

2,4-D metabolism: Pathway of degradation of chlorocatechol by *Arthrobacter* sp. J. Agric. Food Chem. 17:1021-1026.
 Topp, E., D.W. Gutzman, and B. Bourgoin. 1995. Rapid mineralization of the herbicide atrazine in alluvial sediments and enrichment cultures. Environ. Toxicol. Chem. 14:743-747.
 Vanderheyden, V., P. Debongnie, and L. Pussemier. 1997. Accelerated

degradation and mineralization of atrazine in surface and subsurface soil materials. Pestic. Sci. 49:237-242.
 Wolf, D.C., and J.P. Martin. 1975. Microbial decomposition of ring-¹⁴C atrazine, cyanuric acid and 2-chloro-4,6-diamino-s-triazine. J. Environ. Qual. 4:134-139.

Alfalfa Rapidly Remediate Excess Inorganic Nitrogen at a Fertilizer Spill Site

Michael P. Russelle,* JoAnn F. S. Lamb, Bruce R. Montgomery, Donald W. Elsenheimer, Bradley S. Miller, and Carroll P. Vance

ABSTRACT

By 1996, standard remediation techniques had significantly reduced the concentration of nitrate nitrogen (NO_3^- -N) in local ground water at the site of a 1989 anhydrous ammonia spill, but NO_3^- -N concentrations in portions of the site still exceeded the public drinking water standard. Our objective was to determine whether local soil and ground water quality could be improved with alfalfa (*Medicago sativa* L.). A 3-yr study was conducted in replicated plots (24 by 30 m) located hydrologically upgradient of the ground water under the spill site. Three alfalfa entries ['Agate', Ineffective Agate (a non- N_2 -fixing elite germplasm similar to Agate), and MWNC-4 (an experimental germplasm)] were seeded in the spring of 1996. Corn (*Zea mays* L.) or wheat (*Triticum aestivum* L.) was seeded adjacent to the alfalfa each year. Crops were irrigated with N-containing ground water to meet water demand. During the 3-yr period, about 540 kg of inorganic N was removed from the aquifer through irrigation of 4.9 million L water. Cumulative N removal from the site over 3 yr was 972 kg N ha^{-1} in Ineffective Agate alfalfa hay, compared with 287 kg N ha^{-1} for the annual cereal grain. Soil solution NO_3^- concentrations were reduced to low and stable levels by alfalfa, but were more variable under the annual crops. Ground water quality improved, as evidenced by irrigation water N concentration. We do not know how much N was removed by the N_2 -fixing alfalfas, but it appears that either fixing or non- N_2 -fixing alfalfa will effectively remove inorganic N from N-affected sites.

NITROGEN-LADEN ground water is a well-documented human health risk in rural areas of North America. The pervasive and regional nature of NO_3^- -contaminated ground water often makes identification of point sources of these effects difficult. Accidental spills during transport of fertilizer, however, are a known point source of N contamination. In Minnesota during 1998, fertilizer spills occurred from two railroad accidents and 30 other transportation-related accidents (P. Kelly, Minnesota Dep. of Agric., personal communication, 1999). Some spills are relatively easy to clean up; for example, when the materials and soil can be removed from the site and landspread. There is a need, however, for low-

cost remediation techniques when these approaches fail and when inorganic N affects ground water quality. Using agricultural cropping systems to remove the excess N (phytoremediation) could be a practical and cost-effective approach at many sites.

Nitrogen content of crops is a function of dry matter production and crude protein concentration. Many cool-season perennial forage crops have a longer growing season, greater dry matter production, higher water use, higher protein concentrations, and deeper root systems than annual crops. As a result, these perennial forages can remove more N from soil and water than annual crops. If the NO_3^- supply is large, however, some crops will assimilate the N slowly and accumulate NO_3^- . High NO_3^- concentration is not desirable from the standpoint of use as animal feed, because of potential NO_3^- toxicity.

One perennial forage that typically does not accumulate NO_3^- (Howarth, 1988) and is widely adapted in temperate regions is alfalfa. Although alfalfa can obtain most of the N required for growth via symbiotic N_2 fixation, it also is very effective in removing inorganic N from the soil.

Stewart et al. (1968) suggested that alfalfa could be used to prevent ground water contamination by nitrate. For nearly three decades, this idea was supported by indirect evidence—reduced soil NO_3^- contents under alfalfa in semiarid or dry subhumid environments (Schertz and Miller, 1972; Mathers et al., 1975; Muir et al., 1976; Schuman and Elliott, 1978) and very small leaching losses of NO_3^- under tile-drained alfalfa, in contrast with larger losses under annual crops (Bergström, 1987; Randall et al., 1997). However, there remains a widespread misconception that legumes like alfalfa are not particularly effective at absorbing inorganic N from the soil.

The first direct evidence that alfalfa could remove large amounts of subsoil NO_3^- was obtained with ¹⁵N isotope studies. In this and subsequent research (Blumenthal and Russelle, 1996; Blumenthal et al., 1999), the researchers found that ineffectively nodulated alfalfas, which are unable to obtain N from the atmosphere, removed 30 to 40% more subsoil NO_3^- than N_2 -fixing alfalfas. Moreover, these studies confirmed earlier research (Lamb et al., 1995) that symbiotic N_2 fixation in standard alfalfas continued, albeit at reduced rates, even with high NO_3^- supply. This raised the question, Would non- N_2 -fixing alfalfa be the better choice to remove

M.P. Russelle, J.F.S. Lamb, and C.P. Vance, USDA-ARS, 439 Borlaug Hall, Univ. of Minnesota, St. Paul, MN 55108-6028; B.R. Montgomery, Minnesota Dep. of Agriculture, 90 W. Plato Blvd., St. Paul, MN 55107; D.W. Elsenheimer, HDR Engineering, 6190 Golden Hills Dr., Minneapolis, MN 55416; and B.S. Miller, Dakota Growers Pasta Co., Carrington, ND 58421. Joint contribution of the USDA-ARS and the Minn. Agric. Exp. Stn. Paper no. 00125 of the MAES scientific journal series. Received 13 Mar. 2000. *Corresponding author (russelle@soils.umn.edu).

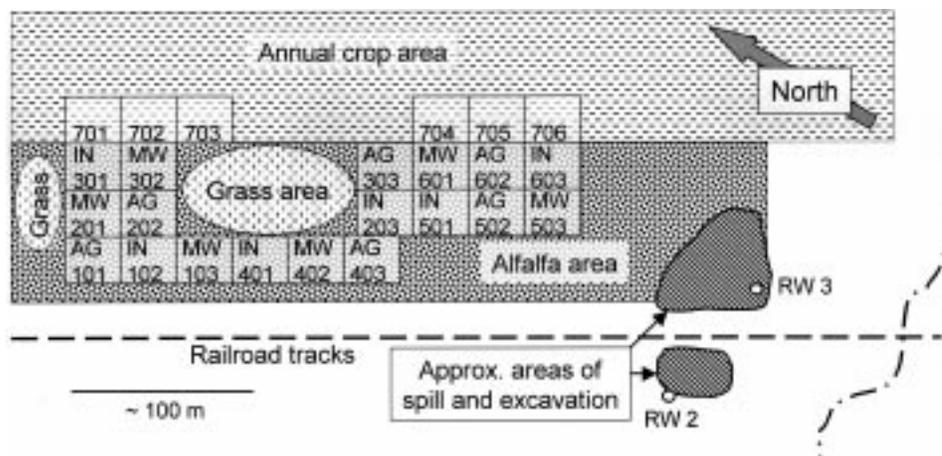


Fig. 1. Schematic diagram of the plot layout near Bordulac, ND. Creek (dotted line) to the south of the spill site drains eastward into Lake George. Abbreviations: AN = annual crop; AG = Agate alfalfa; IN = Ineffective Agate alfalfa; MW = MWNC alfalfa; RW = recovery well.

excess NO_3^- than N_2 -fixing alfalfa? Our primary objective in this research was to evaluate the performance of alfalfa in removing excess inorganic N from contaminated soil and ground water at an actual remediation site. Our secondary objective was to compare N removal by ineffectively nodulated alfalfa and N_2 -fixing alfalfas.

MATERIALS AND METHODS

Site History

In February 1989, a train derailment near the town of Bordulac, ND, resulted in the rupture of three tank cars of anhydrous ammonia (NH_3). Approximately 170 000 L of NH_3 was released. An undetermined quantity of urea fertilizer also was released from five derailed hopper cars, providing an additional source of N at the site. While a portion of the NH_3 was volatilized and transported offsite as vapor, most of the N infiltrated the soil, contaminating soils and shallow ground water at the site.

Initial site cleanup included excavation of about 7000 m^3 of N-affected soil and replacement with clean soil. Excavated soil was removed to about 0.6 m below the 1-m-deep water table and was transported offsite. Ammonia- and NO_3^- -contaminated ground water was withdrawn from two recovery wells downgradient of the spill zone during 1989 and 1990, transported offsite, and discharged to a publicly owned wastewater lagoon. During the following 5 yr, a *pump-and-treat* approach was used by extracting ground water and using it to irrigate annual crops on land adjacent to and upgradient of the spill zone. Although about 30 million L of ground water had been extracted by the end of 1995, these traditional methods of remediation had not restored ground water NO_3^- concentrations to levels below the state mandated cleanup goal of 10 $\text{mg NO}_3^- \text{-N L}^{-1}$, the public drinking water standard.

General Approach

The phytoremediation approach, essentially *pump-and-treat* using fertigation on alfalfa, involved three principal components:

- (i) Plant the derailment site and upgradient field area with alfalfa;
- (ii) Use N-containing ground water to irrigate alfalfa planted in the upgradient field at rates that do not greatly exceed the N uptake capacity of the crop; and
- (iii) Optimize irrigation management to minimize yield re-

ductions from water stress, NO_3^- concentration in the soil solution at the bottom of the root zone, and water movement beneath the alfalfa root zone.

Plot Establishment

Soils at the site are Hamerly loam (fine-loamy, mixed, superactive, frigid Aeric Calciaquoll) and Wyard loam (fine-loamy, mixed, superactive, frigid Typic Endoaquoll). Topsoil samples (0 to 15 cm) were taken on 24 Apr. 1996 from areas of the proposed plots. Air-dried and ground samples were analyzed by the University of Minnesota Soil Testing Laboratory for pH (1:1 soil to water), extractable P by both Olsen and Bray-P1 extractants (Olsen only on samples with pH > 7.5), and exchangeable K (1 M ammonium acetate). Based on these analyses, the site was fertilized on 17 May with 22 kg N ha^{-1} , 48 kg P ha^{-1} , and 112 kg K ha^{-1} (N-containing fertilizer was used, because other sources of P were unavailable). On 22 May, 4 kg ha^{-1} EPTC (*S*-ethyl dipropylthiocarbamate) was applied and incorporated for weed control.

Plots were located north of the original spill site, upgradient with respect to ground water flow. Replicates 2 and 3 were divided to avoid a large, poorly drained area near the center of the site (Fig. 1). The experimental design was a randomized complete block with six replicates, and individual plots were 24 by 30 m in size. Treatments, assigned randomly to plots within replicates, consisted of three alfalfa entries.

Secondary tillage and packing was followed by alfalfa seeding on 29 and 30 May. Seed of the three alfalfas, Agate, Ineffective Agate, and Minnesota experimental MWNC-4, was inoculated with a commercial mixture of *Sinorhizobium meliloti* strains before planting. Agate is a winter dormant cultivar with resistance to root rot caused by *Phytophthora medicoginis* (Barnes and Frosheiser, 1973). Ineffective Agate alfalfa is an elite germplasm selected out of Agate that is genetically incapable of fixing atmospheric N_2 (Barnes et al., 1990). The Minnesota experimental germplasm MWNC-4 has multiple pest resistance and is less dormant and more productive than Agate. Both Agate and MWNC-4 have effective N_2 -fixing symbioses. The remainder of the site was planted with Pioneer 5246,¹ an adapted N_2 -fixing commercial alfalfa cultivar.

¹ Names are necessary to report factually on available data; however, the USDA and the Univ. of Minnesota neither guarantee nor warrant the standard of the product, and the use of the name by the USDA and the Univ. of Minnesota implies no approval of the product to the exclusion of others that may be suitable.

All alfalfa germplasms were planted at a rate of about 550 seeds m^{-2} (about 15 kg ha^{-1}) in rows spaced 15 cm apart. Areas with thin stands were reseeded by hand on 9 and 10 July 1996. After heavy rainfall during midsummer, alfalfa was killed in some areas of several plots where standing water collected. These areas were reseeded on 16 June 1997 and low, frequently wet locations were seeded with 'Palaton' reed canarygrass (*Phalaris arundinacea* L.).

Land adjacent to the site was managed by a local farmer, who planted corn in late May 1996, wheat on 9 June 1997, and corn on 16 May 1998. In each year, hand planting was required in some plots inaccessible to the farmer's large equipment. The farmer reported that he applied about 108 kg N ha^{-1} and 16 kg P ha^{-1} before planting wheat in 1997.

Irrigation and Water Quality

A combination of excessive rainfall and irrigation system difficulties limited the amount of irrigation at the site in 1996 and 1997. During the establishment year, the entire plot area (2.8 ha) was irrigated only twice (in June and September), and one-half of the area was irrigated in early July before heavy rainfall occurred. The moving gun system irrigated a 24-m radius and moved longitudinally between the first two (westernmost) ranks of alfalfa plots and between the alfalfa and annual crop plots. Irrigation water was supplied by a recovery well (RW3, Fig. 1) during the first two years. Water samples were collected from the rain gauges in each plot after volumes were determined, and samples were frozen until analysis.

Irrigation did not begin in 1997 until 31 July, because of equipment problems and wet soil conditions. Wheat was in the late boot to early heading growth stage at this time and was not irrigated to avoid lodging. Rain gauges were used to assess water application rate in each plot. Water samples for analysis were procured at the sprinkler head and were frozen until analysis.

Declining water yield from the recovery wells restricted irrigation in 1998, resulting in irrigation cycles taking much longer than planned. Water yields were reduced by a 1-m drop in the water table elevation and the low hydraulic conductivity of the aquifer matrix. We attempted to improve water yield in RW3 by adding water from RW2 (Fig. 1) during and between irrigation runs. The entire plot area received five cycles of irrigation. Water was sampled as in 1997.

Nine samples of irrigation water were collected in 1996, 18 in 1997, and 114 in 1998. All irrigation water samples were analyzed for NH_4^+-N (Diamond, 1997) and $NO_2^- + NO_3^- - N$ (Wendt, 1997) using flow injection analysis. Although the analytical method determines both $NO_2^- - N$ and $NO_3^- - N$, we refer in this paper to NO_3^- alone, because NO_2^- is typically a very small fraction of oxidized N in soil, plants, and water. Nitrogen removal from the aquifer was based on application rate on each plot and water samples taken at the irrigation gun while each plot was being irrigated.

Dry Matter and Nitrogen Yield

Alfalfa was sampled by removing herbage from one, two, or three 1- m^2 areas per plot a day or two before a cooperating farmer harvested the field. Harvests occurred in August 1996, in June, July, and August 1997, and in May, July, August, and October 1998. Herbage samples were dried at 60°C, weighed, and ground for analysis. No fall harvest was taken in 1996 or 1997 due to wet soil conditions and to assure that the stands would overwinter successfully. Corn stover and grain yields were measured on 16 Oct. 1996 and 4 Nov. 1998 from 3- by 3-m areas, and subsamples were taken for total N and dry matter determination. Aboveground wheat biomass samples

were acquired from 1- m^2 plots in each replicate on 9 Sept. 1997. After oven-drying, grain was separated from cobs or heads and samples were weighed and ground for analysis.

Plant tissue was analyzed for total N by either dry combustion (LECO CN-2000, LECO Analytics, St. Joseph, MI) or flow injection analysis of standard semimicro Kjeldahl digests with a salicylic acid-sodium thiosulfate predigestion for quantitative reduction of NO_3^- (Bremner and Mulvaney, 1982; Diamond, 1992). Separate determinations of $NO_3^- - N$ concentrations were made using flow injection analysis (Sechtig, 1992b) of samples from 1997 and 1998 extracted with 2% (v/v) acetic acid.

Ground Water

Two ground water recovery wells (RW2 and RW3, Fig. 1) had been installed in the source area of the derailment site, which served as the source of water for the irrigation system. Ground water quality within the surficial aquifer was monitored by a network of wells screened between about 1.6 and 2.6 m below the soil surface. One nested well was screened beginning at 3 m below the initial water table surface; all analyses from this well had $NO_3^- - N$ concentrations below the public drinking water standard, which indicated that the derailment affected only the uppermost level of the aquifer. Details of the ground water collection methods and results will be provided elsewhere (Elsenheimer et al., unpublished data, 2000).

Soil Cores and Soil Solution Samples

In May 1996, ceramic suction cup samplers were installed near the center of three of the six replicates, two samplers positioned about 30 cm above and two about 30 cm below the existing saturated zone. Apparent water table depth, and therefore suction cup sampler depth, varied from plot to plot. In alfalfa plots, shallow suction cup samplers were located 1.7, 2.0, and 2.2 m deep in Replicates 1, 5, and 6, respectively, and deep samplers were located 2.3, 2.7, and 2.8 m deep in the respective replicates. In the grain plots, shallow samplers were placed about 2.1 m deep in all three replicates; only one replicate had deep samplers, and these were placed 2.8 m deep.

Each suction cup sampler was comprised of a 60-cm-long polyvinyl chloride (PVC) tube with a 0.05-MPa, round-bottom ceramic cup. Ceramic cups were set in a slurry of soil from the depth of installation, a bentonite plug was added near the top of the samplers, and soil from the access hole was used to fill the remaining hole. Vacuum tubing from the suction cup samplers was sheathed in PVC pipe to protect it from gnawing animals. The open end of the PVC tube was filled with expanding foam to prevent entry of irrigation or precipitation water. Tubing was attached at a convenient height for sampling on a 10- by 10-cm post set in line with the samplers. A standard 10-cm-diam. rain gauge was placed on each post. Soil solution samples were collected approximately every 2 wk during the growing season each year, refrigerated in the field, and frozen until analysis. Samples were diluted 30 times with deionized water to avoid undetermined interferences during flow injection analysis (Diamond, 1997; Wendt, 1997).

Soil cores were obtained from each of the instrumented plots (three replicates, two cores per plot, composited by depth) in May 1996 and two to three cores per plot were obtained from all six replicates in October 1997 and mid-August 1998. No cores were obtained from the grain plots in 1998. Cores were taken to the saturated zone, divided into 30-cm depth increments, oven-dried at about 45°C, and ground. Dry bulk density was determined on separate cores. Mean bulk densities for the plot area were 1.19, 1.13, 1.29, 1.38, 1.40, and 1.56 $g\ cm^{-3}$ for the 30-cm increments beginning

at the soil surface and ending at the 1.8-m depth. Soil samples (3 g) were shaken in 30 mL of 2 M KCl for 1 h and centrifuged. The $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ concentrations in the supernatant were determined by colorimetry on a flow injection analyzer (Sechtig, 1992a; Switala, 1993).

Statistical Analyses

Comparisons among alfalfa treatments were made using standard analysis of variance with a randomized complete block design with three or six replicates. Time was considered a subplot effect for measurements taken over time in the same main plot. Results from grain plots were not analyzed with the alfalfa, because crop species were not randomly allocated within replicate blocks, but were aligned in adjacent fields. Linear regression was used to determine trends with time for some measurements. All statistics were calculated using SAS 6.12 (SAS Institute, 1996).

RESULTS AND DISCUSSION

Irrigation Water Quality and Quantity

Higher than normal rainfall limited the amount of irrigation needed and occasional problems occurred with the irrigation equipment. Total irrigation was limited to 1060 m³ in 1996 and 1160 m³ in 1997. Average water application depths were 1.47, 1.68, 2.01, 1.52, and 1.55 cm for the five irrigation cycles in 1998, totaling 8.20 cm for the season. As expected with a single irrigation gun, large plot-to-plot variation occurred due to wind and occasional equipment malfunction. We applied 2610 m³ of ground water in 1998, still considerably less than the desired ~8500 m³ (10 to 12 ha-cm). Application rates in 1998 were adversely affected by slow aquifer recharge rates; the recovery wells were pumped dry on several occasions.

There were large variations in concentrations of both $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ in irrigation water during each irrigation run and among days (Fig. 2). In 1996, irrigation water contained an average of 83 mg $\text{NH}_4^+\text{-N L}^{-1}$ (standard deviation [SD] = 34 mg L^{-1}) and 52 mg $\text{NO}_3^-\text{-N L}^{-1}$ (SD = 17 mg L^{-1}). Average concentrations in 1997 were 97 mg $\text{NH}_4^+\text{-N L}^{-1}$ (SD = 28 mg L^{-1}) and 69 mg $\text{NO}_3^-\text{-N L}^{-1}$ (SD = 16 mg L^{-1}). Concentrations of $\text{NH}_4^+\text{-N}$ declined rapidly during the first 27 d of irrigation in 1998 (mean rate of decline was 1.9 mg $\text{L}^{-1} \text{d}^{-1}$), then remained at about 32 mg L^{-1} (SD = 17 mg L^{-1}) thereafter. During 1998, $\text{NO}_3^-\text{-N}$ concentrations declined by 0.08 mg $\text{L}^{-1} \text{d}^{-1}$, ending at about 23 mg $\text{NO}_3^-\text{-N L}^{-1}$. We do not believe these declines can be explained by addition of water from the second remediation well to the south of the railroad tracks, which had relatively low and constant concentrations of both NH_4^+ (mean 1.7 mg $\text{NH}_4^+\text{-N L}^{-1}$; SD = 1.0 mg $\text{NH}_4^+\text{-N L}^{-1}$) and NO_3^- (mean 8.5 mg $\text{NO}_3^-\text{-N L}^{-1}$; SD = 3.8 mg $\text{NO}_3^-\text{-N L}^{-1}$) during 1998, but do not have sufficient data on amount of water that this well provided to the aquifer near RW3. Addition of water from RW2 throughout the 1998 growing season was necessary to maintain pumping capacity. Separate samples of water from RW3 were not available in 1998.

In *pump-and-treat* systems, one can expect rapid movement of species like NO_3^- to the remediation well, as they move along hydrologic and concentration gradi-

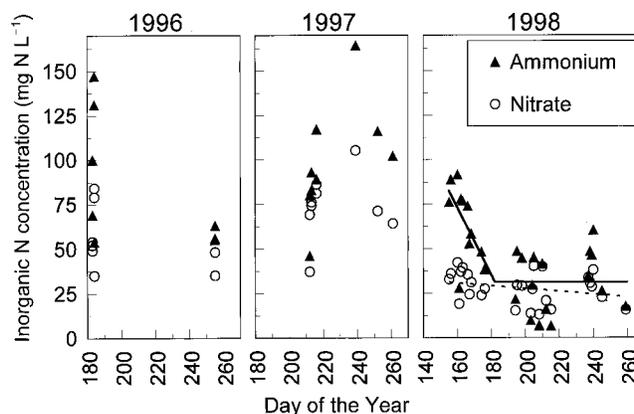


Fig. 2. Ammonium and nitrate concentration in irrigation water during 3 yr of remediation near Bordulac, ND.

ents with relatively little chemical interaction with the soil matrix. Species like NH_4^+ , however, can be expected to be removed much more slowly, as they are sorbed to clays. Both sorption-desorption processes and movement regulated mainly by diffusion slow the replacement of $\text{NH}_4^+\text{-N}$ around the well intake. Thus, the rapidity with which inorganic N can be pumped from a spill site will be related to which ionic species are present. As NH_4^+ is nitrified to NO_3^- , removal rates should increase.

Plots received about 37 to 45 kg N ha^{-1} from ground water in 1996. We estimate that about 90 kg N ha^{-1} was applied via ground water to alfalfa in 1997. Over five irrigation cycles in 1998, we estimate that about 65 kg N ha^{-1} was applied. Thus, a total of 540 kg N was removed from the aquifer over the three cropping years in about 4.9 million L of water applied to about 2.8 ha. With more optimum irrigation design, faster aquifer recharge rates, and normal rainfall in the area, we could have greatly accelerated N removal from the aquifer. For example, using mean inorganic N concentrations in the irrigation water in 1997, 1350 kg N would have been removed in a single season from the aquifer if 10 ha-cm of water had been applied over the site area, resulting in an application rate of 420 kg N ha^{-1} .

Crop Yield and Nitrogen Content

Only one alfalfa harvest was taken during the establishment year and dry matter yields averaged 4.8 Mg ha^{-1} , with no differences among alfalfa entries (Fig. 3). Yields of N_2 -fixing and non- N_2 -fixing alfalfa are expected to be similar when soil inorganic N supplies are high (Lamb et al., 1995). Dry matter yield of corn grain averaged 5.9 Mg ha^{-1} and stover yield was similar. Total N concentration averaged 14.5 g N kg^{-1} in corn grain, 5.3 g kg^{-1} in corn stover, and 28.1 g kg^{-1} in alfalfa herbage. There were no differences in N concentration or N content (average 134 kg N ha^{-1}) among alfalfa entries. Corn N uptake was 85 kg N ha^{-1} in grain and 31 kg N ha^{-1} in stover. Only grain was removed from the corn area.

Alfalfa forage yields in 1997 totaled 11.9 Mg ha^{-1} dry matter after three harvests. Wheat in the adjacent plot area yielded 2.8 Mg dry grain ha^{-1} and 6.0 Mg stubble

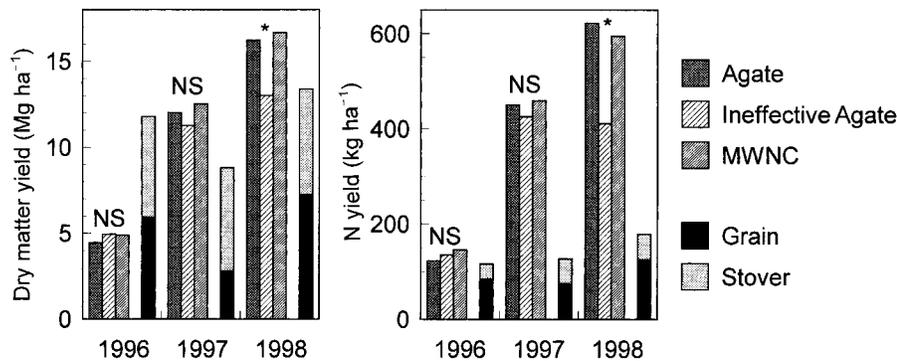


Fig. 3. Annual dry matter yield and N yield in alfalfa herbage and corn (1996 and 1998) and wheat (1997) grain and stover near Bordulac, ND. NS = no statistical difference among alfalfa entries; * = Ineffective Agate mean significantly different from other alfalfa entries ($P < 0.05$).

ha⁻¹. Nitrogen concentration in alfalfa forage in 1997 averaged 36.5 g N kg⁻¹ and about 425 kg N ha⁻¹ was harvested in herbage. Although we left the fall regrowth in the field in order to improve overwintering potential, samples taken in October indicated that an additional 84 kg N ha⁻¹ could have been removed. In contrast, wheat grain contained about 75 kg N ha⁻¹ and an additional 52 kg N ha⁻¹ could have been removed in straw. Wheat grain contained an average of 25.8 g N kg⁻¹ and wheat straw contained an average of 8.3 g N kg⁻¹.

In the first production year (1997), visual differences in alfalfa height and color were noted in some plots on several dates. For example, spring regrowth in Ineffective Agate plots was chlorotic and noticeably shorter than regrowth of the N₂-fixing alfalfa entries on 19 May 1997. However, no statistical differences in yield or N content were detected among the entries, which indicates that N supply (through irrigation and inorganic N in the soil) was sufficient for the non-N₂-fixing alfalfa to match dry matter production of the N₂-fixing types.

Cumulative yield of Ineffective Agate was consistently lower (13.0 Mg ha⁻¹) than that of the N₂-fixing entries (16.5 Mg ha⁻¹) in 1998. Mean grain yield was 7.2 Mg dry matter ha⁻¹ and stover yield averaged 6.1 Mg ha⁻¹. These high corn and alfalfa yields probably were due to lack of water stress and the long growing season, but it is clear that non-N₂-fixing alfalfa yields were limited by inadequate N supply. Non-N₂-fixing alfalfa plots could be identified visually during most of the season by chlorosis. This characteristic of non-N₂-fixing alfalfa would be useful to a site manager not well versed in agronomic management, as an indicator that inorganic N supply in soil and irrigation water are at relatively low levels. The disadvantage is that yield and crude protein concentration of non-N₂-fixing alfalfa suffer with low N supply, which may negatively affect market value.

Total N concentration in alfalfa herbage was 16% higher in the fixing alfalfa (mean 37.0 g kg⁻¹ for the season) than Ineffective Agate (31.8 g kg⁻¹). As a consequence of yield and N concentration differences, total N contained in alfalfa herbage harvested in 1998 was considerably higher for the fixing alfalfas (608 kg N ha⁻¹) than for Ineffective Agate (412 kg N ha⁻¹). Corn grain contained 126 kg N ha⁻¹ and stover contained 53 kg N ha⁻¹ (mean N concentrations of 17.4 and 8.5 g kg⁻¹, respectively). Although total N removal in corn

silage would have been 58 to 74% higher than achieved in the previous 2 yr, total N removal potential with the annual cereal crop remained only 43% as high as removal by Ineffective Agate alfalfa (31% if only corn grain were harvested).

One concern in using plants to remove excess NO₃⁻ is that the resulting forage may contain high concentrations of NO₃⁻, which could be toxic to livestock. Nitrate is conserved in hay, but is decomposed during ensiling. The highest NO₃⁻-N concentrations measured in each of the two production years were 2990 mg kg⁻¹ in 1997 and 2520 mg kg⁻¹ in 1998, which are lower than critical levels for livestock diets composed solely of the analyzed forage (Mayland and Cheeke, 1995). Mean concentrations were similar for the three alfalfas in 1997, but Ineffective Agate contained lower average concentrations (304 mg kg⁻¹) than the two N₂-fixing entries (930 mg kg⁻¹) in 1998. The first two harvests each year contained the highest NO₃ concentrations, and the least was found in the fourth cutting. In no case was there cause for concern in using the hay produced at this site for animal feeding.

Earlier research has shown that estimates of inorganic N uptake in N₂-fixing alfalfa are incorrect when based on the difference in total N contained in herbage of fixing and non-N₂-fixing alfalfas (Blumenthal and Russelle, 1996; Blumenthal et al., 1999). We attempted to assess the proportion of N in the fixing alfalfas derived from N₂ fixation using the ¹⁵N dilution technique, but obtained illogical values because of the highly variable inorganic N supply across the site, which influenced the dilution of added ¹⁵N in the plots (data not shown). Thus, comparison among alfalfa entries for inorganic N removal required evaluation of soil data.

Soil Inorganic Nitrogen—Core Data

In May 1996, NH₄⁺-N concentrations were well within the expected values for soils in this area (mean 5.8 mg kg⁻¹ in the upper 0.3 m and 2.5 mg kg⁻¹ below). Initial soil NO₃⁻-N concentrations in many plots were within the range of what is expected under high fertility conditions under annual cropping, but were higher than expected in some plots (Fig. 4). In some plots, high NO₃⁻-N concentrations were present throughout the sampled soil profile; in others, high concentrations were limited to the upper soil. We do not know why some

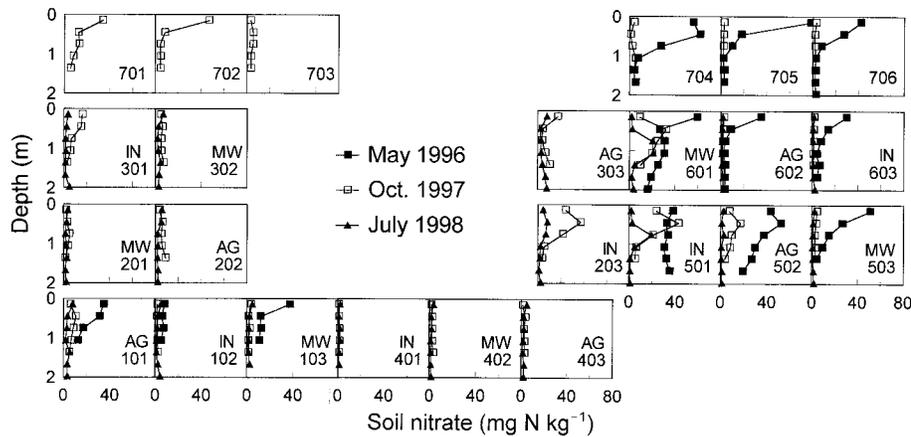


Fig. 4. Bulk soil ammonium and nitrate concentrations in plots at a remediation site near Bordulac, ND. Means of two to three cores removed to the depth of saturated soil. AG = Agate alfalfa; IN = Ineffective Agate alfalfa; MW = MWNC alfalfa.

plots had high NO_3^- -N concentrations. The plots were not located in the immediate area of the derailment site (Fig. 1) and we had expected more uniformly low soil NO_3^- levels. It is possible that some N had been inadvertently moved to the plot area during initial site remediation or by rudimentary irrigation efforts before 1996, that subsequent farming of the plot area had added excess NO_3^- -N, or that a historic source of N existed at the site.

After the first production year (1997), we found no differences among cropping treatments in exchangeable NH_4^+ or NO_3^- concentrations in soil. Ammonium N averaged 3.8 mg N kg^{-1} soil, and did not change appreciably with depth. Nitrate N declined linearly with depth from 5.5 mg kg^{-1} in the 0- to 0.3-m depth to 3.5 mg kg^{-1} in the 1.2- to 1.5-m depth.

A few plots continued to contain rather large amounts of NO_3^- -N (Fig. 4). Estimated amounts of NO_3^- -N in the upper 1.5 m of soil ranged from less than 34 kg N ha^{-1} to about 480 kg N ha^{-1} , with five plots containing more than 340 kg N ha^{-1} . By chance, several of these had not been not selected for the intensive monitoring of soil solutions, soil water content, etc. Most of the NO_3^- was located in the upper part of the soil profile.

Mean total inorganic N content of the soil decreased from 322 to 102 kg N ha^{-1} in the alfalfa plots during the first two seasons, and declined to 67 kg N ha^{-1} during the third. The greatest declines averaged 279 kg N ha^{-1} . The maximum amount of NO_3^- -N remaining in the soil as of mid-August 1998 was 208 kg N ha^{-1} , but most plots contained less than 116 kg NO_3^- -N ha^{-1} . These declines occurred even though the plots were irrigated with N-containing ground water. Due to the spatial heterogeneity of this site, differences in N removal cannot be ascribed to alfalfa type, but these data demonstrate how both N_2 -fixing and non- N_2 -fixing alfalfas can help attain and maintain low soil NO_3^- levels.

Soil Inorganic Nitrogen—Suction Cup Data

Results from the core samples were confirmed by declines in NO_3^- -N concentration of biweekly soil solution samples (Fig. 5). Analyses from deeper suction cup samplers were similar to the shallow samplers and are

not shown here for space considerations. There is evidence of some spatial dependence in NO_3^- -N concentration in subsoil solution. These results correspond well with concentrations of soil NO_3^- -N determined in soil cores taken in fall.

Subsoil NO_3^- -N concentrations declined markedly under all alfalfas. In contrast, soil solution NO_3^- -N concentrations were low initially under corn (1996), but then increased under wheat. Wheat did not receive ground water applications in 1997, so this NO_3^- may have been derived from mineralized soil organic matter N or fertilizer N applied by the farmer.

Differences among samplers were clearly due to specific plot locations (i.e., initial conditions) rather than

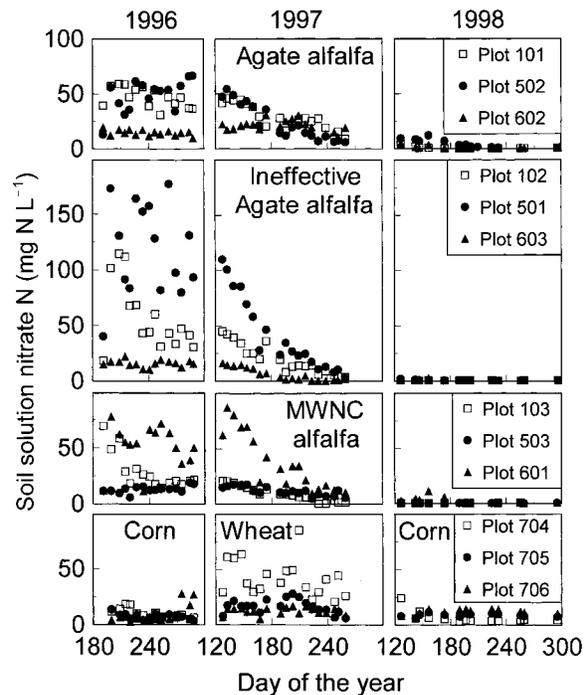


Fig. 5. Soil solution nitrate concentrations obtained in shallow suction cup samplers (1.7 to 2.2 m deep, installed above the water table in 1996) at a remediation site near Bordulac, ND. Most data are means of two samplers per plot, but in some cases only one sampler yielded solution.

effects of the alfalfa entry. However, regardless of how great soil solution NO_3^- concentrations were in summer 1996, they remained below $12 \text{ mg NO}_3^- \text{-N L}^{-1}$ during 1998 in the shallow samplers (1.4 to 1.7 m deep, depending on the replicate, Fig. 5) and below $28 \text{ mg NO}_3^- \text{-N L}^{-1}$ in the deeper soil (1.8 to 2.1 m deep, data not shown).

SUMMARY

Results from this research show that alfalfa is an excellent crop to use for remediation of inorganic N from soil and from ground water via irrigation. Despite problems with the irrigation system and water supply from the remediation wells during the 3 yr of the experiment, we estimate that about 540 kg of inorganic N was removed from the aquifer and applied to the alfalfa and grain crops in about 4.9 million L of water. The average N application rate per unit area was lower than anticipated (200 kg N ha^{-1} over 3 yr) because irrigation was problematic. However, the results clearly show that both total inorganic N in the soil and $\text{NO}_3^- \text{-N}$ concentrations in soil solution declined to low levels under alfalfa.

Total N removal from the site over 3 yr averaged 972 kg N ha^{-1} with the non- N_2 -fixing Ineffective Agate alfalfa hay and was 287 kg N ha^{-1} for the annual cereals (as only grain was harvested). Nitrogen removal in annual cereals could have been 424 kg N ha^{-1} if all above ground material had been harvested. Results from 1998 highlight the conclusion that alfalfa is a better choice for remediation than an annual grain crop. Although total aboveground biomass of Ineffective Agate alfalfa and corn was similar (13 Mg ha^{-1} for Ineffective Agate vs. 13.4 Mg ha^{-1} for corn), N removal by the alfalfa was more than twice as high as corn (412 kg N ha^{-1} vs. 179 kg N ha^{-1}).

Use of ^{15}N isotope dilution was unsuccessful for estimating N_2 -fixation at this site, so we were unable to estimate inorganic N uptake by the N_2 -fixing alfalfas. Given the similarity in decline in soil solution NO_3^- and in soil inorganic N concentrations under all alfalfa entries, it appears that either N_2 -fixing or non- N_2 -fixing alfalfa will perform well in removing inorganic N from contaminated sites. While ineffectively nodulated alfalfa is suited only to sites with very high N supply, strips of this alfalfa planted across any site would indicate when available N supply becomes small. The consistently high yield and forage quality of well-managed N_2 -fixing alfalfa may partially offset expenses associated with the environmental restoration of these sites.

ACKNOWLEDGMENTS

This research was supported in part by a Cooperative Research and Development Agreement between USDA-ARS and the Canadian Pacific Railway. We appreciate the efforts of Dr. D. Klinkebiel, who assisted in plot establishment and conduct of the experiment during the first year; Anne Malan

and Verna Sauer, who managed the plots; Dr. R.A. Henson, who supervised work at the site during the final year; Ms. Lucy Wanschura, who ably coordinated all sample analysis; and to many others who helped collect and process samples.

REFERENCES

- Barnes, D.K., and F.I. Frosheiser. 1973. Registration of Agate alfalfa. *Crop Sci.* 13:768-769.
- Barnes, D.K., G.H. Heichel, C.P. Vance, and R.N. Peaden. 1990. Registration of 'Ineffective Agate' and 'Ineffective Saranac' non- N_2 -fixing alfalfa germplasms. *Crop Sci.* 30:752-753.
- Bergström, L. 1987. Nitrate leaching and drainage from annual and perennial crops in tile-drained plots and lysimeters. *J. Environ. Qual.* 16:11-18.
- Blumenthal, J.M., and M.P. Russelle. 1996. Subsoil nitrate uptake and symbiotic dinitrogen fixation by alfalfa. *Agron. J.* 88:909-915.
- Blumenthal, J.M., M.P. Russelle, and J.F.S. Lamb. 1999. Subsoil nitrate and bromide uptake by contrasting alfalfa entries. *Agron. J.* 91:269-275.
- Bremner, J.M., and C.S. Mulvaney. 1982. Nitrogen—Total, p. 595-624. *In* A.L. Page et al. (ed.) *Methods of soil analysis. Part 2. Chemical and microbiological properties.* 2nd ed. Agron. Monogr. 9. ASA and SSSA, Madison, WI.
- Diamond, D. 1992. Total Kjeldahl nitrogen in soil/plant. QuikChem Method 13-107-06-2-D. Lachat Instruments, Milwaukee, WI.
- Diamond, D. 1997. Determination of ammonia (phenolate) by flow injection analysis. QuikChem Method 10-107-06-1-I. Lachat Instruments, Milwaukee, WI.
- Howarth, R.E. 1988. Antiquity factors and nonnutritive chemical components. p. 493-514. *In* A.A. Hansen et al. (ed.) *Alfalfa and alfalfa improvement.* Agron. Monogr. 29. ASA, CSSA, and SSSA, Madison, WI.
- Lamb, J.F.S., D.K. Barnes, M.P. Russelle, C.P. Vance, G.H. Heichel, and K.I. Henjum. 1995. Ineffectively and effectively nodulated alfalfas demonstrate biological nitrogen fixation continues with high nitrogen fertilization. *Crop Sci.* 35:153-157.
- Mathers, A.C., B.A. Stewart, and B. Blair. 1975. Nitrate-nitrogen removal from soil profiles by alfalfa. *J. Environ. Qual.* 4:403-405.
- Mayland, H.F., and P.R. Cheeke. 1995. Forage-induced animal disorders. p. 121-135. *In* R.F. Barnes et al. (ed.) *Forages. The science of grassland agriculture.* 5th ed. Iowa State Univ. Press, Ames, IA.
- Muir, J., J.S. Boyce, E.C. Seim, P.N. Mosher, E.J. Deibert, and R.A. Olson. 1976. Influence of crop management practices on nutrient movement below the root zone. *J. Environ. Qual.* 5:255-259.
- Randall, G.W., D.R. Huggins, M.P. Russelle, D.J. Fuchs, W.W. Nelson, and J.L. Anderson. 1997. Nitrate losses through subsurface tile drainage in Conservation Reserve Program, alfalfa, and row crop systems. *J. Environ. Qual.* 26:1240-1247.
- SAS Institute. 1996. SAS 6.12. SAS Inst., Cary, NC.
- Schertz, D.L., and D.A. Miller. 1972. Nitrate-N accumulation in the soil profile after alfalfa. *Agron. J.* 64:660-664.
- Schuman, G.E., and L.F. Elliott. 1978. Cropping an abandoned feedlot to prevent deep percolation of nitrate-nitrogen. *Soil Sci.* 126: 237-243.
- Sechtig, A. 1992a. Nitrate in 2 M KCl soil extracts. QuikChem Method 12-107-04-1-B. Lachat Instruments, Milwaukee, WI.
- Sechtig, A. 1992b. Nitrate/nitrite, nitrite in 2% acetic acid plant extracts. QuikChem Method 13-107-04-1-A. Lachat Instruments, Milwaukee, WI.
- Stewart, B.A., F.G. Viets, Jr., and G.L. Hutchinson. 1968. Agriculture's effect on nitrate pollution of groundwater. *J. Soil Water Conserv.* 23:1-15.
- Switala, K. 1993. Ammonia (salicylate) in 2 M KCl soil extracts. QuikChem method 12-107-06-2-A. Lachat Instruments, Milwaukee, WI.
- Wendt, K. 1997. Determination of nitrate/nitrite in surface and wastewaters by flow injection analysis (low flow method). QuikChem Method 10-107-04-1-A. Zellweger Analytics, Milwaukee, WI.