Nitrogen Recovery from ¹⁵N-Labeled Green Manures: II. Recovery by Oat and Soil Two Seasons After Green Manure Incorporation

Jose A. Cueto-Wong Steven J. Guldan William C. Lindemann Marta D. Remmenga

ABSTRACT. Forage legumes in crop rotations are often used to supply N for succeeding crops. However, the residual effect of forage legume green manures for second crops has not been thoroughly investigated in high desert regions of the Southwestern United States. The objective of this study was to evaluate the ¹⁵N recovery by a second subsequent crop (oat [Avena sativa L.]) and soil from microplots amended with ¹⁵N-labeled alfalfa (Medicago sativa L. 'Nitro') and hairy vetch (Vicia villosa Roth 'Madison'). In 1994, two application rates of each legume

Jose A. Cueto-Wong is affiliated with Centro Nacional de Investigacion Disciplinaria en Relacion Agua-Suelo-Planta-Atmosfera (CENID-RASPA-INIFAP), Apartado Postal No. 41, Cd. Lerdo, Durango, Mexico 35150.

Steven J. Guldan is affiliated with Alcalde Sustainable Agriculture Science Center, New Mexico State University, P.O. Box 159, Alcalde, NM 87511.

William C. Lindemann is affiliated the with Department of Agronomy and Horticulture, New Mexico State University, Las Cruces, NM 88003.

Marta D. Remmenga is affiliated with the Department of Economics and International Business, New Mexico State University, Las Cruces, NM 88003.

Address correspondence to: Steven J. Guldan at the above address (E-mail: sguldan @nmsu.edu).

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were incorporated into 1 m² microplots and forage sorghum (*Sorghum bicolor* [L.] Moench) was used to evaluate the ¹⁵N recovery. Aboveground residues were removed from microplots immediately after each of the two sorghum harvests. In 1995, the ¹⁵N recovery by the oat crop and soil was determined. Nitrogen-15 recovery by oat averaged 3% of the ¹⁵N applied as green manure. Of this, 95% was found in tops and 5% in roots. Averaged across application rates, oat recovery of ¹⁵N was 63% greater in treatments amended with alfalfa than with hairy vetch. Green manures did not affect the total N concentration of oat parts. Most of the residual ¹⁵N was found in soil at the 0-0.3 m depth at the end of the oat season. The percent of ¹⁵N recovery by soil in plots amended with alfalfa was 238% higher than with hairy vetch. Green manures applied in 1994 had little residual effect on the N nutrition of oat. [Article copies available for a fee from The Haworth Document Delivery Service: 1-800-342-9678. E-mail address: <getinfo@haworthpressinc.com> Website: http://www.HaworthPress.com © 2001 by The Haworth Press, Inc. All rights reserved.]

KEYWORDS. Green manure, forage legume, ¹⁵N-recovery, hairy vetch, alfalfa, oat

INTRODUCTION

Green-manure forage legumes can be excellent options to improve soil physical and chemical properties, provide forage, and supply N for the succeeding crop. Correctly managed, green manures can replace part or all of the N required for non-leguminous succeeding crops (Blevins et al., 1990; Guldan et al., 1997a; Guldan et al., 1997b; Nelson and King, 1996; Sweeney and Moyer, 1994; Utomo et al., 1990). However, extrapolation of results between regions is difficult because of natural differences in local soil properties and climatic conditions, green manure species and management practices, and cropping systems.

Nitrogen remaining in soil at the end of the cropping season after legume incorporation may vary from 20% up to 80% of the total applied as ¹⁵N-labeled green manure (Ladd et al., 1981; Ladd et al., 1983; Harris et al., 1994; Janzen et al., 1990; Muller, 1988; Muller and Sundman, 1988). Nitrogen recovery by non-leguminous succeeding crops is usually between 5 and 30% of the N applied (Bremer and van Kessel, 1992; Harris and Hesterman, 1990; Harris et al., 1994; Janzen et al., 1990; Muller and Sundman, 1988; Ta and Faris, 1990; Varco et al., 1989). These N recovery percentages are significantly lower than estimates by the difference, apparent recovery, or fertilizer replacement value methods (Blevins et al., 1990; Holderbaum et al., 1990;

Guldan et al., 1997a; Guldan et al., 1997b). The uptake of green manure N by a second succeeding crop has not been investigated under a wide range of environmental conditions and cropping systems.

Studies based on the use of ¹⁵N-labeled legumes indicate that commonly less than 5% of the N applied as green manure is recovered by non-leguminous crops planted the second growing season after legume incorporation (Ladd et al., 1983; Harris and Hesterman, 1990; Harris et al., 1994; Janzen et al., 1990; Jensen, 1994; Ta and Faris, 1990; Varco et al., 1989). In a long-term study carried out under greenhouse conditions, it was found that the N remaining in soil was approximately 30% of the input, eight years after soil amendment with a legume (Ladd et al., 1985).

This investigation was part of a project to evaluate green-manure forage legumes as relay intercrops for sweet corn and chile in north-central New Mexico (Guldan et al., 1996a; Guldan et al., 1996b; Guldan et al., 1997a; Guldan et al., 1997b). In 1994, two rates of ¹⁵N-labeled alfalfa and hairy vetch were incorporated into soil microplots and forage sorghum was used to evaluate the ¹⁵N recovery during the first cropping season (Cueto-Wong et al., 2001). The evaluation of ¹⁵N recovery by a second succeeding crop, oat, and the soil is reported in this paper.

MATERIALS AND METHODS

The experiment was carried out under irrigated conditions at the New Mexico State University Sustainable Agriculture Science Center at Alcalde, NM. The soil was previously described (Cueto-Wong et al., 2001).

¹⁵N Labeling and Incorporation of Alfalfa and Hairy Vetch

Alfalfa and hairy vetch labeled with ¹⁵N were incorporated in late spring 1994 and forage sorghum grown during summer 1994. Plant samples and soil samples were taken to determine recovery of the legume N in sorghum tissue and the ¹⁵N remaining in soil. Details of the methodology are given in Cueto-Wong et al. (2001).

Second Cropping Season

On 26 April 1995, 'Colorado 37' oat was hand-planted across the entire area of each microplot in four 0.18 m rows at a rate of 56 kg seed ha. No nitrogen was applied. Phosphorus was applied to all microplots at planting at a rate of 26 kg P ha. as triple superphosphate and incorporated. Oat was harvested on 13 July at approximately boot stage by taking a representative

sample (0.086 m²) from each microplot where sorghum had been grown the previous year. Plant samples were oven dried 72 h at 65° C to determine moisture content, ground to pass a 250 \square m sieve and analyzed by Dumas combustion with detection of N₂ and CO₂ by thermal conductivity in a carbon-nitrogen-sulfur analyzer. The atom% ¹⁵N was determined on a mass spectrometer (Europa Scientific Ltd., Crewe, Cheshire, UK). Oat N uptake was calculated by multiplying the dry matter yield (tops and roots) by the total N concentration. The percent N derived from the green manures and ¹⁵N recovery by oat plant parts were calculated with the following formulas:

NfGM(%) = (atom% 15 N excess of oat/atom% 15 N excess of legume) × 100

and,

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NREC(%) = [(NfGM)(oat N uptake)/legume-N applied] \times 100

Before planting and after oat harvest, inorganic N (N-NH₄ and N-NO₃) concentrations were measured at four soil depths (0-0.3, 0.3-0.6, 0.6-0.9, and 0.9-1.14 m) in the half of the microplot not planted to sorghum in 1994. NH₄-N and NO₃-N were extracted from field moist samples (2 mm sieve) with 2 M KCl (10:1 solution:soil ratio) and colorimetrically determined (naphthyl ethylene diamine dihydrochloride and sodium salicylate/sodium nitroprusside, respectively) with an autoanalyzer (Technicon, 1973; Technicon, 1974). Soil texture (Bouyoucos hydrometer) and bulk density (soil core) were determined for the same soil depths. Soil samples from 0-0.3 and 0.3-0.6 m depths taken at the end of the oat growing season (from the side of the microplot planted to sorghum in 1994) were air dried and ground to pass a 100 m sieve and analyzed for total C, total N, and atom% 15N content as described above. Soil ¹⁵N recovery was based on the total N content, atom% ¹⁵N concentration, and bulk density of each soil layer. The percent N derived from the green manures and ¹⁵N recovery by the soil were calculated as those for oat plant parts.

Statistical Analyses

Oat dry matter yield, N uptake, total N concentration, NfGM, ¹⁵N concentration, and the ¹⁵N recovery by oat plant parts and soil were analyzed as a randomized complete block design with four replications. Orthogonal contrasts were used to compare the effect of legume species and application rates within species on oat response variables. Total inorganic N levels (NO₃-N + NH₄-N) in soil measured before oat planting and after oat harvest were analyzed as a randomized complete block design with sampling dates and

soil depths as repeated measures variables. Orthogonal contrasts were used to compare treatment effects on total inorganic N between soil depths and sampling dates (SAS Institute, 1990).

RESULTS AND DISCUSSION

Oat Dry Matter Yield

Alfalfa and hairy vetch treatments produced more oat dry matter than the unamended control (Table 1). Averaged across legume species and application rates, the yield of oat tops was 23% higher than the control. Treatments amended with alfalfa produced 22% more dry matter than treatments amended with hairy vetch. Green manured plots averaged 39% higher root biomass than the control, and root dry matter yield in treatments amended with hairy vetch was 25% higher than with alfalfa.

The differences in oat dry matter yield found in this study may be explained in terms of rotational effects associated with the inclusion of the legumes as well as with the legume residual N. Although some studies show that legume green manure can sustain two succeeding crops, it is not clear whether the response is an effect of the residual legume N or other factors

TABLE 1. Oat tops and roots dry matter yield from plots amended with ¹⁵N-labeled alfalfa and hairy vetch.

		Oat p	Oat parts		
Legume	Rate applied	Tops	Roots		
	Mg ha ^{⊡1}	— Dry matter	(kg ha ^{□1}) —		
Alfalfa (Low)	2.7	3670	889		
Alfalfa (High)	4.0	3240	958		
Hairy Vetch (Low)	3.2	2670	1020		
Hairy Vetch (High)	6.4	3000	1290		
Control	0	2560	747		
Contrast					
Legume vs. Control		*	**		
Alfalfa vs. H. Vetch		*	**		
Alfalfa (Low) vs. Alfalfa (High)		NS	NS		
H. Vetch (Low) vs. H. Vetch (High)		NS	**		

^{*, **} Significant at the 0.05 and 0.01 levels of probability, respectively.

(Aflakpui et al., 1993). Studies using ¹⁵N-labeled materials have shown that alfalfa, clovers (*Trifolium* sp.), *Lathyrus* sp., and lentil (*Lens* sp.) usually contribute less than 5% of the N absorbed by the second or third subsequent crop (Harris and Hesterman, 1990; Harris et al., 1994; Janzen et al., 1990; Jensen, 1994; Ta and Faris, 1990), and much of the response may be other than N nutrition.

¹⁵N Concentration in Oat and Soil

All treatments amended with labeled green manures had higher ¹⁵N concentrations in tops and roots than the control (Table 2). This clearly indicates that part of the green manure N applied in 1994 was taken up by the oat in 1995. The ¹⁵N concentration was 138% higher in oat tops in treatments amended with green manures than in the unamended control. Averaged across legume species and application rates, the ¹⁵N concentration in oat roots was 117% higher than in the control. There was also a higher concentration of ¹⁵N in oat tops and roots in the alfalfa treatment compared with the hairy vetch treatment. This suggests that ¹⁵N from alfalfa remained in soil longer than the ¹⁵N from hairy vetch. Additionally, legumes applied at the higher rates increased the concentration of ¹⁵N in oat parts. Averaged across species, the higher legume application rates resulted in 29 and 35% greater concentrations of ¹⁵N in oat tops and roots, respectively (Table 2).

In soil, treatments amended with legumes averaged a 40% higher concentration of ¹⁵N than the unamended control at the 0-0.3 m depth, but no significant differences were found at the 0.3-0.6 m depth (Table 2). The lack of differences in the ¹⁵N concentration at the 0.3-0.6 m soil depth indicates that most of the applied green manure-N that was retained in the soil was retained in the top soil layer, probably as organic N (Harris and Hesterman, 1990; Harris et al., 1994; Ladd et al., 1983), and did not contribute to the N pool of the subsoil. Soil isotopic analyses also showed that the concentration of ¹⁵N was 39% higher for the alfalfa treatment than for the hairy vetch treatment at the 0-0.3 m depth. These results indicate that the alfalfa-¹⁵N had a longer residence time in soil than hairy vetch-¹⁵N despite the larger amount of ¹⁵N applied in the hairy vetch. Westcott et al. (1995) found that alfalfa gave a higher N benefit for a succeeding barley (*Hordeum vulgare* L.) crop than berseem clover (*Trifolium alexandrinum* L.).

Total Nitrogen Concentration and N Uptake in Oat Parts and Soil

Despite the differences in ¹⁵N concentrations found in oat plant parts, no significant differences in total N concentration in oat tops or roots were found between the green manured treatments and the unamended control (Table 3).

TABLE 2. Nitrogen-15 concentration in oat parts and at two soil depths from plots amended with $^{15}{\rm N}$ -labeled alfalfa and hairy vetch.

		Oat parts		Soil depth (m)	
Legume	Rate applied	Tops	Roots	0.0-0.3	0.3-0.6
	Mg ha ^{⊡1}	¹⁵ N (atom%)			
Alfalfa (Low)	2.7	0.88	0.80	0.57	0.41
Alfalfa (High)	4.0	1.09	1.02	0.67	0.43
Hairy Vetch (Low)	3.2	0.71	0.64	0.42	0.40
Hairy Vetch (High)	6.4	0.94	0.92	0.47	0.41
Control	0	0.38	0.39	0.39	0.41
Contrast					
Legume vs. Control		**	**	*	NS
Alfalfa vs. H. Vetch		*	*	**	NS
Alfalfa (Low) vs. Alfalfa (High)		*	**	NS	NS
H. Vetch (Low) vs. H. Vetch (High	n)	*	**	NS	NS

^{*, **} Significant at the 0.05 and 0.01 levels of probability, respectively.

TABLE 3. Total N concentration in oat parts and at two soil depths from plots amended with $^{15}{\rm N}$ -labeled alfalfa and hairy vetch.

		Oat parts		Soil de	Soil depth (m)	
Legume	Rate applied	Tops	Roots	0.0-0.3	0.3-0.6	
	Mg ha ^{⊡1}	— Total N concentration (g kg ^{□t}) —				
Alfalfa (Low)	2.7	14.7	7.5	0.42	0.12	
Alfalfa (High)	4.0	14.4	6.9	0.40	0.13	
Hairy Vetch (Low)	3.2	14.2	7.0	0.41	0.13	
Hairy Vetch (High)	6.4	15.0	6.9	0.42	0.13	
Control	0	15.1	6.9	0.37	0.12	
Contrast						
Legume vs. Control		NS	NS	*	NS	
Alfalfa vs. H. Vetch		NS	NS	NS	NS	
Alfalfa (Low) vs. Alfalfa (High)		NS	NS	NS	NS	
H. Vetch (Low) vs. H. Vetch (High)	NS	NS	NS	NS	

^{*, **} Significant at the 0.05 and 0.01 levels of probability, respectively.

Averaged across legume species and application rates, oat tops and roots contained 14.7 and 7.0 g kg N, respectively. Similar results have been found by others (Ta and Faris, 1990) and indicate that several N sources contributed to the N nutrition of the oat crop. Nitrogen mineralized from the green manure, indigenous organic matter, and that remineralized from the residues of the previous crop (forage sorghum roots and crowns) are the most likely N sources.

Treatments amended with the legumes averaged 12% higher soil N concentration at the 0-0.3 m depth, and no differences were found at the 0.3-0.6 m depth (Table 3). This result is consistent with the results of ¹⁵N concentrations found in the soil at the end of the oat growing season and confirm the negligible contribution of the legumes to the 0.3-0.6 m depth N pool.

Averaged across legume species and application rates, oat tops and roots took up 45 and 7 kg N ha respectively (Table 4). Although oat tops from green manured treatments had a higher concentration of ¹⁵N compared with the control, total N uptake by oat tops was not affected by green manuring.

N Derived from Alfalfa and Hairy Vetch Green Manures

Averaged across legume species and application rates, oat tops and roots derived 10 and 9%, respectively, of their N from the green manures (Table 5).

TABLE 4. Nitrogen uptake in oat tops and roots from plots amended with ¹⁵N-labeled aflalfa and hairy vetch.

		Oat p	Oat parts		
Legume	Rate applied	Tops	Roots		
	Mg ha ^{⊡1}	— N uptake	(kg ha ^{□t}) —		
Alfalfa (Low)	2.7	53.5	6.7		
Alfalfa (High)	4.0	47.0	6.6		
Hairy Vetch (Low)	3.2	38.3	7.1		
Hairy Vetch (High)	6.4	45.3	8.9		
Control	0	38.5	5.2		
Contrast					
Legume vs. Control		NS	**		
Alfalfa vs. H. Vetch		NS	*		
Alfalfa (Low) vs. Alfalfa (High)		NS	NS		
H. Vetch (Low) vs. H. Vetch (High)		NS	*		

^{*, **} Significant at the 0.05 and 0.01 levels of probability, respectively.

TABLE 5. Percent N derived from green manures (NfGM) by oat parts and two soil depths from plots amended with ¹⁵N-labeled alfalfa and hairy vetch.

		Oat parts		Soil depth (m)	
Legume	Rate applied	Tops	Roots	0.0-0.3	0.3-0.6
	Mg ha ^{⊡t}				
Alfalfa (Low)	2.7	8.3	6.9	3.2	0.9
Alfalfa (High)	4.0	12.0	10.7	4.9	1.2
Hairy Vetch (Low)	3.2	7.2	5.7	0.9	0.8
Hairy Vetch (High)	6.4	12.2	11.7	2.2	0.8
Contrast					
Alfalfa vs. H. Vetch		NS	NS	*	NS
Alfalfa (Low) vs. Alfalfa (High)		NS	*	NS	NS
H. Vetch (Low) vs. H. Vetch (High	1)	*	**	NS	NS

^{*, **} Significant at the 0.05 and 0.01 levels of probability, respectively.

In general, more N was derived by oat from treatments amended at the higher legume application rates. Only 6% (Ta and Faris, 1990), and 6-8% (Harris and Hesterman, 1990), of the N in a second-year barley crop came from alfalfa. In our study, approximately 90% of the oat N was taken up from sources other than green manure N.

Only 3 and 1% of the total soil N content came from green manures at the 0-0.3 and 0.3-0.6 m depths, respectively (Table 5). The percent N derived from the green manure was 161% higher in the alfalfa treatment than in the hairy vetch treatment at the 0-0.3 m soil depth. These results are consistent with those of 15 N, and total N concentration discussed above, and indicate that soil retained more N from alfalfa than hairy vetch, and most N remaining was present in the top soil layer (0-0.3 m).

¹⁵N Recovery by Oat Parts and Soil

Averaged across legume species and application rates, ¹⁵N recoveries by oat tops and roots were 3.0 and 0.4%, respectively, of the ¹⁵N applied in 1994 (Table 6). The ¹⁵N recovery by oat tops was 75% higher in treatments amended with alfalfa than with hairy vetch. Nitrogen-15 recovery by oat roots represented 13% of the total oat recovery but no differences were found between treatments. Nitrogen-15 recoveries found in this study by a second crop of oat agree in general with recoveries of others. Recoveries of 4% (Ta and Faris, 1990) and 1% (Harris and Hesterman, 1990) of alfalfa-¹⁵N was found in second crops of barley. Recovery by wheat (*Triticum aestivum* L.)

TABLE 6. Nitrogen-15 recovery by oat parts and at two soil depths from plots amended with ¹⁵N-labeled alfalfa and hairy vetch.

		Oat parts		Soil de	pth (m)
Legume	Rate applied	Tops	Roots	0.0-0.3	0.3-0.6
	Mg ha ^{⊡t}	—— ¹⁵ N recovery (% of the input) ——			
Alfalfa (Low)	2.7	4.3	0.4	57.4	5.7
Alfalfa (High)	4.0	3.4	0.4	54.6	4.2
Hairy Vetch (Low)	3.2	2.1	0.3	13.3	4.0
Hairy Vetch (High)	6.4	2.3	0.4	16.1	2.1
<u>Contrast</u>					
Alfalfa vs. H. Vetch		**	NS	**	NS
Alfalfa (Low) vs. Alfalfa (High)		NS	NS	NS	NS
H. Vetch (Low) vs. H. Vetch (High	٦)	NS	NS	NS	NS

^{*, **} Significant at the 0.05 and 0.01 levels of probability, respectively.

from medic (*Medicago littoralis*) was < 5% in the second year (Ladd et al., 1983). Hairy vetch-¹⁵N recovery by corn (*Zea mays* L.) was 3-7% in the second year (Varco et al., 1989). The ¹⁵N recoveries found in our study also indicate that alfalfa and hairy vetch supplied low amounts of N for a second crop and that N sources other than green manures contributed to the N supply of oat.

Averaged across application rates, 56 and 15% of the green manure-¹⁵N was recovered by the 0-0.3 m soil layer in treatments amended with alfalfa and hairy vetch, respectively (Table 6). The ¹⁵N recovery found in soils in treatments amended with alfalfa are in the range reported by others (Stevenson and van Kessel, 1997; Ladd et al., 1981). The ¹⁵N recovery at the 0.3-0.6 m depth represented 11% of the total recovery by the soil. Similar declines in the ¹⁵N recovery with depth have been reported by others (Harris and Hesterman, 1990; Ladd et al., 1981; Stevenson and van Kessel, 1997). Although most of the ¹⁵N recovered at the 0.3-0.6 m depth was probably in the organic form, this percentage represents about 4% of the input. Results of this study indicate that more N is being lost from hairy vetch than alfalfa green manures.

Inorganic N in Soil Two Crops After Amendment with Alfalfa and Hairy Vetch Green Manures

Total inorganic N concentrations found in soil before planting and after oat harvest were generally low. The repeated measures analysis detected signifi-

cant differences in total inorganic N concentrations between soil depths but not between treatments. Averaged across soil amendments and sampling dates, soil contained 7.0, 6.0, 5.0, and 6.1 mg inorganic N kg at the 0-0.3, 0.3-0.6, 0.6-0.9, and 0.9-1.14 m depths, respectively. Results of a number of studies indicate that large amounts of inorganic N are usually released in less than five weeks after green manure incorporation. Stute and Posner (1995) found that approximately 50% of the N contained in hairy vetch and red clover was released during the four weeks after legume incorporation and low concentrations were measured after ten weeks. High mineralization rates with hairy vetch have been found by others (Sarrantonio and Scott, 1988; Utomo et al., 1990; Varco et al., 1993).

CONCLUSIONS

In summary, alfalfa mineralized slower and supplied more ¹⁵N for a second succeeding crop (oat) than hairy vetch despite the higher amounts of biomass and total N applied with hairy vetch. This conclusion is supported by higher oat ¹⁵N concentrations and ¹⁵N recoveries in plots amended with alfalfa than with hairy vetch. Additional support for this conclusion is shown by the higher soil ¹⁵N concentrations, N derived from green manures, and ¹⁵N recoveries in plots amended with alfalfa than with hairy vetch. Nitrogen-15 recoveries by oat and soil reported here indicate that green manures contributed little to the N nutrition of the second crop and to the soil N status. By comparison, the forage sorghum crop planted after legume incorporation in 1994 had a total ¹⁵N recovery of 17.6% averaged across legumes species and application rates. The ¹⁵N recoveries found in this study suggest that the most important N contribution of green manures is immediately after their incorporation.

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