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Anton Lavrinienko

THE EFFECTS OF EXPOSURE TO RADIONUCLIDE CONTAMINATION ON MICROBIOTA OF WILD MAMMALS

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THE EFFECTS OF EXPOSURE TO RADIONUCLIDE CONTAMINATION ON MICROBIOTA OF WILD MAMMALS

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Abstract

All animals host diverse microbial communities (bacteria, fungi, viruses, known as the microbiota) that inhabit external and internal surfaces of the host. Host-associated microbiota provide essential services to their hosts, such as provision of nutrients, protection against pathogens, and toxic compounds. As changes in the microbiota composition can impact delivery of these functions and thus host health, it is important to understand processes that shape host-associated microbiota. In this thesis, I assess the impacts of environmental contamination on the gut and skin microbiota (bacteria) of small mammals inhabiting areas affected by radionuclides derived from the Chernobyl (Ukraine) and Fukushima (Japan) nuclear accidents. I used marker gene sequencing and field studies to test (I) the effects of exposure to radionuclide contamination on the gut and (II) skin microbiota of the bank vole, *Myodes glareolus*. By conducting a capture-mark-recapture study, I tested (III) the effects of radiation exposure on the temporal dynamics of the bank vole gut microbiota. I also sampled two pairs of mouse species (*Apodemus flavicollis*, *A. sylvaticus*, *A. speciosus*, *A. argenteus*) that occur in sympatry in Chernobyl and Fukushima, to quantify (IV) the general influence of radiation exposure on the gut microbiota of rodents. These data indicate that chronic exposure to radionuclide contamination alters bank vole gut microbiota composition, yet has little notable impact on community composition of the skin microbiota: skin and gut microbiota thus respond to different environmental cues. Longitudinal data indicate that radiation exposure can constrain natural, temporal changes in the bank vole gut microbiota, which is potentially a sign of chronic stress. Also, I show that radiation exposure elicits comparable responses in the gut microbiota of different species of rodents, although host lifestyle can modulate the effects of exposure to radiation on gut microbiota composition. As such, exposure to environmental contaminants has clear potential to alter wildlife microbiota community composition. Given that the microbiota are pivotal to host health, it is important to quantify any microbiota changes in response to diverse anthropogenic disturbances if we are to understand the significance of host-environment interactions in a rapidly changing world.

Keywords: Anna Karenina principle, anthropogenic disturbance, Apodemus, bank vole, capture-mark-recapture, Chernobyl, dysbiosis, environmental contamination, environmental stress, Fukushima, gut microbiome, ionising radiation, Myodes glareolus, nuclear accident, pollution, radiation exposure, S24-7, skin microbiome, stable isotope analysis, stress, TLD, wild rodent, wildlife

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Tiivistelmä

Eläinten mikrobiomi koostuu sen sisällä ja ulkopinnalla elävistä bakteeri-, sieni- ja virusyhteisöistä. Mikrobiomi tuottaa isäntäeliölle olennaisia ravinteita, sekä suojaa taudinaiheuttajia ja myrkyllisiä yhdisteitä vastaan. Koska muutokset mikrobiyhteisöissä voivat vaikuttaa isännän terveyteen, on tärkeää tutkia mikrobiomia muokkaavia tekijöitä. Väitöskirjassani tutkin saastuneen elinympäristön vaikutuksia suoliston ja ihon mikrobiomeihin (bakteerien yhteisöihin) pikkunisäkkäillä Tšernobylin (Ukraina) ja Fukushiman (Japani) ydinvoimalaonnettomuusalueilla. Laajoissa kenttätutkimuksissa selvitin ionisoivalle säteilylle altistumisen vaikutuksia metsämyyrän (*Myodes glareolus*) (I) suoliston ja (II) ihon mikrobiomeihin. Tutkin myös (III) säteilyaltistuksen vaikutusta ajallisiin muutoksiin metsämyyrän suolistomikrobiomissa pyynti-takaisinpyyntimenetelmällä. Lisäksi tutkin (IV) säteilyaltistuksen vaikutuksia laajemmin jyrsijöiden suolistomikrobiomiin käyttäen sympatrisia hiirilajeja Tšernobylissä (*Apodemus flavicollis* ja *A. sylvaticus*) sekä Fukushimassa (*A. speciosus* ja *A. argenteus*). Tulosteni mukaan pitkäaikainen altistuminen ympäristön säteilylle muokkaa metsämyyrien suolistomikrobiomin koostumusta, mutta ei vaikuta merkittävästi ihon mikrobiomiin, osoittaen että ihon ja suoliston mikrobiomeihin vaikuttavat erilaiset ympäristötekijät. Säteilyaltistus voi myös rajoittaa metsämyyräyksilöiden suolistomikrobiomissa tapahtuvia luonnollisia ajallisia muutoksia, mahdollisesti johtuen pitkäaikaisesta stressialtistuksesta. Lisäksi osoitan, että ympäristösäteilylle altistumisen vaikutukset suolistomikrobiomiin ovat samankaltaisia eri jyrsijälajeilla, mutta mikrobiomiin vaikuttavat myös lajien väliset erot elintavoissa. Tutkimukseni siis osoitti, että ympäristön saasteet voivat muokata luonnonvaraisten eläinten mikrobiomien koostumusta. Isäntäeliön ja sen ympäristön vuorovaikutus voi oleellisesti vaikuttaa eläinyksilöiden ja niiden populaatioiden terveyteen ja edelleen niiden kykyyn vastata ihmisen aiheuttamiin ympäristömuutoksiin.

Asiasanat: Anna Karenina -periaate, Apodemus, dysbioosi, Fukushima, ihmistoiminnan aiheuttamat häiriötekijät, ihon mikrobiomi, ionisoiva säteily, isotooppimääritys, jyrsijä, metsämyyrä, Myodes glareolus, pyynti-takaisinpyyntimenetelmä, S24-7, saastuminen, stressi, suolistomikrobiomi, säteilyaltistus, termoluminesenssiannosmittari, Tšernobyl, villieläimet, ydinvoimalaonnettomuus, ympäristösaasteet, ympäristöstressi

To my family

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September 2020 Anton Lavrinienko

Original publications

This thesis is based on the following publications, which are referred throughout the text by their Roman numerals:

I Lavrinienko, A., Mappes, T., Tukalenko, E., Mousseau, T. A., Møller, A. P., Knight, R., Morton, J. T., Thompson, L. R., Watts, P. C. (2018). Environmental radiation alters the gut microbiome of the bank vole *Myodes glareolus*. *ISME Journal*, 12(11), 2801–2806. https://doi.org/10.1038/s41396-018-0214-x

II Lavrinienko, A., Tukalenko, E., Mappes, T., & Watts, P. C. (2018). Skin and gut microbiomes of a wild mammal respond to different environmental cues. *Microbiome*, 6(1), 1–16. https://doi.org/10.1186/s40168-018-0595-0

III Lavrinienko, A., Tukalenko, E., Kesäniemi, J., Kivisaari, K., Masiuk, S., Boratyński, Z., Mousseau, T. A., Milinevsky, G., Mappes, T., Watts, P. C. (2020). Applying the Anna Karenina principle for wild animal gut microbiota: temporal stability of the bank vole gut microbiota in a disturbed environment. *Journal of Animal Ecology*. https://doi.org/10.1111/1365-2656.13342

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Table of author contributions to the original publications.

	I	II	III	IV
Original idea	AL, PCW, TM	AL, PCW, TM	AL, PCW, TM	AL, PCW, TM
Data collection	AL, TM, ET, TAM, APM	AL, TM, ET	AL, TM, ET, KK, ZB, SM, TAM, GM,	AL, TM, KK, ET, ZB
Laboratory work	AL	AL	AL, JK	AL
Data analyses	AL, JTM, LRT, RK PCW	AL	AL	AL, AH, RH
Manuscript preparation	AL, PCW	AL, PCW	AL, PCW	AL, PCW, AH

Anton Lavrinienko (AL), Zbyszek Boratyński (ZB), Anni Hämäläinen (AH), Rasmus Hindström (RH), Jenni Kesäniemi (JK), Kati Kivisaari (KK), Rob Knight (RK), Tapio Mappes (TM), Sergii Masiuk (SM), Gennadi Milinevsky (GM), Anders P. Møller (APM), James T. Morton (JTM), Timothy A. Mousseau (TAM), Luke R. Thompson (LRT), Eugene Tukalenko (ET), Phillip C. Watts (PCW)

Contents

1 Introduction

1.1 Wildlife in a changing world

Environmental changes resulting from human activity impact almost every habitat on earth, making humans one of the world's dominant evolutionary force in the new epoch of Anthropocene (Palumbi, 2001; Pelletier & Coltman, 2018). Anthropogenic transformation of the environment imposes formidable selective pressures upon natural populations, whose persistence typically requires an effective and rapid response to changes in its environment (Carlson, Cunningham, & Westley, 2014). This is important, because anthropogenic habitat impacts can have significant effects on individual health and fitness in wild animal populations (Acevedo-Whitehouse & Duffus, 2009; Pelletier & Coltman, 2018). There is some debate on whether changes in genomic architecture can provide a sufficiently fast response to allow rapid adaptation, especially for many threatened vertebrate species (Merilä & Hendry, 2014). Hence, many populations are thought to rely on phenotypic plasticity to respond rapidly to environmental change (Alberti et al., 2017; Charmantier et al., 2008). The phenotypic plasticity depends on the ability of a genotype to produce distinct phenotypes when exposed to different environments throughout its lifespan (Pigliucci, 2005). However, a growing body of evidence suggests that individual phenotypes are not determined solely by the host genome, but arise from complex interactions between the environment, and combined expression of the host and genomes of associated microorganisms (Bordenstein & Theis, 2015). Within this context, microbial traits are of particular importance as they effectively expand animal biology, modulating host capacity to adapt and survive in a changing environment (Alberdi, Aizpurua, Bohmann, Zepeda-Mendoza, & Gilbert, 2016). Rising human population and concomitant increase in demand for natural resources are likely to further amplify habitat disturbances (*e.g.* pollution, destruction and urbanisation) in future. It is thus imperative that we focus efforts on understanding the ecological and evolutionary impacts of these ongoing human-mediated environmental changes on wildlife and their associated microbes.

1.2 Animals never walk alone

During more than 3 billion years history on Earth, microbes have diversified to occupy virtually all available environments and hence, all animals have evolved under persistent microbial exposure (McFall-Ngai et al., 2013). Rapidly advancing technology have provided the means to identify and count microbes using culture-independent methods. In particular, high-throughput and cost-effective marker gene analyses have led to recognition of the true diversity and ubiquity of host-associated microorganisms (Song et al., 2020; Thompson et al., 2017). Vertebrates engage in symbiotic associations with diverse and complex microbial communities, comprised of bacteria, archaea, viruses, fungi and other eukaryotic microbes, which are often collectively referred to as the *microbiota* (McFall-Ngai et al., 2013). The microbiota and their gene content, or so-called *microbiome*, play fundamental roles in the development, growth and health of their animal hosts (McFall-Ngai et al., 2013; Rosenberg & Zilber-Rosenberg, 2018). Animals are morphologically complex, but many are 'metabolically deficient', thus they rely on microbial metabolic processes for provision of certain key metabolites and nutrients (Douglas, 2018). Microbiome also provide animals with many other services associated with host health, for example, protection against pathogens and modulation of the immune system function. As delivery of these services impacts host physiology, compositional and functional variation of the microbiota holds importance for determining animals' fitness (T. A. Suzuki, 2017). And yet, we lack understanding about how microbiota respond to environmental perturbations, and whether any host-associated microbiota shifts will facilitate or impede overall responses of their animal hosts' (Parfrey, Moreau, & Russell, 2018). At least partly, this is due to the lack of detailed characterisation of microbiota associated with animals in their natural habitat, where many environmental challenges are now commonplace, yet have unknown consequences for most wildlife microbiota (Carthey, Blumstein, Gallagher, Tetu, & Gillings, 2019; Trevelline, Fontaine, Hartup, & Kohl, 2019).

1.3 Microbial habitats within and upon the animal host

1.3.1 Vertebrate gut microbiota

Microbes associate with various host tissues, although one of the most influential and well-studied microbial community is that inhabiting the gastrointestinal tract. Dominated by bacteria, the vertebrate gut microbiota perform important functions

for their hosts. For example, through the process of fermentation of indigestible foods into different metabolites (*e.g.* short-chain fatty acids, vitamins, reviewed by (Lee & Hase, 2014)), gut microbiota provide host with energy and essential molecules that are involved in many regulatory aspects of host physiology (Koh, De Vadder, Kovatcheva-Datchary, & Bäckhed, 2016; Rooks & Garrett, 2016). In addition, gut microbiota are also of key importance to host immunity, and defence against pathogens (T. A. Suzuki, 2017). Beyond these diverse services, the gut microbiota can influence behaviour (Sampson & Mazmanian, 2015) and sociality (Moeller et al., 2016), thereby affecting some complex traits of the animal host.

Given that changes in the gut microbial community can deteriorate host health, it is important to understand processes shaping the vertebrate gut microbiota. Studies of vertebrate microbiota that target laboratory animals and humans have identified how extrinsic and host-associated factors can impact gut microbiota composition. Notably, diet is one of the key environmental determinants of variation in the gut microbiota composition, and holds potential to rapidly and reproducibly alter gut microbiota in humans and mice (Carmody et al., 2015; David et al., 2014; E. D. Sonnenburg et al., 2016). Host genotype (Goodrich et al., 2014), biological sex (de la Cuesta-Zuluaga et al., 2019; Falony et al., 2016) and age (Yatsunenko et al., 2012), also affect the gut microbiota community composition, yet in humans contributions of these host-associated factors in comparison to cumulative effects of environment and lifestyle are relatively weak (Rothschild et al., 2018; Smits et al., 2017). While studies on microbiota of wild animals remain scarce, features of the environment typically also exert a stronger influence on their gut microbiota than intrinsic, host-related factors (*e.g.* age, sex, reproductive status) (Grieneisen et al., 2019; Kolodny et al., 2019; Maurice et al., 2015; Ren et al., 2017). In particular, host diet has a major impact on the gut microbiota community composition in wild animals; for example, gut microbiota exhibit marked seasonal variation due to food availability in red squirrel (Ren et al., 2017), wood mouse (Maurice et al., 2015), gorilla and chimpanzee (Hicks et al., 2018), and other wild mammals (Kartzinel, Hsing, Musili, Brown, & Pringle, 2019). In addition, co-colonisation with gastrointestinal parasites (Kreisinger, Bastien, Hauffe, Marchesi, & Perkins, 2015; Maurice et al., 2015) (*e.g.* nematodes, helminth; but see (Goertz et al., 2019)), biogeography (Goertz et al., 2019; Grond et al., 2019; Linnenbrink et al., 2013) and abiotic properties (soil, temperature, climate) of the habitat (Grieneisen et al., 2019; Sepulveda & Moeller, 2020; Woodhams et al., 2020) can also influence gut microbiota composition in wild mammals. Perhaps not surprisingly, wild animals entering artificial conditions experience drastic changes

in lifestyle, and the gut microbiota of wild and captive animals differ substantially (Clayton et al., 2016). That natural food supplementation promotes a retention of native gut microbiota in captive animals (Martínez-Mota, Kohl, Orr, & Denise Dearing, 2020) reinforces the major effect of diet in structuring gut microbiota in wildlife.

While diet is a key predictor of the gut microbiota composition of any given host species when considered in isolation, in most cases its impact is much smaller than that of host taxonomy when analysing gut microbiota of different species (Amato et al., 2019). Indeed, comparative studies point to a strong signal of host taxonomic identity in structuring gut microbiota of wild birds (Capunitan, Johnson, Terrill, & Hird, 2020; Hird, Sánchez, Carstens, & Brumfield, 2015), primates (Amato et al., 2019) and other mammals (Knowles, Eccles, & Baltrūnaitė, 2019; Lutz et al., 2019). Phylogenetically closely related host species also considered to have more similar gut microbiota; that is, eco-evolutionary pattern known as *phylosymbiosis* (Lim & Bordenstein, 2020). Phylosymbiosis is prevalent, but such evolutionary trend is not universal and vary in strength among animal clades (Grond et al., 2019; Hird et al., 2015; Lutz et al., 2019; Song et al., 2020; Trevelline, Sosa, Hartup, & Kohl, 2020). Several processes, including adaptation to specialised diet or lifestyle could break such cophylogeny patterns, leading to convergence of the gut microbiota in certain host clades regardless of their evolutionary history. The large-scale convergence of the gut microbiota across phylogeny of ant- and termite-eating (myrmecophagous) mammals, due to the extreme diet specialisation (Delsuc et al., 2014); or high similarity of the gut microbiota of birds and bats (Song et al., 2020), potentially due to physiological adaptations associated with true flight, could serve as good examples of such convergence. The implication is that while gut microbiota can be selected by host-specific factors, its composition also reflect host dietary habits and lifestyle.

1.3.2 Vertebrate skin microbiota

Vertebrate skin microbiota has received less research attention than the gut microbiota, with most studies on skin microbiota directed towards humans due to potential biomedical implications (Byrd, Belkaid, & Segre, 2018). Studies of skin microbiota in non-human animals largely directed towards amphibians (Bates et al., 2018) or bats (Lemieux-Labonté, Simard, Willis, & Lapointe, 2017), aimed to prevent worldwide populations decline caused by fungal pathogens. Healthy skin is colonised by a high diversity of symbiotic bacteria (and also fungi, archaea and

viruses, see (Byrd et al., 2018)) that contribute to host fitness by modulating immune response, and by preventing colonisation by pathogens (Belkaid & Segre, 2014; O'Sullivan, Rea, O'Connor, Hill, & Ross, 2019). Depending on the host taxon, skin can be perceived as a microbial habitat that is either (1) dry, sparse in nutrients and covered with fur or plumage (mammals, birds), or (2) moist and coated in sugar-rich mucosal layer (amphibians) (Ross, Rodrigues Hoffmann, & Neufeld, 2019). Consequently, the skin microbiota composition varies across body sites in humans and other animals, reflecting physiological properties of the host skin, and variation in pH, temperature, salt and sebum content (Bewick et al., 2019; Ross et al., 2019).

As the outermost layer of an organism, skin and resident microbiota are in direct contact with the environment, hence the skin microbiota are strongly influenced by various environmental factors (Woodhams et al., 2020). Indeed, geography (Cundell, 2018; Li et al., 2019), habitat type, hygiene and lifestyle (Bouslimani et al., 2019; Clemente et al., 2015; Cundell, 2018; Dimitriu et al., 2019; Hanski et al., 2012), cohabitation status (Song et al., 2013), as well as specific environmental exposures (*e.g.* contact with soil and plant material) (Grönroos et al., 2019) have been shown to structure human skin microbiota. Among host-related factors, some studies on humans reported that host age (Lehtimäki et al., 2017) and sex (Fierer, Hamady, Lauber, & Knight, 2008) explain additional compositional variation in skin microbiota. Substantial work on microbial communities associated with wild amphibians and bats conclude that the skin microbiota are shaped predominantly by local environmental conditions, host species identity and developmental life stage (for amphibians) (Avena et al., 2016; Bletz et al., 2016; Kueneman et al., 2014; Lutz et al., 2019; McKenzie, Bowers, Fierer, Knight, & Lauber, 2012).

Majority of skin microbiota studies conducted on non-human mammals (other than bats) sampled either captive animals from zoos, domestic or companion animals (Ross et al., 2019). Overall, environment and lifestyle (Lehtimäki et al., 2018), body region (Cuscó et al., 2017), host sex (Cuscó et al., 2017; Ross, Müller, Scott Weese, & Neufeld, 2018), health status (Decandia, Leverett, & Vonholdt, 2019; Older et al., 2017), and host phylogeny (Ross et al., 2018) are major factors that determine the skin microbiota composition in non-human mammals. Similarly as with the gut microbiota research, substantially less is known about the factors shaping the skin microbiota of mammals in the wild. In fact, despite about a decade of microbiota research using next-generation sequencing technology (Caporaso et al., 2010), the exhaustive list of studies that have examined skin microbiota of wild

mammals is limited to Tasmanian devils (Cheng et al., 2015), squirrels (Ross et al., 2018), and several marine mammals (Bierlich et al., 2018; Grosser et al., 2019; R. Hooper et al., 2019; Russo et al., 2018). These studies also highlight the overall importance of the environmental factors in structuring skin microbiota composition. However, given a strong impact of captivity on the mammalian skin microbiota (Cheng et al., 2015), it is conceivable that the captive animals may not be representative to those of the same species in the wild. Hence, understanding the relative importance of environmental and host-specific factors in shaping skin microbiota composition requires further studies in wild populations. Nevertheless, a strong biogeography pattern in the skin microbiota variation indicates that often the immediate environment dictates what bacteria are present on the skin of wild animals (Avena et al., 2016; Bletz et al., 2016). At the same time, that the skin microbiota of some species do not simply reflect microbiota found in the surrounding environment (Lutz et al., 2019; McKenzie et al., 2012), and even exhibit host specificity, suggest some host control over recruitment of microbes from the environmental reservoir in the process of skin microbiota assembly.

1.3.3 Wildlife as microbiome study system

An estimated 15% or less of studies on animal microbiota were conducted on wild animals (Pascoe, Hauffe, Marchesi, & Perkins, 2017). Hence, the microbiota of wild animals are poorly-known, and thus may serve as an untapped resource for understanding animal health and fitness. This trend of a large and expanding knowledge gap in microbiomes of wild animals compared with microbiomes of human and non-human (model species, domestic or companion) captive animal hosts is clearly demonstrated by an almost 50-fold difference between the number of published studies indexed in the Web of Knowledge during the last 15 years (Fig. 1). We lack data on composition of microbial communities associated with most vertebrates, particularly in the wild (Hird, 2020).

There is an apparent association between the gut microbiota, host physiology and environment, thus gut microbiota composition differ substantially between wild and captive animals that are living in controlled conditions (Clayton et al., 2016; McKenzie et al., 2017). Such differences are even exacerbated in studies using laboratory animals (Viney, 2019), which due to their associated artefacts (genetic homogenisation) often lack broader ecological reality. Indeed, differences between wild or laboratory-kept animals can contribute to variable or even contradictory results. Together, these distinctions have led to calls for more studies

that examine microbiomes of animals in their natural habitat (Hird, 2017; Pascoe et al., 2017). Collecting samples and metadata from wild animals in the field can be challenging, yet as demonstrated by recent studies in evolutionary biology (Youngblut et al., 2019) and applied biomedical research (Rosshart et al., 2017), incorporating the unique data from wild hosts offers immediate benefits. Finally, many more exploratory and experimental studies on wildlife microbiota are needed, in order to understand processes that assemble and maintain the microbiota community composition under various environmental conditions.

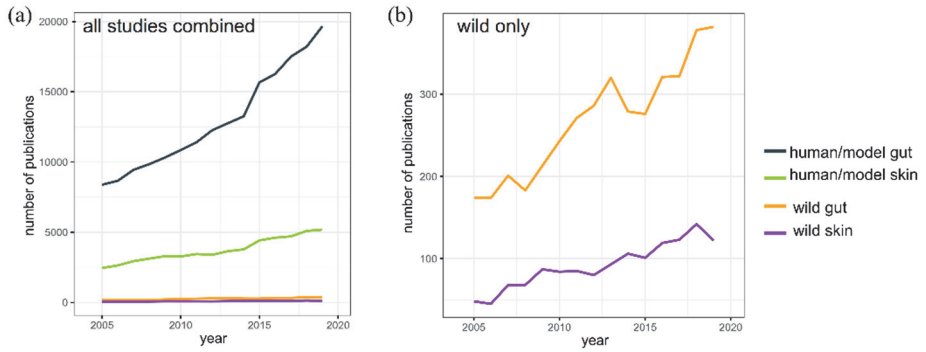

Fig. 1. Knowledge gap in microbiomes of wild animals compared with microbiomes of human and non-human captive animal hosts. Shown are the total number of published microbiota studies indexed in the Web of Knowledge (WOK, accessed 30.03.2020) during 2005-2019, with (a) their distribution across animal hosts (*e.g.* humans/model: human, model species, domestic, companion and other captive animals; wild: wild animals only, plotted separately also in the panel (b)) and the microbiomes studied (skin and gut). A starting point at 2005 was chosen based on seminal work in the field.

1.4 Environmental change and host-microbiota interactions

Recent studies have identified direct associations between gut microbiota profiles and host phenotypic variation that allow host to adapt to novel conditions (Alberdi et al., 2016). For example, populations of woodrats (*Neotoma lepida*) exposed to dietary plant toxins have a gut microbiota community that enables them to digest the toxins in doses that are otherwise lethal to laboratory mice (Kohl, Weiss, Cox, Dale, & Denise Dearing, 2014). In brown bear (*Ursus arctos*), seasonal changes in gut microbiota modulate energy metabolism and fat deposition, helping to prepare the host for hibernation (Sommer et al., 2016). In mice, gut microbiota can confer

cold tolerance upon fluctuations in ambient temperature (Chevalier et al., 2015). The implication is that such host fitness-promoting microbial communities directly influence host's dietary niche breadth, physiology (even gut morphology), and overall adaptive potential, thus likely confer advantage to individuals that harbour them. These studies exemplify that many aspects of host biology cannot be understood without considering microbes, and highlight the importance of studying host-associated microbiota if we are to understand animal host responses to anthropogenic habitat impacts.

1.4.1 Host-associated microbiota and anthropogenic disturbances

Environmental changes derived from human activities have the potential to affect animals and their microbiota (Carthey et al., 2019; Trevelline et al., 2019). The view of each animal as an 'ecosystem' composed of host and their associated microbiota predicts, that certain perturbations and disturbances can result in microbial community instability (often described with an umbrella term dysbiosis) (J. L. Sonnenburg & Sonnenburg, 2019). Such microbiota instability can lead to crash of (bio)diversity accompanied with loss of functions and services, invasion of pathogens and opportunistic microbes, species extinctions, with overall negative consequences for host health. Hence, habitat fragmentation and destruction, pollution, urbanisation and climate change are among some of the most pervasive threats to wildlife health, either directly or indirectly, for example, by altering their associated microbiota (Carthey et al., 2019).

The few studies on microbiota-habitat disturbance interactions indicate that microbiota associated with wildlife can be altered by anthropogenic disturbances (West et al., 2019). For example, black howler (*Alouatta pigra*) and red colobus (*Procolobus gordoronum*) monkeys living in sub-optimal and degraded habitats have altered diet, which associates with loss of the gut microbiota diversity and some associated metabolic functions (*e.g.* pathways necessary to detoxify plant xenobiotics) (Amato et al., 2013; Barelli et al., 2015). Habitat fragmentation altered diet and the gut microbiota of common vampire bats (*Desmodus rotundus*) (Ingala, Becker, Bak Holm, Kristiansen, & Simmons, 2019). Habitat fragmentation can also impact skin microbiota, as for example, in golden lesser treefrog (*Dendropsophus minutus*) deforestation alters the interactions among host, their microbiota and infectious disease (Becker, Longo, Haddad, & Zamudio, 2017). Land-use change due to urbanisation impacts the gut microbiota of birds (house sparrow, *Passer domesticus*) and mammals (eastern grey squirrel, *Sciurus carolinensis*), and might

do so via environmental changes (including dietary shifts) or by altering host physiology (Stothart, Palme, & Newman, 2019; Teyssier et al., 2018). Effects of climate change on animal populations are difficult to predict, but an experimental 2-3 °C warming reduced gut microbiota diversity in the common lizard host (*Zootoca vivipara*), and likely negatively affected lizard survival (Bestion et al., 2017). Loss of sea ice has necessitated polar bears (*Ursus maritimus*) to alter their foraging behaviour, such climate-driven changes were also associated with shifts in the gut microbiota diversity and composition (Watson et al., 2019). Together, these studies demonstrate that the anthropogenic disturbances have clear potential to alter microbial communities associated with wild animals.

1.4.2 Environmental contaminants and host-associated microbiota

Growing evidence indicates that environment is increasingly affected by persistent contaminants that arise from industries, agriculture, transport, and other human activities, at a local and global scale. For example, metal toxicity is one of the most studied threats to the wellbeing of human and wildlife (Assefa & Köhler, 2020). Exposure to toxic metals can perturb microbiota diversity and composition, with the overall negative consequences for host health (Gao et al., 2017; Richardson et al., 2018). Other examples of toxic compounds of environmental health concern include pesticides, xenobiotics (various drugs, persistent organic compounds, and food additives), microplastics and radionuclides, which are widely distributed either due to their deliberate use or accidental release to the environment (Jin, Wu, Zeng, & Fu, 2017; Rosenfeld, 2017; Yuan et al., 2019; A. Zhang & Steen, 2018). All these environmental contaminants have also been shown to alter microbiota composition in laboratory animal models (Chassaing et al., 2015; Goudarzi et al., 2016; Jin, Lu, Tu, Luo, & Fu, 2019; Wang et al., 2020).

In wild animals, however, studies of the impact of any environmental contaminants on microbiota are limited to analyses of the effect of metals on the microbiota of saltwater clams (*Ruditapes philippinarum*) (Milan et al., 2018), toads (*Bufo raddei*) (W. Zhang, Guo, Yang, Ding, & Zhang, 2016), frogs (*Pelophylax perezi*) (Costa, Lopes, Proença, Ribeiro, & Morais, 2016) and deermice (*Peromyscus* spp.) (Coolon, Jones, Narayanan, & Wisely, 2010). Hence, the potential outcomes of exposure to any other type of environmental contamination on microbiota associated with wildlife remain largely unknown. Notably, when it comes to animals in their natural environment, the laboratory observations have little predictive power because (1) microbiota responses are not uniform across

environmental disturbances (Richardson et al., 2018), and (2) laboratory studies rarely use environmentally relevant levels of exposure (*e.g.* intensity, frequency and duration) (Goudarzi et al., 2016; Shuryak, 2019), thus generally lack ecological relevance. This later point is particularly important as animals in their natural environment can differ in their response to similar exposures compared with animals housed in laboratory settings (Rosshart et al., 2017). For example, individuals in their natural habitat are apparently almost ten times more sensitive to radiation exposure than conspecifics tested under controlled laboratory conditions (Garnier-Laplace et al., 2013). While it is difficult to identify particular reason behind such dose-response discrepancies, some studies suggest cumulative effects of additional internal (host-specific) and external (food availability, species interaction) stresses in wild animal populations (Garnier-Laplace et al., 2013).

1.4.3 The definition of stress

One important unresolved issue is whether changes in the host microbiota result from impacts of contaminants on the environment and/or available diet, or because the host is stressed. With this in mind, the definition of stress becomes important. Notably, in addition to the microbiota alterations, exposure to environmental contaminants associates with similar effects on host systemic health. These include, for example, reduced intestinal mucus secretion and compromised intestinal barrier function, increased risk of inflammation, development of allergic and metabolic conditions, and thus general decline of host health (Chassaing et al., 2015; Jin et al., 2019, 2017; A. Zhang & Steen, 2018). From systems biology perspective, environmental challenge becomes a stressor if it results in a failure of the organism to control a critical (for health and fitness) variable (Del Giudice et al., 2018). Given that anthropogenic disturbances, including environmental contamination can lead to a potential disruption of host normal physiology, these factors can be considered as stressors for living organisms. Defining the concept of stress is not an easy task (Del Giudice et al., 2018), yet necessary in order to place the findings from the microbiota research into current theoretical framework. For example, one such framework, the *Anna Karenina principle* (AKP) for animal microbiomes, postulates that exposure to stress destabilises the microbiota, and effectively results in community-wide differences between healthy and dysbiotic individuals (Zaneveld, McMinds, & Thurber, 2017). In analyses of microbial data, AKP effects manifest in high inter-individual variability (stochastic changes and dispersion) in microbiota of stressed animals as compared with healthy (unstressed) animals,

whose microbiota are stable and relatively similar among individuals (Fig. 2). While this concept has already been tested in analyses (*e.g.* the Microbiome Stress Project) focusing on free-living microbial communities and microbiota of laboratory rodents experiencing elevated levels of stress (Rocca et al., 2019), this idea remains largely unexplored in wild vertebrates.

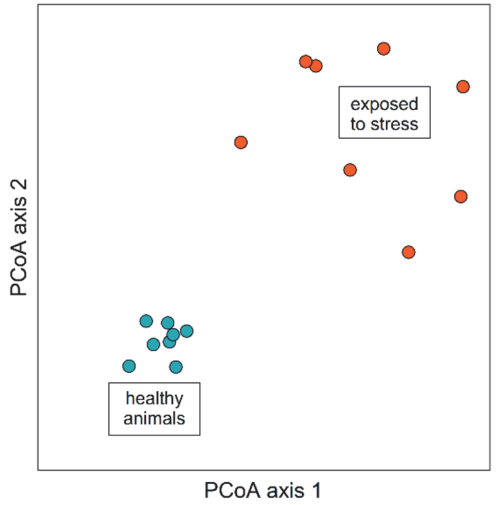

Fig. 2. Hypothetical ordination results for a stressor that alters the microbiota, inducing the *Anna Karenina principle* (AKP) effects. Group of stressed animals (in orange) exhibit high inter-individual microbiome variability (dispersion) as compared with healthy animals (in blue) which harbour microbiomes relatively similar among individuals.

Clearly, consequences of exposure to a majority of anthropogenic stressors for wildlife microbiota remain unknown. That said, environmental disturbances are only about to increase in scale of presence worldwide. Gut microbiota are a vital component of animal host health and it is therefore important to recognise dysbiosis and any variation in response to exposure to environmental stressors. Many of these considerations form the basis of this thesis and my PhD project, which aims to characterise the impacts of environmental disturbances on wildlife and their associated microbiota. Nuclear accidents, such as those at Chernobyl (Ukraine) and Fukushima (Japan), provide unrivalled opportunity to test the effects of environmental stress derived from radionuclide contamination under field conditions. Therefore, in this thesis these two areas were used as a model system and natural laboratories, further details of which are provided below.

1.5 How did Chernobyl and Fukushima become unique natural laboratories?

Accidental release of radionuclides into the environment presents potential stressor with generally negative impacts on health of humans and wildlife (Lourenço, Mendo, & Pereira, 2016; Møller & Mousseau, 2006). Numerous human activities worldwide have contributed to such radionuclide contamination of the environment through, for example, nuclear weapons tests, uranium mining, waste treatment and nuclear accidents (Lourenço et al., 2016). The year 2020 brought with itself the 34th and 9th anniversaries of the nuclear accidents at Chernobyl (Ukraine) and Fukushima (Japan), respectively. On 26 April 1986, reactor 4 of the Chernobyl Nuclear Power Plant (CNPP) exploded, and the exposed reactor core continued to burn for about 10 days resulting in a release of large amounts of radionuclides over much (>200.000 km^2) of Ukraine, Belarus, Russia and parts of Europe (Møller & Mousseau, 2006). Although many of the released radionuclides decayed within days (^{131}Iodine) after accident at the CNPP, other isotopes with long half-lives, such as ^{137}Caesium (^{137}Cs, 30 years), ^{90}Strontium (^{90}Sr, 29 years) and ^{239}Plutonium (^{239}Pu, 24.000 years) are more persistent and will be present in the environment for many years to come (Møller & Mousseau, 2006).

Following the Chernobyl accident, more stringent safety procedures were implemented worldwide and it was assumed that an accident of this scale would never happen again. Then, on 11 March 2011, nuclear accident at the Fukushima Daiichi Nuclear Power Plant (FDNPP) occurred, largely due to the effects of a tsunami that was itself caused by a magnitude-9 Tohoku earthquake at the northern coast of Japan (Aliyu, Evangeliou, Mousseau, Wu, & Ramli, 2015). Similarly as in Chernobyl, accident at the FDNPP resulted in a release of some very short-lived radionuclides such as ^{131}Iodine, or ^{134}Cs with the physical half-life of ~2 years, but also large amounts of ^{137}Cs isotope; these were discharged by either direct release or deposition from the atmosphere into the ocean and the surrounding environment (Aliyu et al., 2015). The Fukushima accident has revived safety concerns and stimulated a growing public and scientific interest to the impacts of such accidents on natural ecosystems, along with the efforts to assess risks of potential future accidents at any of the other 451 nuclear power reactors around the globe that are in operation today (IAEA, 2019; Wheatley, Sovacool, & Sornette, 2017). Notably, Chernobyl and Fukushima are the only two accidents that were classified as maximum of level-7, according to the International Nuclear Events Scale.

In response to the accidents, humans were evacuated from a 4760 km^2 area in Ukraine (and partly in Belarus), and from a 1150 km^2 area in Japan. Access to these evacuation zones remains severely restricted as radiation dose rates in these areas are still above the safety limits for human habitation (Beresford, Scott, & Copplestone, 2020; Harada et al., 2014). Thus, the abandoned areas in Ukraine and Japan are often respectively referred to as the Chernobyl Exclusion Zone (CEZ) and the Fukushima Evacuation/Exclusion Zone (FEZ). Several factors make the CEZ and FEZ unprecedented natural laboratories: (1) environment within both CEZ and FEZ is still contaminated with persistent radionuclides; (2) radionuclide contamination patterns are heterogeneous at a regional scale, securing appropriate control study areas; (3) areas around both accident sites have restrictions for human habitation, but not wildlife; (4) thus, animals inhabiting landscape surrounding both Chernobyl and Fukushima are chronically exposed to elevated levels of radionuclides in their natural environment. Hence, Chernobyl and Fukushima are unique systems for studying biological effects of chronic exposure to radionuclide contamination on wildlife.

1.6 Biological consequences of exposure to radiation

Radionuclides contamination is a potential source of genotoxicity to humans and wildlife (Lourenço et al., 2016). When absorbed by living cells, ionizing radiation can induce significant damage to cell structures, through breakage of chemical bonds of macromolecules (*e.g.* proteins, nucleic acids and lipids), either directly or by reactive oxygen species generated through radiolysis of intracellular water (Azzam, Jay-Gerin, & Pain, 2012; Einor, Bonisoli-Alquati, Costantini, Mousseau, & Møller, 2016). More broadly, outcomes of radiation exposure on living organisms depend on (1) mode of acquisition (external or internal) and (2) intensity of exposure (Beresford, Scott, et al., 2020), (3) weather the absorbed dose is received as a single irradiation (acute) or via prolonged exposure (chronic) (Shuryak, 2019, 2020), and (4) species' radiosensitivity (Real & Garnier-Laplace, 2019). In this context, wildlife inhabiting areas contaminated with radionuclides at Chernobyl and Fukushima accident sites are exposed to low-dose-rate radiation (<0.1 mGy/min, the United Nations Scientific Committee on the Effects of Atomic Radiation, UNSCEAR; see for review (Rühm et al., 2018)), which derives from both external (living in a contaminated area) and internal (ingesting particles) sources, and since radionuclides are persistent over long-term, such exposure is rather chronic than acute. Moreover, much of contaminated with radionuclides

areas within the CEZ and FEZ characterised by ambient radiation dose rates similar to those used as reference values (~4-40 μGy/h, considered by UNSCEAR, ICRP, IAEA international organisations) at which radiation-induced biological effects have been reported in animals and plants with different levels of radiosensitivity (Real & Garnier-Laplace, 2019).

1.6.1 The effects of radiation exposure at the molecular, organismal and population levels

Ecological studies have documented harmful effects of chronic exposure to radionuclides released by the accidents at Chernobyl and Fukushima in many species of wildlife (see for review (Lourenço et al., 2016; Mousseau & Møller, 2014; Strand, Sundell-Bergman, Brown, & Dowdall, 2017)). These research activities can broadly be divided into several focus groups, each examining the effects of exposure to radiation at different biological scales. For example, at the molecular level, an apparent increase in DNA damage (Andrea Bonisoli-Alquati et al., 2010; Hiyama et al., 2012; Nakamura et al., 2017), chromosomal aberrations (Kubota, Tsuji, et al., 2015; Lourenço et al., 2016), oxidative stress (Einor et al., 2016; Urushihara et al., 2016), altered gene expression (Jernfors et al., 2018; Kesäniemi, Jernfors, et al., 2019), telomere and mitochondrial homeostasis (Kesäniemi, Lavrinienko, et al., 2019; Kesäniemi et al., 2020), all have been associated with exposure to radiation in animals inhabiting contaminated areas at Chernobyl and/or Fukushima. At the organismal level, such exposure often manifests in morphological abnormalities and other phenotypic maladies (Ishida, Tanoi, & Nakanishi, 2015; Møller, Mousseau, De Lope, & Saino, 2007), including tumours (Møller, Bonisoli-Alquati, & Mousseau, 2013), aspermy and altered sperm quality (Møller, Bonisoli-Alquati, Mousseau, & Rudolfsen, 2014; Takino et al., 2017), reduced fitness (Mappes et al., 2019), altered haematological parameters (Ochiai et al., 2014), increased frequency of cataracts (Lehmann, Boratyński, Mappes, Mousseau, & Møller, 2016; Mousseau & Møller, 2013), slower growth rates, as well as smaller brain size (Hayama et al., 2017; Møller, Bonisoli-Alquati, Rudolfsen, & Mousseau, 2011). Moreover, surveys of common species from Chernobyl and Fukushima report decrease in abundance of soil invertebrates (Møller & Mousseau, 2018), insects (Møller & Mousseau, 2009; Yoshioka, Mishima, & Fukasawa, 2015), birds (Garnier-Laplace et al., 2015; Morelli, Benedetti, Mousseau, & Møller, 2018), and the density of small mammals (Mappes et al., 2019) with increasing levels of radionuclide contamination. However, other

studies find little notable effects of radiation on the abundance of large-sized mammals within the CEZ and FEZ (Deryabina et al., 2015; Lyons, Okuda, Hamilton, Hinton, & Beasley, 2020; S. C. Webster et al., 2016), suggesting the natural rewilding of the landscape surrounding the nuclear accident sites following human abandonment (Perino et al., 2019). In this context, it is perhaps worth to mention that stability or increase over the population sizes metric do not necessarily imply these animals are unaffected by radiation exposure.

Interestingly, only few studies have actually attempted a direct hypothesis-driven comparisons of the biological effects of radionuclide contamination at the two accident sites, *e.g.* examining wildlife abundance profiles using camera traps (Lyons et al., 2020), and estimating population sizes and DNA damage in birds within the CEZ and FEZ (A. Bonisoli-Alquati et al., 2015; Møller et al., 2012). Therefore, importance of difference in duration of exposure (number of generations) after the accidents at Chernobyl and Fukushima (Hiyama et al., 2012; Møller et al., 2012), variation in levels and composition of radioactive isotopes between the sites (Steinhauser, Brandl, & Johnson, 2014), as well as potential interspecific differences in response to radiation exposure remain largely unknown.

1.6.2 The effects of radiation exposure on free-living and host-associated microorganisms

The surprisingly few studies to quantify the effects of radionuclide contamination on microorganisms have returned conflicting results about the effects of radiation on microbial diversity. For example, the diversity of free-living microbial communities isolated from contaminated areas at Chernobyl was reported to either be reduced (Romanovskaia, Sokolov, Rokitko, & Chernaia, 1998), similar (Hoyos-Hernandez et al., 2019; Ragon, Restoux, Moreira, Møller, & López-García, 2011) or more diverse (Theodorakopoulos et al., 2017) compared with those from control (uncontaminated) areas. Only one study focused on microbial communities at Fukushima, in this study soil samples had similar diversity estimates irrespective of the contamination levels, and rather grouped according to the forest type (Hoyos-Hernandez et al., 2019). That the diversity of free-living microbial communities is not apparently reduced at the contaminated areas compared with elsewhere is consistent with reports of microbes that have evolved resistance to chronic radiation exposure within the CEZ (Ragon et al., 2011; Shuryak, 2019). That said, given that in studies mentioned above samples were either collected at a very local spatial scale (Theodorakopoulos et al., 2017) (*e.g.* within 10 m, from a single radiation

waste disposal trench test site) or bacterial isolates from soil were studied using culturing methods (Romanovskaia et al., 1998) (and thus limited in scope), it is difficult to discern any potential effects of radiation on the environmental microbial reservoirs at Chernobyl and Fukushima.

Microbiota associated with animals inhabiting areas contaminated with radionuclides within the CEZ and FEZ also have potential to be affected by protracted exposure to radiation (A. Zhang & Steen, 2018). For example, the total cultivable bacterial loads from feathers of birds (barn swallows, *Hirundo rustica*) nesting at Chernobyl were negatively correlated with radiation exposure levels (Czirják, Møller, Mousseau, & Heeb, 2010; Ruiz-González et al., 2016). Similarly, the diversity of the gut microbiota of earthworms collected from contaminated areas within the CEZ was lower than in animals from control areas with low contamination, yet also characterised by a site-specific clustering pattern and strongly correlated with the soil pH (Newbold et al., 2019). Hence, only three studies (in birds and earthworms) have examined impacts of radiation upon any host-associated microbiota, and only one of these, used culture-independent sequencing methods to quantify microbial communities (Newbold et al., 2019). Thus at present, from these scattered attempts it is not possible to infer any potential general consequences of radiation exposure on wild animal microbiota.

2 Aims of the study

The aims of this thesis are to characterise the impacts of environmental contamination on wildlife and their associated microbiota, using small mammals inhabiting the areas contaminated with radionuclides at Chernobyl (Ukraine) and Fukushima (Japan) as a model system. To achieve these aims, I integrated marker gene sequencing and field methods to examine effects of radiation exposure upon the host-associated microbiota in wild animals, based on the following research questions:

1. Does exposure to radionuclide contamination at the CEZ alter the gut microbiota of the bank vole, *Myodes glareolus*? If so, which components of the gut microbiota are most affected, *i.e.* community diversity or composition? Can microbial community composition be used as an indicator of radiation exposure? To addressed these questions, I sampled bank voles from spatially separated areas (7 replicates, 10-80 km), thus testing whether association between radionuclide contamination and the bank vole gut microbiota is robust to geographical and habitat variation (**I**).
2. Do different (*i.e.* skin and gut) microbiota associated with an individual host exhibit a convergent response to similar levels of radionuclide contamination? Skin microbiota were selected due to its intrinsic contrast (in niches) with the gut microbiota, and the hypothesised dominant role of the environment (rather than diet) in shaping skin microbiota composition. My sampling scheme afforded an indirect test of the hypothesis that radionuclide contamination impacts the diversity of environmental microbial reservoirs (**II**).
3. Does exposure to radionuclide contamination affect the temporal dynamics of the bank vole gut microbiota? Does any impact of radiation exposure follow the key predictions of the *Anna Karenina principle* (AKP) for animal microbiomes? To address these questions, I used a capture-mark-recapture study of bank voles inhabiting the CEZ, that integrated (1) longitudinal gut microbiota profiling, (2) stable isotope analysis to quantify host diet, and (3) novel radiation dosimetry approach to track intensity of exposure *in vivo* (**III**).
4. Does exposure to radionuclide contamination have a comparable impact on the gut microbiota of other rodents? Are analogous radiation-induced changes identified in the gut microbiota of bank voles from the CEZ consistent across host species and geographical areas, *i.e.* in four *Apodemus* mouse species from the CEZ and FEZ (Fukushima) (**IV**)?

3 Material and methods

3.1 Small mammals as a model system

Small rodents are ideal mammalian model to study the effects of exposure to radionuclide contamination in the wild for several reasons that include: (1) representative species have relatively large population sizes at both Chernobyl and Fukushima accident sites, enabling adequate sampling, (2) they breed well in the laboratory conditions, have limited dispersal abilities and high recapture rate (40-50%), which allow the use of field experiments, (3) most rodents have small home ranges and live close to the soil surface, thus the external exposure of these animals reflects ambient radiation dose rates in their trapping locations. This later point is important, considering the heterogeneous radionuclide contamination that can vary substantially at regional scale. In this thesis, I studied five species of arvicoline and murine rodents (voles and mice) inhabiting the areas around Chernobyl and Fukushima accident sites. Specifically, the bank vole *Myodes glareolus* (formerly known as *Clethrionomys glareolus* Schreber, 1780), yellow-necked mouse *Apodemus flavicollis* (Melchior, 1834) and wood mouse *A. sylvaticus* (Linnaeus, 1758) were studied in Chernobyl, whereas the large and the small Japanese field mice, *A. speciosus* (Temminck, 1844) and *A. argenteus* (Temminck, 1844), respectively, were examined at Fukushima (Fig. 3). While each of these species share most of the aforesaid aspects that justify their use as model species, they also have some unique life history traits and ecology characteristics. Hence, such diverse sampling with several host species provides opportunity for a more general assessment of the effects of exposure to radionuclide contamination upon the host-associated microbiota.

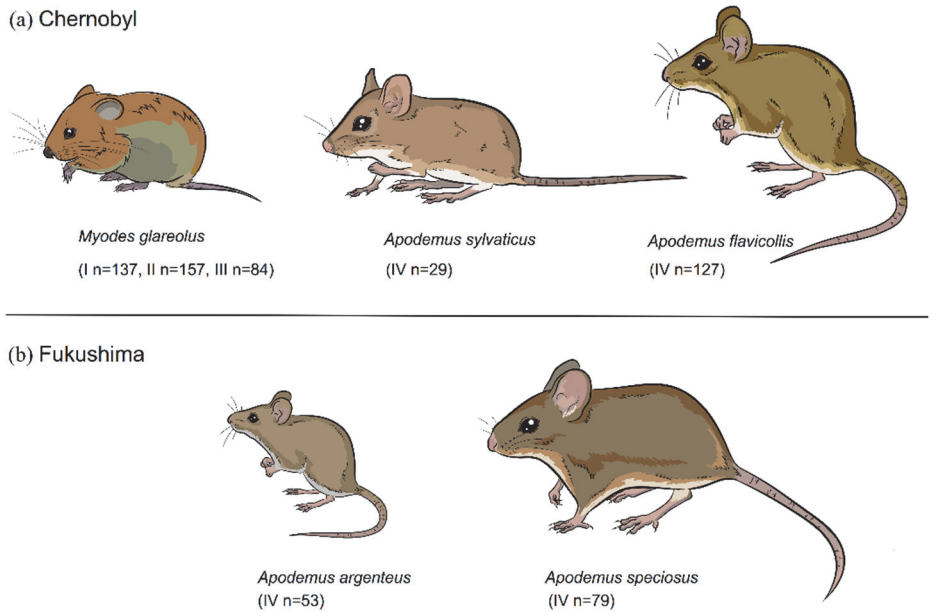

Fig. 3. Summary of the five rodent species studies at (a) Chernobyl, Ukraine and (b) Fukushima, Japan, and their sample sizes in the corresponding studies I-IV presented in this thesis.

3.2 Ecology of the studied species

Species studied in Chernobyl

The bank vole is abundant in much of forest types (broadleaved deciduous and coniferous) across Europe, and one of the most common mammals inhabiting the CEZ (Baker et al., 2017). Several ecological and molecular studies of bank voles have been conducted in the CEZ (Kesäniemi, Jernfors, et al., 2019; Mappes et al., 2019). Curiously, voles were reported to overall be exposed to highest average radiation doses compared with other small mammals common within the CEZ (Chesser et al., 2000). This species characterised by short periods until sexual maturity (*ca.* 20 days), relatively large litter sizes (2-10 pups) and up to four reproduction cycles during breeding season that typically lasts from May to September (Koivula, Koskela, Mappes, & Oksanen, 2003; Mappes & Koskela,

2004). Bank voles have promiscuous mating system, where breeding females are territorial and their home ranges overlap with several males (Koskela, Mappes, & Ylonen, 1997). Consistent with such observations, bank vole home range estimates differ between sexes, but generally are quite small (0.2-0.7 ha) (Bujalska, 1990). Males have larger home ranges than females, and sometimes can disperse on distances of up to 1 km during breeding season (Kozakiewicz, Chołuj, & Kozakiewicz, 2007). Like most other rodents, bank voles are short-lived animals and in the wild, rarely live more than a year (up to 1.5 years) (Innes & Millar, 1994). The bank vole diet usually consists of leaves, grasses, buds, roots, fruits, seeds, fungi, lichens, and small invertebrates (Calandra et al., 2015). The preferred diet and habitats, place bank voles into an intermediate position between other herbivorous/true folivores arvicoline voles (*Microtus* and *Arvicola*) and typical seed- and invertebrate-eating murine rodents like *Apodemus* (Butet & Delettre, 2011). Most vole species are associated with grassland habitats, yet similarly to *Apodemus* species, bank voles prefer mixed woodland habitats with shrubs, low plants and leaf litter. Thus, in the forests within the CEZ these species commonly share similar habitats, and often are sympatric (*i.e.* co-occurring) to the *A. flavicollis* and *A. sylvaticus*. That bank voles and *Apodemus* mice species live in the same habitats enabled their parallel capture and samples collection during the same fieldwork sessions within the CEZ.

Both, *A. flavicollis* and *A. sylvaticus* are common in woodlands throughout Europe and Asia. They are generalist feeders, dominant part of their diet consists of mast seeds (acorns and other nuts) or weed seeds, but also includes invertebrates (Knowles et al., 2019; Ozaki et al., 2018). The two *Apodemus* species are closely related from a phylogenetic, morphological and ecological points of view (Michaux, Libois, & Filippucci, 2005; H. Suzuki et al., 2008), yet some distinctive phenotypic features include, the band of yellow fur around the neck of *A. flavicollis*, and it larger overall body size compared with *A. sylvaticus* (mean 34.27 g compared to 19.24 g, study IV). In these two species, breeding females usually defend their territories and avoid home range overlap with other females (Stradiotto et al., 2009; Tew & Macdonald, 1994). Home ranges of the *A. flavicollis* and *A. sylvaticus* are usually quite small (~0.5 ha) (Godsall, Coulson, & Malo, 2014). During breeding season, average home ranges of males can be substantially larger than those of females, indicating that males invest more energy into movement, as part of their breeding strategy (Tew & Macdonald, 1994). Both sexes can disperse, but typical seasonal dispersal distances rarely exceed 1-1.5 km (Stradiotto et al., 2009). Thus, while *A. flavicollis* and *A. sylvaticus* cover somewhat larger areas than bank voles,

their space use scales are rather comparable. In sympatry, due to similar ecology and diet, the *A. flavicollis* and *A. sylvaticus* are known to compete for space (Montgomery, 1980). However, such interactions are relatively weak and usually lead only to partial spatial segregation of the two species (Hoffmeyer, 1973; Montgomery, 1980, 1981).

Species studied in Fukushima

In Japan, I studied the two other murine rodent species that also belong to the *Apodemus* genus, the large and the small Japanese field mice (*A. speciosus* and *A. argenteus*, respectively). These two species are endemic to Japan, and one of the most common mammals in forested areas around the Fukushima accident site (Kubota, Takahashi, et al., 2015). The two species can be readily distinguished due to the large difference in body size, where on average *A. speciosus* is about twice the size of *A. argenteus* (mean 41.29 g compared to 16.32 g, study IV). Both species are granivorous and feed mostly on acorns, other nuts and seeds (Sato et al., 2018). However, *A. argenteus* has wide niche breadth and is a generalist feeder utilising diversity of plant species, whereas *A. speciosus* is a specialist and largely dependent on acorn-producing tree species (*e.g.* oaks) (Sato et al., 2018). Such observations are consistent with the apparent ability of *A. speciosus* to tolerate toxic effects of tannins contained in acorns, by means of tannin-binding salivary proteins and tannase-producing gut microbiota (Shimada, Saitoh, Sasaki, Nishitani, & Osawa, 2006).

Interestingly, *A. speciosus* and *A. argenteus* also differ in their mating systems, as *A. speciosus* is promiscuous, while *A. argenteus* is monogamous and even has a direct parental care (Oka, 1992). Consequently, the home ranges of *A. speciosus* males overlap with those of both males and multiple females, whereas females defend exclusive areas and are territorial. However, in *A. argenteus* the bond between male and female is tight and rather pairs are territorial. In both species, breeding season lasts from May to October, during which the home ranges of males (~0.2 ha and ~0.12 ha for *A. speciosus* and *A. argenteus*, respectively) are substantially larger than those of females (<0.1 ha for both *A. speciosus* and *A. argenteus*), yet generally home ranges of these two *Apodemus* species are small (Oka, 1992). Notably, the two species are also known to segregate vertically, as *A. argenteus* has a broader vertical spatial niche and exhibits arboreal activity (*i.e.* tree-dwelling, uses tree cavities to give birth and nurse young), whereas *A. speciosus* is largely terrestrial (*i.e.* ground-dwelling) (Oka, 1992). Thus, despite

their overlapping habitats, the large and the small Japanese field mice have strikingly different ecology and life history traits. These differences in dietary characteristics, home ranges and space use are likely important factors that contribute to the niche partitioning and decrease competition between these two sympatric *Apodemus* species.

3.3 Field expeditions and sampling at Chernobyl and Fukushima

The results of this thesis are based on several datasets, with most data collected during fieldwork sessions at the Chernobyl Exclusion Zone (CEZ, Ukraine) and the Fukushima Evacuation/Exclusion Zone (FEZ, Japan) areas in 2015-2016. The data analysed in the studies I-IV, comprises of diverse samples collected from (1) more than 200 bank voles, (2) almost 300 *Apodemus* mice, and also include (3) nearly 150 samples of putative dietary items. These samples include faecal material, skin microbiota swabs, samples of fur and liver tissues; also, herbaceous and woody plants, insects, fungi, mosses and lichens used for a stable isotope survey of potential bank vole diet at Chernobyl. The main procedures and methodologies related to the fieldwork or laboratory analyses are described below. Further details on the methods and other practical features can be found in the individual chapters assigned for each study included in this thesis.

Study areas selection

Within both, CEZ and FEZ, animal trapping locations were selected based on soil radiation levels measured near each trap at 1 cm above the ground using a hand-held Geiger counter (Gamma-Scout GmbH & Co. KG, Germany). Based on such ambient radiation dose rate measurements and in accordance with the recommended benchmark dose rates (~4-40 μGy/h) used in the assessment of potential impacts of radiation on wildlife (Real & Garnier-Laplace, 2019), trapping locations were assigned to one of the contaminated or uncontaminated general study areas. In the studies I-IV, I have given a name to each study area in Ukraine in a systematic way as following, (1) Chernobyl High (CH), (2) Chernobyl Low (CL), and (3) Kyiv Low (KL). Notably, CH locations contained elevated levels of radionuclides compared with both CL and KL, where radiation levels were similar to background measurements elsewhere in northern Ukraine (see Fig. 4, and studies I-IV for more details). By analogy, mice trapping locations in Japan were also assigned either to the Fukushima High (FH) or Fukushima Low (FL) study areas

(Fig. 4) based on the on-site ambient radiation dose rate measurements (study IV). Hence, study areas were designed to contrast in levels of environmental radiation and represent two treatments with high (CH, FH) and low (CL, KL, FL) radionuclide contamination.

Where possible, each study area had several replicate sites that were located in geographically independent areas, separated by distances (10-30 km) that exceed typical dispersal capabilities of the bank vole and the mice species studied (~1-1.5 km) (Kozakiewicz et al., 2007; Stradiotto et al., 2009). That said, clear replicates segregation was difficult to achieve at Fukushima either due to decrease in radionuclide contamination levels north from Namie (FH) or agricultural conversion of the habitats towards east from established areas in both FH and FL (Fig. 4). Thus, rather large number (n=51) of individual trapping locations, each separated by the distance of at least 500 m, were used to introduce spatial heterogeneity in our sampling effort (Fig. 4). Our sampling of spatially separated locations within each study area likely add noise even though all animals were sampled from similar mixed forest habitats. Such design however was important to deconfound potential effects of exposure to radiation from other environmental factors specific to a certain location.

Bank voles and mice trapping

Bank voles and two species of *Apodemus* mice (*A. flavicollis* and *A. sylvaticus*) were caught at 86 sites around northern Ukraine and within the CEZ during summer of 2016, using live trapping (Fig. 4). At each location, 16 Ugglan Special2 traps baited with sunflower seeds and potato were placed in a 4x4 grid, with an inter-trap distance of about 20 m. The trapping period was up to three consecutive nights in each location. Traps were initially set in the late afternoon and animals were collected early each following morning. In Japan, *A. speciosus* and *A. argenteus* mice were caught at 51 location using either Ugglan or Sherman live traps during my expedition to the FEZ in September of 2015 (Fig. 4). The overall mice trapping procedure was similar at Fukushima, yet only 12 traps baited with sunflower seeds and apple were placed in three small quadrants instead of larger grids. In both study areas the minimum distance between trapping locations was always at least 500 m.

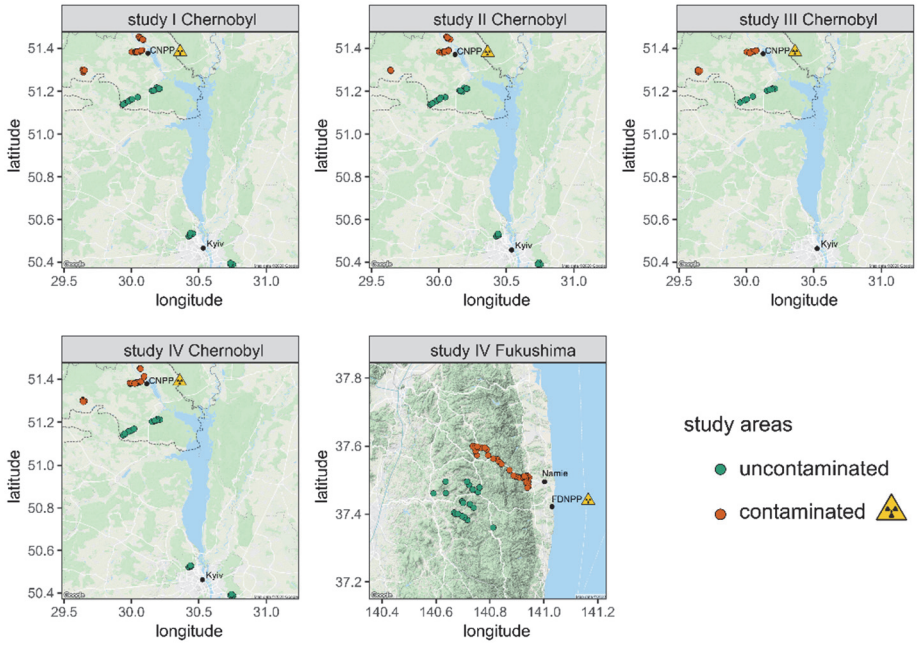

Fig. 4. Maps with the trapping locations of bank voles (upper row) and/or *Apodemus* mice (lower row) in northern Ukraine and in the Fukushima prefecture, Japan, used in the studies I-IV presented in this thesis.

Captured animals were transported to the field laboratories established within the CEZ and FEZ, and depending on the dataset were either sampled immediately upon arrival (study I n=137, II n=157, study III details described below) or were euthanised by cervical dislocation and preserved at -20°C for further processing (study IV n=288). All animals were keyed to species based on the visual assessment of morphological characteristics, and sex, body mass, head width, maturity at capture and other features of animal phenology (*i.e.* descended testes for males, gravid, lactation or with a perforate vagina for females) were recorded for every animal. Individuals of both sexes were used in all, except the study III, where only male bank voles were studied as the anaesthesia procedure (described below) could induce pregnancy complications in females.

The surveys described in the studies I, II and IV were cross-sectional, but the study III uses longitudinal data. In this study, inhalative anaesthesia (using isoflurane, Baxter, Unterschleißheim, Germany) was necessary to implant lithium fluoride thermoluminescent dosimeters (TLD, CHP Dosimetry) under the skin of

each animal to directly measure the absorbed external radiation dose *in vivo*. At the same time, animals were uniquely marked using subcutaneous transponder tags for identification upon recapture. When such experimental work was conducted (study III), animals were housed at the field laboratory at Chernobyl, in individual Makrolon Type III cages (43x26x15 cm) using sawdust and hey for bedding, with rodent food (RM1, Special Diet Services) and water *ad libitum*. After some recovery time from surgery, animals (n=84) were released back to the free-range habitat to their original trapping locations. After nearly five weeks, we have recaptured about 50% of released individuals (n=43), which were processed exactly as described below. Further details on the methods, number of individuals and other statistics associated with the capture-mark-recapture study are described in the study III. I also conducted a capture-mark-recapture pilot study to fit TLDs on *A. flavicollis* (n=10) from contaminated and uncontaminated areas within the CEZ (using same methods as in the study III). The low mice recapture rate (<25%), however, prevented such trials in other species (see study IV for details). All procedures were performed in accordance with international guidelines and regulations for the use of animals in research.

Faecal and swab samples collection

Upon arrival to the field laboratory within the CEZ, bank vole fur was swabbed to sample the skin microbial communities (study II) using Sterile Catch-All Sample Collection Swabs (Epicentre Biotechnologies, Madison, USA). Swabs were firmly pressed against the dorsal thoracic area, rubbed back and forth 20 times, and then immediately placed into MOBIO Power Bead tubes containing buffer solution (MOBIO Laboratories, Carlsbad, USA) and stored at -80°C prior to DNA extraction. After these procedures were completed, animals were placed in individual ethanol-sterilised cages and were monitored for 2 hours and immediately after defecation, faecal pellets were collected for bank vole gut microbiota characterisation (study I-III). Faecal material was frozen at -20°C, and stored at -80°C until further processing. To characterise gut microbiota of *Apodemus* mice captured at both Chernobyl and Fukushima (study IV), animals were dissected and approximately 2 cm section of the distal colon was removed to take gut content samples, which were stored frozen at -80°C until further processing. To avoid potential batch effects and systematic bias, within each study samples from different host species and treatment groups were processed (*e.g.* sampling,

transportation, dissection, storage, and also wet-lab and sequencing procedures described below) at random.

3.4 Quantifying radiation exposure of wildlife

External radiation exposure

In theory, external radiation exposure of an animal can be predicted based on the soil radiation levels at the trapping location measured in the field using simple hand-held dosimeters (Beresford, Scott, et al., 2020; Chesser et al., 2000). This assumption, however, is complicated by the fact that (1) animals are mobile, and (2) habitats can vary in contamination levels at a small spatial scale (sometimes even over distance of ~200 m, see (Beresford, Scott, et al., 2020)), at least within the CEZ, but less so at Fukushima (Møller, Nishiumi, Suzuki, Ueda, & Mousseau, 2013). Thus, relationship between ambient radiation dose rate at a certain location and external exposure of animals that live there is not always straightforward.

In the study III, I established whether soil radionuclide contamination at the trapping locations within the CEZ can represent an animal's radiation exposure using experimental approach by implanting bank voles (n=84) with TLDs (see methods above). The data retrieved from the 43 recaptured bank voles, indeed indicate that the external radiation dose absorbed by bank voles inhabiting the CEZ can be accurately predicted from the ambient radiation dose rate at their trapping locations. Similar trends were also observed in a TLD-based experiment with a limited number of *A. flavicollis* mice in the study IV, thus further highlighting generally close agreement between the dose rates approximated from the on-site soil radiation levels and those estimated from TLDs (at least for soil dwelling rodents that live in close proximity to the ground). The low recapture rate (<25%) of *A. flavicollis* prevented such trials in other mouse species examined in the study IV. There are no studies investigating such relationship in any wild animals from Fukushima. However, given the relatively small home ranges of *A. speciosus* and *A. argenteus*, and rather homogeneous patterns of radiation contamination at the trapping locations (in FH) used in the study IV, the overall correlation strength can be expected to be similar.

Internal radiation exposure

While estimates of external dose rate based on measurements from hand-held dosimeters were proved to be robust in our study system, their use has limitation as they neglect the contribution of the internal radiation exposure to the total absorbed doses. Such exposure derive from consumption of contaminated food and water, as well as ingested soil and dust particles (Chesser et al., 2000). In this thesis, I addressed such limitations using the individual gamma (γ)-spectrometry to estimate the whole-body radionuclide (^{137}Cs) burden and thus internal radiation exposure for sampled bank voles and mice (studies II-IV). The ^{137}Cs activity for each individual was measured using the SAM 940 radionuclide identifier system (Berkeley Nucleonics Corporation, San Rafael, CA, USA). Similarly as with the external doses, animals inhabiting contaminated areas within both CEZ (CH) and FEZ (FH) expose to significantly more internal radiation than animals from uncontaminated areas (CL, KL, FL). In addition, repeated γ-spectrometry measurements (study III) of the same individuals were generally consistent over time, suggesting that studied animals experience a chronic internal radiation exposure. All technical details and methods related to radiation dosimetry can be found in the studies III and IV.

Total radiation exposure of studied species

Consistent with other studies, there was a substantial variation in contribution of external and internal exposures to the total absorbed radiation doses among species, with mice generally having higher (~78% to more than 96%) input from external sources compared with bank voles (~60%) (Beresford, Barnett, et al., 2020; Kubota, Takahashi, et al., 2015; Onuma, Endoh, Ishiniwa, & Tamaoki, 2020). Nevertheless, in all the studied species, the dominant part of the total radiation doses derived from external radiation exposure (studies III and IV), or in other words, animals were exposed to radiation simply by living in a contaminated area within either CEZ or FEZ.

There were some differences in levels of total radiation exposure between studied species. For example, the average total radiation doses for animals captured from contaminated areas either at Chernobyl (CH) or Fukushima (FH) were as following: *A. speciosus* (~0.19 mGy/d) < *A. argenteus* (~0.25 mGy/d) < *A. sylvaticus* (~0.36 mGy/d) < *A. flavicollis* (~0.55 mGy/d) < *M. glareolus* (0.78 mGy/d). Similar radiation doses and extent of variation among studied species were

also reported in other studies conducted at Chernobyl (*e.g.* bank voles have the highest record within the CEZ, see (Chesser et al., 2000)) and Fukushima, and likely reflect some differences in ecology of the studied species (Beresford, Barnett, et al., 2020; Beresford, Scott, et al., 2020; Kubota, Takahashi, et al., 2015; Onuma et al., 2020). While bank voles, on average, seems to be exposed to the highest total radiation dose rates per day, even lower levels estimated for *Apodemus* mice species are substantial and equivalent to several chest radiography scans (2-3 X-ray scans, 0.10-0.15 mGy each) every day (Baker et al., 2017; Brenner & Hall, 2007).

The main conclusion of the dosimetry data is that representatives of all the studied species inhabiting contaminated (CH, FH), but not uncontaminated areas (CL, KL, FL) in both Ukraine and Japan live under chronic radiation exposure derived from both external and internal sources. Such a notable contrast in radiation exposure of animals living in different study areas (treatments), provide further support for appropriate study design and adequate selection of trapping locations.

3.5 Stable isotope analysis

In the capture-mark-recapture study (study III), I have used stable isotope analysis (SIA) to examine potential variation in bank vole diet. At first capture, fur was clipped from the thoracic area of all captured bank voles and stored at room temperature, while liver tissue was sampled from recaptured individuals and stored at -20°C until further processing. To aid interpretation of the bank vole isotopic data, I analysed isotopic composition in putative food items (n=136 samples of herbaceous and woody plants, insects, fungi, mosses and lichens) (Butet & Delettre, 2011), which were sampled from the dominant species present at bank vole trapping locations.

I analysed the stable isotopes of nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) from fur and liver because of the inherent difference in the isotopic turnover rates between these two tissues. Specifically, fur has slow isotopic turnover rate and likely reflects the isotopic signal of bank vole diet in early spring, 1-2 months prior to the first capture (Kurle, Koch, Tershy, & Croll, 2014). That said, the turnover rate in liver tissue is much faster and half-lives for $\delta^{13}C$ and $\delta^{15}N$ in rodents are typically less than 1-2 weeks (Robb, Woodborne, de Bruin, Medger, & Bennett, 2015), which is slow enough to reflect variation in bank vole diet during the release phase (mean 36 days) of the capture-mark-recapture study in early summer. Thus, such sampling scheme allows examining potential changes in stable isotope profiles and thus variation in bank vole dietary preferences over time.

During the sample pre-processing, fur was cut into small pieces and the rest of the samples were homogenised using steel beads and the Qiagen TissueLyser II system. After this step, lipids were removed from fur and liver samples using a 2:1 chloroform to methanol solution (Bligh & Dyer, 1959). All samples were oven dried at 60°C at least for 24 h. Samples were weighed (0.5-1.2 mg), placed into tin cups and analysed for stable isotopes of carbon and nitrogen using a Thermo Finnigan DELTAplus Advantage stable isotope ratio mass spectrometer connected to a Carlo Erba Flash EA1112 elemental analyser at the stable isotope facility of the University of Jyväskylä, Finland. Further details on the methods, internal working standards and analytical precision of the stable isotope analysis are described in the study III.

3.6 DNA isolation and sequencing

Gut and skin microbiota associated with rodents inhabiting areas within the CEZ and FEZ was quantified using the high-throughput marker gene sequencing targeting the V4 region of the 16S ribosomal RNA (rRNA) locus. Total DNA was extracted from faecal material using a PowerFecal DNA Isolation kit (MOBIO Laboratories/Qiagen) following the manufacturer's instructions (studies I, III, and IV). In the study II, DNA from swab samples was extracted using a PowerSoil DNA Isolation kit, with minor modifications to the manufacturer's protocol to increase the efficiency of the microbial cell lysis (Castelino et al., 2017). The extracted DNA was quantified with a Qubit 2.0 fluorometer (Invitrogen).

We employed standardised PCR protocol to amplify the V4 region of the 16S rRNA locus using the original 515F/806R primer pair (Caporaso et al., 2011). For all the samples, library preparation was performed following the Earth Microbiome Project protocol (www.earthmicrobiome.org/protocols-and-standards/). Libraries were sequenced on an Illumina MiSeq to provide 250 bp paired-end reads either at the Beijing Genomics Institute in Hong Kong (BGI, www.bgi.com/global/; studies I and III) or at the Institute for Molecular Medicine Finland (FIMM, www.fimm.fi/; studies II and IV). The technical details on the DNA extraction, library preparation and sequencing are described in the studies I-IV.

3.7 Read data processing

3.7.1 Bioinformatics

Sequence data were de-multiplexed at the sequencing facility (by FIMM or BGI). The data quality was assessed using FASTQC. Reads of low quality, adapter and primer sequences were identified and removed either during a separate data quality control step or within the data processing workflow at the later stages. In the studies I-II, paired-end reads were assembled using PEAR (J. Zhang, Kobert, Flouri, & Stamatakis, 2014), processed and clustered into operational taxonomic units (OTUs) using the combination of SORTMERNA and SUMACLUST methods implemented by the open-reference OTU-picking pipeline within the QIIME1 v1.9 (Caporaso et al., 2010). This pipeline was used to filter out chimeric sequences and sequencing errors, and to cluster reads based on their sequence similarity (*e.g.* 97% identity or above). In the studies III-IV, I processed microbial data using QIIME2 (Bolyen et al., 2019) and the DADA2 de-noising pipeline (Callahan et al., 2016) as these methods have superseded QIIME1 standards. In principle, this pipeline also controls for sequence quality, remove chimeras and assemble paired-end data. However, instead of clustering similar sequences into OTUs, DADA2 uses error profiles to identify subtle nucleotide variation and resolve sequence data into exact sequence features called amplicon sequence variants (ASVs) (Knight et al., 2018). In both cases, the resulting output was a table with counts of observations of different OTUs or ASVs per each sample, to which bacterial taxonomy was assigned against the GREENGENES v.13_8 database (McDonald et al., 2012). To account for differences in read depth between samples, in all the studies microbial data were normalised prior to most downstream analyses by rarefaction to even number of reads (randomly, without replacement) in each sample (Weiss et al., 2017). Sequencing effort was sufficient to recover most of the skin and gut microbiota communities in all the datasets (as rarefaction curves for each sample approached to a saturation plateau), and only few samples with low number of reads were removed from the studies II and IV.

3.7.2 Statistical analyses

Analyses of microbial data to quantify differences among samples can be divided into three main categories: analyses of (1) community diversity within samples or so-called *alpha diversity*, (2) community-wide dissimilarity in taxa abundance and

phylogeny between pairs of samples, known as *beta diversity* or structure, and (3) abundances of OTUs, ASVs or their respective taxonomic groups between samples to identify *differentially abundant* taxa. Here, I provide an overview of key concepts and methods behind these analyses, while further specific details and technical aspects are given in the studies I-IV.

Several indices can be used to measure *alpha diversity*. In my thesis, I used community richness (number of observed OTUs or ASVs), Shannon index (accounts for both richness and evenness in abundances), and Faith's phylogenetic diversity (uses a phylogenetic tree relationships to compute sample diversity) (Knight et al., 2018). Significant differences in mean alpha diversity estimates between groups of samples were identified using non-parametric statistical tests (*e.g.* Kruskal-Wallis, Wilcoxon rank-sum), and potential associations with continues metadata variables were investigated mostly using the Spearman's rank correlation analysis.

Depending on the metric of choice, *beta diversity* comparisons generate a matrix of community-wide distances or dissimilarities between all pairs of samples. Principally, there are two types of beta diversity measures: quantitative metrics (*e.g.* Bray-Curtis, weighted UniFrac) that use taxon abundance information, and thus can detect changes in taxa relative abundance between samples, and qualitative metrics (*e.g.* binary Jaccard, unweighted UniFrac) that use only taxa presence or absence data (Knight et al., 2018). In addition, both weighted and unweighted UniFrac metrics incorporate phylogenetic distances between observed OTUs or ASVs (Hamady, Lozupone, & Knight, 2010). Beta diversity distance matrices are reduced in complexity using Principal Coordinates Analysis (PCoA) to readily visualise differences among samples in two- or three-dimensional ordination space (Vázquez-Baeza, Pirrung, Gonzalez, & Knight, 2013). The permutational analysis of variance (PERMANOVA) using the ADONIS function in the R package VEGAN (Oksanen et al., 2019) was used to assess statistical significance of beta diversity sample clustering patterns between groups.

Using these analyses, I was able to identify groups of samples with distinct community diversities and structures that associated with specific metadata; whereas the *differential abundance* testing aided in identifying taxa that potentially drive the observed patterns. Differential abundance testing was performed using statistical tools such as non-parametric tests, generalised linear modelling (GLM), balances in GNEISS (Morton et al., 2017), and permuted mean difference tests within the DS-FDR (Jiang et al., 2017). In addition, supervised machine-learning algorithm

(*e.g.* RANDOM FOREST) was used to predict categorical sample metadata as a function of microbiota community composition (Bokulich et al., 2018; Breiman, 2001). This analysis also allowed me to identify OTUs/ASVs that have most predictive power, or in other words, the taxa that best discriminate between samples. An assessment of predicted functional composition associated with the bank vole gut microbiota (study I) was made using PICRUST v.1.1.0 tool (Langille et al., 2013).

4 Results and discussion

In this thesis, I asses the effects of exposure to radionuclide contamination upon host-associated microbiota. These data can be summarised as several main conclusions (Table 1): (I) chronic radiation exposure alters the host-associated microbiota composition, although, (II) the response to radiation varies between host microbiomes studied; (III) chronic radiation exposure alters temporal dynamics and can constrain the natural variation in the bank vole gut microbiota over time; (IV) radiation exposure associates with comparable responses across murine and arvicoline rodents, and robust to variation in host species and geographical areas. Together, the findings in this thesis suggest that similar to radionuclide contamination, any other environmental contaminants also have the potential to affect microbiota associated with wild animals.

Table 1. The main results and conclusions in each study (Roman numerals I-IV) presented in this thesis.

Study	Main results	Main conclusions
I	Bank voles inhabiting areas that contrast in the level of radionuclide contamination differ in the gut microbiota composition	Exposure to environmental radiation alters the gut microbiota of a wild mammal
II	Bank vole skin and gut microbiota have distinct responses to radiation exposure and are structured at different spatial scales	Skin and gut microbiota of a wild mammal respond to different environmental cues
III	Bank voles inhabiting areas that contrast in the level of radionuclide contamination differ in their gut microbiota stability over time	Exposure to environmental radiation can constrain the natural temporal variation in the gut microbiota of a wild mammal
IV	Wild mice inhabiting areas contaminated with radionuclides confirm key findings from the bank vole host, and suggest that host lifestyle can modulate effects of radiation exposure	Exposure to environmental radiation associates with comparable gut microbiota responses across wild rodents

4.1 Bank vole gut microbiota changes in response to radionuclide contamination (study I)

In this study, I quantified the effects of exposure to radionuclide contamination upon the bank vole gut microbiota. Bank voles were sampled from replicate study

areas that differed in (1) levels of radionuclide contamination (CH, contaminated; CL/KL, uncontaminated/control, respectively; hereafter, treatment) and (2) proximity to the Chernobyl nuclear power plant (Fig. 4). The key finding in this study is that exposure to radionuclide contamination alters bank vole gut microbiota composition, but have little effect on the gut microbiota alpha diversity.

Host characteristics such as age, sex, body mass or reproductive status had little consistent effect on bank vole gut microbiota composition. However, that the gut microbiota of bank voles inhabiting uncontaminated CL areas within the CEZ and the distant (~80 km, Fig. 4) control KL areas differed from the microbiota of animals from contaminated CH areas highlights the association between radiation exposure and bank vole gut microbiota (Fig. 5). Geographic variation in gut microbiota composition typically co-associates with changes in habitat (*e.g.* habitat type, vegetation, food quality) (Amato et al., 2013; Grieneisen et al., 2019). Thus, ecological variation can be predicted to occur over small spatial scale. Indeed, rodent gut microbiota can differ among populations separated by just a few (1-10) km (Goertz et al., 2019; Knowles et al., 2019). Given the potential for fine scale variation, the similarities between the gut microbiota of bank voles from uncontaminated CL and control KL areas are striking (Fig. 5). The implication is that the gut microbiota of bank voles inhabiting contaminated CH areas within the CEZ are primarily shaped by radionuclide contamination related factors that outweigh the importance of a spatial structure.

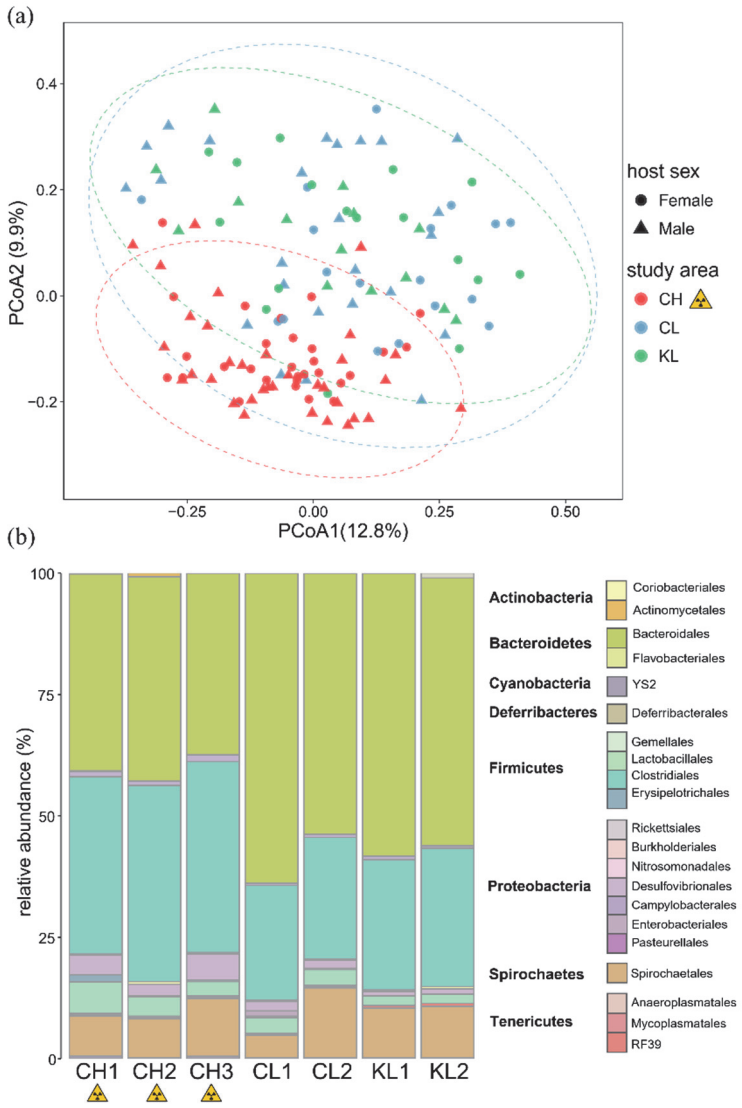

Fig. 5. Differences in bank vole gut microbiota composition and structure associated with exposure to radionuclide contamination. Shown are (a) PCoA based on Bray–Curtis dissimilarity between gut microbiota profiles, and (b) mean relative abundance of bacterial taxa in the gut microbiota of bank voles inhabiting the replicate sites within each study area contaminated (CH1-3) and uncontaminated (CL1-2) with radionuclides within the Chernobyl Exclusion Zone and an uncontaminated area near Kyiv (KL1-2), Ukraine.

Extensive inter-individual variation in gut microbiota profiles reflects natural heterogeneity in hosts and/or their habitat (Knowles et al., 2019; Maurice et al., 2015), and is expected given the deliberately heterogeneous sampling used in this study. Indeed, in the absence of exposure to radiation, bank voles exhibit considerable inter-individual variation in their gut microbiota profiles (Fig. 5). However, bank voles from all the replicates of contaminated areas characterised by a much lower level of inter-individual variation in the gut microbiota (Fig. 5). Thus, exposure to radionuclide contamination appears to reduce natural gut microbiota heterogeneity and select for distinct gut microbiota profiles in CH voles. Variable gut microbiota are thought to be beneficial for host health and fitness in wildlife (Alberdi et al., 2016). Accordingly, high inter-individual variability have been reported in other studies and likely reflects the normal 'healthy' state of wild rodent gut microbiota (Maurice et al., 2015; Ren et al., 2017). The implication is that inhabiting an area with elevated levels of radionuclide contamination associates with the community-wide microbiota perturbations.

Effects of radiation exposure on bank vole gut microbiota were evident as change in proportion of higher taxa, with an almost two-fold increase in the ratio of Firmicutes to Bacteroidetes phyla in areas contaminated with radionuclides (CH) compared with all other uncontaminated areas (CL and KL). The replacement of Bacteroidetes by Firmicutes (F:B ratio shift) was also apparent across taxonomic levels, including changes in the relative abundance of bacteria from the Bacteroidetes family *S24-7* (other proposed names: *Homeothermaceae* or *Muribaculaceae*, see (Lagkouvardos et al., 2019; Ormerod et al., 2016)) and the *Ruminococcacae*, *Lachnospiraceae* and *Lactobacillaceae* groups in Firmicutes. These trends were consistent across replicate sites within each treatment (Fig. 5), thus samples could be readily classified to contaminated or uncontaminated areas based solely on microbial community composition with >90% accuracy. The implication is that the F:B ratio summarises a robust association between radiation exposure and bank vole gut microbiota.

Bacteroidetes and Firmicutes dominate (>80% relative abundance) mammalian gut microbiota, and primarily associated with bacterial metabolic potential and host energy harvest (Ley et al., 2008; Turnbaugh et al., 2006). In a broad context, the F:B ratio is affected by diet in humans and animal models (Carmody et al., 2015; David et al., 2014). Moreover, beyond the phylum level, taxa that drive the F:B ratio shift in bank voles (*e.g. S24-7*, *Ruminococcaceae* and *Lachnospiraceae*) appear to be particularly sensitive to dietary variation in studies of wild animals (Schmidt, Mykytczuk, & Schulte-Hostedde, 2019). Hence, it is

conceivable that a distinct gut microbiota in areas contaminated with radionuclides reflect changes in host diet. There are no detailed biodiversity surveys of the CEZ and surrounding areas, but a dietary change in bank voles can potentially be a secondary consequence of a broader ecological impact of radiation on habitats, affecting availability of certain foods (*e.g.* for arthropods see (Møller & Mousseau, 2009)) at contaminated areas within the CEZ. Alternatively, bank voles inhabiting CH areas actively select some and/or avoid other specific food items present in their environment. This idea is analogous to the studies that have shown that wood mice (*A. sylvaticus*) use food selection as a mean to reduce heavy metals intake in laboratory experiments (Beernaert et al., 2008) and under field conditions in contaminated environment (Ozaki et al., 2018). Whether such dietary changes are adaptive and, similarly as in the case with wood mice, can help bank voles to reduce exposure and/or increase food quality when inhabiting CH areas is unknown. However, apart from nutritional content, dietary changes can also affect microbial functions, abundance of some products of bacterial fermentation (*e.g.* short-chain fatty acids, SCFAs), and thus overall metabolite profiles (Gentile & Weir, 2018; Koh et al., 2016). These microbial metabolites can impact host physiology and health, and have important functions for DNA repair and protection against oxidative stress (Hamer et al., 2007; Rooks & Garrett, 2016). Thus, changes to the SCFAs profiles can be adaptive, for example, to mitigate effects of elevated oxidative stress concomitant with chronic exposure to radiation. This hypothesis warrant further studies that could quantify SCFAs profiles and thoroughly assess host diet.

It is an open question as to whether changes in gut microbiota can be used as a consistent 'biomarker' of exposure to radionuclides or any other environmental contaminants (Tu et al., 2020; A. Zhang & Steen, 2018). Of course, the use of F:B ratio shift as a biomarker of radiation exposure would simplify the far more complex compositional and potentially functional perturbations associated with inhabiting an area contaminated with radionuclides. Also, it is unclear whether these findings are specific to bank voles or represent a more general pattern similar across other mammals (tested in the study IV). However, if similar trend holds true in other host species, changes to the 'wild-type' gut microbiota may be useful in this context, especially as faecal samples can be collected non-invasively.

The results of this study have generated many interesting questions that largely determined direction of the work described in the following chapters of this thesis. I present these findings as published in the *ISME Journal*.

4.2 Bank vole skin microbiota in relation to radiation exposure: distinct microbiota responses within an individual animal host (study II)

In this study, I tested whether different (*i.e.* skin and gut) microbiota associated with an individual host exhibit a convergent response to similar exposure to radionuclide contamination. Using the same study design and sampling areas as in the study I, I examined (1) the impact of exposure to radionuclide contamination on the bank vole skin microbiota, and (2) the effect of spatial separation on the skin microbiota at the small (7-40 km) and large (>80 km) spatial scales (Fig. 4). In addition, given that a set of animals (n=93 out of 157) were sampled for both the skin and the gut microbiota, this study allowed to make (3) a direct comparison between the response of the skin and gut microbiota to the changes in the environment within an individual animal host.

One of the main results in this study is that the bank vole skin microbiota are shaped more by geographic location than level of radionuclide contamination in the environment. Thus, both microbial community diversity and structure were more similar in bank voles within the CEZ, whether contaminated (CH) by radionuclides or not (CL), compared with animals from uncontaminated locations outside the CEZ, near Kyiv (KL). Notably, the factors determining the mammalian skin microbiota composition in natural environment are largely unknown, and this study is one of the first to examine skin microbiota in a non-human mammal (other than bats) in the wild (Ross et al., 2019). However, that the most pronounced differences in the bank vole skin microbiota occurred at a larger spatial scale is consistent with few available studies (on bats, amphibians, domestic animals) that emphasise the importance of the surrounding environment, and potentially its microbial diversity, in shaping vertebrate skin microbiota community composition (Avena et al., 2016; Bletz et al., 2016; Lehtimäki et al., 2018).

The diversity of host-associated microbiota is largely positively associated with host health (Bello, Knight, Gilbert, & Blaser, 2018; Carthey et al., 2019), yet the contribution of host characteristics and environmental factors in modulating skin microbiota diversity in wild animals remain poorly understood. In bank voles, neither levels of soil radionuclide contamination at the trapping location nor individual-level radiation dose estimates (^{137}Cs burden) explained the variation in the skin microbiota diversity. Instead, spatial distance was important, thus voles inhabiting more closely located areas within the CEZ (CH and CL) harboured more diverse skin microbiota than animals from the distantly located KL area. Moreover,

within the CEZ there was a substantial variation among the replicate sites, with bank voles captured in the Red Forest (one of the most radioactive sites within the CEZ) and adjacent areas having the highest number of unique sequences. These results are somewhat surprising, as anthropogenic habitat modifications have widely reported negative impacts on macro- and microorganisms biodiversity (Carthey et al., 2019; Foley et al., 2005), and are expected to have a concomitant effect on the skin microbiota diversity because these communities are sourced primarily from the environment (Clemente et al., 2015; Council et al., 2016; Grönroos et al., 2019; Ross et al., 2018). Indeed, reduced diversity of bank vole skin microbiota may be also predicted based on studies on free-living (Romanovskaia et al., 1998) and host-associated (Czirják et al., 2010; Ruiz-González et al., 2016) microbial communities isolated from contaminated areas within the CEZ, where elevated levels of radionuclides had a negative impact on diversity estimates (but see (Ragon et al., 2011; Theodorakopoulos et al., 2017)). Importantly, these studies were either conducted at a local scale (*e.g.* soil samples collected within 10 m, from a single site) or microbial communities were examined using culturing methods, thus from these studies it is difficult to make a definitive conclusion about radiation effects on microbial diversity within the CEZ. However, the consistently high skin microbiome diversity in bank voles inhabiting the CEZ, and especially the Red Forest, suggests that the radionuclide contamination of the environment does not reduce alpha diversity of environmental microbial reservoirs.

At the individual level, male bank voles harboured more diverse microbial communities on their skin compared with females. That sex-specific differences were also evident in the skin microbiota composition and were consistent across study areas, suggests that sex differences might represent a more general trend due to some physiological properties of the sexes. For example, sexes can differ in skin thickness, secretions, pH and/or hormone profiles (Dao & Kazin, 2007; Fierer et al., 2008). If so, then sex differences in the skin microbiota should be commonly reported also in other animals, yet surprisingly such differences have only been identified in a single study on captive red kangaroos (Ross et al., 2018). That said, the lack of sex documentation is prevalent in skin microbiota studies of many vertebrate clades (largely due to the difficulty of non-invasive sexing methods) (Ross et al., 2019). Thus, sex difference can still be a typical feature of the skin microbial communities, which was simply overlooked in other host species. Alternatively, variation in the skin microbiota between sexes in bank voles reflects a difference in behaviour and/or life history traits that influences the type of environments, and hence microbiota, experienced. For example, as typical of many

small mammals (Lawson Handley & Perrin, 2007), during the breeding season bank vole females are territorial and their home ranges are much smaller than those of males (Kozakiewicz et al., 2007). Consequently, male bank voles tend to move more to forage and/or to find mates. Greater movements may provide the opportunity for male bank voles to acquire a more diverse microbiota. This is somewhat analogous, to an apparent effect of lifestyle (*e.g.* time spent outdoors; inhabiting rural or urban environments; exposure to biodiversity) on skin microbial diversity of other animals and even humans (Grönroos et al., 2019; Hanski et al., 2012; Lehtimäki et al., 2018). How accurately diversity of the skin microbiota reflect the pool of microbes present in the environment is an open question, yet the potential effect of the habitat use on the bank vole skin microbiota reinforces the importance of environment in determining skin microbiota of wild animals.

As a whole, the studies I-II indicate that bank vole gut and skin microbiota respond to different environmental cues. Namely, bank vole skin microbiota are primarily shaped by geographic location, co-associated changes in the surrounding environment and host sex, with community-wide divergence occurring at a larger spatial scale; whereas bank vole gut microbiota are affected more by local variation in level of radionuclide contamination with no apparent influence of spatial structure. That the bank vole skin and gut microbiota are shaped at different spatial scales is consistent with the other study that found a marked effect of geography over similar distance of ~100 km on the skin, but not the gut microbiota of a wild mammal (Tasmanian devils, *Sarcophilus harrisii*) (Cheng et al., 2015). Thus, what is more striking is that exposure to radionuclides has little notable impact on the bank vole skin microbiota, although the individual-level dosimetry data indicate that in the contaminated areas animals were indeed exposed to radiation. This is perhaps where a direct comparison between the skin and gut microbiota within a single bank vole host has its most power and emphasises the distinct responses that the bank vole skin and gut microbiomes have to similar levels of radiation exposure. It is possible that microbiota have adapted to radiation, although it seems unlikely this happened exclusively to the skin microbiota, and/or that gut bacteria are inherently more radiosensitive. On the other hand, the ultimate composition of the skin and gut microbial communities reflect selection within their distinct niches. That is, the skin microbiota are determined largely by environmental diversity, while gut microbiota are strongly influenced by host physiology (immune system, metabolism) and diet (whose relationship to the environment is not always straightforward) (Woodhams et al., 2020). With this in mind, the discrepancy in the

skin and gut microbiota responses may potentially be attributed to spatial variation in environment and dietary sources.

In short, key finding in this study is that bank vole skin and gut microbiomes have distinct responses to similar environmental cues and are structured at different spatial scales. This study demonstrate that defining several microbiomes within an individual host can be useful for better understanding of the host-environment interactions. I present these findings as published in the *Microbiome* journal.

4.3 Temporal dynamics of the bank vole gut microbiota in relation to radiation exposure: applying the *Anna Karenina principle* to wild animals gut microbiota (study III)

The mammalian gut microbiota are pivotal to host health and it is therefore important to recognise microbial community instability, or so-called dysbiosis, especially in relation to environmental disturbance and stress (Rocca et al., 2019; Trevelline et al., 2019). A recent framework to describe impacts of host stress on microbiota structure proposes that exposure to various stressors can disrupt mechanisms of host control over the microbiota composition, leading to high inter-individual microbiome variability and temporal changes in dysbiotic microbial communities (Zaneveld et al., 2017). Conversely, microbiota of healthy animals are thought to be temporally stable and relatively similar among individuals (Fig. 2). In microbial ecology, this concept is known as the *Anna Karenina principle* (AKP), following the opening line of Tolstoy's Anna Karenina novel: '*All happy families are alike; each unhappy family is unhappy in its own way*'. The AKP has proven to be useful, for example, in diagnostics of microbiota dysbiosis under exposure to stress in biomedical settings, and in research on microbiota of threatened coral reefs (Zaneveld et al., 2017). That said, the AKP effects remain largely unexplored in wild mammals living under stress conditions in a disturbed environment.

Given the multiple lines of evidence at the population (Mappes et al., 2019), organismal (Lehmann et al., 2016) and molecular levels (Kesäniemi, Jernfors, et al., 2019), bank voles inhabiting contaminated with radionuclides areas within the CEZ have the reduced ability to regulate critical health-variables, experience elevated levels of stress (Del Giudice et al., 2018), and therefore, provide an interesting test of the AKP in a wild mammal. The studies I and II, indicate that exposure to radionuclides is associated with a marked changes in bank vole gut microbiota, yet these studies have a cross-sectional design and lack one crucial component – time, and thus considerations of temporal variation. In this study, I

test key predictions of the AKP by conducting a capture-mark-recapture survey of bank voles inhabiting areas that contrast in levels of radionuclide contamination (CH and CL within the CEZ, see Fig. 4), to quantify how stress impacts microbiota stability within animal host over time. If the AKP is applicable to my study system, then the gut microbiota of bank voles experiencing stress (radiation exposure) would be characterized by an increase in inter-individual differences and a lack of temporal stability.

The main result in this study is that bank voles inhabiting uncontaminated CL areas within the CEZ harbour variable (increased inter-individual differences) and temporally dynamic gut microbiota, whereas animals exposed to radiation (from CH) host more similar gut microbial communities (within CH, but distinct to CL) that are also remarkably stable over time (Fig. 6). Such contrast in the temporal stability of bank vole gut microbiota in relation to radiation exposure is somewhat unexpected and counter to the key predictions of the AKP. Hence, while environmental stress can alter microbiota community composition, it does not necessarily increase inter-individual differences or temporal variability in the gut microbiota structure (Fig. 6). Together these results highlight two key features of the CEZ bank voles gut microbiota: (1) in the absence of exposure to radiation, their gut microbiota are apparently free to adopt a wide range of community configurations over time, (2) constraint of CH bank voles gut microbiota under chronic exposure to radionuclides.

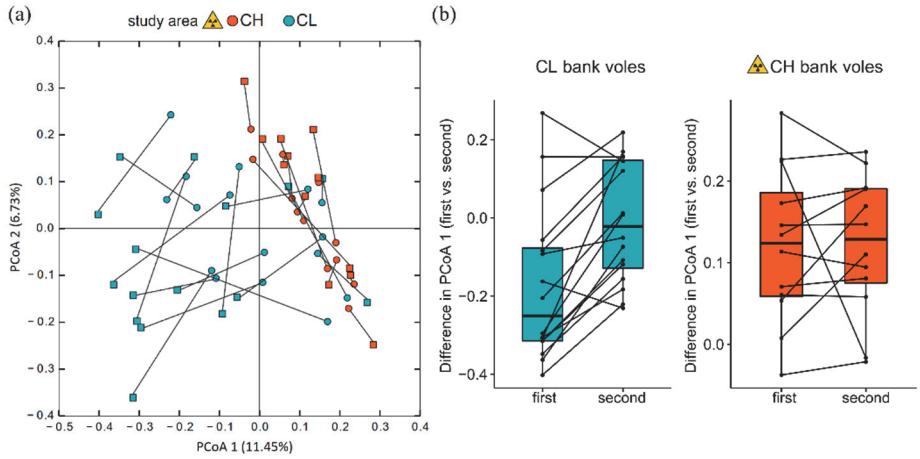

Fig. 6. Differences in temporal dynamics of bank vole gut microbiota in relation to radiation exposure. (a) PCoA based on Bray–Curtis dissimilarity between the gut microbiota profiles of bank voles inhabiting areas that differ in levels of radionuclide contamination. Each point represents a single sample, shape indicate paired first (square) and second (circle) samples from a recaptured individual that are connected by a solid line. (b) Differences in Bray-Curtis dissimilarity PCoA axis 1 (samples from same individual are connected by a solid line) among study areas.

Contrast in the temporal stability of the gut microbiota of bank voles inhabiting contaminated and uncontaminated areas may occur due to a combination of direct and indirect impacts of radiation exposure upon CH bank vole hosts, their microbiota or both, through changes in the environment (Fig. 7, the **six** hypotheses discussed below). **First**, constraints to the gut microbiota of CH, but not CL voles, may just be driven by a strong selection imposed by radiation exposure for certain bacterial taxa due to their radiosensitivity. While the individual-level dosimetry data (*e.g.* TLDs, gamma spectrometry) indicate that CH voles were indeed exposed to about two orders of magnitude higher radiation doses than CL voles (mean 68.7 vs. 0.4 mGy, respectively), strong selection due to such exposure seems unlikely (Shuryak, 2019), and in both areas (and across studies I-II) bank voles maintain similar alpha diversity. **Second**, given that to some extent host-associated microbiota are derived from the pool of microbes available in the environment (Bletz et al., 2016; Perofsky, Lewis, & Meyers, 2019), differences between the gut microbiota of CH and CL voles may be a secondary consequence of a wider ecological impact of radiation on the habitats within the CEZ. However, the few differences in the skin microbiota of bank voles inhabiting contaminated and

uncontaminated areas found in the study II, imply that radionuclide contamination *per se* have little influence on the pool of microbes in the surrounding environment. Thus, given the importance of host-microbe interactions, it is conceivable that the host is more involved in these processes through altered physiology and/or dietary variation.

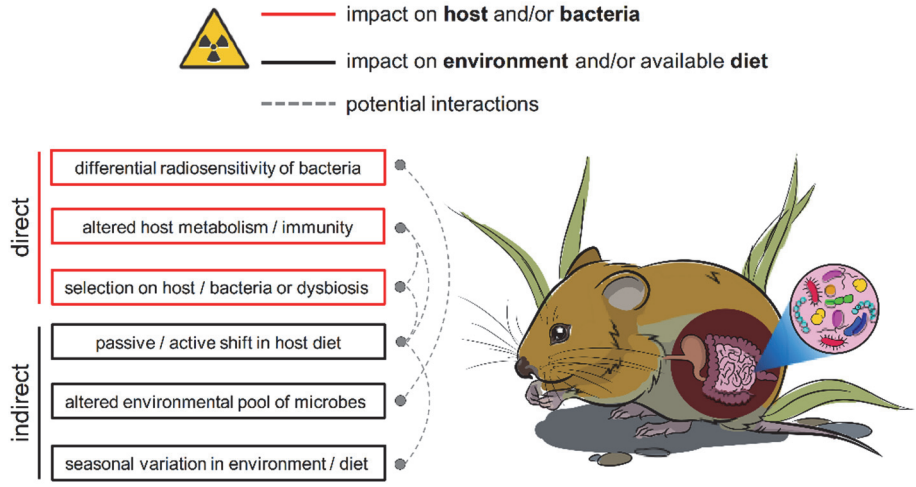

Fig. 7. The hypotheses describing potential direct (on host or bacteria) or indirect (on environment and available diet) impacts of radiation exposure on the bank vole gut microbiota.

Notably, bank voles inhabiting the CEZ have altered metabolic (*e.g.* upregulated lipid metabolism, fatty acid oxidation) and immune (*e.g.* immunosuppression, impaired antigen processing) profiles (Kesäniemi, Jernfors, et al., 2019), the two systems central for the homeostasis and microbiota stability (Gentile & Weir, 2018; L. V. Hooper, Littman, & Macpherson, 2012). Therefore (**third**), constraints on the gut microbiota of CH voles may be driven by the physiology of a stressed host, which prevents natural temporal variation in the gut microbiota composition, typical to animals inhabiting CL areas. This hypothesis is plausible and supported by the liver and spleen transcriptome study (Kesäniemi, Jernfors, et al., 2019), yet resolving the exact mechanisms of such host-microbe interaction requires further studies.

Distinct gut microbiota of bank voles in contaminated areas within the CEZ can also be a sign of a dietary change (**fourth**). Such shifts in diet could either be

passive, due to potential differences in food availability between CH and CL areas (for arthropods see (Møller & Mousseau, 2009)), or active as if bank voles exposed to radiation would actively select food items present in the environment to mitigate effects of harmful exposure (Ozaki et al., 2018). Selective foraging could also be a consequence of the altered host physiology (*e.g.* metabolism) discussed above. In this study, I examined potential variation in bank vole diet between treatments using stable isotope analysis, quantifying variation in stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopes in bank vole tissues (fur and liver, see study III). The stable isotope data from liver tissue that reflect dietary profiles of bank voles during summer season (between captures) indicate no major difference in bank vole diet between CH and CL areas. Moreover, there was little difference in variance of the stable isotope profiles between study areas, in contrast to the variation in the gut microbiota structure (Fig. 6). Thus, variation in bank vole summer diet alone seems unlikely to explain contrast in the temporal stability of the gut microbiota of CH and CL voles. That said, fur stable isotope data suggest some differences in dietary source of carbon (*e.g.* plants) between bank voles from CH and CL areas, in early spring, before the first capture. While it is difficult to directly link inter-tissue (fur and liver) variability in the stable isotope profiles to changes in bank vole diet from early spring to summer, contrast in the magnitude and direction of such variability (see study III) points on potential differential responses of CH and CL voles to seasonal changes in diet.

Difference in the way bank voles from CH and CL areas respond to seasonal changes (**fifth**) may potentially explain contrast in stability of the gut microbiota between treatments over time. Interestingly, several lines of evidence support potential influence of seasonal changes in diet and/or environment on the gut microbiota variability, but only in bank voles inhabiting CL areas. For example, temporal changes in the gut microbiota of CL voles were characterised by a consistent shift (spatially synchronised), which was largely similar in direction and the magnitude of change among individuals (Fig. 6).

Moreover, changes between the first and second capture of bank vole inhabiting CL areas were associated with a reduction in the proportions of specific bacterial taxa (*e.g. S24-7* family) that are notably sensitive to variation in diet and season (Ormerod et al., 2016; Stevenson, Duddleston, & Buck, 2014; van Leeuwen, Mykytczuk, Mastromonaco, & Schulte-Hostedde, 2020). Indeed, high inter-individual variability and temporal changes in composition with season change are consistent with the longitudinal gut microbiota observations in wild rodents (Maurice et al., 2015; Ren et al., 2017), and thus appear to reflect a typical 'healthy'

state in nature. With this in mind, the apparent lack of seasonal changes in the gut microbiota of bank voles inhabiting CH areas is striking and can be a sign of selection (on host and/or microbiota) or microbiota dysbiosis that hampers an appropriate response to natural spatio-temporal variation in resources (**sixth**). For example, selection for radioresistance could target host physiology (metabolism and/or immunity, see (Kesäniemi, Jernfors, et al., 2019)) that imposes constraints on the gut microbiota, or select for specific gut microbiota that provide beneficial services (*e.g.* SCFAs, see study I for detailed discussion). The gut microbiota dysbiosis can potentially have an adverse effect on host health. Notably, bank voles inhabiting CH areas characterised by rather poor body condition (see study III), yet whether these effects are related or such microbiota dysbiosis have any further fitness costs is unknown. Importantly, if radiation exposure indeed induces gut microbiota dysbiosis, in CH voles it manifests itself in a loss of beta diversity and temporal stability of gut microbiota profiles, rather than in stochastic increase in inter-individual differences and temporal changes in microbiota as predicted by the AKP (Zaneveld et al., 2017).

These data on bank vole response to chronic radiation exposure highlight the need to expand the AKP to consider context-dependent variables, in addition to a set of general predictions (Zaneveld et al., 2017). This is because outcomes of stress on microbiome are context-dependent, and environmental stressors vary in type, severity, mode of action and duration (Rocca et al., 2019). This later point can be particularly important, and AKP needs to recognise differences between responses to acute and chronic stress, as in bacteria the mechanisms and physiological adaptations needed to withstand acute vs. chronic stress may not completely overlap (Shuryak, 2019). Moreover, accounting for impacts of stress on host physiology, for seasonal microbiota variation, and the process of selection can be critical in natural populations. Integration of these important variables, however, could be viewed as a positive challenge as it should only broaden ecological reality of the AKP, much-needed in studies of wild animal microbiomes.

It is important to note that the six hypotheses discussed above (Fig. 7) are not mutually exclusive, and in fact, many interactions can be made. The conclusion of the study III is that environmental stress (radiation exposure) can constrain the natural spatial and temporal variation of wild animal gut microbiota. Further field experiments are needed to determine whether environmental radionuclides impose a direct or indirect impact on bank vole gut microbiota. I present these findings as published in the *Journal of Animal Ecology*.

4.4 Comparable responses of wild rodent gut microbiota to radionuclide contamination (study IV)

In this study, I tested whether exposure to radionuclide contamination associates with comparable gut microbiota responses across wild rodents. To obtain a general assessment of the effects of exposure to radionuclide contamination on gut microbiota, I sampled two pairs of mouse species (*Apodemus flavicollis*, *A. sylvaticus*, *A. speciosus*, *A. argenteus*) that occur in sympatry in habitats affected by radionuclides derived from the Chernobyl and Fukushima nuclear accidents (Fig. 4). I compared the patterns of microbiota response to radionuclide contamination among these four mouse species, with the general prediction that patterns observed in the bank vole (study I) should emerge also in mice experiencing similar radiation exposure. More specifically, the gut microbiota of all four mouse species exposed to radiation at the CEZ and FEZ should be characterised by (1) similar alpha diversity estimates, irrespective of host radiation exposure levels, (2) altered community composition, with a marked shift in the ratio of the Firmicutes to Bacteroidetes phyla (F:B), and (3) altered community structure associated with radionuclide contamination.

Although changes resulting from human activity have widely reported negative impacts on biodiversity (Carthey et al., 2019; Foley et al., 2005), the effect of environmental radionuclide contamination on microbial diversity is unclear. That the mice gut microbiota diversity was rather similar at the contaminated with radionuclides areas (CH, FH) compared with that elsewhere (CL, KL, FL) is consistent with the bank vole gut microbiota (studies I-III), and several studies on free-living microbes sampled from the CEZ and FEZ (Hoyos-Hernandez et al., 2019; Ragon et al., 2011; Theodorakopoulos et al., 2017). That said, this result contrasts with soil, bird feather and earthworm microbiota, where a negative association between radiation dose rate and alpha diversity has been observed (Czirják et al., 2010; Newbold et al., 2019; Romanovskaia et al., 1998; Ruiz-González et al., 2016). One possible explanation for such inconsistency in results is a technical variation as only one of these later studies used culture-independent sequencing methods to quantify microbial communities (in earthworms, see (Newbold et al., 2019)). Indeed, the lack of negative impact of low-dose radiation exposure on microbial alpha diversity perhaps is not surprising as many bacteria can withstand chronic radiation exposure (Ragon et al., 2011; Shuryak, 2019). Elevated gut microbiota alpha diversity in *A. speciosus* from Japan is comparable to the patterns reported for soil (Theodorakopoulos et al., 2017) and skin microbiota

of bank voles (see study II) sampled from some of the most contaminated areas within the CEZ. Such increased alpha diversity might reflect an increase in mutation associated with radiation exposure (Shuryak, 2019). That said, it is not known whether the low-dose (~2 mGy/d) radiation experienced by wildlife within the CEZ and FEZ (Beresford, Barnett, et al., 2020; Kubota, Takahashi, et al., 2015) can have such a direct impact on microbiota. Alternatively, an increase in microbiota alpha diversity may reflect a 'rewilding' of the Chernobyl and Fukushima landscapes that followed human abandonment of these sites (Lyons et al., 2020; Perino et al., 2019; S. C. Webster et al., 2016), which widened niche space available to microbes (Gellie, Mills, Breed, & Lowe, 2017). The equivocal impact of rewilding on gut microbial diversity may indicate that environmental diversity *per se* might not be directly relevant for structuring gut microbiota communities as, for example, it is for the skin (see study II, and (Ross et al., 2019)). Effects of radiation exposure on the mouse gut microbiota alpha diversity reinforces patterns found in the bank vole gut microbiota (studies I-III) and suggest that exposure to radionuclide contamination does not reduce gut microbiota diversity in wild rodents.

Exposure to radionuclide contamination was associated with altered gut microbiota composition in three out of four mouse species. Notable exception to this was *A. argenteus* that exhibited similar gut microbiota composition among radionuclide contamination treatments. Among the bacterial ASVs that apparently responded to radiation, about half were assigned to the Bacteroidetes family *S24-7*. In addition, changes in relative abundance of bacteria from the Firmicutes families, including *Ruminococcacae* and *Lachnospiraceae* were also evident in *A. flavicollis* and *A. speciosus* hosts. Hence, similarly as found for bank voles (studies I), members of the Firmicutes and Bacteroidetes phyla drive compositional changes in response to radiation exposure also in mice species studied. Contrary to the pattern observed in the bank vole, however, in mice such changes do not lead to the characteristic shift in Firmicutes to Bacteroidetes (F:B) ratio between treatments. The lack of a systematic increase in F:B ratio in relation to radiation exposure in all mouse species indicates that the F:B ratio is not a general biomarker of exposure to radiation (as hypothesised in the study I). Selection for a more distinct gut microbiota in mice inhabiting radioactively contaminated areas within the CEZ and FEZ thus operate at a finer level of taxonomic hierarchy (species or even strains). Indeed, multiple taxonomic groups characterised by notable differential responses to radiation exposure at the level of individual ASVs. That is, within major bacterial families (*S24-7*, *Ruminococcacae* and *Lachnospiraceae*), individual taxa exhibited

increase and decreases in the proportion in response to radiation exposure. Such differential responses can be overlooked because of agglomeration of ASVs/OTUs into a single taxonomic group that mask any underlying heterogeneity (Ridenhour et al., 2017). Hence, this finding suggests the overall limited utility of simple taxonomic measures, such as F:B ratio, as biomarkers in ecotoxicology (Tu et al., 2020).

Gut microbiota can influence host fitness (Alberdi et al., 2016) and it is therefore important to know whether changes in the gut microbiota of mice exposed to radiation impact host health. Most variation in mice gut microbiota in relation to radiation exposure can be attributed to the *S24-7* family, which exhibit the highest number of differentially abundant ASVs and consistent changes across host species. This bacterial clade is understudied, as it is difficult to culture and only recently genomic content of the *S24-7* has been characterised (Lagkouvardos et al., 2019). Members of the *S24-7* family appear to be versatile with respect to complex carbohydrates degradation, which likely explain their particular affinity to the gut environments of herbivorous and omnivorous rodents, including *Apodemus* mice (Lagkouvardos et al., 2019). The *S24-7* is a large family and genome-resolved metagenomics support presence of almost 700 species (Lagkouvardos et al., 2019). With this diversity in mind, high level of heterogeneity and the lack of a unified response to radiation within the *S24-7* family perhaps are not surprising, and similar differential responses to various external stimuli have been reported in other rodents (Ridenhour et al., 2017; van Leeuwen et al., 2020). Given the presence of multiple trophic guilds within this clade, each having different metabolic capacities utilising either starch, hemicellulose and pectin, or host-derived glycans (Lagkouvardos et al., 2019), it is plausible that the compositional variation in relation to radiation exposure can also affect microbiota functions. Whether variation in functional capacity of the *S24-7* affects host health, especially during seasonal fluctuations in resource (seeds) availability, remain to be quantified.

Animals living in sympatry share the same environment and thus should experience comparable radiation exposure. With this in mind, the lack of response of *A. argenteus* gut microbiota to radionuclide contamination, in contrast to other three mouse species (*e.g. A. flavicollis*, *A. sylvaticus* and *A. speciosus*) and bank voles (studies I-III), is striking and emphasises importance of host ecology and lifestyle in modulating environment-host-microbe interactions even in sympatry (Perofsky et al., 2019). Species may differ in radiosensitivity, and for example among rodents, chinchillas and guinea pigs are highly sensitive to chronic radiation exposure, while mice and rats are thought to be relatively radioresistant (Shuryak,

2020). Whether *A. argenteus* and *A. speciosus* differ in their radiosensisitivity is unknown, yet they do differ in several aspects of ecology and lifestyle that might be relevant in context of radionuclide contamination. For example, arboreal lifestyle of the *A. argenteus* (Oka, 1992) can substantially reduce the average external radiation exposure for this species compared with the soil-dwelling *A. speciosus*, even when sharing exactly same location (Shuryak, 2020; Stark et al., 2017). Indeed, radiation dose rates measured on the ground level can be up to 2-fold higher than those at 1 m above the ground (Kubota, Takahashi, et al., 2015). This is because the majority of radioactive materials derived from the Fukushima accident were deposited in the topsoil layer and leaf litter in forests within the FEZ (Hashimoto et al., 2013). There are no estimates of fraction of time this species spends on trees vs. ground, yet given that I captured *A. argenteus* using live traps placed on the ground (study IV), they at least sometimes descend to the ground when foraging. Thus, an apparent "radiation escape" of *A. argenteus* can be particularly important during early life as this species give birth and raises young in tree cavities (Oka, 1992). Indeed, disruption of the gut microbiota in early life can have lasting effects on microbiota and host health (Knutie, Wilkinson, Kohl, & Rohr, 2017). The implication is that the discrepancy in microbiota responses between sympatric *A. argenteus* and *A. speciosus* may simply reflect *A. argenteus* radiation escape through its unique tree-dwelling lifestyle, rather than any specific radioresistance (Shuryak, 2020).

The total radiation dose received by an organism comprises of combined contributions made by external and internal radiation exposure (Beresford, Barnett, et al., 2020). Notably, differences in host space use described above should not influence internal radiation exposure derived from consumption of contaminated foods as *A. argenteus* and *A. speciosus* share the same habitats and both are expected to forage on contaminated sources. Consistent with such expectations, these species characterised by similar radiation doses from internal sources (see study IV). Importantly, however, in these species internal dose typically accounts for less than 10% of the total absorbed radiation dose (Kubota, Takahashi, et al., 2015), thus a dominant part of the radiation exposure derive from external sources. It is important to note that in this study the estimates of external radiation dose for *A. argenteus* were extrapolated from experimental TLD data on soil-dwelling rodents (studies III-IV, see also (Beresford et al., 2008; Chesser et al., 2000)). Data from TLDs fitted on *A. argenteus* are required to quantify the effect of species-specific variation in space use on radiation exposure intensity (Beresford, Scott, et al., 2020; Shuryak, 2020). The finding of an apparent importance of host lifestyle

offers an interesting counterpoint for future analyses into effects of radiation or any other toxic exposure on host and its associated microbiota.

Besides the value of this study for research focusing on the impacts of radiation exposure on host-associated microbiota, several findings in this work also highlight more general features of wild animal gut microbiota. For example, differences in gut microbiota of *Apodemus* from Ukraine and Japan readily reflect distinct environments at the two study sites. However, differences or similarities in gut microbiota of sympatric species depend on, for example, host species' identity and niche separation (Knowles et al., 2019; Perofsky et al., 2019). That recently diverged (*ca.* 3.1 myo) *A. flavicollis* and *A. sylvaticus* share a similar niche (Michaux et al., 2005) and diet (Butet & Delettre, 2011; Knowles et al., 2019), likely helps to maintain their similar gut microbiota diversity and composition (see study IV). Conversely, *A. speciosus* and *A. argenteus* belong to more distinct (*ca.* 12.4 myo) phylogenetic groups (Kumar, Stecher, Suleski, & Hedges, 2017; H. Suzuki et al., 2008), and exhibit substantial ecological divergence (Oka, 1992; Sato et al., 2018). These Japanese species of *Apodemus* differ in habitat use (*A. speciosus* is terrestrial and *A. argenteus* is arboreal) and diet (*A. argenteus* is a generalist feeder and *A. speciosus* is a specialist). Both diet and habitat niche segregation (Perofsky et al., 2019; Youngblut et al., 2019) are associated with divergence of gut microbiota in other wild mammals and can explain the divergent gut microbiota of these congeners. As such, these data emphasise the importance of host ecology for inter-specific variation and microbial community assembly in wildlife.

In conclusion, sampling of multiple host species from habitats surrounding the Chernobyl and Fukushima nuclear accident sites allowed more general assessment of the effects of exposure to radionuclide contamination on gut microbiota across host species and geographical areas. The main finding in this study is that radiation exposure alters the gut microbiota composition and structure in three out of the four species of *Apodemus* mice. The notable lack of association between the gut microbiota and soil radionuclide contamination in *A. argenteus* likely reflects host radiation escape through its unique tree-dwelling lifestyle. In general agreement with my predictions, these data confirm key patterns found in the bank vole gut microbiota (studies I), and also suggest importance of host ecology in modulating effects of radiation exposure on host and its associated microbiota. I present these findings as a manuscript, submitted for consideration to *Molecular Ecology*.

5 Conclusions and future directions

Anthropogenic habitat impacts convert natural environment and represent a threat to wild animal populations. The human-mediated climate change, habitat destruction, land-use change, disease spread and environment contamination have been shown to have fundamental impacts on biodiversity and ecosystem functioning (Haddad et al., 2015). However, the effects of such environmental stressors on wildlife microbiota are largely unknown (Trevelline et al., 2019), despite the importance of the microbiota for host health (T. A. Suzuki, 2017). In this thesis, I used marker gene sequencing and field studies to examine effects of chronic exposure to anthropogenic radionuclide contamination upon host-associated microbiota. The use of an ecologically relevant exposure and wild mammals as a model system, let me to conclude that environmental stressors (here, in the form of radionuclide contamination) have the potential to alter host-associated (gut) microbiota in wild animals (Table 1, studies I-IV). Such changes in the gut microbiota of exposed individuals seem to be persistent over time (study III), yet with unknown consequences for host fitness. By conducting a comparative study, I confirmed key patterns across multiple host species, and at an independent study site (study IV). I showed that host ecology and lifestyle can potentially modulate effects of radiation exposure, and likely, also exposure to other environmental contaminants (study IV). Together, results in this thesis (studies I-IV) indicate that inhabiting areas contaminated with radionuclides is associated with marked changes in wildlife gut microbiota. Although it remains challenging to identify the exact mechanism behind such changes, they likely derive from a combination of direct and indirect impacts of radiation exposure on bank vole and mouse hosts, their associated microbiota and/or their environment. In the studies III-IV, I have narrowed down these possible scenarios to a set of testable hypotheses (Fig. 7) that could be explored in future studies.

Vertebrate gut microbiota are dynamic and ecologically complex communities, whose variation due to natural processes or environmental perturbations can impact host phenotypes and thus fitness (T. A. Suzuki, 2017). Although a number of studies have shown the extent of gut microbiota variability in wild animal populations, only few have actually linked it to host performance (Kohl et al., 2014; Sommer et al., 2016). Surveys using marker gene sequencing are important and contribute to our understanding of microbial communities across a wide range of taxa, yet it is necessary to integrate additional manipulative experiments and omics' techniques to identify downstream effects of any microbiota changes on host health. For

example, metagenomic, metatranscriptomic sequencing, and metabolomics move beyond the limits of marker gene analyses providing strain-level resolution, and allowing detection of genes, gene expression patterns and metabolites thus profiling functional capacity of an entire microbial community (Knight et al., 2018). These omics' techniques can help to link compositional microbiome variation in wild animals, to variation in their microbiota functional potential, and eventually to variation in host phenotypes and fitness as these are interrelated (T. A. Suzuki, 2017).

One of the most important of the remaining questions in this thesis is whether changes in the gut microbiota composition and community dynamics found in animals exposed to radionuclide contamination have functional significance and concomitant impacts on host health. Although bank voles inhabiting contaminated CH areas within the CEZ show signs of altered metabolic and immune systems functioning (Kesäniemi, Jernfors, et al., 2019), in a broad sense CH bank voles are seemingly healthy and, for example, exhibit no signs of major histological abnormalities or pathological changes in the intestine (colon) tissue (Jernfors, Lavrinienko et al. *unpublished*). Thus, it is plausible that the gut microbiota changes in CH voles are adaptive, as if (for example) certain microbiota community composition is selected to provide host with beneficial services through microbial functions. However, only recently have the metagenomics data from Chernobyl bank voles became available and can potentially provide additional insights along the topic. Preliminary phylogenetic analysis of the metagenome assembled genomes (MAGs) is generally consistent with the marker gene data presented in this thesis (studies I-III), and indicates that bank vole gut microbiota are dominated by members of the Bacteroidetes and Firmicutes phyla (Lavrinienko et al. *unpublished*). Notably, all the Bacteroidetes genomes (n=132) were assigned to the Bacteroidales order. This is interesting as according to the studies I-III, this order in the bank vole gut microbiota seems to almost exclusively (>96%) comprise of the unclassified members of the *S24-7* family. While the MAGs data are not yet resolved at the lower taxonomical levels, diversity within the Bacteroidales order in bank voles indeed most likely is limited to the *S24-7* group. This is in line with other studies that show that bacteria within the *S24-7* dominate rodent gut microbiota, with the highest prevalence and abundance found in wild bank voles and mice (Knowles et al., 2019; Lagkouvardos et al., 2019). Notably, this group seems to be particularly sensitive to various environmental perturbations (*e.g.* captivity, environment, diet and season change) (Ormerod et al., 2016; Schmidt et al., 2019; van Leeuwen et al., 2020). Indeed, at least in the context of radionuclide

contamination, the *S24-7* is responsible for most variation in the bank vole and mouse gut microbiota, with consistent differential abundance between treatments as evident across studies. Interestingly, recent study suggest that based on the metabolic capacities the *S24-7* can be grouped into the three trophic guilds, each utilising a set of different carbohydrate-based substrates (Lagkouvardos et al., 2019). Which guild is more common or whether these different guilds co-occur in bank vole gut microbiota is unknown. However, the low-divergence (shallow) phylogenetic diversity within this group in MAGs data points on potential presence of multiple *S24-7* clades in bank vole gut (Lavrinienko et al. *unpublished*). Given that a change in microbiota composition can elicit a change in its function (Visconti et al., 2019), it is important to quantify the consequences of fluctuations in abundance of the *S24-7* family for bank vole and mouse hosts health. The high prevalence (100%, in >450 samples) and numerical predominance of the *S24-7* in sampled bank voles and mice, as well as their remarkably high variability in relation to environmental factors (including radionuclide contamination), as such make this group an interesting target for future studies focusing on environment-host-microbe interactions.

Several more practical conclusions can be made from work presented in this thesis, which can be translated to suggestions for future studies, either specifically focusing on host-associated microbiota in relation to radionuclide contamination, or studying general effects of (any) environmental stress on wildlife.

Experimental design

Many studies from Chernobyl area have been criticised for the lack of replication in their experimental design (Møller & Mousseau, 2016). This is because studies that use samples collected from a single contaminated and a single uncontaminated (control) area have little statistical power to draw robust conclusions. In this thesis, I have ensured replication by sampling spatially separated locations (geographically independent) within each treatment (studies I-IV). The results in the study I perhaps demonstrate the true value of replication, as it would be difficult to make a clear conclusion without multiple treatment-level replicates within and outside the CEZ (Fig. 4). Such study design makes the case even stronger as radiation effects were apparent across replicates of contaminated (CH) areas, but not in uncontaminated areas within (CL) or outside (KL) the CEZ (study I). Of course, such heterogeneity in sampling effort comes at the cost of additional ‘noise’,

yet is important to deconfound potential effects of exposure to radiation from other environmental factors specific to a certain location. Thus, future studies at Chernobyl and Fukushima, especially with cross-sectional observations, should use replication on spatial scale to ensure rigorous experimental design. For example, samples from parts of Belarus and/or Russia affected by the Chernobyl accident could help to mitigate (potential) autocorrelation associated with spatial clustering of areas contaminated and uncontaminated with radionuclides (*e.g.* allowing star-like or more scattered sampling scheme), if only it would be possible to access these areas to conduct fieldwork. On the larger scale, there is some debate on whether the Chernobyl and Fukushima accident sites themselves can be considered replicates. While comparisons of the biological effects of exposure to radionuclides derived from the two accidents are certainly possible and justifiable due to the presence of large amounts of radionuclides with long half-lives (principally ^{137}Cs) at both sites, they differ in terms of duration of exposure (number of generations) after the accidents (Hiyama et al., 2012; Møller et al., 2012), levels and composition of released radioactive isotopes (Steinhauser et al., 2014), as well as in general environmental conditions. With this in mind, perhaps samples from parts of Belarus and Russia affected by the Chernobyl accident can be more readily comparable, increasing the level of replication (Møller & Mousseau, 2016).

Exposure estimates

It is important to directly measure levels of exposure when studying the effects of any environmental contaminants on animals in their natural environment. In this thesis, I used several radiation dosimetry methods that generally show that animal's radiation exposure can be predicted from the soil radionuclide contamination level at their trapping location (studies III-IV). That said, such relationship conform closely only in soil dwelling rodents that live in close proximity to the ground, as most radionuclides tend to settle in the topsoil layer (Shuryak, 2020). Indeed, in the study IV the total radiation doses for the tree-dwelling *A. argenteus* cannot be resolved without direct measurements in the wild (implanting TLDs). The implication is that host lifestyle can modulate radiation exposure intensity, thus in some species, the apparent lack of response may actually be due to the lack of exposure. This conclusion is likely also applicable to other studies in ecotoxicology, as research in animal populations experiencing environmental stress in the wild differs considerably from the laboratory studies where dosage or intensity of exposure are a subject of experimental design itself. Hence, exposure levels to

radiation or any other environmental contaminants should always be made explicit, preferably using direct measurements from organisms studied. In addition to such apparent effect of spatial segregation on exposure intensity, several other parameters including organism movement (migration, dispersal), life stage (prenatal vs. early life vs. adult), and physiology (metabolism) can also determine outcomes of exposure to environmental contaminants (Stark et al., 2017). Thus, future studies in ecotoxicology should consider organism characteristics, species ecology and life history traits more deeply when quantifying effects of environmental contaminants on wildlife.

A more holistic approach to understanding host-microbiota interactions

Despite the many studies reporting that both gut and skin microbiota are pivotal for host health, these communities are typically studied independently of each other in wild animals, even in biomedical research. The results in this thesis demonstrate that targeting distinct microbiomes within an individual host can yield particularly interesting insights (study II). Indeed, bank vole skin and gut microbiomes have striking contrast in their responses to similar environmental exposures and are structured at different spatial scales. Distinct responses of skin and gut microbiota in various conditions were also reported for amphibian, fish, bat and human hosts. For example, salamander larvae gut and skin microbiomes are generally known to be habitat-specific, however, when hosts were experimentally transferred into novel environment (ponds and streams), skin microbiota matched the microbiota similar to the destination habitat controls, while gut microbiota of the same individuals shifted differentially depending on the directionality of transfer (Bletz et al., 2016). Similar differential responses of skin and gut microbiota were also found in a translocation experiment (with change in environment and diet) of Atlantic salmon (T. M. U. Webster, Rodriguez‐Barreto, et al., 2020), and in context of cohabitation (humans and pets) (Song et al., 2013), temporal dynamics (in social bats) (Kolodny et al., 2019), and environmental stress exposure (in fish) (T. M. U. Webster, Consuegra, & Leaniz, 2020). The implication of such differential responses of the gut and skin microbiota to the same exposures is that these two communities respond to different environmental cues. Thus, comparative analysis of multiple microbiomes with an individual host can both address some specific questions and provide much-needed context. Somewhat analogous to this idea, more studies, particularly focusing on the effects of environmental stress on host-associated microbiota, should employ a comparative method (see study IV),

consistently sampling multiple host species to quantify which trends are general and which are species-specific (Hird, 2020). Hence, when possible, future studies should take a more holistic approach and examine multiple microbiomes within an individual host and sample across host species to improve our understanding of environment-host-microbe interactions.

Defining host diet in the field

Several results in this thesis suggest the potential importance of dietary variation in shaping bank vole gut microbiota. In the study III, I used the stable isotope analysis (in fur and liver) to examine potential variation in bank vole diet. Despite the long history of using stable isotopes in animal tissues as dietary indicators (Tieszen, Boutton, Tesdahl, & Slade, 1983), only few studies have employed this technique linking isotopic ratios to variation in microbiota composition (Ingala et al., 2019). While stable isotope analysis is certainly useful to study short- (1-2 weeks in liver) or long-term (1-2 months in fur) dietary trends (Kurle et al., 2014; Robb et al., 2015), it lacks sensitivity for a more resolved analysis of host diet. That is, while the data in the study III suggest that bank vole diet is relatively similar among study areas during summer season, the exact diet composition of sampled individuals remains unknown. Diet analysis using a DNA-based metabarcoding approach (amplifying marker genes from putative dietary items isolated from host stomach or faecal samples) can provide high resolution of host diet composition (Ozaki et al., 2018; Sato et al., 2018). Indeed, diet analysis for animals with narrow range and specialised diets (*e.g.* herbivores) is fairly straightforward (Kartzinel et al., 2015; Pompanon et al., 2012), yet analysis of complex (omnivorous) diets using metabarcoding associates with difficulties related to barcode choice, and/or potential amplification of non-target species (host) when broad coverage primers are used (De Barba et al., 2014; Pompanon et al., 2012). Recent advances in molecular biology allow to resolve some of these technical aspects, for example, by combining multiple barcode loci in the same sequencing run, and by using blocking primers to reduce amplification of host DNA. However, metabarcoding still largely provide qualitative data, which rather represent a ‘snapshot’ of recently consumed foods (Pompanon et al., 2012). Thus, unless used in the longitudinal settings, metabarcoding alone might not be appropriate method for assessing general patterns in host feeding behaviour. That said, stable isotope analysis and metabarcoding can be combined and applied together (perhaps also with field

surveys of available food resources) to provide sufficient breadth and resolution for studies examining small mammals diet in the field.

Ecology matters

Ecological context is key for predicting outcomes of exposure to environmental stressors on microbiota associated with wildlife. Comprehensive analyses of global microbial diversity (Thompson et al., 2017; Woodhams et al., 2020) suggest that for the microbiota associated with animal hosts, the strongest predictor of diversity and composition is whether the sample originate from animal gut or skin, highlighting the contrast between these biomes presumably due to selection within their distinct niches. Microbial ecology of these two microbiomes matters because it effectively defines the way these communities respond to various external perturbations (study II), and how concomitant compositional and functional changes (if any) affect host health. This idea can be further expanded to the animal level, where host ecology and lifestyle can influence intensity of exposure to environmental contaminants (study IV) and thus, also conditions their associated microbiota experience. The implication is that of the microbiota or the host, but ecological context matters for the environment-host-microbe interactions.

From contigs to causality

While studies presented in this thesis demonstrate clear changes in the gut microbiota of small mammals in relation to radiation exposure, the causal relationship between these observations cannot be verified with the available data. For the assessment of causality, manipulative experiments are needed. One way to advance the studies presented here is to choose the path of the biomedical research and move beyond simple observations by modifying gut microbial communities to test hypotheses generated by field-collected data. This can be achieved either by using faecal microbiota transplants (FMT) of an entire community or some isolates of interest, or by applying antibiotic treatment targeting specific groups of bacteria (T. A. Suzuki, 2017). Indeed, FMT is a powerful approach to resolve causality, yet can be particularly challenging when using in non-model species and under field conditions. The use of antibiotics to manipulate host-associated microbiota can have its own advantages, however this method has been criticised due to the many associated confounding effects (Lundberg, Toft, August, Hansen, & Hansen, 2016). Germ-free systems such as laboratory mice can be particularly useful for FMT

experiments, yet these often lack ecological reality (Greyson-Gaito et al., 2020; Martín, Bermúdez-Humarán, & Langella, 2016). Thus, a particularly powerful alternative approach would be to combine field observations and FMT experiments in a single host species (Kohl et al., 2014). Importantly, even in a single host species we lack some key knowledge about various ecological and evolutionary processes behind the FMT (Greyson-Gaito et al., 2020). The rather early state of this field is reflected in a quote from Professor Rob Knight (University of California at San Diego) who said '*you could transplant a sample from Michael Jordan, but you don't know which aspect of Michael Jordan you will get. Maybe you will just go bald*'. Good news is that manipulative experiments do not always require FMTs, and for example, classical designs in ecology and evolutionary biology, such as reciprocal transplants (*e.g.* host transfer between treatments/areas in full factorial design) can be used to make rigorous test of the generated hypotheses (Møller & Mousseau, 2016). Moreover, a combination of the FMT and host reciprocal transplants may allow to study host-microbe interactions in natural environment thus increasing ecological reality (Greyson-Gaito et al., 2020). That said, such experiments are logistically challenging to complete in the free-range habitat. In certain cases, features of host ecology and life history traits can guide researchers and even simplify experimental design. For example, the knowledge that tree-dwelling *A. argenteus* can build nests even in artificial next boxes (Oka, 1992) can be used as a tool to conduct further experiments in the habitats affected by the Fukushima nuclear accident in Japan (study IV). In most cases, however, some type of outdoor enclosures are needed to conduct host reciprocal transplant experiments. Further analyses and field experiments are underway to quantify functional and health consequences of the radiation-induced microbiota alterations.

Concluding remarks

The main message of this thesis is that similar to radionuclide contamination, other environmental contaminants probably also have the potential to affect microbiota associated with wild animals. Variation in the composition of the host-associated microbiota can affect a variety of host phenotypes, including those related to immunity, digestion and development (T. A. Suzuki, 2017). Therefore, understanding the causes of microbiota variation, and its eventual consequences for host health and fitness is critical for understanding host-environment interactions in a rapidly changing world.

List of references

Acevedo-Whitehouse, K., & Duffus, A. L. J. (2009). Effects of environmental change on wildlife health. *Philosophical Transactions of the Royal Society B: Biological Sciences*, Vol. 364, pp. 3429–3438. https://doi.org/10.1098/rstb.2009.0128

Alberdi, A., Aizpurua, O., Bohmann, K., Zepeda-Mendoza, M. L., & Gilbert, M. T. P. (2016). Do Vertebrate Gut Metagenomes Confer Rapid Ecological Adaptation? *Trends in Ecology and Evolution*, Vol. 31, pp. 689–699. https://doi.org/10.1016/j.tree.2016.06.008

Alberti, M., Correa, C., Marzluff, J. M., Hendry, A. P., Palkovacs, E. P., Gotanda, K. M., … Zhou, Y. (2017). Global urban signatures of phenotypic change in animal and plant populations. *Proceedings of the National Academy of Sciences of the United States of America*, *114*(34), 8951–8956. https://doi.org/10.1073/pnas.1606034114

Aliyu, A. S., Evangeliou, N., Mousseau, T. A., Wu, J., & Ramli, A. T. (2015). An overview of current knowledge concerning the health and environmental consequences of the Fukushima Daiichi Nuclear Power Plant (FDNPP) accident. *Environment International*, Vol. 85, pp. 213–228. https://doi.org/10.1016/j.envint.2015.09.020

Amato, K. R., G. Sanders, J., Song, S. J., Nute, M., Metcalf, J. L., Thompson, L. R., … R. Leigh, S. (2019). Evolutionary trends in host physiology outweigh dietary niche in structuring primate gut microbiomes. *ISME Journal*, *13*(3), 576–587. https://doi.org/10.1038/s41396-018-0175-0

Amato, K. R., Yeoman, C. J., Kent, A., Righini, N., Carbonero, F., Estrada, 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

Assefa, S., & Köhler, G. (2020). Intestinal microbiome and metal toxicity. *Current Opinion in Toxicology*, Vol. 19, pp. 21–27. https://doi.org/10.1016/j.cotox.2019.09.009

Avena, C. V., Parfrey, L. W., Leff, J. W., Archer, H. M., Frick, W. F., Langwig, K. E., … McKenzie, V. J. (2016). Deconstructing the Bat Skin Microbiome: Influences of the Host and the Environment. *Frontiers in Microbiology*, *7*. https://doi.org/10.3389/fmicb.2016.01753

Azzam, E. I., Jay-Gerin, J. P., & Pain, D. (2012). Ionizing radiation-induced metabolic oxidative stress and prolonged cell injury. *Cancer Letters*, Vol. 327, pp. 48–60. https://doi.org/10.1016/j.canlet.2011.12.012

Baker, R. J., Dickins, B., Wickliffe, J. K., Khan, F. A. A., Gaschak, S., Makova, K. D., & Phillips, C. D. (2017). Elevated mitochondrial genome variation after 50 generations of radiation exposure in a wild rodent. *Evolutionary Applications*, *10*(8), 784–791. https://doi.org/10.1111/eva.12475

Barelli, C., Albanese, D., Donati, C., Pindo, M., Dallago, C., Rovero, F., … 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

Bates, K. A., Clare, F. C., O'Hanlon, S., Bosch, J., Brookes, L., Hopkins, K., … Harrison, X. A. (2018). Amphibian chytridiomycosis outbreak dynamics are linked with host skin bacterial community structure. *Nature Communications*, *9*(1). https://doi.org/10.1038/s41467-018-02967-w

Becker, C. G., Longo, A. V., Haddad, C. F. B., & Zamudio, K. R. (2017). Land cover and forest connectivity alter the interactions among host, pathogen and skin microbiome. *Proceedings of the Royal Society B: Biological Sciences*, *284*(1861). https://doi.org/10.1098/rspb.2017.0582

Beernaert, J., Scheirs, J., Van Den Brande, G., Leirs, H., Blust, R., De Meulenaer, B., … Verhagen, R. (2008). Do wood mice (Apodemus sylvaticus L.) use food selection as a means to reduce heavy metal intake? *Environmental Pollution*, *151*(3), 599–607. https://doi.org/10.1016/j.envpol.2007.04.001

Belkaid, Y., & Segre, J. A. (2014). Dialogue between skin microbiota and immunity. *Science*, Vol. 346, pp. 954–959. https://doi.org/10.1126/science.1260144

Bello, M. G. D., Knight, R., Gilbert, J. A., & Blaser, M. J. (2018). Preserving microbial diversity. *Science*, *362*(6410), 33–34. https://doi.org/10.1126/science.aau8816

Beresford, N. A., Barnett, C. L., Gashchak, S., Maksimenko, A., Guliaichenko, E., Wood, M. D., & Izquierdo, M. (2020). Radionuclide transfer to wildlife at a 'Reference site' in the Chernobyl Exclusion Zone and resultant radiation exposures. *Journal of Environmental Radioactivity*, *211*, 105661. https://doi.org/10.1016/j.jenvrad.2018.02.007

Beresford, N. A., Gaschak, S., Barnett, C. L., Howard, B. J., Chizhevsky, I., Strømman, G., … Copplestone, D. (2008). Estimating the exposure of small mammals at three sites within the Chernobyl exclusion zone - a test application of the ERICA Tool. *Journal of Environmental Radioactivity*, *99*(9), 1496–1502. https://doi.org/10.1016/j.jenvrad.2008.03.002

Beresford, N. A., Scott, E. M., & Copplestone, D. (2020). Field effects studies in the Chernobyl Exclusion Zone: Lessons to be learnt. *Journal of Environmental Radioactivity*, *211*, 105893. https://doi.org/10.1016/j.jenvrad.2019.01.005

Bestion, E., Jacob, S., Zinger, L., Di Gesu, L., Richard, M., White, J., & Cote, J. (2017). Climate warming reduces gut microbiota diversity in a vertebrate ectotherm. *Nature Ecology and Evolution*, *1*(6). https://doi.org/10.1038/s41559-017-0161

Bewick, S., Gurarie, E., Weissman, J. L., Beattie, J., Davati, C., Flint, R., … Fagan, W. F. (2019). Trait-based analysis of the human skin microbiome. *Microbiome*, *7*(1), 101. https://doi.org/10.1186/s40168-019-0698-2

Bierlich, K. C., Miller, C., DeForce, E., Friedlaender, A. S., Johnston, D. W., & Apprill, A. (2018). Temporal and regional variability in the skin microbiome of humpback whales along the Western Antarctic Peninsula. *Applied and Environmental Microbiology*, *84*(5). https://doi.org/10.1128/AEM.02574-17

Bletz, M. C., Goedbloed, D. J., Sanchez, E., Reinhardt, T., Tebbe, C. C., Bhuju, S., … Steinfartz, S. (2016). Amphibian gut microbiota shifts differentially in community structure but converges on habitat-specific predicted functions. *Nature Communications*, *7*. https://doi.org/10.1038/ncomms13699

Bligh, E. G., & Dyer, W. J. (1959). A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology*, *37*(8), 911–917. https://doi.org/10.1139/o59-099

Bokulich, N., Dillon, M., Bolyen, E., Kaehler, B., Huttley, G., & Caporaso, J. (2018). q2-sample-classifier: machine-learning tools for microbiome classification and regression. *Journal of Open Source Software*, *3*(30), 934. https://doi.org/10.21105/joss.00934

Bolyen, E., Rideout, J. R., Dillon, M. R., Bokulich, N. A., Abnet, C. C., Al-Ghalith, G. A., … Caporaso, J. G. (2019). Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nature Biotechnology*, Vol. 37, pp. 852–857. https://doi.org/10.1038/s41587-019-0209-9

Bonisoli-Alquati, A., Koyama, K., Tedeschi, D. J., Kitamura, W., Sukuzi, H., Ostermiller, S., … Mousseau, T. A. (2015). Abundance and genetic damage of barn swallows from Fukushima. *Scientific Reports*, *5*(1), 1–8. https://doi.org/10.1038/srep09432

Bonisoli-Alquati, Andrea, Voris, A., Mousseau, T. A., Møller, A. P., Saino, N., & Wyatt, M. D. (2010). DNA damage in barn swallows (Hirundo rustica) from the Chernobyl region detected by use of the comet assay. *Comparative Biochemistry and Physiology - C Toxicology and Pharmacology*, *151*(3), 271–277. https://doi.org/10.1016/j.cbpc.2009.11.006

Bordenstein, S. R., & Theis, K. R. (2015). Host Biology in Light of the Microbiome: Ten Principles of Holobionts and Hologenomes. *PLOS Biology*, *13*(8), e1002226. https://doi.org/10.1371/journal.pbio.1002226

Bouslimani, A., Da Silva, R., Kosciolek, T., Janssen, S., Callewaert, C., Amir, A., … Dorrestein, P. C. (2019). The impact of skin care products on skin chemistry and microbiome dynamics. *BMC Biology*, *17*(1). https://doi.org/10.1186/s12915-019-0660-6

Breiman, L. (2001). Random forests. *Machine Learning*, *45*(1), 5–32. https://doi.org/10.1023/A:1010933404324

Brenner, D. J., & Hall, E. J. (2007). Computed Tomography — An Increasing Source of Radiation Exposure. *New England Journal of Medicine*, *357*(22), 2277–2284. https://doi.org/10.1056/NEJMra072149

Bujalska, G. (1990). Social System of the Bank Vole, Clethrionomys Glareolus. In *Social Systems and Population Cycles in Voles* (pp. 155–167). https://doi.org/10.1007/978-3-0348-6416-9_15

Butet, A., & Delettre, Y. R. (2011). Diet differentiation between European arvicoline and murine rodents. *Acta Theriologica*, Vol. 56, pp. 297–304. https://doi.org/10.1007/s13364-011-0049-6

Byrd, A. L., Belkaid, Y., & Segre, J. A. (2018). The human skin microbiome. *Nature Reviews Microbiology*, Vol. 16, pp. 143–155. https://doi.org/10.1038/nrmicro.2017.157

Calandra, I., Labonne, G., Mathieu, O., Henttonen, H., Lévêque, J., Milloux, M.-J., … Navarro, N. (2015). Isotopic partitioning by small mammals in the subnivium. *Ecology and Evolution*, *5*(18), 4132–4140. https://doi.org/10.1002/ece3.1653

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., & Holmes, S. P. (2016). DADA2: High-resolution sample inference from Illumina amplicon data. *Nature Methods*, *13*(7), 581–583. https://doi.org/10.1038/nmeth.3869

Caporaso, J. G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F. D., Costello, E. K., … Knight, R. (2010). QIIME allows analysis of high-throughput community sequencing data. *Nature Methods*, Vol. 7, pp. 335–336. https://doi.org/10.1038/nmeth.f.303

Caporaso, J. G., Lauber, C. L., Walters, W. A., Berg-Lyons, D., Lozupone, C. A., Turnbaugh, P. J., … Knight, R. (2011). Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. *Proceedings of the National Academy of Sciences of the United States of America*, *108*, 4516–4522. https://doi.org/10.1073/pnas.1000080107

Capunitan, D. C., Johnson, O., Terrill, R. S., & Hird, S. M. (2020). Evolutionary signal in the gut microbiomes of 74 bird species from Equatorial Guinea. *Molecular Ecology*, mec.15354. https://doi.org/10.1111/mec.15354

Carlson, S. M., Cunningham, C. J., & Westley, P. A. H. (2014). Evolutionary rescue in a changing world. *Trends in Ecology and Evolution*, Vol. 29, pp. 521–530. https://doi.org/10.1016/j.tree.2014.06.005

Carmody, R. N., Gerber, G. K., Luevano, J. M., Gatti, D. M., Somes, L., Svenson, K. L., & Turnbaugh, P. J. (2015). Diet dominates host genotype in shaping the murine gut microbiota. *Cell Host and Microbe*, *17*(1), 72–84. https://doi.org/10.1016/j.chom.2014.11.010

Carthey, A. J. R., Blumstein, D. T., Gallagher, R. V., Tetu, S. G., & Gillings, M. R. (2019). Conserving the holobiont. *Functional Ecology*. https://doi.org/10.1111/1365-2435.13504

Castelino, M., Eyre, S., Moat, J., Fox, G., Martin, P., Ho, P., … Barton, A. (2017). Optimisation of methods for bacterial skin microbiome investigation: primer selection and comparison of the 454 versus MiSeq platform. *BMC Microbiology*, *17*(1), 23. https://doi.org/10.1186/s12866-017-0927-4

Charmantier, A., McCleery, R. H., Cole, L. R., Perrins, C., Kruuk, L. E. B., & Sheldon, B. C. (2008). Adaptive phenotypic plasticity in response to climate change in a wild bird population. *Science*, *320*(5877), 800–803. https://doi.org/10.1126/science.1157174

Chassaing, B., Koren, O., Goodrich, J. K., Poole, A. C., Srinivasan, S., Ley, R. E., & Gewirtz, A. T. (2015). Dietary emulsifiers impact the mouse gut microbiota promoting colitis and metabolic syndrome. *Nature*, *519*(7541), 92–96. https://doi.org/10.1038/nature14232

Cheng, Y., Fox, S., Pemberton, D., Hogg, C., Papenfuss, A. T., & Belov, K. (2015). The Tasmanian devil microbiome—implications for conservation and management. *Microbiome*, *3*(1), 76. https://doi.org/10.1186/s40168-015-0143-0

Chesser, R. K., Sugg, D. W., Lomakin, M. D., van den Bussche, R. A., DeWoody, J. A., Jagoe, C. H., … Baker, R. J. (2000). Concentrations and dose rate estimates of 134,137 cesium and 90 strontium in small mammals at Chornobyl, Ukraine. *Environmental Toxicology and Chemistry*, *19*(2), 305–312. https://doi.org/10.1002/etc.5620190209

Chevalier, C., Stojanović, O., Colin, D. J., Suarez-Zamorano, N., Tarallo, V., Veyrat-Durebex, C., … Trajkovski, M. (2015). Gut Microbiota Orchestrates Energy Homeostasis during Cold. *Cell*, *163*(6), 1360–1374. https://doi.org/10.1016/j.cell.2015.11.004

Clayton, J. B., Vangay, P., Huang, H., Ward, T., Hillmann, B. M., Al-Ghalith, G. A., … Knights, D. (2016). Captivity humanizes the primate microbiome. *Proceedings of the National Academy of Sciences of the United States of America*, *113*(37), 10376–10381. https://doi.org/10.1073/pnas.1521835113

Clemente, J. C., Pehrsson, E. C., Blaser, M. J., Sandhu, K., Gao, Z., Wang, B., … Dominguez-Bello, M. G. (2015). The microbiome of uncontacted Amerindians. *Science Advances*, *1*(3). https://doi.org/10.1126/sciadv.1500183

Coolon, J. D., Jones, K. L., Narayanan, S., & Wisely, S. M. (2010). Microbial ecological response of the intestinal flora of Peromyscus maniculatus and P. leucopus to heavy metal contamination. *Molecular Ecology*, *19*, 67–80. https://doi.org/10.1111/j.1365-294X.2009.04485.x

Costa, S., Lopes, I., Proença, D. N., Ribeiro, R., & Morais, P. V. (2016). Diversity of cutaneous microbiome of Pelophylax perezi populations inhabiting different environments. *Science of the Total Environment*, *572*, 995–1004. https://doi.org/10.1016/j.scitotenv.2016.07.230

Council, S. E., Savage, A. M., Urban, J. M., Ehlers, M. E., Pate Skene, J. H., Platt, M. L., … Horvath, J. E. (2016). Diversity and evolution of the primate skin microbiome. *Proceedings of the Royal Society B: Biological Sciences*, *283*(1822). https://doi.org/10.1098/rspb.2015.2586

Cundell, A. M. (2018). Microbial Ecology of the Human Skin. *Microbial Ecology*, *76*(1), 113–120. https://doi.org/10.1007/s00248-016-0789-6

Cuscó, A., Belanger, J. M., Gershony, L., Islas-Trejo, A., Levy, K., Medrano, J. F., … Francino, O. (2017). Individual signatures and environmental factors shape skin microbiota in healthy dogs. *Microbiome*, *5*(1), 139. https://doi.org/10.1186/s40168-017-0355-6

Czirják, G. Á., Møller, A. P., Mousseau, T. A., & Heeb, P. (2010). Microorganisms Associated with Feathers of Barn Swallows in Radioactively Contaminated Areas Around Chernobyl. *Microbial Ecology*, *60*(2), 373–380. https://doi.org/10.1007/s00248-010-9716-4

Dao, H., & Kazin, R. A. (2007). Gender differences in skin: A review of the literature. *Gender Medicine*, *4*(4), 308–328. https://doi.org/10.1016/S1550-8579(07)80061-1

David, L. A., Maurice, C. F., Carmody, R. N., Gootenberg, D. B., Button, J. E., Wolfe, B. E., … Turnbaugh, P. J. (2014). Diet rapidly and reproducibly alters the human gut microbiome. *Nature*, *505*(7484), 559–563. https://doi.org/10.1038/nature12820

De Barba, M., Miquel, C., Boyer, F., Mercier, C., Rioux, D., Coissac, E., & Taberlet, P. (2014). DNA metabarcoding multiplexing and validation of data accuracy for diet assessment: Application to omnivorous diet. *Molecular Ecology Resources*, *14*(2), 306–323. https://doi.org/10.1111/1755-0998.12188

de la Cuesta-Zuluaga, J., Kelley, S. T., Chen, Y., Escobar, J. S., Mueller, N. T., Ley, R. E., … Thackray, V. G. (2019). Age- and Sex-Dependent Patterns of Gut Microbial Diversity in Human Adults. *MSystems*, *4*(4). https://doi.org/10.1128/msystems.00261-19

Decandia, A. L., Leverett, K. N., & Vonholdt, B. M. (2019). Of microbes and mange: Consistent changes in the skin microbiome of three canid species infected with Sarcoptes scabiei mites. *Parasites and Vectors*, *12*(1). https://doi.org/10.1186/s13071-019-3724-0

Del Giudice, M., Buck, C. L., Chaby, L. E., Gormally, B. M., Taff, C. C., Thawley, C. J., … Wada, H. (2018). What Is Stress? A Systems Perspective. *Integrative and Comparative Biology*, *58*(6), 1019–1032. https://doi.org/10.1093/icb/icy114

Delsuc, F., Metcalf, J. L., Wegener Parfrey, L., Song, S. J., González, A., & Knight, R. (2014). Convergence of gut microbiomes in myrmecophagous mammals. *Molecular Ecology*, *23*(6), 1301–1317. https://doi.org/10.1111/mec.12501

Deryabina, T. G., Kuchmel, S. V., Nagorskaya, L. L., Hinton, T. G., Beasley, J. C., Lerebours, A., & Smith, J. T. (2015). Long-term census data reveal abundant wildlife populations at Chernobyl. *Current Biology*, Vol. 25, pp. R824–R826. https://doi.org/10.1016/j.cub.2015.08.017

Dimitriu, P. A., Iker, B., Malik, K., Leung, H., Mohn, W. W., & Hillebrand, G. G. (2019). New insights into the intrinsic and extrinsic factors that shape the human skin microbiome. *MBio*, *10*(4). https://doi.org/10.1128/mBio.00839-19

Douglas, A. E. (2018). *Fundamentals of microbiome science. How microbes shape animal biology*. Princeton, NJ: Princeton University Press.

Einor, D., Bonisoli-Alquati, A., Costantini, D., Mousseau, T. A., & Møller, A. P. (2016). Ionizing radiation, antioxidant response and oxidative damage: A meta-analysis. *Science of the Total Environment*, *548–549*, 463–471. https://doi.org/10.1016/j.scitotenv.2016.01.027

Falony, G., Joossens, M., Vieira-Silva, S., Wang, J., Darzi, Y., Faust, K., … Raes, J. (2016). Population-level analysis of gut microbiome variation. *Science*, *352*(6285), 560–564. https://doi.org/10.1126/science.aad3503

Fierer, N., Hamady, M., Lauber, C. L., & Knight, R. (2008). The influence of sex, handedness, and washing on the diversity of hand surface bacteria. *Proceedings of the National Academy of Sciences of the United States of America*, *105*(46), 17994–17999. https://doi.org/10.1073/pnas.0807920105

Foley, J. A., DeFries, R., Asner, G. P., Barford, C., Bonan, G., Carpenter, S. R., … Snyder, P. K. (2005). Global consequences of land use. *Science*, Vol. 309, pp. 570–574. https://doi.org/10.1126/science.1111772

Gao, B., Chi, L., Mahbub, R., Bian, X., Tu, P., Ru, H., & Lu, K. (2017). Multi-Omics Reveals that Lead Exposure Disturbs Gut Microbiome Development, Key Metabolites, and Metabolic Pathways. *Chemical Research in Toxicology*, *30*(4), 996–1005. https://doi.org/10.1021/acs.chemrestox.6b00401

Garnier-Laplace, J., Beaugelin-Seiller, K., Della-Vedova, C., Métivier, J. M., Ritz, C., Mousseau, T. A., & Møller, A. P. (2015). Radiological dose reconstruction for birds reconciles outcomes of Fukushima with knowledge of dose-effect relationships. *Scientific Reports*, *5*(1), 1–13. https://doi.org/10.1038/srep16594

Garnier-Laplace, J., Geras'kin, S., Della-Vedova, C., Beaugelin-Seiller, K., Hinton, T. G., Real, A., & Oudalova, A. (2013). Are radiosensitivity data derived from natural field conditions consistent with data from controlled exposures? A case study of Chernobyl wildlife chronically exposed to low dose rates. *Journal of Environmental Radioactivity*, *121*, 12–21. https://doi.org/10.1016/j.jenvrad.2012.01.013

Gellie, N. J. C., Mills, J. G., Breed, M. F., & Lowe, A. J. (2017). Revegetation rewilds the soil bacterial microbiome of an old field. *Molecular Ecology*, *26*(11), 2895–2904. https://doi.org/10.1111/mec.14081

Gentile, C. L., & Weir, T. L. (2018). The gut microbiota at the intersection of diet and human health. *Science*, Vol. 362, pp. 776–780. https://doi.org/10.1126/science.aau5812

Godsall, B., Coulson, T., & Malo, A. F. (2014). From physiology to space use: energy reserves and androgenization explain home-range size variation in a woodland rodent. *Journal of Animal Ecology*, *83*(1), 126–135. https://doi.org/10.1111/1365-2656.12116

Goertz, S., de Menezes, A. B., Birtles, R. J., Fenn, J., Lowe, A. E., MacColl, A. D. C., … Taylor, C. H. (2019). Geographical location influences the composition of the gut microbiota in wild house mice (Mus musculus domesticus) at a fine spatial scale. *PLOS ONE*, *14*(9), e0222501. https://doi.org/10.1371/journal.pone.0222501

Goodrich, J. K., Waters, J. L., Poole, A. C., Sutter, J. L., Koren, O., Blekhman, R., … 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

Goudarzi, M., Mak, T. D., Jacobs, J. P., Moon, B.-H., Strawn, S. J., Braun, J., … Li, H.-H. (2016). An Integrated Multi-Omic Approach to Assess Radiation Injury on the Host-Microbiome Axis. *Radiation Research*, *186*(3), 219. https://doi.org/10.1667/rr14306.1

Greyson-Gaito, C. J., Bartley, T. J., Cottenie, K., Jarvis, W. M. C., Newman, A. E. M., & Stothart, M. R. (2020). Into the wild: microbiome transplant studies need broader ecological reality. *Proceedings of the Royal Society B: Biological Sciences*, *287*(1921), 20192834. https://doi.org/10.1098/rspb.2019.2834

Grieneisen, L. E., Charpentier, M. J. E., Alberts, S. C., Blekhman, R., Bradburd, G., Tung, J., & Archie, E. A. (2019). Genes, geology and germs: Gut microbiota across a primate hybrid zone are explained by site soil properties, not host species. *Proceedings of the Royal Society B: Biological Sciences*, *286*(1901). https://doi.org/10.1098/rspb.2019.0431

Grond, K., Bell, K. C., Demboski, J. R., Santos, M., Sullivan, J. M., & Hird, S. M. (2019). No evidence for phylosymbiosis in western chipmunk species. *FEMS Microbiology Ecology*. https://doi.org/10.1093/femsec/fiz182

Grönroos, M., Parajuli, A., Laitinen, O. H., Roslund, M. I., Vari, H. K., Hyöty, H., … Sinkkonen, A. (2019). Short-term direct contact with soil and plant materials leads to an immediate increase in diversity of skin microbiota. *MicrobiologyOpen*, *8*(3), e00645. https://doi.org/10.1002/mbo3.645

Grosser, S., Sauer, J., Paijmans, A. J., Caspers, B. A., Forcada, J., Wolf, J. B. W., & Hoffman, J. I. (2019). Fur seal microbiota are shaped by the social and physical environment, show mother–offspring similarities and are associated with host genetic quality. *Molecular Ecology*, *28*(9), 2406–2422. https://doi.org/10.1111/mec.15070

Haddad, N. M., Brudvig, L. A., Clobert, J., Davies, K. F., Gonzalez, A., Holt, R. D., … Townshend, J. R. (2015). Habitat fragmentation and its lasting impact on Earth's ecosystems. *Science Advances*, *1*(2), e1500052. https://doi.org/10.1126/sciadv.1500052

Hamady, M., Lozupone, C., & Knight, R. (2010). Fast UniFrac: Facilitating high-throughput phylogenetic analyses of microbial communities including analysis of pyrosequencing and PhyloChip data. *ISME Journal*, *4*(1), 17–27. https://doi.org/10.1038/ismej.2009.97

Hamer, H. M., Jonkers, D., Venema, K., Vanhoutvin, S., Troost, F. J., & Brummer, R. J. (2007). Review article: the role of butyrate on colonic function. *Alimentary Pharmacology & Therapeutics*, *27*(2), 104–119. https://doi.org/10.1111/j.1365-2036.2007.03562.x

Hanski, I., Von Hertzen, L., Fyhrquist, N., Koskinen, K., Torppa, K., Laatikainen, T., … Haahtela, T. (2012). Environmental biodiversity, human microbiota, and allergy are interrelated. *Proceedings of the National Academy of Sciences of the United States of America*, *109*(21), 8334–8339. https://doi.org/10.1073/pnas.1205624109

Harada, K. H., Niisoe, T., Imanaka, M., Takahashi, T., Amako, K., Fujii, Y., … Koizumi, A. (2014). Radiation dose rates now and in the future for residents neighboring restricted areas of the Fukushima Daiichi Nuclear Power Plant. *Proceedings of the National Academy of Sciences of the United States of America*, *111*(10), E914–E923. https://doi.org/10.1073/pnas.1315684111

Hashimoto, S., Matsuura, T., Nanko, K., Linkov, I., Shaw, G., & Kaneko, S. (2013). Predicted spatio-temporal dynamics of radiocesium deposited onto forests following the Fukushima nuclear accident. *Scientific Reports*, *3*(1), 1–5. https://doi.org/10.1038/srep02564

Hayama, S. I., Tsuchiya, M., Ochiai, K., Nakiri, S., Nakanishi, S., Ishii, N., … Omi, T. (2017). Small head size and delayed body weight growth in wild Japanese monkey fetuses after the Fukushima Daiichi nuclear disaster. *Scientific Reports*, *7*(1), 1–7. https://doi.org/10.1038/s41598-017-03866-8

Hicks, A. L., Lee, K. J., Couto-Rodriguez, M., Patel, J., Sinha, R., Guo, C., … Williams, B. L. (2018). Gut microbiomes of wild great apes fluctuate seasonally in response to diet. *Nature Communications*, *9*(1). https://doi.org/10.1038/s41467-018-04204-w

Hird, S. M. (2017). Evolutionary biology needs wild microbiomes. *Frontiers in Microbiology*, Vol. 8. https://doi.org/10.3389/fmicb.2017.00725

Hird, S. M. (2020). Context Is Key: Comparative Biology Illuminates the Vertebrate Microbiome. *MBio*, *11*(2). https://doi.org/10.1128/mBio.00153-20

Hird, S. M., Sánchez, C., Carstens, B. C., & Brumfield, R. T. (2015). Comparative Gut Microbiota of 59 Neotropical Bird Species. *Frontiers in Microbiology*, *6*. https://doi.org/10.3389/fmicb.2015.01403

Hiyama, A., Nohara, C., Kinjo, S., Taira, W., Gima, S., Tanahara, A., & Otaki, J. M. (2012). The biological impacts of the Fukushima nuclear accident on the pale grass blue butterfly. *Scientific Reports*, *2*(1), 1–11. https://doi.org/10.1038/srep00570

Hoffmeyer, I. (1973). Interaction and Habitat Selection in the Mice Apodemus Flavicollis and A. Sylvaticus. *Oikos*, *24*(1), 108. https://doi.org/10.2307/3543257

Hooper, R., Brealey, J. C., Valk, T., Alberdi, A., Durban, J. W., Fearnbach, H., … Guschanski, K. (2019). Host-derived population genomics data provides insights into bacterial and diatom composition of the killer whale skin. *Molecular Ecology*, *28*(2), 484–502. https://doi.org/10.1111/mec.14860

Hooper, L. V., Littman, D. R., & Macpherson, A. J. (2012). Interactions between the microbiota and the immune system. *Science*, Vol. 336, pp. 1268–1273. https://doi.org/10.1126/science.1223490

Hoyos-Hernandez, C., Courbert, C., Simonucci, C., David, S., Vogel, T. M., & Larose, C. (2019). Community structure and functional genes in radionuclide contaminated soils in Chernobyl and Fukushima. *FEMS Microbiology Letters*, *366*(21). https://doi.org/10.1093/femsle/fnz180

IAEA. (2019). Nuclear Power Reactors in the World. *Reference Data Series No. 2*. Vienna, Austria: IAEA.

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

Innes, D. G. L., & Millar, J. S. (1994). Life histories of Clethrionomys and Microtus (Microtinae). *Mammal Review*, *24*(4), 179–207. https://doi.org/10.1111/j.1365-2907.1994.tb00142.x

Ishida, K., Tanoi, K., & Nakanishi, T. M. (2015). Monitoring free-living Japanese Bush Warblers (Cettia diphone) in a most highly radiocontaminated area of Fukushima Prefecture, Japan. *Journal of Radiation Research*, *56*(1), i24–i28. https://doi.org/10.1093/jrr/rrv087

Jernfors, T., Kesäniemi, J., Lavrinienko, A., Mappes, T., Milinevsky, G., Møller, A. P., … Watts, P. C. (2018). Transcriptional Upregulation of DNA Damage Response Genes in Bank Voles (Myodes glareolus) Inhabiting the Chernobyl Exclusion Zone. *Frontiers in Environmental Science*, *5*, 95. https://doi.org/10.3389/fenvs.2017.00095

Jiang, L., Amir, A., Morton, J. T., Heller, R., Arias-Castro, E., & Knight, R. (2017). Discrete False-Discovery Rate Improves Identification of Differentially Abundant Microbes. *MSystems*, *2*(6). https://doi.org/10.1128/msystems.00092-17

Jin, Y., Lu, L., Tu, W., Luo, T., & Fu, Z. (2019). Impacts of polystyrene microplastic on the gut barrier, microbiota and metabolism of mice. *Science of the Total Environment*, *649*, 308–317. https://doi.org/10.1016/j.scitotenv.2018.08.353

Jin, Y., Wu, S., Zeng, Z., & Fu, Z. (2017). Effects of environmental pollutants on gut microbiota. *Environmental Pollution*, Vol. 222, pp. 1–9. https://doi.org/10.1016/j.envpol.2016.11.045

Kartzinel, T. R., Chen, P. A., Coverdale, T. C., Erickson, D. L., Kress, W. J., Kuzmina, M. L., … Pringle, R. M. (2015). DNA metabarcoding illuminates dietary niche partitioning by African large herbivores. *Proceedings of the National Academy of Sciences of the United States of America*, *112*(26), 8019–8024. https://doi.org/10.1073/pnas.1503283112

Kartzinel, T. R., Hsing, J. C., Musili, P. M., Brown, B. R. P., & Pringle, R. M. (2019). Covariation of diet and gut microbiome in African megafauna. *Proceedings of the National Academy of Sciences of the United States of America*, *116*(47), 23588–23593. https://doi.org/10.1073/pnas.1905666116

Kesäniemi, J., Jernfors, T., Lavrinienko, A., Kivisaari, K., Kiljunen, M., Mappes, T., & Watts, P. C. (2019). Exposure to environmental radionuclides is associated with altered metabolic and immunity pathways in a wild rodent. *Molecular Ecology*, *28*(20), 4620–4635. https://doi.org/10.1111/mec.15241

Kesäniemi, J., Lavrinienko, A., Tukalenko, E., Boratyński, Z., Kivisaari, K., Mappes, T., … Watts, P. C. (2019). Exposure to environmental radionuclides associates with tissue-specific impacts on telomerase expression and telomere length. *Scientific Reports*, *9*(1), 1–9. https://doi.org/10.1038/s41598-018-37164-8

Kesäniemi, J., Lavrinienko, A., Tukalenko, E., Moutinho, A. F., Mappes, T., Møller, A. P., … Watts, P. C. (2020). Exposure to environmental radionuclides alters mitochondrial DNA maintenance in a wild rodent. *Evolutionary Ecology*, 1–12. https://doi.org/10.1007/s10682-019-10028-x

Knight, R., Vrbanac, A., Taylor, B. C., Aksenov, A., Callewaert, C., Debelius, J., … Dorrestein, P. C. (2018). Best practices for analysing microbiomes. *Nature Reviews Microbiology*, Vol. 16, pp. 410–422. https://doi.org/10.1038/s41579-018-0029-9

Knowles, S. C. L., Eccles, R. M., & Baltrūnaitė, L. (2019). Species identity dominates over environment in shaping the microbiota of small mammals. *Ecology Letters*, *22*(5), 826–837. https://doi.org/10.1111/ele.13240

Knutie, S. A., Wilkinson, C. L., Kohl, K. D., & Rohr, J. R. (2017). Early-life disruption of amphibian microbiota decreases later-life resistance to parasites. *Nature Communications*, *8*(1), 1–8. https://doi.org/10.1038/s41467-017-00119-0

Koh, A., De Vadder, F., Kovatcheva-Datchary, P., & Bäckhed, F. (2016). From dietary fiber to host physiology: Short-chain fatty acids as key bacterial metabolites. *Cell*, Vol. 165, pp. 1332–1345. https://doi.org/10.1016/j.cell.2016.05.041

Kohl, K. D., Weiss, R. B., Cox, J., Dale, C., & Denise Dearing, M. (2014). Gut microbes of mammalian herbivores facilitate intake of plant toxins. *Ecology Letters*, *17*(10), 1238–1246. https://doi.org/10.1111/ele.12329

Koivula, M., Koskela, E., Mappes, T., & Oksanen, T. A. (2003). Cost of reproduction in the wild: manipulation of reproductive effort in the bank vole. *Ecology*, *84*(2), 398–405. https://doi.org/10.1890/0012-9658(2003)084[0398:CORITW]2.0.CO;2

Kolodny, O., Weinberg, M., Reshef, L., Harten, L., Hefetz, A., Gophna, U., … Yovel, Y. (2019). Coordinated change at the colony level in fruit bat fur microbiomes through time. *Nature Ecology and Evolution*, *3*(1), 116–124. https://doi.org/10.1038/s41559-018-0731-z

Koskela, E., Mappes, T., & Ylonen, H. (1997). Territorial Behaviour and Reproductive Success of Bank Vole Clethrionomys glareolus Females. *The Journal of Animal Ecology*, *66*(3), 341. https://doi.org/10.2307/5980

Kozakiewicz, M., Chołuj, A., & Kozakiewicz, A. (2007). Long-distance movements of individuals in a free-living bank vole population: An important element of male breeding strategy. *Acta Theriologica*, *52*(4), 339–348. https://doi.org/10.1007/BF03194231

Kreisinger, J., Bastien, G., Hauffe, H. C., Marchesi, J., & Perkins, S. E. (2015). Interactions between multiple helminths and the gut microbiota in wild rodents. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *370*(1675). https://doi.org/10.1098/rstb.2014.0295

Kubota, Y., Takahashi, H., Watanabe, Y., Fuma, S., Kawaguchi, I., Aoki, M., … Yoshida, S. (2015). Estimation of absorbed radiation dose rates in wild rodents inhabiting a site severely contaminated by the Fukushima Dai-ichi nuclear power plant accident. *Journal of Environmental Radioactivity*, *142*, 124–131. https://doi.org/10.1016/j.jenvrad.2015.01.014

Kubota, Y., Tsuji, H., Kawagoshi, T., Shiomi, N., Takahashi, H., Watanabe, Y., … Yoshida, S. (2015). Chromosomal Aberrations in Wild Mice Captured in Areas Differentially Contaminated by the Fukushima Dai-Ichi Nuclear Power Plant Accident. *Environmental Science and Technology*, *49*(16), 10074–10083. https://doi.org/10.1021/acs.est.5b01554

Kueneman, J. G., Parfrey, L. W., Woodhams, D. C., Archer, H. M., Knight, R., & McKenzie, V. J. (2014). The amphibian skin-associated microbiome across species, space and life history stages. *Molecular Ecology*, *23*(6), 1238–1250. https://doi.org/10.1111/mec.12510

Kumar, S., Stecher, G., Suleski, M., & Hedges, S. B. (2017). TimeTree: A Resource for Timelines, Timetrees, and Divergence Times. *Molecular Biology and Evolution*, *34*(7), 1812–1819. https://doi.org/10.1093/molbev/msx116

Kurle, C. M., Koch, P. L., Tershy, B. R., & Croll, D. A. (2014). The effects of sex, tissue type, and dietary components on stable isotope discrimination factors (Δ^{13}C and Δ^{15}N) in mammalian omnivores. *Isotopes in Environmental and Health Studies*, *50*(3), 307–321. https://doi.org/10.1080/10256016.2014.908872

Lagkouvardos, I., Lesker, T. R., Hitch, T. C. A., Gálvez, E. J. C., Smit, N., Neuhaus, K., … Clavel, T. (2019). Sequence and cultivation study of Muribaculaceae reveals novel species, host preference, and functional potential of this yet undescribed family. *Microbiome*, *7*(1), 28. https://doi.org/10.1186/s40168-019-0637-2

Langille, M. G. I., Zaneveld, J., Caporaso, J. G., McDonald, D., Knights, D., Reyes, J. A., … 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

Lawson Handley, L. J., & Perrin, N. (2007). Advances in our understanding of mammalian sex-biased dispersal. *Molecular Ecology*, *16*(8), 1559–1578. https://doi.org/10.1111/j.1365-294X.2006.03152.x

Lee, W. J., & Hase, K. (2014). Gut microbiota-generated metabolites in animal health and disease. *Nature Chemical Biology*, Vol. 10, pp. 416–424. https://doi.org/10.1038/nchembio.1535

Lehmann, P., Boratyński, Z., Mappes, T., Mousseau, T. A., & Møller, A. P. (2016). Fitness costs of increased cataract frequency and cumulative radiation dose in natural mammalian populations from Chernobyl. *Scientific Reports*, *6*(1), 1–7. https://doi.org/10.1038/srep19974

Lehtimäki, J., Karkman, A., Laatikainen, T., Paalanen, L., Von Hertzen, L., Haahtela, T., … Ruokolainen, L. (2017). Patterns in the skin microbiota differ in children and teenagers between rural and urban environments. *Scientific Reports*, *7*. https://doi.org/10.1038/srep45651

Lehtimäki, J., Sinkko, H., Hielm-Björkman, A., Salmela, E., Tiira, K., Laatikainen, T., … Ruokolainen, L. (2018). Skin microbiota and allergic symptoms associate with exposure to environmental microbes. *Proceedings of the National Academy of Sciences of the United States of America*, *115*(19), 4897–4902. https://doi.org/10.1073/pnas.1719785115

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

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

Li, H., Wang, Y., Yu, Q., Feng, T., Zhou, R., Shao, L., … Zhou, H. (2019). Elevation is Associated with Human Skin Microbiomes. *Microorganisms*, *7*(12), 611. https://doi.org/10.3390/microorganisms7120611

Lim, S. J., & Bordenstein, S. R. (2020). An introduction to phylosymbiosis. *Proceedings of the Royal Society B: Biological Sciences*, *287*(1922), 20192900. https://doi.org/10.1098/rspb.2019.2900

Linnenbrink, M., Wang, J., Hardouin, E. A., Künzel, S., Metzler, D., & Baines, J. F. (2013). The role of biogeography in shaping diversity of the intestinal microbiota in house mice. *Molecular Ecology*, *22*(7), 1904–1916. https://doi.org/10.1111/mec.12206

Lourenço, J., Mendo, S., & Pereira, R. (2016). Radioactively contaminated areas: Bioindicator species and biomarkers of effect in an early warning scheme for a preliminary risk assessment. *Journal of Hazardous Materials*, Vol. 317, pp. 503–542. https://doi.org/10.1016/j.jhazmat.2016.06.020

Lundberg, R., Toft, M. F., August, B., Hansen, A. K., & Hansen, C. H. F. (2016). Antibiotic-treated versus germ-free rodents for microbiota transplantation studies. *Gut Microbes*, *7*(1), 68–74. https://doi.org/10.1080/19490976.2015.1127463

Lutz, H. L., Jackson, E. W., Webala, P. W., Babyesiza, W. S., Kerbis Peterhans, J. C., Demos, T. C., … Gilbert, J. A. (2019). Ecology and Host Identity Outweigh Evolutionary History in Shaping the Bat Microbiome. *MSystems*, *4*(6). https://doi.org/10.1128/msystems.00511-19

Lyons, P. C., Okuda, K., Hamilton, M. T., Hinton, T. G., & Beasley, J. C. (2020). Rewilding of Fukushima's human evacuation zone. *Frontiers in Ecology and the Environment*, fee.2149. https://doi.org/10.1002/fee.2149

Mappes, T., Boratyński, Z., Kivisaari, K., Lavrinienko, A., Milinevsky, G., Mousseau, T. A., … Watts, P. C. (2019). Ecological mechanisms can modify radiation effects in a key forest mammal of Chernobyl. *Ecosphere*, *10*(4), e02667. https://doi.org/10.1002/ecs2.2667

Mappes, T., & Koskela, E. (2004). Genetic basis of the trade-off between offspring number and quality in the bank vole. *Evolution*, *58*(3), 645–650. https://doi.org/10.1111/j.0014-3820.2004.tb01686.x

Martín, R., Bermúdez-Humarán, L. G., & Langella, P. (2016). Gnotobiotic Rodents: An In Vivo Model for the Study of Microbe–Microbe Interactions. *Frontiers in Microbiology*, *7*(MAR), 409. https://doi.org/10.3389/fmicb.2016.00409

Martínez-Mota, R., Kohl, K. D., Orr, T. J., & Denise Dearing, M. (2020). Natural diets promote retention of the native gut microbiota in captive rodents. *ISME Journal*, *14*(1), 67–78. https://doi.org/10.1038/s41396-019-0497-6

Maurice, C. F., Cl Knowles, S., Ladau, J., Pollard, K. S., Fenton, A., Pedersen, A. B., & Turnbaugh, P. J. (2015). Marked seasonal variation in the wild mouse gut microbiota. *ISME Journal*, *9*(11), 2423–2434. https://doi.org/10.1038/ismej.2015.53

McDonald, D., Price, M. N., Goodrich, J., Nawrocki, E. P., Desantis, T. Z., Probst, A., … Hugenholtz, P. (2012). An improved Greengenes taxonomy with explicit ranks for ecological and evolutionary analyses of bacteria and archaea. *ISME Journal*, *6*(3), 610–618. https://doi.org/10.1038/ismej.2011.139

McFall-Ngai, M., Hadfield, M. G., Bosch, T. C. G., Carey, H. V., Domazet-Lošo, T., Douglas, A. E., … Wernegreen, J. J. (2013). Animals in a bacterial world, a new imperative for the life sciences. *Proceedings of the National Academy of Sciences of the United States of America*, Vol. 110, pp. 3229–3236. https://doi.org/10.1073/pnas.1218525110

McKenzie, V. J., Bowers, R. M., Fierer, N., Knight, R., & Lauber, C. L. (2012). Co-habiting amphibian species harbor unique skin bacterial communities in wild populations. *ISME Journal*, *6*(3), 588–596. https://doi.org/10.1038/ismej.2011.129

McKenzie, V. J., Song, S. J., Delsuc, F., Prest, T. L., Oliverio, A. M., Korpita, T. M., … Knight, R. (2017). The Effects of Captivity on the Mammalian Gut Microbiome. *Integrative and Comparative Biology*, *57*(4), 690–704. https://doi.org/10.1093/icb/icx090

Merilä, J., & Hendry, A. P. (2014). Climate change, adaptation, and phenotypic plasticity: the problem and the evidence. *Evolutionary Applications*, *7*(1), 1–14. https://doi.org/10.1111/eva.12137

Michaux, J. R., Libois, R., & Filippucci, M. G. (2005). So close and so different: Comparative phylogeography of two small mammal species, the Yellow-necked fieldmouse (Apodemus flavicollis) and the Woodmouse (Apodemus sylvaticus) in the Western Palearctic region. *Heredity*, *94*(1), 52–63. https://doi.org/10.1038/sj.hdy.6800561

Milan, M., Carraro, L., Fariselli, P., Martino, M. E., Cavalieri, D., Vitali, F., … Cardazzo, B. (2018). Microbiota and environmental stress: how pollution affects microbial communities in Manila clams. *Aquatic Toxicology*, *194*, 195–207. https://doi.org/10.1016/j.aquatox.2017.11.019

Moeller, A. H., Foerster, S., Wilson, M. L., Pusey, A. E., Hahn, B. H., & Ochman, H. (2016). Social behavior shapes the chimpanzee pan-microbiome. *Science Advances*, *2*(1). https://doi.org/10.1126/sciadv.1500997

Møller, A. P., Bonisoli-Alquati, A., & Mousseau, T. A. (2013). High frequency of albinism and tumours in free-living birds around Chernobyl. *Mutation Research - Genetic Toxicology and Environmental Mutagenesis*, *757*(1), 52–59. https://doi.org/10.1016/j.mrgentox.2013.04.019

Møller, A. P., Bonisoli-Alquati, A., Mousseau, T. A., & Rudolfsen, G. (2014). Aspermy, Sperm Quality and Radiation in Chernobyl Birds. *PLoS ONE*, *9*(6), e100296. https://doi.org/10.1371/journal.pone.0100296

Møller, A. P., Bonisoli-Alquati, A., Rudolfsen, G., & Mousseau, T. A. (2011). Chernobyl Birds Have Smaller Brains. *PLoS ONE*, *6*(2), e16862. https://doi.org/10.1371/journal.pone.0016862

Møller, A. P., Hagiwara, A., Matsui, S., Kasahara, S., Kawatsu, K., Nishiumi, I., … Mousseau, T. A. (2012). Abundance of birds in Fukushima as judged from Chernobyl. *Environmental Pollution*, *164*, 36–39. https://doi.org/10.1016/j.envpol.2012.01.008

Møller, A. P., & Mousseau, T. A. (2006). Biological consequences of Chernobyl: 20 years on. *Trends in Ecology and Evolution*, Vol. 21, pp. 200–207. https://doi.org/10.1016/j.tree.2006.01.008

Møller, A. P., & Mousseau, T. A. (2009). Reduced abundance of insects and spiders linked to radiation at Chernobyl 20 years after the accident. *Biology Letters*, *5*(3), 356–359. https://doi.org/10.1098/rsbl.2008.0778

Møller, A. P., & Mousseau, T. A. (2016). Are Organisms Adapting to Ionizing Radiation at Chernobyl? *Trends in Ecology and Evolution*, Vol. 31, pp. 281–289. https://doi.org/10.1016/j.tree.2016.01.005

Møller, A. P., & Mousseau, T. A. (2018). Reduced colonization by soil invertebrates to irradiated decomposing wood in Chernobyl. *Science of the Total Environment*, *645*, 773–779. https://doi.org/10.1016/j.scitotenv.2018.07.195

Møller, A. P., Mousseau, T. A., De Lope, F., & Saino, N. (2007). Elevated frequency of abnormalities in barn swallows from Chernobyl. *Biology Letters*, *3*(4), 414–417. https://doi.org/10.1098/rsbl.2007.0136

Møller, A. P., Nishiumi, I., Suzuki, H., Ueda, K., & Mousseau, T. A. (2013). Differences in effects of radiation on abundance of animals in Fukushima and Chernobyl. *Ecological Indicators*, *24*, 75–81. https://doi.org/10.1016/j.ecolind.2012.06.001

Montgomery, W. I. (1980). Spatial organization in sympatric populations of Apodemus sylvaticus and A. flavicollis (Rodentia: Muridae). *Journal of Zoology*, *192*(3), 379–401. https://doi.org/10.1111/j.1469-7998.1980.tb04239.x

Montgomery, W. I. (1981). A removal experiment with sympatric populations of Apodemus sylvaticus (L.) and A. flavicollis (Melchior) (Rodentia: Muridae). *Oecologia*, *51*(1), 123–132. https://doi.org/10.1007/BF00344662

Morelli, F., Benedetti, Y., Mousseau, T. A., & Møller, A. P. (2018). Ionizing radiation and taxonomic, functional and evolutionary diversity of bird communities. *Journal of Environmental Management*, *220*, 183–190. https://doi.org/10.1016/j.jenvman.2018.05.032

Morton, J. T., Sanders, J., Quinn, R. A., McDonald, D., Gonzalez, A., Vázquez-Baeza, Y., … Knight, R. (2017). Balance Trees Reveal Microbial Niche Differentiation. *MSystems*, *2*(1). https://doi.org/10.1128/msystems.00162-16

Mousseau, T. A., & Møller, A. P. (2013). Elevated Frequency of Cataracts in Birds from Chernobyl. *PLoS ONE*, *8*(7), e66939. https://doi.org/10.1371/journal.pone.0066939

Mousseau, T. A., & Møller, A. P. (2014). Genetic and Ecological Studies of Animals in Chernobyl and Fukushima. *Journal of Heredity*, *105*(5), 704–709. https://doi.org/10.1093/jhered/esu040

Nakamura, A. J., Suzuki, M., Redon, C. E., Kuwahara, Y., Yamashiro, H., Abe, Y., … Fukumoto, M. (2017). The Causal Relationship between DNA Damage Induction in Bovine Lymphocytes and the Fukushima Nuclear Power Plant Accident. *Radiation Research*, *187*(5), 630–636. https://doi.org/10.1667/rr14630.1

Newbold, L. K., Robinson, A., Rasnaca, I., Lahive, E., Soon, G. H., Lapied, E., … Spurgeon, D. J. (2019). Genetic, epigenetic and microbiome characterisation of an earthworm species (Octolasion lacteum) along a radiation exposure gradient at Chernobyl. *Environmental Pollution*, *255*, 113238. https://doi.org/10.1016/j.envpol.2019.113238

O'Sullivan, J. N., Rea, M. C., O'Connor, P. M., Hill, C., & Ross, R. P. (2019). Human skin microbiota is a rich source of bacteriocin-producing staphylococci that kill human pathogens. *FEMS Microbiology Ecology*, *95*(2). https://doi.org/10.1093/femsec/fiy241

Ochiai, K., Hayama, S. I., Nakiri, S., Nakanishi, S., Ishii, N., Uno, T., … Omi, T. (2014). Low blood cell counts in wild Japanese monkeys after the Fukushima Daiichi nuclear disaster. *Scientific Reports*, *4*(1), 1–5. https://doi.org/10.1038/srep05793

Oka, T. (1992). Home range and mating system of two sympatric field mouse species, *Apodemus speciosus* and *Apodemus argenteus*. *Ecological Research*, *7*(2), 163–169. https://doi.org/10.1007/BF02348495

Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., … Helene, W. (2019). vegan: Community Ecology Package. R package version 2.5-6. *Https://CRAN.R-Project.Org/Package=vegan*.

Older, C. E., Diesel, A., Patterson, A. P., Meason-Smith, C., Johnson, T. J., Mansell, J., … Rodrigues Hoffmann, A. (2017). The feline skin microbiota: The bacteria inhabiting the skin of healthy and allergic cats. *PLOS ONE*, *12*(6), e0178555. https://doi.org/10.1371/journal.pone.0178555

Onuma, M., Endoh, D., Ishiniwa, H., & Tamaoki, M. (2020). Estimation of Dose Rate for the Large Japanese Field Mouse (Apodemus speciosus) Distributed in the "Difficult-to-Return Zone" in Fukushima Prefecture. In *Low-Dose Radiation Effects on Animals and Ecosystems* (pp. 17–30). https://doi.org/10.1007/978-981-13-8218-5_2

Ormerod, K. L., Wood, D. L. A., Lachner, N., Gellatly, S. L., Daly, J. N., Parsons, J. D., … Hugenholtz, P. (2016). Genomic characterization of the uncultured Bacteroidales family S24-7 inhabiting the guts of homeothermic animals. *Microbiome*, *4*(1), 1–17. https://doi.org/10.1186/s40168-016-0181-2

Ozaki, S., Fritsch, C., Valot, B., Mora, F., Cornier, T., Scheifler, R., & Raoul, F. (2018). Does pollution influence small mammal diet in the field? A metabarcoding approach in a generalist consumer. *Molecular Ecology*, *27*(18), 3700–3713. https://doi.org/10.1111/mec.14823

Palumbi, S. R. (2001). Humans as the world's greatest evolutionary force. *Science*, Vol. 293, pp. 1786–1790. https://doi.org/10.1126/science.293.5536.1786

Parfrey, L. W., Moreau, C. S., & Russell, J. A. (2018). Introduction: The host-associated microbiome: Pattern, process and function. *Molecular Ecology*, *27*(8), 1749–1765. https://doi.org/10.1111/mec.14706

Pascoe, E. L., Hauffe, H. C., Marchesi, J. R., & Perkins, S. E. (2017). Network analysis of gut microbiota literature: An overview of the research landscape in non-human animal studies. *ISME Journal*, *11*(12), 2644–2651. https://doi.org/10.1038/ismej.2017.133

Pelletier, F., & Coltman, D. W. (2018). Will human influences on evolutionary dynamics in the wild pervade the Anthropocene? *BMC Biology*, Vol. 16, pp. 1–10. https://doi.org/10.1186/s12915-017-0476-1

Perino, A., Pereira, H. M., Navarro, L. M., Fernández, N., Bullock, J. M., Ceauşu, S., … Wheeler, H. C. (2019). Rewilding complex ecosystems. *Science*, Vol. 364. https://doi.org/10.1126/science.aav5570

Perofsky, A. C., Lewis, R. J., & Meyers, L. A. (2019). Terrestriality and bacterial transfer: a comparative study of gut microbiomes in sympatric Malagasy mammals. *ISME Journal*, *13*(1), 50–63. https://doi.org/10.1038/s41396-018-0251-5

Pigliucci, M. (2005). Evolution of phenotypic plasticity: Where are we going now? *Trends in Ecology and Evolution*, *20*(9), 481–486. https://doi.org/10.1016/j.tree.2005.06.001

Pompanon, F., Deagle, B. E., Symondson, W. O. C., Brown, D. S., Jarman, S. N., & Taberlet, P. (2012). Who is eating what: Diet assessment using next generation sequencing. *Molecular Ecology*, Vol. 21, pp. 1931–1950. https://doi.org/10.1111/j.1365-294X.2011.05403.x

Ragon, M., Restoux, G., Moreira, D., Møller, A. P., & López-García, P. (2011). Sunlight-Exposed Biofilm Microbial Communities Are Naturally Resistant to Chernobyl Ionizing-Radiation Levels. *PLoS ONE*, *6*(7), e21764. https://doi.org/10.1371/journal.pone.0021764

Real, A., & Garnier-Laplace, J. (2019). The importance of deriving adequate wildlife benchmark values to optimize radiological protection in various environmental exposure situations. *Journal of Environmental Radioactivity*, *211*, 105902. https://doi.org/10.1016/j.jenvrad.2019.01.014

Ren, T., Boutin, S., Humphries, M. M., Dantzer, B., Gorrell, J. C., Coltman, D. W., … Wu, M. (2017). Seasonal, spatial, and maternal effects on gut microbiome in wild red squirrels. *Microbiome*, *5*(1), 163. https://doi.org/10.1186/s40168-017-0382-3

Richardson, J. B., Dancy, B. C. R., Horton, C. L., Lee, Y. S., Madejczyk, M. S., Xu, Z. Z., … Lewis, J. A. (2018). Exposure to toxic metals triggers unique responses from the rat gut microbiota. *Scientific Reports*, *8*(1), 1–12. https://doi.org/10.1038/s41598-018-24931-w

Ridenhour, B. J., Brooker, S. L., Williams, J. E., Van Leuven, J. T., Miller, A. W., Dearing, M. D., & Remien, C. H. (2017). Modeling time-series data from microbial communities. *ISME Journal*, *11*(11), 2526–2537. https://doi.org/10.1038/ismej.2017.107

Robb, G. N., Woodborne, S., de Bruin, P. R., Medger, K., & Bennett, N. C. (2015). The influence of food quantity on carbon and nitrogen stable isotope values in southern African spiny mice (*Acomys spinosissimus*). *Canadian Journal of Zoology*, *93*(5), 345–351. https://doi.org/10.1139/cjz-2014-0134

Rocca, J. D., Simonin, M., Blaszczak, J. R., Ernakovich, J. G., Gibbons, S. M., Midani, F. S., & Washburne, A. D. (2019). The Microbiome Stress Project: Toward a Global Meta-Analysis of Environmental Stressors and Their Effects on Microbial Communities. *Frontiers in Microbiology*, *9*. https://doi.org/10.3389/fmicb.2018.03272

Romanovskaia, V. A., Sokolov, I. G., Rokitko, P. V, & Chernaia, N. A. (1998). Ecological consequences of radioactive pollution for soil bacteria within the 10-km region around the Chernobyl Atomic Energy Station. *Mikrobiologiia*, *67*(2), 274–280.

Rooks, M. G., & Garrett, W. S. (2016). Gut microbiota, metabolites and host immunity. *Nature Reviews Immunology*, Vol. 16, pp. 341–352. https://doi.org/10.1038/nri.2016.42

Rosenberg, E., & Zilber-Rosenberg, I. (2018). The hologenome concept of evolution after 10 years. *Microbiome*, Vol. 6, p. 78. https://doi.org/10.1186/s40168-018-0457-9

Rosenfeld, C. S. (2017). Gut dysbiosis in animals due to environmental chemical exposures. *Frontiers in Cellular and Infection Microbiology*, Vol. 7, p. 396. https://doi.org/10.3389/fcimb.2017.00396

Ross, A. A., Müller, K. M., Scott Weese, J., & Neufeld, J. D. (2018). Comprehensive skin microbiome analysis reveals the uniqueness of human skin and evidence for phylosymbiosis within the class Mammalia. *Proceedings of the National Academy of Sciences of the United States of America*, *115*(25), E5786–E5795. https://doi.org/10.1073/pnas.1801302115

Ross, A. A., Rodrigues Hoffmann, A., & Neufeld, J. D. (2019). The skin microbiome of vertebrates. *Microbiome*, Vol. 7. https://doi.org/10.1186/s40168-019-0694-6

Rosshart, S. P., Vassallo, B. G., Angeletti, D., Hutchinson, D. S., Morgan, A. P., Takeda, K., … Rehermann, B. (2017). Wild Mouse Gut Microbiota Promotes Host Fitness and Improves Disease Resistance. *Cell*, *171*(5), 1015-1028.e13. https://doi.org/10.1016/j.cell.2017.09.016

Rothschild, D., Weissbrod, O., Barkan, E., Kurilshikov, A., Korem, T., Zeevi, D., … Segal, E. (2018). Environment dominates over host genetics in shaping human gut microbiota. *Nature*, *555*(7695), 210–215. https://doi.org/10.1038/nature25973

Rühm, W., Azizova, T., Bouffler, S., Cullings, H. M., Grosche, B., Little, M. P., … Woloschak, G. E. (2018). Typical doses and dose rates in studies pertinent to radiation risk inference at low doses and low dose rates. *Journal of Radiation Research*, *59*(S2), 1–10. https://doi.org/10.1093/jrr/rrx093

Ruiz-González, M. X., Czirják, G. Á., Genevaux, P., Møller, A. P., Mousseau, T. A., & Heeb, P. (2016). Resistance of Feather-Associated Bacteria to Intermediate Levels of Ionizing Radiation near Chernobyl. *Scientific Reports*, *6*(1), 1–7. https://doi.org/10.1038/srep22969

Russo, C. D., Weller, D. W., Nelson, K. E., Chivers, S. J., Torralba, M., & Grimes, D. J. (2018). Bacterial Species Identified on the Skin of Bottlenose Dolphins Off Southern California via Next Generation Sequencing Techniques. *Microbial Ecology*, *75*(2), 303–309. https://doi.org/10.1007/s00248-017-1071-2

Sampson, T. R., & Mazmanian, S. K. (2015). Control of brain development, function, and behavior by the microbiome. *Cell Host and Microbe*, Vol. 17, pp. 565–576. https://doi.org/10.1016/j.chom.2015.04.011

Sato, J. J., Shimada, T., Kyogoku, D., Komura, T., Uemura, S., Saitoh, T., & Isagi, Y. (2018). Dietary niche partitioning between sympatric wood mouse species (Muridae: Apodemus) revealed by DNA meta-barcoding analysis. *Journal of Mammalogy*, *99*(4), 952–964. https://doi.org/10.1093/jmammal/gyy063

Schmidt, E., Mykytczuk, N., & Schulte-Hostedde, A. I. (2019). Effects of the captive and wild environment on diversity of the gut microbiome of deer mice (Peromyscus maniculatus). *ISME Journal*, *13*(5), 1293–1305. https://doi.org/10.1038/s41396-019-0345-8

Sepulveda, J., & Moeller, A. H. (2020). The Effects of Temperature on Animal Gut Microbiomes. *Frontiers in Microbiology*, *11*, 384. https://doi.org/10.3389/fmicb.2020.00384

Shimada, T., Saitoh, T., Sasaki, E., Nishitani, Y., & Osawa, R. (2006). Role of tannin-binding salivary proteins and tannase-producing bacteria in the acclimation of the Japanese wood mouse to acorn tannins. *Journal of Chemical Ecology*, *32*(6), 1165–1180. https://doi.org/10.1007/s10886-006-9078-z

Shuryak, I. (2019). Review of microbial resistance to chronic ionizing radiation exposure under environmental conditions. *Journal of Environmental Radioactivity*, Vol. 196, pp. 50–63. https://doi.org/10.1016/j.jenvrad.2018.10.012

Shuryak, I. (2020). Review of resistance to chronic ionizing radiation exposure under environmental conditions in multicellular organisms. *Journal of Environmental Radioactivity*, Vol. 212, p. 106128. https://doi.org/10.1016/j.jenvrad.2019.106128

Smits, S. A., Leach, J., Sonnenburg, E. D., Gonzalez, C. G., Lichtman, J. S., Reid, G., … Sonnenburg, J. L. (2017). Seasonal cycling in the gut microbiome of the Hadza hunter-gatherers of Tanzania. *Science*, *357*(6353), 802–805. https://doi.org/10.1126/science.aan4834

Sommer, F., Ståhlman, M., Ilkayeva, O., Arnemo, J. M., Kindberg, J., Josefsson, J., … Bäckhed, F. (2016). The Gut Microbiota Modulates Energy Metabolism in the Hibernating Brown Bear Ursus arctos. *Cell Reports*, *14*(7), 1655–1661. https://doi.org/10.1016/j.celrep.2016.01.026

Song, S. J., Lauber, C., Costello, E. K., Lozupone, C. A., Humphrey, G., Berg-Lyons, D., … Knight, R. (2013). Cohabiting family members share microbiota with one another and with their dogs. *ELife*, *2013*(2). https://doi.org/10.7554/eLife.00458

Song, S. J., Sanders, J. G., Delsuc, F., Metcalf, J., Amato, K., Taylor, M. W., … 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, E. D., Smits, S. A., Tikhonov, M., Higginbottom, S. K., Wingreen, N. S., & Sonnenburg, J. L. (2016). Diet-induced extinctions in the gut microbiota compound over generations. *Nature*, *529*(7585), 212–215. https://doi.org/10.1038/nature16504

Sonnenburg, J. L., & Sonnenburg, E. D. (2019). Vulnerability of the industrialized microbiota. *Science*, Vol. 366. https://doi.org/10.1126/science.aaw9255

Stark, K., Goméz-Ros, J. M., Vives i Batlle, J., Lindbo Hansen, E., Beaugelin-Seiller, K., Kapustka, L. A., … Hinton, T. G. (2017). Dose assessment in environmental radiological protection: State of the art and perspectives. *Journal of Environmental Radioactivity*, Vol. 175–176, pp. 105–114. https://doi.org/10.1016/j.jenvrad.2017.05.001

Steinhauser, G., Brandl, A., & Johnson, T. E. (2014). Comparison of the Chernobyl and Fukushima nuclear accidents: A review of the environmental impacts. *Science of the Total Environment*, Vol. 470–471, pp. 800–817. https://doi.org/10.1016/j.scitotenv.2013.10.029

Stevenson, T. J., Duddleston, K. N., & Buck, C. L. (2014). Effects of season and host physiological state on the diversity, density, and activity of the arctic ground squirrel cecal microbiota. *Applied and Environmental Microbiology*, *80*(18), 5611–5622. https://doi.org/10.1128/AEM.01537-14

Stothart, M. R., Palme, R., & Newman, A. E. M. (2019). It's what's on the inside that counts: Stress physiology and the bacterial microbiome of a wild urban mammal. *Proceedings of the Royal Society B: Biological Sciences*, *286*(1913). https://doi.org/10.1098/rspb.2019.2111

Stradiotto, A., Cagnacci, F., Delahay, R., Tioli, S., Nieder, L., & Rizzoli, A. (2009). Spatial Organization of the Yellow-Necked Mouse: Effects of Density and Resource Availability. *Journal of Mammalogy*, *90*(3), 704–714. https://doi.org/10.1644/08-mamm-a-120r1.1

Strand, P., Sundell-Bergman, S., Brown, J. E., & Dowdall, M. (2017). On the divergences in assessment of environmental impacts from ionising radiation following the Fukushima accident. *Journal of Environmental Radioactivity*, Vol. 169–170, pp. 159–173. https://doi.org/10.1016/j.jenvrad.2016.12.005

Suzuki, H., Filippucci, M. G., Chelomina, G. N., Sato, J. J., Serizawa, K., & Nevo, E. (2008). A biogeographic view of Apodemus in Asia and Europe inferred from nuclear and mitochondrial gene sequences. *Biochemical Genetics*, *46*(5–6), 329–346. https://doi.org/10.1007/s10528-008-9149-7

Suzuki, T. A. (2017). Links between Natural Variation in the Microbiome and Host Fitness in Wild Mammals. *Integrative and Comparative Biology*, *57*(4), 756–769. https://doi.org/10.1093/icb/icx104

Takino, S., Yamashiro, H., Sugano, Y., Fujishima, Y., Nakata, A., Kasai, K., … Fukumoto, M. (2017). Analysis of the Effect of Chronic and Low-Dose Radiation Exposure on Spermatogenic Cells of Male Large Japanese Field Mice (Apodemus speciosus) after the Fukushima Daiichi Nuclear Power Plant Accident. *Radiation Research*, *187*(2), 161. https://doi.org/10.1667/rr14234.1

Tew, T. E., & Macdonald, D. W. (1994). Dynamics of space use and male vigour amongst wood mice, Apodemus sylvaticus, in the cereal ecosystem. *Behavioral Ecology and Sociobiology*, *34*(5), 337–345. https://doi.org/10.1007/BF00197004

Teyssier, A., Rouffaer, L. O., Saleh Hudin, N., Strubbe, D., Matthysen, E., Lens, L., & White, J. (2018). Inside the guts of the city: Urban-induced alterations of the gut microbiota in a wild passerine. *Science of the Total Environment*, *612*, 1276–1286. https://doi.org/10.1016/j.scitotenv.2017.09.035

Theodorakopoulos, N., Evrier, L. F., Barakat, M., Ortet, P., Christen, R., Piette, L., … Chapon, V. (2017). Soil prokaryotic communities in Chernobyl waste disposal trench T22 are modulated by organic matter and radionuclide contamination. *FEMS Microbiology Ecology*, *93*(8). https://doi.org/10.1093/femsec/fix079

Thompson, L. R., Sanders, J. G., McDonald, D., Amir, A., Ladau, J., Locey, K. J., … Zhao, H. (2017). A communal catalogue reveals Earth's multiscale microbial diversity. *Nature*, *551*(7681), 457–463. https://doi.org/10.1038/nature24621

Tieszen, L. L., Boutton, T. W., Tesdahl, K. G., & Slade, N. A. (1983). Fractionation and turnover of stable carbon isotopes in animal tissues: Implications for δ13C analysis of diet. *Oecologia*, *57*(1–2), 32–37. https://doi.org/10.1007/BF00379558

Trevelline, B. K., Fontaine, S. S., Hartup, B. K., & Kohl, K. D. (2019). Conservation biology needs a microbial renaissance: A call for the consideration of host-associated microbiota in wildlife management practices. *Proceedings of the Royal Society B: Biological Sciences*, Vol. 286. https://doi.org/10.1098/rspb.2018.2448

Trevelline, B. K., Sosa, J., Hartup, B. K., & Kohl, K. D. (2020). A bird's-eye view of phylosymbiosis: weak signatures of phylosymbiosis among all 15 species of cranes. *Proceedings of the Royal Society B: Biological Sciences*, *287*(1923), 20192988. https://doi.org/10.1098/rspb.2019.2988

Tu, P., Chi, L., Bodnar, W., Zhang, Z., Gao, B., Bian, X., … Lu, K. (2020). Gut microbiome toxicity: Connecting the environment and gut microbiome-associated diseases. *Toxics*, Vol. 8, p. 19. https://doi.org/10.3390/toxics8010019

Turnbaugh, P. J., Ley, R. E., Mahowald, M. A., Magrini, V., Mardis, E. R., & Gordon, J. I. (2006). An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature*, *444*(7122), 1027–1031. https://doi.org/10.1038/nature05414

Urushihara, Y., Kawasumi, K., Endo, S., Tanaka, K., Hirakawa, Y., Hayashi, G., … Fukumoto, M. (2016). Analysis of Plasma Protein Concentrations and Enzyme Activities in Cattle within the Ex-Evacuation Zone of the Fukushima Daiichi Nuclear Plant Accident. *PLOS ONE*, *11*(5), e0155069. https://doi.org/10.1371/journal.pone.0155069

van Leeuwen, P., Mykytczuk, N., Mastromonaco, G. F., & Schulte-Hostedde, A. I. (2020). Effects of captivity, diet, and relocation on the gut bacterial communities of white-footed mice. *Ecology and Evolution*. https://doi.org/10.1002/ece3.6221

Vázquez-Baeza, Y., Pirrung, M., Gonzalez, A., & Knight, R. (2013). EMPeror: a tool for visualizing high-throughput microbial community data. *GigaScience*, *2*(1), 16. https://doi.org/10.1186/2047-217X-2-16

Viney, M. (2019). The gut microbiota of wild rodents: Challenges and opportunities. *Laboratory Animals*, *53*(3), 252–258. https://doi.org/10.1177/0023677218787538

Visconti, A., Le Roy, C. I., Rosa, F., Rossi, N., Martin, T. C., Mohney, R. P., … Falchi, M. (2019). Interplay between the human gut microbiome and host metabolism. *Nature Communications*, *10*(1), 1–10. https://doi.org/10.1038/s41467-019-12476-z

Wang, G. H., Berdy, B. M., Velasquez, O., Jovanovic, N., Alkhalifa, S., Minbiole, K. P. C., & Brucker, R. M. (2020). Changes in Microbiome Confer Multigenerational Host Resistance after Sub-toxic Pesticide Exposure. *Cell Host and Microbe*, *27*(2), 213-224.e7. https://doi.org/10.1016/j.chom.2020.01.009

Watson, S. E., Hauffe, H. C., Bull, M. J., Atwood, T. C., McKinney, M. A., Pindo, M., & Perkins, S. E. (2019). Global change-driven use of onshore habitat impacts polar bear faecal microbiota. *ISME Journal*, *13*(12), 2916–2926. https://doi.org/10.1038/s41396-019-0480-2

Webster, S. C., Byrne, M. E., Lance, S. L., Love, C. N., Hinton, T. G., Shamovich, D., & Beasley, J. C. (2016). Where the wild things are: Influence of radiation on the distribution of four mammalian species within the Chernobyl Exclusion Zone. *Frontiers in Ecology and the Environment*, *14*(4), 185–190. https://doi.org/10.1002/fee.1227

Webster, T. M. U., Consuegra, S., & Leaniz, C. G. de. (2020). Early life stress causes persistent impacts on the microbiome of Atlantic salmon. *BioRxiv*, 2020.01.16.908939. https://doi.org/10.1101/2020.01.16.908939

Webster, T. M. U., Rodriguez-Barreto, D., Castaldo, G., Gough, P., Consuegra, S., & Garcia de Leaniz, C. (2020). Environmental plasticity and colonisation history in the Atlantic salmon microbiome: A translocation experiment. *Molecular Ecology*, *29*(5), 886–898. https://doi.org/10.1111/mec.15369

Weiss, S., Xu, Z. Z., Peddada, S., Amir, A., Bittinger, K., Gonzalez, A., … Knight, R. (2017). Normalization and microbial differential abundance strategies depend upon data characteristics. *Microbiome*, *5*(1), 27. https://doi.org/10.1186/s40168-017-0237-y

West, A. G., Waite, D. W., Deines, P., Bourne, D. G., Digby, A., McKenzie, V. J., & Taylor, M. W. (2019). The microbiome in threatened species conservation. *Biological Conservation*, Vol. 229, pp. 85–98. https://doi.org/10.1016/j.biocon.2018.11.016

Wheatley, S., Sovacool, B., & Sornette, D. (2017). Of Disasters and Dragon Kings: A Statistical Analysis of Nuclear Power Incidents and Accidents. *Risk Analysis*, *37*(1), 99–115. https://doi.org/10.1111/risa.12587

Woodhams, D. C., Bletz, M. C., Becker, C. G., Bender, H. A., Buitrago-Rosas, D., Diebboll, H., … Whetstone, R. (2020). Host-associated microbiomes are predicted by immune system complexity and climate. *Genome Biology*, *21*(1), 23. https://doi.org/10.1186/s13059-019-1908-8

Yatsunenko, T., Rey, F. E., Manary, M. J., Trehan, I., Dominguez-Bello, M. G., Contreras, M., … Gordon, J. I. (2012). Human gut microbiome viewed across age and geography. *Nature*, Vol. 486, pp. 222–227. https://doi.org/10.1038/nature11053

Yoshioka, A., Mishima, Y., & Fukasawa, K. (2015). Pollinators and Other Flying Insects inside and outside the Fukushima Evacuation Zone. *PLOS ONE*, *10*(11), e0140957. https://doi.org/10.1371/journal.pone.0140957

Youngblut, N. D., Reischer, G. H., Walters, W., Schuster, N., Walzer, C., Stalder, G., … Farnleitner, A. H. (2019). Host diet and evolutionary history explain different aspects of gut microbiome diversity among vertebrate clades. *Nature Communications*, *10*(1). https://doi.org/10.1038/s41467-019-10191-3

Yuan, X., Pan, Z., Jin, C., Ni, Y., Fu, Z., & Jin, Y. (2019). Gut microbiota: An underestimated and unintended recipient for pesticide-induced toxicity. *Chemosphere*, Vol. 227, pp. 425–434. https://doi.org/10.1016/j.chemosphere.2019.04.088

Zaneveld, J. R., McMinds, R., & Thurber, R. V. (2017). Stress and stability: Applying the Anna Karenina principle to animal microbiomes. *Nature Microbiology*, Vol. 2, pp. 1–8. https://doi.org/10.1038/nmicrobiol.2017.121

Zhang, A., & Steen, T. Y. (2018). Gut Microbiomics—A Solution to Unloose the Gordian Knot of Biological Effects of Ionizing Radiation. *Journal of Heredity*, *109*(2), 212–221. https://doi.org/10.1093/jhered/esx059

Zhang, J., Kobert, K., Flouri, T., & Stamatakis, A. (2014). PEAR: a fast and accurate Illumina Paired-End reAd mergeR. *Bioinformatics*, *30*(5), 614–620. https://doi.org/10.1093/bioinformatics/btt593

Zhang, W., Guo, R., Yang, Y., Ding, J., & Zhang, Y. (2016). Long-term effect of heavy-metal pollution on diversity of gastrointestinal microbial community of Bufo raddei. *Toxicology Letters*, *258*, 192–197. https://doi.org/10.1016/j.toxlet.2016.07.003

Original publications

I Lavrinienko, A., Mappes, T., Tukalenko, E., Mousseau, T. A., Møller, A. P., Knight, R., Morton, J. T., Thompson, L. R., Watts, P. C. (2018). Environmental radiation alters the gut microbiome of the bank vole *Myodes glareolus*. *ISME Journal*, 12(11), 2801–2806. https://doi.org/10.1038/s41396-018-0214-x

II Lavrinienko, A., Tukalenko, E., Mappes, T., & Watts, P. C. (2018). Skin and gut microbiomes of a wild mammal respond to different environmental cues. *Microbiome*, 6(1), 1–16. https://doi.org/10.1186/s40168-018-0595-0

III Lavrinienko, A., Tukalenko, E., Kesäniemi, J., Kivisaari, K., Masiuk, S., Boratyński, Z., Mousseau, T. A., Milinevsky, G., Mappes, T., Watts, P. C. (2020). Applying the Anna Karenina principle for wild animal gut microbiota: temporal stability of the bank vole gut microbiota in a disturbed environment. *Journal of Animal Ecology*. https://doi.org/10.1111/1365-2656.13342

IV Lavrinienko, A., Hämäläinen, A., Hindström, R., Tukalenko, E., Boratyński, Z., Kivisaari, K., Mousseau, T. A., Watts, P. C., Mappes, T. (2020). Comparable response of wild rodent gut microbiome to anthropogenic habitat contamination. *Manuscript*.

Original publications are not included in the electronic version of the dissertation.

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