is rare in most clonal plants (Eriksson, 1992). However, different recruitment strategies in this group are known, ranging from species in which seedlings are established only during one initial period ("Initial seedling recruitment") to species presenting continuous recruitment within conspecific adults ("Repeated seedling recruitment"; Eriksson, 1992; Eriksson & Fröborg, 1996). Recruitment events are challenging to investigate in long-lived clonal plants because they can happen at intervals much longer than the average length of field studies but, still, they may be crucial for plant demography and gene flow (Eriksson & Fröborg, 1996). As a consequence, a third and intermediate strategy, called "recruitment at windows of opportunity" (RWO), was proposed for species in which recruitment within conspecific adults is limited spatially or temporally to unpredictable conditions that may happen more than once but at very low frequencies (Jelinski & Cheliak, 1992; Eriksson & Fröborg, 1996).

A classic example of a species following a RWO strategy is the bilberry *Vaccinium myrtillus* (Ericaceae; Eriksson & Fröborg, 1996), a clonal shrub widely distributed in Eurasian temperate and boreal regions (Ritchie, 1956) and a key food for many frugivores with different body sizes, from brown bears *Ursus arctos* to passerines (Honkavaara et al., 2007; García-Rodríguez et al., 2021). Despite their seeds being dispersed in high numbers, usually undamaged, by birds and mammals (Schaumann & Heinken, 2002; Honkavaara et al., 2007; García-Rodríguez et al., 2021), bilberry populations usually expand by clonal propagation, with seedling recruitment being rare within conspecific stands and usually restricted to open gaps with high moisture and organic soil content. Due to this, RWO was proposed as the bilberry' recruitment strategy (Eriksson & Fröborg, 1996). However, this hypothesis relies on sowing experiments and no study has accounted neither for the diverse guild of bilberry dispersers nor for the frequency of these windows of opportunity in natural ecosystems. Specific behaviors of frugivore species, such as defecation in the vicinity of resting sites in the case of bears ("daybeds" hereafter) or at marking points in the case of mesocarnivores, have been proposed as potentially important for bilberry seedling recruitment because they may direct seed dispersal to microhabitats suitable for germination (Schaumann et al., 2002; Steyaert et al., 2019; García-Rodríguez et al., 2021). Still, this hypothesis has never been tested in the field.

Here we challenge the hypothesis that bilberry seedling recruitment always occurs at very low frequencies in natural conditions and, thus, that the species follows a RWO strategy. We assessed bilberry recruitment in a natural ecosystem with a diverse community of dispersers and evaluated how endozoochory by the main disperser guilds in the area -brown bears, mesocarnivores and passerines- affects the frequency of bilberry recruitment.

METHODS

- **Study area**

The study was conducted in Tatra National Park, a 211 km^2 protected area located in the Tatra Mountains – Northwestern Carpathians (southern Poland). The area ranges from 774 to 2,499 metres above sea level (m.a.s.l.). Montane (below 1,550 m.a.s.l.) and subalpine (1,550–1,800 m.a.s.l.) areas are dominated by Norway spruce *Picea abies* and dwarf pine *Pinus mugo* formations, respectively. Montane, subalpine and alpine (up to 2,300 m.a.s.l.) floors are dominated by bilberry, which forms productive patches during the fruiting season, from July to October (up to 1,050 berries per m^2 in some areas, authors' unpublished data; Mirek & Piekos-Mirkowa, 1992). At least 16 frugivorous species consume bilberry fruits in the area, with small to medium-sized passerines (mostly Turdidae), mesocarnivores (red foxes *Vulpes vulpes* and marten *Martes spp*.) and especially brown bears dispersing the majority of seeds (authors' unpublished data).

- **Field sampling**

During the fruiting seasons of 2018 and 2019 we marked 62 faeces containing bilberry seeds belonging to the three main bilberry disperser guilds in the area (33 brown bear and 17 mesocarnivore scats, 12 passerine droppings). All mesocarnivore and passerine faeces and 21 bear scats were found during periodical inspections of animal paths, whereas 12 bear scats were marked at daybeds (n=5) used by GPS-collared bears. Following Steyaert et al. [8], we defined bear daybeds as small dug-out areas with signs of bear presence (scats, hairs), located in areas where bear's GPS relocations clustered for at least 4 h within a range of 50 m. We put each mammal scat in a plastic bag and weighted it with the help of a spring scale. After that, we put each sample back at its original location. We assigned 0.1g as the weight of each passerine dropping based on previous research (authors' unpublished data). We delimited each faecal sample with ropes and metal nails stuck to the ground, recorded its GPS location and assigned an individual ID (Fig. S1).

We revisited each sample a year later, in September 2019 and 2020, using a metal detector to find the marking nails when needed. In each sample found (n = 59 out of 62), we delimited a 1m^2 plot ("sample plot" hereafter) centered at the sample location with the help of wooden measurement sticks. In 2019 we established six additional 1 m^2 "control plots" for 27 bear faecal samples found out of the 29 marked in 2018. Control plots were located at 5, 10 and 30 metres from the marked bear scats in two opposite directions from each sample plot. To avoid overlapping, we established control plots only in one direction from each sample plot when two or more scats were located less than 30 metres away from each other. Thus, for a given location we established one sample plot and a maximum of six controls. We recorded the number of new bilberry seedlings (younger than one year), the abundance of bilberry stands (percentage of ground covered by bilberry ramets), the canopy cover (visual estimate of the percentage of sky covered by tree canopy) and the predominant substrate (vegetation/bryophytes or denuded soil) in each sample and control plot. In 2020, and only for the bear scats marked in 2018, we distinguished new and one year-old seedlings based on morphological differences (new seedlings present entire leaf margins whereas one year-old seedlings already show the toothed shape common in adult shrubs; Fig. S2). In total, we counted bilberry seedling germination in 59 sample and 117 control plots, and seedling survival in 26 sample plots (one out of the 27 plots could not be found in 2020, Table S1).

- **Statistical analysis**

We used Kruskal-Wallis tests to check the effect of the disperser guild (bears, mesocarnivores and passerines) in the number of bilberry seedlings germinating per m^2 and per gram of fresh faeces separately. We performed Dunn tests for pairwise comparisons between guilds. We also used Kruskal-Wallis tests to explore differences in seedling numbers per m^2 and per gram of fresh scat between bear scats located in daybeds and in animal paths. We performed a Generalized Linear Mixed Model (GLMM) fitted to a binary distribution to analyze the effect of the distance to the bear scats on the probability of seedling germination (absence/presence in $1m^2$ plots) and a zero-inflated GLMM fitted to Poisson distribution to analyze the effect of distance on the number of seedlings germinated per m^2. We included data from both sample (only bears, distance = 0 m) and control plots and used their location as a random factor in both models. In the case of bear scats, we performed two Generalized Linear Models (GLMs), fitted to a Poisson and a gamma distribution, to check the combined effects of bilberry stands' abundance, canopy cover and type of substrate in the number of seedlings per m^2 and per gram of fresh scat, separately. We used GLMs fitted to a binomial distribution to assess the proportion of seedlings surviving the first winter after germination in bear scats according to the number of seedlings germinated the previous year per m^2 and per gram of fresh scat, separately. We performed all the analyses in R environment, version 3.4.0 (R Development Core Team, 2017), using the *dunn.test* and *glmmTMB* packages for the implementation of the Dunn tests and zero inflated mixed models (Dinno, 2017; Magnusson et al., 2017).

RESULTS

We found bilberry germination in 100%, 87.5% and 50% of the sample plots associated to bear, mesocarnivore and passerine faeces, respectively. New germination occurred in 77.8% of two-year old bear scats. We detected bilberry germination in 35.9% of controls (43.6%, 41.2% and 23.1% in plots 5, 10 and 30 m away from bear scats, respectively; Table S1). The disperser guild influenced the number of seedlings germinated per m^2

(Kruskal-Wallis chi^2 = 23.374, *p*-value < 0.001; Fig. 1a). The largest numbers of seedlings were associated to bear scats (154.4±237.3 seedlings/m^2), followed by mesocarnivores (18.3±24.4 seedlings/m^2; Fig.1a; Table S2). The number of seedlings germinated per gram in passerine droppings (17.5±31.1 seedlings/g) was larger than in mesocarnivore or bear scats (1.6±1.8 and 0.2±0.2 seedlings/g; Kruskal-Wallis chi^2 = 6.898, *p*-value = 0.032; Fig. 1b; Table S2). More seedlings germinated from bear scats in daybeds than in animal paths, both per m^2 (360.83±277.22 vs 24.05±23.21 seedlings/m^2; Kruskal-Wallis chi^2 = 12.002, *p*-value < 0.001) and per gram of scat (0.32±0.26 vs 0.13±0.13 seedlings/g; Kruskal-Wallis chi^2 = 5.486, *p*-value = 0.019; Fig. 1). Distance to bear scats negatively affected the probability of bilberry germination, but not the number of seedlings per m^2 (Fig. 2, Table S3). Larger abundance of bilberry shrubs, lower canopy covers and denuded soil were associated to larger numbers of seedlings per m^{2,} whereas only the substrate had an effect in seedling germination per gram of fresh scat (Table S4). We detected seedling survival in 84.6% of the bear scats monitored for two years, specifically 91.7% and 78.6% in scats located in daybeds and in animal paths, respectively. In total, 15.7% of all seedlings germinated in 2019 from bear scats were alive in 2020 (Table S1). Neither the number of seedlings per m^2 nor per gram of fresh scat influenced survival rates of one year-old seedlings dispersed by bears (Table S5).

DISCUSSION

Here we have shown that bilberry recruitment is a widespread phenomenon in natural ecosystems when associated to endozoochory and, thus, put in question the RWO as the main recruitment strategy for the species. We detected thousands of bilberry seedlings germinating and surviving from just an insignificant subset of all faeces defecated by frugivores in the study area, which suggests that actual numbers of bilberry seedlings recruited every year are several orders of magnitude higher than the numbers we detected. In line with this prediction, previous research conducted in the area found that more than 50,000 bilberry seeds are dispersed by frugivores per hectare and month upon substrates suitable for bilberry germination (decaying wood, bare soil; authors' unpublished data). According to these numbers, and given that the probability of a bilberry seed becoming an established adult when deposited upon a suitable substrate is around 0.3% (Eriksson & Fröborg, 1996), a minimum of 150 of all bilberry seeds dispersed by frugivores per hectare and month would become part of adult bilberry populations across the entire area. This indicates that bilberry recruitment is not restricted spatially or temporally, even in areas where bilberry stands are common, which implies that recruitment occurs at frequencies much larger than assumed by RWO. Thus, RWO may not be the most appropriate hypothesis of recruitment patterns in bilberry if frugivores are accounted for.

Specific behaviors of frugivores, particularly bear defecation next to their dug-out resting sites and mesocarnivore marking in animal paths (Schaumann & Heinken, 2002), can direct seed dispersal to specific locations suitable for bilberry recruitment. Interestingly, we found bilberry seeds germinating after two years embedded within bear scats, whereas manually extracted bilberry seeds cannot germinate after 45 weeks under controlled moisture conditions (Ranwala & Naylor, 2004). This suggests that the faecal envelope, especially in large faeces as bears', may protect bilberry seeds and allow longer seed viability. We found bilberry seedlings in a third of the control plots, which we believe is most likely a consequence of the presence of passerine droppings scattered throughout the area. The spatiotemporal complementarity of seed dispersal services provided by birds and mammals in the Tatra Mountains, which guarantees the arrival of bilberry seeds to different microsites across the entire fruiting season (authors' unpublished data), together with a good bilberry germination and survival rate from faeces, suggests a repeated seedling recruitment, and not RWO, as the main recruitment strategy for the bilberry in the area. We believe this might also apply to other areas inhabited by a diverse and rich community of dispersers.

Our results point out that endozoochory must be considered when defining plant recruitment strategies in clonal plants. Besides the important contribution of mammals to long-distance seed dispersal events, small birds are also important dispersers which usually mobilize seeds over short distances (Jordano et al., 2007). The relevance of these short-distance dispersal events, together with the small seed size, are two traits usually associated to clonal species showing a repeated seedling recruitment strategy (Eriksson, 1992). As sibling and adult competition is usually stronger next to parent plants, different dispersal strategies might be optimal depending on dispersal distances. For instance, bilberry recruitment far away from parent plants, where sibling competition is expected to be low, might be enhanced by mammal scats containing large seed loads, whereas recruitment next to their parents might benefit from the scattered deposition of many passerine droppings, which usually contains small numbers of seeds. Short-distance seed dispersal may complement clonal propagation by increasing genet numbers, which reduces extinction risk and promotes the persistence of specific genotypes within a population (Eriksson, 1992). In addition to frugivore effects, natural disturbances such as wind storms or bark beetle outbreaks, relevant components of boreal and temperate forest dynamics, may also facilitate bilberry recruitment by increasing the availability of suitable substrates for bilberry establishment (decaying wood or dug-out soil; Eriksson and Fröborg, 1996). This study demonstrates that preserving an intact community of frugivores is essential to guarantee repeated bilberry seedling recruitment and that endozoochory-mediated seed dispersal is an essential component in the assessment of the recruitment strategies of clonal plants.

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AUTHORS' CONTRIBUTIONS

AGR and NS conceived the study, designed the methodology and conducted the fieldwork. AGR performed all statistical analyses and wrote a first draft of the manuscript with significant contributions of NS.

ADDITIONAL INFORMATION

Data availability. The dataset used for this article is provided in Table S1.

Competing interests: The authors declare that they have no competing interests.

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Figure 1. Bilberry recruitment mediated by passerines (n = 12 faeces), mesocarnivores (n = 16) and brown bears (n = 31) indicated as number of seedlings germinated (a) per m^2 and (b) per gram of fresh faeces. Red dots and arrows represent mean values and standard errors, and grey dots the actual observed values. Embedded figures represent the numbers associated to bear scats located in daybeds (n = 12; triangles) and in animal paths (n = 19; circles). Please, note that the Y-axes are represented in different scales.

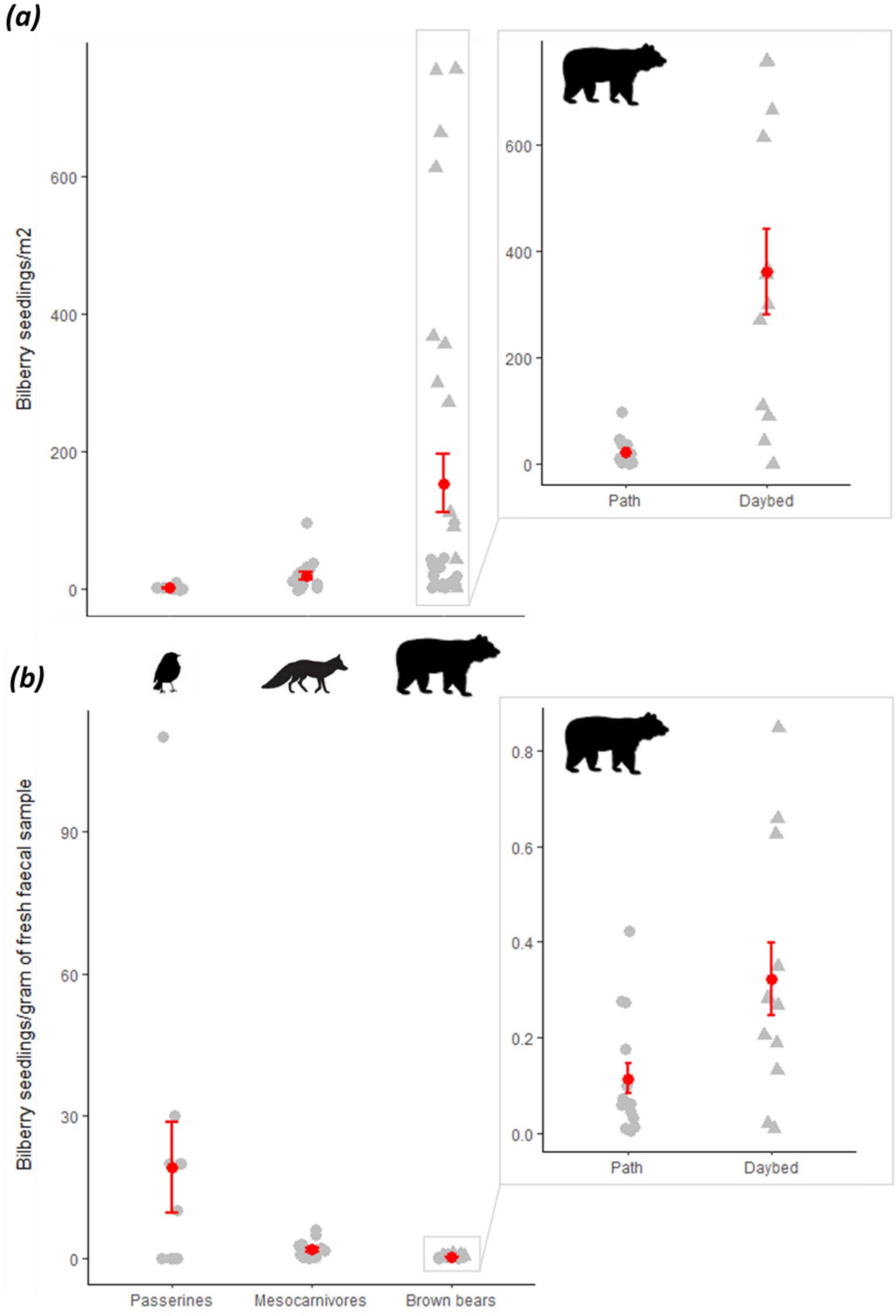
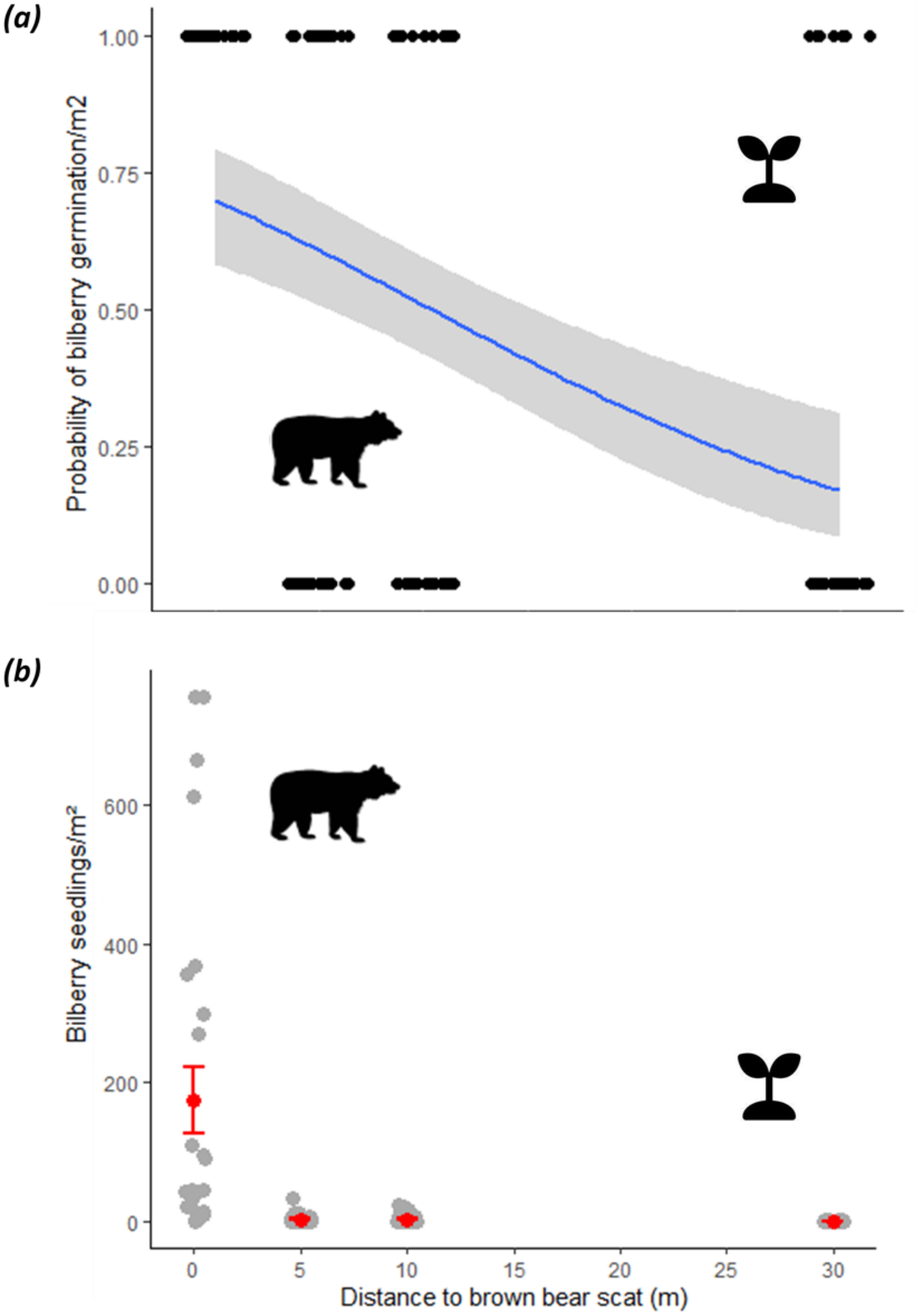


Figure 2. Effects of the distance to brown bear scats on (a) the probability of bilberry germination and (b) the number of bilberry seedlings germinated per m^2. In (a) the blue line and grey-shaded area represent mean and standard error model predictions, and black dots the actual observed values. In (b) red dots and arrows represent mean values and standard errors, and grey dots the actual observed values (distance = 0 represents brown bear scat location).



Supplementary material for:

Constant gardeners: endozoochory promotes repeated seedling recruitment in the bilberry *Vaccinium myrtillus*

Submitted

Alberto García-Rodríguez and Nuria Selva

Figure S1. Picture showing a brown bear scat marked for this study. Bear, mesocarnivore and passerines faeces were delimited with ropes and metal nails stuck to the ground. Picture: Alberto García-Rodríguez.

Figure S2. Pictures showing bilberry seedlings germinating from brown bear scats in the Tatra Mountains (southern Poland). In the upper picture morphological differences between new (narrow leaves with entire margins; yellow circle) and one-year old (wide leaves the toothed margins; red circle) bilberry seedlings are shown. Pictures: Alberto García-Rodríguez and Nuria Selva.

Table S1. Dataset used in this study. The table provides information about the plot ID, location ID (only for bear scats and associated control plots), distance to the marked faeces in metres, canopy cover (visual estimate of the percentage of sky covered by tree canopy), bilberry abundance (percentage of ground covered by bilberry ramets), predominant substrate (vegetation/bryophytes or denuded soil), weight of the faeces in grams, number of new seedlings germinated in one-year old faeces ("New seedlings year 1"), number of seedlings surviving one year after germination ("Surviving seedlings year 1 to 2") and number of new bilberry seedlings germinated from two-year old faeces (only for brown bear scats marked in 2018, which includes Bears–path 1 to Bears–path 15 and Bears–daybed 1 to Bears–daybed 12). NA indicates data not available. Two brown bear and one mesocarnivore scats marked were not found the year after marking them and, thus, they are not included in the analyses.

Plot ID	**Location ID**	**Distance to faeces (m)**	**Canopy cover (%)**	**Bilberry abundance (%)**	**Predominant substrate**	**Weight (g)**	**New seedlings year 1**	**Surviving seedlings year 1 to 2**	**New seedlings year 2**
Bears – path 1	1	0	70	0	Disturbed soil	33	9	9	20
Bears – path 2	2	0	50	0	Disturbed soil	40	11	3	12
Bears – path 3	3	0	0	60	Vegetation	67	3	NA	NA
Bears – path 4	4	0	0	10	Vegetation	81	5	2	1
Bears – path 5	5	0	50	25	Disturbed soil	620	19	9	8
Bears – path 6	6	0	50	20	Vegetation	325	37	1	0
Bears – path 7	7	0	15	0	Vegetation	625	46	5	37
Bears – path 8	8	0	50	10	Disturbed soil	740	45	4	42
Bears – path 9	9	0	30	5	Vegetation	555	97	21	51
Bears – path 10	10	0	10	20	Vegetation	90	38	18	46
Bears – path 11	11	0	0	10	Vegetation	190	2	0	0
Bears – path 12	12	0	0	80	Vegetation	660	8	2	4
Bears – path 13	13	0	20	20	Vegetation	225	22	0	6
Bears – path 14	14	0	50	0	Disturbed soil	240	14	1	0
Bears – path 15	15	0	0	0	Vegetation	1,050	4	0	0
Bears – path 16	NA	0	0	30	Vegetation	50	6	NA	NA
Bears – path 17	NA	0	0	40	Vegetation	250	38	NA	NA
Bears – path 18	NA	0	0	30	Vegetation	70	33	NA	NA
Bears – path 19	NA	0	0	70	Vegetation	230	20	NA	NA
Bears – daybed 1	16	0	60	0	Disturbed soil	139	1	0	0
Bears – daybed 2	17	0	60	60	Disturbed soil	1,020	357	13	1,078
Bears – daybed 3	18	0	30	30	Disturbed soil	890	755	19	512
Bears – daybed 4	19	0	30	50	Disturbed soil	1,060	664	4	242
Bears – daybed 5	20	0	40	50	Disturbed soil	930	613	40	777
Bears – daybed 6	21	0	50	40	Disturbed soil	2,820	367	18	335
Bears – daybed 7	22	0	60	35	Disturbed soil	320	91	2	32
Bears – daybed 8	23	0	40	0	Disturbed soil	2,700	757	11	231

Bears – daybed 9	24	0	50	0	Disturbed soil	1,010	271	6	78
Bears – daybed 10	25	0	50	0	Disturbed soil	1,460	300	69	82
Bears – daybed 11	26	0	65	0	Disturbed soil	590	111	19	83
Bears – daybed 12	27	0	60	0	Disturbed soil	2,310	43	1	3
Mesocarnivores 1	NA	0	50	20	Vegetation	24	0	NA	NA
Mesocarnivores 2	NA	0	60	20	Disturbed soil	7	6	NA	NA
Mesocarnivores 3	NA	0	60	5	Disturbed soil	21	8	NA	NA
Mesocarnivores 4	NA	0	60	0	Disturbed soil	7	21	NA	NA
Mesocarnivores 5	NA	0	30	0	Disturbed soil	5	30	NA	NA
Mesocarnivores 6	NA	0	0	5	Disturbed soil	20	33	NA	NA
Mesocarnivores 7	NA	0	50	15	Vegetation	18	2	NA	NA
Mesocarnivores 8	NA	0	60	0	Disturbed soil	10	25	NA	NA
Mesocarnivores 9	NA	0	50	0	Disturbed soil	15	9	NA	NA
Mesocarnivores 10	NA	0	70	0	Vegetation	10	3	NA	NA
Mesocarnivores 11	NA	0	40	30	Vegetation	7	0	NA	NA
Mesocarnivores 12	NA	0	50	10	Disturbed soil	5	11	NA	NA
Mesocarnivores 13	NA	0	0	0	Disturbed soil	34	9	NA	NA
Mesocarnivores 14	NA	0	0	0	Vegetation	14	1	NA	NA
Mesocarnivores 15	NA	0	0	30	Disturbed soil	20	97	NA	NA
Mesocarnivores 16	NA	0	0	30	Disturbed soil	15	38	NA	NA
Passerines 1	NA	0	0	90	Vegetation	0.1	0	NA	NA
Passerines 2	NA	0	40	10	Disturbed soil	0.1	0	NA	NA
Passerines 3	NA	0	0	80	Vegetation	0.1	0	NA	NA
Passerines 4	NA	0	50	10	Disturbed soil	0.1	2	NA	NA
Passerines 5	NA	0	0	80	Disturbed soil	0.1	0	NA	NA
Passerines 6	NA	0	30	10	Disturbed soil	0.1	11	NA	NA
Passerines 7	NA	0	0	80	Vegetation	0.1	0	NA	NA
Passerines 8	NA	0	0	85	Vegetation	0.1	0	NA	NA
Passerines 9	NA	0	60	10	Disturbed soil	0.1	3	NA	NA
Passerines 10	NA	0	60	0	Disturbed soil	0.1	1	NA	NA
Passerines 11	NA	0	70	25	Disturbed soil	0.1	2	NA	NA
Passerines 12	NA	0	50	0	Disturbed soil	0.1	2	NA	NA
Control 1	1	5	60	0	Disturbed soil	NA	0	NA	NA
Control 2	1	5	50	0	Disturbed soil	NA	1	NA	NA
Control 3	1	10	50	0	Disturbed soil	NA	0	NA	NA
Control 4	1	10	60	0	Disturbed soil	NA	0	NA	NA
Control 5	1	30	50	10	Disturbed soil	NA	0	NA	NA
Control 6	1	30	40	0	Disturbed soil	NA	1	NA	NA
Control 7	2	5	50	0	Vegetation	NA	0	NA	NA
Control 8	2	5	40	0	Vegetation	NA	2	NA	NA
Control 9	2	10	40	0	Vegetation	NA	0	NA	NA
Control 10	2	10	40	0	Vegetation	NA	0	NA	NA
Control 11	2	30	30	0	Vegetation	NA	0	NA	NA
Control 12	2	30	70	10	Vegetation	NA	0	NA	NA
Control 13	3	5	0	95	Vegetation	NA	0	NA	NA

Control 14	3	10	0	15	Vegetation	NA	0	NA	NA
Control 15	3	30	0	10	Vegetation	NA	0	NA	NA
Control 16	4	5	0	25	Vegetation	NA	0	NA	NA
Control 17	4	10	0	60	Vegetation	NA	0	NA	NA
Control 18	4	30	0	100	Vegetation	NA	0	NA	NA
Control 19	5	5	50	10	Vegetation	NA	0	NA	NA
Control 20	5	5	40	10	Vegetation	NA	2	NA	NA
Control 21	5	10	70	0	Vegetation	NA	0	NA	NA
Control 22	5	10	40	10	Vegetation	NA	0	NA	NA
Control 23	5	30	60	0	Vegetation	NA	0	NA	NA
Control 24	5	30	60	0	Vegetation	NA	0	NA	NA
Control 25	6	5	50	5	Disturbed soil	NA	0	NA	NA
Control 26	6	5	70	20	Disturbed soil	NA	0	NA	NA
Control 27	6	10	60	10	Disturbed soil	NA	0	NA	NA
Control 28	6	10	50	20	Disturbed soil	NA	0	NA	NA
Control 29	6	30	70	10	Disturbed soil	NA	0	NA	NA
Control 30	6	30	50	10	Disturbed soil	NA	0	NA	NA
Control 31	7	5	10	0	Vegetation	NA	0	NA	NA
Control 32	7	5	15	0	Vegetation	NA	0	NA	NA
Control 33	7	10	0	50	Vegetation	NA	16	NA	NA
Control 34	7	10	5	5	Vegetation	NA	0	NA	NA
Control 35	7	30	0	15	Vegetation	NA	0	NA	NA
Control 36	7	30	25	15	Vegetation	NA	0	NA	NA
Control 37	8	5	50	0	Vegetation	NA	0	NA	NA
Control 38	8	5	50	5	Vegetation	NA	0	NA	NA
Control 39	8	10	60	15	Vegetation	NA	0	NA	NA
Control 40	8	10	50	15	Vegetation	NA	0	NA	NA
Control 41	8	30	40	30	Vegetation	NA	0	NA	NA
Control 42	8	30	40	0	Vegetation	NA	0	NA	NA
Control 43	9	5	40	0	Vegetation	NA	0	NA	NA
Control 44	9	5	20	0	Vegetation	NA	0	NA	NA
Control 45	9	10	10	0	Vegetation	NA	0	NA	NA
Control 46	9	10	0	0	Vegetation	NA	0	NA	NA
Control 47	9	30	0	0	Vegetation	NA	0	NA	NA
Control 48	9	30	15	0	Vegetation	NA	0	NA	NA
Control 49	10	5	10	5	Vegetation	NA	1	NA	NA
Control 50	10	10	10	5	Vegetation	NA	3	NA	NA
Control 51	10	30	20	1	Vegetation	NA	1	NA	NA
Control 52	11	5	10	5	Vegetation	NA	0	NA	NA
Control 53	11	10	10	5	Vegetation	NA	1	NA	NA
Control 54	11	30	20	0	Vegetation	NA	0	NA	NA
Control 55	12	5	0	90	Vegetation	NA	1	NA	NA
Control 56	12	10	0	95	Vegetation	NA	0	NA	NA
Control 57	12	30	0	10	Vegetation	NA	0	NA	NA
Control 58	13	5	0	65	Vegetation	NA	6	NA	NA

Control 59	13	10	0	80	Vegetation	NA	5	NA	NA
Control 60	13	30	0	50	Vegetation	NA	0	NA	NA
Control 61	14	5	10	20	Vegetation	NA	6	NA	NA
Control 62	14	5	15	0	Vegetation	NA	3	NA	NA
Control 63	14	10	5	5	Vegetation	NA	0	NA	NA
Control 64	14	10	15	0	Vegetation	NA	0	NA	NA
Control 65	14	30	5	10	Vegetation	NA	1	NA	NA
Control 66	14	30	10	0	Vegetation	NA	2	NA	NA
Control 67	15	5	50	0	Vegetation	NA	0	NA	NA
Control 68	15	5	0	0	Vegetation	NA	0	NA	NA
Control 69	15	10	40	0	Vegetation	NA	1	NA	NA
Control 70	15	10	0	0	Vegetation	NA	1	NA	NA
Control 71	15	30	40	30	Vegetation	NA	0	NA	NA
Control 72	15	30	20	15	Vegetation	NA	2	NA	NA
Control 73	16	5	70	10	Disturbed soil	NA	0	NA	NA
Control 74	16	5	60	15	Disturbed soil	NA	0	NA	NA
Control 75	16	10	60	70	Disturbed soil	NA	0	NA	NA
Control 76	16	10	50	15	Disturbed soil	NA	0	NA	NA
Control 77	16	30	40	0	Disturbed soil	NA	0	NA	NA
Control 78	16	30	0	100	Disturbed soil	NA	0	NA	NA
Control 79	17	5	60	5	Disturbed soil	NA	5	NA	NA
Control 80	17	10	15	30	Vegetation	NA	7	NA	NA
Control 81	17	30	20	50	Vegetation	NA	0	NA	NA
Control 82	18	5	40	50	Disturbed soil	NA	6	NA	NA
Control 83	18	10	40	20	Vegetation	NA	3	NA	NA
Control 84	18	30	60	20	Disturbed soil	NA	0	NA	NA
Control 85	19	5	50	35	Disturbed soil	NA	0	NA	NA
Control 86	19	10	25	60	Vegetation	NA	4	NA	NA
Control 87	19	30	70	5	Disturbed soil	NA	2	NA	NA
Control 88	20	5	50	10	Disturbed soil	NA	4	NA	NA
Control 89	20	10	20	40	Vegetation	NA	23	NA	NA
Control 90	20	30	60	30	Disturbed soil	NA	0	NA	NA
Control 91	21	5	60	29	Disturbed soil	NA	11	NA	NA
Control 92	21	10	60	35	Vegetation	NA	7	NA	NA
Control 93	21	30	50	0	Disturbed soil	NA	1	NA	NA
Control 94	22	5	50	25	Vegetation	NA	11	NA	NA
Control 95	22	10	20	70	Vegetation	NA	17	NA	NA
Control 96	22	30	60	60	Disturbed soil	NA	2	NA	NA
Control 97	23	5	30	20	Vegetation	NA	35	NA	NA
Control 98	23	10	40	5	Disturbed soil	NA	3	NA	NA
Control 99	23	30	30	80	Vegetation	NA	0	NA	NA
Control 100	24	5	50	10	Vegetation	NA	8	NA	NA
Control 101	24	10	50	60	Vegetation	NA	0	NA	NA
Control 102	24	30	50	30	Vegetation	NA	1	NA	NA
Control 103	25	5	50	20	Vegetation	NA	6	NA	NA

Control 104	25	10	50	10	Disturbed soil	NA	22	NA	NA
Control 105	25	30	50	25	Vegetation	NA	0	NA	NA
Control 106	26	5	45	10	Disturbed soil	NA	1	NA	NA
Control 107	26	5	55	0	Disturbed soil	NA	0	NA	NA
Control 108	26	10	40	0	Disturbed soil	NA	1	NA	NA
Control 109	26	10	50	0	Disturbed soil	NA	2	NA	NA
Control 110	26	30	50	0	Disturbed soil	NA	0	NA	NA
Control 111	26	30	45	0	Disturbed soil	NA	0	NA	NA
Control 112	27	5	70	0	Disturbed soil	NA	0	NA	NA
Control 113	27	5	60	0	Disturbed soil	NA	0	NA	NA
Control 114	27	10	70	10	Disturbed soil	NA	0	NA	NA
Control 115	27	10	70	10	Disturbed soil	NA	0	NA	NA
Control 116	27	30	60	15	Disturbed soil	NA	0	NA	NA
Control 117	27	30	80	0	Disturbed soil	NA	0	NA	NA

Table S2. Summary statistics of the Dunn tests performed to check differences in the number of bilberry seedlings per m^2 and per gram of fresh faecal sample between different disperser guilds. Asterisks show significant p-values < 0.05.

	Comparison of disperser guilds		**z**	***p-value***
Number of bilberry seedlings/m^2				
	Passerines	Mesocarnivores	-2.498	0.037*
	Passerines	Brown bears	-2.766	<0.001*
	Mesocarnivores	Brown bears	-2.165	0.091
Number of bilberry seedlings/gram of fresh faecal sample				
	Passerines	Mesocarnivores	-0.939	1
	Passerines	Brown bears	1.246	0.638
	Mesocarnivores	Brown bears	2.575	0.030*

Table S3. Summary statistics of the Generalized Linear Mixed Models performed to test the effects of the distance to the brown bear scats (m) on the probability of bilberry germination per m^2 (fitted to a binomial distribution) and on the number of bilberry seedlings per m^2 (fitted to a Poisson distribution and accounting for zero-inflation). Location was included as a random factor. Asterisks show significant p-values < 0.05.

	Probability of bilberry seedling germination/m^2 (Binomial)				**Number of bilberry seedlings/m^2 (Zero-inflated Poisson)**			
Fixed factors	**Estimate**	**St. Error**	**z**	***p-value***	**Estimate**	**St. Error**	**z**	***p-value***
Intercept	1.220	0.414	2.948	0.003*	-1.397	0.395	-3.540	<0.001*
Distance	-0.100	0.022	-4.442	<0.001*	-0.029	0.040	-0.733	0.464
Random factor	**Variance**	**St. Deviation**			**Variance**	**St. Deviation**		
Location	1.408	1.187			3.372	1.836		

Table S4. Summary statistics of the Generalized Linear Models performed to test the effects of bilberry abundance (percentage of ground covered by bilberry ramets), predominant substrate (vegetation/bryophytes or denuded soil) and canopy cover (visual estimate of the percentage of sky covered by tree canopy) in the number of bilberry seedling germinated per m^2 (fitted to a Poisson distribution) and per gram of fresh brown bear scat (fitted to a Gamma distribution). Asterisks show significant p-values < 0.05.

	Number of bilberry seedlings/m^2 (Poisson)				**Number of bilberry seedlings/g of fresh bear scat (Gamma)**			
	Intercept	**St. Error**	**z value**	***p-value***	**Intercept**	**St. Error**	**t value**	***p-value***
Intercept	7.827	0.075	104.58	<0.001	1.117	3.805	0.294	0.772
Bilberry abundance	0.009	0.001	12.39	<0.001*	-0.042	0.039	-1.057	0.301
Substrate (vegetation)	-4.443	0.083	-53.07	<0.001*	9.059	3.861	2.346	0.028*
Canopy cover	-0.052	0.001	-36.34	<0.001*	0.079	0.070	1.131	0.270

Table S5. Summary statistics of the Generalized Linear Models performed to test the effects of the number of bilberry seedlings germinated per m^2 and per gram of fresh bear scat on the probability of bilberry seedlings survival. Both models are fitted to a binomial distribution. Asterisks show significant p-values < 0.05.

		Intercept	**St. Error**	**z value**	***p-value***
Number of bilberry seedlings per m^2					
	Intercept	-1.259	0.631	-1.995	0.046*
	Seedlings/m^2	-0.004	0.004	-0.887	0.375
Number of bilberry seedling per gram of fresh bear scat					
	Intercept	-1.651	0.756	-2.184	0.029*
	Seedlings/g	-0.189	2.552	-0.074	0.941

CONCLUSIONS

Female brown bear with two cubs feeding on bilberry fruits in the study area. Picture: Adam Wajrak

- Fleshy fruits are key food resources for brown bears in all biomes where the species is present, representing a quarter of the total volume consumed. Brown bears eat more than a hundred species of fleshy fruits worldwide. Among them, species belonging to *Rubus*, *Empetrum* and *Vaccinium* are the most commonly consumed. These genera typically form dense vegetation layers at ground level with exceptionally high local fruit abundances, which makes them attractive resources easy to access.

- The bilberry is the second most frequently fleshy-fruited plant species consumed by brown bears worldwide and it is an essential food for most bear populations inhabiting temperate and boreal regions of Eurasia. At local scale, the bilberry is the food item most commonly consumed by Tatra brown bears, present in 42% of all faeces analyzed and during the entire activity period of bears in the region. Additionally, the second most commonly food item consumed by bears in the region throughout the year is also a fleshy-fruited plant species, the raspberry, indicating the vital importance of fleshy fruits for Tatra brown bears. Fleshy fruits, and more specifically bilberry fruits, are especially important for brown bears during hyperphagia (detected in up to 80% of bear faeces collected in August in Tatra National Park), a sensitive period of the feeding annual cycle of bears, when they must fill the energetic demands needed to survive the winter hibernation.

- Brown bears are important bilberry dispersers quantitatively. At local scale, they are responsible of the majority of the seed dispersal in Tatra National Park (up to 85,000 and 110,000 bilberry seeds per ha and month in alpine meadows and coniferous forests, respectively). Brown bears complement the seed dispersal services provided by other important frugivores. For instance, the majority of the bilberry seed dispersal provided by brown bears in Tatra National Park occurs at the end of the bilberry season, when most migrant passerines have already left.

- Brown bear faeces may contain up to several hundred thousand bilberry seeds (mean and maximum detected in Tatra National Park = around 90,000 and 490,000 seeds per faecal sample, respectively). Seeds defecated by brown bears germinate in controlled conditions at higher percentages than those embedded within fruits and at similar percentages than those extracted manually. This also applies for the specific case of bilberry seeds, indicating that, from the bilberry seed perspective, it is positive to be eaten by a brown bear. Bilberry seedling germination in nature is larger associated to brown bear faeces (mean and maximum detected in Tatra National Park = 154 and 1,078 emerging seedlings/m^2, respectively) than associated to mesocarnivore and bird droppings and in control plots. Bilberry seedlings germinating from brown bear faeces may survive in important numbers: seedling survival was detected in 85% of brown bear faeces marked and 16% of all seedlings survived for at least one year. Thus, brown bear faeces provide conditions suitable for bilberry recruitment. Brown bears facilitate bilberry recruitment by defecating in the vicinity of their resting sites. The small excavations and soil removal bears do in these places create conditions suitable for bilberry seedling establishment.

- Human presence *per se* does not necessarily compromise the seed dispersal services provided by brown bears. However, effective management strategies, especially those focused on the spatiotemporal predictability of human presence and on the control of waste disposal, artificial food provisioning to wildlife and the extraction of animal foods by humans (e.g. berry picking by humans), are highly recommended in order to guarantee these ecosystem services in the long term.

- All the above considered, brown bears must be recognized as effective seed dispersers, even in humanized areas. Additionally, they are among the few megafaunal species dispersing viable seeds in non-tropical areas, which implies that they are pivotal in providing long-distance seed dispersal events essential for the colonization of new areas and to enhance genetic diversity among plant populations. Consequently, the decline of these animals may compromise seed dispersal services and plant regeneration processes, altering the functioning of entire ecosystems.

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Female brown bear with two cubs feeding on bilberry fruits in the study area. Picture: Adam Wajrak

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I still get goose bumps when I remember some of the moments I have lived during the field work conducted for this PhD in Tatra National Park: the evening when 7 different bears appeared in the same valley in less than two hours, the marmots playing and taking sunbaths, a wolf crossing the path in front of me and disappearing in less than second like a ghost, the eternal whinnies of black woodpeckers within the spruce forest, my first male capercaillie flying two meters away from me and whose flapping sounded like a helicopter taking off, that roe deer intimidating us, barking and showing his antlers to us … These are only few of the many moments I lived. Many thanks not only to each of these moments, but also to each person who helped me in the field and lived these moments with me: Aida, Danusia, Olga, Marta, Kasia, Max, Carlos, Ismael and Szymon.

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