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Prolonged exposure to manure from livestock administered antibiotics decreases ecosystem carbon-use efficiency and alters nitrogen cycling

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22
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Abstract:

Microbial communities drive soil ecosystem function but are also susceptible to environmental disturbances. We investigated whether exposure to manure sourced from cattle either administered or not administered antibiotics affected microbially-mediated terrestrial ecosystem function. We quantified changes in microbial community composition, and terrestrial elemental cycling via a stable isotope pulse-chase. Exposure to manure from antibiotic-treated cattle caused: *i*) changes in microbial community structure; and *ii*) alterations in elemental cycling throughout the terrestrial system. This exposure caused changes in fungal:bacterial, as well as changes in bacterial community structure. Additionally, exposure to manure from cattle treated with pirlimycin resulted in an approximate two-fold increase in ecosystem respiration of recently fixed-carbon, and a greater proportion of recently-added nitrogen in plant and soil pools compared to the control manure. Manure from antibiotic-treated cattle therefore affects terrestrial ecosystem function via the soil microbiome, causing decreased ecosystem carbon use efficiency, and altered nitrogen cycling.

Introduction:

Use of antibiotics is under heightened scrutiny due to the increased prevalence of antibiotic resistant pathogens (1–3). Antibiotic resistance is a multifaceted problem and although the primary focus is on more stringent use of antibiotics in medical settings, the use of antibiotics in the livestock sector is gaining increased attention (4–13). In the United States, 80% of antibiotics are used in livestock production, representing approximately 15-million kg of antibiotics annually (14, 15); globally livestock antibiotic use is projected to increase by 67% between 2010 and 2030 (4). After dosing, 40-90% of antibiotics are excreted by livestock either intact or as a biologically active metabolite (16–18). Livestock manure either collects in pastures or is applied to cultivated fields as fertilizer, therefore potentially contributing up to 13-million kg of antibiotics to the environment annually (14, 18). This widespread antibiotic exposure can affect human health through the spread of antibiotic resistance, and also has the potential to directly affect soil microbial communities and the ecosystem processes they regulate (19–21).

The effect of antibiotics is an important consideration because microbial communities are key drivers of ecosystem function. Soil microbial communities play an important role in decomposition and elemental cycling in soils (22–25), and impact the composition and productivity of plant communities (26) often through beneficial and detrimental symbioses, and plant-microbe competition for nutrients (27–31). While it is well known that soil microbes compete and signal via antibiotics (32–34), the type and amount of antibiotics that soil microbial communities are exposed to in agroecosystems are often novel and certainly present in amounts far surpassing those found in soils naturally (8, 35).

Evidence is mounting that antibiotics can alter both soil microbial composition through selection by antibiotic pressure, and physiology (21) through a stress response (36) with the potential to affect ecosystem function. For instance, in settings with known exposure to antibiotics microbial efficiency has been shown to decrease, as evidenced by increased microbial mass-specific respiration with a subsequent increase in the abundance of antibiotic resistance genes (21), indicating that the metabolic costs associated with maintaining active antibiotic resistance may reduce microbial efficiency (37). Antibiotic exposure has also been shown to increase methane fluxes from manure (38). In addition to these carbon (C) cycling effects, antibiotic exposure may also affect nutrient cycling. Because production of microbial biomass is more demanding for nutrients (*e.g.* nitrogen; N), the shift away from biomass production towards metabolic pathways associated with a stress response could reduce microbial nutrient immobilization, potentially increasing nutrient losses from ecosystems (36).

To investigate the potential effects of prolonged exposure to manure from livestock treated with antibiotics (hereon these effects are referred to as antibiotic effects) on microbial communities and ecosystem functioning, we applied manure from three groups of cattle (those that received the bactericidal antibiotic

121 cephapirin, those that received the bacteriostatic antibiotic pirlimycin, and control
122 cattle receiving no antibiotics) to grassland plots in a common-garden
123 experiment, along with a no-manure control. The relative impacts of antibiotics on
124 soil microbial communities were examined via determination of fungal:bacterial
125 ratio (hereon F:B) and *16S* and ITS metabarcoding (to assess bacterial and
126 fungal community composition, respectively), and on ecosystem processes via a
127 ^{13}C and ^{15}N stable isotope pulse-chase. We expected that manure itself would
128 positively affect plant growth and lead to an increase in soil C pools. However,
129 when manure was sourced from cattle administered antibiotics, we expected a
130 greater loss of C via respiration, as well as, an overall decrease in ecosystem C-
131 use efficiency due to decreased microbial efficiency (specifically bacterial; 21).
132 Antibiotics are likely to lead to an increase in F:B (39, 40). The implications of this
133 on an ecosystem-scale are subject to debate: given the classical understanding
134 of fungal versus bacterial contribution to biogeochemical processes, we would
135 expect that systems with a higher F:B would retain more C and N (41, 42).
136 Alternatively, recent work has shown that C and N mineralization are unrelated to
137 the relative dominance of bacteria and fungi (43). Therefore, outcomes from this
138 experiment could lend support to either theory in light of recent challenges to the
139 classical understanding of fungal versus bacterial contribution to biogeochemical
140 processes.
141
142 ***Materials and Methods:***
143
144 *Experimental Design:*
145
146 A common garden experiment with a randomized block design (four treatments,
147 n=6) was conducted at Kentland Farm, Blacksburg, VA, USA (37.199490, -
148 80.584659; 547-m elevation; Unison and Braddock cobbly soils; dominant plant
149 cover is grasses, mostly tall fescue, as well as some herbaceous cover including
150 members of the Lamiaceae and Plantaginaceae families). Treatments included
151 three manure additions (manure from cattle given no antibiotics, or manure from
152 cattle given either cephapirin benzathine or pirlimycin hydrochloride) and one
153 control treatment that received no manure. Both antibiotics are commonly used in
154 the prevention of mastitis in dairy cattle, however they vary in a number of ways
155 including their fate in the environment (44) and mode of action. Cephapirin
156 benzathine (Molecular weight = 365.4 g mol^{-1}; pKa = 2.2; water solubility = 3,430
157 mg L^{-1}) is bactericidal, damaging the structural integrity of bacterial cell
158 membranes, whereas pirlimycin hydrochloride (Molecular weight = 447.4 g mol^{-1};
159 pKa = 8.4; water solubility = 64,900 mg L^{-1}) is bacteriostatic, inhibiting protein
160 synthesis. Hereon we refer to these four treatments as no-manure control (NMC),
161 control manure (Con), cephapirin manure (Ceph), and pirlimycin manure (Pir).
162
163 Manure was applied to appropriate treatments at a monthly rate of 648-g-m^{-2} of
164 wet-weight manure starting in October, 2014 until May, 2015 (213 days) –
165 totaling 4,536-g of manure m^{-2}. This amount of manure corresponds with the
166 amount of manure expected given a typical dairy cattle stocking density.

167
168 For information regarding manure properties, sourcing and within-manure
169 antibiotic quantification see supplementary materials.
170
171 *Pulse-chase experiment:*
172
173 Field sampling was conducted in May, 2015. In order to determine whether
174 antibiotic use in dairy cattle affects system-wide elemental cycling, a ^{13}C and ^{15}N
175 stable isotope pulse-chase experiment was conducted. The use of ^{13}C allowed
176 for the tracking of recently photosynthesized C through both above- and
177 belowground C pools. To accomplish ^{13}C-labeling, a ~1-m^2 subplot within each
178 treatment plot was covered with a 0.6-m^3 (0.99-m × 0.99-m × 0.61-m) transparent
179 acrylic chamber (Figure S1). To prevent gas exchange from outside the
180 chamber, the chamber was fitted into a rubber lined wooden base that was
181 trenched 10-cm into the soil. The rubber liner was then adhered to the acrylic
182 glass chamber using silicon grease. $^{13}CO_2$ was introduced into each chamber via
183 gas-tight ports by reacting 1-g sodium carbonate (Na$^{13}CO_3$, 99 atom% ^{13}C,
184 Sigma-Aldrich; CAS number: 9367-48-4; 113-mg of ^{13}C equivalent) with excess
185 hydrochloric acid. Air was circulated within the chambers using a centrally
186 located internal battery-operated fan. Chamber temperature was monitored using
187 an internal thermometer. CO_2 concentrations within the chamber were monitored
188 via a LI-8100 infrared gas analyzer (Li-Cor Biosciences, Lincoln, NE). Chambers
189 were removed after CO_2 levels returned to pre-pulse levels. As temperatures in
190 the chambers can be high during mid-day, pulsing was limited to early morning
191 and late afternoon. The amount of ^{13}C fixed by the plant communities was
192 determined by taking foliar clip samples immediately post-pulse.
193
194 Following the ^{13}C pulse-labeling, each plot was also labeled with ^{15}N ammonium
195 nitrate ($^{15}NH_4$$^{15}NO_3$; 98 atom%; Sigma-Aldrich; CAS Number: 31432-46-9; 67-mg
196 of ^{15}N equivalent) in order to examine N-dynamics in response to manure and
197 antibiotic treatments. Ammonium nitrate (300-mg in 1-L of DI water) was added
198 evenly to the soil surface of each 1-m^2 plot. The amount of ^{15}N, similar to
199 Fraterrigo *et al.* (45), was kept low to avoid a fertilization effect.
200
201 Upon completion of pulse-labeling, we destructively harvested 0.05-m^2 sub-plots
202 within each 1-m^2 plot at 1, 2, and 7-days post-labeling. An additional sub-plot
203 was harvested from each 1-m^2 experimental plot prior to pulse-labeling in order
204 to determine natural abundance of ^{13}C and ^{15}N. Aboveground plant material from
205 each sub-plot was harvested by clipping it at the soil surface. Aboveground plant
206 biomass samples were air-dried, weighed, and milled for elemental and isotope
207 analyses. The belowground portion of the sub-plot was sampled to 10-cm depth;
208 roots and soil were then separated. Root material was initially air-dried and then
209 later washed, air-dried, weighed, and milled for elemental and isotopic analyses.
210 Soils were sieved (4.75-mm), homogenized, and stored at either -80°C, 4°C, or
211 air-dried depending on future analyses (see below).
212

213 For soils, we determined POM and mineral-associated soil C and N, and soil
214 microbial biomass C and N. POM and mineral-associated C and N was
215 determined on air dried soil samples (46). Microbial biomass C and N were
216 determined following the chloroform fumigation extraction (CFE) procedure
217 outlined in Fierer and Schimel (47). Briefly, 40 mls of 0.5M K_2SO_4 was added to
218 one of each 7-g dry mass equivalent soil pair. One of each pair is then exposed
219 to 1-ml of ethanol-free chloroform to lyse microbial cells and accumulate
220 microbial C and N. Samples are capped, and shaken for 4-h. Samples were then
221 allowed to settle before filtration. Microbial biomass was estimated as the
222 difference between the quantity of C and N between the fumigated and un-
223 fumigated samples. Total organic C and N were then calculated for both the
224 fumigated and un-fumigated samples using a Vario TOC Cube (Elemtar,
225 Langenselbold, Germany).
226
227 $\delta^{13}C$ and $\delta^{15}N$ in above- and belowground plant biomass, POM and mineral-
228 associated pools were determined using a Costech ECS 4010 Elemental
229 Analyzer (Costech Analytical, Valencia, CA, USA) paired with an Thermo Delta
230 Plus Advantage Isotope Ratio Mass Spectrometer (IRMS; Thermo Fisher
231 Scientific™, Waltham, MA, USA).
232
233 Prior to each destructive harvest event ecosystem respiration was measured
234 using a LI-8100 Infrared Gas Analyzer (Li-Cor Biosciences, Lincoln, NE, USA).
235 Additionally, two 15-ml subsamples of respired air were captured using a gas
236 syringe and air-evacuated exetainers in order to determine the $\delta^{13}C$ of respired
237 CO_2. The first of two subsamples were collected within the first 15-seconds of a
238 2-minute respiration measurement period, and the second subsample was
239 collected in the final 15-seconds. Both subsamples were then analyzed for $\delta^{13}C$
240 using a GasBench II IRMS (Thermo Fisher Scientific™, Waltham, MA, USA).
241 Data were then recalculated to account for varying heights of soil collars and
242 adjusted to optimize the r^2 of the respiration trend-line from each 2-minute
243 measurement.
244
245 The amount of ^{13}C fixed, respired, and the amount of both ^{13}C and ^{15}N contained
246 in above- and belowground pools was derived using standard isotopic mixing
247 models (48). The amount of C and N derived from ^{13}C and ^{15}N additions was
248 calculated as atom excess in a given C or N pool. The atom% excess of a given
249 pool was then multiplied by the total C or N in that pool, giving the mass of ^{13}C or
250 ^{15}N label. The proportion of label in a given pool was calculated as the mass of
251 ^{13}C or ^{15}N label divided by the total amount of C or N of that specific pool.
252 Cumulative ecosystem respiration was calculated via integration. See
253 Supplemental Methods for details related to additional soil parameters, microbial
254 catabolic response profiles, and microbial community composition measured in
255 conjunction with the pulse-chase experiment.
256
257 *Statistics and analysis:*
258

259 Data was analyzed using linear mixed models (LMM; 'lme4' package; 63) with
260 treatment as a fixed effect and plot nested within block as a random effect to
261 account for sampling of plots across time. Model selection (additive vs.
262 interactive) was determined by lowest Akaike information criterion (AIC) score
263 (50). Normality of variance was tested using a Wilk-Shapiro test. Data with non-
264 normal variance was either log or square-root transformed. If normality
265 assumptions were still not met, generalized linear models (GLM; 'car' package;
266 64) were used using the Gamma family and either the inverse or log link function
267 as all data was continuous and positive (variables containing negative values
268 were standardized). Wald χ^2 tests were used to assess model significance for
269 GLMs. Data was analyzed using the R statistical platform (52). Degrees of
270 freedom for linear mixed models were calculated using Satterthwaite
271 approximations.
272
273 For all analyses, we consider statistical significance at $P < 0.05$, and marginal
274 significance at $P < 0.10$. However, it should be noted that it has typically been
275 deemed acceptable to consider changes in soil C pools at $P < 0.10$ (53, 54),
276 given that soil C is inherently heterogeneous.
277
278 ***Results/Discussion:***
279
280 *Antibiotic effects on active and total microbial biomass:*
281
282 Prolonged manure additions, regardless of antibiotic involvement, increased C
283 mineralization – an estimate of bioavailable soil C – when compared to NMC
284 ($F_{3,15} = 11.8$, $P < 0.001$; Table S1). More surprising was the observation that
285 active microbial biomass – determined via SIR – was differentially affected by the
286 antibiotic status of the manure (again, we are referring to the effect of exposure
287 to manure from cows given an antibiotic, as an antibiotic effect; Figure S2a; $P <$
288 0.01). Specifically, the Ceph treatment exhibited greater active microbial biomass
289 in comparison to the other treatments. Similar to the increase in respiration
290 observed for SIR, increased microbial activity was observed across a range of C-
291 substrates for the Ceph treatment in a catabolic response profile (CRP; Figure
292 S2b). In contrast, we observed a marginally significant treatment effect on total
293 microbial biomass C ($P = 0.07$) and N ($P = 0.08$), primarily driven by a trend
294 towards increased microbial C and N in the Con treatment (Table S2). This
295 contrast between active and total microbial biomass may suggest physiological
296 changes, specifically greater mass-specific activity for the Ceph treatment,
297 consistent with findings from previous investigations (21). As discussed in the
298 corresponding section, the Ceph treatment did not differ from the other antibiotic
299 manure treatment, Pir, in terms of microbial community composition. Therefore,
300 elevated microbial activity could be due to two, non-mutually exclusive, factors: *i)*
301 the increased presence of lysed cellular material from the action of cephapirin, a
302 bactericidal antibiotic, or *ii)* from a stress response of the microbial community,
303 due to the added metabolic cost of maintaining antibiotic resistance (36). This
304 stress response is consistent with previous research on cephapirin use on dairy

305 cattle in pasture systems, and has been suggested as a possible cause of
306 altered ecosystem C cycling, through reduced microbial efficiency (21).
307
308 *Antibiotic effects on fungal:bacterial dominance:*
309
310 As antibiotics detrimentally effect bacteria, we assessed F:B via qPCR to
311 determine shifts in fungi and bacteria. Overall, we observed a significant
312 treatment effect for F:B (Figure 1; $F_{3,63}$ = 6.497$, P$ < 0.001) as has been
313 previously observed (55). Fungal counts increased in the soils receiving manure
314 compared to NMC, as well as in Pir compared to Con. NMC had the lowest ratio,
315 indicating that without manure and associated antibiotics the system is relatively
316 more dominated by bacteria. Notably, within the Pir and Ceph manure
317 treatments, the differences in F:B were due to declines in bacteria (*i.e.* 16S gene
318 abundance), whereas little change in fungi (*i.e.* ITS abundance) was observed.
319 This increase in F:B in the Pir treatment may be driven by pirlimycin's
320 bacteriostatic mode of action: pirlimycin typically reduces bacterial growth but
321 does not induce cell lysis. Conversely, cephapirin – a bactericidal antibiotic –
322 causes cell lysis. Lysed cells, as suggested above, cause an increase in labile
323 resources that potentially favor bacteria in spite of the direct negative effects of
324 the antibiotic (56, 57). This potential net positive effect for bacteria under the
325 Ceph compared to the Pir treatment is supported by a pairwise marginally
326 significant increase in *16S* copies (Figure 1; P = 0.08). It is also possible that
327 decreased inhibition of bacteria in the Ceph treatment can be attributed to
328 cephapirin being relatively more easily degraded than pirlimycin. However,
329 evidence of cephapirin's effect on microbial functional properties was in fact
330 observed, therefore degradability is unlikely to be the explanation for the
331 difference in bacterial effects between antibiotic treatments. Compared to NMC,
332 the addition of manure, regardless of antibiotic involvement, increased the
333 abundance of fungi (*i.e.* ITS copies) in soil ($F_{3,68}$ = 5.868$, P$ < 0.01), with Con,
334 Ceph, and Pir treatments having greater numbers of ITS copies than NMC.
335 Within the manure-addition treatments, Ceph had a marginally lower abundance
336 of fungi compared to Con (P < 0.10), this too could be driven by the mode of
337 action related to this antibiotic.
338
339 The primary fungal effect appeared to be driven by manure itself, given that all
340 manure additions increased ITS copies compared to NMC. This could be
341 attributed in part to coprophilous fungi, which specialize in the decomposition of
342 fecal matter, previously shown to be elevated in conjunction with manure (21).
343 Additionally, the highest counts of *16S* and ITS copies were measured in the Con
344 treatment. This is likely attributed to the influx of manure-derived resources in the
345 absence of antibiotics. Together these results suggest that while manure
346 additions increase F:B, manure from cattle administered antibiotics tends to lead
347 to even greater increases, primarily driven by decreased bacterial abundance.
348
349 *Antibiotic effects on microbial community composition:*
350

351   The results of our community composition assessment largely mirrored the
352   results of the F:B analysis. Bacterial communities changed across our treatments
353   (Figure 2: $pseudo\text{-}F_{3,23}$ = 1.15; P < 0.05; note, the random effect 'block' was
354   dropped from this model because it was non-significant), but fungal communities
355   did not (Figure S3: $pseudo\text{-}F_{3,23}$ = 1.01; P = 0.18). The treatment effect on
356   bacteria was largely driven by differences between Con, and both Pir and Ceph.
357   Notably, a marginally significant pairwise difference was observed between Pir
358   and Ceph (P = 0.064). NMC did not differ from the other three treatments, in fact,
359   as NMC can be viewed as a baseline, shifts in bacterial community composition
360   from manure exposure were dependent on the antibiotic status of the manure. If
361   the manure was sourced from cattle administered an antibiotic, the community
362   shifted to the lower right in ordination space, while control manure caused the
363   community to shift in the opposite direction, with NMC situated between (Figure
364   2a). Additionally, bacterial beta diversity did not differ between treatments (Figure
365   2a; $pseudo\text{-}F_{3,20}$ = 1.12; p = 0.31), suggesting that the microbial communities in
366   our antibiotic treatments are distinct from control environments, and not just more
367   variable in composition. While we did not seek to document the impact of the
368   fecal microbiome on the soil microbiome, previous studies have shown that the
369   fecal microbiome can be impacted by antibiotic exposure (38). Therefore, further
370   research into the quantification of this effect would be beneficial, especially
371   investigations into interactions between the fecal and soil microbiomes.
372
373   To further investigate OTUs that possibly drove treatment differences, we
374   identified potential OTUs of interest via SIMPER that were common across all
375   pairwise treatment comparisons. This resulted in 32 common OTUs of which only
376   6 exhibited significant differences between treatments (Figure 2b). Of these 6
377   OTUs, 4 were associated with Phyla Acidobacteria and γ-Proteobacteria (2 in
378   each), and 2 were associated with the Phyla Bacteroidetes and Verrucomicrobia
379   (1 in each). Interestingly, the two γ-Proteobacteria were associated with families
380   Acinetobacter and Xanthomonadaceae, which are typically associated with the
381   environment but also include members of human health concern (58). In fact,
382   Wepking *et al.* (21) observed a similar increase in the genus Acinetobacter in
383   response to cattle administered a cephalosporin. Our results add further support
384   to the likely influence of antibiotics on soil community structure, and further
385   support the proposition that inputs of manure from cattle given antibiotics can
386   shift soil microbial communities towards organisms that are related to those of
387   human health concern (59, 60).
388
389   Interestingly, though, several OTUs associated with phyla that we expect to be
390   more oligotrophic in nature (*i.e.* Acidobacteria, Verrucomicrobia; 49, 50) also
391   exhibited greater relative abundance in Pir and Ceph. Even some taxa in the
392   family Cytophagaceae could be classed as oligotrophs, especially those involved
393   in cellulose degradation (63). This greater relative abundance of oligotrophic
394   taxa, primarily in Pir, may be due *not* to an increase in these groups but to a
395   decrease in other potentially more copiotrophic groups. That is in the Pir
396   treatment there was an observed decrease in *16S* abundance, suggesting a

397 decline in overall bacterial abundance. Such a decrease, if driven by the
398 antibiotic pirlimycin may have been disproportionate because the antibiotic is
399 bacteriostatic and, as such is likely to have a more detrimental effect on active
400 bacteria (64, 65). Overall, these results suggest that manure from cattle given
401 antibiotics versus those not, has the potential to lead to shifts in soil bacterial
402 community composition and F:B dominance in a relatively short time (*i.e.* ~8
403 months) with implications on microbially-mediated ecosystem function.
404
405 *Antibiotic effects on carbon and nitrogen dynamics:*
406
407 Few differences were observed in most pools of recently fixed C (Table S4), and
408 in the amount of ^{13}C fixed across manure-amended treatments (NMC fixed more
409 ^{13}C relative to total plant C, likely due to the lower plant biomass and identical
410 amount of labeled C added to the chamber; $F_{3,20} = 3.07$, $P = 0.05$). However, we
411 did observe a significant effect of both treatment ($\chi^2 = 18.52$, $df = 3$, $P < 0.001$),
412 and time ($\chi^2 = 87.18$, $df = 2$, $P < 0.001$), as well as a treatment × time interaction
413 ($\chi^2 = 41.13$, $df = 6$, $P < 0.001$), when examining the ecosystem respiration of
414 recently fixed C (Figure 2a). Specifically, the Pir treatment exhibited greater initial
415 respiration of ^{13}C compared to the other treatments, but by day 7 of the
416 experiment, respiration of ^{13}C for this treatment was nearly zero (Figure 3a). The
417 NMC, Pir, and Con treatments exhibited similar respiration dynamics (Figure 3a).
418 Ecosystem respiration dynamics for the Ceph treatment were more constant
419 during the sampling period compared to the other three treatments (Figure 3a). A
420 marginally significant difference in cumulative ^{13}C respired across the entire
421 sampling period was explained by the greatest amount of ^{13}C being respired in
422 the Pir treatment with the NMC and Ceph treatments intermediate, and the Con
423 treatment the lowest ($F_{3,15} = 2.72$; $P = 0.08$; Figure 3a). In fact, nearly twice the
424 amount of newly photosynthesized C was respired – not retained in the soil – in
425 the Pir treatment compared to the Con treatment (Figure 3a).
426
427 These results suggest that manure from cattle administered antibiotics can alter
428 both ecosystem respiration dynamics of recently fixed ^{13}C (*i.e.* Ceph) as well as
429 the total amount of C respired (*i.e.* Pir) compared to manure from antibiotic-free
430 cattle. Manure additions from cattle not administered antibiotics may initially
431 suppress respiration slightly compared to sites receiving no manure, possibly
432 driven by decreased plant demand for nutrients. Additionally, if less recently fixed
433 C is lost from a system via respiration it is likely that more C will be sequestered
434 in that system. This supposition is supported by the significant treatment effect
435 on the proportion of ^{13}C recovered in the mineral pool during the entire
436 experiment (Table S4) with the most ^{13}C recovered in the NMC treatment
437 followed by the Con treatment. Further, at the conclusion of the pulse-chase we
438 observed a marginally significant treatment effect for ^{13}C found in the mineral-
439 associated soil C pool with the most ^{13}C recovered in the Con treatment among
440 the treatments containing manure (Figure 3b; $F_{3,15} = 3.04$, $P = 0.06$). Given the
441 slow turnover of the mineral-associated soil C pool (66), our results suggest that
442 inputs of manure from cattle administered antibiotics may decrease C-

443 sequestration potential. Direct evidence for this potential is the observation of a
444 significant treatment effect for the ratio of fixed ^{13}C to respired ^{13}C (Figure 3c;
445 $F_{3,15}$ = 3.65, P < 0.05), an indicator of ecosystem-scale C-use efficiency (37).
446 Specifically, we observed that the Pir treatment had the lowest overall C-use
447 efficiency, Con had the greatest, and both NMC and Ceph were intermediate.
448 Soils receiving the Con treatment fixed 2.5-fold more C for every unit of C
449 respired than did the Pir treatment. Together these results indicate that manure
450 inputs from animals administered antibiotics have the potential to increase C
451 losses from ecosystems compared to manure inputs from animals not
452 administered antibiotics. However, our results also indicate that this effect on C-
453 cycling may be influenced by the specific choice of antibiotics. Further
454 investigation to examine the ecosystem effects of administering an array of
455 antibiotics is merited, especially as agricultural management practices are
456 increasingly seen as opportunities to mediate global climate change (67).
457
458 A greater proportion of ^{15}N relative to the total N pool was observed with the Pir
459 treatment compared to the other manure-amended plots across all pools
460 sampled (Figure 4, Table S4), but not necessarily in comparison to NMC.
461 Measuring ^{15}N as a proportion of the total N pool addresses potential difference
462 in plant biomass between treatments (68). Within the aboveground biomass ($F_{3,61}$
463 = 8.08, P < 0.001; Figure 4a, Table S4; analyzed as additive model based on
464 quality of model using AIC score) and belowground biomass ($F_{3,55}$ = 4.53, P <
465 0.01; Figure 4b, Table S4) significantly more^{15}N was found in the Pir treatment.
466 In addition, a significant and marginally significant time effect was observed in
467 the proportion of ^{15}N in the aboveground and the belowground biomass,
468 respectively ($F_{2,61}$ = 6.42, P < 0.005,;$F_{2,55}$ = 2.74, P < 0.10, respectively; Table
469 S4; pooled across treatment). This was characterized by an increased in the
470 proportion of ^{15}N in plant biomass across time. We also observed a significant
471 treatment effect for total N in aboveground plant biomass ($F_{3,61}$ = 8.48, P < 0.001;
472 Table S2) and a marginally significant treatment effect for total N in belowground
473 plant biomass ($F_{3,55}$ = 2.55, P = 0.06; Table S2; the former was analyzed as
474 additive model, and the latter as an interactive model based on AIC model
475 score). This effect was likely due to greater biomass in the treatments receiving
476 manure versus NMC (Table S2).
477
478 As observed in plant biomass, a greater proportion of ^{15}N in POM (Treatment:
479 $F_{3,61}$ = 4.77, P < 0.005; Figure 3c, Table S4) and mineral-associated (Treatment:
480 $F_{3,61}$ = 2.49, P < 0.10; Figure 3d, Table S4) soil N pools was observed in the Pir
481 treatment at the conclusion of the experiment compared to the other treatments.
482 The effect of Pir on ^{15}N in the POM N fraction was likely attributable to the root
483 biomass, due to the contribution of plant derived constituents to this pool (69).
484 Abundance of ^{15}N in the mineral pool was also increased with Pir, possibly due to
485 decreased plant-microbe competition for N due to an increased F:B in the
486 microbial community. Microbial communities with a higher F:B typically have a
487 higher C:N due to reduced N demand of fungi (70). With the Pir system being

488 more fungally dominated, the overall microbial demand for N is likely lower than
489 for the other three treatments.
490
491 We see an increased loss of recently fixed C in the Pir treatment but also
492 increased uptake of recently added N in this treatment compared to the other
493 treatments. For C, this is particularly unexpected given the increased F:B
494 associated with the Pir treatment. However, the notion that increased F:B leads
495 to a less leaky C-cycle has been called into question (43, 71). Rousk and Frey
496 (43) found that bacterial dominance is linked to a less leaky C-cycle and a less
497 leaky N-cycle, while we observed the potential for greater plant uptake of N. This
498 disparity between our results and those of Rousk and Frey may be because our
499 research was conducted in a grassland system and theirs in a forest. Of
500 particular relevance – among the many differences between these systems – the
501 uptake of available N is likely greater in grasses and forbs during peak growth,
502 over a short period of time, compared to N uptake in trees. When only
503 considering the microbial community, the Pir treatment (*i.e.* higher F:B) appears
504 to have a leakier N cycle – but when the plant community is also included, this
505 effect is diminished. This highlights the potential for antibiotics to alter plant-
506 microbe interactions and lead to shifts in ecosystem processes.
507
508 Mechanistically, these effects on N dynamics could be due to altered microbe-
509 plant competition for N. In this instance a bacteriostatic antibiotic – pirlimycin –
510 increased F:B leading to a leakier C cycle, but also a decrease in plant-microbe
511 competition for N (evidenced by increased plant N uptake; 52). Another potential
512 mechanism is reduced competition with mycorrhizal fungi for N with the Pir
513 treatment. Given recent evidence suggesting a C cost associated with N uptake
514 (73) for mycorrhizal symbionts, if mycorrhizal N uptake increases with a reduction
515 in bacteria then plant N uptake may increase but more C may be lost from the
516 system. Finally, as this experiment was conducted during peak plant growth and
517 N demand, more N may in fact be lost from the system with decreased plant N
518 demand.
519
520 ***Conclusion:***
521
522 Antibiotics affect not just the soil microbiome but the entire ecosystem; how the
523 ecosystem is affected depends on the antibiotic's mode of action (*i.e.* bactericidal
524 vs. bacteriostatic). Of the two antibiotics investigated, one in particular –
525 pirlimycin – alters both C and N cycling. This is likely due to changes in microbial
526 composition – as demonstrated by increased F:B, and shifts in bacterial
527 community composition. Increased availability of N appears to occur with
528 decreased C retention in the system – subsequently decreased whole ecosystem
529 C-use efficiency. In contrast, cephapirin increases microbial activity as a stress
530 response – in keeping with previously published research which showed
531 decreased microbial efficiency and increased soil C loss (21). While the majority
532 of attention is paid to livestock antibiotic use from the perspective of the
533 proliferation of antibiotic resistant pathogens and antibiotic resistance genes (74,

534 75), the impacts on biogeochemical cycling have been overlooked. With global
535 livestock antibiotic use projected to increase by 67% between 2010 and 2030 (4)
536 combined with increasing atmospheric CO_2 concentrations, understanding and
537 accounting for the effects antibiotics have on soil microbial communities and
538 whole ecosystem function is imperative.
539
540 ***Acknowledgements:***
541

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747

748   *Figures:*
749
750   Figure 1.
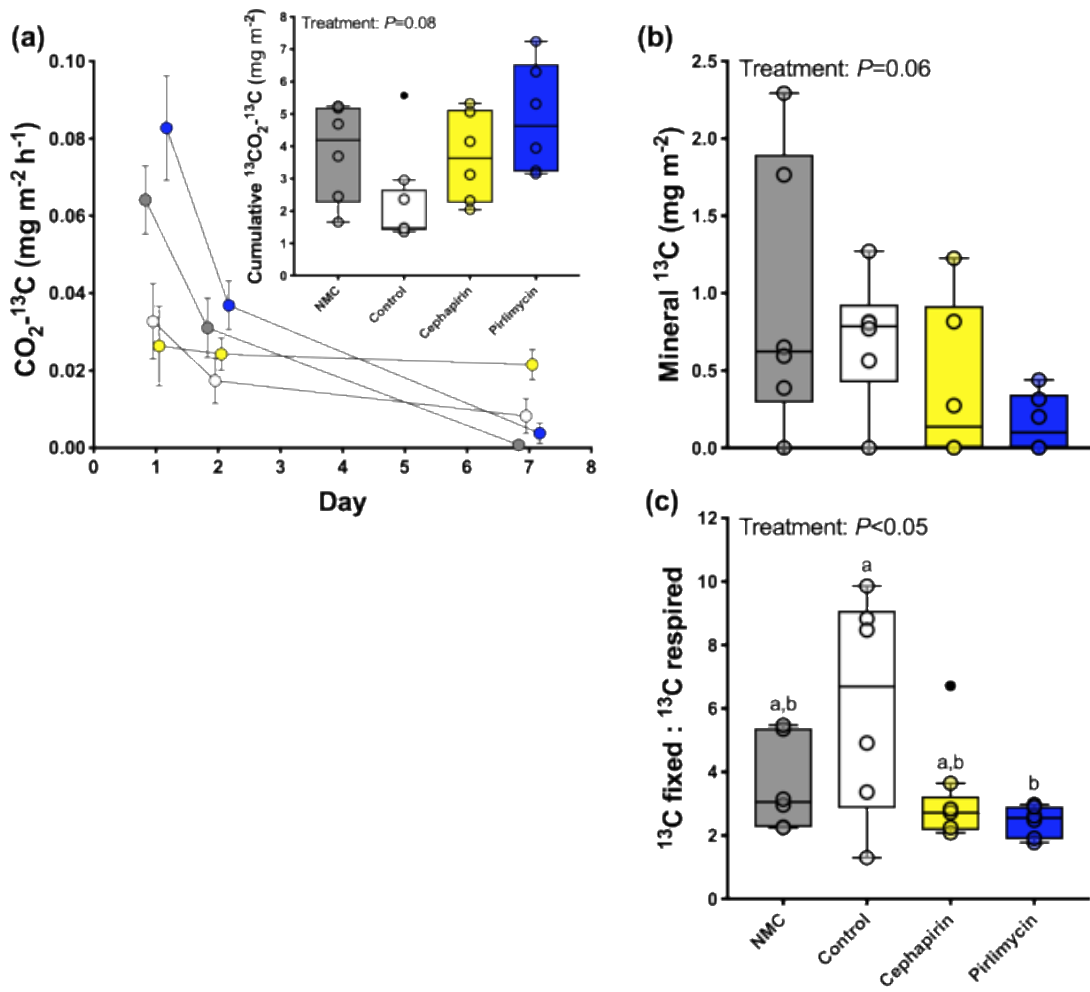751
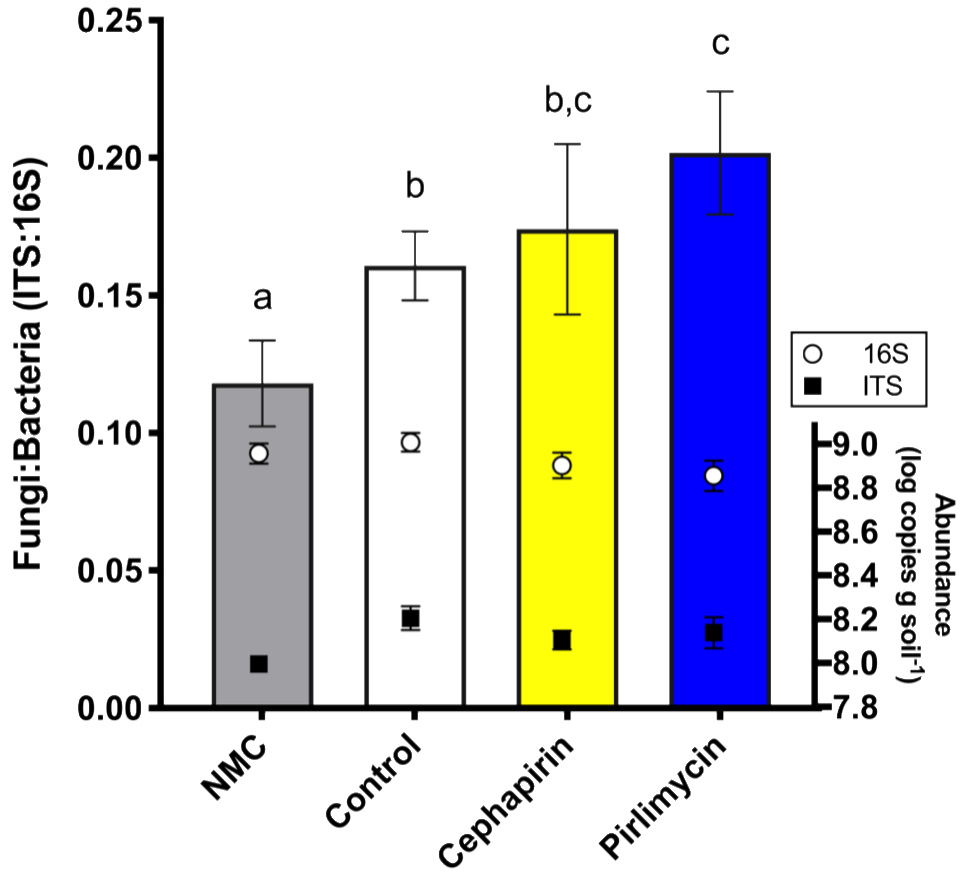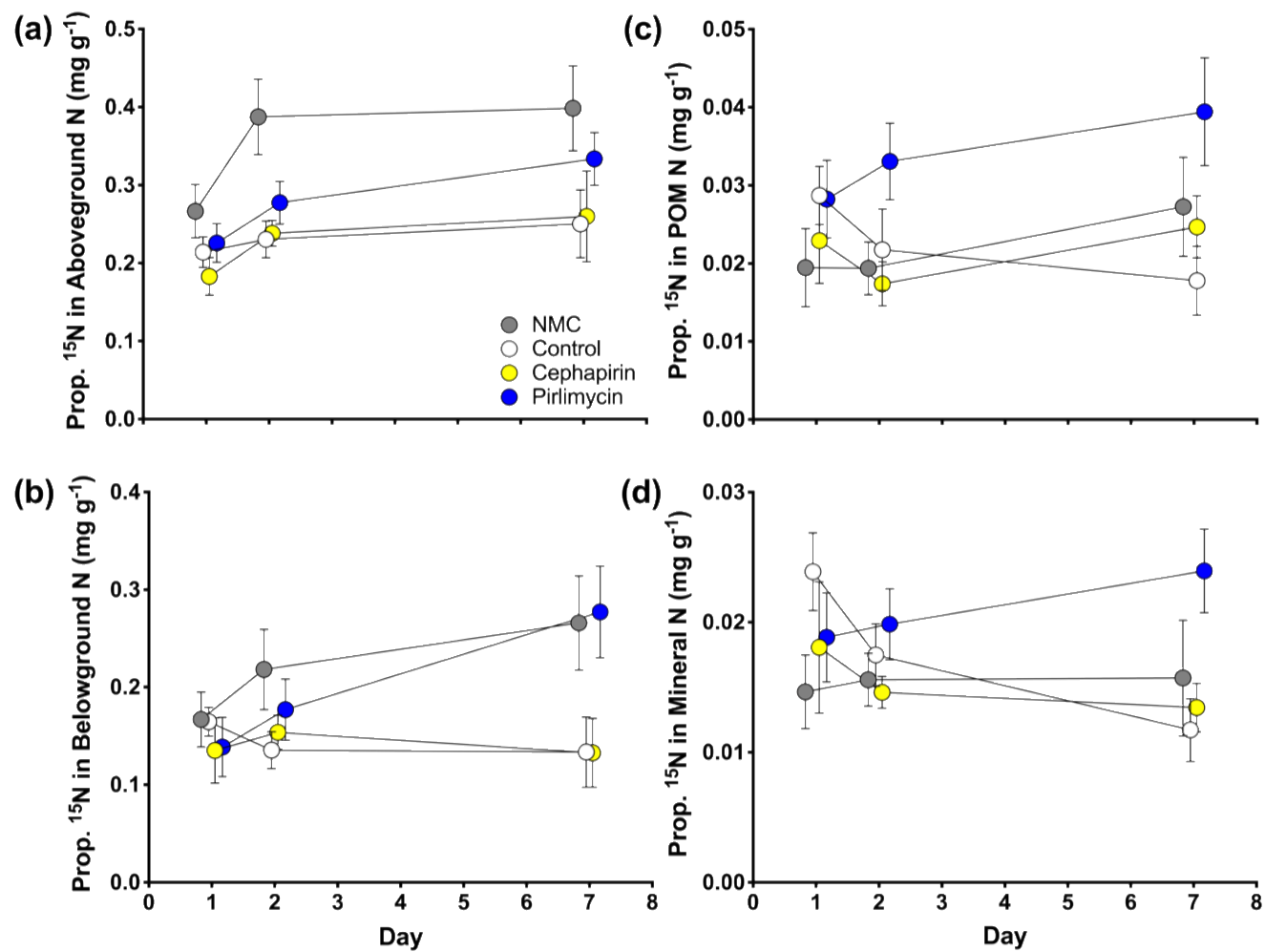

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753   Figure 2
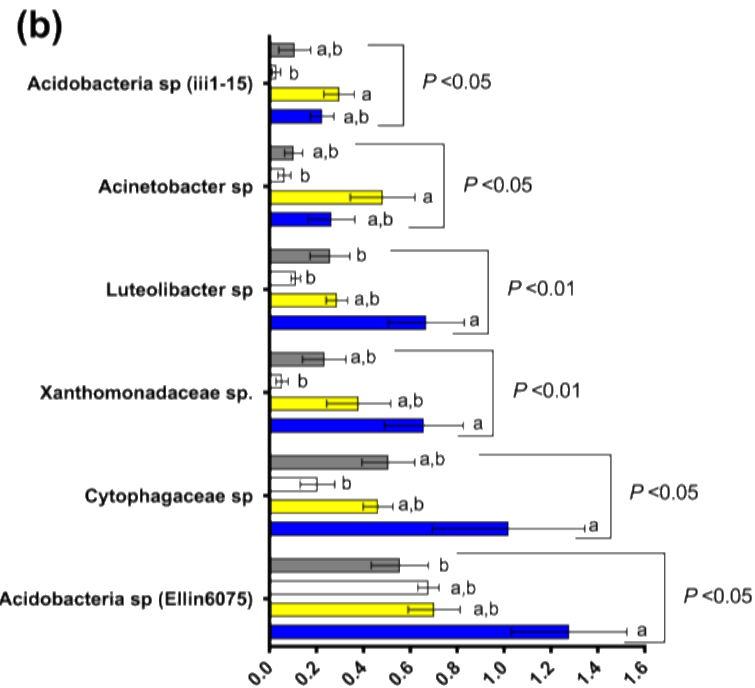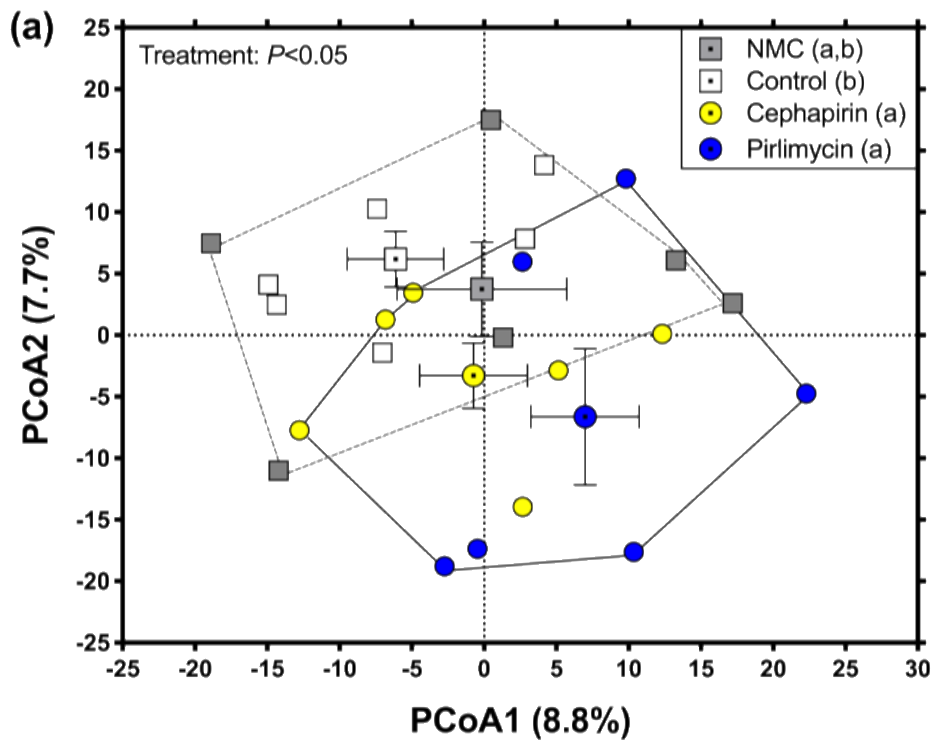
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756   Figure 3
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760   Figure 4
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762

763 **Figure Legends:**
764
765 **Figure 1:** Fungal-to bacterial ratios (F:B) associated with sites receiving manure
766 from cattle administered no antibiotics (Control), administered cephapirin
767 (bactericidal), or pirlimycin (bacteriostatic). Also shown is the ratio for sites
768 receiving no manure (NMC). Bars represent the mean ± 1 SEM. Letters indicate
769 pairwise differences between treatments. *16S* and ITS copies are indicated by
770 open circles and filled squares, respectively.
771
772 **Figure 2:** Effect of manure treatments on soil prokaryotic community
773 composition. **A**) Principal components analysis showing prokaryote community
774 composition associated with the following treatments: Soil amended with no
775 manure (NMC), soil amended with manure from cattle given no antibiotics
776 (Control), and soil amended with manure from cattle given either a bactericidal
777 antibiotic (Cephapirin) or a bacteriostatic antibiotic (Pirlimycin). Centroids are
778 indicated as symbols with central points and shown as the mean ± 1SE.
779 Significant pairwise differences between centroids are denoted by different letters
780 in the key. Additionally, lines connecting points indicate those treatments
781 receiving antibiotics (solid line) versus those that did not receive antibiotics
782 (dashed line). **B**) Relative abundance of OTUs that both contributed to
783 dissimilarity between treatments (as determined via similarity percentages) and
784 were statistically significant. Overall treatment statistical significance is indicated
785 by *P*-values, and significant pairwise differences for within OTU comparisons are
786 denoted by different letters.
787
788 **Figure 3:** Effect of manure and antibiotic treatments on the cycling of C through
789 the above- and belowground pools across the following treatments: soil amended
790 with no manure (NMC), soil amended with manure from cattle given no
791 antibiotics (Control), and soil amended with manure from cattle given either a
792 bactericidal antibiotic (Cephapirin) or a bacteriostatic antibiotic (Pirlimycin). **a)**
793 Ecosystem respiration dynamics across the 7-day sampling period are shown in
794 the main panel. Points represent the mean ± 1 SE (Treatment: $F_{3,61} = 5.3$, $P <$
795 0.005; Time: $F_{2,61} = 26.6$, $P < 0.001$). The panel inset shows a boxplot of the
796 cumulative ^{13}C respired across the entire pulse-chase (Treatment: $\chi^2 = 6.86$, df =
797 3, $P < 0.08$). While the cumulative ^{13}C respired is marginally significant, it
798 represents a doubling of respired CO_2, and is therefore ecologically meaningful.
799 **b)** Total accumulation of ^{13}C in the mineral associated soil fraction by the end of
800 the 7-day pulse chase event (Treatment: $F_{3,15} = 3.04$, $P = 0.06$). **c)** The ratio of
801 ^{13}C fixed to ^{13}C respired, an indicator of whole ecosystem C-use efficiency
802 (Treatment: $F_{3,15} = 3.65$, $P < 0.05$). Letters indicate pairwise differences between
803 treatments.
804
805 **Figure 4:** Effect of manure and antibiotic treatments on the cycling of newly
806 added N through the above- and belowground systems across the following
807 treatments: soil amended with no manure (NMC), soil amended with manure
808 from cattle given no antibiotics (Control), and soil amended with manure from

809   cattle given either a bactericidal antibiotic (Cephapirin) or a bacteriostatic
810   antibiotic (Pirlimycin), and across time. All panels show the proportion of ^{15}N
811   within each respective N pool. Error bars represent ± 1 SEM.

812 ***Supplementary Materials:***

813

814 *Supplemental Methods:*

815

816 *Manure sourcing:*

817

818 Manure collection started by selecting two sets of cattle: 12 healthy, peak
819 lactation dairy cows, and 6 cows at the end of their current lactation cycle (n=18).
820 The latter group was treated with cephapirin (ToMORROW®; Boehringer
821 Ingelheim Vetmedica, Inc., Duluth, GA, USA; intramammary dry cow therapy;
822 single dose of 300-mg into each of four quarters). Half of the former group (n=6)
823 was treated therapeutically with pirlimycin (Pirsue®; Zoetis, Parsippany, NJ, USA;
824 intramammary dose typical for clinical mastitis; two doses of 50-mg each, 24-h
825 apart). The remaining healthy lactating cows (n=6) were used for control manure
826 and therefore were not treated with antibiotics. Experimental cows were selected
827 for homogeneity of body weight and stage of lactation, and none had received
828 previous antibiotic treatment in the current lactation.

829

830 All cattle were offered free choice water and *ad libitum* total mixed ration. Cows
831 assigned to the same treatment were group housed in a single pen located in a
832 free stall barn (*i.e.* total of three pens). On day 1 of the study, cows were treated
833 with the assigned antibiotic. Manure (feces and urine mixed) accumulated over a
834 24-h period was collected from the pen floor on day 2 and 3 post treatment.
835 Manure from each pen was mixed separately to achieve homogeneous manure.
836 All collected manure was then homogenized and stored at -20˚C before being
837 applied to the plots.

838

839 *Manure elemental properties:*

840

841 Some treatment differences in manure %C, %N, and C:N were observed when
842 compared with linear models and ANOVAs (%C: $F_{2,33}$ = 12.525, $P < 0.001$; %N:
843 $F_{2,33}$ = 2.858, $P < 0.072$; CN: $F_{2,33}$ = 4.256, $P < 0.05$; Table S5). Con manure and
844 Pir manure had a significantly greater %C than Ceph manure – although all
845 manures were between 48-50.5% C. For manure %N a marginally significant
846 treatment effect was found, although no significant pairwise differences between
847 treatments were noted – all manures were between 3-4% N. Control manure had
848 the greatest C:N, significantly greater than both of the other manures – all ratios
849 were between 11.9 – 17.4.

850

851 *Quantification of antibiotics in manure:*

852

853 Manure samples were analyzed for cephapirin and pirlimycin using the methods
854 described by Ray *et al.* (76, 77). These methods were modified to make them
855 suitable for the quantification of cephapirin and pirlimycin in manure samples.
856 Manure samples (sample size: 1 g) were extracted using 5 mL of extractant
857 [methanol (70%) and phosphate buffer (50 mM at pH 8.5)]. Extraction was

858   followed by extract clean-up involving solid phase extraction (SPE) using OASIS
859   HLB Plus Short Cartridge (250 mg sorbent; Waters, Milford, MA). An aliquot of 1
860   mL clean extracts was dried to dryness at 35°C under N_2 gas using a Zipvap 20
861   evaporator (Glas-Col, Terre Haute, IN) and dissolved in 1 mL of methanol:water
862   (30:70, v/v) with 0.1% formic acid. Dissolved extracts were filtered through 0.2
863   μm PVDF syringe filter (Fisher, Pittsburgh, PA) into 1.5 mL amber glass HPLC
864   vials and analyzed for cephapirin and pirlimycin using UPLC-MS/MS (Agilent
865   1290 UPLC coupled with Agilent 6490 Triple Quad tandem mass spectrometry).
866   A gradient elution program consisting of two mobile phases (mobile phase A:
867   0.1% formic acid in water; mobile phase B: 0.1% formic acid in methanol) were
868   used at a flow rate of 0.5 mL/min. The concentration of cephapirin and pirlimycin
869   in manure samples was quantified using the calibration curve of seven matrix-
870   matched standards (0.5, 1, 2, 4, 5, 10, and 20 μg L^{-1} matrix solution). Matrix-
871   match standards were prepared using the SPE cleaned-up extracts of blank
872   manure samples.

873   *Microbial community composition and statistical analysis:*

874   In order to determine the effect that antibiotic exposure has on microbial
875   community composition, bacterial and fungal DNA were analyzed. DNA was
876   extracted using DNeasy PowerSoil Kits (Qiagen, Hilden, Germany). The
877   community composition was determined by amplifying the V4 region of the
878   bacterial/archaeal *16S* rRNA gene as well as the ITS1 region of the fungal ITS
879   spacer region using primer pairs 515FB/806RB, and ITS1f/ITS2 respectively.
880   Caporaso *et al.* (78) was followed for amplification of 16S and ITS regions.
881   Multiplexing and sequencing was carried out using an Illumina MiSeq, producing
882   250 base pair paired-end reads (78). A UPARSE pipeline was used for quality
883   filtering and for clustering into OTUs – operational taxonomic units (79).
884   Additionally, all chimeric sequences were identified and removed using UCHIME
885   (80). The Ribosomal Database Project Native Bayesian Classifier was used to
886   assign OTUs to 269 specific taxonomies with the OTU cutoff for clustering of
887   97% (81). This was carried out using the GreenGenes 13.8 reference database
888   for the bacteria and archaea (82) and the UNITE 6.97 database for fungi (83).
889   Rarefication of OTU tables and alpha diversity estimations were carried out using
890   the QIIME pipeline (84).
891
892   For ITS, only forward reads were used due to the size variability of the ITS
893   region. We processed ITS read sequences using the DADA2 pipeline (85), which
894   is designed to resolve exact biological sequences from Illumina sequence data
895   and does not involve sequence clustering (86). Sequences were trimmed to
896   uniform lengths, dereplicated, and the unique sequence pairs were denoised
897   using the 'dada' function, accounting for errors through the model generated with
898   the 'learnErrors' command. We removed chimeras and then assigned taxonomy
899   using the UNITE dynamic general release (ver 01.12.2017; 83) for fungi. To
900   account for differences in sequencing depths, we rarefied fungal samples to
901   36195 sequences per sample.
902

903 We compared microbial community composition using Primer-E (Ver. 7.0.13).
904 Microbial community data were square-root transformed before calculating
905 community dissimilarity between each treatment using Bray-Curtis dissimilarity.
906 These distances were used to generate ordinations (principal coordinates
907 analysis, PCoA) for both bacteria and fungi. Next, we performed PERMANOVA
908 with the community distance matrices to compare community composition using
909 treatments as a fixed effect, and block as a random effect using Primer-E (9999
910 permutations, Ver. 7.0.13; 65). We tested for homogeneity of dispersions from
911 the centroids via betadisper tests (89). To determine the potential OTUs
912 responsible for treatment differences, we first determined the percentage
913 contribution of taxa to overall Bray-Curtis dissimilarity using the SIMPER
914 (similarity percentages) command in Primer. We then identified common OTUs
915 that contributed to the top 20% of dissimilarity between treatment pairs and
916 analyzed each via ANOVA. Analyses were conducted in Primer v6 except
917 ANOVA which was conducted in R.
918

919 *Additional soil and microbial parameters:*

920

921 Soil pH was measured using a SensION+ PH3 laboratory pH probe (Hach,
922 Loveland, CO, USA). In addition to the CFE procedure described above, soil
923 microbial biomass was determined via substrate-induced respiration (SIR). The
924 SIR method is modified from West and Sparling (90) according to Fierer *et al.*
925 (91) and is considered a measure of active microbial biomass, whereas CFE
926 measures the total standing stock of microbial biomass. Briefly, SIR biomass was
927 determined by pre-incubating 4-g of dry weight equivalent soil at 20˚C for 24-h.
928 Next, an excess of autolyzed yeast substrate (792-mg of yeast in dissolved 8-ml
929 of DI water) was added to each sample. The sample was then homogenized and
930 shaken for 1-hour, before the sample was capped and the headspace flushed
931 with CO_2-free air. Samples were then incubated for 5-h at 20˚C, before respired
932 CO_2 accumulated in the headspace of each sample is measured using a gas
933 syringe and a bench-top infrared gas analyzer (IRGA, LI-7000 CO_2 H_2O
934 Analyzer, Li-Cor, Lincoln, NE).

935

936 Using the same basic outline as the SIR protocol, C mineralization (CMin) (46)
937 and catabolic response profile (CRP) (92) were also measured. In order to
938 determine the rate of C mineralization taking place in the soil – a measurement of
939 microbially accessible C – 6-g of dry weight equivalent soil was incubated at
940 20˚C for 60-d. During this time samples were flushed with CO_2-free air and
941 incubated for 24-h at 20˚C. Headspace was then measured using a bench-top
942 IRGA. Over this time water holding capacity was monitored and maintained at
943 approximately 65% - which is advantageous to microbial function. The integrals
944 between these periodic measurements are then extrapolated to determine
945 cumulative C mineralized over the 60-d period.

946

947 Catabolic response profiles (CRP) were used to measure the range of substrate
948 utilization capabilities of a given microbial community. This assay provides a

949 profile of responses to substrates, and helps to describe the metabolic
950 capabilities of a given microbial community. To accomplish this, soils were
951 weighed, substrate added, sample and substrate homogenized, headspace
952 flushed, incubated at 20˚C, and headspace measured in a fashion similar to the
953 SIR protocol. However, instead of an autolyzed yeast solution substrate –
954 glucose, glycine, oxalic acid, cellulose, chitin and water were all used as single
955 substrates in individual assays. Each substrate was pH adjusted to 6, and their
956 respective incubation times varied according to recalcitrance (*i.e.* cellulose and
957 chitin were incubated for 24-h, all others were incubated for 4-h after shaking).
958 Finally, all measurements are standardized to calculate the amount of respired C
959 per quantity of soil and unit of time.
960
961 *Works Cited*
962

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1010
1011

1012   *Supplementary Figures:*

1013

1014   Figure S1.

1015

1016   Figure S2.
1017
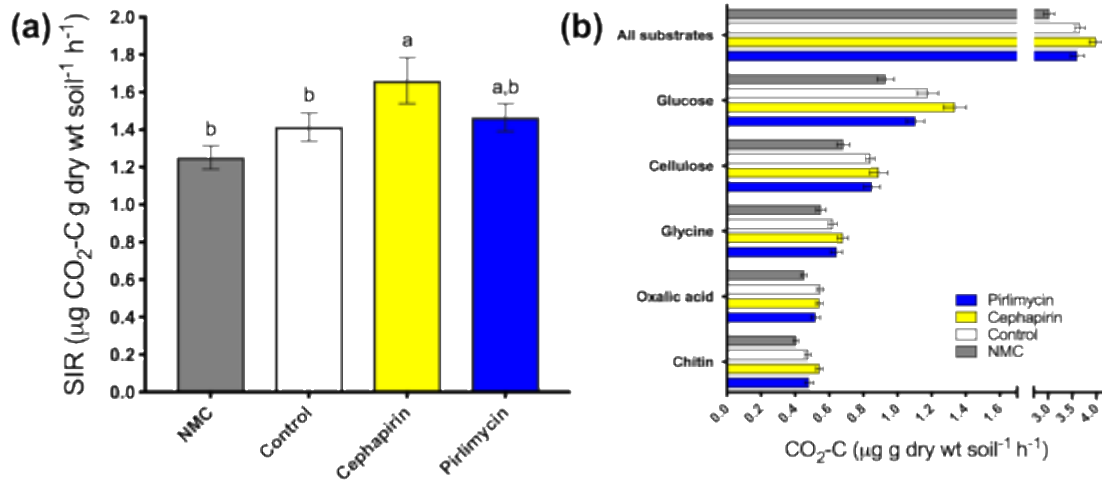
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1020   Figure S3.
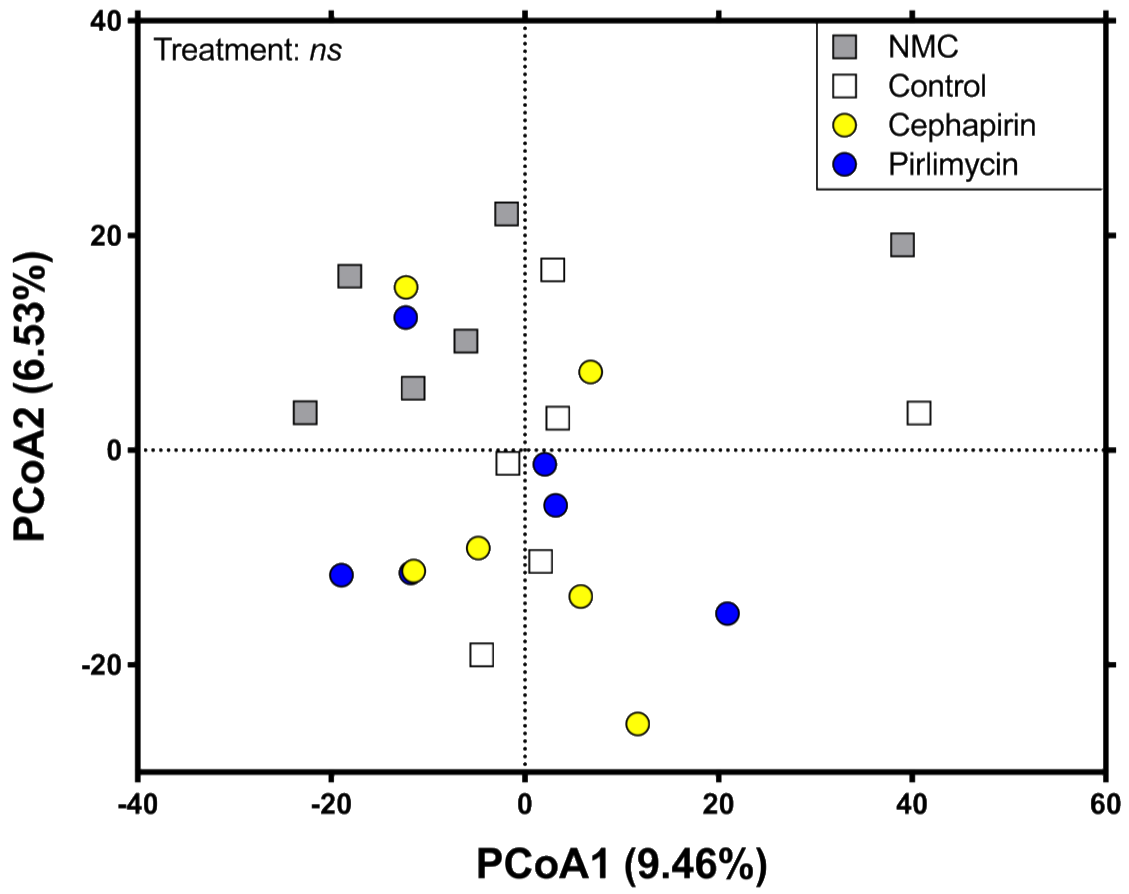
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1024 Supplementary Tables:

1025

1026 Table S1.

Treatment	Cellulose	Chitin	Glucose	Glycine	Oxalic Acid	All Substrates	CMin
NMC	0.68±0.04	0.41±0.02	0.93±0.05	0.55±0.03	0.45±0.02	3.03±0.1	824.69±22.5
Con	0.84±0.03	0.48±0.02	1.18±0.06	0.62±0.03	0.55±0.02	3.66±0.1	1112.29±42.3
Ceph	0.89±0.05	0.54±0.02	1.34±0.07	0.68±0.03	0.54±0.02	4.00±0.1	1190.83±55.6
Pir	0.85±0.05	0.48±0.02	1.11±0.05	0.64±0.03	0.52±0.03	3.61±0.1	1144.89±49.4

1027

1028   Table S2

Treatment	Day	Aboveground – C	Aboveground – N	Aboveground – C:N	Belowground – C	Belowground – N	Belowground – C:N	Microbial – C	Microbial – N	Microbial – C:N	Respiration - C
NMC	1	235.3±33	8.2±0.9	28.3±1.2	327±46	11.9±1.9	27.6±0.9	1.5±0.2	0.3±0.03	7±0.8	2.8±0.3
	2	311.9±33	11.3±1.1	27.6±1.2	353.4±27	13.8±2.5	28±3.0	1.3±0.2	0.2±0.03	8.7±1.1	2.3±0.3
	7	362.7±62	12.6±2.6	29.6±1.0	532.2±63	13.8±2.7	42±5.7	1.5±0.1	0.2±0.03	7.8±0.5	3.6±0.3
Con	1	329.3±56	15.2±2.6	21.8±2.2	347.3±73	15.1±3.6	23.5±1.3	2.0±0.2	0.3±0.04	7.0±0.4	3.0±0.5
	2	377.5±42	16.7±1.7	22.7±1.6	417.1±29	16.5±1.5	25.9±2.1	1.3±0.2	0.2±0.03	8.9±1.3	2.1±0.1
	7	466.2±28	19±2.1	25.2±1.6	597.3±92	20.4±2.5	30.6±5.2	1.8±0.3	0.2±0.05	8.3±1.2	4.5±0.1
Ceph	1	270.3±42	10.9±1.7	25±1.0	318.9±55	13.3±2.3	24.3±1.3	1.9±0.4	0.3±0.06	10.1±2.5	2.6±0.6
	2	426.1±32	18.8±1.5	22.8±0.9	466±55	16.9±1.7	27.3±1.3	1.1±0.2	0.2±0.03	10.2±2.9	1.7±0.2
	7	448.8±62	20.7±1.7	21.4±1.9	497.8±45	20.4±2.1	24.7±1.1	1.5±0.1	0.2±0.02	6.7±0.6	4.9±0.3
Pir	1	308.8±47	13.8±1.6	22.1±1.2	367.2±52	15.5±2.6	24.2±1.3	1.5±0.2	0.2±0.02	11.4±1.4	3.2±0.5
	2	368.8±31	14±1.3	26.5±0.9	427.8±61	17.6±3.0	25.2±1.6	1.3±0.1	0.2±0.02	8.3±0.8	2.3±0.2
	7	495.0±44	19.9±1.4	25.2±2.5	657±109	20±3.5	34.4±4.1	1.6±0.2	0.2±0.03	7.7±0.4	5.4±0.5
Treat	P	*0.047*	*<0.001*	*<0.001*	0.553	*0.065*	*0.022*	*0.071*	*0.080*	χ^2=0.364	*0.044*
Day	P	*<0.001*	*<0.001*	0.624	*<0.001*	*0.018*	*0.001*	*<0.001*	*0.028*	χ^2=0.036	*<0.001*
T*D	P	0.869	--	0.137	--	0.940	--	--	--	--	*0.068*
Treatment Pairwise Significance		Con>NMC Ceph>NMC* Pir>NMC	*All>NMC*	NMC>Con NMC>Pir	--	*Con>NMC Ceph>NMC Pir>NMC**	*NMC>All*	Con>NMC Con>Pir	Con>Pir Ceph>Pir	--	*Pir>NMC Pir>Ceph**

1029

1030   Table S3:

1031

Treatment	Day	POM – C	POM – N	POM –C:N	Mineral - C	Mineral - N	Mineral – C:N
							1032
							1033
NMC	1	708.4±63.9	45.4±4.4	15.7±0.3	1607.8±156.4	166.1±16.9	9.7±0.1
	2	672.6±59.2	45.0±5.2	15.1±0.4	1586.1±150.3	163.7±15.4	9.7±0.1
	7	611.8±52.1	39.7±4.0	15.5±0.2	1419.6±76.2	150.7±7.8	9.4±0.1
Con	1	822.5±72.0	49.1±4.6	16.8±0.3	1440.2±113	148.9±12.2	9.7±0.1
	2	663.5±34.7	40.5±2.6	16.5±0.3	1422.4±71.6	144.5±7.1	9.8±0.1
	7	666.1±63.1	42.4±4.2	15.7±0.2	1453.8±131.8	153.6±11.3	9.4±0.2
Ceph	1	886.8±125.9	54.7±8.7	16.5±0.6	1669.8±109.1	169.7±11.2	9.9±0.1
	2	821.4±86.5	49.0±6.1	17.0±0.7	1489.5±142.8	149.8±13.7	9.9±0.1
	7	508.7±79.5	49.3±6.4	16.7±0.7	1485.6±124.2	148.2±14.3	10.1±0.5
Pir	1	731.6±56.0	42.2±3.6	17.4±0.4	1544.2±109.0	159.6±10.4	9.7±0.1
	2	774.6±55.4	44.0±2.9	17.6±0.2	1467.1±53.0	147.7±6.8	10.0±0.1
	7	756.7±62.0	46.0±4.4	16.6±0.4	1473.3±122.5	152.3±12.2	9.7±0.1
Treat	P	*0.002*	*0.037*	*0.001*	0.150	0.152	$\chi^2=0.182$
Day	P	0.115	0.311	0.168	*0.058*	*0.037*	--
T*D	P	0.575	0.605	0.369	0.459	0.273	--
Treatment Pairwise Significance (* Marginal)		Ceph>NMC Ceph>Pir	Ceph>NMC* Ceph>Pir	*Con>NMC Pir>NMC Pir>Ceph**	--	--	--

1034   Table S4.

Treat	Day	AG-^{13}C-C^{-1}	AG-^{15}N-N^{-1}	BG-^{13}C-C^{-1}	BG-^{15}N-N^{-1}	POM-^{13}C-C^{-1}	POM-^{15}N-N^{-1}	Min-^{13}C-C^{-1}	Min-^{15}N-N^{-1}	Resp-^{13}C-C^{-1}
NMC	1	0.015±1.1E-3	0.267±0.03	2.1E-3±6.3E-4	0.167±0.03	9.9E-4±4.2E-4	0.019±5.0E-3	6.5E-4±2.2E-4	0.015±2.8E-3	0.077±0.1
	2	0.012±1.4E-3	0.387±0.05	2.8E-3±8.2E-4	0.218±0.04	5.6E-4±3.1E-4	0.019±3.4E-3	5.6E-4±2.5E-4	0.016±2.0E-3	1.14±0.1
	7	9.1E-3±1.2E-3	0.398±0.05	1.7E-3±5.6E-4	0.266±0.05	6.0E-4±3.1E-4	0.027±6.3E-3	6.7E-4±2.5E-4	0.016±4.4E-3	1.91±0.5
Con	1	0.017±2.1E-3	0.214±0.02	1.8E-3±4.0E-4	0.165±0.01	2.9E-4±2.1E-4	0.029±3.7E-3	2.6E-4±9.8E-5	0.024±3.0E-3	0.393±0.1
	2	0.012±6.2E-4	0.231±0.20	1.9E-3±6.4E-4	0.135±0.02	4.6E-4±3.5E-4	0.022±5.2E-3	4.4E-4±2.6E-4	0.017±2.4E-3	0.601±0.1
	7	7.3E-3±1.2E-3	0.250±0.04	8.4E-4±4.8E-4	0.134±0.04	3.6E-4±1.3E-4	0.018±4.4E-3	5.2E-4±1.2E-4	0.012±2.4E-3	1.54±0.5
Ceph	1	0.015±1.8E-3	0.183±0.02	3.4E-3±8.9E-4	0.135±0.02	6.3E-4±4.3E-4	0.023±5.4E-3	4.4E-4±2.0E-4	0.018±5.0E-3	0.316±0.1
	2	0.015±2.1E-3	0.238±0.02	3.6E-3±1.5E-3	0.154±0.02	1.1E-3±6.9E-4	0.017±2.8E-3	3.1E-4±1.7E-4	0.015±1.2E-3	0.607±0.2
	7	8.2E-3±1.3E-3	0.260±0.06	1.5E-3±5.5E-4	0.133±0.04	3.2E-4±2.3E-4	0.025±4.0E-3	3.0E-4±1.6E-4	0.013±1.9E-3	2.75±0.4
Pir	1	0.017±5.0E-4	0.226±0.02	1.1E-3±4.3E-4	0.139±0.03	2.2E-4±1.1E-4	0.028±5.0E-3	2.0E-4±9.7E-5	0.019±3.4E-3	0.993±0.2
	2	0.014±2.0E-3	0.277±0.03	3.3E-3±9.2E-4	0.177±0.03	9.0E-4±2.5E-4	0.033±4.9E-3	6.1E-4±9.5E-5	0.020±2.7E-3	1.44±0.2
	7	0.010±1.9E-3	0.334±0.03	1.2E-3±5.6E-4	0.277±0.05	2.1E-4±2.1E-4	0.039±6.9E-3	1.2E-4±5.9E-5	0.024±3.2E-3	2.44±0.5
Treat	P=	0.54	**>0.001**	0.150	**0.009**	0.480	**0.005**	**0.041**	**0.068**	**0.027**
Day	P=	**>0.001**	**>0.005**	**0.010**	**0.088**	0.261	0.397	0.621	0.403	**0.001**
Treatment Pairwise Significance (* Marginal)		--	*NMC>All Pir>Con* Pir>Ceph*	--	*NMC>Con NMC>Ceph Pir>Con Pir>Ceph*	--	*Pir>All*	*NMC>Con* NMC>Ceph NMC>Pir*	*Pir>NMC Pir>Ceph*	*NMC>Con Pir>Con Pir>Ceph*

1035
1036

1037   Table S5.

	%C	pw	%N	pw	C:N	pw	Cephapirin (ng g manure^{-1})	Pirlimycin (ng g manure^{-1})
Con	49.9	b	3.3	a	15.3	b	-	-
Ceph	49.0	a	3.4	a	14.3	a	Below detection (<0.36)	-
Pir	49.6	b	3.5	a	14.3	a	-	149 ± 3.38
SE	0.12		0.06		0.26			

1038

Supplementary Figure and Table Legends:

Figure S1: Plexiglass box for ^{13}C pulse-chase experiment, with wooden frame and rubber liner for sealing the chamber to the ground. The wooden frame was trenched 10-cm into the ground to minimize any leakage of ^{13}C-CO_2 from the pulsing area, as well as any non-labeled CO_2 entering the pulsing area.

Figure S2: Effect of antibiotic exposure on microbial activity and active microbial biomass. **a)** Substrate induced respiration (SIR) by treatment, and **b)** Catabolic response profile (CRP) by substrate and treatment (N = 24 for each treatment). SIR showed a significant treatment effect ($F_{3,87}$ = 4.26, P < 0.01), with treatments receiving manure from cephapirin treated cattle having active microbial biomass significantly greater than the no-manure control (NMC; P < 0.001) and control manure (Con; P < 0.05), and marginally greater than manure from pirlimycin treated cattle (Pir; P = 0.10). A significant treatment effect was observed for glucose ($F_{3,92}$ = 8.27, P < 0.001), cellulose ($F_{3,87}$ = 5.14, P < 0.005), glycine ($F_{3,15}$ = 2.53, P = 0.097), oxalic acid ($F_{3,15}$ = 4.02, P < 0.05), and chitin ($F_{3,87}$ = 8.00, P < 0.001). Across CRP the Ceph treatment is higher than all other treatments, though not consistently significantly different when compared pairwise. This provides evidence that the exposure to manure from cephapirin treated cattle can cause an increase in microbial activity – consistent with previous findings. Increased microbial activity has implications for microbial efficiency and soil C storage.

Figure S3: Nonmetric multidimensional scaling for fungal communities across antibiotic treatments. Distances are based on dissimilarity matrices of sequence-based Bray-Curtis distances. Fungal communities across treatments do not differ significantly from each other (PERMANOVA Fungal: pseudo-$F_{3,15}$ = 1.10, P = 0.18, Stress = 0.16).

Table S1: Catabolic response profile (CRP) average respired C (mean; CO_2-C (μg g dry wt soil^{-1} h^{-1})) by substrate type (cellulose, chitin, glucose, glycine, oxalic acid, and summed respiration across all substrates) and treatment (NMC: no manure control; Con: control manure; Ceph: manure from cephapirin treated cattle; Pir: manure from pirlimycin treated cattle. Additionally, microbially mineralizable C (CMin; CO_2-C (μg g dry wt soil^{-1} h^{-1})). Error listed is standard error.

Table S2: C, N, and C:N of aboveground biomass (Aboveground; g-m^{-2}), belowground biomass (Belowground; g-m^{-2}), microbial biomass (Microbial; g-m^{-2}), and ecosystem respiration (g CO_2-C m^{-2} h^{-1}). These were measured at three time points (Days 1, 2, and 7) across four treatments (NMC: no manure control; Con: control manure; Ceph: manure from cephapirin treated cattle; Pir: manure from pirlimycin treated cattle). The values listed are means and associated standard errors. Linear mixed models (LMM) and type III analysis of variance (ANOVA) were used to analyze the data with the exception of the Mineral-C

1085 which was analyzed using generalized linear model (GLM) and type II ANOVA –
1086 Wald χ^2 Test in order to address failure to pass assumptions necessary for LMM.
1087 As a result, these values are reported as χ^2 as opposed to *P*-values. The additive
1088 models all included treatment (Treat) and time (Day) as fixed effects and Block
1089 as a random effect. Aboveground-N, Belowground-C, Belowground-C:N, and
1090 Microbial-N data were log transformed to meet normality assumptions. Significant
1091 and marginally significant *P*-values are designated by italic and bold font.
1092
1093 **Table S3:** C, N, and C:N of particulate organic matter (POM; g-m^{-2}) and mineral
1094 associated (Mineral; g-m^{-2}) soil pools. These were measured at three time points
1095 (Days 1, 2, and 7) across four treatments (NMC: no manure control; Con: control
1096 manure; Ceph: manure from cephapirin treated cattle; Pir: manure from pirlimycin
1097 treated cattle). The values listed are means and associated standard errors.
1098 Linear mixed models (LMM) and type III analysis of variance (ANOVA) were
1099 used to analyze the data with the exception of the Mineral-C:N which was
1100 analyzed using generalized linear model (GLM) and type II ANOVA – Wald χ^2
1101 Test in order to address failure to pass assumptions necessary for LMM. As a
1102 result, these values are reported as χ^2 as opposed to *P*-values. Models used
1103 were either interactive or nested depending on the best model AIC. For factors
1104 best analyzed with interactive models all *P*-values are reported – for nested
1105 models only treatment *P*-value is reported. For interactive models treatment
1106 (Treat) and time (Day) and interactive (T*D) are used as fixed effects and Block
1107 as a random effect. For the nested model plot is nested within block, thus
1108 accounting for the repeated sampling over time. POM-C:N data were log
1109 transformed to meet normality assumptions. Significant and marginally significant
1110 *P*-values are designated by italic and bold font.
1111
1112 **Table S4:** Showing the fate of added isotopic C (^{13}C) and N(^{15}N) of the various
1113 pools measured: aboveground biomass (AG) belowground biomass (BG)
1114 particulate organic matter (POM), mineral associated (Min), and ecosystem
1115 respiration (for C only). Isotopic content is reported as the amount of label
1116 recovered as a proportion of the total element pool – as a proportion therefore
1117 unitless. These were measured at three time points (days 1, 2, and 7) across four
1118 treatments (NMC: no manure control; Con: control manure; Ceph: manure from
1119 cephapirin treated cattle; Pir: manure from pirlimycin treated cattle). The values
1120 listed are means and associated standard errors. Linear mixed models (LMM)
1121 and type III analysis of variance (ANOVA) were used to analyze the data. The
1122 models all included Treatment and Day as fixed effects and Block as a random
1123 effect. AG-^{15}N-N^{-1} data were log transformed to pass normality assumptions.
1124 Model quality analysis using AIC favored additive models over interactive ones.
1125 Significant *P*-values are designated by bold and italic font.
1126
1127 **Table S5**: Manure characteristics for control (Con), cephapirin (Ceph), and
1128 pirlimycin (Pir) treated cattle. Pairwise comparisons are listed according to *P-*
1129 *values* < 0.05. Model standard error is also noted (SE). Statistical tests were not
1130 performed for antibiotic concentrations.