An analysis of nitrate, ammonium and *E.coli* NAR in tile drain effluent from two adjacent agricultural systems: a mixed poplar-based intercropping system and a monocropped winter wheat system

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Abstract

Tile drain effluent from two adjacent winter wheat (*Triticum aestivum*) agricultural systems (a monocrop and a mixed tree intercrop where hybrid poplar (*Populus spp.* clone DN 177) is the principal species) was collected using ISCO 6700 portable tile drain monitors. An area of 1350 m^2 in each system was subject to application of a mixture of water and a biotracer *E.coli* NAR, an E.coli strain resistant to naladixic acid. This biotracer is a naturally occurring strain of E.coli and has been shown to be safe for introduction into the environment (Joy et al., 1992; Reaume and Joy, 1994). The effluent was sampled 3 times per week; at each sampling time, 12 samples from each treatment for nitrate and ammonium analysis and 6 samples from each treatment for *E.coli* analysis were taken.

The premise of this study is to determine if the safety-net hypothesis is valid in a temperate intercropping system. The theory behind this hypothesis is that the incorporation of trees, especially hybrid poplar, into agricultural systems will allow for a more efficient use of resources. In 2005, during the growing season (April-May)), analysis of water collected from tile drains for NO_3-N indicated that NO_3-N losses from the intercropping and conventional agricultural systems were 44.8 and 46.8 kg NO_3-N ha^{-1}, respectively. These numbers were derived from a paired mini watershed area of 17,200 m^2, following several rainfall events between April and May. The input N via fertilizer was 104 kg ha^{-1} and mineralized N over the period of leachate collection was 116.1 and 104.4 kg ha^{-1} for the intercropping and monocropping systems, respectively. Nitrate-N leaching was reduced by 2 kg N ha^{-1} in the poplar-based intercropping watershed compared to the monocropping watershed. Mean E.coli counts over the sampling period in the poplar-based intercropping and conventional agricultural systems were 844 and 1309 colony formations per 100 ml of tile drain effluent.

Introduction

Leaching of nutrients, particularly nitrate, into groundwater has become a major environmental issue over the past several years. Nitrogen and phosphorus in runoff can cause eutrophication of lakes, rivers and other surface waters (Carpenter et al., 1998). In turn, this can cause toxic algae blooms and fish kills, and loss of biodiversity due to lack of oxygen (Carpenter et al., 1998; Snyder et al., 1998). Excess nitrogen in drinking water can also cause several health effects in infant children and animals because their intestinal tracts can reduce nitrate to nitrite, which when combined with hemoglobin can cause methemoglobinemia or “blue baby syndrome” (U.S. Environmental Protection Agency, 1999; Liang and Mackenzie, 1994).

Another major concern is contamination of drinking water by bacteria such as, *Escherichia coli* (*E.coli*). The recent tragedy in Walkerton, Ontario in which seven people died and thousands became extremely ill, has brought the issue of *E.coli* contamination to a forefront, on a national basis. Studies have shown that more than 25% of rural wells in Ontario are contaminated with levels of nitrate and/or bacteria that are above drinking water limits (Exner and Spalding, 1985; Rudolph and Goss, 1993; Goss et al., 1998).

A potential solution to these problems is the use of tree roots to intercept and absorb nutrients and potentially harmful bacteria, which would have otherwise leached through the system, reducing contamination of ground water. In many alley-cropping systems, crop and tree roots occupy different depths in the soil profile, due to competition, plasticity and predisposed growing patterns, utilizing nutrients from different areas within the soil profile (Schroth, 1995; Schroth, 1999; Lott et al., 1996). Trees that are deep rooted are able to make use of nutrients from soil depths that most agricultural crops cannot (Huxley et al., 1994). This concept is called the safety-net hypothesis.

Although the safety-net hypothesis is mainly applied and studied in tropical climates, it has been shown to be applicable in temperate climates. For example, in a cotton (*Gossypium hirsutum L.*) and pecan (*Carya illinoensis K.*) alley cropping system in the southern United States, cotton roots demonstrated some plasticity, growing at shallower depths when the pecan roots were present (Wanvestraut et al; 2004). Cotton yield did not differ when grown in association with pecan roots, and without. In the same study, there was a 34 % reduction in nitrate levels at 0.9 m depth when there was no barrier impeding cotton roots from the area (Allen, 2003). This indicates that in this system, pecan roots function as a safety net.

There have been limited studies investigating the ability of roots to filter and absorb bacteria. A study comparing the efficiency of various constructed wetlands to filter fecal bacteria found that the system with a larger, more complex rooting system (that of cat tail (*Typha latifolia L.*)) was more effective then the system with a shorter, less complex rooting system (that of fescue (*Festuca arundinacea Schreb.*) (Karathanasis et al., 2003).

This study focuses on the effectiveness of tree roots, including that of a hybrid poplar (*Populus spp.* clone DN 177) and several other species to filter, mitigate, and/or absorb nitrate, ammonium and bacteria such as *E.coli*.

Materials and Methods

The experiments were performed during the spring and summer of 2005 at the Agroforestry Research Station at the University of Guelph in southern Ontario, Canada. The study area is split into two equal sections of approximately 250 x 70 m (1.75 ha) in area: an intercropping system and a monocropped system. Within the intercropping system, tree rows (with mature trees that are approximately 20 years old), which are approximately 1 meter wide, are separated by crop alleys, of which four are 15 meters in width and one is 9 meters (Figure 1). Tree spacing within the row is 6 meters. The crop planted in both fields was winter wheat (*Triticum aestivum)*, to which 200 lbs of urea was added per acre (approximately 225 kg per hectare). The monocropped section was used as a no-tree control.

Figure 1 **Photo of a crop alley separated by rows of mature poplar and maple trees at the Agroforestry Research Station at the University of Guelph**

Each section is tile drained equally and independently. The tile drains run down the center of the alley rows in the intercropping system. Both drainage systems are designed so that all water that enters each tile drain system (either monocropped or intercrop) will flow to separate main drains and will be directed to two ISCO 6700 portable tile drain monitors, where sub-samples can be collected. When flow was detected, the monitors were programmed to collect a 200 mL sample after 10 m^3 of water had passed through the tile drain.

Within each section there is one 50 x 22 m sub-section in which an *E. coli* biotracer (*E.coli* NAR), which is a strain of *E.coli* that is resistant to nalidixic acid, that was applied. It is a naturally occurring strain of *E. coli* (although occurrences are rare), and can be isolated from the environment. There were two applications of the inoculum, the first on April 14th, and the second on May 25th, 2005. The sub-section in the intercropping site had two tree rows running through it; a hybrid poplar row separating two crop rows and a silver maple (*Acer saccharinum L.*) row on the edge of one crop row.

For culture preparation 0.1 mL of frozen stock *E.coli* NAR supplemented with naladixic acid was added to 25 mL of sterile Trypticase Soy Broth (TSB) in a 125 mL Erlenmeyer flask, and incubated at 20°C for 24 hours with gyratory shaking at 200 rpm. 1 mL of culture was then added to 500 mL of sterile TSB in a 1 L Erlenmeyer flask and incubated for 24 hours at 20°C with slow magnetic stirring.

An 18 000 L liquid manure tanker was used to mix the solution. When the tanker was approximately half full, the inoculum was added, and mixed with the tanks churning mechanism while the balance was being filled. The tanker then transported the water/inoculum mixture to the study site.

Approximately 9000 L of the water/inoculum mix was applied to each sub-section. The tanker applied the water/inoculum mix at a constant velocity and output rate, ensuring equal distribution between the two sub-sections.

Ideally the biotracer should be mixed in with liquid manure, however, due to current environmental regulations; the manure could not be transported to the study site. In an effort to simulate liquid manure application, four tones of solid horse manure was applied evenly to each sub-section, using a tractor operated solid manure spreader before the water/inoculum mixture was applied. This simulation was only done for the first application of biotracer.

Prior to application, manure applied to the site, water used to mix with the inoculum, tile drain effluent, and soils within the sub-section were sampled to ensure that they did not contain background *E.coli* NAR.

Samples of tile drain effluent were collected from the tile drain monitors three times a week. For each sampling period, 12 and 3 randomly selected samples were taken for analysis of nitrogen and *E.coli* NAR respectively. Nitrogen analysis included nitrate and ammonium.

Effluent water samples were analyzed for 3 properties: nitrate and ammonium concentrations, and enumeration of *E.coli* NAR. Effluent samples being tested for nitrate and ammonium first had to be filtered for sediment. Samples where then analyzed using an auto analyzer.

Effluent samples being analyzed for *E.coli* NAR had first to be centrifuged at 300 rpm for 5 minutes to separate the bacteria from the sediments. 10 - 100 mL of the sample was then filtered (depending on the concentration) using Gelman sterile cellulose-acetate filters (0.45 μm). The filters were then placed on mTEC agar that had been supplemented with naladixic acid in 60 x 15 mm Petri plates. The plates were then incubated at 37 °C for 24 hours, followed by a recovery period of 1 hour at 20 °C. Colonies appeared purple against the white filter.

Results and Discussion

Nitrate and Ammonium

Nitrate concentration in the tile drain effluent did not consistently differ between treatments. Concentration in Weeks 1 and 6 was significantly greater in the intercropping effluent, whereas in week 3, concentration was significantly greater in the monocropped effluent. In all other weeks the differences were non significant (Table 1).

During the experiment the concentration of ammonium was significantly higher in the intercropping effluent than the monocropped in each week except weeks 1 and 3.

The total amount of water removed by tile drains in the monocropped system (545 m^3) was approximately 4 times that of the intercropping system (135 m^3) during the first week of the study. In the preceding weeks the flow rates for both systems were relatively equal. The tile drain water output from the systems peaked in week 3, with the monocropped output at 2215 m^3 and the intercropping output at 2270 m^3. After this peak, flow began to steadily decrease until it ceased after week 7. May 20th was the last day flow was detected.

Table 1 **Mean concentrations of nitrate and ammonium in the tile drain effluent and the total weekly volume of water running through the tile drains**

	Nitrate Concentration (ppm)		Ammonium Concentration (ppm)		Volume of Water (m^3)	
Week	Mono[1]	Inter[1]	Mono[1]	Inter[1]	Mono[1]	Inter[1]
1	8.193^a	9.516^b	0.145^a	0.168^a	545^b	135^a
2	8.976^a	9.843^a	0.087^a	0.145^b	1945^a	1890^a
3	10.843^b	9.189^a	0.069^a	0.087^a	2215^a	2270^a
4	9.888^a	10.245^a	0.037^a	0.097^b	1730^a	1730^a
5	10.849^a	11.132^a	0.072^a	0.127^b	1890^a	1900^a
6	9.754^a	10.498^b	0.078^a	0.138^b	1730^a	1700^a
7	9.921^a	9.885^a	0.042^a	0.116^b	1100^a	1120^a

Notes [1] Mono – monocropped: Inter - intercropped

[a,b] – comparisons are made for each week across the rows, differences in subscripts indicate a significant difference at $P \leq 0.05$

The nitrate removed from the system was only significantly different in week one (Figure 2). In all other weeks there were no significant differences. In total, 81.85 and 78.41 kg of nitrate were removed from the monocropped and intercropping systems respectively via tile drain effluent during the study period.

The total ammonium removed from the system was significantly different each week (Figure 3). In total, 0.82 and 1.27 kg of ammonium was removed from the monocropped and intercropping system respectively via tile drain effluent during the study period.

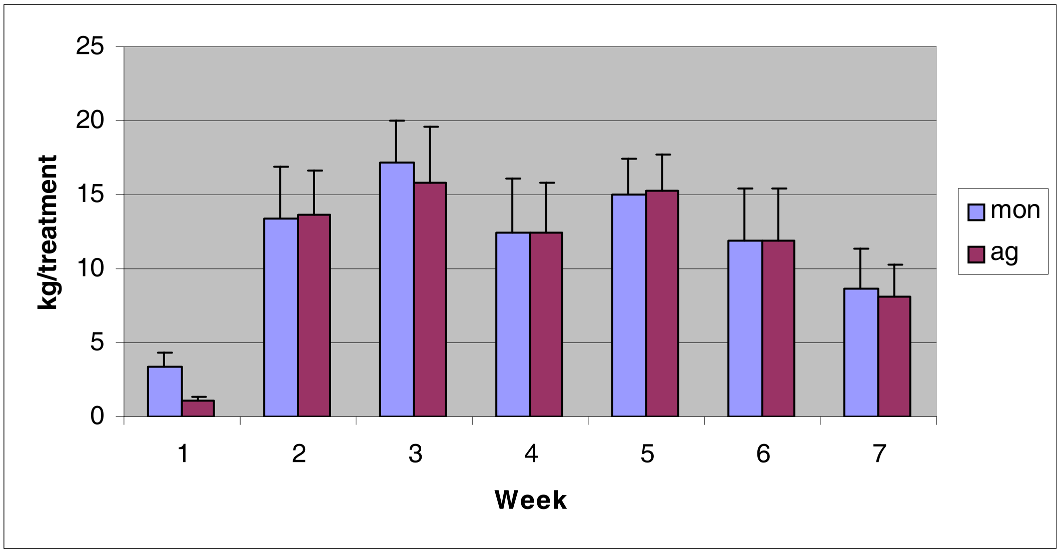

Figure 2 **Total nitrate removed weekly from each system (monocropped (mon) and agroforestry (ag)) via tile drain**

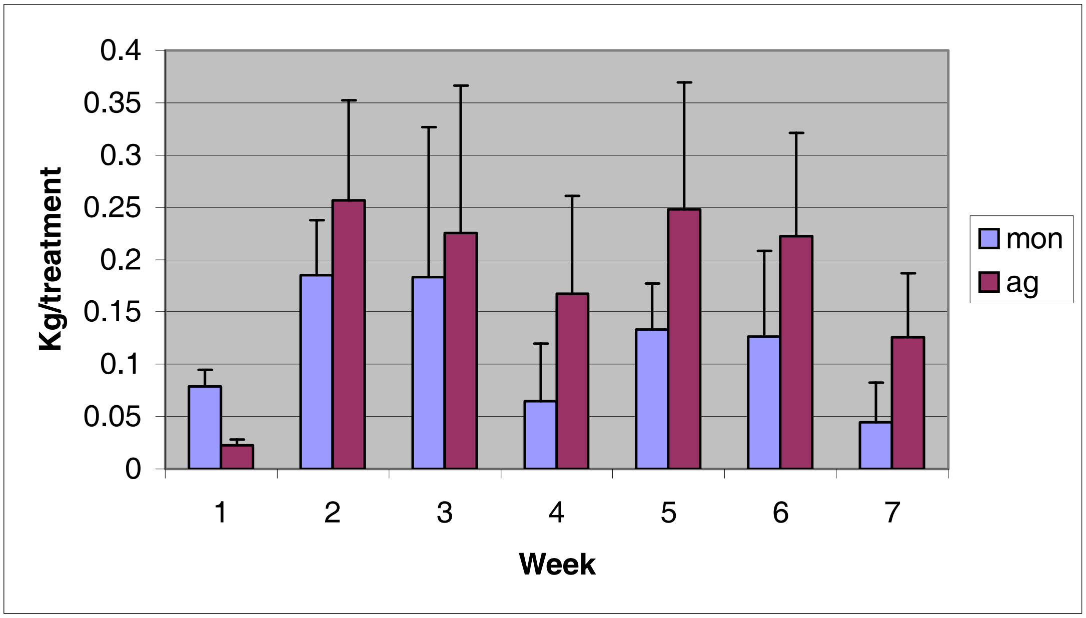

Figure 3 **Total ammonium removed weekly from each system (monocropped (mon) and agroforestry (ag)) via tile drain**

Urea is composed of 46% nitrogen, resulting in an application rate of 106.70 Kg/ha of total nitrogen on the study site. Therefore 186.72 Kg of nitrogen was applied to each section.

E.coli NAR

There were only 4 dates (May 2nd, 9th, 11th, and 16th) in which there were significantly more *E.coli* NAR in the monocropped effluent than the intercropped (Figure 4). However, in most weeks, especially those after the first two weeks of the study, showed visually more *E.coli* NAR in the effluent from the monocropped field. The removal of *E.coli* NAR in the intercropped effluent peaks near the end of week 2 and beginning of week 3. Conversely, the number of colonies in the monocropped effluent follows a similar initial pattern of increasing at the end of week 2, but does not decrease as much in the following sample dates as the intercropped effluent.

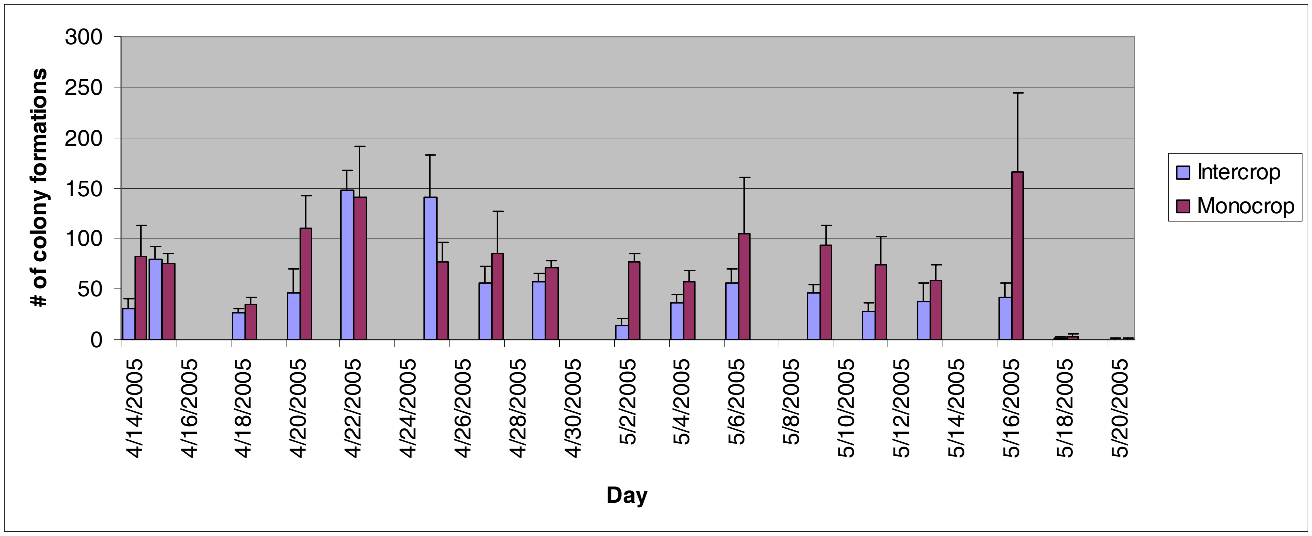

Figure 4 **Number of *E.coli* NAR colonies formed from 100 mL sub-samples of tile drain effluent from the monocropped and intercropped systems**

Conclusions

Several trends were evident in the data which warrant further investigation. Concentrations of nitrate in the tile drain effluent did not appear to differ between either system. During the study time frame, the main determining factor in nitrate loss appears to be the amount of water lost from the system. During week 1, water flow in the monocropped treatment was 4 times that of the intercropping treatment. This is the only week that showed a significant difference in the amount of nitrate leaving the system. Conversely, ammonium concentrations were consistently higher in the intercropping effluent. This resulted in consistently greater amounts of ammonium leaving the intercropping system.

The results of the preliminary studies done on *E.coli* NAR presence in the tile drain effluent suggest that there is little significant difference between the two systems. However, further investigation is necessary because visually it appears poplar roots have an effect on the movement of *E.coli* NAR into tile drain systems. Further and more extensive studies are needed to determine if there is an effect.

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