Methodology

Study site and experimental set-up
We collected soils from an unmaintained field adjacent to the Paradise Ridge reserve which consists of native Palouse prairie. Soils were passed through a 4-mm sieve and screened for fine roots that passed the sieve before placing in mesocosms. Mesocosms contained 250 g wet weight soil and were maintained at 65% water holding capacity (WHC, Strickland et al. 2015) throughout the experiment. Prior to mesocosm construction, 5 g of soil was harvested and stored at -80°C to allow us to assess starting microbial communities.

We assigned mesocosms to either a high or low antibiotic treatment (or control) across 3 different temperatures, 15, 20 or 30°C (3 x 3 factorial). We had 5 replicate mesocosms per treatment per temperature. We used the antibiotic Monensin, a broad-spectrum ionophore antibiotic that inhibits bacterial cell wall transport. Monensin is one of the most commonly administered antibiotics for livestock, as it both promotes growth and treats bacterial infections (Goodrich et al. 1984, Duffield et al. 1998, 2002). Antibiotics were added at a high (0.02 mg/g) or low (0.002 mg/g) concentration. Concentrations represent the maximum (high treatment) or minimum (low treatment) active-product excretion rate for Monensin from dairy cattle. Monensin is excreted primarily in dairy cattle manure, though it can also enter environments through urine (NCBI PubMed). Because Monensin needs to be dissolved in an organic solvent in order to be applied aqueously, dosages were dissolved in 15 ml of EtOH, and then applied to soils. Control soils received 15 ml of EtOH, with no added antibiotic. Antibiotic treatments were applied to soil mesocosm on days 1, 8 and 16. We maintained mesocosms for 21 total days, destructively harvesting the soil communities for subsequent analyses at the end of the experiment.

Respiration measurements
A CO_2/H_2O gas-multiplexer system sampled sequentially air from each mesocosm. Air is sucked from each mesocosm through a 0.2 m tube, where a gas multiplexer and a LICOR 6262 gas-analyzer monitor CO_2 production rates. Each mesocosm was sampled for 60 s, followed by a 30 second flushing to avoid cross-contamination of mesocosms. We recorded mean values over the 60 s period. The gas-analyzer was operated every other day throughout the duration of mesocosm incubation (21 total days).

Microbial community function and soil characteristics
At the end of the 21-day mesocosm antibiotic treatment and incubation period, soil samples were taken to determine microbial community function. We assessed active microbial biomass via substrate induced respiration (SIR) following Strickland et al. (2010). Briefly, we amended 4 g dry weight equivalent soil (1 analytical rep per jar) with 8 mL of an autolyzed yeast solution (12g autolyzed yeast per 1 L H_2O solution, 0.1 mg of substrate g soil^{-1}). After a 1 h pre-incubation with shaking, the soil slurries (i.e., soil and solution combinations) were incubated for 4 h at 20°C. After incubation, respiration for each amendment was determined on an infrared gas analyzer (IRGA; Model LI-7000, LiCor Biosciences, Lincoln, NE, USA) using a static incubation technique (Strickland et al. 2015).

We also determined relative amounts of mineralizable-C, gravimetric soil moisture and soil pH. Mineralizable-C (i.e., bioavailable C) was determined by measuring total CO_2 emissions over the course of a subsequent 60 d incubation. Soils (8 g dry weight) were maintained at 65% water-holding capacity and 20° C, and we determined respiration across this time period using the static incubation procedure described for active microbial biomass assays (soils were measure 6 times in total). Total mineralizable-C was estimated by integrating CO_2 production across time. Gravimetric soil moisture was determined by saturating soil samples, weighing wet samples, drying them at 60°C for 24 hours, and re-weighing dried samples. Soil pH (1:1, soil:H2O by volume) was determined at the end of the experiment on airdried soil samples using a benchtop pH meter.

We analyzed DOC, DON and microbial biomass C and N in each soil sample. To extract dissolved materials, soils were shaken with 0.5 M K2SO4 for 4 h and then filtered using Whatman #42 papers. We estimated microbial biomass C and N using a modified, chloroform-fumigation extraction method as described in Fierer and Schimel (2002, 2003). This method controls for potential soil moisture differences by using soil slurries, and compares the flush of dissolved materials in fumigated samples against non-fumigated controls.

Microbial community composition

Initial soil samples were taken prior to antibiotic treatment to provide a baseline community. Microbial community composition was assessed using 16S/ITS metabarcoding protocol. We extracted DNA from each soil sample using the MoBio© PowerSoil kit (MoBio Laboratories, Inc., Carlsbad, CA, USA), according to the manufacturer's protocols. We amplified ribosomal marker genes using 2 step PCR in accordance with the Earth Microbiome Project protocol for 16S and ITS sequencing (www.earthmicrobiome.org). We used the ITS1F/ITS2 and the 515f/806r primer pairs for fungi and bacteria, respectively. After the first round of PCR, sequences were cleaned using ExoSAP-IT™ PCR cleanup reagent (Affymetrix Inc., Santa Clara, CA, USA), according to the manufacturer's protocol. During the second round of PCR, we attached unique barcoded primers to each sample. After the second round of PCR, samples were cleaned and normalized using SequelPrep™ 96-well plates (Invitrogen, Carlsbad, CA, USA). We pooled equimolar DNA, and sequenced these amplicon pools on an Illumina MiSeq instrument using 2 × 300 bp sequencing kits at the IBEST sequencing facility at the University of Idaho. We used controls throughout the laboratory process to ensure there were no contaminants. Raw sequence data are available at FigShare (doi: TBD).

Raw sequences were first demultiplexed by the IBEST genomic resource core using the program dbcAmplicons (Uribe-Convers et al. 2016). This process also removed barcodes and primers from sequences. We then processed paired sequences using the DADA2 pipeline (Callahan et al. 2016), which is designed to resolve exact biological sequences from Illumina sequence data and does not involve sequence clustering (Leff et al. 2018). Paired sequences were trimmed to uniform lengths, dereplicated, and the unique sequence pairs were denoised using the 'dada' function, accounting for errors through the model generated with the 'learnErrors' command. We merged these paired-end sequences and removed chimeras. We assigned taxonomy assignments the Silva (ver. 132, Quast et al. 2013) and the UNITE dynamic general release (ver 01.12.2017, Abarenkov et al. 2010) databases for bacteria and fungi, respectively. To account for differences in sequencing depths, we rarefied samples to 4413 and 9476 sequences per sample for fungi and bacteria, respectively.

Statistical analyses

We performed most analyses in the R statistical environment (R Core Team 2017), and the packages 'mctoolsr' (http://leffj.github.io/mctoolsr/) and 'phyloseq' were used to facilitate microbial sequencing data manipulation and analyses.

We compared substrate induced respiration (SIR) to assess microbial biomass, soil pH, mass-specific respiration of soils, gravimetric water content (GVM), the amount of "bioavailable" mineralized carbon (MinC), and the relative abundance of taxonomic orders of microbiota (only those with >1.0% relative abundance) using linear models (package *nlme*). We included antibiotic dosage and temperature and their interaction as fixed effects. For all models, we performed nested model reduction based on AIC values and P-values from likelihood ratio tests using the lmer package. We report alpha when different from 0.05.

We compared overall microbial community structure using the software Primer (Ver. 7.0.13) and R (package vegan). We square-root transformed the microbial community data before calculating Bray-Curtis dissimilarity. We used community distance matrices to generate ordinations (non-metric multidimensional scaling or NMDS) for bacteria and fungi. We used PERMANOVA to compare community composition across dosages and temperature, and their interactionse (Anderson 2001, Anderson et al. 2008). We also compared beta diversity across our samples using PERMDISP tests (Anderson 2006, Anderson et al. 2006). PERMDISP tests calculate within group dissimilarity in community composition and then compares the magnitude of dissimilarity among each group (9999 permutations).

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