from the 28, 56, and 112 kg K ha^{-1} treatments rated Very High (182, 245, and 290 mg K kg^{-1}), and the BR samples rated High (88, 92, and 140 mg K kg^{-1}).

SUMMARY

The data clearly indicate nutrient stratification occurring within the row and with soil depth on three soils after 6 years of no-till cotton. Stratification of P was observed in the three soils. Mehlich-1 P was greater in the IR position for two soils, but was greater in the BR position in one soil. Regardless of these results, soil test ratings would not have been affected by sampling position. Although differences in Mehlich-1 P were not of any practical significance, additional time in no-till would be likely to magnify these differences and so might affect fertilizer recommendations. Mehlich-1 K was greater in IR samples and would have affected soil test ratings, depending on the soil and the fertilizer K rate. These data indicate that additional information on sampling techniques is needed to properly assess the nutrient levels of cotton fields after 6 years in no-till production. These data also indicate that additional information is needed to evaluate vertical stratification within different soils.

REFERENCES

Eckert, D.J. 1985. Review: Effects of reduced tillage on the distribution of soil pH and nutrients in soil profiles. J. Fert. Issues 2:86–90.
Howard, D.D., and D.D. Tyler. 1987. Comparison of surface applied phosphorus and potassium rates and in–furrow fertilizer combinations for no–till corn. J. Fert. Issues 4:48–52.
Kitchen, N.R., J.L. Havlin, and D.G. Westfall. 1990. Soil sampling under no–till banded phosphorus. Soil Sci. Soc. Am. J. 54: 1661–1665.
Mehlich, A. 1953. Determination of P, Ca, Mg, K, Na and NH_4. N.C. Soil Testing Div. Memo. N.C. State Univ., Raleigh.
Morrison, J.E., Jr., and F.W. Chichester. 1994. Tillage system effects on soil and plant nutrient distribution on Vertisols. J. Prod. Agric. 7:364–373.
Mullen, M.B., and D.D. Howard. 1992. Vertical and horizontal distribution of soil C,N,P,K, and pH in continuous no–tillage corn production. p. 6–10. *In* M.D. Mullen and B.N. Duck (ed.) Proc. 1992 Southern Conservation Tillage Conference. Tenn. Agric. Exp. Stn. Spec. Publ. 92–01.
Petersen, R.G., and L.D. Calvin. 1986. Sampling. p. 33–51. *In* A. Klute (ed.) Methods of soil analysis. Part 1. 2nd ed. Agron. Monogr. 9. ASA and SSSA, Madison, WL.
Tennessee Department of Agriculture. 1996. Tennessee agriculture, 1996. Tenn. Agric. Stat. Serv., Nashville.
SAS Institute, 1997. SAS/STAT software: Changes and enhancements through release 6.12.SAS Inst., Cary, NC.
Tyler, D.D., and D.D. Howard. 1991. Soil sampling patterns for assessing no–tillage fertilization techniques. J. Fert. Issues 8:52–56.
Watanabe, F.S., and S.R. Olsen. 1965. Test of an ascorbic acid method for determining phosphorus in water and $NaHCO_3$ extracts from soil. Soil Sci. Soc. Am. Proc. 29:677–679.

Subsoil Nitrate and Bromide Uptake by Contrasting Alfalfa Entries

Jürg M. Blumenthal, Michael P. Russelle,* and JoAnn F. S. Lamb

ABSTRACT

Alfalfa (*Medicago sativa* L.) is a deeply rooted perennial legume that can protect the environment by absorbing nitrate (NO_3^-) both better than annual crops and from deeper in the soil. Our objectives were to characterize subsoil NO_3^- removal by eight alfalfa entries differing in symbiotic efficiency, root system architecture, forage quality, and leaf morphology, and to evaluate Br^- as an alternative tracer to ^{15}N for monitoring NO_3^- uptake. Low (≈0.3 m*M*) or high (20 m*M*) NO_3^-–N concentrations were supplied through a subsoil irrigation system installed in a Hubbard loamy sand soil (sandy, mixed Udorthentic Haploboroll) at Becker, MN. Nitrate uptake and N_2 fixation were evaluated during two regrowth periods using ^{15}N. We also added small concentrations of Br^- to the subsoil NO_3^- during three regrowth periods. Subsoil NO_3^-–N removal was similar for the seven N_2–fixing entries, suggesting that selection for traits such as root system architecture, high forage quality, or multiple (>3) leaflets does not necessarily confer an advantage in NO_3^- absorption. Even though 'Ineffective Agate' yielded less herbage than the N_2–fixing entries, this non–N_2–fixing cultivar removed about 38% more subsoil NO_3^- than N_2–fixing entries over the growing season. These results confirm our earlier findings that non–N_2–fixing alfalfa cultivars are likely to be more effective in phytoremediation of NO_3^-–contaminated sites than are standard cultivars. It also confirms our earlier conclusion that the simple difference technique may underestimate rates of symbiotic N_2 fixation when inorganic N supply is large. A strong correlation between ^{15}N and Br^- uptake in the herbage (mg excess $Br^- = 25.2 \times$ mg excess ^{15}N, $r^2 = 0.97$) led us to conclude that Br^- can be used as a tracer of NO_3^- absorption by alfalfa in the field. This provides a new, dual-tracer approach for such studies.

Excessive applications of N have been implicated in contamination of surface water, ground water, and the atmosphere (Power and Schepers, 1989; Matson et al., 1997). Field studies routinely show that soil inorganic N concentrations (Muir et al., 1976; Campbell et al., 1994) and NO_3^- leaching losses (Randall et al., 1997) are smaller under deeply rooted perennial crops such as alfalfa than under annual crops such as corn (*Zea mays* L.).

Are there differences in subsoil NO_3^- uptake among alfalfa cultivars? Lamb et al. (1993) reported preliminary evidence from a field trial that diverse alfalfa entries differed by up to 20% in N uptake from topdressed fertilizer (entries ranged from 34.2 to 41.6% fertilizer N use efficiency). In another field trial, ineffectively nodulated alfalfa, which cannot fix atmospheric N_2, was

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more efficient in subsoil NO_3^- removal than its N_2–fixing parent (Blumenthal and Russelle, 1996). There may be phenotypic traits other than symbiotic effectiveness, however, that coincidentally confer an advantage in NO_3^- uptake. Multileaflet cultivars, for example, may have a greater capacity to reduce and assimilate or to temporarily store in vacuoles the NO_3^- that was not reduced in roots. Cultivars selected for high crude protein concentration in herbage may have a higher total sink capacity for N, thereby conferring improved NO_3^- uptake capacity. Root system architecture may alter uptake of nutrients from soil (Fitter, 1996), although van Noordwijk (1983) argued that relatively small root length densities are sufficient to maximize uptake of mobile nutrients like NO_3^-.

Nitrogen harvested in herbage of standard alfalfa cultivars consists not only of inorganic soil N, but also atmospheric N_2 fixed by *Rhizobium* and N remobilized internally from crowns and roots (Ourry et al., 1994; Russelle et al., 1994). While the use of ^{15}N as a tracer is the only unequivocal way to obtain reliable estimates of NO_3^- removal by alfalfa (Blumenthal and Russelle, 1996), it is an expensive technique. Bromide may be an inexpensive alternative tracer for NO_3^- uptake in legumes (Magarian et al., 1998). Bromide has been used widely as a tracer in investigations dealing with NO_3^- movement in soil (Silvertooth et al., 1992), but Kung (1990) has warned that plant uptake of Br^- may confound both the results and interpretation of such research. Jemison and Fox (1991) showed under both greenhouse and field conditions that Br^- was taken up readily by corn and that application of Br^- at low concentrations did not affect yield. Because there was no competitive inhibition of Br^- on NO_3^-–N uptake or vice versa, uptake rates of these ions may not be correlated. Magarian et al. (1998), however, showed a strong correlation between NO_3^-–N and Br^- uptake in alfalfa under greenhouse conditions and found no evidence that Br^- uptake altered alfalfa growth or yield. If Br^- and NO_3^-–N uptake are correlated, then the two tracers could be used in alternate regrowth cycles, thereby avoiding overestimation of N derived from the source of interest in current herbage regrowth due to remobilization of recently stored N in the root and crown.

The objectives of this study were (i) to compare removal of subsoil NO_3^-–N in herbage of alfalfa entries differing in their symbiotic effectiveness, rooting patterns, herbage quality, and leaf morphology and (ii) to evaluate whether herbage Br^- uptake can be used as an indicator for NO_3^- uptake in alfalfa under field conditions.

MATERIALS AND METHODS

This experiment was conducted at the University of Minnesota Sand Plain Research Farm near Becker, MN (45°23′ N, 93°54′ W). The soil at the site is a Hubbard loamy sand (sandy, mixed Udorthentic Haploboroll) characterized by single-grain structure and underlain by gravel at 95 to 110 cm. During the previous 3 yr, the site was cropped with rye (*Secale cereale* L.). In April 1994, a subirrigation system was installed under the entire plot area by burying soaker hoses (Moisture Master,[1] Aquapore Moisture Systems, Phoenix, AZ) in parallel trenches about 30 cm apart and 50 cm deep. In May 1993, the soil was amended with 112 kg P ha^{-1}, 412 kg K ha^{-1}, 69 kg S ha^{-1}, 3 kg B ha^{-1}, and 6700 kg lime ha^{-1}, based on topsoil tests and Univ. of Minnesota recommendations (Rehm and Schmitt, 1990). All amendments were broadcast applied and disk-incorporated in the upper 20 cm. Trifluralin [2,6-dinitro-*N,N*-dipropyl-4-(trifluoromethyl) benzenamine] was applied preplant at a rate of 0.56 kg a.i. ha^{-1} to control weeds. Postemergence weed control during the experiment was done by hand. In early June 1993, the soil was rototilled and packed, and on 12 June 1994, alfalfa entries (described below) were seeded in 1.8- by 2.4-m plots at a rate of 500 live seeds m^{-2} and a row spacing of 18 cm. The seed was inoculated with commercial *Rhizobium meliloti* (Nitragin, Milwaukee, WI). On 21 Apr. 1995, 45 kg N ha^{-1} as sulfur-coated urea was broadcast-applied to the entire experimental area to supplement the low N supply of this soil.

Experimental Design and Treatments

The experimental design was a randomized complete block in a strip-plot arrangement with seven replicate blocks. Nitrate treatments were applied through the subirrigation system. One half of each replicate received well water amended with $Ca(NO_3)_2$ to 20 m*M* NO_3^-–N from a storage tank, whereas the other half served as the control and received nonamended well water (≈0.3 m*M* NO_3^-–N). The eight different alfalfa entries were planted in strips perpendicular to the NO_3^- treatments. Entries included in this study were: 'Agate'; Ineffective Agate, an ineffectively nodulated, non–N_2–fixing germplasm selected from Agate (Barnes et al., 1990); 'WL-322 HQ', selected for increased forage quality; 'Multi-7', selected for leaves with more than three leaflets; and four experimental germplasms selected from the same genetic source for differences in root system architecture: MWNC-HF_{C2}-BRH (UMN 2966) has high fibrous root mass on a taproot with many lateral roots, MWNC-HF_{C2}-TAP (UMN 2915) has high fibrous root mass on a taproot with no or few lateral roots, MWNC-LF_{C2}-BRH (UMN 2964) has low fibrous root mass on a taproot with many lateral roots, and MWNC-LF_{C2}-TAP (UMN 2963) has low fibrous root mass on a taproot with no or few lateral roots.

Measurements

Herbage was harvested on 19 Aug. and 19 Oct. 1994, on 16 June, 21 July, and 10 Oct. 1995, and on 10 June 1996. At each harvest, herbage from a 1- by 1-m area in each plot was cut by hand about 5 cm above the soil surface and dried in forced-air ovens at about 40°C, and then the dry matter yield was determined. The remainder of the plot was mowed at the same height and cut herbage was removed.

Treatments were applied the first time on 21 Aug. 1994 and continued for the following two growing seasons. Plots were sprinkler irrigated with about 25 mm water, and 2 h later the subsoil treatments were established by applying 25 mm water through the subirrigation system at a rate of about 5 mm h^{-1}. This was done once or twice a week as needed, depending on season and rainfall (Wright and Bergsrud, 1991). When significant rainfall (>20 mm) occurred during a 1- to 2-d period, subsoil treatments were reestablished the following day.

[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.

Each subirrigation event applied ≈71 kg NO_3^-–N ha^{-1} at the 20 m*M* concentration, but we do not know how much of this N remained in the root zone in this shallow, sandy soil, so the amount applied is not appropriate for calculating recovery of added N. The effectively treated depth increment was 40 to 50 cm thick (discussed below). Our objective was to provide a relatively constant concentration of NO_3^- in the subsoil over time, rather than to apply a specific amount of N per unit area.

Subsoil N uptake was quantified using ^{15}N during two herbage regrowth periods. In the summer regrowth of 1995, ^{15}N was added to all five subirrigations between 22 June and 18 July 1995, bringing the added $Ca(NO_3)_2$ to 0.4491 atom % ^{15}N. During two regrowth periods, in spring (six subirrigations) and fall (nine subirrigations) of 1995, Br^- was added at a concentration of 38 μ*M* to the water containing 20 m*M* NO_3^-–N.

We took an approach different from uniform labeling to examine the relationship between $^{15}NO_3^-$ and Br^- tracer uptake. During the spring regrowth in 1996 (six subirrigations), subirrigation water at the high NO_3^- concentration was labeled in three replicate blocks with 0.4491 atom % ^{15}N and 38 μ*M* Br^-, in two replicate blocks with 0.8982 atom % ^{15}N and 75 μ*M* Br^-, and in the final two replicate blocks with 1.3473 atom % ^{15}N and 113 μ*M* Br^-. The same amount and concentration of NO_3^-–N were applied in each case (total ≈ 425 kg NO_3^-–N ha^{-1}). As is the case for the entire experiment, we did not determine how much of this N remained in the rather shallow treated zone of the subsoil. Data from this regrowth were used to compare uptake of the two tracers by the alfalfa entries, and to determine whether alfalfa entries differed in $^{15}NO_3^-$–N uptake.

Herbage samples harvested on 21 July 1995 and 10 June 1996 were ground finely after drying and were analyzed for N and ^{15}N concentrations on a Carlo Erba NA1500 Analyzer (Carlo Erba, Milan, Italy) interfaced with a Tracermass isotope mass spectrometer (Europa Scientific, Cheshire, UK) at the Nitrogen Isotope Laboratory, University of Nebraska, Lincoln. Herbage samples harvested on 16 June and 10 Oct. 1995 and on 10 June 1996 were ground finely after drying and were analyzed for Br^- by energy-dispersive X-ray fluorescence spectroscopy at the University of Nebraska Soil Testing Laboratory.

We used this dual-tracer approach to calculate relative differences in subsoil NO_3^- uptake for an entire growing season. In the second year, we had a direct estimate of NO_3^- uptake based on ^{15}N labeling for the summer regrowth period and indirect estimates based on Br^- uptake for the spring and fall regrowth periods. As in earlier calculations, tracer uptake was corrected for background concentrations before calculating the ratio of uptake in Ineffective Agate divided by uptake in Agate alfalfa. These ratios were then weighted by the proportion of annual herbage N production in Ineffective Agate that was present in each harvest, and results were summed over the three harvests.

Soil samples were taken at about 3, 8, and 13 cm away from subirrigation tubes in two 90-cm-long transects in each subsoil NO_3^- treatment on 25 July 1995, 1 d following a complete irrigation. Inorganic N distribution within the profile was determined for depth increments of 0 to 20, 20 to 40, 40 to 50, 50 to 60, 60 to 70, 70 to 80, and 80 to 90 cm (about the depth of underlying gravel). Both NH_4^+–N and NO_3^-–N were determined colorimetrically by flow injection analysis (Diamond, 1992; Switala, 1993) after 2 *M* KCl extraction of air-dry, ground soil samples (Keeney and Nelson, 1982). We averaged data within each of the two subsoil NO_3^-–N treatments from all cores at distances of 3, 8, and 13 cm from the subirrigation tubes, except that only about 60% of the samples in the control treatment were analyzed, because all were uniformly low in NO_3^-–N concentration. A plot of subsoil NO_3^-–N concentrations was generated using linear interpolation and plotting the isolines as mirror images around the hose.

Because nutrient availability can alter expression of root system architecture, three rows within each subplot were undercut on 4 Oct. 1996 to a depth of about 25 cm, and plants were removed and evaluated for number of lateral roots, fibrous root mass, and taproot diameter 5 cm below the crown as described by Johnson et al. (1996). Root system architecture was not rated in the Ineffective Agate that received the low N treatment, because stands were too poor.

Calculations and Statistical Analysis

The standard isotope equation (Hauck, 1982) was used to calculate NO_3^- uptake. The isotope dilution method (Vose and Victoria, 1986) was used to estimate symbiotic N_2 fixation in the summer regrowth period of 1995. All data were subjected to ANOVA using SAS (SAS Inst., 1987), with both N rate and alfalfa entry considered fixed effects in this strip-plot arrangement. The relationship between uptake of Br^- (in excess of background levels) and ^{15}N (in excess of natural abundance) during the spring regrowth in 1996 was tested using linear regression with SAS. Treatment effects were considered significant for comparisons where the probability of obtaining a larger *F*-statistic by chance alone was less than 5% and LSDs were calculated for comparisons of main effects.

RESULTS AND DISCUSSION

Ammonium-N concentration in soil 1 to 2 d after subirrigation averaged 1.8 mg N kg^{-1} soil and was not influenced by treatment or soil depth. Uniform and small NO_3^- concentrations (<1.7 mg NO_3^-–N kg^{-1} soil) were present after subirrigation with well water (≈0.3 m*M* NO_3^-), indicative of the poor N status of this coarse-textured soil, whereas subirrigation with 20 m*M* NO_3^- solution increased soil NO_3^- concentrations below 45 cm (Fig. 1).

There was a strong gradient in soil NO_3^- concentrations in the 20 m*M* NO_3^- treatment decreasing with distance from the subsoil irrigation hoses. We attribute this gradient to two factors. First, the 30-cm spacing between hoses as recommended by the manufacturer was too large to allow for overlap between adjacent wetting fronts in this coarse-textured soil. These conditions resulted in an area midway between the hoses (about 3 cm wide) where subsoil NO_3^- concentrations did not differ between treatments. Second, the amount of water containing 20 m*M* NO_3^-–N supplied through the subirrigation system was insufficient to completely replace soil water, and so the 20 m*M* NO_3^-–N solution was diluted with soil water increasingly with distance from the subirrigation hose. However, with our subsoil irrigation system, NO_3^- concentration in 97% of the root zone below 45 cm was increased by 2 to 15 times the concentration found in the ≈0.3 m*M* treatment.

Root System Architecture

Alfalfa germplasms selected previously for a fibrous root system (i.e., plants having many roots <2 mm in diameter) had highest scores for fibrousness in both

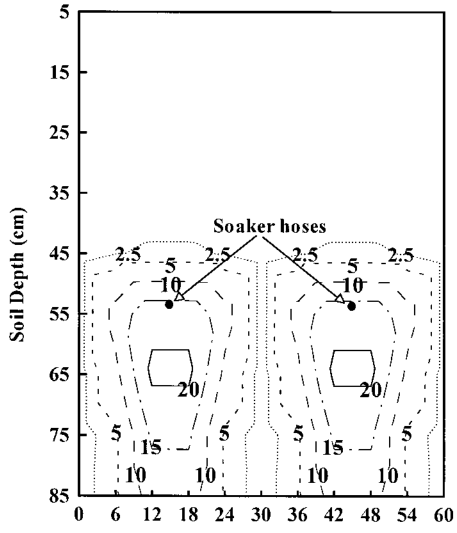

Fig. 1. Nitrate-N concentrations in the soil profile 1 d after subirrigation with 20 m*M* NO_3^-–N solution. Initial data were means of 12 vertical NO_3^-–N distribution profiles measured at 3, 8, and 13 cm from subirrigation hoses. Values between measured points were obtained by linear interpolation and were plotted as mirror images. Values > 2.5 mg NO_3^-–N kg^{-1} soil were different ($P < 0.05$) from plots subirrigated without additional NO_3^-. Concentrations in soil shallower than 45 cm averaged 1.6 mg NO_3^-–N kg^{-1}.

the population selected for having a branched-rooted growth habit and in the population selected for having a strong taproot (Table 1). Lateral root number score of MWNC-LFæC2-TAP was smaller than that of the other experimental germplasms. Agate and WL-322 HQ expressed moderate numbers of fibrous roots and little branching, whereas Multi-7 was more branched and fibrous. Application of subsoil NO_3^- over nearly two growing seasons had no effect on root branching and fibrousness, and increased taproot diameter only marginally compared with alfalfa in plots that received well water only. These data indicate that differences in root characteristics in alfalfa were maintained over the length of the experiment and were not affected by availability of subsoil NO_3^-. Ineffective Agate subirrigated with NO_3^- expressed the same root characteristics as Agate. Evaluation of root system characteristics of the Ineffective Agate that did not receive additional NO_3^- was not possible, because stands were lost by autumn of the third year due to the very low N availability at this site.

Table 1. Root system fibrousness and secondarily thickened lateral root number in 3-yr-old stands of N_2–fixing alfalfa. Values are averaged over two subsoil NO_3^- treatments, because N treatments did not affect root system architecture.

Alfalfa entry†	Fibrousness score‡	Lateral root number§
Agate	2.0	2.0
WL-322 HQ	1.9	1.9
Multi-7	2.8	2.4
MNWC-LF$_{C2}$-TAP	2.2	1.9
MWNC-LF$_{C2}$-BRH	2.2	2.5
MNWC-HF$_{C2}$-TAP	3.5	2.3
MNWC-HF$_{C2}$-BRH	3.5	2.6
LSD (0.05)	0.4	0.3

† Ineffective Agate was not included in the analysis due to stand loss in the control subsoil NO_3^- treatment.
‡ Fibrousness as the number of fibrous roots was scored on a scale of 1 to 5, where 1 = none, 3 = moderate, and 5 = many.
§ Lateral root number per plant was scored on a scale of 1 to 5, where 1 = 0 to 3, 3 = 8 to 11, and 5 = ≥16 lateral roots.

Table 2. Herbage dry matter (DM) yield of contrasting alfalfa entries at two harvest dates during the establishment year. Two subsoil NO_3^- treatments were established, starting 21 Aug. 1994.

Alfalfa entry	DM yield 19 Aug. 1994	DM yield 19 Oct. 1994†
	g m^{-2}	g m^{-2}
Ineffective Agate	72	40
Agate	182	123
WL 322-HQ	180	152
Multi-7	165	142
MNWC-LF$_{C2}$-TAP	204	160
MNWC-LF$_{C2}$-BRH	158	142
MWNC-HF$_{C2}$-TAP	194	152
MNWC-HF$_{C2}$-BRH	168	158
LSD (0.05)	33	16

† Values for the harvest of 19 Oct. 1994 were averaged over two subsoil NO_3^- treatments, because NO_3^- treatments did not affect yield.

Yield

Herbage yield differences were present among N_2–fixing entries during the establishment year (Table 2), but were of minor agronomic importance, because they did not persist after the establishment year. Ineffective Agate yielded about one-third as much as the N_2–fixing alfalfa entries during the establishment year, confirming the very limited N supply capacity of the soil at this site. Application of subsoil NO_3^- to one-half of the plots started on 21 Aug. 1994 and did not affect herbage yield on 19 Oct. 1994. We attribute this lack of NO_3^- response, especially in Ineffective Agate, to the presumably shallow extent of the developing root systems and, therefore, to the very limited capacity of the plants to absorb NO_3^- applied at depth.

As expected, there were marked interactions of subsoil NO_3^- treatment with entry for yields at all harvests of the second and third year after stand establishment, due primarily to the contrasting response of N_2–fixing entries compared with Ineffective Agate. The effect of subsoil NO_3^- on yield of Ineffective Agate increased over time, because this germplasm became increasingly N stressed without subsoil NO_3^- (Fig. 2). Addition of

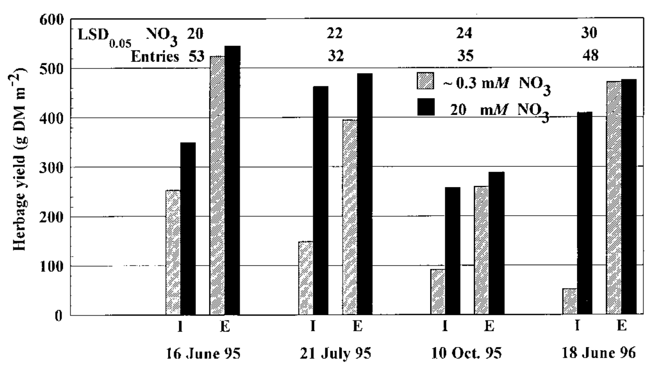

Fig. 2. Mean herbage yield of Ineffective Agate alfalfa (I) and seven N_2–fixing alfalfa (E) entries during the second and third years after planting as affected by concentration of NO_3^-–N in subirrigation water. Yields of the N_2–fixing alfalfa entries were averaged, because they did not differ. LSD (0.05) values are shown for main effects of entry and NO_3^-.

subsoil NO_3^- increased Ineffective Agate yield by 70 to 180% during Year 2 and by 690% in the spring harvest of Year 3. Ineffective Agate supplied with NO_3^- attained the yield of the N_2–fixing entries only in the fall harvest of Year 2 (1995), indicating that Ineffective Agate still was N-limited despite NO_3^- addition to the subsoil.

This result at first seems surprising, because we added between 350 and 640 kg NO_3^-–N ha^{-1} to the subsoil during each regrowth period. Our visual observations at the site, however, indicate that crop roots typically do not penetrate the gravel that underlies the soil (located 95 to 110 cm deep) and, because the water-holding capacity of the coarse-textured subsoil is only 0.03 cm cm^{-1} (Grimes, 1968), we conclude that relatively little of the applied NO_3^-–N remained within the root zone. Ineffective Agate produces yields equal to Agate when N supply is not limiting (Lamb et al., 1995). Comparable yields of Ineffective Agate and the N_2–fixing entries in autumn may be related to lower N requirements for the reduced herbage yield. In contrast, growth and associated N demand in spring and summer apparently were too high to be met by the subsoil NO_3^- supply, resulting in yield differences between Ineffective Agate and the N_2–fixing entries.

In contrast to the large effects observed with Ineffective Agate, subsoil NO_3^- increased total annual forage yield of the N_2–fixing entries by only 12% during Year 2 (Fig. 2). The yield increase due to NO_3^- in these entries probably reflects the combination of limited soil N supply and insufficient rates of symbiotic N_2 fixation. Alfalfa yields commonly are increased with fertilizer N addition at low fertility sites (Feigenbaum and Hadas, 1980; Fishbeck and Phillips, 1981; Trimble et al., 1987), whereas at sites favorable for alfalfa production and with effective inoculation with *Rhizobium*, application of N fertilizer at establishment does not increase yield (Eardly et al., 1985). Lack of yield response to subsoil NO_3^- during spring regrowth of Year 3 indicates that other N supplies (N_2 fixation, mineralized soil N, etc.) were adequate to maximize herbage yield by this time.

Amounts and Sources of Herbage N

Herbage N concentration was 33 to 36 mg N g^{-1} dry matter at all harvests and was not affected by the applied subsoil NO_3^-. In both of the harvests following application of ^{15}N-enriched $Ca(NO_3)_2$, herbage from plots subirrigated with NO_3^- were enriched in ^{15}N, whereas herbage from the other plots was at natural abundance (avg. = 0.3745 atom % ^{15}N at this site), indicating that the ^{15}N label was not absorbed by roots of plants sampled in adjacent plots. Additionally, we observed no pattern of ^{15}N enrichment in labeled herbage across the experimental area, indicating uniform distribution of ^{15}N with this subirrigation system.

Ineffective Agate removed 45% more subsoil NO_3^-–N in herbage than the N_2–fixing entries in the 21 July 1995 harvest, despite having a smaller total N yield than the N_2–fixing entries (Fig. 3). The N_2–fixing entries, however, were not different in accumulation of subsoil NO_3^- in the herbage. During this summer regrowth period, symbiotic N_2 fixation in the N_2–fixing alfalfa entries was reduced by 41% by exposure to subsoil NO_3^-, with no differences in extent of this reduction among N_2–fixing entries. Ineffective Agate and the N_2–fixing entries did not differ in subsoil NO_3^-–N removal in the spring harvest of the third year, but total N yield was smaller in Ineffective Agate than in the N_2–fixing entries (Fig. 4). During this regrowth period, exposure to subsoil NO_3^- reduced symbiotic N_2 fixation in the N_2–fixing entries by 68%, and again no differences were found among N_2–fixing entries in reduction of symbiotic N_2 fixation.

We used the standard isotope equation (Hauck, 1982) to calculate NO_3^- uptake and the isotope dilution method (Vose and Victoria, 1986) to estimate symbiotic N_2 fixation for plants subirrigated with 20 m*M* NO_3^-–N.

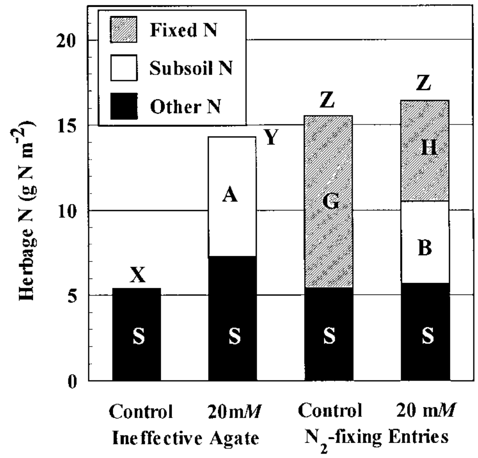

Fig. 3. Amounts and sources of herbage N in the summer harvest of the second year (21 July 1995) as affected by alfalfa entry and subsoil NO_3^- supply. Values of the seven N_2–fixing alfalfa entries were averaged, because they did not differ. Different consecutive letters indicate differences in amount of N derived from the corresponding source or in total N yield. "Other N" includes mineralized soil N, N remobilized from roots and crown, N in the topsoil irrigation water, atmospheric deposition, and other minor sources.

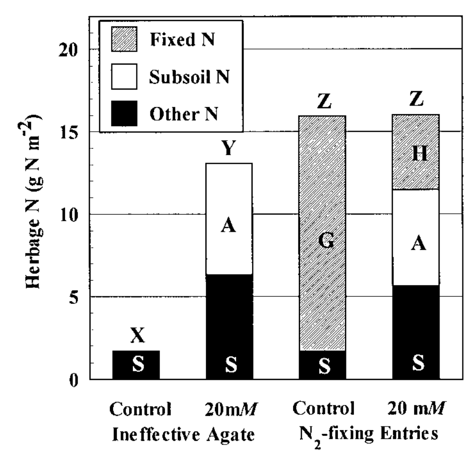

Fig. 4. Amounts and sources of herbage N in the spring harvest of the third year (10 June 1996) as affected by alfalfa entry and subsoil NO_3^- supply. Values of the seven N_2–fixing alfalfa entries were averaged, because they did not differ. Different consecutive letters indicate differences in amount of N derived from the corresponding source or in total N yield.

These are the most accurate techniques to estimate N contribution from different sources, as was demonstrated in an earlier study (Blumenthal and Russelle, 1996). We used the standard difference method to estimate symbiotic N_2 fixation of the plants that were subirrigated with nonamended well water (Weaver, 1986). Under higher soil N supply, such as was provided with subsoil irrigation in this experiment, the difference technique can result in a large underestimation of symbiotic N_2 fixation, even with what appears to be the ideal control plant for these conditions (i.e., a genetically similar non–N_2–fixing control, Ineffective Agate). For example, in Fig. 4, estimated symbiotic N_2 fixation by the difference technique would have been zero, whereas we measured about 5.8 g N m^{-2}. This underestimation results from the incorrect assumption in the difference technique that N uptake from sources other than atmospheric N_2 is similar for both N_2–fixing and non–N_2–fixing crops (Weaver, 1986).

Herbage Br^- Accumulation and its Relation to NO_3^-–N Accumulation

Bromide accumulation in the herbage harvested on 16 June 1995 averaged 22.1 mg Br^- m^{-2} and was the same for all entries. During the fall regrowth of the second year, however, Ineffective Agate accumulated 45% more Br^- in herbage than the N_2–fixing entries (44.4 vs. 30.6 mg Br^- m^{-2}), despite having equal herbage yield.

During the spring regrowth in the third year (1996), subirrigation water containing 20 m*M* NO_3^-–N in the form of $Ca(NO_3)_2$ was labeled with different enrichments of the two tracers in different replicate blocks. We chose this labeling strategy to evaluate how well uptake of ^{15}N and Br^- correlated under the specific fertilization regime we applied in this experiment. Bromide and ^{15}N accumulation in alfalfa herbage were closely related under our field conditions (Fig. 5), confirming what was found under greenhouse conditions

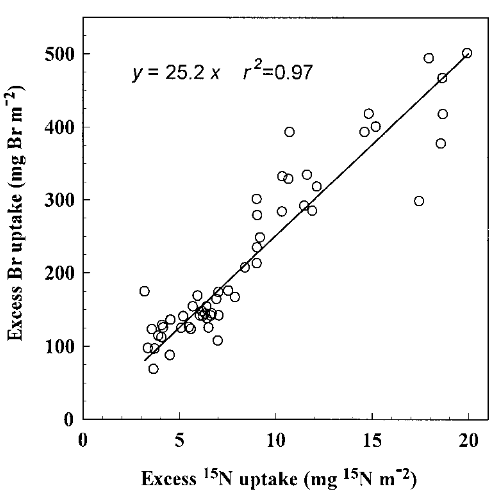

Fig. 5. The linear relationship between excess $^{15}NO_3^-$–N accumulation and excess Br^- accumulation in alfalfa herbage harvested 10 June 1996. Three different enrichments of ^{15}N and Br^- were applied to various groups of replicate plots during spring regrowth.

(Magarian et al., 1998). Taken together, these results indicate that Br^- can be used as a reliable and cost-effective alternative for ^{15}N in NO_3^-–N uptake studies. In addition, alternating these two tracers can largely avoid the confounding problems of shoot N derived from remobilized storage proteins in the roots and crown in experiments designed to estimate current N uptake.

During spring regrowth, Ineffective Agate removed 1.23 times as much subsoil NO_3^- as the N_2–fixing entries (26.4 vs. 21.5 mg Br^- m^{-2}), and herbage at that time contained 0.33 of the total annual N yield (11.4 g N m^{-2} in the spring harvest and 34.8 g N m^{-2} for the year). The product of these ($1.23 \times 0.33 = 0.41$) is the weighted relative subsoil NO_3^-–N uptake for the first harvest. During summer regrowth, Ineffective Agate removed 1.45 times as much subsoil NO_3^- (7.04 vs. 4.84 mg N m^{-2}), and contained 0.41 of annual herbage N (14.3 g N m^{-2}), resulting in a product of 0.60. During the fall harvest, Ineffective Agate also removed 1.46 times as much subsoil NO_3^-–N as the N_2–fixing entries (44.4 vs. 30.4 mg Br^- m^{-2}), but contained only 0.26 of its annual herbage N (9.1 g N m^{-2}), so the product was 0.38. The sum of these weighted products is 1.38; we therefore estimate that Ineffective Agate took up 38% more NO_3^-–N than the N_2–fixing entries during one growing season.

CONCLUSIONS AND IMPLICATIONS

We found that excess Br^- and excess ^{15}N from NO_3^- in alfalfa herbage grown under the described conditions are very strongly correlated. Hence, relative differences found in Br^- accumulation in alfalfa herbage are equal to relative differences in fertilizer NO_3^-–N accumulation. This extends and confirms earlier results obtained in the greenhouse (Magarian et al., 1998). In order to quantify the amount of NO_3^-–N derived from fertilizer, calibration experiments are necessary to establish the relationship between NO_3^-–N and Br^- uptake in the crop of interest under the specific experimental conditions to be used. By establishing this relationship and knowing the Br^- concentrations in the NO_3^- source and crop, total NO_3^-–N uptake can be calculated.

No differences in subsoil NO_3^- uptake were found at any time among diverse N_2–fixing alfalfa entries selected for differences in rooting system architecture, forage quality, and leaf morphology. This indicates that alfalfa entries adapted to the growing conditions of the Upper Midwest of the USA in general may not differ in subsoil NO_3^- uptake, a criterion for which they have not been selected. This contrasts with earlier evidence with topdressed NH_4NO_3, which indicated differences among diverse N_2–fixing alfalfa entries (Lamb et al., 1993). Using the dual-tracer technique, we estimated that Ineffective Agate removed 38% more subsoil NO_3^-–N than several N_2–fixing entries over a growing season, confirming and extending our earlier research (Blumenthal and Russelle, 1996). Providing that N supply in the upper soil profile is sufficient to support vigorous plant growth, these results suggest that ineffectively nodulated alfalfa entries may be the crop of choice for phytoremediation of NO_3^-–contaminated sites.

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REFERENCES

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.

Blumenthal, J.M., and M.P. Russelle. 1996. Subsoil nitrate uptake and symbiotic dinitrogen fixation by alfalfa. Agron J. 88:909–915.

Campbell, C.A., G.P. Lafond, R.P. Zentner, and Y.W. Jame. 1994. Nitrate leaching in a Udic Haploboroll as influenced by fertilization and legumes. J. Environ. Qual. 23:195–201.

Diamond, D. 1992. Total Kjeldahl nitrogen in soil/plant. QuikChem Method 13-107-06-2-D. Lachat Instruments, Milwaukee, WI.

Eardly, B.D., D.B. Hannaway, and P.J. Bottomley. 1985. Nitrogen nutrition and yield of seedling alfalfa as affected by ammonium nitrate fertilization. Agron. J. 77:57–62.

Feigenbaum, S., and A. Hadas. 1980. Utilization of fertilizer nitrogen–nitrogen-15 by field grown alfalfa. Soil Sci. Soc. Am. J. 44: 1006–1010.

Fishbeck, K.A., and D.A. Phillips. 1981. Combined nitrogen and vegetative regrowth of symbiotically grown alfalfa. Agron. J. 73: 975–978.

Fitter, A. 1996. Characteristics and functions of root systems. p. 1–20. *In* Y. Waisel et al. (ed.) Plant roots: The hidden half. 2nd ed. Marcel Dekker, New York.

Grimes, M.F. 1968. Soil survey of Sherburne County, Minnesota. USDA-SCS, U.S. Gov. Print. Office, Washington, DC.

Hauck, R.D. 1982. Nitrogen: Isotope-ratio analysis. p. 735–779. *In* A.L. Page et al. (ed.) Methods of soil analysis. Part 2. 2nd ed. Agron. Monogr. 9. ASA and SSSA, Madison, WI.

Jemison, J.M., Jr., and R.H. Fox. 1991. Corn uptake of bromide under greenhouse and field conditions. Commun. Soil Sci. Plant Anal. 22:283–297.

Johnson, L.D., J.J. Marquez-Ortiz, D.K. Barnes, and J.F.S. Lamb. 1996. Inheritance of root traits in alfalfa. Crop Sci. 36:1482–1487.

Keeney, D.R., and D.W. Nelson. 1982. Nitrogen—Inorganic forms. p. 625–642. *In* A.L. Page et al. (ed.) Methods of soil analysis. Part 2. 2nd ed. Agron. Monogr. 9. ASA and SSSA, Madison, WI.

Kung, K.-J.S. 1990. Influence of plant uptake on the performance of bromide tracer. Soil Sci. Soc. Am. J. 54:975–979.

Lamb, J.F.S., D.K. Barnes, M.P. Russelle, and K.I. Henjum. 1993. Nitrogen uptake efficiency in alfalfa. p. 117. *In* Agronomy abstracts 1993. ASA, 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.

Magarian, D.M., M.P. Russelle, J.F.S. Lamb, and J.M. Blumenthal. 1998. Bromide as a tracer for nitrate-nitrogen uptake in alfalfa herbage. Agron. J. 90:651–657.

Matson, P.A., W.J. Parton, A.G. Power, and M.J. Swift. 1997. Agricultural intensification and ecosystem properties. Science (Washington, DC) 277:504–509.

Muir, J., J.S. Boyce, E.C. Seim, P.N. Mosher, E.J. Diebert, and R.A. Olson. 1976. Influence of crop management practices on nutrient movement below the root zone in Nebraska soils. J. Environ. Qual. 5:255–259.

Ourry, A., T.H. Kim, and J. Boucaud. 1994. Nitrogen reserve mobilization during regrowth of *Medicago sativa* L. Plant Physiol. 105: 831–837.

Power, J.F., and J.S. Schepers. 1989. Nitrate contamination of groundwater in North America. Agric. Ecosys. Environ. 26:165–187.

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.

Rehm, G., and M. Schmitt, 1990. Fertilizer recommendations for agronomic crops in Minnesota. AG-MI-3901. Minn. Ext. Serv., St. Paul.

Russelle, M.P., D.L. Allan, and C.J.P. Gourley. 1994. Direct assessment of symbiotically fixed nitrogen in the rhizosphere of alfalfa. Plant Soil 159:233–243.

SAS Institute. 1987. SAS/STAT guide for personal computers. Version 6 ed. SAS Inst., Cary, NC.

Silvertooth, J.C., J.E. Watson, J.E. Malcuit, and T.A. Doerge. 1992. Bromide and nitrate movement in an irrigated cotton production system. Soil Sci. Soc. Am. J. 56:548–555.

Switala, K. 1993. Ammonia (salicylate) in 2M KCl soil extracts. QuickChem Method 12-107-06-2-A. Lachat Instruments, Milwaukee, WI.

Trimble, M.W., D.K. Barnes, G.H. Heichel, and C.C. Sheaffer. 1987. Forage yield and nitrogen partitioning response in alfalfa to two cutting regimes and three soil nitrogen regimes. Crop Sci. 27: 909–914.

van Noordwijk, M. 1983. Functional interpretation of root densities in the field for nutrient and water uptake. p. 207–226. *In* W. Bohm et al. (ed.) Root ecology and its practical application. Proc. Int. Symp., 1982. Bundesanstalt Gumpenstein, Irdning, Germany.

Vose, P.B., and R.L. Victoria. 1986. Re-examination of the limitations of nitrogen-15 isotope dilution technique for the field measurements of dinitrogen fixation. p. 23–41. *In* R.D. Hauck and R.W. Weaver (ed.) Field measurements of dinitrogen fixation and denitrification. SSSA Spec. Publ. 18. SSSA, Madison, WI.

Weaver, R.W. 1986. Measurement of biological dinitrogen fixation in the field. p. 1–10. *In* R.D. Hauck and R.W. Weaver (ed.) Field measurements of dinitrogen fixation and denitrification. SSSA Spec. Publ. 18. SSSA, Madison, WI.

Wright, J., and F. Bergsrud. 1991. Irrigation scheduling: Checkbook method. AG-FO-1322-G. Minn. Ext. Serv., St. Paul.