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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-Abrán, 2013), influencing migratory decisions (Flack et al., 2016; Plummer, Sirivardena, 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, 2012), and survival (Bakaloudis, Vlachos, Chatzinikos, Bontzorlos, & Papakosta, 2009; Dias et al., 2013). Turtle doves and stock doves feed only on seeds (Browne & Aebischer, 2003; Morton, Westwood, & Isacson, 1964), whereas other columbids will also take leaves and other plant matter (Morton 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 (Morton et al., 1964) to mainly cultivated food plants 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, Sonny, Dewitte, & Murray, 2015) and confirmed in vitro that our new primers (UniplantF [5’-TGTGAATTGCATRATCYMG-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 H2O, 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 (Magoc & 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.full-length” 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.full-length” 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. 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:

\[ O_{jk} = \frac{\sum p_i p_{ik}}{\sqrt{\sum p_i^2 \sum p_{ik}^2}}. \]

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 DISTRIBETREG 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
| Taxonomic unit         | Family            | Category | TD (n = 54) | CD (n = 7) | SD (n = 13) | WP (n = 5) | F     | p     |
|-----------------------|-------------------|----------|-------------|------------|-------------|------------|-------|-------|
| Sambucus nigra        | Adoxaceae         | Natural  | 1.9         | 0          | 0           | 0          |       |       |
| Amaranthus sp.        | Amaranthaceae      | Natural  | 5.6         | 0          | 0           | 0          |       |       |
| 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          |       |       |
| 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          |       |       |
| Salsola sp.           | Amaranthaceae      | Natural  | 11.1        | 0          | 0           | 0          |       |       |
| Suaeda maritima       | Amaranthaceae      | Natural  | 1.9         | 14.3       | 0           | 0          | 1.21  | 0.312 |
| Suaeda sp.            | Amaranthaceae      | Natural  | 1.9         | 0          | 0           | 0          |       |       |
| 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          |       |       |
| Apiaceae              | Apiaceae          | Natural  | 1.9         | 0          | 0           | 0          |       |       |
| Pastinaca sativa      | Apiaceae          | Cultivated| 3.7       | 0          | 7.7         | 0          | 0.504 | 0.681 |
| Achillea millefolium  | Asteraceae        | Natural  | 1.9         | 0          | 0           | 0          |       |       |
| Anthemis cotula       | Asteraceae        | Natural  | 1.9         | 0          | 0           | 0          |       |       |
| Artemisia vulgaris    | Asteraceae        | Natural  | 3.7         | 0          | 0           | 0          |       |       |
| Artemisia vulgaris    | Asteraceae        | Natural  | 3.7         | 0          | 0           | 0          |       |       |
| Cirsium arvense       | Asteraceae        | Natural  | 16.7        | 0          | 0           | 40.0       | 3.549 | 0.018 |
| Cirsium velutum       | Asteraceae        | Natural  | 1.9         | 0          | 0           | 0          |       |       |
| Cirsium vulgare       | Asteraceae        | Natural  | 5.6         | 0          | 7.7         | 0          | 0.536 | 0.659 |
| Guizotia abyssinica   | Asteraceae        | Fed      | 35.2        | 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          |       |       |
| Helminthotheca echoides| Asteraceae       | Natural  | 3.7         | 0          | 0           | 0          |       |       |
| Jacobaea vulgaris     | Asteraceae        | Natural  | 1.9         | 0          | 0           | 0          |       |       |
| Lactuca serriola      | Asteraceae        | Natural  | 0           | 0          | 20.0        |           |       |       |
| Lapsana communis      | Asteraceae        | Natural  | 0           | 0          | 7.7         | 0          |       |       |
| Senecio vulgaris      | Asteraceae        | Natural  | 1.9         | 0          | 0           | 0          |       |       |
| Sonchus arvensis      | Asteraceae        | Natural  | 1.9         | 0          | 0           | 0          |       |       |
| Tripleurospermum inodorum| Asteraceae    | Natural  | 0           | 0          | 0           | 20.0       |       |       |
| Tripleurospermum maritimum| Asteraceae    | Natural  | 1.9         | 0          | 0           | 0          |       |       |
| Tussilago farfara     | Asteraceae        | Natural  | 1.9         | 0          | 0           | 0          |       |       |
| Corylus avellana      | Betulaceae        | Natural  | 0           | 14.3       | 0           | 0          |       |       |
| Boraginaceae          | Boraginaceae      | Natural  | 5.6         | 0          | 0           | 0          |       |       |
| 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          |      |      |
| Symphytum sp.        | Boraginaceae      | Natural  | 25.9        | 71.4       | 7.7         | 0          | 4.109| 0.009|
| Brassica carinata    | Brassicaceae      | Brassica | 1.9         | 0          | 0           | 0          |      |      |
| Brassica juncea      | Brassicaceae      | Brassica | 13.0        | 0          | 0           | 0          |      |      |
| 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          |      |      |
| 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          |      |      |
| Raphanus sativus     | Brassicaceae      | Cultivated| 3.7        | 0          | 0           | 0          |      |      |
| Rorippa sylvestris   | Brassicaceae      | Brassica | 1.9         | 0          | 0           | 0          |      |      |
| 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          |      |      |
| Cerastium glomeratum | Caryophyllaceae   | Natural  | 18.5        | 0          | 0           | 0          |      |      |
| Stellaria pallica    | Caryophyllaceae   | Natural  | 1.9         | 0          | 0           | 0          |      |      |
| Stellaria media      | Caryophyllaceae   | Natural  | 25.9        | 0          | 7.7         | 20.0       | 1.998| 0.122|
| Stellaria neglecta   | Caryophyllaceae   | Natural  | 1.9         | 0          | 0           | 0          |      |      |
| Calystegia sepium    | Convolvulaceae    | Natural  | 5.6         | 14.3       | 0           | 20.0       | 1.272| 0.29 |
| Crassulaceae         | Crassulaceae      | Natural  | 3.7         | 0          | 0           | 0          |      |      |
| Cucumis sp.          | Cucurbitaceae     | Cultivated| 3.7        | 0          | 0           | 20.0       | 1.44 | 0.238|
| Cucurbitaceae        | Cucurbitaceae     | Cultivated| 3.7        | 0          | 0           | 0          |      |      |
| Chamaecyparis lawsoniana | Cupressaceae    | Natural  | 1.9         | 0          | 0           | 0          |      |      |
| Euphorbiaceae        | Euphorbiaceae     | Natural  | 1.9         | 0          | 0           | 0          |      |      |
| Pismum sativum       | Fabaceae          | Cultivated| 1.9        | 0          | 0           | 0          |      |      |
| Vicia hirsuta        | Fabaceae          | Cultivated| 3.7        | 0          | 0           | 0          |      |      |
| Vicia sativa         | Fabaceae          | Cultivated| 1.9        | 0          | 7.7         | 0          | 0.613| 0.608|
| Quercus sp.          | Fagaceae          | Natural  | 0           | 0          | 7.7         | 0          |      |      |
| Geraniaceae          | Geraniaceae       | Natural  | 5.6         | 0          | 0           | 0          |      |      |
| 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          |      |      |
| Geranium pusillum    | Geraniaceae       | Natural  | 7.4         | 0          | 0           | 0          |      |      |
| Linum sp.            | Linaceae          | Cultivated| 3.7        | 0          | 0           | 0          |      |      |
| Epilobium sp.        | Onagraceae        | Natural  | 3.7         | 0          | 0           | 0          |      |      |
| Papaver rhoes         | Papaveraceae      | Natural  | 1.9         | 0          | 0           | 0          |      |      |
| Pinus sp.            | Pinaceae          | Natural  | 1.9         | 0          | 0           | 0          |      |      |
| Plantago lanceolata   | Plantaginaceae    | Natural  | 5.6         | 14.3       | 0           | 40.0       | 0.536| 0.659|
| Agrostis sp.         | Poaceae           | Natural  | 7.4         | 0          | 14.3        | 0          | 2.387| 0.076|
| Agrostis stolonifera | Poaceae           | Natural  | 3.7         | 0          | 0           | 0          |      |      |
| Alopecurus myosuroides| Poaceae           | Natural  | 5.6         | 0          | 0           | 0          |      |      |
| Alopecurus sp.       | Poaceae           | Natural  | 1.9         | 0          | 0           | 0          |      |      |
| Arrhenatherum elatius| Poaceae           | Natural  | 1.9         | 0          | 0           | 0          |      |      |
| Avena sp.            | Poaceae           | Natural  | 3.7         | 0          | 0           | 0          |      |      |
| Cenchrus americanus  | Poaceae           | Fed      | 1.9         | 0          | 0           | 0          |      |      |
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

### 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   |        |
| *Triticaceae*           | Poaceae      | Cultivated | 11.1  | 0          | 0           | 20.0       | 1.954| 0.128  |
| *Triticum aestivum*     | Poaceae      | Cultivated | 7.4   | 0          | 15.4        | 0          | 1.039| 0.38   |
| *Triticum sp.*          | Poaceae      | Cultivated | 7.4   | 0          | 15.4        | 0          | 1.399| 0.38   |
| *Persicaria lapathifolia* | Polygonaceae* | 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*  | 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       | 1.694| 0.328  |
| *Galium aparine*        | Rubiaceae*   | Natural  | 3.7         | 0          | 0           | 0          | b   |        |
| *Citrus sp.*            | Rutaceae*    | Cultivated | 1.9   | 0          | 0           | 0          | b   |        |
| *Acer campestre*        | Sapindaceae* | Natural  | 1.9         | 0          | 0           | 0          | b   |        |
| *Urtica dioica*         | Urticaceae   | Natural  | 33.3        | 14.3       | 15.4        | 0          | 1.947| 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.

*Denotes a family found exclusively in turtle dove diet. Differences not tested statistically as the plant family was only found within one columbid species or in fewer than three individuals.
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 nestling 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 non-independent 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 nestling 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
over 50% of taxonomic units for all species, with estimated numbers of 70–85 and 110 taxonomic units.

Rosaceae (6), Rubiaceae (1), Rutaceae (1), Sapindaceae (1), Urticaceae (1) and Violaceae (2) families and (b) shows taxonomic units within the Adoxaceae (1), Papaveraceae (1), Pinaceae (1), Plantaginaceae (1), Poaceae (27), Polygonaceae (1), Primulaceae (3), Ranunculaceae (1), Rhamnaceae (1), Crassulaceae (1), Cucurbitaceae (2), Cupressaceae (1), Euphorbiaceae (1), Fabaceae (3), Fagaceae (1), Geraniaceae (5), Linaceae (1), Onagraceae (4), Brassicaceae (11), Cannabaceae (1), Caryophyllaceae (5) and Convolvulaceae (1) families, and (b) shows taxonomic units within the Adoxaceae (1), Primulaceae were found in 60% of turtle dove diets and also in stock dove diets (Table 1; Figure 2b). Primulaceae were found in 60% of turtle dove diets and also in stock dove diets (Table 1; Figure 2a). Primulaceae were found in 60% of turtle dove diets and also in stock dove diets (Table 1; Figure 2b). Primulaceae were found in 60% of turtle dove diets and also in stock dove diets (Table 1; Figure 2a).}

**Figure 2** Bileaflet food webs showing dietary overlap between turtle doves, collared doves, stock doves and woodpeckers. In each web, rarefaction analysis on all samples suggests that we detected over 50% of taxonomic units for all species, with estimated numbers of 70–85 and 110 taxonomic units, with seedlings and flowers of Anagallis arvensis. Present in 82% of turtle dove diets, Anagallis arvensis was found in 61% of stock dove diets and in 79% of woodpigeon diets. Anagallis arvensis was found in 70% of turtle dove diets, 83% of collared dove diets and 77% of stock dove diets. In each web, rarefaction analysis on all samples suggests that we detected over 50% of taxonomic units for all species, with estimated numbers of 70–85 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    | 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 | 0.799 | 0.904 | 0.848 | 0.773 |
| Collared dove  | 0.799       |               |            |            |
| Stock dove     | 0.904       |               |            |            |
| Woodpigeon     | 0.848       |               |            |            |

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 $\beta$ | 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 $\beta$ | −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 $\beta$ | 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 $\beta$ | 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: Difference$_F = 10.12$, $p = 0.038$; adult: Difference$_F = 14.835$, $p = 0.005$). Quadratic terms did not improve the fit of either model (nestling: Difference$_F = 7.595$, $p = 0.108$; adult: Difference$_F = 6.504$, $p = 0.165$). 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
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

**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.
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.

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

### TABLE 4

Results of GLMs examining spatiotemporal variation in turtle dove diet

| Variable | Brassica Dev df | p | Cultivated Dev df | p | Fed Dev df | p | Natural 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 |   |
| Day²     | −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 ≤ 0.1) are italicized.

![FIGURE 4](image-url)  

**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.
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 (Crichtley, 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 columbids 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; Crichtley 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 columbids 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 columbids use similar foraging habitats although turtle doves have the greatest dietary range (as suggested by the results of our rarefraction 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 columbids 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,* & Romvari, 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 examina-
tion 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 propor-
tion of dietary components from natural arable plants and turtle
dove nestlings in better condition and a higher proportion of anthropo-
genically provided seed and adults in better condition are ecologi-
cally important. They suggest that habitat management providing
additional sources of fed seeds for adults early in the breeding sea-
son, coupled with habitat rich in accessible seeds of arable plants
(Dunn et al., 2015) once chicks are present, may be crucial to con-
serving 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 addi-
tional 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–ab-
sence data are available from the corresponding author upon reason-
able 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, implemen-
tation and interpretation of molecular analyses and provided valuable
guidance throughout. All authors contributed towards revising the
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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) |
|------|-------------------------------|------------|-------------|-------------------------|---------------------------------------|
|      |                               |            |             |                         | Total samples (amp)                   |
| AH   | Great Wigborough, Essex       | 51°48'10″N | 0°50'18″E   | 2013–2014               | CD 9 (7) SD 13 (13) WP 1 (0)          |
| CHU  | Aldham, Essex                 | 51°53'47″N | 0°46'47″E   | 2011                    | CD 1 (1) WP 1 (0)                     |
| FL   | Stow Maries, Essex            | 51°39'9″N  | 0°38'30″E   | 2014                    | CD 1 (1) WP 1 (0)                     |
| HO   | March, Norfolk                | 52°33'4″N  | 0°5'17″E    | 2013                    | CD 2 (2)                              |
| UJ   | Tolleshant D'Arcy, Essex      | 51°46'19″N | 0°47'39″E   | 2011–2014               | CD 1 (1) SD 12 (12) TD 7 (3)         |
| LO   | Westhorpe, Suffolk            | 52°17'10″N | 0°59'44″E   | 2011–2012               | CD 2 (2) WP 3 (0)                     |
| MA   | Witcham, Cambridgeshire       | 52°23'54″N | 0°8'57″E    | 2011–2013               | CD 1 (1) SD 6 (6) WP 1 (0)           |
| OP   | Ely, Cambridgeshire           | 52°23'58″N | 0°15'43″E   | 2014                    | CD 2 (2) WP 7 (7)                     |
| SI   | Denver, Norfolk               | 52°35'17″N | 0°22'51″E   | 2011–2014               | CD 2 (2) WP 7 (7)                     |
| UH   | Mark's Tey, Essex             | 51°52'34″N | 0°45'51″E   | 2011–2014               | CD 1 (1) SD 3 (3) TD 18 (18) WP 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).

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+a     | Apiales      | Apiaceae      | Cow parsley       | AY548228 and KT948614 |
| Anthemis cotula             | Asterales    | Asteraceae    | Stinking chamomile| EU179216              |
| Carthamus tinctorius+a      | Asterales    | Asteraceae    | Safflower         | JQ230977 and KT948630 |
| Cirsium vulgar               | Asterales    | Asteraceae    | Spear thistle     | JX867638              |
| Guizotia abyssinica+a       | Asterales    | Asteraceae    | Niger seed        | KT948615              |
| Helianthus annuus+a         | Asterales    | Asteraceae    | Sunflower         | JN115024              |
| Helminthotheca echotheca    | Asterales    | Asteraceae    | Bristly ox-tongue | AF528491              |
| Senecio vulgaris+a          | Asterales    | Asteraceae    | Groundsel         | EF538396 and KT948631 |
| Brassica napus+a            | Brassicales  | Brassicaceae  | Oil seed rape     | JQ085860 and KT948616 |
| Capsella bursa-pastoris+a   | Brassicales  | Brassicaceae  | Shepherd’s purse  | DQ310531 and KT948632 |
| Sinapis alba                | Brassicales  | Brassicaceae  | Field mustard     | FJ609733              |
| Reseda lutea+a              | Brassicales  | Resedaceae    | Wild mignonette   | DQ987096a             |
| 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+a           | Caryophyllales| Caryophyllaceae| Chickweed         | JNS89063 and KT948633 |
| Chenopodium album+a         | Caryophyllales| Chenopodiaceae| Fat hen           | FN561552 and KT948617 |
| Atriplex patula             | Caryophyllales| Amaranthaceae | Orache            | HM0058589b            |
| Persicaria maculosa+a       | Caryophyllales| Polygonaceae  | Redshank          | HQ843137 and KT948635 |
| Polygonum aviculare+a       | Caryophyllales| Polygonaceae  | Knotgrass         | KJ025070              |
| Rumex obtusifolius+a        | Caryophyllales| Polygonaceae  | Broad-leaved dock | QQ340059b             |
| Anagallis arvensis+a        | Ericales     | Primulaceae   | Scarlet pimpernel | AY855135 and KT948628 |
| Lotus corniculatus+a        | Fabales      | Fabaceae      | Birds-foot trefoil| DQ312207 and KT948621 |
| Medicago lupulina+a         | 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                    |
| *Gallium 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* | 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 JX576777       |
| *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                    |
| *Fumaria officinalis*    | Ranunculales | Papaveraceae | Common fumitory   | HE603306 and KT948623       |
| *Papaver rhoeas*         | Ranunculales | Papaveraceae | Poppy             | DQ912886                    |
| *Rununculus repens*      | Ranunculales | Ranunculaceae | Creeping buttercup | JN115047b                  |
| *Urtica dioica*          | Rosales     | Urticaceae   | Common nettle     | KF454275 and LF137936       |
| *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 nos 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.

Sequence does not or only partially overlaps forward primer region. lbSequence 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.      | 2                | 149                             | 0                 | 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              | *Suada 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).