Final Report-Phase II

Evaluation of Alternative Causes of Wide-Spread, Low Concentration Perchlorate Impacts to Groundwater

SERDP Project ER-1429

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ABSTRACT

In recent years, a number of potential non-military sources of perchlorate to groundwater have been identified, including Chilean nitrate fertilizers used for production of tobacco and select other crops. Through funding provided by the Department of Defense (DoD) Strategic Environmental Research and Development Program (SERDP), Geosyntec Consultants, Inc. (Geosyntec) and North Carolina State University (NCSU) conducted a study to estimate the potential impacts of historic and current use of Chilean nitrate fertilizers on soil and groundwater quality at two different tobacco research stations in North Carolina.

Baseline analysis of soil and groundwater samples at each of the research stations failed to detect perchlorate, indicating that past Chilean nitrate fertilizer applications at these sites are not causing long-term perchlorate impacts. Analysis of soil and groundwater samples following application of a currently-available Chilean nitrate fertilizer (potassium nitrate) also failed to detect perchlorate, in large part because the amount of perchlorate in the newer available products is orders of magnitude lower than the perchlorate content of the historical Chilean nitrate fertilizer products.

To assess the potential behavior of perchlorate applied historically by standard fertilization practices, nitrate was used as a surrogate for perchlorate. The behavior of perchlorate and nitrate applied to soils using Chilean nitrate fertilizer would be expected to be somewhat similar. Evaluation of nitrate fate and transport demonstrated that nitrate infiltrated into the soil profile, and was also uptaken by tobacco plants. At one research station, the depth of nitrate infiltration was limited by a low-permeability geological layer, preventing underlying groundwater impact. At the second site, nitrate infiltrated to depths capable of impacting shallow groundwater. Assuming historic use of fertilizer products containing higher levels of perchlorate, it could be assumed that surface-applied perchlorate could have contacted shallow groundwater at the second site.

While the results of this study do not conclusively demonstrate perchlorate impacts to soil and groundwater from past or current fertilizer applications, monitoring of applied nitrate suggests that the potential exists/existed to impact groundwater at some sites, depending on site-specific factors. Further research is needed to better understand the potential long-term impacts of historical Chilean nitrate fertilizer application on groundwater systems.

ACKNOWLEDGEMENTS

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LIST OF ACRONYMS

°C	Degrees Celsius
ASL	Analytical Service Laboratory
CAS	Columbia Analytical Services
cm	Centimeters
DO	Dissolved oxygen
DoD	Department of Defense
ft	Feet
ft^2	Square feet
ft bgs	Feet below ground surface
g	Grams
gpm	Gallons per minute
GPS	Global positioning system
HCl	Hydrochloric acid
HSA	Hollow stem augers
KCl	Potassium chloride
kPA	Kilopascals
lbs	Pounds
lbs/acre	Pounds per acre
M	Molar
mg	Milligrams
mg C/L	Milligrams carbon per liter
mg NO_3-N/g	Milligrams nitrate-nitrogen per gram
mg NO_3-N/L	Milligrams nitrate-nitrogen per liter
mg/kg	Milligrams per kilogram
mL	Milliliters
mm	Millimeter
N	Nitrogen
NCSU	North Carolina State University
NO_3	Nitrate
ORP	Oxidation-reduction potential
oz	Ounce
ppb	Parts per billion
ppm	Parts per million
PVC	Polyvinyl chloride
QA/QC	Quality-assurance/quality-control
rpm	Revolutions per minute
SERDP	Strategic Environmental Research and Development Program
UAN	Urea-ammonium-nitrate
µg/L	Micrograms per liter
µg/kg	Micrograms per kilogram
µL	Microliters

1. INTRODUCTION

1.1 Background

The frequency of detection of wide-spread, low concentration perchlorate impacts to groundwater is increasing as regulators nationwide require perchlorate analysis as part of regional groundwater monitoring programs. Over the past few years, various natural and non-military, manmade sources of perchlorate have been identified, including Chilean nitrate fertilizers. Chilean nitrate fertilizers are manufactured from naturally-occurring caliche deposits mined from the Atacama Desert region of Chile (Urbansky et al., 2001a; Urbansky et al., 2001b). Chilean nitrate produced by SQM Corporation and all fertilizers derived partially or completely from Chilean nitrates may contain appreciable perchlorate. Prior to 2002, the estimated perchlorate content in Chilean nitrate fertilizers was 0.2% (Urbansky et al., 2001a), which may be significant relative to the low groundwater action levels in most states.

Chilean nitrate fertilizers were used historically in the United States, particularly in the early part of the twentieth century, for crops such as citrus, cotton, tobacco and corn (Howard, 1931; Goldenwieser, 1919; Mehring, 1943). It is estimated that between 1909 and 1929, 13 million tons of Chilean nitrate was consumed in the U.S. Between 49% and 70% of the imported Chilean nitrate was used as fertilizer, with an average of approximately 65% (Brand, 1930). Assuming an average perchlorate concentration of about 0.2% in the Chilean nitrate, and that 65% of the imported Chilean nitrate (about 12 million tons) was used as fertilizer, then approximately 49 million pounds of perchlorate is likely to have been applied to agricultural soils during this time period.

Chilean nitrate fertilizer was commonly used in the U.S. as a source of nitrogen for tobacco plants, particularly in the early 1900s. Fertilizer application rates for tobacco varied with the season and soil quality; however, application rates of 30 to 40 pounds (lbs) of nitrogen per acre were typically recommended (Bennett et al, 1953). To obtain this amount of nitrogen from nitrate of soda (16% nitrogen), approximately 185 to 250 pounds of nitrate of soda would have been applied per acre of tobacco. This range of application rates is similar to the application rates of nitrate of soda used today for certain tobacco crops (i.e., 3-5 lbs per 100 square yards, or 195-325 lbs/acre). Prior to 2002, this Chilean nitrate fertilizer application rate would correspond to a perchlorate application rate of approximately 0.4 to 0.5 lbs/acre. From 1909 to 1929, Kentucky was the largest producer of tobacco and harvested 10,000,000 acres (http://www.nass.usda.gov/Statistics_by_State/Kentucky/index.asp). North Carolina was the second highest producer of tobacco, harvesting over 9,000,000 acres (http://www.nass.usda.gov/Statistics_by_State/North_Carolina/index.asp).

While the use of Chilean nitrate fertilizers has steadily declined since about the 1930s, there is evidence of continued use through to the present day. For example, imports of fertilizer grade sodium nitrate supplied 27% and 6% of the total nitrogen used as fertilizer in 1939 and 1954, respectively. In 1999, it was estimated that some 75,000 tons of Chilean nitrate fertilizer were used annually in the United States on cotton, tobacco and fruit crops (Urbansky et al., 2001a; Renner, 1999). Through changes in the refinement processes since 2001, the current perchlorate concentration is reported as 0.01% (Urbansky et al., 2001b), which is more than an order of magnitude improvement compared to historic perchlorate contents. However, this amount still represents the potential introduction of more than 15,000 lbs of perchlorate annually to agricultural soils, the fate of which is not well understood.

The application of these perchlorate-containing fertilizers over many decades through the present day (albeit in much lower amounts) may explain the continued presence of low concentrations of perchlorate in soil and groundwater in some agricultural areas and watersheds. The continuing impacts of nitrate to groundwater in former agricultural areas urbanized since the 1940s is evidence of the potential for long lasting impacts of past fertilization practices on some regional watersheds (Fogg et al., 1998).

Through funding provided by the Department of Defense's (DoD) Strategic Environmental Research and Development Program (SERDP), Geosyntec Consultants, Inc. (Geosyntec) and North Carolina State University (NCSU) conducted a study to quantitatively estimate the potential impacts of historic and current use of Chilean nitrate on soil and groundwater quality. The study was conducted at the Oxford Tobacco Research Station, Granville County, NC, and the Border Belt Tobacco Research Station, Columbus County, NC. This Final Report presents the approach, methodology and results of the study.

1.2 Study Objectives

The overall objective of this study was to quantitatively estimate and document the potential perchlorate impacts from historic and continuing application of Chilean nitrate fertilizer to the environment. More specifically, the study was performed to assess two avenues of potential impact, namely:

1. Historic Impacts: The historic use of Chilean nitrate fertilizer has been postulated to be the cause of perchlorate impacts to groundwater in some agricultural areas. However, there is debate at some sites whether perchlorate applied to soils via Chilean nitrate fertilizer decades ago could still be causing impacts to groundwater today. A specific objective of this project was to attempt to quantify the potential impact of perchlorate from Chilean nitrate fertilizer historically applied to two different agricultural sites.

2. Ongoing Impacts: The behaviour (persistence and fate) of perchlorate in soil and groundwater due to Chilean nitrate fertilizer application has not previously been assessed. A specific goal of this study was to track the fate and mass balance of perchlorate applied to a tobacco crop at two different agricultural sites over a single growing season.

At the outset of the project, it was anticipated (based on discussions with NCSU) that it might be possible to procure a sufficient quantity of the historic fertilizer material containing the higher perchlorate content for use in the study. However, during execution of the project, this product could not be procured in sufficient quantity (there appears to be no remaining sources of the historic material). As a result, a potassium nitrate product currently available from SQM was used to address Objective 2 of the project. Previous analyses of various nitrate products by one of the principal investigators (Wayne Robarge; data not shown) suggested that the potassium nitrate substitute would still contain perchlorate, albeit at levels (~0.01 to 0.003%) several orders of magnitude lower than the original nitrate of soda product. Based on these data, the project proceeded with the alternative product.

2. SITE BACKGROUND

The field experiments were conducted at two research stations: the Oxford Tobacco Research Station, Granville County, NC, and the Border Belt Tobacco Research Station, Columbus County, NC. A description of each site is provided in the following sections.

2.1 Oxford Tobacco Research Station

2.1.1 Site Location and Description

The Oxford Tobacco Research Station (Oxford station) is located near Oxford, NC (Granville County) in the Piedmont region of North Carolina. Established in 1910 as a joint effort between the North Carolina Department of Agriculture and the U.S. Department of Agriculture, the station has been dedicated to solving problems related to tobacco production. Scientific investigations throughout nearly a century of research at the stations have included tobacco fertility requirements, cultural practices and insect and disease control. The first flue-cured tobacco varieties with resistance to bacterial wilt, black shank, and root-knot nematode were developed at the Oxford station. Part of the current mission of the research station remains to increase tobacco production efficiency, tobacco quality, and identify production management schemes for tobacco that conserve and protect water.

Currently the research station comprises a total of 426 acres with 110 acres devoted to cropland research and the remainder used for woodlands and infrastructure (Figure 2-A).

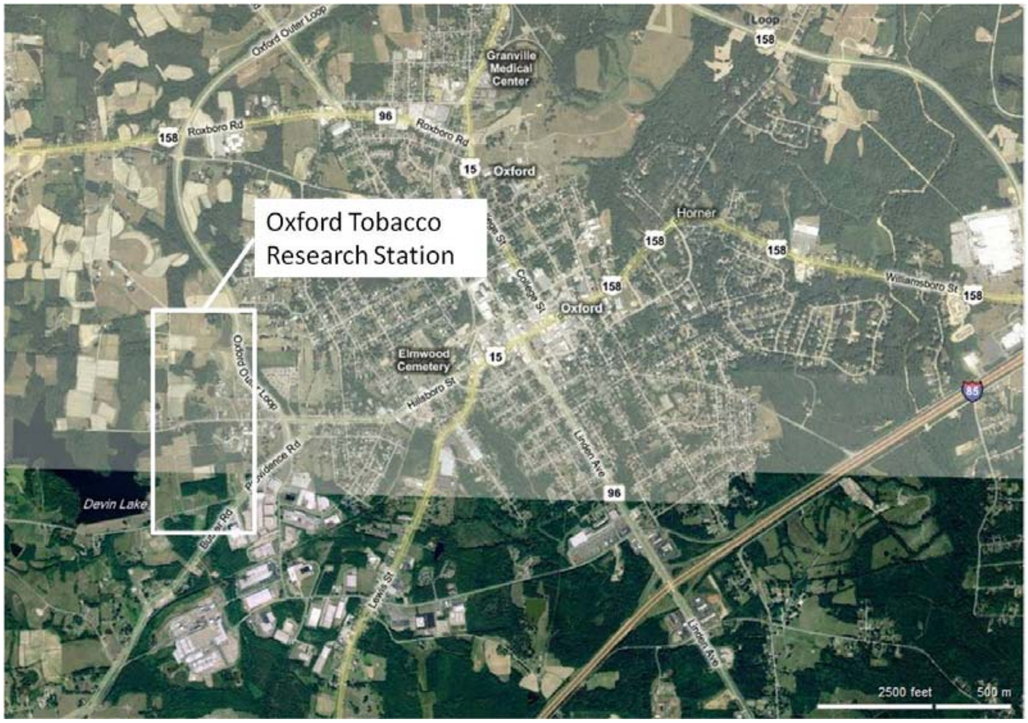

Figure 2-A Oxford Tobacco Research Station (Oxford Station), Oxford, NC (Granville County). (Image courtesy of Microsoft Corporation, 2010)

In addition to staff with extensive experience in tobacco production, the station includes small bulk tobacco barns used for curing, a pack house for storing and sampling tobacco, needed field equipment for planting, fertilizing and harvesting tobacco, greenhouses and maintenance facilities. There are over 9 acres of irrigation ponds at the facility with associated irrigation pumps and piping.

2.1.2 Site Geology and Hydrogeology

The geology of the North Carolina Piedmont is a complex of very old metamorphic and igneous rocks. Sharp boundaries separate many of the major rock bodies and produce abrupt changes in soil materials in relatively short distances. The Oxford station is located on one of the two felsic crystalline systems that comprise part of the North Carolina Piedmont. Typically the bedrock is granite, granite gneiss, mica gneiss and mica schist. Areas of slightly more mafic rock or a complex of felsic rock cut by dikes of gabbro and diorite can also be found. Typical topography in this region varies. Broad gently sloping uplands are common, as are moderately to steeply sloping areas with narrow convex ridges and steep valley slopes (Aull et al., 1978a). Most of the field crop locations are on the gentle upland reaches, but even across these areas, changes of 30 feet (ft) or more in elevation are possible.

The dominant soils used for research at the Oxford station are the Vance (Fine, mixed, semiactive, thermic Typic Hapludults) and Helena (Clayey, mixed, thermic Aquic Hapludults). Typically the Vance and Helena soils occupy more rolling hummocky landscapes, with the Vance soils occurring on convex slopes and ridges, and the Helena soil occupying concave areas along intermittent streams and heads of drains. The fine mixed mineralogy of these soils results in a dense, very firm B horizon that is less permeable to air and water than other less predominant soils found on the station (Aull et al., 1978a). Because of the presence of the denser B horizon, both the Vance and Helena soil series are characterized by the possibility of perched water tables for significant periods of time depending on frequency of seasonal rains. The presence of slowly permeable subsoil horizons together with local topography also present the possibility for sub-surface lateral flow.

Annual precipitation at the Oxford station is approximately 44 inches (average based on 24-year period, 1952-1975), which is distributed roughly equally throughout the year. The wettest month is July and the driest month is either October or November. However, actual rainfall patterns can be highly variable and it is not uncommon to have 2-3 week periods of no rainfall with accompanying high ambient temperatures (large evapotranspiration demand). The State Climate Office of North Carolina maintains a meteorological station at the Oxford station and current and historical records are available.

2.1.3 Assessment of Historic Impacts

Traditionally, the primary emphasis of the Oxford station has been tobacco breeding and production. It is probably safe to assume that all the acreage currently used for station field experiments has at one time been under tobacco production. While there are some historical records regarding field plot trials and sources and amounts of fertilizer applied, these records extended only back to the mid-1970's. The 110 acres actually used for tobacco row crop production at the station are divided into designated land units.

2.2 Border Belt Tobacco Research Station

2.2.1 Site Location and Description

The Border Belt Tobacco Research Station (Whiteville station) is located near Whiteville, NC (Columbus County) in the southeastern portion of the North Carolina Coastal Plain. Founded in 1949, the station moved to its current location in 1956. Historically, the station is concerned primarily with production of flue-cured tobacco, including breeding (Aull et al., 1978b). Today, in addition to tobacco, research projects at the station focus on genetic studies on corn, soybean, and peanuts. Because of its location, the Whiteville station affords an opportunity to study certain diseases and plant pathogens in the field that cannot be duplicated elsewhere.

The Whiteville station consists of 102 acres on level to gently sloping topography (Figure 2-B). As is common in the middle Coastal Plain region of North Carolina, elevation across the station ranges from only 95 to 105 ft. Approximately 50 acres at the station are devoted to research, with the remainder occupied by service roads, buildings and bordering woodland. In addition to the research fields, the station has several greenhouses with hydroponics, curing barns, maintenance shops and a well trained staff in the cultural management practices for tobacco production in this portion of North Carolina.

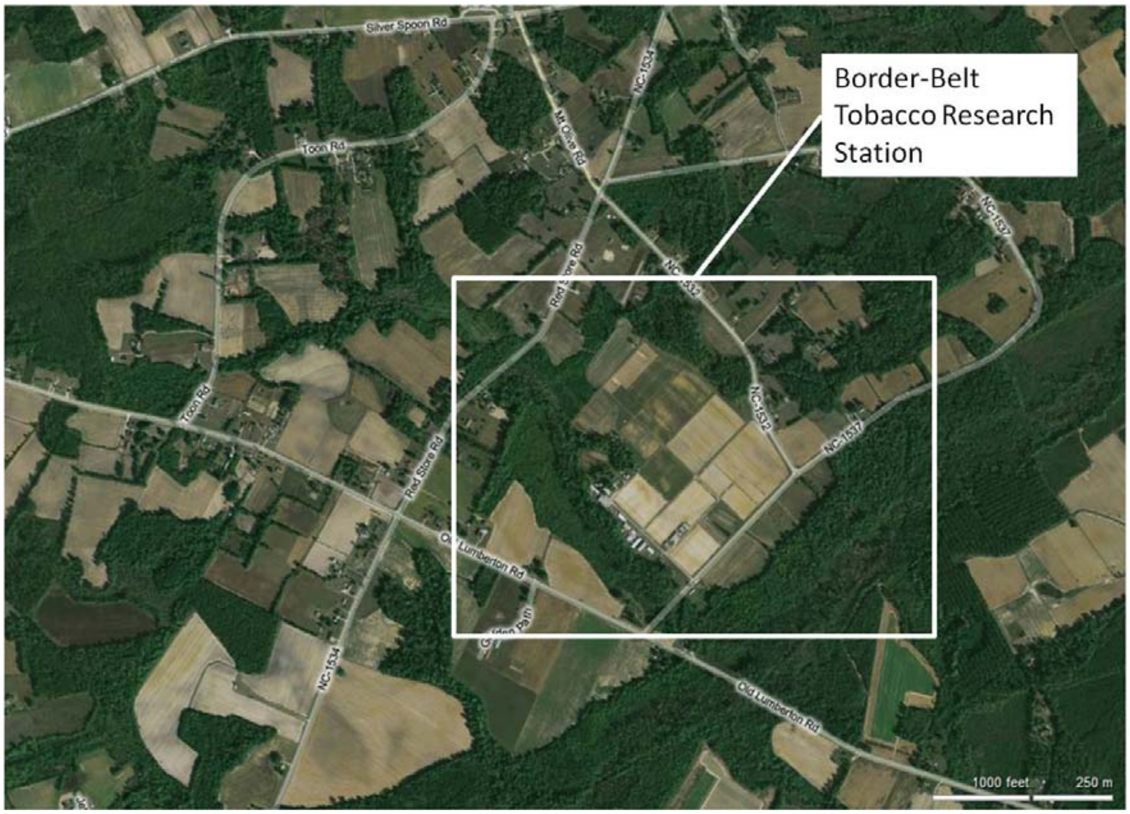

Figure 2-B Border Belt Tobacco Research Station (Whiteville Station), Whiteville, NC (Columbus County). (Image courtesy of Microsoft Corporation, 2010)

2.2.2 Site Geology and Hydrogeology

The geomorphology of the Coastal Plain region of North Carolina is complex, consisting of a series of scarps and deposition events across recent geologic time and accompanying changes in sea level (Aull et a., 1978b). Understanding soil position on the landscape in the Coastal Plain of North Carolina requires understanding drainage of the surrounding land by bisecting streams. Because precipitation exceeds evapotranspiration, the resulting water table under the soil surface varies with distance away from bisecting streams. As such, soil drainage class, which is a dominant factor in soil formation on the Coastal Plain, changes across the landscape with the most well-drained soils occurring at the upper slope position near streams, and more poorly drained soils occurring both in the alluvial areas next to streams and in the broad, nearly level surfaces between streams (Figure 2-C). This is the reverse from what is often expected in more upland situations, such as in the Piedmont or Mountain regions of North Carolina.

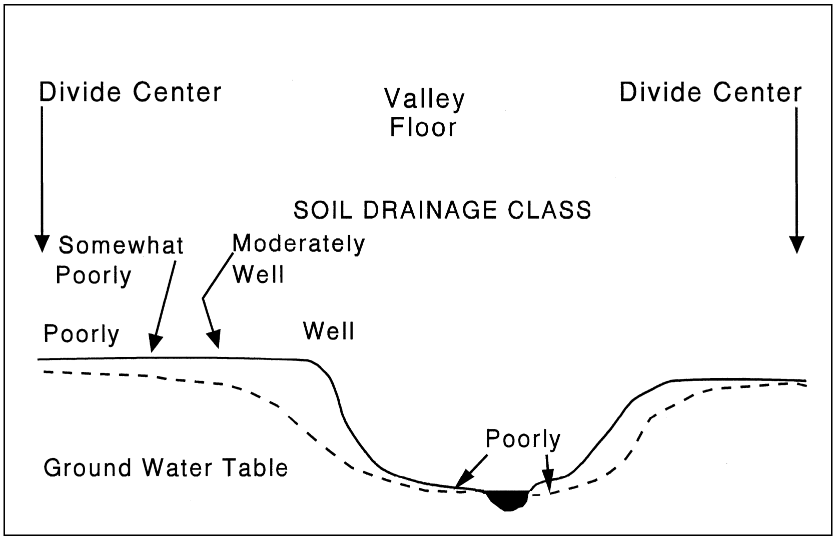

Figure 2-C Relationship of Soil Drainage Class to Topography and Groundwater Depth in the Coastal Plain of North Carolina.

This landscape and accompanying drainage system results in a range of soil series, all influenced by relative depth to the water table. Typically in the well drained positions, depth to seasonal shallow water table is greater than 250 centimeters (cm), and can exceed 300 cm. In moderately drained locations, depth to seasonal shallow water table will range from 50 to 200 cm depending on the time of year. A similar variation occurs in poorly drained areas, except that in many years the water table is at the soil surface during the winter months (periods of no active evapotranspiration). Very poorly drained soils are characterized by seasonal shallow water tables at or just below the soil surface, and as much as 50 to 100 cm below the surface during the driest months (typically October and November). Historical records at the Whiteville station indicate that the mean annual precipitation is 49 inches (annual average for period 1965-1974), with the wettest months being June, July and August. The driest periods appear to be in late October and November. Across the remaining months, the average 30-day rainfall total is between 4–5 inches.

The dominant soil series at the Whiteville station is the Norfolk (Fine-loamy, kaolinitic, thermic Typic Kandiudult), followed by smaller areas classified as the Lynchburg, Goldsboro, Grifton and Wagram. Because of its position on the landscape, the Norfolk soil series is considered a deep (>60 inches), well drained soil with moderate permeability. Historically, the Norfolk soil series has been considered one of the most productive soils for crop production in North Carolina, especially for tobacco.

2.2.3 Assessment of Historic Impacts

As with the Oxford station, the primary emphasis of the Whiteville station has been tobacco breeding and production. It is probably safe to assume that all the acreage currently used for station field experiments has at one time been under tobacco production. As noted above, while some historical records existed regarding field experiments and sources and amounts of fertilizer applied, the extent and accuracy of these records is not known.

3. STUDY DESIGN

3.1 Layout of Test Plots

Three test plots were constructed at each site: a tobacco planting with Chilean nitrate fertilizer treatment (Plot 1); a non-planted area with Chilean nitrate fertilizer treatment (Plot 2); and a tobacco planting without Chilean nitrate fertilizer control (Plot 3). Figures 3-A and 3-B depict the layout of test plots at the Oxford station and Whiteville station, respectively. At both sites, Plots 1 and 3 (plots containing tobacco plants) each consist of four treatment blocks. Plot 2 (no tobacco plants) at both sites consists of only two treatment blocks. The layout of a single treatment block is shown in Figure 3-C. Each treatment block is composed of eight 90-foot rows of tobacco. The two outer rows on either side serve as guard rows that receive the same amount of total nitrogen at transplanting and side dress, but without addition of Chilean nitrate fertilizer.

Within a given treatment block, the total area available for sampling was corrected for edge effects at end of rows. A 5-foot buffer was allowed for edge effects, thus reducing the effective length of each row to 80 ft. This translates to an effective study area in each treatment block of 1,280 ft^2 (each treatment block composed of two sub-blocks each with an effective study area of 640 ft^2).

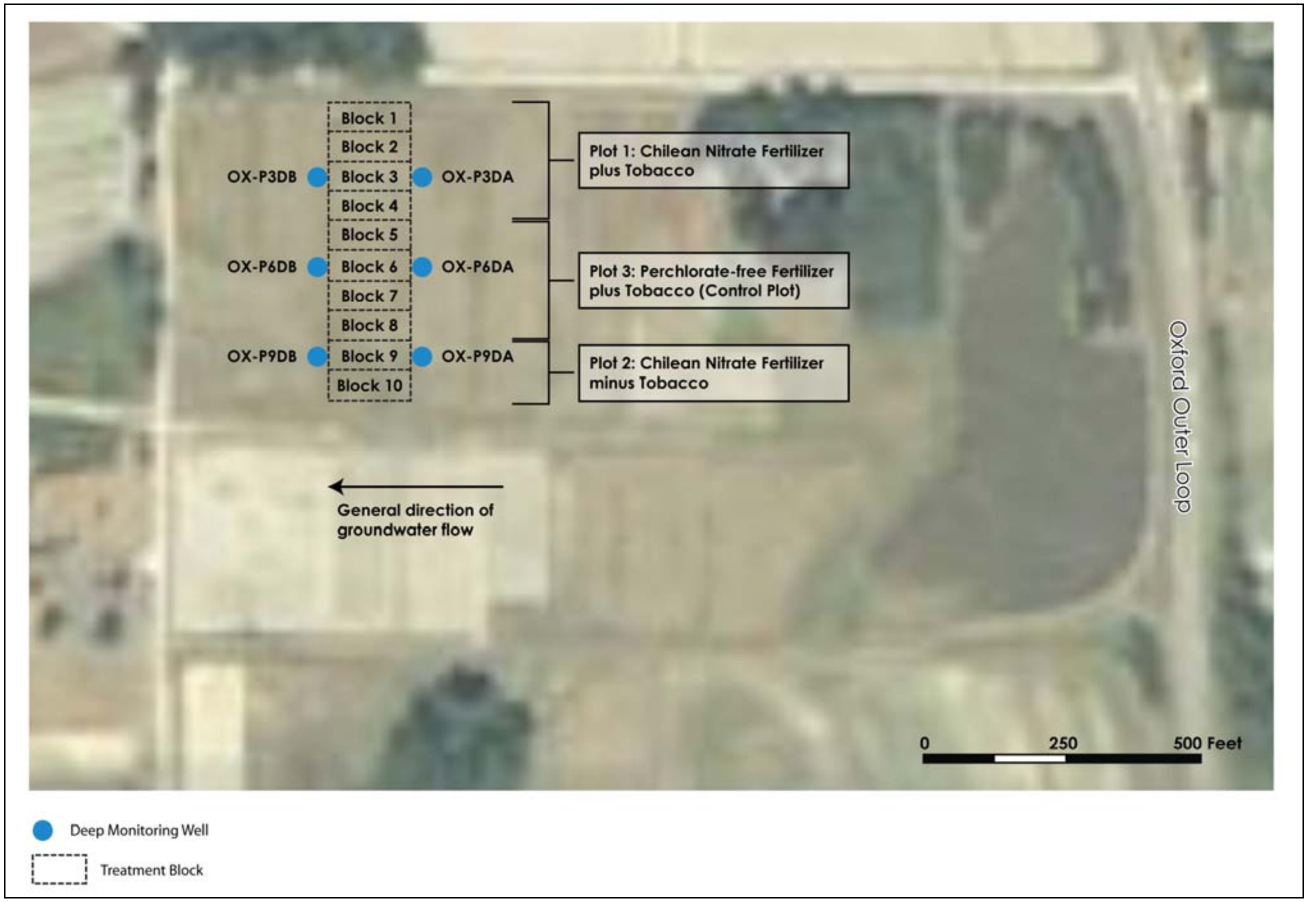

Figure 3-A Layout of Test Plots at the Oxford Station. (Image courtesy of Microsoft Corporation, 2010)

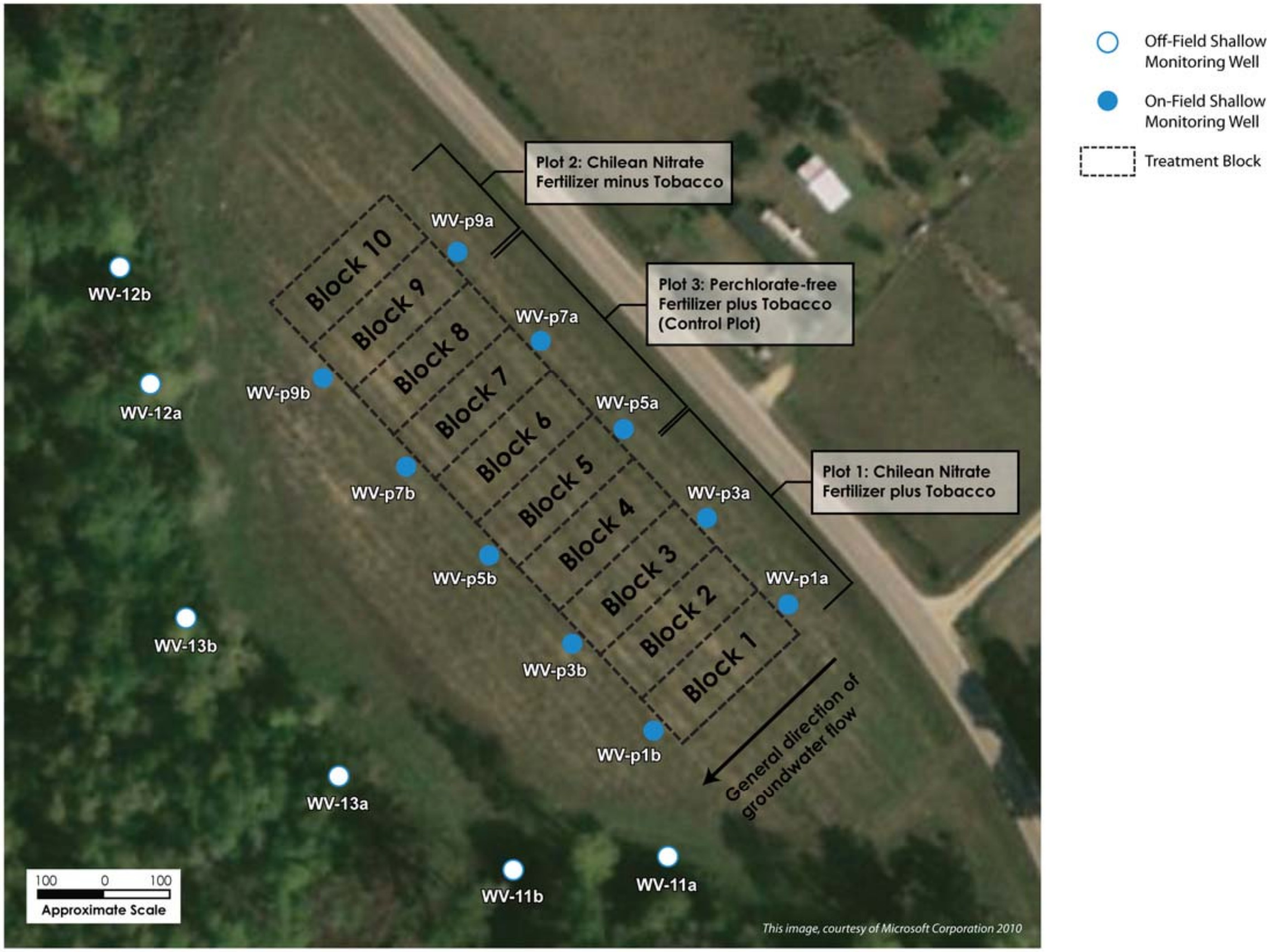

Figure 3-B Layout of Test Plots at the Whiteville Station. (Image courtesy of Microsoft Corporation, 2010)

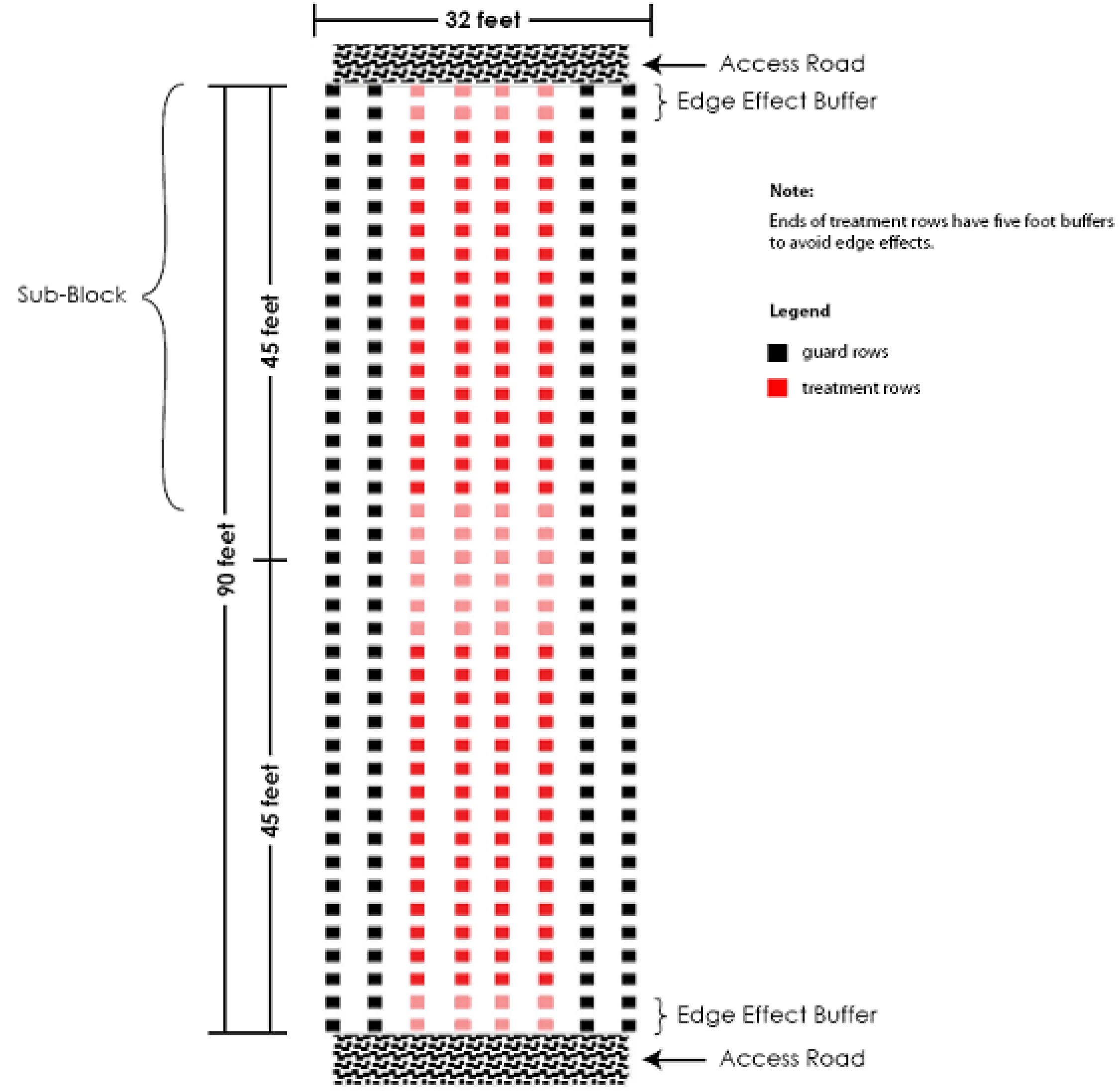

Figure 3-C Layout of Single Treatment Block

4. FIELD ACTIVITIES

4.1 Construction and Installation of Wells

4.1.1 Oxford Station

Six deep groundwater monitoring wells were installed at the station in May 2009 along the upgradient (wells OX-P3DA, OX-P6DA and OX-P9DA) and downgradient (wells OX-P3DB, OX-P6DB and OX-P9DB) edges of treatment blocks 3, 6 and 9. Concern for potential damage to the soil mounds prevented the installation of deep wells within the treatment blocks. Under the direction of Geosyntec, boreholes for the deep wells were drilled to a maximum depth of 27 ft bgs by a licensed driller using hollow stem augers (HSA). Boreholes were drilled to a depth where competent bedrock was encountered. During drilling, samples of the soil cuttings were collected at select borehole locations for the purpose of lithologic characterization. Each deep well was constructed using 2-inch diameter Schedule 40 PVC screen (#10 slot) and Schedule 40 PVC riser. The screened interval for each well measured 5 ft in length, with the bottom of the well screen positioned at the bottom of the borehole. After placement of the well screen and riser casing, the annular space around the casing was filled with uniformly graded, rounded, clean #1 silica sand to a depth of approximately 1-2 ft above the well screen. The height to the top of the sand pack was frequently measured to check that the volume of sand placed in the wells approximated the volume required for the annulus and that no bridging of the filter pack had occurred. An annular seal of bentonite pellets of 2-3 ft in thickness was placed on top of the sandpacks, and the remainder of the annulus was backfilled with grout to a depth of approximately 1 ft bgs. The well risers were allowed to extend approximately 1-2 inches above ground, and were capped with a water tight endcap (J-plug). A length of 4-inch PVC pipe was installed around well riser for protection, and was anchored in the grout for support. Each 4-inch PVC pipe was then capped with a PVC slip-cap. Following installation, the deep wells were developed by standard surging and purging methods.

Soil cuttings collected during well installations revealed the following soil lithology beneath select treatment blocks (borehole logs can be found in Appendix A):

- Treatment Block #3 consists of brown to red-brown, sandy clay grading to clay to a depth of approximately 10 ft bgs. Beneath this lies a l0-ft layer of brown, very fine to fine silty sand with some rock fragments. A brown, fine to medium grained sand and gravel layer was observed from approximately 20 to 25 ft bgs (maximum depth cored).

- Treatment Block #6 consists of brown, clayey silt with some sand to a depth of approximately 5 ft bgs. This is underlain by a 15-ft layer of tan to light brown, silty clay with a trace of rock fragments. A weathered rock layer was encountered from 20 to 21 ft bgs (maximum depth cored).

- Treatment Block #9 consists of a dark brown to tan, sandy/silty clay to a depth of approximately 5 ft bgs. A tan to light brown, very fine to medium grained sand layer with trace silt and clay and some weathered rock fragments was observed from 5 to 9.5 ft bgs (maximum depth cored).

4.1.2 Whiteville Station

Shallow monitoring wells were installed at the Whiteville station in March, 2009. Initially, a subset of the shallow wells planned for the site were installed along the upgradient (wells WV-P1A, WV-P3A, WV-P5A, WV-P7A, and WV-P9A) and downgradient (wells WV-P1B, WV-P3B, WV-P5B, WV-P7B, and WV-P9B) edges of treatment blocks 1, 3, 5, 7 and 9 (Figure 3-B) in order to assess whether shallow groundwater (i.e., <5 ft bgs) was present beneath the test plots. After several weeks of monitoring it was concluded that shallow groundwater was not present and that installation of the remaining shallow wells was unnecessary. However, six off-field shallow monitoring wells (wells WV-11A, WV-11B, WV-12A, WV-12B, WV-13A, and WV-13B) were installed in a drainage area downgradient of the test plots where groundwater could be intercepted with shallow wells. It was anticipated that these wells would aid in assessing the movement of perchlorate from the treatment blocks.

The wells were installed by NCSU using hand augers or mechanical probe truck as necessary. The wells were constructed using a 30 cm (12 in) length of 2-inch diameter Schedule 40 polyvinyl chloride (PVC) screen (#10 slot; 0.01-inch) and Schedule 40 PVC riser. A sand pack consisting of uniformly graded, rounded, clean silica sand was installed in the annulus around the well screen. The annular space above the sand pack was sealed using grout to prevent contamination by surface runoff. Shallow wells were installed to a depth of approximately 1 m (~3 ft), as referenced from the top of the original soil surface. As is typical of the soil profile in this region of North Carolina, a very sandy upper soil layer is typically underlain by a higher-clay sublayer, which acts as a confining layer and often supports a 'perched' water table. Each shallow well was set so that the slotted section was just above this clay layer, resulting in the final depth for the wells varying slightly for any given plot. The well risers for each of the on-field wells were allowed to extend a few inches above ground, and were capped with a PVC slip-cap. The off-field wells were installed using the same design as the on-field wells, with the exception that the average depth of each well was approximately 120 cm (~4 ft). The well risers for each of the off-field wells were allowed to extend approximately 3 ft above ground, and were capped with a PVC slip-cap.

4.2 Installation of Tensiometers

Soil moisture content during the study was monitored using a soil tensiometer, which provided a relative measure of soil water content by sensing soil water tension or pressure. The device consists of a ceramic cup glued to the end of a piece of PVC tubing of appropriate length. The device is buried to a fixed depth in the soil, filled with water and then a rubber septum applied to the non-buried end of the PVC tubing. As water is drawn out of the PVC tubing into the soil via contact between the soil and buried ceramic cup, a tension develops inside the PVC tubing reflecting the relative moisture status of the surrounding soil. As the soil dries, more water is extracted from the tubing, effectively increasing the negative pressure within the PVC tubing. As the surrounding soil wets, water may flow back into the PVC tubing via the ceramic cup, reflecting the change in the surrounding soil water status. The tension (pressure) within the PVC tubing is easily monitored using a pressure gauge that fits over the rubber septum at the unburied end of the PVC tubing.

Soil tensiometers were installed at the Whiteville and Oxford stations in April and May 2009, respectively, following tobacco transplanting and application of the fertilizer side dress treatment (see Sections 4.4 and 4.5). Clusters of tensiometers were installed in all of the plots to three depths. Because the tensiometers were installed in the mounds of soil containing the tobacco plants, the plane of reference for the depth measurements is the top of the mounds, not the original soil surface. Typically the mounds were formed by scooping up surrounding soil in the inter-row and forming a mound approximately 15 cm high relative to the original surface of the soil. Correspondingly, the inter-row between the mounds was ~ 15 cm below the original soil surface. The reported depth measurements therefore are referenced to the top of the mounds containing the tobacco plants, and not to the original soil surface. The three soil depths monitored were 15, 45 and 75 cm. The 15 cm depth corresponds to the bottom of the soil mound, at the original surface of the soil. The 45 and 75 cm depths correspond 30 and 60 cm depths as referenced to the original surface of the soil. By necessity, the tensiometers were installed after the tobacco seedlings had received their initial round of complete fertilizer applications, to avoid damage from the heavy farm equipment necessary to form the mounds. The tensiometers were in place for the second side dress application of either potassium nitrate or calcium nitrate.

4.3 Baseline Soil and Groundwater Sampling

Prior to planting and fertilizer application, soil and groundwater samples were collected from both research stations to establish baseline conditions. A summary of the types of samples collected from both sites throughout the study period is provided in Table 4-A. Table 4-B provides a summary of the analytical methods, container sizes and types, preservation methods, and sample holding times for the parameters analyzed.

4.3.1 Soil Sampling

Initial soil sampling to a depth of 100+ cm at both stations was conducted in the early spring (March, 2009) to determine background levels of soil extractable nitrate and ammonium as well as survey for the possible presence of perchlorate. A combination of hand augers and soil probe truck were used to sample soil with depth. A hand auger was used to sample the top 50 cm of the soil: 0-15, 15-30 and 30-50 cm. Sampling was done by randomly walking across each treatment block and generating composite samples for the 3 depths. A total of 8 sampling points were used to generate the composite samples for each depth of the treatment block. Depths below 50 cm were sampled in 50 cm increments by using a hand auger or soil probe truck. A total of 4 sampling points chosen at random were selected for each treatment block, and the soil from each depth increment was combined to form one composite sample per treatment block. Soil core locations were flagged and referenced using GPS. Following sample collection, composite soil samples were transferred into plastic (polyethylene) bags and then placed into ice chests for transport (for samples analyzed by the Environmental and Agricultural Testing Service [EATS] laboratory) or shipment (for samples analyzed by Columbia Analytical Services [CAS]) to the laboratory. No attempt was made to cool the soil samples due to the volumes of soil collected.

Table 4-A. Summary of Samples Collected at Oxford and Whiteville Stations

Matrix	Analyte(s)	Sample Frequency
Tensiometers	Water potential	Baseline + every 2 weeks for 16 weeks
Shallow well groundwater	Dissolved perchlorate, nitrate, dissolved organic carbon, pH, redox, dissolved oxygen	Baseline + every 2 weeks for 20 weeks
Deep well groundwater	Dissolved perchlorate, nitrate, dissolved organic carbon, pH, redox, dissolved oxygen	Baseline + every 2 weeks for 20 weeks
Soil cores	Water extractable perchlorate, nitrate	Baseline
	Water extractable perchlorate, nitrate	every 2 weeks for 16 weeks during growth phase
	Water extractable perchlorate, nitrate	Post-harvest
	Bulk density	Post-harvest
Plant tissue	Extractable perchlorate	Every 2 weeks for 16 weeks during growth phase + 3 separate harvests

Table 4-B. Summary of Sample Handling and Laboratory Analytical Details

Matrix	Measurements	Analytical Laboratory	Amount Collected	Analytical Method	Method Detection Limit	Sample Container	Sample Preservation$^{(1)}$	Maximum Holding Time$^{(2)}$
Shallow/deep groundwater wells	Perchlorate	EATS	20–50 mL	314.0	3 µg/L	glass	Sulphuric acid to pH<2, cool to 4°C	> 28 days
	Nitrate			4500-NO_3	0.02 mg NO_3-N/L			
	Dissolved organic carbon			5310B	1 mg C/L			
	pH			Field	NA	NA	NA	NA
	Oxidation-reduction potential, dissolved oxygen		Flow-thru cell	Field	NA	NA	NA	NA
Shallow/deep groundwater wells	Perchlorate	CAS	20-125 mL	6850	0.2 µg/L	125 mL, plastic	Cool to 4°C	28 days
Soil	Perchlorate	EATS	100 g	314.0	0.03 mg/kg	Plastic bag/bottle/ aluminum rings	Air dry and store at room temperature	> 28 days
	Nitrate			4500-NO_3	0.2 mg NO_3-N/g			
	Bulk Density		200 g	Blake & Hartge, 1986	NA			
Soil	Perchlorate	CAS	10-20 g	6850	2.1 µg/kg	4 oz, glass	Cool to 4°C	28 days
Plant tissue	Perchlorate	EATS	10-50 g	Ellington & Evans, 2000	3 mg/kg	glass	Cool to 4°C; freeze and freeze-dry; store at 4°C	> 28 days

Notes:

1 - Samples were preserved immediately upon sample collection, if required.

2 - Samples were analyzed as soon as possible after collection. The times listed are the maximum holding times that samples were held before analysis and could still be considered valid. All data obtained beyond the maximum holding times was flagged.

°C – Degrees Celsius

EATS – Environmental and Agricultural Testing Service Laboratory, Department of Soil Science, North Carolina State University

CAS - Columbia Analytical Services, Rochester, NY

g – grams
mL – milliliters
NO_3 - nitrate
mg NO_3-N/L – milligrams nitrate-nitrogen per liter
mg C/L – milligrams carbon per liter
mg NO_3-N/g – milligrams nitrate-nitrogen per gram
µg/L – micrograms per liter
µg/kg – micrograms per kilogram
oz - ounce
mg/kg - milligrams per kilogram

After collection, the composite soil samples were air dried, either by spreading the sample out on a large surface in a controlled environment, or by placement in a forced air convection oven set to ambient temperature. The oven controls are such that operation of the movement of air within the oven is not connected to the oven temperature. Once dried, the soil samples were further processed to break up larger soil clods (typically using a soil grinder) and then passed through a riffler to obtain a suitable subsample. This subsample was then passed through a stainless steel 2 millimeter (mm) sieve, mixed well and then placed into a labeled plastic bottle. The processed air-dried soil samples were stored at room temperature before analysis.

4.3.2 Groundwater Sampling

Prior to collecting groundwater samples for chemical analysis, the stagnant water in the well casing was purged using a peristaltic pump and dedicated tubing. The tubing inlet was placed at the bottom of the well and water was pumped at a rate of <1 gallon per minute (gpm). The water level in the well was measured immediately before purging started. Measurements of groundwater dissolved oxygen (DO) and oxidation-reduction potential (ORP) were conducted during well purging using either a multi-parameter meter (YSI 556 MPS) or individual ORP (Hanna 9025) and DO (YSI 55) meters, and by placing the electrodes into a beaker and pumping a continuous flow of groundwater across them. Following well purging, groundwater samples were collected by transferring groundwater directly from the pump tubing into the sample containers. After sampling, the sample containers were placed in ice chests with ice packs and were transported (for samples analyzed by EATS) or shipped (for samples analyzed by CAS) to the laboratory. Upon receipt by EATS, the samples were acidified with hydrochloric acid (HCl; pH <2) and then stored at 4 Degrees Celsius (ºC) until analysis.

4.4 Tobacco Planting and Growth

Field preparation for tobacco at the research stations consisted of plowing/disking. After disking, the soil was formed into raised beds, approximately 50-60 cm (20-24 inches) high above the soil surface with a 1.2 m (48 inch) inter-row spacing. Use of raised beds for tobacco production is the standard practice used at both the Whiteville and Oxford stations. The raised bed ensures ample soil volume for root growth and aeration. Standing water in fields following major rain events can result in damage to tobacco root growth. Thus, the raised beds ensure that at least a portion of the tobacco root system is sufficiently aerated following major rain events. Tobacco plants also develop adventitious roots; this rooting is encouraged by tillage after transplanting and during fertilization. Beds are essentially reformed after transplanting.

Transplanting of tobacco seedlings at the Whiteville and Oxford stations occurred on April 20 and May 13, 2009, respectively, with populations of about 6,000 plants per acre using 22 inch spacing between plants (Figure 4-A). During transplanting, the top 8-10 inches of the bed was removed and the tobacco seedling placed into the center of the leveled bed. This was accomplished in one operation as the planter moved across the field (Figure 4-B).

Figure 4-A Tobacco Transplanting into Prepared Beds. (Photo by Scott King, Dept. of Soil Science, NCSU)

Once transplanted in the field, the tobacco seedlings progress through 3 phenological stages of varying length. Total time in the field to final harvest is typically 15 weeks. During the first 30 days (3-4 weeks), the seedling begins growth with more leaf development and modest increase in height. Root growth is relatively modest during this period as well. By the 4th week, the crop enters into the exponential growth stage, which continues for approximately 50 days (~7 weeks) ending with flowering. This growth stage results in both extensive growth above and below ground, with the below ground root mass increasing by a factor of 5 or more. Above ground, the crop reaches heights of between 4 to 6 ft, including flowers. The exponential growth stage ends with flowering somewhere between 9-11 weeks following transplanting. By flowering, as much as 80% of the above ground aerial foliage has been produced. Removing the flower bud (topping the tobacco) allows the plant to continue to develop the above ground mass, with harvesting of the lower leaves beginning ~2 weeks later and continuing for perhaps up to 5 to 7 weeks. Tobacco leaves mature from the basal part to the apical part of the plant at the rate of 2 to 4 leaves per week, which means harvesting is usually 2 to 4 leaves per plant per week. The last 30 days of production are considered the third and last phenological stage in tobacco production. A typical tobacco plant can produce over 25 leaves, but the majority of production (~80%) is generated between leaves 5 and 18.

Figure 4-B Transplanted Tobacco with Typical Spacing of 22 inches Between Plants. (Photo by Scott King, Dept. of Soil Science, NCSU)

Following side-dressing with nitrogen, subsequent tillage operations are conducted primarily to control weeds and promote soil aeration until the crop reaches heights of 18 inches or more, depending on the height of the tool bar of the farm implements. Chemical sucker control is sometimes also used, which is applied by farm implements.

4.5 Fertilizer Application

Fertilizer application is typically directed at achieving between 60 to 90 lbs of nitrogen (N) per acre, depending on soil texture. In sandy soils such as at the Whiteville station, the target rate is 90 lbs of N per acre. The rate is closer to 60-70 lbs of N per acre at the Oxford station. In this study, the traditional convention of applying fertilizer was followed, whereby half of the required nitrogen for growth is applied with an initial side dress of complete fertilizer, followed by a side dress with nitrogen-only fertilizer several weeks later. Historically, the nitrogen-only side dress was the soda nitrate (Chilean nitrate) product known to have contained perchlorate. Today, the nitrogen-only side dress is usually urea-ammonium-nitrate (UAN) solution.

In regards to nutrient uptake, nitrogen is critical in that excess nitrogen will result in too much vegetative growth. As such, management of tobacco is oriented to reach maximum nitrogen accumulation in the plant at about week 7. The majority of nitrogen uptake is via mass flow (via transpiration demand), which should also apply to perchlorate. Thus the total mass of perchlorate uptake should be expected to reach a maximum at about week 7, while the concentration of perchlorate in the aerial portion of the canopy should decrease with vegetative growth (assuming no internal destruction of perchlorate within the tobacco plant).

The fertilizer application process used for this study is described in greater detail in the following sections.

4.5.1 Application of Complete Fertilizer

Approximately 1-2 weeks following transplanting, all the plots at Whiteville and Oxford stations were treated with 6-6-18 and 8-8-24 fertilizer, respectively. The complete fertilizer was applied at a rate of ~42 lbs N per acre at the Whiteville station and ~40 lbs N per acre at the Oxford station. At both stations the fertilizer was knifed into the soil 4 inches to the side and 4 inches deep of the plant stalk (4x4 placement pattern). Immediately following application cultivation knifing covered the applied fertilizer nitrogen and the base of the stalk with soil. Final depths were typically 6-8 inches of newly plowed soil added to the top of the existing bed. The intent of the fertilizer is to have the nitrogen available at the start of the exponential growth stage of the crop. The 4x4 placement pattern is to prevent damage to roots that are present, but still have nitrogen readily available to the rapidly expanding root system that will develop during the exponential growth stage. Prior research has indicated that expansion of the roots is necessary before growth of the aerial foliage is triggered.

The same complete nitrogen fertilizer and other practices were also applied to Plot 2 (without tobacco crops) at both stations using the same 4x4 placement pattern described above.

4.5.2 Nitrogen-only Side Dress

As was previously anticipated, locating a sufficient quantity of nitrogen-only (Chilean nitrate) fertilizer that contained historic levels of perchlorate (i.e., ~0.2%) was not possible. Therefore, a Chilean nitrate fertilizer product (potassium nitrate, SQM Corporation) believed to contain ~0.01-0.03% perchlorate was used. The Chilean nitrate fertilizer was applied to Plots 1 and 2 at the Whiteville station approximately 6 weeks following transplanting, at a rate of ~37 lbs N per acre. At the Oxford station, the Chilean nitrate fertilizer was applied to Plots 1 and 2 approximately 5 weeks following transplanting, and was applied at a rate of ~32 lbs N per acre. At both stations the fertilizer was applied using the same 4x4 placement pattern that was used for the complete fertilizer described above. No Chilean nitrate fertilizer was applied in Plot 3 (control) at either station. However, Plot 3 at Oxford and Whiteville stations were side-dressed using calcium nitrate at a rate of 31 lbs N per acre and ammonium nitrate at a rate of 32-34 lbs N per acre, respectively.

4.6 Plant Sampling

4.6.1 Post-Planting/Growth Phase

The tobacco plants were sampled every other week for 16 weeks following application of the potassium nitrate fertilizer side dress to determine the presence of perchlorate due to uptake.

A leaf punch (approximately 2 cm in diameter) was used to collect composite samples from leaves for each treatment block (minimum of 8 tobacco plants). Research station personnel (and other experts in tobacco production) were consulted for proper identification of the various leaf whorls developed as the tobacco plants grew.

A minimum of two heights were sampled 5 weeks after transplanting: mature leaves and newest leaves (top whorl), the later providing a possible index of continued uptake of perchlorate over time. These samples were archived for future analyses.

The discs of plant tissue obtained with the leaf punch were collected in air-tight glass containers in the field. After sampling, the glass containers with samples were placed in ice chests with ice packs to cool the samples during transport to the laboratory. Upon receipt by EATS, the leaf tissue samples were frozen, and then freeze-dried, all within the original glass containers. The freeze-dried sample containers were then stored at 4ºC.

4.6.2 Harvesting

Research station personnel were contacted as to when different whorls on the tobacco plant would be harvested. Prior to harvest, these leaves were sampled using a leaf punch (as described above), producing one composite sample per treatment block, or a total of 8 composite samples per harvest. A total of 3 harvests were performed at the Oxford station, and a total of 4 harvests were performed at the Whiteville station. Total mass of leaves harvested (flue-cured weight at Oxford station; fresh weight at the Whiteville station) was obtained from research station personnel.

4.7 Groundwater Sampling

Following application of the potassium nitrate fertilizer, groundwater samples were collected at both stations every other week for approximately 18 weeks (10 sampling events in total at each station). A summary of the types of samples collected from both sites throughout the study period is provided in Table 4-A. Table 4-B provides a summary of the analytical methods, container sizes and types, preservation methods, and sample holding times for the parameters analyzed. Groundwater samples from both stations were collected using the same sampling protocols that were used during the baseline sampling event (see Section 4.3.2).

4.8 Soil Sampling

4.8.1 Post-Planting/Growth Phase

Following application of the potassium nitrate fertilizer, soil sampling at both stations was performed every other week for 16 weeks during the growth phase. During each sampling event, a hand auger was used to sample the top 100 cm of soil relative to the top of the mound in a row. Samples were collected by coring down through the center of a mound in between two tobacco plants at depth intervals of 0-15, 15-30, 30-50, 50-75 and 75-100 cm. A composite sample was generated for each depth per treatment block by taking 6 cores randomly chosen within the study area of each treatment block (3 cores per sub-block). Soil core locations were flagged and referenced using GPS. The soil sample handling and shipping protocols were the same as those used during baseline sampling event (see Section 4.3.1).

4.8.2 Post-Harvest

Following harvesting of the tobacco, a soil probe truck was used to sample soil within each treatment block at depths below 100 cm. Two locations per treatment block were sampled at depth intervals of 100-150 cm and 150-200 cm. Samples from each location were combined to form composite samples for each depth interval per treatment block. Additional 50 cm depth increments were attempted depending on conditions at the research stations at the time of sampling. Soil bulk density was determined for depth intervals of 0-15, 15-30, 30-50, 50-75 and 75-100 cm using the standard double-cylinder hammer driven core sampler (Blake and Hertge, 1986). One pit was excavated per treatment block and replicate cores were obtained for each soil depth. Estimates of soil bulk density for depth intervals greater than 100 cm were determined from intact cores collected using the soil probe truck.

4.9 Demobilization

At the end of the study, shallow wells at both stations were decommissioned by pulling them from the ground and backfilling the remaining hole with soil. Deep monitoring wells at the Oxford station were decommissioned in accordance with the State of North Carolina decommissioning protocols and procedures.

4.10 Disposal of IDW

All soils generated during installation of the deep wells at the Oxford station were placed on plastic sheeting by the drilling personnel. Samples of the soil were collected and submitted to EATS for analysis of perchlorate. Upon confirmation from the lab that the soil samples did not contain detectable levels of perchlorate, the soil cuttings generated during well installations were spread at ground surface at the station. All purge water and equipment decontamination water during well sampling activities at both stations was containerized in 5-gal pails by the sampling personnel, and was transported to NCSU for disposal by EATS following standard laboratory practices. All other common non-hazardous trash associated with the study was disposed of according to station protocols.

5. ANALYTICAL METHODS

The analytical methods used to analyze groundwater and soil samples are summarized in Table 4-B and discussed in detail below.

5.1 Perchlorate

The perchlorate content of water samples and soil extracts were determined using a Dionex DX-500 ion chromatography system (Sunnyvale, CA). The chromatograph is equipped with a GP50 gradient pump, ED40 electrochemical detector, LC20 chromatographic oven, and AS3500 autosampler. The ED40 unit is equipped with a conductivity cell with built in stabilizer maintained at constant temperature. The chromatograph is equipped with a model ASRSULTRA self-regenerating suppressor (operated at 100 mA) which provides an operating range of 0 – 10 micro Siemens. Anion separation is obtained using an AS16 analytical microbore separation column in tandem with an AS16 guard column, and 60 mM NaOH as eluent. Elution times were typically 12 minutes, but can be varied with changes in eluent as necessary. The typical working range for water and soil extracts was 5-200 micrograms per liter (µg/L) (minimum 5 points per standard curve). Analyses of water and soil extracts were conducted using a 1000 microliter (µL) sample injection loop. All standards, dilutions, and eluent solutions were made using deionized water. Analyses of fertilizer extracts for perchlorate content used the same ion chromatography instrumentation, except the sample loop was reduced to 25 µL, and the standard range was expanded to 5-5000 µg/L. The operating conditions for water and soil extracts were based on US EPA Method 314.0 (Hautman et al., 1999). The operating conditions for fertilizer extracts were derived from Collette et al. (2001).

Air-dried, sieved soil samples were extracted for perchlorate using 0.001M $CaCl_2$ solution. An initial soil:solution ratio of 1:6 was used (5 grams [g] of soil to 30 milliliters [mL] of deionized water). All extracts were carried out in plastic centrifuge tubes with screw caps (e.g., 45 mL Oak Ridge centrifuge tubes). Extractions were carried out overnight (12 hours) at room temperature using a rotary shaker, which prevented settling of the suspension. After equilibration, the suspensions were centrifuged (3,000 revolutions per minute [rpm]) and the supernatants filtered through 0.2 micron Acrodisc® filter disks into glass autosample vials (~1 mL). The filtered solutions were capped and stored at 4ºC until analysis. Typical holding times were 24 hours while a sufficient number of samples were processed to start an analysis by ion chromatography. This procedure is similar to that used by Ellington et al. (2001). All analyses were corrected to oven-dry weight (105ºC) of the air-dried soil samples. Assuming a detection limit with the ion chromatograph of 3 µg/L, a 1:6 soil solution ratio yields a detection limit of 18 µg/kg perchlorate when expressed on an original sample mass basis.

Determination of perchlorate in plant tissue presents a challenging medium due to the presence of other inorganic salts and organic compounds. In this study, the approach of Ellington and Evans (2000) was adopted. This method has been shown to produce suitable extracts of various foodstuffs and plant products for analysis by ion chromatography as described above.

Approximately 600 milligrams (mg) of freeze-dried ground material was weighed into 45 mL Oak Ridge centrifuge tubes with screw caps. Thirty milliliters of deionized water was added to each tube. The capped tubes were then placed in a boiling water bath for 30 minutes to precipitate protein and to saturate the dried plant material. Following heating, the tubes were allowed to cool, and then placed in a 4ºC refrigerator and shaken every 2 hours, and then allowed to settle overnight. The next day, the tubes were spun at 20,000 g in refrigerated centrifuge for 30 minutes, and the supernatant passed through a perchlorate-free coarse filter. The filtrate was again centrifuged at 20,000 g in a refrigerated centrifuge for 30 minutes. The supernatant was then passed through a 0.2 micron Acrodisc® filter, and the filtrate collected in a separate plastic vial and stored at 4ºC until analysis.

For analysis via ion chromatography, 1 mL extracts were added to 500 mg of DD-6 alumina and allowed to react overnight at 4ºC. The suspension was then diluted 1:10 with deionized water, and filtered through a 0.2 micron Acrodisc® filter and a precleaned OnGuard® RP cartridge (Dionex, Sunnyvale, CA). The first 1 mL of eluent was discarded and the remaining eluent used for analysis. For analysis, the freeze-dried plant samples were ground using a Wiley mill with a 1 mm stainless steel screen. The resulting ground sample was mixed and stored in air-tight glass bottle. When not in use, the ground plant samples were stored at 4ºC.

5.2 Nitrate

The nitrate content of water and soil extracts were determined using a standard colorimetric procedure and a Lachat Quik Chem Model 8000 autoanalyzer (www.lachatinstruments.com) (Method 4500-NO_3, 2005). The method is based on the reduction of nitrate to nitrite using a copperized cadmium column with subsequent diazotization with sulfanilamide and coupling to N-(1-naphthyl) ethylenediamine dihydrochloride to form the colored complex. Typical linear operating range is 0.2 – 20 mg NO_3-N/L, however, the sensitivity of the procedure can be expanded by a factor of 10 with selection of larger sample loop (0.02 - 2 mg NO_3-N/L).

Air-dried, sieved soil samples were extracted for nitrate using 1 molar (M) potassium chloride (KCl) solution made from reagent grade KCl and deionized water. Typically, a soil:solution ratio of 1:5 was used (10 g per 50 mL of extracting solution) in plastic 100 mL centrifuge tubes. The tubes were capped, shaken for 1 hour on a rotary shaker, centrifuged (3,000 rpm) and the supernatant passed through Whatman #40 or its equivalent filter paper. The resulting extract was stored in a plastic vial, acidified with HCl ($pH < 2$), and stored at 4ºC until analysis. All analyses were corrected to oven-dry weight (105ºC) of the air-dried soil samples. Assuming a detection limit via colorimetric analysis of 0.2 mg/kg, a 1:5 soil solution ratio yields a detection limit of 1.0 micrograms nitrate-nitrogen per gram (1.0 parts per million [ppm] NO_3-N) when expressed on an original sample mass basis. The reagent blank for this analysis was the

1M KCl extracting solution. All standards for analyses were prepared in 1M KCl due to the refractive nature of the strong salt solution.

5.3 Total Dissolved Organic Carbon

Total dissolved organic carbon in water samples was determined using Method 5310 B (1999) with a typical operating range of 1–8000 milligram carbon per liter (mg C/L).

5.4 Standard Operating Procedures

All analyses were conducted using multi-point calibration curves and appropriate reagent blanks. Each set of analyses included duplicates (minimum 1 duplicate for every 20 samples) and in-house quality control checks. A subset of completed sample analyses was selected for spike recovery tests across the range of observed concentrations.

6. RESULTS AND DISCUSSION

6.1 Perchlorate Content of Potassium Nitrate Side dress

As noted in Section 1.2, previous analyses of various nitrate products by one of the principal investigators (Wayne Robarge; data not shown) had indicated that the potassium nitrate substitute would still contain perchlorate, although at amounts of possibly between 0.01 to 0.03%, or approximately an order magnitude less than the original nitrate of soda product. However, it was also entirely possible that the perchlorate content of the potassium nitrate was even lower, since the previous analyses did not reflect continued efforts by the SQM Corporation to refine and lower the perchlorate content of their nitrate-based products.

Analysis of the potassium nitrate product was originally carried out at North Carolina State University by the principal investigators using water extracts (1:10 solid:solution ratio) and ion chromatography (Collette et al., 2001). The resultant chromatograms displayed the typical shape observed for solid substrates containing primarily soluble salts (Figure 6-A). However, no perchlorate was detected in the extracts using ion chromatography. Addition of spikes of perchlorate to the extracts confirmed that the presence of the high amounts of nitrate were not overloading the analytical column and not inhibiting the detection of perchlorate in the extracts (Figure 6-B). Based on detection limit values, it was estimated that the potassium nitrate product contained somewhere between 0.003 and 0.005% (30 to 50 mg/kg) of perchlorate.

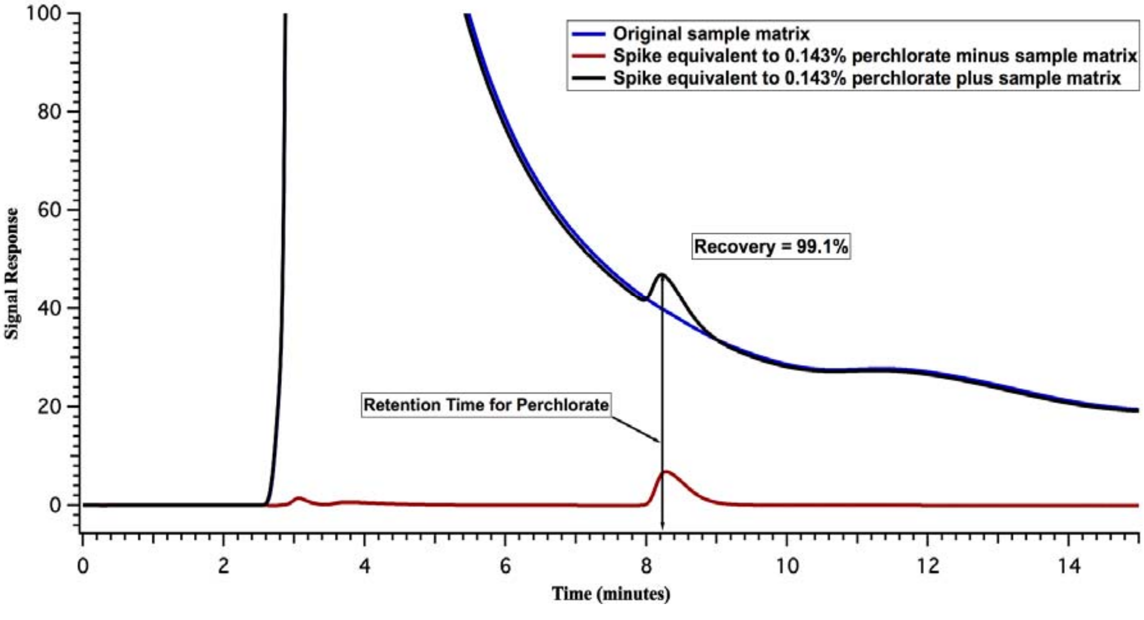

Figure 6-A Example of Chromatogram Obtained in the Analysis of Potassium Nitrate Fertilizer for Perchlorate using Ion Chromatography. (Sample loop 50 microliters, AG-16+AS-16 columns, 40 mM NaOH, 1 mL/min, conductivity detection after suppression with ASRS-300 in recycle mode).

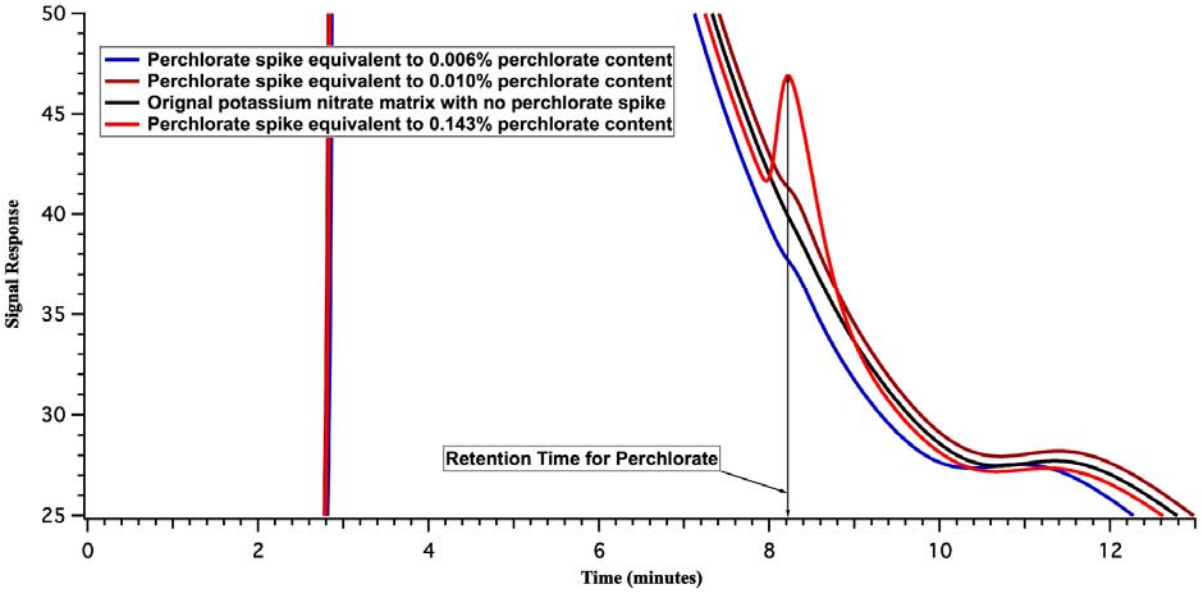

Figure 6-B Example of Chromatogram Obtained in the Analysis of Spiked Potassium Nitrate Fertilizer for Perchlorate using Ion Chromatography. (Sample loop 50 microliters, AG-16+AS-16 columns, 40 mM NaOH, 1 mL/min, conductivity detection after suppression with ASRS-300 in recycle mode).

Samples of the potassium nitrate fertilizer were sent to CAS for analysis using LC-MS-MS, which has detection limits for perchlorate in complicated matrices orders of magnitude lower than that possible using ion chromatography. Results from these analyses indicated that the potassium nitrate contained 0.003% (30 mg/kg) perchlorate. This means that use of the potassium nitrate as the fertilizer source for the side dress application of nitrogen following transplanting of tobacco resulted in ~100x less perchlorate being applied than originally desired. As detailed below, subsequent analyses of soil and plant extracts failed to detect the presence of perchlorate, probably due in large part to the low amounts initially applied. Thus, for this study, potential contamination of underlying groundwater from the historical use of perchlorate-containing fertilizers in tobacco production is inferred in large part based on the fate and transport of nitrate observed in this study. Like perchlorate, nitrate is an anion and known to be relatively mobile in soils.

6.2 Oxford Tobacco Research Station

A visual representation of the development of the tobacco crop at the Oxford Station from May to October 2009 is given in Figures 6-C to 6-G.

Figure 6-C May 29, 2009, Transplanted Tobacco Seedlings, Oxford Station. (Photo by Scott King, Dept. of Soil Science, NCSU)

Figure 6-D June 19, 2009, Tobacco Plants 4 Days Following Second Side Dress Application of Either Potassium Nitrate or Calcium Nitrate, Oxford Station. (Photo by Scott King, Dept. of Soil Science, NCSU)

Figure 6-E July 2, 2009, Tobacco Plots 2+ Weeks Following Second Side Dress Application of Either Potassium Nitrate or Calcium Nitrate, Oxford Station. (Photo by Scott King, Dept. of Soil Science, NCSU)

Figure 6-F July 17, 2009, Tobacco Plants Approximately 1 Month Following Side Dress Application of Either Potassium Nitrate or Calcium Nitrate, Oxford Station. (Photo by Scott King, Dept. of Soil Science, NCSU)

Figure 6-G August 14, 2009, Tobacco plants Approximately 2 Months Following Side Dress Application of Either Potassium Nitrate or Calcium Nitrate, and 7 Days Before First Harvest of Lower Leaves, Oxford Station. (Photo by Scott King, Dept. of Soil Science, NCSU)

6.2.1 Intensity and Temporal Distribution of Rainfall

Rainfall amounts at the Oxford station during the study were determined from the North Carolina State Climate Office's CRONOS weather station network, which has one of its ECONet meteorological towers located at the station. The total amount of rainfall during the 6-month growing season was 18.6 inches (Table 6-A), the largest amount occurring in May and June (>7.9 inches). A substantial portion of this rainfall occurred between the first and second application of fertilizer. Despite the almost 5 inches of rainfall in June, however, the distribution of the rainfall was uneven, resulting in the application of 0.75 inches of irrigation water at the end of month by the research station superintendent. This is the only irrigation event for the entire study period recorded at the Oxford station.

6.2.2 Soil Water Content and Bulk Density

Soil tensiometer readings in Blocks 9-10 (bare soil plots) as a function of date and soil depth are shown in Figure 6-H. Initial readings in early June reflect high soil water content under well drained conditions (~ -0.1 Bar). This status does not change despite a large rain event during the week of June 18th. However, the surface 15cm (soil mound) begins to dry noticeably following the week of June 18th. More rainfall during the weeks of July 10th and 17th appear to maintain the mound water content, but then the soil continues to dry till late September.

Table 6-A. Monthly Rainfall Amounts Recorded at the Oxford Station for April through September, 2009. (Source: NC State Climate Office CRONOS network)

Month	Rainfall Amount (inches)
April	1.26
May	3.54
June	4.96
July	2.87
August	2.52
September	3.46
Average	***3.10***
Total	***18.6***

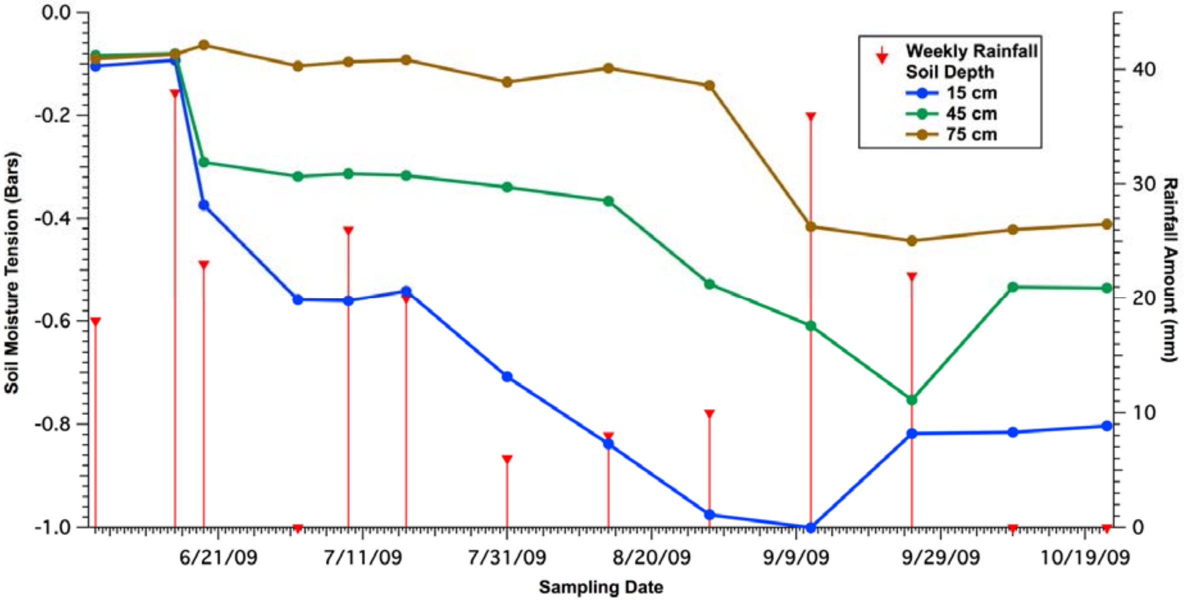

Figure 6-H Mean Soil Tensiometer Readings for Blocks 9-10 as a Function of Date and Soil Depth Together with Weekly Rainfall Amounts, Oxford Station. (Soil depths are referenced to the top of the soil mounds).

The next soil depth increment (45 cm) appears to mimic the pattern at the 15 cm depth somewhat with a noticeable decline after August 1. A mild decline is also observed at the 75 cm depth starting in mid-August. There is some recovery near the end of the study period in the upper two depths in October. Blocks 9-10 contained no plants, thus the increase in soil tension over time reflects water loss through evaporation, despite several rain events. Failure to see recovery following a rain event probably reflects formation of surface crusts on the bare soil, which limited infiltration and favoured lateral surface runoff. Under conditions of relatively high soil water tension, there would be little to no significant vertical transport of water or solutes except as wetting fronts as water moved into the dry soil. A similar pattern in soil tension readings was observed in Blocks 1-8, which did contain tobacco plants. The mean soil tension readings as a function of date and soil depth for Blocks 1-8 are shown in Figure 6-I.

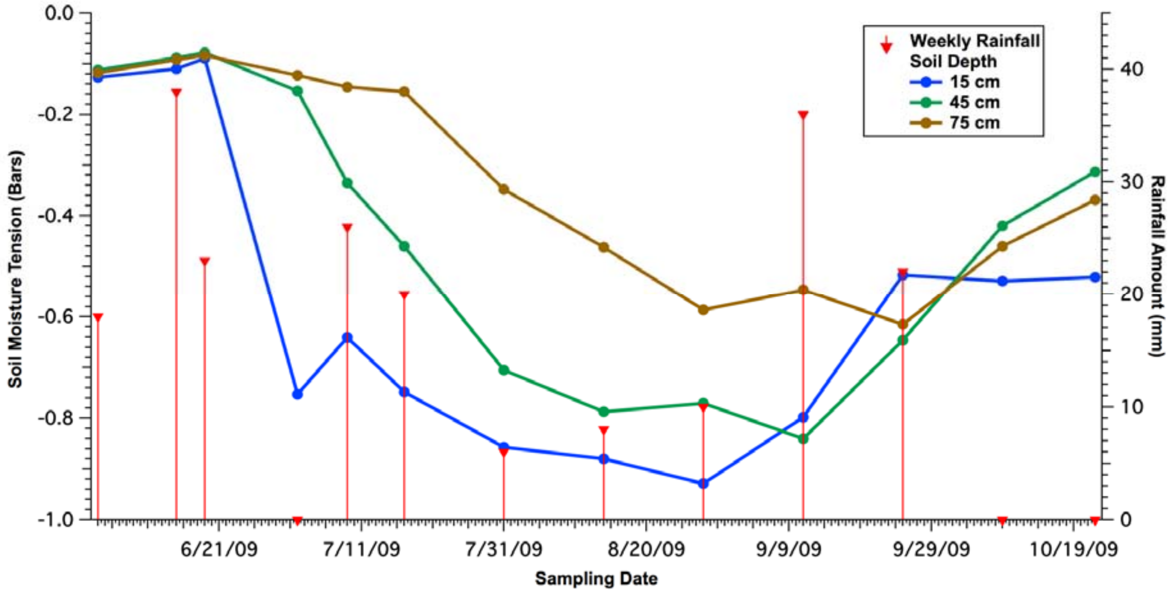

Figure 6-I Mean Soil Tensiometer Readings for Blocks 1-8 as a Function of Date and Soil Depth Together with Weekly Rainfall Amounts, Oxford Station. (Soil depths are referenced to the top of the soil mounds).

With the presence of tobacco plants, the soil in Blocks 1-8 dried relatively faster than the bare soil, with the 75 cm depth beginning to show an increase in soil tension by the first week in July. Driest conditions were reached in early to mid-August across all three-soil depths monitored. As illustrated in Figures 6-F and 6-G, the tobacco plants had reached substantial size by mid-July, and continued to increase in leaf area and mass through mid-August, maintaining a substantial transpiration demand on the soil. Recovery of soil water began in the upper two depths following a relatively large amount of rainfall during the week of August 11th. Due to the size and presence of the plants on the beds, their leaves would act to limit the impact of raindrops forming surface crusts on the soil, and the leaves would also redirect the rain to the inter-rows, which are below the original surface of the soil. The increase in soil water content in the upper two soil depths, especially compared to the bare soil, reflect the ability of the plants to influence soil water infiltration. Apparently the upper soil depths become wet enough by September 25th that the second substantial rain event during this period begins to promote recovery of soil water content at the 75 cm depth relative to the top of the soil mound (Figure 6-I).

Both Figures 6-H and 6-I illustrate that despite the recorded rainfall during the summer of 2009 at the Oxford station, the soil within the research plots began drying and stayed dry throughout much of the study period, and did not begin to recover until September. The presence of the tobacco plants appeared to enhance soil water infiltration as compared to bare soil, promoting both vertical and perhaps lateral transport of water. Vertical transport is reflected in the eventual decrease in soil water tension at the 75 cm depth in late September and the steady decrease in soil water tension at the 45 cm depth. The decrease in soil water tension at the 15 cm depth may reflect lateral movement of water into the soil mound from the surrounding excavated inter-rows. It is anticipated that soil nitrate (and perchlorate) concentrations under these soil moisture conditions would remain relatively static with soil depth, and decrease only due to plant uptake over time.

Soil bulk density was measured as a function of soil depth near the end of the study. Samples were obtained in Blocks 1-10. Samples were taken with reference to the top of the soil mounds. Soil bulk density values as a function of plot and soil depth relative to the top of the soil mound are shown in Table 6-B. The vertical profile of soil bulk density as a function of soil depth relative to the top of the soil mound is shown in Figure 6-J.

Table 6-B. Soil Bulk Density and Mean Soil Porosity as a Function of Depth Relative to the top of the Soil Mound, Oxford Station

Depth range (cm)	Block 1	Block 2	Block 3	Block 4	Block 5	Block 6	Block 7	Block 8	Block 9	Block 10	Mean	Mean Soil Porosity (%)
	(g/cm^3)											
15-0	1.58	1.61	1.59	1.49	1.58	1.54	1.49	1.41	1.62	1.64	**1.56**	**41**
0-15	1.75	1.78	1.71	1.83	1.85	1.68	1.71	1.94	1.71	1.89	**1.79**	**32**
15-35	1.87	1.99	1.88	2.11	1.96	1.95	1.97	1.93	2.03	1.82	**1.95**	**26**
35-60	1.62	1.59	1.55	1.53	1.68	2.07	1.55	1.79	1.89	1.63	**1.69**	**36**
60-85	1.57	1.70	1.69	1.62	1.85	1.69	1.82	1.69	1.85	1.91	**1.74**	**34**

Soil bulk density values as a function of depth were relatively consistent across all 10 blocks. Lowest soil bulk density was measured in the soil mound, which is consistent with being formed by excavation of the surrounding soil earlier in the season. Maximum soil density is observed at a depth of approximately 30 cm below the original surface of the soil (Figure 6-J). This is symptomatic of the presence of a plow pan at approximately 8-12 inches deep in the soil. Such plow pans restrict both water and root movement. The presence of the pan was readily apparent when obtaining soil bulk density samples, offering substantial resistance to penetration of sampling equipment if the soil at this depth was dry, as noted in Figures 6-H and 6-I. Thus the presence of this more dense soil layer would also act to restrict vertical transport of soil nitrate (and perchlorate) in these soils.

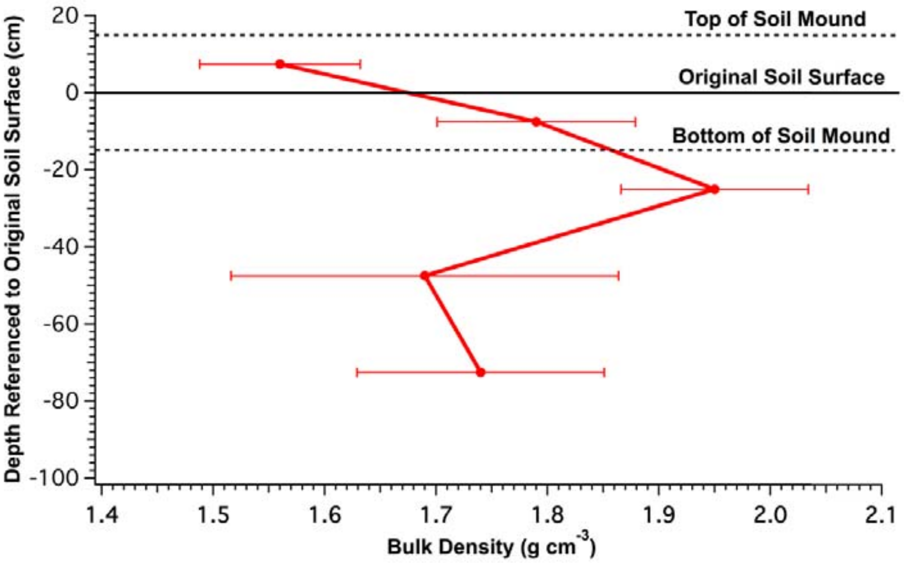

Figure 6-J Mean Soil Bulk Density and Standard Deviation for Block 1-10 as a Function of Soil Depth Relative to the Top of the Soil Mound, Oxford Station.

Another indication of the presence of a plow-pan or at least a restrictive layer present in the soil at ~30 cm depth is the change in soil porosity. Soil porosity is a measure of the bulk soil that is not occupied by soil solids. This value decreases to 26% near the 30 cm depth. Given the clayey nature of the soil at the Oxford station, the majority of this air space at the 30 cm depth will be composed of very fine soil pores, which will also act to restrict water movement (decrease saturated hydraulic conductivity).

6.2.3 Groundwater and Shallow Wells

Most of the deep monitoring wells filled with water within a day of being installed, and all contained water two weeks later when sampled. Averaged across all the wells, depth to groundwater was initially 2.5 m below the surface of the soil and then increased steadily from the end of May through the middle of June reaching of height of 0.75 m, referenced to the original soil surface. This is generally consistent with the soil water tension readings for the 75 cm depth in Figures 6-H and 6-I. The soil water tension readings indicate that the soil was not saturated but at near capacity with enough distance between the surface of the ground water to allow drainage. Rainfall appears to have had little immediate impact on the groundwater depth measurements as there were several occasions where significant rainfall events preceded field sampling with no apparent upward trend in the water table.

The lack in response to major rainfall events in groundwater depth suggests that the restrictive soil layer at 30 cm was limiting the permeability. However, it is significant that the groundwater level reached the 0.75 m depth in early June, when fertilizer was being applied to the plots. The rise in groundwater suggests some communication with rainfall events, even given the presence of the dense clay layer at 30 cm. Whether this communication occurred within the field (soil plots) via potential large cracks in the underlying soil clay layer, or presence of discontinuities in the soil clay layer across the plots, is unknown. The rise in groundwater depth at this time does suggest the possible presence of a more direct route of fertilizer nitrate (and perchlorate) to the underlying groundwater in addition to vertical infiltration through the restrictive clay layer at the 30 cm depth.

Average groundwater pH fluctuated throughout the sampling period from a low of around 7.0 to a high of 8.3, though almost all observations were between 7.0 and 7.5. Part of the observed fluctuations may reflect the difficulty in measuring the pH of water, relatively low in ionic strength, with a near neutral pH value.

Average groundwater temperature gradually increased throughout the summer from 18°C (64° F) in the early spring to about 21°C (70° F) by the late summer. This is to be expected given the heating of the soil, as well as warmer waters that may be reaching the groundwater. The final samples collected in October saw a slight decrease in temperature to 19°C (66°F).

Both redox potential and dissolved oxygen followed the same trend throughout the growing season (Figure 6-K). After installation of the wells, both dissolved oxygen and redox measurements indicated a relatively well-oxygenated environment. To what extent this is an artifact of well installation is not known. It is apparent that even as the groundwater depth increased, reaching a average height of 0.75 m bgs, both the redox potential and dissolved oxygen continued to decrease until reaching relatively constant values between June 20th and July 4th 2009. The steady decline in redox potential and dissolved oxygen content is not necessarily consistent with input of surface waters during the period of increase in groundwater elevation.

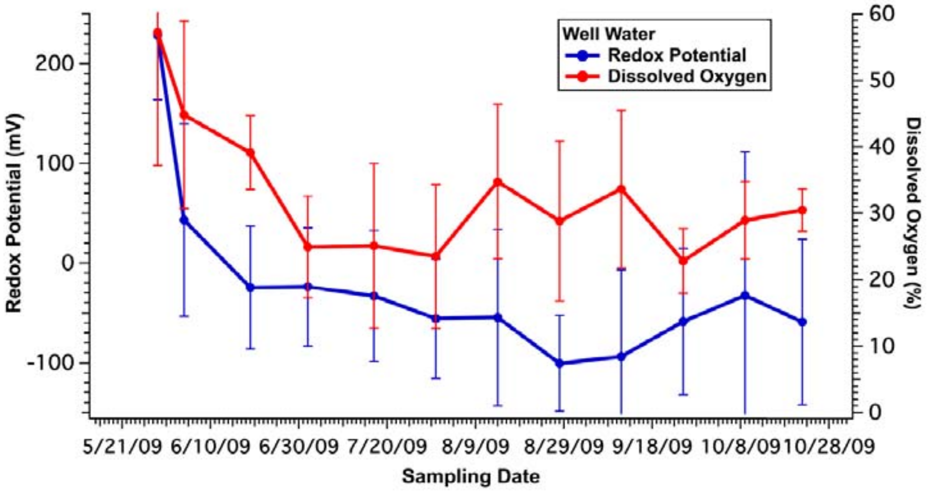

Figure 6-K Average Redox Potential and Dissolved Oxygen Content as a Function of Date for Groundwater from Wells Installed at the Ends of Blocks 3, 6, and 9, Oxford Station. (Vertical lines represent one standard deviation).

Failure of the DO content to reach values of near zero suggests that nitrate could still be present in the groundwater, although the redox potential readings suggests the possible presence of reduced iron in solution as well. More than likely, the groundwater depths at this location mark more of a transition zone across the landscape where conditions vary and concentration of nitrate will vary as well. The readings indicate lack of a presence of a clear demarcation that would indicate destruction of any nitrate that reached the groundwater.

Analysis of the groundwater samples from the deeper wells for perchlorate, chloride and nitrate were carried out using ion chromatography. A qualitative assessment of the presence of sulfates and phosphates in the well water samples was also conducted using ion chromatography. No perchlorate was detected in any of the water samples collected, although the presence of other anions in the water samples was more variable than expected. Figure 6-L illustrates a typical result for anion analyses (chloride, nitrate, phosphate and sulfate) of the water samples.

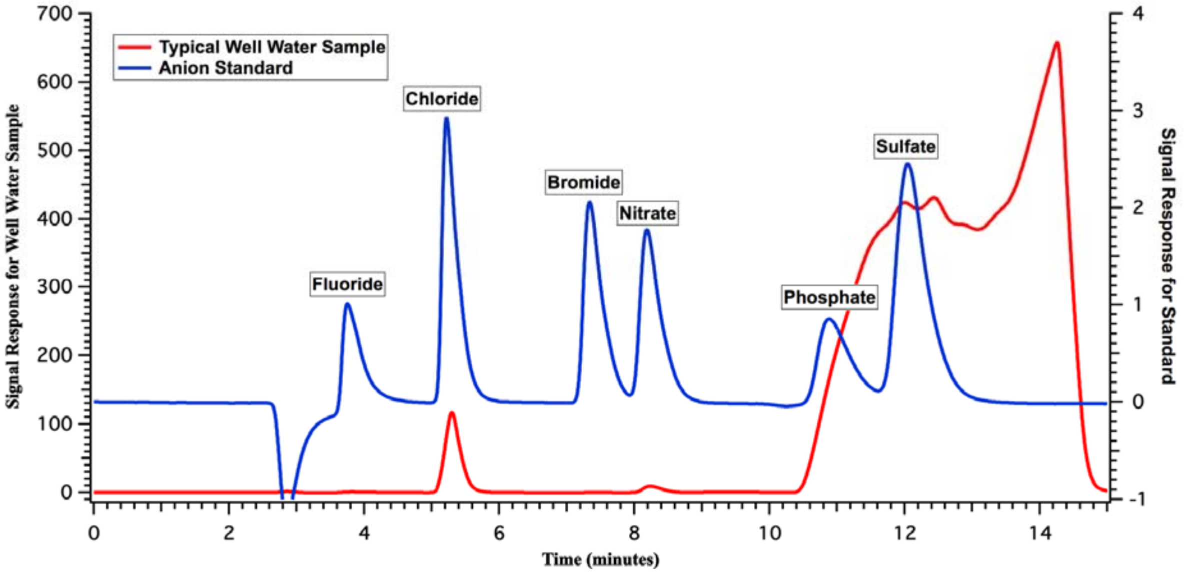

Figure 6-L Standard Anion Analysis of a Typical Well Water Sample Compared to an Anion Standard Using Ion Chromatography, Oxford Station.

Standard anion analysis detected the quantitative presence of chloride and nitrate in the well water samples. Phosphate and sulfate appeared to also be present in the well water samples as well as possible other inorganic or organic species. Note that the scales in Figure 6-L vary by almost a factor of 200x, indicating the presence of substantial amounts of sulfate and possibly phosphate in the groundwater. Over 80% of the chromatograms run for standard anion analyses from both sites (Oxford station and the Whiteville station) had shapes similar to that illustrated in Figure 6-L of varying magnitude.

The presence of the relatively high amounts of dissolved salts in the well water samples effectively raised the detection limit for perchlorate in the well water samples (from ~5 ppb to ~25-50 ppb), in part because the presence of relatively high salts or other anions required a reduction is the injection sample loop from 1 mL to 0.5 mL. Recovery of perchlorate was also reduced due to the presence of the high background in the well water samples (Figure 6-M).

As noted, no perchlorate was detected in the water samples collected during the study using ion chromatography. A small subset of water samples was sent to CAS to confirm analyses of the water samples by ion chromatography (Table 6-C). The method reporting limit for analyses by CAS was 0.2 ppb. Of the 9 separate samples submitted, no perchlorate was detected in 7 samples, 1 sample had a reported value of 1.4 ppb perchlorate, and 1 sample submitted in duplicate yielded a value of 32-36 ppb perchlorate in solution (which exceeded the calibration range of the instrumentation) (Table 6-C). Visual inspection of the chromatograph derived from ion chromatography for the positive detection of perchlorate (sample OX-P9DA) did not indicate the presence of perchlorate, which is not inconsistent with the elevated detection limit using ion chromatography due to the presence of relatively high salts in the water samples. It is not known to what extent these high salts may have impacted the analyses conducted by CAS.

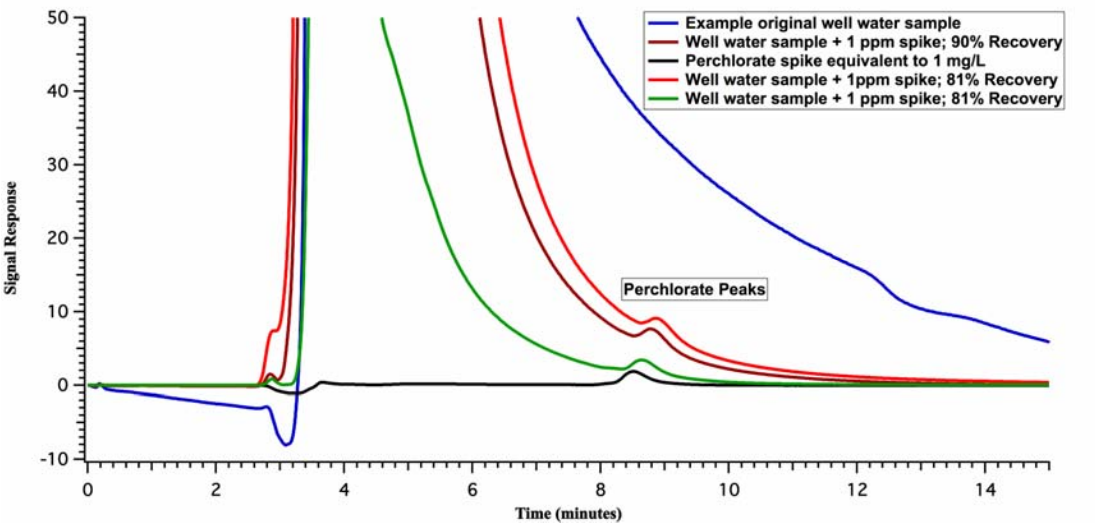

Figure 6-M Example Chromatogram and Spiked Recoveries for Several Well Water Samples for Perchlorate, Oxford Station.

Only two of the groundwater wells were found to consistently contain nitrate in the ground water (well designations OX-P3DA and OX-P9DA). The remaining four wells either had nitrate concentrations below the detection (<0.05 mg NO_3-N/L), or nitrate was detected at concentrations <1 mg NO_3-N/L only during the first several sampling dates. For well OX-P3DA, the concentration of nitrate detected was always low, averaging 0.3 mg NO_3-N/L, with the majority of detections being closer to 0.1 mg NO_3-N/L. The highest nitrate concentration observed for well OX-P3DA was 0.9 mg NO_3-N/L on the first sampling date after installation

(May 14, 2009). Only well OX-P9DA had a relatively consistent elevated concentration of NO_3-N, with a mean concentration of 5.3 mg NO_3-N/L. The highest nitrate concentrations (~8 mg NO_3-N/L) were observed on the first two sampling dates after well installation (May 14 and 29, 2009). For the remainder of the study period, the nitrate concentration in the well was 5 +/- 1 mg NO_3-N/L. There were no apparent temporal trends in the nitrate concentrations in either well OX-P3DA or OX-P9DA.

Table 6-C. Results of Well Water Sample Analyses for Perchlorate by Columbia Analytical Services Laboratory. Oxford Station

Sample	Date	Matrix	Compound	MRL	Adjusted MRL	Result	Flag	Unit
OX-P9DB	06/04/09	WATER	PERCHLORATE	0.2	0.20	0.20	U	µg/L
OX-P3DB	06/19/09	WATER	PERCHLORATE	0.2	0.20	0.20	U	µg/L
OX-P3DA	07/02/09	WATER	PERCHLORATE	0.2	0.20	1.4		µg/L
OX-P6DA	07/31/09	WATER	PERCHLORATE	0.2	0.20	0.20	U	µg/L
OX-P9DA	08/28/09	WATER	PERCHLORATE	0.2	0.20	36	E	µg/L
OX-P9DA-Dup	08/28/09	WATER	PERCHLORATE	0.2	2.0	32	D	µg/L
OX-P6DA	09/25/09	WATER	PERCHLORATE	0.2	0.20	0.20	U	µg/L
OX-P6DB	10/22/09	WATER	PERCHLORATE	0.2	0.20	0.20	U	µg/L
OX-P3DB	10/22/09	WATER	PERCHLORATE	0.2	0.20	0.20	U	µg/L
OX-P9DB	10/22/09	WATER	PERCHLORATE	0.2	0.20	0.20	U	µg/L

Notes:

U – compound was analyzed for but not detected

E – sample concentration exceeded the calibration range

D – compound detected in diluted sample

MRL – method reporting limit

Dup - duplicate

Unlike nitrate, chloride was found to be present in the groundwater sampled from all 6 groundwater wells (Table 6-D). However, the individual chloride concentrations within a well remained relatively unchanged during the sampling period, except possibly for the first sampling date after well installation. This lack in temporal trends in chloride concentrations, as for the two wells with detectable nitrate, suggests that the groundwater being sampled by the six groundwater wells was not directly influenced by the surface applied fertilizers used in this study. The constancy in the observed concentrations suggests that the groundwater being sampled is controlled by a local source in the immediate vicinity of each well.

Table 6-D. Chloride Concentrations in Groundwater, Oxford Station

	OX-P3DA	**OX-P3DB**	**OX-P6DA**	**OX-P6DB**	**OX-P9DA**	**OX-P9DB**
Date	**(mg/L)**					
5/14/09	84	22	102	60	68	110
5/29/09	82	18	89	43	63	105
6/19/09	82	16	90	39	64	103
7/2/09	81	15	87	39	65	104
7/17/09	79	16	87	38	67	104
7/31/09	81	19	92	39	66	107
8/14/09	81	18	88	38	63	107
8/28/09	78	18	85	38	62	106
9/11/09	78	18	86	37	61	104
09/25/09	81	14	87	39	75	81
10/9/09	82	14	88	37	63	107
10/22/09	83	13	88	36	62	103
Mean=	**81**	**17**	**89**	**40**	**65**	**104**
StdDev=	**2**	**3**	**5**	**6**	**4**	**7**
%CV=	**3**	**15**	**5**	**16**	**6**	**7**

6.2.4 Tobacco Production

Tobacco seedlings (variety K326) were originally grown in a greenhouse and transplanted to the field along ~2 foot (61cm) intervals into prepared, raised beds about 12 inches (30cm) high and 4 feet (1.2m) apart. The height of the beds or mounds is actually ~ 15 cm compared to the original soil surface. Roughly 15 cm of soil is removed from the inter-rows to form the beds, giving a net height from the soil surface in the inter-row to the top of the bed or mound of 30 cm.

The transplanted tobacco grows relatively slowly over the first 4 weeks, due in part to the shock of being transplanted from the greenhouse. Once acclimated, the tobacco plants enter a rapid growth stage and a second side dress of an all-nitrogen fertilizer is applied (200 lbs/ac of 15.5-0-0) in the same position with regards to the plants as the initial side dress.

Harvesting of the lower tobacco leaves began at about 13 weeks from the transplant date. Subsequent harvests occurred at 16 and 20 weeks from transplant and were based upon the station manager's judgment as to the quality of the leaves. Weather plays a major factor in leaf quality and harvest timing - hot temperatures with no rain immediately prior to the harvest being most important. The harvest weights collected from Blocks 1-8 were considered by the station manager to be typical of harvests in the area for 2009. The amount of tobacco leaves harvested increased with each harvest. The mass of leaves removed during the third and final harvest was ~3x each of the early two harvests. The weights of harvested tobacco leaves from Blocks 1-8 are summarized in Table 6-E.

Table 6-E. Mass of Harvested Tobacco Leaves from Blocks 1-8, Oxford Station. (Results are for cured tobacco, moisture content 12-15%)

	Harvest 1 8/21/2009	**Harvest 2 9/11/2009**	**Harvest 3 10/9/2009**	**Totals**
	(kg)			
Block 1	14	22	73	**110**
Block 2	17	22	67	**106**
Block 3	19	22	57	**98**
Block 4	20	24	51	**96**
Mean	***17.5***	***22.5***	***62.0***	***102.5***
Block 5	16	21	66	**103**
Block 6	18	24	64	**107**
Block 7	21	22	60	**103**
Block 8	15	24	70	**110**
Mean	***17.5***	***22.8***	***65.0***	***105.8***
Total	**141**	**182**	**509**	**832**

The total mass of tobacco leaves produced appeared not to be influenced by the source of fertilizer used for the second side dress application (potassium nitrate versus calcium nitrate). The mean of the amount harvested from the sets of four blocks per treatment were essentially identical for the first two harvests, with the mean removed from Blocks 5-8 (calcium nitrate source) being slightly higher in the third harvest. However, the absolute difference in the means is well within the variation within the respective plots such that the differences are not statistically significant.

6.2.5 Soil Chemical Analysis – Perchlorate

Pre-planting soil samples were obtained from Blocks 1–10 on March 27, 2009. The samples were taken over the depth interval of 0-200 cm when possible. Restrictive layers at depths >100 cm prevented sampling below 100 cm in some blocks. Scheduled biweekly sampling was initiated on June 19, 2009 and continued throughout the growing season with the last set of samples recovered on September 25, 2009. The regular biweekly soil sampling was over the depth interval of 100 cm from the top of the mound, divided into 5 increments. A total of 454 soil samples were collected from the Oxford station location (Table 6-F1).

Table 6-F1. Number of Soil Samples Collected During 2009 Field Study, Oxford Station

Date	Depth Interval (cm)	Reference	Number of Depths Sampled	Count
3/27/09	0-200	Original soil surface	5-6	54
6/19/09	0-100	Top of mound	5	50
7/02/09	0-100	Top of mound	5	50
7/17/09	0-100	Top of mound	5	50
7/31/09	0-100	Top of mound	5	50
8/14/09	0-100	Top of mound	5	50
8/28/09	0-100	Top of mound	5	50
9/11/09	0-100	Top of mound	5	50
9/25/09	0-100	Top of mound	5	50
			Total Number of Soil Samples =	454

All 454 soil samples obtained from the field study at the Oxford station were extracted with the 0.001M $CaCl_2$ solution and the filtered extracts analyzed for perchlorate. No perchlorate was detected in any of the 454 soil samples. Each chromatogram was reviewed to ensure that the integration software for the ion chromatograph was functioning correctly and had not inadvertently excluded perchlorate peaks in the analysis output. Failure to detect perchlorate in the soil extracts was not totally unexpected given the possibility that the amount of perchlorate added during side dress with the SQM potassium nitrate was lower than expected. As discussed above, the amount of perchlorate was substantially less than originally projected for the experiment.

A total of 6 soil samples were submitted to CAS for analysis of extractable perchlorate (Table 6-F2). The soil samples were chosen at random from the various plots and sampling dates. Perchlorate was below the reporting limit (2 µg/kg) for 5 of the 6 soil samples. One soil sample (obtained June 19, 2009; 0-15 cm depth) was found to have a concentration of 4.7 µg/kg perchlorate.

Table 6-F2. Results of Soil Analyses for Perchlorate by Columbia Analytical Services Laboratory. Oxford Station

Sample	Date	Matrix	Compound	MRL	Adjusted MRL	Result	Flag	Unit
OX-P3-50-100	06/01/09	SOIL	PERCHLORATE	2.0	2.0	2.0	U	µg/kg
OX-P1-0-15	06/19/09	SOIL	PERCHLORATE	2.0	2.1	4.7		µg/kg
OX-P8-30-50	07/02/09	SOIL	PERCHLORATE	2.0	2.0	2.0	U	µg/kg
OX-P2-30-50	07/31/09	SOIL	PERCHLORATE	2.0	2.0	2.0	U	µg/kg
OX-P3-15-30	08/28/09	SOIL	PERCHLORATE	2.0	2.0	2.0	U	µg/kg
OX-P9-30-50	09/25/09	SOIL	PERCHLORATE	2.0	2.1	2.1	U	µg/kg

Notes:

U – compound was analyzed for but not detected

MRL – method reporting limit

6.2.6 Soil Chemical Analysis – Ammonium and Nitrate

All 454 soil samples obtained from the Oxford station were analyzed for extractable NH_4-N and NO_3-N. Pre-plant soil samples obtained on March 27, 2009 had very low background levels of extractable NO_3-N and NH_4-N (Figure 6-N). This was consistent throughout the 0-200 cm sampling depth. Overall, no significant differences in pre-plant soil concentrations of NO_3-N or NH_4-N were determined to exist, and the grand mean from Blocks 1-10 as a function of depth increment sampled was used to compare to results from post-planting soil samples.

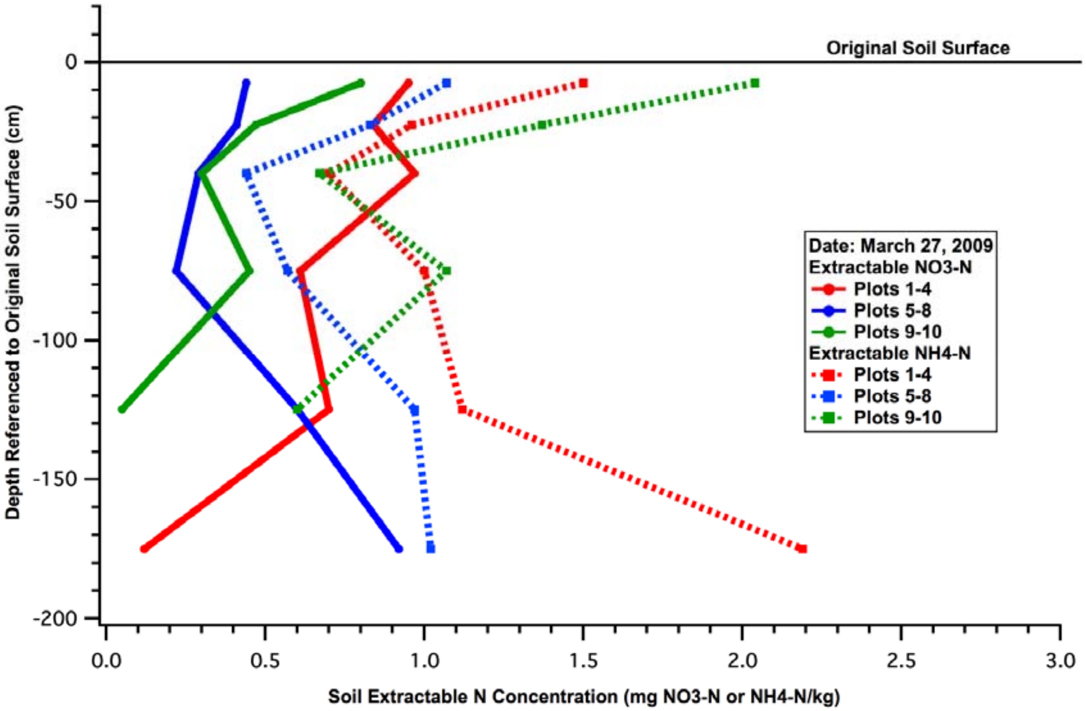

Figure 6-N Mean Soil Extractable NO3-N and NH4-N Concentrations as a Function of Sampled Depth Increment for Blocks 1-10, Oxford Station. (March 27, 2009. Mean values are plotted at mid-point of depth increment sampled).

The mean soil concentrations and calculated standard deviations of extractable NO_3-N grouped by treatment plots for the soil samples collected post-planting as a function of depth increment sampled are provided in Table 6-G. As expected, the range of extractable soil NO_3-N varied substantially both with soil depth and date of sampling. Individual coefficients of variation typically had values of > 50 to > 100%. This is to be expected given that the emphasis in this study and the soil sampling protocol adopted focused on perchlorate transport.

As illustrated in Figure 6-N, background levels of extractable NO_3-N at transplanting were most likely very low, thus the fertilizer bands applied during the study were for all intents and purposes the only source of NO_3-N in the soil mounds. The distribution of this soil NO_3-N was not uniform, and migrated away from the fertilizer band where it was initially applied, depending on time and rainfall amounts. Thus, it is reasonable to expect that with time, the level of NO_3-N intercepted when obtaining soil cores from the center of the soil mounds would increase, as NO_3-N moved and dispersed both laterally within the mound and vertically into the underlying soil. Apparent concentrations of extractable soil NO_3-N should increase initially across the various depth increments sampled until reaching an apparent maximum and then begin to decrease due to uptake of the N by the growing tobacco plants, and continued vertical transport of the N with continued rainfall events throughout the growing season.

Table 6-G. Mean Soil Concentrations of Extractable NO_3-N as a Function of Depth Increment Sampled from June 19, 2009 to September 25, 2009, Oxford Station. (Values in parentheses are calculated standard deviation for plots sampled. Depth increments are referenced to top of soil mounds formed for tobacco transplants)

Depth Increment (cm)	**Sampling Date**							
	6/19/09	**7/2/09**	**7/17/09**	**7/31/09**	**8/14/09**	**8/28/09**	**9/11/09**	**9/25/09**
	(mg NO_3-N/kg)							
Blocks 1-4 (Potassium Nitrate plus Tobacco)								
0-15	18.3 (11.2)	41.5 (19.4)	25.1 (15.9)	19.5 (13.8)	10.3 (7.4)	5.2 (3.0)	2.1 (3.0)	0.6 (1.2)
15-30	12.4 (5.6)	24.9 (13.6)	14.9 (9.2)	36.0 (29.1)	24.6 (21.0)	3.5 (2.1)	2.9 (2.1)	1.8 (2.1)
30-50	7.0 (3.9)	5.4 (3.2)	4.0 (2.1)	3.1 (1.7)	2.5 (2.3)	2.0 (1.2)	1.6 (1.2)	1.0 (0.8)
50-75	2.8 (1.3)	3.7 (2.1)	3.4 (2.8)	2.4 (1.2)	1.9 (1.6)	1.7 (1.1)	1.4 (1.1)	0.5 (0.7)
75-100	1.4 (1.0)	3.0 (2.2)	2.2 (1.1)	2.2 (1.4)	2.1 (1.9)	2.1 (1.8)	1.3 (1.8)	0.5 (0.7)
Blocks 5-8 (Calcium Nitrate plus Tobacco)								
0-15	23.9 (18.4)	66.8 (7.5)	41.9 (26.0)	35.4 (16.4)	28.4 (11.6)	14.9 (7.9)	10.8 (7.9)	2.9 (1.4)
15-30	23.2 (5.7)	24.2 (9.3)	29.9 (23.2)	56.6 (30.2)	24.0 (10.4)	14.4 (12.3)	9.4 (9.8)	2.0 (0.5)
30-50	7.8 (2.0)	3.0 (0.8)	2.6 (1.1)	8.2 (6.3)	5.5 (5.9)	4.6 (5.6)	4.9 (6.7)	2.7 (3.7)
50-75	3.3 (1.4)	1.5 (0.4)	1.8 (0.9)	2.8 (1.3)	2.4 (2.3)	2.5 (1.3)	1.3 (0.2)	1.3 (0.9)
75-100	1.4 (0.4)	1.2 (0.6)	1.2 (0.2)	2.0 (1.3)	2.5 (2.2)	2.8 (1.9)	1.2 (0.4)	1.0 (0.4)
Blocks 9-10 (Potassium Nitrate minus Tobacco)								
0-15	41.0 (10.9)	73.3 (1.7)	49.0 (7.5)	53.5 (18.9)	39.6 (29.5)	35.3 (12.6)	20.4 (8.8)	10.3 (10.1)
15-30	16.7 (7.1)	20.1 (6.9)	32.4 (12.0)	59.6 (19.5)	21.4 (7.5)	18.6 (11.9)	28.2 (0.8)	13.5 (8.2)
30-50	8.1 (0.5)	14.2 (14.7)	13.4 (1.0)	5.6 (1.3)	3.3 (2.5)	11.5 (6.2)	2.9 (2.4)	2.5 (2.9)
50-75	3.0 (1.4)	1.9 (0.5)	2.5 (1.4)	0.7 (0.4)	2.4 (2.0)	2.3 (0.5)	1.9 (0.9)	0.5 (0.4)
75-100	3.7 (0.9)	2.2 (0.7)	1.5 (0.1)	0.5 (0.3)	0.9 (0.1)	1.5 (1.2)	0.9 (0.1)	0.5 (0.3)

In general, this expected trend in extractable soil NO_3-N concentrations is evident in Table 6-G, where the highest concentrations of soil NO_3-N were observed in early and mid July, followed by an increase in NO_3-N at the lower soil depth increments sampled.

A review of the data in Table 6-G suggests a number of reactions occurring with soil NO_3-N throughout the growing season in Blocks 1-4. The first is that the soil volume in the top 15 cm of the soil mound increases in NO_3-N as nitrate diffuses away from the fertilizer band. This concentration of NO_3-N then decreases steadily over time, probably due to a combination of vertical leaching and plant uptake. The extent of vertical transport is evident in the 15-30 cm sampling increment, where NO_3-N continues to increase for at least a month after the initial sampling date of June 19, 2009. The 15-30 cm depth then experiences a rather rapid decrease in NO_3-N over the following month, mostly likely due to plant uptake as the tobacco plant develops and its root system expands within the soil mound.

There was a noticeable amount of NO_3-N extracted from the 30-50 cm sampling increment with the first soil sampling of June 19, 2009 from Blocks 1-4 and 5-8. Blocks 5-8 exhibited the same trend in the concentration of NO_3-N in the 0-15 cm sampling increment (top of the mound) as Blocks 1-4, and also exhibited the maximum concentration in soil NO_3-N in the 15-30 cm sampling increment (bottom of the soil mound) on July 31, 2009. After this date, there was the expected decline in NO_3-N in the 15-30 cm depth as exhibited by Blocks 1-4, but not to the same extent, with approximately twice the concentration of NO_3-N remaining across the 0-30 cm depth in Blocks 5-8 than Blocks 1-4. The concentration of NO_3-N in the 30-50 cm depth increment also remained constant and somewhat higher than observed in Blocks 1-4, although the difference is probably not significant given the variation in observed concentrations (Table 6-G).

The similar patterns observed in soil NO_3-N concentration as a function of date and sampling increment support the conclusion that nitrate supplied by the two different sources used in this study (potassium nitrate for Blocks 1-4 and calcium nitrate for Blocks 5-8) behaved similarly and was not influenced by the differences in the associated cations of the two nitrate salts (monovalent potassium versus divalent calcium). The mean values as a function of date and sampling increment for Blocks 1-8 were therefore calculated and plotted in Figure 6-O to facilitate comparison to the soil NO_3-N patterns observed for the bare soil plots (Blocks 9-10; Figure 6-P).

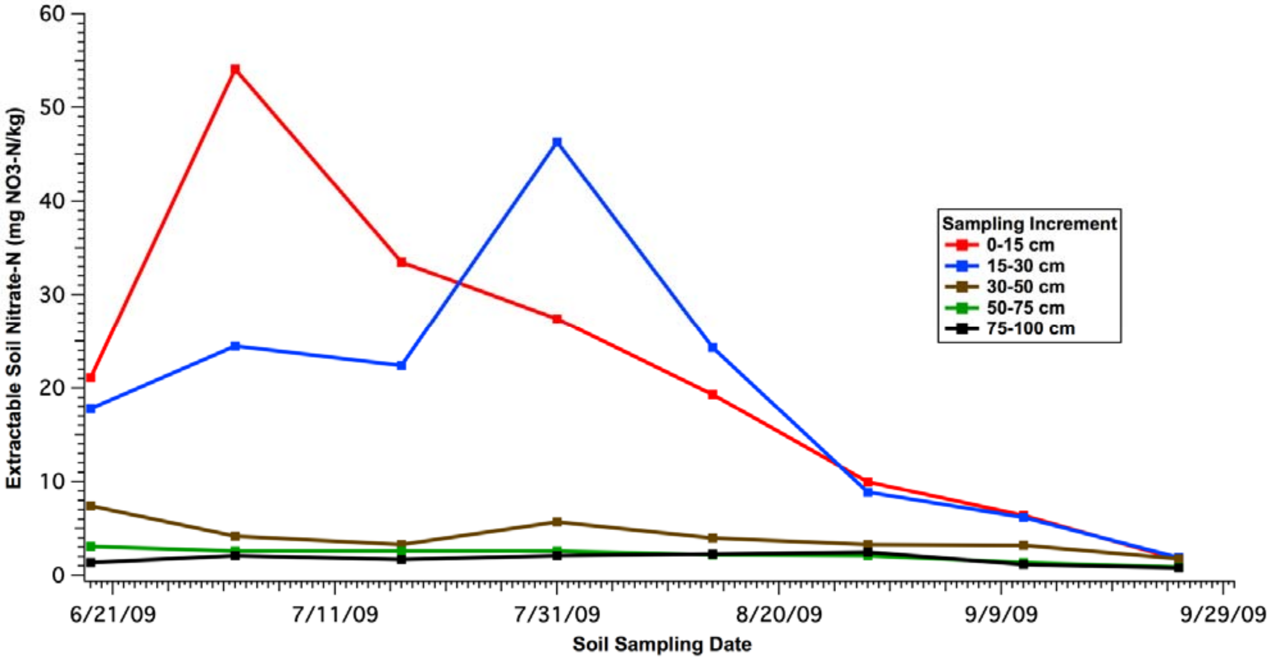

Figure 6-O Mean Concentrations of Soil Extractable NO3-N for Blocks 1-8 as a Function of Sampling Date and Sampling Depth Increment, Oxford Station. (Sampling depth increments are referenced to top of soil mounds).

Comparison of Figures 6-O and 6-P indicates that the patterns in NO_3-N in the presence of tobacco were also evident in Blocks 9-10 in the absence of tobacco. Soil NO_3-N increased in the top of the mound (0-15 cm sampling increment) and reached a maximum on July 2, 2009. There then was a steady decline in the concentration of NO_3-N. Maximum soil NO_3-N concentration in the 15-30 cm sampling increment (bottom half of the soil mound) was also observed on July 31, 2009. The major difference between Blocks 9-10 versus Blocks 1-8 is the relative magnitude of the soil NO_3-N concentrations, with Blocks 9-10 demonstrating relatively higher NO_3-N concentrations throughout the growing season. The rapid increase in NO_3-N in the 30-50 cm depth increment is duplicated in Blocks 9-10, with the suggestion that the concentration of NO_3-N across this depth increment remains elevated throughout the growing season.

A consistent theme across all 10 blocks was the apparent stability of the soil NO_3-N concentrations at the 50-75 and 75-100 depth increments. The NO_3-N concentrations at these deeper soil depths appeared to remain constant or decrease slightly during the growing season (Table 6-G, Figures 6-O and 6-P).

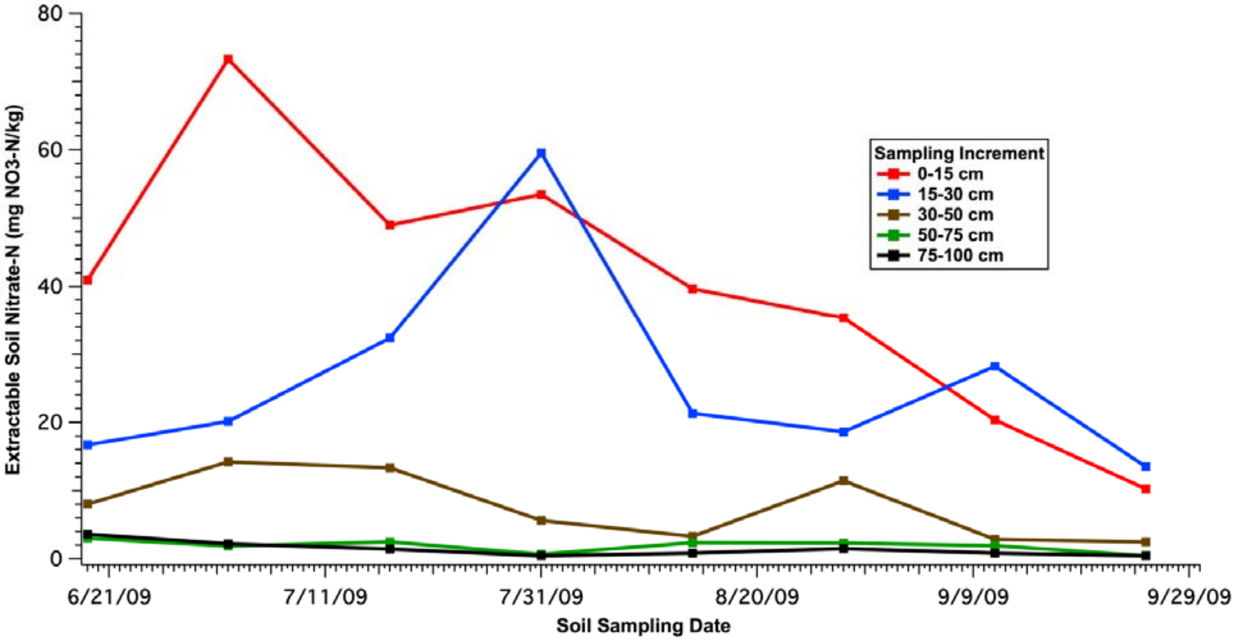

Figure 6-P Mean Concentrations of Soil Extractable NO_3-N for Blocks 9-10 as a Function of Sampling Date and Sampling Depth Increment, Oxford Station. (Sampling depth increments are referenced to top of soil mounds).

The bulk of extractable soil NO_3-N movement in the soil mound was relatively independent of the presence of the tobacco crop. Vertical movement was restricted by the presence of a dense soil layer at the 30-50 cm depth, relative to the original soil surface (Table 6-B). The absolute magnitude of NO_3-N in the soil mound was influenced by the presence of the tobacco crop, especially after July 31, 2009, when there is a distinct, consistent removal of NO_3-N from the top 30 cm occupied by the soil mound. By September 25, 2009, NO_3-N concentrations in Blocks 1-8 were approaching those observed in the background soil samples collected on March 27, 2009, while concentrations of NO_3-N in Blocks 9-10 remained relatively elevated, at least to the 50 cm depth as referenced to the original soil surface.

6.2.7 Tobacco Nitrogen Content

Leaf punches were obtained from tobacco plants in Blocks 1-8 throughout the growing season. Percent moisture content ranged from 67 to 87 % regardless of age of the leaf (Table 6-H). Percent N content varied with age of leaf, with highest N content found in the youngest leaves, and lowest N content found in harvested leaves. Overall, the N content between harvests and blocks showed similar trends reinforcing the conclusion that there was no significant difference in tobacco growth at the Oxford station using either KNO_3 or $Ca(NO_3)_2$ as the N source for the second side dress with N.

Table 6-H. Nitrogen and Moisture Content of Tobacco Samples During 2009 Field Study, Oxford Station

Date	Sample Type	Lower Leaves		Upper Leaves	
		N-Content Wet-Wt. Basis (%)	**Moisture Content Wet-Wt. Basis (%)**	**N-Content Wet-Wt. Basis (%)**	**Moisture Content Wet-Wt. Basis (%)**
Blocks 1-4 Nitrogen Source: Potassium Nitrate					
15-JUN-09	Leaf	0.579	86.6	-	-
19-JUN-09	Leaf	0.488	83.0	0.906	79.4
2-JUL-09	Leaf	0.540	78.7	1.100	72.1
17-JUL-09	Leaf	0.630	81.3	0.972	78.1
31-JUL-09	Leaf	0.442	79.4	1.014	70.9
14-AUG-09	Leaf	0.428	79.8	0.845	67.1
21-AUG-09	Harvest	0.420	80.2	-	-
28-AUG-09	Leaf	-	-	0.894	68.3
11-SEP-09	Leaf	-	-	0.706	70.8
11-SEP-09	Harvest	-	-	0.442	79.9
9-OCT-09	Harvest	-	-	0.596	74.2
Blocks 5-8 Nitrogen Source: Calcium Nitrate					
15-JUN-09	Leaf	0.587	85.1	-	-
19-JUN-09	Leaf	0.544	82.7	0.820	80.5
2-JUL-09	Leaf	0.682	75.6	1.172	73.5
17-JUL-09	Leaf	0.531	82.1	0.948	80.4
31-JUL-09	Leaf	0.411	79.6	1.009	72.5
14-AUG-09	Leaf	0.508	81.3	0.890	68.5
21-AUG-09	Harvest	0.370	83.1	-	-
28-AUG-09	Leaf	-	-	0.878	67.6
11-SEP-09	Leaf	-	-	0.763	70.7
11-SEP-09	Harvest	-	-	0.412	80.1
9-OCT-09	Harvest	-	-	0.653	72.7

An estimate of N recovered by the harvested tobacco leaves is presented in Table 6-I, based on the provided mass of leaves harvested reported by the Oxford station staff (Table 6-D), and the analyses of leaf punches obtained from the harvested leaves (Table 6-H). An average of 13.5% moisture content was assumed for the cured-tobacco (Table 6-D) in order to express the calculated results on an oven-dry basis.

The calculated recoveries of fertilizer N added average 91 and 98% for Blocks 1-4 and 5-8, respectively. While such high recoveries are consistent with the patterns of NO_3-N observed in the soil mounds during the growing season (i.e. the mass of NO_3-N applied appeared to remain in the 30 cm depth occupied by the soil mound with relatively little downward leaching of N), recoveries exceeding 90% for N utilization by crops are not typically observed. More typically N uptake recoveries vary from 30 to possibly 50%. Thus the results in Table 6-I should be considered atypical and possibly biased because of the way leaf sampling was conducted during the study.

Table 6-I. Mass of Nitrogen Recovered in Harvested Tobacco Leaves, Oxford Station. (All calculations normalized to an oven-dry weight basis)

Harvest Date	Harvested Leaf Mass (kg/block)	Percent N Concentration (%)	Mass of N Recovered	
			(g N/block)	(lbs N/block)
Blocks 1-4 Nitrogen Source: Potassium Nitrate (Total N Applied: 2.2 kg N/block; 4.8 lbs N/block)				
21-AUG-09	15.1	2.12	320	0.71
11-SEP-09	19.5	2.20	429	0.94
9-OCT-09	53.6	2.31	1238	2.73
		Totals=	**1987**	**4.38**
Blocks 5-8 Nitrogen Source: Calcium Nitrate (Total N Applied: 2.1 kg N/block; 4.7 lbs N/block)				
21-AUG-09	15.1	2.19	331	0.73
11-SEP-09	19.7	2.07	408	0.90
9-OCT-09	56.2	2.40	1349	2.97
		Totals=	**2088**	**4.60**

Leaf samples used to make the calculations in Table 6-I are based on leaf punches and not total leaf samples gathered each time the crop was sampled. Leaf punches will favour the fleshy portion of the leaf and avoid the mid-rib and other larger leaf veins, which will typically be low in N content, but will contribute to the total leaf mass. Thus one explanation for the high recovery of N in Table 6-I, is that the %N concentration is biased high due to the preferential sampling of the fleshy portion of the tobacco leaves. In other words, the harvested mass of tobacco leaves needs to be corrected downward to eliminate the contribution in mass from leaf veins and mid-rib. Another possible explanation for the high recoveries is that the mass of harvested leaves per plot reported by the research station staff had a higher moisture content than cited for the provided data (Table 6-E). This would also act to lower the mass of tobacco harvested and thus the estimate of the percent N recovered.

6.2.8 Summary of Pertinent Results

Perchlorate was not detected in any of the soil or groundwater samples collected during the baseline event, thus it was not possible to quantify the potential impact of perchlorate from Chilean nitrate fertilizer historically applied to tobacco crops at the Oxford station.

Perchlorate was also not detected in any of the groundwater or soil samples collected following application of the fertilizers and analyzed by EATS (although perchlorate was detected at low concentrations in one soil sample and two groundwater samples submitted to CAS for confirmatory analysis). Due to the absence of perchlorate in the groundwater and soil samples, the study could not adequately assess the fate of perchlorate at this research station. Instead, nitrate was used as a surrogate to assess potential transport of perchlorate through the soil matrix and into the underlying groundwater.

The lack in temporal trends in nitrate and chloride concentrations in groundwater samples suggests that the deep groundwater immediately upgradient and downgradient of the test plot was not directly influenced by the surface-applied fertilizers used in this study. The constancy in the observed concentrations suggests that the deep groundwater being sampled is controlled by a local source in the immediate vicinity of the wells.

Pre-plant soil samples had very low background levels of nitrate. Following fertilizer application, nitrate was observed to initially increase in the top of the soil mounds after application of the fertilizer side dress, but then decrease, likely as a result of plant uptake. The bulk of extractable soil NO_3-N movement in the soil mound was relatively independent of the presence of the tobacco crop. Vertical movement was restricted by the presence of a dense soil layer at the 30-50 cm depth, relative to the original soil surface. However, the absolute magnitude of NO_3-N in the soil mound was influenced by the presence of the tobacco crop, especially after July 31, 2009, when there is a distinct, consistent removal of NO_3-N from the top 30 cm occupied by the soil mound. By the end of the study, NO_3-N concentrations in the blocks containing tobacco crop were approaching those observed in the background soil samples, while concentrations of NO_3-N in the non-tobacco blocks remained relatively elevated, at least to the 50 cm depth as referenced to the original soil surface.

Based on these results, had the applied potassium nitrate fertilizer contained perchlorate at levels significantly greater than those used in this study, it is probable that movement of perchlorate through the soil matrix would have mimicked that of nitrate, with perchlorate being transported through the upper 30 cm of soil within the blocks containing tobacco crop, and as deep at 50 cm within the non-tobacco blocks. However it is unlikely that downward vertical migration of perchlorate into the deep groundwater would have occurred, due to site-specific geological constraints.

6.3 Whiteville Tobacco Research Station

A visual representation of the development of the tobacco crop at the Whiteville station from April to September 2009 is given in Figures 6-Q to 6-U.

6.3.1 Intensity and Temporal Distribution of Rainfall

Rainfall amounts at the Whiteville station during the study were determined from the North Carolina State Climate Office's CRONOS weather station network, which has one of its ECONet meteorological towers located at the station. The total amount of rainfall during the 6-month growing season was ~ 635 mm (25.0 inches) (Table 6-J), the largest amount occurring in April and May (~ 290 mm; ~11.4 inches). As at the Oxford station, a substantial portion of this rainfall occurred between the first and second application of fertilizer. June was relatively dry receiving only 61 mm (2.4 inches) of rainfall. Rainfall totals in July and August were more normal averaging ~ 120 mm or 4.7 inches. September was again relatively dry. Because of the adequate amount of rainfall during the study period, no supplemental irrigation was used at the Whiteville station in support of this project.

Figure 6-Q April 10, 2009, Prepared Soil Mounds Across Research Plots, Whiteville Station.

Figure 6-R May 27, 2009, Tobacco Plants Following Transplanting and Approximately 30 Days After First Application of Fertilizer, Whiteville Station.

Figure 6-S June 27, 2009, Tobacco Plants Approximately 9 Weeks Since Transplanting and 3 Weeks Following Application of Second Side Dress of Nitrogen Fertilizers, Whiteville Station.

Figure 6-T August 31, 2009, Tobacco Plants Approximately 17 Weeks Since Transplanting and After First Harvest, Whiteville Station.

Figure 6-U September 28, 2009, Representative Picture of Research Plots After Final Harvest, Demonstrating Soil Mounds are Still Intact at the End of the Sampling Period, Whiteville Station.

Table 6-J. Monthly Rainfall Amounts Recorded at the Whiteville Station for April through September, 2009. (Source: NC State Climate Office CRONOS network)

Month	Rainfall Amount (mm)	Rainfall Amount (inches)
April	116	4.57
May	171	6.73
June	61	2.40
July	101	3.98
August	144	5.67
September	41	1.61
Average	***106***	***4.16***
Total	***634***	***25.0***

6.3.2 Soil Water Content and Bulk Density

Clusters of tensiometers were installed in all of the plots to three depths (15, 45 and 75 cm), referenced to the top of the soil mound rather than the original soil surface. The height of the soil mounds were approximately 15 cm above the original soil surface, and the inter-row areas were approximately 15 cm below the height of the original soil surface. The 15 cm depth corresponds to the bottom of the soil mound, at the original surface of the soil. The 45 and 75 cm depths correspond 30 and 60 cm depths as referenced to the original surface of the soil. The tensiometers were installed after the tobacco seedlings had received their initial round of complete fertilizer applications, to avoid damage from the heavy farm equipment necessary to form the mounds. The tensiometers were in place for the second side dress application of either potassium nitrate or ammonium nitrate.

Soil tensiometer readings in Blocks 9-10 (bare soil plots) as a function of date and soil depth are shown in Figure 6-V along with rainfall amounts for given precipitation events. From May through about mid-July, the soil moisture content across the top 75 cm of the soil as measured from the top of the soil mound remained fairly uniform, showing a slight decrease with time. This is probably a result of adequate rainfall amounts, especially in April and in May. After July 15, the top 15 cm of the soil mound began to dry out, probably due to an increase in evapotranspiration demand with increased air temperatures. This dry down of the top 15 cm of the soil mound continues despite a large rain event between July 22 and 29, 2009. The top 15 cm of the soil mound eventually becomes very dry in mid-August and remains so through September.

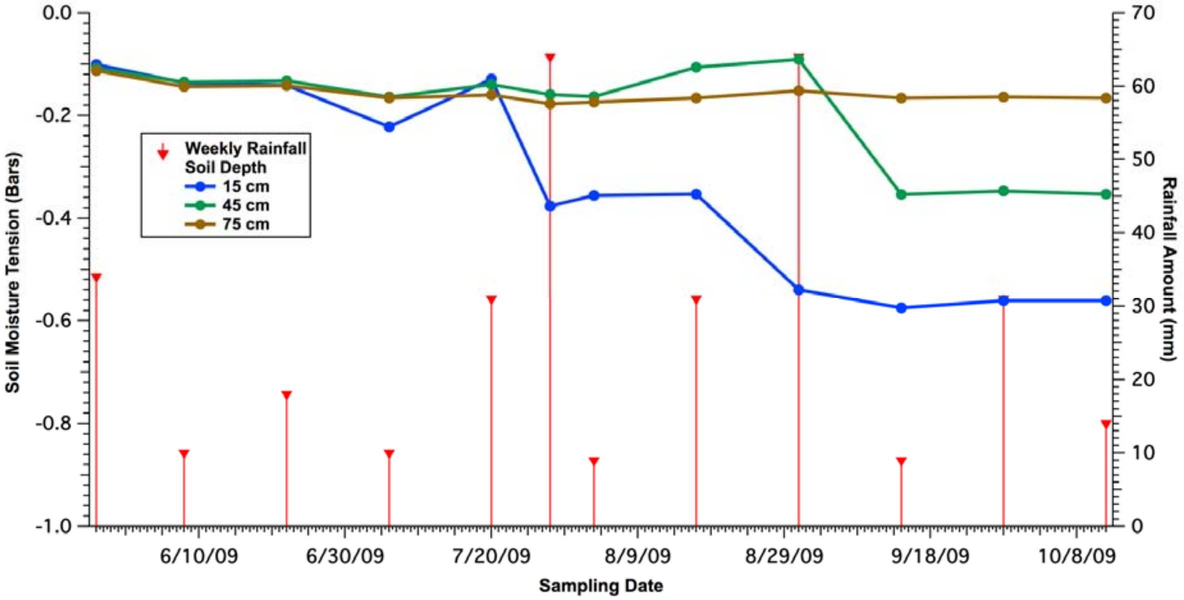

Figure 6-V Mean Soil Tensiometer Readings for Blocks 9-10 as a Function of Date and Soil Depth Together with Weekly Rainfall Amounts, Whiteville Station. (Soil depths are referenced to the top of the soil mounds).

The 45 cm soil depth increment appears to mimic the pattern at the 15 cm depth with a noticeable decline after August 26th. There is some recovery of moisture content at this depth due to several large rain events after July 22, 2009 and around August 19, 2009. Beyond August

26, 2009, the 45 cm depth increment appears to reach a steady state in terms of soil moisture content. Throughout the growing season, there is a mild decline in the moisture content at the 75 cm depth, but overall the moisture content of the lower portion of the soil profile appeared to remain relatively constant.

Theoretically, Blocks 9-10 contained no plants, thus the increase in soil tension over time reflects water loss through evaporation, despite several rain events. Failure to see recovery following a rain event probably reflects formation of surface crusts on the bare soil, which limited infiltration and favored lateral surface runoff. Under conditions of relatively high soil water tension, there would be little to no significant vertical transport of water or solutes except as wetting fronts as water moves into the dry soil. Herbicide was sprayed several times on Blocks 9-10 to control weeds, so some water loss evident in the top 15 cm of the soil mound may have been due to weed growth.

The presence of the tobacco plants altered the observed pattern in soil moisture content for Blocks 1-8 as compared to Blocks 9-10, at least for the 15 and 45 cm depths (Figure 6-W). There was an overall slight decline in soil moisture content through June 24, 2009. Following this date, there was a significant and relatively rapid decline in moisture content within the 15 and 45 cm depths, as referenced to the top of the soil mound. This dip in soil moisture content is also present in Blocks 9-10 for the 15 cm depth (Figure 6-W). There was a recovery in moisture content at these two depths following an apparent rain event between July 15, 2009 and July 22, 2009. The subsequent larger rainfall event between July 22, 2009 and July 29, 2009 appeared to increase the moisture content of the top 15 cm even further, although the 45 cm interval seemed to first decline and then subsequently recover for the remainder of the growing season.

After August 5, 2009, the top 15 cm depth declined to an apparent steady state for the rest of the growing season. At the 75 cm depth, the soil moisture content showed a steady decline until the relatively large rain event between July 22, 2009 and July 29, 2009. After this event, the soil moisture content at the 75 cm depth appeared to remain constant, or at least achieve a steady state.

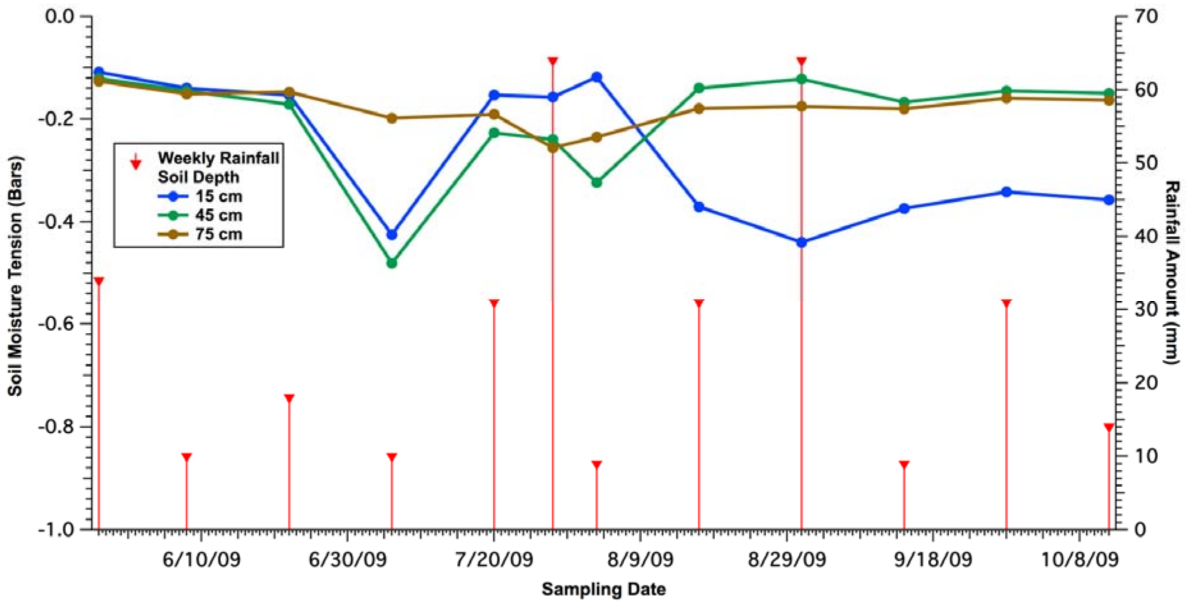

Figure 6-W Mean Soil Tensiometer Readings for Blocks 1-8 as a Function of Date and Soil Depth Together with Weekly Rainfall Amounts, Whiteville Station. (Soil depths are referenced to the top of the soil mounds).

The data in Figure 6-W suggest some movement of water following rain events in the soil profile both laterally and vertically, at least for two rain events between July 15, 2009 and July 29, 2009. Prior to July 15, 2009, there appeared to be considerable evapotranspiration demand on the tobacco crop, resulting in depletion of soil moisture down to at least the 45 cm depth, if not some effect down to the 75 cm depth. Recharge of the soil moisture status at all three depths suggests definite vertical movement of water to at least the 75 cm depth shortly after July 29, 2009. Additional lateral movement of water may have occurred into the base of the soil mounds as well. Presence of the tobacco crop would act to shield the top of the soil mound from direct contact with rainfall. However, the leaves would redirect the rainfall to the inter rows, where lateral infiltration of water would occur at the base of the soil mounds. This lateral infiltration would move both across the soil mound as well as potentially vertically upward, resulting in a recharge of the soil moisture content throughout the soil profile, as measured from the top of the soil mound.

After this recharge of soil moisture content, subsequent plant growth and evapotranspiration demand acted to lower the soil moisture content of the top 15 cm of the soil mound, but additional rain events provided sufficient moisture to maintain a steady state in the soil moisture content of the 15 cm depth, and to prevent significant draw down of soil moisture from the 45 and 75 cm depths. Under such conditions, it can be assumed there was likely little vertical transport of water through the soil profile after August 12, 2009.

Soil bulk density was measured as a function of soil depth near the end of the study in Blocks 1-10. Samples were taken with reference to the top of the soil mounds. Soil bulk density values as a function of block and soil depth relative to the top of the soil mound are shown in Table 6-K. The vertical profile of soil bulk density as a function of soil depth relative to the top of the soil mound is shown in Figure 6-X.

Table 6-K. Soil Bulk Density and Mean Soil Porosity as a Function of Depth Relative to the top of the Soil Mound, Whiteville Station

Depth range (cm)	Block 1	Block 2	Block 3	Block 4	Block 5	Block 6	Block 7	Block 8	Block 91	Block 10	Average	Mean Soil Porosity (%)
	(g/cm^3)											
0-15	1.53	1.41	1.55	1.47	1.52	1.47	1.44	1.54	1.44	1.45	**1.48**	**44.2**
15-30	1.70	1.71	1.63	1.68	1.77	1.56	1.75	1.59	1.54	1.62	**1.66**	**37.3**
30-50	1.70	1.74	1.80	1.68	1.74	1.72	1.76	1.72	1.56	1.75	**1.72**	**35.1**
50-75	1.54	1.46	1.40	1.46	1.56	1.63	1.54	1.53	1.54	1.54	**1.52**	**42.6**
75-100	1.69	1.62	1.72	1.80	1.77	1.74	1.65	1.65	1.55	1.56	**1.67**	**36.9**

The average bulk densities measured for Blocks 1-10 were generally consistent, and illustrated a general uniformity in soil physical structure across the research site. The presence of a restricted layer was suggested at the 30-50 cm depth increment relative to the top of the soil mound. This is evident by both the increase in bulk density to ~1.7 grams per cubic centimeter, and a corresponding decrease on soil porosity. However, unlike the presence of the restricted layer at the Oxford station, the restricted layer is still rather porous and probably not a major impediment to vertical transport of water.

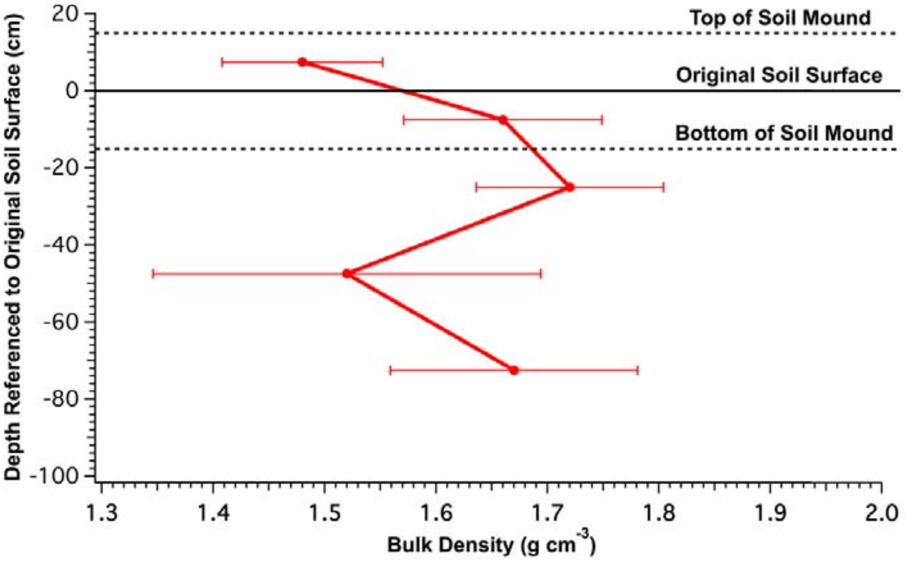

Figure 6-X Mean Soil Bulk Density and Standard Deviation for Blocks 1-10 as a Function of Soil Depth Relative to the Top of the Soil Mound, Whiteville Station.

6.3.3 Groundwater and Shallow Wells

Water levels were checked on every site visit for soil sampling and/or leaf tissue collection. No water was ever detected in the on-field wells, suggesting that at this particular location any water that may have accumulated at the top of the subsoil clay layer either succeeded in penetrating the clay layer and/or moved laterally out of the plots. Vertical movement through the subsoil clay layer is supported by the observed recharge of soil moisture at the 75 cm depth (Figure 6-W), and soil porosity for this location (Table 6-K).

The off-field wells were located at the edge of the experimental field and then oriented in upslope and downslope positions relative to the stream and surrounding marshy areas that separated the field site from the rest of the research station (Figure 3-B). Total distance to the field edge was approximately 30 m (~100 ft). The assumption was made that subsurface lateral movement from the experimental plots would be toward the lower-elevation marsh area.

Sampling of the off-field wells began on April 10, 2009 and ended October 12, 2009. The shallow well samples were collected before, concurrently, and after the 8 soil sampling events. Groundwater was analyzed in the field for oxidation/reduction potential (ORP; mV), dissolved oxygen (DO; %), pH, and temperature (°C). Depth to groundwater was determined using a Solinst-brand water level meter.

Average water depth in the off-field wells observed during the growing season varied between 60 cm to ~120 cm. The decline in water table depth was relatively gradual from April to October, with two noticeable recharge events occurring in late May and late August. These coincide with a 32 mm (1.3") event on May 26, 2009 (data not shown) and a 30 mm (1.2") event on August 30, 2009 (Figure 6-W). There is a suggestion of an impact from a relatively large event at the end of June, 2009 (Figure 6-W) as well.

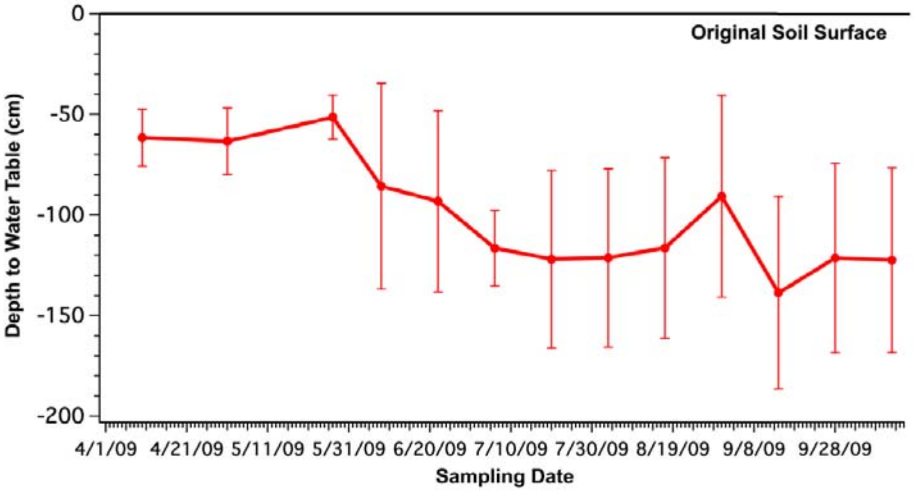

Figure 6-Y Average Depth to Groundwater as a Function of Date for Off-Field Wells, Whiteville Station. (Vertical lines represent one standard deviation).

The transient rise in water depth observed for the two rain events suggests that the shallow water table at these locations responds relatively rapidly to large rainfall events capable of generating sufficient soil water infiltration to produce vertical transport and possibly sub-lateral flow. However, the observations in Figure 6-Y are only based on bi-weekly observations and it would appear the elevations in water table height were of short duration. This may explain why no apparent changes in height were observed for other large rainfall events such as occurred towards the end of July 2009 (Figure 6-W). The observed water table depths are also probably closely tied to the level of stream flow in the nearby creek, which would explain the gradual lowering in water table depth during the growing season as stream flow receded and the surrounding marsh land drained. The average water table depth at the Whiteville site remained relatively unchanged from July to October 2009, except for transient inputs from rain events at least 25 mm (~ 1") in total.

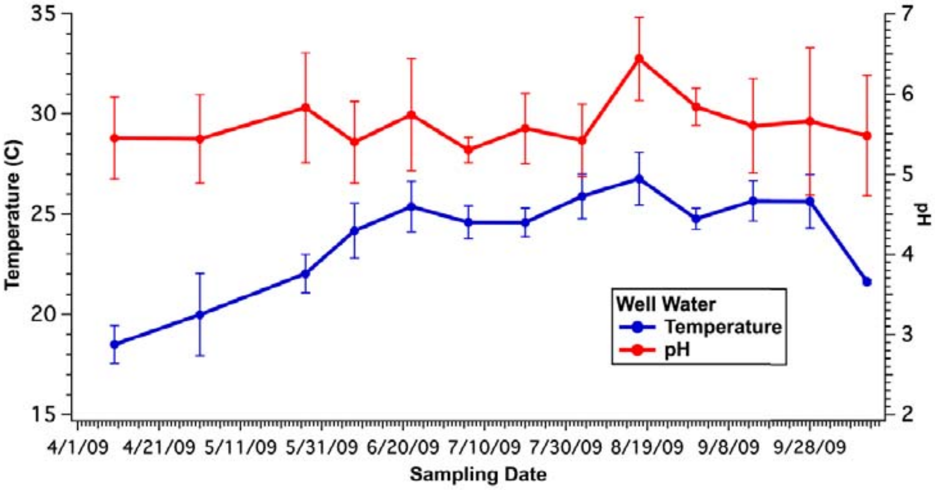

Figure 6-Z Average pH and Temperature as a Function of Date for Off-Field Wells, Whiteville Station. (Vertical lines represent one standard deviation).

Temperature of the shallow groundwater gradually increased during the growing season from ~20°C to a maximum of ~27°C on August 17, 2009 (Figure 6-Z). The maximum of 27°C appeared to be related to a short term rise in water table height (Figure 6-Z) and provides further support for direct communication between water infiltrating through upper surface layers of soil and eventually contacting the underlying groundwater. Overall, the groundwater temperature remained relatively constant at 25°C from late June to the end of September. There was a noticeable decline in water temperature at the last sampling date in October.

Water pH remained relatively constant between 5.5 and 6.0 throughout the measurement period. The only rise in pH (~pH 6.5) occurred with rise in water temperature on the August 17, 2009 sampling date. This rise in pH may reflect impact of soil water draining from upper soil layers and coming in contact with the underlying groundwater.

The measured ORP for the shallow groundwater was variable and displayed a cyclic pattern during the study period (Figure 6-AA). The highest ORP was observed right after well installation. There was no consistent trend between the cyclic pattern in the ORP readings and rainfall events (Figure 6-Y).

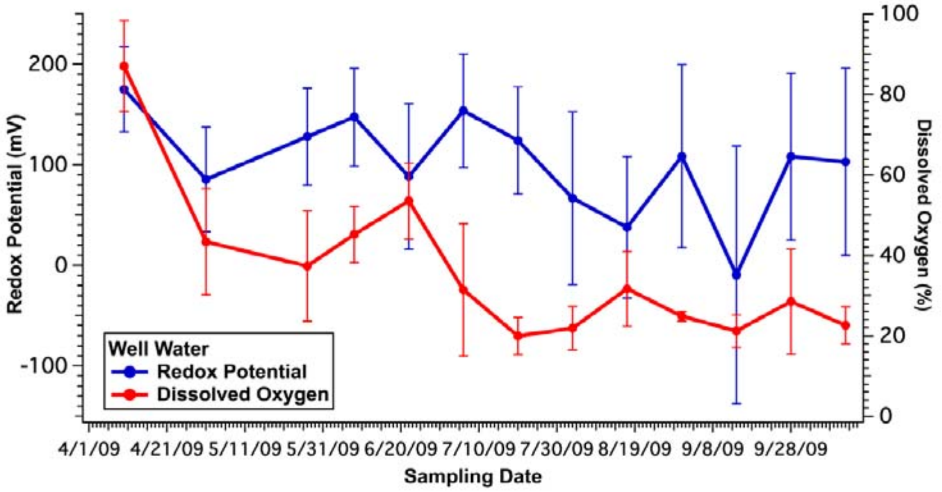

Figure 6-AA Average Redox Potential and Dissolved Oxygen Content as a Function of Date for Off-Field Wells, Whiteville Station. (Vertical lines represent one standard deviation).

DO in the shallow groundwater declined during the growing season. There are periodic increases in DO during the gradual decline that are perhaps related to rainfall events and reflect input of oxygenated soil water moving downward through the soil profile. There is, however, no direct correspondence to actual rainfall events, possibly due to the bi-weekly nature of sampling the shallow groundwater, as well as time of transport of water through the soil profile.

Analysis of water samples from the off-field wells for perchlorate, chloride and nitrate were carried out using ion chromatography. A qualitative assessment of the presence of sulfates and phosphates in the well water samples was also conducted using ion chromatography. As was observed at the Oxford station, perchlorate was not detected in any of the well water samples collected using ion chromatography. The presence of other anions in groundwater samples was more variable than expected. A detailed discussion of the attempt to detect perchlorate in water samples from both the Oxford and Whiteville sites is presented in Section 6.2.3 and will not be repeated here.

Upon further discussion with the superintendent at the Whiteville station, and consultation of station records, it was determined that the field location used for this study at the Whiteville station had a long history of peanut trials, the latest being 2 years before this study. Visual confirmation of peanut hulls in the soil across the plots confirmed this conclusion. Peanut production employs relatively large applications of gypsum, which accounts for the relatively large concentration of sulfate detected in the shallow groundwater samples. Sulfate would be retained by iron oxides associated with the clay minerals deeper within the soil profile, and would not be easily removed from the soil profile by subsequent rainfall events. As noted in Section 6.2.3, the presence of sulfate in the shallow groundwater raised the effective detection limit for perchlorate via ion chromatography.

A subset of groundwater samples was sent to CAS to confirm analyses of the well water samples by ion chromatography (Table 6-L). The method reporting limit for the analyses was 0.2 ppb. Of the 6 separate samples submitted, no perchlorate was detected in 4 samples, 1 sample had a reported value of 1.3 ppb perchlorate, and 1 sample had a reported value of 0.27 ppb perchlorate (Table 6-L).

The detection of perchlorate in the shallow groundwater samples is consistent with timing of fertilizer applications at the Whiteville station, although the concentrations observed could also have been the result of direct rainwater input. The results for well 12B sampled on May 1, 2009, support the conclusion that there were no significant background levels of perchlorate at the site prior to the start of the experiment.

Nitrate was detected in all six of the off-field wells (Table 6-M). Well 11A consistently had nitrate present if there was water present in the well (mean NO_3-N concentration ~12 mg NO_3-N/L). Groundwater collected from well 11B also contained nitrate although the concentration dropped below the detection limit midway through the growing season. The remaining wells had relatively low to no nitrate detected in the water samples. Well 12B demonstrated a similar pattern to well 11A in that nitrate was present in all the water samples collected, however the majority of concentrations measured were <2 mg NO_3-N/L. Higher concentrations of nitrate in wells 11A and 11B are consistent with their position relative to the field plots of the study and the general overall direction of drainage at the site, but the highest NO_3-N concentration observed for well 11A was April 10, 2009, which is inconsistent with the first application of fertilizer in late April 2009. In addition, the consistency in NO_3-N readings throughout the growing season suggests a different source of NO_3-N was controlling the presence of NO_3-N in the wells in addition to local variation in ORP and DO.

Table 6-L. Results of Well Water Sample Analyses for Perchlorate by Columbia Analytical Services Laboratory. Whiteville Station

Sample	Date	Matrix	Compound	MRL	Adjusted MRL	Result	Flag	Unit
WV-12B	05/01/09	WATER	PERCHLORATE	0.2	0.20	0.20	U	µg/L
WV-13A	06/22/09	WATER	PERCHLORATE	0.2	0.20	1.3		µg/L
WV-13B	07/20/09	WATER	PERCHLORATE	0.2	0.20	0.27		µg/L
WV-12B	08/17/09	WATER	PERCHLORATE	0.2	0.20	0.20	U	µg/L
WV-12B	09/14/09	WATER	PERCHLORATE	0.2	0.20	0.20	U	µg/L
WV-11B	10/12/09	WATER	PERCHLORATE	0.2	0.20	0.20	U	µg/L

Notes:

U – compound was analyzed for but not detected

MRL – method reporting limit

Table 6-M. Nitrate Concentrations in Groundwater, Whiteville Station

Date	WV-11A	WV-11B	WV-12A	WV-12B	WV-13A	WV-13B
	(mg NO_3-N/L)					
4/10/09	16.4	6.6	0.1	2.7	DW	DW
5/1/09	12.3	9.7	<0.05	3.8	DW	DW
5/27/09	0.9	9.6	0.9	6.0	<0.05	2.7
6/8/09	15.4	7.1	<0.05	2.4	3.1	6.7
6/22/09	11.4	2.6	<0.05	1.4	1.8	4.3
7/8/09	11.7	4.6	0.1	2.4	1.8	0.4
7/20/09	13.0	2.1	<0.05	1.4	0.1	0.6
8/3/09	DW	<0.05	<0.05	1.5	0.1	DW
8/17/09	12.7	0.1	<0.05	0.6	<0.05	<0.05
8/31/09	12.7	<0.05	<0.05	0.8	<0.05	0.2
9/14/09	DW	<0.05	<0.05	0.5	<0.05	<0.05
9/28/09	10.7	<0.05	0.1	0.4	0.3	DW
10/12/09	DW	<0.05	<0.05	0.2	<0.05	DW
Mean=	**11.7**	**5.3**	**0.3**	**1.9**	**1.2**	**2.5**
StdDev=	**4.2**	**3.5**	**0.4**	**1.6**	**1.2**	**2.6**
%CV=	**36**	**67**	**143**	**88**	**102**	**106**

DW – dry well

Chloride was detected in groundwater from all 6 wells (Table 6-N). However, the individual chloride concentrations within a well remained relatively unchanged during the sampling period, except possibly for the first sampling dates after well installation. This lack in temporal trends in chloride concentrations, as observed for nitrate, suggests that the groundwater was not directly influenced by the fertilizer used in this study. Elevated chloride was detected in wells 11A, 11B and 12A on May 27, 2009, which was approximately 30 days after the first application of fertilizer that contained potassium chloride. The appearance of chloride coincides with a preceding relatively large rainfall event. However, if the elevated chloride values were the result of a pulse of chloride moving off the plots, the elevated chloride concentrations are superimposed upon an apparent background level of chloride that impacts the wells differently across the site. As with the apparent background levels of sulfate in the deeper subsoil, there may be an elevated level of chloride in the deeper subsoil that is impacting the wells due to long term usage of potassium chloride as a fertilizer at the station.

Table 6-N. Chloride Concentrations in Groundwater, Whiteville Station

Date	WV-11A	WV-11B	WV-12A	WV-12B	WV-13A	WV-13B
	(mg NO_3-N/L)					
4/10/09	74.8	32.8	34.1	35.4	DW	DW
5/1/09	70.6	30.2	31.9	34.6	DW	DW
5/27/09	84.9	65.1	59.4	24.7	32.4	35.2
6/8/09	59.6	27.2	31.3	33.9	18.4	21.4
6/22/09	49.3	25.1	30.5	31.6	16.5	21.4
7/8/09	47.6	25.6	29.3	29.9	32.0	28.7
7/20/09	46.0	22.4	19.3	32.3	6.1	15.1
8/3/09	DW	18.2	26.1	33.6	10.7	DW
8/17/09	45.8	38.2	25.8	30.4	10.2	13.8
8/31/09	55.1	33.9	27.5	31.3	8.9	14.6
9/14/09	DW	34.9	31.5	32.6	21.5	21.8
9/28/09	48.8	24.9	31.0	33.3	12.1	DW
10/12/09	DW	17.5	30.5	33.5	13.1	DW
Mean=	**58.2**	**30.5**	**31.4**	**32.1**	**16.5**	**21.5**
StdDev=	**13.9**	**12.2**	**9.2**	**2.7**	**8.9**	**7.4**
%CV=	**24**	**40**	**29.3**	**9**	**54**	**35**

DW – dry well

6.3.4 Tobacco Production

Harvesting of the lower tobacco leaves began at about 13 weeks (July 27, 2009) from the transplant date. Subsequent harvests occurred at 16 weeks (August 17, 2009), 20 weeks (September 17, 2009), and finally at 21 weeks (September 24, 2009) from transplant and were based on the station superintendent's judgment as to the quality of the leaves. The harvest weights collected from Blocks 1-8 were typical of harvests in the area that year according to the station superintendent (Table 6-O). The overall weight was 3,423 Kg (the equivalent of 51,773 Kg/ha or 114,160 lbs/ac). The mass of leaves harvested increases each subsequent harvest as the size of the leaves increases further up the stalks.

There was a noticeable difference in mass of leaves harvested from Blocks 1-4 versus Blocks 5-8. This difference was consistent throughout the growing season, especially for Blocks 1-3 where the smaller plant heights were observed for the entire blocks over the course of the season. There was no obvious explanation for this reduction in plant growth in Blocks 1-4. Consultation with the station superintendent indicated that there were no problems during transplanting or with fertilizer applications for these blocks. Transplant tobacco seedlings came from the same greenhouse on site and were essentially chosen at random, so it is unlikely the observed differences arose at planting. All other treatment variables were consistent across Blocks 1-10: rainfall amounts, soil amendments, and pest control applications. There were also no observed differences in soil compaction or soil texture. It is possible Blocks 1-4 experienced a residual effect from previous field trials at the study site location. It was assumed that the observed differences were not due to the use of potassium nitrate versus ammonium nitrate in the second side dress of nitrogen. Results at the Oxford station indicated that the potassium nitrate product does not have a negative impact on tobacco growth (Table 6-E). Unlike the Oxford station harvests, the mass of tobacco leaves harvested at the Whiteville station were determined in the field by use of a portable scale. A subset of stalks were sampled within each plot, and then the mass determined scaled to total stalk count per plot. The apparent mass of leaves harvested from the Whiteville versus the Oxford station is greater because of the difference in moisture contents between freshly harvested leaves (typically 70-80%) versus cured leaves (12-15%).

Table 6-O. Mass of Harvested Tobacco Leaves from Blocks 1-8, Whiteville Station. (Results are for field-harvested tobacco, moisture content 70-80%)

	Harvest 1 7/27/2009	**Harvest 2 8/17/2009**	**Harvest 3 9/17/2009**	**Harvest 4 9/24/2009**	**Totals**
	(kg)				
Block 1	78	123	103	105	**409**
Block 2	66	70	96	69	**300**
Block 3	78	97	110	105	**390**
Block 4	91	89	139	123	**443**
Means	***78.2***	***94.8***	***112.0***	***100.5***	***385.5***
Block 5	99	101	135	142	**476**
Block 6	89	109	117	146	**460**
Block 7	87	102	142	174	**504**
Block 8	90	117	119	114	**441**
Means	***91.2***	***107.2***	***128.2***	***144.0***	***470.2***
Totals	***677***	***808***	***960***	***978***	***3423***

6.3.5 Soil Chemical Analysis – Perchlorate

Pre-planting soil samples were obtained from Blocks 1–10 on March 9 and 11, 2009. The samples were taken over the depth interval of 0-200 cm using a hand auger as referenced from the surface of the original soil. Scheduled biweekly sampling was initiated on June 8, 2009 and continued throughout the growing season with the last set of samples recovered on September 14, 2009. A total of 460 soil samples were collected from the Whiteville station (Table 6-P) and processed as outlined in Section 5.1.

All 460 soil samples were extracted with 0.001M $CaCl_2$ and the cleared supernatant analyzed for perchlorate using ion chromatography. No perchlorate was detected in any of the soil extracts. A detailed description of the analysis of the soil extracts by ion chromatography can be found in Section 6.2.5.

A total of 5 soil samples were submitted to CAS for analysis of extractable perchlorate (Table 6-Q). The soil samples were chosen at random from the various plots and sampling dates. No perchlorate above the method reporting limit of 2 ppb was detected in the soils.

Table 6-P. Number of Soil Samples Collected During 2009 Field Study, Whiteville Station

Date	Depth Interval (cm)	Reference	Number Depths Sampled	Count
3/9/09	0-200	Original soil surface	6	60
6/8/09	0-100	Top of mound	5	50
6/22/09	0-100	Top of mound	5	50
7/6/09	0-100	Top of mound	5	50
7/20/09	0-100	Top of mound	5	50
8/3/09	0-100	Top of mound	5	50
8/17/09	0-100	Top of mound	5	50
8/31/09	0-100	Top of mound	5	50
9/14/09	0-100	Top of mound	5	50
Total Number of Soil Samples =				460

6.3.6 Soil Chemical Analysis – Ammonium and Nitrate

All 460 samples were analyzed for extractable NH_4-N and NO_3-N. The pre-planting soil samples showed two separate patterns for the distribution of extractable NH_4-N and NO_3-N with depth (Figure 6-BB). Ammonium-N appeared to be relatively uniform with depth, with Blocks 1-8 having extractable NH_4-N from 1.5 to 2 mg NH_4-N/kg. The surface and deepest depth sampled had extractable NH_4-N values >3 mg NH_4-N/kg for Blocks 9-10, but the mid-depth concentrations of NH_4-N were similar to values for Blocks 1-8.

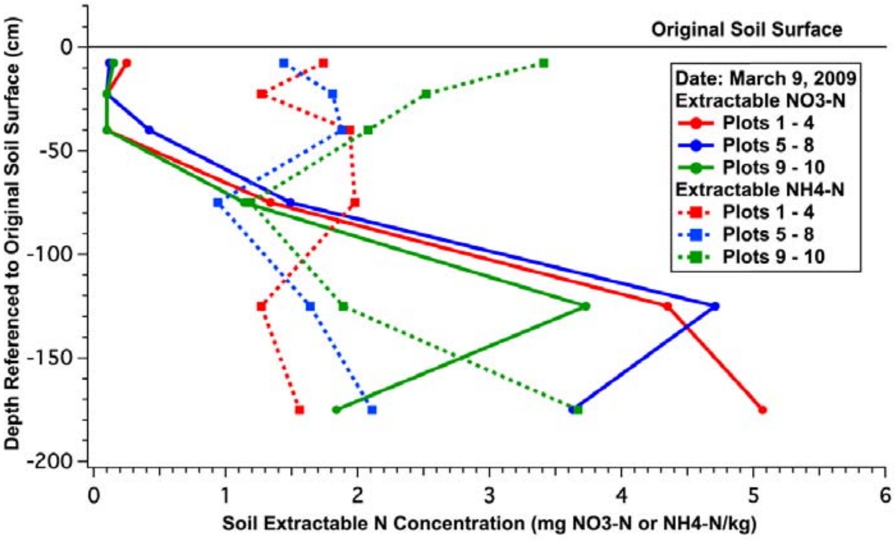

Figure 6-BB Mean Soil Extractable NO_3-N and NH_4-N Concentrations as a Function of Sampled Depth Increment for Blocks 1-10, Whiteville Station. (March 9-11, 2009. Mean values are plotted at mid-point of depth increment sampled).

Table 6-Q. Results of Soil Analyses for Perchlorate by Columbia Analytical Services Laboratory. Whiteville Station

Sample	Date	Matrix	Compound	MRL	Adjusted MRL	Result	Flag	Unit
WV-P1-15-30	03/09/09	SOIL	PERCHLORATE	2.0	2.0	2.0	U	µg/kg
WV-P6-0-15	06/22/09	SOIL	PERCHLORATE	2.0	2.0	2.0	U	µg/kg
WV-P9-15-30	07/20/09	SOIL	PERCHLORATE	2.0	2.1	2.1	U	µg/kg
WV-P2-75-100	08/17/09	SOIL	PERCHLORATE	2.0	2.0	2.0	U	µg/kg
WV-P4-30-50	09/14/09	SOIL	PERCHLORATE	2.0	2.1	2.1	U	µg/kg

Notes:

U – compound was analyzed for but not detected

MRL – method reporting limit

Extractable NO_3-N demonstrated a distinctly different pattern with the lowest concentrations of NO_3-N at the soil surface (<0.5 mg NO_3-N/kg), and then soil concentrations increasing up to 3.5–5 mg NO_3-N/kg at the 150 cm depth. Below this depth the range in concentrations across the blocks was more variable, but still exceeded 5 mg NO_3-N/kg for Blocks 1-4. The overall distribution of soil NO_3-N is consistent with a possible layer restricting water movement at the 100-150 cm depth.

Table 6-R. Mean Soil Concentrations of Extractable NO_3-N as a Function of Depth Increment Sampled from June 8, 2009 to September 14, 2009, Whiteville Station. (Values in parentheses are calculated standard deviation for blocks sampled. Depth increments are referenced to top of soil mounds formed for tobacco transplants)

Depth Increment (cm)	**Sampling Date**							
	6/08/09	**6/22/09**	**7/06/09**	**7/20/09**	**8/03/09**	**8/17/09**	**8/31/09**	**9/14/09**
	(mg NO_3-N/kg)							
Blocks 1-4 (Potassium Nitrate plus Tobacco)								
0-15	4.4 (2.8)	0.74 (0.7)	2.0 (0.6)	0.54 (0.7)	1.6 (1.1)	2.1 (0.9)	0.92 (0.8)	0.72 (0.3)
15-30	3.7 (1.1)	4.2 (4.4)	3.0 (1.6)	0.75 (0.5)	0.86 (0.3)	1.6 (0.9)	1.8 (1.2)	0.52 (0.4)
30-50	6.5 (3.0)	4.5 (2.1)	5.2 (1.6)	1.1 (0.2)	0.70 (0.3)	0.69 (0.5)	0.72 (0.9)	0.50 (0.4)
50-75	3.3 (1.2)	6.8 (2.7)	3.7 (0.7)	2.4 (1.4)	1.6 (1.7)	0.91 (0.8)	0.21 (0.1)	0.45 (0.4)
75-100	1.6 (0.6)	3.5 (0.8)	2.7 (0.8)	1.1 (0.7)	1.5 (1.0)	1.9 (1.6)	0.36 (0.5)	1.7 (1.6)
Blocks 5-8 (Ammonium Nitrate plus Tobacco)								
0-15	5.1 (5.0)	2.4 (1.6)	3.0 (1.5)	0.54 (0.5)	1.1 (0.2)	1.6 (0.5)	2.2 (1.4)	4.6 (1.5)
15-30	7.1 (5.6)	5.0 (0.5)	4.7 (2.4)	2.0 (1.8)	0.76 (0.5)	1.3 (0.2)	2.9 (1.1)	5.9 (1.4)
30-50	8.9 (3.2)	5.8 (0.4)	4.6 (2.2)	1.8 (0.6)	0.75 (0.3)	0.55 (0.3)	1.6 (0.7)	2.6 (0.1)
50-75	2.8 (0.7)	3.4 (0.7)	2.4 (1.5)	2.5 (2.7)	0.76 (0.8)	0.79 (0.5)	0.59 (0.8)	0.17 (0.1)
75-100	2.6 (0.9)	1.6 (0.5)	1.9 (0.8)	1.5 (1.3)	1.0 (0.2)	0.97 (0.8)	0.44 (0.6)	0.15 (0.1)
Blocks 9-10 (Potassium Nitrate minus Tobacco)								
0-15	6.4 (3.1)	15.1 (9.7)	11.4 (1.2)	4.3 (2.0)	0.65 (0.2)	0.77 (0.4)	1.7 (1.0)	2.8 (1.6)
15-30	15.5 (11.0)	8.2 (0.6)	5.8 (0.6)	7.8 (2.4)	2.0 (1.1)	2.9 (0.5)	5.3 (3.7)	4.7 (2.7)
30-50	12.9 (8.7)	9.5 (3.8)	9.3 (0.2)	9.5 (1.8)	6.5 (0.4)	5.5 (1.2)	9.8 (3.3)	5.4 (1.3)
50-75	4.7 (0.9)	8.3 (2.0)	5.3 (0.3)	6.5 (4.0)	5.4 (1.3)	9.7 (7.7)	8.8 (7.5)	3.0 (1.7)
75-100	2.5 (0.1)	5.0 (3.5)	4.2 (0.8)	3.1 (1.4)	1.6 (0.7)	7.0 (5.2)	6.0 (3.9)	2.3 (0.4)

The mean soil concentrations and calculated standard deviations of extractable NO_3-N grouped by treatment blocks for the soil samples collected post-planting as a function of depth are provided in Table 6-R. While the range of extractable soil NO_3-N varied substantially both with soil depth and date of sampling, the absolute magnitude of the extractable soil NO_3-N concentrations were in general an order of magnitude less than those observed at the Oxford station (Table 6-G). This suggests that there had been a rapid diffusion/dispersion of NO_3-N away from the initial fertilizer bands, perhaps the result of a rain event, occurring right before the first soil sampling (Figure 6-V).

The mean concentration of extractable NO_3-N for Blocks 1-4 as a function of sampling date is provided in Figure 6-CC. For the 0-15 cm depth (top of the soil mound), the highest concentration of NO_3-N was observed on the first sampling date (June 8, 2009). Subsequent samplings for the 0-15 cm depth, suggest that either the fertilizer N had become uniformly distributed in the top of the soil mound, or, more likely, the majority of fertilizer N had moved vertically away from the top of the soil mound or was removed by crop growth.

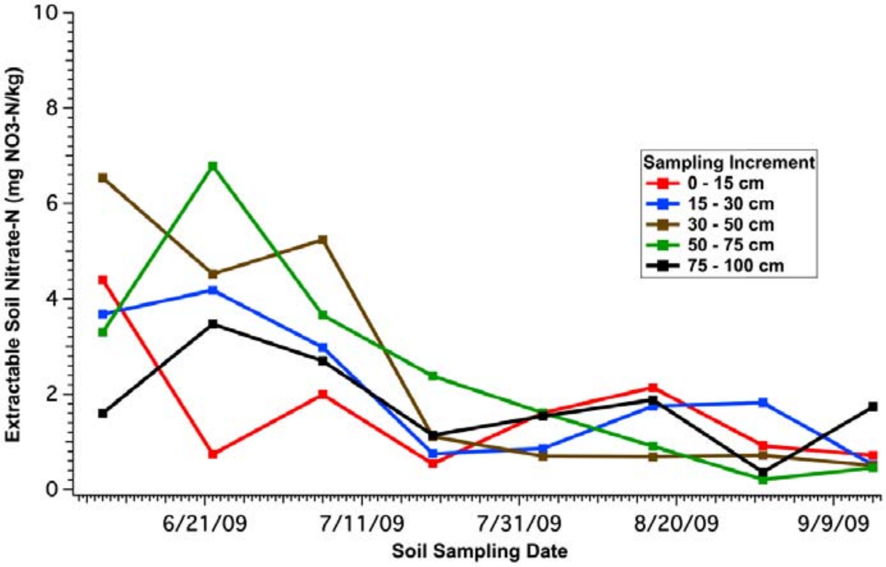

Figure 6-CC Mean Concentrations of Soil Extractable NO_3-N for Blocks 1-4 as a Function of Sampling Date and Sampling Depth Increment, Whiteville Station. (Sampling depth increments are referenced to top of soil mounds).

For the remaining soil depths sampled, the patterns in extractable NO_3-N with soil sampling date suggest fairly rapid movement of NO_3-N through the soil column, with even an increase in soil NO_3-N concentration being detected at 75-100 cm depth (relative to the top of the soil mound) by the second sampling date, June 22, 2009. For the remaining soil sampling dates, extractable NO_3-N declined in a similar pattern across the deeper depths samples with values reaching <2 mg NO_3-N/kg by July 31, 2009.

The mean concentration of extractable NO_3-N for Blocks 5-8 as a function of sampling date is provided in Figure 6-DD. By the first soil sampling date, the mean concentrations of extractable NO_3-N where already higher in the 15-30 (bottom of the soil mound) and 30-50 cm depths than in the top of the soil mound (0-15 cm depth). There was also elevated NO_3-N at the 50-75 and 75-100 cm depths, suggesting there was rapid movement of NO_3-N through the soil profile.

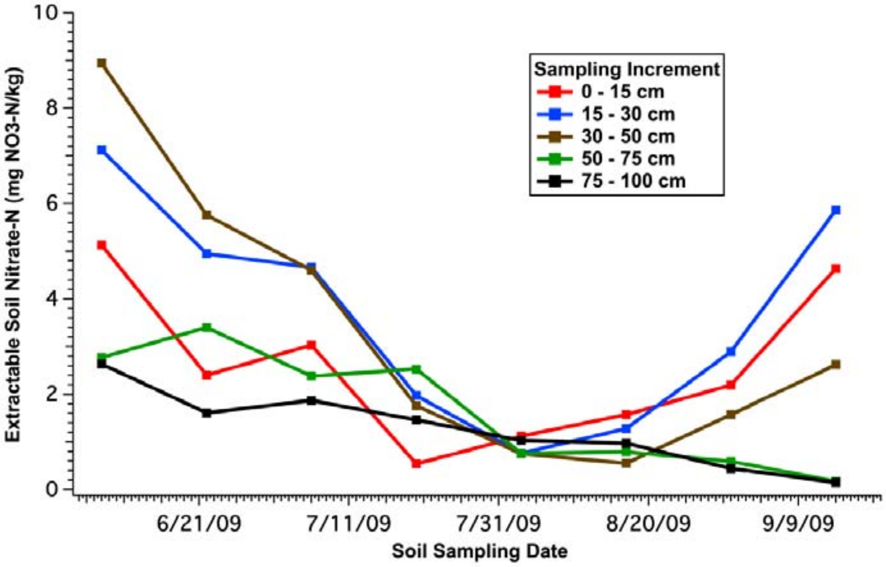

Figure 6-DD Mean Concentrations of Soil Extractable NO_3-N for Blocks 5-8 as a Function of Sampling Date and Sampling Depth Increment, Whiteville Station. (Sampling depth increments are referenced to top of soil mounds).

Soil extractable NO_3-N declined uniformly with depth and sampling date in Blocks 5-8, reaching minimum values on August 3, 2009. Beyond this date, soil NO_3-N continued to decline at the 50-75 and 75-100 cm depths, but increased substantially in the upper 0-50 cm of the soil profile. This rise in soil NO_3-N appeared to coincide with the first and second harvest dates for the tobacco crop (Table 6-O). A possible source of the increase in NO_3-N could be the death of part of the plant root system due to loss of leaves from harvesting, resulting in mineralization and release of NH_4-N, which in turn was converted to NO_3-N. Such a scenario is consistent with the increase in NO_3-N being restricted to the 0-50 cm depth relative to the top of the soil mound. It is not known why a similar pattern was not observed for Blocks 1-4 if indeed the source of the new NO_3-N is due to mineralization of dead roots.

The mean concentration of extractable NO_3-N for Blocks 9-10 as a function of sampling date is provided in Figure 6-EE. In the absence of tobacco plants, the pattern in NO_3-N in the top of the soil mound (0-15 cm) displayed a more consistent trend with extractable soil NO_3-N reaching a maximum by the second soil sampling and then showing a general decline for most of the remainder of the growing season. However, rapid communication with the deeper depths sampled is still evident perhaps even to the 75-100 cm depth. There is some decline in NO_3-N across the 0-50 cm depths during the growing season and a strong suggestion of consistent NO_3-N movement to deeper within the soil profile, especially after the end of July, 2009. Across the 50-100 cm depth, relative to the top of the soil mound, NO_3-N begins to decrease at last harvest data (September 14, 2009), suggesting further movement of NO_3-N beyond the 100 cm depth.

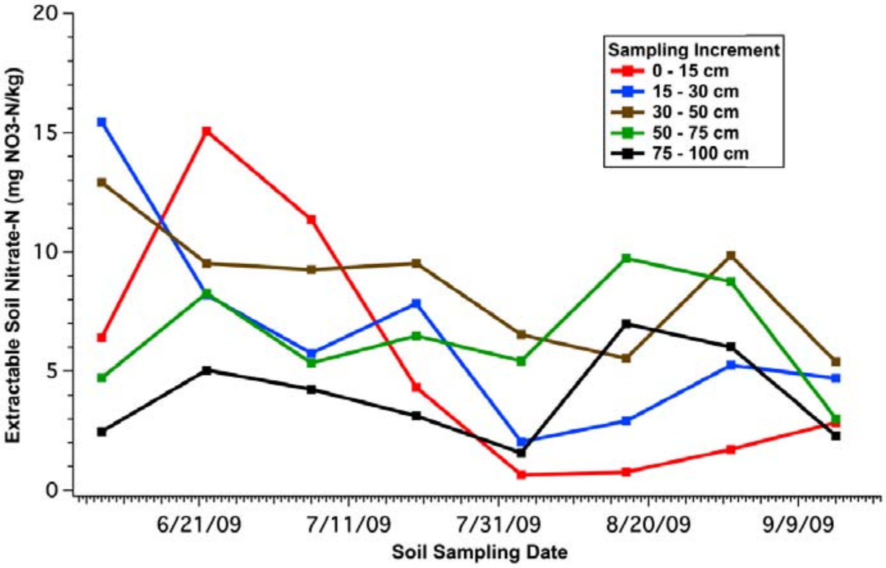

Figure 6-EE Mean Concentrations of Soil Extractable NO_3-N for Blocks 9-10 as a Function of Sampling Date and Sampling Depth Increment, Whiteville Station. (Sampling depth increments are referenced to top of soil mounds).

There is a suggestion of an increase in soil NO_3-N in the 0-15 and 15-30 cm soil depths, similar to that observed in Blocks 5-8. Although this increase cannot be attributed to mineralization of dead tobacco roots, weeds were present on Blocks 9-10 and herbicide was used several times during the growing season to control weed growth. Thus, the increase in soil NO_3-N may be due to the mineralization of weed roots, which may have penetrated only the 0-30 cm depth of the soil mound.

The data support the conclusion that extractable soil NO_3-N was relatively mobile in the upper 100 cm of soil at the Whiteville site, and a substantial fraction had already moved past the bottom of the soil mound by the first soil sampling on June 8, 2009. The NO_3-N detected, however, has two potential sources since two additions of fertilizer N were applied at approximately the same position in the soil mound. The initial application of fertilizer N contained both NO_3-N and NH_4-N. Ammonium-N would mineralize in the soil to form NO_3-N. It is reasonable to assume, therefore, that a substantial fraction of the NO_3-N detected below the bottom of the soil mound was derived from the first application of fertilizer. The second application of fertilizer N in the form of the nitrate salts represents a second pulse of NO_3-N that would move or diffuse away from the fertilizer band. This may explain why there is more apparent variability in NO_3-N in the top of the soil mound. As discussed in the next section, despite the relatively low extractable concentrations of NO_3-N at the Whiteville site versus the Oxford site, there was apparently sufficient N present to meet the needs of the tobacco crop.

6.3.7 Tobacco Nitrogen Content

A total of 4 harvests were obtained from the field site at the Whiteville station in 2009 (Table 6-S). The moisture content ranged from 70-80% in the harvested leaves, and the %N concentration ranged from 0.46 to 0.64%, with the majority of values across Blocks 1-8 <0.5%N in the harvested leaves. No significant differences between Blocks 1-4 and 5-8 were noted in moisture content or %N concentration in the harvested leaves.

Table 6-S. Nitrogen and Moisture Content of Tobacco Samples During 2009 Field Study, Whiteville Station

Date	Sample Type	Lower Leaves		Upper Leaves	
		N-Content Wet-Wt. Basis (%)	Moisture Content Wet-Wt. Basis (%)	N-Content Wet-Wt. Basis (%)	Moisture Content Wet-Wt. Basis (%)
Blocks 1-4 Nitrogen Source: Potassium Nitrate					
8-JUN-09	Leaf	0.585	77.5	-	-
22-JUN-09	Leaf	0.441	84.0	0.894	79.4
6-JUL-09	Leaf	0.588	80.2	1.058	68.3
20-JUL-09	Leaf	0.440	78.8	0.906	69.6
28-JUL-09	Harvest	0.576	71.6	-	-
3-AUG-09	Leaf	0.653	69.0	0.850	64.8
17-AUG-09	Leaf	-	-	0.689	67.9
17-AUG-09	Harvest	0.495	75.2	-	-
31-AUG-09	Leaf	-	-	0.739	65.2
14-SEP-09	Leaf	-	-	0.669	70.3
17-SEP-09	Harvest	-	-	0.495	75.2
24-SEP-09	Harvest	-	-	0.463	79.1
Blocks 5-8 Nitrogen Source: Ammonium Nitrate					
8-JUN-09	Leaf	0.908	75.4	-	-
22-JUN-0	Leaf	0.595	74.6	1.371	76.5
6-JUL-09	Leaf	0.475	76.6	1.370	68.9
20-JUL-09	Leaf	0.466	78.9	0.971	68.8
28-JUL-09	Harvest	0.662	71.4	-	-
3-AUG-09	Leaf	0.645	70.1	0.862	66.1
17-AUG-09	Leaf	-	-	0.733	66.5
17-AUG-09	Harvest	0.470	78.8	-	-
31-AUG-09	Leaf	-	-	0.591	74.9
14-SEP-09	Leaf	-	-	0.730	67.8
17-SEP-09	Harvest	-	-	0.470	78.8
24-SEP-09	Harvest	-	-	0.471	78.8

An estimate of N recovered by the harvested tobacco leaves is presented in Table 6-T, based on the provided mass of leaves harvested as determined by the Whiteville station staff in the field (Table 6-O), and the analyses of leaf punches obtained from the harvested leaves (Table 6-

S). The percent moisture content for each harvest (Table 6-S) was used to express the calculated results on an oven-dry weight basis.

As for the calculated N recovery for the Oxford station (Table 6-D), the calculated recoveries of fertilizer N added for the Whiteville station were high, averaging 80 and 107% for Blocks 1-4 and 5-8, respectively. Such values are unrealistic and reflect the presence of a large positive bias in the calculation of N recovered.

Table 6-T. Mass of Nitrogen Recovered in Harvested Tobacco Leaves, Whiteville Station. (All calculations normalized to an oven-dry weight basis)

Harvest Date	**Harvested Leaf Mass (kg/block)**	**Percent N Concentration (%)**	**Mass of N Recovered**	
			(g N/block)	**(lbs N/block)**
Blocks 1-4 Nitrogen Source: Potassium Nitrate (Total N Applied: 2.4 kg N/plot; 5.2 lbs N/plot)				
28-JUL-09	22.2	2.03	451	0.99
17-AUG-09	23.5	1.74	409	0.90
17-SEP-09	27.8	1.99	553	1.22
24-SEP-09	21.0	2.22	466	1.03
Totals=			***1879***	***4.14***
Blocks 5-8 Nitrogen Source: Ammonium Nitrate (Total N Applied: 2.3 kg N/plot; 5.0 lbs N/plot)				
28-JUL-09	26.1	2.30	600	1.32
17-AUG-09	22.7	2.41	547	1.20
17-SEP-09	27.2	2.22	604	1.33
24-SEP-09	30.5	2.23	680	1.50
Totals=			***2431***	***5.35***

The source of this large positive bias is currently unknown. While use of the leaf punches to determine the %N content of whole leaves is probably responsible for part of the systematic bias (as noted in section 6.2.7), it was assumed this potential source of error would be less at the Whiteville station because the harvest masses were taken during the harvest operation at field moisture content, and were not based on cured tobacco masses provided by the station superintendent as at the Oxford station. Other possible explanations such as an additional source of N already present in the soil or mistakes in N application rates seem highly unlikely. Results for extractable soil N for soil samples collected prior to planting were low, and a mistake in N application rates seems unlikely by both the Oxford and the Whiteville station staff. In addition, the %N content for the harvested leaves were in the range anticipated, suggesting the targeted N application rates were achieved and were applied correctly.

6.3.8 Summary of Pertinent Results

As was observed at the Oxford station, perchlorate was not detected in any of the soil or groundwater samples collected during the baseline event at the Whiteville station. Thus, it was not possible to quantify the potential impact of perchlorate from Chilean nitrate fertilizer historically applied to tobacco crops at the Whiteville station.

Perchlorate was also not detected in any of the groundwater or soil samples collected following application of the fertilizers and analyzed by EATS (although perchlorate was detected at low concentrations in two groundwater samples submitted to CAS for confirmatory analysis). Due to the absence of perchlorate in the groundwater and soil samples, nitrate was used as a surrogate to assess potential transport of perchlorate through the soil matrix and into the underlying groundwater.

The lack in temporal trends in nitrate and chloride concentrations in groundwater samples suggests that the shallow off-site groundwater was not directly influenced by the fertilizer used in this study.

Pre-plant soil samples had very low background levels of nitrate at the soil surface, but nitrate concentrations increased with depth to a depth of approximately 150 cm. Following fertilizer application, the data suggest fairly rapid movement of nitrate through the soil column to a depth of approximately 100 cm, and possibly beyond. As was observed at the Oxford station, the presence of the tobacco crop at the Whiteville station influenced the magnitude of NO_3-N, with higher NO_3-N concentrations being observed in the blocks without tobacco crop. This would suggest that removal of NO_3-N from soils in the blocks that contained tobacco crops was also attributed to plant uptake.

Based on these results, had the applied potassium nitrate fertilizer contained perchlorate at levels significantly greater than those used in this study, it is probable that movement of perchlorate through the soil matrix would have mimicked that of the nitrate, with perchlorate being transported through the upper 100 cm of soil (and possibly deeper). It is also probable that migration of perchlorate into the shallow groundwater would have occurred as the depth to groundwater was observed to vary between 60 cm to ~120 cm, which is within the observed vertical migration range of the nitrate.

7. CONCLUSIONS AND IMPLICATIONS FOR FUTURE RESEARCH

A study on the use of Chilean nitrate fertilizer was performed to address the possible presence of perchlorate in agricultural sites with historical production practices for growing tobacco. Historically, it was standard practice to use Chilean nitrate fertilizer as the nitrogen source for flue-cured tobacco production in NC following two to three weeks after transplanting of the tobacco seedlings. This practice lead to the suggestion of whether the current issue of wide-spread perchlorate contamination to groundwater in various agricultural areas of the United States can be linked to the historical use of Chilean nitrate fertilizer.

This study was designed to determine whether any perchlorate may still be present in soils, and underlying groundwater, historically used for tobacco production in NC, and to assess the behaviour of perchlorate resulting from the application of perchlorate-containing fertilizer to tobacco crops at two different agricultural sites over a single growing season. It was hypothesized that perchlorate could still be detected in soil extracts and water samples even though the current refined Chilean nitrate fertilizer contains an estimate of only 0.01% of perchlorate or less (Urbansky et al., 2001b). Unfortunately, the original Chilean nitrate product containing higher perchlorate levels is no longer available, and as such, a potassium nitrate product containing lower perchlorate content was used (by necessity) for the study.

The study was designed to assess the fate and persistence of perchlorate in the applicable media, including groundwater, soil and soil pore water, as well as potential uptake by plants. When it was determined that the perchlorate content of the available product was very low, nitrate was evaluated as a possible surrogate for predicting the movement of perchlorate in the subsurface.

The analysis of potassium nitrate fertilizer performed at NCSU found no detectable perchlorate. A subsequent analysis performed by CAS indicated that the fertilizer contained only 0.003% perchlorate. Analyses of soil and plant tissues using ion chromatography were unable to show the presence of any detectable perchlorate, probably due to the low amount of perchlorate actually applied using the potassium nitrate product. Perchlorate above background levels was detected in only one well water sample sent to CAS for confirmation analyses. Therefore, in order to fulfill the objectives of the study, fate and transport of nitrate at the study sites was used as a surrogate for perchlorate, as indicated above.

Perchlorate and nitrate are known to be readily soluble and subject to leaching depending on rain amounts and frequency, soil water content and soil physical properties. Thus, it was necessary to monitor the soil moisture content at each study site throughout the study and to compare measured soil moisture contents to the intensity and temporal distribution of rainfall with changes in soil water tension, as well as to evaluate possible trends of water movement based on soil properties such as bulk density and the percentage of soil pore space.

Based on the data of soil moisture at a depth of 0-75 cm (referenced to the top of the beds) for each plot at the Oxford station, it can be assumed that soil nitrate and perchlorate concentrations under these soil moisture conditions remained relatively static with soil depth and decreased only due to plant uptake over time. However, the data of the average depth to groundwater suggested that the nitrate (and perchlorate) from the fertilizer could possibly reach the underlying groundwater, although the impact of the rainfall events on movement of nitrate was not immediately evident. The existence of a restrictive clay layer at the 30 cm depth appeared to limit vertical movement of water to lower soil depths.

Soil moisture data for the Whiteville station showed that the vertical movement was prominent compared to the Oxford station. This can be explained by the differences in soil properties and the average mean of rainfall intensity at both sites. Temporal changes in soil moisture content at the Whiteville station suggested that the frequency and amounts of rainfall during the growing season were sufficient to keep parts of the soil profile near field capacity. Under these conditions, excess water would drain through the soil profile, acting to dilute and transport soluble anions like nitrate to deeper depths. This was evident in the lower extractable soil nitrate concentrations at the Whiteville station versus the Oxford station. However, no water was ever detected in the on-site shallow wells at the Whiteville station at the interface with a more restrictive clay layer at 100+ cm, suggesting nitrate moved to deeper soil depths due to rainfall, moved laterally offsite, or penetrated to deeper depths due to inconsistencies in the clay layer at 100+ cm.

The results from the well water sampling in this study support the conclusion that there is not a reservoir of perchlorate in soil or groundwater underlying these sites historically used for tobacco production. The extent to which perchlorate may have been destroyed through redox processes in the groundwater is not known. However, Shrout and Parkin (2006) have demonstrated that bacterial degradation of perchlorate is dependent primarily on the concentration of available carbon. Thus, it is possible that historically-applied perchlorate has degraded through naturally-occurring biological processes.

Perchlorate was not detected in any soil extracts obtained from soils collected from either research station. Excellent recovery values of spiked perchlorate using randomly picked soil extracts confirmed these results, as did soil samples picked at random and submitted to an external laboratory (CAS). Extractable NO_3-N data obtained for soil samples from the Oxford station showed almost similar patterns for Blocks 1-4 and Blocks 5-8 regardless the different type of fertilizers used: potassium nitrate versus calcium nitrate. Blocks 9-10 displayed trends similar to Blocks 1-8 except there is evidence of some further movement in the mass of NO_3-N deeper into the soil probably due to the absence of tobacco crop.

Extractable soil NO_3-N concentrations for the Whiteville station were in general an order of magnitude less than those observed at the Oxford station and the data showed different patterns for Blocks 1-4 and 5-8. In general, the mass of NO_3-N concentrations in the upper 0-50 cm of the soil profile for Blocks 5-8 after the first and second harvest dates tended to increase relative to

Blocks 1-4. No immediate explanation is available, although overall tobacco yields were also lower for Blocks 1-4. Lower yields may be due to differences in drainage across the blocks even though the study site looked fairly uniform to the naked eye. Tobacco is susceptible to poor drainage conditions. Poor soil drainage may also have promoted denitrification but on a time scale not detectable with the bi-weekly observations used in this study. The data for Blocks 9-10 indicate considerable variability in NO_3-N concentrations, but with possible further movement in the mass of NO_3-N deeper into the soil due to the absence of tobacco crop.

Based on these results, had the applied potassium nitrate fertilizer contained perchlorate at levels significantly greater than those used in this study, it is likely that movement of perchlorate through the soil matrix would have mimicked that of the nitrate, with perchlorate being transported throughout the upper 50 cm of soil at the Oxford station, and to depths potentially beyond 100 cm at the Whiteville station. At the Oxford station, it is unlikely that downward vertical migration of perchlorate into the deep groundwater would have occurred. At the Whiteville station, it is likely that migration of perchlorate into the shallow groundwater would have occurred since migration of nitrate was observed to depths below the range of seasonal groundwater table elevations.

Based on the application rate of potassium nitrate fertilizer for both sites, the potential concentration of perchlorate applied to the sites at the Oxford station and Whiteville station can be projected as within the range of 208-347 mg ClO_4/block (7,910–13,232 mg ClO_4/ha) and 238-397 mg ClO_4/block (9,092–15,204 mg ClO_4/ha), respectively, assuming the perchlorate content of the potassium nitrate ranged from 0.003 to 0.005%.

Although from this study, a significant relationship cannot be shown to exist between the low-level wide-spread contamination of perchlorate in the environment and the application of Chilean nitrate fertilizer for agricultural practices, the idea of Chilean nitrate fertilizer as one of the possible sources of perchlorate cannot be completely rejected.

Having a sampling site with adequate historical data on sources and amount of fertilizer used is crucial to predict the fate of perchlorate from the historical application of Chilean nitrate fertilizer. However, this is not a simple task as most of the research stations have only data that extend back to the mid-1970's.

Relevant models for more reliable projection on perchlorate movement with considerable attention on nitrate due to the nature of fertilizer itself as a nitrogen source for crops may be useful to compensate for the lack of available historical data for the assessment of perchlorate contamination, and for further understanding on the fate of perchlorate in the environment.

Information generated from this study is useful in understanding the potential environmental contamination of perchlorate from the application of Chilean nitrate fertilizer in tobacco production and to emphasize the need for further research, possibly incorporating spiked fertilizers to increase the amount of perchlorate to mimic historical levels and/or the use of other non-regulated anion tracers such as bromide, whose presence and transport though the soil would not be confounded by other soil processes, and which would not pose a threat to groundwater.

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APPENDIX A
BOREHOLE LOGS

Geosyntec consultants

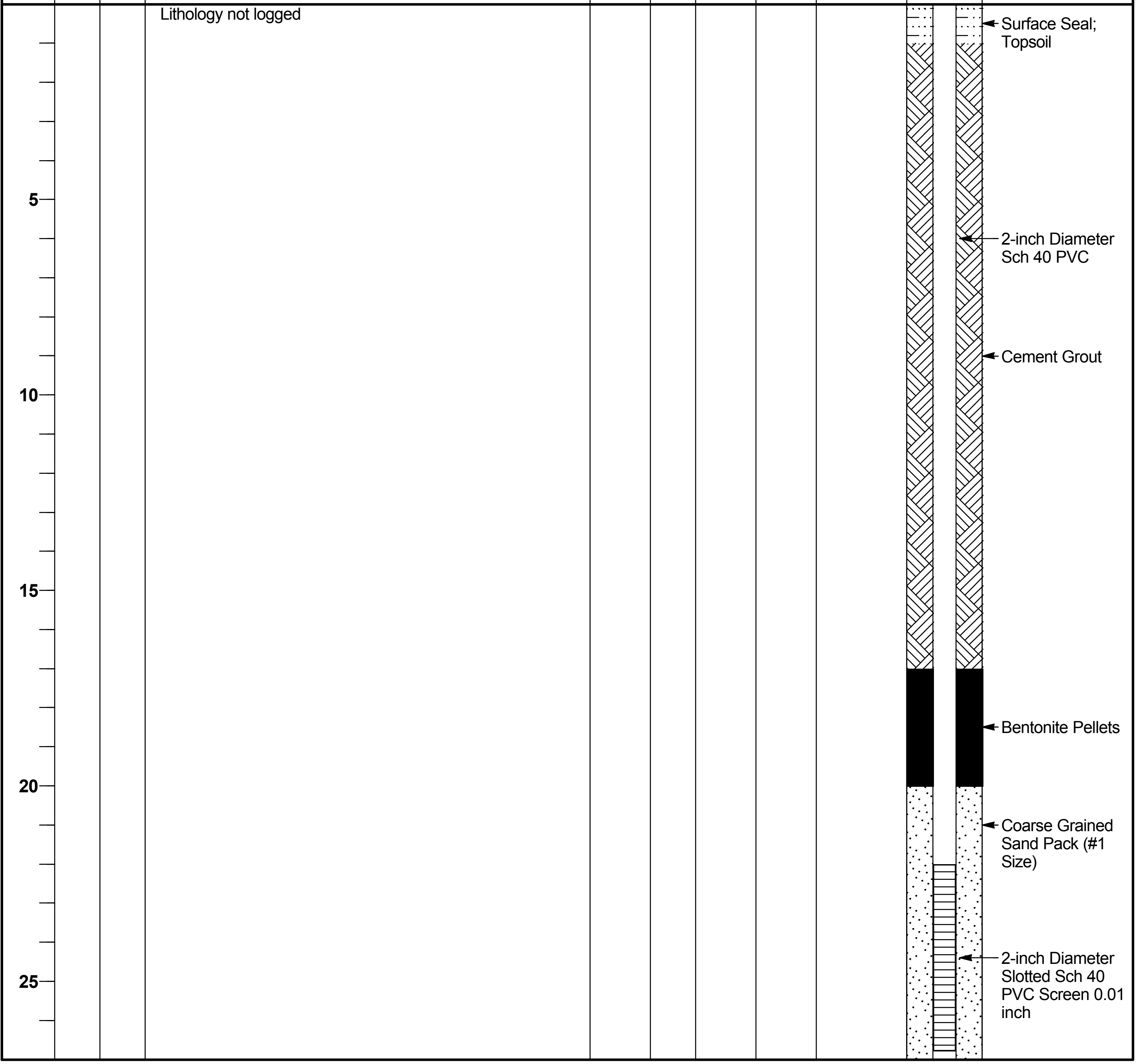
Borehole No. P3DA

Page 1 of 1

Borehole Log

Project No.:	TR0197A	**Location:**	Oxford Tobacco Research Station, NC
Client:	SERDP	**Coordinates:**	Not Surveyed
Logged By:	Stepen W. Randall	**Borehole Diameter:**	8 inches
Reviewed By:	Mark Watling	**Site Datum:**	Not Surveyed
Drilling Company:	Parratt-Wolff inc.	**Ground Surface Elevation:**	Not Surveyed
Drilling Method:	Hollow Stem Auger	**Top PVC Casing Elevation:**	Not Surveyed
Well Material:	Schedule 40 PVC	**Completion Date:**	12 May 2009

Depth (ft bgs)	Water Level	Stratigraphy	**Lithologic Description**	**Geologic Samples**					**Well Configu-ration**	**Comments**
				Unified Soil Classification	Recovery (%)	Blowcount	PID (ppmv)	Soil Sample ID		
			Lithology not logged							



Borehole depth 27.0 ft

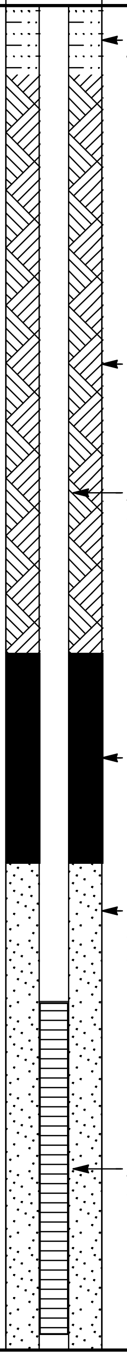
Borehole No. P6DA

Borehole Log

Project No.:	TR0197A	**Location:**	Oxford Tobacco Research Station, NC
Client:	SERDP	**Coordinates:**	Not Surveyed
Logged By:	Stepen W. Randall	**Borehole Diameter:**	8 inches
Reviewed By:	Mark Watling	**Site Datum:**	Not Surveyed
Drilling Company:	Parratt-Wolff inc.	**Ground Surface Elevation:**	Not Surveyed
Drilling Method:	Hollow Stem Auger	**Top PVC Casing Elevation:**	Not Surveyed
Well Material:	Schedule 40 PVC	**Completion Date:**	12 May 2009

Depth (ft bgs)	Water Level	Stratigraphy	Lithologic Description	Geologic Samples					Well Configu-ration	Comments
				Unified Soil Classification	Recovery (%)	Blowcount	PID (ppmv)	Soil Sample ID		
			Lithology not logged							

Depth (ft bgs): 5, 10, 15



Surface Seal; Topsoil

Cement Grout

2-inch Diameter Sch 40 PVC

Bentonite Pellets

Coarse Grained Sand Pack (#1 Size)

2-inch Diameter Slotted Sch 40 PVC Screen 0.01 inch

Borehole depth 19.3 ft

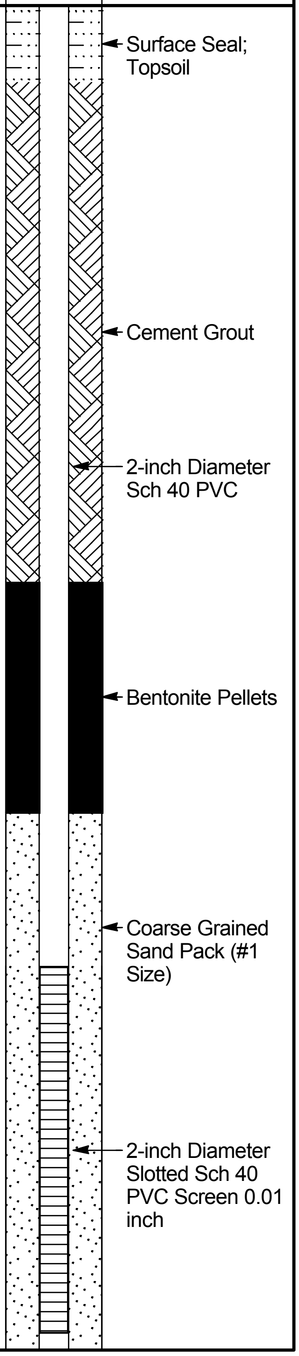
Borehole No. P9DA

Page 1 of 1

Borehole Log

Project No.:	TR0197A	**Location:**	Oxford Tobacco Research Station, NC
Client:	SERDP	**Coordinates:**	Not Surveyed
Logged By:	Stepen W. Randall	**Borehole Diameter:**	8 inches
Reviewed By:	Mark Watling	**Site Datum:**	Not Surveyed
Drilling Company:	Parratt-Wolff inc.	**Ground Surface Elevation:**	Not Surveyed
Drilling Method:	Hollow Stem Auger	**Top PVC Casing Elevation:**	Not Surveyed
Well Material:	Schedule 40 PVC	**Completion Date:**	12 May 2009

Depth (ft bgs)	Water Level	Stratigraphy	Lithologic Description	Geologic Samples					Well Configu-ration	Comments
				Unified Soil Classification	Recovery (%)	Blowcount	PID (ppmv)	Soil Sample ID		
			Lithology not logged							
5										
10										
15										



Borehole depth 17.5 ft

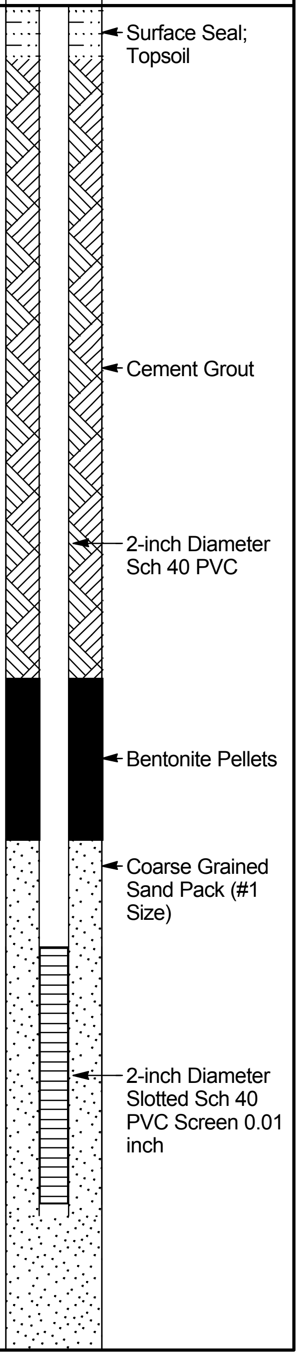
Borehole No. P3DB Page 1 of 1 Borehole Log

Project No.:	TR0197A	**Location:**	Oxford Tobacco Research Station, NC
Client:	SERDP	**Coordinates:**	Not Surveyed
Logged By:	Stepen W. Randall	**Borehole Diameter:**	8 inches
Reviewed By:	Mark Watling	**Site Datum:**	Not Surveyed
Drilling Company:	Parratt-Wolff inc.	**Ground Surface Elevation:**	Not Surveyed
Drilling Method:	Hollow Stem Auger	**Top PVC Casing Elevation:**	Not Surveyed
Well Material:	Schedule 40 PVC	**Completion Date:**	14 May 2009

Depth (ft bgs)	Water Level	Stratigraphy	Lithologic Description	Geologic Samples: Unified Soil Classification	Recovery (%)	Blowcount	PID (ppmv)	Soil Sample ID
			Lithology logged from soil cuttings					
		▨	CLAY (CL); brown to reddish brown; very fine to fine-grained sand with minor silt; medium plasticity; occasional quartz grains	CL				
5		▨	CLAY (CL); brown; low plasticity; grading to clayey sand with depth	CL/SC				
10			SAND (SM); brown; very fine to fine grained sand with minor medium grained sand and silt; loose; frequent 1 cm size quartz aggregates	SM				
15			SAND (SM); brown; very fine to medium rock fragments content (25-30%)	SM				
20		●	SAND AND GRAVEL (SM/GM); Brown; fine to medium grained sand with rock fragments (40-50%)	SM/GM				

Borehole depth 25.0 ft

Well Configuration



Borehole No. P6DB

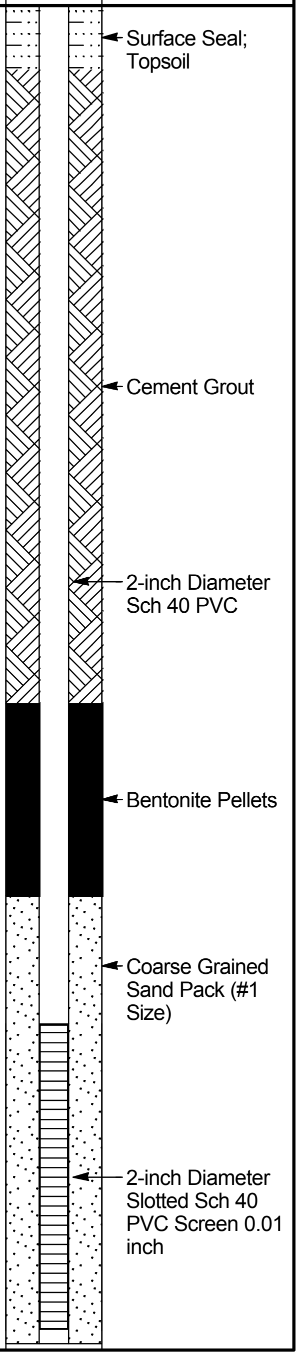
Page 1 of 1

Borehole Log

Project No.:	TR0197A	**Location:**	Oxford Tobacco Research Station, NC
Client:	SERDP	**Coordinates:**	Not Surveyed
Logged By:	Stepen W. Randall	**Borehole Diameter:**	8 inches
Reviewed By:	Mark Watling	**Site Datum:**	Not Surveyed
Drilling Company:	Parratt-Wolff inc.	**Ground Surface Elevation:**	Not Surveyed
Drilling Method:	Hollow Stem Auger	**Top PVC Casing Elevation:**	Not Surveyed
Well Material:	Schedule 40 PVC	**Completion Date:**	13 May 2009

Depth (ft bgs)	Water Level	Stratigraphy	Lithologic Description	Geologic Samples: Unified Soil Classification	Recovery (%)	Blowcount	PID (ppmv)	Soil Sample ID
			Lithology logged from soil cuttings					
		░	SILT (MC/CL); brown; moist; clayey with minor sand content; medium plasticity	MC/CL				
5		▨	CLAY (CL); tan to light brown; damp; silty; medium plasticity	CL				
10		▨	CLAY (CL); tan to light brown; dry; silty with trace quartz grains (5-10%); medium plasticity	CL				
15		▨	CLAY (CL); tan to light brown; silty with trace granite rock fragments (5-10%); medium plasticity	CL				
20		▩	ROCK ; quartz rich weathered fragments; granite					

Well Configuration / **Comments**



Borehole depth 21.0 ft

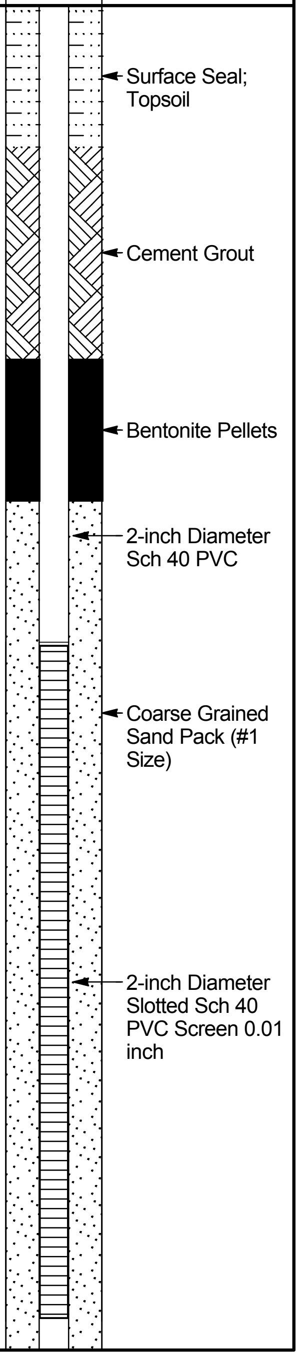
Borehole No. P9DB

Page 1 of 1

Borehole Log

Project No.:	TR0197A	**Location:**	Oxford Tobacco Research Station, NC
Client:	SERDP	**Coordinates:**	Not Surveyed
Logged By:	Stepen W. Randall	**Borehole Diameter:**	8 inches
Reviewed By:	Mark Watling	**Site Datum:**	Not Surveyed
Drilling Company:	Parratt-Wolff inc.	**Ground Surface Elevation:**	Not Surveyed
Drilling Method:	Hollow Stem Auger	**Top PVC Casing Elevation:**	Not Surveyed
Well Material:	Schedule 40 PVC	**Completion Date:**	13 May 2009

Depth (ft bgs)	Water Level	Stratigraphy	**Lithologic Description**	**Geologic Samples**					**Well Configuration**	**Comments**
				Unified Soil Classification	Recovery (%)	Blowcount	PID (ppmv)	Soil Sample ID		
			Lithology logged from soil cuttings							
		▨	CLAY (CL/SC); dark brown to tan; moist; very fine to fine grained sand with silt; medium plasticity	CL/SC						
5		░	SAND (SC/SP); tan to light brown; very fine to medium grained sand with minor silt content; clayey; medium plasticity; loose with weathered rock fragments and quartz	SC/SP						



Borehole depth 9.5 ft