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1 The role of cultivated vs. wild seeds in the diet of European turtle
2 doves (*Streptopelia turtur*) across European breeding and African
3 wintering grounds

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7 Running title

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46 bioinformatic pipeline); AM (acquisition of data - high throughout sequencing)

47 iii) **Writing of the manuscript:** REY

48

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53 Data will be made available via Dryad repository following acceptance of the manuscript.

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55 We confirm that no authors have any conflicts of interest to disclose.

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65

66

67 Abstract

68 Agricultural intensification is a major driver in species declines, with changes in land use
69 resulting in widespread alteration of resource availability. An increase in anthropogenic food
70 resources, alongside decreasing natural resources, has resulted in species undergoing dietary
71 changes that can have important ecological consequences, particularly for declining species.
72 Here we use high throughput sequencing to analyse the diet of the migrant European turtle
73 dove (*Streptopelia turtur*), a species that has experienced significant population decline
74 throughout its European range. We analyse the diet of this species on both breeding and
75 wintering grounds to gain an understanding of resource use throughout the annual cycle, and
76 compare areas of more and less intensive agriculture in western and eastern Europe,
77 respectively. We examine associations with body condition, spatiotemporal variation and the
78 source of food (wild or cultivated). We identified 121 taxonomic units in the diet, with
79 significant variation across sampling seasons, and very little overlap between the breeding and
80 wintering seasons, as well as high levels of cultivated food resources in the diet of turtle doves
81 in both breeding and wintering grounds, with the highest proportion of wild seeds in the diet
82 occurring in birds caught in Hungary, where agricultural intensity was lowest. We detected no
83 association between body condition and the consumption of cultivated food resources. We
84 demonstrate the importance of wild resources in birds on the wintering grounds as they
85 approach migration, where body condition increased as the season progressed, concurrent with
86 an increased consumption of wild seeds. These findings indicate the importance of habitats rich
87 in wild seeds and the need to consider food availability on the wintering grounds, as well as
88 the breeding grounds in turtle dove conservation strategies.

89

90 1 Introduction

91 Diet is a central aspect of an animal's ecology, with an appropriate diet key to ensuring they
92 receive the required nutrients to maintain a healthy body condition for survival, breeding and
93 migration (Martin 1987; Newton 2004a). However, there are increasing instances of species
94 changing diet and consuming novel food resources, driven by two anthropogenic influences:
95 an increased availability of novel food resources, or a decrease in natural food resource
96 availability (Dunn *et al.* 2018). Some common factors resulting in an increase in novel foods
97 are supplementary feeding, human food waste and an increase in farmed crops (Browne and
98 Aebischer 2003a; Newsome and Van Eeden 2017; Robb, McDonald, Chamberlain and
99 Bearhop 2008). Farmed crops not only increase the availability of novel foods in the

100 environment, but may also, depending on farming practices, remove many of the natural food
101 resources from the environment. The removal of natural flora and invertebrates heavily impacts
102 farmland biodiversity, affecting species feeding on a range of organisms and resulting in
103 widespread species declines (Benton, Vickery and Wilson 2003; Donald, Green and Heath
104 2001; Potts, 1991).

105 Dietary switches do not necessarily have a detrimental effect. Across the world, species such
106 as Red Foxes (*Vulpes vulpes*) and Black-headed Gulls (*Chroicocephalus ridibundus*) thrive in
107 urban environments (Contesse, Hegglin, Gloor, Bontadina and Deplazes 2004; Scott, Duncan
108 and Green 2015), thanks to an abundance of calorifically rich food (McCleery, Moorman and
109 Peterson 2014). However, species' declines are commonly associated with dietary switches
110 and nutritional stress, as alternative food resources are often of poorer quality than optimal
111 natural resources (Moorhouse-Gann, Kean, Parry, Valladares, Chadwick 2020; Will *et al.*
112 2015), potentially resulting in diminished survival rate and reproductive success (Litzow, Piatt,
113 Prichard and Roby 2002; Wanless, Harris, Redman and Speakman 2005).

114 Agricultural intensification is a leading cause of dietary shifts, with changing farming practices
115 dramatically increasing crop yields, but depleting natural food resources (Chamberlain, Fuller,
116 Bunce, Duckworth and Shrubbs 2000). For example, in the UK, removal of traditional
117 hedgerows and weedy habitats in favour of cultivated crops, extensive use of herbicides
118 reducing wild plant cover and seed abundance, and a shift to autumn-sown cereals decreasing
119 the fallow period between seasons all contribute to a depletion in food resources (Browne,
120 Aebischer and Crick 2005; Gillings, Newson, Nobel and Vickery 2005; Marshall *et al.* 2003).
121 Intensity of agriculture varies geographically, for example between western and eastern
122 European countries, with a greater extent of traditional, less intense farming methods used in
123 eastern Europe (Donald *et al.* 2001).

124 The European turtle dove *Streptopelia turtur* (hereafter turtle dove), has experienced long-term
125 population declines across its European breeding range (Burns *et al.* 2020), with a decline of
126 33% since 1998 leading to the species being classified as 'Vulnerable' to extinction (BirdLife
127 International. 2019; Lormée *et al.* 2020). Various studies in the UK have shown that, in that
128 country, the main cause of these declines is agricultural intensification, which has led to shifts
129 in diet, fewer nesting attempts and lower annual productivity (Browne and Aebischer 2003a;
130 Browne and Aebischer 2004), but agricultural intensification has occurred on both breeding and
131 wintering grounds (Binswanger-Mkhize and Savastano 2017; Brink and Eva, 2009; Browne,

132 Aebischer, Yfantis and Marchant 2004; Chamberlain *et al.* 2000; Zwarts, Bijlsma and van der
133 Kamp 2018). As the only obligate granivorous Afro-Palaearctic migrant in Europe, the turtle
134 dove is ecologically unique and is particularly vulnerable, due to a lack of mitigation for
135 depleted natural food resources, with most agri-environment schemes targeting invertebrate
136 food. Being a migratory species, female turtle doves require a good food source at a very
137 specific time, early in the breeding season, to rapidly increase body condition after migration
138 in preparation for egg-laying; however, most agri-environment schemes focus on providing
139 habitat for invertebrates rather than early-seeding plants (Robinson and Sutherland 1999).
140 Other granivorous species are similarly experiencing severe declines. In Africa, dramatic
141 declines have been observed in seed-eating species, including the loss of the three most
142 abundant granivorous species, the Sudan golden sparrow (*Passer luteus*), the black-crowned
143 (*Eremopterix nigriceps*) and chestnut-backed (*Eremopterix leucotis*) sparrow larks from parts
144 of North West Senegal (Zwarts *et al.* 2018). Furthermore, Zwarts *et al.* (2018) found an average
145 decline of 80% in seed-eating resident birds between the early 1960s and 2014/2015, estimating
146 a loss of 24 million African granivorous birds. An even larger relative decline was observed in
147 the European turtle dove, attributed to a combination of adverse effects on wintering and
148 breeding grounds (Zwarts *et al.* 2009; Zwarts *et al.* 2018).

149 There has been a shift in the diet of turtle doves in the UK since the 1960s. The prevalence of
150 seeds of non-cultivated arable plants declined from 95% in the 1960s (Murton, Westwood, and
151 Isaacson 1964), to just 40% in the 2000s (Brown and Aebischer 2003), replaced by the seeds
152 of cultivated crops, principally wheat (*Triticum* sp.) and rape (*Brassica*). Furthermore, seeds
153 such as niger (*Guizotia abyssinica*), sorghum (*Sorghum bicolor*) and hemp (*Cannabis sativa*),
154 common components in garden bird food, have only recently become prevalent in turtle doves'
155 diet in the UK (Dunn *et al.* 2018).

156 In the UK, a higher proportion of cultivated seeds in adult turtle doves' diet was associated
157 with better body condition, but the opposite was true for nestlings, which were in better
158 condition when fed a higher proportion of wild seed species (Dunn *et al.* 2018), when they
159 were subsequently more likely to survive 30 days post-fledging (Dunn, Morris and Grice 2017).
160 Together, these findings indicate that, whilst anthropogenic food resources may benefit adult
161 birds early in the breeding season in food-limited areas like the UK, the availability of wild
162 food resources is important in increasing the likelihood of successfully fledging chicks. It
163 would also be important to assess whether these patterns are maintained in other areas. The diet
164 of turtle doves on the wintering grounds also consists of a mixture of wild and cultivated seeds

165 (Jarry & Baillon 1991), but little is known of the relative effects of each on body condition and
166 survival. A better understanding of turtle dove diet across its range, and their use of wild and
167 cultivated resources, will help inform the development of appropriate conservation measures
168 for this species (Newton 2004a).

169 Unlike other dietary studies focussed solely on the breeding grounds (Browne and Aebischer
170 2003a; Dunn *et al.* 2018; Gutiérrez-Galán and Alonso 2016; Mansouri *et al.* 2019), this study
171 includes sites on both the breeding and wintering grounds. We used high throughput
172 sequencing (HTS) to reveal the food resources used by turtle doves across a range of
173 environments, testing the following hypotheses: i) Dietary resources used will differ between
174 breeding and wintering grounds, ii) Turtle doves will feed on a higher proportion of wild seeds
175 in a country with less intensive agriculture; iii) Birds feeding on a higher proportion of
176 cultivated seeds will be in better condition than those feeding on more wild seeds.

177 2 Methods

178 2.1 Sample collection

179 Faecal samples were collected in three countries, across both wintering and breeding grounds
180 (Figure 1). A wintering population of turtle doves was sampled at the Beer Sheba Project (Beer
181 Sheba Project, 2002), an agricultural resource centre encompassing a 100 ha *Acacia* woodland
182 near Sandiara (14°22'N, 16°48'W), Senegal between November and March over four winters
183 (2014/2015 to 2017/2018). Mist nets were used to catch birds when they returned to the site to
184 roost in the evening. Birds were caught at two different European breeding locations, in
185 western Europe (France) and eastern Europe (Hungary) in order to sample geographically
186 distinct populations using different migratory flyways (Marx, Korner-Nievergelt and Quillfeldt
187 2016), early in the breeding season. In France, samples were collected in spring 2017 (15th-30th
188 May) from two mainland sites, comprising large areas of deciduous forest: Chizé Forest
189 (46°07'N, 0°25'W) and Aulnay Forest (46°02'N, 0°14'W), and a more open and varied habitat
190 on Ile d'Oléron (45°93'N, 01°28'W), an island just off the west coast. In Hungary birds were
191 sampled in spring 2018 (20th May – 7th June) from Balotaszállás-Öttömos (46°16'N, 19°35'W),
192 consisting of a mix of plantations and native forest (Appendix A: sampling location
193 information). Whilst there was variation in the surrounding environment, all sites were located
194 within a landscape containing a mix of agricultural land and small towns or villages, often
195 interspersed with natural grassland. As we aim to compare two breeding grounds with varying
196 levels of agricultural intensity (between more intense agriculture observed in western Europe

197 and less intense in eastern Europe) the extent of agriculture around the breeding grounds field
198 sites was determined using land use maps (CLC, 2018) by calculating the proportion of land
199 within a 10km radius accounted for by different land use types. In France, potter traps were
200 baited with bird-seed mix (species composition identified by DNA sequencing; see Appendix
201 B: bait composition), and in Hungary, the capture site, where mist nets were used, was baited
202 with ground maize. Baiting frequency and regimes varied between sites, with bait being
203 provided daily in Chizé, every other day in Aulnay and on Ile d'Oléron, and every day for the
204 week of 26th May – 2nd June, then every other day after this date in Hungary. Further
205 information of baiting methods are provided in Appendix B: delivery of bait.

206 Faecal samples were collected from sterile bird bags within which birds were temporarily held
207 after capture, and dried to preserve plant DNA (Rayé *et al.* 2011). In the first three sampling
208 seasons in Senegal (2014/15-2016/17), faecal samples were air-dried; in all other sampling
209 seasons, faecal samples were dried using self-indicating silica gel beads (2.5-6mm, Fisher
210 Scientific). Silica gel was added to samples, which were stored at room temperature. Samples
211 were checked after 12-24 hours and if all silica gel had turned from orange to clear, indicating
212 moisture absorption, silica gel was poured out and replaced. This process was repeated until
213 silica stopped changing colour. Samples were frozen as soon as possible following arrival in
214 the UK (Appendix C: Sample storage). Due to field conditions and time between sample
215 collection and import into the UK, the time to freezing varied, and was a maximum of 38 days
216 for the majority of samples. However, for samples collected between November 2015 and
217 March 2016, this time was considerably longer (241-346 days), due to import restrictions.

218 Each bird was fitted with a standard metal ornithological ring and a range of morphometric
219 measurements taken, including weight, and maximum flattened wing chord (Svensson, 1992).
220 In Senegal, birds were aged (Baker 1993) as either adult, first winter or unknown. Birds caught
221 in Europe were not aged, but all were at least one year old. All bird handling was conducted
222 by licensed ringers.

223 2.2 DNA extraction and PCR amplification

224 2.2.1 Faecal samples for dietary analysis

225 DNA was extracted using either a QIAamp DNA Stool Mini Kit, or QIAamp Fast Stool Mini
226 Kit (Qiagen, Hilden, Germany), following manufacturers protocols with minor modifications,
227 as detailed in Dunn *et al.* (2016). Two extraction negatives, containing nuclease free water

228 instead of sample DNA, were included in every extraction batch to detect potential
229 contamination.

230 PCR amplification of the ITS2 region was carried out using the UniPlant primer pair (F: 5'-
231 TGTGAATTGCARRATYCMG-3'; R: 5'- CCCGHYTGAYYTGRGGTDCDC-3')
232 (Moorhouse-Gann *et al.* 2018), specifically designed for short amplicons (187-387bp) to
233 maximise amplification of degraded plant DNA from faecal samples. Both forward and reverse
234 primers were labelled with MID-tags (Multiple Identifiers) to provide a unique combination of
235 tags for each DNA sample. PCRs were carried out in 25µl reaction volumes, consisting of
236 12.5µl Multiplex PCR Master Mix (Qiagen), 2.5µl nuclease free water, 2.5µl each of 0.01M
237 UniPlantF and UniPlantR MID-tagged primers (Eurofins, Ebersberg, Germany), and 5µl
238 template DNA. Within each 96-well plate, 11 extraction negatives, one PCR negative, two
239 unused MID-tag combinations and two PCR positives (DNA extracted from plants endemic to
240 Mauritius, thus not occurring within our study system) were included as controls (Taberlet,
241 Bonin, Zinger and Coissac 2018). Replicates were included for a total of 16 samples to assess
242 the consistency of sequencing.

243 Reaction conditions were initial denaturation at 95°C (15 minutes); 40 cycles of 95°C (30
244 seconds), 58°C (90 seconds), 72°C (90 seconds); final extension at 72°C (10 minutes).
245 Concentration of amplified DNA was determined using a DNA fast analysis cartridge with
246 QIAxcel (Qiagen). Each plate of MID-tagged PCR product was combined into a single pool of
247 uniquely identifiable samples, with approximately equal concentration per sample. Plates were
248 pooled individually due to unavoidable variation between QIAxcel runs. Following pooling of
249 samples, the quality and size of amplicons in each pool was checked via TapeStation2200
250 (Agilent, Santa Clara, USA) and pools cleaned using SPRIselect beads (Beckman Coulter,
251 Brea, USA) following manufacturer's instructions, at a ratio of 1:1.1, to retain fragments of the
252 desired size range (207-400bp). Once cleaned, the concentration of DNA was measured for
253 each pool using a Qubit dsDNA High-sensitivity Assay (ThermoFisher Scientific) and pools
254 were combined into a single equimolar pool.

255 The library preparation for Illumina sequencing was carried out on this pool using NEXTflex
256 Rapid DNA-seq Kit (Bioo Scientific, Austin, USA), following the manufacturer's protocols.
257 The library was sequenced using a V2 chip, with 2x250bp paired-end reads on an Illumina
258 MiSeq sequencer (Illumina, San Diego, CA).

259

260 2.3 Bioinformatic analysis

261 Bioinformatic analyses were carried out using a custom pipeline designed for analysis of diet
262 metabarcoding data from Illumina MiSeq (Drake *et al.* 2021). Briefly, sequences were
263 trimmed, aligned and quality checked using FastP (Chen, Zhou, Chen and Gu. 2018), using a
264 minimum sequence length of 170bp and quality threshold (Q) of 33. Sequences were
265 demultiplexed and assigned to their sample ID, prior to removal of MID-tag and primer, using
266 Mothur v1.39.5 (Schloss *et al.* 2009). Following demultiplexing, samples were dereplicated
267 and error-corrected via Unoise3, in Usearch 11, to remove sequencing and PCR errors and
268 chimeras, producing sequence outputs of zero-radius taxonomic units (zOTUs) (Edgar 2016).
269 BLASTn v2.7.1 (Camacho *et al.* 2009) was used to assign sequences to taxa using the GenBank
270 BLAST database (Altschul, Gish, Miller, Myers and Lipman, 2015), with the minimum percent
271 identity of 95%. BLAST results were filtered using the ‘dplyr’ package in R to retain only the
272 top BIT score for each zOTU, calculated by combining the percent ID and e-value. These
273 BLAST outputs were analysed using MEGAN v6.15.2 (Huson *et al.* 2016) to assign taxonomic
274 names to each zOTU.

275 Following taxonomic assignment, results were manually checked to identify zOTUs not
276 assigned to species level, generally resulting from variations in nomenclature or erroneous
277 sequences on GenBank. Where possible, sequences were assigned to species level; however,
278 where there were multiple top hits on GenBank, they were assigned to the highest taxonomic
279 rank in common. The geographic range of all taxa identified was checked using Kew Science
280 ‘plants of the world online’ (POWO, 2019). We identified 15 species assignments which did
281 not occur within our sampling range, these were thus assigned the most appropriate taxonomic
282 level occurring within our study region. The final step was aggregation, whereby all zOTUs
283 assigned to the same taxon were aggregated in R using the ‘aggregate’ function.

284 Data cleaning was carried out as per Drake *et al.* (2021), using complementary filtering
285 methods of removing maximum contamination (based on negative controls and unused MID-
286 tag combinations) and removal of uncommon sequences, to remove both exogenous
287 contaminants, resulting from lab procedures, and internal contamination, resulting from
288 sequencing errors, respectively.

289 For the removal of uncommon sequences, the filtering threshold was based on frequency of
290 occurrence of taxa per sample; accounting for variation in read depth between samples (in this
291 case, read depth/sample ranges from 4276-172171), facilitating a standardised clean up across

292 all samples. Different filtering thresholds, determined based on the frequency of non-target
293 DNA occurring in positive controls, were tested to assess the efficiency of removal of false
294 positives from positive controls, as per Drake *et al.* (2021), with a threshold of 4% being
295 selected. (Appendix D: Filtering threshold selection).

296 Following data filtering, fungal and bacterial taxa were removed, as were any taxa which were
297 not identified to at least family level.

298 2.4 Statistical analysis

299 All statistical analyses were carried out in R, version 4.0.1 (R Core Team 2020). Due to
300 differences in the amplification efficiency of DNA from different plant species, as
301 demonstrated during development and testing of the primer pair used in this analysis
302 (Moorhouse-Gann *et al.* 2018), presence or absence of each taxonomic unit within each
303 sampling unit, rather than read count, was used in all statistical analyses (frequency of
304 occurrence) (Lamb *et al.* 2018).

305 As sample size varied across countries, with a relatively small number of birds being caught in
306 Hungary, we assessed sampling completeness using nonparametric Chao estimators using the
307 package *vegan* (Oksanen *et al.* 2020). The ‘specpool’ function was used for to extrapolate the
308 species richness in each country sampled, using incidence-based estimates, using the default
309 Chao equation. The extrapolated species richness was used to estimate the proportion of total
310 taxonomic units of the diet likely to have been detected in each sampling site. Repeatability of
311 sequencing was assessed using Jaccard distance, to assess the dissimilarity between data sets
312 of the repeated sampling runs, using the ‘vegdist’ function in the package *vegan*.

313 2.4.1 Dietary variation in turtle doves

314 Two GLMs with Poisson errors and logarithmic link functions were fitted to assess differences
315 in dietary species richness (number of taxa found within a faecal sample) between: i) sampling
316 seasons and ii) years. The latter just used data for Senegal, as this was the only site sampled
317 more than once (four consecutive winters). An additional independent variable of the number
318 of days between sample collection and freezing, and the interaction between this and sampling
319 season was included to account for potential effects of the variation in time that samples were
320 stored in the field. For all GLMs, models were fitted using the ‘glm’ function in base R, and
321 goodness of fit assessed by comparing the residual and fitted values, and with Q-Q plots.
322 Significant pairwise differences were determined using Tukey’s post-hoc test, using the
323 *multcomp* package in R (Hothorn, Bretz and Westfall. 2008). Most analyses carried out

324 included the supplementary seed provided to birds as bait, as this was considered an important
325 aspect of the diet of birds sampled in France. However, some additional analyses were
326 conducted to assess the effect of removing bait seed from the analysis.

327 An index of bird body condition (hereafter referred to as ‘condition’) was calculated using
328 linear regression, and model assumptions of normality were checked as previously described.
329 Bird weight was the dependent variable, and wing length and time of day were independent
330 variables, the latter to account for within-day variation in weight as there was an approximate
331 linear increase in weight through the daily sampling period. Whilst date of capture may be a
332 factor in body condition, in particular for birds returning from migration, this was not included
333 as a variable in calculating body condition because birds returning from migration land on the
334 breeding grounds over a period of approximately one month, and there was no way for us to
335 know how soon after migration birds were captured. Residuals from the model were used as a
336 proxy for condition of individual birds, as this residual variation in weight not accounted for
337 by wing length is associated with relative fat mass in adults, a commonly used estimate of body
338 condition in birds (Labocha and Hayes, 2012). Body weight can be also influenced by the
339 amount of seed in a bird’s crop. This would have a greater effect on the weight of birds captured
340 in France, as they were caught in baited potter traps, rather than mist nets as in other sites. As
341 this variation could not be corrected for, birds sampled in France were not included in
342 assessment of body condition. Variation in condition between the five remaining sampling
343 seasons was assessed using a one-way ANOVA, modelling condition as the dependent and
344 sampling season as the independent variables.

345 Multivariate Generalised Linear Models (MGLMs) were fitted using the *mvabund* package
346 (Wang, Naumann, Wright and Warton 2012) to investigate variation in the diet of turtle doves.
347 As not all dietary items were differentiated to species, the presence of taxa at genus level was
348 used for this analysis, including 85 different genera. Senegal data had unique attributes of
349 multiple years of sampling, a mix of identifiable adult and first year birds, and sampling over
350 a five-month period, therefore two MGLMs were fitted to address different questions. All
351 models including birds captured in France were run twice, once using the full dietary data set
352 and one with the bait seeds omitted, in order to assess the effect of bait seed on the analysis. In
353 all instances, the ‘*manyglm*’ function was used to create an MGLM using the binomial family
354 and complementary log-log link function. An ‘*mvabund*’ object was created using the
355 presence-absence matrix of dietary items, which was the dependent variable in the models.
356 Using a subset containing only data from Senegal, the following terms and interactions were

357 included as independent variables: year, age, condition, Julian date (calculated as number of
358 days from November 1st), and the following interactions: year:age, year:condition, year:Julian
359 date, condition:age and condition:Julian date. As there was no evidence of a linear trend in
360 Julian date across winter, Julian date was fitted as a quadratic term to allow for basic non-
361 linearity. Using the full dataset, containing all birds, the only independent variable was
362 sampling season, as the use of bait in France meant that body condition was not comparable
363 across all samples, thus it was not included as a variable in this model. Because different study
364 sites were sampled in different years, year and country could not be distinguished in the model:
365 instead, ‘sampling season’, comprising the six combinations of year and country, were used.
366 The fit of the model was checked using the ‘plot.manyglm’ function, modelling residuals
367 against fitted values and generating Q-Q plots.

368 Model selection was carried out based on Akaike’s Information Criterion (AIC), using R’s
369 ‘step’ command. The significance of terms in the final models was assessed using the ‘anova’
370 function. To examine which taxa contributed to dietary variation, univariate tests were run on
371 each dietary item separately using the ‘p.uni= “adjusted”’ argument in the ‘anova.manyglm’
372 function. Two methods were used to visualise distribution of consumed genera: i) genus level
373 bipartite plots were created using the *bipartite* package (Dormann 2019) to illustrate the overlap
374 of genera consumed between countries; and ii) differences in genera consumed between
375 sampling seasons were visualised using non-metric multidimensional scaling (NMDS). For
376 NMDS analysis, genera occurring in a single dietary sample were removed, as this analysis is
377 liable to outliers skewing results. NMDS used the ‘metaMDS’ command from the *vegan*
378 package (Oksanen *et al.* 2020) based on using Jaccard dissimilarities. ‘Ordspider’ was used to
379 visualise results from nMDS via plotting with *ggplot2* (Wickham 2011).

380 2.4.2 The effect of cultivated vs. wild seeds on bird condition

381 To test if variation in food types consumed in the different sampling countries influenced turtle
382 dove condition, plants were categorised into “cultivated” (34 taxonomic units), consisting of
383 crop plants, those cultivated for animal fodder and seeds commonly provided in supplementary
384 feed, and “wild” (95 taxonomic units). For the most part, wild/cultivated status did not differ
385 across sampling sites, with the exception of *Panicum* sp., which occur naturally in Senegal, but
386 primarily as a constituent of bird feed in Europe. The genus *Brassica* occurred in one instance
387 where it could not be identified to species level. Due to the diversity of this genus including
388 both wild and cultivated species within our study area, this taxonomic unit was excluded from
389 the analysis of food type.

390 These classifications were used to analyse the effect of the type of food consumed on body
391 condition. Condition and the proportion of wild seed taxa consumed were compared between
392 sampling seasons by generating an analysis of covariance, with Gaussian family and identity
393 link function, using body condition as the dependent variable and sampling season, proportion
394 of dietary items accounted for by wild seed and their interaction as predictor variables. Model
395 selection was carried out using 'step'. In order to assess whether body condition changed as
396 winter progressed, the same analysis was carried out on a subset of data consisting of only birds
397 sampled in Senegal, including Julian date (with quadratic transformation), and interactions
398 between Julian date and year as additional dependent variables. The distribution of sampling
399 dates in each year was visualised in order to demonstrate variation between years, to consider
400 if this could be driving between-year trends (Appendix E: Bird capture date across sampling
401 seasons).

402 All mean values are reported \pm standard error.

403 3 Results

404 3.1 DNA amplification, sequencing and taxon identification

405 DNA was successfully amplified from 203 turtle dove faecal samples across three countries,
406 including four winter sampling seasons and two spring sampling seasons (Table 1). The
407 Illumina sequencing run yielded 17.1 million paired-end reads, which was reduced to 9.7
408 million reads following data processing (Appendix F: processing of Illumina MiSeq output)
409 The average read count per sequenced faecal sample was $43,586 \pm 1,322$ reads (min=323,
410 max=136,843). A total of 121 taxonomic units were present in the diet of turtle doves in this
411 study (N=203). Of these, 57.9% (n=70) were identified to species level, another 38.8% (n=47)
412 to genus, 2.5% (n=3) to tribe or subtribe and the remaining 0.8% (n=1) to family (Appendix G:
413 taxonomic units identified).

414 When assessing sampling completeness, species richness of estimated available sampling units
415 varied considerably between the three countries sampled, with the lowest richness predicted in
416 Hungary (67.365 ± 28.80) and the highest in France (143.575 ± 55.11) (Appendix H, Table 1).
417 This analysis suggests that we detected 46% of available taxonomic units in Senegal, 34% in
418 France and 39% in Hungary. Neither the number of days samples were stored prior to freezing,
419 or the interaction between days to freezing and sampling year had a significant effect on species
420 richness (GLM: $Dev_{3,120} = 0.100$, $p = 0.751$; GLM: $Dev_{3,120} = 1.489$, $p = 0.685$, respectively).

421 3.2 Land use around field sites

422 A 10km radius was plotted around each capture site to determine the land use in the
423 surrounding areas (Appendix I: European land use). In France, 50% of the land use was
424 attributed to agriculture, including non-irrigated arable land, vineyards and fruit tree
425 plantations and 21% was accounted for by suitable natural habitat, including broad-leaved
426 forest, coniferous forest and transitional woodland and shrub. A further 2% was accounted
427 for by pastures, which often have appropriate foraging habitat for turtle doves. The remaining
428 27% was accounted for by water, marshes and intertidal flats and urban areas, which are not
429 suitable foraging habitats for turtle doves. In Hungary, 39% of land was cultivated, and the
430 majority of the remaining land was also suitable for turtle dove foraging, with 45% being
431 accounted for by forest and woodland, 12% being pasture and only 4% being accounted for
432 by marshes, water bodies and urban areas.

433 3.3 Dietary variation

434 Mean species richness was 2.827 ± 0.288 taxa consumed per individual (range 1-7; Table 1).
435 Species richness in samples from Senegal differed between years (GLM: $Dev_{3,124} = 12.676$,
436 $p = 0.005$); and across all data, species richness varied between sampling seasons (GLM:
437 $Dev_{5,197} = 30.199$, $p < 0.001$; Table 1). Birds sampled from Senegal in the winter of 2017/18 had
438 the lowest mean species richness and birds in Hungary had the highest mean species richness
439 (Table 1).

440 When supplementary food from bait was excluded in France, faeces from birds caught in potter
441 traps had the lowest average species richness, and lowest maximum species richness within a
442 single individual, indicating a less varied use of natural resources in the diet of birds visiting
443 potter traps supplemented with bait.

444 The most frequently occurring taxa in the diet of turtle doves differed across countries sampled.
445 In Senegal, the taxa consumed by the greatest proportion of birds sampled were *Cenchrus*
446 *americanus* (pearl millet) and *Sorghum* sp., each being detected in 32.0% (n=41) of birds
447 sampled. Cenchrinae, the subtribe to which *C. americanus* belongs, was detected in a further
448 14.1% (n=18) of birds sampled. In France, the most prevalent taxa were *Triticum* sp. (74.6%,
449 n=47), *Helianthus* sp. (41.3%, n=26), Triticeae (the subtribe within which *Triticum* falls)
450 (36.5%, n=23), and *Sorghum* sp. (33.3%, n=21), all of which were present in the seed-mix used
451 to bait traps. *Triticum monococcum* (einkorn wheat) was detected in 22.2% of birds (n=14).
452 The species of *Triticum* present in the seed mix could not be differentiated between three top
453 hits on GenBank (*T. turgidum*, *T. aestivum* and *T. dicoccoides*), but as *T. monococcum* is grown

454 in France, this seed may have come from the environment or from garden bird feeders.
455 Excluding items known, or likely, to be fed to birds, the most frequently detected food resource
456 was *Geranium molle* (dove's foot cranesbill), an arable weed commonly occurring across
457 Europe. In Hungary, the three most frequently detected taxa identified were *Euphorbia* sp.
458 (50%, n=6), *Euphorbia pseudoesula* (Figert's spurge) (50%, n=6), and *Euphorbia cyparissias*
459 (Cypress spurge) (33.3%, n=4), all of which are naturally occurring species.

460 At the family level, Poaceae was the most frequently consumed taxon in Senegal and France,
461 occurring in the diet of 86.7% (n=111) and 90.5% (n=57) of turtle doves, respectively. When
462 bait seeds were removed from the diet of birds sampled in France, Poaceae was detected in the
463 diet of a considerably smaller proportion of birds sampled (24%, n=9), but this was still
464 comparatively common, being the second most detected taxa in birds sampled. Of the 14
465 taxonomic units within this family detected in Senegal, 28% were cultivated, including *C.*
466 *americanus* and *Sorghum* sp.. *Panicum* sp. and *Echinochloa* sp. were consumed by 22.6%
467 (n=29) and 18.0% (n=23) of birds sampled respectively; both are widespread genera in the
468 grass family, native to Africa. In France, 50% of taxonomic units within the Poaceae family
469 were cultivated (n=6), including *Sorghum* sp., *Triticum* sp. and *Panicum miliaceum*. This
470 frequent occurrence of cultivated seed is expected due to the trapping method, and when bait
471 taxa were excluded from analysis, the wild genera in this family (*Argrotis* sp., *Lolium* sp., *Poa*
472 sp. and the subtribe *Loliinae*) appeared in the diet of a quarter of birds sampled. Poaceae was
473 the second most commonly consumed family in Hungary, appearing in the diet of 41.7% of
474 birds sampled (n=5). Fewer taxonomic units from this family were identified in Hungary (n=5),
475 but of those present 60% were cultivated.

476 In initial analysis, Hungary was the only country where the dominant family consisted solely
477 of wild food resources. Euphorbiaceae were detected in the diet of 58.3% (n=7) of birds, and
478 consisted of five different taxonomic units: *Euphorbia* sp. and four distinct species of
479 *Euphorbia*, all of which are naturally occurring. Despite being used to bait capture sites in
480 Hungary, maize was not detected in the diet of birds in this location. When bait seed was
481 excluded from samples collected in France, Euphorbiaceae was the second most frequently
482 consumed taxa, being detected in the diet of 24% (n=9) of birds sampled, equal to the
483 proportion of birds consuming wild Poaceae. When analysed with bait seed excluded, the diet
484 of birds in France was also dominated by wild resources, with the dominant family being
485 consumed by individuals retained in the sample being Geraniaceae, predominantly *Geranium*
486 *molle*, which was present in 39% (n=15) of samples.

487 Asteraceae occurred in the top three families consumed by individuals in all countries sampled,
488 being the third most commonly detected in Senegal (10.9%) and Hungary (25.0%) and second
489 in France (41.3%). In France, this was dominated by *Helianthus* sp., a component of the bait
490 used and common element in bird-seed, which was detected in 41.3% of all birds sampled.
491 When bait was removed from analysis, Asteraceae occurred in the diet of 8% (n=3) of birds
492 retained in analysis, with *Carduus* sp. (true thistles) accounting for the wild genera consumed
493 from this family. In contrast, in Hungary, *Helianthus* sp. was only identified in one individual,
494 and the other two, naturally occurring genera: *Ambrosia* sp. (ragweeds) and *Carduus* sp.,
495 accounted for most of the occurrence of the Asteraceae family. In Senegal, the most commonly
496 occurring member of the Asteraceae family was *Blainvillea*, another wild genus.

497 Following model selection using step, the only variable retained in the model assessing dietary
498 variation between years in Senegal was Julian date (MGLM: $LRT_2=344.6$, $p < 0.001$). In the
499 full data set, sampling season was significantly associated with genera consumed (MGLM:
500 $LRT_5=647.4$, $p < 0.001$; Figure 2). There was little overlap of consumed taxa between countries
501 sampled (Figure 3). After adjusting for multiple testing, sampling season had a significant
502 effect on 13 dietary items (N=85 genera), indicating that these genera were responsible for a
503 large proportion of the seasonal variation (Appendix J: Univariate test results). Of these, eight
504 occurred only in Senegal (*Cenchrus*, *Echinochloa*, *Dactyloctenium*, *Senna*, *Balinvillea*,
505 *Melochia*, *Nymphaea* and *Urochloa*) and two occurred only in France (*Triticum* and
506 *Geranium*), supporting the idea that country drove the observed dietary variation.

507 When investigating the effect of removal of bait genera from analysis of the genera consumed
508 by turtle doves, the significant terms in the model did not change, with both the full data set
509 and that with bait excluded producing the same level of significance for the association between
510 sampling season and dietary items consumed by turtle doves (Appendix K: Effects of removing
511 bait, Table 1). There was a small amount of variation between the two data sets when adjusting
512 for multiple testing, with differences in the genera consumed responsible for the largest portion
513 of dietary variation (Appendix K: Effects of removing bait, Table 2). Two items found in bait
514 (*Triticum* sp. and *Helianthus* sp.) significantly contributed to dietary variation when bait was
515 included, but these were no longer significant following the exclusion of bait. One genus which
516 did not significantly drive variation when bait was included did have an effect when bait was
517 excluded, this was a wild seed found only in France (*Mercurialis* sp.).

518 3.4 Food type and its effect on body condition

519 Hungary had the greatest proportion of wild taxonomic units recorded in the diet (80.6%; Table
520 22), whilst France had the highest proportion of cultivated seeds recorded in the diet (72.0% of
521 taxonomic units detected), with cultivated taxa present in the diet of 95.2% of birds sampled
522 (Table 2). This is unsurprising due to the use of bait to capture birds. When excluding seeds
523 present in the bird seed mix, 25 individuals (39.7%) sampled in France had no taxonomic units
524 recorded in their diet, indicating that this feed mix is an important part of the recent diet of
525 birds caught in potter traps, as expected.

526 Only sampling season (GLM: $F_{4,130}=10.154$, $p<0.001$) affected turtle dove body condition.
527 Proportion of wild taxa consumed (GLM: $F_{1,134}=0.003$, $p<0.955$) and the interaction between
528 these two terms (GLM: $F_{4,126}=1.557$, $p=0.190$) were not significant. Birds sampled in Senegal
529 in winters 1 and 3 (2014/15 and 2016/17, respectively) had the lowest body condition scores,
530 both of which were significantly less than the body condition score for birds sampled in
531 Hungary and in Senegal in winter 4 (2017/18).

532 In Senegal, year (GLM: $F_{3,120}=8.048$, $p<0.001$) and Julian date (GLM: $F_{2,118}=14.354$, $p<0.001$)
533 both influenced body condition, but the proportion of the diet comprised of wild genera did not
534 have a significant effect on body condition (GLM: $F_{1,123}=2.045$, $p=0.155$). Body condition
535 increased as the winter progressed (Figure 5A), as did the proportion of wild taxa consumed
536 (Figure 5B), indicating increased use of wild seeds later in the season. There were two periods
537 of sampling in W2, W3 and W4 (November and March) but only one in W1 (January).

538 3.5 Repeatability

539 Sixteen repeated faecal DNA samples were included to assess the consistency of sequencing
540 within a single run. The average Jaccard distance was 0.390, and dissimilarity scores ranged
541 from 0.019 to 0.785 (Table 4). The range of species richness and average species richness
542 detected was slightly higher in repeat 2, but the proportion of taxonomic units accounted for
543 by wild and cultivated seeds, and two of the most abundant families (*Poaceae* and *Asteraceae*)
544 were relatively consistent between repeats (Table 3). The number of taxa recorded for repeated
545 samples, as well as the number of taxa in common between the two repeats is presented in
546 Table 4. Seven of the 16 samples had identical results between repeats. All taxa occurring in
547 only one of the two repeats after cleaning occurred at relatively low read counts (Appendix L:
548 Sequencing repeats raw data). Variation between repeats could not be mitigated by altering
549 threshold for data cleaning.

550 4 Discussion

551 We detected a wide range of dietary items consumed by turtle doves across breeding and
552 wintering grounds, with 121 taxonomic units identified, although only a small number
553 accounted for the majority of the diet, with most observed infrequently. The mean number of
554 different taxonomic units detected in the diet of individuals (2.827 ± 0.288) was lower than
555 previously described when analysing turtle dove diet using HTS (Dunn *et al.* 2018). There was
556 little overlap between the genera consumed in different countries. The only genera found in all
557 three regions were *Panicum* sp., *Sorghum* sp., *Cannabis* sp. and *Helianthus* sp. With the
558 exception of *Panicum* sp., which is native to West Africa (National Research Council 1996),
559 these genera are not naturally occurring within the regions, rather they are cultivated crops or
560 common components of bird seed.

561 4.1 Turtle dove diet in the wintering grounds

562 A mix of cultivated and wild seeds were found in individuals sampled in Senegal, although the
563 former were more frequent (between 60.8% and 96.4%). The most common seeds consumed
564 in this study were *Cenchrus americanus* (pearl millet) and *Sorghum* sp., common crops in the
565 landscape surrounding the roost site where the birds were caught (*personal observation*). Field
566 observations of a high proportion of caught birds having *Sorghum*, millet or both in their crop
567 support these findings (*unpublished data*). Both of these species have previously been shown
568 to be important constituents of turtle doves' diets (Morel & Morel 1979, Morel & Roux 1966),
569 and the annual production of these crops (along with rice *Oryza* sp.) in west Africa has been
570 correlated with the annual survival of turtle doves (Eraud *et al.* 2009). However, cultivated rice
571 itself, commonly regarded as important to turtle doves (Jarry & Baillon 1991), was absent in
572 the present study. This probably reflects the geographical variation in the different crops grown,
573 as very few areas of rice cultivation were present in our study area (*personal observation*).

574 Wild grass seeds were also an important constituent of turtle doves' diet in Senegal, especially
575 *Panicum* and *Echinochloa* sp. which, although often harvested for food (National Research
576 Council, 1996), are not cultivated crops. As there is currently no ITS2 region sequence for *P.*
577 *laetum* on Genbank, this species could not be confirmed in the present study; however, it is
578 commonly found in Senegal, and has been shown to be an important food source for turtle
579 doves on the wintering grounds (Jarry & Baillon 1991, Morel 1987). *P. laetum* was present in
580 previously wet areas around Beer Sheba, but it was not abundant, and was more abundant at
581 sites along the Senegal river than at our study site (*unpublished data*). Two more wild grasses,
582 *Dactyloctenium aegyptium* and *Urochloa mosambicensis* were also detected relatively

583 frequently in the diet, further suggesting that wild grass seeds are an important element of the
584 diet of turtle doves in their wintering grounds. The abundance of wild grass seeds in the diet of
585 turtle doves is consistent with the findings of Morel and Morel (1974), that resident doves
586 studied in the Sahelian region, including African collared dove (*Streptopelia roseogrisea*),
587 Senegal dove (*Streptopelia senegalensis*) and vinaceous dove (*Streptopelia vinacea*) fed
588 largely on the seeds of wild grasses, including *Panicum laetum*, *Brachiaria hagerupii* and
589 *Dactyloctenium aegyptium*.

590 The proportion of cultivated seed species in the diet increased with each winter sampled,
591 potentially a result of variation in the sampling between years. Average Julian date was earlier
592 in W3 and W4 than W1 and W2, as a result of varied capture success and DNA extraction or
593 PCR failure. The two most prevalent cultivated crops, millet and sorghum, are harvested
594 September-November in Senegal (GIEWS 2020), resulting in an abundance of spilt grain
595 earlier in the season, which decreases as these resources are consumed post-harvest. As more
596 birds were sampled during this earlier period in W3 and W4, this may, at least in part, explain
597 the higher prevalence of cultivated seed in the diet in these years. Furthermore, as the wintering
598 season progressed, it appears birds became more heavily reliant on wild seeds than cultivated,
599 as the proportion of wild seeds in the diet increased with Julian date.

600 4.2 Turtle dove diet in the breeding grounds

601 The relative contributions of cultivated and wild seeds to the diet differed between the countries
602 surveyed, although variation in methods of trapping may account for those differences. While
603 turtle doves sampled in France consumed the highest proportion of cultivated seeds, much of
604 this would have consisted of the bait used to attract birds as, pre-harvest, when trapping
605 occurred, these seeds would not have been readily available in the surrounding farmland. Due
606 to the necessity of using bait to capture birds in this region, and the repeated provisioning of
607 sites each year for long-term monitoring purposes, it is difficult to discern the extent to which
608 birds would utilise alternative cultivated versus wild resources if this feed were not provided.
609 Whilst significant quantities of bait are provided in France (Appendix B: delivery of bait), other
610 species, including wild boar (*Sus scrofa*) and roe deer (*Capreolus capreolus*), are known to eat
611 large quantities of this. Although bait (maize) was used in Hungary, it was not detected in their
612 diet, despite evidence that UniPlant primers detect this species, both in *in vitro* primer testing,
613 and in HTS studies (Dunn *et al.* 2018, Moorhouse-Gann, 2017). Unlike in France, the site in
614 Hungary was not baited to attract birds every year, and the bait was not put out every day during

615 sampling. In addition, birds trapped in mist-nests are not necessarily attracted to bait, and maize
616 is a less attractive bait for turtle doves than alternatives such as sunflower seeds.

617 In addition to the cultivated seeds present, a diverse range of wild seeds was also detected in
618 the diet, with 33 wild taxonomic units identified in France and 20 wild taxonomic units
619 identified in Hungary. This may reflect the higher floral diversity in the forest habitat where
620 they were caught, compared to heavily managed agricultural land. Turtle doves captured in
621 forests in Spain also consumed a greater number of wild species than previously recorded from
622 farmland habitats (Gutiérrez-Galán and Alonso 2016). However, turtle doves forage over
623 distances of several kilometres (Browne and Aebischer 2003a), thus would likely also feed in
624 non-forest habitats, such as agricultural fields.

625 The most commonly detected wild species in France was *Geranium molle*, which was present
626 in 20.6% of birds sampled. This annual herbaceous plant occurs across Europe, and has
627 previously been detected in the diet of turtle doves in Spain (Gutiérrez-Galán and Alonso 2016)
628 and the UK (Dunn *et al.* 2018). Gutiérrez-Galán and Alonso (2016) found *G. molle* to be among
629 the most frequently consumed species in their study, and recognised it as a potential species of
630 importance due to its availability early in the breeding season. Other wild seeds which were
631 previously reported as important in the diet of turtle doves in a UK study were *Fumaria*
632 *officinalis* (common fumitory) and *Stellaria media* (common chickweed) (Murton *et al.* 1964).
633 In contrast *F. officinalis* was not detected in this study, and *S. media* only in the diet of two
634 birds in France (3.2%), similar to findings of recent studies in the UK (Dunn *et al.* 2018). These
635 plant species, once common in arable land across Europe, are declining as farming practices
636 reduce arable plant biodiversity across Europe (Andreasen, Stryhn and Streibig 1996; Dunn *et*
637 *al.* 2015; Storkey, Meyer, Still and Leuschner 2012). For example, *Stellaria media*, previously
638 one of the most abundant arable weeds, declined significantly between 1979 and 2009 in one
639 study in France (Fried, Petit, Dessaint and Reboud 2009).

640 The proportion of the diet accounted for by wild seeds was highest in Hungary (80.6%), with
641 wild seeds being detected in the diet of over 90% of birds sampled, as opposed to cultivated
642 seeds, which only occurred in the diet of one third of birds sampled. This is supported by a
643 previous study in Hungary (Haraszthy 1998). When investigating the gut contents of 36 turtle
644 doves, wild seeds such as *Sinapsis arvensis*, *Chenopodium* sp., *Stellaria* sp. and *Fumaria* sp.
645 dominated the diet, accounting for 83% of seeds identified, whilst common crops and animal
646 fodder such as *Alfalfa* sp. and wheat accounted for just 13% of seeds present (Haraszthy 1998).

647 Birds in Hungary also had, on average, the greatest richness of taxonomic units per individual
648 (3.583 ± 0.288), indicating a more varied diet, although this was still lower than previously
649 observed in turtle doves in the UK (10.400 ± 0.61) (Dunn *et al.* 2018). This may reflect a higher
650 diversity of food available in the UK than our study sites, or variation caused by different
651 bioinformatic pipelines and methods of data cleaning used in the two studies, illustrating the
652 need for a more standardised approach to data cleaning (Drake *et al.* 2021).

653 *Euphorbia* species were the most abundant taxonomic units identified in Hungary, including
654 *E. pseudoesula*, *E. cyparissias*, *E. seguieriana* and *E. stepposa*. Taxa from the *Euphorbia* genus
655 and Euphorbiaceae family have previously been detected in the diet of turtle doves in small
656 amounts in the UK and France (Dubois 2002; Dunn *et al.* 2018; Murton *et al.* 1964), but to our
657 knowledge, this is the first study that has identified this genus as a dominant food resource.
658 Despite a relatively small sample size in Hungary in this study, sampling completion analysis
659 predicted relatively consistent level of available dietary items being detected across all three
660 study sites (between 34% and 46%), with 39% of estimated available taxonomic units being
661 detected in samples from Hungary.

662 The frequency of wild seeds in the diet in Hungary is consistent with the hypothesis that birds
663 would consume a higher proportion of wild food in areas of lower agricultural intensity.
664 Agricultural intensity tends to be lower in central and eastern European countries, which are
665 either not members, or new members of the EU (Donald *et al.* 2001). Traditional farming
666 methods, such as smaller field sizes, lower use of chemical herbicides, and retention of semi-
667 natural field margins (Tryjanowski *et al.* 2011), may have contributed to smaller declines in
668 farmland bird populations than observed in western Europe. However, intensification is
669 increasing across Europe (Verhulst, Báldi and Kleijn 2004), so it is important that appropriate
670 agri-environment and other land management measures are implemented to ensure the declines
671 observed across western Europe do not spread across the whole continent.

672 4.3 Body condition

673 When considering just birds caught without the use of bait in Hungary and Senegal, there was
674 no correlation between the proportion of dietary items accounted for by cultivated seeds and
675 body condition, therefore not supporting the hypothesis that a higher proportion of cultivated
676 seed in the diet would result in better body condition.

677 A study in the UK indicated that an important contributor to the decline of turtle doves is a
678 reduction in breeding attempts, with a 61% decline in the number of young successfully fledged

679 per pair observed between the 1960's and 2000 (Browne and Aebischer 2004). This has been
680 suggested to be linked to reduced food availability, making it harder for adults to reach and
681 maintain adequate body condition for breeding, resulting in a shorter breeding season (Browne
682 and Aebischer 2001). Whilst this has not been proven in turtle doves (Browne and Aebischer
683 2003b), a reduction in wild food availability early in the breeding season, when nutritional
684 demands to reach breeding condition are highest, occurred concurrently with a dietary switch
685 to a greater proportion of cultivated seed (Browne and Aebischer 2003a), making this a
686 plausible cause for reduced breeding attempts in the UK. As crop plants do not provide seeds
687 until later in the breeding season, restoration of early-flowering wild plants in the environment
688 is essential for the species to provide seed-rich habitats. This is supported by a recent review
689 of turtle dove habitat use (Carboneras *et al.* 2022), and the findings of Dunn *et al.* (2018) that
690 nestlings in their study were in better condition when the diet included a higher proportion of
691 naturally occurring weed seeds. In areas where it may be difficult to restore natural weed seed
692 resources, such as the UK, provision of supplemental feed may be a potential strategy for
693 species management, to help make up for the shortfall of wild food availability early in spring,
694 although the latter should be considered as an “emergency action” rather than a continued
695 strategy (Fischer, *et al.*, 2018).

696 During the breeding season, when there are energy demands for producing and laying eggs
697 (Robb *et al.* 2008), and pre-migration when birds need to fuel their migratory journeys (Newton
698 2006), it benefits birds to have higher fat reserves. However, in the non-breeding season, birds
699 often carry less weight, as they only need to obtain enough food to satisfy their own subsistence
700 needs to survive, and lower weight can enable more effective predator avoidance (Kullberg,
701 Fransson and Jakobsson 1996). We observed birds with lower condition scores in winter,
702 however, in Senegal, body condition increased with Julian date, suggesting that birds were
703 investing more in improving body condition later in the season in preparation for migration,
704 one of the most energetically demanding periods of the annual cycle (Cooper, Sherry, Marra
705 and Inouye 2015). This increase was concurrent with an increase in consumption of wild
706 resources. This may be a result of a lack of cultivated seeds in the environment following
707 harvest earlier in the season. Alternatively, turtle doves may be utilising wild seeds for their
708 nutritional value to increase fat reserves in preparation for migration. The latter argument is in
709 concordance with what has been observed in turtle dove nestlings in the UK (Dunn *et al.* 2018).

710 Annual variation in environmental factors may influence the availability of wild and cultivated
711 resources, potentially affecting body condition as a result. Whilst investigation of this is outside

712 the scope of this work, it would merit future research to understand how changing weather
713 conditions globally may impact resource availability and wildlife health.

714 4.4 The inclusion of bait in dietary analysis

715 This study uses metabarcoding to provide a more detailed insight into the variation in the diet
716 of a migratory species, and demonstrates the effectiveness of using recently developed, plant-
717 specific primers to identify a wide range of taxa consumed within a granivorous diet
718 (Moorhouse-Gann *et al.* 2018). In addition, a key limitation of diet analysis is addressed in this
719 study.

720 The need to use bait to attract rare species for capture in order to analyse diet comes with the
721 inherent problem of dealing with bait items occurring in the diet. In some studies, bait items
722 are simply removed from analysis as a known item that was fed to individuals (da Silva *et al.*
723 2020). Whilst we acknowledge that this may be the most appropriate response in some study
724 systems, this is not always the case. The abundance of these bait seeds in the birds sampled in
725 France highlight that this is an important component of the diet of birds caught in potter traps,
726 although it is not possible to conclude how important it is for the population as a whole. A
727 previous dietary study of turtle doves in the UK found that 89% of birds sampled had consumed
728 seed commonly occurring in garden bird feed (Dunn *et al.* 2018), showing that turtle doves are
729 commonly consuming this type of resource, even when it is not used as bait, at least in certain
730 areas. It is arguable that turtle doves would not need to use such resources if an abundance of
731 wild resources were available in their environment, although it cannot be excluded either that
732 such food may be favoured when available, given the usually higher fat contents of seeds used
733 as bait, and the predictability of its occurrence.

734 Whilst we discuss the drawbacks of the need to use bait to attract birds to capture sites in this
735 study, we acknowledge that supplementary food is a potentially important aspect of the diet in
736 situations where wild food resources are scarce, particularly regarding the management of
737 declining species, as reflected in several other studies related to supplementary feeding. In New
738 Zealand, supplementary food is provided for the critically endangered Kakapo (*Strigops*
739 *habroptilus*) to increase productivity, and the Mauritian pink pigeon (*Columba mayeri*) is
740 highly reliant on the provision of supplementary food due to severe habitat degradation as a
741 result of extensive deforestation (Edmunds, Bunbury, Sawmy, Jones and Bell 2008, Elliot,
742 Murton and Jansen 2001). Furthermore, in a study by Newton (2004b), a lack of food resources
743 resulting from the widespread switch to autumn-sown crops and increased use of herbicides
744 was determined to be a key driver in the decline of 10 seed-eating farmland birds in the UK.

745 As demonstrated by these, and many other examples, there is an argument for supplementary
746 feeding in the management of declining species, when habitat conversion and degradation lead
747 to a lack of natural resources. Such is the case for many farmland birds in the UK, including
748 turtle doves, resulting in a number of agri-environmental schemes being put in place (Newton
749 2004b). These are designed to combat the loss of natural food resources resulting from
750 extensive agricultural intensification, and includes a supplementary food scheme specifically
751 targeting turtle doves in England (UK Government, 2021).

752 We conducted analysis both with and without the bait seed included to assess the impacts this
753 provisioned food may have on ecological inferences made in dietary studies. Overall, the
754 inclusion of bait did not affect the level of significance of terms in the full model. Some
755 variation was observed in specific dietary items driving the majority of the variation in
756 sampling season, however, the majority of the seeds determined to be driving the dietary
757 variation remained the same. *Triticum* and *Helianthus* were two components of bait which
758 significantly drove dietary variation. When bait was excluded, *Triticum* was absent from
759 analysis as this was only consumed from bait. *Helianthus* was consumed in Hungary and
760 Senegal as well as from bait, but when bait was excluded from analysis, the consumption of
761 this genus no longer significantly contributed to dietary variation. The biggest ecological
762 difference observed when removing bait was that of the similarity between diets, as represented
763 by NMDS. When bait was removed, there was greater difference evident from plots between
764 the diets of birds sampled in Europe and Africa, which will be driven by the use of seeds which
765 are widespread in the environment in Senegal (*Sorghum* and *Panicum*) in bait used in France.

766 Whilst there is some variation in the results, the comparison of data with and without bait
767 indicates that the dietary variation between sites was not heavily driven by the provision of
768 bait, and taxonomic groups driving variation between the sites were primarily wild resources.
769 Due to the abundance of bait in the diet detected in this study, and that of Dunn et al. (2018),
770 as well as the known switch to an increase in consumption of cultivated seed in absence of wild
771 resources observed in turtle doves (Browne and Aebischer 2003a), we suggest that ecological
772 inferences of diet would be less representative of the true nature of the foraging behaviour of
773 turtle doves if bait were excluded. The prevalence of cultivated seed in the diet of turtle doves
774 in these studies suggests that this is a favourable food source when available, making it a useful
775 short-term management tool in areas, such as the UK, where wild food resources are heavily
776 depleted.

777 Another important consideration in using metabarcoding for diet analysis is the repeatability
778 of the sequencing process, which we assessed by the inclusion of 16 pairs of replicate samples.
779 The mean Jaccard dissimilarity between paired replicates was 0.309. We did find some
780 inconsistencies in the absence/present of taxa between repeats. This is consistent with the
781 findings of previous studies assessing the repeatability of diet metabarcoding (Jedlicka, Vo,
782 and Almeida, 2017, Shutt *et al.* 2020), indicating it would be advisable to conduct repeat
783 extraction and sequencing runs for avian dietary analysis where possible. However, we do show
784 a reasonable level of repeatability in sequencing runs, suggesting that reliable results can still
785 be obtained where this level of replication is not possible or practical.

786 We have shown significant variation in the diet of turtle doves not only between the breeding
787 and wintering grounds, but also between two different breeding sites within Europe with
788 differing levels of agricultural intensity, with a greater abundance of wild seeds in the diet of
789 birds sampled from the eastern European site, where agricultural intensity is lower. It would
790 be beneficial to further investigate the diet of turtle doves in a wider range of countries
791 subject to less intense agriculture. We did not find support for our hypothesis that birds
792 feeding on a high proportion of cultivated seeds would be in better condition, as there was no
793 significant effect of the proportion of wild or cultivated seeds in the diet on the body
794 condition. The concurrent increase in the consumption of wild seed and body condition prior
795 to migration in birds sampled in Senegal suggests that wild seeds may play a role in
796 increasing body condition in preparation for spring migration. Further investigation into the
797 importance of dietary diversity in the context of body condition would be useful in informing
798 management plans to deliver optimal food resources to support his declining species.

799

800 5 References

- 801 Altschul, S.F., Gish, W., Brouwer, J., Miller, W., Myers, E.W. and Lipman, D.J. (1990). Basic
802 local alignment search tool. *Journal of Molecular Biology* 215, 403-410.
803 [https://doi.org/10.1016/S0022-2836\(05\)80360-2](https://doi.org/10.1016/S0022-2836(05)80360-2)
- 804 Andreassen, C., Stryhn, H. and Streibig, J.C. (1996). Decline of the flora in Danish arable
805 fields. *Journal of Applied Ecology*, 619-626. <https://doi.org/10.2307/2404990>
- 806 Baker, K. (1993). *Identification Guide to European Non-Passerines*. British Trust for
807 Ornithology.
- 808 Beer Sheba Project (2002). The Beer Sheba Project. Published on the internet;
809 <http://www.beershebaproject.org> Retrieved 2 June 2021
- 810 Benton, T.G., Vickery, J.A. and Wilson, J.D. (2003). Farmland biodiversity: Is habitat
811 heterogeneity the key? *Trends in Ecology and Evolution* 18, 182–188.
812 [https://doi.org/10.1016/S0169-5347\(03\)00011-9](https://doi.org/10.1016/S0169-5347(03)00011-9)
- 813 Binswanger-Mkhize, H.P. and Savastano, S. (2017). Agricultural intensification: The status in
814 six African countries. *Food Policy* 67, 26–40. <https://doi.org/10.1016/j.foodpol.2016.09.021>
- 815 Birdlife international (2019). *Streptopelia turtur*. The IUCN Red List of Threatened Species
816 2019. Published on the internet www.iucnredlist.org/species/22690419/154373407 Retrieved
817 3 June 2021
- 818 Brink, A.B. and Eva, H.D. (2009). Monitoring 25 years of land cover change dynamics in
819 Africa: A sample based remote sensing approach. *Applied Geography* 29, 501–512.
820 <https://doi.org/10.1016/j.apgeog.2008.10.004>
- 821 Browne, S. and Aebischer, N. (2001). The Role of Agricultural Intensification in the Decline
822 of the Turtle Dove *Streptopelia turtur*. English Nature Research Report Number 421.
823 Peterborough: English Nature.
- 824 Browne, S. and Aebischer, N. (2003a). Habitat use, foraging ecology and diet of Turtle Doves
825 *Streptopelia turtur* in Britain. *Ibis* 145, 572–582. [https://doi.org/10.1046/j.1474-](https://doi.org/10.1046/j.1474-919X.2003.00185.x)
826 [919X.2003.00185.x](https://doi.org/10.1046/j.1474-919X.2003.00185.x)
- 827 Browne, S. and Aebischer, N. (2003b). Temporal variation in the biometrics of turtle doves
828 *Streptopelia turtur* caught in Britain between 1956 and 2000. *Ringing and Migration* 21, 203-
829 208. <https://doi.org/10.1080/03078698.2003.9674293>

830 Browne, S. and Aebischer, N. (2004). Temporal changes in the breeding ecology of European
831 turtle doves *Streptopelia turtur* in Britain, and implications for conservation. *Ibis* 146, 125-
832 137. <https://doi.org/10.1111/j.1474-919X.2004.00235.x>

833 Browne, S., Aebischer, N. and Crick, H. (2005). Breeding ecology of Turtle Doves *Streptopelia*
834 *turtur* in Britain during the period 1941-2000: An analysis of BTO nest record cards. *Bird Study*
835 52, 1–9. <https://doi.org/10.1080/00063650509461368>

836 Browne, S.J., Aebischer, N.J., Yfantis, G. and Marchant, J.H. (2004). Habitat availability and
837 use by Turtle Doves *Streptopelia turtur* between 1965 and 1995: an analysis of Common Birds
838 Census data. *Bird Study* 51, 1–11. <https://doi.org/10.1080/00063650409461326>

839 Burns, F., Eaton, M., Balmer, D., Banks, A., Caldow, R., Donelan, J., Duigan, C., Foster, S.,
840 Frost, T., Grice, P.V., Hall, C., Hanmer, H.J., Harris, S. J., Johnstone, I., Lindley, P.,
841 McCulloch, N., Nobel, D.G., Risley, K., Robinson, R.A. and Wootton, S. (2020). The State of
842 UK’s Birds report 2020.

843 Carboneras, C., Moreno-Zarate, L. & Arroyo, B. (2022). The European Turtle Dove in the
844 ecotone between woodland and farmland: multi-scale habitat associations and implications for
845 the design of management interventions. *Journal of Ornithology* 163, 339–355
846 <https://doi.org/10.1007/s10336-021-01946-1>

847 Camacho, C., Coulouris, G., Avagyan, V., Ma, N., Papadopoulos, J., Bealer, K. and Madden,
848 T.L. (2009). BLAST+: architecture and applications. *BMC Bioinformatics* 10, 421.
849 <https://doi.org/10.1186/1471-2105-10-421>

850 Chamberlain, D.E., Fuller, R.J., Bunce, R.G.H., Duckworth, J.C. and Shrubbs, M. (2000).
851 Changes in the abundance of farmland birds in relation to the timing of agricultural
852 intensification in England and Wales. *Journal of Applied Ecology* 37, 771–788.
853 <https://doi.org/10.1046/j.1365-2664.2000.00548.x>

854 Chen, S., Zhou, Y., Chen, Y. and Gu, J. (2018). Fastp: an ultra-fast all-in-one FASTQ
855 preprocessor. *Bioinformatics* 34:i884–i890. <https://doi.org/10.1093/bioinformatics/bty560>

856 CLC (2018). Corine Land Cover 2018, v.2020_20u1, published on the internet CLC 2018 —
857 Copernicus Land Monitoring Service, retrieved 29 December 2022

858 Contesse, P., Hegglin, D., Gloor, S., Bontadina, F. and Deplazes, P. (2004). The diet of urban
859 foxes (*Vulpes vulpes*) and the availability of anthropogenic food in the city of Zurich,
860 Switzerland. *Mammalian Biology* 69, 81–95. <https://doi.org/10.1078/1616-5047-00123>

861 Cooper, N.W., Sherry, T.W., Marra, P.P. and Inouye, B.D. (2015). Experimental reduction of
862 winter food decreases body condition and delays migration in a long-distance migratory bird.
863 *Ecology* 96, 1933–1942. <https://doi.org/10.1890/14-1365.1>

864 Donald, P.F., Green, R.E. and Heath, M.F. (2001). Agricultural intensification and the collapse
865 of Europe’s farmland bird populations. *Proceedings of the Royal Society B: Biological Sciences*
866 268, 25–29. <https://doi.org/10.1098/rspb.2000.1325>

867 Dormann, C.F. (2019). Using bipartite to describe and plot two-mode networks in R. *R package*
868 2020

869 Drake, L., Cuff, J., Young, R., Marchbank, A., Chadwick, E. and Symondson, W. (2021). An
870 assessment of minimum sequence copy thresholds for identifying and reducing the
871 prevalence of artefacts in dietary metabarcoding data. *Methods in Ecology and Evolution*.
872 <https://doi.org/10.1111/2041-210X.13780>

873 Dubois, M. (2002). Contribution a l’etude de la tourterelle des bois (*Streptopelia turtur*):
874 biologie, zoologie, chasse.

875 Dunn, J.C., Morris, A.J. and Grice, P.V. (2017). Post-fledging habitat selection in a rapidly
876 declining farmland bird, the European Turtle Dove *Streptopelia turtur*. *Bird Conservation*
877 *International* 27, 45–57. <https://doi.org/10.1017/S0959270916000022>

878 Dunn, J.C., Stockdale, J.E., McCubbin, A., Thomas, R.C., Goodman, S.J., Grice, P.V., Morris,
879 A.J., Hamer, K.C., and Symondson, W.O.C. (2016). Non-cultured faecal and gastrointestinal
880 seed samples fail to detect Trichomonad infection in clinically and sub-clinically infected
881 columbid birds. *Conservation genetics resources* 8, 97–99. [https://doi.org/10.1007/s12686-](https://doi.org/10.1007/s12686-016-0518-y)
882 016-0518-y

883 Dunn, J.C., Stockdale, J.E., Moorhouse-Gann, R.J., McCubbin, A., Hipperson, H., Morris,
884 A.J., Grice, P.V. and Symondson, W.O.C. (2018). The decline of the Turtle Dove: Dietary
885 associations with body condition and competition with other columbids analysed using high-
886 throughput sequencing. *Molecular Ecology* 27, 3386–3407.
887 <https://doi.org/10.1111/mec.14766>

888 Edgar, R. (2016). UNOISE2: improved error-correction for Illumina 16S and ITS amplicon
889 sequencing. *bioRxiv:081257*.

890 Edmunds, K., Bunbury, N., Sawmy, S., Jones, C.G. and Bell, D.J. (2008). Restoring avian
891 island endemics: use of supplementary food by the endangered Pink Pigeon (*Columba*
892 *mayeri*). *Emu-Austral Ornithology 108:74–80*. <https://doi.org/10.1071/MU06056>

893 Elliott, G.P., Merton, D. V. and Jansen, P.W. (2001). Intensive management of a critically
894 endangered species: the kakapo. *Biological Conservation 99:121–133*.
895 [https://doi.org/10.1016/S0006-3207\(00\)00191-9](https://doi.org/10.1016/S0006-3207(00)00191-9)

896 Eraud, C., Boutin, J.M., Riviere, M., Brun, J., Barbraud, C. and Lormee, H. (2009). Survival
897 of Turtle Doves *Streptopelia turtur* in relation to western Africa environmental conditions. *Ibis*
898 *151*, 186–190.

899 Fischer, I., Ashpole, J., Scallan, D., Poroud, T. and Carboneras C. (2018) European
900 Commission, Directorate-General for Environment, (2018). *International single species action*
901 *plan for the conservation of the European turtle-dove Streptopelia turtur (2018 to 2028)*,
902 Publications Office. <https://data.europa.eu/doi/10.2779/743376>

903 Fried, G., Petit, S., Dessaint, F. and Reboud, X., 2009. Arable weed decline in Northern France:
904 crop edges as refugia for weed conservation? *Biological Conservation, 142*, 238-243.
905 <https://doi.org/10.1016/j.biocon.2008.09.029>

906 GIEWS (2020). Global Information and Early Warning System, facilitated by the Food and
907 Agriculture Organization of the United Nations. Published on the internet;
908 <http://www.fao.org/giews/countrybrief/country.jsp?code=SEN&lang=en> Retrieved 16 March
909 2021

910 Gillings, S., Newson, S., Noble, D.G. and Vickery, J.A. (2005). Winter availability of cereal
911 stubbles attracts declining farmland birds and positively influences breeding population trends.
912 *Proceedings of the Royal Society B: Biological Sciences 272*, 733–739.
913 <https://doi.org/10.1098/rspb.2004.3010>

914 Guar, D., Peach, W. and Taylor, R. (2003) Summer diet and body condition of song thrushes
915 *Turdus philomelos* in stable and declining farmland populations. *Ibis 145*, 637-649.
916 <https://doi.org/10.1046/j.1474-919X.2003.00202.x>

917 Guti rrez-Gal n, A. and Alonso, C. (2016). European Turtle Dove *Streptopelia turtur* diet
918 composition in Southern Spain: the role of wild seeds in Mediterranean forest areas. *Bird Study*
919 *63*, 490–499. <https://doi.org/10.1080/00063657.2016.1236070>

920 Haraszthy, L. 1998. Magyarország madarai. Mezőgazda Kiadó, Budapest

921 Hothorn, T., Bretz, F. and Westfall, P. (2008). Simultaneous inference in general parametric
922 models. *Biometrical Journal* *50*, 346–363. <https://doi.org/10.1002/bimj.200810425>

923 Huson, D.H., Beier, S., Flade, I., G rska, A., El-Hadidi, M., Mitra, S., Ruscheweyh, H.J. and
924 Tappu, E. (2016). MEGAN Community Edition - Interactive Exploration and Analysis of
925 Large-Scale Microbiome Sequencing Data. *PLoS Computational Biology* *12*.
926 <https://doi.org/10.1371/journal.pcbi.1004957>

927 Jarry, G. and Baillon, F. (1991). Wintering of the turtledove (*Streptopelia turtur*) in Senegal:
928 study of a population in the region of Nianing. *Internal report. Paris: Centre of Research on*
929 *the population biology of birds.*

930 Jedlicka, J. A., Vo, A.-T.- E., & Almeida, R. P. (2017). Molecular scatology and high-
931 throughput sequencing reveal predominately herbivorous insects in the diets of adult and
932 nestling Western Bluebirds (*Sialia mexicana*) in California vineyards. *The Auk*, *134(1)*, 116–
933 127. <https://doi.org/10.1642/AUK-16-103.1>

934 Kullber, c., Fransson, T. and Jakobsson, S. (1996). Impared predator evasion in fat blackcaps
935 (*Sylvia atricapilla*) *Proceedings of the Roayal Society of London. Series B: Biological Sciences*,
936 *263*, 1671-1675. <https://doi.org/10.1098/rspb.1996.0244>

937 Labocha, M.K., Hayes, J.P. Morphometric indices of body condition in birds: a review. *Journal*
938 *of Ornithology* **153**, 1–22 (2012). <https://doi.org/10.1007/s10336-011-0706-1>

939 Lamb, P., Hunter, E., Pinnegar, J., Creer, S., Davies, R. and Taylor, M. (2018). How
940 quantitative is metabarcoding: A meta-analytical approach. *Molecular Ecology* *28*, 420–430.
941 <https://doi.org/10.1111/mec.14920>

942 Litzow, M.A., Piatt, J.F., Prichard, A.K. and Roby, D.D. (2002). Response of pigeon guillemots
943 to variable abundance of high-lipid and low-lipid prey. *Oecologia* *132*, 286–295.
944 <https://doi.org/10.1007/s00442-002-0945-1>

945 Lormée, H., Barbraud, C., Peach, W., Carboneras, C., Lebreton, J., Moreno-Zarate, L., Bacon,
946 L. and Eraud, C. (2020). Assessing the sustainability of harvest of the European Turtle-dove
947 along the European western flyway. *Bird Conservation International*, 30(4), 506-521.
948 doi:10.1017/S0959270919000479

949 Mansouri, I., Al-Sadoon, M.K., Rochdi, M., Paray, B.A., Dakki, M. and Elghadraoui, L.
950 (2019). Diversity of feeding habitats and diet composition in the turtle doves *Streptopelia turtur*
951 to buffer loss and modification of natural habitats during breeding season. *Saudi Journal of*
952 *Biological Sciences* 26, 957–962. <https://doi.org/10.1016/j.sjbs.2018.11.006>

953 Marshall, E.J.P., Brown, V.K., Boatman, N.D., Lutman, P.J.W., Squire, G.R. and Ward, L.K.
954 (2003). The role of weeds in supporting biological diversity within crop fields*. *Weed Research*
955 43, 77–89. <https://doi.org/10.1046/j.1365-3180.2003.00326.x>

956 Martin, T.E. (1987). Food as a Limit on Breeding Birds: A Life-History Perspective. *Annual*
957 *Review of Ecology and Systematics* 18, 453–487.

958 Marx, M., Korner-Nievergelt, F. and Quillfeldt, P. (2016). Analysis of Ring Recoveries of
959 European Turtle Doves *Streptopelia turtur* — Flyways, Migration Timing and Origin Areas of
960 Hunted Birds. *Acta Ornithologica* 51, 55–70.
961 <https://doi.org/10.3161/00016454AO2016.51.1.005>

962 McCleery, R.A., Moorman, C.E. and Peterson, M.N. (2014). *Urban Wildlife Conservation :
963 Theory and Practice*. Springer.

964 Moorhouse-Gann, R.J. (2017). Ecological replacement as a restoration tool: disentangling the
965 impacts of Aldabra giant tortoises (*Aldbrachelys gigantea*) using DNA metabarcoding.
966 (Doctoral dissertation, Cardiff Univeristy).

967 Moorhouse-Gann, R.J., Dunn, J.C., De Vere, N., Goder, M., Cole, N., Hipperson, H. and
968 Symondson, W.O.C. (2018). New universal ITS2 primers for high-resolution herbivory
969 analyses using DNA metabarcoding in both tropical and temperate zones. *Scientific Reports* 8,
970 1–15. <https://doi.org/10.1038/s41598-018-26648-2>

971 Moorhouse-Gann, R.J., Kean, E.F., Parry, G., Valladares, S. and Chadwick, E.A. (2020).
972 Dietary complexity and hidden costs of prey switching in a generalist top predator. *Ecology*
973 *and Evolution* 10, 6395–6408. <https://doi.org/10.1002/ece3.6375>

974 Morel, G. (1987). La Touterelles des Bois, *Streptopelia turtur*, dans l'ouste african:
975 mouvements migratoires et régime alimentaire. *Malimbus 9*, 23-42

976 Morel, G. and Morel, M.Y. (1974) Recherches écologiques sur une savane sahélienne du Ferlo
977 septentrional, Sénégal : influence de la sécheresse de l'année 1972-1973 sur l'avifaune. Revue
978 d'Ecologie, Terre et Vie, Société nationale de protection de la nature, 1974, pp.95-123.

979 Morel, G. and Morel, M.Y. (1979). La Touterelle des bois dans l'extrême ouest-african.
980 *Malimbus 1*, 66-67

981 Morel, G. and Roux, F. (1966) Les migrateurs Paléarctiques au Sénégal. *Terre et Vie 113*, 19-
982 72, 163-176

983 Murton, R.K., Westwood, N.J. and Isaacson, A.J. (1964). The Feeding Habits of the
984 Woodpigeon *Columba palumbus*, Stock Dove *C. oenas* and Turtle Dove *Streptopelia turtur*.
985 *Ibis 106*, 174–188. <https://doi.org/10.1111/j.1474-919X.1964.tb03694.x>

986 National Research Council, (1996). *Lost Crops of Africa: Volume I: Grains*. National
987 Academic Press.

988 Newsome, T.M. and Van Eeden, L.M. (2017). Food waste is still an underappreciated threat to
989 wildlife. *Animal Conservation 20*, 405–406.

990 Newton, I. (2006). Can conditions experienced during migration limit the population levels of
991 birds? *Journal of Ornithology 147*, 146–166. doi:10.1007/s10336-006-0058-4

992 Newton, I. (2004a). Population limitation in migrants. *Ibis 146*, 197–226.
993 <https://doi.org/10.1111/j.1474-919X.2004.00293.x>

994 Newton, I. (2004b). The recent declines of farmland bird populations in Britain: An appraisal
995 of causal factors and conservation actions. *Ibis 146*, 579–600. <https://doi.org/10.1111/j.1474-919X.2004.00375.x>

997 Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., Mcglinn, D., Minchin, P.R.,
998 O'hara, R.B., Simpson, G.L., Solymos, P. and Henry, M. (2020). *Package 'vegan' Title*
999 *Community Ecology Package Version 2.5-7*.

1000 PECBMS (2017) Pan-European Common Bird Monitoring Scheme. Published on the internet;
1001 <http://www.ebcc.info/index.php?ID=631> Retrieved 13 March 2018

1002 Potts G.R. (1991) The environmental and ecological importance of cereal fields. In The
1003 Ecology of Temperate Cereal Fields (eds L.G. Firbank, N. Carter, J.F. Darbyshire & G.R.
1004 Potts), pp. 3-21. Blackwell Scientific Publications, Oxford.

1005 POWO (2019). Plants of the World Online, facilitated by the Royal Botanical Gardens, Kew.
1006 Published on the internet; <http://www.plantsoftheworldonline.org/> Retrieved 16 March 2021

1007 R Core Team (2020). R: A language and environment for statistical computing. R Foundation
1008 for Statistical Computing, Vienna, Austria. URL <https://www.R-project.org/>.

1009 Rayé, G., Miquel, C., Coissac E., Redjadj, C., Loison, A. and Taberlet, P. (2011). New insights
1010 on diet variability revealed by DNA barcoding and high-throughput pyrosequencing: chamois
1011 diet in autumn as a case study. *Ecological Research* 26, 265–276.
1012 <https://doi.org/10.1007/s11284-010-0780-5>

1013 Robb, G., McDonald, R., Chamberlain, D. and Bearhop, S. (2008). Food for thought:
1014 supplementary feeding as a driver of ecological change in avian populations. *Frontiers in*
1015 *Ecology and the Environment* 6, 476–484. <https://doi.org/10.1890/060152>

1016 Robinson, R. and Sutherland, W. (1999). The winter distribution of seed-eating birds: habitat
1017 structure, seed density and seasonal depletion. *Ecography* 22, 447–454.
1018 <https://doi.org/10.1111/j.1600-0587.1999.tb00581.x>

1019 Scott, P., Duncan, P. and Green, J.A. (2015). Food preference of the Black-headed Gull
1020 *Chroicocephalus ridibundus* differs along a rural–urban gradient. *Bird Study* 62, 56–63.
1021 <https://doi.org/10.1080/00063657.2014.984655>

1022 Schloss, P.D., Westcott, S.L., Ryabin, T., Hall, J.R., Hartmann, M., Hollister, E.B.,
1023 Lesniewski, R.A., *et al.* (2009). Introducing mothur: Open-Source, Platform-Independent,
1024 Community-Supported Software for Describing and Comparing Microbial Communities.
1025 *Applied and Environmental Microbiology* 75, 7537–7541.
1026 <https://doi.org/10.1128/AEM.01541-09>

1027 Shutt, J.D., Nicholls, J.A., Trivedi, U.H., Burgess, M.D., Stone, G.N., Hadfield, J.D. and
1028 Phillimore, A.B., 2020. Gradients in richness and turnover of a forest passerine's diet prior to
1029 breeding: A mixed model approach applied to faecal metabarcoding data. *Molecular*
1030 *ecology*, 29(6), 1199-1213. <https://doi.org/10.1111/mec.15394>

1031 Storkey, J., Meyer, S., Still, K.S. and Leuschner, C., 2012. The impact of agricultural
1032 intensification and land-use change on the European arable flora. *Proceedings of the Royal*
1033 *Society B: Biological Sciences* 279,1421-1429. <https://doi.org/10.1098/rspb.2011.1686>

1034 Svensson, L. (1992) *Identification Guide to European Passerines*. Published privately.
1035 Stockholm, Swededn.

1036 Taberlet, P., Boulin, A., Zinger, L. and Coissac, E. (2018) *Environemntal DNA: For*
1037 *biodiversity research and monitoring*. Oxford Univeristy Press.

1038 Tryjanowski, P., Hartel, T., Báldi, A., Szymański, P., Tobolka, M., Herzon, I., Goławski, A.,
1039 Konvička, M., Hromada, M., Jerzak, L. and Kujawa, K. (2011). Conservation of farmland birds
1040 faces different challenges in Western and Central-Eastern Europe. *Acta Ornithologica* 46, 1–
1041 12. <https://doi.org/10.3161/000164511X589857>

1042 UK Government (2021). *SP9: Threatened Species Supplement - GOV.UK* [Online]. Available
1043 at: [https://www.gov.uk/countryside-stewardship-grants/threatened-species-supplement-](https://www.gov.uk/countryside-stewardship-grants/threatened-species-supplement-sp9#using-this-supplement-for-turtle-dove)
1044 [sp9#using-this-supplement-for-turtle-dove](https://www.gov.uk/countryside-stewardship-grants/threatened-species-supplement-sp9#using-this-supplement-for-turtle-dove) [Accessed: 1 December 2023].

1045 Verhulst, J., Báldi, A. and Kleijn, D. (2004). Relationship between land-use intensity and
1046 species richness and abundance of birds in Hungary. *Agriculture, Ecosystems and Environment*
1047 *104*, 465–473. <https://doi.org/10.1016/j.agee.2004.01.043>

1048 Wang, Y., Naumann, U., Wright, S.T. and Warton, D.I. (2012). Mvabund- an R package for
1049 model-based analysis of multivariate abundance data. *Methods in Ecology and Evolution* 3,
1050 471–474.

1051 Wanless, S., Harris, M.P., Redman, P. and Speakman, J.R. (2005). Low energy values of fish
1052 as a probable cause of a major seabird breeding failure in the North Sea. *Marine Ecology*
1053 *Progress Series* 294, 1–8. doi:10.3354/meps294001

1054 Wickamn, H. (2011) “ggplot2”. Wiley Interdisciplinary Reviews: Computational Statistics, 3,
1055 180-185

1056 Will, A., Watanuki, Y., Kikuchi, D.M., Sato, N., Ito, M., Callahan, M., Wynne-Edwards, K.,
1057 Hatch, S., Elliot, K., Slater, L. and Takahashi, A. (2015). Feather corticosterone reveals stress
1058 associated with dietary changes in a breeding seabird. *Ecology and Evolution* 5, 4221–4232.
1059 <https://doi.org/10.1002/ece3.1694>

1060 Zwarts, L., Bijlsma, R., Van der Kamp, J. and Wymenga, E. (2009). *Living on the Edge:*
1061 *Wetlands and Birds in a Changing Sahel*. Zeist: KNNV Publishing.

1062 Zwarts, L., Bijlsma, R.G. and van der Kamp, J. (2018). Large decline of birds in Sahelian
1063 rangelands due to loss of woody cover and soil seed bank. *Journal of Arid Environments* 155,
1064 1–15. <https://doi.org/10.1016/j.jaridenv.2018.01.013>

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Country	Year	Season	Sample size	SR range	Mean SR \pm S.E.	Significant pairwise differences
Senegal	2014/15	Winter 1 (W1)	25	1-5	2.920 \pm 0.237	AB
Senegal	2015/16	Winter 2 (W2)	14	1-6	3.214 \pm 0.482	A
Senegal	2016/17	Winter 3 (W3)	28	1-5	2.536 \pm 0.260	AB
Senegal	2017/18	Winter 4 (W4)	61	1-6	1.918 \pm 0.147	B
Senegal	Total		128	1-6	2.391 \pm 0.121	
France	2018	Spring 1 (S1)	63	1-7	3.381 \pm 0.350	A
Hungary	2019	Spring 2 (S2)	12	1-6	3.583 \pm 0.288	A
Overall			203	1-7	2.768 \pm 0.100	
France (bait removed)	2018	Spring 1 (S1)	38	1-4	1.711 \pm 0.155	

Table 1: Species richness (SR) detected from faecal samples, per country and year sampled. Mean SR differed between years sampled in Senegal, (GLM; $Dev_{3,124}=12.676$, $p=0.005$, $N=128$), and between sampling seasons, (GLM; $Dev_{5,197}=30.199$, $p<0.001$, $N=203$). Letters (A-B) show significant difference according to Tukey's post-hoc test: species richness was lower in W4 than in S1, S2 and W2. Species richness for France was also included following removal of taxa found in bait.

	% Individuals sampled with wild/cultivated taxa detected in diet		% Individuals sampled with only wild/cultivated taxa detected in diet		% Dietary items accounted for by wild/cultivated taxa	
	Wild	Cultivated	Only wild	Only cultivated	Wild	Cultivated
W1	88.0%	68.0%	32.0%	12.0%	67.2±6.6	32.8±6.6
W2	85.7%	78.6%	21.4%	14.3%	58.9±8.6	41.1±8.6
W3	67.9%	96.4%	3.6%	32.1%	36.3±5.6	63.7±5.6
W4	45.9%	86.9%	13.1%	54.1%	30.7±4.7	69.3±4.8
S1 (full data set)	91.3%	95.2%	4.8%	39.7%	28.0±3.6	72.0±3.7
S1 (bait removed)	100.0%	10.5%	89.5%	0.0%	95.4±2.1	3.9±2.0
S2	91.7%	33.3%	66.7%	8.3%	80.6±9.9	19.4±9.9

1066

1067 Table 2: Summary of levels of wild plants (n=93) and cultivated plants (n=30) consumed in each sampling season in Senegal (W1-W4), France
1068 (S1) and Hungary (S2). First two columns represent the percentage of individual birds in which wild or cultivated taxonomic units were detected.
1069 Middle two columns show the percentage of individual birds in which the diet consisted solely of either wild or cultivated seeds. Last two columns
1070 show mean percentage of diet (±S.E) accounted for by wild and cultivated plants.

	SR range	Mean SR \pm S.E.	Proportion wild taxa	Proportion cultivated taxa	Proportion Asteraceae	Proportion Poaceae
Repeat 1	1-5	2.583 \pm 0.417	0.581	0.419	0.194	0.419
Repeat 2	1-6	3.083 \pm 0.379	0.541	0.459	0.194	0.581

1071

1072 Table 3: Variation in species richness and proportion of taxonomic units accounted for by
1073 wild and cultivated taxa, and two of the dominant families detected between repeated samples
1074 to assess consistency of Illumina sequencing.

	Jaccard distance		N. taxa recorded	N. taxa in common
B02	0.288	Repeat 1	4	4
		Repeat 2	5	
B24	0.426	Repeat 1	3	3
		Repeat 2	3	
EG82510	0.215	Repeat 1	4	4
		Repeat 2	4	
EP38521	0.164	Repeat 1	1	1
		Repeat 2	1	
EP38582	0.276	Repeat 1	3	2
		Repeat 2	2	
EY46975	0.785	Repeat 1	1	1
		Repeat 2	2	
EZ11354	0.786	Repeat 1	1	1
		Repeat 2	2	
EZ11358	0.490	Repeat 1	5	4
		Repeat 2	4	
EZ11376	0.413	Repeat 1	2	2
		Repeat 2	3	
GY149801	0.130	Repeat 1	1	1
		Repeat 2	1	
GY149826	0.504	Repeat 1	2	2
		Repeat 2	3	
GY154243	0.461	Repeat 1	4	4
		Repeat 2	6	
GY154862	0.494	Repeat 1	4	4
		Repeat 2	4	
GY161023	0.274	Repeat 1	4	4
		Repeat 2	4	
GY161034	0.591	Repeat 1	1	1
		Repeat 2	3	
HA70752	0.019	Repeat 1	3	3

		Repeat 2	3
1075			
1076	Table 4: Jaccard distance and the number of taxa recorded in each repeated sample to assess		
1077	repeatability of Illumina sequencing		