



Influence of Selected Land Application Strategies on the Fate and Transport of Antimicrobials and Antimicrobial Resistance Genes

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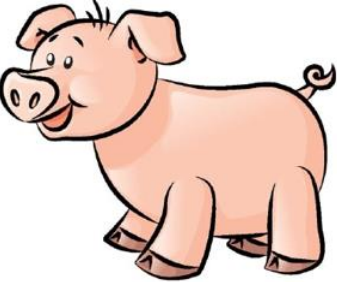


Project Team

- University of Nebraska-Lincoln
 - Dr. Xu Li, Civil Engineering
 - Dr. Shannon Bartelt-Hunt, Civil Engineering
 - Dr. Daniel Snow, Nebraska Water Center
 - Stacey Joy, Bhavneet Soni
- United States Department of Agriculture
 - Dr. John Gilley
 - Dr. Bryan Woodbury
- Funding provided by the National Pork Board



Background



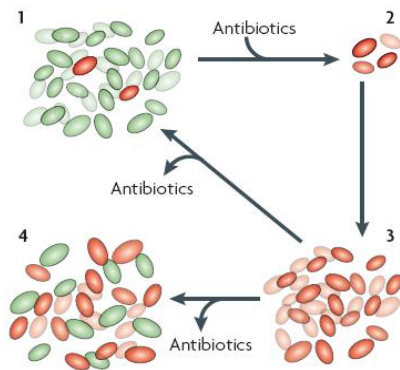
Antimicrobials and Antimicrobial Resistance

❑ Antimicrobials in livestock production

- Therapeutic level: disease treatment
- Sub-therapeutic level: prophylaxes and growth promotion
- A substantial amount not absorbed and released with wastes

❑ Antimicrobial resistance

- Emerging in hospitals
- Emerging in the livestock gut and the environment
- Commensal and pathogenic bacteria
- Antimicrobial resistance infections: \$20 billion in excess health care costs and 8 million additional hospital days



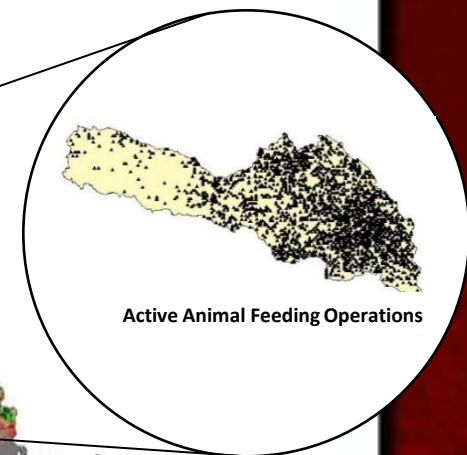
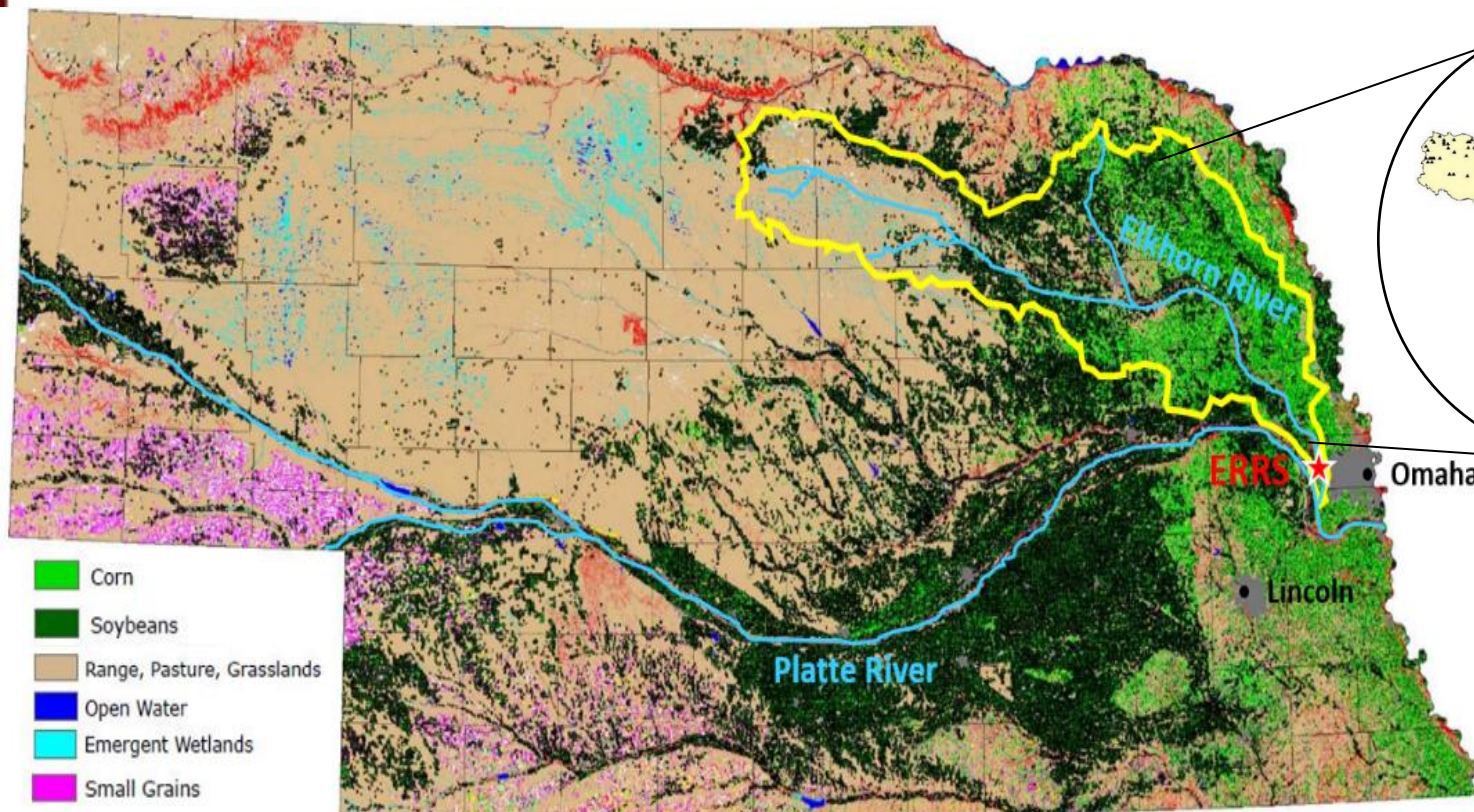
Antimicrobial pressure



Relative abundance of
resistant bacteria



Animal production and crop production in Nebraska



Motivation

- Runoff from fields where manure is land applied can contain antimicrobials and antimicrobial resistance genes (ARG)
- Limited studies investigating the influence of land application strategy and mitigation strategies such as grass buffer strips on antimicrobial and ARG transport
- Limited studies have investigated the fate of both antimicrobial and ARG concurrently



Research Objectives

- (1) Evaluate the fate of antimicrobial and ARG after land application of swine manure as a function of land application method
- (1) Evaluate removal of antimicrobial and ARG in runoff by a narrow grass hedge



AB and ARGs in runoff – study design

- Manure was collected from US Meat Animal Research Center (USDA) from various animal production barns using different antimicrobials: chlortetracycline, tylosin and bacitracin

| Manure slurry | Antimicrobial | | ARG | |
|---------------|---------------|------------|-------------------------------|-------------------------------|
| | (mg/kg ww) | (mg/kg dw) | (copy/mL) | |
| CTC-manure | CTC | | <i>tet(Q)</i> | <i>tet(X)</i> |
| | 3.3 ± 1.6 | 404 ± 138 | (2.5 ± 1.3) × 10 ⁴ | (1.3 ± 0.7) × 10 ³ |
| TYL-manure | TYL | | <i>erm(B)</i> | <i>erm(F)</i> |
| | 0.29 ± 0.12 | 32.5 ± 7.2 | (1.6 ± 1.1) × 10 ⁴ | (1.4 ± 0.5) × 10 ² |
| BAC-manure | BAC | | <i>bcrA, bcrB, bcrC</i> | <i>bceA, bceR</i> |
| | 0.78 ± 0.75 | 320 ± 31.5 | ND [#] | ND |



Land Application Methods

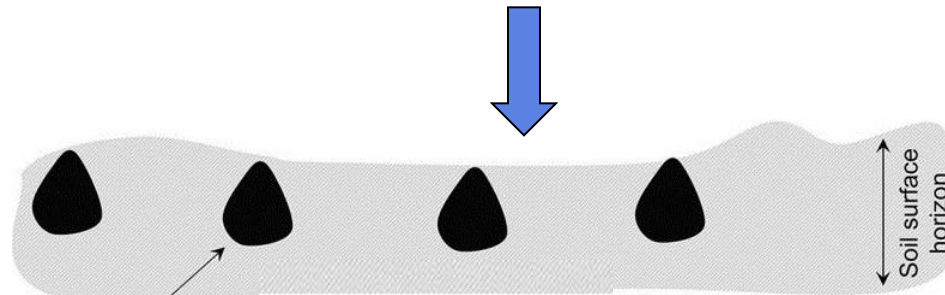
Broadcast



Incorporation



Injection



Manure slurry

Antimicrobial loading through land application of manure is estimated at the kg/hectare level (Winckler & Grafe, 2001)



Experimental Methods

Manure slurry collected at the USDA Meat Animal Research Center (MARC)



Field testing site at the UNL Roger's Memorial Farm



Experimental design allowed for statistical analysis of the data

| | | | | | | | |
|--------------------------|----------------------|----------------------|----------------------|----------------------|----------------------|--------------------------|--------------------------|
| Broadcast BAC | Broadcast CTC | Broadcast CONTROL | Broadcast TYL | | | | |
| Incorporation CONTROL | Incorporation CTC | Incorporation TYL | Incorporation BAC | Injection TYL | Injection BAC | Injection CONTROL | Injection CTC |
| Injection CTC | Injection BAC | Injection TYL | Injection CONTROL | Incorporation TYL | Incorporation CTC | Incorporation CONTROL | Incorporation BAC |
| Broadcast BAC | Broadcast CTC | Broadcast CONTROL | Broadcast TYL | Incorporation BAC | Incorporation TYL | Incorporation CTC | Incorporation CONTROL |
| Broadcast TYL | Broadcast BAC | Broadcast CONTROL | Broadcast CTC | Injection TYL | Injection CONTROL | Injection BAC | Injection CTC |

Evaluated in a randomized block design on fields that had never received manure application

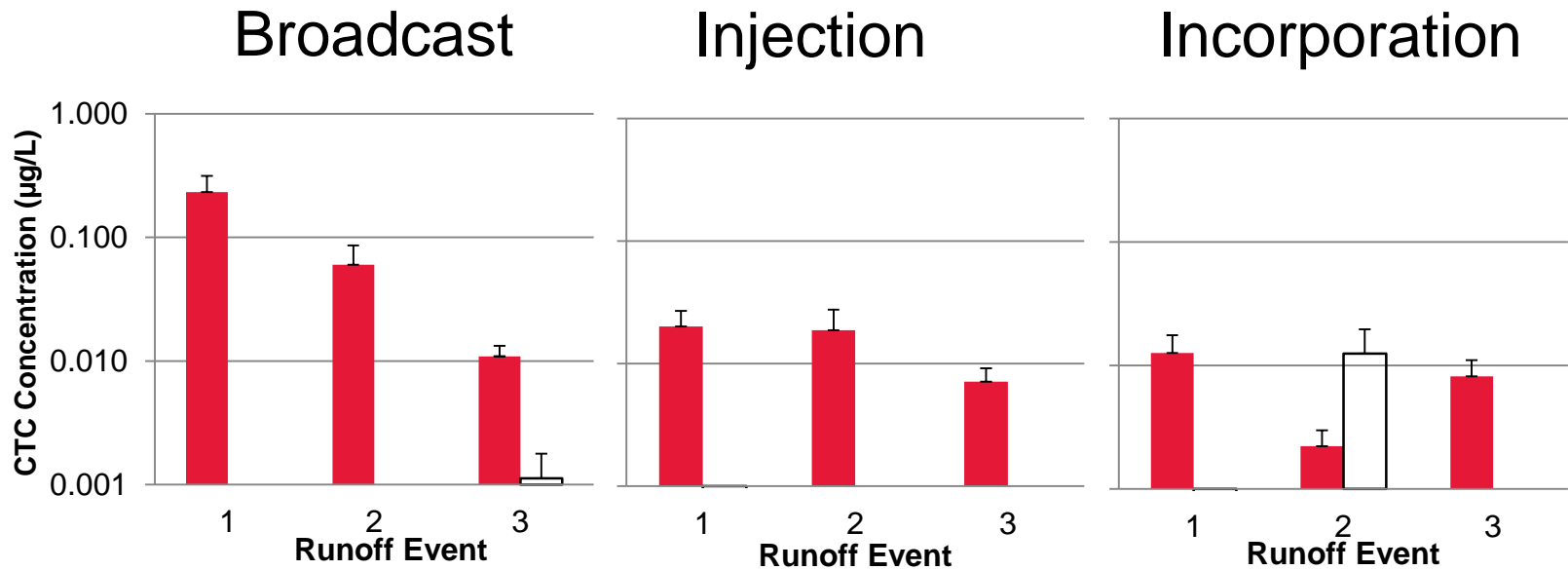


Rainfall Simulation Experiments

- Simulated rainfall intensity 70 mm/hr
 - 3 sequential events (#1, 2, and 3)
- All runoff collected and a composite sample taken
 - Stored at -20°C until analysis
- Soil cores collected before and after rainfall simulation



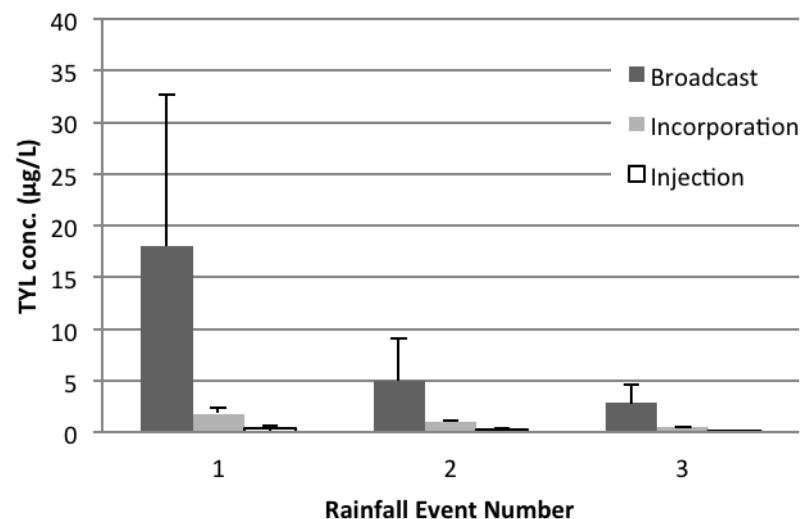
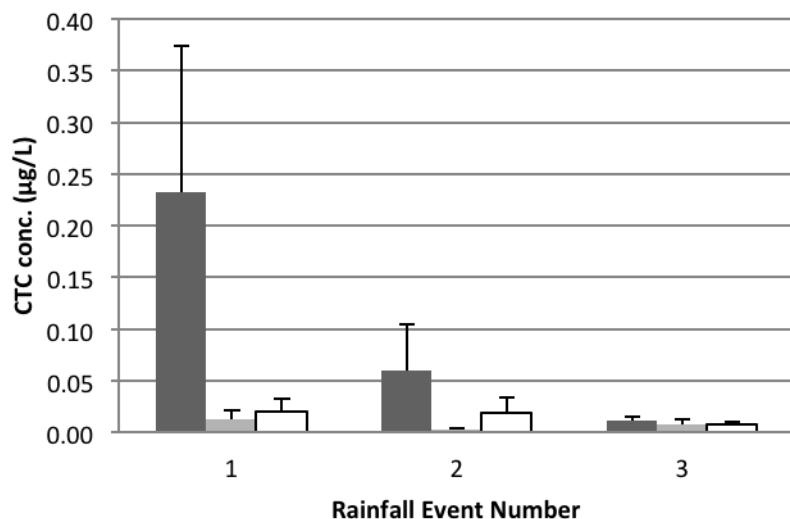
CTC in Runoff



| Run | Mass Loading (average \pm std.error, $\mu\text{g}/\text{m}^2$) | | |
|-------------------|---|-----------------|-----------------|
| | Broadcast | Injection | Incorporation |
| 1 | 4.54 ± 1.65 | 0.30 ± 0.14 | 0.23 ± 0.11 |
| 2 | 1.11 ± 0.50 | 0.27 ± 0.14 | 0.06 ± 0.02 |
| 3 | 0.15 ± 0.03 | 0.09 ± 0.03 | 0.14 ± 0.04 |
| Sum | 5.80 | 0.66 | 0.43 |
| Fraction in Run 1 | 0.78 | 0.45 | 0.55 |



Comparison of antimicrobials in runoff

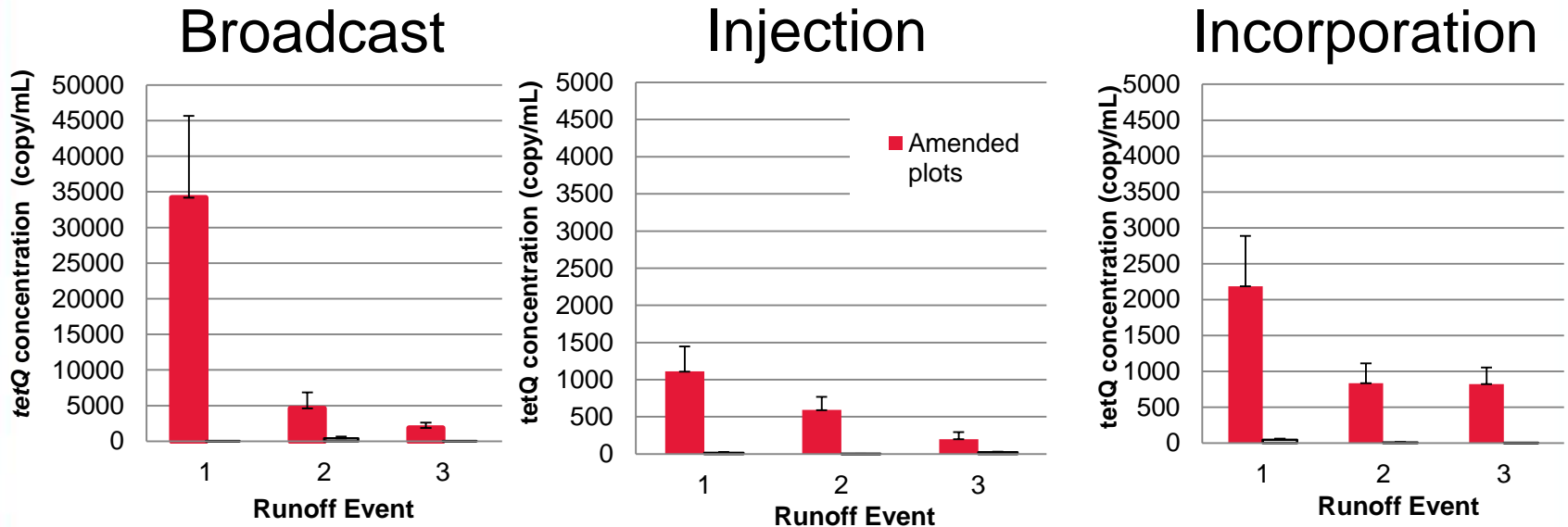


Tylosin mass transport in runoff

| Run | Mass Loading (average \pm std.error, $\mu\text{g}/\text{m}^2$) | | |
|-------------------|---|-----------------|-------------------|
| | Broadcast | Injection | Incorporation |
| 1 | 280.41 \pm 213.66 | 5.23 \pm 2.28 | 33.56 \pm 22.45 |
| 2 | 89.02 \pm 70.28 | 4.59 \pm 1.84 | 11.55 \pm 1.64 |
| 3 | 56.37 \pm 34.12 | 1.73 \pm 1.25 | 4.50 \pm 0.56 |
| Sum | 425.90 | 11.55 | 49.61 |
| Fraction in Run 1 | 0.66 | 0.45 | 0.68 |



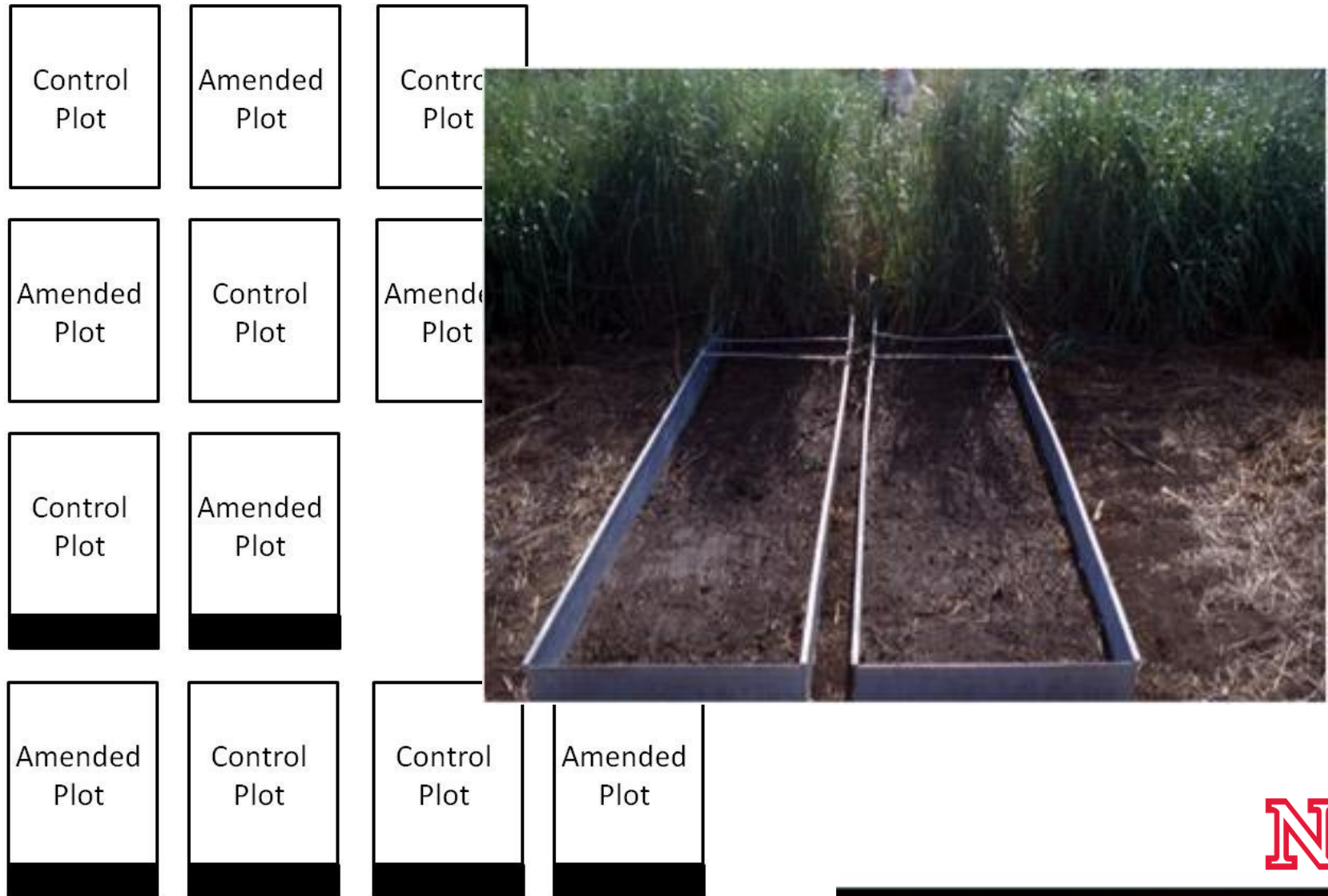
ARGs in Runoff



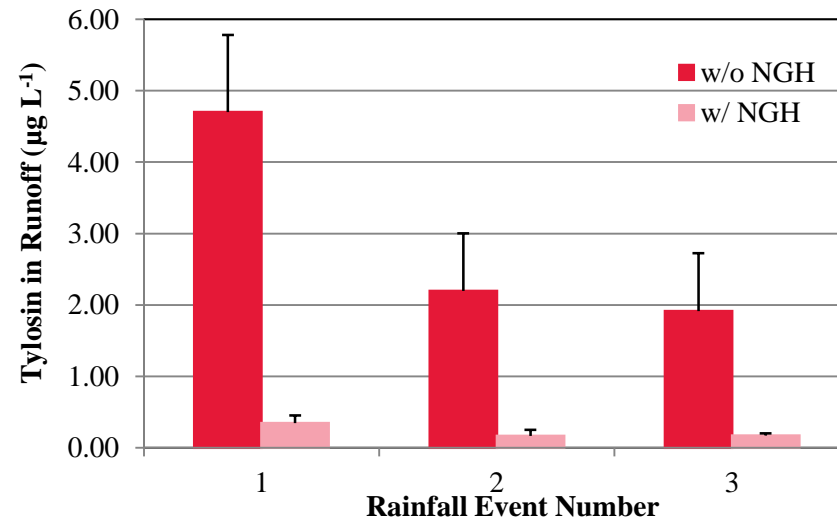
- Highest loads of antimicrobials and ARGs occurred in the runoff during the 1st rain event.
- Broadcast application leads to more antimicrobial and ARGs in runoff than injection and incorporation.



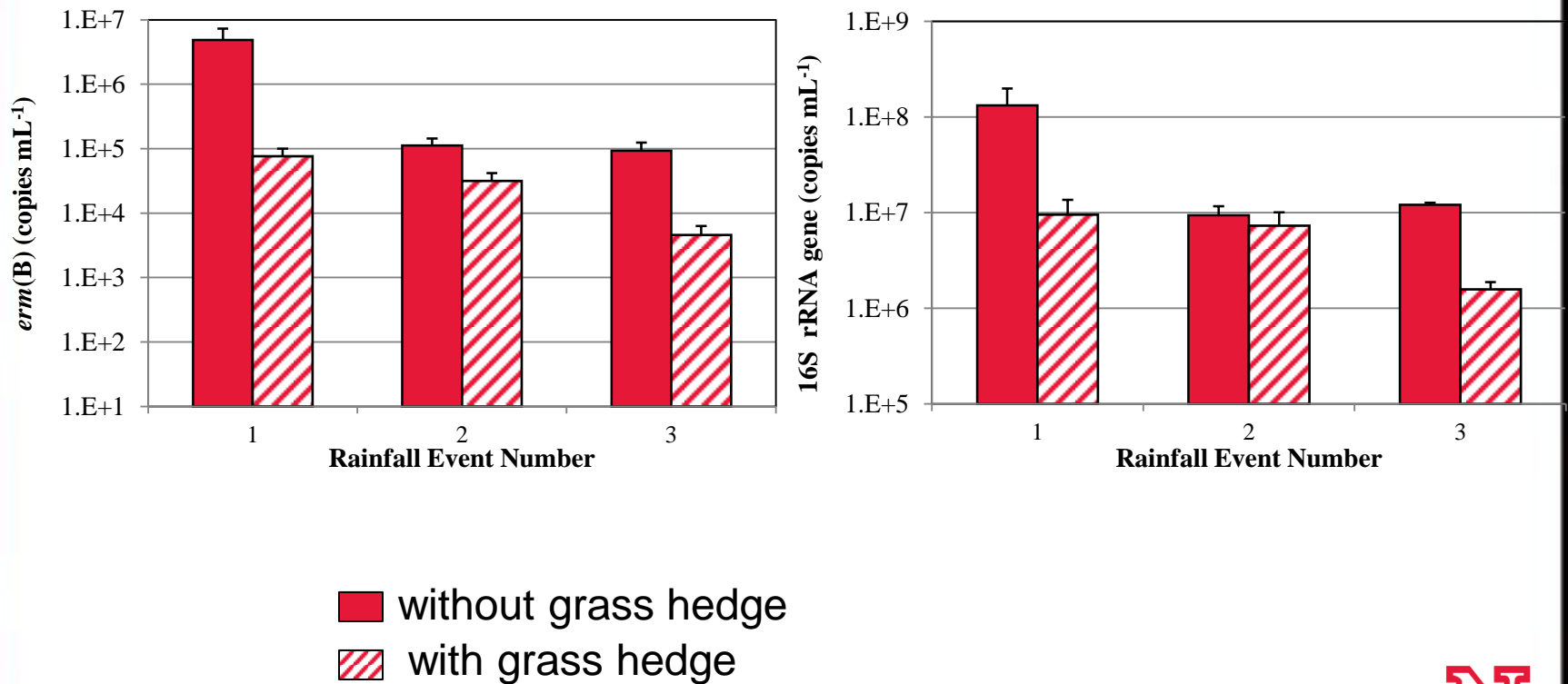
Narrow Grass Hedges – Study Design



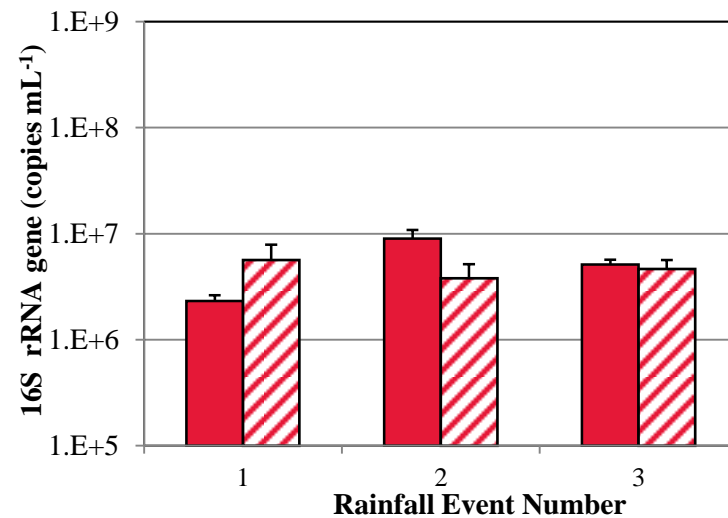
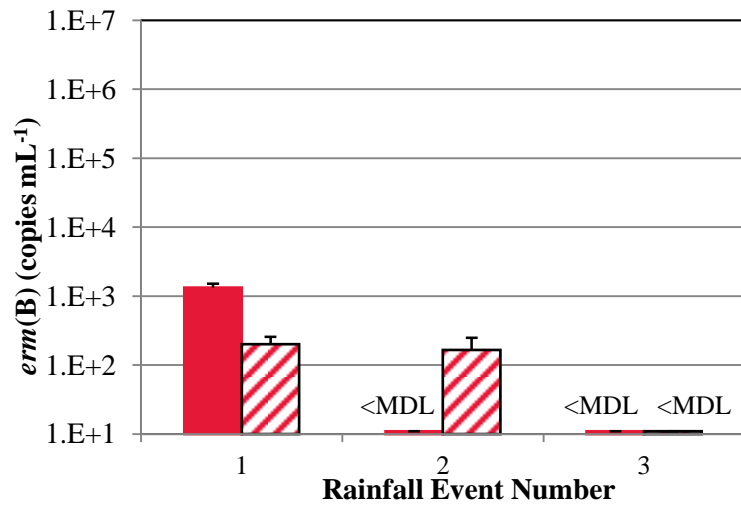
Antibiotic removal by grass hedge



ARG removal by grass hedge



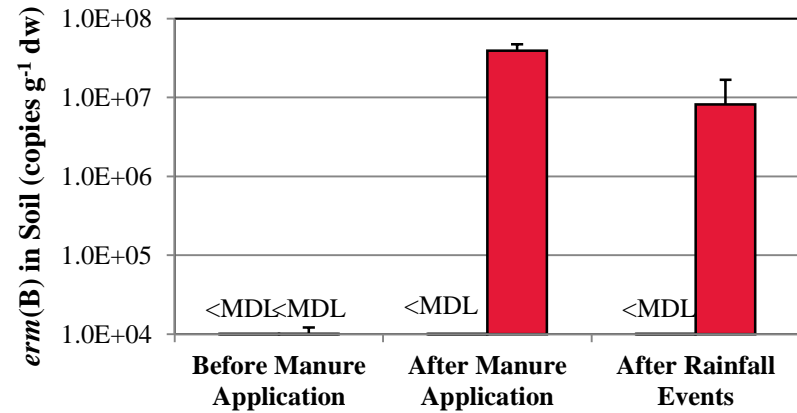
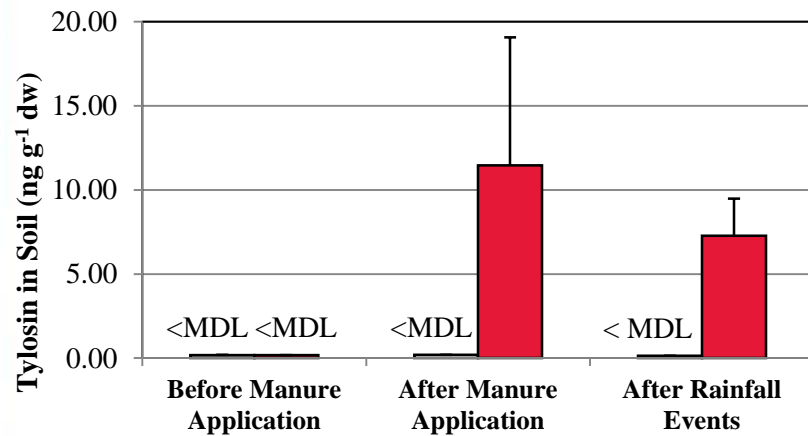
ARG occurrence in check plots



■ without grass hedge
▨ with grass hedge



AB and ARG occurrence in soils



■ Control plot
■ Amended plot



Conclusions

- Narrow grass hedges reduced tylosin in runoff by over an order of magnitude, likely due to enhanced infiltration or sorption
- *erm(B)* and 16S RNA were also removed by grass hedges
 - Consistent with prior work on removal of suspended sediment and fecal coliforms
- Tylosin and *erm(B)* occurrence in soil can be attributable to manure application



Publications Resulting from this Work

Soni, B.; Bartelt-Hunt, S.L.; Snow, D.D.; Gilley, J.; Marx, D.; Woodbury, B.; Li, X. (2015). Effect of Narrow Grass Hedges on the Transport of Antimicrobial and Antimicrobial Resistance Genes in Runoff Following Land Application of Swine Slurry. Journal of Environmental Quality.

Joy, S.R.; Li, X.; Snow, D.D.; Gilley, J.E.; Woodbury, B.; Bartelt-Hunt, S.L. (2014). Fate of Antimicrobials and Antimicrobial Resistance Genes in Simulated Swine Manure Storage. Science of the Total Environment, 481: 69-74.

Gilley, J.E.; Bartelt-Hunt, S.L.; Li, X.; Marx, D.B.; Snow, D.D.; Parker, D.B.; Woodbury, B.L. (2013). Runoff nutrient transport as affected by land application method, swine growth stage and runoff rate, Transactions of ASABE, 56: 1295-1303.

Joy, S.R.; Bartelt-Hunt, S.L.; Snow D.D.; Gilley, J.; Woodbury, B; Parker D.; Marx, D.; Li, X. (2013). Fate and transport of antimicrobials and antimicrobial resistance genes in soil and runoff following land application of swine manure slurry. Environmental Science and Technology, 47(21): 12081-12088.

Gilley, J.E.; Bartelt-Hunt, S.L.; Li, X.; Marx, D.B.; Snow, D.D.; Parker, D.B.; Woodbury, B.L. (2013). Narrow Grass Hedge Effects on Nutrient Transport Following Swine Slurry Application, Transactions of ASABE, 56: 1441-1450.





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