

# Azores Woodpigeon's diet and its spatio-temporal variation - a preliminary evaluation based on non-invasive sampling methods

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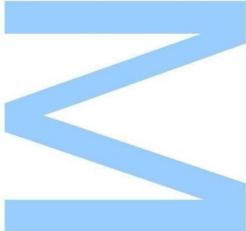
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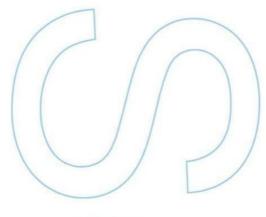
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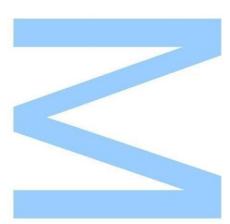


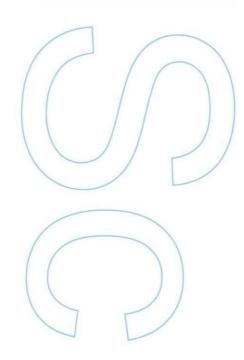




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Rui Filipe Moreira dos Santos

Paredes, 30 de setembro de 2022

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### Summary

The Columbidae family is composed of pigeons and doves which play an important ecological role as seed dispersers of a wide variety of plants, from grasses to large-sized fruit species. Furthermore, they colonise and restore isolated and fragmented areas, such as the Azores. The woodpigeon (*Columba palumbus*) colonised the archipelago between 70,000 and 380,000 years ago, back when the Azores' landscape was dominated by the laurel forest, composed of endemic species from Macaronesia.

The relevance of endemic pigeons for laurel forest as seed dispersers has been shown in other archipelagos of Macaronesia, but in the Azores, the ecological relationship of the Azores woodpigeon (*C. p. azorica*) with the plant resources of the native forests remains unclear. The same applies to its role in the dispersal of native plants and its impact on several crops because it may consume exotic and native species. The nominate subspecies, *C. p. palumbus*, feeds on plant material and, occasionally, on invertebrates. Recently, a genetic and morphological comparative study between *C. p. palumbus* and the endemic *C. p. azorica* confirmed a slight differentiation.

Our work aims to study the trophic relation of *C. p. azorica* with the plant species on Pico Island, in the Azores archipelago. The niche of the endemic subspecies and its ecological role are yet to be known. We intend to investigate about *C. p. azorica* influence on native and exotic plant species and on spatio-temporal diet variation.

The faecal samples were collected twice per season from May 2021 until January 2022, in 50 transects. Two primers were selected for the molecular analysis, the fwh1F1-R1 (CO1), which validated the sample host, and the UniplantF-R (ITS2), which amplified any plant sequences present in the faeces. Cumulative curves were used to evaluate

*C. p. azorica* diet niche-width. Generalised linear models were used to assess the seasonal and land cover effects on diet average richness and composition. Finally, a Fourth Corner Analysis was also used to provide insights for relationships between OTUs and season, and OTUs and land cover. The amplified fragments of CO1 suggested that 52.1% of the 365 collected samples corresponded to *C. p. azorica*. The amplified samples of ITS2 produced 111 unique OTUs, ofwhich, one was identified to the family level, 36 to the genus level and 74 to the species level.

Regarding richness, the seasons influenced the niche-width, i.e., diet showed lower diversity in winter than in summer or autumn. The average richness was influenced by land cover in every season except winter, i.e., in summer, agriculture area negatively affected the average richness, opposite to urban land area which positively affected the average richness.

In autumn, native forest positively affected the average richness, while no land cover type had influence on average richness in winter.

Relating to composition, in summer, diet presented differences related with urban land and natural land, with the abundance of the exotic weed *Crepis* sp1. inversely proportional to urban land area. In Autumn, diet showed differences related to native forest, with the native tree *llex perado*, and to natural land, with the exotic weed *Poa* sp1. Both plant species showed abundances proportional to their respective land cover area. In winter, native forest, with *Myrsine africana*, and agriculture, with *Ranunculus repens*, were more frequent in their respective land cover than in others.

Our work provides insights on the Azores Woodpigeon's diet, concerning the influence of season and land cover in diet richness and composition. Thus, *C. p. azorica* conservation measures can be directed to the depleted native plant resources, including weeds (e.g. *Epilobium obscurum, Rumex acetosella* and *Holcus lanatus*), shrubs (e.g. *Erica azorica* and *Viburnum treleasei*), and trees (*Morella faya* and *Ilex perado*). We also found that, in addition to native plants, C. p. azorica diet also includes exotic plants. Consequently, Azores Woodpigeon may be contributing significantly to the dispersion of both type of plants. Further research on seed dispersal is necessary to better evaluate the impact of the endemic Azorean woodpigeon in the island landscape.

Keywords: Azores, Columba palumbus, diet, ecology, endemism, land cover, metabarcoding, season.

### Resumo

A família Columbidae é composta por pombos e rolas, que desempenham um papel ecológico fundamental enquanto dispersores de sementes de uma grande variedade de plantas, desde gramíneas a árvores frutíferas de grande porte. Além disso, colonizam e restauram áreas isoladas e fragmentadas, como os Açores. O pombo-torcaz (*Columba palumbus*) colonizou o arquipélago entre 70 000 e 380 000 anos atrás, quando a paisagem dos Açores era dominada pela floresta laurissilva, composta por espécies endémicas da Macaronésia.

A importância dos pombos endémicos para a floresta laurissilva enquanto dispersores de sementes foi demonstrada noutros arquipélagos da Macaronésia, mas nos Açores, a relação ecológica do pombo-torcaz-dos-Açores (*C. p. azorica*) com os recursos vegetais da floresta nativa permanece pouco clara. O mesmo se aplica ao seu papel na dispersão de plantas nativas e ao seu impacto em várias culturas, porque poderá consumir tanto espécies nativas como exóticas. A subespécie nominal, *C. p. palumbus*, alimenta-se de matéria vegetal e, ocasionalmente, de invertebrados. Recentemente, um estudo genético e morfológico comparativo entre *C. p. palumbus* e o endemismo *C. p. azorica* confirmou uma ligeira diferenciação.

O nosso trabalho visa estudar a relação trófica de *C. p. azorica* com as espécies vegetais da Ilha do Pico, no arquipélago dos Açores. O nicho da subespécie endémica e o seu papel ecológico ainda estão por desvendar. Pretendemos investigar sobre a influência de *C. p. azorica* nas plantas nativas e exóticas e a variação espaciotemporal da dieta.

As amostras fecais foram recolhidas duas vezes por estação entre maio de 2021 e janeiro de 2022, ao longo de 50 transectos. Foram selecionados dois primers para a análise molecular, o fwh1F1-R1 (CO1), que validou o hospedeiro da amostra, e o UniplantF-R (ITS2), que amplificou quaisquer sequências de plantas presentes nas fezes. Recorreu-se a curvas cumulativas para avaliar a diversidade da dieta ("amplitude de nicho") de C. p. azorica. Utilizaram-se modelos lineares para avaliar os efeitos das estações e da cobertura do solo sobre a riqueza média e a composição da dieta. Por fim, foram realizadas duas análises de quadrantes ("Fourth Corner Analysis") para obter informações sobre as relações entre as OTUs (sigla em inglês para Unidades Taxonómicas Operacionais) e as estações do ano e também entre as OTUs e a cobertura do solo. Os fragmentos amplificadospelo CO1 sugeriram que 52,1% das 365 amostras fecais recolhidas correspondiam a *C. p. azorica*. As amostras amplificadas pelo ITS2 produziram 111 OTUs únicas, das quais, uma foi identificada à família, 36 ao género e 74 à espécie.

Relativamente à riqueza, as estações influenciaram a diversidade, ou seja, a dieta apresentou um valor de diversidade inferior no inverno comparativamente ao verão ou ao outono. A riqueza média foi influenciada pela cobertura do solo em todas as estações do ano, exceto no inverno. No verão, a agricultura afetou negativamente a riqueza média, ao contrário do terreno urbano que afetou positivamente a riqueza média. No outono, a floresta nativa afetou positivamente a riqueza média, enquanto nenhuma cobertura do solo afetou a riqueza média no inverno.

Relativamente à composição, no verão, a dieta apresentou diferenças relacionadas com o terreno urbano e terreno natural, com a abundância da erva exótica *Crepis* sp1. inversamente proporcional à área do terreno urbano. No outono, observaram-se diferenças relacionadas com a floresta nativa, com a árvore nativa *llex perado*, e com o terreno natural, com a erva daninha exótica *Poa* sp1. Ambas as espécies vegetais mostraram abundâncias proporcionais às áreas das respetivas coberturas do solo. No inverno, o arbusto exótico, *Myrsine africana*, na floresta nativa, e a erva exótica, *Ranunculus repens*, na agricultura, apresentaram abundâncias superiores nas suas respetivas áreas de cobertura do solo em relação a outras coberturas.

O nosso trabalho fornece informações sobre a influência da estação do ano e da cobertura do solo na riqueza e na composição da dieta do pombo-torcaz-dos-Açores. Assim, as medidas de conservação destinadas ao *C. p. azorica* podem ser dirigidas aos recursos vegetais nativos, incluindo ervas daninhas (*Epilobium obscurum*, *Rumex acetosella* e *Holcus lanatus*), arbustos (*Erica azorica* e *Viburnum treleasei*) e árvores (*Morella faya* e *llex perado*). Também constatamos que, para além de plantas nativas, a dieta do C. p. azorica também inclui plantas exóticas. Consequentemente, o pombo-torcaz-dos-Açores pode estar a contribuir significativamente para a dispersão de ambos os tipos de plantas. É necessária mais investigação sobre a dispersão de sementes para melhor avaliar o impacto do pombo-torcaz-dos-Açores na paisagem da ilha.

Palavras-chave: Açores, cobertura do solo, *Columba palumbus*, dieta, ecologia, endemismo, estação, *metabarcoding*.

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### List of Abbreviations

ANOVA – Analysis of Variance CO1 - Cytochrome C Oxidase Subunit I COSA2018 - Carta de Ocupação do Solo da Região Autónoma dos Açores 2018 ESVs - Exact Sequence Variants GIS - Geographical Information System GLM - Generalised Linear Model IFRAA 2007 - Inventário Florestal da Região Autónoma dos Açores 2007 ITS2 - Internal Transcribed Spacer 2 OGTR - Office of the Gene Technology Regulator OTUs - Operational Taxonomic Units ZOTUs - Zero-radius Operational Taxonomic Units

### 1. Introduction

#### 1.1. Literature Review

The Columbidae family is constituted of pigeons and doves which occur worldwide except in Antarctica (Gibbs et al., 2001; Winkler et al. 2020). They play an important ecological role as seed dispersers of a wide variety of plants, from grasses to large-sized fruit species, due to their strong flying ability and medium to large size, associated with a diversified diet (Gibbs et al., 2001). Pigeons fulfil a particularly important task for plant colonisation and restoration of isolated and fragmented areas, favouring the arrival and establishment of plant species even on remote islands, such as the Azores. The woodpigeon (Columba palumbus) occurs in the Palearctic Region and subdivides into five subspecies, among which thenominate (C. p. palumbus) and the endemic Azores woodpigeon (C. p. azorica). In the Azores archipelago, over the last few centuries, the area occupied by native land covershas regressed and the laurel forest has been particularly diminished for agriculture, grazing and timber production (Fernández-Palacios et al., 2011). The primordial evergreen humid forest composed of endemic species (Elias et al., 2016; Pavão et al., 2019) - dominated most of the Azores islands' landscape until the arrival of human settlers in the 15<sup>th</sup> century. Currently, it is highly fragmented and consequently, it is listed in the EU Habitat Directive AnnexI due to its area reduction estimated at 98% (Guimarães & Olmeda, 2008).

The importance of endemic pigeons for laurel forest as seed dispersers has been shown in other Macaronesian archipelagos: *Columba trocaz* in Madeira, *C. bollii* and *C. junoniae* in the Canary Islands (Oliveira et al., 2002; Marrero et al., 2004; Marrero, 2009). In the Azores, the ecological relationship of the endemic *C. p. azorica* with the plant food resources of the natural forests remains unclear, although it has long been assumed that pigeons might have been important vectors of tree diaspores to the islands by endozoochory (Guppy, 1917).

A study by Gonzalez et al. (2009) pointed to two distinct events of colonization of the Canary Islands by pigeons, the first originated *C. junoniae* (20 Mya) and the other originated *C. bollii* (5 Mya). Another study by Dourado et al. (2014) proposed a splitting event to explain the non-monophyletic pattern for the insular endemic species, based on mitochondrial (cytochrome b and cytochrome c oxidase I) and nuclear ( $\beta$ -fibrinogen intron 7) genetic markers. This is, the event must have originated two clades, one common ancestor originated

*C. bollii* in the Canary Islands and *C. trocaz* in Madeira, while other common ancestor derived *C. p. palumbus* in mainland Europe and *C. p. azorica* in the Azores.

The Woodpigeon colonised the archipelago between 70,000 and 380,000 years ago (Valente et al., 2017), therefore, the native laurel forest was its original habitat in the Azores. The Azorean population was considered to represent an endemic subspecies in 1905 (Hartert

& Ogilvie-Grant, 1905) due to morphological differences with the continental C. p. palumbus. Recently, patterns of genetic and morphological divergence between these two taxa were assessed (Andrade et al., 2016; Cataldo, 2017; Andrade et al., 2021), confirming that C. p. azorica has already undergone slight differentiation. Two other columbids occur in the Azores, the Rock pigeon (C. livia), whose populations are constituted by feral individuals; and the Eurasian collared dove (Streptopelia decaocto), first recorded at the archipelago in 2006 supposedly as a consequence of a natural expansion from the continent and currently breeding in Pico and other Azorean islands (Rodrigues et al., 2010). C. p. azorica was a game species until 1992 when it was included in the EU Birds Directive Annex I. It was classified as "data deficient" in the Portuguese Red Data Book, which pointed to illegal hunting and habitat loss as the main threats (Almeida et al., 2005). A survey from 2003 suggested that it was more abundant than previously thought (Dickens & Neves, 2005). In 2014, an annual survey was implemented on all islands to evaluate population trends (Fontaine et al., 2015). This showed that the bird was very common on all the islands of the central group and São Miguel and less abundant on Santa Maria, Flores and Corvo, and that it occurs mostly at low and midelevations (rarely above 800 metres above sea level) in a wide array of habitats such as natural forests, exotic woodland, agricultural fields and urban areas. A study of habitat use patterns on Terceira also suggested an altitudinal zonation, with a decrease in abundance withelevation and a preference for Eucalyptus globulus and Cryptomeria japonica plantations and cultivated areas, which are among the more common land covers in the island (Santos, 2017). The C. p. azorica low abundance in natural areas was likely explained by the presence of thisland cover mostly at high elevations (Santos, 2017). The first data on breeding biology was also gathered in 2014 on Terceira, revealing low values of breeding success and suggesting that the species does not depend on natural forests for nesting (Fontaine et al., 2019). Low breeding success was later confirmed by a 2-year nest monitoring on Pico, which highlighted a preference for fire tree (Morella faya) forests for nesting (Fontaine et al. 2021).

Over the recent years, *C. p. azorica* has been often associated with crop damage, especially in vineyards which, in the Azores, currently cover an area of about 1389 ha (SREA, 2019). This promoted further investigations on Pico to evaluate the impact of the species on vineyards (Fontaine et al., 2018). In 2017 and 2018, vineyards were monitored during the ripening stage; in both years, 67% of all bunches under survey had at least one grape consumed and, overall, about 10% of all grapes were consumed (Fontaine et al., 2018). Data from trail cameras suggest that the Azores Woodpigeon rarely consumed grapes, contrasting with winegrowers' opinion (Fontaine et al., 2018). Grape damages were mostly attributed to *Teira dugesii, Rattus* sp., *Turdus merula azorensis* and *Fringilla coelebs moreletti* (Fontaine et al., 2018). Another study by Lamelas-López & Marco (2021), in Terceira (2016 - 2017), reached similar results.

Other crops might also constitute an important food resource for the Azores woodpigeon. Pastures currently cover *ca.* 42% of the area of the archipelago (Monteiro et al., 2008) which is almost 100,000 ha. Corn (mostly maize-forage) constitutes the main temporary crop, with a production area of 10,668 ha (SREA, 2019). The species is commonly seen feeding on pastures and cornfields, sometimes gathering in large groups that can reach hundreds of individuals. Feeding on pastures and several cereal crops was commonly found for the *C. p. palumbus* in mainland Europe, considered to have increased in numbers and range following the development of agriculture and balancing its diet according to food availability (Negrier et al., 2021). In Madeira, the endemic *Columba trocaz* consumes fruits in winter and autumn (when fruit availability in laurel forest is higher) and leaves in spring and summer – and tends to specialise in crops, far from forest areas (Marrero et al., 2004), with important implications for conservation and management given damages caused on cabbage crops.

The feeding habits of *C. p. palumbus* have been well documented along its native distribution. The nominal subspecies' diet varies with season and locality (Cramp, 1998; Gutiérrez et al., 2017) and is mostly based on plant material such as green leaves, seeds, berries, buds, flowers and root crops and it occasionally feeds on invertebrates (Rouxel & Czajkowski, 2004; Gutiérrez et al., 2017). In winter, *C. p. palumbus* usually feeds on clover leaves, lettuce, cabbage, peas and sugar beet (Cramp, 1998; Gutiérrez et al., 2017). In spring, the diet includes buds and flowers of ash (*Fraxinus* spp.), beech (*Fagus* spp.), hawthorn (*Crataegus* spp.) or plum (*Prunus* spp.), and in cultivated areas, cereal and legume sowings (Cramp, 1998; Gutiérrez et al., 2017). In summer, it mostly feeds on ripe cereals from standing crops or stubbles but also consumes berries and drupes (Cramp, 1998; Rouxel & Czajkowski,2004; Gutiérrez et al., 2017). In autumn, the diet includes ripe tree fruits such as beech mastsand acorns (Murton et al., 1964; Gutiérrez et al., 2017).

Considering the actual knowledge acquired about the feeding habits of *C. p. palumbus* in mainland Europe, and the observations made in the Azores, it is expected for *C. p. azorica* to have a diversified and plastic diet and not dependent on natural vegetation. The Azorean subspecies may consume both invasive species, such as *Pittosporum undulatum*, currently problematic in the Azores (Silva et al., 2008) and native species that produce fleshy fruits, such as *Laurus azorica*, *Morella faya* and *Picconia azorica*. Therefore, it is expected for *C. p. azorica* to be either a disperser of invasive species or an agent in the regeneration of native forest remains, depending on its feeding habitats.

The *C. p. azorica* diet was assessed for the first time using non-invasive samples (faeces) by molecular DNA metabarcoding (DNA barcodes coupled with next-generation sequencing).

This method has been increasingly used in dietary analysis to study trophic interactions givenits precision and cost-effectiveness (Moorhouse-Gann et al., 2018). It has been successfully applied to non-invasive samples to describe the diet of many animals, including pigeons and doves (Ando et al., 2013; Dunn et al., 2017).

#### 1.2. Objectives

The main objective of this dissertation is to develop a preliminary study on *C. p. azorica* diet and its trophic interactions, with emphasis on native forests, exotic woodland and agricultural fields. In particular, this work aims to:

- Investigate the *C. p. azorica* diet and its temporal variation, resorting to non- invasive methods, specifically, the analysis of faecal samples collected for almost oneyear (from May 2021 to January 2022) on Pico Island.
- Relate the *C. p. azorica* diet to land cover to better understand the *C. p. azorica* trophic ecology and evaluate food selection and seasonal fluctuations in diet diversity.

### 2. Methods

#### 2.1. Study area

The study was conducted on Pico Island located in the Azores archipelago, which consists of nine islands of volcanic origin located in the North Atlantic, at *ca.* 1,600 km west of mainland Portugal. The islands are distributed along the Terceira rift in a northwest-southeast orientation and are geographically divided into three groups: the Western Group (Flores and Corvo), the Central Group (Pico, Faial, São Jorge, Graciosa and Terceira) and the Eastern Group (São Miguel and Santa Maria). Pico Island is the second largest of the archipelago, with about 44 500 ha, an oval shape, elongated in the east-west direction and a coastline of 151.8 km (Gil et al., 2017).

Pico Island offers adequate features to conduct this study as, for example, it holds 41% of the total area of native forest on the Azores (Gaspar et al., 2008) and an abundant *C. p. azorica* population (Fontaine et al., 2015). Additionally, Pico's wine producers frequently complain about crop damage, presumably due to *C. p. azorica* consuming grapes. Vineyards are one of the main regional crops in terms of cultivated area and production and the majority of vineyards are located on Pico Island.

#### 2.2. Sample collection

For the collection of faecal samples, we established transects using open source GIS software QGIS and two complementary digital cartographies: 2018 Regional Land Cover Map (Carta de Ocupação do Solo da Região Autónoma dos Açores – COSA2018; DRRF, 2018) and 2007 Regional Forest Inventory (Inventário Florestal da Região Autónoma dos Açores – IFRAA2007; DRRF, 2007). The habitats above 900 metres altitude and not used regularly by pigeons were not included, as *C. p. azorica* was not observed in those sites on Pico Island in a previous study (Fontaine et al., 2015). Three main land covers were considered: native forests, exotic forests and agricultural areas.

For native forests, the classification proposed by Elias et al. (2016) was followed: *Erica-Morella* coastal woodlands (occurring between 0~100m above sea level), *Picconia-Morella* lowland forests (between 100~300m a.s.l.), *Laurus* submontane forests (between 300~600m a.s.l.) and *Juniperus-Ilex* montane forests (between 600~900m a.s.l.). Given that these natural land covers were not mapped and did not have equivalent classes in COSA2018 and IFRAA2007, we crossed their altitudinal zonation with natural forests ("3.1.3. Florestas naturais") and scrublands ("3.1.6. Matos") of COSA2018 to estimate their distributions. Native land covers at higher elevations were not included given the very low occurrence of *C. p. azorica* in the past years during field observations for other studies (Fontaine et al., 2015; Santos, 2017).

For exotic forests, three classes were considered according to the dominant tree species: *Pittosporum undulatum* (Pittosporum forest), *Cryptomeria japonica* (Cryptomeria forest) and *Pinus* spp. (Pine forest). Data about dominant tree species were retrieved from IFRAA 2007 and overlaid to "exotic forest" classes of COSA2018.

Agricultural areas included three land covers: vineyards (the main permanent crop of the Azores), cornfields (the main temporary crop) and pastures, which currently cover about 18% of the area of Pico Island. We considered the following COSA2018 classes: permanent cultures ("2.1.2 Culturas permanentes") for vineyards; arable lands ("2.1.1 Terras aráveis") for cornfields; and pastures ("2.1.3 Prados/pastagens") and natural herbaceous vegetation ("3.1.5 Vegetação herbácea natural").

A total of 50 transects were defined proportionally to the area occupied by each land cover on the island (Figure 1), therefore, between four and six transects were randomly sorted per land cover (see Attachment - Table S1). Transects were set to 100 metres, due to the highly fragmented Azorean landscape. The feasibility of the transects was verified (access, type of land cover) and, when necessary, their location was adjusted.

Each transect was walked twice per season from May 2021 until January 2022, except in the bimester September-October 2021 when only one visit occurred (Figure 2). Three

seasons were considered based on bimesters: summer (corresponding to sample collection of June and July), autumn (corresponding to sample collection of September and October) and winter (corresponding to sample collection of December and January). In each visit to a transect, the surrounding areas were also prospected for faeces. Additionally, faeces found by spotting birds when travelling between transects were also collected.

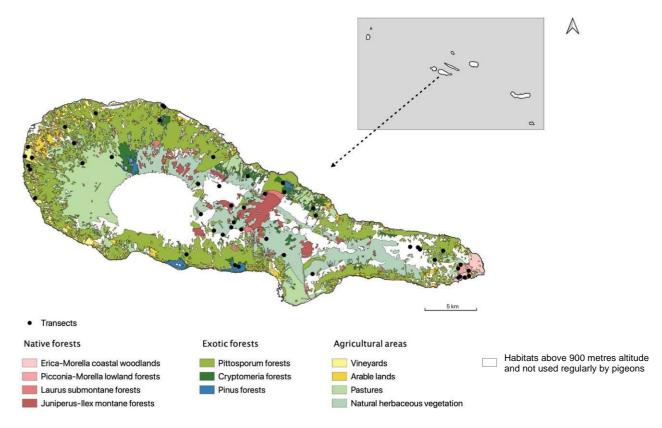


Figure 1. Location of Pico Island on the Central Group of the Azores Archipelago. Land cover types are represented by different colours and the location of the transects are represented by black circles. White areas represent habitats and elevations not used by pigeons.

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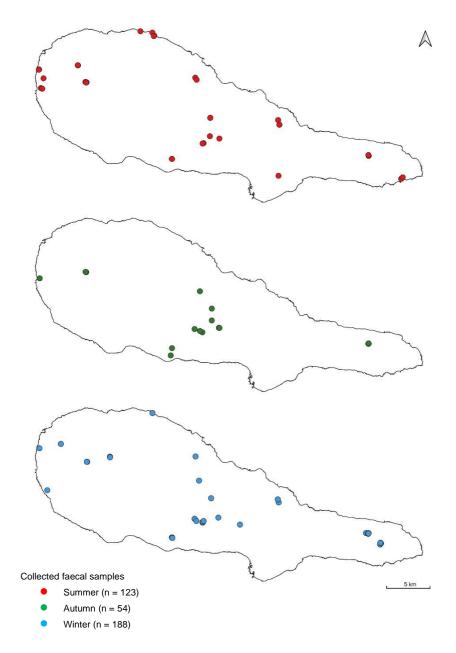


Figure 2. Location of the collected faecal samples on Pico Island in each season.

The faecal samples were collected in a non-invasive way, i.e., the faeces were collected without causing disturbance to C. p. azorica. 365 faecal samples were collected from the ground and stonewalls with tweezers, using a scalpel to remove any material in contact with the substrate. To avoid DNA contamination, those instruments were sterilised between each sample collection by flaming after immersion in 96% ethanol. Only faeces whose size (large) and/or shape (spiralon an edge) suggested that they came from C. p. azorica were collected. The coordinates of each sample were registered using a GPS device and their locations were compiled in a Geographical Information System (GIS). Samples were labelled and preserved in 8 mL tubes with 96% ethanol at room temperature until laboratory processing.

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#### 2.3. Laboratory work

DNA was extracted from samples in a non-invasive dedicated room to avoid contamination from high-quality samples. Equipment was sterilised with bleach and exposed to UV light before and after usage. Samples were extracted using the NORGEN Stool DNA Isolation Kit to maximise the amount of DNA obtained while reducing the amount of PCR inhibitors. Each extraction batch was composed of 23 samples plus a negative control tube, containing everything except the sample, to exclude procedural contamination. Since pellets were quite big and compact, each one was grinded against the tube wall and homogenised with a sterilised spatula before a subsample was removed for DNA extraction. Extraction procedures followed the manufacturer's recommendations, except that two elutions of 50  $\mu$ L were done.

To confidently assign the identity of the collected samples to *C. p. azorica*, a fragment of the cytochrome c oxidase subunit I (CO1) gene was amplified using the fwhF1-R1 primers (Vamos et al., 2017), modified with Illumina adaptors. PCR reactions consisted of 5  $\mu$ L of QIAGEN Multiplex PCR Master Mix, 0.3  $\mu$ L of each forward and reverse primer, 2.4  $\mu$ L of water, and 3  $\mu$ L of DNA. PCR cycling conditions consisted in an initial denaturation step at 95°C for 15 min, followed by 45 cycles of 95°C denaturing for 30 s, annealing at 56°C for 30 s, extension at 72°C for 30 s, and a final extension at 72°C for 10 min. To determine plant diet composition, the Internal transcribed spacer 2 (ITS2) region was amplified using the UniplantF-R primers (Moorhouse-Gann et al., 2018; Silva et al., 2019), also modified with Illumina adaptors. PCR reactions were as before, while cycling conditions consisted in an initial denaturation step at 95°C for 30 s, annealing at 56°C for 30 s, annealing at 56°C for 30 s, annealing at 56°C for 15 min, followed by 45 cycles of 95°C denaturing conditions consisted in an initial denaturation step at 95°C for 15 min, followed by 45 cycles of 95°C denaturing for 30 s, annealing at 56°C for 10 min. Amplification success and quality were checked by running the PCR products in 2% agarose gels stained with GelRed.

PCR products were diluted 1:4 with water and indexed with distinct combinations of custom 7 bp long P5 and P7 indexes. PCR reactions consisted in 7 µL of KAPA HiFi HotStart ReadyMix, 0.7 µL of each P5 and P7 index, 2.8 µL of water, and 2.8 µL of diluted PCR product. PCR cycling conditions consisted in an initial denaturation step at 95°C for 3 min, followed by 10 cycles of 95°C denaturing for 30 s, annealing at 55°C for 30 s, extension at 72°C for 30 s, and a final extension at 72°C for 5 min. Indexing success was checked by running a subset of PCR products before and after the indexing reaction in 2% agarose gels stained with GelRed. Indexed PCR products were cleaned with Agencourt AMPure XP beads to remove primers, nucleotides, and enzyme leftovers that might interfere with sequencing. Cleaned PCR products were then quantified with Epoch® Microplate Spectrophotometer and pooled equimolarly per PCR plate. DNA concentration from each pool was then estimated using qPCR (KAPA Library

Quant Kit qPCR Mix, Rocher). To prepare the two libraries (fwh1 and uniplant), the respective pools of each gene were again pooled equimorlaly and their quality was assessed in TapeStation®, i.e., to confirm fragment size distribution and the absence of primer dimer. Finally, both libraries were again quantified with qPCR and sequenced in a MiSeq® System using a MiSeq Reagent Kit v3 (2x250 bp) together with libraries from other projects, with a target depth of 3 k and 20 k reads for fwh1 and uniplant, respectively.

#### 2.4. Bioinformatics

Pairwise alignment of paired-end reads was done using OBITools (Boyer et al., 2016) with the command 'illuminapairedend', discarding reads whenever the overlapping quality score was <40 (Taberlet et al., 2018). The command 'ngsfilter' was used to assign the sample's information to the reads, and to remove forward and reverse primer sequences, allowing a total of four mismatches to the expected primer sequence. Afterwards, the commands 'obiunig' and 'obiannotate' were used to collapse reads into exact sequence variants (ESVs), and remove singletons, while keeping only relevant information from each sample. Reads of each sample were combined per primer set and denoised using VSEARCH (Rognes et al., 2016) command '--cluster unoise', while also removing sequences without the expected length (fwh1: 155-200 bp; uniplant: >187 bp). Resulting Zero-radius Operational Taxonomic Units (ZOTUs) were inspected for chimeras with the command 'uchime3\_denovo' and then clustered with 99% similarity with 'cluster size'. Reads were then mapped back to the retained Operational Taxonomic Units (OTUs) using the command '--usearch global' with an identity level of 99%. Finally, LULU (Frøslev et al., 2017) was used to merge similar OTUs (identity >84%) with high co-occurrence levels (>95% of samples), this way greatly reducing the number of retained PCR artefacts, sequencing errors, as well as nuclear copies of the mitochondria, which tend to artificially inflate the number of OTUs present in each sample.

Retained OTUs were compared against the online database of the National Center for Biotechnology Information (NCBI; https://www.ncbi.nlm.nih.gov/) using the Basic Local Alignment Search Tool (BLAST) algorithm. In case an OTU matched several species, genera or families at similar identity levels, the higher taxonomic category was selected, i.e., if an OTU matched two species of the same genus with the same percentage of similarity, that OTU would be identified to the genus level. Also, sequences with > 90% similarity would be assigned to family level, > 95% similarity would be assigned to genus level, and > 98% would be assigned to species level. Whenever the OTU did not match any valid sequence, it would be assigned as a "no match". This could be due to organisms not being sequenced yet or badly recognisable sequences.

Samples were allocated to C. p. azorica if they had simultaneously at least 250 reads

for fwh1, and > 80% of them assigned to *C. p. azorica.* For Uniplant data, the number of reads observed per OTU present in extraction and PCR blanks was subtracted from the respective samples associated with each extraction batch and PCR plate, to remove potential lab contaminations. Additionally, taxa that did not belong to the kingdom Plantae were considered not diet and excluded. Finally, OTUs representing less than 1% of the total number of dietary reads of each sample were also excluded to reduce false positives arising from crosstalk events.

#### 2.5. Data Analysis

The dietary data was analysed in two components, the richness, i.e., the number of different OTUs, and OTU composition, i.e., the identity of the OTUs detected, hereafter referred only as richness and composition, respectively. All analyses were performed in Rv4.2.0 (R Core Team, 2020), using a conservative approach with binary presence/absence dietary data (Nichols et al., 2018; Mata, 2020).

Given that the majority of the faecal samples were found outside the transects, when prospecting and spotting birds in the surrounding areas, to evaluate the effects of the different land covers in the *C. p. azorica* diet, we considered a 1 km radius buffer, corresponding to 314 ha, around each faecal sample, using GIS software QGIS, to get the relative abundance (area occupied) of different land covers. The size of the buffer was chosen taking into account that the landscape at Pico Island is highly fragmented, and the home range for the nominal subspecies of *C. palumbus* was estimated at 254 ha for non-migrant birds (Haynes et al., 2003).

Besides the three main land cover categories previously described for the establishment of transects (native forests, exotic forests and agricultural areas), other two, complementary were considered (obtained from COSA2018), to better represent the vegetation cover at the buffers: "urban land" and "natural land". Urban land is composed of artificial matrices, such as public gardens and infrastructures in habitational and industrial areas. Natural land is composed of wetlands and marginal habitats. Therefore, land cover categories correspond mostly to COSA2018 level 1 categories (e.g., urban land, agriculture, forests and natural land), with the exception of forests which were divided into native forest andexotic forest. To use the percentage of each land cover type in the models, the percentage was transformed to its arcsine using the built-in R trigonometric function 'asin'. The correlation between all land covers was evaluated using the base R function 'cor' and visualised using the function 'corrplot' from the package corrplot (Wei & Simko, 2021). All land covers with more than 0.6 of correlation were removed from further analysis, giving priority to the most abundant land cover (Figure 3).

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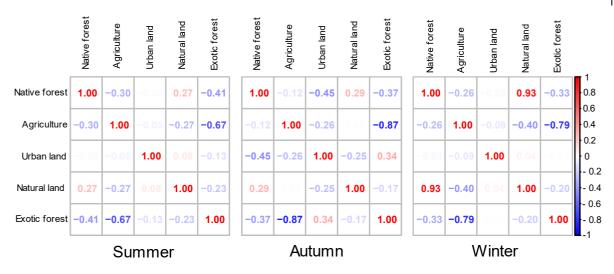


Figure 3. Correlation values between pairs of land cover types for each season. The intensity of the colours relates to stronger correlations between pairs of land covers.

Given its high correlation with agriculture (which presents 210% more area covered), exotic forest was not considered for diet analysis. To evaluate the seasonal effect on the diet, the season was considered a categorical explanatory variable, with three levels corresponding to the sampling seasons (summer, autumn and winter). Afterwards, variables were assessed for multicollinearity based on variance inflation factors (VIFs) using the function 'vif' of the package car (Fox & Weisberg, 2019) with a limit value of 3 (Kock & Lynn, 2012).

To evaluate the total richness, i.e., the niche width, for each season we built rarefaction curves based on Hill numbers (Chao et al., 2014). The rarefaction curves were obtained with the function 'iNEXT' of the package iNEXT (Chao et al., 2014). The comparisons between seasons were made at the same sample coverage, using as reference value, the double of the sample size of the season with less sample coverage, to avoid extrapolation biases. Seasons were considered to differ if the 95% confidence intervals did not overlap. Generalised linear models (GLM) with a Poisson distribution and a log link function were used with the base R function 'glm' to compare the average richness detected in the faeces between seasons. The significance of explanatory variables was assessed using the 'Anova' function of the package car. The function 'glht' of the package multcomp (Hothorn et al., 2008) was used to perform pairwise comparisons to identify in which pairs the observed differences occurred.

To evaluate the diet composition for differences across seasons, a GLM for multivariate data was performed with the base function 'mvabund' of the package mvabund (Wang et al., 2012). The significance of the univariate tests was evaluated using the function 'manyglm' and the significance of the explanatory variable was tested using the function 'anova.manyglm' from the mentioned package. Furthermore, a Fourth Corner Analysis was performed to model for species abundance as a function of season and the interaction between land cover types and species abundance, using the function 'traitglm' of the mvabund package.

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As a strong effect of the season is expected in diet richness and composition, the influence of the land cover was tested independently for the three sampled seasons.

### 3. Results

#### 3.1. Sample Collection and Sequence Amplification

A total of 365 faecal samples were submitted to DNA extraction. Fragments were successfully amplified in 235 extracted samples with fwh1 primers, and in 141 samples with Uniplant primers. The results from fwh1 did not amplify invertebrate sequences and confirmed that 190 faecal samples corresponded to *C. p. azorica* (52.1% of the dataset). The remaining samples were either "inconclusive" for 130 faecal samples (35.6% of the dataset) or assigned to *Turdus merula azorensis* for 45 faecal samples (12.3% of the dataset).

### 3.2. Diet Characterization

After the filtering step, a total of 111 unique OTUs were retained (with and average of 17.3 k reads per sample in a total of ~2.8 M reads). The phylum Tracheophyta was the only phylum identified within the OTUs, of which: one OTU was assigned to family level (family Polygonaceae); 36 OTUs were assigned to genus level; and 74 OTUs were assigned to species level. The assigned OTUs belonged to one phylum, three classes, 21 orders, 38 families, 36 genera and 74 species. Also, the plants were classified concerning plant habit, crop exploration and status in the Azores (Attachment - Table S2). Autumn had 41 OTUs;Summer had 69 OTUs; Winter had 64 OTUs. The most consumed plants in per cent of total occurrences per season are listed in Table 1.

Relating to the most frequent OTUs in descending per cent of total occurrences per season: in summer, *Ipomoea* sp1., *Ipomoea trifida*, *Ipomoea* sp2., *Crepis* sp1. and *Trifolium repens* were the most frequent. Also, *Morella faya* was present. In autumn, *Trifolium repens*, *Rumex sanguineus*, *Amaranthus* sp2., *Rumex* sp1. and *Rumex conglomeratus* were the most frequent, followed by *Morella faya* and *Ilex perado*. In winter, *Rumex sanguineus*, *Rumex* sp1., *Trifolium repens* and *Crepis* sp1. were the most frequent, and *Ilex perado* was also present.

OTUs	Summer (n = 196)	Autumn (n = 114)	Winter (n = 389)	Total (n = 699)
Rumex sanguineus	1.5	12.3	16.7	11.7
Trifolium repens	5.1	15.8	10.3	9.7
Rumex conglomeratus	1.5	7.0	10.8	7.6
Rumex sp1.	1.5	7.9	10.3	7.4
Crepis sp1.	5.1	0.9	6.4	5.2
Leontodon saxatilis	2.6	0.9	4.9	3.6
<i>Ipomoea</i> sp1.	7.7	0.9	0.8	2.7
Agrostis sp1.	2.0	3.5	2.6	2.6
Rumex acetosella	0.0	0.0	4.4	2.4
Amaranthus sp2.	1.0	9.6	0.5	2.1
Ipomoea trifida	7.1	0.0	0.3	2.1
Poa trivialis	0.5	0.0	3.6	2.1
Crepis capillaris	1.0	0.0	3.1	2.0
Ipomoea sp2.	5.1	0.9	0.3	1.7
Polygonaceae	0.0	0.0	2.8	1.6
lpomoea batatas	4.1	0.9	0.3	1.4
Poa sp2.	1.0	0.9	1.8	1.4
llex perado	0.5	2.6	1.3	1.3
Morella faya	2.0	3.5	0.3	1.3
Rubus sp1.	2.0	2.6	0.3	1.1

**Table 1.** Percentage of occurrence of the most frequent OTUs (> 1%) for each season and in total. 'n' refers to the total number of plant detections, i.e., the sum of different taxa detections across all samples.

#### 3.3. Species richness

The estimated niche-width at similar sampling coverage differed between seasons, with winter showing a lower niche-width than summer and autumn (Figure 4A). Concerning average richness (Figure 4B), there were significant differences between seasons, with summer showing the highest diversity, followed by winter, and autumn with the lowest diversity (LR  $\chi^2$ =10.903; p=0.004).

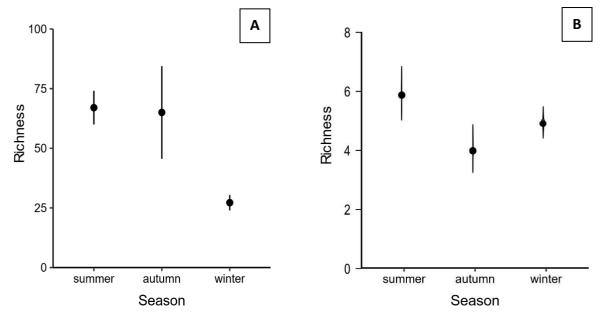


Figure 4. A - The diet niche-width at each season. B - Average richness per season estimated from GLM. The point corresponds to the mean value and the bar is the 95% confidence interval.

Land covers had a significant impact on average diet richness in every season studied except winter (Figure 5). In summer, the average richness observed per sample was negatively affected by the area covered by agriculture (LR  $\chi^2$ =19.276; p<0.001) and positively affected by the extent of urban land (LR  $\chi^2$ =13.381; p<0.001) (see Table 2).

Season	Land cover	LR Chisq	Df	Pr(>Chisq)
Summer				
	Native forest	0.291	1	0.589
	Agriculture	19.276	1	<0.001
	Urban land	13.381	1	<0.001
	Natural land	0.011	1	0.918
Autumn				
	Native forest	5.653	1	0.017
	Agriculture	0.024	1	0.877
	Urban land	2.686	1	0.101
	Natural land	2.829	1	0.092
Winter				
	Native forest	2.281	1	0.131
	Agriculture	0.084	1	0.772
	Urban	1.154	1	0.283

**Table 2**. Analysis of deviance table of the richness in all seasons.

In autumn, the average richness observed per sample was positively affected by the area covered by native forest (LR  $\chi^2$ =5.653; p=0.017)(Table 2), while in winter it was not affected by the area covered by any of the land cover types (Table 2).

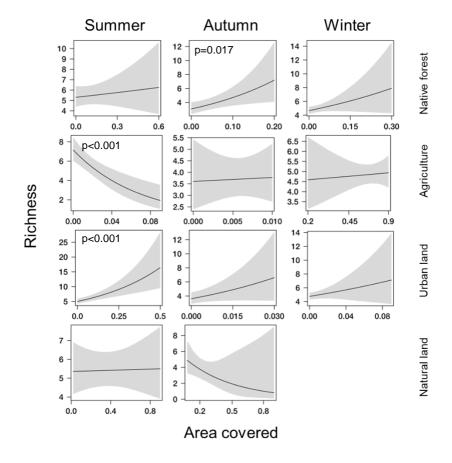


Figure 5. Effect of land covers and seasons in the expression of richness by the area covered.

#### 3.4. Diet composition

Diet composition was significantly influenced by season (see Attachment – Table S3) and by land cover (see Attachment – Table S4).

The GLM results showed that, in summer, there was evidence of a significant effect of urban area (p=0.001) and natural land (p=0.006) on diet composition, with *Crepis* sp1. being significantly less consumed in sites with a higher area of urban land (p=0.048). In autumn, diet composition was affected by the extent of native forest (p=0.005) and natural land (p=0.024), with *Ilex perado* (p=0.002) and *Poa* sp1. (p=0.049) being significantly more consumed in sites with higher cover of native forest. In winter, diet composition was significantly affected by the area of native forest (p=0.004) and agriculture (p=0.019), with *Myrsine africana* (p=0.002) being more frequently consumed in areas more covered with native forest and *Ranunculus repens* (p=0.001) in sites with higher agricultural area.

The associations between the OTUs and season (obtained from Fourth Corner analysis; Figure 6) suggested that *Lantana camara*, *L. hodgei*, *Phillyrea angustifolia*, *Trifolium micranthum*, *Ipomoea batatas*, *Ipomoea* sp1., *Ipomoea* sp2. and *Ipomoea trifida* presented positive associations with summer; the opposite of what was observed with *Agrostis* sp1., *Rumex conglomeratus*, *Rumex sanguineus*, *Rumex* sp1. and *Trifolium repens*. *Amaranthus* sp2., *Poa* sp1. and *Trifolium repens* showed positive associations with autumn, in contrast to *Crepis* sp1 which showed a negative association. *Crepis capillaris*, *Crepis* sp1., *Leontodon saxatilis*, *Poa trivialis*, Polygonaceae, *Ranunculus repens*, *Rumex conglomeratus*, *Rumex longifolius*, *Rumex sanguineus* and *Rumex* sp1. presented positive associations with winter, and *Agrostis* sp2., *Anagallis arvensis*, *Juniperus* sp1., *Morella faya* and *Rubus* sp1 negative associations.

The associations between OTUs and land cover (obtained from Fourth Corner analysis; Figure 7) suggested that, in summer, *Ipomoea batatas, Ipomoea* sp1., *Ipomoea* sp2. and *Ipomoea trifida* were positively associated with native forest. *Agrostis* sp2. and *Rumex conglomeratus* showed stronger positive associations with agriculture area, the opposite of *Phillyrea angustifolia* and *Plantago lanceolata*. *Lantana camara* and *Lantana hodgei* showed stronger positive associations with urban land, the opposite of what was observed with *Trifolium repens* and *Crepis* sp1. *Amaranthus* sp1., *Amaranthus* sp2., Bromus sp1., Cerastium sp1. presented positive associations with natural land, but *Ipomoea batatas, Ipomoea* sp2. and *Ipomoea trifida* showed the opposite. In autumn, *Ilex perado* and *Poa* sp1. showed positive associations with native forest, contrary to *Anagallis arvensis* and *Rumex sanguineus*. *Trifolium repens* was positively associated to agricultural area, opposite to *Rumex conglomeratus*. *Amaranthus* sp2. and *Rumex sanguineus*.

urban land. *Pittosporum* sp1., *Pittosporum undulatum* and *Psidium cattleyanum* were positively associated with natural land. In winter, *Myrsine africana* was positively associated with native forest, the opposite of *Rumex conglomeratus*. *Ranunculus repens* was positively associated to agricultural land, unlike *Rumex pulcher*. *Lonicera japonica* and *Lonicera macranthoides* were positively associated with urban land, contrary to *Rumex conglomeratus*.

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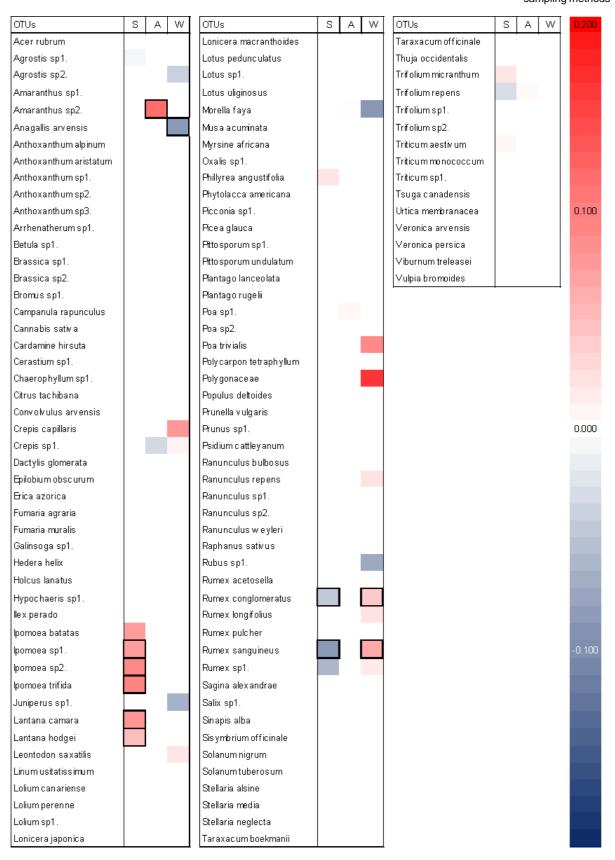
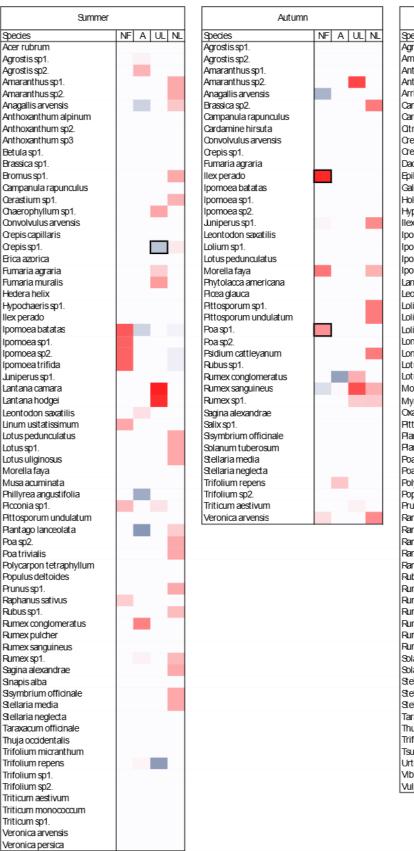


Figure 6. Fourth corner modelling results with standardized coefficients where season–prey interaction terms are presented, from a Lasso Regularization GLM. Darker squares show stronger associations than brighter squares. Positive associations are represented in red and negative associations are represented in blue. The seasons are listed as "S" for summer, "A" for autumn and "W" for winter. The OTUs that differed significantly between seasons (Table S3) are highlighted in a black box.

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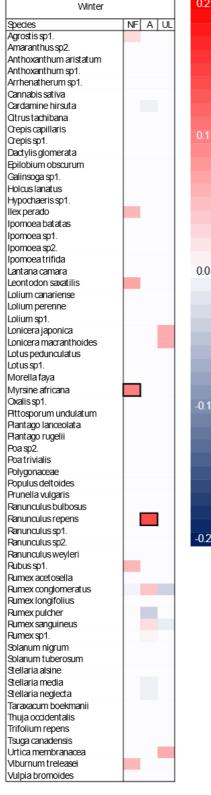


Figure 7. Fourth corner modelling results with standardized coefficients where land cover-prey interaction terms are presented, from a Lasso Regularization GLM. Darker squares show stronger associations than brighter squares. Positive associations are represented in red and negative associations are represented in blue. The land covers are listed as "NF" for native forest, "A" for agriculture, "UL" for urban land and "NL" for natural land. The OTUs that differed significantly between land covers in each season (Table S4) are highlighted in a black box.

### 4. Discussion

To our knowledge, this is the first evaluation of the diet of *Columba palumbus azorica*. The results confirm the ecological plasticity of this subspecies, similar to the nominal subspecies. As expected, there was evidence of differences between seasons and land covers in species richness and composition of the diet. Land cover had an important role in resource availability. Areas devoted to agriculture should provide a high resource availability and diversity. Nonetheless, this resource availability and diversity should also be influenced by the agricultural practices, i.e., rotative regimen provided a greater diversity of crops compared with permanent cultures, and pastures usually contain cattle feeders (DRRF, 2018), which may deliver easy access of grains and cereals to *C. p. azorica* from livestock feed. A previous study by Santos (2017) suggested that *Cryptomeria japonica* plantations, included in the exotic forest, and agricultural areas, both present on Pico Island, were important niches for *C. p. azorica*, however, the exotic forest could not be considered in this diet analysis as it had a high correlation with agriculture (see Figure 3), likely because, both forest plantations and agriculture, are developed in accessible areas of Pico Island.

Regarding the niche-width, OTU diversity in winter was lower than the OTU diversity in summer and autumn. This result is in agreement with that obtained in a previous work, by Gutiérrez et al. (2017), with *C. p. palumbus*, that suggested a far more diversified diet in summer and autumn than in winter. The last season coincides with the period when cultivated fields have a decreased seed availability (Negrier et al., 2021).

Regarding the average richness, in summer, the relationships with agriculture and urban land suggest relevant associations with the abundance of ripening cereals (Gutiérrez et al., 2017) and crops cultivated in agricultural areas and, in parallel, the abundance of spontaneous weeds in urban areas, such as public and residential gardens. In autumn, the relationship with native forest suggests, on the one hand, a lower resource availability in agricultural areas and, on the other, the abundant fructification of endemic plants was attracting pigeons to the native forest. In winter, the average richness was not influenced by the area covered by any land cover, suggesting similar resource diversity available across the different land covers.

In what concerns composition, in summer, the differences in urban land and natural land were assigned to within-season differences. The OTU *Crepis* sp1. was negatively associated with urban land, which suggested that it was less consumed in urban land than in other land cover. In autumn, the differences in native forest, associated with *Ilex perado*, and natural land, associated with *Poa* sp1., were assigned to within season differences, i.e., these plants were more consumed in the land covers where they were expected to be more abundant. In winter, the differences in native forest, associated with *Myrsine africana*, and in

agriculture, associated with *Ranunculus repens*, were assigned to within-season differences; both OTUs were more consumed in their associated land cover than in other land cover.

The diet characterization suggested that the most common OTUs (the most frequently consumed) were not always responsible for differences in diet composition across land covers. For instance, the most common OTUs in summer were Ipomoea sp1., Ipomoea trifida, Ipomoea sp2., Crepis sp1. and Trifolium repens; so, Ipomoea spp. and Trifolium repens composed a substantial part of the occurrences (25.0%), but do not present a significant variation among land covers, Morella faya, a native shrub, was also present in summer, when the drupes were immature (Silva & Tavares, 1995). Thus, its seed dispersal by C. p. azorica may not be successful in summer. The most common OTUs in autumn were *Trifolium repens*, Rumex sanguineus, Amaranthus sp2., Rumex sp1. and Rumex conglomeratus. These OTUs corresponded to exotic weeds in the Azores with serious eradication problems, specially the genus Amaranthus (Silva & Smith, 2006; OGTR, 2021), and C. p. azorica may favour their seed dispersion. The natives Morella faya and llex perado were also present in autumn when drupes reached the peak of maturation (Silva & Tavares, 1995); consequently, C. p. azorica may facilitate their seed dispersion. The most common OTUs in winter were Rumex sanguineus, Rumex conglomeratus, Rumex sp1., Trifolium repens and Crepis sp1. which correspond to exotic weeds in the Azores (Silva & Smith, 2006). The native tree *llex perado* was also present in winter, thus C. p. azorica may favour its seed dispersion.

The establishment of *Pittosporum undulatum* in Pico Island and its dominance in the landscape highlighted its invasive status (Silva & Smith, 2006; Fontaine et al., 2021). In the present work *Pittosporum undulatum* and *Pittosporum* sp1. represented less than 1% of the total occurrences. In contrast, an exploratory work (Santos et al. not published) involving *C. p. azorica* faecal samples analysis for seed detection (identified by morphological traits) showed that *Pittosporum* seeds - either intact or fragmented - were present in 13% of the samples (total samples analysed: 351). The apparent underestimation by the molecular analysis may have resulted from the resinous tegument that surrounds *Pittosporum* seeds (Negrelle et al., 2018), which contain bio inhibitors with toxic properties (Costa et al., 2016) - saponin and leucoanthocyanin – therefore, reducing the PCR efficiency and further OTU detectability.

*Pittosporum undulatum*, *Cryptomeria japonica* and *Pinus* spp., that represent the class of exotic forests, were not among the OTUs detected on *C. p. azorica* faeces. Thus, those habitats may be used for other activities other than feeding (Fontaine et al., 2021).

There was no evidence of *C. p. azorica* feeding on vineyards since the corresponding OTU was absent from our results, even for summer season, when the grapes ripen. This finding complements the results of studies carried out in the archipelago, on the islands of Pico (Fontaine et al. 2019) and Terceira (Lamelas-López & Marco (2021), which showed that the

*C. p. azorica* is not an important consumer of grapes. Our work seems to point that, even outside the ripening season of the grapes, the vine will also be little consumed by the *C. p. azorica*. The impact of *C. p. azorica* may extend to other crops with repercussions on management and conservation of the subspecies, similarly to what was observed in Madeira, with the *C. trocaz* damaging crops (Marrero et al., 2004). Several plants with commercial interest were present in *C. p. azorica* diet - cruciferous vegetables (*Brassica* spp.), tangerine tree (*Citrus tachibana*), sweet potato (*Ipomoea* spp.), banana plant (*Musa acuminata*), fruit trees (*Prunus* spp.), potato (*Solanum tuberosum*), radish (*Raphanus sativus*), cannabis (*Cannabis sativa*) and wheat (*Triticum* spp.). Thus, the crop productivity could be affected if the crop buds and/or flowers are consumed (Cramp, 1998; Gutiérrez et al., 2017).

### 5. Conclusion

This work suggested the influence of season and land cover on species richness and diet composition of *C. p. azorica* diet. Relating to richness, the variation on niche-width suggested lower diversity in winter than in summer and autumn. Also, the variation on average richness showed that the abundance of crops and weeds retained associations with agriculture and urban land in summer. In autumn, the fruitful endemic plants attracted *C. p. azorica* to the native forest. In winter, no association was observed, suggesting similar resource diversity across land covers.

Regarding composition, in summer, urban land and natural land showed differences, with *Crepis* sp1. abundance being inversely proportional to urban land area. In autumn, *llex perado*, associated with native forest, and *Poa* sp1, associated with natural land, presented higher abundances in their associated land cover than in others. In winter, *Myrsine africana*, associated with native forest, and *Ranunculus repens*, associated with in agriculture, were more consumed in their associated land cover than in other land covers.

Overall, DNA metabarcoding provided a cost-effective and expedient method to characterise the diet of C. p. azorica and may even be applied to research on other columbids diet. Comparing the results of DNA metabarcoding of ITS2 region with previous studies on diet of *C. p. palumbus*, we observed a clear difference in the taxonomic resolution of the OTUs detected. The most frequent OTUs present in *C. p. azorica* were successfully assigned to the species level. The OTUs corresponded mostly to grasses with exotic status in the Azores (e.g. *Rumex sanguineus, Trifolium repens, Rumex conglomeratus, Rumex* sp1., *Crepis* sp1., *Leontodon saxatilis, Agrostis* sp1., *Amaranthus* sp2., Poa trivialis, *Crepis capillaris, Poa* sp2., *Rubus* sp1.), to crops (*Ipomoea* sp1., *Ipomoea trifida, Ipomoea* sp2., *Ipomoea batatas*) and to native trees (*Ilex perado* and *Morella faya*). Furthermore, it was possible to trace several plants with commercial interest in the dietary analysis, which raises issues of conservation nature, since the Azores Woodpigeon may be affecting the crop productivity by consuming buds and/or flowers and, ultimately, may be in conflict with farmers.

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### Attachment

Land cover	Land cover composition	Number of transects
Native forest	Erica-Morella	4
	Picconia-Morella	4
	Laurus	4
	Juniperus-Ilex	4
	Natural herbaceous vegetation	6
Agriculturo	Arable lands	4
Agriculture	Permanent cultures	4
	Pastures	6
	Pittosporum undulatum	6
Exotic forest	<i>Pinus</i> sp.	4
	Cryptomeria japonica	4

**Table S1**. Distribution of the 50 transects across the area occupied by each land cover on Pico Island.

Table S2. Characteristics of the plants (OTUs present on C. p. azorica diet) regarding their habit, crop exploration and status in the Azores.

OTUs	Habit	Crop	Status	OTUs	Habit	Crop	Status
Acer rubrum	tree	no	exotic	Phillyrea angustifolia	shrub	no	exotic
Agrostis sp1.	weed	no	exotic	Phytolacca americana	weed	no	exotic
Agrostis sp2.	weed	no	exotic	Picconia sp1.	tree	no	native
Amaranthus sp1.	weed	no	exotic	Picea glauca	tree	no	exotic
Amaranthus sp2.	weed	no	exotic	Pittosporum sp1.	shrub	no	exotic
Anagallis arvensis	weed	no	exotic	Pittosporum undulatum	shrub	no	exotic
Anthoxanthum alpinum	weed	no	exotic	Plantago lanceolata	weed	no	exotic
Anthoxanthum aristatum	weed	no	exotic	Plantago rugelii	weed	no	exotic
Anthoxanthum sp1.	weed	no	exotic	Poa sp1.	weed	no	exotic
Anthoxanthum sp2.	weed	no	exotic	Poa sp2.	weed	no	exotic
Anthoxanthum sp3.	weed	no	exotic	Poa trivialis	weed	no	exotic
Arrhenatherum sp1.	weed	no	exotic	Polycarpon tetraphyllum	weed	no	exotic
Betula sp1.	tree	no	exotic	Polygonaceae	weed	no	exotic
Brassica sp1.	weed	yes	exotic	Populus deltoides	tree	no	exotic
Brassica sp2.	weed	yes	exotic	Prunella vulgaris	weed	no	native
Bromus sp1.	weed	no	exotic	Prunus sp1.	tree	yes	exotic
Campanula rapunculus	weed	no	exotic	Psidium cattleyanum	shrub	no	exotic
Cannabis sativa	weed	yes	exotic	Ranunculus bulbosus	weed	no	native
Cardamine hirsuta	weed	no	exotic	Ranunculus repens	weed	no	exotic
Cerastium sp1.	weed	no	exotic	Ranunculus sp1.	weed	no	exotic
Chaerophyllum sp1.	weed	no	native	Ranunculus sp2.	weed	no	exotic
Citrus tachibana	tree	yes	exotic	Ranunculus weyleri	weed	no	exotic
Convolvulus arvensis	weed	no	exotic	Raphanus sativus	weed	no	exotic
Crepis capillaris	weed	no	exotic	Rubus sp1.	shrub	no	exotic
Crepis sp1.	weed	no	exotic	Rumex acetosella	weed	no	native
Dactylis glomerata	weed	no	exotic	Rumex conglomeratus	weed	no	exotic
Epilobium obscurum	weed	no	native	Rumex longifolius	weed	no	exotic
Erica azorica	shrub	no	native	Rumex pulcher	weed	no	exotic
Fumaria agraria	weed	no	exotic	Rumex sanguineus	weed	no	exotic
Fumaria muralis	weed	no	exotic	Rumex sp1.	weed	no	exotic
Galinsoga sp1.	weed	no	exotic	Sagina alexandrae	weed	no	exotic
Hedera helix	vine	no	exotic	Salix sp1.	tree	no	exotic
Holcus lanatus			native	Sinapis alba			exotic
	weed weed	no	exotic	Sinapis alba Sisymbrium officinale	weed	no	exotic
Hypochaeris sp1.		no	native	Solanum nigrum	weed	no	exotic
llex perado	tree	no			weed	no	
Ipomoea batatas	weed	yes	exotic	Solanum tuberosum Stellaria alsine	weed	yes	exotic
<i>Ipomoea</i> sp1.	weed	yes	exotic		weed	no	native
lpomoea sp2.	weed	yes	exotic	Stellaria media	weed	no	exotic
Ipomoea trifida	weed	yes	exotic	Stellaria neglecta	weed	no	exotic
Juniperus sp1.	tree	no	exotic	Taraxacum boekmanii	weed	no	exotic
Lantana camara	shrub	no	exotic	Taraxacum officinale	weed	no	exotic
Lantana hodgei	shrub	no	exotic	Thuja occidentalis	tree	no	exotic
Leontodon saxatilis	weed	no	exotic	Trifolium micranthum	weed	no	exotic
Linum usitatissimum	weed	no	exotic	Trifolium repens	weed	no	exotic
Lolium canariense	weed	no	exotic	Trifolium sp1.	weed	no	exotic
Lolium perenne	weed	no	exotic	Trifolium sp2.	weed	no	exotic
Lolium sp1.	weed	no	exotic	Triticum aestivum	weed	yes	exotic
Lonicera japonica	vine	no	exotic	Triticum monococcum	weed	yes	exotic
Lonicera macranthoides	vine	no	exotic	<i>Triticum</i> sp1.	weed	yes	exotic
Lotus pedunculatus	weed	no	exotic	Tsuga canadensis	tree	no	exotic
Lotus sp1.	weed	no	exotic	Urtica membranacea	weed	no	exotic
Lotus uliginosus	weed	no	exotic	Veronica arvensis	weed	no	exotic
Morella faya	shrub	no	native	Veronica persica	weed	no	exotic
Musa acuminata	weed	yes	exotic	Viburnum treleasei	shrub	no	native
Myrsine africana	shrub	no	exotic	Vulpia bromoides	weed	no	exotic
Oxalis sp1.	weed	no	exotic	-			

Table S3. Analysis of deviance table for seasonal composition. The significant p-values ( $\alpha$ =0.05) are in bold.

OTUs	Season (p-value)	OTUs	Season (p-value
Acer rubrum	1.000	Phytolacca americana	0.994
Agrostis sp1.	1.000	<i>Picconia</i> sp1.	0.721
Agrostis sp2.	0.213	Picea glauca	1.000
Amaranthus sp1.	0.812	Pittosporum sp1.	0.997
Amaranthus sp2.	0.001	Pittosporum undulatum	1.000
Anagallis arvensis	0.023	Plantago lanceolata	0.926
Anthoxanthum alpinum	1.000	Plantago rugelii	1.000
Anthoxanthum aristatum	1.000	Poa sp1.	0.530
Anthoxanthum sp1.	1.000	Poa sp2.	1.000
Anthoxanthum sp2.	1.000	Poa trivialis	0.079
Anthoxanthum sp3.	1.000	Polycarpon tetraphyllum	1.000
-	1.000		
Arrhenatherum sp1.		Polygonaceae	0.051
Betula sp1.	1.000	Populus deltoides	1.000
Brassica sp1.	1.000	Prunella vulgaris	1.000
Brassica sp2.	0.997	Prunus sp1.	0.749
Bromus sp1.	0.749	Psidium cattleyanum	0.997
Campanula rapunculus	0.965	Ranunculus bulbosus	1.000
Cannabis sativa	1.000	Ranunculus repens	0.849
Cardamine hirsuta	1.000	Ranunculus sp1.	1.000
Cerastium sp1.	0.744	Ranunculus sp2.	1.000
Chaerophyllum sp1.	1.000	Ranunculus weyleri	1.000
Citrus tachibana	1.000	Raphanus sativus	0.724
Convolvulus arvensis	0.982	Rubus sp1.	0.301
Crepis capillaris	0.244	Rumex acetosella	0.901
Crepis sp1.	0.792	Rumex conglomeratus	0.023
Dactylis glomerata	1.000	Rumex longifolius	0.849
Epilobium obscurum	1.000	Rumex pulcher	1.000
Erica azorica	1.000		0.001
		Rumex sanguineus	
Fumaria agraria	0.828	Rumex sp1.	0.124
Fumaria muralis	1.000	Sagina alexandrae	0.812
Galinsoga sp1.	1.000	Salix sp1.	0.994
Hedera helix	1.000	Sinapis alba	1.000
Holcus lanatus	1.000	Sisymbrium officinale	0.952
Hypochaeris sp1.	1.000	Solanum nigrum	1.000
llex perado	1.000	Solanum tuberosum	1.000
Ipomoea batatas	0.055	Stellaria alsine	1.000
Ipomoea sp1.	0.014	Stellaria media	1.000
Ipomoea sp2.	0.014	Stellaria neglecta	1.000
Ipomoea trifida	0.002	Taraxacum boekmanii	1.000
Juniperus sp1.	0.058	Taraxacum officinale	1.000
Lantana camara	0.014	Thuja occidentalis	1.000
Lantana hodgei	0.035	Linum usitatissimum	1.000
Leontodon saxatilis	1.000	Trifolium micranthum	0.185
Lolium canariense		Trifolium repens	
	1.000	1	1.000
Lolium perenne	1.000	Trifolium sp1.	0.721
Lolium sp1.	1.000	Trifolium sp2.	0.965
Lonicera japonica	1.000	Triticum aestivum	0.278
Lonicera macranthoides	1.000	Triticum monococcum	1.000
Lotus pedunculatus	1.000	<i>Triticum</i> sp1.	1.000
Lotus sp1.	1.000	Tsuga canadensis	1.000
Lotus uliginosus	1.000	Urtica membranacea	1.000
Morella faya	0.149	Veronica arvensis	0.792
Musa acuminata	1.000	Veronica persica	1.000
Myrsine africana	1.000	Viburnum treleasei	1.000
Oxalis sp1.	1.000	Vulpia bromoides	1.000
Phillyrea angustifolia	0.213		1.000

**Table S4**. Analysis of deviance table of composition in relation to land cover (per season). The significant p-values ( $\alpha$ =0.05) are in bold.

Summe	er (part I)		Summe	r (part II)	
OTUs		UL NL	OTUs	NF A	UL NL
Acer rubrum	0.994 0.991 0	.996 1.000	Phillyrea angustifolia	0.521 1.000	0.996 0.996
Agrostis sp1.	1.000 0.929 0	.996 1.000	Phytolacca americana	1.000 1.000	1.000 1.000
Agrostis sp2.	1.000 0.929 0	.965 1.000	Picconia sp1.	0.956 1.000	0.168 1.000
Amaranthus sp1.	1.000 0.800 0	.322 1.000	Picea glauca	1.000 1.000	1.000 1.000
Amaranthus sp2.	1.000 0.808 0	.812 0.996	Pittosporum sp1.	1.000 1.000	1.000 1.000
Anagallis arvensis	1.000 0.984 0	.812 1.000	Pittosporum undulatum	1.000 1.000	
Anthoxanthum alpinum	1.000 1.000 0		Plantago lanceolata	1.000 0.326	0.377 1.000
Anthoxanthum aristatum	1.000 1.000 1	.000 1.000	Plantago rugelii	1.000 1.000	1.000 1.000
Anthoxanthum sp1.	1.000 1.000 1		Poa sp1.	1.000 1.000	1.000 1.000
Anthoxanthum sp2.	1.000 1.000 0		Poa sp2.		0.322 1.000
Anthoxanthum sp3.	1.000 1.000 0		Poa trivialis		0.812 1.000
Arrhenatherum sp1.	1.000 1.000 1		Polycarpon tetraphyllum		
Betula sp1.	1.000 1.000 0		Polygonaceae		1.000 1.000
Brassica sp1.	1.000 1.000 0		Populus deltoides		0.997 0.738
Brassica sp2.	1.000 1.000 1		Prunella vulgaris		1.000 1.000
Bromus sp1.	1.000 0.800 0		Prunus sp1.		0.322 1.000
Campanula rapunculus	1.000 1.000 0		Psidium cattleyanum		1.000 1.000
Cannabis sativa	1.000 1.000 1		Ranunculus bulbosus		1.000 1.000
Cardamine hirsuta	1.000 1.000 1		Ranunculus repens		1.000 1.000
Cerastium sp1.	1.000 1.000 0		Ranunculus sp1.		1.000 1.000
Chaerophyllum sp1.	1.000 1.000 0		Ranunculus sp2.		1.000 1.000
Citrus tachibana	1.000 1.000 1		Ranunculus weyleri		1.000 1.000
Convolvulus arvensis	1.000 1.000 0		Raphanus sativus Rubus sp1.	0.997 1.000	
Crepis capillaris Crepis sp1.	1.000 1.000 0 1.000 1.000 <b>0</b>		Rumex acetosella		0.668 1.000 1.000
Dactylis glomerata	1.000 1.000 1		Rumex conglomeratus		0.997 1.000
Epilobium obscurum	1.000 1.000 1		Rumex longifolius		1.000 1.000
Erica azorica	1.000 1.000 0		Rumex pulcher	0.592 1.000	
Fumaria agraria	1.000 1.000 0		Rumex sanguineus	1.000 1.000	
Fumaria muralis	1.000 1.000 0		Rumex sp1.		0.086 0.985
Galinsoga sp1.	1.000 1.000 1		Sagina alexandrae		0.322 1.000
Hedera helix	1.000 0.994 0		Salix sp1.		1.000 1.000
Holcus lanatus	1.000 1.000 1		Sinapis alba		0.997 0.738
Hypochaeris sp1.	0.994 1.000 0		Sisymbrium officinale		0.812 1.000
llex perado	1.000 0.994 0	.997 1.000	Solanum nigrum	1.000 1.000	1.000 1.000
lpomoea batatas	0.427 1.000 0	.997 0.994	Solanum tuberosum	1.000 1.000	1.000 1.000
<i>Ipomoea</i> sp1.	0.524 1.000 0	.934 1.000	Stellaria alsine	1.000 1.000	1.000 1.000
<i>Ipomoea</i> sp2.	0.510 1.000 0	.996 0.994	Stellaria media	1.000 1.000	0.812 1.000
Ipomoea trifida	0.510 1.000 0	.996 0.994	Stellaria neglecta	1.000 1.000	0.997 0.990
<i>Juniperus</i> sp1.	1.000 1.000 0		Taraxacum boekmanii		1.000 1.000
Lantana camara	0.995 1.000 0	.812 1.000	Taraxacum officinale	1.000 1.000	0.997 0.994
Lantana hodgei	0.923 1.000 0	.743 1.000	Thuja occidentalis	1.000 1.000	0.997 0.990
Leontodon saxatilis	1.000 0.992 0		Trifolium micranthum		0.997 0.998
Linum usitatissimum	0.340 1.000 0		Trifolium repens		0.322 0.998
Lolium canariense	1.000 1.000 1		Trifolium sp1.		0.997 1.000
Lolium perenne	1.000 1.000 1		Trifolium sp2.		0.997 0.990
Lolium sp1.	1.000 1.000 1		Triticum aestivum		0.997 1.000
Lonicera japonica	1.000 1.000 1		Triticum monococcum		0.997 1.000
Lonicera macranthoides			Triticum sp1.		0.997 0.991
Lotus pedunculatus	1.000 1.000 0		Tsuga canadensis		1.000 1.000
Lotus sp1.	1.000 1.000 0		Urtica membranacea		1.000 1.000
Lotus uliginosus	1.000 1.000 0		Veronica arvensis		0.997 1.000
Morella faya	1.000 1.000 0		Veronica persica	0.995 1.000	
Musa acuminata	0.994 0.991 0		Viburnum treleasei		1.000 1.000
Myrsine africana	1.000 1.000 1		Vulpia bromoides	1.000 1.000	1.000 1.000
Oxalis sp1.	1.000 1.000 1	.000 1.000		-	

Table S4 (Cont.) Analysis of deviance table of composition in relation to land cover (per season). The significant p-values ( $\alpha$ =0.05) are in bold.

Διιτιιτ	Autumn (part I)				n (part II)		—
OTUs	NF A	UL	NL	OTUs	NF A	UL NL	_
Acer rubrum	1.000 1.000	-		Phillyrea angustifolia	1.000 1.000	-	
Agrostis sp1.	0.947 1.000			Phytolacca americana	0.995 1.000		
Agrostis sp2.	0.886 1.000			Picconia sp1.	1.000 1.000		
Amaranthus sp1.	0.346 0.993			Picea glauca	0.832 1.000		
Amaranthus sp2.	0.995 1.000			Pittosporum sp1.	0.926 0.994		
Anagallis arvensis	0.637 0.903			Pittosporum undulatum	0.926 0.994		
Anthoxanthum alpinum	1.000 1.000			Plantago lanceolata	1.000 1.000		
Anthoxanthum aristatum				Plantago rugelii	1.000 1.000		
Anthoxanthum sp1.	1.000 1.000	1.000	1.000	Poa sp1.	<b>0.049</b> 0.877		
Anthoxanthum sp2.	1.000 1.000	1.000	1.000	Poa sp2.	0.995 1.000		
Anthoxanthum sp3.	1.000 1.000	1.000	1.000	Poa trivialis	1.000 1.000		
Arrhenatherum sp1.	1.000 1.000	1.000	1.000	Polycarpon tetraphyllum	1.000 1.000	1.000 1.00	00
Betula sp1.	1.000 1.000	1.000	1.000	Polygonaceae	1.000 1.000		
Brassica sp1.	1.000 1.000	1.000	1.000	Populus deltoides	1.000 1.000	1.000 1.00	00
<i>Brassica</i> sp2.	0.926 0.994	1.000	0.376	Prunella vulgaris	1.000 1.000	1.000 1.00	00
Bromus sp1.	1.000 1.000	1.000	1.000	Prunus sp1.	1.000 1.000	1.000 1.00	)0
Campanula rapunculus	0.582 1.000	1.000	0.975	Psidium cattleyanum	0.926 0.994	1.000 0.37	76
Cannabis sativa	1.000 1.000	1.000	1.000	Ranunculus bulbosus	1.000 1.000	1.000 1.00	)0
Cardamine hirsuta	0.582 1.000			Ranunculus repens	1.000 1.000	1.000 1.00	)0
Cerastium sp1.	1.000 1.000	1.000	1.000	Ranunculus sp1.	1.000 1.000	1.000 1.00	)0
Chaerophyllum sp1.	1.000 1.000			Ranunculus sp2.	1.000 1.000	1.000 1.00	)0
Citrus tachibana	1.000 1.000	1.000	1.000	Ranunculus weyleri	1.000 1.000	1.000 1.00	)0
Convolvulus arvensis	0.995 1.000	0.928	0.930	Raphanus sativus	1.000 1.000		
Crepis capillaris	1.000 1.000			Rubus sp1.	0.974 0.768	0.928 0.93	30
Crepis sp1.	0.346 0.993			Rumex acetosella	1.000 1.000		
Dactylis glomerata	1.000 1.000			Rumex conglomeratus	0.996 0.900		
Epilobium obscurum	1.000 1.000			Rumex longifolius	1.000 1.000		
Erica azorica	1.000 1.000			Rumex pulcher	1.000 1.000		
Fumaria agraria	0.582 1.000			Rumex sanguineus	0.974 1.000		
Fumaria muralis	1.000 1.000			Rumex sp1.	0.990 1.000		
Galinsoga sp1.	1.000 1.000			Sagina alexandrae	0.346 0.993		
Hedera helix	1.000 1.000			Salix sp1.	0.995 1.000		
Holcus lanatus	1.000 1.000			Sinapis alba	1.000 1.000		
Hypochaeris sp1.	1.000 1.000			Sisymbrium officinale	0.346 0.993		
llex perado	<b>0.002</b> 1.000 0.582 1.000			Solanum nigrum	1.000 1.000 0.549 0.972		
Ipomoea batatas	0.582 1.000			Solanum tuberosum Stellaria alsine			
Ipomoea sp1.	0.582 1.000			Stellaria media	1.000 1.000 0.582 1.000		
lpomoea sp2. Ipomoea trifida	1.000 1.000			Stellaria neglecta	0.582 1.000		
Juniperus sp1.	0.660 1.000			Taraxacum boekmanii	1.000 1.000		
Lantana camara	1.000 1.000			Taraxacum officinale	1.000 1.000		
Lantana hodgei	1.000 1.000			Thuja occidentalis	1.000 1.000		
Leontodon saxatilis	0.995 1.000			Trifolium micranthum	1.000 1.000		
Linum usitatissimum	1.000 1.000			Trifolium repens	0.995 0.790		
Lolium canariense	1.000 1.000			Trifolium sp1.	1.000 1.000		
Lolium perenne	1.000 1.000			Trifolium sp2.	0.582 1.000		
Lolium sp1.	0.346 0.993			Triticum aestivum	0.995 1.000		
Lonicera japonica	1.000 1.000			Triticum monococcum	1.000 1.000		
Lonicera macranthoides				Triticum sp1.	1.000 1.000		
Lotus pedunculatus	0.832 1.000			Tsuga canadensis	1.000 1.000		
Lotus sp1.	1.000 1.000			Urtica membranacea	1.000 1.000		
Lotus uliginosus	1.000 1.000			Veronica arvensis	0.447 0.993		
Morella faya	0.174 1.000			Veronica persica	1.000 1.000		-
Musa acuminata	1.000 1.000			Viburnum treleasei	1.000 1.000		
Myrsine africana	1.000 1.000			Vulpia bromoides	1.000 1.000		
Oxalis sp1.	1.000 1.000	1.000	1.000		_		
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Table S4 (Cont.) Analysis of deviance table of composition in relation to land cover (per season). The significant p-values ( $\alpha$ =0.05) are in bold.

Winter (pa	art I)		Winter (part II)			
OTUs	NF	A UL	OTUs	NF	A U	L
Acer rubrum	1.000 1	.000 1.000	Phillyrea angustifolia	1.000 1	1.000 1.0	000
Agrostis sp1.	0.995 1	.000 1.000	Phytolacca americana	1.000 1	1.000 1.0	000
Agrostis sp2.	1.000 1	.000 1.000	Picconia sp1.	1.000 1	1.000 1.0	000
Amaranthus sp1.	1.000 1	.000 1.000	Picea glauca	1.000 1	1.000 1.0	000
Amaranthus sp2.	1.000 0	.983 1.000	Pittosporum sp1.	1.000 1	1.000 1.0	000
Anagallis arvensis	1.000 1	.000 1.000	Pittosporum undulatum	0.737 (	0.999 1.0	000
Anthoxanthum alpinum	1.000 1	.000 1.000	Plantago lanceolata	1.000 (	0.999 1.0	000
Anthoxanthum aristatum	1.000 1	.000 1.000	Plantago rugelii	1.000 1	1.000 1.0	)00
Anthoxanthum sp1.		.000 1.000	<i>Poa</i> sp1.		1.000 1.0	
Anthoxanthum sp2.	1.000 1	.000 1.000	Poa sp2.	1.000 1	1.000 1.0	)00
Anthoxanthum sp3.	1.000 1	.000 1.000	Poa trivialis	0.999 1	1.000 1.0	)00
Arrhenatherum sp1.		.000 1.000	Polycarpon tetraphyllum	1.000 1	1.000 1.0	)00
<i>Betula</i> sp1.	1.000 1	.000 1.000	Polygonaceae	0.995 (	0.999 1.0	)00
Brassica sp1.	1.000 1	.000 1.000	Populus deltoides		1.000 1.0	
Brassica sp2.		.000 1.000	Prunella vulgaris		1.000 1.0	
Bromus sp1.		.000 1.000	Prunus sp1.		1.000 1.0	
Campanula rapunculus		.000 1.000	Psidium cattleyanum		1.000 1.0	
Cannabis sativa		.000 1.000	Ranunculus bulbosus		0.954 1.0	
Cardamine hirsuta		.998 1.000	Ranunculus repens		<b>0.001</b> 1.0	
Cerastium sp1.		.000 1.000	Ranunculus sp1.		0.807 1.0	
Chaerophyllum sp1.		.000 1.000	Ranunculus sp2.		0.807 1.0	
Citrus tachibana		.000 0.991	Ranunculus weyleri		0.807 1.0	
Convolvulus arvensis		.000 1.000	Raphanus sativus		1.000 1.0	
Crepis capillaris		.000 1.000	Rubus sp1.		1.000 1.0	
Crepis sp1.		.000 1.000	Rumex acetosella		1.000 1.0	
Dactylis glomerata		0.427 1.000	Rumex conglomeratus		0.983 0.8	
Epilobium obscurum		000 1 000	Rumex longifolius		0.983 1.0	
Erica azorica		.000 1.000	Rumex pulcher		0.999 0.9	
Fumaria agraria Fumaria muralis		.000 1.000	<i>Rumex sanguineus</i> <i>Rumex</i> sp1.		1.000 1.0 1.000 1.0	
Galinsoga sp1.		.000 0.935	Sagina alexandrae		1.000 1.0	
Hedera helix		.000 1.000	Salix sp1.		1.000 1.0	
Holcus lanatus		.983 1.000	Sinapis alba		1.000 1.0	
Hypochaeris sp1.		.000 1.000	Sisymbrium officinale		1.000 1.0	
llex perado		.000 1.000	Solanum nigrum		1.000 1.0	
Ipomoea batatas		.000 1.000	Solanum tuberosum		1.000 0.9	
Ipomoea sp1.		.000 0.996	Stellaria alsine		).648 1.0	
Ipomoea sp2.		.000 1.000	Stellaria media		0.998 1.0	
Ipomoea trifida		.000 1.000	Stellaria neglecta		0.998 1.0	
Juniperus sp1.	1.000 1	.000 1.000	Taraxacum boekmanii		1.000 1.0	
Lantana camara	0.778 1	.000 1.000	Taraxacum officinale	1.000 1	1.000 1.0	000
Lantana hodgei	1.000 1	.000 1.000	Thuja occidentalis	1.000 1	1.000 1.0	000
Leontodon saxatilis	0.802 1	.000 1.000	Trifolium micranthum		1.000 1.0	
Linum usitatissimum	1.000 1	.000 1.000	Trifolium repens	1.000 1	1.000 1.0	000
Lolium canariense	0.994 1	.000 0.991	Trifolium sp1.	1.000 1	1.000 1.0	000
Lolium perenne	1.000 1	.000 0.972	Trifolium sp2.	1.000 1	1.000 1.0	000
Lolium sp1.	1.000 1	.000 0.972	Triticum aestivum	1.000 1	1.000 1.0	)00
Lonicera japonica		.000 0.251	Triticum monococcum		1.000 1.0	
Lonicera macranthoides			<i>Triticum</i> sp1.		1.000 1.0	
Lotus pedunculatus		.000 1.000	Tsuga canadensis		1.000 0.9	
Lotus sp1.		.000 1.000	Urtica membranacea		1.000 0.1	
Lotus uliginosus		.000 1.000	Veronica arvensis		1.000 1.0	
Morella faya		.000 0.972	Veronica persica		1.000 1.0	
Musa acuminata		.000 1.000	Viburnum treleasei		1.000 1.0	
Myrsine africana		.000 1.000	Vulpia bromoides	1.000 1	1.000 1.0	)00
Oxalis sp1.	1.000 1	.000 0.972		-		