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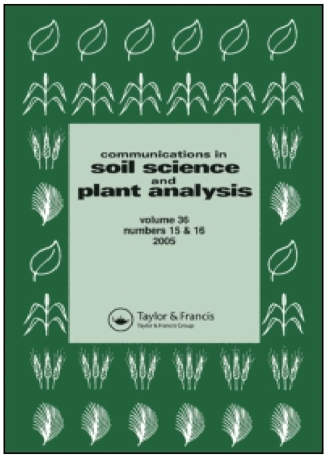

Communications in Soil Science and Plant Analysis

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597241

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To cite this Article: , 'Ability of Forage Grasses Exposed to Atrazine and Isoxaflutole to Reduce Nutrient Levels in Soils and Shallow Groundwater', Communications in Soil Science and Plant Analysis, 38:9, 1119 - 1136
To link to this article: DOI: 10.1080/00103620701327976
URL: http://dx.doi.org/10.1080/00103620701327976

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Communications in Soil Science and Plant Analysis, 38: 1119–1136, 2007
Copyright © Taylor & Francis Group, LLC
ISSN 0010-3624 print/1532-2416 online
DOI: 10.1080/00103620701327976

Ability of Forage Grasses Exposed to Atrazine and Isoxaflutole to Reduce Nutrient Levels in Soils and Shallow Groundwater

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Abstract: Successful implementation of vegetative buffers requires inclusion of plant species that facilitate rapid dissipation of deposited contaminants before they have a chance to be transported in surface runoff or to shallow groundwater. Thirty-six field lysimeters with six different ground covers [bare ground, orchardgrass (*Dactylis*

Received 20 October 2005, Accepted 12 June 2006

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glomerata L.), tall fescue (*Festuca arundinacea* Schreb.), smooth bromegrass (*Bromus inermis* Leyss.), timothy (*Phleum pratense* L.), and switchgrass (*Panicum virgatum* L.)] were established to evaluate the ability of grasses to reduce nutrient levels in soils and shallow groundwater. Nitrate (NO_3^-) and orthophosphate (PO_4^{3-}) were uniformly applied to each lysimeter. In addition, half of the lysimeters received an application of atrazine, and the other half received isoxaflutole (Balance^{TM}) at levels indicative of surface runoff from cropland. The leachate from each lysimeter was collected after major rainfall events during a 25-day period, and soil was collected from each lysimeter at the end of the 25-day period. Water samples were analyzed for NO_3-N and PO_4-P, and soil samples were analyzed for NO_3-N. Grass treatments reduced NO_3-N levels in leachate by 74.5 to 99.7% compared to the bare ground control, but timothy was significantly less effective at reducing NO_3-N leaching than the other grasses. Grass treatments reduced residual soil NO_3-N levels by 40.9 to 91.2% compared to the control, with tall fescue, smooth bromegrass, and switchgrass having the lowest residual levels. Switchgrass decreased PO_4-P leaching to the greatest extent, reducing it by 60.0 to 74.2% compared to the control. The ability of the forage grasses to reduce nutrient levels in soil or shallow groundwater were not significant between herbicide treatments. Quantification of microbial NO_3^- dissipation rates in soil suggested that denitrification was greatest in switchgrass, smooth bromegrass, and tall fescue treatments. The overall performance of these three grasses indicated that they are the most suitable for use in vegetative buffers because of their superior ability to dissipate soil NO_3^- and reduce nutrient transport to shallow groundwater.

Keywords: Bioremediation, denitrification, herbicides, lysimeter, riparian buffer

INTRODUCTION

Nutrients have been identified as important nonpoint-source agricultural pollutants in surface and groundwaters. High nitrate-N (NO_3-N) concentrations have been found in surface runoff and shallow groundwater sampled in the Midwest (USDA 1995). A USDA Management Systems Evaluation Areas report (USDA 1994) noted that NO_3-N concentrations in surface runoff commonly exceeded the 10 mg/L maximum contamination limit for about 40 days following fertilizer application (USDA 1995). Concentrations of NO_3-N in surface runoff and topsoils can be as high as 40–60 mg/L (USDA 1994). Kitchen et al. (1997) reported that 25% of tested wells in a glacial-till aquifer in northern Missouri had NO_3-N concentrations greater than 10 mg/L. Among 123,656 wells sampled throughout the United States in the early 1980s, about 6.4% had NO_3-N concentrations greater than the 10 mg/L standard (Madison and Burnett 1985).

Multispecies riparian buffer systems have been recognized as one of the most cost-effective bioremediation approaches to alleviate nonpoint sources of agricultural pollutants on adjacent croplands (Schultz et al. 1995, 1991). Sediment retention and increased infiltration are the initial mechanisms that reduce nutrient loads in surface runoff. It has been generally observed that 50 to 90% of the sediments and the nutrients attached to the sediment are trapped by vegetative buffers (Mendez, Dillaha, and Mostaghimi 1999; Schmitt, Dosskey, and Hoagland 1999). In a runoff simulation study, 6-m grass buffers removed from 38 to 47% of NO_3-N and 39 to 46% of orthophosphate (PO_4-P) from surface runoff (Lee et al. 1997). In the same study, switchgrass (*Panicum virgatum*) buffers removed significantly more NO_3-N and PO_4-P than cool-season grass buffers consisting of bromegrass (*Bromus inermis*), timothy (*Phleum pratense*), and fescue (*Festuca* sp.). Parsons et al. (1991) found that buffers with a mixture of crab grass (*Digitaria* sp.) and bermudagrass (*Cyndon dactylon*) removed 50% of PO_4-P and 50% of total N from cropland runoff. Blanco-Canqui et al. (2004) also reported a comparable effectiveness in reducing N and P losses between fescue buffers and a buffer composed of a switchgrass barrier in combination with native grass and forb species. The effectiveness of both buffers increased with distance, and more than 71% of the nutrients in the surface runoff were retained within the first 4 m of the buffers. The addition of a 0.7-m switchgrass hedge to fescue buffers was found to be 15% more effective than fescue buffers alone for reducing losses of organic N, NO_3-N, NH_4-N, particulate P, and PO_4-P.

Once the initial retention of nutrients has occurred, plant uptake and/or denitrification are the major mechanisms that reduce nutrient levels within buffers. Carlson, Hunt, and Delancy (1974) found that the N content of grass harvested from a buffer accounted for 31% of the applied N. Prayer and Weil (1987) reported that reed canarygrass (*Phalaris arundinacea*) can take up about 45% of applied P. In a nutrient uptake study using a mixture of bermudagrass and ryegrass (*Lolium perenne* L.), the total N uptake ranged from 461 kg/ha, without applied N, to 1024 kg/ha with N applied as lagoon effluent (Liu et al. 1997). In their study, forage N uptake was found to parallel yield. In general, C_3 species exhibit higher NO_3^- uptake capacity than C_4 species. Sehtiya and Goyal (2000) reported a 55 to 91% higher NO_3^- uptake capacity in C_3 plants as compared to C_4 plants.

Microbial denitrification, the biological reduction of NO_3^- to N_2, NO, NO_2, or other N gases, has been reported as the dominant process for the removal of NO_3^- in a saturated, anaerobic riparian zone (Reddy, Sacco, and Graetz 1980; Simmons, Gold, and Groffman 1992). Reddy, Sacco, and Graetz (1980) reported that about 97% of applied $^{15}NO_3$-N was lost through denitrification, 2.5% was reduced to $^{15}NH_4$-N, and 0.6% was immobilized into the organic-^{15}N fraction. Most denitrifying bacteria exist in the topsoil (0–30 cm) with the number decreasing exponentially down to 120–150 cm (Parkin and Meisinger 1989). The denitrification process is limited by redox conditions and available carbon in soils. A number of authors have shown that increased denitrification was associated with organic carbon (C) inputs derived from the presence of vegetation (Groffman et al. 1991; Lowrance Vellidis, and Hubbard 1995; Reddy, Sacco, and Graetz 1980; Stefanson 1972). Among different vegetation covers, denitrification was significantly higher in grasslands than in either hardwood or pine forest areas (Lowrance, Vellidis, and Hubbard 1995). Groffman et al. (1991) reported a significantly higher denitrification rate in tall fescue (*Festuca arundinacea* Schreb.) (51% denitrified) and reed canarygrass (*Phalaris arundinacea*) buffers (29% denitrified) than a forest buffer (1.3 to 4.5% denitrified).

Under agronomic conditions, it is common to detect many herbicide compounds along with nutrients in surface runoff. Certain herbicides currently used in agricultural operations could greatly influence the bioremediation capacity of vegetative buffers either through inhibited forage growth or alteration of N transformation processes in the rhizosphere. A number of studies have investigated the influence of pesticides and their metabolites on the microbial denitrification process. Many pesticides, such as captan (N-trichloromethylmercapto-4-cyclohexene-1,2-dicarboximide), MANEB (manganous ethylenebisdithiocarbamate), and 2,4-D (2,4-dichlorophenoxyacetic acid) cause strong inhibition of the respiratory NO_3^- reduction processes (Lowrance, Vellidis, and Hubbard 1995). McElhannon, Mills, and Bush (1984) also found that many s-triazines including simazine (2-chloro-4,6-biethylamino-m-triazine) and atrazine (ATR) (2-chloro-4-ethylamino-6-isopropylamino-s-triazine) interfered with the denitrification process, resulting in a accumulation of NO_3^- in soil. Balance^{TM} (isoxaflutole (IXF) [5-cyclopropyl-4-(2-methylsulfonyl-4-trifluoromethylbenzoyl)-isoxazole]) belongs to the new class of isoxazole herbicides introduced by Rhone-Poulenc Agrichemical Co. (Research Triangle Park, NC). The effects of this new compound on forage growth, microbial activity, and nitrogen transformation are not well understood.

Successful implementation of agroforestry multispecies buffers requires ground cover species that can effectively remove nutrients trapped within the buffers before they reach groundwater or are redischarged to surface water. In addition, they need to be able to tolerate the shade cast by the woody species near the river banks. Information derived from a shade screening trial has helped to identify 15 ground cover species or cultivars that have significant potential for agroforestry multispecies buffer strips (Lin et al. 1998). Five of these species were selected for additional study because of their moderate to good tolerance of the shade. The objectives of this study were to evaluate the effectiveness of these five forage grass species for their ability to reduce nutrient levels in soil and shallow groundwater while exposed to ATR and IXF herbicides at levels typical of surface runoff from cropland.

MATERIALS AND METHODS

Experimental Design

Thirty-six 1 m wide and 0.5 m deep lysimeters were established with six different ground covers. These included bare ground, orchardgrass (*Dactylis* *glomerata* L.) (C_3), tall fescue (*Festuca arundinacea* Schreb.) (C_3), smooth bromegrass (*Bromus inermis* Leyss.) (C_3), timothy (*Phleum pratense* L.) (C_3), and switchgrass (*Panicum virgatum* L.) (C_4). Ground covers were established in 1998 at the University of Missouri Horticulture and Agroforestry Research Center, New Franklin, MO (longitude 92°46′ W; latitude 39°1′ N). These lysimeters were arranged as a completely randomized design with three replications. Each lysimeter was filled with a sandy loam soil with average pH of 7.0, organic content of 0.72%, and cation exchange capacity of 3.0 meq/100 g. The interior surface of each lysimeter was fluorinated, and each lysimeter was attached to a 5-cm drain line that ends in an enclosed collection facility. Three liters of fertilizer (1.5 g/L, Peters 20:20:20) solution was applied to each lysimeter every 2 weeks from April through July 1998 for a total of 1800 mg of N and 1800 mg of P. In September 1998, 3-L solutions containing 50 mg/L NO_3-N along with either ATR (500 μg/L) or IXF (80 μg/L) were uniformly applied to each lysimeter. The applied concentrations were representative of those expected in the surface runoff in northern Missouri (USDA 1995). On an area basis, herbicide application to the lysimeters corresponded to 0.9% of the maximum atrazine field application rate and 1.9% of the maximum IXF field application rate. The inclusion of the herbicide treatments was to compare any indirect effects that may compromise the ability of the grasses to take up nutrients or effect nutrient transformation in the rhizosphere of the grass treatments between the two classes of herbicides. The leachate from each lysimeter was collected in 13-L high-density polypropylene tanks after every major rainfall event for 25 days following herbicide and NO_3-N application. The leachate volumes were recorded, and each sample was filtered through 0.45-μm nylon filters and stored at 4°C until analysis.

Soil and plant samples were collected at the end of the 25-day period. The aboveground plant material was cut and harvested, and the dry weight determined after drying the samples at 43°C for 4 days. Five 35-cm-deep soil cores were collected from each lysimeter. The soils were homogenized and subsampled for nutrient and microbial biomass C analyses.

Analysis of Nitrate in Soil and Water

Nitrate-N and PO_4-P in leachate or soil extracts were determined by standard colorimetric procedures (Lachat QuikChem 1993) using Lachat QuikChem automated flow-injection ion analyzer (Lachat Instruments, Loveland, CO). For soil NO_3-N analysis, 10 g of soil (dry-weight basis) was subsampled from each lysimeter and extracted with 25 mL of 2 M potassium chloride (KCl). The suspension was shaken for 5 min at 200 oscillations per minute and filtered through Whatman No. 42 filter paper, and the filtrate was transferred to a 10-mL test tube. Nutrient concentrations were expressed on an elemental basis (i.e., NO_3-N and PO_4-P).

Determination of Total Transpiration Rate

Transpiration flux density (mmol $H_2O \cdot m^{-2} \cdot s^{-1}$) of each forage was measured, on a leaf area basis, in the field on a clear day using a LiCOR 6400 Portable Photosynthesis System. Light intensity was 1715 μmol photons $\cdot$ m^{-2} s^{-1} of photosynthetically active photon flux density, relative humidity was 18%, and air temperature was 21°C. Ten readings were recorded during each measurement made around midday in mid-Missouri. To determine the total transpiration rate (mmol H_2O m^{-2} s^{-1}) of each vegetated lysimeter, flux density was multiplied by the estimated total leaf area (m^2). Total leaf area was calculated by multiplying total aboveground dry weight by the specific leaf area (i.e., cm^2 leaf area/leaf dry weight). Specific leaf area was determined by the measurement of leaf area and dry weight of 20 leaves subsampled from each lysimeter. The leaf area was measured using a LI-COR 3000 leaf area meter.

Evaluation of Microbial Nitrate Dissipation Rates

Nitrate dissipation rates of ground cover treatments were also measured. Samples of moist lysimeter soil containing a dry-weight equivalent of 20 g were adjusted to 10% moisture content. This was accomplished by measuring the dry-weight content of a subsample of the lysimeter soil. Eight samples from each lysimeter were placed into specimen cups and then saturated with 10 mL of 50 mg/L NO_3-N solution. As noted before, this concentration is representative of that observed in the surface runoff near cornfields (USDA 1995). Cups were cap-sealed and incubated in the dark at 28°C for periods of 0, 6, 12, 24, 48, 72, 96, or 138 h. At the end of each incubation period, cups were removed from the incubation chamber and extracted with 50 mL of 2 M KCl. The resulting suspension was filtered through filter paper, and the filtrate was analyzed for NO_3-N as described previously.

Determination of Microbial Biomass Carbon

Soil microbial biomass C was determined by a modified chloroform fumigation and direct extraction method (Jordan and Beare 1991). Twenty grams of moist soil collected from each lysimeter were used and adjusted to 10% moisture on an oven-dry-weight basis. Samples were either fumigated with chloroform under vacuum or not fumigated for 24 h. Soil microbial biomass C from fumigated and unfumigated samples was extracted with 80 ml of 0.5 M potassium sulfate (K_2SO_4) after shaking for 30 min on a rotary shaker at 320 rpm. Biomass C of the extractant was evolved as carbon dioxide (CO_2) by persulfate digestion. Carbon dioxide was trapped in 1 mL of 0.1 M sodium hydroxide (NaOH) and titrated with 0.01 M hydrochloric acid (HCl). One percent phenolphthalein was used as an indicator. A volume of 100 μL of 1 N barium chloride ($BaCl_2$) was added to the NaOH solution and then titrated to a colorless endpoint. The amount of C trapped in the NaOH was determined from a standard curve constructed from a series of glucose standards (0, 100, 200, 300, 400, and 500 μg of C). Soil microbial biomass C was calculated as the difference between duplicate fumigated and unfumigated samples. A soil mineralization constant ($Kc = 0.41$) was used as a conversion factor (Voroney and Paul 1984).

RESULTS AND DISCUSSION

Nutrient Leaching and Residual Soil Nitrate

Forage treatments showed significantly lower NO_3-N levels in leachate compared to the bare ground control (Figure 1). Cumulative NO_3-N mass in

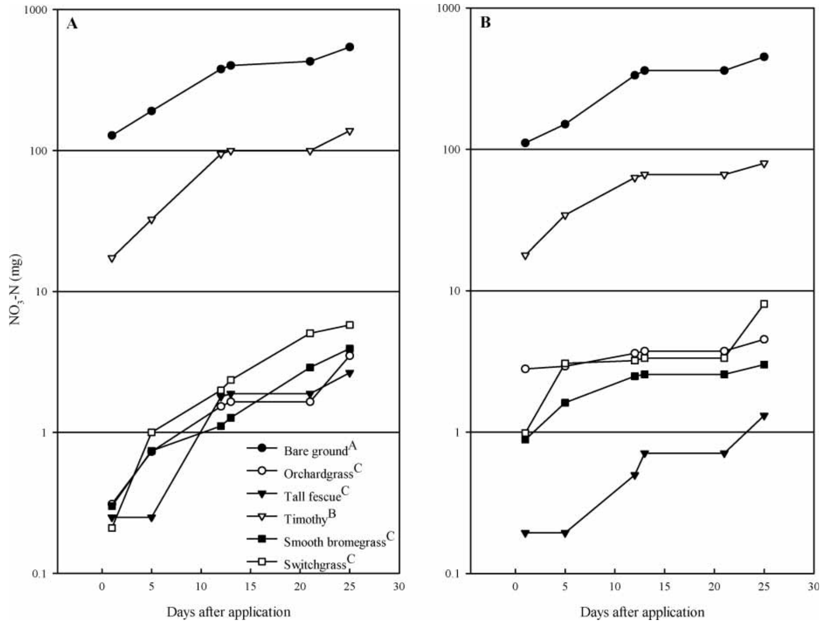

Figure 1. Cumulative NO_3-N in leachate for (A) atrazine-treated and (B) isoxaflutole-treated lysimeters ($n = 3$). Means followed by the same letter did not significantly differ from each other at 10% level of probability using the LSD test. The effects of herbicides ($P = 0.28$) and their interaction with grass treatments ($P = 0.76$) were not significant.

leachate from orchardgrass, tall fescue, smooth bromegrass, and switchgrass treatments was reduced by 98.2 to 99.7% compared to bare ground during the experimental period. Timothy-treated lysimeters reduced NO_3-N from 74.5 to 82.3%. Herbicide treatment did not significantly affect the ability of the forage grasses to reduce NO_3-N in leachate ($P = 0.28$).

The reduction of PO_4-P in leachate by grass treatments was not as dramatic as that for NO_3-N (Figure 2). Switchgrass (a C_4 species) displayed a significantly greater capacity to reduce PO_4-P levels in leachate compared to the cool-season grasses (C_3 species). Switchgrass treatments reduced PO_4-P from 60.0 to 74.2% compared to the control. When data for both herbicide treatments were combined, PO_4-P mass in leachate from orchardgrass, smooth bromegrass, and tall fescue treatments was reduced 14.9 to 48.0% compared to the control. It should be noted that for timothy, the PO_4-P reduction was not consistent between the two herbicide treatments. This indicated a possible herbicide effect for timothy with respect to PO_4-P in leachate.

Residual soil NO_3-N was also significantly reduced by the grass treatments 25 days after fertilizer application (Table 1). Smooth bromegrass,

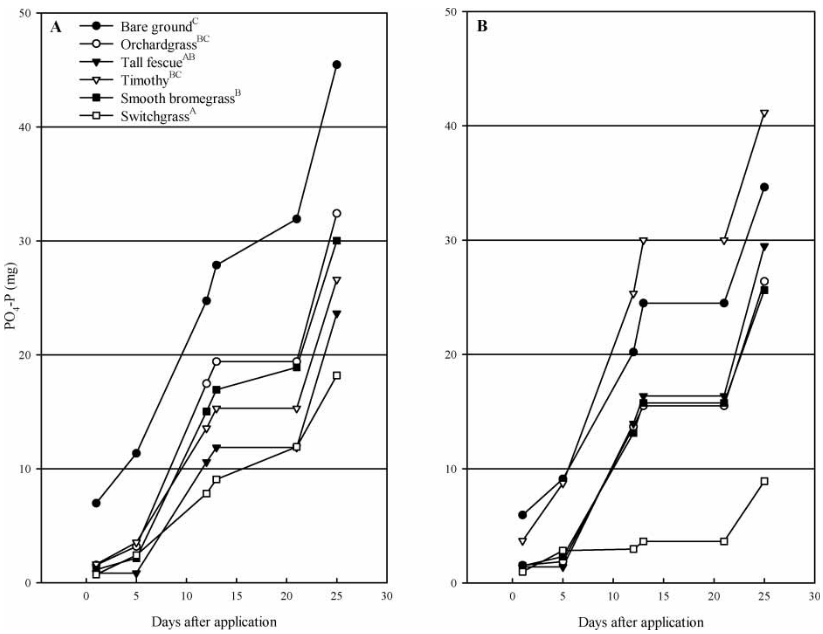

Figure 2. Cumulative PO_4-P in leachate for (A) atrazine-treated and (B) isoxaflutole-treated lysimeters ($n = 3$). Means followed by the same letter did not significantly differ from each other at 10% level of probability using the LSD test. The effects of herbicides ($P = 0.86$) and their interaction with grass treatments ($P = 0.49$) were not significant.

Table 1. Residual NO_3-N mass (mg) in atrazine and isoxaflutole-treated lysimeter soils (n = 3)

Treatment	Bare ground^A	Orchard-grass ^{B}	Tall Fescue^C	Timothy^{BC}	Smooth bromegrass^C	Switchgrass^C
Atrazine^D	338	217	32.0	114	51.0	43.0
Balance^D	408	217	33.0	118	21.0	35.0

Note: Means followed by the same letter did not significantly differ from each other at 10% level of probability using the LSD test. The effects of herbicides (P = 0.77) and their interaction with grass treatments (P = 0.93) were not significant.

switch grass, and tall fescue exhibited the highest capacity to remove NO_3-N from soil. Relative to the NO_3-N level in the bare ground control, tall fescue, smooth bromegrass, switchgrass, timothy, and orchardgrass reduced NO_3-N in the soils by 91.2, 89.8, 89.3, 79.0, and 40.9%, respectively. As was the case for NO_3-N in leachate, no significant differences between herbicide treatments were observed with respect to residual NO_3-N in soil ($P = 0.77$).

Nitrate Dissipation in Soil

Evaluation of the NO_3^- dissipation rates indicated that the grass treatments significantly enhanced NO_3^- loss from soil (Figure 3). The average NO_3^- dissipation rates were found to be greatest in soil collected from switchgrass, tall fescue, and smooth bromegrass treatments ($P < 0.1$). However, NO_3^- dissipation rates between bare ground treatment and other grass treatments were not significantly different ($P > 0.1$). Dissipation rates were similar between ATR and IXF treatments. The maximum rate of NO_3-N dissipation was observed between 72 and 96 h after incubation. Elevated NO_3-N levels at 138 h are likely to be associated with the beginning of nitrification of organic soil N. It has been shown that under experimental conditions similar to those used here, nitrification usually begins approximately 120 h into the incubation period (Tchobanoglous and Schroeder 1987). For ATR-treated lysimeters, the maximum dissipation rate strongly correlated with elevated microbial biomass C in forage treatments (Figure 4A). On the other hand, this correlation was poor in the soil collected from IXF -treated lysimeters (Figure 4B). Smith and Tiedje (1979) reported faster denitrification rates in planted soil than unplanted. However, much faster NO_3-N dissipation rates were reported in a similar study conducted by Reddy, Sacco, and Graetz (1980) using an organic soil (45.1% total C content). In their work, almost 80% of initial NO_3-N was lost within 48 h after incubation. In contrast, the microbial NO_3-N reduction rates for all the treatments in the work reported here were much lower, and the maximum reduction rate occurred at a later incubation time. The low C source of the soil (a sandy loam soil with

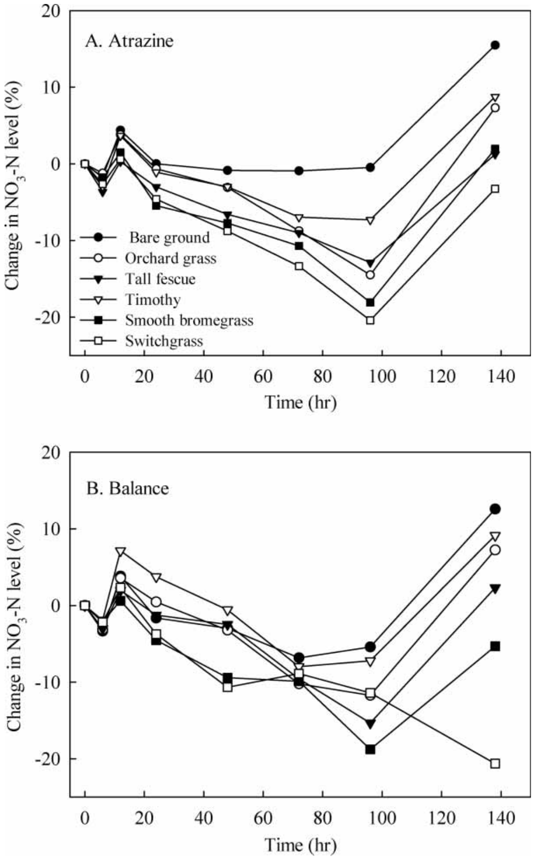

Figure 3. Microbial NO_3^- dissipation rates in soils collected from (A) atrazine-and (B) isoxaflutole-treated lysimeter soil (n = 3).

organic content of 0.72%) used here is believed to be the major factor accounting for the low dissipation rates. For example, low C availability can dramatically decrease denitrification rates in soils (Reddy, Sacco, and Graetz 1980).

The reduction of NO_3-N in soils and leachate of the forage treatments was likely achieved by a combination of enhanced denitrification activity, increased denitrifier population, plant uptake, and NO_3^- assimilation into microbial biomass (Reddy, Sacco, and Graetz 1980; Liu et al. 1997). Qian, Doran, and Walters (1997) reported a 1.5-fold greater maximum denitrification rate and 77% greater cumulative denitrification loss during early growth stages in planted soil as compared to bare soil. The higher microbial NO_3^- dissipation found in soils collected from switchgrass, tall fescue, and smooth bromegrass coincided with their greater capacity to remove NO_3^- from the system. Additional evidence of enhanced microbial activity in the

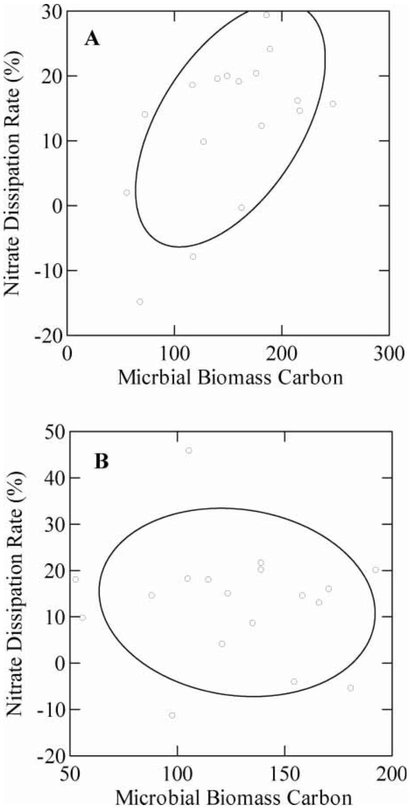

Figure 4. Maximum microbial NO_3^- dissipation rates versus microbial biomass carbon for (A) atrazine-treated (correlation coefficient = 0.53; P = 0.026) and (B) isoxaflutole-treated lysimeters (correlation coefficient = −0.117; P = 0.643). The size of the ellipse is defined by one standard deviation (probability value = 0.6827).

rhizosphere of these same experimental units was previously reported (Lin et al. 2004, 2003). Degradation of atrazine by microbial dealkylation was increased 45–55% in grass treatments compared to the bare ground control. Moreover, microbial biomass C of ATR-treated forage grasses was significantly correlated to overall atrazine degradation in this study. Compared to the control, forage treatments showed an increase in microbial biomass C of 83 to 119% when exposed to ATR and 114% to 185% when exposed to IXF. However, the difference in microbial biomass C between forage treatments was not significant at the tested confidence level ($\alpha = 0.1$). Although the population of denitrifiers was not directly quantified in this work, the enhanced microbial biomass C and microbial NO_3^- dissipation rates strongly suggested increased denitrification activity and/or increased denitrifier populations. According to previous studies conducted in a similar system, denitrification may account for 12.5 to 38% of total NO_3^- loss in forage grass treatments (Horwath et al. 1998; Pu et al. 2001).

The physiological and morphological differences between species may dictate the predominant NO_3^- removal mechanisms in the lysimeter system. C_4 species partition more of the plant total fresh weight to roots and exhibit greater root NO_3^- reductase activity and N utilization efficiency than C_3 grasses (Dhawan and Goyal 2004; Jiang, Hull, and Sullivan 2002). Greater partitioning of carbohydrates to the root system enhances root-derived, water-soluble, and bioavailable organic C in soils and stimulates microbial denitrifier populations and activities in C_4 plantations (Corre, Schnabel, and Shaffer 1999; Pu et al. 2001; Qian, Doran, and Walters 1997). As a result, C_4 plants harbor higher numbers of bacteria and actinomycetes than C_3 plants (Mahmood and Renuka 1990; Qian, Doran, and Walters 1997). It has been estimated that about 15% of the microbial biomass is contributed from root-derived C under maize (*Zea mays* L.) production (Mahmood and Renuka 1990; Qian, Doran, and Walters 1997). C_3 species generally require more NO_3^- to grow and display higher NO_3^- uptake and transpiration rates than C_4 species (Jiang, Hull, and Sullivan 2002; Sehtiya and Goyal 2000). Higher transpiration rates were also observed in the C_3 species treatments in this study (Table 2). Therefore, plant uptake is expected to be the predominant NO_3^- mitigation mechanism in the tall fescue and smooth bromegrass lysimeters. Bartholomew, Nelson, and Werkman (1950) and Hamid (1972) observed this behavior in ^{15}N-labeled studies conducted on a number of C_3 species. Although switchgrass has a lower N requirement and NO_3^- uptake rate than the C_3 species, its greater root NO_3^- reductase and microbial denitrifier activities facilitated the greater NO_3^- dissipation rates observed in this and other studies (Jiang, Hull, and Sullivan 2002; Lin et al. 2001; Mitchell et al. 1997; Sehtiya and Goyal 2000). Preliminary results from a growth chamber study indicated that soil enzymatic activities (dehydrogenase, β-glucosidase, and fluorescein diacetate hydrolysis) and soil mineralization rates of ATR

Table 2. Average total transpiration rates and leachate volumes collected over the 25-day experimental period (n = 6)

Parmeter	Bare ground	Orchardgrass	Tall fescue	Timothy	Smooth bromegrass	Switchgrass
Average total transpiration rate (mmol $H_2O \cdot s^{-1}$)	—	15.1^B	13.1^B	5.5^C	21.7^A	2.1^C
Average leachate volume (L)	63.8^A	37.2^B	30.4^B	36.6^B	42.9^B	49.6^B

Note: Means followed by the same letter in the same rows did not significantly differ from each other at 10% level of probability using the LSD test.

were significantly higher in the rhizosphere of C_4 species than C_3 species (Lin et al. 2005).

The dry-matter yields between two herbicide treatments were similar (Figure 5), and no herbicide injury was observed. The poor performance of timothy was mainly attributed to its sensitivity to the heat stress that may have occurred during the experiment (Lin et al. 2004). In general, C_3 species are more sensitive to ATR than C_4 species, whereas switchgrass is more sensitive to IXF than C_3 grasses (Lin et al. 2004). In a greenhouse study, injury symptoms and growth inhibition were not observed until the soil herbicide concentrations reached 1000 μg/kg for ATR and 200 μg/kg for IXF (Lin et al. 2004). The concentrations applied in this study are representative of those expected in surface runoff from cropland in northern Missouri (USDA 1995). In this study, the herbicide treatments received the equivalent of about 5 μg/kg of ATR and 0.8 μg/kg of IXF in the lysimeter soils. These soil herbicide concentrations are much lower than the concentrations required to affect photosynthesis and other physiological processes in mature plants and/or interfere with denitrification in the rhizosphere (Cervelli and Rolston 1983; Lin 2004, 2003; Somda, Phatak, and Mills 1990; Yeomans and Bremner 1987). However, accumulation of herbicides due to multiple surface runoff events in agronomic field conditions may significantly affect the physiological development of grasses and, therefore, compromise their ability to take up NO_3^-. Herbicide injury or growth inhibition is often observed in grasses along waterways in traditional agronomic cropping systems (personal field observations). When data for all species were pooled, both grass dry-matter yield and microbial biomass C were negatively correlated with NO_3-N levels in leachate, soil, and leachate plus soil for both herbicide treatments (Table 3). This correlation was more significant in

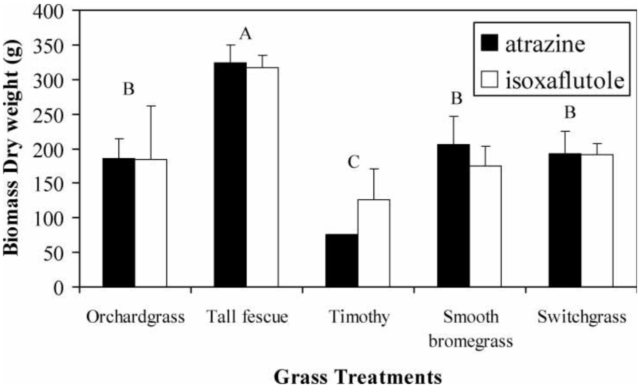

Figure 5. Total aboveground biomass dry weight of five forage treatments. Means followed by the same letter did not significantly differ from each other at 10% level of probability using the LSD test. The effects of herbicides and their interaction with grass treatments were not significant.

Table 3. Correlation coefficients of grass dry-matter yield or microbial biomass carbon with NO_3-N level in lysimeter leachate and soil treated with atrazine or isoxaflutole

	Atrazine			Isoxaflutole		
Parameter	NO_3-N in leachate	NO_3-N in soil	NO_3-N in soil + leachate	NO_3-N in leachate	NO_3-N in Soil	NO_3-N in soil + leachate
Microbial biomass carbon	-0.83^{***}	-0.57^{**}	-0.78^{***}	-0.61^{**}	-0.41^{*}	-0.56^{*}
Grass dry weight	-0.82^{***}	-0.60^{**}	-0.84^{***}	-0.72^{**}	-0.58^{**}	-0.68^{**}

Note: Significance levels: $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$.

ATR treatments than in IXF treatments. The reduced leachate volume resulting from higher transpiration rates in the grass treatments also partially accounted for the reductions in NO_3-N and PO_4-P levels in leachate (Table 2) (Goderya et al. 1996; Lin et al. 2003; Yoneyama 1984). In summary, switchgrass, smooth bromegrass, and tall fescue appear to be desirable forages for incorporation into multispecies riparian buffer strip systems to remove deposited nutrients in the rhizosphere. The incorporation of these forage species will substantially reduce the amount of NO_3^- and PO_4^{3-} reaching shallow aquifers or discharged to surface runoff.

CONCLUSIONS

This study demonstrated that forage treatments significantly reduced nutrient leaching to shallow groundwater and residual soil NO_3-N when exposed to ATR and IXF at levels representing surface runoff from cropland. Switchgrass, smooth bromegrass, and tall fescue exhibited the greatest ability to remove NO_3^- from the soil–water system. Among the grass treatments, switchgrass and smooth bromegrass also exhibited the highest soil NO_3^- dissipation rates. Thus, two C_3 species and the one C_4 species evaluated showed a superior potential to reduce offsite nutrient transport. This has important implications for optimizing the design of vegetative buffers. In the midwestern United States, the highest seasonal NO_3^- loss in surface runoff would be expected in April and May following N fertilizer application to row-crop fields, and the highest seasonal NO_3^- leaching will occur in fall because of groundwater recharge. Thus, the early and late season transport of nutrients coincides with the enhanced growth period of C_3 species. However, at midseason, surface runoff events will correspond to the enhanced growth period of C_4 species. Therefore, from a management standpoint, field implementation of a grass buffer system that employs strips of C_3 and C_4 grasses would appear to be more effective than either grass type alone. Further information such as competition for light and other resources, as well as stand persistence, is required to better understand the performance of such a system under field conditions.

ACKNOWLEDGMENTS

The authors thank the Center for Agroforestry at the University of Missouri for funding this research project. Disclaimer: This work was funded through the University of Missouri Center for Agroforestry under cooperative agreement 58-6227-1-004 with the Agricultural Research Service (ARS). Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the authors and do not necessarily reflect the view of the U.S. Department of Agriculture (USDA). Mention of specific companies, products, or trade names is made only to provide information to the reader and does not constitute endorsement by the USDA—Agricultural Research Service.

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