Supplementary Figures

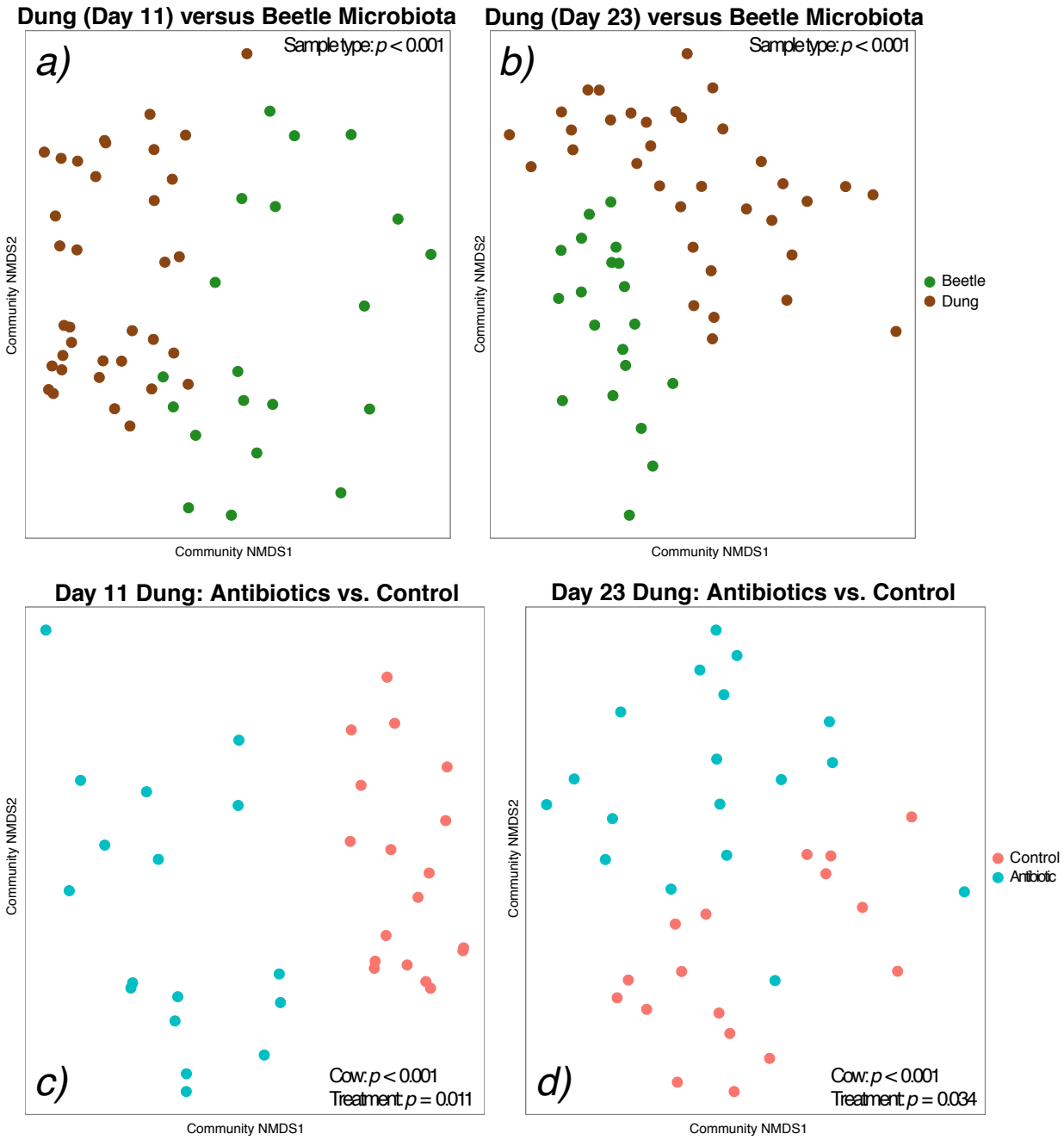

Figure S1. Regardless of the sampling point (number of days into the experiment), dung microbiota are distinct from dung beetle microbiota, and remain affected by antibiotic treatment and cow individual. Shown are non-metric multidimensional scaling ordinations of microbial communities in dung versus beetles (both treatments combined; *a* and *b*), and in dung from control cows versus antibiotic-treated cows (*c* and *d*). The ordinations visually represent Bray-Curtis dissimilarities among samples in two dimensions. In the corners of each plot, we show P values associated with each tested predictor variable from permutational multivariate ANOVAs.

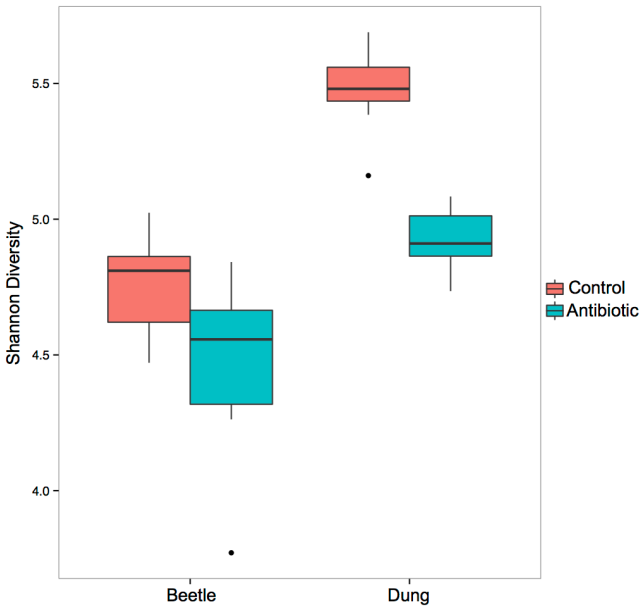

Figure S2. Microbial community diversity (Shannon index) is lower in beetles than in dung, and is also lowered by antibiotic treatment. Shown are boxplots with bold horizontal lines indicating the median, box limits showing first and third quartiles, whiskers extending to the most extreme values within 1.5 * the inter-quartile range, and dots showing outlying data points.

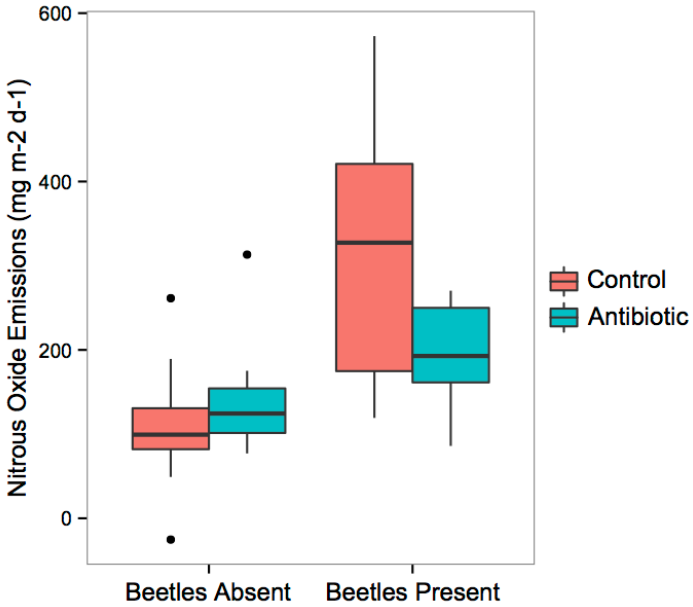

Figure S3. Total dung pat emissions of nitrous oxide are generally raised by the presence of dung beetles, but to an extent that is mediated by antibiotic treatment. Antibiotics limit the degree to which beetles increase nitrous oxide fluxes. Shown are boxplots with bold horizontal lines indicating the median, box limits showing first and third quartiles, whiskers extending to the most extreme values within 1.5 * the inter-quartile range, and dots showing outlying data points.

Supplementary Methods

Experimental setup

On June 7th 2014, ten cows were randomly assigned to two treatments: five were given a standard three-day course of tetracycline (Engemycin LA vet 100 mg/ml, manufacturer Intervet International B.V., dosage 10 mg/kg by intravenous injection administered once daily, "AB+") and five were left as controls ("AB-"). In the control group, only one cow had received antibiotic therapy in its lifetime, whereas the others had never been treated with antibiotics. Eleven months prior to the experiment, this cow had received "dry cow therapy" (DCT), a mixture of penethamate hydroiodide, benethamine penicillin, and framycetin administered locally as a single, low-level dose to the udder, with low absorption. In the antibiotic group, three of the cows had previously been treated with antibiotics: one with a course of penicillin six months prior to the experiment, the second with penicillin six weeks prior and DCT 11 months prior, and the third with two courses of penicillin and two DCT three years prior. A permit for medicating healthy cows was obtained from the Animal Experiment Board in Finland (ELLA) in accordance with the Finnish Act on Animal Experimentation (6/2/2006). The ten cows ranged in weight from 580 to 810 kg and were housed in a shed at the Viikki Experimental Farm at the University of Helsinki and grazed outdoors daily.

From each cow, we collected 7-10 kg of dung rectally into a clean 20 L tub. To minimize variation, we collected all dung within a single day (June 9, 2014), between 10 AM and 2 PM, beginning one hour after the last administration of antibiotics. That afternoon, we started the experiment by first homogenizing and separating the dung from each cow into six 1 L pats using a scoop replaced between cows. Each pat was placed in a mesocosm under semi-natural field conditions. These mesocosms were constructed from open-bottom plastic buckets (58 cm diameter, height 32cm) that had been dug into the soil in May 2014. To keep the grass at a length typical of pastures, it was cut inside and outside the mesocosms. We also included four control mesocosms without dung, which were used to measure background fluxes of carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O) from the soil.

To examine the effects of antibiotics on dung beetle microbiota, on the performance of beetles, and on beetle-mediated effects on gas fluxes from cow pats, we focused on the dung beetle *Aphodius fossor* (L.). *A. fossor* is a regionally widespread and locally common species (Roslin 2001), and its ecology and interactions with dung have been extensively studied (e.g., Vessby 2001, Penttilä et al. 2013, Slade et al. 2015). The beetles were collected in the field in early June and stored in moist paper at 4°C until they were added to dung pats. Beetles from different localities were randomized among mesocosms.

Dung beetles were added to four of the six dung pats produced by each cow (randomly chosen). Gas measurements and dung samples for microbial analysis were taken from two intact pats with beetles and two without beetles. The two additional pats with beetles were used for more invasive sampling of beetles for microbial characterization and to measure beetle reproduction and development. Based on beetle densities recorded in the field and on a previous study (Vessby 2001), we added 12 beetles to each pat, maintaining a sex ratio of 1:1. Each mesocosm was covered by a mesh lid for the full course of the experiment to prevent beetle escape and corvid predation.

Gas flux measurements

Gas fluxes of CO_2 were measured in the field with an EGM-4 Environmental Gas Monitor for CO_2, and a SRC-1 soil respiration chamber (PP Systems, Amesbury, USA). For each sampling event, the gas flux chamber was pushed 5 cm into the ground around the pat. The sampling time was 80 seconds and the measuring interval 4.8 seconds. Temperature inside the chamber was recorded during the sampling of all gases, for later scaling of gas fluxes to temperatures.

For N_2O and CH_4, we took four sequential samples 5, 10, 20 and 30 minutes after pushing the chamber into the ground and sealing it. Samples were taken with syringes into 3-ml Exetainer® vials with double-wadded septa (Labco Ltd., Lampeter, UK). Gas flux measurements of N_2O and CH_4 were taken on days 1, 5, 10, 24 and 43 of the experiment (experimental setup being conducted on day 0). CO_2 measurements were taken on the same days apart from the first, when we had to measure CO_2 on day 2 instead due to rain. The composition of gas samples was quantified within five days of collection with a gas chromatograph (Agilent 7890B Series Custom GC GHG-Analyser, Agilent Technologies, Santa Clara, USA). Net gas fluxes over the course of the experiment were calculated as in Penttilä et al. 2013.

Dung and beetle sampling

All dung and beetle samples were preserved for subsequent characterization of microbiota using 95% ethanol, which is an effective storage medium for microbial community analysis (Hammer et al. 2015). On days 2, 11, and 23 of the experiment, samples of approx. 1 ml were taken from the underside of pats (to avoid breaking the hard crust surface) and stored in ethanol. On day 2 we collected one sample per mesocosm, and on the other days four samples were taken and later pooled before DNA extraction.

To test whether antibiotic treatment affected beetle microbiota, two parent beetles from each of the two beetle-sampling pats per cow were collected and preserved in ethanol on day 7 of the experiment. To measure antibiotic effects on beetle size, reproduction and survival, we sampled pats on day 43 (for half-grown larvae) and days 71 and 73 (for the next generation of adult beetles). Larvae recovered were weighed while fresh and the width of their head capsule was measured. Total offspring counts by the end of the experiment were used as an integrated measure of both adult reproduction and offspring survival.

Molecular protocol and sequence data processing

To characterize the overall microbial community associated with dung beetles, whole adult beetles were thoroughly homogenized with autoclaved, stainless steel beads in a shaker (SPEX Geno/Grinder), and DNA was extracted from ~100 mg of homogenate with the MoBio PowerSoil kit, following similar studies (Hammer et al. 2014, 2015). Dung samples were drained of ethanol before subsampling ~100 mg for DNA extraction with the same kit. Using barcoded primers, we PCR-amplified the ~300 bp V4 region of the bacterial and archaeal 16S rRNA gene, with the amplicons sequenced on an Illumina MiSeq platform as previously described (Ramirez et al. 2014, Hammer et al. 2015).

Paired-end sequence reads were merged, quality-filtered and clustered into operational taxonomic units ("OTUs") using the UPARSE pipeline (Edgar 2013; see Ramirez et al. 2014 for more detail). Taxonomic affiliation was assigned using the RDP classifier (Wang et al. 2007) and the August 2013 GreenGenes database (McDonald et al. 2012). OTUs that were relatively abundant in DNA extraction blanks and PCR no-template controls were removed, as were OTUs

identified as mitochondria or chloroplasts. For the most abundant OTUs in beetles and dung (Fig. 2), we used the representative sequence of each OTU and RDP SeqMatch (Cole et al. 2009) to refine genus-level identities. Prior to all statistical analyses, we rarefied (randomly subsampled) each sample to an even sequencing depth of 5000 reads/sample. Quality filtering and rarefaction resulted in dropping one beetle sample and 16 dung samples from subsequent analyses, leaving 39 beetles and 103 total dung samples.

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