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

Article

Accepted Version

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

Authors: Carl Wepking[a,b,*], Brian Badgley[c], John E. Barrett[b], Katharine F. Knowlton[d], Jane M. Lucas[e], Kevan Minick[f], Partha P. Ray[g], Sarah Shawver[c], Michael S. Strickland[e*]

[a] School of Global Environmental Sustainability, Colorado State University, Fort Collins, CO, 80523; [b] Department of Biological, Virginia Tech, Blacksburg, VA, 24061; [c] Sciences Department of Crop and Soil Environmental Sciences, Virginia Tech, Blacksburg, VA, 24061; [d] Department of Dairy Science, Virginia Tech, Blacksburg, VA, 24060; [e] Department of Soil and Water Systems, University of Idaho, Moscow, ID, 83844; [f] Department of Forestry and Environmental Resources, NC State University, Raleigh, NC, 27695; [g] Animal, Dairy and Food Chain Sciences, School of Agriculture, Policy and Development, University of Reading, Reading RG6 6AR

ORCID: CW: 0000-0003-1910-5999; MSS: 0000-0001-5349-0363

Keywords: elemental cycles, microbial ecology, stable isotopes, ecosystem function, antibiotics, agroecology

Authorship Statement: C.W. and M.S.S. designed research. C.W., B.B., J.E.B., K.F.K., K.M., P.P.R., S.S., and M.S.S. performed research. C.W., J.M.L and M.S.S. analyzed the data; C.W., and M.S.S. wrote the manuscript; all authors participated in editing and revision of the manuscript.

Data Statement: The data that support the findings of this study are available at https://datadryad.org/resource/doi:xxxxxx (placeholder until this Dryad dataset is official).

Number of words in abstract:	147
Number of words in main text:	4954
Number of figures:	4 (additionally, 3 supplementary figures and 5 supplementary tables)
Number of references:	75 (plus 17 in supplementary materials)

*Corresponding authors and contact information:

Carl Wepking
School of Global Environmental Sustainability – Colorado State University
108 Johnson Hall
Fort Colllins, CO 80523
carl.wepking@colostate.edu
phone: 970-491-7647 / fax: 970-492-4130

Michael S. Strickland
Department of Soil and Water Systems
University of Idaho
875 Perimeter Dr. MS 2340
Moscow, ID 83844-2340
mstrickland@uidaho.edu
phone: 208-885-0960

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

cephapirin, those that received the bacteriostatic antibiotic pirlimycin, and control cattle receiving no antibiotics) to grassland plots in a common-garden experiment, along with a no-manure control. The relative impacts of antibiotics on soil microbial communities were examined via determination of fungal:bacterial ratio (hereon F:B) and *16S* and ITS metabarcoding (to assess bacterial and fungal community composition, respectively), and on ecosystem processes via a ^{13}C and ^{15}N stable isotope pulse-chase. We expected that manure itself would positively affect plant growth and lead to an increase in soil C pools. However, when manure was sourced from cattle administered antibiotics, we expected a greater loss of C via respiration, as well as, an overall decrease in ecosystem C-use efficiency due to decreased microbial efficiency (specifically bacterial; 21). Antibiotics are likely to lead to an increase in F:B (39, 40). The implications of this on an ecosystem-scale are subject to debate: given the classical understanding of fungal versus bacterial contribution to biogeochemical processes, we would expect that systems with a higher F:B would retain more C and N (41, 42). Alternatively, recent work has shown that C and N mineralization are unrelated to the relative dominance of bacteria and fungi (43). Therefore, outcomes from this experiment could lend support to either theory in light of recent challenges to the classical understanding of fungal versus bacterial contribution to biogeochemical processes.

Materials and Methods:

Experimental Design:

A common garden experiment with a randomized block design (four treatments, n=6) was conducted at Kentland Farm, Blacksburg, VA, USA (37.199490, -80.584659; 547-m elevation; Unison and Braddock cobbly soils; dominant plant cover is grasses, mostly tall fescue, as well as some herbaceous cover including members of the Lamiaceae and Plantaginaceae families). Treatments included three manure additions (manure from cattle given no antibiotics, or manure from cattle given either cephapirin benzathine or pirlimycin hydrochloride) and one control treatment that received no manure. Both antibiotics are commonly used in the prevention of mastitis in dairy cattle, however they vary in a number of ways including their fate in the environment (44) and mode of action. Cephapirin benzathine (Molecular weight = 365.4 g mol^{-1}; pKa = 2.2; water solubility = 3,430 mg L^{-1}) is bactericidal, damaging the structural integrity of bacterial cell membranes, whereas pirlimycin hydrochloride (Molecular weight = 447.4 g mol^{-1}; pKa = 8.4; water solubility = 64,900 mg L^{-1}) is bacteriostatic, inhibiting protein synthesis. Hereon we refer to these four treatments as no-manure control (NMC), control manure (Con), cephapirin manure (Ceph), and pirlimycin manure (Pir).

Manure was applied to appropriate treatments at a monthly rate of 648-g-m^{-2} of wet-weight manure starting in October, 2014 until May, 2015 (213 days) – totaling 4,536-g of manure m^{-2}. This amount of manure corresponds with the amount of manure expected given a typical dairy cattle stocking density.

For information regarding manure properties, sourcing and within-manure antibiotic quantification see supplementary materials.

Pulse-chase experiment:

Field sampling was conducted in May, 2015. In order to determine whether antibiotic use in dairy cattle affects system-wide elemental cycling, a ^{13}C and ^{15}N stable isotope pulse-chase experiment was conducted. The use of ^{13}C allowed for the tracking of recently photosynthesized C through both above- and belowground C pools. To accomplish ^{13}C-labeling, a ~1-m^2 subplot within each treatment plot was covered with a 0.6-m^3 (0.99-m × 0.99-m × 0.61-m) transparent acrylic chamber (Figure S1). To prevent gas exchange from outside the chamber, the chamber was fitted into a rubber lined wooden base that was trenched 10-cm into the soil. The rubber liner was then adhered to the acrylic glass chamber using silicon grease. $^{13}CO_2$ was introduced into each chamber via gas-tight ports by reacting 1-g sodium carbonate ($Na^{13}CO_3$, 99 atom% ^{13}C, Sigma-Aldrich; CAS number: 9367-48-4; 113-mg of ^{13}C equivalent) with excess hydrochloric acid. Air was circulated within the chambers using a centrally located internal battery-operated fan. Chamber temperature was monitored using an internal thermometer. CO_2 concentrations within the chamber were monitored via a LI-8100 infrared gas analyzer (Li-Cor Biosciences, Lincoln, NE). Chambers were removed after CO_2 levels returned to pre-pulse levels. As temperatures in the chambers can be high during mid-day, pulsing was limited to early morning and late afternoon. The amount of ^{13}C fixed by the plant communities was determined by taking foliar clip samples immediately post-pulse.

Following the ^{13}C pulse-labeling, each plot was also labeled with ^{15}N ammonium nitrate ($^{15}NH_4{}^{15}NO_3$; 98 atom%; Sigma-Aldrich; CAS Number: 31432-46-9; 67-mg of ^{15}N equivalent) in order to examine N-dynamics in response to manure and antibiotic treatments. Ammonium nitrate (300-mg in 1-L of DI water) was added evenly to the soil surface of each 1-m^2 plot. The amount of ^{15}N, similar to Fraterrigo *et al.* (45), was kept low to avoid a fertilization effect.

Upon completion of pulse-labeling, we destructively harvested 0.05-m^2 sub-plots within each 1-m^2 plot at 1, 2, and 7-days post-labeling. An additional sub-plot was harvested from each 1-m^2 experimental plot prior to pulse-labeling in order to determine natural abundance of ^{13}C and ^{15}N. Aboveground plant material from each sub-plot was harvested by clipping it at the soil surface. Aboveground plant biomass samples were air-dried, weighed, and milled for elemental and isotope analyses. The belowground portion of the sub-plot was sampled to 10-cm depth; roots and soil were then separated. Root material was initially air-dried and then later washed, air-dried, weighed, and milled for elemental and isotopic analyses. Soils were sieved (4.75-mm), homogenized, and stored at either -80°C, 4°C, or air-dried depending on future analyses (see below).

For soils, we determined POM and mineral-associated soil C and N, and soil microbial biomass C and N. POM and mineral-associated C and N was determined on air dried soil samples (46). Microbial biomass C and N were determined following the chloroform fumigation extraction (CFE) procedure outlined in Fierer and Schimel (47). Briefly, 40 mls of 0.5M K_2SO_4 was added to one of each 7-g dry mass equivalent soil pair. One of each pair is then exposed to 1-ml of ethanol-free chloroform to lyse microbial cells and accumulate microbial C and N. Samples are capped, and shaken for 4-h. Samples were then allowed to settle before filtration. Microbial biomass was estimated as the difference between the quantity of C and N between the fumigated and un-fumigated samples. Total organic C and N were then calculated for both the fumigated and un-fumigated samples using a Vario TOC Cube (Elemtar, Langenselbold, Germany).

$\delta^{13}C$ and $\delta^{15}N$ in above- and belowground plant biomass, POM and mineral-associated pools were determined using a Costech ECS 4010 Elemental Analyzer (Costech Analytical, Valencia, CA, USA) paired with an Thermo Delta Plus Advantage Isotope Ratio Mass Spectrometer (IRMS; Thermo Fisher Scientific™, Waltham, MA, USA).

Prior to each destructive harvest event ecosystem respiration was measured using a LI-8100 Infrared Gas Analyzer (Li-Cor Biosciences, Lincoln, NE, USA). Additionally, two 15-ml subsamples of respired air were captured using a gas syringe and air-evacuated exetainers in order to determine the $\delta^{13}C$ of respired CO_2. The first of two subsamples were collected within the first 15-seconds of a 2-minute respiration measurement period, and the second subsample was collected in the final 15-seconds. Both subsamples were then analyzed for $\delta^{13}C$ using a GasBench II IRMS (Thermo Fisher Scientific™, Waltham, MA, USA). Data were then recalculated to account for varying heights of soil collars and adjusted to optimize the r^2 of the respiration trend-line from each 2-minute measurement.

The amount of ^{13}C fixed, respired, and the amount of both ^{13}C and ^{15}N contained in above- and belowground pools was derived using standard isotopic mixing models (48). The amount of C and N derived from ^{13}C and ^{15}N additions was calculated as atom excess in a given C or N pool. The atom% excess of a given pool was then multiplied by the total C or N in that pool, giving the mass of ^{13}C or ^{15}N label. The proportion of label in a given pool was calculated as the mass of ^{13}C or ^{15}N label divided by the total amount of C or N of that specific pool. Cumulative ecosystem respiration was calculated via integration. See Supplemental Methods for details related to additional soil parameters, microbial catabolic response profiles, and microbial community composition measured in conjunction with the pulse-chase experiment.

Statistics and analysis:

Data was analyzed using linear mixed models (LMM; 'lme4' package; 63) with treatment as a fixed effect and plot nested within block as a random effect to account for sampling of plots across time. Model selection (additive vs. interactive) was determined by lowest Akaike information criterion (AIC) score (50). Normality of variance was tested using a Wilk-Shapiro test. Data with non-normal variance was either log or square-root transformed. If normality assumptions were still not met, generalized linear models (GLM; 'car' package; 64) were used using the Gamma family and either the inverse or log link function as all data was continuous and positive (variables containing negative values were standardized). Wald χ^2 tests were used to assess model significance for GLMs. Data was analyzed using the R statistical platform (52). Degrees of freedom for linear mixed models were calculated using Satterthwaite approximations.

For all analyses, we consider statistical significance at $P < 0.05$, and marginal significance at $P < 0.10$. However, it should be noted that it has typically been deemed acceptable to consider changes in soil C pools at $P < 0.10$ (53, 54), given that soil C is inherently heterogeneous.

Results/Discussion:

Antibiotic effects on active and total microbial biomass:

Prolonged manure additions, regardless of antibiotic involvement, increased C mineralization – an estimate of bioavailable soil C – when compared to NMC ($F_{3,15} = 11.8$, $P < 0.001$; Table S1). More surprising was the observation that active microbial biomass – determined via SIR – was differentially affected by the antibiotic status of the manure (again, we are referring to the effect of exposure to manure from cows given an antibiotic, as an antibiotic effect; Figure S2a; $P < 0.01$). Specifically, the Ceph treatment exhibited greater active microbial biomass in comparison to the other treatments. Similar to the increase in respiration observed for SIR, increased microbial activity was observed across a range of C-substrates for the Ceph treatment in a catabolic response profile (CRP; Figure S2b). In contrast, we observed a marginally significant treatment effect on total microbial biomass C ($P = 0.07$) and N ($P = 0.08$), primarily driven by a trend towards increased microbial C and N in the Con treatment (Table S2). This contrast between active and total microbial biomass may suggest physiological changes, specifically greater mass-specific activity for the Ceph treatment, consistent with findings from previous investigations (21). As discussed in the corresponding section, the Ceph treatment did not differ from the other antibiotic manure treatment, Pir, in terms of microbial community composition. Therefore, elevated microbial activity could be due to two, non-mutually exclusive, factors: *i*) the increased presence of lysed cellular material from the action of cephapirin, a bactericidal antibiotic, or *ii*) from a stress response of the microbial community, due to the added metabolic cost of maintaining antibiotic resistance (36). This stress response is consistent with previous research on cephapirin use on dairy

cattle in pasture systems, and has been suggested as a possible cause of altered ecosystem C cycling, through reduced microbial efficiency (21).

Antibiotic effects on fungal:bacterial dominance:

As antibiotics detrimentally effect bacteria, we assessed F:B via qPCR to determine shifts in fungi and bacteria. Overall, we observed a significant treatment effect for F:B (Figure 1; $F_{3,63}$ = 6.497, P < 0.001) as has been previously observed (55). Fungal counts increased in the soils receiving manure compared to NMC, as well as in Pir compared to Con. NMC had the lowest ratio, indicating that without manure and associated antibiotics the system is relatively more dominated by bacteria. Notably, within the Pir and Ceph manure treatments, the differences in F:B were due to declines in bacteria (*i.e.* 16S gene abundance), whereas little change in fungi (*i.e.* ITS abundance) was observed. This increase in F:B in the Pir treatment may be driven by pirlimycin's bacteriostatic mode of action: pirlimycin typically reduces bacterial growth but does not induce cell lysis. Conversely, cephapirin – a bactericidal antibiotic – causes cell lysis. Lysed cells, as suggested above, cause an increase in labile resources that potentially favor bacteria in spite of the direct negative effects of the antibiotic (56, 57). This potential net positive effect for bacteria under the Ceph compared to the Pir treatment is supported by a pairwise marginally significant increase in *16S* copies (Figure 1; P = 0.08). It is also possible that decreased inhibition of bacteria in the Ceph treatment can be attributed to cephapirin being relatively more easily degraded than pirlimycin. However, evidence of cephapirin's effect on microbial functional properties was in fact observed, therefore degradability is unlikely to be the explanation for the difference in bacterial effects between antibiotic treatments. Compared to NMC, the addition of manure, regardless of antibiotic involvement, increased the abundance of fungi (*i.e.* ITS copies) in soil ($F_{3,68}$ = 5.868, P < 0.01), with Con, Ceph, and Pir treatments having greater numbers of ITS copies than NMC. Within the manure-addition treatments, Ceph had a marginally lower abundance of fungi compared to Con (P < 0.10), this too could be driven by the mode of action related to this antibiotic.

The primary fungal effect appeared to be driven by manure itself, given that all manure additions increased ITS copies compared to NMC. This could be attributed in part to coprophilous fungi, which specialize in the decomposition of fecal matter, previously shown to be elevated in conjunction with manure (21). Additionally, the highest counts of *16S* and ITS copies were measured in the Con treatment. This is likely attributed to the influx of manure-derived resources in the absence of antibiotics. Together these results suggest that while manure additions increase F:B, manure from cattle administered antibiotics tends to lead to even greater increases, primarily driven by decreased bacterial abundance.

Antibiotic effects on microbial community composition:

The results of our community composition assessment largely mirrored the results of the F:B analysis. Bacterial communities changed across our treatments (Figure 2: *pseudo-*$F_{3,23}$ = 1.15*; P* < 0.05; note, the random effect 'block' was dropped from this model because it was non-significant), but fungal communities did not (Figure S3: *pseudo-*$F_{3,23}$ = 1.01*; P* = 0.18). The treatment effect on bacteria was largely driven by differences between Con, and both Pir and Ceph. Notably, a marginally significant pairwise difference was observed between Pir and Ceph (*P* = 0.064). NMC did not differ from the other three treatments, in fact, as NMC can be viewed as a baseline, shifts in bacterial community composition from manure exposure were dependent on the antibiotic status of the manure. If the manure was sourced from cattle administered an antibiotic, the community shifted to the lower right in ordination space, while control manure caused the community to shift in the opposite direction, with NMC situated between (Figure 2a). Additionally, bacterial beta diversity did not differ between treatments (Figure 2a; *pseudo-*$F_{3,20}$ = 1.12; *p* = 0.31), suggesting that the microbial communities in our antibiotic treatments are distinct from control environments, and not just more variable in composition. While we did not seek to document the impact of the fecal microbiome on the soil microbiome, previous studies have shown that the fecal microbiome can be impacted by antibiotic exposure (38). Therefore, further research into the quantification of this effect would be beneficial, especially investigations into interactions between the fecal and soil microbiomes.

To further investigate OTUs that possibly drove treatment differences, we identified potential OTUs of interest via SIMPER that were common across all pairwise treatment comparisons. This resulted in 32 common OTUs of which only 6 exhibited significant differences between treatments (Figure 2b). Of these 6 OTUs, 4 were associated with Phyla Acidobacteria and γ-Proteobacteria (2 in each), and 2 were associated with the Phyla Bacteroidetes and Verrucomicrobia (1 in each). Interestingly, the two γ-Proteobacteria were associated with families Acinetobacter and Xanthomonadaceae, which are typically associated with the environment but also include members of human health concern (58). In fact, Wepking *et al.* (21) observed a similar increase in the genus Acinetobacter in response to cattle administered a cephalosporin. Our results add further support to the likely influence of antibiotics on soil community structure, and further support the proposition that inputs of manure from cattle given antibiotics can shift soil microbial communities towards organisms that are related to those of human health concern (59, 60).

Interestingly, though, several OTUs associated with phyla that we expect to be more oligotrophic in nature (*i.e*. Acidobacteria, Verrucomicrobia; 49, 50) also exhibited greater relative abundance in Pir and Ceph. Even some taxa in the family Cytophagaceae could be classed as oligotrophs, especially those involved in cellulose degradation (63). This greater relative abundance of oligotrophic taxa, primarily in Pir, may be due *not* to an increase in these groups but to a decrease in other potentially more copiotrophic groups. That is in the Pir treatment there was an observed decrease in *16S* abundance, suggesting a

decline in overall bacterial abundance. Such a decrease, if driven by the antibiotic pirlimycin may have been disproportionate because the antibiotic is bacteriostatic and, as such is likely to have a more detrimental effect on active bacteria (64, 65). Overall, these results suggest that manure from cattle given antibiotics versus those not, has the potential to lead to shifts in soil bacterial community composition and F:B dominance in a relatively short time (*i.e.* ~8 months) with implications on microbially-mediated ecosystem function.

Antibiotic effects on carbon and nitrogen dynamics:

Few differences were observed in most pools of recently fixed C (Table S4), and in the amount of ^{13}C fixed across manure-amended treatments (NMC fixed more ^{13}C relative to total plant C, likely due to the lower plant biomass and identical amount of labeled C added to the chamber; $F_{3,20} = 3.07$, $P = 0.05$). However, we did observe a significant effect of both treatment ($\chi^2 = 18.52$, $df = 3$, $P < 0.001$), and time ($\chi^2 = 87.18$, $df = 2$, $P < 0.001$), as well as a treatment × time interaction ($\chi^2 = 41.13$, $df = 6$, $P < 0.001$), when examining the ecosystem respiration of recently fixed C (Figure 2a). Specifically, the Pir treatment exhibited greater initial respiration of ^{13}C compared to the other treatments, but by day 7 of the experiment, respiration of ^{13}C for this treatment was nearly zero (Figure 3a). The NMC, Pir, and Con treatments exhibited similar respiration dynamics (Figure 3a). Ecosystem respiration dynamics for the Ceph treatment were more constant during the sampling period compared to the other three treatments (Figure 3a). A marginally significant difference in cumulative ^{13}C respired across the entire sampling period was explained by the greatest amount of ^{13}C being respired in the Pir treatment with the NMC and Ceph treatments intermediate, and the Con treatment the lowest ($F_{3,15} = 2.72$; $P = 0.08$; Figure 3a). In fact, nearly twice the amount of newly photosynthesized C was respired – not retained in the soil – in the Pir treatment compared to the Con treatment (Figure 3a).

These results suggest that manure from cattle administered antibiotics can alter both ecosystem respiration dynamics of recently fixed ^{13}C (*i.e.* Ceph) as well as the total amount of C respired (*i.e.* Pir) compared to manure from antibiotic-free cattle. Manure additions from cattle not administered antibiotics may initially suppress respiration slightly compared to sites receiving no manure, possibly driven by decreased plant demand for nutrients. Additionally, if less recently fixed C is lost from a system via respiration it is likely that more C will be sequestered in that system. This supposition is supported by the significant treatment effect on the proportion of ^{13}C recovered in the mineral pool during the entire experiment (Table S4) with the most ^{13}C recovered in the NMC treatment followed by the Con treatment. Further, at the conclusion of the pulse-chase we observed a marginally significant treatment effect for ^{13}C found in the mineral-associated soil C pool with the most ^{13}C recovered in the Con treatment among the treatments containing manure (Figure 3b; $F_{3,15} = 3.04$, $P = 0.06$). Given the slow turnover of the mineral-associated soil C pool (66), our results suggest that inputs of manure from cattle administered antibiotics may decrease C-

sequestration potential. Direct evidence for this potential is the observation of a significant treatment effect for the ratio of fixed ^{13}C to respired ^{13}C (Figure 3c; $F_{3,15} = 3.65$, $P < 0.05$), an indicator of ecosystem-scale C-use efficiency (37). Specifically, we observed that the Pir treatment had the lowest overall C-use efficiency, Con had the greatest, and both NMC and Ceph were intermediate. Soils receiving the Con treatment fixed 2.5-fold more C for every unit of C respired than did the Pir treatment. Together these results indicate that manure inputs from animals administered antibiotics have the potential to increase C losses from ecosystems compared to manure inputs from animals not administered antibiotics. However, our results also indicate that this effect on C-cycling may be influenced by the specific choice of antibiotics. Further investigation to examine the ecosystem effects of administering an array of antibiotics is merited, especially as agricultural management practices are increasingly seen as opportunities to mediate global climate change (67).

A greater proportion of ^{15}N relative to the total N pool was observed with the Pir treatment compared to the other manure-amended plots across all pools sampled (Figure 4, Table S4), but not necessarily in comparison to NMC. Measuring ^{15}N as a proportion of the total N pool addresses potential difference in plant biomass between treatments (68). Within the aboveground biomass ($F_{3,61} = 8.08$, $P < 0.001$; Figure 4a, Table S4; analyzed as additive model based on quality of model using AIC score) and belowground biomass ($F_{3,55} = 4.53$, $P < 0.01$; Figure 4b, Table S4) significantly more^{15}N was found in the Pir treatment. In addition, a significant and marginally significant time effect was observed in the proportion of ^{15}N in the aboveground and the belowground biomass, respectively ($F_{2,61} = 6.42$, $P < 0.005$,;$F_{2,55} = 2.74$, $P < 0.10$, respectively; Table S4; pooled across treatment). This was characterized by an increased in the proportion of ^{15}N in plant biomass across time. We also observed a significant treatment effect for total N in aboveground plant biomass ($F_{3,61} = 8.48$, $P < 0.001$; Table S2) and a marginally significant treatment effect for total N in belowground plant biomass ($F_{3,55} = 2.55$, $P = 0.06$; Table S2; the former was analyzed as additive model, and the latter as an interactive model based on AIC model score). This effect was likely due to greater biomass in the treatments receiving manure versus NMC (Table S2).

As observed in plant biomass, a greater proportion of ^{15}N in POM (Treatment: $F_{3,61} = 4.77$, $P < 0.005$; Figure 3c, Table S4) and mineral-associated (Treatment: $F_{3,61} = 2.49$, $P < 0.10$; Figure 3d, Table S4) soil N pools was observed in the Pir treatment at the conclusion of the experiment compared to the other treatments. The effect of Pir on ^{15}N in the POM N fraction was likely attributable to the root biomass, due to the contribution of plant derived constituents to this pool (69). Abundance of ^{15}N in the mineral pool was also increased with Pir, possibly due to decreased plant-microbe competition for N due to an increased F:B in the microbial community. Microbial communities with a higher F:B typically have a higher C:N due to reduced N demand of fungi (70). With the Pir system being

more fungally dominated, the overall microbial demand for N is likely lower than for the other three treatments.

We see an increased loss of recently fixed C in the Pir treatment but also increased uptake of recently added N in this treatment compared to the other treatments. For C, this is particularly unexpected given the increased F:B associated with the Pir treatment. However, the notion that increased F:B leads to a less leaky C-cycle has been called into question (43, 71). Rousk and Frey (43) found that bacterial dominance is linked to a less leaky C-cycle and a less leaky N-cycle, while we observed the potential for greater plant uptake of N. This disparity between our results and those of Rousk and Frey may be because our research was conducted in a grassland system and theirs in a forest. Of particular relevance – among the many differences between these systems – the uptake of available N is likely greater in grasses and forbs during peak growth, over a short period of time, compared to N uptake in trees. When only considering the microbial community, the Pir treatment (*i.e.* higher F:B) appears to have a leakier N cycle – but when the plant community is also included, this effect is diminished. This highlights the potential for antibiotics to alter plant-microbe interactions and lead to shifts in ecosystem processes.

Mechanistically, these effects on N dynamics could be due to altered microbe-plant competition for N. In this instance a bacteriostatic antibiotic – pirlimycin – increased F:B leading to a leakier C cycle, but also a decrease in plant-microbe competition for N (evidenced by increased plant N uptake; 52). Another potential mechanism is reduced competition with mycorrhizal fungi for N with the Pir treatment. Given recent evidence suggesting a C cost associated with N uptake (73) for mycorrhizal symbionts, if mycorrhizal N uptake increases with a reduction in bacteria then plant N uptake may increase but more C may be lost from the system. Finally, as this experiment was conducted during peak plant growth and N demand, more N may in fact be lost from the system with decreased plant N demand.

Conclusion:

Antibiotics affect not just the soil microbiome but the entire ecosystem; how the ecosystem is affected depends on the antibiotic's mode of action (*i.e.* bactericidal vs. bacteriostatic). Of the two antibiotics investigated, one in particular – pirlimycin – alters both C and N cycling. This is likely due to changes in microbial composition – as demonstrated by increased F:B, and shifts in bacterial community composition. Increased availability of N appears to occur with decreased C retention in the system – subsequently decreased whole ecosystem C-use efficiency. In contrast, cephapirin increases microbial activity as a stress response – in keeping with previously published research which showed decreased microbial efficiency and increased soil C loss (21). While the majority of attention is paid to livestock antibiotic use from the perspective of the proliferation of antibiotic resistant pathogens and antibiotic resistance genes (74,

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

Acknowledgements:

We thank the University of Georgia Stable Isotope Ecology Laboratory, and the Yale Analytical and Stable Isotope Center for analysis of stable isotopic samples. Thanks as well to the many graduate and undergraduate researchers who helped with the field and lab work associated with this study.

Funding:

USDA-NIFA: Award number: 2013-67019-21363
NSF: Award number: 1832888

Works Cited:

1. O'Neill J (2016) *Tackling drug-resistant infections globally: final report and recommendations. The review on antimicrobial resistance.*
2. Rubin RJ, et al. (1999) The economic impact of Staphylococcus aureus infection in New York City hospitals. *Emerg Infect Dis* 5(1):9–17.
3. Klein E, Smith DL, Laxminarayan R (2007) Hospitalizations and deaths caused by methicillin-resistant Staphylococcus aureus, United States, 1999-2005. *Emerg Infect Dis* 13(12):1840–1846.
4. Van Boeckel TP, et al. (2015) Global trends in antimicrobial use in food animals. *Proc Natl Acad Sci U S A* 112(18):5649–5654.
5. Koch BJ, Hungate BA, Price LB (2017) Food-animal production and the spread of antibiotic resistance: the role of ecology. *Front Ecol Environ* 15(6):309–318.
6. Van Boeckel TP, et al. (2017) Reducing antimicrobial use in food animals. *Science (80-)* 357(6358):1350–1352.
7. Kemper N (2008) Veterinary antibiotics in the aquatic and terrestrial environment. *Ecol Indic* 8(1):1–13.
8. Chee-Sanford JC, et al. (2009) Fate and transport of antibiotic residues and antibiotic resistance genes following land application of manure waste. *J Environ Qual* 38(3):1086.
9. Zhu Y-G, et al. (2013) Diverse and abundant antibiotic resistance genes in Chinese swine farms. *Proc Natl Acad Sci* 110(9):3435–3440.
10. Jechalke S, Heuer H, Siemens J, Amelung W, Smalla K (2014) Fate and effects of veterinary antibiotics in soil. *Trends Microbiol* 22(9):536–545.
11. Ghosh S, LaPara TM (2007) The effects of subtherapeutic antibiotic use in farm animals on the proliferation and persistence of antibiotic resistance among soil bacteria. *ISME J* 1(3):191–203.
12. Forsberg KJ, et al. (2012) The shared antibiotic resistome of soil bacteria and human pathogens. *Science (80-)* 337(6098):1107–1111.
13. Popowska M, et al. (2012) Influence of soil use on prevalence of tetracycline, streptomycin, and erythromycin resistance and associated resistance genes. *Antimicrob Agents Chemother* 56(3):1434–1443.
14. FDA (2013) *2013 summary report on antimicrobials sold or distributed for use in food-producing animals*.
15. Lipsitch M, Singer RS, Levin BR (2002) Antibiotics in agriculture: When is it time to close the barn door? *Proc Natl Acad Sci* 99(9):5752–5754.
16. Toth JD, Feng Y, Dou Z (2011) Veterinary antibiotics at environmentally relevant concentrations inhibit soil iron reduction and nitrification. *Soil Biol Biochem* 43(12):2470–2472.
17. Gutiérrez IR, Watanabe N, Harter T, Glaser B, Radke M (2010) Effect of sulfonamide antibiotics on microbial diversity and activity in a Californian Mollic Haploxeralf. *J Soils Sediments* 10(3):537–544.
18. Sarmah AK, Meyer MT, Boxall ABA (2006) A global perspective on the use, sales, exposure pathways, occurrence, fate and effects of veterinary antibiotics (VAs) in the environment. *Chemosphere* 65(5):725–759.

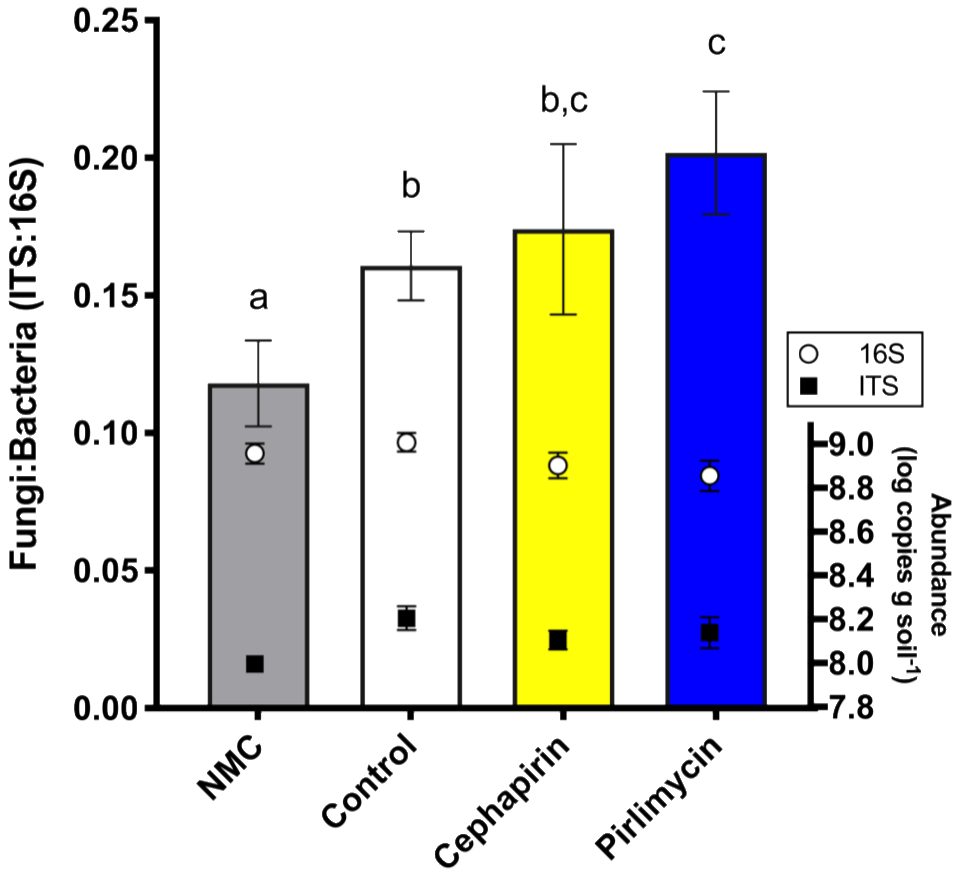
19. Roose-Amsaleg C, Laverman AM (2016) Do antibiotics have environmental side-effects? Impact of synthetic antibiotics on biogeochemical processes. *Environ Sci Pollut Res* 23(5):4000–4012.
20. Grenni P, Ancona V, Barra Caracciolo A (2018) Ecological effects of antibiotics on natural ecosystems: A review. *Microchem J* 136:25–39.
21. Wepking C, et al. (2017) Exposure to dairy manure leads to greater antibiotic resistance and increased mass-specific respiration in soil microbial communities. *Proc R Soc B Biol Sci* 284:1–9.
22. Zak DR, Blackwood CB, Waldrop MP (2006) A molecular dawn for biogeochemistry. *Trends Ecol Evol* 21(6):288–295.
23. Schimel JP, Schaeffer SM (2012) Microbial control over carbon cycling in soil. *Front Microbiol* 3(348):1–11.
24. Philippot L, et al. (2013) Loss in microbial diversity affects nitrogen cycling in soil. *ISME J* 7(8):1609–1619.
25. Graham EB, et al. (2014) Do we need to understand microbial communities to predict ecosystem function? A comparison of statistical models of nitrogen cycling processes. *Soil Biol Biochem* 68:279–282.
26. van der Heijden MGA, Bardgett RD, van Straalen NM (2008) The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. *Ecol Lett* 11(3):296–310.
27. Bonfante P, Anca I-A (2009) Plants, mycorrhizal fungi, and bacteria: a network of interactions. *Annu Rev Microbiol* 63(1):363–383.
28. Raaijmakers JM, Paulitz TC, Steinberg C, Alabouvette C, Moënne-Loccoz Y (2009) The rhizosphere: a playground and battlefield for soilborne pathogens and beneficial microorganisms. *Plant Soil* 321(1–2):341–361.
29. van der Heijden MGA, Wagg C (2013) Soil microbial diversity and agro-ecosystem functioning. *Plant Soil* 363(1–2):1–5.
30. Dessaux Y, Grandclément C, Faure D (2016) Engineering the rhizosphere. *Trends Plant Sci* 21(3):266–278.
31. Raaijmakers JM, Mazzola M (2016) Soil immune responses. *Science (80-)* 352(6292):1392–1393.
32. Waksman S, Woodruff HB (1940) The soil as a source of microorganisms antagonistic to disease-producing bacteria. *J Bacteriol* 40(4):581–600.
33. Martínez JL (2008) Antibiotics and antibiotic resistance genes in natural environments. *Science (80-)* 321(5887):365–367.
34. Fajardo A, Martínez JL (2008) Antibiotics as signals that trigger specific bacterial responses. *Curr Opin Microbiol* 11(2):161–167.
35. Aust MO, et al. (2008) Distribution of sulfamethazine, chlortetracycline and tylosin in manure and soil of Canadian feedlots after subtherapeutic use in cattle. *Environ Pollut* 156(3):1243–1251.
36. Schimel J, Balser TC, Wallenstein M (2007) Microbial stress-response physiology and its implications for ecosystem function. *Ecology* 88(6):1386–1394.
37. Geyer KM, Kyker-Snowman E, Grandy AS, Frey SD (2016) Microbial carbon use efficiency: accounting for population, community, and ecosystem-scale controls over the fate of metabolized organic matter.

Biogeochemistry 127(2–3):173–188.
38. Hammer TJ, et al. (2016) Treating cattle with antibiotics affects greenhouse gas emissions, and microbiota in dung and dung beetles. *Proc R Soc B Biol Sci* 283(1831):20160150.
39. Thiele-Bruhn S, Beck IC (2005) Effects of sulfonamide and tetracycline antibiotics on soil microbial activity and microbial biomass. *Chemosphere* 59(4):457–465.
40. Demoling LA, Bååth E, Greve G, Wouterse M, Schmitt H (2009) Effects of sulfamethoxazole on soil microbial communities after adding substrate. *Soil Biol Biochem* 41(4):840–848.
41. Bardgett RD, McAlister E (1999) The measurement of soil fungal:bacterial biomass ratios as an indicator of ecosystem self-regulation in temperate meadow grasslands. *Biol Fertil Soils* 29(3):282–290.
42. Malik AA, et al. (2016) Soil fungal:bacterial ratios are linked to altered carbon cycling. *Front Microbiol* 7(AUG):1–11.
43. Rousk J, Frey SD (2015) Revisiting the hypothesis that fungal-to-bacterial dominance characterizes turnover of soil organic matter and nutrients. *Ecol Monogr* 85(3):457–472.
44. Wind L, et al. (2018) Fate of pirlimycin and antibiotic-resistant fecal coliforms in field plots amended with dairy manure or compost during vegetable cultivation. *J Environ Qual* 47(3):436–444.
45. Fraterrigo JM, Strickland MS, Keiser AD, Bradford MA (2011) Nitrogen uptake and preference in a forest understory following invasion by an exotic grass. *Oecologia* 167(3):781–791.
46. Lal R (2001) *Assessment methods for soil carbon* (Lewis Publishers, Boca Raton).
47. Fierer N, Schimel JP (2002) Effects of drying–rewetting frequency on soil carbon and nitrogen transformations. *Soil Biol Biochem* 34(6):777–787.
48. Fry B (2006) *Stable Isotope Ecology* (Springer New York, New York, NY) doi:10.1007/0-387-33745-8.
49. Bates D, Maechler Martin, Walker S (2016) Package “lme4.” *CRAN Repos*:113.
50. Akaike H (1973) Information theory and an extension of the maximum likelihood principle. *Second International Symposium on Information Theory* doi:10.1007/978-1-4612-1694-0_15.
51. Fox J, et al. (2016) Package ‘car.’ *CRAN Repos*. doi:10.1177/0049124105277200.
52. R Core Team (2017) R: A language and environment for statistical computing. *R Found Stat Comput Vienna, Austria*:R Foundation for Statistical Computing.
53. Carney KM, Hungate BA, Drake BG, Megonigal JP (2007) Altered soil microbial community at elevated CO2 leads to loss of soil carbon. *Proc Natl Acad Sci* 104(12):4990–4995.
54. Strickland MS, Devore JL, Maerz JC, Bradford MA (2010) Grass invasion of a hardwood forest is associated with declines in belowground carbon pools. *Glob Chang Biol* 16(4):1338–1350.

689 55. Thiele-Bruhn S, Beck I-C (2005) Effects of sulfonamide and tetracycline
690 antibiotics on soil microbial activity and microbial biomass. *Chemosphere*
691 59(4):457–465.
692 56. Hendrix PF, et al. (1986) Detritus food webs in conventional and no-tillage
693 agroecosystems. *Bioscience* 36(6):374–380.
694 57. Rousk J, Bååth E (2007) Fungal and bacterial growth in soil with plant
695 materials of different C/N ratios. *FEMS Microbiol Ecol* 62(3):258–267.
696 58. Berendonk TU, et al. (2015) Tackling antibiotic resistance: the
697 environmental framework. *Nat Rev Microbiol* 13(5):310–317.
698 59. Marti R, et al. (2013) Impact of manure fertilization on the abundance of
699 antibiotic-resistant bacteria and frequency of detection of antibiotic
700 resistance genes in soil and on vegetables at harvest. *Appl Environ*
701 *Microbiol* 79(18):5701–5709.
702 60. Wind L, et al. (2018) Fate of pirlimycin and antibiotic-resistant fecal
703 coliforms in field plots amended with dairy manure or compost during
704 vegetable cultivation. *J Environ Qual* 47(3). doi:10.2134/jeq2017.12.0491.
705 61. Fierer N, et al. (2012) Comparative metagenomic, phylogenetic and
706 physiological analyses of soil microbial communities across nitrogen
707 gradients. *ISME J* 6(5):1007–1017.
708 62. Bergmann GT, et al. (2011) The under-recognized dominance of
709 Verrucomicrobia in soil bacterial communities. *Soil Biol Biochem*
710 43(7):1450–1455.
711 63. McBride MJ, Liu W, Lu X, Zhu Y, Zhang W (2014) The family
712 cytophagaceae. *The Prokaryotes: Other Major Lineages of Bacteria and*
713 *The Archaea* doi:10.1007/978-3-642-38954-2_382.
714 64. Ding C, He J (2010) Effect of antibiotics in the environment on microbial
715 populations. *Appl Microbiol Biotechnol* 87(3):925–941.
716 65. Lobritz MA, et al. (2015) Antibiotic efficacy is linked to bacterial cellular
717 respiration. *Proc Natl Acad Sci* 112(27):8173–8180.
718 66. Schlesinger WH, Lichter J (2001) Limited carbon storage in soil and litter of
719 experimental forest plots under increased atmospheric CO2. *Nature*
720 411:466–469.
721 67. Griscom BW, et al. (2017) Natural climate solutions. *Proc Natl Acad Sci*
722 114(44):11645–11650.
723 68. Fraterrigo JM, Strickland MS, Keiser AD, Bradford MA (2011) Nitrogen
724 uptake and preference in a forest understory following invasion by an
725 exotic grass. *Oecologia*. doi:10.1007/s00442-011-2030-0.
726 69. Grandy AS, Neff JC (2008) Molecular C dynamics downstream: The
727 biochemical decomposition sequence and its impact on soil organic matter
728 structure and function. *Sci Total Environ* 404(2–3):297–307.
729 70. Mouginot C, et al. (2014) Elemental stoichiometry of fungi and bacteria
730 strains from grassland leaf litter. *Soil Biol Biochem* 76:278–285.
731 71. Strickland MS, Rousk J (2010) Considering fungal:bacterial dominance in
732 soils – Methods, controls, and ecosystem implications. *Soil Biol Biochem*
733 42(9):1385–1395.
734 72. Schimel JP, Bennett J (2004) Nitrogen mineralization: Challenges of a

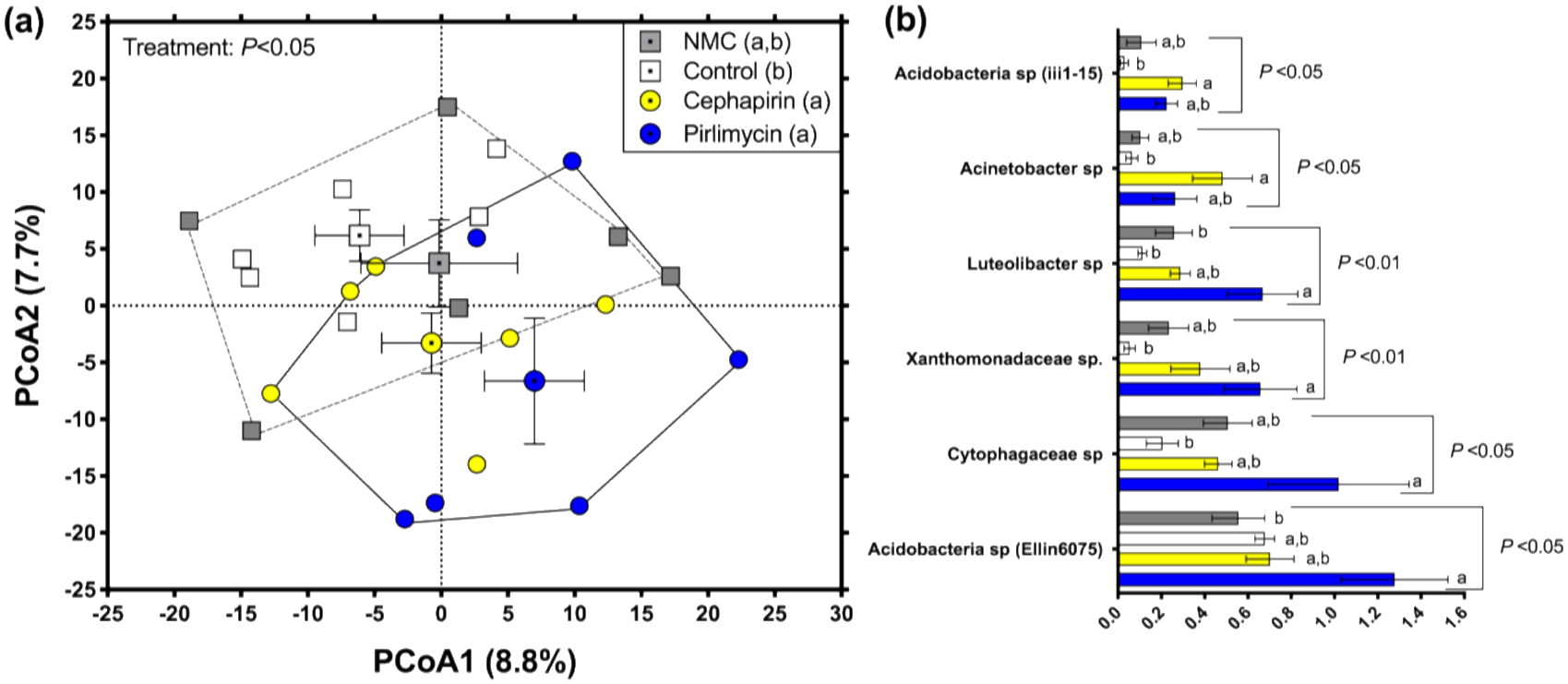
changing paradigm. *Ecology*. doi:10.1890/03-8002.
73. Shi M, Fisher JB, Brzostek ER, Phillips RP (2016) Carbon cost of plant nitrogen acquisition: global carbon cycle impact from an improved plant nitrogen cycle in the Community Land Model. *Glob Chang Biol* 22(3):1299–1314.
74. Udikovic-Kolic N, Wichmann F, Broderick NA, Handelsman J (2014) Bloom of resident antibiotic-resistant bacteria in soil following manure fertilization. *Proc Natl Acad Sci* 111(42):15202–15207.
75. McEachran AD, et al. (2015) Antibiotics, bacteria, and antibiotic resistance genes: Aerial transport from cattle feed yards via particulate matter. *Environ Health Perspect* 123(4):337–343.

748 *Figures:*
749
750 Figure 1.
751



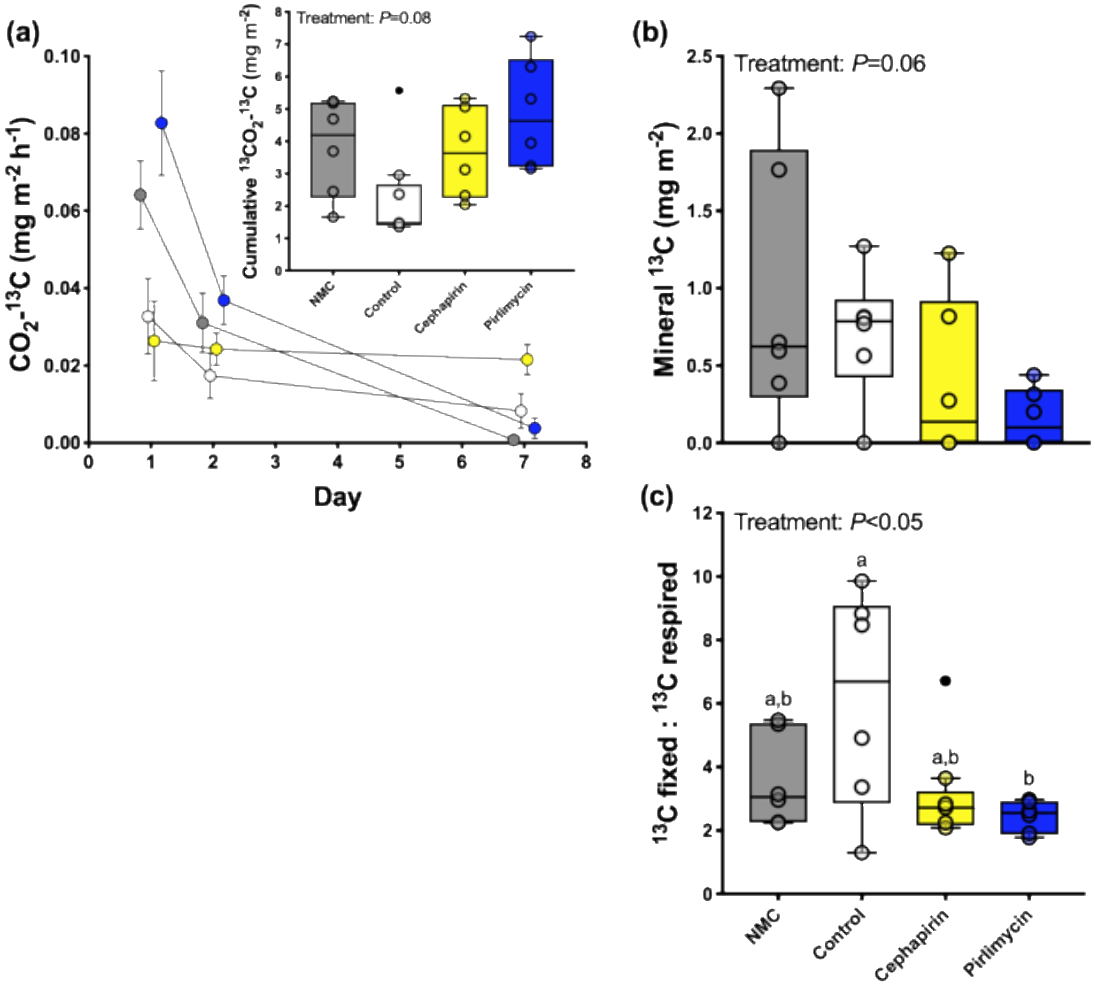
752

753 Figure 2

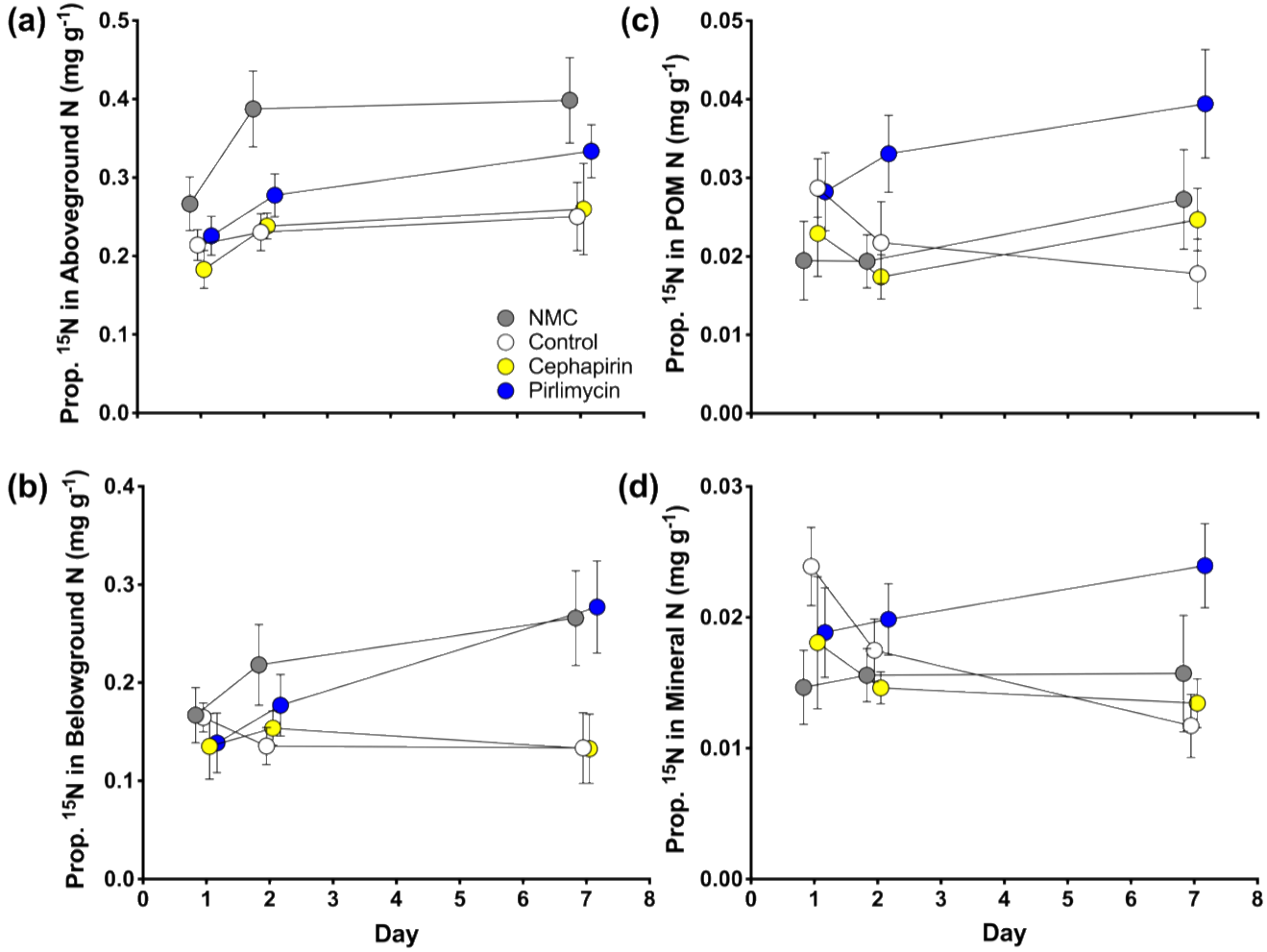


754
755

Figure 3


(a)
CO_2-^{13}C (mg m^{-2} h^{-1})
Day
Cumulative $^{13}CO_2$-^{13}C (mg m^{-2})
Treatment: P=0.08
NMC
Control
Cephapirin
Pirlimycin
(b)
Mineral ^{13}C (mg m^{-2})
Treatment: P=0.06
(c)
^{13}C fixed : ^{13}C respired
Treatment: P<0.05
a
a,b
a,b
b
NMC
Control
Cephapirin
Pirlimycin

760 Figure 4
761



762

Figure Legends:

Figure 1: Fungal-to bacterial ratios (F:B) associated with sites receiving manure from cattle administered no antibiotics (Control), administered cephapirin (bactericidal), or pirlimycin (bacteriostatic). Also shown is the ratio for sites receiving no manure (NMC). Bars represent the mean ± 1 SEM. Letters indicate pairwise differences between treatments. *16S* and ITS copies are indicated by open circles and filled squares, respectively.

Figure 2: Effect of manure treatments on soil prokaryotic community composition. **A**) Principal components analysis showing prokaryote community composition associated with the following treatments: Soil amended with no manure (NMC), soil amended with manure from cattle given no antibiotics (Control), and soil amended with manure from cattle given either a bactericidal antibiotic (Cephapirin) or a bacteriostatic antibiotic (Pirlimycin). Centroids are indicated as symbols with central points and shown as the mean ± 1SE. Significant pairwise differences between centroids are denoted by different letters in the key. Additionally, lines connecting points indicate those treatments receiving antibiotics (solid line) versus those that did not receive antibiotics (dashed line). **B**) Relative abundance of OTUs that both contributed to dissimilarity between treatments (as determined via similarity percentages) and were statistically significant. Overall treatment statistical significance is indicated by *P*-values, and significant pairwise differences for within OTU comparisons are denoted by different letters.

Figure 3: Effect of manure and antibiotic treatments on the cycling of C through the above- and belowground pools across the following treatments: soil amended with no manure (NMC), soil amended with manure from cattle given no antibiotics (Control), and soil amended with manure from cattle given either a bactericidal antibiotic (Cephapirin) or a bacteriostatic antibiotic (Pirlimycin). **a)** Ecosystem respiration dynamics across the 7-day sampling period are shown in the main panel. Points represent the mean ± 1 SE (Treatment: $F_{3,61} = 5.3$, $P < 0.005$; Time: $F_{2,61} = 26.6$, $P < 0.001$). The panel inset shows a boxplot of the cumulative ^{13}C respired across the entire pulse-chase (Treatment: $\chi^2 = 6.86$, df = 3, $P < 0.08$). While the cumulative ^{13}C respired is marginally significant, it represents a doubling of respired CO_2, and is therefore ecologically meaningful. **b)** Total accumulation of ^{13}C in the mineral associated soil fraction by the end of the 7-day pulse chase event (Treatment: $F_{3,15} = 3.04$, $P = 0.06$). **c)** The ratio of ^{13}C fixed to ^{13}C respired, an indicator of whole ecosystem C-use efficiency (Treatment: $F_{3,15} = 3.65$, $P < 0.05$). Letters indicate pairwise differences between treatments.

Figure 4: Effect of manure and antibiotic treatments on the cycling of newly added N through the above- and belowground systems across the following treatments: soil amended with no manure (NMC), soil amended with manure 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.

Supplementary Materials:

Supplemental Methods:

Manure sourcing:

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

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

Manure elemental properties:

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

Quantification of antibiotics in manure:

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

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

Microbial community composition and statistical analysis:

In order to determine the effect that antibiotic exposure has on microbial community composition, bacterial and fungal DNA were analyzed. DNA was extracted using DNeasy PowerSoil Kits (Qiagen, Hilden, Germany). The community composition was determined by amplifying the V4 region of the bacterial/archaeal *16S* rRNA gene as well as the ITS1 region of the fungal ITS spacer region using primer pairs 515FB/806RB, and ITS1f/ITS2 respectively. Caporaso *et al.* (78) was followed for amplification of 16S and ITS regions. Multiplexing and sequencing was carried out using an Illumina MiSeq, producing 250 base pair paired-end reads (78). A UPARSE pipeline was used for quality filtering and for clustering into OTUs – operational taxonomic units (79). Additionally, all chimeric sequences were identified and removed using UCHIME (80). The Ribosomal Database Project Native Bayesian Classifier was used to assign OTUs to 269 specific taxonomies with the OTU cutoff for clustering of 97% (81). This was carried out using the GreenGenes 13.8 reference database for the bacteria and archaea (82) and the UNITE 6.97 database for fungi (83). Rarefication of OTU tables and alpha diversity estimations were carried out using the QIIME pipeline (84).

For ITS, only forward reads were used due to the size variability of the ITS region. We processed ITS read sequences using the DADA2 pipeline (85), which is designed to resolve exact biological sequences from Illumina sequence data and does not involve sequence clustering (86). 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 removed chimeras and then assigned taxonomy using the UNITE dynamic general release (ver 01.12.2017; 83) for fungi. To account for differences in sequencing depths, we rarefied fungal samples to 36195 sequences per sample.

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

Additional soil and microbial parameters:

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

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

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

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

Works Cited

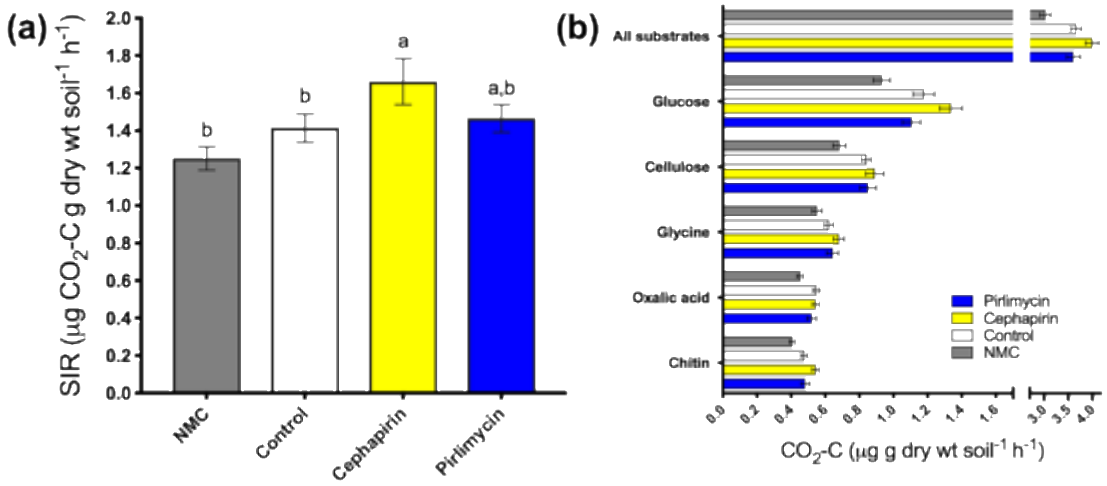
46. Lal R (2001) Assessment methods for soil carbon (Lewis Publishers, Boca Raton).
76. Ray P, Knowlton KF, Shang C, Xia K (2014) Development and validation of a UPLC-MS/MS method to monitor cephapirin excretion in dairy cows following intramammary infusion. PLoS One 9(11):e112343.
77. Ray P, Knowlton KF, Shang C, Xia K (2014) Method development and validation: solid phase extraction-ultra performance liquid chromatography-tandem mass spectrometry quantification of pirlimycin in bovine feces and urine. J AOAC Int 97(6):1730–1736.
78. Caporaso JG, et al. (2012) Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. ISME J 6(8):1621–1624.
79. Edgar RC (2013) UPARSE: highly accurate OTU sequences from microbial amplicon reads. Nat Methods 10:996.
80. Edgar RC, Haas BJ, Clemente JC, Quince C, Knight R (2011) UCHIME improves sensitivity and speed of chimera detection. Bioinformatics 27(16):2194–2200.
81. Wang Q, Garrity GM, Tiedje JM, Cole JR (2007) Naïve Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Appl Environ Microbiol 73(16):5261–5267.
82. DeSantis TZ, et al. (2006) Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. Appl Environ Microbiol 72(7):5069–5072.
83. Nilsson RH, et al. (2019) The UNITE database for molecular identification of fungi: Handling dark taxa and parallel taxonomic classifications. Nucleic Acids Res 47(D1):D259–D264.
84. Caporaso JG, et al. (2010) QIIME allows analysis of high-throughput community sequencing data. Nat Methods 7(5):335–336.
85. Callahan BJ, et al. (2016) DADA2: High-resolution sample inference from Illumina amplicon data. Nat Methods 13(7):581–583.
86. Leff JW, et al. (2018) Predicting the structure of soil communities from plant community taxonomy, phylogeny, and traits. ISME J 12(7):1794–1805.

87. Abarenkov K, et al. (2010) The UNITE database for molecular identification of fungi - recent updates and future perspectives. New Phytol 186(2):281–285.
88. Anderson MJ, Gorley RN, Clarke KR (2008) PERMANOVA+ for PRIMER: Guide to Software and Statistical Methods. Plymouth, UK doi:10.13564/j.cnki.issn.1672-9382.2013.01.010.
89. Clarke KR, Gorley RN (2006) PRIMER v6: User Manual/Tutorial. Prim Plymouth UK. doi:10.1111/j.1442-9993.1993.tb00438.x.
90. West AW, Sparling GP (1986) Modifications to the substrate-induced respiration method to permit measurement of microbial biomass in soils of differing water contents. J Microbiol Methods 5(3–4):177–189.
91. Fierer N, Schimel JP, Holden PA (2003) Variations in microbial community composition through two soil depth profiles. Soil Biol Biochem 35(1):167–176.
92. Degens BP, Harris J a. (1997) Development of a physiological approach to measuring the catabolic diversity of soil microbial communities. Soil Biol Biochem 29(9–10):1309–1320.

Supplementary Figures:

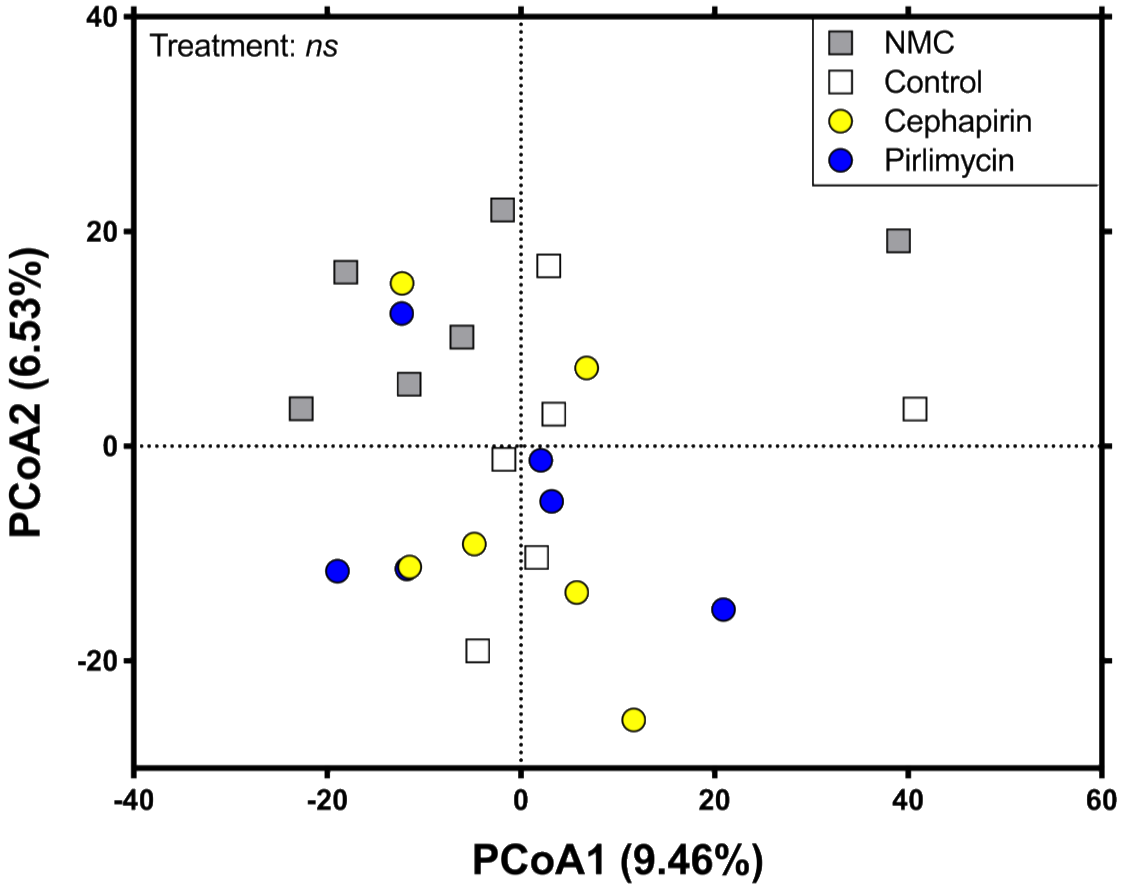
Figure S1.

1016 Figure S2.
1017



1018
1019

1020 Figure S3.



1021
1022
1023

Supplementary Tables:

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

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***	$\chi^{2=}$0.364	***0.044***
Day	*P*	***<0.001***	***<0.001***	0.624	***<0.001***	***0.018***	***0.001***	***<0.001***	***0.028***	$\chi^{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

Table S3:

Treatment	Day	POM – C	POM – N	POM –C:N	Mineral - C	Mineral - N	Mineral – C:N
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**	--	--	--

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*

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

which was analyzed using generalized linear model (GLM) and type II ANOVA – Wald χ^2 Test in order to address failure to pass assumptions necessary for LMM. As a result, these values are reported as χ^2 as opposed to *P*-values. The additive models all included treatment (Treat) and time (Day) as fixed effects and Block as a random effect. Aboveground-N, Belowground-C, Belowground-C:N, and Microbial-N data were log transformed to meet normality assumptions. Significant and marginally significant *P*-values are designated by italic and bold font.

Table S3: C, N, and C:N of particulate organic matter (POM; g-m^{-2}) and mineral associated (Mineral; g-m^{-2}) soil pools. 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:N which was analyzed using generalized linear model (GLM) and type II ANOVA – Wald χ^2 Test in order to address failure to pass assumptions necessary for LMM. As a result, these values are reported as χ^2 as opposed to *P*-values. Models used were either interactive or nested depending on the best model AIC. For factors best analyzed with interactive models all *P*-values are reported – for nested models only treatment *P*-value is reported. For interactive models treatment (Treat) and time (Day) and interactive (T*D) are used as fixed effects and Block as a random effect. For the nested model plot is nested within block, thus accounting for the repeated sampling over time. POM-C:N data were log transformed to meet normality assumptions. Significant and marginally significant *P*-values are designated by italic and bold font.

Table S4: Showing the fate of added isotopic C (^{13}C) and N(^{15}N) of the various pools measured: aboveground biomass (AG) belowground biomass (BG) particulate organic matter (POM), mineral associated (Min), and ecosystem respiration (for C only). Isotopic content is reported as the amount of label recovered as a proportion of the total element pool – as a proportion therefore unitless. 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. The models all included Treatment and Day as fixed effects and Block as a random effect. AG-^{15}N-N^{-1} data were log transformed to pass normality assumptions. Model quality analysis using AIC favored additive models over interactive ones. Significant *P*-values are designated by bold and italic font.

Table S5: Manure characteristics for control (Con), cephapirin (Ceph), and pirlimycin (Pir) treated cattle. Pairwise comparisons are listed according to *P-values* < 0.05. Model standard error is also noted (SE). Statistical tests were not performed for antibiotic concentrations.