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Changes in the Skin Microbiota in Two Bare-nosed Wombats (*Vombatus ursinus*) with Differing Recovery Trajectories following Treatment for Sarcoptic Mange

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ABSTRACT: We report tracking of bacterial skin microbiota for two bare-nosed wombats (*Vombatus ursinus*) following in situ treatment for sarcoptic mange. *Sarcoptes scabiei*, the etiologic agent, has dramatic effects on skin microbiota. Our case reports show differing disease trajectory and bacterial beta diversity between the two treated individuals.

Key words: Bare-nosed wombat, microbiota, recovery, restoration, sarcoptic mange, *Sarcoptes scabiei*, treatment *Vombatus ursinus*.

The skin can be viewed as an ecosystem comprised of host tissue, a microbial community, and sometimes other organisms (Grice and Segre 2011). Some diseases disrupt this epidermal microbiota or are caused by microbial imbalances, such as impetigo, atopic dermatitis, and psoriasis. Despite a general understanding of which microbes are associated with various skin diseases (e.g., *Staphylococcus* in atopic dermatitis; Yang et al. 2022), there is little information on whether microbial community changes associated with disease are restored by treatment or if treatment leads to an alternative microbial state.

Sarcoptic mange (scabies in humans) is a skin disease caused by *Sarcoptes scabiei* and is among the most widespread and impactful of mammalian ectoparasites. Sarcoptic mange been documented in ca. 150 mammal species and occurs on every continent except Antarctica (Escobar et al. 2021). *Sarcoptes scabiei* infestation disrupts microbial communities in the skin as seen in multiple species, including humans (Brook 1995; Bernigaud et al. 2021), canids (DeCandia et al. 2019), pigs (Swe et al. 2014), and wombats (Næsborg-Nielsen et al. 2022). Typical impacts are reductions of microbial diversity and increased potentially pathogenic microbial taxa (e.g., *Staphylococcus*, *Streptococcus*). Sarcoptic mange can be resolved using acaricides. However, the effects of treatment on *S. scabiei*–induced microbial community changes are poorly understood.

Herein, we used a field treatment trial of *S. scabiei* in bare-nosed wombats (*Vombatus ursinus* [BNWs]), which suffer severe crusted mange (Martin et al. 2018). They are treated in situ for sarcoptic mange throughout their range in southeast Australian range, predominantly with ivermectin (Ruykys et al. 2013), moxidectin (Martin et al. 2019), or fluralaner (Wilkinson et al. 2021). We opportunistically collected repeat skin swabs from two mange-impacted individuals before and after fluralaner treatment at Cape Portland, Tasmania (2021–22) and assessed microbial community changes relative to known microbial community states from healthy and mangy individuals (Næsborg-Nielsen et al. 2022; hereafter, background wombat data).

Tracking and assessing the recovery of free-living mammals after treatment is difficult. Our case-study wombats were both adult males of unknown age and had contrasting disease trajectories (recovery vs. reinfestation). Captures were made by hand netting, with ear tagging on initial capture; scoring of sarcoptic mange severity is as described previously (Simpson et al. 2016). In brief, swabs moistened with sterile saline were collected in triplicate from each flank (left and right) and the groin and then stored at −20 C.

Wombat 1 was captured and treated five times with fluralaner (Bravecto, MSD Animal

Health, Merck & Co., Kenilworth, New Jersey, USA) at 45-, 45-, 85-, 45-, and 45-mg/kg doses in March, May, June, July, September, and January, respectively, owing to slow recovery and signs of reinfestation or recrudescence (Fig. 1A). Wombat 2 was captured twice and treated with 85 and 45 mg/kg fluralaner in July and September and exhibited clear signs of recovery (Fig. 1A).

From each swab, DNA was extracted by the Garvan Institute of Medical Research by using the QIAmp Cell and Tissue extraction kit (QIAGEN, Sydney, Australia), including 20 sterile saline swabs as negative controls. Bacterial 16S rRNA and fungal internal transcribed spacer 2 (ITS2) rDNA were PCR-amplified using primers 515F/806r and fITS7/ITS4, respectively, and barcoded for next-generation sequencing. The DNA sequencing was performed on an MiSeq v2 system (Illumina, Sydney, Australia) at the Ramaciotti Center for Genomics (University of New South Wales, Sydney, Australia). The DNA sequencing data from the BNWs, 20 negative controls, and background wombat data were processed and analyzed using the microbiome bioinformatics platform QIIME 2 2022.11 (Bolyen et al. 2019). Jupyter notebooks containing all code are available for 16SV4 and ITS2 in Github (https://github.com/ChrisNaes/BNW_microbiome_recovery). The initial dataset contained 3,137,709 raw reads across 124 samples (mean, 20,966) across 11 animals for 16S and 4,718,584 raw reads across 123 samples (mean, 38,362) across 11 animals for ITS2. The raw reads are available in BioProject PRJNA1002826 (National Center for Biotechnology Information, Bethesda, Maryland). Forward and reverse reads (R1+R2) were imported into QIIME2 (Bolyen et al. 2019) and denoised with DADA2 (via q2-dada2; Callahan et al. 2016) with a trim length of 240 bp for 16S. The trim length for ITS2 was 0 due to the potential of removing important information in the highly variable ITS2 region (Yang et al. 2018). Sequences were assigned taxonomy by using the feature-classifier (Bokulich et al. 2018) plugin (naïve Bayesian) on a pretrained SILVA 138 classifier (Robeson et al. 2020) for 16S and on a trained UNITE v9 29.11.2022 classifier (Abarenkov et al. 2022) for ITS and clustered at 99% similarity. A phylogenetic tree was created using the fragment-insertion plugin in combination with SEPP (Janssen et al. 2018) for 16S data.

The data from QIIME2 were sorted with the filter_taxa function from the R package metacoder (Foster et al. 2017); contaminants were identified using decontam's prevalence-based method (Davis et al. 2018) and removed. The feature tables were rarefied using metacoder (Foster et al. 2017) with a depth rounded down to 10,000 for both 16S and ITS2. Rarefaction depth was determined from the sample with the lowest number of reads after removing contaminants. The final analyzed dataset comprised 2,982,632 reads (mean, 28,406) for 16S and 3,879,685 reads (mean, 43,107) for ITS2 (36 samples for wombat 1 and 18 samples for wombat 2).

For both bacterial and fungal microbiota data, we calculated alpha diversity for all time points and body sites by using the inverted Simpson index from the vegan R package (Dixon 2003) and beta diversity based on species abundance by using metacoder (Foster et al. 2017). Community structure (beta diversity) was visualized via multidimensional scaling of Bray-Curtis dissimilarity index (Bolyen et al. 2019). In preliminary analyses, we evaluated the effect on body site (flanks vs. groin) by using a repeated measures analysis of variance (wombat ID as the repeated measure, because 6–12 samples came from the same individual, with 3 samples per body site). Diversity did not differ among body site groups ($P>0.05$); data were therefore combined for further analyses (Fig. 1B, C).

The wombats had differing recovery trajectories based on bacterial beta diversity (Fig. 1B). Wombat 2 exhibited a microbial and mange severity score trajectory toward recovery, whereas wombat 1's scores suggested initial recovery followed by reexposure or recrudescence (not possible to distinguish) and relapse into severe disease (Fig. 1A). The bacterial phyla with the greatest abundance in both wombats were Actinobacteriota and Firmicutes. Eighteen bacterial families accounted for ca. 90% of skin bacteria, and the remaining taxa were pooled as "rare" (Fig. 1C). Variation of bacterial communities differed through time and by individual (Fig. 1C). Wombat 1 had a continuous presence of Staphylococcaceae, Corynebacteriaceae, Streptococcaceae and Brevibacteriaceae, with no

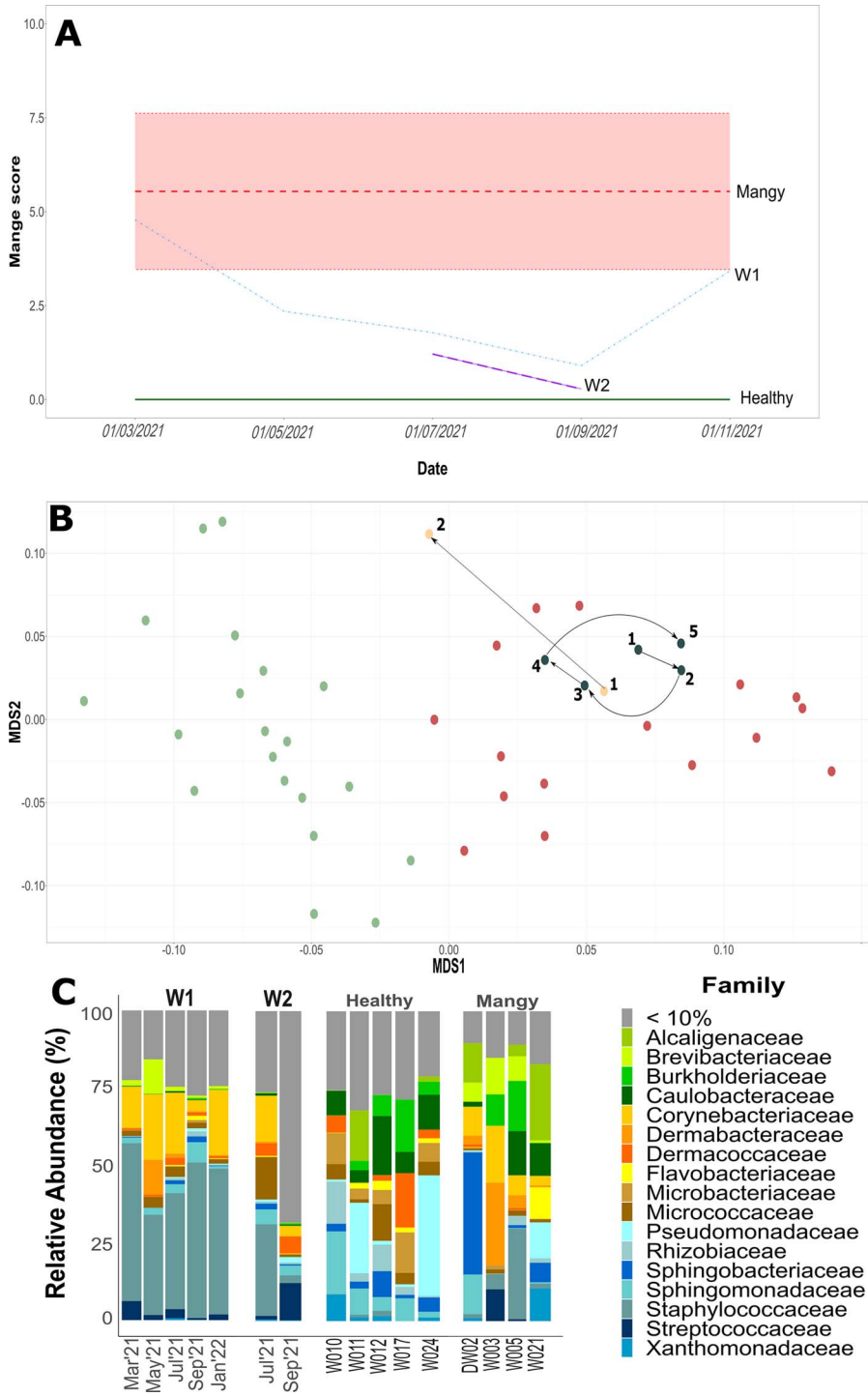

FIGURE 1. (A) Line plot of mange scores for wombat 1 (W1), wombat 2 (W2), and the background wombat data (healthy and mangy). Wombats W1 and W2 are shown as average mange severity scores in the sampled months, and background wombat data are shown as a mean mange score and SD calculated based on the mange score of all wombats included in healthy and mangy respectively. (B) Multidimensional scaling plot of 16S V4 bacterial data by using the Bray-Curtis dissimilarity index. Each dot in dark blue or yellow represents a

obvious variation in relative abundance. Wombat 2 initially (July) had abundant Staphylococcaceae, Micrococcaceae, and Corynebacteriaceae as well as a smaller presence of Streptococcaceae and Dermacoccaceae. Relative bacterial abundance changed from September to having a larger presence of rare taxa (gray color in Fig. 1C) as well as continuous smaller presence of Staphylococcaceae, Dermacoccoceae and Corynebacteriaceae. Our fungal dataset (https://github.com/ChrisNaes/BNW_microbiome_recovery) exhibited more variance in both the background data and for the two individuals examined.

Sarcoptes scabiei–induced changes in skin microbiota from the two wombats in this study are consistent with those of previous reports (Swe et al. 2014; DeCandia et al. 2019; Bernigaud et al. 2021; Næsborg-Nielsen et al. 2022). Consistent with our previous research (Næsborg-Nielsen et al. 2022), potentially pathogenic Staphylococcaceae and Corynebacteriaceae were present in our wombats, with degree associated with mange severity (Fig. 1A). The relative abundance of dominant bacterial taxa remained consistent over time in wombat 1, except for a slight decrease of *Corynebacterium* in September 2021 when wombat 1 had a lower mange score (Fig. 1A). By contrast, wombat 2 shifted from having a similar bacterial composition to wombat 1 at initial sampling toward being more similar to the healthy controls, with an increased proportion of taxa classified as rare, reduced potentially pathogenic bacteria, and intermediate beta diversity relative to healthy and mangy controls (Fig. 1B). Whether this is an indication that restoration to healthy microbiota was occurring, but was not complete, or that an alternative state may be attained after treatment needs further investigation.

Few studies have investigated the recovery of skin microbiotas, but gut studies have provided evidence that microbial diversity can progressively recover over a period of 6 mo after antibiotic disturbance, but full restoration of bacterial richness may take longer or be permanently lost (Palleja et al. 2018). Our sampling of wombat 2 indicates that if recolonization of skin microbial communities was occurring for this individual, it was taking >2 mo.

We cannot be certain of the cause(s) of the different recovery trajectories of the wombats in this study (e.g., reexposure, recrudescence, impacts of duration of infection, age of animal, and other health factors at the time of treatment). Continuous and severe infections may coalesce into a chronic state of immune suppression with persistent and reoccurring secondary infections (Delano and Ward 2016). This phenomenon could potentially explain why wombat 1 seemingly had an improved mange score in July and September 2021, but no substantial change in bacterial beta diversity, or relative abundance of *Staphylococcus* or *Corynebacterium*. Further research is needed into the effects of prolonged sarcoptic mange infestations on host immune pathways.

Obtaining repeat samples from wombats in situ is not trivial, and we caution overinterpretation of our results given the small sample size. Our results provide preliminary insight into host's bacterial community changes in association with sarcoptic mange treatment. Further investigations are warranted because the proportion of *Staphylococcus* on the skin might influence disease progression and treatment outcomes; tracking disease recovery through the epidermal microbiota might provide us with more in-depth knowledge of potential disease drivers and therapeutic advances.

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wombat, and each dot in green and red represents a sample. The numbers from 1 to 5 represents the different months and year (e.g., March 2021, May 2021, July 2021, September 2021, and January 2022) in which W1 was caught. The numbers 1 and 2 represent W2 in July 2021 and September 2021, respectively. The arrows link the samples across time. Green and red symbolize the healthy and mangy background wombat data, respectively, from Næsborg-Nielsen et al. (2022). (C) Family-level microbial taxonomic bar chart of 16S V4 bacterial data collapsed by wombat ID and sectioned into the month and year that the samples were collected and wombat data mange status.

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