The blame game: Using eDNA to identify species-specific tree browsing by red deer (*Cervus elaphus*) and roe deer (*Capreolus capreolus*) in a temperate forest

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ABSTRACT

Increasing deer populations in many temperate regions can affect tree regeneration, resulting in severe long-term impacts on forest structure, composition and diversity. Of the most common deer species in Europe — red deer (*Cervus elaphus*) and roe deer (*Capreolus capreolus*) — roe deer are generally thought to have the highest impact on palatable tree species owing to their feeding niche. Although browsing and its potential consequences are well researched, less is known about the influence of specific deer species within multi-species ungulate communities on specific tree species. Environmental DNA (eDNA) allows the determination of species-specific browsing habits without the need for direct observations, facilitating effective targeting of management interventions. In this study eDNA was used to elucidate the browsing patterns of these two deer species in the temperate forest of the Bavarian Forest National Park, Germany and analysed the influence of tree species, management type and height of browsing, on the success rate of the method. Samples were collected from twigs used in feeding trials from enclosures containing red deer or roe deer and from naturally browsed twigs in three different management types within the national park. eDNA was successfully amplified from 98% of the feeding trial samples, and the correct deer species was identified for all samples. eDNA was successfully amplified from approximately 50% of the naturally browsed samples. Neither management type, tree species, nor height of browsing had any significant influence on the success of the method. For silver fir and rowan, no significant difference was found in the proportion of browsing events attributable to roe or red deer, when the two deer species occur at similar densities. These results indicate that roe deer might not always be disproportionately responsible for the browsing of palatable tree species as expected from its food niche. Roe deer were significantly more responsible for browsing at lower heights than red deer. Although not statistically significant, roe deer were more responsible for browsing in intact forest compared to bark-beetle-impact forest, with the opposite relationship for red deer.

1. Introduction

In many temperate regions, deer populations have increased greatly in the last decades (Burbaitė and Csányi, 2009, 2010; Kanda et al., 2005; Rooney, 2001; Ward, 2005). Increased population densities and expanding ranges of ungulate species can result in profound changes in forest ecosystems (Ramirez et al., 2018). By negatively affecting tree regeneration, deer browsing can have severe long-term impacts on forest structure and composition and lead to reductions in tree species diversity through the loss of the most palatable tree species (Côté et al., 2004; Didion et al., 2009; Ramirez et al., 2019; Schulze et al., 2014). Furthermore, deer browsing can have other cascading effects on forests,

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e.g. by affecting the diversity or abundance of birds, mammals, invertebrates and herbaceous understory plants (Chollet and Martin, 2013; Holt et al., 2011; Nuttle et al., 2014; Parsons et al., 2013; Sakai et al., 2012).

Furthermore, deer browsing sometimes severely affects forestry by reducing growth rates of commercially grown tree species, thereby reducing forest productivity (Scott et al., 2009; White, 2012). However, the full economic impact of deer browsing has been difficult to quantify (Clasen et al., 2011; Kaien, 2006; Ward et al., 2004). Moreover, increased browsing by deer has presented a major constraint to the adoption of alternative silvicultural systems that promote the use of mixed species stands or continuous canopy cover (Clasen et al., 2011; Ficko et al., 2016; Mason et al., 2018).

Although browsing and its potential consequences are well researched, less is known about the influence of specific deer species within multi-species ungulate communities on specific tree species. For example, the relative contribution of a particular deer species to tree browsing in areas with multiple deer species cannot be predicted solely on the densities of the deer species present, which poses difficulties for effective targeting of management interventions (Nichols and Spong, 2014). Hunting is often used to reduce deer densities and has sometimes shown to be effective in reducing browsing damage (Beguin et al., 2016; Hothorn and Müller, 2010). However, in multi-species deer communities, hunting might remove a part of the deer population that only has a proportionally small browsing impact. Furthermore, reducing deer densities through hunting can be costly, time consuming and ineffective in reducing the browsing levels of the most palatable tree species (Kamler et al., 2010), so improvements in efficiency and effectiveness are needed. Additionally, highly protected areas that aim to maintain ecological functions and processes with minimal management intervention may wish to reduce hunting effort to the minimum needed to maintain sensitive plant communities (Möst et al., 2015). To overcome this, effective targeting of the deer species most responsible for browsing damage is required.

Therefore, it is necessary to determine the browsing habits of a specific deer species, which is challenging in multi-ungulate communities inhabiting forested habitats. Typically, this has been done through the use of macroscopic analysis or DNA techniques to identify plant material recovered from ungulate rumens or faeces (Nichols et al., 2016). However, these methods have been problematic because there are difficulties in quantifying the different components of ungulate diet and/or a large amount of the plant material is often impossible to identify (Nichols et al., 2016). Furthermore, these methods do not allow observed browsing to be attributed to a specific species of ungulate. More recently, an environmental DNA (eDNA) method has been developed that overcomes these problems by allowing the identification of the species responsible for a particular browsing event using the genetic material from the deer's saliva left behind on browsed twigs (Nichols et al., 2012). This method has already been successfully applied in Scandinavia to investigate browsing patterns in multi-ungulate communities and has demonstrated large overlap among ungulate species in the tree species browsed (Nichols et al., 2015). However, the eDNA method has thus far only been utilized in a limited area of the boreal region, which are characterised by low temperatures and precipitation. The effectiveness of the technique under different environmental conditions in other regions may vary. Furthermore, the method has thus far been little used to elucidate the browsing patterns of common deer species in a forest management context. Improving understanding of these browsing patterns in multi-species deer communities may help reduce the impact of deer on sensitive plant communities by allowing managers to improve the efficiency and effectiveness of wildlife management.

The most common deer species in Europe — roe deer (*Capreolus capreolus*) and red deer (*Cervus elaphus*) — dominate a wide range of habitats from Mediterranean to boreal biomes (Apollonio et al., 2010). Analyses of diet preferences have typically led to viewing red deer as intermediate feeders that forage on a wide range of woody and non-woody vegetation (Gebert and Verheyden-Tixier, 2001; Krojerová-Prokešová et al., 2010; Latham et al., 1999; Storms et al., 2008) and roe deer as more selective browsers that feed on more concentrated food sources (Hofmann, 1989; Latham et al., 1999; Storms et al., 2008; Tixier et al., 2009; Tixier and Duncan, 1996). As a result, it is generally perceived that roe deer are disproportionately responsible for the browsing of palatable tree species (Senn and Suter, 2003). However, there appears to be large variation in the diet preferences of both deer species depending on season and available food sources, and roe deer and red deer diets often overlap (Obidziński et al., 2013; Storms et al., 2008). Additionally, some studies have found that red deer have a greater preference for foraging in more open habitats within forest stands than roe deer (Kuijper et al., 2009; Latham et al., 1996). However, other studies have not found a difference in foraging habitat preference between the two deer species (Borkowski and Ukalska, 2008).

Silver fir (*Abies alba*) and rowan (*Sorbus aucuparia*) are a highly attractive food of ungulates in temperate forests (Edenius and Ericsson, 2015; Motta, 2003; Senn and Suter, 2003), probably because of their greater palatability. Silver fir is a valued species in Central European montane forests due to its importance as a stabilizing element in protection forests, resistance to the bark beetle (*Ips typographus*) and association with high levels of biodiversity (Senn and Suter, 2003). Rowan is important for biodiversity in subalpine spruce forest ecosystems in Central Europe as it produces fruit that is nutritionally important for birds and other species (Guitián and Munilla, 2010; Raspé et al., 2000). Rowan is of additional ecological value as a species that can germinate in old Norway spruce (*Picea abies*) stands (Emmer et al., 1998), and it is a major component of regeneration in spruce forests following natural disturbance (Zeppenfeld et al., 2015).

Populations of silver fir have sharply declined over the last 200 years, and widespread low recruitment rates have been observed (Ficko et al., 2016, 2011; Senn and Suter, 2003; van der Knaap et al., 2019). Alongside silvicultural practices, browsing is likely a key factor in the decline of silver fir (Ammer, 1996; Caudullo et al., 2003; Didion et al., 2009; Feurdean and Willis, 2008; Klopcic et al., 2009) and can also prevent the establishment of rowan in the tree layer (Heroldová et al., 2003; Linder et al., 1997; Motta, 2003).

This study used the eDNA method to obtain fundamental data to inform deer management practices. The effectiveness of the method in a temperate forest and the factors influencing it were tested. The study also analysed whether the two ecologically and/or economically important tree species, silver fir and rowan, are browsed disproportionately by the deer species present and hypothesized that roe deer have a disproportionately high impact on these palatable tree species. Lastly, the influence of tree species, height of browsing (cm) and management type on roe deer and red deer browsing were analysed. It was hypothesised that roe deer are less likely to be responsible for browsing in management types with more open habitat and at higher heights compared to red deer.

2. Methods

2.1. Study area

The Bavarian Forest National Park (24,250 ha) is a strictly protected area in southeast Germany, adjacent to the border with the Czech Republic (48.9595 °N, 13.3949 °E). It covers an area ranging in elevation from 600 to 1,450 m a. s. l. Across this elevation gradient, mean annual temperatures vary from 3 to 6.5 °C, and mean annual precipitation ranges from 830 to 2,230 mm, much of which falls as snow. Snow cover persists for 5–8 months each year depending on elevation (Heurich et al., 2010). This elevation gradient maintains a variety of forest types, which can be split into three broad categories. Above 1,100 m.a.s.l., sub-alpine Norway spruce forests dominate, with rowan
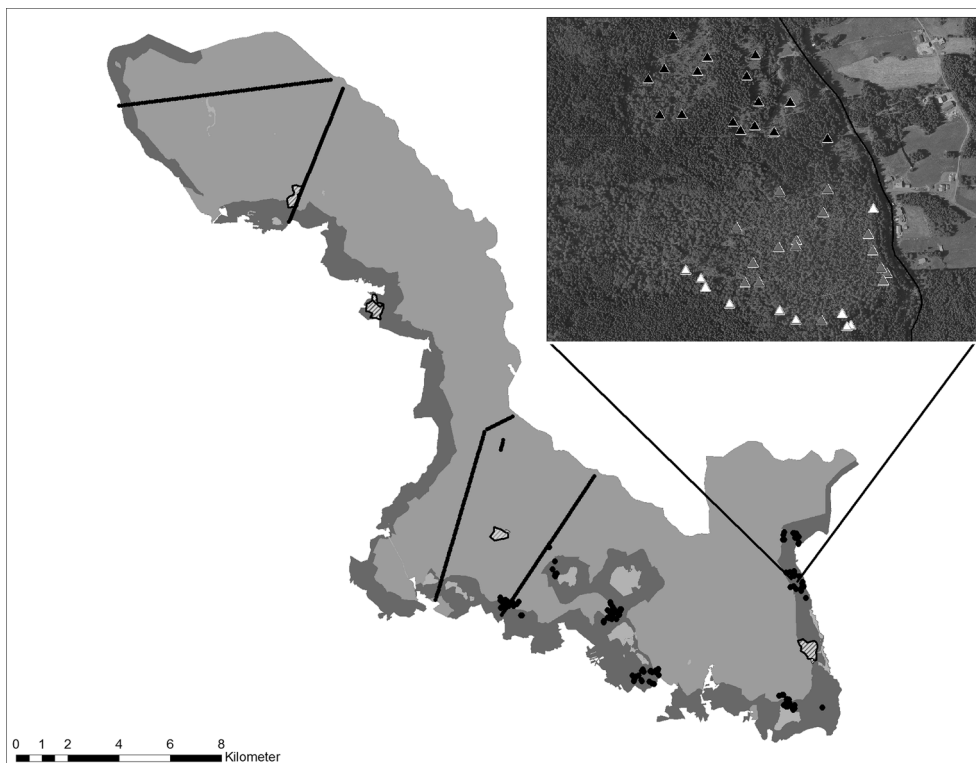
Fig. 1. Sample collection within the Bavarian Forest National Park. The black dots represent the 300 browsed twig samples collected in the field. The black lines represent the transect on which dung pellet counts took place and the diagonal lined polygons represent the four winter enclosures. The darker grey area within the national park indicates the buffer zone. Inset: The samples were collected in three different management types: bark-beetle-impacted forest (black triangles), managed forest (grey triangles) and intact forest (white triangles).

as a minor component. At 600–1,100 m.a.s.l., mixed forests of Norway spruce, silver fir, European beech (*Fagus sylvatica*) and sycamore (*Acer pseudoplatanus*) dominate. In cold and wet depressions at the bottoms of valleys, forests are dominated by Norway spruce, rowan and birch (*Betula pendula* and *Betula pubescens*) (Cailleret et al., 2014).

A buffer zone is situated along the national park border, where conservation management is implemented to protect areas adjacent to the national park from potential damages caused by the non-intervention strategy implemented within the core zone of the national park (Fig. 1). Bark beetle infested trees are felled and removed within the buffer zone to prevent spread to adjacent areas, as described within the national nark management plan (Nationalpark Bayerischer Wald, 2010). No management takes place within the core zone of the national park. Herbivores are managed in the buffer zone of the national park to reduce both impacts on the understory and conflicts over adjacent privately-owned commercial forests and agricultural areas. Each year, a part of the red deer population migrates to four winter enclosures within the national park, where supplementary food is given to prevent migrations into adjacent forests. Red deer are shot when they are contained within these winter enclosures (Rivrud et al., 2016). Between 2001 and 2018 approximately 86–130 red deer were shot each year within the national park to compensate for the mortality previously provided by predators. Numbers are decided based on an estimated number of offspring present, which is based on the number of female red deer counted within and outside the winter enclosures. Each year, both male and female deer are shot. However, only males up-to three years are allowed to be shot within the national park, due to a ban on trophy hunting. Since 2012, following the re-establishment of Eurasian lynx (*lynx lynx*) in the national park in the 1980 s, roe deer are no longer managed in the study area and only a few migrate into the winter enclosures. Between 2001 and 2012, 49–142 roe deer were shot each year within the national park (Heurich et al., 2012). Available evidence suggests that current roe deer mortality rates are likely similar the those prior to the re-establishment of Eurasian lynx and the cessation of roe deer management (Heurich et al., 2012).

2.2. Sample collection

To test whether eDNA can be effectively extracted from browsed twigs in temperate regions, a feeding trial was conducted to collect a total of 49 known-browser twig samples from roe deer (n = 20) and red deer (n = 29), within the winter enclosures located in the national park. Samples were collected from multiple silver fir that were placed within each winter enclosure. Freshly browsed samples were collected from different individual trees to maximize the likelihood that these samples were browsed by different individuals. Each sample was collected by clipping the top 2 cm of browsed twigs into small empty tubes. To avoid cross contamination, the clipped twigs were handled with flame-sterilized secateurs. After field collection, the samples were frozen at − 40 °C and shipped on dry ice to the Swedish University of Agricultural Sciences Umeå (SLU) for eDNA extraction.

To directly quantify fine-scale resource use by ungulates, a total of 300 naturally browsed twig samples were collected in March 2016 across the national park (Fig. 1). Sample collection was conducted within the mixed forest between 600 and 1,000 m.a.s.l. and within the forests in the valley bottoms. Only freshly browsed apex shoots were browsed, identified by their lighter colour compared to older growth. These shoots were collected following the above-described protocol in an unbalanced random block design in seven different stands across the Rachel-Lusen area of the national park (Fig. 1). Each of these stands covered three different management types, namely bark-beetle-impacted forest, managed forest and intact forest, which were assigned through visual assessment in the field. Bark-beetle-impacted forest had large numbers of bark-beetle-impacted Norway spruce, either standing or lying. Managed forest had signs of timber harvesting, i.e. tree stumps. Intact forest had no indication of timber harvesting or bark beetle infestation. Stand selection was based on the presence of winter enclosures and the home range size of the two deer species, roe deer and red deer. Close to the winter enclosures, the probability of red deer browsing was higher compared to roe deer browsing. Stands were placed at least 2 km from all winter enclosures and from each other based on roe deer and red deer home range size. Average roe deer home

range size ranges between 0.55 and 0.77 km^2 (Morellet et al., 2013) and red deer home range sizes ranges from $3.84 - 11.1$ km^2 (Bevanda et al., 2015). Approximating a circular home range with a radius between 0.42 and 0.50 km for roe deer and 1.11–1.88 km for red deer, selected stands were at least 2 km apart to ensure that different stands were visited by different individuals and were therefore independent.

A total of 300 samples were collected from the two tree species, rowan (n = 150) and silver fir (n = 150). Samples of rowan and silver fir were collected within each of the three management types: bark-beetle-impacted (n = 50 for each tree species), managed (n = 50) and intact (n = 50). Last, within each management type, rowan and silver fir samples were collected from trees at 10–60 cm in height (n = 25) and between 75 and 150 cm in height (n = 25), with the exact height of browsing being recorded for each sample. Deer do not browse uniformly or completely randomly throughout a stand, but browsing is often clustered (Gill, 1992). To decrease the chance that sampled trees were browsed by the same individual, sampled trees of the same species were approximately 50 m apart. Trees of the two different species were sampled within a distance of 50 m when present, as an indication of forage selection of the two deer species.

2.3. Genetic analyses

All samples were analysed in the molecular ecology laboratory at the Department of Wildlife, Fish, and Environmental Studies, SLU. This laboratory is set up specifically to work with low-quality DNA samples. To minimize the risk of contamination, all samples followed a uni-directional flow, in which eDNA extraction, PCR set-up, and PCR cycling and Fluidigm analyses were performed in separate rooms with designated equipment. All handling of open samples took place in UV hoods, and DNA free filter tips were used for all pipetting.

Twigs were first sonicated in phosphate-buffered saline solution (PBS buffer) to increase the eDNA yield. After removing the twigs the samples were centrifuged and the supernatant discarded. eDNA was then extracted using the Qiasymphony DNA Investigator kit (Qiagen, Netherlands) according to the manufacturer's instructions.

eDNA was assigned to roe deer or red deer according to the results of ten mitochondrial single nucleotide polymorphism (SNP) assays. These assays were monomorphic within species (with the exception of one marker for roe deer), but polymorphic between species groups. Across the full set, each species generated a unique haplotypic SNP profile, with a minimum of two differences between any given species pair. eDNA samples were amplified and run on an integrated fluidic circuit (IFC) on a Fluidigm Biomark (Fluidigm, USA) following the manufacturer's instructions, with the addition of a pre-amplification step of 40 cycles.

2.4. Relative deer densities

The relative densities of the deer species needs to be accounted for when testing whether rowan and silver fir are browsed disproportionately by the deer species present. The relative densities of both deer species were estimated inside the national park using pellet group counts. On 3–17 May 2017, we counted the number of dung pellet groups on plots along four linear transects that included the full range of management practices and forest structures present within the Bavarian Forest National Park (Bässler et al., 2009; Fig. 1). Transects were selected using a stratified random scheme and were balanced in order to avoid autocorrelation in forest structure. Each transect is 5.7–8.4 km long, with 100 m^2 circular plots placed every 200 m. Pellet groups were assigned to either roe deer or red deer according to shape and pellet size. Highly decomposed pellet groups, of which the ungulate species could not be identified anymore, were excluded from analyses. As pellet group counts were conducted shortly after snow melt, the number of decomposed pellet groups was minimal. The number of pellet groups from each species was used as a relative index of the

density of both deer species. This assumes that the defecation rates were the same for both species. Published defecation rates for both deer species vary, but also show considerable overlap (Dobiáš et al., 1996; Mitchell and McCowan, 1984; Mitchell et al., 1985; Neff, 1968). This method estimated that roe deer made up 39% of the total population of both deer species.

2.5. Statistical analyses

The 49 samples collected within the deer enclosures were sent as blind samples to the laboratory in Sweden for eDNA analysis. The percentage of samples that successfully identified species-specific browsing was calculated.

All statistical analyses were conducted in R 3.4.1 (R Core Team, 2017). We first analysed the factors influencing the predictive ability of the method. For each apex shoot sample, a value of 1 was given when the browsed twigs amplified eDNA during PCR, and a value of 0 was given when the method was unable to successfully identify the deer species responsible for browsing (hereafter called success rate). We used a generalized linear mixed model from the lme4 package (Bates et al., 2014) with success rate as the binomial response variable. The following three variables were used as fixed effects: tree species (silver fir and rowan), height of browsing (cm) and management type (bark-beetle-impacted forest, managed forest and intact forest). Further, we tested whether a spatial autocorrelation structure needed to be added using the DHARMa package (Hartig, 2019) and found that the collected samples were spatially independent. However, to account for differences between the different stands, they were included as the random effect.

We then tested whether the number of browsing events of each deer species differed from the expected browsing based on the relative deer densities estimated from pellet counts, using an exact binomial test from the stats package. For this, two different models were created, one for silver fir and one for rowan. For each model, the number of successes was the number of successfully identified browsing events of roe deer on the respective tree species, and the number of trials was the total number of browsing events of roe deer and red deer on the respective tree species. The expected probability was set at 0.39 for roe deer, based on the estimated relative difference between roe deer and red deer densities.

Lastly, we tested which environmental variables influenced deer-species-specific browsing. Again, we used a generalized linear mixed model from the lme4 package with tree species (silver fir and rowan), height of browsing (cm) and management type (bark-beetle-impacted forest, managed forest and intact forest) as fixed effects. Browsing by either roe deer or red deer was added as the binomial response variable and to account for the differences between stands, stands were included as a random effect.

3. Results

3.1. Predictive ability of DNA amplification method

Of the 49 twig samples that were collected during feeding trials, deer eDNA was successfully amplified from 48 samples (98%). The deer species responsible for the browsing was correctly identified for all of these amplified samples. Of the 300 naturally browsed twig samples collected in the field, deer DNA was successfully extracted and amplified from 151 samples (50.3%); of these, 88 were from red deer and 63 were from roe deer. Amplification of deer DNA tended to be slightly higher from twigs collected in the intact forest than in the bark-beetle-impacted forest, but the difference was not statistically significant (0.546 ± 0.292, z = 1.87, p = 0.061). No significant difference was found in the success of amplification of deer eDNA from twigs collected in the managed forest and twigs collected in the bark-beetle-impacted forest (-0.065 ± 0.290, z = -0.23, p = 0.822). There was also no
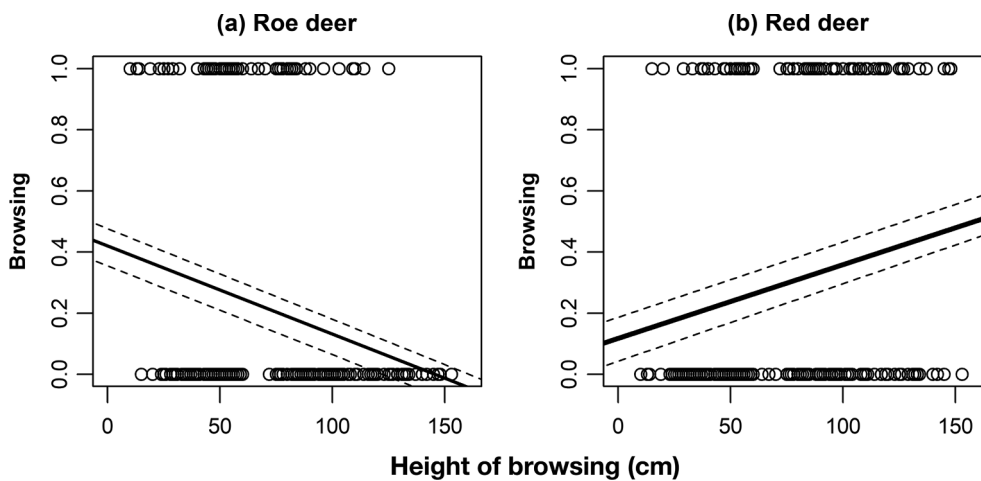
(a) Roe deer   **(b) Red deer**

Fig. 2. Probability that a particular browsing event is attributable to (a) roe deer and (b) red deer with increasing height at which the browsing occurred on trees, as determined by amplification of eDNA left behind on the browsed twigs. Red deer were significantly more likely to be responsible for browsing at higher heights than roe deer.

significant difference in the success of amplification of deer eDNA between twigs collected from the two tree species (-0.064 ± 0.262, $z = -0.25$, $p = 0.807$) nor the success of amplification of twigs collected from different heights (-0.051 ± 0.120, $z = -0.43$, $p = 0.671$).

3.2. Deer species responsible for tree species browsing

Browsing on rowan [$p = 0.810$, 95% CI (0.289, 0.525)] and silver fir [$p = 0.567$, 95% CI (0.315, 0.540)] had the same probability of being attributable to roe deer and red deer.

3.3. Variables influencing browsing of specific tree species

The probability that red deer were responsible for browsing increased with height of browsing (0.810 ± 0.214, $z = 3.79$, $p < 0.001$; Fig. 2). Furthermore, the probability of red deer browsing tended to be lower in the intact forest than in the bark-beetle-impacted forest, although this was not statistically significant (0.865 ± 0.457, $z = 1.89$, $p = 0.058$; Fig. 3). No significant difference in the probability of red deer browsing was found between the managed forest and the bark-beetle-impacted forest (-0.396 ± 0.498, $z = -80.795$, $p = 0.426$; Fig. 3). Lastly, no significant difference was found in the probability of
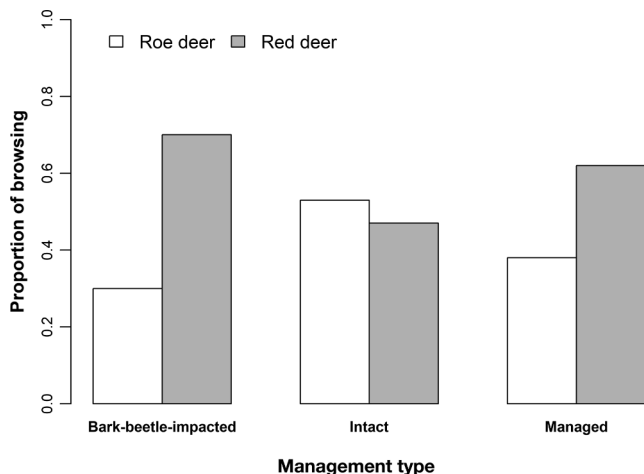

Fig. 3. Proportion of browsing events attributable to roe deer or red deer in three management types within the Bavarian Forest National Park, as determined by amplification of eDNA left behind on the browsed twigs. The probability of roe deer being responsible for browsing was higher in intact forest than bark-beetle-impacted, although this relationship was not statistically significant.

browsing between the two different tree species (0.063 ± 0.387, $z = 0.162$, $p = 0.871$).

4. Discussion

4.1. Factors influencing the eDNA method

The successful extraction of eDNA from 98% of the feeding trial browsed twig samples and identification of the correct deer species in all of these samples demonstrated the feasibility of the method in identifying the deer species responsible for browsing. The success of deer eDNA extraction and amplification did not differ between the two deer species tested, indicating that there is unlikely to be bias in the eDNA degradation rate between the two deer species. However, successful extraction of deer DNA from naturally browsed samples was lower, around 50%. This lower success rate most likely reflects a longer elapsed time between browsing and collection of the naturally browsed samples. For example, Nichols et al. (2012) found that amplification success rate of eDNA recovered from browsed twigs decreased by around 50% after 12 weeks. Browsed twigs from the enclosures were very fresh, i.e. less than 3 days old, whereas the exact time since browsing of the naturally browsed twigs is unknown and could be longer in some cases. Environmental factors, such as temperature, precipitation and exposure to UV radiation, largely determine the rate of degradation of eDNA (Nichols et al., 2012). Therefore, browsed twigs are increasingly exposed to environmental conditions with time, increasing degradation of the eDNA present. However, as the degradation rate of roe deer and red deer eDNA is the same, this is unlikely to be a strong limitation on the use of the technique- assuming that only freshly browsed twigs are sampled and a large enough sample is collected.

Although not statistically significant, the amplification success rate from samples from intact forests was slightly higher compared to the amplification success in bark-beetle-infected forest and managed forest. This may be explained by a lower eDNA degradation rate resulting from a reduced exposure to UV radiation and precipitation and constant cooler temperatures with fewer extremes under the higher canopy cover. To delineate the limits of the extraction and amplification of eDNA, the method needs to be tested under a broader range of environmental conditions, where factors such as temperature, precipitation and UV exposure have been better quantified. Nevertheless, the results from this temperate forest indicate that eDNA amplification could be used to elucidate the browsing patterns of ungulate species without the need for direct observations.

4.2. Relative impact on palatable tree species

Dietary analysis, including studies conducted in the Bavarian Forest

National Park, suggest that roe deer are typical concentrate browsers that predominantly feed on high-concentration, low-fibre food items, whereas red deer are intermediate feeders whose diets also contain a high proportion of high-fibre food items, such as grasses (Barančeková et al., 2009; Krojerová-Prokešová et al., 2010). As such, it was hypothesised that roe deer would be disproportionately more responsible for the browsing of the palatable tree species, rowan and silver fir, compared to red deer. However, we found that silver fir and rowan are just as likely to be browsed by roe deer as by red deer.

These results therefore appear to contrast with those expected based on the feeding niches of the two deer species. However, whilst considerable evidence supports the deer's respective feeding niches, large variations in the diet of both species in response to a wide range of environmental and habitat variables is often observed (Bugalho and Milne, 2003; Cornelis et al., 1999; Storms et al., 2008, Storms et al. (2006)). The exact contribution of roe deer and red deer to the browsing of palatable tree species may therefore also experience a great deal of variation, both temporally as well as between locations. Furthermore, studies often show a large degree of overlap in the diet of both species, which sometimes appears to be greatest during the winter-when the samples used for this study were collected (Dumont et al., 2005; Latham et al., 1999; Mátrai and Kabai, 1989; Obidziński et al., 2013; Storms et al., 2008). For example, in Central Europe, silver fir appears to be a favoured food source of both deer species primarily during winter, when other food sources are lacking (Häsler and Senn, 2012), which could explain why roe deer were not disproportionately responsible for the browsing on silver fir. Additionally, red deer can also have strong impacts on the regeneration of silver fir and rowan, which suggests that their browsing levels on these tree species can be high in some circumstances (Klopcic et al., 2009; Speed et al., 2013).

The ecological and behavioural drivers behind the results of this study and the reasons for their contrast with the expected results based on feeding niches remain speculative. Predicting the relative impact of a particular deer species on a particular plant species based solely on their expected feeding niche may always be difficult. This is demonstrated by the results of this study, which indicate that a concentrate browser such as roe deer does not necessarily have a greater browsing impact on tree species assumed to be the most palatable. This result further demonstrates the usefulness of eDNA amplification for elucidating complex browsing patterns, which could influence wildlife management practices. Further studies utilizing this method in other regions would demonstrate whether the findings of this study are found more broadly.

4.3. Variables influencing browsing

The results of this study demonstrate that roe deer were more likely to be responsible for browsing at lower heights, while red deer were more likely to be responsible for browsing at higher heights. These results are consistent with the results of Nichols et al. (2015), who used the same eDNA method and demonstrated that, although there was no minimum height browsed, the mean height of red deer browsing was significantly higher than that of roe deer, which reflects the larger body size of red deer and their ability to reach higher branches.

Although not statistically significant, the results of this study found some differences among roe and red deer in the use of forest management types for foraging. Roe deer were more likely to be responsible for browsing in intact forest compared to other habitats. Both red deer and roe deer prefer to forage along forest edges, in small gaps or in younger forests with a lower canopy cover (Gill et al., 1996; Staines and Welch, 2011; Tufto et al., 1996). Red deer occupy both pastures that offer abundant forage and forested areas, and they alternate between the two areas depending on season and time of day (Godvik et al., 2009; Schmidt, 1993; Suter et al., 2004). Although not statistically significant, the results of this study appear to be consistent with those that have found red deer to have a greater preference for foraging in open

habitats within forest stands compared to roe deer (Kuijper et al., 2009; Latham et al., 1996). However, caution is needed when interpreting the results of this study. As well as not being statistically significant, the level of cover and forage within each of the three management types has not been directly quantified. It is therefore difficult to interpret the drivers behind differences in the proportion of browsing events attributable to each deer species in the three management types.

5. Conclusion

Our results have implications for game management in Central European forests, in which it is believed that a lack of regeneration of palatable species such as silver fir and rowan is due to browsing by deer. In contrast to expectations based on the different feeding niches of red deer and roe deer, our results suggest that in forests where both deer species are present, neither species may be disproportionately responsible for the browsing of palatable tree species. Therefore, increasing the number of roe deer removed by hunting relative to the number of red deer, would not likely lead to greater reductions in tree browsing than if both deer species were targeted equally. Further studies are needed to determine whether the observed pattern holds true under different environmental conditions and in different forest ecosystems and different abundances of deer. The success of this eDNA amplification method for elucidating the browsing habits of ungulates in temperate forests suggests that it can be used as a tool to improve the efficiency and effectiveness of deer management in multispecies ungulate communities to reach silvicultural objectives or conserve sensitive plant communities. This might assist in increasing the efficiency and effectiveness of deer management and in reducing human disturbance of deer populations in strictly protected areas that aim to conserve natural processes. However, the results of this study seem to confirm that the likelihood of successful amplification may reduce rapidly with time, suggesting that successful use of the technique may currently rely on a large sample size of freshly browsed twigs. Further studies utilizing this technique in a broader range of environmental conditions may allow the limits of the technique to be better determined.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foreco.2019.117483.

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