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Managing Nitrogen Contaminated Soils: Benefits of N_2–Fixing Alfalfa

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ABSTRACT

Perennial forage crops offer an effective, low-cost method for remediating excess soil N. Where it is agronomically adapted, alfalfa (*Medicago sativa* L.) is a particularly desirable species for remediation of excess soil N because it has high dry matter (DM) yield and N uptake potential, it can absorb nitrate (NO_3) from depths beyond those attainable by most annual crops, and its market value is usually higher than grass forages. On the basis of previous research, we hypothesized that non-N_2–fixing alfalfa would remove more inorganic soil N than standard, N_2–fixing alfalfa and tested this hypothesis at an abandoned barnyard on a Richford sandy loam (mixed, mesic Psammentic Hapludalfs) in central Wisconsin, USA. Duplicate plots (30 by 60 m) of both N_2–fixing and nonfixing alfalfa were seeded in August 1998 and 1999. Nonfixing alfalfa produced lower DM and N yield and showed greater variability than standard, N_2–fixing alfalfa. Yield, N concentration, and stand declined in the nonfixing type where inorganic N supply was inadequate. Average maximum estimated recovery of soil and manure N was about 200 kg N ha^{-1} annually for plots seeded with N_2–fixing alfalfa. During 2 yr when inorganic N uptake was estimated by the ^{15}N natural abundance technique, N_2–fixing alfalfa removed nearly 60% more soil and manure N than nonfixing alfalfa, but weeds in the nonfixing alfalfa plots made up the difference. Neither alfalfa prevented ground water contamination by NO_3 and concentrations increased similarly under both alfalfas. Because declines in yield and protein concentration may be expected for nonfixing alfalfa on sites with patchy available N distribution, economic remediation of these sites will be promoted by using an adapted cultivar of N_2–fixing alfalfa.

Excess soil N can result from overapplication of fertilizer, animal manures, or other by-products, inadvertent spills of N-containing materials, and accumulation of eroded topsoil. Left untreated, such sites may contaminate ground and surface water with inorganic N. Phytoremediation may be used to reduce the oversupply of N if site conditions do not limit aboveground plant yields.

At least three decades ago, alfalfa was recommended for removal of excess NO_3–N from soil, in particular from abandoned feedlots (Stewart et al., 1968; Schuman and Elliott, 1978). Alfalfa has many characteristics that make it suitable for effective NO_3 removal, including deep rooting potential, a large N requirement, long periods of N and water uptake, and perennial growth habit (Russelle et al., 2001), and its value in the market typically is higher than grasses, making it more profitable. Nitrogen removal rates are often two to four times higher with alfalfa than with annual crops like corn (*Zea mays* L.) or wheat (*Triticum aestivum* L.; Russelle et al., 2001). In contrast to grasses and other nonlegumes, however, alfalfa does not accumulate high NO_3–N concentrations in the shoot during later phases of regrowth when grown with high N supply (Howarth, 1988; Schmidt et al., 2003), making it safer to use as livestock feed.

In N_2–fixing 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 in soil to the crop (Allos and Bartholomew, 1959; Streeter, 1988). This facultative nature of N_2 fixation may therefore be considered a means of buffering variable inorganic N supply in the environment while sustaining high crop yields. On the other hand, the relatively high internal N status of N_2–fixing plants may reduce their ability to absorb inorganic N. We found, for example, that nonfixing alfalfa was more effective than normal, N_2–fixing alfalfa in removing large amounts of NO_3–N applied during growth (Lamb et al., 1995; Blumenthal and Russelle, 1996; Blumenthal et al., 1999).

We hypothesized that nonfixing alfalfa would be more effective than standard, N_2–fixing alfalfa for remediating excess soil N that mineralizes from soil and manure, and that this difference should lead to lower NO_3 contamination of ground water under nonfixing alfalfa. To test this hypothesis, we selected a site with a heterogeneous, patchy N supply, typical of many situations where excess organic materials have been applied to land or where fertilizer spills have occurred.

MATERIALS AND METHODS

Site Description

The experiment was located on a Richford loamy sand (0–6% slope) in Portage County, Wisconsin, USA (approx. 44°30′ N, 89°34′ W). Dairy heifers had been fed year-round on 4 to 6 ha that reportedly had been nearly devoid of vegetation for about 15 yr. About 100 heifers were fed at the site initially, but numbers increased over the years to about 280 head. The farmers reported that they had moved the feeding stations occasionally to improve manure distribution. The earthen feedlot was abandoned in summer 1998.

Alfalfa Establishment and Sampling

Soil sampling, seedbed preparation, and seeding of alfalfa were initiated in summer 1998 (Table 1). Two replicate blocks of two 30- by 60-m plots were oriented parallel to the direction

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Abbreviations: DM, dry matter; fNdfa, fraction of N derived from the atmosphere; fNdfs, fraction of N derived from the soil; Ndfa, mass of N derived from the air, kg N ha^{-1}; Ndfs, mass of N derived from the soil, kg N ha^{-1}.

Table 1. Schedule of planting, harvest, and sampling activities at an abandoned barnyard site. Herbage samples for natural abundance ^{15}N analysis were taken on the same dates as harvest samples for yield. Ground water sampling was about monthly during the growing season and once in winter.

Year	Block 1	Block 2
1998	Grid topsoil sampling (July) Seeded (late July) Random deep soil coring (November)	Grid topsoil sampling (July) Seeded (late July—stand failure) Random deep soil coring (November)
1999	*First production year* 3 harvests for yield (27 May, 7 July, 11 August) Difference method for fNdfa† Ground water sampling (began June)	Seeded in August Ground water sampling (began June)
2000	*Second production year* Grid topsoil sampling (June) 3 harvests for yield (29 May, 6 July, 14 August) Natural abundance paired plots for fNdfa Root measurements (October) Ground water sampling	*First production year* Grid topsoil sampling (June) 3 harvests for yield (29 May, 6 July, 14 August) Natural abundance paired plots for fNdfa Root measurements (October) Ground water sampling
2001	*Third production year* Grid topsoil sampling (May) 3 harvests (23 May, 10 July, 9 August) Natural abundance paired plots for fNdfa Ground water sampling	*Second production year* Grid topsoil sampling (May) 3 harvests for yield (23 May, 10 July, 9 August) Natural abundance paired plots for fNdfa Ground water sampling
2002	Hay production continued by farmer Ground water sampling	Hay production continued by farmer Ground water sampling

† fNdfa, fraction of N derived from the atmosphere.

of ground water flow at the site, as determined by temporary 3.2-cm-diam. piezometers (Fig. 1). Large plots were necessary to allow assessment of ground water quality impacts. Topsoil analyses showed high levels of Bray P1-extractable P (170 mg P kg^{-1} soil) and Bray P1-exchangeable K (525 mg K kg^{-1} soil) (see Kelling et al., 1998, for extraction method), low organic matter (14 g kg^{-1} soil), and near-neutral pH (6.5). The remainder of the field around the experimental plots was seeded with a mixture of commercially available alfalfa and perennial grass in August 1998.

Each large plot was seeded in late July 1998 with either standard N_2–fixing alfalfa, 'Agate,' or Ineffective Agate, an experimental nonfixing alfalfa germplasm. Stands of both treatments in Block 2 failed due to incorrect adjustment of the seeder, and it was reseeded in August 1999 with another experimental nonfixing germplasm, Ineffective Saranac, and the related N_2–fixing cultivar Saranac. Both nonfixing germplasms nodulate with *Sinorhizobium meliloti*, but do not establish an N_2–fixing symbiosis because of apparent single-gene mutations in the plants (Barnes et al., 1990). These two nonfixing germplasms have similar response to fertilizer N, and neither Agate nor Saranac showed yield or N content responses to fertilizer N (Lamb et al., 1995). Seed was inoculated with recommended rates of commercial rhizobial inoculum immediately before planting. To improve growth of the nonfixing alfalfa, we applied 87 kg N ha^{-1} in a slow release formulation to all plots in June 1999.

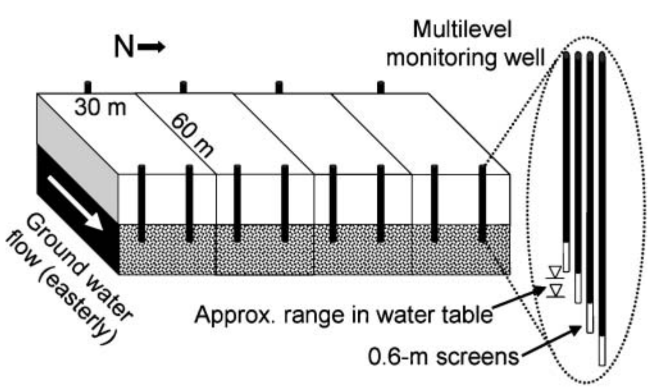

Fig. 1. Schematic diagram of plot layout with well nests. Down-gradient wells were actually located a few meters within the plots, but were drawn on the border to show their relationship to the water table.

In this experiment, we compared plant responses using stands of similar age in these large plots. The first production year was defined as 1999 for Block 1 (Agate entries) and as 2000 for Block 2 (Saranac entries; Table 1). Therefore, block effects for yield, N concentration, and N content in this experiment included year of establishment (1998 for Block 1 and 1999 for Block 2) and parental germplasm type (Agate for Block 1 and Saranac for Block 2).

In 1999, 2000, and 2001, 5 to 13 herbage samples (depending on labor availability) were collected per plot from 1-m^2 sections located in areas of contrasting soil fertility (see description of topsoil sampling in a later section) at each of three harvests per year, a few days before the field was harvested by the farmer. These sites were selected randomly at each harvest in 1999, but additional paired plots along the borders between the N_2–fixing and nonfixing plots were sampled the following 2 yr. Shoots were cut 5- to 10-cm above the soil surface, alfalfa and weeds were separated, oven dried, ground, and analyzed for total N by direct combustion.

Treatment differences were discerned by ANOVA using PROC MIXED in SAS (SAS Institute, 2006) in a split-plot arrangement in an RCB design, with replicate block as a random variable, N_2 fixation capacity (fixing vs. nonfixing) as the subplot, and harvest within year as the repeated sub-subplot. In all PROC MIXED analyses, the type of covariance structure was selected to minimize the absolute value of the information criteria, within sets of rational covariance structures. For the annual time scale, the ANOVA was a split-plot arrangement in a RCB with age of stand as the main plot and N_2 fixation capacity as the subplot.

Alfalfa roots were sampled in 7.5-cm-diam. soil cores taken to a depth of 1.2 m at six locations within each large plot in October 2000. Sampling sites were limited to nearly weed-free areas to avoid weed roots, which restricted randomization in the nonfixing plots to locations with more vigorous alfalfa growth. Duplicate cores at each site were divided into depth increments, combined by depth, and samples were stored in closed plastic bags under refrigeration until processing. Roots were separated from soil using a hydropneumatic elutriator (Smucker et al., 1982). Fine roots (<1-mm diam.) were separated from thick roots by hand. The dry mass of each root fraction was determined after oven drying at 65°C. Fine root length was determined on a set of washed, moist subsamples, and the relationship between length and dry mass (cm root = 6.73 × mg root, $r^2 = 0.85$) was used to estimate fine root length in the remaining samples. Means were tested using PROC MIXED, using N_2 fixation capacity as the main plot, depth as a repeated measure, and blocks as a random variable.

Estimation of Inorganic Nitrogen Uptake and N_2 Fixation

We used the ^{15}N natural abundance technique to estimate inorganic N uptake from soil and manure and symbiotic N_2

fixation in N_2–fixing alfalfa during 2000 and 2001 (Weaver and Danso, 1994). Six paired plots were located near the borders between the N_2–fixing and nonfixing plots in each replicate. Locations were selected to represent some of the variability in original soil N supply, as indicated by an index of potential N mineralization capacity of the soil (Fig. 2). Herbage was removed from 1-m^2 subplots as described above. Dry mass was determined, herbage was ground in a cyclone mill, total N was determined by direct combustion, and subsamples were analyzed for ^{15}N at the Stable Isotope Laboratory at the University of California–Davis.

The amount of N derived from the atmosphere (Ndfa) was calculated as the product of fNdfa and total plant N content. The remaining plant N was considered to be the faction of N derived from the soil (fNdfs); therefore, fNdfs = 1 − fNdfa. The amount of N derived from the soil (Ndfs) was equal to the product of fNdfs and total plant N. In both cases, we use the shorthand "soil" to include manure-derived N. The fraction of N derived from the atmosphere (fNdfa) was calculated as

$$\text{fNdfa} = \left(\frac{\delta^{15}\text{N}_o - \delta^{15}\text{N}_t}{\delta^{15}\text{N}_o - \delta^{15}\text{N}_a}\right)$$

where $\delta^{15}N$ is the per mil (‰) departure from the ^{15}N concentration of the atmosphere for the reference nonfixing plant (subscript *o*), the N_2–fixing plant grown under field conditions in which both soil and atmospheric N are available (subscript *t*), and the N_2–fixing plant grown under conditions in which only atmospheric N is available (subscript *a*; Shearer and Kohl, 1986). To estimate $\delta^{15}N_a$, we first determined the maximum observed fNdfa using the difference technique based on (i) total N content (g m^{-2}) of the N_2–fixing alfalfa sample with the lowest $\delta^{15}N$ (that is, the greatest fNdfa), and (ii) total N content of weeds of the paired nonfixing plot (representing N uptake from soil and manure). We solved the equation above for $\delta^{15}N_a$ using this maximum observed fNdfa (0.923) and the $\delta^{15}N_o$ and $\delta^{15}N_t$ for this pair of plots. The resulting $\delta^{15}N_a$ value ($-1.0079\ \delta^{15}N$) was used for all subsequent calculations.

We intended to use nonfixing alfalfa as the reference plant, but many surviving alfalfa plants in these plots apparently were fixing N_2, based on visual inspection (lack of N-deficiency chlorosis in the shoots), tissue analysis (high total N concentration), presence of pink nodules (indication of oxygenated leghemoglobin), and lower $\delta^{15}N$ compared with weeds in the same plots (presumptive evidence of atmospheric N assimilation by the alfalfa). Barnes et al. (1990) stated that a small percentage of normal, N_2–fixing plants are present in the ineffective germplasm seed sources. These N_2–fixing plants apparently survived better than nonfixing alfalfa at this site, especially in areas of smaller soil N supply. Therefore, we used the $\delta^{15}N$ in weeds, rather than nonfixing alfalfa, for $\delta^{15}N_o$ (Rochester et al., 1998), except for five plots at the first harvest in 2000 that contained too few weeds.

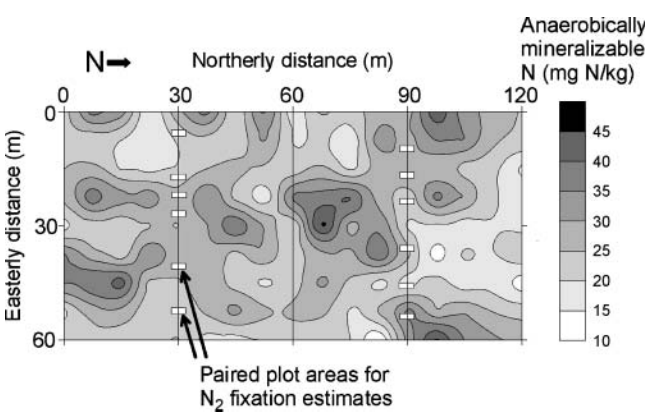

Fig. 2. Kriged contours of anaerobically mineralizable N in topsoil (0–0.15 m) over the plot area in July 1998. Paired plots for plant sampling are indicated by the 12 clear rectangles.

Although we did not begin this sampling during the first production year of Block 1 (1999), we extended sampling through the third production year of this replicate (2001). Therefore, this dataset included six observations from the first forage production year (Block 2 in 2000), 12 from the second production year (Block 1 in 2000 and Block 2 in 2001), and 6 from the third production year (Block 1 in 2001), thereby broadening the region of inference. Because distances between adjacent pairs of plots were >6 m, we considered them to be independent. Relationships were characterized using regression analysis. Means comparisons of Ndfs between N_2–fixing and nonfixing plots were made using PROC MIXED, with N_2–fixation capacity (as seeded) as main plots, year as subplots, and blocks as random.

Soil Sampling

To evaluate initial soil characteristics, we obtained 144 soil samples (0–0.15 m) in a 7.6-m regular grid pattern from the plot area in July 1998 and analyzed them for ammonium (NH_4)-N and NO_3–N [2 *M* KCl extractant analyzed by flow injection (Sechtig, 1992; Switala, 1993)], potentially mineralizable N [7-d anaerobic incubation (Hart et al., 1994) analyzed by flow injection (Diamond, 1997)], and total N by direct combustion. We sampled the plot area again in spring 2000 and 2001 and extended the sampling grid by 7.6 m around the plots (209 sampling points). Concentrations of NH_4–N and NO_3–N in the topsoil were measured each year, with duplicates from earlier analyses rerun in subsequent years to assure consistency. Total N concentrations were determined by direct combustion for all samples at the end of the experiment. All concentrations were tested for samples within the plots (excluding borders) for the effect of alfalfa entry, blocks, years, and entry × year interaction using ANOVA in a split plot arrangement with years as subplots with PROC MIXED. Spatial distribution of N mass was visualized using kriged grid files generated by SURFER (Golden Software, 1997).

To obtain an estimate of N mineralization potential that might be related to root growth, topsoil samples (0–0.15 m) were procured at the same time and location as root samples. We estimated potentially mineralizable N using both the 7-d anaerobic and 28-d aerobic batch incubation (Hart et al., 1994), followed by 2 *M* KCl extraction and flow injection analysis.

Thirty-six soil cores (nine per plot) were collected to 0.9 m in November 1998. Cores were divided into 0.15-m increments to 90 cm, then into 0.3-m increments to the depth of sampling. Samples were dried (45°C), ground, extracted with 2 *M* KCl, and analyzed for inorganic N.

Ground Water Monitoring

Ground water quality was assessed by a vertical series of well screens located up- and down-gradient with respect to ground water flow direction. Single nests of 1.9-cm-diam. polyvinyl chloride multilevel monitoring wells were established a few meters up-gradient of the plots and duplicate nests were installed within each large plot a few meters from the down-gradient border, allowing us potentially to sample the upper 2.4 m of the aquifer in four 0.6-m-deep increments (Fig. 1). Holes were bored with a 7.6-cm-diam. auger to the desired depth, wells were inserted, then the holes were backfilled with sand and topped with a bentonite seal in the upper 30 cm.

Depth to the water table and water samples were collected approximately monthly from March through October and once in winter. The water table gradient averaged 0.007 m m^{-1}. At least three volumes of water were purged from each well before collecting samples using a peristaltic pump and polypropylene tubing. Water samples were passed through an in-line filtering cassette containing a 0.45-μm membrane filter. One subsample was preserved with sulfuric acid and the other was not amended. Samples were transported on ice to the state certified Environmental Task Force Laboratory at the University of Wisconsin–Stevens Point. Analyses included NO_3–N + nitrite-N and chloride (Cl) on all sample dates plus two samples per year for NH_4–N.

RESULTS AND DISCUSSION

Monthly temperature data from the Central Division of Wisconsin was provided by the Western Regional Climate Center, and monthly precipitation data was from the National Weather Service Station in Waupaca (18 km NE of the site) (Fig. 3), although our research site may have experienced different weather. Air temperatures during the experiment were similar to the 30-yr means, except for the unusually warm winter of 2001–2002. Growing season precipitation is especially important for crop growth on these coarse-textured soils. Relative to the 30-yr mean of 57.9 cm, total rainfall at Waupaca from April through September was 1 and 10% lower in 1998 and 1999, and 11 and 6% greater in 2000 and 2001, respectively.

Soil NO_3 distribution with depth over the plot area was heterogeneous before alfalfa planting (Fig. 4), indicating that NO_3 leaching had occurred before abandonment of the feedlot. In some areas, high concentrations of NO_3 were present near the surface and extended to depth, whereas in others, NO_3 was present at depth, but not near the surface. This implies that our attempt

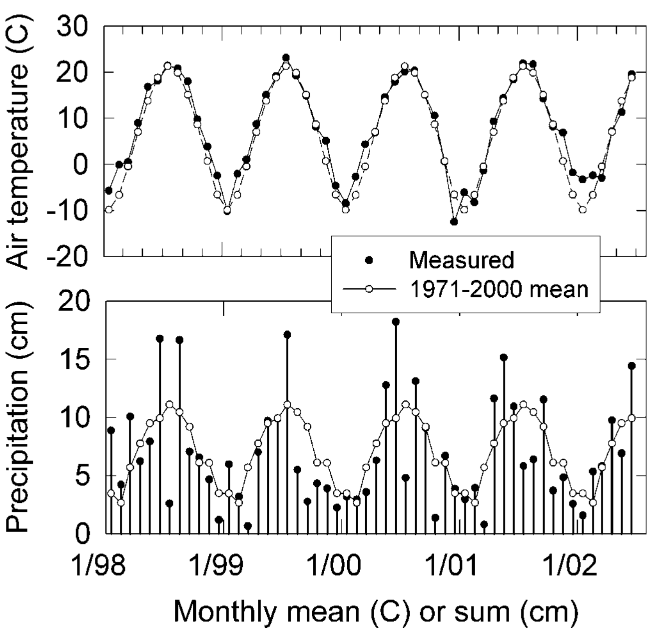

Fig. 3. Mean monthly air temperature in the Central District of Wisconsin and monthly precipitation at Waupaca, WI, during the study period, and the 1971–2000 long-term means of these data.

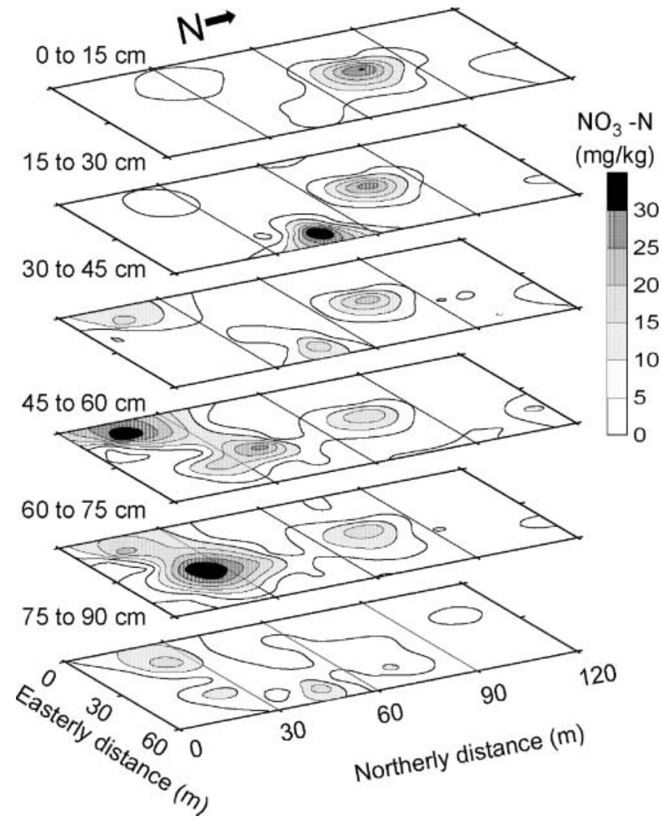

Fig. 4. Spatial distribution of soil nitrate N at the feedlot site in November 1998. Soil depth increments are represented by different planes in the figure, with kriged surface contours shown as in the two-dimensional maps in other figures.

to characterize initial soil N supply may have been inadequate by restricting intensive grid sampling to the topsoil only. On the other hand, residence time of NO_3 in the root zone is likely to be short in this soil, which is a loamy sand with moderately rapid permeability overlaying a sand at 1 to 1.5 m that has rapid permeability (Soil Survey Staff, 2005). Although some loss of NO_3 may have occurred by denitrification, these results tend to support the observation that NO_3 leaching can occur under active outdoor feedlots on coarse-textured soils (Maulé and Fonstad, 2000), in contrast with finer-textured soils (Mielke and Mazurak, 1976; McCullough et al., 2001). As seen in the ground water analyses at this site (discussed below), significant degradation of ground water by NO_3 may occur before feedlot abandonment. For sites on coarse-textured soils, therefore, rapid establishment of plants after feedlot abandonment may be necessary if they are to limit subsequent ground water contamination.

Alfalfa Growth and Nitrogen Uptake

Standard, N_2–fixing alfalfa produced more forage DM than nonfixing alfalfa in all harvests each production year (Fig. 5), resulting in 76% more yield. Effectively nodulated alfalfa produced nearly twice the yield of nonfixing alfalfa at the first harvest and about 63% more yield during the remainder of the season. Much of this difference in forage yield presumably was due to

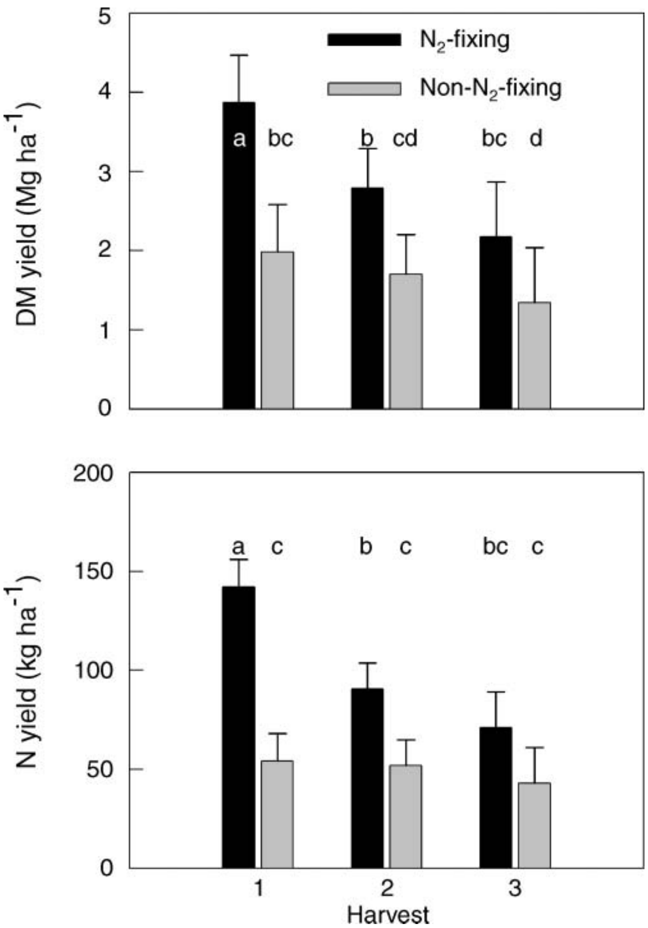

Fig. 5. Mean and SE of alfalfa herbage dry matter (DM) yield and N yield for N_2–fixing (black bars) and non-N_2–fixing (gray bars) alfalfa by harvest at an abandoned feedlot (averaged across 2 forage production years). Bars with the same letter did not differ ($P > 0.05$).

poorer growth related to N deficiency in the nonfixing alfalfa in areas with low N supply, as Lamb et al. (1995) observed using a range of fertilizer N rates.

Evidence for N deficiency was provided by plant N concentration, which was lower in nonfixing than in N_2–fixing alfalfa (29.5 vs. 34.8 g N kg^{-1}, respectively, averaged across all harvests). Most of this difference was due to effects of the first harvest each year, when N_2–fixing alfalfa contained about one-third higher N concentration than nonfixing alfalfa (37.2 vs. 27.9 g N kg^{-1}, respectively). Shoot N concentrations averaged only 7% higher in N_2–fixing than nonfixing alfalfa for the second and third harvests. Nonfixing alfalfa plants were chlorotic in much of the field after the first year, although there were areas without chlorosis. Furthermore, N concentration in the nonfixing alfalfa was more variable than in N_2–fixing alfalfa. In nine of 12 harvest comparisons, the CV for N concentration in the nonfixing alfalfa ranged from 1.7 to 3.5 times larger than the CV of the N_2–fixing alfalfa. Correspondingly, the CV in dry mass yield of nonfixing alfalfa within each block typically was >40% in the first production year and reached 90% the second, whereas N_2–fixing alfalfa had DM CVs of 25% or less throughout the experiment. These results confirm that the wide variation in N supply across the plot area was reflected more strongly in nonfixing plants.

As a consequence of higher DM production and higher N concentration, harvested forage of N_2–fixing alfalfa contained more N than nonfixing alfalfa in the first two harvests (Fig. 5), resulting in a two-fold difference between harvested N over two forage production years (610 vs. 300 kg N ha^{-1}, respectively).

Alfalfa roots were present throughout the upper 1.2 m of soil in October 2000 (Fig. 6). Root distributions were similar for N_2–fixing and nonfixing alfalfa. The mass of thick roots declined to <0.5 kg m^{-3} below the topmost increment (0–7.5 cm), with no differences among deeper depths. Total root mass also did not change below 0.15 m and averaged 5.1 Mg ha^{-1} for the upper 1.2 m of soil. Fine root length densities were constant in the upper 0.15 m, declined rapidly with depth, but remained >0.9 m m^{-3} in the subsoil. We found no relationship between root mass and soil N mineralization potential measured at the sampling sites. These results support earlier conclusions that N_2–fixing and nonfixing alfalfa did not differ in root system architecture (Goins and Russelle, 1996) and that NO_3 supply did not affect root system architecture (Blumenthal et al., 1999).

Soil Nitrogen Supply and N_2 Fixation

The ^{15}N natural abundance technique is predicated on the assumptions that the nonfixing and N_2–fixing plants are acquiring inorganic N with temporally and spatially similar patterns (Shearer and Kohl, 1986) and that isotopic discrimination between ^{14}N and ^{15}N among plant organs is similar (Ledgard, 1989). Within-plant isotopic discrimination, $\delta^{15}N_a$ or "B value," is notoriously variable and is affected by host species and cultivar, rhizobial strain, plant age, plant part, and growth envi-

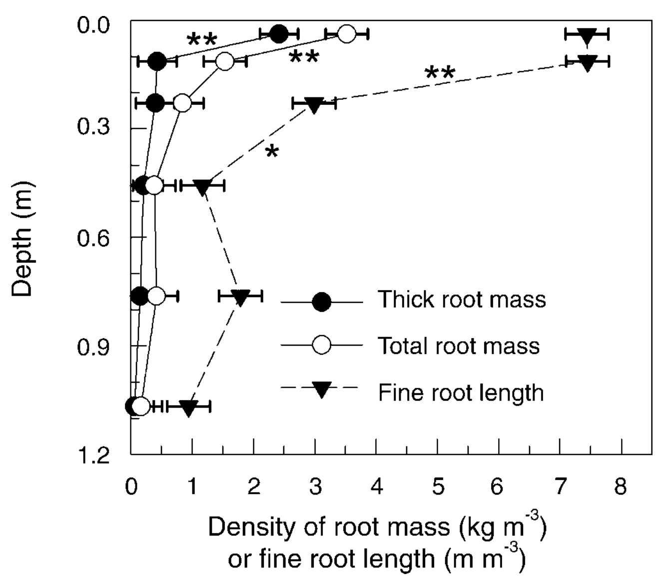

Fig. 6. Mean and SE of average fine root length and root mass by depth for alfalfa. Fine roots had diam. < 1 mm. No differences were detected among alfalfa entries or for the interaction of depth and N_2 fixation status. Differences between adjacent depth increments are indicated by the asterisks (* $P < 0.05$; ** $P < 0.01$).

ronment (Ledgard, 1989; Unkovich et al., 1994; Okito et al., 2004). Estimates of $\delta^{15}N_a$ range from −3.18 to 1.9 $\delta^{15}N$ for alfalfa (Houssain et al., 1995; Walley et al., 1996). When the first value was used with our data, estimates of fNdfa were reduced by 25%, whereas the second resulted in irrational values (i.e., average fNdfa = 1, many plots having fNdfa >> 1). Although our approach to estimating $\delta^{15}N_a$ was prone to error, we suggest that this error was likely no greater than what would be engendered by more traditional methods (Ledgard, 1989; Unkovich et al., 1994; Okito et al., 2004). In particular, our approach avoided biases that arise from deriving B values with one set of rhizobial strains that differ from those that inhabit root nodules in the field (Byun et al., 2004).

Summed across harvests within years, annual Ndfs in N_2–fixing alfalfa (137 kg N ha^{-1}) was greater than Ndfs in nonfixing alfalfa alone (87 kg N ha^{-1}), but equal to Ndfs of nonfixing alfalfa plus weeds (144 kg Nha^{-1}). In the nonfixing plots, weeds contributed between 22 and 46% of the Ndfs harvested. Better weed control in the nonfixing plots to improve the economic value of the forage crop would have reduced competition for soil + manure N. Although this may have improved total Ndfs uptake by nonfixing alfalfa, it may not have counterbalanced the decline in nonfixing alfalfa population we observed.

Nonfixing alfalfa had removed more NO_3 in experiments in which NO_3 supply was large and frequently applied (Lamb et al., 1995; Blumenthal and Russelle, 1996; Blumenthal et al., 1999). In those cases, N supply was sufficient for high yields of the nonfixing alfalfas, whereas in this experiment, N supply from soil and manure often was not. Because all known seed sources of nonfixing alfalfa contain a small proportion of symbiotically effective plants (Barnes et al., 1990), this experiment represents the change in effectiveness from mainly nonfixing to mainly N_2–fixing that can be expected with these germplasms, as nonfixing plants disappear in low-N environments.

The estimated fNdfa in N_2–fixing alfalfa was unrelated to shoot yield or N content and ranged from 0.02 to 0.92 (Fig. 7), with the maximum set by our approach to estimating $\delta^{15}N_a$. Only three of 72 estimates in N_2–fixing plots during the 2 yr were irrational (fNdfa < 0) and these occurred in only two plots. Total annual herbage N apparently increased with improved soil + manure N uptake, although this relationship explained only 25% of the variability in shoot N content (Fig. 8). At the same time, fNdfa declined as expected because estimated fNdfa = 1 − fNdfs when other sources, such as seed N, are insignificant.

Alfalfa yields have responded to manure or fertilizer N application (Raun et al., 1999; Lloveras et al., 2004), but such responses cannot as yet be predicted. In addition to direct N uptake and the reduced photosynthate required to assimilate NO_3 as compared with N_2 (Cetto and Spallacci, 2006), other factors may have affected alfalfa yield in some areas of the field (e.g., macro- or micronutrient availability or better water retention in sites with more residual manure or feed residues). The

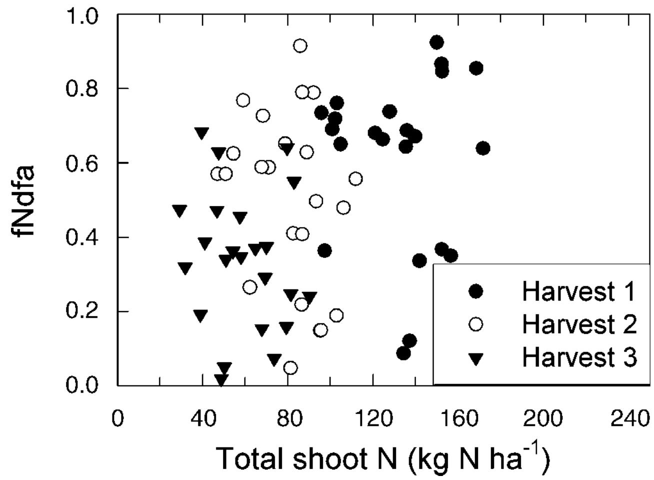

Fig. 7. Plot of the fraction of N derived from the atmosphere (fNdfa) against total harvested N in N_2–fixing alfalfa shoots. Data are from paired 1-m^2 plots across two growing seasons; fNdfa (fraction of N derived from the atmosphere) was estimated by the natural abundance ^{15}N method.

increase in total N yield of N_2–fixing alfalfa with inorganic N supply was due to Harvests 2 and 3 at this site, rather than to the first harvest of each year (data not shown). Greater moisture stress during summer regrowth in the sandy soil at this site may have been ameliorated in some areas by greater manure accumulation and subsequent improved soil water holding capacity. Both yield potential and nodule function can be expected to improve when water deficits are reduced (Serraj et al., 1999).

We show the estimated Ndfa (amount of N derived from the air) plotted against inorganic N uptake (Fig. 8) to highlight the facultative nature of symbiotic N_2 fixation, although Ndfa was estimated from the same data used to estimate soil + manure N uptake. These results confirm that N_2 fixation served to regulate total N supply to the plant (Allos and Bartholomew, 1959; Streeter, 1988), and highlight the resilience conferred by this process in sites with heterogeneous N supply. In

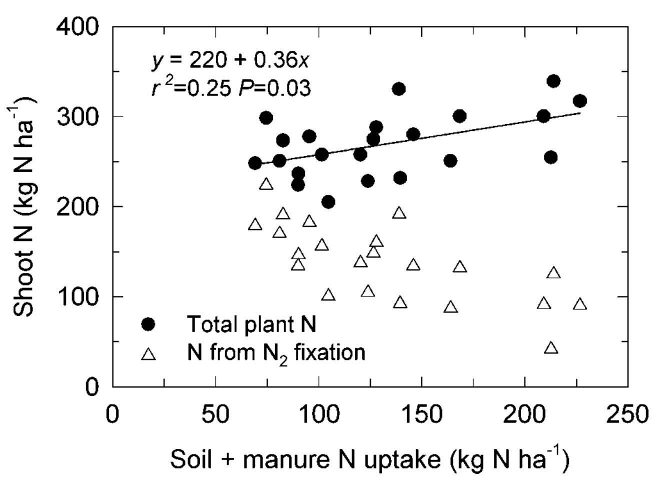

Fig. 8. Response of total N (closed symbols) and fixed N (open symbols) in N_2–fixing alfalfa herbage to soil and manure N uptake in an abandoned feedlot. Data are from paired 1-m^2 plots during two growing seasons based on the natural abundance ^{15}N method.

areas with low N supply, N_2–fixing alfalfa produced high N harvests comprised mainly of fixed N, whereas the crop removed large amounts of N from other areas, and N_2 fixation declined concomitantly. Nonfixing plants do not have this capacity.

Inorganic soil N removal potential can be estimated as the maximum inorganic N removal in the paired 1-m^2 plots, which was 225 kg N ha^{-1} in 2000 and 180 kg N ha^{-1} in 2001. Total N accumulation in nonfixing alfalfa in 1999 averaged 250 kg N ha^{-1}, but this likely was increased by uptake of the slow release N fertilizer applied in June 1999. If we assume a typical fertilizer N use efficiency of 0.5, recovery of soil + manure N in 1999 was similar to later years. Therefore, we estimate that average *maximum* annual soil N removal by established, N_2–fixing alfalfa at this site was about 200 kg N ha^{-1}. This compares with maxima > 400 kg N ha^{-1} 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 ground water containing elevated concentrations of both NH_4–N and NO_3–N (Russelle et al., 2001). Annual yields of irrigated nonfixing Ineffective Agate alfalfa averaged 12 Mg DM ha^{-1} at the North Dakota site, compared with <10 Mg DM ha^{-1} by established N_2–fixing alfalfa in this study. The capacity for N removal by N_2–fixing alfalfa at this Wisconsin feedlot remediation site was likely limited by a combination of moderate yield (plant N demand) and reduced rooting depth, presumably caused by low herbage yield (photosynthate supply) and dry soil conditions, which increased penetration resistance of the soil.

Changes in Soil Nitrogen

There was a rapid decline in topsoil (0–0.15 m) inorganic N across time, and slower change in total topsoil N (Fig. 9). We detected no difference among alfalfa entries in NH_4–N or total-N in the topsoil. Average NH_4–N concentration declined from 14.8 mg N kg^{-1} in 1998 to 4.5 mg N kg^{-1} in 2001, approximately equivalent to 22 kg N ha^{-1} (soil dry bulk density from 0 to 0.15 m = 1.39 g cm^{-3}). During the same time interval, NO_3–N concentration dropped by about 33 kg N ha^{-1} in the N_2–fixing plots and by 44 kg N ha^{-1} in the nonfixing plots, reflecting less than one-third of the Ndfs removal in herbage measured in the paired plots. Total soil N declined from 0.96 g kg^{-1} in 1998 and 2000 (no statistical difference between these 2 yr) to 0.84 g kg^{-1} in 2001, a drop of about 170 kg N ha^{-1}, about 30 kg N ha^{-1} more than was accounted for in harvested alfalfa herbage.

Ground Water Quality

Water quality in most wells was impaired by nutrient leaching from animal manure to ground water at the time of our earliest sampling in June and August 1999. Nitrate-N concentrations in down-gradient wells ranged from 8.8 to 88 mg N L^{-1}, chloride concentrations ranged from 6 to 116 mg Cl L^{-1}, and concentrations of these two ions were correlated ([Cl] = 1.33 [NO_3–N]; r^2 = 0.72), supporting the conclusion that livestock manure, rather than fertilizer N, was the source of NO_3 in the ground water (Stites and Kraft, 2001). Ammonium N concentrations were < 0.03 mg N L^{-1} (except for a single measurement of 0.14 mg N L^{-1}). We could not determine whether these manure-related impacts on ground water quality were due to the long history of the site or to more recent effects, but impacts from leaching to ground water under sandy soils in this region tend to be rapid. Rapid NO_3 leaching in these sandy soils may have contributed to N deficiency in the nonfixing alfalfa.

The foregoing data do not include anomalous values from the northernmost up-gradient and down-gradient wells, which generally had higher NO_3–N concentrations in 1999 than other wells (mean = 50 mg N L^{-1}) and

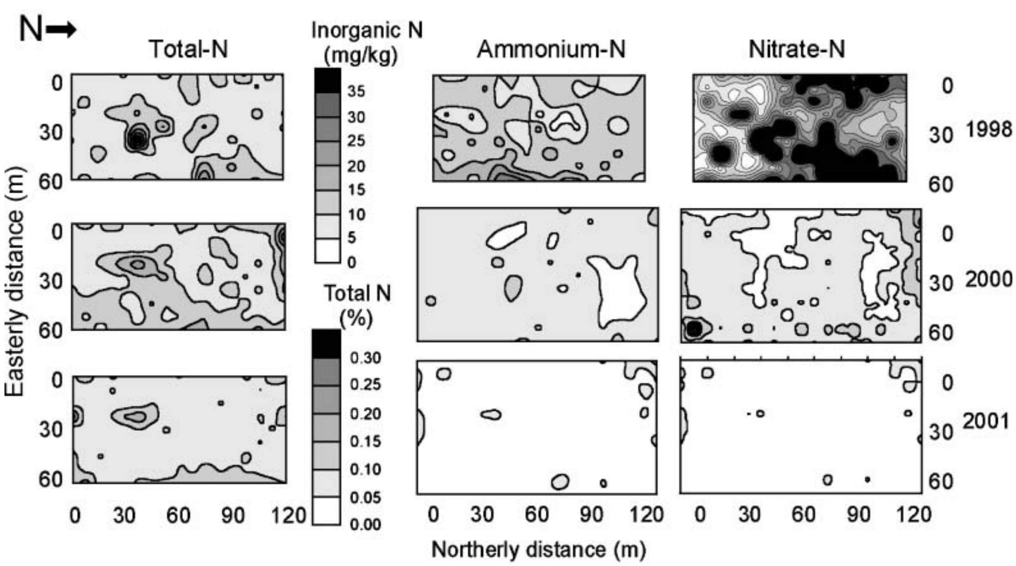

Fig. 9. Kriged contour diagrams of total N, NH_4–N, and NO_3–N in topsoil (0–0.15 m) across time at the abandoned feedlot site. In 1998, NO_3–N concentrations ranged up to 120 mg N kg^{-1} soil. Figures are shown in consistent scale, but the sampling frame for inorganic N was expanded by 7.6 m in all directions in 2000 and 2001.

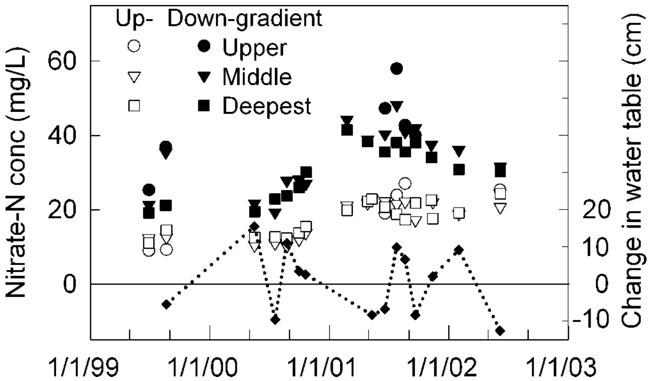

Fig. 10. Mean nitrate N concentrations and mean change in water table depth in all monitoring well ports (except two wells affected by focused recharge, discussed in the text), 1999–2002. Each sampling depth was screened over adjacent 0.6-m intervals. The shallowest of the four installed wells was dry during the study period, and the next shallowest (Upper) was dry on many dates.

showed no change over time (data not shown). These wells were located near an area that received surface runoff from the up-gradient feedlot. We infer that focused surface runoff from up-gradient land use degraded ground water quality on this coarse-textured soil.

Nitrate-N concentration in the remaining three up-gradient wells was slightly more than 10 N mg L^{-1} from June 1999 through July 2000, then increased during the next 8 mo until reaching a plateau in April 2001 at about 21 mg N L^{-1} (Fig. 10). We interpret this increase as an indication that water quality flowing toward the plot area was being affected by the adjacent up-gradient land use, which included a poorly vegetated feedlot area.

A total of 266 observations were used from down-gradient sampling wells. These had a mean NO_3–N of 32.9 mg N L^{-1} (SE = 1.0 mg N L^{-1}). In contrast with up-gradient sampling sites, NO_3–N concentration measured in the upper 1.5 m of the ground water at down-gradient wells rose from a mean of about 20 mg N L^{-1} in June 1999 to about 45 mg N L^{-1} in July 2001, before declining to 30 mg N L^{-1} 11 mo later (Fig. 10), averaging 15 mg NO_3–N L^{-1} higher than the up-gradient wells (paired t test $P < 0.0001$). We did not sufficiently characterize ground water recharge events (although some are apparent in Fig. 10) or flow rate, which limits us to making qualitative inferences about the net addition of NO_3–N from the plot areas. From the these data, however, it appears that that NO_3 leaching occurred from the study plots, in spite of substantial inorganic N removal in alfalfa herbage.

CONCLUSIONS

Nitrate leaching to ground water occurred at this site, with all wells exceeding the 10 mg N L^{-1} public drinking water standard for NO_3–N. Both nonpoint and focused recharge leaching were apparent, highlighting the need for better livestock manure handling and storage, and perhaps engineering of feedlots (e.g., installation of a liner to restrict water percolation and installation of a pond for runoff collection), to minimize both leaching and runoff on these soils. This propensity for NO_3 leaching suggests that remediation strategies may require active ground water cleanup (Russelle et al., 2001), companion seeding of fast growing cereal crops, direct seeding techniques to minimize tillage-induced N mineralization, or removal of nutrient-laden topsoil (Uusi-Kamppa, 2002).

We found that soil and manure N uptake was greater for N_2–fixing than nonfixing alfalfa at this site, in contrast to research on sites with higher overall inorganic N supply. Where mineralized N is insufficient to support vigorous growth of a nonfixing crop, lower yield, lower crude protein concentration, weed incursion, and stand decline reduce the economic value of the crop. Protein content is a major determinant of hay quality. Weed incursion will result in reduced buyer preference for alfalfa hay (Ward, 1987), increased weed seed bank, or higher weed control costs.

This research demonstrated how symbiotic N_2 fixation provides both efficient N removal and yield resilience on a site with patchy inorganic N supply, which would economically benefit a phytoremediation effort.

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