The Effects of Veterinary Antibiotics on Soil Microbial Community Function in Vegetative Filter Strips and Cropland

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Introduction:

•Veterinary antibiotics are used in confined feeding operations (CAFOs) for: the therapeutic treatment of sick animals; illness prevention; enhanced growth rates; and increased feed efficiency[10].

•Thirty to eighty percent of an antibiotic dose can rapidly pass through the G.I. tract of an animal in an unaltered state [5, 6, 8]; antibiotics are introduced into agricultural ecosystems via land application of animal waste.

•The presence of these compounds in the environment may adversely affect soil microbial communities, diminish water quality, and increase the spread of antibiotic resistant bacteria[1, 3, 7, 9].

•Recently, investigators at the UM Center for Agroforestry have been exploring the use of agroforestry and grass filter strips to mitigate the spread of antibiotics in the environment.

•A complimentary, and essential, aspect that requires investigation is the effect of infiltrating antibiotics on soil microbial communities and functions within the rhizosphere environment.

Objectives:

•To measure the effect of veterinary antibiotics in soil from agroforestry and grass filter strips and cropped areas on soil microbial community function.

•To determine changes in microbial community characteristics immediately following antibiotic application as well as recovery time.

Methods:

Study Site

•University of Missouri's Greenley Memorial Research Center, Novelty MO (40°01'N, 92°11'W), Paired Watershed Study Site (Fig. 1)

- •Agroforestry Filter Strip Watershed = 4.44 ha
- •Grass Filter Strip Watershed = 3.16 ha
- •Control = no filter strips, corn-soybean rotation = 1.65 ha

Figure 1: Study location in state of Missouri, watershed map (K. Veum) and areal view of watershed (Google Earth). Grey bands indicate location of grass or agroforestry filter strips; ▪ indicate sampling locations.

•Agroforestry and grass filter strips were established in 1997. Prior to this date, these watersheds were under a corn-soybean rotation with no-till management. Filter-strips for both watersheds are 4.5 m wide and 36.5 m apart.

•June 1997 – all filter strips were planted with redtop (*Agrostis gigantea* Roth), brome grass (*Bromus* spp.) and birdsfoot trefoil (*Lotus corniculatus* L.).

•November 1997 – pin oak (*Quercus palustris* Muenchh), swamp white oak (*Quercus bicolor* Willd.) and bur oak (*Quercus macrocarpa* (Michaux)were planted 3m apart in the center of the Agroforestry filter strips.

Soil Sampling:

•Bulk soil samples (3 per watershed) were collected in October 2009 from all watersheds at the same landscape position (Fig. 1).

•Samples were moist sieved and added to incubation jars (total jars = 566).
•Three treatments were applied:
- i) an untreated control
- ii) oxytetracycline (oxy) at concentrations of 5, 50, and 200 mg kg^{-1} soil
- iii) and lincomycin (lin) at concentrations of 5, 50, and 200 mg kg^{-1} soil

•Samples were incubated for 0, 3, 7, 14, 21, 28, 35, 49 and 63 days.
•At each time step, three sample jars per treatment and land management type were removed from the incubation experiment.

Assessment of Microbial Community Function:

•Biolog ECO microplates:
i) Average Well Color Development (AWCD) = measures species activity and density, and the ability of the microbial community to respond to a particular substrate
ii) Diversity ($H = -\Sigma\ p_i\ (\ln p_i)$, where p_i = the ratio of the activity on a particular substrate to the sum of activities on all substrates)
iii) Richness (total number of positive responses,i.e., OD > 0.10)
iv) Evenness (E = H/log S, where S = richness)

•Dehydrogenase and fluorescein diacetate hydrolysis (FDA) enzyme assays.

Data Analysis:

ANOVA (PROC GLM) to examine the effects of filter strip, antibiotic treatment (type and concentration) and time on AWCD, diversity, richness, evenness, dehydrogenase activity and FDA activity.

Results:

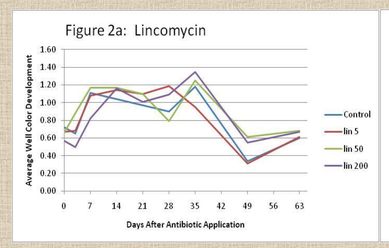
•Significant treatment*time effects were observed for all variables except microbial community evenness. Land*time effects were observed for richness, evenness and dehydrogenase activity; while land*treatment effects were observed for diversity and dehydrogenase activity (Table 1).

•Similar patterns were observed for both antibiotics for AWCD (Fig. 2), richness (Fig. 3), and diversity (Fig. 4). This pattern showed an early decline in response, followed by a rapid recovery with peak levels occurring around 35d. Response dropped sharply by 49d but then recovered to pre-treatment levels by 63d.

•Enzyme assays also showed an early decline and recovery pattern. Dehydrogenase activity shows a another decline at day 35 but activity at day 63 is greater then pre-treatment levels (Fig. 5). FDA activity shows a more dramatic early decline at day 7, followed by a rebound to pre-treatment activities (Fig. 6).

Table 1: ANOVA Results

	AWCD		Richness		Diversity		Evenness		Dehydro		FDA	
	F	p-value	F	p-value	F	p-value	F	p-value	F	p-value	F	p-value
Land	0.72	0.52	1.40	0.32	1.88	0.23	1.87	0.23	**11.44**	**0.009**	**13.22**	**0.006**
Treatment	1.11	0.36	1.10	0.36	**2.25**	**0.04**	0.36	0.90	**9.87**	**<0.0001**	1.45	0.20
Land * Treatment	1.04	0.42	0.89	0.55	**2.29**	**0.008**	0.87	0.58	**3.75**	**<0.0001**	0.82	0.63
Time	**72.18**	**<0.0001**	**67.65**	**<0.0001**	**53.25**	**<0.0001**	**6.55**	**<0.0001**	**111.46**	**<0.0001**	**52.35**	**<0.0001**
Land * Time	1.38	0.15	**2.15**	**0.006**	1.35	0.17	**3.38**	**<0.0001**	**4.89**	**<0.0001**	0.64	0.85
Treatment * Time	**2.46**	**<0.0001**	**2.30**	**<0.0001**	**2.54**	**<0.0001**	0.65	0.97	**1.68**	**0.005**	**1.71**	**0.004**
Land * Trt * Time	0.68	0.99	0.75	0.95	0.98	0.53	0.65	0.99	0.83	0.86	0.51	0.99



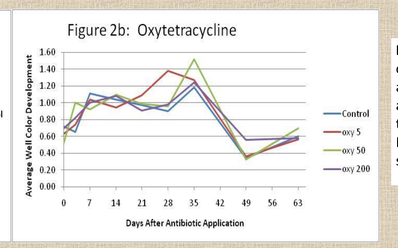
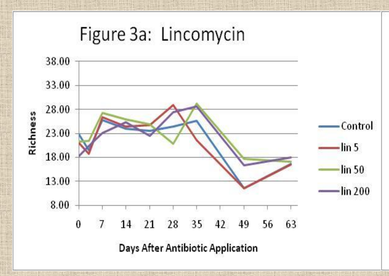


Figure 2: Average well color development for a) lincomycin treatments and b) oxytetracycline treatments over time. Means ± 0.085 are not significantly different.



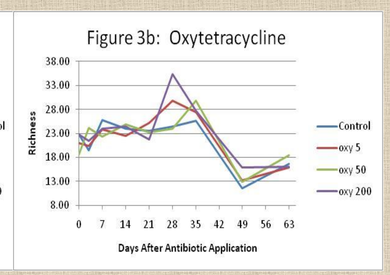
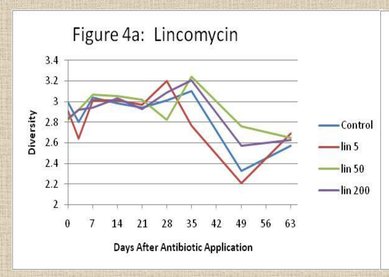


Figure 3: Microbial species richness for a) lincomycin treatments and b) oxytetracycline treatments over time. Means ± 1.38 are not significantly different.



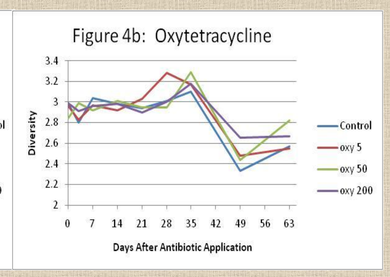


Figure 4: Microbial species diversity for a) lincomycin treatments and b) oxytetracycline treatments over time. Means ± 0.074 are not significantly different.

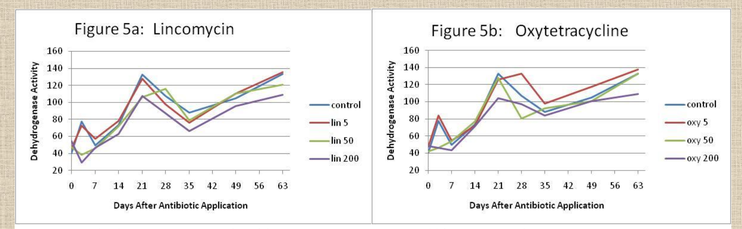


Figure 5: Dehydrogenase activity for a) lincomycin treatments and b) oxytetracycline treatments over time. Means ± 8.59 are not significantly different.

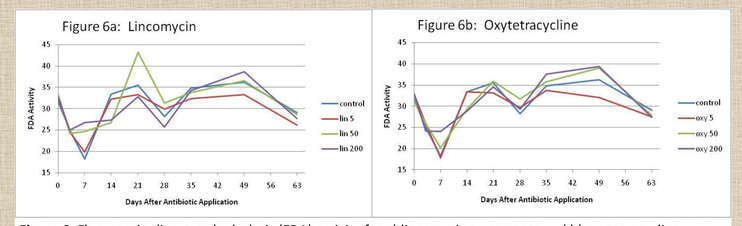


Figure 6: Fluorescein diacetate hydrolysis (FDA) activity for a) lincomycin treatments and b) oxytetracycline treatments over time. Means ± 1.89 are not significantly different.

Discussion:

•Lincomycin and oxytetracycline had an initial inhibitory effect on the soil microbial communities; however, this inhibitory effect was quickly mitigated.

•Microbial community function recovers to pre-treatment levels by 63d.

•Antibiotics entering the soil environment face three primary fates: sorption, leaching or degradation[9].

•Oxytetracycline seems susceptible to strong sorption[2] and rapid degradation[9].
- •Sorption is related to the high clay content of these soils (data not shown).
- •Degradation is related to the relatively simple structure of these compounds.

•Lack of differences between the action of lincoymcin and oxytetracycline in this study suggest that lincomycin also readily sorbs to soil surfaces.

•Once sorption occurs, these antibiotics could become substrates for some members of the soil microbial community[4].

•A lack of concentration effect may be related to the fast rate of sorption and degradation.

•Response curves suggest a density-dependent regulation mechanism.

Conclusions and Future Work:

•The soil microbial communities of these filter strips and crop system seem robust to the effects of lincomycin and oxytetracycline at test concentrations. These results may be due to rapid sorption and microbial degradation.

•Microbial community structure will be assessed by phospholipid fatty acid analysis. Structural community changes may be observed despite lack of functional changes.

•The development of antibiotic resistance in these soil microbial communities is also under investigation.

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