

Livestock manure and antibiotics alter extracellular enzyme activity

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ABSTRACT

Soil enzyme activities are commonly used for inferring microbial processes and nutrient limitations. In agroecosystems these enzymes can also be used to determine management effects on soil quality. Here we report the effect of dairy manure inputs and veterinary antibiotics (cephapirin and pirlimycin) on soil enzyme activities, using both a nationwide survey and a controlled field experiment. We found that manure from dairy cows increased α -glucosidase (67.5%), β -D-cellulobiosidase (51.4%), β -xylosidase (48.5%), and total measured enzyme activity (34.0%). Manure reduced mass-specific enzyme activity of 5 of the 6 measured enzymes and relieved microbial phosphorus limitation, as β -glucosidase:acid phosphatase activity was 34% higher in the manure treatment. Veterinary antibiotics administered to livestock decreased the activity of individual soil enzymes, yet only pirlimycin elicited a significant decrease in activity for β -D-cellulobiosidase (48.1%), leucine aminopeptidase (24.1%), β -xylosidase (41.9%), and total measured enzyme activity (18.6%). We found that microbial resource allocation was largely unchanged by antibiotic treatment; however, mass-specific leucine aminopeptidase was marginally higher (21.4%) in the control treatment than in the cephalosporin treatment, potentially linking antibiotics to microbial resource allocation strategies. Our results suggest that administering antibiotics to livestock affects gross ecosystem processes - *i.e.* decomposition rate - through effects on microbial biomass. Furthermore, manure directly impacts microbial resource allocation while antibiotics administered to livestock appears to have a less pronounced impact on microbial resource allocation. Taken together, administration of antibiotics to livestock can affect overall ecosystem process rates but is unlikely to affect microbial resource allocation.

1. Introduction

Soil enzyme activities are commonly used to interpret both ecosystem processes and microbial resource allocation (Allison et al., 2010; Das and Varma, 2010; McBride and Strickland, 2019). For example, increased elemental cycling rates are often attributed to higher soil enzyme activity (Sinsabaugh et al., 2008), or higher microbial effort allocated to decomposition of complex substrates (Allison and Vitousek, 2005). Due to their sensitivity to environmental perturbation, soil enzymes are considered a good indicator of soil quality, *i.e.* high enzyme activity is indicative of high soil quality (Das and Varma, 2010; Stewart et al., 2018). Increased enzyme activity is also thought to free nutrients contained in soil organic matter which can increase plant nitrogen (N) and phosphorous (P) availability (Alkorta

et al., 2003). These effects make enzymes particularly well suited for determining how experimental manipulations and management practices affect soils in agroecosystems.

Livestock management has important ramifications for agroecosystems due to livestock alteration of ecosystem functions (Manier and Hobbs, 2007; Reeder and Schuman, 2002). A substantial amount of manure enters agroecosystems through animals grazing on pasture, or as fertilizer. Manure inputs increase soil quality by increasing plant available nutrients, microbial biomass and soil enzyme activity (Parham et al., 2002; Rochette and Gregorich, 1998). Additionally, manure can induce functional and compositional changes to resident soil microorganisms (Kumar et al., 2017; Lin et al., 2019; Peacock et al., 2001). These microbial changes drive numerous soil processes (Graham et al., 2016; Schimel et al., 2007; Strickland et al., 2009), including greenhouse gas production, and carbon (C) se-

Abbreviations: Con, control manure (no antibiotics administered to the cows); Ceph, manure from cows administered cephalosporin benzathine; Pir, manure from cows administered pirlimycin hydrochloride.

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questration (Owen et al., 2015; Xia et al., 2017). However, much of the manure that enters agroecosystems contains antibiotics - or biologically active antibiotic metabolites - due to administration of antibiotics to livestock (Sarmah et al., 2006).

Antibiotics are commonly used in livestock management as growth promoters and for treatment or prevention of disease (Sneeringer et al., 2017). While it is common to find antibiotics in soils absent any human influence (Waksman, 1961), the amount (Toth et al., 2011), type (Lucas et al., 2019), and diversity (Grenni et al., 2018) of antibiotics entering soils in managed ecosystems is likely markedly different from unmanaged ecosystems (Kemper, 2008). Belowground antibiotics are capable of altering microbial processes in the soil (Roose-Amaleg and Laverman, 2016), e.g. exposure to livestock antibiotics alters microbial community structure and physiology, in turn affecting microbial efficiency (Wepking et al., 2017) as well as the cycling of C and N in terrestrial systems (Wepking et al., 2019). Veterinary antibiotics can also change soil ecosystems through decreasing soil enzyme activity (Chen et al., 2013; Liu et al., 2015, 2009), and reducing microbial biomass (Hammesfahr et al., 2008; Hund-Rinke et al., 2004; Thiele-Bruhn and Beck, 2005), thus mitigating the positive effects of manure on these soil characteristics (Parham et al., 2002; Rochette and Gregorich, 1998).

While manure and antibiotics have seemingly counteracting effects on soil microbial biomass and enzyme activity, these two factors are intertwined in agroecosystems. While, several studies have investigated the effects of manure additions, and direct additions of antibiotics on soil enzymes activities we are not aware of any studies that have investigated the effects of these two factors under simulation of common management practices. In this study, we aimed to determine the effects of livestock manure and livestock administered antibiotics on soil hydrolytic enzyme activity and microbial resource allocation by measuring soil enzyme activity and mass-specific enzyme activity. We conducted two separate studies focused on dairy operations: *i*) a nationwide survey of paired sites receiving manure inputs from cattle-administered antibiotics as well as nearby reference sites; and *ii*) a controlled field experiment using manure from cattle that either received no antibiotics or one of two commonly administered antibiotics (cephapirin, or pirlimycin). We predicted that manure would increase soil enzyme activity, however since manure alleviates C and nutrient limitations (particularly P limitation) this would lead to a reduction in mass-specific enzyme activity. We also predicted that manure from cows treated with antibiotics would reduce enzyme activity through direct effects on microbial biomass and microbial activity by killing soil bacteria (*i.e.* cephalosporin), or halting bacterial metabolism (*i.e.* pirlimycin). We expect that antibiotic treatment will decrease mass-specific enzyme activity for both enzymes, through different mechanisms - *i.e.* cephalosporin kills soil bacteria and releases labile microbially available cellular contents into the soil matrix, reducing the need for soil enzyme production in living bacteria. Alternatively, since pirlimycin halts microbial protein production, fewer extracellular enzymes will be produced by the community as a whole.

2. Materials and methods

2.1. Nationwide survey - Field description

Paired soil samples were collected from manure-impacted and reference sites at 11 dairy operations across the United States between 21 November 2013 and 1 January 2014. These soil samples were from pastures, and areas adjacent to pastures (in the case of reference soils) that had recently held cattle treated with the mastitis-prevention antibiotic cephalosporin benzathine (discussed in greater detail below). Triplicate soil samples were collected to a depth of 5-cm, and compiled into one composite soil sample for each sample type and location. Samples were immediately sent to Virginia Polytechnic Institute and State Uni-

versity, Blacksburg, VA, USA for further processing (sieved with a 4 mm sieve, homogenized, and stored at -80°C for further enzyme analysis). For more information regarding this study see Wepking et al. (2017).

2.2. Controlled experiment - Field description

A common garden field experiment (three treatments, $n = 6$) was constructed in autumn of 2014 with a randomized block design. This experiment was constructed at Kentland Farm, a research farm associated with Virginia Polytechnic Institute and State University (Blacksburg, VA, USA; 37.199490, 80.584659; 547-m elevation; Unison and Braddock cobbly soils; dominant plant cover is grasses, mostly tall fescue, and some herbaceous cover). Soil enzyme activity was tested across three treatments. The control manure treatment (hereon 'Con') came from cows *not* administered an antibiotic. The final two treatments were from cows administered either the antibiotic cephalosporin benzathine (hereon 'Ceph'; Molecular weight = $365.4\text{-g}\cdot\text{mol}^{-1}$; $\text{pKa} = 2.2$; water solubility = $3430\text{-mg}\cdot\text{L}^{-1}$) or pirlimycin hydrochloride (hereon 'Pir'; Molecular weight = $447.4\text{-g}\cdot\text{mol}^{-1}$; $\text{pKa} = 8.4$; water solubility = $64,900\text{-mg}\cdot\text{L}^{-1}$). While these antibiotics are both common mastitis prevention medications, they vary in their mode of action. Ceph is bactericidal, damaging the stability of cell walls and killing susceptible bacteria, while Pir is bacteriostatic, inhibiting the synthesis of proteins.

Manure application occurred monthly from October 2014 through May 2015 (213 days) at a rate of $648\text{-g}\cdot\text{m}^{-2}$ of wet-weight manure. Cumulatively this amounts to $4536\text{-g}\cdot\text{m}^{-2}$ and represents an amount of manure equivalent to what would be expected with a typical stocking density of dairy cattle. Following approximately eight months of manure additions, four soil samples were collected from each plot. These four sub-samples were collected as monoliths consisting of the top 10-cm of soil within a 0.05-m^2 subplot. The monoliths were then divided between plant biomass and bulk soil, sieved using a 4-mm sieve, homogenized and stored at 4°C or -80°C for further analysis. For more information regarding this experiment see Wepking et al. (2019).

2.3. Microbial biomass and soil enzymes

We determined microbial C, N, and P, as well as soil C, N, and P by simultaneous chloroform fumigation extraction (Fierer and Schimel, 2002), in previous studies (Table S1; Wepking et al., 2019, 2017). Extracts were analyzed for dissolved organic C (DOC) and microbial C with an Elementar Variocube TOC/TN (Elementar Americas Inc., Mt. Laurel, NJ, USA), and total dissolved N and P, and microbial N and P were measured using a Lachat QuikChem flow injection analyzer (Hach Company, Loveland, CO, USA). We measured seven hydrolytic extracel-

Table 1

Extracellular enzymes assayed in the two studies. NS = nationwide survey, FE = field experiment.

Enzyme	Abbreviation	Enzyme Function	Study
α -Glucosidase	AG	Cellulose degradation	NS
β -Glucosidase	BG	Disaccharide decomposition	NS, FE
β -Xylosidase	XYL	Hemicellulose degradation	NS, FE
β -D-Cellubiosidase	CBH	Cellulose degradation	NS, FE
Acid phosphatase	AP	Phosphorus mineralization	NS, FE
Leucine aminopeptidase	LAP	Protein depolymerization	FE
N-acetyl- β -glucosaminidase	NAG	Chitin degradation	NS, FE

lular enzymes involved in C, N, and P cycling (Table 1; Osburn et al., 2018; Saiya-Cork et al., 2002). Fresh soil (~0.25 g) was homogenized in pH-adjusted buffer (120 mL). For acidic soils (pH < 6) 50 mM sodium acetate buffer was used, while for circumneutral soils (pH 6–8) a modified universal buffer (Niemi and Vepsäläinen, 2005) was used. While mixing continuously, 200 µl of the soil-buffer solution was added to a 96-well microplate containing fluorescently labeled 7-amino-4-methylcoumarin (AMC), or 4-methylumbelliferone (MUB) – used for LAP activity and all other enzymes, respectively. Each assay was paired with a 10 µM AMC or MUB standard. Both the assay and the standard were performed eight times to account for soil heterogeneity. Fluorescence was quantified with a Tecan infinite M200 microplate reader (Tecan Group Ltd, Mannedorf, Switzerland) at excitation and emission wavelengths of 365-nm and 450-nm, respectively. In order to determine overall ecosystem effects of the treatments we measured potential soil enzyme activity, corrected for dry mass equivalents of soil. We expressed potential soil enzyme activity per unit microbial biomass C in order to identify if the treatments impacted microbial resource allocation (mass-specific enzyme activity). C:N acquiring enzymes were calculated using the ratio of BG:NAG, and C:P acquiring enzymes using the ratio of BG:AP. We interpreted lower BG:NAG, or BG:AP as indicative of relative N and P limitation, respectively (Sinsabaugh et al., 2008).

2.4. Statistical analysis

Data were analyzed in R (R core Development Team, 2017) using linear mixed effects models. If the resulting model did not meet the assumptions of a linear mixed effects model we used generalized linear mixed effects models with a gamma distribution (*lme4* package; Bates et al., 2016). In the nationwide study, treatment was a fixed effect and site was a random effect, while for the common garden experiment, antibiotic treatment was a fixed effect, and block treated as a random effect. (Table S1). Dunnett's test (*emmeans* package) was used for pairwise comparisons in order to determine differences between each antibiotic treatment and the control (Lenth et al., 2019). Missing data points were imputed (*mice* package) using predictive mean matching (van Buuren and Groothuis-Oudshoorn, 2011; supplementary methods).

3. Results

3.1. Nationwide survey

Across the 11 sites, enzyme activity was higher in the soils exposed to cattle manure. Specifically, AG ($\chi_1^2 = 7.05$; $P = .008$), CBH ($\chi_1^2 = 5.80$; $P = .016$), and XYL ($\chi_1^2 = 4.61$; $P = .032$) activity was 207.9, 106.0, and 94.4% higher, respectively, in the manure-exposed sites than in the reference locations (Table 2). There was no significant effect of cattle input on AP ($\chi_1^2 = 0.39$; $P = .53$), BG ($\chi_1^2 = 2.61$; $P = .11$), and NAG ($\chi_1^2 = 1.03$; $P = .31$), however their activities were 17.9, 70.7, and 20.2% greater in the manure-exposed sites than the reference sites, respectively (Table 2). Mass-specific enzyme activity

was higher in the reference sites. The mass-specific activity of AP ($\chi_1^2 = 35.45$; $P < .001$), BG ($\chi_1^2 = 2.75$; $P = .097$), NAG ($\chi_1^2 = 7.52$; $P = .006$), and XYL ($\chi_1^2 = 2.81$; $P = .094$) were 59.8, 36.8, 50.6, and 26.6% higher, respectively. Mass-specific CBH activity ($\chi_1^2 = 2.40$; $P = .12$) was 17.7% higher in the reference sites than the manure exposed sites, and mass-specific AG ($\chi_1^2 = 0.06$; $P = .81$) was 4.5% higher in manure-exposed sites.

The manure-exposed sites had ~51.5% greater total enzyme activity ($\chi_1^2 = 2.85$; $P = .091$) than the reference sites (Fig. 1a), but the reference sites had 45.1% greater mass-specific enzyme activity (Fig. 1b). C:N enzymes (BG:NAG; $\chi_1^2 = 0.34$; $P = .56$), and C:P enzymes (BG:AP; $\chi_1^2 = 4.66$; $P = .031$) were 30.0% and 51.8% lower in the reference sites, respectively.

3.2. Field experiment

Generally, enzyme activity was lower in the antibiotic treated plots than in the control manure plots, with Pir having a greater decrease in activity than Ceph. However, this effect was only significant for LAP ($\chi_2^2 = 8.39$; $P = .015$), CBH ($\chi_2^2 = 5.26$; $P = .072$), and XYL ($\chi_2^2 = 12.71$; $P = .002$), while there was no significant difference for AP ($\chi_2^2 = 1.10$; $P = .58$), NAG ($\chi_2^2 = 0.48$; $P = .79$), and BG ($\chi_2^2 = 1.02$; $P = .60$; Table 3). Additionally, total enzyme activity was significantly different between treatments ($\chi_2^2 = 8.33$; $P = .016$; Fig. 2). Although not all were significant, there was a general trend for reduced enzyme activity for the plots receiving manure from cows administered antibiotics. Ceph had lower enzyme activity than Con for NAG (7.9%; $P = .91$), BG (9.4%; $P = .71$), CBH (35.6%; $P = .23$), LAP (16.4%; $P = .15$), and total activity (7.6%; $P = .44$; Table 3; Fig. 2a). However, AP (7.1%; $P = .92$) and XYL (0.1%; $P = .94$) activity were lower in Con than Ceph (Table 3). Pir had lower enzyme activity than Con for all enzymes measured, NAG (16.6%; $P = .72$), AP (17.3%; $P = .71$), BG (11.7%; $P = .51$), CBH (48.1%; $P = .09$), LAP (24.1%; $P = .03$), XYL (41.9%; $P < .01$), and total enzyme activity (18.6%; $P = .03$; Table 3; Fig. 2a).

Mass-specific enzyme activity was largely unchanged between the two treatments and the control, with no detectable effect on mass-specific AP, BG, CBH, NAG, XYL, and total enzyme activity. However, mass-specific LAP activity was different between treatments ($\chi_2^2 = 6.28$; $P = .043$); it was significantly reduced by Ceph (21.4%; $P = .071$) but not Pir (4.7%; $P = .82$), compared to Con (Table 3; Fig. 2B). Additionally, there were no significant treatment effects for functional stoichiometry (i.e. BG:NAG, BG:AP; Table 3).

4. Discussion

In the nationwide survey, manure exposure generally led to an increase in enzyme activity (Table 2; Fig. 1A). These results are similar to other studies that found manure increased soil enzyme activity in various ecosystems (Liang et al., 2005; Neufeld et al., 2017; Parham et al., 2002; Shi et al., 2018). However, it appears that these results are almost entirely driven by an increase in microbial biomass, since the reference sites have significantly higher mass-spe-

Table 2

Enzyme activities, and mass-specific enzyme activities of the 6 measured enzymes: α -glucosidase (AG), β -glucosidase (BG), β -xylosidase (XYL), β -D-cellulobiosidase (CBH), Acid Phosphatase (AP), Leucine aminopeptidase (LAP), and *N*-acetyl- β -glucosaminidase (NAG) from the nationwide survey. Listed are activities \pm the standard error. * $P < .05$, ** $P < .01$.

Source	AG	BG	XYL	CBH	AP	NAG	BG:NAG	BG:AP
Enzyme activity (nmol g ⁻¹ soil h ⁻¹)								
Manure	329 \pm 120 **	1302 \pm 282	342 \pm 104*	592 \pm 162*	1413 \pm 494	555 \pm 173	–	–
Reference	107 \pm 34	763 \pm 179	176 \pm 52	287 \pm 71	1198 \pm 368	461 \pm 130	–	–
Mass-specific enzyme activity (nmol g ⁻¹ microbial C h ⁻¹)								
Manure	718 \pm 342	3962 \pm 975	850 \pm 314	1450 \pm 403	3584 \pm 1124**	1399 \pm 331	4.5 \pm 2.1	1.8 \pm 0.5*
Reference	407 \pm 101	4934 \pm 1253	741 \pm 159	1330 \pm 264	6905 \pm 2313	2358 \pm 728	3.5 \pm 1.0	1.2 \pm 0.3

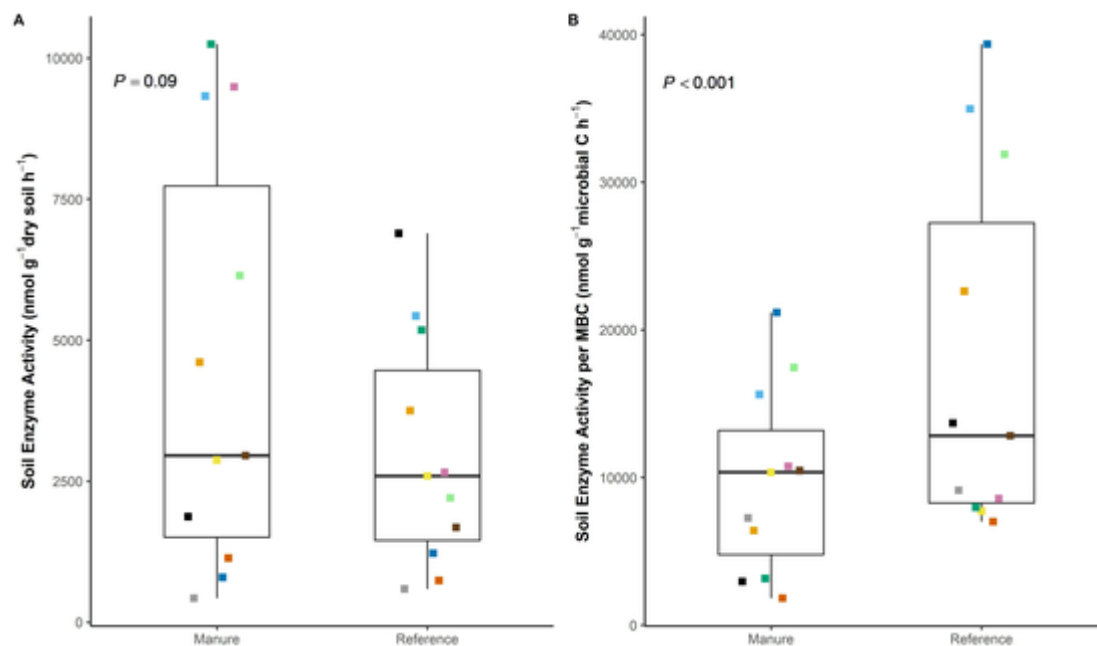


Fig. 1. Total enzyme activity (A) and total enzyme activity expressed per unit microbial biomass C (B) for the Nationwide survey. Colored squares indicate paired sites between the manure and reference.

Table 3

Enzyme activities and mass-specific enzyme activities of the 6 measured enzymes: α -glucosidase (AG), β -glucosidase (BG), β -xylosidase (XYL), β -D-cellubiosidase (CBH), Acid Phosphatase (AP), Leucine aminopeptidase (LAP), and *N*-acetyl- β -glucosaminidase (NAG) from the field experiment. Listed are activities \pm the standard error. Pairwise differences from the control determined using Dunnett's test * $P < .1$, ** $P < .05$, *** $P < .01$.

Source	NAG	AP	BG	CBH	LAP	XYL	BG:NAG	BG:AP
Enzyme Activity ($\text{nmol g}^{-1} \text{ soil h}^{-1}$)								
Con	108 \pm 16	183 \pm 42	255 \pm 31	42 \pm 8	127 \pm 14	13 \pm 2	–	–
Ceph	100 \pm 23	197 \pm 25	231 \pm 17	27 \pm 4	106 \pm 20	13 \pm 3	–	–
Pir	90 \pm 15	151 \pm 27	226 \pm 32	22 \pm 7**	97 \pm 11**	8 \pm 2***	–	–
Biomass corrected Enzyme Activity ($\text{nmol g}^{-1} \text{ microbial C h}^{-1}$)								
Con	7255 \pm 1440	10,978 \pm 1762	17,069 \pm 2836	2669 \pm 280	7963 \pm 565 ^a	861 \pm 207	2.5 \pm 0.3	1.9 \pm 0.4
Ceph	6001 \pm 1161	13,422 \pm 2938	15,463 \pm 2197	1804 \pm 459	6259 \pm 530 ^b	828 \pm 221	3.3 \pm 1	1.3 \pm 0.2
Pir	7232 \pm 1204	12,086 \pm 2589	18,601 \pm 3616	1870 \pm 632	7591 \pm 687 ^a	640 \pm 230	3.1 \pm 0.9	2.8 \pm 1.6

sific enzyme activity (Fig. 1B). Likewise, some of these studies noted concomitant increases of microbial biomass and enzyme activity (Neufeld et al., 2017; Shi et al., 2018). This is not surprising given microbial biomass is a major driver of extracellular enzyme activity (Osburn et al., 2018). These results suggest that manure inputs cause net increases to ecosystem process rates while manure provides readily available nutrients to soil microbes alleviating the resource limitations that the microbes experience in soil without manure additions.

The addition of manure reduced mass-specific activity of the C acquiring enzymes BG, XYL, and CBH by 36.8, 26.6 and 17.7%, respectively. Since cow manure contains an abundance of microbially accessible C it is likely that the soil microbes no longer needed to invest resources into enzymes for C-acquisition (Allison and Vitousek, 2005). Additionally, manure inputs reduced N and P limitation. Mass-specific NAG and AP activity were 50.6% and 59.8% higher in the reference sites, indicating an increased microbial demand for N and P. The apparent P limitation was confirmed with BG:AP activity - a method for determining microbial stoichiometric needs (Sinsabaugh et al., 2009) - which was 51.8% lower in the reference sites. Indeed, the microbial biomass C:P was 132.9% higher in the reference sites (Table S1). It is not surprising that manure alleviates P limitation in these organisms which also allows for increased microbial growth (Elser et al., 2003

), partially explaining why biomass increases with manure addition. The effects of manure inputs have implications for both biogeochemical cycling and microbial resource allocation. Increased available C and nutrients reduce the need for microbes to invest resources in extracellular enzymes while increasing overall biomass. This addition of biomass results in higher total enzyme activity which increases the overall rate of decomposition.

In the field experiment we were able to isolate antibiotic effects from manure effects; we found that manure from cows receiving antibiotics generally decreased enzyme activity below that of manure from cows that were antibiotic free. This antibiotic effect was more pronounced in the Pir treatment (total activity; 18.6%) than the Ceph treatment (total activity; 7.6%). As previously noted, while antibiotics are common to soils (Waksman, 1961), the amount and type are novel in agroecosystems influenced by veterinary practices. Therefore, either their relative novelty or their varying modes of action could be a contributing factor to the differences in enzymatic responses between treatments. Additionally, this reduced enzyme activity could be attributed to a number of factors including microbial community shifts (F:B as well as community composition), and physiological changes in the microbial community (Wepking et al., 2019). Specifically, Wepking et al. (2019) provided evidence that plant-microbe interactions

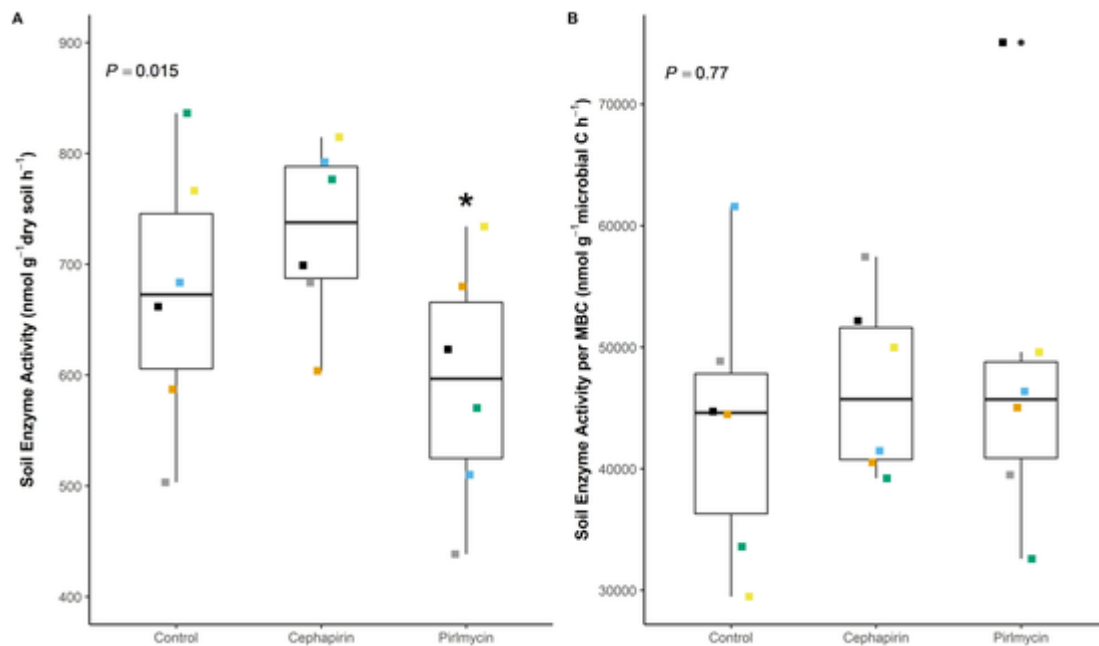


Fig. 2. Total enzyme activity (A) and total enzyme activity expressed per unit microbial biomass C (B) for the field experiment. Colored squared indicate paired blocks between the three manure treatments. *Indicates significant pairwise differences between the control manure and treatment manure using Dunnett's test; $P < .05$.

were affected by antibiotic exposure based on observed changes in terrestrial elemental cycling. This reduction in enzyme activity under antibiotic treatment has been observed previously (Chen et al., 2013; Liu et al., 2015, 2009), and since soil enzymes are the proximate agents of organic matter decomposition, antibiotics could be slowing down this ecosystem process. Indeed, previous studies show that Ceph and Pir decreased C sequestration potential by reducing C use efficiency (Wepking et al., 2019, 2017). Our results suggest that antibiotics have a generally negative effect on microbial enzyme activity, and indicate that gross processing of macromolecules may be reduced by the addition of antibiotics, mitigating the positive manure effect (Fig. 2).

Our controlled field study found that alteration to enzyme activity was driven largely by a decrease in microbial biomass in both the Ceph and Pir treatments. This biomass effect is unsurprising as the negative effects of antibiotics on microbial biomass are well documented (Hammesfahr et al., 2008; Hund-Rinke et al., 2004; Thiele-Bruhn and Beck, 2005), and indicates that antibiotics may affect the rate of decomposition. However, there were no significant differences for mass-specific enzyme activity, for 5 of the 6 assayed enzymes (Table 2); mass-specific LAP activity was marginally reduced in the Ceph treatment compared to Con ($P = .089$). It is unclear why LAP activity would be affected while other enzymes were not. One possibility is that antibiotic effects on membrane permeability affect the release of quorum sensing molecules (Skindersoe et al., 2008), and the inhibition of quorum sensing has previously been shown to reduce mass-specific enzyme activity of AP, NAG, LAP (McBride and Strickland, 2019). Alternatively, antibiotic induced stress has previously been shown to alter microbial resource allocation (Bollenbach et al., 2009). Additionally, there were no significant differences in BG:NAG activity or BG:AP activity, indicating that none of the treatments were more N or P limited than another (Sinsabaugh et al., 2009). These results suggest that although antibiotics reduce total enzyme activity they do not generally affect microbial resource allocation.

5. Conclusion

This study determined the effects of cattle manure and livestock antibiotics on soil enzyme activity. We found that exposure to both ma-

nure and antibiotics affect soil enzyme activity through changes in soil microbial biomass. This has potential implications for management practices, as enzyme activity and microbial biomass are both indicators of soil quality (Stewart et al., 2018). Our results suggest caution should be taken when interpreting enzyme activity in soils. Specifically, when interpreting ecosystem scale processes enzyme activities alone are sufficient, e.g. an increase in β -glucosidase activity is indicative of greater cellulose decomposition. However, in order to interpret changes in microbial resource allocation, mass-specific enzyme activity should be used, e.g. an increase in β -glucosidase activity that does not coincide with an increase in mass-specific β -glucosidase activity would not indicate an increase in microbial C acquisition. Furthermore, manure positively affects indicators of soil health by increasing microbial biomass and enzyme activity, while antibiotics found in livestock manure negatively affect soil health by reducing both of these indicators. Our results further indicate that a composite indicator such as mass-specific enzyme activity could be more useful for interpreting the effects of management on soil health, since these types of indicators give additional information that cannot be obtained from the individual metrics, i.e. microbial resource allocation. This study adds to the growing body of evidence that not all manure is created equal – instead, the antibiotic dosing history of livestock can have consequences on microbially mediated ecosystem function, although microbial resource allocation remains the same.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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