Captivity Reduces Diversity and Shifts Composition of the Brown Kiwi Microbiome

Priscilla A San Juan (psanjuan@stanford.edu)
Stanford University https://orcid.org/0000-0001-5341-6622
Isabel Castro
Massey University
Manpreet Kaur Dhami
Manaaki Whenua: Landcare Research New Zealand

Short report

Keywords: Captive rearing, animals , gut microbiota, Brown Kiwi

Posted Date: November 20th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-111427/v1

Abstract

Background

Captive rearing is often critical for animals that are vulnerable to extinction in the wild. However, few studies have investigated the extent at which captivity impacts hosts and their gut microbiota, despite mounting evidence indicating that host health is affected by gut microbes. We assessed the influence of captivity on the gut microbiome of the Brown Kiwi (*Apteryx mantelli*), a flightless bird endemic to New Zealand. We collected wild (n = 68) and captive (n = 38) kiwi feces at seven sites on the north island of New Zealand.

Results

Using bacterial 16S rRNA and fungal ITS gene profiling, we found that captivity was a significant predictor of the kiwi gut bacterial and fungal communities. Captive samples had lower microbial diversity and different composition when compared to wild samples. History of coccidiosis, a gut parasite primarily affecting captive kiwi, showed a marginally significant effect.

Conclusions

Our findings demonstrate captivity's potential to shape the Brown Kiwi gut microbiome, but further investigation is needed to elucidate the effects of these differences on welfare.

Background

Diet, behavior, habitat type, and environmental species pools can all influence the composition and diversity of gut microbiomes [1–4]. However, few studies have investigated the impact of captivity, a severe lifestyle shift, on gut microbiota. Reports on mice (*Peromyscus maniculatus*), horses (*Equus ferus przewalskii* and *E. f. caballus*), and non-human primates demonstrate captivity's potential to impact gut microbiomes. Even fewer studies have investigated bird hosts particularly across spatially independent sites that vary by climate and vegetation, which are expected to differ in microbial species pools, a potential source for gut microbes [5–8].

Captive rearing is often necessary to conserve populations of threatened animal species. In the case of the Brown Kiwi (*Apteryx mantelli*), predation from introduced mammals have made it imperative for some chicks to be raised in captivity until individuals are large enough to defend themselves [9]. Although successful in increasing population size, consequences to kiwi health via modification of the gut microbiome remain largely unknown. Factors pervasive in captivity such as artificial diets, sterile built environments, human interaction, and medical intervention [6, 10, 11] may cause changes in the microbiome, but such changes have not been well described.

Altering microbial communities may have costs to host health as microbes continue to be recognized for their roles in immune function, pathogen defense, and digestion [12]. Coccidiosis, a gut parasite caused

by protozoan *Eimeria spp.*, is a common disease in captive kiwi [13]. However, the relationship between captivity, coccidia, and gut microbial communities has garnered little attention. We sought to compare gut bacteria and fungi between captive and wild kiwi. We tested the hypothesis that captivity status and history of coccidiosis would decrease diversity and modify composition of the gut microbiome.

Results

Fresh fecal samples were collected from seven sites on the north island of New Zealand (Fig. 1a, Supplementary Table 1) during January – April 2019. Bacterial 16S rRNA (V4 region) [14] and fungal ITS genes [15] were amplified using isolated DNA from captive (n = 38) and wild kiwi fecal samples (n = 68).

To determine whether captivity influences kiwi gut microbiota, we used PERMANOVA and NMDS analyses. Bacterial communities clustered by captivity across spatially independent sites with little overlap between the ellipses (Fig. 1b, PERMANOVA, $r^2 = 0.07$, $p = 0.001$). Captivity significantly decreased alpha diversity of bacteria (Fig. 2a, ANOVA, $p < 0.005$) and fungi (Supplementary Fig. 1, ANOVA, $p = 0.012$) by 33% and 74% respectively. To assess the spread of variation among kiwi microbiomes in captive and wild treatments, we calculated distance to centroid, a metric for beta diversity. No discernible pattern was observed for bacteria (Supplementary Fig. 2a, ANOVA, $p = 0.948$), but a marginally significant pattern was detected in fungal communities (Supplementary Fig. 2b, ANOVA, $p = 0.051$). We also tested if site (a factor nested within captivity status) and history of coccidiosis (positive or negative) had an influence on variation in microbial communities using PERMANOVA (Supplementary Table 2). Site showed a significant effect on bacteria ($r^2 = 0.129$, $p = 0.001$) and fungi ($r^2 = 0.183$, $p = 0.001$). Coccidiosis history showed a marginally significant trend with bacteria (Fig. 2b, $r^2 = 0.048$, $p = 0.095$) and fungi ($r^2 = 0.074$, $p = 0.087$). Although bacterial phyla composition was variable within and across treatments, Firmicutes was more prevalent in wild kiwi, while Proteobacteria dominated in captive kiwi (Fig. 2c).

We conducted a simper analysis [16] to determine the most influential OTUs that differentiate captive and wild kiwi samples for both bacteria and fungi. Eighty-seven bacterial OTUs and fifteen fungal OTUs accounted for about 70% of the differences between wild and captive samples (Supplementary Tables 3 and 4). Using a multinomial species classification method (clamtest) [16] we categorized OTUs into four classes: rare, generalist, wild specialist, and captive specialist. For bacterial OTUs, 10% were classed as generalist, 53% as rare, 20% as wild specialist, and 17% as captive specialist (Fig. 3a, Supplementary Table 5). For fungal OTUs, 0% were classed as generalist, 47% as rare, 27% as wild specialist, and 27% as captive specialist (Fig. 3b, Supplementary Table 5).

Discussion

Our results indicate that captivity changes both bacterial and fungal communities in the Brown Kiwi gut. Bacterial composition clustered by captivity (Fig. 1b), suggesting that kiwi from the wild are more similar to each other than their captive counterparts, even across geographically distinct sites. The consequences of reduced microbial diversity between wild and captive kiwi remain unclear, but several

studies have linked dysbiosis to higher disease prevalence in a variety of animals from humans to corals [17, 18]. Coccidiosis history showed a marginally significant effect. However, these results may be affected by small sample size. Our results suggest a potential link between changes in the microbiome to disease states that requires further exploration.

The shift in dominant bacterial phyla, Firmicutes to Proteobacteria, from wild to captive samples may be caused by sterile captive facilities and probiotic supplementation, a common practice particularly after antibiotics. Frequent surface disinfection [10] and probiotic treatment [19] have been shown to increase *Proteobacteria* in human subjects. *Lactobacillus* (OTU 49), a common genus in probiotics, was grouped as a captive specialist. Other captive-associated taxa include *Corynebacterium* (OTU 62), which has been found in the cloaca of penguins and the preen gland of turkeys [20], and *Bacteroides* (OTU 544), normally found in animal hosts but can include potential pathogens [21]. One wild specialist was *Bacillaceae* (OTU 101), a family associated with soil and plant pathogen defense [22], suggesting kiwi are ingesting environmental microbes when foraging [23]. *Blautia* (OTU 290) is a genus found in the human gut and associated with visceral fat accumulation [24]. *Faecalitalea cylindroides* (OTU 687), a butryrate producing microbe, has been detected in chicken [25]. These taxa may be indicative of nutrient acquisition in the wild, where food may be less available.

No fungal OTUs were categorized as generalists suggesting fungi in kiwi reflect their local environment. Some captive specialists include *Cladosporium* (OTU 151) and *Aureobasidium* (OTU 2), both associated with indoor environments and plant material [26, 27], implicating the contribution of added soil and ferns to enclosures. *Trichosporon* (OTU 171), another captive specialist, is a common human skin taxa [28], suggesting close human interaction may shape kiwi fungi. One wild specialist, *Rhizopogon* (OTU 159), has been identified as a dietary component of small mammals, suggesting kiwi may be consuming and dispersing these fungi [29]. *Preussia* (OTU 181) and *Saitozyma podzolica* (OTU 37), both associated with soil and litter, were grouped as wild specialists [30, 31].

Conclusions

In captivity, factors that shape gut microbial communities may include artificial diet, sterile built environments, and human interaction [6, 10]. A follow-up study investigating the establishment of gut microbes throughout kiwi development with sampling of the captive environment can elucidate how factors inherent to captivity contribute to the kiwi gut microbiome. Overall, our data suggest that captivity influences the gut microbiome of the Brown Kiwi with potential for health and disease assessment for captive-reared individuals.

Methods

Study system

Captive

The National Kiwi Hatchery is located at the Rainbow Springs Nature Park in Rotorua, New Zealand. It is the leading facility in kiwi husbandry, egg incubation, and kiwi rearing. The facility has hatched and reared nearly 2000 kiwi eggs. Otorohanga Kiwi House is located in Otorohanga, New Zealand. Both facilities are a part of the Operation Nest Egg (ONE) program.

Wild

Ponui Island is located 30 kilometers east of Auckland, New Zealand. 14 Brown Kiwi were introduced to the island by the New Zealand Wildlife Service in 1964, where populations have been increasing, establishing one of the densest populations of kiwi at an estimated 1500 individuals. Motuarohia Island is located in the Bay of Islands, 4 kilometers northeast of Russell, New Zealand. Moturua Island is east of Motuarohia in the Bay of Islands. Puketi Forest is located in the Northland region of New Zealand. Rakaumangamanga is located near the Bay of Islands. All these sites are home to a number of Brown Kiwi individuals.

Sample collection

Fresh fecal samples (n = 108) were collected using sterile spatulas. The interior of the fecal pellet was collected to ensure minimal environmental exposure. Fecal samples were stored in 5 mL Eppendorf tubes suspended in molecular grade ethanol and when accessible refrigerated in -4ºC. DNA was extracted using Macherey-Nagel NucleoSpin Soil Kit (Macherey-Nagel, Duren, Germany) on Agilent extraction robot, suspended in TE buffer, and stored in -4ºC.

Metabarcoding

Using a metabarcoding approach, we amplified a highly variable region (V4) of the bacterial 16S rRNA gene using 515F/806R primers [14] and the fungal ITS gene [15]. We used a SeraMag solution to clean PCR products to isolate bacterial and fungal DNA [32]. We pooled DNA according to the number of samples. Qubit (dsDNA HS Assay Kit, Invitrogen, Carlsbad, United States) was used to quantify DNA concentration and libraries were diluted to 4 nM prior to final pooling. We used LabChip GX Touch Nucleic Acid Analyzer to determine DNA concentration and assess quality. Samples were sequenced using Illumina MiSeq platform at Auckland Genomics Facility (University of Auckland), phiX spike 10%, 250 × 2 cycles. Bioinformatics pipeline Claident was used to demultiplex raw sequences. Pear merged paired end reads. VSEARCH filtered noisy reads, removed chimeras, and clustered sequences into operational taxonomic units (OTUs). Claident generated an OTU and assigned taxonomy with RDP classifier database [33].

Statistical analysis

We calculated Shannon index to test for a relationship between microbial alpha diversity and captivity. To better understand the intraspecific variation in microbial composition and assess if certain bird species have higher variance than others, we calculated beta diversity using a multivariate version of Levene's test for homogeneity of variances. We reported the distance to centroid value.

We used R packages *phyloseq* and *vegan* [16, 34]. We used non-metric dimensional scaling (NDMS) with Bray-Curtis dissimilarity matrices to reduce multivariate data and spatially visualize microbial communities. NMDS was used to find patterns across captivity. We used permutational analysis of the variance (PERMANOVA) also with Bray-Curtis distance matrices to determine whether different factors, such as captivity status (wild/captive), site (geographic area), microsite (i.e. in brooder box, soil, etc.), age (days old of captive individuals), weight (mass in grams for captive individuals), collection date, and history of coccidiosis (positive/negative) can explain microbial community variance. We conducted a simper analysis [16] to determine which OTUs explain over 70% of the differences between treatments. We used a clamtest [16] to categorize bacterial and fungal OTUs into groups: generalist, too rare, and treatment specialist (wild-, captive-, positive-, negative-). Positive and negative correspond to individual kiwi who have had a history of coccidiosis.

Abbreviations

OTU: Operational taxonomic unit; PCR: Polymerase chain reaction; DNA: Deoxyribonucleic nucleic acid; rRNA: Ribosomal ribonucleic acid; PERMANOVA: permutational analysis of the variance; NMDS: non-metric multidimensional scaling

Declarations

Acknowledgements

We thank Ana Podolyan, Caroline Mitchell, Katherine Trought, Eva Weber, and Carina Davis at Manaaki Whenua Landcare Research for their expertise in the lab. Thank you to Malin Undin, Janneke Klute, Yi Luo, Emily Thompson, and Natasha Bansal for their help in field sampling. We appreciate the Chamberlain family for access to kiwi on their property. Ngai Tai (Ponui Island), Te Patukeha and Ngati Kuta (Rakaumangamanga, Moturua and Motuarohia) and Ngati Toro (Puketi) provided support and permission to collect fecal samples in their rohe (tribal areas) used in this study. Thank you to Emma Bean and Helen McCormick at the National Kiwi Hatchery and Ngāi Tahu for their continued support of the National Kiwi Hatchery and Jo Russell at the Otorohanga Kiwi House. Thank you to the Fukami Lab at Stanford University for their helpful feedback on the manuscript.

Author’s contributions

PASJ, MKD, and IC designed the study. PASJ and IC collected fecal samples. PASJ performed the molecular work including DNA extractions, PCR, and library preparation. PASJ and MKD completed the bioinformatics. PASJ conducted the statistical analyses. PASJ wrote the manuscript with feedback from MKD and IC.

Funding

This work was supported by Birds New Zealand grant for PASJ & MKD, MBIE Strategic Science Investment Fund supported MKD, and the Stanford Center for Conservation Biology for PASJ. All captive research performed is covered under the Zoo and Aquarium Association of New Zealand permit and wild kiwi research is covered under the Department of Conservation permits 38796-FAU and 70826-CAP.

Availability of data and materials

The datasets generated and/or analyzed during the current study are available in a github repository, https://github.com/psanjuan/kiwi_microbiome_2019.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no conflict of interest.

References

1. Hicks AL, Lee KJ, Couto-Rodriguez M, Patel J, Sinha R, Guo C, et al. Gut microbiomes of wild great apes fluctuate seasonally in response to diet. Nat Commun. 2018;9. doi:10.1038/s41467-018-04204-w.
2. Ezenwa VO, Gerardo NM, Inouye DW, Medina M, Xavier JB. Animal Behavior and the Microbiome. Science (80-). 2012;338:198–9. doi:10.1126/science.1227412.
3. San Juan PA. Land-use change has host-speci fi c in fl uences on avian gut microbiomes. ISME J. 2020;14:318–21.
4. Miller ET, Svanbäck R, Bohannan BJM. Microbiomes as Metacommunities: Understanding Host-Associated Microbes through Metacommunity Ecology. Trends Ecol Evol. 2018;33:926–35. doi:10.1016/j.tree.2018.09.002.
5. Schmidt E, Mykytczuk N, Schulte-Hostedde AI. Effects of the captive and wild environment on diversity of the gut microbiome of deer mice (Peromyscus maniculatus). ISME J. 2019;13:1293–305. doi:10.1038/s41396-019-0345-8.

6. Clayton JB, Vangay P, Huang H, Ward T, Hillmann BM, Al-Ghalith GA, et al. Captivity humanizes the primate microbiome. Proc Natl Acad Sci. 2016;113:10376–81. doi:10.1073/pnas.1521835113.
7. Metcalf JL, Song SJ, Morton JT, Weiss S, Seguin- A, Joly F, et al. Evaluating the impact of domestication and captivity on the horse gut microbiome. Sci Rep. 2017;7:1–9.
8. Department of Conservation. https://www.doc.govt.nz/nature/habitats/. Accessed 29 Oct 2020.
9. Colbourne R, Bassett S, Billing T, McCormick H, McLennan J, Nelson A, et al. The development of Operation Nest Egg as a tool in the conservation management of kiwi. Sci Conserv. 2005;:1–24.
10. Mahnert A, Moissl-Eichinger C, Zojer M, Bogumil D, Mizrahi I, Rattei T, et al. Man-made microbial resistances in built environments. Nat Commun. 2019;10:1–12. doi:10.1038/s41467-019-08864-0.
11. Bokulich NA, Chung J, Battaglia T, Henderson N, Jay M, Li H, et al. Antibiotics, birth mode, and diet shape microbiome maturation during early life. Sci Transl Med. 2016;8:343ra82. doi:10.1126/scitranslmed.aad7121.
12. Sommer F, Bäckhed F. The gut microbiota — masters of host development and physiology. Nat Rev Microbiol. 2013;11:227–38. doi:10.1038/nrmicro2974.
13. Morgan KJ, Alley MR, Pomroy WE, Castro I, Howe L. Enteric coccidiosis in the brown kiwi (Apteryx mantelli). Parasitol Res. 2012;111:1689–99.
14. Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Huntley J, Fierer N, et al. Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. ISME J. 2012;6:1621–4.
15. Toju H, Tanabe AS, Yamamoto S, Sato H. High-coverage ITS primers for the DNA-based identification of ascomycetes and basidiomycetes in environmental samples. PLoS One. 2012;7:1–11.
16. Oksanen J, Blanchet F, Friendly M, Kindt R, Legendre P, McGlinn D. vegan: Community Ecology Package. R package version. 2017.
17. Kriss M, Hazleton KZ, Nusbacher NM, Martin CG, Lozupone CA. Low Diversity Gut Microbiota Dysbiosis: Drivers, Functional Implications and Recovery. Curr Opin Microbiol. 2019;44:34–40.
18. Bass D, Stentiford GD, Wang HC, Koskella B, Tyler CR. The Pathobiome in Animal and Plant Diseases. Trends Ecol Evol. 2019;34:996–1008. doi:10.1016/j.tree.2019.07.012.
19. Ferrario C, Taverniti V, Milani C, Fiore W, Laureati M, De Noni I, et al. Modulation of fecal clostridiales bacteria and butyrate by probiotic intervention with Lactobacillus paracasei DG varies among healthy adults. J Nutr. 2014;144:1787–96.
20. Braun MS, Zimmermann S, Danner M, Rashid H or, Wink M. Corynebacterium uropygiale sp. nov., isolated from the preen gland of Turkeys (Meleagris gallopavo). Syst Appl Microbiol. 2016;39:88–92. doi:10.1016/j.syapm.2015.12.001.
21. Grond K, Sandercock BK, Jumpponen A, Zeglin LH. The Avian Gut Microbiota: Community, Physiology and Function in Wild Birds. J Avian Biol. 2018;e01788:1–19. doi:10.1111/jav.01788.
22. Mandic-Mulec I, Stefanic P, van Elsas JD. Ecology of Bacillaceae. Microbiol Spectr. 2015;3.
23. Cunningham SJ, Castro I. The secret life of wild brown kiwi: Studying behaviour of a cryptic species by direct observation. N Z J Ecol. 2011;35:209–19.

24. Ozato N, Saito S, Yamaguchi T, Katashima M, Tokuda I, Sawada K, et al. Blautia genus associated with visceral fat accumulation in adults 20–76 years of age. npj Biofilms Microbiomes. 2019;5:1–9. doi:10.1038/s41522-019-0101-x.
25. De Maesschalck C, Van Immerseel F, Eeckhaut V, De Baere SD, Cnockaert M, Croubels S, et al. Faecalicoccus acidiformans gen. nov., Sp. nov., Isolated from the chicken caecum, And reclassification of streptococcus pleomorphus (barnes et al. 1977), Eubacterium biforme (eggerth 1935) and eubacterium cylindroides (cato et al. 1974) as faecalicoccus p. Int J Syst Evol Microbiol. 2014;64:3877–84.
26. Bensch K, Groenewald JZ, Meijer M, Dijksterhuis J, Jurjević, Andersen B, et al. Cladosporium species in indoor environments. Stud Mycol. 2018;89:177–301.
27. van Nieuwenhuijzen EJ. Aureobasidium. Encycl Food Microbiol Second Ed. 2014;1:105–9.
28. Mariné M, Brown NA, Riaño-Pachón DM, Goldman GH. On and Under the Skin: Emerging Basidiomycetous Yeast Infections Caused by Trichosporon Species. PLoS Pathog. 2015;11:1–9.
29. Colgan W, Claridge AW. Mycorrhizal effectiveness of Rhizopogon spores recovered from faecal pellets of small forest-dwelling mammals. Mycol Res. 2002;106:314–20.
30. Arenal F, Platas G, Peláez F. Two new Preussia species defined based on morphological and molecular evidence Two new Preussia species defined based on morphological and molecular evidence Introduction species of bitunicate ascomycetes with non-ostiolate ascomata , containing dark. 2014; November 2004.
31. Mašínová T, Bahnmann BD, Větrovský T, Tomšovský M, Merunková K, Baldrian P. Drivers of yeast community composition in the litter and soil of a temperate forest. FEMS Microbiol Ecol. 2017;93:1–10.
32. Rohland N, Reich D. Cost-effective , high-throughput DNA sequencing. Genome Res. 2011;:939–46.
33. Tanabe AS, Toju H. Two New Computational Methods for Universal DNA Barcoding: A Benchmark Using Barcode Sequences of Bacteria, Archaea, Animals, Fungi, and Land Plants. PLoS One. 2013;8.
34. McMurdie PJ, Holmes S. Phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. PLoS One. 2013;8.

Figures

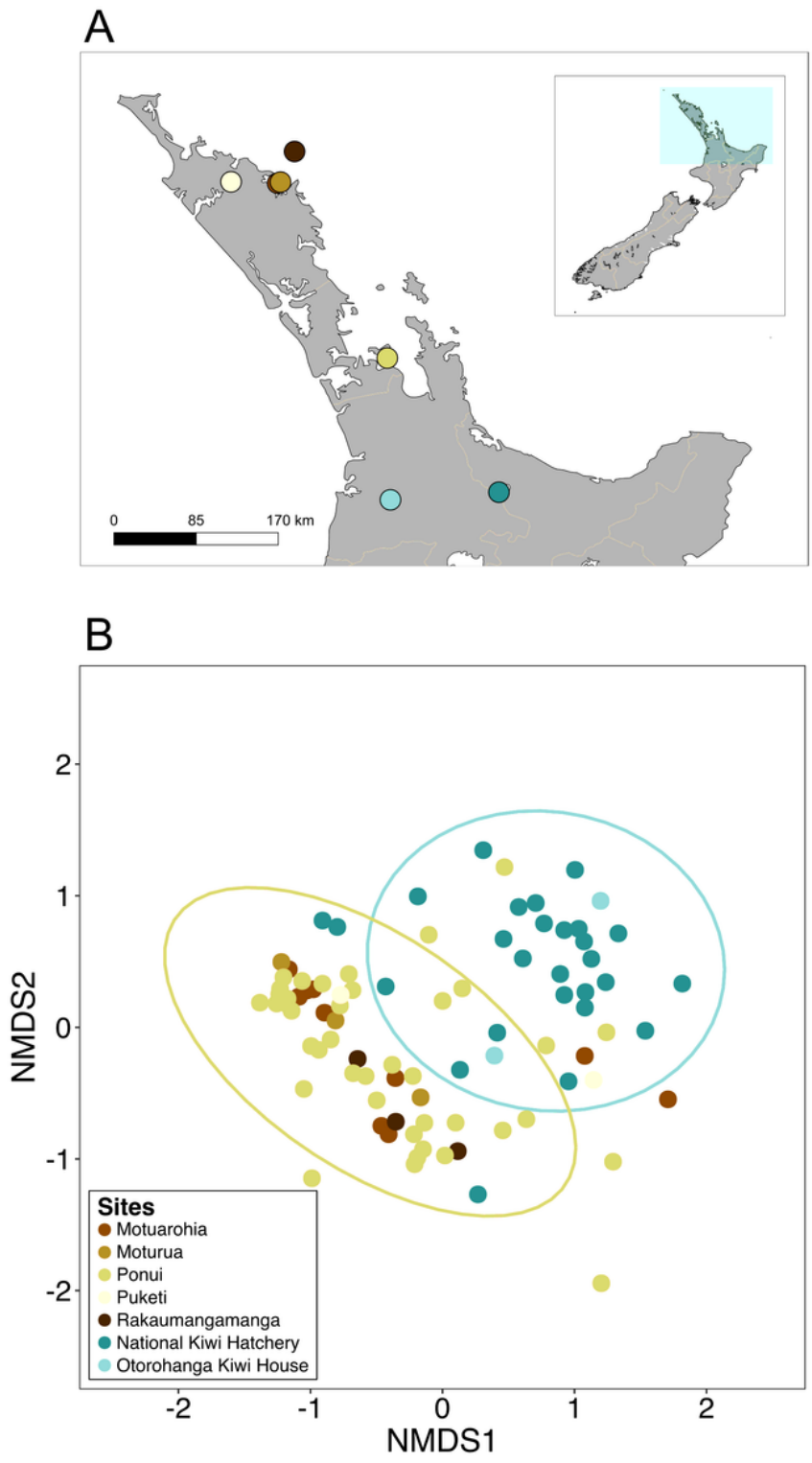

Figure 1

Captivity but not geography shifts the Brown Kiwi gut bacterial composition. (A) Map of collection sites where colors correspond to key in panel b. Teal colors correspond to captive sites, while brown colors correspond to wild sites. (B) NMDS plot shows samples clustering by captivity status with little overlap between the groups (permANOVA, r2 = 0.07, p = 0.001). Ellipses denote 95% confidence level.

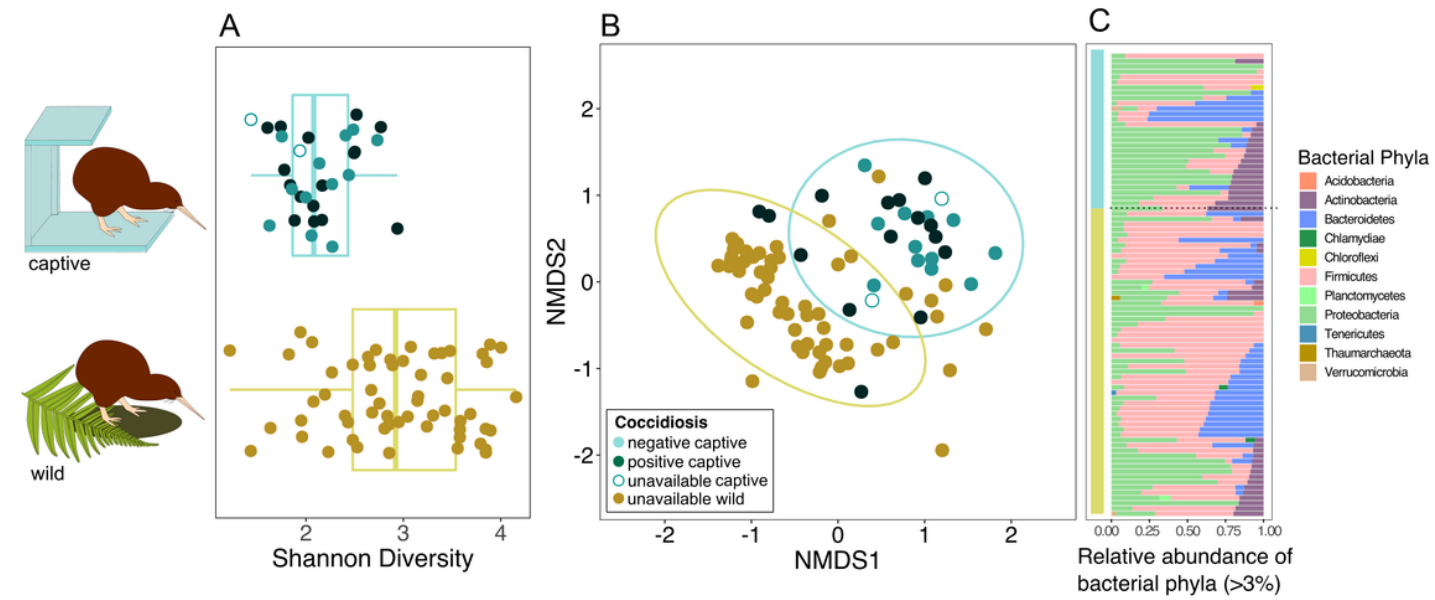

Figure 2

The Brown Kiwi bacterial community differs both in diversity and composition due to captivity status. (A) Alpha diversity of captive kiwi is significantly reduced compared to wild individuals (B) NMDS shows distinct clusters between wild and captive samples and differences between individuals with and without a history of coccidiosis (C) Relative abundances of bacterial phyla between captive and wild kiwi.

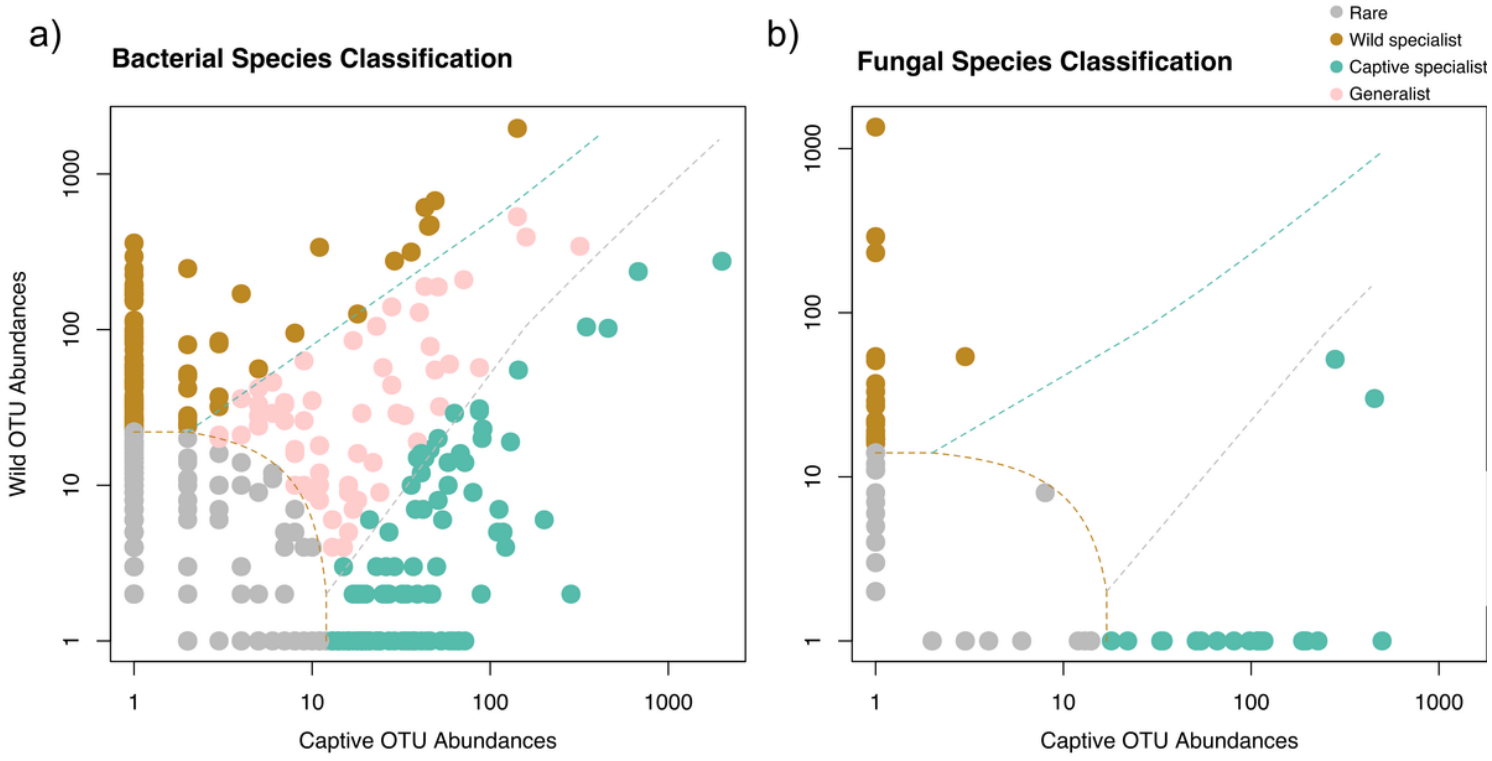

Figure 3

A multinomial species classification method (clamtest) categorized bacterial and fungal OTUs into one of four classes: rare, generalist, wild specialist, and captive specialist. (A) For bacterial OTUs, 9.9% were classed as generalist, 53% as rare, 19.7% as wild specialist, and 17.4% as captive specialist. (B) For fungal OTUs, 0% were classed as generalist, 46.7% as rare, 26.7% as wild specialist, and 26.7% as captive specialist.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SanJuanetalGraphicalAbstractAnimalmicrobiomecolormod.png
- SanJuanetalSI.docx
- SanJuanetalSupplementarytable1.pdf
- SanJuanetalSupplementarytable2.pdf
- SanJuanetalSupplementarytable3.pdf
- SanJuanetalSupplementarytable4.pdf
- SanJuanetalSupplementarytable5.pdf
- SanJuanetalSupplementarytable6.pdf
- SanJuanetalSupplementaryFig1.pdf
- SanJuanetalSupplementaryFig2.tiff