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Endozoochorous seed dispersal by cattle and horse in a spatially heterogeneous landscape

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Abstract

Seed dispersal has become an important issue in plant ecology and restoration management. In this paper we examined dung germinating seed content and seed deposition patterns of horses (Shetland and Konik breeds) and Scottish Highland cattle grazing two coastal dune nature reserves. Two times 2.5 l of fresh dung from each type of herbivore were collected during seven sessions in the main fruiting season. Dung samples were placed under greenhouse conditions after drying and cooling. Animal defecation patterns were derived from a study of herbivore activities during 6 h observation sessions 8 times a month. One hundred and seventeen plant species i.e. 27% of all species occurring in the study area, were recorded as seedlings emerging from the dung samples. The most abundantly and frequently recorded plant species were *Urtica dioica*, *Juncus* spp. and different species of *Poaceae* and *Caryophyllaceae*. In general seedling density is high (1158 seedlings/dung sample). Seedling density and species richness were further analysed in order to detect temporal variability and possible animal and site related characteristics. Dung deposition patterns reflect a non-random use of habitats and hence a non-random seed deposition among habitats. Calculated seed deposition per square meter ranged from a few (< 10 germinating seeds) to more than 100 in the most frequently selected habitats. From the herbivores' selective habitat use and their estimated mean retention time we can further assume their ability for inter-habitat endozoochorous seed dispersal. This characteristic of large herbivores is further discussed in the light of nature management and restoration.

Introduction

Plant diversity is affected by herbivores through their impact on dominant plant species, plant regeneration opportunities and propagule transport (Olff and Ritchie 1998). The latter has become an important issue in plant ecology in general (Primack and Miao 1992) and restoration management in particular. The re-establishment of characteristic semi-natural plant communities sometimes fails due to unsuitable abiotic conditions for the target species or because of biotic constraints (Bakker 1998; Bakker and Berendse 1999). Many plant species cannot rely on a long-term persistent seedbank for regeneration after their disappearance from the relict vegetation. Seed dispersal then becomes a serious bottle-neck in restoration management (Verhagen et al. 2001; Pywell et al. 2002). Therefore studies on possible seed dispersal mechanisms are of key interest in the understanding of

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the colonisation abilities of plants at the landscape scale. Despite the increasing demand for reliable autoecological information on dispersal related topics of the temperate, native European flora most of this information is still anecdotal (Grime et al. 1988). It certainly cannot be derived adequately just from assumed morphological adaptations to specific dispersal mechanisms.

In the framework of nature conservation and restoration seed dispersal by livestock was examined by Welch (1985), Bakker (1989), Malo and Suárez (1995) and Stender et al. (1997) who show the potential of endozoochorous seed dispersal in semi-natural landscapes. Moreover those studies indicate that many more plant species might be successfully dispersed through endozoochory than previously thought (Pakeman et al. 2002).

Seed dispersal patterns are not simply a function of distance from the parent plant. It can also be affected by animal behaviour (Nathan and Muller-Landau 2000). Although selective habitat use is a well known feature of mammalian herbivores (e.g. Duncan 1983; Putman 1986; Gordon 1989), there has been almost no attention so far for its potential importance for seed dispersal patterns in West European situations. Only Malo et al. (2000) demonstrate non-random deposition of endozoochorously dispersed seeds among four different *dehesa* habitats. Malo et al. (1995) further mentioned rabbits acting as directional seed dispersers in Mediterranean pastures.

With the establishment of a year round, low density grazing regime, plant ecologists and nature managers expect that through selective grazing, herbivores will create a mosaic of different vegetation communities varying in structure and plant species composition. It is believed that such a mosaic in the end will guarantee a high degree of biodiversity. However the role of herbivores in the dispersal and establishment of plant species is poorly understood (van Wieren and Bakker 1998). Particularly, field data on endozoochory in a North-West European context are still rather scarce. The specific conditions, i.e. high degree of plant species and community richness due to a considerable variation in abiotic conditions, of the coastal dune landscape offered unique opportunities to extend the existing knowledge on plant species that can take advantage of endozoochory. Furthermore the heterogeneous distribution of plant communities and formations (further called habitats) across the landscape forced us to think about the possible impact of selective habitat use on seed deposition patterns. With this paper we try to combine data on germinating seed content and seed deposition patterns to produce a description of the potential role of endozoochorous seed dispersal within a medium-sized fragment of the semi-natural coastal landscape. Therefore we aim to

1. describe and quantify the germinable seed content of herbivore dung in a temperate coastal dune ecosystem during the main fruiting season;
2. compare dung germinating seed content characteristics between different animal species grazing the same site (animal effect) and the same animal species grazing different sites (site effect);
3. determine seed deposition characteristics among different habitats.

Materials and methods

Study area and species

This study was conducted in two dune nature reserves along the Belgian coast: Westhoek North (51°05′12″N, 2°35′27″E) and Westhoek South (51°04′50″N, 2°34′19″E). Both sites have a sandy soil varying in soil moisture content (dry to wet), soil organic matter (purely mineral sand to humus rich sand) and soil reaction (acid to neutral pH). The dune landscape is dominated by scrubs of *Hippophae rhamnoides* and *Ligustrum vulgare*. Though, grassland covers at least one-third of the area, part of the grassland has scattered the scrub as small and mostly species poor remnants of dune grassland or as species poor *Calamagrostis epigejos* dominated patches, which were recently established after scrub degradation (Table 1). Flowering and fruiting of vegetation is concentrated from April to October. The selected dune nature reserves are stocked with small numbers of two species of domestic livestock: 4 Scottish Highland cattle and 7 Konik ponies at Westhoek North (54 ha), 4 Scottish Highland cattle and 19 Shetland ponies at Westhoek South (61 ha). The maximum possible distance individuals could move in a straight line between two locations within the fenced area of Westhoek North and Westhoek South is 1380 and 1400 m, respectively.

Table 1. Main habitats with brief description of the vegetation characteristics and with indication of surface proportion and the total number of plant species recorded within both study sites (1990–2000).

Study sites: Habitat: code + description	Westhoek South Area %	Westhoek North Area %
A/T: White dunes with *Ammophila arenaria*, *Festuca juncifolia* (A) or grey dunes i.e. mosses and lichens rich dunes with scattered *C. arenaria* and annuals (T)	1.70	4.11
	7.13	5.24
Ud: Vegetation on dry soils dominated by tall herb patches (e.g. *Eupatorium cannabinum*, *Cirsium arvense*, *Senecio jacobaea*) scatterd within grass layer (*Holcus lanatus*)	5.62	8.23
Uw: Vegetation on wet soils dominated by tall herb patches (e.g. *E. cannabinum*, *Lythrum salicaria*, *Lycopus europaeus*) scattered within grass layer (*Poa trivialis*, *Holcus lanatus*)	3.76	5.39
Gd: Dune grassland on dry soils with a high plant species diversity (e.g. dicotyledons) incl. *Rosa pimpinellifolia* dominated grassland	4.09	5.12
Gw: Wet dune slack vegetations including short pioneer vegetation with *Carex* spp., *Juncus* spp.; young *Salix repens* and *Hippophae rhamnoides* scrubs and herb rich later succession phases	8.30	3.64
C: Almost monospecific grassland dominated by *Calamagrostis epigejos* or *C. canescens*. Frequently under deteriorating scrub	5.36	10.95
S: Scrub dominated either by *Ligustrum vulgare*, *Hippophae rhamnoides*, *Salix repens* or mixed with other shrub species + sometimes herb layer with *Claytonia perfoliata*	48.6	56.21
F: Forest *Populus* spp. or *Alnus glutinosa* dominated wood patches	15.4	0.31
Paths: pioneer vegetation of dry or wet situations	0.50	1.45
Area (ha)	61	54
Total # of plant species	422	402

Dung collection and treatment

Dung of horses and cattle was collected during 7 sessions in a period of 3 months (18 July, 1 and 16 August, 12 and 25 September and 9 October 2000) at both study sites. During each session we observed the animals' defecation behaviour. Freshly deposited dung was immediately collected after defecation occurred, leaving behind the lowermost part of the dung to avoid contamination of seeds on the soil surface. Collected dung of different animals was put together to obtain 2 times 2.5 l samples of each herbivore species. Immediately after dung collection, samples were spread out in trays ($40 \times 40 \times 2$ cm) and put in a greenhouse (< 35 °C) to dry for 2 weeks.

After sun-drying in the greenhouse, dung samples were kept at 4 °C for at least 2 weeks. After grinding in a Retsch-mill (type SK 100 – without sieve plate), the samples were spread out over a sterilised sand/peat substrate ($40 \times 40 \times 2$ cm, 1:1 ratio) in a layer of about 0.75 cm. Grinding could have damaged large sized seeds. However, this treatment enabled us to spread out dung samples in a thin layer guaranteeing all seeds receiving enough light to germinate within a short time period (Ter Heerdt et al. 1996).

To detect possible germination from the potting soil substrate and contamination in the greenhouse, 15 trays with only sterilised sand/peat substrate were also set up. To maintain humidity, sample trays were watered twice a day during the whole germination period. Greenhouse conditions were kept at 20–25 °C with a relative humidity of 50–60% during 16 h of light (280–410 μmol m^{-2} s^{-1}) and at 10–15 °C and 80–90% relative humidity during 8 h of darkness. Seedlings were counted as soon as identification was possible (within 2 months) and was continued for 6 months. During the last 2 months very few seedlings emerged. Counted seedlings were immediately removed to avoid competition between seedlings and to prevent flowering.

Only in a few cases, seedlings could not be identified accurately. This was the case for *Erodium cicutarium* and *E. lebelii*, *Sagina apetala* and *S. procumbens*, *Veronica chamaedrys* and

V. arvensis, respectively. They were lumped in three 'pseudospecies'. We used the genus name for those seedlings that could only be identified at the genus level, e.g. *Epilobium* spp. and *Rumex* spp. and considered them as a 'species' in the data analysis. Nomenclature follows Lambinon et al. (1998).

Herbivore and site related effects on dung germinating seed density and plant species richness was determined by documenting of dung germinating seed content of the different herbivore species at each site (animal effect) and of the same herbivore species grazing both study sites (site effect). Because of non-normality and lack of homogeneity of variance we used the non-parametric Wilcoxon signed ranks test for statistical analysis of these data (Siegel and Castellan 1988). This method allowed us to test for differences between paired samples i.e. cattle–horse dung samples at each sampling date, which will cope well with the temporal variability. Similarity in plant species composition of horse and cattle dung samples was expressed as Jaccard similarity i.e. $c/(a + b + c)$, where c is the number of plant species in common, a and b is the number of plant species only present in sample a or b.

Animal defecation behaviour

Herbivore species behaviour data (e.g. movements, grazing and defecating) were extracted from our nutritional ecology research programme (Cosyns et al. 2001). During 6 h sessions (6–12, 12–18, 18–24, 0–6 h), 8 times a month, continuous time budget of herbivore activity was registered by one observer close to the focal animal (within a distance of less than 3 m). This animal was randomly chosen before each session started. During the observations all plant species and plant parts consumed were recorded as well as the plant community in which the activities occurred. Each focal animal's position was recorded on a detail aerial map every 15 min.

To estimate the total volume of dung produced during the sampling period and at each site we counted defecation frequency per animal species during focal observations and estimated the mean dung pile volume of each animal species by carefully measuring the volume of 20 freshly deposited piles per animal species. Differences in mean dung volume were analysed using an independent samples T-test (Sokal and Rohlf 1997).

Summer defecation frequencies were used to calculate the animals species' proportional defecation pattern over all habitats (June–October 1999 – Shetlandpony; 2000 – Konik; or 2001 – Cattle). A chi-square analysis, with Yates correction, was used to determine whether herbivores utilised all habitat types in proportion to their availability (Siegel and Castellan 1988). If chi-square analysis indicated a significant difference existed between habitat use for defecating and availability, significant disproportional use for individual habitat types was determined using the Bonferroni Z simultaneous confidence interval (CI) approach revised by Byers and Steinhorst (1984). Grazing and defecation activity data (%), observed in each of the different habitats were subjected to a Spearman correlation to indicate whether both activities were correlated. All statistical tests were carried out using SPSS software 11.01 (SPSS, 2001) except for the computation of the CI approach (Byers and Steinhorst 1984) which was conducted in Excel (MS Office, 1997).

Results

Germinating seed content of herbivore dung

A total of 59,049 seedlings emerged from 51 dung samples. The mean number of seedlings per sample was 1158 ± 722. One hundred and seventeen different plant species (incl. three pseudospecies) or 27% of all species ever recorded in both study areas were represented. The mean number of plant species per sample of 2.5 l of excrements was 31 ± 6.

A majority of 72 plant species (62%) is present in no more than 20 dung samples. Only 30 plant species (25%) occur in more than 40% of all dung samples (Table 2, Appendix A). Seventy seven plant species (66%) were represented with on average less than 1 seedling per dung sample. Twenty plant species (17%) emerged with on average more than five seedlings per sample.

Urtica dioica, *Juncus bufonius* and *J. articulatus* were among the most abundant species emerging from the dung samples. Seedlings of *Poa trivialis*, *Veronica chamaedrys/arvensis*, *Cerastium fontanum*, *Poa pratensis*, *Agrostis stolonifera* and *Sagina procumbens/apetala* appeared very frequently and

Table 2. Alphabetic ordered list of 68 plant species, within 3 functional groups, which were recorded at least 5 times from different dung samples of large herbivores (2.5 l) at Westhoek North and South with the indication of their mean number of seedlings in dung samples, their overall mean number of seedlings (mean #) and their frequency of occurrence (F_{req}) across all dung samples (maximum = 51).

Species	Westhoek North			Westhoek South			Mean # 2.5 l	F_{req} max. = 51
	Cover %	Konik	Cattle	Cover %	Shetland	Cattle		
Graminoids								
Agrostis capillaris	0.03	0.2	18.7	0.53	6.0	99.0	29.0	26
Agrostis stolonifera	0.89	24.0	39.5	2.90	28.4	18.9	27.4	47
Aira praecox	0.02	0.4	1.2	0.72	2.9	1.2	1.4	14
Calamagrostis epigejos	1.20	0.1	0.0	13.54	0.4	0.6	0.3	10
Carex arenaria	3.62	3.3	20.0	3.70	1.8	9.2	7.9	39
Carex flacca	0.27	1.8	1.0	0.90	0.0	0.0	0.7	8
Festuca rubra	3.58	0.2	0.7	3.62	0.0	1.3	0.5	8
Holcus lanatus	2.07	6.6	18.9	7.85	19.9	25.9	17.5	47
Juncus articulatus	0.32	356.6	148.5	0.03	40.8	28.8	147.9	50
Juncus bufonius	0.08	228.4	139.0	0.03	102.1	334.0	199.3	51
Luzula campestris	0.27	0.4	1.5	0.56	0.4	0.2	0.6	17
Phleum pratense	0.50	1.6	0.5	0.50	0.6	1.0	0.9	23
Poa annua	0.06	20.4	4.9	0.02	22.8	6.9	14.5	46
Poa pratensis	0.75	28.7	64.3	1.58	22.1	17.8	32.0	49
Poa trivialis	1.21	81.5	188.5	2.00	32.4	93.4	93.9	50
Scirpus setaceus	0.01	0.1	0.0	0.01	1.9	1.4	0.9	16
Forbs								
Arenaria serpyllifolia	0.40	17.2	3.5	0.72	9.6	14.6	11.5	44
Cardamine hirsuta	0.14	1.3	0.5	0.02	0.5	0.4	0.7	21
Centaurium erythraea	0.05	5.6	0.0	0.29	0.9	1.3	2.1	15
Centaurium littorale	0.01	0.5	0.1	0.01	0.1	0.3	0.3	9
Cerastium fontanum	0.48	28.9	32.0	0.45	29.1	48.3	34.2	51
Cirsium arvense	5.53	0.1	0.5	2.16	0.0	0.1	0.2	6
Conyza canadensis	0.18	0.9	0.0	0.20	0.1	0.2	0.3	9
Crepis capillaris	1.61	0.3	0.0	0.27	1.1	4.6	1.5	10
Dicotyl spp		0.0	0.9		0.0	0.4	0.3	8
Epilobium ciliatum	0.13	0.9	0.0	0.06	0.0	0.5	0.4	10
Epilobium hirsutum	0.06	0.3	0.3	0.06	0.4	1.1	0.5	9
Epilobium roseum	0.97	1.2	6.7	0.09	0.3	4.3	2.9	32
Epilobium sp.		2.1	0.2		2.7	1.0	1.6	31
Erodium cicutarium/lebelii	1.59	0.1	0.2	0.55	0.1	0.0	0.1	5
Eupatorium cannabinum	7.44	3.8	0.0	1.57	0.4	0.3	1.2	19
Galium aparine	0.41	1.9	0.5	0.28	0.1	0.8	0.9	13
Galium mollugo	0.04	0.0	0.2	0.66	1.2	1.0	0.6	6
Galium palustre	0.01	0.6	0.2	0.07	0.1	0.3	0.3	11
Galium uliginosum	0.37	14.6	0.5	1.30	0.5	0.6	4.4	14
Galium verum	4.67	5.0	2.4	2.41	0.1	0.4	2.0	15
Geranium molle	0.03	0.1	1.2	0.13	1.6	1.8	1.2	27
Gnaphalium uliginosum	0.01	3.6	0.0	0.00	0.0	0.0	1.0	10
Helianthemum nummularium	0.97	0.0	0.6	0.00	0.0	0.0	0.2	5
Hydrocotyle vulgaris	1.12	1.1	0.1	1.15	0.1	0.3	0.5	14
Lycopus europaeus	0.28	4.6	180.8	0.01	0.5	1.2	40.7	18
Lythrum salicaria	4.66	10.6	7.2	1.06	1.6	2.7	5.5	28
Mentha aquatica	1.16	2.4	7.3	1.00	0.7	2.3	3.0	18
Oenothera glazioviana	0.20	0.2	0.1	0.09	0.5	2.3	0.7	10
Oxalis spec.		0.6	0.4		0.2	0.2	0.4	6
Plantago lanceolata	0.05	0.9	3.7	1.58	0.1	0.2	1.1	14
Plantago major	0.04	15.9	1.8	0.00	7.3	4.2	7.7	37
Potentilla reptans	0.02	1.0	1.5	0.62	1.3	4.4	2.0	26
Prunella vulgaris	0.06	1.1	0.7	0.06	1.6	7.4	2.6	22

Table 2. Continued.

Species	Westhoek North			Westhoek South			Mean # 2.5 l	F_{req} max. = 51
	Cover %	Konik	Cattle	Cover %	Shetland	Cattle		
Ranunculus repens	0.06	1.5	1.5	1.36	6.9	46.8	13.6	39
Ranunculus sceleratus	0.05	0.2	0.0	0.01	0.0	0.2	0.1	5
Rumex acetosella	0.05	0.1	1.8	0.05	0.0	2.9	1.1	5
Rumex conglomeratus	0.10	0.3	0.2	0.10	0.9	2.7	1.0	14
Rumex crispus	0.10	0.1	0.5	0.10	0.5	0.2	0.3	8
Sagina procumbens/apetala	0.20	16.1	1.6	0.07	43.1	28.8	23.4	44
Samolus valerandi	0.03	1.1	0.0	0.01	0.0	0.0	0.3	6
Senecio jacobaea	0.33	1.6	0.6	0.73	7.9	3.4	3.5	26
Solanum nigrum	0.01	0.9	0.0	0.00	0.0	0.1	0.3	5
Sonchus oleraceus	0.09	0.7	0.6	0.00	0.0	0.0	0.3	12
Stellaria media	0.10	11.5	19.4	0.10	0.9	5.3	8.8	39
Trifolium campestre	0.00	0.0	0.0	0.10	0.1	1.6	0.4	6
Trifolium dubium	0.02	0.2	0.4	0.06	4.5	1.0	1.6	22
Trifolium repens	0.06	3.3	2.4	1.66	19.2	19.2	11.2	39
Urtica dioica	0.62	42.3	1144.8	0.14	226.6	16.7	324.7	51
Veronica arvensis/chamaedrys	0.16	102.6	35.1	0.76	41.1	18.6	51.4	51
Veronica officinalis	0.00	4.8	4.6	0.10	1.5	1.3	3.0	34
Veronica serpyllifolia	0.01	0.2	0.1	0.05	0.2	0.1	0.2	6
Woody								
Rubus caesius	7.58	1.5	0.9	3.91	1.0	1.4	1.2	22

The estimated total cover (%) of the plant species in the whole area is obtained by taking into account the average cover from at least 5 relevés in each of the habitats and the relative surface of each habitat. A list of 49 less frequent recorded plant species can be found in Appendix A.

were reasonably abundant in the dung. Other plant species showed notable numbers of seedlings in part of the samples e.g. *Lycopus europaeus*, *Ranunculus repens* and *Agrostis capillaris*. A considerable number of plant species was recorded regularly (> 40% of all samples) but showed on average low mean seedling densities (< 5) e.g. *Cardamine hirsuta*, *Epilobium*, *Geranium molle*, *Phleum pratense*, *Potentilla reptans*, *Rubus caesius* and *Veronica officinalis* (Table 2).

Herbivore and site-related effect on dung germinating seed content

At both sites seedling densities were at least as high as or higher in cattle than in horse dung across all sampling dates (Table 3 and Figure 1a, b). Seedling density differs only significantly between Highland cattle and Konik at Westhoek North (Wilcoxon Z: -2.22, $p < 0.026$).

The dung of both animal species contained germinable seeds of a considerable number of plant species e.g. Konik (max. 43), cattle (Westhoek South, max. 40). Mean plant species richness varies significantly between animal species at both sites. At least as many or more plant species are counted from Konik dung than of cattle at Westhoek North (Wilcoxon Z: -2.65, $p < 0.008$) and from cattle than from Shetland pony at Westhoek South (Wilcoxon Z: -2.39, $p < 0.017$) (Table 3, Figure 1c, d). Overall Jaccard similarity of dung germinating seed composition from both animal species is 0.52 and 0.66 at Westhoek North and South respectively, suggesting a considerable number of plant species common (Table 3). On general similar values were obtained at each sampling date at both sites, though a much higher value was found in October at Westhoek South (Figure 1c, d).

Seedling density was significantly higher in cattle dung at Westhoek North than in cattle dung at Westhoek South (Wilcoxon Z: -2.58, $p < 0.001$). A similar pattern was observed for horse dung (Wilcoxon Z: -1.97, $p < 0.047$) (Figure 1a, b). Species richness was significantly higher in horse dung at Westhoek North than in horse dung at Westhoek South (Wilcoxon Z: -2.72, $p < 0.023$) but did not differ between cattle dung samples (Figure 1c, d).

Table 3. Total number (#) of seeds and plant species germinated from all dung samples, mean (±S.D.) and maximum seed density and species richness in 2.5 l dung samples of ponies and cattle.

	Westhoek North		Westhoek South	
	Konik	Cattle	Shetland	Cattle
Total # germinated seeds	15098	23290	9814	10811
Mean seed density	1078 ± 444	2118 ± 862	701 ± 243	901 ± 355
Maximum seed density	1782	3622	1308	1507
Minimum seed density	465	665	351	313
Total # plant spp.	90	67	67	82
Mean species richness	34 ± 5	28.5 ± 5	29 ± 5	33 ± 5
Maximum species richness	43	34	38	40
Minimum species richness	27	23	22	19
Jaccard similarity (%)	52		66	
# Samples (2.5 l)	14	11	14	12

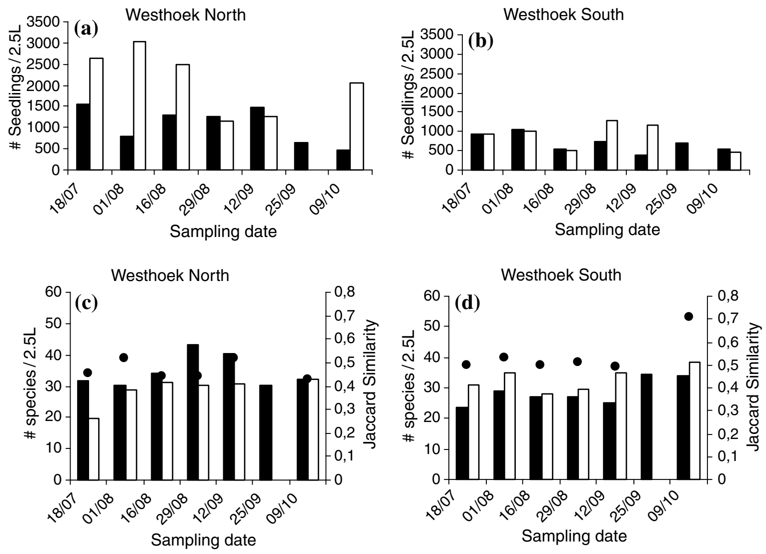

Figure 1. Temporal variability of seedling density (a, b), plant species number and Jaccard similarity (c, d) in horse and cattle dung samples which were collected in two coastal dune nature reserves (Westhoek North and Westhoek South). White bars represent cattle and black bars represent the horse breeds. Filled circles indicate the Jaccard similarity.

Defecation frequencies and seed numbers dispersed during the fruiting season

Within sites both herbivore species show comparable defecation rates (Table 4). Given the mean dung germinating seed content, mean dung volume and the calculated number of defecations during the fruiting season the potential importance of endozoochory becomes obvious (Table 4). Konik and Highland cattle at Westhoek North potentially disperse the greatest number of germinating seeds per animal during this period, hence contributing potentially to a considerable seed dispersal service at this site.

Defecation patterns and seed deposition among habitats

At both sites all herbivore species show a disproportional use of habitats in relation to their area for dung deposition. In general observed defecation frequencies of all herbivores were significantly higher than expected in habitats dominated by tall herbs on dry or wet soils (Ud and Uw resp., Table 5). At Westhoek North, Highland cattle defecated more than expected in dune grasslands on dry soil (Gd) and in the small sized forest (F). At Westhoek South cattle deposited more dung than expected in monospecific, *Calamagrostis epigejos*

Table 4. The potential number (#) of seeds that is on average endozoochorously dispersed by an individual horse and cow during a whole summer (92 days) in a coastal dune area.

	Westhoek North		Westhoek South	
	Konik	Cattle	Shetland	Cattle
Mean dung volume (l) ± S.D.	1.6^{***} ± 0.7	0.8 ± 0.2	0.8 ± 0.4	0.8 ± 0.2
(*n*)	(20)	(20)	(20)	(20)
Mean # defecations/h ± S.D.	0.8 ± 0.3	0.8 ± 0.5	0.6 ± 0.2	0.6 ± 0.2
(*n*)	(18)	(20)	(20)	(14)
Mean # seeds dispersed/h	552	542	135	173
Mean # seeds/summer and individual	±1,200,000	±1,200,000	±291,000	±382,000

Numbers are based on mean defecation frequency (i.e. number of defecations per hour, h), mean seed content of dung and mean dung volume (l). *** Indicates a significant higher mean dung volume of Konik vs. cattle at Westhoek North ($T = 4.768$, df: 38). Means were calculated from the number of samples (*n*) indicated which were obtained from random selected adult individuals of each animal species.

grassland (Cl). In several habitats dung deposition was lower than expected e.g. in Scrub (S), white and grey dunes (AT), the exception being cattle at Westhoek South. Neither horse breed defecated in forest (F) (Table 5).

Time spent grazing was distributed across habitats in much the same way as defecations. Spearman correlation of the % of grazing activity by habitat and % of defecation activity by habitat is positive and significant (Figure 2).

Both herbivore species tend to intensify seed deposition in preferred habitats, mainly vegetation dominated by tall herbs on dry (Ud) and wet soils (Uw), resulting in a calculated mean seed deposition of about 100–200 seeds per square meter at Westhoek North and a clearly lower number (15–60) at Westhoek South (Figure 3). A relative high seed input was calculated for forest habitat (144 seeds/m^2) at Westhoek North.

Discussion

Estimating the germinating seed content of herbivore dung

In this study we used a method which is very comparable to recommended seed bank estimation through germination (Thompson et al. 1997). Nonetheless some bias and underestimation of real germinable seed content is possible. Grinding the samples in order to be able to easily spread them out in a thin layer could have damaged larger sized seeds. This could be a reason why some plant

Table 5. Expected (exp.) and observed (obs.) defecation frequencies by different herbivore species in 9 habitats (see Table 1) at both study sites and calculated chi-square (χ_8) values for 8 degrees of freedom with its probability (P).

Habitats	Westhoek North				Westhoek South			
	Konik		Cattle		Shetland		Cattle	
	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.
AT	4.0	1*	9.9	2*	5.5	0*	6.6	7
Gd	2.2	7	5.4	16*	2.5	9	3.1	0*
Gw	1.6	2	3.9	2	5.1	12	6.2	7
Cl	4.7	0*	11.6	9	3.3	7	4.0	14*
Ud	3.5	14*	8.7	25*	3.5	25*	4.2	18*
Uw	2.3	17*	5.7	31*	2.3	1	2.8	12*
S	24.2	1*	59.6	11*	30.1	4*	36.5	10*
F	0.1	0*	0.3	6*	9.3	0*	11.3	7
Path	0.6	1	1.5	4	0.3	4	0.4	0*
χ_8	150.9		283.9		219.4		112.9	
p	< 0.001		< 0.001		< 0.001		< 0.001	

A significant difference between expected and observed defecation frequency in a certain habitat, based on Bonferroni *Z* simultaneous confidence interval approach (Byers and Steinhorst 1984).

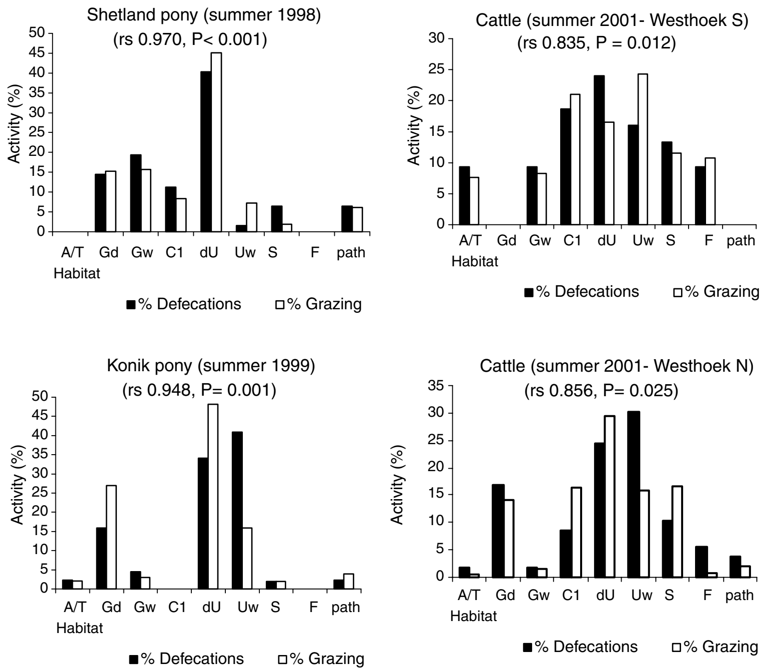

Figure 2. The mean proportional distribution of grazing time and defecation frequency is shown for each herbivore species over the different habitats at each study site. These behavioural parameters can be interpreted as a measure for the expected contribution of each habitat to dung plant seed composition and the chance that dung will be dropped in a specific habitat. Spearman rank correlation (r_s) between grazing time (%) and defecation frequency (%) is also shown. AT: white and grey dunes; Ud, Uw: vegetation dominated by tall herbs on dry and wet soils respectively; Gd, Gw: dune grassland on dry and wet soils respectively; Cl *Calamagrostis epigejos* dominated grassland, S: scrub; F: forest.

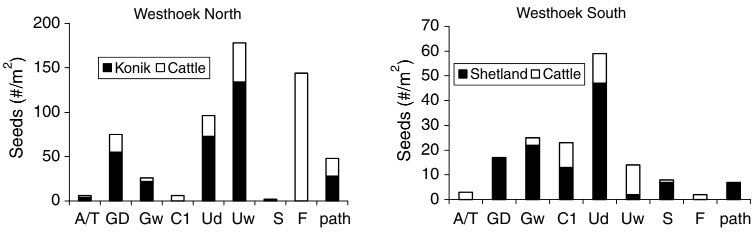

Figure 3. Estimated number of seeds deposited per m^2 in each habitat, during summer, by all adult individuals of both large herbivore species at Westhoek North and Westhoek South. Calculations are based on mean dung seed content and defecation frequency in each habitat during summer. AT: white and grey dunes; Ud, Uw: vegetation dominated by tall herbs on dry and wet soils respectively; Gd, Gw: dune grassland on dry and wet soils respectively; Cl *Calamagrostis epigejos* dominated grassland, S: scrub; F: forest.

species, although the fruits have been seen consumed, did not emerge from the dung samples e.g. *Rosa pimpinellifolia*, *R. canina*, *Ligustrum vulgare*. Further evidence for such damage came from a comparable greenhouse experiment without grinding dung samples in which several *Ligustrum vulgare* seedlings emerged from horse dung.

The experiment was conducted in greenhouse conditions. which are assumed to be favourable for most plant species. But germination conditions can differ between plant species (Grime et al. 1988), hence comparison of frequency distribution of different plant species may be biased this way (Malo 2000).

The experiment was stopped after 6 months because at that moment no significant further germination was detected. Nonetheless, from the results of Müller (1955) one could expect viable seeds still to be present. Malo (2000) recently showed the need for longer germination periods for seed bank and dung samples to allow more plant species and seedlings to emerge so avoiding important biases in the results.

It should be kept in mind though that we aimed at detecting the *potential* contribution of endozoochory to seed dispersal. Therefore it was justified to follow germination in semi-optimal greenhouse conditions. However, germination should also be tested under field conditions to be able to estimate the true contribution of endozoochory to dispersal, taking into account the less favourable conditions in the field caused by factors such as competition, climate, physical conditions of the dung, herbivory, etc.

The germinating seed content of herbivore dung

The results of this study stress the potential of endozoochory by large herbivores as an important seed dispersal mechanism with a large number of seeds of a considerable number of plant species dispersed during the fruiting season. The relevance of this mechanism is shown in a qualitative way by the fact that at least 27% of all plant species which were recorded from both study sites can potentially be dispersed endozoochorically, although only a fraction of them in large amounts. When adding the knowledge on endozoochorically dispersed plant species from other studies e.g. Welch (1985); Malo and Suárez (1995); Dai (2000) and Pakeman et al. (2002), a still larger fraction of plant species are candidates for this mode of dispersal (41%).

Quantitatively the potential of endozoochory becomes obvious when taking into account the animal species' defecation frequencies and mean dung volumes during the fruiting season. Horses and cattle are both capable, depending on site and animal related characteristics, to disperse large numbers of germinable seeds. A simple calculation, based on mean number of germinating seeds per liter dung, mean dung volume per defecation and number of defecations during the fruiting seasons, gives an estimate of the potential number of viable seeds dispersed by both ungulates in one study site of approximately 1,200,000 seeds per season (Table 4).

Subtle variation in grazing behaviour and diet selection could explain some of the observed differences in germinating seed content between cattle and horse dung. Cattle and horses prefer grazing of graminoid rich habitats (Duncan 1983; Menard et al. 2002; Table 5). Within habitats horses will select very short grass dominated patches, whereas cattle prefer higher vegetation patches (Menard et al. 2002, Cosyns unpubl.). In the higher patches some graminoid species will be able to produce more seeds as compared to the short grazed patches. This could explain the observed differences in seed content of horse and cattle dung in case of *Agrostis*, *Holcus* and *Poa* spp. (Table 2). In contrast, other plant species may occur more frequently or produce more seeds in short swards e.g. *Sagina procumbens*, *Plantago major*, *Poa annua*. Their seeds were found more abundantly in the horse dung compared to the cattle dung samples. The large difference in numbers of *Lycopus europaeus* and *Urtica dioica* seedlings found in dung of Highland cattle (Westhoek North) vs. Konik are mainly the result of the observed frequent consumption of *Lycopus* and *Urtica* by cattle and avoidance of it by Konik (Cosyns 2004). Because of its scarcity in the species pool of Westhoek south, *L. europaeus* was almost absent from cattle and pony dung at this site. So *Lycopus* can be considered a very illustrative species to demonstrate both the possible effects of diet selection and seed availability on the process of endozoochory.

Besides the above mentioned frequently occurring species of which several can be indicated as ruderals or ruderal-competitive species, we were able to detect a considerable number of plant species occurring only in minor densities and frequencies of which several are to a certain level, stress tolerating grassland species e.g. *Galium verum*, *Veronica officinalis*, *Luzula campestris*, *Trifolium* spp. Their scarceness is at least partly due to their low availability in the species pool though seedlings of e.g. *Helianthemum nummularium* and *Galium mollugo* on dung were also mentioned by Müller-Schneider (1954) and *H. nummularium*, *G. verum*, *Plantago lanceolata* and*Trifolium campestre* by Dai (2000), suggesting their ability to survive the gastro-intestinal tract.

The observed variations in species richness (mean, maxima/sample and total species richness) between dung samples of different herbivore species mainly resulted from the accidental presence of in most cases rare plant species. Within herbivore species, dung samples in general consist of a core of 'commonly present' plant species (ca. 15–20 species) complemented with a varying selection and number of infrequent plant species. Between herbivore species the size of this infrequent plant species pool tend to vary according to the observed total species richness.

Defecation patterns and its possible influence on grassland dynamics

In this study plant species growing preferably in dry grassland (habitats Gd and Ud) and to some extent also in wet (Uw) or monospecific grasslands (Cl) generally have the best chance of being dispersed into the same, and presumably better, habitat (Figure 3). Plant species related of other habitats (e.g. scrub and forest) have a minor chance of being dispersed endozoochorically in an identical habitat. On the other hand, whenever grazed they have a far higher chance being dispersed to grass dominated habitats. It is plausible to suggest that the most preferred habitats receive the largest proportion of viable seeds from plants that grow in other habitats. It can be hypothesised that these habitats therefore are more 'vulnerable' for invasion by new species. Although seed arrival is no guarantee of recruitment, the non-random dispersal to other habitats might induce directional succession, if late successional species are dispersed into earlier successive phases.

Of course even within a potentially suitable habitat there is no guarantee that deposited seeds will encounter the right germination conditions. On the other hand not all plant species are restricted to one particular habitat. Species characteristics can differ and some species behave rather opportunistically, while others depend on narrowly defined environmental conditions. For this reason a species based approach can add further specific information about the significance of endozoochorous seed transport through the landscape, also in relation to other dispersal mechanisms. The hypothesis that non-random dispersal of late-successive species towards earlier successive phases could enhance succession, should be further investigated as well.

Because of the size and scattered distribution of the preferred habitats within the landscape large herbivores will regularly move through both habitats and the landscape. Taking into account mean retention time i.e. 36–41 h (Shetlandpony resp. Konik) and 74 h (cattle), as calculated from Illius and Gordon (1992), and animal behaviour data we found indications for potential movements – measured in a straight line from the origin to a given point after of several hundreds of metres. Such distances were regularly travelled even within relatively short periods, such as 6 h. Taking into account the potential of all herbivore species to cover most parts of the fenced area after MRT, inter-habitat endozoochorous dispersal therefore is perfectly possible. The more detailed effects are still to be examined but the above mentioned evidence clearly shows the potential importance of endozoochory as a non-random, long distance seed dispersal mechanism.

Conclusions in relation to ecosystem management and rehabilitation

From this study it is clear that dispersal by mammalian herbivores is a relevant dispersal mechanism for a significant proportion of species in dune grasslands. When epizoochory is also taken into account, half of the number of plant species is supposed to have a reasonable chance of being dispersed by animals and hence the contribution of this mechanism to the plant species' dispersability will probably be important.

Secondly there is important evidence from this study that domesticated large herbivores can contribute significantly to medium long-distance seed dispersal. Free ranging, domesticated herbivores studied here show a general tendency to select preferentially grass dominated habitats to feed (e.g. Duncan 1983; Putman 1986; Gordon 1989). This suggest that, not only within heterogeneous dune landscapes, but also in other parts of the degraded and highly fragmented semi-natural NW European landscape, large herbivores can play an important role as seed dispersal agents by moving seeds between isolated patches of comparable habitats. In other words and as already suggested by Bonn and Poschlod (1998), they can act as important dynamic ecological 'corridors'.

Several authors have suggested the (re-)introduction of livestock in 'nature reserves' in order to enhance species richness of highly impoverished habitats within a well defined landscape. A large number of examples is concerned with species poor 'target units' that could be connected with species rich 'source units' by means of zoochorous seed dispersal from the latter. Examples included calcareous grasslands (Hillegers 1993) and heathland (Büllow Olsen 1980; Bakker 1989). From this study we can derive furthermore important evidence for seed input of true grassland species into actually species poor grassland habitats (Cl, Ud and Uw). Whether this would result in the establishment of species rich grassland communities needs further study of germination under field conditions.

A comparable use of livestock is the use of cattle and sheep as 'sowing machines' to improve grasslands (Lowry 1997; Ghassali et al. 1998). In the same context we would therefore argue to consider the opportunities which arise from the movements of livestock between parcels or small nature reserves. Knowing their potential as seed dispersing agents one can try to outline a grazing management which can help to maintain or enhance plant species diversity at the local scale. Therefore grazing of species rich sites should precede grazing of species poor sites with the same herd of individuals. Others have stressed the importance of maintaining and re-establishing seed dispersal processes on a larger spatial scale by re-introducing shepherding 'transhumance' (Fischer et al. 1996; Bonn and Poschlod 1998; Zobel et al. 1998), the traditional system of moving domestic livestock, particularly sheep, over large distances permitting the complementary use of resources between the highlands and lowlands (Ruiz and Ruiz 1986).

Bonn and Poschlod (1998) and Bruun and Fritzbøger (2002) rely on strong historical evidence to derive and state a high degree of connectivity between the different units of the traditionally used man-made i.e. semi-natural landscape such as grassland, arable field, heathland, villages as a result of the presence of many dispersal vectors and processes of which several can be attributed to livestock grazing practices. They argue the loss and decrease of dispersal processes and vectors in today's man-made landscape as an important cause of local extinction of species in the remnants of the semi-natural landscape. Therefore, nature conservation and restoration management should regard this fact and include the restoration or a simulation of these dispersal processes (Bonn and Poschlod 1998).

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Appendix A. Alphabetic ordered list of 49 plant species, within two functional groups, which were recorded less than 5 times from different dung samples of large herbivores (2.5 l) at Westhoek North and South.

Forbs				
Achillea millefolium	*Claytonia perfoliata*	*Lotus corniculatus*	*Potentilla anserina*	*Silene latifolia*
Anagallis arvensis	*Epilobium obscurum*	*Lotus pedunculatus*	*Ranunculus bulbosus*	*Solanum dulcamara*
Anchusa officinalis	*Epilobium parviflorum*	*Lychnis flos-cuculi*	*Ranunculus trichophyllus*	*Sonchus asper*
Anthriscus caucalis	*Fallopia convolvulus*	*Lysimachia vulgaris*	*Rumex obtusifolius*	*Taraxacum sp.*
Aphanes inexpecta	*Festuca arundinacea*	*Medicago arabica*	*Rumex sp.*	*Trifolium arvense*
Arabidopsis thaliana	*Geranium robertianum*	*Medicago lupulina*	*Sagina nodosa*	
Cerastium semidecandrum	*Hypericum perforatum*	*Ononis repens*	*Sedum acre*	
Chenopodium album	*Hypericum tetrapterum*	*Plantago coronopus*	*Senecio sylvaticus*	
Chenopodium rubrum	*Leontodon saxatilis*	*Polygonum aviculare*	*Senecio vulgaris*	
Graminoids				
Anthoxanthum odoratum	*Carex trinervis*	*Juncus inflexus*	*Poaceae indet*	
Calamagrostris canescens	*Carex viridula*	*Juncus subnodulosus*		

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