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FORUM

Some observations on meaningful and objective inference in radioecological field studies

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Abstract

1. Anthropogenic releases of radiation are of ongoing importance for environmental protection, but the radiation doses at which natural systems begin to show effects are controversial. More certainty is required in this area to achieve optimal regulation for radioactive substances. We recently carried out a large survey (268 sampled animals and 20 sites) of the association between environmental radiation exposures and small mammal gut-associated microbiomes (fungal and bacterial) in the Chornobyl Exclusion zone (CEZ). Using individual measurements of total absorbed dose rates and a study design and analyses that accounted for spatial non-independence, we found no, or only limited, association.
2. Watts et al. have criticised our study: for not filtering candidate non-resident components prior to our fungal microbiome analyses, for our qualified speculations on the relative merits of faecal and gut samples, and for the design of our study which they felt lacked sufficient replication.
3. The advantage of filtering non-resident-fungal taxa is not clear and it would not have changed the null (spatially adjusted) association we found between radioactive dose and mycobiome composition because the most discriminatory fungal taxa with regard to dose were non-resident taxa.
4. We maintain that it was legitimate for us to make qualified discussion comments on the differences in results between our faecal and gut microbiome analyses and on the relative merits of these sample types.
5. Most importantly, the criticism of our study design by Watts et al. and the designs and analysis of their recent studies in the CEZ show a misunderstanding of the true nature of independent replication in field studies. Recognising the importance of spatial non-independence is essential in the design and analysis of radioecological field surveys.

KEYWORDS
Chernobyl, Chornobyl, dose rate, faeces, microbiome, radiation, red Forest, small mammal

1 | INTRODUCTION

Approaching four decades since the world's worst nuclear accident occurred at the Chornobyl Nuclear Power Station in the former Soviet Union (present day Ukraine), there is an ongoing debate on the extent to which radiation has impacted, and continues to impact, the natural environment (Beresford, Horemans, et al., 2020; Chesser & Baker, 2006; Mothersill et al., 2020; Mousseau & Møller, 2011; Smith, 2020). This debate is the most significant source of controversy within radioecology and, by extension, environmental radiation protection. Such controversy influences the development of, and stakeholder perceptions regarding, robust regulation for radioactive substances internationally. Therefore, it is essential that research on the wildlife of the Chornobyl Exclusion Zone adopts robust and replicable study designs, provides openly accessible datasets that others can independently analyse and fosters a transparent approach to determining the dose-effect response of wildlife to radiation.

Over recent years, there has been a growing interest in the relationship between animal microbiomes and exposure to pollutants (Antwis et al., 2021; Jin et al., 2017), recognising the crucial role that microbiomes play in host health and function (McFall-Ngai et al., 2013; McKenney et al., 2018). This has led to a number of studies on the impact of radiation on microbiomes within 'natural laboratories', such as the Chornobyl and Fukushima exclusion zones (e.g. Antwis et al., 2021; Lavrinienko, Hämäläinen, et al., 2021; Lavrinienko, Mappes, et al., 2018; Lavrinienko, Tukalenko, et al., 2018; Newbold et al., 2019; Videvall et al., 2022). Collectively, these studies have considered both the microbiomes of a range of organisms and of environmental substrates, reaching differing conclusions on relationships between radiation and microbiome composition.

In Antwis et al. (2021), we published our findings of an extensive study on the gut microbiome of small mammals (striped field mouse, yellow-necked mouse, wood mouse and bank vole) in the Chornobyl Exclusion Zone. That study, spanning 2 years (2017 and 2018), characterised the microbiome of 268 individuals from various locations in the Chornobyl Exclusion Zone across a range of ambient radiation dose rates (gamma dose rates measured in air using handheld dosimeters). Our subsequent analysis of these microbial datasets used the estimated total individual absorbed dose rate of each animal (i.e. quantifying both the external and internal absorbed radiation dose rate for each individual animal), providing an opportunity to explore dose-effect associations in the small mammal microbiome. One other group has undertaken a number of studies on the small mammal gut microbiome for animals in Chornobyl and, latterly, Fukushima (e.g. Lavrinienko et al., 2020; Lavrinienko, Hämäläinen, et al., 2021; Lavrinienko, Mappes, et al., 2018; Lavrinienko, Tukalenko, et al., 2018). Their studies have consistently identified associations between radiation and a response within the gut microbiome. The study we report in Antwis et al. (2021) reached different conclusions to the studies by this group, finding that associations between radiation exposure and gut microbiome composition were not robust against geographical variation.

In response to our original paper (Antwis et al., 2021), Lavrinienko, Scholier, et al. (2021) and, subsequently, Watts et al. (2022) have utilised the openly accessible data that we shared to undertake a reanalysis. We welcome their engagement in this constructive and open approach to scientific research. Their reanalysis provides a valuable opportunity for further discussion on some key aspects of gut microbiome analysis and of radiation effects studies utilising the Chornobyl Exclusion Zone. Watts et al. (2022) have been critical of certain aspects of our original study (Antwis et al., 2021). Here we discuss each of their main points in turn, with the section on 'Study design' being the most important as it relates to crucial issues in the design of field surveys of radiological effects in wildlife.

2 | IMPLICATIONS OF FUNGAL TAXA FILTERING

In their reanalysis of our original data, Watts et al. (2022) and Lavrinienko, Scholier, et al. (2021) suggest that the fungal component of the gut microbiome identified by Antwis et al. (2021) included some non-gut-resident fungi originating from the diet. This is likely true although, as we explain below, the methodological solution proposed by Lavrinienko, Scholier, et al. (2021) and Watts et al. (2022) is of uncertain value and its effect on our analyses of radiation would likely be negligible. Considering sequencing-based microbiome studies more generally, it is worth noting that uncertainty about the non-resident component is likely to apply to gut bacteria also. For bacterial gut and faeces communities in wild mammals, filtering candidate non-resident taxa is not routinely carried out (e.g. Alberdi et al., 2021; Clayton et al., 2016; Cui et al., 2021; Maurice et al., 2015; Weldon et al., 2015), probably due to a lack of information on which to base this. Lavrinienko, Scholier, et al. (2021) suggest that diet-derived bacteria in faeces are negligible, citing human gut microbiota calculations in Derrien and van Hylckama Vlieg (2015). However, the assumptions made in these calculations might not be applicable to small mammals which likely have a colonic volume several orders of magnitude smaller than humans and which might ingest more bacteria than a typical human (e.g. in the gut of prey, in carrion or through coprophagy). Moreover, it is possible that some non-gut-residents might have transient functional roles in the microbiome (Derrien & van Hylckama Vlieg, 2015; Zhang et al., 2016) and thus might be informative to include in overall analyses.

To demonstrate their point, Watts et al. (2022) identified candidate resident and non-resident taxa using Microfungi Collections Consortium (www.microfungi.org/table1) and FUNGUILD v.1.2 (Nguyen et al., 2016). They then filtered possible non-resident fungi from the fungal dataset published by Antwis et al. (2021). Although this might be analytically useful, Watts et al. miss a much more important point when making their criticism. Crucially,

variation due to non-resident taxa is likely to be fundamentally linked to the community composition of residents. This is because the diet, including the non-resident fungal components, will almost inevitably be a key driver of resident microbiome structure (Zhang et al., 2010). Filtering candidate non-resident taxa may thus, perhaps, help incrementally, but does not remove the influence of diet from a dataset.

Whether such filtering helps or hinders an analysis depends on the quality of the information on which it is based. As noted by Lavrinienko, Scholier, et al. (2021), uncritical filtering based on traits of microbial taxa has previously led to erroneous removal of novel resident microbiota. There are likely to be some limitations to the applicable fungal databases. Many sequences will be unannotated and there will inevitably be errors or inconsistencies in annotation (Abarenkov et al., 2018; Nguyen et al., 2016; Nilsson et al., 2012, 2019). This will be compounded by a lack of precision with taxonomic assignments inherent in current metabarcoding methods (Abarenkov et al., 2018; Hleap et al., 2021; Lücking et al., 2020). Watts et al. (2022) and Lavrinienko, Scholier, et al. (2021) found that their filtering made some changes in relation to associations with radiation. Watts et al. (2022) noted changes in data dispersion and Lavrinienko, Scholier, et al. (2021), using permutational multivariate analysis of variance (PERMANOVA), found a change in R^2 for radiation from 0.007 in an unfiltered analysis to 0.006 for resident taxa and 0.007 for non-resident taxa. However, it is highly unlikely that any reanalysis would change the ultimate conclusions of Antwis et al. (2021) about null associations of the fungal assemblage with radioactivity (when spatially adjusted). This is because the most discriminatory taxa, as radiation exposure increased, were those defined by Watts et al. (2022) and Lavrinienko, Scholier, et al. (2021) as non-resident taxa and thus removing them would further reinforce the null result. We note, additionally, that although Lavrinienko, Scholier, et al. (2021) reported associations with radiation in their PERMANOVA reanalyses, the models lacked any spatial representation (see Section 5) and the detected effects are likely due to habitat-related spatial confounding as suggested by our analysis in Antwis et al. (2021). It is difficult to evaluate taxonomic decisions by Watts et al. (2022) and Lavrinienko, Scholier, et al. (2021) as open data on these were not provided.

Watts et al. (2022) propose that '*informed filtering provides a more detailed assessment of the biological signal in the data than simply overlooking the ecology of fungi and animal hosts*'. However, based on our discussion above, we suggest that a holistic analysis, that includes all fungal components and recognises that the diet and resident mycobiome components are inherently intertwined is just as likely to provide useful insights. Furthermore, for the purposes of radioecological studies, the use of the microbiome including non-resident biota may inform understanding of differences in dietary composition between organisms. In Antwis et al. (2021), we observed associations between total absorbed dose rates of individuals and two fungal families that Watts et al. (2022) suggest are non-resident taxa. Given that diet determines internal radiation exposure, these taxa may be biomarkers of radiation exposure as we proposed in Antwis et al. (2021).

3 | FAECES VERSUS GUT SAMPLING IN MICROBIOME STUDIES

Antwis et al. (2021) observed differing associations between microbiome composition and radiation dose rate in gut samples compared to in faecal samples. Watts et al. (2022) discount this observation stating that the gut and faeces samples were unmatched (and thus subject to confounding variation). In fact, we explicitly acknowledged that our faeces and gut samples were not matched and this is reflected in the quote that Watts et al. provide in their criticism (i.e. '*… bank vole gut samples were collected in 2018 from across the CEZ, whereas the faeces samples collected in 2017 were all from inside the Red Forest (including from a number of sites that had been recently burnt), which may also be influencing the observed differences between the gut and faecal samples*.').

Despite the lack of sample matching, we maintain that it is appropriate to note the contrasts in the radiological correlations for faecal and gut samples, as this is indicative of a lack of consistency. Such inconsistency could be due to other variation (as we fully recognised), but could also be due to sample type. While faecal samples are indispensable for longitudinal studies and no doubt carry an important signal from the gut microbiome, nonetheless they may also be more influenced by the diet effects alluded to in the point above. Thus, faecal bacteria are likely to contain more unattached cells passing through the gut lumen and influenced by the diet. On the other hand, bacteria from the gastrointestinal tract itself are more likely to include attached, resident cells that are in closer contact with the host's immune system and physiology and thus more likely to be a sensitive indicator of the symbiotic interactions between the host and microbiome.

In summary, we agree with Watts et al. (2022) that faecal samples may be indispensable for longitudinal studies, or where destructive sampling is otherwise not logistically or ethically possible. We acknowledge that the value of faecal sampling could have been further discussed within Antwis et al. (2021). We also do not dispute that faeces will carry some useful signal about the composition of the gastrointestinal microbiota. However, in the case of a study design where destructive sampling is possible, we maintain our recommendation that samples are taken from the gut directly. Ingala et al. (2018) discuss the merits of faecal sampling versus sampling from the gastrointestinal tract. These authors conclude that gastrointestinal tract and faecal microbiomes record different information about the host, with faecal sampling likely being ideal for studies seeking to analyse the microbiome in context of host diet, whereas intestinal samples may be better suited for answering questions framed in the context of host evolution.

4 | EXPOSURE ESTIMATES

For ease, many previous observational surveys conducted in the Chornobyl Exclusion Zone relate biological variation (often termed 'effects') to ambient dose rates recorded using handheld dosimeters

(e.g. Lehmann et al., 2016; Møller & Mousseau, 2009, 2013). If the area surveyed is representative of the home range of the study species, the measurement of ambient dose rate is likely to give a reasonable estimate of the external dose rate (largely from radionuclides present in soil) from the gamma-emitting radioisotope ^{137}Cs (see discussion in Antwis et al., 2021). However, it ignores internal exposure (from radionuclides taken up into the animal's body) which contributes a significant proportion of the total dose rate for most organisms in the Chornobyl Exclusion Zone (Beresford, Barnett, et al., 2020); ecological variables will mean that there is not a consistent relationship between internal and external exposures across different sites. To best interpret radiation effects studies and to derive meaningful effects–dose rate relationships, absorbed dose rates for study organisms need to be estimated; this view is widely supported by the international community (see recommendations by, e.g. Beaugelin-Seiller et al., 2020; Beresford, Horemans, et al., 2020; Chesser & Baker, 2006; Lecomte-Pradines et al., 2020; Mothersill et al., 2020).

In Antwis et al. (2021), we state that '*we present the first analyses of small mammal faecal and gut microbial communities from the CEZ for which individual total absorbed dose rates have been estimated*'. Watts et al. refute this statement, suggesting that we had ignored their previous studies reported in Lavrinienko, Tukalenko, et al. (2018) and Lavrinienko et al. (2020). We note that in both of those papers the authors determined ^{137}Cs activity concentrations in their study animals to estimate internal dose. While this is to be applauded, it does not take into account internal exposure from ^{90}Sr. We estimated internal exposure from ^{90}Sr in Antwis et al. (2021); for a number of animals, ^{90}Sr contributed more to the internal dose rate than did ^{137}Cs and for some animals it contributed 50% or more of the total absorbed dose rate (i.e. internal and external dose rates combined). We thereby stand by our statement that we did present the first analyses of small mammal faecal and gut microbial communities from the CEZ for which individual total absorbed dose rates have been estimated.

In Antwis et al. (2021), we show a relatively poor correlation between ambient dose rate and total dose rates for small mammals. Furthermore, analysing the microbiome data against ambient dose rate gave different results compared to when using total absorbed dose rate.

5 | STUDY DESIGN

Watts et al. (2022) criticise the sampling design adopted by Antwis et al. (2021). This raises fundamental issues in the design of many types of field radioecological survey. Watts et al. (2022) suggest that 'replication of sites' is lacking in Antwis et al. (2021), although their spatial sampling design in the CEZ, which is the basis for multiple publications discussed below (Lavrinienko et al., 2020; Lavrinienko, Mappes, et al., 2018; Lavrinienko, Tukalenko, et al., 2018), looks similar to our own, except more dispersed and on a SW-NE axis instead of a NW-SE axis (see figure 1 in Watts et al., 2022). While they have more nominal sites than in the design adopted by Antwis et al. (2021), as we develop below, they do not represent the discreteness of these sites in their analyses and thus their extended spatial sampling is likely a source of magnified confounding more than anything else. Above all, in their criticism of Antwis et al. (2021) and in the design and/or analysis of their own recent works, Watts et al. miss the crucial point, which is of statistical independence of sampling and how non-independent (grouped) sampling is represented in analyses. In other words, what does, and what does not, constitute genuine replication.

Grouped samples cannot automatically be treated as genuine replicates because, due to their grouping, they may be influenced by similar extraneous factors and thus be biased. In particular, spatial location is a significant source of non-independence because, typically, many important components of the environment will vary spatially. A crucial further point to be made with regard to the Antwis et al.'s (2021) study is that it employs individual measures of total absorbed dose rate from live-monitoring individual animals. When different spatial sites are represented appropriately in subsequent statistical analyses (as they have been by Antwis et al., 2021), this means that each animal approximates to a genuine replicate with regard to radiation effects—with each unique value of the response (in this case, microbiome metrics) corresponding to a unique value of the predictor (in this case, total absorbed dose rate).

Watts et al. suggest that, as bank voles can potentially migrate 'several km' in a year, this effectively reduces some of our more clustered sites to a single site, but this is unrealistic. In fact, the citation used to support their suggested migration distance contains direct observations only of unconstrained long-term population, and not individual, movement (upper estimate 2.63 km/year, = *c.* 50 m/week) at the wave front of a biological invasion (White et al., 2012). Individual bank voles and other arvicoline and apodemine rodents typically maintain some level of site fidelity over weeks, months and even years (with the life spans of most animals measured in weeks or months) and home ranges with diameters of a few hundreds of metres at most (e.g. Attuquayefio et al., 1986; Lee & Rhim, 2016). This is amply evidenced by the wide popularity of these types of animal for mark–recapture studies (e.g. Fenton et al., 2014; Hammond & Anthony, 2006; Turner et al., 2014) which would otherwise be impractical. Although some dispersal may occur over extended timescales, this will be made up of sporadic events and it is reasonable to expect that an animal trapped at one site will usually reflect the general environment within a few 100 m of that site. For example, in a meta-analysis of 100 arvicoline species, Le Galliard et al. (2012) found average dispersal events to mostly be 'of the order of a few 10 m'. It is important to note, also, that in the case of radioactive dose, our individual measurements reflect the experience of the individual animal.

The appropriate application of individual total absorbed dose rate measurements contrasts with the case where ambient dose rate measurement is used and all animals at one site are effectively assumed to have the same exposure (e.g. Lavrinienko, Mappes, et al., 2018). Here, within a site, variation in the response variable

between individual animals cannot be linked to variation in the radiation dose absorbed by those individual animals and so the variation between the different animals—if the site is associated with a unique set of environmental confounders—holds no statistically recoverable information about the effects of dose. Within-site replication, in this case, is only truly useful for the estimation of a site mean. In such an ambient dose rate study, because all of the animals within a site are grouped and share the same myriad site-specific effects, the real level of biological replication is the site, with the site means, rather than individual values, being the true replicates. This has implications for study design, sample size and statistical power; in radioecology or in ecology more generally, any analysis that attempts to compare biological variation in individuals between sites (unless the overall effect of site itself is the actual interest), as if they were independent replicates, will inevitably be prone to confounding and false positives. For example, in the Lavrinienko, Mappes, et al.'s (2018) study, rather than their analyses having (where this is quoted) >100 degrees of freedom, in fact, because of spatial dependence, they only have three true, independent biological replicates with respect to radiation exposure because of the geographical clustering of the high, low and control exposure groups they use in analyses. (Here the groupings in their analysis correspond to the two SW–NE scatters of samples in the CEZ shown in Watts et al. figure 1, the more northerly of which has higher dosage, and control sites *c*. 100 km away in the Kyiv area.) Where site replication is included in an individual-level study, such as Antwis et al. (2021), this may largely be for representativeness (to avoid bias by selecting a single or small number of unusual sites, or to represent a range of different environments) and not for the statistical value of the site replication per se. Multiple sites may also be necessary to enable a greater range in individual total absorbed dose rates to be evaluated.

In the Antwis et al.'s (2021) study, individual sites are represented as a multi-level factor in PERMANOVA analyses, and as a spatial matrix in partial Mantel analyses. In fact, there is wide variation in individual dose rate within many sites, and there is wide overlap between many sites, and the inclusion of site in the analyses (with each site representing a small area, a trapping grid or line) accounts for spatial non-independence and between-site differences. In contrast, in the studies cited by Watts et al. as examples of individual measurement, although individual exposures were estimated (see discussion above), these measurements are not used effectively in analyses. In Lavrinienko, Tukalenko, et al. (2018), sampling is spread out highly non-randomly in two long SW–NE scatters in the CEZ (see Watts et al., figure 1), each of which is 10s of km long and separated from the other by 10s of km, with the other samples collected *c*. 100 km to the southeast around Kyiv. The individual doses are analysed in a PERMANOVA model with a two-level geographical factor for the Kyiv area versus the CEZ, or by a Spearman's correlation. In the former case, the spatial factor is simply not granular enough to account for non-independence in the spatial sampling pattern. In the Spearman's correlation, there is no accounting for spatially non-independent sampling at all. Lavrinienko et al. (2020) use the same sampling sites as Lavrinienko, Tukalenko, et al. (2018) and the same approach for PERMANOVA analyses; they also carry out balancing analyses employing four site levels for the sampling in the CEZ. As the sampling is spread out (27 sites are mentioned), this is again unlikely to be granular enough to account for spatial non-independence, leaving great uncertainty about study conclusions. Other potentially influential studies from the same group have been even more optimistic in their approach to study design and interpretation. For example, Kesäniemi et al. (2019) collected 10 bank voles at each of 2 sites in the CEZ and 2 sites around Kyiv and then analysed these for differential gene expression seemingly using a two-level comparison (Kyiv vs. CEZ), making extensive conclusions about the effects of radiation on metabolism and immunosuppression. However, with effectively no true biological replication for radiation exposure, as geography is totally confounded with radiation exposure, this study would not be expected to yield anything beyond the most tentative inference at best. Furthermore, it is worth noting that the CEZ lies in the Polesia woodland ecological zone, but that Kyiv is located at the southern edge of this zone, where it meets the East European forest steppe biome (Fileccia et al., 2014). Kyiv (*c*. 100 km away from the CEZ) thus represents a poor control site, even if it were incorporated in a suitable study design.

We hope this discussion illustrates the advantages of individual dose rate measurements, provided that they are incorporated in analyses that represent spatial variation sufficiently. Whatever the scale of spatial sampling that is undertaken, the most important consideration is to design and analyse studies in a way that takes spatial variation into account and that does not confound the variables of interest.

6 | CONSIDERATIONS ON STUDIES IN RADIOLOGICALLY CONTAMINATED SITES

In Antwis et al. (2021), we make a comment that '*any study that uses the Red Forest as a location for radiation effect studies on wildlife needs to consider the historical impacts of radiation and other stressors (e.g. wildfires) on this area*'. Watts et al. (2022) appear to challenge this opinion. The Red Forest is a virtually unique area within the Chornobyl Exclusion Zone. In 1986, radiation dose rates were sufficient to kill pine trees, with soil invertebrates and small mammals also being recorded as severely impacted in the area (Geras'kin et al., 2008). Although the Red Forest has slowly been recolonised by less radiosensitive deciduous tree species and understorey vegetation, it has poor habitat quality. In a recent paper (Beresford et al., 2022), we report that soil biological activity (invertebrates) is comparatively low in the Red Forest and suggest that this may be a residual consequence of the acute high exposure the ecosystem experience in 1986. We recognised that studies will continue to use the Red Forest as a study site because of the high dose rates organisms receive there. However, we stand by our comment that studies conducted in the Red Forest need to consider historical impacts and this view has been supported by many scientists internationally (Beresford, Barnett, et al., 2020; Lecomte-Pradines et al., 2020).

There are many studies (e.g. Mappes et al., 2019; Møller & Mousseau, 2009, 2011, 2013) reporting effects on organisms in the Chornobyl Exclusion Zone at what appear extremely low-dose rates compared to natural background exposure and what are considered no effects exposure dose rates for regulatory assessment (Beresford, Horemans, et al., 2020). Unfortunately, the underlying data for such papers is rarely published preventing independent reanalyses.

Watts et al. (2022) suggest, in our view erroneously as we have demonstrated above, that Antwis et al.'s (2021) study is '*yet another controversy that does not appear to be justified*'. While we disagree with this, we would agree that there are many papers making conclusions on the effects of radiation at contaminated sites that are not justified. An example is Mappes et al. (2019) who, with respect to the consequences of the Chornobyl accident, state that '*entire ecosystems, are likely to have been affected across perhaps 200,000 km^2 in Eastern, Northern, and even Central Europe*' yet present no robust evidence to support this statement. Smith (2020) points out that such 'findings', which often generate significant media and public impact, could severely hinder the very difficult process of recovery of the communities living in areas affected by the Chornobyl and Fukushima accidents, potentially damaging people's health and wellbeing. Researchers have a responsibility to ensure that their conclusions are defensible and fully justified based on the evidence presented.

There is perhaps a tendency for some scientists investigating the effects of radiation at contaminated field sites to feel they need to find a selective explanation for their results; it seems to be difficult for some to say 'we do not understand why'. Watts et al. (2022) cite Lavrinienko, Hämäläinen, et al. (2021) as demonstrating impacts of exposure to radionuclides in the gut microbiota of three, out of four, *Apodemus species* studied in the Chornobyl Exclusion Zone and Fukushima area. The species which showed no effect was *Apodemus argenteus*, one of two species studied at Fukushima (the other species being *A. speciosus*). The authors conclude that '*The notable lack of gut microbiota response to radiation in one mouse species from Fukushima* (i.e. A. argenteus) *may be due to host escape from most radiation exposure through its unique tree-dwelling lifestyle*' also suggesting that '*the microbiota species escape radiation*'. However, *A. argenteus* is, as the authors acknowledge, only semi-arboreal (Nakamura-Kojo et al., 2016) with some reports indicating that it can nest underground (Odachi et al., 2009), which would increase exposure. Anderson et al. (2021) conducted a large sampling of *A. speciosus* and *A. argenteus* in the Fukushima area finding little difference in the internal dose rate of the two species, a finding in agreement with Lavrinienko, Hämäläinen, et al. (2021). However, external exposure is the largest contributor to total dose in the Fukushima area. Anderson et al. (2021) estimated the external exposure of *A. argenteus* for two scenarios: (a) assuming the species spends 100% of its time on the ground surface; and (b) assuming it spends 50% of time in trees. When 50% occupancy in trees was assumed, the estimated dose rate was only about 8% lower than when 100% occupancy on the ground surface was assumed. Therefore, the suggestion of Lavrinienko, Hämäläinen, et al. (2021) to explain their finding does not appear plausible.

7 | CONCLUSIONS

In overall conclusion, Antwis et al. (2021) presented a large study (268 sampled animals and 20 sites), with analyses that effectively adjusted for spatial distribution, robustly demonstrating that individual radiation dose has no association or limited association with gut and faecal microbiomes of small mammals within the CEZ. We are unconvinced that filtering non-resident-fungal taxa would have improved our fungal microbiome analyses or altered our main conclusions with regard to associations with dose rate. Although we acknowledge that the value of faecal sampling could have been further discussed within Antwis et al. (2021), we maintain that it was appropriate for us to comment on the differences in results between our faecal and gut microbiome analyses and on the relative merits of these sample types. Most importantly, we find that the criticism of our study design by Watts et al. (2022) and the designs and analysis of their recent studies in the CEZ show a misunderstanding of the true nature of replication in field studies. Recognising the importance of spatial non-independence is essential in the design of radioecological field surveys—but this does not necessarily entail evermore extended spatial sampling—rather it is important to carry out representative studies that generate sufficient genuine biological replication for the features of interest.

AUTHORS' CONTRIBUTIONS

J.A.J., R.E.A., N.A.B. and M.D.W. contributed to writing the manuscript and gave final approval for publication.

CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

DATA AVAILABILITY STATEMENT

This article did not use any datasets.

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