Antibiotic Resistance in Manure-Amended Agricultural Soils

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Summary with Implications

Manure application to agricultural land benefits soil health and agronomic yields. However, as antibiotic resistance becomes a more serious threat to public health, there is concern that antibiotic resistance originating from livestock manure could impact human health through contamination of the environment or food. This study sought to quantify this risk by monitoring concentrations of antibiotic resistance bacteria and genes in fallow soil during the period of October through April, representing fall manure application through spring planting. Resistance to three common antibiotics—tylosin, azithromycin and tetracycline—was monitored following application of fresh, stockpiled, or composted beef feedlot manure, or inorganic fertilizer. Overall, concentrations of all monitored resistant bacteria were below the detection limit for enumeration. Results indicate that while all the manure treatments increased at least one measure of antibiotic resistance during the sampling period, by the final sampling day antibiotic resistance prevalence and concentrations in manured plots were not significantly different from soil receiving no fertilizer treatments.

Procedures

This study was conducted at the University of Nebraska's Rogers Memorial Farm (RMF) east of Lincoln, NE. The RMF is a no-till crop research farm, the soil at this site was an Aksarben silty clay loam had no recent history of manure application; the field had been planted in soybeans

Table 1. Properties of fertilizer amendments

Treatment Type[1]	Fresh Beef Feedlot Manure	Composted Beef Feedlot Manure	Stockpiled Beef Feedlot Manure	Inorganic Fertilizer (15-23-10)	Control
Application Rate	20 ton/ac	20 ton/ac	20 ton/ac	900 lb/ac	N/A
N Rate (lbs/ac)	110	28	28	141	0
P_2O_5 Rate (lbs/ac)	460	600	780	216	0
K_2O Rate (lbs/ac)	600	660	680	94	0
Prevalence AR Bacteria (%)	100	6–12	0–30	0	0
Concentration 16S (log copies g^{-1} d.w.)	nd	8.9	8.7	0	0
Concentration *ermB* (log copies g^{-1} d.w.)	nd	3.6	4.3	0	0
Concentration *tetO* (log copies g^{-1} d.w.)	nd	4.2	4.3	0	0
Concentration *tetQ*[1] (log copies g^{-1} d.w.)	nd	4.8	4.7	0	0

[1] Concentrations of AR genes and bacteria in amendments as reported in preceding studies. PCR was not conducted on fresh manure samples so there is no direct measure of AR genes in the samples, but fresh manure was assessed for presence of AR bacteria. AR *E.coli* and *Enterococci* were found in all of the 50+ samples of fresh manure analyzed prior to land application.

in 2018, as the first year of a four-year rotation of soybeans, corn, winter wheat, and sorghum (milo). Twenty plots (10 ft × 15 ft) were randomly assigned to one of five experimental treatments: fresh beef feedlot manure (20 tons/ac), composted beef manure (20 tons/ac), stockpiled beef manure (20 tons/ac), inorganic fertilizer (N:P:K at 15-23-10 sufficient to apply 140 lb/ac), and a control (no amendment). Fresh manure for the study was sourced from the feedlot at the Eastern Nebraska Research and Extension Center (ENREC) near Mead, NE from animals that had been fed tylosin (90 mg steer[-1] day^{-1}) for disease prevention. The stockpiled manure and composted manure originated at the USDA US Meat Animal Research Center (USMARC) near Clay Center, NE from previous a study monitoring antibiotic resistance levels in manure during manure storage. All of the treatments were broadcast by hand to the surfaces of the study plots according to the mass/area measurements described in Table 1 and left unincorporated.

Soil was sampled from all plots once before and after treatment applications

in October and then monthly through April. Each sample consisted of a composite of four 4-in deep cores obtained at random locations of each plot using a soil probe (2-in diameter), crop residue and treatment applications was brushed away before collecting soil with the soil probe. Soil probes were sterilized between each plot using a 70% ethanol solution. Samples were analyzed for prevalence (proportion of samples containing resistant species) and enumeration (total number of resistant cells or genes within the sample) of both live resistant bacteria [azithromycin (AZ[R])- and tetracycline (TET[R])-resistant *Escherichia coli* and tylosin (TY[R])- and TET[R]-resistant *Enterococci*] and genes that convey resistance [*tetO, tetQ, ermB*].

Results and Discussion

Throughout the study, samples from control plots consistently contained antibiotic resistant (AR) bacteria and AR genes, which is expected since these elements are naturally occurring in the soil environment.

The prevalence of AR bacteria increased immediately following application of fresh and stockpiled manure treatments to the soil but returned to the same prevalence as control plots by the end of the study. Moreover, because all the genes and AR bacteria considered in this study were also observed in soil from control plots, it becomes more challenging to determine the true AR contribution of the treatments. Possibly the increasing changes observed were fluctuations in the native resistant populations responding to environmental conditions and an influx of nutrients in the fertilizers, especially in the carbon-rich manures. Future work should conduct background studies of the native fluctuations of resistance species responding to the crop management and environmental conditions which could provide more insight into the source and nature of the resistance in soil at the site.

The only treatment that significantly impacted AR genes was composted manure, which increased overall *ermB* concentration. However, as with AR bacteria, the AR gene concentration in plots receiving composted manure returned to control levels by the end of the study. Further studies should consider why the plots receiving composted manure had the highest prevalence of *ermB* despite composted manure having the lowest initial concentration of *ermB* genes of any of the manure treatments applied (Table 1). This may be because the cells that managed to survive the composting process had other survival mechanisms, such as endospore formation, that made them more capable of surviving in the harsher soil environment than other native fecal bacteria. Future research should thus incorporate metagenomic analysis to determined which species were responsible for transfer of genes to soil bacteria form manure.

Implications/Conclusions

Soils, whether influenced by human actions or not, contain naturally-occurring antibiotic resistant bacteria and antibiotic resistance genes. Application of carbon-rich manures may initially increase AR indicators in agricultural soils, but the effect lessens over time. Based on the results of this study, a fall application of manure would not significantly increase the risk of transferring AR bacteria or genes to crops planted in the spring.

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