Estimating Alfalfa's Impact on Regional N Budgets in the Santa Clara & Central Valley's of California Using the ¹⁵N Stable Isotope Natural Abundance Method



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Introduction

- Alfalfa will utilize residual soil nitrogen (N) over N fixation when there is N present in the soil.
- Because of its deep root system which has been measured as far as 15ft below the soil surface, alfalfa has the potential to prevent or alleviate nitrate leaching into ground water

Site ID	Site Name	Location	Soil Type	Manure/Wastewater History	Year Planted
1	Burrel	Burrel, Fresno County	Colpien loam	Dairy manure consistently from 1975- 2008, Lagoon H ₂ 0 4 years prior	2008
2	RY-4	Visalia, Tulare County	Tranquility, wet, complex, saline sodic (Clay)	Dairy manure 2007, Lagoon H ₂ 0 Feb 2011	Fall 2007
3	Johns 80	Tulare, Tulare County	Nord fine sandy loam	2012 & 2011 Chicken manure, 2010 Cow manure	2010
4	Taggart 80	Tulare, Tulare County	Colpien loam	2012 & 2011 Chicken manure, 2010 Cow manure, receives periodic lagoon H ₂ 0	2010
5	Tipton	Tipton, Tulare County	Colpien loam	Last manure app 3 years prior, 5 years prior 7 ton/ac, 6 years prior 8 ton/ac.	2010
6	Los Banos	Los Banos, Merced County	Colpien loam	No Dry manure, receives lagoon water irrigations	Oct. 2010
7	Hanford1	Hanford, Kings County	Kimberlina fine sandy loam, saline-alkali	Dairy manure spring 2012, 1 truckload per 3 ac.	Feb. 2012
8	Hanford2	Hanford, King County	Remnoy very fine sandy loam	Dairy manure spring 2012, 1 truckload per 3 ac.	Feb. 2012
9	Roza4	Tulare, Tulare County	Lakeside clay loam, drained	10 years prior dry manure, frequent lagoon water irrigations for many years	2012
10	Roza2	Tulare, Tulare County	Lakeside clay loam, drained	10-20 ton dry manure spring 2012	2012



sources.

- Dairy manure is an invaluable nutrient source that is commonly applied to crops in California (CA).
- When manure is applied to fields there is a loss of N during ammonia volatilization. Often greater amounts of the lighter ¹⁴N isotope is lost, leaving the soil enriched in ¹⁵N.
- The natural abundance stable isotope method can be used to determine how well the alfalfa 'cleans' up the soil by determining the percentage of N derived from the atmosphere (%Ndfa).

Purpose

In order to effectively use the BNF habits of legumes, it is important to have methods to quantify the amount of biologically fixed N.

Quantitatively determining the amount of N obtained from soil and from atmosphere can help estimate effects of field practices on N_2 fixation.

Figure 1. δ^{15} N content of alfalfa tissue in fields with and without a history of manure/wastewater applications.



Figure 2. Percent N derived from the atmosphere from fields with and without a history of manure applications.

Objective

Determine N content and N isotope ratios on alfalfa and reference plant samples in order to estimate the %Ndfa.

Methods

- Study conducted within Fresno, Kings, Merced, Tulare and Santa Clara Counties, California
- Complete Randomized Design
- Treatments: Manured fields: 10 replications Non-manured fields: 7 replications
- 3, 3 ft x 3 ft plots of sudan grass planted in random locations at each site (sub-sample)
- Samples were taken two times during growing season, 1st from June to August & 2nd from September to October
- Sampled with 2 ft. x 2 ft. frame from the crown of the plant. Samples were taken as close as possible to harvest

Calculations

•The equation used in determining $\delta^{15}N$, is $0^{0}/_{00}$ (parts per thousand) on the δ scale, where $R = {}^{15}N/{}^{14}N$ and R standard is the standard of air R = 0.0036765:

 $\delta^{15}N = 1000(R Sample-R Standard/R Standard)$

•The equation used for estimating the relative concentration of nitrogen fixed from the atmosphere in relation to the total nitrogen in plants:

 $\% \text{Ndfa} = (\delta^{15} \text{N}_{\text{ref}} - \delta^{15} \text{N}_{\text{fix}}) / (\delta^{15} \text{N}_{\text{ref}} - \delta^{15} \text{N}_{\text{of N2}}) - (\delta^{15} \text{N}_{\text{ref}} - \delta^{15} \text{N}_{2 \text{ fix}}) / (\delta^{15} \text{N}_{\text{ref}} + 0.68)$



Results

- The age of the alfalfa stand did not have an influence on the $\delta^{15}N$ content, %Ndfa and N content of alfalfa tissue.
- There was not a significant difference in the %Ndfa and δ¹⁵N content between the 1st and 2nd sampling dates, although the N content in the 2nd sampling dates was higher than in the 1st.
- There was not a significant difference of N content of alfalfa tissue (ug/g of sample) between the fields with a history of manure/wastewater applications and fields with no manure application history.
- There was a significant difference in the N content of alfalfa tissue amongst the fields with a manure applications history.



date

- Alfalfa samples were taken in close proximity to non legume reference plant sample
- Plant samples were ground to a fine powder and weighed to 3.5-4.0 mg on a microbalance
- Samples submitted to UC Davis Stable Isotope lab
- Combustion technique used to determine N content
- Isotope ratio mass spectrometry used to measure ¹⁵N/¹⁴N isotope ratio

Photograph of sudan grass reference plot in
alfalfa field.Red markers represent fields with a manure
application history, yellow markers represent
fields with no manure application history.

There variation of the results in the manured fields is most likely due to the difference in the field's manure application history.
The natural abundance method can be used in estimating small amounts of ¹⁵N via isotope ratio mass spectrometry.
Alfalfa can utilize residual soil N from manure giving it the potential to prevent nitrate leaching.
Further studies could be conducted looking at the δ¹⁵ N content at different levels in the soil.