

# **Report as of FY2006 for 2005SD29B: "The Influence of Manure Placement on Crop Yields and the Transport and Fate of Nutrient and Antibiotics"**

## **Publications**

- Conference Proceedings:
  - Hoese, A., S. Gibson, V. Brozel, R. Thaler, and S. Clay. 2006. The influence of antimicrobial chemicals on soil and herbicide degrading bacteria. ASA meetings, November. Indianapolis, IN
  - Reicks, G, C.G. Carlson, and D.E. Clay. 2006. The Impact of Deep Manure Injection on Nutrient Distribution and Corn Yield. SE. Farm field days. September 6, 2006.
  - Reese, C. L., D. E. Clay, D. Beck, J. Kleinjan, C. G. Carlson and S. Clay. Lessons learned from implementing management zones and participatory research in production fields. Eighth International Conference on Precision Agriculture. 23 26 July 2006. Marriott Hotel, Minneapolis, Minnesota.
- Other Publications:
  - Reiman, M. 2006. The impact of deep manure injection on water infiltration, nutrient distribution, and corn yield. M.S. Thesis, SDSU Brookings, SD.
  - Lehnert, K., V. Brözel, S. Gibson, and S. Clay. 2006. Antibacterials in manure influence soil microbial activity. Poster for NC Amer. Microbiology Society Meeting. Brookings, SD. October, 2006.
  - Lehnert, K., S. A. Clay, S. Gibson and V. Brozel. 2007. Detecting shifts in soil microbial community structure and function post landsread of manure or biosolids containing antimicrobial chemicals. ASA meetings, November, New Orleans, LA (submitted)
  - Graduate student seminars. 2005, 2006, and 2007. Ms. Lehnert, Mr. Reiman, and Mr. Hoese presented seminars for faculty and students in Biology and Plant Science Departments as part of their ongoing graduate training.
  - Carlson, C.G. D.E. Clay, and C. Reese. 2007. Deep manure injection. North and South Dakota Watershed Manager Training. Feb 12, 2007. Aberdeen, SD.
  - Clay, D.E., C.G. Carlson, and S.A. Clay. 2007. Precision deep manure applications. SD EPA319 task force planning meeting. March 20, 2007, Pierre SD.
  - Kleinjan, J., C.G. Carlson, and D.E. Clay. 2006. Precision deep manure application (poster) Brookings field day. June 2006. Brookings SD.

## **Report Follows**

## Completion Report for FY06 USGS 104b Project

**Title:** The influence of manure placement on crop yields and the transport and fate of nutrient and antibiotics.

**Principal investigators:** S.A. Clay, SDSU, Brookings, SD  
D.E. Clay, SDSU, Brookings, SD  
C.G. Carlson, SDSU, Brookings, SD

### Introduction

Wind and water soil erosion are processes that transports P, N, microorganisms, and antibiotics contained within manure from agricultural fields to surface waters and drainage networks. Erosion reduces land productivity and contaminants may impair water quality for planned uses. Erosion and runoff are likely to occur whenever there is excess water on a slope that cannot be absorbed or trapped in soil. In addition, the antimicrobial chemicals tylosin (Tyl) and chlortetracycline (CTC) are used as growth promoters in animal production and can be excreted as the parent compound. Landspreading manure can move these chemicals into the soil. To meet water quality goals, techniques that reduce the potential for off-site transport of microorganisms, nutrients, and antibiotics contained within the manure while simultaneously increasing yields and reducing erosion are needed. Previous experience indicated that for SD producers to voluntarily adopt a best management practice (BMP), the practice must generate revenue, fit into their operation, pay for the costs associated with adopting the BMP, make sense in their operation, and the operator must be committed to make the management practice work. Revenue can be generated by two general approaches, direct payment or increased profitability.

This project was integrated into an EPA 319 funded project and a special EPA project so that infiltration and runoff of N, P, and antibiotics could be monitored as well as some of the effects on native soil microorganisms. The South Dakota Precision Farming Consortium works to develop management tools that reduce the adverse impacts of agriculture on the environment, improve water quality, and maintain land productivity. Consortium membership includes: scientists; producers; consultants; and USDA-NRCS, USDA-ARS, and government agency personnel. The consortium has held annual planning and informational meetings where priorities for the upcoming year are identified, review of research occurs, develops training materials such as the "Site Specific Management Guidelines" (available at <http://www.ppi-far.org/ssmg>), sponsors workshops, helps producers conduct on-farm-research, and assists in the development of public education segments that can be aired on Today's Ag. The research conducted in this proposal was the direct outcome of a producer/scientist focus group held in January 2002.

The goals of the overall 2-year projects were to determine the impact of precision manure management/placement on the fate and transport of water, carbon, organisms, and antibiotics contained within manure to non-target areas. The hypothesis of this project is that by integrating soil and other landscape information manure can be

managed such that adverse effects are minimized and profitability is increased. Fields selected for implementation have the following characteristics: (i) evidence of substantial erosion; (ii) glacial till parent materials; and (iii) have been identified as high risk by TMDL and water monitoring studies. This project 1) field tested management approaches that were designed to simultaneously increase productivity and reduce the impact manure on the surface and ground water quality and 2) conducted laboratory studies to observe the impact of manure on soil process including microbial activity. Specific experiments within this overall project investigated 1) the effect of deep manure placement on infiltration, nutrient availability to the crop, and runoff potential of manure and manure contaminants; 2) the effect of antimicrobials alone on isolated soil microbes; 3) the effect antimicrobials on the activity of specific isolated herbicide degraders; and 4) the effect of antimicrobials on 'normal' herbicide degradative soil processes.

### **Research Objectives**

This project was a multi-faceted project that bridged several projects to examine the environmental fate of manure, the impact of manure and its contaminants on soil microbial activity, and test an application technique that would minimize the negative aspects of manure application and maximize its nutrient crop value as a source of N and P and as a soil amendment.

Specific objectives included:

1. To determine the influence of precision manure placement and landscape position on tylosin and chlortetracycline transport and fate.
2. To determine the influence of precision manure placement and landscape position on water budgets and nutrient (C, N, and P) cycling.
3. To determine the influence of manure placement and landscape position on crop yields, runoff, and P transport.
4. Train undergraduate and graduate students in water resources.
5. To share research findings with the general public and the scientific community.

The management practice tested was precision deep manure placement. It was hypothesized that deep manure placement in highly eroded glacial till soils would increase water infiltration, help build a deep soil profile, and reduce surface transport of P, N, microorganisms, and antibiotics from fields to non-target areas.

### **Methodology**

Field research was initiated at two South Dakota sites in 2004. Yields, water infiltration, P, and N transformations were measured for the two years following manure injection. Treatments were deep manure injection (45cm), shallow manure injection (15 cm), and conventional fertilizer-based management. At each site, treatments were replicated three times. Soil samples to a depth of 90 cm were collected and analyzed for Bray P, ammonium-N, and nitrate-N. Apparent saturated hydraulic conductivity and crop grain yields were measured. In the fall of 2004, several replicated deep manure placement studies in 2 producer's fields were initiated. Funding for this project was provided by EPA-319. The goals of the EPA 319 funded project were to field test the impact of deep manure placement on P transport and yields in producer's fields. This project expanded the scope of this project to include the impact of precision deep manure placement on water budgets and the transport and fate of antibiotics, carbon, N, and organisms contained within the manure.

Figure 1. Manure applicator with shanks 15 to 18” deep for application.



Double ring infiltration (Figure 2) was done at 3 sites and sprinkler infiltration (Figure 3) was conducted at two sites and infiltration rates measured. Infiltration rates using the double ring techniques was significantly greater (36 to 139%) in deep or 6” injection treatments vs surface applied or typical farm management areas. One area was tested in early summer and September and the magnitudes of infiltration differences for the treatments were measured. similar at each time indicating a fairly long term affect. Sprinkler infiltration was statistically greater at one site and numerically greater at another with deep manure injection.

In another related study, swine were fed with rations that contained no antibiotic, or tylosin or chlortetracycline (CTC) at labeled rates. Manure and manure from treated swine were placed on the soil surface of a silty clay loam soil. A microsprinkler system was used to water treated areas for about 45 min 1-d after application to determine runoff/run-in of manure and chemicals. Runoff was collected in about 500 ml increments from the area and each runoff increment was analyzed for antimicrobial concentration. The area was allowed to dry for several days and soil samples were taken in about 15 cm increments to a depth of about 45 cm with soil extracted to determine the concentration of antimicrobials in each soil increment.



Figure 2. Double ring infiltrometer to measure infiltration rates after manure application.



Figure 3. Microsprinkler system used to examine infiltration and antibiotic runoff from soil after manure application.

In addition to field studies, laboratory studies were conducted. In one study, we examined the susceptibility of microbes plated from soil (10 strains) and swine manure (5

strains) to tylosin and CTC. In another the soil microbial diversity and community shifts were examined using several techniques including most probable number (MPN) and denaturing gradient gel electrophoresis (DGGE) of the V3 region of the 16S rRNA gene pool at 0, 7, 28, and 42 days after treatment (DAT) of manure with or without antibiotic present and the herbicide, 2,4-dichlorophenoxyacetic acid (2,4-D). The degradation of 2,4-D and atrazine were also examined using pure known degrading cultures of microbes that were used alone or with CTC or tylosin.

### **Principal findings and significance**

Replicated field studies were managed by collaborating farmers. Students (both graduate and undergraduate) assisted and conducted independent field, laboratory, simulation studies, and were trained in sampling techniques and laboratory procedures. Students obtained hands-on experience in water quality research and gained an understanding in land and resource stewardship when addressing environmental issues. Fact sheets, workshops, seminars, posters, and scientific papers are continuing to be prepared by undergraduate and graduate students with the assistance of the project coordinator and faculty members.

Manure application rates based on N requirements can result in P build up in soil (Figure 4). With increasing N fertilizer costs, the ammonia in the manure is more important than ever. Adopting techniques designed to reduce ammonia volatilization losses may reduce the total amount of manure required to meet the plants N requirement. Deep manure injected increased apparent saturated hydraulic conductivity. However, this increase was short lived, and after two years, apparent hydraulic saturated hydraulic conductivity in deep manure and conventionally managed treatments were almost identical. For the two years after manure injection, deep manure treatments contained more inorganic N than shallow manure treatments (Figures 5 and 6). These results were attributed to deep injection reducing organic N mineralization rates, nitrification rates, and N losses. Deep manure injection increased corn (*Zea mays*) yields if N limited growth. Results from this study suggest that deep placement of manure should be considered as a technique to reduce N losses from manure, which in turn can reduce N fertilizer requirements and amount of manure required to meet the crops N requirement.

When differences occurred among treatments, corn and soybean yield were greater in areas when manure was deep injected into the soil, compared to surface application of manure and conventional management with inorganic fertilizers (Table 1). This response may have been due to higher N levels retained in soil or greater water infiltration rates.

Figure 4. The influence of manure placement at Flandreau on the distribution of Bray-P between the corn rows 7 and 12 months after application. (Reiman, 2006)

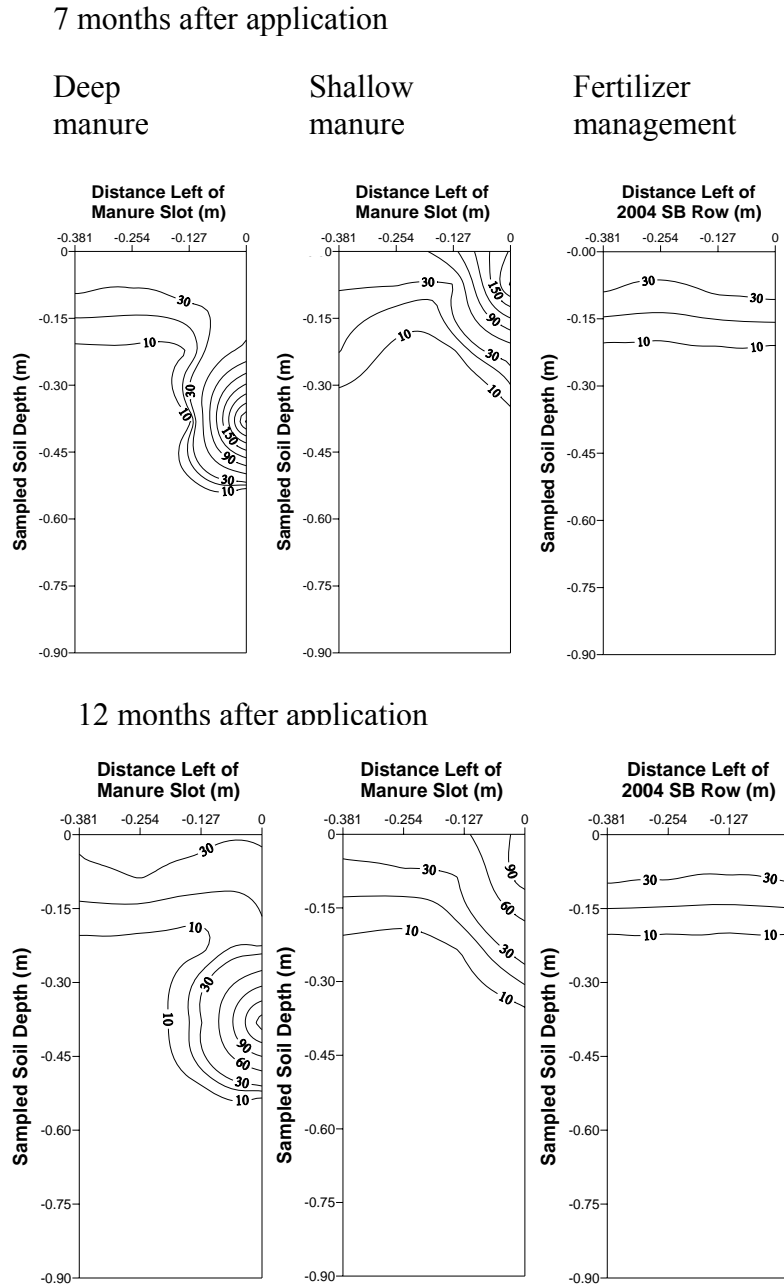
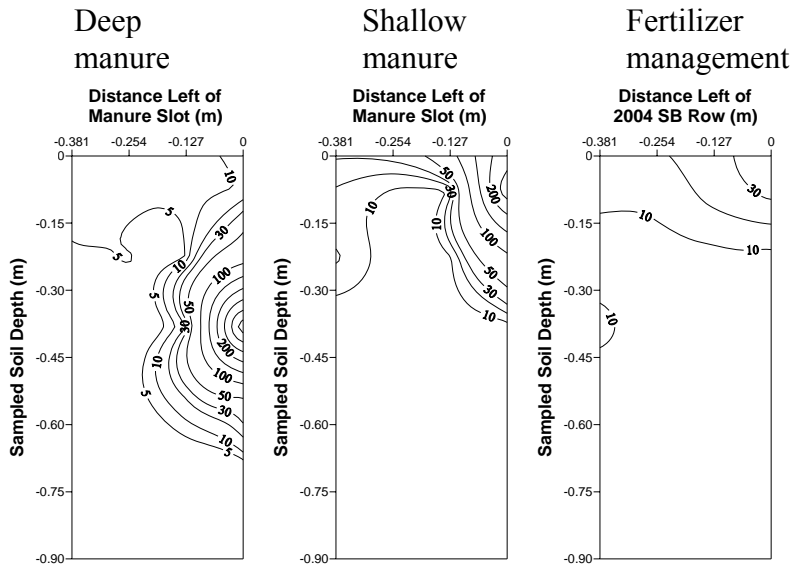


Figure 5. The influence of manure placement at Flandreau on the ammonia concentration in the zone between the corn rows 7 and 12 months after application. (Reiman,2006)

7 months after application



12 months after application

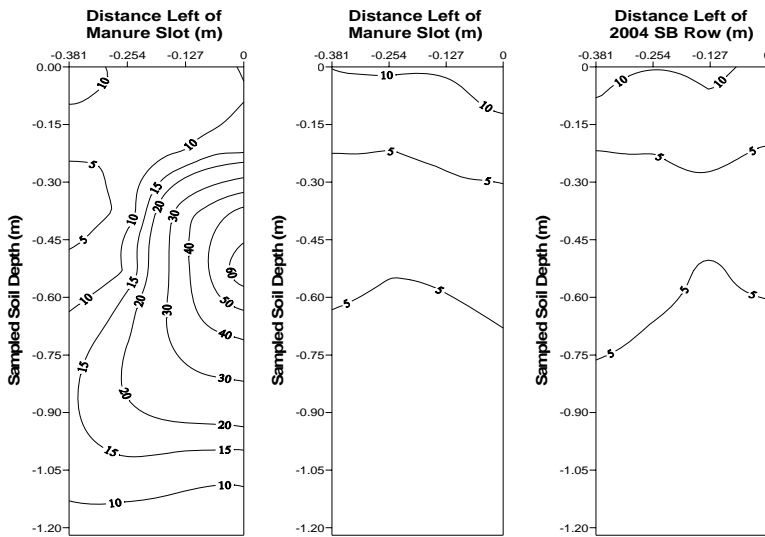
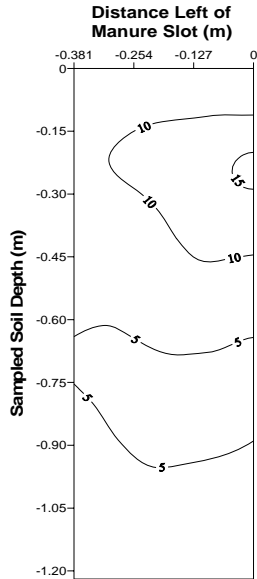




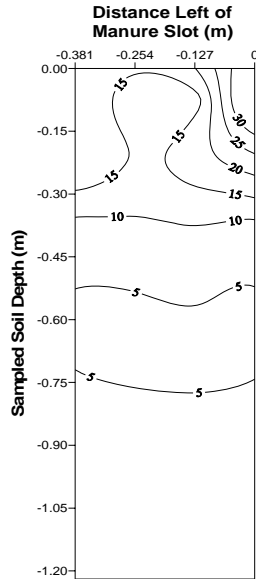
Figure 6. The influence of manure placement at Flandreau on nitrate-N (ppm) concentration in the area between the corn rows 7 and 12 months after application. (Reiman, 2006)

7 months after application

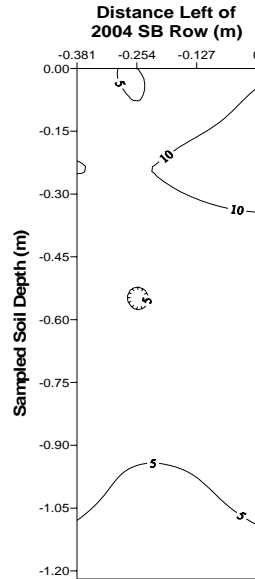
Deep manure



Shallow manure



Fertilizer management



12 months after application

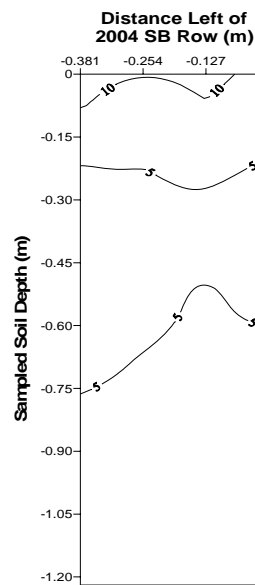
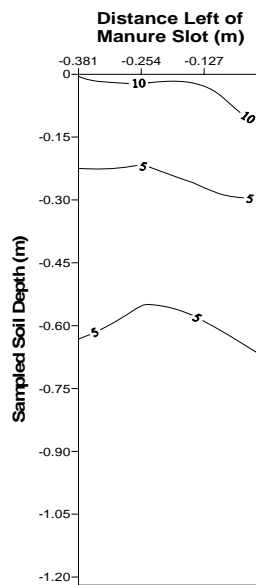
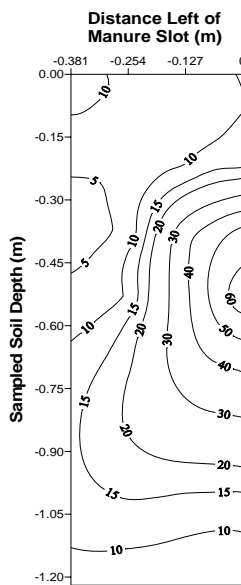


Table 1. Influence of manure on Flandreau and Volga corn and soybean yields in 2005 and 2006. (Reiman, 2006)

Treatment	Flandreau		Volga	
	Corn 2005	Corn 2006	Corn 2005	Soybean 2006
			kg/ha	
DI	13,500	12,000	9,200	4,090
SA			8,890	4,040
SI	13,100	11,500	9,140	3,960
CM	12,700	11,300	9,010	3,760
P	0.078	0.022	0.09	0.12
LSD <sub>(0.10)</sub>	530	440	188	

<sup>†</sup>DI = 38 cm manure injection; SA = surface manure application; CM = conventional management with inorganic fertilizers, SI = 15 cm manure injection.

In areas treated with surface manure applications, runoff was much greater than in untreated areas due to sealing of the soil surface. The amount of CTC in manure was 118 mg per treatment whereas the amount of tylosin in the same amount of manure addition was 0.11 mg. CTC was present in runoff water and ranged from 0.9 to 3.5% of the applied amount and the amount was 1.8 to 4.2 mg (Table 2 and Figure 7). The total amount of tylosin in runoff was much lower ranging from 18 to 27  $\mu$ g but the percentage of tylosin was much greater ranging from 9 to 12% of the amount applied (Table 3 and Figure 8).

Table 2. Runoff amounts and chlortetracycline (CTC) content in runoff after manure application in 2005. The amount of manure applied to the microinfiltration sprinkler area contained a total of 118 mg chlortetracycline. (authors unpublished data)

Location	Water Applied	Water Recovered	CTC runoff	
	(L)	(%)	(mg)	(%)
Brkg1	4.26	95.0	1.80	1.55
Brkg16	5.45	89.7	4.20	3.54
Aurora	5.54	93.9	1.00	0.86

### CTC Runoff

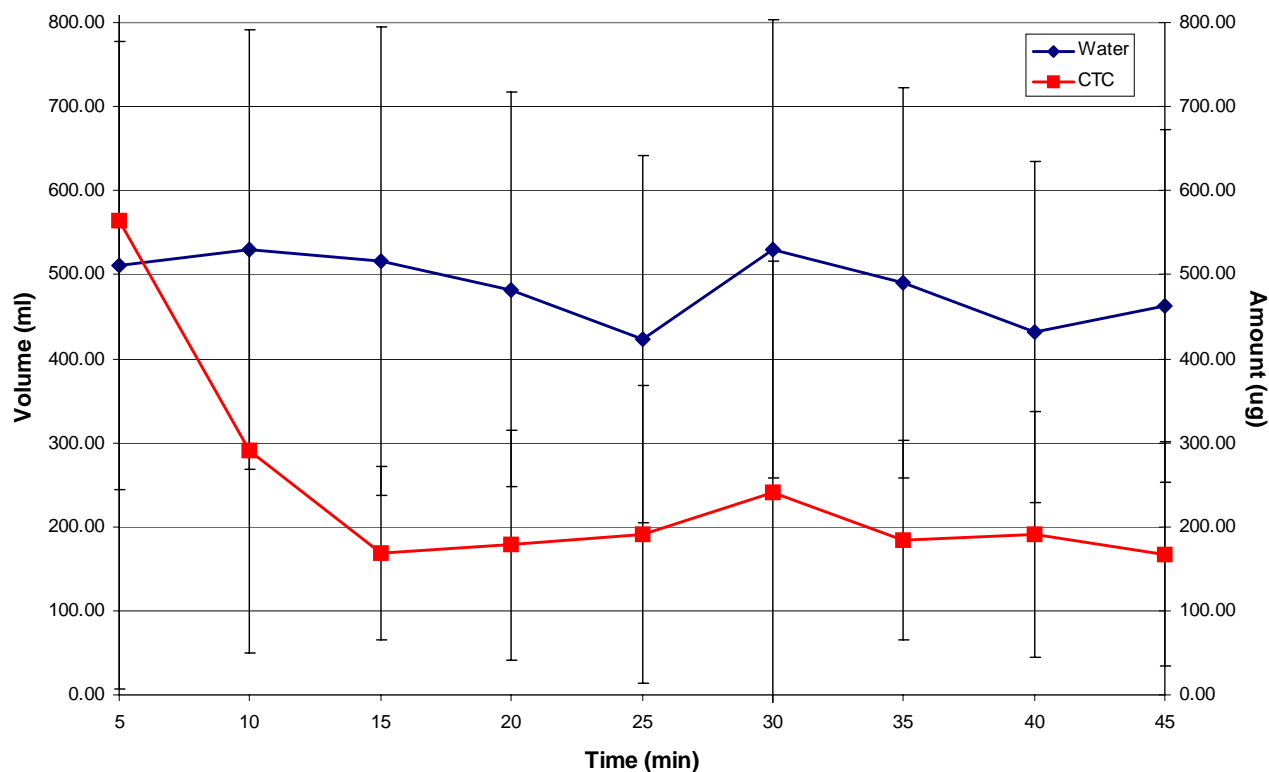


Figure 7. Runoff volume and CTC amount in runoff after manure application and sprinkler infiltration. (authors unpublished data)

Location	Water Applied	Water Recovered	Tylosin runoff	
	(L)	(%)	(µg)	(%)
Brkg1	5.73	92.9	27.80	12.07
Brkg16	4.23	89.7	19.20	8.40
Aurora	5.73	88.3	20.60	8.98

Table 3. Runoff amounts and tylosin content in runoff after manure application in 2005. The amount of manure applied to the microinfiltration sprinkler area contained a total of 0.11 mg tylosin. (authors unpublished data)

### Tylosin Runoff

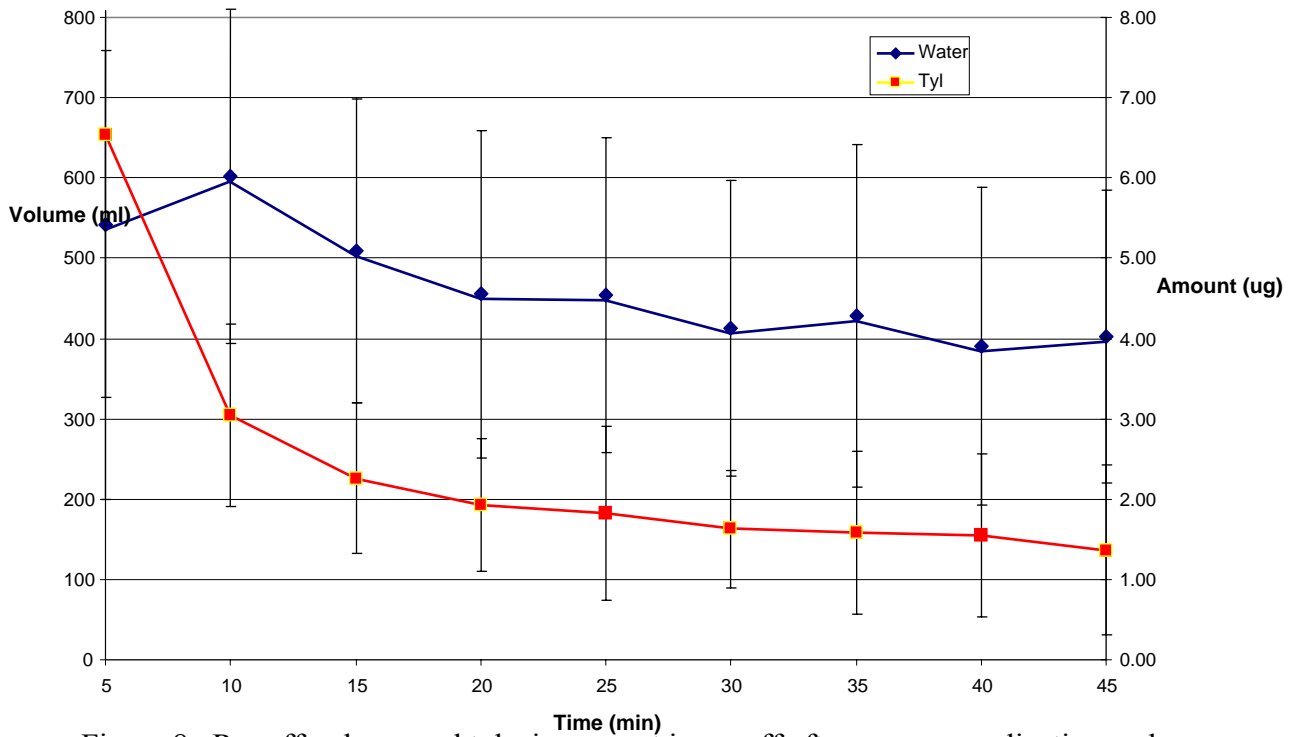


Figure 8. Runoff volume and tylosin amount in runoff after manure application and sprinkler infiltration. (authors unpublished data)

In 2006, this same study was repeated. Swine were fed at the same antibiotic concentration, and slurry was collected and a similar amount of slurry was applied. However, the concentration of antibiotic was much lower in 2006 than 2005. The amount of CTC present in slurry was only about 14 mg applied and tylosin, while it was detectable in the slurry it was low enough so that quantification was not possible. The results were that runoff from either set of antibiotic plots at all locations had no detectable antibiotic levels, even though the amount of water applied were similar between years. Another difference between the two years of application was that the initial water content of soil prior to manure application was about 20% in 2005 but as low as 10% in 2006 due to dry conditions. This may have allowed for more antibiotic to sorb to the dry soil. When soil samples were taken to examine the depth of infiltration, the amount of antibiotic in either treatment was not detectable at any depth in either year.

Growth of soil microbes could be inhibited by very low amounts of CTC (<5 ppb) but not tylosin (Figures 9 and 10). Microbial growth from strains purified from swine manure were not affected by CTC up to 50 ppb.

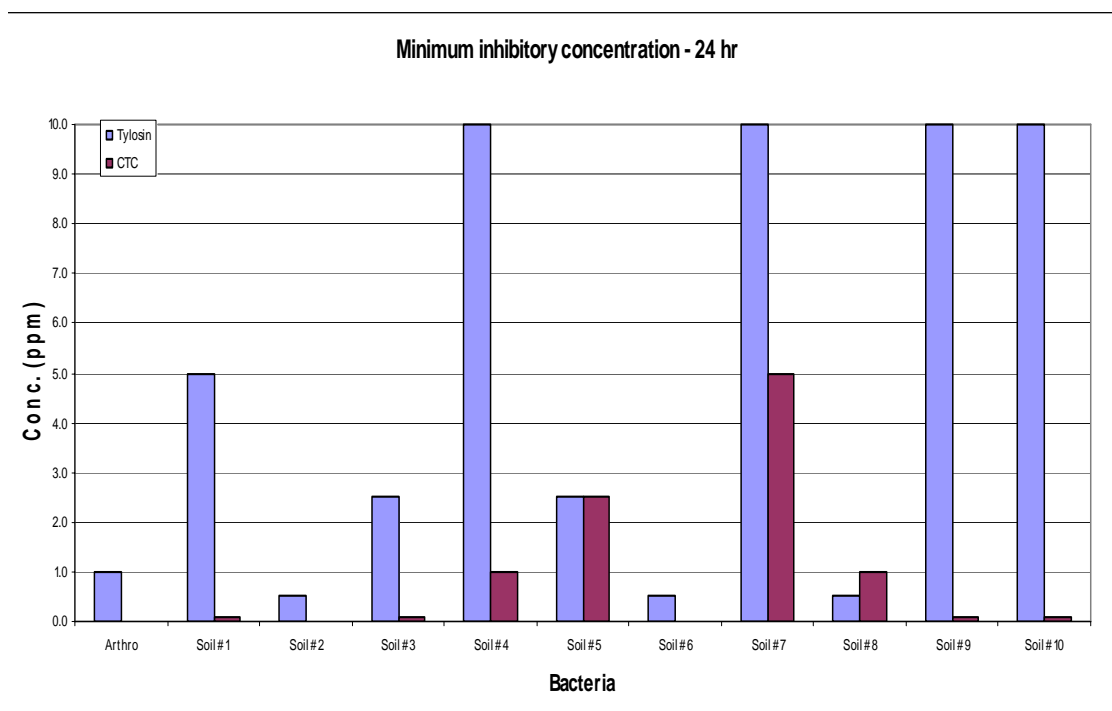
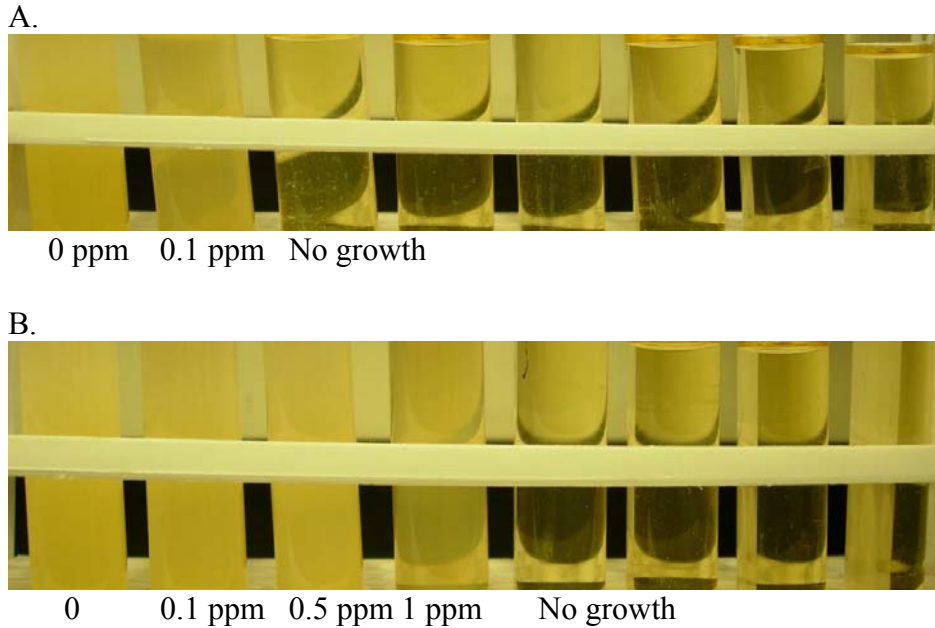


Figure 9. Minimum inhibitory concentrations of tylosin and chlortetracycline (CTC) after 24 hours of growth to 10 strains of bacteria, most of which were isolated from soil. Most isolated soil bacteria were very sensitive to CTC with concentrations of <5ppb inhibiting growth. Strains were not as sensitive to tylosin. (authors unpublished data)

When using microbial strains that were specific herbicide degraders, atrazine was fully degraded after 24 h in the control and tylosin cultures and after 72 h in the chlortetracycline culture. About 88% of the 2,4-D in control treatments was mineralized by the 2,4-D degraders 96 h after treatment, whereas tylosin slowed the initial degradation with only 4% mineralized by 10 days after treatment (DAT). However by 14 DAT, recovery occurred and the amount mineralized in the tylosin treatment was similar to the control. Chlortetracycline substantially decreased the activity of *S. herbicidovorans* with only 12% of the added 2,4-D mineralized 14 DAT. Manure decreased microbial mineralization of atrazine compared to soil. When manure contained chlortetracycline (when soil was treated with manure from swine feeding trials), the amount of atrazine mineralized was significantly less (Figure 11). Soil amended with biosolid or biosolid that contained tetracycline had amounts of atrazine mineralization that were similar to soil alone.

Figure 10. Example of sensitive and nonsensitive microbes to CTC. In example A, no growth of bacteria occurred after 24 hr in tubes that contained more than 0.1 ppm CTC. In example B, growth of bacteria could be seen in concentrations up to 1 ppm CTC. (authors unpublished data)



Colony forming units (CFU) of heterotrophic microorganisms culturable under aerobic conditions on R2A agar plates were 10-fold higher 7DAT in soil treated with manure containing CTC compared with all other treatments but it is unclear if the increase in CFU was due to native soil or manure organisms. The density of 2,4-D degrading microorganisms using the MPN method indicate a nearly 20 fold increase in number of 2,4-D degraders after adding 2,4-D to soil samples compared to soil samples without the application. The addition of manure with or without antibiotics did not influence the numbers of 2,4-D degrading organisms. DGGE analysis allowed for representation of all microorganisms present, culturable or not. Significant shifts in the 16S rRNA gene pool occurred within and among the different treatments over time.

## Atrazine Mineralization Study

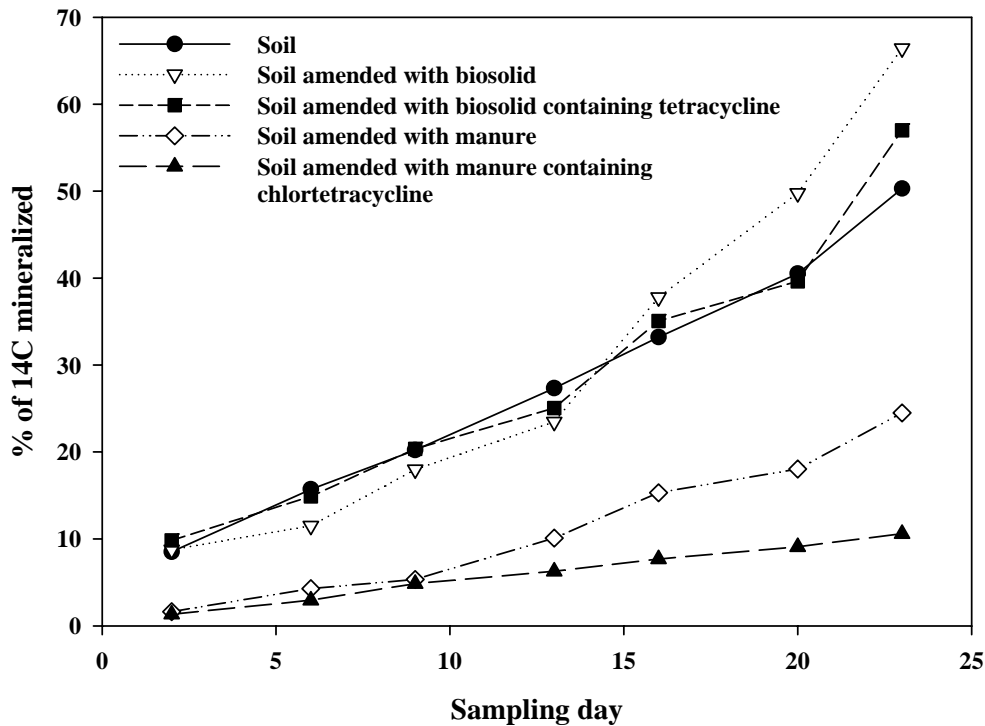


Figure 11. Atrazine mineralization in soil amended with soil, biosolid, and manure either alone or treated with antibiotics (tetracycline or chlortetracycline). (authors unpublished data)

These data suggest that deep application of manure in appropriate soils (i.e. in areas where large rocks would not interfere with application or other areas where deep tillage could be done) would 1) help conserve nutrients, especially N, 2) will not negatively affect corn or soybean yield, and 3) may be a method to reduce the effects of antimicrobial and nutrient movement in runoff waters to nontarget areas. Just adding manure affected microbial mineralization of atrazine, but less so than in the manure contained chlortetracycline. Placing manure off the soil surface may also reduce impacts on the normal biological processes of surface soil. The use of antimicrobials in some stages of swine development is often mandatory from pork processors. Choice of antimicrobial compound may also be of importance to minimize the disruption of normal soil processes after landspreading manure. For example, tylosin was found to have less inhibitory effect on native soil microbes and soil processes (i.e. herbicide mineralization) than an antibiotic such as chlortetracycline.

## **Publications, citations, and information transfer.**

### ***Completed***

Reiman, M. 2006. The impact of deep manure injection on water infiltration, nutrient distribution, and corn yield. M.S. Thesis, SDSU Brookings, SD.

Lehnert, K., V. Brözel, S. Gibson, and S. Clay. 2006. Antibacterials in manure influence soil microbial activity. Poster for NC Amer. Microbiology Society Meeting. Brookings, SD. October, 2006.

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Graduate student seminars. 2005, 2006, and 2007. Ms. Lehnert, Mr. Reiman, and Mr. Hoese presented seminars for faculty and students in Biology and Plant Science Departments as part of their ongoing graduate training.

Carlson, C.G. D.E. Clay, and C. Reese. 2007. Deep manure injection. North and South Dakota Watershed Manager Training. Feb 12, 2007. Aberdeen, SD.

Clay, D.E., C.G. Carlson, and S.A. Clay. 2007. Precision deep manure applications. SD EPA319 task force planning meeting. March 20, 2007, Pierre SD.

Kleinjan, J., C.G. Carlson, and D.E. Clay. 2006. Precision deep manure application (poster) Brookings field day. June 2006. Brookings SD.

Kleinjan, J., C.G. Carlson, and D.E. Clay. 2005. Precision deep manure application (poster) Brookings field day. June 2005. Brookings SD.

Reicks, G, C.G. Carlson, and D.E. Clay. 2006. The Impact of Deep Manure Injection on Nutrient Distribution and Corn Yield. SE. Farm field days. September 6, 2006.

Reese, C. L., D. E. Clay, D. Beck, J. Kleinjan, C. G. Carlson and S. Clay. Lessons learned from implementing management zones and participatory research in production fields. Eighth International Conference on Precision Agriculture. 23 – 26 July 2006. Marriott Hotel, Minneapolis, Minnesota.

Reese, C., L., D. E. Clay, S. A. Clay, C. G. Carlson and J. Kleinjan. 2005. South Dakota State Report. Upper Midwest Aerospace Consortium Annual Meeting. 3-5 February 2005. Grand Forks, ND.



Clay, D.E., S.A. Clay, C.G. Carlson. 2005. Is precision farming a BMP. National 406 water quality meeting. San Diego California, February 7-10, 2005

Carlson, C. G. and D. E. Clay, 2005. Precision Manure Management, East Central conference on integrating emerging technology into agriculture, 26 January, Sioux Falls.

Carlson, C. G. and D. E. Clay, 2005, South East Field Days. Deep tillage update, 1 September, Beresford

Carlson, C.G. 2005. Precision manure management for improved profitability. East central conference on integrating emerging technologies into agriculture. Sioux Fall SD, January 26, 2005

Clay, S.A. 2005. Manure effects on factors other than nutrients. East central conference on integrating emerging technologies into agriculture. Sioux Fall SD, January 26, 2005

Carlson, C.G. 2005. Precision deep fertilizer placement. SD Professional Soil Scientists. Huron, SD March 10, 2005

### **Web-page development**

A web-site that highlights research activities has been developed. The site is available at <http://plantsci.sdstate.edu/precisionfarm/index.aspx>. This site contains a virtual tour of the manure Flandreau deep manure research experiment.

### **Symposiums**

A day long symposium on integrating emerging technologies into production systems was held in Sioux Fall, SD on January 26, 2005 and in Aberdeen SD on March 8, 2005. At these meetings approximately 100 and 250 producers attended the Sioux Falls and Aberdeen meetings, respectively. The Sioux Fall meeting discussed manure management and ethanol production from corn. A discussion of deep manure management was presented at the Aberdeen meeting.

### ***In preparation***

Clay, D.E., C.G. Carlson, and M. Reiman. 2008. Deep manure impact on soil nutrient concentrations and corn yields. A draft has been prepared. This paper is ready for internal review which will be followed up with submittal to the Journal of Environ. Qual.

Hoese, A. 2007 (in progress). Tylosin and chlortetracycline, soil runoff and impact on soil microbes. M.S. Thesis. SDSU, Brookings.

SDSU Research Farm Field Days. Brookings and Beresford, SD. June, September 2007. Information presented by: Graig Reicks.

### **Student support:**

Graduate student involvement (3): Mark Reiman, Aaron Hoese, Kelly Lehnert

Undergraduate or high school student involvement (7): Dan Clay, Mitch Olson, Steve Biersbach, Kelsey Wick, Elissa Olson, Neal Bainbridge, Jesse Hall

### **Notable Awards and Achievements**

Lehnert, K. 2006. Monitoring soil diversity after tylosin and chlortetracycline laden manure treatments. First Place. SDSU Sigma Xi Graduate Student Proposal Contest

Reiman, M. 2005. The impact of deep manure injection on water infiltration, nutrient distribution, and corn yield. Second Place. SDSU Sigma Xi Graduate Student Proposal Contest

Based on some of the research with manure and antibiotics, an NSF grant that is a joint effort between SDSU and SDSMT was funded in 2006 to investigate the fate of antibiotics in manure and its effects on manure prior to land application.