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Body condition, diet and ecosystem function of red deer (*Cervus elaphus*) in a fenced nature reserve



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ABSTRACT

Body condition, as a sign of animal welfare, is of management concern in rewilding projects where fenced animals are subject to winter starvation, which may conflict with animal welfare legislation. Investigating the relationship between body condition, age, sex, diet quality and diet composition is therefore relevant to increase understanding of herbivores' ecosystem function and to inform management. In this study, we focused on red deer, *Cervus elaphus*, in a fenced nature reserve in Denmark, where the deer are managed as ecosystem engineers to contribute to biodiversity conservation. We measured body mass and body size of 91 culled red deer, and determined diet composition using DNA metabarcoding and diet quality using fecal nitrogen on 246 fecal samples. We found that body condition was predicted by age and diet composition, but not diet quality. We also found that individuals of different body condition had different diets, i.e., the fecal samples of red deer in poorer body condition contained significantly more Ericaceae sequences than red deer in good body condition. This may imply that certain functions of red deer in ecosystems, such as regeneration of heather by grazing, may depend on variation in body condition within the population. Our findings call for the need to consider the consequences of management practices, including culling or supplemental feeding, on the outcomes of habitat restoration, and more broadly underline the importance of preserving the overall breath of herbivore ecosystem functions for effective biodiversity conservation. © 2017 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

1. Introduction

Animal welfare becomes a nature conservation issue when large mammals are fenced for ecosystem function. Large herbivores are increasingly used as ecosystem engineers in nature conservation and nature restoration projects (referred to as rewilding, Soule and Noss, 1998). Herbivores contribute to creating vegetation diversity and thereby to biodiversity conservation (Danell, 2006; Sandom et al., 2014). For example, red deer affect ecosystems through grazing, browsing, trampling, nutrient cycling, and seed dispersal (Iravani et al., 2011; Murray et al., 2013; Virtanen et al., 2002). Their density, spatial distribution and feeding preferences are therefore the key to their ecosystem function. However, this effect requires herbivores at relatively high densities that lead to conflicts with agriculture, forestry and other land uses in cultural landscapes. To avoid such conflicts and ensure large herbivore ecosystem function, nature reserves are often fenced. But managing fenced

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wildlife introduces a new set of challenges including animal welfare concerns (Hayward and Kerley, 2009; Woodroffe et al., 2014).

Seasonal weight loss in wild populations of ungulates can be up to 30% (Anderson et al., 1972; Mitchell et al., 1976), but for fenced wildlife, managers feel a certain responsibility and often also have a legal obligation to assure the animals' welfare. Body condition, assumed to be positively related to the animal's fitness and welfare and the relative amounts of stored fat and protein (Clutton-Brock et al., 1982) is generally used to assess the animals and the necessity and urgency of intervening, e.g. by regulating population size or supplying supplementary fodder (ICMO, 2006; Putman and Staines, 2004). Thereby, the animals' body condition can have implications for the intensity of management, the ecosystem function of the animals and ultimately for the biodiversity conservation of the nature area. Understanding what affects body condition of large herbivores in fenced nature reserves is therefore key to ecosystem function and more self-sustainable nature management.

In red deer, body condition can be related to age and sex as a result of differences between the biology and ecology of the two sexes, the individual's social rank and reproductive status, which results in different nutritional requirements, which again may be manifested in differences in diet (Appleby, 1980; Clutton-Brock et al., 1982). However, diet also reflects habitat choice, season and opportunity (Cornelis et al., 1999; Zweifel-Schielly et al., 2012). Although the link between diet and body condition is highly relevant for managing large herbivores, especially in fenced ecosystems with limited resource availability, to our knowledge, it has never been investigated in the context of ecosystem function.

Variations in wild ungulate food intake can be difficult to quantify as this would require constant observation yet without disturbing the animals. Researchers have therefore resorted to feeding trials with controlled food intake (e.g. Hodgman et al., 1996 for mule deer; Monteith et al., 2014 for white-tailed deer; Mould and Robbins, 1981 for North American red deer). Although, such studies are extremely valuable for understanding ungulate diet, they are often based on relatively small sample sizes of animals under highly unnatural conditions. Non-invasive methods of estimating diet quality from fecal Nitrogen (FN) make it possible to estimate diet quality from feces collected from wild animals without disturbing them (Carpio et al., 2015; Leslie et al., 2008; Leslie and Starkey, 1987). Now, herbivore diet composition can also be investigated non-invasively based on DNA metabarcoding (Taberlet et al., 2012) by analyzing chloroplast DNA-fragments in the feces (Lopes et al., 2015; Pompanon et al., 2012; Valentini et al., 2009a). Compared to the commonly used method of determining diet based on visual inspection of plant material (macroscopy) in rumen or fecal samples (e.g., Jensen, 1968), DNA metabarcoding is less time consuming and potentially allows a higher taxonomic resolution (Nichols et al., 2016). An integrated approach, combining the use of DNA metabarcoding and fecal nitrogen analyses could prove particularly useful for studying the link between diet and body condition because it provides information on specific food items consumed as well as aspects of diet quality.

In this study, we investigate the relationship between body condition and diet of red deer, *Cervus elaphus*, in a fenced nature reserve in Denmark, where the deer are managed primarily as ecosystem engineers to contribute to biodiversity conservation. Animal welfare considerations result in population regulations by culling within the hunting season and a minimum of supplementary feeding to ensure acceptable body condition during winter. For the deer culled, we evaluate body condition as indicated by the relationship between body mass and body size (Cannon bone length) and how it is predicted by age and sex classes. We focus on two aspects of red deer diet, i.e., diet quality estimated from FN and variation in diet composition based on plant taxa in feces identified by DNA metabarcoding. Lastly, we discuss the implications of our findings for the management of red deer and other large herbivores and for ecosystem function.

2. Methods

2.1. Study area and population

Klelund Deer Park is a 14 km² "biodiversity deer park", a new legal concept for deer parks in Denmark where biodiversity conservation is the main focus. Here, red deer are primarily seen as a management tool that contributes to diversification of the area through grazing, trampling, and by providing dung and carcasses. Klelund Deer Park was fenced in 2010 and enclosed all animals naturally present in the area. Before the fence was erected the deer had access to a typical cultural landscape with fields with nutrient rich crops surrounding Klelund. After fencing, the animals have been restricted to the nature areas within the park, i.e., their ecosystem function is also focused on the nature areas instead of causing damages to crops in the surroundings.

The site quality is relatively low and heath, moor and coniferous plantation are widespread within the area. Half the area of the deer park is forested with mainly coniferous plantation. Forestry ceased in 2010 and the plantations are undergoing conversion to natural forests with native species and forest glades. Heathlands cover c. 3 km² and have also been subjected to intensive management, such as stripping the vegetation and top soil to renew the heathlands. There is some mosaic structure between the different vegetation types, but the former plantation is located in the middle and south of the park and heathlands are widespread in the northern and western parts of the park. There is a watercourse and Natura 2000 area in the southern part of the park. Topographic variation is low and the highest point is c. 70 m asl. Vegetation changes are monitored in permanent plots throughout the park. Forage availability for red deer is expected to increase in the process of conversion from plantation to nature reserve.

Red deer are the most numerous grazers in the park with c. 300–400 individuals in spring stock but there are also some roe deer (*Capreolus capreolus*) (c. 150 individuals). Because of the fence, animal welfare laws (animal husbandry laws) that

require animals to maintain good body condition throughout winter still apply. Animal welfare is a high priority and there is a wish to limit starvation and winter die-off. Therefore, winter fodder is provided from December onwards. About 150 red deer are hunted every year in the hunting season (September–January). The goal of hunting is population regulation to limit winter die-off and assuring acceptable body condition and is based on a demographic plan with a natural distribution in sex and age classes and increasing the number of old stags. The pre-harvest sex and age distribution reflects this, i.e., more or less even distribution between the sexes, regulation focused on the younger age classes, and reserving the older stags (Appendix 1).

From April 2013 to May 2014 three young stags (2–3 years) in the deer park were GPS-collared. They moved around the whole study area during a year (minimum convex polygon would therefore be c. 14 km²), but 95% Kernel Density in Spatial Analyst in ArcGIS 10.2 estimated their home ranges at 1.62–2.24 km². An early study of red deer movement and home range patterns from a similar landscape also located in Jutland, Denmark, showed hinds have home range sizes (minimum area estimates) that vary from c. 0.2–4 km² depending on disturbance and season (Jeppesen, 1987). When disturbed by hunter, tourist etc. mean flight length to cover was c. 3.5 km (Jeppesen, 1987).

2.2. Red deer trait data and fecal samples

A subset of the individuals shot during the hunting season September 2013 to January 2014 ($n = 92$) were all weighed (guted weight), the length of the Cannon bone was measured as a proxy for body size ($n = 91$, Cannon bone measurement was missing for one individual), and sex and age was determined. Body mass distribution among the three age classes (calf, hind/stag <2 years and hind/stag ≥ 2 years) showed unequivocal variation in the oldest age class. Further specification of age for hinds was difficult, but for stags it was possible based on antlers. To obtain a more even distribution of variation of body mass among the age classes, we therefore used the following age classes for hinds: calves (0), hinds <2 years (1) and hinds ≥ 2 years (2), and for stags: calves (0), stags <2 years (1), stags = 2 years (2) and stags ≥ 3 years (3) (see also Fig. 1). Fecal samples were collected from the rectum of the deer.

For the period of the hunting season, fecal samples were collected from dung found distributed throughout the deer park ($n = 154$). Only fresh (<1 week based on appearance) dung was sampled and c. 30 samples were collected each month. When possible, single pellets were collected from inside mounts of pellets and undisturbed by insects. Dung that was notably disturbed by insects was avoided. These samples will be referred to as field samples.

All fecal samples were collected using disposable forceps and divided into two sub-samples for DNA metabarcoding and fecal nitrogen analysis, respectively. Samples used for DNA analysis were placed in 20 mL scintillation vials containing silica desiccant and stored in a freezer at c. -18°C , then shipped to the lab with silica desiccant at room temperature. Fecal samples for N analysis were collected in foil containers and stored in a freezer until the end of the collection period.

2.3. Fecal nitrogen analysis

Fecal nitrogen content was measured as percentage nitrogen in dried and homogenized fecal samples. Analyses were performed by Elementar Vario El Cube chromatograph.

2.4. DNA-metabarcoding analysis

DNA metabarcoding analysis for identifying plant species in the fecal samples was performed by amplifying and sequencing on an Illumina HiSeq 2500 platform (Illumina Inc., San Diego, CA, USA) the P6 loop of the chloroplast *trnL* (UAA) intron in angiosperms and gymnosperms (Taberlet et al., 2007). DNA extraction, PCR amplification, sequencing, sequence data filtering and analysis, and taxonomic identification were carried out following published protocols (De Barba et al., 2014; Taberlet et al., 2012; Valentini et al., 2009a). Detailed description of all steps of the DNA metabarcoding analysis is provided in Supplementary Material.

2.5. Statistical analysis

All analyses were performed in R version 3.3.0 (R Core Team, 2016). To estimate and visualize the variation in diet composition of red deer, we used the first three axes of a 4-dimensional Nonmetric Multidimensional Scaling (NMS, Zuur et al. (2007)) using function metaMDS in the vegan package (Oksanen et al., 2007) based on frequency of sequences at the lowest possible taxonomic level (LPTU), i.e., the percentage of sequences in a sample identified to the taxonomic level at which sequences can be unambiguously and discretely identified (see Rayé et al. (2011)). In practice this means that for two sequences identified as e.g., *Calluna vulgaris* and *Calluna*, respectively, the LPTU is *Calluna*. Both field samples and samples from culled deer were included in the NMS ($n = 246$). To illustrate the plant composition and FN that explains most of the variation in the ordination, vectors of sequences of major plant families and FN were fitted to the NMS (function envfit of package vegan).

Ln-transformed body mass (lnBM) was used as the response variable and Cannon bone length (CB), age class (Age), Sex, FN, the first three NMS axes, representing variation in diet composition, and Days (a count of days since the beginning of the

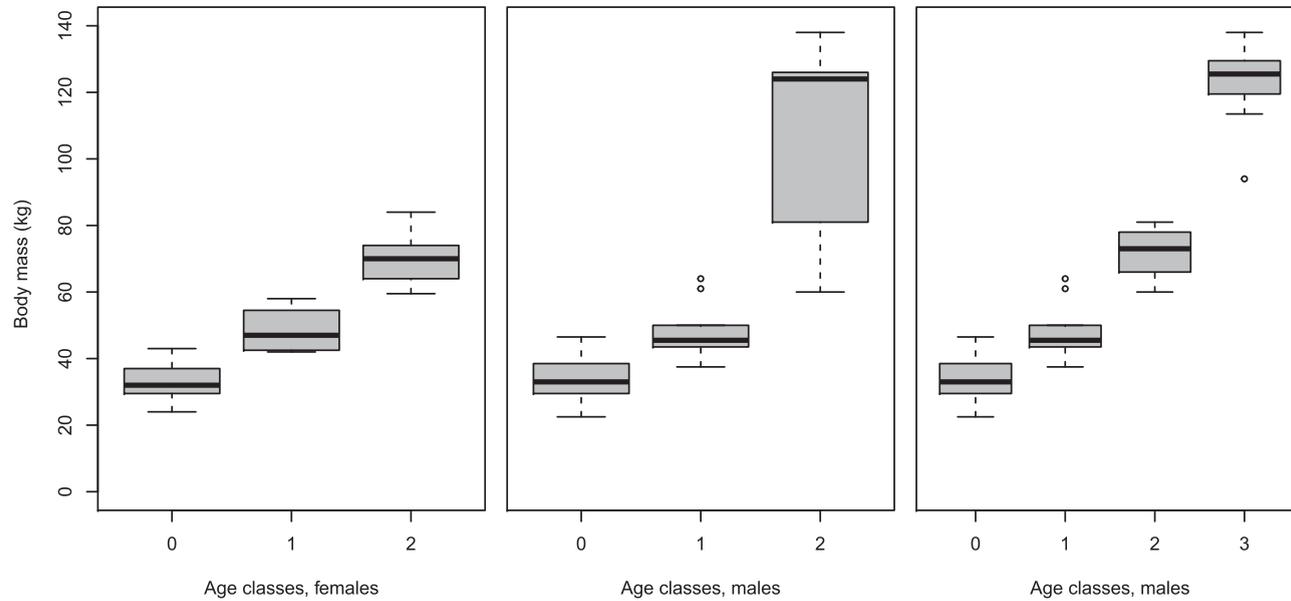


Fig. 1. Variation in body mass amongst individuals in the different age classes. A. The use of the three age classes for hinds show an even distribution in variation in body mass amongst the age groups. B. Using the same three age classes for stags as for hinds (i.e., stags >2 years are age class 2) shows that there is notable more variation in age class 2 than in the others. C. Using four age classes for stags shows a more even distribution in body mass variation amongst the age classes. Thick line shows the median, boxes represent the interquartile range (IQR) and the whiskers extend 1.5 times the IQR below and above the first and third quartiles.

hunting season and representing a general deterioration in climatic conditions and food availability with the onset of winter) were included as explanatory variables ($n = 91$). Data exploration steps showed that only CB, Age and NMS-axis 1 were significant predictors of lnBM. To obtain one variable for diet composition, that best fits to the residuals variation in the body mass after CB and Age were accounted for, we performed a procrustes rotation (function `procrustes` in `vegan`) to superimpose and fit the three NMS-axes to the residuals of an ANOVA (function `aov`) of lnBM on CB and Age. We called the output Diet and used it as an explanatory variable representing the diet selection and resulting diet composition.

We then assessed the relationship between lnBM and CB, Age, Sex, Days, Diet and FN using ANOVA. Model selection was performed based on Akaike Information Criterion (AIC), i.e., insignificant variables were removed and AIC was checked to ensure a decrease in AIC with model reduction. Normality and heterogeneity were assessed by visual inspection of qq-plots, residual plots and histograms of residuals.

To understand the magnitude of the effect of diet on body mass, we calculated the Diet effect based on the non-standardized coefficients of ANOVA.

Correlations between NMS-axes, lnBM and the continuous predictors were reported using Pairwise Person's correlations.

To further explore the relationship between body condition and ecosystem function, we investigated the relationship between body condition and the most common plants ingested. We checked for significant correlations between the frequency of plant sequences in the fecal samples and the body condition estimated by the residuals of an ANOVA of lnBM on CB and Age.

3. Results

Culled red deer included a wide distribution of individuals of different sex and age classes, i.e. 34 calves, 4 hinds <2 years, 12 stags <2 years, 19 hinds \geq , 6 stags = 2 years, and 14 stags >3 years (see also [Figs. 1 and 2](#)). Body mass, which ranged 20–140 kg (gutted weight) from calves to adult males, varied within age classes and the variation was most pronounced for adult males >2 years. This variation was notably reduced when adding another age class, >3 years, for the males resulting in a more even distribution in variation in body mass ([Fig. 1](#)). Cannon bone length ranged 19.2–29 cm. Body mass was closely and

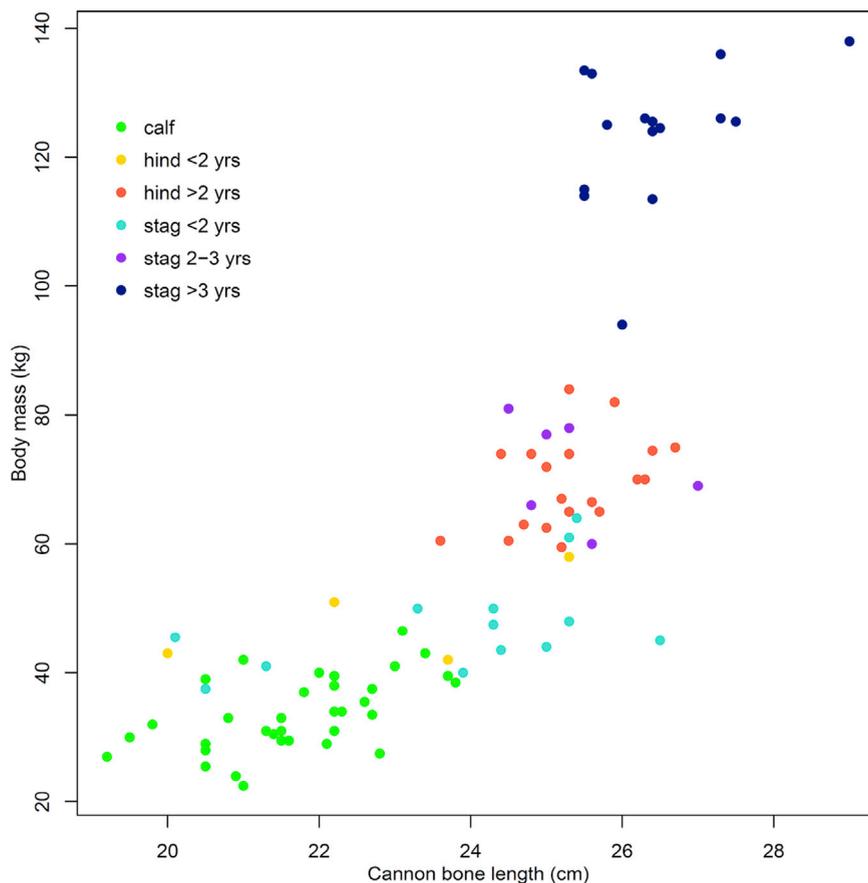


Fig. 2. Plot of body mass and body size (Cannon bone length, $n = 91$) shows a non-linear relationship and that the relationship changes amongst age and sex classes. Body condition has previously been estimated by the ratio between body mass and body size or by the residuals of a model of body mass fit to body size.

positively associated with body size (CB), although the relationship was not linear and varied among age and sex classes (Fig. 2).

DNA-analysis of the 246 fecal samples (92 from culled red deer and 154 field samples) resulted in c. 3 million sequences that were identified as belonging to 90 different plant taxonomic units ranging in resolution from species to orders. Summarizing sequences at the lowest possible taxonomic unit (see methods) identified 40 unambiguous and discrete taxonomic units ranging from genera to order. Summarizing the sequences at family (or higher level) identified 26 plant families and 3 orders. The most common plant families, occurring in most samples (frequency of occurrence across all samples), were grass, heather and pine, detected in more than 77% of the samples. Also common were beech, legumes and sedges, which occurred in more than 28% of all samples (Table 1).

FN ranged from 1.11 to 3.45 %N ($x = 2.33$, $SE = 0.044$, $n = 92$) for samples from hunted red deer and 1.00–4.34 %N ($x = 2.13$, $SE = 0.044$, $n = 154$) for field samples.

Ordination of diet composition based on DNA metabarcoding revealed great variation in diet among different age and sex classes, where the plant families that explained most of the variation in diet composition ($r^2 > 0.2$ and $p \leq 0.05$) were Poaceae (grass), Betulaceae (birch), Pinaceae (pine), Ericaceae (heather), Roseaceae (roses) and Fagaceae (beech) (Fig. 3). FN also explained significant variation in the diet composition (Fig. 3).

ANOVA of body condition investigated as lnBM as a response variable and CB as a predictor alongside age, sex, days, Diet and FN revealed that only CB, Diet and age were significant predictors for body condition (Table 2). Model prediction was high ($R^2 = 0.94$) and remained so throughout model selection and evaluation was therefore based on reduction of AIC and a principle of model simplicity over complexity. Visual inspection of qq-plots, residual plots and histograms of residuals indicated normality and heterogeneity in the final model.

The effect of diet was pronounced in the oldest age class (stags >3 years), where diet had an average positive effect of c. 4.7 kg on the body mass of individuals, i.e. a stag >3 years with a certain Cannon bone length will on average weight 4.7 kg more due to its Diet than expected based on age and CB alone. The effect was less pronounced for the other age classes, and even negative in the calves and hinds/stags <2 years, i.e. a hind/stag <2 years weighs c. 1.2 kg less because of its diet than expected from its CB and age alone. The effect of Diet on body mass in the final model showed a pronounced positive effect of diet in the old males (>3 years) and a much less noticeable effect in the younger age classes (Fig. 4).

Pairwise Pearson's correlations showed that there were some, although not notably strong, correlations between NMS1 and FN and Days (Table 3). lnBM and CB were highly correlated ($r = 0.854$) and FN and Days were slightly negatively correlated. The procrustes rotation, Diet, correlated most with the first of the three ordination axes and with lnBM.

Table 1

Frequency of occurrence of sequences (proportion of samples where the sequence was present) belonging to the different plant families in the samples ($n = 246$) from hunted deer and field samples.

Family	Frequency of occurrence
Poaceae	1.000
Ericaceae	0.854
Pinaceae	0.776
Fabaceae	0.329
Fagaceae	0.325
Cyperaceae	0.289
Juncaceae	0.187
Roseaceae	0.171
Dryopteridaceae	0.154
Saliceae	0.118
Betulaceae	0.102
Caryophyllales	0.102
Asterales	0.093
Hypnales	0.053
Polygonaceae	0.037
Myricaceae	0.037
Rubiaceae	0.024
Dicranaceae	0.016
Polytrichaceae	0.016
Cypressaceae	0.012
Malvaceae	0.012
Typhaceae	0.008
Rhamnaceae	0.008
Ranunculaceae	0.008
Lanceolaceae	0.008
Plantaginaceae	0.004
Onagraceae	0.004
Apiaceae	0.004
Geraniaceae	0.004

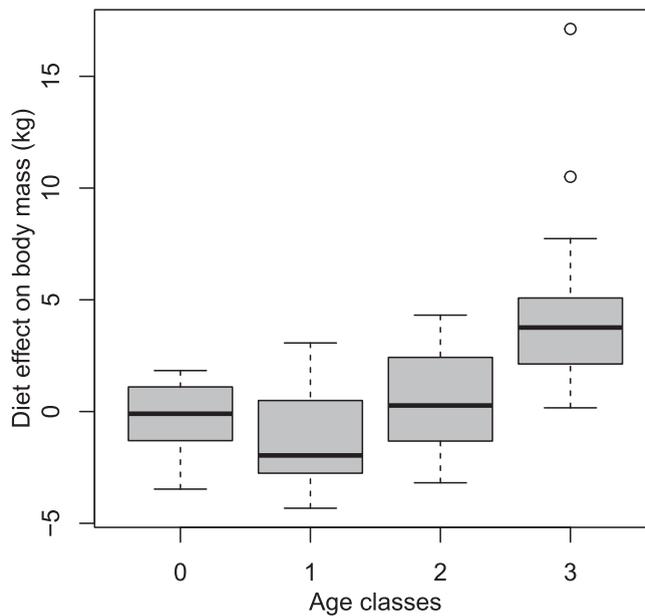


Fig. 4. The median effect of diet on body mass for the four age classes (0 = calves, 1 = hinds/stags <2 years, 2 = hinds >2 years/stags 2–3 years, 3 = stags > 3 years) calculated based on coefficients in the final model with non-standardized variables. Boxes represent the interquartile range (IQR) and the whiskers extend 1.5 times the IQR below and above the first and third quartiles.

Table 3

Pairwise Pearson's correlations between NMS-axes, body mass, continuous predictor variables and the diet effect on body mass.

	NMS2	NMS3	lnBM	CB	FN	Days	Diet
NMS1	0.021	0.254	0.459	0.295	0.292	-0.437	0.566
NMS2		0.107	-0.321	-0.239	0.017	0.219	-0.078
NMS3			0.115	0.046	0.030	-0.022	-0.083
lnBM				0.854	0.103	-0.342	0.436
CB					-0.001	-0.293	0.331
FN						-0.263	0.161
Days							0.048

Table 4

Pairwise Pearson's correlations with significance levels (ns, * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$) between body condition (residuals of ANOVA of lnBM on CB and Age) and frequency of sequences of the most common plant families.

	Poaceae	Ericaceae	Pinaceae	Fabaceae	Fagaceae
Body condition	0.087 ns	-0.272**	0.192 ns	-0.047 ns	-0.018 ns

high, near-natural densities of herbivores and their impact on the vegetation. Investigating the relationship between body condition, age, sex, diet quality and diet composition is therefore relevant to increase understanding of red deer ecosystem function and to inform management. We found that body condition was predicted by age and diet composition, but not diet quality as estimated by fecal nitrogen. We also found that individuals of different body condition had different diets, i.e., the fecal samples of red deer in poorer body condition contained significantly more Ericaceae sequences than red deer in good body condition.

4.1. Relationship between body condition and diet of red deer

To avoid violating assumptions in estimating body condition as an index of body mass and body size, as has otherwise been common practice, but also criticized (see Green, 2001), we have chosen a simple and more transparent approach, where we modeled ln-transformed body mass as a response variable and body size (CB) as an explanatory variable alongside the other variables. We thereby found that body condition was predicted by Age and Diet and that the effect of Diet was most pronounced in stags >3 years, which is in accordance with social rank in red deer stags determining the quality of diet consumed (Appleby, 1980) and may also reflect different strategies depending on sex and life history, i.e., calves focus on allocating

energy to growth and reproduction while lactation reduces body condition of hinds (Mitchell et al., 1976). Our study period follows the rut, where stags are known to eat into body reserves and reduce their body condition (Clutton-Brock et al., 1982). Stags have been found to increase their consumption in this period possibly to re-fuel (Groot Bruinderink and Hazebroek, 1995), but a more selective diet could also contribute to meeting the stags' nutritional requirements in this period. Anecdotal evidence from the game keeper suggests that the largest stags take residence in the southern part of the reserve, where site quality is higher and there is less heath and more deciduous forest. This is further supported by the ordination showing that older stags are generally positioned opposite the vector for Ericaceae and towards the vector of Fagaceae.

Interestingly, Sex did not significantly explain body condition. However, the differences between the sexes could at least partly be represented by Age, i.e., age class 3 being older stags. Sex differences may also be mirrored in the animals' diet selection. Few studies have investigated this aspect of diet selection and their findings were not unequivocal. In a review of European red deer diet composition, Gebert and Verheyden-Tixier (2001) found that only few diet studies had given information on the sex, but from those studies, they found no significant differences in the diet composition of the two sexes. Red deer in Holland showed no differences in diet composition, but in consumed quantities between the sexes indicating that consumption reflects metabolic demands (Groot Bruinderink and Hazebroek, 1995). Similarly, diets of Mediterranean hinds and stags was found to vary with the seasons likely reflecting inter- and intra-specific competition in seasons with scarce resources as well as reproduction and physiological status (Azorit et al., 2012).

The use of DNA metabarcoding in investigating complex ecological questions is still relatively new and its possibilities and limitations are still under investigation (Valentini et al., 2009b), but it is increasingly used to study diet composition in mammals (De Barba et al., 2014; Elfström et al., 2014; Kartzinel et al., 2015; Kowalczyk et al., 2011; Lopes et al., 2015; Rayé et al., 2011). Sequence read counts and their frequencies have limitations for accurate quantification of food consumed due to various sources of variation, including differences in DNA content across species and tissues, differential digestion efficiency among consumer species, and analytical bias (i.e. amplification and bioinformatic bias) (Deagle et al., 2013; Pompanon et al., 2012; Thomas et al., 2014). However, a number of studies using the DNA metabarcoding approach and the trnL P6 loop marker provided evidence that read frequencies obtained can confidently approximate relative amount of food ingested (Kartzinel et al., 2015; Soininen et al., 2009; Willerslev et al., 2014) supporting their use for comparative analyses (Pompanon et al., 2012) for describing inter- and intra-specific diet variations (Kartzinel et al., 2015; Kowalczyk et al., 2011; Lopes et al., 2015; Rayé et al., 2011).

FN as an indicator of diet quality has been developed and explored over the last decades and its use is now well-established (Carpio et al., 2015; Leslie et al., 2008). The novel integration of these two complementary methods allowed us to gain a more comprehensive understanding of the relationship between deer diet and body condition. FN explained some of the variation in the diet composition. Other explanations of variation in diet composition not investigated here may be related to opportunity, habitat selection, secondary metabolites in plant tissues, and sugar content in food items (Forsyth et al., 2005; Gebert and Verheyden-Tixier, 2001; Tixier et al., 1997). Whereas Diet significantly explained variation in body condition, FN did not, probably because the aspect of FN in diet selection was incorporated in the variable Diet.

Body condition is affected by relatively short term environmental conditions and seasonal changes in behavior, whereas the Diet based on fecal DNA gives somewhat of a snapshot of the red deer diet as most food items pass through in 2–4 days (Gill (1961) referenced in Jensen (1968)). However, the importance of Diet for body condition indicated that Diet also reflects more long-term/seasonal nutritional requirements and social rank that result in certain preferences or competition for food items or habitats (Appleby, 1980; Barboza and Bowyer, 2000; Ceacero et al., 2012; Gebert and Verheyden-Tixier, 2001; Thouless, 1990) resulting in the sample reflecting characteristics of a more long-term diet composition, e.g., an animal roaming primarily the heaths will generally consume a lot of heather, which is likely to be represented by many Ericaceae sequences in a given fecal sample from that individual.

DNA metabarcoding confirmed red deer as an intermediate feeder in Klelund deer park, i.e., the major plant families eaten were grasses, sedges, pine, heather, beech and legumes (Table 1). The diet showed similarities with deer diets reported elsewhere (Gębczyńska, 1980; Gebert and Verheyden-Tixier, 2001; Groot Bruinderink and Hazebroek, 1995; Jensen, 1968). But there were also some differences, e.g., the importance of beech, which was less pronounced in the other studies. The diet reflected the habitats available within the fence, i.e., heath, moor and coniferous plantation are widespread within the area and beech has recently been planted in many clearings of former coniferous plantation and is therefore available in a good browsing height for red deer.

A study on bison diet specifically investigated effects of supplementary feeding (Kowalczyk et al., 2011). This was more challenging to do in our study due to the composition of the supplementary fodder. Most of the fodder consisted of bale silage from hay grown within the deer park and therefore difficult to distinguish both because it had the same plant composition as found elsewhere in the park and because the DNA-content may be reduced due to fermentation, which breaks down DNA as other digestive and decomposition processes. The hay was supplemented with whole oats, which are largely made up of starch molecules and therefore contain relatively little DNA compared to other plant parts. Supplementary fodder was provided from December onwards and may therefore have influenced the diet and body condition of the samples collected in Dec–Jan (25 out of the 91 samples from culled deer). The effect of fodder is probably minor as it was a mild winter and the animals will make more use of the fodder towards the end of the winter, when resources become scarce. However, if as for European bison, supplementary fodder changes the diet selection of the animals, the effect will be reflected in the Diet variable.

As in many other wildlife studies, the available data stems from hunted individuals and we must be aware what bias this may entail in interpretation of our results (see e.g., [Leclerc et al., 2016](#) for bias in data from hunted bears). The data from Klelund is relatively unbiased as the individuals culled are selected following a demographic model for the population with a natural distribution in sex and age classes. In line with this, we find congruence between the diet composition in the samples taken from hunted deer and those collected in the field ([Fig. 3](#)). This indicates that the results presented here are representative of the population.

4.2. Ecosystem function and management implications

The ecosystem function of red deer is versatile and includes much more than grazing in the strict sense. Red deer may reduce tree cover and encroachment, maintaining plant species richness of grasslands and contributing to mosaic structure in vegetation ([Tschöpe et al., 2011](#); [Virtanen et al., 2002](#)). This also includes transformation of foliage to dung, which creates spatial heterogeneity in nutrient cycling, reduces litter cover, and changes soil properties compared to ungrazed systems ([Murray et al., 2013](#); [Peco et al., 2006](#)), particularly when large herbivore density varies in space and time. Red deer provide anthelmintic-free dung for coprophilic insects ([Beynon et al., 2012](#)) and carcasses for numerous species of specialized decomposing invertebrates and fungi ([Barton et al., 2013](#); [Melis et al., 2004](#)) as well as scavenging birds and mammals ([Selva, 2004](#)) and contribute to variation with local mineral enrichment of the soil ([Barton et al., 2013](#)). While bark-stripping is considered damaging to forest plantations (see e.g., [Jarnemo et al., 2014](#)), it is an important function of red deer in a nature reserve with focus on biodiversity. Damage to trees induce colonization by fungi, insects, epiphytes, bats and birds as the trees grow to veteran trees and grazing in the forest understory may significantly increase the life span of ancient giant trees, otherwise susceptible to shading by young vigorous trees ([Rackham, 2008](#); [Read, 2000](#)).

Maintaining the full array of ecosystem function of red deer may be challenging notably in fenced rewilding projects where animal welfare and animal husbandry legislation ([Jepson, 2016](#)) may result in management practices, which in the end obstruct natural ecosystem function. A strict concern for body condition of large herbivores may have implications for the management intensity and ecosystem function of the animals. Animals in poor body condition often results in intervention by supplementary feeding or reactive culling (e.g., see the reports on managing wildlife in Oostvaardersplassen, [ICMO2 2010](#); [ICMO, 2006](#)). However, our findings suggest that such actions may have important consequences on ecosystems. It has already been reviewed that supplementary feeding contributes to domestication and alters the natural grazing patterns ([Myrsterud, 2010](#); [Putman and Staines, 2004](#)). However, how supplementary feeding influences red deer diet is at best equivocal and is influenced by a complex set of site-specific conditions ([Putman and Staines, 2004](#)).

Supplementary feeding may influence the winter diet of red deer depending on the composition of the fodder and can both increase and decrease the consumption of woody plants (e.g., [Rajský et al., 2008](#)). Here, it is important to focus on the management goal. A feeding regime that leads to reduced consumption of woody plants would be less desirable in the case of Klelund “biodiversity deer park”, where the management goal is to increase biodiversity in an area that until recently was dominated by plantation and encroached heathland. Here, bark-stripping, reducing tree cover and rejuvenation of heathland by grazing are desirable functions of red deer. However, reducing the consumption of woody plants by red deer may be desirable where conservation is focused more narrowly on tree species ([Palmer and Truscott, 2003](#)) or specific woodland types ([Putman and Moore, 1998](#)). A conversion from plantation to natural open forest mosaic would generally increase the availability of forage, but vegetation development (e.g. succession, changes in species composition) over longer time scales may cause a reduction in available forage. Given the broad biodiversity goals at Klelund, reducing the numbers of red deer may be more suitable than increasing the supplementary feeding. Securing alternative forage in the shrub and field layer has been shown to reduce the need for supplementary feeding in plantations ([Jarnemo et al., 2014](#)). However, although heather and bilberry are both widespread at Klelund it is still doubtful that these items in the absence of winter fodder can sustain the red deer at body conditions in accordance with animal welfare and husbandry legislation. Lastly, it is important to monitor vegetation, red deer densities and possible other taxa or proxies for a better overview of biodiversity effects.

The population we investigated was well below carrying capacity and some supplementary feeding was provided, yet, we showed that there were pronounced variations in body condition related to the diet. This effect of diet varied among age and sex classes and particularly older stags appear to take advantage of access to supplementary feeding and improved grassland within the reserve. From this we can infer, that ecosystem function will depend on the individuals' sex, age and possibly social status.

We found that samples from red deer in poorer body condition contained more Ericaceae sequences than red deer in good body condition indicating that a variation in body condition among a population of red deer is related to the utilization of a wider aspect of food items. We cannot conclude on the direction of the causal relationship, i.e., whether poor body condition causes higher intake of heather or whether high intake of heather causes poor body condition. However, it is likely that animals in poor body condition, i.e. stressed by competition and food shortages, resort to utilizing alternative resources and thereby have a different effect on ecosystem function, such as more effectively reducing tree and shrub encroachment on open vegetation types. This result may also entail that a common conservation goal such as regeneration of heathlands by grazing ([Rosa García et al., 2013](#)) may require that some individuals of the population are in poorer body condition.

In addition to this, our results, showing differential food consumption by animals with low body condition, now warns on the need for considering the effects of herbivores' management strategies including culling or supplemental feeding on the

outcomes of habitat restoration. More broadly, our results call for the importance of preserving the overall breath of herbivore ecosystem functions for effective biodiversity conservation.

Despite our empirical contribution, there is still a need for a better understanding of the impact of winter food shortage on ecosystem function of large herbivores. Ideally, this should be investigated experimentally by looking at populations of red deer and other herbivores that are at or near carrying capacity and that are not provided with supplementary fodder. However, except Oostvaardersplassen, such populations are hard to find in Europe and we therefore concur with the suggestion to establish a network of experimental rewilding sites to inform wildlife and biodiversity management (Jepson, 2016; Lorimer and Driessen, 2014).

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.gecco.2017.07.003>.

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