Tree Species Diversity and Forest Edge Density Jointly Shape the Gut Microbiota Composition in Juvenile Great Tits (Parus major)

Evi Goossens1†, Roschong Boonyarittichaikij1,2†, Daan Dekeukeleire3, Lionel Hertzog3, Sarah Van Praet1, Frank Pasmans1, Dries Bonte3, Kris Verheyen4, Luc Lens3, An Martel1‡ and Elin Verbrugghe1*

1 Department of Pathology, Bacteriology and Avian Diseases, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium, 2 Department of Clinical Sciences and Public Health, Faculty of Veterinary Science, Mahidol University, Nakhon Pathom, Thailand, 3 Terrestrial Ecology Unit, Department of Biology, Ghent University, Ghent, Belgium, 4 Forest & Nature Lab, Department of Environment, Ghent University, Ghent, Belgium

Despite the microbiome’s key role in health and fitness, little is known about the environmental factors shaping the gut microbiome of wild birds. With habitat fragmentation being recognised as a major threat to biological diversity, we here determined how forest structure influences the bacterial species richness and diversity of wild great tit nestlings (Parus major). Using an Illumina metabarcoding approach which amplifies the 16S bacterial ribosomal RNA gene, we measured gut microbiota diversity and composition from 49 great tit nestlings, originating from 23 different nests that were located in 22 different study plots across a gradient of forest fragmentation and tree species diversity. Per nest, an average microbiome was determined on which the influence of tree species (composition and richness) and forest fragmentation (fragment area and edge density) was examined and whether this was linked to host characteristics (body condition and fledging success). We found an interaction effect of edge density with tree species richness or composition on both the microbial richness (alpha diversity: Chao1 and Shannon) and community structure (beta diversity: weighted and unweighted UniFrac). No significant short-term impact was observed of the overall faecal microbiome on host characteristics, but rather an adverse effect of specific bacterial genera on fledging success. These results highlight the influence of environmental factors on the microbial richness as well as the phylogenetic diversity during a life stage where the birds’ microbiota is shaped, which could lead to long-term consequences for host fitness.

Keywords: great tits (Parus major), faeces, microbiota, tree species diversity, forest fragmentation

INTRODUCTION

During the past two decades, the gut microbiome has attracted considerable research attention because of its role in a host’s physiology and health status. The microbiota of the gastrointestinal tract plays a fundamental role in food digestion, pathogen defence, stimulation of the immune system, gut and central nervous system functioning, life-history traits and behaviour...
fitness, especially for bird survival after fledgling (Monróš
et al., 2003; Rodriguez et al., 2016; Teyssier et al., 2018a).
With the transition from nestling to fledgling being a key
moment in the development of altricial birds and with gut
microbiota characteristics and nestling body condition in
great tits being linked to each other (Teyssier et al., 2018a),
we also explored the hypothesis that forest-driven changes
in the microbiome can affect important host parameters
including body condition and bird survival after fledgling.
More specifically, we tested (1) if tits located in areas
with greater tree species richness and diversity displayed
higher faecal microbial richness and microbial community
structure, (2) if habitats with larger areas defined as forest
edges, which are associated with a greater abundance and
diversity of arthropods, were associated with higher microbial
diversity and (3) whether this affects host characteristics
including body condition and fledging success of the great
tits.

MATERIALS AND METHODS

Study Site

We performed a gut microbiota study of 23 great tit nests in
22 study plots (30 × 30 m) located in 11 mature (>60 years)
deciduous forest fragments in the south of Ghent (coordinates:
50°57′19″N, 3°43′31″E), northern Belgium (Figure 1; see De
Groote et al., 2017 for more details). These study plots were
established in 2014 to study effects of tree species diversity and
forest fragmentation on food web dynamics (Hertzog et al.,
2019). Plots varied in tree species composition [Pedunculate oak
(Q. robur), Red oak (Quercus rubra), and Beech (F. sylvatica)]
in monocultures, two species mixtures or three species mixtures
(Figure 1 and Supplementary Table 1). Plots of all tree species
compositions are replicated along a fragmentation gradient,
with some plots being situated in larger fragments far from the
forest edge, and other in smaller forest fragments, close
to the forest edge. All plots are located in forest stands
with similar land-use history (continually wooded since at
least 1850), management history (mature stands where no
forestry management took place in the last decade) and soil
(dry sandy loam).

As explained in Dekeukeleire et al. (2019), 12 fragmentation
measures were collected (Supplementary Table 2) of which
fragment-level forest area and plot-level edge density were
selected as key components of habitat fragmentation (Ewers
et al., 2007; Fisher and Lindenmayer, 2007). Fragment area
was calculated as the total surface area of the forest fragment in
which a study plot is situated (range: 10.74–90.36 ha), based on
detailed land use GIS layers (Vriens et al., 2011; Dekeukeleire
et al., 2019). Edge density quantifies the intensity of edge effects
on individual plots, with higher values being characteristic for
more fragmented forests. Edge density was calculated as the
total length of all edges of the forest with other land use classes
(e.g., agricultural land or residential areas) within a radius of
300 m of the plot (range: 655–2932.40 m) (Dekeukeleire et al.,
2019). Previously these plots were used to analyse the effects
of forest fragmentation and tree species composition on the

Initially the gut microbiota is shaped by external bacteria
present in the nesting environment and by parental feeding (Hird
et al., 2014; Ding et al., 2017; Teyssier et al., 2018a). Food-
associated microbial communities may, however, vary by location
and the food quality can pose a differential selection pressure on
the gut microbiota (Grond et al., 2018; Davidson et al., 2020). As
such, variation in habitat can determine microbiota composition,
which may have implications on nestling body condition and
long-term consequences for host fitness. For example, Teyssier
et al. (2018b) showed that adult house sparrows from urban areas
undergoing substantial changes (Zhu et al., 2017; Teyssier et al.,
2018).

In temperate zone forests, tree species composition and
diversity shape arthropod abundance and by parental feeding (Hird
et al., 2014; Ding et al., 2017; Teyssier et al., 2018a). Other
environmental factors including forest fragmentation also
impact the availability of food resources (Burke and Nol, 1998;
Zanette et al., 2000). Great tits (Parus major) were shown to
feed their nestlings less caterpillars in small fragments compared
to larger fragments (Bueno-Enciso et al., 2016). The effects
of forest fragmentation can, however, depend on the local tree
species composition. In northern Belgium, breeding success
of great tits decreases with fragment area in Fagus sylvatica
forests but does not vary with fragment size in insect-rich
Quercus robur forests (Dekeukeleire et al., 2019). It can thus
be expected that habitat characteristics such as tree species
composition and forest fragmentation also jointly affect the
avian gut microbiota.

In this study, we assessed the individual and interacting
effects of environmental factors such as tree species (composition
and richness) and forest fragmentation (fragment area and
edge density) on the gut microbiota of wild birds. We
use great tits, an insectivorous forest bird during breeding
season, as a model species by sampling the gut microbiota
in 49 great tit nestlings originating from 23 different nests
to determine the average nest microbiome. The nests were
located in 22 different forest fragments in the south of Ghent,
northern Belgium, with a varying fragmentation gradient and
tree species composition. Taking into account both forest
composition and forest fragmentation allowed us to address
the question whether alpha and beta diversity are shaped
by forest structure and whether it has consequences for
bird health and fitness. Previously, it has been shown that
body condition of great tits is an important factor for bird
fitness, especially for bird survival after fledgling (Monróš
et al., 2003; Kamada et al., 2013; Zanette et al., 2018; Macke et al., 2017;
Grizotte-Lake et al., 2018). Despite its pivotal role in host health,
research on the gut microbiota of avian species has lagged
behind mammalian research and is dominated by studies of
agriculturally important birds as well as birds of conservation
interest (Grond et al., 2018). In wild bird populations, studies
examining the gastrointestinal flora are rather scarce and mostly
focusing on juvenile birds of which the gut microbiota is still
undergoing substantial changes (Zhu et al., 2017; Teyssier et al.,
2018a).

Initially the gut microbiota is shaped by external bacteria
present in the nesting environment and by parental feeding (Hird
et al., 2014; Ding et al., 2017; Teyssier et al., 2018a). Food-
associated microbial communities may, however, vary by location
and the food quality can pose a differential selection pressure on
the gut microbiota (Grond et al., 2018; Davidson et al., 2020). As
such, variation in habitat can determine microbiota composition,
which may have implications on nestling body condition and
long-term consequences for host fitness. For example, Teyssier
et al. (2018b) showed that adult house sparrows from urban areas
undergoing substantial changes (Zhu et al., 2017; Teyssier et al.,
2018). Great tits (Parus major) were shown to
feed their nestlings less caterpillars in small fragments compared
to larger fragments (Bueno-Enciso et al., 2016). The effects
of forest fragmentation can, however, depend on the local tree
species composition. In northern Belgium, breeding success
of great tits decreases with fragment area in Fagus sylvatica
forests but does not vary with fragment size in insect-rich
Quercus robur forests (Dekeukeleire et al., 2019). It can thus
be expected that habitat characteristics such as tree species
composition and forest fragmentation also jointly affect the
avian gut microbiota.

In this study, we assessed the individual and interacting
effects of environmental factors such as tree species (composition
and richness) and forest fragmentation (fragment area and
edge density) on the gut microbiota of wild birds. We
use great tits, an insectivorous forest bird during breeding
season, as a model species by sampling the gut microbiota
in 49 great tit nestlings originating from 23 different nests
to determine the average nest microbiome. The nests were
located in 22 different forest fragments in the south of Ghent,
northern Belgium, with a varying fragmentation gradient and
tree species composition. Taking into account both forest
composition and forest fragmentation allowed us to address
the question whether alpha and beta diversity are shaped
by forest structure and whether it has consequences for
bird health and fitness. Previously, it has been shown that
body condition of great tits is an important factor for bird
fitness, especially for bird survival after fledgling (Monróš
et al., 2003; Rodriguez et al., 2016; Teyssier et al., 2018a).

With the transition from nestling to fledgling being a key
moment in the development of altricial birds and with gut
microbiota characteristics and nestling body condition in
great tits being linked to each other (Teyssier et al., 2018a),
we also explored the hypothesis that forest-driven changes
in the microbiome can affect important host parameters
including body condition and bird survival after fledgling.
More specifically, we tested (1) if tits located in areas
with greater tree species richness and diversity displayed
higher faecal microbial richness and microbial community
structure, (2) if habitats with larger areas defined as forest
edges, which are associated with a greater abundance and
diversity of arthropods, were associated with higher microbial
diversity and (3) whether this affects host characteristics
including body condition and fledging success of the great
tits.
breeding performance and body condition of Great and Blue tits (Dekeukeleire et al., 2019).

**Sample Collection**

For full details on the field work procedures we refer to Dekeukeleire et al. (2019). Briefly, at the corner of each plot, four standard nest boxes for great tits (dimensions $23 \times 9 \times 12$ cm, entrance $32$ mm) were installed at a height of $1.5$ m, in the autumn of 2014. During the breeding season (April–June 2015), all nest boxes were checked at least twice a week to determine occupancy by great tits and collect breeding data. At 14–15 days of age, the juveniles were fitted with a metal ring from the Belgian Ringing scheme, measured [weight (g) and tarsus length (mm)] and a faecal sample was collected. The body condition was calculated using the scaled-mass index (SMI). This adjusts the mass of all individuals to that which they would have obtained if they all had the same body size, using the equation of the linear regression of ln-body mass on ln-tarsus length estimated by type-II (standardised major axis; SMA) regression (Peig and Green, 2009; Supplementary Table 3). Faecal samples from 49 great tit nestlings, originating from 23 different nests were collected by placing the individual animals in a sterilised cotton bag. The birds were regularly checked to see if they defecated, with a maximum of a 30 min incubation period. If the birds defecated, the faecal samples were collected into Eppendorf tubes while wearing sterile gloves. All samples were kept in sterile Eppendorf tubes at $-20^\circ C$ until further analysis. The individual samples ($n = 49$) were sequenced and analysed on nest level ($n = 23$).
DNA Extraction

Total community DNA was isolated from great tit faecal samples \((n = 49)\) using the CTAB method modified from Kowalchuk et al. (1999) and Griffiths et al. (2000). Briefly, we added 0.5 ml CTAB buffer (hexadecyltrimethylammonium bromide 5% (w/v), 0.35 M NaCl, 120 mM K_2HPO_4) and 0.5 ml phenol-chloroform-isoamyl alcohol (25:24:1). The mixture was homogenised by grinding \((2 \times)\) with 0.5 g unwashed glass beads (Sigma-Aldrich, Overyse, Belgium) in a bead beater (1.5 min, 22.5 Hz; TissueLyser; Qiagen, Hilden, Germany) with a 30 s interval between shakings. After centrifugation (10 min, 8000 g), 300 µl of the supernatant was transferred to a new tube. A re-extraction from the remaining content was performed by adding 0.25 mL CTAB buffer. After homogenisation as described above, samples were centrifuged (10 min, 8000 g) and 300 µl of supernatant was added to the first 300 µl. An equal volume (600 µL) of chloroform-isoamyl alcohol (24:1) was added to the supernatant collected in order to remove the phenol from the samples. The mixture was further centrifuged at 16 000 g for 10 s and the aqueous phase was transferred to a new Eppendorf tube. Nucleic acids were precipitated with two volumes of PEG-6000 solution [polyethylene glycol 30% (w/v), 1.6 M NaCl] for 2 h at room temperature. After centrifugation (20 min, 13000 g), the pellet was rinsed with 1 ml of ice-cold, 70% (v/v) ethanol. The pellet was dried and resuspended in 100 µl RNAse free water. The quality and the concentration of the DNA was examined spectrophotometrically (NanoDrop, Thermo Scientific, Waltham, MA, United States). The isolated DNA from the individual samples \((n = 49)\) were individually sequenced, but analysed on nest level \((n = 23)\).

PCR Amplification and High-Throughput Sequencing

Following the recommendations of Klindworth et al. (2013), the V3-V4 hypervariable region of the 16s rRNA gene was amplified using the gene-specific primers S-D-Bact-0341-b-S-17 \((5′-CCTACGGGNGGCWGCAG-3′)\) and S-D-Bact-0785-a-A-21 \((5′-GACTACHVGGGTATCTAACTC-3′)\). A 25 µL PCR reaction contained 2.5 µL DNA \((~20 ng/µL)\), 0.2 µM of forward and reverse primers and 12.5 µL 2 × KAPA HiFi HotStart ReadyMix (Roche, Diegem, Belgium). PCR conditions were as follows: initial denaturation at 95°C for 3 min, followed by 25 cycles of 95°C for 30 s, 55°C for 30 s, 72°C for 30 s, and a final extension at 72°C for 5 min. Subsequently, the PCR products were purified using CleanNGS beads (CleanNA) and the DNA quality and quality was analysed spectrophotometrically (NanoDrop) and by agarose gel electrophoresis (1.5% agarose). In a second PCR, dual indices were attached to the 16S V3-V4 fragment. This 50 µL PCR reaction contained 5 µL of purified PCR product, 2 × KAPA HiFi HotStart ReadyMix (25 µL) and 2.5 µl index primer 1 (N7xx) (10 µM stock) and index primer 2 (S5xx) (10 µM stock). PCR conditions were as follows: initial denaturation at 95°C for 3 min, followed by eight cycles of 95°C for 30 s, 55°C for 30 s, 72°C for 30 s and a final extension at 72°C for 5 min. The final PCR products were purified and the concentration was determined using the Quantrus fluorimeter (Promega, Leiden, Netherlands). The final barcoded libraries were combined to an equimolar 5 nM pool and sequenced with 30% PhiX spike-in using the Illumina MiSeq v3 technology \((2 \times 300 \text{ bp, paired-end})\) at the Oklahoma Medical Research center (Oklahoma City, OK, United States).

Bioinformatic Processing of 16S rRNA Data

Demultiplexing of the ampiclon dataset and deletion of the barcodes was done by the sequencing provider. Quality of the raw sequence data was checked with the FastQC quality-control tool (Babraham Bioinformatics, Cambridge, United Kingdom) followed by initial quality filtering using Trimmomatic v0.38 by cutting reads with an average quality per base below 15 using a 4-base sliding window and discarding reads with a minimum length of 200 bp (Bolger et al., 2014). The paired-end sequences were assembled and primers were removed using PANDAseq (Masella et al., 2012), with a quality threshold of 0.9 and length cut-off values for the merged sequences between 390 and 430 bp. Chimeric sequences were removed using UCHIME (Edgar et al., 2011). Open-reference operational taxonomic unit (OTU) picking was performed at 97% sequence similarity using USEARCH (v6.1) (Edgar, 2010) and OTU taxonomy was assigned against the Silva database (v128, clustered at 97% identity) (Quast et al., 2013). OTUs with a total abundance below 0.01% of the total sequences were discarded (Bokulich et al., 2013), resulting in an average of approximately 13288 reads per sample. Alpha rarefaction curves were generated using the QIIME “alpha_rarefaction.py” script and a subsampling depth of 7800 reads was selected, which was used for all subsequent analyses. Any sequences of mitochondrial or chloroplastic origins were removed before further analysis.

Statistical Analyses

All statistical analyses were performed in R (v3.5.1) (R Core Team, 2018). Due to non-convergence of LMM when analysing the microbiome on bird level \((n = 49)\), and to normalise for a possible nestbox effect and difference in the number of individuals sampled per nest \((Supplementary Table 3)\), nestlings sampled from the same nest box were treated as one biological replicate by averaging the OTUs per nest. As such, the microbiome analysis was performed on nest level \((n = 23)\). Microbiota alpha diversity (Chao1 richness estimator and Shannon diversity estimator) measures were calculated using the phyloseq package (McMurdie and Holmes, 2013) and showed a Gaussian distribution. Linear models were used to test the effect of different forest parameters (tree species richness, tree species composition, fragment area, or edge density) on the microbial alpha diversity, as well as the resulting effects on fledging success (number of fledglings/number of nestlings) or fledging body condition (SMI). In accordance with the OTU’s, the fledging SMI and fledging success were averaged on nest level \((Supplementary Table 3)\). Models were run for each alpha diversity metric separately. In each model the explanatory variables were: a tree species measure (composition or richness),

1http://www.bioinformatics.babraham.ac.uk/projects/fastqc/
a measure of forest fragmentation (edge density or fragment area) and the interaction between both. As established in Dekeukeleire et al. (2019), there is a high degree of collinearity between the individual measures. Therefore, all models were run with one tree species measure (composition or richness; categorical variable) and one fragmentation measure (fragment area or edge density; continuous variable; Pearson's $R = -0.43$) at a time. For all models, significant effects were determined by ANOVA (type I sum of squares). The significance of the fragmentation effects for the different tree richness or tree composition levels was assessed using the addSE packages (Hertzog, 2018). The effect of microbiota diversity on host characteristics was assessed by including either nestbox fledging success (binomial distribution) or average fledgling SMI per nestbox (Gaussian distribution) as response variable. In models including body condition, the number of nestlings was included as an additional fixed covariate.

Beta diversity was studied using phyloseq, taking Jaccard (presence/absence), Bray–Curtis (presence/absence, as well as abundance), unweighted UniFrac (phylogenetic distance) and weighted UniFrac (phylogenetic distance, as well as abundance) dissimilarities into account. The effect of either tree species composition or tree species richness and forest fragmentation (fragment area or edge density) on the microbial community composition was assessed via permutational multivariate analysis of variance (PERMANOVA) using the adonis function from the vegan package (Dixon, 2003). The effect of the microbial community composition on either nestbox fledging success or average fledgling SMI per nestbox, was assessed using linear models. Models were run for each beta diversity metric separately, with either fledging success or body condition as response variable, and the first two principal coordinates from the beta diversity dissimilarity matrix as explanatory variables.

DESeq2 analysis was performed on the genus level abundance data to identify bacterial genera that (1) are driving the difference in microbial community composition in monocultures with varying edge densities by taking into account the interaction between the tree species richness and the edge density and (2) are linked to host characteristics by considering either average fledgling SMI per nestbox or nestbox fledging success. Significant differences were obtained using a Wald test followed by a Benjamini–Hochberg multiple hypothesis correction. For all tests, a $p$-value $< 0.05$ was considered significant.

**Ethical Considerations**

Bird captures and handling were carried out under licence and guidelines of the Belgian Ringing Scheme and the Flemish authorities (Agentschap voor Natuur en Bos; ANB/BL-FF/V15-00034). All trapping and sampling protocols of great tits were approved and permitted by the Ethical Committee VIB (the Flanders Institute for Biotechnology) Ghent site (EC2015-023) with permissions of all site owners.

**RESULTS**

**Taxonomic Composition of Microbiota**

The microbial taxonomic composition of the juvenile great tit faecal samples was characterised by a predominance of the phyla Firmicutes (50.96 $\pm$ 14.63%), Proteobacteria (27.58 $\pm$ 8.44%), and Actinobacteria (17.90 $\pm$ 12.38%). Bacteroidetes (1.16 $\pm$ 1.09%), Tenericutes (1.49 $\pm$ 4.33%), and other phyla (0.91 $\pm$ 1.28%) showed a lower abundance (Figure 2 and Supplementary Data 1, 2). When selecting the 5 most abundant genera in all nests, the genera *Staphyloccocus, Bacillus, Lactobacillus*, and *Carnobacterium* belonging to the Class Bacilli, together with genus *Burkholderia-Paraburkholderia* belonging to the Class Betaproteobacteria were most prevalent in the great tit microbiome (Supplementary Data 3).

**Influence of Tree Species Diversity and Forest Fragmentation on the Faecal Microbial Composition of Great Tits**

**Alpha Diversity**

Faecal samples harboured on average 456 $\pm$ 92.89 OTUs (min = 268, max = 603 observed OTUs). When taking the interaction between tree species and edge density into account, a significant influence was observed on the alpha diversity, indicating an edge density effect on microbial richness with a direction depending on the particular tree species richness or composition (Supplementary Table 4). A significant interaction effect of tree species richness with edge density on Chao1 (ANOVA: $F$-value = 4.157; $DF = 2$; $p$-value = 0.034) and tree species composition with edge density on the Shannon diversity (ANOVA: $F$-value = 7.717; $DF = 6$; $p$-value = 0.004).
was observed (Supplementary Table 4). More specifically a significant increase in OTU richness with edge density was observed in monoculture forests [Figure 3 and Supplementary Table 4 (AddSE): Chao1 slope: 0.191, 95% CI: 0.040/0.342]. Fragment area as a fragmentation metric in combination with tree species diversity did not significantly impact the alpha diversity (Supplementary Table 4).

**Beta Diversity**
Permutational multivariate analysis of variance analysis on the beta diversity showed that the interaction between tree species composition and edge density significantly affected the UniFrac dissimilarity indexes, and accounted for 36.2% (unweighted UniFrac, \( p = 0.041 \)) to 37.4% (weighted UniFrac, \( p = 0.055 \)) of the variation observed between the nests. The combined effects of tree species richness and edge density could explain 23.2% (unweighted UniFrac, \( p \)-value = 0.001) to 19.7% (weighted UniFrac, \( p \)-value = 0.007) of the variation (Supplementary Table 5 and Supplementary Figure 1). The Jaccard and Bray–Curtis indexes were not significantly influenced by tree species and/or fragmentation metrics (\( p \)-value > 0.05).

**Abundance Changes of Bacterial Genera That Are Linked to the Interaction Effect of Edge Density With Tree Species Richness**
Using DESeq2 analysis, 12 genera were identified as having a significant (adjusted \( p \)-value < 0.05) differential abundance in monocultures when the edge density varies (Table 1). When selecting the most abundant genera (baseMean > 100) *Lactococcus* (Class: Bacilli) was show to be most prevalent in monoculture plots characterised by a small edge density. *Pseudarthrobacter*, *Arthrobacter* (Class: Actinobacteria), and *Erysipelatoclostridium* (Class: Erysipelotrichia) showed a higher abundance in monoculture plots characterised by a high density of edges (Table 1).

**Influence of the Microbiome on Body Condition and Fledging Success of Great Tits**

**Microbial Diversity**
The great tits showed an overall SMI ± SD of 18.06 ± 1.61 and an overall fledging success ± SD of 80.72 ± 29.38%. On nest level, the global microbiome diversity did not affect the host characteristics as neither the alpha diversity, nor the beta diversity significantly impacted the average fledgling SMI per nestbox or nestbox fledging success (Supplementary Tables 6, 7).

**Abundance Changes of Bacterial Genera That Are Linked to the Body Condition or Fledging Success**
On nest level, no specific genera were associated with the average SMI per nestbox. When examining the driving genera that are linked to fledging success, 19 genera were negatively associated with fledging success (Table 2). Nestboxes having a higher fledging success were associated with decreased numbers (baseMean > 100) of *Brachybacterium*, *Brevibacterium* (Class: Actinobacteria), *Serratia* (Class: Gammaproteobacteria), *Sphingomonas* (Class: Alphaproteobacteria), *Carnobacterium*,...
Jeotgalicoccus (Class: Bacilli), and Tyzzerella 3 (Class: Clostridia), with fold changes (log$_2$) that vary between −2.12 and −30.00 (Table 2).

DISCUSSION

The taxonomic composition of great tits' faecal microbial community was characterised by a predominance of the phyla Firmicutes, Proteobacteria, and Actinobacteria, in line with previous studies (kropáčková et al., 2017; Teyssier et al., 2018a). On genus level, the great tit microbiome was dominated by Staphylococcus, Bacillus, Lactobacillus, Carnobacterium, and Burkholderia-Paraburkholderia which corresponds with previous research (Goossens et al., 2021). Although some species belonging to these genera (e.g., Staphylococcus) can be pathogenic in birds, these most common genera contain a multitude of beneficial bacteria (Benskin et al., 2009; Grond et al., 2018).
| Phylum          | Class          | Order           | Family          | Genus     | baseMean | log₂ fold change | lfcSE | padj      |
|-----------------|----------------|-----------------|-----------------|-----------|-----------|------------------|-------|-----------|
| Actinobacteria  | Actinobacteria | Micrococcales   | Microbacteriaceae | Frondihabitans | 57.97086 | -4.96097      | 1.54935 | 0.01465   |
| Actinobacteria  | Actinobacteria | Micrococcales   | Micrococcaceae   | Micrococcus  | 97.60023 | -5.38600      | 1.25073 | 0.00514   |
| Actinobacteria  | Actinobacteria | Micrococcales   | Micrococcaceae   | Kocuria     | 13.95047 | -5.69727      | 1.90552 | 0.00591   |
| Actinobacteria  | Actinobacteria | Micrococcales   | Dermabacteraceae | Brachybacterium | 144.81196 | -5.63555      | 1.58524 | 0.00553   |
| Actinobacteria  | Actinobacteria | Micrococcales   | Brevibacteriaceae | Brevibacterium | 233.08480 | -6.05455      | 1.16893 | 0.00002   |
| Actinobacteria  | Actinobacteria | Corynebacteriales | Nocardiaceae   | Williamsia  | 56.78876 | -7.79145      | 2.27969 | 0.00782   |
| Actinobacteria  | Actinobacteria | Dietziaceae     | Dietzia         |            | 33.74808 | -7.00390      | 2.17538 | 0.00022   |
| Actinobacteria  | Thermoleophilia | Solibrobacteriales | Patulibacteriaceae | Patulibacter | 11.39001 | -5.57505      | 1.75776 | 0.01525   |
| Proteobacteria  | Gammaproteobacteria | Legionellales   | Coxiellaceae   | Diplorickettsia | 15.20776 | -3.41905      | 1.20995 | 0.09997   |
| Proteobacteria  | Gammaproteobacteria | Enterobacteriales | Enterobacteriaceae | Serasia | 343.56698 | -16.86702     | 5.17961 | 0.01297   |
| Proteobacteria  | Alphaproteobacteria | Sphingomonadales | Sphingomonadaceae | Sphingomonas | 736.07041 | -2.11996      | 0.59077 | 0.00536   |
| Proteobacteria  | Alphaproteobacteria | Rickettsiales   | Anaplasmataceae | Wolbachia  | 13.89976 | -21.25073     | 5.77625 | 0.00471   |
| Saccharibacteria | uncultured bacterium | uncultured bacterium | uncultured bacterium | uncultured bacterium | 40.93864 | -7.19182      | 1.94072 | 0.00471   |
| Firmicutes      | Bacilli         | Bacillales      | Bacillaceae     | Oceanobacillus | 7.84285 | -18.19205     | 5.80793 | 0.01643   |
| Firmicutes      | Bacilli         | Bacillales      | Listeriaceae    | Brochothrix  | 8.75038     | -8.00489      | 2.57606 | 0.01688   |
| Firmicutes      | Bacilli         | Lactobacillales | Carnobacteriaceae | Desernzia  | 8.45595      | -12.15891     | 3.00312 | 0.00166   |
| Firmicutes      | Bacilli         | Lactobacillales | Carnobacteriaceae | Carnobacterium | 1659.98838 | -3.66081      | 0.89935 | 0.00166   |
| Firmicutes      | Bacilli         | Bacillales      | Staphylococcaceae | Jeotgalicoccus | 243.77101 | -5.78625      | 1.49281 | 0.00285   |
| Firmicutes      | Clostridia      | Clostridales    | Lachnospiraceae | Tyzzerella 3 | 116.39970 | -30.00000     | 5.80680 | 0.00002   |

The faecal microbiome of great tits were analysed using DESeq2 analysis to identify differentially abundant taxa that are associated with fledging success. Significant differences in genus level abundance (adjusted p-value < 0.05) in the faecal microbiota from great tits that are linked to fledging success. The taxonomic classification, the baseMean, the log₂ fold change, the log fold change Standard Error (lfcSE), and adjusted p-values of the DESeq2 normalised abundance of each genus are shown.
showed that when taking both tree species and edge density into account, a significant effect on alpha and beta diversity was observed, but with a direction and intensity depending on the local tree species richness or tree species composition. Wild birds and their habitat are inextricably linked to human activities as their natural environment is subjected to an increasing pressure of fragmentation. Despite relevant proof in mammals (Amato et al., 2013; Fackelmann et al., 2021) and data indicating that habitat type can influence the microbiome of certain birds (San Juan et al., 2020), it remains largely unknown whether the gut microbiomes of passerines are affected by habitat fragmentation and/or whether other factors are also involved. Our data now show that combined changes in forest structure also have the potential to shift gut microbial communities in passerines.

Microbial recruitment to young bird guts may occur through various routes. Parental effects are the most likely explanation for the patterns observed. Although parents feed insect prey directly, without ingestion and regurgitation, parents can influence their offspring's gut microbiota through saliva transfer or through variable prey selection (Pagani-Núñez et al., 2015). In forest fragments, the abundance and diversity of arthropods is higher at edges (e.g., De Smedt et al., 2019; van Schrojenstein Lantman et al., 2019). Changes in food resources for great tits could thus explain the changes in the great tit microbiome in habitats having a higher edge density. Possibly, in monocultures which have a resource-poor status (Yahya et al., 2017), more forest edges and thus more insect preys, results in an increase in OTU richness (Chao1), whereas in mixed forest stands this is not the case due to a higher biodiversity. These data are in line with previously published work where a positive edge effect was described on arthropod abundance in monocultures, whereas this relationship was negated in habitats having a higher tree diversity (van Schrojenstein Lantman et al., 2019). With about 29% of Europe's forests only being composed of a single tree species (Barsoum et al., 2016) and great tits being widespread and common forest birds, our data highlight that changes in the landscape may affect the gut microbiome of wild birds.

Due to the small sample size in this study, we were not able to reliably determine the edge effect per tree species. However, abundance, richness, and diversity of insects may differ greatly depending on the tree identity. Native Q. robur is characterised by a species-rich arthropod community (Southwood et al., 2004) and in native F. sylvatica monocultures a higher arthropod abundance has been described closer to the edges (van Schrojenstein Lantman et al., 2019). Q. rubra is an invasive species in Belgium, and only supports a low abundance and diversity of herbivorous insects (Goßner, 2004). This species forms a dense shrub layer, especially if light is available (Dey and Parker, 1996). It is therefore very likely that in monocultures, edge density influences the microbial diversity differently depending on the local tree species type and that changes in food resources are linked to the observed shifts in the microbiome (Bodawatta et al., 2021).

Besides habitat-driven dietary changes, the observed patterns could also be linked to differences in nest material or even hatching date. Nest material can shape the bacterial community in the nest, which in turn can colonise the gut of nestlings (van Veelen et al., 2017). In great tits, nest material composition, weight and size have previously been found to vary with local tree species composition (Alvarez et al., 2013). Tit nests can also contain anthropogenic material and hairs of domestic animals (Reynolds et al., 2019), which could be more common in small forest fragments or at edges between forests and residential areas. Thus, potentially tree species composition and forest edges can jointly shape material and thus the bacterial community in the nest. Differences in hatching date could possibly also influence the great tit microbiome (Kreisinger et al., 2018). In our study, Julian dates ranged from 120 to 131 (Supplementary Table 3) and did not significantly impact the alpha (LM p-values > 0.05), nor the beta diversity (PERMANOVA p-values > 0.05). However, previous studies have shown that Julian date can be a confounding factor influencing the microbiome in passerines (Kreisinger et al., 2018).

When looking at abundance of genera, DESeq2 analysis showed that monoculture plots characterised by more edges show a decrease in Lactococcus. This genus comprises gram-positive lactic acid bacteria that act as probiotics, stimulate the immune system and aid in the digestion and absorption of nutrients (Salminen et al., 2004). A significant increase of Pseudarthrobacter, Arthrobacter, and Erysipelatoclostridium was observed in monoculture plots having more edges. The genus Arthrobacter has previously been linked to diet changes in great tits (Davidson et al., 2020) and the genus Pseudarthrobacter was shown to be increased in pine monocultures in which thinning practices led to the formation of a native understory vegetation (Trentini et al., 2020). As such, the increased abundance of Pseudarthrobacter is possibly linked to more solar radiation at the edges which results in more favourable conditions for the underlying forest layers. The genus Erysipelatoclostridium belongs to the normal gut microbiome of birds (Zhao et al., 2019), but it is also considered an opportunistic pathogen in humans (Shao et al., 2017).

The exact impact of these microbial changes or how gut microbiota affect the health of wild birds in general remains largely unknown (Grond et al., 2018). In great tit nestlings, a higher diversity and stability in microbiota composition were associated with better bird condition (Teyssier et al., 2018a), whereas in house sparrows, hosting a diverse and abundant microbiota flora limits the growth of developing nestlings (Kohl et al., 2018). In our study, the alpha and beta diversity did not significantly impact fledging success of the great tits on nest level. However, other than the lack of an overall microbiome effect, specific bacterial genera were shown to be linked to the fledging success. Tyzzerella 3 showed a fold change (Log2) of ~30, indicating that this genus is strongly associated with a reduction in fledging success. Tyzzerella 3 has previously been linked to diabetes (Yue et al., 2019; Ma et al., 2020), cardiovascular diseases (Kelly et al., 2016) and even reduced disease resistance in poultry (Cazals et al., 2022). The abundance of Brachybacterium, Brevisbacterium, Serratia, and Sphingomonas, was also negatively linked to fledging success. In general, these genera are not described as common avian pathogenic genera, but they comprise known pathogenic species in many
animals, including humans and birds (Pascual and Collins, 1999; Saidenberg et al., 2007; Saeb et al., 2014; Tamai et al., 2018). These data hint towards an adverse health effect of specific bacterial genera, rather than a major effect of the overall microbial richness and diversity. In this study, we did not observe an effect of the microbial changes on the average fledging SMI per nestbox. However, since we only analysed a short-term effect of the microbial changes on the body condition of the great tits, we cannot rule out whether the observed microbial changes bare no consequences on the long-term. Therefore, further studies are needed to identify the long-term effects of the environmental-driven changes in alpha and beta diversity on host characteristics of great tits. Summarised, we showed that more habitat edges in combination with changes in tree species diversity can influence the microbial richness and phylogenetic diversity in great tits during a life stage where the birds’ microbiota is shaped, which can lead to long-term consequences for host fitness.

**DATA AVAILABILITY STATEMENT**

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://www.ncbi.nlm.nih.gov/, PRJNA615317.

**ETHICS STATEMENT**

The animal study was reviewed and approved by Ethical Committee VIB (the Flanders Institute for Biotechnology) Ghent site (EC2015-023).

**REFERENCES**

Alvarez, E., Belda, E. J., Verdejo, J., and Barba, E. (2013). Variation in Great Tit Nest Mass and Composition and Its Breeding Consequences: a Comparative Study in four Mediterranean Habitats. *Avian Biol. Res.* 6, 39–46. doi: 10.3184/175815513X1360951787237

Amato, K. R., Yeoman, C., Kent, A., Righini, N., Carbonero, F., Alejandro, E., et al. (2013). Habitat degradation impacts black howler monkey (*Alouatta pigra*) gastrointestinal microbiomes. *ISME J.* 7, 1344–1353. doi: 10.1038/ismej.2013.16

Barsoum, N., Coote, L., Eycott, A. E., Fuller, L., Kiewitt, A., and Davies, R. G. (2016). Diversity, functional structure and functional redundancy of woodland plant communities: how do mixed tree species plantations compare with monocultures? *For. Ecol. Manag.* 382, 244–256. doi: 10.1016/j.foreco.2016.10.005

Benskin, C. M. H., Wilson, K., Jones, K., and Hartley, I. A. (2009). Bacterial pathogens in wild birds: a review of the frequency and effects of infection. *Biol. Rev.* 84, 349–373. doi: 10.1111/j.1469-185X.2008.00076.x

Bodawatta, K. H., Freiberga, I., Puzejova, K., Sam, K., Poulsen, M., and Jonsson, K. A. (2021). Flexibility and resilience of great tit (*Parus major*) gut microbiome to changing diets. *Anim. Microbiome* 3:20. doi: 10.1186/s42523-021-00076-6

Bokulich, N. A., Subramanian, S., Faith, J. I., Gevers, D., Gordon, J. I., Knight, R., et al. (2013). Quality-filtering vastly improves diversity estimates from illumine amplicon sequencing. *Nat. Methods* 10, 57–59. doi: 10.1038/nmeth.2276

**AUTHOR CONTRIBUTIONS**

AM, LL, KV, DB, FP, DD, and EV conceived the study and participated in its design and coordination. RB and DD performed material preparation and sample collection. SVP, EG, and EV performed the sample analysis. EG, EV, and LH performed the statistical analysis. EV wrote the first draft of the manuscript. All authors commented on previous versions of the manuscript, and read and approved the final manuscript.

**FUNDING**

Financial support for this research was provided via the UGent GOA project Scaling up Functional Biodiversity Research: from Individuals to Landscapes and Back (TREEWEB). EV and EG were supported by the Research Foundation Flanders [FWO grants 12E6616N/1507119N and 12W8919N, respectively].

**SUPPLEMENTARY MATERIAL**

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2022.790189/full#supplementary-material

Bolger, A. M., Lohse, M., and Usadel, B. (2014). Trimomatic: a flexible trimmer for illumina sequence data. *Bioinformatics* 30, 2144–2145. doi: 10.1093/bioinformatics/btu170

Bueno-Enciso, J., Ferrer, E. S., Barrientos, R., Serrano-Davies, E., and Sanz, J. J. (2016). Habitat fragmentation influences nestling growth in Mediterranean blue and great tits. *Acta Oecol.* 70, 129–137. doi: 10.1016/j.actao.2015.12.008

Burke, D. M., and Nol, E. (1998). Influence of Food Abundance, Nest-Site Habitat, and forest fragmentation on breeding ovenbirds. *Auk* 115, 96–104. doi: 10.2307/4089115

Cazaús, A., Estellé, J., Bruneau, N., Coville, J.-L., Menanteau, P., Rossignol, M.-N., et al. (2022). Differences in caecal microbiota composition and *Salmonella* carriage between experimentally infected inbred lines of chickens. *Genet. Sel. Evol.* 54:7. doi: 10.1186/s12711-022-00699-6

Cenit, M. C., Sanz, Y., and Codoñer-Franch, P. (2017). Influence of gut microbiota on neuropsychiatric disorders. *World J. Gastroenterol.* 23, 5486–5498. doi: 10.3748/wjg.v23.i30.5486

Davidson, G. L., Wiley, N., Cooke, A. C., Johnson, C. N., Fouhy, F., Reichert, M. S., et al. (2020). Diet induces parallel changes to the gut microbiota and problem solving performance in a wild bird. *Sci. Rep.* 10:20783. doi: 10.1038/s41598-020-07726-y

De Groote, S. R., van Schrojenstein, L., Irene, M., Sercu, B. K., Dekeukeleire, D., Boonyarittichaikij, R., et al. (2017). Tree species identity outweighs the effects of tree species diversity and forest fragmentation on understory diversity and composition. *Plant Ecol. Evol.* 150, 229–239. doi: 10.5091/plcevo.2017.1331

**ACKNOWLEDGMENTS**

We thank the private forest owners and the Flemish Forest and Nature Agency (ANB) for allowing this research on their property, and we thank Robbe De Beelde, Lieze Rouffaer, Bram Sercu, Irene Van Schrojenstein Lantman, and Pieter Vantieghem for their help with the fieldwork.
Kelly, T. N., Bazzano, L. A., Ajami, N. J., He, H., Zhao, J., Petrosino, J. F., et al. (2016). Gut microbiome associates with lifetime cardiovascular disease risk profile among bogalusa heart study participants. Circ. Res. 119, 956-964. doi: 10.1161/CIRCRESAHA.116.309219

Klindworth, A., Pruesse, E., Schweer, T., Peplies, J., Quast, C., Horn, M., et al. (2013). Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. Nucleic Acids Res. 41:e1. doi: 10.1093/nar/gks088

Kohl, K. D., Antonio, B., Bordenstein, S. R., Caviedes-vidal, E., and Karasov, W. H. (2018). Gut microbes limit growth in house sparrow nestlings (Passer domesticus) but not through limitations in digestive capacity. Ingr. Zool. 13, 139–151. doi: 10.11171/1477-4877.12289

Kovalchuk, G. A., Naoumenko, Z. S., Deriks, P. J. L., Felske, A., Stephen, J. R., and Arkhipchenko, I. A. (1999). Molecular analysis of ammonia-oxidizing bacteria of the beta subdivision of the class Proteobacteria in compost and composted materials. Appl. Environ. Microbiol. 65, 396–403. doi: 10.1128/AEM.65.2.396-403.1999

Kreisinger, J., Schmiedlová, L., Petříčková, A., Tomášek, O., Adámková, M., and Michálek, R. (2018). Fecal microbiota associated with phytohaemagglutinin-induced immune response in nestlings of a passerine bird. Ecol. Evol. 8, 9793–9802. doi: 10.1002/ece3.4545

Kropáčková, K., Pechmanová, H., Vinkler, M., Svobodová, J., Velová, H., Těšíček, M., et al. (2017). Variation between the oral and faecal microbiota in a free-living passerine bird, the great tit (Parus major). PLoS One 12:e0179945. doi: 10.1371/journal.pone.0179945

Ma, S., You, Y., Huang, L., Song, Z., Zhang, J., Guo, C., et al. (2020). Alterations in gut microbiota of gestational diabetes patients during the first trimester of pregnancy. Front. Cell. Infect. Microbiol. 10:58. doi: 10.3389/fcimb.2020.00058

Macke, E., Tasiemski, A., Massol, F., Callens, M., and Daccaesteck, E. (2017). Life history and eco-evolutionary dynamics in light of the gut microbiota. Oikos 126, 508–531. doi: 10.1111/oik.03900

Macpherson, A. J., and Harris, N. L. (2004). Interactions between commensal intestinal bacteria and the immune system. Nat. Rev. Immunol. 4, 478–485. doi: 10.1038/nri1373

Masella, A. P., Bartram, A. K., Truszkoski, J. M., Brown, D. G., and Neufeld, J. D. (2012). PANDAseq: a paired-end assembler for illumine sequences. BMC Bioinformatics 13:31. doi: 10.1186/1471-2105-13-31

McMurdie, P. J., and Holmes, S. (2013). Phylseq: an R package for reproducible interactive analysis and graphics of microbiome census data. PLoS One 8:e61217. doi: 10.1371/journal.pone.0061217

Monró, S. J., Belda, E. J., and Emilio, B. (2003). Post-fledging survival of individual great tits: the effect of hatching date and fledging mass. Oikos 99, 481–488. doi: 10.1034/j.1600-0706.2002.11909.x

Naef-Daenzer, B. (2006). Taxon time allocation and patch sampling by foraging birds. Behav. Ecol. Sociobiol. 50, 1527–1530. doi: 10.1007/s00265-004-0867-9

Nizard, J., Ibáñez-Álamo, J. D., Sumasguther, P., and Mainwaring, M. C. (2019). Urbanisation and nest building in birds: a review of threats and opportunities. J. Ornithol. 160, 841–860. doi: 10.1007/s10336-021-01884-y

Peig, J., and Green, A. J. (2009). New perspectives for estimating body condition from mass/length data: the scaled mass index as an alternative method. J. Avian Biol. 40, 178–185. doi: 10.1111/j.0908-8857.2009.04576.x

Peñuelas, J., Gallardo, A., and postfledging survival in great tits (Parus major): be large and be early (but not too much). Ecol. Evol. 13, 4458–4467. doi: 10.1002/ece3.2192

Peñuelas, J., Gallardo, A., and postfledging survival in great tits (Parus major): be large and be early (but not too much). Ecol. Evol. 13, 4458–4467. doi: 10.1002/ece3.2192
van Veelen, H. P., Salles, J. D., and Tieleman, B. I. (2017). Multi-level
microbiology and functional aspects. New York: CRC press.
San Juan, P. A., Hendershot, J. N., Daily, G. C., and Fukami, T. (2020). Land-
use change has host-specific influences on avian gut microbiomes. ISME J. 14, 318–321. doi: 10.1038/s41396-019-0535-4
Shao, T. J., Shao, L., Li, H. C., Xie, Z. J., He, Z. X., and Wen, C. P. (2017). Combined
signature of the fecal microbiome and metabolome in patient with gout. Front. Microbiol. 8:268. doi: 10.3389/fmicb.2017.00268
Shutt, J., Bolton, M., Cabello, I. B., Burgess, M. D., and Phillimore, A. (2018). The
effects of woodland habitat and biogeography on blue tit Cyanistes caeruleus
territory occupancy and productivity along a 220 km transect. Ecology 41, 1967–1978. doi: 10.1111/ecog.03573
Southwood, T. R. E., Wint, G. R. W., Kennedy, C. E. J., and Greenwood, S. R. (2004). Seasonality, abundance, species richness and specificity of the phytophagous guild of insects on oak (Quercus) canopies. Eur. J. Entomol. 101, 43–50.
Tamai, K., Akashi, Y., Yoshimoto, Y., Yaguchi, Y., Takeuchi, Y., Shiiai, M., et al. (2018). First case of a bloodstream infection caused by the genus Brachybacterium. J. Infect. Chemother. 24, 998–1003. doi: 10.1016/j.jiac.2018.06.005
Teyssier, A., Lens, L., Matthysen, E., and White, J. (2018a). Dynamics of gut
microbiota during the early development of an avian host: evidence from a
cross-foster experiment. Front. Microbiol. 9:1524. doi: 10.3389/fmicb.2018.01524
Teyssier, A., Rouffaer, L. O., Hudin, N. S., Strubbe, D., Matthysen, E., Lens, L., et al. (2018b). Inside the guts of the city: urban-induced alterations of the gut microbiota in a wild passerine. Sci. Total Environ. 612, 1276–1286. doi: 10.1016/j.scitotenv.2017.09.033
Trentini, C. P., Campanello, P. I., Villagrasa, M., Ferreras, J., and Hartmann, M. (2020). Thinning Partially Mitigates the Impact of Atlantic Forest Replacement by Pine Monocultures on the Soil Microbiome. Front. Microbiol. 11:1491. doi: 10.3389/fmicb.2020.01491
van Schrojenstein Lantman, I. M., Hertzog, L. R., Vandeghuchte, M. L., Martel, A., Verheyen, K., Lens, L., et al. (2019). Forest edges, tree diversity and tree identity change leaf miner diversity in a temperate forest. Insect Conserv. Divers. 13, 10–22. doi: 10.1111/icad.12358
van Veenen, H. P. J., Salles, J. D., and Tieleman, B. I. (2017). Multi-level
comparisons of cloacal, skin, feather and nest-associated microbiota suggest
considerable influence of horizontal acquisition on the microbiota assembly of
sympatric woodlarks and Skylarks. Microbiome 5:156. doi: 10.1186/s40168-017-0371-6
Vriens, L., Bosch, H., De Knijf, G., De Saeger, S., Guelinckx, R., Oosterlynck, P., et al. (2011). De biologische waarderingkaart. Biotopen en hun verspreiding in Vlaanderen en het Brussels Hoofdstedelijk Gewest. Mededelingen van het Instituut voor Natuur- en Bosonderzoek. INBO/M. J. Brussel: Mededelingen van het Instituut voor Natuur- en Bosonderzoek, 416.
Yahya, M. S., Syafiq, M., Ashton-Butt, A., Ghazali, A., Asmah, S., and Azhar, B. (2017). Switching from monoculture to polyculture farming benefits birds in oil palm production landscapes: evidence from mist netting data. Ecol. Evol. 16, 6314–6325. doi: 10.1002/ece3.3205
Yue, S., Zhao, D., Peng, C., Tan, C., Wang, Q., and Gong, J. (2019). Effects of theabrownin on serum metabolites and gut microbiome in rats with a high-sugar diet. Food Funct. 10, 7063–7080. doi: 10.1039/C9FO00334B
Zanette, L., Doyle, P., and Trémont, S. M. (2000). Food shortage in small fragments:
evidence from an area-sensitive passerine. Ecology 81, 1654–1666. doi: 10.2307/177314
Zhao, Y., Li, K., Luo, H., Duan, L., Wei, C., Wang, M., et al. (2019). Comparison of the intestinal microbial community in duck reared differently through high-throughput sequencing. Biomed Res. Int. 2019, 1–14. doi: 10.1155/2019/901504
Zhu, X., Han, Y., Du, J., Liu, R., Jin, K., and Yi, W. (2017). Microbiota-gut-brain axis and the central nervous system. Oncotarget 8, 53829–53838. doi: 10.18632/oncotarget.17754
Conflict of Interest: The authors declare that the research was conducted in the
absence of any commercial or financial relationships that could be construed as a
potential conflict of interest.
Publisher's Note: All claims expressed in this article are solely those of the authors
and do not necessarily represent those of their affiliated organizations, or those of the
publisher, the editors and the reviewers. Any product that may be evaluated in
this article, or claim that may be made by its manufacturer, is not guaranteed or
endorsed by the publisher.
Copyright © 2022 Goossens, Boonyarittichakjik, Dekeukeleire, Hertzog, Van Prael, Pasmans, Bonte, Verheyen, Lens, Martel and Verbrugghe. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.