Report for 2003AR47B: Antibiotic resistance and the relationship between enzyme activity and P in runoff from poultry litter amended soil

- Other Publications:
  - Tomlinson, P.J., and M.C. Savin, 2004, Antibiotic Resistance in Runoff and Soil Receiving Poultry Litter, Annual Meetings Abstracts [CD-ROM], ASA, CSSA,a nd SSSA, Madison, WI, in press.
  - Tomlinson, P.J., and M.C. Savin, 2004, Poultry Litter, An Influence on Antibiotic Resistance in Soil,? Annual Meeting Abstracts, Southern Branch of the American Society of Agronomy, Biloxi, MS, June 27-29,2004 in press.
  - Tomlinson, P.J., K.R. Payne, K.R. Brye, and M.C. Savin, 2003, Microbial Dynamics in Long-Term Research Plots Receiving Alum-Treated and Untreated Poultry Litter, Annual Meetings Abstracts [CD-ROM], ASA, CSSA, and SSSA, Madison, WI.

Report Follows

PROBLEMS AND RESEARCH OBJECTIVES

While poultry litter amendments have provided a means to improve pastures, concern about runoff water quality is escalating. Phosphatase enzymes release P from organic sources and may be contributing to the subsequent P contamination of runoff from soils receiving long-term applications of poultry litter. Some previous studies have suggested that organic P in animal wastes is much more mobile than inorganic P. However, there is some question as to whether elevated phosphatase enzymes will be sustained after multiple years of poultry litter inputs. If phosphatase enzyme activity is high in soils receiving animal wastes, then this could provide valuable insight into soluble P concentrations found in runoff.

Previous research has also indicated that antibiotic resistant microbes are present in poultry litter and antibiotic resistance in general is increasing. However, it is not clear to what extent antibiotics in poultry litter-amended soil influence the development and transport of antibiotic resistant bacteria from soil into runoff waters.

Objectives

The primary objective of this research is to investigate the effect of poultry litter amendments on environmental microbial community functions and potential contributions to pollution of surface waters. Specific objectives include the following:

1. Determine contribution of poultry litter land applications to antibiotic resistance development in microbial communities transported in surface runoff.
2. Determine if there is a relationship between phosphatase enzyme activities in surface soil amended with poultry litter and dissolved P concentrations in runoff water.

METHODOLOGY

Runoff water and soil samples were collected in 2003 from 20 plots (4 replications per treatment) growing tall fescue and receiving annual inputs of poultry litter since 1995. Plots are located at the University of Arkansas Main Agricultural Experiment Station, Fayetteville, AR. Treatments included unfertilized, control plots (C), plots receiving alum-treated litter applied at 2.24 Mg/ha (A1), alum-treated litter applied at 8.98 Mg/ha (A4), untreated litter at 2.24 Mg/ha (L1), and untreated litter applied at 8.98 Mg/ha (L4). Litter was added to soil in May and soil samples were collected in May, June, July, and November. Soil microbial biomass was measured using chloroform-fumigation-extraction. Acid and alkaline phosphatase enzyme activities were measured colorimetrically after incubation of soil in buffered sodium p-nitrophenyl phosphate solutions.

Runoff was collected in July (3 time points), September, and November. Runoff collection troughs, covered with plexiglass to minimize inputs other than from runoff, were fitted at the bottom of each plot (5% slope). Runoff samples were collected after filtration in the field through fiberglass mesh and a 20-um filter into previously sterilized, plastic bottles placed in enclosed collection bins following rain events significant enough to generate runoff.

A small portion of the water sample was removed, filtered, acidified, and analyzed colorimetrically for soluble reactive P. Because of the filtration system used in the field to eliminate grazers, a subsample of runoff was extracted for total DNA. Another subsample was serially diluted and plated onto 0.1x tryptic soy agar (TSA) plates. Cultivated bacteria were then replica plated onto 0.1x TSA plates containing known concentrations of monensin, bacitracin, or tetracycline (November only). Antibiotic concentrations tested were as follows: 0, 10, 50, 250 µg/ml monensin, 0, 0.5, 2.5, 12.5 units/ml bacitracin, and 0, 10, 50 and 100 µg/ml tetracycline. Resistance was measured by the loss of bacterial growth at each antibiotic concentration as compared to replica plates containing no antibiotics. DNA from antibiotic resistant cultured bacteria was extracted and amplified by polymerase chain reaction (PCR) for 16S rRNA gene fragments. Amplified DNA was separated on polyacrylamide gels using denaturant gradient gel electrophoresis (DGGE) to generate profiles of bacterial community structure.

PRINCIPAL FINDINGS AND SIGNIFICANCE

Deeper insight into biological properties, such as antibiotic resistance, and processes, such as nutrient cycling, are necessary before we can consistently and effectively enhance the positive, and ameliorate the negative, environmental consequences of land management practices on surface runoff water quality. In this project we investigated the relationship between microbial activity in the soil and its impact on runoff water. The information collected in this research project will enhance the current state of knowledge concerning biological processes controlling the environmental impact of poultry litter amendments on soil and surface water quality.

Relatively few rain events were significant enough to generate runoff from our plots. It was not until autumn that natural runoff was collected from all 20 plots. Generally, rain had to be close to or exceed 1.5 cm to generate runoff. However, large rain amounts did not guarantee runoff. For example, at the end of August there were two rain events in less than one week where over 4 cm of rain fell each time, but no runoff was generated from our plots.

Objective 1.

We had hypothesized that antibiotic resistance would be higher in runoff from soil receiving high rates of poultry litter inputs. Our data, in fact, do not support that hypothesis. Runoff collected from four rain events (7/12-7/13, 7/22, 9/13, and 11/18) was screened for antibiotic resistance. We found very little difference in expression of antibiotic resistance from bacteria cultivated from runoff of the controls and four litter application treatments. Levels of antibiotic resistance were related to antibiotic tested, with use of monensin resulting in higher levels of antibiotic resistance as compared to bacitracin. Resistance to monensin was high at each sampling time, with greater than 80% resistance measured in all runoff treatments as well as in bacteria isolated from the litter itself. Bacitracin resistance was always greater than 50% in all treatments at all sampling dates with no consistent differences among treatments across sampling dates. These results contrast to resistance expressed by bacteria cultivated from the litter itself. Resistance to bacitracin among litter bacteria was 20% at a concentration of 2 units/ml.

Tetracycline was screened in November because a collaborator in Indiana found higher resistance to tetracycline-related antibiotics in soil receiving swine wastes than untreated soil. In our runoff communities, controls showed resistance in 30% of isolates whereas resistance in runoff from litter treated soils was less than 10% at the highest tetracycline concentration tested.

Preliminary results of DGGE profiles of soil bacterial communities growing on each of the antibiotics suggest that community structures of tetracycline resistant bacteria are not the same as communities growing on monensin and bacitracin. Additionally, community structures of bacteria isolated from control soils expressing resistance to tetracycline appear to be different from litter treated soils. We are continuing to analyze bacterial community structures by DGGE for soil and runoff bacterial isolates.

Objective 2.

It was hypothesized that phosphatase levels in surface soils can serve as positive indicators of potential soluble P concentrations in runoff waters. To assess this possibility we measured soluble reactive P (SRP) in runoff and alkaline and acid phosphatase activities in soil. Because visual inspection of samples indicated that differing amounts of dissolved organic carbon (DOC) were present in runoff samples, we measured DOC in each of the SRP runoff samples. Microbial biomass and soil DOC were also measured in June, July and November. Scatter plots of runoff SRP plotted as a function of runoff DOC suggested that SRP was linearly related to DOC concentration ($R^2 = 0.69$ for all points, $R^2 = 0.93$ with 3 outliers removed). When analyzing each treatment separately, runoff SRP was linearly related to runoff DOC in C ($R^2 = 0.99$), less so in runoff from alum-treated soil treatments A1 ($R^2 = 0.69$), and A4 ($R^2 = 0.73$). Preliminary analysis suggests that there was not a clear linear relationship in either L1 ($R^2 = 0.20$) or L4 ($R^2 = 0.37$).

November was the only sampling time that both runoff and phosphatase activity data were collected for all plots. Runoff SRP did not appear to be directly related to either soil acid or alkaline phosphatase activities at that sampling time.

Analysis of data is continuing in 2004. However, preliminary results suggest that antibiotic resistance expressed in bacterial communities isolated from runoff depends on the particular antibiotic, and, for at least some antibiotics, the sampling time and/or soil treatments. Soluble reactive P appears to be related to the amount of dissolved organic C in runoff. Neither acid nor alkaline phosphatase activity six months after litter applications appeared to be related to runoff SRP. We are currently analyzing runoff collected in the spring 2004 before and after litter application to determine if soil phosphatase activity is related to runoff SRP in the spring.