Carcasses for Nature:

An investigation into the consequences of the provisioning of deer carcasses on the biodiversity of a temperate woodland ecosystem in the UK

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Abstract

Large carcasses serve as a key resource for a variety of species within most terrestrial ecosystems, with broader ecosystem benefits by promoting nutrient cycling and wider biodiversity. In recent decades, humans have greatly impacted the availability of large carcasses, both directly and indirectly, with dire consequences for the diversity and functioning of ecosystems. Therefore, this has promoted interest in the provisioning of large carcasses to ecosystems where they are less abundant. In the UK in particular, the combination of a complete absence of apex predators and the management of deer populations have resulted in a reduction of large carcasses.

Therefore, in the present study, the consequences of the carcasses of two native deer species on the biodiversity of a temperate forest ecosystem in the North York Moors were investigated. Specifically, the impacts of carcasses, and equivalent control sites, on the diversity and composition of four broad groups were examined, with these consisting of vertebrates, invertebrates, plants and soil fungi.

The summary of findings included the activity of vertebrates shown to be higher at carcass sites compared to controls, plus vertebrate composition varied significantly between site types. Moreover, the species richness and diversity of invertebrates differed significantly between carcass and control sites. In contrast, no evidence was found for any effect of carcasses on the diversity and composition of plants or soil fungi.

Future research into carcass provisioning in the UK should focus on different types of ecosystems and other geographical regions, as well as replicating the unpredictable spatiotemporal distribution of carcasses produced by natural predation. Studies should also aim to examine for any long-term impacts of carcasses on plants and soil fungi that were not revealed in the current study. Furthermore, potential impacts of domestic dogs on carcass consumption, proximity to human-activity and risks of mesoscavenger dominance should be considered.

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List of Abbreviations

Abbreviation	Definition
AIC	Akaike Information Criterion
ANOVA	Analysis of Variance
ASV	Amplicon Sequence Variant
bp	Base Pairs
CI	Confidence Interval
DADA	Divisive Amplicon Denoising Algorithm
eDNA	Environmental DNA
GLM	Generalised Linear Model
GLMM	Generalised Linear Mixed Model
ITS	Internal Transcribed Spacer
LM	Linear Model
LMM	Linear Mixed Model
NMDS	Non-metric Multidimensional Scaling
OTU	Operational Taxonomic Unit
PERMANOVA	Permutational Multivariate Analysis of Variance
PCR	Polymerase Chain Reaction
REML	Restricted Maximum Likelihood
SE	Standard Error
SSSI	Site of Special Scientific Interest
w_i	Akaike Weight

Chapter 1: Introduction

1.1. The Recycling and Decomposition of Carcasses in Ecosystems

Within all known ecosystems, the decomposition of organic matter is fundamental to the cycling of energy and nutrients, and subsequent ecosystem function (Swift, Heal and Anderson, 1979; Moore *et al.*, 2004). A key subset of organic matter within ecosystems are carcasses, with all animals ultimately dying and a significant proportion of their mass returning back to the nutrient cycle via both decomposition and consumption (Figure 1.1; Barton, Cunningham, Lindenmayer, *et al.*, 2013). The process of carcass decomposition is carried out by a varied array of organisms, including microbes, invertebrates and vertebrates, collectively referred to as the necrobiome (Benbow *et al.*, 2019). In recent years, there has been growing acknowledgement of the vital role of carcasses in conserving the diversity of these necrophagous species, as well as the wider biodiversity of species more loosely associated with the necrobiome (Wilson and Wolkovich, 2011; Moleón and Sánchez-Zapata, 2015).

Carcasses only account for a small proportion of the overall detrital biomass of terrestrial ecosystems in comparison to plant detritus (Parmenter and MacMahon, 2009); however, they have a disproportionate role in nutrient cycling and ecosystem functioning, primarily based on the high density of key nutrients they return to the environment (Moore *et al.*, 2004). Of particular importance are carcasses of large mammals (defined here as mammal species where the average mass of an individual is greater than 10 kg), with this group representing the majority of vertebrate biomass across global populations of wild animals (Greenspoon *et al.*, 2023). Thus, within this study the focus is on large carcasses, with these defined as those of mammals exceeding a mass of 10 kg (Moleón *et al.*, 2015; Greenspoon *et al.*, 2023).

The high-nutrient content of large carcasses considerably alters local soil biogeochemistry (Macdonald *et al.*, 2014), where these changes to soils can affect the diversity of microbial communities (Metcalf *et al.*, 2016; Risch *et al.*, 2020), as well as the growth and diversity of plants (Towne, 2000; Bump *et al.*, 2009; Barton *et al.*, 2016). Carcasses also impact the diversity of scavengers in terrestrial ecosystems, including both invertebrate and vertebrate species (Parmenter and MacMahon, 2009; Wilson and Wolkovich, 2011). Both obligate and facultative scavengers rely on carcasses as a valuable food resource, where facultative scavenging is both taxonomically and globally widespread (Foltan *et al.*, 2005). Scavengers help to improve ecosystem stability by increasing connectivity between food web trophic levels (DeVault, Rhodes and Shivik, 2003; Moleón *et al.*, 2014) and by facilitating the distribution of nutrients across the broader landscape (Moleón and Sánchez-Zapata, 2015).

Large carcasses have been denoted as discrete and ephemeral “hotspots” of both chemical and biological activity (Finn, 2001; Carter, Yellowlees and Tibbett, 2007). This refers to their distinct and unpredictable nature, both spatially and temporally, as resources that contribute to a disproportionate amount of heterotrophic activity, with this more prominent as carcass size increases (Barton, Cunningham, Lindenmayer, *et al.*, 2013). Therefore, through direct carcass influences on ecosystems, as well as wider indirect impacts, large carcasses contribute to the overall diversity and composition of ecosystems communities.

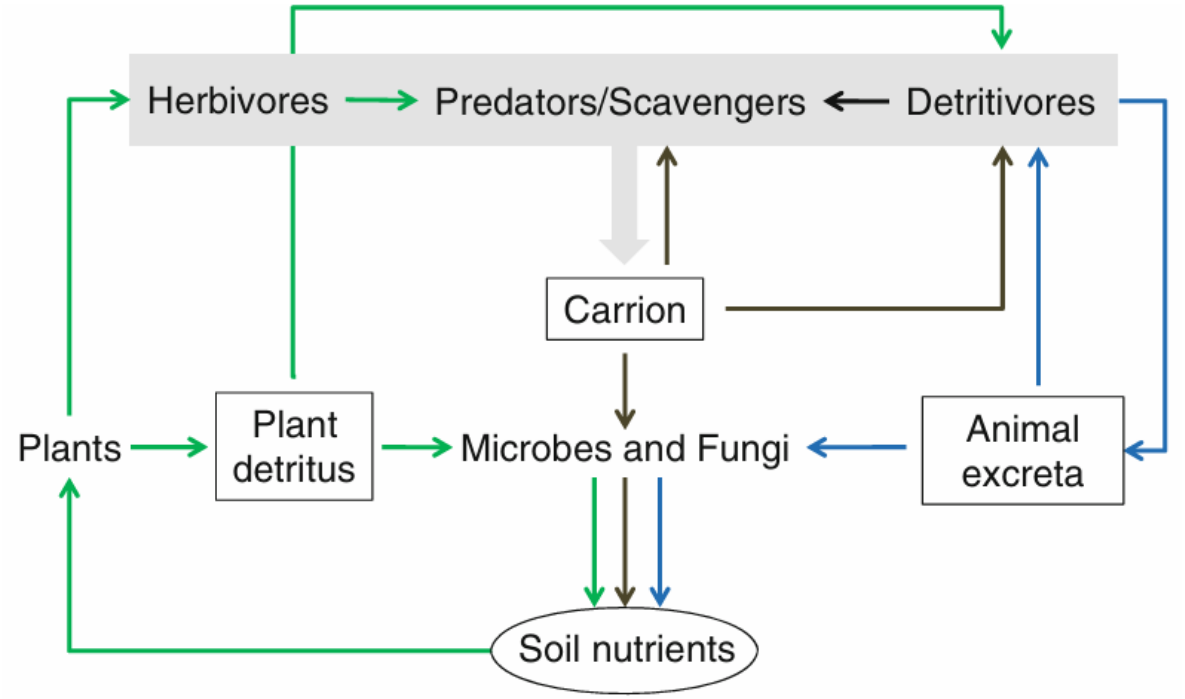

Figure 1.1: Summary of pathways of energy flow in a carcass-centred food web. *Grey box*: living animals and their potential input to the carcass pool upon their death. *Brown arrows*: flow of energy from carcasses through scavengers and detritivores, and the flow of nutrients through microbes into the soil. *Blue arrows*: the transfer of energy from live animals to excreta to detritivores and microbes. *Green arrows*: the flow of nutrients via plants. Taken from Barton, Cunningham, Lindenmayer, *et al*. (2013).

1.2. Loss and Shifts to the Availability and Distribution of Carcasses

In recent decades, there have been numerous global declines in populations of large carnivores (Estes *et al.*, 2011; Ripple *et al.*, 2014; Mateo-Tomás *et al.*, 2015), predominantly due to both the fragmentation and destruction of their habitats as well as direct persecution (Ripple *et al.*, 2014). This has resulted in losses and shifts to the spatial and temporal availability of predator-killed large carcasses (Wilson and Wolkovich, 2011; Cunningham *et al.*, 2019), particularly evident in ecosystems where there is a complete absence of any apex predator, with subsequent decreasing numbers of many vertebrate scavenger species (Whitfield *et al.*, 2008; Margalida *et al.*, 2010). The absence and declines of predators and scavengers have been shown to have dire consequences for ecosystems and the services they provide to humans (O'Bryan *et al.*, 2018). Particularly, carcass removal rates are likely to be lower in the an absence of large predators and/or scavengers, compared to ecosystems with intact scavenger guilds where ecosystem services are met with the efficient removal of carcasses (Figure 1.2; Newsome *et al.*, 2021).

Furthermore, in many countries the availability of carcasses is worsened by the management of wild ungulates. In countries where apex predators are nearly or entirely absent, it is common practice for wild ungulates to be culled to control their populations, with carcasses typically removed from the environment and often used to supply meat for human consumption. This reduces the number of ungulates that will die of natural causes, especially when culls often target infirm individuals (Fielding *et al.*, 2014; Torres-Porras *et al.*, 2014), and subsequently decreases carcass availability (Wilson and Wolkovich, 2011). This severely impacts the wider ecosystem, with nutrients lost from carcasses that would have otherwise been cycled back into the ecosystem (Wilson and Wolkovich, 2011; Beasley, Olson and Devault, 2012; Ferraro and Hirst, 2024).

Finally, legislation in many countries regarding the management of livestock dictates that when an animal dies it must be removed from the environment, further decreasing the abundance of large carcasses. In Europe, this is primarily due to the risk of disease for other livestock and the public (Council of the European Union, 2002), with the only exemptions being animals that died in the most inaccessible areas, such as remote uplands (Margalida *et al.*, 2010).

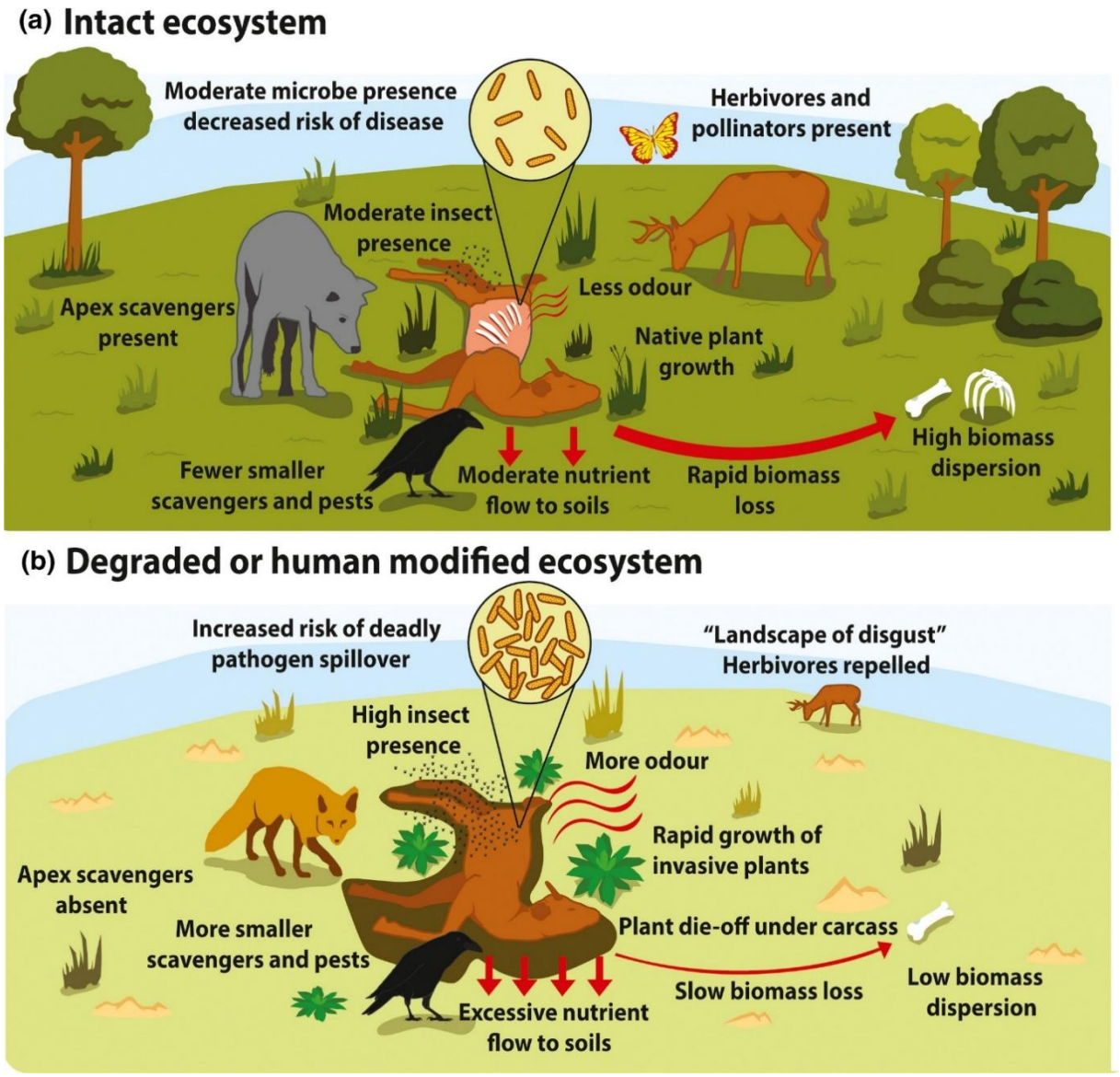

Figure 1.2: Proposed interactions around large carcasses in (a) an intact and functioning ecosystem with apex predators/scavengers present, compared to (b) a degraded and human-modified ecosystem where apex predators/scavengers are absent. Taken from Newsome *et al.* (2021).

1.3. Growing Interest in Carcass Provisioning

In many ecosystems where there is an apparent reduction in the availability of large carcasses, there has been greater awareness of the associated detrimental impacts leading to an increased interest in the practice of the provisioning of large carcasses (Fielding *et al.*, 2014). Examples of carcass provisioning have generally been with the overarching aim of maintaining biodiversity by providing vital food sources for endangered vertebrate scavenger species, often various vulture species (Gilbert *et al.*, 2007; Cortés-Avizanda, Carrete and Donázar, 2010).

An example of carcass provisioning is for the conservation of griffon vultures (*Gyps fulvus*) in South West Europe, where the legal removal of livestock carcasses led to declines in vulture populations (Margalida *et al.*, 2010). This prompted legislation changes allowing the deployment of large carcasses within fenced feeding stations, plus some carcasses outside of fenced zones, as a resource for avian scavengers (Cortés-Avizanda, Carrete and Donázar, 2010). In addition to noticeable benefits for biodiversity, there were evident economic motivations, with carcass removal by scavengers estimated to save between €900,000–1,500,000 that would have otherwise been spent on disposal (Margalida and Colomer, 2012).

However, across other European nature reserves the provisioning of large carcasses has often proved to be a controversial practice. As although it is legal to leave carcasses of wild ungulates in situ there can be strong public opposition, such as at the Oostvaardersplassen “rewilding experiment” in the Netherlands where this led to interventions to reduce the abundance of large carcasses that were left out in the reserve (Colijn, 2014; Fielding *et al.*, 2014).

1.4. A Carcass-depleted Landscape – the UK as a Case Study

In the UK, for several hundred years there has been a complete absence of any terrestrial apex predator, where anthropogenic impacts led to the local extinction of the grey wolf (*Canis lupus*; Ritchie, 1920), brown bear (*Ursus arctos*; O'Regan, 2018) and Eurasian lynx (*Lynx lynx*; Hetherington, Lord and Jacobi, 2006). In contrast, there are several species of deer with large populations and increasing ranges (Ward, 2005), where the absence of apex predators is a significant driver of deer overpopulation (Côté *et al.*, 2004). These deer species include two native species, red deer (*Cervus elaphus*) and roe deer (*Capreolus capreolus*), one naturalised species, fallow deer (*Dama dama*), and three non-native species, Chinese water deer (*Hydropotes inermis*), Reeve's muntjac (*Muntiacus reevesi*) and sika deer (*Cervus nippon*).

The populations of deer in the UK have led to numerous negative impacts for both commercial forestry and natural ecosystems via excessive browsing and grazing (Welch *et al.*, 1991; Gill, 1992; Putman and Moore, 1998; Reimoser and Putman, 2011). Therefore, deer populations across the UK are typically managed by culling, with culled individuals removed from the landscape to be sold as venison (Deer Working Group, 2019). Between 2010 and 2021 in Scotland alone, approximately 2.1 million deer were culled and removed from the environment, removing a significant amount of nutrients that would otherwise have been returned via decomposition and scavenging (Ferraro and Hirst, 2024).

Landowners in some parts of Scotland have been known to leave deer carcasses as a food source for birds of prey, primarily golden eagles (*Aquila chrysaetos*) and white-tailed eagles (*Haliaeetus albicilla*) (John Muir Trust, 2021). Yet, there is a lack of research into the consequences of carcass provisioning for these birds, let alone investigations into the consequences of carcass provisioning for a variety of taxa within ecosystems. Even in countries other than the UK, there has been limited empirical research into the impacts of large carcasses on the diversity of multiple levels of an ecosystem, with a need to further the understanding of their holistic contribution to local biodiversity (Parmenter and MacMahon, 2009; Wilson and Wolkovich, 2011; Barton, Cunningham, Lindenmayer, *et al.*, 2013).

Therefore, this bodes the question – what are the wider impacts of the provisioning of large carcasses on the biodiversity of terrestrial ecosystems? Specifically, with a focus on the UK in this study.

1.5. Study Aims and Thesis Structure

The aim of this project was to quantify the impacts of large carcasses, specifically of two native deer species (red and roe deer), on the biodiversity of a temperate forest ecosystem in North East England from early winter to early summer. Specifically, how carcasses affected the diversity and composition of four broad groups: vertebrates (plus activity of this group), invertebrates, plants and soil fungi. These were measured at sites with either a roe or red deer carcass present, as well as un-baited control sites. It was predicted that the red deer carcasses, being larger than roe deer carcasses, would generally host a higher biodiversity across these four broad groups, due to the increased biomass they provide as an ephemeral resource. With roe deer carcasses having a lower biomass than red deer carcasses, they would host a slightly lower biodiversity of these similar species assemblages compared to red deer carcasses, but they would host a significantly greater diversity than the un-baited control sites. It was also predicted that the composition of these broad groups would likely differ between carcass and control sites, with carcasses offering more resource opportunities for necrophagous vertebrates and invertebrates, and creating altered soil biogeochemical states for different plants and fungi to exploit.

From this study, it is hoped that motivation and advice is provided for the conception of other similar studies investigating the effects of carcass provisioning in other areas and different ecosystems of the UK. In the long-term, it is hoped that this study, and the results of similar investigations, will provide greater insight to land managers and policy makers of the overall consequences of carcass provisioning for the biodiversity and ecosystem function of a variety of terrestrial ecosystems across the UK and beyond.

The structure of this thesis is as follows:

Chapter 1 – Introduction:

This chapter introduces the role of large carcasses in the nutrient cycle of ecosystems, and how they contribute to local biodiversity and ecosystem functioning. The ways in which human-induced changes to the availability and distribution of large carcasses is explored and how this has encouraged interest in carcass provisioning. Finally, the present state of large carcass abundance in the UK is discussed and hence the subsequent motivation for and aims of this study, as well as introducing the study site.

Chapter 2 – Assessing the impacts of deer carcasses on vertebrate, invertebrate and plant diversity:

Using camera traps, pitfall traps and quadrats, the impacts of large carcasses on the diversity and composition of vertebrates (plus activity), invertebrates and plants were quantified. Potential influences of habitat type, changes over time, and increasing distance from carcasses (for invertebrates and plants only) were examined.

Chapter 3 – Investigating the effects of deer carcasses on the diversity and composition of soil fungi using eDNA approaches:

Using environmental DNA (eDNA) approaches, the impacts of large carcasses on the diversity and composition of soil fungi were explored. Soil samples were taken at several sites prior to carcass placement, as well as corresponding control sites, followed by further soil samples extracted from the same sites after considerable carcass decomposition. Subsequent laboratory work was carried out to extract, amplify and sequence fungal DNA, in order to derive taxonomic lists for comparisons of fungal diversity and composition between the initial and final samples, and between the carcass and control sites.

Chapter 4 – Discussion: Implications of, and advice for, carcass provisioning for the biodiversity of ecosystems in the UK:

In the final chapter, the overall findings of this study of the consequences of large carcasses on the biodiversity of a temperate woodland in the UK are considered. Limitations of the present study are discussed, and guidance offered for future studies to build upon the findings with the long-term aim of influencing land management and policy regarding large carcass provisioning across the UK.

1.6. The Study Site

1.6.1. Newtondale SSSI

The study was carried out in the Newtondale Site of Special Scientific Interest (SSSI, 54°20' N, 0°44' W; Figure 1.3) within Cropton Forest, in the south of the North York Moors National Park, the UK. Newtondale SSSI is managed by Forestry England and covers roughly 4.7 km^2, of which approximately 4.1 km^2 is forested with around 35% covered by broadleaf woodland and the other 65% by coniferous plantations. The dominant coniferous species are Sitka spruce (*Picea sitchensis*), Scots pine (*Pinus sylvestris*) and Norway spruce (*Picea abies*), respectively. Whereas the dominant broadleaf species is birch (*Betula pubescens* and *B. pendula*), followed by oak (*Quercus spp.*) and ash (*Fraxinus excelsior*). The SSSI is within a valley, with the sides of the valley mostly forested, with the upper areas becoming more open with patches of bracken (*Pteridium spp.*) and heather (*Calluna vulgaris*), as well as areas of upland grass moorland and scrub (Forestry England, 2022).

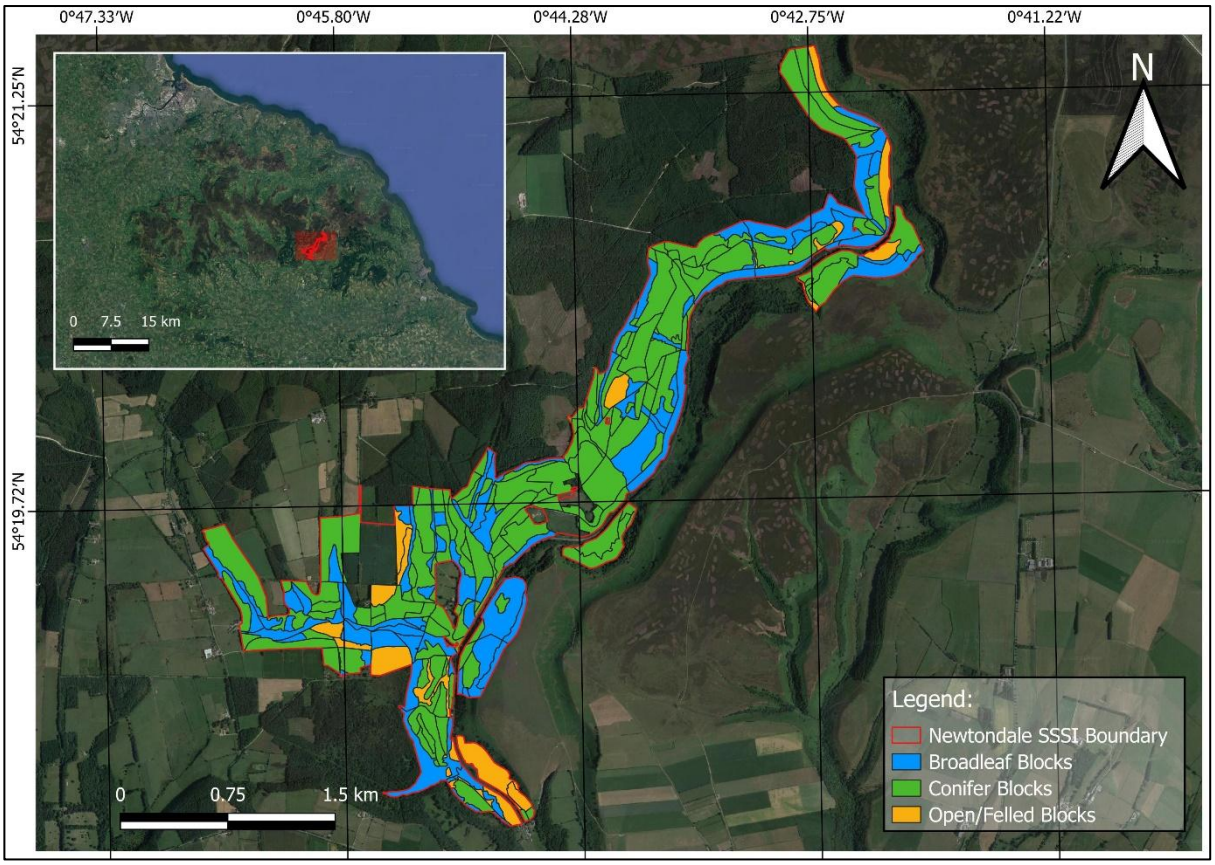

Figure 1.3: Map of Newtondale SSSI (with red line indicating the site boundary), with an inset map of its location in the wider North York Moors. Distinct blocks of the three main habitat types are shown, i.e., broadleaf, conifer and open/felled. Map produced using QGIS.

The narrow valley of Newtondale has a small river, Pickering Beck, and the North York Moors steam railway track running through. The area has multiple tracks for access by Forestry England staff, logging vehicles and for access to several of the private properties within the forest., plus numerous public footpaths and bridleways.

The area supports a diverse range of wildlife, including a range of nationally important birds such as nightjars (*Caprimulgus europaeus*), turtle doves (*Streptopelia turtur*) and woodcocks (*Scolopax rusticola*), as well as over one thousand Northern hairy wood ant (*Formica lugubris*) nests (Forestry England, 2022). There are also populations of both red deer and roe deer, where these are managed through culling by Forestry England.

The climate of the area local to Newtondale typically consists of long and cold winters, and short, cool summers, with a yearly average maximum temperature of 11.5°C and average minimum temperature of 5.0°C. The yearly average rainfall is 980 mm, with the highest monthly amount falling in November (119 mm) and the lowest in May (56 mm). This climate data was obtained from Fylingdales weather station which lies roughly 5 km due north-east of Newtondale.

1.6.2. Site Locations

Based on the availability of carcasses able to be supplied by collaborators at Forestry England, six roe deer and six red deer carcasses were obtained. Across the study area, there are three distinct habitat types of interest to the study – coniferous woodland, broadleaf woodland and open areas which were felled in the past year or two. Therefore, based on the number of carcasses and the different habitat types, an appropriate site design of clusters of three sites was formulated, with one red deer carcass, one roe deer carcass and a shared control at each. Thus, this resulted in six clusters comprising 18 sites in total, with 12 study sites and six controls. These clusters were then distributed across the three habitat types, based on a stratified sampling approach.

Although all three sites within a cluster were located within the same broad habitat type, several biotic and abiotic factors were generally controlled for, including slope aspect, ground vegetation cover and tree canopy cover. Hence, the distance between sites of a cluster needed to be large enough to avoid direct and in-direct carcass effects on neighbouring sites, but small enough so that the fine-scale habitat was not markedly different. A distance of 100 m between sites of a cluster was chosen, with this an appropriate distance to prevent odour bouquets from carcasses at adjacent sites overlapping (von Hoermann *et al.*, 2018; Schwegmann *et al.*, 2022). Moreover, a minimum distance of 500 m

between the central point of adjacent clusters was selected, with this large enough to ensure neighbouring clusters do not influence each other dramatically but small enough that clusters could be positioned within the defined study area. The exact distance between clusters depended on the areas of each habitat type, and the topography and overall area of the study site.

In order to identify the precise locations of clusters, stratified sampling of the whole study area was undertaken based on the total areas of each of the three habitat types. QGIS was used to plan the locations of sites, with the necessary information for each distinct block provided by Forestry England, including the general habitat type (i.e., forested or open) and the dominant tree species. From this, areas were defined into either coniferous, broadleaf or felled areas, as well as calculating the total area of each of these. Several blocks had to be excluded from these calculations including those located on the eastern side of the railway track and those present on the steep sides of the valley, with these areas not safely accessible.

Out of the suitable blocks, coniferous woodland was the dominant habitat type by area, followed by broadleaf and then open habitats; thus, this resulted in four clusters being positioned in coniferous blocks, and one each in broadleaf and felled areas. Four coniferous blocks were then randomly selected as the ones for clusters to be located within. If a block was randomly chosen and was within 500 m of another cluster, then this random allocation was repeated to avoid adjacent clusters being too close to one another. This process was then repeated for both the suitable broadleaf blocks, and then the felled blocks, with one block of each selected.

The exact location of clusters was then determined, with a cluster design of an equilateral triangle, with the three sites positioned at the three vertices, deemed appropriate. A distance of 50 m between sites for all clusters had to be chosen, where this was the largest distance possible in order for clusters to be able to fit within a distinct block. The centre points of cluster triangles were randomly located within each block. If the point was initially positioned too close to the block edge (so that at least one site was thus located outside of the block), then the location was randomly chosen again. This was also for the avoidance of any sites being located too close to any of the forest paths or tracks, so that members of the public walking by were less likely to encounter any of the sites. Moreover, a cluster’s location would also be reassigned if it was within 500 m of one of the private properties present within the study area.

The position of the three sites of each cluster was next determined, with a random bearing chosen to establish the orientation of the cluster triangle around the cluster centre. Finally, ,

the type of site (e.g., red deer carcass, roe deer carcass or control) was then randomly assigned (Figure 1.4). This was important so that any edge effects at each cluster, with one or two sites potentially being closer to the block edge, were randomised between the three site categories. The final locations of the 18 sites were established, with these distributed across the study site and between the three habitat types (Figure 1.5 and Table 1.1).

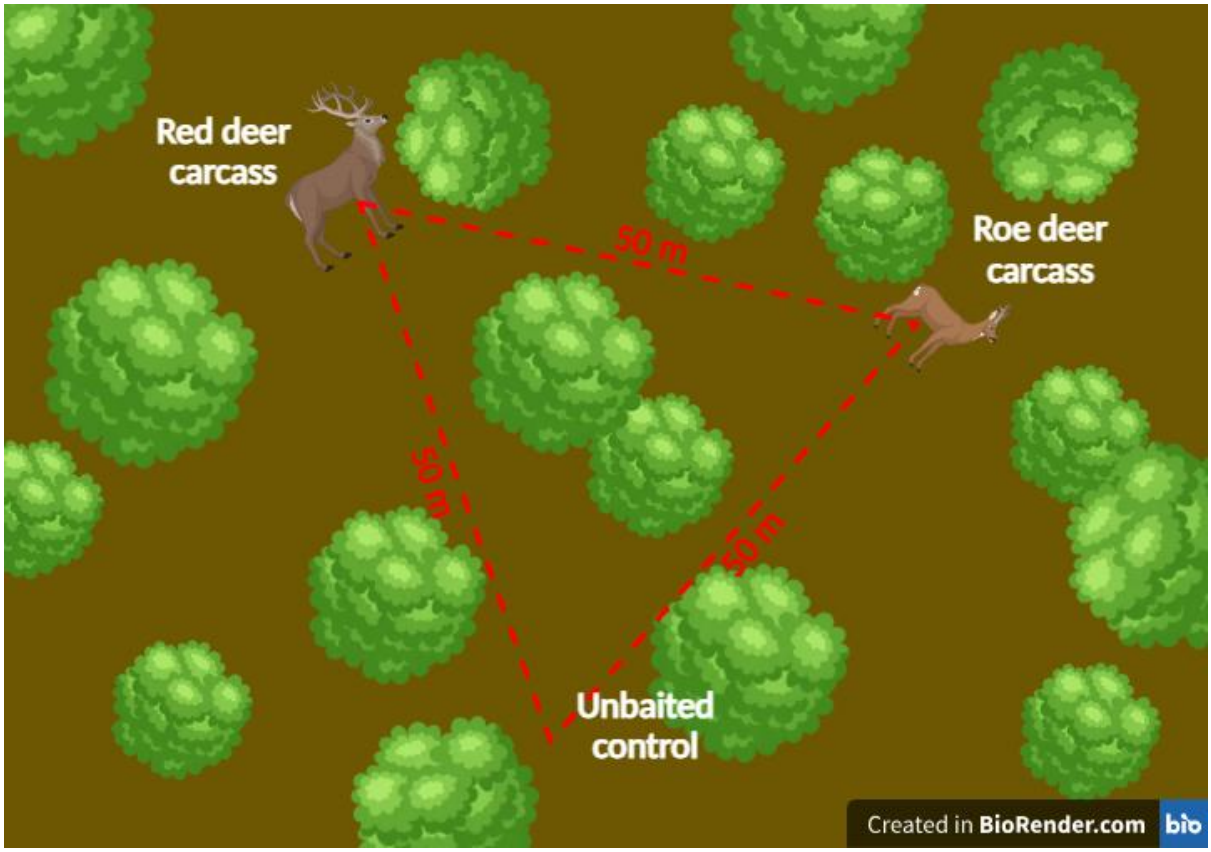

Figure 1.4: Schematic of the experimental design of a cluster of the three site types – red deer carcass, roe deer carcass, and un-baited control. The three site types were arranged in triangular formation for each cluster, with each site separated by a distance of 50 m from the other two sites. The orientation of the cluster triangle and the position of each site on the triangle, were both randomised. Figure produced using BioRender.com.

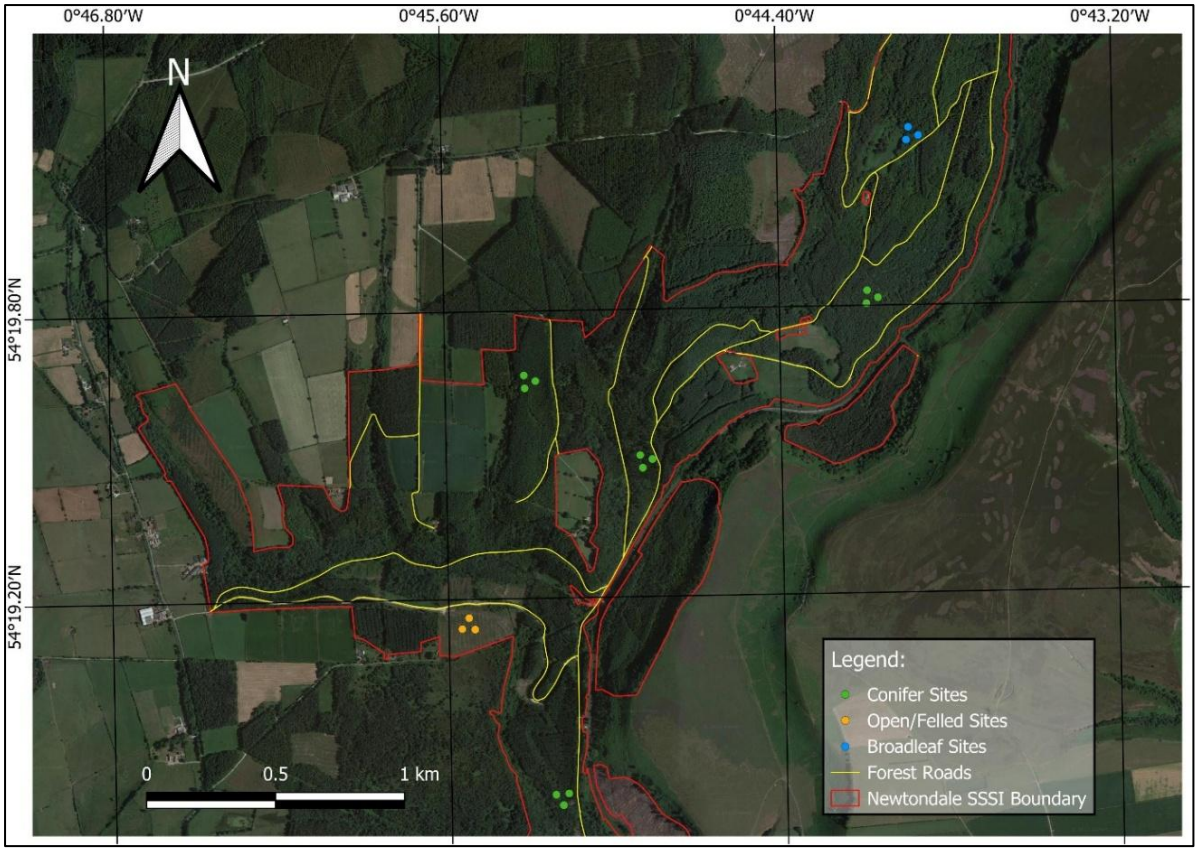

Figure 1.5: Map of the study area with the location of all sites, with these coloured by habitat type. The main forest tracks are denoted by the yellow lines. Map produced in QGIS.

Table 1.1: Summary information for the 18 sites, including the broad habitat type of the block a site is present within, dominant tree species, the site type (red, roe or control), and site coordinates.

Cluster ID	Habitat Type (with *dominant tree species*)	Site ID	Site Type	Latitude	Longitude
1	Broadleaf (*European ash*)	1	Control	54.336180	-0.732134
		2	Roe	54.335889	-0.731547
		3	Red	54.335738	-0.732271
2	Conifer (*Sitka spruce*)	4	Red	54.330860	-0.734729
		5	Control	54.330419	-0.734573
		6	Roe	54.330542	-0.735305
3	Conifer (*Norway spruce*)	7	Control	54.324533	-0.748915
		8	Roe	54.324086	-0.748714
		9	Red	54.324413	-0.748179
4	Conifer (*Sitka spruce*)	10	Control	54.327907	-0.755436
		11	Red	54.327707	-0.754743
		12	Roe	54.327447	-0.755409
5	Conifer (*Sitka spruce*)	13	Red	54.313053	-0.753230
		14	Roe	54.312645	-0.753369
		15	Control	54.312942	-0.753879
6	Felled (*felled in 2022, and restocked with sessile oak, Quercus petraea*)	16	Red	54.319343	-0.759071
		17	Roe	54.318971	-0.759516
		18	Control	54.318934	-0.758760

1.6.3. Site Setup and Surveys

After the determination of precise site locations, site recces were undertaken in late November 2023 to assess if the identified locations were suitable, mainly regarding accessibility. These were all deemed appropriate with adequately safe access to these on foot from adjacent paths or tracks. Sites were then set up, with this being carried out over several days at the beginning of December 2023, with 4-week intervals between visits to sites following on from this. Monthly site visits would typically run over two consecutive days with relevant samples collected and surveys undertaken.

Deer carcasses were obtained from population culls carried out by Forestry England rangers across Cropton Forest over the autumn. The six roe deer carcasses supplied were all a similar size and mass, where the average mass of an adult roe deer is between 10 and 25 kg. Similarly, the red deer carcasses were all of comparable mass, where the average mass of carcasses was between 70 and 100 kg. Over the site set up days, all carcasses were positioned in place at their sites, with the carcasses firstly brought in by Forestry rangers in their vehicles and then transported as close to sites as possible along the forest tracks. Next, they were either dragged to the exact site location by hand (i.e., for roe deer carcasses) or by quad bike (i.e., for red deer carcasses), with this handling work undertaken by Forestry England rangers.

Chapter 2: Assessing the Impacts of Deer Carcasses on Vertebrate, Invertebrate and Plant Diversity

2.1. Introduction

Large carcasses are an essential resource for species across various trophic levels in terrestrial ecosystems, with one of these key groups being vertebrate scavengers. Scavenging of large carcasses by vertebrates has been shown to be a globally widespread behaviour (Wilson and Wolkovich, 2011), whereby most of these scavenger species will facultatively feed on carcasses to complement their overall diet (DeVault, Rhodes and Shivik, 2003; Selva *et al.*, 2005), especially during times when carcass availability increases or when other food sources are rare (Selva *et al.*, 2005; Olson, Beasley and Jr, 2016). Only vultures are known to depend solely on carcasses, thus representing the only obligate scavengers (Moleón *et al.*, 2014). Facultative scavengers include many large carnivores, such as many species in the families Canidae, Felidae and Hyaenidae, as well as smaller facultative scavengers, deemed mesoscavengers, including crows (*Corvus spp.*) and foxes (*Vulpes spp.*) (Mateo-Tomás *et al.*, 2017), with all of these groups important components of a diverse vertebrate scavenger guild. Large carcasses in particular have been reported as supporting a higher diversity of vertebrate scavengers compared to small carcasses, due to supplying a greater abundance of resources for an increased period of time (Selva *et al.*, 2005; Moleón and Sánchez-Zapata, 2015). Furthermore, large carnivores and mesopredators have been shown to have a preference towards larger carcasses (Moleón *et al.*, 2015; Turner *et al.*, 2017; Stiegler *et al.*, 2020); therefore, it is evident that large carcasses are of critical importance for supporting a diversity of vertebrate scavengers.

Invertebrate scavengers are another group reliant on large carcasses, with invertebrates the most diverse group of organisms present at carcasses, excluding the microbial community (Braack, 1987; Benbow *et al.*, 2019). They also play a key role in the decomposition of carrion, with carcass decomposition rates shown to be significantly lower when invertebrates are experimentally excluded (Payne, King and Beinhart, 1968; Parmenter and MacMahon, 2009; Pechal *et al.*, 2014; Barton and Evans, 2017). Once again, there is evidence to suggest large carcasses are able to support a greater diversity of invertebrate species due to the increased variety and abundance of resources available (Schoenly and Reid, 1983); nevertheless, even evisceration residues of small ungulates have been revealed to be able to host a significant diversity of invertebrate scavengers (Schwegmann *et al.*, 2022). Concerning empirical research, there has been a limited number of studies that have investigated the effects of large carcasses on invertebrates in a biodiversity context, with greater research emphasis on their application to forensics and succession (Benecke, 2001; Michaud, Schoenly and Moreau, 2015). Studies that have examined the invertebrate diversity around large carcasses have mostly focussed on necrophagous invertebrates,

typically the families Diptera and Coleoptera (e.g.; Melis *et al.*, 2004; von Hoermann *et al.*, 2018; Barry *et al.*, 2019; Schwegmann *et al.*, 2022).

Large carcasses are not exclusively a resource for scavenging vertebrates and invertebrates; they have also been shown to be opportunistically utilised by typically non-scavenging species, either of the carcass itself or indirectly on resources which the carcass promotes. Examples include ungulates feeding on the bones, hair and skin of carcasses (Wenting, Rinzema and van Langevelde, 2022), non-corvid passerine birds feeding on abundant invertebrates (Moreno-Opo and Margalida, 2013; Baruzzi *et al.*, 2018; Wenting, Rinzema and van Langevelde, 2022), and increased abundances of herbivorous invertebrates associated with increased plant biomass (van Klink *et al.*, 2020).

Plants are another trophic group that are impacted by large carcasses in terrestrial ecosystems, where the action of soil microbial communities in carcass decomposition results in an increased localised availability of nutrients, thus influencing plant growth rates and diversity (De Deyn and van der Putten, 2005; Wardle, 2013). Studies have researched the increased nutrient loads into soils from carcasses, the nutrient uptake of these by plants, and subsequent influences on plant growth rates and biomass (Danell, Berteaux and Bråthen, 2002; Melis *et al.*, 2007; Bump *et al.*, 2009; Bump, Peterson and Vucetich, 2009; Barton, Cunningham, Macdonald, *et al.*, 2013; van Klink *et al.*, 2020). However, there has been limited research into the effects of large carcasses on plant community composition and diversity (except see: Towne, 2000; Bump *et al.*, 2009; Barton *et al.*, 2016).

There is a clear need to not just focus on the consequences of large carcasses on vertebrate scavenger guilds, necrophagous invertebrate families, or the diversity of plants, but instead to integrate these in order to gain a better understanding of the impacts of large carcasses on the wider biodiversity of an ecosystem. Hence, in this chapter, the overall effects of large carcasses on the diversity and composition of vertebrates, invertebrates and plants in a temperate woodland ecosystem were investigated. In addition to examining differences in diversity between carcass and control sites, the influence of several other factors was considered, including habitat type, the time period (separated out as monthly intervals from early winter until early summer), and for invertebrates and plants the effect of increasing distance from carcasses (or equivalent control).

The first hypothesis was that the diversity of vertebrates would be similar at both red and roe carcasses, but higher than the diversity at control sites. This is due to carcasses likely attracting several of the facultative scavengers found in the study area, as well as likely occurrences of common non-scavenging species. Whereas at control sites there would likely be fewer species observed, particularly only the more common mammalian species, with a

lower likelihood of any avian species occurring. It was also predicted that the activity of vertebrates would be highest at red deer carcasses, followed by roe carcasses and then control sites, due to the corresponding decrease in the mass of resources available at each site type (Sebastián-González *et al.*, 2021). Overall invertebrate diversity of sites was predicted to be highest at carcass sites due to the greater abundance of a variety of necrophagous invertebrate species in addition to the more widespread invertebrate species common to the woodland, with only these more common species likely present at control sites (Van Klink *et al.*, 2020). Moreover, invertebrate diversity would likely be highest at the closer sampling distances at carcass sites, due to the carcass itself and its immediate surroundings being the focal point of any invertebrate activity (Sawyer and Bloch, 2020). Finally, plant diversity was not expected to vary significantly between the carcass and control sites of the same broad habitat type, due to the impact of increased soil nutrients from the decomposing carcasses not likely to have any effect on plant growth or diversity until at the earliest during the following growing season (Towne, 2000).

2.2. Methods

2.2.1. Vertebrates

Automatic motion-triggered camera traps in view of carcasses were used, and at control sites, to measure the activity of vertebrates, primarily mammalian and avian species. At each site, a single camera was set up (Bushnell Prime Low Glow), with these attached to trees approximately 2 m away from carcasses, or equivalent marked control point. In the absence of trees at the felled sites, cameras were attached to actively placed wooden poles of about 1.5 m in height and 10 cm in diameter, with these securely hammered into the ground. All cameras were positioned between a height of 0.5 to 1 m and typically angled slightly bent forward to ensure that carcasses were fully in view. They were also positioned so that they faced a general northward direction to reduce glare from the sun leading to false triggers and secured to trees/posts using a padlocked cable to deter any tampering or thefts.

Cameras were programmed to capture three consecutive photos per trigger, with a time delay of 1 minute before it could be triggered again. This was to prevent an excessive number of photos being captured of animals present at sites for a prolonged period (e.g., a scavenger feeding on a carcass), but a short enough period so even brief visitors to sites were be captured. During monthly site visits, camera traps were checked to ensure they were still securely in place and functioning correctly, with SD cards removed and replaced with an empty one for the next four weeks of recording. Batteries were replaced as necessary, and two cameras were replaced, with one stolen and the other severely damaged during the project. All photos captured on cameras were then downloaded from the retrieved SD cards and backed-up for later annotation and analysis, with SD cards then formatted to be swapped back into cameras the next month. Prior to analysis, all photos were sorted through with all relevant information recorded, including site ID and site details, photo timestamps, and species captured.

Activity of vertebrates was the key metric being recorded at sites by camera traps, with this simply the number of photos per unit of time of individuals of a species. This will help to inform of the different activity levels of the diversity of species recorded at the different site types, and in the different habitats. It is common for studies investigating vertebrate activity at carcasses to focus specifically on scavenging species, whereas in this study observations of all species recorded were included. This allows for comparisons against observations of species present at control sites to determine if there is a clear effect of carcass presence on vertebrate species activity and diversity.

2.2.2. Invertebrates

Pitfall traps were used to measure the invertebrate diversity at sites. These traps consisted of a sturdy, transparent plastic cup, of roughly 12 cm in depth and 8 cm diameter of the opening, with these placed into a hole dug in the ground so that the cup rims were flush with the ground's surface.

Roughly 50 ml of a solution composed of three-parts water to one-part ethylene glycol was added to each trap, in order to trap all invertebrates that fell or flew in, and kill and preserve these specimens. A small drop of non-scented detergent was also added to each cup to break the surface tension of the fluid. Square pieces of metal wire mesh, with side lengths of 10 cm and holes with diameter 2 cm, were placed over the openings of the cups and secured in place with metal pegs. The mesh holes were large enough to allow invertebrates to pass through but small enough to prevent non-invertebrate species from falling into traps, specifically small mammals and amphibians (van Klink *et al.*, 2020). Finally, square, flat pieces of wood, with side lengths of about 10 cm, were placed on top of several wooden tees to act as roofs to keep out rain and prevent traps from flooding. These roofs were held in place using metal pegs to prevent them being knocked off by larger vertebrates.

At each site, three pitfall traps were positioned to investigate the effect of carcasses on the diversity of invertebrates, but to also to examine for an effect of increasing distance from the carcass (or control site) on diversity. Therefore, the three traps were placed at equidistant points along transects extending out from the carcass/control, with the first trap positioned 1 m away from the carcass/control centre, the next 3 m away and the third 5 m away. The first trap was located 1 m away, rather than immediately adjacent to carcasses, so that they were not overridden by fly larvae migrating away from carcasses in late spring and summer (van Klink *et al.*, 2020). The orientation of the transects at each site was randomly assigned based on a random number between 0 and 359, with this representing the compass orientation for the transect to be directed in. Reassignment of this direction was carried out if there were clear obstacles in the way, i.e., a tree in the centre of the proposed transect.

Traps were left open for the entirety of 4-week sampling periods and all invertebrates caught in the traps over that time were then extracted during the following site visit. Invertebrates were placed into small containers labelled with the corresponding site ID and distance group, containing 70% ethanol solution to preserve specimens for later identification (Schwegmann *et al.*, 2022).

All specimens were identified to the lowest taxonomic rank possible based on morphology. For certain invertebrate orders, particularly Coleoptera and Diptera, it was extremely difficult

to identify as low as genus or species in most cases, with many specimens only identified to as low as family or even order. In situations where there were several specimens from the same trap that varied in morphology but could only be identified down to the same taxonomic level, these were recorded as rove beetle A and B, for example, which is sufficient for calculating diversity metrics. Identification was carried out with the use of a light microscope and relevant invertebrate field guides (Chinery, 1993, 2009; Roberts, 2001). For each container representing a single trap, the number of individuals of each taxon was recorded, along with the relevant site details and collection date.

2.2.3. Plants

Plants were surveyed to compare relative plant species diversity between carcass and control sites. In addition, it was explored to see if carcasses had a distance type effect on plant diversity with increasing distance from carcasses. A 0.5 m gridded quadrat, with 100 squares, was used to carry out plant surveys, with these placed along a transect extending out from the centre of carcass or control sites. Quadrats were placed along transects with the closest edge of the quadrat located 1 m, 3 m and 5 m away from the site centre points. The quadrats were always placed above the tape measure, with the measurement markings facing the right way up. During each site visit the orientation of the transect was randomised with a random number between 0 and 359 selected, with this representing the compass bearing. This is to provide a representative cover of the area within a 5 m radius of site centres over the whole study period.

For each quadrat survey, estimates were made for the total percentage cover of plant species that were rooted within a quadrat, with the use of several plant guides to aid in the identification of species (Fitter, Fitter and Farrer, 1984; Sykes, 1993; Rose and O'Reilly, 2006; British Bryological Society, 2010; Streeter, 2016). The functional group of each species was also recorded (i.e., ferns (with horsetails), forbs, graminoids, mosses, saplings and shrubs), as well as the quadrat distance group, site ID, bearing and the date. Initial plant surveys were undertaken during the site set up period to establish baseline measures for all sites, with surveys then undertaken accordingly during each monthly site visits.

2.2.4. Data Analysis

To investigate the effects of carcasses on vertebrate activity and diversity, firstly an activity metric was considered which was defined as the number of observations of a species per day at each site. The second metric was vertebrate species richness, calculated as the total

number of species recorded at each site per sampling period. A diversity metric was also considered, specifically the Shannon-Wiener diversity index (*H*; Shannon, 1948), which was calculated as follows using the number of observations of each species (with *n* equalling the observations for each species at each site per sampling period and *N* equalling the total number of observations of all species at each site per sampling period):

$$H = -\sum \frac{n}{N}\left(\ln \frac{n}{N}\right)$$

Considering both invertebrate and plant diversity next, three different metrics were chosen: species richness, Shannon-Wiener diversity index and Simpson's diversity index (*D*; Simpson, 1949). These two diversity metrics were chosen as they are commonly used measures of diversity that incorporate both richness and abundance of species (Morris *et al.*, 2014) with Shannon-Wiener weighted more towards species richness and Simpson's with more weight on species evenness (Kim *et al.*, 2017). These diversity metrics were calculated for the total invertebrate diversity at a site by combining species recorded across the three traps at a site per period, and as well for the diversity per distance group (i.e., per trap). The same was calculated for these plant diversity metrics with a total diversity based on combined species across the three distance quadrats, plus diversity per distance group. For invertebrates, the Shannon-Wiener diversity index was calculated using the previously described equation, with *n* equalling the number of individuals for each species, and *N* equalling the total number of all individuals of all species, whilst Simpson's diversity index was calculated with the following equation:

$$D = 1 - \sum \frac{n(n-1)}{N(N-1)}$$

For plants, the Shannon-Wiener diversity index was also calculated using the previous equation, but with *n* equalling the cover percentage per quadrat for each species and *N* equalling the total cover of all species within a quadrat. Simpson's diversity index was calculated slightly differently with the following equation:

$$D = 1 - \sum \left(\frac{n}{N}\right)^2$$

The activity metrics for vertebrates and the diversity metrics for vertebrates, invertebrates and plants were all assessed for normality before running initial analyses. Shapiro-Wilk's tests showed that these were all were all non-normally distributed, thus non-parametric analyses were undertaken. Kruskal-Wallis tests were carried out to examine any differences in overall activity and diversity metrics between the different site types, as well as between

the different distance groups for invertebrate and plant diversity, with any post-hoc analysis performed using Dunn's multiple comparison tests with Holm adjustments (Dunn, 1964).

To further examine the effects of site type on the activity metrics for vertebrates, and the diversity metrics for vertebrates, invertebrates and plants, a range of different models were employed, where this approach enabled the effects of other variables on activity and diversity to also be investigated. These included the time period (i.e., defined as the seven 4-week sampling periods between site visits), broad habitat type (i.e., broadleaf, conifer and felled) and distance from carcasses or equivalent control (i.e., 1 m, 3 m or 5 m) for both invertebrates and plants. In these models, the activity and diversity metrics were the response variables, where these are the outcome being predicted, with the independent variables, including site type, habitat type, time period and distance, being the predictor variables which are thought to influence the response variables. For models with the vertebrate activity as response variable, this metric is count data and was shown to be highly over dispersed (i.e., the conditional variance far exceeded the conditional mean), hence it was appropriate to run generalised linear mixed models (GLMMs) and generalised linear models (GLMs) with a negative binomial distribution (with log link). For species richness of vertebrates, invertebrates and plants as response variables, both GLMMs and GLMs with Poisson distributions were used, due to these also being count data but not being significantly over-dispersed. However, for both Shannon-Wiener and Simpson's diversity index values of vertebrates, invertebrates and plants as response variables, both linear models (LMs) and linear mixed models (LMMs) were run, as a Gaussian distribution is suitable for continuous data.

Prior to running any models, any outliers were removed, with the only ones identified being invertebrate diversity metrics that had been skewed by extreme group sizes of *Formica lugubris* (with multiple group sizes significantly greater than 100) due to the close proximity of a nest to the site. In mixed models, a nested random effect was included alongside the predictor variables as fixed effects, with the random effect being the site ID factor nested within the cluster ID in order to account for spatial autocorrelation of sites of the same cluster (Elbroch *et al.*, 2017). Moreover, all fixed and random factors were assessed for collinearity, with all factors correlated at $r < 0.2$ ($p < 0.001$), except only the habitat variable being collinear with both the site and cluster variables for the vertebrate, invertebrate and plant data ($r > 0.8$, $p < 0.001$). Therefore, no mixed-effect models were undertaken with habitat as a fixed effect. With each of the diversity metrics employed as response variable, for all three groups of focus, several a prior models were formulated with different combinations of the fixed and random effects to investigate which of these best fit each metric, as well as to include only informative parameters (Arnold, 2010). The residuals of models were all

checked for heteroscedasticity to ensure model assumptions were met. To compare models, for each an Akaike's Information Criterion (AIC) value was calculated, plus the relative difference between a model's AIC and the best model's AIC (ΔAIC), relative likelihood and Akaike weights (w_i, another measure of relative likelihood) (Burnham and Anderson, 2004). Top-performing models were defined as those with the lowest AIC values, with any models within an ΔAIC score of less than two from the top-performing model also considered. Type III analysis of variance (ANOVA) tests were performed for top models to test the significance of variables, where for LMs and GLMs *F*-tests were calculated and for LMMs and GLMMs Wald chi-squared tests were calculated. Any significant parameter estimates were reported for significant variables of top-performing models, along with their corresponding odds ratios, as well as reporting conditional R^2 values to further assess model performance.

Finally, to investigate how community composition varied between the different site types for the three groups, non-metric multidimensional scaling plots (NMDS) were produced, with Bray-Curtis dissimilarity as distance metric. Ellipses were drawn for each of the site type groups with these calculated based on the standard deviation of points and with a confidence interval (CI) of 95%. Permutational multivariate analysis of variance tests (PERMANOVA) were also carried out to investigate for any statistically significant differences in the community compositions between the site types. For vertebrates, scores were calculated based on the total number of observations of different species for each site type of each period, with only species with at least a total of 50 observations included in the analysis. For invertebrates, the total abundance of each invertebrate family for each site across all time periods was used, with the inclusion of families with a minimum total abundance of 50 individuals. Lastly, for plants, this was based on the total cover percentage of each functional group per site, across all time periods.

All statistics were conducted using R Statistical Software v4.4.1 (R Core Team, 2024). Several packages were employed for running models, including *nlme* for LMMs (Pinheiro, Bates and R Core Team, 2023), *lme4* for GLMs and GLMMs with Poisson distributions and GLMMs with negative binomial distributions (Bates *et al.*, 2015), and *MASS* for GLMs with negative binomial distributions (Venables and Ripley, 2002). Type III ANOVAs were calculated using the *car* package (Fox and Weisberg, 2019). Conditional R^2 values for all models were determined using the *MuMIn* package (Nakagawa, Johnson and Schielzeth, 2017). For GLMs and GLMMs, conditional R^2 values were calculated using the *trigamma* function, where this provides the most accurate estimates of the variance for distributions associated with log link, such as Poisson (Nakagawa, Johnson and Schielzeth, 2017). Lastly, the *vegan* package was used for producing NMDS plots and PERMANOVA analyses (Oksanen *et al.*, 2024).

2.3. Results

Fieldwork ran for a total of 204 days from early December 2023 until late June 2024, with a total of seven site visits (not including the initial site set up and surveys) undertaken every four weeks. All in situ equipment, including camera traps and pitfall traps were removed from sites during the final site visits, with only what remained of carcasses left in place.

Of the 12 carcasses employed in the study, six carcasses were either consumed entirely (with only bones and remnants of skin and hair remaining) or removed from site locations by scavengers before the end of the study period. One roe carcass was removed entirely from its position (at site 8) 49 days into the study, with this likely removed by a red fox (*Vulpes vulpes*) as this was the last animal captured before the carcass was removed; thus, any photos captured at this site after carcass removal were not included in the analysis. The red deer carcass at site 9 was removed by domestic dogs (*Canis familiaris*) 84 days into the study, with it then being consumed down to the bones about 20 m away from its original position over the next month. The red deer carcass at site 11 was also consumed nearly entirely by the action of domestic dogs and red foxes after 66 days, as was the roe deer at site 12 after 91 days. The red carcass at site 4 and the roe carcass at site 6 were consumed nearly entirely mainly by the action of red foxes after 140 and 165 days, respectively.

There were also several camera faults and thefts throughout the project. Firstly, the camera at the red deer site 3 was stolen during the first month of recording and subsequently replaced with the camera at control site 1 (with this then replaced by a new camera the following month). Moreover, the camera at the control site 15 was damaged by human action and consequently not replaced.

2.3.1. Vertebrate Activity, Diversity and Composition

Across the 18 cameras and a total of 205 camera trap days, a total of 9,318 observations were obtained, comprised of 22 different species of 15 families, with the number of observations per species, including the total and per site type, shown in Appendix Table 1. The number of observations per site type for the eight species with at least 100 overall observations are shown in Figure 2.1, with the four most observed (Figure 2.2) being red fox (3,430 observations and 36.2% of total observations), domestic dog (2,723 and 28.7%), grey squirrel (*Sciurus carolinensis*; 1,313 and 13.8%) and carrion crow (*Corvus corone*; 891 and 9.4%).

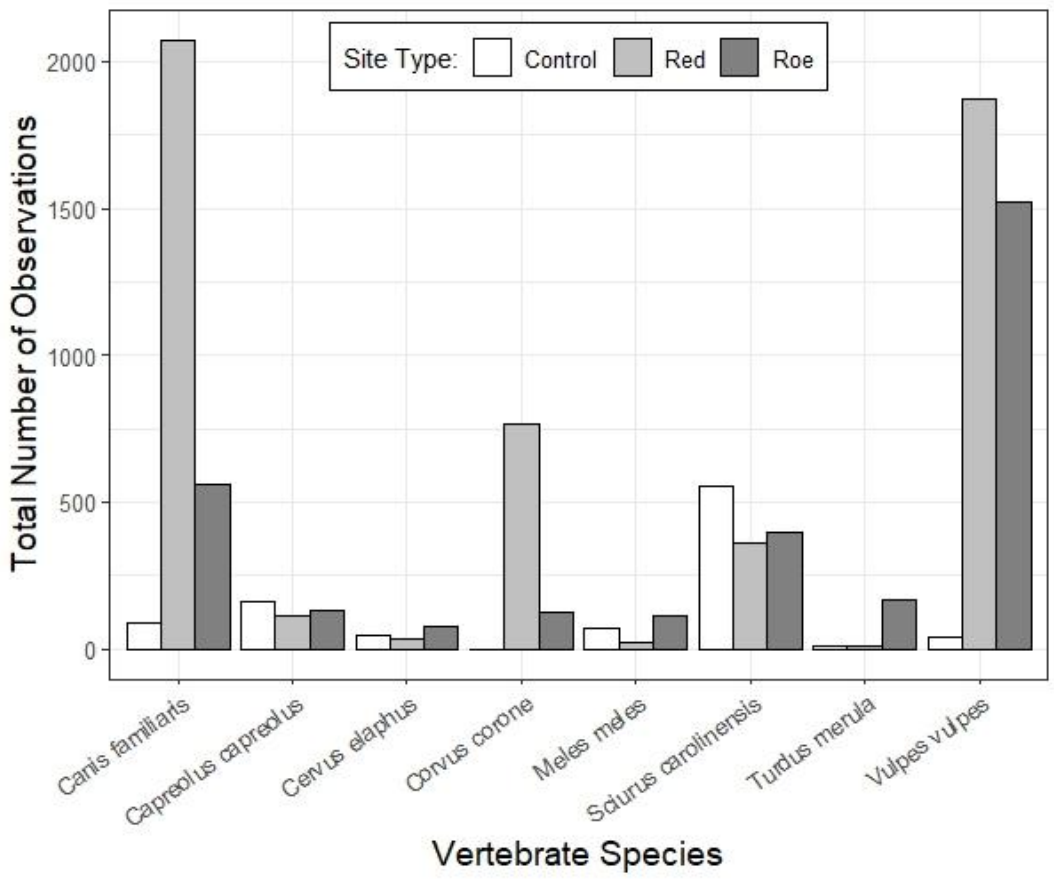

Figure 2.1: The number of observations per site of the eight vertebrate species with at least 100 observations across all sites and periods.

Figure 2.2: Camera trap images taken during the study of the four most observed vertebrate species across all sites and the whole study duration: red fox (*Vulpes vulpes*; A), domestic dog (*Canis familiaris*; B), grey squirrel (*Sciurus carolinensis*; C) and carrion crow (*Corvus corone*; D).

The activity, i.e., the number of observations per day, for each of the top four most observed species between the different site types are shown in Figure 2.3. Comparisons for the activity of each species between the three site types were undertaken by Kruskal-Wallis tests, and any necessary post-hoc tests with Dunn's tests (Table 2.1).

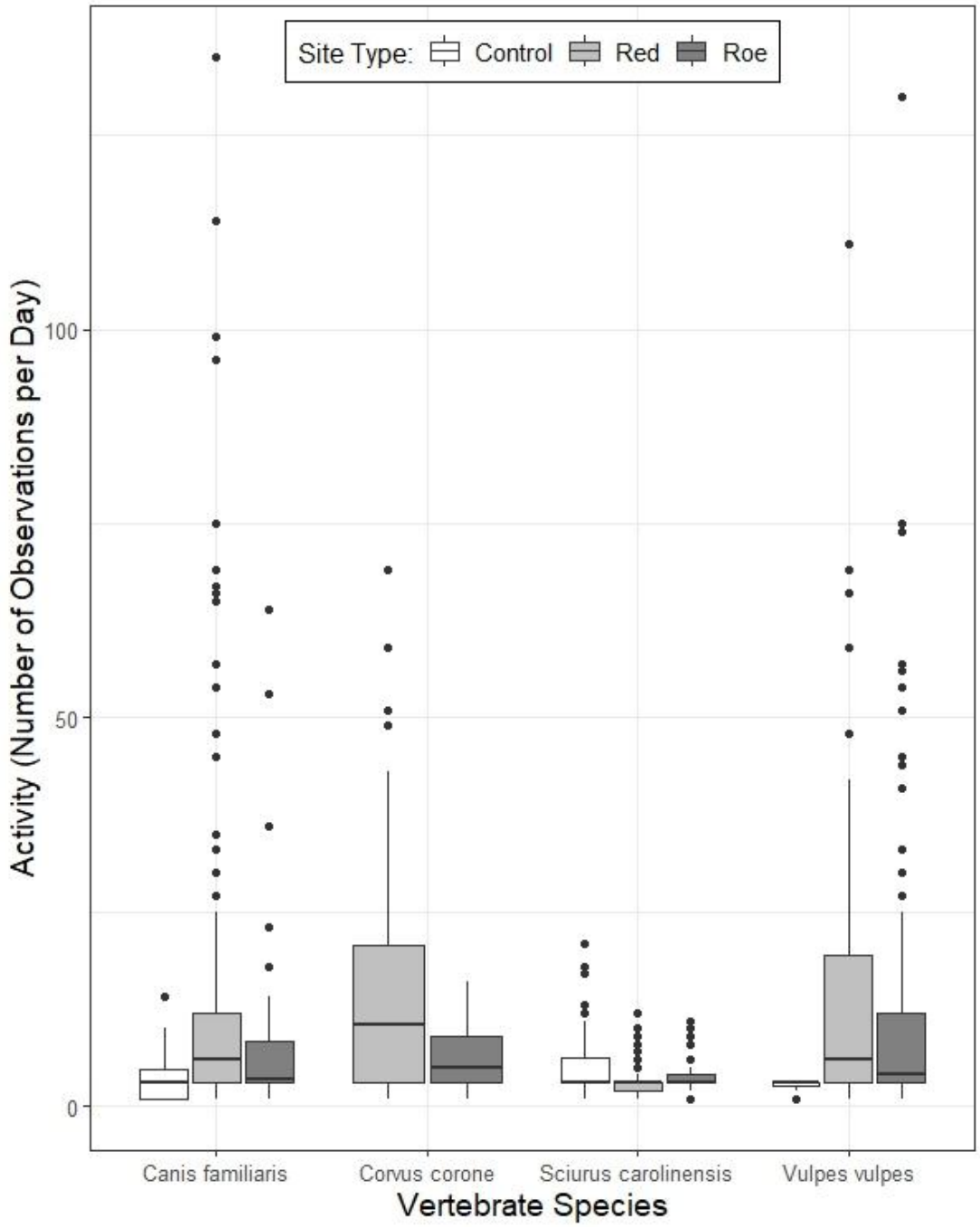

Figure 2.3: Boxplot of the activity (number of observations of a species per day) of the four most observed species – domestic dog (*Canis familiaris*), carrion crow (*Corvus corone*), grey squirrel (*Sciurus carolinensis*) and red fox (*Vulpes vulpes*) – separated by site type. The top of boxes

represents the 75th percentile, the bottom the 25th percentile and the middle line the median. The whiskers correspond to the range, excluding outliers which are shown by points.

Table 2.1: Summary table of the Kruskal-Wallis tests comparing the activity of the four most observed species between the three site types, with Kruskal-Wallis test statistics (*H*) shown along with *p*-values (*p*). *P*-values of Dunn's multiple comparisons tests between the site type types are also shown (with no values available for *Corvus corone* due to no observations of this species at control sites). Results that are significant at the 5% significance level are shown in bold.

Vertebrate Species	Kruskal-Wallis Tests		Dunn's Multiple Comparisons Tests		
			Control - Red	Control - Roe	Red - Roe
	H_2	*p*	*p*	*p*	*p*
Canis familiaris	18.76	**< 0.001**	**< 0.001**	**0.006**	0.105
Corvus corone	4.63	**0.031**	-	-	-
Sciurus carolinensis	28.38	**< 0.001**	**< 0.001**	**0.002**	**0.049**
Vulpes vulpes	20.56	**< 0.001**	**< 0.001**	**0.001**	**0.043**

Specifically, the activity of red fox was significantly different between all three site types, with differences between control sites with both deer carcasses, but only a slight significant difference between red and roe carcasses. The activity of red fox was highest at red deer carcasses (median = 6, IQR = 3–21), followed by roe carcasses (median = 4, IQR = 3–12) and then controls (median = 3, IQR = 2–3). The activity of domestic dogs was different between the three sites, but only significantly different between control with both deer carcasses, with no difference between red and roe carcasses. Similarly to red fox, the activity of dogs was highest at red deer carcasses (median = 6, IQR = 3–12), followed by roe carcasses (median = 3.5, IQR = 3–8) and then controls (median = 3, IQR = 1–5). For grey squirrels, activity levels were also significantly different between site types, with these differences significant between all site combinations. Although the median activity levels were equal for all site types (median = 3), there was slight variation in the IQRs indicating that grey squirrels were most active at control compared to carcass sites – control (IQR = 3–6), red deer (IQR = 2–3) and roe deer (IQR = 3–4). Finally, the activity of carrion crows was significantly different between red and roe carcasses, with activity significantly higher at red (median = 10.5, IQR = 3–21) compared to roe deer (median = 5, IQR = 3–9). No carrion crows were recorded at any control sites.

Considering the species richness of vertebrates based on the number of species recorded at each site per monthly time period, there were no significant differences between the different site types (H_2 = 3.97, p = 0.138; Figure 2.4). There was also revealed to be no significant differences between the different site types for the vertebrate Shannon-Wiener diversity

index values, with these generated from the number of observations per species and all species at each site per time period (H_2 = 1.20, p = 0.550; Figure 2.5).

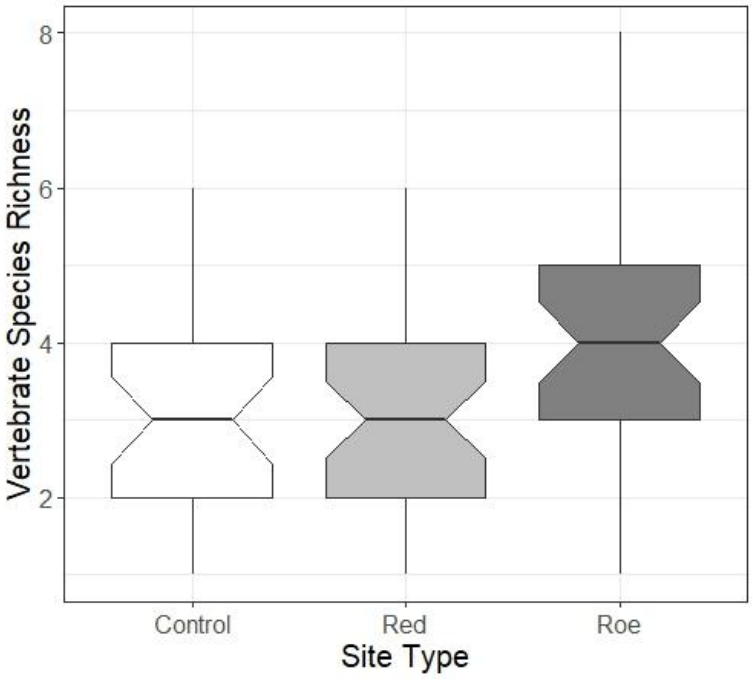

Figure 2.4: Variation of vertebrate species richness based on site type. The top of boxes represents the 75th percentile, the bottom the 25th percentile and the middle line the median, whilst whiskers correspond to the range. Notches are useful to identify any significant differences between group medians.

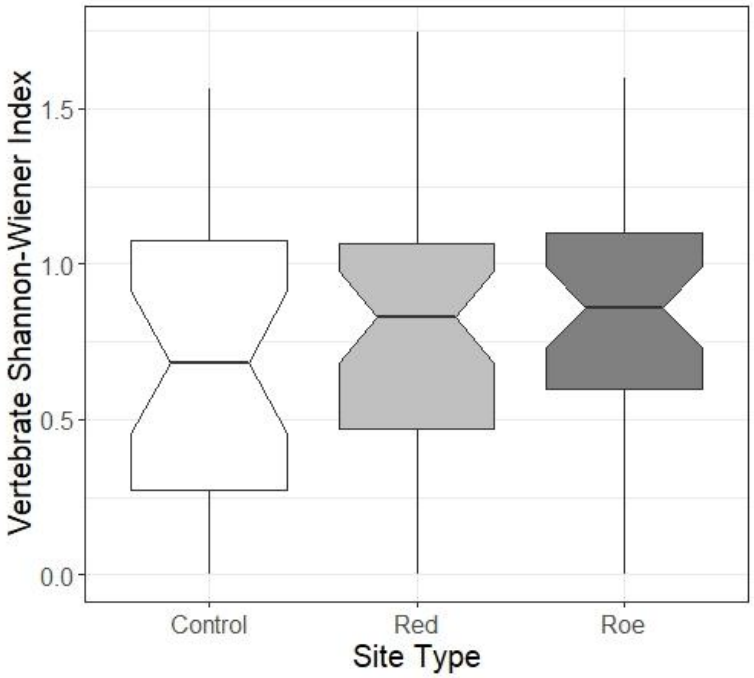

Figure 2.5: Variation of the vertebrate Shannon-Wiener diversity index by site type. The top of boxes represents the 75th percentile, the bottom the 25th percentile and the middle line the median, whilst whiskers correspond to the range.

The first set of models ran were several GLMMs and GLMs with activity as the response variable, to reveal what predictor variables had a significant effect on the activity of vertebrates. Only one top model was identified, *Activity ~ Period + Site Type + rand (Cluster/Site ID)*, which was responsible for 93.6% of the w_i and with a conditional R^2 value that explained 21.9% of the variation in the data (Table 2.2). Both model variables were shown to be significant at the 5% significance level (*Period*: X^2 (6) = 104.10, $p < 0.001$; *Site Type*: X^2 (2) = 11.92, $p = 0.002$) and their parameter estimates along with odds ratios are summarised in Table 2.3.

The next set of models ran were GLMMs and GLMs, with Poisson distributions, with vertebrate species richness as response variable (Table 2.4). The top performing model here was *Richness ~ Habitat*, with this responsible for 49.9% of the w_i; however, the *Habitat* variable was not deemed significant (X^2 (2) = 4.41, $p = 0.110$). The second-best performing model *Richness ~ Site Type + Habitat* (with an ΔAIC value of < 2 from the top model) had a w_i value of 24.4% but neither of these variables were deemed significant (*Site Type*: X^2 (2) = 2.57, $p = .277$; *Habitat*: X^2 (2) = 4.40, $p = 0.111$). Finally, the third-best performing model (also with an ΔAIC value of < 2 from the top model) was *Richness ~ Site Type* with a w_i value of 20.0%, but again this variable was not significant (X^2 (2) = 2.58, $p = 0.275$).

Table 2.2: Model comparisons for the GLMMs and GLMs with vertebrate activity as response variable. Models are ranked from best to worst fit based on AIC values, with selected models in bold.

Models	AIC	ΔAIC	Likelihood	Akaike Weights (w_i)
Period + Site Type + rand (Cluster/Site ID)	**6444.407**	**0.000**	**1.000**	**0.936**
Period + rand (Cluster/Site ID)	6449.774	5.367	0.068	0.064
Period + Site Type + Habitat	6489.122	44.714	0.000	0.000
Period + Site Type	6519.262	74.855	0.000	0.000
Site Type + rand (Cluster/Site ID)	6535.228	90.821	0.000	0.000

Table 2.3: Parameter estimates for the GLMM, *Activity ~ Period + Site Type*. Standard errors for the parameter estimates (SE), *p*-values, odds ratios and their 95% confidence interval (CI) are also shown. Parameters that are significant at the 5% significance level are shown in bold.

Fixed Effects	Parameter Estimate	*SE*	*p*-value Pr(>|z|)	Odds Ratio	*95% CI*
Period: 2	0.073	*0.110*	0.503	1.076	*0.868–1.335*
Period: 3	**1.109**	***0.123***	**< 0.001**	**3.031**	***2.382–3.859***
Period: 4	0.142	*0.111*	0.202	1.153	*0.926–1.434*
Period: 5	**0.448**	***0.122***	**< 0.001**	**1.565**	***1.232–1.988***
Period: 6	**0.493**	***0.117***	**< 0.001**	**1.637**	***1.303–2.057***
Period: 7	0.011	*0.116*	0.926	1.011	*0.805–1.270*
Site Type: Red	**0.799**	***0.243***	**0.001**	**2.224**	***1.380–3.583***
Site Type: Roe	**0.652**	***0.245***	**0.008**	**1.920**	***1.188–3.101***

Table 2.4: Model comparisons for the GLMMs and GLMs with vertebrate species richness as response variable. Models are ranked based on AIC values, with selected models in bold.

Models	AIC	ΔAIC	Likelihood	Akaike Weights (w_i)
Habitat	**399.497**	**0.000**	**1.000**	**0.499**
Site Type + Habitat	**400.929**	**1.432**	**0.489**	**0.244**
Site Type	**401.326**	**1.829**	**0.401**	**0.200**
Site Type + rand (Cluster/Site ID)	404.695	5.199	0.074	0.037
Period + Habitat	405.930	6.433	0.040	0.020

The final set of vertebrate models ran were LMMs and LMs with vertebrate Shannon-Wiener diversity as response variable (Table 2.5). The top model here, *Shannon-Wiener ~ Habitat*, had an w_i. of 70.9% with this variable deemed significant ($F_{2, 106} = 3.16$, $p = 0.047$). The only significant parameter estimate was the felled habitat type, with a value of -0.367 (± 0.147 SE; $p = 0.014$) and associated odds ratio of 0.693 (95% CI = 0.519–0.925). The conditional R^2 value for this model performed very poorly, with a value of 5.5%.

Table 2.5: Model comparisons for the LMMs and LMs with vertebrate Shannon-Wiener diversity indices as response variable. Models are ranked based on AIC values, with selected models in bold.

Models	AIC	ΔAIC	Likelihood	Akaike Weights (w_i)
Habitat	**135.944**	**0.000**	**1.000**	**0.709**
Site Type + Habitat	138.357	2.413	0.299	0.212
Site Type	140.768	4.823	0.090	0.064
Period + Habitat	144.157	8.212	0.016	0.012
Site Type + rand (Cluster/Site ID)	146.279	10.334	0.006	0.004

Finally, a comparison between the community compositions of the different site types by NMDS was undertaken based on the total number of observations of species across all sites and the whole surveying duration (n = 54; Figure 2.6). Moderate clustering of sites of each site type was revealed, with some clear overlapping but also some significant separation between these clusters. A PERMANOVA suggested that the observed separation between the community compositions for the three site types was significant ($F_{2, 111} = 3.70$, $p = 0.001$).

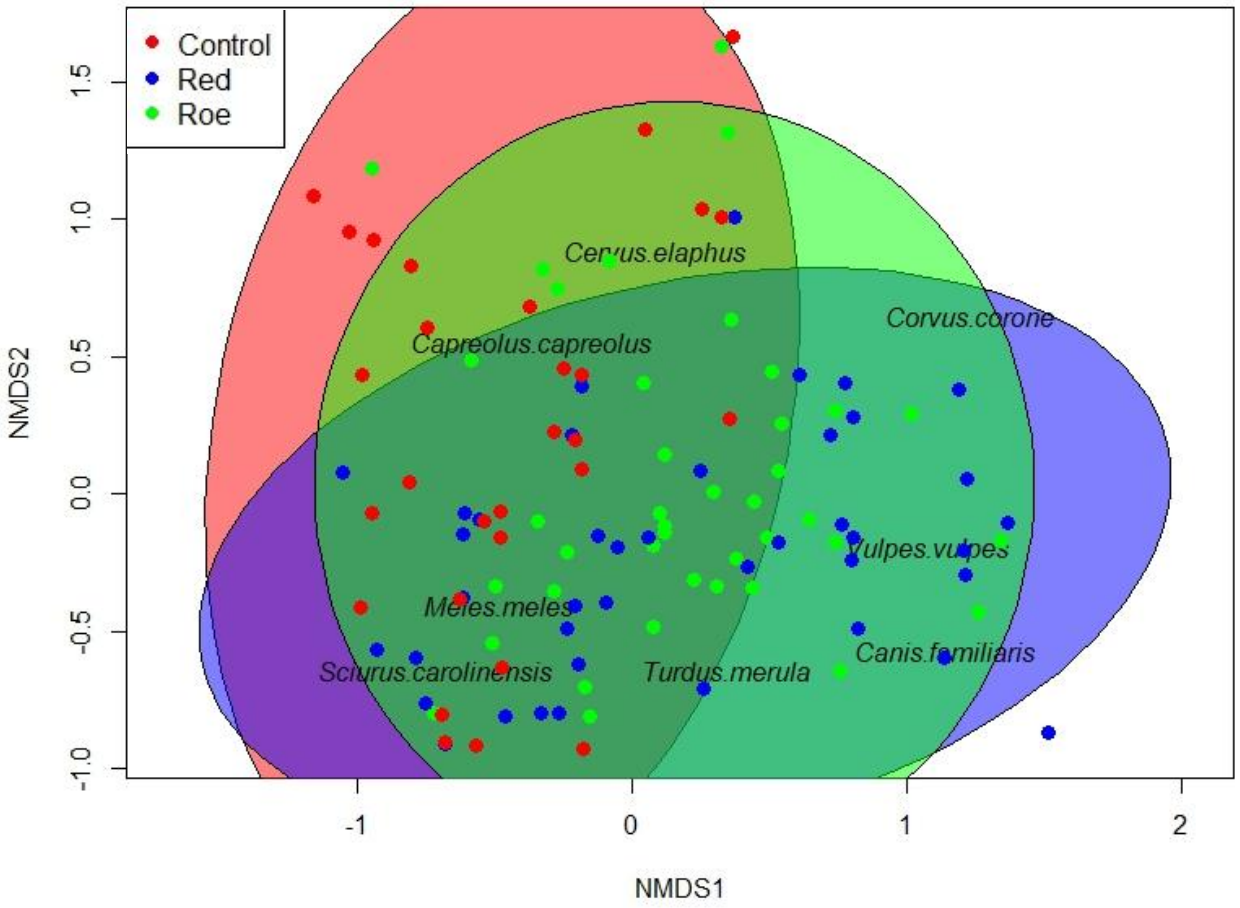

Figure 2.6: Non-metric multidimensional scaling (NMDS) plot of vertebrate species community composition between the different site types – control (n = 18), red (n = 18) and roe (n = 18) – with 95% confidence interval (CI) ellipses for each group. Points were calculated based on the number of observations of each species for each site per time period, with these coloured by site type, with species positions also shown. The number of dimensions (k) = 4, and stress value = 0.114.

2.3.2. Invertebrate Diversity and Composition

Across the seven months of pitfall trap sampling, a total of 46,826 invertebrate specimens were collected, with these spanning across 60 families of 22 orders (see Appendix Table 2). Considering site type, 12,772 specimens were collected from control sites, 16,616 from roe deer sites and 17,438 from red deer sites. The most abundant family was ants, Formicidae, with a total of 17,984 (38.4% of total specimens), followed by the rove beetles, Staphylinidae, with 5,429 individuals (11.6% of total) and thirdly woodlice, Oniscidae, with 3,025 specimens (6.5% of total). The top ten most abundant families, including a group representing the order Diptera for which specimens could not be allocated to a family, are shown in Figure 2.7, where the number of individuals has been separated per site type for each family.

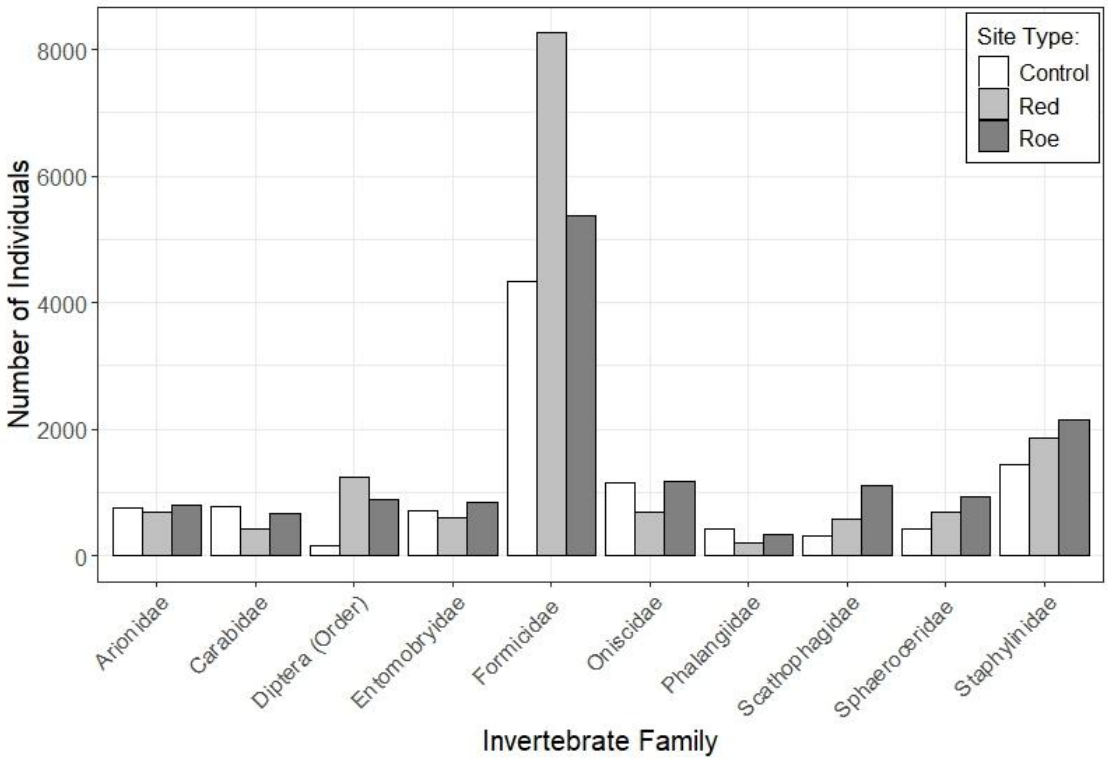

Figure 2.7: The total number of individuals of the ten most abundant invertebrate families per site type, including a group representing individuals in the order Diptera that could not be identified to family.

Considering invertebrate species richness first, it was revealed for the overall richness per site type that there is no significant difference (H_2 = 4.61, p = 0.100; Figure 2.8). When the species richness for each site type was separated into the three distance groups (Figure 2.9), further Kruskall-Wallis tests showed there were no significant differences between the distance groups for the control site (H_2 = 0.74, p = 0.689) or the red deer site (H_2 = 2.68, p = 0.262). However, there was evidence of a significant difference between the distance groups for the roe deer sites (H_2 = 7.99, p = 0.018), with Dunn's test indicating that this was only significant between the 1 m and 5 m groups (p = 0.023).

For the overall invertebrate Shannon-Wiener diversity index per site type, there was shown to be a significant difference between these (H_2 = 8.21, p = 0.016; Figure 2.10). A Dunn's test implied that there was only a significant difference between the red deer and control site types (p = 0.012). Further analysis to investigate any differences between the distance groups for each site type (Figure 2.11) revealed no significant differences for either the control (H_2 = 1.62, p = 0.444), red deer (H_2 = 1.60, p = 0.450) or roe deer sites (H_2 = 4.85, p = 0.089). Finally, for overall Simpson's diversity index per site type, a Kruskal-Wallis test revealed no significant differences between site types (H_2 = 5.80, p = 0.055; Figure 2.12).

Once again, no significant differences were found for the distance groups (Figure 2.13) of either the control (H_2 = 2.43, p = 0.296), red deer (H_2 = 4.48, p = 0.107) or roe deer sites (H_2 = 2.14, p = 0.344).

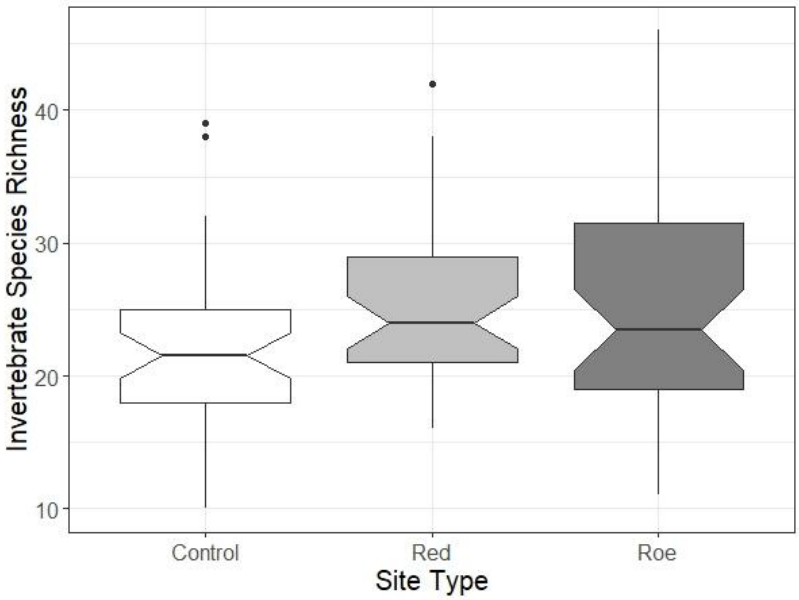

Figure 2.8: Variation of the overall invertebrate species richness per site type. The top of boxes represents the 75th percentile, the bottom the 25th percentile and the middle line the median, whilst whiskers correspond to the range. Outliers are represented by points.

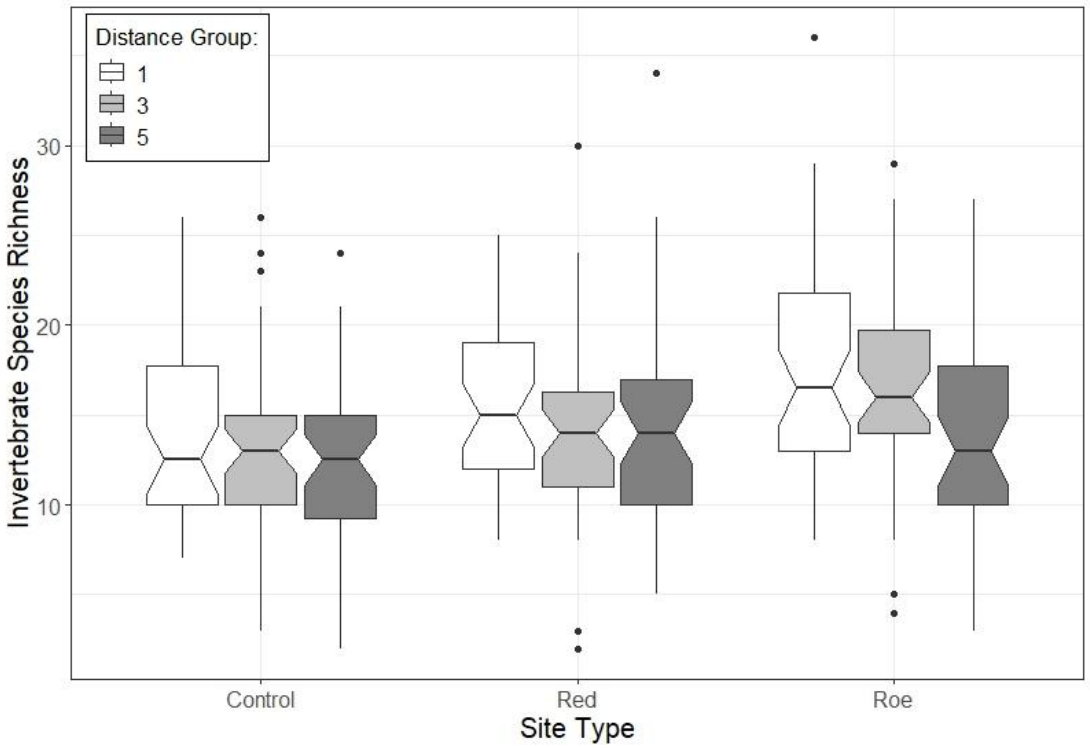

Figure 2.9: Variation of invertebrate species richness per site type, further separated into the three distance groups – 1 m, 3 m and 5 m. The top of boxes represents the 75th percentile, the bottom the 25th percentile and the middle line the median, whilst whiskers correspond to the range. Outliers are represented by points.

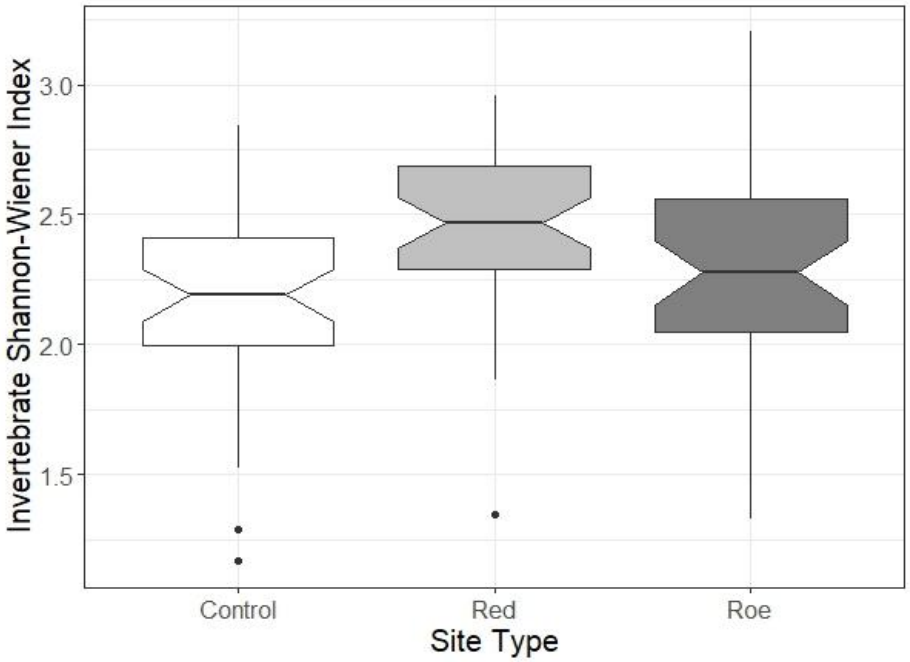

Figure 2.10: Variation of the overall invertebrate Shannon-Wiener diversity index per site type. The top of boxes represents the 75th percentile, the bottom the 25th percentile and the middle line the median, whilst whiskers correspond to the range. Outliers are represented by points.

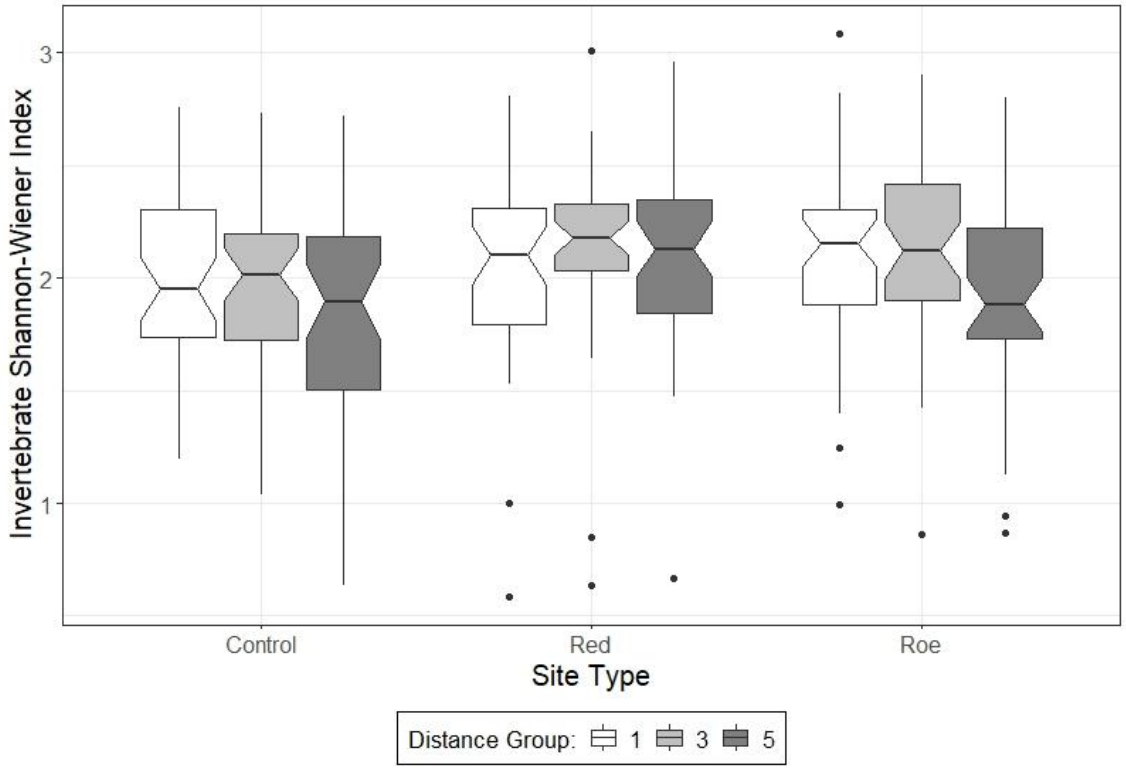

Figure 2.11: Variation of invertebrate Shannon-Wiener diversity index per site type, with this further separated into the three distance groups. The top of boxes represents the 75th percentile, the bottom the 25th percentile and the middle line the median, whilst whiskers correspond to the range. Outliers are represented by points.

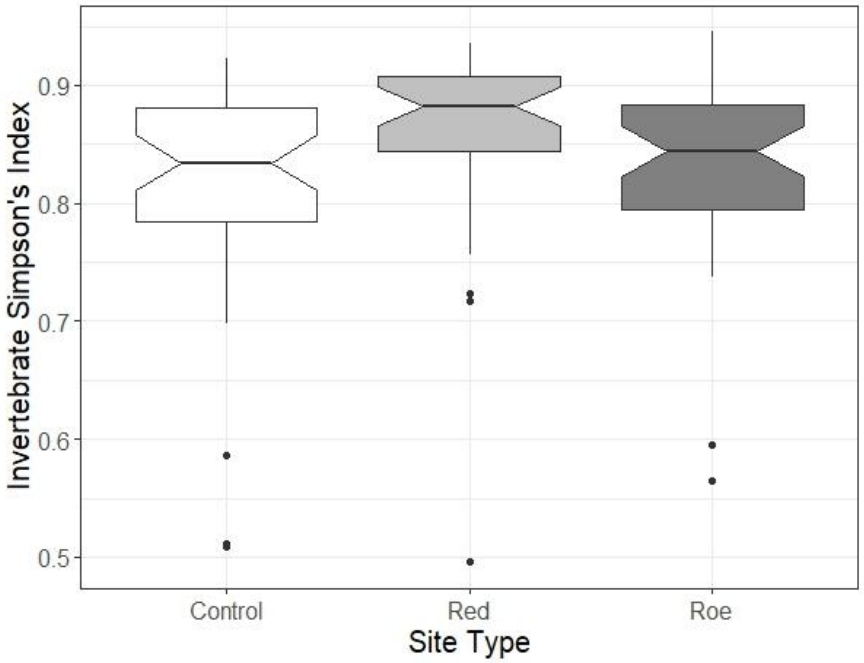

Figure 2.12: Variation of the overall invertebrate Simpson's diversity index per site type. The top of boxes represents the 75th percentile, the bottom the 25th percentile and the middle line the median, whilst whiskers correspond to the range. Outliers are represented by points.

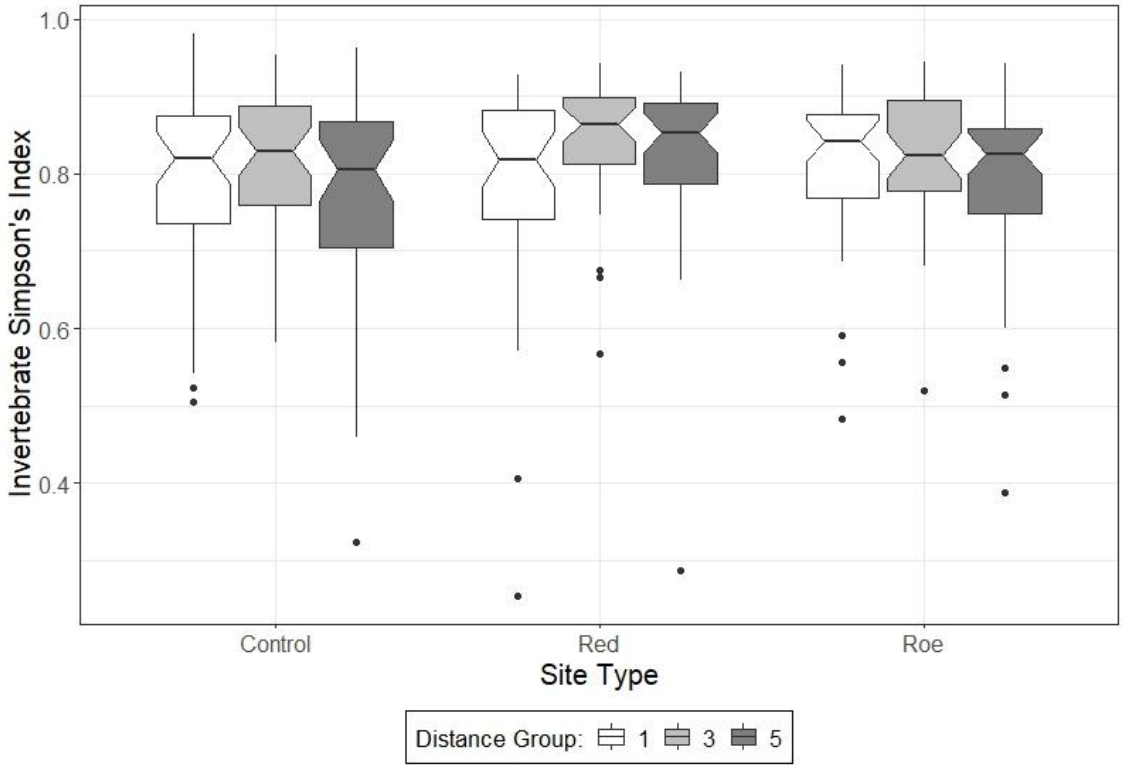

Figure 2.13: Variation of invertebrate Simpson's diversity index per site type, with this separated into the three distance groups. The top of boxes represents the 75th percentile, the bottom the 25th percentile and the middle line the median, whilst whiskers correspond to the range. Outliers are represented by points.

For the first set of models, several LMMs and LMs were ran with Shannon-Wiener diversity index as the response variable with the top five models listed in Table 2.6.

Table 2.6: Model comparisons for the LMMs and LMs with invertebrate Shannon-Wiener diversity index as response variable. Models are ranked based on AIC values, with selected models in bold.

Models	AIC	ΔAIC	Likelihood	Akaike Weights (w_i)
Period + Site Type + Habitat + Distance	**326.584**	**0.000**	**1.000**	**0.875**
Period + Site Type + Habitat	330.520	3.935	0.140	0.122
Period + Habitat + Distance	339.391	12.807	0.002	0.001
Period + rand (Cluster/Site ID)	340.849	14.265	0.001	0.001
Period + Habitat	342.895	16.310	0.000	0.000

The top model here based on AIC values, *Shannon-Wiener ~ Period + Site Type + Habitat + Distance*, explained 87.5% of the Akaike weight (w_i). All four model variables were shown to be significant (*Period*: $F_{6, 360} = 5.35$, $p < 0.001$; *Site Type*: $F_{2, 360} = 8.30$, $p < 0.001$; *Habitat*: $F_{2, 360} = 43.76$, $p < 0.001$; *Distance*: $F_{2, 360} = 3.87$, $p = 0.022$). Six significant parameter estimates were identified at the 5% significance level (Table 2.7). The model performed moderately well based on its conditional R^2 value, with this explaining 28.0% of the variation in data.

Table 2.7: Parameter estimates for the LM, *Shannon-Wiener ~ Period + Site Type + Habitat + Distance*. Included are the standard errors for the parameter estimates (SE), *p*-values, odds ratios and their 95% confidence interval (CI). Parameters that are significant at the 5% significance level are shown in bold.

Fixed Effects	Parameter Estimate	*SE*	*p*-value Pr(>|t|)	Odds Ratio	*95% CI*
Period: 2	-0.064	*0.071*	0.369	0.938	*0.815–1.079*
Period: 3	-0.114	*0.071*	0.107	0.892	*0.776–1.025*
Period: 4	0.061	*0.071*	0.388	1.063	*0.925–1.221*
Period: 5	**0.143**	***0.071***	**0.044**	**1.153**	***1.004–1.325***
Period: 6	0.016	*0.071*	0.827	1.016	*0.884–1.167*
Period: 7	**0.227**	***0.072***	**0.002**	**1.255**	***1.090–1.444***
Site Type: Red	**0.187**	***0.047***	**< 0.001**	**1.205**	***1.099–1.321***
Site Type: Roe	**0.125**	***0.046***	**0.007**	**1.133**	***1.035–1.241***
Habitat: Conifer	**-0.458**	***0.054***	**< 0.001**	**0.632**	***0.569–0.703***
Habitat: Felled	**-0.576**	***0.067***	**< 0.001**	**0.562**	***0.493–0.642***

For the second set of models, LMMs and LMs with Simpson's diversity index as the response variable were employed, with the top five performing models listed in Table 2.8.

Table 2.8: Model comparisons for the top five LMMs and LMs with invertebrate Simpson's diversity index as response variable. Models are ranked based on AIC values, with selected models in bold.

Models	AIC	ΔAIC	Likelihood	Akaike Weights (wi)
Site Type + Habitat + Distance	**-602.986**	**0.000**	**1.000**	**0.381**
Distance + rand (Cluster/Site ID)	**-602.068**	**0.918**	**0.632**	**0.241**
Period + Site Type + Habitat + Distance	**-601.717**	**1.269**	**0.530**	**0.202**
Habitat + Distance	-600.570	2.416	0.299	0.114
Site Type + Habitat	-599.403	3.583	0.167	0.063

There were three top models identified (with the top model and two others within 2 ΔAIC from the top model), with various significant parameter estimates for these (Table 2.9). The top model, *Simpson's ~ Site Type + Habitat + Distance*, was responsible for 38.1% of the w_i, and all three variables were significant (*Site Type*: $F_{2, 366} = 3.18$, $p = 0.043$; *Habitat*: $F_{2, 366} = 15.18$, $p < 0.001$; *Distance*: $F_{2, 366} = 3.76$, $p = 0.024$). Four significant parameter estimates were identified and its conditional R^2 value was 10.4%. The second-ranked model, *Simpson's ~ Distance + rand (Cluster/Site ID)*, had a w_i of 24.1%, with this *Distance* variable shown to be significant (X^2 (2) = 8.77, $p = 0.012$), along with only one significant parameter estimate and a conditional R^2 value of 22.3%. Finally, the third-ranked model, *Simpson's ~ Period + Site Type + Habitat + Distance*, was responsible for 20.2% of the w_i, but only three of the variables were shown to be significant (*Site Type*: $F_{2, 360} = 3.35$, $p = 0.036$; *Habitat*: $F_{2, 360} = 15.82$, $p < 0.001$; *Distance*: $F_{2, 360} = 3.82$, $p = 0.023$). From these significant variables, four significant parameter estimates were identified and the models conditional R^2 value was shown to be 12.7%.

Table 9: Parameter estimates for the top three performing models from the LMMs and LMs ran with invertebrate Simpson's diversity as response variable. The top model is *Simpson's ~ Site Type + Habitat + Distance*, followed by *Simpson's ~ Distance + rand (Cluster/Site ID)* and *Simpson's ~ Period + Site Type + Habitat + Distance*. The standard errors for the parameter estimates (SE), *p*-values, odds ratios and the 95% confidence interval (CI) for the odds ratios are also included. Parameters that are significant at the 5% significance level are shown in bold.

Model	Fixed Effects	Parameter Estimate	*SE*	*p*-value Pr(>|z|)	Odds Ratio	*95% CI*
1. Site Type + Habitat + Distance	**Site Type: Red**	**0.034**	***0.014***	**0.012**	**1.035**	***1.008–1.063***
	Site Type: Roe	0.016	*0.013*	0.243	1.016	*0.989–1.043*
	Habitat: Conifer	**-0.081**	***0.016***	**< 0.001**	**0.922**	***0.894–0.950***
	Habitat: Felled	**-0.093**	***0.019***	**< 0.001**	**0.911**	***0.877–0.947***
	Distance: 3 m	**0.028**	***0.014***	**0.036**	**1.029**	***1.002–1.056***
	Distance: 5 m	-0.006	*0.014*	0.636	0.994	*0.968–1.020*
2. Distance + rand (Cluster/Site ID)	**Distance: 3 m**	**0.029**	***0.013***	**0.023**	**1.029**	***1.004–1.055***
	Distance: 5 m	-0.006	*0.013*	0.622	0.994	*0.969–1.019*
3. Period + Site Type + Habitat + Distance	Site Type: Roe	0.016	*0.013*	0.241	1.016	*0.990–1.043*
	Site Type: Red	**0.035**	***0.013***	**0.010**	**1.036**	***1.009–1.063***
	Habitat: Conifer	**-0.083**	***0.016***	**< 0.001**	**0.920**	***0.893–0.949***
	Habitat: Felled	**-0.094**	***0.019***	**< 0.001**	**0.910**	***0.876–0.945***
	Distance: 3 m	**0.028**	***0.013***	**0.035**	**1.029**	***1.002–1.056***
	Distance: 5 m	-0.007	*0.013*	0.628	0.994	*0.968–1.020*

For the final set of models, GLMMs and GLMs were run with species richness as response variable with the top performing models shown in Table 2.10. The top model here was *Richness ~ Period + Site Type + Habitat + Distance*, with this responsible for 99.2% of the w_i. and all four model variables shown to be significant (*Period*: X^2 (6) = 217.95, $p < 0.001$; *Site Type*: X^2 (2) = 28.93, $p < 0.001$; *Habitat*: X^2 (2) = 123.88, $p < 0.001$; *Distance*: X^2 (2) = 18.46, $p < 0.001$). There were multiple significant parameters identified at the 5% significance level (Table 2.11). This model performed reasonably well based on its conditional R^2 value with this explaining 50.5% of the variation in data.

Table 2.10: Model comparisons for both the GLMMs and GLMs with invertebrate species richness as response variable. Models are ranked based on AIC values, with selected models in bold.

Models	AIC	ΔAIC	Likelihood	Akaike Weights (wi)
Period + Site Type + Habitat + Distance	**2049.227**	**0.000**	**1.000**	**0.992**
Period + Site Type + Distance + rand (Cluster/Site ID)	2059.268	10.041	0.007	0.007
Period + Site Type + Habitat	2063.683	14.456	0.000	0.000
Period + Distance + rand (Cluster/Site ID)	2063.702	14.475	0.000	0.000
Period + Site Type + rand (Cluster/Site ID)	2073.689	24.462	0.000	0.000

Table 2.11: Parameter estimates for the GLM, *Richness ~ Period + Site Type + Habitat + Distance*. Also included are the standard errors for the parameter estimates (SE), *p*-values, odds ratios and the 95% confidence interval for the odds ratios (CI). Parameters that are significant at the 5% significance level are shown in bold.

Fixed Effects	Parameter Estimate	*SE*	*p*-value Pr(>|z|)	Odds Ratio	*95% CI*
Period: 2	**-0.129**	***0.058***	**0.027**	**0.879**	***0.784–0.985***
Period: 3	-0.023	*0.056*	0.684	0.978	*0.876–1.090*
Period: 4	**0.171**	***0.053***	**0.001**	**1.187**	***1.069–1.318***
Period: 5	**0.302**	***0.052***	**< 0.001**	**1.352**	***1.222–1.497***
Period: 6	**0.353**	***0.051***	**< 0.001**	**1.424**	***1.288–1.574***
Period: 7	**0.461**	***0.051***	**< 0.001**	**1.585**	***1.435–1.751***
Site Type: Red	**0.128**	***0.034***	**< 0.001**	**1.136**	***1.063–1.215***
Site Type: Roe	**0.173**	***0.033***	**< 0.001**	**1.189**	***1.114–1.269***
Habitat: Conifer	**-0.323**	***0.034***	**< 0.001**	**0.724**	***0.677–0.774***
Habitat: Felled	**-0.495**	***0.047***	**< 0.001**	**0.609**	***0.556–0.669***
Distance: 3 m	**-0.070**	***0.033***	**0.032**	**0.932**	***0.874–0.994***
Distance: 5 m	**-0.143**	***0.033***	**< 0.001**	**0.867**	***0.812–0.925***

Finally, a community composition comparison of the invertebrate families between the different site types was undertaken by NMDS (Figure 2.14). The plot revealed considerable overlapping of the distinct clusters for each site type, with a PERMANOVA test showing there to be no significant differences between the invertebrate community compositions for the three site types ($F_{2, 17} = 0.84$, $p = 0.622$).

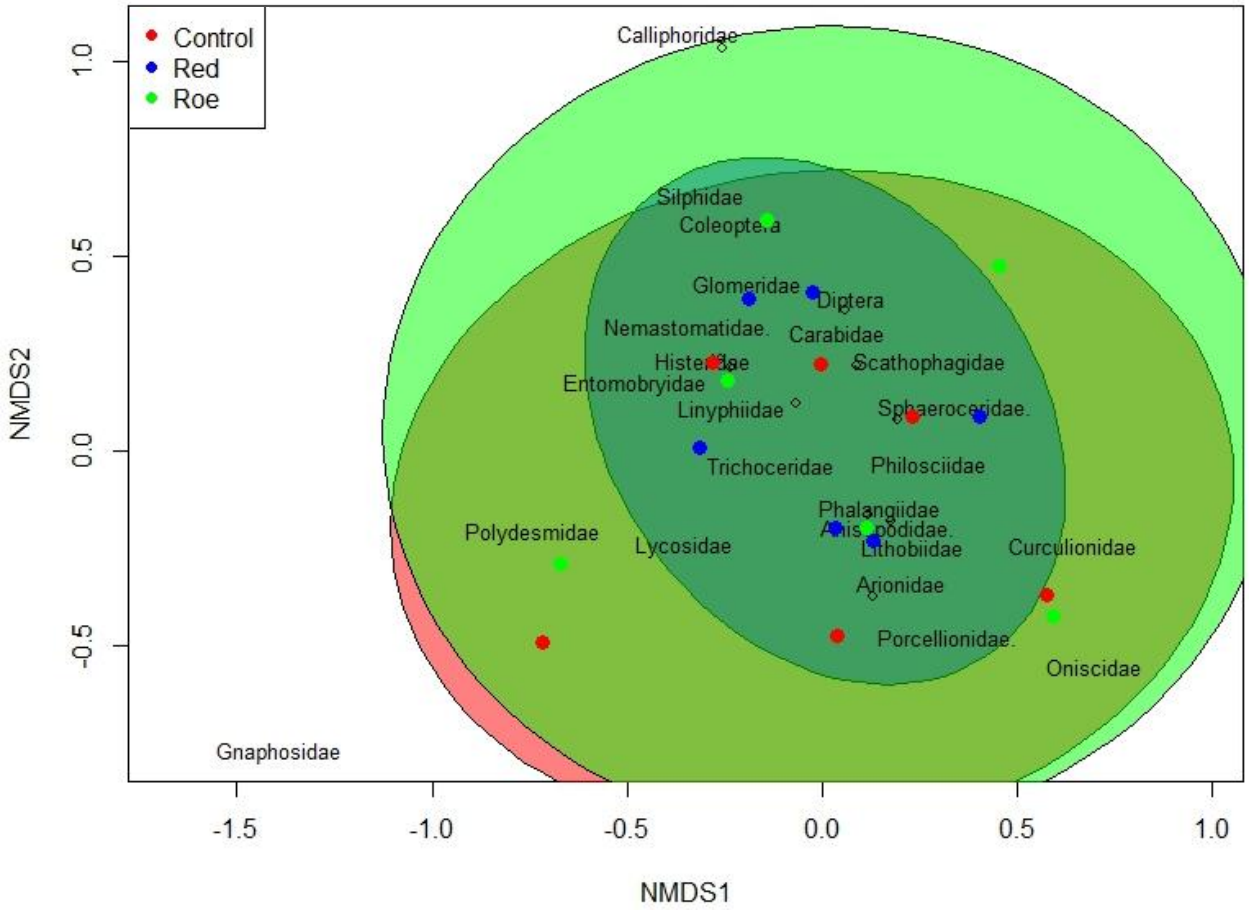

Figure 2.14: NMDS plot of invertebrate family community composition between the different site types – control (n = 6), red (n = 6) and roe (n = 6). Ellipses for each group represent the 95% confidence interval (CI). Points represent each site, with these coloured by site type, with the positions of invertebrate families overlayed. Number of dimensions (k) = 4, and stress value = 0.039.

2.3.3. Plant Diversity and Composition

Across the eight surveying periods (including the initial quadrat surveys undertaken during site set-up), a total of 54 plant species were identified, from 36 families and 23 orders. The total cover percentage (i.e., cumulative percentage of the cover for each species across all time periods and sites) and occurrence (i.e., number of quadrats a species was observed in) per species across all sites and all surveying periods can be viewed in Appendix Table 3, as well as the total cover and occurrence per site type. The top ten species based on total cover are shown in Figure 2.15, with these separated out by site type, with the most abundant species by cover being rough-stalked feather-moss (*Brachythecium rutabulum*; 10,278%), followed by wavy hair-grass (*Deschampsia flexuosa*; 2,746%) and Yorkshire fog (*Holcus lanatus*; 1,496%). Considering total occurrence, the top ten most common species are shown in Figure 2.16, with the first and second again being *B. rutabulum* (314) and *D. flexuosa* (63), with the third being narrow buckler-fern (*Dryopteris carthusiana*; 57).

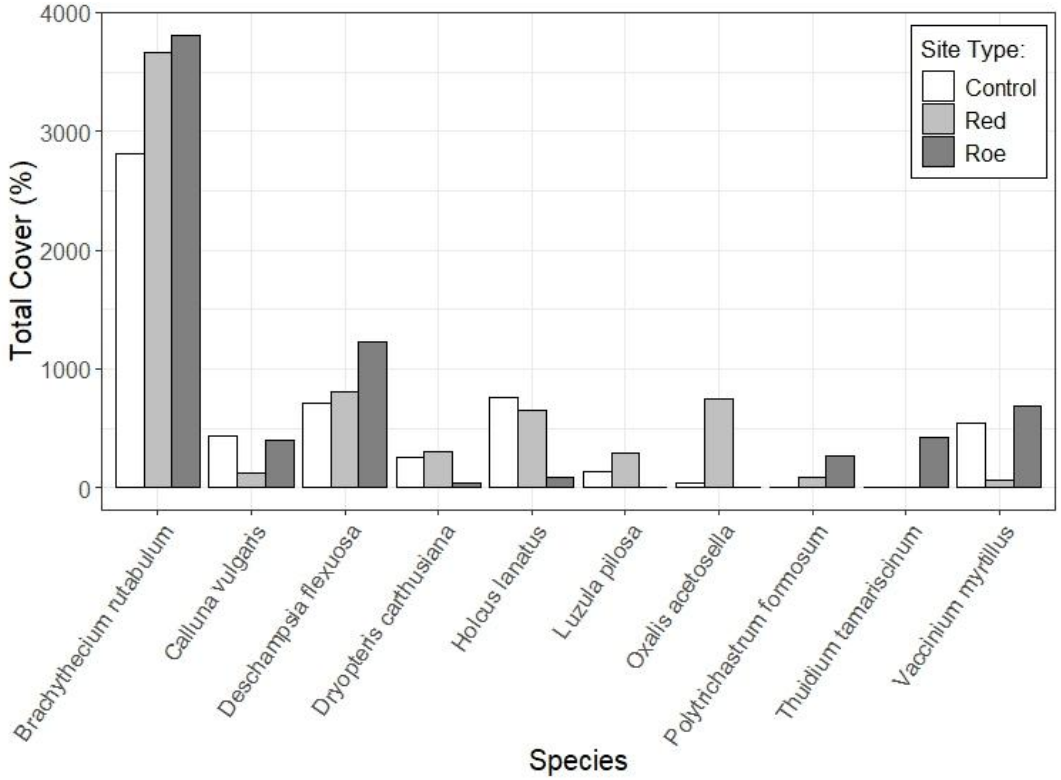

Figure 2.15: The total cover percentage of the ten plant species with the highest overall cover, per site type.

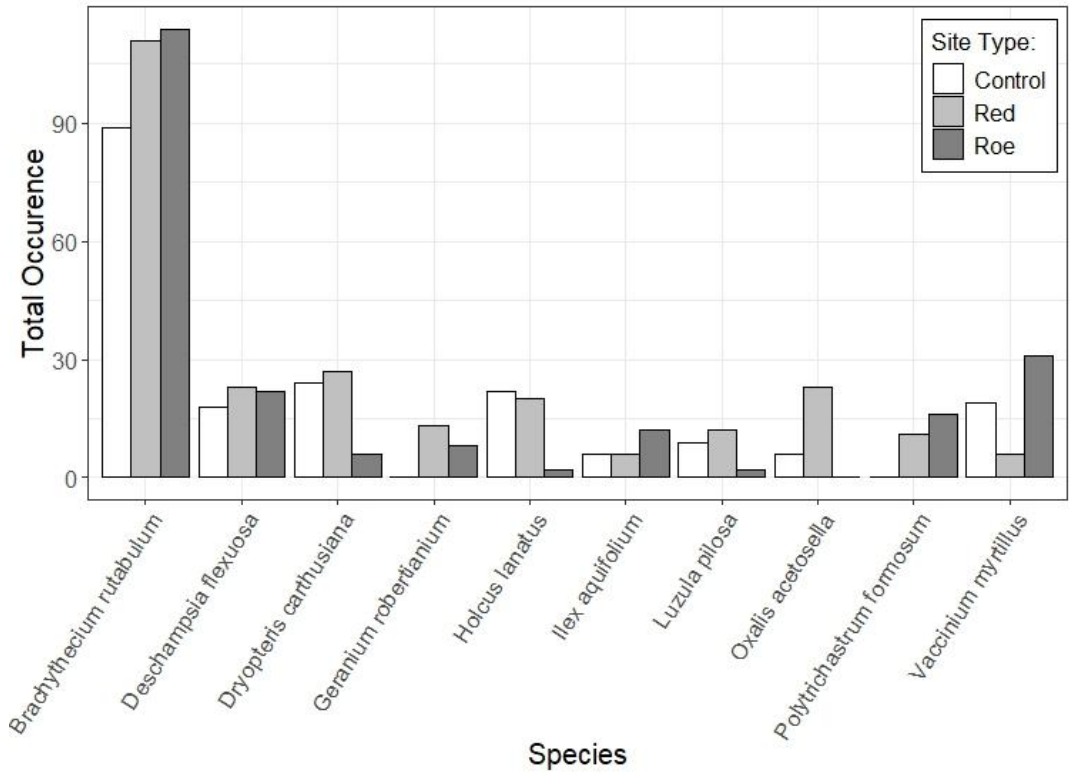

Figure 2.16: The total occurrence, i.e., number of quadrats a species was observed in across the whole study, of the ten plant species with the highest occurrence, separated out into site type.

Considering species richness first, a Kruskal-Wallis test revealed no significant difference for the overall species richness per site type (H_2 = 0.43, p = 0.808; Figure 2.17). When considering the species richness of the three distance groups for each site type independently (Figure 2.18), further Kruskall-Wallis tests showed there were no significant differences between the distance groups for control sites (H_2 = 3.97, p = 0.138), red deer sites (H_2 = 0. 27, p = 0.874) or roe deer sites (H_2 = 1.81, p = 0.404).

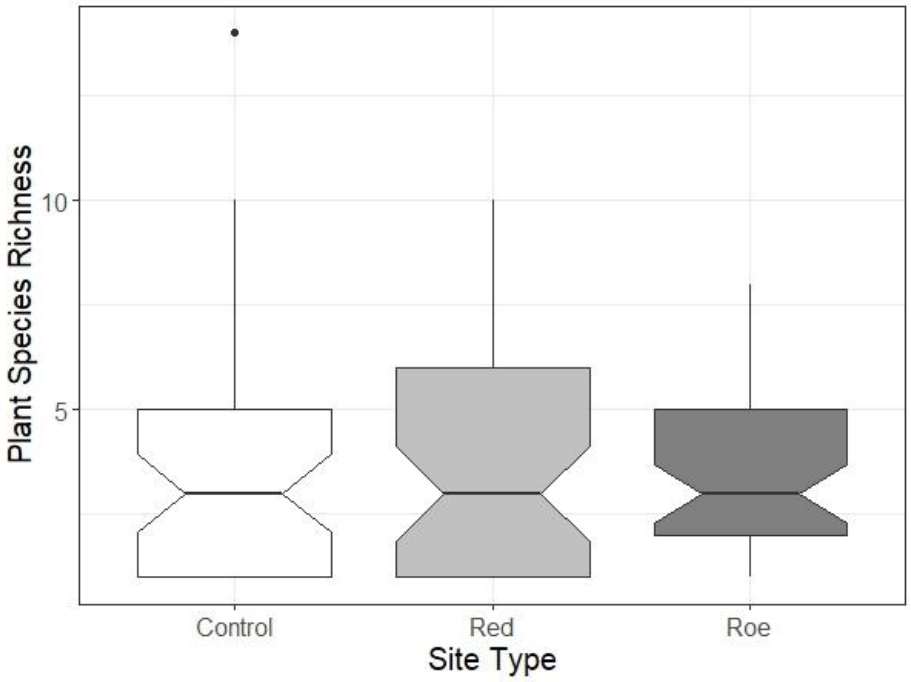

Figure 2.17: Variation of the overall plant species richness per site type. The top of boxes represents the 75th percentile, the bottom the 25th percentile and the middle line the median, whilst whiskers correspond to the range. Outliers are represented by points.

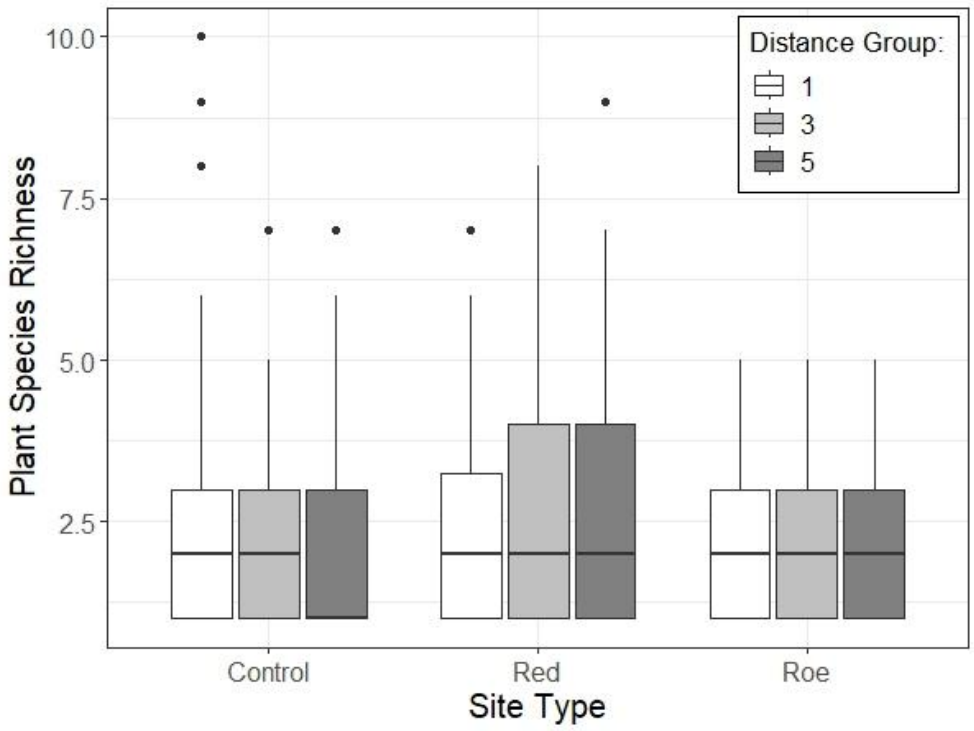

Figure 2.18: Variation of plant species richness per site type, with this separated into the three distance groups – 1 m, 3 m and 5 m. The top of boxes represents the 75th percentile, the bottom the 25th percentile and the middle line the median, whilst whiskers correspond to the range. Outliers are represented by points.

For the overall plant Shannon-Wiener indices between site types, there was no evidence of a significant difference (H_2 = 1.55, p = 0.461; Figure 2.19). Comparing the three distance groups for each site type (Figure 2.20), there was also shown to be no significant differences between these for control sites (H_2 = 3.07, p = 0.215), red deer sites (H_2 = 0.10, p = 0.949) or roe deer sites (H_2 = 1.77, p = 0.412).

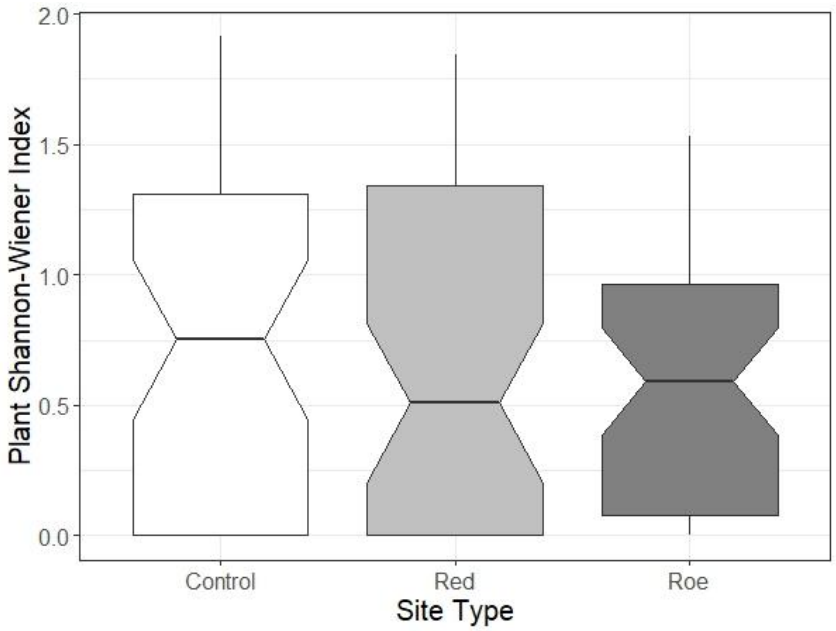

Figure 2.19: Variation of the overall plant Shannon-Wiener diversity indices per site type. The top of boxes represents the 75th percentile, the bottom the 25th percentile and the middle line the median, whilst whiskers correspond to the range.

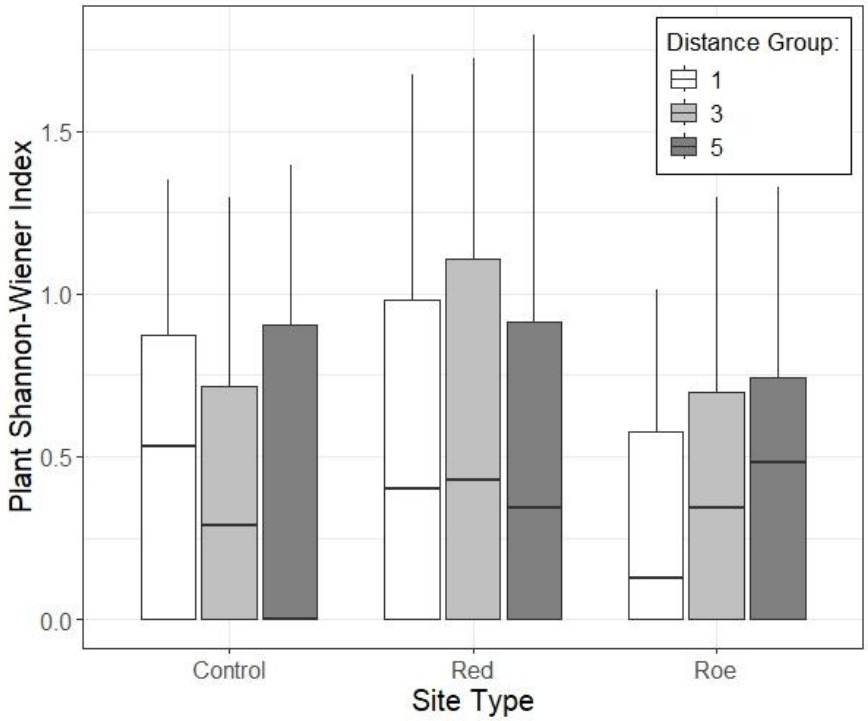

Figure 2.20: Variation of plant Shannon-Wiener indices per site type, further separated into the three distance groups. The top of boxes represents the 75th percentile, the bottom the 25th percentile and the middle line the median, whilst whiskers correspond to the range.

Finally, for the overall plant Simpson's indices between site types, a Kruskal-Wallis test revealed no significant difference (H_2 = 1.58, p = 0.454; Figure 2.21). There was shown to be no significant differences between the Simpson's indices between the three distance groups (Figure 2.22) for the control (H_2 = 2.55, p = 0.279), red deer (H_2 = 0.03, p = 0.987) or roe deer sites (H_2 = 1.39, p = 0.499).

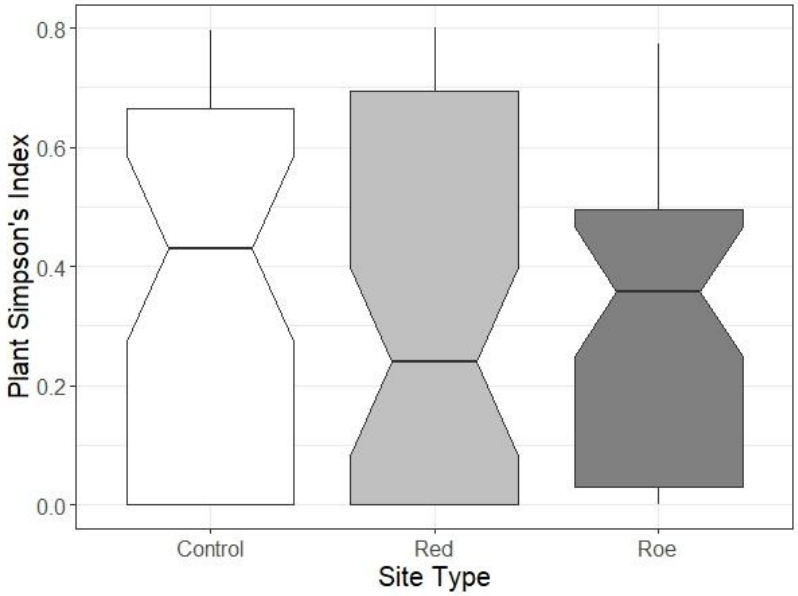

Figure 2.21: Variation of the overall plant Simpson's diversity indices per site type. The top of boxes represents the 75th percentile, the bottom the 25th percentile and the middle line the median, whilst whiskers correspond to the range.

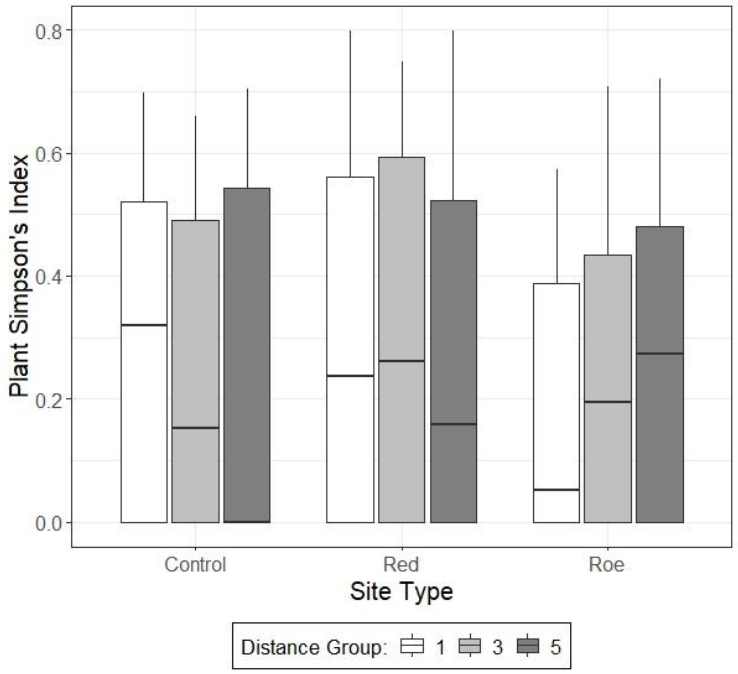

Figure 2.22: Variation of plant Simpson's indices per site type, separated into the three distance groups. The top of boxes represents the 75th percentile, the bottom the 25th percentile and the middle line the median, whilst whiskers correspond to the range.

Several LMMs and LMs were ran with plant Shannon-Wiener diversity as the response variable (Table 2.12). The top model based on AIC scores, *Shannon-Wiener ~ Site Type + rand (Cluster/Site ID)*, was responsible for 93.2% of the w_i but the *Site Type* variable was deemed not significant (X^2 (2) = 0.90, p = 0.638).

Table 2.12: Model comparisons for the LMs and LMMs with plant Shannon-Wiener diversity index as response variable. Models are ranked based on AIC values, with selected models in bold.

Models	AIC	ΔAIC	Likelihood	Akaike Weights (w_i)
Site Type + rand (Cluster/Site ID)	**252.122**	**0.000**	**1.000**	**0.932**
Distance + rand (Cluster/Site ID)	257.416	5.294	0.071	0.066
Site Type + Distance + rand (Cluster/Site ID)	265.214	13.091	0.001	0.001
Period + rand (Cluster/Site ID)	269.793	17.671	0.000	0.000
Period + Site Type + rand (Cluster/Site ID)	277.506	25.384	0.000	0.000

For the next set of models, again LMs and LMMs were employed with Simpson's diversity index as the response variable (Table 2.13). The top model, *Simpson's ~ Site Type + rand (Cluster/Site ID)*, was responsible for the majority of the w_i (90.4%) but again the *Site Type* variable was not significant (X^2 (2) = 0.79, p = 0.675).

Table 2.13: Model comparisons for the LMMs with plant Simpson's diversity index as response variable. Models are ranked based on AIC values, with selected models in bold.

Models	AIC	ΔAIC	Likelihood	Akaike Weights (w_i)
Site Type + rand (Cluster/Site ID)	**-180.556**	**0.000**	**1.000**	**0.904**
Distance + rand (Cluster/Site ID)	-176.070	4.486	0.106	0.096
Site Type + Distance + rand (Cluster/Site ID)	-165.172	15.384	0.000	0.000
Period + rand (Cluster/Site ID)	-155.979	24.576	0.000	0.000
Period + Site Type + rand (Cluster/Site ID)	-145.154	35.402	0.000	0.000

For the final set of models, GLMMs and GLMs were ran with species richness as response variable (Table 2.14). Three top models were identified (the top model and two with an ΔAIC

value of < 2 from the top model), with the first, *Richness ~ Site Type + rand (Cluster/Site ID)*, responsible for 30.8% of the w_i but this variable was shown to not be significant (X^2 (2) = 0.35, p = 0.842). The second and third ranked models, *Richness ~ Distance + rand (Cluster/Site ID)* and *Richness ~ Period + rand (Cluster/Site ID)*, respectively, were both responsible for 27.8% of the w_i but also neither of these variables were significant (*Distance*: X^2 (2) = 0.14, p = 0.934; *Period*: X^2 (7) = 9.90, p = 0.194).

Table 2.14: Model comparisons for the GLMs and GLMMs with plant species richness as the response variable. Models are ranked based on AIC values, with selected models in bold.

Models	AIC	ΔAIC	Likelihood	Akaike Weights (w_i)
Site Type + rand (Cluster/Site ID)	**1197.974**	**0.000**	**1.000**	**0.308**
Distance + rand (Cluster/Site ID)	**1198.179**	**0.205**	**0.902**	**0.278**
Period + rand (Cluster/Site ID)	**1198.181**	**0.208**	**0.901**	**0.278**
Period + Site Type + rand (Cluster/Site ID)	1201.807	3.834	0.147	0.045
Site Type + Distance + rand (Cluster/Site ID)	1201.840	3.866	0.145	0.045

Finally, comparison of the community composition, based on plant functional groups, between the three site types was undertaken by NMDS analysis (Figure 2.23). No clear tight clustering of the site types was revealed, with ellipses also overlapping considerably. An absence of any significant differences of the plant functional group community compositions between the three site types was confirmed by a PERMANOVA ($F_{2, 53}$ = 1.27, p = 0.222).

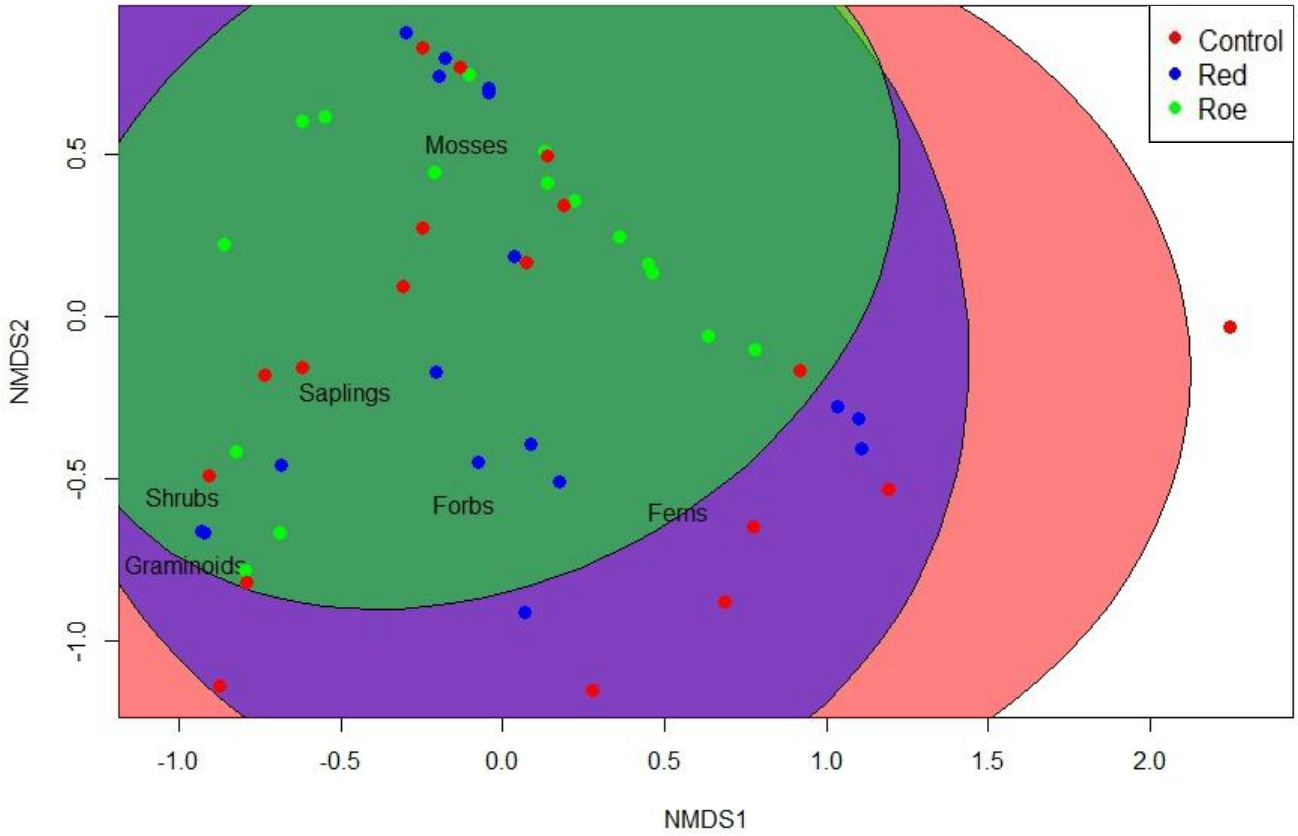

Figure 2.23: NMDS plot of plant functional group community composition between the different site types – control (n = 18), red (n = 18) and roe (n = 18). Ellipses for each group represent the 95% confidence interval (CI). Points represent each distance group per site, coloured by site type, with plant functional group positions overlayed. Number of dimensions (k) = 4, and stress value = 0.062.

2.4. Discussion

2.4.1. Vertebrates

Carcass size and type:

Clear evidence was found to support an effect of carcasses on vertebrate activity compared to control sites, with this particularly evident for the four vertebrate species with highest recorded activity – red fox, domestic dog, grey squirrel and carrion crow. These species, except grey squirrels, are deemed facultative scavengers where this was evident in the median activity for each of these scavengers having the highest levels of activity at carcasses compared to control sites.

From modelling analyses for the activity of vertebrates, it was revealed that site type had a significant effect on activity, with both carcass types shown to promote significantly higher activity compared to controls. No clear effects of site type were found for the diversity of vertebrates; however, site type influenced vertebrate community composition based on activity between site types. Several species were shown to be ubiquitous across site types, including grey squirrel, badger (*Meles meles*), roe deer and red deer; yet other species were far more prevalent at carcasses, including the facultative scavengers of red fox, domestic dogs and carrion crow, as well as blackbirds (*Turdus merula*).

These results provide evidence to support the greater impact of large carcasses on vertebrate activity, especially that of facultative scavengers. Although no directly significant effect of carcasses was found for vertebrate diversity, the results of other studies have found evidence for this. Specifically, it has been suggested that larger carcasses offer more resources, in both biomass and resource type, which are likely to persist for an extended period of time and hence promote increased diversity of species (Selva and Fortuna, 2007; Moleón *et al.*, 2015; Turner *et al.*, 2017; Stiegler *et al.*, 2020). This is reinforced by a meta-analysis that investigated factors affecting patterns of vertebrate scavenger diversity across ecosystems, with carcass size the only variable that consistently affected both diversity and richness, with large carcasses generally increasing richness (Sebastián-González *et al.*, 2019).

Effects of the seasons and habitat type:

There was found to be a significant effect of the time period on vertebrate activity. Specifically, the time periods approximately corresponding to the months February, April and May were shown to be significant. These time periods were shown to promote higher activity, especially the February time period. With February being at the end of winter in the

UK, an increase in vertebrate activity at carcasses during this period highlights the importance of carcasses as a resource during winter where carcass consumption has been shown to increase as winter advances (Selva *et al.*, 2003). In this study, no significant effect of time period, and hence seasonality, was observed for the richness or diversity of vertebrates; however, similar studies in temperate forests have identified increased richness and diversity during winter (Selva *et al.*, 2003, 2005; DeVault, Brisbin, Jr. and Rhodes, Jr., 2004; Stiegler *et al.*, 2020). The general increase in vertebrate activity and diversity during colder periods can be attributed to several factors, including decreased competition with invertebrates and microbes (Ray, Seibold and Heurich, 2014; Inagaki *et al.*, 2022) and an absence of other food resources (Selva *et al.*, 2003). The longer carcass persistence time during cold periods may drive an increased diversity of vertebrates, particularly some more typically non-necrophagous species (Turner *et al.*, 2017).

Concerning habitat type, the top-performing model with Shannon-Wiener diversity as response variable had habitat type as the only significant predictor variable. Specifically, the felled habitat was shown to result in a lower diversity than the baseline broadleaf woodland . This could perhaps be related to the dominance of carrion crows at the felled sites in the later time periods. Carcasses present in open habitats are likely frequented more by avian scavengers (Selva *et al.*, 2003, 2005) due to birds relying on visual cues for foraging (Ruxton and Houston, 2004).

Findings from studies in the UK and similar European temperate forests:

There is an apparent lack of research on the impact of carcasses on vertebrate activity and diversity in the UK. One of the only studies looked at the role of vertebrate scavengers in urban areas (Inger *et al.*, 2016), finding a low species richness recorded at carcasses, with only three species observed scavenging, i.e., red foxes, carrion crows and magpies (*Pica pica*). However, there are clear limitations when comparing against their findings, namely that the carcasses were of small rodents and the urban location. Therefore, it is more appropriate to compare against studies undertaken in temperate forest ecosystems of mainland Europe with larger carcasses.

Compared to other biomes globally, scavenger guilds of temperate forests of the Northern Hemisphere are typically less diverse with no obligate scavengers; however, there are still an array of mammalian and avian species that forage on or around carcasses (Selva *et al.*, 2003), particularly mesoscavengers and birds (Mateo-Tomás *et al.*, 2015). Findings from different studies examining carcass effects on vertebrates in European temperate forests reveal great variation in the guild of vertebrates observed at carcasses. Similarly to the present study, several studies found red foxes to be the most observed vertebrate at

carcasses (Ray, Seibold and Heurich, 2014; Stiegler *et al.*, 2020) or one of the most observed (Jędrzejewski *et al.*, 1993; Selva *et al.*, 2003, 2005), highlighting the importance of carcasses for red foxes especially during colder winter periods, with estimates of carrion accounting for 30% of their winter diet, compared to nearly 0% in summer (Jędrzejewski and Jędrzejewska, 1992).

Out of the other vertebrate species recorded in the present study at carcasses, several of these were recorded at carcasses in similar studies, including badgers (Selva *et al.*, 2005), carrion crow (Wenting, Rinzema and van Langevelde, 2022), buzzards (Selva *et al.*, 2003, 2005) and even domestic dogs (Selva *et al.*, 2003). In the current study, badgers were observed at both carcass types as well as control sites, with badgers known to feed on carcasses facultatively (Asprea and Marinis, 2005). However, the only observations of buzzards were from a single red deer carcasses (Figure 2.24), thus indicating the value of carcasses for birds of prey.

Figure 2.24: Camera trap observation of a buzzard (*Buteo buteo*) at a red deer carcass (site 3).

Carcass-use by non-necrophagous vertebrates:

As discussed in this chapter's introduction, there are many typically non-necrophagous vertebrates that have been revealed to exploit carcass resources, either directly or indirectly. In the present study, this was predominantly of non-corvid passerine birds including blackbirds, song thrush (*Turdus philomelos*) and great tit (*Parus major*). The two thrush species (*Turdus spp.*) were observed mainly at carcass sites, and during the later time periods of the study where the increased abundance of invertebrates provided a localised

and copious food resource (Campobasso, Di Vella and Introna, 2001; Moreno-Opo and Margalida, 2013; Wenting, Rinzema and van Langevelde, 2022).

There was one observation of great tit with an individual recorded at a red deer carcasses appearing to gather tufts of carcass hair (Figure 2.25). Great tits have been recorded at carcasses with individuals observed feeding on invertebrates as well as gathering hairs (Selva *et al.*, 2003; Wenting, Rinzema and van Langevelde, 2022), . It is suggested that gathered hairs are used for nest building (Ondrušová and Adamík, 2013), with similar behaviours shown for corvids including ravens (*Corvus corax*) and chough (*Pyrrhocorax pyrrhocorax*; Riney, 1951; Margalida and Bertrán, 2000).

Figure 2.25: Camera trap observation of a great tit (*Parus major*), highlighted by the red circle, at a red deer carcass after significant carcass removal (site 9).

The absence of apex scavengers and subsequent mesoscavenger domination of carcasses:

Apex scavengers refer to obligate scavengers or large carnivores that are known to scavenge facultatively, with these species able to rapidly locate and efficiently consume large carcasses (Newsome *et al.*, 2021). They also promote more efficient carcass removal by signalling the location of carcasses, as well as opening carcasses to allow greater access, to more subordinate scavengers (Moleón *et al.*, 2014; Kane and Kendall, 2017; Sebastián-González *et al.*, 2021). With global declines of numerous apex scavengers (Estes *et al.*, 2011; Ripple *et al.*, 2014; O'Bryan *et al.*, 2018), this could impact the functionality of

scavenger guilds (Newsome *et al.*, 2021) and possibly lead to the domination of mesoscavengers at carcasses.

In the UK there is a complete absence of any apex scavengers, with some consequences of this apparent in the present study where mesoscavengers, i.e., red fox and carrion crows, dominated activity and therefore many carcasses persisted for the majority of the study. However, the activity of domestic dogs was very high at several carcasses leading to rapid carcass removal – yet, domestic dogs cannot be deemed a sufficient substitute for a wild scavenger. The observed carcass consumption by domestic dogs was potentially quite unique to this study, due to the vast majority of scavenging undertaken by a group of three large dogs seemingly local to the study site. The impact of carcass consumption by domestic dogs may have implications for public health so this must be investigated further in the future if carcass provisioning is to be carried out in areas of the UK openly accessible to the public.

2.4.2. Invertebrates

Carcass size and type:

There was shown to be a clear effect of site type on the diversity and richness of invertebrates, with modelling analyses revealing a significant increased invertebrate diversity and richness at carcasses compared to controls, with this particularly evident at red deer carcasses. Further analyses undertaken to investigate for any differences between the invertebrate community composition between site types revealed no significant variance.

These results indicate the clear effects of carcasses promoting higher invertebrate richness and diversity, compared to a control site of the same environmental conditions. This is likely influenced heavily by the greater abundance and richness of necrophagous invertebrates found at carcasses. Invertebrates of the orders Diptera and Coleoptera generally make up the majority of invertebrates associated with carcasses (Byrd and Tomberlin, 2010; Barton, Cunningham, Lindenmayer, *et al.*, 2013; von Hoermann *et al.*, 2018), with species either consuming carcasses directly such as blow flies (family Calliphoridae; Campobasso, Di Vella and Introna, 2001), or using carcasses indirectly such as carrion beetles (family Silphidae) feeding on other invertebrates or using carcasses as a place to reproduce (Trumbo, 1992; Gibbs and Stanton, 2001). Due to their known association with carrion, studies have examined the effect of carcasses solely on the richness and diversity of these necrophagous invertebrate families. For example, Schwegmann *et al.* (2022) studied the differences in abundance and richness of Dipterans and Coleopterans between roe deer carcasses, carcass eviscerations and control sites in the Black Forest, Germany. They found a clear

trend of significantly increased abundances and richness of both groups at carcass sites compared to evisceration sites, and also increased abundances of each at evisceration sites compared to controls.

Some studies have even investigated the effects of carcasses solely on Coleopterans, such as Barry *et al*. (2019) who investigated differences in the diversity of beetle assemblages between puma-killed (*Puma concolor*) carcasses and control sites in Yellowstone, USA. They revealed that carcasses typically increased the abundance, richness and diversity of beetles compared to control sites. Many of the carcass-associated beetle families observed by Barry *et al*. (2019) were also identified disproportionately at carcasses in the present study, including Silphidae, Histeridae, Curculionidae and Geotrupidae.

The current study indicated that red deer carcasses had a stronger effect promoting invertebrate richness and diversity than the smaller roe carcasses. Other studies have also noted effects of carcass size, with larger carcasses shown to be able to host a higher abundance of invertebrates, especially more blowfly larvae which then serve as prey to additional invertebrates (Gu *et al.*, 2014). Due to the decomposition of larger carcasses often taking an extended period of time, they are able to provide a more stable resource for a greater abundance and diversity of invertebrates (van Klink *et al.*, 2020), as well as being able to provide resources for several generations (Gu *et al.*, 2014).

Effects of habitat type:

There was shown to be multiple effects of habitat type on the richness and diversity of invertebrates, with both the conifer and felled habitats shown to imply a reduced diversity compared to the baseline broadleaf woodland. Concerning these observed impacts of habitat, one study examined the effects of habitat types of a British temperate woodland on the diversity and composition of carabid beetles, as an indicator species of invertebrates more broadly (Fuller, Oliver and Leather, 2008). They compared the diversity and community composition between five main forest habitats, three of which are comparable to the habitats in the present study. They found that diversity was not significantly different between habitats, except the clear-felled habitat which had significantly lower diversity. Regarding community assemblages, only the deciduous woodland had significant numbers of defined forest specialist species. These findings indicate the importance of considering environmental variables, such as habitat, alongside the impacts of carcasses themselves.

Impacts of the seasons:

There was also shown to be several impacts of the time period, and thus indirectly seasonality, on invertebrate richness and diversity, with evidence of the later time periods

resulting in both increased richness and Shannon-Wiener diversity. The effect of higher average temperatures during these later time periods were likely partly responsible for the observed increases to diversity and richness.

Similar trends have been observed, including Farwig *et al*. (2014) who examined the impact of mice carcasses (*Mus musculus*) on the abundance and richness of invertebrate scavengers in the Bohemian Forest, Germany, where they deemed a decreasing temperature gradient between sites to be a major factor in the observed decrease in species richness. Benbow *et al*. (2013), who investigated the consequences of domestic pig carcasses (*Sus scrofa domesticus*) on necrophagous invertebrates in a temperate forest in Ohio, revealed richness increased through spring into summer alongside corresponding increasing average temperature and with progressing carcass decomposition.

The effect of increasing distance from carcasses:

Concerning the effect of increasing distance from carcasses and control sites on invertebrate diversity and richness, it was implied that Simpson's diversity was highest at the 3 m distance group compared to the baseline 1 m group, with species richness highest at the 1 m distance.

There seems to be only one other study that investigated an effect of increasing distance from carcasses on the diversity of arthropods (Sawyer and Bloch, 2020). Specifically, they examined the effect of carrion of domestic pig heads on the abundance, richness and diversity of both necrophagous and non-necrophagous arthropods at distances of 0 m, 1.5 m and 3 m from carrion in a temperate forest in Massachusetts, USA. They found that the diversity of necrophagous arthropods was highest immediately adjacent to carcasses. For the non-necrophagous arthropods, both abundance and diversity were comparable across the distance groups, , whereas the richness was highest at the 3 m group. It is difficult to compare these results to those of the present study, due to the richness and diversity measures calculated with both necrophagous and non-necrophagous invertebrates considered. Interestingly, unlike many similar studies, they investigated the impact of carcasses on both necrophagous and non-necrophagous arthropods, with their results indicating that changes in richness and diversity were driven by both groups, highlighting the importance of considering not just carrion-specific invertebrates.

Carcass-use by non-necrophagous invertebrates:

It is important to consider the overall invertebrate community when investigating carcass effects on diversity, where very few studies have examined how carcasses affect wider invertebrate diversity when both necrophagous and non-necrophagous invertebrates are

considered, with the understanding of the importance of large carcasses for non-necrophagous invertebrates particularly limited (Gu *et al.*, 2014).

One study investigated the impacts of red deer carcasses, and equivalent control sites, on arthropod diversity and community composition at grassland sites in the Oostvaardersplassen rewilding reserve (van Klink *et al.*, 2020). They evaluated the immediate carcass effects on various arthropod functional groups, namely carcass-associated, dung-associated, predatory, herbivorous and detritivorous, as well as delayed carcass effects 5 months post-carcass placement. Their findings at the immediate sampling stage indicated there were no significant differences in overall species richness between carcass and control sites; however, the species richness of the carcass and dung-associated arthropods were both significantly greater at carcass sites. In contrast, the findings 5-months after carcass placement revealed that the overall species richness was over 2.5 times higher at carcass sites than control sites. Moreover, both carcass and dung-associated arthropods were present in such low abundances that no comparisons between richness could be made between carcass and controls. Contrastingly, there were significant differences in species richness revealed between the other functional groups, including higher richness of predators (2.7 times), herbivores (two times) and detritivores (five times) at carcass sites compared to controls. It was suggested that the delayed increases in richness of these groups at carcasses were due to the observed five-fold increase in plant biomass at carcasses. Therefore, these results highlight the consequences of large carcasses not just on the immediate diversity of necrophagous invertebrates, but also the delayed and indirect impacts on the diversity of non-necrophagous taxa.

In terms of the more rare and less discussed non-necrophagous invertebrates observed at large carcasses, Gu *et al*. (2014) aimed to provide a greater insight into the wider invertebrate community associated with large carcasses, particularly more unique taxa and behaviours. For example, 14 species of Lepidoptera were observed “puddling” on carcasses (Downes, 1973) with this suggested as either a reproductive strategy (Pivnick and McNeil, 1987), or simply to obtain nutrients. Species of Hymenoptera were observed feeding directly on carcasses, or on other carcass-associated invertebrates (Braack, 1987). Bees of several genera were observed feeding on carcasses likely to ingest water and nutrients (Baumgartner and Roubik, 1989). These observations of both direct and indirect carcass-use by typically non-necrophagous invertebrates emphasises the impacts of large carcasses on the richness and diversity of the wider invertebrate community.

2.4.4. Plants

Site type and distance effects:

The investigations into the effects of carcasses on plant richness, diversity and community composition, revealed no significant findings. Despite the lack of significant findings in the present study, there are some well-studied impacts of carcasses on plants. These mostly include the impacts of dramatic localised inputs of carcass-derived nutrients which affect the soil biogeochemistry (Macdonald *et al.*, 2014), and subsequent growing conditions for plants (Melis *et al.*, 2007; Bump, Peterson and Vucetich, 2009; Parmenter and MacMahon, 2009; Van Klink *et al.*, 2020). There has, nevertheless, been far less research undertaken into the consequences of carcasses for the composition and diversity of localised plant assemblages, with less than a handful of studies.

Carcass impacts on plant diversity and composition from equivalent studies:

One such study investigated the long-term changes in composition and diversity of plants surrounding large carcasses in a North American grassland prairie (Towne, 2000). They discovered that in the first year after carcass placement that richness and diversity were lowest. However, both richness and diversity increased significantly over the next two years before reaching a stable composition, which remained distinct compared to surrounding vegetation over 5 m away from carcasses after 5 years. These results indicate that large carcasses can introduce long-lasting heterogeneity, of both species composition and structural complexity, to grassland ecosystems.

Another study examined the effects of white-tailed deer (*Odocoileus virginianus*) carcasses on the composition of the herbaceous layer, and subsequent effects on tree seedling germination, in a temperate forest of Michigan (Bump *et al.*, 2009). They found that even mostly consumed carcasses significantly reduced the cover of herbaceous species due to the high concentrations of nutrients released into the soil, with nutrient levels remaining high for at least 2 years post-mortem. Lower cover of herbaceous species at carcasses led to increased germination and growth rates of tree seedlings, thus indicating how large carcasses can introduce greater heterogeneity of forest plant communities.

When carcasses are often left largely intact, either due to an absence of apex predators and scavengers or other instances of high carcass abundance in the landscape, it can lead to more intense inputs of nutrients into the soil, impacting plant growth more (Bump *et al.*, 2009). This was true for several of the carcasses in the present study, which had limited consumption by vertebrates, and therefore most of the carcass biomass entered the soil with decomposition. Particularly, this was evident for the carcass sites present in the broadleaf

woodland and felled habitats, with ground vegetation largely absent in the coniferous woodland sites. At these sites after significant decomposition, there were visible exclusion zones of plants around carcasses (Figure 2.26), where the carcasses had smothered plants or bodily fluids had killed the plants immediately adjacent to carcasses (Towne, 2000). Therefore, it is probable that any impacts on plant growth, composition and diversity at carcasses in the present study may be more pronounced in subsequent growing seasons. Specifically, in the immediate locality of carcasses with the greatest influx of carcass nutrients, these areas of exposed ground may be favoured by opportunistic plant species, or even tree seedlings, promoting their growth and thus may introduce species diversity and heterogeneity to the wider area.

Figure 2.26: Areas devoid of plants around red deer carcasses at the felled site (A; site 16) and the broadleaf site (B; site 3), both photos taken in late June 2024 approximately 204 days after carcass placement.

2.5. Conclusion

Comparisons of vertebrate activity between site types revealed clear differences, with a likely effect of increasing carcass size promoting higher vertebrate activity. The composition of vertebrate species found between the site types also showed significant differences. Vertebrate diversity was found not to vary between carcass and controls, perhaps due to low general vertebrate diversity in the landscape; however, in other similar studies in woodland ecosystems across Europe an effect of carcasses on diversity has been shown. Several occurrences of typically non-necrophagous vertebrates at carcasses were observed, and some consequences of the absence of apex predators/scavengers in the UK were alluded to, with these discussed further in Chapter 4.

For invertebrates, there was shown to be a significant impact of site type, with both carcass sites elevating richness and diversity. It was also suggested that carcasses likely affected the diversity of both necrophagous and non-necrophagous invertebrates. There were clear influences of the broad habitat type, seasons and distance from carcasses on the richness and diversity of invertebrates.

No effects of carcasses were found for plant richness, diversity or community composition, with this probably due to the shortened timespan of the study. The observed plant exclusion zones around carcasses in the broadleaf and felled habitats may promote new growth in the following years, thus providing motivation for further research.

Chapter 3: Investigating the Effects of Deer Carcasses on the Diversity and Composition of Soil Fungi Using eDNA Approaches

3.1. Introduction

Accounting for the most diverse group of organisms associated with terrestrial carcasses, soil microbes are critical for carcass decomposition and subsequent nutrient cycling (Madsen, 2011; Mason, Taylor and DeBruyn, 2023). With the death of an animal and onset of decomposition, there is an influx of bodily fluids into the surrounding soil that radically alters its biogeochemistry, particularly due to inputs of high concentrations of carbon and nitrogen (Carter, Yellowlees and Tibbett, 2007; Mason, Taylor and DeBruyn, 2023), with resulting shifts to the pH and establishment of an increasingly anaerobic environment (Parmenter and MacMahon, 2009; Metcalf *et al.*, 2016; Keenan *et al.*, 2018). The formation of these disturbed areas of soils impacts the local diversity and abundance of soil microbes, especially of bacteria and fungi, where the composition of microbes native to soils shifts in response to these altered local biogeochemical conditions as well as due to the influx of carcass-derived microbes (Mason, Taylor and DeBruyn, 2023). The ecosystem services of soil microbes are highlighted by their absence resulting in extreme reduced rates of carcasses decomposition, and the build-up of potentially dangerous biogeochemical waste (Gilbert and Neufeld, 2014).

Within the literature on carcasses and soil microbes, the majority of studies have been undertaken with a focus on applications for human forensics (e.g., Vass *et al.*, 2002; Breton *et al.*, 2016; Burcham *et al.*, 2019). However, various research has investigated the impacts of carcasses on soil microbes from an ecological perspective; yet many of these studies employ either humans or small domestic animals, such as rats and piglets, as their study carcass and investigations are generally undertaken in controlled experimental systems. In contrast, there have been even fewer studies that have investigated the diversity and composition of soil microbes associated with large, wild mammal carcasses in natural systems, with Bump, Peterson and Vucetich (2009) and Risch *et al.* (2020) as limited examples. Therefore, there remains a lack of understanding into the impact of large carcasses of wild animals on the diversity and composition of soil microbes, and the importance of understanding how soil microbes in natural systems contribute to ecosystem functioning (Risch *et al.*, 2020).

With upwards of 3 million species estimated, fungi are an extremely diverse kingdom of heterotrophic organisms (Hawksworth and Lücking, 2017), with a critical role as one of the main groups of decomposers (Mason, Taylor and DeBruyn, 2023). However, there has generally been more research undertaken into the consequences of carcasses on the diversity and succession of bacteria (e.g., Cobaugh, Schaeffer and DeBruyn, 2015;

Adserias-Garriga *et al.*, 2017; Singh *et al.*, 2018), as opposed to fungi (e.g., Breton *et al.*, 2016; Metcalf *et al.*, 2016; Fu *et al.*, 2019).

Considering empirical research that has investigated the effects of large carcasses of wild animals on the diversity of soil fungi, research by Risch *et al*. (2020) appears to be the sole study, where they compared soil fungal (and bacterial) communities between predated elk (*Cervus canadensis*) and bison carcasses in Yellowstone National Park, USA. They revealed differences in soil fungi diversity and composition between elk and bison carcasses, and that changes in soil microbial communities associated with carcass decomposition are not as generalisable as once thought. As a consequence, the aims of the present study were to investigate and quantify the impacts of carcasses of two different ungulate species, namely red and roe deer, on the diversity and composition of soil fungi in a temperate woodland in the North East of the UK in order to see if the findings of Risch *et al*. (2020) are applicable to a different ecosystem. Using eDNA metabarcoding approaches, the fungal diversity and composition of different soil samples were determined, where the use of eDNA metabarcoding is an effective tool to determine the diversity of microbial communities such as fungi (Taberlet *et al.*, 2018; Giampaoli *et al.*, 2020; Gemmellaro *et al.*, 2023; Shumskaya *et al.*, 2023). Differences were compared between samples taken at sites prior to the placement of carcasses with samples taken at the same sites around six months later, after significant carcass decomposition had occurred. Comparisons of the soil fungal diversity and composition between the two types of deer carcasses were also explored, as well as with the diversity and composition found at equivalent control sites without a carcass.

The first hypothesis for this chapter was that the diversity of soil fungi would not vary hugely between the initial pre-carcass samples and the samples taken after six months of carcass decomposition, but instead that the community composition between these would vary. This is due to the influx of carcass-derived nutrients and microbes influencing the existing fungal community by leading to the proliferation of some types of fungi that are better suited to the altered biogeochemical conditions, whilst these novel conditions would be disadvantageous for other types of fungi (Bump, Peterson and Vucetich, 2009; Finley *et al.*, 2016). The second hypothesis was similar to the first, with the prediction that the diversity of fungi between red deer carcasses, roe deer carcasses and control sites would not differ considerably, but the fungal community composition between them would, based on the findings from Risch *et al*. (2020) indicating differences in the fungal taxa associated with elk and bison carcasses.

3.2. Methods

Soil samples were collected from sites to investigate the effects of large ungulate carcasses on soil microbial community composition and diversity. Specifically, fungi was chosen as a suitable group to represent the soil microbial community, based on previous research undertaken by collaborators at Forestry England in recent years into the array of fungal diversity of soils from forests across North Yorkshire (with results currently unpublished). Therefore, with the eventual publication of their research, specifically the list of soil fungi identified, this will be useful as a baseline of taxa to compare with the findings of the present study.

3.2.1. Sampling

Due to limited funds, it was only possible to collect a maximum of 20 soil samples for analysis. Hence, it was decided that samples would be collected from nine sites (i.e., three clusters) at the start of the study, with samples taken from the same nine sites around 6 months later to compare changes to fungal diversity and composition over time between the different site types. Also, to gain an insight into differences between the three habitat types, the nine chosen sites were from the broadleaf cluster (sites 1, 2 and 3), the felled cluster (sites 16, 17 and 18), as well as one of the coniferous clusters with this selected randomly (sites 13, 14 and 15). The initial samples were obtained from these sites during the site set up days prior to carcass placement (5th and 6th of December 2023), with the final samples collected 176 days later post-carcass placement (29th and 30th of May 2024). The final samples could not be obtained any later due to a period of several months needed to carry out the DNA extraction and amplification, external sequencing and bioinformatic analyses.

Soil samples were obtained using a metal trowel, which was used to dig equal volume soil cores, approximately 15 cm in depth and 5 cm in diameter. Any leaf litter and other debris was first brushed away, and the first 1 to 3 mm of soil on the surface scratched away to remove any fungal spores randomly present on the soil surface (Krah and March-Salas, 2022). At each site, four separate cores were collected all located 0.5 m away from the site centre point. The exact position of these was based on a random compass bearing defining the placement of one core, with the other three then positioned 90° apart. The four samples from each site were gathered into a sterile plastic bag and mixed to form a representative composite sample. All composite samples were placed immediately at around 4°C in an ice-filled cool box, and then returned to the lab for storage in a freezer at -20°C within 2 to 4 hours following collection (Nuñez *et al.*, 2021). Sterile measures were necessary to avoid

DNA contamination between soil samples at different sites, including the wearing of sterile nitrile gloves during the collection of soil samples, with new ones worn for each site. Moreover, the trowel was thoroughly cleaned to remove any soil and DNA residue between sites, with 20% bleach solution used to destroy any traces of DNA and any bleach rinsed off with distilled water and paper towels.

3.2.2. eDNA Extraction, Analysis and Sequencing

DNA Extraction:

Soil samples were defrosted before being mixed thoroughly. Using sterile forceps, 0.25 g of soil from each sample was measured out, with this the minimum mass to ensure a sufficient volume of fungal DNA could be extracted (Li *et al.*, 2023).

DNA extraction was carried out using Qiagen DNeasy PowerLyzer PowerSoil kits, with this kit developed to extract eDNA from soil types which are typically more difficult to extract eDNA from, such as very loamy or high-sediment soils. This is achieved via the first step of the extraction process, where the soil is added to tubes containing 0.1 mm glass beads, which ensures thorough homogenisation and cell lysis of the soil particles when vortexed. The procedure was followed as per the kit protocol (Appendix A), with several adjustments. In step 4, a horizontal vortex was used to homogenise samples; in step 5, samples were centrifuged for 3 minutes as opposed to 1 minute to ensure the soil was completed pelleted; then in step 8, the centrifuge time was increased, from 1 minute to 2 minutes, for complete pellet formation.

Extractions were carried out for all 18 soil samples, including an additional negative control (i.e., no material added) to check for cross-contamination and a positive control (i.e., a pure sample of fungi, with 0.25 g of a button mushroom (*Agaricus bisporus*) obtained from a supermarket) to verify the DNA extraction process. For all extractions, DNA yields and purities were verified using a NanoDrop™ 2000 Spectrophotometer (Thermo Fisher Scientific), with a summary of yields and purities available (Appendix Table 4).

Polymerase Chain Reaction (PCR):

The advised universal priming region for fungi is the internal transcribed spacer (ITS) regions of the 18S rRNA gene, with this region highly conserved across fungi (Schoch *et al.*, 2012). Thus, the primer Fung02 was used, which corresponds to the ITS1 region of the nuclear rDNA, with this primer highly appropriate for metabarcoding analyses as well as effectively dealing with degraded DNA (Taberlet *et al.*, 2018). The forward primer of Fung02 is 5’-

GGAAGTAAAAGTCGTAACAAGG (White *et al.*, 1990), and the reverse primer is 5'-CAAGAGATCCGTTGYTGAAAGTK (Taberlet *et al.*, 2018). The minimum metabarcode length for this primer is 68 base pairs (bp) and the maximum 919 bp (Taberlet *et al.*, 2018). Additionally, primers incorporated 5' overhang adaptor sequences which are essential for Illumina sequencing of fungal metabarcodes, with these as follows: 5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG- (forward overhang) and 5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG– (reverse overhang).

The initial PCR was based on a protocol from a study investigating the fungal community in topsoil using the Fung02 primer (Guerrieri *et al.*, 2023). Thus, following their protocol PCRs of all samples, plus a negative control, were run where these consisted of 20 µL reactions with the following components: 10 µL of AmpliTaq Gold 360 Master Mix (Applied Biosystems), 2 µL of both the forward and reverse primer working stock (5 µM concentration), 2 µL of undiluted eDNA, and 4 µL of distilled water (or 6 µL of distilled water and no eDNA for the negative control). The PCR consisted of 45 amplification cycles with the following profiles: initial step of 10 minutes at 95°C; then 45 cycles of 30 seconds denaturation at 95°C, 30 seconds annealing at 56°C, and 90 seconds elongation at 72°C; with a final elongation at 72°C for 7 minutes.

Verification of this PCR protocol was undertaken by a gel electrophoresis of a random selection of samples to verify fragment lengths and check for primer dimers. This led to some necessary optimisation, specifically an increase to the annealing temperature from 56°C to 60°C, and a decrease in the number of cycles from 45 to 40. From this amended PCR protocol, three separate PCR replicates were produced for each sample, with these then pooled prior to sending off for sequencing.

Sequencing:

Next-generation sequencing was carried out by first normalising and pooling libraries according to Illumina's protocol, then samples were sequenced using Illumina's paired-end MiSeq V2 platform. This produced a set of Illumina-sequenced paired-end fastq files that were split by sample with Illumina barcodes removed.

3.2.3. Bioinformatics and Data Analysis

Bioinformatics was carried out using the Divisive Amplicon Denoising Algorithm 2 (DADA2) open-source software (Callahan *et al.*, 2016), which was produced to model and correct Illumina-sequenced amplicon errors without constructing operational taxonomic units (OTUs). Specifically, we followed the DADA2 ITS-specific pipeline for Illumina-sequenced

fungi samples. Primers were firstly identified and removed, with read quality profiles then inspected. All reads were next filtered and trimmed based on the standard filtering parameters. The core sample inference algorithm was applied to the data, before the forward and reverse paired reads were all merged. An amplicon sequence variant (ASV) table was produced, with this a higher-resolution version of the traditional OTU tables, with chimeras then removed. Finally, taxonomic assignment was carried out using the UNITE ITS database, specifically the General FASTA release files for fungi (Abarenkov *et al.*, 2024).

Prior to data analysis, the number of reads in each sample were normalised using median sequencing depth (Lundin *et al.*, 2012), with the total number of reads used for normalisation being 2,125,052 and the median number of reads across samples being 117,743. Fungi community composition was first investigated, with bar plots produced to show the relative abundance of different fungal taxon, specifically at the phylum and class levels, between the 18 different samples. Samples were then grouped by both site type and time period resulting in six groups (control A, control B, red A, red B, roe A and roe B), with the top five most abundant fungal families compared between each of these groups. To investigate how community composition varied between the different combinations of site types and time periods, NMDS were produced with Bray-Curtis dissimilarity as the distance measure. A PERMANOVA was run to investigate if any differences in community composition were statistically significant between the groups. Finally, fungal diversity was considered for site type alone, with first the species richness for each site type calculated based on the Chao1 index, which provides an estimate of microbial species richness and considers rare taxa (Chao, 1984). Two diversity metrics were also calculated, specifically Shannon-Wiener (more weight on species richness) and Simpson's diversity (more weight on species evenness) (Kim *et al.*, 2017). Kruskal–Wallis tests were employed to compare the differences in species diversity among the three site types for each of the three metrics.

All statistics were conducted using R Statistical Software v4.4.1 (R Core Team, 2024). The *phyloseq* package was used for producing bar plots of the relative abundance of different fungal taxa and for statistical analysis comparing species richness between the different site types (McMurdie and Holmes, 2013). The *vegan* package was used for producing NMDS plots and PERMANOVA analyses (Oksanen *et al.*, 2024).

3.3. Results

Across the 18 samples, a total of approximately 2.1 million fungal taxonomic reads were recorded, with a mean number of reads per sample of 118,058 and a range of 95,857 (sample 14B) to 150,261 (sample 3B). A total of 2,884 unique fungal taxa were identified, with these stemming from 12 phyla and 40 classes. The three phyla with the highest numbers of unique taxa were Ascomycota (1,448), Basidiomycota (869) and Mortierellomycota (118). The relative abundance of the different fungal phyla for each sample is shown in Figure 3.1, where these are also separated out by site type. Regarding the fungal classes, the three with the highest numbers of unique taxa were Agaricomycetes (615; phylum Basidiomycota), Leotiomycetes (474; phylum Ascomycota) and Sordariomycetes (466; phylum Ascomycota). The relative abundance of the different fungal classes for each sample, again separated by site type, is shown in Figure 3.2.

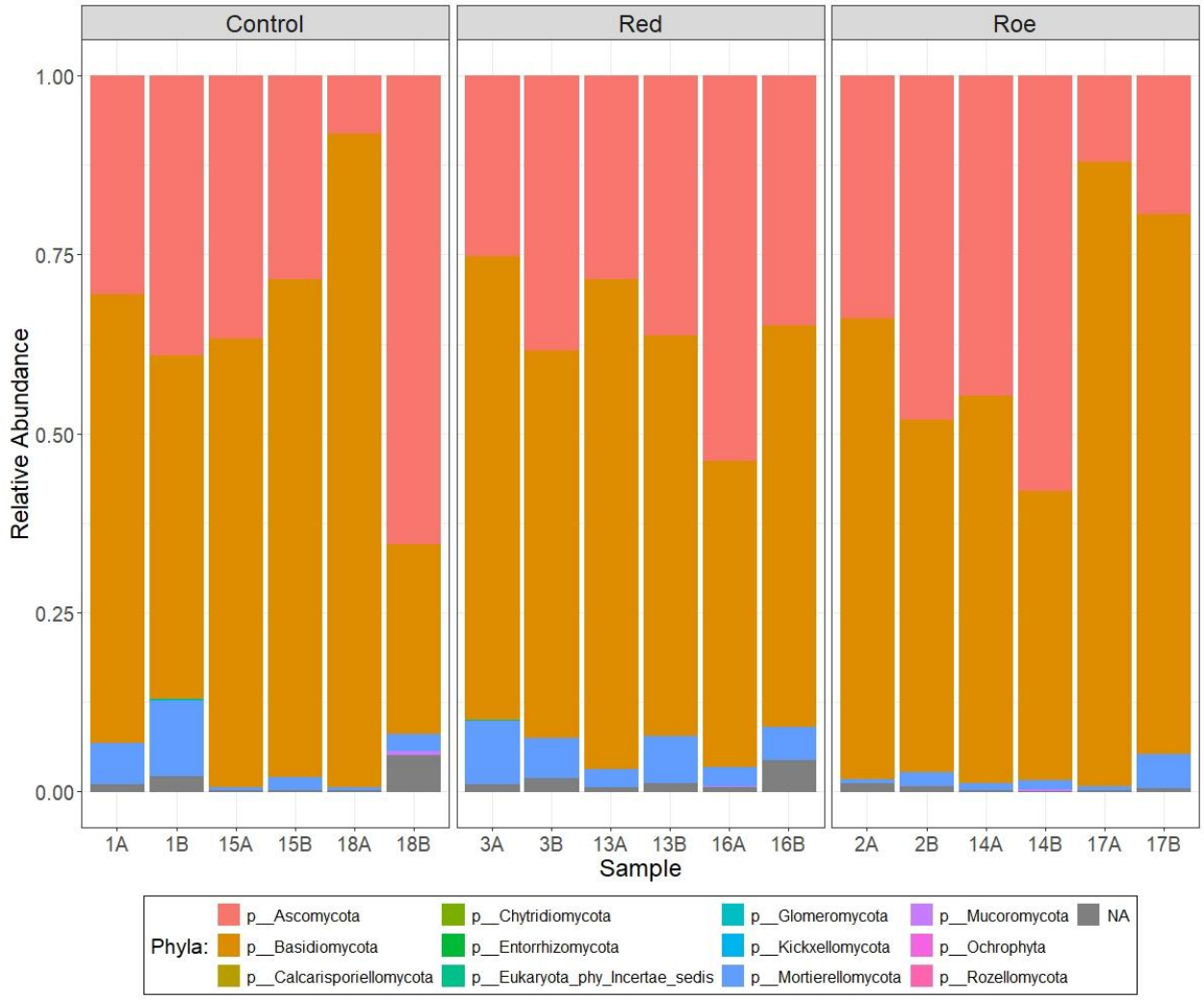

Figure 3.1: The relative abundance of the different fungal phyla for each of the soil samples collected. These are grouped based on the pairings of the two time periods that samples were taken for each site (e.g., 1A and 1B), with samples also separated by site type (i.e., control, red or roe).

Figure 3.2: The relative abundance of the different fungal classes for each of the soil samples. These are grouped based on the pairings of the two time periods that samples were taken for each site (e.g., 1A and 1B), with samples also separated by site type (i.e., control, red or roe).

The top five most abundant taxa, ranked by family, for each of the six groups of the different combinations of site types (i.e., control, red and roe) and time period (A and B) are shown in Figure 3.3. For the control site type, the most abundant family for the time period A was Thelephoraceae (class Agaricomycetes) and for the time period B was Trimorphomycetaceae (class Tremellomycetes). The most abundant family for the red A group was Russulaceae (class Agaricomycetes), whereas for the red B group it was Aspergillaceae (class Eurotiomycetes). Finally, for both the roe A and B groups, the most abundant family was Thelephoraceae.

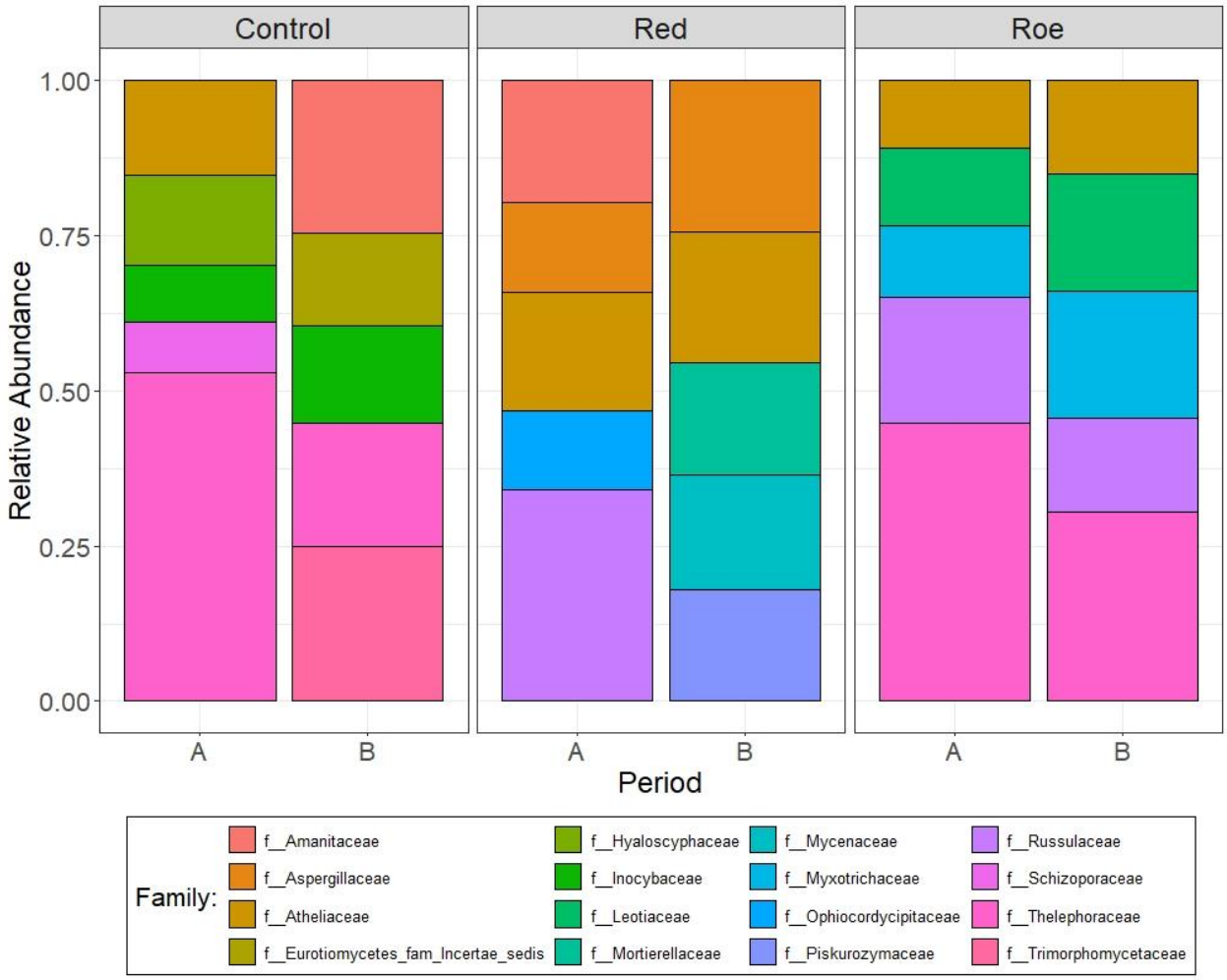

Figure 3.3: The top five most abundant fungal families for each of the six groups of site type and time period combinations, with these shown by their relative abundance.

Comparison of the fungal community composition between the six groups of site type and time period combinations was undertaken by NMDS analysis (Figure 3.4), with a PERMANOVA test revealing no significant differences in the fungal community composition between the six groups ($F_{5, 17} = 0.79$, $p = 0.971$).

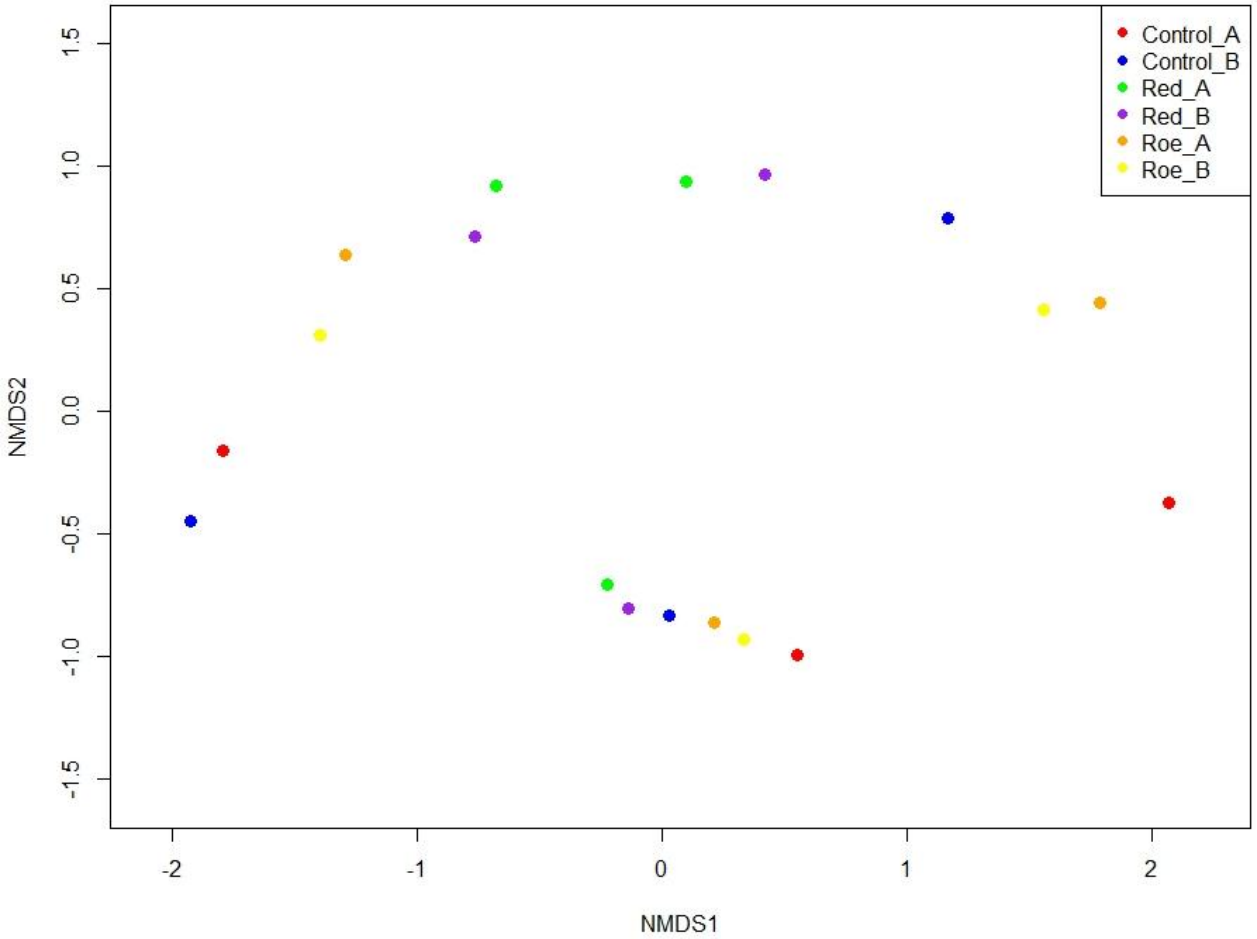

Figure 3.4: NMDS plot of fungi community composition between the 6 groups (n = 3 for each group) – control A, control B, red A, red B, roe A and roe B. Number of dimensions (k) = 4, and stress value = 0.054.

Finally, when comparing the different metrics of fungal species diversity between the three site types, there was no evidence of any significant differences found by Kruskal-Wallis tests: Chao1 index (H_2 = 3.66, p = 0.160; Figure 3.5); Shannon-Wiener index (H_2 = 3.89, p = 0.143; Figure 3.6); and Simpson's index (H_2 = 2.68, p = 0.262; Figure 3.7).

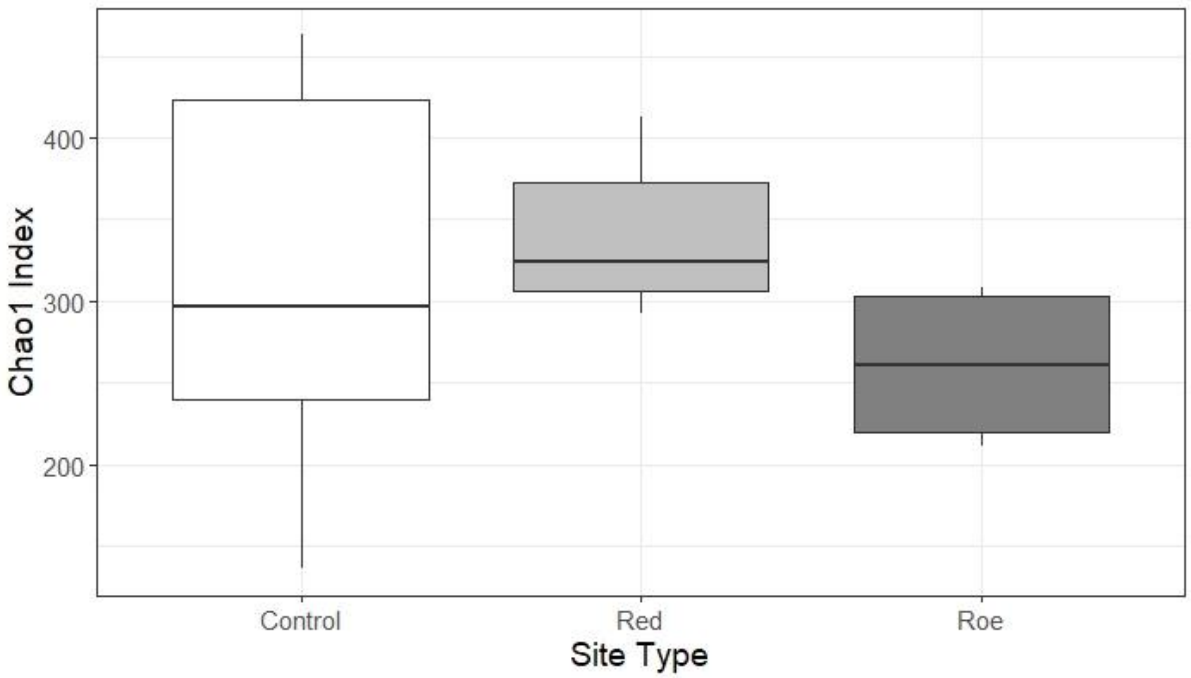

Figure 3.5: Variation of the fungal Chao1 index values per site type. The top of boxes represents the 75th percentile, the bottom the 25th percentile and the middle line the median, whilst whiskers correspond to the range.

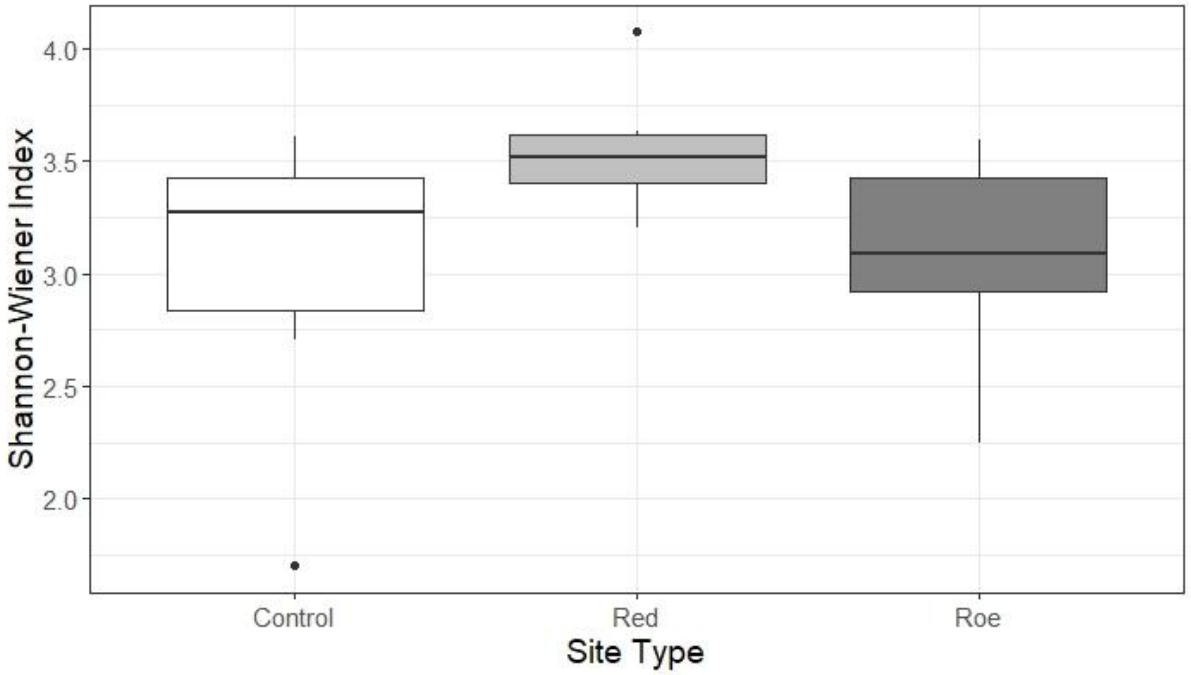

Figure 3.6: Variation of the fungal Shannon-Wiener diversity index values per site type. The top of boxes represents the 75th percentile, the bottom the 25th percentile and the middle line the median, whilst whiskers correspond to the range. Outliers are represented by points.

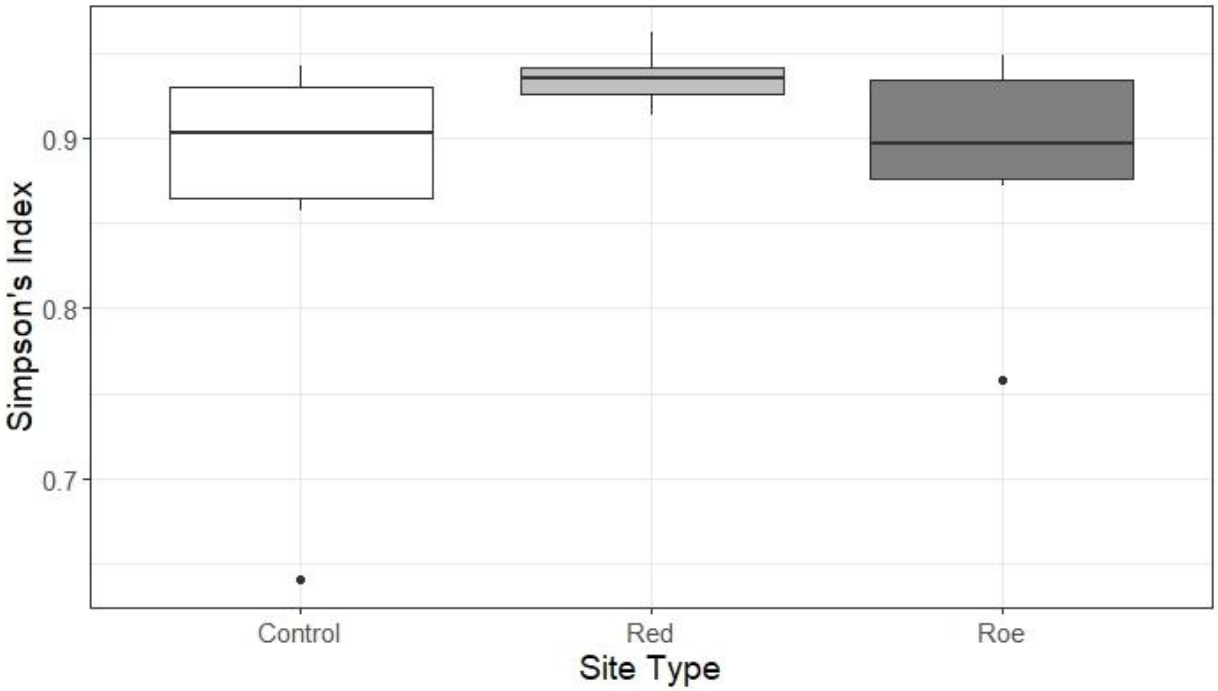

Figure 3.7: Variation of the fungal Simpson's diversity index values per site type. The top of boxes represents the 75th percentile, the bottom the 25th percentile and the middle line the median, whilst whiskers correspond to the range. Outliers are represented by points.

3.4. Discussion

3.4.1 Impact of carcasses on soil fungal diversity

As predicted by the hypothesis regarding the richness and diversity of soil fungi between site types, there was shown to be no significant differences between the red deer, roe deer and control site types. No comparison could be made between the two time periods (i.e., soil samples taken pre-carcass placement and samples taken six months post-carcass placement) for each of the site types, due to a lack of replicates for each with only three samples for each of the six group combinations (i.e., control A, control B, red A, etc.).

Other studies have attempted to understand more about the impacts of carcasses on the richness and diversity of soil fungi, including an examination of the successional patterns of soil fungi associated with decomposing juvenile pig carcasses in a grassland area in Hunan, China, with comparisons to fungi in control plots (Fu *et al.*, 2019). Carcass soils were shown to have significantly lower fungal Shannon-Wiener diversity than control soils, but Chao1 diversity levels were similar between the two soil types – these differences in diversity metrics were attributed to carcass decomposition not impacting soil fungal species richness but decreasing species evenness. Specifically, only several fungal taxa were abundant across carcass soils (including Chaetothyriales species of the class Eurotiomycetes, and both *Candida catenulate* and *Yarrowia lipolytica* of the class Saccharomycetes) compared to around sixty fungal taxa abundant across control soils. There appears to be only one study which has assessed the diversity and composition of soil fungal communities associated with carcasses of large wild mammals (Risch *et al.*, 2020) – hence, this is the most informative research to inform the present study. They investigated the diversity and composition of soil microbial communities of wolf-killed bison and elk (*Cervus canadensis*) carcasses in comparison to control sites, in grassland and scrub habitats of Yellowstone National Park, USA. Regarding fungal diversity, they showed that elk carcasses in particular reduced fungal species richness implying that elk carcasses may have altered soil conditions leading to fungi that thrive in nutrient-rich, or anaerobic conditions, outcompeting less-suited taxa. Moreover, it was revealed that the diversity and richness of soil fungi at carcasses, again specifically elk carcasses, varied across the wider landscape indicating the importance of considering ecosystem and soil types when assessing the soil microbial communities of carcasses, even when other studies have concluded that carcass soil fungal communities did not differ significantly between three broadly different ecosystems (desert, grassland and subalpine forests; Metcalf *et al.*, 2016). Therefore, even though the current study found no effect of carcasses on soil fungi, it could be interpreted from results of other studies that large carcasses often result in a decreased diversity of fungal soil taxa, but where this is not

necessarily related to a decrease in species richness between carcass and control soils but a decreased species evenness with carcass soils dominated by fewer fungi that thrive in the carcass-altered soil conditions.

3.4.2. The composition of soil fungal communities around carcasses

Concerning the composition of the soil fungi communities associated with the six groups in the present study (i.e., the different combinations of the three site types and two time periods), there was found to be no clear significant differences between these. This is in contrast to our hypothesis where we expected the composition of soil fungi communities to vary between site types and the two time periods. The results of other studies have noted clear differences in the composition of soil fungi between carcass and control sites, different carcass types, and between pre-carcass/early decomposition soils with later decomposition soils. Furthermore, the fungal taxa commonly associated with carcass decomposition, and how these change as decomposition progresses, have been discussed in numerous studies.

One such study aimed to investigate the succession of soil microbes, including fungi as part of a wider eukaryotic group, of pig carcasses and control sites across summer and winter in a North American grassland (Carter *et al.*, 2015). They found that the composition of eukaryotes within control sites did not vary between the summer and winter. Contrastingly, eukaryotic communities in carcass soils were found to significantly differ with control soils in summer, but no difference was detected in winter. Despite this, the impact of fungi alone on these differences in eukaryotic composition cannot be determined, with the phylum Nematoda likely largely responsible. Several fungi taxa did, however, have significantly greater abundances in carcass soils, specifically the classes Eurotiomycetes (phylum Ascomycota) and Tremellomycetes (phylum Basidiomycota) in summer, and Dothideomycetes in winter (phylum Ascomycota) (Tibbett and Carter, 2003; Sagara, Yamanaka and Tibbett, 2008).

In the study by Lauber *et al*. (2014), as discussed in the previous section, the most abundant fungal taxa associated with the untreated carcass soils was the subphylum Mucoromycotina, with this mostly absent from the sterilised carcass soils. Fungi within this taxa are known to inhabit nutrient-rich environments associated with decomposing plant material (Voříšková and Baldrian, 2013), with their observation indicating this group is potentially associated with decomposing animal carcasses as well. In contrast, as also mentioned previously, the most abundant fungal taxa associated with the pig carcasses in the study by Fu *et al*. (2019) were the order Chaetothyriales, but predominantly two species within the class Saccharomycetes

(*C. catenulate* and *Y. lipolytica*) which increased in abundance in carcass soils considerably throughout decomposition – they were present in control soils, but their abundance did not change over time. Both of these are species of yeasts, with *Y. lipolytica* generally found in protein- or lipid-rich environments (Nicaud, 2012), as well as having a recorded synergistic relationship with *C. catenulate* (van de Voorde and van Dijck, 1982; Gkatzionis *et al.*, 2014); hence, it is probable that these species increased in abundance due to the influx of nutrient-rich carcass fluids.

Generally, however, the results of the fungal taxa associated with the soils of decomposing carcasses clearly differ greatly across different studies, with numerous other factors influencing soil fungi composition, including the specific soil type and condition, the carcass species and size, plus other environmental factors (Ramette and Tiedje, 2007).It has been shown that carcass decomposition affects the abundances of fungi taxa of the phyla Ascomycota and Basidiomycota (Mason, Taylor and DeBruyn, 2023); However, one study observed increased abundances of both phyla throughout decomposition (Forger *et al.*, 2019), yet another observed increases in Ascomycota (and Zygomycota) but decreases in Basidiomycota (Metcalf *et al.*, 2016). Concerning the most abundant fungal taxa associated with both the carcass soils and the control soils in the current study, a variety of fungal taxa were recorded. The most abundant fungal family at the red deer carcass sites from the later time period samples was Aspergillaceae (Ascomycota, Eurotiomycetes) with the class Eurotiomycetes shown to be abundant in carcass soils in other studies (Lauber *et al.*, 2014; Carter *et al.*, 2015; Fu *et al.*, 2019). The family Mortierellaceae of the subphylum Mucoromycotina was also one of the top five most abundant in the late time period red deer carcass soils, similar to the results of Lauber *et al*. (2014). For the roe deer carcass soils, the same five fungal families were most abundant in the pre-carcass and later time period samples, with the family Thelephoraceae (Basidiomycota, Agaricomycetes) the most abundant in both.

Considering again the study by Risch *et al*. (2020), it was observed that the soil fungal communities of both the carcass types were significantly different compared to the control soil fungal communities. Furthermore, the fungi communities between the bison and elk carcasses were deemed considerably different, where this variation between carcass types could potentially be attributed to differences in carcass mass (Parmenter and MacMahon, 2009; Turner *et al*., 2017), tissue composition (Meyer *et al.*, 1998) or even dietary preferences, with bison grazers and elk generally browsers. In terms of the specific fungal taxa present at carcasses in their study, they identified only three indicator taxa at bison carcasses but 53 associated with elk carcasses (with indicator taxa defined as OTUs with over 50 sequences, thus ignoring rare taxa). Of the fungal indicator taxa at elk carcasses,

these were mostly from the phylum Ascomycota, particularly from the four classes Dothideomycetes, Eurotiomyceetes, Leotiomycetes and Sordariomycetes. Although they found the majority of these indicator fungi to be negatively associated with carcasses, regardless of many of these taxa being saprotrophic (Goodwin, 2014; Zhang and Wang, 2015). It was implied that many of these taxa are found ubiquitously across the landscape, hence why they were recorded across both control and carcass soils.

Examining how the results of Risch *et al*. (2020), in particular, inform the results of the current study, even though both red and roe deer belong to the same taxonomic family (Cervidae), they vary significantly in body mass and size, as well as their broad dietary preferences. Therefore, it might be expected that soil fungi composition would differ between these two carcass types, and even more so with the soil fungi communities at control sites. A major limitation in the present study was the low number of sites sampled for their soil fungi communities, with only six carcasses and three control sites sampled (two soil samples taken at each for the two time periods). This lack of replicates for each group type means that the findings here cannot be deemed truly representative, and future studies with soil samples taken from a greater number of carcasses of the different species, plus more samples taken across the time span of decomposition, would aid in producing a better understanding of the impacts of carcasses of these two deer species on the composition and diversity of soil fungi across woodland habitats.

Another clear limitation of this study was the lack of consideration of the broad habitat type that carcass and control sites were in, and the subsequent impact these would have had on the diversity and composition of soil fungi. Soil samples were taken across all three habitat types defined in this study (broadleaf, coniferous and felled woodland), with one cluster of each of the three site types from each habitat sampled. However, no consideration was made of the effects these habitats would have on soil fungal diversity, with the general native soil fungal communities found in these different habitats likely varying significantly (Ramette and Tiedje, 2007; Shi *et al.*, 2014). In future research, in order to ensure increased comparability between the different soil fungal communities of different carcasses and control sites, either the habitat type should be kept consistent across all carcass and control sites, or more replicates of carcass and control sites within different habitats should be employed.

3.5. Conclusion

Overall, a lack of replicates meant limited conclusions could be drawn regarding the impacts of carcasses on soil fungal diversity and composition, including both any differences between the two carcass types and control sites and the effect of sampling time (i.e., pre-carcass or post-carcass decomposition). The influence of habitat type on soil fungi could also not be determined, again due to insufficient replicates. Future work should ensure that soil samples from a greater number of replicates of both carcass and control sites should be taken, so that more informed conclusions can be obtained for a more complete picture of the impacts of carcasses on soil fungi in similar ecosystems.

Chapter 4: Discussion – Implications of, and Advice for, Carcass Provisioning for the Biodiversity of Ecosystems in the UK

4.1. Introduction

The current study provides some preliminary findings of the implications of the provisioning of large carcasses on the biodiversity of a temperate woodland in the UK. This included observed impacts on the activity and composition of mammalian and avian species, and the diversity and composition of invertebrates, plus some indicative findings for the consequences of carcasses on both plant and soil fungi composition and diversity which could be developed with further research.

In this final chapter, a more holistic view of the present study's conclusions is discussed, with the observed findings of the separate groups considered more wholly. Further considerations of the impacts of carcass provisioning from other research are made, as well as recommendations for effective provisioning to promote biodiversity. Specifically, the implications of, and advice for, carcass provisioning are reviewed whilst referring to how these might apply to ecosystems of the UK, particularly concerning the consequences of a complete absence of any apex predator and/or scavengers. Finally, the limitations of the present study are considered, along with advice and motivation for future research investigating the consequences of large carcasses on the wider biodiversity of temperate woodland ecosystems in the UK.

4.2. The Overall Impacts of Large Carcasses on a Temperate Woodland and Ecosystem Services Provided

The present study appears to be the sole piece of research that has examined the impacts of large carcasses on the diversity and composition of four broad groups, ranging from vertebrates all the way down to a component of the soil microbial community, as well as invertebrates and local plant assemblages. Other research has focussed explicitly on the effect of carcasses on one of these groups, and sometimes two (e.g., van Klink *et al.*, 2020); however, the incorporation of multiple different levels of an ecosystem's community into one study allows for a greater overall understanding of the consequences of large carcasses. Specifically, this was achieved by examination of the carcass impacts on each study group across the same study carcass and control sites, where this mimics the natural conditions carcasses would be under without experimentally excluding another group, such as the exclusion of vertebrates in some studies examining carcass effects on invertebrates (e.g., Benbow *et al.*, 2013; Sawyer and Bloch, 2020; Schwegmann *et al.*, 2022).

One of the key findings from this study, along with conclusions drawn from other research, was the indication that larger carcasses (here, of red deer) promote higher levels of activity of vertebrates and increased diversity of invertebrates compared to smaller carcasses (roe deer). This is primarily due to larger carcasses generally persisting for a longer period of time, and thus typically supporting an increased diversity of both vertebrates and invertebrates by allowing less-specialised species to utilise carcasses as a resource (Baruzzi *et al.*, 2018). However, when linking the diversity of vertebrates, invertebrates, and even microbial decomposers, to the efficiency of carcass removal and decomposition as a critical ecosystem service, there is conflicting evidence across the literature.

Several studies have observed increasing diversity of scavenger and decomposer species – whether they be of vertebrates, invertebrates or microbes – to be associated with increased rates of carcass decomposition and removal (Moreno-Opo and Margalida, 2013; Moleón *et al.*, 2014; Abernethy *et al.*, 2016; Baruzzi *et al.*, 2018). Some potential mechanisms underlying this may be niche complementarity, where different species will utilise different parts of carcasses as a resource, such as Dermestid beetles feeding on carcass skin and Calliphoridae flies feeding on flesh (Braack, 1987); thus, increased diversity of species exploiting different parts of a carcass will promote more efficient consumption. Another mechanism could be the facilitation of carcass consumption by one group for another, such as larger vertebrates opening carcasses to allow access for smaller vertebrates (Sebastián-González *et al.*, 2021), invertebrates and microbes (Meehan, Seminet-Reneau and Quinn, 2005).

Contrastingly, many other studies have noted that larger carcasses increasing the diversity of vertebrates, invertebrates and/or microbes actually had the opposite effect of leading to slower consumption and decomposition rates (Selva and Fortuna, 2007; Sutherland *et al.*, 2013; Barton and Evans, 2017; Wenting, Rinzema and van Langevelde, 2022). It has been implied that carcass consumption/decomposition by fewer more efficient, and more dominant, scavengers promotes faster carcass removal, for both invertebrates (Barton and Evans, 2017) and vertebrates (Wenting, Rinzema and van Langevelde, 2022). Moreover, it has also been suggested that the absence of dominant vertebrate scavengers and instead the dominance of less-efficient scavengers, due to density or hostile behaviour for example, could slow the rate of decomposition (Gessner *et al.*, 2010). Therefore, this underlies the importance of a diverse and complete scavenger guild, including the presence of apex scavengers and/or predators, for effective carcass removal as a critical ecosystem service.

4.3. Carcass Provisioning in the Absence of Apex Predators

In the remote uplands of Scotland, the provisioning of large carcasses has been promoted in order to attract enigmatic, but threatened, vertebrate scavengers, particularly golden and white-tailed eagles, to try and enhance their populations (The Scottish Government, 2011). In the Uk, where there is a complete lack of any apex scavengers and/or predators, it is necessary to provide large carcasses to aid in the conservation of these vulnerable species where there is otherwise a lack of naturally produced carcasses. However, there needs to be further motivation for many landowners, such as the generation of local economic benefits from wildlife tourism (Fielding *et al.*, 2014), where difficulty and costs associated with carcass removal often deter culling.

Nevertheless, the results of the present study provide motivation for further research and consideration into the provisioning of large carcasses in ecosystems of the UK, with benefits of carcass provisioning revealed for the wider biodiversity of ecosystems. However, there are several key considerations for similar studies and conservation schemes in the UK where multiple large carcasses will be placed within an ecosystem, particularly concerning the absence of apex predators and subsequent management approaches. Primarily, the spatiotemporal distribution of provisioned carcasses across the landscape being akin to a natural distribution, as would be produced through predation, is critical to consider (Moreno-Opo and Margalida, 2013). The provisioning of carcasses by humans without consideration for this more random distribution would not generate the unpredictable distribution that would otherwise be created by wild predators (Wilmers and Post, 2006; Ferraro and Hirst, 2024). Studies examining the impacts of carcasses as feeding stations, generally for vulnerable avian scavengers (e.g., Cortés-Avizanda *et al.*, 2012), have found that predictability of these resources leads to dominant species outcompeting those more subordinate, hence these carcasses becoming “ecological traps” (Gilroy and Sutherland, 2007).The diversity of vertebrate scavengers was also found to be higher at wolf-killed carcasses compared to those produced by hunters, with this suggested to be due to the more aggregated hunter-produced carcasses being located predictably and thus controlled by dominant scavengers (Wilmers *et al.*, 2003). Therefore, this further highlights the need for randomness in both time and space when provisioning a resource that would be randomly distributed under natural scenarios. Furthermore, it has been suggested that multiple carcasses at different stages of decomposition, and of different species, would support a greater diversity of species than carcasses of the same species at the same stage of decomposition (Barton, Cunningham, Lindenmayer, *et al.*, 2013).

The importance of apex predators in producing carcasses, particularly of larger ungulates, cannot be undermined with their tendency to generate carcasses dispersed with unpredictable distributions. Furthermore, carcasses produced by predators themselves have been shown to be generally preferred by many vertebrates compared to provisioned carcasses or non-predated natural carcasses (Selva *et al.*, 2005). One study investigating the impacts of carcasses on vertebrate and invertebrate scavengers even simulated natural lynx-killed carcasses by inflicting wounds on road-kill carcasses and positioning them similarly to how lynx would, in order to specifically explore the impacts of “predator-killed” carcasses (Ray, Seibold and Heurich, 2014).

Therefore, in ecosystems where there is a decline or even complete absence of apex predators, such as in the present study landscape, there are multiple negative consequences for the recycling of carcasses. Firstly, with a lack of apex predators the temporal distribution of naturally occurring large carcasses will likely be more pulsed, with the production of carcasses dictated by a combination of disease, old age and resource limitation (Wilmers and Getz, 2004). Thus, due to these factors it is likely that populations of large ungulates, for example, in the absence of any predators will likely suffer higher incidences of natural mortality during winter. The spatial distribution of large carcasses will also be affected with a lack of regulation by predators, with carcasses likely to be more condensed in space (Wilmers *et al.*, 2003).

An absence of apex predators and scavengers as the dominant vertebrate consumers of carcasses would likely lead to a shift to mesoscavenger dominance at carcasses (O’Bryan *et al.*, 2018; Cunningham *et al.*, 2019), such as the dominance of foxes and domestic dogs observed in this study. This could cause mesoscavenger populations to increase, potentially leading to other indirect consequences for ecosystems, similar to those described after increases in populations of mesopredators (Prugh *et al.*, 2009). These species generally consume less carcass mass than apex predators due to their lower energetic requirements (Mateo-Tomás et al., 2017), resulting in the increased persistence of carcasses (Cunningham *et al.*, 2019). Consequently, there are heightened disease risks associated with long-lasting carcasses, whereby disease-causing bacteria have a higher probability of establishing on these carcasses and thus, the risks of disease spreading to wild animals, or even livestock and humans, are raised (Markandya *et al.*, 2008; Buechley and Şekercioğlu, 2016). Accordingly, it has been suggested that in order to establish effective carcass cycling, there needs to be focussed conservation efforts to protect or restore complete and fully functioning native scavenger guilds, particularly reintroductions of absent species such as the missing apex predators in the UK (Bartel *et al.*, 2024).

4.5. Study Limitations and Advice for Future Research

In order to build upon the findings of the present study, there are a number of limitations which are addressed here to better inform future research. As well as further recommendations for similar studies going forward to build on the current understanding of carcass impacts on woodland ecosystems in the UK.

One significant limitation concerned the distribution of sites across the study area, specifically the distance between sites and clusters, but also the area of the habitat blocks that sites were within. The distance between sites of the same cluster of 50 m was not adequate to achieve site independence, with a minimum distance of 200 m between carcasses suggested for site independence for flying invertebrates (Müller and Brandl, 2009), and a minimum distance of 1 km between carcasses for vertebrates (Turner *et al.*, 2017). This also implies that the minimum distance between clusters of 500 m was likely not sufficient to ensure these were independent of each other when considering vertebrates. However, the average size of different habitat blocks of the study area restricted the maximum distance between sites without these being located in considerably different broad habitat types, plus the total area of the study site limited the maximum distance between clusters. It has also been suggested that a minimum habitat block of 10 hectares in area is necessary to lower any bias towards vertebrate edge specialists (Turner *et al.*, 2017), again indicating that the habitat blocks in the present study were generally too small. Therefore, future studies should ensure that distinct habitat blocks within a study area are large enough so that sites can be separated by appropriate distances and are far enough away from habitat edges.

Another constraint of the study was the impact of domestic dogs on the removal of carcasses and their dominance at several carcasses. The actions of domestic dogs, specifically what appeared to be a group of three dogs, led to the rapid removal and consumption of several carcasses early on in the study. A lack of any cord to secure carcasses in place was a limitation here, as it meant these carcasses were generally dragged out of view from camera traps so observations of the activity of vertebrates was restricted. The consequences of carcass provisioning on domestic dogs cannot be fully discerned from this study, as it could be more of a localised issue due to the nature of the study area having several tracks and footpaths, and residences within the wider woodland – thus, the presence and impacts of dogs was high. In future study areas in the UK, it is critical to consider the potential impacts of carcasses on domestic dogs, particularly the proximity of sites to human activity such as footpaths and determining how well used these are.

In addition, the identification of invertebrates was somewhat inadequate due to the absence of specialist assistance, with identification undertaken by the author who had no specific training and used basic invertebrate guides. Particularly, many specimens of ubiquitous and large invertebrate groups, such as the orders Coleoptera and Diptera, were not identified to very low taxonomic levels, with most only identified to family level and others only to order. However, when concerning diversity and richness metrics, the concept of morphospecies was sufficient with this referring to, for example, two fly species that cannot be explicitly classified but are likely separate species based on morphology and are thus counted as two distinct taxa. Nevertheless, the support of specialist invertebrate identification for future projects investigating invertebrate assemblages associated with carcasses cannot be undermined.

Finally, there are several additional considerations for future research investigating the impacts of large carcass on the biodiversity of ecosystems in the UK. Firstly, other studies should aim to undertake research in other regions of the UK, specifically, where there are differences in the assemblages of mammalian and avian species, particularly those deemed facultative scavengers, including, but not limited to, golden and white-tailed eagles, wild boar (*Sus scrofa*), red kite (*Milvus milvus*), pine marten (*Martes martes*), ravens, etc. Other broad habitats could also be considered, from remote uplands, a variety of woodlands and grasslands, to examine if there is variation in the impacts of carcass provisioning in these different habitats. Moreover, the timing of carcass provisioning should be investigated, with carcasses placed out at different times during the year to assess what impacts this has on the diversity of the four broad groups studied. For example, carcasses placed out in summer will likely be dominated by invertebrates and microbes, hence the consequences of this for vertebrates could be studied.

4.6. Conclusion

In summary, the present study revealed that the provisioning of large carcasses of two native deer species impacted the biodiversity of a woodland ecosystem. Specifically, the activity of mammalian and avian species was shown to be higher at carcass sites compared to controls, and vertebrate composition varied significantly between the site types. The species richness and diversity of invertebrates differed significantly between carcasses and controls, with the highest levels of richness and diversity typically recorded at the larger red carcasses. Influences of habitat type, seasonality and distance from carcasses were also shown for invertebrates. However, no substantial evidence was found for any effect of carcasses on the diversity and composition of plants or soil fungi, potentially due to a shortened study timespan and lack of carcass and control replicates, respectively.

Regarding the implications for the management of deer populations in the UK and subsequent carcass provisioning, there are several key considerations. Due to the complete absence of apex predators in the UK, populations of deer are dictated by non-predatory natural processes and human management, thus these both impact the distribution of large carcasses. Therefore, when considering carcass provisioning it is critical to attempt to replicate the unpredictable distribution of carcasses that would be produced by predation, both in space and time. The lack of apex predators will also potentially lead to mesoscavenger dominance at carcasses, where the consequences of this needed to be deliberated. Moreover, the density of large carcasses in an area needs to be considered, where high densities of carcasses may lead to incomplete consumption and carcass persistence, and subsequently these may become reservoirs of disease. Finally, the location where carcasses are planned to be placed, specifically concerning their proximity to human activity, is essential to consider, with increased likelihood of exposure to humans and domestic dogs where carcasses are closer to areas of higher human activity.

It is hoped that this study will provide motivation for the synthesis of similar studies investigating the consequences of the provisioning of large carcasses on the biodiversity of ecosystems in the UK, with the identified limitations and recommendations discussed used to inform further research. Foremost, the sites of the present study could be investigated further to reveal any long-term carcass effects on the diversity and composition of both plants and soil fungi. Other studies should aim to examine the consequences of large carcasses in different regions and ecosystems of the UK, with a focus on investigating different densities and spatiotemporal distributions of carcasses, particularly replicating the unpredictable distributions of carcasses derived by natural predation.

References

Abarenkov, K. *et al.* (2024) 'UNITE general FASTA release for Fungi'. UNITE Community. Available at: https://doi.org/10.15156/BIO/2959332.

Abernethy, E.F. *et al.* (2016) 'Carcasses of invasive species are predominantly utilized by invasive scavengers in an island ecosystem', *Ecosphere*, 7(10), p. e01496. Available at: https://doi.org/10.1002/ecs2.1496.

Adserias-Garriga, J. *et al.* (2017) 'Daily thanatomicrobiome changes in soil as an approach of postmortem interval estimation: An ecological perspective', *Forensic Science International*, 278, pp. 388–395. Available at: https://doi.org/10.1016/j.forsciint.2017.07.017.

Arnold, T.W. (2010) 'Uninformative Parameters and Model Selection Using Akaike's Information Criterion', *The Journal of Wildlife Management*, 74(6), pp. 1175–1178. Available at: https://doi.org/10.1111/j.1937-2817.2010.tb01236.x.

Asprea, A. and Marinis, A.M.D. (2005) 'The diet of the badger *Meles meles* (Mustelidae, Carnivora) on the Apennines (Central Italy)', *Mammalia*, 69(1), pp. 89–95. Available at: https://doi.org/10.1515/mamm.2005.009.

Barry, J.M. *et al.* (2019) 'Pumas as ecosystem engineers: ungulate carcasses support beetle assemblages in the Greater Yellowstone Ecosystem', *Oecologia*, 189(3), pp. 577–586. Available at: https://doi.org/10.1007/s00442-018-4315-z.

Bartel, S.L. *et al.* (2024) 'Global change influences scavenging and carrion decomposition', *Trends in Ecology & Evolution*, 39(2), pp. 152–164. Available at: https://doi.org/10.1016/j.tree.2023.09.008.

Barton, P.S., Cunningham, S.A., Macdonald, B.C.T., *et al.* (2013) 'Species Traits Predict Assemblage Dynamics at Ephemeral Resource Patches Created by Carrion', *PLOS ONE*, 8(1), p. e53961. Available at: https://doi.org/10.1371/journal.pone.0053961.

Barton, P.S., Cunningham, S.A., Lindenmayer, D.B., *et al.* (2013) 'The role of carrion in maintaining biodiversity and ecological processes in terrestrial ecosystems', *Oecologia*, 171(4), pp. 761–772. Available at: https://doi.org/10.1007/s00442-012-2460-3.

Barton, P.S. *et al.* (2016) 'Substantial long-term effects of carcass addition on soil and plants in a grassy eucalypt woodland', *Ecosphere*, 7(10), p. e01537. Available at: https://doi.org/10.1002/ecs2.1537.

Barton, P.S. and Evans, M.J. (2017) 'Insect biodiversity meets ecosystem function: differential effects of habitat and insects on carrion decomposition', *Ecological Entomology*, 42(3), pp. 364–374. Available at: https://doi.org/10.1111/een.12395.

Baruzzi, C. *et al.* (2018) 'Effects of increasing carrion biomass on food webs', *Food Webs*, 17, p. e00096. Available at: https://doi.org/10.1016/j.fooweb.2018.e00096.

Bates, D. *et al.* (2015) 'Fitting Linear Mixed-Effects Models using lme4', *Journal of Statistical Software*, 67(1), pp. 1–48. Available at: https://doi.org/10.18637/jss.v067.i01.

Baumgartner, D.L. and Roubik, D.W. (1989) 'Ecology of Necrophilous and Filth-Gathering Stingless Bees (Apidae: Meliponinae) of Peru', *Journal of the Kansas Entomological Society*, 62(1), pp. 11–22.

Beasley, J.C., Olson, Z.H. and Devault, T.L. (2012) 'Carrion cycling in food webs: comparisons among terrestrial and marine ecosystems', *Oikos*, 121(7), pp. 1021–1026. Available at: https://doi.org/10.1111/j.1600-0706.2012.20353.x.

Benbow, M.E. *et al.* (2013) 'Seasonal Necrophagous Insect Community Assembly During Vertebrate Carrion Decomposition', *Journal of Medical Entomology*, 50(2), pp. 440–450. Available at: https://doi.org/10.1603/ME12194.

Benbow, M.E. *et al.* (2019) 'Necrobiome framework for bridging decomposition ecology of autotrophically and heterotrophically derived organic matter', *Ecological Monographs*, 89(1), p. e01331. Available at: https://doi.org/10.1002/ecm.1331.

Benecke, M. (2001) 'A brief history of forensic entomology', *Forensic Science International*, 120(1), pp. 2–14. Available at: https://doi.org/10.1016/S0379-0738(01)00409-1.

Braack, L.E.O. (1987) 'Community dynamics of carrion-attendant arthropods in tropical African woodland', *Oecologia*, 72(3), pp. 402–409. Available at: https://doi.org/10.1007/BF00377571.

Breton, H. *et al.* (2016) 'The impact of carrion decomposition on the fatty acid methyl ester (FAME) profiles of soil microbial communities in southern Canada', *Canadian Society of Forensic Science Journal*, 49(1), pp. 1–18. Available at: https://doi.org/10.1080/00085030.2015.1108036.

British Bryological Society (2010) *Mosses and Liverworts of Britain and Ireland – A Field Guide*. Edited by I. Atherton, S. Bosanquet, and M. Lawley. Latimer Trend & Co.

Buechley, E.R. and Şekercioğlu, Ç.H. (2016) 'The avian scavenger crisis: Looming extinctions, trophic cascades, and loss of critical ecosystem functions', *Biological Conservation*, 198, pp. 220–228. Available at: https://doi.org/10.1016/j.biocon.2016.04.001.

Bump, J.K. *et al.* (2009) 'Ungulate Carcasses Perforate Ecological Filters and Create Biogeochemical Hotspots in Forest Herbaceous Layers Allowing Trees a Competitive Advantage', *Ecosystems*, 12(6), pp. 996–1007. Available at: https://doi.org/10.1007/s10021-009-9274-0.

Bump, J.K., Peterson, R.O. and Vucetich, J.A. (2009) 'Wolves modulate soil nutrient heterogeneity and foliar nitrogen by configuring the distribution of ungulate carcasses', *Ecology*, 90(11), pp. 3159–3167. Available at: https://doi.org/10.1890/09-0292.1.

Burcham, Z.M. *et al.* (2019) 'Total RNA Analysis of Bacterial Community Structural and Functional Shifts Throughout Vertebrate Decomposition', *Journal of Forensic Sciences*, 64(6), pp. 1707–1719. Available at: https://doi.org/10.1111/1556-4029.14083.

Burnham, K.P. and Anderson, D.R. (2004) *Model Selection and Multimodel Inference: A Practical Information-Theoretic Approach*. 2nd edn. New York, NY: Springer. Available at: https://doi.org/10.1007/b97636.

Byrd, J.H. and Tomberlin, J.K. (eds) (2010) *Forensic Entomology: The Utility of Arthropods in Legal Investigations*. 3rd edn. Boca Raton, FL, USA: CRC Press.

Callahan, B.J. *et al.* (2016) 'DADA2: High-resolution sample inference from Illumina amplicon data', *Nature Methods*, 13(7), pp. 581–583. Available at: https://doi.org/10.1038/nmeth.3869.

Campobasso, C.P., Di Vella, G. and Introna, F. (2001) 'Factors affecting decomposition and Diptera colonization', *Forensic Science International*, 120(1), pp. 18–27. Available at: https://doi.org/10.1016/S0379-0738(01)00411-X.

Carter, D.O. *et al.* (2015) 'Seasonal variation of postmortem microbial communities', *Forensic Science, Medicine, and Pathology*, 11(2), pp. 202–207. Available at: https://doi.org/10.1007/s12024-015-9667-7.

Carter, D.O., Yellowlees, D. and Tibbett, M. (2007) 'Cadaver decomposition in terrestrial ecosystems', *Naturwissenschaften*, 94(1), pp. 12–24. Available at: https://doi.org/10.1007/s00114-006-0159-1.

Chao, A. (1984) ‘Nonparametric Estimation of the Number of Classes in a Population’, *Scandinavian Journal of Statistics*, 11(4), pp. 265–270.

Chimutsa, M. *et al.* (2015) ‘Soil fungal community shift evaluation as a potential cadaver decomposition indicator’, *Forensic Science International*, 257, pp. 155–159. Available at: https://doi.org/10.1016/j.forsciint.2015.08.005.

Chinery, M. (1993) *Insects of Britain and Northern Europe (Collins Field Guide)*. 3rd edn. London, UK: HarperCollins.

Chinery, M. (2009) *Collins Complete Guide to British Insects*. London, UK: HarperCollins.

Cobaugh, K.L., Schaeffer, S.M. and DeBruyn, J.M. (2015) ‘Functional and Structural Succession of Soil Microbial Communities below Decomposing Human Cadavers’, *PLOS ONE*, 10(6), p. e0130201. Available at: https://doi.org/10.1371/journal.pone.0130201.

Colijn, E.O. (2014) ‘Kevers op kadavers in Nederland, de stand van zaken’, *Entomologische Berichten*, 74(1–2), pp. 60–67.

Cortés-Avizanda, A. *et al.* (2012) ‘Resource unpredictability promotes species diversity and coexistence in an avian scavenger guild: a field experiment’, *Ecology*, 93(12), pp. 2570–2579. Available at: https://doi.org/10.1890/12-0221.1.

Cortés-Avizanda, A., Carrete, M. and Donázar, J.A. (2010) ‘Managing supplementary feeding for avian scavengers: Guidelines for optimal design using ecological criteria’, *Biological Conservation*, 143(7), pp. 1707–1715. Available at: https://doi.org/10.1016/j.biocon.2010.04.016.

Côté, S.D. *et al.* (2004) ‘Ecological Impacts of Deer Overabundance’, *Annual Review of Ecology, Evolution, and Systematics*, 35(Volume 35, 2004), pp. 113–147. Available at: https://doi.org/10.1146/annurev.ecolsys.35.021103.105725.

Council of the European Union (2002) *Regulation (EC) No 1774/2002 of the European Parliament and of the Council of 3 October 2002 laying down health rules concerning animal by-products not intended for human consumption*. 1774/2002.

Cunningham, C.X. *et al.* (2019) ‘Top carnivore decline has cascading effects on scavengers and carrion persistence’, *Proceedings of the Royal Society B: Biological Sciences*, 285(1892), p. 20181582. Available at: https://doi.org/10.1098/rspb.2018.1582.

Danell, K., Berteaux, D. and Bråthen, K.A. (2002) ‘Effect of Muskox Carcasses on Nitrogen Concentration in Tundra Vegetation’, *Arctic*, 55(4), pp. 389–392.

De Deyn, G.B. and van der Putten, W.H. (2005) ‘Linking aboveground and belowground diversity’, *Trends in Ecology & Evolution*, 20(11), pp. 625–633. Available at: https://doi.org/10.1016/j.tree.2005.08.009.

Deer Working Group (2019) *The management of wild deer in Scotland: Deer Working Group report*. Environment and Forestry Directorate. Available at: https://www.gov.scot/publications/management-wild-deer-scotland/.

DeVault, T.L., Brisbin, Jr., I.L. and Rhodes, Jr., O.E. (2004) ‘Factors influencing the acquisition of rodent carrion by vertebrate scavengers and decomposers’, *Canadian Journal of Zoology*, 82(3), pp. 502–509. Available at: https://doi.org/10.1139/z04-022.

DeVault, T.L., Rhodes, Jr., O.E. and Shivik, J.A. (2003) ‘Scavenging by vertebrates: behavioral, ecological, and evolutionary perspectives on an important energy transfer pathway in terrestrial ecosystems’, *Oikos*, 102(2), pp. 225–234. Available at: https://doi.org/10.1034/j.1600-0706.2003.12378.x.

Downes, J.A. (1973) ‘Lepidoptera feeding at puddle-margins, dung, and carrion’, *Journal of the Lepidopterists‘ Society*, 27, pp. 89–99.

Dunn, O.J. (1964) ‘Multiple Comparisons Using Rank Sums’, *Technometrics*, 6(3), pp. 241–252. Available at: https://doi.org/10.1080/00401706.1964.10490181.

Elbroch, L.M. *et al.* (2017) ‘Vertebrate diversity benefiting from carrion provided by pumas and other subordinate, apex felids’, *Biological Conservation*, 215, pp. 123–131. Available at: https://doi.org/10.1016/j.biocon.2017.08.026.

Estes, J.A. *et al.* (2011) ‘Trophic Downgrading of Planet Earth’, *Science*, 333(6040), pp. 301–306. Available at: https://doi.org/10.1126/science.1205106.

Ferraro, K.M. and Hirst, C. (2024) ‘Missing carcasses, lost nutrients: Quantifying nutrient losses from deer culling practices in Scotland’, *Ecological Solutions and Evidence*, 5(3), p. e12356. Available at: https://doi.org/10.1002/2688-8319.12356.

Fielding, D. *et al.* (2014) ‘Carcass Provisioning to Support Scavengers: Evaluating a Controversial Nature Conservation Practice’, *AMBIO*, 43(6), pp. 810–819. Available at: https://doi.org/10.1007/s13280-013-0469-4.

Finley, S.J. *et al.* (2016) ‘Microbial Signatures of Cadaver Gravesoil During Decomposition’, *Microbial Ecology*, 71(3), pp. 524–529. Available at: https://doi.org/10.1007/s00248-015-0725-1.

Finn, J.A. (2001) ‘Ephemeral Resource Patches as Model Systems for Diversity-Function Experiments’, *Oikos*, 92(2), pp. 363–366.

Fitter, R.S.R., Fitter, A. and Farrer, A. (1984) *Grasses, Sedges, Rushes and Ferns of Britain and Northern Europe*. London, UK: HarperCollins.

Foltan, P. *et al.* (2005) ‘The significance of facultative scavenging in generalist predator nutrition: detecting decayed prey in the guts of predators using PCR’, *Molecular Ecology*, 14(13), pp. 4147–4158. Available at: https://doi.org/10.1111/j.1365-294X.2005.02732.x.

Forestry England (2022) ‘Cropton Forest Plan (FP 11)’. Available at: https://consult.forestryengland.uk/forest-districts/cropton_forest_plan-1/user_uploads/text-combined-pdf.pdf.

Forger, L.V. *et al.* (2019) ‘A eukaryotic community succession based method for postmortem interval (PMI) estimation of decomposing porcine remains’, *Forensic Science International*, 302, p. 109838. Available at: https://doi.org/10.1016/j.forsciint.2019.05.054.

Fox, J. and Weisberg, S. (2019) *An R Companion to Applied Regression*. 3rd edn. Thousand Oaks, CA, USA: Sage. Available at: https://socialsciences.mcmaster.ca/jfox/Books/Companion/.

Fu, X. *et al.* (2019) ‘Fungal succession during mammalian cadaver decomposition and potential forensic implications’, *Scientific Reports*, 9(1), p. 12907. Available at: https://doi.org/10.1038/s41598-019-49361-0.

Fuller, R.J., Oliver, T.H. and Leather, S.R. (2008) ‘Forest management effects on carabid beetle communities in coniferous and broadleaved forests: implications for conservation’, *Insect Conservation and Diversity*, 1(4), pp. 242–252. Available at: https://doi.org/10.1111/j.1752-4598.2008.00032.x.

Gemmellaro, M.D. *et al.* (2023) ‘Assessment of Fungal Succession in Decomposing Swine Carcasses (*Sus scrofa L.*) Using DNA Metabarcoding’, *Journal of Fungi*, 9(9), p. 866. Available at: https://doi.org/10.3390/jof9090866.

Gessner, M.O. *et al.* (2010) 'Diversity meets decomposition', *Trends in Ecology & Evolution*, 25(6), pp. 372–380. Available at: https://doi.org/10.1016/j.tree.2010.01.010.

Giampaoli, S. *et al.* (2020) 'A semi-automated protocol for NGS metabarcoding and fungal analysis in forensic', *Forensic Science International*, 306, p. 110052. Available at: https://doi.org/10.1016/j.forsciint.2019.110052.

Gibbs, J.P. and Stanton, E.J. (2001) 'Habitat Fragmentation and Arthropod Community Change: Carrion Beetles, Phoretic Mites, and Flies', *Ecological Applications*, 11(1), pp. 79–85. Available at: https://doi.org/10.1890/1051-0761(2001)011[0079:HFAACC]2.0.CO;2.

Gilbert, J.A. and Neufeld, J.D. (2014) 'Life in a World without Microbes', *PLOS Biology*, 12(12), p. e1002020. Available at: https://doi.org/10.1371/journal.pbio.1002020.

Gilbert, M. *et al.* (2007) 'Vulture restaurants and their role in reducing diclofenac exposure in Asian vultures', *Bird Conservation International*, 17(1), pp. 63–77. Available at: https://doi.org/10.1017/S0959270906000621.

Gill, R.M.A. (1992) 'A Review of Damage by Mammals in North Temperate Forests: 1. Deer', *Forestry: An International Journal of Forest Research*, 65(2), pp. 145–169. Available at: https://doi.org/10.1093/forestry/65.2.145.

Gilroy, J.J. and Sutherland, W.J. (2007) 'Beyond ecological traps: perceptual errors and undervalued resources', *Trends in Ecology & Evolution*, 22(7), pp. 351–356. Available at: https://doi.org/10.1016/j.tree.2007.03.014.

Gkatzionis, K. *et al.* (2014) 'Diversity and activities of yeasts from different parts of a Stilton cheese', *International Journal of Food Microbiology*, 177, pp. 109–116. Available at: https://doi.org/10.1016/j.ijfoodmicro.2014.02.016.

Goodwin, S.B. (2014) 'Dothideomycetes: Plant Pathogens, Saprobes, and Extremophiles', in F. Martin (ed.) *The Ecological Genomics of Fungi*. Oxford, UK: Wiley Blackwell, pp. 119–148. Available at: https://doi.org/10.1002/9781118735893.ch6.

Greenspoon, L. *et al.* (2023) 'The global biomass of wild mammals', *Proceedings of the National Academy of Sciences*, 120(10), p. e2204892120. Available at: https://doi.org/10.1073/pnas.2204892120.

Gu, X. *et al.* (2014) 'Carcass ecology – more than just beetles', *Entomologische Berichten*, 74(1–2), pp. 68–74.

Guerrieri, A. *et al.* (2023) 'Metabarcoding data reveal vertical multitaxa variation in topsoil communities during the colonization of deglaciated forelands', *Molecular Ecology*, 32(23), pp. 6304–6319. Available at: https://doi.org/10.1111/mec.16669.

Hawksworth, D.L. and Lücking, R. (2017) 'Fungal Diversity Revisited: 2.2 to 3.8 Million Species', *Microbiology Spectrum*, 5(4), p. 10.1128/microbiolspec.funk-0052–2016. Available at: https://doi.org/10.1128/microbiolspec.funk-0052-2016.

Hetherington, D.A., Lord, T.C. and Jacobi, R.M. (2006) 'New evidence for the occurrence of Eurasian lynx (*Lynx lynx*) in medieval Britain', *Journal of Quaternary Science*, 21(1), pp. 3–8. Available at: https://doi.org/10.1002/jqs.960.

Inagaki, A. *et al.* (2022) 'Carcass detection and consumption by facultative scavengers in forest ecosystem highlights the value of their ecosystem services', *Scientific Reports*, 12(1), p. 16451. Available at: https://doi.org/10.1038/s41598-022-20465-4.

Inger, R. *et al.* (2016) 'Ecological role of vertebrate scavengers in urban ecosystems in the UK', *Ecology and Evolution*, 6(19), pp. 7015–7023. Available at: https://doi.org/10.1002/ece3.2414.

Jędrzejewski, W. *et al.* (1993) 'Foraging by lynx and its role in ungulate mortality: the local (Białowieża Forest) and the Palaearctic viewpoints', *Acta Theriologica*, 38(4), pp. 385–403.

Jędrzejewski, W. and Jędrzejewska, B. (1992) 'Foraging and diet of the red fox *Vulpes vulpes* in relation to variable food resources in Biatowieza National Park, Poland', *Ecography*, 15(2), pp. 212–220. Available at: https://doi.org/10.1111/j.1600-0587.1992.tb00027.x.

John Muir Trust (2021) 'Wild land management standards'. Available at: https://www.johnmuirtrust.org/resources/category/12-wild-land-management-standards (Accessed: 4 September 2024).

Kane, A. and Kendall, C.J. (2017) 'Understanding how mammalian scavengers use information from avian scavengers: cue from above', *Journal of Animal Ecology*, 86(4), pp. 837–846.

Keenan, S.W. *et al.* (2018) 'Mortality hotspots: Nitrogen cycling in forest soils during vertebrate decomposition', *Soil Biology and Biochemistry*, 121, pp. 165–176. Available at: https://doi.org/10.1016/j.soilbio.2018.03.005.

Kim, B.-R. *et al.* (2017) 'Deciphering Diversity Indices for a Better Understanding of Microbial Communities', *Journal of Microbiology and Biotechnology*, 27(12), pp. 2089–2093. Available at: https://doi.org/10.4014/jmb.1709.09027.

Krah, F.-S. and March-Salas, M. (2022) 'eDNA metabarcoding reveals high soil fungal diversity and variation in community composition among Spanish cliffs', *Ecology and Evolution*, 12(12), p. e9594. Available at: https://doi.org/10.1002/ece3.9594.

Lauber, C.L. *et al.* (2014) 'Vertebrate Decomposition Is Accelerated by Soil Microbes', *Applied and Environmental Microbiology*, 80(16), pp. 4920–4929. Available at: https://doi.org/10.1128/AEM.00957-14.

Li, T. *et al.* (2023) 'Soil sample sizes for DNA extraction substantially affect the examination of microbial diversity and co-occurrence patterns but not abundance', *Soil Biology and Biochemistry*, 177, p. 108902. Available at: https://doi.org/10.1016/j.soilbio.2022.108902.

Lundin, D. *et al.* (2012) 'Which sequencing depth is sufficient to describe patterns in bacterial α- and β-diversity?', *Environmental Microbiology Reports*, 4(3), pp. 367–372. Available at: https://doi.org/10.1111/j.1758-2229.2012.00345.x.

Macdonald, B.C.T. *et al.* (2014) 'Carrion decomposition causes large and lasting effects on soil amino acid and peptide flux', *Soil Biology and Biochemistry*, 69, pp. 132–140. Available at: https://doi.org/10.1016/j.soilbio.2013.10.042.

Madsen, E.L. (2011) 'Microorganisms and their roles in fundamental biogeochemical cycles', *Current Opinion in Biotechnology*, 22(3), pp. 456–464. Available at: https://doi.org/10.1016/j.copbio.2011.01.008.

Margalida, A. *et al.* (2010) 'Sanitary versus environmental policies: fitting together two pieces of the puzzle of European vulture conservation', *Journal of Applied Ecology*, 47(4), pp. 931–935. Available at: https://doi.org/10.1111/j.1365-2664.2010.01835.x.

Margalida, A. and Bertrán, J. (2000) 'Nest-building behaviour of the bearded vulture *Gypaetus barbatus*', *Ardea*, 88(2), pp. 259–264.

Margalida, A. and Colomer, M.À. (2012) 'Modelling the effects of sanitary policies on European vulture conservation', *Scientific Reports*, 2(1), p. 753. Available at: https://doi.org/10.1038/srep00753.

Markandya, A. *et al.* (2008) 'Counting the cost of vulture decline—An appraisal of the human health and other benefits of vultures in India', *Ecological Economics*, 67(2), pp. 194–204. Available at: https://doi.org/10.1016/j.ecolecon.2008.04.020.

Mason, A.R., Taylor, L.S. and DeBruyn, J.M. (2023) 'Microbial ecology of vertebrate decomposition in terrestrial ecosystems', *FEMS Microbiology Ecology*, 99(2), p. fiad006. Available at: https://doi.org/10.1093/femsec/fiad006.

Mateo-Tomás, P. *et al.* (2015) 'From regional to global patterns in vertebrate scavenger communities subsidized by big game hunting', *Diversity and Distributions*, 21(8), pp. 913–924. Available at: https://doi.org/10.1111/ddi.12330.

Mateo-Tomás, P. *et al.* (2017) 'Both rare and common species support ecosystem services in scavenger communities', *Global Ecology and Biogeography*, 26(12), pp. 1459–1470. Available at: https://doi.org/10.1111/geb.12673.

McMurdie, P.J. and Holmes, S. (2013) 'phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data', *PLOS ONE*, 8(4), p. e61217. Available at: https://doi.org/10.1371/journal.pone.0061217.

Meehan, E.P., Seminet-Reneau, E.E. and Quinn, T.P. (2005) 'Bear Predation on Pacific Salmon Facilitates Colonization of Carcasses by Fly Maggots', *The American Midland Naturalist*, 153(1), pp. 142–151. Available at: https://doi.org/10.1674/0003-0031(2005)153[0142:BPOPSF]2.0.CO;2.

Melis, C. *et al.* (2004) 'Influence of a deer carcass on Coleopteran diversity in a Scandinavian boreal forest: a preliminary study', *European Journal of Wildlife Research*, 50(3), pp. 146–149. Available at: https://doi.org/10.1007/s10344-004-0051-2.

Melis, C. *et al.* (2007) 'Soil and vegetation nutrient response to bison carcasses in Białowieża Primeval Forest, Poland', *Ecological Research*, 22(5), pp. 807–813. Available at: https://doi.org/10.1007/s11284-006-0321-4.

Metcalf, J.L. *et al.* (2016) 'Microbial community assembly and metabolic function during mammalian corpse decomposition', *Science*, 351(6269), pp. 158–162. Available at: https://doi.org/10.1126/science.aad2646.

Meyer, H.H.D. *et al.* (1998) 'Accumulation of polyunsaturated fatty acids by concentrate selecting ruminants', *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, 120(2), pp. 263–268. Available at: https://doi.org/10.1016/S1095-6433(98)00028-2.

Michaud, J.-P., Schoenly, K.G. and Moreau, G. (2015) 'Rewriting ecological succession history: did carrion ecologists get there first?', *The Quarterly Review of Biology*, 90(1), pp. 45–66. Available at: https://doi.org/10.1086/679763.

Moleón, M. *et al.* (2014) 'Inter-specific interactions linking predation and scavenging in terrestrial vertebrate assemblages', *Biological Reviews*, 89(4), pp. 1042–1054. Available at: https://doi.org/10.1111/brv.12097.

Moleón, M. *et al.* (2015) 'Carcass size shapes the structure and functioning of an African scavenging assemblage', *Oikos*, 124(10), pp. 1391–1403. Available at: https://doi.org/10.1111/oik.02222.

Moleón, M. and Sánchez-Zapata, J.A. (2015) 'The Living Dead: Time to Integrate Scavenging into Ecological Teaching', *BioScience*, 65(10), pp. 1003–1010. Available at: https://doi.org/10.1093/biosci/biv101.

Moore, J.C. *et al.* (2004) 'Detritus, trophic dynamics and biodiversity', *Ecology Letters*, 7(7), pp. 584–600. Available at: https://doi.org/10.1111/j.1461-0248.2004.00606.x.

Moreno-Opo, R. and Margalida, A. (2013) 'Carcasses provide resources not exclusively to scavengers: patterns of carrion exploitation by passerine birds', *Ecosphere*, 4(8), p. art105. Available at: https://doi.org/10.1890/ES13-00108.1.

Morris, E.K. *et al.* (2014) 'Choosing and using diversity indices: insights for ecological applications from the German Biodiversity Exploratories', *Ecology and Evolution*, 4(18), pp. 3514–3524. Available at: https://doi.org/10.1002/ece3.1155.

Müller, J. and Brandl, R. (2009) 'Assessing biodiversity by remote sensing in mountainous terrain: the potential of LiDAR to predict forest beetle assemblages', *Journal of Applied Ecology*, 46(4), pp. 897–905. Available at: https://doi.org/10.1111/j.1365-2664.2009.01677.x.

Nakagawa, S., Johnson, P.C.D. and Schielzeth, H. (2017) 'The coefficient of determination R^2 and intra-class correlation coefficient from generalized linear mixed-effects models revisited and expanded', *Journal of The Royal Society Interface*, 14(134), p. 20170213. Available at: https://doi.org/10.1098/rsif.2017.0213.

Newsome, T.M. *et al.* (2021) 'Monitoring the dead as an ecosystem indicator', *Ecology and Evolution*, 11(11), pp. 5844–5856. Available at: https://doi.org/10.1002/ece3.7542.

Nicaud, J.-M. (2012) '*Yarrowia lipolytica*', *Yeast*, 29(10), pp. 409–418. Available at: https://doi.org/10.1002/yea.2921.

Nuñez, N.F. *et al.* (2021) 'Potential of high-throughput eDNA sequencing of soil fungi and bacteria for monitoring ecological restoration in ultramafic substrates: The case study of the New Caledonian biodiversity hotspot', *Ecological Engineering*, 173, p. 106416. Available at: https://doi.org/10.1016/j.ecoleng.2021.106416.

O'Bryan, C.J. *et al.* (2018) 'The contribution of predators and scavengers to human well-being', *Nature Ecology & Evolution*, 2(2), pp. 229–236. Available at: https://doi.org/10.1038/s41559-017-0421-2.

Oksanen, J. *et al.* (2024) 'vegan: Community Ecology Package'. Available at: https://CRAN.R-project.org/package=vegan.

Olson, Z.H., Beasley, J.C. and Jr, O.E.R. (2016) 'Carcass Type Affects Local Scavenger Guilds More than Habitat Connectivity', *PLOS ONE*, 11(2), p. e0147798. Available at: https://doi.org/10.1371/journal.pone.0147798.

Ondrušová, K. and Adamík, P. (2013) 'Characterizing the mammalian hair present in Great Tit (*Parus major*) nests', *Bird Study*, 60(3), pp. 428–431. Available at: https://doi.org/10.1080/00063657.2013.818935.

O'Regan, H.J. (2018) 'The presence of the brown bear *Ursus arctos* in Holocene Britain: a review of the evidence', *Mammal Review*, 48(4), pp. 229–244. Available at: https://doi.org/10.1111/mam.12127.

Parmenter, R.R. and MacMahon, J.A. (2009) 'Carrion decomposition and nutrient cycling in a semiarid shrub–steppe ecosystem', *Ecological Monographs*, 79(4), pp. 637–661. Available at: https://doi.org/10.1890/08-0972.1.

Payne, J.A., King, E.W. and Beinhart, G. (1968) 'Arthropod Succession and Decomposition of Buried Pigs', *Nature*, 219(5159), pp. 1180–1181. Available at: https://doi.org/10.1038/2191180a0.

Pechal, J.L. *et al.* (2014) 'Delayed insect access alters carrion decomposition and necrophagous insect community assembly', *Ecosphere*, 5(4), pp. 1–21. Available at: https://doi.org/10.1890/ES14-00022.1.

Pinheiro, J., Bates, D. and R Core Team (2023) 'nlme: Linear and Nonlinear Mixed Effects Models'. Available at: https://CRAN.R-project.org/package=nlme.

Pivnick, K.A. and McNeil, J.N. (1987) 'Puddling in butterflies: sodium affects reproductive success in *Thymelicus lineola*', *Physiological Entomology*, 12(4), pp. 461–472. Available at: https://doi.org/10.1111/j.1365-3032.1987.tb00773.x.

Prugh, L.R. *et al.* (2009) 'The Rise of the Mesopredator', *BioScience*, 59(9), pp. 779–791. Available at: https://doi.org/10.1525/bio.2009.59.9.9.

Putman, R.J. and Moore, N.P. (1998) 'Impact of deer in lowland Britain on agriculture, forestry and conservation habitats', *Mammal Review*, 28(4), pp. 141–164. Available at: https://doi.org/10.1046/j.1365-2907.1998.00031.x.

R Core Team (2024) 'R: A Language and Environment for Statistical Computing'. Vienna, Austria: R Foundation for Statistical Computing. Available at: https://www.R-project.org/.

Ramette, A. and Tiedje, J.M. (2007) 'Multiscale responses of microbial life to spatial distance and environmental heterogeneity in a patchy ecosystem', *Proceedings of the National Academy of Sciences*, 104(8), pp. 2761–2766. Available at: https://doi.org/10.1073/pnas.0610671104.

Ray, R.-R., Seibold, H. and Heurich, M. (2014) 'Invertebrates outcompete vertebrate facultative scavengers in simulated lynx kills in the Bavarian Forest National Park, Germany', *Animal Biodiversity and Conservation*, 37(1), pp. 77–88. Available at: https://doi.org/10.5167/uzh-130585.

Reimoser, F. and Putman, R.J. (2011) 'Impacts of wild ungulates on vegetation: Costs and benefits', in R.J. Putman, R. Apollonio, and R. Andersen (eds) *Ungulate management in Europe*. Cambridge, UK: Cambridge University Press, pp. 144–191.

Riney, T. (1951) 'Relationships between Birds and Deer', *The Condor*, 53(4), pp. 178–185. Available at: https://doi.org/10.2307/1364874.

Ripple, W.J. *et al.* (2014) 'Status and Ecological Effects of the World's Largest Carnivores', *Science*, 343(6167), p. 1241484. Available at: https://doi.org/10.1126/science.1241484.

Risch, A.C. *et al.* (2020) 'Effects of elk and bison carcasses on soil microbial communities and ecosystem functions in Yellowstone, USA', *Functional Ecology*, 34(9), pp. 1933–1944. Available at: https://doi.org/10.1111/1365-2435.13611.

Ritchie, J. (1920) *The Influence of Man on Animal Life in Scotland*. Cambridge, UK: Cambridge University Press.

Roberts, M., J. (2001) *Spiders of Britain and Northern Europe (Collins Field Guide)*. London: HarperCollins.

Rose, F. and O'Reilly, C. (2006) *The Wild Flower Key*. 2nd edn. London, UK: Frederick Warne Books.

Ruxton, G.D. and Houston, D.C. (2004) 'Obligate vertebrate scavengers must be large soaring fliers', *Journal of Theoretical Biology*, 228(3), pp. 431–436. Available at: https://doi.org/10.1016/j.jtbi.2004.02.005.

Sagara, N., Yamanaka, T. and Tibbett, M. (2008) 'Soil Fungi Associated with Graves and Latrines: Toward a Forensic Mycology', in M. Tibbett and D.O. Carter (eds) *Soil Analysis in Forensic Taphonomy*. Boca Raton, FL, USA: CRC Press, pp. 67–108.

Sawyer, S.J. and Bloch, C.P. (2020) 'Effects of carrion decomposition on litter arthropod assemblages', *Ecological Entomology*, 45(6), pp. 1499–1503. Available at: https://doi.org/10.1111/een.12910.

Schoch, C.L. *et al.* (2012) 'Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi', *Proceedings of the National Academy of Sciences*, 109(16), pp. 6241–6246. Available at: https://doi.org/10.1073/pnas.1117018109.

Schoenly, K. and Reid, W. (1983) 'Community structure of carrion arthropods in the Chihuahuan Desert', *Journal of Arid Environments*, 6(3), pp. 253–263. Available at: https://doi.org/10.1016/S0140-1963(18)31510-6.

Schwegmann, S. *et al.* (2022) 'Evisceration residues from hunted roe deer as a resource for necrophagous insect fauna in the Black Forest, Germany: a preliminary study', *Wildlife Biology*, 2022(6), p. e01055. Available at: https://doi.org/10.1002/wlb3.01055.

Sebastián-González, E. *et al.* (2019) 'Scavenging in the Anthropocene: Human impact drives vertebrate scavenger species richness at a global scale', *Global Change Biology*, 25(9), pp. 3005–3017. Available at: https://doi.org/10.1111/gcb.14708.

Sebastián-González, E. *et al.* (2021) 'Functional traits driving species role in the structure of terrestrial vertebrate scavenger networks', *Ecology*, 102(12), p. e03519. Available at: https://doi.org/10.1002/ecy.3519.

Selva, N. *et al.* (2003) 'Scavenging on European bison carcasses in Białowieża Primeval Forest (eastern Poland)', *Écoscience*, 10(3), pp. 303–311. Available at: https://doi.org/10.1080/11956860.2003.11682778.

Selva, N. *et al.* (2005) 'Factors affecting carcass use by a guild of scavengers in European temperate woodland', *Canadian Journal of Zoology*, 83(12), pp. 1590–1601. Available at: https://doi.org/10.1139/z05-158.

Selva, N. and Fortuna, M.A. (2007) 'The nested structure of a scavenger community', *Proceedings of the Royal Society B: Biological Sciences*, 274(1613), pp. 1101–1108. Available at: https://doi.org/10.1098/rspb.2006.0232.

Shannon, C.E. (1948) 'A mathematical theory of communication', *The Bell System Technical Journal*, 27(3), pp. 379–423.

Shi, L.-L. *et al.* (2014) 'Variation in forest soil fungal diversity along a latitudinal gradient', *Fungal Diversity*, 64(1), pp. 305–315. Available at: https://doi.org/10.1007/s13225-013-0270-5.

Shumskaya, M. *et al.* (2023) 'MycoPins: a metabarcoding-based method to monitor fungal colonization of fine woody debris', *MycoKeys*, 96, pp. 77–95. Available at: https://doi.org/10.3897/mycokeys.96.101033.

Simpson, E.H. (1949) 'Measurement of diversity', *Nature*, 163, p. 688.

Singh, B. *et al.* (2018) 'Temporal and Spatial Impact of Human Cadaver Decomposition on Soil Bacterial and Arthropod Community Structure and Function', *Frontiers in Microbiology*, 8, p. 2616.

Stiegler, J. *et al.* (2020) 'Carcass provisioning for scavenger conservation in a temperate forest ecosystem', *Ecosphere*, 11(4), p. e03063. Available at: https://doi.org/10.1002/ecs2.3063.

Streeter, D. (2016) *Collins Wild Flower Guide*. 2nd edn. London, UK: HarperCollins.

Sutherland, A. *et al.* (2013) 'The effect of body size on the rate of decomposition in a temperate region of South Africa', *Forensic Science International*, 231(1), pp. 257–262. Available at: https://doi.org/10.1016/j.forsciint.2013.05.035.

Swift, M.J., Heal, O.W. and Anderson, J.M. (1979) *Decomposition in Terrestrial Ecosystems*. Oxford, UK: Blackwell Scientific Publications.

Sykes, N. (1993) *Wild Plants and Their Habitats in the North York Moors: Comprehensive Flora of the North York Moors National Park*. North York Moors National Park.

Taberlet, P. *et al.* (2018) *Environmental DNA: For Biodiversity Research and Monitoring*. Oxford University Press. Available at: https://doi.org/10.1093/oso/9780198767220.001.0001.

The Scottish Government (2011) *Supplementary Food Provision for Raptors*. Available at: https://webarchive.nrscotland.gov.uk/20201111102345/http://www2.gov.scot/Topics/farmingrural/SRDP/RuralPriorities/Options/FoodProvisionForRaptors (Accessed: 16 December 2024).

Thermo Fisher Scientific (2010) *Thermo Scientific NanoDrop Spectrophotometers: Nucleic Acid*. T104. Wilmington, Delaware, USA, pp. 1–30. Available at: https://assets.thermofisher.com/TFS-Assets/CAD/manuals/ts-nanodrop-nucleicacid-olv-r2.pdf (Accessed: 20 June 2024).

Tibbett, M. and Carter, D.O. (2003) 'Mushrooms and taphonomy: the fungi that mark woodland graves', *Mycologist*, 17(1), pp. 20–24. Available at: https://doi.org/10.1017/S0269-915X(03)00115-0.

Torres-Porras, J. *et al.* (2014) 'The tragedy of the commons: unsustainable population structure of Iberian red deer in hunting estates', *European Journal of Wildlife Research*, 60(2), pp. 351–357. Available at: https://doi.org/10.1007/s10344-013-0793-9.

Towne, E.G. (2000) 'Prairie vegetation and soil nutrient responses to ungulate carcasses', *Oecologia*, 122(2), pp. 232–239. Available at: https://doi.org/10.1007/PL00008851.

Trumbo, S.T. (1992) 'Monogamy to communal breeding: exploitation of a broad resource base by burying beetles (*Nicrophorus*)', *Ecological Entomology*, 17(3), pp. 289–298. Available at: https://doi.org/10.1111/j.1365-2311.1992.tb01060.x.

Turner, K.L. *et al.* (2017) 'Abiotic and biotic factors modulate carrion fate and vertebrate scavenging communities', *Ecology*, 98(9), pp. 2413–2424. Available at: https://doi.org/10.1002/ecy.1930.

van de Voorde, H. and van Dijck, P.J. (1982) 'Determination of the time of death by fungal growth', *Zeitschrift für Rechtsmedizin*, 89(2), pp. 75–80. Available at: https://doi.org/10.1007/BF02092372.

van Klink, R. *et al.* (2020) 'Rewilding with large herbivores: Positive direct and delayed effects of carrion on plant and arthropod communities', *PLOS ONE*, 15(1), p. e0226946. Available at: https://doi.org/10.1371/journal.pone.0226946.

Vass, A.A. *et al.* (2002) 'Decomposition Chemistry of Human Remains: A New Methodology for Determining the Postmortem Interval', *Journal of Forensic Sciences*, 47(3), pp. 542–553. Available at: https://doi.org/10.1520/JFS15294J.

Venables, W.N. and Ripley, B.D. (2002) *Modern Applied Statistics with S*. 4th edn. New York, NY: Springer. Available at: https://www.stats.ox.ac.uk/pub/MASS4/.

von Hoermann, C. *et al.* (2018) 'Effects of abiotic environmental factors and land use on the diversity of carrion-visiting silphid beetles (Coleoptera: Silphidae): A large scale carrion study', *PLOS ONE*, 13(5), p. e0196839. Available at: https://doi.org/10.1371/journal.pone.0196839.

Voříšková, J. and Baldrian, P. (2013) 'Fungal community on decomposing leaf litter undergoes rapid successional changes', *The ISME Journal*, 7(3), pp. 477–486. Available at: https://doi.org/10.1038/ismej.2012.116.

Ward, A.I. (2005) 'Expanding ranges of wild and feral deer in Great Britain', *Mammal Review*, 35(2), pp. 165–173. Available at: https://doi.org/10.1111/j.1365-2907.2005.00060.x.

Wardle, D.A. (2013) 'Communities and Ecosystems: Linking the Aboveground and Belowground Components (MPB-34)', in *Communities and Ecosystems*. Princeton, New Jersey, USA: Princeton University Press. Available at: https://doi.org/10.1515/9781400847297.

Welch, D. *et al.* (1991) 'Leader Browsing by Red and Roe Deer on Young Sitka Spruce Trees in Western Scotland I. Damage Rates and the Influence of Habitat Factors', *Forestry: An International Journal of Forest Research*, 64(1), pp. 61–82. Available at: https://doi.org/10.1093/forestry/64.1.61.

Wenting, E., Rinzema, S.C.Y. and van Langevelde, F. (2022) 'Functional differences in scavenger communities and the speed of carcass decomposition', *Ecology and Evolution*, 12(2), p. e8576. Available at: https://doi.org/10.1002/ece3.8576.

White, T.J. *et al.* (1990) 'Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics', in M. Innis *et al*. (eds) *PCR Protocols*. San Diego: Academic Press, pp. 315–322.

Whitfield, D.P. *et al.* (2008) *A conservation framework for golden eagles: implications for their conservation and management in Scotland*. Report 193. Scottish National Heritage. Available at: https://e-space.mmu.ac.uk/96477/ (Accessed: 12 February 2024).

Wilmers, C.C. *et al.* (2003) 'Resource dispersion and consumer dominance: scavenging at wolf- and hunter-killed carcasses in Greater Yellowstone, USA', *Ecology Letters*, 6(11), pp. 996–1003. Available at: https://doi.org/10.1046/j.1461-0248.2003.00522.x.

Wilmers, C.C. and Getz, W.M. (2004) 'Simulating the effects of wolf-elk population dynamics on resource flow to scavengers', *Ecological Modelling*, 177(1), pp. 193–208. Available at: https://doi.org/10.1016/j.ecolmodel.2004.02.007.

Wilmers, C.C. and Post, E. (2006) 'Predicting the influence of wolf-provided carrion on scavenger community dynamics under climate change scenarios', *Global Change Biology*, 12(2), pp. 403–409. Available at: https://doi.org/10.1111/j.1365-2486.2005.01094.x.

Wilson, E.E. and Wolkovich, E.M. (2011) 'Scavenging: how carnivores and carrion structure communities', *Trends in Ecology & Evolution*, 26(3), pp. 129–135. Available at: https://doi.org/10.1016/j.tree.2010.12.011.

Zhang, N. and Wang, Z. (2015) 'Pezizomycotina: Sordariomycetes and Leotiomycetes', in D.J. McLaughlin and J.W. Spatafora (eds) *Systematics and Evolution: Part B*. Berlin, Heidelberg, Germany: Springer (The Mycota), pp. 57–88. Available at: https://doi.org/10.1007/978-3-662-46011-5_3.

Appendix

Appendix A: Experienced User Protocol for Qiagen DNeasy PowerLyzer PowerSoil Kit:

Important points before starting:

- Perform all centrifugation steps at room temperature (15–25°C).
- If Solution C1 has precipitated, heat at 60°C until precipitate dissolves.
- Shake to mix Solution C4 before use.

Procedure:

1. Add up to 0.25 g of soil sample to the PowerBead Tube provided.
2. Add 750 µl of PowerBead Solution to the PowerBead Tube.
3. Add 60 µl of Solution C1 and invert several times or vortex briefly.
4. Bead beating options:
    - PowerLyzer 24 Homogenizer: Place the PowerBead Tubes into the tube holder for the PowerLyzer 24 Homogenizer. The PowerBead Tubes must be balanced in the tube holder. Run the samples for a time and RPM suitable for your soil type. Note: For clay soils, 4,000 RPM for 45 s is the best starting point. For loose, granular and high organic soils, 2,500 RPM for 45 s will provide an optimal result.
    - Vortex: Secure the PowerBead Tubes horizontally using a Vortex Adapter (cat. no. 13000-V1-24). Vortex at maximum speed for 10 min. Note: If you are using a 24-place Vortex Adapter for more than 12 preps, increase the vortex time by 5–10 min.
5. Make sure the PowerBead Tubes rotate freely in the centrifuge without rubbing. Centrifuge at 10,000 x g for 30 s. Do not exceed 10,000 x g. Note: Centrifuge for 3 min at 10,000 x g for clay soils or if your soil is not completely pelleted after 30 s.
6. Transfer the supernatant to a clean 2 ml Collection Tube (provided). Note: Expect 400–500 µl. Supernatant may still contain some soil particles.
7. Add 250 µl of Solution C2 and vortex for 5 s. Incubate at 2–8°C for 5 min. Note: You can skip the 5 min incubation. However, if you have already validated the PowerSoil extractions with the incubation we recommend you retain the step.
8. Centrifuge the tubes for 1 min at 10,000 x g. Avoiding the pellet, transfer up to 600 µl of supernatant to a clean 2 ml Collection Tube (provided).
9. Add 200 µl of Solution C3 and vortex briefly. Incubate at 2–8°C for 5 min. Note: You can skip the 5 min incubation. However, if you have already validated the PowerSoil extractions with the incubation we recommend you retain the step.
10. Centrifuge the tubes for 1 min at 10,000 x g. Avoiding the pellet, transfer up to 750 µl of supernatant into a clean 2 ml Collection Tube (provided).
11. Add 1200 µl of Solution C4 to the supernatant and vortex for 5 s.
12. Load 675 µl of the supernatant onto an MB Spin Column and centrifuge at 10,000 x g for 1 min. Discard the flow-through and add an additional 675 µl of supernatant.
13. Centrifuge at 10,000 x g for 1 minute. Load the remaining supernatant onto the MB Spin Column and centrifuge at 10,000 x g for 1 min. Note: A total of three loads for each sample processed is required.
14. Add 500 µl of Solution C5 and centrifuge for 30 s at 10,000 x g.
15. Discard the flow-through. Centrifuge again for 1 min at 10,000 x g.
16. Carefully place the MB Spin Column in a clean 2 ml Collection Tube (provided). Avoid splashing any Solution C5 onto the MB Spin Column.
17. Add 100 µl of Solution C6 to the center of the white filter membrane. Alternatively, you may use sterile DNA-free PCR-grade water (cat. no. 17000-10) or TE buffer.
18. Centrifuge for 30 s at 10,000 x g. Discard the MB Spin Column.

19. The DNA is now ready for downstream applications. Note: We recommend storing DNA frozen (–90°C to –15°C) as Solution C6 does not contain EDTA. To concentrate DNA, see the Troubleshooting Guide.

Appendix Table 1: List of vertebrate species captured across all sites from the whole study duration, with these in descending order from the highest to lowest activity. The activity for each species is also broken down into the different site types – control, red and roe.

Class	Order	Family	Species	Activity			
				Control	Red	Roe	Total
Mammalia	Carnivora	Canidae	*Vulpes vulpes*	40	1871	1519	3430
Mammalia	Carnivora	Canidae	*Canis familiaris*	90	2072	561	2723
Mammalia	Rodentia	Sciuridae	*Sciurus carolinensis*	552	361	400	1313
Aves	Passeriformes	Corvidae	*Corvus corone*	0	766	125	891
Mammalia	Artiodactyla	Cervidae	*Capreolus capreolus*	159	115	134	408
Mammalia	Carnivora	Mustelidae	*Meles meles*	71	24	110	205
Aves	Passeriformes	Turdidae	*Turdus merula*	9	12	168	189
Mammalia	Artiodactyla	Cervidae	*Cervus elaphus*	45	36	78	159
Aves	Passeriformes	Turdidae	*Turdus philomelos*	0	3	32	35
Aves	Galliformes	Phasianidae	*Phasianus colchicus*	7	9	12	28
Mammalia	Lagomorpha	Leporidae	*Lepus europaeus*	21	4	0	25
Aves	Accipitriformes	Accipitridae	*Buteo buteo*	0	21	0	21
Aves	Charadriiformes	Scolopacidae	*Erithacus rubecula*	0	17	0	17
Aves	Passeriformes	Muscicapidae	*Scolopax rusticola*	9	3	0	12
Mammalia	Lagomorpha	Leporidae	*Oryctolagus cuniculus*	0	0	7	7
Aves	Columbiformes	Columbidae	*Columba palumbus*	3	3	0	6
Aves	Passeriformes	Paridae	*Parus major*	0	3	0	3
Aves	Strigiformes	Strigidae	*Strix aluco*	0	3	0	3
Aves	Accipitriformes	Accipitridae	*Accipiter gentilis*	2	0	0	2
Aves	Passeriformes	Corvidae	*Garrulus glandarius*	0	0	2	2
Mammalia	Rodentia	Muridae	*Apodemus sylvaticus*	0	0	2	2
			Totals	**1008**	**5323**	**3150**	**9481**

Appendix Table 2: List of invertebrate families sampled from pitfall traps across the whole study, with these in descending order from the highest total count down to the lowest. The counts for each family are also broken down into the counts across the different site types – control, red and roe.

Phylum	Class	Order	Family	Count			
				Control	Red	Roe	Total
Arthropoda	Insecta	Hymenoptera	Formicidae	4339	8259	5386	**17984**
Arthropoda	Insecta	Coleoptera	Staphylinidae	1433	1852	2144	**5429**

Phylum	Class	Order	Family	Count			
				Control	Red	Roe	Total
Arthropoda	Malacostraca	Isopoda	Oniscidae	1151	687	1187	**3025**
Arthropoda	Insecta	Diptera		164	1243	897	**2304**
Mollusca	Gastropoda	Stylommatophora	Arionidae	749	693	810	**2252**
Arthropoda	Collembola		Entomobryidae	710	595	840	**2145**
Arthropoda	Insecta	Diptera	Sphaeroceridae	426	692	944	**2062**
Arthropoda	Insecta	Diptera	Scathophagidae	318	590	1104	**2012**
Arthropoda	Insecta	Coleoptera	Carabidae	775	419	673	**1867**
Arthropoda	Arachnida	Opiliones	Phalangiidae	430	210	344	**984**
Arthropoda	Malacostraca	Isopoda	Philosciidae	548	91	120	**759**
Arthropoda	Diplopoda	Polydesmida	Polydesmidae	209	203	206	**618**
Arthropoda	Arachnida	Araneae	Linyphiidae	197	168	233	**598**
Arthropoda	Insecta	Coleoptera		130	251	146	**527**
Arthropoda	Diplopoda	Julida	Julidae	158	153	134	**445**
Arthropoda	Insecta	Coleoptera	Silphidae	21	138	196	**355**
Arthropoda	Malacostraca	Isopoda	Porcellionidae	95	96	150	**341**
Arthropoda	Insecta	Diptera	Muscidae	109	58	111	**278**
Arthropoda	Insecta	Diptera	Mycetophilidae	147	53	66	**266**
Arthropoda	Arachnida	Araneae	Gnaphosidae	80	15	137	**232**
Arthropoda	Diplopoda	Glomerida	Glomeridae	60	147	18	**225**
Arthropoda	Insecta	Coleoptera	Histeridae	1	140	81	**222**
Arthropoda	Insecta	Diptera	Sciaridae	33	100	75	**208**
Arthropoda	Insecta	Diptera	Anisopodidae	75	75	50	**200**
Arthropoda	Insecta	Diptera	Trichoceridae	57	44	72	**173**
Arthropoda	Arachnida	Opiliones	Nemastomatidae	62	80	30	**172**
Mollusca	Gastropoda	Stylommatophora	Gastrodontidae	53	56	44	**153**
Arthropoda	Chilopoda	Lithobiomorpha	Lithobiidae	37	50	62	**149**
Arthropoda	Insecta	Diptera	Heleomyzidae	33	30	56	**119**
Arthropoda	Insecta	Coleoptera	Curculionidae	18	36	43	**97**
Arthropoda	Insecta	Diptera	Calliphoridae	0	18	70	**88**
Annelida	Clitellata	Opisthopora	Lumbricidae	29	27	17	**73**
Arthropoda	Arachnida	Araneae	Lycosidae	25	25	23	**73**
Arthropoda	Insecta	Coleoptera	Geotrupidae	9	27	35	**71**
Arthropoda	Diplopoda	Chordeumatida		5	19	22	**46**
Arthropoda	Chilopoda	Geophilomorpha	Geophilidae	17	12	6	**35**
Arthropoda	Insecta	Lepidoptera		8	16	6	**30**
Arthropoda	Insecta	Diptera	Bibionidae	6	5	14	**25**
Arthropoda	Insecta	Coleoptera	Elateridae	2	7	9	**18**
Arthropoda	Insecta	Hymenoptera	Proctotrupidae	2	7	9	**18**
Mollusca	Gastropoda	Stylommatophora	Clausiliidae	4	9	5	**18**
Arthropoda	Arachnida	Araneae	Clubionidae	7	2	8	**17**
Arthropoda	Insecta	Diptera	Stratiomyidae	4	7	4	**15**
Arthropoda	Insecta	Hymenoptera	Ichneumonidae	1	13	1	**15**

Phylum	Class	Order	Family	Count			
				Control	Red	Roe	Total
Arthropoda	Arachnida	Trombidiformes	Trombidiidae	2	9	0	**11**
Arthropoda	Insecta	Diptera	Empididae	11	0	0	**11**
Arthropoda	Insecta	Hymenoptera	Apidae	1	1	9	**11**
Arthropoda	Arachnida	Ixodida	Ixodidae	8	2	0	**10**
Arthropoda	Insecta	Dermaptera	Forficulidae	2	1	7	**10**
Arthropoda	Insecta	Lepidoptera	Noctuidae	0	4	1	**5**
Arthropoda	Insecta	Diptera	Dolichopodidae	0	1	3	**4**
Arthropoda	Arachnida	Araneae	Thomisidae	1	0	2	**3**
Arthropoda	Insecta	Hymenoptera		2	1	0	**3**
Arthropoda	Arachnida	Mesostigmata	Parasitidae	2	0	0	**2**
Arthropoda	Arachnida	Trombidiformes	Calyptostomatidae	1	0	1	**2**
Arthropoda	Insecta	Hemiptera	Psyllidae	1	0	1	**2**
Arthropoda	Arachnida	Araneae	Tetragnathidae	0	0	1	**1**
Arthropoda	Arachnida	Pseudoscorpiones	Chthoniidae	1	0	0	**1**
Arthropoda	Insecta	Coleoptera	Scarabaeidae	0	0	1	**1**
Arthropoda	Insecta	Coleoptera	Scirtidae	1	0	0	**1**
Arthropoda	Insecta	Diptera	Limoniidae	0	1	0	**1**
Arthropoda	Insecta	Diptera	Lonchopteridae	1	0	0	**1**
Arthropoda	Insecta	Diptera	Tephritidae	0	0	1	**1**
Arthropoda	Insecta	Neuroptera	Chrysopidae	0	0	1	**1**
Mollusca	Gastropoda	Stylommatophora	Helicidae	1	0	0	**1**
			Totals	**12772**	**17438**	**16616**	**46826**

Appendix Table 3: List of plant species sampled at all sites across the whole study, with these in descending order from the highest overall cover percentage. Included as well is the occurrence of the number of quadrats each species was identified in. The cover and occurrence for each species is also broken down into the different site types – control, red and roe.

Functional Group	Species	Cover (%)				Occurrence			
		Control	Red	Roe	Total	Control	Red	Roe	Total
Moss	*Brachythecium rutabulum*	2807	3660	3811	10278	89	111	114	314
Graminoid	*Deschampsia flexuosa*	713	807	1226	2746	18	23	22	63
Graminoid	*Holcus lanatus*	755	656	85	1496	22	20	2	44
Shrub	*Vaccinium myrtillus*	541	59	691	1291	19	6	31	56
Shrub	*Calluna vulgaris*	438	126	405	969	5	2	6	13
Forb	*Oxalis acetosella*	36	743	0	779	6	23	0	29
Ferns	*Dryopteris carthusiana*	250	305	36	591	24	27	6	57
Moss	*Thuidium tamariscinum*	8	0	423	431	2	0	11	13
Graminoid	*Luzula pilosa*	130	289	4	423	9	12	2	23
Moss	*Polytrichastrum formosum*	0	84	270	354	0	11	16	27

Functional Group	Species	Cover (%)				Occurrence			
		Control	Red	Roe	Total	Control	Red	Roe	Total
Graminoid	*Poa trivialis*	252	0	0	252	5	0	0	5
Forb	*Rabelera holostea*	96	137	0	233	7	9	0	16
Forb	*Veronica serpyllifolia*	4	150	5	159	2	8	1	11
Shrub	*Ilex aquifolium*	42	17	84	143	6	6	12	24
Forb	*Circaea lutetiana*	0	138	0	138	0	11	0	11
Forb	*Geranium robertianium*	0	111	20	131	0	13	8	21
Graminoid	*Juncus effusus*	0	127	0	127	0	7	0	7
Forb	*Viola riviniana*	118	5	0	123	13	2	0	15
Moss	*Plagiomnium undulatum*	0	2	107	109	0	1	4	5
Forb	*Galium aparine*	5	100	2	107	4	7	2	13
Moss	*Hypnum cupressiforme*	24	0	62	86	2	0	4	6
Forb	*Geum urbanum*	31	52	0	83	4	5	0	9
Forb	*Potentilla sterilis*	59	9	0	68	6	2	0	8
Ferns	*Pteridium aquilinum*	50	0	17	67	4	0	2	6
Moss	*Rhytidiadelphus squarrosus*	0	0	63	63	0	0	1	1
Forb	*Chamerion angustifolium*	0	59	0	59	0	12	0	12
Sapling	*Picea abies*	11	0	48	59	1	0	16	17
Moss	*Dicranum scoparium*	10	3	44	57	3	1	7	11
Shrub	*Rubus fruticosus*	35	17	1	53	9	5	1	15
Graminoid	*Carex sylvatica*	43	0	6	49	1	0	1	2
Shrub	*Lonicera periclymenum*	3	26	5	34	2	7	2	11
Moss	*Dicranoweisia cirrata*	0	6	24	30	0	1	1	2
Sapling	*Crataegus monogyna*	29	0	1	30	10	0	1	11
Sapling	*Fagus sylvatica*	1	16	12	29	1	1	2	4
Sapling	*Fraxinus excelsior*	0	24	0	24	0	2	0	2
Forb	*Taraxacum officinale*	15	0	0	15	3	0	0	3
Moss	*Campylopus flexuosus*	11	3	0	14	1	1	0	2
Forb	*Stachys sylvatica*	0	12	0	12	0	2	0	2
Sapling	*Prunus padus*	0	12	0	12	0	1	0	1
Forb	*Hyacinthoides non-scripta*	0	6	0	6	0	2	0	2
Forb	*Galium saxatile*	0	6	0	6	0	1	0	1
Forb	*Hypericum perforatum*	0	6	0	6	0	1	0	1
Sapling	*Quercus petraea*	0	4	1	5	0	1	1	2
Sapling	*Sorbus aucuparia*	0	0	5	5	0	0	1	1
Sapling	*Picea sitchensis*	0	0	4	4	0	0	2	2
Shrub	*Cytisus scoparius*	0	0	3	3	0	0	1	1
Ferns	*Blechnum spicant*	2	0	0	2	1	0	0	1
Forb	*Cirsium spp.*	2	0	0	2	1	0	0	1
Forb	*Valeriana dioica*	0	0	2	2	0	0	1	1
Forb	*Teucrium scorodonia*	0	0	2	2	0	0	2	2
Ferns	*Equisetum sylvaticum*	0	0	1	1	0	0	1	1
Forb	*Achillea ptarmica*	0	1	0	1	0	1	0	1

Functional Group	Species	Cover (%)				Occurrence			
		Control	Red	Roe	Total	Control	Red	Roe	Total
Forb	*Rumex acetosa*	1	0	0	1	1	0	0	1
Forb	*Digitalis purpurea*	0	1	0	1	0	1	0	1
	Totals	**6522**	**7779**	**7470**	**21771**	**281**	**346**	**284**	**911**

Appendix Table 4: Summary of DNA yields and purities of soil fungi eDNA extractions. The numbers of the sample IDs represent the site ID, with the letter corresponding to the date of collection, i.e., initial (A) or final (B). The two purity ratios indicate sample quality, with a 260/280 ratio of ~1.8 and a 260/230 ratio of 1.8–2.2 indicative of pure samples for DNA (Thermo Fisher Scientific, 2010).

Sample ID	DNA Yield (ng/µl)	260/280 Ratio	260/230 Ratio
1A	162.1	1.90	2.17
2A	62.7	1.87	2.04
3A	28.9	1.77	1.44
13A	39.6	1.82	1.62
14A	36.5	1.82	1.37
15A	20.7	1.85	1.40
16A	68.3	1.87	2.01
17A	54.7	1.73	1.51
18A	66.0	1.73	1.36
1B	102.5	1.84	1.73
2B	73.7	1.89	2.09
3B	70.0	1.84	1.84
13B	31.5	1.84	1.66
14B	34.5	1.84	1.70
15B	58.4	2.04	2.77
16B	71.6	2.00	2.62
17B	32.9	2.10	2.70
18B	54.6	2.00	2.50
Negative control	*0.5*	*-1.00*	*0.44*
Positive control	*11.0*	*1.92*	*1.07*