1  **Linking functional group richness and ecosystem functions of dung beetles -**
2  **an experimental quantification**
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9      **Abstract**

10     Dung beetles form an insect group that fulfils important functions in terrestrial ecosystems throughout

11     the world. These include nutrient cycling through dung removal, soil bioturbation, plant growth,

12     secondary seed dispersal and parasite control. We conducted field experiments at two sites in the

13     northern hemisphere temperate region in which dung removal and secondary seed dispersal were

14     assessed. Dung beetles were classified in three functional groups, depending on their size and dung

15     manipulation method: dwellers, large and small tunnelers. Other soil inhabiting fauna were included as a

16     fourth functional group. Dung removal and seed dispersal by each individual functional group and

17     combinations thereof were estimated in exclusion experiments using different dung types. Dwellers were

18     the most diverse and abundant group, but tunnelers were dominant in terms of biomass. All dung beetle

19     functional groups had a clear preference for fresh dung. The ecosystem services in dung removal and

20     secondary seed dispersal provided by dung beetles were significant and differed between functional

21     groups. Although in absolute numbers more dwellers were found, large tunnelers were disproportionally

22     important for dung burial and seed removal. In the absence of dung beetles, other soil inhabiting fauna,

23     such as earthworms, partly took over the dung decomposing role of dung beetles while most dung was

24     processed when all native functional groups were present. Our results, therefore, emphasize the need to

25     conserve functionally complete dung ecosystems in order to maintain full ecosystem functioning.


26     **Keywords**: dung beetles, dung removal, secondary seed dispersal, functional groups

**Introduction**

In the last decades, there has been an increasing interest in ecosystem functions and services and their value for human economy and well-being (Millennium Ecosystem Assessment 2005, Naeem et al. 2002). The signs of a new human induced species extinction wave (Barnosky et al. 2011) resulted in a multitude of studies of the relation between biodiversity and ecosystem functioning (Cardinale et al. 2002, Reiss et al. 2009) in an attempt to model the future effects of performance of incomplete ecosystems and to find solutions to biodiversity loss. Many of these studies have shown the importance of preserving biodiversity to maintain ecosystem functioning and the resulting services (e.g., Schwartz et al. 2000, Srivastava and Vellend 2005, Balvanera et al. 2006). Often, mixtures of species outperform the best functioning monoculture for a particular function through facilitation or niche complementarity (so-called 'transgressive overyielding') (Cardinale et al. 2002, Schmid et al. 2008). However, an often posed criticism on biodiversity and ecosystem functioning research is that many of these studies used artificially composed and restricted sets of species and were performed under strictly controlled abiotic conditions (Finn 2001, Naeem and Wright 2003, Slade et al. 2007). Field studies in which specific species or groups of species are experimentally included or excluded in the measurement of ecosystem functions can overcome this problem. The use of existing species assemblages without manipulating the environmental conditions is therefore recommended in order to study specific ecosystem functions. Dung beetles are an example of suitable model organisms in ecosystem functioning research as they are globally distributed and abundantly present (Hanski and Cambefort 1991). Most of the ecosystem functions provided by dung beetles are linked to the removal and underground burial of dung (Losey and Vaughan 2006, Nichols et al. 2008), which results in bioturbation increase and enhanced nutrient cycling. While digging their nests, dung beetles perturb the soil, aerate it, change the hydrological properties (Brown et al. 2010) and altogether alter the structure of the soil top layers (Bang et al. 2005). In Western Europe for instance, the presence of the tunnelling species *Typhaeus typhoeus* on its own is estimated to move 450 kg of soil per hectare annually (Brussaard and Visser 1987).

Dung beetles play an important role in nutrient cycling as they remove and bury vertebrate faeces containing considerable amounts of nutrients (Hanski 1987), which increases the availability of these

54    nutrients in the ecosystem (Yamada et al. 2007), plant growth (Borghesio et al. 1999) and crop yield

55    (Miranda et al. 2000) and reduces the emission of the greenhouse gas methane (Penttila et al. 2013). An

56    indirect effect of dung beetle activity is their role as pest control agents. Dung plays an important role in

57    the lifecycle of many parasites infecting vertebrates, such as flatworms and nematodes, but also attracts

58    external pest species such as flies. By reducing the aboveground amount of dung, dung beetles have a

59    negative impact on the reproduction success of these pest species (Bryan 1973) and reduce the infection

60    rate of grazing vertebrates (Fincher 1973, 1975). Overall, a great economic value has been attributed to

61    dung beetles. Losey and Vaughan (2006) estimate the annual economic value of dung beetles in the

62    United States at 380 million dollars, remarking that this number is an underestimate.

63    Next to these ecosystem functions with direct economic benefits, dung beetles play an important role in

64    plant ecology through the process of secondary seed dispersal (Feer 1999, Andresen 2002b).

65    Endozoochory, or dispersal of propagules by herbivores after gut passage, is a commonly found dispersal

66    mechanism and enables long-distance dispersal of plant seeds (Nathan et al. 2008). Dung beetles can act

67    as secondary dispersers of the seeds already present in dung and reshape plant communities (Andresen

68    2003, Nichols et al. 2008). Secondary seed dispersal by dung beetles may result in a higher seedling

69    establishment by reducing the risks of predation and mortality, directing dispersal to more favourable

70    locations for germination and decreasing scramble competition for space and nutrients by seedlings

71    (Andresen 1999, Andresen and Levey 2004, Andresen and Feer 2005, Nichols et al. 2008). Given their

72    ecological and economic importance, it is essential to understand the implications of a changing

73    community composition for the ecosystem functions dung beetles fulfil, also because they are sensitive to

74    habitat modification (Nichols et al. 2008), which is a global problem. In comparison with tropical biomes,

75    few studies were published on dung beetle ecology in temperate regions, and the knowledge of their role

76    in ecosystem functions and services in Western Europe is rather fragmentary. Nevertheless, as dung

77    beetles are abundantly present beyond the tropics, they are very well suited for biodiversity and

78    ecosystem functioning research in many ecosystems. Furthermore, it is easy to measure rates of dung and

79    seed removal and dung pats which are spatially and temporally separated patches can simply be

80    replicated and manipulated (Finn, 2001).

81    As a division into functional groups based on body size and method of dung exploitation can be made, the

82    effects of excluding certain groups can be assessed experimentally. One common way of classifying dung

83    beetles is based on functional traits according to the beetles' nesting behaviour (Doube 1990). Telocoprids

84    (rollers) make dung balls and roll it away from the dung pile before burying it, while paracoprids

85    (tunnelers) bury dung below the dung pile and endocoprids (dwellers) do not significantly relocate dung

86    (Hanski and Cambefort 1991). The use of functional groups is relevant as changes in certain ecosystem

87    functions can be greater when an entire functional group is lost compared to the loss of an equivalent

88    number of species spread over all functional groups (Larsen et al. 2005, Slade et al. 2007). Since the

89    amount of dung that is removed strongly correlates to the mean female body size (Horgan 2001) and

90    larger beetle species tend to be more extinction prone and functionally efficient (Larsen et al. 2005) an

91    extra division based on body size is appropriate.

92    In this study, we aim to link dung beetle assemblages with dung removal and secondary seed dispersal in

93    the temperate Atlantic biogeographical region. As we expect that dung removal and secondary seed

94    removal are closely linked to nesting behaviour and beetle size, we designed a field experiment in which

95    different combinations of functional groups were granted or denied access to experimental dung piles.

96    We also hypothesize that dung beetle assemblages, and therefore the ecosystem services provided by

97    dung beetles, vary between sites and seasons and that dung originating from different herbivore species

98    might attract different sets of dung beetle species. Therefore, species diversity and abundance were

99    recorded during the experiments and the study was simultaneously performed in two locations using

100    different dung types and was replicated in two seasons.

## Materials and methods

*Site description*

The study took place in two nature reserves situated within the Atlantic biogeographical region in Belgium: the 'Zwindunes and Zwinpolders' and 'De Zoom-Kalmthoutse heide'. The 'Zwindunes and Zwinpolders' (further referred to as 'The Zwin') are situated in the coastal dune ecoregion at the most north-eastern section of the Flemish coast between the centre of Knokke-Heist and the Dutch-Belgian border. 'De Zoom-Kalmthoutse heide' (further referred to as 'Kalmthout') is located in the Campine ecoregion and is a cross-border park on the Dutch-Belgian border near the centre of Kalmthout (Electronic supplementary material, ESM 1) (Couvreur et al. 2004). Free-ranging semi-wild grazers were introduced in The Zwin area in 2002 and in Kalmthout in 1997. During this study, the grazed areas in The Zwin and Kalmthout respectively had a surface area of 98 ha and 639 ha. Grazing pressure and animal species and breeds differ between sites with Highland cattle (*Bos taurus*, 0.52 animal units (AU)/ha), Konik horses (*Equus ferus caballus*, 0.15 AU/ha) and Flemish sheep (*Ovis aries*, 0.20 AU/ha) in The Zwin area and Galloway cattle (*Bos taurus*, 0.20 AU/ha) in Kalmthout. Furthermore, a herded flock of Drenthe sheep (*Ovis aries*, 0.20 AU/ha) is present in Kalmthout.

In each study site an experimental area of approximately 300m² was fenced out in order to avoid disturbance by grazers during the field experiment, but allowing access of dung beetles associated with the large herbivores in the area. The exclosure in The Zwin was located in the subarea 'Kleyne Vlakte' (51°21'21"N, 3°20'41"E, elevation 3 m a.s.l.) which is a relatively open landscape consisting mainly of grassland (most affinity with *Arrhenatherion* grassland communities), while the exclosure in Kalmthout was located in the subarea 'Biezenkuilen' (51°24'54"N, 4°25'10"E, elevation 18 m a.s.l.) and is covered by heathland (*Genisto anglicae-Callunetum typicum*). The soil in both areas consists predominantly of sand: 85.7% sand, 7.6% silt and 6.7% clay (USDA texture class loamy sand) in The Zwin and 97.4% sand, 1.5% silt and 1.1% clay (USDA texture class sand) in Kalmthout.

*Functional groups*

126　We focussed on quantifying two ecosystem functions implemented by different functional groups of

127　coprophagous fauna: dung removal and the redistribution of endozoochorously dispersed seeds

128　(secondary seed dispersal). Dung beetles were classified in three functional groups according to their

129　dung processing behaviour: rollers, tunnelers, and dwellers. A further classification was made based on

130　body size. Tunnelers and rollers were classified as either small or large depending on their ability to move

131　through a 1 cm² mesh (Slade et al. 2007). Dwellers are generally small beetles and as they do not actively

132　move dung, no further size differentiation was made. Furthermore, other soil fauna, such as earthworms,

133　are often major dung decomposers in Northern Europe (Gittings et al. 1994, Gittings and Giller 1999).

134　Therefore, the dung removing behaviour of all other soil fauna able to go through 1 cm² mesh but

135　stopped by 1 mm² mesh was measured as well (ESM 2).

136　*Experimental design*

137　The experiment was replicated using three dung types of domestic grazers present in or close-by the

138　study sites: cattle, horses, and sheep. Three sections were marked within each fenced exclosure, one for

139　each dung type. In each section, the experimental units and sampling units were put in a fully randomized

140　design (ESM 3). Individual experimental units and sampling units were on a spacing of 60 cm, and

141　different sections were at a distance of 2 m.

142　Eleven types of experimental units were designed by combining different kinds of ground screens, vertical

143　'walls' and/or 'ceilings' in order to include or exclude dung removal activities by the distinguished

144　functional groups (ESM 4). Each experimental unit had a square ground surface of 40 cm by 40 cm and

145　walls were 15 cm high. Ground screens were used to exclude tunneler activity, while vertical walls

146　prevented dung removal by rolling species. Ground screens and walls were made of plastic mesh, of which

147　the mesh size determined the size class of beetles that were allowed to enter the experimental unit: 1

148　cm² to include small beetles, but to exclude large beetles, and 1 mm² to exclude all beetle activity. In case

149　no ground screen or wall was used, respectively tunnelers and rollers of all size classes were able to move

150　dung. As dwellers do not relocate dung, dung removal by this group could only be prevented by excluding

151　all dung beetle activity in a combination of walls and a ceiling in fine mesh material or by combining a

152  fine-meshed ground screen, walls and a ceiling (respectively treatments 10 and 11 in ESM 4). In

153  treatments using a coarse meshed ground screen or no ground screen, the dung removing activities of

154  ground fauna other than dung beetles and unable to move through 1 mm² mesh were inevitably included.

155  Dung removal by the latter was measured in treatment 10 (ESM 4). Each type of experimental unit was

156  replicated six times for each dung type, which resulted in a total number of 198 experimental units in

157  each study area (ESM 3).

158  *Experimental setup*

159  Before starting the experiment the vegetation within the exclosures was cut to a height of ca. 3 cm in

160  order to enable constructing the experimental units on a level surface. Fresh dung was collected on site

161  from animals that had not been treated with anthelmintics in at least the preceding 6 weeks. Drug-free

162  dung was preferred as anthelmintics have been proven to alter the attractiveness of dung and to have a

163  negative impact on dung beetles (Wardhaugh and Mahon 1991, Ridsdill-Smith 1993, Holter et al. 1993).

164  Once collected, the dung was homogenized and divided into standard amounts of 300 g for cattle dung

165  and 200 g for horse and sheep dung and frozen at -20°C for at least two days (as in e.g., Slade et al. 2007)

166  in order to kill all groups of biota that consume a considerable part of dung (e.g., dung beetles,

167  earthworms, fly larvae,…) or interfere with the experiment in a different way (e.g., predatory beetles

168  hunting for fly or dung beetle larvae). Seeds of *Galium aparine* and caryopses of *Alopecurus myosuroides*

169  and *Poa pratensis* were used as proxies in the seed dispersal experiment. This selection of species was

170  based on their differing shape and size, which is elongated and large (6 mm), spherical and medium-sized

171  (3 mm) and elongated and small (1 mm) for *A. myosuroides*, *G. aparine,* and *P. pratensis* respectively. All

172  seeds were purchased in a specialized seed web shop ([www.herbiseed.com](www.herbiseed.com)). In order to avoid seed loss

173  caused by germinating seeds during the experiment, seeds were, prior to the experiment, sterilized by dry

174  heating at 80°C during 7 days. As after 60 days, no seedlings emerged in the subsequent germinability test

175  on 1% water agar in lab conditions, we assumed that no germination during the field experiment would

176  occur. Each seed species was spray-painted in distinct fluorescent colours in order to increase visibility.

177    Ten seeds of each species were mixed with the homogenized dung portions (either cattle dung (300 g),

178    horse dung (200 g) or sheep dung (200 g)).

179    At the start of the experiment, 1 dung portion was put in the centre of each experimental unit and left on

180    site during 4 weeks. The dung removal experiment was replicated in 2 seasons, summer and autumn, with

181    July 30th 2014 and September 16th 2014 as the respective starting dates. The seed dispersal experiment

182    was conducted once, simultaneously with the dung removal experiment in summer.

183    *Dung beetle sampling*

184    During each experimental run, the dung beetle assemblage was sampled in each study area. To achieve a

185    complete view on dung beetle diversity, two types of pitfall traps were used (ESM 5). The first trap type

186    consists of one large 1 l container covered by hexagonal chicken wire (mesh size 25 mm) and a nylon bag

187    filled with dung in the middle (Larsen and Forsyth 2005). The second trap type consists of five smaller 0.2 l

188    containers surrounding a central dung pile of approximately 300 g (D'hondt et al., 2008). In both cases, 2

189    stacked containers were used to ease the emptying, and the upper rim of the containers was levelled with

190    the soil surface. A saturated water-salt solution (365 g/l NaCl with some drops of unscented detergent)

191    was used as fixation fluid. The traps were placed randomly between the experimental units in a

192    randomized pattern with six replicates per trap type (ESM 3). As the dung beetle diversity and activity is

193    known to differ between dung types (Finn and Giller 2002), each trap was baited with one of the used

194    dung types in the experiments: cattle, horses, and sheep. In order to avoid interference with the dung and

195    seed removal experiments, traps were put in operation one week after the start of the experiment. All

196    traps were emptied weekly during the experiments in order to measure dung beetle abundance and

197    diversity with ageing dung. Scarabaeoidea species were identified using Janssens (1960), Jessop (1986)

198    and Baraud (1992). Five specimens of each dung beetle species were dried at 60 °C during 5 days in order

199    to measure average dry biomass. As some members of the Hydrophilidae (mainly *Sphaeridium* and

200    *Cercyon* species) are commonly found in dung in temperate regions (Finn et al. 1999), the number of

201    individuals in this group was counted as an estimate of their abundance. However, as species in this group

202    are not coprophagous during their entire life cycle and do not move dung laterally or vertically, this group

203 was further not considered in the analyses and dung beetles were strictly defined as beetles belonging to

204 the Scarabaeidae and Geotrupidae families. Dung beetle assemblage was defined by species diversity

205 (number of species), total abundance and Shannon diversity index (Jost 2006). Total biomass was

206 calculated per dung type, experimental period and study site by multiplying and summating species

207 abundance and specific biomass.

208 *Measurements*

209 Of each dung type, three reference samples were taken from the same dung batch that was used in the

210 experiments. The fresh reference samples were oven-dried at 60 °C and their dry weight was

211 subsequently measured for use as a proxy of the wet: dry ratio of fresh dung. At the end of each

212 experimental period, the remaining dung in the experimental units was collected and oven-dried at 60 °C.

213 Once completely dry, each sample's weight was recorded and the amount of seeds left in the summer

214 samples was counted. To facilitate the counting, samples were pulverized using a blender in short pulses

215 during 30 s. A black light was used to visualize the fluorescent seeds among the dung particles.

216 Dung removal was calculated as:

$$M_{removed} = \frac{M_{reference} - M_{sample}}{M_{reference}} \ x \ 100$$

217 where $M_{reference}$ is the average dry mass of the reference samples put in the units at the start of the

218 experiment and $M_{sample}$ is the dry mass of the remaining dung at the end of the experiment. Seed dispersal

219 was expressed similarly as:

$$S_{dispersed} = \frac{S_{initial} - S_{retrieved}}{S_{initial}} \ x \ 100$$

220 where $S_{initial}$ is the number of seeds put in the dung samples and $S_{retrieved}$ is the number of seeds retrieved

221 from the samples at the end of the experiment.

222 *Statistics*

223    All statistical analyses were performed in R version 3.2.2 (R Core Team 2015). Dung preference for each

224    individual species was specified using chi-squared tests for each season and study site. Species diversity

225    and abundance data were analysed in relation to dung age using generalized mixed-effects models

226    (GLMMs, Zuur et al. (2009)) with dung type, trap type, season, study site and dung age as fixed effects and

227    a nested trap location : trap type term as random effect. Species diversity was fitted using the glmer

228    function in the lme4 package (Bates et al. 2014) with Poisson error structure. The abundance count data

229    were over-dispersed and were therefore fitted with a quasi-Poisson error structure using the glmmPQL

230    function in the MASS package (Venables and Ripley 2002). Biomass was similarly analysed using a linear

231    mixed-effects model with the lme function in the nlme package (Pinheiro et al. 2015). Prior to the

232    analyses, biomass values were square root transformed in order to meet the requirements of normal

233    distribution of the residuals. Models were built for the entire dung beetle assemblage, and each of the

234    functional groups. The resulting models were simplified using a backward selection process in which

235    covariates were eliminated based on AIC values (Bolker et al. 2009). Significant differences between dung

236    types were determined using Tukey posthoc tests with package multcomp (Hothorn et al. 2008).

237    As no rollers were found and treatments that differ only in the inclusion or exclusion of rollers did not

238    differ significantly ($F_{4,6}$= 0.636, $P$=0.701), the number of treatment classes was reduced to five classes:

239    complete assemblage (complete), complete assemblage with the exclusion of large tunnelers (complete-

240    T), dwellers (dwellers), soil fauna (soilF) and negative control (control) (table 1). Differences between

241    treatments, study sites, dung types and seasons were analysed using ANOVA and Tukey posthoc tests.

242    Transformations were applied to dung removal and seed dispersal ratios in order to meet the

243    requirements for parametric tests (log(x+1) for dung removal, large seed, and small seed dispersal;

244    arcsine square root for medium seeds).

245    We used hierarchical partitioning (Chevan and Sutherland 1991) to examine the independent effect of

246    each functional group on dung removal and seed dispersal. Hierarchical partitioning is a multiple

247    regression technique in which all possible models are jointly considered in an attempt to identify the most

248    likely causal factors (Mac Nally 2000, Chevan and Sutherland 1991). A binomial error structure was

249    implemented in the models and significance levels were achieved after running randomization tests with

250    1000 iterations. Hierarchical partitioning and randomization tests were implemented using the hier.part

251    package in the R environment (Walsh and Mac Nally 2013). The relation between seed dispersal and dung

252    removal was assessed by a multiple regression including seed sizes and study sites.

**Results**

*Dung beetle fauna*

In total 7845 dung beetles were sampled representing 17 species, of which 2 species were classified as large tunnelers, 2 species as small tunnelers and 13 species as dwellers (table 2). No rollers were found at either study site. The large tunnelling species *Typhaeus typhoeus* was found exclusively in Kalmthout in relatively large numbers during the autumn experiment in cattle and sheep dung. In Kalmthout, the relative abundance of functional groups largely varies between seasons in summer 60% of the sampled dung beetles are tunnelers this number drops to 5% in autumn. Nevertheless, the increase in dwellers during the autumn experiment in Kalmthout can almost entirely be attributed to *Nimbus contaminatus* and *Melinopterus prodromus* which make up respectively 73 and 26% of the total number of dwellers sampled in autumn (table 2). Some of the rare species were found exclusively in The Zwin (*Onthophagus coenobita* (2), *Agrilinus ater* (1) and *Melinopterus consputus* (1)) or Kalmthout (*Chilothorax distinctus* (6)). More species were found during the autumn experiment (*Wald Z-test:* z= 0.082, *P*<0.001), *Typhaeus typhoeus*, *Chilothorax distinctus*, *Melinopterus consputus*, *Melinopterus sphacelatus*, *Melinopterus prodromus* and *Nimbus contaminatus* were trapped exclusively in autumn (table 2). For *M. prodromus* and *N. contaminatus*, we collected the largest number of individuals: 1736 and 5098 respectively.

Overall, more dung beetle species were found in The Zwin (*Wald Z-test:* z= -3.512, *P*<0.001), although dung beetles were collected more abundantly in Kalmthout (*Wald t-test:* t= -6.630, *P*<0.001) and especially in autumn (*Wald t-test:* t= -8.019, *P*<0.001) (table 3). Similarly, more biomass of both dweller and tunneller species was collected in Kalmthout (*Wald t-test:* t= -11.014, *P*<0.001) and during the autumn experiment (*Wald t-test:* t= -7.683, *P*<0.001). None of the used dung types had a significant effect on tunneler diversity, while more dweller species were found in sheep dung compared to cattle dung (*Wald Z-test:* z= 2.567, *P*= 0.028). Dung beetles clearly preferred horse dung over cattle (*Wald Z-test:* z= -6.443, *P*<0.001) or sheep dung (*Wald Z-test:* z= -5.490, *P*<0.001). Overall, a higher abundance and diversity of dwellers was found and both diversity and abundance decreased with ageing dung for all considered functional groups (figure 1).

*Dung removal and seed dispersal*

280     On average, more dung was removed in The Zwin than in Kalmthout, especially in summer (tables 4, 5). In

281     The Zwin significantly more dung was removed during the summer experiments while in Kalmthout no

282     seasonal differences have been found. In summer, more horse dung was removed compared to cattle and

283     sheep dung in The Zwin, while significantly more sheep dung was removed in Kalmthout. In both areas,

284     significant differences between treatments were found (table 4). Dung removal by soil fauna was

285     significant in The Zwin (summer and autumn) and in Kalmthout during the autumn experiment (figure 2).

286     Large tunnelers removed a large amount of dung of all tested dung types in Kalmthout during the autumn

287     experiment, while this effect was only significant for horse dung in the summer experiment. In The Zwin

288     large tunnelers removed more dung during the summer experiment; while in the autumn experiment

289     both large and small tunnelers were equally important dung removers. In both study sites and seasons,

290     the contribution of dwellers in dung removal was insignificantly small (figure 2). Seed removal was

291     positively correlated with dung removal, although differences between study sites and seed sizes were

292     found (tables 4, 6). More large, medium and small seeds were removed in The Zwin compared to

293     Kalmthout, which is in line with our results on dung removal (table 5). Large seeds were primarily

294     dispersed by ground fauna other than dung beetles in both study areas (figure 2). Generally, the role of

295     each functional group in seed dispersal was similar to their importance in dung removal. However,

296     dwellers dispersed large amounts of small seeds in cattle and horse dung in Kalmthout, while their effect

297     on dung removal was limited. Also, small tunnelers dispersed more large seeds compared to large

298     tunnelers in Kalmthout, while the opposite was found in dung removal (figure 2).

**Discussion**

*Dung beetle diversity and abundance*

Overall, dung beetle assemblages in both study sites are dominated by dweller species which corresponds to what may be expected at this latitude. The functional composition of dung beetle assemblages is known to vary between biogeographical regions with a shift from dweller dominated assemblages in the northern climatic zones towards a dominance of larger tunnelers and rollers in southern biogeographical regions (Hanski and Cambefort 1991, Verdú and Lobo 2008, Hortal et al. 2011). *M. prodromus*, *M. sphacelatus* (Gittings and Giller 1997) and *N. contaminatus* (Hanski 1980) generally do not breed in dung. Still, as in our study and previous studies (Hutton and Giller 2003, Finn et al. 1999) these species with generalist saprophagous larvae were found abundantly in dung baited traps, dung may be an important food source for the imago's of these species. Mass occurrences of generalist dweller species such as *M. prodromus* and *N. contaminatus* in our study have been reported widely in northern temperate ecosystems, but it seems to occur infrequently (Finn and Gittings 2003, Holter 1982, Hanski and Cambefort 1991). Although mass occurrence events certainly lead to reproduction failure in other dweller species due to the rapid dung decomposition associated with these events, the mechanism of mass occurrences is not yet revealed (Finn and Gittings 2003). We should also keep in mind that relatively large abundances of *Cercyon* and *Sphaeridium* species have been trapped which suggests that these species have their share in dung removal. Despite the fact that the larvae of these Hydrophilids are predators on fly larvae in dung (Sowig 1997, Hanski and Koskela 1979), adults have a similar feeding strategy as dung beetles (Holter 2004) and oviposit in dung, which makes them functionally equivalent to dwelling dung beetles sensu stricto. As our experimental setup did not allow for discrimination between the activity of Hydrophilidae and Scarabaeidae species, ecological functions provided by dwellers should be interpreted as the sum of the action of both groups.

*Dung as a food source*

Dung beetles clearly prefer fresh dung as both the diversity and the abundance of the sampled dung beetles rapidly declines after the first week. Previous research in the temperate climate zone also found a

15

325    clear preference for fresh dung (Finn and Giller 2002) which might be related to the loss of scent

326    (Dormont et al. 2004, 2007) and water content (Holter and Scholtz 2007) of older dung. Our results

327    suggest that tunnelers remove most dung in the first days following dung deposition and dwellers make

328    chambers and oviposit in fresh dung pats.

329    Furthermore, most species prefer a certain type of dung, but this effect is not always straightforward

330    when comparing seasons and study sites. In most cases species either prefer dung from hindgut

331    fermenters (horses) or ruminants (cattle and sheep). This preference can be addressed to the different

332    consistency of both dung types as dung of hindgut fermenters mostly consists of light, uncompressed

333    dung containing large plant particles (Steuer et al. 2013) while ruminant dung has a smaller average

334    particle size and is much denser. As 40-50% of the dry fraction of fresh cattle, horse and sheep dung

335    contains particles small enough for dung beetle ingestion (Holter 2000), the preference for a certain dung

336    type is most likely not driven by a shortage of food resources but rather by nesting strategies. Therefore,

337    the use of different herbivore species in nature management results in more diverse dung beetle

338    assemblages, but on the other hand, reduced dung beetle diversity may lead to slower dung degradation

339    as certain specialized species might be missing.

340    *Dung removal rate*

341    Tunnelers, and especially large tunnelers, removed a large proportion of dung whereas dwellers had no

342    significant effect on dung removal. In addition to dung beetles, the presence of epigeic earthworms, dung

343    flies and their combinations can largely alter the decomposition of dung (O'Hea et al. 2010). While the

344    dung removing activity of coprophagous dipteran larvae resembles the behaviour of dwellers,

345    earthworms actively move dung through tunnels beneath dung pats which is comparable to the action of

346    tunnelers. Our set-up did not allow differentiating dung removal by dwellers and flying larvae, but as in

347    treatment soilF only digging macro-invertebrates were able to reach the dung pat, we make an estimate

348    of the impact of this group. In both areas, digging soil macro-invertebrates other than dung beetles were

349    important dung removers. According to Gittings et al. (1994) and Gittings and Giller (1999), other soil-

350    inhabiting macro-invertebrates, such as earthworms, can fulfil an important role in dung decomposition in

northern Europe. In an extensive review of competitive interactions between dung fauna, Finn and Gittings (2003) also classify earthworms as a separate functional group for which dung is an important food source in both the adult and larval stage. Especially in cool, wet weather, earthworms can even remove an entire dung pat before dweller larvae have completed their development (Gittings and Giller 1999). Although we did not quantify them, earthworms were indeed abundantly present in dung at both study sites. The high dung removal in the treatments in which only soil organisms acted as dung manipulating fauna suggests that earthworms fulfil an equal role in dung removal as tunnelling dung beetles. The fact that equal portions of dung are removed by earthworms alone and the complete functional assemblage suggests that competition takes place between large tunnelers and earthworms and opposes the findings of Holter (1983) who concludes that dung with beetles is more attractive to earthworms than dung without dung beetles. On the other hand, Rosenlew and Roslin (2008) conclude that large tunnelers exceed earthworms in dung removal. In our study, large tunnelers were important dung removers as well, but the amount of dung removed by this group differs between sites and seasons. Although large tunnelers were clearly more abundantly present in Kalmthout, dung removal by this group was lower but still rather high in the Zwin. Furthermore, dung beetle biomass is relatively high at both sites which is in line with the positive correlation between tunneler biomass and dung removal found earlier (Horgan 2005) and which is most apparent for large fast-burying tunnelers on sandy soils (Davis 1996) such as our study sites.

*Secondary seed dispersal*

In tropical biomes dung beetles generally do not treat dung with seeds differently than seed-free dung, given that the seeds are relatively small in comparison to the beetle (Andresen 2002a, Slade et al. 2007, Braga et al. 2013). Our results suggest similar behaviour of dung beetles in temperate ecosystems as a clear linear relation between dung removal and seed dispersal was found, although this was not the case for all seed size classes. Tunnelers and soil fauna are the main dispersers of large and medium seeds, while these patterns were less apparent for the smallest seed class. As high removal rates were found in the control treatments and the treatments including dwellers which are not known to move dung, these results should be interpreted with caution. Despite the efforts put in the recollection of seeds, it is

probable that a portion of seeds was overlooked or lost due to other reasons, such as seed loss following heavy rain. Using a larger amount of seeds as a proxy could overcome this problem. Unlike in the tropics, dung beetle assemblages in the northern temperate region are mainly composed by dwelling and tunnelling species and generally lack rolling species. As a result, seeds are almost exclusively dispersed in a vertical direction instead of the combination of horizontal and vertical dispersal in case rollers are present. Seed burial is often considered beneficial for seed survival as it is hidden for seed predators such as rodents (Shepherd and Chapman 1998). On the other hand, germination and establishment probabilities decrease when seeds are buried too deep. Unlike the numerous examples of positive effects of dung beetle activity on seed germination and seedling establishment in the tropics (e.g., Andresen and Levey 2004, Shepherd and Chapman 1998)), D'hondt et al. (2008) found a negative correlation in a coastal grassland in the northern temperate zone; they ascribe this to large tunnelling species that bury seeds too deep. As also earthworms are known to dig deep (Ojha and Devkota 2014) and body size and tunnel depth are positively correlated in tunnelers (Gregory et al. 2015), seedling establishment could be similarly affected as in the study of D'hondt et al. (2008).

We conclude that ecological functions performed by dung beetles are of great importance in northern temperate ecosystems and differ between functional groups. Although more dweller species were found, large tunnelers are disproportionally important in dung burial and seed removal. The dung decomposing activities of small tunnelers and other soil inhabiting fauna, such as earthworms, is of prime importance as well, which stresses the need for conservation of functionally complete dung ecosystems.

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586 **Figure captions**

587 **Fig. 1** Abundance (number of individuals per sampling) in relation to dung age by functional groups

588 (tunnelers and dwellers) and by dung type (cattle, horse and sheep) in summer and autumn. Symbol sizes

589 reflect species richness. Lines are fitted from GLMM's with trap location and trap type as random effect

590 **Fig. 2** Hierarchical partitioning results for dung removal (summer and autumn experiment) and dispersal

591 of large, medium and small seeds by each functional group: dwellers (D), large tunnelers (T), small

592 tunnelers (t) and other soil inhabiting fauna (S). The percentage of independent effect contributed by

593 each of the functional groups is plotted for the studied dung types. Symbols indicate whether the

594 contribution of each functional group is significant ($p < 0.05$) for cattle (asterisks), horse (hash tags) and

595 sheep dung (filled dots) after evaluation of the Z-scores of randomization tests with 1000 iterations

**Figures**
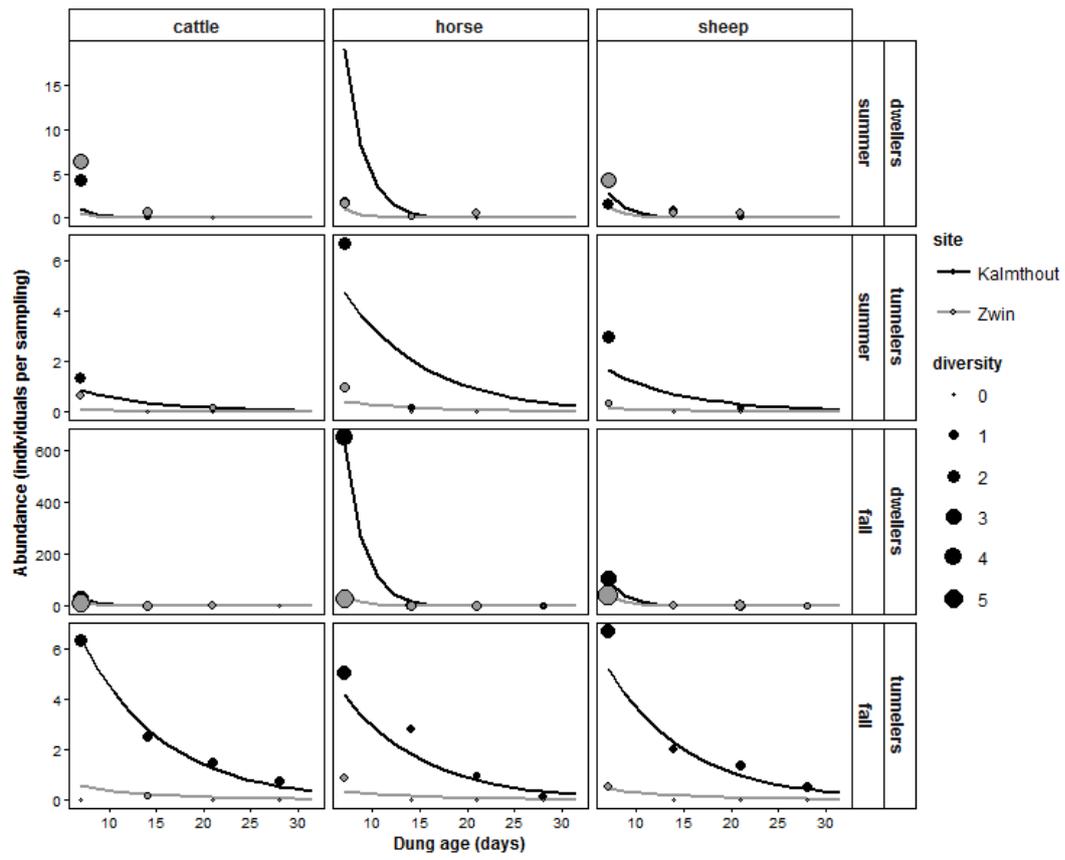


Fig. 1
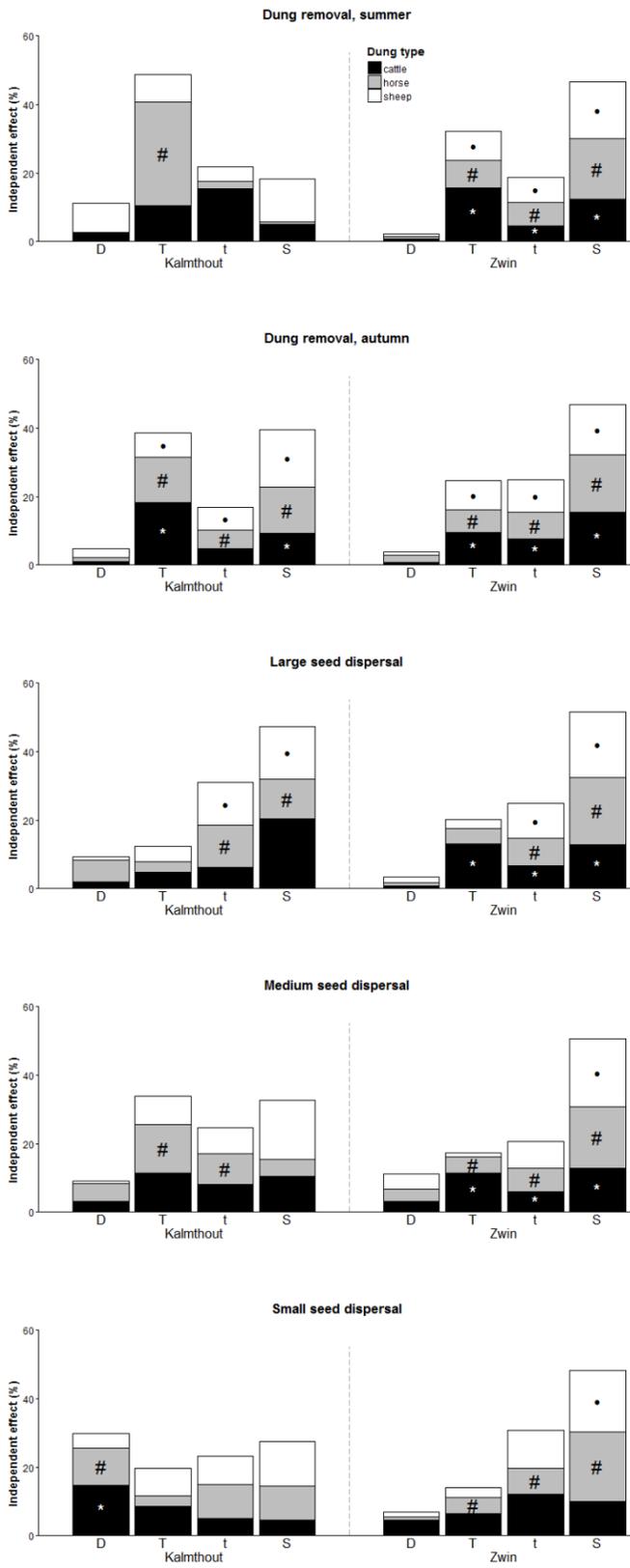
Fig. 2

**Tables**

**Table 1** Treatments defined in the dung removal and seed dispersal experiments, with the functional groups able to process dung, the experimental unit types and the number of replicates for each treatment using 1 dung type (N). The inclusion or exclusion of functional groups is marked with respectively + and -. A full description of the experimental unit types can be found in ESM 4.

| | functional groups | | | | experimental unit | |
| treatment | dwellers | large tunnelers | small tunnelers | soil fauna | type | N |
|---|---|---|---|---|---|---|
| complete | + | + | + | + | 1, 4, 5 | 18 |
| complete-T | + | - | + | + | 3, 7, 9 | 18 |
| dwellers | + | - | - | - | 2, 6, 8 | 18 |
| soilF | - | - | - | + | 10 | 6 |
| control | - | - | - | - | 11 | 6 |

**Table 2** Total number of individuals of each species sampled by study area, season and used dung bait. Asterisks indicate significant differences between dung types after chi-square tests with ***: $P<0.001$, **: $P<0.01$, *: $P<0.05$.

| Sampled species | Functional group | Individual biomass (g) | Kalmthout | | | | | | Zwin | | | | | |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| | | | Summer | | | Autumn | | | Summer | | | Autumn | | |
| | | | cattle | horse | Sheep | cattle | horse | sheep | cattle | horse | sheep | cattle | horse | sheep |
| **Geotrupidae Latreille, 1802** | | | | | | | | | | | | | | |
| *Geotrupes spiniger* (Marsh., 1802) | tunneler (large) | 0.3662 | 9 | 34 | 19*** | 3 | 21 | 25*** | 1 | 3 | 1 | 3 | 3 | 0 |
| *Typhaeus typhoeus* (L., 1758) | tunneler (large) | 0.1111 | 0 | 0 | 0 | 149 | 45 | 79*** | 0 | 0 | 0 | 0 | 0 | 0 |
| **Scarabaeidae Latreille, 1802** | | | | | | | | | | | | | | |
| *Onthophagus coenobita* (Hbst., 1783) | tunneler (small) | 0.0154 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| *Onthophagus similis* (Scriba, 1790) | tunneler (small) | 0.0044 | 4 | 60 | 14*** | 4 | 15 | 9* | 6 | 4 | 2 | 0 | 9 | 7* |
| *Acrossus rufipes* (L., 1758) | dweller | 0.0255 | 29 | 12 | 11** | 1 | 2 | 11** | 17 | 0 | 17*** | 20 | 9 | 28** |
| *Agrilinus ater* (DeGeer, 1774) | dweller | 0.0032 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| *Aphodius fimetarius* (L., 1758) | dweller | 0.0098 | 0 | 0 | 1 | 3 | 0 | 0* | 1 | 0 | 1 | 0 | 0 | 0 |
| *Aphodius foetens* (F., 1787) | dweller | 0.0121 | 4 | 7 | 7 | 2 | 3 | 1 | 7 | 9 | 7 | 2 | 8 | 1* |
| *Bodilopsis rufa* (Moll., 1782) | dweller | 0.0031 | 4 | 1 | 12** | 0 | 0 | 7*** | 8 | 0 | 17*** | 18 | 0 | 18** |
| *Chilothorax distinctus* (Müll., 1776) | dweller | 0.0001 | 0 | 0 | 0 | 0 | 4 | 2 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Melinopterus consputus* (Creutz., 1799) | dweller | 0.0016 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| *Melinopterus prodromus* (Brahm, 1790) | dweller | 0.0030 | 0 | 0 | 0 | 132 | 1152 | 363*** | 0 | 0 | 0 | 16 | 28 | 45*** |
| *Melinopterus sphacelatus* (Panz., 1798) | dweller | 0.0027 | 0 | 0 | 0 | 4 | 15 | 3** | 0 | 0 | 0 | 9 | 31 | 28** |
| *Nimbus contaminatus* (Hbst., 1783) | dweller | 0.0029 | 0 | 0 | 0 | 116 | 4161 | 386*** | 0 | 0 | 0 | 49 | 211 | 175*** |
| *Otophorus haemorrhoidalis* (L., 1758) | dweller | 0.0024 | 1 | 0 | 0 | 0 | 0 | 0 | 10 | 0 | 7*** | 2 | 0 | 6* |
| *Teuchestes fossor* (L., 1758) | dweller | 0.0257 | 2 | 0 | 2 | 0 | 0 | 0 | 4 | 0 | 3 | 0 | 0 | 0 |
| *Volinus sticticus* (Panz., 1798) | dweller | 0.0024 | 0 | 0 | 0 | 1 | 6 | 2 | 2 | 10 | 4* | 3 | 8 | 3 |
| **Hydrophilidae Latreille, 1802** | | | | | | | | | | | | | | |
| *Cercyon* spec. | other | | 33 | 51 | 40 | 71 | 30 | 64*** | 52 | 59 | 62 | 15 | 6 | 14 |
| *Sphaeridium* spec. | other | | 1 | 7 | 9* | 4 | 13 | 8 | 31 | 0 | 3*** | 0 | 7 | 1** |
| **Total number**[1] | | | 53 | 114 | 66 | 415 | 5424 | 888 | 57 | 26 | 60 | 122 | 307 | 313 |
| **Biomass (g)** [1] | | | 4.167 | 13.111 | 7.484 | 18.486 | 28.281 | 20.482 | 1.079 | 1.249 | 1.074 | 1.913 | 2.257 | 1.562 |
| **Species richness**[1] | | | 7 | 5 | 7 | 10 | 10 | 11 | 10 | 4 | 10 | 9 | 8 | 11 |
| **Shannon entropy**[1] | | | 1.414 | 1.149 | 1.703 | 1.304 | 0.646 | 1.237 | 1.953 | 1.272 | 1.864 | 1.721 | 1.150 | 1.460 |

[1] calculated using Geotrupidae and Scarabaeidae species only.

**Table 3** Results of the generalized linear mixed-effects models (GLMM) with diversity and abundance as response variables, and the linear mixed-effects model with biomass as response variable. In each model the nested term trap location : trap type was used as random effect.

| Functional group | Fixed effects | df | Diversity Wald $\chi^2$ | Diversity P | Abundance Wald $\chi^2$ | Abundance P | Biomass Wald $\chi^2$ | Biomass P |
|---|---|---|---|---|---|---|---|---|
| all | dung age | 1 | 396.891 | <0.001 | 90.485 | <0.001 | 177.694 | <0.001 |
| | study site | 1 | 12.171 | <0.002 | 40.580 | <0.001 | 124.019 | <0.001 |
| | trap type | 1 | 12.426 | <0.003 | 20.582 | <0.001 | 3.210 | 0.073 |
| | season | 1 | 90.301 | <0.004 | 49.726 | <0.001 | 60.346 | <0.001 |
| | dung type | 2 | 7.362 | 0.0252 | 59.216 | <0.001 | 0.880 | 0.644 |
| | study site : dung type | 2 | 2.646 | 0.266 | 11.048 | 0.004 | 0.799 | 0.671 |
| | season : dung type | 2 | 3.855 | 0.146 | 5.667 | 0.059 | 1.267 | 0.531 |
| | trap type : dung type | 2 | 6.651 | 0.0360 | 0.513 | 0.774 | 1.052 | 0.591 |
| tunnelers | dung age | 1 | 76.488 | <0.001 | 125.586 | <0.001 | 78.434 | <0.001 |
| | study site | 1 | 80.462 | <0.001 | 97.665 | <0.001 | 140.675 | <0.001 |
| | trap type | 1 | 16.622 | <0.001 | 0.094 | 0.759 | 0.237 | 0.627 |
| | season | 1 | 0.166 | 0.684 | 20.707 | <0.001 | 32.965 | <0.001 |
| | dung type | 2 | 1.764 | 0.414 | 2.032 | 0.362 | 0.010 | 0.995 |
| | study site : dung type | 2 | 0.256 | 0.880 | 0.820 | 0.664 | 0.776 | 0.678 |
| | season : dung type | 2 | 3.861 | 0.145 | 36.106 | <0.001 | 8.826 | 0.012 |
| | trap type : dung type | 2 | 1.544 | 0.462 | 0.545 | 0.762 | 1.361 | 0.506 |
| dwellers | dung age | 1 | 313.053 | <0.001 | 32.591 | <0.001 | 547.605 | <0.001 |
| | study site | 1 | 6.143 | 0.013 | 19.722 | <0.001 | 3.092 | 0.079 |
| | trap type | 1 | 65.828 | <0.001 | 15.311 | <0.001 | 10.885 | <0.001 |
| | season | 1 | 15.537 | <0.001 | 14.072 | <0.001 | 92.347 | <0.001 |
| | dung type | 2 | 6.154 | 0.046 | 37.233 | <0.001 | 9.286 | 0.010 |
| | study site : dung type | 2 | 2.990 | 0.224 | 7.648 | 0.022 | 9.001 | 0.011 |
| | season : dung type | 2 | 14.623 | <0.001 | 3.434 | 0.180 | 21.034 | <0.001 |
| | trap type : dung type | 2 | 5.396 | 0.067 | 0.339 | 0.844 | 1.717 | 0.424 |

**Table 4** ANOVA results for the removal of dung and dispersal of large, medium and small seeds.

| Factor | Df | Dung removal | | Large seeds | | Medium seeds | | Small seeds | |
|---|---|---|---|---|---|---|---|---|---|
| | | F value | *P* | F value | *P* | F value | *P* | F value | *P* |
| study site | 1 | 155.307 | <0.001 | 60.313 | <0.001 | 212.062 | <0.001 | 127.330 | <0.001 |
| season | 1 | 21.817 | <0.001 | | | | | | |
| dung type | 2 | 7.712 | <0.001 | 14.139 | <0.001 | 18.408 | <0.001 | 3.200 | 0.042 |
| treatment | 4 | 64.342 | <0.001 | 24.870 | <0.001 | 22.093 | <0.001 | 5.483 | <0.001 |
| study site: season | 1 | 13.118 | <0.001 | | | | | | |
| study site: dung type | 2 | 9.446 | <0.001 | 4.551 | 0.011 | 20.772 | <0.001 | 16.240 | <0.001 |
| dung type : season | 2 | 15.307 | <0.001 | | | | | | |
| study site : treatment | 4 | 9.887 | <0.001 | 3.635 | 0.006 | 4.572 | 0.001 | 4.600 | 0.001 |
| dung type : treatment | 8 | 0.940 | 0.482 | 0.618 | 0.763 | 1.874 | 0.062 | 1.638 | 0.113 |

**Table 5** Average values and standard errors for dung removal (%) and seed dispersal (%) by study site and dung type. Different letters in the same column indicate significant differences between treatments within each measured variable and season (ANOVA and Tukey posthoc tests). Asterisks indicate significant differences between sites (column measured variable) or seasons (column season).

| Measured variable | Season | Treatment | Kalmthout | | | Zwin | | |
|---|---|---|---|---|---|---|---|---|
| | | | cattle | horse | sheep | cattle | horse | sheep |
| dung removal* | summer* | complete | 29.1±3.0a | 46.2±4.2q | 44.9±3.6x | 87.8±1.5a | 78.4±2.6q | 60.7±3.9x |
| | | complete-T | 34.2±1.9a | 38.0±2.5q | 42.7±3.2x | 46.5±3.9b | 64.2±2.2r | 50.7±2.5xy |
| | | dwellers | 28.0±3.0a | 41.6±0.7q | 41.3±0.9x | 26.6±2.4c | 45.6±2.6s | 38.2±2.7y |
| | | soilF | 27.2±3.9a | 42.0±1.6q | 47.0±5.8x | 76.7±9.4a | 77.6±3.5q | 57.3±8.0x |
| | | control | 32.0±2.8a | 41.2±1.8q | 43.1±1.7x | 21.7±4.5c | 41.9±1.8s | 34.5±5.0y |
| | autumn* | complete | 53.6±7.6a | 46.9±5.0q | 48.1±4.9x | 53.8±1.5a | 52.1±1.8q | 51.9±2.3x |
| | | complete-T | 35.0±1.7ab | 31.6±1.7qr | 32.9±2.0y | 46.9±1.8b | 48.4±1.3qr | 45.7±1.0xy |
| | | dwellers | 36.0±2.1ab | 28.8±1.3r | 28.9±2.5y | 38.0±0.8c | 42.8±1.5rs | 34.9±1.3z |
| | | soilF | 51.2±12.9ab | 50.0±9.7q | 64.9±10.4x | 50.3±2.8ab | 50.0±2.0qr | 46.0±3.3xy |
| | | control | 26.1±1.5b | 21.5±2.0r | 21.4±1.3y | 38.0±1.6c | 37.5±0.8s | 35.4±4.8yz |
| large seeds* | summer | complete | 17.8±3.6a | 45.0±6.9q | 48.8±6.2x | 72.2±6.2a | 66.7±5.8q | 56.7±6.0x |
| | | complete-T | 20.6±5.1a | 42.8±4.9q | 45.6±6.4x | 43.9±6.8bc | 57.2±5.9q | 55.6±6.1x |
| | | dwellers | 17.2±3.1a | 22.8±3.3r | 27.6±4.2x | 20.7±3.2bc | 30.6±5.8r | 36.7±5.0x |
| | | soilF | 23.3±6.1a | 26.0±4.0qr | 40.0±8.6x | 53.3±12.8ab | 70.0±8.9q | 56.0±4.0x |
| | | control | 13.3±5.6a | 6.0±4.0r | 28.3±7.5x | 13.3±5.6c | 30.0±5.2r | 28.3±8.7x |
| medium seeds* | summer | complete | 22.8±4.1a | 60.6±6.1q | 50.6±6.4x | 95.6±1.5a | 94.4±2.3q | 63.9±6.1x |
| | | complete-T | 15.6±2.0a | 45.6±6.2qr | 43.1±7.1x | 69.4±4.7b | 83.3±3.7qr | 64.4±5.7x |
| | | dwellers | 17.8±3.0a | 36.7±4.6r | 33.5±4.4x | 41.3±7.4c | 66.1±7.1rs | 51.7±6.5x |
| | | soilF | 28.3±6.0a | 30.0±11.4r | 48.3±10.1x | 90.0±10.0ab | 91.7±3.1qr | 72.0±12.4x |
| | | control | 15.0±3.4a | 28.0±4.9r | 30.0±8.6x | 60.0±12.4bc | 41.7±17.0s | 31.7±9.8x |
| small seeds* | summer | complete | 29.4±4.3a | 50.6±6.7q | 65.0±6.2x | 85.0±4.0a | 86.7±4.2q | 71.7±5.1x |
| | | complete-T | 38.9±3.6ab | 46.1±4.4qr | 59.4±3.8x | 78.9±6.1ab | 77.2±5.0q | 70.6±4.7x |
| | | dwellers | 33.9±4.3ab | 47.2±4.6qr | 52.9±6.3x | 60.7±6.9b | 56.1±6.2r | 56.1±5.4x |
| | | soilF | 36.7±6.7ab | 16.0±2.4r | 58.3±4.8x | 75.0±14.5ab | 90.0±4.5q | 72.0±9.2x |
| | | control | 55.0±4.3b | 44.0±9.8qr | 45.0±6.2x | 76.7±6.1ab | 46.7±6.1r | 60.0±8.9x |

**Table 6** Regression coefficients of the multiple regression between seed dispersal and dung removal by seed size and study site ($F_{5,1150}$ = 1535, $P$<0.001, $R^2$=0.854).

| factor | estimate±SE | t value | *P* |
|---|---|---|---|
| dung removal | 0.70±0.04 | 19.341 | <0.001 |
| large seeds | -0.84±1.91 | -0.439 | 0.660 |
| medium seeds | 12.33±1.91 | 6.441 | <0.001 |
| small seeds | 18.80±1.91 | 9.820 | <0.001 |
| site Zwin | 14.02±1.45 | 9.689 | <0.001 |