Remediation of Soil and Groundwater using Effectively and Ineffectively Nodulated Alfalfa

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University of Wisconsin System Project 01-REM-4 and DATCP Project 00-04

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Location of Research: University of Wisconsin-Stevens Point, Univ of MN St Paul and Dopp dairy farm Portage County

Duration of UWS Funding: July 1, 2000 to June 30, 2002

Project Summary

Background:
Federal drinking water standards are exceeded for nitrate-N in 10% of the private wells in Wisconsin and over 40% in some Portage County townships. In addition, P leaching is becoming a recognized concern on soils that receive large applications of livestock manure.

This study was designed to test whether normal, N2-fixing (effectively nodulated) alfalfa or special non-N2-fixing (ineffectively nodulated) alfalfa can remove excess N and P from an abandoned barnyard. Nutrient removal, yield, and persistence of these plants will help determine the feasibility of their use in this and other agricultural activities in medium-to-coarse textured soils common to Wisconsin and other Midwest states.

Objective:
The primary objective of this study was to monitor changes that occur in groundwater quality and soil fertility in an abandoned barnyard planted with effectively and ineffectively nodulated alfalfa cultivars (Agate and Saranac) for possible use in phytoremediation. This project consists of several components; Variables analyzed included groundwater chemistry, soil characteristics, and differences between alfalfa cultivars for yield and plant nutrient content.

Methods:
Groundwater monitoring wells (up- and down-gradient) of the 60 m-long plots were sampled monthly through the growing season, from March through October, plus a winter sampling, totaling nine sample dates per year. Analyses included NO2+NO3-N and Cl- on all sample dates plus two samples per year for total reactive P, K, and NH4-N.

Spatially-referenced samples from the upper 1.5 m of soil were collected at the end of each growing season in 1998 and 2000 for analysis of NO2+NO3-N, NH4-N, and extractable P. Spatially-referenced topsoil samples were obtained in spring of each year and analyzed for inorganic N and extractable P. Eight spatially-referenced herbage samples were collected from each plot every harvest for determination of biomass and N and P content. Topsoil samples taken at the same locations were analyzed for inorganic N and extractable P.
Results:
Both Agate and Saranac cultivars of alfalfa are capable of taking up as much as 400 kg/ha of N from these sandy soils. The maximum uptake was 380 kg/ha by effective Agate in 1999, whereas Ineffective Agate removed 250 kg/ha. Yield and N uptake were reduced in 2000 and 2001 due to drier conditions.

This research demonstrates that N₂ fixation uptake is facultative with less fixation occurring when N supply is large. Ineffective cultivars did not remove as much total N as their effective pairs, because available N was rapidly leached in these soils, limiting growth and yield of the non-N₂-fixing alfalfas.

Nitrate leaching to ground water was very significant with all down-gradient wells exceeding the 10 mg/L nitrate-N standard. Values as high as 88 mg/L were found in the down-gradient wells in 2002. The average nitrate-N concentrations in the upper 1.8 m of the water table down-gradient of the plots in 2001 was 47 mg/L. This concentration of N would amount is equivalent to about 250 kg/ha in the upper 1.8 meters of the aquifer. We did not find any evidence of significant groundwater impacts from P, ammonium N or K in this study, but P leaching was apparent in the upper soil profile.

Conclusions and Recommendations:

This research has shown that in sites with sandy soils and groundwater recharge of approximately 25 cm per year that leaching of N from cultivated barnyard soils is more rapid than is the establishment and N uptake alfalfa.

Further research to optimize N uptake in remediation projects should focus of companion crops to alfalfa that would provide more rapid uptake of N during alfalfa establishment. Direct seeding techniques that would not require cultivation should be used to minimize oxidation of organic N compounds. Cultivation results in the destruction of any compacted layer, which in turn, encourages both rapid mineralization of organic N and leaching. Cultivation may be unavoidable, however, if compaction is too high to allow rapid root elongation.

Key Words: phytoremediation, nitrate leaching, ineffective alfalfa, groundwater nitrate, nitrogen fixation

Funds for this study were provided by University of Wisconsin System, Wisconsin Department of Agriculture, Trade, and Consumer Protection, USDA Agricultural Research Service, and the University of Wisconsin-Stevens Point. This project has been a cooperative venture between University of Wisconsin-Stevens Point (UWSP), USDA’s Agricultural Research Service (ARS), Portage and Waupaca County Land Conservation Districts (LCD), the Tomorrow/Waupaca River Priority Watershed Project (TWRP), and the Dopp family farm.
Remediation of Soil and Groundwater using Effectively and Ineffectively Nodulated Alfalfa

Introduction

Water quality concerns relative to agricultural activities have been increasing nationwide. Point sources are largely being controlled, yet water quality in many areas continues to deteriorate. This is especially true in much of central Wisconsin and other areas dominated by sandy soil where groundwater contamination has become a serious problem, including Portage County where this project is located.

Portage County lies within the Central Sands region of Wisconsin, and is dominated by medium-to-coarse textured glacial outwash soils along with a shallow water table. This combination makes the groundwater highly susceptible to contamination from local land use which is predominantly irrigated and non-irrigated cash crop and dairy-based agriculture, with small urban centers. Federal drinking water standards are exceeded for nitrate-N in 10% of the private wells in Wisconsin and over 40% in some Portage County townships (UWSP Groundwater Database, Unpub. Data). Studies indicate that 90% of groundwater contaminants in Wisconsin are a result of agriculture (Shaw, 1994; Mechenich and Stites and Kraft, 1997).

Buildup of excessive P levels by manure addition to agricultural soils threatens surface water quality (Sharpley et al., 1996) and, in some instances, groundwater quality (Sims et al., 1998). Although P is generally considered immobile in soil, there are increasing reports of significant P leaching when livestock manure rates have been high.

In earlier research conducted near Stevens Point, groundwater down-gradient of an abandoned barnyard was monitored and found to contain nitrate above the drinking water quality standard. Following abandonment and breakup of the hoof compacted layer, levels of nitrate-N exceeded 130 mg/L in the upper part of the aquifer (Shaw and Turyk, 1992). Eleven years after the abandonment, down-gradient nitrate-N levels in the upper aquifer were still approximately 20 mg/L (Shaw and Turyk, 1996). Similar results have been found by other research on abandoned barnyards.

In this research, we compared the removal of excess N and P by effective (N₂ fixing) and ineffective (non-N₂ fixing) alfalfas to protect groundwater. The suggestion that alfalfa can be used to remove excess nitrate-N from soil, and in particular from abandoned feedlots, was made at least 30 years ago (Stewart et al., 1968). There are limits to how much N can be absorbed by alfalfa, but removal rates are typically 2 to 4 times higher than with most annual crops, such as corn, and there generally is little worry about high nitrate-N concentrations in the resulting alfalfa forage, in contrast to forage grasses (Howarth, 1988).

In effectively nodulated legumes, the relative proportion of N derived from the atmosphere and from inorganic N depends largely on the quantity and type of inorganic N available to the crop (Allos and Bartholomew, 1959; Streeter, 1988). Since nitrate moves readily through the soil, alfalfa’s deep rootedness, high capacity for and longer period of N and water uptake, and perennial growth habit are characteristics that make it suitable in situations where N removal is desired.

The study site lies within the Tomorrow/Waupaca River Watershed, which was designated as one of Wisconsin’s Priority Watershed Projects. Goals for this watershed include the reduction
of nutrients to the river system via groundwater inflow and surface runoff and protection of groundwater quality.

Surface drainage at the study site is generally to the northeast. The soil is a Richford loamy sand with 0 to 6 percent slope, which has become very compacted over the years. Runoff from the lot channelizes at the northeast corner of the site and travels northeast 1.3 km until it drains into Radley Creek. Localized groundwater at the site appears to be flowing to the northeast less than 4 to 7 m from the surface and eventually feeds into Radley Creek as groundwater inflow. Aquifer materials in this region are generally homogeneous, making it ideal to analyze differences that may exist between cultivars.

Dairy young stock had been heavily concentrated on these four to six hectares, which had been devoid of vegetation for the last 15 years. Initially, there were approximately 100 head at the site with numbers gradually increasing to about 280 head. As part of the Priority Watershed efforts, the earthen barnyard at this site was abandoned in the fall of 1998 and moved further from Radley Creek. The abandoned earthen barnyard made an ideal site to test the effectiveness of the alfalfa in reducing the movement of N to groundwater.

**Methods**

Soil and groundwater sampling, preliminary piezometer installation, and seeding of alfalfa were initiated in summer of 1998; groundwater monitoring well nests were installed in 1999 after funds were allocated through the Wisconsin DNR’s Runoff Management Section. Preliminary piezometers were installed to determine the depth to groundwater and direction of flow to orient the alfalfa plots parallel to groundwater flow (Fig. 1). Prior to seeding, soil samples were taken at depths up to 1 m to determine texture and nutrient content. Two replicate 30 x 60 m plots of standard N$_2$-fixing alfalfas (Agate and Saranac) and two of non-N$_2$-fixing alfalfa (Ineffective Agate and Ineffective Saranac), germplasms that nodulate with *Sinorhizobium meliloti* but do not

![Figure 1. Layout of research plots relative to groundwater flow and general groundwater monitoring design.](image-url)
establish an effective N-fixing symbiosis, were established at the site. The Agate types were seeded in August 1998 and the Saranac types were seeded in August 1999. Due to limited seed we used both of these alfalfa germplasms. These tasks were accomplished in cooperation with the collaborators at UWSP, ARS, the TWRP, and the Dopp Family Farm. Local land use impacts to groundwater can be measured using a vertical series of well screens located down gradient of the land use. Groundwater chemistry due to up-gradient land use can be measured using wells located up-gradient of the land use being studied. Single nests of multi-level monitoring wells were established up-gradient and duplicate nests were installed down-gradient of the alfalfa research plots, resulting in the ability to sample the upper 2.4 m of the aquifer in 0.6 m increments (Fig.1). Each nest consisted of four wells. Wells were established at depths covering the entire range of groundwater elevations expected in wet and dry periods, with the shallow wells placed 0.3 m above the water table at the time of installation. Turyk and Shaw have used this design successfully for similar studies in this area.

Groundwater was sampled in June and August 1999 to determine current status of groundwater quality. All wells show animal waste impact to groundwater; nitrate-N concentrations in down-gradient wells ranged from 11 to 74 mg/L and chloride concentrations ranged from 7 to 80 mg/L. NH4-N concentrations were all less than 0.3 mg/L and concentrations of reactive P ranged from below detection to 0.27 mg/L.

**Groundwater Monitoring** Sampling frequency was conducted approximately monthly from March through October, plus a winter sampling. Samples were obtained from wells using a peristaltic pump and polypropylene tubing one sample was preserved with H2SO the other unpreserved. Samples were transported on ice to UWSP’s state certified Environmental Task Force Lab. Analyses included NO2+NO3-N and Cl- on all sample dates plus two samples per year for total reactive P, K, and NH4-N.

**Soil sampling** We obtained 144 samples from the plot area in July 1998 and analyzed them for NH4-N and potentially mineralizable-N. These data were kriged using SURFER, which showed large differences in these characteristics within the plot area. We also analyzed these samples for Bray-extractable P. We sampled the plot area in spring 2000 and 2001 to determine how the barnyard changed with time when cropped to alfalfa. Our hypothesis was that total soil C, total soil N, and extractable P would decline slowly with time after inputs of manure ceased. We were unable to complete the analyses of these topsoil samples for total N and C, due to long-term problems with the ARS combustion analyzer. Grant funds were insufficient to support analysis at a fee-for-service laboratory.

Thirty-six soil cores (9/plot) were collected to the 1.8 m depth in November 1998. These cores were divided into 15 cm increments to 90 cm, then into 30 cm increments to 1.8 m, were dried, ground, and analyzed for inorganic N. The heterogeneity of nitrate distribution with depth over the plot area was quite large. In some areas, high concentrations of nitrate were present near the surface and extended to depth, whereas in others, nitrate was present at depth, but not near the surface. Soil cores (10/plot) were procured again in October 2000. These were divided into 15-cm increments in the upper 30 cm, then 30-cm increments to the 1.2-m depth.

Statistical comparisons of soil Bray P concentrations at specific depth increments in 1998 and 2000 were made using log-transformed values, due to the non-normal distribution of the data. Averages of adjacent 15 cm increments in 1998 were used in the comparison of 30 cm increments taken in 2000.
Plant sampling. Eight samples were obtained per plot from 1 m² sections located in areas of contrasting soil fertility at each of three alfalfa harvests per year. Alfalfa and weeds were separated, oven dried, ground and analyzed for total N. Nutrient content (yield) was determined by multiplying concentration by dry matter yield in each sampling site. Soil and plant analysis was performed at USDA ARS in St. Paul, Minnesota.

We used the $^{15}$N natural abundance technique to estimate symbiotic N$_2$ fixation by effective alfalfa during 2000 and 2001. Six paired plots were located near the border between the effective and ineffective germplasms of each alfalfa cultivar. Locations were chosen to cover the variability in original soil N supply, as indicated by mineralizable N. Herbage was removed from 1-m² subplots a few days before field harvest at 5 to 10 cm above the soil surface. Dry mass was determined, herbage was ground to powder in a cyclone mill, total N was determined on a LECO CN2000 analyzer, and tissue samples were analyzed for $^{15}$N at the Stable Isotope Laboratory at the University of California – Davis.

The fraction of N derived from the atmosphere ($f_{Ndfa}$) was calculated as

$$\%Ndfa = 100 \left( \frac{\delta^{15}N_o - \delta^{15}N_t}{\delta^{15}N_o - \delta^{15}N_a} \right)$$

where $\delta^{15}N$ is the per mil (‰) departure from the $^{15}$N concentration of the atmosphere, $o$ is the reference plant, $t$ is the fixing plant grown under field conditions in which both soil and atmospheric N are available, and $a$ is the fixing plant grown under conditions in which only atmospheric N is available (Shearer and Kohl, 1986). We estimated the latter value by selecting the single effective alfalfa plot with the lowest $^{15}$N concentration during the experiment, subtracting the total N uptake in weeds in the paired ineffective plot (as an estimate of N available from soil and manure, and then calculating the $\delta^{15}N$ to produce the resulting $f_{Ndfa}$ (0.923). This value (-1.0079 $\delta^{15}N$) was used for all subsequent calculations. Although we had intended to use the ineffective alfalfa as the non-fixing reference plant, it was clear that many of the alfalfa plants in the ineffective plots were fixing N$_2$, based on visual inspection (lack of N deficiency chlorosis), tissue analysis (high total N concentration), presence of pink nodules, and low $^{15}$N concentration relative to weeds in the same plots. Therefore, we used the $^{15}$N concentration in weeds for $\delta^{15}N_o$, except for five plots at the first harvest in 2000, where there were insufficient weeds to sample and we used the $^{15}$N concentration in the respective ineffective alfalfa plants.

Results and Discussion

Results of dry matter yield and total N removal for both Agate and Saranac alfalfa cultivars are presented in Table 1. These results clearly indicate that plots containing effective alfalfa cultivars produce more dry matter and N than plots that contain ineffective alfalfa. Much of this difference is believed to be due to poorer growth related to N deficiencies in the ineffective plots as a result of nitrate leaching. Nitrogen leaching in these sandy soils was apparently occurring more rapidly than was alfalfa uptake or root growth.
Table 1: Yield and total N removal by alfalfa cultivars for 1999, 2000 and 2001 (values in kg/ha). Derived by difference between the effective and ineffective germplasms in 1999 and by the natural abundance ^15^N isotope technique in 2000 and 2001, using non-fixing weeds in the ineffective alfalfa plots as the controls.

<table>
<thead>
<tr>
<th>Alfalfa germplasm</th>
<th>Effective Agate</th>
<th>Ineffective Agate</th>
<th>Effective Saranac</th>
<th>Ineffective Saranac</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1999</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yield</td>
<td>11580</td>
<td>8370</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N harvested</td>
<td>380</td>
<td>250</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N removed from soil†</td>
<td>130</td>
<td>250</td>
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<tr>
<td><strong>2000</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Yield</td>
<td>8200</td>
<td>4580</td>
<td>6170</td>
<td>2560</td>
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<tr>
<td>N harvested</td>
<td>310</td>
<td>150</td>
<td>230</td>
<td>70</td>
</tr>
<tr>
<td>N removed from soil</td>
<td>180</td>
<td>130</td>
<td>110</td>
<td>70</td>
</tr>
<tr>
<td><strong>2001</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yield</td>
<td>9160</td>
<td>4540</td>
<td>7870</td>
<td>3240</td>
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<tr>
<td>N harvested</td>
<td>290</td>
<td>130</td>
<td>270</td>
<td>90</td>
</tr>
<tr>
<td>N removed from soil</td>
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<td>90</td>
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<td><strong>1999-2001</strong></td>
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<tr>
<td>Yield</td>
<td>28940</td>
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<td>5800</td>
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<tr>
<td>N harvested</td>
<td>980</td>
<td>530</td>
<td>500</td>
<td>160</td>
</tr>
<tr>
<td>N removed from soil</td>
<td>460</td>
<td>450</td>
<td>200</td>
<td>130</td>
</tr>
</tbody>
</table>

In the last two years of the experiment, we estimated N$_2$ fixation using the $^{15}$N natural abundance technique. Estimated fNdfa (fraction of herbage N derived from the atmosphere by symbiotic N$_2$ fixation) ranged from 0.02 to 0.92. There was no relationship between total herbage N and estimated fNdfa (Fig. 2), demonstrating that high N yield of alfalfa does not necessarily assure either high N$_2$ fixation or large removal of inorganic N. However, the facultative nature of symbiotic N$_2$ fixation is shown by the linear decline in fNdfa with increasing availability of inorganic N, as evidenced by plant uptake (Fig. 3). This relationship was affected by growth period during the year, but not by alfalfa cultivar or by year (stand age and environment). This changing sensitivity to inorganic N is directly related to the total N requirement of the alfalfa crop. The average amount of harvested N declined from 131 at the first harvest, to 87 at the second, to 54 kg N/ha at the third in this droughty site. The relationship between inorganic N uptake and fNdfa was closest in the first growth period ($r^2=0.97$) and weakest in the third ($r^2=0.44$). This was due, in part to the increasing CV for N yield during the year (19% to 29%), but also to increasing variation in the estimate of fNdfa (CV=38, 47, and 54% for harvests 1, 2, and 3, respectively). Average amounts of N removed from inorganic sources (mineralized soil and manure N) in both 2000 and 2001 were 200 kg N/ha for Saranac and 330 kg N/ha for Agate. Total N harvested over the two years averaged 500 kg N/ha in Saranac and 590 kg N/ha in Agate. Because fNdfa in these alfalfa cultivars responded similarly to inorganic N uptake, we attribute the lower inorganic N uptake in Saranac to its delayed development due to later seeding. This was evident in the lower total N yield in 2000, the first production year for Saranac, when it yielded 230 kg N/ha compared to the well established Agate (310 kg N/ha). In contrast, total N yield in 2001 was similar for the two cultivars, with Saranac yielding 270 kg N/ha and Agate yielding 280 kg N/ha. We conclude that these two cultivars had similar capacities to absorb
inorganic N from the soil and, by extension, it is likely that most alfalfa cultivars that are well adapted to a given site would remove inorganic N similarly.

![Graph](https://example.com/graph1.png)

Figure 2. No relationship was observed between harvested herbage N and the proportion of plant N derived from the atmosphere (fNdfa) in Saranac and Agate alfalfa in 2000 and 2001 at the barnyard remediation site.

![Graph](https://example.com/graph2.png)

Figure 3. Absorption of inorganic N from soil and manure reduced the fraction of plant N derived from the atmosphere (fNdfa) in Saranac and Agate alfalfa in 2000 and 2001 at the barnyard remediation site. The relationship varied with growth interval during the year with fNdfa declining by 7.4, 12.4, and 17.2 % Ndfa per kg N/ha absorbed by harvest 1, 2, and 3, respectively.

We also estimated the amount of N derived from the atmosphere and from soil in the ineffective alfalfa stands. Despite careful seed production techniques, seed of ineffective alfalfa typically contains a small percentage of effective seed. When N supply is low, these individuals will flourish, while the majority of the stand will suffer. Due to progressive stand decline of non-N₂-fixing individuals in the ineffective germplasms and development of larger effective plants in the stand, some N was derived from N₂ fixation in the ineffective alfalfa plots. These results make it clear that planting a normal, N₂-fixing alfalfa is preferable to a non-fixing alfalfa when N supply from the contaminated site is unreliable.
The maximum inorganic N removal measured in effective alfalfa in the paired 1-m² plots was 440 kg N/ha over two growing seasons, and the second greatest was 420 kg N/ha. These are underestimates of N removal from the remediation site, because they do not include uptake in 1999. Total N uptake by Ineffective Agate in 1999 averaged 250 kg N/ha, implying that annual removal by established alfalfa at this site could total at least 200 kg N/ha in areas with high N supply. This compares with over 400 kg N/ha removed annually by established Ineffective Agate at a site in North Dakota, where alfalfa had access to inorganic N in the soil and was irrigated with groundwater containing both NH₄⁺-N and NO₃⁻-N (Russelle et al., 2001). Yields of irrigated non-fixing alfalfa averaged 12 Mg DM/ha at the North Dakota site, compared to less than 10 Mg DM/ha annually by established Agate alfalfa at this barnyard remediation site in Wisconsin. The capacity for N removal by alfalfa at this barnyard remediation site was limited by both the yield (plant N demand) and soil N supply.

Significant differences in the distribution of Bray P in the soil were apparent (Fig. 4). Soil P concentration decreased in the upper 15 cm, but increased in the 30 to 90 cm depth increment. Total Bray-extractable P remained unchanged at about 1300 kg P/ha. These results suggest that significant leaching of Bray-extractable P occurred in this soil after abandonment of the barnyard (Fig. 4). Decline in Bray P in the topsoil was also detectable in the intensive grid samples (Appendix). Some of the apparent discrepancies between samples in 1998 and 2000 are due in part to different grid sampling points.

![Figure 4. Median values of Bray-extractable P in the abandoned barnyard in November 1998 and October 2000. Statistical differences (P<0.05) in the log-transformed data were present for the 0 to 15 cm, 30 to 60 cm, and 60 to 90 cm depth increments, indicative of leaching in these coarse materials.](image)

Although we do not have total P analyses of the harvested plant tissue, other research has concluded that alfalfa rarely contains more than 3.5 mg P/kg dry matter (Russelle et al., 2000). With a total herbage yield in 1999 and 2000 of about 22 Mg/ha, agate alfalfa removed only 78 kg P/ha. Phytoremediation of sites with excessive P will require many years, even with high-yielding crops. On sandy soils with low P adsorption capacity, this may result in eventual leaching of P to groundwater.
Groundwater Results

Groundwater monitoring up-gradient and down-gradient of each treatment included nitrate-N, Cl, P, ammonium-N and K. No significant concentration of P, ammonium or K was found in any of the wells. Both nitrate and Cl were found in high concentrations from leaching of animal waste. The ratio of nitrate to Cl was very consistent throughout the study area and over time (mean=0.92, SD=0.32), suggesting that little or no denitrification was occurring in the soil or groundwater underlying these sites. During the study, groundwater elevations fluctuated by approximately 120 cm (Fig. 5).

Figure 5. Water table elevations for monitoring well E1B during the project.

Figure 6 shows the nitrate concentrations from 1999 to 2002 for the up-gradient and down-gradient wells for each treatment. Nitrate concentrations in most up-gradient wells remained fairly constant at 15 to 20 mg/L or increased slightly over time. Down-gradient wells showed much larger increases over time, especially in 2001.
Figure 6. Nitrate Concentrations in all monitoring well ports. 1999-2002.
Table 2 shows the average nitrate-N by treatment for the two upper well ports, yielding samples, for the 3-year study period and for 2001. From these data it is apparent that significant leaching occurred from the study plots throughout the project in spite of significant N removal by both the effective and ineffective alfalfa cultivars. We feel the large amount of leaching that occurred in 2001 is a result of organic N mineralization, a consequence of the cultivation of the barnyard soils when alfalfa was planted and adequate rainfall in 2001 to leach the released nitrate to groundwater. Soil nitrate concentrations in the upper 1.5 m also declined during this time period due to both leaching and plant uptake.

Table 2: Average nitrate concentrations in upper 1.8 m of the aquifer up-gradient and down-gradient of each treatment for 1999-2001 and for 2001. (Values in mg/L as nitrate-N)

<table>
<thead>
<tr>
<th></th>
<th>1999-2001</th>
<th>2001</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Down-Gradient</td>
<td>Up-Gradient</td>
</tr>
<tr>
<td>Effective 1</td>
<td>35</td>
<td>15</td>
</tr>
<tr>
<td>Ineffective 1</td>
<td>32</td>
<td>17</td>
</tr>
<tr>
<td>Effective 2</td>
<td>41</td>
<td>16</td>
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<tr>
<td>Ineffective 2</td>
<td>47</td>
<td>54</td>
</tr>
<tr>
<td>Average</td>
<td>39</td>
<td>25</td>
</tr>
</tbody>
</table>

Groundwater concentrations of 47 mg/L as found down gradient of the plots in 2001 would require about 50 kg of N to leach with the 25 cm of groundwater recharge typical in this area. Another way of looking at the amount of N leached is to quantify the amount in the upper 1.8 meters of the aquifer sampled by the upper two well ports. Using 30 percent porosity the 47 mg/L would amount to 280 kg of N per ha. Some of this is from up gradient sources but certainly less than half based on the up gradient nitrate concentrations.

Conclusions and Recommendations

Both Agate and Saranac alfalfa are capable of taking up as much as 400 kg/ha of N from these sandy soils. The maximum uptake was 380 kg N/ha by effective Agate in 1999, whereas Ineffective Agate removed 250 kg N/ha.

This research demonstrated how the facultative nature of symbiotic N$_2$ fixation is a benefit for economic phytoremediation. In sites that have heterogenous accumulations of nitrate, N-fixing alfalfa will yield well across the site, yet remove inorganic N effectively. Non-N$_2$ fixing alfalfa did not remove as much total N as their effective pairs at this site, because available N was rapidly leached, thereby limiting growth and yield in the ineffective plots. Dry growing conditions in 2000 and 2001 also limited yield compared to 1999. This suggests that strategic and moderate additions of water via irrigation on these soils may promote successful remediation in dry years.
Nitrate leaching to groundwater was very significant at this site with all wells exceeding the 10 mg/L nitrate-N standard. Values as high as 80 mg/L nitrate-N were found in down-gradient wells in 2001. The average nitrate-N concentrations in the upper 1.8 m of the water table down-gradient of the plots in 2001 was 47 mg/L. This concentration of N would amount to about 250 kg/ha in the upper 1.8 m of the aquifer.

It is apparent that in sites with sandy soils and groundwater recharge of approximately 25 cm per year that leaching of N from abandoned, cultivated barnyard soils is more rapid than is the establishment and N uptake by alfalfa.

Further research to optimize N uptake in remediation sites should focus on companion crops to alfalfa that would provide more first year uptake of N while alfalfa develops its root system. Direct seeding techniques that would not require cultivation should be used where previous soil compaction will not limit root growth. Cultivation results in the destruction of any compacted layer which in turn, encourages rapid mineralization of organic N and leaching.

Although it is known from other research that alfalfa can prevent or greatly diminish nitrate losses from soil, in soils of high hydraulic conductivity it may be desirable to plant a fast growing crop like wheat or oats with the alfalfa to utilize nitrate during alfalfa establishment. This cereal crop can be harvested for silage at the soft dough growth stage to provide feed and to limit competition with alfalfa. Farmers who use this forage should note that ensiling will reduce excess nitrate in the stored forage, whereas drying for hay will not.

With all phytoremediation approaches, one must recognize the limitations and potential risks of the system. For example, the risk of stand loss of alfalfa over winter is present throughout the northern USA. This can be managed to some extent by selecting winter-hardy cultivars, by providing sufficient potassium and alleviating other growth-limiting conditions, and by harvesting less frequently than is possible in the region (i.e., three rather than four harvests per year in central Wisconsin) (Barnes and Sheaffer, 1985). In addition, one should recognize that in highly 'leaky' soils, plants may not generate sufficient N demand quickly enough to prevent significant leaching losses. These situations may require that active remediation techniques are employed to prevent spread of the ground water contamination (Russelle et al., 2001).
References

UWSP Groundwater Database. Unpublished data. Central Wisconsin Groundwater Center, University of Wisconsin-Stevens Point.
Appendix
Two-dimensional distribution of total N (2000), mineralizable N (1998), ammonium-N, and nitrate-N at the barnyard site. Distribution of nitrate-N in 1998 was determined at a lower resolution than all other graphs.
Spatial distribution of soil N at the barnyard site in 1998. Soil depth increments are represented by different planes in the figure, with surface distribution shown as in the two-dimensional maps in other figures.
Bray-extractable P in topsoil (0-15 cm) in the experimental area, interpolated by kriging. The sampling frame was expanded by 25 feet (7.6 m) in all directions in 2000 and 2001, but only alternate sampling points of the regular grid were analyzed for P.
GROUNDWATER QUALITY DATA