Received: 4 August 2025 | Accepted: 13 July 2026

DOI: 10.1002/2688-8319.70308

Ecological Solutions and Evidence

RESEARCH ARTICLE

Multi-tool monitoring: Integrating non-invasive methods to assess vertebrate richness, functional diversity and trophic complexity

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Handling Editor: Christian Rossi

Abstract

1. Effective biodiversity monitoring is essential for understanding taxonomic, functional and trophic dynamics in changing ecosystems. Non-invasive tools such as environmental DNA (eDNA) metabarcoding, passive acoustic monitoring (PAM) and camera trapping offer complementary strengths, yet their relative effectiveness for assessing biodiversity, in particular functional diversity and trophic structure, remains underexplored. These approaches are particularly valuable in rewilding landscapes, where restoration actions create heterogeneous, rapidly changing habitats that require scalable monitoring solutions.
2. We applied an integrated monitoring approach at a rewilding site in Scotland, comparing vertebrate diversity detected by metabarcoding four eDNA sample types (water, soil, tree rolling and scat) with PAM and camera traps. Each method was evaluated for taxonomic richness, community composition, functional diversity via trait-based analyses and trophic complexity through co-occurrence networks, metrics rarely assessed together across methods.
3. In total, 79 vertebrate taxa were detected: PAM captured 52 taxa, eDNA 44 and camera traps 12. Of the eDNA substrates, water and tree-rolling samples had the highest richness. Each method detected unique species, with birds and bats best covered by PAM, and small mammals by eDNA. Community composition varied between methods, with eDNA substrates capturing broader communities. PAM revealed the strongest community differences between wooded and open habitats.
4. Tree rolling and water eDNA captured the greatest functional diversity, while PAM showed higher redundancy. No single method captured all functional traits. Integrating eDNA methods with PAM produced the most complete trophic network. PAM provided the most complete network but missed key terrestrial interactions that were filled by eDNA.
5. *Practical implication*. Rewilding and restoration programmes require an integrated multi-method 'toolkit' to comprehensively capture vertebrate richness, functional

diversity and trophic complexity. We recommend combining PAM with either tree rolling or water eDNA sampling as an optimal monitoring strategy, balancing their complementary strengths in taxonomic and functional coverage.

KEYWORDS
biodiversity monitoring, camera traps, eDNA, functional diversity, passive acoustic monitoring, rewilding, trophic complexity

1 | INTRODUCTION

Effective biodiversity monitoring is essential to understand how ecosystems respond to environmental pressures. Global frameworks such as the Kunming-Montreal Global Biodiversity Framework (CBD, 2022) emphasise the need for scalable, repeatable methods to assess biodiversity change. Monitoring must capture multiple dimensions of biodiversity, including taxonomic richness, functional traits and ecological interactions, as these processes underpin ecosystem resilience and stability (Cadotte et al., 2011; Mori et al., 2013; Thompson et al., 2012). Developing approaches that can reliably measure these dimensions across heterogeneous terrestrial environments is therefore a central challenge for ecological monitoring.

Rewilding provides an applied context in which robust biodiversity monitoring is particularly valuable. Rewilding can restore ecosystems and reverse biodiversity declines by encouraging wilder, diverse landscapes. It aims to restore self-sustaining ecosystems with increased resilience and complexity by letting nature take the lead through approaches like land abandonment and species translocations (Carver et al., 2021; Perino et al., 2019). Despite growing popularity, empirical evidence on the ecological impacts of rewilding remains limited (Hart et al., 2023). Unlike traditional restoration, rewilding can form unpredictable ecosystems over unknown timescales (Pettorelli & Bullock, 2023), requiring systematic monitoring. Effective monitoring must therefore capture whole-ecosystem dynamics, such as trophic complexity, disturbance and connectivity, while remaining cost-effective (Torres et al., 2018).

Recent advances in non-invasive biodiversity monitoring offer scalable solutions for tracking ecological change while minimising disturbance. Camera traps are widely regarded as the go-to option for monitoring terrestrial mammals, providing reliable data on species occurrence, activity and behaviour, though detections are often biased towards larger-bodied species (Burton et al., 2015). Passive acoustic monitoring (PAM) enables minimally invasive surveys of sound-producing taxa and can be deployed over extended periods, though is restricted to species producing identifiable sounds (Hoefer et al., 2023). Finally, eDNA metabarcoding detects organisms from genetic material present in environmental samples, such as water, soil and air (Taberlet et al., 2012). However, efficacy varies among substrates and ecosystems (Ruppert et al., 2019), and applications in terrestrial systems lag behind those in aquatic habitats (Cowgill et al., 2025). Differences in eDNA origin, transport and persistence create substrate-specific challenges (Newton et al., 2025), although vertebrates can be detected from diverse substrates, including water, soil, air and vegetation surfaces (reviewed by Cowgill et al., 2025).

Despite growing adoption, the relative performance of different vertebrate detection methods remains uncertain. Comparisons between eDNA metabarcoding and camera trapping show mixed results (Cowgill et al., 2025). While eDNA from water samples can outperform camera traps for mammal detections (Sales et al., 2020), results vary across contexts (Mena et al., 2021). Soil-derived eDNA may detect small mammals missed by camera traps but can perform poorly for other vertebrates (Leempoel et al., 2020). Combining PAM and eDNA metabarcoding could leverage their complementary strengths across taxa and reduce costs (Bell & Malerba, 2025). However, few empirical comparisons exist between PAM and terrestrial eDNA metabarcoding (Cowgill et al., 2025). Moreover, the optimal combination of methods for vertebrate monitoring remains unknown, and detection is strongly context-dependent, with the same method performing differently across ecosystems (Newton et al., 2025).

Studies using eDNA metabarcoding and PAM have primarily focused on species richness and community composition, with functional analyses remaining underexplored (Cowgill et al., 2025; Sugai et al., 2019). Functional diversity, encompassing the ecological traits within communities, is crucial for understanding ecosystem processes (Cadotte et al., 2011). Likewise, trophic networks based on potential interactions (metawebs; Dunne, 2005) offer a framework to understand ecosystem recovery (Boyse et al., 2025). While direct observation best captures local trophic dynamics, this can be impractical; emerging non-invasive technologies can support analysis of larger, more complex networks and help overcome key data collection constraints (Adhurya & Park, 2025; Hartig et al., 2024). However, the effectiveness of different methods for these metrics is poorly understood for terrestrial ecosystems.

In this study, we compare camera trapping, PAM and eDNA metabarcoding of four substrates (soil, water, tree rolling and scat) for assessing vertebrate diversity within a heterogeneous rewilding landscape. The relative efficacy of these methods for capturing taxonomic, functional and trophic dimensions of biodiversity remains poorly understood. This uncertainty is particularly relevant in rewilding landscapes, where restoration trajectories are inherently uncertain and monitoring must capture multiple dimensions of biodiversity change. We therefore address two questions: (1) Is there an optimal combination of non-invasive methods

to capture vertebrate taxonomic, functional and trophic diversity? (2) Is a single eDNA substrate sufficient to characterise vertebrate diversity, or is a combination required? We hypothesise that methods will detect different components of the vertebrate community due to differences in species ecology and detection processes: cameras will primarily detect larger terrestrial mammals, PAM will be biased towards aerial and vocal taxa, and eDNA substrates will capture small, elusive species, with substrate-specific functional biases (e.g. terrestrial or aquatic signals). We expect each method to reveal distinct facets of taxonomic, functional and trophic diversity, and combining methods will provide the most complete assessment of community structure and function. Our findings aim to inform best practice for scalable biodiversity monitoring and identify complementary approaches for monitoring in restoration landscapes.

2 | MATERIALS AND METHODS

2.1 | Study site

Data were collected at the Natural Capital Laboratory (NCL), a 42 ha rewilding site near Loch Ness, Scotland (Figure 1), established in 2019 as a demonstration site for ecological restoration and long-term natural capital monitoring. The site comprises regenerating birch woodland, conifer plantation, felled/open ground and peatland under restoration. Formerly dominated by non-native conifers and drained peat, it is now undergoing native woodland regeneration, conifer thinning, peatland rewetting, species reintroductions and deer exclusion. The habitat mosaic supports a diverse vertebrate community, with regional records indicating the presence of 25 mammal, 122 bird, 3 amphibian and 3 reptile taxa (Database S1).

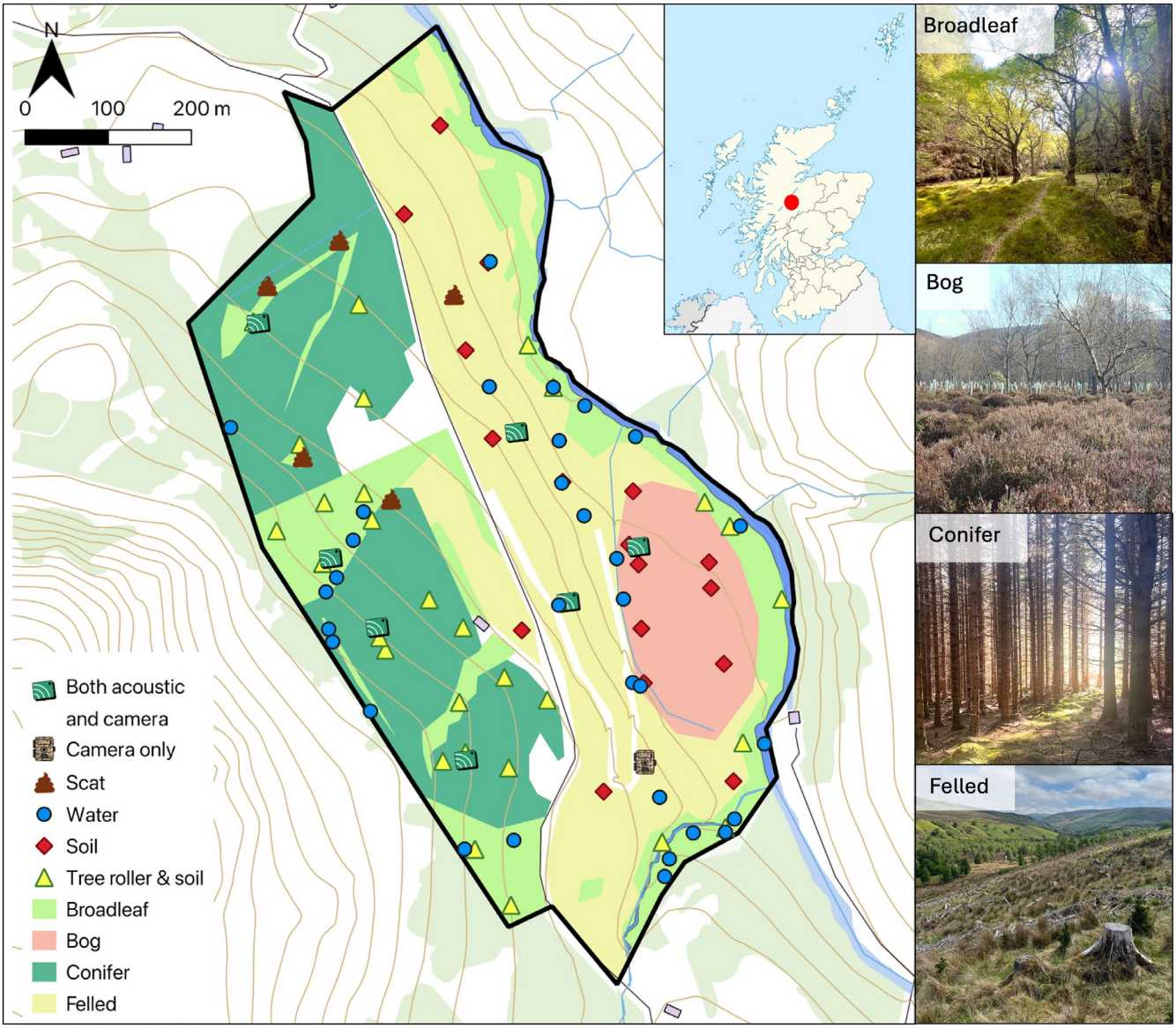

FIGURE 1 Map of the Natural Capital Laboratory rewilding site (57.1879, −4.4883) and sampling locations. Colour identifies habitats, with eDNA sampling points and passive camera and acoustic monitoring points marked. Images show habitat examples.

Fieldwork was conducted with landowner permission. No additional licences or permits were required.

2.2 | PAM and camera monitoring

Two trail cameras (Browning BTC-8E-H4) were deployed at eight locations. Camera locations were selected using a stratified random sampling approach to provide representative coverage of the sites' major habitat types (peatbog, conifer plantation, birch woodland, open grassland). Within each habitat type, camera locations were randomised to minimise placement bias and better reflect true habitat use. Each location hosted two cameras facing opposite directions at different heights: one low (0.3–0.5 m) targeting small ground-dwelling mammals and one higher (0.8–1.2 m) targeting medium-to-large species, creating distinct detection zones. The dual configuration also provided redundancy against vegetation, glare, weather or equipment failure, and the cameras were therefore treated as independent deployments. Photographs were manually audited, with ambiguous records verified on iNaturalist (https://www.inaturalist.org/). Records were considered independent if the species was not spotted for 10 min prior.

AudioMoth recorders (v1.2.0; Open Acoustic Devices, UK) at the same eight locations recorded 30 s hourly at 192 kHz, a sampling rate sufficient to detect all bat species expected at the site. Each recorder was housed in an AudioMoth IPX7 Waterproof Case (Open Acoustic Devices, UK) for weather protection. One device was excluded due to technical errors. Birds were identified using BirdNET Analyzer (Kahl et al., 2021) with location filtering for 193 potential species. Identifications were manually verified for at least 20 recordings per species per week using visual sonogram inspection and audio checking. Small-mammal calls were processed using the BTO Acoustic Pipeline (version 5.502; https://www.bto.org/our-work/science/research-areas/acoustic-monitoring), with manual verification of sonograms for all calls.

For comparison with eDNA sampling, autonomous recording data from 1 May to 13 June 2023 was analysed, covering 5 weeks prior to and including the week of eDNA sampling. This period allowed PAM and camera traps to exploit their primary strength of extended autonomous deployment while retaining temporal overlap with eDNA sampling. Direct temporal equivalence is challenging since camera traps and PAM provide temporally explicit detections (Sugai et al., 2019), whereas eDNA integrates biological signals over substrate-specific persistence windows that can range from days in water to months in soil (Guthrie et al., 2024; Mauvisseau et al., 2022). Comparisons therefore reflected the practical application of each approach rather than identical sampling durations.

2.3 | eDNA sampling

Sampling was designed to cover the whole site while maximising feasible replication for each method. Soil and tree-roller sampling followed a shared stratified random design across habitats: 50 soil and 30 tree-roller samples were selected to provide sufficient replication while remaining within field and laboratory capacity, with woodland points split evenly between broadleaf and conifer. Non-woodland points were allocated in proportion to habitat area (8 bog and 12 felled). Random sampling points were generated within each habitat with a minimum 25 m spacing, in addition to sampling at each camera/PAM station. Due to dry weather, incidental sampling was used for all available waterbodies (e.g. puddles, ditches and streams).

We collected 116 samples: 50 soil, 30 tree-roller, 31 water, and 5 scat samples between 6 and 11 June 2023, coinciding with peak activity levels for many vertebrates during the breeding season. Sampling followed contamination control procedures using sterile gloves, bleach-sterilised equipment and daily field blanks for water and tree-roller samples. Samples were double-bagged separately by sample type and habitat.

Water samples (~1 L) were collected using Whirl-Pak® bags, filtered on the day of collection using manifold units, through two 0.45 µm cellulose ester membrane filters (47 mm; Whatman, UK) per sample, and stored in sterile 5 mL tubes at −20°C.

At each sampling point, 50 mL soil samples from the top 5 cm were collected from four subsamples within a 1 m^2 quadrat, stored in 50 mL Falcon tubes and frozen within a few hours of collection.

Tree-roller samples were collected in wooded habitats, following the approach of (Valentin et al., 2021) to collect DNA from the surface of branches. Bleach-sterilised paint rollers were wetted with purified water and rolled over branches for 30 s. Rollers were then soaked in purified water, and this solution was vacuum-filtered and stored in the same way as the water samples, with one filter used per sample.

Scat samples found incidentally ($n = 5$) were collected in 50 mL Falcon tubes and frozen within a few hours of collection.

2.4 | eDNA laboratory methods

Full details regarding field, laboratory and bioinformatics workflows are provided on protocols.io (https://doi.org/10.17504/protocols.io.eq2lyqm8mvx9/v1). DNA was extracted using the Mu-DNA method (Sellers et al., 2018), a modular lysis and silica-binding protocol adaptable across diverse environmental substrates, applying the water protocol to water/tree-roller samples and the soil protocol for soil/scat samples. Tree-roller samples were eluted in 50 µL buffer rather than the standard 100 µL to increase concentration. Soil and scat samples (2.5 g) were homogenised using a TissueLyser II (Qiagen Ltd. UK) in 10 mL jars with 5.5 mL lysis buffer. Two replicates per soil/scat sample were processed separately. Extraction blanks (buffers only) were included, with additional lysis blanks for soil/scat samples. DNA extracts were stored at −20°C.

We used nested two-step metabarcoding with dual MID-tagging (Griffiths et al., 2023), targeting the vertebrate 12S mitochondrial region with primers 12S-V5-F and 12S-V5-R (Kelly et al., 2014; Riaz et al., 2011). These primers provide broad vertebrate coverage and species-level resolution for most UK vertebrates. First-round PCRs used Q5 High-Fidelity polymerase (Qiagen Ltd. UK) for water/tree samples and MyTaq HS (Meridian Bioscience, UK) for soil/scat.

Sub-libraries included blanks, PCR negatives and positive controls (*Astatotilapia calliptera*, 0.05 ng/μL). Triplicate PCRs were pooled by band intensity on 2% agarose gels, and samples showing signs of inhibition by poor amplification were diluted 1:10 and re-amplified. Amplicons were purified using magnetic bead cleanup (0.9× and 0.15× Mag-BIND® beads, OmegaBiotek). Second round PCR added Illumina Unique Dual Indexes (Illumina Cambridge Ltd. UK) and underwent bead cleanup (0.7× and 0.15× Mag-BIND® beads). Sub-libraries were quantified using Qubit (dsDNA HS Assay Kit, Thermo Fisher Scientific Ltd. UK) and qPCR (NEBNext Library Quant Kit, New England Biolabs, UK), pooled in equal concentrations and diluted to 4 nM. Libraries were sequenced on an Illumina MiSeq (with 2 × 300 bp v3 chemistry) with 10% PhiX.

Raw reads were processed with the Tapirs workflow (https://github.com/EvoHull/Tapirs). Sequences were trimmed, quality-filtered (Q30) and merged using fastp (Chen, 2023), then clustered with VSEARCH (Rognes et al., 2016). Taxonomic assignment used a UK vertebrate 12S database (Harper et al., 2018; see data repository), applying a majority lowest common ancestor approach (≥98% identity, ≥90% coverage; ≥80% agreement among top 2% BLAST hits). To assess marker coverage, we compiled a list of terrestrial and semi-aquatic vertebrates recorded within 5 km of the site from NBN Atlas records up to 2023. Most recorded taxa were represented in the database at species level, with the remainder represented at genus level (Database S1). Sample-type-specific species-level limits of detection (LOD) were calculated based on control reads:

$$\mathrm{LOD} = \mathrm{mean}_{\text{blank reads}} + \left(3 \times \mathrm{SD}_{\text{blank reads}}\right)$$

LOD thresholds were applied per species and sample type. Read counts below the LOD were set to zero. Low frequency noise was removed using a 0.1% read threshold per sample. Human and domestic species (*Homo sapiens*, *Sus scrofa*, *Ovis aries*, *Bos taurus*, *Canis lupus*) were removed since these DNA sources commonly enter samples through anthropogenic contamination. Fish detections were also excluded as non-target taxa. The number of samples with no vertebrate detections following quality filtering is summarised in Table S1.

2.5 | Data analysis

A presence–absence matrix was generated for species detections in each eDNA sample or week of camera trap and PAM data for each device. Weekly aggregation helped align passive monitoring data with the temporal resolution of eDNA samples, particularly given the short duration of daily recordings (12 min per day) and the relatively stable, non-migratory communities expected during the study period. Taxa identified at multiple taxonomic levels were consolidated at the lowest consistently detected level (Table S2), with detections merged at the most precise level when both species and higher ranks were found. PAM and camera methods typically resolved taxa to species level, with higher-level identifications more common in eDNA data.

Data analysis was performed in RStudio version 4.4.2 (R Core Team, 2025); see Table S3 for package references. Data were aggregated into weekly units using 'lubridate' and visualised with 'ggplot2'. Taxonomic richness was modelled using Poisson generalised linear models (GLMs) fitted with 'MASS'. Model comparisons showed method type was a stronger predictor of richness than habitat or location (Table S4). Species rarefaction curves were generated with 'iNEXT', for eDNA samples and daily pooled PAM and camera-trap data (16 cameras and 7 Audiomoths over 44 days, 660 and 308 deployment days, respectively). Community composition was analysed using non-metric multidimensional scaling (NMDS) with Jaccard dissimilarity in 'vegan', both across methods and methods to assess habitat-specific distinctions. Community differences were assessed with pairwise PERMANOVAs ('pairwiseAdonis').

Functional diversity was based on body mass, lifestyle and trophic traits from published sources, with additional sources to fill data gaps (Tables S5 and S6; Database S2). Samples with ≥3 species were included in this analysis. Functional diversity was quantified using Rao's quadratic entropy (RaoQ; the average functional difference between two species based on pairwise trait dissimilarities, Botta-Dukát, 2005) and Functional Redundancy (FRed, the difference between species diversity and RaoQ; de Bello et al., 2016). Metrics were calculated with 'SYNCSA', using Gower's trait dissimilarities (Aglieri et al., 2021). Dunn tests ('dunn.test') compared survey methods, and trait variation was visualised using principal coordinates analysis (PCoA).

Trophic complexity was assessed using a metawebs, constructing potential trophic interaction networks from species co-occurrence and known trophic links (Dunne, 2005). Trophic interactions from the GLOBI database were used (Poelen et al., 2014) and supplemented with additional sources (Database S3). Species trophic levels were calculated using PreyAveragedTL ('cheddar'), increased by one to account for the absence of primary producers. Co-occurrence of taxa was defined as detection at the same site within a sliding window of ±7 days for camera and PAM data, or within the same eDNA sample. This window was chosen to capture ecologically meaningful co-occurrences while limiting sensitivity to daily variation in detectability. Metawebs were constructed for each method and the combined dataset, and visualised using 'igraph'. Network metrics included linkage density (mean links per taxon), connectance (proportion of realised to possible links), robustness (proportion of species removed before 50% of the network remains), modularity (strength of division into subgroups) and mean trophic level (average PreyAveragedTL). Scat samples were excluded due to small sample size and their focus on diet rather than co-occurrence, which could inflate connectance.

3 | RESULTS

After quality control, eDNA data consisted of 17,517,345 paired-end reads, with an average read depth of 151,012 reads per sample. Of these, 12,074,565 reads were successfully assigned to taxa. Low-level contamination was observed in some controls and is detailed in Table S7.

Following manual verification of 2589 acoustic records (21.6% of automated assignments), 680 records were removed as false identifications.

3.1 | Alpha diversity

Seventy-nine vertebrate taxa were detected across all methods. PAM detected 53 species over 308 deployment days, predicted to approach a plateau of around 70 species after 1000 days (Figure 2a). Camera traps produced 477 independent records and detected 12 species from 4063 photographs. Combining acoustic and camera data ('Both', Figure 2a) increased total detections to 60.

After quality control, 44 vertebrate taxa were detected across eDNA methods (Figure 2b). Water samples showed the highest richness (34 taxa), although extrapolation predicted tree rolling could exceed this (Figure 2b). Approximately 25 water or tree-roller samples recovered comparable richness (~32 species) to 50 PAM days. Soil sampling detected 28 taxa, requiring ~250 samples to match water sampling richness. Scat samples had the lowest richness, reflecting the small sample size (three *Martes martes* scats, one *Buteo buteo*, one with no detectable predator DNA). Maximum richness was achieved by combining all eDNA samples (Figure 2b), though ~50 water or tree-roller samples showed potential to detect most vertebrates.

Median taxonomic richness differed among methods (Kruskal–Wallis: $\chi^2 = 197.9$, $p < 0.001$; Figure 2c). PAM showed the highest median richness, and cameras the lowest (Figure 2c). Median richness of water and tree-rolling samples did not differ significantly (Dunn's test, $p = 0.435$), though both exceeded soil and scat sample richness (Dunn's tests, all $p < 0.016$; Table S8).

3.2 | Community composition

Across methods, 22 mammal, 54 bird and 3 amphibian taxa were detected (Figure 3a). Amphibians were found only with eDNA, although the PAM recording period fell outside the vocal activity associated with amphibian breeding. Of the mammals, 18 were detected with eDNA, six with cameras and four with PAM, with no mammals detected by all three methods. Small terrestrial mammals were exclusive to eDNA, except common shrew (*Sorex araneus*), also detected acoustically. Five mammal taxa were detected by eDNA and cameras, *Vulpes vulpes* only with cameras and bats only with PAM. Of the 54 bird taxa, 48 were found with PAM (Figure 3b). Four bird taxa were detected with all three methods, and 13 additionally with both PAM and eDNA. Four birds were exclusive to eDNA, including semi-aquatic *Cinclus cinclus*. *Accipiter gentilis* was detected with soil eDNA and cameras but not PAM.

Of the 44 taxa detected with eDNA, five were found across all substrates, with 14 also shared across water, soil and tree-rollers (Figure 3c). Water detected 77% of eDNA-taxa, tree-rollers 70%, 64% with soil and 16% with scat samples (Figure 3c). Each environmental substrate detected at least three unique taxa, reflecting substrate-specific habitats, for example *Dendrocopos major* only found with tree rolling, *Talpa europaea* with soil and *Neomys fodiens* in water.

Communities differed between methods (PERMANOVA: $R^2 = 0.313$, $p < 0.001$; Figure 4). Pairwise differences were greatest between PAM

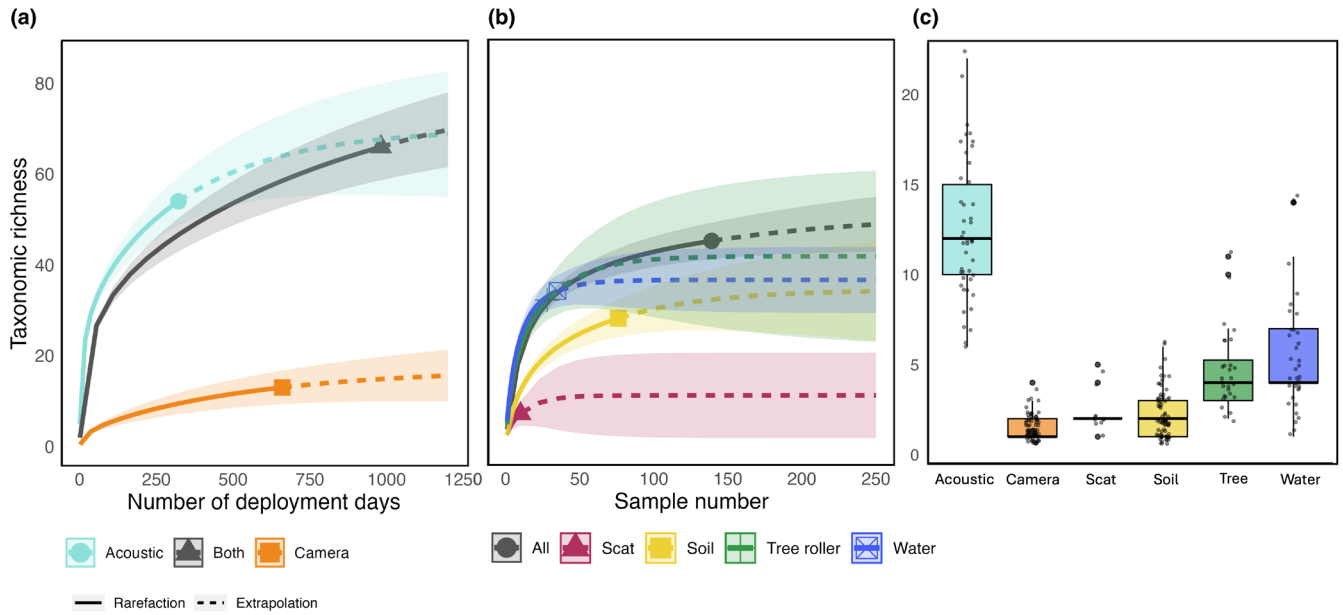

FIGURE 2 Species rarefaction curves (95% confidence intervals), calculated with Chao II estimator and 999 bootstrap permutations. (a) Camera trap (blue) and acoustic recorders (orange), plotted against deployment days, pooled data in grey (Both). (b) Environmental DNA (eDNA) substrates against number of samples. 'All' refers to pooled all environmental substrates (soil, tree-rolling and water). (c) Taxonomic richness for each method, points equate to one sample (eDNA) or week of monitoring (other methods).

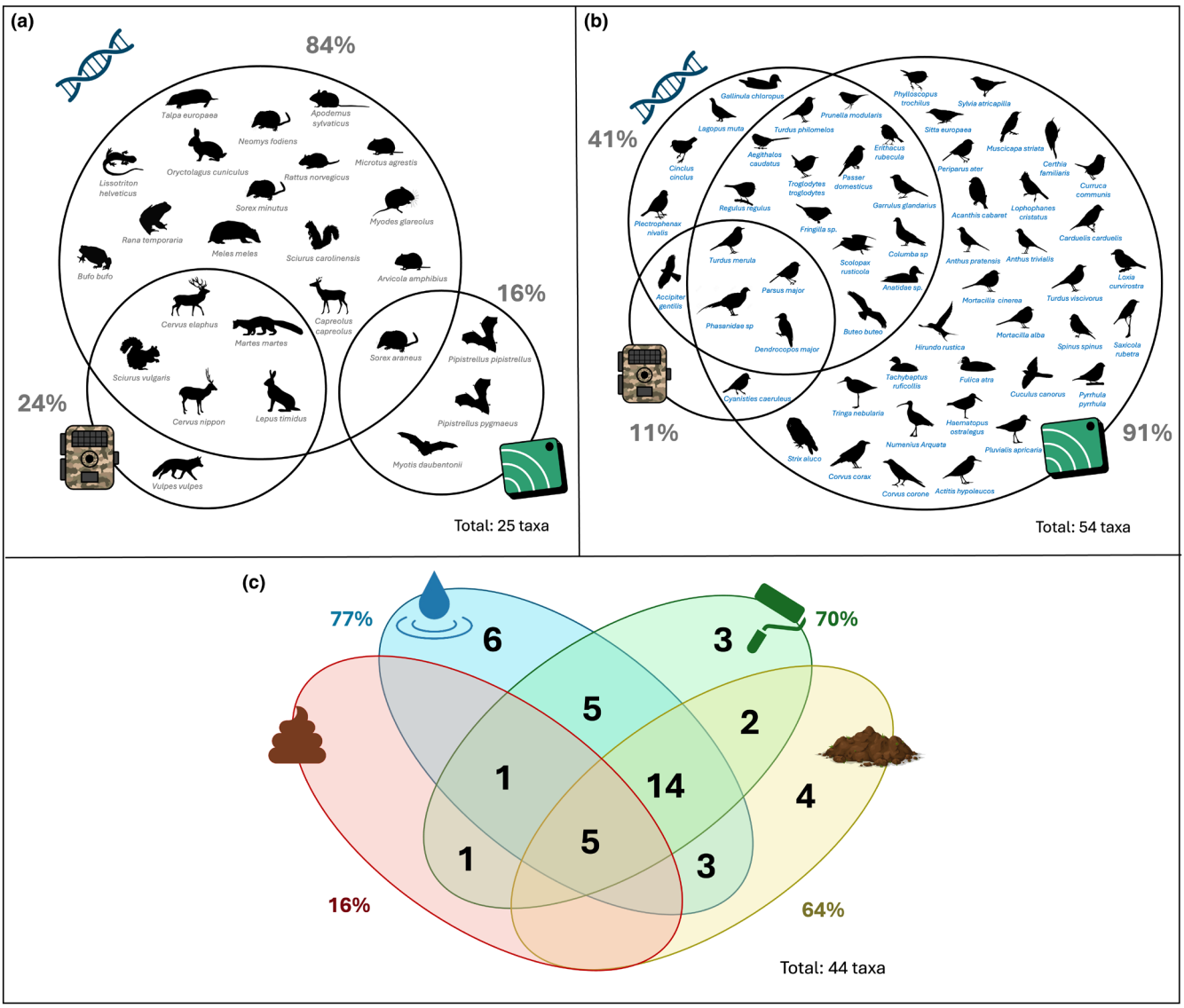

FIGURE 3 Taxa detected with environmental DNA (eDNA), camera trapping and passive acoustic monitoring (PAM) for (a) mammals and amphibians, and (b) birds. (c) Taxa detected by each eDNA substrate and overlap. Labels show the percentage of species detected per method. Silhouettes from phylopic https://www.phylopic.org.

and camera traps (Pairwise PERMANOVA: $R^2=0.49$, $p=0.015$), and smallest among eDNA substrates (all $R^2<0.09$; Table S9). Soil samples showed the lowest pairwise differences with other methods.

PAM showed the strongest community distinctions between habitats ($R^2=0.318$, $p<0.001$), revealing differences between wooded and open habitats (Figure 5a; Table S10). Water samples distinguished between conifer and felled habitats ($R^2=0.24$, $p=0.006$; Figure 5d). Soil samples revealed community differences between all wooded and non-wooded habitats ($R^2=0.13$, $p<0.001$; Figure 5e), reflecting the site's spatial pattern of woodland in the west and open habitats to the east. Tree rolling was the only method to detect a significant, albeit small, difference between wooded habitats ($R^2=0.07$, $p=0.007$).

3.3 | Functional diversity

Functional diversity (RaoQ) differed between methods (Kruskal–Wallis: $\chi^2=54.51$, $p<0.001$, Figure 6a; Table S10). Tree rolling and water samples revealed greater functional diversity than soil, PAM or cameras (Figure 6a; Dunn test: $p<0.008$). PAM showed higher functional redundancy than other methods (Figure 6b; Dunn test: $p<0.001$), detecting many species with similar functions.

PCoA showed PAM detected smaller, arboreal invertivores, whereas others detected larger, terrestrial folivores and omnivores (Figure 6c). Within eDNA samples, tree rolling was associated with arboreal granivores and water samples with more semi-aquatic taxa.

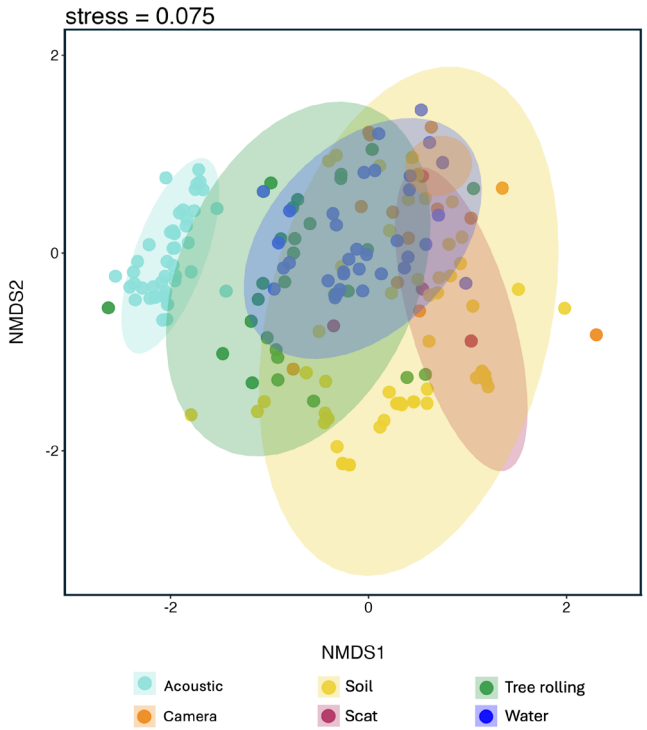

FIGURE 4 Non-metric multidimensional scaling (NMDS) plot (Jaccard dissimilarity) of vertebrate community composition. Ellipses show 95% confidence intervals.

3.4 | Trophic complexity

The combined co-occurrence metaweb revealed 179 trophic links (Figure 7). PAM recovered 76% of links, weighted towards birds (Figure 7a,e), while camera traps detected 3% (Figure 7b,e). Combined eDNA methods recovered 29%, including interactions involving mammals missed by PAM (Figure 7c). Water, tree rolling and soil samples recovered 16%, 15% and 6% of links, respectively (Figure S1). PAM and tree rolling together captured 89% of links, the highest coverage of any two-method combination. Adding soil or water increased coverage to over 90%, and combining PAM with all eDNA substrates captured 98% of links.

PAM showed highest linkage density and connectance, exceeding even the combined network (Figure 7d,f). Acoustics and tree rolling produced the most robust networks (Figure 7g), while soil showed highest modularity, detecting relatively disconnected interactions (Figure 7h). Cameras had a large proportion of species occupying high-trophic positions (Figure 7i). Variation in these network metrics among individual samples is shown in Figure S2.

4 | DISCUSSION

Different methods revealed complementary subsets of vertebrate biodiversity. Combining PAM with eDNA metabarcoding delivered

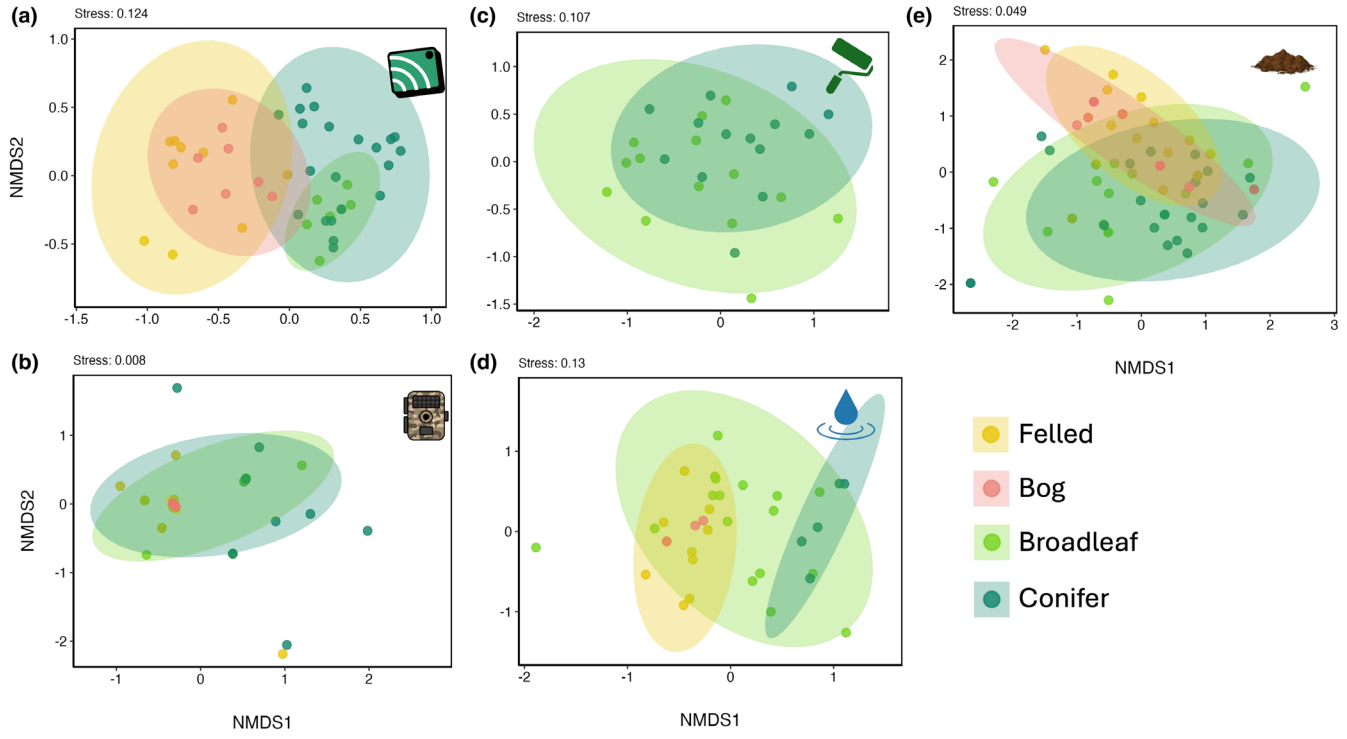

FIGURE 5 Non-metric multidimensional scaling (NMDS) plots of vertebrate community composition across habitats for each method (Jaccard dissimilarity). For passive sampling (a, b), points represent weekly data per device. For eDNA (c–e), points represent samples.

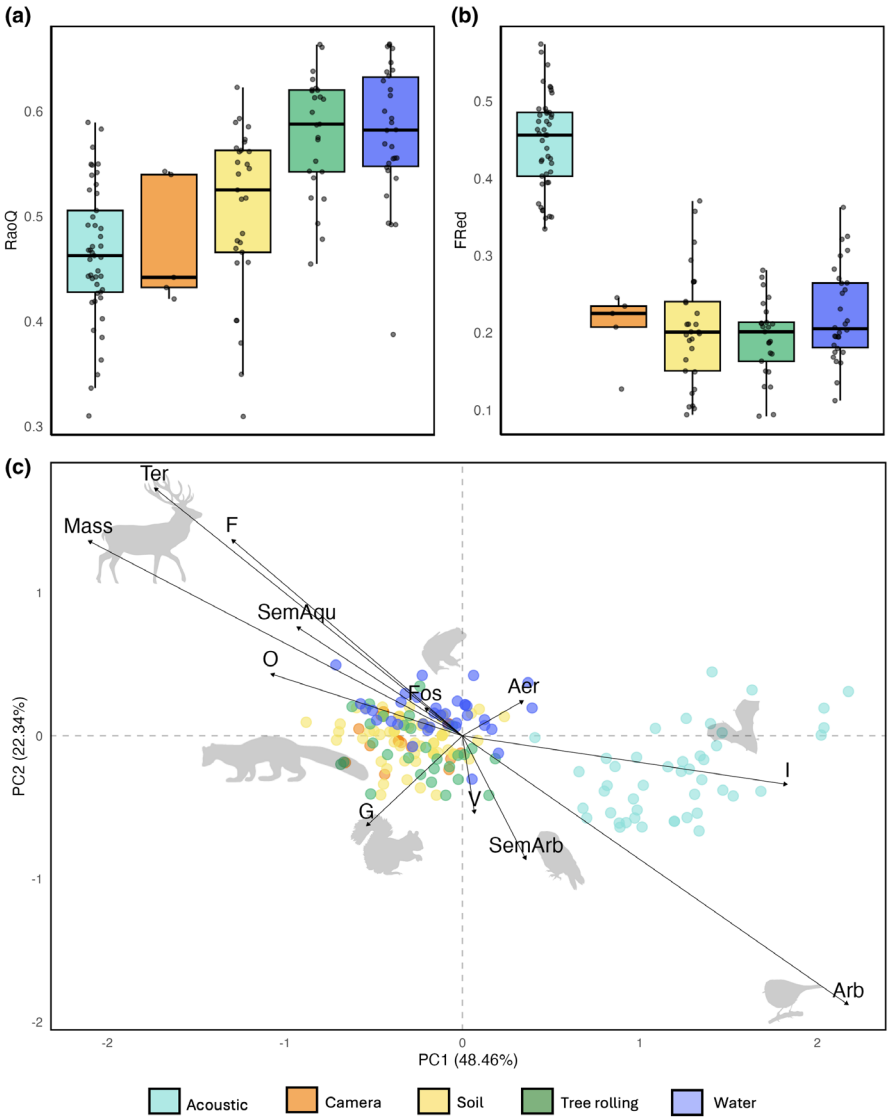

FIGURE 6 Functional diversity estimates per method: (a) Rao's quadratic entropy and (b) functional redundancy. (c) Principal coordinates analysis (PCoA) of functional traits detected by different methods. Arrows indicate trait direction and magnitude. Functional traits: Mass (log); F = Folivore, G = Granivore, O = Omnivore, I = Invertivore, V = Vertivore; SemAqu = Semi-Aquatic, Fos = Fossorial, Ter = Terrestrial, SemArb = Semi-Arboreal, Arb = Arboreal, Aer = Aerial. Silhouettes show species examples.

the most comprehensive assessments of taxonomic, functional and trophic diversity. Among eDNA substrates, tree-roller and water samples detected the richest assemblages. These findings support integrated monitoring of vertebrate diversity in rewilding contexts and terrestrial systems more broadly.

4.1 | Taxonomic richness and community composition

Methods differed in terms of species detections. PAM recorded the highest taxonomic richness, with most (49/53) being birds, the most species-rich UK vertebrates (McInerny et al., 2022). PAM achieved this with comparatively low sampling effort, emphasising its efficiency for long-term monitoring. eDNA metabarcoding captured 44 taxa, including small mammals and amphibians missed by other approaches. Camera traps detected only 15% of taxa due to selectivity for large-bodied mammals, of which the UK has few species (Mathews et al., 2018), unlike in high-diversity regions where camera traps can match eDNA (Mena et al., 2021). This likely reflected our camera-trap configuration, which was biased towards large-mammal detections; dedicated small-mammal camera-trapping approaches can improve detection of rodents and shrews (Littlewood et al., 2021). Scat sampling, though effective for low-density species (van der Heyde et al., 2020), was limited here by sample availability. These findings align with a recent meta-analysis showing that comparative eDNA performance varies across contexts for vertebrate surveys (Cowgill et al., 2025), with detections influenced by species' body size and visitation frequency (Ryan et al., 2022). To our knowledge, this is the first direct comparison of terrestrial eDNA and PAM, highlighting their complementarity: PAM rapidly detects sound-producing taxa, whereas eDNA captures less vocal and elusive species.

Strategic substrate selection may be more cost-effective than comprehensive multi-substrate sampling for vertebrate eDNA monitoring. Combining all eDNA substrates did not substantially increase richness beyond that potentially achieved by 50 water or tree-roller samples, challenging the assumption that more substrates yield higher richness (Cowgill et al., 2025). Although rarefaction curves for

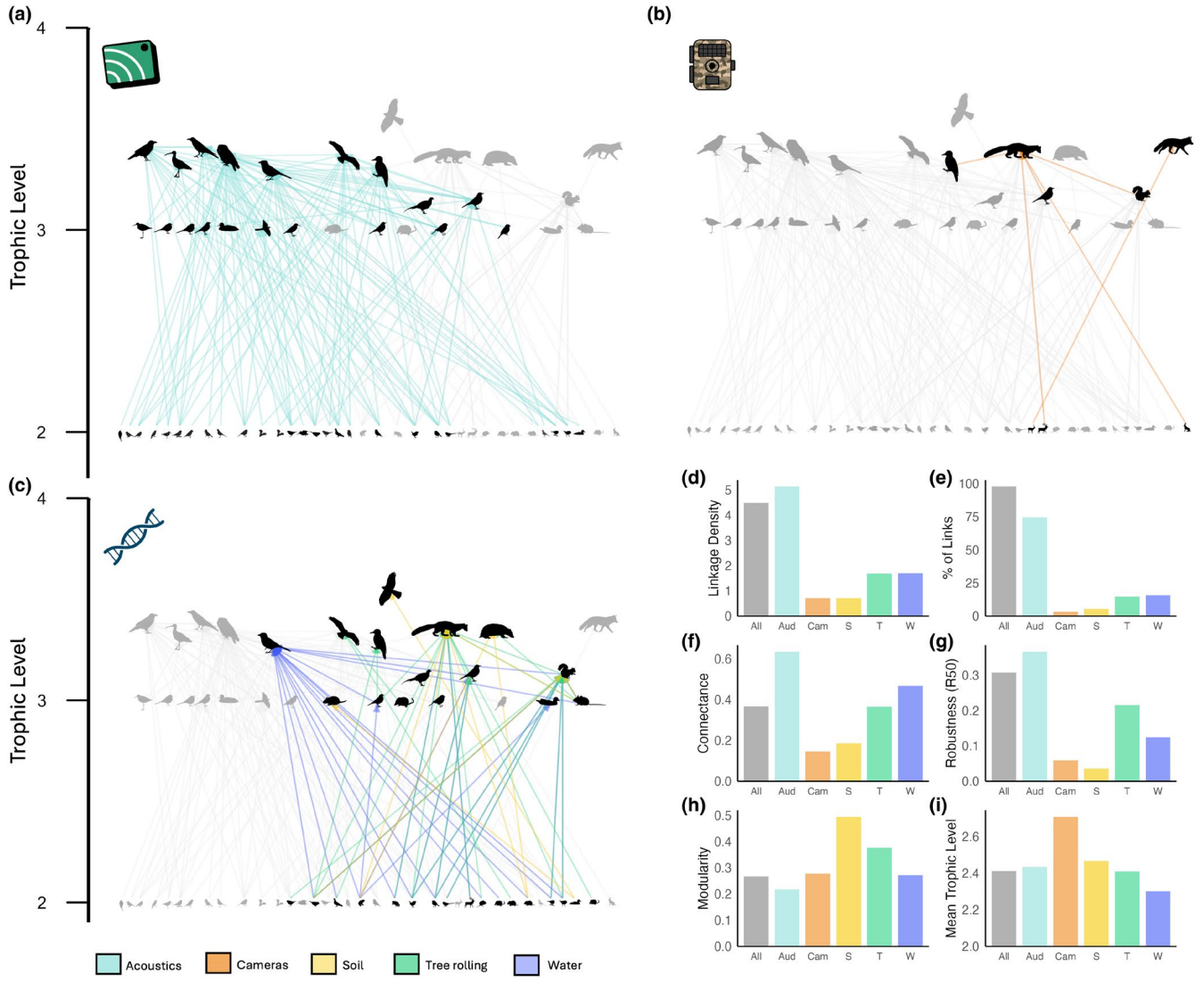

FIGURE 7 Co-occurrence metawebs of species with known trophic interactions detected using (a) passive acoustic monitoring, (b) camera traps and (c) eDNA substrates (links found by multiple eDNA substrates shown by overlaying colours). Nodes and links not detected by the highlighted method are shown in grey. (d–i) show network metrics for each method and the combined dataset: (d) Linkage density; (e) Percentage of detected links; (f) Connectance; (g) Robustness; (h) Modularity; (i) Mean trophic level.

soil eDNA remained non-plateauing, supporting continued sampling, the lower richness likely reflects the heterogeneous distribution of mammal DNA and the large sample volumes and replication required to stabilise richness (Leempoel et al., 2020). As this level of effort is generally impractical for routine monitoring, the lower soil richness observed here is likely representative of what can realistically be recovered. This supports focusing on fewer, higher-yield substrates, although additional genetic markers or MOTU-level analyses could increase apparent richness.

While most historically recorded taxa were represented within the 12S reference database used for eDNA taxonomic inference (Database S1), eDNA metabarcoding remains subject to biases arising from marker choice, amplification efficiency and taxonomic resolution (Ruppert et al., 2019). These factors may have contributed to the lower representation of birds in the eDNA datasets relative to PAM, particularly as several avian taxa could only be resolved to genus level within the reference database. The differences between PAM and eDNA communities likely reflect both ecological differences in the taxa detected and methodological biases in eDNA metabarcoding. Future studies could improve taxonomic coverage by incorporating additional genetic markers alongside complementary monitoring approaches (Cowgill et al., 2025).

Discriminating between habitats is important to track changes in community composition and connectivity. Methods varied in distinguishing vertebrate communities by habitat. PAM clearly distinguished open from wooded habitats, likely due to differences in sound propagation and community composition (Winiarska et al., 2024). Soil eDNA was also effective for distinguishing open and wooded habitats, though distinctions were smaller. Only tree rolling differentiated between coniferous and broadleaf woodland communities. Detection capabilities could be influenced by

habitat-specific factors affecting sound propagation or DNA preservation. Tree rolling is limited to wooded areas, capturing DNA from species that contact tree surfaces (Allen et al., 2023), with signals likely influenced by woodland composition and structure. Water eDNA can lack fine-scale spatial specificity as DNA may originate from distant sources (Carraro et al., 2020), while UV degradation may be higher in open habitats (Guthrie et al., 2024), driving community distinctions. These patterns should, however, be interpreted in light of uneven sampling effort across vegetation types, as sample numbers were stratified by habitat and water samples were restricted to available waterbodies. Pooling data from different methods risks masking ecological patterns because each approach may respond differently to habitat variation, contributing unique information that could be lost if combined without consideration. These taxonomic distinctions also shape the range of ecological roles captured by each method, underpinning differences in functional diversity.

4.2 | Functional diversity

High taxonomic richness does not necessarily translate into high functional richness, particularly in systems with substantial ecological redundancy. Ecosystem functioning is driven by traits of abundant or high-biomass taxa (Smith et al., 2020), and methods missing functionally dominant species may misrepresent functional complexity. Tree rolling and water eDNA sampling captured the highest functional diversity, despite PAM detecting more species. These methods sample across niches: tree rolling captures arboreal and terrestrial taxa (Allen et al., 2023), while water integrates DNA across spatial scales via runoff (Carraro et al., 2020). This echoes findings from aquatic systems, where eDNA outperforms traditional methods for detecting functional diversity (Aglieri et al., 2021). In contrast, PAM detected many small arboreal taxa while omitting larger terrestrial species. This bias towards sound-producing taxa meant functionally important mammals that vocalise infrequently remain undetected, potentially misrepresenting community functioning.

However, trait-based approaches have their limitations. Many taxa lack published trait data, traits are often generalised across regions or life stages, and eDNA methods often fail to resolve taxa to species level (Condachou et al., 2023). Although eDNA read counts or acoustic detection rates may correlate with abundance to an extent (Di Muri et al., 2020; Doser et al., 2021), these cannot currently estimate abundance reliably, limiting the use of metrics like RaoQ (Aglieri et al., 2021). Such limitations, combined with no single method detecting the full range of functional traits, caution against overreliance on trait databases. However, acoustic and eDNA data can be archived and reanalysed as reference databases and classifiers improve (Condachou et al., 2023; Darras et al., 2025), potentially expanding their functional scope in future. Method-specific biases therefore shape the ecological roles detected and, consequently, how trophic interactions are represented within food webs.

4.3 | Trophic complexity

Constructing complete trophic networks requires integrating methods that capture different components of community structure. PAM generated the most connected network, reflecting comprehensive coverage of bird taxa across trophic positions, but missed most mammalian interactions, which were primarily detected with eDNA. Camera traps contributed few interactions and were biased towards large, high-trophic-level species, consistent with known body size-trophic position relationships (Brose et al., 2019). Soil eDNA underperformed overall but uniquely detected *Accipiter gentilis*, the top predator. These method-specific biases reduced linkage density and robustness in single-method networks. Integrating methods proved critical: PAM and tree rolling together captured nearly 90% of observed links, rising to 98% when all eDNA substrates were included, demonstrating the value of method integration to avoid underestimation of trophic complexity.

Differences in temporal resolution of methods further influence inferred trophic interactions across methods. PAM and camera traps provide temporally explicit records (Sugai et al., 2019), enabling fine-scale inference of co-occurrence. By contrast, eDNA integrates signals over longer periods: days to weeks in water (Mauvisseau et al., 2022), weeks to months in soil (Guthrie et al., 2024), and likely intermediate durations on tree surfaces (Valentin et al., 2021). These broader temporal windows may dilute fine-scale patterns of interactions or inflate co-occurrence by capturing species present at different times. This is especially relevant in rewilding landscapes, where species are often transient, and trophic interactions are dynamic (Torres et al., 2018). Understanding these temporal mismatches is essential for interpreting network structure.

4.4 | Implications for restoration monitoring

Rewilding projects require monitoring approaches capable of capturing change across multiple taxa, ecological functions and trophic levels. Our results suggest that integrating PAM with targeted eDNA sampling provides a practical balance between biodiversity coverage and monitoring effort. Although combining multiple eDNA substrates maximised detections, water and tree-rolling samples captured much of the richness recovered by more intensive sampling designs, potentially reducing monitoring costs. In contrast, soil eDNA required substantially greater replication to characterise vertebrate communities, consistent with recent assessments questioning its cost-efficiency for large-scale vertebrate monitoring (Broadhurst et al., 2025; Zhao & Andermann, 2026). Equally, recent advances in automated acoustic and image classification have improved the scalability of autonomous monitoring (Sugai et al., 2019), although 26% of audited PAM detections were incorrect, highlighting the continued need for quality control. Emerging approaches such as airborne eDNA may further expand monitoring toolkits

by providing landscape-scale community assessments (Tournayre et al., 2025), while multi-marker metabarcoding could improve taxonomic coverage. Future studies would benefit from repeated eDNA sampling alongside continuous monitoring approaches to assess how temporal variation influences biodiversity estimates and monitoring recommendations.

5 | CONCLUSIONS

Integrating non-invasive monitoring methods provides a more complete picture of vertebrate biodiversity by combining complementary taxonomic, functional and trophic information. PAM excels for taxonomic richness and trophic complexity, while eDNA captures broader functional diversity. However, eDNA substrate selection needs consideration. Soil and water substrates integrate signals from terrestrial, aquatic and subterranean environments, while tree-rolling detects arboreal species.

Method selection should align with monitoring goals, prioritising complementarity over redundancy. Combining PAM with one eDNA method provides sufficient coverage. For detecting habitat-level variation, combining PAM with soil sampling best captures community differences. For trophic complexity, combining PAM with tree rolling suits wooded habitats, while soil or water eDNA may better suit other contexts. Although not compared here, airborne eDNA methods also show promise for landscape-scale monitoring, capturing homogenised community signals (Tournayre et al., 2025) and should be considered in future research.

Around 1000 days of PAM alongside 50 tree rolling or water samples achieved optimal taxonomic coverage. However, single-timepoint eDNA sampling may have underestimated eDNA potential compared with continuous PAM data, and further method comparisons would benefit from temporal replication. Continuous PAM suits monitoring annual trends, while intensive eDNA sampling provides multitrophic snapshots of functional diversity. Adopting multi-tool monitoring that explicitly integrates taxonomic, functional and trophic perspectives will enable more comprehensive monitoring for ecosystem restoration and rewilding.

AUTHOR CONTRIBUTIONS

Clare Cowgill, Lori Lawson Handley, James D. J. Gilbert and Ian Convery conceived the study. Clare Cowgill conducted eDNA sampling, lab work and analyses. Acoustic and camera data formed part of Ian Convery and Volker Deecke's ongoing monitoring. Graham Sellers provided bioinformatics support. Clare Cowgill and Lori Lawson Handley led writing; Lori Lawson Handley and James D. J. Gilbert advised on analyses. All authors edited and approved the final manuscript. The authors declare no conflicts of interest.

ACKNOWLEDGEMENTS

We thank Emilia and Roger Leese for access to the Natural Capital Laboratory and Kristy Adaway for field assistance. This work was funded by the University of Hull REWILD research cluster.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

PEER REVIEW

The peer review history for this article is available at https://www.webofscience.com/api/gateway/wos/peer-review/10.1002/2688-8319.70308.

DATA AVAILABILITY STATEMENT

Data, code, DNA sequences and media files are available via Zenodo (Cowgill et al., 2026; https://doi.org/10.5281/zenodo.15723454) and GitHub (https://github.com/clarecowgill/Cowgill-et-al.-2025-Multi-tool-monitoring). Raw sequence data are also archived on the NCBI Sequence Read Archive (SRA) under BioProject accession PRJNA1345715. DNA sampling and sequencing protocol is on protocols.io: https://doi.org/10.17504/protocols.io.eq2lyqm8mvx9/v1.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Data S1: Reference database used for taxonomic assignment of vertebrate 12S sequences, including species recorded within 5 km of the Natural Capital Laboratory and their representation in the reference database.

Data S2: Functional trait database used to calculate functional diversity metrics, including body mass, trophic and lifestyle traits compiled from published sources for all vertebrate taxa included in the analyses.

Data S3: Sources of trophic interactions used to construct the vertebrate metaweb, including interactions obtained from GLOBI and additional published literature.

Figure S1: Separate trophic co-occurrence networks for the eDNA sampling substrates: (A) Tree-rolling, (B) Water, (C) Soil.

Figure S2: Network metrics calculated for each method, shown by individual sample. Note that camera and acoustic networks were generated using a rolling ±7-day detection window (i.e. each point represents detections within a 14-day window centred on that time point), which results in non-independent observations across samples.

Table S1: Limits of Detection (LOD) read thresholds for each sample type and species combination where contamination was detected in corresponding blanks.

Table S2: Taxonomic consolidation in the dataset for analyses across different methods.

Table S3: Presence and absence of each species for each method (shaded for presence), along with their functional traits used for analyses. Trophic niches: G (granivore), F (folivore), O (omnivore), I (invertivore), V (vertivore).

Table S4: Kruskal–Wallis and Dunn test results of species richness. *p* values adjusted with Benjamini–Hochberg.

Table S5: Pairwise PERMANOVA results for the community composition detected with each monitoring method using Jaccard dissimilarity. R^2 values first, with adjusted *p* value (Bonferroni) after.

Table S6: PERMANOVA results for the community composition detected within each habitat for each monitoring method using Jaccard dissimilarity.

Table S7: Kruskal–Wallis and Dunn test results of functional diversity metrics. *p* values adjusted with Bonferroni.

Table S8: AIC scores for each of the tested models for predicting taxonomic richness per sample. The model in bold was chosen as the best fitting model.

Table S9: Coefficients of the GLM predicting taxonomic richness based on sampling method type. Estimates represent the log difference in taxonomic richness compared with the reference method (Acoustics).

Table S10: The number of samples (either DNA samples or week of passive monitoring) which did not detect any terrestrial vertebrate taxa.

Table S11: R packages used and their citations.

Table S12: Sources of functional trait data used to compile the trait database for analysis, including database names and references.

How to cite this article: Cowgill, C., Gilbert, J. D. J., Convery, I., Deecke, V., Sellers, G., & Lawson Handley, L. (2026). Multi-tool monitoring: Integrating non-invasive methods to assess vertebrate richness, functional diversity and trophic complexity. *Ecological Solutions and Evidence*, *7*, e70308. https://doi.org/10.1002/2688-8319.70308