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Drivers of change and stability in the gut microbiota of an omnivorous avian migrant exposed to artificial food supplementation

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Abstract

Human activities shape resources available to wild animals, impacting diet and probably altering their microbiota and overall health. We examined drivers shaping microbiota profiles of common cranes (*Grus grus*) in agricultural habitats by comparing gut microbiota and crane movement patterns (GPS-tracking) over three periods of their migratory cycle, and by analysing the effect of artificially supplemented food provided as part of a crane-agriculture management programme. We sampled faecal droppings in Russia (nonsupplemented, premigration) and in Israel in late autumn (nonsupplemented, postmigration) and winter (supplemented and nonsupplemented, wintering). As supplemented food is typically homogenous, we predicted lower microbiota diversity and different composition in birds relying on supplementary feeding. We did not observe changes in microbial diversity with food supplementation, as diversity differed only in samples from nonsupplemented wintering sites. However, both food supplementation and season affected bacterial community composition and led to increased abundance of specific genera (mostly Firmicutes). Cranes from the nonsupplemented groups spent most of their time in agricultural fields, probably feeding on residual grain when available, while food-supplemented cranes spent most of their time at the feeding station. Thus, nonsupplemented and food-supplemented diets probably diverge only in winter, when crop rotation and depletion of anthropogenic resources may lead to a more variable diet in nonsupplemented sites. Our results support the role of diet in structuring bacterial communities and show that they

Sasha Pekarsky and Ammon Corl: Equal contribution.

undergo both seasonal and human-induced shifts. Movement analyses provide important clues regarding host diet and behaviour towards understanding how human-induced changes shape the gut microbiota in wild animals.

KEYWORDS
birds, crane, diet, GPS-tracking, gut microbiome, supplementary feeding

1 | INTRODUCTION

The communities of microbes occupying the gastrointestinal tracts of animals play a fundamental role in immune function, metabolism and development of the host (Claus et al., 2008; Gould et al., 2018; Hooper et al., 2012; Kau et al., 2011; Rowland et al., 2018; Sommer & Bäckhed, 2013; Thaiss et al., 2016). An individual's microbiota is shaped in turn by co-evolution with the host, host ecology and behaviour, and the environment (Benson et al., 2010; Colman et al., 2012; Grond et al., 2018, 2019; Muegge et al., 2011; Waite & Taylor, 2014; Youngblut et al., 2019). For example, in mammals, diversity in diet has been associated with diversity of the gut microbiota, which influences gut function and can thereby alter host health (Claesson et al., 2012; Faith et al., 2011; Suzuki & Ley, 2020; Zmora et al., 2019). While strong links have been observed between the diet of mammals and their microbiota, some studies of avian gut microbiota suggest that diet may be only weakly correlated with diversity of the microbiota, with other host factors (e.g., phylogeny, migration, locality) playing a more significant role (Hird et al., 2015; Song et al., 2020). However, avian host-associated microbiota remain poorly understood compared to advances in knowledge gained over the past decade for mammals (Grond et al., 2020; Suzuki & Ley, 2020), and recent studies suggest stronger connections between avian diets and the microbiome (Xiao et al. 2021). Many avian studies were designed to examine differences across taxonomic groups or dietary shifts among poultry or other domesticated species (Hird et al., 2015; Leung et al., 2019; Waite & Taylor, 2015, Xiao et al. 2021). Studies of the impact of dietary shifts on the gut microbiota in wild birds are scarce (but see Lewis et al., 2017) and usually require holding the birds in captivity (Bodawatta et al., 2021; Davidson et al., 2020; Teyssier et al., 2020). However, conducting studies on wild species is essential to understand the importance of diet in mediating gut microbiota diversity in natural ecological settings among avian species. Addressing this challenge necessitates information about the movement of individual wild birds that represent the population under study at the relevant spatial and temporal scales, motivating the use of advanced wildlife tracking tools such as ATLAS (Advanced Tracking and Localization of Animals in real-life Systems; Toledo et al., 2020) for locally foraging species (Corl et al., 2020) and GPS devices for migrating species (Nathan et al., 2008).

Human-induced alterations of resource availability, quality and quantity can affect the host microbiota by changing available foraging habitats (Amato et al., 2013; Chang et al., 2016; Ingala et al., 2019; Knutie et al., 2019; Teyssier et al., 2018). The influence of human alterations on host microbiota is likely to vary with host sensitivity to disturbance, along with variation in food availability and quality. For example, ground finches (*Geospiza fortis*) foraging in areas that overlap with human use had less diverse microbiota than those that did not (Knutie et al., 2019). Similarly, urban house sparrows (*Passer domesticus*) had reduced microbiota diversity compared to rural conspecifics (Teyssier et al., 2018). In contrast, urban white-crowned sparrows (*Zonotrichia leucophrys*) had higher microbiota diversity in comparison with rural conspecifics (Berlow et al., 2020).

Many large migratory species, such as geese and cranes, whose diet outside the breeding season is mainly herbivorous, rely more heavily on food resources available in agricultural fields rather than natural vegetation (Austin et al., 2018; Fox et al., 2017). This dependence, together with rising numbers of some migratory species, frequently causes extensive agricultural damage along migratory routes and this in turn leads to human–wildlife conflicts (Fox et al., 2017; Nilsson et al., 2016). A common practice for alleviating such conflicts is diversionary feeding, in which food supplementation is used to divert the target species from actions that result in agricultural damage (Kubasiewicz et al., 2016). While supplementary feeding may increase survival and reproduction by minimizing the starvation risk of target animals (Spiegel et al., 2013), it may also lead to decreased immunity and increased rates of disease transmission (Blanco et al., 2011; Milner et al., 2014).

The microbiota may affect host immunity and health (Hooper et al., 2012; Suzuki & Ley, 2020). Thus, it is important to understand whether there are large and long-term changes in gut microbiota due to supplementary feeding. In addition, empirically examining the effects of supplementary feeding on the gut microbiota could be an accessible tool for assessing the effects of management practices (Trevelline et al., 2019). We examined the effects of food supplementation on the gut microbiota to explore the spatiotemporal drivers shaping gut microbiota diversity of an omnivorous migratory avian species in areas with varying degrees of human-induced habitat alteration. Specifically, we examined the gut microbiota and movement patterns of free-ranging common cranes (*Grus grus*) during different stages of their annual migration cycle, with (namely a dedicated feeding station) and without food supplementation (Figure 1). We emphasize that these comparisons both between sites and within a single site during different periods were required to control for other potential factors driving gut microbiota differences and were possible through GPS tracking of free-ranging cranes representing the study population across all relevant spatiotemporal scales.

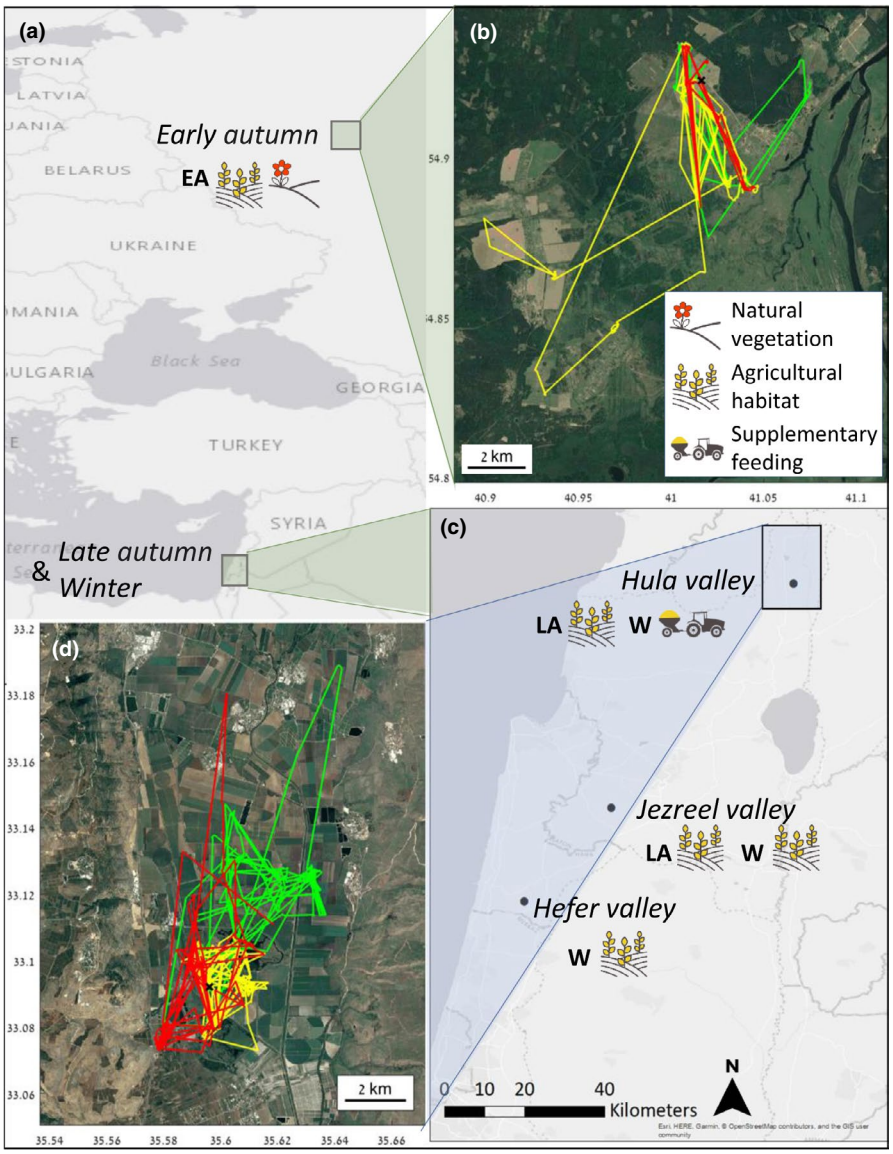

FIGURE 1 Map of the study sites. (a) The general locations of the study sites along the migration route of the cranes: early autumn at the premigration flocking site in Ryazan area in Russia, late autumn and winter at wintering and staging sites in Israel. (c) Zoomed in map of the sampling locations in Israel which are dominated by intensive farming. The letter EA (early autumn), LA (late autumn) and W (winter) indicate the sampling season in each location and the icons indicate the dominant habitat for the location and season (see Table 1 for more details). Colour zoomed in insets show examples of sampling locations (black X) and tracks of cranes that visited the location around the time of sampling (colours indicate different individuals). The Ryazan area (b), near the Oka River, is characterized by a patchy agricultural–natural matrix and the Hula Valley (d), the main wintering site in Israel, is characterized by intensified agriculture which together with large numbers of wintering cranes led to conflicts with local farmers. Diversionary feeding of corn from December to March (winter) is meant to keep them away from sensitive crops [Colour figure can be viewed at wileyonlinelibrary.com]

We hypothesized that microbiota diversity would vary by season and especially with food supplementation during winter, primarily because diversionary feeding in winter provides an excess of homogenous food. Specifically, we predicted lower bacterial diversity in samples collected from cranes at the homogenous food-supplemented site during winter, in line with the positive relationship between microbial diversity and greater diet and foraging site diversity found in both mammals (Amato et al., 2013; Claesson et al., 2012; De Filippo et al., 2010) and birds (Corl et al., 2020; Fuirst et al., 2018; Teyssier et al., 2018). In contrast, some recent evidence suggests that diets with a wider variety of macronutrients and plant diversity (Kartzinel et al., 2019) might not alter microbiome diversity (Bodawatta et al., 2021; Kartzinel et al., 2019) or even lead to lower diversity in some cases (Sugden et al. 2020). Thus, an alternative hypothesis was that the diversionary feeding would not lead to reduction in microbial diversity, but would instead shift the composition of the bacterial community due to the change in food resources. We also predicted that habitat characteristics and crane movement patterns, together with food supplementation, would result in differences in microbiota composition among the samples collected at the premigration areas and those collected at postmigration areas during late autumn and winter. This hypothesis follows from evidence that differences in foraging habitats can affect host microbial communities in some bird species (Berlow et al., 2020; San Juan et al., 2020).

2 | METHODS

2.1 | Study system

Cranes are long-distance, omnivorous migrants that typically feed on plant material (e.g., acorns and cereals) and invertebrates (Johnsgard, 1983). Outside the breeding season, most cranes nowadays depend on food available in agricultural habitats (Deinet et al., 2013; Harris & Mirande, 2013). This, together with their gregarious

TABLE 1 Sampling locations and periods

Stage	Food supplementation	Period	Location	Samples
Early autumn	None	August 20–September 20	Ryazan area, Russia	37
Late autumn	None	October 15–November 16	Hula Valley (+Jezreel Valley), Israel	40 (+10)
Winter	None	December 26–February 08	Jezreel & Hefer Valleys, Israel	40
Winter	Yes	December 26–February 08	Hula Valley, feeding station, Israel	40

Note: All locations are agricultural areas, without food supplementation except the feeding station (last row) where food supplementation is provided as part of the management programme.

behaviour during migration and wintering, has led to conflicts with local farmers and the subsequent establishment of various management schemes along the migration flyways (Austin et al., 2018).

We studied two areas along the common crane East European migration route (Figure 1a): a premigration staging area in western Russia (Ryazan area; 54°56′N, 41°02′E) and an important stopover and wintering area in Israel (Leito et al., 2015; Pekarsky et al., 2015). The premigration staging area is used by several hundred cranes after the breeding period. The main wintering site of these cranes is in the Hula Valley (33°06′N, 35°36′E) in northern Israel where up to 50,000 individual cranes can be found (Pekarsky et al., 2021). Several thousand cranes also occupy other sites along Israel's valleys (Levi & Yom-Tov, 1991), with the primary sites outside of the Hula Valley being the Hefer (32°25′N, 34°58′E) and Jezreel (32°31′N, 35°15′E) valleys in central Israel (Y. Davidson, pers. Comm; Figure 1c).

During the premigration period (early autumn, Russia), cranes flock in the stubble of harvested cereal fields for several weeks before the onset of their autumn migration (Johnsgard, 1983; Leito et al., 2015). Human activity in this area of Russia is considerably lower compared to the sites in Israel, with a higher proportion of noncultivated land, compared to the sites in Israel. Human impact in Israel encompasses intensive agriculture (corn, peanuts, wheat and orchards), and a designated 85-ha diversionary feeding station at the Hula Valley where corn is usually spread daily between December 1 and March 15, to prevent agricultural damage to the newly sown fields (Pekarsky et al., 2021).

2.2 | Crane trapping and tagging

We caught and tagged 29 cranes at the premigration staging site in western Russia during the summers of 2016 and 2017. The cranes were trapped using bait mixed with alpha-chlorolose, an oral sedation technique which is routinely applied to capture cranes in North America and Russia and is associated with low morbidity and mortality rates (Hartup et al., 2014; Markin, 2013) and processed in accordance with protocols approved by the Department of Environment of the Ryazan District, Russia (permit CK19-7154). Captured birds were colour-ringed and fitted with leg-mounted, solar-powered, GPS-GSM transmitters (28 OrniTrack-L40: Ornitela; one e-obs GmbH). The maximal total mass of a transmitter plus rings used for attachment was (mean ± *SD*) 0.8 ± 0.09% (range: 0.7%–1%; 35–42 g) of the captured cranes' average body mass. Three-dimensional GPS positions were recorded every 3–30 min depending on battery status. All data were downloaded remotely through a GSM network connection.

2.3 | Movement analysis

We focused our movement analyses on tagged cranes that visited sampled fields up to 3 days prior to faecal sample collection for host-associated bacterial analysis. The 3-day buffer was allowed because the tagged individuals were not always present on the same days as the big flocks chosen for faecal sampling. Food at the supplementary feeding station was provided only from dawn to 16:00 hours, and thus only cranes visiting the area during this time were included in the analysis. After identifying GPS-tagged focal birds for each sampling event and location, we extracted movement data for the 2 weeks prior to the specific sampling event. To circumvent potential behavioural changes caused by trapping, we excluded data obtained in Russia during the first 5 days after trapping from our analysis. To represent crane movements in sufficiently high temporal resolution, we used only days with GPS sampling interval <6 min.

We classified the habitats in which tagged birds were observed into three types: (i) crop—cultivated or harvested agricultural land; (ii) orchard—*Prunus* spp. that cranes use for foraging; and (iii) noncultivated—meadows and other noncultivated land. Habitats in Israel were classified based on GIS information provided by the Ministry of Agriculture and Rural Development. In Russia, where such information was unavailable, habitats were classified using satellite imagery from Sentinel-2 (Modified Copernicus Sentinel Hub, Sinergise Ltd) on a cloud-free day within the sampling period (September 1, 2017). The classification was performed using QGIS (version 2.18, QGIS Development Team, 2019) and subsequently verified by a local team.

We calculated the time spent by each GPS-tagged cranes in each habitat and divided this figure by the total number of GPS positions recorded for that individual during the entire period to analyse the proportion of time spent in the three habitats. We performed identical calculations to obtain the proportion of time that the cranes spent in the feeding station during the period of feeding station operation. Since cranes forage during the day (Nilsson et al., 2018), we only used daytime positions (from the time the birds departed the roost until they returned at night) in these calculations. Movement analysis was performed using MATLAB R2020a (The Mathworks Inc.).

To assess the effect of time period on proportion of time spent in each habitat, we applied an aligned rank-transformed ANOVA (ART-ANOVA) for nonparametric, factorial analyses with crane ID as a random factor and three period categories (early autumn, late autumn or winter), using the ARTOOL package (Wobbrock et al., 2011) in R version 3.5.1 (R Core Team 2020). We conducted within-group comparisons using the ARTOOL pair-wise contrast function and between-group comparisons using Mann–Whitney *U* tests with Bonferroni corrected *p*-values.

2.4 | Faecal sample collection

Faecal samples were collected in Russia and Israel from August 20, 2017 to February 8, 2018. Sampling locations were chosen based on foraging movements of tagged cranes at specific points of the annual cycle (premigration, postmigration, wintering) and in habitats subject to varying types of human influence (agricultural, diversionary feeding; Figure 1). Sample locations were selected where multiple cranes were observed both on the day of and several days before sampling. Only locations visited by at least one tagged individual within 3 days of a sampling event were included in this study.

During the premigration period ("early autumn" Russia, Figure 1), faecal samples were collected over four consecutive weeks (Table 1; Table SU1, Dataset S1). The seasonal sampling in Israel ("late autumn & winter," Figure 1) was divided into two segments: (i) "late autumn," a postmigration period during which migratory and wintering cranes are present but the diversionary feeding station, in the Hula Valley, was not operational (Figure 1c,d) and (ii) "winter," after the completion of the autumn migration and before the beginning of the spring migration (Figure 1c). Sampling during early (Russia) and late autumn (Hula and Jezreel Valleys, Israel) was performed only in agricultural (nonsupplemented) areas, while during the winter, it was performed in both the feeding station (supplemented) in the Hula Valley and the alternative agricultural (nonsupplemented) areas in the Jezreel and Hefer Valleys (Table 1, Figure 1; Table SU1, Dataset S1). Sampling in the Hula Valley during winter was performed in the feeding station to ensure that the sampled individuals were actually feeding on the supplementary grain. However, cranes foraging outside of the feeding station cannot be assigned to a nonsupplementary group with high probability because most of the wintering cranes in the Hula Valley use the supplementary feeding station at least for some of their foraging (Pekarsky et al., 2021). Thus, alternative wintering areas outside the Hula Valley were used to more confidently sample a nonsupplemented group of cranes (Figure 1c). During one week in late autumn (November 15–16), samples were collected in the Hula (main site) and Jezreel (alternative site) Valleys before the onset of diversionary feeding in the Hula Valley, to allow for baseline comparisons between the two sites when food supplementation was not yet available. Sampling in the Hefer Valley was not performed during autumn because the cranes do not use this site until later in the season.

We employed a noninvasive, four-step sampling protocol: (i) identification of a flock of cranes in a relevant sampling site; (ii) scattering of cranes to a new location by approaching them; (iii) collection of individual droppings (>30 cm apart) from the inner part of the dropping (to minimize contamination) using sterilized plastic spoon faeces tubes (Sarstedt) or sterilized cotton swabs (Deltalab), and then placing these in 1.5-ml Eppendorf tubes with 95% EtOH; and (iv) storage of faecal samples in a mobile −20°C freezer prior to transfer to long-term storage at −80°C. Given that cranes are large birds, we were able to typically fill most of the tube with the faecal sample (~1 ml or more of sample).

2.5 | Microbial DNA extraction and sequencing

We used a Qiagen PowerLyzer PowerSoil DNA kit to extract DNA from the faecal samples using methods detailed in Corl et al. (2020). In brief, faecal samples were transferred to bead tubes using flame sterilized forceps; three forceps full of faecal material were added to each tube, and the tubes were heated to 65°C for 10 min and then bead-beaten using a PowerLyzer homogenizer set at 3,500 rpm for 16 cycles of 30 s on and 30 s off. We processed randomized sets of faecal samples, because contaminants unique to a kit might confound biological effects or cause spurious groups if sets of similar samples were all processed together (Salter et al., 2014; Weiss et al., 2014). We also processed three negative control samples that went through the same DNA extraction process as the samples, but did not contain any faecal material. Extracted DNA was concentrated with a Centrivap vacuum to 40 μl, and half of each sample was submitted to the Argonne Sequencing Center at Argonne National Laboratory for polymerase chain reaction (PCR) amplification, library preparation and sequencing. We also submitted two blank samples containing Invitrogen UltraPure DNase/RNase-Free water to control for any contaminants acquired during PCR of the samples. We PCR-amplified the V4 region of the 16S rRNA gene using the primers 515F and 806R that were linked to the adapter sequences necessary for Illumina sequencing; the forward primer also contained Golay barcodes to allow pooling of the samples (Caporaso et al., 2011). Each PCR had 200 pM of each primer, 9.5 μl Mobio certified DNA-free water, 12.5 μl of AccuStart II PCR ToughMix (QuantaBio) and 1 μl of DNA (or more if the initial amplification failed). Thermal cycler settings were as follows: 94°C for 3 min; 35 cycles of 94°C for 45 s, 50°C for 60 s and 72°C for 90 s; and 72°C for 10 min. Each sample underwent three independent PCRs, the PCRs were combined and then equimolar amounts of each sample were pooled. Samples were sequenced on a 151-bp paired-end run of an Illumina MiSeq.

2.6 | Bioinformatic analyses

QIIME 2 (Bolyen et al., 2019) was used to demultiplex the raw sequence data. We followed Callahan et al.'s (2016) protocol for processing microbiome amplicon data in R. After trimming the first 10 bases of the reads, DADA2 (Callahan et al., 2016) was used to infer amplicon sequence variants (ASVs). We inferred ASVs from the pooled

sequencing reads of all samples, which meant that ASVs that were rare within samples but observed in other samples were maintained (i.e., DADA2 only excluded singleton ASVs that were supported by just a single read in the pool of all samples). Forward and reverse reads were merged, chimeric sequences were filtered out, and then taxonomic classifications were made with the SILVA taxonomy database (Glöckner et al., 2017; Pruesse et al., 2007; Quast et al., 2012) via a SILVA version 132 derived training set (Callahan, 2018). The sequences were aligned using the R package DECIPHER (Wright, 2015), and a maximum likelihood phylogeny was inferred with the R package PHANGORN (Schliep, 2011). Finally, we used the R package PHYLOSEQ (McMurdie & Holmes, 2013) to join the ASV table, sequence taxonomies, phylogenetic tree and metadata for subsequent downstream analyses.

2.7 | Sample and ASV filtering

In total, 167 samples were sequenced, yielding a total of 5,123 ASVs. Five outlier samples were identified based on: (i) them clustering together far from the rest of the samples in the ordination space, regardless of distance measure employed; and (ii) based on our fieldnotes that these faecal samples were dry when they were collected (Table SU1, Dataset S1). The outlier samples were removed because time-to-collection following defecation can affect bacterial PCR-amplification success (Nearing et al., 2021). We used the DECONTAM R package (Davis et al., 2018) to identify and remove contaminants using the prevalence method with a threshold of 0.5 to identify any ASV that was more common within the three negative controls and two PCR-blank samples than within all the other crane samples (Table SU5, Dataset S1). This resulted in the removal of 23 ASVs. We also removed sequences that could not be assigned to a phylum ($n = 74$) and those that were assigned to mitochondria, chloroplast or kingdoms other than bacteria ($n = 152$).

The average number of reads across all samples was 13,962 (range: 5,547–25,670) per individual. After examining rarefaction curves (Figure SU2, Dataset S1), a minimum read depth of 8,000 was chosen to optimize the trade-off between read depth and sample size. As a result, 14 of the original 162 samples were excluded due to low read depths. We then standardized our sequencing effort by rarefying the remaining samples to the minimum sequencing depth (8,158; random seed: 999) (Weiss et al., 2014). Rarefaction reduced the total number of ASVs from 4,666 to 3,697, with 148 individuals remaining in the data set (Tables SU1, SU2, Dataset S1).

2.8 | Statistical analysis

We analysed bacterial alpha diversity by calculating Shannon's diversity index and the Chao1 richness index using the PHYLOSEQ R package and by calculating Faith's phylogenetic diversity (Faith, 1992) using the R package PICANTE (Kembel et al., 2010). Differences in community diversity were assessed with Kruskal–Wallis tests (Kruskal & Wallis, 1952), due to lack of normality in the data, followed by pairwise comparisons (Mann–Whitney *U* test) with a Bonferroni correction (Dunn, 1961).

We visualized the bacterial community composition (beta diversity) using principal coordinates analysis (PCoA). We tested for differences between groups using the adonis function in the VEGAN package (Oksanen et al., 2019) in R by conducting a permutational multivariate analysis of variance (PERMANOVA) followed by pairwise comparisons with Bonferroni corrections. We applied the ordination on Bray–Curtis, Jaccard, weighted UniFrac and unweighted UniFrac distances between the microbial communities of the samples. UniFrac distance measures take into account evolutionary distances between the ASVs. Bray-Curtis and weighted UniFrac are affected by ASV relative abundance (Knight et al., 2018) whereas the other two distance measures focus solely on presence/absence of ASVs. We used betadisper in the VEGAN package (Oksanen et al., 2019) in R to assess homogeneity of dispersion between groups prior to running PERMANOVA, because significant differences observed with the PERMANOVA tests could potentially result from differences in dispersion rather than differences in location.

To identify bacterial taxa that differed in abundance between groups, we performed an analysis of composition of microbiomes (ANCOM) using the ANCOM 2.1 R package (Mandal et al., 2015), including the week of sampling as a random factor. We used an adjusted *p*-value cutoff of <.01 with a Benjamini–Hochberg correction for reducing false positives when multiple comparisons are made (Benjamini & Hochberg, 1995; Chen et al., 2017). We excluded ASVs present in less than 10% of the samples, in order to exclude rare taxa, thus focusing our analyses on differences in the potentially more stable taxa. Taxa were considered differentially abundant if the W statistic was at or above the 85th percentile. We conducted the analysis on rarefied data at the genus level because rarefaction reduces false positives and is considered a more conservative approach (Weiss et al., 2017). For differentially abundant genera detected by ANCOM, Mann–Whitney *U* pairwise tests were conducted with a Bonferroni correction.

3 | RESULTS

3.1 | Movement patterns

We analysed the GPS tracks of 28 cranes over a period of 108 days. The number of tagged cranes foraging in the selected sampling fields ranged from one to 17; on average, tracks from 6.8 ± 1.5 (mean ± *SE*) individuals were analysed for each location and date (see Table S1, Appendix S1). Of 20 tagged cranes in Israel, the Hula Valley served as the sole wintering site for 17 individuals and the main wintering site for an additional two individuals. One crane used the secondary wintering sites (Jezreel and Hefer Valleys) exclusively. The three different wintering sites in Israel were partially connected, with two of the tagged cranes using more than one site. However, movement between the wintering sites did not occur on a daily basis; cranes spent

on average 24.1 successive days (and at least two successive days) at each wintering site (Figure 2e; Figure S1, Appendix S1). Our movement analysis also showed that cranes visiting the feeding station around the sampling dates spent most of their time in the station during the 14 days prior to the sampling event, whereas cranes from the alternative wintering sites did not visit the Hula feeding station during the 14 days prior to sampling.

On average, cranes spent around 80% of their time in agricultural fields when diversionary feeding was unavailable (Figure 2a). Most of the cranes shifted their foraging behaviour drastically once the feeding station became operational (Pekarsky et al., 2021), spending 74% of their time at the station and only 18% in agricultural fields during the 2 weeks prior to sampling (ART-ANOVA: $F_{3,51} = 27.11$, $p < .001$, Figure 2a,d). During early autumn, in the premigration flocking areas, cranes spent significantly more time in noncultivated areas (ART-ANOVA and *post hoc* tests with Bonferroni correction: $F_{3,51} = 13.03$, $p_{adj} < .001$, Figure 2b), though orchards were not available in the Russian habitat. During the winter, cranes wintering at alternative areas with no supplementary feeding used pecan and almond orchards significantly more than cranes wintering near the feeding station (ART-ANOVA: $F_{2,37} = 3.46$, $p_{adj} < .05$, Figure 2c); however, the proportion of time spent at orchards was not significantly different

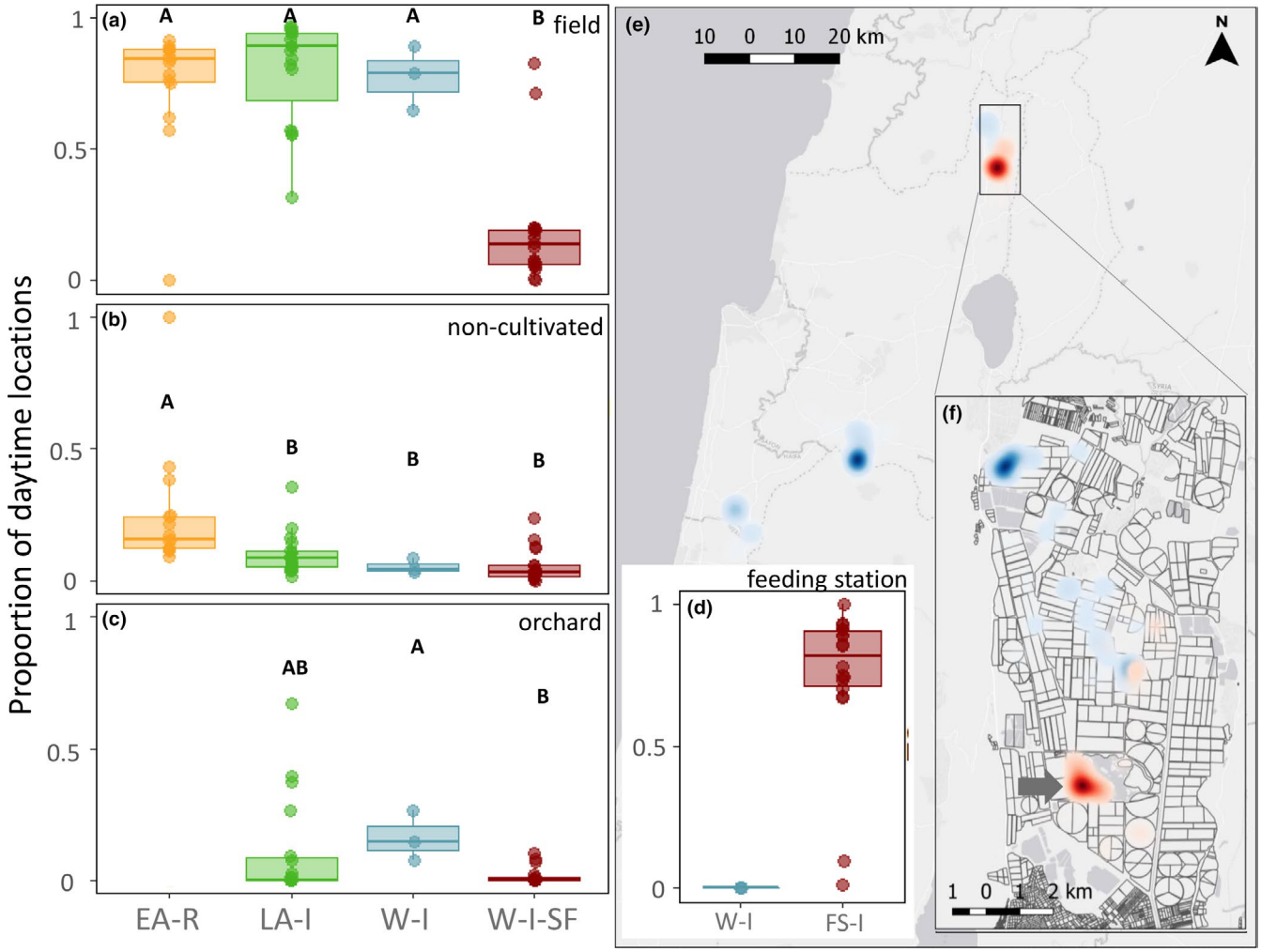

FIGURE 2 Proportion of time spent by tagged cranes at various habitats. Analysis included only individuals that visited the sampling location around the time of sampling (see also Figure 1); the boxplots present the median, and 25th and 75th percentiles and the points show the proportion of daytime locations per individual crane in (a) agricultural fields, (b) noncultivated land, (c) *Prunus* spp. orchards and the (d) feeding station. Letters indicate significant differences based on ART-ANOVA followed by pairwise *post hoc* tests with Bonferroni correction ($p < .05$). Orange = early autumn at premigration agricultural flocking areas (EA-R), green = late autumn in agricultural habitat in the Hula Valley, Israel (LA-I), blue = winter at nonsupplemented agricultural wintering areas (W-I), and red = winter at the food-supplemented wintering area in the Hula Valley, Israel (W-I-SF). The proportion of time spent in the feeding station is presented only during winter because it was operational only during this period. (e) Map showing the density of GPS locations 2 weeks prior to the cranes visiting sampled fields at nonsupplemented agricultural wintering areas (blue shading) and food-supplemented wintering areas in the Hula Valley (red shading) and (f) a zoomed in panel of Hula Valley. Note that even though one of the cranes from the nonsupplemented wintering areas presented also in the Hula valley (Figure S1) it did not feed in the feeding station. The intensity of colour corresponds to higher density of GPS locations. Grey arrow indicats the feeding station [Colour figure can be viewed at wileyonlinelibrary.com]

between cranes during autumn and the nonsupplemented group of cranes during winter.

3.2 | Microbiota composition

A total of 3,697 ASVs were identified from 148 rarefied samples which were sampled over three periods at agricultural habitats (with no food supplementation: early autumn, $n = 36$; late autumn, $n = 33$ Hula Valley +9 in Jezreel Valley; winter, $n = 32$) and from an artificial diversionary feeding station with food supplementation (winter, $n = 38$; Tables SU2, SU3; Dataset S1). The average prevalence of ASVs was low (4.1%), implying low ASV overlap between samples. Only nine ASVs occurred in at least 90% of the samples, most of which belonged to the genus *Lactobacillus* in the phylum Firmicutes (Table 2; Table SU4, Dataset S1).

The most abundant phyla (mean relative abundance >1% of the total sequences) across all faecal samples collected were Firmicutes (mean ± *SE*: 74.32 ± 1.3%), Proteobacteria (7.9 ± 1.2%), Fusobacteria (6.8 ± 1.3%), Tenericutes (5.9 ± 0.4%), Epsilonbacteraeota (2.1 ± 0.4%) and Actinobacteria (2.0 ± 0.2%). Within the Firmicutes, the classes Bacilli and Clostridia were the most abundant, and Gammaproteobacteria and Alphaproteobacteria were more abundant in Proteobacteria. The most abundant genera across samples were *Lactobacillus* (relative abundance: 33.5 ± 2.0%, prevalence 100%) and *Catellicoccus* (relative abundance: 21.1 ± 2.0%, prevalence 99%). Four other genera also had high prevalence: *Campylobacter* (95%), *Turicibacter* (91%), *Enterococcus* (91%) and *Fusobacterium* (85%), but a relative abundance under 5%.

3.3 | Microbiota differences and similarities

Samples were compared both temporally (early autumn, late autumn and winter) and in relation to the intensity of human impact (feeding supplementation status in winter; Table 1). During winter, samples collected at nonsupplemented sites (Jezreel and Hefer Valleys) had significantly higher Faith's phylogenetic diversity (PD; $\chi^2 = 22.81$, $df = 3$, $p_{adj} < .01$) and Chao1 richness ($\chi^2 = 33.95$, $df = 3$, $p_{adj} < .01$) than samples from the Hula Valley feeding station (food-supplemented) but similar Shannon diversity ($\chi^2 = 29.93$, $df = 3$, $p_{adj} = 1$; Figure 3a). Samples collected at the food-supplemented site during winter had generally similar bacterial diversity (Chao1 richness, Shannon diversity, PD) to samples collected in early autumn in Russia during premigration flocking and to samples from the late autumn collected in Israel. The sole potential difference was that the feeding station samples had (slightly) higher Chao1 richness compared to samples collected in late autumn in Israel (Figure 3a). This pattern appears to result from the fact that control samples (nonsupplemented sites during late autumn) collected in the Jezreel Valley had richness and diversity lower than those collected in the Hula Valley during the same week ($p_{adj} < .01$). This difference cannot be attributed to supplementary feeding (which only occurred later in the Hula Valley), nor can it be considered to result from outliers (sampling points are well distributed within the observed range; Figure 3a; empty symbols, Figure S3, Appendix S1). When the samples collected at the Jezreel Valley were excluded, there was no significant difference between Chao1 richness in the Hula Valley during the late autumn (nonsupplemented) and winter (supplemented).

We observed temporal changes in the microbiota beta diversity, because all groups' bacterial communities were significantly different from one another using the Bray–Curtis ($p < .001$), Jaccard ($p < .001$) and unweighted UniFrac distances ($p < .001$; Figure 4a,b,d). With weighted UniFrac distances, the two autumn samples (pre- and postmigration) were not significantly different from one another ($p_{adj} = 0.2$), but they differed significantly from the two winter samples ($p_{adj} < .01$), with the nonsupplemented and supplemented winter samples also differing significantly from one another ($p_{adj} < .001$; Figure 4c). A very large effect of food supplementation was apparent when Bray–Curtis and Jaccard distances were used, because the supplemented group clustered substantially farther from the nonsupplemented groups across all seasons (Figure 4a,b). Homogeneity

TABLE 2 Prevalence, relative abundance ± *SE* and classification (phylum, family and genera) of the nine most prevalent ASVs found in cranes sampled in all seasons

ASV ID	Prevalence (%)	Mean relative abundance (%)	Phylum	Family	Genus
1	100.0	19.2 ± 1.4	Firmicutes	Lactobacillaceae	*Lactobacillus*
6	100.0	5.2 ± 0.5	Firmicutes	Lactobacillaceae	*Lactobacillus*
2	97.3	11.4 ± 1.3	Firmicutes	Enterococcaceae	*Catellicoccus*
52	97.3	0.7 ± 0.1	Firmicutes	Lactobacillaceae	*Lactobacillus*
26	95.9	2.0 ± 0.3	Firmicutes	Enterococcaceae	*Catellicoccus*
114	95.9	0.3 ± 0.0	Firmicutes	Lactobacillaceae	*Lactobacillus*
47	95.3	0.8 ± 0.1	Firmicutes	Lactobacillaceae	*Lactobacillus*
25	91.9	1.9 ± 0.3	Firmicutes	Lactobacillaceae	*Lactobacillus*
4	90.5	4.6 ± 0.8	Firmicutes	Erysipelotrichaceae	*Turicibacter*

Note: These ASVs made up 46% of the total abundance of the crane microbiome

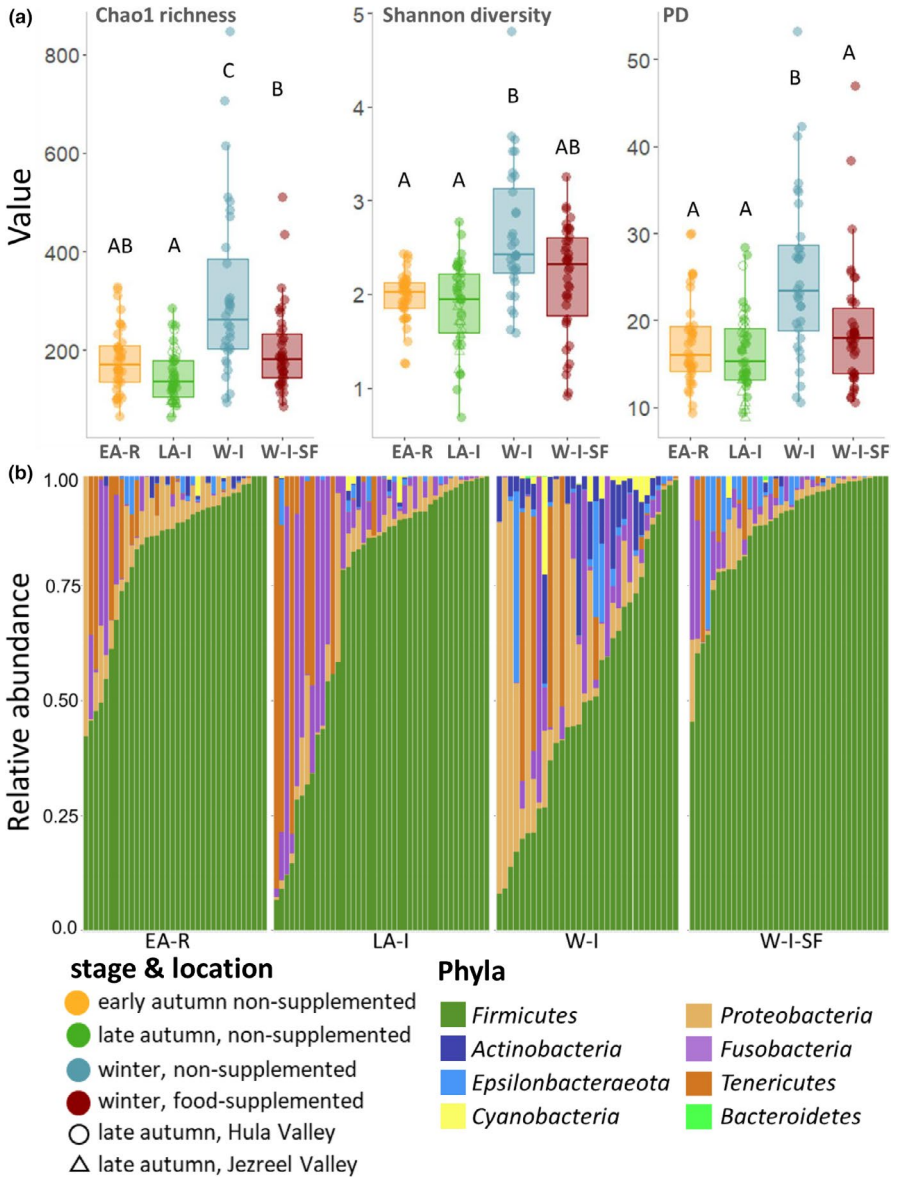

FIGURE 3 Alpha-diversity and composition of faecal samples collected in agricultural habitats and at the feeding station. (a) Differences in measures of Chao1 richness, Shannon diversity and Faith's phylogenetic diversity (PD) between groups. Significantly different groups ($p_{adj} < .05$) are indicated by different letters. The *p*-value is adjusted according to the Bonferroni correction; orange = early autumn at premigration agricultural flocking areas (EF-R), green = late autumn in agricultural habitat in the Hula Valley, Israel (LF-I), blue = winter at nonsupplemented agricultural wintering areas (W-I), and red = winter at the food-supplemented wintering area in the Hula Valley, Israel (W-I-SF). Open circles indicate samples collected during same week in late autumn, both in the Hula Valley and the Jezreel Valley, to control for the differences before the onset of the diversionary feeding (see Figure S3, Appendix S1 for more details). (b) Relative abundance of the most abundant bacterial phyla (>0.1% of the total sequences) across individuals, sorted by season and diet (see Figure S4, Appendix S1 for details of samples collected during late autumn). Samples are arranged by relative abundance of the dominant phylum, Firmicutes [Colour figure can be viewed at wileyonlinelibrary.com]

of dispersion was rejected for Bray–Curtis and Jaccard distances (Figure S4, Appendix S1), but there is clearly a difference in location of the food-supplemented group in addition to differences in dispersion among groups (Figure 4a,b). The effect of food supplementation was still apparent, but not as extreme, once phylogenetic information was added (Figure 4c,d), with no significant differences in dispersion across the groups when weighted and unweighted UniFrac distances were used. Prior to operation of the feeding station, the composition of the gut microbiota of cranes did not differ between the Hula Valley and the alternative wintering sites with weighted UniFrac ($p_{adj} = .75$; Figure 4c, empty circles and triangles). However, with unweighted UniFrac these samples differed from each other ($p_{adj} < .01$) and were located in the PCoA between the samples collected at the agricultural areas and at the feeding station (Figure 4d, empty circles and triangles).

Samples collected during winter at nonsupplemented sites featured higher relative abundances of Proteobacteria (nonsupplemented: 20.3% vs. supplemented: 2.7%) and Actinobacteria (6.7% vs. 0.5%) but lower abundances of Firmicutes (51.8% vs. 87.5%) compared with samples collected at the food-supplemented site (Figure 3b; Table 3). Samples collected in the Jezreel Valley during late autumn had higher relative abundances of Fusobacteria (33.1% vs. 9%) relative to samples collected during the same week in the Hula Valley (Figure S5).

Differential abundance analysis (ANCOM2) identified 33 genera that differed significantly among the groups (cut-off of 0.85, W > 125; Table S2; Figure S6, Appendix S1; Figure 5 shows the 10 most abundant genera). Cranes sampled in Russia during early autumn and in Israel during winter with no food supplementation had higher abundance of three genera in the phylum Actinobacteria and four genera in the phylum Proteobacteria. The nonsupplemented group during winter had an additional 10 genera with relatively high abundance, most belonging to Actinobacteria or Proteobacteria. The food-supplemented group had higher abundance of 10 genera, nine of which belonged to the phylum Firmicutes, specifically within the classes Bacilli, Erysipelotrichia

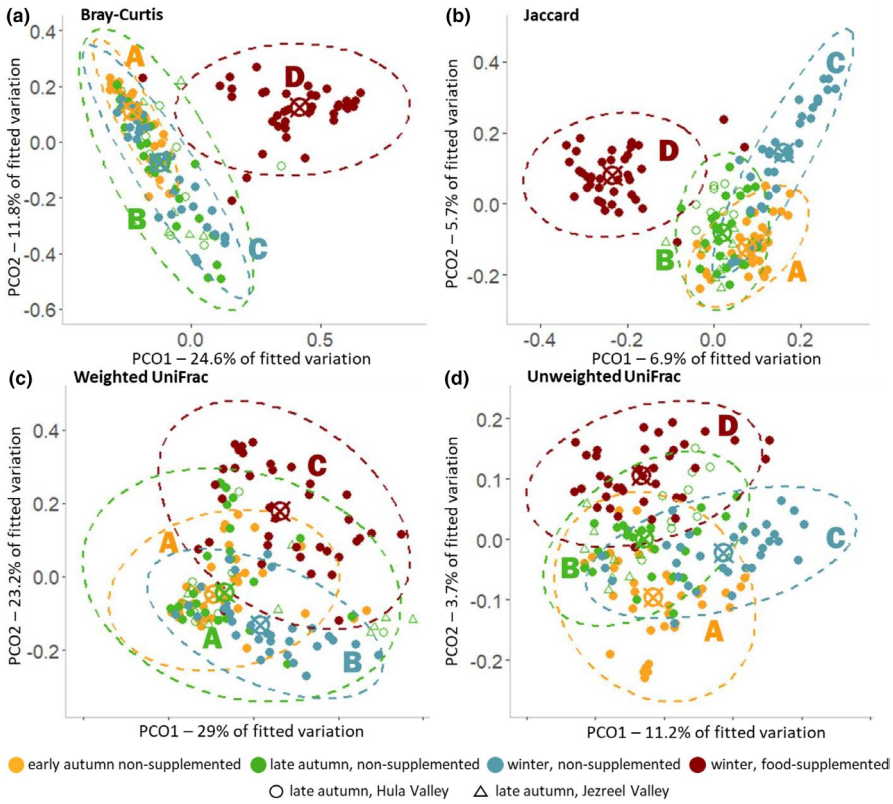

FIGURE 4 Bacterial community composition of faecal samples collected in agricultural habitats and at the feeding station. PCoA ordination based on (a) Bray–Curtis, (b) Jaccard (c) weighted and (d) unweighted UniFrac distances, revealing the influence of season and artificial feeding on the community structure of gut microbiota across individual cranes. Ellipses indicate 95% confidence around centroids. Centroids are shown as open, crossed circles; letters indicate significant differences based on pairwise *post hoc* tests with Bonferroni correction ($p < .01$); orange = early autumn at premigration agricultural flocking areas (EA-R), green = late autumn in agricultural habitat in the Hula Valley, Israel (LA-I), blue = winter at nonsupplemented agricultural wintering areas (W-I), and red = winter at the food-supplemented wintering area in the Hula Valley, Israel (W-I-SF). Open circles indicate samples collected during same week in late autumn, both in the Hula Valley and the Jezreel Valley, to control for the differences before the onset of the diversionary feeding (see Figure S2 for more details) [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 3 Relative mean abundance ± *SE* (%) during different seasons for the most abundant phyla (>0.1% of the total sequences)

Phylum	All	Early autumn Russia	Late autumn Israel	Winter Israel	Feeding station
Firmicutes	74.3 ± 1.3	82.5 ± 2.7	72.4 ± 4.5	51.8 ± 4.9	87.5 ± 4.9
Proteobacteria	7.9 ± 1.2	6.9 ± 1.6	4.0 ± 1.0	20.3 ± 4.8	2.7 ± 4.8
Fusobacteria	6.8 ± 1.3	3.4 ± 1.5	11.8 ± 3.1	8.2 ± 2.7	3.5 ± 2.7
Tenericutes	5.9 ± 0.4	4.7 ± 1.8	9.5 ± 3.5	7.1 ± 3.1	2.1 ± 3.1
Epsilonbacteraeota	2.1 ± 0.4	1.0 ± 0.3	1.0 ± 0.3	3.5 ± 1.5	3.2 ± 1.5
Actinobacteria	2.0 ± 0.2	1.1 ± 0.2	0.6 ± 0.2	6.7 ± 1.3	0.5 ± 1.3
Cyanobacteria	0.7 ± 0.0	0.3 ± 0.2	0.6 ± 0.2	2.0 ± 0.7	0.3 ± 0.7
Bacteroidetes	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.1	0.1 ± 0.1

and Clostridia. Among the most abundant genera (mean relative abundance >0.02%; Figure 5), *Clostridium sensu stricto*, *Escherichia*, *Romboutsia*, *Streptococcus* and *Turicibacter* were enriched in the food-supplemented group, whereas *Catellicoccus* and *Lactobacillus* were depleted in this group.

4 | DISCUSSION

Humans have dramatically altered the habitats and food resources available to animals, with many consequences for their ecology. For example, contemporary agriculture can potentially provide

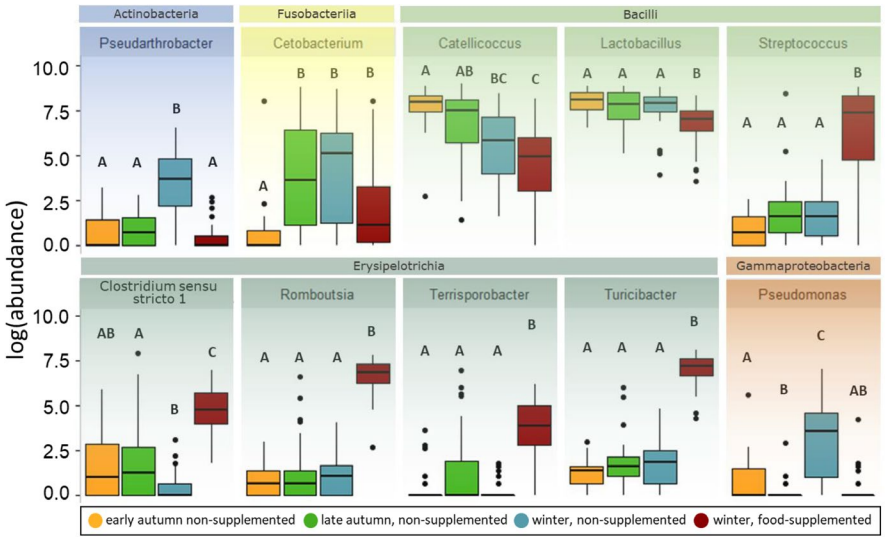

FIGURE 5 Differential abundance of genera across crane faecal samples. Differentially abundant genera among those most generally abundant (>.01%; see Figure S6, Appendix S1 for all genera) in crane samples collected during early autumn at premigration agricultural flocking areas (orange), late autumn in agricultural habitat in the Hula Valley, Israel (green), winter at nonsupplemented agricultural wintering areas (blue), and winter at food-supplemented areas in the Hula Valley, Israel (red), as indicated by analysis of composition of microbiomes (ANCOM2). Boxplots present the median, 25th and 75th percentiles and, if applicable, outliers for log relative abundance. Lower text bands indicate genera and upper bands denote class. Genera are shaded to highlight them belonging to Actinobacteria (blue), Firmicutes (two shades of green for two classes of bacteria within the Firmicutes), Fusobacteria (yellow) and Proteobacteria (brown). Significantly different groups (Mann–Whitney *U* test pairwise comparisons with Bonferroni correction, $p_{adj} < .05$) are indicated by different letters [Colour figure can be viewed at wileyonlinelibrary.com]

alternative food resources, and management of animals sometimes involves food supplementation (Emmerson et al., 2016; Fox & Abraham, 2017; Murray et al., 2016). We sought to explore how food supplementation affects the gut microbiota of wild birds and whether there are unanticipated consequences of this common management practice. We found that food supplementation did result in large shifts in the gut microbial communities (Figure 4) but did not result in loss of overall bacterial diversity (Figure 3). Thus, the cranes maintain a diverse set of bacteria in their gut despite a homogeneous diet of corn, and their microbiota composition shifts in response to the available food resources. Our data suggest that food supplementation does not result in permanent changes to the crane's microbiota.

Tracking the movements of the cranes made it possible for us to link landscape use with patterns in the gut microbial data, which is often challenging for wild species. Movement analysis revealed that cranes foraging in nonsupplemented areas spent the vast majority of their time in agricultural fields during all seasons (Figure 2). Thus, residual grain left on the landscape from agricultural monocultures appears to be an important source of food for cranes foraging in both Russia and Israel Austin et al., 2018. This pattern of land use suggests that cranes might maintain generally similar diets and microbiota despite foraging in widely divergent geographical locations. The shift to higher bacterial richness/diversity in the winter (Figure 3) could be explained by shifts in movement patterns and diet. We observed that at the nonsupplemented sites in winter, the cranes spent a slightly higher proportion of time in orchards than during late autumn (Figure 2c), probably relying on almonds and pecans and perhaps also on more "natural" food resources such as nut grass tubers (*Cyperus rotundus*) that are available in the area. This shift in foraging behaviour during winter could be due to prior intensive foraging in agricultural fields in conjunction with decomposition and crop rotation, leading to depletion of food resources as winter approaches (Nilsson et al., 2018). Such a behavioural change has been observed in cranes in Spain, which switched to feeding on acorns and bulbs once cereal grain was no longer available to them (Avilés et al., 2002). The movement data also showed that although cranes wintering in the food-supplemented site spent most of their time at the supplementary feeding station, they still spent around 20% of their foraging time in agricultural fields. Thus, food-supplemented cranes are unlikely to have a completely homogeneous diet, because they probably diversify their diet with invertebrates outside of the feeding station. This is consistent with previous research suggesting that animals constitute up to 10% of the diet of cranes (Avilés et al., 2002; Bart & Jonathan, 2000; Reinecke & Krapu, 1986). Therefore, food-supplemented cranes are likely to maintain some dietary diversity, which could help explain why alpha-diversity of the gut microbiome did not differ for this group from nonsupplemented groups in the autumn (Figure 3) in accordance with our alternative hypothesis.

Cranes at the food-supplemented site had very distinct microbial communities (i.e., beta-diversity, Figure 4), with many genera in the phylum Firmicutes being differentially abundant in these individuals (Figure 5) and the overall proportion of Firmicutes increasing with food supplementation (Figure 3b, Table 3). Firmicutes were the most abundant phyla in crane microbiota in accordance with previous studies of cranes (Trevelline et al., 2020) and other avian hosts

(Bodawatta et al., 2018; Hird et al., 2015; Waite & Taylor, 2014). The increase in Firmicutes abundance in food-supplemented cranes is expected due to their known role in digestion, where they play an essential role in the breakdown of dietary polysaccharides and carbohydrates that are abundant in grain-rich diets such as corn (Grond et al., 2018). Similar patterns to ours were found in a study of hooded cranes (*Grus monacha*), which had higher relative abundance of Firmicutes in the autumn when corn is a primary food resource than in the spring when the grain is depleted (Zhang et al., 2020). Although many genera within Firmicutes increased in abundance with food supplementation, two (*Lactobacillus* and *Catellicoccus*) decreased in abundance (Figure 5), demonstrating that the effects of dietary change can differ even amongst genera within a larger group of bacteria. When phylogeny was not included in beta-diversity analyses, the food-supplemented group was better separated from other groups and had lower dispersion. This further suggests that the food-supplemented group had a specific set of bacteria common to most individuals, and that they might be closely related phylogenetically.

Our extensive sampling let us assess whether temporal and regional changes, at nonsupplemented sites, affected the microbiota of the cranes. We found that the early autumn, late autumn and winter groups generally differed from one another (Figure 4), suggesting the microbiota community may change because of temporal changes in the cranes' diet or changes in the seasonal abundance of particular bacteria. However, cranes in the early autumn in Russia did not differ significantly in microbiota from cranes in the late autumn in Israel as measured by weighted UniFrac (Figure 4c) and had relatively low differentiation with the other metrics of community composition (Figure 4a,b,d). Thus, migratory status (pre- vs. post-migration in autumn) and the sampling region (Russia vs. Israel) did not have large effects on the microbial communities compared to the differences observed between autumn and winter or supplemented and nonsupplemented areas. This is in contrast to several bird studies that show a higher association with migratory status and locality than diet (Grond et al., 2019; Turjeman et al. 2020), but is in line with other studies that found overall community composition to be similar among co-occurring migrants and residents (Risely et al., 2018) and influenced by local food availability at stopover sites (Lewis et al., 2017; Xiao et al. 2021). Cranes sampled during autumn in Russia and Israel also did not differ in microbiota richness and diversity (Figure 3) despite their different migratory status in these sites. Interestingly, the samples collected in Jezreel Valley during autumn had significantly lower diversity than samples collected during the same week at the geographically close Hula valley. The Jezreel Valley is a staging site for much lower numbers of cranes, which could lead to accidental sampling of birds belonging to the same flock (less probable in the Hula Valley where large number of cranes mix). In that case, the lower diversity found in birds sampled in the Jezreel Valley could be because of the flock origin (Grond et al., 2019) or because it was sampled shortly after arrival, as migration is known to alter microbial communities (Lewis et al., 2017; Risely et al., 2018).

Nonsupplemented cranes during winter showed increased ASV richness and phylogenetic diversity (Figure 3), as well as an increase in overall proportion of Proteobacteria (Figure 3b; Table 3) with several genera in this phylum being differentially abundant in this group compared to nonsupplemented cranes during autumn (Figure 5). During winter, the relative abundance of Proteobacteria was more similar to levels reported for other crane species (Dong et al., 2019; Wang et al., 2020; Xie et al., 2016; Zhao et al., 2017). An increase in Proteobacteria relative abundance was observed also in hooded cranes during spring when availability of corn decreased (Zhang et al., 2020). While ASV richness increased during winter, there was no seasonal difference in Shannon diversity between the groups, indicating the differences were probably attributed to a higher incidence of rare species in this group. Higher gut microbial richness is thought to be related to the diversity of food consumed by the host and its tendency towards an omnivorous diet (Moles & Otaegui, 2020; Yun et al., 2014). However, microbial responses vary not only due to physical components of the diet but also due to the macronutrient composition (Bodawatta et al., 2021; Conlon & Bird, 2014; O'Grady & Shanahan, 2021).

In Russia, the dominant crops available post-harvest are barley, wheat and corn, while in Israel during autumn, in addition to corn, peanut crops and almond orchards are also available. Interestingly, while peanuts and nuts have higher protein and fat content than grains, we found similar bacterial richness in birds from these sites. The depletion of agricultural sources during winter could lead to more diverse foraging strategies (Avilés et al., 2002), which may explain their higher bacterial diversity and higher interindividual differences due to consumption of diets with more diverse macronutrients. For example, an increase in dietary fibre leads to a more diverse microbiome both in humans and in wild mammals (Dahl et al., 2020; Makki et al., 2018; O'Grady & Shanahan, 2021), suggesting a similar process may be happening with cranes. The finding that such a seasonal effect was not apparent in food-supplemented sites during winter suggests that the dietary diversity of cranes feeding at these sites is comparable to nonsupplemented sites during autumn. In a similar manner, microbial diversity in house sparrows remained constant in urban settings, probably due to high reliance on anthropogenic food resources between seasons, but shifted in rural conspecifics due to seasonal fluctuations in resource availability in their environment (Teyssier et al., 2018). However, while the addition of movement data enabled the testing of our interpretations more thoroughly, specific dietary items eaten by cranes at the different sites were unknown and thus our conclusions regarding dietary features that contribute to differences observed in crane microbiota remain suggestive.

The pronounced shifts in microbial community composition with food supplementation suggest that it is worth considering whether these changes might impact the health of the cranes. The bacterial genus *Lactobacillus* had the highest abundance and prevalence across our samples, in line with previous studies of the gut microbiota of black-necked and hooded cranes (Fu et al., 2020; Wang et al., 2020; Zhao et al., 2017) and other birds and mammals (Heeney et al.,

2018; Hird et al., 2015). This genus was less abundant in our food-supplemented group compared to the nonsupplemented group in all seasons. *Lactobacillus* is thought to have protective qualities and is widely used as a probiotic to promote host growth and resistance to pathogens (Bernardeau et al., 2006; Khan et al., 2007; Li et al., 2009). Another change in the food-supplemented group was the higher abundance of *Clostridium sensu stricto* and *Escherichia*, genera which include several pathogenic species (Benskin et al., 2009; Laviad-Shitrit et al., 2019). We lack the strain-level resolution in our data that is needed to study diseases, but studying whether cranes in food-supplemented sites harbour more pathogenic bacteria is a worthwhile avenue for future research. The supplementary feeding station results in exceptionally large and dense congregations of cranes, so there is high potential for the spread of pathogens. Our study provides a foundation of knowledge for future study of the bacteria of food-supplemented cranes and how they spread among individuals.

5 | CONCLUSIONS AND FUTURE DIRECTIONS

We found that microbiota sampling paired with movement data is useful for elucidating the effects of human activities on the foraging habitats of omnivorous migrant cranes. First, we observed that although food-supplemented cranes had different microbial communities, they did not have lower bacterial alpha diversity in their gut, which could stem from them foraging away from the feeding station part of the time and thus not having a completely homogeneous diet. Second, cranes in pre- and postmigration sites showed similarities in their microbiome despite being separated by vast distances, which could be due to the birds foraging on similar agricultural crops and thus having comparable diets. Third, nonsupplemented cranes in winter had different microbial diversity and community composition, which could be due to the birds shifting their foraging patterns to a less grain-dominated diet (Avilés et al., 2002; Bart & Jonathan, 2000). Microbiota sampling alone would not have yielded the same inferences without integration of movement data, highlighting the need for further integration of host microbiota and animal movement studies (see also Corl et al., 2020). Further work is needed in this system to gain more direct assessments of the diets of the cranes, which could be achieved by analysing the relative nutrients of different food items and determining the dietary diversity from DNA metabarcoding of faecal samples.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

DATA AVAILABILITY STATEMENT

The 16S amplicon sequence data underlying this study are available on in the Sequence Read Archive (BioProject ID: PRJNA578383, biosample accession numbers: SAMN20111390 - SAMN20111560; https://www.ncbi.nlm.nih.gov/sra/PRJNA578383) along with associated metadata (Pekarsky et al., 2021). All data sets analyzed during the current study are available on Dryad https://doi.org/10.5061/dryad.02v6wwq3m, including the R scripts used for microbiome and movement data analysis and metadata for the archived sequence data.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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