

Title	A continuous feast of bramble: <i>Rubus fruticosus</i> agg. is a key cross#seasonal dietary resource for a fallow deer population
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Supporting Information: A continuous feast of bramble: *Rubus fruticosus* agg. is a key cross-seasonal dietary resource for a UK fallow deer population

Methodology

Ground flora survey protocol

The surveyed ground flora included scrambling shrubs such as bramble and lianas such as ivy and honeysuckle, although only ground cover was surveyed (i.e. the vertical cover of lianas climbing tree stems were not recorded). Woody plants less than 30 cm tall (such as tree seedlings) were excluded. There was at least one month between successive woodland surveys and the order was randomised. This ensured sufficient time had elapsed to observe seasonal change and that the survey order would not influence any temporal trends. Fifteen-metre radius circular sample plots were established in each woodland. For each survey, eight 0.25-m² quadrats were located randomly within each plot (Figure 1). Each quadrat was subdivided into 25 x 0.01-m² squares. The position of the quadrat was selected using polar coordinates from a randomly generated compass bearing and a random number of paces (0-15) from the plot centre. If a tree obstructed the placement, the quadrat was moved a step further out from the centre (not exceeding the plot perimeter) until a position was found where the quadrat could be placed. The percentage cover of all plant taxa in each quadrat was assessed by counting the number of 0.01-m² squares containing each plant taxon.

Faecal sample preparation

The workbench was first cleaned with 10% bleach solution and all pipettes, racks and pipette tips were exposed to UV light in a hood for at least 20 minutes. Faecal samples were defrosted at room temperature. A scalpel and tweezers were dipped in 70% ethanol and held in a Bunsen burner flame after each sample and periodically cleaned with 10% bleach. Faecal pellets were placed on a clean glass tile and, at first, the external part of the pellet was cut off, the centre extracted using the tweezers and the external parts discarded. This was to minimise any contamination from exterior sources such as aerial pollen or soil. However, this process was deemed inefficient as it greatly lengthened the amount of time spent processing each sample. Therefore, the final method involved removing any obvious contaminants such as pieces of vegetation using the tweezers and scalpel but retaining whole pellets. Each sample was then homogenised using a mortar and pestle. Each mortar and pestle set was cleaned with 10% bleach between samples. A portion of 0.04 – 0.08 g of crushed faecal material was weighed out in a plastic weigh boat. The remaining faecal material was then returned to storage at -20 °C in their original containers unless the container was particularly soiled by invertebrates that had emerged from the sample when it was first frozen, in which case the remaining material was frozen in a new falcon tube.

Where there was no time to perform DNA extractions following sample preparation, samples were refrozen in 1.5 millilitre microcentrifuge tubes.

DNA extraction protocol

Summary: The lysis solution was added to the sample tubes, which were then put on a bead-beater for four minutes at 1800 rpm, followed by a 30-minute incubation period at 65 °C. Samples were then put through a Qiagen spin column in several washing steps with buffer

and then 100% ethanol to remove any staining from the column membrane. The DNA was then eluted in 60 µl of AE buffer. The elution step was repeated by putting the AE buffer through the column a second time to increase the yield.

Prior to beginning protocol:

- UV racks, tips, tubes etc.
- UV ethanol
- Soak steel beads in bleach, rinse in water then soak in ethanol. Rinse in water before use.
- Warm buffer AP1 to 65°C on heat block
- Remember to include a tube for negative control!
- Make lysis solution:
 - Buffer AP1 @ 400 ul per sample
 - RNase A (100mg/ml) @ 1ul per sample *spin down RNase A
 - Proteinase K (20mg/ml) @ 4ul per sample *defrost & vortex to mix

Protocol:

1. Place a steel bead inside the tube with sample
2. Add 405 µl lysis solution to sample
3. Place lid locks on all samples / tape lids if no lid locks
4. Beat sample for 4 mins / 30hz / 1800 rpm
5. Remove lid locks / tape
6. Incubate samples @ 65°C for 30 mins
7. Cool tubes to RT (can store samples overnight)
8. Micro centrifuge tubes (few secs)
9. Add Buffer P3 @ 130 ul per sample *Change tip each time
10. Shake all samples up and down for 15 secs
11. Micro centrifuge for a few seconds
12. Incubate sample in – 20°C for 10 mins
13. Centrifuge @ **14,000** rpm for 5 mins
14. Transfer liquid from each sample into new 1.5ml micro centrifuge tube
15. Centrifuge again @ **14,000** rpm for 5 mins
16. Transfer liquid from each sample into new 1.5ml micro centrifuge tube
17. Add 600 ul Buffer AW1 to each sample (this should be ~1.5 x volume therefore 600 ul)
18. Shake vigorously up and down for 15 secs
19. Centrifuge @ 3000rpm
20. Transfer 650 ul of this mixture into a labelled DNeasy mini spin column placed in a 2 ml collection tube
21. Centrifuge for 1 min @ **14,000** rpm
22. Discard the flowthrough
23. Repeat steps (21-22) until samples are empty
24. Place spin column into new 2ml collection tube
25. Add 500 ul Buffer AW2
26. Centrifuge for 1 min @ 14,000 rpm
27. Discard the flow through
28. Add another 500 ul Buffer AW2

29. Centrifuge for 2 mins @ 14,000 rpm
30. Discard the flow through
31. Add 500 μ l ethanol (96-100%)
32. Centrifuge for 2 mins @ 14,000 rpm
33. Remove spin column carefully so it doesn't come into contact with flow through
34. Transfer the spin column into a new 1.5ml micro centrifuge tube with no lid.
35. **Elution step:** Add 60 μ l Buffer AE
36. Incubate @ RT for 10 mins
37. Centrifuge for 1 min @ 8000 rpm
38. Transfer the elution back into the spin column on to the membrane
39. Incubate @ RT for 10 mins
40. Centrifuge for 1 min @ 8000 rpm
41. Discard the spin column
42. Aliquot DNA from snipped 1.5ml tubes into a new 1.5ml tube
43. If using Q-BIT aliquot out accordingly
44. Store samples at -20°C
45. Remove steel beads from original tubes and soak in 50% bleach

Round 1 PCR protocol

All primers were diluted from a starting stock of 4 nmol to 10 μ mol. The first round PCR amplification was carried out in 25 μ l sample volumes using 12.5 μ l of Qiagen Multiplex PCR Master Mix (2X) (containing HotStarTaq DNA Polymerase, multiplex PCR buffer and dNTP mix), 2 μ M of forward and reverse primers, 1 μ l of DNA template and 10.5 μ l of DNase/RNase-free water. Thermocycling conditions began with an initial activation step of 95 °C for 15 minutes, followed by 35 cycles of the following program: 94 °C for 30 seconds, annealing at 50 °C for 90 seconds, extension at 72 °C for 1 minute. The final extension step was 10 minutes at 72 °C.

The first round PCR products were then cleaned using magnetic beads (Agencourt® AMPure® XP, Beckman Coulter) at a volume of 0.7 x the original PCR reaction volume (17.5 μ l of beads added to 25 μ l of PCR product) to remove unused primers and primer dimers.

Bead clean-up protocol for 25 ul volume

1. Leave the Agencourt AMPure XP bottle at room temperature for 30 minutes.
2. Gently shake the AMPure XP bottle to re-suspend any magnetic particles that may have settled. Check the bottom of the bottle to see if the beads are stuck to it. Vortex a little and tap to “spin down”.
3. Aliquot out some to prevent future contamination (number of samples + extra for pipetting error + extra again for pipetting into strip).
4. Split the beads into an 8-tube strip for the multi-channel pipette according to the number of rows you are doing.
5. Add **17.5 µl** of Agencourt AMPure XP to **25 µl** of Round 1 PCR product (0.7x)
6. Mix reagent and PCR product thoroughly by pipette mixing 15 times. Let the mixed samples incubate for **5 minutes** at room temperature for maximum recovery.
7. Quick spin (optional < 500rpm) and place the 96-well plate onto an DynaMag-96 Side Magnet for **5 minutes** to separate beads from the solution.
8. Using 200ul or 100ul tips, remove very slowly the cleared solution (around 43 µl) from the 96 well PCR plate, making sure not to disturb the ring of separated magnetic beads (or remove from one side if using a strip). Discard the cleared solution.
9. With the PCR plate still on the magnetic plate, add **200 µl** of freshly prepared 80% ethanol (made fresh each day, in a 15ml falcon tube 8ml 100% ethanol and 2ml distilled water) to each well and incubate for 30 seconds at room temperature.
10. Using 200 ul tips, very slowly remove the ethanol and discard.
11. Add **200 µl** of freshly prepared 80% ethanol to each well and incubate for 30 seconds at room temperature.
12. Using 200 ul tips, very slowly remove the ethanol and discard. For the second ethanol wash, be sure to remove all the ethanol from the bottom of the well as it is a known PCR inhibitor. Use 10ul tips to remove any final drops.
13. Still on the magnetic plate, dry for 3 minutes at room temperature to ensure all traces of ethanol are removed. Watch for ethanol droplets and make sure they have evaporated before proceeding. Don't leave for much longer than 4 mins as the beads will dry out (but this is variable).
14. Take the PCR plate off the magnetic plate, then add **26ul µl of PCR water** to each well of the 96-well plate and pipette mix 10 times. Make sure that the beads are put back in suspension and well mixed.
15. Incubate on bench for 5 minutes, spin down (optional: incubate at 37°C in PCR machine if beads are not well mixed, make sure lid is at 37°C).
16. Place the PCR plate onto an DynaMag-96 Side Magnet for 5 minute to separate beads from the solution.
17. Using 200 ul tips, transfer 25 µl of the eluent to individual PCR tubes, leaving a few microlitres behind if necessary, to ensure no beads are in the final elution. If beads are drawn out, leave a few microliters behind. If beads are present in the pipette tip, resuspend the beads and try again.

Round 2 PCR protocol

The second round of PCR was also carried out in a 25 µl volume using 12.5 µl of Qiagen Multiplex PCR Master Mix (2X), 2 µM of the Illumina Fi5 and Ri7 primers in unique combinations for each sample to enable individual sample identification during bioinformatics analysis, 6.5 µl of DNase/RNase-free water and 5 µl of cleaned first round PCR product. Thermocycling conditions began with 95 °C for 15 minutes, followed by 15 cycles of the following programme: 98 °C for 10 seconds, 65 °C for 30 seconds, 72 °C for 30 seconds. The final extension step was 5 minutes at 72 °C.

Sample pooling and size selection

The second round PCR products were electrophoresed on a 96-well 1% agarose gel and categorised by DNA concentration according to brightness of each band. The second round PCR products were then combined into pools of the same concentration category at 10 µl per sample (**Table S6**). Samples of the strongest concentration category were combined within PCR plates only; samples in the medium, weak and no band categories were combined across plates. This gave a total of 11 pools for ITS2 and 11 pools for *rbcL*. The negative controls for extractions and PCRs were added into the strongest pools of each plate at 1 µl per control. The positive controls were added into their respective concentration category pool at 1 µl per control. For *rbcL*, ten samples were not sequenced as they did not produce a band after second round PCR and quantification using a high-sensitivity Qubit (Invitrogen, USA) assay found no detectable DNA.

The fragment sizes and their relative concentrations were analysed on a 4150 TapeStation System (Agilent), following the manufacturer's instructions. The pools were combined

according to the concentration of the target band to obtain a final volume of 325 µl each for ITS2 and *rbcL* (**Table S7**). The two weakest pools were limited to 100 µl within these final volumes to ensure they were represented during sequencing but did not overdilute pools with stronger concentrations. Pooling in equimolar concentrations was not possible, as this would overdilute the final pool.

The two final 325 µl pools were isolated using a Pippin Prep (Sage Science) to remove any non-target bands, following manufacturer's instructions. For ITS2, the size range selected was 500 – 700 bp, and for *rbcL*, 600 – 800 bp, terminating with a final elution of the sequencing library into 40 µl.

Processing of sequencing data

Data processing was carried out in R version 4.1.0 (R Core Team, 2021) using the Supercomputing Wales (SCW) facility. Initially, the ITS2 and *rbcL* primers were removed using *cutadapt* (Martin, 2011). This step also removed any sequences where the primers were not present. Based on the fastqc quality scores (QC) of the reads, a QC score of 20 was used as the threshold for downstream processing. The sequences were filtered and trimmed using the *DADA2* package (Callahan *et al.*, 2016). For the *filterAndTrim* function, we specified a *truncQ* score of 20 and a max error rate of 2 for the forward reads and 5 for the reverse reads, with a *truncLen* value of 0. For the *mergePairs* function, we specified a maximum mismatch of 1 and a minimum overlap of 10. Any detected chimeras were also removed. For *rbcL* sequences, the *filterAndTrim* step resulted in heavy losses of reads, therefore we took the decision to use unmerged forward reads for taxonomy assignment. In contrast, the ITS2 marker performed well during merging, therefore we used the merged reads for taxonomy assignment.

The Amplicon Sequence Variants (ASVs) from each sample were then blasted against a curated ITS2 and *rbcL* plant database from Barcode UK (Jones *et al.*, 2021) using the *blastn* (Madden & Camacho, 2008) function on the SCW platform. This assigned plant species codes to each ASV.

Supplementary tables and figures

Table S1. Sampling occasions for fallow deer faecal samples per site and month over two years (2019-2021) and number of faecal samples collected per month.

Study Site	Month	Sampling occasions	Number of samples
1	January	2	11
	February	2	12
	March	2	11
	April	1	5
	May	1	5
	June	1	6
	July	2	13
	August	2	11
	September	2	12
	October	2	10
	November	2	10
	December	2	10
	Total	21	116
2	January	2	10
	February	2	11
	March	2	12
	April	1	6
	May	1	5
	June	1	5
	July	2	10
	August	2	11
	September	2	12
	October	2	15
	November	2	11
	December	2	11
	Total	21	119
3	January	2	10
	February	2	11
	March	2	7
	April	1	5
	May	1	6
	June	1	6
	July	2	13
	August	2	11
	September	2	11
	October	2	11
	November	2	11
	December	2	10
	Total	21	112
Zoo	December	1	6

Table S2. Area and 15 metre-radius sample plot replication in the ten woodland study sites surveyed for ground flora and woody vegetation.

Study Site	Sample plot quantity	Site area (ha)
1	8	11
2	10	64
3	7	11
4	6	5
5	6	12
6	10	20
7	8	10
8	5	6
9	7	12
10	4	2

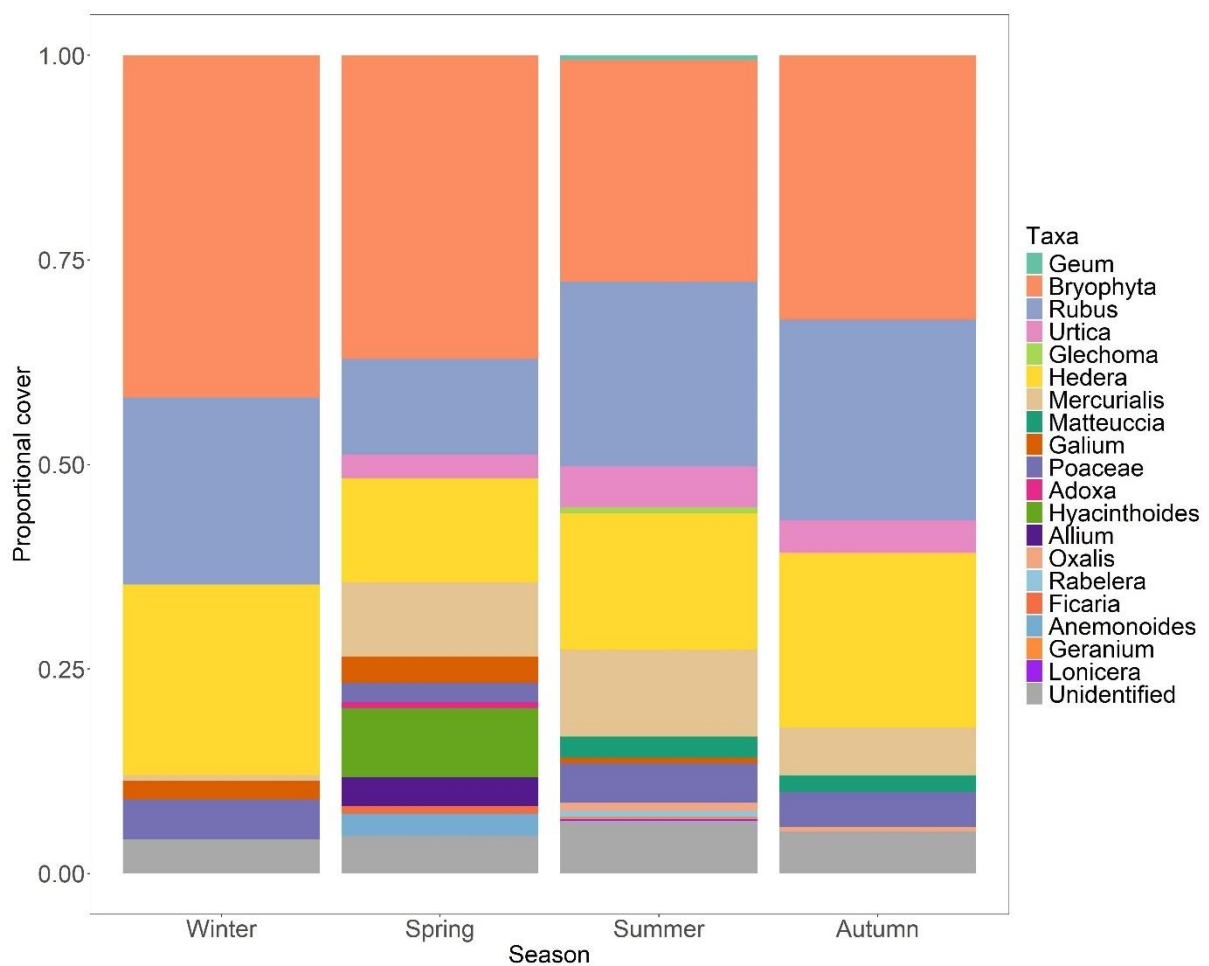


Figure S3: The proportional ground cover of taxa identified in the ground flora surveys per season across the ten woodlands surveyed. Bare ground was not included in this plot.

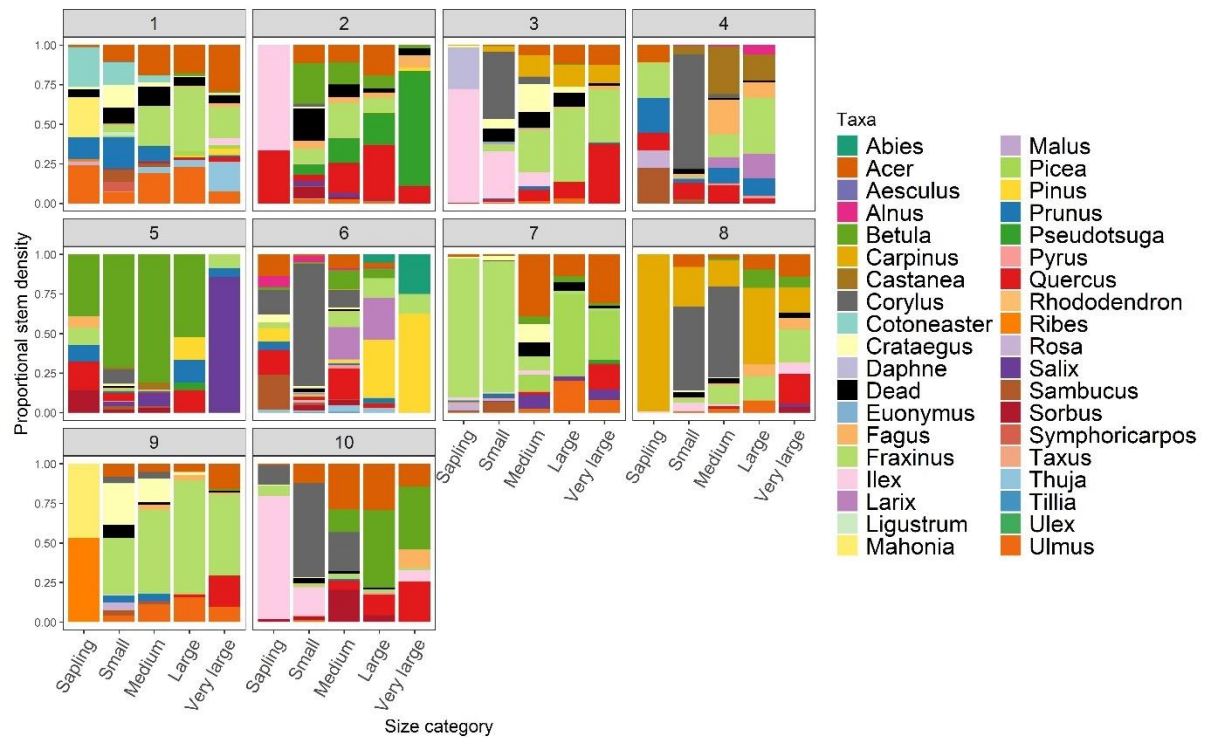


Figure S4: Proportional stem density of tree and shrub taxa identified in the woodland surveys, grouped by stem size category. The “Dead” category indicates the proportion of stems that were dead (not included in diet preference analysis). Plots are numbered by woodland ID. Woodlands 1-3 were the woodlands surveyed for diet samples. Stem size was categorized as follows: Sapling: > 30 cm height, < 1.3 m height; Small = > 1.3 m height, < 10 cm DBH; Medium: 10-20 cm DBH; Large: 21-30 cm DBH; Very large: >=31 cm DBH.

Table S5: Primer sequences. The length of amplicons was estimated using the results from analysis on a 4150 TapeStation System (Agilent) (Table S4).

Marker	Primer position	Primer name	Primer sequence (5'-3')	5' universal tail	Reference	Est. amplicon length
ITS2	Forward	ITS2F	ATGCGATACTT GGTGTGAAT	ACACTCTTTCC CTACACGACGC TCTTCCGATCT- NNNNNN	Chen et al., (2010)	300-400bp
ITS2	Reverse	Unipla ntR	CCCGHYTGAYY TGRGGTCDC	GTGACTGGAGT TCAGACGTGTG CTCTTCCGATCT	Moorhouse-Gann et al., (2018).	
<i>rbcL</i>	Forward	<i>rbcLa</i> - F	ATGTCACCACA AACAGAGACT AAAGC	ACACTCTTTCC CTACACGACGC TCTTCCGATCT- NNNNNN	Khanam et al., (2016)	500-560bp
<i>rbcL</i>	Reverse	<i>rbcLr</i> 5 06	AGGGGACGAC CATACTTG TTC A	GTGACTGGAGT TCAGACGTGTG CTCTTCCGATCT	de Vere et al., (2012)	

Table S6: List of sample pools for ITS2 and *rbcL*. Number of samples includes positive controls (one per plate), first and second round PCR controls (one each per plate) and negative controls for DNA extractions (2-3 per plate). The peak size indicates the average base pair length for the PCR amplicons in each pool. The peak size and concentration were recorded on the 4150 TapeStation System (Agilent).

Marker	Pool ID	Pool contents	Number of samples	Peak size	Peak conc ng/ul
ITS2	Pool 1	No band plate 1 + 2	31	494	0.60
ITS2	Pool 2	No band plate 3 + 4	25	509	3.70
ITS2	Pool 3	Weak plate 1 + 4	43	510	4.94
ITS2	Pool 4	Weak plate 2 + 3	24	505	1.85
ITS2	Pool 5	Medium plate 1 + 4	38	507	6.32
ITS2	Pool 6	Medium plate 2 + 3	40	508	2.90
ITS2	Pool 7	Strong plate 1	33	501	9.99
ITS2	Pool 8	Strong plate 2	31	518	2.49
ITS2	Pool 9	Strong plate 3 A	36	515	18.10
ITS2	Pool 10	Strong plate 3 B	35	508	11.70
ITS2	Pool 11	Strong plate 4	27	509	12.00
<i>rbcL</i>	Pool 12	No band plate 1 + 2	33	666	0.33
<i>rbcL</i>	Pool 13	No band plate 3 + 4	34	666	1.44
<i>rbcL</i>	Pool 14	Weak plate 1 + 2	35	669	2.42
<i>rbcL</i>	Pool 15	Weak plate 3 + 4	32	664	5.42
<i>rbcL</i>	Pool 16	Medium plate 1 + 3	26	670	9.08
<i>rbcL</i>	Pool 17	Medium plate 2 + 4	31	675	9.78
<i>rbcL</i>	Pool 18	Strong plate 1	29	667	15.60
<i>rbcL</i>	Pool 19	Strong plate 2 A	26	679	6.21
<i>rbcL</i>	Pool 20	Strong plate 2 B	27	688	6.05
<i>rbcL</i>	Pool 21	Strong plate 3	40	666	19.60
<i>rbcL</i>	Pool 22	Strong plate 4	38	672	24.70

Table S7: Details of the volume of each plate pool added to the two final pools for ITS2 and *rbcL* for sequencing. The two weakest pools were limited to 100 µl within these final volumes to ensure they were represented during sequencing but did not overdilute pools with stronger concentrations. Pooling in equimolar concentrations was not possible, as this would overdilute the final pool.

Marker	Pool ID	Pool	ng/ul	n Samples	ng per sample	Volume	ng in pool
ITS2	Pool 1	No band plate 1 + 2	0.60	31	1.10	57.4	34.21
ITS2	Pool 4	Weak plate 2 + 3	1.85	24	3.43	44.4	82.22
ITS2	Pool 8	Strong plate 2	2.49	31	1.82	22.6	56.39
ITS2	Pool 6	Medium plate 2 + 3	2.90	40	2.12	29.2	84.74
ITS2	Pool 2	No band plate 3 + 4	3.70	25	2.70	18.3	67.57
ITS2	Pool 3	Weak plate 1 + 4	4.94	43	3.61	31.4	155.18
ITS2	Pool 5	Medium plate 1 + 4	6.32	38	4.62	27.8	175.44
ITS2	Pool 7	Strong plate 1	9.99	33	7.30	24.1	240.83
ITS2	Pool 10	Strong plate 3 B	11.70	35	8.55	25.6	299.15
ITS2	Pool 11	Strong plate 4	12.00	27	8.77	19.7	236.69
ITS2	Pool 9	Strong plate 3 A	18.10	36	13.22	26.3	476.01
<i>rbcL</i>	Pool 12	No band plate 1 + 2	0.33	33	0.43	42.9	14.14
<i>rbcL</i>	Pool 13	No band plate 3 + 4	1.44	34	1.87	44.2	63.58
<i>rbcL</i>	Pool 14	Weak plate 1 + 2	2.42	35	1.92	27.7	67.10
<i>rbcL</i>	Pool 15	Weak plate 3 + 4	5.42	32	4.29	25.4	137.41
<i>rbcL</i>	Pool 16	Medium plate 1 + 3	9.08	26	7.19	20.6	187.04
<i>rbcL</i>	Pool 17	Medium plate 2 + 4	9.78	31	7.75	24.6	240.20
<i>rbcL</i>	Pool 18	Strong plate 1	15.60	29	12.36	23.0	358.42
<i>rbcL</i>	Pool 19	Strong plate 2 A	6.21	26	4.92	20.6	127.92
<i>rbcL</i>	Pool 20	Strong plate 2 B	6.05	27	4.79	21.4	129.41
<i>rbcL</i>	Pool 21	Strong plate 3	19.60	40	15.53	31.7	621.13
<i>rbcL</i>	Pool 22	Strong plate 4	24.70	38	19.57	30.1	743.61

Table S8: All taxa present in one or more faecal samples, classified by group

Taxon	Group
<i>Acer</i>	Broadleaf tree
<i>Achillea</i>	Herbaceous
<i>Adoxaceae</i>	Shrub
<i>Aesculus</i>	Broadleaf tree
<i>Alchemilla</i>	Herbaceous
<i>Alliaria</i>	Herbaceous
<i>Allium</i>	Herbaceous
<i>Angelica</i>	Herbaceous
<i>Apiaceae</i>	Herbaceous
<i>Arbutus</i>	Shrub
<i>Arenaria</i>	Herbaceous
<i>Asteraceae</i>	Herbaceous
<i>Atrichum</i>	Moss
<i>Atriplex</i>	Shrub
<i>Aucuba</i>	Shrub
<i>Bellis</i>	Herbaceous
<i>Bergenia</i>	Herbaceous
<i>Beta</i>	Herbaceous
<i>Betula</i>	Broadleaf tree
<i>Betulaceae</i>	Broadleaf tree
<i>Brassica</i>	Herbaceous
<i>Brassicaceae</i>	Herbaceous
<i>Bryophyta</i>	Moss
<i>Buddleja</i>	Shrub
<i>Caltha</i>	Herbaceous
<i>Campanula</i>	Herbaceous
<i>Capsella</i>	Herbaceous
<i>Cardamine</i>	Herbaceous
<i>Carex</i>	Sedge
<i>Carpinus</i>	Broadleaf tree
<i>Caryophyllaceae</i>	Herbaceous
<i>Castanea</i>	Broadleaf tree
<i>Centaurea</i>	Herbaceous
<i>Cerastium</i>	Herbaceous
<i>Chaenomeles</i>	Shrub
<i>Chaerophyllum</i>	Herbaceous
<i>Chamaenerion</i>	Herbaceous
<i>Chenopodium</i>	Herbaceous
<i>Chrysosplenium</i>	Herbaceous
<i>Cicerbita</i>	Herbaceous
<i>Circaea</i>	Herbaceous
<i>Cirsium</i>	Herbaceous
<i>Clematis</i>	Liana
<i>Convolvulus</i>	Herbaceous
<i>Cornus</i>	Shrub

<i>Corylus</i>	Broadleaf tree
<i>Crataegus</i>	Broadleaf tree
<i>Crepis</i>	Herbaceous
<i>Crocosmia</i>	Herbaceous
<i>Crocus</i>	Herbaceous
<i>Cruciata</i>	Herbaceous
<i>Deutzia</i>	Herbaceous
<i>Epilobium</i>	Herbaceous
<i>Erica</i>	Shrub
<i>Ericaceae</i>	Shrub
<i>Eucalyptus</i>	Broadleaf tree
<i>Euonymus</i>	Broadleaf tree
<i>Fabaceae</i>	Herbaceous
<i>Fagopyrum</i>	Herbaceous
<i>Fagus</i>	Broadleaf tree
<i>Ficaria</i>	Herbaceous
<i>Ficus</i>	Broadleaf tree
<i>Filipendula</i>	Herbaceous
<i>Fraxinus</i>	Broadleaf tree
<i>Frullania</i>	Liverwort
<i>Fuchsia</i>	Shrub
<i>Fumaria</i>	Herbaceous
<i>Galium</i>	Herbaceous
<i>Geranium</i>	Herbaceous
<i>Geum</i>	Herbaceous
<i>Gunnera</i>	Herbaceous
<i>Hedera</i>	Liana
<i>Helianthemum</i>	Herbaceous
<i>Heracleum</i>	Herbaceous
<i>Hyacinthoides</i>	Herbaceous
<i>Hydrangea</i>	Shrub
<i>Hypericum</i>	Herbaceous
<i>Ilex</i>	Shrub
<i>Jasminum</i>	Liana
<i>Juncus</i>	Rush
<i>Lathyrus</i>	Herbaceous
<i>Lepidium</i>	Herbaceous
<i>Ligusticum</i>	Herbaceous
<i>Ligustrum</i>	Shrub
<i>Lilium</i>	Herbaceous
<i>Lonicera</i>	Liana
<i>Lotus</i>	Herbaceous
<i>Luzula</i>	Rush
<i>Lysimachia</i>	Herbaceous
<i>Lythrum</i>	Herbaceous
<i>Medicago</i>	Herbaceous
<i>Mercurialis</i>	Herbaceous
<i>Oenanthe</i>	Herbaceous

<i>Orchidaceae</i>	Herbaceous
<i>Orobanche</i>	Herbaceous
<i>Oxalis</i>	Herbaceous
<i>Persicaria</i>	Herbaceous
<i>Pinaceae</i>	Coniferous tree
<i>Pinus</i>	Coniferous tree
<i>Plantago</i>	Herbaceous
<i>Poaceae</i>	Grass
<i>Polygonum</i>	Herbaceous
<i>Polypodium</i>	Fern
<i>Polystichum</i>	Fern
<i>Populus</i>	Broadleaf tree
<i>Potentilla</i>	Herbaceous
<i>Primula</i>	Herbaceous
<i>Prunus</i>	Broadleaf tree
<i>Pseudotsuga</i>	Coniferous tree
<i>Pteris</i>	Fern
<i>Quercus</i>	Broadleaf tree
<i>Ranunculus</i>	Herbaceous
<i>Rheum</i>	Herbaceous
<i>Rhynchosstegium</i>	Moss
<i>Ribes</i>	Shrub
<i>Rodgersia</i>	Herbaceous
<i>Rosa</i>	Scrambling shrub
<i>Rosaceae</i>	Scrambling shrub
<i>Rubia</i>	Herbaceous
<i>Rubus</i>	Scrambling shrub
<i>Rumex</i>	Herbaceous
<i>Salix</i>	Broadleaf tree
<i>Salsola</i>	Shrub
<i>Salvia</i>	Herbaceous
<i>Sambucus</i>	Shrub
<i>Saussurea</i>	Herbaceous
<i>Saxifraga</i>	Herbaceous
<i>Silene</i>	Herbaceous
<i>Sinapis</i>	Herbaceous
<i>Sorbus</i>	Broadleaf tree
<i>Sparganium</i>	Reed
<i>Spiraea</i>	Herbaceous
<i>Stellaria</i>	Herbaceous
<i>Symphoricarpos</i>	Shrub
<i>Taraxacum</i>	Herbaceous
<i>Taxus</i>	Coniferous tree
<i>Tilia</i>	Broadleaf tree
<i>Torilis</i>	Herbaceous
<i>Trifolium</i>	Herbaceous
<i>Tuberaria</i>	Herbaceous
<i>Typhaceae</i>	Bulrush

<i>Ulex</i>	Shrub
<i>Ulmus</i>	Broadleaf tree
<i>Umbilicus</i>	Herbaceous
<i>Urtica</i>	Herbaceous
<i>Valeriana</i>	Herbaceous
<i>Veronica</i>	Herbaceous
<i>Viburnum</i>	Shrub
<i>Vicia</i>	Herbaceous
<i>Vinca</i>	Herbaceous
<i>Viola</i>	Herbaceous
<i>Weigela</i>	Shrub

Table S9: Results from ground flora preference analysis. The column “Test” indicates whether a preference for a given resource was detected, and if it was stronger or weaker than expected.

Resource	Observed	Null	Lower.95.CL	Upper.95.CL	Test	SES
<i>Cirsium</i>	2	0.552	0	2	ns	2.528503
<i>Epilobium</i>	5	0.794	0	3	Stronger	5.25403
<i>Geranium</i>	5	3.512	1	7	ns	0.982345
<i>Hedera</i>	71	95.544	89	102	Weaker	-7.65483
<i>Mercurialis</i>	2	1.302	0	4	ns	0.664008
<i>Poaceae</i>	39	47.228	40	54	Weaker	-2.27312
<i>Rubus</i>	119	91.48	84	99	Stronger	7.701968
<i>Rumex</i>	8	4.598	3	6.525	Stronger	3.672504
<i>Stellaria</i>	2	2.16	0	4	ns	-0.1643
<i>Taraxacum</i>	1	1.012	1	1	ns	-0.1101
<i>Urtica</i>	4	9.818	7	13	Weaker	-3.59842

Table S10. Results from woody plant preference analysis. The column “Test” indicates whether a preference for a given resource was detected, and if it was stronger or weaker than expected.

Resource	Observed	Null	Lower.95 .CL	Upper.95 .CL	Test	SES
<i>Acer</i>	56	34.134	25	44	Stronger	4.295894
<i>Aesculus</i>	4	0.064	0	1	Stronger	15.55451
<i>Betula</i>	13	76.544	64	89	Weaker	-9.94848
<i>Carpinus</i>	2	17.048	10	25	Weaker	-3.77411
<i>Corylus</i>	15	96.588	82	112	Weaker	-11.1062
<i>Crataegus</i>	23	22.804	14	32	ns	0.041985
<i>Euonymus</i>	1	0.626	0	2	ns	0.507443
<i>Fagus</i>	9	9.214	4	15	ns	-0.07424
<i>Fraxinus</i>	76	160.032	147	172	Weaker	-12.33
<i>Ilex</i>	6	29.504	20	40	Weaker	-4.5294
<i>Pinus</i>	2	4.408	1	9	ns	-1.18149
<i>Prunus</i>	96	16.118	10	23	Stronger	21.95144
<i>Pseudotsuga</i>	1	9.982	5	16	Weaker	-2.98609
<i>Quercus</i>	100	30.07	22	40	Stronger	14.90332
<i>Ribes</i>	3	3.134	0	7	ns	-0.0736
<i>Rosa</i>	82	9.902	4	16	Stronger	23.88632
<i>Salix</i>	15	7.652	3	13	Stronger	2.794761
<i>Sambucus</i>	3	20.294	13	29	Weaker	-4.12573
<i>Sorbus</i>	3	9.386	5	15.525	Weaker	-2.21533
<i>Symphoricarpos</i>	1	1.272	0	4	ns	-0.24951
<i>Taxus</i>	1	0.14	0	1	ns	2.361129
<i>Ulmus</i>	60	13.084	6	20	Stronger	13.59069

Supporting Information References

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