

Clean Water Act Section 319(h) Nonpoint Source Pollution Control Program

Efficient Nitrogen Fertilization: Accounting for Field Nitrogen Mineralization

***TSSWCB Project Number 08-04
Revision #3***

Quality Assurance Project Plan

Texas State Soil and Water Conservation Board

prepared by

Texas AgriLife Research
Texas Water Resources Institute

Effective Period: Upon Approval through August 2012
(with annual updates required)

Questions concerning this quality assurance project plan should be directed to:

Lucas Gregory
Quality Assurance Officer
Texas A&M AgriLife
Texas Water Resources Institute
2118 TAMU
College Station, Texas 77843-2118
lfgregory@ag.tamu.edu
(979) 845-7869

A1 APPROVAL PAGE

Quality Assurance Project Plan for *Efficient Nitrogen Fertilization: Accounting for Field Nitrogen Mineralization*.

United States Environmental Protection Agency (EPA), Region VI

Name: Curry Jones
Title: EPA Chief; State/Tribal Programs Section

Signature: _____ Date: _____

Name: Henry Brewer
Title: EPA Texas Nonpoint Source Project Manager

Signature: _____ Date: _____

Texas State Soil and Water Conservation Board (TSSWCB)

Name: Ashley Alexander
Title: TSSWCB Project Manager (PM)

Signature: _____ Date: _____

Name: Mitch Conine
Title: TSSWCB Quality Assurance Officer (QAO)

Signature: _____ Date: _____

United States Department of Agriculture – Agricultural Research Service (ARS)

Name: Rick Haney
Title: Soil Scientist; ARS Project Co-Lead / Lab Manager

Signature: _____ Date: _____

Name: Daren Harmel
Title: Agricultural Engineer; ARS Project Co-Lead / Project Manager

Signature: _____ Date: _____

Texas AgriLife Research, Texas Water Resources Institute (TWRI)

Name: Lucas Gregory
Title: TWRI Quality Assurance Officer (QAO)

Signature: _____ Date: _____

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A3 DISTRIBUTION LIST

Organizations, and individuals within, which will receive copies of the approved QAPP and any subsequent revisions include:

**U.S. Environmental Protection Agency Region 6
1445 Ross Avenue, Suite # 1200; Dallas, TX 75202-2733**

Name: Henry Brewer
Title: Texas NPS Project Manager, Water Quality Division

**Texas State Soil and Water Conservation Board (TSSWCB)
P.O. Box 658; Temple, Texas 76503**

Name: Ashley Alexander
Title: TSSWCB PM

Name: Mitch Conine
Title: TSSWCB QAO

**U.S. Department of Agriculture – Agricultural Research Service (ARS)
808 Blackland Rd., Temple, TX 76502**

Name: Rick Haney
Title: ARS Project Co-Lead / Lab Manager

Name: Daren Harmel
Title: ARS Project Co-Lead / Project Manager

**Texas AgriLife Research, Texas Water Resources Institute (TWRI)
2118 TAMU; College Station, TX 77843-2118**

Name: Lucas Gregory
Title: TWRI QAO

List of Acronyms

ACS	American Chemical Society
ARS	U.S. Department of Agriculture – Agricultural Research Service
CAR	Corrective Action Report
CD	Compact Disk
CO ₂ -C	Carbon Dioxide - Carbon
COC	Chain of Custody
DQO	Data Quality Objectives
EPA	Environmental Protection Agency
Lbs	Pounds
MDL	Method detection limit
N	Nitrogen
NH ₄ -N	Ammonium - Nitrogen
NIST	National Institute of Standards and Technology
NO ₃ -N	Nitrate - Nitrogen
PM	Project Manager
PO ₄ -P	Phosphate - Phosphorus
QA	Quality Assurance
QC	Quality Control
QAO	Quality Assurance Officer
QAPP	Quality Assurance Project Plan
RPD	Relative Percent Difference
S.D.	Standard Deviation
SOPs	Standard Operating Procedures
ST	Total Samples
SV	Valid Samples
TAMU	Texas A&M University
TCEQ	Texas Commission on Environmental Quality
TSSWCB	Texas State Soil and Water Conservation Board
TWRI	Texas Water Resources Institute

A4 PROJECT/TASK ORGANIZATION

The following is a list of individuals and organizations participating in the project with their specific roles and responsibilities:

U.S. Environmental Protection Agency Region 6

Henry Brewer, EPA Project Officer

Responsible for managing the project for EPA. Reviews project progress and reviews and approves QAPP and QAPP amendments.

Texas State Soil and Water Conservation Board (TSSWCB)

Ashley Alexander, TSSWCB Project Manager

Responsible for ensuring that the project delivers data of known quality, quantity, and type on schedule to achieve project objectives. Provides the primary point of contact between ARS and the TSSWCB. Tracks and reviews deliverables to ensure that tasks in the work plan are completed as specified in the contract. Notifies the TSSWCB QAO of significant project nonconformances and corrective actions taken as documented in quarterly progress reports from the ARS Project Co-Lead / Project Manager.

Mitch Conine, TSSWCB Quality Assurance Officer

Reviews and approves QAPP and any amendments or revisions and ensures distribution of approved/revised QAPPs to TSSWCB participants. Responsible for verifying that the QAPP is followed by ARS. Assists the TSSWCB Project Manager on QA-related issues. Coordinates reviews and approvals of QAPPs and amendments or revisions. Conveys QA problems to appropriate TSSWCB management. Monitors implementation of corrective actions. Coordinates and conducts audits

United States Department of Agriculture – Agricultural Research Service (ARS)

Rick Haney, ARS Project Co-Lead / Lab Manager

Manage Temple and private landowner demonstration sites, conduct soil test analysis, assist with soil sample collection, and make fertilizer recommendations. Responsible for supervision of laboratory personnel involved in generating analytical data for the project. Responsible for ensuring that laboratory personnel involved in generating analytical data have adequate training and thorough knowledge of the QAPP and all SOPs specific to the analyses or task performed. Responsible for oversight of all laboratory operations ensuring that all QA/QC requirements are met, documentation related to the analysis is complete and adequately maintained, and that results are reported accurately. Responsible for ensuring that corrective actions are implemented, documented, reported and verified. Monitors implementation of the measures within the laboratory to ensure complete compliance with project data quality objectives in the QAPP. Conducts in-house audits to ensure compliance with written SOPs and identify potential problems.

Daren Harmel, ARS Project Co-Lead / Project Manager

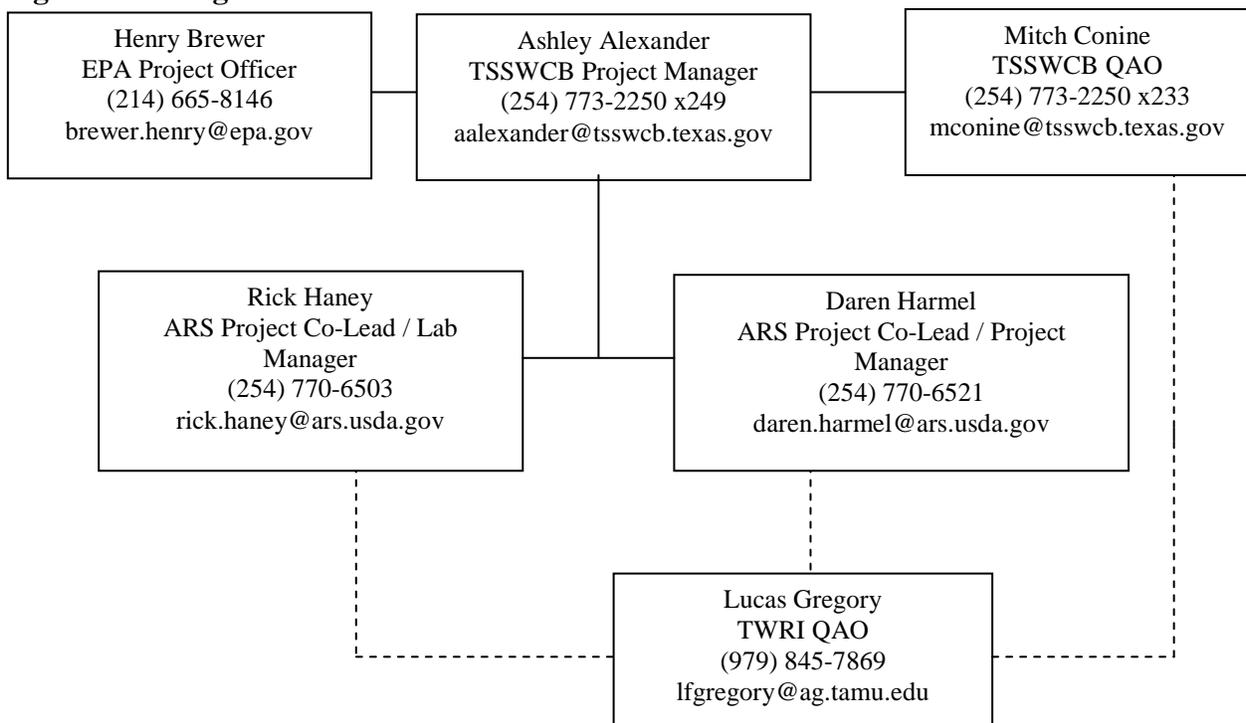
Manage Riesel demonstration sites and assist with soil sample collection. Responsible for reporting. Responsible for ensuring that tasks and other requirements in the contract are executed on time and with the quality assurance/quality control requirements in the system as defined by the contract and in the project QAPP; assessing the quality of subcontractor/participant work; and submitting accurate and timely deliverables to the TSSWCB Project Manager. Responsible for ensuring adequate training and supervision of all activities involved in generating analytical and field data.

Texas AgriLife Research, Texas Water Resources Institute (TWRI)

Lucas Gregory, TWRI Quality Assurance Officer (QAO)

Responsible for coordinating the development and implementation of the project’s QA program including writing, maintaining and distributing the QAPP and any appendices and amendments, and monitoring its implementation. Responsible for identifying, receiving, and maintaining project quality assurance records; coordinating with the TSSWCB to resolve QA-related issues; and notifying the ARS Project Co-Leads and TSSWCB Project Manager of particular circumstances which may adversely affect the quality of data. Coordinates the research and review of technical QA material and data related to water quality monitoring system design and analytical techniques. Responsible for the facilitation of audits and the implementation, documentation, verification and reporting of corrective actions. Provides copies of QAPP and any amendments or revisions to each project participant.

Figure A4.1 Organization Chart



A5 PROBLEM DEFINITION/BACKGROUND

The Texas Nonpoint Source Management Program (TCEQ and TSSWCB, 2005) states that “Nutrients, pesticides, and other pollutants can come from a variety of sources including over-fertilized fields, runoff from improperly managed animal operations and waste applications, inaccurate pesticide sprayer settings, and dozens of other sources.” This project is directly aimed at reducing the potential for overapplying nitrogen (N) fertilizer based on current soil test methodology in Texas.

Traditional soil nitrogen tests determine only the inorganic N in soil in the form of $\text{NO}_3\text{-N}$, but fail to account for plant available $\text{NH}_4\text{-N}$, plus a mineralizable portion of the soil organic N pool. Organic matter in the soil provides plant-available N when soil microbes mineralize organic C. Since organic C and organic N are highly linked, organic N is broken down to plant available N. This very important component of soil microbiology has been traditionally under-appreciated because of the difficulty of accurately assessing mineralization with lab techniques, especially its contribution to providing N to enhance crop production. Since traditional soil tests do not recognize the contribution of available $\text{NH}_4\text{-N}$ or mineralizable soil N in the estimation of plant available N, current soil test recommendations are often higher than necessary, which result in overapplication of N fertilizer.

This excess application increases N inputs into Texas rivers and lakes, which can accelerate eutrophication and substantially increase water treatment costs. Excess N in the Mississippi River, some of which is contributed by Texas watersheds, contributes to a major environmental problem - Gulf of Mexico hypoxia. Steve DiMarco, a Texas A&M researcher, has also recently claimed the existence of a Texas Gulf Coast hypoxic zone. Such hypoxic (low oxygen) areas are absent of most marine life and threaten to inexorably damage important ecosystems.

The Texas Commission on Environmental Quality (TCEQ) is currently in the process of revising Texas Surface Water Quality Standards for the 2009 triennial review. Major revisions to the Standards are being drafted, including the establishment of numeric nutrient criteria for reservoirs and modifications to contact recreation use and bacteria criteria. Numeric nutrient criteria will also be established for major rivers and small streams over the next decade. As a result numerous Texas water bodies which currently have concerns for nutrients will likely be impaired once the nutrient criteria are adopted.

In addition to adverse environmental effects, excess N fertilizer application increases input costs for agricultural producers. Overapplying N fertilizer wastes money on unnecessary inputs and reduces profitability. The problem is that traditional soil test procedures and resulting recommendations fail to account for mineralizable N in the soil that is released and made plant available. Thus, farmers do not knowingly apply excess N; they apply at the recommended N rates. The issue lies with fertilizer recommendations based on conventional soil test results.

Although agriculture is not the only contributor to the problem of excess N in our Nation's waters, agriculture should do its part to reduce N loading. Basing fertilizer application rates on

soil tests that more accurately account for the total amount of plant available N in the soil, including mineralizable N, could have tremendous socio-economic and environmental benefits.

The innovative soil test methodology, demonstrated in this project, represents an important agronomic advancement with the potential for major socio-economic and environmental benefits. The environment will benefit as less N will be introduced into streams and rivers. Similarly, input costs will decrease as N fertilizer inputs are reduced. The cost savings should result in increased profitability. The economic incentive associated with the enhanced soil test methodology will increase the broadscale adoption of the methodology by laboratories and landowners alike and thus measurable improvements in runoff water quality. Additional benefits of reduced N application include reduced market demand for N thereby reducing petroleum inputs required to generate N fertilizer.

A6 PROJECT/TASK DESCRIPTION

Current soil test procedures and fertilizer N recommendations will be adjusted in this project by the inclusion of $\text{NH}_4\text{-N}$ analysis and a new method (1-day $\text{CO}_2\text{-C}$), which uses soil microbial activity to rapidly estimate N mineralization. Since the majority of soil nutrients are cycled through the soil microbial biomass, testing soil microbial activity provides an excellent snapshot of the soil health prior to fertilization. Over many years of research, this method has reliably separated soils by their fertility. The more fertile the soil, the more $\text{CO}_2\text{-C}$ produced in a 24 hour period. Consequently, microbial ability to mineralize N from organic N is linked to the fertility of a given soil.

The current project will demonstrate this enhanced soil test methodology that accounts for all sources of plant available N in the soil, including $\text{NO}_3\text{-N}$, available $\text{NH}_4\text{-N}$, and mineralizable N (Task 5). These soil N sources provide N to crops and represent N that is not adequately accounted for by producers. The project will demonstrate the potential for reduced N runoff due to reduced N application based on this soil test methodology by establishing demonstration sites on research facilities (Tasks 2, 3) and on private land (Task 4). Crop yield, economic throughput, fertilizer cost, and water quality data (Task 6) data will be presented (Task 7) at local and national producer and scientific meetings.

This project is based on the principle that voluntary, practical, and cost-efficient management alternatives can effectively solve nonpoint source problems. Substantial producer buy-in (Tasks 4, 7) is expected based on the potential for increased profitability when using the improved plant available N methodology to adjust N fertilizer recommendations. The practical nature of this enhancement should also appeal to producers; it will simply result in less fertilizer N applied.

A 20-50% reduction in agricultural fertilizer use would have been unthinkable without recent increases in fuel and fertilizer costs. However, dramatic increases in input costs have now forced farmers to consider input costs. Prior to recent increases, fuel and fertilizer costs were relatively low. As a result, farmers assumed that maximizing yield maximized profit and thus applied N fertilizer at rates to ensure N deficiency did not limit yields. In the current economic climate, a more appropriate strategy for maximizing profit and maintaining productivity is balancing input costs with expected yield and commodity prices. This project will demonstrate an innovative soil test methodology for achieving this balance.

Through a separate project, not funded by this or other Clean Water Act §319(h) funds, the water quality impacts of reduced N fertilizer application on demonstration sites (Tasks 3) will be evaluated. Storm and baseflow water quality samples will be collected from USDA-ARS watersheds in Riesel and analyzed for $\text{NO}_3\text{-N}$, $\text{NH}_4\text{-N}$, and $\text{PO}_4\text{-P}$. An expected 10-20% reduction in N runoff will be evaluated with these corroboratory data (Task 3).

In order to produce results in a timely manner, the project will follow the timeline described in Table A6.1.

Table A6.1. Project Plan Milestones

Task	Project Milestones	Agency	Start	End
1.1	Prepare & submit quarterly reports to TSSWCB & participants	ARS	09/08	08/12
1.2	Perform accounting functions	ARS	09/08	08/12
1.3	Host coordination meetings with TSSWCB & partners	ARS	09/08	08/12
1.4	Develop project final report	ARS	03/11	08/12
2.1	Establish 10 demo sites at Temple	ARS	01/09	08/12
2.2	Gather land mgt, crop yield, and economic data on demonstration sites	ARS	01/09	03/12
2.3	Collect annual soil samples on Temple demonstration sites	ARS	01/09	03/12
3.1	Establish 8 demonstration sites at Riesel	ARS	01/09	08/12
3.2	Gather land mgt, crop yield, and economic data on demonstration sites	ARS	01/09	03/12
3.3	Collect annual soil samples on Riesel demonstration sites	ARS	01/09	03/12
3.4	Collect and analyze runoff from Riesel demonstration sites	ARS	01/09	03/12
4.1	Establish 10-20 demonstration sites on private land	ARS	01/09	08/12
4.2	Gather land mgt, crop yield, and economic data on demonstration sites	ARS	01/09	03/12
4.3	Collect annual soil samples on private land demonstration sites	ARS	01/09	03/12
4.4	Compensate cooperators for demonstration site establishment	ARS	01/09	08/12
5.1	Soil processing and testing	ARS	01/09	07/12
5.2	Comparison of N soil test methods	ARS	01/09	08/12
6.1	Develop QAPP	TWRI	09/08	12/08
6.2	QAPP Annual Revisions	TWRI	01/09	08/12
6.3	Develop and maintain project website	TWRI	03/09	08/12
7.1	Conduct field days at demonstration sites	ARS	03/09	08/12
7.2	Make presentations at scientific meetings	ARS	09/08	08/12
7.3	Make presentations at producer meetings	ARS	09/08	08/12
7.4	Prepare refereed publication	ARS	10/10	08/12

A7 QUALITY OBJECTIVES AND CRITERIA

The project objective is to demonstrate (1) enhanced soil test methodology that accounts for all sources of plant available N in the soil, including NO₃-N, available NH₄-N, and mineralizable N and (2) the potential for reduced N runoff resulting from the reduced N application based on this soil test methodology. The measurement performance specifications to support the project objective are specified in Table A7.1. Laboratory Measurement Quality Control Requirements and Acceptability Criteria are provided in Section B5.

Table A7.1. Measurement Performance Specifications

Parameter	Units	Extractant	Analysis Method	Method Reference	Reproducibility Limits ⁵	Precision Limits ⁶	Percent Complete
Plant available phosphate	lbs P ₂ O ₅ / acre	H ³ A	ICP	Haney et al. 2006 ¹	1 S.D. Mean	RPD<10%	90%
1-day CO ₂ -C	N/A	N/A	Solvita	Haney et al. 2008 ²	1 S.D. Mean	RPD<10%	90%
Mineralizable N	lbs N / acre	N/A	0.5 x 1-day CO ₂ -C	Haney et al. 2001 ³	1 S.D. Mean	RPD<10%	90%
Total Inorganic N NO ₃ -N+NH ₄ -N	lbs N / acre	H ³ A	RFA	Haney et al. 2006 ¹	1 S.D. Mean	RPD<10%	90%
Total N	lbs N / acre	N/A	Mineralizable N + Total Inorganic N	Haney et al. 2004 ⁴	1 S.D. Mean	RPD<10%	90%

¹ Haney, R.L., E.B. Haney, L.R. Hossner, and J.G. Arnold. 2006. Development of a new soil extractant for simultaneous phosphorus, ammonium, and nitrate analysis. *Communications in Soil Science and Plant Analysis*, 37: 1511-1523, 2006. (Appendix C1)

² Haney, R.L., W.F. Brinton, and E. Evans. 2008. Soil CO₂ respiration: comparison of chemical titration, CO₂ IRGA analysis and the Solvita gel system. *Renewable Agriculture and Food Systems*: 23(2); 171-176. (Appendix C2)

³ Haney R.L., F.M. Hons, M.A. Sanderson, and A.J. Franzluebbers. 2001. A rapid procedure for estimating nitrogen mineralization in manured soil. *Biol. Fertil Soils* (2001) 33:100-104. (Appendix C3)

⁴ Haney R.L., A.J. Franzluebbers, E.B. Porter, F.M. Hons, and D.A. Zuberer. 2004. Soil carbon and nitrogen mineralization: influence of drying temperature. *Soil Sci. Soc. Am. J.* 68:489-492 (2004). (Appendix C4)

⁵ 1 S.D. Mean = standard deviation of laboratory check sample means where mean value that will be obtained from the continued analysis of laboratory check samples

⁶ RPD = relative percent deviation

Precision

The precision of data is a measure of the reproducibility of a measurement when an analysis is repeated. It is strictly defined as the degree of mutual agreement among independent measurements as the result of repeated application of the same process under similar conditions. The precision of all analyses will be determined by analyzing a standard laboratory check sample once per batch or once per 30 samples, whichever is the greater frequency. The laboratory check sample will be subjected to analytical steps subjected to the unknown samples. Relative percent difference (RPD) of duplicate analyses (X₁ and X₂) will be calculated with the formula with the precision limits indicated in Table A7-1:

$$\text{Relative Percent Difference} = \frac{(X_1 - X_2)}{(X_1 + X_2)/2} \times 100\%$$

Accuracy

Accuracy is the degree of conformity with a standard. Accuracy relates to the quality of a result, and is distinguished from precision, which relates to the quality of the operation by which the result is obtained. The relative accuracy of the analytical process will be monitored via comparison of the laboratory check sample(s) data. This differs from traditional accuracy assessments since there is no proper procedure for spiking soil to add a known to the sample. Due to the inherent heterogeneity of soil, a variety of reactions can occur, making accuracy difficult to determine. These reactions may include precipitation, anion exchange, and cation exchange. Instead, reproducibility will be used.

Reproducibility

Reproducibility will be determined by evaluation of a laboratory soil check sample within each sub-batch of 30 samples. Recovery of critical data for each check sample will be compared to the historic project data associated with the laboratory soil check sample. Values with greater than one standard deviation of the mean will be determined to be substandard and all extracted solutions between the previous acceptable laboratory soil check sample and the next acceptable laboratory soil check sample will be re-analyzed.

Database checks for validity will be performed on an on-going basis by the ARS Project Co-Lead / Lab Manager. Data will be reviewed for abnormalities or any unusual results. Any unusual results will be traced for error sources. In the event no error is found, the data will be assumed normal and appropriate for decision determinations. If an error is found and cannot be resolved, the raw samples will be prepared again and reanalyzed. If there is not sufficient raw sample for preparation, the data will be discarded based upon the decisions of the ARS Project Co-Leads and TWRI QAO.

Representativeness

Site selection and sampling of the soil using accepted sampling methods will assure that data represents the conditions at the site. Representativeness also depends on the number of samples taken to accurately reflect the technological effectiveness at a given site. In order to ensure that sufficient numbers of samples are collected to represent each field, the following minimum sample numbers will be employed:

- Fields <10 acres = A minimum of 10 cores (i.e. 1 core/acre minimum) will be composited into 1 sample for each experimental unit.
- Fields 10-100 acres = A minimum of 10-20 cores (i.e. 1-5 core/acre minimum) will be composited into 1 sample for each experimental unit.
- Fields >100 acres = A minimum of 20 cores will be composited into 1 sample for each experimental unit.

Comparability

Confidence in the comparability of data sets from this project to those for similar uses is based on the commitment of project staff to use only accepted sampling and analysis methods and QA/QC protocols in accordance with quality system requirements and as described in this QAPP and project SOPs (Appendix C). Comparability is also guaranteed by reporting data in standard units, by using accepted rules for rounding figures, and by reporting data in a standard format. The ARS Project Co-Leads will closely coordinate activities to ensure that proper protocols are utilized.

Completeness

Although 100 percent of collected data should be available, accidents, insufficient sample volume, or other problems must be expected. A goal of 90 percent data completeness will be required for data usage. Should less than 90 percent data completeness occur, the ARS Project Co-Leads will initiate corrective action. Data completeness will be calculated as a percent value and evaluated with the following formula:

$$\% \text{ completeness} = \frac{SV}{ST} \times 100$$

where: SV = number of samples with a valid analytical report
 ST = total number of samples collected

A8 SPECIAL TRAINING/CERTIFICATION

Laboratory analysts have a combination of experience, education, and training to demonstrate knowledge of their function. In addition, all personnel involved in sampling, sample analyses, and statistical analyses have received the appropriate education and training required to adequately perform their duties.

A9 DOCUMENTS AND RECORDS

Hard copies of all field data sheets, chain of custody forms (COCs), laboratory data files, field data entry sheets, and corrective action reports (CARs) will be archived by the ARS Project Co-Leads for at least five years from the end of the project. Instrument (general maintenance records) logs will be maintained by the ARS Project Co-Lead / Lab Manager. All electronic data are backed up on a DVD monthly and are simultaneously saved in an external network folder and the computer's hard drive. In addition, the ARS Project Co-Leads will archive electronic forms of all project data for at least five years from the end of the project. A CAR form is presented in Appendix A and a copy of a COC is presented in Appendix B.

Quarterly progress reports will note activities conducted in connection with the project's soil analyses, items or areas identified as potential problems, and any variations or supplements to the QAPP. CARs will be utilized when necessary. CARs will be maintained in an accessible location for reference at ARS. CARs that result in any changes or variations from the QAPP will be made known to pertinent project personnel and documented in an update or amendment to the QAPP. All quarterly progress reports and QAPP revisions will be distributed to personnel listed in Section A3. Finally, the TSSWCB may elect to take possession of records at the conclusion of the specified retention period.

QAPP Revision and Amendments

Until the work described is completed, this QAPP shall be revised as necessary and reissued annually on the anniversary date, or revised and reissued within 120 days of significant changes, whichever is sooner. The last approved versions of QAPPs shall remain in effect until revised versions have been fully approved; the revision must be submitted to the TSSWCB for approval before the last approved version has expired. If the entire QAPP is current, valid, and accurately reflects the project goals and the organization's policy, the annual re-issuance may be done by a certification that the plan is current. This will be accomplished by submitting a cover letter stating the status of the QAPP and a copy of new, signed approval pages for the QAPP.

QAPP amendments may be necessary to reflect changes in project organization, tasks, schedules, objectives and methods; address deficiencies and nonconformances; improve operational efficiency; and/or accommodate unique or unanticipated circumstances. Written requests for amendments are directed from the TWRI QAO to the TSSWCB PM and are effective immediately upon approval by the TSSWCB PM and QAO. Amendments to the QAPP and the reasons for the changes will be documented and distributed to all individuals on the QAPP distribution list by the TWRI QAO. Amendments shall be reviewed, approved, and incorporated into a revised QAPP during the annual revision process.

B1 SAMPLING PROCESS DESIGN

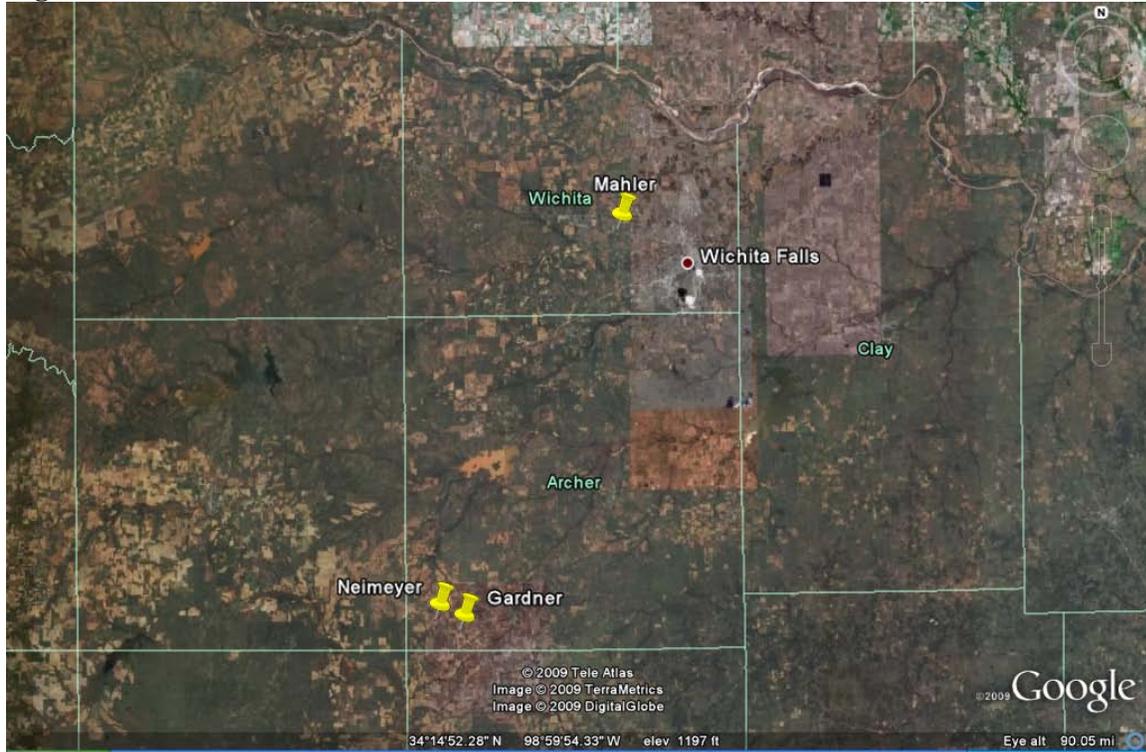
The primary project objective is to demonstrate enhanced soil test methodology that accounts for all sources of plant available N in the soil, including $\text{NO}_3\text{-N}$, available $\text{NH}_4\text{-N}$, and mineralizable N. The enhanced soil test methodology will be demonstrated by establishing demonstration sites on ARS research facilities at Riesel and Temple, as well as on private land. Table A7.1 lists the parameters to be tested. All parameters are considered “critical” to achieving the objectives of the project.

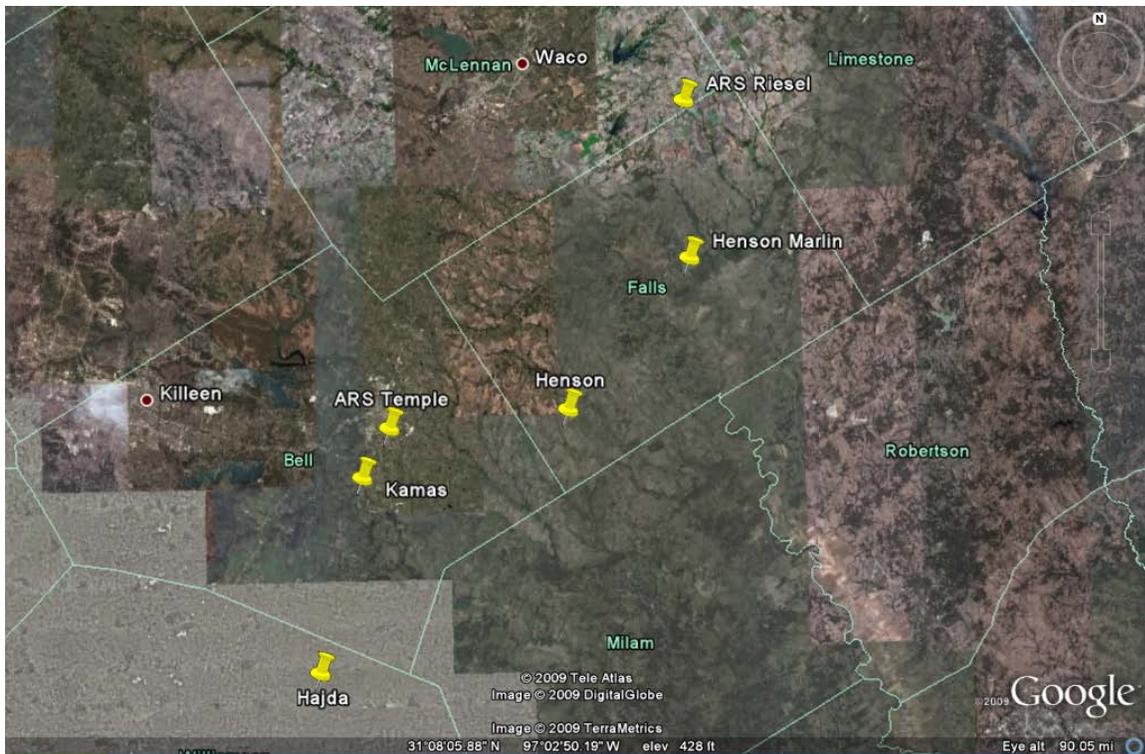
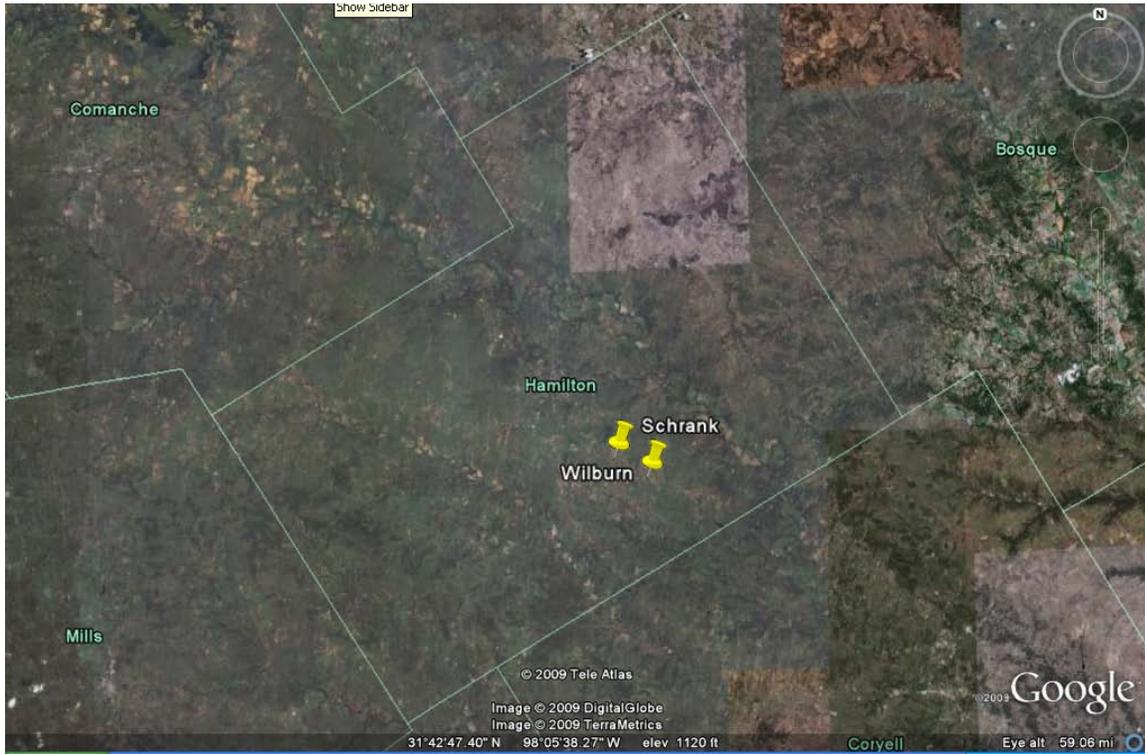
ARS will establish 10 demonstration sites, including 5 control sites, at the Temple ARS research facility to demonstrate the enhanced soil test method and its ability to predict plant available N resulting in reduced N application. On each site, tillage, weed and insect control, crop production, and fertilizer application including both organic and inorganic formulations, will be performed. The control sites will be treated the same as the other sites, except will receive no fertilizer. ARS will gather and record land management, crop yield, and economic data to demonstrate the economic benefits of reduced N application resulting from use of the enhanced soil test methodology. ARS will collect annual soil samples for testing to determine plant available N prior to fertilization. In addition, monthly soil samples may also be collected to track within year plant available N changes at selected sites.

ARS will establish 8 demonstration sites, including a control site, at the Riesel ARS research facility to demonstrate the enhanced soil test method and its ability to predict plant available N resulting in reduced N application. Riesel sites Y6, Y8, Y10, Y13, W12, W13, and SW16 will receive fertilization based on the enhanced soil test methodology. On each site, tillage, weed and insect control, crop production, and fertilizer application including both organic and inorganic formulations, will be performed. Site 7-1 at Riesel will serve as the control site and will be treated the same as the other sites, except it will receive no fertilizer. ARS will gather and record land management, crop yield, and economic data to demonstrate the economic benefits of reduced N application resulting from use of the enhanced soil test methodology. ARS will collect annual soil samples for testing to determine plant available N prior to fertilization. Also, monthly soil samples may be collected to track within year plant available N changes at selected sites.

ARS will establish 10-20 sites on private land to demonstrate the ability of the enhanced soil test method to determine plant available N. Cooperators will perform tillage, weed and insect control, fertilizer application, and crop production on demonstration sites. All cooperators will set up at least one control plot from which to determine plant available N contributed by the soil with no fertilizer addition. Cooperators may also choose to establish plots that will be fertilized with N rates based on the enhanced N soil test. Cooperators will gather and record land management and crop yield data for the demonstration sites. Cooperators or ARS personnel will collect annual soil samples for soil test analysis to determine plant available N.

Figure B1.1 Locations of Demonstration Sites





B2 SAMPLING METHODS

Soil samples will be collected at least annually from each demonstration site using a soil corer. Sampling protocol will involve removing any plant debris through moving, raking and etc.; collecting each soil core to a depth of 6 inches (15 cm); compositing the cores in a 5 gallon bucket; transferring a subsample of the composite to a Ziploc bag; and transporting the sample to the ARS Lab in Temple. In order to ensure that sufficient numbers of samples are collected to represent each field, the following minimum sample numbers will be employed:

- Fields <10 acres = A minimum of 10 cores (i.e. 1 core/acre minimum) will be composited into 1 sample for each experimental unit.
- Fields 10-100 acres = A minimum of 10-20 cores (i.e. 1-5 core/acre minimum) will be composited into 1 sample for each experimental unit.
- Fields >100 acres = A minimum of 20 cores will be composited into 1 sample for each experimental unit.

Recording Data

For the purposes of this section and subsequent sections, all field and laboratory personnel will: (1) write legibly in indelible, waterproof ink with no modifications, write-overs or cross-outs; (2) correct errors with a single line followed by an initial and date; and (3) close-out incomplete pages with an initialed and dated diagonal line.

Deviations from Sampling Method Requirements or Sample Design, and Corrective Action

Examples of deviations from sampling method requirements include inadequate sample volume collected, failure to preserve samples appropriately, contamination of sample bottle during collection, storage temperature and holding time exceedance, and sampling at the wrong site. Deviations invalidate resulting data and may require corrective action including samples being discarded and re-collected. It is the responsibility of the ARS Project Co-Leads and TWRI QAO to ensure that the actions and resolutions to the problems are documented and that records are maintained in accordance with this QAPP. In addition, these actions and resolutions will be conveyed to the TSSWCB PM both verbally and in writing in progress reports and by completion of a corrective action report (CAR) as shown in Appendix A. CARs will be included with project progress reports. In addition, significant conditions (i.e., situations which, if uncorrected, could have a serious effect on safety or on the validity or integrity of data) will be reported to the TSSWCB immediately both verbally and in writing.

B3 SAMPLE HANDLING AND CUSTODY

Chain-of-Custody

Proper sample handling and custody procedures ensure the custody and integrity of samples beginning at the time of sampling and continuing through transport, sample receipt, preparation, and analysis. The chain-of-custody (COC) form is used to document sample handling during transfer from the field to the laboratory. The sample number, location, date, changes in possession and other pertinent data will be recorded in indelible ink on the COC. The sample collector will sign the COC and transport it with the sample to the laboratory. At the laboratory, samples are inventoried against the accompanying COC. Any discrepancies will be noted at that time and the COC will be signed for acceptance of custody. In the instance that the field sample collector and laboratory sample processor are one in the same, a field-to-lab COC will be unnecessary. A copy of a blank COC form used on this project is included in Appendix B.

Sample Labeling

Samples will be labeled on the container with an indelible, waterproof marker. Label information will include site identification, date, sampler's initials, and time of sampling. The COC form will accompany all sets of sample containers.

Sample Handling

Following collection, samples will be transported to the laboratory and stored at ambient temperature until analysis. The ARS Project Co-Lead / Lab Manager has the responsibility to ensure that holding times are met with all samples. Any problem will be documented with a CAR.

Failures in Chain-of-Custody and Corrective Action

All failures associated with chain-of-custody procedures as described in this QAPP are immediately reported to the TWRI QAO and ARS Project Co-Leads. These include such items as delays in transfer, resulting in holding time violations; violations of sample preservation requirements; incomplete documentation, including signatures; possible tampering of samples; broken or spilled samples, etc. The TWRI QAO and ARS Project Co-Leads will determine if the procedural violation may have compromised the validity of the resulting data. Any failures that have reasonable potential to compromise data validity will invalidate data and the sampling event should be repeated. The resolution of the situation will be reported to the TSSWCB Project Manager in the project progress report. Corrective action reports will be prepared by the TWRI QAO and submitted to the TSSWCB Project Manager along with project progress report.

B4 ANALYTICAL METHODS

Within 2 weeks of arrival at the lab (and typically less than 1 week), each soil sample is dried at 40°C for 24-48 hours (depending on moisture level) and ground to pass through a 5-mm sieve. The parameters and analytical methods are listed in Table A7.1 and described in detail in Appendix C. In the event of a failure in the analytical system, the ARS Project Co-Leads will be notified. The TWRI Quality Assurance Officer and ARS Project Co-Leads will then determine if the existing sample integrity is intact, if re-sampling should and/or can be done, or if the data should be omitted.

Failures in Measurement Systems and Corrective Actions

Failures in field and laboratory measurement systems involve, but are not limited to such things as instrument malfunctions, failures in calibration, blank contamination, quality control samples outside QAPP defined limits, etc. In many cases, the field technician or lab analyst will be able to correct the problem. If the problem is resolvable by the field technician or lab analyst, then they will document the problem on the field data sheet or laboratory record and complete the analysis. If the problem is not resolvable, then it is conveyed to the ARS Project Co-Leads, who will make the determination in coordination with the TWRI QAO. If the analytical system failure may compromise the sample results, the resulting data will not be reported to the TSSWCB as part of this project. The nature and disposition of the problem is reported on the data report. The TWRI QAO will include this information in the CAR and the ARS Project Co-Lead / Project Manager will submit it with the Progress Report which is sent to the TSSWCB Project Manager.

B5 QUALITY CONTROL

The ARS lab will determine the precision of their analyses. Annual laboratory audits, sampling site audits, and quality assurance of field sampling methods will be conducted by the TSSWCB or their designee. Table B5.1 outlines the required analytical quality control for the parameters of interest. No spiked sample analyses will be performed in the course of this project due to the varied adsorptive capacities of different soil types in relation to the majority of elements being evaluated. Adding elements to soils would always yield varying returns due to the chemical properties of the soils. The spiking of soil samples risks precipitation of those parameters. Matrix blanks, and known standards not used in the calibration of the instrument, will be employed in place of spiked samples to insure accurate and proper recovery of each parameter. All standards with added concentrations of elements or compounds to be analyzed will be comprised of purchased NIST solutions whenever possible and practical. These matrix blanks and/or standards will be included in each batch of samples analyzed. Recovery of each parameter in the non-calibration standards must be within 10% of known value.

Table B5.1. Required Quality Control Analyses

Soil Parameters	Blank	Standard	Duplicate
Plant available phosphate	A	A	B
1-day CO ₂ -C	A	A	B
Mineralizable N	A	A	B
Total Inorganic N NO ₃ -N+NH ₄ -N	A	A	B
Total N	A	A	B

A - Where specified, blanks and standards shall be performed each day that samples are analyzed.

B - Where specified, duplicate analyses of the laboratory soil check sample extract shall be performed every 30 samples each day that samples are analyzed. At least two laboratory soil check samples will be extracted every day.

In the database, missing values will be left as blanks. The ARS Project Co-Leads will graphically screen data to highlight questionable data points. Questionable data will be traced through the COC forms, CARs, and, as necessary, through research laboratory notebooks and field data sheets to ensure that data are properly entered. Changes will be made only if an error is found in transcription into the database. Values determined to be below laboratory method detection limits (RFA 0.1 ppm; ICP 0.01 ppm; Solvita 0.5 ppm; C/N analyzer 0.1 ppm) will be noted in the comment column of the database and used in statistical analyses as one-half the method detection limit (MDL), as recommended by Gilliom and Helsel (1968) and Ward et al. (1988). Values that are greater than the upper method detection limit will be diluted and reanalyzed.

It is the responsibility of the ARS Project Co-Leads to verify that the data are representative. The use of peer reviewed sampling and analytical methods will ensure that measured data accurately represent field conditions. The data's precision, accuracy, and comparability generated in the ARS Lab will be the responsibility of the ARS Project Co-Lead / Lab Manager. The ARS Project Co-Leads also have the responsibility of determining that the 90 percent completeness criteria is met, or will justify acceptance of a lesser percentage.

Failures in Quality Control and Corrective Action

Corrective action will involve identification of the possible cause (where possible) of the contamination failure. Any failure that has potential to compromise data validity will invalidate data, and the sampling event should be repeated. The resolution of the situation will be reported to the TSSWCB in the quarterly progress report. The CAR's will be maintained by the ARS Project Co-Leads and the TSSWCB PM.

B6 INSTRUMENT/EQUIPMENT TESTING, INSPECTION AND MAINTENANCE

Manufacturers' recommendations for scheduling testing, inspection, and maintenance of each piece of equipment will be followed or exceeded. Maintenance and inspection logs will be kept on each piece of laboratory equipment. The ARS Project Co-Lead / Lab Manager will routinely review laboratory instrument logbooks for maintenance and operational irregularities.

To minimize downtime of all measurement systems, all field sampling equipment and laboratory equipment, must be maintained in a working condition. Also, backup equipment or common spare parts will be made available if any piece of equipment fails during use. This will ensure that repairs or replacements can be made quickly, allowing measurement tasks to be resumed. All staff who use chemicals, reagents, or equipment whose parts require periodic replacement and other consumable supplies receive instruction concerning the remaining quantity (unique for each supply) which should prompt a request to order additional supplies.

B7 INSTRUMENT/EQUIPMENT CALIBRATION AND FREQUENCY

All instruments or devices used in obtaining data will be used according to appropriate laboratory or field practices. Standards and purchased solutions used for instrument or method calibrations shall be of known purity and be National Institute for Standards and Testing (NIST) traceable whenever possible. When NIST traceability is not available, standards shall be of American Chemical Society (ACS) or reagent grade quality, or of the best attainable grade. All certified standards will be maintained traceable with certificates on file in the laboratory. Dilutions from all primary standards will be recorded in the standards log book and given unique identification numbers. The date, analyst initials, stock standards sources with lot number and manufacturer, and the dilution concentrations/ratios will also be recorded in the standards log book and be identified by a unique standards number which will also be placed on the standards bottle.

Calibrations for the ICP are performed with a minimum of four standards of increasing concentrations and a reagent blank. Standards shall not exceed the linear range of the instrument or method. Calibration shall be verified immediately after a set of standards is analyzed and continuously throughout an analytical run, every 44 samples, and at the end of an analysis to verify that the instrument or method has not drifted or changed more than 10% since calibration. The initial calibration verification and continuing calibration verification will be matched to the generated standard curve and screened for acceptability. If the values are not acceptable, the samples, within the group of 44 samples not passing, will be re-analyzed. Laboratory equipment and devices needing calibration and recalibration are numerous and varied. All equipment will have verifiable calibration documentation maintained and available for inspection in the laboratory. Laboratory standards will be checked to verify that the concentrations are those which are prescribed for the analytical method.

All instruments or devices used in obtaining data will be calibrated prior to use. Each instrument has a specialized procedure for calibration and a specific type of standard used to verify calibration. All calibration procedures will meet the requirements that are specified by the equipment manufacturer, as well as any instructions specified by applicable analytical methods. All information concerning required data calibration will be recorded in the project laboratory book by the person performing the calibration and will be accessible for verification during either a laboratory or field audit.

All calibration procedures used in the field or laboratory will meet or exceed the calibration frequencies published in the test methods used for this project. Additional calibration procedures may be conducted if laboratory personnel determine additional calibration is warranted as beneficial to this project. Instruments and laboratory equipment used in the analyses of these that require calibration prior to use will be calibrated before each day's analyses.

B8 INSPECTION/ACCEPTANCE OF SUPPLIES AND CONSUMABLES

All supplies and consumables received by ARS are inspected upon receipt for damage, missing parts, expiration date, and storage and handling requirements. Labels on reagents, chemicals, and standards are examined to ensure they are of appropriate quality, the packing slip is initialed by staff member and marked with receipt date. Volumetric glassware is inspected to ensure class "A" classification, where required.

B9 NON-DIRECT MEASUREMENTS

Through a separate project, not funded by this or other Clean Water Act §319(h) funds, the water quality impacts of reduced N fertilizer application on demonstration sites (Tasks 3) will be evaluated. Storm and baseflow water quality samples will be collected from USDA-ARS watersheds in Riesel and analyzed for $\text{NO}_3\text{-N}$, $\text{NH}_4\text{-N}$, and $\text{PO}_4\text{-P}$. An expected 10-20% reduction in N runoff will be evaluated with these corroboratory data (Task 3).

B10 DATA MANAGEMENT

Field Collection and Management of Routine Samples

All field collection will be completed as described in Section B2 of the QAPP. A field notebook is filled out in the field for each site visit. Samples collected will be labeled and transported to the laboratory. A COC form will be used. Site name, time of collection, comments, and other pertinent data are copied from the field notebook to the COC.

Laboratory Data

Once the samples are received at the ARS lab, samples are logged and stored at ambient temperature until processed. The COC will be checked for number of samples, proper and exact I.D. number, signatures, dates, and type of analysis specified. If any discrepancy is found, proper corrections will be made. The COC and accompanying sample bags/bottles are submitted to the ARS laboratory analyst, with relinquishing and receiving personnel both signing and dating the COC. All COC and soils data will be manually entered into an electronic spreadsheet. The electronic spreadsheet will be created in Microsoft Excel software on an IBM-compatible microcomputer with a Windows Operating System. The project spreadsheet will be maintained on the computer's hard drive, which is also simultaneously saved in a network folder. Data manually entered in the database will be reviewed for accuracy by the ARS Project Co-Leads to ensure that there are no transcription errors. Hard copies of data will be printed and housed in the laboratory for a period of five years. Any COC's and analysis records related to QA/QC of lab procedures will be housed at the ARS Lab. All pertinent data files will be backed up monthly on an external hard drive. Current data files will be backed up on an external hard drive monthly and stored in separate area away from the computer. Original data recorded on paper files will be stored for at least five years. Electronic data files will be archived to CD after approximately the end of the project, and then stored with the paper files for the remaining 4 years.

Data Validation

Following review of laboratory data, any data entry that is not representative of environmental conditions, because it was generated through poor field or laboratory practices, will not be submitted to the TSSWCB. This determination will be made by the ARS Project Co-Leads, TWRI QAO, TSSWCB QAO, and other personnel having direct experience with the data collection effort. This coordination is essential for the identification of valid data and the proper evaluation of that data. The validation will include the checks specified in Table D2.1.

Data Dissemination

At the conclusion of the project, the ARS Project Co-Leads will provide a copy of the complete project electronic spreadsheet via recordable CD to the TSSWCB PM, along with the final report. The TSSWCB may elect to take possession of all project records. However, summaries of the data will be presented in the final project report.

C1 ASSESSMENTS AND RESPONSE ACTIONS

The following table presents types of assessments and response actions for data collection activities applicable to the QAPP.

Table C1.1 Assessments and Response Actions

Assessment Activity	Approximate Schedule	Responsible Party	Scope	Response Requirements
Status Monitoring Oversight, etc.	Continuous	ARS Project Co-Lead / Project Manager	Monitoring of project status and records to ensure requirements are being fulfilled. Monitoring and review of laboratory performance and data quality	Report to TSSWCB in Quarterly Report. Ensure project requirements are being fulfilled.
Laboratory Inspections	Dates to be determined by TSSWCB QAO	TSSWCB QAO	Analytical and quality control procedures employed at laboratory	30 days to respond in writing to TSSWCB to address corrective actions
Monitoring Systems Audit	Dates to be determined by TSSWCB	TSSWCB QAO	Field sampling, handling and measurement; facility review; and data management as they relate to project	30 days to respond in writing to TSSWCB to address corrective actions

Corrective Action

The TWRI QAO and ARS Project Co-Leads are responsible for implementing and tracking corrective action procedures as a result of audit findings. Records of audit findings and corrective actions are maintained by the TSSWCB Project Manager and TWRI QAO. Corrective action documentation will be submitted to the TSSWCB Project Manager with the progress report. If audit findings and corrective actions cannot be resolved, then the authority and responsibility for terminating work is specified in agreements or contracts between participating organizations.

C2 REPORTS TO MANAGEMENT

Quarterly progress reports will be generated by ARS personnel and will note activities conducted in connection with the water quality monitoring program, items or areas identified as potential problems, and any variation or supplement to the QAPP. The CARs forms will be utilized when necessary and will be maintained in an accessible location for reference at ARS and TWRI. The CARs that result in changes or variations from the QAPP will be made known to pertinent project personnel, documented in an update or amendment to the QAPP and distributed to personnel listed in Section A3. Following any audit performed by the ARS or TSSWCB, a report of findings, recommendations and responses are sent to the TSSWCB Project Manager in the quarterly/monthly progress report.

Field measurements and all sampling for the project will be done according to the QAPP. However, if the procedures and guidelines established in this QAPP are not successful, corrective action is required to ensure that conditions adverse to quality data will be identified promptly and corrected as soon as possible. Corrective actions include identification of root causes of problems and successful correction of identified problems. The CARs will be filled out to document the problems and the remedial action taken.

Laboratory data reports contain the results of all analyses, as well as specified QC measures listed in section B5. This information is reviewed by the ARS Project Co-Leads and compared to the pre-specified acceptance criteria to determine acceptability of data. This information is available for inspection by the TSSWCB.

D1 DATA REVIEW, VERIFICATION AND VALIDATION

All data obtained from field and laboratory measurements will be reviewed and verified for conformance to project requirements, and then validated against the data quality objectives which are listed in Section A7. Only those data which are supported by appropriate quality control data and meet the data quality objectives defined for this project will be considered acceptable. This data will be submitted to the TSSWCB.

The procedures for verification and validation of data are described in Section D2, below. The ARS Project Co-Leads are responsible for ensuring that field data are properly reviewed and verified for integrity. The ARS Project Co-Lead / Lab Manager is responsible for ensuring that laboratory data are scientifically valid, defensible, of acceptable precision and accuracy, and reviewed for integrity. The ARS Project Co-Lead / Project Manager will be responsible for ensuring that all data are properly reviewed and verified, validated, and submitted in the required format as described by the TSSWCB Project Manager. Finally, the TWRI QAO is responsible for validating that all data to be reported meet the objectives of the project and are suitable for reporting to TSSWCB.

D2 VERIFICATION AND VALIDATION METHODS

All field and laboratory data will be reviewed, verified and validated to ensure they conform to project specifications and meet the conditions of end use as described in Section A7. The staff and management of the respective field, laboratory, and data management tasks are responsible for the integrity, validation and verification of the data each task generates or handles throughout each process. The field and laboratory tasks ensure the verification of raw data, electronically generated data, and data on chain-of-custody forms and hard copy output from instruments.

Verification, validation and integrity review of data will be performed using self-assessments and peer review, as appropriate, followed by technical review by the ARS Project Co-Leads. The data to be verified (listed by task in Table D2.1) are evaluated against project specifications (Section A7) and are checked for errors, especially errors in transcription, calculations, and data input. Potential outliers are identified by examination for unreasonable data. If a question arises or an error or potential outlier is identified, the manager of the task responsible for generating the data is contacted to resolve the issue. Issues which can be corrected are corrected and documented electronically or by initialing and dating the associated paperwork. If an issue cannot be corrected, the ARS Project Co-Leads consult with the TWRI QAO and TSSWCB PM to establish the appropriate course of action, or the data associated with the issue are rejected.

The ARS Project Co-Leads, with assistance from the TWRI QAO, are responsible for validating that the verified data are scientifically valid, legally defensible, of known precision, accuracy, integrity, meet the data quality objectives of the project, and are reportable to TSSWCB. One element of the validation process involves evaluating the data for anomalies. The ARS Project Co-Leads may designate other experienced experts to perform this evaluation. Any suspected errors or anomalous data must be addressed by the manager of the task associated with the data, before data validation can be completed.

A second element of the validation process is consideration of any findings identified during the monitoring systems audit conducted by the TWRI QAO or TSSWCB QAO assigned to the project. Any issues requiring corrective action must be addressed, and the potential impact of these issues on previously collected data will be assessed. Finally, the ARS Project Co-Leads validate that the data meet the data quality objectives of the project and are suitable for reporting to the TSSWCB.

Table D2.1. Data Verification Procedures

Data to be Verified	TWRI QAO	ARS Project Co-Leads	TSSWCB PM/QAO
Analysis techniques consistent with SOPs and QAPP	X	X	X
Instrument calibration data complete	X	X	X
Bacteriological records complete		X	X
Sample documentation complete	X	X	X
Sample identifications	X	X	X
Chain of custody complete/acceptable	X	X	X
Sample preservation and handling	X	X	X
Holding times	X	X	X
QC samples analyzed at required frequencies		X	X
QC samples within acceptance limits		X	X
Instrument readings/printouts	X	X	X
Calculations	X	X	X
Laboratory data verification for integrity, precision, accuracy, and validation		X	X
Laboratory data reports		X	X
Data entered in required format	X	X	X
Site ID number assigned			X
Absence of transcription error	X	X	X
Reasonableness of data	X	X	X
Electronic submittal errors	X	X	X
Sampling and analytical data gaps	X	X	X

D3 RECONCILIATION WITH USER REQUIREMENTS

Data produced by this project will be evaluated against the established DQOs and user requirements to determine if any reconciliation is needed. Reconciliation concerning the quality, quantity or usability of the data will be reconciled with the user during the data acceptance process. Corrective Action Reports will be initiated in cases where invalid or incorrect data have been detected. Data that have been reviewed, verified, and validated will be summarized for their ability to meet the data quality objectives of the project and the informational needs of decision-makers and cooperators.

The final data for the project will be reviewed to ensure that it meets the requirements as described in this QAPP. Data summaries along with descriptions of any limitations on data use will be included in the final report. Only data that has met the data quality objectives described in this QAPP will be reported and included in the final project report. Data and information produced thru this project will be used to demonstrate enhanced soil testing methodology that accounts for all sources of plant available N in the soil, including $\text{NO}_3\text{-N}$, available $\text{NH}_4\text{-N}$, and mineralizable N. Ultimately, producers will use the information produced by this project for determining proper fertilizer needs and reduce nutrient runoff from their fields and pastures.

APPENDIX A. CORRECTIVE ACTION REPORT

Corrective Action Report

CAR #: _____

Date: _____

Area/Location: _____

Reported by: _____

Activity: _____

State the nature of the problem, nonconformance, or out-of-control situation:

Possible causes:

Recommended corrective action:

CAR routed to: _____

Received by: _____

Corrective Actions taken:

Has problem been corrected? YES NO

ARS Project Co-Lead / Lab Manager: _____

ARS Project Co-Lead / Project Manager: _____

TWRI Quality Assurance Officer: _____

APPENDIX B. CHAIN-OF-CUSTODY FORM

USDA-ARS LAB CHAIN OF CUSTODY RECORD

Project Name:					# of containers	Analyses Required											Sample ID	
Station ID	Date	Time (24hr)	Matrix	Description														
Relinquished by: (Signature)			Date:	Time:	Received by: (Signature)			Date:	Time:	Laboratory remarks:								
Relinquished by: (Signature)			Date:	Time:	Received by: (Signature)			Date:	Time:									
Relinquished by: (Signature)			Date:	Time:	Received for lab by: (Signature)			Date:	Time:	Laboratory Name: ARS Lab								

APPENDIX C.

Standard Operating Procedures

- C1: Development of a new soil extractant for simultaneous phosphorus, ammonium, and nitrate analysis¹
- C2: Soil CO₂ respiration: comparison of chemical titration, CO₂ IRGA analysis and the Solvita gel system²
- C3: A rapid procedure for estimating nitrogen mineralization in manured soil³
- C4: Soil carbon and nitrogen mineralization: influence of drying temperature⁴

¹ Haney, R.L., E.B. Haney, L.R. Hossner, and J.G. Arnold. 2006. Development of a new soil extractant for simultaneous phosphorus, ammonium, and nitrate analysis. *Communications in Soil Science and Plant Analysis*, 37: 1511-1523, 2006.

² Haney, R.L., W.F. Brinton, and E. Evans. 2008. Soil CO₂ respiration: comparison of chemical titration, CO₂ IRGA analysis and the Solvita gel system. *Renewable Agriculture and Food Systems*: 23(2); 171-176.

³ Haney R.L., F.M. Hons, M.A. Sanderson, and A.J. Franzluebbbers. 2001. A rapid procedure for estimating nitrogen mineralization in manured soil. *Biol. Fertil Soils* (2001) 33:100-104.

⁴ Haney R.L., A.J. Franzluebbbers, E.B. Porter, F.M. Hons, and D.A. Zuberer. 2004. Soil carbon and nitrogen mineralization: influence of drying temperature. *Soil Sci. Soc. Am. J.* 68:489-492 (2004).

Appendix C1

Development of a new soil extractant for simultaneous phosphorus, ammonium, and nitrate analysis

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Development of a New Soil Extractant for Simultaneous Phosphorus, Ammonium, and Nitrate Analysis

R. L. Haney

United States Department of Agriculture, Agricultural Research Service,
Temple, Texas, USA

E. B. Haney

Railroad Commission of Texas, Surface Mining and Reclamation
Division, Austin, Texas, USA

L. R. Hossner

Department of Soil & Crop Sciences, Texas A&M University, Texas
Agricultural Experiment Station, College Station, Texas, USA

J. G. Arnold

United States Department of Agriculture, Agricultural Research Service,
Temple, Texas, USA

Abstract: A new soil extractant (H^3A) with the ability to extract NH_4 , NO_3 , and P from soil was developed and tested against 32 soils, which varied greatly in clay content, organic carbon (C), and soil pH. The extractant (H^3A) eliminates the need for separate phosphorus (P) extractants for acid and calcareous soils and maintains the extract pH, on average, within one unit of the soil pH. The extractant is composed of organic root exudates, lithium citrate, and two synthetic chelators (DTPA, EDTA). The new soil extractant was tested against Mehlich 3, Olsen, and water for extractable P, and 1M KCl and water-extractable NH_4 and NO_2/NO_3 . The pH of the extractant after adding soil, shaking, and filtration was measured for each soil sample (5 extractants \times 2 reps \times 32 soils = 320 samples) and was shown to be

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Address correspondence to R. L. Haney, United States Department of Agriculture,
Agricultural Research Service, 808 E. Blackland Road, Temple, Texas 76502, USA.
E-mail: rhaney@spa.ars.usda.gov

highly influential on extractable P but has no effect on extractable NH_4 or NO_2/NO_3 . H^3A was highly correlated with soil-extractable inorganic N (NH_4 , NO_2/NO_3) from both water ($r = 0.98$) and 1 M KCl ($r = 0.97$), as well as being significantly correlated with water ($r = 0.71$), Mehlich 3 ($r = 0.83$), and Olsen ($r = 0.84$) for extractable P.

Keywords: Chelate, extractant pH, Mehlich 3, Olsen, soil extractant, soil pH

INTRODUCTION

Soil nutrient data from soil testing and research laboratories is a valuable tool available to producers and research scientists. Currently, soil test procedures require at least two soil extractants to analyze for ammonium (NH_4), nitrate (NO_2)/nitrite (NO_3), and phosphorus (P). Generally, 1–2 M KCl or water is used for $\text{NH}_4/\text{NO}_2/\text{NO}_3$, and Bray, Mehlich 3, and Olsen for P, although others can be used depending on soil type, soil pH, and climatic conditions. The use of a single extractant would increase laboratory productivity and decrease analysis cost. Few of the soil extractants currently available are capable of multinutrient extraction without sacrificing accuracy for one nutrient or another (Holford 1980). Soils are highly variable and complex; therefore, developing a multinutrient extractant that does an acceptable job of accurately identifying plant-available nutrients is difficult and time consuming. However, the need for such an extractant does exist.

Mehlich 3 is currently a popular multinutrient extractant because of its ability to extract a number of nutrients (with the exceptions of ammonium and nitrate/nitrite) and was primarily developed for neutral to acid soils (Mehlich 1984). The Olsen extractant was developed primarily for calcareous soils (Olsen et al. 1954).

An extractant that has the ability to extract nutrients near the soil pH is a desirable trait because soil pH and P solubility are highly interrelated (Golterman 1998, Sharply 1993). After a literature review, we decided that a good soil extractant would mimic the soil environment that has actively growing roots, because the target for fertilizer recommendations is plant yield. While it would be impossible to understand all the processes that occur in the rhizosphere, we chose to focus on plant root exudates to develop a soil extractant. Plants have the ability to deliver organic exudates to the soil solution to acquire necessary nutrients (Rengel 2002; Baudoin, Benizri, and Guckert 2003). Under certain conditions, plants can increase production of root exudates to overcome nutrient deficiencies such as phosphorus, iron, zinc, and manganese (Azaizeh et al. 1995; Rengal 1997; Subbarao, Ae, and Otani 1997). Ion toxicity and pathogen attack can also stimulate an exudates response from plants (Ryan et al. 1997, Zheng and Ma 1998; Mehta, Sharma, and Sindhan 1992). When plants encounter phosphorus deficiency, they have the ability to exude a wide range of both organic and inorganic compounds to increase the availability of phosphorus in the soil

New Soil Extractant

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solution (Rengel 2002). The objective of this study was to develop a soil extractant that meets the following initial criteria: 1) it should contain compounds that have been identified as common organic root exudates; 2) it should be able to extract soil, on average, within one unit of the soil pH; 3) it should be compatible with colorimetric and ICP methods for determining nutrient concentration; and 4) it should be significantly correlated with results of currently used soil test methods.

MATERIALS AND METHODS

Extractant Development

Different chemicals were experimented with, in many combinations and concentrations, including hydroquinone, citric acid, oxalic acid, acetic acid, lithium citrate, sucrose, instant tea (for tannins), sodium citrate, malic acid, ethylenedinitrilotetraacetic acid (EDTA), diethylenetriaminepentaacetic acid (DTPA), ascorbic acid, aluminum chloride, and lithium chloride. The soft drink 7-Up was even tried, and it actually compared quite well with Olsen and Mehlich 3 for extractable P.

It was reasoned that the organic compounds contained in root exudates were the most important, but we did not want to add potassium (K), sodium (Na), or calcium (Ca) in the extractant because metals, and other nutrients as well, might eventually be extracted. Lithium citrate was chosen because lithium is rarely tested in soil and lithium would act somewhat like K in KCl for replacing NH_4 from exchange sites. In the soils tested, lithium citrate alone was an excellent extractant in calcareous soils for P. The amount of lithium citrate in the extractant was proportional to the amount of extractable P in soil with pH greater than 7.

Next, three organic acids were added to the extractant that plants most commonly use to overcome deficiencies of various nutrients; oxalic acid, malic acid, and citric acid (Rengel 2002; Baudoin, Benizri, and Guckert 2003; Shenker, Hadar, and Chen 1999) and balanced the acidic solution with lithium citrate. The lithium citrate acted as a weak buffer when the acids were added, and it was a delicate balancing act to determine the proportion of the three acids to lithium citrate. It was known that lithium citrate was working well for extractable P in calcareous soils. However, some of the extracting power was lost after the acids were added for calcareous soils, but the addition of the organic acids made the extractant more flexible for use across a wider range of soil pH. Combinations of chemicals were chosen based on extractant pH. EDTA and DTPA were also added to help with the extraction of P and possibly other metals. Chelators help protect certain cations such as iron (Fe), zinc (Zn), copper (Cu), and manganese (Mn) from reacting with soil by forming chelate complexes, which can be taken up by plants. The aim was to create a soil extractant

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based on organic acids (citric and oxalic acid are chelators), lithium citrate, and two synthetic chelators (EDTA and DTPA) that would extract nutrients near the soil pH. Therefore, the final combination of chemicals were based on extractant pH. The extractant H³A (Haney, Haney, Hossner, Arnold) was designated, which is produced by dissolving the following ingredients in one liter of water. Molarities are also stated. Lithium citrate: 5.0 g = 0.02 M; citric acid: 0.5 g = 0.0024 M; malic acid: 0.5 g = 0.004 M; oxalic acid: 0.5 g = 0.004 M; EDTA: 0.25 g = 0.002 M; and DTPA: 0.25 g = 0.001 M.

Soils

Soils were collected from ten states in the USA. They were from California (3), Texas (6), Illinois (6), Oklahoma (5), Pennsylvania (4), Colorado (4), Arizona (1), Alaska (1), Mississippi (1), and Wyoming (1) for a total of 32 soil samples. Soil characteristics are listed in Table 1. These soils were collected from pastureland (5 soils) and croplands, with the majority in conventional tillage (23 soils) and some in no-till (4 soils). The soils had a wide range in soil pH (4.7–8.4), organic C (0.1–2.6 g C kg⁻¹), and clay content (6–59%).

Extraction and Analytical Methods

Each soil was dried at 55°C for 24 h and ground to pass a 2-mm sieve. Each soil was weighed (4 g) in duplicate in 50-mL plastic centrifuge tubes and extracted with 40 mL using five extractants (1 M KCl, water, Olsen, Mehlich 3, and H³A) for a total of 320 samples. Samples were shaken for 30 min (5 min for Mehlich 3), centrifuged at 3000 rpm for 8 min, and filtered through Whatman 2V pleated filter paper. Each of the 320 soil extracts was tested for pH. The samples were then analyzed for NH₄-N, NO₂/NO₃-N, and PO₄-P on an OI Analytical, Flow IV, rapid-flow colorimetric analyzer.

RESULTS AND DISCUSSION

The amount of P extracted from soil is dependent upon many factors. Among those are soil pH, clay content, and concentrations of calcium, iron, and aluminum (Cox 2001). Extractable P is strongly influenced by soil pH but is also highly influenced by the pH of the soil extractant (Golterman 1988). In an effort to demonstrate this phenomenon, it was decided to manipulate the pH of an extractant on four acid soils (soil pH 5.5–6.8). Organic acids were used to drop the extractant pH to 2.6, and then we increased the pH incrementally to pH 9.0 with a combination of acids and lithium citrate and lithium citrate alone (details in Table 2). Extractable P ranged from 60–95 ppm at

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Table 1. Soil characteristics

Soil number	Soil series	% Clay content	% Soil organic C	Soil pH
1	Perkins	15	0.59	4.7
2	Pella	32	1.37	5.4
3	Ellis	15	2.54	5.7
4	Chelsea	12	0.83	5.9
5	Bresser	15	3.19	5.9
6	Mardin	34	2.17	6.0
7	Kichatna	11	2.58	6.0
8	Hagerstown	34	1.78	6.1
9	Platner	23	0.95	6.1
10	Berks	31	2.41	6.2
11	Gilford	15	2.13	6.2
12	Anton	23	0.94	6.2
13	Lindon	23	1.00	6.3
14	Rosetta	22	1.11	6.3
15	San Ysidro	20	0.52	6.3
16	Leland	13	0.59	6.3
17	Morocco	10	0.57	6.4
18	Belmond	18	1.84	6.6
19	Chelsea	12	0.17	6.7
20	Griffy	17	1.12	6.9
21	Wheatwood	20	1.32	7.4
22	Beckman	42	0.96	7.6
23	Houston pasture	59	1.77	7.8
24	Ardep	6	0.78	8.0
25	Casa grande	13	1.08	8.0
26	Houston pasture fertilized	50	1.80	8.0
27	Quinlan	12	0.35	8.2
28	Houston con-till corn	55	1.38	8.2
29	Weswood con-till com	28	0.72	8.3
30	Pratt	13	0.56	8.3
31	Weswood con-till sorghum	28	0.37	8.3
32	Houston no-till corn	52	1.64	8.4

an extractant pH of 2.6 for the four acid soils and decreased to less than 15 ppm at an extracting pH of 8.5 (Figure 1). The pH of the extracting solution had a considerable impact on the amount of extractable P. In addition, we included two soils, one with a soil pH of 8.2 (low organic C, low nutrients) and one with a soil pH of 6.5 (high organic C, high nutrients), and repeated the process of manipulating the extractant pH to demonstrate the impact of extractant pH on extractable phosphorus. The pH

Table 2. Manipulation of soil-extracting solution pH by varying organic acid and lithium citrate concentrations

Extractant	Ingredients: dissolved in 1 l	pH
1	5.0 g lithium citrate	9.0
2	2.5 g lithium citrate	8.0
3	5.0 g lithium citrate, 0.25 g malic acid	7.5
4	5.0 g lithium citrate, 0.5 g malic acid	7.0
5	2.5 g lithium citrate, 0.5 g citric acid	6.5
6	5.0 g lithium citrate, 0.5 g malic acid, 0.5 g oxalic acid	6.0
7	5.0 g lithium citrate, 0.5 g malic acid, 0.5 g oxalic acid, 0.5 g citric acid	5.5
8	2.5 g lithium citrate, 0.5 g citric acid, 0.5 g malic acid, 0.5 g oxalic acid	4.5
9	1.5 g lithium citrate, 0.5 g citric acid, 0.5 g malic acid, 0.5 g oxalic acid	4.2
10	1.5 g lithium citrate, 0.75 g citric acid, 0.75 g malic acid, 0.75 g Oxalic acid	3.5
11	0.5 g citric acid, 0.5 g malic acid, 0.5 g oxalic acid	2.6
12	0.75 g citric acid, 0.75 g malic acid, 0.75 g oxalic acid	2.35

6.5 soil increased from 20 ppm extractable P to 95 ppm as the extractant pH decreased, while the soil pH 8.2 increased from 2 ppm extractable P to 3.8 ppm as the extractant pH decreased (Figure 2). These results indicate that we can manipulate the extractable phosphorus by increasing or decreasing the pH of the soil extractant. Because Mehlich 3 and Olsen extract at such low and high pH, respectively, it seems that extracting the soil near the pH (more

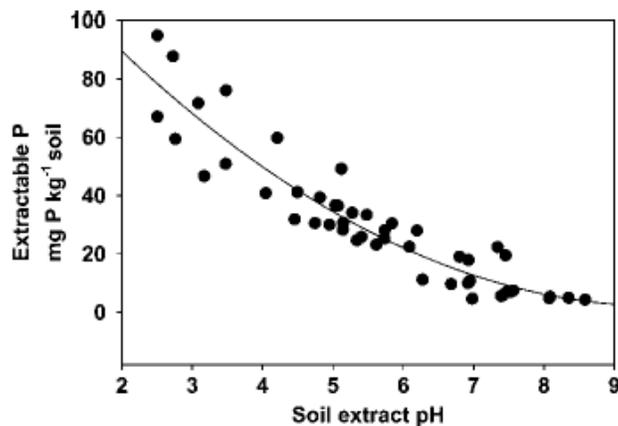


Figure 1. Impact of soil extract pH (2.4–9.0) on extractable P from four acid soils (pH 5.5–6.5).

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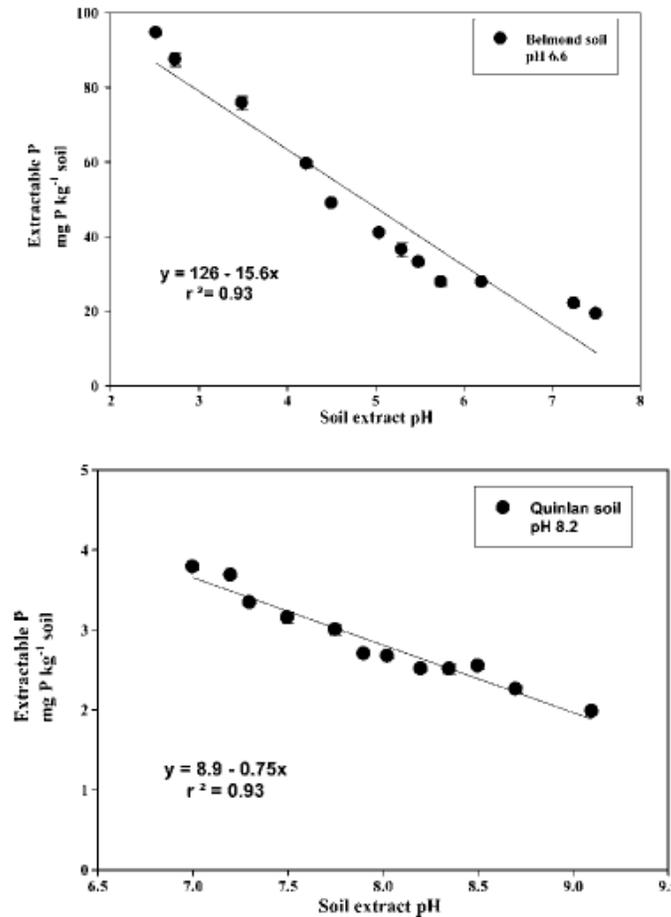


Figure 2. Effect of changing extract pH on extractable P from a low- and high-pH soil (changes described in Table 1). Error bars indicate one standard deviation.

accurately representing field conditions) of the soil would increase the accuracy of estimating plant-available phosphorus.

The soil extractants we tested differed in their ability to extract soil P depending upon the soil pH and the pH of the extractant. The effect of extractant pH on extractable P is marked by the acidity of the extractant. For example, Mehlich 3 releases significantly more P than the other three extractants we tested because of its ability to dissolve iron, aluminum, and calcium phosphates (Nelson, Mehlich, and Winters 1953). The range of extractable P from Mehlich 3 was 0–80 ppm with a mean of 34.3, H³A 0–40 ppm with a mean of 16.6, Olsen 0–20 ppm with a mean of 9.9, and water 0–10 ppm with a mean of 3.3 on the same soils. Using 3D graphing and comparing soil pH, soil extract pH, and extractable P reveals an interesting picture of the interaction

between the three components (Figure 3). Mehlich 3 extracted soil P in the 2.9–4.2 pH range (mean = 3.4) regardless of the soil pH value and extracted more than twice as much P as H³A, almost four times that of Olsen and eight times as much as water. H³A and water extracted P in the 5.0–8.5 pH range (H³A mean = 6.2, water mean = 6.7); however, H³A tended to produce soil extracts from acid to neutral soils in the 5.0–5.5 range until soil pH increased above 7.5 where soil extracts had higher pH values (6.5–8.0), demonstrating an increased sensitivity to soil pH. The pH of the water extract followed the soil pH very closely, as we would expect. The Olsen soil extract pH range was 8.3–9.0 (mean = 8.6) regardless of soil pH and was completely opposite Mehlich 3 in extract pH (Figure 3). Interestingly, H³A is almost exactly between Mehlich 3 and Olsen in soil extract pH and extractable P. In the soils we tested, water would be the ideal extractant to extract nutrients near the soil pH; however, the water-extractable P among

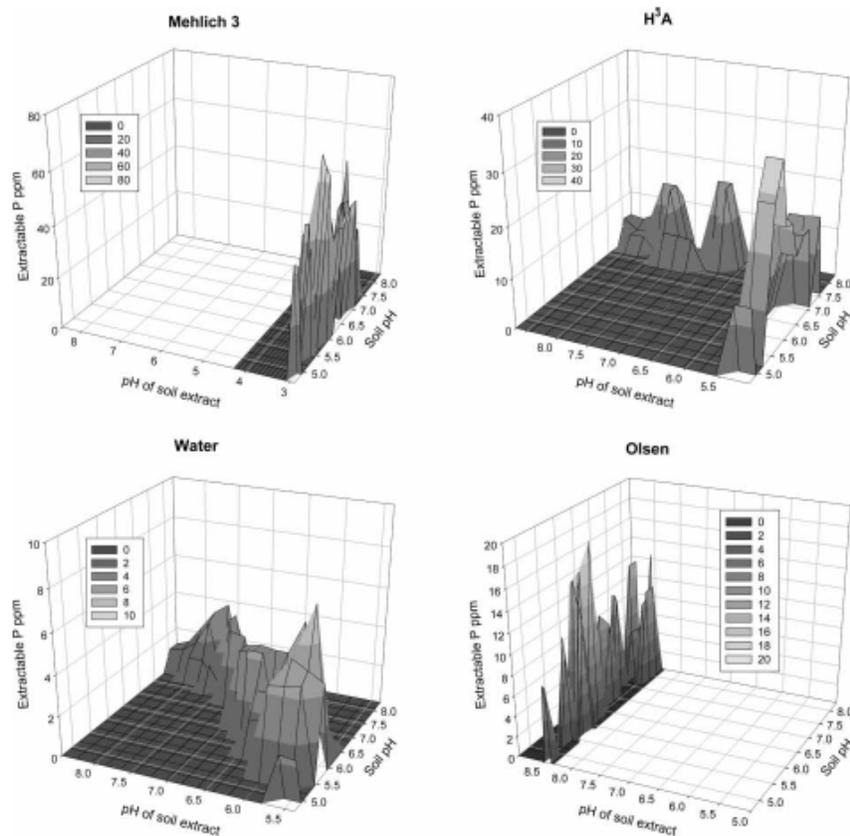


Figure 3. Interaction of soil pH and pH of soil extract on soil phosphorus from the four different extractants.

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different soils is usually quite low and only moderately correlated with Olsen ($r = 0.63$) and Mehlich 3 ($r = 0.52$) extractable P (Table 2).

Water-extractable P was significantly correlated with Mehlich 3, Olsen, and H³A (Table 3), strongest with H³A and weakest with Mehlich 3. Mehlich 3 and Olsen were better correlated with each other than Mehlich 3 and H³A, although the differences were slight ($r = 0.84$ vs. $r = 0.83$). H³A extracted roughly half the P as Mehlich 3. Olsen extracted roughly one-fifth the P as that of M3. Olsen extractable P had the best relationship with H³A followed by Mehlich 3 and water. Although good correlations are observed between all four extractants, the concentration of extractable P varies greatly with each extractant.

Based on the chemical composition of Mehlich 3, H³A, and Olsen, soil-extractable P may be defined based on acidity or alkalinity of the extractant and the ability of the extractant to respond to soil conditions. Using Mehlich 3 on calcareous soils may overestimate available P, and using Olsen on acid soils may underestimate available P because of the buffering capacity of the soil. Water-extractable P does not take into account the action of plant root exudates upon soil P, whereas H³A better simulates the soil solution when plants are present without artificially driving the extract pH too low (Mehlich 3) or too high (Olsen). The data in Figure 4 illustrate the effect of extract pH to extractable P. Figure 4a shows the proximity of the soil extractant pH to the actual soil pH (0-line). The soil pH becomes more alkaline as the samples move from left to right (Figure 4a). As the soil pH increases, the Mehlich 3 extract pH deviates further from the original soil pH. The average of soil extract pH deviation for the 32 soils we tested using Mehlich 3 was 3.4 pH units away from the soil pH. Olsen is just the opposite; soil extract pH is over 3 pH units from the soil pH for low pH soils and slowly becomes more similar to the soil pH for high pH soils, with an average of 1.8 pH units from the soil pH. Water and H³A extract pH tend to be close to the soil pH; however, H³A deviates as much as 1.5 units at soil pH of 7.5. H³A contains both dilute organic

Table 3. Correlation matrix for extractable P by various solutions and their associated regression equations

	Olsen (y)	Water (y)	H ³ A (y)
Mehlich 3 (x)	0.84*** $y = 2.9 + 0.2x$	0.52*** $y = 1.2 + 0.06x$	0.83*** $y = 1.1 + 0.45x$
Olsen (x)		0.63*** $y = 0.14 + 0.33x$	0.84*** $y = -2.3 + 1.9x$
Water (x)			0.71*** $y = 6.2 + 3.2x$

***Indicates $p < 0.001$.

Notes: (x) and (y) are for the regression equations. N = 64 (32 soils, 2 reps).

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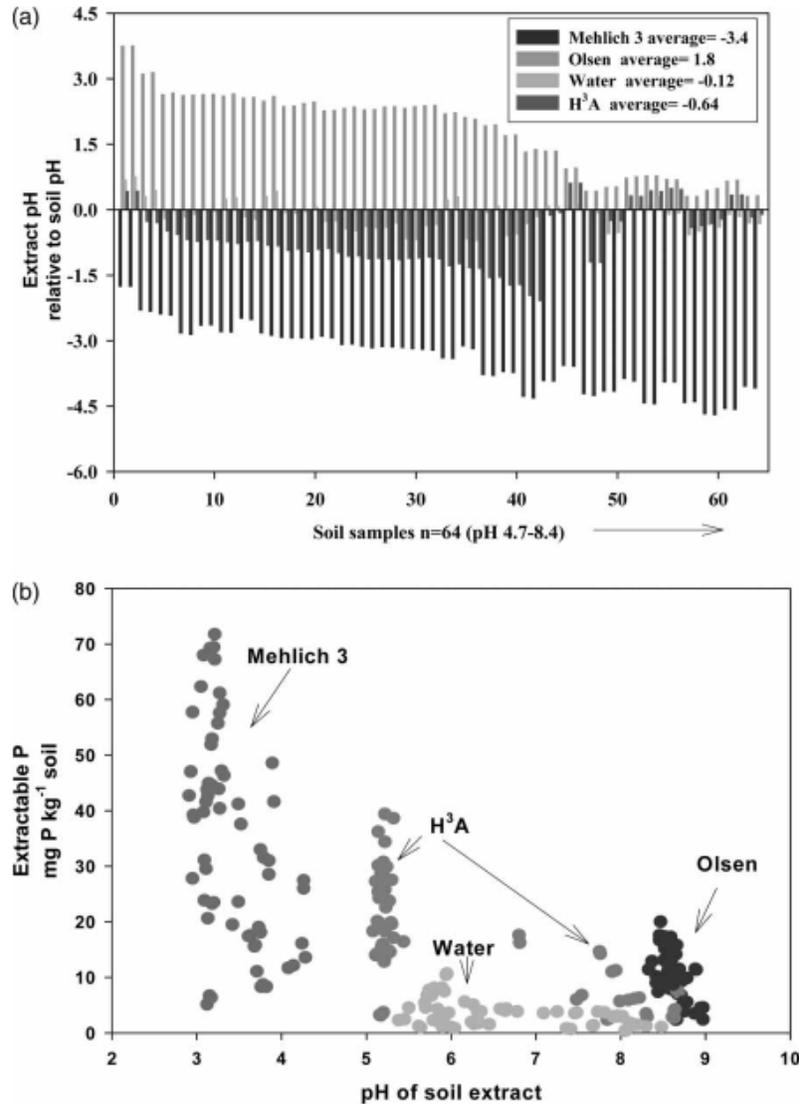


Figure 4. Proximity of extractant pH to soil pH and impact on extractable P. (a) Soil extract proximity to soil pH; (b) Soil phosphorus study soil pH 4.7–8.5, 32 soils, 4 extractants, and 2 reps.

acids and lithium citrate. Lithium citrate alone (5 g) has an extractant pH of 8.4; however, when 1.5 g of acid are added in addition to the lithium citrate, the pH falls to 5.5 (extractant 7, Table 1). Therefore, the acids dominate the extract pH until soil pH reaches 7.5 and above, where the alkalinity of the soil overwhelms the organic acid concentration and the extract pH in soil

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increases sharply and is similar to the soil pH. Conversely, the lithium citrate appears to keep H³A from acidifying the soil as strong as Mehlich 3 does, because the organic acids alone in H³A have an extractant pH of 2.6 (extractant 11, Table 1). These factors make H³A more flexible in extracting P near the soil pH, but extractable P is much higher than for water. The average proximity of extract pH to soil pH is 0.64 for H³A and 0.12 for water (Figure 4a). H³A extract pH was within one pH unit of the soil pH for 86% of the 32 soils, water 100%, Olsen 23% and Mehlich 30%.

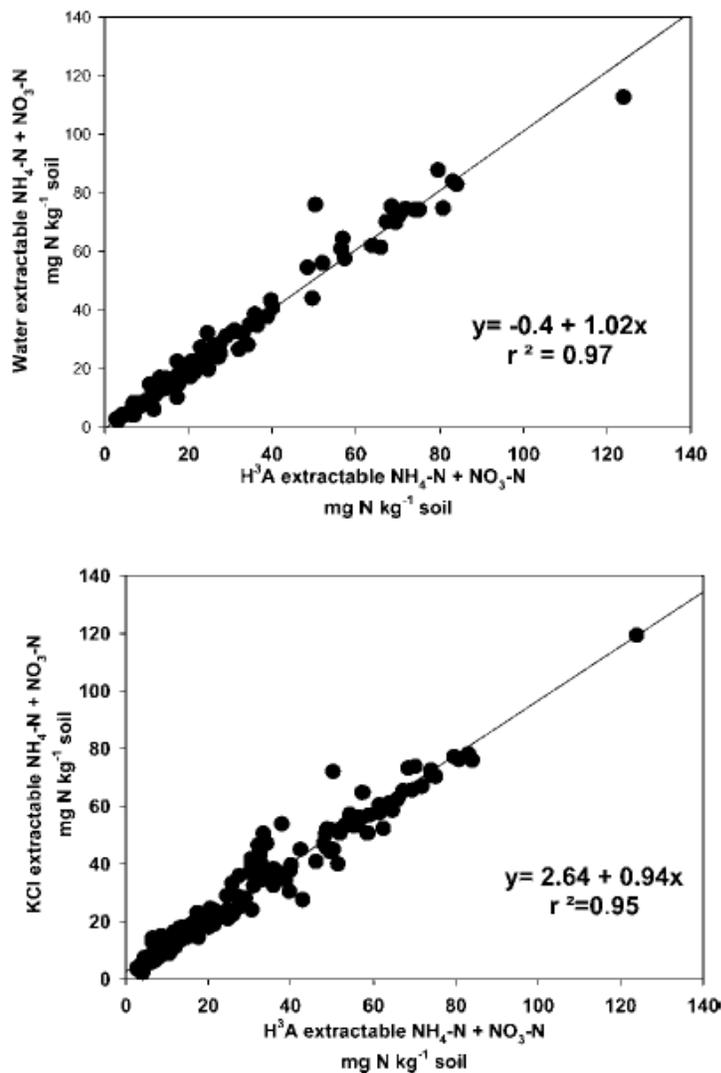


Figure 5. Relationships for soil extractable NH₄ plus NO₃ from H³A compared to 1 M KCl and water.

Soil-extractable ammonium and nitrate were highly correlated between H³A and water and H³A and 1 M KCl ($r^2 = 0.97$ and 0.95 , respectively) (Figure 5). H³A and water consistently extracted 5–10% more nitrate than KCl; however, KCl usually extracted more ammonium than H³A or water. When added together, the extractable NH₄ + NO₃ was nearly identical for the three extractants, with H³A extracting slightly more nitrogen than water but slightly less than KCl (Figure 5). The pH of the soil extractant using KCl, water, and H³A had no significant effect on the extracting ability of soil NH₄ and NO₂/NO₃ (data not shown).

CONCLUSIONS

The soils used for this preliminary test of a new soil extractant based on organic acid root exudates had a wide range of soil pH, organic C, and clay content. H³A was highly correlated with soil-extractable inorganic N from both water and 1 M KCl, as well as being highly correlated with water, Mehlich 3, and Olsen extractable P. These results indicate that H³A may be used as a limited multinutrient (inorganic N and P) extractant, which would eliminate the need for two extractants to test for plant-available NH₄, NO₃, and P. Because soil-extractable P is highly influenced by soil pH and pH of the soil extract, extracting soil within one unit of the soil pH would be a desirable attribute of a soil extractant. On average, H³A extract pH was within one pH unit of the soil pH for 86% of the 32 soils, water 100%, Olsen 23%, and Mehlich 30%. Based on this data, extracting near the soil pH could provide a more reliable estimate of plant available inorganic N and P without overestimating soil P on calcareous soils and underestimating P on acid soils.

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Appendix C2

Soil CO₂ respiration: comparison of chemical titration, CO₂ IRGA analysis and the Solvita gel system

Soil CO₂ respiration: Comparison of chemical titration, CO₂ IRGA analysis and the Solvita gel system

R.L. Haney^{1,*}, W.F. Brinton² and E. Evans²

¹USDA-ARS, 808 E Blackland Rd, Temple, TX 76502 USA.

²Woods End Laboratories, Inc., 290 Belgrade Road, Mt Vernon, ME 04352, USA.

*Corresponding author: rhaney@spa.ars.usda.gov

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Abstract

The measurement of soil carbon dioxide respiration is a means to gauge biological soil fertility. Test methods for respiration employed in the laboratory vary somewhat, and to date the equipment and labor required have somewhat limited more widespread adoption of such methodologies. The purpose of this research is to compare the results of measured soil CO₂ respiration using three methods: (1) titration method; (2) infrared gas analysis (IRGA); and (3) the Solvita gel system for soil CO₂ analysis. We acquired 36 soil samples from across the USA for comparison, which ranged in pH from 4.5 to 8.5, organic C from 0.8 to 4.6% and the clay content from 6 to 62%. All three methods were highly correlated with each other after 24-h of incubation (titration and Solvita $r^2 = 0.82$, respirometer and Solvita $r^2 = 0.79$ and titration versus respirometer $r^2 = 0.95$). The 24-h (1-day) CO₂ release from all three methods was also highly correlated to both basal soil respiration (7–28 days) and cumulative 28-day CO₂ respiration. An additional 24 soil samples were acquired and added to the original 36, for a total of 60 soil samples. These samples were used for calibration of the Solvita gel digital color reader results using CO₂-titration results and regression analysis. Regression analysis resulted in the equation $y = 20.6 * (\text{Solvita number}) - 16.5$ with an r^2 of 0.83. The data suggest that the Solvita gel system for soil CO₂ analysis could be a simple and easily used method to quantify soil microbial activity. Applications may also exist for the gel system for *in situ* measurements in surface gas chambers. Once standardized soil sampling and laboratory analysis protocols are established, the Solvita method could be easily adapted to commercial soil testing labs as an index of soil microbial activity.

Key words: chemical titration, soil CO₂ respiration, infrared gas analysis, soil microbial activity

Introduction

Soil respiration is an important aspect of soil-quality and an indicator of soil fertility¹. As early as 1931, Smith and Humfeld² noted that during decomposition of green manures, the numbers of bacteria followed CO₂ evolution, which rose rapidly during the first 4 days and then declined to a fairly constant level. Even earlier, Gainey³ noticed a parallel formation of CO₂, NH₄-N and NO₃-N in soil. In 1924, Lebedjantzev⁴ stated that drying soil at low temperature appeared to increase the fertility of the soil which, he noticed, also occurred in nature. For roughly 90 years, CO₂ respiration from soil has been used as an indicator of the relative fertility of various soils^{3–5}. Soil CO₂ respiration has been widely used for many years to quantify the impact on soil microbial activity of various treatment and management inputs. The purpose of many of these studies are mainly concerned with the rates of C, N or

P mineralization in an effort to gain a clearer understanding of these natural processes. A clear understanding of nutrient cycling is essential to developing accurate computer models and could have a tremendous impact upon the soil testing industry.

Chemical titration for soil CO₂ respiration is an effective means whereby different soils can be compared for microbial activity. Soils are incubated along with an aqueous solution of KOH or NaOH in a small vial. The alkali reacts chemically with CO₂ and BaCl₂ and can be back-titrated with HCl to a phenolphthalein endpoint which is relative to the amount of CO₂ released by soil microorganisms⁶. A control vial with no soil is included in the incubation to correct for the CO₂ in the jar at the initiation of the incubation. An equation is then employed to arrive at mg CO₂-Ckg⁻¹ soil. Soil CO₂ respiration can also be measured with a gas chromatograph or an infrared gas analyzer (IRGA) for CO₂ detector. Although chemical

titration has avenues for error associated with the procedure, it is a fairly simple and straightforward method. However, the method requires mixing the alkali, assumption that the control is accurate, care in titration, and accurately hitting the endpoint, which can induce error.

More recently, soil laboratories have been reviewing early methods in view of environmental disposal concerns, such as in the use of dichromate for soil organic carbon digestion. The presence of BaCl₂ in the CO₂ titration procedure would qualify for such concern. To render unreacted BaCl₂ harmless after titration requires the additional step of adding an equimolar or greater amount of a soluble source of sulfate ions, producing insoluble BaSO₄. Such steps add to the complexity of the procedure.

The Solvita gel system was designed as a complete procedure to quantify the relative differences between varying types of compost in terms of the amount of CO₂ evolved in a short time period. This is interpreted as an indication of the completeness of active degradation, also called a maturity index⁷. In this research, a similar principle of CO₂ respiration is being applied to soil respiration. Soils differ from compost in that the gross amount of respiration is likely to be less than soils, since soils typically have 1/10th–1/20th the amount of carbon. The Solvita gel system is a new tool to evaluate soil microbial respiration rate in an efficient and cost-effective manner, without the need for reagent handling and standardization. A pH-sensitive gel (paddle) is embedded in a one-piece plastic holder that narrows to a point so that it can be pushed into the soil. After a specified time-period, the paddle can be removed from the incubation jar and analyzed with a digital color reader (DCR) developed specifically for the test. This process takes a minimum of time and labor. The USDA Soil Quality Institute has listed the Solvita kit as an alternate soil respiration procedure in its national soil-quality test kit program which released a full soil quality test document. This application of the Solvita gel-system was found suitable since it was able to detect meaningful changes in surface gas chambers CO₂ concentrations (John Doran, personal communication, October 2007). Solvita has been reported to have compared sensitivity to Dräger tubes when employed in compost chamber tests⁸. The Solvita chemistry gel technology is different from alkali traps in that it does not absorb all the CO₂ but absorbs a relative concentration of CO₂. Since its inception, the visual color strips used to interpret the reaction have been upgraded by the DCR in which the intensity of red, green and blue (RGB) emissions from the gel is read by a diode array detector (DAD) assembly within the DCR. Using this approach permits very rapid measurement of accumulated CO₂ within the Solvita gel at any time during incubation, and improves reliability and significantly increases accuracy. The reactive gel with DAD appears to closely obey Beer–Lambert's optical law over a wide range of concentration of CO₂ and suffers only small interference from volatile fatty acids which form a positive response with CO₂ gels, consistent with an unstable compost condition.

The Solvita system is almost error free, since it involves placing the paddle in the soil and removing it after the allotted time-period, placing it in the reader and pressing a button. Soil CO₂ respiration is a common and simple measure of biological activity in soil. Soil microbial activity as measured by CO₂ respiration is a function of substrate availability, which is related to the amount or quality of organic C and N. The purpose of this research is twofold: first, to compare the soil CO₂ release from the titration method, IRGA and the Solvita gel system, and secondly, to investigate the possibility that the release of CO₂ can be adapted to soil testing labs to provide a biological method that could discern differences in soil microbial activity which might provide an additional insight to the relative activity of different soils.

Materials and Methods

Experiment 1

Thirty-six soil samples were collected from Texas, Oklahoma, Georgia, Mississippi, Idaho, Wyoming and Illinois. The range in soil pH was 5.0–8.3, soil organic C 0.65–4.52%, and clay content 10–55%. All soils were ground to pass a 5-mm sieve, dried at 40°C and weighed into 50 ml plastic beakers. All soils were wetted to approximately 50% water-filled pore space.

Titration. Forty grams of wetted soil was placed in a 1 pint mason jar along with a vial of 10 ml of 1 M KOH. The alkali traps were changed and titrated at days 1, 3, 7, 14, 21 and 28. Unreacted alkali in the KOH traps was back-titrated with 1 N HCl to determine CO₂-C⁶. Basal soil respiration was calculated by subtracting the cumulative 7-day CO₂-C from the cumulative 28-day CO₂-C.

IRGA. Forty grams of wetted soil samples were placed in 8 oz jars and capped. Each jar was connected to the IRGA via twin solenoids which open simultaneously to allow CO₂-free air to purge the jar of CO₂ and direct it to the analyzer (ADC model 225) at a rate of 400 ml min⁻¹ for 3 min. Eight soil samples and two controls were used in the 10 sample system. Each glass jar was sampled for 3 min and then closed (Fig. 1). The samples were analyzed every hour for 24 h.

Solvita. Forty grams of wetted soil samples were placed in 8 oz glass jars with a Solvita gel paddle. At the end of 24 h each paddle was placed in the DCR for analysis (Fig. 2). A simple regression analysis was used to assess the correlation between 24-h CO₂ evolution from titration versus the Solvita gel and CO₂-C from IRGA.

Experiment 2

An additional 24 soil samples from Utah, Washington, California, Montana, New Mexico, North Carolina, Maine, Pennsylvania and Ohio were acquired and added to the original 36 in dry form. All 60 samples were wetted as described above and incubated for 24 h. The titration method and the Solvita gel system were used for 1-day

Soil CO₂ respiration



Figure 1. Closed system soil respirometer.

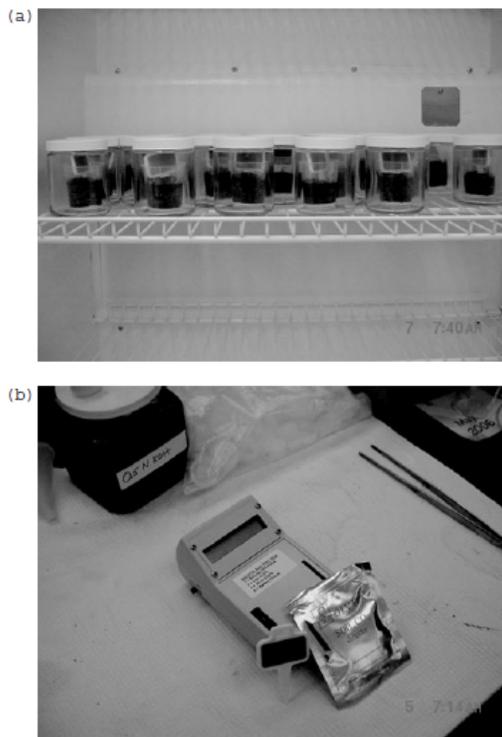


Figure 2. (a) Solvita gel paddles in soil and (b) Solvita digital reader.

CO₂-C analysis to calibrate the DCR to the CO₂-C from titration.

Experiment 3

Since the Solvita gel system does not absorb all the CO₂ within the container but rather absorbs a relative amount,

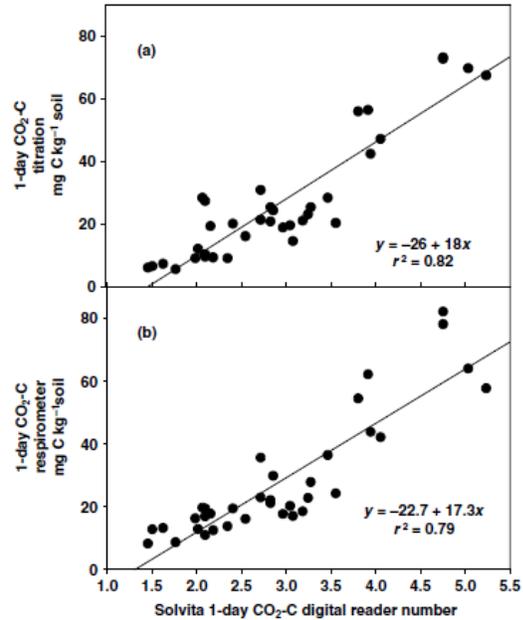


Figure 3. Solvita 24-h CO₂ versus (a) 24-h CO₂-C titration and (b) 24-h CO₂-C closed system respirometer.

we chose 20 soil subsamples to study the influence of container volume on CO₂ respiration by the Solvita gel system. Twenty grams of soil samples were weighed into 50 ml plastic beakers, rewetted as described above, and placed into 8, 16 and 32 oz glass jars with gel paddles in each jar. After 24 h of incubation the paddles were removed and analyzed with the DCR.

Results and Discussion

Experiment 1

The Solvita number from the DCR was compared to the CO₂-C from both the titration method and the CO₂-C from the closed system respirometer (IRGA) glass after 24-h (1-day) incubation. Regression analysis established a highly significant relationship between CO₂ evolution from the Solvita number and titration ($r^2 = 0.82$, Fig. 3a) and the Solvita number and the CO₂-C from the respirometer ($r^2 = 0.79$, Fig. 3b). There was also a highly significant relationship between titration and the respirometer methods after a 24-h incubation ($r^2 = 0.95$, Fig. 4). The strong correlations between these methods suggest that any of the three methods could rapidly quantify soil microbial activity, although the Solvita method would be the simplest and least labor intensive. Since most of the 36 soils were in a dry state when they arrived at our lab, we chose to incubate the soils for 28 days after rewetting. We calculated basal soil respiration as the cumulative 28-day minus the

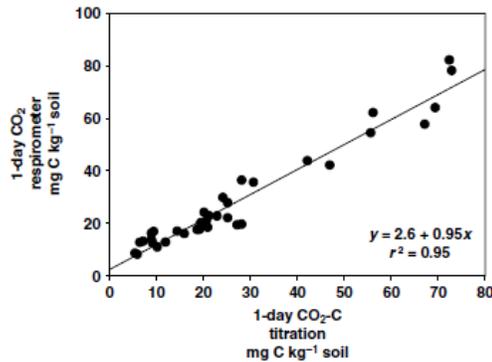


Figure 4. 24-h CO₂-C titration versus 24-h CO₂-C respirometer.

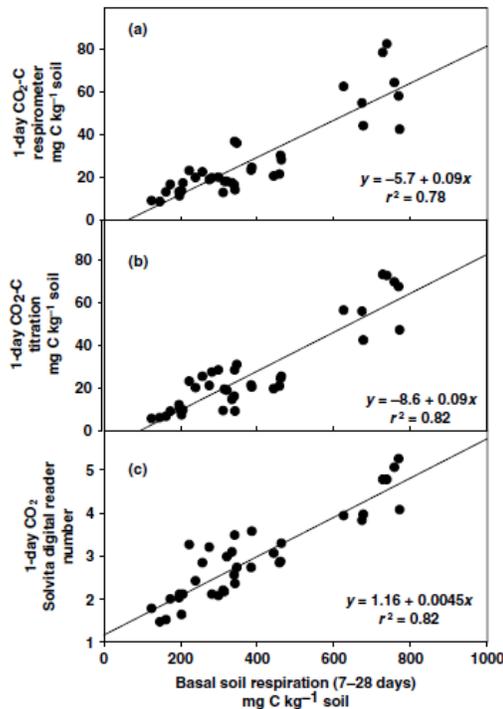


Figure 5. Basal soil respiration (7–28 days cumulative) versus (a) 24-h CO₂-C closed system respirometer, (b) 24-h CO₂-C titration and (c) Solvita CO₂ digital reader number.

initial 7-day period for CO₂-C after rewetting. A paper by Franzluebbers⁹ indicated that a 7-day incubation period was adequate to overcome the elevated release of CO₂-C from the drying-rewetting effect. Therefore, we compared basal soil respiration (7–28 days) against the 1-day CO₂ value from titration, respirometer, and Solvita to explore possible changes in microbial activity after removing the drying/rewetting flush of CO₂. The relationships of each 1-day

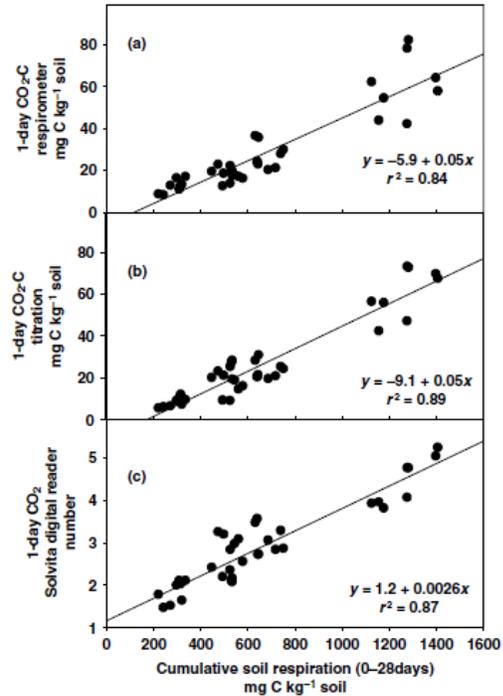


Figure 6. Cumulative soil respiration (0–28 days) versus (a) 24-h CO₂-C closed system respirometer, (b) 24-h CO₂-C titration and (c) Solvita CO₂ digital reader number.

method to basal soil respiration are shown in Figure 5. The respirometer data for 1 day had an $r^2 = 0.78$ (Fig. 5a), titration exhibited an $r^2 = 0.82$ (Fig. 5b), and Solvita an $r^2 = 0.82$ (Fig. 5c) with basal soil respiration. Again, each method proved to be adequate at predicting basal soil respiration even though the 1-day CO₂ release was taken during the greatest portion of the CO₂ release from the drying/rewetting process^{10,11}. We also compared the 1-day CO₂ release after drying/rewetting with the cumulative 28-day CO₂ evolved including the flush of CO₂ from drying/rewetting. The relationships between 1-day CO₂ and 28-day CO₂ showed only slightly better correlations compared with 1-day CO₂ and the basal rate. The respirometer data had an $r^2 = 0.84$ (Fig. 6a), titration an $r^2 = 0.89$ (Fig. 6b), and Solvita an $r^2 = 0.87$ (Fig. 6c) with cumulative 28-day CO₂-C.

Experiment 2

The soil CO₂ released, after soil drying/rewetting and incubating for 24 h, from 60 soils was determined using the Solvita gel system with a DCR and was highly related to 24-h soil CO₂ measured using the titration method (Fig. 7). Although drying soil is not a prerequisite to using the system; we used dried soil to start all the soils in the experiment from an equal state. We also wanted to

Soil CO₂ respiration

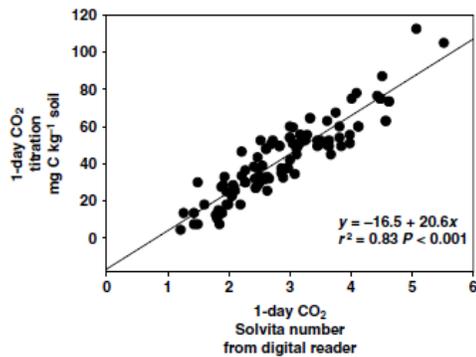


Figure 7. 1-day CO₂ Solvita versus 1-day CO₂ titration. Sixty soil samples from US, pH range 4.5–8.5, soil organic C range 0.8–4.6% clay content range 15–62%.

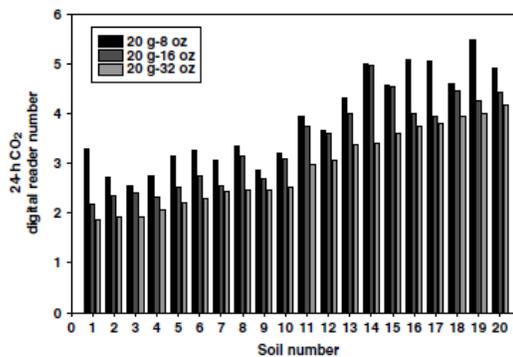


Figure 8. Influence of chamber volume on digital reader number. Twenty grams of soil subsamples were used for each chamber. Chamber size was 8, 16 and 32 oz.

accommodate soil testing protocols since most soil testing labs dry and grind their soil samples prior to analysis. The above-mentioned relationship suggests that the Solvita soil system can be equally as effective as the titration method as an index of microbial activity in order to quantify changes or differences in soil respiration from various soils. The equation $y = 20.6 * (\text{Solvita number}) - 16.5$ can be used to convert the DCR number to CO₂-C, which is commonly reported with the titration method (Fig. 7).

Experiment 3

When high soil CO₂ respiration is expected, it is possible to increase the container volume, which will dilute the relative amount of CO₂ in equilibrium with the gel. This provides flexibility to measure soils with recent manure or compost additions without overwhelming the system with carbon dioxide. The analogous limit with standard CO₂ titration methods is when the base (KOH or NaOH) becomes overwhelmed with excess carbonate, and the appropriate

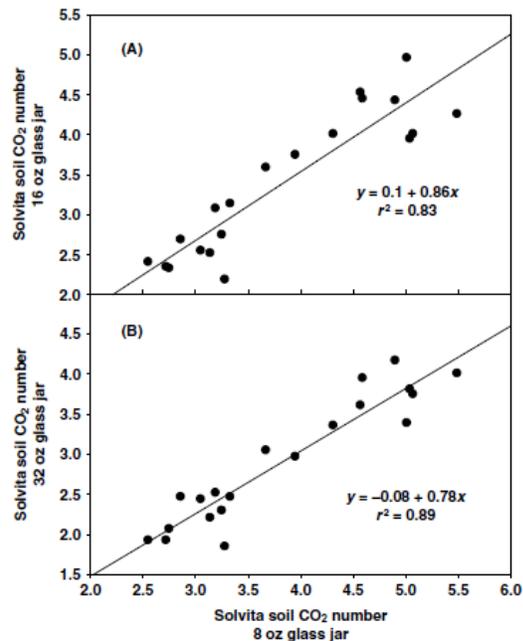


Figure 9. Chamber volume relationships on soil CO₂.

recourse is to increase the amount of alkali, or raise its concentration. When we compared various volumes, the mean Solvita number across all 20 soils for the 8 oz jar was 3.84 with a standard deviation of 0.22, mean for the 16 oz jar was 3.40 with a standard deviation of 0.20 and the mean for the 32 oz jar was 2.91 with a standard deviation of 0.18 (Fig. 8). The linear regression relationships between chamber volumes are illustrated in Figure 9. Twenty soils samples of 20 g were used for each chamber volume. The 20 g soil 8 oz glass jar volume is compared to both the 16 and 32 oz glass jar volumes. The data indicate that it is feasible to use greater volumes to dilute the CO₂ when incubating soil samples that are expected to produce a high output of soil CO₂. We chose to use the 8 oz glass jar since it had the strongest relationship with CO₂ from both titration and IRGA compared to the 16 and 32 oz jars (data not shown).

Conclusion

The methods we compared were well correlated with each other and offer promise in utilizing soil CO₂ data as an index of microbial activity. However, a concentrated effort would be needed to further this research and develop a standardized method for microbial activity which could be readily adapted by soil testing labs. The Solvita gel measurement of soil CO₂ is a simple and rapid method which can quantify microbial activity from various soils. Since soil fertility is a relative estimate between soils, the

introduction of a rapid and accurate method for soil testing labs, which could separate soils based on microbial activity, could find an application in tracking management changes for either conventional or organic farming systems. In addition, we recommend using the 8 oz glass jar unless soils contain recent addition of manure and/or compost and high CO₂ is expected, in which case the use of 16 or 32 oz glass jars can then be substituted without loss of accuracy.

If soil fertility is reflected in the microbial community and one soil is more fertile than another, the more fertile soil should have higher yield potential than the other. Therefore, if we can make connections between soil fertility and soil microbial respiration, we can apply this information to our benefit as stewards of the land. This additional information may enable us to make better management decisions, give us direction in making more accurate fertilizer recommendations or give us a starting place with which to monitor our performance in our soil management strategies.

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Appendix C3

A rapid procedure for estimating nitrogen mineralization in manured soil

ORIGINAL PAPER

R.L. Haney · F.M. Hons · M.A. Sanderson
A.J. Franzluebbers

A rapid procedure for estimating nitrogen mineralization in manured soil

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Abstract A routine soil testing procedure for soil N mineralization is needed that is rapid and precise. Not accounting for N mineralization can result in the over-application of N, especially in soils with a history of manure application. Our objectives were to compare results from a recently proposed rapid laboratory procedure with: (1) long-term N mineralization under standard laboratory conditions, and (2) actual forage N uptake from soil receiving dairy cattle (*Bos taurus*) manure in a 2-year field study. The rapid procedure is based on the quantity of CO₂-C evolved during 24 h under optimum laboratory conditions following the rewetting of dried soil. Dairy cattle manure was surface applied beginning in 1992 at annual rates of 0, 112, 224, or 448 kg N ha⁻¹ to field plots on a Windthorst fine sandy loam soil (fine, mixed, thermic Udic Paleustalf) near Stephenville, Texas (32°N, 98°W). Results of the one-day CO₂ procedure were highly correlated with soil N mineralized from samples collected in March of 1995 ($P=0.004$) and 1996 ($P<0.001$) and with forage N uptake ($P<0.001$) both years of the study. Residual inorganic N in the same soil samples was poorly correlated with soil N mineralization and forage N uptake.

Keywords Carbon mineralization · Nitrogen mineralization · Soil testing · Manure · Nitrogen uptake

Introduction

Accurate prediction of the quantity of N that is mineralized from soil organic matter during a growing season would result in more efficient use of N fertilizers and manure and decrease the potential for surface and groundwater contamination. Several soil N mineralization procedures have been advocated (Stanford and Smith 1972; Keeney 1982), but these procedures generally are not suited for routine soil testing because of the lengthy time periods they require. The Stanford and Smith (1972) method also underestimates N mineralization in soils normally exposed to repeated wetting and drying cycles (Campbell et al. 1988) because it does not account for the flush of N mineralization that occurs when dry soil is rewetted (Birch 1958; Cabrera 1993).

The concept of decay series has been used to estimate N mineralized from animal manure over several cropping seasons. Methods used to estimate decay series are often indirect, usually involving N uptake by successive crops following manure application. Klausner et al. (1994) reported a decay series of 0.21, 0.09, 0.03, 0.03, and 0.02 for five growing seasons following a dairy cattle manure application in New York state. The first number in the series represents the fraction of N that is mineralized the first year, the second represents the fraction of residual organic N mineralized during the second year, and so on. Decay series estimates are often site specific, may not be sensitive to changing soil conditions, and require an accurate history of previous manure applications.

Computer programs are also available for predicting nutrient release from animal manures. Twelve computer programs evaluated by Thompson et al. (1997), however, used potential N availabilities from manures ranging from 0 to 100% in the year of application, with an

R.L. Haney · F.M. Hons (✉)
Department of Soil and Crop Sciences, Texas Agricultural
Experiment Station, Texas A. and M. University, 2474 TAMU,
College Station, TX 77843-2474, USA
e-mail: f-hons@tamu.edu
Fax: +1-409-8450456

M.A. Sanderson
USDA-ARS Pasture Systems and Watershed Management
Research Laboratory, Curtin Road, University Park,
PA 16802-3702, USA

A.J. Franzluebbers
USDA-ARS J. Phil Campbell, Sr. Natural Resources
Conservation Center, 1420 Experiment Station Road,
Watkinsville, GA 30677-2373, USA

average of 37%. Chang and Janzen (1996) experimentally estimated that 56% of N added in beef feedlot manure was mineralized in their study, while Sanderson and Jones (1997) estimated that 25% of added dairy cattle manure N was removed by coastal bermudagrass [*Cynodon dactylon* (L.) Pers.] in their research.

Residual inorganic soil N, predominantly NO_3^- , has been a useful tool for predicting crop N needs in low rainfall areas, but has generally been less successful in more humid regions because of NO_3^- losses via leaching and denitrification (Schmitt and Randall 1994). The pre-sidedress soil NO_3^- test was developed by Magdoff et al. (1984) based on the premise that not sampling until corn (*Zea mays* L.) reaches a specified growth stage allows N mineralization and N losses to occur as long as possible before a N fertilizer decision is made. This test is widely used in the northeastern USA (Jokela 1989) and is being adopted in some midwestern states (Blackmer et al. 1991). Research in Maryland (Meisinger et al. 1992) and Pennsylvania (Fox et al. 1989), however, showed poor correlations between grain yield and in-season NO_3^- values.

Blackmer et al. (1989) stated that reducing N-fertilizer input by identifying non-responsive fields was an important use of this test in Iowa. Soil NO_3^- content measures N available at the time of sampling and does not necessarily indicate the ability of soils to mineralize additional N, which can be significant in soils receiving manures or other organic wastes.

Organic N is mineralized because of organic-C mineralization, and CO_2 evolution accordingly has been studied as a predictor of soil N mineralization. Castellanos and Pratt (1981) demonstrated that C released as CO_2 during a 1-week aerobic incubation of ten manures was a satisfactory index for estimating manure-N availability in a 10-month greenhouse trial. They hypothesized that a 2- or 3-day incubation might provide an equally satisfactory relationship. Gilmour et al. (1985) suggested that CO_2 evolution might predict net N mineralization from plant residues added to soil, while Gilmour et al. (1996) showed that a 7-day incubation of biosolids could be used to predict decomposition at >60 days. Franzluebbers et al. (1996a) recently reported that net soil N mineralization and soil microbial biomass were related to soil C mineralized as CO_2 in as little as 1 day. The coefficient of determination (r^2) for CO_2 evolved during the first day after rewetting dried soil and net N mineralization after 21 days was 0.85 for eight diverse soils.

The prediction of organic C and N dynamics from CO_2 evolved after rewetting dried soil has a strong theoretical basis. Moderate drying of soil kills a portion of the soil microbial biomass (Jenkinson 1966) as well as rendering a portion of soil organic matter mineralizable because of physical disturbance (Van Gestel et al. 1991). The flush of microbial activity soon after rewetting probably reflects the contribution of both soil microbial biomass and other active organic matter pools that are easily mineralizable. Elliot (1986) hypothesized

that drying/rewetting is one mechanism by which each soil N pool is replenished from successively more recalcitrant or physically protected N pools. Inubushi and Wada (1987) found that air-drying and rewetting soil not only increased the easily mineralizable soil N pool, but also increased the size of a more stable pool that mineralized more slowly. Most biologically-based procedures normally advocate the use of field-moist soil, an additional limitation for routine soil testing that commonly requires dried soil. A procedure that rapidly and precisely estimates N mineralization from dried soil would increase adoption by soil-testing laboratories.

The objective of our study was to determine relationships between the flush of CO_2 evolved during 1 day following rewetting of dried soil, and potential soil N mineralization in the laboratory and forage N uptake in the field from manure-amended soil.

Materials and methods

Field plot design and soil sampling

A dryland field experiment utilizing coastal bermudagrass and bermudagrass overseeded with wheat (*Triticum aestivum* L.) was established near Stephenville, Texas (32°N, 98°W) in May 1992 (Sanderson and Jones 1997). The soil was a Windthorst fine sandy loam (fine, mixed, thermic Udic Paleustalf) with pH approximately 6.5 and containing 120 g kg^{-1} and 660 g kg^{-1} of clay and sand, respectively, in the surface 7.5 cm. Mean soil organic C and total N in this same soil depth were 14.1 g kg^{-1} and 1.3 g kg^{-1} soil. The average annual air temperature is 18°C and annual precipitation averages 750 mm. The experimental design was a 2 (cropping system) \times 4 (manure N rate) factorial within a randomized complete block with four replicates; berms separated the blocks to prevent the overland flow of applied amendments. Each plot was 3 \times 6 m. Dairy cattle manure (0, 112, 224, or 448 kg N ha^{-1} year $^{-1}$) was surface applied in four equal applications each year to the two cropping systems, beginning in February 1992 through 1996. Concentrations of N, P, and K in the manure averaged 20.0, 5.6, and 16.6 g kg^{-1} , respectively. Five harvests were made in 1995, one from wheat and four from coastal bermudagrass. Forage was harvested 5 times from both bermudagrass and bermudagrass overseeded with wheat in 1996. A sickle-bar mower was used to harvest a 1 \times 6-m strip at a 5-cm height from the center of each plot at each harvest.

Forage samples for chemical analysis were hand-clipped from each plot at each harvest to avoid contamination with manure, rinsed with deionized water, and dried at 55°C for 48 h before grinding to pass a 1-mm mesh. Bermudagrass N concentration was determined using a near-infrared reflectance spectrometer (Shenk and Westerhaus 1991). N concentrations of wheat samples were determined by wet chemical procedures (Baethgen and Alley 1989). Forage N uptake was calculated by multiplying dry matter yield and N concentration.

Thirty soil cores (2.5 cm diameter, 0 to 7.5-cm depth) were composited monthly from each plot from February through July in 1995; this was repeated in 1996. Samples were dried in a forced-draft oven at 40°C for 24 h and passed through a 5-mm sieve (Franzluebbers et al. 1996a). Only results for March samplings are reported because this month generally resulted in the highest correlations, and this is also the period that producers in this region would sample soils to allow sufficient time to apply additional fertilizers or manure prior to the spring growth of bermudagrass.

Soil C and N mineralization

Soil C and N mineralization were determined from three 40-g subsamples moistened to 55% water-filled pore space and incubated in 1-l glass jars. C mineralization was determined from CO₂ trapped in 10 ml of 1 M KOH during 24 days of incubation at 25 °C (Franzluebbers et al. 1996a). Alkali traps were titrated with standardized HCl to a phenolphthalein endpoint (Anderson 1982). A vial containing 10 ml water was also placed in each jar to maintain humidity.

N mineralization was determined from soil inorganic-N concentrations at 0 and 24 days of incubation. Subsamples were oven dried at 60 °C for 24 h, and ground to pass a 2-mm sieve. A 7-g portion was shaken with 28 ml of 2 M KCl for 30 min, with the filtered extract analyzed for NH₄⁺-N and NO₂⁻-N plus NO₃⁻-N using autoanalyzer techniques and the modified indophenol blue (Technicon Industrial Systems 1977a) and Cd reduction methods (Technicon Industrial Systems 1977b), respectively. Residual inorganic soil N was defined as the sum of the extracted forms of N given above determined immediately prior to incubation.

Statistical analyses

Linear regression and correlation were used to determine relationships among soil properties and forage N uptake (SigmaStat for Windows version 2.0, 1992–1995; Jandel, San Rafael, Calif.).

Results and discussion

C mineralized in 1 day from dried and rewetted soil was highly correlated with potential N mineralized in 24 days from soil samples collected in March 1995 and 1996 (Fig. 1a). March is the normal time for soil sampling for N recommendations for warm-season forages in this region, and residual soil NO₃⁻ is the soil N test most commonly utilized. Wheat overseeded into bermudagrass and actively growing during soil sampling did not significantly affect the observed relationships (Fig. 1a).

Residual inorganic soil N was poorly correlated with potential N mineralized in 24 days for either year (Fig. 1b). Since mineralization of added manure N should be a principal source of residual inorganic N in this study, it was thought that residual inorganic soil N should correlate with N mineralized in laboratory incubations. Residual inorganic N from early spring soil samples, however, apparently would not be an adequate estimator of potential N mineralization in this manured soil.

Soil N mineralized in 24 days in the laboratory in both study years was highly related with forage N uptake in the field (Fig. 2a). This procedure is too time consuming, however, to be used as a routine soil test. Forage N uptake both years was also very highly correlated with C mineralized in 1 day from dried and rewetted soil (Fig. 2c). The 1-day C mineralization procedure is sufficiently rapid to be used as a routine soil test and explained a slightly greater proportion of the variation in crop N uptake than did potential N mineralization. Slopes of the regressions for C mineralized in 1 day after rewetting dried soil vs. forage N uptake were very

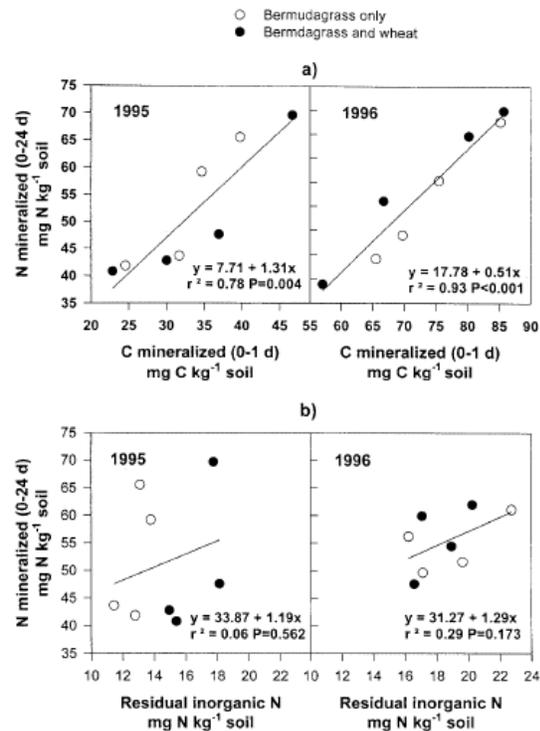


Fig. 1 Relationships of soil N mineralized in 1995 and 1996 with a) C mineralized in 1 day from dried and rewetted soil, and b) soil residual inorganic N

similar for both study years, whereas slopes for 24-day soil N mineralization vs. forage N uptake varied three-fold between years. Wheat overseeded into bermudagrass vs. bermudagrass only did not influence the observed relationships. Residual inorganic soil N was poorly correlated with forage N uptake both years (Fig. 2b).

Thicke et al. (1993) reported that 1 week of aerobic incubation for N contributed as much as a 12-week incubation to models of corn grain yield and total N uptake as determined by stepwise multiple regression. Acid permanganate-, autoclave-, and glucose-extractable N and anaerobic incubation did not consistently contribute to the models. The authors found, however, that although the initial experiments with aerobic incubation resulted in promising relationships, results of field validation experiments were not reliably predicted probably because of yearly weather variation. Other authors have reported moderate correlations between results of chemical tests that extract a fraction of soil organic N and plant N uptake in the greenhouse or soil N mineralization in the laboratory (Keeney and Bremner 1966; Lathwell et al. 1972), but poorer results under field conditions (Fox and Piekielek 1978).

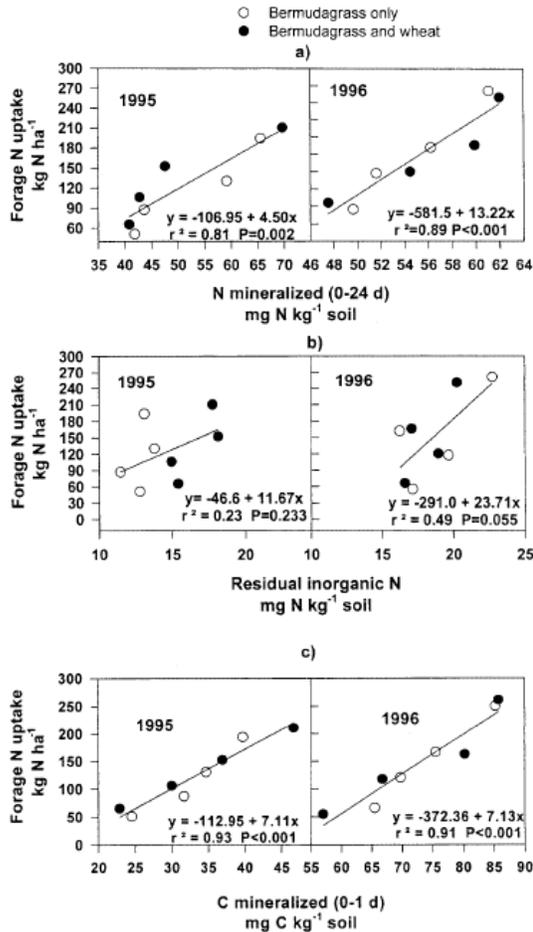


Fig. 2 Relationships of forage N uptake in 1995 and 1996 with **a** soil N mineralized in 24 days, **b** soil residual inorganic N, and **c** C mineralized in 1 day from dried and rewetted soil

The flush of CO₂ in 1 day after rewetting dried soil appeared to adequately represent the contribution of the active soil microbial biomass and soil organic matter pools that were readily mineralizable (Franzuebbers et al. 1996b), based on relationships with laboratory N mineralization and forage N uptake. Partial desiccation of microbial biomass due to drying and rewetting and subsequent release of the desiccated microbial biomass as CO₂ may have contributed to these results. The Windthorst soil is naturally exposed to temperatures of >40°C, the drying temperature used in this study, during summer months. Unpublished data from our laboratory shows only slightly greater C mineralization (2–3%) from soils dried at 40°C vs. continuously moist soils, and the values are highly correlated ($r^2 > 0.90$). Therefore, the flush of CO₂ following the rewetting of

soil dried at 40°C may mimic the natural CO₂ flush of soil in the field from the partial desiccation and release of microbial biomass.

In summary, quantities of CO₂-C evolved during the first day after rewetting dried soil were closely related to longer-term soil N mineralization and forage N uptake from soil receiving dairy cattle manure. Because of its relative simplicity, rapidity, and reliability, we recommend that this procedure be considered as a rapid test to estimate potential net N mineralization in manure-amended soils.

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Appendix C4

Soil carbon and nitrogen mineralization: influence of drying temperature

DIVISION S-3—SOIL BIOLOGY & BIOCHEMISTRY

Soil Carbon and Nitrogen Mineralization: Influence of Drying Temperature

R. L. Haney,* A. J. Franzluebbers, E. B. Porter, F. M. Hons, and D. A. Zuberer

ABSTRACT

Carbon and N mineralization in dried soils that are rewetted has been proposed as a rapid index of C and N mineralization potential and to reflect soil management, but further research is needed on effects of soil type and drying temperature for this approach. The objective of this study was to determine the effect of maintaining soil field moisture or drying soil at 40, 60, or 100°C followed by rewetting and a 3-d incubation on C and N mineralization across diverse soil types. Strong correlations between C mineralized in 24 d from field moist soils vs. C mineralized in 24 h from soils dried at 40 or 60°C were observed. Carbon mineralization values for 24 vs. 3 d resulted in nearly linear relationships for all drying treatments. Nitrogen mineralization in 24 d from moist vs. dried at 40 or 60°C and rewetted soils were also highly correlated with field moist N mineralization. The drying and rewetting pre-incubation of soil followed by a 3-d incubation was shown to be a useful indicator of longer-term (24 d) C mineralization potential. Nitrogen mineralization potential may also be obtained after drying/rewetting at 40 or 60°C without the need for keeping soil in a continuously field-moist state.

CARBON AND N MINERALIZATION can be a useful tool for quantifying the impact of various organic and inorganic amendments on soil functions. Carbon mineralization is generally determined by monitoring CO₂ fluxes from field-moist samples that are wetted to roughly 50% of field capacity and subsequently incubated in the laboratory for various periods of time. The incubation period is generally several weeks long, depending on the objective of the study. Short incubations and air-drying soil facilitate routine soil testing procedures and for certain tests air-drying avoids biochemical artifacts that could occur if soils are kept moist before analysis.

Adopting a technique that uses dried soil may significantly reduce variability within the same soil sample and reduce the amount of refrigerated space necessary for storage of moist soils. Soil samples from the same site can vary greatly in moisture content, depending on season or short-term weather patterns. Drying soil holds potential to minimize this variability. Drying and rewetting soil may also permit researchers to determine C and N mineralization potentials on dry, archived soil

samples. When a soil analysis requires field-moist soil, a pre-incubation time of 7 to 10 d after rewetting dried soil may be used to equilibrate the samples before analysis (Franzluebbers et al., 1996).

Rewetting dried soil is thought to alter the soil physicochemical environment and make it an unrealistic treatment (Martens, 1995). On the other hand, laboratory drying and rewetting tends to produce a uniform release of C and N and is a natural process that occurs under field conditions (Birch, 1958, 1959, 1960). Furthermore, short-term C mineralization (1–3 d) of soil after drying (40 or 60°C) followed by rewetting correlates strongly with longer-term (100-d) CO₂ evolution and soil microbial biomass C (Franzluebbers et al., 2000; Haney et al., 1999).

Marumoto et al. (1982) and Sparling et al. (1995) have shown that it may be possible to estimate soil C and N mineralization potential by monitoring the fluxes of CO₂ following the rewetting of dried soil. Other authors have stated that the amount and quality of substrates available for mineralization may be quantified using CO₂ evolution (Sorensen, 1974; Sparling and Ross, 1988). Anderson and Domsch (1978) suggested that the size of the soil microbial biomass is reflected by the short-term flush of CO₂ after amending labile substrates. This is the basis for substrate induced respiration (SIR) method for determination of soil microbial biomass. If the evolution of CO₂ following rewetting of dried soils can be related to soil microbial biomass and potential mineralizable C and N for different soils under different environments then this method might serve as a rapid indicator of potential C and N mineralization.

Chemical and physical disturbances of soil organic matter have been proposed as mechanisms for increasing the flush of CO₂ associated with soil drying and rewetting (van Gestel et al., 1991). For example, Franzluebbers and Arshad (1999) ground dry soils to a powder then rewetted the soils and trapped evolved CO₂. This treatment resulted in a greater flush of CO₂ than from undisturbed soil. However, C mineralized in 3 d from the disturbed soils was strongly correlated with 24-d C mineralization in undisturbed samples. Similar results were observed when comparing N mineralization as affected by drying temperature.

Currently, most soil incubations use field-moist soil. The flush of C and N after drying and rewetting could possibly become a rapid assessment tool for monitoring changes in C and N mineralization potential due to organic or inorganic inputs as well as inputs from different management strategies. The objective of this study was to compare soil C and N mineralization after drying and rewetting with those maintained field-moist to ex-

R.L. Haney, USDA-ARS, 808 E. Blackland Rd, Temple, TX 76502; E.B. Porter, F.M. Hons, and D.A. Zuberer, Dep. of Soil & Crop Sciences, Texas A&M University, Texas Agricultural Experiment Station, College Station, TX 77843; A.J. Franzluebbers, USDA-ARS, 1420 Experiment Station Road, Watkinsville, GA 30677-2373. Received 15 Jan. 2002. *Corresponding author (rhaney@spa.ars.usda.gov).

Table 1. Soil location, classification, and land management.

Location	Soil Classification	Land Management	pH	Clay	Organic C
				%	g C kg ⁻¹ soil
Weslaco, TX	Hidalgo sandy clay loam (fine-loamy, mixed, active, hyperthermic Typic Calcustoll)	Irrigated maize (<i>Zea mays</i> L.) under no-tillage	8.0	28	9.3
Corpus Christi, TX	Victoria clay (Fine, smectitic, hyperthermic Udic Haplustert)	Sorghum [<i>Sorghum bicolor</i> (L.) Moench], conventional tillage with 60 kg N ha ⁻¹	6.5	41	11.5
Overton, TX	Bowie fine sandy loam (Fine-loamy, siliceous, semiactive, thermic Plinthic Paleudult)	Bermuda grass hay [<i>Cynodon dactylon</i> (L.) Pers.] receiving 100 kg N ha ⁻¹ as poultry litter	5.9	6	6.5
Stephenville, TX	Windthorst fine sandy loam (Fine, mixed, active, thermic Udic Paleustalf)	Bermuda grass hay receiving 400 kg N ha ⁻¹ as dairy manure	6.3	13	13.0
Lubbock, TX	Acuff loam (Fine-loamy, mixed, superactive, thermic Aridic Paleustoll)	Sorghum receiving 200 kg N ha ⁻¹	7.4	22	9.3
Clinton, LA	Providence silt loam (Fine-silty, mixed, active, thermic Oxyaquic Fragludalf)	Alamo switch grass	6.6	18	22.5
Kenai, AK	Kenai silt loam (Medial over loamy, mixed, superactive Typic Haplocryod)	Bluegrass pasture	7.2	19	48.3
Oakwood, OK	Lincoln loamy fine sand (Sandy, mixed, thermic Typic Ustifluvent)	Wheat (<i>Triticum aestivum</i> L.), continuous tillage with 20 kg N ha ⁻¹	6.5	10	10.8

plore the possibility of using of dried soil in routine incubations as opposed to field-moist soil.

MATERIALS AND METHODS

Soil samples were collected from four states. Five of the soils were from Texas (Windthorst, Acuff, Hidalgo, Bowie, Victoria series), and one each was from Alaska (Kenai), Louisiana (Providence), and Oklahoma (Lincoln) (see Table 1 for soil descriptions). Sampling depth in each case was 0 to 7.5 cm. (Table 1). Soil pH was measured with 2:1 water/soil ratio. Soil texture was determined by the hydrometer method and soil organic C from the modified Mebius method (Thomas, 1996).

Each sample was homogenized and passed through a 5-mm sieve, and then split into four treatment groups with each soil sample having 160 g on an oven-dried basis. All soil samples were incubated for 7 d at about 50% of field capacity (range of water addition was 6–14 mL per 40 g of soil). After that, four treatments (three replicates each) were imposed: 24 h drying at 40, 60, 100°C, or continuously moist at 50% field capacity.

Dried soil treatments were subsequently rewetted with water to approximately 50% field capacity. After rewetting, soil samples were incubated at 25°C in 1-L glass jars with an alkali trap containing 10 mL of 1 M KOH to adsorb CO₂ and a container with 10 mL water to maintain humidity. Traps were changed at 1, 2, 3, 4, 5, 7, 14, and 24 d after incubation began and titrated with 1 M HCl (Anderson, 1982).

Nitrogen mineralization was determined by subtracting the initial inorganic N concentration (NH₄⁺-N and NO₃⁻-N) of nonincubated soil samples from soil N extracted after 24-d of incubation. Inorganic N was extracted from 7-g soil subsamples using 28 mL of 2 M KCl. Samples were shaken for 30 min on a reciprocal shaker, filtered, and the extracts analyzed for NH₄⁺-N and NO₃⁻ plus NO₂⁻-N using an autoanalyzer (Technicon Industrial Systems, 1977a, 1977b). The sum of the above N forms was designated inorganic N.

The experiment was analyzed as a completely randomized factorial design with eight soil types and four incubation treatments. Linear regression was determined to show the strength of relationships. We used SigmaStat ver. 2.03 (SPSS Inc. Chicago, IL.) for all the statistical work.

RESULTS AND DISCUSSION

Increasing the drying temperature of soils from 40 to 100°C caused a substantial change in the evolution of CO₂ during the first day after soil rewetting. Of the eight soils studied, seven reached their peak rate of CO₂ evolution on or before the second day of incubation (Fig. 1). The flush of CO₂ from drying and rewetting was essentially complete for all soils by the fourth day of incubation.

The quantity of CO₂ produced (C mineralized) in the first 4 d of the incubation was highly correlated to drying temperature ($r = 0.98$ data not shown). This suggests that short-term CO₂ flux after soil drying and rewetting may provide a stable index for comparative analysis. Results for continuously moist soils vs. soils dried at 40°C (data not shown) were not significantly different from one another at $P < 0.05$, as analyzed by ANOVA.

We observed close correlations between 1-d CO₂ evolution following rewetting of soils dried at 40 or 60°C and 24-d cumulative CO₂ release from continuously moist soils, with r values of 0.99 and 0.98, respectively (Table 2). This CO₂ evolution may partially be due to reestablishment of the microbial population following microbial death due to drying (Sorensen, 1974) and osmotic shock following rewetting (Kieft et al., 1987).

Soils dried at 100°C exhibited both increased C mineralization and greater variability as compared with the 40 or 60°C treatments (Table 2). Birch (1959) used various soils that were air-dried (25°C) or dried at 100°C (with soil drying lasting for 24 to 48 h) and observed a flush of C and N after rewetting. He stated that desiccation of microbial biomass likely occurred during drying. It is likely that the difference in the flush of CO₂ from soils following drying and rewetting originates predominantly from killed soil microbial biomass that is quickly mineralized by the remaining heat-resistant or protected microorganisms. The poor correlation of 1-d CO₂ to

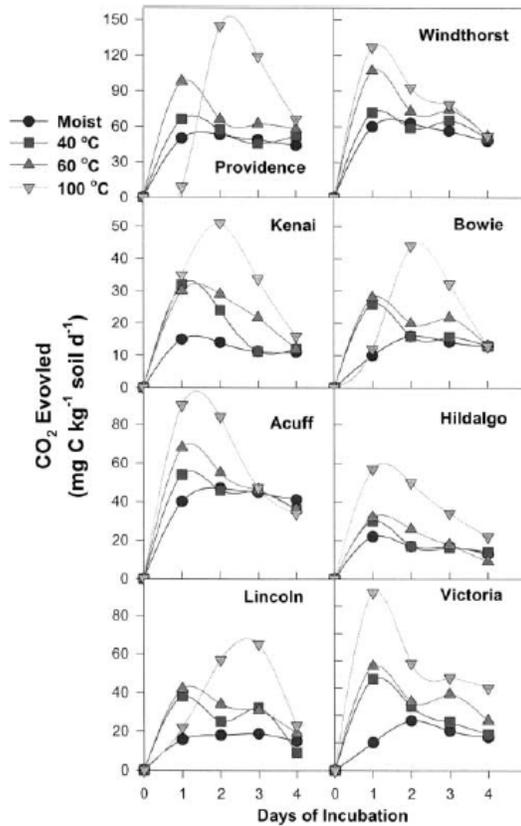


Fig. 1. Rate of CO₂ evolution during the first 4 d of incubation after drying at 40, 60, and 100°C and then rewetting.

24-d CO₂ shown in Table 2 for the 100°C drying treatment was most likely due to a greater portion of the indigenous microbial community affected by 100°C over the 40 or 60°C treatments. By Day 3 of the incubation, however, the evolution of CO₂ from all dried treatments was strongly correlated with field moist 24-d C mineralization, indicating the relatively rapid recovery of the more heat-sensitive microorganisms even when dried at 100°C (Table 2). Therefore, a 24-h recovery period after drying at 100°C and rewetting was insufficient to estimate the complete flush of CO₂.

Nitrogen mineralized after 24 d from soils that were dried at different temperatures and then rewetted was strongly related to N mineralized after a 24-d incubation of continuously moist soils (Fig. 2). Essentially a 1:1 relationship in N mineralized was observed for soils dried at 40°C and rewetted compared with soils kept continuously moist. As the drying temperature increased, the relationships between N mineralized in dried/rewetted vs. moist soils became more variable, although they were still significantly correlated. Nitrogen immobilization due to microbial uptake or N volatilization may have occurred following the higher temper-

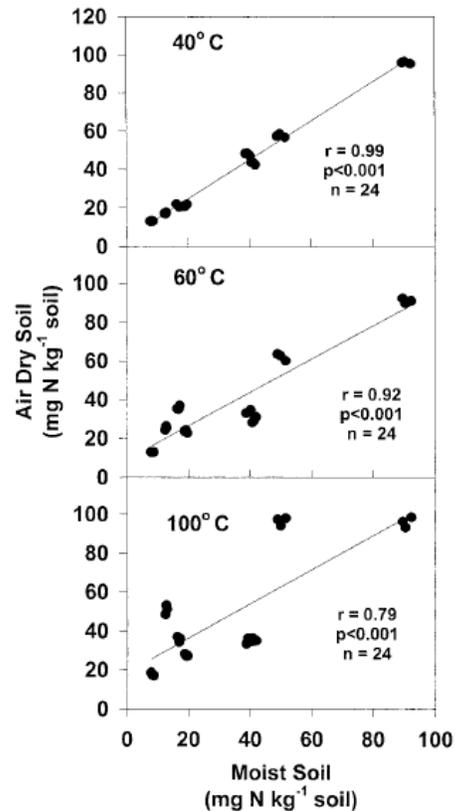


Fig. 2. Nitrogen mineralized after 24 d in soils dried at 40, 60, or 100°C following rewetting compared with 24-d N mineralization in soils that were kept continuously moist.

ature treatments during the 24-d incubation and may possibly explain the increasing variability with increasing drying temperature.

The intercepts of the regression lines increased as soil drying temperature increased, suggesting that the pool of mineralizable N was larger in soils that were dried and rewetted than in field-moist soils (data not shown). One possible explanation may be that protein denaturation begins around 60°C, which suggests that proteins may have been degraded to amino acids and NH₄⁺ by

Table 2. Correlation coefficients of potential C mineralization from soils with pretreatment drying and rewetting followed by 3-d incubation with 24-d incubation of field-moist soil (*n* = 24).

Incubation time	Temperature	Correlation coefficient
d	°C	<i>r</i>
1	40	0.99***
1	60	0.99***
1	100	0.54 ^{NS} †
3	40	0.98***
3	60	0.99***
3	100	0.98***

*** Significant at *P* < 0.001.

† Not significant at *P* < 0.05.

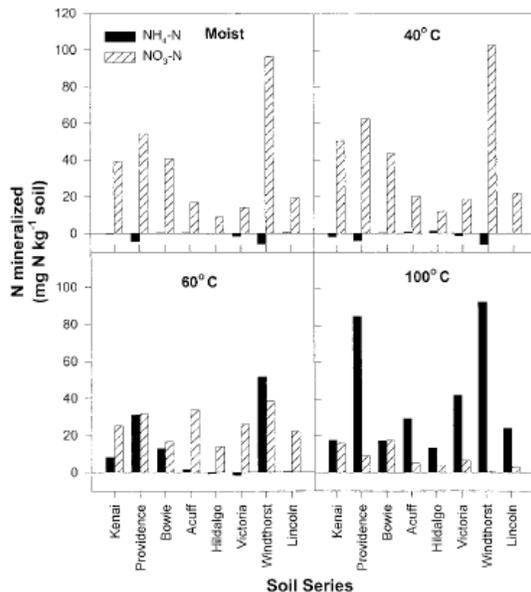


Fig. 3. Nitrogen mineralized ($\text{NH}_4^+\text{-N}$, $\text{NO}_2^-\text{-N}$, $\text{NO}_3^-\text{-N}$) after 24 d in soils kept continuously moist vs. soils that were dried and rewetted.

the drying process. As drying temperature was increased from 40 to 100°C, $\text{NH}_4^+\text{-N}$ also became more prevalent than $\text{NO}_3^-\text{-N}$ (Fig. 3). Total inorganic N concentrations from summing both $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ were similar in all drying treatments, but $\text{NH}_4^+\text{-N}$ increased with increasing temperature, indicating that soil-nitrifying bacteria were more sensitive to desiccation at higher drying temperatures, but were not completely eliminated even at 100°C. It is also interesting to note that soils dried at 40°C and rewetted, total N mineralization and individual quantities of NH_4^+ and NO_3^- were not significantly different compared with continuously moist samples. Therefore, as long as standardized laboratory techniques are followed, the use of soil dried at 40°C and rewetted should be useful to evaluate potential N mineralization across a wide range of soils without having to maintain soil in a field-moist state.

CONCLUSIONS

The flush of CO_2 after 3 d of incubation from soils that were dried at 40, 60, or even 100°C and rewetted were highly correlated to 24-d incubations with soil that was incubated in a field-moist state. Drying soil at 100°C, rewetting, and then determining CO_2 evolution for 1 d was not reliable for estimating longer-term C mineralization (24 d). However, even when dried at 100°C, soil microbial respiration rebounded sufficiently within 3 d of rewetting to strongly correlate with longer-term C mineralization. Drying at increasing temperatures then rewetting soils produced a near linear increase in CO_2 release across a variety of soil types. The flush of CO_2 after drying and rewetting reaches its peak on the first

or second day of incubation, depending on drying temperature, however, we recommend a 3-d, as opposed to a 1-d incubation following drying and rewetting, to ensure a more complete recovery of the CO_2 flush.

For potential N mineralization, soil dried at 40°C was highly related to soil kept at field-moist conditions and no significant differences were detected in the amount of $\text{NH}_4^+\text{-N}$ or $\text{NO}_3^-\text{-N}$ when comparing dried (40°C) vs. field-moist soil.

Drying and rewetting may serve as a useful alternative to maintaining soils in a field-moist state for estimating potential C and N mineralization.

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