

# BIOAG PROJECT REPORT TEMPLATE

FINAL REPORT JUNE 30, 2018

TITLE: MONITORING UPTAKE OF LEGUME N BY APPLE TREES USING NITROGEN ISOTOPE DISCRIMINATION

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## ABSTRACT

Intercropped legumes can supply nitrogen to fruit trees and reduce the need for purchased nitrogen fertilizer, potentially reducing costs and environmental footprint. The project evaluated nitrogen isotope techniques (comparing the  $^{15}\text{N}$  and  $^{14}\text{N}$  signal from the legume N versus other sources) to monitor the degree of uptake by apple trees of legume N grown in the orchard. The project involved two components: 1) a greenhouse study using  $^{15}\text{N}$ -labeled clover residue added to potted apple trees to quantify legume-N uptake and a comparison to values using nitrogen isotope natural abundance; 2) a field study in an existing orchard legume cover crop trial using natural abundance for a qualitative evaluation of legume N and the ability to detect this signal in an ambient field environment. The  $^{15}\text{N}$  enrichment technique is known to be more sensitive than the natural isotope abundance technique, but is not suitable for routine field use by growers or consultants. The objective of the project was to determine the uptake of legume-derived N in a quantitative manner and compare it with a more qualitative method to assess its utility on farms. Lower tree leaf  $^{15}\text{N}$  atom% with legume alleys versus grass was detected in several instances in both trials, indicating that the natural isotope abundance method does have potential use for monitoring the timing of legume uptake by trees.

## PROJECT DESCRIPTION

### Objectives.

Trial 1. Compare legume-N uptake by potted apple trees using the  $^{15}\text{N}$  enrichment technique versus the natural isotope abundance technique.

Trial 2. Monitor natural isotope abundance of legumes, soil, and apple trees in an existing legume cover crop trial to determine the magnitude of the signal difference in the different orchard components.

### Methods.

Trial 1 was repeated using potted trees under shade cloth outdoors, after the initial trial (2014) was compromised by saline water in the greenhouse. Trees ('Golden Delicious'/B.9) were planted on 9 April 2015 in 20" diameter pots filled with virgin Warden silt loam soil (no history of cropping). Forty-five trees were planted; 20 for each phase of the trial, with 5 extras. The trees were grown through the 2015 season without treatment due to the lack of enough  $^{15}\text{N}$  labeled clover, and then they were placed in cold storage for the winter. There was not enough labeled 'Alice' white clover biomass to do spring 2016 treatments either, so trees and clover were grown through the summer. Fall treatments were applied on 9/13/16 to 20 trees. Prior to this, soil samples (0-10") and tree leaves (10 per tree) were collected for a pre-treatment baseline. All trees had received a Hoagland's nutrient solution during 2015 and 2016 in order for them to grow, and there appears to be a small amount of  $^{15}\text{N}$  enrichment in this material that

showed up in the trees from the 2014 pot trial. Trees were sampled again in October 2016, and then they were put in storage for the winter after they were dormant. Trees came out of storage in on 4/21/17. Spring treatments were applied to 20 trees on 6/7/17, with baseline soil and leaf samples taken that day. Forty grams of clover powder was applied to the soil surface of each clover treatment pot and very lightly incorporated to prevent it from blowing away. A solution of 0.1 g  $^{15}\text{N}$  labeled urea and deionized water was applied to urea treatment pots. Trees were watered on Monday, Wednesday, and Friday with Roza irrigation water. All fruit was removed from trees on June 13. An application of 1 L Hoagland solution (mixed without nitrogen) was added to each pot in July.

Leaf samples from all treatments (15 leaves per tree) were collected on 7/7/17 and 8/7/17, dried and ground. A soil sample was taken on 8/7/17, compositing three 10" cores per pot.

Trees from both the fall and spring treatments were destructively harvested on 9/11/17. For each tree, all leaves were placed in a paper bag and dried at 65C for a minimum of 24 h , and then ground in a Wiley mill. Trunks and first year shoots were separated, dried, and ground. Roots were extracted from pots by washing and dried at 65C.

All samples from the fall and spring treatments were sent together to the USDA lab (Pullman WA) for total N and  $^{15}\text{N}$  analysis using dry combustion and mass spectroscopy.

The following treatments were used:

- A) control; no fertilizer, no clover residue (Hoagland solution used to keep trees alive).
- B)  $^{15}\text{N}$  labelled urea (10% atom) with no clover residue at 25 kg N/ha rate.
- C)  $^{15}\text{N}$ -labeled clover residue from greenhouse.
- D) unlabeled clover residue from Alvarez Orchard field trial.

The trial used single-tree plots (1 pot) replicated 5 times.

Growing the labeled clover proved to be a large challenge and resulted in the project delay. It also led to application of unequal amounts of N since multiple lots of clover were used, at different times, and there was not enough lead time to get the required analysis in advance of their application to the pots. The N content and the amount applied are presented in Tables 1 and 2.

Table 1. Total N and  $^{15}\text{N}$  content of clovers and urea.

Material	Application Use	Total N (%)	$^{15}\text{N}$ (Atom %)
Regular clover	Fall application	3.57	0.3732
Enriched clover	Fall application	4.30	1.5135
Regular clover	Spring application	3.51	0.3686
Enriched clover	Spring application	3.54	1.8705
Enriched urea	Spring and fall	46	10.21

Table 2. Amount of N and  $^{15}\text{N}$  applied per pot.

Treatment	Rate	Amount N Applied (g/pot)	Amount $^{15}\text{N}$ Applied (g/pot)
Fall control	0	0	0
Fall clover	40 g	1.428	0.14928
Fall $^{15}\text{N}$ clover	40 g	1.72	0.6054
Fall $^{15}\text{N}$ urea	0.1 g	0.046	0.01021
Spring control	0	0	0
Spring clover	40 g	1.404	0.14764
Spring $^{15}\text{N}$ clover	40 g	1.416	0.7482
Spring $^{15}\text{N}$ urea	0.1 g	0.046	0.01021



Photo of spring treatment application on June 7, 2017 (D. Granatstein).



Photo of incorporation of  $^{15}\text{N}$  clover amendment (D. Granatstein).

## Results.

Soil data from the second pot trial were only available from the 8/7/17 sampling from all pots. These are summarized in Figure 1. The  $^{15}\text{N}$  atom% values show that the soil was clearly enriched by the labeled materials. For the natural abundance comparison, soil with the unlabeled field harvested clover (CloAlv) added should have a lower  $^{15}\text{N}$  value than the control (Ctl) soil, which occurred for the fall applied treatments (not significantly different) but not the spring. A similar pattern for lower values of  $\delta^{15}\text{N}$  with legumes growing was observed in the Alvarez field soil (Fig. 2) but again differences were not significant. This is because fixed N in the legume gives its tissue a  $^{15}\text{N}$  value closer to that of the atmosphere and lower than soil, and thus when added to soil it will dilute the natural  $^{15}\text{N}$  enrichment and lower the  $^{15}\text{N}$  value. Given that the clover was added to the surface of the soil in the pots, and very lightly incorporated ( $<1$  cm), it is likely that it was not evenly distributed through the soil from which a sample core was taken. Thus, the difference between the control and unlabeled clover soils might have been larger if only a surface 2-3 cm sample had been taken. However, the small amount of legume N added to the much larger mass of soil was unlikely to alter the soil natural abundance signature.

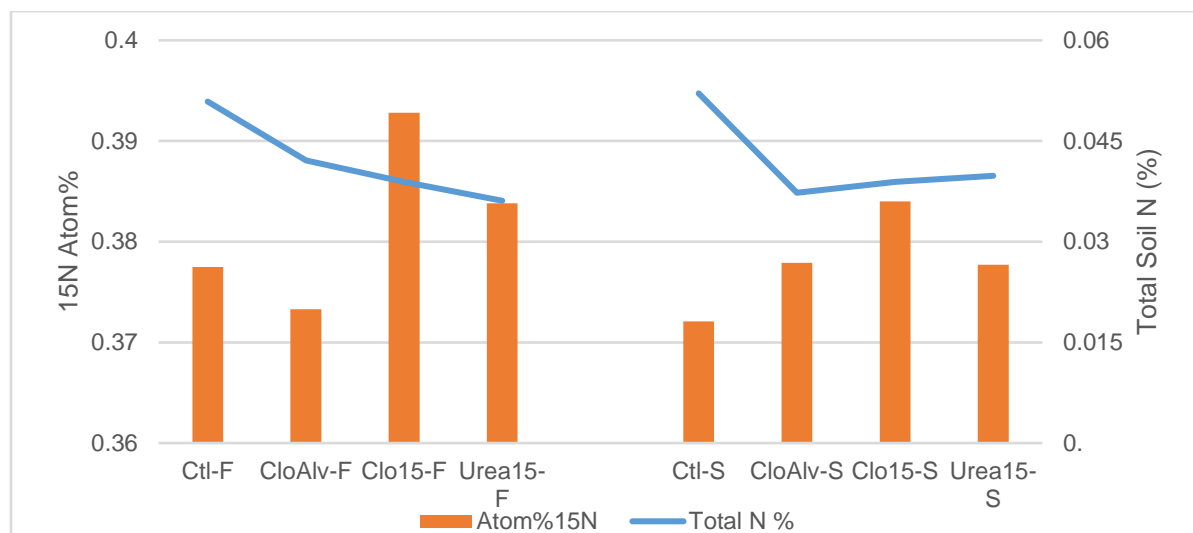


Figure 1. Total N (%) and  $^{15}\text{N}$  atom% for soils from potted trees for fall (F) and spring (S) applied treatments, sampled on 8/7/17. Ctl=control. CloAlv=field grown white clover. Clo15= $^{15}\text{N}$  labeled white clover. Urea15= $^{15}\text{N}$  labeled urea.

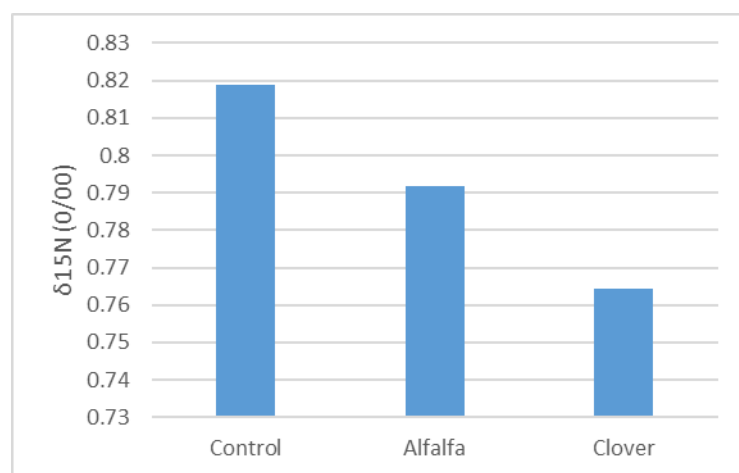


Figure 2.  $\delta^{15}\text{N}$  for soils from Alvarez orchard with grass (control), alfalfa, or clover vegetation in alleys.

The  $^{15}\text{N}$  data for the apple leaves provide the most useful results to look at (Fig. 3). For both fall and spring applied treatments, total N declined over the season as is typical. There were few treatment effects for the fall applied treatments. Leaf N was lowest in the control, while spring-applied labeled clover had the highest leaf N in August and September. The fall labeled clover led to consistently higher  $^{15}\text{N}$  than the other treatments, followed by the fall labeled urea. Thus the clover N was clearly being taken up by the trees. There were no differences in  $^{15}\text{N}$  between the control and the unlabeled clover for the fall treatment tree leaves. For the spring applied treatments, there were few statistical differences. The unlabeled clover led to lower  $^{15}\text{N}$  values

than the control for most dates (but not statistically different), suggesting that the natural abundance method was detecting evidence of legume uptake by the trees as early as one month after application.

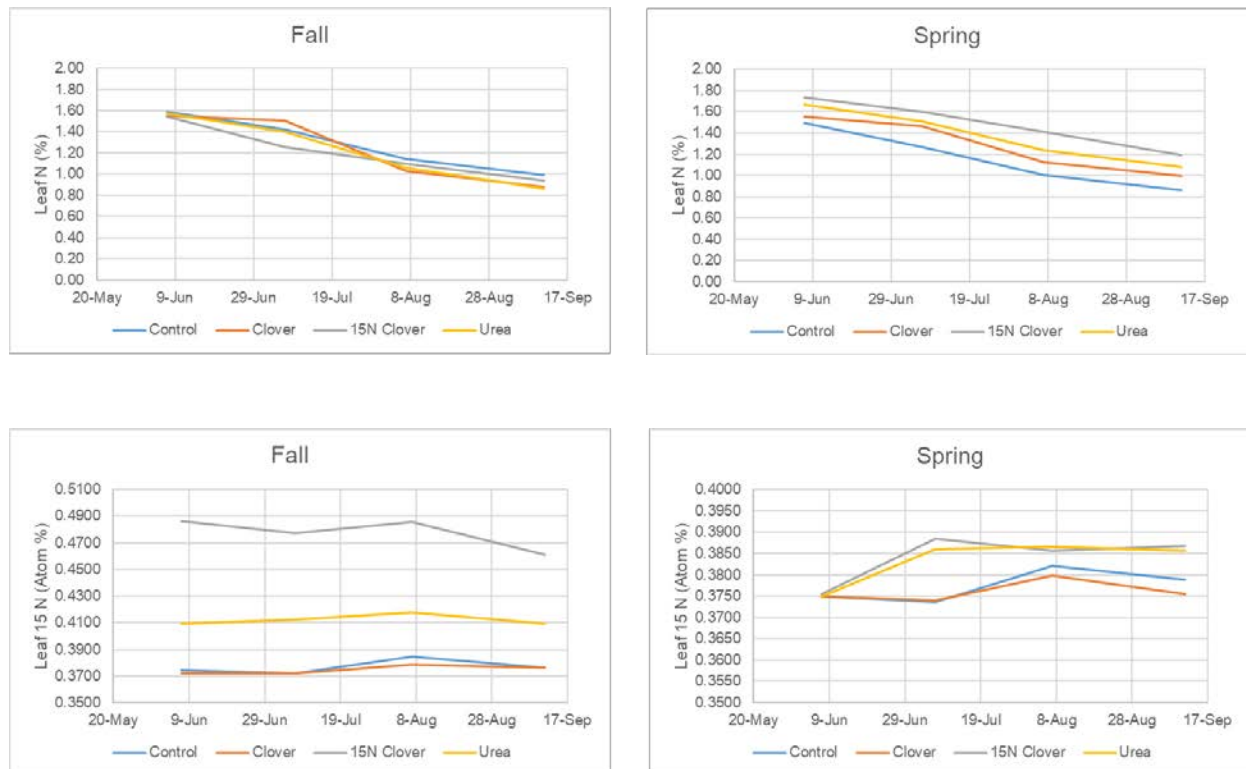


Figure 3. Apple leaf total N % (top charts) and  $^{15}\text{N}$  atom% (bottom charts) for Fall or Spring applied treatments at sampling dates in 2017.

The data from the 2014 pot trial show a similar trend of the tree leaves from unlabeled clover treatment having lower  $^{15}\text{N}$  than the control trees (see the June 30, 2017 progress report).

For the orchard trials using natural abundance, apple leaves with alfalfa alleys had lower  $^{15}\text{N}$  than the grass control for Alvarez in July and for Tudor in July and August. This reinforces the pot study finding that the natural abundance method can detect legume-derived N taken up by trees in the field (see the June 30, 2017 progress report).

The trees in the pot study were destructively sampled and all plant parts separated and measured for dry matter, % N, quantity of N, and quantity of  $^{15}\text{N}$ .

There were no treatment effects on the dry matter weight of different plant parts when both fall and spring were combined. Trees with fall applied treatments did grow more trunk and shoot dry matter than trees with spring applied treatments (Fig. 4).

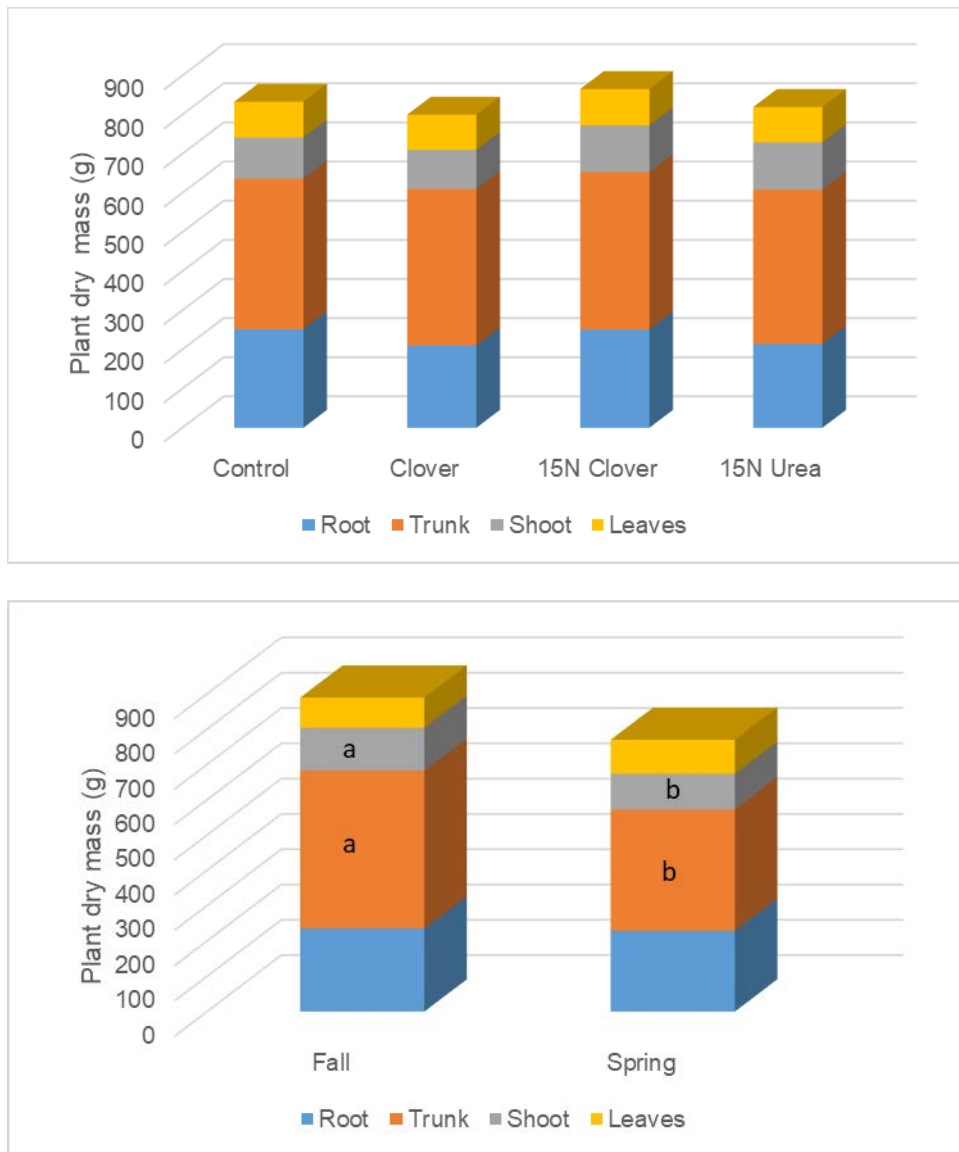


Figure 4. Tree dry matter partition by treatment (top chart) and season of application (bottom chart).

Total N % in the different tree parts was significantly higher in the roots of the control than of the urea trees when pooled over fall and spring treatments, but not for other plant parts (Table 3). When pooled over treatment, spring treated trees has higher total N % in shoots and leaves than fall treated. When separated by fall and spring treatment application, there were only significant differences for leaves, with leaves from spring applied  $^{15}\text{N}$  clover having higher total N than from fall applied  $^{15}\text{N}$  clover or urea (data not shown).

Table 3. Total N % in different tree parts at destructive sampling, by treatment and by season of treatment application.

Treatment	Root	Trunk	Shoot	Leaves
	----- % N -----			
Control	0.556 a	0.267	0.582	0.923
Clover	0.488 ab	0.264	0.594	0.930
Clover <sup>15</sup> N	0.525 ab	0.291	0.609	1.059
Urea	0.450 b	0.273	0.617	0.947
<b>Season</b>				
Fall	0.489	0.256	0.552 b	0.899 b
Spring	0.523	0.292	0.651 a	1.031 a

From plant dry matter and percent N, the actual grams of N in each plant part were calculated (Fig. 5). Trees with fall treatments tended to have higher total g N than the spring-treated trees, except for spring Clover <sup>15</sup>N. This was mostly due to more g N in the trunks.

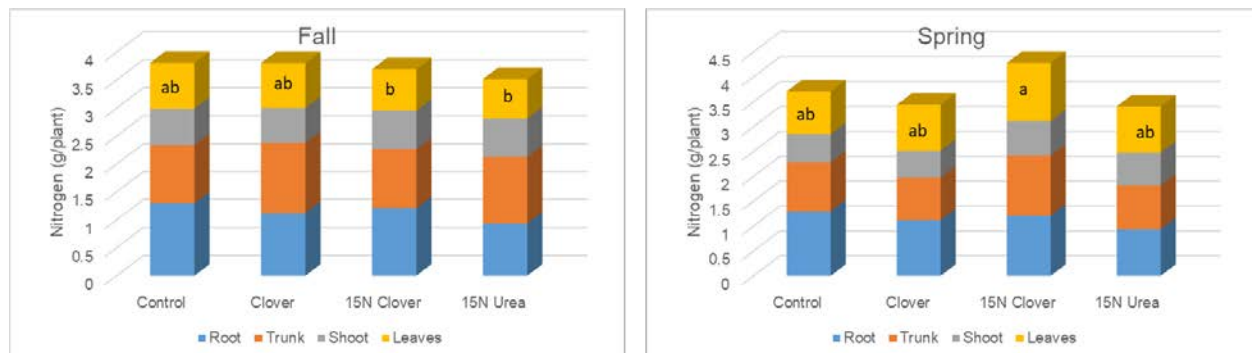


Figure 5. Nitrogen content (g) of plant parts at destructive harvest for fall and spring applied treatments.

Plant tissue from treatments with labeled <sup>15</sup>N were clearly elevated compared to the control (Table 4) as expected. The fall treated trees with Clover <sup>15</sup>N were more enriched than other treatments, and more enriched than the spring treated Clover <sup>15</sup>N trees. The unlabeled clover showed a slight numerical reduction in <sup>15</sup>N atom% relative to the control for some plant parts, but this was not significant.

Table 4. <sup>15</sup>N atom% of plant parts of trees at destructive harvest.

	<b>Treatment</b>	Root	Trunk		Shoot		Leaves
	Control	0.3800	0.3807		0.3778		0.3792
	Clover	0.3799	0.3805		0.3767		0.3777
	Clover <sup>15</sup> N	0.4377	0.4043		0.4178		0.4259
	Urea	0.4093	0.3915		0.3941		0.3989
	<b>Season</b>						
	Fall	0.4090	0.3939		0.3982		0.4069
	Spring	0.3944	0.3846		0.3851		0.3840
Fall	Control	0.3761	0.3782	D	0.3753	D	0.3760
	Clover	0.3798	0.3805	CD	0.3765	CD	0.3782
	Clover <sup>15</sup> N	0.4689	0.4206	A	0.4443	A	0.4631
	Urea	0.4113	0.3962	B	0.3965	B	0.4102
Spring	Control	0.3839	0.3831	CD	0.3804	BCD	0.3772
	Clover	0.3800	0.3804	CD	0.3770	CD	0.3824
	Clover <sup>15</sup> N	0.4065	0.3880	BC	0.3913	BC	0.3886
	Urea	0.4073	0.3868	C	0.3917	BC	0.3875

The results generally show more <sup>15</sup>N from the fall applied treatments was present in the trees. These trees had additional time for uptake compared with spring applied treatments. For both treatment dates, the amount of <sup>15</sup>N applied in the labeled clover was much higher than that in the urea, and also in the unlabeled clover. Thus, higher <sup>15</sup>N levels in the tree parts from the clover <sup>15</sup>N treatment is an unintended artifact of this difference that resulted from the treatment application needing to occur before lab results were available for more precise N calculations.

Urea was taken up more efficiently than labeled clover whether applied during spring or fall, probably due to the complexity of the N source for the clover compared to urea (Fig. 6). However, uptake efficiencies were low for both sources indicating slow uptake of both sources of N and/or losses from the system. These percent uptakes are considerably lower than the estimates made by Mullinix and Granatstein (2011) using surface applied alfalfa in the tree row and mineralization tubes.

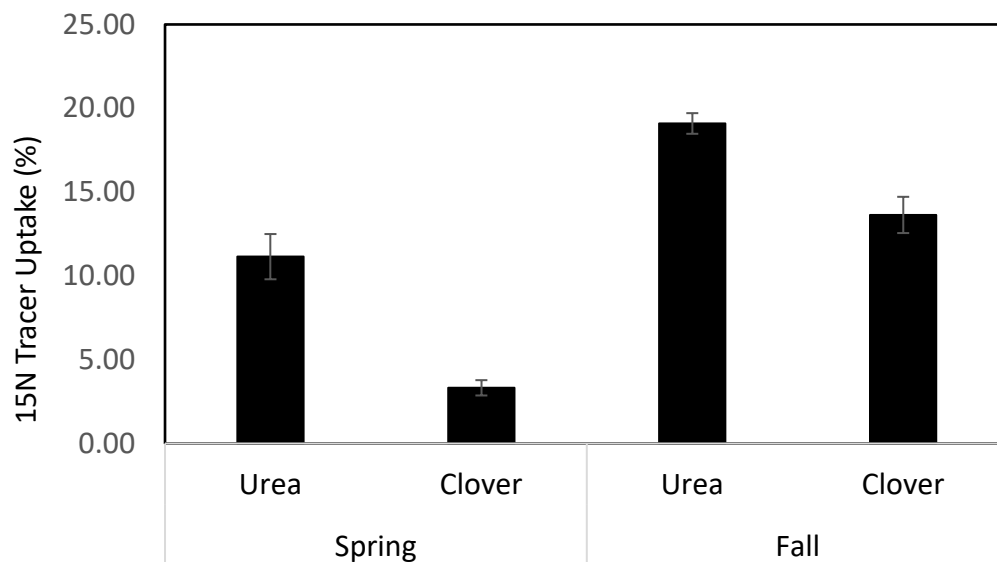


Figure 6. <sup>15</sup>N tracer nitrogen uptake efficiency (%) for apple where either urea or clover was applied in either the spring or fall. Error bars denote standard error (N=5)

More spring applied N stayed in the roots and less was transported to the shoots whereas for the fall application, more ended up in new growth and less in the roots (Fig. 7). Urea N had greater allocation to new growth than the clover or untreated control. Spring applied N produced a greater growth response in roots and new growth compared to fall applied N, and the root:shoot biomass ratio was lower for urea compared to clover and untreated control.

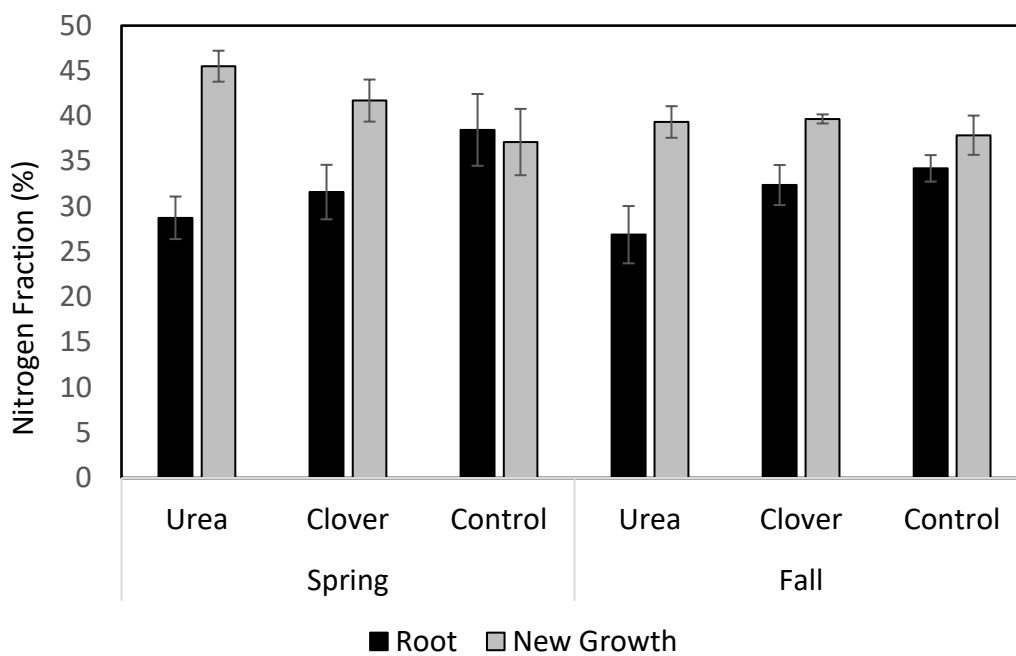


Figure 7. Estimates of total nitrogen fractions (%) in roots or new growth of apple where either urea or clover was applied either in the spring of the fall compared to an untreated control. Error bars denote standard error (N=5)

**Trial 2.** This study compared natural isotope abundance of N in two orchards that had contrasting grass and legume alley vegetation to see if the method might work under field conditions.

At the Alvarez Orchard (mature semi-dwarf ‘Golden Delicious’ apple), apple leaf N tended to be lower with a grass alley than with the legume (alfalfa or clover). Leaves were borderline N deficient in the July sampling (1.9-2.0% N). In July, leaves from trees with alfalfa alleys showed higher total N (not significant) and lower  $^{15}\text{N}$  atom%, indicating that the trees had taken up nitrogen fixed by the cover crop. The total N of the legume cover crops was significantly higher than the grass in July (Table 5).

Table 5. Tissue analysis of cover crop and apple leaf at Alvarez orchard, 2014.

	Total N %		$^{15}\text{N}$ Atom %	
<b>Cover Crop</b>	May	July	May	July
Grass		2.15 b		0.368 ab
Alfalfa	3.67 a	4.16 a	0.367 a	0.367 b
Clover	3.37 a	3.56 a	0.367 a	0.3703 a
P=	0.278	0.006		0.056
<b>Tree Leaf</b>	May	July	May	July
Grass	2.39 a	1.89 a	0.3686 a	0.3695 a
Alfalfa	2.49 a	2.04 a	0.3698 a	0.3685 b
Clover	2.50 a	1.93 a	0.3687 a	0.3695 a
P=	0.34	0.51	0.96	0.037

There were no differences in total N or the N isotope ratio for soils from the alley of the grass, alfalfa, and clover (Table 6). Two years may be insufficient time for the isotope signature of the soil to change.

Table 6. Total N and  $^{15}\text{N}$  atom% for soil in May 2014, Alvarez orchard.

	Total N %	$^{15}\text{N}$ Atom %
Control	0.105 a	0.3693 a
Alfalfa	0.097 a	0.3692 a

Clover	0.115 a	0.3691 a
P=	0.33	0.41

At the Tudor Orchard (4-yr old ‘Gala’ apple on M.9 rootstock), the alfalfa had significantly higher total N than grass on both sample dates. However, there were no differences in tree leaf N (Table 7). The alfalfa tissue did show a significantly lower <sup>15</sup>N atom% than the grass on both dates, and the same pattern was seen for the tree leaf <sup>15</sup>N atom%, again providing evidence of uptake of legume N.

Table 7. Tissue analysis of cover crop and apple leaf at Tudor orchard, 2014.

	Total N %		<sup>15</sup> N Atom %	
<b>Cover Crop</b>	July	August	July	August
Grass	3.43 b	3.67 b	0.3694 a	0.3683 a
Alfalfa	3.58 a	5.48 a	0.3688 b	0.3678 b
P=	0.005		0.05	
<b>Tree Leaf</b>	July	August	July	August
Grass	2.31 a	1.91 a	0.3837 a	0.3700 a
Alfalfa	2.40 a	1.88 a	0.3730 b	0.3695 b
P=	0.60		0.01	



Photo of Tudor orchard with alfalfa cover crop in alley. (D. Granatstein)

### **Conclusions.**

This study verified that apple trees are able to take up nitrogen from legumes grown in orchards for the purpose of nitrogen fixation, a potentially more sustainable source of this nutrient. The uptake was verified through the pot studies with  $^{15}\text{N}$  labeled clover, as well as the depressed  $^{15}\text{N}$  atom% in the leaves of field-grown trees with legumes in the alley versus grass. These results corroborate what was reported by Culumber (2016) for peaches in Utah with either grass or birdsfoot trefoil alley vegetation. Both the fall and spring applied treatments resulted in legume derived N present in the tree leaves approximately one month after application. This may be a fast enough response for the method to be useful in the field for monitoring tree N status where legumes are an important N source. However, the natural abundance method will not likely allow for a quantitative estimate of the % legume N taken up by the crop.

### **References.**

Culumber, C.M. 2016. Soil Nutrient Cycling and Water Use in Response to Orchard Floor Management in Stone-Fruit Orchards in the Intermountain West. Ph.D. Dissertation, Utah St. Univ., Logan, UT. <https://digitalcommons.usu.edu/cgi/viewcontent.cgi?article=6071&context=etd>  
 Mullinix, M.K., and Granatstein, D. 2012. 2011. Potential nitrogen contributions from legumes in Pacific Northwest apple orchards. Intl. J. Fruit Science 11:74-87.

### **OUTPUTS**

- Work Completed: Two trials completed.
- Methods, Results, and Discussion (discussion for final reports only): see text above
- Publications, Handouts, Other Text & Web Products: Final report. A journal article is in preparation in collaboration with Dr. Lee Kalcsits.

- Outreach & Education Activities: Discussed results at several grower meetings in 2018. Field day held in Prosser in summer 2014.

#### IMPACTS

- Short-Term: Results suggest this method could be useful to growers for estimating plant use of legume derived nitrogen
- Intermediate-Term: none
- Long-Term: none

ADDITIONAL FUNDING APPLIED FOR / SECURED. NONE

GRADUATE STUDENTS FUNDED. NONE

RECOMMENDATIONS FOR FUTURE RESEARCH. EXPLORE THE USE OF THIS TECHNIQUE WITH OTHER CROPS.