UAB
Universitat Autònoma de Barcelona

Human footprints on bat's health: exploring anthropogenic impacts

Lourdes Lobato Bailón

ADVERTIMENT. L'accés als continguts d'aquesta tesi queda condicionat a l'acceptació de les condicions d'ús establertes per la següent llicència Creative Commons: https://creativecommons.org/licenses/?lang=ca

ADVERTENCIA. El acceso a los contenidos de esta tesis queda condicionado a la aceptación de las condiciones de uso establecidas por la siguiente licencia Creative Commons: https://creativecommons.org/licenses/?lang=es

WARNING. The access to the contents of this doctoral thesis it is limited to the acceptance of the use conditions set by the following Creative Commons license: https://creativecommons.org/licenses/?lang=en

UAB
Universitat Autònoma de Barcelona

Human footprints on bat's health: exploring anthropogenic impacts

Author
Lourdes Lobato Bailón

Supervisors
Oscar Cabezón
Ignasi Marco
Jaime Martínez Urtaza

Tutor
Ignasi Marco

PhD in Animal Medicine and Health
Department of Animal Medicine and Surgery
Veterinary Faculty
Autonomous University of Barcelona
2024

Wildlife Conservation Medicine
Research group

UAB
Universitat Autònoma de Barcelona

Els doctors **Oscar Cabezón Ponsoda**, investigador del Departament de Medicina i Cirurgia Animals de la UAB, **Ignasi Marco Sánchez**, catedràtic d'universitat del Departament de Medicina i Cirurgia Animals de la UAB, **Jaime Martínez Urtaza**, catedràtic d'universitat del Departament de Genètica i de Microbiologia de la UAB

Informen:

Que la memòria titulada "**Human footprints on bat's health: exploring anthropogenic impacts**", presentada per la **Lourdes Lobato Bailón** per a la obtenció del títol de Doctora per la Universitat Autònoma de Barcelona, s'ha realitzat sota la nostra direcció i, un cop considerada satisfactòriament finalitzada, autoritzem la seva presentació per tal que sigui avalada per la comissió corresponent.

I perquè així consti als efectes que siguin oportuns, firmem el present informe a Bellaterra, 18 juny de 2024.

Oscar Cabezón Ponsoda
Director

Ignasi Marco Sánchez
Director i Tutor

Jaime Martínez Urtaza
Director

Lourdes Lobato Bailón
Doctoranda

Akwata empola atuuka wala,

Akwata empola atuuka wala* - em va dir enmig d'un sotabosc de falgueres, bambú i enfiladisses esquinçades pel pas de goril·les de muntanya.

Voldria prendre aquest proverbi com la pedra angular d'aquest treball, i aprofitar l'avinentesa per expressar el meu sincer agraïment a totes les persones que han contribuït de manera significativa a l'assoliment d'aquesta tesi doctoral.

Per començar, gràcies als meus tres *Súper*-visors de tesi: la brúixola que sempre apunta el Nord i amb un somriure; el company de batalles i mestre d'idees esbojarrades; i l'engranatge silenciós, "máis listo que un allo". Perquè, malgrat tot l'estrès inherent a aquesta etapa professional, heu suposat per mi un equip tècnic i personal immillorable! Sense la vostra guia, el vostre bon rotllo i la vostra expertesa, la culminació d'aquesta tesi no seria possible. Qui ho sabia al 2019, que estudiar la taxa de filtració de les nàiades m'obriria un camí tan interessant i que em permetria conèixer els indrets més macos de Catalunya, a la llum de les estrelles i entre centenars de ratpenats; que em permetria treballar amb experts de tantes disciplines; tenir cura d'una colònia de ratpenats en captivitat, descobrir el meu restaurant tailandès preferit; i capturar ratpenats al Parc Nacional de la Selva Impenetrable de Bwindi. A tots tres, gràcies per creure i confiar en mi, delegar-me responsabilitats, ensenyar-me a relativitzar i fer-me sentir una peça important de l'equip.

Companys de doctorat i residència, companys de 1000 i 1 nits d'estiu entre puces de ratpenats i tritons del Pirineu, companya de batalles per Uganda: gràcies. Ha estat una gran sort compartir aquests anys amb vosaltres! El vostre suport, complicitat, companyia i motivació han estat fonamentals durant aquesta intensa etapa acadèmica i personal. Especialment gràcies a vosaltres, aquests anys seran recordats amb nostàlgia. Guardo al meu cor i amb especial carinyo certs moments durant les restriccions per la pandèmia: permisos per sortir a mostrejar, zooms interminables i "etogrames" a les cales de Roses. Espero seguir compartint amb tots vosaltres moments surrealistes de 24h seguides, plenes de fauna salvatge, d'ironia i humor, i alguna que altra birra entre cartes del Wingspan. Amics, us desitjo molta sort en el vostre camí, que la vostra passió per la recerca i el coneixement us guiï cap a camins plens d'èxits i realitzacions personals. Per mi, sou i sereu sempre *fabulous* and *fantastic*!

Gràcies a la resta d'estudiants, visitants i companys d'expedicions ratpenateres que han passat per WildCoM al llarg d'aquests 5 anys. El vostre interès, la vostra

* *Quan una persona s'apassiona per alguna cosa, encara que sigui difícil, ho aconsegueix.*
(Proverbi Ugandès, llengua Luganda)

energia i completa disponibilitat per sortir a camp totes les nits d'estiu i enllaçar amb necròpsies de ratpenats i macerats de teixits a l'endemà, han estat essencials per mantenir-me al peu del canó amb motivació. M'omple de felicitat mantenir el contacte amb molts de vosaltres i veure com, mica en mica, aneu trobant el vostre camí. Molta sort!

L'efecte que un bon professor pot tenir sobre l'estudiant és immesurable. Gràcies a la resta de professors, tècnics i estudiants de la Facultat de Veterinària, Facultat de Biociències de la UAB i l'IRTA-CReSa que heu col·laborat en la meva formació específica de benestar animal, laboratori, estadística i seqüenciació. Gràcies a la vostra paciència, crítica constructiva (sempre seràs el meu estadístic preferit, irremplaçable) i art en l'ensenyança heu despertat el meu interès en àmbits que desconeixia i que espero continuar aprenent.

Em sento extremadament afortunada d'haver pogut treballar mà a mà, estiu rere estiu, amb els ratpanateros i biòlegs del Centre de Ciència i Tecnologia de Catalunya i del Museu de Ciències Naturals de Granollers. La vostra expertesa, ganes de compartir coneixement i amor pels ratpenats han estat essencials pel desenvolupament d'aquesta tesi i pel meu aprenentatge en maneig de ratpenats. La vostra passió per aquestes bestioles se'm va enganxar la primera sortida a les mines de Mequinença, i sé que mai podré treure-me-la de sobre! Malgrat les expedicions amb tot l'equip per Catalunya en busca de ratpenats s'han convertit en excursions de petit comitè de Vic a Girona, i de Girona a Sabadell, és una alegria per mi tenir-vos encara al meu costat. Gràcies pel vostre suport i amistat!

Un agraïment profund a les meves amigues d'infància, adolescència i Universitat que encara formen part de la meva vida. Dones fortes, valentes i intel·ligents que m'han inspirat, m'han ajudat a superar-me i m'han donat suport continuadament al llarg d'aquest viatge. Gràcies per entendre tots els angles possibles de la meva personalitat, respectar les meves necessitats extremes durant aquests 5 anys i continuar recolzant-me dia a dia. Gràcies també a aquell amic excepcional que, malgrat no comprendre completament la meva motivació i passió envers el treball, continua recolzant-me de manera incondicional en cada situació de la meva vida. Setanta pujades farragoses a la Mola, unes (moltes) birres i trenta-tres plors. Gràcies per ajudar-me a forjar unes cames i *cor d'acer*!

Per últim, vull dedicar un espai d'agraïment especial a la meva família més propera, els meus pares, la meva germana i el meu company de vida. No em puc sentir més afortunada de comptar amb el suport i amor incondicional de tots vosaltres. Pares i germana, gràcies per educar-me en la seguretat i confiança de mi mateixa, en la paciència, en mantenir la il·lusió per allò que et fa feliç amb petites dosis de realisme, i en perdre la por a equivocar-me. Tata, has estat i seràs el meu pilar de recolçament i inspiració de millora per la resta de la meva vida, gràcies mil vegades. I gràcies al meu company de vida, que jo he escollit, i que ell m'ha escollit a mi tal com sóc. Gràcies per redirigir sempre la meva energia i els meus pensaments cap a un lloc menys fosc i més reconfortant. Al teu costat, sento que sóc capaç de tot. Us estimo família, molt.

Fent el balanç final, m'he n'adono més que mai que aquesta tesi ha suposat una transformació personal i professional tant extrema que serà molt difícil de superar en els propers anys. He après de manera inimaginable, he rigut tant com per tenir mal als queixals i la panxa, m'he desfet i plorat davant de molts de vosaltres, i gràcies a tots vosaltres he trobat forces per seguir endavant i convertir la meva passió en una realitat enriquidora. Gràcies per fer-me costat en aquesta meravellosa etapa de la meva vida, una forta abraçada.

Index

Index ..... **I**
List of abbreviations ..... **III**
Summary ..... **V**
Resum ..... **VII**
Justification ..... **IX**
Introduction **1**
1. The order Chiroptera **2**
1.1. Bats' history and culture 2
1.2. Taxonomy and morphology 3
1.2.1. Insectivorous bats 5
1.3. Why do bats matter? 6
2. Anthropogenic impacts on bat populations **10**
2.1. Chemical pollutants 11
2.2. Persecution and consumption 11
2.3. Climate change 12
2.4. Habitat loss and encroachment 14
2.5. Scientific practice 17
2.6. Diseases 19
3. The microbiome **22**
3.1. The microbiome in health and disease 24
3.2. The gut and fecal bacterial microbiome 25
3.3. Current knowledge of bats' fecal microbiome 27
4. Methods of microbiome analysis **30**
4.1. Historical evolution of sequencing technologies 30
4.2. Sequencing approaches to the study of microbial communities 34

4.3. Bioinformatics tools and software 36

4.4. Statistical analysis and visualization methods used in microbiome projects 38

5. Workflow overview for the full-length 16S rRNA gene metabarcoding using the MinION™ sequencer 41

Hypotheses 45

Objectives 46

Study 1. Reappraising the use of forearm rings for bat species 47

Supplementary material: S1 69

Study 2. The fecal bacterial microbiome of the Kuhl's pipistrelle (*Pipistrellus kuhlii*) reflects landscape anthropogenic pressure 75

Availability of data and materials 98

Supplementary material: S2 99

Study 3. Assessing the relationship of the fecal bacterial microbiome of five bat species from Catalonia (NE Spain) and their fecal bacterial pathogens with types of land use 105

Results summary 132

Supplementary material: S3 142

General discussion 159

Healthy wings for a thriving life 160

"To tag or not to tag": ethical decision-making in individual marking tools for insectivorous bats 161

A promising non-invasive technique for the study of insectivorous bat's health: the fecal microbiome 164

Understanding and managing the risks associated with potential pathogenic bacteria identified through sequencing methods in bat feces 166

Conclusions 169

Future directions 170

Bibliography 171

Annex 223

List of abbreviations

ACE2: Angiotensin Converting Enzyme 2
ANOSIM: Analysis of Similarities
ASVs Amplicon Sequence Variants
ATP: Adenosine Triphosphate
CMOS: Complementary Metal-Oxide Semiconductor
DDT: Dichlorodiphenyltrichloroethane
DMR: Dirichlet-multinomial regression
DNA: Deoxyribonucleic Acid
dNTP: Deoxynucleotide Triphosphate
EM algorithm: expectation-maximization algorithm
HTS: High-Throughput Sequencing
IBD: Inflammatory Bowel Disease
LDA: Linear discriminant analysis
LEfSE: Linear discriminant analysis Effect Size
MERS: Middle East Respiratory Syndrome
MDR: Modified (microbiome) Dysbiosis Ratio
NGS: Next-Generation Sequencing
NMDS: Non-Metric Multidimensional Scaling
ONT: Oxford Nanopore sequencing technology
PCA: Principal Component Analysis
PCoA: Principal Coordinate Analysis
PERMANOVA: Permutational Multivariate Analysis of Variance
PPB: potential pathogenic bacteria
RNA: Ribonucleic Acid
rRNA: ribosomal Ribonucleic Acid
SARS: Severe Acute Respiratory Syndrome
SARS-CoV-2: Severe Acute Respiratory Syndrome Coronavirus 2
SBS: Sequence-By-Synthesis
SMRT: Single-Molecule Real Time sequencing
SMS: Single-Molecule Sequencing

Summary

European bat populations have experienced significant declines for the past 50 years. Consequently, nearly a quarter of these species are classified as Endangered (EN) or Vulnerable (VU) by the International Union for Conservation of Nature (IUCN). This urgent need for protection has led to increased research focused on understanding the drivers of such negative trends in their populations. In this context, millions of bats across Europe have been individually marked, mostly with forearm rings. Although these capture-mark-recapture studies have contributed significantly to our understanding of bats' biology and ecology, the use of forearm rings in insectivorous bats has been repeatedly associated with negative health effects and even some mass mortality events. Additionally, these long-term studies have rarely incorporated bats' health aspects, which currently represents an unexplored area requiring much more attention. For instance, fecal microbiome analysis is a valuable method to assess human and animal health, yet it remains underdeveloped for bat species. We hypothesized that the health of free-ranging European bat populations may be negatively affected by a range of anthropogenic activities, such as individual marking activities and human encroachment into bats' habitats.

In **Study 1**, we assessed the health impact of the two most used marking techniques in bat species, the forearm ring and the Passive Integrated Transponder (PIT) tag, collecting available information from the main bat research institutions from Catalonia (NE-Spain) and testing both techniques in captivity. We reported 55.1% (435/790) of recaptured free-ranging bats with forearm rings presenting skin lesions associated with this technique. No PIT-tagged bat showed lesions (n=446). In captivity, all 22 banded *Carollia perspicillata* showed skin lesions and discomfort behaviours due to forearm rings, while none of the PIT-tagged bats (n=21) exhibited such issues throughout the study. These results call for establishing an ethics committee within the framework of bat research involving individual marking activities. Particularly, the safety of forearm rings should be explored for each bat species and under captive conditions, where full recapture rates are possible.

In **Studies 2** and **3**, we assessed the impact of human activities and types of land use on the structure and composition of the fecal bacterial microbiome, and on the occurrence and abundance of potential pathogenic bacteria (PPB) of five free-ranging European bat species (*Miniopterus schreibersii, Nyctalus leisleri, Pipistrellus kuhlii, P. pipistrellus* and *P. pygmaeus*). We used the full-length 16S rRNA gene metabarcoding with the nanopore long-read sequencer MinION™ to perform these analyses. Taxonomic assignment for the analyses of the microbiome structure, composition and diversity were performed at bacterial genus level, while the assessment of PPB was performed at bacterial species level. Both studies showed that samples from urban landscapes exhibited the lowest alpha diversity value compared to well-preserved landscapes (natural or extensive agriculture areas). Analyses at the bacterial species level showed 222 PPB, including some recognized animal and human pathogens (e.g. *Brucella abortus* and *Francisella tularensis*) and others (e.g. *Yersinia enterocolitica*) previously isolated from pathological samples of deceased European bats. No correlation was found between the land use type and the occurrence and abundance of PPB. These results suggest that well-preserved landscapes promote more resilient fecal bacterial microbiomes in the five studied bat species. We further highlighted the potential of microbiome studies reaching bacterial species-level resolution to be used in bat health studies. Owing to its non-invasive nature and simplicity of sampling, the fecal microbiome analysis has the potential to be used as a health indicator for bat species.

This thesis also concludes that to gain knowledge about bats' health while supporting bat conservation efforts, future studies must enhance their commitment to adhering to ethical standards and prioritize non-invasive techniques when working with bats.

Resum

Els ratpenats europeus han experimentat importants davallades poblacionals durant els últims 50 anys. Gairebé una quarta part d'aquestes espècies estan classificades com En Perill (*EN*) o Vulnerables (*VU*) per la Unió Internacional per a la Conservació de la Natura (UICN). La urgència per protegir aquestes espècies ha comportat un augment d'estudis enfocats a comprendre els desencadenants de les seves davallades poblacionals. Conseqüentment, milions de ratpenats europeus han estat marcats individualment amb anelles d'avantbraç. Encara que aquests estudis de captura-marcatge-recaptura han contribuït significativament al nostre coneixement sobre biologia i ecologia de ratpenats, l'ús d'anelles s'ha associat de manera repetida amb efectes negatius sobre la seva salut i fins i tot amb alguns esdeveniments de mortalitat massiva. A més, aquests estudis rarament incorporen aspectes sobre salut poblacional, àrea de coneixement que actualment es troba pràcticament inexplorada i que requeriria molta més atenció. Per exemple, l'anàlisi del microbioma fecal està considerat un mètode valuós des de fa anys per a avaluar la salut humana i animal, però en els ratpenats només s'ha començat a explorar recentment. Hipotetitzem que la salut de les poblacions de ratpenats podria veure's deteriorada per diverses activitats antropogèniques, com ara el marcatge individual i la intrusió de l'ésser humà en els seus hàbitats.

A l'**Estudi 1**, vam avaluar l'impacte de les dues tècniques de marcatge més utilitzades en ratpenats, l'anella d'avantbraç i el microxip subcutani, recopilant la informació disponible de les principals institucions de recerca de ratpenats de Catalunya i avaluant totes dues tècniques en captivitat. El 55.1% (435/790) dels ratpenats salvatges recapturats amb anelles van presentar lesions associades amb aquesta tècnica. Cap ratpenat microxipat va mostrar lesions (n=446). Els 22 exemplars de *Carollia perspicillata* anellats en captivitat van mostrar lesions i comportaments de malestar degut a aquest mètode de marcatge, mentre que cap dels ratpenats microxipats (n=21) va mostrar tals problemes. Aquests resultats advoquen per l'establiment d'un comitè d'ètica referent al marcatge individual de ratpenats. Especialment, la innocuïtat de les anelles s'hauria

d'avaluar per a cada espècie de ratpenat i en condicions de captivitat, on una taxa de recaptura completa és possible.

Als **Estudis 2** i **3**, vam avaluar l'impacte de diverses activitats antropogèniques sobre l'estructura i composició del microbioma bacterià fecal, i sobre l'ocurrència i abundància de potencials bacteris patògens (*PPB*) de cinc espècies de ratpenats europeus (*Miniopterus schreibersii, Nyctalus leisleri, Pipistrellus kuhlii, P. pipistrellus* i *P. pygmaeus*). Per aquest motiu, vam realitzar la seqüenciació completa del gen 16S rRNA mitjançant l'ús del seqüenciador MinION™. L'assignació taxonòmica per l'anàlisi del microbioma es va realitzar a nivell de gènere bacterià, mentre que l'avaluació de les PPB es va realitzar a nivell d'espècie bacteriana. Tots dos estudis van mostrar que els ratpenats de paisatges urbans exhibien una menor diversitat alfa en comparació amb els de paisatges ben preservats (àrees naturals o d'agricultura extensiva). Vam identificar 222 *PPB*, incloent alguns patògens animals i humans reconeguts (*Brucella abortus*, *Francisella tularensis*) i altres (*Yersinia enterocolitica*) prèviament aïllats de mostres patològiques de cadàvers de ratpenats. No es va trobar correlació entre el tipus d'ús del sòl i l'ocurrència i abundància de PPB. Aquests resultats suggereixen que els paisatges ben preservats promouen microbiomes bacterians fecals més resilients en les espècies de ratpenats estudiades. Degut a la seva naturalesa no invasiva i facilitat de mostreig, l'anàlisi del microbioma fecal té el potencial de ser emprat com a indicador de salut per espècies de ratpenats insectívors.

Amb l'objectiu de donar suport a les mesures de conservació dels ratpenats, els estudis en aquestes espècies han de mostrar un major compromís amb els codis de conducta ètica i prioritzar l'ús de tècniques de mostreig no invasives.

Justification

More than half of bat species around the globe are under threat (IUCN, 2023). Particularly in Catalonia, there are 30 insectivorous bat species (**Annex: Table A1**), of which four are classified as critically endangered and 13 as vulnerable, but all are protected by law ("Decret legislatiu 2/2008, de 15 d'abril, Llei de protecció dels animals"; "Llei estatal 42/2007, de 13 de desembre"; and "Real Decreto 139/2011: Lista y Catálogo Nacional de especies amenazadas") (Generalitat de Catalunya, 2023).

According to the "Estat de la natura a Catalunya" report from 2020, animal populations from Catalonia have suffered a significant decline (25% average) across all landscapes between 2002 and 2019, particularly those species inhabiting freshwater biomes. The main driver of this biodiversity decline in Catalonia, as well as in Europe, has been attributed to land-use changes for human development (Brotons et al., 2020). Whereas other factors, such as introduction of invasive species (i.e. Sistema d'Informació d'Espècies Exòtiques de Catalunya, CREAF) and climate change effect, have been monitored and quantified for years (Brotons et al., 2020), there is a lack of analysis regarding the short and long-term health trends in most wildlife populations. Such is the case of bat species in Catalonia, whose populations are being monitored across their different habitats (Museu de Ciències Naturals de Granollers et al., 2024), but without consideration of their health status or potential pathological processes that may affect them.

Apart from the white-nose syndrome in North American bats [>700 documents by June 2024, Scopus keywords: (Pseudogymnoascus AND destructans) OR (white-nose AND syndrome) OR (Geomyces AND destructans)], there has been little research conducted on the health and diseases affecting bat populations worldwide. Furthermore, the available information is limited to necropsy findings or descriptions of pathogens (**Annex: Table A2**), lacking a detailed study of their impact on bat populations dynamics. Bats offer crucial ecosystem services and significantly contribute to biodiversity (IUCN, 2023; Ramirez-Francel et al., 2022). Therefore, it is crucial to develop research focused on obtaining baseline health

data for bat species, exploring health bioindicators, and investigating diseases affecting bat populations.

The aim of this doctoral thesis is to enhance the global understanding of the health of insectivorous bat populations. By establishing a foundational level of knowledge, this thesis intends to encourage future health-related research on these species and the use of cutting-edge and non-invasive technologies to expedite its progress.

At the start of this doctoral research journey, and after discussions with bat conservationists and researchers, we came across an important issue that needed immediate attention but continued to be overlooked by the scientific community: the impact of individual marking tools on insectivorous bats. This issue raised ethical concerns that needed to be addressed before we could move forward with any other studies. Therefore, **Study 1** encompasses a comprehensive review of existing bat recapture data, combined with a controlled investigation under captive conditions using *Carollia perspicillata* kept in a zoological institution. In the following studies, we focus on providing knowledge on the impact of anthropogenic activities on the health and fitness of insectivorous bats from Catalonia (NE Spain), applying non-invasive sampling and next-generation-sequencing technologies. In **Study 2**, we analyse the impact of landscape anthropogenic pressure on the diversity and composition of the fecal bacterial microbiome of Kuhl's pipistrelles inhabiting different ecosystems of Catalonia (NE Spain) with the use of the MinION™ sequencer. In **Study 3**, we investigate the bacterial microbiome and the occurrence of potential pathogenic bacteria in fecal samples of five bat species inhabiting Catalonia and their relationship with the presence of domesticated animals and high human populated areas. By using the MinION™ sequencer and the new computational software "Emu" we improve the taxonomic resolution at the species level.

Introduction

1. The order Chiroptera

1.1. Bats' history and culture

All bats on the planet belong to a single order known as Chiroptera, which represents one of the largest and most diverse animal groups. As of March 2023, there are 1462 identified bat species, comprising nearly a quarter of extant mammal species (Simmons & Cirranello, 2023). Bats inhabit all the continents except Antarctica and live in extremely differing environments, as reflected in the wide range of individual adaptations they have undergone (Hutson et al., 2001). Echolocation abilities and the faculty of powered flight comprise two unique features of this taxonomic group, although the evolutionary pathways that lead to these adaptations are poorly understood. The most primitive bat fossil found, from the Early Eocene Green River Formation of Wyoming (USA), dates back to over 50 million years ago and suggests that flight may had evolved first than echolocation. Nonetheless, bat fossils documenting this transition are still lacking (Simmons et al., 2008).

As happens with other nocturnal species, bats have triggered many superstitions and raised irrational fears throughout the history of many cultures. While Asia-Pacific civilizations seem to have far more positive associations with bats, Western cultural associations of bats with ill omens and evil spirits, particularly in the Christian tradition, date back to the fourteenth century (Sieradzki & Mikkola, 2022) (**Fig. 1**).

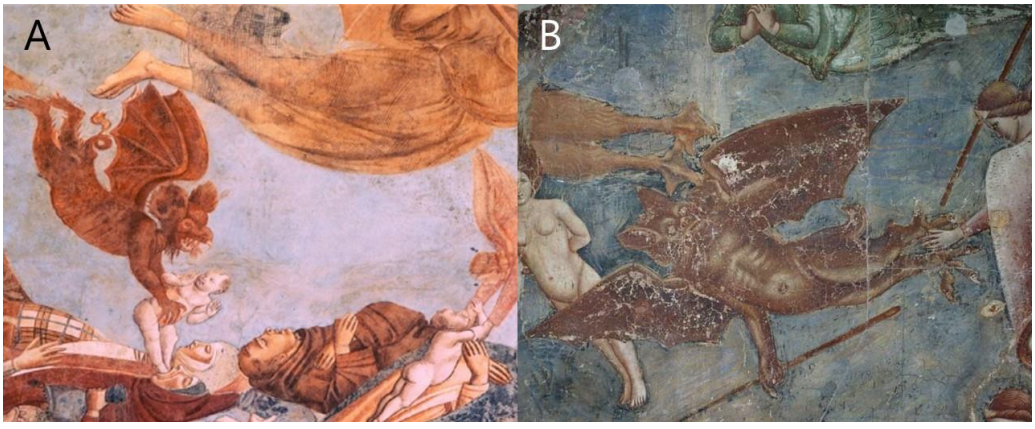


Figure 1. Buonamico Buffalmacco fresco entitled 'Triumph of Death' (1336-1341). Demons are represented as bat-like creatures. Sources: MindestensM-stock.adobe.com (A) and akg.images (B).

Global negative perceptions of bats in the culture and folklore, along with conflicts in the human-bat interface and the widespread sensationalist media about bats and zoonosis, represent significant boundaries to bat conservation strategies around the globe.

1.2. Taxonomy and morphology

Historically, bats have been classified into two suborders based on morphology and behavior. Bats with echolocation abilities were classified in the suborder Microchiroptera, while the rest were classified as Megachiroptera (which only comprised the family Pteropodidae). However, recent phylogenomic analyses have revealed a new classification (**Fig. 2**): suborders Yinpterochiroptera (comprised by the families Pteropodidade and superfamily Rhinolophoidea) and Yangochiroptera (the old Microchiroptera suborder excluding the superfamily Rhinolophoidea) (Lei & Dong, 2016; Teeling et al., 2002).

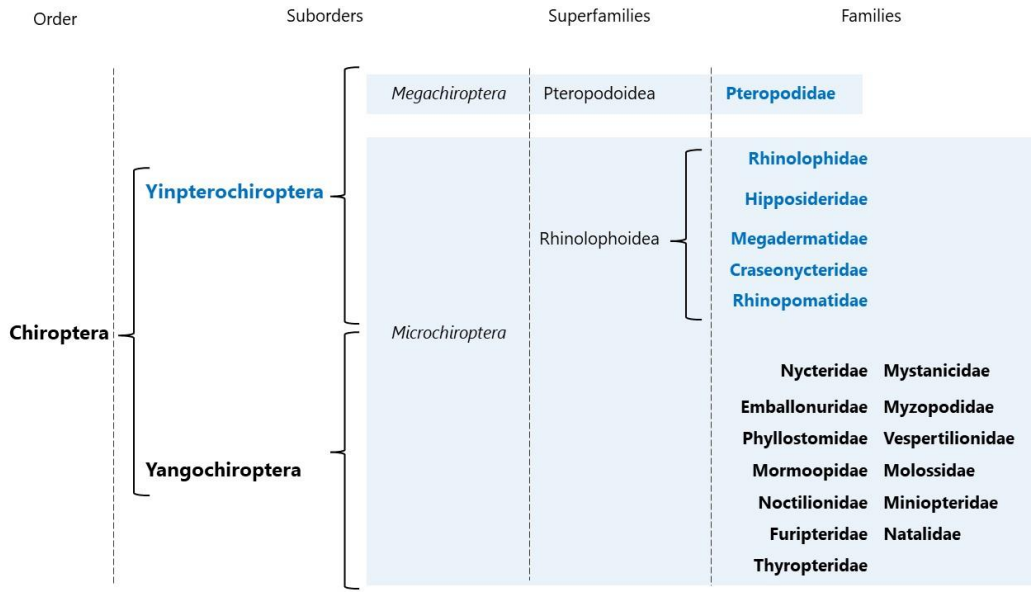


Figure 2. Subdivisions within the order Chiroptera. The old classification (suborders) is depicted in italics and embedded in light blue squares. The current classification is depicted in bold letters: Yinpterochiroptera (blue letters) and Yangochiroptera (black letters).

Morphological features vary strongly among bat species, with size being the most striking difference. Their range spans from just a few grams of body weight (e.g soprano pipistrelle) (**Fig. 3**) to a substantial body weight of 1.6 kg (e.g. Indian flying fox) (IUCN, 2023).

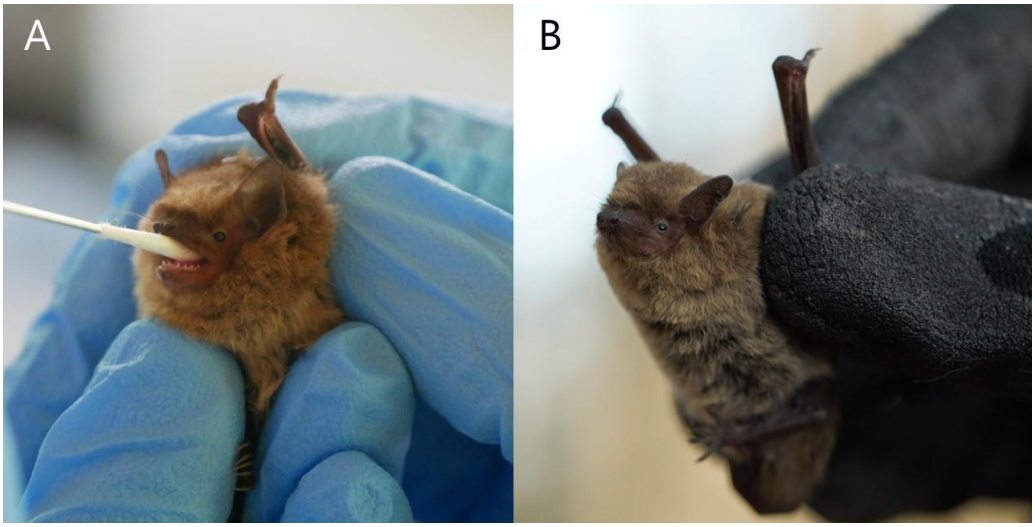


Figure 3. Soprano pipistrelle (*Pipistrellus pygmaeus*). See mini-swab and handler's fingers for bat size reference. **A**. Oral sampling with a mini cotton-tipped swab (deltalab©, Barcelona). Credit: Ignasi Marco (2019). **B**. Grip handling technique, useful for a closer examination of small-sized bats. Credit: Laura Torrent (2019).

Additionally, the order Chiroptera shows many different trophic adaptations and diversity: the majority of bat species (around 70%) prey on insects, less than 10% supplement their insectivorous diet with small vertebrates (referred to as 'carnivore' species), around 10% of species feed on fruits and nectar, and only three species feed exclusively on blood. These trophic adaptations have led to considerable variations in their morphology, including between species sharing similar dietary niches (**Fig. 4E and EF**). For instance, pteropodids (distributed in subtropical and tropical areas of southern and central Asia, Australia and Africa) feed only on fruits and nectar from flowers, playing an important role in seed dispersion and pollination (Mickleburgh et al., 1992). Some of these nectar-feeding pteropodids and also several phyllostomids (collectively known as American leaf-nosed bats, which are native to tropical and subtropical areas of the Americas) have developed protrusible tongues, some of them covered with long hair-like papillae, which may even exceed their total body length and that are used as a pumping mechanism for nectar (Tschapka et al., 2015). On the other side, the common vampire bat (*Desmodus rotundus*) shows a unique and exceptional dietary adaptation to blood consumption. They have a very well-developed olfactory system and the ability to sense infrared radiation to find their prey. Furthermore, to cope with the large amount of blood they consume every day, their stomachs withstand large distensions, and they are adapted to low carbohydrate and high iron content intake as compared to other carnivore bat species (Blumer et al., 2022).

1.2.1. Insectivorous bats

The majority of bat species worldwide are insectivorous, while some of these species may include small mammals, birds, small fish and amphibians as a supplementary part of their diet (Hutson et al., 2001). They are considerable small size, although some species present a long forearm of above 60 mm length, such as the Greater Noctule bat (*Nyctalus lasiopterus*) (**Fig. 4A**), the largest vespertilionid bat species present in Europe (Juste, 2007a). The second digit of insectivorous bats lacks a claw and runs very near to the third digit. The interfemoral membrane (uropatagium) is usually well developed across all the species (**Fig. 4B**), which also accompanies a relatively long tail that can end up free in a few species, such as the European free-tailed bat (*Tadarida teniotis*) (**Fig. 4C**) (Hutson et al., 2001).

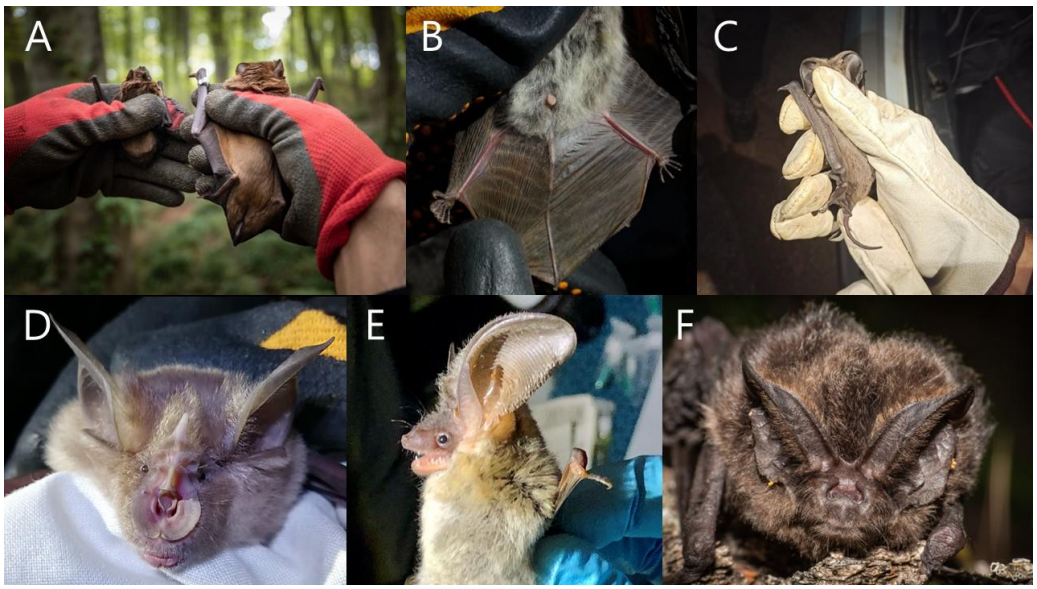


Figure 4. Morphological variation among some European insectivorous bats. **A.** Size comparison between *Nyctalus leisleri* (left) and *N. lasiopterus* (right). Both individuals were captured in La Fageda d'en Jordà, one of the few reported places in Catalonia where *N. lasiopterus* roosts and breeds. Credit: Xavier Florensa (2019). **B.** Well-developed interfemoral membrane (uropatagium) from a common long-eared bat (*Plecotus auritus*). **C.** Long and free tail from a European free-tailed bat (*Tadarida teniotis*). **D.** Closer look at the prominent noseleaves of a lesser horseshoe bat (*Rhinolophus hipposideos*). **E.** Long pinna and tragus of a common long-eared bat, which are linked to its ability to glean insects from surfaces by discriminating very fine details and receiving subtle insect movements. **F.** The short and broad ears, joined at the top of the forefront, give the western barbastelle (*Barbastella barbastellus*) a unique ear morphology among European insectivorous bats. Credit: Xavier Florensa (2019).

All insectivorous bats are nocturnal and orientate and hunt using ultrasonic sounds originated in the larynx and emitted through the nose or the mouth,

depending on the species. This mechanism is known as echolocation and involves ultrasonic echoes hearing and species-specific emission of ultrasonic vocalizations ranging from above 20 to over 200 kHz (Davies et al., 2013). Most nasal-emitting echolocators have a dermal overgrowth above the nostrils (commonly known as noseleaves) (**Fig. 4D**) or around the lips, which seems to have a role in shaping and steering the echoes (Vanderelst et al., 2010). Furthermore, they have also evolved several inner ear structural adaptations for detecting ultrasonic echoes, such as enlarged cochleae and more cochlear turns (Davies et al., 2013).

1.3. Why do bats matter?

Bats play an important ecological role in the proper functioning of natural and human-modified landscapes. On the one hand, they highly contribute to biodiversity with more than 1400 different species (IUCN, 2023). From a purely moral perspective, the conservation of nature and its biodiversity should be driven by the aim of making it accessible to future human generations. Biodiversity supports manifold functions and provides many services which are essential to life on earth, the so-called ecosystem services (Mengist et al., 2020). Proper stewardship of biodiversity is linked to an improvement of community's resilience, and the loss of its resources and services could trigger ecosystem destabilization and subsequent health and economic consequences. Furthermore, there is strong scientific evidence of the health benefits for humans derived from interacting with natural and biodiverse environments, helping in treatments for depression and anxiety, and improving children's mental and sensory development (World Health Organization et al., 2015).

Economic arguments linked to bat conservation strategies are gaining in popularity throughout low and high-income countries. Bats are often considered keystone species due to their large contribution to environmental stability by means of soil fertility and nutrient distribution, seed dispersal, pollination, and insect regulation (**Fig. 5**) (Voigt & Kingston, 2015).

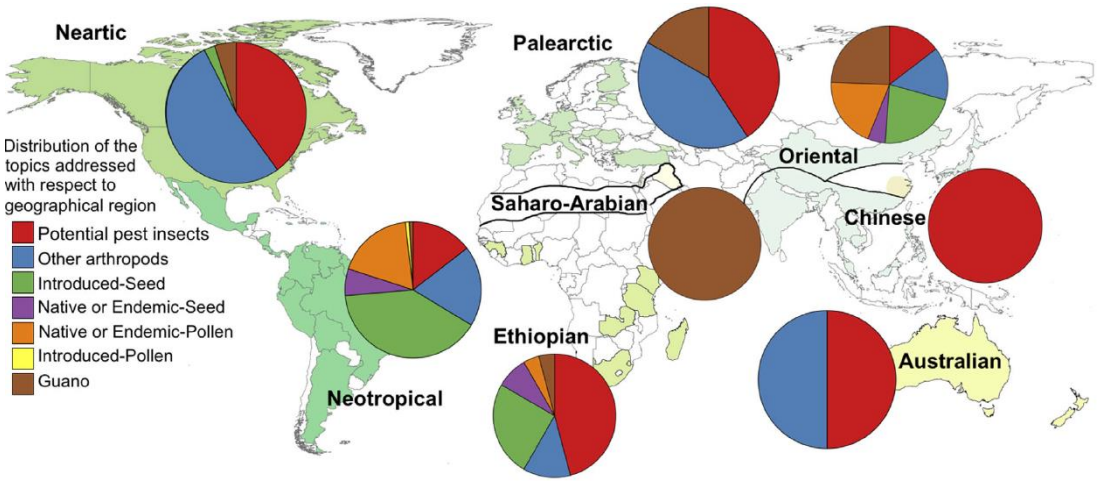


Figure 5. Studies assessing ecosystem services (see legend for details) provided by bats worldwide, depicted for each biogeographical region. Potential pest insects: bats acting as agricultural pest controllers. Other arthropods: bats consuming other arthropods different from agricultural pests. Introduced-Seed: bats acting as seed dispersers of introduced or exotic plants. Native or Endemic-Seed: bats acting as seed dispersers of native plants. Native or Endemic-Pollen: bats acting as pollinators of endemic plants. Introduced-Pollen: bats acting as pollinators of introduced or exotic plants. Guano: studies assessing bat guano as a natural fertilizer. Adaptation from (Ramirez-Francel et al. 2022).

Insectivorous bats are top-down regulators of several insect orders, some of which include agricultural pests, such as *Coleoptera*, *Diptera*, *Hemiptera*, *Isoptera* and *Lepidoptera*. In this sense, they contribute to significantly reduce human interventions for pest control, such as insecticide use, leading to substantial cost savings (Ramirez-Francel et al., 2022). This ecosystem service has been particularly demonstrated in Texas (USA), where it has been estimated that the Mexican free-tailed bat (*Tadarida brasiliensis*) has a value of $741,000 per year as a pest controller (Cleveland et al., 2006). Recently, several studies from Australia, Europe and Asia have highlighted the benefits of insectivorous bats in agricultural settings (Kolkert et al., 2020; Puig-Montserrat et al., 2015; Wanger et al., 2014). Contrastingly, studies in Africa are scarce and mainly focused on the presence of bats in crops, suggesting a potential role as pest-controlling agents (Noer et al., 2012; Weier et al., 2018). The direct linkage between their presence and their derived economic benefits still needs to be elucidated.

Additionally, the undigested remains of insects in guano compose a concentrated nutrient source of nitrogen, phosphates, potassium, sodium, magnesium, and calcium (Shahack-Gross et al., 2004). On one hand, bats are highly mobile and therefore, they facilitate long-distance nutrient transfer through their droppings. On the other hand, many species congregate in caves

and shelters, creating big guano piles underneath (Ramirez-Francel et al., 2022). Mining the guano from caves is a significant economic activity in Asia, where they use and sell it as a high-quality crop fertilizer while contributing to the local economy (Leelapaibul et al., 2005; Sothearen et al., 2014). In Texas, guano produced by large colonies of Mexican free-tailed bats (*Tadarida brasiliensis*) from the Bracken Cave is also extracted and commercialized as a natural fertilizer, and retail sales currently range from $2.86 to $12.10 per kg (Cleveland et al., 2006).

The paramount importance of fruit bats in long-distance seed dispersion and pollination is widely acknowledged, some of them being considered keystone species in isolated islands due to their unique role as seed dispersers (Voigt & Kingston, 2015). Particularly, the consumption of a wide variety of plant species (over 1,000) has been reported in Pteropodids worldwide, including flowers, nectar, pollen, leaves and fruits. Up to 29 pteropodid species have been confirmed to act as seed dispersers and 16 more species as pollinators (Aziz et al., 2021). Furthermore, many of these seed-dispersed and pollinated plant species are considered of economic importance, such as the *Agave* spp. plant in Mexico (Trejo-Salazar et al., 2016), the durian fruit in Polynesia (Sheherazade et al., 2019), and several timber species in Africa (Muscarella & Fleming, 2007).

At the other end, disservices provided by vampire bats (which exclusively feed on mammalian blood) are obvious, contributing to substantial economic losses due to rabies-related cattle deaths in Latin America (Voigt & Kingston, 2015). Nonetheless, the saliva of the common vampire bat (*Desmodus rotundus*) contains a highly potent plasminogen activator, known as DSPA, which represents a new and superior class of thrombolytic agent. This compound has gained considerable attention from the medical and scientific community due to its promising use as a stroke treatment (Reddrop et al., 2005).

Lastly, bats have been positioned as excellent bioindicators due to their sensitivity to changes in the environment and their long lifespan, which expose them to long-term environmental stressors and bioaccumulation of pollutants (Jones et al., 2009). Furthermore, the order Chiroptera has a wide geographic range and a rich trophic diversity, which enables a genuine representation of specific ecological contexts such as urban landscapes and its pollutants, pristine environments such as mature forests and riverine areas, and landscape changes for farming practices among others (Jones et al., 2009; Russo et al., 2021). Using

bats as bioindicators offers the potential for assessing the health of an ecosystem before it becomes functionally compromised, which in turn can both inform policymakers and strengthen bat conservation strategies.

2. Anthropogenic impacts on bat populations

The International Union for Conservation of Nature (IUCN) classifies over a third of bat species worldwide as threatened or data deficient (**Fig. 6**), which means that the assessment of their risk of extinction is inadequate (IUCN, 2023). In this sense, bats' ecology (e.g. roosting in inaccessible locations), biology (flying creatures and most of them small-sized) and fast decomposition of carcasses pose a serious challenge for both their monitoring and post-mortem investigation (Mühldorfer et al., 2011). Nonetheless, bat populations worldwide are believed to be in continuous decline because of myriad human activities such as habitat loss and fragmentation, deliberate persecution, and release of pollutants into the environment among others (Haysom et al., 2013; Ingersoll et al., 2013; Preble & Vincenot, 2021). Particularly in Europe, bat populations declines have been reported since the second half of the 20th century for the abovementioned reasons (Haysom et al., 2013). Surprisingly though, some species have undergone a significant population recovery, reflecting the impact of several national and European conservation strategies, mostly under the EUROBATS agreement (Hutson et al., 2015). However, a multidisciplinary approach -including infectious diseases investigation and large spatial and temporal monitoring- is still lacking but essential to understand the driving forces of bat populations declines.

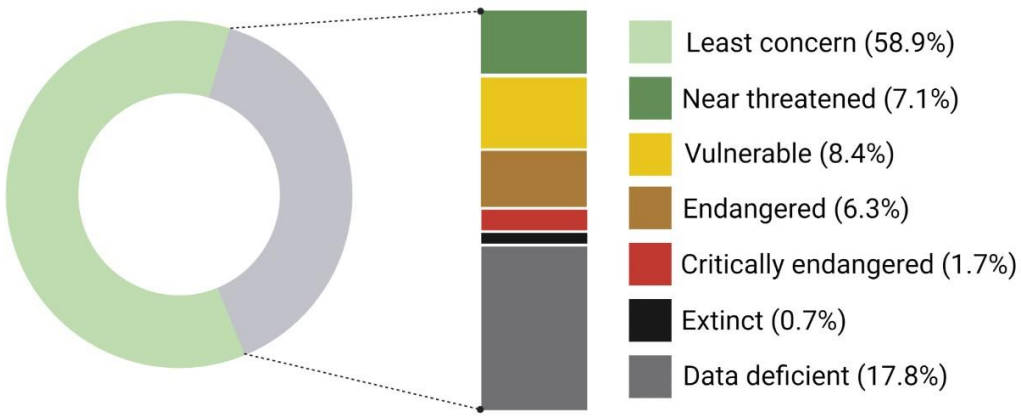


Figure 6. Red List Category of bat species worldwide. Almost half of the total species are threatened or data deficient (IUCN, 2023). Figure created with BioRender.com.

2.1. Chemical pollutants

Bats frequently forage in farmland. As a consequence, they are potentially exposed to pesticides through ingestion of contaminated prey and contact with water sources (Stahlschmidt et al., 2017). Most of the knowledge about the impacts of pesticides on bats is related to persistent organochlorine compounds, particularly to the use of dichlorodiphenyltrichloroethane (DDT) in North America (Bayat et al., 2014). Even several years after this pesticide use was banned (1972 in USA and 1986 in Europe), DDT residues have been detected in bat tissues worldwide (Eidels et al., 2007; Hernández et al., 1993; Luftl et al., 2005). Although high concentrations and direct exposure to pesticides can lead to lethal effects (Berny, 2007), chronic and sub-lethal exposure can lead to long-term disorders such as immunotoxicity, endocrine disruption and reproductive failure, among many others, which may impact bat populations survival (Bayat et al., 2014).

Little is known about the effects of sub-lethal pesticide exposure on bats and currently, there are no specific regulations to include bats in the European Union pesticide risk assessment, assuming that the scheme for birds and mammals also covers bats (European Food Safety Authority, 2009). However, a recent scientific statement published by the European Food Safety Authority (EFSA) has concluded that this assumption may be overlooking several characteristics of this taxonomic group and therefore, they may not be adequately covered by the current risk assessment scheme (Hernández-Jerez et al., 2019). The panel suggested including a focus on pesticide exposure through insect consumption and grooming, as well as dermal exposure, and bioaccumulation in milk and subsequent exposure in newborns.

Furthermore, high pesticide use in intensive agriculture has been correlated with declines in insect populations (van der Sluijs, 2020), which may have severe implications for bat populations' fitness and survival.

2.2. Persecution and consumption

As stated in a previous section (1.1.), bats are worldwide persecuted and killed for several reason, including misconceived fear by cultural beliefs, linkage with

zoonotic diseases, roosting in human settlements and feeding on crops (Frick et al., 2020, Lim et al., 2021).

In addition to persecution, 13% of bat species are severely threatened by bushmeat practices and traditional medicine, particularly across Southeast Asia and West and Central Africa (Frick et al., 2020; Mickleburgh et al., 2009; Wandera, 2018). Large frugivorous bats are the most affected species by bushmeat practices but also by illegal trade. Their vulnerability is mostly linked to their roost fidelity (caves and trees), which makes them usually visible and predictable targets (Mickleburgh et al., 2009). Nonetheless, the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) only includes the genus *Pteropus* (65 species), *Platyrrhinus* (1 species) and *Acerodon* (5 species) in its appendix for control and trade ban (CITES, 2020).

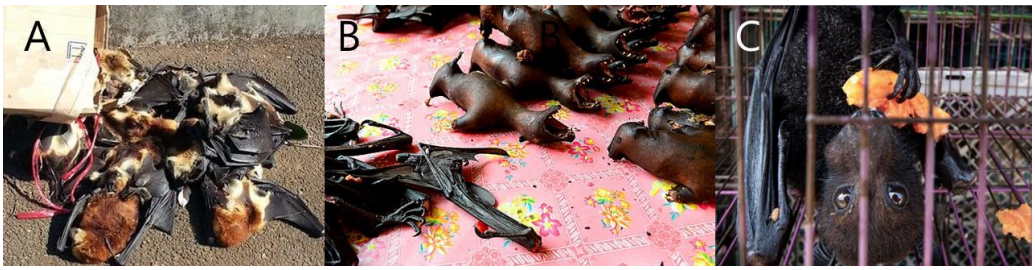


Figure 7. **A.** Mauritius flying foxes (*Pteropus niger*) culled as a part of the Mauritius Government approved strategy for controlling fruit crops damage in 2015. Credit: Zahirah Abdooraman (batcon.org; August 2023). **B.** Rosted carcasses of flying foxes (unknown spp.) for sale at a bushmeat market in the north of Suawesi, Indonesia (2020). Credit: Enrique Lopez-Tapia (naturepl.com; August 2023). **C.** Juvenile *Pteropus* spp. bat being sold as an exotic pet in a Chinese market. Credit: thetimes.co.uk; June 2020.

2.3. Climate change

Based on distribution models, climate change is recognized as a significant threat to global biodiversity, whose effects could reach comparable levels with those of historical land use by 2070 (Newbold, 2018). To date, not much is known about the specific effects of climate change on bat populations. However, climate disruptions may alter bats' food and shelter resources and their availability, having a huge impact on their eco-physiology, distribution, and survival (Frick et al., 2020).

Fragmentation and local extinction of animal populations may be one of the major impacts of climate change, due to animal movements to new suitable areas (Thomas et al., 2004). In this sense, bats could face more rapid range

expansion in response to climate change than most mammals due to their flight ability (Frick et al., 2020). However, bat populations from semi-arid and dry regions are likely to suffer a reduced species richness and range contraction. In a recent study of a future predicted scenario (2061-2080), 50% of Mediterranean Western Palearctic bat species and 53% of Western North American bat species showed to be at high risk of contracting their distribution due to reduced climatically suitable regions (Piccioli Cappelli et al., 2021).

Moreover, climate change may likely alter biotic interactions such as insect prey-predator dynamics and pollination through alterations in flowering phenology and shifts in geographical distribution (Frick et al., 2020). By modelling the future bat-plant pollination network (years 2050 and 2070) from central Mexico to the southern USA, the overlap between agave plants and one of their main bat pollinators, *Leptonycteris nivalis*, will be reduced at least by 75%. This may have severe implications for bat populations' survival, as well as for the reproduction and genetic variability of agave plants (Gómez-Ruiz & Lacher, 2019). Regarding insectivorous bats, the worst-case scenario is likely to be for aerial-hawking species (between 38 to 47 European and north-west African bat species), which are reliant on temporally and spatially variable insect prey sources (Sherwin et al., 2013).

Changes in temperature and precipitation are predicted to alter the health, fitness, and survival of bat populations worldwide, many of which have narrow thermodynamic tolerances. Reductions in reproductive output (Adams, 2010; Amorim et al., 2015) and heatwave-induced bat mass mortality events (Welbergen et al., 2008) have been related to climate change effects. Furthermore, climate change may be playing an important role in the emergence of infectious diseases and bat's response to these, such as the case of the white-nose syndrome from bat populations in North America. Conditions outside the hibernacula -where the infection occurs- may modulate the survival of infected bats by means of insect availability and energy expenditure from premature arousals (Flory et al., 2012).

Unfortunately, European bats are often neglected in climate change effect assessments, and strategies to monitor their response have not been set at a European level. In response to that, a COST Action (Climate change and bats: from science to conservation, CLIMBATS) was approved to assess the

responsiveness of insectivorous bat species from Europe to climate change, with the aim to inform future conservation strategies for bat species and those regions more at risk (COST Action CA18107, 2018, 2021). A recent delivery from this COST Action summarizes the current state of knowledge regarding climate change effects on bat species and identifies research gaps for future projects. The most reported responses to climate change were range shifting and changes in diversity patterns. Authors from this systematic review highlight the need for long-term studies and standardized study designs (Festa et al., 2023). Eventually, bats may likely be used as indicators of climate change effect, particularly because of their temperature sensitivity, which is crucial for physiological phases such as hibernation and reproduction (Haysom et al., 2013).

2.4. Habitat loss and encroachment

Habitat loss, mainly driven by landscape modifications for agriculture and urbanization, entails one of the main threats to biodiversity globally (Pimm & Raven, 2000). Degradation of natural ecosystems may lead to habitat fragmentation, which implies the division of a given habitat into smaller and isolated fragments, which sometimes may constitute an "ecological trap" in which the remaining population of certain species is unable to thrive (Haddad et al., 2015). Moreover, habitat loss and fragmentation can severely affect species interactions and the subsequent function of the ecosystem, by means of loss of keystone species or resources, and the reduction in overlapping between them (Fischer & Lindenmayer, 2007). For example, habitat loss by agricultural activities has caused a reduction in plant richness in several tropical ecosystems and with this, a reduction in fruit resources and impoverishment of bat species diversity (Hernández-Montero et al., 2015; Laurindo et al., 2019). Furthermore, logging activities and changes in the structure of the forest, such as the removal of standing dead trees for silvicultural or agricultural practices, strongly compromise bat diversity and richness worldwide (Frick et al., 2020).

Landscape modification typically results in human encroachment into natural areas, increasing related human-wildlife conflicts, which negatively affect both humans and animals (Mekonen, 2020) (**Fig. 8**). Humans have been using caves since the beginnings of history and the earliest direct evidence of cave-living ancestors dates back above 700.000 BP. Nowadays, humans rely on caves for shelter, burial, or storage facilities only in a few parts of the world, but caves are

still considered frequent destinations for recreational and touristic activities. It is estimated that roughly 40% of bat species worldwide strongly depend on subterranean cavities, such as caves and mines (Voigt & Kingston, 2015). Recreational activities may bring unintentional -and sometimes intentional- disturbance to roosting bats. Continuous visits, alterations of the cavity or acts of vandalism are widely regarded as significant causes of abandonment of the cavity or roost, premature arousals during hibernation and suspected derived mortality from that. Particularly young bats are very vulnerable to cave disturbance as they depend upon the energy transfer from the mother and the thermoregulatory benefits from the roost. Either a reduced number of bats frequenting the cave roost, or an increasing energy expenditure of females can be critical for the appropriate growth and survival of young individuals (Mühldorfer et al., 2011).



Figure 8. The outbreaks of Nipah virus illustrate the adverse effects of human encroachment into natural habitats on wildlife protection, domestic animals health and human's health and economy. **A.** Abandoned pig farm (Negeri Sembilan, Malaysa) exhibiting a noticeable proximity and encroachment into the forest, which creates a favorable environment for the contact between bats, pigs and humans. Credit: Sanjit Das (npr.org). **B.** More than 1 million pigs were culled to control the Nipah virus outbreak in 1999 in Malaysia, causing a substantial economic impact. Credit: Andy Wong (npr.org). **C.** The putative natural reservoirs for Nipah virus in Malaysia are *Pteropus hypomelanus* [left; credit: Pacel Kirillov (Flickr)] and *P. vampyrus* [right; credit: Cyril Ruoso (pixels.com)]. Thousands of bats, including some threatened species, have been culled in numerous areas where Nipah virus outbreaks have occurred, driven by fear.

The development of renewable energy sources has increased globally in response to concerning greenhouse gas emissions form fossil fuel and their impact on climate change. The use of wind turbines as an alternative energy source has been a major contributor to reducing these carbon emissions but has also been associated with important habitat fragmentation and severe impact on wild birds and bats (Allison et al., 2019). Unprecedented rates of bat mortality derived from wind turbines have been recorded worldwide, but particularly in Europe and the United States (Guest et al., 2022; Kunz et al., 2007; Rydell et al., 2010; Voigt & Kingston, 2015) (**Fig. 9**). Two main causes of bat fatalities from

wind energy infrastructures have been confirmed: direct collision and barotrauma. While direct collision has been evidenced with thermal infrared cameras (resulting in physical injuries such as broken wings, skull and column), barotrauma occurs without direct turbine contact. Histological examination of bat carcasses affected by barotrauma indicates that the drop in atmospheric pressure along the top of the blade of the turbine may severely damage the lungs and other organs (causing fatal hemorrhages) of bats that fly through this pressure change (Grodsky et al., 2011).

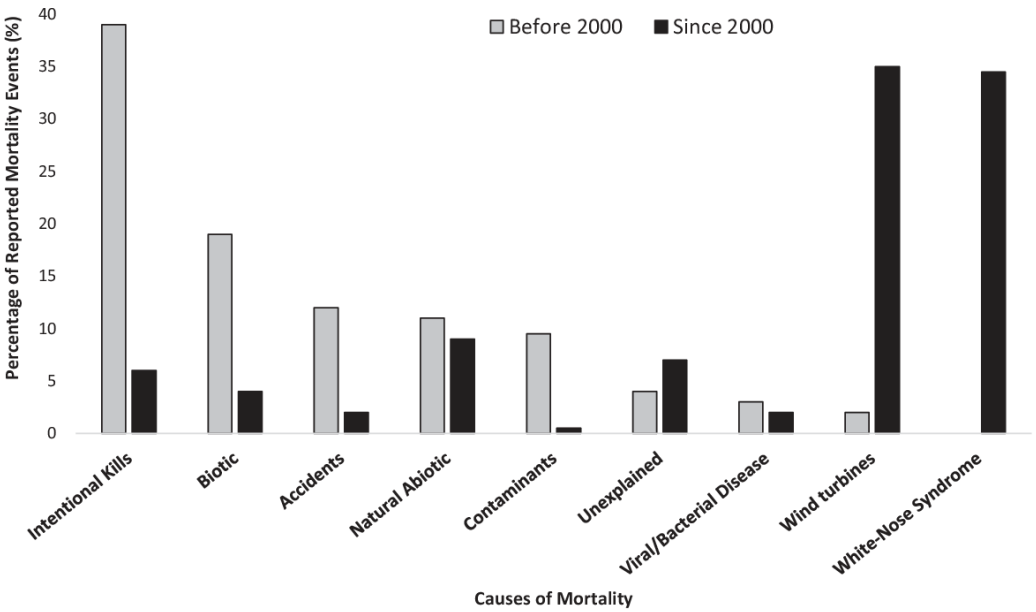


Figure 9. The percentages of reported multiple mortality events in bats, categorized by cause before and after 2000, show that wing turbines are the most significant cause, closely followed by white-nose syndrome (O'Shea et al., 2016).

Beyond the effects of physical infrastructures and ecosystem degradation, light and noise pollution from urban landscapes have received recent attention given their impact on bats' foraging efficiency (Frick et al., 2020). While some species, such as the common pipistrelle (*Pipistrellus pipistrellus*) and the soprano pipistrelle seem to be attracted by insects concentrated under light spots, other species such as horseshoe bats (*Rhinolophus* spp.) and mouse-eared bats (*Myotis* spp.) may display reduced foraging activity due to light aversion (Browning et al., 2021). Furthermore, artificial lighting may trigger changes in the circadian rhythm of bats and therefore, interfere with roosting emerge, hibernation and breeding timing (Stone et al., 2015).

As most insectivorous bats rely upon returning ultrasonic echoes of their calls to detect prey, orientate and communicate, traffic and different sorts of urban noise may interfere with their perception of the surroundings and therefore, have an impact on their fitness (Lim et al., 2021). Nonetheless, the impact of anthropogenic noise may vary across species, mostly depending upon their foraging ecology and echoes spectrum. Through passive acoustic surveys at gas wells with and without compressor noise, the Mexican free-tailed bat showed a significant decrease in their activity when noise was present, in comparison to other species such as the little brown bat (*Myotis lucifugus*) which didn't show differences between both scenarios (Bunkley et al., 2015). Beyond the echoes being masked and overlapped in noisy environments, noise by itself seems to frighten bats away. Luo et al. (2015) studied the foraging activity of Daubenton's bats (*Myotis daubentonii*) under traffic noise conditions. Although its echoes don't overlap with traffic noise, the total foraging flights under traffic sounds decreased in comparison with those under silence conditions (Luo et al., 2015). Overall, noise and light pollution seem to act as an aversive stimulus and reduce the foraging efficiency of echolocating bats.

2.5. Scientific practice

Ecological field and experimental research have generated a wealth of valuable information about bats, much of which informs conservation efforts (Feijó et al., 2019; Lim et al., 2021). However, ethical considerations may arise when such research disturbs, harms or endangers the individual, the species or the population of bats (Parris et al., 2010). Common practices in bat field research include roost disturbing, capturing, handling, sampling and photographing individuals amongst others. While the impact of some research activities on bat populations, such as disturbing hibernating bats or maternity roosts, have been reported and discussed among scientific communities (EUROBATS, 2006; Martin et al., 2000; Mitchell-Jones, 2004; Thomas et al., 2016), yet the scientific literature contains little explicit information of the impact of other activities such as professional photography, capture or sampling techniques on the welfare of bats.

Free-ranging bats are usually captured with mist-nets, hand-nets, harps (**Fig. 10A**) or directly captured by hand in bat-boxes. While correct training in handling and trap setting may prevent most of the damage, unintentional

trauma may arise during the extraction of bats from the net, or due to bats colliding with the net as it happens in bird netting (Spotswood et al., 2012) (**Fig. 10**). Furthermore, accidental predation of bats tangled in nets has also been reported (Serra-Gonçalves et al., 2017). Unintentional injuries derived from sampling activities, such as significant hemorrhages or blood loss after venipuncture (Eshar & Weinberg, 2010), may also be prevented with proper training but not completely avoided. These events are considered sporadic but still they should be considered in the risk assessment of the scientific activity. Furthermore, researchers should be trained on decision making in bat welfare when dealing with injured bats: releasing back into the wild, transferring the bat to a rehabilitation centre or performing authorized humanitarian euthanasia.

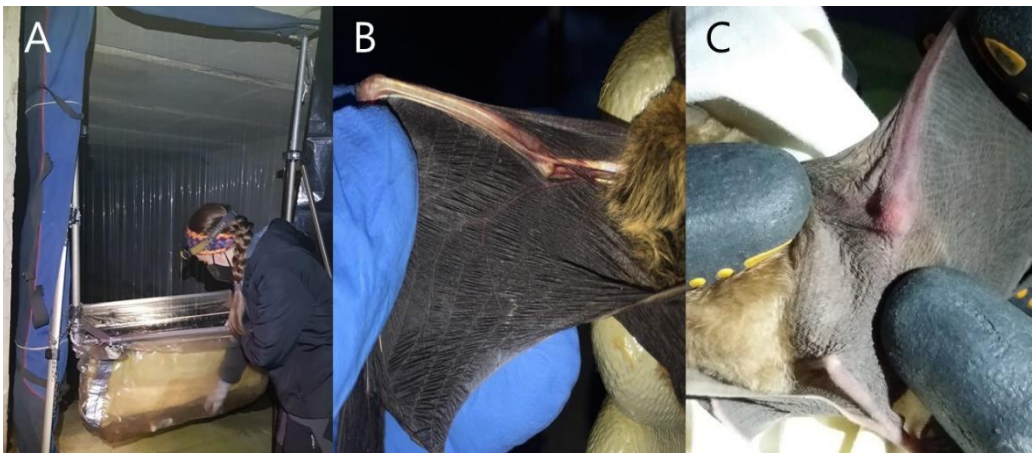


Figure 10. A. Harp trap set at the entrance of a tunnel. Bats collide with two roads of fine thread and slide down into a padded and slippery bag, which keeps them warm while preventing them from scaping (by flying or climbing). **B.** Haematoma in the inner elbow of a Kuh'l pipistrelle (*Pipistrellus kuhlii*) resulting from crashing into the threads of the harp. **C.** The elbow of a horseshoe bat collected from the harp trap bag appears swollen and erythematous, likely due to trauma from colliding into the harp trap.

The impact of photographing bats professionally is rarely addressed because of its assumed negligible effects on the welfare of bats and because of its expected educational benefit. However, photophobia and negative impacts of light pollution are well documented in nocturnal animals, including bat species (Spoelstra et al., 2017; Straka et al., 2020; Voigt & Kingston, 2015), and bat photography sessions often require multiple shoot trials with the use of flashguns (Tuttle, 2019). Given the potential variance in species-specific susceptibility to photic damage, prudent care in employing flash during nocturnal photography is imperative.

Whereas incidents from the previous activities are usually reported as single events, scientific bat banding activities and roost disturbing have been shown to negatively impact the health and fitness of large numbers of bats and even linked to mortality events (Baker et al., 2001; Dietz et al., 2006; Ellison, 2008; Hutterer et al., 2005; Jensen & Baagoe, 1984; Martin et al., 2000; Veith, 1996; Zambelli et al., 2009), clearly representing a threat for bat populations. The ethics of acceptable practice for bat research are still poorly defined and call for an integrative approach of veterinary medicine, conservation biology and ethical policy.

2.6. Diseases

Similar to what happens with the anthropogenic-mediated introduction of nonnative fauna and flora into new geographic areas, the introduction of pathogens into new ecosystems and to naïve hosts poses a substantial threat to biodiversity (Cunningham et al., 2003; Daszak et al., 2000). This mechanism of invasion, termed 'pathogen pollution', has been involved in numerous events of disease emergence in wildlife populations and severe population declines (Cunningham et al., 2003; Daszak et al., 2000). One of the examples is the death of millions of cave-roosting bats in North America since 2006 following the infection with *Pseudogymnoascus destructans* (the causative agent of white-nose syndrome, WNS) (**Fig. 11A**), which entails the second most prevalent cause of mass mortality event in bats during the last decade (**Fig. 9**) (O'Shea et al., 2014). This fungus has been found in bat species from Europe, Asia and North America, although it does not affect all species equally. Bats from Europe and Asia exhibit higher tolerance to the infection and disease than North American bat species, nine of which have shown to be severely affected (*Myotis lucifugus, M. grisescens, M. septentrionalis, M. sodalis, M. leibii, M. austroriparius, M. velifer, Eptesicus fuscus* and *Perimyotis subflavus*) (Gómez-Rodríguez et al., 2022). Scientific evidence suggests that the fungus is native to Europe and was introduced to naïve bat populations from North America through recreational cave exploration and/or research activities in Howe Cave (USA) in 2006. Human clothing and equipment act as fomites for the movement of *P. destructans*, but infected bats' movement between roosts may pose a risk for the dispersal of the fungus too (Ballmann et al., 2017). Additionally, an infected hibernaculum (cave) may serve as a reservoir of WNS for up to two years, under certain environmental conditions (Reynolds & Barton, 2013). Currently, white-nose syndrome is increasingly spreading its geographic area (as of June 2024, 40 states and eight Canadian provinces have

been affected) and affecting species of vespertilionid North American bats at unprecedented rates (**Fig. 11B**).

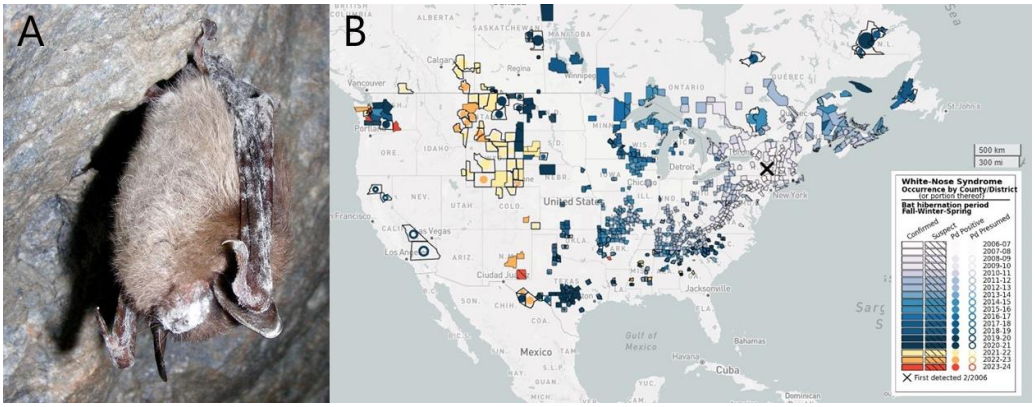


Figure 11. **A.** A little brown bat (*Myotis lucifugus*) with white-nose syndrome. A white fuzz can be seen on the bat's muzzle, ears and wings. Credit: Marvin Moriarty. **B.** Occurrence of white-nose syndrome or *Pseudogymnoascus destructans* (*Pd*) in North America, as of June 2024. The black cross indicates the first detection of the disease/fungus in 2006, whereas red polygons indicate the most recent outbreaks or detection of *Pd*. Credit: U.S. Fish and Wildlife Service (2024).

The emergence of diseases in wildlife poses a challenge to conservation due to their rapid spread (especially among gregarious species such as bats) and their occasionally high mortality rate (Daszak et al., 2000). However, the chiropteran immune system significantly differs from those of other mammal species, conferring them some ability to cope with pathogens without becoming ill (Hayman et al., 2013). Their increased metabolism and consequent increase in body temperature during flight may have played a role in bat's indirect evolved mechanisms to control viral replication and promote a high viral diversity and high virulent viruses (O'Shea et al., 2014). For this reason, bat research has been historically focused on bat-borne diseases affecting humans and domestic animals, while studies on diseases affecting bats or impacting the health of bat populations have received significantly less attention. Owing to several aspects of their evolution, ecology and biology, bats are implicated as hosts of many zoonotic (and potentially zoonotic) pathogens, such as rabies (Han et al., 2015). Furthermore, several highly lethal emerging and re-emerging zoonoses from the last decades are known to have spillover from bats into humans, including Ebola, Nipah, MERS (Middle East Respiratory Syndrome) and SARS (Severe Acute Respiratory Syndrome) (Khan et al., 2022). The recent pandemic caused by SARS-CoV-2 has taken center stage and with that, bats as reservoirs for emerging viruses. Although bats have been suggested as the original host of SARS-CoV-2

precursor, this hypothesis remains unproved (Andersen et al., 2020). Furthermore, the low binding affinity of SARS-CoV-2 virus for bat ACE2 (angiotensin converting enzyme 2), the main receptor for SARS-CoV-2 spike protein, strongly supports the need for a long-term intermediate host to allow it to adapt to humans (Piplani et al., 2021).

While several eco-biological and evolutive features may enable bats to carry a diversity of pathogens (particularly viruses) (Van Brussel & Holmes, 2022), viral spillovers are driven by multiple and complex ecological processes that increase the opportunity for pathogens to be transmitted between bats and intermediary hosts (livestock and wildlife) or directly humans (Daszak et al., 2000). Enabling conditions for bat-viruses spillover include the following conditions to be connected in time and space: bats must be present, infected and shedding the pathogen; the virus must survive in the environment for the recipient host to get in contact with (if indirect contact); and the recipient host must be susceptible to its infection and be exposed to enough viral particles for an infection to get established (Plowright et al., 2014). Therefore, disease emergence depends on multifactorial drivers and is set by the complex relationships between the hosts and the environment (**Fig. 12**) (Daszak et al., 2000). For instance, changes in land-use and climate change effect may promote the emergence of bat-associated infections in humans and livestock, supported by an increase in encroachment on bat habitats (Eby et al., 2023).

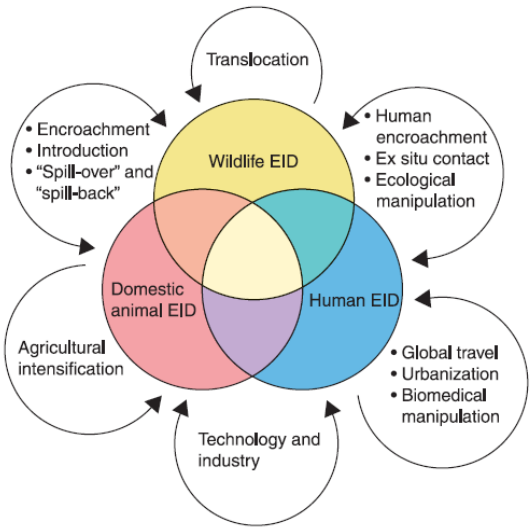


Figure 12. Host-pathogen interactions in the human, domestic and wildlife interface and drivers of disease emergence, taken from Daszak et al. (2000).

3. The microbiome

Microbiome research has become a topic of great scientific interest in the last decades. However, there are many microbiome definitions that have been used since the 20th century, as a consequence of the large variety of perspectives applied to the term. In response to this, Berg et al. (2020) published an article based on the outcomes of the MicrobiomeSupport project workshop, which took place in March 2019 in Austria, with the aim to establish international research standards and a consensual definition of the term microbiome.

Currently, the most accepted definition is an adaptation from the one postulated by Lederberg and collegues in 2001 (Prescott, 2017). The term microbiome describes a community of microorganisms (commensal, symbiotic, and pathogenic) within a defined environment or body space, and their "theater of activity" (**Fig. 13**).

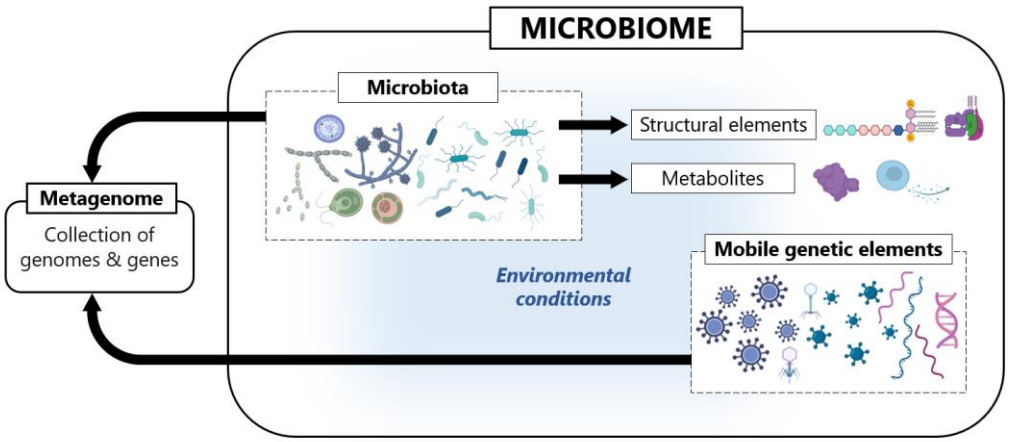


Figure 13. Schematic representation of the microbiome composition and metagenome. The microbiota is composed by a community of microorganisms (bacteria, archaea, fungi, algae, and small protists). Structural elements refer to polysaccharides, proteins, peptides and lipids. Metabolites refer to toxins, organic, inorganic and signaling molecules. Mobile genetic elements refer to viruses, phages and extracellular DNA. The collection of genomes and genes from the community of microorganisms (microbiota) and the mobile genetic elements is known as metagenome. Figure created with BioRender.com.

The community of microorganisms is referred as the microbiota, and comprises all living members of the microbiome, including bacteria, fungi, algae, archaea and small protists. The microbiome also includes mobile genetic elements such as viruses, plasmids, prions or phages in the defined environment, but they are

not included in the microbiota compartment as they are not considered living organisms. An integral ecological view of the microbiome contemplates the so called "theater of activity", which refers to a whole spectrum of molecules produced by the microbiota, such as toxins or signaling molecules, as well as their structural elements such as polysaccharides, proteins and nucleic acids. On the other hand, the microbiome should not be confused with the metagenome, which refers to the combination of genomes and genes from the microbiota and the mobile genetic elements of a given microbiome (Berg et al., 2020). Other terminology frequently used in microbiome research can be found in **Table 1**.

Table 1. Terminology frequently used in microbiome research.

Terminology	Definition	References
Epigenome	Collection of all the chemical modifications to DNA and histone proteins that regulate the expression of genes within the genome, occurring without intrinsic changes in the primary DNA sequence (e.g. DNA methylation).	(Morgensztern et al., 2018)
Epigenetics	Study of the epigenetic modifications that occur in single genes or sets of genes.	(Sharma et al., 2020)
Epigenomics	Study of genome-wide profiles of epigenetic modifications and their effects.	(Kato & Natarajan, 2019)
Genomics	Systematic study of an organism's whole genomes.	(Manzoni et al., 2018)
Metabolome	Complete set of all metabolites present in any given strain, cell or tissue at a given time.	(Marchesi & Ravel, 2015)
Metabolomics	Study of the analytical approaches used to determine the metabolite profile in any given strain, cell or tissue at a given time under specified conditions.	(Marchesi & Ravel, 2015)
Metagenomics	Characterization of the pool of genes from members of a given microbiota.	(Lanaspa et al., 2017)
Metaproteomics	Study of the set of proteins being expressed in a specific cell or tissue and present in the environment at a given time under specified conditions.	(Marchesi & Ravel, 2015)
Mycobiome	Collection of the entire genomic content of fungi in a particular environment or body space.	(Lanaspa et al., 2017)
Proteome	Collection of the entire set of proteins in a given cell, body space or environment, at a given time.	(Barnett et al., 2015)
Transcriptome	Set of messenger RNA (mRNA) and noncoding RNA (ncRNA) transcripts expressed by a group of genes. Characterization of the transcriptome is usually done through mRNA conversion into complementary DNA (cDNA) and subsequent sequencing.	(Morgensztern et al., 2018)
Transcriptomics	Study of the transcriptome produced by a given genome at a given time under specified conditions.	(Manzoni et al., 2018)
Virome	Collection of the entire genomic content of viruses in a particular environment or body space.	(Lanaspa et al., 2017)
Viromics	Study of the virome present in a given environment o body space. Also dubbed viral metagenomics.	(Ramamurthy et al., 2017)

3.1. The microbiome in health and disease

A significant number of studies on the human microbiome suggest that the microbiota and its 'theater of activity' may be playing a crucial role in health and disease (Turnbaugh et al., 2007). Bacterial communities colonize the host shortly after birth and continue to develop progressively during the growing period until they reach stable host-bacterial beneficial relationships, which ensures the proper functioning of the host's biological activities. In fact, the microbiome affects the host physiology to a great extent by means of increasing the energy extraction from food or building physical barriers to foreign pathogens amongst many other pathways (Wang et al., 2017).

Within a 'healthy' host, specific and relatively stable composition of the different microbiomes contributes to its homeostasis (Peixoto et al., 2021). Furthermore, optimized resources' specializing from a species-rich microbiome promotes a more efficient functioning and enhances resilience, which means the ability to adapt and cope with changing environments (Bäckhed et al., 2012; Lozupone et al., 2012). Perturbations of these balanced microbiomes can potentially lead to a state of dysbiosis, in which the microbiome community modifies its metabolic state and usually results in a decreased bacterial diversity. In turn, these changes eventually may give rise to pathogen invasion (Bass et al., 2019). Transition from a healthy microbiome to a dysbiosis state can be triggered by many factors, including but not limited to host resistance and immunity, antimicrobial and other drugs use, reproductive status, and environmental stressors (Bass et al., 2019).

Most of the current knowledge on microbiome in health and disease is related to the human gut (Turnbaugh et al., 2007). However, other body spaces and animal models are gaining attention in the last decade as a response to alarming declines in the abundance of some species or taxonomic orders that may be suffering from imbalances in their microbiomes. For instance, the skin is considered an important barrier between the host and the environment and is comprised of a highly diverse microbial community (Peixoto et al., 2021). Amphibian skin studies suggest that the natural microbiome composition of some species may contain molecules (antimicrobial peptides) that can prevent or retard the growth of the fatal pathogenic fungus *Batrachochytrium dendrobatidis* (Loudon et al., 2020). Furthermore, antifungal bacterial species,

such as *Janthinobacterium lividum*, have shown to prevent morbidity and mortality derived from *B. dendrobatidis* in several amphibian species under experimental conditions (Harris et al., 2009). The recent emergence of other fatal skin fungal disease in bats from North America, the white nose syndrome (WNS), highlights the important role of skin microbiome on health. Susceptibility to *P. destructans* invasion varies by host species. On one hand, the skin fungal assemblages on WNS-susceptible bat species seem to have lower microbial diversity and abundance than WNS-resistant species (Vanderwolf et al., 2021). Furthermore, WNS-surviving bats may have a shift in their skin microbiota, which may eventually be enriched with specific taxa, recognized for their antifungal activity or even for their ability to inhibit *P. destructans* (Lemieux-Labonté et al., 2017).

3.2. The gut and fecal bacterial microbiome

Extensive research on human microbiomes estimates that about half the cell population in a human body is indeed represented by microbial cells. Particularly, the gut microbiota comprises the largest and richest of these microbial communities (Leviatan & Segal, 2020). Indeed, combined genomes of these gut microbial communities may outnumber the host genetic material by two orders of magnitude (Gill et al., 2006). Most gut microbial species reside in the lumen rather than in the epithelium, and the vast majority of them are found in the colon. Whereas revised estimates of total human cells accounts for 3.0×10^{13}, the cecum concentrates up to 10^{8} bacteria/g and stool samples may concentrate over 10^{11} bacteria/g (Sender et al., 2016). As fecal samples are richer in composition and easier to collect, most of the current studies have focused on the fecal microbiome.

Owing to the large representation of intestinal microbial communities in the host, their metabolic and biochemical activities play an important role in the physiology of the host, including but not limited to host immune defense (Belkaid & Hand, 2014), brain function (Rogers et al., 2016) and hormones secretion (Martin et al., 2019). For example, in the last decades, the gut microbiota has been a focal point of study in metabolic and inflammatory diseases research. Alterations in the gut microbiota composition and its metabolic activity -known as dysbiosis- has been associated with an increasing

incidence of cancer, liver diseases, IBD, obesity, Crohn's disease, cardiovascular diseases, and Type 2 Diabetes among others (Turnbaugh et al., 2007).

Gut microbes also comprise a crucial reservoir of metabolic capabilities otherwise inaccessible for the host. These include the conversion of specific nutrient sources into readily absorbable metabolites, the synthesis of vitamins and the metabolism of xenobiotics amongst others. For example, gastrointestinal microbes from ruminants and marsupial herbivores are essential for food conversion and nutrient assimilation, as well as for detoxification of specific plants included in their diet (Peixoto et al., 2021). Furthermore, microbes have the ability to alter the expression of the host genes encoding antimicrobial compounds which may decrease the host susceptibility to enteropathogens colonization (Turnbaugh et al., 2007).

Many complex factors can influence the host's microbiome composition, including age (Koenig et al., 2011), host genetics (Goodrich et al., 2014), diet (Ley et al., 2008; Muegge, 2013), geography (Yatsunenko et al., 2012), urbanization (Winglee et al., 2017; Zuo et al., 2018), microbial-derived metabolites (Agus et al., 2021), and the use of antibiotics (Jernberg et al., 2007). Among these, diet is believed to be a major driver of the gut's microbiome composition, able to induce acute changes within 24-48 hours of a dietary intervention (Sonnenburg & Bäckhed, 2016). However, the ability of the microbiome to rapidly respond to different macronutrients intake is shaped and maintained by a combination of the above-mentioned extrinsic and intrinsic factors. For instance, separating seasonal environmental changes from the associated seasonal changes in diet may be very difficult to assess (Leeming et al., 2019).

Although the composition and function of healthy people's gut microbiota have not been defined at taxonomic resolution, overall, high microbial diversity and richness, and stable microbiome cores are associated to healthy gut microbial communities (The Human Microbiome Project Consortium, 2012). According to studies on Inflammatory Bowel Disease (IBD), chronically low microbiota richness is associated with a pro-inflammatory host immune system, low microbial fitness, and consequent reduction in resistance to pathogens colonization (Contijoch et al., 2019). However, the assumption of a healthy microbiome in animal models entails the need for species-specific research, as microbial and microbiome

compositions are unique to taxonomic orders and even individuals (Fan & Pedersen, 2021).

3.3. Current knowledge of bats' fecal microbiome

Over the past decade, there has been an increased interest in the fecal microbiome of bats, with a growing body of research seeking to understand their diversity and the ecological roles of their microbial communities in different bat species. While the dominant bacterial phylum in mammals' microbiome is *Bacteriodetes*, the dominant phylum in bats is represented by *Proteobacteria* and followed by *Firmicutes*, *Actinobacteria* and a relatively poor abundance of *Bacteriodetes* (Dong-Lei et al., 2020). In this sense, bats host gut's microbiomes resemble more to those of birds than other mammals, suggesting a convergent physiological adaptation to flight (Song et al., 2020).

Additional observations have revealed that the gut microbiome of bats is highly diverse and variable, showing significant differences between species (Gerbáčová et al., 2020; Vengust et al., 2018), development and reproductive state (Li et al., 2021; Philips et al., 2012; Yin et al., 2020), geographical distances and habitats (Alpízar et al., 2021; Gaona et al., 2020; Víquez-R et al., 2021), seasons (G. Xiao et al., 2019), and dietary preferences or shifts among others (Aizpurua et al., 2021; Alpízar et al., 2021; Banskar et al., 2016; Galicia et al., 2014; Gong et al., 2021; Ingala et al., 2019; Li et al., 2018).

The role of bat-associated microbiota in zoonotic disease transmission has also been a subject of recent interest. Several studies have investigated the presence of bacteria with zoonotic potential in bat's feces or guano (Banskar et al., 2016; Gerbáčová et al., 2020; Núñez-Montero et al., 2021; Saputra et al., 2018; Veikkolainen et al., 2014; Wolkers-Rooijackers et al., 2018). Other microbiome studies not oriented to zoonoses have also identified potential pathogenic bacteria in bat's feces (Dietrich et al., 2017; Galicia et al., 2014; Vengust et al., 2018), highlighting the complex nature of the bat's microbiome. However, the relevance of these findings regarding public health concerns remains a challenge. Difficulties associated with interpreting all these results include the challenge of achieving species-level differentiation within the detected bacteria (particularly enteropathogenic bacteria such as *Clostridium* or *Escherichia* spp.) and the unlikelihood of human exposure to many of these pathogens (e.g. arthropod-borne pathogens such as *Rickettsia*) (Vengust et al., 2018). Therefore, caution must be exercised when drawing conclusions. Moreover, despite decades of research on bat-associated bacteria have revealed that bacterial

pathogens are widely present in bats, our understanding of their pathogenic effects and transmission pathways on bat species is very limited (Szentivanyi et al., 2023).

Since bats harbor several RNA viruses of public and domestic animal health concern, they represent an optimal model for studying pathogen-microbiome interactions. Accordingly, several studies have investigated the potential of the bat's microbiome in antiviral response (Liu et al., 2022; Luo et al., 2021; Popov et al., 2021; Wasimuddin et al., 2018), and gut microbiome and bat's immune responses (e.g. Major Histocompatibility Complex) to viral infections ((Fleischer et al., 2022). For instance, Liu et al. (2022) demonstrated that mice with transplanted gut microbiota from wild bats decreased their inflammatory response to H1N1 infection and increased their survival. These studies may expand the scope of antiviral response and provide a new paradigm for the prevention of emerging infectious diseases.

Conversely, limited research has investigated the influence of anthropogenic activities, such as pollution, deforestation, habitat fragmentation or urbanization on the composition of bat's fecal microbiome. The study of Ingala et al. (2019) analyzed the consequences of land-use change on the microbiota composition of free-ranging common vampire bats (*Desmodus rotundus*). Their results showed that forest fragmentation and consequent diet homogenization led to shifts in some core bacteria associated with innate immune function, highlighting the potential negative impact of habitat loss on bat's immunity. More recently, Alpízar et al. (2021) revealed that intensive management and use of pesticides in banana plantations simplified the gut's microbiota (lower microbial diversity) of nectar-feeding bats *Glossophaga soricine* in comparison to bats of the same species feeding in organic banana plantations, suggesting that intensive management practices in agriculture promote less diverse and potentially dysbiotic microbiomes. The interpretation of the consequences of microbiome diversity dilution and composition on bat's health may not be so straightforward since certain taxa may have positive or negative effects depending on the context. For instance, *Neoromicia nana* bats foraging at wastewater treatment sites (contaminated with pollutants, pathogens and influents from domestic, agriculture and industrial sources) exhibited larger gut microbial diversity and specific taxa with pollutant-removal properties than those foraging outside these areas (Mehl et al., 2021). Additionally, as more studies are conducted, the adaptable and dynamic nature of the gut's microbiome is becoming increasingly evident. Among others, the structure of the fecal microbiome of bats seems to rapidly converge in captivity regardless of their social history, environmental or

geographic origin (Edenborough et al., 2020; Y. Xiao et al., 2019; Yarlagadda et al., 2021).

While recent advances in sequencing technologies and bioinformatics have enabled to investigate the gut's microbiome of bats in greater detail, studies with higher resolution methods and larger sample sizes are needed to deepen our understanding of the functional roles of bat microbiomes, the presence of bacterial pathogens (at species and strain levels) and their implications for bats' health, disease transmission and ecological interactions.

4. Methods of microbiome analysis

The study of the microbiome has seen a rapid expansion due to the development of culture-independent methods, and particularly after the emergence of next-generation sequencing (NGS). NGS methods have changed the nature of microbiome research as they enable the identification of uncultured species and the determination of low-abundance species and others previously unknown (Cénit et al., 2014). These advances provide a better qualitative and quantitative picture of microbiome communities.

4.1. Historical evolution of sequencing technologies

Since the beginnings of DNA sequencing in 1977 with the development of the 'dideoxy chain-termination' method by Fred Sanger (Sanger et al., 1977), NGS technologies have significatively been enhanced in terms of data output and efficiency. Fred Sanger based his method on the natural process of DNA replication (sequencing by synthesis, SBS) by creating radioactively labeled DNA strands complementary to the template strand. This method, known as 'Sanger-sequencing' or 'first-generation sequencing', required a long process, including DNA preparation, gel (later capillary) electrophoresis and interpretation of results, which first was performed manually by reading base per base and entering them into the computer (Hu et al., 2021; Sanger et al., 1977). About three decades later, new and significantly improved sequencing methods were presented under the name of NGS or high-throughput sequencing (HTS) technologies. Whereas both terms are often used interchangeably, NGS encompasses several DNA sequencing technologies, and HTS refers to the specific capability of generating fast and large volumes of sequencing data using these NGS platforms.

Now dubbed as 'second-generation sequencing', the first NGS platform, produced by 454 Life Sciences and later purchased by Roche (already discontinued), used the pyrosequencing method. This consisted of a two-enzyme process where ATP sulfurylase converted pyrophosphate into ATP, used later as the substrate for luciferase and therefore, producing light proportional to the amount of pyrophosphate. In this sense, the sequence was inferred by measuring pyrophosphate production by each nucleotide (Heather & Chain, 2016). This new sequencing technology allowed for massively parallel

sequencing of many independent DNA fragments, which significantly increased the data output by more than three orders of magnitude (Tucker et al., 2009). Sanger and pyrophosphate sequencing methods are both included in the category of 'sequence-by-synthesis (SBS)', as they require the DNA polymerase for output visualizing. However, pyrophosphate sequencing allowed for the complete sequencing of a single human's genome much faster than Craig Venter's team using Sanger sequencing (Heather & Chain, 2016).

Following the success of the 454 sequencer (Life Sciences), several parallel sequencing technologies appeared, being the Solexa method the major player. The latter, acquired by Illumina, also followed a SBS method, but sequencing was based on a technique known as 'bridge amplification' of DNA molecules. Illumina technology used fluorescent 'reversible-terminator' dNTPs and was initially only capable of producing short reads (up to 35 bp long) using the standard Genome Analyzer (GAIIx). Later, this was replaced first by the HiSeq (greater length read and depth) and then MiSeq, which had lower throughput but longer read lengths and faster turnaround (Heather & Chain, 2016). Several other sequencing methodologies were developed (and others also discontinued) in the early years of second-generation sequencing, such as Ion Torrent system (sold by Thermo-Fischer) (**Table 2**). The Ion Torrent platform differed from the latter in terms of using neither fluorescence nor luminescence but using the difference of pH caused by the release of protons during DNA polymerization. This method was made possible by the development of complementary metal-oxide semiconductor (CMOS) chips (microprocessor chips) and has been one of the most successful sequencing platforms in recent years (Heather & Chain, 2016).

The development of third-generation sequencing methods overcame some limitations of the second-generation by sequencing a single molecule of DNA (single-molecule sequencing, SMS) without the need for amplification. This reduced the sequencing error rate and produced long reads with less run time (Pervez et al., 2022). Furthermore, third-generation sequencing methods had the advantage of building better consensus accuracy for identifying rare variants and required a smaller amount of sample, gaining popularity in clinical diagnostics. The most notorious third-generation sequencing approach is Single-Molecule Real-Time Sequencing (SMRT) by Pacific BioSciences (PacBio), which is included in the category of SBS methods and uses the same fluorescent labelling as most second-generation sequencing methods (Kchouk et al., 2017).

Table 2. Comparative of the four most used next-generation sequencing (NGS) platforms. Adaptation from Hu et al. (2021). The quality score represents the error rate of the platform: reaching a Q30 quality score virtually represents that all the reads are perfect, without errors or ambiguities (ie. the accuracy is >99.9%). Q30 represents a benchmark for quality in NGS. *Since 2021, MinION users can achieve >99% raw read accuracy using the kit 12 chemistry and R10 flowcell. SBS: sequencing-by-synthesis; SMS: single-molecule sequencing.

Company	**Illumina**	**Thermofisher**	**Pacific Biosciences**	**Oxford Nanopore**
Platforms	iSeq, Minseq, MiSeg, NextSeq550, nEXTsEQ 1000&2000, NovaSeq 6000, MiSeqDX, NextSeq550 Dx	GeneStudio S5, Genexus, Ion PGM-Dx	Sequel, Sequel II, Sequel IIe	Flonge, MinION, GridION, PromethION
Method	SBS	SBS	SM	SM
Read length	Short-read	Short-read	Long-read	Long-read
Detection	Fluorescent	Fluorescent	Fluorescent	Electric conductivity
Max. read length (bases)	2x150bp - 2x 300 bp	200bp - 600 bp	300kb	>4Mb
Output/flow cell	1 - 3000Gb	1 - 24Gb	75 - 600Gb	1 - 400Gb
Quality score/Accuracy	Q30	Q30	>99%	>97.5 - 99.999%
Run time	5 - 56h	4 - 31h	20 - 30h	1min - 72 h
Presentation	Benchtop	Benchtop	Benchtop	Portable and benchtop
Advantages	High accuracy; good coverage depth	High accuracy; good coverage depth	No DNA amplification required; long reads resolve ambiguities; fast running time	Faster running time; real-time data analysis; best for *de novo* assembly and identifying low abundance sequences within a sample; portability; low-cost equipment
Disadvantages	Long running time	Long running time	Expensive; higher error rate (but continues to improve)	*Higher error rate (but continues to improve)

Few years later to the development of PacBio, in 2014, a new third generation sequencing instrument was developed, known as nanopore technology. MinION (Oxford Nanopore sequencing technology, ONT) was the first commercial application of nanopore technology, developed for identifying the order of nucleotides in a DNA sequence (Hu et al., 2021). Unlike the PacBio platform, ONT performs sequencing in real-time and DNA synthesis is not required. ONT

process involves the identification of nucleotides by their distinguished electric signals that are generated while passing through protein nanoscale holes (2 mm diameter) of a thin membrane (e.g., flow cell). This change of electric signal is then decoded into their respective bases: adenine (A), guanine (G), cytosine (C) and thymine (T) (**Fig. 14**).

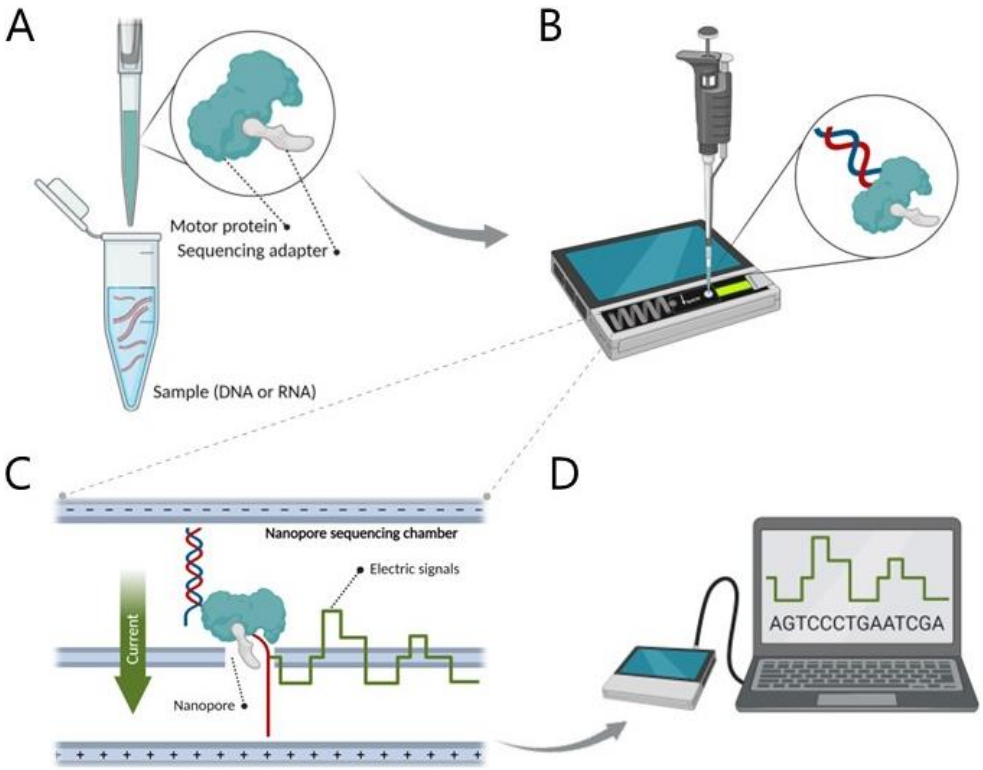


Figure 14. Basic steps involved in nanopore sequencing. **A.** Library preparation: binding of a sequencing adapter and a motor protein at each end of the DNA or RNA fragment of the sample. **B.** The DNA or RNA is introduced into the sequencing chamber through the sample inlet. **C.** Forced by an electric field, the DNA or RNA strand is translocated through the nanopore. Different current magnitudes are generated by the different nucleotides as the strand passes through the nanopore. **D.** This change of signal generated is converted in the computer into their respective bases: adenine (A), guanine (G), cytosine (C) and thymine (T). Figure created with BioRender.com.

Nanopore technologies can sequence whole genomes as they read the entire length of the DNA/RNA fragments presented to the nanopores. The longest read so far exceeds 4 Mb (Oxford Nanopore Technologies, 2022). The long read length provided by nanopore technology allows for more sequence overlap and therefore, is best for *de novo* assembly than the short-length sequencing technology (Lin et al., 2021). On the other side, ONT's limitations include its lower

output and higher cost compared to short-read sequencing platforms such as Illumina (Hu et al., 2021). Nonetheless, growing improvements in nanopore technology accuracy (currently >99%) combined with its convenience in terms of portability (Hu et al., 2021; Wang et al., 2021), will favor the replacement of the previous generation of sequencing devices such as Illumina.

4.2. Sequencing approaches to the study of microbial communities

In microbiome studies, microbial communities can be analyzed using two approaches: shotgun sequencing and amplicon sequencing (Durazzi et al., 2021). The first step in shotgun sequencing is random DNA fragmentation. The obtained DNA fragments are then sequenced individually, and their respective DNA sequence reads are assembled in order, based on their overlapping, to reconstitute the genome or complete sequence. With this approach, all genomic DNA in a sample can be read, which means that many types of microorganisms (bacteria, fungi, viruses,…) can be identified. Additionally, shotgun sequencing can provide information about functional profiles, since all regions of the gene can potentially be read (Quince et al., 2017).

On the other hand, amplicon sequencing specifically targets a single gene, such the 16S ribosomal RNA (rRNA) gene. The 16S rRNA amplicon sequencing, therefore, targets the full-length or some specific variable regions of the 16S rRNA gene (Johnson et al., 2019). The 16S rRNA gene is a 1500 bp long gene present and conserved in most bacteria and archaea. This gene comprises nine variable regions (V1-V9) separated by highly conserved sequences across different taxa (**Fig. 15**). The combination of the slow evolution rates of the conserved regions and the fast evolution rates of the variable regions of the 16S rRNA gene enables the design of universal primers that can differentiate between bacterial species (Johnson et al., 2019; Patel, 2001). Using specific (universal) primers, different sub-regions of this gene can be single-targeted (e.g. V4), or targeted in combination (e.g. V3-V4), for bacterial identification and taxonomic comparison (Johnson et al., 2019; Kameoka et al., 2021). Since this gene is universal in bacteria, it is very informative for revealing the bacterial taxonomic composition of a microbiome. However, 16S rRNA amplicon sequencing excludes non-bacterial and archeal members of the microbiota and

does not provide direct evidence of the community's metabolic and functional capabilities (Langille et al., 2013).

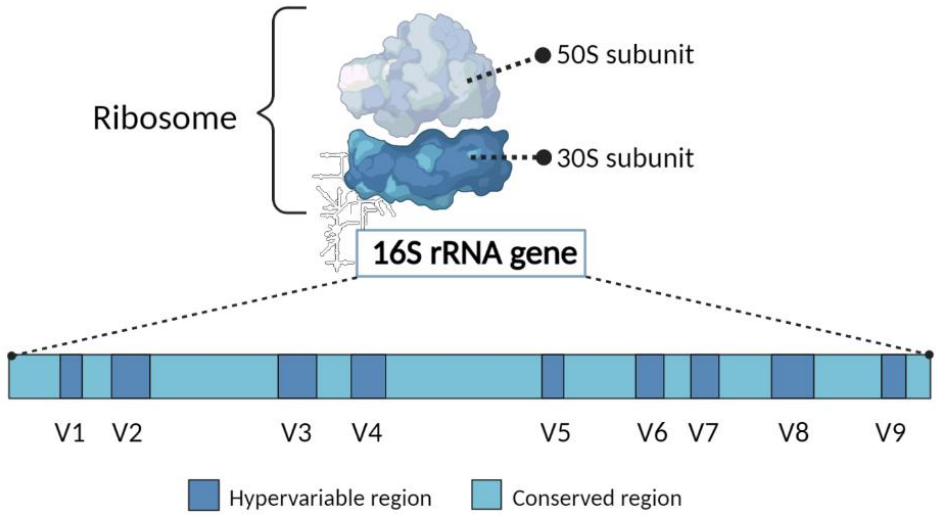


Figure 15. Schematic representation of the 16S rRNA gene, contained in the ribosomal 30S (small) subunit. Figure created with BioRender.com.

Until the development of nanopore technologies, the vast majority of 16S rRNA studies performed sequencing of targeted sub-regions because of read length restrictions of the Illumina platform (Sadowsky et al., 2017). This platform was widely used because of its reduced cost burden and effort compared to Sanger sequencing. However, it may produce chimeric sequences during PCR amplification for library construction. The latter and the low resolution of short amplicons limit the microbial classification at the genus level and lead a large proportion of the 16S rRNA gene database in GenBank to be labeled as unclassified (Jeong et al., 2021).

Conversely, advanced PacBio and Oxford Nanopore sequencing platforms can produce reads of >1500bp, therefore are capable of sequencing the full-length 16S rRNA gene (Curry et al., 2022). For instance, nanopore technologies allow for identifying bacteria with a similar or higher taxonomic resolution at genus and species levels than short-read sequencers and with a reduced turnaround time for data processing (Heikema et al., 2020; Matsuo et al., 2021). The level of taxonomy assignment obtained with the MinION™ platform can vary from genus to species, or down to species (i.e. strains) due to several reasons (Leidenfrost et al., 2020). For example, differentiation between *Escherichia* and *Shigella* is challenging even when using the full-length 16S rRNA gene because of their

close genetic relatedness (Matsuo et al., 2021). In this situation, targeting other phylogenetic markers such as *rpoB* and *mdh* genes could provide better resolution for their identification (Devanga Ragupathi et al., 2018). Since there are no universal PCR primers for amplifying the whole 16S rRNA gene, their specific choice may also introduce some bias in the results, as they may offer variable resolution for different bacteria. To address this issue, a combination of PCR primer pairs could be used (Fuks et al., 2018). Additionally, primers are designed to specifically target regions of the 16S rRNA gene. If a sample contains fragmented DNA sequences with some of these regions missing, primers may not be able to bind effectively, therefore, leading to an unsuccessful amplification (Fuks et al., 2018). The use of different reference databases in sequencing studies, which are often incomplete or contain a limited number of sequences, can also lead to varying outcomes in the taxonomic assignment (Terrón-Camero et al., 2022).

Until recently, the MinION™ platform had some drawbacks compared to Illumina or other sequencing platforms, such as its higher error rate (Jeong et al., 2021; Matsuo et al., 2021). However, new generation of kits and flow cells have introduced a level of accuracy similar to Illumina. Furthermore, analytical strategies like cluster analysis (pooling together all the reads with high similarity and generating a consensus sequence) can reduce the impact of errors on results (Curry et al., 2022). Therefore, integrating the full-length 16S rRNA gene amplification into nanopore sequencing can be particularly useful for microbiome profiling and fast pathogen identification (Huggins et al., 2022; Jahan et al., 2021; Kai et al., 2019; Matsuo et al., 2021). Overall, microbiome analyses require making fundamental decisions at every step in order to obtain accurate results and make them reproducible. More focus should be directed toward refining experimental designs incorporating the full-length 16S rRNA gene sequencing using the MinION™ platform to standardise and enhance control over each step of the process.

4.3. Bioinformatics tools and software

Microbiome analysis using NGS is a very complex assignment since it generates large amounts of data and requires the researcher to make a set of essential decisions in order to obtain reliable and reproducible results. To avoid data errors when executing each of the steps (and being able to control them),

researchers use bioinformatics pipelines, which allow for the automation of the whole process (Terrón-Camero et al., 2022).

Since NGS generates such an amount of data, pre-processing is usually first performed and considered an essential step. By data pre-processing, low-quality sequences, undesirable sequences, and host sequences can be removed. Furthermore, sequencer adapters are also discarded. Following this quality control, taxonomic identification can be directly performed, generally through two procedures: taxonomic classification or de novo assembly (Terrón-Camero et al., 2022). De novo assembly is based on the alignment of contiguous sequences (overlapping DNA fragments), known as contigs, followed by the alignment of these contigs into longer sequences, known as scaffolds, which contain some gaps (unknown DNA fragments). This method is useful when reference databases are not large enough (Meng et al., 2022).

On the other hand, taxonomic classification is a method that compares the reads against a reference database of microbial genes (e.g., GenBank). Many computational tools have been developed for this purpose, such as Kraken2 (Kraken), Braken or Centrifuge among many, each of which follows different strategies (Terrón-Camero et al., 2022). For example, Kraken uses an algorithm that associates short genomic sequences (also dubbed *k*-mers) with their lowest common ancestor taxa (Wood et al., 2019). Until the emergence of the latest generation of ONT, distinguishing sequencing errors from true different reads represented one of the main challenges of this single-pass sequencing platform. Nowadays, new generation of kits and flow cells have solved this issue. Furthermore, newer software tools are being developed. For instance, Emu software, which is a two-stage algorithm developed to refine the taxonomic profiling to the species level by accounting for these error rates, and at reduced cost. The first stage of the process involves aligning the sample reads with the reference database. In the second stage, an expectation-maximization (EM) algorithm is performed with each of the obtained sequences. The EM algorithm is an error-correction iterative process based on estimating the relative abundance of different sequences and adjusting these taxonomic assignments by accounting for sequencing errors or artifacts. With these two steps, Emu is able to distinguish between signal (real sequence) and error with fewer false positives and negatives than alternative methods. Furthermore, it can differentiate genomically similar species, such as *Salmonella enterica* and *S.*

bongori, which have an average nucleotide identity of 97%. However, Emu has currently some limitations that are open for future development, such as its arbitrary cut-off (its minimum abundance threshold to determine true assignments) and its higher requirements in terms of time and computing memory (Curry et al., 2022).

Most essential output files that come from the amplicon and shot-gun analysis workflow are the taxonomic and functional tables. This data needs to be further analysed in order to answer the researcher's questions, such as identifying types and relative abundances of specific microbes, or finding differences in microbes' composition between experimental groups (Lu et al., 2022). These downstream analyses may include statistics and visualization methods, both of which can and are usually performed with web applications, such as Pavian or Microbiome Analyst among many (Breitwieser & Salzberg, 2020; Dhariwal et al., 2017).

4.4. Statistical analysis and visualization methods used in microbiome projects

The most common approach to microbiome analysis is to look for taxa abundance (in general and differentially abundant taxa across the comparison groups of the data set) and look at differences in microbial diversity and community composition between groups (Galloway-Peña & Hanson, 2020; Kers & Saccenti, 2022; Lutz et al., 2022). Given the complexity of microbiome data, visualization methods usually take precedence in the initial stages of the analysis. A common way to visualize the microbiome composition is by using stacked bar plots, which allows for comparing categories of data and discovering potential associations within the dataset (Galloway-Peña & Hanson, 2020). Outcomes from these visualization methods help to design the following more rigorous and specific statistical analyses.

One of the most popular areas of interest in microbiome profiling comprises detecting and quantifying differentially abundant taxa across the comparison groups, known as differential abundance analysis. The aim of this method is to look for quantitative differences in taxa between the comparison groups, assigning statistical significance values to each comparison (Cappellato et al., 2022; Lutz et al., 2022). Common statistical methods used for differential

abundance analyses are the Linear discriminant analysis (LDA) Effect Size (LEfSe) and varying methods based on log-linear regression models (Segata et al., 2011).

Diversity measures are used for describing the complexity of microbiomes, in terms of richness (number of different taxa), evenness (relative abundance of taxa), or both. Differences in richness and evenness can be evaluated at two levels: within samples (alpha diversity) and between samples (beta diversity). Common metrics used for alpha diversity analyses include Shannon's index, observed number of amplicon sequence variants (ASVs), Chao1, and Simpson's index, among others (Galloway-Peña & Hanson, 2020). Metrics that consider both richness and evenness, such as Shannon's and Simpson's indices, are considered more robust than richness-alone estimators (i.e. ASVs). However, they differ from each other in terms of placing greater weight on richness (Shannon's index) or evenness (Simpson's) in their equations (Kers & Saccenti, 2022). It is also common to analyse differences in alpha diversity between the comparison groups, for which conventional statistical tests such as ANOVA or Kruskal-Wallis test are used (Galloway-Peña & Hanson, 2020).

Beta diversity compares the diversity between the different groups in the dataset. Usually, beta diversity quantifies the dissimilarity between the comparison groups and is reflected in a distance matrix. Conventional distance methods for calculating beta diversity are the Bray-Curtis dissimilarity, unweighted UniFrac, weighted UniFrac, and Jaccard's index (which comprises a similarity coefficient). While some of these metrics consider taxa abundance (i.e., Bray-Curtis dissimilarity), others account only for the presence-absence of taxa but not for their relative abundances (i.e., unweighted UniFrac, Jaccard's index) (Kers & Saccenti, 2022). Statistical tests used to analyse this dissimilarity (or similarity) are Permutational Multivariate Analysis of Variance (PERMANOVA) and Analysis of Similarities (ANOSIM) among others. Different ordination methods are used to visualize the relationship between the comparison groups, such as Principal Coordinate Analysis (PCoA), Non-Metric Multidimensional Scaling (NMDS), or Principal Component Analysis (PCA). For instance, PCoA fits the raw distance values onto the axes of the plot sequentially, representing the pairwise distances between data points while preserving the dissimilarity structure. The amount of variance captured by the axes indicates the true distances represented in the plot. On the other side, NMDS is a non-metric alternative to PCoA, which means that it does not use the original (raw) dissimilarity distance values. Instead, it

converts these values into ranks (ranked differences) and uses them in its algorithm. Furthermore, NMDS uses an iterative process that runs until the objects are best repositioned in the ordination space. This final outcome is dictated by a stress function, where stress values inferior to 0.15 are considered acceptable. Whereas NMDS may return different outcomes on re-analysis of the same data set, PCoA gives a unique analytical solution (Paliy & Shankar, 2016; Ramette, 2007). There are many different analytical algorithms to analyse large-scale data sets. The decision on which ordination method to use will be dictated by the type of data (i.e., null values are not optimal for NMDS, zero values are not optimal for PCA), their assumed relationship (i.e. PCoA assumes a linear relationship between data and distances), and the dissimilarity distance used (i.e. PCoA requires a distance matrix, NMDS works with any distance measure), among many other factors. When different ordination methods are available for the same data set, procrustes or co-inertia analyses can help to evaluate whether both methods give similar results (Paliy & Shankar, 2016).

The set of taxa consistently found within a given habitat (i.e., human intestine) is defined as the core microbiome. In microbiome projects, core microbiomes are usually referred to as the shared taxa between the comparison groups (two or more groups/samples). This metric can be measured with different methods such as the abundance-based method (accounting for the top 75% of the total reads without considering their consistent presence across the comparison groups/samples), the occupancy-based method (accounting for taxa present in more than 50% of the comparison groups/samples), a combination of abundance and occupancy-based method, and the Venn diagram (accounting for taxa present in all the comparison groups/samples) (Custer et al., 2023).

Overall, the nature of the microbiome project will dictate the most convenient metric and statistical method to use. For instance, numerous studies have revealed the association between dietary covariates and certain taxa implicated in the development of chronic inflammatory and metabolic diseases (Turnbaugh et al., 2007). In such cases, discovering associations between taxonomy and covariates requires additional integrative analyses, such as the Dirichlet-multinomial regression (DMR) (Lutz et al., 2022). Statistical techniques for microbiome analysis are continuously developing due to the inherent complexity of the microbiome data set and the need for a better understanding of the intricate connections between the microbiome, health, and disease.

5. Workflow overview for the full-length 16S rRNA gene metabarcoding using the MinION™ sequencer

The full-length 16S rRNA gene of different samples can be amplified and pooled together for sequencing via the MinION™ platform, while still maintaining their distinct identities. The process of bacterial identification in a mixed sample (bulk DNA) via next-generation sequencing of a DNA sequence region is dubbed metabarcoding. This method differs from simple DNA barcoding (historically performed with Sanger sequencing to identify single specimens) because the taxonomy of many species present in the same sample can be rapidly assigned (Baloğlu et al., 2021; Zhang et al., 2018). Metabarcoding can be extremely useful when working with samples with less data than the total amount that can be generated from a single flow cell. Therefore, it allows the pooling of multiple samples (already extracted DNA) and sequencing them together.

The library preparation in 16S rRNA metabarcoding studies relies on a PCR that starts with amplifying the full-length 16S rRNA gene (regions V1-V9) by using specific primers, such as 27F and 1492R. This step is followed by the addition of specific barcodes and adapter tags. The latter will allow the attachment of the sequencing adapters, a step known as library preparation (**Fig. 16**) (Baloğlu et al., 2021). Libraries are loaded into the MinION™ sequencer, which will produce real-time data visualization. Within a brief timeframe (1min-72h), this data can be transferred to a computer via a USB stick, making it readily available for subsequent bioinformatics analysis (Hu et al., 2021).

Nanopore data output is obtained in fast5 files, which show the raw current signal obtained by nucleic acid translocation through the protein nanopore of the MinION™. This raw data is then transformed into fastQ files, which show the sequences of bases already decoded. This process is called basecalling and is a critical step since almost all downstream steps depend on it (Pagès-Gallego & de Ridder, 2023). Following steps to improve the quality of the data include trimming certain parts of a sequence and remove short or low-quality reads. For instance, for 16S rRNA gene analyses, we filter reads by a desired length of more than 1500 bp. Adapters and barcodes that were added to our sample DNA fragments during the library preparation are also trimmed from the sequences (de Coster et al., 2018).

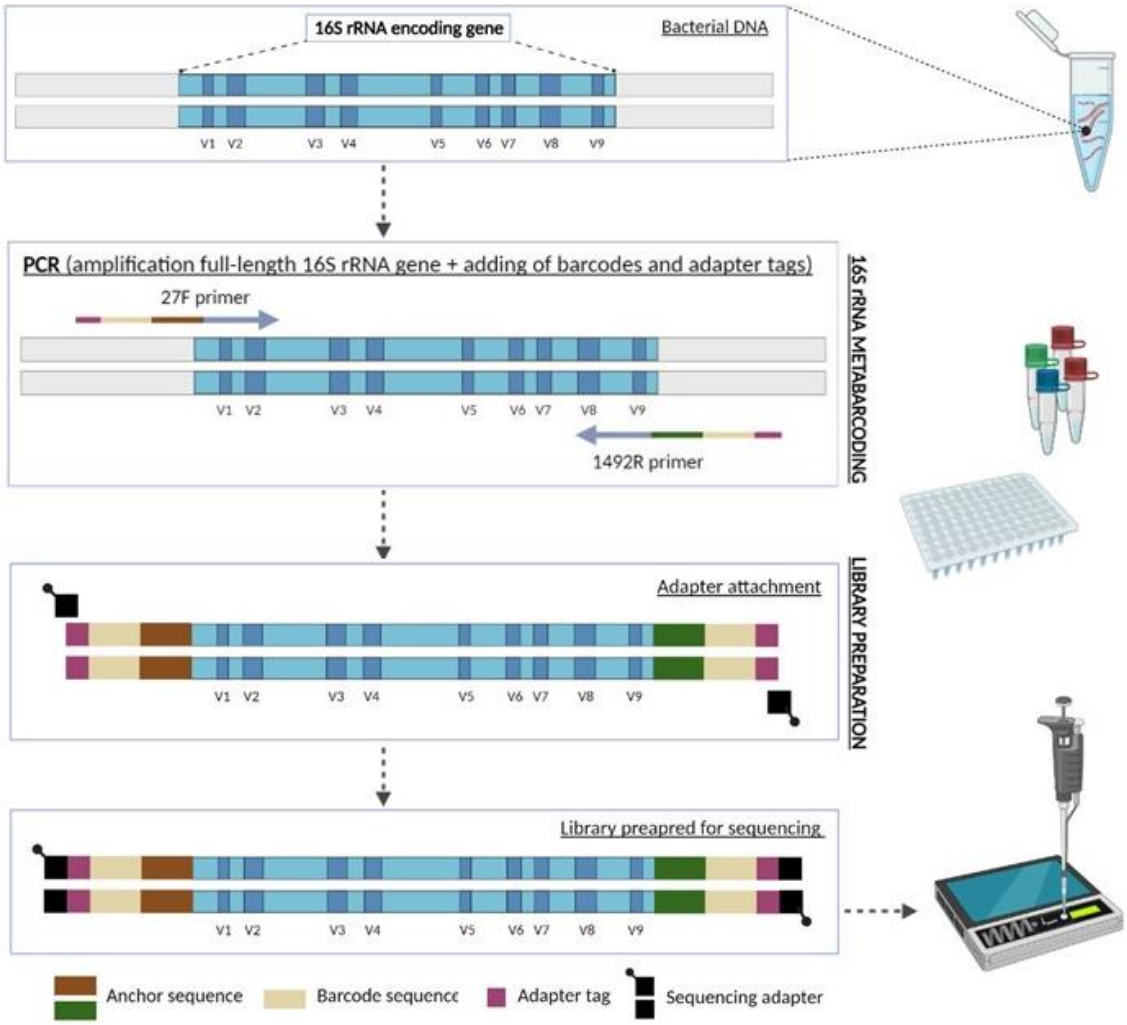


Figure 16. Workflow of the library preparation for the 16S rRNA gene metabarcoding using the MinION™ platform. Sequencing libraries are generated by adding a sequencing adapter to the DNA sequences after the amplification of the full-length 16S rRNA gene and the addition of barcodes. Figure created with BioRender.com.

Taxonomic assignment (from phylum and commonly up to genus level) can be carried out with several metagenomics classifiers (i.e., Centrifuge, Kraken) using varying reference databases (i.e., Silva, Ribosomal Database Project (RDP), Greengenes,..).

Finally, data can be filtered by removing single read counts and by setting a minimum read count with a desired percentage of prevalence in the sample (i.e.

minimum read count of 2 with a 20% prevalence). The data obtained can be then analysed and plotted with several software tools, such as Pavian or MicrobiomeAnalyst, which support comprehensive statistics and data visualization (Salazar et al., 2020; Wang et al., 2021).

Hypotheses

The general hypothesis of this thesis is that the health status of free-ranging bat populations may be adversely affected by a range of anthropogenic activities, primarily associated with human encroachment into bat's dwelling areas.

In recent years, there is evidence of injuries associated with the use of forearm rings in bats, and until now no in-depth follow-up has been carried out to evaluate its impact on bat's health. In this context, we hypothesize that individual marking techniques might be jeopardizing the well-being and health of free-ranging bat populations.

A growing body of evidence highlights the role of the microbiome as an early indicator of individual and population health in wildlife species. Furthermore, host-associated microbiomes have been shown to quickly reflect anthropogenic disturbances and environmental degradation. Currently, there are no available data regarding the health status of insectivorous bat populations in Europe, nor about the effect of environmental degradation on their fecal microbiome. Within this scope, we first hypothesize that anthropogenic activities, such as intensification of agriculture or urbanization, could negatively affect the quality of the intestinal bacterial microbiome of free-ranging bats. Secondly, we hypothesize that the same environmental factors would modulate the occurrence and abundance of potential pathogenic bacterial communities within their fecal bacterial microbiomes.

Objectives

The objectives of the present thesis were:

1. To analyze available information regarding the health impact of forearm rings and Passive Integrated Transponder (PIT) tags on free-ranging bats captured in Catalonia (NE-Spain) from 2009 to 2021.
2. To assess, under controlled conditions, the health impact of the two most used marking techniques for bat species: the forearm ring and the PIT tag.
3. To assess the composition, structure and diversity of the fecal bacterial microbiome of five species of free-ranging insectivorous bats (*Miniopterus schreibersii*, *Nyctalus leisleri, Pipistrellus kuhlii*, *P. pipistrellus* and *P. pygmaeus*) captured in Catalonia and assess the relationship between these microbiome parameters and varying levels of anthropogenic environmental degradation.
4. To identify potential pathogenic bacteria (PPB) at the level of bacterial species in five species of free-ranging insectivorous bats (*Miniopterus schreibersii*, *Nyctalus leisleri, Pipistrellus kuhlii*, *P. pipistrellus* and *P. pygmaeus*) captured in Catalonia and assess the relationship, in terms of occurrence and abundance, of these PPB with land use types.

Study 1. **Reappraising the use of forearm rings for bat species**

Biological Conservation, Volume 286, October 2023
DOI: 10.1016/j.biocon.2023.110268

Lourdes Lobato-Bailón, Adrià López-Baucells, David Guixé, Carles Flaquer, Jordi Camprodon, Xavier Florensa-Rius, Maria Mas, Laura Torrent, Laura Ordeix, Oriol Tallo-Parra, Maria P. Ribas, Ignasi Marco, Annaïs Carvajal, Manel López-Bejar, Sebastian Napp, Lola Pailler-García, Johan Espunyes, Oscar Cabezón

Abstract

Long-term mark-recapture studies are essential for bat conservation. Over the last decades, millions of bats across Europe and America have been marked with forearm rings for this purpose. Although it is considered a cost-effective method compared to Passive Integrated Transponders (PIT) tags, direct injuries from using forearm rings have been reported since its very first use. Yet, their impact on bats' welfare has not been systematically evaluated and remains a highly controversial issue among the scientific community and policymakers. Here we assess the impact of forearm rings and PIT tags on the health of different bat species. We reviewed 12 years of the existing recapture data of free-ranging bats from NE Spain and evaluated the impact of both marking tools in a captive colony of *Carollia perspicillata*, by assessing the development of skin lesions and levels of cortisol metabolites in guano (CG) after marking. We report that 55.1% (435/790) of the recaptured free-ranging bats with forearm rings presented skin lesions. All banded *C. perspicillata* (n=22, 100%) developed skin lesions, whereas none of the PIT-tagged (n=21) presented lesions. Levels of CG were significantly higher after marking with forearm rings only for one group. Banded *C. perspicillata* exhibited discomfort-associated behaviours due to forearm rings. Under the "precautionary principle", we recommend the ban of forearm rings for all bat species until species-specific studies under controlled conditions are performed and approved by a legally constituted ethics committee. Consideration of other long-term marking tools is mandatory to align with global bat conservation strategies.

Background

Bat populations have experienced significant declines over the past century, leading the International Union for Conservation of Nature (IUCN) to classify nearly half of the species as threatened or near threatened globally (IUCN, 2023). In Europe, bat populations have shown downward trends for the past 50 years, especially throughout Western Europe (Hutson et al., 2001). At present, 22% of the European bat species are classified as Endangered (EN) or Vulnerable (VU) by the IUCN (Hutson et al., 2001; IUCN, 2023) but strict species and habitat protection, accompanied by investments in research to improve conservation strategies, have stabilized populations for a number of species (Browning et al., 2021; Haysom et al., 2013). However, population trends and key threatening processes are not fully understood for the vast majority of species.

The Agreement on the Conservation of Populations of European Bats (UNEP/EUROBATS) was set up in 1991 to cooperate towards the conservation of bats throughout Europe. The Agreement provides guidelines to be implemented by European institutions and conservationists on diverse topics, including the capture and marking of bats (Hutson et al., 2001; UNEP/EUROBATS, 2023). For decades, the capture-mark-recapture technique has been used in wildlife for research and conservation (Cody & Smallwood, 1996; Gaisler & Chytil, 2002; Shenbrot et al., 2010). Particularly, the recognised challenge of providing information about bat ecology, population dynamics and social behaviour identifies long-term mark-recapture studies as an important tool for bat conservation (Locatelli et al., 2019). At present, most of these long-term bat monitoring studies are undertaken using two marking tools: forearm rings (also dubbed bands) and Passive Integrated Transponder (PIT) tags (Kunz & Parsons, 2009; Mitchell-Jones & McLeish, 2004). Tagging with forearm rings is a cost-effective method for marking bats, compared to PIT tags, and no technical device is required to read it. These attributes have given bands a rising popularity among bat researchers and conservationists.

Millions of bats have been marked with forearm rings worldwide, especially in Europe and North America, over the last decades (Ellison, 2008; Hutson et al., 2001; Hutterer et al., 2005). However, direct injuries from using bands have been

reported, suggesting that this marking technique could silently compromise the conservation efforts of bat populations worldwide (Baker et al., 2001; Balmori & Quetglas, 2000; Ellison, 2008; Hutterer et al., 2005). This issue has become highly controversial among the scientific community and policymakers. The recommended ring sizes and designs in Europe are based on field recapture experience from Bulgaria, Germany, The Netherlands, Portugal, Ukraine, and the United Kingdom (UNEP/EUROBATS, 2003). However, the UNEP/EUROBATS Agreement recognises that banding is an invasive procedure and that the degree of tolerance to this marking tool varies among species and populations of the same species (UNEP/EUROBATS, 2018). For this reason, some countries (i.e. USA, Australia and France) have implemented moratoriums on the use of forearm rings in bat research based on field studies that reported direct physical injuries in bat wings produced by this marking tool (Baker et al., 2001; Ellison, 2008; Hutterer et al., 2005). Currently, the knowledge of the impact of marking tools on bats' health is mainly based on field studies with low recapture rates (Ellison, 2008; Hutterer et al., 2005; Steffens et al., 2007). As a consequence, the direct effects and the magnitude of the impact might be largely underestimated. Furthermore, few studies have examined the impact of marking tools on survival rates and sublethal effects (e.g. reduction in body condition) in free-ranging bats (Baker et al., 2001; Locatelli et al., 2019; Mellado et al., 2022). These field studies, although valuable, are unable to explore other collateral effects such as alterations in social or reproductive behavior, or increased stress and susceptibility to disease.

Measurement of cortisol metabolites in feces has been broadly used to evaluate stress in animals, including bats (Hernández-Arciga et al., 2020; Kelm et al., 2016). Sustained high cortisol concentrations have been associated with poor body condition, reduced immunocompetence, decreased reproductive success, and ultimately fitness disruption (Cain & Cidlowski, 2017; Edwards et al., 2019). Research on bat marking tools under controlled conditions to document and understand these acute and long-term effects on the health and survival of these species is crucial. Insights from such controlled studies can help to develop evidence-based national and international policies to guarantee animal welfare and support current bat conservation strategies.

The aim of the present study was to assess the impact of forearm rings and Passive Integrated Transponder (PIT) tags on the health of a selected group of

bat species by 1) reviewing the existing recapture data of wild insectivorous bats from Catalonia (NE Spain) from 12 years (2009 to 2021) and 2) monitoring skin injuries, assessing cortisol metabolites in guano and observing alterations in the behaviour of captive *Carollia perspicillata*, derived from both marking tools, under captive conditions in a zoological institution.

Methods

Assessment of the impact of forearm rings in free-ranging recaptured bats from Catalonia, NE Spain

We analysed data from bat recaptures in NE-Spain, from the last 12 years (2009-2021), on forearm rings and PIT tags (**Supplementary material: Table S1.1**) collected by two institutions for the research of bats in Catalonia (BiBio Research Group - Natural Sciences Museum of Granollers and Centre de Ciència i Tecnologia Forestal de Catalunya).

We calculated the proportion of bats detected with injuries from the total recaptured per species and marking method, with exact confidence intervals of 95%. First, considering all the recaptured bats, we tested whether there was a statistically significant difference in the frequency of lesions between bats marked with forearm rings and bats marked with PIT tags using the Fisher's exact test. Then, for recaptured bats marked with forearm rings, we also evaluated whether there were statistically significant differences in the frequency of lesions between the different species of bats. To do that, we used pairwise Fisher's exact tests with the Holm's correction to account for multiple comparisons. Only those species for which there were at least seven individuals were included in the comparison.

In a small subset of the bats marked with forearm rings (n=304), besides the data on the species, there was supplementary information collected on the age, sex and reproductive state. The effect of age could not be evaluated because all animals included in the study were adults. To evaluate whether the other factors had a significant effect on the probability of developing a lesion, a logistic regression model was built with the variables species, sex, and reproductive state.

Assessment of the impact of tagging microbats in captivity

We tested the impact of forearm rings and PIT tags on a captive colony of adult Seba's short-tailed bats (*Carollia perspicillata*), housed at Butterfly Park (license number G25-00119), a small zoo established in Empuriabrava (NE Spain). These animals were already required to be individually identified for the zoo collection register. All bats were born in captivity and adapted to the routine institutional management strategies: daily cleansing of the facilities between 9:00 and 10:00 and food supply between 18:00 to 19:00 hours.

The Seba's short-tailed bat is a widespread Phyllostomidae bat species inhabiting Central and South America. This species is classified as Least Concern by the IUCN Red List (Barquez et al., 2015) and is exhibited in many zoological institutions due to its small size, diet specifications (mainly frugivorous species) and its gregarious behaviour, roosting in groups up to hundreds of individuals (Rasweiler IV et al., 2009). Roosts can be organised as a harem (1 adult male with large aggregations of females) and as a bachelor (large aggregations of adult and subadult males without females) (Martínez-Medina et al., 2018). The phenotypic characteristics of this species (similar in size and morphology to several species of European bats) and its adaptation to a captive environment (Rasweiler IV et al., 2009) made it suitable for the purpose of this study.

An intentional sampling method was used to select 32 adult females and 11 adult males from the captive colony of Seba's short-tailed bats (n=150), excluding individuals with scars, solved fractures or any other integument abnormalities. Bats were captured with a telescopic net bag (BTHK Tree-Roost net bag, NHBS). Best practices for the care and handling of bats (Miller, 2016) were followed throughout the study and at least one European Diplomate in Veterinary Medicine (Wildlife Population Health and/or Veterinary Dermatology) and a bat specialist were present during all procedures. Bats were distributed in four groups: group 1 (one male and eight females) and group 2 (one male and 12 females) were marked with forearm rings, and group 3 (one male and 12 females) and group 4 (8 males) were fitted with PIT tags. Each group was tested independently and housed at different times in a separate enclosure of 50 square meters and 3 meters high, meeting the environmental requirements of *C. perspicillata* (relative humidity of 80% and average minimum and maximum temperature of 20–26°C) (Bat Taxonomic Advisory Group, 2004; Martínez-

Medina et al., 2018). The enclosure provided a green semi-opened area and an artificial cave for light insulation during daylight hours. Additionally, bats were isolated from external stressors such as visitors and educational activities for the whole duration of the study. The adaptation period for each group spanned four weeks, after which a marking tool (forearm ring or PIT tag) was implemented and tested for six consecutive weeks. Forearm rings were withdrawn at the end of the sixth week in groups 1 and 2 and then those bats were PIT-tagged and returned to their original enclosure.

Forearm rings used for groups 1 and 2 were distributed by the SECEMU Association (Spanish Association for the Conservation and Research of Bats, Spain) and approved for their use in Spain. They contained a unique alpha-numerical code, were omega-shaped, 4.2 mm in diameter and made of a light alloy of aluminum and magnesium (model 3X, 0.103g of weight). Bats were banded (**Supplementary material: Fig. S1.1**) by highly experienced personnel. Fingers were used to gently close the ring, maintaining their round shape and leading to ~1 mm wide opening between the tips so that it was free to slide up and down the forearm but prevented finger bones from becoming trapped.

Bats from groups 3 and 4 were PIT-tagged using a sterilised 10 mm x 1.4 mm transponder of 0.036 g of weight (FrenChip Mini, Avid®, California, USA), injected subcutaneously in the dorsum so that the tag rested between the shoulder blades, as described previously in the literature (van Harten et al., 2020). Next, a drop of surgical glue (3M™ VetBond™) was applied to the injection site to minimise tag loss, and the animal was restrained for a few seconds until the complete drying of the adhesive.

Physical impact assessment

All bats were physically examined prior to the study and at the end of the adaptation period to ensure they were physically fit and healthy before any tag was implanted. Particular attention was given to fur and patagia. The evaluation of skin lesions caused by both marking tools was carried out by a Diplomate specialist from The European College of Veterinary Dermatology (ECVD) at the end of the six-week period in all four groups. Cutaneous lesions found after marking with forearm rings were classified in order of increasing severity in Stage 1, Stage 2, and Stage 3 (**Supplementary material: Table S1.2**). Stage 1 was

assigned to any mild lesion in the sense that the epidermis was not damaged, and no treatment was required after the ring withdrawal. Stage 2 referred to moderate cutaneous lesions associated with a superficial breakdown in the continuity of the epidermis. Topical treatment may improve and boost the patient's recovery time assigned to this stage. Stage 3 included injuries with significant skin tissue and fluid loss, exposure of the dermis and subcutis, and epidermal ischemia. Topical, parenteral, and surgical treatment may be necessary for some presentations of Stage 3 to avoid the development of sepsis or loss of extremity function (Miller et al., 2012).

Complementary animal welfare monitoring

All bats were continuously monitored by two infrared video cameras (Foscam FI9926P®) located at the entrance of the artificial cave of the experimental facility and in front of the feeding plates, respectively. Video cameras allowed the live view of the animals and stored a continuous recording for delayed observation and assessment of behavioural indicators of welfare. Furthermore, zoo caretakers reported any abnormal conduct detected during feeding and cleansing times.

Additionally, to explore the indirect effects of forearm rings and PIT tags on the welfare of bats, we measured cortisol metabolites in guano (CG) from each group, twice a week during the adaptation and marking period. Hormone metabolites were extracted following a methanol extraction protocol previously described (Palme et al., 2013; Tallo-Parra et al., 2014) (**Supplementary material: S1.4. Cortisol analysis**).

Statistical analyses

A Fisher's exact test was applied to compare the frequencies of lesions between groups marked with forearm rings and PIT tags.

To evaluate whether the marking tool (forearm ring or PIT tag) influenced the levels of CG from the animals in the different groups, a linear regression model was built considering CG as the response variable, and the time point (sampling time) and the interaction between period (adaptation or marking) and group (1-4) as the covariates. The comparison between the estimated marginal means was

computed. We hypothesised that CG might be modulated by the stress response produced by being tagged. Therefore, intragroup CG concentrations after tagging were expected to be significantly higher than those during the adaptation period, particularly in the two groups with forearm rings.

All the analyses were performed using R programming (R Core Team, 2022) language and RStudio (RStudio Team, 2022) environment. We use the package lsmeans (Least-Squares Means) for the analyses (Russell V, 2016) and ggplot2 (Wickham, 2016) and tidyverse (Wickham et al., 2019) for the figures.

Results

Physical impact of tagging free-ranging bats with forearm rings

Information regarding 1236 marked free-ranging bats was analysed, 790 of which had forearm rings and 446 had PIT tags (Table 1). Recaptured bats included the following species: *Miniopterus schreibersii, Myotis capaccinii, M. daubentonii, M. myotis, Nyctalus lasiopterus, N. leisleri, N. noctula* and *Pipistrellus pygmaeus*. A total of 435 recaptured bats (55.1%, CI95%: 51.5-58.6) showed skin lesions derived from the forearm ring, whereas no skin lesions (0%, CI95%: 0.0-0.8) were detected in any of the 446 bats recaptured with PIT tags (**Table 3**). The frequency of lesions was significantly higher in bats marked with forearm rings compared with bats marked with PIT tags (p-value < 0.001). Statistically significant differences between species are shown in **Fig. 17**.

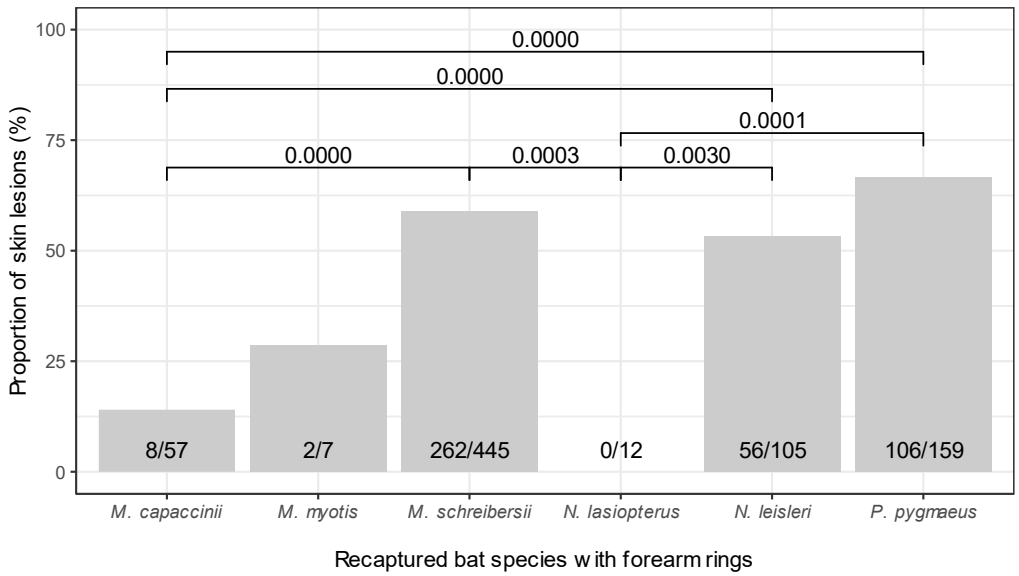


Figure 17. Differences between proportions of skin lesions found in free-ranging recaptured bats with forearm rings (pairwise Fisher's exact tests with Holm's correction, p-value <0.05). The number of bats detected with skin lesions from the total recaptured is depicted in each species box.

Table 3. Percentage of injured bats due to forearm rings and PIT tags. Data obtained during the last 12 years (2009-2021), from bats recaptured in Catalonia (NE-Spain).

Species	Marking tool	Bats recaptured	Bats with injuries	Injuries occurrence % ($CI_{95\%}$)
M. schreibersii	Forearm ring	445	262	58.9 (54.2-63.5)
	PIT tag	116	0	0.0 (0.0-3.1)
M. capaccinii	Forearm ring	57	8	14.0 (6.3-25.8)
M. daubentonii	Forearm ring	3	1	33.3 (0.8-90.6)
M. myotis	Forearm ring	7	2	28.6 (3.7-71.0)
N. lasiopterus	Forearm ring	12	0	0.0 (0.0-26.5)
	PIT tag	5	0	0.0 (0.0-52.2)
N. leisleri	Forearm ring	105	56	53.3 (43.3-63.1)
	PIT tag	79	0	0.0 (0.0-4.6)
N. noctula	Forearm ring	2	0	0.0 (0.0-84.2)
P. pygmaeus	Forearm ring	159	106	66.7 (58.8-74.0)
	PIT tag	246	0	0.0 (0.0-1.5)
Total	Forearm ring	790	435	55.1 (51.5-58.6)
	PIT tag	446	0	0.0 (0.0-0.8)

The presence or absence of skin lesions was reported for all recaptured species, but the classification or description of the lesions was only provided for the following four species. The most reported injury in *M. schreibersii* and *N. leisleri* was a callus formation (55.2% and 66.7% respectively) (**Fig. 18A**), followed by an ulcerated callus (21.2% and 14.6% respectively). Ulcerated callus (**Fig. 18B**) was the most described lesion (29.4%%) in *P. pygmaeus*, followed by callus formation (20.3%), ulcers and extended erosions (19.6%), and ring embedding (13.1%) (**Fig. 18C**). The recovered *M. daubentonii* with skin lesions showed an extended area of skin thinning and depigmentation, and an erythematous plaque in the distal carp (**Fig. 18D**).

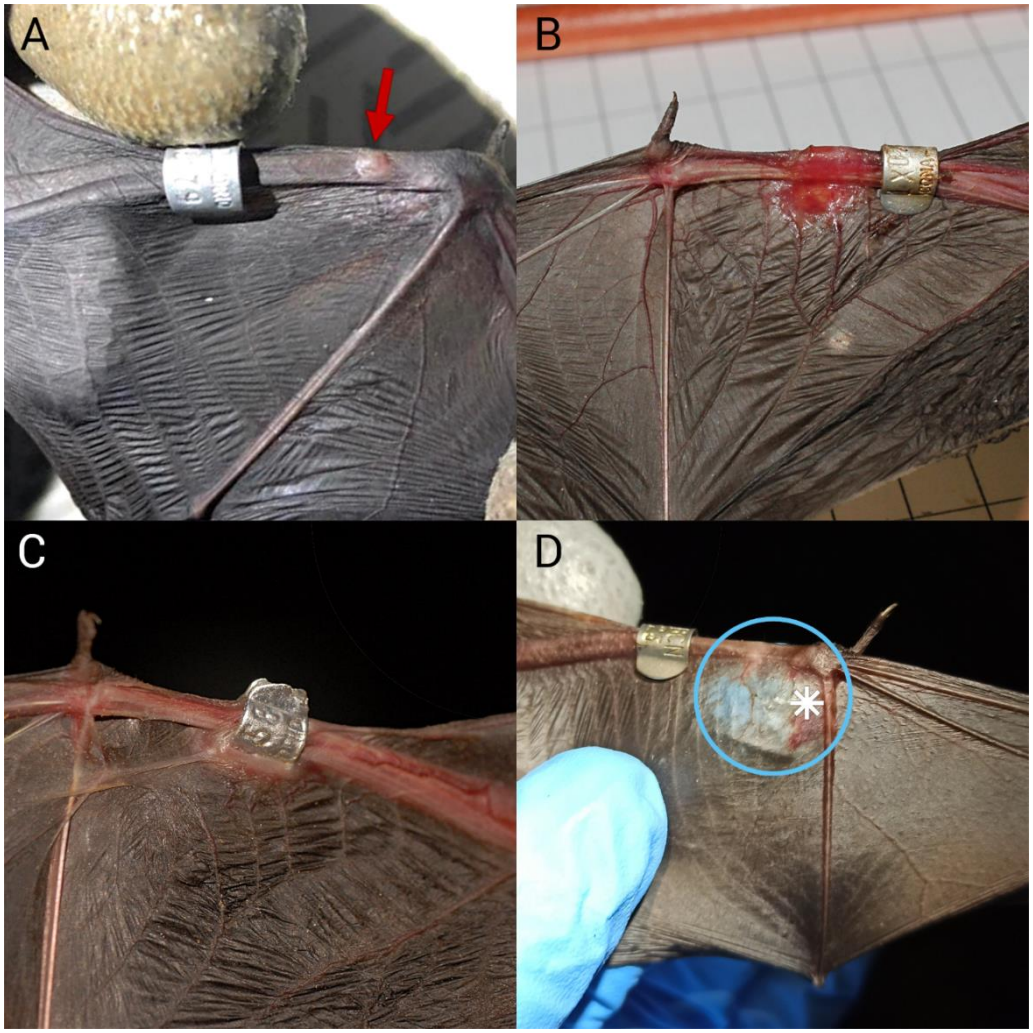


Figure 18. Skin lesions found in free-ranging bats recovered with forearm rings in Catalonia. **A.** Callus formation (red arrow) in the dorsal aspect of the forearm of a *Nyctalus leisleri*. **B.** Ulcerated callus found in the forearm of a *Pipistrellus pygmaeus*. **C.** Marks of ring bite and ring embedding in the forearm of a *Pipistrellus pygmaeus*. **D.** Extended area of skin thinning and depigmentation (encircled), and an erythematous plaque (white star), in the distal carp of a banded *Myotis daubentonii*.

Additional information on sex and reproductive state (for the latter see **Fig. 19)** was available only for a subset of 304 bats marked with forearm rings: *M. schreibersii* (n=163), *N. leisleri* (n=17), and *P. pygmaeus* (n=124). The results of the logistic regression model using the previous subset of the dataset showed that the sex of the bats did not have a significant effect on the probability of lesions. In contrast, the reproductive state did, showing that bats in a passive reproductive state were less likely to develop skin lesions than those in an active reproductive state (OR=0.09, p-value= 0.018) (**Table 4**). The logistic regression

model showed that *P. pygmaeus* had a significantly lower risk of developing skin lesions derived from the use of forearm rings than *M. schreibersii* (OR=0.38, p-value= 0.003) (**Table 4**).

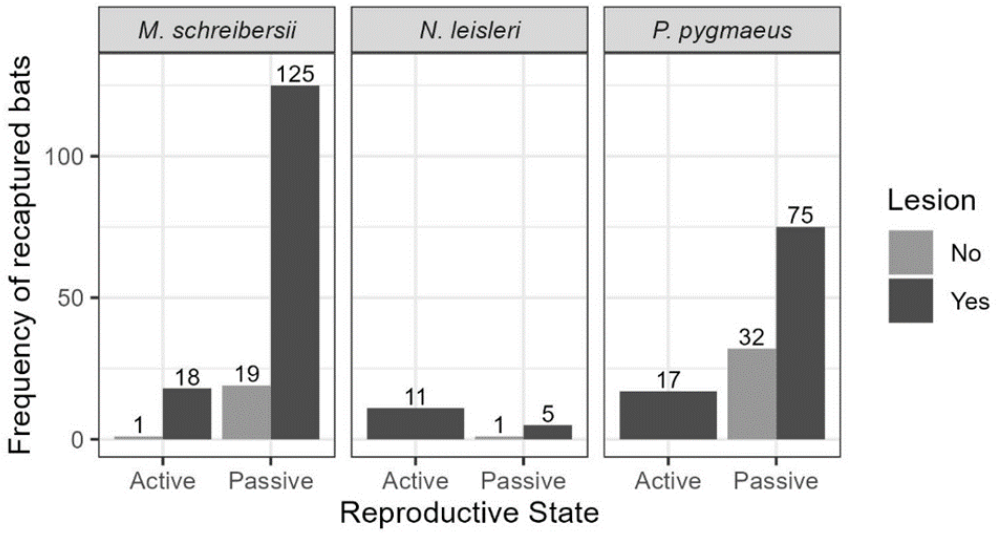


Figure 19. Frequency of skin lesions derived from the use of forearm rings in recaptured free-ranging bats (Catalonia, 2009 – 2021) by species and reproductive state. Both male and female bats are included.

Table 4. Summary of the logistic regression model with presence of lesion as the response and, species and reproductive state as predictors. The coefficients of the model are presented in terms of odds ratio along with their 95% confidence interval. *Miniopterus schreibersii* and active reproductive state are the reference categories for species and reproductive state, respectively. Statistically significant values are indicated with *.

Variable	OR	95%CI	p-value	
Intercept	72.87	[14.31, 1342.91]	0.0000	*
Nyctalus leisleri	0.97	[0.15, 18.77]	0.9763	
Pipistrellus pygmaeus	0.38	[0.20, 0.71]	0.0026	*
Passive reproductive state	0.09	[0.00, 0.43]	0.0182	*

Impact of tagging *C. perspicillata* in captivity

Skin lesions

All banded bats (n=22, 100%) developed skin lesions of varying degrees of severity. Forearm rings were found displaced, piercing the dorsal and ventral aspects of the distal forearm in most banded *C. perspicillata* (n=15, 68.2%). The

most frequently found lesions were skin depigmentation and thinning (n=19, 86.4 %), erythema (n=16, 72.7%) (**Fig. 20A**) and skin neovascularisation (n=13, 59.1%) (**Fig. 20**). The area of skin depigmentation and thinning coincided with the location of ring displacement along the forearm ring, dorsally and ventrally to the radius and ulna. Erythematous plaques (n=12, 54.5%) (**Fig. 20B**) were visible along the same area of skin depigmentation and thinning, presumably in the most proximal area where the ring could be displaced. These erythematous plaques were commonly surrounded by neovascularisation, newly formed capillaries arising from the brachial vein (**Fig. 20B**). Erosions (**Fig. 20C**) were present in six bats, mostly on the edges of the propatagium close to the carp and in the same area of the erythematous plaques, due to forearm ring rubbing. Four (18.2%) bats developed severe cutaneous lesions, corresponding with ulcers (**Fig. 20D**) located dorsally and ventrally to the radius and ulna, and also in the most proximal area where the ring could be displaced. One forearm ring was found displaced from the propatagium, completely closed around the radius and ulna, and embedded in the skin (**Fig. 20E**). After the removal, granulomatous tissue and inflammation were evidenced, and the subcutis and muscles from the radius and ulna were exposed (**Fig. 20F**). Following the previous classification, 22 (n=22, 100%) bats showed cutaneous lesions derived from the use of forearm rings, of which six (27.3%) had at least one mild lesion (Stage 1), 12 (54.5%) presented at least one moderate lesion (Stage 2), and four (18.2%) showed at least one severe lesion (Stage 3). All cutaneous lesions (**Supplementary material: Table S1.2**) were treated as needed and monitored until their resolution. We examined all forearm rings (n=22) after their removal and recorded any signs of biting. Sixteen (72,7%) forearm rings showed clear marks of bites (**Supplementary material: Fig. S1.2**).

All tags were read correctly by the microchip scanner and found by palpation and skin transparency between the shoulder blades (**Supplementary material**: **Fig. S1.3**) at the end of the study. None of the PIT-tagged bats (n=21, 0%) showed cutaneous lesions by the end of the study. Bats marked with forearm rings presented a significantly higher frequency of lesions than PIT-tagged bats (p-value < 0.0001).

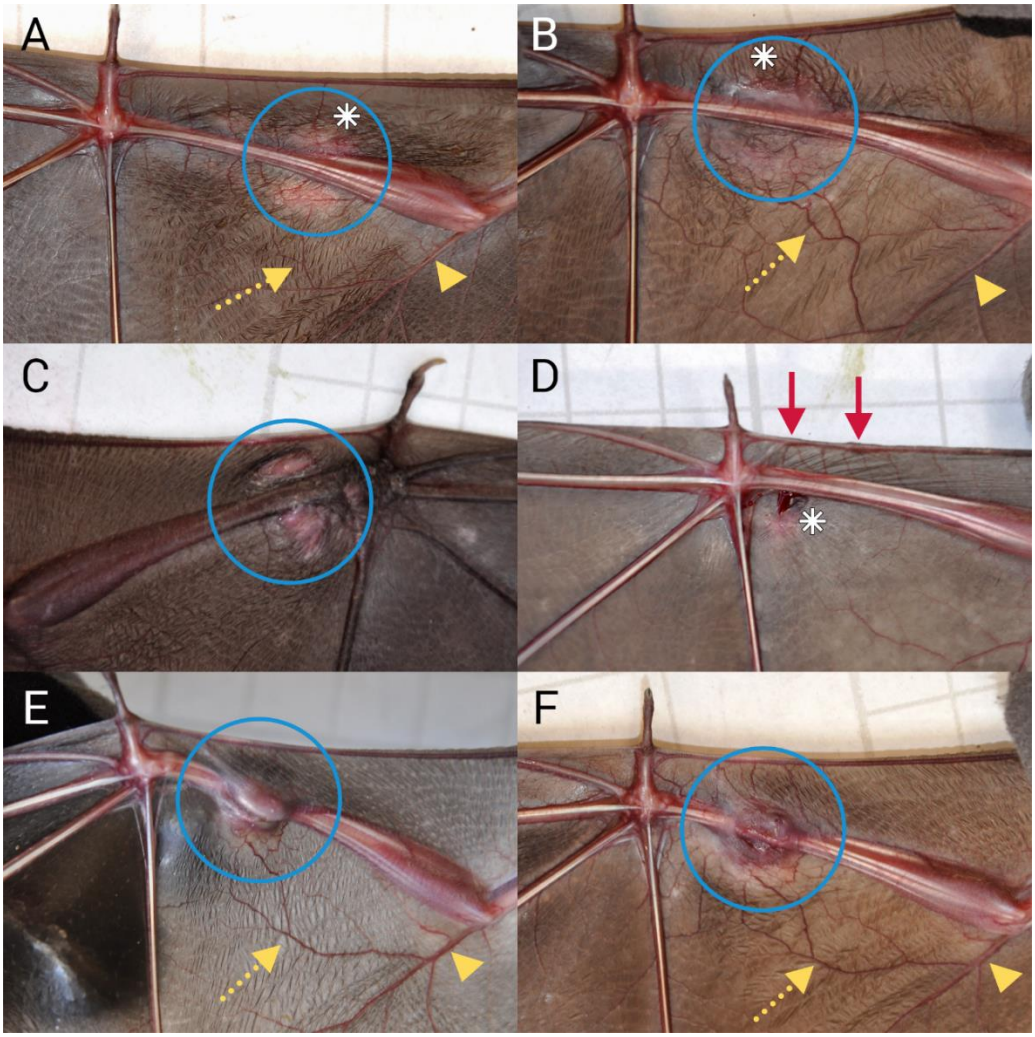


Figure 20. Skin lesions found in captive *Carollia perspicillata* due to marking with forearm rings. Areas of neovascularisation are indicated with yellow dashed arrows, and their respective brachial veins marked with arrow heads. **A**. Skin depigmentation and thinning, dorsally and ventrally to the radius and ulna (encircled). An area of erythema is indicated with a white star. **B.** Skin depigmentation and thinning, dorsally and ventrally to the radius and ulna (encircled). An erythematous plaque is indicated with a white star. **C.** Dorsal cutaneous erosions, dorsally and ventrally to the radius and ulna (encircled). **D.** Cutaneous ulcer ventrally to the radius and ulna with active bleeding (white star) manifested after the forearm ring removal. Additionally, two punctiform erosions (red arrows) were present on the edges of the propatagium. **E.** Forearm ring displaced from the propatagium, firmly closed, and embedded in the skin (encircled). **F.** The same individual than figure 3E, after the forearm ring removal and disinfection of the wound. The subcutis and muscles from the radius and ulna were exposed and the cutaneous tissue around the ulcer was swollen and erythematous (encircled).

Behavioural changes and levels of cortisol metabolites in guano

We observed some of the banded bats biting the ring (**Supplementary video C1, Appendix C: doi: 10.1016/j.biocon.2023.110268**) and scratching it with their hindlimbs in an attempt to remove it (**Supplementary video C2, Appendix C: doi: 10.1016/j.biocon.2023.110268**). In contrast, no behaviours indicative of tagging-related discomfort were observed in PIT-tagged bats.

A statistically significant difference between the levels of CG before and after the intervention was found for group 1 (forearm ring, p-value= 0.013) (**Supplementary material: Table S1.3 and Fig. S1.4**). In the remaining groups, the differences were not statistically significant (**Supplementary material**: **Fig. S1.4**).

Discussion

Decades of individually marking bats with forearm rings have helped us to understand many aspects of their ecology and biology (Ellison, 2008; Hutterer et al., 2005). However, the proportion of recovered bats is usually less than 1% (Steffens et al., 2007), except for studies that work in roosting sites with high-fidelity species (Gaisler & Chytil, 2002). Therefore, recapture data accuracy relies on capturing and marking a relatively large proportion of the population (Ellison, 2008; Hutterer et al., 2005).

Knowledge of the physical impact of forearm rings has been obtained by banding reports (Baker et al., 2001; Ellison, 2008; Hutterer et al., 2005; Steffens et al., 2007), systematic studies in the wild (Mellado et al., 2022; Zambelli et al., 2009), and other studies using bat bands whose authors noticed negative associated consequences and decided to report them (Balmori & Quetglas, 2000; Dietz et al., 2006; O'Shea et al., 2004; Sendor & Simon, 2003). Our study confirms the health impact of forearm rings in free-ranging bats, showing that a high percentage (55.1%) of bats recaptured in Catalonia over the last decade had injuries derived from this marking tool. Moreover, considering the overall low recapture rate in bat mark-recapture studies, we must be cautious in extrapolating the results to the whole population of bats. For instance, it is likely that some of the non-recaptured bats had suffered severe injuries from marking tools and died. This assumption is reinforced by the high percentages of *C. perspicillata* from our study in captivity that presented moderate (54.5%) and severe (18.2%) lesions derived from the use of forearm rings.

Factors reported to affect the level of injury caused by forearm rings are the type of metal used, the accuracy of banders when placing the ring and the size of the ring ((Baker et al., 2001; Dietz et al., 2006; Zambelli et al., 2009). Zambelli et al. (2009) reported skin lesions caused by forearm rings in *N. leisleri*. They concluded that an incorrect band size was the most likely reason for the injuries and suggested the use of slightly larger band sizes in case of doubt or in the absence of species-specific recommendations. Although we used a larger band size for *N. leisleri* bats, we still detected a high injury rate (53.3%), even larger than the 11.2% reported by Zambelli et al. (2009). Furthermore, certain morphological

factors, such as the shape and width of the propatagium, have been targeted as potential defining features for sensibility to bands (Dietz et al., 2006). However, these factors have not been accurately validated for European bats using long-term studies under captivity conditions and conclusions were based on field observations. Notably, a high injury rate was reported by Dietz et al. (2006) in banded *Rhinolophus ferrumequinum, R. euryale* and *R. mehelyi*, regardless of the size of the band or the existence of well-established recommendations. The authors of the study concluded that the higher incidence of injuries in these three species compared with those of banded vespertilionids resulted from increased ring rubbing along the frontal margin of the propatagium due to a wider propatagium. In our study, only vespertilionid species were assessed, but still, we found a concerning proportion of injured bats. Since some of them are classified as cave-roosting species (e.g., *M. schreibersii*) and others as crevice-dwelling species (e.g., *P. pygmaeus*), a larger impact was expected to be found in the latter (Palomo et al., 2007). However, when the proportions of skin lesions were compared among our bat species, we found three species, *N. leisleri, P. pygmaeus* and *M. schreibersii*, exhibiting significantly worse responses to bands than the rest. For some of the species evaluated the sample sizes were really small, so larger studies are needed. The logistic regression model accounting for the effect of sex and reproductive state showed that *P. pygmaeus*, our smallest crevice-dwelling species assessed, was 62% less likely to develop skin lesions due to forearm rings than *M. schreibersii*. This result contrasts with the lack of a statistically significant difference between the two species in the study using the complete dataset. This contrast can be attributed to the different methodologies employed and/or differences between the complete dataset and the subpopulation used in the logistic regression model, involving factors that influence the risk of developing lesions. A comprehensive understanding of the factors and variables influencing the development of skin lesions is essential for a nuanced interpretation of results. Future studies should incorporate larger datasets with a comprehensive range of variables. Unlike males, female *N. leisleri* exhibit long-distance migratory behaviour (Alcalde et al., 2013), a trait that was previously assumed to cause worse consequences than sedentary habits. However, our results showed that sex does not seem to have a significant effect on the probability of developing lesions. In contrast, we found that bats in an active reproductive state were more likely to develop skin lesions than those in a passive state. All these findings emphasize that forearm rings have a significant impact on the health of insectivorous bats, regardless of their ecology, size, or

sex. Furthermore, it highlights that during the reproductive season, they may be particularly vulnerable to banding activities.

Carollia perspicillata is one of the most studied bat species in the laboratory and other captive settings due to its adaptability to captivity. Furthermore, it is an abundant species in its indigenous habitat, so it is also widely used in ecological studies (Rasweiler IV et al., 2009). Because these studies may need to identify the bats individually, several externally attached tools have been employed, including forearm bands (Alviz & Pérez–torres, 2020; Mellado et al., 2022; Monteiro et al., 2019; Sánchez et al., 2007), plastic splits (Knörnschild et al., 2013; Rex et al., 2011), and collars (Mellado et al., 2022; Monteiro et al., 2019; Rodríguez-Posada & Santa-Sepúlveda, 2013). However, despite the large body of scientific studies where the individual marking of *C. perspicillata* is witnessed, there is often a lack of information regarding the design or manufacturer reference (Alviz & Pérez–torres, 2020; Sánchez et al., 2007) and size (Alviz & Pérez–torres, 2020; Monteiro et al., 2019; Rex et al., 2011); and their physical impact or absence of injuries is hardly ever reported. All these factors significantly hinder making an optimal choice in marking tools for *C. perspicillata* studies. Similarly to Dietz et al. (2006), our study with captive *C. perspicillata* suggests that a wider propatagium may interfere with a proper slide of the band along the forearm. Slightly splitting the plagiopatagium and passing the ring through it has been used as an alternative for bats with large propatagia, such as *C. perspicillata* (Monteiro et al., 2019). However, this marking method is highly harmful, as bats are essentially left with an open wound, susceptible to bacterial colonisation. Recently, Mellado et al. (2022) reported a significant occurrence of skin lesions in *C. perspicillata* resulting from this method. The healing process (inflammatory, proliferative and remodeling phases) may be prolonged and easily complicated when splitting the plagiopatagium, as friction between the wound edges and the ring is expected to be maintained as long as the ring is fitted (Schultz et al., 2011).

In our study with *C. perspicillata*, we found a significant difference in the levels of CG between the adaptation and the marking period in group 1, which was marked with forearm rings. However, the higher levels of CG after the marking period cannot be explained by the marking tool alone because bats from group 2 (also marked with forearm rings) did not show the same tendency as group 1. Because cortisol secretion is a dynamic process (Busch & Hayward, 2009) and is

individually modulated by intrinsic factors such as age, reproductive state, or social status (Palme et al., 2013), many factors may have jeopardised correct data interpretation of the pooled group samples. We collected all fecal samples at the same hour (in the morning) and CG in each sample represented an average of the defecating period (12 hours). During this period, different stressful events, such as social disputes, may have randomly happened. Moreover, the temporal stability of cortisol metabolites shows significant variations among species (Donini et al., 2022). For instance, the fast intestinal transit of *C. perspicillata* (Laska, 1990), coupled with differences in time deposition of feces or low stability of cortisol metabolites after deposition, could explain the absence of correlation in the remaining groups (Donini et al., 2022). Therefore, as a first step, we recommend investigating the species-specific stability of cortisol metabolites in feces before any physiological study is performed. Additionally, increasing the sample size and testing cortisol metabolites levels individually -if possible- may be helpful to ameliorate the problem of high data variance in longitudinal studies where cortisol changes in relation to an event are evaluated.

Remarkably, there is no upper limit on the acceptable amount of physical damage and pain that bats may experience due to marking tools. EUROBATS recognizes that banding bats is an invasive procedure that can seriously harm or even kill the bat if performed incorrectly. While recommendations of ring sizes by species are given, the Agreement also states that sensitivity to bands may vary between species and also between individuals of the same species or population (UNEP/EUROBATS, 2018). Nonetheless, the decision of banding or not banding bats rests on the researcher, as the establishment of a tolerable damage limit does. If we would follow the recommendation of Baker et al. (2001) to discontinue banding bats if injury rates surpass 2%, banding activities would need to be terminated in most, if not all, insectivorous bat species. However, clinging to the principles of ethical research with animal subjects (Ethical Guidelines for the Use of Animals in Research, 2018), bat banding activities continue regardless of the evidence of physical injuries because of the expected benefit obtained by this technique (UNEP/EUROBATS, 2018). However, assumptions from these activities such as that marks are not lost with time or do not interfere with survival may introduce significant biases in bat population studies. Furthermore, researchers must minimise the risk of disturbance and suffering of bats and be responsible for considering options that may improve the animal's welfare while still obtaining the same benefit from the research

(NENT, 2018; UNEP/EUROBATS, 2018). In our results and the reviewed literature, the band injury rate for several species exceeded 10% (Baker et al., 2001; Dietz et al., 2006; Mellado et al., 2022; Zambelli et al., 2009), which points to considering other ethically acceptable alternatives.

In contrast, recent studies using PIT tags in free-ranging bats have proved to be suitable for individual recognition (Locatelli et al., 2019; van Harten et al., 2020), assessing bat movements (van Harten et al., 2019, 2022), reproductive success (Rigby et al., 2012), body condition (Locatelli et al., 2019; Rigby et al., 2012), and survival estimates (Ellison et al., 2007; van Harten et al., 2022). This method has several advantages in comparison with the use of other traditional and long-term marking techniques. For instance, it provides higher encounter probabilities with no handling or physical disturbance for species roosting in caves or bat boxes (Godinho et al., 2015; van Harten et al., 2022). Furthermore, there is no evidence linked to detrimental effects on bats' health, reproductive or survival success (Edmonds et al., 2017; Locatelli et al., 2019; Rigby et al., 2012; van Harten et al., 2020). This is supported by the results obtained in the present study. The use of a sterilised small-sized PIT tag injected subcutaneously in the dorsum caused no physical impact in any of our captive *C. perspicillata* bats. Four free-ranging vespertilionid species from Catalonia, including *P. pygmaeus*, whose body size does not exceed 4 grams, neither showed lesions associated with this marking tool. The present study highlights the potential of this technique to be used in bat species regardless of their morphological differences or body size. Nevertheless, appropriate theoretical and practical training is crucial before implementing any marking method.

Due to the high injury rates found in free-ranging and captive bats, banding activities are at odds with conservation goals and call into question the scientific value of recapture data obtained with this method. Our study concludes that the use of forearm rings should be banned under the 'precautionary principle' for all bat species until systematic studies under controlled conditions are performed. Particularly, efforts should be made to include indicators of indirect effects such as changes in behaviour or reduced fitness. While decisions on marking tools for bat species must be on a case-by-case basis, new technological advances may provide efficient solutions and tools, such as PIT tags, to achieve multi-species tolerance.

Supplementary material: S1

Table S1.1. Specificities about bands and PIT tags used in free-ranging bats from Catalunya over the last decade. Bands were distributed by the SECEMU Association (Spanish Association for the Conservation and Research of Bats, Spain) and approved for their use in Spain.

Marking tool	Model	Material	Size	Weight (g)	Species
Ω-shape band	1X	Light alloy of Al and Mg	2.5 mm Ø	0.038 g	*Pipistrellus pygmaeus*
Ω-shape band	2X		3 mm Ø	0.046 g	*Myotis daubentonii* *Myotis capaccinii*
Ω-shape band	3X		4 mm Ø	0.103 g	*Myotis myotis* *Miniopterus schreibersii* *Nyctalus lasiopterus* *Nyctalus leisleri* *Nyctalus noctula*
PIT tag	MUSICC Identification System, Avid®	Glass capsule coated with Parylene C	8 x 2.1m	0.028 g	*Pipistrellus pygmaeus* *Miniopterus schreibersii* *Nyctalus lasiopterus* *Nyctalus leisleri*

Table S1.2. Description of skin lesions resulting from the use of forearm rings in captive *Carollia perspicillata* and classification by their degree of severity. Adapted from W. Miller *et al.* (2012).

Stage	Presenting sign	Description
1	Skin depigmentation and thinning	Post-inflammatory loss of normal pigmentation and reduced skin thickness.
	Neovascularisation	Formation of new blood vessels in the form of microvascular networks which supply blood to damaged skin.
	Alopecia	Skin area with hair loss due to constant friction with the band. This alopecia can be present along the forearm or chest, resulting from the contact between the forearm ring and the chest while hanging upside down with folded wings.
	Erythema	Skin area of redness, usually accompanied by an increase in temperature.
2	Erythematous plaque	Solid and well-demarcated raised area of skin, wider than high, accompanied by erythema.
	Blister formation	Free accumulation of inflammatory liquid under the skin due to a constant increased blood flow into the area.
	Erosion	Superficial loss of epidermal continuity along the forearm, resulting from a sharp friction of the band with the skin.

Table S1.2. *Continued.*

Stage	Presenting sign	Description
3	Ulcer	Deep breakdown in the continuity of the epidermis, usually exposing the dermis and involving minimal bleeding, due to external factors such as the repeated friction caused by the band over an extended period. Crust formation can be present.
	Acute constriction and distal swelling	The ring may be found closed around the forearm as a consequence of a bite. The distal aspect of the wing and adjacent to the ring may be swollen due to an improper venous return.
	Embedded forearm ring	The forearm ring is partially or completely covered by swollen soft flesh from the forearm. Ring biting (causing constriction) and ulcers are predisposing factors.

Table S1.3. Cortisol metabolites concentrations in guano from captive *Carollia perspicillata* during the adaptation and marking periods from groups 1 and 2 (forearm ring), 3 and 4 (PIT tags). * = Cortisol metabolites concentration in guano from the first 12h after marking.

		Cortisol (ng/g)			
	Guano sample	**Group 1**	**Group 2**	**Group 3**	**Group 4**
Adaptation period	**1**	3.37	2.743	4.15	3.85
	2	1.39	2.298	2.65	2.02
	3	1.59	1.706	1.64	2.41
	4	0.96	1.352	1.06	1.63
	5	1.64	0.742	1.36	1.67
	6	1.9	1.304	1.46	2.61
	7	0.65	0.531	1.25	2.67
	8	0.81	0.285	1.66	1.56
Marking period	**9**	1.22*	0.834*	4.92*	1.34*
	10	1.61	0.654	3.09	3.43
	11	2.49	1.253	1.72	1.7
	12	1.97	0.686	0.78	3.24
	13	2.66	2.218	2.4	0.77
	14	2.49	2.097	1.99	2.66
	15	6.13	1.347	2.53	0.41
	16	7.84	2.544	2.45	1.2
	17	9.09	0.841	3.54	1.19
	18	5.21	1.186	2.65	4.28
	19	11.17	0.942	4.44	1.15
	20	8.16	1.541	na	0.75

S1.4. Cortisol analysis

Feces were pooled and collected in 15 mL Falcon® tubes between 1 and 2 times a week for the entire duration of the study and kept frozen until steroid extraction. For that purpose, a clean and new polyethylene cover sheet was placed under the bats' resting area the evening prior to sample collection. The polyethylene cover sheet remained for 12 hours on all occasions, after which the guano was collected, coinciding with feeding and cleansing activities.

Before steroid extraction, fecal samples were dried in an oven (Heraeus model T6; Kendro®LaboratoryProducts, Langenselbold, Germany) at 60º C for three to four days. A protocol previously described by Palme et al. (2013) was applied with modification for the guano matrix. Samples were individually grounded with a Mixer Mill (MM2 type; Retsch®, Haan, Germany) until a homogenous powder of less than 3 mm particles was obtained. Cortisol was extracted adding 5 ml of 80% methanol (methanol reagent grade 99.9%, Sharlab, S.L., Sentmenat, Spain) in 500 mg of each sample previously aliquoted into a 15 ml conical tube and vortexed (VortexMixer S0200-230 V-EU; Labnet International Inc., Edison, NJ, USA) for 30 minutes. Briefly, samples were centrifuged (Hermle Z300K; Hermle®Labortechnik, Wehingen, Germany) at 4500 g for 15 minutes and the supernatant was transferred into a microtube and immediately frozen at -20ºC until steroid determination.

A commercial enzyme immunoassay (EIA) kit (Cortisol ELISA KIT; Neogen® Corporation, Ayr, U.K.) was used for steroids analysis and validated biochemically for *C. perspicillata*. The guano matrix was validated by verifying: 1) Precision (intra-assay coefficient of variation (CV) from all samples analyzed, run by triplicate and, inter-assay CV from samples run in the different tests); 2) Sensitivity (smallest steroid concentration analyzed); 3) Specificity (evaluated with the linearity of dilution, determined by using 1:1, 1:2, 1:5 and 1:10 dilutions of pools with EIA buffer); 4) Accuracy (through the spike-and-recovery test, calculated by adding different amounts of guano pool to different volumes of pure standard cortisol solution: 0.44 ng/ml, 1.11 ng/ml and 1.80 ng/ml).

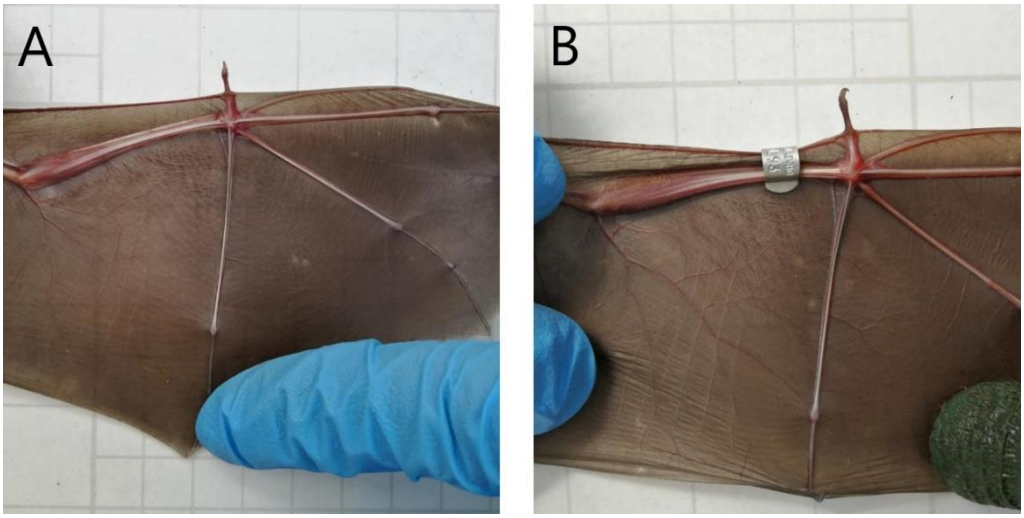


Figure S1.1. Appearance of *Carollia perspicillata* patagia before (**A**) and after (**B**) forearm ring fitting.



Figure S1.2. Marks of bites in the forearm ring of a banded *Carollia perspicillata*.



Figure S1.3. Subcutaneous transparency of the PIT-tag in a *Carollia perspicillata*, located between the shoulder blades, after six weeks of the implementation.

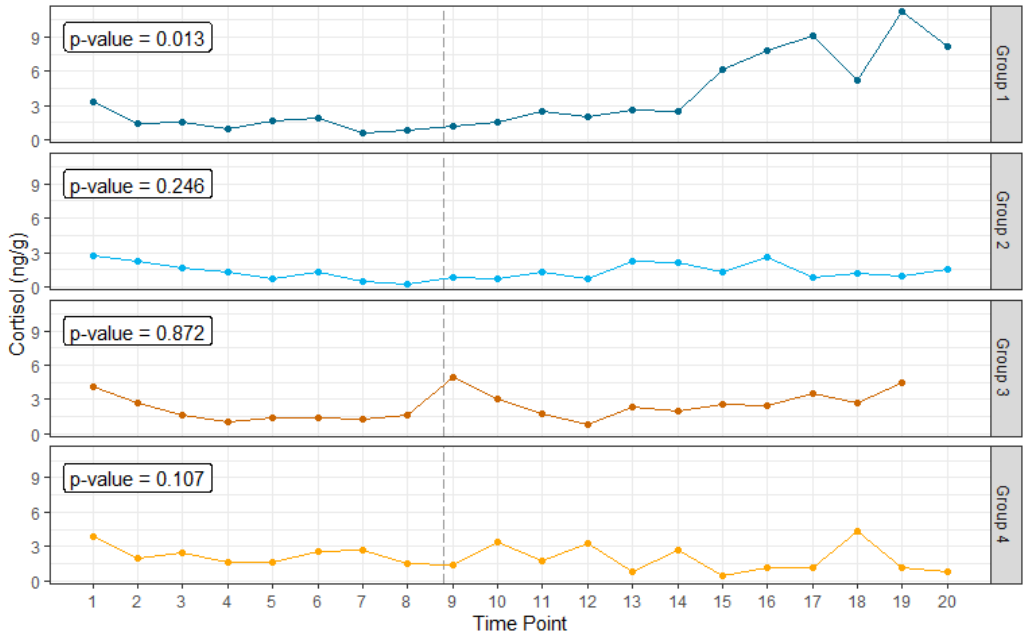


Figure S1.4. Cortisol metabolites concentrations in guano from captive *Carollia perspicillata*: groups 1 and 2 were marked with forearm rings, while groups 3 and 4 were marked with PIT tags. The vertical dashed grey line indicates the placement of the marking tool, therefore time point 9 reflects cortisol metabolites concentrations in guano 12 hours after marking. A p-value < 0.05 indicates a significant difference in cortisol metabolites concentrations between the adaptation period (from sample points 1 to 8) and the marking period (from sample points 9 to 20).

Study 2. **The fecal bacterial microbiome of the Kuhl's pipistrelle (*Pipistrellus kuhlii*) reflects landscape anthropogenic pressure**

Animal Microbiome, Volume 5, February 2023
DOI: 10.1186/s42523-023-00229-9

Lourdes Lobato-Bailón, Manuel García-Ulloa, Andrés Santos, David Guixé, Jordi Camprodon, Xavier Florensa-Rius, Raúl Molleda, Robert Manzano, Maria P. Ribas, Johan Espunyes, Andrea Dias-Alves, Ignasi Marco, Lourdes Migura-Garcia, Jaime Martínez-Urtaza, Oscar Cabezón

Abstract

Anthropogenic disturbance has the potential to negatively affect wildlife health by altering food availability and diet composition, increasing the exposure to agrochemicals, and intensifying the contact with humans, domestic animals, and their pathogens. However, the impact of these factors on the fecal microbiome composition of wildlife hosts and its link to host health modulation remains barely explored. Here we investigated the composition of the fecal bacterial microbiome of the insectivorous bat Kuhl's pipistrelle (*Pipistrellus kuhlii*) dwelling in four environmental contexts with different levels of anthropogenic pressure. We analyzed their microbiome composition, structure and diversity through full-length 16S rRNA metabarcoding using the nanopore long-read sequencer MinION™. We hypothesized that the bacterial community structure of fecal samples would vary across the different scenarios, showing a decreased diversity and richness in samples from disturbed ecosystems. The fecal microbiomes of 31 bats from 4 scenarios were sequenced. A total of 4,829,302 reads were obtained with a taxonomic assignment percentage of 99.9% at genus level. Most abundant genera across all scenarios were *Enterococcus*, *Escherichia/Shigella*, *Bacillus* and *Enterobacter*. Alpha diversity varied significantly between the four scenarios ($p < 0.05$), showing the lowest Shannon index in bats from urban and intensive agriculture landscapes, while the highest alpha diversity value was found in near pristine landscapes. Beta diversity obtained by Bray–Curtis distance showed weak statistical differentiation of bacterial taxonomic profiles among scenarios. Furthermore, core community analysis showed that 1,293 genera were shared among localities. Differential abundance analyses showed that the highest differentially abundant taxa were found in near pristine landscapes, with the exception of the family Alcaligenaceae, which was also overrepresented in urban and intensive agriculture landscapes. This study suggests that near pristine and undisturbed landscapes could promote a more resilient gut microbiome in wild populations of *P. kuhlii*.

Background

The majority of Earth's ecosystems are dominated by human activity and suffer significant and continuously growing disturbances. Consequently, we are witnessing a global biodiversity crisis in which current species extinction rates far exceed background estimates (Cardillo et al., 2005; Powers & Jetz, 2019). In turn, biodiversity sustains many ecosystem services needed by humans, and its loss is entailing clear ecological, public health and economic costs at a global scale (Hosseini et al., 2016).

The Order Chiroptera contributes to worldwide biodiversity with more than 1400 different species, making up one-fourth of terrestrial mammals (Hutson et al., 2001). This diverse group of mammals inhabits all continents except Antarctica and is responsible to promote and support ecosystem health by means of pollination, seed dispersal and insect and vector-borne diseases regulations (Ramirez-Francel et al., 2022). Nonetheless, it is among the most endangered group of mammals with more than 200 bat species around the world considered threatened by the International Union for the Conservation of Nature (Hutson et al., 2001).

Most of the current research efforts relevant to bat conservation worldwide are linked to trends in species diversity and abundance, and distribution patterns (Feijó et al., 2019; Lim et al., 2021; Preble & Vincenot, 2021). Furthermore, investigation into the threats affecting bat species has focused mainly on climate change, habitat degradation and other related human disturbances such as over-hunting, pollution, or collisions with wind energy turbines (Frick et al., 2019). Except for the white-nose syndrome, which has given rise to extensive research, very little investigation has been done on bat health in contrast to other endangered species (Feijó et al., 2019; Hutson et al., 2001). In Europe, bat populations have declined considerably over the last decades presumably due to multiple factors of anthropogenic origin (e.g., pesticides use in agriculture, wind turbines or habitat loss and fragmentation) (Frick et al., 2019). Understanding the factors that contribute to such declines and the differential responses of bat species to habitat disturbance is critical for worldwide bat conservation.

The bacterial communities that inhabit the gut of all animal species constitute the intestinal bacterial microbiota. The genetic and structural elements (e.g., lipids, proteins) and metabolites (e.g., signaling molecules, inorganic and organic molecules, toxins) produced by these organisms in a specific environment, and their theatre of activity, are referred to as the bacterial microbiome (Berg et al., 2020). Most of the knowledge about the normal gastrointestinal microbial community of bats comes from traditional microbiological studies (di Bella et al., 2003; Jarzembowski, 2002) and they are particularly focused on the presence of infectious agents with zoonotic potential (Mühldorfer, 2013; Wolkers-Rooijackers et al., 2018). Current studies on the metagenomic profiling for fecal bacterial communities of bat species have contributed to broadening the knowledge of species-specific microbial diversity, zoonotic pathogens (Banskar et al., 2016; de Leon et al., 2018; de Mandal et al., 2015; Dong-Lei et al., 2020; Gerbáčová et al., 2020; Núñez-Montero et al., 2021; Selvin et al., 2019; Vengust et al., 2018), diet and niche adaptation, and evolution (Banskar et al., 2016; Dietrich et al., 2017; Ingala et al., 2021).

Two decades of microbiome studies in a wide range of species suggest that intestinal microbiota may contribute not only to the gut's health but to the overall host's immunity (Fan & Pedersen, 2021; Peixoto et al., 2021). Among other characteristics, species-rich communities in a given microbial system appear to be more resilient and to prevent the establishment of exogenous microbes -including pathogens- than species-poor communities (Bäckhed et al., 2012). This bacterial richness further promotes a better functioning of the community by resource specializing and, in turn, using limited resources more efficiently (Lozupone et al., 2012). As it happens in macroecosystems (Oliver et al., 2015), the inherent properties of resource specializing and resilience from bacterial species-rich communities may be reflected in the overall health status of the host. Given that the microbiome is composed of a dynamic community of bacteria, it is constantly susceptible to change due to age, diet, environment, and diseases among other factors (Barko et al., 2017). Hence, substantial changes in species proportions or richness within the gastrointestinal microbial community of a host may lead to dysbiosis, which has been associated to digestive, neurologic, metabolic, and respiratory affections in mammals (Zheng et al., 2020).

Land-use changes for agricultural use and urbanization can negatively affect wildlife health, especially by altering food availability and diet composition, increasing the exposure to agrochemicals, and increasing the contact with humans, domestic animals, and their pathogens. These factors may disrupt the normal gut microbiota and, in consequence, increase the incidence of pathogens which may contribute to the emergence of diseases (Rosenfeld, 2017; Zuo et al., 2018). Particularly, agriculture development has been shown to create selective pressure on invertebrates (Seibold et al., 2019) and soil microbes (Lupatini et al., 2017) through intensification practices and pesticide use. Their impact on the nutrient cycling of soil and its microbes further affects the food webs of ecosystems (de Ruiter et al., 1994; de Vries et al., 2013), which eventually may alter the intestinal microbiome of animals. However, evidence for an association between habitat degradation and gut microbiota changes in bat species has barely been explored (Alpízar et al., 2021; Ingala et al., 2019). Furthermore, information on bat microbial communities -especially these detected in feces- and their role in bat's health remain scarce. Understanding the complex relationship between host habitat and fecal bacterial composition could contribute to developing a new operational framework for the assessment of bats' health which can be applied to guide future conservation decisions.

Here, we contribute to the information gathered worldwide about the fecal microbiome of insectivorous bats by exploring the hypothesis that land-use changes and anthropogenic disturbances could shape differences in the composition of the fecal bacterial microbiome of *Pipistrellus kuhlii*. Because bats dwelling in intensive agriculture and urban landscapes may have access to a less diverse diet and be more exposed to pesticides and pollutants, we hypothesized that bats residing in near pristine ecosystems would show an increased alpha diversity of fecal samples in comparison with bats dwelling in human-disturbed ecosystems. We also expected to find significant differences in the structure of the fecal bacterial community between the different scenarios.

Methods

Study areas

Our study areas were located in Catalonia, in the northeast of the Iberian Peninsula. We selected four environmental contexts with different levels of anthropogenic environmental degradation and separated by a minimum of 10 km from each other (**Fig. 21; Supplementary material: Table S2.1**): mature and old-growth forest (D0), extensive farming and agriculture (D1), immature and secondary forest (D2), and urban and intensive agriculture landscape (D3).

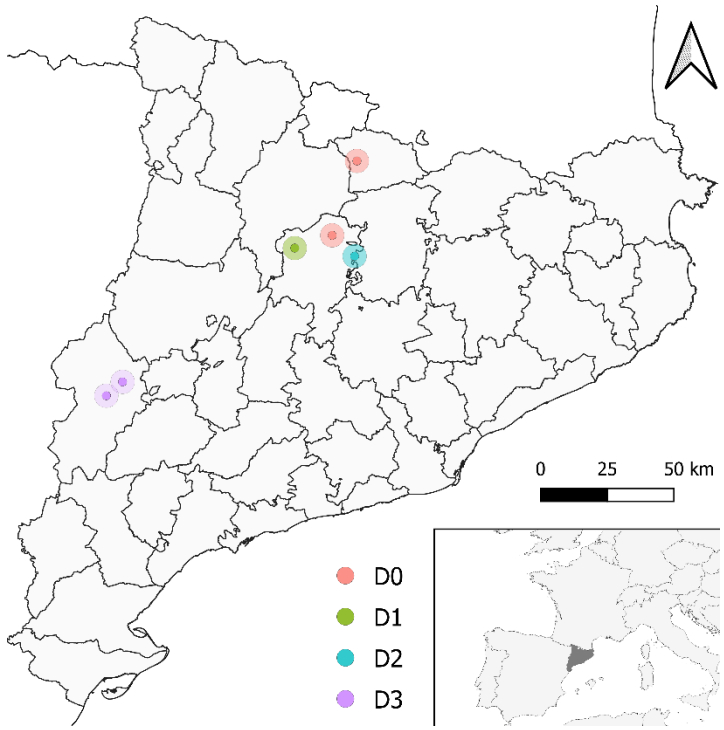


Figure 21. Map of sampling locations. Localities and their associated scenarios are depicted in the map with colored dots covering the foraging range of Pipistrellus kuhlii (4.5km): D0 (mature and old-growth forest), D1 (extensive farming and agriculture), D2 (immature and secondary forest), and D3 (urban and intensive agriculture landscape).

Mature forests from this study were composed of large trees with abundant suitable roosts for bats and were assumed to be free of human disturbance. The selected youth forest had been under logging pressure and habitat

fragmentation for decades and was represented by a less complex vegetation structure than mature forests and scarce suitable roosts for bats. In scenario D1, the main use of the soil was pastureland for bovine grazing and crop fields with traditional pest management and moderate pesticide use. The urban and intensive agriculture landscape selected (D3) was located in the Segrià County, which comprises one of the largest intensive pig industry and intensive agriculture area of Spain, and Europe (Generalitat de Catalunya, 2020; Gilbert et al., 2018).

Animals and samples

We selected the Kuhl's pipistrelle (*Pipistrellus kuhlii*), a sedentary and synanthropic bat species in NE-Spain, as a study model. This species indistinctively inhabits open forests and anthropogenic landscapes from the Mediterranean basin and extends throughout Europe. From what is known so far, the home range of this species is less than 2 km^2, and foraging sites can be as far as 4.5 km (Amichai & Korine, 2020). Its ability to dwell in pristine and altered ecosystems makes this species an interesting model for assessing the impact of anthropogenic disturbance on fecal bacterial composition.

Thirty-one Kuhl's pipistrelles were captured across the four studied landscapes from mid-July to early September 2021 (**Supplementary material: Table S2.2**). Bats were captured using harp traps and mist nets and placed in individual and clean cloth-holding bags until sampling. Fecal samples were collected directly from bats or from cloth-holding bags using sterile forceps. Samples were individually placed in sterile Phosphate Buffered Saline (PBS) (Lonza, Basel, Switzerland) and immediately deposited in dry ice. Once in the laboratory facilities, they were stored at -80ºC until DNA extraction.

Bats included in this study were individually marked with a circular wing biopsy (3 mm punch) which was used for other research purposes but also allowed us to avoid resampling. All sampling procedures followed the EUROBATS best practices (Battersby, 2010) and at least one wildlife veterinarian was present in all the captures in order to guarantee the welfare of the captured individuals. No bats resulted harmed or died during the performance of the study.

DNA extraction, generation of 16S rRNA gene amplicon sequences and library preparation for next-generation sequencing

Fecal DNA was extracted using QIAamp PowerFecal Pro DNA Kit (Qiagen, Hilden, Germany) following manufacturer's instructions. Initial DNA of the samples was quantified by Qubit Fluorometric Quantification High Sensitivity Assay (Invitrogen, California, USA). 16S rRNA was selectively amplified from genomic DNA by the polymerase chain reaction (PCR) according the SQK-RAB201 Nanopore Kit using universal bacterial primers 27F (5'-AGA GTT TGA TCC TGG CTC AG-3') and 1492R (5'-GGT TAC CTT GTT ACG ACT T-3'), enabling the amplification of approximately 1500 bp of the 16S rRNA gene. PCR amplification was performed in 50 µl of PCR mix comprising 25 µl mix reaction buffer 2 × (LongAmp Taq 2X master mix, New England Biolabs); 14 µl of ultra-pure water; 1 µl of each primer 10 µM; and 10 µl of DNA. The temperature and cycling conditions were as follows: first, preheating at 95 °C for 1 min; then 25 cycles at 95 °C for 20 secs; 55 °C for 30 secs; 75 °C for 2 min; and a final incubation at 65 °C for 5 min. Library construction was performed using the Rapid 16S Amplicon Barcoding Kit (SQK-RAB201) from Oxford Nanopore Technologies (ONT, Oxford, United Kingdom). Two sequencing runs of 20 and 11 multiplexed samples were carried out on a MinION sequencer (ONT) using a brand new R9.6 flow cell.

Data analysis

All 16S rRNA sequences were obtained by the MinKNOW suite (Baloğlu et al., 2021) and basecalled with Guppy 3.0. (ONT). Reads were filtered by length (> 1500 bp) and quality (> 10) using NanoFilt 1.1.0 (de Coster et al., 2018); adapters and barcodes were trimmed with qcat-1.1.0 (ONT). Taxonomic assignment at genus level was carried out with Centrifuge 10.3-beta (Kim et al., 2016), using Silva 132 database (Quast et al., 2012) based on a 95% of identity threshold. Afterwards, taxa with single read counts were removed. In addition, low count filter was set to a minimum read count of 4 with a 20% prevalence in the samples. Finally, filtered data was normalized using total sum scaling (TSS). Plots and analysis of microbiomes structure and diversity were made with Pavian-0.3 (Breitwieser & Salzberg, 2020) and MicrobiomeAnalyst (Dhariwal et al., 2017).

Statistical analysis

Alpha diversity of each sample was estimated by the Shannon index. Differences between alpha diversity indices were assessed using the Kruskal–Wallis test (Kruskal & Wallis, 1952) with a significance threshold set at $p < 0.05$. Additionally, beta diversity was determined using Bray–Curtis distances and localities were compared using the nonparametric analysis of similarities (ANOSIM) test (Chang et al., 2022). Regarding the study of differential taxa among scenarios, a Linear Discriminant Analysis Effect Size (LEfSe) (Chang et al., 2022) algorithm with a LDA effect size threshold of 2 (on a log10 scale) was applied at phylum, family, and genus levels. Moreover, core (taxa shared by 100% of samples), accessory (taxa shared by samples from 2 or 3 scenarios) and exclusive microbiomes (taxa found exclusively in one scenario) were identified using the R package vegan (Oksanen et al., 2007); and the Venn diagram was obtained using the VennDiagram package (Chen & Boutros, 2011). Abundance threshold for the core microbiome analysis was set to 0.01. Random effects of sex and locality on alpha and beta diversity were tested with Kruskal–Wallis and a Permutational Mantel test (9999 permutations) based on Spearman's rank correlation rho, respectively. All statistical analyses were performed using R version 4.1.3 (R Core Team, 2022).

Results

The entire 16S gene (≈ 1.5 kb) from the fecal microbiomes of 31 bats was sequenced, for which a total of 4,829,302 reads (7.2 Gb) were obtained with an average of 114,988 ± 43,577 reads per sample. The percentage of taxonomic assignment at the genus level was 99.9% of the total sequences obtained and rarefaction curves of richness against sequence sample size reached asymptotic growth (**Fig. 22**).

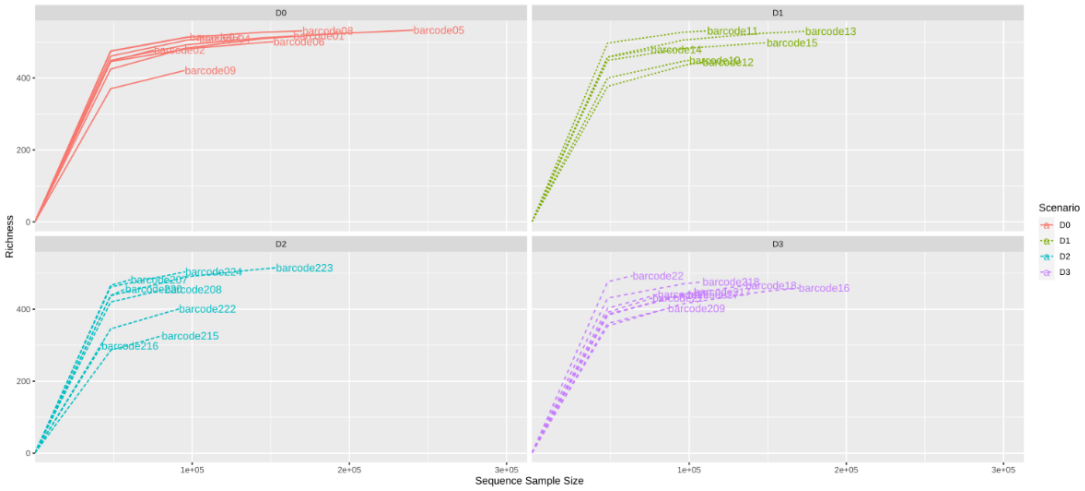


Figure 22. Rarefaction curves of each sample, separated by scenario. Sequence sample size (number of reads generated) and genus richness are depicted on the X and Y-axis, respectively.

Taxonomic composition

Firmicutes and Proteobacteria were the most abundant phyla in all scenarios (**Fig. 23**). Firmicutes showed a relative abundance of 58.6% ± 31.1 in scenario D0, 70.63% ± 33.7 in scenario D1, 45.3% ± 28.8 in scenario D2 and 58.1% ± 22 in scenario D3. On the other hand, Proteobacteria showed an abundance of 39.88% ± 31.9 in scenario D0, 28.32% ± 33.8 in scenario D1, 54.3% ± 29 in scenario D2 and 41.4% ± 22 in scenario D3. Actinobacteria was the third most abundant phyla in all the samples. Nevertheless, its relative abundance was < 1% for all scenarios, ranging from 0.09% in scenario D2 to 0.40% in scenario D0.

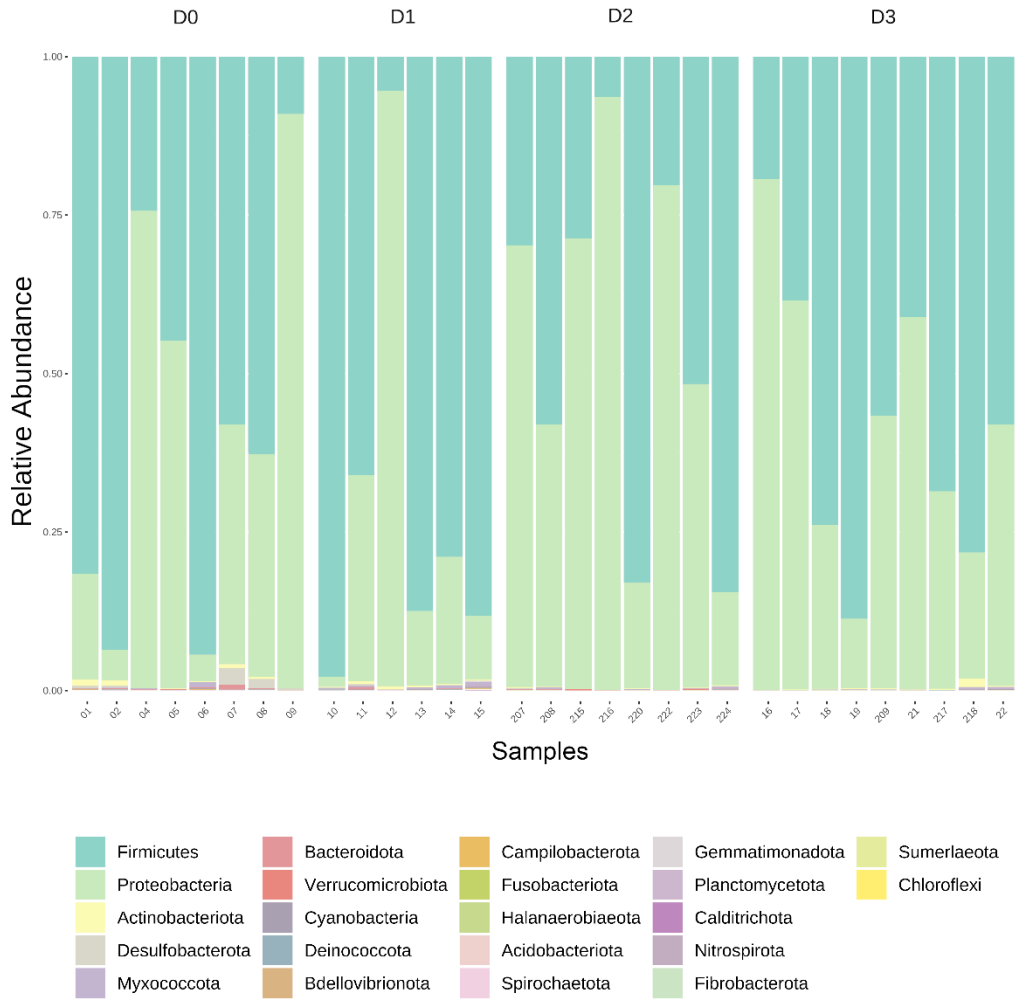


Figure 23. Stacked bar plot of relative abundances of phyla from scenarios D0 (mature and old growth forest), D1 (extensive farming and agriculture), D2 (immature and secondary forest), and D3 (urban and intensive agriculture). Fecal samples are displayed on the bottom and scenarios displayed at the top.

Although there was not a strict pattern of relative abundance across all scenarios, *Enterococcus*, *Escherichia/Shigella*, *Bacillus* and *Enterobacter* were consistently found as the most abundant genera, showing relative abundances (among scenarios) of 15.4% ± 15.1 for *Enterococcus*, 10.5% ± 10 for *Escherichia/Shigella*, 6.7% ± 7 for *Bacillus* and 5.5% ± 6 for *Enterobacter* (**Fig. 24**). *Enterobacter* displayed both high and low dominance across samples with a range of 0.02% to 20%. The most abundant genera in particular scenarios included *Serratia* (4.56% ± 0.61), *Lachnoclostridium* (4.13% ± 0.25), *Candidatus* Soleaferrea (4.12% ± 0.29), *Pseudomonas* (3.7% ± 0.59), and *Carnobacterium* (3.38% ± 0.56) in D0; *Staphylococcus* (11.7% ± 3), and *Vespertiliibacter* (2.59% ± 3) in D1; *Serratia*

(4.23% ± 0.32), *Lonsdalea* (3.58% ± 0.6), *Hafnia* (3.38% ± 0.7), *Ricketsiella* (3.1% ± 0.8) and *Klebsiella* (2.8% ± 0.56) in D2; and *Lactococcus* (8.9% ± 0.12), *Carnobacterium* (3.51% ± 0.85) and *Klebsiella* (3% ± 0.6) in D3.



Figure 24. Stacked bar plot of relative abundances of the top 30 most abundant genera from scenarios D0, D1, D2 and D3. Fecal samples are displayed on the bottom and scenarios displayed at the top.

Diversity analyses

Alpha diversity analyses (**Fig. 25A**) revealed differential diversity patterns for the studied scenarios, showing Shannon index values varying between 2.8 (Scenario D3) and 3.4 (Scenario D0). Scenario D0 showed the highest mean alpha diversity

(Shannon's Index = 3.45 ± 0.38), followed by D1 (3.14 ± 0.57), D2 (3.1 ± 0.38) and D3 (2.7 ± 0.38). Furthermore, Shannon index varied significantly between the four scenarios (H: 7.935; $p < 0.05$), while no significant variance was found between localities (H: 10.45, $p > 0.05$) and the sex of the bats (H: 105, $p > 0.05$). Pair-wise comparisons of Shannon values showed significant differentiation between scenarios D0 and D3 (**Supplementary material: Table S2.3**). On the other hand, beta diversity obtained by Bray–Curtis distance showed weak statistical differentiation of community structure across scenarios (**Fig. 25B**; ANOSIM's R: 0.12; $p < 0.02$), and pair-wise comparison showed significant differentiation between D0-D3 and D1-D3 (**Supplementary material: Table S2.3**). No significant correlation between beta diversity and geographic distance was found (Mantel's r: 0.013, $p > 0.05$).

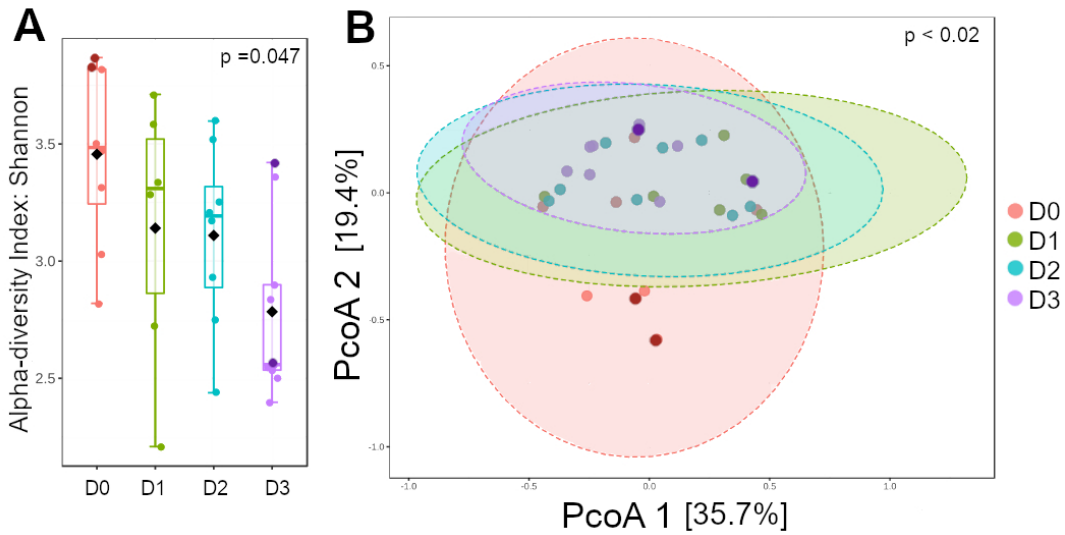


Figure 25. Alpha (**A**) and beta (**B**) diversity measures of the four scenarios: D0, D1, D2 and D3. Boxplot of Shannon's Diversity Index (Kruskal–Wallis statistic: 7.935; $p = 0.047$) is depicted in panel (A). A PcoA of Bray–Curtis distance matrix (ANOSIM's R: 0.12; $p < 0.02$) is shown in panel (B). Different color intensities in dots from scenarios D0 and D3 are used to indicate the different localities sampled within each scenario (**Fig. 21; Supplementary material: Table S2.1 and S2.2**)

Scenarios D0 and D3 displayed the greatest differentiation between each other, both in terms of composition regardless of taxonomic hierarchy (**Figs. 23** and **24**), and alpha and beta diversities (**Fig. 26**). Because only scenario D0 included lactating females, we tested whether these samples were influencing the overall results. No significant differentiation in alpha (Kruskal–Wallis statistic: 7; $p = 1$) and beta diversity (ANOSIM's R: -0.16923; $p = 0.783$) were found between males

and females (all lactating) within scenario D0 (**Supplementary material: Fig. S2.1**).

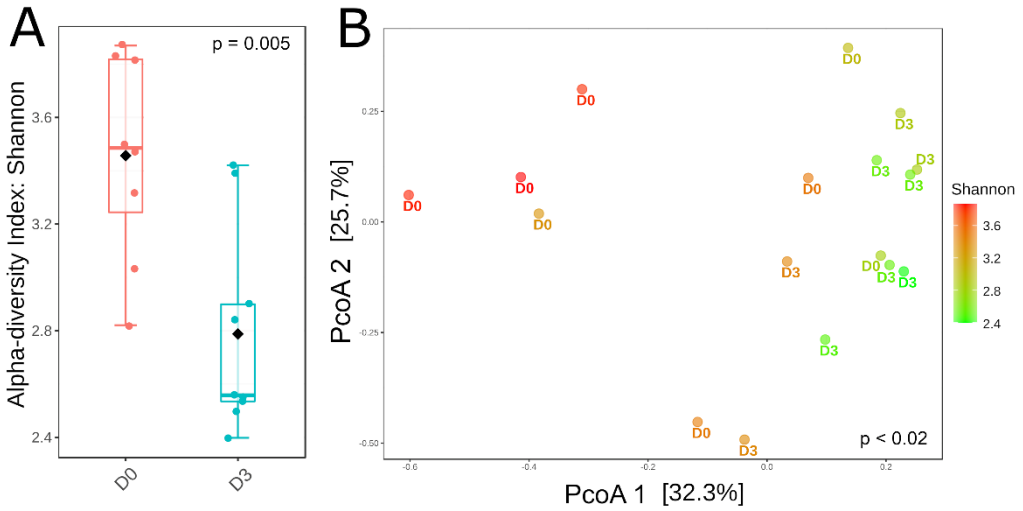


Figure 26. Alpha (**A**) and beta (**B**) diversity measures of scenarios D0 and D3. Shannon's Diversity Index for D0 and D3 is shown in panel A (Kruskal–Wallis statistic: 64; p = 0.005). Bray–Curtis distance matrix (ANOSIM's R: 0.28; p < 0.002) for scenarios D0 and D3 is illustrated in panel (B). Alpha diversity is also shown in the ordination plot through a color gradient from green (low value) to red (high value).

Differential abundance analyses

Linear discriminant analyses effect size (LEfSe) of all scenarios showed a distinct pattern of differentially abundant taxa, which were found to be significantly more abundant in scenarios D0 and D1 than D2 and D3 (**Fig. 27**). Phylum Gemmatimonadota; orders Burkholderiales, Xanthomonadales, Bdellovibrionales and Gemmatimonadales; and families Xanthomonadaceae, Oxalobacteraceae, Bdellovibrionaceae, Nitrosomonadaceae, Alcaligenaceae, Nocardioidaceae, Gemmatimonadaceae and Rhodocyclaceae were significantly more abundant in scenario D0. Phylum Spirochaetota; orders Spirochaetales and Coxiellales; and families Spirochaetaceae and Coxiellaceae were significantly more abundant in scenario D1. With the exception of Alcaligenaceae, which appeared in high-to-moderate abundance in scenario D3 and low-to-moderate abundance in D1, all aforementioned taxa were found in low abundance in scenarios D2 and D3. No significant taxa were found at the class and genus levels.

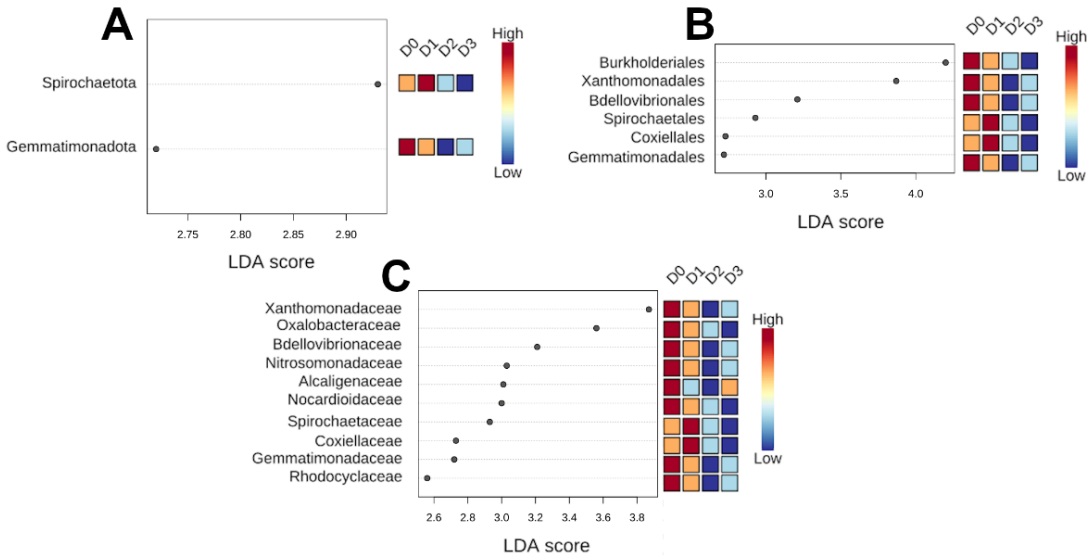


Figure 27. Linear discriminant analysis effect size (LEfSe) of all scenarios. Phylum, order and family taxonomic levels are respectively shown in panels (**A, B** and **C**). LEfSe threshold of 2 (on a log10 scale) and a significance threshold of 0.05 were set. p values were adjusted for false discovery rate (FDR) method.

Core microbiome

Core community analysis indicated that 1,293 genera were shared by all sites (**Fig. 28A**). Scenario D0 contained the largest number of exclusive genera (n = 239), while scenario D2 comprised the least (n = 50). A plot of the distance matrix calculated from binary data (presence/absence of genera) roughly depicted two partially overlapping clusters composed of Scenarios D0-D1 and D2-D3 (**Fig. 28B**).

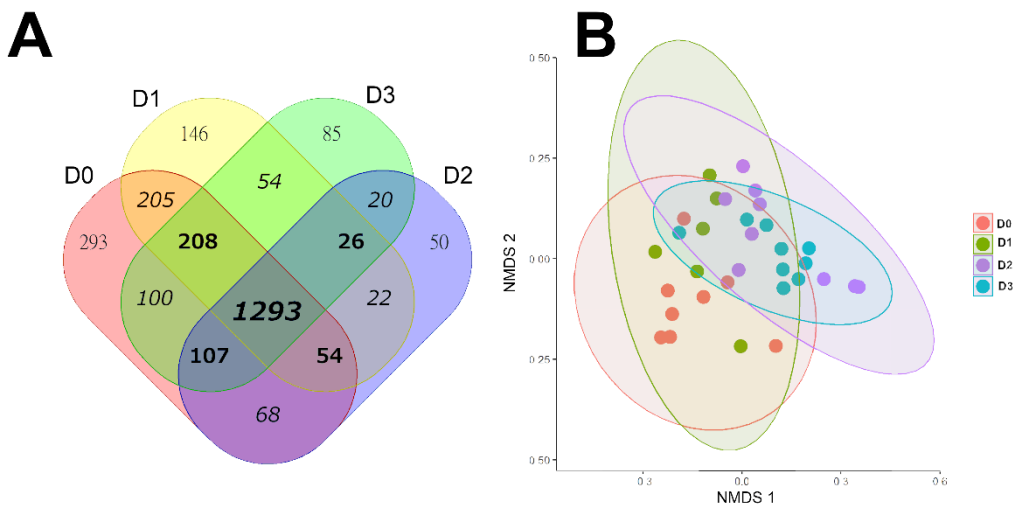


Figure 28. Core community analysis of the four scenarios studied. A Venn Diagram of genera shared across and exclusive to localities is shown in panel (**A**). A Non-metric Multidimensional Scaling plot from a Jaccard distance matrix of presence/absence data is depicted in panel (**B**).

Discussion

Similar fecal microbial communities are shared among *P. kuhlii* and other insectivorous bats, regardless of the quality of the environment

According to the literature and supported by our results, chiropteran's gut microbiota is mainly represented by Firmicutes and Proteobacteria, while the anaerobic phylum Bacteroidetes is underrepresented in bats, but highly dominant in other terrestrial mammals (Dong-Lei et al., 2020). The fast gastrointestinal transit of bats and their adaptation to flight is believed to be responsible for this Proteobacteria-dominated gut microbiota, as also observed in birds (Jones et al., 2022).

Although the fecal microbiome is strongly influenced by gut microbial communities, guano samples may also be strongly influenced by environmental factors (Fofanov et al., 2018). Nonetheless, the most abundant genera found in fresh feces of *P. kuhlii* from the four scenarios of our study are consistent with the most common taxa identified by NGS in guano samples from different bat species around the globe, particularly *Enterococcus* and *Bacillus* genera (Banskar et al., 2016; de Leon et al., 2018; Li et al., 2018).

Bacteriological analyses showed the presence of *Escherichia adecarboxylata*, *Citrobacter freundii*, *Klebsiella oxytoca*, *Enterobacter agglomerans*, *E. aerogenes*, *E. gergoviae*, *Proteus vulgaris* and *Streptococcus faecalis* in feces of *P. kuhlii* from Italy (di Bella et al., 2003). While *Enterobacter*, *Klebsiella*, *Streptococcus*, *Escherichia*, and *Citrobacter* genera were also present in our analyses, different isolation and detection techniques could have contributed to different findings such as the high abundance of *Enterococcus* genera across all our studied scenarios. Since most microbes remain unculturable, using culture-independent analyses in microbiome studies is essential to better understand and characterize bacterial communities and their functions (Bodor et al., 2020). As demonstrated by Newman et al. in 2018, the observation of a high abundance of the genus *Vespertiliibacter* in scenario D1 from our study would have been neglected by culture-dependent analyses. This Pasteurellaceae was isolated for the first time

in Germany in the upper respiratory tract of three different bat species of the family Vespertilionidae, including one *P. pipistrellus* (Mühldorfer et al., 2014). More recently, it has also been isolated in guano samples from a maternal colony of *Tadarida brasiliensis* (family Molossidae) in New Mexico (US), showing a greater presence on fresh guano samples over surface or subsurface guano samples (Bodor et al., 2020). However, the role of the genus *Vespertiliibacter* as a commensal or opportunistic pathogen for bats is yet to be elucidated. As a dominant genus in samples from scenario D1, it may be responsible of meaningful functions in the microbiome of these bats.

Despite partial overlapping of fecal microbial communities exists between bats with different trophic strategies (Banskar et al., 2016), diet specialization is generally linked with specific microbiota capable of nutrient assimilation (Ley et al., 2008). In this sense, significant differences in microbiome composition between frugivorous, insectivorous and piscivorous bats have been reported by several authors (Aizpurua et al., 2021; Cláudio et al., 2018; Li et al., 2018; Lutz et al., 2019). More recently, different bat dietary habits have also been linked to differences in metagenome functions which may be linked to specific metabolic pathways (Ingala et al., 2021). Several most abundant genera from our study (*Carnobacterium, Serratia, Hafnia, Enterococcus* and *Lonsdalea*) were also found to be significantly abundant in some insectivorous bat species across Europe, China, Israel, Mexico and Costa Rica, compared to piscivorous bat species (Aizpurua et al., 2021). Notably, we found that *Serratia*, *Hafnia* and *Lonsdalea* genera were differentially abundant in *P. kuhlii* from scenario D2. Insectivorous bat species from the former study included *P. kuhlii*, other vespertilionid and rhinolophid bats, but specific characteristics of the sampling localities were not specified. Moreover, none of the most differentially abundant genera present in piscivorous bats were represented in our study (Aizpurua et al., 2021), further supporting the current scientific data about the significant impact of trophic guilds on fecal bacterial communities (Ley et al., 2008). Additionally, the predominant bacterial genera in fecal samples from scenario D0 were found to particularly overlap with fecal samples of *P. pipistrellus* and other insectivorous bats from the Netherlands, sharing *Carnobacterium*, *Serratia* and *Pseudomonas* as the most abundant genera (Wolkers-Rooijackers et al., 2018). Interestingly, we would have anticipated greater similarities between either scenario D1, D2 or D3 since the bats from the latter study were captured in lime-stone mines near villages rather than in near pristine habitats. These counterintuitive results may

be explained by the fact that mines are usually located in rural areas and, once mine activities cease, sites tend to be partially restored to pre-mining conditions, with minimal human presence (Environmental Law Alliance Worldwide, 2010). However, persistent environmental pollutants of different mining activities can also alter the fecal microbiome of bats and their impacts still need to be clarified.

Intensive agriculture and urban landscapes may be enhancing intestinal bacterial lineages with pollutant-degrading properties

By linear discriminant analyses effect size (LEfSe), differential abundance taxa, including the order Burkholderiales, appeared in high abundance in scenario D0. The order Burkholderiales constitutes a metabolic and ecologically diverse bacterial linage, which includes human opportunistic bacteria (mostly nosocomial), animal and plant pathogens, bacteria present in wastewater and sludge, and bacteria naturally present in soil, freshwater and sediment (Pérez-Pantoja et al., 2012; Rosenberg et al., 2006). This order comprises the families Alcaligenaceae, Burkholderiaceae, Comamonadaceae, Oxalobacteraceae, Sutterellaceae, Sphaerotilaceae and Burkholderiales genera incertae sedis (Schoch et al., 2020). Our results showed that, while Oxalobacteraceae and Alcaligenaceae were highly abundant in scenario D0, Alcaligenaceae was also the only family overrepresented in scenario D3.

Species belonging to the family Oxalobacteraceae have shown the ability to invade and persist in different niches, such as plant tissues, Antarctic soil, rivers and lakes, groundwater and contaminated soils among others (Rosenberg et al., 2006). Moreover, species belonging to this family have been recovered from patients with clinical disease and are considered mild and opportunistic human pathogens (Rosenberg et al., 2006). On the other hand, the family Alcaligenaceae includes some well-recognized primary animal and human pathogens such as the genus *Bordetella* (Rosenberg et al., 2006) and *Taylorella* (World Animal Health et al., 2015). Bacteria from the family Alcaligenaceae have also been isolated from biogas slurry (genus *Advenella*) (Wang et al., 2016), activated sludge (genera *Caenimicrobium* and *Pusillimonas*) (Felföldi et al., 2017; Koh et al., 2019) and other samples from wastewater treatment plants (genera *Parapusullimonas* and *Pigmentiphaga*) (Kim et al., 2010; Yoon et al., 2007). Other bacteria from this family have been isolated from freshwater sources (genus

Parvibium) (Chen et al., 2018) or have shown the ability to thrive in different environments such as the genus *Pusillimonas*, isolated from wastewater treatment plants, farm soil and poultry manure among others (Koh et al., 2019; Lee et al., 2010; Lin et al., 2022). Although the ecological function of bacteria shouldn't be defined solely by their source of isolation, many genera from the family Alcaligenaceae have shown the ability to degrade Polychlorobiphenyl (PCB) (Fedi et al., 2001), neonicotinoids (Wang et al., 2013) and aromatic hydrocarbons (Pérez-Pantoja et al., 2012; Seo et al., 2009), pointing out their potential in removing environmental pollutants. The high abundance of Alcaligenaceae in bats from scenario D3 may be explained by the gut bacterial community's response to pesticides and other pollutants exposure, promoting the growth of detoxifying bacteria. The differentially high abundance of this family in scenario D0 in contrast to D1 and D2 may not be so straightforward. Evidence of atmospheric transport and deposition of pollutants into mountain ecosystems (cold trapping effect) have been reported for several pollutants, including microplastics (Allen et al., 2019) and semi-volatile organic chemical pollutants (Fernandez et al., 2021). Lles de Cerdanya, one of the sampled localities in scenario D0, is located in a montane area of the southeastern Pyrenees. Therefore, despite being far from major populations or industrial centers, the high elevation of this village may aid the deposition of air chemical pollutants through rain, snow, fog, or wind.

The family Alcaligenaceae has been described in the fecal microbiome of different bat species ((Dong-Lei et al., 2020; Gaona et al., 2019; Vengust et al., 2018), regardless of their dietary habits or geographic distribution. Furthermore, this family of proteobacteria was found to be characteristic of Palla's Long-tongued bats (*Glossophaga soricine*) foraging in natural forests in comparison to those foraging in organic or conventional banana monocultures where pesticides were used (Alpízar et al., 2021). As a highly diverse bacterial linage, further research is needed to understand Alcaligenaceae's functional significance in the gut microbiome of bats.

Multiple variables may explain why healthier ecosystems support more resilient fecal bacterial microbiomes

We demonstrated that the richness and diversity of the fecal bacterial microbiome of *P. kuhlii* bats were affected by the level of anthropogenic

disturbance (Shanon Index; $p < 0.05$), while gender and geographical distance between sampling sites had no significant impacts on the microbiome composition (Mantel test; $p > 0.05$). Variation in gut microbial diversity of bats has been linked to shifts in season (mainly pre- and post-hibernation) and specific reproductive states such as lactation or pregnancy (Dietrich et al., 2018; G. Xiao et al., 2019). Three females from scenario D0 were captured during the lactation period, when microbial diversity often increases (Gaona et al., 2019). However, no significant differences in alpha and beta diversity were found between these lactating females and the rest of the bats from the same scenario, thereby ruling out any potential effect of lactation on the overall microbial diversity.

In our study, *P. kuhlii* inhabiting pristine forests displayed richer fecal bacterial microbiomes than those dwelling in degraded environments such as farmlands and urban landscapes. Defining what represents a healthy gut microbiome has proven difficult, particularly due to the high interindividual variability (McBurney et al., 2019). However, resistance and resilience to external perturbations are among the crucial characteristics of a healthy microbiome (Dogra et al., 2020; Lloyd-Price et al., 2016) and both are positively affected by high microbial diversity (Lozupone et al., 2012). Accordingly, the higher alpha diversity displayed by feces of Kuhl's pipistrelles from undisturbed forests may be indicative of a more resilient gut microbiome compared to that of bats dwelling in disturbed environments.

The gut microbiota at the population level is supposed to be evolutionarily selected and to remain relatively stable across individuals of the same species (Jalanka-Tuovinen et al., 2011). Nevertheless, environmental factors can mediate bacterial community structure as demonstrated in human and other animal studies. Such is the case of industrialization, which has been related to shifts in the relative abundance of bacterial lineages and reduced microbiota diversity in human populations (Clemente et al., 2015; Smits et al., 2017). Human activities have also modified how wild animals move through the landscape and interact with its biological components (Lewis et al., 2021). In this sense, shifts in intestinal microbiota composition and diversity from wild animals have been reported as consequences of habitat fragmentation and encroachment (Amato et al., 2013; Chang et al., 2016). Human-disturbed and fragmented landscapes were also associated with significantly lower alpha diversity in the gut microbiome of

Tome's spiny rats (*Proechimys semispinosus*) (Fackelmann et al., 2021). However, when considering habitat fragmentation without additional anthropogenic disturbances such as contact with humans and domesticated animals, no impacts were detected. Similar to our study, Barelli et al. (2015) showed that colobus monkeys (*Procolobus gordonorum*) inhabiting undisturbed forests from Tanzania had significantly higher alpha diversity than those from disturbed forests. Through functional analyses they concluded that this variation was associated with dietary changes and diversity derived from anthropogenic habitat degradation (Barelli et al., 2015).

The Kuhl's pipistrelle is considered a selective-opportunist feeder, targeting larger preys over the smaller ones. The most important orders of insects in the diet of *P. kuhlii* are *Coleoptera*, *Diptera*, *Hemiptera*, *Heteroptera*, *Hymenoptera* and *Lepidoptera*. In the Iberian Peninsula, *Diptera* and *Lepidoptera* account for the main representative orders of their diet, and these include several agricultural pests such as *Pectinophora gossypiella* (i.e. cotton pest) (Amichai & Korine, 2020). However, as an opportunistic feeder, variation in insect diversity and volumetric representation may be encountered in the diet of Kuhl's pipistrelles across different types of landscape. Further explorations of *P. kuhlii*'s diet across the different scenarios from this study could provide a better overview of how insects respond to these specific anthropogenic changes and their potential effect on the fecal microbiome of this bat species. Land use changes, particularly urbanization and agriculture, are probably responsible for the recent decline in insect abundance and biotic homogenization (Mata et al., 2014; McKinney, 2006; Uhler et al., 2021). Future research assessing the potential effects of diet homogenization on the health of insectivorous bats may highlight the relevance of specific habitat conservation strategies.

Another consequence of human activities is the release of toxic substances into air, water and land at harmful rates for living beings (Gheorghe & Ion, 2011). Therefore, wildlife that manages to thrive and adapt to urban and agricultural landscapes faces constant or temporary exposure to pollutants and pesticides. A growing body of evidence indicates that pesticides may induce changes in the gut's microbiota composition and lead to dysbiosis or alteration of the homeostasis in several host species (Giambò et al., 2021). Insectivorous and frugivorous bats forage equally in plantations -feeding on pests or crops respectively- as well as in forests or natural areas. Alpízar et al. (2021) revealed

that nectar-feeding bats *Glossophaga soricine* foraging intensively managed banana plantations had a significantly lower alpha diversity than those foraging organic banana plantations, and far lower than bats foraging natural forests. Although a less diverse diet may explain this association, no differences in diet were seen among bats foraging intensively managed plantations and those foraging organic plantations, suggesting that reduction in gut microbiota diversity may be caused by other factors such as the use of pesticides (Alpízar et al., 2021). The use of pesticides in the studied agricultural, logged, and urban landscapes from Catalonia is a common practice (personal observation). Therefore, the lower alpha diversity of Kuhl's pipistrelles from these scenarios may be further explained by similar factors to those suggested in *G. soricine* (Alpízar et al., 2021).

Our study demonstrates a correlation, rather than causal relationship, between anthropogenic pressure and the fecal bacterial microbiome of Kuhl's pipistrelles. Nonetheless, the inverse correlation found between the level of anthropogenic disturbance and the fecal bacterial microbiome richness and diversity of *P. kuhlii* indicates that near pristine and undisturbed landscapes could promote more resilient gut microbiomes. Studies on physiological parameters for different bat species during their variable and complex life cycle are needed to establish reference values and, in turn, to be able to link microbiome changes to health and disease status.

Availability of data and materials

The dataset supporting the conclusions of this article is available in the GenBank repository (https://www.ncbi.nlm.nih.gov/biosample/). Biosample numbers are available in **Supplementary material: Table S2.4**

Supplementary material: S2

Table S2.1. Environmental information of the scenarios selected.

Scenario	**D0**		**D1**	**D2**	**D3**	
Locality	Lles de Cerdanya	Sant Llorenç de Morunys	Lladurs	L'Espunyola	Lleida city	Lleida outskirts
District	Baixa Cerdanya	Solsonès	Solsonès	Berguedà	Segrià	Segrià
X,Y coordinates	42.388056,1.687222	42.136111,1.580833	42.09256,1.41260	42.06748,1.41260	41.63102,0.64231	41.57864,0.64231
[1]Elevation (m)	1471	925	834	803	155	130
[1]Surface area (km^2)	102.79	4.34	128.03	35.46	212.30	10.46*
[1]Population in year 2021 (total hab.)	288	985	187	258	140,080	2,224*
[1]Declared industrial waste in tonnes (last year of declaration)	0.01 (2019)	3 (2020)	1 (2014)	10 (2015)	24,607.22 (2020)	1 (2020)*
[2]Main land use/cover	Forest and high mountain meadows	Mature forest (±meadows)	Extensive farming and immature forest	Forest for logging and extensive farming	Intensive agriculture and urban land	Intensive agriculture and urban land
[2]Main tree cover	Red pine and oak	Red pine and oak and Holm oak	Pine and Holm oak	Pine (±Holm oak)	NA	NA
[1]Main livestock	NA	NA	Cattle, pig and poultry	Pig and poultry	Pig, poultry, cattle and sheep	Pig and poultry*

NA refers to 'non-applicable' information. *****The closest municipality to Lleida outskirts sampling point (1.5 km apart) is Albatàrrec, which has a total population of 2,224 habitants (last update in 2021) and is located 4 km apart from Lleida city. Values indicated with * apply to this municipality. **[1]***Institut d'Estadística de Catalunya (idescat), Generalitat de Catalunya*. **[2]***Cartografia dels hàbitats a Catalunya, versió 2 (2018), Generalitat de Catalunya.* Land use/cover within a 4km-buffer around each sampling point. **[3]***Agencia Estatal de Meteorología (AEMET)* and *Institut d'Estadística de Catalunya (idescat), Generalitat de Catalunya.* Annual values and averages recovered from official annual weather maps (data obtained from *Servei Meteorològic de Catalunya, SMC*).

Table S2.1. *Continued.*

Scenario	**D0**		**D1**	**D2**	**D3**	
Locality	Lles de Cerdanya	Sant Llorenç de Morunys	Lladurs	L'Espunyola	Lleida city	Lleida outskirts
[3]Average 2021 temperature (0ºC)	8	10	12	12	15	15
[3]Average 2021 minimum temperature (0ºC)	2.5	5	5	7.5	7.5	7.5
[3]Average 2021 maximum temperature (0ºC)	12.5	12.5	17.5	15	22.5	22.5
[3]Cumulative rainfall in 2020 (mm)	700	550	400	550	250	250
[3]Average frost days in 2020	100	100	50	50	25	25
[3]Average snow days in 2021	20	10	3	5	1	1
[4]Closest Air Quality station (district)	Bellver de Cerdanya	Berga	Berga	Berga	Lleida city	Lleida city
[4]Average PM2,5 detection in 2021 (ug/m3)	17.05	17.98	17.98	17.98	21.57	21.57
[4]Average PM2,5 detection in 2021 (ug/m3)	8.75	9.27	9.27	9.27	13.79	13.79

[3]*Agencia Estatal de Meteorología (AEMET)* and *Institut d'Estadística de Catalunya (idescat), Generalitat de Catalunya.* Annual values and averages recovered from official annual weather maps (data obtained from *Servei Meteorològic de Catalunya, SMC*). **[4]***Xarxa de Vigilància i Previsió de la Contaminació Atmosfèrica (dades obertes Catalunya), Generalitat de Catalunya. The European Union determines that the assessment of the air quality must be done by 'air quality zones' because measuring every point of the territory is impossible. This system implies dividing the territory (Catalonia) into zones where pollutant levels are believed to be similar. In Catalonia, the territory is divided into 14 air quality zones (that have similar emissions and dispersion conditions), and each municipality is included in one of the 14 zones. Particular differences between the sampled localities cannot be depicted; hence, values of the closest air quality station to each locality have been illustrated in the table.*

Table S2.2. Biological data of sampled bats, time and location of sampling.

ID sample	Gender	Age	Body weight (g)	Forearm length (mm)	BMI	Sampling date	Scenario	Location	X, Y coordinates
01*	F	A	6.5	33.30	0.20	07-2021	D0	St. Llorenç de Morunys	42.136111, 1.580833
02	M	A	4.7	33.64	0.14	07-2021	D0	St. Llorenç de Morunys	42.136111, 1.580833
04	M	A	4.5	32.46	0.14	07-2021	D0	Lles	42.388056, 1.687222
05	M	A	6.0	35.28	0.17	07-2021	D0	Lles	42.388056, 1.687222
06	M	A	5.5	33.11	0.17	07-2021	D0	Lles	42.388056, 1.687222
07	M	A	7.0	32.46	0.22	07-2021	D0	Lles	42.388056, 1.687222
08*	F	A	7.5	34.89	0.21	07-2021	D0	Lles	42.388056, 1.687222
09*	F	A	7.0	34.61	0.20	07-2021	D0	Lles	42.388056, 1.687222
10	M	J	5.5	32.79	0.17	08-2021	D1	Lladurs	42.09256, 1.41260
11	F	J	5.5	34.25	0.16	08-2021	D1	Lladurs	42.09256, 1.41260
12	F	A	6.0	35.60	0.17	08-2021	D1	Lladurs	42.09256, 1.41260
13	F	A	6.0	35.25	0.17	08-2021	D1	Lladurs	42.09256, 1.41260
14	F	A	6.5	33.54	0.19	08-2021	D1	Lladurs	42.09256, 1.41260
15	F	A	6.0	35.00	0.17	08-2021	D1	Lladurs	42.09256, 1.41260
207	F	A	6.0	35.36	0.17	09-2021	D2	L'Espunyola	42.06748, 1.41260
208	F	A	6.0	33.69	0.18	09-2021	D2	L'Espunyola	42.06748, 1.41260
215	M	A	5.0	33.65	0.15	09-2021	D2	L'Espunyola	42.06748, 1.41260
216	F	A	5.0	32.53	0.15	09-2021	D2	L'Espunyola	42.06748, 1.41260
220	F	A	6.5	34.81	0.19	09-2021	D2	L'Espunyola	42.06748, 1.41260
222	F	A	5.0	32.22	0.16	09-2021	D2	L'Espunyola	42.06748, 1.41260

*Lactating females. M: male; F: female; A: adult; J: juvenile (yearling). The age of the bats was assessed by transilluminating bat wings, showing a translucent epiphyseal cartilage of the metacarpal-phalangeal joint at the 4th finger in juveniles, and rounded bony finger joint in adults. The Body Mass Index (BMI) was calculated by dividing the body weight between the forearm length.

Table S2.2. *Continued*.

ID sample	Gender	Age	Body weight (g)	Forearm length (mm)	BMI	Sampling date	Scenario	Location	X, Y coordinates
223	F	A	6.5	34.91	0.19	09-2021	D2	L'Espunyola	42.06748, 1.41260
224	M	A	6.5	36.98	0.18	09-2021	D2	L'Espunyola	42.06748, 1.41260
16	F	A	4.5	34.37	0.13	09-2021	D3	Lleida outskirts	41.57864, 0.64231
17	F	A	6.5	35.72	0.18	09-2021	D3	Lleida outskirts	41.57864, 0.64231
18	F	A	6.5	33.88	0.19	09-2021	D3	Lleida outskirts	41.57864, 0.64231
19	F	A	5.0	33.30	0.15	09-2021	D3	Lleida outskirts	41.57864, 0.64231
209	F	A	5.5	35.50	0.15	09-2021	D3	Lleida outskirts	41.57864, 0.64231
21	M	A	6.5	34.18	0.19	09-2021	D3	Lleida outskirts	41.57864, 0.64231
217	F	A	5.5	31.61	0.17	09-2021	D3	Lleida outskirts	41.57864, 0.64231
218	M	A	5.0	33.17	0.15	09-2021	D3	Lleida city	41.63102, 0.64231
22	M	A	6.5	33.79	0.19	09-2021	D3	Lleida city	41.63102, 0.64231

*Lactating females. M: male; F: female; A: adult; J: juvenile (yearling). The age of the bats was assessed by transilluminating bat wings, showing a translucent epiphyseal cartilage of the metacarpal-phalangeal joint at the 4th finger in juveniles, and rounded bony finger joint in adults. The Body Mass Index (BMI) was calculated by dividing the body weight between the forearm length.

Table S2.3 Pair-wise comparison of alpha and beta diversities between scenarios. Significant results are signaled with *.

	Snannon's diversity		Bray-Curtis distance	
	Kruskall-Wallis's s	**p-value**	**ANOSIM's R**	**p-value**
D0_D1	33	0.28	0.15	0.1
D0_D2	47	0.13	0.18	0.08
D0_D3	64	0.005*	0.28	0.002*
D1_D2	28	0.66	-0.04	0.57
D1_D3	37	0.27	0.22	0.045*
D2_D3	52	0.14	0.15	0.09

Table S2.4. Biosample accession numbers for datasets obtained in Study 2.

Biosample	Sample Barcode	Sample correspondence
SAMN27615841	barcode01	01
SAMN27615842	barcode02	02
SAMN27615843	barcode04	04
SAMN27615844	barcode05	05
SAMN27615845	barcode06	06
SAMN27615846	barcode07	07
SAMN27615847	barcode08	08
SAMN27615848	barcode09	09
SAMN27615849	barcode10	10
SAMN27615850	barcode11	11
SAMN27615851	barcode12	12
SAMN27615852	barcode13	13
SAMN27615853	barcode14	14
SAMN27615854	barcode15	15
SAMN27615855	barcode16	16
SAMN27615856	barcode209	209
SAMN27615857	barcode17	17
SAMN27615858	barcode18	18
SAMN27615859	barcode19	19
SAMN27615860	barcode21	21
SAMN27615861	barcode22	22
SAMN27615862	barcode217	217
SAMN27615863	barcode218	218
SAMN27615864	barcode220	220
SAMN27615865	barcode207	207
SAMN27615866	barcode222	222
SAMN27615867	barcode223	223
SAMN27615868	barcode224	224
SAMN27615870	barcode215	215
SAMN27615871	barcode208	208
SAMN27615872	barcode216	216

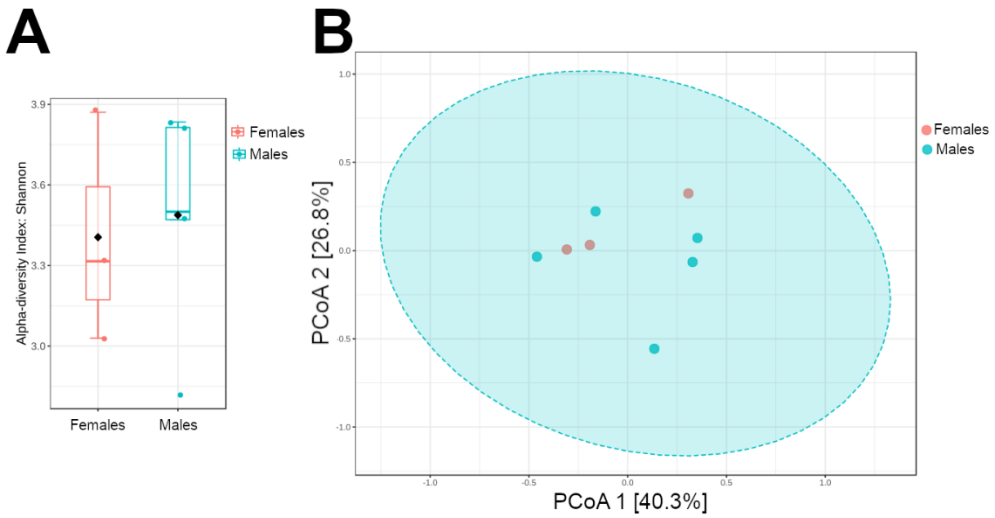


Figure S2.1. Comparison of diversity between males and lactating females from scenario D0. No significant differentiation in alpha diversity given by Shannon's Index (Panel A; Kruskal-Wallis statistic: 7; p = 1) and beta diversity given by Bray-Curtis distance (Panel B; ANOSIM's R: -0.16923; p = 0.783) were found.

Study 3. **Assessing the relationship of the fecal bacterial microbiome of five bat species from Catalonia (NE Spain) and their fecal bacterial pathogens with types of land use**

Abstract

Land use changes encompassed by other anthropogenic activities, such as urban and industrial pollution, increase the chances for wildlife to come into contact with naïve pathogens, including bacteria, from human and domestic animal origins. Additionally, these human-mediated landscape alterations have been identified as a significant contributing factor to the emergence and prevalence of several pathogens within bat populations. However, despite the evidence of bacterial microorganisms being implicated in bat disease outbreaks, there has been minimal research on this subject. In this study, we analysed the fecal bacterial microbiome of five insectivorous bat species (*Miniopterus schreibersii*, n= 21; *Nyctalus leisleri*, n= 18; *Pipistrellus kuhlii*, n= 50; *P. pipistrellus*, n= 18; *P. pygmaeus*, n= 19) from Catalonia (NE Spain), sampled in different types of land use (extensive agriculture, intensive agriculture, natural area and urban area). The main objectives were to assess the composition and structure of their fecal bacterial microbiome at the level of bacterial genus, to identify potential pathogenic bacteria (PPB) at the level of bacterial species, and to explore their association with types of land use. For this purpose, the full-length 16S rRNA of their fecal samples were analysed using the nanopore long-read sequencer MinION™. First, we performed the taxonomic assignment at the level of bacterial genus. This dataset was used to assess the structure and composition of bats' fecal bacterial microbiomes and perform diversity analyses. We found a weak differentiation in bat's fecal community structure (at the level of bacterial genus) regardless of their species. However, *M. schreibersii* samples appeared more distant from the rest of bat samples, and also showed a significantly lower alpha diversity value than the rest of bat species. In contrast, *P. kuhlii* was notable for exhibiting the highest alpha diversity value and the highest number of differentially abundant taxa. Overall, significant differentiation in alpha diversity values was found between extensive agriculture and urban landscapes. Extensive agriculture landscapes showed the highest bacterial diversity, whereas urban landscapes showed the lowest. Secondly, we repeated the taxonomic assignment but at the level of bacterial species using EMU software. We used this new dataset to identify PPB at the species level present within the fecal samples and explore the relationship between their occurrence and abundance with land use types. Additionally, we explored the same set of bats to identify potential dysbiotic microbiomes. We found 222 PPB, of which 76 were classified as recognized pathogens. The three most abundant PPB across all bat species were *Rickettsiella massiliensis*, *Enterococcus faecalis* and *Serratia fonticola*. Alpha

diversity analysis revealed no differentiation of observed PPB for each type of land use. However, alpha diversity analysis revealed different observed PPB for the five bat species assessed: *P. kuhlii* and *P. pygmaeus* exhibited statistically significant higher observed PPB than both *N. leisleri* and *M. schreibersii*. Beta diversity obtained by Bray-Curtis distance showed weak differentiation of PPB community structure across the different types of land use and the five bat species assessed. Lastly, we found 32 bats with potential dysbiotic microbiomes. Overall, we found a weaker differentiation of microbial community structure across the different sampled landscapes than what we found across the sampled bat species. Additionally, we found no correlation between the type of land use and the occurrence and abundance of PPB. However, several bacterial species overrepresented in the potentially dysbiotic microbiomes (e.g. *Yersinia enterocolitica*) have previously been isolated from pathologic samples of deceased European bats. Given the limited existing knowledge in this area, we strongly encourage the expansion of research in bat microbiology, including factors -other than types of land use- that modulate their microbiome structure and composition, as well as the impact of specific pathogenic bacteria on their health.

Background

Bacterial pathogens are considered ubiquitous in bats, but limited data exist regarding their occurrence, distribution, and transmission pathways. Furthermore, despite decades of research into pathogens carried by bats, knowledge regarding the impact of bacterial microorganisms on bat populations' health remains limited (Szentivanyi et al., 2023). Evidence of bacterial diseases in bats has been reported during necropsy procedures in European insectivorous bats (Hajkova & Pikula, 2007; Evans et al., 2009; Mühldorfer et al., 2013, 2011), Australian flying foxes (Van Brussel et al., 2023), vampire bats maintained in captivity for research purposes (Mioni et al., 2018), and in mass mortality events in bats involving *Yersinia enterocolitica* (Imnadze et al., 2020), *Y. pseudotuberculosis* (Childs-Sanford et al., 2009; Hahn et al., 2021) and *Pasteurella multocida* infections (Blehert et al., 2014).

Bats have huge variable genotypes, which correlates with the large phenotypic and behavioural diversity found across their order, which currently comprises 1,462 species (Simmons & Cirranello, 2023). Similarly, bats from different species and geographic areas exhibit large differences in their gut microbiome (Gerbáčová et al., 2020; Vengust et al., 2018), which usually correlate with their specific diet and lifestyle adaptations (Corduneanu et al., 2023). Additionally, interaction with the surrounding environment may also shape differences in their microbiome structure and composition, as demonstrated by studies under captive conditions (Edenborough et al., 2020) and in the wild (Alpízar et al., 2021; Ingala et al., 2019; Mehl et al., 2021) (**Study 2** of this thesis). Some of these interactions may have negative consequences for the host and their microbiome functioning, such as the reduced gut microbial richness and diversity found in bats from intensive agricultural landscapes in comparison to natural forests (Alpízar et al., 2021). The negative impact of urbanization on the structure and composition of the gut microbiome has also been reported, both in animal and human models (Fackelmann et al., 2021; Nurkolis et al., 2022; Tasnim et al., 2017). Microbiome changes due to urbanization, mostly characterized by reduced microbial diversity and richness, have been linked to the use of antibiotics, increased sanitation, and reduced soil microbial diversity as a consequence of intensive land practices (Tasnim et al., 2017).

Landscape modification has been identified as a significant contributing factor to the emergence and prevalence of several pathogens within wildlife

populations (Brearley et al., 2013). Particularly, recent viral pandemic events have driven global research interests into the impact of human disturbances on virus assemblages in bat populations (White & Razgour, 2020). For instance, land use changes have been shown to impact the prevalence of coronavirus in bat populations, being agriculture, deforestation, and mining the most significant factors contributing to an overall higher prevalence (Warmuth et al., 2023).

Anthropogenic activities additionally increase the chances for wildlife to be in contact with naïve pathogens from human and domestic animals origin, consequences from which have been devastating for some wildlife populations (De Garine-Wichatitsky et al., 2013; Scheele et al., 2019). Several authors have recently suggested that the habitat also influences the assembly of bacterial pathogens in several wildlife species. For instance, Fackelmann et al. (2021) reported an over-representation of DNA sequences belonging taxa associated with animal pathogens in Tome's spiny rats (*Proechimys semispinosus*) from human-disturbed landscapes in comparison to those from undisturbed landscapes. Also, other studies with non-human primates, such as the red colobus monkey and the black howler monkey, have shown similar results (Amato et al., 2013; Barelli et al., 2015). In these studies, monkeys from fragments of disturbed forests showed higher proportions of potential pathogenic bacteria than monkeys from undisturbed forests.

Influenced by the predominant focus on the role of bats as reservoir hosts for many zoonotic viruses (Nabi et al., 2021; Streicker & Gilbert, 2020), the occurrence and drivers of bacterial pathogens in bat populations regarding anthropogenic disturbances remain to be explored (Szentivanyi et al., 2023). Moreover, the health implications arising from these alterations in microbiome composition and relative abundances of pathogens are yet to be fully understood.

Due to bats' large diversity (Simmons & Cirranello, 2023), data obtained from one species cannot be applied to other species. Therefore, the first step to identifying microbiome features implicated in disease is cataloging numerous sets of microbiomes (including structure, composition, and microbial diversity) across individuals, bat species, and habitats. Additionally, exploring the impact of anthropogenic activities on the occurrence of bacterial pathogens in bat species may shed light on bacterial transmission pathways in these populations and elucidate the level of connectivity in the human-domestic animals-wildlife interface.

The objectives of the present study were 1) to assess the composition of the fecal bacterial microbiome; 2) to identify potential pathogenic bacteria at the species level; 3) to analyse their association, in terms of occurrence and abundance, with types of land use; and 4) to individually identify potential dysbiotic microbiomes, in five insectivorous bat species from Catalonia (NE Spain). First, we expected to find a similar general composition and structure of the fecal bacterial microbiome than we found in bats from **Study 1** of this thesis. Secondly, we hypothesized that bats dwelling in intensive agriculture and urban landscapes may show a higher occurrence of potential pathogenic bacteria in their fecal samples than, particularly, bats from natural landscapes.

Methods

Animals and samples

From mid-July to early October 2021, 127 bats of five different species were sampled across Catalonia, NE Spain (**Supplementary material, Table S3.1**): *Miniopterus schreibersii* (n= 21), *Nyctalus leisleri* (n= 18), *Pipistrellus kuhlii* (n= 50), *P. pipistrellus* (n= 18) and *P. pygmaeus* (n= 19). Bats were captured using mist nets and harp traps, and placed in individual and clean cloth holding bags for five to ten minutes, leaving enough time for bats to defecate. Fecal samples were collected with sterile forceps directly from their perineal area or from the cloth-holding bags and placed individually in 500 µl of sterile Phosphate Buffered Saline (PBS) (Lonza, Basel, Switzerland). They were immediately deposited in dry ice and afterward brought to the laboratory facilities, where they were stored at -80ºC until DNA extraction.

Sampled bats were weighted, their forearms were measured, and all were marked with a circular wing biopsy (3 mm) which was used for other research purposes and avoided resampling. All procedures were performed by highly experienced personnel following best practices in bat capture, handling and sampling (Mitchell-Jones & McLeish, 2004). No bats resulted harmed or died during the study. The capture and sampling of the bats were previously authorized by the Government of Catalonia (authorization code SF/0087/21).

Sampling locations

Sampling locations were classified into the following land uses: extensive agriculture (E) (n= 51), intensive agriculture (I) (n= 16), natural (N) (n= 36) and urban (U) (n= 23). Categories were created based on three landscape parameters: livestock capacity (including bovine, ovine, caprine and swine), human population and their main land cover (farming, immature and mature forests, bush and urbanisation) (**Supplementary material, Table S3.1** and **Fig. S3.1**). Locations with high livestock capacity and farming activities were classified as intensive agriculture, whereas those forested lands (immature and young forests) with high and medium livestock capacity were classified as extensive agriculture. Locations of mature and old-growth forests and bush, devoid of settlements, and without livestock presence or scattered through the landscape,

were classified as natural. Urbanized locations with a high human population density were classified as urban. Data was provided by the *Departament d'Acció Climàtica, Alimentació i Agenda Rural* (i.e. Department of Climate Action, Food and Rural Agenda) of the Government of Catalonia.

DNA extraction, 16S rRNA gene amplification and library preparation for next-generation sequencing

Fecal DNA was extracted and purified using QIAamp PowerFecal Pro DNA Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. The obtained DNA was quantified by Qubit Fluorometric Quantification High Sensitivity Assay (Invitrogen, California, USA). The 16S rRNA was selectively amplified from genomic DNA by the polymerase chain reaction (PCR) according the SQK-RAB201 Nanopore Kit using universal bacterial primers 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′), enabling the amplification of the whole 16S rRNA gene. DNA amplification was performed using 50 µl of PCR mix comprising 25 µl mix reaction buffer 2× (LongAmp Taq 2X master mix, New England Biolabs); 14 µl of ultra-pure water; 1 µl of each primer 10 µM; and 10 µl of DNA. The temperature and cycling conditions consisted of 1 min preheating at 95 °C; then 25 cycles of 20s at 95 °C; 30s at 55 °C; 2 min at 75 °C; and a final incubation of 5 min at 65 °C. Library construction was performed using the Rapid 16S Amplicon Barcoding Kit (SQK-RAB201) from Oxford Nanopore Technologies (ONT, Oxford, United Kingdom). Six sequencing runs of 24 multiplexed samples were carried out on a MinION sequencer (ONT) using a brand new R9.6 flow cell until the sample was exhausted (**Fig. 29**).

Data analysis

We used Porechop to trim the adapter sequences from the reads (Wick et al., 2017). Reads were filtered by length (over 1,000 bp; max length on 1,900 bp) and quality (> 9) using Nanofilt v2.8.0 (de Coster et al., 2018). Direct taxonomic assignment at genus level was performed using Centrifuge v1.0.4 against the SILVA 138 SSU database, using default parameters (Kim et al., 2016). Taxa with single read counts were removed and low count filter for analyses at the genus level was set to a minimum read count of 2 with a 20% prevalence in the samples (**Fig. 29**).

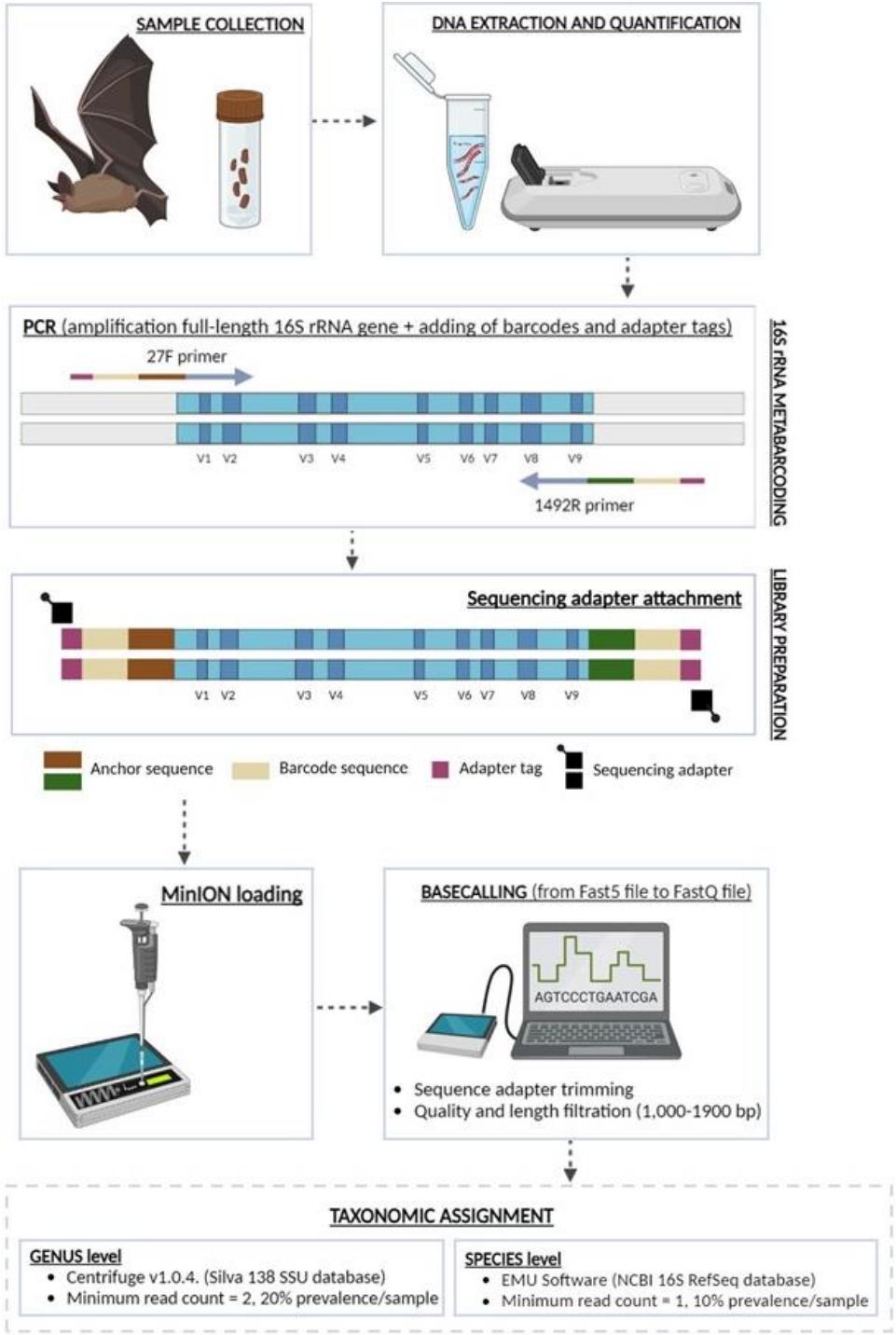


Figure 29. Workflow from sample collection (bat feces) to taxonomic assignment for the analyses at genus level and species level. Figure created with BioRender.com.

Filtered data was normalized using total sum scaling (TSS). Plots and analysis of microbiomes structure and diversity were made with MicrobiomeAnalyst (Dhariwal et al., 2017) and using R 4.3.3 and the package phyloseq (McMurdie & Holmes, 2013; R Core Team, 2022). Additionally, we used the online Venn diagram software tool for visualizing the intersections between different sets (Bioinformatics & Evolutionary Genomics (VIB-UGent), 2023).

We used the EMU software to conduct the analyses at bacterial species level (Curry et al., 2022), employing the –keep-counts option and the NCBI 16S RefSeq database (O'Leary et al., 2016). Low count filter was set to a minimum read count of 1 with a 10% prevalence in the samples (**Fig. 29**). Filtered data was also normalized using total sum scaling (TSS). Plots and analysis of microbiomes structure and diversity were made with MicrobiomeAnalyst (Dhariwal et al., 2017). We used the same online Venn diagram software tool to visualize the intersections between different sets (Bioinformatics & Evolutionary Genomics (VIB-UGent), 2023).

Statistical analysis

Alpha diversity was estimated by the Shannon index, and differences between alpha diversity indices were assessed using the Kruskal–Wallis test (Kruskal & Wallis, 1952) with a significance threshold set at $p < 0.05$. Non-Metric Multidimensional Scaling (NMDS) plot from Bray-Curtis distances was used to determine the beta diversity (Oksanen et al., 2007). Significant different taxa (at phylum, family, genus and species level) among groups were obtained by a Linear Discriminant Analysis Effect Size (LEfSe) (Chang et al., 2022) algorithm with a LDA effect size threshold of 2 on a log10 scale. All statistical analyses were performed using R version 4.3.3 (R Core Team, 2022).

Potential pathogenic bacteria (PPB) selection

Potential pathogenic bacteria (PPB) were selected based on the World Organisation for Animal Health (WOAH, founded as OIE) list of notifiable diseases (WOAH, 2020), bacterial diseases listed by the European Centre for Disease Prevention and Control (ECDC) (ECDC, 2023), waterborne bacteria classified as potential health concerns by the WHO (Lightfoot, 2003), animal bacterial pathogens listed in the Pathogen Host Interaction (PHI)-base (Winnenburg, 2006), necropsy reports of European bats with organ lesions associated to bacteria (Imnadze et al., 2020; Mühldorfer et al., 2011, 2010) and

other bacteria included in the *Red Nacional de Vigilancia Epidemiológica* (RENAVE) from Spain (RENAVE, 2013). Additionally, the dataset of bacteria obtained in our analyses was examined to identify taxa known to have caused at least one case of disease or reported from a clinical sample (Reimer et al., 2022). These bacterial species were also considered as PPB in the statistical analyses (**Supplementary material, Table S3.2)**.

Individual assessment of PPB

We individually calculated the modified microbiome dysbiosis ratio (MDR) by dividing the number of PPB by non-PPB (PPB/non-PPB) (Maslennikov et al., 2021). Since there is little information regarding specific beneficial and harmful bacteria for bat species (Szentivanyi et al., 2023), we used PPB as "harmful bacteria" (independent from their classification of recognized or opportunistic pathogens) and the rest as "beneficial bacteria". We selected bats showing an MDR inferior to 0.25 for which at least one PPB accounted for more than 20% of the total counts. With these criteria, we aimed to find potential dysbiotic microbiomes, where one PPB had a significant representation from the complete set of bacteria.

Furthermore, we investigated the relationship between dysbiotic microbiomes and bats' body condition index (BCI). First, to test whether sex had an effect on bat's BCI we performed a Fischer's exact test. The Body Condition Index (BCI) was calculated individually by dividing the weight (g) by the forearm length (mm). Afterwards, the mean BCI and their ±1.5 standard deviation (SD) were calculated for each bat species to classify bats by "poor" or "good" BCI. A "poor" BCI was given to bats with a BCI inferior to the BCI minus their 1.5 SD, and a "good" BCI was given to bats with a BCI superior to that.

Results

Fecal bacterial microbiome composition of five bat species from Catalonia

We obtained a total of 13,348,818 reads (20 Gb), with an average of 105,943 ± 96,676 reads per sample. Rarefaction curves of richness against sequence sample size reached asymptotic growth for all samples except for PM084, PM104 and PM105 (**Supplementary material, Fig. S3.2**), which were removed from the microbiome analysis at genus level.

Taxonomic composition: bat species

At the phylum level, Firmicutes and Proteobacteria were the two most abundant taxa across all bat species (**Fig. 30**). The relative abundances of Firmicutes were 55.65% ± 36.62 for *P. pygmaeus*, 44.12% ± 32.50 for *P. pipistrellus*, 56.67% ± 2.55 for *P. kuhlii*, 74.92% ± 13.65 for *N. leisleri*, and 60.64% ± 20.44 for *M. schreibersii*. The relative abundances for Proteobacteria were 43.66% ± 36.90 for *P. pygmaeus*, 55.13% ± 32.59 for *P. pipistrellus*, 40.56% ± 3.46 for *P. kuhlii*, 23.98% ± 13.36 for *N. leisleri*, and 32.16% ± 15.19 for *M. schreibersii*. On the other hand, *M. schreibersii* displayed a relatively higher abundance of Actinobacteriota (2.99% ± 3.51) compared to the other bat species.

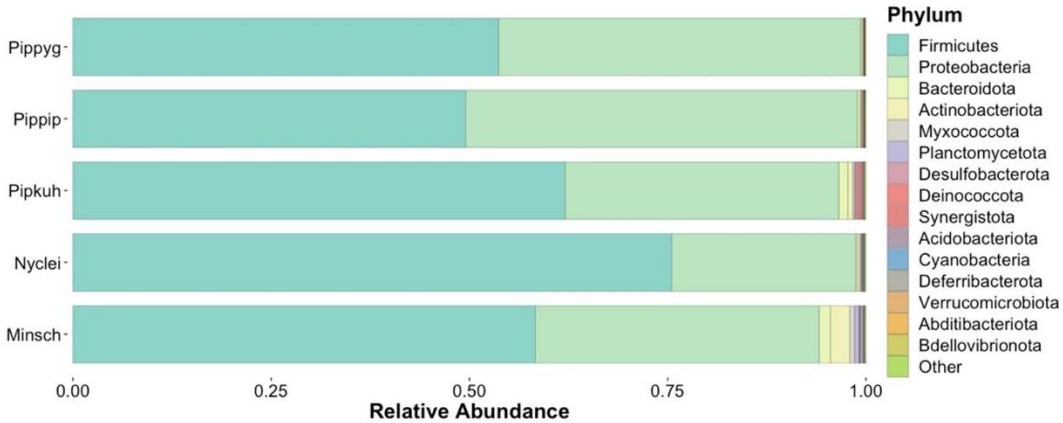


Figure 30. Stacked bar plot of relative abundances of phyla, merging small taxa <1, from each bat species (Pippyg= *Pipistrellus pygmaeus*; Pippip= *P. pipistrellus*; Pipkuh= *P. kuhlii*; Nyclei= *Nyctalus leisleri*; Minsch= *Miniopterus schreibersii*).

The most abundant genus across all bat species were *Enterococcus* (14.66% ± 18.29), *Escherichia/Shigella* (9.32% ± 13.05), *Bacillus* (7.20% ± 10.09) and *Lactococcus* (5.21% ± 13.10) (**Fig. 31**). The three specific genera with the highest relative abundances for each bat species were: *Escherichia/Shigella* (17.04% ± 23.17), *Enterococcus* (10.43% ± 16.68) and *Lactococcus* (10.42% ± 18.09) for *P. pygmaeus*; *Enterococcus* (16.50% ± 20.16), *Rickettsiella* (10.16% ± 29.58) and *Clostridium* sensu stricto 1 (8.21% ± 23.63) for *P. pipistrellus; Enterococcus* (13.39% ± 14.91), *Escherichia/Shigella* (7.73% ± 7.90) and *Bacillus* (6.57% ± 6.81) for *P. kuhlii*; *Enterococcus* (19.38% ± 22.69), *Lactococcus* (13.14% ± 23.26) and *Carnobacterium* (5.80% ± 10.28) for *N. leisleri;* and *Bacillus* (15.76% ± 17.21), *Enterococcus* (14.22% ± 20.46) and Escherichia/Shigella (12.23% ± 15.38) for *M. schreibersii*.

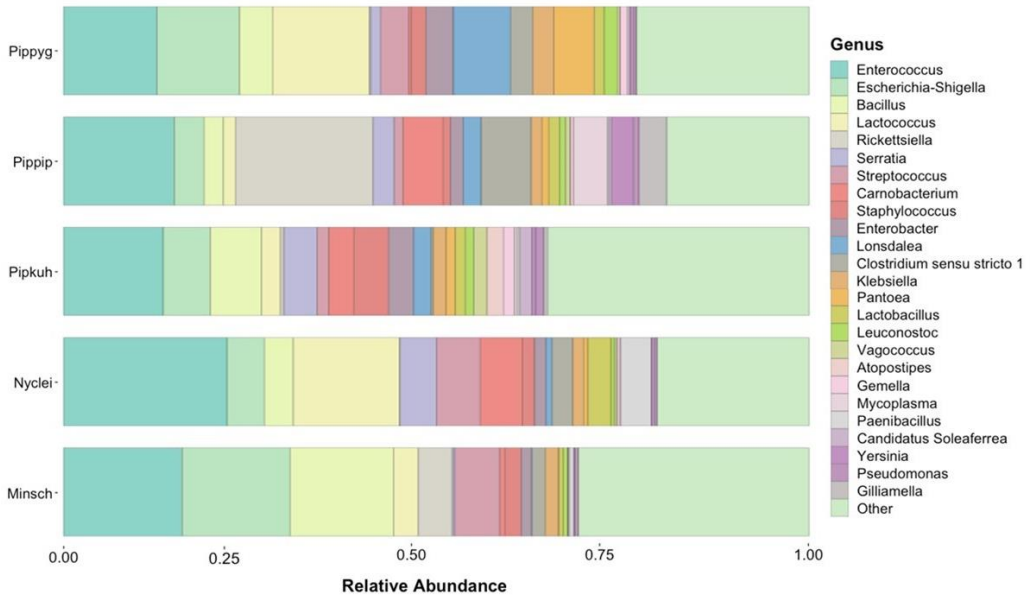


Figure 31. Stacked bar plot of relative abundances of the top 25 genera from each bat species (Pippyg= *Pipistrellus pygmaeus*; Pippip= *P. pipistrellus*; Pipkuh= *P. kuhlii*; Nyclei= *Nyctalus leisleri*; Minsch= *Miniopterus schreibersii*).

Core analysis: bat species

Core community analysis showed 1,277 (39.20%) out of 3,258 genera shared by all bat species (**Fig. 32**). *Pipistrellus kuhlii* showed the largest number of exclusive genera (n=270), followed by *M. schreibersii* (n=62), *N. leisleri* (n=38), *P. pipistrellus* (n=36) and *P. pygmaeus* (n=15).

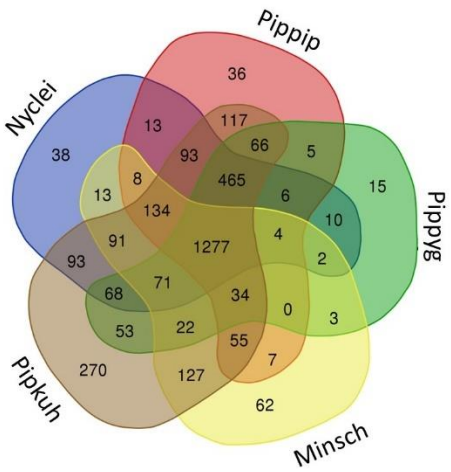


Figure 32. A Venn Diagram showing bacterial taxa at the genus level shared across and exclusive to bat species from the present study (Minsch= *Miniopterus schreibersii*; Nyclei= *Nyctalus leisleri*; Pipkuh= *Pipistrellus kuhlii*; Pippip= *Pipistrellus pipistrellus*; Pippyg= *Pipistrellus pygmaeus*).

Fecal bacterial microbiome composition, structure and diversity of five bat species from Catalonia

Diversity analyses: bat species and types of land use

We found differential diversity patterns for all bat species, being *P. kuhlii* the species with the highest mean alpha diversity, and *M. schreibersii* the species with the lowest mean alpha diversity (**Fig. 33**). Shannon index values varied between the different bat species (Kruskal-Wallis statistic: 28.79; p-value= 8.6253e-06): 2.06 ± 0.79 for *M. schreibersii*, 2.20 ± 0.67 for *N. leisleri*, 3.22 ± 0.53 for *P. kuhlii*, 2.54 ± 0.97 for *P. pipistrellus*, and 2.54 ± 0.65 for *P. pygmaeus*. A pair-wise comparison of Shannon indices from all bat species showed statistically significant differences between *P. kuhlii* and the rest of the species.

Pair-wise comparisons of Shannon index values showed significant differentiation between bats from extensive agriculture and urban landscapes (p-value= 0.014) (**Supplementary material, Fig. S3.3**). The urban landscape showed the lowest Shannon index value (2.25 ± 0.77), whereas the extensive agriculture landscape showed the highest value (2.88 ± 0.70). We also found significant differences in Shannon index values when comparing the different sampling locations (Kruskal-Wallis statistic: 28.248, p-value= 0.029). Shannon index values did not vary between the sexes of the bats (Kruskal-Wallis statistic: 0.62675, p-value= 0.73098).

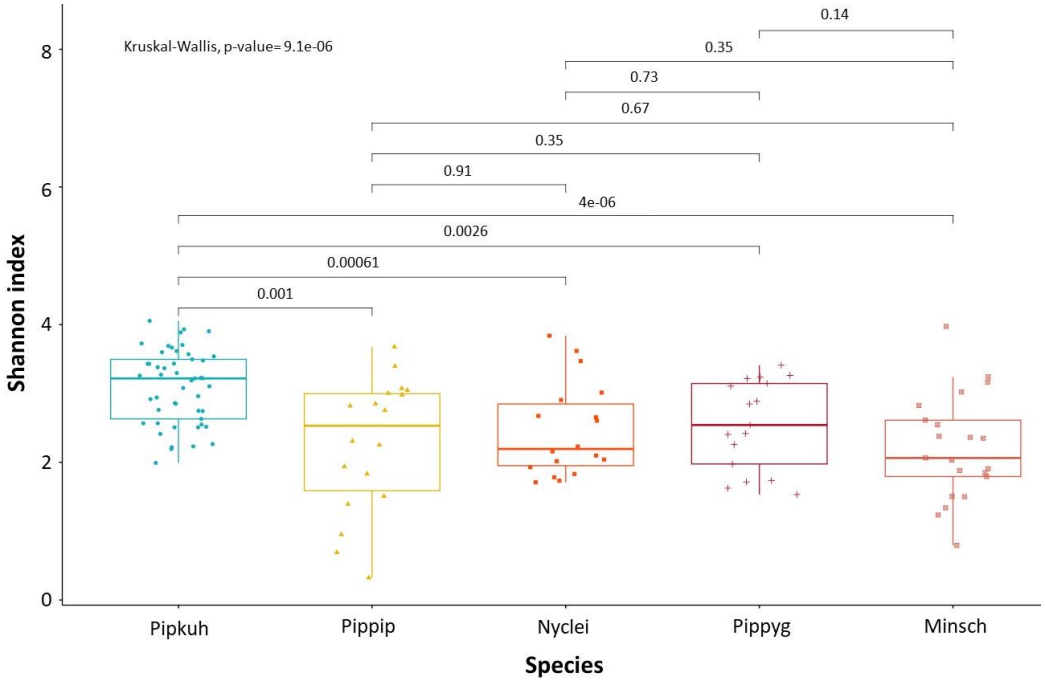


Figure 33. A boxplot of Shannon's Diversity Index (Kruskal-Wallis statistic: 28.79; p-value= 8.6253e-06) for the five studied bat species: Minsch= *Miniopterus schreibersii*; Nyclei= *Nyctalus leisleri*; Pipkuh= *Pipistrellus kuhlii*; Pippip= *Pipistrellus pipistrellus*; and Pippyg= *Pipistrellus pygmaeus*. Significant values are those with a p-value <0.05.

Since we had a large sample size of *P. kuhlii* (n=50), we assessed the alpha diversity profiling of this species with regard to the different land uses. We found significant differences between the types of land use (Kruskal-Wallis statistic: 6.9368; p-value= 0.031167). A pair-wise comparison showed that the natural landscape (3.55 ± 0.42) had a significantly higher Shannon index value than the intensive agriculture landscape (H= 2.72 ± 0.48; p-value= 0.007) and the extensive agriculture landscape (H= 3.21 ± 0.55; p-value= 0.017), respectively.

We identified weak variability in the bacterial community structure among all bat species (PERMANOVA's R: 0.089; p-value= 0.001) (**Fig. 34**). Additionally, only 5.4% of the total variability could be attributed to the different land uses (PERMANOVA's R: 0.054; p-value= 0.001) (**Supplementary material, Fig. S3.4**).

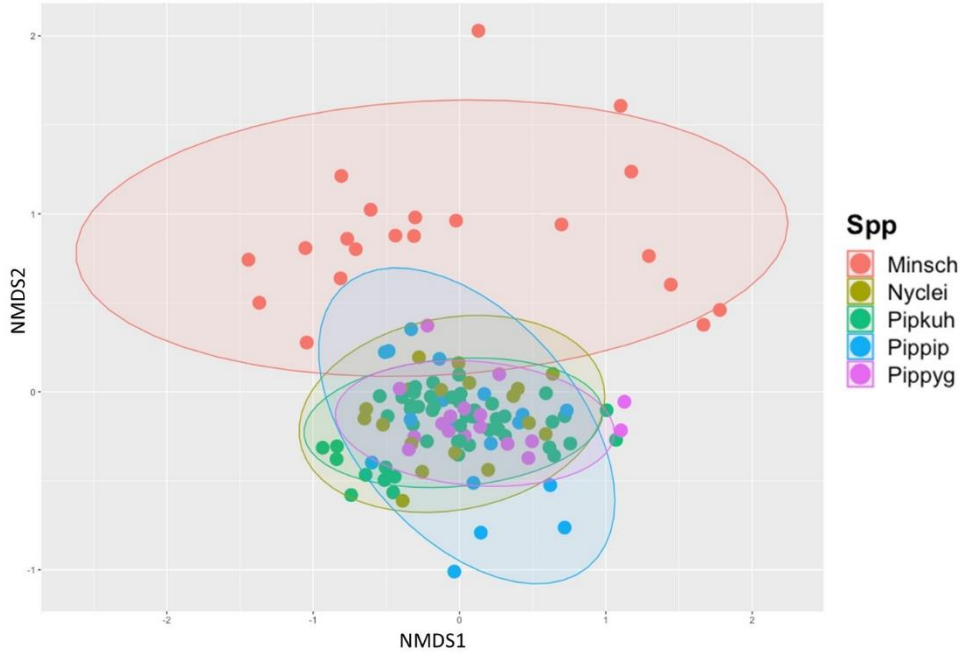


Figure 34. Non-Metric Multidimensional Scaling (NMDS) plot from Bray-Curtis distances for bat species (PERMANOVA's R: 0.089; p-value= 0.001). Pippyg= *Pipistrellus pygmaeus*; Pippip= *P. pipistrellus*; Pipkuh= *P. kuhlii*; Nyclei= *Nyctalus leisleri*; Minsch= *Miniopterus schreibersii*.

Differential abundant taxa at the level of bacterial genus: bat species and types of land use

A total of 15 significant phyla and 449 significant genera were found across the five bat species (log LDA score <2.0; p-value cutoff= 0.05). Among them, *Miniopterus schreibersii* significantly showed the least differentially abundant genera (**Fig. 35**).

A total of 5 differentially abundant genera were found for the different types of land uses (log LDA score <2.0; p-value cutoff= 0.05): *Clostridium* sensu stricto 1 and *Paraclostridium* for urban landscapes; *Enterococcus* and *Carnobacterium* for natural landscapes; and *Lonsdalea* for extensive agriculture landscapes. Intensive agriculture landscapes showed no differentially abundant genera.

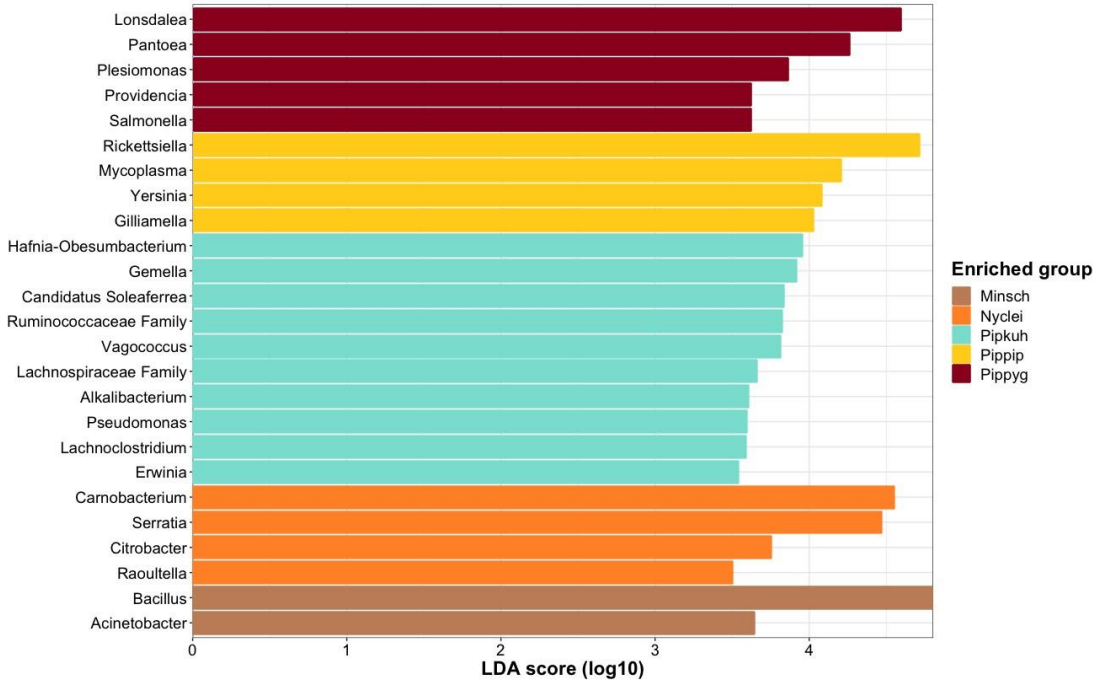


Figure 35. Linear discriminant analysis effect size (LEfSe) of the five sampled bat species at the genus level, showing the top 25 genera. LEfSe threshold of 2 (on a log10 scale) and a significance threshold of 0.05 were set. P-values were adjusted for false discovery rate (FDR) method. Pippyg= *Pipistrellus pygmaeus*; Pippip= *P. pipistrellus*; Pipkuh= *P. kuhlii*; Nyclei= *Nyctalus leisleri*; Minsch= *Miniopterus schreibersii*.

Identification of potential pathogenic bacteria at the level of bacterial species

Taxonomic composition: types of land use and bat species

Results at bacterial species level showed a total of 3,564 bacteria. We identified 222 (6.23%) species from the complete data set as potential pathogenic bacteria (PPB). Seventy-six PPB were classified as recognized pathogens (15 affecting exclusively animals, 25 affecting exclusively humans, and 36 affecting both humans and animals), due to their inclusion in either WOAH/WHO/ECDC/RENAVE lists, or their direct and habitual association with specific symptoms or pathology (**Supplementary material, Table S3.2: the identified PPB are highlighted in bold in the table**). Particularly, nine of the identified species are included in the WOAH listed diseases: *Bacillus anthracis, Brucella abortus, Brucella melitensis, Coxiella burnetii, Francisella tularensis,*

Mycoplasma capricolum, Mycoplasma mycoides, Pasteurella multocida and *Salmonella enterica*.

The most abundant PPB per type of land use, considering all bat species together, were the following (**Fig. 36**): *Rickettsiella massiliensis* (15.49% ± 21.20), *Enterococcus faecalis* (15.31% ± 22.23) and *Hafnia alvei* (12.67% ± 19.86) for extensive agriculture landscapes; *Rickettsiella massiliensis* (32.06% ± 25.74), *Enterobacter cloacae* (7.62% ± 21.41) and *Serratia marcescens* (7.30% ± 12.07) for intensive agriculture landscapes; *Serratia fonticola* (28.46% ± 32.46), *Enterococcus faecalis* (9.12% ± 23.85) and *Raoultella ornithinolytica* (6.00% ± 13.89) for natural landscapes; and *Escherichia coli* (28.32% ± 30.64), *Enterococcus faecalis* (13.70% ± 27.91) and *Clostridium perfringens* (13.09% ± 22.45) for urban landscapes.

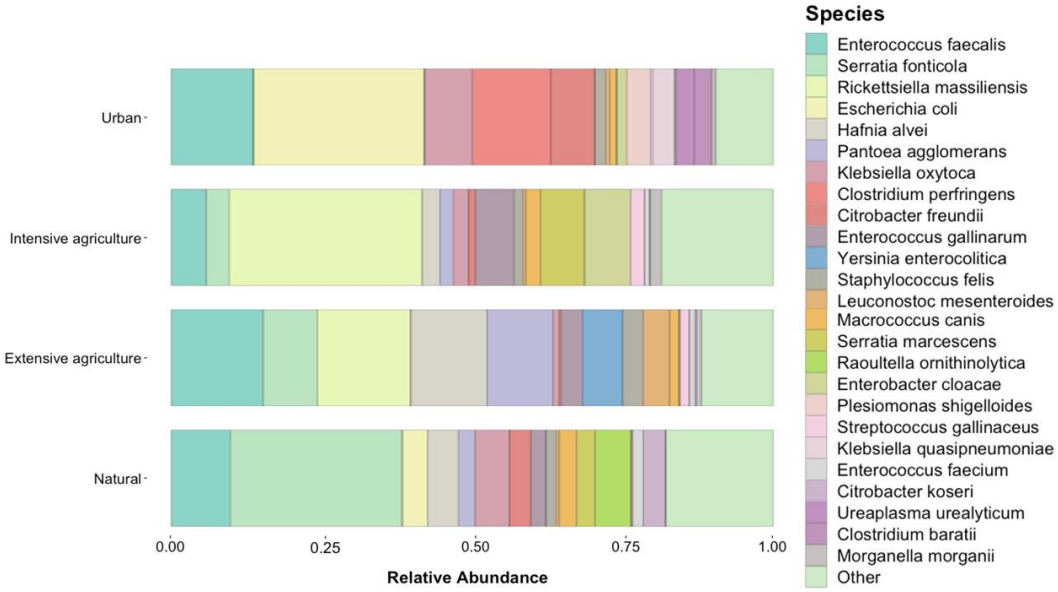


Figure 36. Stacked bar plot of relative abundances of the top 25 potentially pathogenic bacteria at the species level for type of land use (urban, intensive agriculture, extensive agriculture and natural).

The three most abundant PPB across all bat species were *Rickettsiella massiliensis* (14.87 % ± 18.75), *Enterococcus faecalis* (13.51 % ± 10.01) and *Serratia fonticola* (9.48 % ± 10.77). The three specific PPB with the highest relative abundances for each bat species were (**Fig. 37**): *Escherichia coli* (31.21 % ± 33.07), *Enterococcus faecalis* (15.01 % ± 26.26) and *Pantoea agglomerans* (13.72 % ± 25.12) for *P. pygmaeus*; *Rickettsiella massiliensis* (39.99 % ± 31.98), *Yersinia enterocolitica* (15.01 % ± 23.44) and *Clostridium perfringens* (8.15 % ± 20.74) for *P. pipistrellus; Serratia fonticola* (21.06 % ± 23.32), *Hafnia alvei* (15.72 % ± 19.63) and *Enterococcus faecalis* (9.62 % ± 20.56) for *P. kuhlii*; *Enterococcus faecalis* (30.43 %

± 32.32), *Serratia fonticola* (22.26 % ± 39.10) and *Raoultella ornithinolytica* (8.39 % ± 19.65) for *N. leisleri; Rickettsiella massiliensis* (17.95 % ± 20.81), *Klebsiella oxytoca* (12.35 % ± 20.29) and *Ureaplasma urealyticum* (10.44 % ± 13.20) for *M. schreibersii*.

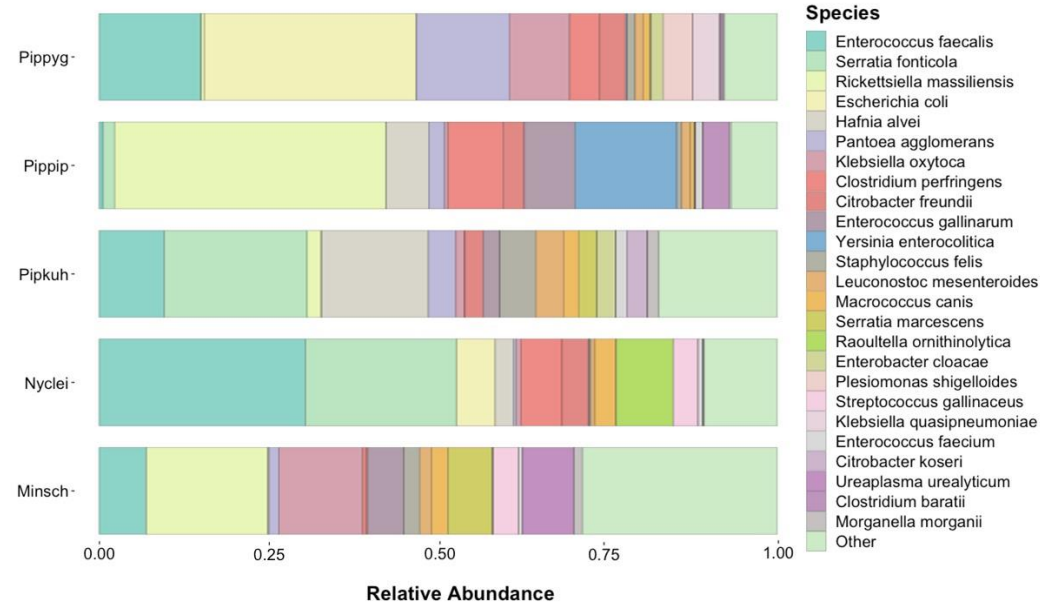


Figure 37. Stacked bar plot of relative abundances of the top 25 potentially pathogenic bacteria at the species level for each bat species (Pippyg= *Pipistrellus pygmaeus*; Pippip= *P. pipistrellus*; Pipkuh= *P. kuhlii*; Nyclei= *Nyctalus leisleri*; Minsch= *Miniopterus schreibersii*).

When comparing PPB across the different bat species and land uses, we also found differences in their taxonomic composition: different bat species from the same type of land use displayed different taxonomic composition (**Fig. 38**). Similarly, the same bat species from different types of land use displayed different taxonomic composition (**Fig. 38**).

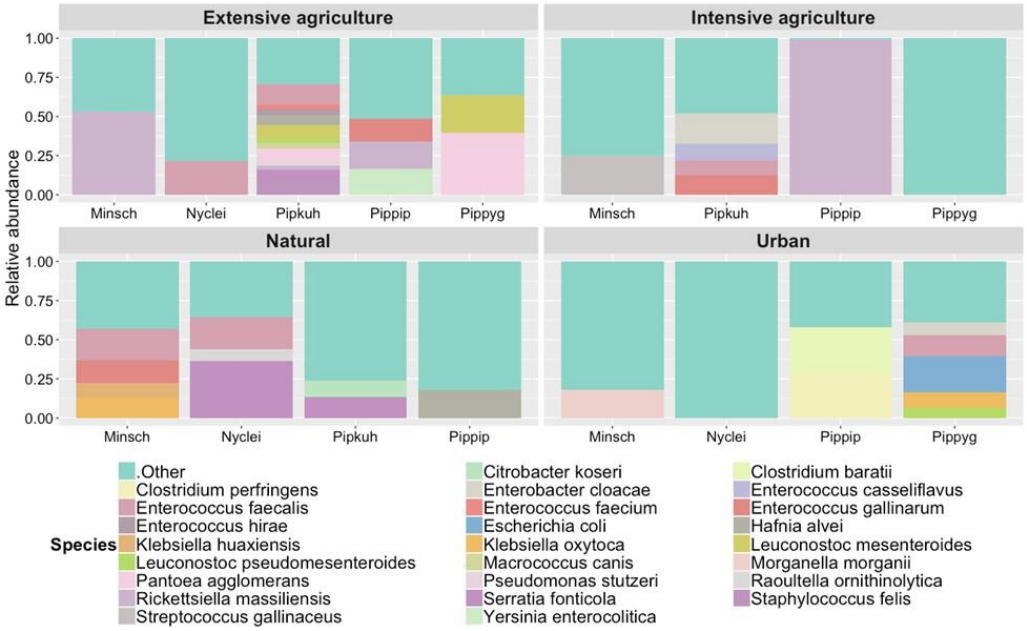


Figure 38. Stacked bar plot of relative abundances of the top 25 potentially pathogenic bacteria at the species level for each bat species (Pippyg= *Pipistrellus pygmaeus*; Pippip= *Pipistrellus pipistrellus*; Pipkuh= *Pipistrellus kuhlii*; Nyclei= *Nyctalus leisleri*; Minsch= *Miniopterus schreibersii*) and type of land use (extensive agriculture, intensive agriculture, natural and urban).

Core community analyses of PPB: types of land use and bat species

Core analyses revealed that 27.93% (n=62) of PPB were shared among all types of land use (**Fig. 39A**). Samples from natural landscapes showed the largest exclusive microbiome-associated PPB (n=29), whereas samples from urban landscapes showed the smallest (n=9). Overall, the core microbiome-associated PPB (n=62) for the four different land uses showed 32.26% (n=20) of PPB classified as recognized pathogens for humans and other animals. We found 38 PPB classified as recognized pathogens in natural landscapes, 26 in extensive agriculture landscapes, 12 in urban landscapes and 11 in intensive agriculture landscapes.

Additionally, a Venn diagram of PPB across the different bat species revealed that 22.52% (50/222) of PPB were found in all the categories (**Fig. 39B**). *Pipistrellus kuhlii* (n=29) and *M. schreibersii* (n=26) showed the largest exclusive

and accessory (n=12) microbiome-associated PPB, while *P. pygmaeus* showed the smallest (n=3).

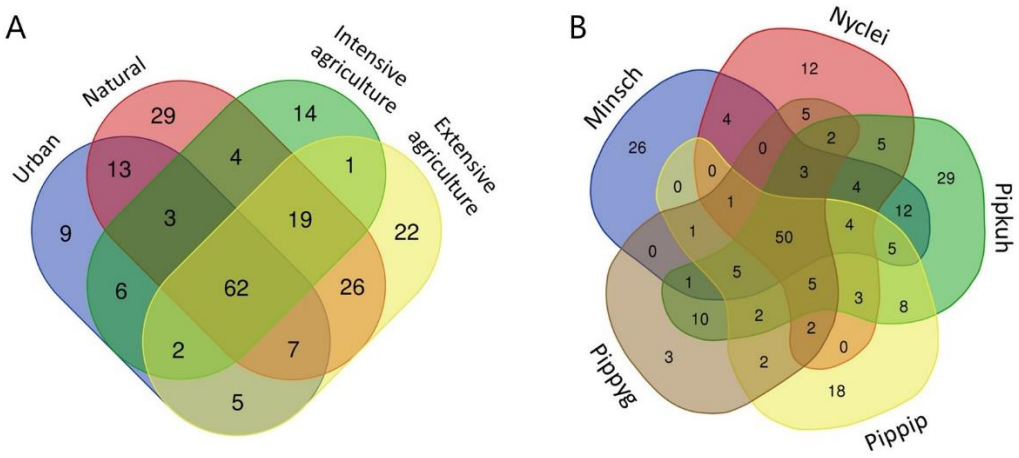


Figure 39. A. Venn diagram of potential pathogenic bacteria across the different land uses: urban, natural, intensive agriculture and extensive agriculture. **B.** Venn diagram of potential pathogenic bacteria across the different bat species: Minsch= *Miniopterus schreibersii;* Nyclei= *Nyctalus leisleri;* Pipkuh= *Pipistrellus kuhlii;* Pippip= *P. pipistrellus;* Pippy= *P. pygmaeus*.

Diversity analyses of PPB: types of land use and bat species

Alpha diversity analysis revealed no differentiation of observed PPB for each type of land use (Kruskal-Wallis statistic: 3.2018; p-value= 0.36155), although their values were different: 25 ± 10.62 for extensive agriculture landscapes, 20 ± 11.01 for natural landscapes, 23 ± 8.41 for intensive agriculture landscapes, and 25 ± 9.78 for urban landscapes (**Fig. 40**).

However, alpha diversity analysis revealed different observed PPB for the five bat species assessed (Kruskal-Wallis statistic: 15.171; p-value= 0.0074) (**Fig. 41**): 17.50 ± 8.58 for *M. schreibersii*, 17.00 ± 8.68 for *N. leisleri*, 27.50 ± 10.75 for *P. kuhlii*, 22.00 ± 9.66 for *P. pipistrellus* and 26.00 ± 9.37 for *P. pygmaeus*. *Pipistrellus pygmaeus* and *P. kuhlii* displayed the highest means of observed PPB diversity, whereas *M. schreibersii* showed the lowest. A pair-wise comparison showed *P. kuhlii* and *P. pygmaeus* exhibiting statistically significant higher observed PPB than both *N. leisleri* and *M. schreibersii*.

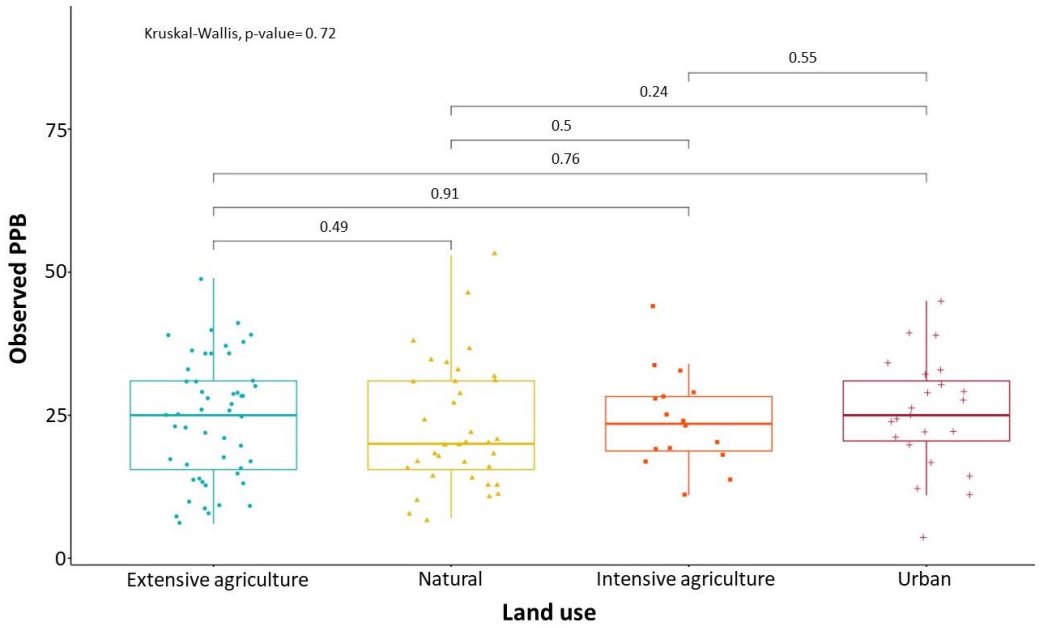


Figure 40. A boxplot of observed PPB diversity (Kruskal-Wallis statistic: 3.2018; p-value= 0.36155) across the different land uses (extensive agriculture, intensive agriculture, natural and urban).

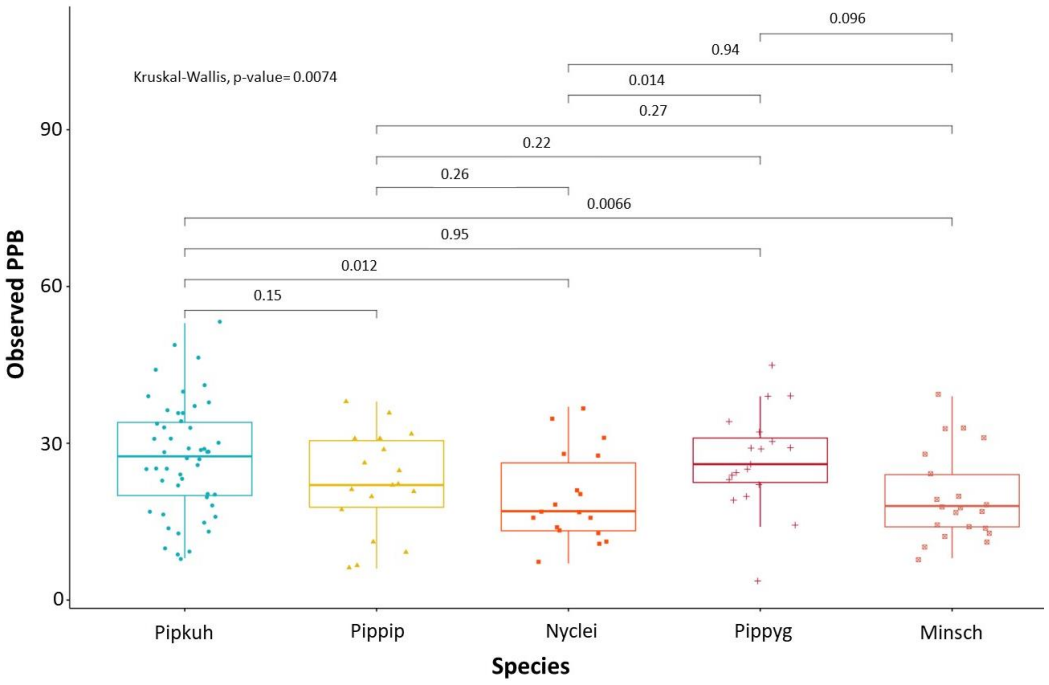


Figure 41. A boxplot of observed potential pathogenic bacteria (PPB) diversity (Kruskal-Wallis statistic: 15.171; p-value= 0.0074) for the five studied bat species: Minsch= *Miniopterus schreibersii*; Nyclei= *Nyctalus leisleri*; Pipkuh= *Pipistrellus kuhlii*; Pippip= *Pipistrellus pipistrellus*; and Pippyg= *Pipistrellus pygmaeus*. Significant values are those with a p-value <0.05.

We performed the same alpha diversity analyses but only considering *P. kuhlii* (n=50). No significant differences were found between the different land uses in numbers of PPB (Kruskal-Wallis statistic: 0.070588; p-value= 0.96532).

Beta diversity obtained by Bray-Curtis distance showed weak differentiation of PPB community structure across the different types of land use (PERMANOVA's R: 0.057; p-value= 0.001) and the five bat species assessed (PERMANOVA's R: 0.064; p-value= 0.001).

Differential abundance analyses of PPB: types of land use and bat species

We found 18 differentially abundant taxa when comparing the different types of land use (**Table 5**). Extensive agriculture and urban landscapes each showed five significant PPB respectively, whereas intensive agriculture and natural landscapes each showed four significant PPB. We found five significatively abundant PPB classified as recognized pathogens, of which four were found in urban landscapes (**Table 5**). Additionally, we found a total of 12 differentially abundant PPB across all bat species (**Fig. 42**): *M. schreibersii* (n=4), *N. leisleri* (n=1), *P. kuhlii* (n=3), *P. pipistrellus* (n=2) and *P. pygmaeus* (n=2).

Table 5. Significant taxa at the species level found by linear discriminant analysis effect size (LEfSe; threshold of 2 on a log10 scale) of the different land uses (Extensive= extensive agriculture; Intensive= intensive agriculture; Natural and Urban) with p-values adjusted for false discovery rate (FDR) method. Pathogenic relevance accounts for O (opportunistic pathogens) and R (recognized pathogens) (Lightfoot, 2003; WOAH, 2020). H= human; A=animal.

Species	Pathogenic relevance	Primary host/s	Land use (significant)
Bacillus cytotoxicus	R	H	Extensive
Leuconostoc mesenteroides	O	H	Extensive
Macrococcus canis	O	A (dogs)	Extensive
Serratia fonticola	O	H	Extensive
Staphylococcus felis	O	H, A (cats)	Extensive
Citrobacter braakii	O	H	Intensive
Enterobacter cloacae	O	H	Intensive
Enterococcus casseliflavus	O	H	Intensive
Enterococcus gallinarum	O	H, A (mice)	Intensive
Hafnia alvei	O	H, A (cattle, poultry, ducks, others)	Natural
Serratia liquefaciens	O	H, A (cattle)	Natural
Serratia plymuthica	O	H	Natural
Yersinia frederiksenii	O	H	Natural

Table 5. *Continued.*

Species	Pathogenic relevance	Primary host/s	Land use (significant)
Bacillus cereus	R	H, A (cattle, swine, poultry, psittacines, bats)	Urban
Citrobacter freundii	O	H, A (small ruminants, swine, poultry, reptiles and aquatic animals)	Urban
Clostridium baratii	R	H	Urban
Clostridium perfringens	R	H, A (cattle, swine, poultry, horses)	Urban
Klebsiella pneumoniae	R	H, A (cattle, swine, others)	Urban

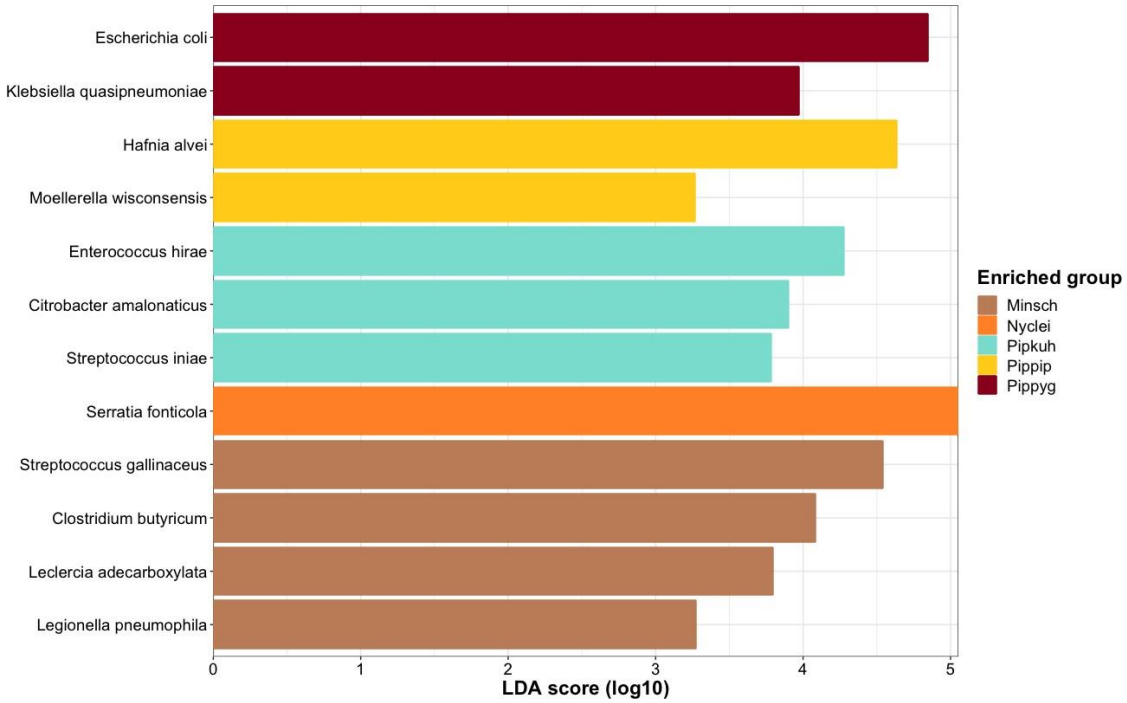


Fig. 42. Linear discriminant analysis effect size (LEfSe) of potentially pathogenic bacteria at species level across the studied bat species (Minsch= *Miniopterus schreibersii*; Nyclei= *Nyctalus leisleri*; Pipkuh= *Pipistrellus kuhlii*; Pippip= *Pipistrellus pipistrellus*; and Pippyg= *Pipistrellus pygmaeus*). LEfSe threshold of 2 (on a log10 scale) and a significance threshold of 0.05 were set. P-values were adjusted for false discovery rate (FDR) method.

Individual assessment of the modified microbiome dysbiosis ratio (MDR)

We found no statistically significant differences in body condition indices (BCIs) between males and females (p-value= 0.34). Values of "poor" BCIs for each bat species were: *M. schreibersii* (BCI ≤0.26), *N. leisleri* (BCI ≤ 0.27), *P. kuhlii* (BCI ≤ 0.15), *P. pipistrellus* (BCI ≤ 0.12), and *P. pygmaeus* (BCI ≤ 0.13).

A total of 32 bats showed an MDR lower than 0.25 with at least one PPB accounting for more than 20% of their total bacterial species counts (**Table 6**). We identified 16 PPB under these circumstances: *Citrobacter freundii, C. koseri, Enterococcus faecalis, E. gallinarum, Escherichia coli, Hafnia alvei, Klebsiella huaxiensis, K. oxytoca, K. quasipneumoniae, Pantoea agglomerans, Plesiomonas shigelloides, Rickettsiella massiliensis, Serratia fonticola, Ureaplasma parvum, U. urealyticum* and *Yersinia enterocolitica*.

Table 6. Bats with a Microbiome Dysbiosis Ratio (MDR) lower than 0.25 with at least one PPB accounting for more than the 20% of their total species counts. BCI= Body Condition Index. N= natural; U= urban; I= intensive agriculture; E= extensive agriculture. Obs.= total number of observed bacterial species. PPB (MDR)= number of potential pathogenic bacteria identified from the total observed (Microbial Dysbiosis Ratio expressed in %). Total counts= absolute number of counts obtained with species-level taxonomic assignment Emu software. PPB counts (%) = total number of counts (and relative abundance from the total counts) obtained with species-level taxonomic assignment EMU corresponding to the specific bacteria indicated in the same row.

Spp	BCI	Land use	Obs.	PPB (MDR)	Total counts	Bacteria	PPB counts (%)
Pipkuh	Good	E	124	9 (7.3%)	70237	*Enterococcus faecalis*	14987 (21.3%)
Pippyg	na	U	108	12 (11.1%)	134748	*Klebsiella quasipneumoniae*	45367 (33.7%)
Pipkuh	Good	E	130	8 (6.2%)	109058	*Rickettsiella massiliensis*	22080 (20.2%)
Minsch	Good	N	75	11 (14.7%)	124995	*Klebsiella huaxiensis*	28931 (23.1%)
Nyclei	Poor	N	48	5 (10.4%)	102126	*Serratia fonticola*	23954 (23.5%)
Pipkuh	Good	N	323	11 (3.4%)	164372	*Citrobacter koseri*	40996 (24.9%)
Pipkuh	Good	E	111	10 (9.0%)	201386	*Enterococcus faecalis*	51100 (25.4%)
Nyclei	Good	U	106	11 (10.38%)	89401	*Citrobacter freundii*	23875 (26.7%)
Minsch	Good	U	51	5 (9.8%)	77665	*Ureaplasma parvum*	23100 (29.7%)
						Ureaplasma urealyticum	38701 (49.8%)

Table 6. *Continued.*

Spp	BCI	Land use	Obs.	PPB (MDR)	Total counts	Bacteria	PPB counts (%)
Minsch	Good	N	39	9 (23.1%)	183671	*Klebsiella oxytoca*	57414 (31.3%)
Pippyg	Good	U	154	11 (7.1%)	159929	*Plesiomonas shigelloides*	50752 (31.7%)
Pippip	Good	N	191	9 (4.7%)	127886	*Hafnia alvei*	47704 (37.3%)
Nyclei	Good	N	66	8 (12.1%)	87631	*Serratia fonticola*	33842 (38.6%)
Minsch	Good	E	85	8 (9.4%)	181314	*Rickettsiella massiliensis*	72758 (40.1%)
Pipkuh	Good	N	262	11 (4.2%)	306860	*Serratia fonticola*	133880 (43.6%)
Pippip	Poor	E	137	10 (7.3%)	134281	*Enterococcus gallinarum*	60501 (45.1%)
Nyclei	Good	N	25	6 (24.0%)	97855	*Escherichia coli*	44924 (45.9%)
						Enterococcus faecalis	46681 (47.7%)
Pippyg	Good	U	97	9 (9.3%)	131025	*Hafnia alvei*	62160 (47.4%)
Pipkuh	Good	E	58	4 (6.9%)	27970	*Serratia fonticola*	13549 (48.4%)
Pippip	Good	U	79	10 (12.7%)	51348	*Citrobacter freundii*	25268 (49.2%)
Pipkuh	Poor	E	53	7 (13.2%)	100274	*Hafnia alvei*	62160 (62.0%)
Pippyg	Good	U	48	10 (20.8%)	147031	*Escherichia coli*	98988 (67.3%)
Pippip	Good	E	156	4 (2.6%)	185165	*Yersinia enterocolitica*	124996 (67.5%)
Pipkuh	Good	E	40	5 (12.5%)	195046	*Serratia fonticola*	137938 (70.7%)
Pippyg	Good	U	66	7 (10.6%)	108850	*Escherichia coli*	77983 (71.6%)
Pippyg	Good	U	98	10 (10.2%)	192329	*Escherichia coli*	144566 (75.2%)
Pippyg	Good	E	173	8 (4.2%)	205353	*Pantoea agglomerans*	154391 (75.2%)
Nyclei	na	E	64	7 (10.9%)	195117	*Enterococcus faecalis*	152116 (78.0%)
Pipkuh	na	E	57	9 (15.8%)	185301	*Hafnia alvei*	153748 (83.0%)
Pippip	Good	E	90	12 (13.3%)	200200	*Rickettsiella massiliensis*	170088 (85.0%)
Pippip	Good	I	44	10 (22.7%)	179339	*Rickettsiella massiliensis*	162844 (90.8%)
Nyclei	Good	N	76	7 (9.2%)	104552	*Serratia fonticola*	96832 (92.6%)

We found nine out of 23 (39.13%) bats with the same criteria (MDR lower than 0.25 with at least one PPB accounting for more than 20% of their total species counts) belonging to urban landscapes, 13 out of 51 (25.49%) to extensive agriculture landscapes, nine out of 36 (25.00%) to natural landscapes and one out of 16 (8.25%) to intensive agriculture landscapes. All bats were in good body condition, except for three individuals that were in poor body condition: one *N. leisleri* from a natural landscape, and one *P. kuhlii* and one *P. pipistrellus* from extensive agriculture landscapes.

Two bats sampled in natural landscapes and with a good body condition exhibited respectively two PPBs with a total representation of more than 25% each: *M. schreibersii* (29.7% *Ureaplasma paryum* and 49.8% *U. urealyticum*), and *N. leisleri* (45.9% *Escherichia coli* and 47.7% *Enterococcus faecalis*).

Seven bats showed a unique PPB accounting for more than 75% of the total bacterial counts, of which seven belonged to extensive agriculture landscapes, one to an intensive agriculture landscape, one to a natural landscape and one to an urban landscape.

Results summary

Fecal microbiome structure, composition and diversity analyses at the level of bacterial genus

Bat species

- Taxonomic composition: the four most abundant bacterial genera across all bat species were *Enterococcus* (14.66% ± 18.29), *Escherichia/Shigella* (9.32% ± 13.05), *Bacillus* (7.20% ± 10.09) and *Lactococcus* (5.21% ± 13.10).
- Core community analysis: 39.20% of all bacterial genera were shared by all bat species. *Pipistrellus kuhlii* showed the largest number of exclusive genera (n=270), followed by *M. schreibersii* (n=62), *N. leisleri* (n=38), *P. pipistrellus* (n=36) and *P. pygmaeus* (n=15).
- Alpha diversity analysis: *P. kuhlii* showed the significantly highest Shannon index (H) value (3.22 ± 0.53). The other bat species showed the next H values: 2.54 ± 0.97 for *P. pipistrellus*, 2.54 ± 0.65 for *P. pygmaeus*, 2.20 ± 0.67 for *N. leisleri* and 2.06 ± 0.79 for *M. schreibersii*.
- Beta diversity analysis: the bacterial community structure among all bat species showed a weak variability (only 8.9% of the total variability could be attributed to the different bat species).
- Differentially abundant genera: *Miniopterus schreibersii* significantly showed the least differentially abundant genera (n=2), while *P. kuhlii* showed the most (n=10).

Types of land use

- Alpha diversity analysis: the urban landscape showed the statistically significant lowest H value (2.25 ± 0.77), while the extensive agriculture landscape showed the highest H value (2.88 ± 0.70).
- Beta diversity analysis: the bacterial community structure among all types of land use showed a weak variability (only 5.4% of the total variability could be attributed to the different land uses).
- Differentially abundant genera: a total of 5 genera were significantly identified as differentially abundant across urban (n=2), natural (n=2) and extensive agriculture (n=1) landscapes. No differentially abundant genera were found in intensive agriculture landscapes.

Potential pathogenic bacteria (PPB) identified at the level of bacterial species

- A total of 222 PPB were identified, of which 66 were classified as recognized pathogens.
- Nine of these 66 PPB are included in the WOAH listed diseases: *Bacillus anthracis, Brucella abortus, Brucella melitensis, Coxiella burnetii, Francisella tularensis, Mycoplasma capricolum, Mycoplasma mycoides, Pasteurella multocida* and *Salmonella enterica*.

Bat species

- Taxonomic composition: the three most abundant PPB across all bat species were *Rickettsiella massiliensis* (14.87 % ± 18.75), *Enterococcus faecalis* (13.51 % ± 10.01) and *Serratia fonticola* (9.48 % ± 10.77).
- Core analyses: 22.52% (n=50) of PPB were shared among all bat species.
- Alpha diversity analysis: *P. kuhlii* (27.50 ± 10.75) and *P. pygmaeus* (26.00 ± 9.37) exhibited statistically significant higher observed PPB than both *N. leisleri* (17.00 ± 8.68) and *M. schreibersii* (17.50 ± 8.58).
- Beta diversity analysis: the PPB community structure among all bat species showed a weak variability (only 6.4% of the total variability could be attributed to the different land uses).
- Differentially abundant genera: a total of 12 differentially abundant PPB were found across all bat species: *M. schreibersii* (n=4), *N. leisleri* (n=1), *P. kuhlii* (n=3), *P. pipistrellus* (n=2) and *P. pygmaeus* (n=2).

Types of land use

- Taxonomic composition: The most abundant PPB per type of land use were::
    - Extensive agriculture landscapes: *Rickettsiella massiliensis* (15.49% ± 21.20), *Enterococcus faecalis* (15.31% ± 22.23) and *Hafnia alvei* (12.67% ± 19.86)
    - Intensive agriculture landscapes: *Rickettsiella massiliensis* (32.06% ± 25.74), *Enterobacter cloacae* (7.62% ± 21.41) and *Serratia marcescens* (7.30% ± 12.07).
    - Natural landscapes: *Serratia fonticola* (28.46% ± 32.46), *Enterococcus faecalis* (9.12% ± 23.85) and *Raoultella ornithinolytica* (6.00% ± 13.89)
    - Urban landscapes: *Escherichia coli* (28.32% ± 30.64), *Enterococcus faecalis* (13.70% ± 27.91) and *Clostridium perfringens*.
- Core community analyses: 27.93% (n=62) of PPB were shared among all land use types.
- Alpha diversity analysis: no differentiation of observed PPB for each type of land use was found. However, their values of observed PPB were the following:
    - Extensive agriculture landscapes: 25 ± 10.62.
    - Intensive agriculture landscapes: 23 ± 8.41.
    - Natural landscapes: 20 ± 11.01.
    - Urban landscapes: 25 ± 9.78.
- Beta diversity analysis: the PPB community structure among all types of land use showed a weak variability (only 5.7% of the total variability could be attributed to the different land uses).
- Differentially abundant genera: a total of 18 PPB were significantly identified as differentially abundant across all types of land uses. Four out of the five differentially abundant PPB classified as recognized pathogens were found in urban landscapes.

Individual assessment of the modified microbiome dysbiosis ratio (MDR)

- Identification of potentially dysbiotic microbiomes: a total of 32 bats showed a MDR lower than 0.25 and one (or two) PPB overrepresented (accounting more than 20% of their total bacterial species counts), distributed as follows:
    - Extensive agriculture: 13 out of 51 (25.49%) bats with potentially dysbiotic microbiomes.
    - Intensive agriculture: 1 out of 16 (6.25%) bats with potentially dysbiotic microbiomes.
    - Natural landscapes: 9 out of 36 (25.00%) bats with potentially dysbiotic microbiomes.
    - Urban landscapes: 9 out of 23 (39.13%) bats with potentially dysbiotic microbiomes.
- In these bats, overrepresented PPB were the following: *Citrobacter freundii, C. koseri, Enterococcus faecalis, E. gallinarum, Escherichia coli, Hafnia alvei, Klebsiella huaxiensis, K. oxytoca, K. quasipneumoniae, Pantoea agglomerans, Plesiomonas shigelloides, Rickettsiella massiliensis, Serratia fonticola, Ureaplasma parvum, U. urealyticum* and *Yersinia enterocolitica*.

Discussion

In this study, we examined the fecal microbiome composition of five insectivorous bat species with different roosting and diet preferences and dwelling in areas with different land uses. The overall most abundant bacterial genera found in our samples were *Enterococcus, Escherichia/Shigella, Bacillus and Lactococcus,* which correlates with other microbiome studies in insectivorous bats (Aizpurua et al., 2021; di Bella et al., 2003; Gerbáčová et al., 2020; Wolkers-Rooijackers et al., 2018). Several fecal microbiome studies on bat species have revealed that diet seems to play a crucial role in shaping their fecal microbiome (Corduneanu et al., 2023; Ingala et al., 2021). Although all five bat species are insectivorous and in the Iberian Peninsula their diet is mainly dominated by Lepidoptera and Diptera, they consume a variable proportion of invertebrate taxa (Amichai & Korine, 2020; Museu de Ciències Naturals de Granollers, 2023). However, almost 40% of all bacterial genera identified were found among all samples, showing a solid core microbiome across the different bat species. These findings may suggest that the diets of our sampled insectivorous bats may not differ significantly among them, leading to minimal microbiome differentiation between them. Most probably, marked variations in dietary strategies such as consumption of fish, insects, or fruits, may result in the establishment of specific bacterial communities tailored to the digestion of these respective types of food, and therefore, lead to stronger microbiome differentiation (Corduneanu et al., 2023; Ingala et al., 2021). Beta diversity analyses also supported the solid core microbiome found in our study, since we found a weak differentiation in bat's fecal community structure regardless of their species (PERMANOVA's R: 0.089; p-value= 0.001). However, our NMDS plot from Bray-Curtis distances for bat species depicted *M. schreibersii* samples more distant from the rest of the bat species of this study. In fact, *M. schreibersii* exhibited also a significantly lower alpha diversity value than the other bat species and the lowest proportion of differentially abundant genera. In contrast, *P. kuhlii* was notable for exhibiting the highest alpha diversity value and the highest number of differentially abundant taxa.

Anthropogenic activities, such as urbanization and agriculture, have been recognized as significant drivers of alterations in the microbiome composition of various wildlife species (Amato et al., 2013; Fackelmann et al., 2021), including bats (Alpízar et al., 2021; Ingala et al., 2019; **Study 2** of this thesis). In order to elucidate the reason behind the marked differences between *P. kuhlii and M.*

schreibersii, we explored the impact of land use on the fecal microbiome diversity, structure and composition of our studied bat species. Overall, significant differentiation was found between extensive agriculture and urban landscapes. Extensive agriculture landscapes showed the highest bacterial diversity, whereas urban landscapes showed the lowest. Both bat species, *M. schreibersii* and *P. kuhlii* were sampled in extensive agriculture and natural areas, but the former was also sampled in urban areas and the latter in intensive agriculture landscapes. Since *M. schreibersii* was sampled in the significantly less diverse landscape, the urban, performing the analyses intraspecifically would have been the most appropriate approach to assess the effect of this variable in the overall results. However, we did not have enough sampled *M. schreibersii* bats in each type of landscape to perform such analyses. In contrast, we had a large sample size of *P. kuhlii* and samples were homogenously distributed across extensive agriculture, intensive agriculture, and natural landscapes. We found that both extensive and intensive agriculture landscapes showed a significantly less diverse microbial diversity than natural landscapes, which supports the results from the **Study 2** of this thesis but differs from the results of the present study accounting for all bat species (extensive agriculture being the more diverse landscape). Overall, we found a weaker differentiation of microbial community structure across the different sampled landscapes than what we found across the sampled bat species. This suggests that other factors, rather than the type of landscape where bats dwell, may be behind the reason for the bacterial community differences found between bat species. For instance, migratory behaviour has been found to affect the gut microbiome composition and diversity of several bird species (Góngora et al., 2021; Obrochta et al., 2022; Risely et al., 2018). Since migratory birds are expected to get into contact with different environments and possibly diet, a higher fecal alpha diversity is also expected to be found (Góngora et al., 2021). However, contrary to this theory, some migratory bird species such as the Canada geese (*Branta canadensis*) have been shown to exhibit higher fecal diversity values in sedentary individuals than in migratory individuals (Obrochta et al., 2022). In our study, both migratory species, *M. schreibersii* and *N. leisleri*, also showed less diverse fecal microbiomes than the rest of the bat species. In the specific case of *N. leisleri* from Catalonia, only females migrate for hibernation to northern areas of Europe, whereas males stay in the same territory all year around (Alcalde et al., 2013). However, no differences were found between males and females from our study. Mechanisms of adaptation and responses of the gut microbiome of bat species to migration still need to be elucidated.

In addition to the loss of bacterial diversity and the convergence of the microbiome composition of urban wildlife with the human microbiome, bacterial spillover from humans into wildlife has been suggested by several authors to occur in urban areas (Dillard et al., 2022; Fackelmann et al., 2021). In our study, we identified 222 potential pathogenic bacterial species, nearly half of which are classified as recognized human and domestic animal pathogens (ECDC, 2023; Lightfoot, 2003; WOAH, 2020). Consequently, we expected to find a correlation between the type of land use and the occurrence and type of potential pathogenic bacteria (PPB). However, core analyses revealed a large group of PPB shared between all types of land uses, meaning that the occurrence of many PPB was not defined by landscape anthropogenic pressure. Additionally, alpha diversity analyses showed no significant differences between the different land uses, and differentially abundant analyses showed almost the same proportion of PPB across the various land uses. However, four out of five significantly abundant PPB classified as recognized pathogens for humans and domestic animals were detected in urban landscapes (ECDC, 2023; Lightfoot, 2003; WOAH, 2020). Although human activities can contribute to the spread of bacteria present in the environment (Han et al., 2013; Mu et al., 2020; Xu et al., 2012), in the present study, the bacterial species identified as differentially abundant in bats from urban areas have been previously reported in bat fecal samples, irrespective of their habitat of origin (Banskar al., 2016; Daniel et al., 2013; de Leon et al., 2018; Núñez-Montero et al., 2021). Only one PPB (*Bacillus cytotoxicus*) identified in extensive agriculture landscapes was classified as well as a recognized pathogen, whereas the rest of the differentially abundant taxa were classified as opportunistic bacteria. The use of manure for fertilization and soil irrigation with treated wastewater in agricultural landscapes are the most impactful sources of soil pollution with enterobacteria (some pathogenic) and antibiotic-resistant bacteria (Li et al., 2023). Three out of four bacteria identified in samples from intensive agriculture landscapes (*Enterobacter cloacae*, *Enterococcus casseliflavus,* and *E. gallinarum*) are considered commensals from the gastrointestinal tract of humans and domestic animals, but they are also some of the most common bacteria involved in nosocomial infections (Palmer et al., 2012; Reid et al., 2001). Additionally, *E. casseliflavus* and *E. gallinarum* are known to exhibit a high resistance level to Vancomycin (Reid et al., 2001), an antibiotic currently forbidden in agricultural settings due to its emerging threat to human and animal therapeutics success (European Medicines Agency, 2020). The significative occurrence of these bacteria in samples from intensive agriculture landscapes may suggest just a reflection of the bat's commensal microbiome. Still, the influence of landscape contamination resulting from domestic animal excretions and human wastewater irrigation cannot be

discounted. Different *Serratia* species were found to be significantly abundant in samples from extensive agriculture (*S. fonticola*) and natural areas (*S. liquefaciens* and *S. plymuthica*), both landscapes where the human impact is less pronounced. Broadly, *Serratia* species are commonly found in natural environments (water, plants and soil) (Mahlen, 2011), and their presence contributes to ecological processes such as the cycling of nutrients and breaking down complex organic compounds, such as chitin (Petersen & Tisa, 2013). However, they were included in our list of potential pathogenic bacteria since they can also act as important opportunistic pathogens for humans and animals, including insects (Mahlen, 2011; Petersen & Tisa, 2013).

Analyses of PPB among the different bat species showed little differentiation in terms of diversity and abundance. However, differences were again contrasting between *P. kuhlii* and *M. schreibersii.* A Venn diagram of PPB showed an overall large core microbiome and that both bat species previously mentioned had the largest exclusive PPB-associated microbiomes. Additionally, like alpha diversity analyses at the genus level, *P. kuhlii* bats exhibited a significantly higher value of mean PPB occurrence, in contrast to *M. schreibersii* and *N. leisleri*. The higher occurrence of PPB within *P. kuhlii* samples may mirror their intrinsic greater alpha diversity at the genus level, suggesting that within their bacterial diversity profile, PPB were also included. When assessing the abundance of specific PPB, *M. schreibersii* exhibited the highest number of significantly abundant taxa (n=4), followed by *P. kuhlii* (n=3). This finding contrasts with the results at the genus level, where *M. schreibersii* showed the lowest number of significantly abundant taxa. Nonetheless, most significantly abundant PPB found in both *M. schreibersii* and *P. kuhlii* are known to primarily function as commensal bacteria (i.e. *Clostridium butyricum,* significant taxon in *M. schreibersii; Enterococcus hirae,* significant taxon in *P. kuhlii*), yet under specific conditions, they may exhibit opportunistic pathogenic behavior (Cassir et al., 2016; Nakamura et al., 2021; Vela et al., 2010). On the contrary, *Streptococcus gallinaceus*, identified as a significant taxon in *M. schreibersii* samples, is a pathogenic bacterium for chickens and is currently considered a zoonotic *Streptococcus*, since it has been recovered from febrile abattoir workers and humans with prior exposure to chickens (Patel et al., 2023). *Miniopterus schreibersii* also exhibited *Legionella pneumophila* as a significantly abundant PPB. Naturally found in freshwater sources, *L. pneumophila* is the etiologic agent of Legionnaire's disease, which causes serious pneumonia in humans but is extremely rare in animals (e.g. a single recorded case of the disease in a calf from Italy and laboratory-exposed animals) (Baskerville et al., 1981; CDC, 2021; Fabbi et al., 1998). The impact of this

bacteria on bat's health, as well as the potential role of bats as its reservoir requires further investigation.

Differential abundant analyses identify bacteria whose abundance correlates with a specific variable, such as bat species or land use. While they can provide valuable insights into the identification of taxa with biological significance and the exploration of potential biomarkers (e.g., of disease), further tools are needed to explore the status of microbiomes at the individual level (Yang & Chen, 2022). For instance, the microbial dysbiosis ratio (i.e., abundance ratio of "good and bad bacteria") has been used to determine the status of individual microbiomes from cohorts of healthy and diseased human patients (Zha et al., 2021). These studies use the abundance of beneficial bacteria and the abundance of potentially harmful bacteria, expressed as in the following ratio: "beneficial bacteria/harmful bacteria". Results obtained from such a formula show a decreased value for aggravated dysbiotic microbiomes. Other studies are currently using a modified dysbiosis ratio (MDR) in which harmful bacteria is placed in the numerator and beneficial bacteria in the denominator (harmful bacteria/beneficial bacteria), resulting in a less confusing interpretation (Maslennikov et al., 2021). In the present study, we used this MDR to individually determine the dysbiotic status of the sampled bats (Maslennikov et al., 2021; Zha et al., 2021). Similar to humans with Chron's disease and ulcerative colitis, which seem to show more than a 10% increase of fecal Enterobacteriaceae, the overrepresented PPB (>20% of the total counts) found in our samples could be playing a significant (and potentially negative) role within bats' microbiome (Khorsand et al., 2022). Contrary to popular belief, current research suggests that bats are susceptible to bacterial infections much like other mammals (Szentivanyi et al., 2023). In fact, several bacterial species overrepresented in the potentially dysbiotic microbiomes from the present study have been also isolated from pathologic samples of deceased European bats (Mühldorfer et al., 2011, 2010). For instance, *Enterococcus faecalis* was isolated from histologic lesions in *P. pipistrellus* with mild to severe enteritis and *Myotis mystacinus* with mild bile duct proliferation (Mühldorfer et al., 2011). *Escherichia coli* was recovered from European deceased bats with nephritis and cystitis. *Klebsiella oxytoca* and *E. coli* were associated with systemic disease and pneumonia in bats from Germany (Mühldorfer et al., 2010). In 2018, *Yersinia enterocolitica* was recovered from *M. schreibersii* carcasses after a massive bat die-off event in Georgia (Imnadze et al., 2020). Additionally, we identified *Rickettsiella massiliensis* (basonym: *Diplorickettsia massiliensis*) as a significant PPB in four potential dysbiotic microbiomes from the present study. First isolated from *Ixodes ricinus* ticks in Slovak republic in 2006, *R. massiliensis* is an obligate intracellular and gram-negative bacterium (family Coxiellaceae) and is currently

a recognized human pathogen (Mathew et al., 2012). Although *Coxiella burnetii*, which belongs to the same family, has already been described in several bat species and their ectoparasites around the world (Ikeda et al., 2021), to our knowledge, this is the first time that *R. massiliensis* has been detected in bats. Similar to other pathogenic bacteria such as *Borrelia*, *Francisella*, *Brucella* and *Yersinia* species, the clinical and pathological impacts of *Rickettsiella massiliensis* in bat species are still to be explored (Szentivanyi et al., 2023). Since many of the PPB found in the present study can cause pathological processes in humans, other mammals and birds (CDC, 2021; Mathew et al., 2012; Primavilla et al., 2023; Reid et al., 2001), they should be a matter of attention for bat health research as well. In our study, most bats with overrepresented PPBs exhibited a good body condition index and no external signs of disease were recorded during sampling. Therefore, additional health indicators and further characterization of pathogenic properties of the identified bacteria are needed to supplement and understand these findings. Given the limited knowledge about most pathogenic bacteria in bats, we strongly encourage the expansion of research in bat microbiology and targeted surveillance. Results from such studies will establish a fundamental baseline for conducting bat's health and microbiome investigations at both individual and population levels.

Supplementary material: S3

Table S3.1. Information regarding the sampling locations and sampled bats (Minsch= *Miniopterus schreibersii*; Nyclei= *Nyctalus leisleri*; Pipkuh= *Pipistrellus kuhlii*; Pippip= *P. pipistrellus*; Pippyg= *P. pygmaeus*). LC= Livestock capacity; HP= Human population; M= medium; L= low; H= high; Extensive= Extensive agriculture; Intensive= Intensive agriculture.

Sample	Location name	Location ID	Land cover*	LC*	HP*	Land use	Species	Sex
PM001	Cap del Pla	E	Immature forest	M	L	Extensive	Pipkuh	F
PM002	Cap del Pla	E	Immature forest	M	L	Extensive	Pipkuh	F
PM003	Cap del Pla	E	Immature forest	M	L	Extensive	Pipkuh	F
PM004	Cap del Pla	E	Immature forest	M	L	Extensive	Pippip	NA
PM005	Cap del Pla	E	Immature forest	M	L	Extensive	Pipkuh	F
PM006	Cap del Pla	E	Immature forest	M	L	Extensive	Pippip	F
PM007	St. Llorenç de Morunys	Q	Mature Forest	L	L	Natural	Pipkuh	F
PM008	St. Llorenç de Morunys	Q	Mature Forest	L	L	Natural	Pipkuh	M
PM009	Cal Pares	D	Farming	H	L	Intensive	Pipkuh	M
PM010	Cal Pares	D	Farming	H	L	Intensive	Pippip	F
PM011	Lles de Cerdanya	J	Mature Forest	M	L	Natural	Pipkuh	M
PM012	Lles de Cerdanya	J	Mature Forest	M	L	Natural	Pipkuh	M
PM013	Lles de Cerdanya	J	Mature Forest	M	L	Natural	Pipkuh	M
PM014	Lles de Cerdanya	J	Mature Forest	M	L	Natural	Pipkuh	M
PM015	Lles de Cerdanya	J	Mature Forest	M	L	Natural	Pipkuh	F
PM016	Lles de Cerdanya	J	Mature Forest	M	L	Natural	Pipkuh	F
PM017	Lles de Cerdanya	J	Mature Forest	M	L	Natural	Pippip	F
PM018	Refugi de Conangles	P	Mature Forest	L	L	Natural	Nyclei	M
PM019	Refugi de Conangles	P	Mature Forest	L	L	Natural	Nyclei	M
PM020	Refugi de Conangles	P	Mature Forest	L	L	Natural	Pippip	F
PM021	Refugi de Conangles	P	Mature Forest	L	L	Natural	Nyclei	M
PM022	Refugi de Conangles	P	Mature Forest	L	L	Natural	Nyclei	M
PM023	Refugi de Conangles	P	Mature Forest	L	L	Natural	Nyclei	M
PM024	Refugi de Conangles	P	Mature Forest	L	L	Natural	Nyclei	M
PM025	Refugi de Conangles	P	Mature Forest	L	L	Natural	Nyclei	M
PM026	Artiga de Lin	B	Mature Forest	L	L	Natural	Pippip	F
PM027	Artiga de Lin	B	Mature Forest	L	L	Natural	Pippip	M
PM028	Artiga de Lin	B	Mature Forest	L	L	Natural	Pippip	M
PM029	Artiga de Lin	B	Mature Forest	L	L	Natural	Nyclei	M
PM030	Artiga de Lin	B	Mature Forest	L	L	Natural	Nyclei	M
PM031	Artiga de Lin	B	Mature Forest	L	L	Natural	Nyclei	M
PM032	Artiga de Lin	B	Mature Forest	L	L	Natural	Nyclei	M
PM033	Montpol	M	Immature forest	M	L	Extensive	Pipkuh	M
PM034	Montpol	M	Immature forest	M	L	Extensive	Pippip	M
PM035	Montpol	M	Immature forest	M	L	Extensive	Pipkuh	F
PM036	Montpol	M	Immature forest	M	L	Extensive	Pipkuh	F

*Data was provided by the Departament d'Acció Climàtica, Alimentació i Agenda Rural (i.e. Department of Climate Action, Food and Rural Agenda) of the Government of Catalonia.

Table S3.1. *Continued.*

Sample	Location name	Location ID	Land cover*	LC*	HP*	Land use	Species	Sex
PM037	Montpol	M	Immature forest	M	L	Extensive	Pipkuh	F
PM038	Montpol	M	Immature forest	M	L	Extensive	Pippip	M
PM039	Montpol	M	Immature forest	M	L	Extensive	Pipkuh	F
PM040	Montpol	M	Immature forest	M	L	Extensive	Pipkuh	F
PM041	Montpol	M	Immature forest	M	L	Extensive	Pippip	F
PM042	Montpol	M	Immature forest	M	L	Extensive	Pipkuh	F
PM043	Montpol	M	Immature forest	M	L	Extensive	Pipkuh	F
PM044	Montpol	M	Immature forest	M	L	Extensive	Pipkuh	F
PM045	Aiguamolls de Rufea	A	Farming	H	L	Intensive	Pippyg	F
PM046	Aiguamolls de Rufea	A	Farming	H	L	Intensive	Pipkuh	F
PM047	Aiguamolls de Rufea	A	Farming	H	L	Intensive	Pipkuh	F
PM048	Aiguamolls de Rufea	A	Farming	H	L	Intensive	Pipkuh	F
PM049	Aiguamolls de Rufea	A	Farming	H	L	Intensive	Pipkuh	F
PM050	Aiguamolls de Rufea	A	Farming	H	L	Intensive	Pipkuh	F
PM051	Aiguamolls de Rufea	A	Farming	H	L	Intensive	Pipkuh	M
PM052	Aiguamolls de Rufea	A	Farming	H	L	Intensive	Pipkuh	F
PM053	Parc de la Mitjana	N	Farming	H	L	Intensive	Pipkuh	M
PM054	Parc de la Mitjana	N	Farming	H	L	Intensive	Pipkuh	M
PM055	L'Espunyola	I	Immature forest	H	L	Extensive	Pipkuh	F
PM056	L'Espunyola	I	Immature forest	H	L	Extensive	Pipkuh	F
PM057	L'Espunyola	I	Immature forest	H	L	Extensive	Pipkuh	F
PM058	L'Espunyola	I	Immature forest	H	L	Extensive	Pipkuh	F
PM059	L'Espunyola	I	Immature forest	H	L	Extensive	Pippyg	M
PM060	L'Espunyola	I	Immature forest	H	L	Extensive	Pippyg	F
PM061	L'Espunyola	I	Immature forest	H	L	Extensive	Pippip	F
PM062	L'Espunyola	I	Immature forest	H	L	Extensive	Pippyg	M
PM063	L'Espunyola	I	Immature forest	H	L	Extensive	Nyclei	M
PM064	L'Espunyola	I	Immature forest	H	L	Extensive	Pipkuh	F
PM065	L'Espunyola	I	Immature forest	H	L	Extensive	Pipkuh	F
PM066	L'Espunyola	I	Immature forest	H	L	Extensive	Pipkuh	M
PM067	L'Espunyola	I	Immature forest	H	L	Extensive	Pipkuh	F
PM068	L'Espunyola	I	Immature forest	H	L	Extensive	Pipkuh	F
PM069	L'Espunyola	I	Immature forest	H	L	Extensive	Pipkuh	F
PM070	L'Espunyola	I	Immature forest	H	L	Extensive	Nyclei	M
PM071	L'Espunyola	I	Immature forest	H	L	Extensive	Pipkuh	F
PM072	L'Espunyola	I	Immature forest	H	L	Extensive	Pipkuh	F
PM073	L'Espunyola	I	Immature forest	H	L	Extensive	Pipkuh	M
PM074	L'Espunyola	I	Immature forest	H	L	Extensive	Pipkuh	F
PM075	L'Espunyola	I	Immature forest	H	L	Extensive	Pipkuh	F
PM076	L'Espunyola	I	Immature forest	H	L	Extensive	Pipkuh	NA
PM077	L'Espunyola	I	Immature forest	H	L	Extensive	Pippyg	NA
PM078	L'Espunyola	I	Immature forest	H	L	Extensive	Nyclei	F
PM079	L'Espunyola	I	Immature forest	H	L	Extensive	Nyclei	F
PM080	L'Espunyola	I	Immature forest	H	L	Extensive	Nyclei	F
PM081	L'Espunyola	I	Immature forest	H	L	Extensive	Pipkuh	F

*Data was provided by the Departament d'Acció Climàtica, Alimentació i Agenda Rural (i.e. Department of Climate Action, Food and Rural Agenda) of the Government of Catalonia.

Table S3.1. *Continued.*

Sample	Location name	Location ID	Land cover*	LC*	HP*	Land use	Species	Sex
PM082	L'Espunyola	I	Immature forest	H	L	Extensive	Pipkuh	M
PM083	L'Espunyola	I	Immature forest	H	L	Extensive	Pipkuh	F
PM084	L'Espunyola	I	Immature forest	H	L	Extensive	Nyclei	F
PM085	Margalef	G	Bush	L	L	Natural	Pippip	M
PM086	Margalef	G	Bush	L	L	Natural	Pippip	M
PM087	Margalef	G	Bush	L	L	Natural	Pippip	F
PM088	Barcelona	C	Urbanisation	L	H	Urban	Pippyg	M
PM089	Barcelona	C	Urbanisation	L	H	Urban	Nyclei	M
PM090	Prat de Llobregat	O	Urbanisation	L	H	Urban	Pippip	F
PM091	Prat de Llobregat	O	Urbanisation	L	H	Urban	Pippyg	F
PM092	Prat de Llobregat	O	Urbanisation	L	H	Urban	Pippyg	M
PM093	Prat de Llobregat	O	Urbanisation	L	H	Urban	Pippip	M
PM094	Prat de Llobregat	O	Urbanisation	L	H	Urban	Pippyg	M
PM095	Prat de Llobregat	O	Urbanisation	L	H	Urban	Pippyg	F
PM096	Prat de Llobregat	O	Urbanisation	L	H	Urban	Pippyg	M
PM097	Prat de Llobregat	O	Urbanisation	L	H	Urban	Pippip	F
PM098	Prat de Llobregat	O	Urbanisation	L	H	Urban	Pippyg	F
PM099	Prat de Llobregat	O	Urbanisation	L	H	Urban	Pippyg	M
PM100	Prat de Llobregat	O	Urbanisation	L	H	Urban	Pippyg	F
PM101	Prat de Llobregat	O	Urbanisation	L	H	Urban	Pippyg	M
PM102	Prat de Llobregat	O	Urbanisation	L	H	Urban	Pippyg	M
PM103	Prat de Llobregat	O	Urbanisation	L	H	Urban	Pippyg	F
PM104	Prat de Llobregat	O	Urbanisation	L	H	Urban	Pippyg	M
PM105	Prat de Llobregat	O	Urbanisation	L	H	Urban	Pippyg	F
PM106	Saverneda	H	Immature forest	M	L	Extensive	Minsch	F
PM107	Saverneda	H	Immature forest	M	L	Extensive	Minsch	M
PM108	Saverneda	H	Immature forest	M	L	Extensive	Minsch	M
PM109	Mequinensa	L	Farming	H	L	Extensive	Minsch	M
PM110	Mequinensa	L	Farming	H	L	Extensive	Minsch	F
PM111	Mequinensa	L	Farming	H	L	Extensive	Minsch	M
PM112	Mequinensa	L	Farming	H	L	Extensive	Minsch	M
PM113	Margalef	G	Bush	L	L	Natural	Minsch	M
PM114	Margalef	G	Bush	L	L	Natural	Minsch	M
PM115	Margalef	G	Bush	L	L	Natural	Minsch	F
PM116	Montrebei	F	Immature forest	L	L	Natural	Minsch	F
PM117	Malgrat de Mar	K	Urbanisation	L	H	Urban	Minsch	M
PM118	Malgrat de Mar	K	Urbanisation	L	H	Urban	Minsch	M
PM119	Montrebei	F	Immature forest	L	H	Natural	Minsch	F
PM120	Malgrat de Mar	K	Urbanisation	L	H	Urban	Minsch	M
PM121	Malgrat de Mar	K	Urbanisation	L	H	Urban	Minsch	M
PM122	Malgrat de Mar	K	Urbanisation	L	H	Urban	Minsch	F
PM123	Montrebei	F	Immature forest	L	L	Natural	Minsch	F
PM124	Montrebei	F	Immature forest	L	L	Natural	Minsch	F
PM125	Montrebei	F	Immature forest	L	L	Natural	Minsch	F
PM126	Montrebei	F	Immature forest	L	L	Natural	Minsch	F

*Data was provided by the Departament d'Acció Climàtica, Alimentació i Agenda Rural (i.e. Department of Climate Action, Food and Rural Agenda) of the Government of Catalonia.

Table S3.2. List of potential pathogenic bacteria (PPB) used in the microbiome-associated PPB analyses at species level. PPB identified by EMU software from the five bat species studied (*Miniopterus schreibersii*, *Nyctalus leisleri*, *Pipistrellus kuhlii*, *P. pipistrellus* and *P. pygmaeus*) are highlighted in bold. PPB are classified by their relevance as a potential/opportunistic (O) or recognized (R) human (H) or animal (A) pathogen (Lightfoot, 2003; WOAH, 2020; ECDC, 2023; Winnenburg, 2006; Mühldorfer et al, 2011). *Bacteria from WOAH Listed Diseases (WOAH); waterborne bacteria classified as "of potential health concern" by the WHO (WHO); bacteria included in "Red Nacional de Vigilancia Epidemiológica" (RENAVE) from Spain; European Centre for Disease Prevention and Control (ECDC); and bacteria identified from organ lesions of European deceased bats (Bat necropsies).

Bacteria species	Pathogenic relevance	Primary host	Disease/Syndrome and notes	*List
Aerococcus viridans	O	H, A (bats, cattle, swine)	Severe enteritis in bats.	Bat necropsies.
Aeromonas hydrophila	R	H, A (swine)		WHO
Achromobacter xylosoxidans	O	H		
Acinetobacter baumannii	O	H, A (cattle, swine, horses, dogs, cats, gulls and songbirds)		WHO
Acinetobacter haemolyticus	O	H		WHO
Acinetobacter nosocomialis	O	H		WHO
Acinetobacter pittii	O	H		WHO
Actinobacillus lignieresii	R	A (cattle)	Wooden tongue.	
Actinobacillus pleuropneumoniae	R	A (swine)	Pleuropneumonia.	
Aerococcus urinae	O	H		
Aerococcus viridans	O	H, A (*Eptesicus serotinus*)	Report of a severe enteritis in one *E. serotinus*.	
Aeromonas caviae	R	H, A (cattle, swine, other mammals and cold-blooded animals)		WHO
Aeromonas hydrophila	R	H, A (Cattle, swine and amphibians)		WHO
Arcobacter suis	O	H	Foodborne emerging pathogen.	
Anaplasma marginale	R	H, A (cattle)	Bovine anaplasmosis.	WOAH
Bacillus anthracis	R	H, A (domestic and wild herbivores, swine)	Anthrax (toxin).	WOAH, WHO, RENAVE, ECDC
Bacillus cereus	R	H, A (bats, cattle, swine, poultry, psittacine)	Foodborne and local/systemic infections.	Bat necropsies
Bacillus cytotoxicus	R	H	Foodborne. Member of the *Bacillus cereus* group.	

Table S3.2. *Continued.*

Bacteria species	Pathogenic relevance	Primary host	Disease/Syndrome and notes	*List
Bacillus subtilis	O	H	Immunocompromised patients.	
Bacillus thuringiensis	O	H, A (insects)	Foodborne, rare.	
Bacteroides fragilis	O	H		
Bartonella ancashensis	R	H	Verruga peruana.	
Bartonella bacilliformis	R	H	Verruga peruana.	
Bartonella clarridgeiae	R	H, A (dogs, cats)	Cat scratch disease.	
Bartonella elizabethae	R	H, A (warm-blooded)	Rodents are reservoirs; fleas are the vectors.	
Bartonella grahamii	R	H	Cat scratch disease. Rodents are reservoirs; *Ixodes ricinus* the vector.	
Bartonella henselae	R	H, A (dogs, cats)	Cat scratch disease.	
Bartonella quintana	R	H	Trench fever.	
Bartonella vinsonii	O	H		
Bordetella parapertussis	R	H	Mid whooping cough.	
Bordetella pertussis	R	H	Whooping cough.	RENAVE, ECDC
Bordetella petrii	O	H		
Bordetella trematum	O	H		
Borrelia burgdorferi	R	H, A (dog, horse)	Lyme disease.	RENAVE, ECDC
Borrelia hispanica	R	H	Lyme disease.	RENAVE
Bosea thiooxidans	O	H		
Brucella abortus	R	H, A (cattle)	Brucellosis or undulant fever.	WOAH, WHO, ECDC
Brucella melitensis	R	H, A (small ruminants)	Malta fever.	WOAH, WHO, RENAVE, ECDC
Brucella ovis	R	A (small ruminants)	Ovine epididymitis.	WOAH
Brucella suis	R	H, A (swine)	Brucellosis in swine.	WOAH, ECDC
Burkholderia ambifaria	O	H		
Burkholderia cenocepacia	O	H		
Burkholderia cepacia	O	H		
Burkholderia mallei	R	H, A (horse)	Glanders.	WOAH
Burkholderia multivorans	O	H		
Burkholderia pseudomallei	R	H, A (cattle, swine, poultry and other mammals, fish and reptiles)	Considered a welfare agent.	WHO

Table S3.2. *Continued.*

Bacteria species	Pathogenic relevance	Primary host	Disease/Syndrome and notes	*List
Burkholderia pseudomultivorans	O	H		
Burkholderia pyrrocinia	O	H		
Burkholderia stabilis	O	H		
Burkholderia thailandensis	O	H		
Burkholderia vietnamiensis	O	H		
Campylobacter coli	R	H, A (cattle and ostriches but rare)	Enteric campylobacteriosis in humans.	WHO, RENAVE
Campylobacter fetus	R	A (cattle)	Bovine genital campylobacteriosis.	WOAH
Campylobacter hyointestinalis	R	H, A (swine, cattle and other mammals)	Porcine proliferative enteritis.	
Campylobacter jejuni	R	H, A (cattle and ostriches but rare)	Enteric campylobacteriosis in humans.	WHO, RENAVE, ECDC
Cedecea davisae	O	H, A (bats)	Bile duct proliferation in a sick (death) bat.	Bat necropsies.
Chlamydia abortus	R (O for humans)	H, A (small ruminants)	Ovine chlamydiosis.	WOAH
Chlamydia psittaci	R	H, A (poultry, cattle, swine, horse)	Psittacosis	WOAH
Chlamydia trachomatis	R	H	Chlamydiosis.	RENAVE, ECDC
Citrobacter amalonaticus	O	H		
Citrobacter braakii	O	H		
Citrobacter farmeri	O	H		
Citrobacter freundii	O	H, A (small ruminants, swine, poultry, reptiles and aquatic animals)		WHO
Citrobacter koseri	O	H, A (cattle)		WHO
Clostridioides difficile	R	H, A (cattle, swine, horses and other mammals)	Pseudomembranous colitis (toxins).	ECDC
Clostridium baratii	R	H	Spores.	
Clostridium botulinum	R	H, A (cattle, poultry and other mammals)	Botulism (toxins). Considered a welfare agent. Spores.	WHO, RENAVE, ECDC
Clostridium butyricum	O	H	Spores.	
Clostridium colinum	R	A (quail)	Ulcerative enteritis ("quail disease").	

Table S3.2. *Continued.*

Bacteria species	Pathogenic relevance	Primary host	Disease/Syndrome and notes	*List
Clostridium haemolyticum	R	H, A (cattle)	Bacillary haemoglobinuria in cattle. Toxins.	
Clostridium perfringens	R	H, A (cattle, swine, poultry, horses)	Toxins.	
Clostridium septicum	O	H	Toxins.	
Clostridium sordellii	O	H, A (bats, cattle, horse)	Bile duct proliferation in a sick (death) bat. Gas gangrene in humans. Sudden Death Syndrome in cattle.	Bat necropsies.
Clostridium spiroforme	R	A (rabbits)	Enteritis (toxins).	
Clostridium tetani	R	H, A (mammals)	Tetanus (toxins).	RENAVE, ECDC
Comamonas kerstersii	O	H		
Comamonas testosteroni	O	H		
Corynebacterium diphtheriae	R	H	Diphtheria.	RENAVE, ECDC
Corynebacterium provencense	O	A (cat)		
Corynebacterium pseudotuberculosis	R	H, A (cattle, horse)	Caseous lymphadenitis.	RENAVE
Corynebacterium renale	R	A (cattle)		
Corynebacterium stationis	O	H		
Corynebacterium ulcerans	R	H, A (cattle, cat, dog)	Diphtheria.	RENAVE, ECDC
Coxiella burnetii	R	H, A (cattle, swine and many mammals, including wildlife)	Q fever.	WOAH, ECDC
Ehrlichia chaffeensis	R	H	Human monocytotropic ehrlichiosis.	
Ehrlichia minasensis	R	H, A (cattle)	Bovine ehrlichiosis	
Ehrlichia muris	R	H	Human ehrlichiosis (CDC, USA).	
Ehrlichia ruminantium	R	A (cattle)	Cowdriosis.	
Enterobacter cloacae	O	H		
Enterobacter cloacae complex sp.	O	H		
Enterococcus casseliflavus	O	H		
Enterococcus cecorum	O	H, A (poultry and other mammals)		
Enterococcus faecalis	O	H, A (cattle, bats)	Mastitis in cattle. Severe enteritis in bats.	Bat necropsies.
Enterococcus faecium	O	H, A (cattle)	Mastitis in cattle.	

Table S3.2. *Continued.*

Bacteria species	Pathogenic relevance	Primary host	Disease/Syndrome and notes	*List
Enterococcus gallinarum	O	H, A (mice)	Systemic lupus erythematous in mice.	
Enterococcus hirae	O	H, A (cattle, swine, poultry)		
Erysipelothrix rhusiopathiae	R	H, A (sheep, swine, poultry)		
Erysipelothrix tonsillarum	O	A (swine)	Rare swine erysipelas.	
Escherichia albertii	R	H, A (mammals and birds)	Emerging enteropathogen (diarrhoea) of humans and many avian species. It has also been reported in pigs, cats, dogs, bats, raccoons, penguins, and seals. Strains that affect wild birds have the potential to infect humans, livestock and domestic animals.	
Escherichia coli	R	H, A (cattle, swine, poultry, bats, many others)	Particularly enteropathogenic, enterotoxigenic or verotoxigenic.	WHO, RENAVE, ECDC, Bat necropsies
Francisella philomiragia	R	H		
Francisella tularensis	R	H, A (small ruminants, swine, rabbits, hares, rodents, beavers, cats, horses, other wildlife).	Considered a welfare agent.	WOAH, WHO, RENAVE, ECDC
Fusobacterium varium	O	H		
Gemella bergeri	O	H		
Gemella cuniculi	O	A (rabbit)	Isolated from an abscess.	
Gemella haemolysans	O	H		
Gemella morbillorum	O	H		
Gemella sanguinis	O	H		
Haemophilus haemolyticus	O	H		
Haemophilus influenzae	R	H		RENAVE, ECDC
Haemophilus parahaemolyticus	R	A (swine)	Pleuropneumonia.	
Haemophilus parainfluenzae	O	H		
Hafnia alvei	O	H, A (cattle, poultry, ducks, others)		

Table S3.2. *Continued.*

Bacteria species	Pathogenic relevance	Primary host	Disease/Syndrome and notes	*List
Hathewaya histolytica	O	H, A (horses, cattle)		
Helicobacter hepaticus	O	H, A (mice)		
Helicobacter pylori	O	H		
Ignatzschineria indica	O	H		
Ignatzschineria ureiclastica	O	H		
Klebsiella aerogenes	O	H, A (many)		WHO
Klebsiella grimontii	O	H		WHO
Klebsiella huaxiensis	O	H		WHO
Klebsiella michiganensis	O	H		WHO
Klebsiella oxytoca	R	H, A (bats, cattle)	Second most common cause of *Klebsiella* infections in humans.	WHO, Bat necropsies
Klebsiella pneumoniae	R	H, A (cattle, swine, others)	Primary pathogen capable of causing UTIs, liver abscess and pneumonia in healthy people.	WHO
Klebsiella quasipneumoniae	O	H		
Klebsiella variicola	O	H		
Lawsonia intracellularis	R	A (swine)	Porcine proliferative enteritis (PPE).	
Leclercia adecarboxylata	O	H		
Legionella longbeachae	R	H	Legionellosis.	
Legionella pneumophila	R	H	Legionellosis.	RENAVE, ECDC
Leptospira interrogans	R	H, A (mammals, amphibians, reptiles)	Leptospirosis.	RENAVE, ECDC
Leuconostoc mesenteroides	O	H		
Leuconostoc pseudomesenteroides	O	H		
Listeria monocytogenes	R	H, A (cattle, poultry, others)	Listeriosis.	RENAVE, ECDC
Listeria seeligeri	O	H	Foodborne.	
Macrococcus canis	O	A (dog)		
Macrococcus caseolyticus	O	A (cattle, swine, poultry)		
Mannheimia glucosida	O	H, A (cattle and sheep)		
Mannheimia haemolytica	R	A (cattle)	Bovine pneumonic pasteurellosis (mannheimiosis).	
Mannheimia varigena	O	A (cattle, swine)		
Massilia oculi	O	H		

Table S3.2. *Continued.*

Bacteria species	Pathogenic relevance	Primary host	Disease/Syndrome and notes	*List
Massilia timonae	O	H		
Mesomycoplasma bovoculi	O	A (cattle)	Infectious bovine keratoconjunctivitis.	
Mesomycoplasma dispar	O	A (cattle)		
Mesomycoplasma hyopneumoniae	R	A (swine)	Most important respiratory pathogen.	
Mesomycoplasma hyorhinis	R	A (swine)	Respiratory disease and arthritis.	
Moellerella wisconsensis	O	H		
Moraxella bovis	R (O for humans)	H, A (cattle)	Infectious bovine keratoconjunctivitis (most common).	
Moraxella bovoculi	O	A (cattle)	Infectious bovine keratoconjunctivitis.	
Morganella morganii	O	H		
Mycobacterium alsense	O	H		
Mycobacterium barrassiae	O	H		
Mycobacterium bovis	R	H, A (cattle, many)	Bovine tuberculosis.	WOAH, WHO, RENAVE, ECDC
Mycobacterium caprae	R	H, A (small ruminants)	Caprine tuberculosis.	WOAH
Mycobacterium goodii	O	H		
Mycobacterium koreense	O	H		
Mycobacterium leprae	R	H	Leprosy.	RENAVE
Mycobacterium paratuberculosis (*M. avium* spp. *paratuberculosis)*	R	H, A (cattle, small ruminants, swine, other mammals)	Johne's disease (cattle) and Crohn's disease (humans).	WOAH, WHO
Mycobacterium senegalense	O	H		
Mycobacterium talmoniae	O	H		
Mycobacterium tuberculosis	R	H, A (non-human primates)	Human tuberculosis.	WOAH, WHO, ECDC
Mycobacterium virginiense	O	H		
Mycobacterium xenopi	R	H		WHO
Mycoplasma agalactiae	R	A (small ruminants)	Contagious agalactia.	WOAH
Mycoplasma anserisalpingitidis	R	A (geese, ducks, other waterfowl)	The most important pathogenic *Mycoplasma* species of waterfowl in Europe and Asia.	
Mycoplasma capricolum	R	A (caprine)	Contagious caprine pleuropneumonia (CCPP).	OIE

Table S3.2. *Continued.*

Bacteria species	Pathogenic relevance	Primary host	Disease/Syndrome and notes	*List
Mycoplasma mycoides	R	A (cattle)	Subsp. *mycoides* causes contagious bovine pleuropneumonia (CBPP).	OIE
Mycoplasma nasistruthionis	O	A (ostriches)		
Mycoplasma struthionis	O	A (ostriches)		
Mycoplasmopsis canis	O	A		
Mycoplasmopsis felis	O	A (cats, horses)		
Mycoplasma gallisepticum	R	A (poultry)	Avian mycoplasmosis.	WOAH
Mycoplasmopsis pulmonis	O	A (rat)		
Mycoplasma synoviae	R	A (poultry)	Avian mycoplasmosis.	WOAH
Neisseria animaloris	O	H		
Neisseria gonorrhoeae	R	H	Gonorrhoea.	RENAVE
Neisseria meningitidis	R	H	Meningococcal disease.	RENAVE, ECDC
Nocardia asteroides	O	H		
Nocardia farcinica	O	H		
Nocardiopsis dassonvillei	O	H		
Pandoraea apista	O	H		
Pandoraea pnomenusa	O	H		
Pandoraea pulmonicola	O	H		
Pantoea agglomerans	O	H		
Paracoccus yeei	O	H		
Pasteurella multocida	R	H, A (many)		WOAH, WHO, Bat necropsies.
Plesiomonas shigelloides	O	H		
Proteus mirabilis	O	H, A (cattle, swine, poultry)		
Proteus vulgaris	O	H, A (many)		
Pseudomonas aeruginosa	R	H, A (cattle, swine, poultry)		WHO
Pseudomonas fluorescens	O	H		
Pseudomonas luteola	O	H		
Pseudomonas mendocina	O	H		
Pseudomonas putida	O	H		
Pseudomonas simiae	O	A (monkey)	Detected in a monkey (*Callithrix geoffroyi*) with pneumonitis and pneumonia.	
Pseudomonas stutzeri	O	H		
Raoultella ornithinolytica	O	H		

Table S3.2. *Continued.*

Bacteria species	Pathogenic relevance	Primary host	Disease/Syndrome and notes	*List
Raoultella planticola	O	H		
Raoultella terrigena	O	H		
Rhodococcus equi	R	H, A (cattle, swine, others)	Pyogranulomatous pneumonia in foals, lymphadenitis in swine.	
Rickettsia akari	R	H	Considered a welfare agent.	WHO
Rickettsia asiatica	O	A	Considered a welfare agent.	WHO
Rickettsia bellii	O	H	Considered a welfare agent. Ancestral group (AG)	WHO
Rickettsia canadensis	O	H	Considered a welfare agent. Ancestral group (AG)	WHO
Rickettsia felis	O	H	Considered a welfare agent.	WHO
Rickettsia japonica	O	H	Considered a welfare agent. Japanese spotted fever.	WHO
Rickettsia massiliae	R	H	Considered a welfare agent. Spotted fever group (SFG). Transmitted by: *Rhipicephalus turanicus, R. sanguineus, R. bursa, R. pusillus, Ixodes ricinus*. Spotted fever in Europe.	WHO
Rickettsia slovaca	R	H	Considered a welfare agent. Tick borne lymphadenopathy (TIBOLA) or Dermacentor borne, necrosis, erythema, lymphadenopathy (DEBONEL). Transmitted by: *Dermacentor marginatus, D. reticulatus* in Europe.	WHO
Rickettsiella massiliensis	O	H		
Ruminococcus gnavus	O	H		
Salmonella abortusovis (*S. enterica* serovar Abortusovis)	R	A (small ruminants)	Salmonellosis.	WOAH
Salmonella bongori	R	H, A (many)	Salmonellosis.	WHO
Salmonella enterica	R	H, A (many)		WOAH, WHO, REAVE, Bat necropsies
Salmonella paratyphi (*S. enterica* serovar Paratyphi)	R	H, A (mammals, birds, reptiles)	Paratyphoid fever in humans.	WHO, ECDC

Table S3.2. *Continued.*

Bacteria species	Pathogenic relevance	Primary host	Disease/Syndrome and notes	*List
Salmonella Typhi (*S. enterica* serovar Typhi)	R	H	Typhoid fever in humans.	WHO, ECDC
Salmonella Typhimurium (*S. enterica* serovar Typhimurium)	R	H, A (mammals, poultry, passerines, reptiles, amphibians)	Gastroenteritis (usually self-limiting) in humans. Avian salmonellosis.	WOAH
Serratia fonticola	O	H		
Serratia liquefaciens	O	H, A (cattle)	Mastitis.	
Serratia marcescens	O	H, A (cattle)	Mastitis.	
Serratia odorifera	O	H		
Serratia plymuthica	O	H		
Serratia rubidaea	O	H, A		
Shigella boydii	R	H	Shigellosis.	WHO, RENAVE, ECDC
Shigella dysenteriae	R	H	Shigellosis.	WHO, RENAVE, ECDC
Shigella flexneri	R	H	Shigellosis.	WHO, RENAVE, ECDC
Shigella sonnei	R	H	Shigellosis.	WHO, RENAVE, ECDC
Staphylococcus aureus	R	H, A (bats, cattle, swine, poultry rabbits, others)	Enterotoxin B.	WHO, Bat necropsies
Staphylococcus caprae	O	H, A (cattle, swine, horses)		
Staphylococcus chromogenes	O	A (cattle, swine)		
Staphylococcus cohnii	O	H		
Staphylococcus delphini	O	A		
Staphylococcus epidermidis	O	H, A (swine, rodents)		
Staphylococcus felis	O	H, A (cats)		
Staphylococcus haemolyticus	O	H, A (small ruminants,)		
Staphylococcus hominis	O	H		
Staphylococcus hyicus	R	A (cattle, swine)	Swine exudative epidermidis (EE).	
Staphylococcus massiliensis	O	H		
Staphylococcus pseudintermedius	O	H, A (cattle, sheep, caprine, dogs)		

Table S3.2. *Continued.*

Bacteria species	Pathogenic relevance	Primary host	Disease/Syndrome and notes	*List
Stenotrophomonas maltophilia	O	H, A (cattle, sheep, caprine, swine)		
Streptococcus agalactiae	O	H, A (cattle)		
Streptococcus cristatus	O	H		
Streptococcus equi	R	A (horses, cattle)	Strangles in horses.	
Streptococcus equinus	O	H, A (horses)		
Streptococcus gallinaceus	O	H, A (poultry)		
Streptococcus iniae	O	H		
Streptococcus parauberis	O	A (cattle)	Mastitis.	
Streptococcus pneumoniae	R	H	Invasive pneumococcal disease.	RENAVE, ECDC
Streptococcus pyogenes	R	H	Severe tonsilitis, particularly in children.	
Streptococcus suis	R	H, A (swine)	Meningitis, septicaemia, and arthritis in swine.	
Streptococcus uberis	O	A (cattle)	Mastitis.	
Taylorella equigenitalis	R	A (horse)	Contagious equine metritis.	WOAH
Treponema pallidum	R	H, A (non-human primates)	Syphilis.	RENAVE
Tsukamurella paurometabola	O	H		
Tsukamurella strandjordii	O	H		
Ureaplasma diversum	O	A (cattle)		
Ureaplasma parvum	O	H		
Ureaplasma urealyticum	O	H		
Vibrio cholerae	R	H	Cholera.	WHO, RENAVE, ECDC
Vibrio parahaemolyticus	R	H	Most common species causing disease in humans from USA.	WHO
Vibrio vulnificus	R	H	Most common species causing disease in humans from USA.	WHO

Table S3.2. *Continued.*

Bacteria species	Pathogenic relevance	Primary host	Disease/Syndrome and notes	*List
Yersinia enterocolitica	R	H, A (bats, cattle, swine)	Yersiniosis.	WHO, RENAVE, ECDC, Bat necropsies
Yersinia frederiksenii	O	H		
Yersinia pestis	R	H, A (cattle, cats, dogs, lagomorphs, mustelids, and occasionally ungulates)	Plague.	WHO, ECDC
Yersinia pseudotuberculosis	R	H, A (cattle, sheep, caprine, deer, dogs, cats, wildlife, turkeys, other birds)	Pseudotuberculosis.	WHO, RENAVE, ECDC, Bat necropsies

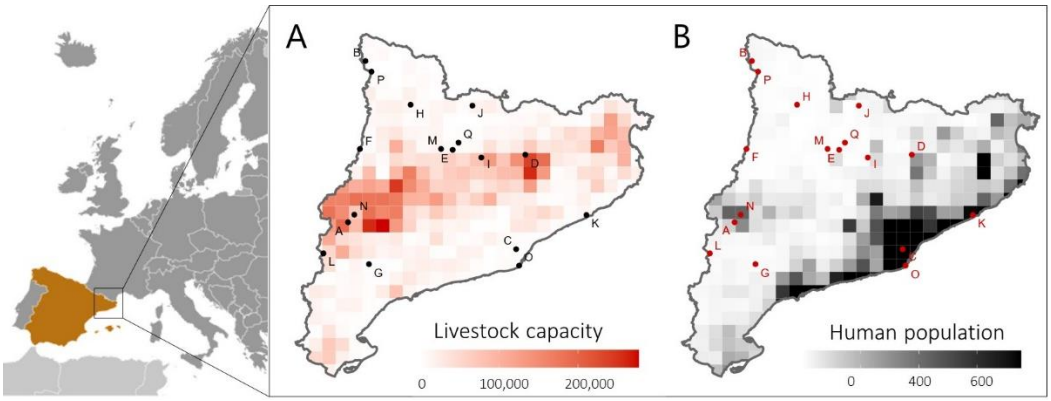


Figure S3.1. A. Livestock capacity, including bovine, ovine, caprine and swine, from Catalonia (NE Spain) represented in a 10 x 10 km grid. **B.** Human population from Catalonia (NE Spain) represented in a 10 x 10 km grid. High colour intensities reflect higher values in both A and B figures. Sampling locations are depicted with letters from A to Q, whose corresponding localities can be found in **Table S3.1 (Supplementary material)**.

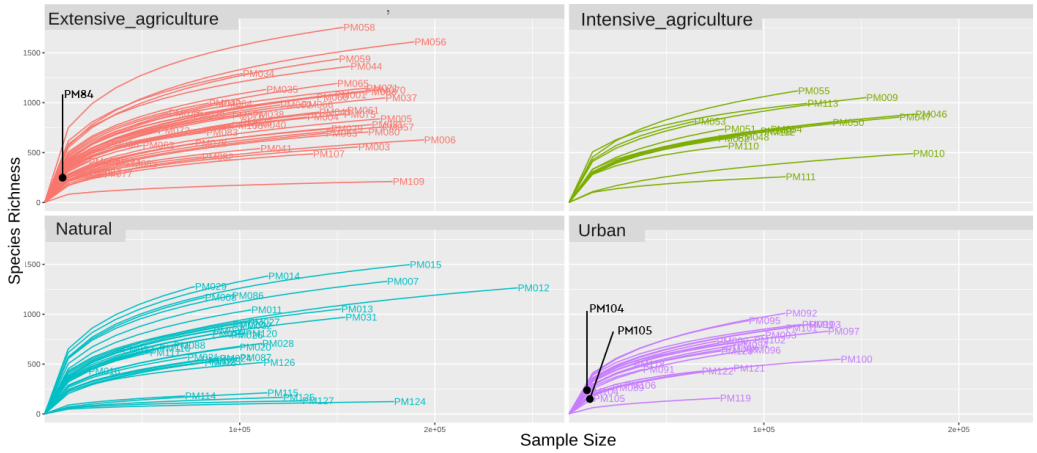


Figure S3.2. Rarefaction curves of bat faecal samples sample, separated by land uses. Sequence sample size (number of reads generated) and genus richness are respectively depicted on the X and Y-axis. Rarefaction curves of richness against sequence sample size reached asymptotic growth for all samples except for PM084 (extensive agriculture), and PM104 and PM105 (urban).

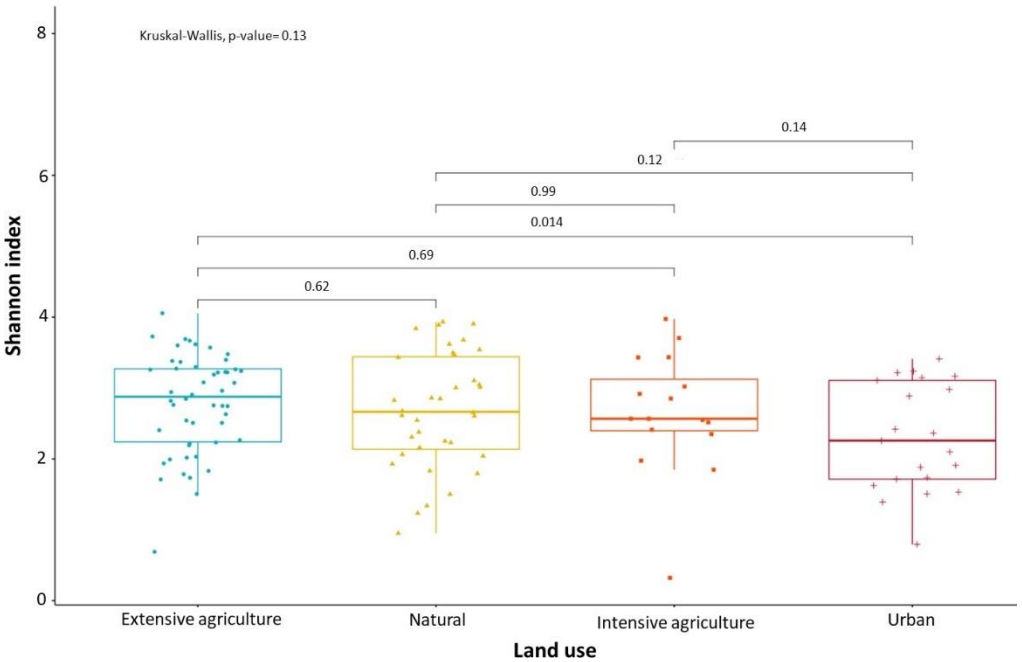


Figure S3.3. A boxplot of Shannon's Diversity Index (Kruskal-Wallis statistic: 11.144, p-value= 0.13) for the different land uses (extensive agriculture, natural, intensive agriculture and urban). Significant values are those with a p-value <0.05.

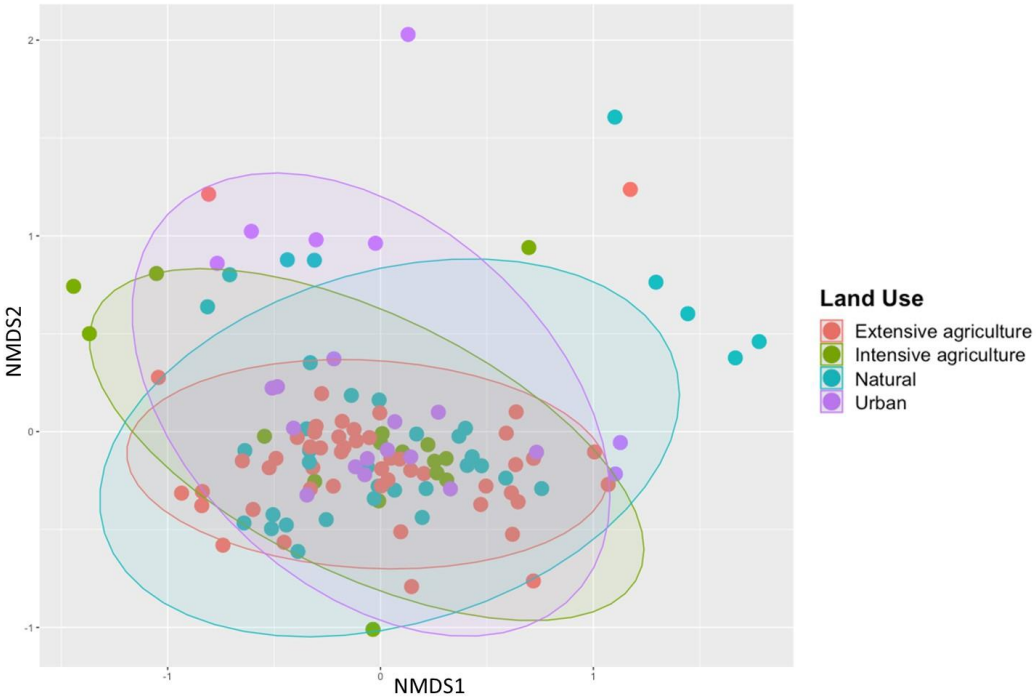


Figure S3.4. Non-Metric Multidimensional Scaling (NMDS) plot from Bray-Curtis distances for the different land uses (PERMANOVA's R: 0.054; p-value= 0.001).

General discussion

This thesis highlights the significance of meticulously designed studies and the establishment of an ethics committee within the framework of bat research in order to support current and worldwide bat conservation strategies. Additionally, it accentuates the promise of non-invasive methodologies, specifically the examination of the fecal microbiome, as a valuable approach for evaluating the health of insectivorous bat populations. Overall, this thesis advocates for the initiation of studies inquiring into bat populations' health while also prioritizing bat welfare.

Healthy wings for a thriving life

Bat wings are composed of a bilayered skin, referred to as a membrane, structured with abundant elastin and neurovascular bundles (Cheney et al., 2017) and skeletal muscles that actively participate in the bat flight control system (Cheney et al., 2014). Each membrane section has a particular mechanical behaviour: the distal parts (dactylopatagium and uropatagium) function mostly in propulsion, while the proximal sections (propatagium and plagiopatagium) are involved in the lifting process (Zubaid et al., 2006; Vaughan, 1970). For instance, the stiffness of the propatagium enables the air to pass above and under the wing at a specific angle (known as the angle of attack) to develop lift effectively (Vaughan, 1970). In **Study 1**, all 22 Seba's short-tailed bats developed injuries in the propatagium after six weeks of being marked with forearm rings. Even mildly affected bats showed structural changes in that area, characterized by skin thinning and increased blood flow. More severe lesions implied the loss of the normal continuity of the skin tissue. Although the damaged areas of the skin were not examined histologically, changes in the normal composition and function of collagen and elastin fibres are expected from these injuries ((Ceballos-Vasquez et al., 2015; Eming et al., 2014; Schultz et al., 2011). In birds, injuries in the propatagium can make the bird flightless because it is a crucial component in the wing extension mechanism (Samour, 2016). Despite propatagium damage is acknowledged by bat banding specialists, and supported by our results from **Study 1**, it is unemphasized in the published literature and the consequences of such damage have barely been explored. Similar to birds, impaired contraction capabilities and stiffness of the propatagium may have negative impacts on wing physiology and flight performance. For instance, the flight performance and metabolic rates from two species of free-ranging bats (*Myotis albescens* and *M. nigricans*) affected and not

affected by injured wing membranes were compared in captivity (Voigt, 2013). Although all bats flew at a similar speed, bats with damaged wing membranes performed fewer U-turns in a circular flight area, possibly translating to lower maneuverability. Overall, many questions remain unanswered regarding the negative effects associated with flight membrane damage, such as reduced foraging or reproductive success.

In our analysis of bat recaptures in NE-Spain over the past 12 years, we found an occurrence of wing damage exceeding 10% in eight different bat species. Skin lesion development and healing due to forearm rings may be influenced by specific reproductive behaviour, hormonal fluctuations, immune system changes, or energy allocation during the reproductive season (Ruoss et al., 2019). Particularly, in our **Study 1**, we emphasized the increased susceptibility of reproductively active bats to develop skin lesions from forearm rings. Bats have a low annual reproductive output, compensated by a significantly longer life span than other mammal species (O'Shea et al., 2004). Accordingly, any reduction in their reproductive success resulting from the consequences of being banded may risk the bat population's survival (Davy et al., 2022; Schorcht et al., 2009).

Besides being the bat's primary mode of locomotion, wings are also crucial components for bat's thermoregulation (Reichard et al., 2010) and water balance (Webb et al., 1995). Therefore, intentional (i.e. wing biopsy) and unintentional human activities (i.e. banding activities) that may cause damage to wing membranes and adversely affect their physiological functions should be investigated in further detail.

"To tag or not to tag": ethical decision-making in individual marking tools for insectivorous bats

According to the Terrestrial Code of the World Organisation for Animal Health (WOAH), "animal welfare" means "the physical and mental state of an animal in relation to the conditions in which it lives and dies". Animal welfare subject is one of the priorities of the WOAH, and has the following principles known as "Five Freedoms": freedom from hunger, malnutrition and thirst; freedom from fear and distress; freedom from heat stress or physical discomfort; freedom from pain, injury and disease; and freedom to express normal patterns of behaviour (WOAH, 2023). Irrespective of the objective, any intervention on a wild animal

(capture, handling, marking…) can compromise its welfare at different levels (Lindsjö et al., 2019). However, the lack of clear welfare indicators in most wild animal species complicates management and regulation efforts (Soulsbury et al., 2020). Moreover, varying levels of legal protection for wildlife species (some are more protected than others) hinder the control and regulation of wildlife activities from a welfare perspective (Lindsjö et al., 2019). Notably, all European bat species are protected by law, both at local and international scales (Hutson et al., 2001; UNEP/EUROBATS, 2002). Despite several guidelines outlining best practices for bat handling have been developed (Battersby, 2010; Institutional Animal Care and Use Committee, 2023), research involving bats is often conducted in the field, with challenging conditions such as bad weather, poor light, and difficult access to the capture location (Voigt & Kingston, 2015). Therefore, these guidelines are not directly linked to bat welfare but to practicality, as happens with most wildlife species guidelines (Soulsbury et al., 2020). For instance, invasive procedures such as skin biopsies, may usually take place in non-sterile environments, with limited technical resources (e.g. nonstable working surface) and deficient post-handling monitoring.

Concerning individual marking techniques for insectivorous bat species, the same concept of practicability has been applied since the beginning of marking activities in the nineteenth century. Overall, the most used device for individually identifying bats are forearm rings, which are significantly cheap and easy to place (Stebbings, 2004). While initially considered a promising and non-invasive method, detrimental impacts of this technique have been reported worldwide since its implementation (Baker et al., 2001; Balmori & Quetglas, 2000; Dietz et al., 2006; Ellison, 2008; Hutterer et al., 2005; O'Shea et al., 2004; Sendor & Simon, 2003; Steffens et al., 2007). Contrary to what should be expected, forearm rings are still the first choice for many bat specialists and researchers, who claim that banding activities cause no harm to bat populations or continue regardless of harm evidence because of the expected benefit obtained by this technique (Ethical Guidelines for the Use of Animals in Research, 2018; UNEP/EUROBATS, 2018). Overall, in our **Study 1** we found much larger proportions of skin lesions compared to previous studies involving *P. pygmaeus* (Dietz et al., 2006; Sendor & Simon, 2003), *M. schreibersii* (Baker et al., 2001) and *N. leisleri* (Zambelli et al., 2009). Lack of pathology and bat welfare knowledge, and variable dimensions of injury perception may lead to different conclusions between researchers, particularly if their studies omit to report injuries. However, from a welfare point

of view, no skin lesion, regardless of its severity, should be neglected but recognized as a source of physical discomfort to bats. Furthermore, a standard ethical approach to the justification of research methods is to balance the harm to research results (NENT, 2018; UNEP/EUROBATS, 2018). In this sense, bat researchers must explore alternatives, such as but not limited to PIT tags, which prove to be suitable for individual recognition without causing harm (Edmonds et al., 2017; Locatelli et al., 2019; Rigby et al., 2012; van Harten et al., 2020).

Before this thesis was submitted and in response to the publication of **Study 1** in a peer-reviewed journal, we as authors were questioned by part of the bat scientific community, attributing the high percentage of band injuries to a lack of technique or training. However, currently, there is no formal training on this topic at the European level, and training is based on repeated field manipulations where animals are released and cannot be monitored. In this sense, many people, regardless of their level of expertise or knowledge, are allowed to mark bats with this method. Field technicians from **Study 1** have extensive experience in bat handling and banding, so their technique should not be questioned following the aforementioned argument. As scientists, our marking methods of choice should not be those whose success depends so heavily on the experience of the handler, but rather those that inherently cause no adverse effects, regardless of the level of training required. In this context, the requirement for formal training and qualified personnel (e.g., veterinarians) should not be a limiting factor in promoting the use of certain marking methods over others.

At the international level, better coordination is needed among institutions to implement evidence-based strategies on bat research without compromising the provision and exchange of information, particularly for migratory bat species. Decisions on the acceptable rate of injuries derived from marking tools should be standardised following ethical principles and evaluated by competent authorities and recognised institutions. We recommend that policymakers ban the authorisation of marking techniques -particularly the use of forearm rings- in bat species until adequate studies to evaluate their impact have been performed under controlled conditions. In any case, all studies carrying out marking activities on bats should be submitted to a legally constituted ethics committee for consideration, guidance, and approval.

A promising non-invasive technique for the study of insectivorous bat's health: the fecal microbiome

Health can be broadly defined as a state of physical and psychological well-being and the subsequent ability to adapt and cope with a changing environment (Ryser-Degiorgis, 2013). Measuring the health of a group of individuals or populations, particularly in free-ranging species such as insectivorous bats, can be challenging. Health indices, such as body condition or hematological values, are quantifiable parameters used to refer to the health state of a group of animals or species (Ryser-Degiorgis, 2013). However, a specific framework to assess this particular issue in populations of insectivorous bats around the globe has not yet been developed. Difficulties inherent to this broad taxonomic group are not only the requirement of species-specific knowledge of ecology, anatomy, disease susceptibility and pathology, but also arduous sampling conditions often entailing inaccessible sites or technical training (Frick et al., 2019; Mühldorfer et al., 2011). Overall, capturing and sampling bats require specialized technical training to ensure the safe setting of traps and the appropriate and safe handling of bats, minimizing the risk of injury to both bats and the handlers. Furthermore, the researcher must be ready to work in inclement weather conditions and difficult-to-access terrains. For **Studies 2** and **3** we employed various capture methods, including mist nets and harp nets for night captures, as well as checking bat-boxes during the day. Some sampling locations were located in remote areas, such as Montrebei and Margalef, which were difficult to access. For instance, reaching these locations and setting the capture traps required physical fitness, and wearing personal protective equipment such as harnesses and helmets was necessary for safety reasons. All these challenges justify the urgent need to investigate biological samples that are easy to collect in the field and can provide us with information about bats' health without requiring fieldwork essentials such as stable tables and chairs, or specialized technical equipment such as a mobile anaesthesia unit.

Currently, health surveillance programs for insectivorous bats' health worldwide are mostly limited to white-nose syndrome (WNS) analysis in the USA, assessing body condition (regardless of many technical limitations) and, to a lesser extent and primarily in dead bats, evaluating exposure to pesticides and toxins (Segers et al., 2021; Torquetti et al., 2021; U.S. Fish and Wildlife Service, 2024). Particularly, body condition and exposure to pesticides and toxins are also believed to

directly correlate with the degree of habitat degradation (Segers et al., 2021). In **Study 3**, we explored the body condition index of all sampled bats and found no significant differences between them, regardless of the habitat degradation level at their sampling locations. However, in both **Study 2** and **3**, we found significant differences in microbiome community structure between landscapes poorly preserved, such as urban areas, and better preserved, such as natural areas or areas dedicated to extensive agriculture. These differences were not explained by geographic distance or different reproductive status, highlighting the potential impact of human activities on the fecal microbiome composition of insectivorous bats. Furthermore, we found that the fecal bacterial richness of five free-ranging insectivorous bat species decreased along a gradient of increasing urbanization and other anthropogenic disturbances such as the high presence of domesticated animals. Since the loss of microbiota diversity opens up niches for opportunistic pathogens (Zheng et al., 2020), results from both **Study 2** and **3** suggest that anthropogenic disturbance may impact the gut microbiome composition (and indirectly their functionality) of insectivorous bats and pose a risk to their health.

The state of the human gut microbiome (bacterial diversity, relative abundances of "good" and "bad" bacteria, etc.) is currently being used as an indicator for assessing human health (Gut Microbiome Wellness Index, formerly Gut Microbiome Health Index) (Chang et al., 2023; Gupta et al., 2020). On the contrary, the study of the gut/fecal microbiome in free-ranging insectivorous bats is in its infancy, with much still to be explored. However, the field of bat population health is receiving increasing recognition for its significance in informing conservation strategies worldwide (Fagre & Malmlov, 2021). Owing to its non-invasive nature and simplicity of sampling, the study of the fecal bacterial microbiome seems a promising technique to be used as a health indicator for populations of free-ranging insectivorous bats. Nevertheless, larger sample sizes and experimental and longitudinal studies will be required to explore the dynamics of bats' gastrointestinal microbiome through life and its adaptation processes to environmental disturbances, with the aim of establishing a fundamental baseline. Like human microbiome research (Proctor et al., 2019), bat microbiome studies should incorporate the investigation of possible deleterious effects arising from changes in the fecal bacterial microbiome composition, structure and diversity, such as weakened immunity system, impaired nutrient acquisition during digestion, and development of diseases.

Understanding and managing the risks associated with potential pathogenic bacteria identified through sequencing methods in bat feces

Advances in sequencing technologies, accompanied by an increased interest in studying pathogens in bats, have facilitated the publication of more sophisticated bacteriological studies on these species (Berman et al., 2023; Fleischer et al., 2024; Grisnik et al., 2023). Results from such studies have significantly expanded the database of bacteria found in bats. For instance, in **Study 2,** we found over 1,200 different bacterial genera, some of which cannot be recovered from routine microbiological cultures such as those from the Coxiellaceae family (Zhang et al., 2022). Nonetheless, microbiome analyses performed at the genus level mask the variations among different bacterial species within the same genus that can have different metabolism and functionality within the host's microbiome (Yu et al., 2017). *Pseudomonas* was found the fourth most abundant genus present in fecal samples from bats dwelling in mature and old-growth forests (well-preserved ecosystems) from **Study 2**. This is an example of a genus that includes hundreds of different species, with some being recognized as relevant pathogens, while others play crucial ecological roles in the environment and demonstrate significant potential in biotechnological applications (Silby et al., 2011).

Results from **Study 3** show, for the first time in the literature, bats' fecal microbiome analysis at the level of bacterial species using nanopore technologies. Although we identified over 3,000 bacterial species, we only selected potential pathogenic bacteria (PPB) (n=222) from the complete data set to perform our analyses at the bacterial species level. With this strategy, we looked for the identification of a more accurate (species-level) list of potential pathogenic bacteria, rather than replicate the microbiome analysis performed already at the genus level. Indirectly, we highlighted the potential of new taxonomic assignment software, such as EMU, to be used in bat microbiome studies looking for a higher bacterial resolution (Curry et al., 2022). Although promising, the field of bat (and other wildlife) microbiome research is urging standardized protocols to aid comparisons across studies (Combrink et al., 2023). We believe one of the next steps in bats' bacterial microbiome investigation will

implicate a shift in the taxonomic assignment of metagenomic sequences toward species-level identification.

It is worth noting that many bacterial species can have both pathogenic and commensal strains. In this context, the analysis of the 16S rRNA gene may offer limited insight into bacterial functionality (including pathogenicity) or antimicrobial susceptibility (Gupta et al., 2019). For the microbiome-associated PPB analysis of **Study 3,** we included bacterial species that are known to have both pathogenic and commensal strains, such as the case of *Escherichia coli*. This species exhibits commensal strains (which are members of the normal intestinal microbiota of mammal species) and pathogenic strains, such as enteropathogenic *E. coli* (EPEC) or enterohaemorrhagic *E. coli* (EHEC) besides many others (Kaper et al., 2004). Overall, strain-level (or lower taxonomic level) investigation of bacteria requires sequencing the whole bacterial genome and/or targeting specific genes that are known for encoding virulence factors (e.g. VirulenceFinder database) (Bogaerts et al., 2021; D'Onofrio et al., 2023). However, the objective of the research should not be overlooked, because it will dictate the most appropriate taxonomic level to use in sequencing analyses. While strain-level profiling can be useful for an outbreak investigation, functional insight, or defining bacterial isolates relatedness (bacterial strain typing), bacterial profiling at both the genus and species levels are essential for understanding broad ecological patterns of a host's microbiome (Bogaerts et al., 2021; Duval et al., 2023; Marsland et al., 2020).

In the context of Public Health, the need for bacterial surveillance in bat species has recently become more evident, since several bacteria have been involved in bat-human transmission. For instance, *Borrelia johnsonii* was detected in a bat tick and a human patient with borreliosis in the USA (Kingry et al., 2018); *Bartonella rousetti* was detected in Egyptian fruit bats from a Nigerian cave and several people entering the same cave (Bai et al., 2018); and several *Bartonella* genotypes from European bats are known to be closely related to (cluster with) the genotypes identified from forest workers in Poland (Urushadze et al., 2017). Regardless of the evidence of potentially zoonotic and pathogenic bacteria detected in bats, the ecological drivers of these bacterial pathogens' occurrence, excretion, and inter- and intra-species transmission are still not recognized (Mühldorfer, 2013; Szentivanyi et al., 2023). Additionally, since bacterial pathogen surveillance data obtained by sequencing technologies identifies bacterial DNA regardless of its viability, it is not sufficient to reliably prove that

the host is actively excreting infective/viable bacteria or is suffering from a disease (Emerson et al., 2017). For this reason, the identification of recognized human or animal bacterial pathogens, such as *Francisella tularensis* or *Brucella abortus* detected in bats from **Study 3** of this thesis, should not excessively alarm us regarding their zoonotic risk or their implication in bat diseases. However, it warrants implementing basic biosecurity measures, such as using sterile nitrile gloves when working with bats or their secretions/excretions (Aguilar-Setién et al., 2022). Furthermore, cloth-holding bags, handling gloves, skin biopsy punches, sterile nitrile gloves, mist nets, forceps, and other fieldwork instruments should be cleaned and sterilized (or replaced) between uses to mitigate cross-contamination of samples and to avoid any possible iatrogenic transmission among bats (Aguilar-Setién et al., 2022).

Conclusions

1. The use of forearm rings in bat species should be banned by policymakers and avoided by scientists until adequate studies to evaluate their impact have been performed under controlled conditions.
2. Studies carrying out individual marking activities on bats should be submitted to a legally constituted ethics committee for consideration, guidance, and previous approval.
3. The use of Passive Integrated Transponders (PIT) tags has been found to cause no adverse effects on the health of the studied bat species, thereby establishing it as a safe and reliable marking tool for them.
4. Well-preserved landscapes promote more resilient fecal bacterial microbiomes in five species of free-ranging bats (*Miniopterus schreibersii*, *Nyctalus leisleri, Pipistrellus kuhlii*, *P. pipistrellus* and *P. pygmaeus*) captured in Catalonia.
5. The occurrence of potential pathogenic bacteria (PPB) within the studied bats' fecal samples may mirror their intrinsic alpha diversity value, rather than the type of land use from where they were collected.

Future directions

In the field of bat research, the pursuit of scientific knowledge intersects with the urgent need for species conservation. In order to address both objectives, it is imperative to carefully consider the welfare and ethical dimensions involved in such research. Bat research must strive to enhance our understanding of these species while safeguarding their overall welfare.

Therefore, given 1) the legal protection of all bat species; 2) the current unawareness of the health status of insectivorous bat species worldwide; and 3) the limited knowledge about methods for health assessment in free-ranging bat populations, we strongly encourage the expansion of research in the following areas:

- Ethical and standardized methods for individually marking bats, particularly insectivorous bats.
- Standardized and non-invasive methods of physical and welfare assessment for free-ranging insectivorous bat species: the development of standardized protocols using non-invasive methods can stimulate researchers to collect data regarding bats' health during their activities, regardless of their primary research objective.
- Basic bat bacteriology and targeted surveillance with the aim of establishing a fundamental baseline for conducting bat health investigations, including (but not limited to) the assessment of morbidity/mortality associated with bacterial infections.
- Bat's microbiome analysis: refinement and standardization of methods and development of more complex computational analysis. Furthermore, the integration of knowledge about morbidity/mortality associated with bacterial infections in bats will likely result in a significant shift in the approach to microbiome studies in these species.

Bibliography

Adams, R. A. (2010). Bat reproduction declines when conditions mimic climate change projections for western North America. In *Ecology* (Vol. 91, Issue 8).

Aguilar-Setién, A., Aréchiga-Ceballos, N., Balsamo, G. A., Behrman, A. J., Frank, H. K., Fujimoto, G. R., Gilman Duane, E., Hudson, T. W., Jones, S. M., Ochoa Carrera, L. A., Powell, G. L., Smith, C. A., Triantis Van Sickle, J., & Vleck, S. E. (2022). Biosafety practices when working with bats: A guide to field research considerations. *Applied Biosafety*, *27*(3), 169–190. https://doi.org/10.1089/apb.2022.0019

Agus, A., Clément, K., & Sokol, H. (2021). Gut microbiota-derived metabolites as central regulators in metabolic disorders. In *Gut* (Vol. 70, Issue 6, pp. 1174–1182). BMJ Publishing Group. https://doi.org/10.1136/gutjnl-2020-323071

Aizpurua, O., Nyholm, L., Morris, E., Chaverri, G., Herrera Montalvo, L. G., Flores-Martinez, J. J., Lin, A., Razgour, O., Gilbert, M. T. P., & Alberdi, A. (2021). The role of the gut microbiota in the dietary niche expansion of fishing bats. *Animal Microbiome*, *3*(1). https://doi.org/10.1186/s42523-021-00137-w

Alcalde, J. T., Ibáñez, C., Antón, I., & Nyssen, P. (2013). First case of migration of a Leisler's bat (*Nyctalus leisleri*) between Spain and Belgium. *Le Rhinolophe*, *19*, 87–88.

Allen, S., Allen, D., Phoenix, V. R., Le Roux, G., Durántez Jiménez, P., Simonneau, A., Binet, S., & Galop, D. (2019). Atmospheric transport and deposition of microplastics in a remote mountain catchment. *Nature Geoscience*, *12*(5), 339–344. https://doi.org/10.1038/s41561-019-0335-5

Allison, T. D., Diffendorfer, J. E., Baerwald, E. F., Beston, J. A., Drake, D., Hale, A. M., Hein, C. D., Huso, M. M., Loss, S. R., Lovich, J. E., Strickland, M. D., Williams, K. A., & Winder, V. L. (2019). *Impacts to wildlife of wind energy siting and operation in the United States*. www.esa.org

Alpízar, P., Risely, A., Tschapka, M., & Sommer, S. (2021). Agricultural fast food: bats feeding in banana monocultures are heavier but have less diverse gut microbiota. *Frontiers in Ecology and Evolution*, *9*. https://doi.org/10.3389/fevo.2021.746783

Alviz, Á., & Pérez–torres, J. (2020). A difference between sexes: temporal variation in the diet of *Carollia perspicillata* (Chiroptera, Phyllostomidae) at

the Macaregua cave, Santander (Colombia). *Animal Biodiversity and Conservation*, *43*(1), 27–35. https://doi.org/10.32800/abc.2020.43.0027

Amato, K. R., Yeoman, C. J., Kent, A., Righini, N., Carbonero, F., Estrada, A., Gaskins, R. H., Stumpf, R. M., Yildirim, S., Torralba, M., Gillis, M., Wilson, B. A., Nelson, K. E., White, B. A., & Leigh, S. R. (2013). Habitat degradation impacts black howler monkey (*Alouatta pigra*) gastrointestinal microbiomes. *ISME Journal*, *7*(7), 1344–1353. https://doi.org/10.1038/ismej.2013.16

Amichai, E., & Korine, C. (2020). *Kuhl's Pipistrelle* Pipistrellus kuhlii *(Kuhl, 1817)* (pp. 1–19). https://doi.org/10.1007/978-3-319-65038-8_69-1

Amorim, F., Mata, V. A., Beja, P., & Rebelo, H. (2015). Effects of a drought episode on the reproductive success of European free-tailed bats (*Tadarida teniotis*). *Mammalian Biology*, *80*(3), 228–236. https://doi.org/10.1016/j.mambio.2015.01.005

Andersen, K. G., Rambaut, A., Lipkin, W. I., Holmes, E. C., & Garry, R. F. (2020). The proximal origin of SARS-CoV-2. *Nature Medicine*, *26*(4), 450–452. https://doi.org/10.1038/s41591-020-0820-9

Aziz, S. A., McConkey, K. R., Tanalgo, K., Sritongchuay, T., Low, M. R., Yong, J. Y., Mildenstein, T. L., Nuevo-Diego, C. E., Lim, V. C., & Racey, P. A. (2021). The critical importance of Old World Fruit Bats for healthy ecosystems and economies. In *Frontiers in Ecology and Evolution* (Vol. 9). Frontiers Media S.A. https://doi.org/10.3389/fevo.2021.641411

Bäckhed, F., Fraser, C. M., Ringel, Y., Sanders, M. E., Sartor, R. B., Sherman, P. M., Versalovic, J., Young, V., & Finlay, B. B. (2012). Defining a healthy human gut microbiome: Current concepts, future directions, and clinical applications. In *Cell Host and Microbe* (Vol. 12, Issue 5, pp. 611–622). https://doi.org/10.1016/j.chom.2012.10.012

Bai, Y., Osinubi, M. O. V., Osikowicz, L., McKee, C., Vora, N. M., Rizzo, M. R., Recuenco, S., Davis, L., Niezgoda, M., Ehimiyein, A. M., Kia, G. S. N., Oyemakinde, A., Adeniyi, O. S., Gbadegesin, Y. H., Saliman, O. A., Ogunniyi, A., Ogunkoya, A. B., Kosoy, M. Y., & Idanre Bat Festival Investigation Team (2018). Human exposure to novel *Bartonella* species from contact with fruit bats. *Emerging Infectious Diseases*, *24*(12), 2317–2323. https://doi.org/10.3201/eid2412.181204

Baker, G. B., Lumsden, L. F., Dettmann, E. B., Schedvin, N. K., Schulz, M., Watkins, D., & Jansen, L. (2001). The effect of forearm bands on insectivorous bats

(Microchiroptera) in Australia. *Wildlife Research*, *28*(3), 229–237. https://doi.org/10.1071/WR99068

Ballmann, A. E., Torkelson, M. R., Bohuski, E. A., Russell, R. E., & Blehert, D. S. (2017). Dispersal hazards of *Pseudogymnoascus destructans* by bats and human activity at hibernacula in summer. *Journal of Wildlife Diseases*, *53*(4), 725–735. https://doi.org/10.7589/2016-09-206

Balmori, A., & Quetglas, J. (2000). *Análisis de los daños por anillamiento en murciélagos. Barbastella, 1*: 22-26.

Baloğlu, B., Chen, Z., Elbrecht, V., Braukmann, T., MacDonald, S., & Steinke, D. (2021). A workflow for accurate metabarcoding using nanopore MinION sequencing. *Methods in Ecology and Evolution*, *12*(5), 794–804. https://doi.org/10.1111/2041-210X.13561

Banskar, S., Bhute, S. S., Suryavanshi, M. V., Punekar, S., & Shouche, Y. S. (2016). Microbiome analysis reveals the abundance of bacterial pathogens in *Rousettus leschenaultii* guano. *Scientific Reports*, *6*. https://doi.org/10.1038/srep36948

Banskar, S., Mourya, D. T., & Shouche, Y. S. (2016). Bacterial diversity indicates dietary overlap among bats of different feeding habits. *Microbiological Research*, *182*, 99–108. https://doi.org/10.1016/j.micres.2015.10.006

Banyard, A. C., Johnson, N., Voller, K., Hicks, D., Nunez, A., Hartley, M., & Fooks, A. R. (2009). Repeated detection of European bat lyssavirus type 2 in dead bats found at a single roost site in the UK. *Archives of Virology*, *154*(11), 1847–1850. https://doi.org/10.1007/s00705-009-0504-8

Barelli, C., Albanese, D., Donati, C., Pindo, M., Dallago, C., Rovero, F., Cavalieri, D., Michael Tuohy, K., Christine Hauffe, H., & De Filippo, C. (2015). Habitat fragmentation is associated to gut microbiota diversity of an endangered primate: Implications for conservation. *Scientific Reports*, *5*. https://doi.org/10.1038/srep14862

Barko, P. C., McMichael, M. A., Swanson, K. S., & Williams, D. A. (2017). *The gastrointestinal microbiome: a review*. https://doi.org/10.1111/jvim.14875

Barlow, A., Jolliffe, T., Tomlin, M., Worledge, L., & Miller, H. (2011). Mycotic dermatitis in a vagrant parti-coloured bat (*Vespertilio murinus*) in Great Britain. In *Veterinary Record* (Vol. 169, Issue 23, p. 614). https://doi.org/10.1136/vr.d7783

Barnett, M., Young, W., Cooney, J., & Roy, N. (2015). Metabolomics and proteomics, and what to do with all these "Omes": Insights from nutrigenomic investigations in New Zealand. In *Journal of Nutrigenetics*

and Nutrigenomics (Vol. 7, pp. 274–282). S. Karger AG. https://doi.org/10.1159/000381349

Barquez, R., Perez, S., Miller, B., & Diaz, M. (2015). *Carollia perspicillata. The IUCN Red List of Threatened Species 2015: e.T3905A22133716*. https://doi.org/10.2305/IUCN.UK.2015

Barrett, J., Carlisle, M., & Prociv, P. (2002). Neuro-angiostrongylosis in wild Black and Grey-headed flying foxes (*Pteropus* spp). In *Scientific Aust Vet J* (Vol. 80, Issue 9).

Baskerville, A., Broster, M., Fitzgeorge, R. B., Hambleton, P., & Dennis, P. J. (1981). Experimental transmission of Legionnaires' disease by exposure to aerosols of *Legionella pneumophila*. *The Lancet*, *318*(8260–8261), 1389–1390. https://doi.org/10.1016/S0140-6736(81)92803-8

Bass, D., Stentiford, G. D., Wang, H. C., Koskella, B., & Tyler, C. R. (2019). The pathobiome in animal and plant diseases. In *Trends in Ecology and Evolution* (Vol. 34, Issue 11, pp. 996–1008). Elsevier Ltd. https://doi.org/10.1016/j.tree.2019.07.012

Bat Taxonomic Advisory Group. (2004). *AZA Standardized care guidelines for fruit bats*.

Battersby, J. (2010). Guidelines for surveillance and monitoring of European bats. *EUROBATS Publication Series No. 5*, *Series No.5*, 0–95.

Bayat, S., Geiser, F., Kristiansen, P., & Wilson, S. C. (2014). Organic contaminants in bats: Trends and new issues. In *Environment International* (Vol. 63, pp. 40–52). Elsevier Ltd. https://doi.org/10.1016/j.envint.2013.10.009

Belkaid, Y., & Hand, T. W. (2014). Role of the microbiota in immunity and inflammation. In *Cell* (Vol. 157, Issue 1, pp. 121–141). Elsevier B.V. https://doi.org/10.1016/j.cell.2014.03.011

Berg, G., Rybakova, D., Fischer, D., Cernava, T., Vergès, M.-C. C., Charles, T., Chen, X., Cocolin, L., Eversole, K., Corral, G. H., Kazou, M., Kinkel, L., Lange, L., Lima, N., Loy, A., Macklin, J. A., Maguin, E., Mauchline, T., McClure, R., Mitter, B., Ryan, M., Sarand, I., Smidt, H., Schelkle, B., Roume, H., Kiran, G. S., Selvin, J., Souza, R. S. C., van Overbeek, L., Singh, B. K., Wagner,. M., Walsh, A., Sessitsch, A., & Schloter, M. (2020). Microbiome definition re-visited: old concepts and new challenges. *Microbiome*, *8*(1), 103. https://doi.org/10.1186/s40168-020-00875-0

Berman, T. S., Weinberg, M., Moreno, K. R., Czirják, G. Á., & Yovel, Y. (2023). In sickness and in health: the dynamics of the fruit bat gut microbiota under a bacterial antigen challenge and its association with the immune

response. *Frontiers in Immunology*, *14*. https://doi.org/10.3389/fimmu.2023.1152107

Berny, P. (2007). Pesticides and the intoxication of wild animals. *J. Vet. Pharmacol. Therap.*, 93–100. http://faostat.fao.org/faostat/

Berzunza-Cruz, M., Rodríguez-Moreno, Á., Gutiérrez-Granados, G., González-Salazar, C., Stephens, C. R., Hidalgo-Mihart, M., Marina, C. F., Rebollar-Téllez, E. A., Bailón-Martínez, D., Balcells, C. D., Ibarra-Cerdeña, C. N., Sánchez-Cordero, V., & Becker, I. (2015). *Leishmania* (*L.*) *mexicana* infected bats in Mexico: novel potential reservoirs. *PLoS Neglected Tropical Diseases*, *9*(1). https://doi.org/10.1371/journal.pntd.0003438

Bioinformatics & Evolutionary Genomics (VIB-UGent). (2023, May). *Calculate and draw custom Venn diagrams*. Version 2023-5. https://bioinformatics.psb.ugent.be/webtools/Venn/

Blehert, D. S., Maluping, R. P., Earl Green, D., Berlowski-Zier, B. M., Ballmann, A. E., & Langenberg, J. A. (2014). Acute pasteurellosis in wild big brown bats (*Eptesicus fuscus*). In *Journal of Wildlife Diseases* (Vol. 50, Issue 1, pp. 136–139). https://doi.org/10.7589/2012-02-063

Blumer, M., Brown, T., Freitas, M. B., Destro, A. L., Oliveira, J. A., Morales, A. E., Schell, T., Greve, C., Pippel, M., Jebb, D., Hecker, N., Ahmed, A.-W., Kirilenko, B. M., Foote, M., Janke, A., Lim, B. K., & Hiller, M. (2022). Gene losses in the common vampire bat illuminate molecular adaptations to blood feeding. In *Sci. Adv* (Vol. 8). https://www.science.org

Bodor, A., Bounedjoum, N., Vincze, G. E., Erdeiné Kis, Á., Laczi, K., Bende, G., Szilágyi, Á., Kovács, T., Perei, K., & Rákhely, G. (2020). Challenges of unculturable bacteria: environmental perspectives. In *Reviews in Environmental Science and Biotechnology* (Vol. 19, Issue 1). Springer. https://doi.org/10.1007/s11157-020-09522-4

Bogaerts, B., Nouws, S., Verhaegen, B., Denayer, S., Van Braekel, J., Winand, R., Fu, Q., Crombé, F., Piérard, D., Marchal, K., Roosens, N. H. C., De Keersmaecker, S. C. J., & Vanneste, K. (2021). Validation strategy of a bioinformatics whole genome sequencing workflow for Shiga toxin-producing *Escherichia coli* using a reference collection extensively characterized with conventional methods. *Microbial Genomics*, *7*(3). https://doi.org/10.1099/mgen.0.000531

Botella, P., & Esteban, J. G. (1995). Histopathology of the stomach lesion caused by *Physaloptera brevivaginata* (Nematoda: Physalopteridae) in bats in Spain. *Folia Parasitologica*, *42*, 143–148.

Brearley, G., Rhodes, J., Bradley, A., Baxter, G., Seabrook, L., Lunney, D., Liu, Y., & Mcalpine, C. (2013). Wildlife disease prevalence in human-modified landscapes. *Biological Reviews*, *88*(2), 427–442. https://doi.org/10.1111/brv.12009

Breitwieser, F., & Salzberg, S. (2020). Pavian: interactive analysis of metagenomics data for microbiome studies and pathogen identification. *Bioinformatics*, *36*(1303–4).

Brock, A. P., Cortés-Hinojosa, G., Plummer, C. E., Conway, J. A., Roff, S. R., Childress, A. L., & Wellehan, J. F. X. (2013). A novel gammaherpesvirus in a large flying fox (*Pteropus vampyrus*) with blepharitis. *Journal of Veterinary Diagnostic Investigation*, *25*(3), 433–437. https://doi.org/10.1177/1040638713486645

Brotons, L., Pou, N., Herrando, S., Bota, G., Villero, D., Garrabou, J., Ordóñez, J., Anton, M., Gual, G., Recorder, L., Alcaraz, J., Pla, M., Sainz de la Maza, P., Pont, S., & Pino, J. (2020). *Estat de la natura a Catalunya 2020*.

Browning, E., Barlow, K. E., Burns, F., Hawkins, C., & Boughey, K. (2021). Drivers of European bat population change: a review reveals evidence gaps. In *Mammal Review* (Vol. 51, Issue 3, pp. 353–368). Blackwell Publishing Ltd. https://doi.org/10.1111/mam.12239

Bunkley, J. P., McClure, C. J. W., Kleist, N. J., Francis, C. D., & Barber, J. R. (2015). Anthropogenic noise alters bat activity levels and echolocation calls. *Global Ecology and Conservation*, *3*, 62–71. https://doi.org/10.1016/j.gecco.2014.11.002

Busch, D. S., & Hayward, L. S. (2009). Stress in a conservation context: a discussion of glucocorticoid actions and how levels change with conservation-relevant variables. *Biological Conservation*, *142*(12), 2844–2853. https://doi.org/10.1016/j.biocon.2009.08.013

Cain, D. W., & Cidlowski, J. A. (2017). Immune regulation by glucocorticoids. In *Nature Reviews Immunology* (Vol. 17, Issue 4, pp. 233–247). Nature Publishing Group. https://doi.org/10.1038/nri.2017.1

Cappellato, M., Baruzzo, G., & Di Camillo, B. (2022). Investigating differential abundance methods in microbiome data: a benchmark study. *PLOS Computational Biology*, *18*(9), e1010467. https://doi.org/10.1371/journal.pcbi.1010467

Cárdenas-Canales, E. M., Gigante, C. M., Greenberg, L., Velasco-Villa, A., Ellison, J. A., Satheshkumar, P. S., Medina-Magües, L. G., Griesser, R., Falendysz, E., Amezcua, I., Osorio, J. E., & Rocke, T. E. (2020). Clinical presentation and

serologic response during a rabies epizootic in captive common vampire bats (*Desmodus rotundus*). *Tropical Medicine and Infectious Disease*, *5*(1). https://doi.org/10.3390/tropicalmed5010034

Cardillo, M., Mace, G. M., Jones, K. E., Bielby, J., Bininda-Emonds, O. R. P., Sechrest, W., Orme, C. D. L, & Purvis, A. (2005). Multiple causes of high extinction risk in large mammal species. *Philos. Trans. R. Soc. London Ser. B*, *2*, 379. https://doi.org/10.1111/j.1420-9101

Cassir, N., Benamar, S., & La Scola, B. (2016). *Clostridium butyricum*: from beneficial to a new emerging pathogen. *Clinical Microbiology and Infection*, *22*(1), 37–45. https://doi.org/10.1016/j.cmi.2015.10.014

CDC. (2021, March 25). *Legionella (Legionnaires' Disease and Pontiac Fever)*. Health Topics A-Z. https://www.cdc.gov/legionella/about/

Ceballos-Vasquez, A., Caldwell, J. R., & Faure, P. A. (2015). Seasonal and reproductive effects on wound healing in the flight membranes of captive big brown bats. *Biology Open*, *4*(1), 95–103. https://doi.org/10.1242/bio.201410264

Cénit, M. C., Matzaraki, V., Tigchelaar, E. F., & Zhernakova, A. (2014). Rapidly expanding knowledge on the role of the gut microbiome in health and disease. In *Biochimica et Biophysica Acta - Molecular Basis of Disease* (Vol. 1842, Issue 10, pp. 1981–1992). Elsevier. https://doi.org/10.1016/j.bbadis.2014.05.023

Chang, C. W., Huang, B. H., Lin, S. M., Huang, C. L., & Liao, P. C. (2016). Changes of diet and dominant intestinal microbes in farmland frogs. *BMC Microbiology*, *16*(1). https://doi.org/10.1186/s12866-016-0660-4

Chang, D., Gupta, V. K., Hur, B., Cunningham, K. Y., & Sung, J. (2023). GMWI-webtool: a user-friendly browser application for assessing health through metagenomic gut microbiome profiling. *Bioinformatics*, *39*(2). https://doi.org/10.1093/bioinformatics/btad061

Chang, F., He, S., & Dang, C. (2022). Assisted selection of biomarkers by Linear Discriminant Analysis Effect Size (LEfSe) in microbiome data. *Journal of Visualized Experiments: JoVE*, *183*. https://doi.org/10.3791/61715

Chen, H., & Boutros, P. C. (2011). VennDiagram: A package for the generation of highly customizable Venn and Euler diagrams in R. *BMC Bioinformatics*, *12*. https://doi.org/10.1186/1471-2105-12-35

Chen, W. M., Xie, P. B., Hsu, M. Y., & Sheu, S. Y. (2018). *Parvibium lacunae* gen. nov., sp. nov., a new member of the family Alcaligenaceae isolated from a

freshwater pond. *International Journal of Systematic and Evolutionary Microbiology*, *68*(4), 1291–1299. https://doi.org/10.1099/ijsem.0.002667

Cheney, J. A., Allen, J. J., & Swartz, S. M. (2017). Diversity in the organization of elastin bundles and intramembranous muscles in bat wings. *Journal of Anatomy*, *230*(4), 510–523. https://doi.org/10.1111/joa.12580

Cheney, J. A., Konow, N., Middleton, K. M., Breuer, K. S., Roberts, T. J., Giblin, E. L., & Swartz, S. M. (2014). Membrane muscle function in the compliant wings of bats. *Bioinspiration and Biomimetics*, *9*(2). https://doi.org/10.1088/1748-3182/9/2/025007

Childs-Sanford, S. E., Kollias, G. V., Abou-Madi, N., McDonough, P. L., Garner, M. M., & Mohammed, H. O. (2009). *Yersinia pseudotuberculosis* in a closed colony of Egyptian Fruit Bats (*Rousettus aegyptiacus*). *Journal of Zoo and Wildlife Medicine*, *40*(1), 8–14. https://doi.org/10.1638/2007-0033.1

CITES. (2020). *Checklist of CITES Species. Chiroptera.* http://checklist.cites.org.

Cláudio, V. C., Gonzalez, I., Barbosa, G., Rocha, V., Moratelli, R., & Rassy, F. (2018). Bacteria richness and antibiotic-resistance in bats from a protected area in the Atlantic Forest of Southeastern Brazil. *PLoS ONE*, *13*(9). https://doi.org/10.1371/journal.pone.0203411

Clemente, J. C., Pehrsson, E. C., Blaser, M. J., Sandhu, K., Gao, Z., Wang, B., Magris, M., Hidalgo, G., Contreras, M., Noya-Alarcón, Ó., Lander, O., McDonald, J., Cox, M., Walter, J., Oh, P. L., Ruiz, J. F., Rodriguez, S., Shen, N., Song, S. J., Metcalf, J., Knight, R., Dantas, G., & Dominguez-Bello, M. G. (2015). The microbiome of uncontacted Amerindians. *Science Advances*, *1*(3). https://doi.org/10.1126/sciadv.1500183

Cleveland, C. J., Betke, M., Federico, P., Frank, J. D., Hallam, T. G., Horn, J., López, J. D., McCracken, G. F., Medellín, R. A., Moreno-Valdez, A., Sansone, C. G., Westbrook, J. K., & Kunz, T. H. (2006). Economic value of the pest control service provided by Brazilian free-tailed bats in south-central Texas. *Frontiers in Ecology and the Environment*, *4*(5), 238–243. https://doi.org/10.1890/1540-9295(2006)004[0238:EVOTPC]2.0.CO;2

Cody, M., & Smallwood, J. (1996). *Long-term studies of vertebrate communities* (M. L. Cody & J. A. Smallwood, Eds.). Academic Press.

Cogswell-Hawkinson, A., Bowen, R., James, S., Gardiner, D., Calisher, C. H., Adams, R., & Schountz, T. (2012). Tacaribe Virus causes fatal infection of an ostensible reservoir host, the Jamaican Fruit Bat. *Journal of Virology*, *86*(10), 5791–5799. https://doi.org/10.1128/jvi.00201-12

Combrink, L., Humphreys, I. R., Washburn, Q., Arnold, H. K., Stagaman, K., Kasschau, K. D., Jolles, A. E., Beechler, B. R., & Sharpton, T. J. (2023). Best practice for wildlife gut microbiome research: A comprehensive review of methodology for 16S rRNA gene investigations. In *Frontiers in Microbiology* (Vol. 14). Frontiers Media S.A. https://doi.org/10.3389/fmicb.2023.1092216

Contijoch, E. J., Britton, G. J., Yang, C., Mogno, I., Li, Z., Ng, R., Llewellyn, S. R., Hira, S., Johnson, C., Rabinowitz, K. M., Barkan, R., Dotan, I., Hirten, R. P., Fu, S. C., Luo, Y., Yang, N., Luong, T., Labrias, P. R., Lira, S., Peter, I., Grinspan, A., Clemente, J. C., Kosoy, R., Kim-Schulze, S., Qin, X., Castillo, A., Hurley, A., Atreja, A., Rogers, J., Fasihuddin, F., Saliaj, M., Nolan, A., Reyes-Mercedes, P., Rodriguez, C., Aly, S., Santa-Cruz, K., Peters, L., Suárez-Fariñas, M., Huang, R., Hao, K., Zhu, J., Zhang, B., Losic, B., Irizar, H., Song, W. M., Di Narzo, A., Wang, W., Cohen, B. L., DiMaio, C., Greenwald, D., Itzkowitz, S., Lucas, A., Marion, J., Maser, E., Ungaro, R., Naymagon, S., Novak, J., Shah, B., Ullman, T., Rubin, P., George, J., Legnani, P., Telesco, S. E., Friedman, J. R., Brodmerkel, C., Plevy, S., Cho, J. H., Colombel, J. F., Schadt, E. E., Argmann, C., Dubinsky, M., Kasarskis, A., Sands, B., & Faith, J. J. (2019). Gut microbiota density influences host physiology and is shaped by host and microbial factors. *ELife*, *8*. https://doi.org/10.7554/eLife.40553

Corduneanu, A., Wu-Chuang, A., Maitre, A., Obregon, D., Sándor, A. D., & Cabezas-Cruz, A. (2023). Structural differences in the gut microbiome of bats using terrestrial vs. aquatic feeding resources. *BMC Microbiology*, *23*(1), 93. https://doi.org/10.1186/s12866-023-02836-7

COST Action CA18107. (2018). Climate Change and Bats: from science to conservation (CLIMBATS). In *COST European Cooperation in Science & Technology: Vol. COST 088/18*.

COST Action CA18107. (2021). *Progress Report at 24 months. Climate change and bats: from science to conservation*.

Cunningham, A. A., Daszak, P., & Rodríguez, J. P. (2003). Pathogen pollution: defining a parasitological threat to biodiversity conservation. *Journal of Parasitology*, S78–S83. https://www.researchgate.net/publication/284061023

Curry, K. D., Wang, Q., Nute, M. G., Tyshaieva, A., Reeves, E., Soriano, S., Wu, Q., Graeber, E., Finzer, P., Mendling, W., Savidge, T., Villapol, S., Dilthey, A., & Treangen, T. J. (2022). Emu: species-level microbial community profiling of full-length 16S rRNA Oxford Nanopore sequencing data. *Nature Methods*, *19*(7), 845–853. https://doi.org/10.1038/s41592-022-01520-4

Custer, G. F., Gans, M., van Diepen, L. T. A., Dini-Andreote, F., & Buerkle, C. A. (2023). Comparative analysis of core microbiome assignments: implications for ecological synthesis. *MSystems*, *8*(1). https://doi.org/10.1128/msystems.01066-22

Daniel, D. S., Ng, Y. K., Chua, E. L., Arumugam, Y., Wong, W. L., & Kumaran, J. V. (2013). Isolation and identification of gastrointestinal microbiota from the short-nosed fruit bat *Cynopterus brachyotis brachyotis*. *Microbiological Research*, *168*(8), 485–496. https://doi.org/10.1016/j.micres.2013.04.001

Daszak, P., Cunningham, A. A., & Hyatt, A. D. (2000). Emerging infectious diseases of wildlife-threats to biodiversity and human health. *Science*, *287*. www.sciencemag.org

Davies, K. T. J., Maryanto, I., & Rossiter, S. J. (2013). Evolutionary origins of ultrasonic hearing and laryngeal echolocation in bats inferred from morphological analyses of the inner ear. *Frontiers in Zoology*, *10*(1). https://doi.org/10.1186/1742-9994-10-2

Davis, A., Gordy, P., Rudd, R., Jarvis, J. A., & Bowen, R. A. (2012). Naturally acquired rabies virus infections in wild-caught bats. *Vector-Borne and Zoonotic Diseases*, *12*(1), 55–60. https://doi.org/10.1089/vbz.2011.0674

Davy, C. M., von Zuben, V., Kukka, P. M., Gerber, B. D., Slough, B. G., & Jung, T. S. (2022). Rapidly declining body size in an insectivorous bat is associated with increased precipitation and decreased survival. *Ecological Applications*, *32*(7). https://doi.org/10.1002/eap.2639

de Coster, W., D'Hert, S., Schultz, D., Cruts, M., & van Broeckhoven, C. (2018). Nano- Pack: visualizing and processing long-read sequencing data. *Bioinformatics*, *34*(2666–9).

De Garine-Wichatitsky, M., Caron, A., Kock, R., Tschopp, R., Munyeme, M., Hofmeyr, M., & Michel, A. (2013). A review of bovine tuberculosis at the wildlife-livestock-human interface in sub-Saharan Africa. In *Epidemiology and Infection* (Vol. 141, Issue 7, pp. 1342–1356). https://doi.org/10.1017/S0950268813000708

de Leon, M. P., Montecillo, A. D., Pinili, D. S., Siringan, M. A. T., & Park, D. S. (2018). Bacterial diversity of bat guano from Cabalyorisa Cave, Mabini, Pangasinan, Philippines: A first report on the metagenome of Philippine bat guano. *PLoS ONE*, *13*(7). https://doi.org/10.1371/journal.pone.0200095

de Mandal, S., Zothansanga, Panda, A. K., Bisht, S. S., & Senthil Kumar, N. (2015). First report of bacterial community from a Bat Guano using Illumina next-

generation sequencing. *Genomics Data*, *4*, 99–101. https://doi.org/10.1016/j.gdata.2015.04.001

de Ruiter, P. C., Neutel, A.-M., & Moore, J. C. (1994). Modelling food webs and nutrient cycling in agro-ecosystems. *Trends Ecol Evol*.

de Vries, F. T., Thébault, E., Liiri, M., Birkhofer, K., Tsiafouli, M. A., Bjørnlund, L., Jørgensen, H. B., Brady, M. V., Christensen, S., de Ruiter, P. C., D'Hertefeldt, T., Frouz, J., Hedlund, K., Hemerik, L., Gera Hol, W. H., Hotes, S., Mortimer, S. R., Setälä, H., Sgardelis, S. P., Uteseny, K., van der Putten, W. H., Wolters, V., & Bardgett, R. D. (2013). Soil food web properties explain ecosystem services across European land use systems. *Proceedings of the National Academy of Sciences of the United States of America*, *110*(35), 14296–14301. https://doi.org/10.1073/pnas.1305198110

Devanga Ragupathi, N. K., Muthuirulandi Sethuvel, D. P., Inbanathan, F. Y., & Veeraraghavan, B. (2018). Accurate differentiation of *Escherichia coli* and *Shigella* serogroups: challenges and strategies. *New Microbes and New Infections*, *21*, 58–62. https://doi.org/10.1016/j.nmni.2017.09.003

Dhariwal, A., Chong, J., Habib, S., King, I., Agellon, L., & Xia, J. (2017). Microbiome analyst: a web-based tool for comprehensive statistical, visual and meta-analysis of microbiome data. *Nucleic Acids Res*, *45*(W180–8).

di Bella, C., Piraino, C., & Bella, D. C. (2003). Enteric microflora in Italian Chiroptera. In *J. Mt. Ecol* (Vol. 7). https://www.researchgate.net/publication/235953222

Dietrich, M., Kearney, T., Seamark, E. C. J., & Markotter, W. (2017). The excreted microbiota of bats: Evidence of niche specialisation based on multiple body habitats. In *FEMS Microbiology Letters* (Vol. 364, Issue 1). Oxford University Press. https://doi.org/10.1093/femsle/fnw284

Dietrich, M., Kearney, T., Seamark, E. C. J., Paweska, J. T., & Markotter, W. (2018). Synchronized shift of oral, faecal and urinary microbiotas in bats and natural infection dynamics during seasonal reproduction. *Royal Society Open Science*, *5*(5). https://doi.org/10.1098/rsos.180041

Dietz, C., Dietz, I., Ivanova, T., & Siemers, B. M. (2006). Effects of forearm bands on horseshoe bats (Chiroptera: Rhinolophidae). *Acta Chiropterologica*, *8*(2), 523–535. https://doi.org/10.3161/1733-5329(2006)8[523:EOFBOH]2.0.CO;2

Dillard, B. A., Chung, A. K., Gunderson, A. R., Campbell-Staton, S. C., & Moeller, A. H. (2022). Humanization of wildlife gut microbiota in urban environments. *ELife*, *11*. https://doi.org/10.7554/eLife.76381

Dogra, S. K., Doré, J., & Damak, S. (2020). Gut microbiota resilience: Definition, link to health and strategies for intervention. In *Frontiers in Microbiology* (Vol. 11). Frontiers Media S.A. https://doi.org/10.3389/fmicb.2020.572921

Dong-Lei, S., Yi-Zhou, G., Xing-Yi, G., Zheng-Li, S., & Ning-Yi, Z. (2020). Special features of bat microbiota differ from those of terrestrial mammals. *Frontiers in Microbiology*, *11*(1040).

Donini, V., Iacona, E., Pedrotti, L., Macho-Maschler, S., Palme, R., & Corlatti, L. (2022). Temporal stability of fecal cortisol metabolites in mountain-dwelling ungulates. *Science of Nature*, *109*(2). https://doi.org/10.1007/s00114-022-01792-y

D'Onofrio, V., Cartuyvels, R., Messiaen, P. E. A., Barišić, I., & Gyssens, I. C. (2023). Virulence factor genes in invasive *Escherichia coli* are associated with clinical outcomes and disease severity in patients with sepsis: A prospective observational cohort study. *Microorganisms*, *11*(7). https://doi.org/10.3390/microorganisms11071827

Durazzi, F., Sala, C., Castellani, G., Manfreda, G., Remondini, D., & De Cesare, A. (2021). Comparison between 16S rRNA and shotgun sequencing data for the taxonomic characterization of the gut microbiota. *Scientific Reports*, *11*(1). https://doi.org/10.1038/s41598-021-82726-y

Duval, A., Opatowski, L., & Brisse, S. (2023). Defining genomic epidemiology thresholds for common-source bacterial outbreaks: a modelling study. *The Lancet Microbe*, *4*(5), e349–e357. https://doi.org/10.1016/S2666-5247(22)00380-9

Eby, P., Peel, A. J., Hoegh, A., Madden, W., Giles, J. R., Hudson, P. J., & Plowright, R. K. (2023). Pathogen spillover driven by rapid changes in bat ecology. *Nature*, *613*(7943), 340–344. https://doi.org/10.1038/s41586-022-05506-2

ECDC. (2023). *A-Z disease list*. European Centre for Disease Prevention and Control. https://www.ecdc.europa.eu/

Edenborough, K. M., Mu, A., Mühldorfer, K., Lechner, J., Lander, A., Bokelmann, M., Couacy-Hymann, E., Radonic, A., & Kurth, A. (2020). Microbiomes in the insectivorous bat species *Mops condylurus* rapidly converge in captivity. *PLoS ONE*, *15*(3). https://doi.org/10.1371/journal.pone.0223629

Edmonds, H., Pryde, M., & O'Donnell, C. F. J. (2017). Survival of PIT-tagged lesser short-tailed bats (*Mystacina tuberculata*) through an aerial 1080 pest control operation. *New Zealand Journal of Ecology*, *41*(2). https://doi.org/10.20417/nzjecol.41.20

Edwards, K. L., Edes, A. N., & Brown, J. L. (2019). Stress, well-being and reproductive success. In *Advances in Experimental Medicine and Biology* (Vol. 1200, pp. 91–162). Springer New York LLC. https://doi.org/10.1007/978-3-030-23633-5_5

Eidels, R. R., Whitaker, J. O., & Sparks, D. W. (2007). Insecticide residues in bats and guano from Indiana. In *Proceedings of the Indiana Academy of Science* (Vol. 1, Issue 1).

Ellison, L. E. (2008). *Summary and analysis of the U.S. Government Bat Banding Program: U.S. Geological Survey Open-File Report 2008-1363*. http://www.usgs.gov/

Ellison, L. E., O'shea, T. J., Daniel J, Pearce, M. A., & Roger D. (2007). A comparison of conventional capture versus PIT reader techniques for estimating survival and capture probabilities of big brown bats (*Eptesicus fuscus*). *Acta Chiropterologica*, *9*(1), 149–160. https://doi.org/10.3161/1733

Emerson, G. L., Nordhausen, R., Garner, M. M., Huckabee, J. R., Johnson, S., Wohrle, R. D., Davidson, W. B., Wilkins, K., Li, Y., Doty, J. B., Gallardo-Romero, N. F., Metcalfe, M. G., Karem, K. L., Damon, I. K., & Carroll, D. S. (2013). Novel poxvirus in big brown bats, northwestern United States. *Emerging Infectious Diseases*, *19*(6), 1002–1004. https://doi.org/10.3201/eid1906.121713

Emerson, J. B., Adams, R. I., Román, C. M. B., Brooks, B., Coil, D. A., Dahlhausen, K., Ganz, H. H., Hartmann, E. M., Hsu, T., Justice, N. B., Paulino-Lima, I. G., Luongo, J. C., Lymperopoulou, D. S., Gomez-Silvan, C., Rothschild-Mancinelli, B., Balk, M., Huttenhower, C., Nocker, A., Vaishampayan, P., & Rothschild, L. J. (2017). Schrödinger's microbes: Tools for distinguishing the living from the dead in microbial ecosystems. In *Microbiome* (Vol. 5, Issue 1, p. 86). https://doi.org/10.1186/s40168-017-0285-3

Eming, S. A., Martin, P., & Tomic-Canic, M. (2014). Wound repair and regeneration: Mechanisms, signaling, and translation. *Science Translational Medicine*, *6*(265). https://doi.org/10.1126/scitranslmed.3009337

Environmental Law Alliance Worldwide. (2010). Overview of mining and its impacts. In *Guidebook for evaluating mining projects EIAs: Vol. Chapter 1* (pp. 1–16).

Eshar, D., & Weinberg, M. (2010). Venipuncture in bats. *Lab Animal*, *39*(6), 175–176. https://doi.org/10.1038/laban0610-175

EUROBATS. (2006). Protecting and managing underground sites for bats. In *5th Session of the Meeting of the Parties*. EUROBATS.

European Food Safety Authority. (2009). Guidance document on risk assessment for birds & mammals on request from EFSA. *EFSA Journal*, *7*(12). https://doi.org/10.2903/j.efsa.2009.1438

European Medicines Agency. (2020). Categorisation of antibiotics in the European Union. In *European Medicines Agency*.

Evans, N. J., Bown, K., Timofte, D., Simpson, V. R., & Birtles, R. J. (2009). Fatal borreliosis in bat caused by relapsing fever spirochete, United Kingdom. *Emerging Infectious Diseases*, *15*(8), 1331–1333. https://doi.org/10.3201/eid1508.090475

Fabbi, M., Pastoris, M. C., Scanziani, E., Magnino, S., & Di Matteo, L. (1998). Epidemiological and environmental investigations of *Legionella pneumophila* infection in cattle and case report of fatal pneumonia in a calf. *Journal of Clinical Microbiology*, *36*(7), 1942–1947. https://doi.org/10.1128/JCM.36.7.1942-1947.1998

Fackelmann, G., Gillingham, M. A. F., Schmid, J., Heni, A. C., Wilhelm, K., Schwensow, N., & Sommer, S. (2021). Human encroachment into wildlife gut microbiomes. *Communications Biology*, *4*(1), 800. https://doi.org/10.1038/s42003-021-02315-7

Fagre, A., & Malmlov, A. (2021). *Bat Health Foundation*. https://www.bathealthfoundation.org/html/about.html

Fan, Y., & Pedersen, O. (2021). Gut microbiota in human metabolic health and disease. *Nature Reviews Microbiology*, *19*, 55–71. https://doi.org/10.1038/s41579-020-0433-9

Fedi, S., Carnevali, M., Andracchio, A., Zappoli, S., & Zannoni, D. (2001). Polychlorinated biphenyl degradation activities and hybridization analyses of fifteen aerobic strains isolated from a PCB-contaminated site. In *Res. Microbiol* (Vol. 152).

Feijó, A., Wang, Y., Sun, J., Li, F., Wen, Z., Ge, D., Xia, L., & Yang, Q. (2019). Research trends on bats in China: A twenty-first century review. *Mammalian Biology*, *98*, 163–172. https://doi.org/10.1016/j.mambio.2019.09.002

Felföldi, T., Schumann, P., Mentes, A., Kéki, Z., Máthé, I., & Tóth, E. M. (2017). *Caenimicrobium hargitense* gen. nov., sp. nov., a new member of the family Alcaligenaceae (Betaproteobacteria) isolated from activated sludge. In *International Journal of Systematic and Evolutionary Microbiology* (Vol. 67, Issue 3, pp. 627–632). Microbiology Society. https://doi.org/10.1099/ijsem.0.001674

Fernandez, P., van Drooge, B. L., Arellano, L., & Grimalt, J. O. (2021). Atmospheric deposition of semivolatile organic pollutants in European high mountains: Sources, settling and chemical degradation. *Science of the Total Environment*, *784*. https://doi.org/10.1016/j.scitotenv.2021.147099

Festa, F., Ancillotto, L., Santini, L., Pacifici, M., Rocha, R., Toshkova, N., Amorim, F., Benítez-López, A., Domer, A., Hamidović, D., Kramer-Schadt, S., Mathews, F., Radchuk, V., Rebelo, H., Ruczynski, I., Solem, E., Tsoar, A., Russo, D., & Razgour, O. (2023). Bat responses to climate change: a systematic review. *Biological Reviews*, *98*(1), 19–33. https://doi.org/10.1111/brv.12893

Fischer, J., & Lindenmayer, D. B. (2007). Landscape modification and habitat fragmentation: a synthesis. *Global Ecology and Biogeography*, *16*, 265–280. https://doi.org/10.1111/j.1466-8238.2006.00287.x

Fleischer, R., Jones, C., Ledezma-Campos, P., Czirják, G. Á., Sommer, S., Gillespie, T. R., & Vicente-Santos, A. (2024). Gut microbial shifts in vampire bats linked to immunity due to changed diet in human disturbed landscapes. *Science of The Total Environment*, *907*, 167815. https://doi.org/10.1016/j.scitotenv.2023.167815

Fleischer, R., Schmid, D. W., Wasimuddin, Brändel, S. D., Rasche, A., Corman, V. M., Drosten, C., Tschapka, M., & Sommer, S. (2022). Interaction between MHC diversity and constitution, gut microbiota and Astrovirus infections in a neotropical bat. *Molecular Ecology*, *31*(12), 3342–3359. https://doi.org/10.1111/mec.16491

Flory, A. R., Kumar, S., Stohlgren, T. J., & Cryan, P. M. (2012). Environmental conditions associated with bat white-nose syndrome mortality in the north-eastern United States. *Journal of Applied Ecology*, *49*(3), 680–689. https://doi.org/10.1111/j.1365-2664.2012.02129.x

Fofanov, V. Y., Furstenau, T. N., Sanchez, D., Hepp, C. M., Cocking, J., Sobek, C., Pagel, N., Walker, F., & Chambers, C. L. (2018). Guano exposed: Impact of aerobic conditions on bat fecal microbiota. *Ecology and Evolution*, *8*(11), 5563–5574. https://doi.org/10.1002/ece3.4084

Fraser, G. C., Hooper, P. T., Lunt, R. A., Gould, A. R., Gleeson, L. J., Hyatt, A. D., Russell, G. M., & Kattenbelt, J. A. (1996). Encephalitis caused by a Lyssavirus in Australia. *Emerging Infectious Diseases*, *2*(4).

Frick, W. F., Kingston, T., & Flanders, J. (2020). A review of the major threats and challenges to global bat conservation. In *Annals of the New York Academy*

of Sciences (Vol. 1469, Issue 1, pp. 5–25). Blackwell Publishing Inc. https://doi.org/10.1111/nyas.14045

Fuks, G., Elgart, M., Amir, A., Zeisel, A., Turnbaugh, P. J., Soen, Y., & Shental, N. (2018). Combining 16S rRNA gene variable regions enables high-resolution microbial community profiling. *Microbiome*, *6*(1), 17. https://doi.org/10.1186/s40168-017-0396-x

Gaisler, J., & Chytil, J. (2002). Mark-recapture results and changes in bat abundance at the cave of Na Turoldu, Czech Republic. *Folia Zool.*, *51*(1), 1–10. https://www.researchgate.net/publication/237369915

Galicia, M. M., Buenrostro, A., & García, J. (2014). Diversidad específica bacteriana en murciélagos de distintos gremios alimenticios de la Sierra Sur de Oaxaca, México. *Rev. Biol. Trop.*, *62*(4), 1673–1681.

Galloway-Peña, J., & Hanson, B. (2020). Tools for analysis of the microbiome. *Digestive Diseases and Sciences*, *65*(3), 674–685. https://doi.org/10.1007/s10620-020-06091-y

Gaona, O., Cerqueda-García, D., Moya, A., Neri-Barrios, X., & Falcón, L. I. (2020). Geographical separation and physiology drive differentiation of microbial communities of two discrete populations of the bat *Leptonycteris yerbabuenae*. *MicrobiologyOpen*, *9*(6), 1113–1127. https://doi.org/10.1002/mbo3.1022

Gaona, O., Gómez-Acata, E. S., Cerqueda-García, D., Neri-Barrios, C. X., & Falcón, L. I. (2019). Fecal microbiota of different reproductive stages of the central population of the lesser-long nosed bat, *Leptonycteris yerbabuenae*. *PLoS ONE*, *14*(7). https://doi.org/10.1371/journal.pone.0219982

Generalitat de Catalunya. (2020). *Explotaciones ganaderas por especies. Comarcas y Aran, ámbitos y provincias.* https:// www. idesc at. cat/ pub/? id= aec&n= 454& lang= es.

Generalitat de Catalunya. (2023, February 3). *Fauna autòctona protegida: ratpenats*. Generalitat de Catalunya, Departament de Medi Ambient i Sostenibilitat.

Gerbáčová, K., Maliničová, L., Kisková, J., Maslišová, V., Uhrin, M., & Pristaš, P. (2020). The faecal microbiome of building-dwelling insectivorous bats (*Myotis myotis* and *Rhinolophus hipposideros*) also contains antibiotic-resistant bacterial representatives. *Current Microbiology*, *77*(9), 2333–2344. https://doi.org/10.1007/s00284-020-02095-z

Gheorghe, I. F., & Ion, B. (2011). The effects of air pollutants on vegetation and the role of vegetation in reducing atmospheric pollution. In *The Impact of*

Air Pollution on Health, Economy, Environment and Agricultural Sources: Vol. Chapter 12 (pp. 241–280). www.intechopen.com

Giambò, F., Teodoro, M., Costa, C., & Fenga, C. (2021). Toxicology and microbiota: how do pesticides influence gut microbiota? A review. *International Journal of Environmental Research and Public Health*, *18*(11), 5510. https://doi.org/10.3390/ijerph18115510

Gilbert, M., Nicolas, G., Cinardi, G., Van Boeckel, T. P., Vanwambeke, S. O., Wint, G. R. W., & Robinson, T. P. (2018). Global distribution data for cattle, buffaloes, horses, sheep, goats, pigs, chickens and ducks in 2010. *Scientific Data*, *5*(1), 180227. https://doi.org/10.1038/sdata.2018.227

Gill, S. R., Pop, M., DeBoy, R. T., Eckburg, P. B., Turnbaugh, P. J., Samuel, B. S., Gordon, J. I., Relman, D. A., Fraser-Liggett, C. M., & Nelson, K. E. (2006). Metagenomic analysis of the human distal gut microbiome. *Science*, *312*(5778), 1355–1359. https://doi.org/10.1126/science.1124234

Godinho, L. N., Lumsden, L. F., Coulson, G., & Griffiths, S. R. (2015). Network analysis reveals cryptic seasonal patterns of association in Gould's wattled bats (*Chalinolobus gouldii*) roosting in bat-boxes. *Behaviour*, *152*(15), 2079–2105. https://doi.org/10.1163/1568539X-00003315

Gómez-Rodríguez, R. A., Sánchez-Cordero, V., Boyer, D., Schondube, J. E., Rodríguez-Moreno, Á., & Gutiérrez-Granados, G. (2022). Risk of infection of white-nose syndrome in North American vespertilionid bats in Mexico. *Ecological Informatics*, *72*. https://doi.org/10.1016/j.ecoinf.2022.101869

Gómez-Ruiz, E. P., & Lacher, T. E. (2019). Climate change, range shifts, and the disruption of a pollinator-plant complex. *Scientific Reports*, *9*(1), 14048. https://doi.org/10.1038/s41598-019-50059-6

Gong, L., Liu, B., Wu, H., Feng, J., & Jiang, T. (2021). Seasonal dietary shifts alter the gut microbiota of avivorous bats: implication for adaptation to energy harvest and nutritional utilization. *MSphere*, *6*(4). https://doi.org/10.1128/msphere.00467-21

Góngora, E., Elliott, K. H., & Whyte, L. (2021). Gut microbiome is affected by inter-sexual and inter-seasonal variation in diet for thick-billed murres (*Uria lomvia*). *Scientific Reports*, *11*(1), 1200. https://doi.org/10.1038/s41598-020-80557-x

Goodrich, J. K., Waters, J. L., Poole, A. C., Sutter, J. L., Koren, O., Blekhman, R., Beaumont, M., van Treuren, W., Knight, R., Bell, J. T., Spector, T. D., Clark, A. G., & Ley, R. E. (2014). Human genetics shape the gut microbiome. *Cell*, *159*(4), 789–799. https://doi.org/10.1016/j.cell.2014.09.053

Greer, D. L., & McMurray, D. N. (1981). Pathogenesis of experimental histoplasmosis in the bat *Artibeus lituratus*. *Am. J. Trop. Med.*, *30*, 653–659.

Grisnik, M., Grinath, J. B., Munafo, J. P., & Walker, D. M. (2023). Functional redundancy in bat microbial assemblage in the presence of the white-nose pathogen. *Microbial Ecology*, *86*(1), 713–726. https://doi.org/10.1007/s00248-022-02098-2

Grodsky, S. M., Behr, M. J., Gendler, A., Drake, D., Dieterle, B. D., Rudd, R. J., & Walrath, N. L. (2011). Investigating the causes of death for wind turbine-associated bat fatalities. *Journal of Mammalogy*, *92*(5), 917–925. https://doi.org/10.1644/10-MAMM-A-404.1

Guest, E. E., Stamps, B. F., Durish, N. D., Hale, A. M., Hein, C. D., Morton, B. P., Weaver, S. P., & Fritts, S. R. (2022). An updated review of hypotheses regarding bat attraction to wind turbines. *Animals*, *12*(3). https://doi.org/10.3390/ani12030343

Gupta, S., Mortensen, M. S., Schjørring, S., Trivedi, U., Vestergaard, G., Stokholm, J., Bisgaard, H., Krogfelt, K. A., & Sørensen, S. J. (2019). Amplicon sequencing provides more accurate microbiome information in healthy children compared to culturing. *Communications Biology*, *2*(1). https://doi.org/10.1038/s42003-019-0540-1

Gupta, V. K., Kim, M., Bakshi, U., Cunningham, K. Y., Davis, J. M., Lazaridis, K. N., Nelson, H., Chia, N., & Sung, J. (2020). A predictive index for health status using species-level gut microbiome profiling. *Nature Communications*, *11*(1). https://doi.org/10.1038/s41467-020-18476-8

Haddad, N. M., Brudvig, L. A., Clobert, J., Davies, K. F., Gonzalez, A., Holt, R. D., Lovejoy, T. E., Sexton, J. O., Austin, M. P., Collins, C. D., Cook, W. M., Damschen, E. I., Ewers, R. M., Foster, B. L., Jenkins, C. N., King, A. J., Laurance, W. F., Levey, D. J., Margules, C. R., Melbourne, B. A., Nicholls, A. O., Orrock, J. L., Song, D., & Townshend, J. R. (2015). Habitat fragmentation and its lasting impact on Earth's ecosystems. *Science Advances*, *1*(2). https://doi.org/10.1126/sciadv.1500052

Hahn, K., Veiga, I. B., Schediwy, M., Wiederkehr, D., Meniri, M., Schneeberger, M., den Broek, P. R., Gurtner, C., Fasel, N. J., Kittl, S., Fredriksson-Ahomaa, M., Schmitt, S., & Stokar-Regenscheit, N. (2021). *Yersinia pseudotuberculosis* serotype O:1 infection in a captive Seba's short tailed-fruit bat (*Carollia perspicillata*) colony in Switzerland. *BMC Veterinary Research*, *17*(1), 92. https://doi.org/10.1186/s12917-021-02796-y

Hajkova, P., & Pikula, J. (2007). Veterinary treatment of evening bats (Vespertilionidae) in the Czech Republic. *Veterinary Record*, *161*(4), 139–140. https://doi.org/10.1136/vr.161.4.139

Han, H. J., Wen, H. ling, Zhou, C. M., Chen, F. F., Luo, L. M., Liu, J. wei, & Yu, X. J. (2015). Bats as reservoirs of severe emerging infectious diseases. *Virus Research*, *205*, 1–6. https://doi.org/10.1016/j.virusres.2015.05.006

Han, Z. Y., Weng, W. G., & Huang, Q. Y. (2013). Characterizations of particle size distribution of the droplets exhaled by sneeze. *Journal of The Royal Society Interface*, *10*(88), 20130560. https://doi.org/10.1098/rsif.2013.0560

Harris, R. N., Brucker, R. M., Walke, J. B., Becker, M. H., Schwantes, C. R., Flaherty, D. C., Lam, B. A., Woodhams, D. C., Briggs, C. J., Vredenburg, V. T., & Minbiole, K. P. C. (2009). Skin microbes on frogs prevent morbidity and mortality caused by a lethal skin fungus. *ISME Journal*, *3*(7), 818–824. https://doi.org/10.1038/ismej.2009.27

Hayman, D. T. S., Bowen, R. A., Cryan, P. M., McCracken, G. F., O'Shea, T. J., Peel, A. J., Gilbert, A., Webb, C. T., & Wood, J. L. N. (2013). Ecology of zoonotic infectious diseases in bats: current knowledge and future directions. *Zoonoses and Public Health*, *60*(1), 2–21. https://doi.org/10.1111/zph.12000

Haysom, K., Dekker, J., Russ, J., van de Meij, T., & van Strein, A. (2013). *European bat population trends: A prototype biodiversity indicator*.

Heather, J. M., & Chain, B. (2016). The sequence of sequencers: The history of sequencing DNA. In *Genomics* (Vol. 107, Issue 1, pp. 1–8). Academic Press Inc. https://doi.org/10.1016/j.ygeno.2015.11.003

Heikema, A. P., Horst-Kreft, D., Boers, S. A., Jansen, R., Hiltemann, S. D., de Koning, W., Kraaij, R., de Ridder, M. A. J., van Houten, C. B., Bont, L. J., Stubbs, A. P., & Hays, J. P. (2020). Comparison of Illumina versus nanopore 16s rRNA gene sequencing of the human nasal microbiota. *Genes*, *11*(9), 1–17. https://doi.org/10.3390/genes11091105

Hernández, L. M., Ibáñez, C., Fernández, M. A., Guillén, A., González, M. J., & Pérez, J. L. (1993). Organochlorine insecticide and PCB residues in two bat species from four localities in Spain. *Bulletin of Environmental Contamination and Toxicology*, *50*(6), 871–877. https://doi.org/10.1007/BF00209952

Hernández-Arciga, U., Herrera, L. G. M., Königsberg, M., Valdez, R. A., Flores-Martínez, J. J., & Romano, M. C. (2020). Synergetic effects of immune challenge and stress depress cortisol, inflammatory response and

antioxidant activity in fish-eating *Myotis*. *Journal of Experimental Biology*, *223*(24). https://doi.org/10.1242/jeb.234914

Hernández-Jerez, A., Adriaanse, P., Aldrich, A., Berny, P., Coja, T., Duquesne, S., Gimsing, A. L., Marina, M., Millet, M., Pelkonen, O., Pieper, S., Tiktak, A., Tzoulaki, I., Widenfalk, A., Wolterink, G., Russo, D., Streissl, F., & Topping, C. (2019). Scientific statement on the coverage of bats by the current pesticide risk assessment for birds and mammals. *EFSA Journal*, *17*(7). https://doi.org/10.2903/j.efsa.2019.5758

Hernández-Montero, J. R., Saldaña-Vázquez, R. A., Galindo-González, J., & Sosa, V. J. (2015). Bat-fruit interactions are more specialized in shaded-coffee plantations than in tropical mountain cloud forest fragments. *PLoS ONE*, *10*(5). https://doi.org/10.1371/journal.pone.0126084

Hosseini, P. R., Roche, B., Mills, J. N., Ne Prieur-Richard, A.-H., Ezenwa, V. O., Bailly, X., Rizzoli, A., Suzán, G., Vittecoq, M., García-Peña, G. E., Daszak, P., & Guégan, J.F. (2016). Does the impact of biodiversity differ between emerging and endemic pathogens? The need to separate the concepts of hazard and risk. *Philosophical Transactions B*, *372*(20160129). https://doi.org/10.1098/rstb.2016.0129

Hu, T., Chitnis, N., Monos, D., & Dinh, A. (2021). Next-generation sequencing technologies: an overview. *Human Immunology*, *82*(11), 801–811. https://doi.org/10.1016/j.humimm.2021.02.012

Huggins, L. G., Colella, V., Atapattu, U., Koehler, A. V., & Traub, R. J. (2022). Nanopore sequencing using the full-length 16S rRNA gene for detection of blood-borne bacteria in dogs reveals a novel species of hemotropic *Mycoplasma*. *Microbiology Spectrum*, *10*(6). https://doi.org/10.1128/spectrum.03088-22

Hutson, A. M., Marnell, F., & Tõrv, T. (2015). *A guide to the implementation of the Agreement on the Conservation of Populations of European Bats (EUROBATS)*. www.eurobats.org

Hutson, A., Mickleburgh, S. P., & Racey, P. A. (2001). Microchiropteran bats: global status survey and conservation action plan. *IUCN/SSC Chiroptera Specialist Group. IUCN, Gland, Switzerland and Cambridge, UK*, 258.

Hutterer, R., Ivanova, T., Meyer-Cords, C., & Rodrigues, L. (2005). *Bat migration in europe. A review of banding data and literature* (28; Vol. 28). Federal Agency for Nature Conservation in Germany. https://www.researchgate.net/publication/311443225

Ikeda, P., Torres, J. M., Placa, A. J. V., Mello, V. V. C. de, Lourenço, E. C., Herrera, H. M., Oliveira, C. E. de, Hemsley, C., Titball, R. W., Machado, R. Z., & André, M. R. (2021). Molecular survey of Anaplasmataceae agents and Coxiellaceae in non-hematophagous bats and associated ectoparasites from Brazil. *Parasitologia*, *1*(4), 197–209. https://doi.org/10.3390/parasitologia1040021

Imnadze, T., Natradze, I., Zhgenti, E., Malania, L., Abazashvili, N., Sidamonidze, K., Khmaladze, E., Zakalashvili, M., Imnadze, P., Arner, R. J., Motin, V., & Kosoy, M. (2020). Identification of a novel *Yersinia enterocolitica* strain from bats in association with a bat die-off that occurred in Georgia (Caucasus). *Microorganisms*, *8*(7), 1000. https://doi.org/10.3390/microorganisms8071000

Ingala, M., B Simmons, N., Dunbar, M., Wultsch, C., Krampis, K., & L Perkins, S. (2021). You are more than what you eat: differential enrichment of microbiome functions across bat dietary guilds. *Animal Microbiome*, *82*.

Ingala, M. R., Becker, D. J., Bak Holm, J., Kristiansen, K., & Simmons, N. B. (2019). Habitat fragmentation is associated with dietary shifts and microbiota variability in common vampire bats. *Ecology and Evolution*, *9*(11), 6508–6523. https://doi.org/10.1002/ece3.5228

Ingersoll, T. E., Sewall, B. J., & Amelon, S. K. (2013). Improved analysis of long-term monitoring data demonstrates marked regional declines of bat populations in the eastern United States. *PLoS ONE*, *8*(6). https://doi.org/10.1371/journal.pone.0065907

Institutional Animal Care and Use Committee. (2023). *Standard Operating Procedure for the Study of Bats in the Field*.

IUCN. (2023, May). *Order Chiroptera. IUCN Red List of Threatened Species*. Version 2023-8.

Jahan, N. A., Lindsey, L. L., Kipp, E. J., Reinschmidt, A., Heins, B. J., Runck, A. M., & Larsen, P. A. (2021). Nanopore-based surveillance of zoonotic bacterial pathogens in farm-dwelling peridomestic rodents. *Pathogens*, *10*(9). https://doi.org/10.3390/pathogens10091183

Jalanka-Tuovinen, J., Salonen, A., Nikkilä, J., Immonen, O., Kekkonen, R., Lahti, L., Palva, A., & de Vos, W. M. (2011). Intestinal microbiota in healthy adults: temporal analysis reveals individual and common core and relation to intestinal symptoms. *PLoS ONE*, *6*(7), e23035. https://doi.org/10.1371/journal.pone.0023035

Jarzembowski, T. (2002). Commensal aerobic bacterial flora of the gastrointestinal tract of *Pipistrellus nathusii* (Chiroptera: Vespertilionidae): Lack of *Escherichia coli* in fecal samples. *Acta Chiropterologica*, *4*(1), 103–106. https://doi.org/10.3161/001.004.0112

Jensen, B., & Baagoe, H. (1984). Overvintrende flagermuspa Kronborg Slot 1958 – 83. *Flora Og Fauna*, *90*, 65–69.

Jeong, J., Yun, K., Mun, S., Chung, W. H., Choi, S. Y., Nam, Y. do, Lim, M. Y., Hong, C. P., Park, C. H., Ahn, Y., & Han, K. (2021). The effect of taxonomic classification by full-length 16S rRNA sequencing with a synthetic long-read technology. *Scientific Reports*, *11*(1). https://doi.org/10.1038/s41598-020-80826-9

Jernberg, C., Löfmark, S., Edlund, C., & Jansson, J. K. (2007). Long-term ecological impacts of antibiotic administration on the human intestinal microbiota. *ISME Journal*, *1*(1), 56–66. https://doi.org/10.1038/ismej.2007.3

Johnson, J. S., Spakowicz, D. J., Hong, B. Y., Petersen, L. M., Demkowicz, P., Chen, L., Leopold, S. R., Hanson, B. M., Agresta, H. O., Gerstein, M., Sodergren, E., & Weinstock, G. M. (2019). Evaluation of 16S rRNA gene sequencing for species and strain-level microbiome analysis. *Nature Communications*, *10*(1). https://doi.org/10.1038/s41467-019-13036-1

Jones, D. N., Ravelomanantsoa, N. A. F., Yeoman, C. J., Plowright, R. K., & Brook, C. E. (2022). Do gastrointestinal microbiomes play a role in bats' unique viral hosting capacity? *Trends in Microbiology*, *30*(7), 632–642. https://doi.org/10.1016/j.tim.2021.12.009

Jones, G., Jacobs, D. S., Kunz, T. H., Wilig, M. R., & Racey, P. A. (2009). Carpe noctem: the importance of bats as bioindicators. In *Endangered Species Research* (Vol. 8, Issues 1–2, pp. 93–115). https://doi.org/10.3354/esr00182

Juste, J. (2007). *Nyctalus lasiopterus* Shcreber, 1780. In L. J. Palomo, J. Gisbert, & J. C. Blanco (Eds.), *Atlas y Libro Rojo de los Mamíferos Terrestres de España* (pp. 233–236). Dirección General para la Biodiversidad -SECEM-SECEMU.

Kai, S., Matsuo, Y., Nakagawa, S., Kryukov, K., Matsukawa, S., Tanaka, H., Iwai, T., Imanishi, T., & Hirota, K. (2019). Rapid bacterial identification by direct PCR amplification of 16S rRNA genes using the MinION™ nanopore sequencer. *FEBS Open Bio*, *9*(3), 548–557. https://doi.org/10.1002/2211-5463.12590

Kameoka, S., Motooka, D., Watanabe, S., Kubo, R., Jung, N., Midorikawa, Y., Shinozaki, N. O., Sawai, Y., Takeda, A. K., & Nakamura, S. (2021). Benchmark of 16S rRNA gene amplicon sequencing using Japanese gut microbiome

data from the V1–V2 and V3–V4 primer sets. *BMC Genomics*, *22*(1). https://doi.org/10.1186/s12864-021-07746-4

Kaper, J. B., Nataro, J. P., & Mobley, H. L. T. (2004). Pathogenic *Escherichia coli*. In *Nature Reviews Microbiology* (Vol. 2, Issue 2, pp. 123–140). https://doi.org/10.1038/nrmicro818

Kato, M., & Natarajan, R. (2019). Epigenetics and epigenomics in diabetic kidney disease and metabolic memory. In *Nature Reviews Nephrology* (Vol. 15, Issue 6, pp. 327–345). Nature Publishing Group. https://doi.org/10.1038/s41581-019-0135-6

Kchouk, M., Gibrat, J. F., & Elloumi, M. (2017). Generations of sequencing technologies: from First to Next Generation. *Biology and Medicine*, *09*(03). https://doi.org/10.4172/0974-8369.1000395

Kelm, D. H., Popa-Lisseanu, A. G., Dehnhard, M., & Ibáñez, C. (2016). Non-invasive monitoring of stress hormones in the bat *Eptesicus isabellinus* - Do fecal glucocorticoid metabolite concentrations correlate with survival? *General and Comparative Endocrinology*, *226*, 27–35. https://doi.org/10.1016/j.ygcen.2015.12.003

Kemenesi, G., Kurucz, K., Dallos, B., Zana, B., Földes, F., Boldogh, S., Görföl, T., Carroll, M. W., & Jakab, F. (2018). Re-emergence of Lloviu virus in *Miniopterus schreibersii* bats, Hungary, 2016. In *Emerging Microbes and Infections* (Vol. 7, Issue 1). Nature Publishing Group. https://doi.org/10.1038/s41426-018-0067-4

Kemenesi, G., Tóth, G. E., Mayora-Neto, M., Scott, S., Temperton, N., Wright, E., Mühlberger, E., Hume, A. J., Suder, E. L., Zana, B., Boldogh, S. A., Görföl, T., Estók, P., Lanszki, Z., Somogyi, B. A., Nagy, Á., Pereszlényi, C. I., Dudás, G., Földes, F., Kurucz, K., Madai, M., Zeghbib, S., Maes, P., Vanmechelen, B., & Jakab, F. (2022). Isolation of infectious Lloviu virus from Schreiber's bats in Hungary. *Nature Communications*, *13*(1). https://doi.org/10.1038/s41467-022-29298-1

Kers, J. G., & Saccenti, E. (2022). The power of microbiome studies: some considerations on which alpha and beta metrics to use and how to report results. *Frontiers in Microbiology*, *12*. https://doi.org/10.3389/fmicb.2021.796025

Khan, S. A., Imtiaz, M. A., Islam, M. M., Tanzin, A. Z., Islam, A., & Hassan, M. M. (2022). Major bat-borne zoonotic viral epidemics in Asia and Africa: A systematic review and meta-analysis. In *Veterinary Medicine and Science*

(Vol. 8, Issue 4, pp. 1787–1801). John Wiley and Sons Inc. https://doi.org/10.1002/vms3.835

Khorsand, B., Asadzadeh Aghdaei, H., Nazemalhosseini-Mojarad, E., Nadalian, B., Nadalian, B., & Houri, H. (2022). Overrepresentation of Enterobacteriaceae and *Escherichia coli* is the major gut microbiome signature in Crohn's disease and ulcerative colitis; a comprehensive metagenomic analysis of IBDMDB datasets. *Frontiers in Cellular and Infection Microbiology*, *12*. https://doi.org/10.3389/fcimb.2022.1015890

Kim, D., Song, L., Breitwieser, F. P., & Salzberg, S. L. (2016). Centrifuge: rapid and sensitive classification of metagenomic sequences. *Genome Research*, *26*(12), 1721–1729. https://doi.org/10.1101/gr.210641.116

Kim, Y. J., Kim, M. K., Im, W. T., Srinivasan, S., & Yang, D. C. (2010). *Parapusillimonas granuli* gen. nov., sp. nov., isolated from granules from a wastewater-treatment bioreactor. *International Journal of Systematic and Evolutionary Microbiology*, *60*(6), 1401–1406. https://doi.org/10.1099/ijs.0.009720-0

Kingry, L. C., Anacker, M., Pritt, B., Bjork, J., Respicio-Kingry, L., Liu, G., Sheldon, S., Boxrud, D., Strain, A., Oatman, S., Berry, J., Sloan, L., Mead, P., Neitzel, D., Kugeler, K. J., & Petersen, J. M. (2018). Surveillance for and discovery of *Borrelia* species in US patients suspected of tickborne illness. *Clinical Infectious Diseases*, *66*(12), 1864–1871. https://doi.org/10.1093/cid/cix1107

Knörnschild, M., Feifel, M., & Kalko, E. K. V. (2013). Mother-offspring recognition in the bat *Carollia perspicillata*. *Animal Behaviour*, *86*(5), 941–948. https://doi.org/10.1016/j.anbehav.2013.08.011

Koenig, J. E., Spor, A., Scalfone, N., Fricker, A. D., Stombaugh, J., Knight, R., Angenent, L. T., & Ley, R. E. (2011). Succession of microbial consortia in the developing infant gut microbiome. *Proceedings of the National Academy of Sciences of the United States of America*, *108*(SUPPL. 1), 4578–4585. https://doi.org/10.1073/pnas.1000081107

Koh, H. W., Song, M. S., Do, K. T., Kim, H., & Park, S. J. (2019). *Pusillimonas thiosulfatoxidans* sp. Nov., a thiosulfate oxidizer isolated from activated sludge. *International Journal of Systematic and Evolutionary Microbiology*, *69*(4), 1041–1046. https://doi.org/10.1099/ijsem.0.003266

Kolkert, H., Andrew, R., Smith, R., Rader, R., & Reid, N. (2020). Insectivorous bats selectively source moths and eat mostly pest insects on dryland and

irrigated cotton farms. *Ecology and Evolution*, *10*(1), 371–388. https://doi.org/10.1002/ece3.5901

Kruskal, W. H., & Wallis, W. A. (1952). Use of ranks in one-criterion variance analysis. In *Source: Journal of the American Statistical Association* (Vol. 47, Issue 260).

Kübber-Heiss, A., & Reifinger, M. (1999). Cardiopulmonary filariosis in bats (Microchiroptera) in Austria. *Erkrankungen Der Zootiere* , *39*, 423–427.

Kunz, T. H., Arnett, E. B., Cooper, B. M., Erickson, W. P., Larkin, R. P., Mabee, T., Morrison, M. L., Strickland, M. D., & Szewczak, J. M. (2007). Assessing Impacts of wind-energy development on nocturnally active birds and bats: A guidance document. *Journal of Wildlife Management*, *71*(8), 2449–2486. https://doi.org/10.2193/2007-270

Kunz, T. H., & Parsons, S. (Eds.). (2009). *Ecological and behavioral methods for the study of bats* (2nd ed.). Johns Hopkins University Press.

Lanaspa, M., Bassat, Q., Medeiros, M. M., & Muñoz-Almagro, C. (2017). Respiratory microbiota and lower respiratory tract disease. In *Expert Review of Anti-Infective Therapy* (Vol. 15, Issue 7, pp. 703–711). Taylor and Francis Ltd. https://doi.org/10.1080/14787210.2017.1349609

Langille, M. G. I., Zaneveld, J., Caporaso, J. G., McDonald, D., Knights, D., Reyes, J. A., Clemente, J. C., Burkepile, D. E., Vega Thurber, R. L., Knight, R., Beiko, R. G., & Huttenhower, C. (2013). Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. *Nature Biotechnology*, *31*(9), 814–821. https://doi.org/10.1038/nbt.2676

Laska, M. (1990). Food transit times and carbohydrate use in three Phyllostomid bat species. *Z. Säugetierkunde*, *55*, 49–54. http://www.biodiversitylibrary.org/

Laurindo, R. S., Novaes, R. L. M., Vizentin-Bugoni, J., & Gregorin, R. (2019). The effects of habitat loss on bat-fruit networks. *Biodiversity and Conservation*, *28*(3), 589–601. https://doi.org/10.1007/s10531-018-1676-x

Lee, M., Woo, S. G., Chae, M., & Ten, L. N. (2010). Pusillimonas soli sp. nov., isolated from farm soil. *International Journal of Systematic and Evolutionary Microbiology*, *60*(10), 2326–2330. https://doi.org/10.1099/ijs.0.020404-0

Leelapaibul, W., Bumrungsri, S., & Pattanawiboon, A. (2005). Diet of wrinkle-lipped free-tailed bat (*Tadarida plicata* Buchannan, 1800) in central Thailand: Insectivorous bats potentially act as biological pest control agents. *Acta Chiropterologica*, *7*(1), 111–119. https://doi.org/10.3161/1733-5329(2005)7[111:DOWFBT]2.0.CO;2

Leeming, E. R., Johnson, A. J., Spector, T. D., & Roy, C. I. L. (2019). Effect of diet on the gut microbiota: Rethinking intervention duration. In *Nutrients* (Vol. 11, Issue 12). MDPI AG. https://doi.org/10.3390/nu11122862

Lei, M., & Dong, D. (2016). Phylogenomic analyses of bat subordinal relationships based on transcriptome data. *Scientific Reports*, *6*. https://doi.org/10.1038/srep27726

Leidenfrost, R. M., Pöther, D. C., Jäckel, U., & Wünschiers, R. (2020). Benchmarking the MinION: evaluating long reads for microbial profiling. *Scientific Reports*, *10*(1). https://doi.org/10.1038/s41598-020-61989-x

Lemieux-Labonté, V., Simard, A., Willis, C. K. R., & Lapointe, F. J. (2017). Enrichment of beneficial bacteria in the skin microbiota of bats persisting with white-nose syndrome. *Microbiome*, *5*(1), 115. https://doi.org/10.1186/s40168-017-0334-y

Leviatan, S., & Segal, E. (2020). Identifying gut microbes that affect human health-leer corta nota. *Nature*, *587*.

Lewis, J. S., Spaulding, S., Swanson, H., Keeley, W., Gramza, A. R., VandeWoude, S., & Crooks, K. R. (2021). Human activity influences wildlife populations and activity patterns: implications for spatial and temporal refuges. *Ecosphere*, *12*(5). https://doi.org/10.1002/ecs2.3487

Ley, R. E., Hamady, M., Lozupone, C., Turnbaugh, P. J., Ramey, R. R., Bircher, J. S., Schlegel, M. L., Tucker, T. A., Schrenzel, M. D., Knight, R., & Gordon, J. I. (2008). Evolution of mammals and their gut microbes. *Science*, *320*(5883), 1647–1651. https://doi.org/10.1126/science.1155725

Li, J., Chu, Y., Yao, W., Wu, H., & Feng, J. (2021). Differences in diet and gut microbiota between lactating and non-lactating Asian Particolored Bats (*Vespertilio sinensis*): implication for a connection between diet and gut microbiota. *Frontiers in Microbiology*, *12*. https://doi.org/10.3389/fmicb.2021.735122

Li, J., Li, L., Jiang, H., Yuan, L., Zhang, L., Jing-E Ma, ·, Zhang, · Xiujuan, Cheng, M., & Chen, J. (2018). Fecal bacteriome and mycobiome in bats with diverse diets in South China. *Current Microbiology*, *75*, 1352–1361. https://doi.org/10.1007/s00284-018-1530-0

Li, S., Ondon, B. S., Ho, S.-H., & Li, F. (2023). Emerging soil contamination of antibiotics resistance bacteria (ARB) carrying genes (ARGs): New challenges for soil remediation and conservation. *Environmental Research*, *219*, 115132. https://doi.org/10.1016/j.envres.2022.115132

Lightfoot, N. (2003). Bacteria of potential health concern. In J. Bartram, J. Cotruvo, M. Exner, C. Fricker, & A. Glasmacher (Eds.), *Heterotrophic plate counts and drinking-water safety* (p. 256). World Health Organization, World Health Organization (WHO).

Lim, B. K., Brock, · M, Brigham, F. R. M., Mistry, S., Kurta, A., Gillam, E. H., Russell, A., & Ortega, J. (2021). *50 Years of Bat Research: Foundations and New Frontiers* (B. K. Lim, M. B. Fenton, R. M. Brigham, S. Mistry, A. Kurta, E. H. Gillam, A. Russell, & J. Ortega, Eds.). Springer. http://www.springer.com/series/15408

Lin, B., Hui, J., & Mao, H. (2021). Nanopore technology and its applications in gene sequencing. In *Biosensors* (Vol. 11, Issue 7). MDPI. https://doi.org/10.3390/bios11070214

Lin, S. Y., Hameed, A., Tsai, C. F., Tang, Y. S., & Young, C. C. (2022). *Pusillimonas faecipullorum* sp. nov., isolated from the poultry manure. *Archives of Microbiology*, *204*(5). https://doi.org/10.1007/s00203-022-02859-0

Lindsjö, J., Cvek, K., Spangenberg, E. M. F., Olsson, J. N. G., & Stéen, M. (2019). The dividing line between wildlife research and management: Implications for animal welfare. *Frontiers in Veterinary Science*, *6*. https://doi.org/10.3389/fvets.2019.00013

Liu, B., Chen, X., Zhou, L., Li, J., Wang, D., Yang, W., Wu, H., Yao, J., Yang, G., Wang, C., Feng, J., & Jiang, T. (2022). The gut microbiota of bats confers tolerance to influenza virus (H1N1) infection in mice. *Transboundary and Emerging Diseases*, *69*(5). https://doi.org/10.1111/tbed.14478

Lloyd-Price, J., Abu-Ali, G., & Huttenhower, C. (2016). The healthy human microbiome. In *Genome Medicine* (Vol. 8, Issue 1). BioMed Central Ltd. https://doi.org/10.1186/s13073-016-0307-y

Locatelli, A. G., Ciuti, S., Presetnik, P., Toffoli, R., & Teeling, E. (2019). Long-term monitoring of the effects of weather and marking techniques on body condition in the Kuhl's pipistrelle bat, *Pipistrellus kuhlii*. *Acta Chiropterologica*, *21*(1), 87–102. https://doi.org/10.3161/15081109ACC2019.21.1.007

Lorch, J. M., Minnis, A. M., Meteyer, C. U., Redell, J. A., Paul White, J., Kaarakka, H. M., Muller, L. K., Lindner, D. L., Verant, M. L., Shearn-Bochsler, V., & Blehert, D. S. (2015). The fungus *Trichophyton redellii* sp. nov. causes skin infections that resemble white-nose syndrome of hibernating bats. *Journal of Wildlife Diseases*, *51*(1), 36–47. https://doi.org/10.7589/2014-05-134

Loudon, A. H., Kurtz, A., Esposito, E., Umile, T. P., Minbiole, K. P. C., Parfrey, L. W., & Sheafor, B. A. (2020). Columbia spotted frogs (*Rana luteiventris*) have characteristic skin microbiota that may be shaped by cutaneous skin peptides and the environment. *FEMS Microbiology Ecology*, *96*(10). https://doi.org/10.1093/femsec/fiaa168

Lozupone, C. A., Stombaugh, J. I., Gordon, J. I., Jansson, J. K., & Knight, R. (2012). Diversity, stability and resilience of the human gut microbiota. *Nature*, *489*(7415), 220–230. https://doi.org/10.1038/nature11550

Lu, J., Rincon, N., Wood, D. E., Breitwieser, F. P., Pockrandt, C., Langmead, B., Salzberg, S. L., & Steinegger, M. (2022). Metagenome analysis using the Kraken software suite. *Nature Protocols*, *17*(12), 2815–2839. https://doi.org/10.1038/s41596-022-00738-y

Luftl, S., Freitag, B., Deutz, A., Steineck, T., & Tataruch F. (2005). Concentrations of organochlorine pesticides and PCBs in the liver of European bats (Michrochiroptera). *Fresenius Environ Bull* , *14*.

Luo, J., Liang, S., & Jin, F. (2021). Gut microbiota in antiviral strategy from bats to humans: a missing link in COVID-19. In *Science China Life Sciences* (Vol. 64, Issue 6, pp. 942–956). Science in China Press. https://doi.org/10.1007/s11427-020-1847-7

Luo, J., Siemers, B. M., & Koselj, K. (2015). How anthropogenic noise affects foraging. *Global Change Biology*, *21*(9), 3278–3289. https://doi.org/10.1111/gcb.12997

Lupatini, M., Korthals, G. W., de Hollander, M., Janssens, T. K. S., & Kuramae, E. E. (2017). Soil microbiome is more heterogeneous in organic than in conventional farming system. *Frontiers in Microbiology*, *7*(JAN). https://doi.org/10.3389/fmicb.2016.02064

Lutz, H. L., Jackson, E. W., Webala, P. W., Babyesiza, W. S., Kerbis Peterhans, J. C., Demos, T. C., Patterson, B. D., & Gilbert, J. A. (2019). Ecology and host identity outweigh evolutionary history in shaping the bat microbiome. *American Society for Microbiology*, *4*(6). https://doi.org/10.1128/mSystems

Lutz, K. C., Jiang, S., Neugent, M. L., De Nisco, N. J., Zhan, X., & Li, Q. (2022). A survey of statistical methods for microbiome data analysis. *Frontiers in Applied Mathematics and Statistics*, *8*. https://doi.org/10.3389/fams.2022.884810

Mahlen, S. D. (2011). *Serratia* infections: from military experiments to current practice. *Clinical Microbiology Reviews*, *24*(4), 755–791. https://doi.org/10.1128/CMR.00017-11

Manzoni, C., Kia, D. A., Vandrovcova, J., Hardy, J., Wood, N. W., Lewis, P. A., & Ferrari, R. (2018). Genome, transcriptome and proteome: The rise of omics data and their integration in biomedical sciences. *Briefings in Bioinformatics*, *19*(2), 286–302. https://doi.org/10.1093/BIB/BBW114

Marchesi, J. R., & Ravel, J. (2015). The vocabulary of microbiome research: a proposal. *Microbiome*, *3*(1). https://doi.org/10.1186/s40168-015-0094-5

Marsland, R., Cui, W., & Mehta, P. (2020). A minimal model for microbial biodiversity can reproduce experimentally observed ecological patterns. *Scientific Reports*, *10*(1). https://doi.org/10.1038/s41598-020-60130-2

Martin, A. M., Sun, E. W., Rogers, G. B., & Keating, D. J. (2019). The influence of the gut microbiome on host metabolism through the regulation of gut hormone release. *Frontiers in Physiology*, *10*(MAR). https://doi.org/10.3389/fphys.2019.00428

Martin, K., Puckette, W., Hensley, S., & Leslie, D. (2000). Internal cave gating as a means of protecting cave-dwelling bat populations in Eastern Oklahoma. *Proc. Okla. Acad. Sci.*, *80*, 133–137.

Martínez-Medina, D., Pérez-Torres, J., & Martínez-Luque, L. (2018). Apuntes sobre la estructura social de *Carollia perspicillata* (Chiroptera, Phyllostomidae) in the Macaregua Cave, Santander, Colombia. *Rev. Biodivers. Neotrop.*, *8*(1), 14–21. https://doi.org/http://dx.doi.org/10.18636/bioneotropical.v8i1.687

Maslennikov, R., Ivashkin, V., Efremova, I., Alieva, A., Kashuh, E., Tsvetaeva, E., Poluektova, E., Shirokova, E., & Ivashkin, K. (2021). Gut dysbiosis is associated with poorer long-term prognosis in cirrhosis. *World Journal of Hepatology*, *13*(5), 557–570. https://doi.org/10.4254/wjh.v13.i5.557

Mata, L., Goula, M., & Hahs, A. K. (2014). Conserving insect assemblages in urban landscapes: accounting for species-specific responses and imperfect detection. *Journal of Insect Conservation*, *18*(5), 885–894. https://doi.org/10.1007/s10841-014-9696-7

Mathew, M. J., Subramanian, G., Nguyen, T.-T., Robert, C., Mediannikov, O., Fournier, P.-E., & Raoult, D. (2012). Genome sequence of *Diplorickettsia massiliensis*, an emerging *Ixodes ricinus* associated human pathogen. *Journal of Bacteriology*, *194*(12), 3287–3287. https://doi.org/10.1128/JB.00448-12

Matsuo, Y., Komiya, S., Yasumizu, Y., Yasuoka, Y., Mizushima, K., Takagi, T., Kryukov, K., Fukuda, A., Morimoto, Y., Naito, Y., Okada, H., Bono, H., Nakagawa, S., & Hirota, K. (2021). Full-length 16S rRNA gene amplicon

analysis of human gut microbiota using MinION™ nanopore sequencing confers species-level resolution. *BMC Microbiology*, *21*(1). https://doi.org/10.1186/s12866-021-02094-5

McBurney, M. I., Davis, C., Fraser, C. M., Schneeman, B. O., Huttenhower, C., Verbeke, K., Walter, J., & Latulippe, M. E. (2019). Establishing what constitutes a healthy human gut microbiome: state of the science, regulatory considerations, and future directions. In *Journal of Nutrition* (Vol. 149, Issue 11, pp. 1882–1895). Oxford University Press. https://doi.org/10.1093/jn/nxz154

McKinney, M. L. (2006). Urbanization as a major cause of biotic homogenization. *Biological Conservation*, *127*(3), 247–260. https://doi.org/10.1016/j.biocon.2005.09.005

McLelland, D. J., Reardon, T., Bourne, S., Dickason, C., Kessell, A., & Boardman, W. (2013). Outbreak of skin nodules associated with *Riouxgolvania beveridgei* (Nematoda: Muspiceida) in the Southern Bentwing bat (*Miniopterus schreibersii bassanii*), South Australia. *Journal of Wildlife Diseases*, *49*(4), 1009–1013. https://doi.org/10.7589/2012-11-288

McMurdie, P. J., & Holmes, S. (2013). phyloseq: An R package for reproducible interactive analysis and graphics of microbiome census data. *PLoS ONE*, *8*(4), e61217. https://doi.org/10.1371/journal.pone.0061217

Mehl, C., Schoeman, M. C., Sanko, T. J., Bezuidenhout, C., Mienie, C. M. S., Preiser, W., & Vosloo, D. (2021). Wastewater treatment works change the intestinal microbiomes of insectivorous bats. *PLoS ONE*, *16*(3 March). https://doi.org/10.1371/journal.pone.0247475

Mekonen, S. (2020). Coexistence between human and wildlife: The nature, causes and mitigations of human wildlife conflict around Bale Mountains National Park, Southeast Ethiopia. *BMC Ecology*, *20*(1). https://doi.org/10.1186/s12898-020-00319-1

Mellado, B., Carneiro, L. de O., Nogueira, M. R., & Monteiro, L. R. (2022). The impacts of marking on bats: mark-recapture models for assessing injury rates and tag loss. *Journal of Mammalogy*, *103*(1), 100–110. https://doi.org/10.1093/jmammal/gyab153

Meng, Y., Lei, Y., Gao, J., Liu, Y., Ma, E., Ding, Y., Bian, Y., Zu, H., Dong, Y., & Zhu, X. (2022). Genome sequence assembly algorithms and misassembly identification methods. *Molecular Biology Reports*, *49*(11), 11133–11148. https://doi.org/10.1007/s11033-022-07919-8

Mengist, W., Soromessa, T., & Feyisa, G. L. (2020). A global view of regulatory ecosystem services: existed knowledge, trends, and research gaps. In *Ecological Processes* (Vol. 9, Issue 1). Springer. https://doi.org/10.1186/s13717-020-00241-w

Mickleburgh, S. P., Hutson, A. Michael., & Racey, P. A. (1992). *Old World fruit bats: an action plan for their conservation*. IUCN International Union for Conservation of Nature and Natural Resources.

Mickleburgh, S., Waylen, K., & Racey, P. (2009). Bats as bushmeat: A global review. In *ORYX* (Vol. 43, Issue 2, pp. 217–234). https://doi.org/10.1017/S0030605308000938

Miller, H. (Ed.). (2016). *Bat Care Guidelines* (2nd edn). Bat Conservation Trust. http://www.bats.org.uk/pages/bat_care_supporters.html

Miller, W., Griffin, C., & Kampbell, K. (2012). *Muller & Kirk's Small animal dermatology* (7th ed.). Saunders-Elsevier.

Mioni, M. D. S. R., Castro, F. F. C., Moreno, L. Z., Apolinário, C. M., Belaz, L. D., Peres, M. G., Ribeiro, B. L. D., Castro, M. J. D. S., Ferreira, A. M., Cortez, A., Moreno, A. M., Heinemann, M. B., & Megid, J. (2018). Septicemia due to *Streptococcus dysgalactiae* subspecies *dysgalactiae* in vampire bats (*Desmodus rotundus*). *Scientific Reports*, *8*(1). https://doi.org/10.1038/s41598-018-28061-1

Mitchell-Jones, A., & McLeish, A. (2004). *Bat workers' manual* (A. J. Mitchell-Jones & A. P. McLeish, Eds.; 3r Edition). Joint Nature Conservation Committee.

Mitchell-Jones, T. (2004). *Bat mitigation guidelines*. English Nature .

Monteiro, L. R., Mellado, B., Nogueira, M. R., & de Morais-Jr, M. M. (2019). Individual asymmetry as a predictor of fitness in the bat *Carollia perspicillata*. *Journal of Evolutionary Biology*, *32*(11), 1207–1229. https://doi.org/10.1111/jeb.13522

Morgensztern, D., Devarakonda, S., Mitsudomi, T., Maher, C., & Govindan, R. (2018). Mutational events in lung cancer. In *IASLC Thoracic Oncology* (pp. 95-103.e2). Elsevier. https://doi.org/10.1016/b978-0-323-52357-8.00011-1

Mu, F., Li, Y., Lu, R., Qi, Y., Xie, W., & Bai, W. (2020). Source identification of airborne bacteria in the mountainous area and the urban areas. *Atmospheric Research*, *231*, 104676. https://doi.org/10.1016/j.atmosres.2019.104676

Muegge, B. D. (2013). *The influence of diet on the mammalian gut microbiome* [Washington University, Division of Biology and Biomedical Sciences]. https://openscholarship.wustl.edu/etd/1064

Mühldorfer, K. (2013). Bats and bacterial pathogens: a review. *Zoonoses and Public Health*, *60*(1), 93–103. https://doi.org/10.1111/j.1863-2378.2012.01536.x

Mühldorfer, K., Speck, S., Kurth, A., Lesnik, R., Freuling, C., Müller, T., Kramer-Schadt, S., & Wibbelt, G. (2011). Diseases and causes of death in European bats: dynamics in disease susceptibility and infection rates. *PLoS ONE*, *6*(12), e29773. https://doi.org/10.1371/journal.pone.0029773

Mühldorfer, K., Speck, S., & Wibbelt, G. (2011). Diseases in free-ranging bats from Germany. *BMC Veterinary Research*, *7*, 61. https://doi.org/10.1186/1746-6148-7-61

Mühldorfer, K., Speck, S., & Wibbelt, G. (2014). Proposal of *Vespertiliibacter pulmonis* gen. nov., sp. nov. and two genomospecies as new members of the family Pasteurellaceae isolated from European bats. *International Journal of Systematic and Evolutionary Microbiology*, *64*(PART 7), 2424–2430. https://doi.org/10.1099/ijs.0.062786-0

Mühldorfer, K., Wibbelt, G., Haensel, J., Riehm, J., & Speck, S. (2010). *Yersinia* species isolated from bats, Germany. In *Emerging Infectious Diseases* (Vol. 16, Issue 3, pp. 576–578). https://doi.org/10.3201/eid1603.090996

Muscarella, R., & Fleming, T. H. (2007). The role of frugivorous bats in tropical forest succession. In *Biological Reviews* (Vol. 82, Issue 4, pp. 573–590). https://doi.org/10.1111/j.1469-185X.2007.00026.x

Museu de Ciències Naturals de Granollers. (2023). *Espècies de ratpenats de Catalunya*. https://www.ratpenats.org/especies/

Museu de Ciències Naturals de Granollers, Generalitat de Catalunya & Diputació de Barcelona. (2024). *Programa de seguiment de ratpenats*. https://www.ratpenats.org/

Nabi, G., Wang, Y., Lü, L., Jiang, C., Ahmad, S., Wu, Y., & Li, D. (2021). Bats and birds as viral reservoirs: A physiological and ecological perspective. *Science of the Total Environment*, *754*. https://doi.org/10.1016/j.scitotenv.2020.142372

Nakamura, S., Settai, S., Hayashidani, H., Urabe, T., Namai, S., & Une, Y. (2013). Outbreak of yersiniosis in Egyptian Rousette Bats (*Rousettus aegyptiacus*) caused by *Yersinia pseudotuberculosis* serotype 4b. *Journal of Comparative Pathology*, *148*(4), 410–413. https://doi.org/10.1016/j.jcpa.2012.07.007

Nakamura, T., Ishikawa, K., Matsuo, T., Kawai, F., Uehara, Y., & Mori, N. (2021). *Enterococcus hirae* bacteremia associated with acute pyelonephritis in a patient with alcoholic cirrhosis: a case report and literature review. *BMC Infectious Diseases*, *21*(1), 999. https://doi.org/10.1186/s12879-021-06707-2

Negredo, A., Palacios, G., Vázquez-Morón, S., González, F., Dopazo, H., Molero, F., Juste, J., Quetglas, J., Savji, N., de la Cruz Martínez, M., Herrera, J. E., Pizarro, M., Hutchison, S. K., Echevarría, J. E., Lipkin, W. I., & Tenorio, A. (2011). Discovery of an ebolavirus-like filovirus in Europe. *PLoS Pathogens*, *7*(10). https://doi.org/10.1371/journal.ppat.1002304

NENT. (2018). *Ethical guidelines for the use of animals in research* (1st ed.). The Norwegian National Research Ethics Committees. www.etikkom.no

Newbold, T. (2018). Future effects of climate and land-use change on terrestrial vertebrate community diversity under different scenarios. *Proceedings of the Royal Society B: Biological Sciences*, *285*(1881). https://doi.org/10.1098/rspb.2018.0792

Newman, M. M., Kloepper, L. N., Duncan, M., McInroy, J. A., & Kloepper, J. W. (2018). Variation in bat guano bacterial community composition with depth. *Frontiers in Microbiology*, *9* (May). https://doi.org/10.3389/fmicb.2018.00914

Noer, C. L., Dabelsteen, T., Bohmann, K., & Monadjem, A. (2012). Molossid bats in an African agro-ecosystem select sugarcane fields as foraging habitat. *African Zoology*, *47*(1), 1–11. https://doi.org/10.3377/004.047.0120

Núñez-Montero, K., Santos, A., Quezada-Solís, D., Martínez-Urtaza, J., Lisón, F., & Barrientos, L. (2021). Bacterial communities in fecal samples of *Myotis chiloensis* from Southern Chile. *Int. J. Morphol.*, *39*(1), 57–63.

Nurkolis, F., Mayulu, N., Yasmine, N., Puspaningtyas, D. S., & Taslim, N. A. (2022). Human activities and changes in the gut microbiome: A perspective. *Human Nutrition & Metabolism*, *30*, 200165. https://doi.org/10.1016/j.hnm.2022.200165

Obrochta, S., Savo Sardaro, M. L., Amato, K. R., & Murray, M. H. (2022). Relationships between migration and microbiome composition and diversity in urban Canada geese. *Frontiers in Ecology and Evolution*, *10*. https://doi.org/10.3389/fevo.2022.742369

Oksanen, J., Blanchet, F. G., Kindt, R., Legendre, P., Minchin, P. R., O'Hara, R. B., Simpson, G. L., Solymos, P., Stevens, M. H. H., & Wagner, H. (2007). *The vegan package. Community ecology package*. *10*(719), 631–637.

O'Leary, N. A., Wright, M. W., Brister, J. R., Ciufo, S., Haddad, D., McVeigh, R., Rajput, B., Robbertse, B., Smith-White, B., Ako-Adjei, D., Astashyn, A., Badretdin, A., Bao, Y., Blinkova, O., Brover, V., Chetvernin, V., Choi, J., Cox, E., Ermolaeva, O., Farrell, C. M., Goldfard, T., Gupta, T., Haft, D., Hatcher, E., Hlavina, W., Joardar, V. S., Kodali, V. K., Li, W., Maglott, D., Masterson, P., McGarvey, K. M., Murphy, M. R., O'Neill, K., Pujar, S., Rangwala, S. H., Rausch, D., Riddick, L. D., Schoch, C., Shkeda, A., Storz, S. S., Sun, H., Thibaud-Nissen, F., Tolstoy, I., Tully, R. E., Vatsan, A. R., Wallin, C., Webb, D., Wu, W., Landrum, M. J., Kimchi, A., Tatusova, T., DiCuccio, M., Kitts, P., Murphy, T. D., & Pruitt, K. D. (2016). Reference sequence (RefSeq) database at NCBI: current status, taxonomic expansion, and functional annotation. *Nucleic Acids Research*, *44*(D1), D733–D745. https://doi.org/10.1093/nar/gkv1189

Oliver, T. H., Heard, M. S., Isaac, N. J. B., Roy, D. B., Procter, D., Eigenbrod, F., Freckleton, R., Hector, A., Orme, C. D. L., Petchey, O. L., Proença, V., Raffaelli, D., Suttle, K. B., Mace, G. M., Martín-López, B., Woodcock, B. A., & Bullock, J. M. (2015). Biodiversity and resilience of ecosystem functions. In *Trends in Ecology and Evolution* (Vol. 30, Issue 11, pp. 673–684). Elsevier Ltd. https://doi.org/10.1016/j.tree.2015.08.009

O'Shea, T. J., Cryan, P. M., Cunningham, A. A., Fooks, A. R., Hayman, D. T., Luis, A. D., Peel, A. J., Plowright, R. K., & Wood, J. L. (2014). Bat flight and zoonotic viruses. *Emerging Infectious Diseases*, *20*(5), 741–745. https://doi.org/10.1128/CMR

O'Shea, T. J., Cryan, P. M., Hayman, D. T. S., Plowright, R. K., & Streicker, D. G. (2016). Multiple mortality events in bats: A global review. In *Mammal Review* (Vol. 46, Issue 3, pp. 175–190). Blackwell Publishing Ltd. https://doi.org/10.1111/mam.12064

O'Shea, T. J., Ellison, L. E., & Stanley, T. R. (2004). Survival estimation in bats: historical overview, critical appraisal, and suggestions for new approaches. In *Sampling rare or elusive species: concepts, designs, and techniques for estimating population parameters* (pp. 297–336).

Oxford Nanopore Technologies. (2022). *Nanopore sequencing — how it works* (pp. 1–19). www.nanoporetech.com

Pagès-Gallego, M., & de Ridder, J. (2023). Comprehensive benchmark and architectural analysis of deep learning models for nanopore sequencing basecalling. *Genome Biology*, *24*(1), 71. https://doi.org/10.1186/s13059-023-02903-2

Paliy, O., & Shankar, V. (2016). Application of multivariate statistical techniques in microbial ecology. *Molecular Ecology*, *25*(5), 1032–1057. https://doi.org/10.1111/mec.13536

Palme, R., Touma, C., Arias, N., Dominchin, M. F., & Lepschy, M. (2013). Steroid extraction: get de best out of faecal samples. *Wiener Tierärztliche Monatsschrift*, *100*.

Palmer, K. L., Godfrey, P., Griggs, A., Kos, V. N., Zucker, J., Desjardins, C., Cerqueira, G., Gevers, D., Walker, S., Wortman, J., Feldgarden, M., Haas, B., Birren, B., & Gilmore, M. S. (2012). Comparative genomics of Enterococci: variation in *Enterococcus faecalis*, clade structure in *E. faecium*, and defining characteristics of *E. gallinarum* and *E. casseliflavus*. *MBio*, *3*(1). https://doi.org/10.1128/mBio.00318-11

Palomo, L. J., Gisbert, J., & Blanco, J. C. (2007). Atlas y Libro Rojo de los Mamíferos Terrestres de España. *Dirección General Para La Biodiversidad-SECEM-SECEMU*, 1–588.

Parris, K. M., McCall, S. C., McCarthy, M. A., Minteer, B. A., Steele, K., Bekessy, S., & Medvecky, F. (2010). Assessing ethical trade-offs in ecological field studies. *Journal of Applied Ecology*, *47*(1), 227–234. https://doi.org/10.1111/j.1365-2664.2009.01755.x

Patel, J. (2001). 16S rRNA gene sequencing for bacterial pathogen identification in the clinical laboratory. *Molecular Diagnosis*, *6*(4), 313–321. https://doi.org/10.1054/modi.2001.29158

Patel, J., Murin, P., Sharif, N., & Animalu, C. (2023). Disseminated *Streptococcus gallinaceus* infection. A new breed of zoonotic *Streptococcus*. *Journal of the National Medical Association*, *115*(3), 298–301. https://doi.org/10.1016/j.jnma.2023.02.009

Peixoto, R. S., Harkins, D. M., & Nelson, K. E. (2021). Advances in Microbiome Research for Animal Health. *Annual Review of Animal Biosciences*. https://doi.org/10.1146/annurev-animal-091020

Pérez-Pantoja, D., Donoso, R., Agulló, L., Córdova, M., Seeger, M., Pieper, D. H., & González, B. (2012). Genomic analysis of the potential for aromatic compounds biodegradation in Burkholderiales. In *Environmental Microbiology* (Vol. 14, Issue 5, pp. 1091–1117). https://doi.org/10.1111/j.1462-2920.2011.02613.x

Pervez, M. T., Hasnain, M. J. U., Abbas, S. H., Moustafa, M. F., Aslam, N., & Shah, S. S. M. (2022). A comprehensive review of performance of Next-

Generation sequencing platforms. In *BioMed Research International* (Vol. 2022). Hindawi Limited. https://doi.org/10.1155/2022/3457806

Petersen, L. M., & Tisa, L. S. (2013). Friend or foe? A review of the mechanisms that drive *Serratia* towards diverse lifestyles. *Canadian Journal of Microbiology*, *59*(9), 627–640. https://doi.org/10.1139/cjm-2013-0343

Phillips, C. D., Phelan, G., Dowd, S. E., McDonough, M. M., Ferguson, A. W., Delton Hanson, J., Siles, L., OrdO'Ñez-Garza, N., San Francisco, M., & Baker, R. J. (2012). Microbiome analysis among bats describes influences of host phylogeny, life history, physiology and geography. *Molecular Ecology*, *21*(11), 2617–2627. https://doi.org/10.1111/j.1365-294X.2012.05568.x

Piccioli Cappelli, M., Blakey, R. v., Taylor, D., Flanders, J., Badeen, T., Butts, S., Frick, W. F., & Rebelo, H. (2021). Limited refugia and high velocity range-shifts predicted for bat communities in drought-risk areas of the Northern Hemisphere. *Global Ecology and Conservation*, *28*. https://doi.org/10.1016/j.gecco.2021.e01608

Pimm, S. L., & Raven, P. (2000). Extinction by numbers. *Nature*, *403*.

Piplani, S., Singh, P. K., Winkler, D. A., & Petrovsky, N. (2021). In silico comparison of SARS-CoV-2 spike protein-ACE2 binding affinities across species and implications for virus origin. *Scientific Reports*, *11*(1). https://doi.org/10.1038/s41598-021-92388-5

Plowright, R. K., Eby, P., Hudson, P. J., Smith, I. L., Westcott, D., Bryden, W. L., Middleton, D., Reid, P. A., McFarlane, R. A., Martin, G., Tabor, G. M., Skerratt, L. F., Anderson, D. L., Crameri, G., Quammen, D., Jordan, D., Freeman, P., Wang, L. F., Epstein, J. H., Marsh, G. A., Kung, N. Y., & McCallum, H. (2014). Ecological dynamics of emerging bat virus spillover. In *Proceedings of the Royal Society B: Biological Sciences* (Vol. 282, Issue 1798). Royal Society of London. https://doi.org/10.1098/rspb.2014.2124

Popov, I. v., Mazanko, M. S., Kulaeva, E. D., Golovin, S. N., Malinovkin, A. v., Aleshukina, I. S., Aleshukina, A. v., Prazdnova, E. v., Tverdokhlebova, T. I., Chikindas, M. L., & Ermakov, A. M. (2021). Gut microbiota of bats: pro-mutagenic properties and possible frontiers in preventing emerging disease. *Scientific Reports*, *11*(1). https://doi.org/10.1038/s41598-021-00604-z

Powers, R. P., & Jetz, W. (2019). Global habitat loss and extinction risk of terrestrial vertebrates under future land-use-change scenarios. *Nature Climate Change*, *9*, 323–329. https://doi.org/10.1038/s41558-019-0406-z

Preble, J. H., & Vincenot, C. E. (2021). In the shadow of the rising sun: a systematic review of Japanese bat research and conservation. *Mammal Review*, *51*, 109–126. https://doi.org/10.1111/mam.12226

Prescott, S. L. (2017). History of medicine: Origin of the term microbiome and why it matters. *Human Microbiome Journal*, *4*, 24–25. https://doi.org/10.1016/j.humic.2017.05.004

Primavilla, S., Farneti, S., Roila, R., Branciari, R., Altissimi, C., Valiani, A., & Ranucci, D. (2023). Retrospective study on the prevalence of *Yersinia enterocolitica* in food collected in Umbria region (central Italy). *Italian Journal of Food Safety*, *12*(1). https://doi.org/10.4081/ijfs.2023.10996

Proctor, L. M., Creasy, H. H., Fettweis, J. M., Lloyd-Price, J., Mahurkar, A., Zhou, W., Buck, G. A., Snyder, M. P., Strauss, J. F., Weinstock, G. M., White, O., & Huttenhower, C. (2019). The Integrative Human Microbiome Project. *Nature*, *569*(7758), 641–648. https://doi.org/10.1038/s41586-019-1238-8

Puig-Montserrat, X., Torre, I., López-Baucells, A., Guerrieri, E., Monti, M. M., Ràfols-García, R., Ferrer, X., Gisbert, D., & Flaquer, C. (2015). Pest control service provided by bats in Mediterranean rice paddies: Linking agroecosystems structure to ecological functions. *Mammalian Biology*, *80*(3), 237–245. https://doi.org/10.1016/j.mambio.2015.03.008

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., & Glöckner, F. O. (2012). The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Research*, *41*(D1), D590–D596. https://doi.org/10.1093/nar/gks1219

Quince, C., Walker, A. W., Simpson, J. T., Loman, N. J., & Segata, N. (2017). Shotgun metagenomics, from sampling to analysis. In *Nature Biotechnology* (Vol. 35, Issue 9, pp. 833–844). Nature Publishing Group. https://doi.org/10.1038/nbt.3935

R Core Team. (2022). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing. https://www.R-project.org/

Ramamurthy, M., Sankar, S., Kannangai, R., Nandagopal, B., & Sridharan, G. (2017). Application of viromics: a new approach to the understanding of viral infections in humans. *VirusDisease*, *28*(4), 349–359. https://doi.org/10.1007/s13337-017-0415-3

Ramette, A. (2007). Multivariate analyses in microbial ecology. *FEMS Microbiology Ecology*, *62*(2), 142–160. https://doi.org/10.1111/j.1574-6941.2007.00375.x

Ramirez-Francel, L. A., Garcia-Herrera, L. V., Losada-prado, S., Reinoso-flórez, G., Sánchez-hernández, A., Estrada-villegas, S., Lim, B. K., & Guevara, G. (2022). Bats and their vital ecosystem services: a global review. *Integrative Zoology*, *17*, 2–23. https://doi.org/10.1111/1749-4877.12552

Rasweiler IV, J. J., Cretekos, C. J., & Behringer, R. R. (2009). The short-tailed fruit bat *Carollia perspicillata*: a model for studies in reproduction and development. *Cold Spring Harbor Protocols*, *4*(3). https://doi.org/10.1101/pdb.emo118

Reddrop, C., Moldrich, R. X., Beart, P. M., Farso, M., Liberatore, G. T., Howells, D. W., Petersen, K. U., Schleuning, W. D., & Medcalf, R. L. (2005). Vampire bat salivary plasminogen activator (desmoteplase) inhibits tissue-type plasminogen activator-induced potentiation of excitotoxic injury. *Stroke*, *36*(6), 1241–1246. https://doi.org/10.1161/01.STR.0000166050.84056.48

Reichard, J. D., Fellows, S. R., Frank, A. J., & Kunz, T. H. (2010). Thermoregulation during flight: body temperature and sensible heat transfer in free-ranging Brazilian free-tailed bats (*Tadarida brasiliensis*). *Physiological and Biochemical Zoology*, *83*(6), 885–897. https://doi.org/10.1086/657253

Reid, K. C., Cockerill, F. R., & Patel, R. (2001). Clinical and epidemiological features of *Enterococcus casseliflavus/flavescens* and *Enterococcus gallinarum* bacteremia: a report of 20 cases. *Clinical Infectious Diseases*, *32*(11), 1540–1546. https://doi.org/10.1086/320542

Reimer, L. C., Sardà Carbasse, J., Koblitz, J., Ebeling, C., Podstawka, A., & Overmann, J. (2022). *BacDive in 2022: the knowledge base for standardized bacterial and archaeal data*. Nucleic Acids Research. https://bacdive.dsmz.de/

RENAVE. (2013). *Protocolos de la Red Nacional de Vigilancia Epidemiológica*. Centro Nacional de Epidemiología. Instituto de Salud Carlos III.; Ministerio de Economía y Competitividad. Ministerio de Sanidad, Servicios Sociales e Igualdad .

Rex, K., Michener, R., Kunz, T. H., & Voigt, C. C. (2011). Vertical stratification of Neotropical leaf-nosed bats (Chiroptera: *Phyllostomidae*) revealed by stable carbon isotopes. *Journal of Tropical Ecology*, *27*(3), 211–222. https://doi.org/10.1017/S0266467411000022

Reynolds, H. T., & Barton, H. A. (2013). White-nose syndrome: human activity in the emergence of an extirpating mycosis. *Microbiology Spectrum*, *1*(2). https://doi.org/10.1128/microbiolspec.oh-0008-2012

Rigby, E. L., Aegerter, J., Brash, M., & Altringham, J. D. (2012). Impact of PIT tagging on recapture rates, body condition and reproductive success of wild Daubenton's bats (*Myotis daubentonii*). *Veterinary Record*, *170*(4), 101. https://doi.org/10.1136/vr.100075

Risely, A., Waite, D. W., Ujvari, B., Hoye, B. J., & Klaassen, M. (2018). Active migration is associated with specific and consistent changes to gut microbiota in *Calidris* shorebirds. *Journal of Animal Ecology*, *87*(2), 428–437. https://doi.org/10.1111/1365-2656.12784

Robardet, E., Borel, C., Moinet, M., Jouan, D., Wasniewski, M., Barrat, J., Boué, F., Montchâtre-Leroy, E., Servat, A., Gimenez, O., Cliquet, F., & Picard-Meyer, E. (2017). Longitudinal survey of two serotine bat (*Eptesicus serotinus*) maternity colonies exposed to EBLV-1 (European Bat Lyssavirus type 1): assessment of survival and serological status variations using capture-recapture models. *PLoS Neglected Tropical Diseases*, *11*(11). https://doi.org/10.1371/journal.pntd.0006048

Rodríguez-Posada, M. E., & Santa-Sepúlveda, M. A. (2013). Reporte de lesiones en murciélagos causadas por el uso incorrecto de collares plásticos como método de marcaje. *Therya*, *4*(2), 395–400. https://doi.org/10.12933/therya-13-124

Rogers, G. B., Keating, D. J., Young, R. L., Wong, M. L., Licinio, J., & Wesselingh, S. (2016). From gut dysbiosis to altered brain function and mental illness: mechanisms and pathways. In *Molecular Psychiatry* (Vol. 21, Issue 6, pp. 738–748). Nature Publishing Group. https://doi.org/10.1038/mp.2016.50

Rosenberg, E., Delong, E. F., Lory, S., Stackebrandt, E., & Thompson, F. (Eds.). (2006). *The Prokaryotes* (Fourth Edition).

Rosenfeld, C. S. (2017). Gut dysbiosis in animals due to environmental chemical exposures. In *Frontiers in Cellular and Infection Microbiology* (Vol. 7, Issue SEP). Frontiers Media S.A. https://doi.org/10.3389/fcimb.2017.00396

RStudio Team. (2022). *RStudio: Integrated Development Environment for R*. RStudio, PBC. http://www.rstudio.com/

Ruoss, S., Becker, N. I., Otto, M. S., Czirják, G., & Encarnação, J. A. (2019). Effect of sex and reproductive status on the immunity of the temperate bat *Myotis daubentonii*. *Mammalian Biology*, *94*, 120–126. https://doi.org/10.1016/j.mambio.2018.05.010

Russell V, L. (2016). Least-Squares Means: The R Package lsmeans. *Journal of Statistical Software*, *69*(1), 1–33. https://doi.org/10.18637/jss.v069.i01

Russo, D., Salinas-Ramos, V. B., Cistrone, L., Smeraldo, S., Bosso, L., & Ancillotto, L. (2021). Do we need to use bats as bioindicators? *Biology*, *10*(8). https://doi.org/10.3390/biology10080693

Rydell, J., Bach, L., Dubourg-Savage, M. J., Green, M., Rodrigues, L., & Hedenström, A. (2010). Bat mortality at wind turbines in northwestern Europe. *Acta Chiropterologica*, *12*(2), 261–274. https://doi.org/10.3161/150811010X537846

Ryser-Degiorgis, M.-P. (2013). Wildlife health investigations: needs, challenges and recommendations. *BMC Veterinary Research*, *9*(223). http://www.biomedcentral.com/1746-6148/9/223

Sadowsky, M. J., Staley, C., Heiner, C., Hall, R., Kelly, C. R., Brandt, L., & Khoruts, A. (2017). Analysis of gut microbiota: An ever changing landscape. *Gut Microbes*, *8*(3), 268–275. https://doi.org/10.1080/19490976.2016.1277313

Salazar, A. N., Nobrega, F. L., Anyansi, C., Aparicio-Maldonado, C., Costa, A. R., Haagsma, A. C., Hiralal, A., Mahfouz, A., McKenzie, R. E., van Rossum, T., Brouns, S. J. J., & Abeel, T. (2020). An educational guide for nanopore sequencing in the classroom. *PLOS Computational Biology*, *16*(1), e1007314. https://doi.org/10.1371/journal.pcbi.1007314

Samour, J. (Ed.). (2016). *Avian medicine* (3rd ed.). Elsevier.

Sánchez, F., Alvarez, J., Ariza, C., & Cadena, A. (2007). Bat assemblage structure in two dry forests of Colombia: composition, species richness, and relative abundance. *Mammalian Biology*, *72*(2), 82–92. https://doi.org/10.1016/j.mambio.2006.08.003

Sanger, F., Nicklen, S., & Coulson, A. R. (1977). DNA sequencing with chain-terminating inhibitors. *Proceedings of the National Academy of Sciences of the United States of America*, *74*(12), 5463–5467. https://doi.org/10.1073/pnas.74.12.5463

Sangster, C. R., Gordon, A. N., & Hayes, D. (2012). Systemic toxoplasmosis in captive flying-foxes. *Aust. Vet. J.*, *90*, 140–142.

Saputra, F., Sulsia, N., Dwi Agustin, A. L., & Kholik. (2018, November). Isolation and identification of gram-negative bacterial pathogens of bat guano from Liang Bukal and Liang Petang cave on Sumbawa Island. *Proc. of the 20thFAVA CONGRESS & The 15thKIVNAS PDHI*. https://www.researchgate.net/publication/339753902

Scheele, B. C., Pasmans, F., Skerratt, L. F., Berger, L., Martel, A., Beukema, W., Acevedo, A. A., Burrowes, P. A., Carvalho, T., Catenazzi, A., De la Riva, I., Fisher, M. C., Flechas, S. V, Foster, C. N., Frías-Álvarez, P., J Garner, T. W.,

Gratwicke, B., Guayasamin, J. M., Hirschfeld, M., Kolby, J. E., Kosch, T. A., La Marca, E., Lindenmayer, D. B., Lips, K. R., Longo, A. V., Maneyro, R., McDonald, C. A., Mendelson, J. 3rd, Palacios-Rodriguez, P., Parra-Olea, G., Richards-Zawacki, C. L., Rödel, M. O., Rovito, S. M. , Soto-Azat, C., Toledo, L. F., Voyles, J., Weldon, C., Whitfield, S. M., Wilkinson, M., Zamudio, K. R., & Canessa, S. (2019). Amphibian fungal panzootic causes catastrophic and ongoing loss of biodiversity. *Science*, *363*, 1459–1463. https://www.science.org

Schoch, C. L., Ciufo, S., Domrachev, M., Hotton, C. L., Kannan, S., Khovanskaya, R., Leipe, D., Mcveigh, R., O'Neill, K., Robbertse, B., Sharma, S., Soussov, V., P Sullivan, J., Sun, L., Turner, S., & Karsch-Mizrachi, I. (2020). *NCBI Taxonomy: a comprehensive update on curation, resources and tools. The order Burkholderiales*. Database (Oxford). https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Tree&id=80840&lvl=3&keep=1&srchmode=1&unlock

Schorcht, W., Bontadina, F., & Schaub, M. (2009). Variation of adult survival drives population dynamics in a migrating forest bat. *Journal of Animal Ecology*, *78*(6), 1182–1190. https://doi.org/10.1111/j.1365-2656.2009.01577.x

Schultz, G. S., Chin, G. A., Moldawer, L., & Diegelmann, R. F. (2011). Principles of wound healing. In *Mechanisms of Vascular Disease: A Reference Book for Vascular Specialists* (Vol. 23). University of Adelaide Press.

Segata, N., Izard, J., Waldron, L., Gevers, D., Miropolsky, L., Garrett, W. S., & Huttenhower, C. (2011). Metagenomic biomarker discovery and explanation. *Genome Biology*, *12*(6), R60. https://doi.org/10.1186/gb-2011-12-6-r60

Segers, J., Mcburney, S., Jones, M., & Zimmer, P. (2021). *The Canadian Wildlife Health Cooperative National Bat Health Report-2021*. www.cwhc-rcsf.ca

Seibold, S., Gossner, M. M., Simons, N. K., Blüthgen, N., Müller, J., Ambarlı, D., Ammer, C., Bauhus, J., Fischer, M., Habel, J. C., Linsenmair, K. E., Nauss, T., Penone, C., Prati, D., Schall, P., Schulze, E. D., Vogt, J., Wöllauer, S., & Weisser, W. W. (2019). Arthropod decline in grasslands and forests is associated with landscape-level drivers. *Nature*, *574*(7780), 671–674. https://doi.org/10.1038/s41586-019-1684-3

Selvin, J., Lanong, S., Syiem, D., De Mandal, S., Kayang, H., Kumar, N. S., & Kiran, G. S. (2019). Culture-dependent and metagenomic analysis of lesser horseshoe bats' gut microbiome revealing unique bacterial diversity and

signatures of potential human pathogens. *Microbial Pathogenesis*, *137*. https://doi.org/10.1016/j.micpath.2019.103675

Sender, R., Fuchs, S., & Milo, R. (2016). Revised estimates for the number of human and bacteria cells in the body. *PLoS Biology*, *14*(8). https://doi.org/10.1371/journal.pbio.1002533

Sendor, T., & Simon, M. (2003). Population dynamics of the pipistrelle bat: effects of sex, age and winter weather on seasonal survival. *Journal of Animal Ecology*, *72*(2), 308–320. https://doi.org/10.1046/j.1365-2656.2003.00702.x

Seo, J. S., Keum, Y. S., & Li, Q. X. (2009). Bacterial degradation of aromatic compounds. In *International Journal of Environmental Research and Public Health* (Vol. 6, Issue 1, pp. 278–309). MDPI. https://doi.org/10.3390/ijerph6010278

Serra-Gonçalves, C., López-Baucells, A., & Rocha, R. (2017). Opportunistic predation of a silky short-tailed bat (*Carollia brevicauda*) by a tawny-bellied screech-owl (*Megascops watsonii*), with a compilation of predation events upon bats entangled in mist-nets. *Barbastella*, *10*(1). https://doi.org/10.14709/barbj.10.1.2017.07

Shahack-Gross, R., Berna, F., Karkanas, P., & Weiner, S. (2004). Bat guano and preservation of archaeological remains in cave sites. *Journal of Archaeological Science*, *31*(9), 1259–1272. https://doi.org/10.1016/j.jas.2004.02.004

Sharma, M., Li, Y., Stoll, M. L., & Tollefsbol, T. O. (2020). The Epigenetic Connection Between the Gut Microbiome in Obesity and Diabetes. In *Frontiers in Genetics* (Vol. 10). Frontiers Media S.A. https://doi.org/10.3389/fgene.2019.01329

Sheherazade, Ober, H. K., & Tsang, S. M. (2019). Contributions of bats to the local economy through durian pollination in Sulawesi, Indonesia. *Biotropica*, *51*(6), 913–922. https://doi.org/10.1111/btp.12712

Shenbrot, G., Krasnov, B., & Burdelov, S. (2010). Long-term study of population dynamics and habitat selection of rodents in the Negev Desert. *Journal of Mammalogy*, *91*(4), 776–786. https://doi.org/10.1644/09-MAMM-S-162.1

Sherwin, H. A., Montgomery, W. I., & Lundy, M. G. (2013). The impact and implications of climate change for bats. In *Mammal Review* (Vol. 43, Issue 3, pp. 171–182). Blackwell Publishing Ltd. https://doi.org/10.1111/j.1365-2907.2012.00214.x

Sieradzki, A., & Mikkola, H. (2022). Bats in folklore and culture: A review of historical perceptions around the world. In *Bats: Disease-Prone but Beneficial*. IntechOpen. https://doi.org/10.5772/intechopen.102368

Silby, M. W., Winstanley, C., Godfrey, S. A. C., Levy, S. B., & Jackson, R. W. (2011). Pseudomonas genomes: Diverse and adaptable. *FEMS Microbiology Reviews*, *35*(4), 652–680. https://doi.org/10.1111/j.1574-6976.2011.00269.x

Simmons, N. B., & Cirranello, A. L. (2023). *Bat species of the world: a taxonomic and geographic database*. https://batnames.org/

Simmons, N. B., Seymour, K. L., Habersetzer, J., & Gunnell, G. F. (2008). Primitive Early Eocene bat from Wyoming and the evolution of flight and echolocation. *Nature*, *451*(7180), 818–821. https://doi.org/10.1038/nature06549

Simpson, V. R., Borman, A. M., Fox, R. I., & Mathews, F. (2013). Cutaneous mycosis in a Barbastelle bat (*Barbastella barbastellus*) caused by *Hyphopichia burtonii*. *Journal of Veterinary Diagnostic Investigation*, *25*(4), 551–554. https://doi.org/10.1177/1040638713493780

Smits, S. A., Leach, J., Sonnenburg, E. D., Gonzalez, C. G., Lichtman, J. S., Reid, G., Knight, R., Manjurano, A., Changalucha, J., Elias, J. E., Dominguez-Bello, M. G., & Sonnenburg, J. L. (2017). Seasonal cycling in the gut microbiome of the Hadza hunter-gatherers of Tanzania. *Science*, *357*(6353), 802–806. https://doi.org/10.1126/science.aan4834

Song, S. J., Sanders, J. G., Delsuc, F., Metcalf, J., Amato, K., Taylor, M. W., Mazel, F., Lutz, H. L., Winker, K., Graves, G. R., Humphrey, G., Gilbert, J. A., Hackett, S. J., White, K. P., Skeen, H. R., Kurtis, S. M., Withrow, J., Braile, T., Miller, M., McCracken, K. G., Maley, J. M., Ezenwa, V. O., Williams, A., Blanton, J. M., McKenzie, V. J., & Knight, R. (2020). Comparative analyses of vertebrate gut microbiomes reveal convergence between birds and bats. *MBio*, *11*(1). https://doi.org/10.1128/mBio.02901-19

Sonnenburg, J. L., & Bäckhed, F. (2016). Diet-microbiota interactions as moderators of human metabolism. In *Nature* (Vol. 535, Issue 7610, pp. 56–64). Nature Publishing Group. https://doi.org/10.1038/nature18846

Sothearen, T., M. FUrey, N., & A. Jurgens, J. (2014). Effect of bat guano on the growth of five economically important plant species. *Journal of Tropical Agriculture*, *52*(2), 169–173.

Soulsbury, C. D., Gray, H. E., Smith, L. M., Braithwaite, V., Cotter, S. C., Elwood, R. W., Wilkinson, A., & Collins, L. M. (2020). The welfare and ethics of research

involving wild animals: A primer. *Methods in Ecology and Evolution*, *11*(10), 1164–1181. https://doi.org/10.1111/2041-210X.13435

Souto, E. P. F., Oliveira, A. M., Hoffmann, A. R., Mota, R. A., Galiza, G. J. N., & Dantas, A. F. M. (2023). Cutaneous phaeohyphomycosis in a greater bulldog bat (*Noctilio leporinus*) in northeastern Brazil. *Journal of Comparative Pathology*, *201*, 37–40. https://doi.org/10.1016/j.jcpa.2022.12.013

Spoelstra, K., van Grunsven, R. H. A., Ramakers, J. J. C., Ferguson, K. B., Raap, T., Donners, M., Veenendaal, E. M., & Visser, M. E. (2017). Response of bats to light with different spectra: light-shy and agile bat presence is affected by white and green, but not red light. *Proceedings of the Royal Society B: Biological Sciences*, *284*(1855). https://doi.org/10.1098/rspb.2017.0075

Spotswood, E. N., Goodman, K. R., Carlisle, J., Cormier, R. L., Humple, D. L., Rousseau, J., Guers, S. L., & Barton, G. G. (2012). How safe is mist netting? Evaluating the risk of injury and mortality to birds. *Methods in Ecology and Evolution*, *3*(1), 29–38. https://doi.org/10.1111/j.2041-210X.2011.00123.x

Stahlschmidt, P., Hahn, M., & Brühl, C. A. (2017). Nocturnal risks-high bat activity in the agricultural landscape indicates potential pesticide exposure. *Frontiers in Environmental Science*, *5*(OCT). https://doi.org/10.3389/fenvs.2017.00062

Stebbings, R. (2004). Ringing and marking. In A. Mitchell-Jones & A. McLeish (Eds.), *Bat Workers' Manual* (3d ed.). Joint Nature Conservation Committee.

Steffens, R., Zöphel, U., Brockmann, D., & Depture of Nature Landscape Soil. (2007). *40th Anniversary Bat Marking Centre Dresden-Evaluation of methods and overview of results*. www.umwelt.sachsen.de/lfug

Stone, E. L., Harris, S., & Jones, G. (2015). Impacts of artificial lighting on bats: A review of challenges and solutions. In *Mammalian Biology* (Vol. 80, Issue 3, pp. 213–219). Elsevier GmbH. https://doi.org/10.1016/j.mambio.2015.02.004

Straka, T. M., Greif, S., Schultz, S., Goerlitz, H. R., & Voigt, C. C. (2020). The effect of cave illumination on bats. *Global Ecology and Conservation*, *21*. https://doi.org/10.1016/j.gecco.2019.e00808

Streicker, D. G., & Gilbert, A. T. (2020). Contextualizing bats as viral reservoirs. *Science*, *370*(6513), 172–173. https://doi.org/10.1126/science.abd4559

Szentivanyi, T., McKee, C., Jones, G., & Foster, J. T. (2023). Trends in bacterial pathogens of bats: global distribution and knowledge gaps. *Transboundary and Emerging Diseases*, *2023*, 1–17. https://doi.org/10.1155/2023/9285855

Tallo-Parra, O., Manteca, X., Sabes-Alsina, M., Carbajal, A., & Lopez-Bejar, M. (2014). Hair cortisol detection in dairy cattle by using EIA: Protocol validation and correlation with faecal cortisol metabolites. *Animal*, *9*(6), 1059–1064. https://doi.org/10.1017/S1751731115000294

Tasnim, N., Abulizi, N., Pither, J., Hart, M. M., & Gibson, D. L. (2017). Linking the gut microbial ecosystem with the environment: does gut health depend on where we live? *Frontiers in Microbiology*, *8*. https://doi.org/10.3389/fmicb.2017.01935

Teeling, E. C., Madsen, O., Van Den Bussche, R. A., de Jong, W. W., Stanhope, M. J., & Springer, M. S. (2002). Microbat paraphyly and the convergent evolution of a key innovation in Old World rhinolophoid microbats. *Proceedings of the National Academy of Sciences*, *99*(3), 1431–1436. https://doi.org/10.1073/pnas.022477199

Terrón-Camero, L. C., Gordillo-González, F., Salas-Espejo, E., & Andrés-León, E. (2022). Comparison of metagenomics and metatranscriptomics tools: a guide to making the right choice. *Genes*, *13*(12), 2280. https://doi.org/10.3390/genes13122280

The Human Microbiome Project Consortium. (2012). Structure, function and diversity of the healthy human microbiome. *Nature*, *486*(7402), 207–214. https://doi.org/10.1038/nature11234

Thomas, C. D., Cameron, A., Green, R. E., Bakkenes, M., Beaumont, L. J., Collingham, Y. C., Erasmus, B. F. N., Ferreira De Siqueira, M., Grainger, A., Hannah, L., Hughes, L., Huntley, B., van Jaarsveld, A. S., Midgley, G. F., Miles, L., Ortega-Huerta, M. A., Peterson, A. T., Phillips, O. L., & Williams, S. E. (2004). Extinction risk from climate change. *Nature*. www.nature.com/nature

Thomas, S., Ingersoll, T., Jernigan, J., & Moore, B. (2016). *A Protocol for monitoring bats at cave roosts in the Cumberland Piedmont Network - Version 2.0*.

Torquetti, C. G., Guimarães, A. T. B., & Soto-Blanco, B. (2021). Exposure to pesticides in bats. *Science of The Total Environment*, *755*, 142509. https://doi.org/10.1016/j.scitotenv.2020.142509

Trejo-Salazar, R. E., Eguiarte, L. E., Suro-Piñera, D., & Medellin, R. A. (2016). Save our bats, save our tequila: Industry and science join forces to help bats and agaves. *Natural Areas Journal*, *36*(4), 523–530. https://doi.org/10.3375/043.036.0417

Tschapka, M., Gonzalez-Terrazas, T. P., & Knörnschild, M. (2015). Nectar uptake in bats using a pumping-tongue mechanism. *Science Advances*, *1*(8). https://doi.org/10.1126/sciadv.1500525

Tucker, T., Marra, M., & Friedman, J. M. (2009). Massively parallel sequencing: the next big thing in genetic medicine. In *American Journal of Human Genetics* (Vol. 85, Issue 2, pp. 142–154). https://doi.org/10.1016/j.ajhg.2009.06.022

Turnbaugh, P. J., Ley, R. E., Hamady, M., Fraser-Liggett, C. M., Knight, R., & Gordon, J. I. (2007). The human microbiome project. In *Nature* (Vol. 449, Issue 7164, pp. 804–810). Nature Publishing Group. https://doi.org/10.1038/nature06244

Tuttle, M. (2019, September 14). *How I photograph bats*. Merlin Tuttle's Bat Conservation. https://www.merlintuttle.org/how-i-photograph-bats/

Uhler, J., Redlich, S., Zhang, J., Hothorn, T., Tobisch, C., Ewald, J., Thorn, S., Seibold, S., Mitesser, O., Morinière, J., Bozicevic, V., Benjamin, C. S., Englmeier, J., Fricke, U., Ganuza, C., Haensel, M., Riebl, R., Rojas-Botero, S., Rummler, T., Uphus, L., Schmidt, S., Steffan-Dewenter, I., & Müller, J. (2021). Relationship of insect biomass and richness with land use along a climate gradient. *Nature Communications*, *12*(1), 5946. https://doi.org/10.1038/s41467-021-26181-3

UNEP/EUROBATS. (2002). *2nd Amendment to the Agreement on the conservation of bats in Europe: Vol. Treaty Series No. 49*.

UNEP/EUROBATS. (2003). *4th Session of the meeting of parties. Resolution No. 4.6 Guidelines for the issue of permits for the capture and study of captured wild bats*.

UNEP/EUROBATS. (2018). *Record of the 23rd meeting of the Advisory Committee*.

Urushadze, L., Bai, Y., Osikowicz, L., McKee, C., Sidamonidze, K., Putkaradze, D., Imnadze, P., Kandaurov, A., Kuzmin, I., & Kosoy, M. (2017). Prevalence, diversity, and host associations of *Bartonella* strains in bats from Georgia (Caucasus). *PLoS Neglected Tropical Diseases*, *11*(4). https://doi.org/10.1371/journal.pntd.0005428

U.S. Fish and Wildlife Service. (2024). *White-Nose Syndrome response team*. https://whitenosesyndrome.org/

Van Brussel, K., & Holmes, E. C. (2022). Zoonotic disease and virome diversity in bats. In *Current Opinion in Virology* (Vol. 52, pp. 192–202). Elsevier B.V. https://doi.org/10.1016/j.coviro.2021.12.008

Van Brussel, K., Mahar, J. E., Hall, J., Bender, H., Ortiz-Baez, A. S., Chang, W.-S., Holmes, E. C., & Rose, K. (2023). Gammaretroviruses, novel viruses and pathogenic bacteria in Australian bats with neurological signs, pneumonia and skin lesions. *Virology*. https://doi.org/10.1016/j.virol.2023.07.011

van der Sluijs, J. P. (2020). Insect decline, an emerging global environmental risk. In *Current Opinion in Environmental Sustainability* (Vol. 46, pp. 39–42). https://doi.org/10.1016/j.cosust.2013.05.007

van Harten, E., Lawrence, R., Lumsden, L. F., Reardon, T., & Prowse, T. A. A. (2022). Novel passive detection approach reveals low breeding season survival and apparent lactation cost in a critically endangered cave bat. *Scientific Reports*, *12*(1). https://doi.org/10.1038/s41598-022-11404-4

van Harten, E., Reardon, T., Holz, P. H., Lawrence, R., Prowse, T. A. A., & Lumsden, L. F. (2020). Recovery of southern bent-winged bats (*Miniopterus orianae bassanii*) after PIT-tagging and the use of surgical adhesive. *Australian Mammalogy*, *42*(2), 216–219. https://doi.org/10.1071/AM19024

van Harten, E., Reardon, T., Lumsden, L. F., Meyers, N., Prowse, T. A. A., Weyland, J., & Lawrence, R. (2019). High detectability with low impact: optimizing large PIT tracking systems for cave-dwelling bats. *Ecology and Evolution*, *9*(19), 10916–10928. https://doi.org/10.1002/ece3.5482

Vanderelst, D., de Mey, F., Peremans, H., Geipel, I., Kalko, E., & Firzlaff, U. (2010). What noseleaves do for FM bats depends on their degree of sensorial specialization. *PLoS ONE*, *5*(8). https://doi.org/10.1371/journal.pone.0011893

Vanderwolf, K. J., Campbell, L. J., Goldberg, T. L., Blehert, D. S., & Lorch, J. M. (2021). Skin fungal assemblages of bats vary based on susceptibility to white-nose syndrome. *ISME Journal*, *15*(3), 909–920. https://doi.org/10.1038/s41396-020-00821-w

Vaughan, T. A. (1970). Adaptations for flight in bats. *Fondren Science Series*, *1*(11), 127–143. https://scholar.smu.edu/fondrenscienceseries/vol1/iss11/8

Veikkolainen, V., Vesterinen, E. J., Lilley, T. M., & Pulliainen, A. T. (2014). Bats as reservoir hosts of human bacterial pathogen, *Bartonella mayotimonensis*. *Emerging Infectious Diseases*, *20*(6), 960–967. https://doi.org/10.3201/eid2006.130956

Veith, M. (1996). Qualitative und quantitative Veränderungeneiner Lebensgemeinschaft überwinternder Fledermäuse (Mammalia, Chiroptera): Ergebnisse von sechs Jahr-zehnten Erfassung. *Fauna Og Flora* , *21*, 95–105.

Vela, A. I., Fernández, A., Moreno, B., Casamayor, A., Chacón, G., Villa, A., Comenge, J., & Fernández-Garayzábal, J. F. (2010). Isolation of *Enterococcus hirae* from suckling rabbits with diarrhoea. *Veterinary Record*, *167*(9), 345–346. https://doi.org/10.1136/vr.c4460

Vengust, M., Knapic, T., & Weese, J. S. (2018). The fecal bacterial microbiota of bats; Slovenia. *PLoS ONE*, *13*(5). https://doi.org/10.1371/journal.pone.0196728

Víquez-R, L., Speer, K., Wilhelm, K., Simmons, N., Medellín, R. A., Sommer, S., & Tschapka, M. (2021). A faithful gut: core features of gastrointestinal microbiota of long-distance migratory bats remain stable despite dietary shifts driving differences in specific bacterial taxa. *Microbiology Spectrum*, *9*(3). https://doi.org/10.1128/spectrum.01525-21

Voigt, C. C. (2013). Bat flight with bad wings: is flight metabolism affected by damaged wings? *Journal of Experimental Biology*, *216*(8), 1516–1521. https://doi.org/10.1242/jeb.079509

Voigt, C. C., & Kingston, T. (2015). Bats in the Anthropocene: conservation of bats in a changing world. In *Bats in the Anthropocene: Conservation of Bats in a Changing World*. Springer International Publishing. https://doi.org/10.1007/978-3-319-25220-9

Wandera, J. (2018). *Assessment of the people's knowledge, attitude and practices regarding bats and health risks associated with exposure. Case study of Bananywa subcounty in Kyankwanzi district, Uganda.* . Makerere University.

Wang, B., Yao, M., Lv, L., Ling, Z., & Li, L. (2017). The Human Microbiota in Health and Disease. In *Engineering* (Vol. 3, Issue 1, pp. 71–82). Elsevier Ltd. https://doi.org/10.1016/J.ENG.2017.01.008

Wang, G., Yue, W., Liu, Y., Li, F., Xiong, M., & Zhang, H. (2013). Biodegradation of the neonicotinoid insecticide Acetamiprid by bacterium *Pigmentiphaga* sp. strain AAP-1 isolated from soil. *Bioresource Technology*, *138*, 359–368. https://doi.org/10.1016/j.biortech.2013.03.193

Wang, H., Zhou, S., Wang, Y., Kong, D., Guo, X., Zhu, J., Dong, W., & Ruan, Z. (2016). *Advenella alkanexedens* sp. nov., an alkane-degrading bacterium isolated from biogas slurry samples. *International Journal of Systematic and Evolutionary Microbiology*, *66*(2), 906–911. https://doi.org/10.1099/ijsem.0.000811

Wang, Y., Zhao, Y., Bollas, A., Wang, Y., & Au, K. F. (2021). Nanopore sequencing technology, bioinformatics and applications. In *Nature Biotechnology* (Vol.

39, Issue 11, pp. 1348–1365). Nature Research. https://doi.org/10.1038/s41587-021-01108-x

Wanger, T. C., Darras, K., Bumrungsri, S., Tscharntke, T., & Klein, A. M. (2014). Bat pest control contributes to food security in Thailand. *Biological Conservation*, *171*, 220–223. https://doi.org/10.1016/j.biocon.2014.01.030

Warmuth, V. M., Metzler, D., & Zamora-Gutierrez, V. (2023). Human disturbance increases coronavirus prevalence in bats. *Science Advances*, *9*(eadd0688). https://www.science.org

Wasimuddin, Brändel, S. D., Tschapka, M., Page, R., Rasche, A., Corman, V. M., Drosten, C., & Sommer, S. (2018). Astrovirus infections induce age-dependent dysbiosis in gut microbiomes of bats. *ISME Journal*, *12*(12), 2883–2893. https://doi.org/10.1038/s41396-018-0239-1

Webb, P. I., Speakman, J. R., & Racey, P. A. (1995). Evaporative water loss in two sympatric species of vespertilionid bat, *Plecotus auritus* and *Myotis daubentoni*: relation to foraging mode and implications for roost site selection. *J. Zool*, *235*, 269–278.

Weier, S. M., Grass, I., Linden, V. M. G., Tscharntke, T., & Taylor, P. J. (2018). Natural vegetation and bug abundance promote insectivorous bat activity in macadamia orchards, South Africa. *Biological Conservation*, *226*, 16–23. https://doi.org/10.1016/j.biocon.2018.07.017

Welbergen, J. A., Klose, S. M., Markus, N., & Eby, P. (2008). Climate change and the effects of temperature extremes on Australian flying-foxes. *Proceedings of the Royal Society B: Biological Sciences*, *275*(1633), 419–425. https://doi.org/10.1098/rspb.2007.1385

White, R. J., & Razgour, O. (2020). Emerging zoonotic diseases originating in mammals: a systematic review of effects of anthropogenic land-use change. In *Mammal Review* (Vol. 50, Issue 4, pp. 336–352). Blackwell Publishing Ltd. https://doi.org/10.1111/mam.12201

Wick, R. R., Judd, L. M., Gorrie, C. L., & Holt, K. E. (2017). Completing bacterial genome assemblies with multiplex MinION sequencing. *Microbial Genomics*, *3*(10). https://doi.org/10.1099/mgen.0.000132

Wickham, H. (2016). *ggplot2: Elegant Graphics for Data Analysis*. Springer-Verlag. https://ggplot2.tidyverse.org

Wickham, H., Averick, M., Bryan, J., Chang, W., McGowan, L., François, R., Grolemund, G., Hayes, A., Henry, L., Hester, J., Kuhn, M., Pedersen, T., Miller, E., Bache, S., Müller, K., Ooms, J., Robinson, D., Seidel, D., Spinu, V., Takahashi, K., Vaughan, D., Wilke, C., Woo, K., & Yutani, H. (2019). Welcome

to the tidyverse. *Journal of Open Source Software*, *4*(43). https://doi.org/10.21105/joss.01686

Winglee, K., Green Howard, A., Sha, W., Gharaibeh, R. Z., Liu, J., Jin, D., Fodor, A. A., & Gordon-Larsen, P. (2017). Recent urbanization in China is correlated with a Westernized microbiome encoding increased virulence and antibiotic resistance genes. *Microbiome*, *5*(121), 1–13. https://doi.org/10.1186/s40168-017-0338-7

Winnenburg, R. (2006). PHI-base: a new database for pathogen host interactions. *Nucleic Acids Research*, *34*(90001), D459–D464. https://doi.org/10.1093/nar/gkj047

WOAH. (2020). *World Organisation for Animal Health listed diseases*. Animal Health Surveillance. www.animalhealthsurveillance.agriculture.gov.ie/oielisteddiseases/

WOAH. (2023). *Animal health and welfare*. https://www.woah.org/en/what-we-do/animal-health-and-welfare/animal-welfare/

Wolkers-Rooijackers, J. C. M., Rebmann, K., Bosch, T., & Hazeleger, W. C. (2018). Fecal bacterial communities in insectivorous bats from the Netherlands and their tole as a possible vector for foodborne diseases. *Acta Chiropterologica*, *20*(2), 475–483. https://doi.org/10.3161/15081109ACC2018.20.2.017

Wood, D. E., Lu, J., & Langmead, B. (2019). Improved metagenomic analysis with Kraken 2. *Genome Biology*, *20*(1), 257. https://doi.org/10.1186/s13059-019-1891-0

World Animal Health, The center for food security & public health, Institute for International Cooperation in Animal Biologics, Iowa State University, & U.S. Department of Agriculture. (2015). *Contagious Equine Metritis* (pp. 1–5). www.cfsph.iastate.edu

World Health Organization, UNEP, & Convention on Biological Diversity. (2015). *Connecting global priorities: biodiversity and human health. A state of knowledge review*.

Wünschmann, A., Wellehan, J. F. X., Armien, A., Bemrick, W. J., Barnes, D., Averbeck, G. A., Roback, R., Schwabenlander, M., D'almeida, E., Joki, R., Childress, A. L., Cortinas, R., Gardiner, C. H., & Greiner, E. C. (2010). Renal infection by a new coccidian genus in Big Brown bats (*Eptesicus fuscus*). *Journal of Parasitology*, *96*(1), 178–183. https://doi.org/10.1645/GE-2250.1

Xiao, G., Liu, S., Xiao, Y., Zhu, Y., Zhao, H., Li, A., Li, Z., & Feng, J. (2019). Seasonal changes in gut microbiota diversity and composition in the greater

horseshoe bat. *Frontiers in Microbiology*, *10*. https://doi.org/10.3389/fmicb.2019.02247

Xiao, Y., Xiao, G., Liu, H., Zhao, X., Sun, C., Tan, X., Sun, K., Liu, S., & Feng, J. (2019). Captivity causes taxonomic and functional convergence of gut microbial communities in bats. *PeerJ*, *2019*(4). https://doi.org/10.7717/peerj.6844

Xu, Z., Shen, F., Li, X., Wu, Y., Chen, Q., Jie, X., & Yao, M. (2012). Molecular and microscopic analysis of bacteria and viruses in exhaled breath collected using a simple impaction and condensing method. *PLoS ONE*, *7*(7), e41137. https://doi.org/10.1371/journal.pone.0041137

Yang, L., & Chen, J. (2022). A comprehensive evaluation of microbial differential abundance analysis methods: current status and potential solutions. *Microbiome*, *10*(1). https://doi.org/10.1186/s40168-022-01320-0

Yarlagadda, K., Razik, I., Malhi, R. S., & Carter, G. G. (2021). Social convergence of gut microbiomes in vampire bats. *Biology Letters*, *17*(11). https://doi.org/10.1098/rsbl.2021.0389

Yatsunenko, T., Rey, F. E., Manary, M. J., Trehan, I., Dominguez-Bello, M. G., Contreras, M., Magris, M., Hidalgo, G., Baldassano, R. N., Anokhin, A. P., Heath, A. C., Warner, B., Reeder, J., Kuczynski, J., Caporaso, J. G., Lozupone, C. A., Lauber, C., Clemente, J. C., Knights, D., … Gordon, J. I. (2012). Human gut microbiome viewed across age and geography. In *Nature* (Vol. 486, Issue 7402, pp. 222–227). https://doi.org/10.1038/nature11053

Yin, Z., Sun, K., Li, A., Sun, D., Li, Z., Xiao, G., & Feng, J. (2020). Changes in the gut microbiota during Asian particolored bat (*Vespertilio sinensis*) development. *PeerJ*, *2020*(3). https://doi.org/10.7717/peerj.9003

Yoon, J. H., Kang, S. J., Kim, W., & Oh, T. K. (2007). *Pigmentiphaga daeguensis* sp. nov., isolated from wastewater of a dye works, and emended description of the genus *Pigmentiphaga*. *International Journal of Systematic and Evolutionary Microbiology*, *57*(6), 1188–1191. https://doi.org/10.1099/ijs.0.64901-0

Yu, J., Song, Y., Ren, Y., Qing, Y., Liu, W., & Sun, Z. (2017). Genome-level comparisons provide insight into the phylogeny and metabolic diversity of species within the genus Lactococcus. *BMC Microbiology*, *17*(1). https://doi.org/10.1186/s12866-017-1120-5

Zambelli, N., Moretti, M., Mattei-Roesli, M., & Bontadina, F. (2009). Negative consequences of forearm bands that are too small for bats. *Acta Chiropterologica*, *11*(1), 216–219. https://doi.org/10.3161/150811009X465857

Zha, H., Liu, F., Ling, Z., Chang, K., Yang, J., & Li, L. (2021). Multiple bacteria associated with the more dysbiotic genitourinary microbiomes in patients with type 2 diabetes mellitus. *Scientific Reports*, *11*(1), 1824. https://doi.org/10.1038/s41598-021-81507-x

Zhang, G. K., Chain, F. J. J., Abbott, C. L., & Cristescu, M. E. (2018). Metabarcoding using multiplexed markers increases species detection in complex zooplankton communities. *Evolutionary Applications*, *11*(10), 1901–1914. https://doi.org/10.1111/eva.12694

Zhang, J., Hao, Y., Wang, Z., & Yang, Q. (2022). Diagnosis of *Coxiella burnetii* infection via metagenomic next-generation sequencing: a case report. *BMC Infectious Diseases*, *22*(1). https://doi.org/10.1186/s12879-022-07309-2

Zheng, D., Liwinski, T., & Elinav, E. (2020). Interaction between microbiota and immunity in health and disease. *Nature Cell Research*, *30*, 492–506. https://doi.org/10.1038/s41422-020-0332-7

Zubaid, A., McCracken, G. F., & Kunz, T. H. (Eds.). (2006). *Functional and evolutionary ecology of bats*. Oxford Unversity Press, Inc.

Zuo, T., Kamm, M. A., Colombel, J. F., & Ng, S. C. (2018). Urbanization and the gut microbiota in health and inflammatory bowel disease. In *Nature Reviews Gastroenterology and Hepatology* (Vol. 15, Issue 7, pp. 440–452). Nature Publishing Group. https://doi.org/10.1038/s41575-018-0003-z

Annex

Table A1. Classification of bat species present in Catalonia by their legal and specific protection (LSP). Common names are given in Catalan. 1= species protected by law in Catalonia ("Decret legislatiu 2/2008, de 15 d'abril, Llei de protecció dels animals"; "Llei estatal 42/2007, de 13 de desembre"; and "Real Decreto 139/2011: Lista y Catálogo Nacional de especies amenazadas"). 2= species classified as vulnerable in Catalonia; 3= species classified as critically endangered in Catalonia.

Family	Species	Common name	LSP
Molossidae	*Tadarida teniotis*	Ratpeant cuallarg	1
Rhinolophydae	*Rhinolophus euryale*	Ratpenat de ferradura mediterrani	1, 3
	Rhinolophus ferrumequinum	Ratpenat de ferradura gran	1, 2
	Rhinolophus hipposideros	Ratpenat de ferradura petit	1
	Rhinolophus mehelyi	Ratpenat de ferradura mitjà	1
Vespertilionidae	*Barbastella barbastellus*	Ratpenat de bosc	1, 2
	Eptesicus serotinus	Ratpenat dels graners	1
	Hypsugo savii	Ratpenat muntanyenc	1
	Miniopterus schreibersii	Ratpenat de cova	1, 2
	Myotis alcathoe	Ratpenat de bigotis petit	1, 3
	Myotis bechsteinii	Ratpenat de Bechstein	1, 3
	Myotis blythii	Ratpenat de mussel agut	1, 2
	Myotis capaccinii	Ratpenat de peus grans	1, 3
	Myotis crypticus	Ratpenat gris itàlic	1
	Myotis daubentonii	Ratpenat d'aigua	1
	Myotis emarginatus	Ratpenat d'orelles dentades	1, 2
	Myotis escalerai	Ratpenat de doble serrell	1
	Myotis myotis	Ratpenat de mussel llarg	1, 2
	Myotis mystacinus	Ratpenat de bigotis	1, 2
	Nyctalus lasiopterus	Nòctul gegant	1, 2
	Nyctalus leisleri	Nòctul petit	1
	Nyctalus noctula	Nòctul gros	1, 2
	Pipistrellus kuhlii	Ratpenat de vores clares	1
	Pipistrellus nathusii	Ratpenat de Nathusius	1, 2
	Pipistrellus pipistrellus	Ratpenat comú	1
	Pipistrellus pygmaeus	Ratpenat soprano	1
	Plecotus auritus	Ratpenat orellut septentrional	1, 2
	Plecotus austriacus	Ratpenat orellut meridional	1
	Plecotus macrobullaris	Ratpenat orellut alpí	1, 2
	Vespertilio murinus	Ratpenat aregentat	1, 2

Table A2. Summary of causes of mortality, histopathological and aetiological findings in bat species worldwide. Only aetiologies for which pathology or death events have been associated are included in this table. Note that many more metazoan, bacterial species and virus (as well as ectoparasites) have been described in chiropteran species, but pathological processes associated with them have not been investigated.

Aetiology	Bat species	Pathology/Clinical signs	Country/State	Reference
Bacterial				
Aerococcus viridans	*Eptesicus nilssoni*	Enteritis	Germany	(Mühldorfer et al., 2011)
Bacillus spp.	not specified (European bat)	Pneumonia	Germany	(Mühldorfer et al., 2011)
Borrelia spp. (relapsing fever spirochete)	*Pipistrellus* spp.	Fatal hepatitis and septicemia	England (UK)	(Evans et al., 2009)
Cedecea davisae	*Myotis daubentonii*	Interstitial pneumonia	Germany	(Mühldorfer et al., 2011)
Clostridium perfringens	*Nyctalus noctula*	Haemorrhagic enteritis	Czech Republic	(Hajkova & Pikula, 2007)
Clostridium sordellii	not specified (European bat)	Haemorrhagic enteritis	Germany	(Mühldorfer et al., 2011)
Enterobacter spp.	*Vespertilio murinus*	Fibrinous suppurative peri-epicarditis	Germany	(Mühldorfer et al., 2011)
Enterococcus faecalis	*Pipistrellus pipistrellus*	Enteritis	Germany	(Mühldorfer et al., 2011)
	Myotis mystacinus	Bile duct proliferation	Germany	
Enterococcus faecium	*Eptesicus nilssoni*	Interstitial pneumonia	Germany	(Mühldorfer et al., 2011)
Escherichia coli	not specified (European bat)	Systemic infection, pneumonia, nephritis and cystitis	Germany	(Mühldorfer et al., 2011)
Klebsiella mobilis	not specified (European bat)	Systemic infection and pneumonia	Germany	(Mühldorfer et al., 2011)
Klebsiella oxytoca	not specified (European bat)	Systemic infection and pneumonia	Germany	(Mühldorfer et al., 2011)

Table A2. *Continued*.

Aetiology	Bat species	Pathology/Clinical signs	Country/State	Reference
Bacterial				
Pasteurella multocida	*Pipistrellus pipistrellus*	Interstitial pneumonia	Germany	(Mühldorfer et al., 2011)
	Plecotus auritus	Interstitial pneumonia	Germany	
	Vespertilio murinus	Interstitial pneumonia	Germany	
	Myotis nattereri	Interstitial pneumonia	Germany	
	Eptesicus fuscus	Fatal septicemia with interstitial pneumonia (mass mortality event; >100 bats).	Wisconsin (USA)	(Blehert et al., 2014)
Salmonella enterica Enteriditis	not specified (European bat)	Systemic infection, pneumonia and wound infection.	Germany	(Mühldorfer et al., 2011)
Salmonella enterica Thyphimurium	not specified (European bat)	Systemic infection, pneumonia and meningitis.	Germany	(Mühldorfer et al., 2011)
Salmonella spp.	*Nyctalus noctula*	Interstitial pneumonia.	Germany	(Mühldorfer et al., 2011)
Serratia liquefaciens	not specified (European bat)	Systemic infection, pneumonia and wound infection.	Germany	(Mühldorfer et al., 2011)
Serratia marcescens	not specified (European bat)	Systemic infection and pneumonia.	Germany	(Mühldorfer et al., 2011)
Staphylococcus aureus	*Pipistrellus nathusi*	Liver and kidney necroses.	Germany	(Mühldorfer et al., 2011)
Yersinia enterocolitica	*Miniopterus schreibersii*	Mass mortality event	Georgia	(Imnadze et al., 2020)
Yersinia pseudotuberculosis	*Myotis myotis*	Hemoperitoneum, multifocal severe necrotizing hepatitis and splenitis, and interstitial pneumonia.	Germany	(Mühldorfer et al., 2010)
	Carollia perspicillata (captive)	Necrosuppurative pneumonia, hepatitis, splenitis, enterocolitis and endometritis.	Switzerland	(Hahn et al., 2021)
	Rousettus aegyptiacus	Necrosuppurative splenitis, hepatitis, pyelonephritis and pneumonia.	Kanto Region (Japan)	(Nakamura et al., 2013)
Fungal				
Cladosporium spp.	*Vespertilio murinus*	Necrotizing dermatitis with intralesional fungal hyphae.	Scotland (UK)	(Barlow et al., 2011)
Histoplasma capsulatum	*Artibeus lituratus*	Fatal histoplasmosis, but also chronic systemic infection (reservoir).	Colombia	(Greer & McMurray, 1981)

Table A2. *Continued*.

Aetiology	Bat species	Pathology/Clinical signs	Country/State	Reference
Fungal				
Hyphopichia burtonii	*Barbastella barbastellus*	Skin crusting and thickening, ulceration and formation of skin nodules, associated to septate fungal hyphae and aggregates of ovoid spores.	Devon (UK)	(Simpson et al., 2013)
Phaeohyphomycosis (Cladosporium *spp.*, Curvularia *spp.*, Exserohilum *spp.*, Bipolaris *spp. and* Alternaria *spp.*)	*Noctilio leporinus*	Multifocal to coalescent grey, crusted, dry, scaly cutaneous lesions over the thorax, abdomen, muzzle, ears and wings. Histologically, fungal organisms are associated with multifocal areas of skin ulcers but not with an inflammatory response.	Brazil	(Souto et al., 2023)
Pseudogymnoascus destructans	*Myotis lucifugus, M. grisescens, M. septentrionalis, M. sodalis, M. leibii, M. austroriparius, M. velifer, Eptesicus fuscus* and *Perimyotis subflavus*	White fungi growth on the muzzle, ears and wing membranes. Ultimately there is emaciation, dehydration and death. Histologically, aggregates of curved arthroconidia are seen over the stratum corneum of the skin, associated to ulcers but not to inflammatory response.	38 states of USA and 8 of Canada	(Gómez-Rodríguez et al., 2022)
Trichophyton redellii	*Myotis lucifugus* and *M. velifer*	Gross lesions in wings, ears and legs (not the muzzle) similar to *P. desctructans*, and ring-like lesions on the skin. Histologically, a neutrophilic pustular response is seen associated to conidia.	Wisconsin (USA)	(Lorch et al., 2015)
Metazoa				
Angiostrongylus cantonensis	*Pteropus alecto, P. scapulatus and P. poliocephalus*	Granulomatous meningitis or meningoencephalitis, with malacia.	Australia	(Barrett et al., 2002)
Physaloptera brevivaginata	*Myotis sp.*	Erosions and gastric ulcerations associated with attached parasites in the mucosa-submucosal junction, with mixed inflammatory response and fibrosis.	Spain	(Botella & Esteban, 1995)

Table A2. *Continued*.

Aetiology	Bat species	Pathology/Clinical signs	Country/State	Reference
Metazoa				
Riouxgolvania beveridgei	*Miniopterus schreibersii bassanii*	White cutaneous nodules (granulomas) in thoracic and pelvic limbs, associated to nematodes.	Naracoorte (Australia)	(McLelland et al., 2013)
Unspecified filaria-like	*Nyctalus noctula*	Cardiopulmonary filariosis.	Austria	(Kübber-Heiss & Reifinger, 1999)
Coccidian				
Nephroisospora eptesicii	*Eptesicus fuscus*	Multifocal and well-demarcated cortical renal lesion (<1 mm in diameter). Histologically, cystic and dilated renal tubules with numerous coccidian parasites within the tubular epithelial cells and the lumen are seen.	Minnesota (USA)	(Wünschmann et al., 2010)
Toxoplasma gondii	*Pteropus scapulatus*	Pyogranulomatous inflammation in lungs, brain, peritoneum, intestines, heart and skeletal muscle.	Australia	(Sangster et al., 2012)
Leishmania mexicana	*Artibeus lituratus*	Raised ulcerated mass on the patagium.	Brazil	(Berzunza-Cruz et al., 2015)
Viral				
Australian Bat Lyssavirus (ABLV)	*Pteropus alecto*	Nonsuppurative encephalitis.	Australia	(Fraser et al., 1996)
Classical rabies virus (RABV)	*Eptesicus fuscus*	Described as "clinical rabies", symptoms not specified.	Texas (USA)	(Davis et al., 1996)
	Desmodus rotundus	Increased vocalization, aggression within the group. Paralysis, non-responsiveness, respiratory distress, lethargy and dull mentation. Others showed hyper-salivation, aggression, teeth chattering, irritability to sound and light sources, and excessive vocalization. Sudden death.	Madison (bat's origin: Mexico)	(Cárdenas-Canales et al., 2020)

Table A2. *Continued*.

Aetiology	Bat species	Pathology/Clinical signs	Country/State	Reference
Viral				
European Bat Lyssavirus 1 (EBLV-1)	*Eptesicus serotinus*	Juveniles found dead (suspected cause of death, no histopathology performed).	France	(Robardet et al., 2017)
European Bat Lyssavirus 2 (EBLV-2)	*Myotis daubentonii*	Dead bats found repeatedly at a single roost (qRT-PCR and detection of viral antigen by immunohistochemistry).	UK	(Banyard et al., 2009)
Gammaherpesvirus (novel)	*Pteropus vampyrus* (captive)	Lymphoplasmacytic blepharitis and conjunctivitis, and Meibomian gland adenitis (relationship between virus and lesions still discussed).	Florida (USA)	(Brock et al., 2013)
Lloviu virus (LLOV)	*Miniopterus schreibersii*	Detected in lungs of bats from mass mortality events in Europe (Spain and Hungary). Relationship between the virus and the casualty of disease is still discussed, but not ruled out.	Spain and Hungary	(Kemenesi et al., 2018, 2022; Negredo et al., 2011)
Poxvirus (*Eptesipox virus)	*Miniopterus schreibersii bassanii*	Epidermal hyperplasia with intracytoplasmatic inclusion bodies in the skin.	Australia	(McLelland et al., 2013)
	**Eptesicus fuscus*	Joint swelling and necrosuppurative osteomyelitis and tenosynovitis in long and facial bones.	Washington (USA)	(Emerson et al., 2013)
Tacaribe virus	*Artibeus* spp. (experimentally infected)	Rabies-like symptoms, including tremors, group isolation and bad appearance of haircoat. Histologically, neutrophilic interstitial pneumonia, adrenocortical and hepatocellular necrosis and degeneration, neutrophilic enteritis and splenitis, lymphocitic leptomeningitis, neutrophilic encephalitis and gliosis are seen.	Trinidad and Tobago	(Cogswell-Hawkinson et al., 2012)



Stelaluna tenía miedo, pero se soltó del árbol y se lanzó al cielo azul oscuro. Janen Canoll (1993)