Title: From pixels to plants: linking remotely sensed habitat data to roe deer diet from DNA metabarcoding in Scottish forest landscapes

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Abstract

Understanding how landscape characteristics influence herbivore diet selection is fundamental to predicting ecological impacts and developing effective wildlife management strategies. We linked remotely sensed habitat data and dietary DNA metabarcoding to test whether landcover classifications can explain variation in European roe deer (*Capreolus capreolus*) diet across Scottish forest landscapes. We characterised the diets of 198 roe deer from six sites, integrating remotely sensed habitat mapping of cull locations for 190 individuals with location data. Our results revealed that site explained nearly twice as much variation in diet composition (18%) as seasonal effects (10%), emphasising the primacy of spatial over temporal factors. Localities with similar habitat composition supported more similar diets, with these associations strongest in winter and summer. Habitat composition explained a consistent but partial share of dietary variation, the remainder likely reflecting individual selectivity, forage quality and fine-scale vegetation structure. Woodland habitats generally showed positive associations with particular plant genera, while open and wet habitats show predominantly negative relationships. These findings demonstrate that landscape configuration influences roe deer foraging, with clear management applications: for example, the extent of riverine and fen scrub within a locality was associated with reduced winter browsing on commercially valuable conifers, indicating where browsing pressure is likely to concentrate and where protection or deer management effort should be targeted. More broadly, linking freely available landcover data to molecular dietary analysis at the scale of individual foraging localities offers a transferable approach for anticipating herbivore impacts across forested landscapes.

Key words: Capreolus capreolus; DNA metabarcoding; remote sensing; herbivore; diet analysis; deer management

1. Introduction

Understanding the mechanisms that shape herbivore diet selection is fundamental to predicting their impacts on ecosystems and informing wildlife management strategies (Vangilder et al., 1982; Hanley, 1997; Illius et al., 2002; Tanentzap et al., 2009; Proffitt et al., 2016). For concentrate selectors like roe deer (*Capreolus capreolus*), dietary choices reflect complex interactions between animal preferences, nutritional requirements and resource availability across landscapes (de Jong et al., 1995; Tixier et al., 1997; Duncan et al., 1998; Cornelis et al., 1999; Minder, 2012; Freschi et al., 2021; Yuan et al., 2022; Hirst et al., 2026). As the most numerous wild ungulate species in Europe (Apollonio et al., 2010; Lovari et al., 2016), the European roe deer exerts significant ecological and economic impacts, making the prediction of their dietary patterns particularly important. In forestry settings, intense browsing by roe deer and other ungulates leads to economic losses through decreased timber quality, reduced tree growth, and increased tree mortality (Welch et al., 1992; Gill 1992; Reimoser, 2000). In natural and protected areas, over-browsing is likewise a concern (Watson, 1983; Putman and Moore, 1998; Putman and Staines, 2004) and can lead to the extirpation of native plant species (Rooney and Dress, 1997) and shifts in plant species composition toward communities dominated by unpalatable or browse-tolerant plant species (Augustine and Mcnaughton, 1998; Hidding et al., 2013).

The challenge of predicting roe deer diet selection is magnified by the diverse environments they inhabit. As selective feeders, roe deer utilise a wide range of locally available plant species but typically concentrate their consumption on a smaller subset based on availability and nutritional value (Tixier et al., 1997), thereby adapting their diet preferences to spatiotemporal variations in food availability (Freschi et al., 2021). Throughout their extensive range, roe deer encounter substantial variations in food resources, habitat types, climatic conditions, and interspecific competition (Linnell et al., 1998; Cornelis et al., 1999). These factors, combined with seasonal changes, lead to considerable variation in diet composition (Cornelis et al., 1999), varying according to season, predominant habitat type, and biogeographical region (Tixier and Duncan 1996; Cornelis et al., 1999; Spitzer et al., 2020, Hirst et al., 2026). This variability, coupled with their concentrate-selecting strategy of targeting nutritious plant parts across a broad range of species rather than exhibiting fixed preferences for particular taxa, makes characterising population-level dietary patterns particularly challenging.

Previous research has established that a critical determinant of roe deer diets is plant availability within their local environment (de Jong et al., 1995; Tixier et al., 1997; Duncan et al., 1998; Cornelis et al., 1999; Minder, 2012; Freschi et al., 2021; Yuan et al., 2022). However, directly quantifying plant availability across landscapes presents substantial logistical challenges, requiring considerable manpower, botanical expertise, and time investment. Traditional methods like vegetation quadrats (Daubenmire, 1959; Bonham et al., 2004; Leonard et al., 2017) are typically limited to smaller spatial scales, making landscape-level assessments impractical.

Two methodological advances have created new opportunities for investigating habitat-diet relationships at landscape scales. First, advances in molecular techniques have significantly enhanced our ability to analyse wildlife diets with greater precision (Soininen et al., 2009; Pompanon et al., 2012; Taberlet et al., 2012; Casey et al., 2023) and have been widely applied to ungulate diet studies, including various deer species (Czernik et al., 2013; Nichols et al., 2016; Erickson et al., 2017; Ratkiewicz et al., 2024; Gresham et al., 2025). DNA metabarcoding offers advantages over traditional microhistological analysis, enabling faster and more accurate taxonomic identification of consumed plants (Soininen et al., 2009; Czernik et al., 2013; Nichols et al., 2016). This enhanced resolution allows for a more comprehensive assessment of diet composition and diversity, facilitating robust statistical analysis of factors driving dietary variation across landscapes and seasons.

Although several investigations have utilised DNA metabarcoding to study the diets of European roe deer (Czernik et al., 2013; Nichols et al., 2016; Spitzer, 2019; Ratkiewicz et al., 2024) and Siberian roe deer (*Capreolus pygarus*) (Adhikari et al., 2016; Liu and Huang 2023), this has not yet been applied to British populations. Additionally, the Barcode UK project (Jones et al., 2021), provides a comprehensive reference database that enables precise taxonomic identification of plant material in British herbivore diets (de Vere et al., 2012; Gresham et al., 2025), representing an extensive effort to barcode more than 6,100 specimens from 1,482 species of herbarium specimens and collections throughout Britain,.

Second, high-resolution habitat classification through remote sensing offers a promising alternative approach for estimating potential plant resources available to herbivores. This approach leverages the ecological principle that plant species assemble into vegetation

communities through evolutionary and environmental processes (Velázquez et al., 2016; Getaneh et al., 2023), which further aggregate into recognisable habitat types. The specific habitats available to herbivores lead to differences in the plant species encountered and nutrients gained from foraging (Mysterud et al., 2001; Storms et al., 2006; Krojerová-Prokešová et al., 2010; Yuan et al., 2022; Dahl et al., 2023). Since roe deer habitat selection is intrinsically linked to foraging opportunities (Pettorelli et al., 2001; Mancinelli et al., 2015), local habitat composition may serve as a valuable proxy for predicting diet composition. Modern remote sensing datasets, such as the Scotland 2022 Landcover Map with European Nature Information System (EUNIS) level 2 habitat classifications (https://www.space-intelligence.com/scotland-landcover/), now enable researchers to precisely characterise habitat distributions across extensive landscapes in Scotland.

The convergence of these methodological advancements - DNA metabarcoding and remote sensing habitat classification - creates a unique opportunity to link detailed habitat characteristics to dietary composition in wild herbivore populations at landscape scales. Traditional approaches have often relied on coarse habitat classifications, frequently characterising entire study sites by dominant tree species or vegetation types, failing to capture the fine-grained heterogeneity that herbivores encounter across complex vegetation mosaics. By moving beyond site-level classifications toward spatially explicit habitat characterisation at the scale of individual localities, we can better understand how the unique mosaic of habitats encountered by each individual influences their diet selection.

This study aimed to determine whether habitat characteristics can predict roe deer diet composition. The specific objectives were to: (1) quantify the relative contribution of spatial

(site) and temporal (seasonal) factors to variation in roe deer diet composition; (2) examine relationships between habitat metrics (richness, diversity, composition) and corresponding dietary metrics within roe deer localities; (3) identify season-specific associations between habitat types and the occurrence of plant genera in roe deer diets; and (4) develop models to predict the presence and relative abundance of plant genera in roe deer diets based on habitat composition. By integrating high-resolution remote sensing data with molecular dietary analysis through DNA metabarcoding, we sought to develop a predictive framework for understanding how landscape configuration influences herbivore foraging ecology.

2. Methods

This study employed a multi-faceted approach to analyse roe deer diet composition and its relationship to an individual's immediate surroundings. Our methodology encompassed four main components: (1) field collection of rumen samples from culled deer across Scotland, (2) DNA metabarcoding of these samples to identify plant taxa in rumen contents, (3) geospatial analysis of habitat composition around cull locations, and (4) statistical analyses to examine relationships between diet and the local environment. Each component was designed to enable robust investigation of seasonal and spatial patterns in roe deer foraging ecology.

2.1. Study Areas

Sampling was conducted across six sites (Figure 1) within three ecologically distinct regions of Scotland; the Cairngorms, the Trossachs, and the Scottish Borders - selected to represent a spectrum of Scotland's major habitat types and land management contexts.

The Cairngorms sites were located within the Cairngorms Connect Partnership area, a landscape-scale restoration partnership covering approximately 600 km² of the Cairngorms (Gullett et al., 2023). Abernethy (57°24'N, 3°7'W) is a 136 km² RSPB nature reserve encompassing one of the largest remnant Caledonian pine forest fragments in Scotland, dominated by Scots pine (*Pinus sylvestris*) with an understorey of heather (*Calluna vulgaris*) and *Vaccinium* spp., alongside areas of upland birchwood, blanket bog and montane heath. Like Glenfeshie, Abernethy has undergone sustained woodland restoration through coordinated deer management, with natural regeneration expanding woodland cover over recent decades (Gullett et al., 2023). Glenfeshie (57°03'N, 3°9'W) covers over 315 km² of the western Cairngorms, with a valley floor mosaic of grasslands and riparian woodland giving way to semi-natural Scots pine, birch woodlands and shrub heathland on the montane slopes, following extensive woodland restoration since 2006.

The Scottish Borders sites were both commercial upland conifer forests managed by Forestry and Land Scotland. Craik Forest (55°21'N, 3°2'W) is a 42.4 km² plantation dominated by Sitka spruce (*Picea sitchensis*) with areas of blanket bog, heathland and improved grassland. Glentress Forest (55°66'N, 3°1'W) is a 16 km² forest established in 1920, similarly dominated by Sitka and Norway spruce (*Picea abies*), bordered by dry heath and agricultural grassland.

The Trossachs sites represented contrasting land uses within the Loch Lomond and Trossachs National Park. Glen Finglas (56°27'N, 4°4'W) is a 48.8 km² Woodland Trust estate characterised by diverse open habitats including native oak woodlands, a range of grassland types, wet heaths, fens and montane scrub. The Trossachs Forests (56°N, 4°W) comprise

several commercial conifer plantations dominated by Sitka spruce and Scots pine, with ancient oak woodlands and native broadleaved woodland persisting along watercourses.

2.2. Sample collection

Rumen samples were collected from 200 roe deer (63 females, 137 males) culled by professional deer stalkers using hunting rifles across six sites from three regions in Scotland between April 2021 and January 2023. Samples were primarily obtained during legal hunting seasons (1st April to the 20th October for males; 21st October to the 31st March for females), with some collected under out-of-season licences. Only deer >1 year of age were sampled to avoid collecting rumen contents from unweaned individuals.

Immediately after culling, approximately 150 ml of rumen content was collected and frozen at -20°C. Sex and age class (yearling, subadult, adult and old adult) were recorded for each individual, whereas exact location data was only available for 190 individuals. Samples were distributed across seasons (autumn= 38, spring= 58, summer= 53, winter= 51) and sites from the Cairngorms (Glenfeshie=63, Abernethy=41) Trossachs (Glen Finglas=30, Trossachs Forests=20) and Borders (Craik = 35, Glentress = 11) regions of Scotland. Due to hunting season timing, females were sampled primarily in autumn (n=24) and winter (n=32), with few in spring (n=7) and none in summer. In contrast, males were sampled in all seasons, with the most in spring (n=51) and summer (n=53) and the least in winter (n=19) and autumn (n=14).

2.3. DNA metabarcoding dietary analysis

We employed DNA metabarcoding to identify the plant genera present in rumen samples. This molecular approach allowed for the high-resolution identification of dietary components while avoiding the limitations of traditional microhistological analysis. Total genomic DNA was extracted from 100 mg of homogenised rumen content using the Qiagen plant mini kit with a modified protocol incorporating 10 µL of lysozyme (20 mg/ml) added to the AP1 lysis buffer, along with RNase A. Samples were incubated at 37°C for 5 minutes, followed by 67°C for 15 minutes. DNA was eluted in 75 µL of buffer AE alongside an extraction blank to control for extraction kit contaminants. DNA concentrations were quantified using the Qubit dsDNA BR Assay Kit on a Qubit 4 Fluorometer (Thermo Fisher Scientific) with 2 µL of DNA. The extracted DNA samples were standardised to 5-20 ng/µL by dilution in nuclease-free water (Qiagen) before downstream polymerase chain reaction (PCR).

Plant taxa were identified using two DNA markers: a region of the ribulose bisphosphate carboxylase (*rbcL*) gene within the chloroplast genome and part of the internal transcribed spacer 2 (*ITS2*) region of nuclear ribosomal DNA. The *rbcL* and *ITS2* regions were amplified using the primers *rbcL-a* (Kress and Erickson, 2007) and *rcbLr590* (de Vere et al., 2012) and the primers *ITS2F* and *ITS3R* (Chen et al., 2010), respectively. Reactions were carried out in 25 µL volumes containing 2 µL of extracted DNA, 12.5 µL of 2X Simplifi HS Mix (Meridian Bioscience Inc.), 0.25 µL of forward and reverse primers, and 10 µL of nuclease-free water (Qiagen). Reactions were performed in duplicate for each sample and marker. Amplification was confirmed by visualising 3 µL of PCR product on a 2% agarose gel stained with SYBR® Safe (Thermo Scientific) alongside a GeneRuler 100 bp ladder (Thermo Scientific). Where

both duplicates produced visible amplicons of the expected size, they were pooled. Exact primer sequences and cycling conditions are provided in Supplementary Materials S1.

2.4. Library preparation and sequencing

PCR primers included Illumina TruSeq adapter sequences to enable multiplexing. Amplicons were normalised to equimolar concentrations (median 114.61 nM) based on Qubit quantification and expected amplicon size (*rbcL*: 580 bp; *ITS2*: 520 bp). Normalised pools were purified using Ampure beads (1:1 ratio) and submitted to Eurofins Genomics for library preparation and Illumina MiSeq sequencing (2x300 bp paired-end). Of 203 submitted samples (200 deer samples, 2 technical repeats, 1 extraction blank), libraries were successfully constructed and sequenced for 198 deer samples across five MiSeq runs. Library construction failed for two deer samples and the extraction blank due to insufficient DNA.

2.5. Bioinformatics analysis

Paired-end fastq files were demultiplexed by marker using *cutadapt* (v.1.16) (Martin, 2011) utilising the `—discard-untrimmed` option to search for occurrences of the forward and reverse primer sequences within each fastq file, retaining only properly paired reads containing the expected primer sequences. This primer search and filtering process was performed separately for each marker to produce marker-specific paired-end fastq files for downstream analysis.

ITS2 data were imported into QIIME2 using the PairedEndFastqManifestPhred33V2 option, while *rbcL* data required single-end processing using the SingleEndFastqManifestPhred33V2 option due to amplicon length constraints with 2x300 bp sequencing. Imported data were

used to create summary artefacts detailing sequence count summaries and quality plots. Quality filtering parameters were standardised for each marker across sequencing runs (*ITS2* 260 forward, 220 reverse; *rbcL* 260 forward) based on quality score profiles. Sequences were processed using QIIME2's DADA2 plugin for quality filtering, error correction, and dereplication (Callahan et al., 2016), with data from five sequencing runs merged by marker for downstream analysis.

2.6. Taxonomic Classification and Data Processing

Sequences were classified using the BarcodeUK database (Jones et al., 2021) and the *RESCRIPT* pipeline (Robeson et al., 2021) to download and format additional *Pinales* sequence records from NCBI for each marker to include important non-native species not covered in BarcodeUK. The combined Barcode UK and NCBI *Pinales* classifier was trained using the Naïve Bayes method implemented in QIIME2 (Bokulich et al., 2018) with a confidence level of 0.7 as per previous studies using *ITS2* and *rbcL* markers (Arstingstall et al., 2021, 2023; Walker et al., 2022; Turk Dermastia et al., 2023). The QIIME2 phylogenetic plugin was used to construct phylogenetic trees with *MAFFT 7.3* (Katoh and Standley, 2013) and *FastTree 2.1* (Price et al., 2010). The phylogenetic tree, feature table, and taxonomy table were exported for downstream analysis in R (v.4.3.2).

Taxonomic data were collapsed to genus level using the `glom` function in the *phyloseq* R package (v.3.18) (McMurdie and Holmes, 2013). Relative read abundance (RRA) transformations were applied to normalising read counts for each taxon within a sample, with genera retained only if they represented ≥ 1% of reads in a sample. This approach was

307 chosen over rarefaction to preserve data while accounting for varying sequencing depths
308 (Erickson et al., 2017; McInnes et al., 2017; Deagle et al., 2019; Michel et al., 2023).
309
310 2.7. Geospatial analysis and habitat assessment
311 To connect our dietary analyses to local environments, we conducted detailed geospatial
312 analyses of the habitats surrounding each culling location to understand the local conditions
313 influencing deer foraging behaviour. Habitats were analysed using the Scotland 2022
314 Landcover Map (Space Intelligence/ NatureScot https://www.space-intelligence.com/
315 scotland-landcover/), which uses satellite remote sensing datasets, including Optical
316 Sentinel 2 (S2), Synthetic Aperture Radar (SAR), Sentinel-1 descending and ascending ALOS-
317 PALSAR 2 (SpatialData.gov.uk, 2023), cross-verified with high-resolution imagery, providing
318 EUNIS level 2 classifications at 20 x 20 m^2 resolution. For the 190 roe deer samples with
319 available cull locations, coordinates were processed in QGIS (v.3.24.1) and R (v.4.3.2) to
320 create buffer zones around cull locations using the *raster* (v. 3.6-26) (Hijmans et al., 2023)
321 and *sf* (v. 1.0-16) (Pebesma et al., 2024) R packages. Given the variation in roe deer home
322 range sizes within the UK (4–68 ha; Hewison and Staines, 2008), including recent GPS-
323 derived estimates from northeast Scotland averaging 65 ha (range 32–123 ha; Marshall et
324 al., 2026), buffer zones were created to characterise local habitat mosaics rather than
325 approximate individual home ranges. Buffer radii of 150m (7 ha), 250m (20 ha), 500m (78
326 ha), 750m (176 ha) and 1000m (314 ha) were evaluated using species-area curve analysis to
327 determine the optimal radius for characterising habitats around localities.
328
329 2.8. Statistical analysis

To integrate our dietary and habitat data, we employed a multi-tiered statistical approach that examined both individual patterns and broader ecological relationships. Our analyses progressed from basic diversity metrics to more complex models of diet-habitat associations.

2.8.1. Assessing the frequency of dietary items: Frequency of occurrence

Frequency of occurrence (FOO) and RRA represent complementary measures of diet composition: FOO captures the proportion of individuals in which a taxon was detected, reflecting dietary breadth across a population, while RRA reflects the proportional contribution of a taxon within a sample, providing an indication of relative dietary abundance at the individual level. To provide an overview of roe deer diet, we combined FOO data from both *rbcL* and *ITS2* markers. Genera detected in only a single sample were excluded as likely rare or incidental occurrences. Within this set, genera present in more than 5% of samples were additionally identified as commonly consumed taxa, reducing exaggeration of the importance of rarer items (Pansu et al., 2019) while still capturing a wide range of dietary components. When calculating the number of genera present in a particular season or at a specific site, an additional filtering step was implemented in which a genus was only considered present if it was detected in more than one sample within that seasonal or site-specific subset. To assess how well RRA associates with FOO, Spearman's correlations were performed between marker-specific RRA and FOO, as well as between the RRA of each marker with the combined FOO across both markers.

2.8.2. Diversity and compositional analyses

Alpha diversity metrics (richness and Shannon's diversity index; Shannon, 1948) were calculated for diet (genus-level) data using the `get_alphaindex` function in the *MicrobiotaProcess* R package (v.1.14.0) (Xu et al., 2023), and for locality (20 x 20 m² habitat classification) data using the *vegan* R package (v.2.6-4) (Dixon, 2003), which were then compared across seasons, sites and demographic factors (diet only) using Kruskal–Wallis tests, followed by pairwise Wilcoxon rank-sum tests with Benjamini–Hochberg correction (Benjamini and Hochberg, 1995).

Compositional differences among diets and localities (beta diversity) were evaluated using Bray-Curtis dissimilarity (Bray and Curtis, 1957) and visualised via Non-Metric Multidimensional Scaling (NMDS). Permutational multivariate analysis of variance (PERMANOVA; Anderson, 2001) with 999 permutations, conducted using the `adonis2` function in the *vegan* R package (v.2.6-4) (Dixon, 2003) tested the effects of intrinsic (sex, age) and extrinsic (season, site) factors on diet composition (*Bray–Curtis dissimilarity ~ season + location + season*location + sex + age*), with a separate model evaluating variance in the habitat composition of localities (*Bray–Curtis dissimilarity (locality) ~ season + location + season*location*). Significant effects were further examined using pairwise comparisons with the R package *pairwiseAdonis* (v.0.4.1) (Martinez, 2017), applying the Bonferroni method (Rice 1989). Beta dispersion was analysed using ANOVA with post-hoc Tukey's HSD tests to explore pairwise differences in multivariate dispersion between factor levels.

Consistency between *rbcL* and *ITS2* markers was evaluated using Spearman's correlations for alpha diversity measures and Mantel tests for beta diversity matrices. Relationships between locality and dietary alpha diversity were examined using linear regression, with the locality

alpha diversity measures as independent variables. Mantel tests were also performed to evaluate correlations between the habitat and dietary beta diversity matrices for each plant marker (*rbcL* and *ITS2*). Separate analyses were conducted by season to investigate context-specific patterns.

2.8.3. Predicting diet using habitat data

To identify associations between the proportional area of habitat types within localities and the relative abundance of plant genera in the diet, Spearman's correlation analyses were conducted on seasonal subsets of the data, as the relationship between habitats and plants will likely change seasonally due to plant phenology. Only genera and habitats present in ≥ 10% of the seasonal samples were included (10% of samples: spring=6 samples, summer=4, autumn=3, winter=5) to minimise rare occurrences. The analyses used *rbcL* data exclusively to simplify interpretation. Correlations with Spearman's rank coefficient (r_s) $|r_s| \geq 0.3$ were considered robust correlations for further analysis.

Building on these correlations, we used logistic regression for season-specific plant-habitat correlations meeting the $|r_s| \geq 0.3$ threshold to examine the relationships between the presence/absence of a plant genus and the proportional area of a habitat type within a specific season. These models quantified the probability of a plant genus being present or absent in each habitat type within a certain season, using habitat area as the predictor variable. By accounting for the non-linear nature of binary data, logistic regressions assessed the strength and direction of the relationship between the predictor (habitat abundance) and the binary response (plant genus presence/absence) (Crawley, 2007).

Finally, for significant habitat-plant relationships identified through the presence/absence modelling, we analysed habitat effects on dietary quantities using logistic regression with a binomial error structure. The response variable was the proportion of the plant genus in the diet, converted to a percentage (multiplied by 100), with the proportional area of the corresponding habitat type as the predictor. This approach examined whether or not the proportion of diet components varied with habitat area within seasons.

3. Results

3.1. Diet analysis

Using *rbcL* and *ITS2* amplicon sequencing, the diet composition of 198 roe deer rumen samples was characterised across six sites in Scotland; library construction failed for the remaining two samples due to insufficient DNA. After quality filtering, denoising and chimera removal in DADA2, an average of 14,282±351 (*rbcL*) and 20,681±519 (*ITS2*) reads were retained per sample, yielding 548 and 1,740 unique Amplicon Sequence Variants (ASVs) across 4,089,647 and 5,917,925 total reads, respectively. The extraction blank failed library construction due to insufficient DNA, indicating negligible laboratory contamination. Full sequencing and read-processing statistics are provided in the Supplementary Material S2. Sequenced samples were distributed across seasons (spring n=58, summer n=53, autumn n=37, winter n=50) and sites (Glenfeshie n=62, Abernethy n=40, Craik n=35, Glen Finglas n=30, Trossachs Forests n=20, Glentress n=11).

To assess methodological consistency, two technical replicates (BF19/2BF19, WL21/2WL21) were processed independently through the entire workflow. Both pairs shared 100% of plant genera, had identical taxonomic richness, near-identical Shannon diversity (differing by

<2.2%), and very low Bray–Curtis dissimilarity (*rbcL* 0.02, *ITS2* 0.04), with RRAs highly correlated between replicates for both markers (rs ≥ 0.99, p<0.001).

Having established the reliability of our methodology, we next examined the consistency between the two markers. Richness and diversity metrics from both *rbcL* and *ITS2* markers were significantly positively correlated (richness Spearman's r_s=0.62, p<0.001; Shannon's Spearman's r_s=0.35, p <0.001), indicating consistency between markers in assessing plant genera diversity. Mantel tests revealed a strong positive correlation between *rbcL* and *ITS2* markers in dietary composition (Mantel r=0.79, p=0.001, Figure 7A), indicating that both markers effectively capture similar patterns of variation in the structural composition of diets among individuals.

A strong positive correlation was observed between the marker-relative RRA and FOO of genera detected using the *rbcL* (Spearman's rs=0.93, p<0.001), and *ITS2* markers (Spearman's rs=0.80, p<0.001), indicating that genera with higher RRA are also more frequently detected across samples. To assess the consistency between RRA and FOO measures across markers, we examined correlations between marker-specific RRA and the combined marker FOO. The strong correlation for *rbcL* (Spearman's rs=0.72, p<0.001) and moderate correlation for *ITS2* (Spearman's rs=0.50, p<0.001) indicate that abundance data from *rbcL* more closely matches the overall presence patterns detected across both markers.

3.2. Overall diet composition

Molecular analysis using *rbcL* and *ITS2* revealed 130 unique plant genera in roe deer diets, of which 53 (41%) were detected by both markers, with 38 and 39 detected exclusively by *rbcL*

and ITS2, respectively. Combining FOO data from both markers, 59 genera were detected in more than one sample and considered dietary components; the remaining 71 genera, each detected in only a single sample, likely represent rarely consumed taxa or incidental ingestion and were excluded to avoid overstating the importance of rare items. Of the 59 genera, 28 occurred in 5% or fewer of samples, leaving 31 commonly consumed genera (>5% of samples; Supplementary Table S3 to S5). The most frequently occurring genera were Vaccinium, present in 91% of samples, *Calluna* (83%), *Potentilla* (37%), *Rumex* (35%) and *Betula* (34%). Hereafter, FOO is used to describe the occurrence of genera across the population, while RRA is used for diversity and compositional analyses and habitat-based predictions.

3.3. Intrinsic factors influencing the diet of roe deer

While sex explained a small but significant proportion of variance in diet composition for *ITS2* (R^2=0.04, p=0.001), no significant effect was detected for *rbcL* (R^2=0.01, p=0.174). Age had no significant effect on diet composition for either marker (*rbcL*: p=0.142; *ITS2*: p=0.150), and although Shannon's diversity varied across age classes for *ITS2* (p=0.038), no pairwise differences between age classes were significant. Given the negligible variance explained by intrinsic factors, and the confounding of sex with season arising from hunting season timing, subsequent analyses pooled samples across sexes and age classes.

3.4. Seasonal variation in diet

Both markers detected significant seasonal variation in the richness (*rbcL* p<0.001, *ITS2* p<0.001) and Shannon’s diversity (*rbcL* p=0.022, *ITS2* p<0.001) of plant genera in roe deer

diets (Figure 2 C-D = *rbcL*, E-F = *ITS2*). While the significance of pairwise seasonal comparisons varied between them (Table 1), both markers identified summer as the season with the highest dietary richness (mean= 9±0.4 genera overall, *rbcL*=7±0.4, *ITS2*= 7±0.3) and diversity (mean Shannon's *rbcL*=1.02±0.07, *ITS2* 1.2±0.05). A total of 43 genera were detected in summer diets, eight of which were unique to this season. The most prevalent summer genera were *Vaccinium* (present in 87% of samples), *Potentilla* (70%), *Calluna* (68%), *Rumex* (68%), and *Betula* (58%). Potentilla and Rumex were detected in roughly twice the proportion of samples compared with the dataset overall (37% and 35%, respectively), indicating a marked summer increase in the number of individuals consuming these herbaceous genera.

In contrast, both markers found that winter diets had the lowest dietary richness (mean= 6±0.4 genera overall, *rbcL*= 5±0.3, ITS2= 4±0.3). Winter diversity was also lowest for *ITS2* (mean Shannon's=0.71±0.05), although for *rbcL* the lowest diversity occurred in spring (0.75±0.06) rather than winter (0.87±0.05). Only 29 genera were recorded in winter, with just two unique to this season. *Vaccinium* was present in all winter samples, while *Calluna* occurred in 98%, followed by *Alnus* (46%), *Pinus* (46%), and *Myrica* (42%).

Spring diets included 43 genera present in more than one sample, with a mean of 7±0.5 genera per individual, significantly less rich than summer diets (*rbcL* p= 0.008). Five genera were unique to this season, with *Vaccinium* (90% of samples), *Calluna* (81%), *Rumex* (36%), *Epilobium* (33%), and *Erica* (29%) being the most prevalent. In autumn, 32 genera were present in more than one sample, with a mean of 8±0.6 genera per individual. Only one genus was unique to autumn diets. *Calluna* and *Vaccinium* were the most frequently

detected, occurring in 86% of samples, followed by *Potentilla* (51%), *Betula* (49%), and *Alnus* (37%).

Table 1. Pairwise comparisons of roe deer diet between seasons for both markers. Alpha diversity columns give p values from pairwise Wilcoxon rank sum tests on genus richness and Shannon diversity, adjusted using the Benjamini–Hochberg method. Beta diversity columns give R^2 and adjusted p values from pairwise PERMANOVA on Bray–Curtis dissimilarities (Bonferroni-adjusted), and BD p.adj gives Tukey's HSD p values for pairwise differences in multivariate dispersion (distance to group centroid). Overall dispersion differed significantly among seasons for *ITS2* (permutest p=0.005) but not *rbcL* (p=0.637). * = $p < 0.05$.

	rbcL					***ITS2***				
	Alpha diversity (p)		**Beta diversity**			**Alpha diversity (p)**		**Beta diversity**		
Comparison	**Richness**	**Shannon**	**R^2**	**p.adj**	**BD p.adj**	**Richness**	**Shannon**	**R^2**	**p.adj**	**BD p.adj**
Spring vs Summer	0.008*	0.020*	0.03	0.126	0.950	0.001*	<0.001*	0.07	0.006*	0.683
Summer vs Autumn	0.038*	0.170	0.05	0.012*	0.995	0.168	0.017*	0.06	0.006*	0.658
Autumn vs Winter	0.169	0.770	0.05	0.018*	0.639	<0.001*	<0.001*	0.05	0.012*	0.125
Spring vs Autumn	0.621	0.400	0.03	0.210	0.888	0.082	0.331	0.02	0.750	0.998
Spring vs Winter	0.281	0.170	0.09	0.006*	0.949	0.002*	0.005*	0.08	0.006*	0.042*
Summer vs Winter	<0.001*	0.170	0.13	0.006*	0.724	<0.001*	<0.001*	0.17	0.006*	0.002*

509 Season significantly influenced plant composition in roe deer diets for both markers (*rbcL*:
510 R^2=0.09, p=0.001, Figure 3B; *ITS2*: R^2=0.12, p=0.001, Figure 3C), explaining approximately
511 10% of the observed variance. Winter diets were significantly distinct from all other seasons
512 for both markers (Table 1), reflecting seasonal shifts in both the range of plant genera
513 consumed and their relative contributions.
514
515 Analysis of within-season dietary variability using *ITS2* revealed significant differences
516 across seasons (p=0.003, Figure 3F). Winter *ITS2* diets were significantly more uniform
517 (average distance to centroid = 0.41) than spring (0.50, p=0.042) and summer diets (0.54,
518 p=0.002); autumn was intermediate (0.49) and did not differ significantly from winter (Table
519 1). In contrast, *rbcL* showed no significant difference in within-season dietary variability
520 (p=0.62, Figure 3E), with similar levels of individual variation across seasons (autumn=0.50,
521 spring=0.47, summer=0.49, and winter=0.45).
522
523 3.5. Spatial variation in diet
524 Diet richness (*rbcL* p<0.001, *ITS2* p<0.001) and Shannon's diversity (*rbcL* p<0.001, *ITS2*
525 p=0.035) varied significantly across sites for both markers (Table 2, Figure 4 C-D= *rbcL*, E-F =
526 *ITS2*). The two Cairngorms sites showed contrasting dietary patterns despite their
527 geographic proximity. Abernethy showed the lowest dietary richness of all sites (5±0.5
528 genera/individual overall, 4±0.4 *rbcL*, 4±0.3 *ITS2*), with 22 genera present in more than one
529 sample and two unique genera. *Vaccinium* and *Calluna* dominated (100% and 98%
530 occurrence), followed by *Potentilla* (40%), *Pinus* (35%), and *Betula* (25%). In contrast,
531 Glenfeshie supported the most genera detected across individuals (45) and the most unique
532 genera (11), though individual diet richness remained moderate (8±0.5 genera/individual

overall, 5±0.4 *rbcL*, 6±0.4 *ITS2*), primarily composed of *Calluna* and *Vaccinium* (92% each), followed by *Alnus* (54%), *Betula* (44%), and *Myrica* (40%).

In the Trossachs, Trossachs Forests exhibited the richest individual diets of all sites (10±0.7 genera/individual overall, 8±0.5 *rbcL*, 7±0.5 *ITS2*) despite having only 26 genera detected and one unique site-level genus. These diets were distinguished by a high prevalence of *Rubus* (95%), *Vaccinium* (90%), *Calluna* (80%), *Betula* (60%), and *Epilobium* (55%). Glen Finglas showed markedly lower individual diet richness (6±0.5 genera/individual overall, 4±0.3 *rbcL*, 5±0.4 *ITS2*), with 24 genera detected and one unique genus. Diets were dominated by *Vaccinium* (100%), *Calluna* (67%), *Potentilla* (60%), *Betula* (37%), and *Alnus* (33%).

The Borders sites showed similar dietary patterns, consistent with their habitat similarity. Craik had 30 genera with three unique genera and moderate individual diet richness (8±0.5 genera/individual overall, 7±0.3 *rbcL*, 5±0.4 *ITS2*), with diets distinguished by high prevalence of *Epilobium* (78%), Rumex (71%), *Chamaenerion* (67%), and *Vaccinium* (67%). Glentress had the fewest genera present (17) with no unique genera and comparable individual richness (9±1 genera per individual overall, 7±0.6 *rbcL*, 6±0.1 *ITS2*), with diets characterised by 100% prevalence of *Vaccinium*, and high occurrences of *Calluna* and *Oxalis* (83% each), followed by *Epilobium* (67%), and *Erica*, *Picea*, and *Rubus* (58% each). Although the diversity of diets varied significantly for *rbcL* (ten out of 15 significant pairwise comparisons), only Trossachs Forests and Abernethy diets differed in diversity for *ITS2*.

Table 2. Comparisons of alpha diversity indices of habitats within localities and plant genera detected by *rbcL* and *ITS2* between sites. P values were obtained from pairwise Wilcoxon rank sum tests and were adjusted using the Benjamini–Hochberg method.

	Localities		***rbcL***		***ITS2***	
Comparison	**Richness**	**Shannon**	**Richness**	**Shannon**	**Richness**	**Shannon**
Craik vs Abernethy	0.003*	0.041*	<0.001*	<0.001*	0.014	0.466
Glen Finglas vs Abernethy	0.025*	0.371	0.416	0.17	0.012	0.308
Glen Finglas vs Craik	<0.001*	0.001*	<0.001*	<0.001*	0.988	0.818
Glenfeshie vs Abernethy	<0.001*	<0.001*	0.003*	0.030*	0.012	0.082
Glenfeshie vs Craik	<0.001*	0.002*	0.002*	0.030*	0.988	0.466
Glenfeshie vs Glen Finglas	<0.001*	<0.001*	0.043*	0.001*	0.988	0.669
Glentress vs Abernethy	0.189	0.084	<0.001*	0.030*	0.03	0.162
Glentress vs Craik	0.396	0.621	0.99	0.971	0.789	0.378
Glentress vs Glen Finglas	<0.001*	0.002*	<0.001*	0.002*	0.782	0.391
Glentress vs Glenfeshie	0.003*	0.065	0.022*	0.236	0.691	0.574
Trossachs Forests vs Abernethy	0.010*	0.206	<0.001 *	<0.001 *	<0.001 *	0.049
Trossachs Forests vs Craik	0.494	0.788	0.141	0.188	0.15	0.316
Trossachs Forests vs Glen Finglas	<0.001*	0.049*	<0.001*	<0.001*	0.154	0.378
Trossachs Forests vs Glenfeshie	0.015*	0.002*	<0.001*	0.002*	0.183	0.474
Trossachs Forests vs Glentress	0.424	0.352	0.342	0.31	0.719	0.893

Site significantly influenced plant composition in roe deer diets for both markers (*rbcL*: R^2=0.18, p=0.001, Figure 5B; *ITS2*: R^2=0.18, p=0.001, Figure 5C), explaining nearly twice the variance of seasonal effects. The interaction between season and site was also significant for both markers (*rbcL*: R^2=0.09, p=0.001; *ITS2*: R^2=0.09, p=0.001), indicating that seasonal shifts in diet composition varied in magnitude among sites. Based on pairwise comparisons (Table 3), in the Cairngorms, Glenfeshie and Abernethy diets differed significantly in composition for both markers (*rbcL* R^2=0.08, p=0.015; *ITS2* R^2=0.09, p=0.015), demonstrating within-region dietary variation**.** Abernethy diets were particularly distinctive, differing from all other sites for *ITS2* and showing the greatest cross-regional dissimilarity with Trossachs Forests (R^2=0.24). In the Trossachs, both sites showed distinct dietary compositions from most other locations. Glen Finglas diets differed from all other sites for *ITS2*, while Trossachs Forests diets differed from all other sites for *rbcL*. The greatest dissimilarity for *rbcL* occurred

between the two geographically distant sites Glen Finglas (Trossachs) and Craik (Borders) (R^2=0.23). In the Borders, Craik and Glentress showed contrasting patterns between markers: diets did not differ significantly for *ITS2* (R^2=0.04, p=1.0) but did differ for *rbcL* (R^2=0.08, p=0.045) suggesting marker-specific sensitivity to compositional variation. Craik diets were notably distinctive for *rbcL*, differing significantly from all other sites.

Within-site dietary variability - that is, the degree of variation in diet composition among individual deer within a site - differed significantly across sites (*rbcL* p<0.001, Figure 5E; *ITS2* p<0.001, Figure 5F). Abernethy consistently showed the most uniform diets (distance to centroid: *rbcL*=0.33, *ITS2*=0.31), significantly more uniform than Craik (0.54), Glenfeshie (0.49) and Trossachs Forests (0.49) for *rbcL*, and all other sites for *ITS2*. Glen Finglas diets were also more uniform than Craik and Glenfeshie with the *rbcL* marker.

Table 3. Pairwise comparisons of Bray–Curtis dissimilarities between sites for localities, *rbcL* and *ITS2*. p.adj=adjusted p value, BD p.dj=adjusted p value for beta dispersion. Values in bold represent pairwise comparisons that were significant for both diet markers.

	Localities			***rbcL***			***ITS2***		
Comparison	**R^2**	**p.adj**	**BD p.adj**	**R^2**	**p.adj**	**BD p.adj**	**R^2**	**p.adj**	**BD p.adj**
Abernethy vs Glen Finglas	0.25	0.015*	0.982	0.15	0.015*	0.1	0.16	0.015*	0.001*
Abernethy vs Trossachs Forests	0.12	0.015*	0.486	0.19	0.015*	0.017*	0.24	0.015*	0.018*
Craik vs Abernethy	0.13	0.015*	<0.001*	0.21	0.015*	<0.001*	0.21	0.015*	<0.001*
Craik vs Glen Finglas	0.43	0.015*	0.015*	0.23	0.015*	<0.001*	0.2	0.015*	0.916
Craik vs Glenfeshie	0.35	0.015*	0.09	0.13	0.015*	0.874	0.12	0.015*	0.1
Craik vs Glentress	0.06	1	0.902	0.08	0.045*	0.299	0.04	1	0.98
Craik vs Trossachs Forests	0.09	0.030*	0.495	0.08	0.045*	0.967	0.13	0.015*	0.852
Glenfeshie vs Abernethy	0.19	0.015*	0.317	0.08	0.015*	<0.001*	0.09	0.015*	<0.001*
Glenfeshie vs Glen Finglas	0.24	0.015*	0.859	0.06	0.015*	0.004*	0.07	0.015*	0.843
Glenfeshie vs Glentress	0.22	0.015*	0.028*	0.04	0.135	0.686	0.04	0.135	0.962
Glenfeshie vs Trossachs Forests	0.18	0.015*	0.1	0.09	0.015*	1	0.11	0.015*	0.77
Glentress vs Abernethy	0.08	0.135	<0.001*	0.08	0.24	0.803	0.1	0.045*	0.043*
Glentress vs Glen Finglas	0.37	0.015*	0.005*	0.08	0.225	0.903	0.11	0.015*	1
Glentress vs Trossachs Forests	0.1	0.075	0.163	0.12	0.045*	0.788	0.09	0.255	0.1

3.6. Habitat characteristics across sites

Habitat richness stabilised at a 500m buffer radius (14.9 at 150m, 15.0 at 250m, 15.3 at 500m, 15.3 at 750m, 15.7 at 1000m), which was therefore selected for all analyses. The area enclosed by this radius (78.5 ha) falls within the range of recent GPS-derived estimates of roe deer home range size in northeast Scotland (weighted mean 65.3 ha, range 32.2–122.5 ha; Marshall et al., 2026). Within the 190 localities (500m buffers), we identified 21 different EUNIS level 2 habitat categories, dominated by “G3 Coniferous woodland”, “F4 Temperate shrub heathland”, “E2 Mesic grasslands”, “G1 Broadleaved deciduous woodland”, and “E1 Dry grasslands” (Figure 6).

3.7. Spatial variation in habitat diversity

The richness and Shannon's diversity of habitats within localities varied significantly across sites (richness $p<0.001$, Shannon's $p<0.001$, Table 2, Figure 4A-B). Glenfeshie demonstrated the highest habitat richness (mean richness=11.8±0.4) and diversity (mean Shannon's=1.6±0.06) of all sites, with 19 habitats, dominated by "G1 Broadleaved deciduous woodland" (mean proportion = 26%), "F4 Temperate shrub heathland" (25%), and "E2 Mesic Grasslands" (12%). Within the Cairngorms, Abernethy localities were significantly less rich and diverse than those at Glenfeshie (mean richness=7.4±0.5: mean Shannon's=1.04±0.09, both $p<0.001$) and comprised 20 habitats dominated by "G3 Coniferous woodland" (33%) and "F4 Temperate shrub heathland" (30%), with no other habitat type exceeding 5%. Although Abernethy localities were compositionally more concentrated, with only two habitat types exceeding 5% compared with six at Glenfeshie, the number of habitat types recorded was comparable between the two sites (20 and 19 respectively), indicating a difference in the evenness rather than the range of habitats sampled. In the Trossachs, Glen Finglas consistently showed significantly lower habitat richness and diversity (mean richness=6.8±0.3: mean Shannon's=1.0±0.06) compared to all other sites and was comprised of 17 habitats dominated by "F4 Temperate shrub heathland" (38%), "E1 Dry grasslands" (25%), and "E3 Seasonally wet and wet grasslands" (25%). In contrast, the two Borders sites (Craik and Glentress) showed similar habitat characteristics ($p=0.396$ for richness, $p=0.621$ for Shannon's), representing the only site pair within a region that did not differ significantly.

3.8. Spatial variation in habitat composition

Site significantly influenced habitat composition (beta diversity) within localities ($R^2=0.36$, $p=0.001$), explaining 36% of the observed variation (Figure 5A). Season ($R^2=0.02$, $p=0.024$,

Figure 3A) and the interaction between site and season (R^2=0.08, p=0.002) also accounted for significant variation, though pairwise seasonal comparisons showed no significant differences.

In the Cairngorms, Glenfeshie and Abernethy localities differed significantly in composition (R^2=0.19, p=0.015), reinforcing that even sites within the same region exhibited distinct habitat configurations**.** Glenfeshie localities showed the most pronounced differences from other sites across Scotland (five significant pairwise differences). In the Trossachs, both sites differed markedly from most other locations, with Glen Finglas localities being compositionally distinct from all other sites. The greatest dissimilarity between any site pair occurred between Glen Finglas and Craik (R^2=0.43). In the Borders, Craik and Glentress localities did not differ significantly in composition (R^2=0.06, p=1.0), representing the only regional site pair that showed compositional similarity. Craik consisted of 17 habitats dominated by "G3 Coniferous woodland" (45%), "O Bare after post" (14%), and "G5 Lines of trees, small anthropogenic woodlands, recently felled woodlands, early-stage woodland, and coppice" (14%). Cross-regional comparisons revealed that Glentress (Borders) and Trossachs Forests showed no significant compositional differences (R^2=0.10, p=0.075), while Craik and Trossachs Forests differed only weakly (R^2=0.09, p=0.030), again reinforcing that habitat composition patterns do not align strictly with regional boundaries.

3.9. Within-site habitat heterogeneity

Analysis of within-site variation revealed significant differences in the uniformity of habitat types and their relative proportions across sites (p<0.001) (Table 3, Figure 5D). In the Cairngorms, both Glenfeshie and Abernethy localities showed relatively high variability in

habitat composition (distances to centroid: 0.37 and 0.43, respectively), with no significant difference in dispersion between them (BD p.adj=0.317), indicating similar levels of within-site heterogeneity. In the Trossachs, Glen Finglas localities were highly variable (0.41), while Trossachs Forests localities were more uniform (0.36), though this within-region difference in dispersion was not statistically significant. The Borders showed the most uniform habitat compositions overall, with both Craik (0.29) and Glentress (0.25) exhibiting low within-site variation and no significant difference in dispersion between them (BD p.adj=0.902). Glentress localities demonstrated the most uniform habitat composition of all sites, differing significantly in dispersion from the more heterogeneous Cairngorms sites (Abernethy BD p.adj<0.001, Glenfeshie BD p.adj=0.028) and from Glen Finglas (BD p.adj=0.005). Similarly, Craik localities showed significantly greater uniformity than the more variable Abernethy (BD p.adj<0.001) localities. This pattern suggests that the Borders sites not only share similar habitat types (as shown by compositional analyses) but also exhibit consistently low within-site habitat heterogeneity, while the Cairngorms and Trossachs display greater landscape complexity at fine spatial scales.

3.10. Seasonal variation in habitat diversity

Both the richness and Shannon's diversity measures of habitats within localities varied significantly across seasons (richness p=0.028, Shannon's p=0.019). Winter localities exhibited significantly higher richness (mean richness=10.7±0.5, p=0.018) and diversity (mean Shannon's=1.5±0.07, p=0.012) compared to spring localities (mean richness=8.8±0.4: Shannon's=1.2±0.06) (Figure 2A-B). This pattern reflects seasonal differences in where deer were culled rather than temporal change in the landscape itself; habitat classifications are derived from a single static landcover map.

682

683 3.11. Using habitat characteristics to predict deer diet

684 3.11.1. Habitat and diet richness patterns

685 Habitat richness showed a weak but significant positive relationship with dietary richness for
686 *rbcL* (Est= 0.3±0.084; R^2=0.04; p=0.003) but not for *ITS2* or the combined marker richness.
687 The relationship strengthened in summer for *ITS2* (Est=0.6±0.189; R^2=0.18; p=0.001),
688 suggesting 0.6 unique plant genera were detected per additional habitat in a locality. No
689 other seasonal or site-specific relationships were detected, and the diversity of habitats
690 within localities showed no significant relationship with diet diversity.

691

692 3.11.2. Habitat and diet compositional correlations

693 Mantel tests revealed significant positive correlations between habitats and diet dissimilarity
694 matrices for both markers (*rbcL* Mantel r=0.13, p=0.001, *ITS2* Mantel r=0.13, p=0.001),
695 suggesting that deer culled from localities with similar habitat compositions tended to have
696 more similar diets than those from localities with divergent habitat compositions.
697 Correlations were strongest during winter (*rbcL* Mantel r=0.26, p=0.001, *ITS2* Mantel r=0.24,
698 p=0.001) and summer (*rbcL* Mantel r=0.20, p=0.001, *ITS2* Mantel r=0.33, p=0.001), weaker in
699 autumn (*rbcL* only Mantel r=0.20, p=0.001), and weakest during spring (*ITS2* only Mantel
700 r=0.11, p=0.02).

701

702 3.11.3. Habitat-based predictions of plant presence in deer diets

703 Focusing on the 21 plant genera and 18 habitats associations which met the screening
704 threshold (|rs| ≥0.3; Supplementary Table S7), logistic regression analysis revealed 30
705 significant season-specific relationships between habitat area within localities and the

presence of specific plant genera in diets. These involved 11 unique habitats and 14 unique plant genera, with an equal number of positive and negative associations (Table 4). Summer and autumn showed the most relationships (11 and 10), while winter and spring showed fewer (5 and 4). Summer relationships were predominantly positive (7/11), suggesting that increased habitat abundance during this season generally corresponds to a higher likelihood of related plants appearing in the diet.

Open and wet habitats such as “D1 Raised and blanket bog”, “D2 Valley mires, poor fens and transition mires”, “E1 Dry grasslands”, and “E3 Seasonally wet and wet grasslands” exhibited exclusively negative relationships, while woodland habitats tended to show more positive relationships, with habitats like “G1 Broadleaved deciduous woodland” and “G4 Mixed deciduous and coniferous woodland” showing only positive relationships.

Oxalis showed the most habitat relationships (6), followed by *Chamaenerion*, *Myrica*, *Rubus*, and *Quercus* (3 each). *Alnus*, *Rubus*, *Rumex* and *Epilobium* showed consistently positive relationships, indicating that their presence in the diet is positively related to the abundance of certain habitats, while *Calluna*, *Picea*, *Pinus*, *Potentilla* and *Salix* showed negative relationships, suggesting that their presence in the diet decreased as the area of certain habitats increased.

The strongest positive relationships occurred between habitat “E2 Mesic grasslands” and *Quercus* in autumn (22x higher odds per 20m^2), between habitat “G5 Lines of trees, small anthropogenic woodlands, recently felled woodland, early-stage woodland and coppice” and *Chamaenerion* in summer (17.65x higher odds per 20m^2), and between habitat “G3

730 Coniferous woodland" and *Myrica* in winter (14x higher odds per 20m²) (Table 4).
731 Conversely, the strongest negative relationships were found between habitat "D1 Raised and
732 blanket bog" and *Quercus* in autumn (97% decrease in the odds of Quercus presence per
733 20m²), between habitat "F9 Riverine and fen scrubs" and *Pinus* in winter (96.4% decrease in
734 odds per 20m²), and between habitat "D2 Valley mires, poor fens and transition mires" and
735 *Picea* in winter (94.7% decrease in odds per 20m²).
736
737 3.11.4. Habitat-based predictions of plant abundance in deer diets
738 Of the 30 seasonally specific relationships between the proportional area of habitat types
739 within localities (500m buffers) and plant genera presence in deer diets, 21 showed
740 significant effects on the proportion of these plants when present in the diet (Table 4), with
741 negative relationships (13) outnumbering positive ones (8). Autumn and summer showed
742 the most significant associations (7 and 6), followed by winter (5) and spring (3). Negative
743 relationships dominated in autumn (5/7) and winter (4/5).
744
745 Habitats "D1 Raised and blanket bog", "D2 Valley mires, poor fens, and transition mires", "E1
746 Dry grasslands", and "G3 Coniferous woodland" were associated with decreased proportions
747 of certain plants in the diet as habitat area increased. In contrast, mixed and deciduous
748 habitats ("G1 Broadleaved deciduous woodland" and "G4 mixed deciduous and coniferous
749 woodland") tended to show positive relationships.
750
751 *Rubus*, *Myrica*, and *Quercus* each demonstrated three significant relationships with habitat
752 area. *Rubus* showed responsiveness across spring, summer and different habitat types. In
753 contrast, *Myrica* and *Quercus* showed strong seasonal specificity (winter and autumn,

754 respectively) across different habitat types. The strongest positive relationships were
755 between "E5 Woodland fringes and clearings and tall forb stands" and *Rubus* (spring, +7.7%
756 prevalence in diets per 20m² increase) and *Quercus* (autumn, +4.2% per 20m²). The
757 strongest negative relationship occurred between "F9 Riverine and fen scrubs" and *Pinus*
758 (winter, -13.2% per 20m²). Notably, some plant-habitat combinations showed opposing
759 trends between presence/absence and proportional analyses (highlighted in bold in Table 4),
760 suggesting complex foraging strategies where habitat abundance influences diet
761 composition through multiple mechanisms.
762
763 Table 4. Logistic regression results for significant season-specific relationships between
764 proportional habitat area within localities (500m buffers) and (a) the presence and (b) the
765 relative abundance (proportion) of plant genera in roe deer diets. Proportion models were
766 fitted only for relationships significant in the presence/absence models; n.s. = not significant.
767 Odds ratios (OR) greater than 1 indicate an increased likelihood (presence model) or
768 proportion (proportion model) of the plant genus in the diet as habitat area increases;
769 values less than 1 indicate a decrease. Rows in bold indicate relationships with opposing
770 directions between the presence and proportion models. CI = confidence interval.

			Presence/absence model				**Proportion model**			
Habitat	**Plant genus**	**Season**	**OR**	**+/–**	**CI**	**P val**	**OR**	**+/–**	**CI**	**P val**
D1 Raised and blanket bog	*Hypericum*	Autumn	0.12	–	1.855	0.025	n.s.			
D1 Raised and blanket bog	*Oxalis*	Autumn	0.14	–	1.654	0.018	0.98	–	0.013	<0.001
D1 Raised and blanket bog	*Oxalis*	Summer	0.11	–	1.739	0.012	n.s.			
D1 Raised and blanket	*Quercus*	Autumn	0.03	–	2.306	0.003	0.96	–	0.020	<0.001

bog										
D2 Valley mires, poor fens and transition mires	*Chamaenerion*	Spring	0.16	–	1.398	0.009	n.s.			
D2 Valley mires, poor fens and transition mires	*Oxalis*	Autumn	0.11	–	2.208	0.049	n.s.			
D2 Valley mires, poor fens and transition mires	*Picea*	Winter	0.05	–	2.169	0.008	0.98	–	0.007	<0.001
E1 Dry grasslands	*Oxalis*	Summer	0.11	–	1.226	0.020	n.s.			
E1 Dry grasslands	*Salix*	Autumn	0.14	–	1.724	0.025	0.99	–	0.012	<0.001
E2 Mesic grasslands	*Calluna*	Autumn	0.08	–	2.283	0.032	0.99	–	0.001	<0.001
E2 Mesic grasslands	*Quercus*	Autumn	22.00	+	2.261	0.007	1.01	+	0.002	<0.001
E2 Mesic grasslands	***Rumex***	**Summer**	**5.95**	**+**	**1.617**	**0.031**	**0.99**	**–**	**0.001**	**<0.001**
E3 Seasonally wet and wet grasslands	*Oxalis*	Summer	0.18	–	1.419	0.017	n.s.			
E5 Woodland fringes, clearings, and tall forb stands	***Calluna***	**Spring**	**2.40**	**+**	**1.418**	**0.049**	**0.98**	**–**	**0.001**	**<0.001**
E5 Woodland fringes, clearings, and tall forb stands	*Oxalis*	Autumn	8.75	+	1.773	0.017	n.s.			
E5 Woodland fringes, clearings, and tall forb stands	*Quercus*	Autumn	6.67	+	1.783	0.037	1.04	+	0.008	<0.001
E5 Woodland fringes, clearings, and tall forb stands	*Rubus*	Spring	4.17	+	1.418	0.049	1.08	+	0.022	<0.001
F9 Riverine and fen scrubs	***Alnus***	**Autumn**	**12.00**	**+**	**1.914**	**0.011**	**0.97**	**–**	**0.011**	**<0.001**
F9 Riverine and fen scrubs	*Myrica*	Winter	5.28	+	1.289	0.011	1.03	+	0.009	<0.001
F9 Riverine and fen scrubs	*Pinus*	Winter	0.04	–	2.143	0.002	0.89	–	0.107	<0.001
G1 Broadleaved	*Rumex*	Summer	6.03	+	1.508	0.020	1.00*	+	0.001	<0.001

deciduous woodland										
G3 Coniferous woodland	***Myrica***	**Winter**	**14.00**	**+**	**2.191**	**0.018**	**0.99**	**–**	**0.001**	**<0.001**
G3 Coniferous woodland	*Potentilla*	Summer	0.18	–	1.420	0.019	0.99	–	0.001	<0.001
G3 Coniferous woodland	***Rubus***	**Summer**	**5.85**	**+**	**1.619**	**0.033**	**0.99**	**–**	**0.004**	**<0.001**
G4 Mixed deciduous and coniferous woodland	*Hypericum*	Summer	5.33	+	1.258	0.033	n.s.			
G4 Mixed deciduous and coniferous woodland	*Rubus*	Summer	6.75	+	1.537	0.003	1.01	+	0.001	<0.001
G5 Lines of trees, small anthropogenic woodlands, recently felled woodland, early-stage woodland and coppice	*Chamaenerion*	Spring	11.25	+	2.097	0.025	1.00*	+	0.001	<0.001
G5 Lines of trees, small anthropogenic woodlands, recently felled woodland, early-stage woodland and coppice	*Chamaenerion*	Summer	17.65	+	2.117	0.008	1.00*	+	0.001	<0.001
G5 Lines of trees, small anthropogenic woodlands, recently felled woodland, early-stage woodland and coppice	*Epilobium*	Summer	10.23	+	2.117	0.032	n.s.			
G5 Lines of trees, small anthropogenic woodlands, recently felled woodland, early-stage woodland and coppice	*Myrica*	Winter	0.11	–	1.537	0.003	0.99	–	0.004	<0.001

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4. Discussion

4.1. Reliability of molecular dietary analysis

Before interpreting our findings, it is important to assess the reliability of our metabarcoding approach. Technical replicates processed independently through separate laboratory workflows and sequencing runs showed identical taxonomic richness, near-identical diversity indices, and minimal compositional dissimilarity, demonstrating high methodological reproducibility. The two complementary markers were significantly positively correlated in both alpha and beta diversity, while each detected unique taxa, enhancing overall taxonomic coverage. The ecological coherence of the results - consistent site-specific diets and seasonal variation aligning with known phenological changes - further indicates that the data captured ecological processes rather than methodological artefacts. Together, these lines of evidence indicate that our molecular data provide a reproducible and representative assessment of roe deer diets across the study sites.

4.2. Spatial primacy in diet determination

Previous research throughout Britain has documented strong spatial variation in roe deer diet (Hosey, 1981; Calder, 1994; de Jong et al., 1995; Latham et al., 1999; de Jong et al., 2004), typically attributing these differences to local forage availability (Maizeret & Sung, 1984; Calder, 1994; Tixier et al., 1997; Minder, 2006). Concurrently, researchers have identified seasonal fluctuations in diet composition throughout Europe (Tixier and Duncan, 1996; Cornelis et al., 1999; Hirst et al., 2026). In the Scottish populations studied, site location explained nearly twice as much variation in diet composition (18%) as seasonal effects (10%). This highlights the predominance of spatial over temporal factors in shaping roe deer foraging ecology, suggesting that landscape configuration and local habitat

composition exert a stronger influence on diet selection than seasonal shifts in plant phenology and nutritional requirements. Moreover, the significant season-by-site interaction indicates that even seasonal dietary shifts were not uniform across Scotland, further underscoring the role of local landscape context in shaping foraging patterns (see Supplementary Materials S6).

The spatial structuring of diet operated at the site rather than the regional scale: sites within the same region sometimes differed as markedly in diet composition as sites in different regions (e.g. Glenfeshie and Abernethy in the Cairngorms), whereas some cross-regional site pairs were compositionally similar (e.g. Glentress and Trossachs Forests). Dietary differentiation therefore reflects local habitat composition rather than broad regional context, mirroring the equivalent pattern in habitat composition itself. This spatial dominance contrasts with the traditional emphasis on seasonality in roe deer dietary studies and underscores the importance of considering fine-scale habitat heterogeneity when predicting and managing roe deer foraging patterns.

4.3. Seasonal variation in roe deer diets

Roe deer diets exhibit significant seasonal variations (see reviews: Tixier and Duncan, 1996; Cornelis et al., 1999; Hirst et al., 2026), and our data reflected this in dietary richness, diversity and composition. Both molecular markers detected significant seasonal shifts in the richness and Shannon's diversity of plant genera in roe deer diets, with summer diets exhibiting the highest diversity and winter diets showing the lowest. The 43 genera detected in summer diets, including eight unique to this season, contrasted markedly with winter diets comprising only 29 genera and just two unique to this period. This seasonal pattern

aligns with plant phenology and availability across Scottish landscapes, where summer offers the greatest abundance and diversity of high-quality herbaceous forage resources (Henry, 1978; Jackson, 1980; Hinge, 1986; Calder, 1994; Latham et al., 1999), and winter diets shift towards a less diverse browse-based diet when forage availability is limited (Hosey, 1974; Henry, 1975; Calder, 1994; Tixier et al., 1997; Latham et al., 1999).

Season significantly influenced plant genera composition in roe deer diets for both markers, with winter diets significantly distinct from all other seasons. These compositional differences reflect not only seasonal availability but potentially shifting nutritional requirements and foraging strategies throughout the year (Tixier and Duncan, 1996; Moser et al., 2006). *ITS2* data revealed significantly more uniform winter diets compared to the more variable spring and summer diets, suggesting reduced dietary flexibility during resource-limited periods. This reduced flexibility likely arises because high-quality winter forage is scarce and patchily distributed across the landscape, concentrating foraging within the habitat patches that retain viable resources (Mysterud et al., 1999; Storms et al., 2008).

4.4. Habitat heterogeneity across study sites

The composition and configuration of available habitats shape herbivore foraging opportunities through differences in the plant species encountered and their nutritional value (Mysterud et al., 2001; Storms et al., 2006; Krojerová-Prokešová et al., 2010; Yuan et al., 2022; Dahl et al., 2023), and plant availability is an established determinant of roe deer diet selection (de Jong et al., 1995; Tixier et al., 1997; Duncan et al., 1998; Cornelis et al., 1999; Minder, 2012; Freschi et al., 2021; Yuan et al., 2022).

Site location explained over a third of the variation in habitat composition within localities, and this variation was structured by the contrasting character of the three regions. The Borders sites, Craik and Glentress, are commercial conifer plantations, and were correspondingly the most internally homogeneous: localities shared similar habitat suites dominated by coniferous woodland, felled ground and improved grassland, and the two sites formed the only pair within any region that did not differ significantly in habitat composition.

The Cairngorms sites, by contrast, differed as much from each other as from sites in other regions despite their proximity and their shared status as landscape-scale restoration projects (Gullett et al., 2023). Localities sampled at Abernethy were dominated by Caledonian pinewood with an ericaceous understorey, whereas those at Glenfeshie spanned broadleaved woodland, heathland and grassland mosaics and had the highest habitat richness of any site. Both reserves contain a comparable range of habitat types at the whole-site scale, so this contrast reflects where deer were culled rather than the habitats each reserve contains. The Trossachs region contained the study's most divergent pair of land uses: Glen Finglas, a largely unwooded estate of heathland and grassland with the lowest habitat richness of all sites, and the Trossachs Forests, commercial plantations compositionally more similar to the distant Borders sites than to their regional neighbour. Habitat structure therefore did not follow regional boundaries but management context - plantation forestry produced convergent, uniform habitat mosaics wherever it occurred, while semi-natural and restoration landscapes generated the greatest heterogeneity, both within and between localities. These differences in habitat availability provide a structural

basis for the site-based dietary variation described above; we next examine how directly habitat metrics predict diet.

4.5. Linking habitat metrics to diet patterns

The relationship between habitat metrics and corresponding dietary patterns revealed complex connections between landscape structure and roe deer foraging. We found that habitat richness showed a weak but significant positive relationship with dietary richness for *rbcL*, suggesting that greater habitat variety moderately increased the total number of plant genera consumed. However, this relationship was not detected for *ITS2* or the combined richness of markers, indicating marker-specific sensitivity to habitat-diet connections. This marker-specific difference likely reflects the inherent taxonomic biases of each barcode region (Kress and Erickson, 2007; Chen et al., 2010; de Vere et al., 2012). The *rbcL* marker typically performed better at detecting and distinguishing woody plant taxa, including deciduous and coniferous trees that characterise different woodland habitats, making it more sensitive to habitat-level distinctions. In contrast, *ITS2* appeared to recover higher proportions of herbaceous taxa, which are often more ephemeral and less habitat-specific across landscapes.

We observed no significant relationship between habitat diversity (Shannon's index) and diet diversity across the entire dataset, suggesting that the number of habitat types, rather than their relative proportions, better predicts dietary patterns in roe deer. When analysing compositional similarities, significant positive correlations were revealed between habitat and diet dissimilarity matrices for both markers, demonstrating that localities with similar habitat compositions tended to support more similar diets. These compositional

relationships were stronger than the relationships between habitat richness and dietary richness, suggesting that the specific combination and relative proportions of habitats better reflect dietary composition than simple habitat count metrics. This compositional correspondence aligns with previous research suggesting that roe deer diet reflects the spatial arrangement and availability of plant communities (de Jong et al., 1995; Tixier et al., 1997; Freschi et al., 2021). Despite the statistical significance of these correlations, their moderate strength suggests that while habitat composition influences dietary composition, other factors such as individual selectivity, plant nutritional quality, and fine-scale resource distribution likely play substantial roles in determining the specific plant genera consumed by roe deer in different landscapes (Mysterud et al., 1999; Obidziński et al., 2013; Dupke et al., 2017).

4.6. Seasonal modulation of habitat-diet relationships

The relationship between habitat and diet metrics exhibited distinct seasonal patterns, revealing when landscape characteristics most strongly predict roe deer foraging choices. Habitat-diet compositional correlations varied markedly across seasons, showing substantially stronger associations during winter and summer. These seasonal peaks exceed the overall habitat-diet compositional correlation, indicating that temporal context significantly influences the predictive power of habitat composition for diet.

The stronger winter correspondence between habitat and diet composition is consistent with this concentration of foraging within remaining resource patches: with fewer alternatives available, individual winter diets more closely tracked the proportional availability of habitats within localities, despite the reduced dietary diversity of this season.

Similarly, the pronounced correlation between habitat composition and diet composition during summer suggests that even amid resource abundance, roe deer diets remain closely tethered to local habitat structure. This pattern aligns with observations that roe deer exhibit highly selective foraging behaviour during summer, preferentially targeting nutritionally optimal forage items that are differentially distributed across habitat types (Mussa et al., 2003). The summer relationship may also reflect reduced movement patterns and more stable, resource-driven habitat selection during this period of abundance (Kjellander et al., 2004; Morellet et al., 2013), further enhancing the predictive power of local habitat composition for dietary outcomes.

Beyond compositional relationships, the particularly strong association between habitat and dietary richness during summer for *ITS2*, explaining four times more variance than the overall habitat-richness relationship, reinforces our understanding that during peak growing season, roe deer capitalise on habitat richness by incorporating a wider range of forage items into their diet. The absence of this relationship in other seasons suggests that dietary richness becomes increasingly decoupled from habitat richness as plant phenology advances beyond peak growing season or recedes during winter dormancy, when fewer plant species remain viable food sources regardless of habitat diversity.

These contrasting mechanisms - resource limitation in winter versus selective optimisation in summer (Andersen et al., 1998; Morellet et al., 2011) - mean that both the strength and the nature of habitat–diet relationships shift across the annual cycle (Tixier et al., 1997; Abbas et al., 2011). Models predicting roe deer diet from habitat characteristics therefore

941 require seasonal stratification, and management interventions based on such predictions
942 must be seasonally targeted.
943
944 4.7. Habitat-specific plant associations and contradictory patterns
945 Our habitat-based predictions of plant presence and abundance in roe deer diets revealed
946 complex, season-specific relationships with important implications for understanding
947 foraging behaviour. Logistic regression identified 30 significant plant-habitat relationships,
948 evenly split between positive and negative associations, which were further explored
949 through proportion-based analyses that yielded 21 significant results.
950
951 The complementary nature of our presence/absence and proportional analyses provides a
952 more comprehensive understanding of habitat-diet relationships than either approach
953 alone. Presence/absence models identify habitats influencing the likelihood of plants being
954 consumed, capturing broader landscape patterns, while proportion-based models reveal
955 how habitat abundance affects the relative importance of plants within the diet.
956
957 Five plant-habitat combinations showed opposing directions between the presence and
958 proportion models. For example, while *Myrica* in winter and Rubus in summer showed
959 positive relationships with the presence of "G3 Coniferous woodland", their proportional
960 contribution to diets decreased as this habitat became more dominant. These opposing
961 patterns likely reflect complex spatial habitat co-occurrences rather than contradictions.
962 *Myrica* typically occurs in wetlands and bogs (Waterhouse et al., 1998), which often intermix
963 with coniferous plantations in Scottish uplands (Smith and Charman, 1988). Similarly, *Rubus*
964 - a highly preferred roe deer forage (Jackson, 1980; Hosey, 1981; González Hernández and

965 Silva-Pando, 1996; Bergquist et al., 1999) - showed a strong positive association with “E5
966 Woodland fringes, clearings and tall forb stands” but decreased proportionally with greater
967 coniferous woodland cover as previously reported (Gill et al., 1996), reflecting its ecological
968 preference for forest edges and clearings.
969
970 The strongest negative relationship observed was between *Pinus* consumption and "F9
971 Riverine and fen scrubs" during winter (-13.2% per 20m² increase), suggesting these habitats
972 may provide alternative forage sources like *Myrica*, which increased proportionally in diets
973 (+3.3% per 20m²) with greater fen scrub coverage. Overall, these patterns indicate that roe
974 deer foraging reflects the distribution of both preferred and less preferred forage across
975 habitat types (Moser et al., 2006; Ward et al. 2008).
976
977 4.8. Management implications
978 Habitat characteristics provide useful, albeit imperfect, predictors of roe deer diet
979 composition. Managers can make moderate inferences about likely diet from habitat data
980 alone, and these inferences will be most accurate when seasonal context is incorporated.
981 Such inferences complement, rather than replace, direct field assessment of herbivore
982 impacts (Armstrong et al., 2025).
983
984 From a practical management perspective, our results indicate that landscape composition
985 particularly influences the presence and abundance of certain key plant genera in roe deer
986 diets. First, promoting habitat richness within landscapes may increase dietary diversity,
987 potentially diluting browsing pressure on any single plant genus. Our summer data showed
988 that each additional habitat type corresponded to approximately 0.6 additional plant genera

in the diet (R^2=0.18), suggesting that heterogeneous landscapes may distribute herbivory impacts more broadly across plant communities.

The negative relationship between *Pinus* consumption and riverine and fen scrub habitats during winter suggests that where such habitats already occur within or adjacent to plantations, they may provide alternative winter forage (e.g. *Myrica*) that reduces browsing pressure on commercial conifers. This is consistent with wider evidence that increasing the availability of alternative winter forage shrubs in commercial conifer woodlands can reduce browsing pressure on coniferous trees (Welch et al., 1991; Ward et al., 2008). Deliberately creating wetland habitat within forestry is unlikely to be practicable - afforestation of deep peat is now discouraged on carbon and biodiversity grounds - but these habitat–diet relationships offer predictive value: stands lacking alternative winter forage in the surrounding mosaic can be identified as being at elevated browsing risk, indicating where tree protection or deer control effort is most needed. Retaining and restoring existing riparian and fen scrub during forest design may nonetheless deliver browsing-mitigation benefits alongside their established biodiversity value.

However, the moderate strength of habitat-diet correlations and the complexity of seasonal relationships indicate important limitations. Our models explain only a portion of the observed dietary variation, suggesting that other factors such as plant nutritional quality, fine-scale vegetation structure, and individual selection behaviour also substantially influence foraging decisions.

These limitations are partly inherent to the remote-sensing approach. Landcover

classification at 20 x 20 m resolution captures the composition and configuration of habitat mosaics, but not the understorey structure, sward height or forage quality that determine what an individual actually encounters and selects within a habitat patch. Nor does a single static classification capture within-habitat phenological change. Against this, the practical advantages are considerable: the classification used here is freely available, consistent across the whole of Scotland, and required no field vegetation survey - the constraint that has historically restricted plant availability data to small spatial extents (Daubenmire, 1959; Bonham et al., 2004; Leonard et al., 2017). Field-based quadrat surveys deliver far finer resolution but cannot realistically be extended across the landscape scales at which deer management operates. Remote-sensed habitat data therefore offer a pragmatic compromise: moderate predictive power obtained at negligible marginal cost, over areas where direct vegetation survey would be prohibitive. For managers, the relevant question is not whether habitat data predict diet perfectly, but whether they predict it well enough to inform decisions that would otherwise be made with no dietary information at all.

Beyond the six sites studied, the specific plant–habitat associations we report are likely to be contingent on Scottish upland vegetation and the particular habitat mosaics sampled and should not be transferred directly to other regions. The general framework, however, is portable: EUNIS-classified landcover data are available across Europe, DNA reference libraries increasingly span national floras, and the core finding - that local habitat composition predicts diet better than season, with predictive power varying seasonally - is testable in any roe deer population. Applying this approach in lowland agricultural landscapes, where crops constitute an important seasonal food source (Hosey, 1974; Henry,

1975; Calder, 1994; Latham et al., 1999), or in continental European forest systems would establish which associations are general and which are locally contingent.

Future research should address the unexplained dietary variation through field-based vegetation survey at a subset of localities, which would also allow the predictive limits of landcover data to be quantified directly. The integration of GPS tracking (e.g. Marshall et al., 2026) with dietary analysis could help clarify the spatial scale at which habitat–diet relationships operate most strongly.

4.9. Conclusion

While habitat characteristics alone cannot fully predict roe deer diets, they provide meaningful insights into foraging patterns that can inform landscape-level management strategies. By understanding how habitat influences diet across seasons, managers can better anticipate where browsing pressure on commercially valuable species will concentrate, and target protection and deer management accordingly. The approach developed here provides a basis for predicting herbivore diets from readily available habitat data, and its predictive power should improve as finer-scale vegetation and movement data become available.

Ethics statement

No animals were killed for the purposes of this study. Rumen samples were collected post-mortem from roe deer culled by professional deer stalkers as part of routine deer management operations, conducted during legal open seasons or under out-of-season licences issued by NatureScot.

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CRediT authorship contribution statement

Christopher Hirst: Conceptualisation, Methodology, Investigation, Formal analysis, Data curation, Resources, Project administration, Visualization, Writing - original draft, Writing - review & editing. **Darren J. Shaw:** Methodology, Supervision, Writing -review & editing. **Rory Putman:** Supervision, Writing - review & editing. **Laura Glendinning:** Methodology, Supervision, Writing - review & editing. **Robin Gill:** Supervision, Writing - review & editing. **Richard A. Ennos:** Methodology, Supervision, Writing - review & editing. **Gerry McLachlan:** Methodology, Supervision, Writing - review & editing. Rob Ogden: Methodology, Supervision, Writing -review & editing.

Data availability

The genus-level dietary data (*rbcL* and *ITS2*), habitat composition data for each locality, and sample metadata used for all analyses are available at Zenodo: https://doi.org/10.5281/zenodo.21982423. Precise cull locations are withheld to protect landowner interests; the habitat variables derived from them are provided in full, so all analyses reported here can be reproduced. The Scotland 2022 Landcover Map is available from Space Intelligence/NatureScot (https://www.space-intelligence.com/scotland-landcover/).

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References

Abbas, F., Morellet, N., Hewison, A. J. M., Merlet, J., Cargnelutti, B., Lourtet, B., Angibault, J. M., Daufresne, T., Aulagnier, S., and Verheyden, H. (2011). Landscape fragmentation generates spatial variation of diet composition and quality in a generalist herbivore. Oecologia, 167, 401–411.

Adhikari, P., Park, S.-M., Kim, T.-W., Lee, J.-W., Kim, G.-R., Han, S.-H., and Oh, H.-S. (2016). Seasonal and altitudinal variation in roe deer (Capreolus pygargus tianschanicus) diet on Jeju Island, South Korea. Journal of Asia-Pacific Biodiversity, 9, 422–428.

Anderson, M.J. (2001). A new method for non-parametric multivariate analysis of variance. *Austral Ecology* 26:32-46.

Andersen, R., Duncan, P., Linnell, J.D.C. (Eds.) (1998). The European Roe Deer: The Biology of Success. Scandinavian University Press, Oslo

Apollonio, M., Andersen, R., and Putman, R. (2010). European Ungulates and Their Management in the 21st Century. Cambridge University Press.

Armstrong, H., Black, B., Holl, K., Thompson, R., 2025. The Woodland Herbivore Impact Assessment Method: User Guide. Scottish Forestry. https://www.forestry.gov.scot/publications/woodland-herbivore-impact-assessment-method-user-guide (accessed [12/08/2026])

Arstingstall, K. A., DeBano, S. J., Li, X., Wooster, D. E., Rowland, M. M., Burrows, S., and Frost, K. (2021). Capabilities and limitations of using DNA metabarcoding to study plant–pollinator interactions. Molecular Ecology, 30, 5266–5297.

Arstingstall, K. A., DeBano, S. J., Li, X., Wooster, D. E., Rowland, M. M., Burrows, S., and Frost, K. (2023). Investigating the use of pollen DNA metabarcoding to quantify bee foraging and effects of threshold selection. PLoS ONE, 18, e0282715.

Augustine, D., and Mcnaughton, S. (1998). Ungulate Effects on The Functional Species Composition of Plant Communities: Herbivore Selectivity and Plant Tolerance. Journal of Wildlife Management, 62, 1165–1183.

1128 Benjamini, Y., and Hochberg, Y. (1995). Controlling the False Discovery Rate: A Practical and
1129 Powerful Approach to Multiple Testing. Journal of the Royal Statistical Society: Series B
1130 (Methodological), 57, 289–300.
1131
1132 Bergquist, J., Örlander, G., and Nilsson, U. (1999). Deer browsing and slash removal affect
1133 field vegetation on south Swedish clearcuts. Forest Ecology and Management, 115, 171–182.
1134
1135 Bokulich, N. A., Kaehler, B. D., Rideout, J. R., Dillon, M., Bolyen, E., Knight, R., Huttley, G. A.,
1136 and Caporaso, J. G. (2018). Optimizing taxonomic classification of marker-gene amplicon
1137 sequences with QIIME 2's q2-feature-classifier plugin. Microbiome, 6, 90.
1138
1139 Bonham, C. D., Mergen, D. E., and Montoya, S. (2004). Plant Cover Estimation: A Contiguous
1140 Daubenmire Frame, 26, 17–22.
1141
1142 Bray, J.R. and Curtis, J.T. (1957) An Ordination of the Upland Forest Communities of Southern
1143 Wisconsin. Ecological Monographs, 27, 325-349.
1144
1145 Calder, C. J. (1994). Population performance and feeding ecology of roe deer in farm
1146 woodland. PhD Thesis, University of Aberdeen.
1147
1148 Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., and Holmes, S. P.
1149 (2016). DADA2: High-resolution sample inference from Illumina amplicon data. Nature
1150 Methods, 13, 581-583.
1151

1152 Casey, F., Old, J. M., and Stannard, H. J. (2023). Assessment of the diet of the critically
1153 endangered northern hairy-nosed wombat (Lasiorhinus krefftii) using DNA metabarcoding.
1154 Ecology and Evolution, 13, e10469.
1155
1156 Chen, S., et al. (2010). Validation of the ITS2 region as a novel DNA barcode for identifying
1157 medicinal plant species. PLoS ONE, 5, 1–8.
1158
1159 Cornelis, J., Casaer, J., and Hermy, M. (1999). Impact of season, habitat and research
1160 techniques on diet composition of roe deer (Capreolus capreolus): A review. Journal of
1161 Zoology, 248, 195–207.
1162
1163 Crawley, M. J. (2007). The R Book. First edition. John Wiley & Sons, Ltd.
1164
1165 Czernik, M., Taberlet, P., Świsłocka, M., Czajkowska, M., Duda, N., and Ratkiewicz, M. (2013).
1166 Fast and efficient DNA-based method for winter diet analysis from stools of three cervids:
1167 Moose, red deer, and roe deer. Acta Theriologica, 58, 379–386.
1168
1169 Dahl, SA., Seifert, J., Camarinha-Silva, A. et al. (2023). Microbiota and Nutrient Portraits of
1170 European Roe Deer (Capreolus capreolus) Rumen Contents in Characteristic Southern
1171 German Habitats. Microb Ecol, **86**, 3082–3096 https://doi.org/10.1007/s00248-023-02308-5
1172
1173
1174 Daubenmire, F. R. (1959). A canopy-coverage method of vegetational analysis. Northwest
1175 Science, 33, 43–64.

Deagle, B. E., Thomas, A. C., McInnes, J. C., Clarke, L. J., Vesterinen, E. J., Clare, E. L., Kartzinel, T. R., and Eveson, J. P. (2019). Counting with DNA in metabarcoding studies: How should we convert sequence reads to dietary data? Molecular Ecology, 28, 391–406.

Dixon, P. (2003). VEGAN, a package of R functions for community ecology. Journal of Vegetation Science, 14, 927–930.

Duncan, P., Tixier, H., Hofmann, R. R., and Lechner-Doll, M. (1998). Feeding strategies and the physiology of digestion in roe deer. In The European Roe Deer: the biology of success. Scandinavian University Press, Oslo, Norway.

Dupke, C., Bonenfant, C., Reineking, B., Hable, R., Zeppenfeld, T., Ewald, M., Heurich, M. (2017). Habitat selection by a large herbivore at multiple spatial and temporal scales is primarily governed by food resources. Ecography, 40, 1014–1027.

Erickson, D. L., Reed, E., Ramachandran, P., Bourg, N. A., McShea, W. J., and Ottesen, A. (2017). Reconstructing a herbivore's diet using a novel rbcL DNA mini-barcode for plants. AoB PLANTS, 9.

Freschi, P., Fascetti, S., Riga, F., Rizzardini, G., Musto, M., and Cosentino, C. (2021). Feeding Preferences of the Italian Roe Deer Mediterranean Environment. Animals, 11, 308.

Getaneh, Z. A., Demissew, S., Woldu, Z., and Aynekulu, E. (2023). Determinants of plant community along environmental gradients in Geramo forest, the western escarpment of the rift valley of Ethiopia. PLoS ONE, 18, e0294324.

Gill, R. M. A. (1992). A review of damage by mammals in north temperate forests: 3. Impact on trees and forests. Forestry, 65, 363–388.

Gill, R. M. A., Johnson, A. L., Francis, A., Hiscocks, K., and Peace, A. J. (1996). Changes in roe deer (Capreolus capreolus L.) population density in response to forest habitat succession. Forest Ecology and Management, 88, 31–41.

González Hernández, M. P., and Silva-Pando, F. J. (1996). Grazing effects of ungulates in a Galician oak forest (northwest Spain). Forest Ecology and Management, 88, 65–70.

Gresham, A., Pillay, K., Healey, J. R., Eichhorn, M. P., Ellison, A., Lowe, A., Cordes, L. S., Creer, S., & Shannon, G. (2025). A continuous feast of bramble: Rubus fruticosus agg. is a key cross-seasonal dietary resource for a fallow deer population. Ecological Solutions and Evidence, 6, e70008. https://doi.org/10.1002/2688-8319.70008

Gullett, P. R., Leslie, C., Mason, R., Ratcliffe, P., Sargent, I., Beck, A., Cameron, T., Cowie, N. R., Hetherington, D., MacDonell, T., Moat, T., Moore, P., Teuten, E., & Hancock, M. H. (2023). Woodland expansion in the presence of deer: 30 years of evidence from the Cairngorms Connect landscape restoration partnership. Journal of Applied Ecology, 60, 2298–2308. https://doi.org/10.1111/1365-2664.14501

1222 Hanley, T. A. (1997). A Nutritional View of Understanding and Complexity in the Problem of
1223 Diet Selection by Deer (Cervidae). Oikos, 79, 209–218.
1224
1225 Henry, B. A. M. (1975). Dispersion, diet and criteria of age of roe deer (Capreolus capreolus
1226 L.) in Hamsterley forest, county Durham. PhD Thesis, Durham University.
1227
1228 Hewison, A.J.M., Staines, B.W. (2008). Roe deer. In: Harris, S., Yalden, D.W. (Eds.), Mammals
1229 of the British Isles: Handbook, 4th ed. The Mammal Society, Southampton, pp. 605–617.
1230
1231 Hidding, B., Tremblay, J.-P., and Côté, S. D. (2013). A large herbivore triggers alternative
1232 successional trajectories in the boreal forest. Ecology, 94, 2852–2860.
1233
1234 Hijmans, R. J., et al. (2023). raster: Geographic Data Analysis and Modeling. R package
1235 version 3.6-26. https://CRAN.R-project.org/package=raster
1236
1237 Hinge, M. D. C. (1986). Ecology of red and roe deer in a mixed-age conifer plantation:
1238 comparative studies on habitat selection, ranging behaviour and feeding strategies. PhD
1239 Theses, University of Edinburgh.
1240
1241 Hirst, C., Gill, R., Ogden, R., et al. (2026). Characterising the dietary patterns of the European
1242 roe deer across biogeographical regions. European Journal of Wildlife Research, 72, 6.
1243 https://doi.org/10.1007/s10344-025-02038-8
1244

1245 Hosey, G. R. (1974). The food and feeding ecology of roe deer (Capreolus capreoulus). PhD
1246 Thesis, University of Manchester.
1247
1248 Hosey, G. R. (1981). Annual foods of the Roe deer (Capreolus capreolus) in the south of
1249 England. Journal of Zoology, 194, 276–278.
1250
1251 Illius, A. W., Duncan, P., Richard, C., and Mesochina, P. (2002). Mechanisms of functional
1252 response and resource exploitation in browsing roe deer. Journal of Animal Ecology, 71, 723–
1253 734.
1254
1255 Jackson, J. (1980). The annual diet of the Roe deer (Capreolus capreolus) in the New Forest,
1256 Hampshire, as determined by rumen content analysis. Journal of Zoology, 192, 71–83.
1257
1258 Jones, L., Twyford, A. D., Ford, C. R., Rich, T. C. G., Davies, H., Forrest, L. L., Hart, M. L.,
1259 McHaffie, H., Brown, M. R., Hollingsworth, P. M., and de Vere, N. (2021). Barcode UK: A
1260 complete DNA barcoding resource for the flowering plants and conifers of the United
1261 Kingdom. Molecular Ecology Resources, 21, 2050–2062.
1262
1263 de Jong, C. B., van Wieren, S. E., Gill, R. M. A., and Munro, R. (2004). Relationship between
1264 diet and liver carcinomas in roe deer in Kielder Forest and Galloway Forest. Veterinary
1265 Record, 155, 197–200.
1266

1267 de Jong, C. B., Gill, R. M. A., van Wieren, S. E., and Burlton, F. W. E. (1995). Diet selection by
1268 roe deer Capreolus capreolus in Kielder Forest in relation to plant cover. Forest Ecology and
1269 Management, 79, 91-97.
1270
1271 Katoh, K., and Standley, D. M. (2013). MAFFT Multiple Sequence Alignment Software Version
1272 7: Improvements in Performance and Usability. Molecular Biology and Evolution, 30, 772–
1273 780.
1274
1275 Kjellander, P., Hewison, A. J. M., Liberg, O., Angibault, J.-M., Bideau, E., and Cargnelutti, B.
1276 (2004). Experimental evidence for density-dependence of home-range size in roe deer
1277 (Capreolus capreolus L.): a comparison of two long-term studies. Oecologia, 139, 478–485.
1278
1279 Kress, W. J., and Erickson, D. L. (2007). A Two-Locus Global DNA Barcode for Land Plants: The
1280 Coding rbcL Gene Complements the Non-Coding trnH-psbA Spacer Region. PLoS ONE, 2,
1281 e508.
1282
1283 Krojerova-Prokešova, J., Barančekova, M., Šustr, P., and Heurich, M. (2010). Feeding patterns
1284 of red deer Cervus elaphus along an altitudinal gradient in the Bohemian Forest: effect of
1285 habitat and season. Wildlife Biology, **16**, 173–184.
1286
1287 Latham, J., Staines, B. W., and Gorman, M. L. (1999). Comparative feeding ecology of red
1288 (Cervus elaphus) and roe deer (Capreolus capreolus) in Scottish plantation forests. Journal of
1289 Zoology, 247, 409–418.
1290

1291 Leonard, J. L., Perkins, L. B., Lammers, D. J., and Jenks, J. A. (2017). Are Bison Intermediate
1292 Feeders? Unveiling Summer Diet Selection at the Northern Fringe of Historical Distribution.
1293 Rangeland Ecology and Management, 70, 405–410.
1294
1295 Linnell, J. D. C., Duncan, P., and Andersen, R. (1998). Chapter 1. The European roe deer: A
1296 portrait of a successful species. In The European Roe Deer: The Biology of Success.
1297 Scandinavian University Press.
1298
1299 Liu, Y., and Huang, N. (2023). Comparison of dietary diversity and niche overlap of sympatric
1300 sika deer and roe deer based on DNA barcoding in Northeast China. European Journal of
1301 Wildlife Research, 69, 115.
1302
1303 Lovari, S., Herrero, J., Masseti, M., Ambarli, H., Lorenzini, R., and Giannatos, G. (2016).
1304 Capreolus capreolus. The IUCN Red List of Threatened Species 2016: e.T42395A22161386.
1305 The IUCN Red List of Threatened Species, 8235, 1–12.
1306
1307 Maizeret, C., and Tran Manh Sung, D. (1984). Etude du régime alimentaire et recherche du
1308 déterminisme fonctionnel de la sélectivité chez le chevreuil des Landes de Gascogne. Gibier
1309 Faune Sauvage, 1, 63–103.
1310
1311 Mancinelli, S., Peters, W., Boitani, L., Hebblewhite, M., and Cagnacci, F. (2015). Roe deer
1312 summer habitat selection at multiple spatio-temporal scales in an Alpine environment.
1313 Hystrix, the Italian Journal of Mammalogy, 26, 132-140.
1314

Marshall, B.M., Gilbert, L., Boyle, J., Lhamine, V.V., Greener, M.S., Gill, R., Millins, C., Morrison, T.A. (2026). Roe deer show an affinity for woodland and reluctance to cross roads. EcoEvoRxiv. https://doi.org/10.32942/X2JD47

Martin, M. (2011). Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet.journal, 17, 10–12.

Martinez, A. P. (2017). pairwiseAdonis: Pairwise Multilevel Comparison using Adonis. R package version 0.4.1.

McInnes, J. C., Alderman, R., Lea, M.-A., Raymond, B., Deagle, B. E., Phillips, R. A., Stanworth, A., Thompson, D. R., Catry, P., Weimerskirch, H., Suazo, C. G., Gras, M., and Jarman, S. N. (2017). High occurrence of jellyfish predation by black-browed and Campbell albatross identified by DNA metabarcoding. Molecular Ecology, 26, 4831–4845.

McMurdie, P. J., and Holmes, S. (2013). phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. PLoS ONE, 8, e61217.

Michel, A., Minocher, R., Niehoff, P.-P., Li, Y., Nota, K., Gadhvi, M. A., Su, J., Iyer, N., Porter, A., Ngobobo-As-Ibungu, U., Binyinyi, E., Nishuli Pekeyake, R., Parducci, L., Caillaud, D., and Guschanski, K. (2023). Isolated Grauer's gorilla populations differ in diet and gut microbiome. Molecular Ecology, 32, 6523–6542.

1338 Minder, I. (2006). Adaptive parameters of the diet of roe deer in a coastal Mediterranean
1339 area. Facoltà di Scienze Matematiche, Fisiche e Naturali Dottorato.
1340
1341 Minder, I. (2012). Local and seasonal variations of roe deer diet in relation to food resource
1342 availability in a Mediterranean environment. European Journal of Wildlife Research, 58,
1343 215–225.
1344
1345 Morellet, N., Bonenfant, C., Börger, L., Ossi, F., Cagnacci, F., Heurich, M., Kjellander, P.,
1346 Linnell, J. D. C., Nicoloso, S., Sustr, P., Urbano, F., and Mysterud, A. (2013). Seasonality,
1347 weather and climate affect home range size in roe deer across a wide latitudinal gradient
1348 within Europe. Journal of Animal Ecology, 82, 1326–1339.
1349
1350 Morellet, N., Van Moorter, B., Cargnelutti, B., Angibault, J.M., Lourtet, B., Merlet, J., Ladet,
1351 S., Hewison, A.J.M. (2011). Landscape composition influences roe deer habitat selection at
1352 both home range and landscape scales. Landscape Ecology, 26, 999–1010.
1353
1354 Moser, B., Schütz, M., and Hindenlang, K. E. (2006). Importance of alternative food resources
1355 for browsing by roe deer on deciduous trees: The role of food availability and species quality.
1356 Forest Ecology and Management, 226, 248–255.
1357
1358 Mussa, P. P., Aceto, P., Abba, C., Sterpone, L., and Meineri, G. (2003). Preliminary study on
1359 the feeding habits of roe deer (Capreolus capreolus) in the western Alps. Journal of Animal
1360 Physiology and Animal Nutrition, 87, 105–108.
1361

1362 Mysterud, A., Langvatn, R., Yoccoz, N. G., and Stenseth, N. C. (2001). Plant Phenology,
1363 Migration and Geographical Variation in Body Weight of a Large Herbivore: The Effect of a
1364 Variable Topography. Journal of Animal Ecology, 70, 915–923.
1365
1366 Mysterud, A., Lian, L. B., and Hjermann, D. Ø. (1999). Scale-dependent trade-offs in foraging
1367 by European roe deer (Capreolus capreolus) during winter. Canadian Journal of Zoology, 77,
1368 1486–1493.
1369
1370 Nichols, R. V., Åkesson, M., and Kjellander, P. (2016). Diet Assessment Based on Rumen
1371 Contents: A Comparison between DNA Metabarcoding and Macroscopy. PLoS ONE, 11,
1372 e0157977.
1373
1374 Obidziński, A., Kiełtyk, P., Borkowski, J., Bolibok, L., and Remuszko, K. (2013). Autumn-winter
1375 diet overlap of fallow, red, and roe deer in forest ecosystems, Southern Poland. Central
1376 European Journal of Biology, **8**, 8–17.
1377
1378 Pansu, J., Guyton, J. A., Potter, A. B., Atkins, J. L., Daskin, J. H., Wursten, B., Kartzinel, T. R.,
1379 and Pringle, R. M. (2019). Trophic ecology of large herbivores in a reassembling African
1380 ecosystem. Journal of Ecology, 107, 1355–1376.
1381
1382 Pebesma, E., et al. (2024). sf: Simple Features for R. R package version 1.0-16.
1383 https://CRAN.R-project.org/package=sf
1384

1385 Pettorelli, N., Gaillard, J.-M., Duncan, P., Ouellet, J.-P., and Van Laere, G. (2001). Population
1386 density and small-scale variation in habitat quality affect phenotypic quality in roe deer.
1387 Oecologia, 128, 400–405.
1388
1389 Pompanon, F., Deagle, B. E., Symondson, W. O. C., Brown, D. S., Jarman, S. N., and Taberlet,
1390 P. (2012). Who is eating what: diet assessment using next generation sequencing. Molecular
1391 Ecology, 21, 1931–1950.
1392
1393 Price, M. N., Dehal, P. S., and Arkin, A. P. (2010). FastTree 2 -Approximately Maximum-
1394 Likelihood Trees for Large Alignments. PLoS ONE, 5, e9490.
1395
1396 Proffitt, K. M., Hebblewhite, M., Peters, W., Hupp, N., and Shamhart, J. (2016). Linking
1397 landscapescale differences in forage to ungulate nutritional ecology. Ecological Applications,
1398 26, 2156–2174.
1399
1400 Putman, R. J., and Moore, N. P. (1998). Impact of deer in lowland Britain on agriculture,
1401 forestry and conservation habitats. Mammal Review, 28, 141–164.
1402
1403 Putman, R. J., and Staines, B. W. (2004). Supplementary winter feeding of wild red deer
1404 Cervus elaphus in Europe and North America: justifications, feeding practice and
1405 effectiveness. Mammal Review, 34, 285–306.
1406
1407 Ratkiewicz, M., Coissac, E., Świsłocka, M., Czajkowska, M., Kowalczyk, R., Czernik, M., and
1408 Taberlet, P. (2024). Winter diet overlap among moose, roe deer and red deer in coniferous

and mixed forests depends on snow cover depth. Forest Ecology and Management, 556, 121710.

Reimoser, F. (2000). Income from hunting in mountain forests of the Alps. In Forests in Sustainable Mountain Development: A State-of-Knowledge Report for 2000. CABI Publishing, New York, 346–353.

Rice, W. R. (1989). Analyzing Tables of Statistical Tests. American Sociological Review, 78, 1–3.

Robeson, M. S., O'Rourke, D. R., Kaehler, B. D., Ziemski, M., Dillon, M. R., Foster, J. T., and Bokulich, N. A. (2021). RESCRIPt: Reproducible sequence taxonomy reference database management. PLoS Computational Biology, 17, e1009581.

Rooney, T., and Dress, W. (1997). Species loss over sixty-six years in the ground-layer vegetation of heart's content, and old-growth forest in Pennsylvania, USA. Natural Areas Journal, 17, 297–305.

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

Smith, R. S., and Charman, D. J. (1988). The Vegetation of Upland Mires Within Conifer Plantations in Northumberland, Northern England. Journal of Applied Ecology, 25, 579–594.

1433 Soininen, E. M., Valentini, A., Coissac, E., Miquel, C., Gielly, L., Brochmann, C., Brysting, A. K.,
1434 Sønstebø, J. H., Ims, R. A., Yoccoz, N. G., and Taberlet, P. (2009). Analysing diet of small
1435 herbivores: The efficiency of DNA barcoding coupled with high-throughput pyrosequencing
1436 for deciphering the composition of complex plant mixtures. Frontiers in Zoology, 6, 1–9.
1437
1438 Spitzer, R. (2019). Trophic resource use and partitioning in multispecies ungulate
1439 communities. PhD Thesis. Swedish University of Agricultural Sciences.
1440
1441 Spitzer, R., Felton, A., Landman, M., Singh, N. J., Widemo, F., and Cromsigt, J. P. G. M. (2020).
1442 Fifty years of European ungulate dietary studies: a synthesis. Oikos, 129, 1668-1680.
1443
1444 Storms, D., Aubry, P., Hamann, J.-L., Saïd, S., Fritz, H., Saint-Andrieux, C., and Klein, F. (2008).
1445 Seasonal variation in diet composition and similarity of sympatric red deer Cervus elaphus
1446 and roe deer Capreolus capreolus. Wildlife Biology, 14, 237–250.
1447
1448 Storms, D., Said, S., Fritz, H., Hamann, J. L., Saint-Andrieux, C., and Klein, F. (2006). Influence
1449 of hurricane Lothar on red and roe deer winter diets in the Northern Vosges, France. Forest
1450 Ecology and Management, 237, 164–169.
1451
1452 Taberlet, P., Coissac, E., Pompanon, F., Brochmann, C., and Willerslev, E. (2012). Towards
1453 nextgeneration biodiversity assessment using DNA metabarcoding. Molecular Ecology, 21,
1454 2045–2050.
1455

Tanentzap, A. J., Burrows, L. E., Lee, W. G., Nugent, G., Maxwell, J. M., and Coomes, D. A. (2009).
Landscape-level vegetation recovery from herbivory: Progress after four decades of invasive red deer control. *Journal of Applied Ecology*, **46**, 1064–1072.

Tixier, H., and Duncan, P. (1996). Are European roe deer browsers? A review of variations in the composition of their diets. Revue d'Ecologie, 51.

Tixier, H., Duncan, P., Scehovic, J., Yant, A., Gleizes, M., and Lila, M. (1997). Food selection by European roe deer (Capreolus capreolus): effects of plant chemistry, and consequences for the nutritional value of their diets. Journal of Zoology, 242, 229–245.

Turk Dermastia, T., Vascotto, I., Francé, J., Stanković, D., and Mozetič, P. (2023). Evaluation of the rbcL marker for metabarcoding of marine diatoms and inference of population structure of selected genera. Frontiers in Microbiology, 14.

UK (England) 1 F Pannonian Czech feeding habits of the roe deer in winter in central Finland. Acta Theriologica, 25, 395-402.

Vangilder, L. D., Torgerson, O., and Porath, W. R. (1982). Factors Influencing Diet Selection by WhiteTailed Deer. The Journal of Wildlife Management, 46, 711–718.

Velázquez, A., Medina García, C., Durán Medina, E., Amador, A., and Gopar Merino, L. F. (2016). Background on Vegetation Classification Systems. In: Velázquez, A., Medina García,

C., Durán Medina, E., Amador, A., Gopar Merino, L. F. (Eds.), Standardized Hierarchical Vegetation Classification: Mexican and Global Patterns. Springer, Cham.

de Vere, N., Rich, T. C. G., Ford, C. R., Trinder, S. A., Long, C., Moore, C. W., Satterthwaite, D., Davies, H., Allainguillaume, J., Ronca, S., Tatarinova, T., Garbett, H., Walker, K., and Wilkinson, M. J. (2012). DNA Barcoding the Native Flowering Plants and Conifers of Wales. *PLoS ONE*, **7**, e37945.

Walker, F. M., Sanchez, D. E., Froehlich, E. M., Federman, E. L., Lyman, J. A., Owens, M., and Lear, K. (2022). Endangered Nectar-Feeding Bat Detected by Environmental DNA on Flowers. Animals, 12, 3075.

Ward, A. I., White, P. C. L., Walker, N. J., and Critchley, C. H. (2008). Conifer leader browsing by roe deer in English upland forests: Effects of deer density and understorey vegetation. Forest Ecology and Management, 256, 1333–1338.

Waterhouse, E. C., Sporring, S., and Bayfield, N. G. (1998). Sustainable harvesting of bog myrtle (Myrica gale L.) in Scotland. Botanical Journal of Scotland, 50, 11–20.

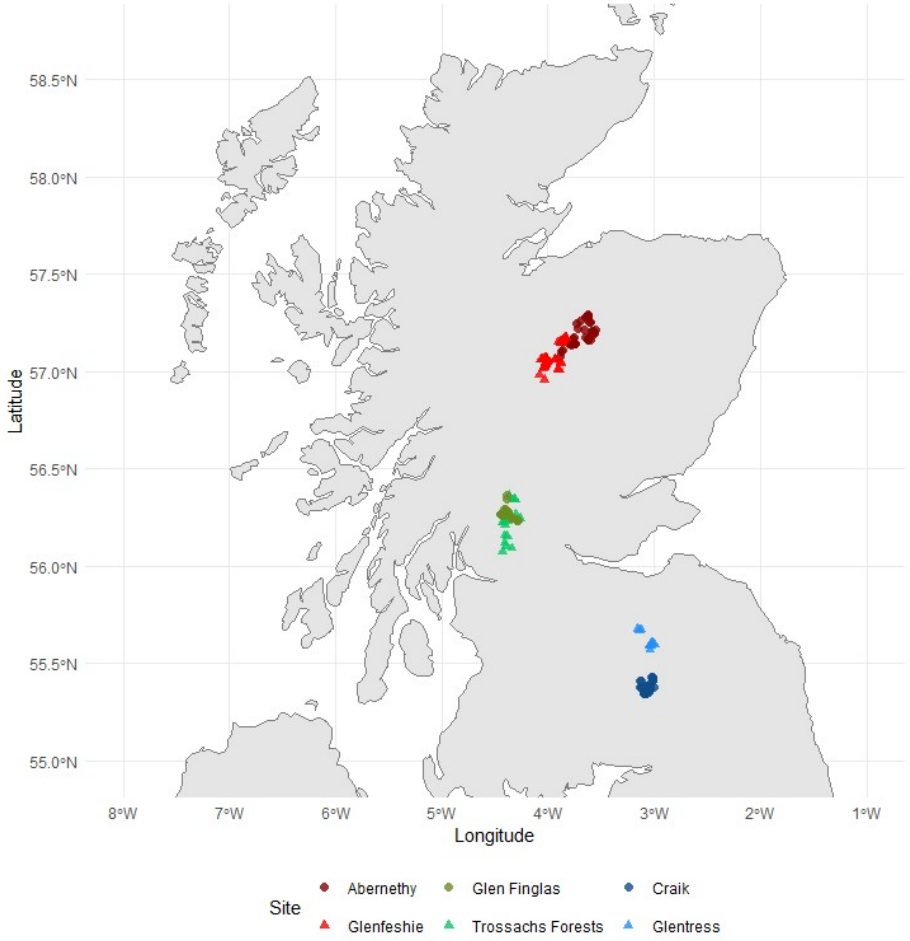
Watson, A. (1983). Eighteenth century deer numbers and pine regeneration near Braemar, Scotland. Biological Conservation, 25, 289–305.

Welch, D., Staines, B. W., Scott, D., French, D. D., and Catt, D. C. (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, 61–82.

Xu, S., Zhan, L., Tang, W., Wang, Q., Dai, Z., Zhou, L., Feng, T., Chen, M., Wu, T., Hu, E., Yu, G. (2023). MicrobiotaProcess: a comprehensive R package for deep mining microbiome. The Innovation, 4, 100388.

Yuan, Z., Zhong, L., Du, H., Feng, J., Liu, X., Yuan, H., Guo, J., Liu, P., and Zhang, M. (2022). Effects of vegetation type differences induced by human disturbance on the nutrition strategy and gut microbiota of Siberian roe deer. Molecular Ecology, 32, 2534-2550.



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1518 **Figure 1**. Map of cull locations across Scotland where roe deer rumen samples were

1519 collected. The six sampling sites were Abernethy (RSPB) and Glenfeshie (WildLand Limited)

1520 in the Cairngorms region; Glen Finglas (Woodland Trust) and Trossachs Forests (Forestry and

1521 Land Scotland) in the Trossachs region; and Craik and Glentress (Both Forestry and Land

1522 Scotland) in the Scottish Borders. Point colour and shape denote sampling site.

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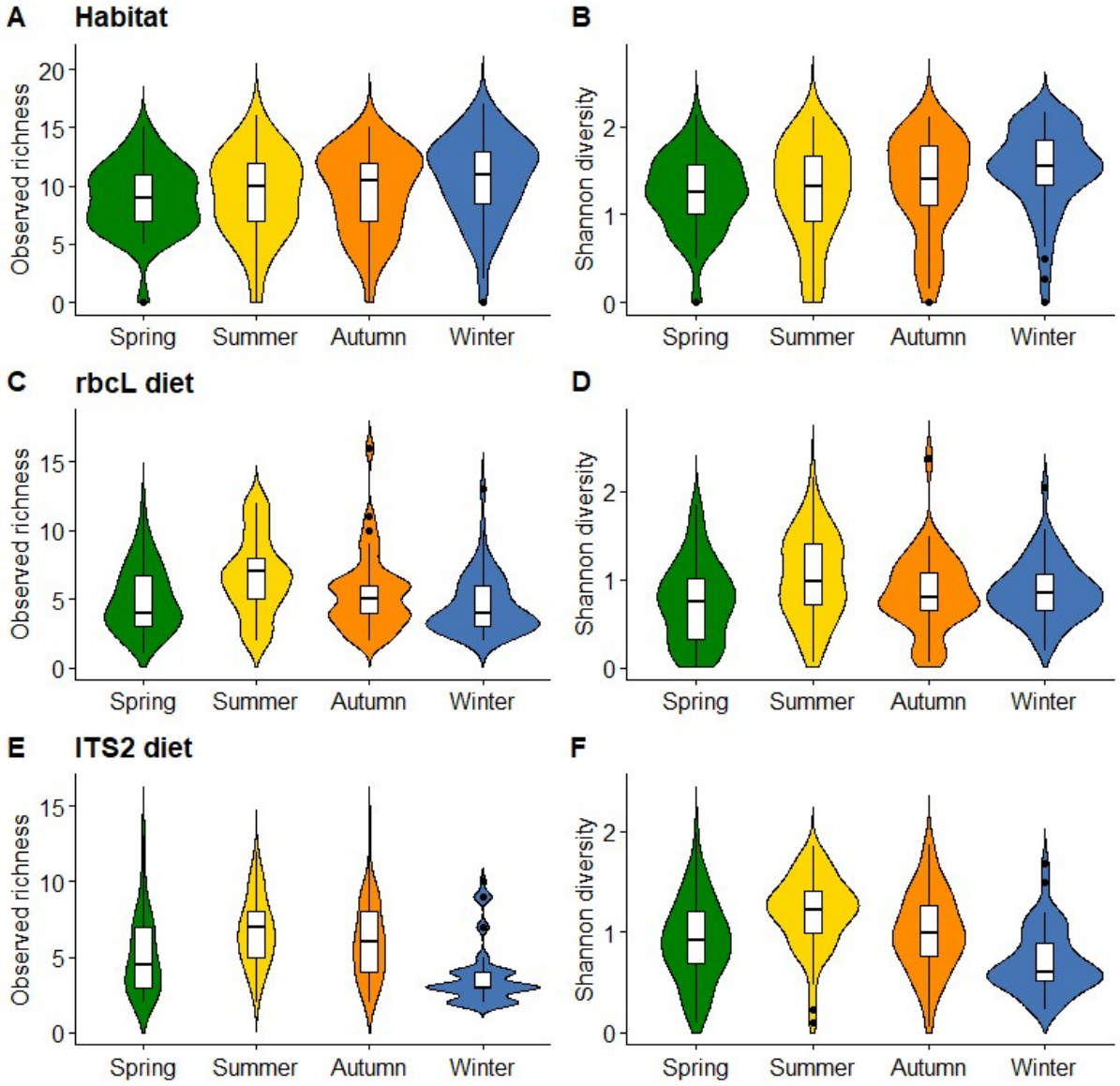

Figure 2. Seasonal variation in habitat diversity and diet diversity of roe deer. Violin plots showing (A-B) habitat richness and diversity, (C-D) *rbcL*-detected diet richness and diversity (genus-level), and (E-F) *ITS2*-detected diet richness and diversity (genus-level) across seasons. Left panels (A, C, E) show observed richness (number of unique habitat types or plant genera); right panels (B, D, F) show Shannon diversity index. Boxplots within violins indicate median (centre line), interquartile range (box), and 1.5× IQR (whiskers). Spring n=58, summer n=53, autumn n=37, winter n=50.

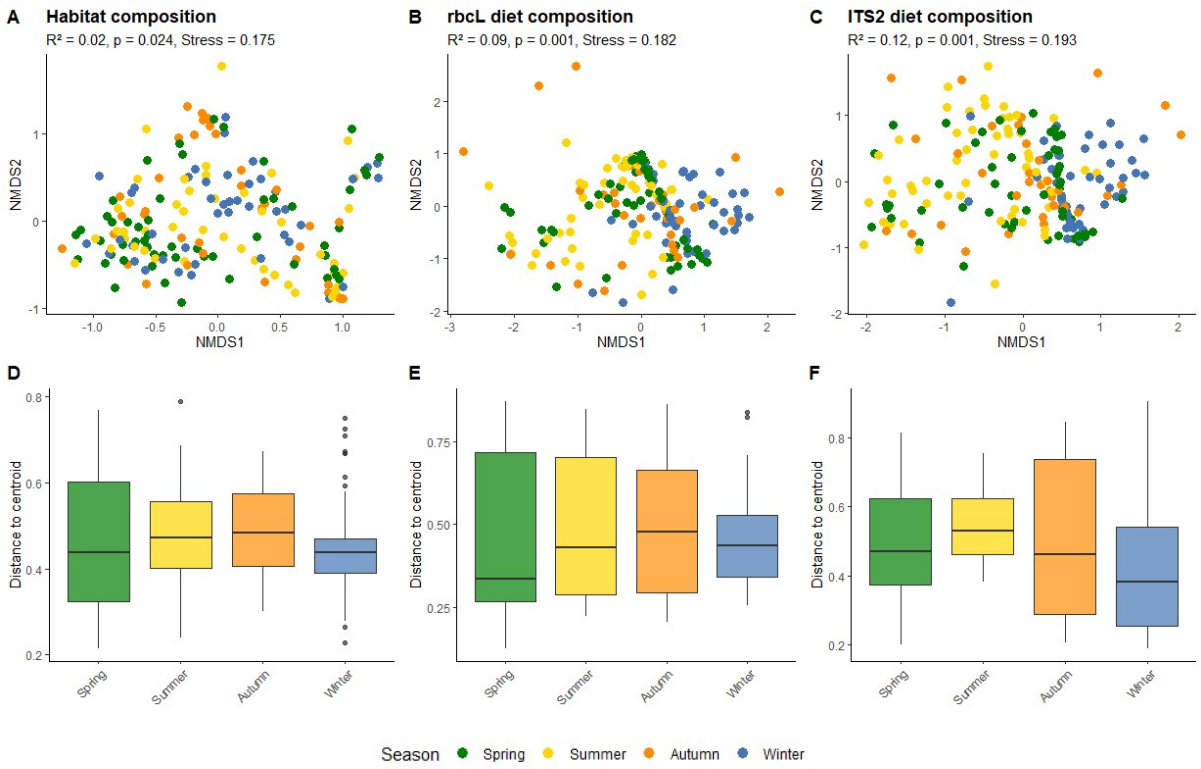

Figure 3. Seasonal differences in habitat and diet composition of roe deer. Non-metric multidimensional scaling (NMDS) ordinations (top row) and betadispersion analyses (bottom row) for (A,D) habitat composition, (B,E) *rbcL*-detected diet, and (C,F) *ITS2*-detected diet across seasons. NMDS plots (A-C) show Bray-Curtis dissimilarity between samples. Beta dispersion boxplots (D-F) show within-group variability (distance to centroid) for each season. NMDS stress values are shown on each panel; R^2 and p values are from PERMANOVA.

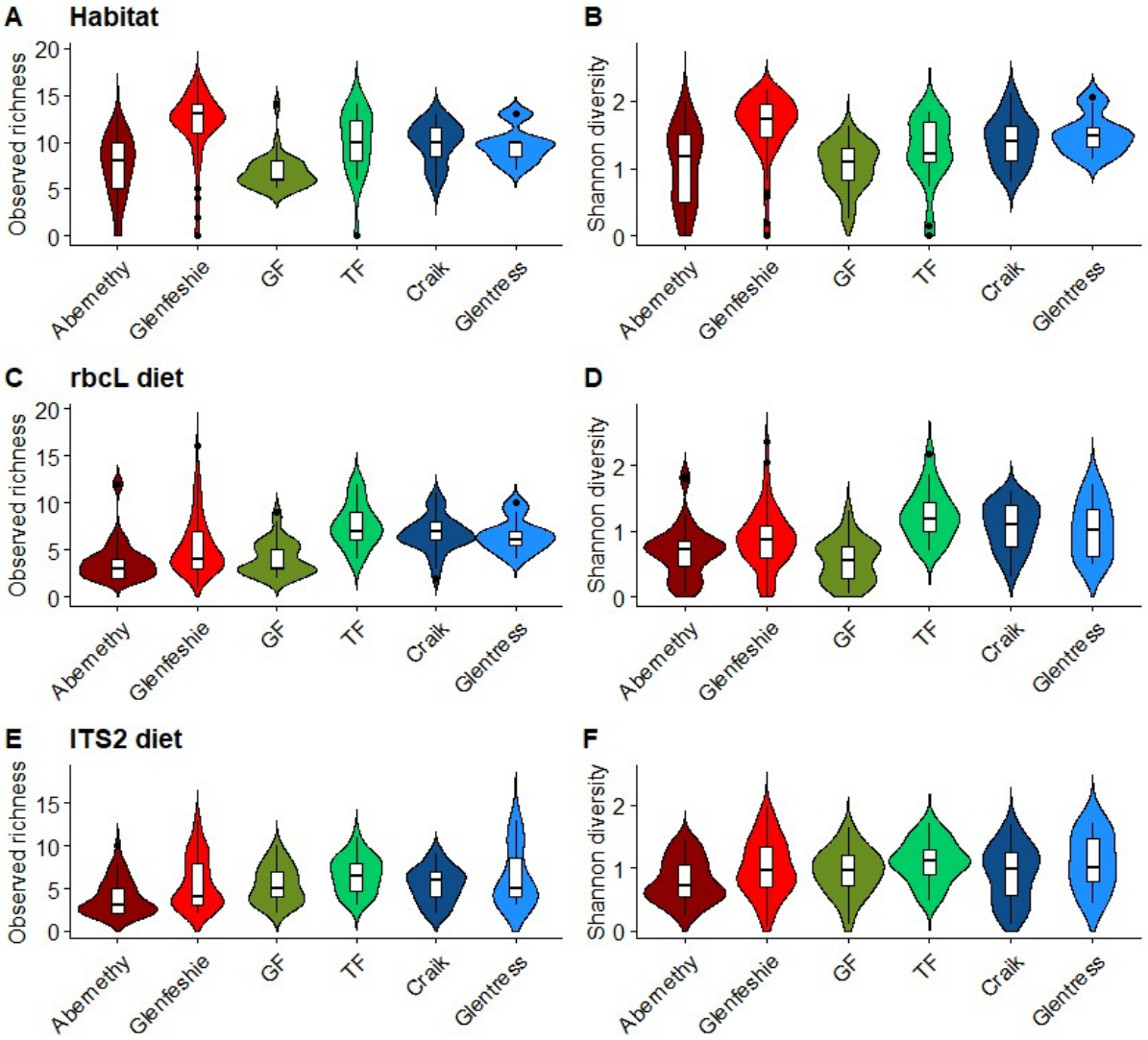

Figure 4. Site variation in habitat diversity and diet diversity of roe deer. Violin plots showing (A-B) habitat richness and diversity, (C-D) *rbcL*-detected diet richness and diversity (genus-level), and (E-F) *ITS2*-detected diet richness and diversity (genus-level) across six sites. Left panels (A, C, E) show observed richness (number of unique habitat types or plant genera); right panels (B, D, F) show Shannon diversity index. Boxplots within violins indicate median (centre line), interquartile range (box), and 1.5× IQR (whiskers). GF = Glen Finglas, TF = Trossachs Forests. Glenfeshie n=62, Abernethy n=40, Craik n=35, Glen Finglas n=30, Trossachs Forests n=20, Glentress n=11.

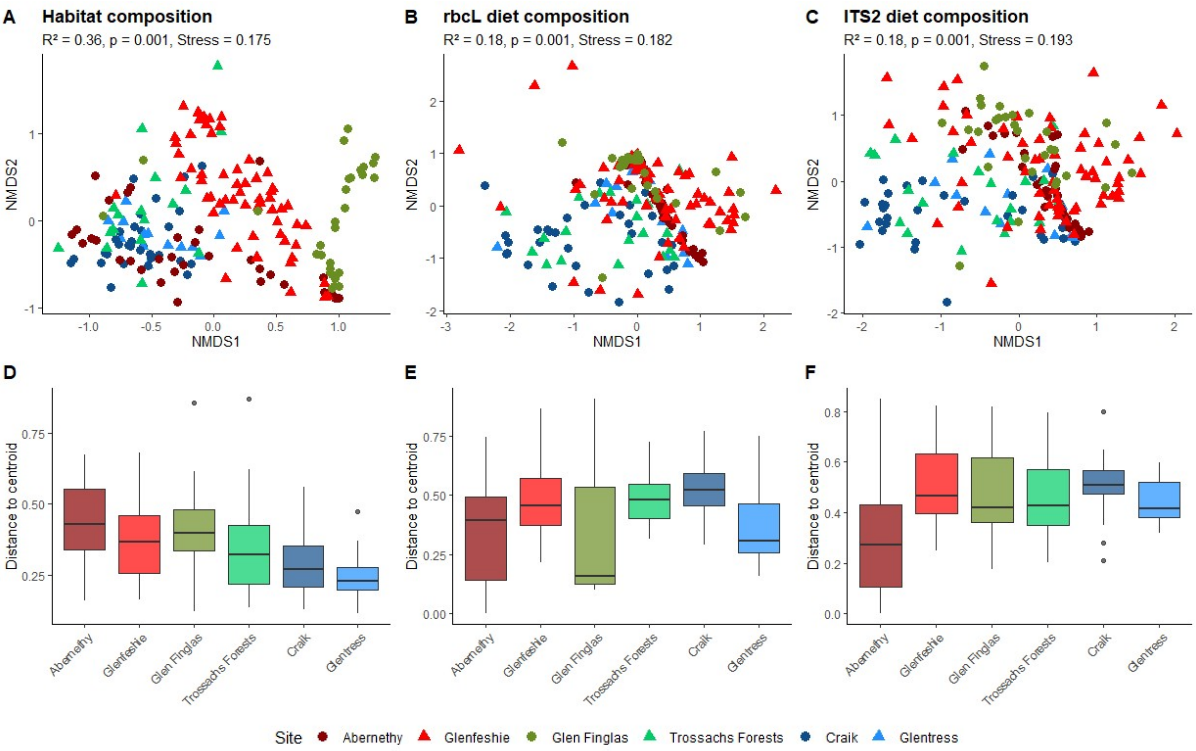

Figure 5. Site differences in habitat and diet composition of roe deer. Non-metric multidimensional scaling (NMDS) ordinations (top row) and betadispersion analyses (bottom row) for (A,D) habitat composition, (B,E) *rbcL*-detected diet, and (C,F) *ITS2*-detected diet across six sites. NMDS plots (A-C) show Bray-Curtis dissimilarity between samples. Beta dispersion boxplots (D-F) show within-group variability (distance to centroid) for each site. NMDS stress values are shown on each panel; R^2 and p values are from PERMANOVA.

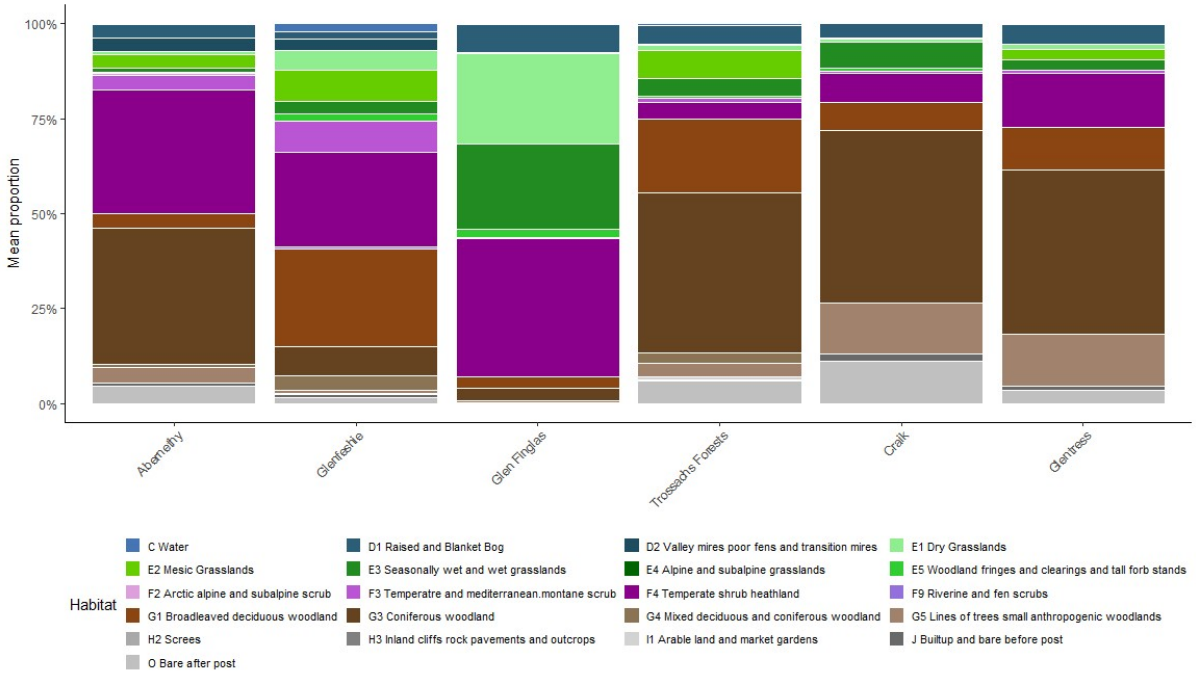

Figure 6. Habitat composition within 500m buffers of roe deer sampling locations across six study sites in Scotland. Stacked bar chart showing mean proportions of habitat types (based on EUNIS classification) for samples from each site. Habitat types are grouped by ecological category: water and wetlands (C, D - blues), grasslands (E - greens), scrub and heathland (F - purples), woodlands (G - browns), and bare/built areas (H, I, J, O -greys). Bars represent mean proportions across all samples from each site and sum to 100%.

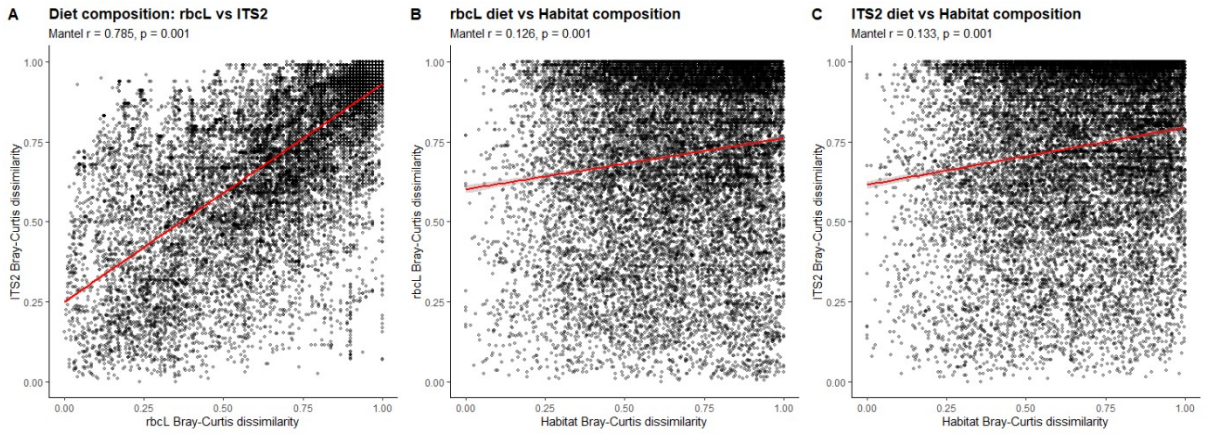

Figure 7. Diet and habitat compositional correlations. Mantel test scatter plots comparing Bray-Curtis dissimilarities between (A) *rbcL* and *ITS2* diets, (B) *rbcL* diet and habitat, and (C) *ITS2* diet and habitat. Each point represents a sample pair; lines show linear fits with 95% CI. A) uses all 198 sequenced samples; (B) and (C) use the 190 with habitat data.

Supplementary Material

From Pixels to Plants: Habitat-Based Predictions of Roe Deer Foraging

Christopher Hirst, Darren J. Shaw, Rory J. Putman, Laura Glendinning, Robin Gill, Richard A. Ennos, Gerry McLachlan, Rob Ogden

Contents: Supplementary Methods S1-S2; Supplementary Tables S1-S5; Supplementary Figure S1.

S1. Primer sequences and PCR conditions

Marker	Primer	Primer sequence 5'→3'	Expected size	Source
ITS2	*ITS2*F	ATGCGATACTTGGTGTGAAT	520 bp	Chen et al. (2010)
	*ITS2*R	GACGCTTCTCCAGACTACAAT		
rbcL	*rbcL*-a	ATGTCACCACAAACAGAGACTAAAGC	580 bp	Kress and Erickson (2007)
	*rbcL*r590	AGTCCACCGCGTAGACATTCAT		de Vere et al. (2012)

PCRs were performed in 25 µL reactions containing 2 µL of extracted DNA, 12.5 µL of 2X Simplifi HS Mix (Meridian Bioscience Inc.), 0.25 µL each of forward and reverse primers, and 10 µL of nuclease-free water (Qiagen). Reactions were performed in duplicate for each sample and marker. Cycling conditions for *rbcL* were: 95°C for 2 min; 45 cycles of 95°C for 30 s, 50°C for 90 s, and 72°C for 40 s; final extension 72°C for 5 min. Cycling conditions for *ITS2* were: 95°C for 1 min; 40 cycles of 95°C for 30 s, 56°C for 30 s, and 72°C for 1 min; final extension 72°C for 5 min. Amplification was confirmed by visualising 3 µL of PCR product on a 2% agarose gel stained with SYBR® (Thermo Scientific) alongside a GeneRuler 100 bp ladder (Thermo Scientific); duplicates producing visible amplicons were pooled.

All PCR primers included Illumina TruSeq adapter sequences to enable multiplexing: forward 5'-ACACTCTTTCCCTACACGACGCTCTTCCGATCT-3'; reverse 5'-GACTGGAGTTCAGACGTGTGCTCTTCCGATCT-3'.

S2. Sequencing and read-processing statistics

Paired-end reads were demultiplexed and primer-trimmed prior to processing; "input reads" below refers to the reads entering the DADA2 pipeline after this step. Across all 198 samples, a total of 5,547,512 and 9,447,695 input reads were processed in DADA2 for *rbcL* and *ITS2*, respectively, averaging 19,129±489 reads per sample for *rbcL* and 32,578±802 for *ITS2*. For *rbcL*, an average of 17,996±448 reads per sample passed quality filtering (89-97% of input reads across samples); for *ITS2*, an average of 75%±0.3% of reads passed quality filtering (range 62-85%). After denoising, an average of 17,891±446 reads per sample were obtained for *rbcL* (range 4,238-45,462) and 24,029±571 for *ITS2* (range 2,837-55,617). For *ITS2*, an average of 23,664±563 reads per sample were successfully merged (73%±0.3% of input reads); *rbcL* amplicons were processed as single-end reads due to amplicon length constraints and did not require merging. The resulting non-chimeric reads averaged 14,282±351 per sample for *rbcL* (76%±0.7% of input reads) and 20,681±519 per sample for *ITS2* (64%±0.5% of input reads). After filtering, a total of 548 unique Amplicon Sequence Variants (ASVs) were observed across 4,089,647 total reads for *rbcL*, and 1,740 unique ASVs across 5,917,925 total reads for *ITS2*.

Table S3. Frequency of occurrence (FOO, % of samples) and mean relative read abundance (RRA, %) of the 31 plant genera detected in more than 5% of roe deer rumen samples (n = 198), ordered by combined FOO. FOO combined counts a genus as present if detected by either marker. Values of 0.0 indicate the genus was not detected by that marker.

Genus	**FOO combined (%)**	**FOO *rbcL* (%)**	**FOO *ITS2* (%)**	**Mean RRA *rbcL* (%)**	**Mean RRA *ITS2* (%)**
Vaccinium	90.9	89.4	86.9	36.8	20.2
Calluna	82.8	79.8	79.3	22.1	30.7
Potentilla	37.4	22.2	36.9	2.0	6.7
Rumex	34.8	32.8	21.7	3.0	1.5
Betula	34.3	0.0	34.3	0.0	3.4
Rubus	31.8	23.2	31.8	3.6	6.1
Epilobium	30.3	14.6	30.3	0.7	8.8
Salix	28.3	22.7	27.8	1.5	1.8
Alnus	27.3	7.1	24.7	0.9	8.8
Viola	23.7	11.6	23.2	0.3	1.3
Chamaenerion	22.2	22.2	0.0	7.2	0.0
Erica	21.2	15.7	21.2	0.8	1.4
Oxalis	21.2	21.2	0.0	0.8	0.0
Myrica	19.2	19.2	0.0	8.2	0.0
Filipendula	18.7	15.2	17.7	1.2	2.7
Pinus	18.2	18.2	0.0	1.2	0.0
Picea	15.7	15.7	1.5	2.5	0.2
Hedlundia	12.6	0.0	12.6	0.0	0.3
Ranunculus	12.6	12.6	0.5	0.3	0.1
Hypericum	11.6	11.6	0.5	0.4	0.0
Plantago	9.1	2.5	8.6	0.0	0.4
Geum	8.1	5.1	7.6	0.1	0.3
Aria	6.6	6.6	0.5	0.9	0.0
Larix	6.6	6.6	0.0	0.5	0.0
Corylus	6.1	3.0	6.1	0.0	0.1
Lysimachia	6.1	2.5	6.1	0.0	0.1

Genus	FOO combined (%)	FOO *rbcL* (%)	FOO *ITS2* (%)	Mean RRA *rbcL* (%)	Mean RRA *ITS2* (%)
Quercus	6.1	6.1	0.0	1.8	0.0
Arctostaphylos	5.1	0.0	5.1	0.0	0.4
Galium	5.1	5.1	0.0	0.1	0.0
Lathyrus	5.1	2.0	4.5	0.0	0.1
Prunus	5.1	3.5	4.0	0.3	0.5

Table S4. Frequency of occurrence (%) of plant genera in roe deer diets by season (combined markers). A genus was counted as present within a season only if detected in more than one sample of that seasonal subset; dashes indicate genera not meeting this criterion. Sample sizes: spring n = 58, summer n = 53, autumn n = 37, winter n = 50. The 31 genera exceeding 5% overall FOO (Table S1) are listed first in that order, followed by the remaining genera detected in more than one sample.

Genus	Spring	Summer	Autumn	Winter
Vaccinium	89.7	86.8	86.5	100.0
Calluna	81.0	67.9	86.5	98.0
Potentilla	25.9	69.8	51.4	6.0
Rumex	36.2	67.9	24.3	6.0
Betula	25.9	58.5	48.6	8.0
Rubus	27.6	50.9	37.8	12.0
Epilobium	32.8	56.6	21.6	6.0
Salix	19.0	47.2	35.1	14.0
Alnus	12.1	18.9	37.8	46.0
Viola	17.2	41.5	37.8	-
Chamaenerion	27.6	39.6	16.2	-
Erica	29.3	9.4	27.0	20.0
Oxalis	15.5	32.1	24.3	14.0
Myrica	8.6	13.2	13.5	42.0
Filipendula	27.6	30.2	8.1	4.0
Pinus	10.3	5.7	10.8	46.0
Picea	17.2	9.4	16.2	20.0
Hedlundia	12.1	20.8	13.5	4.0
Ranunculus	17.2	13.2	10.8	8.0
Hypericum	5.2	17.0	18.9	8.0
Plantago	6.9	13.2	16.2	-
Geum	10.3	11.3	5.4	4.0
Aria	6.9	15.1	-	-

Larix	6.9	9.4	-	8.0
Corylus	10.3	5.7	8.1	-
Lysimachia	8.6	5.7	5.4	4.0
Quercus	-	3.8	21.6	4.0
Arctostaphylos	8.6	-	10.8	-
Galium	3.4	-	5.4	12.0
Lathyrus	8.6	7.5	-	-
Prunus	5.2	-	10.8	4.0
Acer	3.4	-	8.1	-
Alchemilla	-	5.7	5.4	-
Anemone	3.4	-	-	-
Angelica	-	3.8	-	-
Chrysosplenium	5.2	3.8	-	4.0
Cicuta	-	3.8	-	-
Cirsium	-	-	-	6.0
Comarum	3.4	5.7	-	-
Conopodium	3.4	-	-	-
Empetrum	5.2	-	-	-
Fagus	3.4	3.8	8.1	-
Ficaria	6.9	-	-	-
Geranium	3.4	5.7	-	6.0
Helianthemum	-	3.8	-	-
Lotus	3.4	5.7	-	-
Melampyrum	-	3.8	-	-
Mercurialis	-	-	-	4.0
Narthecium	-	7.5	-	-
Primula	3.4	-	-	4.0
Rhinanthus	-	3.8	-	-
Rosa	3.4	5.7	-	-
Stellaria	3.4	-	-	-

Succisa	-	5.7	-	-
Trientalis	-	3.8	-	-
Veronica	-	-	5.4	-

Table S5. Frequency of occurrence (%) of plant genera in roe deer diets by site (combined markers). A genus was counted as present at a site only if detected in more than one sample of that site subset; dashes indicate genera not meeting this criterion. Sample sizes: Glenfeshie n = 62, Abernethy n = 40, Craik n = 35, Glen Finglas n = 30, Trossachs Forests n = 20, Glentress n = 11. The 31 genera exceeding 5% overall FOO (Table S1) are listed first in that order, followed by the remaining genera detected in more than one sample.

Genus	Glenfeshie	Abernethy	Craik	Glen Finglas	Trossachs Forests	Glentress
Vaccinium	91.9	100.0	68.6	100.0	90.0	100.0
Calluna	91.9	97.5	65.7	66.7	80.0	81.8
Potentilla	35.5	40.0	20.0	60.0	40.0	27.3
Rumex	25.8	15.0	71.4	30.0	45.0	36.4
Betula	43.5	25.0	5.7	36.7	60.0	54.5
Rubus	17.7	12.5	45.7	16.7	95.0	63.6
Epilobium	17.7	5.0	77.1	6.7	55.0	63.6
Salix	12.9	12.5	65.7	26.7	50.0	18.2
Alnus	54.8	15.0	5.7	33.3	10.0	-
Viola	33.9	10.0	14.3	26.7	30.0	27.3
Chamaenerion	9.7	-	68.6	-	45.0	45.5
Erica	22.6	22.5	-	20.0	30.0	63.6
Oxalis	19.4	15.0	14.3	-	45.0	90.9
Myrica	40.3	12.5	-	23.3	-	-
Filipendula	9.7	-	45.7	30.0	15.0	27.3
Pinus	17.7	35.0	14.3	-	10.0	27.3
Picea	-	-	40.0	-	45.0	63.6
Hedlundia	16.1	15.0	-	26.7	-	-
Ranunculus	14.5	-	42.9	-	-	-
Hypericum	16.1	15.0	-	6.7	20.0	-
Plantago	17.7	5.0	-	10.0	-	-
Geum	-	-	25.7	10.0	-	-
Aria	3.2	-	11.4	6.7	10.0	18.2
Larix	4.8	-	11.4	-	10.0	36.4

Genus	Glenfeshie	Abernethy	Craik	Glen Finglas	Trossachs Forests	Glentress
Corylus	3.2	-	8.6	10.0	15.0	-
Lysimachia	4.8	-	-	10.0	15.0	-
Quercus	14.5	-	-	-	10.0	-
Arctostaphylos	8.1	12.5	-	-	-	-
Galium	-	-	14.3	-	10.0	-
Lathyrus	4.8	-	14.3	-	-	-
Prunus	9.7	-	-	-	-	-
Acer	6.5	-	-	-	10.0	-
Alchemilla	3.2	5.0	5.7	-	-	-
Anemone	-	-	-	6.7	-	-
Angelica	-	-	5.7	-	-	-
Chrysosplenium	3.2	-	5.7	-	-	-
Cicuta	-	-	5.7	-	-	-
Cirsium	3.2	-	5.7	-	-	-
Comarum	6.5	-	-	-	-	-
Conopodium	4.8	-	-	-	-	-
Cytisus	3.2	-	-	-	-	-
Empetrum	-	7.5	-	10.0	-	-
Fagus	3.2	-	11.4	-	10.0	-
Ficaria	-	-	11.4	-	-	-
Geranium	4.8	-	5.7	6.7	-	-
Lotus	-	-	-	-	20.0	-
Mercurialis	3.2	-	-	-	-	-
Narthecium	-	7.5	-	-	-	-
Populus	4.8	-	-	-	-	-
Primula	4.8	-	-	-	-	-
Rhinanthus	3.2	-	-	-	-	-
Rosa	6.5	-	-	-	-	-
Succisa	4.8	-	-	6.7	-	-
Tilia	3.2	-	-	-	-	-

Genus	Glenfeshie	Abernethy	Craik	Glen Finglas	Trossachs Forests	Glentress
Trientalis	-	5.0	-	-	-	-
Veronica	4.8	-	-	-	-	-

Table S6. Pairwise comparisons of Bray-Curtis dissimilarities between seasons within each site, for both markers, illustrating the season × site interaction in diet composition. Comparisons where either season contained fewer than three samples were not tested (-). p.adj = p value adjusted using the Bonferroni method within each site; * = p.adj < 0.05.

			rbcL		*ITS2*	
Site	**Comparison**	**n**	**R^2**	**p.adj**	**R^2**	**p.adj**
Glenfeshie	Spring vs Summer	11 / 10	0.04	1.000	0.09	0.306
Glenfeshie	Spring vs Autumn	11 / 18	0.07	0.396	0.04	1.000
Glenfeshie	Spring vs Winter	11 / 23	0.11	0.138	0.11	0.042*
Glenfeshie	Summer vs Autumn	10 / 18	0.06	0.774	0.04	1.000
Glenfeshie	Summer vs Winter	10 / 23	0.10	0.114	0.14	0.006*
Glenfeshie	Autumn vs Winter	18 / 23	0.10	0.018*	0.06	0.276
Abernethy	Spring vs Summer	13 / 10	0.09	0.774	0.24	0.030*
Abernethy	Spring vs Autumn	13 / 7	0.01	1.000	0.10	0.780
Abernethy	Spring vs Winter	13 / 10	0.19	0.258	0.18	0.198
Abernethy	Summer vs Autumn	10 / 7	0.14	0.390	0.16	0.330
Abernethy	Summer vs Winter	10 / 10	0.49	0.006*	0.46	0.006*
Abernethy	Autumn vs Winter	7 / 10	0.35	0.030*	0.34	0.006*
Craik	Spring vs Summer	14 / 12	0.07	0.372	0.10	0.183
Craik	Spring vs Autumn	14 / 2	–	–	–	–
Craik	Spring vs Winter	14 / 7	0.16	0.066	0.12	0.231
Craik	Summer vs Autumn	12 / 2	–	–	–	–
Craik	Summer vs Winter	12 / 7	0.29	0.003*	0.34	0.006*
Craik	Autumn vs Winter	2 / 7	–	–	–	–
Glen Finglas	Spring vs Summer	13 / 11	0.10	0.072	0.23	0.006*
Glen Finglas	Spring vs Autumn	13 / 1	–	–	–	–
Glen Finglas	Spring vs Winter	13 / 5	0.49	0.003*	0.35	0.003*
Glen Finglas	Summer vs Autumn	11 / 1	–	–	–	–
Glen Finglas	Summer vs Winter	11 / 5	0.59	0.003*	0.53	0.003*
Glen Finglas	Autumn vs Winter	1 / 5	–	–	–	–
Trossachs Forests	Spring vs Summer	3 / 7	0.14	0.825	0.18	0.393

Trossachs Forests	Spring vs Autumn	3 / 9	0.08	1.000	0.08	1.000
Trossachs Forests	Spring vs Winter	3 / 1	–	–	–	–
Trossachs Forests	Summer vs Autumn	7 / 9	0.16	0.132	0.20	0.033*
Trossachs Forests	Summer vs Winter	7 / 1	–	–	–	–
Trossachs Forests	Autumn vs Winter	9 / 1	–	–	–	–
Glentress	Spring vs Summer	4 / 3	0.07	1.000	0.21	0.924
Glentress	Spring vs Autumn	4 / 0	–	–	–	–
Glentress	Spring vs Winter	4 / 4	0.49	0.156	0.41	0.093
Glentress	Summer vs Autumn	3 / 0	–	–	–	–
Glentress	Summer vs Winter	3 / 4	0.47	0.138	0.54	0.102
Glentress	Autumn vs Winter	0 / 4	–	–	–	–

Table S7. Season-specific Spearman rank correlations between the proportional area of habitat types within localities (500 m buffers) and the relative read abundance (*rbcL*) of plant genera in roe deer diets, for all 162 combinations meeting the |rs| ≥ 0.3 screening threshold - screening was based on effect size rather than statistical significance; some retained combinations therefore have p > 0.05. Genera and habitats were included where present in ≥ 10% of that season's samples. These combinations were carried forward to the logistic regression models reported in the main text.

Habitat	**Plant genus**	**Season**	**rs**	**p**
C Water	*Alnus*	Autumn	0.470	0.003
C Water	*Calluna*	Autumn	-0.369	0.025
C Water	*Myrica*	Summer	0.380	0.005
C Water	*Picea*	Autumn	-0.329	0.047
C Water	*Pinus*	Winter	-0.302	0.033
C Water	*Prunus*	Autumn	0.340	0.040
C Water	*Quercus*	Autumn	0.456	0.005
C Water	*Ranunculus*	Autumn	0.440	0.006
C Water	*Vaccinium*	Autumn	-0.357	0.030
D1 Raised and blanket bog	*Epilobium*	Autumn	0.324	0.051
D1 Raised and blanket bog	*Hypericum*	Autumn	-0.320	0.054
D1 Raised and blanket bog	*Oxalis*	Autumn	-0.366	0.026
D1 Raised and blanket bog	*Oxalis*	Summer	-0.472	<0.001
D1 Raised and blanket bog	*Quercus*	Autumn	-0.487	0.002
D1 Raised and blanket bog	*Rubus*	Summer	-0.300	0.029
D1 Raised and blanket bog	*Salix*	Winter	0.307	0.030
D1 Raised and blanket bog	*Vaccinium*	Autumn	0.306	0.065
D2 Valley mires, poor fens and transition mires	*Chamaenerion*	Spring	-0.359	0.006
D2 Valley mires, poor fens and transition mires	*Myrica*	Autumn	0.397	0.015
D2 Valley mires, poor fens and transition mires	*Oxalis*	Autumn	-0.321	0.052
D2 Valley mires, poor fens and transition mires	*Oxalis*	Summer	-0.307	0.025
D2 Valley mires, poor fens and transition mires	*Picea*	Autumn	-0.363	0.027
D2 Valley mires, poor fens and transition mires	*Picea*	Winter	-0.388	0.005

Habitat	Plant genus	Season	rs	p
E1 Dry grasslands	*Chamaenerion*	Spring	-0.321	0.014
E1 Dry grasslands	*Myrica*	Winter	0.395	0.004
E1 Dry grasslands	*Oxalis*	Summer	-0.317	0.021
E1 Dry grasslands	*Rubus*	Summer	-0.301	0.028
E1 Dry grasslands	*Salix*	Autumn	-0.396	0.015
E1 Dry grasslands	*Vaccinium*	Spring	0.476	<0.001
E2 Mesic grasslands	*Alnus*	Autumn	0.321	0.053
E2 Mesic grasslands	*Calluna*	Autumn	-0.534	<0.001
E2 Mesic grasslands	*Erica*	Autumn	-0.331	0.045
E2 Mesic grasslands	*Filipendula*	Summer	0.317	0.021
E2 Mesic grasslands	*Oxalis*	Autumn	0.338	0.041
E2 Mesic grasslands	*Quercus*	Autumn	0.593	<0.001
E2 Mesic grasslands	*Ranunculus*	Autumn	0.318	0.055
E2 Mesic grasslands	*Rumex*	Summer	0.327	0.017
E2 Mesic grasslands	*Vaccinium*	Autumn	-0.373	0.023
E2 Mesic grasslands	*Viola*	Autumn	0.348	0.035
E3 Seasonally wet and wet grasslands	*Alnus*	Autumn	0.374	0.023
E3 Seasonally wet and wet grasslands	*Calluna*	Autumn	-0.500	0.002
E3 Seasonally wet and wet grasslands	*Calluna*	Spring	-0.354	0.006
E3 Seasonally wet and wet grasslands	*Calluna*	Summer	-0.426	0.001
E3 Seasonally wet and wet grasslands	*Calluna*	Winter	-0.430	0.002
E3 Seasonally wet and wet grasslands	*Filipendula*	Summer	0.329	0.016
E3 Seasonally wet and wet grasslands	*Myrica*	Winter	0.365	0.009
E3 Seasonally wet and wet grasslands	*Oxalis*	Summer	-0.369	0.007
E3 Seasonally wet and wet grasslands	*Prunus*	Autumn	0.365	0.026
E3 Seasonally wet and wet grasslands	*Rubus*	Spring	0.315	0.016
E5 Woodland fringes, clearings and tall forb stands	*Calluna*	Spring	-0.317	0.015
E5 Woodland fringes, clearings and tall forb stands	*Calluna*	Winter	-0.396	0.004
E5 Woodland fringes, clearings and tall forb stands	*Myrica*	Autumn	0.428	0.008
E5 Woodland fringes, clearings and tall forb stands	*Myrica*	Winter	0.329	0.020

Habitat	Plant genus	Season	rs	p
E5 Woodland fringes, clearings and tall forb stands	*Oxalis*	Autumn	0.499	0.002
E5 Woodland fringes, clearings and tall forb stands	*Pinus*	Autumn	0.437	0.007
E5 Woodland fringes, clearings and tall forb stands	*Quercus*	Autumn	0.364	0.027
E5 Woodland fringes, clearings and tall forb stands	*Ranunculus*	Summer	0.307	0.025
E5 Woodland fringes, clearings and tall forb stands	*Rubus*	Spring	0.323	0.013
E5 Woodland fringes, clearings and tall forb stands	*Rumex*	Autumn	-0.351	0.033
E5 Woodland fringes, clearings and tall forb stands	*Rumex*	Summer	0.440	<0.001
E5 Woodland fringes, clearings and tall forb stands	*Salix*	Autumn	-0.305	0.066
F2 Arctic, alpine and subalpine scrub	*Vaccinium*	Autumn	0.398	0.015
F3 Temperate and Mediterranean montane scrub	*Erica*	Autumn	-0.390	0.017
F3 Temperate and Mediterranean montane scrub	*Galium*	Winter	-0.310	0.028
F3 Temperate and Mediterranean montane scrub	*Myrica*	Autumn	0.354	0.032
F3 Temperate and Mediterranean montane scrub	*Myrica*	Summer	0.350	0.010
F3 Temperate and Mediterranean montane scrub	*Picea*	Autumn	-0.357	0.030
F3 Temperate and Mediterranean montane scrub	*Picea*	Winter	-0.455	<0.001
F3 Temperate and Mediterranean montane scrub	*Quercus*	Autumn	0.319	0.054
F4 Temperate shrub heathland	*Alnus*	Autumn	-0.396	0.015
F4 Temperate shrub heathland	*Calluna*	Autumn	0.384	0.019
F4 Temperate shrub heathland	*Chamaenerion*	Summer	-0.508	<0.001
F4 Temperate shrub heathland	*Epilobium*	Summer	-0.490	<0.001
F4 Temperate shrub heathland	*Myrica*	Autumn	0.382	0.019
F4 Temperate shrub heathland	*Oxalis*	Autumn	-0.351	0.033
F4 Temperate shrub heathland	*Oxalis*	Spring	0.336	0.010
F4 Temperate shrub heathland	*Oxalis*	Summer	-0.312	0.023
F4 Temperate shrub heathland	*Picea*	Autumn	-0.417	0.010
F4 Temperate shrub heathland	*Quercus*	Autumn	-0.459	0.004
F4 Temperate shrub heathland	*Ranunculus*	Autumn	-0.330	0.046
F4 Temperate shrub heathland	*Rubus*	Autumn	-0.378	0.021
F4 Temperate shrub heathland	*Rubus*	Summer	-0.377	0.005
F4 Temperate shrub heathland	*Salix*	Summer	-0.401	0.003

Habitat	**Plant genus**	**Season**	**rs**	**p**
F4 Temperate shrub heathland	*Vaccinium*	Autumn	0.455	0.005
F4 Temperate shrub heathland	*Vaccinium*	Summer	0.485	<0.001
F4 Temperate shrub heathland	*Viola*	Autumn	-0.308	0.063
F9 Riverine and fen scrubs	*Alnus*	Autumn	0.495	0.002
F9 Riverine and fen scrubs	*Myrica*	Winter	0.393	0.005
F9 Riverine and fen scrubs	*Picea*	Winter	-0.332	0.018
F9 Riverine and fen scrubs	*Pinus*	Winter	-0.533	<0.001
G1 Broadleaved deciduous woodland	*Calluna*	Autumn	-0.411	0.011
G1 Broadleaved deciduous woodland	*Calluna*	Spring	-0.321	0.014
G1 Broadleaved deciduous woodland	*Calluna*	Winter	-0.312	0.028
G1 Broadleaved deciduous woodland	*Chamaenerion*	Summer	0.353	0.010
G1 Broadleaved deciduous woodland	*Potentilla*	Summer	-0.334	0.015
G1 Broadleaved deciduous woodland	*Quercus*	Autumn	0.404	0.013
G1 Broadleaved deciduous woodland	*Ranunculus*	Summer	0.306	0.026
G1 Broadleaved deciduous woodland	*Rubus*	Spring	0.315	0.016
G1 Broadleaved deciduous woodland	*Rubus*	Summer	0.464	<0.001
G1 Broadleaved deciduous woodland	*Rumex*	Summer	0.495	<0.001
G1 Broadleaved deciduous woodland	*Vaccinium*	Autumn	-0.504	0.001
G1 Broadleaved deciduous woodland	*Viola*	Spring	0.361	0.005
G3 Coniferous woodland	*Calluna*	Winter	0.554	<0.001
G3 Coniferous woodland	*Chamaenerion*	Spring	0.373	0.004
G3 Coniferous woodland	*Chamaenerion*	Summer	0.641	<0.001
G3 Coniferous woodland	*Epilobium*	Autumn	0.323	0.051
G3 Coniferous woodland	*Erica*	Autumn	0.311	0.061
G3 Coniferous woodland	*Filipendula*	Summer	0.331	0.015
G3 Coniferous woodland	*Myrica*	Autumn	-0.347	0.035
G3 Coniferous woodland	*Myrica*	Winter	-0.589	<0.001
G3 Coniferous woodland	*Picea*	Autumn	0.405	0.013
G3 Coniferous woodland	*Picea*	Spring	0.343	0.008
G3 Coniferous woodland	*Picea*	Winter	0.420	0.002

Habitat	**Plant genus**	**Season**	**rs**	**p**
G3 Coniferous woodland	*Pinus*	Winter	0.303	0.032
G3 Coniferous woodland	*Potentilla*	Summer	-0.444	<0.001
G3 Coniferous woodland	*Rubus*	Autumn	0.316	0.057
G3 Coniferous woodland	*Rubus*	Summer	0.404	0.003
G3 Coniferous woodland	*Vaccinium*	Summer	-0.397	0.003
G4 Mixed deciduous and coniferous woodland	*Alnus*	Autumn	0.312	0.060
G4 Mixed deciduous and coniferous woodland	*Calluna*	Autumn	-0.413	0.011
G4 Mixed deciduous and coniferous woodland	*Hypericum*	Summer	0.332	0.015
G4 Mixed deciduous and coniferous woodland	*Oxalis*	Autumn	0.384	0.019
G4 Mixed deciduous and coniferous woodland	*Quercus*	Autumn	0.594	<0.001
G4 Mixed deciduous and coniferous woodland	*Ranunculus*	Autumn	0.375	0.022
G4 Mixed deciduous and coniferous woodland	*Rubus*	Summer	0.495	<0.001
G4 Mixed deciduous and coniferous woodland	*Vaccinium*	Autumn	-0.334	0.044
G5 Lines of trees, small anthropogenic woodlands	*Calluna*	Winter	0.375	0.007
G5 Lines of trees, small anthropogenic woodlands	*Chamaenerion*	Autumn	0.345	0.037
G5 Lines of trees, small anthropogenic woodlands	*Chamaenerion*	Spring	0.452	<0.001
G5 Lines of trees, small anthropogenic woodlands	*Chamaenerion*	Summer	0.580	<0.001
G5 Lines of trees, small anthropogenic woodlands	*Epilobium*	Autumn	0.469	0.003
G5 Lines of trees, small anthropogenic woodlands	*Myrica*	Winter	-0.622	<0.001
G5 Lines of trees, small anthropogenic woodlands	*Picea*	Spring	0.405	0.002
G5 Lines of trees, small anthropogenic woodlands	*Picea*	Winter	0.543	<0.001
G5 Lines of trees, small anthropogenic woodlands	*Potentilla*	Summer	-0.314	0.022
G5 Lines of trees, small anthropogenic woodlands	*Ranunculus*	Spring	0.336	0.010
G5 Lines of trees, small anthropogenic woodlands	*Rubus*	Autumn	0.329	0.047
G5 Lines of trees, small anthropogenic woodlands	*Rumex*	Spring	0.346	0.008
G5 Lines of trees, small anthropogenic woodlands	*Vaccinium*	Spring	-0.450	<0.001
G5 Lines of trees, small anthropogenic woodlands	*Vaccinium*	Summer	-0.348	0.011
I1 Arable land and market gardens	*Hypericum*	Summer	0.322	0.019
I1 Arable land and market gardens	*Pinus*	Autumn	0.306	0.066
I1 Arable land and market gardens	*Rubus*	Autumn	0.300	0.071

Habitat	Plant genus	Season	rs	p
J Built-up and bare (before post)	*Chamaenerion*	Autumn	0.425	0.009
J Built-up and bare (before post)	*Chamaenerion*	Spring	0.511	<0.001
J Built-up and bare (before post)	*Chamaenerion*	Summer	0.336	0.014
J Built-up and bare (before post)	*Myrica*	Winter	-0.362	0.010
J Built-up and bare (before post)	*Picea*	Winter	0.368	0.009
J Built-up and bare (before post)	*Potentilla*	Summer	-0.421	0.002
J Built-up and bare (before post)	*Rubus*	Summer	0.358	0.009
J Built-up and bare (before post)	*Rumex*	Autumn	0.621	<0.001
J Built-up and bare (before post)	*Salix*	Autumn	0.326	0.049
J Built-up and bare (before post)	*Vaccinium*	Summer	-0.310	0.024
O Bare (after post)	*Chamaenerion*	Autumn	0.351	0.033
O Bare (after post)	*Chamaenerion*	Spring	0.411	0.001
O Bare (after post)	*Chamaenerion*	Summer	0.467	<0.001
O Bare (after post)	*Epilobium*	Spring	0.350	0.007
O Bare (after post)	*Rubus*	Summer	0.413	0.002
O Bare (after post)	*Rumex*	Autumn	0.318	0.055
O Bare (after post)	*Rumex*	Spring	0.353	0.006
O Bare (after post)	*Vaccinium*	Spring	-0.426	<0.001
O Bare (after post)	*Vaccinium*	Summer	-0.333	0.015