CDFA FREP Project Suggestion: ***EVALUATION OF A 24 HOUR SOIL CO_2 TEST FOR ESTIMATING POTENTIAL N-MINERALIZATION TO REASSESS FERTILIZER N RECOMMENDATIONS.***

Project location: ***State of California***
Project duration: ***Three years***

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CDFA Funding Request Amount/Other Funding

FREP Budget request: Year 1: $49,990; year 2: $49,985; year 3: $49,996; Total: $149,971

Extra-mural or in-kind sources: $ 25,632; committed salary of Dr. Horwath over 3 years of project

Executive Summary

Estimating growing season soil N availability using a soil test remains a problem with few solutions. As a result, fertilizer recommendations are often made without an accurate assessment of the amount of N that becomes available through soil N mineralization during the crop growing season, but rather it is based on soil inorganic N levels, e.g. the pre-plant nitrate test, total soil N or previous crop recommendation. However, these tests cannot estimate the amount of growing season soil N mineralization. This is critical since experiments with an isotope of N show that growing season soil N mineralization accounts for a minimum of 50% of crop N uptake. The amount of mineralized N is similar independent of low or high organic matter soils. The lack of a suitable test to estimate mineralizable N has troubled the soil test industry for decades. After 60 years of research on the soil N cycle and soil N tests to predict soil N mineralization, we still have no testing procedures the soil testing industry can adopt. Though studies have shown that tests, such as hot KCl or phosphate-borate extractions, and total soil N are fairly accurate under highly controlled research conditions, these tests have not worked well in practice. The failure of these tests is due, in part, to the wide variability associated with soil mineralogy and texture, soil management history, crop planting dates, etc.

Biological based tests, i.e. incubations, have been shown to predict soil N availability better than chemical assays. These incubations rely on the soil microbial community to mineralize soil N from various soil organic matter fractions, ranging from easily decomposable to resistant. However, from a soil testing perspective, incubations are time consuming, space demanding and labor intensive, and, therefore, not suited for high output requirements of soil test labs. Recent studies have shown the flush of CO_2 following drying and rewetting of soil to mimic natural processes and characteristics of long-term incubations, correlate with N-supply potential in soils with a wide range of organic mater contents. In some studies, this short-term flush of soil CO_2 explained 97% of the variability in N mineralization among different soils over several weeks.

We will evaluate whether the flush of CO_2 from soils can predict growing season soil N mineralization across a range of soils that vary in fertilizer N requirements, soil amendments (crop residues and manures and composts), organic matter contents and other agronomic practices. We will develop correlations to other tests such as total soil N, total soil organic matter, crop N uptake and pre-crop nitrate levels to predict soil N mineralization potential with the main goal to reassess fertilizer N applications for important California crops. Finally, we will evaluate the cost effectiveness of implementing biologically based soil assays and procedures in commercial soil test labs. The criteria for project success include the development of a soil test to predict in season soil N availability and its evaluation by the soil test labs.

The target audience ranges from California producers, soil laboratories, soil consultants and the fertilizer industry. Of most importance is to demonstrate a cost-effective biologically –based test that can be readily implemented by soil test labs, which routinely perform soil tests to estimate soil N contribution for crop uptake to optimize fertilizer N recommendations.

Justification

Problem

The development of suitable soil tests to estimate growing season soil N mineralization for crop uptake has not seen much success despite decades of research into the topic. This is particularly disturbing in light of the fact that all the enzymatic processes involved in the N cycle have been discovered. Despite knowing the enzymology associated with all N cycling processes, we have failed to use this understanding in predicting available soil N. The failure to predict soil N mineralization has resulted in a guessing game based on perceived crop N needs estimated from fertilizer rate trials or long-term yield studies. The result is that fertilizer recommendations are made without an accurate assessment of the amount of N that becomes available through soil N mineralization during the crop growing season. After 60 years of research on the soil N cycle and soil N tests to predict soil N mineralization, we still have no suitable tests that the soil testing industry can adopt. As a result, based on a mix of economic decisions and determination to achieve maximum productivity, N application rates may be above crop need. Evidence of over-applications of fertilizer N has been documented in recent reports from the Water Resources Center and Agricultural Sustainability Institute California N Assessment at the University of California's (Viers et al. 2012; http://asi.ucdavis.edu/research/nitrogen). The conclusions of these reports suggest agricultural fertilizers and animal wastes are by far the largest contributors to nitrate (NO_3^-) groundwater contamination. For this reason, there is an immediate need to develop a soil test that will predict soil N availability during the growing season. Once a soil's growing season N mineralization potential is known, fertilizer N application rates can be reassessed accordingly. The impact on the statewide level will be to reduce nitrate loading to groundwater, improve crop N use efficiency, reduce nitrous oxide emission and decrease grower input costs.

CDFA/FREP goals

The proposed research addresses 6 of the 7 priority research areas in CDFA/FREP. This project addresses the "Emerging issues" area by addressing the economics of fertilizer use through the development of methodologies for minimizing fertilizer losses; "Crop nutrient" uptake by assessing growing season soil available N availability; "Improving efficiency" by promoting efficient fertilizing practices to ensure the right rate of application; "Precision crop management" by demonstrating and quantifying fertilizer applications for precision crop management; "Developing tools" by developing field and laboratory tests for crop nutrient response to aid in fertilizer recommendations; and "Education and Outreach" through implementing educational activities that result in on-farm changes for more efficient fertilizer use.

In addition, this research addresses two of the five recommendations of the CDFA Nitrate Working Group:

1. Develop best management practices to be incorporated into local nitrate management programs.

2. Establish a research and demonstration project on nitrate control through irrigation, fertilizer and manure management.

Impact

The development of a quick and accurate test to determine growing season soil N availability will provide the following benefits:

1. Accurate N application rate recommendations through accounting for growing season soil N mineralization. This will increase fertilizer N use efficiency by the crop.
2. Reduction of fertilizer N input cost for growers.
3. The optimal fertilizer N application rate will reduce nitrate leaching and nitrous oxide emission.
4. When broadly implemented by soil test labs, tests to predict in growing season soil N mineralization will be widely available and their use will address local, regional and statewide issues of fertilizer N and animal manure nitrate contamination of ground water.

The implementation of a growing season soil N test to predict N mineralization may be especially successful in combination with other standard tests such as precrop soil nitrate, total soil N, and soil organic matter content.

Long-term solutions

The search for a suitable soil test to estimate growing season soil N mineralization has been elusive. As mentioned above, much research has been done in this area with no suitable technology transfer to the soil test industry. Notably missing from the arsenal of soil tests are tests based on the biological potential of soil to mineralize soil N. This fact is intriguing since an accurate soil test should reflect the biological potential of the soil to mineralize N. This is understandable since previous soil tests based on biological potential usually involve soil incubations. Soil incubations have shown to be reliable and accurate when done under highly controlled conditions, such as those found in agricultural research labs. The incubations often involve very specific soil conditions such as ideal temperature and moisture, leaching in columns, interpretation of rate data on N mineralization and other factors. With all these attributes, the incubation assays do not meet the fast turn around requirements of soil test labs.

Regardless of the shortcomings of the incubation approach, the basic premise is sound in that it does remarkably well in predicting soil N mineralization. The problem is finding a way to port the technology to the soil test lab that is labor and space efficient and easy to interpret. Recent research (explained below) has shown that shorter incubations can replace long-term incubations in predicting soil N mineralization. In addition, the flush of CO_2 from soils has been shown to correlate to soil N mineralization across a range of soil organic matter contents.

Related research

<u>Background</u>
Nitrogen occupies a unique position among the soil-derived elements essential for plant growth because of its complex biogeochemistry and the rather large amounts required by most agricultural crops in comparison to other elements. In plants, N is a constituent of chlorophyll, all

proteins, including the enzymes, and many other compounds. Without the input of N fertilizer and or amendments, crops would not attain maximum yield potential. While insufficient application of N can have serious economic consequences for the farmer, excessive fertilization increases the risk of environmental pollution, because the N cycle in soils has many loss pathways. Losses occur through leaching, denitrification, volatilization, erosion, and in agricultural systems through crop removal. The leaching of nitrate in agriculture has received considerable attention in recent years because of possible pollution of groundwater (Viers et al 2012). Therefore, there is a strong need to develop a soil test to account for the contribution of soil N mineralization to adjust the inputs of fertilizer N sources to avoid environmental impact.

The large need of plants for N and the limited ability of soils to supply it cause this nutrient to be the most limiting for crop production on a global basis (Foth and Ellis, 1997). The need to achieve maximum yield is critical, as future human population growth will require an ever-expanding food supply. Although many productive mineral soils contain several thousand kilograms of N per hectare, about 90% of the soil N is unavailable in the form of organic matter, and most of the remainder exists as fixed ammonium (NH_4^+) in clays (Foth and Ellis, 1997). Only a small fraction of the N in soils, generally less than 0.1%, exists in plant-available mineral compounds, such as NO_3^- and exchangeable NH_4^+, at any one time, and no more than 1-2% of the total soil N will be available to plants during a growing season (Stevenson and Cole, 1999). However, the amount of soil N mineralized can be significant and therefore should be accounted from a nutrient management perspective.

The soil microbial biomass plays a critical role in controlling the supply of N to crops by mineralizing soil organic N. This is likely the reason that long-term incubations reflect the potential to mineralize soil N. It is well known that crops are dependent on N from inorganic fertilizers. The mineralization of soil N often accounts for more than 50% of total crop N uptake, as indicated by studies using isotopically labeled fertilizers (Kramer et al 2002; Doane et al 2009). These studies were done on soils with about 1% soil C, typical of California agriculture. Therefore, the rate of soil biological activity should serve as a reliable index of the soil's capacity to supply N and perhaps other nutrients such as P to crops.

Biological based soil N tests

In biological tests, a soil sample is incubated under temperature and moisture conditions conductive to N mineralization. After a given time period, the total mineral N produced is measured. Many variations of this basic procedure exist, but in general, biological methods are divided into two major groups: aerobic and anaerobic incubations. The anaerobic incubation has performed well for forest and grassland soils and shows good correlation to N availability (Powers 1980). However, anaerobic incubations have not performed well in agricultural soils.

Many procedures have been proposed for aerobic incubations. They differ in their incubation time, temperature and whether mineral N is determined destructively or by repeated leaching of a sample. Correlations between biological procedures and greenhouse results are generally good, but when testing progresses to field conditions, the correlations are usually considerably lower or non-existent. The following reasons may be responsible:

1. Measurements of N mineralized from disturbed soil samples often overestimate field N availability due to stimulation of mineralization by drying, crushing and sieving the soil (Bundy and Meisinger, 1994; Cabrera and Kissel, 1988). Bremner (1965) found that short-term aerobic incubations are dependent on methods used in pretreating the soil before incubation. Even with the use of rigorously standardized methods, results of short-term incubations do not necessarily reflect the potential, long-term N supplying capacities of soils (Stanford and Smith, 1972). Nitrogen mineralized during incubation of undisturbed soil cores can provide a more reliable assessment of N availability. However, a relatively large number of undisturbed soil cores is needed due to soil spatial variability which often makes this approach impractical for field-scale N mineralization predictions by soil test labs (Cabrera and Kissel, 1988).

2. Nitrogen mineralized in short periods under aerobic conditions may be influenced by the N derived from decomposition of recently incorporated residues and microbial tissues (Stanford et al., 1975). On the other hand, N immobilization due to recently incorporated residues with a high C/N ratio may lead to an under-prediction of the N mineralization potential of the soil (Chichester et al., 1975). Longer-term incubation suffers less from this issue as the effect of C/N ratio lessens with incubation length. The issue can also be mitigated in short-term incubations if careful care is taken to remove recently deposited crop residues. These issues will be examined in this research.

Long-term incubations estimate the N mineralization potential of soils better, but this approach is expensive and time-consuming (Haney et al 2008a). The most serious drawback of this approach is that the long incubation time does not allow for growing season estimation of N fertilizer application rates. Despite these limitations, it is generally recognized that aerobic incubations that produce NO_3^--N and NH_4^+-N provide a sound relative measure of the N mineralization potential of a soil. This is because the soil organic N is released by the same biological processes active under field conditions.

As the C and N cycles in soil are closely linked, CO_2 evolution has been found to correlate with N mineralization (Franzluebbers, 2000). Aerobic incubations allow for the determination of both mineral N and CO_2, which may reveal interesting aspects of the decomposition process. Recent studies have shown the flush of CO_2 following drying and rewetting of soil with a wide range of organic mater contents mimics some natural processes and characteristics of long-term incubations. In addition, the CO_2 flush has been observed to correlate with N-supply potential. Some studies have shown that this short-term flush of soil CO_2 explained 97% of the variability in N mineralization over several weeks (Franzluebbers et al 1996; Haney et al. 2008a,b).

Recent innovations in performing soil CO_2 response include reducing the time of analysis (Haney et al 2008a). A high degree of correlation has been demonstrated between longer term (28-day, 7-day) and shorter-term (3-day and 1-day) procedures such that a significant reduction in lab encumbrances in routinely performing tests has been accomplished. Many commercial labs are now employing a commercial 24-hr test that is pre-calibrated (Solvita®) and can be read with a hand-held spectrometer (Haney et al 2008b). Therefore a significant advance in ability to meet grower soil quantity needs at the commercial lab level will be addressed in this study.

Contribution to knowledge base

The proposed project will explore the possibility of using a biologically based soil tests to more accurately reflect the contribution of soil N mineralization for crop uptake. As said above, there exists a strong relationship between the amount of C and N mineralized from soils. However, this relationship has not been assessed for a wide range of soils necessary to develop predictive relationships to estimate the amount of soil N mineralized. **The intent of this project is to develop data necessary to validate the correlation between short-term C mineralization and soil N mineralization potential across a range of California agricultural soils.** A broad dataset is necessary for adoption of the procedure by soil test laboratories that prefer minimal interpretation of the results. However, the test must be useful for a diverse set of soils, crops and regions within California.

Respirometry methods have not attracted serious attention by commercial soil labs due to the high cost of required labor, specifics of reagent handling and data interpretation. We will assess a 24-hr CO_2 burst from a rapid-rewetting procedure that can simplify laboratory processing of soils. As mentioned above, previous research has shown a strong correlation between C and N mineralization in soils. If successful, the test would provide an index of soil N mineralization potential and allow for reassessing fertilizer N application rates.

We will collaborate with the USDA Agricultural Research Service Soil Lab in Texas to compare our results and for quality control to commercial versions available for use in soil test labs. The USDA lab will be testing a commercial version of the 24-hr CO_2 burst from a rapid-rewetting procedure. Here at UC Davis, we will be validating the procedure from a research perspective to determine if the test is suitable for California soils. Our collaboration will include exchanging soils from Texas as soils (arid environment and low soil organic matter) and some cropping system practices are similar between states. To make one point clear, any well equipped soil lab can complete the procedure without making use of the commercial test. Standard gas chromatography and titration techniques can substitute for the commercial product. However, from a standardization perspective, the commercial product is simple and requires less interpretation.

Grower use

A tool to account for soil N mineralization contribution to crop N uptake would benefit growers in several ways. The soil test would allow them to reassess fertilizer N application rates by taking soil N mineralization into account. Using an optimized rate of N fertilizer and would increase N use efficiency. The reassessment of fertilizer N application rates would also provide for the reduction of gaseous N and nitrate leaching losses. Adoption of this new soil test, combined with traditional tests, will allow growers maintaining crop yield potential while minimizing environmentally harmful N losses, such as nitrous oxide emissions and groundwater contamination.

Objectives

The criteria for the success of this project include the development of a biological soil test to predict in season soil N availability. We will work with soil test labs to determine whether it is feasible both from a scientific and analytical standpoint.

The main objective of this research is to

1. Evaluate whether the flush of CO_2 from soils can predict growing season soil N mineralization across a range of soils that vary in fertilizer N requirements, soil amendments (crop residues and manures and composts), organic matter contents and other agronomic practices.
2. Develop correlations to other tests such as total soil N, total soil organic matter, crop N uptake and pre-crop nitrate levels to predict soil N mineralization potential with the main goal of reassessing fertilizer N applications for important California crops.
3. Evaluate the cost-effectiveness of implementing biologically based soil assays and procedures in commercial soil test labs.

Work Plans and Methods

Task 1. Conduct a literature review on the use of soil respiration (CO_2 output) and soil organic matter (SOM) based tests for estimating active carbon, biomass, and potentially available N. Though literature is cited in this proposal, we will conduct a further review to include fundamental and applied knowledge in the area of soil C and N mineralization. This meta-analysis is required in order to develop consistently reliable soil testing protocols that are based on sound principles and can be used to assess N mineralization potential in a wide range of soils and cropping systems. The evaluation of soil C and N mineralization became a routine analysis beginning in the 1950s as a way to establish microbial activity (Bremmer 1965). During the 1960s, soil C and N mineralization were closely linked and the association with microbial activity was validated (Jenkinson et al 1985; Paul and Juma 1981). In the 1970s, soil C and N mineralization was used to estimate the size of the microbial biomass, soil organic mater maintenance and potential to predict N mineralization under field conditions (Jenkinson et al 1985; McGill et al 1986, Ladd and Paul 1973; Stanford and Smith 1972). During the 1980s through 1990s numerous soil C and N mineralization studies validated the concept that incubations could estimate a soils potential to mineralize both C and N under lab and field conditions (Bonde and Rosswell 1987; Paul et al. 1995). More recent studies have continued to evaluate the use of lab incubations in predicting both soil C and N mineralization under field conditions (Franzluebbers et al 2000, Haney 2008a,b). Evaluation of existing methods will include assessing the feasibility, reliability and ease and cost of laboratory analyses of soil samples.

The literature review will be completed by July 2013. The interim product of this task is to provide the scientific basis to validate the results of the lab incubations, which will be conducted through July 2015.

Task 2. Develop sampling protocols and analyze a range of target soils for a variety of soil properties including total-carbon (C), water-soluble carbon (WSOC), water-soluble nitrogen (WSN) and other standard chemical properties (pH, color, texture).

Task 2a. Survey soils from major cropping systems in California.

Soils from the major cropping systems will be sampled and assayed. Soil samples will be taken from 0 to 6 inches for the incubation studies. Additional soil to 24 inches depending on cropping system will be taken to determine soil total C and N and mineral N. The soils will be air dried and analyzed within 3 weeks to mimic possible conditions in a soil test lab during the spring where soil testing is at its peak. Longer-term storage of the soils up to 6 months does not generally affect the outcome of incubation studies. This will be critical to demonstrate that off growing season soil samples can be assayed to predict in growing season N mineralization. We realize that soils samples from fall versus spring may provide different results. Our research will determine if this is a constraint to when soil samples can be taken. The major cropping systems are presented in the following table 1.

Table 1. Representative soil samples will be sampled from the following commodities. Commodity area and value for California are also presented.

Commodity	Area (1,000 acres)	Value ($1,000)
Corn	180	182,520
Lettuce	207	1,642,249
Tomatoes (processing)	270	878,006
Cotton	303	610,042
Wheat	455	226,268

The intent of the soil sampling will be to assemble a wide range of soil texture and total C and N contents. A key element of the research will be to define the influence of soil texture and total soil C and N content on potentially mineralizable C and N. The gathering of soil samples will be completed by July 2014.

Task 2b. Analyze the soil samples for various soil properties.

The following describes methods for soil sampling and methods to characterize soil properties during the first and second year of the project.

Soil sampling and preparation

Soil samples will be taken in the field with a 1.2 cm auger. At least five cores will be taken per plot and stored in an ice chest. In the lab, the samples will be air dried at room temperature. The dry samples will be ground to pass a 2-mm sieve and stored in plastic bags.

Gravimetric soil moisture content

A portion of all soil samples will be used to determine oven dry weight at 105° C. All concentrations of soil properties and constituents will be reported on an oven dry (OD) basis.

Water holding capacity

The water holding capacity of soil samples will be determined by the funnel method. Filter paper will be placed in a funnel with about 20 g of soil. The funnel is placed into a beaker filled with water. The water is left to imbibe into the soil until the surface of the soil glistened brightly. The funnel is then removed from the beaker and drained for 30 minutes after which a spoonful of soil will be removed and placed on a tin pan. The pan and soil will be weighed and placed into an oven maintained at 105° C for 24 h and weighed again. All incubations will be performed at 55% of water holding capacity.

Particle size analysis

The particle size distribution of the soil samples will be determined using the pipet method (Gee and Bauder, 1986). The method provides for the quantitative determination of the sand, silt and clay fractions.

pH/EC

Soil pH will be measured in a 1:1 soil:water solution (Thomas, 1996). Soil and DI water will be added to a 50 mL centrifuge tube and shaken for 15 min on a reciprocal shaker. Before measuring solution pH, the samples are allowed to equilibrate for 30 min. The same solution will be used to measure electrical conductivity (Rhoades, 1996).

Total carbon and nitrogen

Total C and N of the soil samples will be determined by the dry combustion method (Nelson and Sommers, 1996; Bremner, 1996). Subsamples are finely ground on a ball mill and weighed into tin cups. Total C and N will be analyzed on a CNS analyzer (Costec Inc., Lake Zurich, IL).

Extractable nitrogen

Soil samples are weighed into 50-ml centrifuge tubes, 2M KCl solution is added (KCl solution to soil ratio 5:1) and the tubes shaken on a reciprocal shaker for one hour. After shaking, the suspension is filtered through a filter paper (Fisherbrand, Q5; 12.5 cm diameter). Prior to use, the filter papers will be leached with 15 mL of 2M KCl solution to remove any NH_4^+ (Mulvaney, 1996).

The NO_3^- and NH_4^+ concentrations in the filtrate will be analyzed colorimetrically on a spectrophotometer (model UV mini 1240, Shimadzu). Nitrate is analyzed using a single reagent that reduces nitrate to nitrite, which is complexed by sulfanilamide. Further reaction with N(1-naphthyl)ethylenediamine produces a red dye that is quantitated colorimetrically at 540 nm (Doane and Horwath, 2003).

The NH_4^+ concentration is determined by the salicylate method (Verdouw et al. 1978). This method is based on the Berthelot reaction in which ammonium reacts with phenol and hypochlorite to form a green indophenol compound whose concentration is determined colorimetrically at 650 nm. The reagents described by Foster (1995).

Water Soluble C and N

Water soluble C and N will be extracted as described for extractable N, except that DI water will be used instead of KCl. Water soluble C will be analyzed on a UV-Persulfate Total Organic C Analyzer (model Phoenix 8000, Tekmar Dohrmann^{TM}, Cincinnati, Ohio). Water soluble N will be determined with the alkaline persulfate oxidation method in which the extract is mixed with an equal amount of an oxidizing reagent (Cabrera and Beare, 1993), heated in a boiling water bath for 2 h, and analyzed for NO_3^- as described above.

This task will be completed by March 2015.

Task 3. Validate the "24 hr CO_2 evolution test" against long-term soil incubations to confirm estimates of soil N mineralization potential

Aerobic soil incubations will be carried out on air dry and sieved soil (Bundy and Meisinger, 1994). Water will be added to attain 55% water holding capacity determined by the funnel method described above. Samples will be kept at 22° C in the dark. Soils will be incubated for up to 100 days. Soil sampling for CO_2, NO_3^- and NH_4^+ will occur at 1, 5, 12, 20, 35, 50, 75, 100 days approximately. Soil sampling at 24 hr is required to validate the 24 hr CO_2 evolution test and the result extracted from the longer-term incubation. Nitrate and NH_4^+ will be determined as described above. Net N mineralization will be calculated by subtracting the mineral N at day zero from the mineral N determined after a certain incubation time. Periodic measurement of the mineralized N will allow for description of the relation between cumulative N mineralization (N_t) and time of incubation (t, in days) based on the following first-order equation (Stanford and Smith, 1972):

$$N_t = N_0 (1\text{-}e^{-kt})$$

Where N_0 is the N mineralization potential in units of mass (e.g. mg/kg of soil) and k is the mineralization rate constant (i.e. the specific rate of mineralization as a function of N_0).

The measurement of CO_2 evolution will occur simultaneously with the determination of N mineralization described above. The containers described above will contain a septum for headspace sampling. Headspace CO_2 will be analyzed on a Qubit CO_2 analyzer (model S-151, Qubit Systems Inc., Kingston, Canada). After each analysis, the containers will be opened and air exchanged for 3 minutes before closing them and returning to incubation conditions. A blank (no soil) will be used to correct for background CO_2. The amount of C respired per unit mass of soil will be calculated from the headspace concentration of CO_2, using the ideal gas law (Zibilske, 1994). The results will be used to correlate between the 24 hr results with those produced up to 100 days. A good correlation (r^2>0.80) is required to assure the 24 hr response is valid.

This task will be completed by March of 2015.

Task 4.Field validate "24 hr CO_2 evolution test" against field N application rates and crop N uptake at across varying sites, including sites in Texas, with a range of soil organic matter contents with objective of reassessing fertilizer N recommendations.

The potential usefulness of a "Quick Soil CO_2 Test" in evaluating contributions of soil N from soil biological activity and from soil amendments will be evaluated in field trials. We will select up to 20 fields of each of the five model cropping systems (tomato, corn, lettuce, wheat, cotton) that vary in terms of fertilizer N requirements, inputs of soil amendments (crop residues and manures and composts) and other management such as crop rotation and tillage. We will perform a preplant soil N test to 60 cm to assess plant-available N and the 24 h CO_2 evolution test to predict the amount of N that will become available during the growing season and wherever possible will adjust the fertilizer N application accordingly in a portion of the grower fields. Furthermore, wherever possible we will install a zero N treatment plot to determine the uptake of soil N in the absence of fertilizer. Yield and biomass N will be measured at harvest to assess whether this approach will lead to achieving a crop's full yield potential and greater N use efficiency. Additionally, on a number of grower fields (where permissible) soil samples will be taken at the end of the growing season to assess post harvest inorganic N levels.

As part of this task, we will exchange soils with the USDA in Texas to for inter laboratory quality control determinations.

The analysis (24 hr Soil CO_2 test, long-term soil incubations for C and N mineralization, soluble C and N analysis) of the soil samples from Task 2a will be correlated against a number of field observations, described above. The data will be used to develop correlations to other tests such as total soil N, total soil organic matter, pre-crop nitrate levels and total crop N uptake.

This Task will begin April/May 2013 and end April 2015.

Task 5. Construct guidelines for soil test labs for performing the "24 hr CO_2 evolution test".

Once the datasets have been evaluated, principal trends of soil tests have been validated, and a correlation developed to long-term incubations and field response data (preseason NO_3^-, end season NO_3^- and crop N uptake), we will construct guidelines to inform where fertilizer N application should be reevaluated. These guidelines will include information from Tasks 1, 2, 3 and 4. A simplified envisioned version of the guidelines incorporated into the charts is shown in Figures 1 and 2.

These example charts are based on preliminary findings, using the outcome of the correlation analysis based on the 24 hr soil test against crop N uptake, and long-term incubations done on long-term studies at UC Davis (Horwath, unpublished data). According to our present knowledge, as discussed earlier, a strong relationship exists between soil incubations and their ability to predict mineralizable N. Key to the success of this project will be whether a 24 hr soil test based on the CO_2 flush of air dry soil is predictive of potential soil N mineralization.

The guidelines for the testing labs and regulatory agencies will include all the information about a the range of responses and the accuracy of the correlations among the 24 hr soil test and long-term incubation and crop N uptake. As part of this task we will evaluate the cost-effectiveness of implementing biologically based soil assays and procedures in commercial soil test labs. Basically this will entail a detailed assessment of cost, labor and time need to perform the analysis.

This task will begin April 2015 and end December 2015.

Figure 1. Conceptual diagram depicting the relationship between soil N mineralization and crop N demand. As soil N mineralization increases the amount of fertilizer N application can be decreased. The extent of decrease depends on the amount of soil N mineralized and the certainty of the N mineralization results of specific soils and crops. For example, the ranges depicted by A, B and C represent decreasing need for fertilizer N inputs.

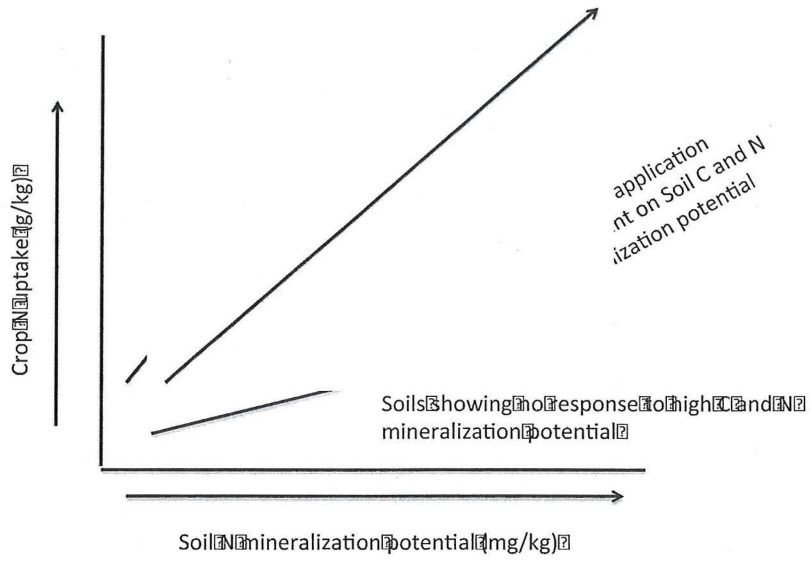
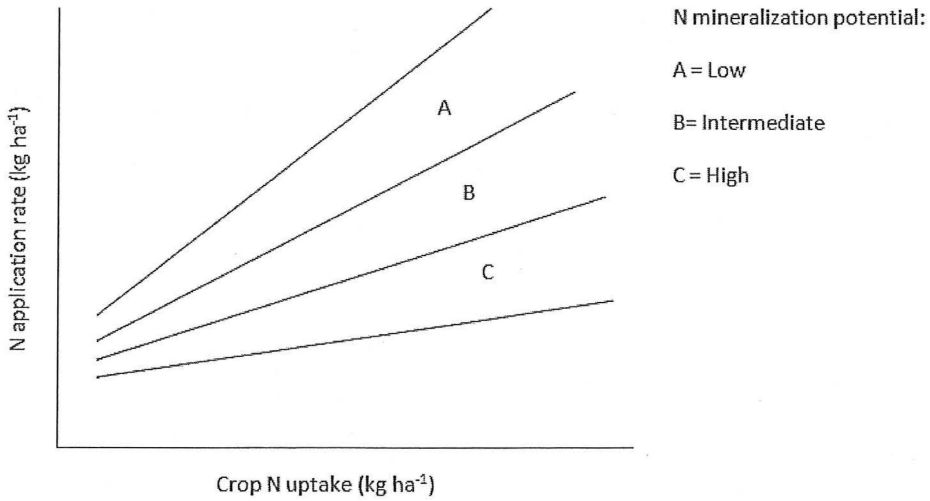


Figure 2. A more simplified version correlating N mineralization potential with crop N uptake.



Task 6. Conduct an outreach program to educate soil test labs and growers on the usefulness of the "24 hr CO_2 evolution test" in reassessing fertilizer N recommendation rates.

The purpose of the outreach program is to educate soil test labs, regulatory agencies and growers to understand the concept of a biologically based soil test to predict growing season soil N mineralization. In addition, the practicality of implementing the test in soil test labs will be evaluated through cross comparison of soil samples with UC Davis and the three participating soil test labs. The information and reports from this research will be accessible to the general public and as publications in peered reviewed journals. Publication in peer-reviewed journals is key to establishing its scientific legitimacy for adopting as a soil test for growing season N availability.

We will engage in 3 outreach activities per year during the duration of the project. However, during the first year and much of the second year, the activities are primarily associated with soil sampling and testing. We will likely ask for permission to allow more outreach events in years two and three as information on the successfulness of the test becomes available.

First, we will present our findings at the fall FREP Conference and submit our presentations for publication in the Conference Proceedings. Second, we will participate in outreach events and symposia sponsored by University of California, Western Plant Health Association, Soil Science Society of America and Western Soil Science Society, the Annual South Sacramento Valley Processing Tomato Production Meeting in Woodland, Small Grains Field Day organized by UC Davis extension, Monterey County Annual Irrigation and Management Meeting and Western Plant Health Association Meeting (120 participants), among others. Third, we will make available short summaries of our work to be published in the in the form of newsletters, technical articles and others. Fourth, we will present at the California Plant and Soil Conference and will submit an article to the Conference Proceedings. Fifth, we will write articles for the Agriculture and Natural Resources (ANR) Extension Bulletin and trade news outlets like the California Farmer. Sixth, at the end of the project we will submit at least one article for publication in a scientific journal.

Project Management, Evaluation, and Outreach

William R. Horwath. Dr. Horwath will be primarily responsible for all analytical procedures, field work, data analysis, reporting, development of guidelines for the 24 hr CO_2 Soil Test and assessment of the procedure with soil test labs. Dr. Horwath has published numerous articles on agronomy and crop N use for numerous crops ranging from rice to grapes. Drs. Hanely and Brinton are collaborators on the project and will assist in experimental design and interpretation of results. In addition, Dr. Hanely will run complimentary analyses of soils between California and Texas. He will also test the commercial version of the 24 hr CO_2 soil test. Dr. Horwath will also be responsible for publishing the results for scientific journals.

Stuart Pettygrove and Jeffery Mitchell will work with us to obtain growers from growers. Dr. Pettygrove will work with dairies and Sacramento Valley farmers. Jeff Mitchell will work with farmers in the San Joaquin Valley. Dr. Pettygrove and Dr. Horwath will also write the news bulletin and extension article for the project.

The proposed work will be coordinated through regular meetings with Dr. Horwath and Dr. Burger and the requested post doc. A meeting with the collaborators is also anticipated, probably in year 3. In year three we will hold a meeting with the supporting soil test labs to discuss the feasibility of implementing the test as an industry protocol to introduce a biologically based soil test to assess growing season N mineralization (see below for supporting test labs). As data is collected and interpreted more meetings will be held to discuss the outreach and education components of the project (See **Task 6**). Dr. Horwath will be primarily responsible for coordinating these meetings and reporting of results.

Project Supporters

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Dellavalle Laboratory, Inc., 1910 W. McKinley Ave Suite #110, Fresno, CA 93728. http://dellavallelab.com/aboutus.html

Task 6 will begin July 2015 and end December 2015.

Evaluation

The potential usefulness of a "Quick Soil CO_2 Test" in evaluating contributions of soil N from soil biological activity and from soil amendments will be evaluated. We intend to evaluate a range (up to 100) of row and specialty crop soils that vary in fertilizer N requirements, inputs of soil amendments (crop residues and manures and composts) and other management such as crop rotation and tillage. The data will be used to develop correlations to other tests such as total soil N, total soil organic matter, pre-crop nitrate levels and total crop N uptake. We will cross compare soils with the USDA ARS in TX to incorporate more soils and for quality control. The success of the "Quick Soil CO_2 Test" will be determined by its good correlation with soil N mineralization potential and growing season crop N uptake. We will also evaluate the cost effectiveness of performing a rapid assay that offers a simple and rapid solution as opposed to the more complicated systems of biologically based soil incubations that commercial labs hesitate to adopt. Such a test if used by more soil labs and soil consultants could aid in more efficient fertilizer application rates and improve upon our understanding of the role of soil biological activity in fertility management.

Outreach See Task 6.

Budget justification 2013-2015

Category A. Personnel Expenses

Funds are requested for a 75% post doc ($30,000; with $6,120 benefits @ 20.4% benefit rate) for year one. The post doc salary request increases 5% annually with a combined increase in the benefit rate to 21.4% by year 3 for a total 3 year request of $94,575 in salary and $19, 846 in benefits. The post doc will perform the necessary analytical assays, field sampling and project management along with W. Horwath. An undergraduate student assistant to assist the post doc in both lab and field work is requested at $4,000 per year for a total of $12,000.

Category B. Operating Expenses including travel

Chemical reagents and miscellaneous lab and field supplies of $6,400 are requested for the duration of the project. These supplies are intended to support lab and field efforts for soil collection and prep, crop sampling and items needed for lab incubations. A request of $7,490 is required for lab incubations to determine potentially mineralizable C and N. This includes incubation containers, carrier gas and standards for gas chromatograph supplies, cuvettes for N analysis, reagents and expendable for dissolved carbon and nitrogen analysis, carrier gas and supplies for the elemental combustion analyzer, etc. Hazardous waste disposal ($810) is requested to dispose of hazardous waste generated from soil analysis and the elemental analyzer. Travel ($4,500) within year one and two is primarily for collecting of soil and crop biomass samples needed to conduct lab assays for potential C and N mineralization and to validate the results of the 24hr soil CO_2 test in relationship to crop yield. We expect to require 45 days of travel for initial soil sampling and for final crop biomass estimates at a cost of $100 per day during the first two years. The travel sites have yet to be determined. Travel ($850) in the third is requested for additional biomass estimates and outreach activities to be determined. We expect about 8 to 10 trips during the third year. Outreach activity ($3,500) is requested for publications and meetings with growers and soil test labs. A sum of $750 will be set aside to compensate soil test lab for running soil tests to validate the 24 hr soil CO_2 test and for quality control development.

Other funding sources

Dr. Horwath will contribute $8,544 annually for a total of $25, 632.

Total request $149,971

Total Budget $175,603

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