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The decline of the Turtle Dove: Dietary associations with body condition and competition with other columbids analysed using high-throughput sequencing

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Abstract

Dietary changes linked to the availability of anthropogenic food resources can have complex implications for species and ecosystems, especially when species are in decline. Here, we use recently developed primers targeting the ITS2 region of plants to characterize diet from faecal samples of four UK columbids, with particular focus on the European turtle dove (*Streptopelia turtur*), a rapidly declining obligate granivore. We examine dietary overlap between species (potential competition), associations with body condition in turtle doves and spatiotemporal variation in diet. We identified 143 taxonomic units, of which we classified 55% to species, another 34% to genus and the remaining 11% to family. We found significant dietary overlap between all columbid species, with the highest between turtle doves and stock doves (*Columba oenas*), then between turtle doves and woodpigeons (*Columba palumbus*). The lowest overlap was between woodpigeons and collared doves (*Streptopelia decaocto*). We show considerable change in columbid diets compared to previous studies, probably reflecting opportunistic foraging behaviour by columbids within a highly anthropogenically modified landscape, although our data for nonturtle doves should be considered preliminary. Nestling turtle doves in better condition had a higher dietary proportion of taxonomic units from natural arable plant species and a lower proportion of taxonomic units from anthropogenic food resources such as garden bird seed mixes and brassicas. This suggests that breeding ground conservation strategies for turtle doves should include provision of anthropogenic seeds for adults early in the breeding season, coupled with habitat rich in accessible seeds from arable plants once chicks have hatched.

KEYWORDS

anthropogenic food resources, dietary switching, high-throughput sequencing, ITS2, molecular analysis of diet, next-generation sequencing, wildlife management

1 | INTRODUCTION

Dietary changes linked to the availability of anthropogenic food resources (such as crop plants and artificially provided food) can have broad ecological effects (Oro, Genovart, Tavecchia, Fowler, & Martínez-Abraín, 2013), influencing migratory decisions (Flack et al., 2016; Plummer, Siriwardena, Conway, Risely, & Toms, 2015), body condition (Auman, Meathrel, & Richardson, 2008; Romano, Piatt, & Roby, 2006), productivity (Plummer, Bearhop, Leech, Chamberlain, & Blount, 2013; Robb, Mcdonald, Chamberlain, Reynolds, et al., 2008) and population size (Duhem, Roche, Vidal, & Tatoni, 2008). These impacts can be beneficial, reducing energy expenditure, improving body condition and increasing breeding performance (e.g., Auman et al., 2008; Flack et al., 2016). However, when the novel diet replacing natural foods is of poorer quality, this can cause nutritional stress (Will et al., 2015), reduce nestling growth, both fledgling (Österblom, Casini, Olsson, & Bignert, 2006) and adult body mass (Rosen & Trites, 2000), and also be linked to population declines (Kitaysky, Kitaiskaia, Piatt, & Wingfield, 2006).

Dietary switching can have ecosystem-scale impacts on food webs through trophic cascades (e.g., Estes, Tinker, Williams, & Doak, 1998; Estes et al., 2011; Rodewald, Kearns, & Shustack, 2011) and altered community structure (Fuller, Warren, Armsworth, Barbosa, & Gaston, 2008). There are two, mutually nonexclusive, drivers of dietary switches: either an increase in abundance of a novel food type (Grémillet et al., 2008) or a reduction in the availability of a preferred food type forcing habitat or dietary change (e.g., Boates & Goss-Custard, 1989; Smart & Gill, 2003). Declining species are frequently food-limited with implications for both productivity (e.g., Hart et al., 2006) and survival (e.g., Siriwardena, Calbrade, & Vickery, 2008), and habitat or dietary switching may be a warning of ecological changes prior to changes in demographic rates and population declines (Smart & Gill, 2003).

The European turtle dove (hereon referred to as turtle dove) is the UK's and one of Europe's fastest declining breeding bird species (Hayhow et al., 2017; PECBMS 2015). It is classified as a farmland specialist in the UK, although elsewhere it is also associated with open woodlands and forest borders (e.g., Bakaloudis, Vlachos, Chatzinikos, Bontzorlos, & Papakosta, 2009; Dias et al., 2013). Turtle doves and stock doves feed only on seeds (Browne & Aebischer, 2003; Murton, Westwood, & Isaacson, 1964), whereas other columbids will also take leaves and other plant matter (Murton et al., 1964; Wilson, Morris, Arroyo, Clark, & Bradbury, 1999). Previous microscopic analysis of faecal samples has shown that the diet of the turtle dove changed from mainly noncultivated (natural) arable plants in the 1960s (Murton et al., 1964) to mainly cultivated food resources (mostly wheat [*Triticum aestivum*] and oilseed rape [*Brassica napus*]) in the 1990s (Browne & Aebischer, 2003). The turtle dove diet switch occurred concurrently with decreases in the abundance of many natural arable plants (Storkey, Meyer, Still, & Leuschner, 2012), along with a decrease in reproductive effort and a rapid population decline (Browne & Aebischer, 2004). It is postulated that this dietary switch may be associated with a reduction in food availability during key periods of the breeding season when seeding natural arable plants have become scarce as a result of agricultural change (Browne & Aebischer, 2004). For example, increases in autumn-sown crops, with associated fertilizer and herbicide applications and a consequent reduction in the area of overwinter fallow, have adversely affected populations of natural arable plants that persist overwinter in fallow land or germinate after spring tillage, thus reducing the availability of accessible seed for breeding birds (Smart, Firbank, Bunce, & Watkins, 2000). There is also uncertainty about the dietary quality for turtle doves of the anthropogenic foods that have largely replaced natural arable plant seeds (Pruitt, Hewitt, Silvy, & Benn, 2008).

Recent developments in genetic analysis of diet have led to the possibility of using molecular barcodes amplified from faecal DNA and analysed using high-throughput sequencing (HTS), a method with higher resolution and improved accuracy when compared to traditional microscopic methods (Ando et al., 2013; Galimberti et al., 2016). Standard barcode analyses of plant species use parts of the *rbcL* and *matK* genes, which can provide species-level discrimination of 75% when combined (de Vere et al., 2012). However, limitations on amplicon length in HTS (current maximum of 2×300 base pair reads on Illumina Miseq; Illumina 2016), as well as the need to design primers that will amplify shorter barcodes to detect degraded DNA in faecal samples (Ando et al., 2013; King, Read, Traugott, & Symondson, 2008; Pompanon et al., 2012), have meant in practice that these gene regions provide limited discriminatory powers for analysis of faecal samples from herbivores (Pompanon et al., 2012).

The ITS2 nuclear gene has been proposed as a target for the design of short-length barcodes suitable for dietary analysis (Bradley et al., 2007) with a high species-level discrimination for identifying medicinal plants (92.7%; Chen et al., 2010) and herbivorous insect gut contents (61.6% for the Zingiberales order; García-Robledo, Erickson, Staines, Erwin, & Kress, 2013), suggesting ITS2 may have higher resolution than more widely used short-length barcodes (Hollingsworth, Graham, & Little, 2011). A major criticism of ITS2 is the lack of reference sequences available for this region (Hollingsworth et al., 2011); however, the latest update to the ITS2 database has doubled the number of reference sequences available to 711,172, of which 208,822 belong to the Chloroplastida (Ankenbrand, Keller, Wolf, Schultz, & Förster, 2015). This figure does not include a new database for the majority of UK plants that has recently been made available on GenBank (N. de Vere, C. R. Ford, H. Davies, E. Brittain, L. Jones, P. Hollingsworth, L. Forrest & M. Hart, unpublished data). Novel universal primers targeting the ITS2 region have recently been developed, with product lengths ranging from 187 to 380 base pairs (Moorhouse-Gann et al., 2018), short enough to encompass the most variable region within the gene and take advantage of paired-end Illumina MiSeq sequencing technology. A comprehensive in silico analysis of these primers suggested that 88% of plant species (n = 1,111 species from 148 families tested) are amplified and that of these, 99.4% could be identified to the genus level (Moorhouse-

Gann et al., 2018). This is considerably higher than either trnL or rbcL short-amplicon primers (which identify 34% and 42% of plant sequences, respectively, to genus level; Pompanon et al., 2012) and avoids the need to use multiple gene targets to maximize identification. In practice, in vitro tests of 202 UK and tropical plant species showed that 99% were amplified by the Moorhouse-Gann et al. (2018) primers, despite mismatches.

Here, our aim was to apply HTS to identify dietary components from columbid faecal samples and test three hypotheses:

1. Turtle dove diet currently shows strong overlap with that of other UK columbids, suggesting competition for limited food resources.
2. Anthropogenic food resources, such as cultivated crops and artificially provided food for songbirds at bird tables, are associated with poorer condition in both adult and nestling turtle doves.
3. Turtle dove diet shows both inter- and intra-annual variation, with anthropogenic food resources more important early in the turtle dove breeding season.

2 | METHODS

2.1 | Sites and field collection

Faecal samples were collected from adult and nestling columbids (turtle doves, collared doves, stock doves and woodpigeons), as part of a 4-year autecological study of turtle dove breeding ecology at 12 farmland sites across Essex, Suffolk, Cambridgeshire and Norfolk, UK. During 2011–2012, faecal samples were collected at sites described in Dunn, Morris, and Grice (2015); seven sites where turtle doves no longer bred were replaced with new sites during 2013–2014 (Figure 1; Appendix 1).

Adult columbids were caught using whoosh and mist nets (Redfern & Clark, 2001) at temporarily baited sites in areas either where birds had previously been seen feeding, or where farmers provided grain, during May, June and July 2011–2014. Thus, we expected a small amount of mixed seed to be present in faecal samples of adult columbids if they were regularly using baited sites. When caught, birds were weighed and maximum wing chord measured (Redfern & Clark, 2001). Adult turtle doves were fitted with tail-mounted Pip3 radio-tags (Biotrack, Dorset, UK) weighing 1.7 g (<1.5% of body mass), to help in locating nests. All adults were caught prior to them having chicks in the nest, ensuring we were identifying components of adult diet, rather than seeds collected for regurgitation to nestlings. As well as adult turtle doves (n = 26), we also collected faecal samples from adult collared doves (n = 6) and stock doves (n = 12). Faecal samples were collected either directly from the bird or from the inside of clean bird bags within which the birds were temporarily held after capture. All faecal samples were frozen at −20°C as soon as possible after collection (1–8 hrs) until subsequent analysis.

Nests were located by monitoring the movements of radio-tagged turtle doves and by cold-searching suitable habitat for all columbid species. Nests were checked every 2 days, and when nestlings were seven (turtle dove n = 66 and collared dove n = 5) or 10–14 days old (stock dove n = 3 and woodpigeon n = 22), they were ringed, weighed and faecal samples collected. Different sampling ages were due to different nestling growth rates between species (Robertson, 1988), precluding the sampling of turtle doves later than 7 days old when they were capable of leaving the nest prematurely. At this age, nestlings are fed seeds and not crop milk (confirmed by

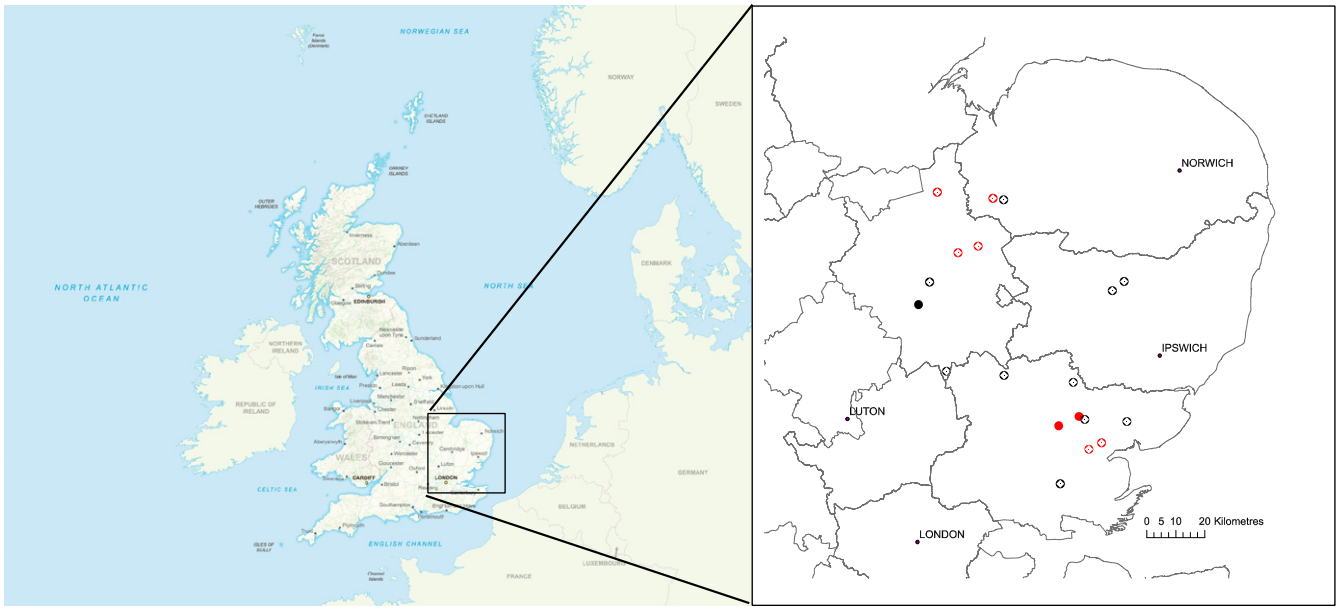

FIGURE 1 Locations of study sites from where faecal samples were collected. Sites where only nonturtle dove faecal samples were collected are shown as black dots, although turtle doves were also present at these sites; red dots denote sites from which turtle dove faecal samples were collected in addition to those of other columbids. Further site and faecal sample collection details are provided in Appendix 1. Contains Ordnance Survey data © Crown copyright and database right 2017 [Colour figure can be viewed at wileyonlinelibrary.com]

examining the crop contents of three nestlings found dead under their nests at 3–5 days old; J. Dunn, personal observation). Multiple faecal samples from nestmates were processed separately and data subsequently pooled for statistical analyses. Faecal samples from nestlings were collected between June and September, 2011–2014.

2.2 | Construction of a DNA barcode reference library

Seeds were collected in the field from 24 plant species, supplemented by seeds from nine species known to be commonly present within commercial seed mixes (Appendix 2). We downloaded sequences from an additional 19 species from GenBank to ensure that all species previously recorded in turtle dove diet (Browne & Aebischer, 2003; Murton et al., 1964), as well as other plant species commonly found at our field sites, were included in the barcode library (Appendix 2; Moorhouse-Gann et al., 2018). We extracted DNA from all species using a standard salting-out protocol (Randall, Sornay, Dewitte, & Murray, 2015) and confirmed in vitro that our new primers (UniPlantF [5′-TGTGAATTGCARRATYCMG-3′] and UniPlantR [5′-CCCGHYTGAYYTGRGGTCDC-3′]) amplified all our target species (Moorhouse-Gann et al., 2018), with no nontarget amplicons. PCRs were carried out in 10 μl reaction volumes containing 5 μl multiplex buffer (Qiagen, Manchester, UK), 2.6 μl H_2O, 0.2 μl each primer (10 μM) and 2 μl DNA. Reaction conditions were initial denaturation at 95°C for 15 min; 40 cycles of 95°C for 30 s, 56°C for 30 s, 72°C for 1 min; final extension of 72°C for 10 min.

2.3 | Faecal analysis

DNA was extracted from approximately 200 mg of each faecal sample using a QIAamp DNA Stool Mini Kit (Qiagen) with slight modifications to the manufacturer's instructions detailed in Dunn et al. (2016), using negative extraction controls (n = 6) throughout. We used primers UniPlantF and UniPlantR to amplify a 187- to 380-bp region encompassing the ITS2 region of plant nuclear DNA and labelled each sample with a unique combination of forward and reverse MID tags (Brown et al., 2014). The PCR recipe and thermal profile are as described above. Samples were pooled according to intensity of the PCR product on a 1% agarose gel stained with SYBR®Safe (Thermo Fisher Scientific, Paisley, UK) when compared to a standardized 100-bp ladder and subsequently quantified using a BioAnalyzer (Agilent Technologies, Santa Clara, CA, USA) to check peak amplicon size and DNA concentration. Only samples where a clear band was visible following electrophoresis were processed further. Samples were purified in pools of similar DNA concentration using a QIAquick PCR Purification kit (Qiagen), quantified using a Qubit (Thermo Fisher Scientific, Waltham, MA, USA) and pools subsequently combined to provide an approximately equal amount of amplicon DNA from each faecal sample.

The pool of individually tagged amplicons was used to prepare a library for paired-end sequencing using the NEBNext Ultra DNA Library Prep Kit for Illumina (New England Biolabs, Ipswich, MA, USA). The library was sequenced using 250-bp paired-end reads on a MiSeq desktop sequencer (Illumina, San Diego, CA, USA).

2.4 | Identification of plant species

Our Illumina run resulted in 12,592,989 paired-end reads, which were filtered for quality using Trimmomatic v0.32 (Bolger, Lohse, & Usadel, 2014) with a minimum quality score of 20 over a sliding window of 4 bp, retaining sequences with a minimum length of 135 bp resulting in 10,138,058 sequences. These were aligned using FLASH (Magoč & Salzberg, 2011), resulting in 9,921,248 aligned sequences. These were demultiplexed into faecal sample-specific files using the MID tag sequence with the "trim_seqs" command in Mothur (Schloss et al., 2009), which also removes the MID and primer sequences from the reads. After eliminating reads without an exact match to primer sequences and MID tags, 6,105,478 sequences remained (mean ± *SE* for samples: 42,917 ± 2,871; for negatives and unused tag combinations: 1,930 ± 382). We then used the "derep_fulllength" and "uchime2_denovo" commands in the USEARCH software v9.2.64 (Edgar, 2010) to remove any sequences with fewer than 10 copies within a faecal sample and any potential chimeric sequences, resulting in 12,608 unique sequences. Analysis of species discrimination at the ITS2 region (Moorhouse-Gann et al., 2018) suggests this region to be unsuitable for an approach of clustering similar sequences into molecular operational taxonomic units (MOTUs) due to the loss of ability to distinguish between species prior to the grouping of multiple polymorphisms within some plant species. Therefore, we adopted a closest matching sequence approach to identify species within our samples (e.g., Hawkins et al., 2015).

We took a sequence read-number approach to deal with any background contamination. First, we examined sequences found only in samples with unused MID combinations (n = 20) as these could only be attributed to background contaminants or "tag jumping" (Kircher, Sawyer, & Meyer, 2012; Schnell, Bohmann, & Gilbert, 2015). The highest number of reads for any of these sequences was 139, so we re-ran our initial dereplication step (using "derep_fulllength" in USEARCH) with this new sequence read threshold. This resulted in 1,192 unique sequences, which we then assigned to taxonomic unit using the BLAST algorithm (Altschul et al., 1997) to search GenBank, combined with new sequences from our barcode library (GenBank Accession nos KT948614–KT948638). If a sequence had the smallest *e*-value matching only one species on GenBank, with >99% sequence identity, we assigned the sequence to that species (Hawkins et al., 2015). If the sequence matched more than one species from the same genus, tribe or family, we assigned the sequence to the lowest common taxonomic unit up to the family level. Any sequence with <90% match to the closest matching species on GenBank, or for which BLAST returned no significant match (n = 80), was discarded, as was any sequence for which the closest match included a bacterium or fungus (n = 64). Next, to deal with any specific contaminants within our samples, we examined each unique sequence found in a negative sample, including unused MID combinations, PCR negatives (n = 2) and extraction negatives (n = 6).

For each sequence, we identified the highest read number within a negative sample and removed this sequence from any sample where the read number was below this threshold (detailed in Appendix 3). Five sequences had their highest read numbers in negative samples (n = 5; Appendix 3) and were thus discarded. Finally, we combined our 1,043 remaining sequences within each of 143 taxonomic units. We briefly discuss the possible effects of faecal or plant inhibitors and secondary predation in the Supporting Information.

Where we had multiple faecal samples from two nestlings within the same nest (no nest contained more than two nestlings), we combined these into sampling units for subsequent analysis.

2.5 | Statistical analysis

For dietary overlap analyses and subsequent statistical analyses, we used the presence or absence of each taxonomic unit in each sampling unit. For morphometric analysis of nestlings at the level of the sampling unit, we averaged data from both nestlings to avoid pseudoreplication due to nonindependence of nestmates. All statistical analyses were carried out in R version 3.1.2 "Pumpkin Helmet" for Mac (R Core Team 2016) unless otherwise stated.

2.6 | Dietary breadth and overlap between columbid species

To determine whether species showed differences in the number of taxonomic units in their diet, we constructed a generalized linear model using the number of taxonomic units per sampling unit as the response variable and the columbid species as a fixed factor, allowing for a Poisson distribution corrected for overdispersion. We tested the significance of the species term by comparison of this model with a null model using likelihood ratio tests.

To calculate dietary overlap of each species pair at the taxonomic unit level, we calculated Pianka's measure of overlap (Pianka, 1986) in EcoSimR (Gotelli & Ellison, 2013) using the equation:

$$O_{jk} = \frac{\sum p_{ij} p_{ik}}{\sqrt{\sum p_{ij}^2 p_{ik}^2}},$$

where O_{jk} is Pianka's measure of overlap between species j and species k, p_{ij} is the proportion of total resources that resource i is for species j, and p_{ik} is the proportion of total resources that resource i is for species k. O ranges from 0, where two species have no resources in common, to 1, where there is complete overlap in resource use. To portray dietary overlap between species, we constructed bipartite food webs using the BIPARTITE package (Dormann, Gruber, & Fruend, 2008).

Finally we assessed the diets of different columbid species at the level of both the taxonomic unit and the plant family. For each taxonomic unit (n = 129) or plant family (n = 34) where the taxonomic unit or family was found in the diet of more than one columbid species (taxonomic unit: n = 52; family: n = 19), we ran a binomial GLM corrected for overdispersion, comparing the proportions of diets from each family (calculated as the proportion of individuals within each columbid species whose diet contains each taxonomic unit and plant family separately), carrying out Tukey HSD post hoc tests to identify differences between turtle doves and other columbids.

As our sample sizes for nonturtle dove columbids is relatively small, we carried out rarefaction analysis using the package VEGAN (Oksanen et al., 2016) to estimate the proportion of total taxonomic units in the diet of each species that we are likely to have detected. For our larger turtle dove sample, we created four subsets of our data, each with n = 13 and carried out rarefaction analysis on each subset separately to confirm differences in estimated numbers of taxonomic units between species.

2.7 | Associations between diet and condition in turtle doves

To identify whether relative proportions of taxonomic units in diet were associated with condition in adult or nestling turtle doves, we categorized dietary components into four broad categories according to likely source (detailed in Table 1): "fed" (eight taxonomic units) contained seeds likely to be found in the vicinity of bird tables and supplementary food sources such as game bird feeders or grain tailings; "cultivated" crop plants as well as those widely cultivated as components of seed mixes sown to provide seed for game or wild birds within our study area (16 taxonomic units; excluding wheat, as this was widely available as supplementary food at our study sites); "natural" contained any wild plant species (109 taxonomic units). We considered "brassica" (Brassicaceae; 11 taxonomic units) as a separate category as this plant family forms components of provisioned bird seed as well as being widely cultivated within our study area and also contains several naturally occurring wild species.

We used residuals from a linear regression of mean nestling body mass on mean nestling tarsus length at 7 days old to give an index of mean nestling condition within each nest whilst controlling for the nonindependence of nestmates (Labocha & Hayes, 2012). We used tarsus length because wing length is not easily measured on nestlings with limited primary feather growth. To obtain an index of adult condition at capture, we used residuals from a linear regression of body mass on wing length (Labocha & Hayes, 2012). We then used the DIRICHLETREG package (Maier, 2015) to carry out Dirichlet regressions for compositional diet data (Sánchez & Dos Santos, 2015) to identify how the relative proportions of taxonomic units within each dietary category are associated with adult and nestling turtle dove condition separately.

2.8 | Temporal variation in turtle dove diet

We carried out analyses of temporal variation in dietary importance for each of our four broad dietary component categories. For each dietary category, we constructed a Binomial GLM corrected for underdispersion (dispersion parameters of noncorrected binomial GLMs: brassica 0.07; cultivated 0.03; fed 0.07; natural 0.07) with the

TABLE 1 Presence of taxonomic units of plants in the diet of each columbid species (TD: turtle dove; CD: collared dove; SD: stock dove; WP: woodpigeon), with results from GLMs (*F* statistics and *p* values) testing for differences in the mean proportion of total taxonomic units within diet (which are preliminary due to smaller sample sizes for three species)

Taxonomic unit	Family	Category	TD (*n* = 54)	CD (*n* = 7)	SD (*n* = 13)	WP (*n* = 5)	*F*	*p*
Sambucus nigra	Adoxaceae[a]	Natural	1.9	0	0	0	[b]	
Amaranthaceae	Amaranthaceae	Natural	0	14.3	0	0	[b]	
Amaranthus sp.	Amaranthaceae	Natural	5.6	0	0	0	[b]	
Atriplex sp.	Amaranthaceae	Natural	16.7	14.3	15.4	40.0	0.479	0.698
Chenopodium album	Amaranthaceae	Natural	1.9	0	0	0	[b]	
Chenopodium polyspermum	Amaranthaceae	Natural	5.6	0	7.7	0	0.536	0.659
Chenopodium sp.	Amaranthaceae	Natural	18.5	14.3	0	20.0	1.822	0.15
Halimione sp.	Amaranthaceae	Natural	0	14.3	0	0	[b]	
Salicornia sp.	Amaranthaceae	Natural	11.1	0	0	0	[b]	
Suaeda maritima	Amaranthaceae	Natural	1.9	14.3	0	0	1.21	0.312
Suaeda sp.	Amaranthaceae	Natural	1.9	0	0	0	[b]	
Anthriscus sp.	Apiaceae	Natural	11.1	14.3	7.7	20.0	0.178	0.911
Anthriscus sylvestris	Apiaceae	Natural	0	14.3	0	0	[b]	
Apiaceae	Apiaceae	Natural	1.9	0	0	0	[b]	
Pastinaca sativa	Apiaceae	Cultivated	3.7	0	7.7	0	0.504	0.681
Achillea millefolium	Asteraceae	Natural	1.9	0	0	0	[b]	
Anthemis cotula	Asteraceae	Natural	1.9	0	0	0	[b]	
Artemesia vulgaris	Asteraceae	Natural	3.7	0	0	0	[b]	
Asteraceae	Asteraceae	Natural	1.9	0	0	20.0	1.564	0.205
Bellis perennis	Asteraceae	Natural	5.6	0	0	20.0	1.476	0.228
Carduus crispus	Asteraceae	Natural	0	0	0	20.0	[b]	
Carthamus glaucus	Asteraceae	Natural	1.9	0	0	0	[b]	
Carthamus sp.	Asteraceae	Natural	1.9	0	0	0	[b]	
Carthamus tinctorius	Asteraceae	Fed	3.7	14.3	0	0	1.09	0.359
Centaurea sp.	Asteraceae	Natural	1.9	0	0	0	[b]	
Chromolaena odorata	Asteraceae	Natural	1.9	0	0	0	[b]	
Cirsium arvense	Asteraceae	Natural	16.7	0	0	40.0	**3.549**	**0.018**
Cirsium velatum	Asteraceae	Natural	1.9	0	0	0	[b]	
Cirsium vulgare	Asteraceae	Natural	5.6	0	7.7	0	0.536	0.659
Guizotia abyssinica	Asteraceae	Fed	35.2	0	15.4	40.0	*2.556*	*0.062*
Helianthus annuus	Asteraceae	Fed	13.0	14.3	7.7	0	0.536	0.659
Helianthus argophyllus	Asteraceae	Fed	1.9	0	0	0	[b]	
Helminthotheca echioides	Asteraceae	Natural	3.7	0	0	0	[b]	
Jacobaea vulgaris	Asteraceae	Natural	1.9	0	0	0	[b]	
Lactuca serriola	Asteraceae	Natural	0	0	0	20.0	[b]	
Lapsana communis	Asteraceae	Natural	0	0	7.7	0	[b]	
Senecio vulgaris	Asteraceae	Natural	1.9	0	0	0	[b]	
Sonchus arvensis	Asteraceae	Natural	1.9	0	0	0	[b]	
Tripleurospermum inodorum	Asteraceae	Natural	0	0	0	20.0	[b]	
Tripleurospermum maritimum	Asteraceae	Natural	1.9	0	0	0	[b]	
Tussilago farfara	Asteraceae	Natural	1.9	0	0	0	[b]	
Corylus avellana	Betulaceae	Natural	0	14.3	0	0	[b]	
Boraginaceae	Boraginaceae	Natural	5.6	0	0	0	[b]	
Borago officinalis	Boraginaceae	Cultivated	96.3	85.7	61.5	80.0	**3.436**	**0.021**

(Continues)

TABLE 1 (Continued)

Taxonomic unit	Family	Category	TD (*n* = 54)	CD (*n* = 7)	SD (*n* = 13)	WP (*n* = 5)	*F*	*p*
Echium plantagineum	Boraginaceae	Natural	22.2	0	0	0	[b]	
Symphytum sp.	Boraginaceae	Natural	25.9	71.4	7.7	0	**4.109**	**0.009**
Brassica carinata	Brassicaceae	Brassica	1.9	0	0	0	[b]	
Brassica juncea	Brassicaceae	Brassica	13.0	0	0	0	[b]	
Brassica napus	Brassicaceae	Brassica	25.9	28.6	38.5	40.0	0.337	0.799
Brassica oleracea	Brassicaceae	Brassica	24.1	14.3	7.7	20.0	0.699	0.556
Brassica rapa	Brassicaceae	Brassica	1.9	0	0	0	[b]	
Brassica sp.	Brassicaceae	Brassica	88.9	71.4	61.5	80.0	1.719	0.17
Brassicaceae	Brassicaceae	Brassica	53.7	0	46.2	40.0	**3.459**	**0.021**
Capsella bursa-pastoris	Brassicaceae	Brassica	1.9	0	0	0	[b]	
Raphanus sativus	Brassicaceae	Cultivated	3.7	0	0	0	[b]	
Rorippa sylvestris	Brassicaceae	Brassica	1.9	0	0	0	[b]	
Thlaspi arvense	Brassicaceae	Brassica	3.7	14.3	7.7	0	0.594	0.621
Cannabis sativa	Cannabaceae	Fed	18.5	0	7.7	0	1.853	0.145
Caryophyllaceae	Caryophyllaceae	Natural	3.7	0	0	0	[b]	
Cerastium glomeratum	Caryophyllaceae	Natural	18.5	0	0	0	[b]	
Stellaria pallida	Caryophyllaceae	Natural	1.9	0	0	0	[b]	
Stellaria media	Caryophyllaceae	Natural	25.9	0	7.7	20.0	1.998	0.122
Stellaria neglecta	Caryophyllaceae	Natural	1.9	0	0	0	[b]	
Calystegia sepium	Convolvulaceae	Natural	5.6	14.3	0	20.0	1.272	0.29
Crassulaceae	Crassulaceae[a]	Natural	3.7	0	0	0	[b]	
Cucumis sp.	Cucurbitaceae	Cultivated	3.7	0	0	20.0	1.44	0.238
Cucurbitaceae	Cucurbitaceae	Cultivated	3.7	0	0	0	[b]	
Chamaecyparis lawsoniana	Cupressaceae[a]	Natural	1.9	0	0	0	[b]	
Euphorbiaceae	Euphorbiaceae[a]	Natural	1.9	0	0	0	[b]	
Pisum sativum	Fabaceae	Cultivated	1.9	0	0	0	[b]	
Vicia hirsuta	Fabaceae	Cultivated	3.7	0	0	0	[b]	
Vicia sativa	Fabaceae	Cultivated	1.9	0	7.7	0	0.613	0.608
Quercus sp.	Fagaceae	Natural	0	0	7.7	0	[b]	
Geraniaceae	Geraniaceae	Natural	5.6	0	0	0	[b]	
Geranium dissectum	Geraniaceae	Natural	51.9	14.3	30.8	60.0	1.769	0.16
Geranium lucidum	Geraniaceae	Natural	5.6	0	7.7	0	0.536	0.659
Geranium molle	Geraniaceae	Natural	3.7	0	0	0	[b]	
Geranium pusillum	Geraniaceae	Natural	7.4	0	0	0	[b]	
Linum sp.	Linaceae[a]	Cultivated	3.7	0	0	0	[b]	
Epilobium sp.	Onagraceae[a]	Natural	3.7	0	0	0	[b]	
Papaver rhoeas	Papaveraceae[a]	Natural	1.9	0	0	0	[b]	
Pinus sp.	Pinaceae[a]	Natural	1.9	0	0	0	[b]	
Plantago lanceolata	Plantaginaceae	Natural	5.6	0	7.7	0	0.536	0.659
Agrostis sp.	Poaceae	Natural	7.4	14.3	0	40.0	*2.387*	*0.076*
Agrostis stolonifera	Poaceae	Natural	3.7	0	0	0	[b]	
Alopecurus myosuroides	Poaceae	Natural	5.6	0	0	0	[b]	
Alopecurus sp.	Poaceae	Natural	1.9	0	0	0	[b]	
Arrhenatherum elatius	Poaceae	Natural	1.9	0	0	0	[b]	
Avena sp.	Poaceae	Natural	3.7	0	0	0	[b]	
Cenchrus americanus	Poaceae	Fed	1.9	0	0	0	[b]	

(Continues)

TABLE 1 (Continued)

Taxonomic unit	Family	Category	TD (n = 54)	CD (n = 7)	SD (n = 13)	WP (n = 5)	F	p
Dactylis glomerata	Poaceae	Natural	83.3	28.6	30.8	40.0	**6.42**	**<0.001**
Dactyloctenium aegyptium	Poaceae	Natural	5.6	0	0	0	[b]	
Elymus repens	Poaceae	Natural	3.7	14.3	0	0	1.09	0.359
Festuca sp.	Poaceae	Natural	1.9	0	0	0	[b]	
Holcus lanatus	Poaceae	Natural	1.9	0	0	0	[b]	
Holcus sp.	Poaceae	Natural	1.9	0	0	0	[b]	
Hordeum sp.	Poaceae	Cultivated	1.9	0	7.7	0	0.613	0.608
Hordeum vulgare	Poaceae	Cultivated	5.6	0	0	0	[b]	
Lolium sp.	Poaceae	Natural	1.9	0	7.7	0	1.853	0.145
Panicum miliaceum	Poaceae	Fed	87.0	42.9	61.5	60.0	**3.014**	**0.035**
Phalaris sp.	Poaceae	Natural	1.9	0	0	0	[b]	
Poa annua	Poaceae	Natural	1.9	0	7.7	0	0.613	0.608
Poa infirma	Poaceae	Natural	16.7	14.3	7.7	20.0	0.269	0.848
Poa sp.	Poaceae	Natural	11.1	0	15.4	0	1.106	0.352
Poa trivialis	Poaceae	Natural	9.3	0	0	20.0	1.756	0.163
Poaceae	Poaceae	Natural	33.3	28.6	38.5	40.0	0.094	0.963
Sorghum sp.	Poaceae	Fed	9.3	0	0	0	[b]	
Triticeae	Poaceae	Cultivated	11.1	0	0	0	[b]	
Triticum aestivum	Poaceae	Cultivated	11.1	0	0	20.0	1.954	0.128
Triticum sp.	Poaceae	Cultivated	7.4	0	15.4	0	1.039	0.38
Persicaria lapathifolia	Polygonaceae[a]	Natural	1.9	0	0	0	[b]	
Anagallis arvensis	Primulaceae	Natural	81.5	85.7	84.6	60.0	0.446	0.721
Anagallis sp.	Primulaceae	Natural	3.7	0	0	20.0	1.44	0.238
Primulaceae	Primulaceae	Natural	24.1	14.3	7.7	0	1.484	0.226
Clematis vitalba	Ranunculaceae	Natural	5.6	0	7.7	0	0.536	0.659
Reseda lutea	Resedaceae	Natural	0	0	15.4	20.0	**12.977**	**<0.001**
Ziziphus spina-christi	Rhamnaceae[a]	Natural	1.9	0	0	0	[b]	
Geum urbanum	Rosaceae	Natural	7.4	0	0	0	[b]	
Potentilla sp.	Rosaceae	Natural	3.7	0	0	0	[b]	
Prunus sp.	Rosaceae	Natural	20.4	14.3	7.7	0	1.103	0.354
Rosa sp.	Rosaceae	Natural	20.4	14.3	0	0	**2.857**	**0.043**
Rosaceae	Rosaceae	Natural	3.7	0	7.7	0	0.504	0.681
Rubus sp.	Rosaceae	Natural	50.0	28.6	30.8	20.0	1.169	0.328
Galium aparine	Rubiaceae[a]	Natural	3.7	0	0	0	[b]	
Citrus sp.	Rutaceae[a]	Cultivated	1.9	0	0	0	[b]	
Acer campestre	Sapindaceae[a]	Natural	1.9	0	0	0	[b]	
Urtica dioica	Urticaceae	Natural	33.3	14.3	15.4	0	1.974	0.125
Viola arvensis	Violaceae	Natural	29.6	71.4	7.7	20.0	**2.924**	**0.039**
Violaceae	Violaceae	Natural	1.9	0	0	0	[b]	

Notes. Percentage of taxonomic units for each family is presented for each columbid species; those highlighted in bold differ from those of turtle doves at $p < 0.05$ and those in italics at $p < 0.1$.

[a]Denotes a family found exclusively in turtle dove diet. [b]Differences not tested statistically as the plant family was only found within one columbid species or in fewer than three individuals.

proportion of dietary taxonomic units comprising the relevant component within each sampling unit as a response variable. Fixed terms were as follows: mean-centred Julian day specified to test for both linear and quadratic relationships (range of day is from 22nd May to 4th September); age (adult or nestling); year (n = 4, as a categorical variable); and site (n = 6, with three farms in Norfolk combined due

to small sample sizes). To determine the importance of each term within the model, we removed each term in turn and compared the fit of the model with and without each term using chi-squared tests. We retained all terms in the final model from which we made predictions, to control for our unbalanced sampling design as not all sites were sampled in all years (Appendix 1). We then used Tukey HSD post hoc tests to identify where factor levels differed from each other.

We had data from nine nests where we also have data from one (n = 8 nests, n = 6 adults) or both (n = 1 nest, n = 2 adults) of the adults at the nest. However, all adults were caught a minimum of 27 days before their respective nestlings were sampled (mean ± *SE*: 45.8 ± 14.3 days). As there were temporal differences between adult and nestling samples, and between sequential nesting attempts from the same adult (n = 2 adults, two nesting attempts each), we treated these as independent data points for the purposes of the spatiotemporal analysis models described above as we had insufficient nonindependent samples to allow a mixed-effects model (including a "Family" term) to converge. However, to examine whether related adults and nestlings have more similar diets than unrelated adults and nestlings, we examined a subset of our data involving adults for whom we also had nestling samples and sampling units from sequential nesting attempts by the same adult where we did not have an adult faecal sample. We tested the effect of "Family" on the proportion of each dietary component category, as defined above, using a GLM with quasi-binomial error structure to allow for underdispersed proportion data.

3 | RESULTS

We successfully amplified DNA from 121 samples from 98 individual birds, forming a total of 79 independent sampling units (turtle doves: 26 adult sampling units, 28 nestling sampling units (including two for which morphometric measurements were not collected); collared doves: three adult sampling units, four nestling sampling units; stock dove: 10 adult sampling units, three nestling sampling units; and five woodpigeon nestling sampling units).

3.1 | Diet composition and overlap between columbid species

We identified 55% of sequences to species (62.9% of taxonomic units), an additional 34% to genus (26.6% of taxonomic units) and the remaining 11% to family level (10.5% of taxonomic units). Sixty-eight taxonomic units were found only in turtle doves, 10 taxonomic units were found only in nonturtle doves, and 51 taxonomic units were shared between turtle doves and other columbids (Figure 2). The remaining 14 taxonomic units were found in faecal samples from nests, which we do not consider further in this study (n = 20 samples).

We found significant differences between columbid species in the number of taxonomic units per faecal sample (GLM: F_3 = 2.77, p = 0.04; Table 2), with turtle doves having more taxonomic units per faecal sample than collared doves (t = 2.25, p = 0.03; Table 2) and marginally more than stock doves (t = −1.75, p = 0.08). Pianka's measure suggested significant dietary overlap between all four species ($p < 0.001$ for all pairwise comparisons; Table 2) with values ranging from 0.70 to 0.90. The highest dietary overlap was between turtle dove and stock dove, then between turtle dove and woodpigeon, and the lowest overlap between collared dove and woodpigeon (Table 2).

All taxonomic units were assigned to one of 34 plant families, and we examined differences in the mean proportion of diet comprised of each plant family between columbid species. Thirty-one families were found in turtle dove diet, of which 13 families were found exclusively in turtle dove diet (Table 1). None of these families constituted more than 1% of taxonomic units in turtle dove diets.

We examined the proportion of diets from each columbid species that contained each family, and each taxonomic unit (Table 1), and summarize ecologically important observations here (detailed findings are provided in the Supporting Information). Taxonomic units from the Asteraceae were found in a higher proportion of turtle dove diets than either collared dove or stock dove diets, with niger seed (*Guizotia abyssinica*), a common seed in garden bird seed mixes, present in 35% of turtle dove diets, 15% of stock dove diets and 40% of woodpigeon diets but not recorded in collared dove diet (Table 1; Figure 2a). Also found in more than 10% of turtle dove diets were Creeping thistle (*Cirsium arvense*), a natural arable plant, and sunflower (*Helianthus annuus*), another seed commonly provided in garden seed mixes (Table 1). Taxonomic units from the Boraginaceae were found in a higher proportion of turtle dove diets than in stock dove diets (Table 1; Figure 2a), with borage (*Borago officinalis*) found in 96% of turtle dove diets, 86% of collared dove diets, 62% of stock dove diets and 80% of woodpigeon diets (Table 1; Figure 2a).

Caryophyllaceae taxonomic units were found in a marginally higher proportion of turtle dove diets than stock dove diets: Common chickweed (*Stellaria media*) was found in 26% of turtle dove diets compared to 20% of woodpigeons and 8% of stock doves (Table 1; Figure 2a). Brassicas (Brassicaceae) were found in 86–100% of species' diets, but did not differ in consumption between species. Oilseed rape and various brassica cultivars (*Brassica oleracea*) were found in 25%–40% and 8%–24% of species' diets, respectively, whilst Chinese mustard (*Brassica juncea*) was found in 13% of turtle dove diets but not any other species (Table 1; Figure 2a). Amaranths (Amaranthaceae) were found in the diet of all species, with goosefoot species (*Chenopodium* sp.) being found in more than 10% of turtle dove diets (Table 1; Figure 2a). Geraniums (Geraniaceae) were found in 14–60% of species' diets, but their prevalence did not differ between species. Cut-leaved cranesbill (*Geranium dissectum*) was found in the diets of all species and had been consumed by 52% of turtle doves (Table 1; Figure 2b).

Cannabaceae, comprising a single taxonomic unit of hemp (*Cannabis sativa*), a common component of bird seed mixes, was found in

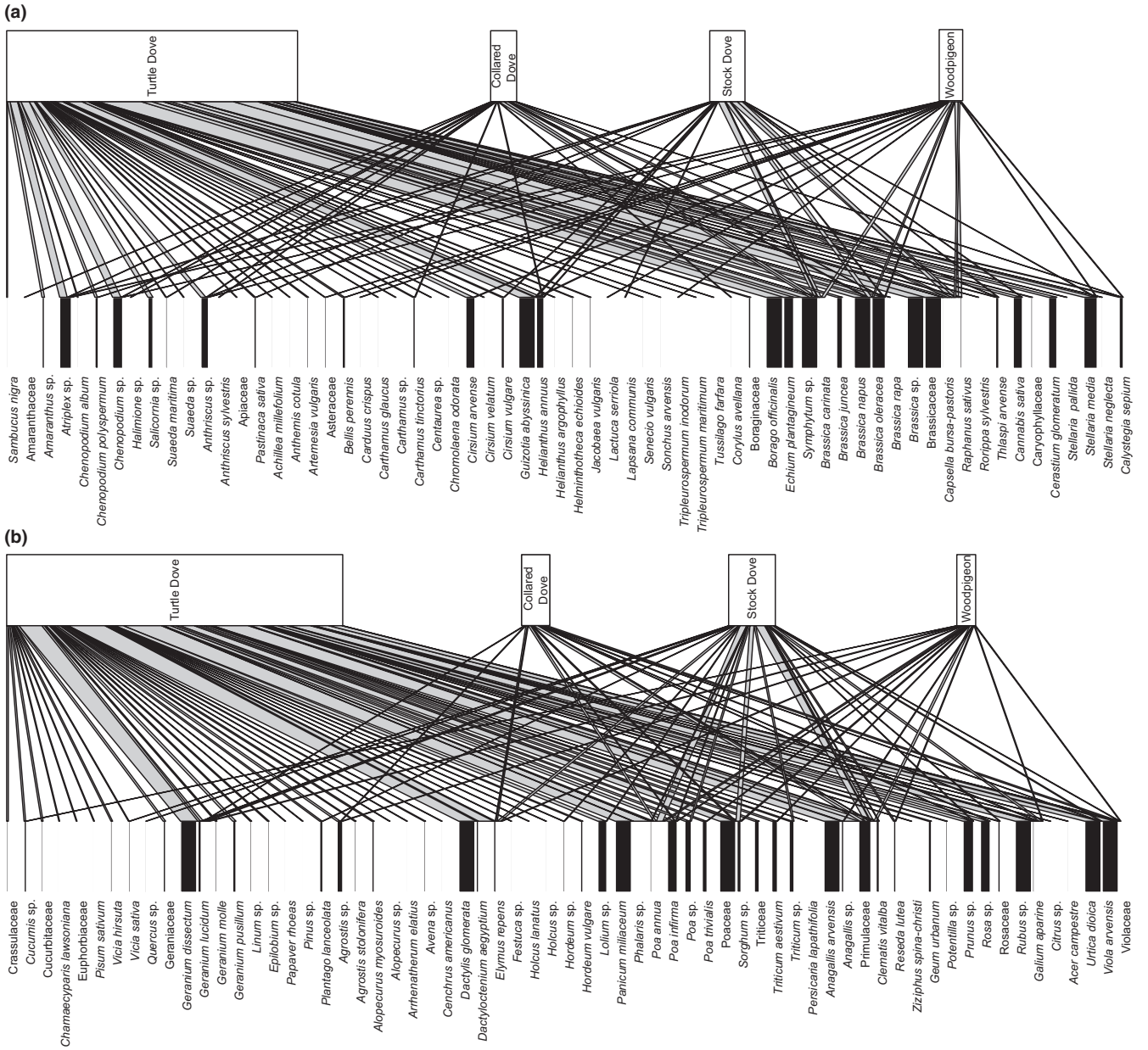

FIGURE 2 Bipartite food webs showing dietary overlap between turtle doves, collared doves, stock doves and woodpigeons. In each web, the upper bars represent columbid species and the lower bars represent taxonomic units. The width of each bar represents the number of samples from (upper bar) or containing (lower bar) that species or taxonomic unit. Interactions between species are shown by lines between bars; thicker lines represent commoner interactions. For clarity due to the number of taxonomic units found in columbid diets, (a) shows taxonomic units within the Adoxaceae (n = 1 taxonomic unit), Amaranthaceae (10), Apiaceae (4), Asteraceae (26), Betulaceae (1) Boraginaceae (4), Brassicaceae (11), Cannabaceae (1), Caryophyllaceae (5) and Convolvulaceae (1) families, and (b) shows taxonomic units within the Crassulaceae (1), Cucurbitaceae (2), Cupressaceae (1), Euphorbiaceae (1), Fabaceae (3), Fagaceae (1), Geraniaceae (5), Linaceae (1), Onagraceae (1), Papaveraceae (1), Pinaceae (1), Plantaginaceae (1), Poaceae (27), Polygonaceae (1), Primulaceae (3), Ranunculaceae (1), Rhamnaceae (1), Rosaceae (6), Rubiaceae (1), Rutaceae (1), Sapindaceae (1), Urticaceae (1) and Violaceae (2) families

19% of turtle dove diets and also in stock dove diets (Table 1; Figure 2a). Primulaceae were found in 60%–92% of species' diets, with scarlet pimpernel (*Anagallis arvensis*), present in 81% of turtle dove diets (Table 1; Figure 2b).

Rarefaction analysis on all samples suggests that we detected over 50% of taxonomic units for all species, with estimated numbers of 110 taxonomic units in 50 diets for turtle doves compared to 50–60 taxonomic units for all three other species (Appendix 4). Our rarefaction analysis on four subsamples each containing data from 13 turtle dove samples predicted consistently higher estimates of taxonomic unit numbers in 50 samples than for the other three species, predicting 70, 80, 85 and 110 taxonomic units.

TABLE 2 Dietary breadth (number of taxonomic units per sampling unit), Pianka's measure of dietary overlap (using the proportion of diets within which each taxonomic unit occurs) for each columbid species pairing

Species	Turtle dove	Collared dove	Stock dove	Woodpigeon
Sample size (adult; nestling sampling units)	54 (26; 28)	7 (3; 4)	13 (10; 3)	5 (0; 5)
Mean ± *SE* taxonomic units per faecal sample	10.40 ± 0.61	6.55 ± 0.69	7.62 ± 0.94	10.20 ± 2.06
Pianka's measure of dietary overlap				
Collared dove	0.799			
Stock dove	0.904	0.773		
Woodpigeon	0.848	0.703	0.827	

Note. Pianka's measure was significant at $p < 0.001$ for every species pair.

3.2 | Dietary associations with turtle dove body condition

We found significant associations between diet composition and both adult and nestling turtle dove body condition (Table 3). The proportion of fed taxonomic units in nestling diet was negatively associated with condition, with the diet of nestlings in the best condition containing half the proportion of fed items than those in the poorest condition (Table 3a; Figure 3a). On the contrary, the diets of nestlings in better condition contained a higher proportion of natural taxonomic units and a slightly (but significantly) lower proportion of brassicas (Table 3a; Figure 3a).

Adults in better condition had a higher proportion of both brassicas and cultivated taxonomic units in their diet (Table 3b; Figure 3b). An increase in the proportion of fed taxonomic units was also associated with a marginally significant increase in adult condition (Table 3b; Figure 3b).

TABLE 3 Results from models examining associations between diet composition and (a) nestling and (b) adult condition

Variable	Statistic	Brassica	Cultivated	Fed	Natural
(a) Nestling condition					
Intercept	β	1.405	1.228	1.164	2.728
	z	8.143	7.076	6.675	16.241
	p	<0.001	<0.001	<0.001	<0.001
Condition	β	**−0.043**	*−0.038*	**−0.057**	**−0.032**
	z	**−2.100**	*−1.797*	**−3.318**	**−2.046**
	p	**0.036**	*0.072*	**<0.001**	**0.041**
(b) Adult condition					
Intercept	β	0.982	0.897	0.878	2.303
	z	5.467	4.978	4.870	13.236
	p	<0.001	<0.001	<0.001	<0.001
Condition	β	**0.030**	**0.030**	*0.025*	0.007
	z	**2.494**	**2.250**	*1.942*	0.547
	p	**0.013**	**0.024**	*0.052*	0.585

Note. Both models were significantly improved by the addition of dietary component as a multivariate linear explanatory variable (nestling: $\text{Difference}_4 = 10.12$, $p = 0.038$; adult: $\text{Difference}_4 = 14.835$, $p = 0.005$). Quadratic terms did not improve the fit of either model (nestling: $\text{Difference}_4 = 7.595$, $p = 0.108$; adult: $\text{Difference}_4 = 6.504$, $p = 0.165$). Terms significant at $p < 0.05$ are highlighted in bold; marginally significant terms ($0.05 < p \leq 0.1$) are italicized.

3.3 | Spatiotemporal variation in turtle dove diet

We found no evidence for differences in diet composition between adult and nestling turtle doves or between sites (Table 4). The proportion of brassica in diet was higher in 2011 than in any other year, whereas the proportion of natural dietary components was lower in 2011 than in either 2012 or 2013 (Table 4; Figure 4a). The proportion of cultivated dietary components was marginally lower in 2011 and 2014 than in 2013 (Table 4; Figure 4a). Only the proportion of brassica taxonomic units in diet showed any intra-annual variation, with the proportion of dietary taxonomic units decreasing throughout the breeding season (Table 4; Figure 4b).

Families differed in the proportion of cultivated species in diet ($F_{8,12} = 3.76$, $p = 0.02$; Appendix 5), but other dietary categories did not differ (Brassica: $F_{8,12} = 1.49$, $p = 0.26$; Fed: $F_{8,12} = 1.18$, $p = 0.38$; Natural: $F_{8,12} = 1.48$, $p = 0.26$).

4 | DISCUSSION

Dietary switching can have complex implications for species and ecosystems. Here, we use, for the first time in an ecological study, universal plant primers (Moorhouse-Gann et al., 2018) targeting the ITS2 region of plants, to characterize and compare the diet of UK columbids. We found a high degree of dietary overlap between all four columbid species, with inclusion of anthropogenic plant species found at bird feeders and/or cultivated within our study region and not previously recorded in UK columbid diet suggesting ongoing dietary change, although as sample sizes were low our findings for nonturtle doves should be considered preliminary. We found dietary associations with body condition in both adult and nestling turtle doves, with a higher proportion of anthropogenically fed taxonomic units associated with better condition in adults, and poorer condition in nestlings.

4.1 | Dietary overlap and composition in UK columbids

The high dietary overlap between all four columbid species suggests shared resources are important, although we also found significant differences in dietary composition. In contrast to the rapidly

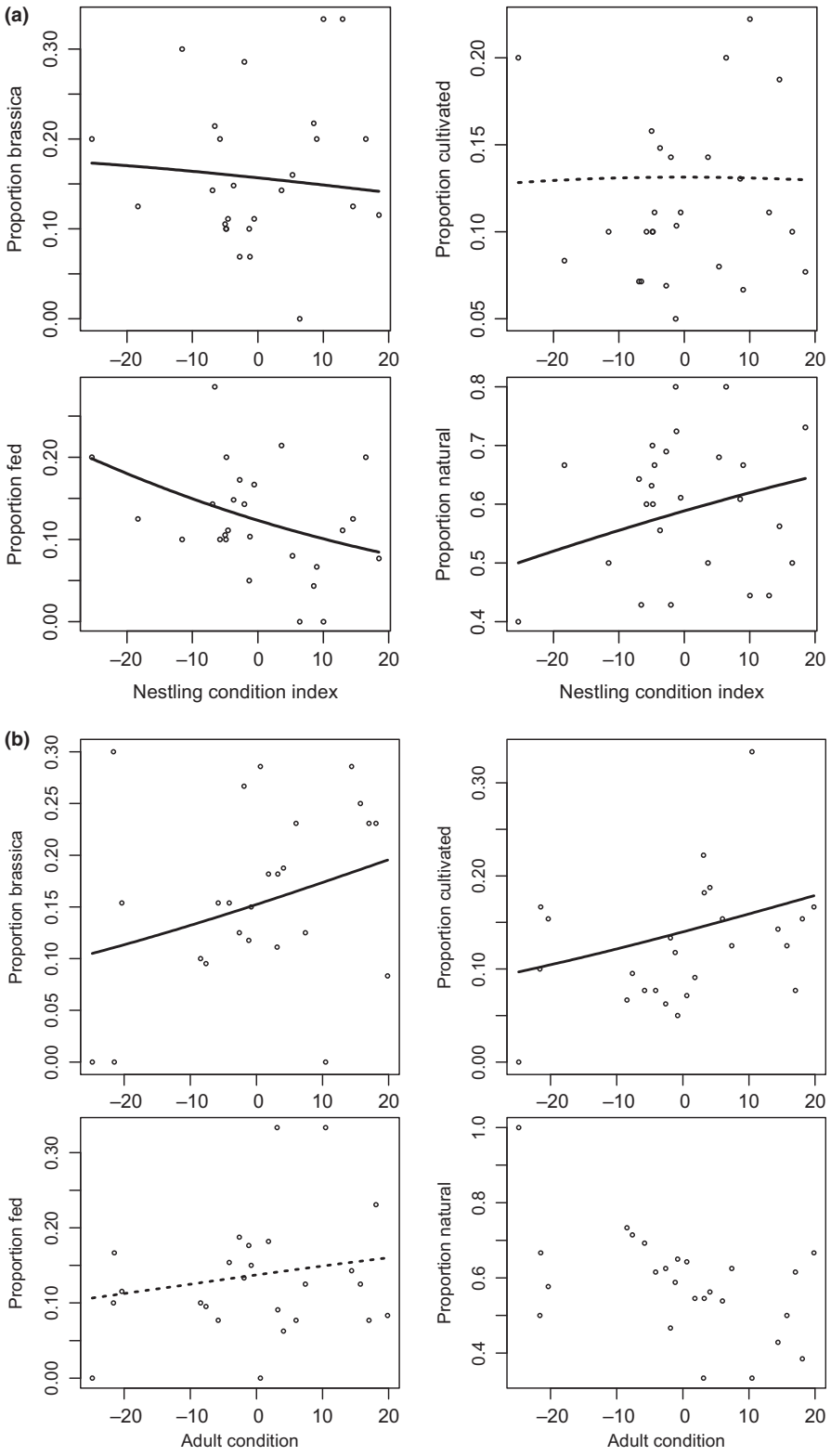

FIGURE 3 Associations between diet composition (in terms of proportion of taxonomic units present) and condition for (a) nestling (n = 26 nests) and (b) adult (n = 26) turtle doves. Nestling condition indices are residuals from a linear regression of mean nestling body mass on mean nestling tarsus length at 7 days old for each nest, and adult condition indices are residuals from a linear regression of body mass on wing length at capture. Solid lines show trends significant at $p < 0.05$; dotted lines show marginally significant trends ($p < 0.1$). Statistical details are provided in the legend to Table 3

declining turtle dove (1970–2014 UK population trend −97%; Hayhow et al., 2017), collared dove, stock dove and woodpigeon populations are all increasing (327%, 116% and 124% population increase, respectively; Hayhow et al., 2017). Turtle doves and stock doves showed the highest dietary overlap, consistent with a previous dietary study suggesting that both are weed seed specialists (Murton et al., 1964). Competition between turtle doves and the recently colonized collared dove has been speculated as contributing to the turtle

TABLE 4 Results of GLMs examining spatiotemporal variation in turtle dove diet

	Brassica			Cultivated			Fed			Natural		
Variable	Dev	df	*p*	Dev	df	*p*	Dev	df	*p*	Dev	df	*p*
Age	−0.04	1, 40	0.394	−0.01	1, 40	0.883	−0.01	1, 40	0.829	−0.02	1, 40	0.586
Year	**−0.71**	**3, 40**	**0.007**	*−0.19*	*3, 40*	*0.054*	−0.03	3, 40	0.924	**−0.83**	**3, 40**	**0.004**
Day	*−0.17*	*1, 40*	*0.054*	−0.03	1, 40	0.269	−0.01	1, 40	0.876	*−0.23*	*1, 40*	*0.057*
Day^2	−0.10	1, 40	0.182	−0.02	1, 40	0.381	−0.06	1, 40	0.305	−0.10	1, 40	0.212
Farm	−0.44	5, 40	0.182	−0.23	5, 40	0.090	−0.02	5, 40	0.997	−0.53	5, 40	0.131

Note. Statistics presented are from comparison of the global model with and without each term (presented as Deviance, degrees of freedom and *p* value). Terms significant at $p < 0.05$ are highlighted in bold; marginally significant terms ($0.05 < p \leq 0.1$) are italicized.

dove population decline (Rocha & Hidalgo De Trucios, 2000), but our data do not support this suggestion as collared doves showed the least overlap with all three other columbid species. Previous dietary studies have shown woodpigeons utilize green vegetation (as opposed to seeds alone; Murton, 1966; Ó hUallachain & Dunne, 2013) and can specialize on Brassicaceae crops when widely available (Inglis, Isaacson, Smith, Haynes, & Thearle, 1997). However, as this study shows relatively high dietary overlap between columbids, it is possible that different species may be feeding on different parts of the same plant species.

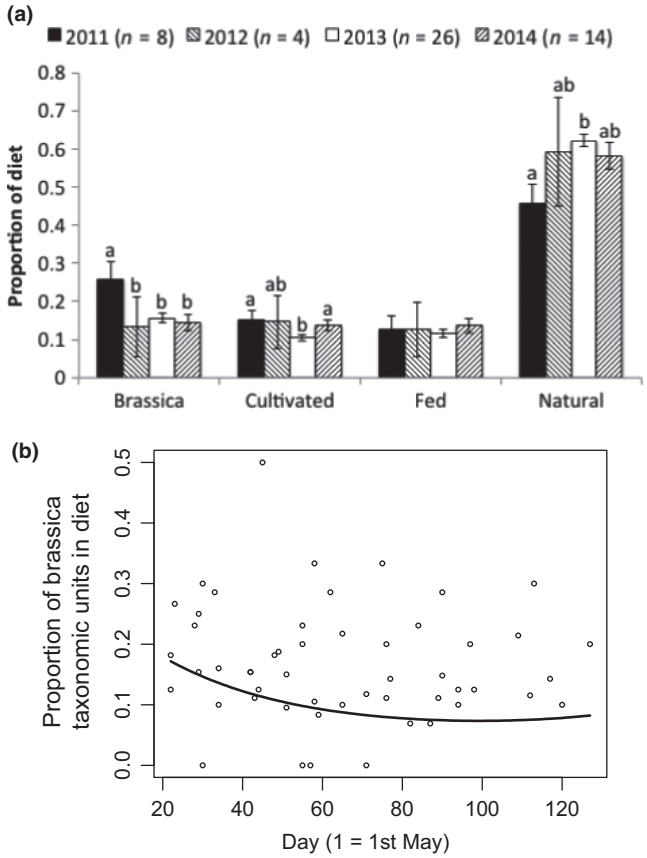

FIGURE 4 (a) Diet composition showed interannual variation and (b) the proportion of brassica in diet varied within year. For (a) bars show mean ± 1 *SE* proportion and differing letters above bars indicate significant differences in dietary composition between sites or years at $p < 0.05$. For (b) points show raw data, and the line is predicted from the model (Table 4) for adult birds in 2013 at Mark's Tey, Essex

The concept of dietary competition relies on the assumption that shared food resources are limiting when in fact, species may be taking advantage of patchy but abundant resources (e.g., Pérez & Bulla, 2000), or using different foraging habitats (e.g., Emrich, Clare, Symondson, Koenig, & Fenton, 2014). Within our system, however, competition for seeds from limited and declining populations (Potts, Ewald, & Aebischer, 2010) of noncultivated plants remains likely (Browne & Aebischer, 2003). Here, it is important to look at diet as a whole, rather than examining the presence of individual taxonomic units or species groups: a single species may be present in a range of foraging situations or habitats, and taking diet as a whole (as we have done with our categorization of dietary components for turtle dove-specific analyses) may provide greater insight into foraging habitats. For example, during the breeding season, wheat or brassica seeds may be provided as a component of bird seed mixes in gardens or through supplementary feeding of songbirds or game birds. Wheat and brassica seeds may also be found as a consequence of grain spillages during harvest or transportation. Wheat and brassica leaves may be taken year-round from growing crops, and, as crops ripen, fallen seeds may be acquired from the ground (or in situ from the standing crop—although turtle doves rarely use this method of foraging). All these sources would result in the same presence of wheat and brassica taxonomic units in faecal samples, but the source would have very different ecological implications in terms of resource availability and dietary competition.

We found a wide range of seeds in columbid diet that is likely to have originated from seed mixes provided for wild birds in gardens or on farmland. Whilst our more sensitive methodology might be able to detect and discriminate between a wider range of species than microscopic methods used by previous studies (Ando et al., 2013; Galimberti et al., 2016), seeds such as niger and hemp have a distinctive husk that should be readily detectable through microscopic analysis of faecal samples. Seed components such as hemp, niger and sorghum have not previously been recorded in turtle dove diet in the UK (Browne & Aebischer, 2003; Cramp & Perrins, 1994; Murton et al., 1964), but our findings concur with an increase in the feeding of birds with seed mixes that include these species, and

anecdotal reports of an increase in this species being seen under bird feeders in gardens. The positive associations between turtle dove condition and the proportion of fed, cultivated and brassica taxonomic units in the diet of adult turtle doves suggest that anthropogenic food makes up for a shortfall in availability of other food resources, especially prior to the onset of breeding (when adult birds were sampled). The addition of wild bird seed mixes to turtle dove diet may have had further consequences, with the possibility of increased exposure to parasites such as *Trichomonas gallinae* (Stockdale et al., 2015), a parasite transmitted at shared food and water resources (Stabler, 1954), linked to feeding on resources commonly shared with other species (Lennon et al., 2013). However, the negative relationship between fed and brassica dietary components and nestling condition, and positive association with natural dietary components, suggests that reproductive success is still reliant upon the availability of natural food resources. Elsewhere, we show that nestlings in better condition have a better chance of survival postfledging (Dunn, Morris, & Grice, 2017).

We found evidence for widespread usage of cultivated crops by columbids, notably borage. Borage is a relatively widespread crop within our study region, cultivated for the high gamma-linolenic acid content of its seeds (Asadi-Samani, Bahmani, & Rafieian-Kopaei, 2014). These high-energy oily seeds may be valuable for breeding birds, as well as providing an open-habitat structure with potentially higher abundance of broad-leaved weeds than more widespread but densely structured graminid crops and oilseed rape. Despite this apparent adoption of additional cultivated crops and components of anthropogenically fed bird seed into the diet of UK columbids, evidence from other systems as well as our finding of a positive association between the diversity of natural taxonomic units in nestling diet and body condition suggests that native seeds may be crucial in ensuring breeding success. For example, Pruitt et al. (2008) found lower fledging success and fledgling weight in white-winged doves (*Zenaida asiatica*) fed only agricultural grains compared to those fed a mixture of agricultural grains and native seeds, concluding that agricultural grains had insufficient protein content to support normal productivity.

The availability of seeds from natural arable plants has declined as a result of changes in farming practice, and their availability to ground-feeding birds is limited, especially early in the breeding season. Agri-environment schemes within farmland do offer options designed to ameliorate this to some extent (Critchley, Allen, Fowbert, Mole, & Gundrey, 2004; Natural England 2015; Walker et al., 2007) but seed-rich habitat created within these schemes is usually aimed at providing forage for wintering birds (Henderson, Vickery, & Carter, 2004) or nectar for pollinating insects (Carvell, Meek, Pywell, Goulson, & Nowakowski, 2007) and often creates too dense a sward to be accessible by foraging doves in the breeding season (Dunn et al., 2015). Despite this reduction in overall abundance of arable weeds (Potts et al., 2010), we found several species present within columbid diet, most notably within turtle and stock doves. Among the annual arable weeds commonly present in the diet of turtle doves (and other columbids), scarlet pimpernel and common chickweed are widespread but declining species on regularly tilled arable land within the UK and across Europe (Andreasen, Stryhn, & Streibig, 1996; Critchley et al., 2004; Fried, Petit, Dessaint, & Reboud, 2009; Sutcliffe & Kay, 2000; Walker et al., 2007). Chickweed was previously one of the most important components of turtle dove diet (>30% of adult diet: Murton et al., 1964; 10% of adult diet: Browne & Aebischer, 2003). Species within the *Geranium* genus, along with goosefoot (*Chenopodium polyspermum* and *C. album*) and thistle species (*Cirsium arvense* and *C. vulgare*) are often associated with disturbed, uncropped land and have increased in abundance in the UK (Potts et al., 2010; Sutcliffe & Kay, 2000): Whilst not previously widely recorded in columbid diet in the UK (Cramp & Perrins, 1994), their widespread availability may have led to their increased exploitation as a food resource. Indeed, *Chenopodium* sp. are a relatively common component of turtle dove diet in Portugal and Spain (e.g., Dias & Fontoura, 1996; Gutiérrez-Galán & Alonso, 2016).

Overall, it appears that all four columbid species use similar foraging habitats although turtle doves have the greatest dietary range (as suggested by the results of our rarefaction analyses) and forage within a wider range of semi-natural habitats than their heterospecifics, but are more constrained by their inability to exploit green matter and in situ seed from tall vegetation. All four species eat anthropogenically fed seed probably sourced from gardens and farmyards: In the same way, high levels of dietary overlap were found in four co-existing columbid species in Venezuela, where Pérez and Bulla (2000) concluded that these closely related doves foraged opportunistically but randomly from the same available seed pool. The same may occur within our system, especially early in the summer before natural seed resources become widely available: We do not know the degree to which dietary overlap is driven by food availability, and our data allow only limited insight into temporal variation in diet.

4.2 | Associations between diet and condition, and spatiotemporal variation in diet

We predicted that the consumption of anthropogenic food resources such as cultivated crops, and food provided for game and songbirds, would be associated with poor condition in both adult and nestling turtle doves, which have evolved to exploit other types of seed. This hypothesis was supported in nestlings by a negative association between the proportion of fed and brassica taxonomic units and body condition, and a positive effect of natural taxonomic units. Contrary to our predictions, adult condition was positively associated with brassica and cultivated taxonomic units; anthropogenically fed taxonomic units showed a marginally significant positive association. Given the higher calorific value of seeds such as hemp and sunflower (Hullar, Meleg, Fekete, & Romvar, 1999), this may be a beneficial side effect of a forced change in foraging ecology resulting from the background decline in availability of alternative, natural, food sources. However, any potential benefits of provisioned seed need to be balanced with potential negative impacts (e.g., increased risk of predation or parasite transmission) where high densities of

birds congregate (Eraud, Jacquet, & Legagneux, 2011; Lennon et al., 2013; Robb, Mcdonald, Chamberlain, & Bearhop, 2008).

We found no evidence for systematic geographic variation in diet. Given the relative landscape-scale homogeneity across our study sites, this is not surprising and adds validity to our examination of dietary overlap at multiple sites within our study area when we were not always able to sample from multiple species at each site. We predicted that diet would show both inter- and intra-annual variation with anthropogenic food resources more important early in the breeding season. We did find that brassica consumption decreased sharply from mid-May to mid-June, possibly reflecting a reduction in availability of oilseed rape tailings at our sites over this time period. We found no evidence for systematic trends in diet composition between years, although interannual differences in diet are likely to represent variability in seed abundance driven by changes in weather patterns. For example, natural seed formed a lower proportion of diet in 2011 compared to other years: 2011 had a very dry spring, and thus, it is possible that brassica (which formed a higher proportion of diet in 2011 compared to other years), likely acquired through tailings, filled a gap in food availability early in 2011.

Samples from adults prebreeding and their chicks, or multiple nests from the same adult, showed a tendency for consistency in the proportion of cultivated food within their diet. This may be a consequence of adults specializing on certain foraging habitat types as adult and nestling samples, as well as samples from consecutive nesting attempts, were temporally separated, although larger sample sizes would be required to test this rigorously.

Our findings of positive associations between a higher proportion of dietary components from natural arable plants and turtle dove nestlings in better condition and a higher proportion of anthropogenically provided seed and adults in better condition are ecologically important. They suggest that habitat management providing additional sources of fed seeds for adults early in the breeding season, coupled with habitat rich in accessible seeds of arable plants (Dunn et al., 2015) once chicks are present, may be crucial to conserving the species.

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DATA ACCESSIBILITY

New sequences generated from this study have been deposited in GenBank under Accession nos KT948614–KT948638 inclusive. Raw MiSeq data for all samples described in this manuscript and additional faecal samples from turtle dove nests have been uploaded to the NCBI Sequence Read Archive under SRA Accession no. SRP136381. Detailed individual-level taxonomic unit presence–absence data are available from the corresponding author upon reasonable request.

AUTHOR CONTRIBUTIONS

This work was part of a wider study led by J.C.D. and overseen by A.J.M. and P.V.G., testing conservation interventions for European turtle doves in UK farmland. R.J.M.-G. helped with primer design and developed methods for analysis of HTS data for the ITS2 region alongside J.C.D. and H.H. J.C.D. led and carried out fieldwork and collected samples alongside field-based research assistants. J.C.D., J.E.S. and A.M. performed molecular analyses and J.C.D. and HH analysed resulting HTS data. J.C.D. performed statistical analyses and wrote the manuscript. W.O.C.S. oversaw the design, implementation and interpretation of molecular analyses and provided valuable guidance throughout. All authors contributed towards revising the manuscript.

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SUPPORTING INFORMATION

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

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APPENDIX 1 Details of sites from which faecal samples were collected, along with the location of nearest town, number of faecal samples collected and number of faecal samples from which DNA was successfully amplified

Site	Nearest town	Latitude	Longitude	Years samples collected	Number of faecal samples (amplified)			
					CD	SD	TD	WP
AH	Great Wigborough, Essex	51°48′10″N	0°50′18″E	2013–2014		9 (7)	13 (13)	
CHU	Aldham, Essex	51°53′47″N	0°46′47″E	2011				1 (0)
FL	Stow Maries, Essex	51°39′9″N	0°38′30″E	2014		1 (1)		
HO[a]	March, Norfolk	52°33′4″N	0°5′17″E	2013			2 (2)	
LI	Tolleshunt D'Arcy, Essex	51°46′19″N	0°47′39″E	2011–2014	1 (1)		12 (12)	7 (3)
LO	Westhorpe, Suffolk	52°17′10″N	0°59′44″E	2011–2012		2 (2)		3 (0)
MA	Witcham, Cambridgeshire	52°23′54″N	0°8′57″E	2011–2013	1 (1)		6 (6)	1 (0)
OP[a]	Ely, Cambridgeshire	52°23′58″N	0°15′43″E	2014			2 (2)	
PG	Silver End, Essex	51°50′50″N	0°37′26″E	2011–2014	5 (5)		17 (16)	7 (2)
SI[a]	Denver, Norfolk	52°35′17″N	0°22′51″E	2011–2014	2 (2)		7 (7)	1 (0)
UH	Mark's Tey, Essex	51°52′34″N	0°45′51″E	2011–2014	1 (1)	3 (3)	18 (18)	2 (0)

Notes. This omits eight sites shown in Figure 1 from which no faecal samples were acquired. Samples were collected in 2011 (n = 18), 2012 (n = 11), 2013 (n = 49) and 2014 (n = 46).
[a]Sites that were combined for statistical analysis due to small sample sizes.

APPENDIX 2 Seeds collected from the field and used to construct the barcode library, along with Order, Family and common name

Species	Order	Family	Common name	Genbank accession nos
Anthriscus sylvestris[+]	Apiales	Apiaceae	Cow parsley	AY548228 and KT948614
Anthemis cotula	Asterales	Asteraceae	Stinking chamomile	EU179216
Carthamus tinctorius[+]	Asterales	Asteraceae	Safflower	JQ230977 and KT948630
Cirsium vulgare	Asterales	Asteraceae	Spear thistle	JX867638
Guizotia abyssinica[+a]	Asterales	Asteraceae	Niger seed	KT948615
Helianthus annuus[+]	Asterales	Asteraceae	Sunflower	JN115024
Helminthotheca echoides	Asterales	Asteraceae	Bristly ox-tongue	AF528491
Senecio vulgaris[+]	Asterales	Asteraceae	Groundsel	EF538396 and KT948631
Brassica napus[+]	Brassicales	Brassicaceae	Oil seed rape	JQ085860 and KT948616
Capsella bursa-pastoris[+]	Brassicales	Brassicaceae	Shepherd's purse	DQ310531 and KT948632
Sinapsis alba	Brassicales	Brassicaceae	Field mustard	FJ609733
Reseda lutea[a]	Brassicales	Resedaceae	Wild mignonette	DQ987096[b]
Cerastium fontanum	Caryophyllales	Caryophyllaceae	Common mouse-ear	GU444015
Silene latifolia subsp. alba	Caryophyllales	Caryophyllaceae	White campion	AY594308
Silene vulgaris	Caryophyllales	Caryophyllaceae	Bladder campion	FN821149
Spergula arvensis	Caryophyllales	Caryophyllaceae	Corn spurrey	JX274532
Stellaria graminea	Caryophyllales	Caryophyllaceae	Lesser stitchwort	AY594304
Stellaria media[+]	Caryophyllales	Caryophyllaceae	Chickweed	JN589063 and KT948633
Chenopodium album[+]	Caryophyllales	Chenopodiaceae	Fat hen	FN561552 and KT948617
Atriplex patula	Caryophyllales	Amaranthaceae	Orache	HM005859[b]
Persicaria maculosa[+]	Caryophyllales	Polygonaceae	Redshank	HQ843137 and KT948635
Polygonum aviculare[+]	Caryophyllales	Polygonaceae	Knotgrass	KJ025070
Rumex obtusifolius[+]	Caryophyllales	Polygonaceae	Broad-leaved dock	GQ340059[b]
Anagallis arvensis[+]	Ericales	Primulaceae	Scarlet pimpernel	AY855135 and KT948628
Lotus corniculatus[+]	Fabales	Fabaceae	Birds-foot trefoil	DQ312207 and KT948621
Medicago lupulina[+]	Fabales	Fabaceae	Black medick	DQ311980

(Continues)

APPENDIX 2 (Continued)

Species	Order	Family	Common name	Genbank accession nos
Trifolium pratense[+]	Fabales	Fabaceae	Red clover	AF053171 and KT948619
Trifolium repens[+]	Fabales	Fabaceae	White clover	DQ311962 and KT948620
Vicia sativa[+]	Fabales	Fabaceae	Common vetch	KJ787165
Galium aparine[+]	Gentianales	Rubiaceae	Goosegrass	DQ006036
Geranium dissectum[+]	Geraniales	Geraniaceae	Cut-leaved cranesbill	AY944413 and KT948622
Veronica persica[+]	Lamiales	Plantaginaceae	Common field speedwell	AF313001 and KT948624
Kickxia spuria	Lamiales	Scrophulariaceae	Round-leaf fluellen	AF513880
Euphorbia esula	Malpighiales	Euphorbiaceae	Green spurge	JN010042
Viola arvensis[+]	Malpighiales	Violaceae	Field pansy	DQ005347 and KT948636
Viola tricolor	Malpighiales	Violaceae	Heartsease	DQ055406
Alopecurus myosuroides[+a]	Poales	Poaceae	Black grass	KT948627
Festuca pratensis	Poales	Poaceae	Meadow fescue	KJ598995
Hordeum vulgare[+]	Poales	Poaceae	Barley	KM217265 and KT948626
Panicum miliaceum[+]	Poales	Poaceae	Millet	KT948629 and JX576677
Poa annua[+]	Poales	Poaceae	Meadow grass	KJ599003 and KT948634
Poa trivialis	Poales	Poaceae	Rough meadow-grass	KJ598983
Sorghum bicolor[+]	Poales	Poaceae	White sorghum	GQ856358
Triticum aestivum[+]	Poales	Poaceae	Wheat	KF482086 and KT948625
Zea mays[+]	Poales	Poaceae	Maize	DQ683016[b]
Fumaria officinalis[+]	Ranunculales	Papaveraceae	Common fumitory	HE603306 and KT948623
Papaver rhoeas	Ranunculales	Papaveraceae	Poppy	DQ912886
Ranunculus repens	Ranunculales	Ranunculaceae	Creeping buttercup	JN115047[b]
Urtica dioica	Rosales	Urticaceae	Common nettle	KF454275 and KF137936
Convolvulus arvensis[+]	Solanales	Convolvulaceae	Field bindweed	AY558826

Notes. This table is also found in Moorhouse-Gann et al. (2018) and was used in primer design. Accession noss beginning KT9486 are those uploaded from this study, and the rest were downloaded from GenBank. All species were either known from previous studies of turtle dove diet (Browne & Aebischer, 2003; Murton et al., 1964) or common at our field sites or in supplementary or planted seed mixes (e.g. Dunn et al., 2015). Where multiple Accession nos are provided, these sequences were stitched together to cover the entire ITS2 and primer binding regions.

[+]denotes species for which we extracted DNA from field-collected specimens.

[a]Sequence does not or only partially overlaps forward primer region. [b]Sequence does not or only partially overlaps reverse primer region.

APPENDIX 3 Details of sequences found in negative controls showing the number of negative samples within which the sequence was found (negative samples), the cut-off threshold used for each sequence, the number of samples in which the sequence was found (number of samples) and the number of samples for which the sequence had a read number below the threshold and was removed (sequence removed)

Sequence number	Taxonomic unit	Negative samples	Cut-off threshold (read number)	Number of samples	Sequence removed
1	*Borago officinalis*	1	1,919	58	37
2	*Borago officinalis*	1	150	2	0
3	*Brassica oleracea*	2	158	20	0
4	*Cirsium arvense*	2	150	1	0
5	*Dactylis glomerata*	15	318	100	7
6	*Poa trivialis*	2	162	1	0
7	*Viola arvensis*	2	153	26	0
8	*Agrostis* sp.	2	162	3	0
9	*Alopecurus myosuroides*	4	155	3	0
10	*Anagallis arvensis*	12	247	38	0
11	*Anthriscus* sp.	4	152	8	0
12	*Borago officinalis*	2	154	21	0

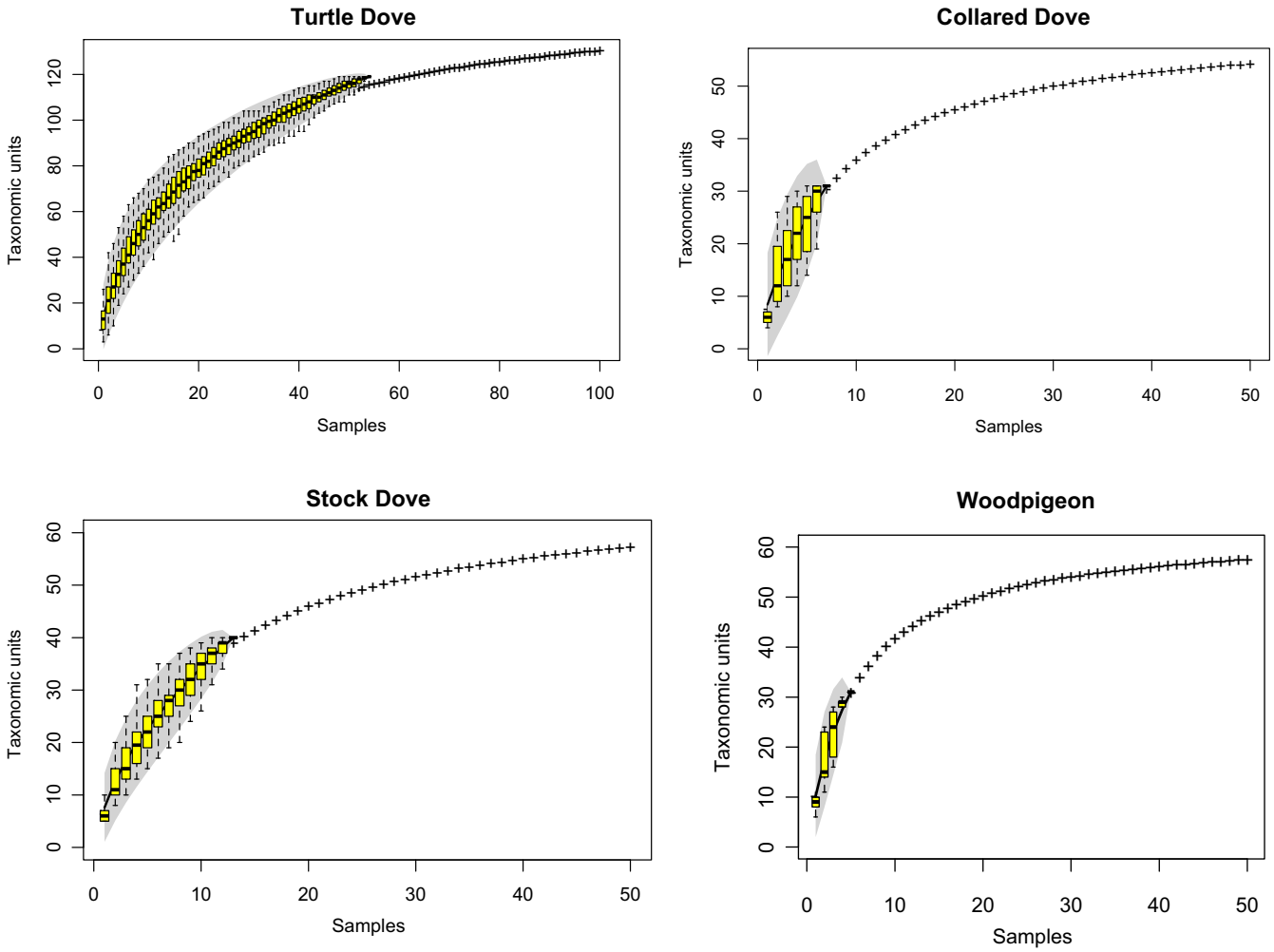
(Continues)

APPENDIX 3 (Continued)

Sequence number	Taxonomic unit	Negative samples	Cut-off threshold (read number)	Number of samples	Sequence removed
13	*Borago officinalis*	16	336	108	6
14	*Borago officinalis*	1	149	14	0
15	*Borago officinalis*	1	1,914	**3**	**3**
16	*Brassica* sp.	1	149	**0**	**0**
17	*Brassica* sp.	8	166	66	0
18	*Brassica* sp.	2	414	13	6
19	*Brassica* sp.	3	156	1	0
20	*Cucumis* sp.	3	150	3	0
21	*Guizotia abyssinica*	2	155	1	0
22	*Panicum miliaceum*	16	334	93	13
23	*Panicum miliaceum*	5	166	5	0
24	*Rubus* sp.	17	1,108	117	66
25	*Salicornia* sp.	1	606	**0**	**0**
26	*Stellaria media*	2	165	1	0
27	*Suaeda maritima*	1	152	**0**	**0**
28	Primulaceae	1	280	**0**	**0**
29	Brassicaceae	1	1,227	52	36
30	Poaceae	5	200	11	0

Note. Bold highlights sequences not remaining in any samples following removal of contaminant levels of the sequence (n = 5 sequences).

APPENDIX 4 Predicted species accumulation curves for each columbid species based on the accumulation of taxonomic units. Predicted points, denoted by "+," are overlaid by confidence intervals (grey shading) and barplots from raw data based on 100 permutations of adding samples in a random order.



APPENDIX 5 Boxplot showing differences in the proportion of cultivated components between families. Boxplots show range (whiskers), interquartile range (box) and median (thick line).