ABSTRACT

MAHMUD, AINUL FAIZAH. Reaction of Contaminants in Agricultural Soils: Perchlorate and Cadmium. (Under the direction of Dr. Wayne P. Robarge.)

Flue-cured tobacco has long been an important part of North Carolina agriculture. Until ~ 10 years ago, nitrate-based fertilizers derived from caliche ore constituted a significant source of nitrogen (N) used in flue-cured tobacco production. Fertilizers derived from caliche ore contain perchlorate. Exposure to perchlorate in drinking water/foodstuffs can negatively impact thyroid function. Perchlorate contamination of groundwater from anthropogenic-sources (primarily as a component of solid rocket fuel and explosives) has lead to the establishment of exposure limits by the U.S. EPA. This study was designed to provide insight into the potential for groundwater contamination because of the historical use of perchlorate-containing fertilizers in tobacco production. Flue-cure tobacco was grown at the Oxford Tobacco Research Station (Oxford, NC; thermic Typic Hapludults) and the Border Belt Tobacco Research Station (Whiteville, NC; thermic Typic Kandiudults) as representatives of two different physiographic regions in NC that have a long history of tobacco production. Perchlorate-containing fertilizer (potassium nitrate; SQM Corporation) and non-perchlorate containing fertilizer (ammonium nitrate or calcium nitrate) were applied at the recommended rate (30-40 kg N ha^{-1}). The traditional source of N fertilizer (nitrate of soda, ~ 0.1 – 0.2% perchlorate) was not available, and the substitute (potassium nitrate) proved relatively low in perchlorate (~0.003%). As a result, perchlorate was not detected in soil extracts, monitoring wells or plant tissue samples. Implications for potential historical movement of perchlorate were instead based on soil nitrate (NO_3-N). Highest soil NO_3-N was found in the soil beds constructed for production. At the Oxford station, soil NO_3-N did appear to move vertically with time, but soil NO_3-N concentrations were substantially lower below the 30-cm depth, possibly due to in part to a compacted layer (bulk density 1.95 +/- 0.08 g/cc; mean porosity 26%). At the Whiteville station, soil NO_3-N was substantially lower

than observed at the Oxford site, but still demonstrated a gradient of decreasing NO_3-N with depth. Tobacco yields and N contents were similar at both sites suggesting no deficiency in N for plant growth. Nitrate concentrations varied among monitoring wells perhaps due to variations in redox conditions that may have favored denitrification. Well water at both locations also contained substantial amounts of chlorides, sulfates and phosphates. Perchlorate in highly mobile in soils and should mimic the movement of nitrate. Results from this study suggest it is reasonable to assume that some fraction of historically applied perchlorate through the use of nitrate-of-soda products derived from Chilean caliche migrated out of the rooting zone and into contact with surficial water layers and deeper groundwater in NC. However, no residual perchlorate in groundwater was detected in this study. This suggests there is not a reservoir of perchlorate in NC groundwater systems due to the historical use of perchlorate-containing fertilizers in tobacco production.

Cadmium is one of the toxic elements related to environmental problems. The adverse effect of cadmium on humans is the major impetus for continued study of its behavior and fate in the environment. A limited literature review was conducted pertaining to the occurrence of cadmium in the environment, with emphasis on cadmium accumulation in tobacco due to the application of phosphate fertilizer. Although the ability of tobacco to accumulate cadmium has been proven and cadmium accumulation in soil via application of phosphate fertilizers is likely, further investigation to better understand the interactions between soil factors and the available fraction of cadmium in soils is needed. This can be achieved via field experiments for a better assessment on the behavior of cadmium in the environment. Soil extraction methods also need to better estimate of cadmium bio-availability irrespective of differences in soil conditions and type of plants.

Reaction of Contaminants in Agricultural Soils: Perchlorate and Cadmium

by
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DEDICATION

To me……..finally.

BIOGRAPHY

Ainul Faizah Mahmud was born in Kuala Lumpur, Malaysia on March 3, 1985. She received her Bachelor of Science degree in Industrial Chemistry from Universiti Putra Malaysia in 2007. After her B.S. degree, she joined a local university in Malaysia and was subsequently awarded a scholarship under the "Young Lecturer Scheme" program to continue her studies for a Masters of Science degree in Soil Science. In spring 2009, Ainul was admitted to North Carolina State University as a Master of Science student in the Department of Soil Science. She has since worked under the guidance of Dr. Wayne Robarge. Upon her return to Malaysia, she will continue with her current employment where she assists senior lecturers in teaching several soil science courses at the undergraduate level. It is her intention to continue her professional development and to pursue a Doctor of Philosophy in Soil Science in the near future. She plans to continue her focus in the areas of soil chemistry and environmental chemistry.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ACRONYMS

°C	Degrees Celsius
EATS	Environmental and Agricultural Testing Service
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
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
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. CHAPTER 1: LITERATURE REVIEW (PERCHLORATE)

1.1 Introduction

Soil is a very complex heterogeneous material that covers the earth's surface and can acts as a sink for various kinds of organic and inorganic substances in the terrestrial system. Whether these substances are introduced by natural or anthropogenic sources, their fate and behaviour are greatly influenced by chemical, physical and biological processes in soil. These substances are considered as contaminants when they are present in substantial concentration with potential adverse effects to the environment (Peijnenburg, 2004).

Although soil serves as the main reservoir for numerous kinds of elements, other systems in the environment such as the groundwater and freshwater systems including plants can be affected if soils did not have the ability to retain these chemical elements (Adriano et al., 2005). These elements can be transported vertically and reach the groundwaters or be discharged to the surface waters via lateral water movement. These events may eventually lead to environmental problems such as contaminated drinking water supplies. Therefore it is important to understand the processes responsible for regulating the fate and behavior of chemical substances in a terrestrial ecosystem. These include the ability of certain soil properties in influencing the mobility of chemical elements and their availability for plant uptake as well as the possible degradation by soil microorganisms.

The main purpose of this study is to improve the understanding on the fate and behaviour of inorganic contaminants in soil, particularly perchlorate and cadmium, including potential plant uptake and impacts on groundwater and surface waters. Although they have different chemistry and properties, both perchlorate and cadmium

are known to be accumulated in tobacco via the application of certain fertilizers (Ellington et al., 2001; Lugon-Moulin et al., 2006).

The assessment on perchlorate contamination and the related study are covered in this chapter and the second chapter of this thesis, whereas a review on cadmium contamination in soil and tobacco is addressed in chapter three.

1.2 **Objectives**

The objectives of this study were to:

1. Quantify the potential impact of perchlorate from caliche-based nitrate fertilizer historically applied to two different agricultural sites. The historic use of caliche-based nitrate fertilizer has been postulated to be the cause of perchlorate impacts to groundwater in some agricultural areas. However, there is a debate as to whether perchlorate applied to soils via caliche-based nitrate fertilizer decades ago could still be causing impacts to groundwater today.

2. Attempt to track the fate of perchlorate applied to a tobacco crop at two different agricultural sites over a single growing season in order to increase our understanding of the behavior (e.g. persistence) of perchlorate in soil and groundwater due to the historical use of caliche-based nitrate fertilizer.

3. Conduct a literature review to further our understanding of the potential sources and subsequent soil reactions that influence cadmium uptake by agricultural crops, in particular tobacco. As a non-nutrient element, cadmium is applied to agricultural soils as a trace contaminant in various soil amendments (phosphate fertilizers, lime, animal manures) as well as in bio-solids from industrial and municipal sources. Uptake of cadmium is an example of how plant bio-availability of a chemical species is a function of a number of soil variables.

1.3 **Perchlorate**

For the past several decades, there has been a debate within the United States on the apparent wide spread contamination of soil, sediment, groundwater and surface waters in many regions of the country from perchlorate (Aziz et al., 2009). Concerns about potential impacts to human health and the sources of the apparent perchlorate contamination have led to numerous studies to investigate the matter thoroughly, and to provide more reliable and accurate data for a better understanding on possible risks imposed by this substance to the environment (Gu, B. and J. D. Coates, 2006; Sellers, K., 2007).

The sources of perchlorate contamination can be divided into two categories: anthropogenic and non-anthropogenic. The anthropogenic sources are typically related to the use and disposal of rocket propellant (which contains ammonium perchlorate) in the defense and aerospace industries. Ammonium perchlorate is also used in fireworks and road flares. Contamination from naturally occurring perchlorate has mostly been attributed to the use of caliche-based nitrate fertilizer in crop and vegetable production (Aziz and Hatzinger, 2009). However, there are also studies suggesting that atmospheric processes active in the formation of perchlorate are present in today's atmosphere, such that atmospheric deposition (especially by rainfall) is one possible source for the detection of low levels of perchlorate in groundwater in certain areas of the United States (Dasgupta et al., 2005; Rajagopalan et al., 2006).

Perchlorate is a negatively charged ion that consists of one chlorine atom and four oxygen atoms (ClO_4^-). Due to its weak association with a positively charged ion, perchlorate salts are extremely soluble in water. Perchlorate is generally a non-complexing anion that is a poor nucleophile and is also kinetically inert to oxidation and reduction. It also has limited sorption to mineral or organic surfaces. Thus, once perchlorate is released into the environment, it will persist and be readily mobile. It can

easily be spread across relatively large distances via the flow of surface or groundwater (Brown and Gu, 2006; Aziz et al., 2009).

One potential adverse effect of perchlorate on human health is that it can interfere with hormone production by competing with the uptake of iodide by the thyroid. One example of the effect of the competition between perchlorate and iodide is through the sodium (Na^+) / iodide (I^-) symporter (NIS) molecule. NIS is the protein responsible for transporting iodide into the thyroid gland for the production of triiodothyronine[T3] and thyroxine [T4]. The reduction of these hormones can lead to potential risks of fetal and neonatal development, although no specific cases have been reported to date (Clarkson et al., 2006; Charnley, 2008).

Perchlorate uptake by skin is minimal (NRC, 2005) and due to the low vapor pressure of the salts, uptake by inhalation is considered as negligible except probably for certain occupational conditions with high exposure to perchlorate dust or particles (NRC, 2005). The main route for perchlorate uptake in human is via ingestion (oral consumption) because of its rapid uptake from the gastrointestinal tract (Clarkson et al., 2006).

1.4 **History of Regulatory Actions**

According to the National Research Council, perchlorate contamination was discovered in wells at California Superfund sites in 1985, but only gained serious media and regulatory attention in 1997. The U.S. Environmental Protection Agency US EPA) reported that from the data obtained for the Unregulated Contaminant Monitoring Regulation (UCMR) 1, almost 11 million people have perchlorate in their public drinking-water supplies at concentrations of at least 4 ppb (4 µg/L) (NRC 2005). Since then, this topic continues to be debated between several *United States government*

agencies such as the Department of Defense (DOD), the Department of Energy (DOE), and the National Aeronautics and Space Administration (NASA) as to which agency will possibly assume the responsibility for cleaning up or developing treatment processes at sites deemed contaminated with perchlorate. Russel et al. (2009) estimated the potential treatment cost required to meet a proposed national primary drinking water regulation (NPDWR) to be between $76 million and $140 million/year at a maximum contaminant level (MCL) of 4 μg/L.

The controversy on the potential toxicity of perchlorate on human health has led the US EPA to develop a precautionary limit on lifetime exposure purposely to avoid any adverse effects on sensitive populations such as infants and people with hypothyroidism or iodide deficiencies. Although the numerical value of that exposure limit is debated by some, US EPA adopted the Reference Dose (RfD) of 0.0007 mg/kg of body weight per day recommended by National Academy of Sciences (NAS) for perchlorate and perchlorate salt in February 2005 (NRC, 2005). For example, in order for an adult with body weight of 70 kg to reach the RfD of 0.0007 mg/kg of body weight per day, he or she needs to consume more than 12 liters of water that contained 4 μg/L of perchlorate. According to the Drinking Water Equivalent Level (DWEL) by EPA, assuming a 70 kg body weight with 2 liters per day consumption of drinking water, it takes 24.5 ug/L of perchlorate to achieve the stated exposure limit (AWWA, 2005). Therefore it can be assumed that the current RfD for perchlorate is reasonable even when considering the most vulnerable population.

The US EPA has taken a series of actions pertaining to the establishment of regulations on perchlorate level in drinking water. On March 2, 1998, US EPA included perchlorate on the first Contaminant Candidate List (CCL) and also in the following second and third CCL that were published in the Federal Register on February 24, 2005, and October 8, 2009, respectively. On February 2011, US EPA decided to regulate perchlorate under the Safe Drinking Water Act (SDWA). This action will initiate a

process to develop and establish a national primary drinking water regulation (NPDWR) (US EPA, February 2011).

Prior to the various actions taken by USEPA on perchlorate contamination, some states have already established their own regulations. In 1997, The California Department of Health Services (CDHS) initiated a perchlorate action level of 18µg/L; in 2004, it was changed to 6µg/L (Tikkanen, 2006). The Massachusetts Department of Environmental Protection (MA DEP) established a perchlorate Drinking Water Standard (DWS) of 2 µg/L in 2006 (Zewdie et al., 2010).

1.5 **Chilean Nitrate Fertilizer**

Portions of this thesis will focus on nitrate fertilizer produced from caliche ore in Chile (Chilean nitrate fertilizer) as one of the potential sources responsible for the occurrences of wide spread, low level concentrations of perchlorate found in US. Chilean nitrate fertilizer is manufactured from caliche deposits mined from the Atacama Desert region of Chile that is known to contain naturally occurring perchlorate (Schilt, 1979; Ericksen, 1983). Caliche, also known as calcrete, can be defined as terrestrial materials composed of calcium carbonate and can be found mainly in semiarid to arid areas (Dixon, J.C. 2009).

Caliche deposits contain sodium nitrate ($NaNO_3$) that can be processed into nitrate-based fertilizer such as sodium nitrate (N-P-K ratio: 16-0-0). Sodium nitrate, also known as nitrate of soda, was commonly used as a nitrogen source for the production of tobacco, citrus fruits, cotton, and some vegetable crops in the United States during the first half of the 20th century. This product was mainly produced and distributed for US consumption under the commercial name of Bulldog Soda by Chilean Nitrate Corporation, the North America subsidiary of SQM Company (http://www.sqm.com) based in Chile (Aziz et al., 2006; Urbansky et al., 2001).

Although the consumption of Chilean nitrate fertilizer in United States has declined during recent decades, the potential that the historical use of Chilean nitrate fertilizer is a source of current perchlorate contamination in groundwater cannot be excluded from consideration as perchlorate is known to have the ability to resist any changes in the environment. Urbansky et al. (2001) showed that the products of Chiliean nitrate fertilizer containing approximately 0.5 - 2 mg/g of perchlorate anion can still be found in the United States, particularly in niche markets. Even though the SQM Company continues to improve the refinement process for their fertilizer production to ensure lower concentrations of perchlorate in their products (Lauterbach, 2004), it can be assumed that perchlorate is still being potentially introduced to the environment from these materials even today.

1.6 **Analytical Methods for Analysis of Perchlorate**

Due to perchlorate's water solubility and high mobility in soils, most of the early studies on its fate in the environment focused on potential impacts to surface water and the underlying groundwater, particularly to assess possible perchlorate contamination in drinking water supplies. In 1999, US EPA provided a standard method known as Method 314.0 for determination of perchlorate in drinking water using ion chromatography (US EPA, 1999). Prior to the release of Method 314.0, the Sanitation and Radiation Laboratory of California Department of Health Services (CDHS) released a method based on ion chromatography to determine trace concentrations of perchlorate in drinking water samples (CDHS, 1997).

The low level detection limit of perchlorate can be related to the enhanced performance of ion chromatography (IC). Based on the method developed by CDHS, it was reported that the method detection limit (MDL) and the minimum reporting level

(MRL) in groundwater and surface water were ≤ 1µg/L and 4 µg/L, respectively (CDHS, 1997) compare to the earlier detection limit of 400 µg/L (Motzer, 2001).

Ion chromatography (IC) has been the most widely used technique for perchlorate determination (Jackson et al., 1999). A primary reason lies within the chemistry of perchlorate itself. Perchlorate anion has low hydration energy or less hydrophilic compared to other inorganic anions. Therefore, perchlorate is strongly retained to the resin in the chromatographic column selected for the analysis, and is eluted later compared to other more hydrophilic ions (Brown and Gu, 2006)

Basic ion chromatograph consists of a stationary phase and a mobile phase. The stationary phase is the column which contains resin, while, the mobile phase or eluent is the ion extraction liquid. Carbonate/bicarbonate or hydroxide is the most common eluent used. Ion chromatograph measures the concentrations of ionic species based on their affinity for the chromatographic column. The eluent carries the liquid samples onto the stationary phase (column). When the liquid sample is loaded onto the column, the analytes of interest are retained on the column allowing separation from the original sample liquid matrix. The analytes are then eluted from the column by a similarly charged species. The exchange processes lead to a different retention times for each analyte. A conductivity detector is used to differentiate the ionic species based on their retention times and the peaks shown in the chromatogram represents the relative amount of each ion. This amount can be determined by comparing the area under each peak against a known standard.

Although the earlier methods were designed to determined perchlorate in surface water, groundwater and drinking water supplies, the sensitivity and the robustness of analytical methods on perchlorate analysis are being improved continually to adapt to different types of sample matrices to fulfill the needs for reliable data for various studies. Synder et al. (2005) used liquid chromatography-tandem triple-quadrupole mass

spectrometry (LC-MS/MS) system with minimum reporting level of 50 ng/L to determine perchlorate in surface waters, groundwaters, and commercially bottled waters. In January 2007, the USEPA Office of Solid Waste (OSW) published Methods 6850 and 6860 that use high performance liquid chromatography/electrospray ionization/mass spectrometry (HPLC/ESI/MS) and ion chromatography/electrospray ionization/mass spectrometry (IC/ESI/MS), respectively, to determine perchlorate concentration in various samples matrices such as soil, sludge, wastewater and high salt water (US EPA (OSW), 2007). Therefore it can be assumed more widespread detection of low level perchlorate will be discovered in the environment following the continuous advancement in analytical methods.

1.7 **Perchlorate in the Environment**

Parker et al. (2008) showed that 109 samples from 326 groundwater samples taken from domestic, agricultural, and recreational wells in the United States, contained <1000 ng/L perchlorate and 28 samples contained 1000 to 10,400 ng/L perchlorate. More than half of the 28 samples with perchlorate concentration >1000 ng/L were located on or in close proximity to active agricultural land. It was suggested that the detection of perchlorate can be associated with the historical use of Chilean nitrate fertilizer in those agricultural sites.

Munster and Hanson (2009) found that the average concentrations of perchlorate measured in soil waters beneath sites treated with substances classed as organic fertilizers was significantly higher compared to the sites treated with more common chemical fertilizers or sites with no fertilizer applied. Therefore, the application of perchlorate-containing fertilizers was predicted to potentially affect the groundwater quality as the perchlorate detected in soil water at 100 cm can be leached through subsoils and can eventually reach groundwater. Further studies by several researchers have shown no significant retention of perchlorate by soils and that the transport of

perchlorate in soil is highly influenced by hydrologic factors (Tipton et al., 2003; Urbansky and Brown, 2003).

In addition to perchlorate contamination in groundwater and soil, considerable attention has been given to understanding its potential uptake by plants. Several species of plants are known to take up and even accumulate perchlorate, such as salt cedar *(Tamarix ramosissima* Ledeb) (Urbansky et al., 2000), and tobacco (*Nicotiana tabacum* L.) (Ellington et al., 2001).

There has also been great concern shown on the elevated perchlorate exposure in the food chain based on data from surveys conducted by US Food and Drug Administration (FDA) in 2004/ 2005 to examine the perchlorate concentration in several foods including milk in United States. It was found that the milk samples contained up to 11 µg/L of perchlorate, with a mean value of 5.76 µg/L (US FDA, 2004). Subsequent studies found that perchlorate can also be present in human breast milk with higher concentrations compared to milk from cows (Kirk et al., 2005,) ; Borjan et al., 2011).

1.8 **Nitrate-based Fertilizers from Chile and Tobacco**

Nitrate-based fertilizer from Chile was a common fertilizer used as a source of nitrogen for tobacco production in the early and mid-20th century. Nitrogen is an important nutrient as lack of nitrogen directly influences the yield and the quality of tobacco. Inadequate amounts of applied nitrogen can reduce the yield, while excessive N can lead to difficulties in harvesting process such as a delay in maturity and an extension in curing time. The total amount of N recommended for maximum production varies between 56 to 90 kgN/ha, with approximately half applied at transplanting and the remainder added as a sidedress 3-4 weeks later. However, the recommended rate of N application is dependent on soil type, the previous crop, and a leaching adjustment factor

if necessary (Peedin, 1993; Smith, 2010). Nitrogen efficiency can also be influenced by the method used to apply the N fertilizer. Broadcast versus side-dressed has been shown to result in different rates of nitrogen recovery: 36.6% versus 43-54% of the applied N, respectively (MacKown and Sutton, 1997).

Chilean nitrate fertilizer (nitrate of soda) with NPK ratio of 16:0:0 was commonly used as the side dress fertilizer application to supply the remaining nitrogen needed for optimum growth. Although the application of nitrate of soda has declined significantly after late 50's (Sheridan, 1979), it was still being recommended by the North Carolina Cooperative Extensive Service of North Carolina State University in 1992 to be used as side dress fertilizer, along with calcium nitrate or ammonium nitrate. Based on a survey of county Extension agents, nitrate of soda was the most common sidedress fertilizer used in 1992 for tobacco production in North Carolina. (Peedin, G.F., 1993). In 1992, North Carolina had the highest acreage of tobacco in the U.S. (U.S. Census of Agriculture, 1997). However, since 2005, the North Carolina Cooperative Extensive Service has been recommending other types of side dress fertilizer due to the limited supply of nitrate of soda and its increasing cost (Smith and Wood, 2005).

The mechanism of perchlorate uptake, translocation and accumulation in tobacco plants are not completely understood. Ellington et al. (2001) found that tobacco leaf lamina can contain 96.0 ± 0.6 mg/kg dry weight (14.6 ± 0.1 mg/kg fresh weight) of perchlorate when grown with perchlorate added with N fertilizer. It was suggested that the perchlorate applied was translocated from the roots to the leaves and accumulated there. Published results from other studies suggests that perchlorate uptake in tobacco plants occurs predominately via mass flow driven by transpiration (passive transport) (Tan et al., 2004; Sundberg et al., 2003).

1.9 **Nitrate as Possible Surrogate for Perchlorate**

As mentioned above, the main purpose of using Chilean nitrate fertilizer for tobacco production is to provide a source of nitrogen. Although the perchlorate is added unintentionally as the fertilizer is applied, it can be said that both anions (nitrate and perchlorate) can have a somewhat similar fate in the environment. Nitrate ion is very soluble in water and prone to be lost through leaching and surface runoff. Nitrate also moves via mass flow or diffusion for plant uptake (Powlson and Addiscott, 2005). The behavior of perchlorate in the environment is not fully known and it is even harder to assess the fate of low concentrations of perchlorate as they can be influenced by many factors such as distance between fertilizer application and surface and groundwater, hydrologic factors, plant uptake and microbial activities (Tan et al., 2004; Tipton et al., 2003). Therefore, it is reasonable to make predictions on the fate of perchlorate based on the behavior of nitrate. This may be important since studies done to predict the movement of perchlorate from the use of Chilean nitrate fertilizer in the environment may be limited in the future due to the difficulty in obtaining the perchlorate-containing fertilizers for research purposes.

1.10 **Potential Remediation of Perchlorate**

Common technologies used for treating perchlorate contamination in drinking water, groundwater, and soil can be divided into two main categories: *in situ* or *ex situ* remediation technologies. US EPA (May 2005) has listed several approaches for each category as follow:

***Ex Situ* Remediation**

- Ion Exchange

- Bioreactor
- Liquid Phase Carbon Adsorption
- Composting

***In Situ* Remediation**

- Bioremediation
- Phytotechnology
- Permeable Reactive Barrier
- Membrane Technologies (Electrodialysis and Reverse Osmosis)

The most common technique used for *ex situ* remediation is by using ion-exchange resins developed for removal of perchlorate, with the anion present in the ion exchange resin replacing the perchlorate. However, this technique can be expensive to operate whether by using the single-pass systems that will generate resin needing to be disposed of regularly, or renewable systems that will produce perchlorate-containing brine solutions that need to be treated properly before disposal (Gu et al., 2002) (US EPA, May 2005).

Bioremediation and phytotechnology have been greatly considered as effective options for *in situ* remediation of perchlorate. Bioremediation involves the use of microorganisms capable of reducing perchlorate to chloride and oxygen under anaerobic conditions by supplying appropriate substrates for microbial growth. Some bacteria species are known to use perchlorate as an electron acceptor for cellular respiration and perchlorate is degraded to chloride ion (Logan, 2001; Urbansky and Schock, 1999; Xu et al., 2003)

Phytotechnology or phytoremediation is the use of plants to remove contaminants in soils and shallow groundwater by natural processes occurring within the plant body (Schnoor et al., 1995). Phytotechnology includes various mechanisms such as rhizosphere biodegradation, phyto-degradation, phytovolatilization, phytoaccumulation and phytostabilization However, phytodegradation and rhizodegradation are the two most important mechanisms of phytoremediation of perchlorate. Phytodegradation is the process of degrading the contaminants within plants tissues using enzymes, while rhizodegradation involves the biodegradation of contaminants by microorganisms that utilize the nutrients released by plants roots (Nzengung et al., 1999; Nzengung et al., 2004; FRTR 2005). Certain plants species such as smartweed (*Polygonumpunctatum* Elliott), water-lily (*Nymphaea odorata* Aiton*)* and tobacco (*Nicotiana tabacum* L.) have shown potential for phytoremediation of perchlorate (Sundberg et al., 2003; Susarla et al., 2000).

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2. CHAPTER 2: PERCHLORATE FROM THE USE OF CHILEAN NITRATE FERTILIZER

2.1 Introduction

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, anthropogenic 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 contain appreciable perchlorate. Prior to 2002, the estimated perchlorate content in Chilean nitrate fertilizers was 0.2% (Urbansky et al., 2001a), which is significant relative to the low groundwater action levels in most states.

Large quantities of Chilean nitrate fertilizers were used historically in the U. S., 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 were consumed in the United States. 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) were used as

fertilizer, then approximately 48 million pounds of perchlorate are likely to have been applied to agricultural soils during this time period.

Chilean nitrate fertilizer was commonly used in the United States as a source of nitrogen for tobacco plants, particularly in the early 1900s. Fertilizer application rates for tobacco varied with the season and soil type; however, application rates of 30 to 40 lbs of nitrogen per acre were typically recommended (Bennett et al, 1953). This application rate is common for flue-cured tobacco in North Carolina when adding a side dress of N fertilizer several weeks after transplanting. 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 (208-281kg/ha). 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; www.ncagr.com/agronomi/stnote2.htm). 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 (0.5-0.6 kg/ha). 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), although not all of this was flue-cured tobacco.

While the use of Chilean nitrate fertilizers has steadily declined since around 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 U.S. 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 lower compared to historic perchlorate contents. However, this amount still represents the potential introduction of more than 15,000 lbs (6800 kg) 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 are clear 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.

The overall initial 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. However, due to the following reason, the course of this study was redirected to accommodate the change in material used and at the same time, ensure the objectives listed could be achieved.

The SQM Corporation (www.sqm.com) is a known producer of nitrate of soda fertilizer products that at one time were used in tobacco production. These fertilizers were produced from caliche ore. Representatives of SQM North America Corporation

(Atlanta, GA) were contacted and informed as to the nature of the planned research, and asked of their willingness to donate sufficient amounts of their current nitrate of soda product in support of this project. It was learned, however, that the SQM Corporation no longer markets nitrate of soda products for tobacco production in the United States. In addition, representatives from SQM Corporation noted that substantial improvements in their production of nitrate of soda have substantially lowered the perchlorate content of the product.

As an alternative, the SQM North America Corporation generously offered to donate another nitrate product (potassium nitrate), and provide sufficient amounts needed for both research sites. In addition, SQM North America Corporation would send the potassium nitrate product directly to the research stations. After consultation with the project program managers, SQM North America Corporation's offer to use the potassium nitrate product as the source of fertilizer for the sidedress application of nitrogen to tobacco was accepted. The potassium nitrate fertilizer will also be addressed as Chilean nitrate fertilizer in following sections.

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 0.003%, or approximately an of order magnitude less than the original nitrate of soda product. It was also possible that the perchlorate content 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.

It was debated as to whether the potassium nitrate should be spiked with perchlorate salts to add an adequate amount of perchlorate in the sidedress application

to ensure detectable amounts in both soil and tobacco foliage. The final decision was not to spike the potassium nitrate product, but to use the transport of nitrate within the soil as a surrogate for predicting perchlorate movement if necessary. As perchlorate, nitrate is an anion that is known to be relatively mobile in soils.

2.2 **Oxford Tobacco Research Station**

2.2.1 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. Throughout nearly a century, scientific investigations on tobacco fertilization, cultural practices and insect and disease control have been conducted (Aull et al., 1978a). 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 its current mission remains to increase tobacco production efficiency, tobacco quality, and identify production management schemes for tobacco that conserve and protect soil and water.

Figure 2-1. Oxford Tobacco Research Station, Oxford, NC (Granville County)
(Source: Aull et al., 1978a.)

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 2-1). 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, field equipment for planting, fertilizing and harvesting tobacco, greenhouses and maintenance facilities. There are over 9 acres of irrigation ponds with associated irrigation equipment.

2.2.2 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 from broad gently sloping uplands to moderate to steep sloping areas with narrow convex ridges and steep valley slopes (Aull et al., 1978a).

Figure 2-2 illustrates the typical changes in slope. 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.

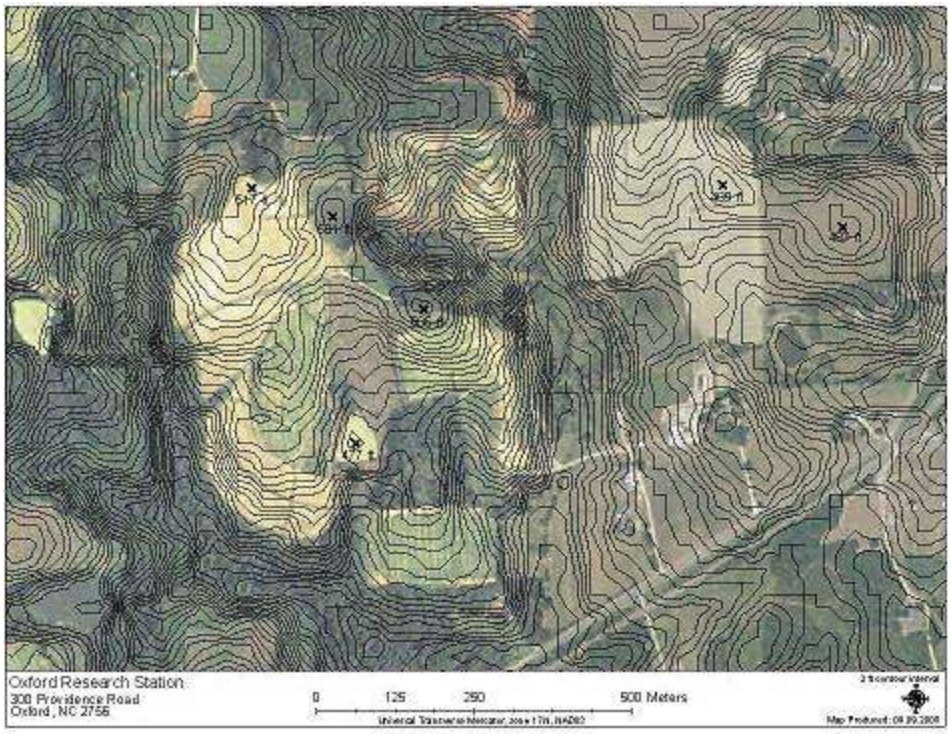

Figure 2-2. Countor Overlay for the Oxford Station (Source: Aull et al., 1978a.)

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) (Figure 2-3). Typically the Vance and Helena soils have landscapes of gently sloping or moderately sloping 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 presents the possibility for sub-surface lateral flow.

Figure 2-3. Aerial photo of the Oxford Station showing the dominant soil series (Source: Aull et al., 1978a.)

Annual precipitation at the Oxford station is approximately 112 cm (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.

2.2.3 Assessment of Historic Impacts of Land Use

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 experiments and fertilization, these records may only extend back to the mid-1970's. The 44.5 ha (110 acres) actually used for tobacco production at the station are divided into designated land units (e.g. Figure 2-4).

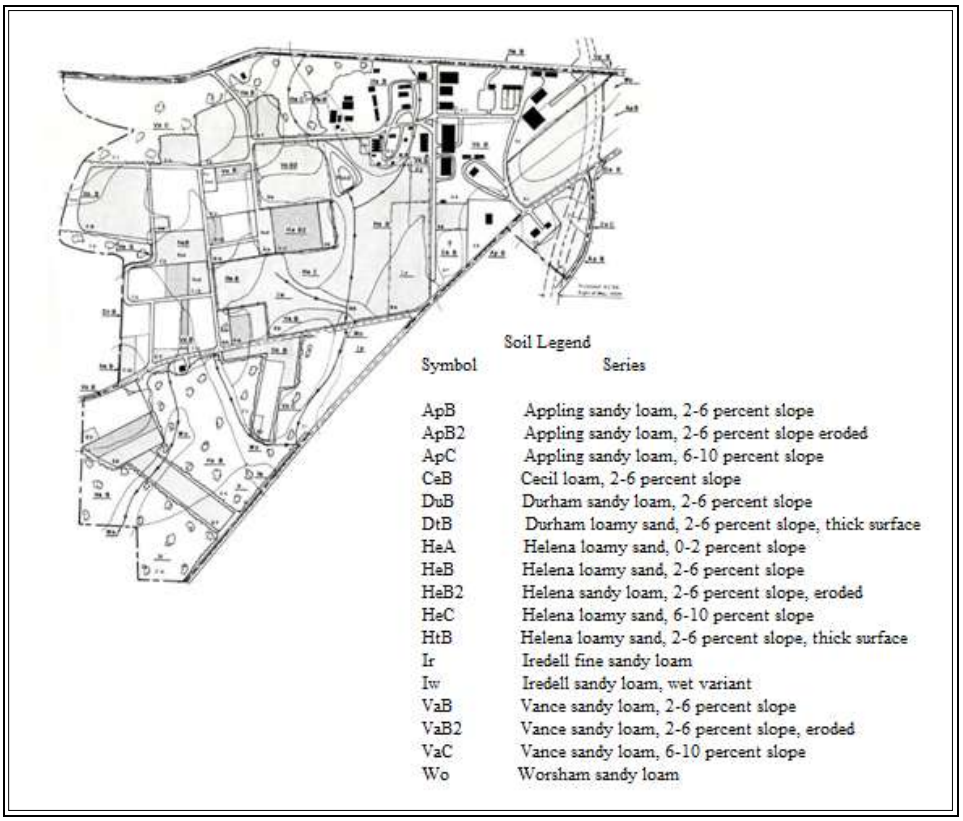

Figure 2-4. Map of the Oxford Station showing field units and related soil series (Source: Aull et al., 1978a).

2.3 **Border Belt Tobacco Research Station**

2.3.1 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 all aspects of flue-cured tobacco production, including breeding (Aull et al., 1978b). Today, in addition to tobacco, research projects focus on genetic studies under corn, soybean, and peanut production. 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 2-5). As is common in the middle Coastal Plain region of North Carolina, elevation across the station ranges from only 29 to 32 m (95 to 105 ft) (Figure 2-6). Approximately 20 ha (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 2-5. Border Belt Tobacco Research Station (Whiteville Station), Whiteville, NC (Columbus County) (Source: Aull et al., 1978b.)

Figure 2-6**.** Contour Overlay for the Whiteville Station (Source: Aull et al., 1978b.)

2.3.2 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 al., 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 2-7). This is the reverse from what is often expected in more upland situations, such as in the Piedmont or Mountain regions of North Carolina.

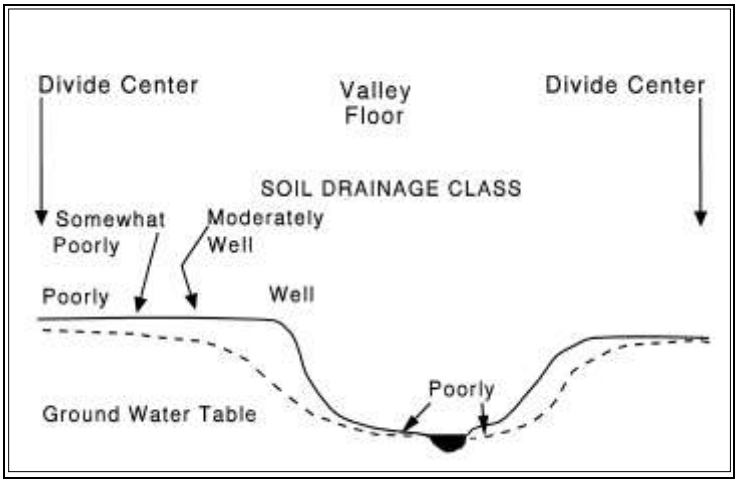

Figure 2-7. Relationship of soil drainage class to topography and groundwater depth in the Coastal Plain of North Carolina (Source: Aull et al., 1978b.)

This landscape and accompanying drainage system result in a range of soil series influenced by relative depth to the water table. Typically in the well-drained positions, depth to a 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.

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 124 cm (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 10 - 13 cm (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 (Fine-loamy, siliceous, semiactive, thermic Aeric Paleaquults), Goldsboro (Fine-loamy, siliceous, subactive, thermic Aquic Paleudults) and Wagram (Loamy, kaolinitic, thermic Arenic Kandiudults) (Figure 2-8). Because of its position on the landscape, the Norfolk soil series is considered a deep (>150 cm), well-drained soil with moderate permeability. Historically, the Norfolk soil series is considered one of the most productive soils for crop production in North Carolina, especially for flue-cured tobacco.

Figure 2-8. Aerial photo of the Whiteville Station showing the dominant soil series (Source: Aull et al., 1978b.)

2.3.3 Assessment of Historic Impacts of Land Use

As with the Oxford station, the primary emphasis at 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 exist regarding field experiments and sources and fertilization, the extent and accuracy of these records is not known. Current crop production field for the Whiteville station are shown in Figure 2-9.

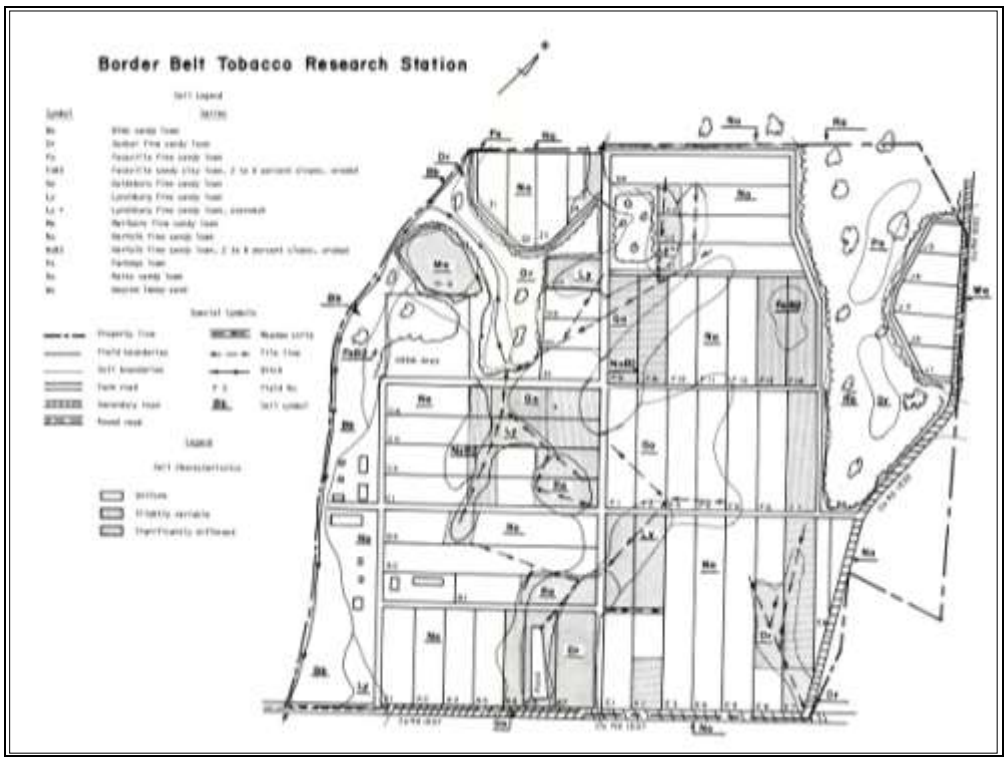

Figure 2-9. Map of the Whiteville Station showing field units and related soil series (Source: Aull et al., 1978b.)

2.4 **Study Design**

2.4.1 Layout of Test Plots

The standard row length for a tobacco research plot at both research stations is ~ 27.5 meter (90 ft.). Typically the entire 90-foot length is planted with subsequent fertilizer applications. Then an alley is placed by removing plants to provide two 13. 7 meter (45 ft.) long sections.

Three treatments were imposed at each site: a tobacco sidedressed with Chilean nitrate fertilizer (Treatment 1); a non-planted area fertilized with Chilean nitrate

fertilizer (Treatment 2); and tobacco without Chilean nitrate fertilizer (Treatment 3-Control). Figure 2-10 depicts the layout of treatments at the Oxford station and Whiteville station. At both sites, Treatments 1 and 3 consisted of four plots for each treatment. Treatment 2 at both sites consisted of only two plots. The layout for Treatment 1 and 3 is shown in Figure 2-11. Each plot 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. Two guard rows will serve two adjacent plots. Plants mid-way down the rows were removed to provide an alleyway for access within each plot.

All 4 plots were adjacent to each other, the number of rows of tobacco planted to yield 4 plots with 4 treated rows of tobacco per plot is 26. With 1.22 meter (48-inch) centers and 2.75 meter (90-ft) long rows, the minimum area occupied by one treatment was 870 square meters (m^2) (9,360 ft^2). For a minimum of 2 treatments (control and historical side dress rate of Chilean nitrate fertilizer), the total area required was 1740 m^2 (18,720 ft^2). It was equivalent to ~ 0.174 hectare (~0.43 acre) of tobacco, with each treatment occupied a total of ~0.085 hectare (~0.21 acre).

Within the given plot, the total area available for sampling was corrected for edge effects, especially at end of rows and near the alleyway in center of the plot. A 1.5 meter (5-ft) buffer was allowed for edge effects, thus reducing the effective length of each row to 21.5 meter (70 ft), with an alleyway in the middle of each plot. This translates to an effective study area in each plot of 104 m^2 (1,120 ft^2). Each plot composed of two sub-plots each with an effective study area of 52 m^2 (560 ft^2). For one treatment with 4 plots, the total effective study area was 416 m^2 (4,480 ft^2) or 0.04 hectare (0.1 acre).

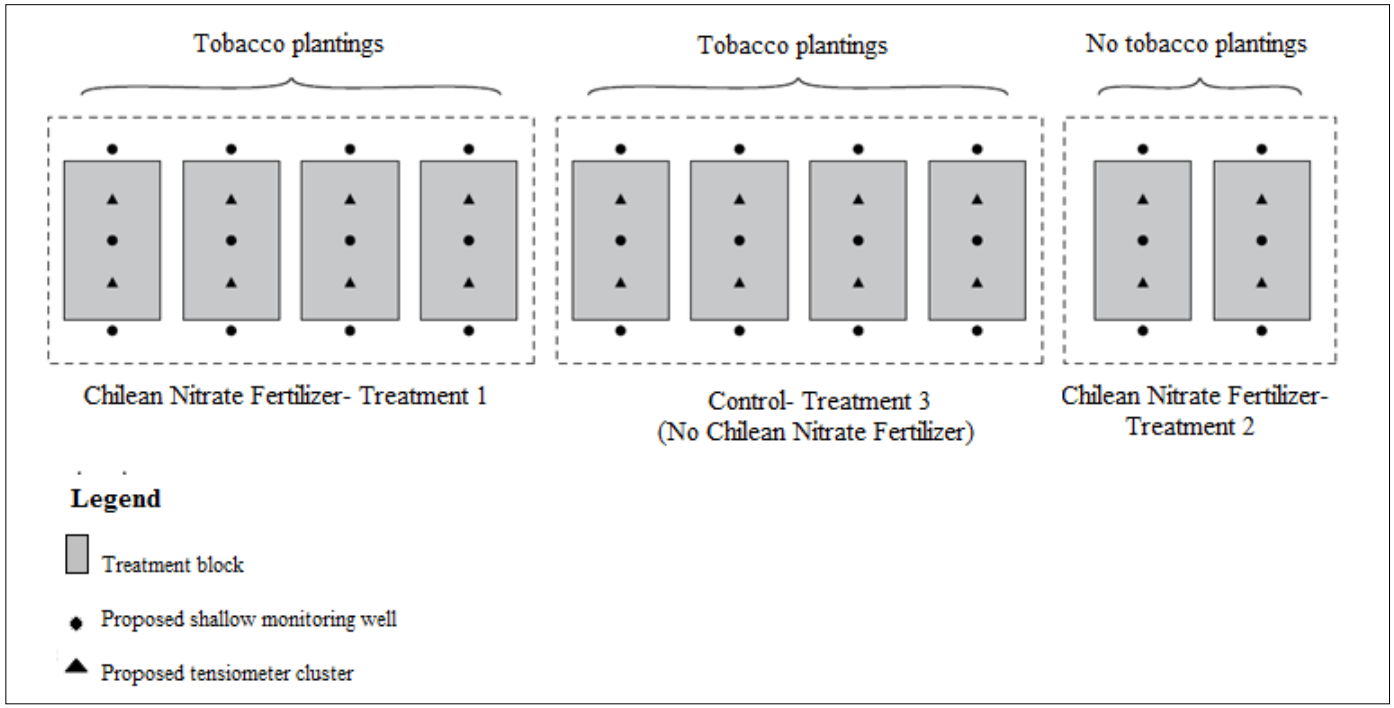

Figure 2-10. Layout of Treatments at the Oxford and Whiteville Stations.

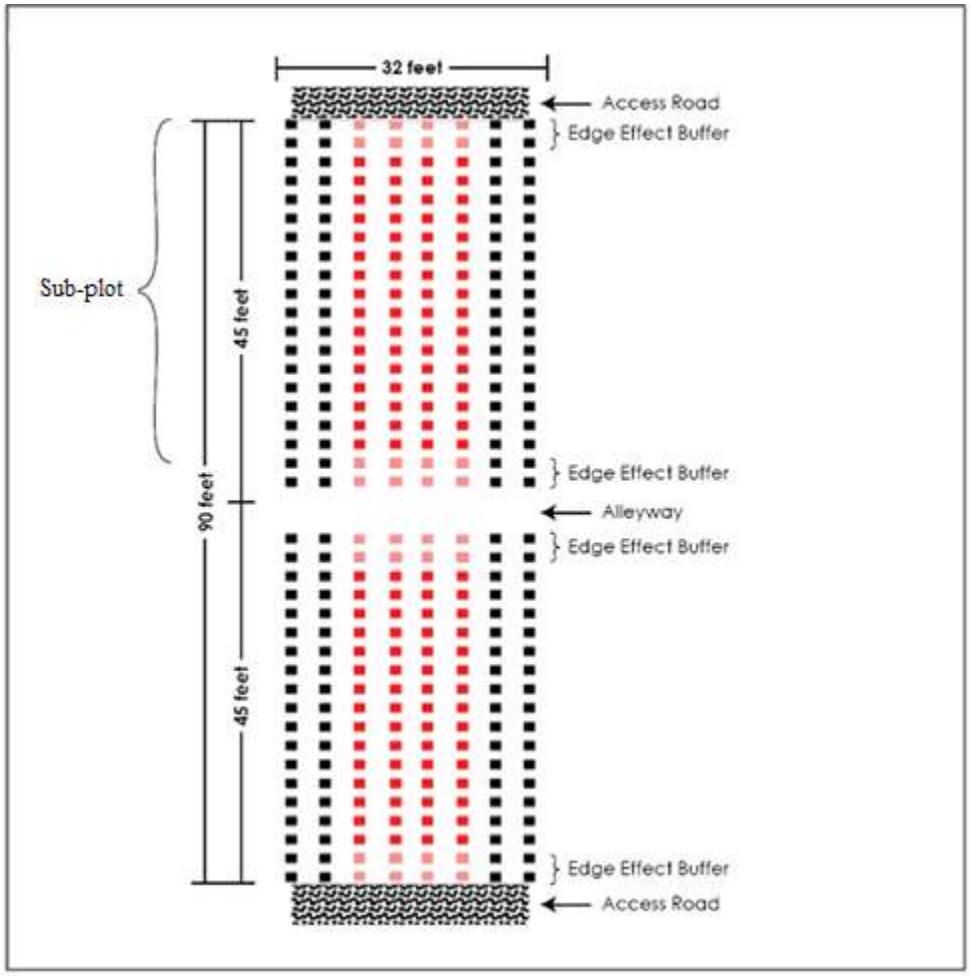

Figure 2-11. Layout of Single Plot for Treatments 1 and 3.

2.5 **Material and Methods (Field activities)**

2.5.1 Construction and Installation of Wells

Groundwater monitoring wells were installed at both sites to allow sampling of the shallow and deep (Oxford station only) groundwater and to estimate the gradient in the groundwater zones across the treatment blocks.

2.5.2 Oxford Station

Shallow groundwater monitoring wells were installed at the Oxford station in April, 2009. Initially, a subset of the thirty shallow wells planned for the site were installed along the up-gradient and down-gradient edges of treatment blocks (Figure 2-10) in order to assess whether shallow groundwater (i.e., < 1.5 meter (5 ft) below ground surface [m 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. The wells were installed by NCSU using hand augers or a mechanical probe truck as necessary. Wells were constructed using 5 cm (2-inch) diameter Schedule 40 polyvinyl chloride (PVC) screen (#10 slot; 0.25 mm (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. The well risers for each shallow well were allowed to extend approximately 3-5 cm (1-2 inches) above ground and were capped with a PVC slip-cap.

Six deep groundwater monitoring wells were installed at the station in May, 2009 along the upgradient (wells P3DA, P6DA and P9DA) and downgradient (wells

P3DB, P6DB and P9DB) edges of treatment blocks P3, P6 and P9. Concern for potential damage to the bedded rows prevented the installation of deep wells within treatment areas. Under the direction of Geosyntec, boreholes for the deep wells were drilled to a maximum depth of 8.2 m bgs (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 5 cm (2-inch) diameter Schedule 40 PVC screen (#10 slot) and Schedule 40 PVC riser. The screened interval for each well measured 1.5 m (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 30-60 cm (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 60-90 cm (2-3 ft) in thickness was placed on top of the packed sand, and the remainder of the annulus was backfilled with grout to a depth of approximately 30 m bgs (1 ft bgs). The well risers for each deep well were allowed to extend approximately 30-60 cm (1-2 inches) above ground and were capped with a water tight endcap (J-plug). A length of 10 cm (4-inch) PVC pipe was installed around the well riser for protection, and was anchored in the ground for support. Each 4-inch PVC pipe was then capped with a PVC slip-cap. Well construction details are summarized in Table 2-1. Following installation, the deep wells were developed by standard surging and purging methods.

Soil lithology beneath select treatment blocks (provided by drilling company under the direction of Geosyntec).

Treatment block	Soil Lithology
P3	• consisted of brown to red-brown, sandy clay grading to clay to a depth of approximately 3 m bgs (10 ft bgs). Beneath this was a 3 m (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 6 to 7.5 m bgs (20-25 ft bgs) (maximum depth cored).
P6	• consisted of brown, clayey silt with some sand to a depth of approximately 1.5 m bgs (5 ft bgs). This is underlain by a 4.6 m (15-ft) layer of tan to light brown, silty clay with a trace of rock fragments. A weathered rock layer was encountered from 6 to 7.5 m bgs (20-25 ft bgs) (maximum depth cored).
P9	• consisted of a dark brown to tan, sandy/silty clay to a depth of approximately 1.5 m bgs (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 1.5 to 2.9 m bgs (5-9.5 ft bgs) (maximum depth cored).

Table 2-1. Summary of well construction details

Research Station	Well ID	Installation Date	Drilling Method	Total Depth (m bgs)	Top of Screen (m bgs)	Bottom of Screen (m bgs)	Borehole Diameter (cm)	PVC Screen/Casing Diameter (cm)	PVC Screen Slot Size (mm)
Oxford	P3DA	5/12/ 2009	HSA	8.3	6.7	8.3	20	5	0.25
	P3DB	5/14/ 2009	HSA	7.6	5.3	6.9	20	5	0.25
	P6DA	5/12/ 2009	HSA	5.9	4.4	5.9	20	5	0.25
	P6DB	5/13/ 2009	HSA	6.4	4.9	6.4	20	5	0.25
	P9DA	5/12/ 2009	HSA	5.3	3.8	5.3	20	5	0.25
	P9DB	5/13/ 2009	HSA	3	4.5	3	20	5	0.25
Whiteville	11A	Third week of March, 2009	HA/MPT	2	1.1	1.7	20	5	0.25
	11B		HA/MPT	2	1.1	1.7	20	5	0.25
	12A		HA/MPT	2	1.0	1.7	20	5	0.25
	12B		HA/MPT	2	1.6	1.7	20	5	0.25
	13A		HA/MPT	2	1.2	1.7	20	5	0.25
	13B		HA/MPT	2	1.1	1.7	20	5	0.25

Notes:

HSA – Hollow Stem Augers, HA/MPT- hand augers or mechanical probe truck

m bgs – meter below ground surface

2.5.3 Whiteville Station

Shallow monitoring wells were installed at the Whiteville station in March and April, 2009. Initially, a subset of the shallow wells planned for the site were installed along the up-gradient and down-gradient edges of treatment blocks (Figure 2-10) in order to assess whether shallow groundwater (i.e., < 1.5 m 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 were installed in a drainage area down gradient of the experiments where shallow groundwater was expected. 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 a mechanical probe truck as necessary. The wells were constructed using 5 cm (2-inch) diameter Schedule 40 PVC screen (#10 slot; 0.25 mm (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. The well risers for each of the on-field wells were allowed to extend approximately 3-5 cm (1-2) inches above ground, and were capped with a PVC slip-cap. The well risers for each of the off-field wells were allowed to extend approximately 90 cm (3 ft) above ground and were capped with a PVC slip-cap. Well construction details are summarized in Table 2-1.

2.5.4 Installation of Tensiometers

Soil tensiometers at several depths below the root zone of the tobacco were used to measure the gradient in soil water potential and therefore the movement of soil water under the crop throughout the growing season.

Soil tensiometers were installed at the Whiteville and Oxford stations in April and May, 2009 respectively, following tobacco transplanting and application of the fertilizer sidedress treatment (see Sections 2.5.8 and 2.5.9). Construction of the soil tensiometers was similar to those used by Young and Sisson (2002). Tensiometer bodies were constructed from standard ½ inch Schedule 80 PVC tubing cut to a length 15 cm greater than the desired measurement depth. The tensiometer body was fit at one end with sight tubing (3/16 x 7/16 inch clear PVC tubing) exposed to a length of 6.35 cm and fixed with standard PVC cement. The other end of the tensiometer body was fit with a porous ceramic cup and fixed with epoxy. Prior to field installation, tensiometers were tested to ensure that a potential >80 kilopascals (kPa) could be maintained for at least 24 hours.

The completed tensiometers were installed in the field by preparing a hole, similar in diameter to the tensiometers, with a driving rod and a hand auger. Water was added to the bottom of the hole to create a slurry before the tensiometer was inserted. Tensiometers were filled with de-aired water, and then allowed to stand open while the ceramic cup at the other end of the PVC tubing became saturated. The tensiometers were then refilled with de-aired water and the clear site-tubing sealed with a septum stopper.

The soil tensiometers were installed in groups of three in the center of the bedded row, within the same row approximately 6-7 m (22 inches) apart in between tobacco plants. Target depths were 30, 60 and 90 cm below ground surface. Two clusters were installed per treatment block (one cluster within each sub-block) for a total of 8 clusters per treatment, or n=8 replicates for each depth in each treatment. Following installation,

the location of each soil tensiometer was referenced using global positioning system (GPS) hardware and software.

A tensiometer was used to read the soil water potential (Marthaler et al., 1983). The tensiometer was calibrated with a mercury manometer prior to field use. De-aired water was added to the tensiometers to maintain the proper water level within the tensiometer as determined through visual observation through the site tubing. A PVC cap was placed over each tensiometer to provide thermal protection until the tobacco canopy closed (Butters and Cardon, 1998).

2.5.5 Baseline Soil and Groundwater Sampling

Prior to planting and fertilizer application, soil and groundwater samples were collected from both research stations to establish baseline concentrations. A summary of the sample types, locations, and number of samples collected during the baseline sampling event is provided in Table 2-2. Table 2-3 provides a summary of the analytical methods, container sizes and types, preservation methods, and sample holding times for the parameters analyzed.

2.5.6 Soil Sampling

A combination of hand augers and a soil probe truck were used to sample soil with depth. A hand auger was used to sample the top 50 cm of the soil across three depth increments: 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 to the depth of 200 cm 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 Environmental and Agricultural Tests Service [EATS], Department of Soil Science, NC State University, Raleigh, NC; www.soil.ncsu/services/asl/) or shipment (for samples analyzed by Columbia Analytical Services [CAS]; www.caslab.com) to the laboratory. No attempt was made to cool the soil samples due to the volumes of soil collected.

Table 2-2. Total number and types of samples collected.

Matrix	Parameter	Sample Frequency	Total Number of Sample Events	Number of Sample Locations	Number of Depths	Total Samples Per Station[1]
Tensiometers	Water potential	Baseline + every 2 weeks for 16 weeks	9	20	3	540
Off-field Shallow groundwater (Whiteville)	Dissolved perchlorate, nitrate, pH, redox, dissolved oxygen	Baseline + every 2 weeks for 20 weeks	11	6	1	66
Deep groundwater (Oxford)	Dissolved perchlorate, nitrate, pH, redox, dissolved oxygen	Baseline + every 2 weeks for 20 weeks	11	6	1	66
Soil cores	Water extractable perchlorate, nitrate	Baseline	1	10[3]	6	60
	Water extractable perchlorate, nitrate	every 2 weeks for 16 weeks during growth phase	8	10[3]	5	400
	Water extractable perchlorate, nitrate	Post-harvest	1	10[3]	2	20
	Bulk density	Post-harvest	1	10	7	140
Plant tissue	Extractable perchlorate,nitrate	Every 2 weeks for 16 weeks during growth phase + 3 separate harvests	11	8[3]	N/A	88

Notes:

1 – Types of sample collected i.e. not including quality-assurance/quality-control (QA/QC) samples.

3 – Projected numbers of composite samples for each location.

Table 2-3. 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	ASL	20–50 mL	US EPA Method 314.0	3 µg/L	glass	Sulfuric acid to pH<2, cool to 4°C	> 28 days
	Nitrate-N			4500-NO_3	0.02 mg NO_3-N/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	US EPA Method 6850	0.2 µg/L	125 mL, plastic	Cool to 4°C	28 days
Soil	Perchlorate	ASL	100 g	US EPA Method 314.0	0.03 mg/kg	Plastic bag/bottle/ aluminum rings	Air dry and store at room temperature	> 28 days
	Nitrate-N			4500-NO_3	0.2 mg NO_3-N/kg			
	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	ASL	10-50 g	Ellington & Evans, 2000	3 mg/kg	glass	Cool to 4°C; freeze and freeze-dry; store at 4°C	> 28 days

Table 2-3 Continued

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 were flagged.

°C – Degrees Celsius
ASL - Analytical Service Laboratory, Department of Soil Science, North Carolina State University
CAS - Columbia Analytical Services, Rochester, NY
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
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 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.

2.5.7 Groundwater Sampling

Due to the absence of shallow groundwater at both stations, groundwater samples could only be collected from the off-field shallow monitoring wells at the Whiteville station, and from the deep monitoring wells at the Oxford station.

Prior to collecting groundwater samples for chemical analysis, the stagnant water in the well casing was pumped empty by inserting an appropriate length of acrylic tubing and applying vacuum. 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; www.ysi.com) or individual ORP (Hanna 9025; www.hannainst.com) 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 sample containers. After sampling, sample containers were placed in ice chests with ice packs and 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 (pH < 2) and then stored at 4 (ºC) until analysis.

2.5.8 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.

Tobacco transplanting at the Whiteville and Oxford stations occurred on April 20 and May 13, 2009, respectively, with populations of about 14,800 plants per hectare (6,000 plants per acre) using 56 cm (22 inch) spacing between plants (Figure 2-12). During transplanting, the top 20- 25 cm (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 2-13).

Figure 2-12. Tobacco transplanting into prepared beds.
(Photo by Scott King, Dept. of Soil Science, NCSU)

Figure 2-13. Transplanted tobacco with typical spacing of 22 inches between plants.
(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 4^{th} 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 has reached heights of between 1.2 to 1.8 m (4-6 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.

Following side-dressing with nitrogen, subsequent tillage operations are conducted primarily to control weeds and promote soil aeration until the crop reaches heights of 0.5 m (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.

2.5.9 Fertilizer Application

Fertilizer application is typically directed at achieving between 67 to 101 kg of nitrogen (N) per hectare (60-90 lbs N/acre), depending on soil texture. In sandy soils such as at the Whiteville station, the target rate is 101 kg N/ha (90 lbs N/acre). The rate is closer to 67 to 79 kg N/ha (60-70 lbs N/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 application 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.

2.5.10 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 ~ 47 kg of N per hectare (~ 42 lbs N/acre) at the Whiteville station and ~ 45 kg of N per hectare (~ 40 lbs N/acre) at the Oxford station. At both stations the fertilizer was knifed into the soil 10 cm (4 inches) to the side and 10 cm (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 15 to 20 cm (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 (Goetjaga et al., 1989).

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

2.5.11 Nitrogen-Only Sidedress

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 Treatments 1 and 2 at the Whiteville station approximately 6 weeks following transplanting, at a rate of ~ 42 kg of N per hectare (~ 37 lbs N/acre). At the Oxford station, the Chilean nitrate fertilizer was applied to Treatments 1 and 2 approximately 5 weeks following transplanting, and was applied at a rate of ~ 36 kg of N per hectare (~ 32 lbs N/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 Treatment 3 (control) at either station. However, Treatment 3 at Oxford and Whiteville stations were sidedressed using calcium nitrate at a rate of 35 kg of N per hectare (31 lbs N/acre) and ammonium nitrate at a rate of 36-38 kg of N per hectare (32-34 lbs N/acre), respectively.

2.5.12 Plant Sampling (Post-Planting/Growth Phase)

The tobacco plants were sampled every other week for 16 weeks following application of the Chilean nitrate fertilizer sidedress 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 randomly 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 stored in air-tight glass containers in the field. After sampling, the glass containers were placed in ice chests with ice packs to cool 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.

2.5.13 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.

2.5.14 Groundwater Sampling

Following application of the Chilean 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 sample types, locations, and number of samples collected is provided in Table 2-2.

Unfortunately, most of the shallow wells at the Oxford station were damaged during farming activities in June, 2009. However, because of the absence of shallow groundwater at the site it was not practical to replace these wells. Instead, the deep monitoring wells were used to collect groundwater samples during subsequent sampling events. The absence of shallow groundwater at the Whiteville station prevented the collection of samples from the in-field shallow monitoring wells. Thus, groundwater samples were only collected from the off-field shallow monitoring wells. Groundwater samples from both stations were collected using the same sampling protocols that were used during the baseline sampling event (see Section 2.5.7).

2.5.15 Soil Sampling (Post-Planting/Growth Phase)

Following application of the Chilean 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 bedded row. Samples were collected by coring down through the center of a bed 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 2.5.6).

2.5.16 Post-Harvest

Following tobacco harvesting, 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.

2.6 **Analytical Methods**

The analytical methods used to analyze groundwater and soil samples are presented in Table 3-3.

2.6.1 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 nanograms per gram (18 parts per billion [ppb] 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.

2.6.2 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). 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.

2.6.3 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.

2.7 **Results and Discussion**

2.7.1 Perchlorate Content of Potassium Nitrate Sidedress

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 (Fig. 2-14). 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 (Fig. 2-15). 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.

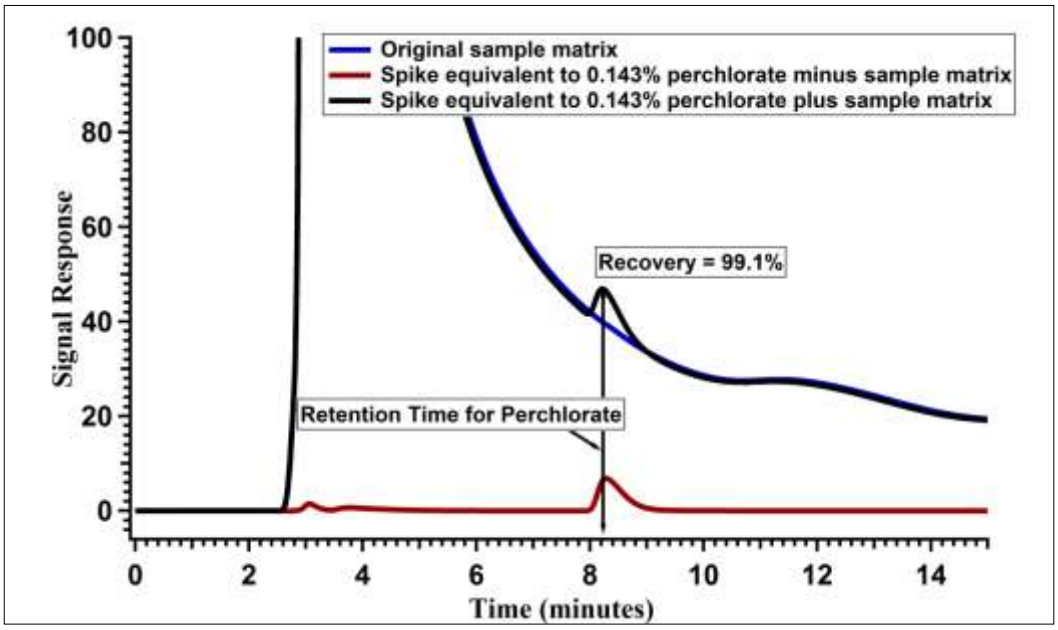

Figure 2-14. Example of chromatogram obtained in the analysis of potassium nitrate fertilizer for perchlorate using ion chromatography. (Sample loop 50 microliters, AG-16+AS-16 columns, 40 mM NaOH, 1 mL/min, conductivity detection after suppression with ASRS-300 in recycle mode).

Samples of the potassium nitrate fertilizer were sent to Columbia Analytical Services in Rochester, New York, 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 sidedress application of nitrogen following transplanting of tobacco resulted in ~ 100 x less perchlorate 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 potential contamination of underlying groundwaters 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.

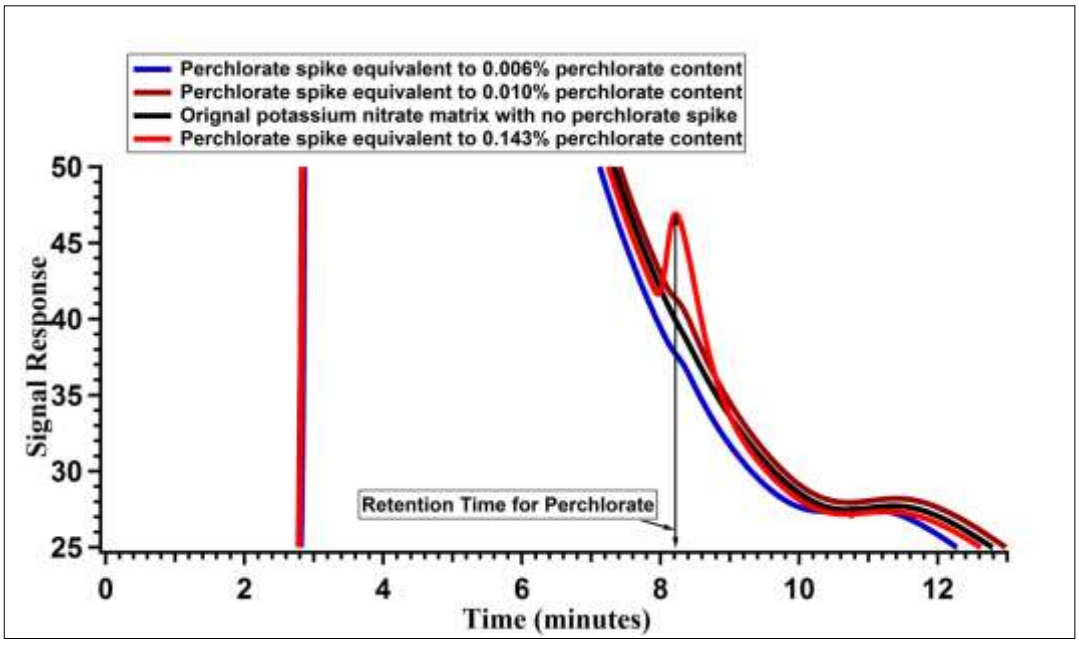

Figure 2-15. Example of chromatogram obtained in the analysis of spiked potassium nitrate fertilizer for perchlorate using ion chromatography. (Sample loop 50 microliters, AG-16+AS-16 columns, 40 mM NaOH, 1 mL/min, conductivity detection after suppression with ASRS-300 in recycle mode).

2.7.2 Oxford Tobacco Research Station

Plot locations for the experiment were determined in early 2009 in consultation with the research station manager. Initial soil sampling to a depth of 100+ cm was conducted in the early spring (March 29, 2009) to determine background levels of soil extractable nitrate and ammonium as well as survey for the possible presence of

perchlorate. Tobacco seedlings (flue-cured variety K326) were transplanted to the research plots on May 11 and 12, 2009. On May 26, 2009, the complete fertilizer with the formulation of 8-8-24 was applied in each bed adjacent to the tobacco plants; the target application rate was 45.6 kg N/ha (40 lbs N/acre), or 1.2 kg N/plot (2.64 lbs N/plot). Unlike other agricultural row crops, it is not common practice to broadcast fertilizer to the entire surface area of the field at planting, or transplanting. Instead, fertilizer is only applied twice via a sidedress application in the bed along side of the tobacco plant. The objective is to place both applications of fertilizer in the same position in the soil relative to the tobacco plant. Thus it is reasonable to assume that following application of the fertilizer, the majority of nitrate (or perchlorate) would remain in the volume of soil composing the bed, and would move vertically into the underlying soil over time.

One June 15, 2009, the sidedress of N-containing fertilizer was applied. The potassium nitrate source material was applied at a rate of 36.5 kg N/ha (32 lbs N/acre) or 0.96 kg N/plot (2.12 lbs N/plot) to plots 1-4 (Treatment 1), which were designated as the perchlorate treatment plots. The non-perchlorate treated plots 5-8 (Treatment 3) were sidedressed using calcium nitrate at a rate of 35.3 kg N/ha (31 lbs N/acre) or 0.93 kg N/plot (2.05 lbs N/plot). Plots 9-10 (Treatment 2) were treated exactly the same as plots 1-4, except these plots were kept bare (no tobacco plants). Plots 9-10 were sprayed several times during the season with a herbicide to prevent weed growth. Soil and plant sampling started following the second sidedress with fertilizer. A visual representation of the development of the tobacco crop at the Oxford Research Station from May to October 2009 is given in Figures 2-16 to 2-20.

Figure 2-16. May 29, 2009, Oxford Research Station. Transplanted tobacco.
(Photo by Scott King, Dept. of Soil Science, NCSU)

Figure 2-17. June 19, 2009, Oxford Research Station. Tobacco 4 days following sidedress application of either potassium nitrate or calcium nitrate.
(Photo by Scott King, Dept. of Soil Science, NCSU)

Figure 2-18. July 2, 2009. Oxford Research Station. Tobacco 2+ weeks following sidedress application of either potassium nitrate or calcium nitrate.
(Photo by Scott King, Dept. of Soil Science, NCSU)

Figure 2-19. July 17, 2009, Oxford Research Station. Tobacco plants approximately 1 month following sidedress application of either potassium nitrate or calcium nitrate.
(Photo by Scott King, Dept. of Soil Science, NCSU)

Figure 2-20. August 14, 2009, Oxford Research Station. Tobacco plants approximately 2 months following sidedress application of either potassium nitrate or calcium nitrate, and 7 days before first harvest of lower leaves.
(Photo by Scott King, Dept. of Soil Science, NCSU)

2.7.2.1 Intensity and Temporal Distribution of Rainfall

Rainfall amounts at the Oxford Research Station during the study were determined from the North Carolina State Climate Office's CRONOS weather station network (www.nc-climate.ncsu.edu/cronos/), which has one of its ECONet meteorological towers located at the station. The total amount of rainfall during the 6-month growing season was 473 mm (Table 2-4), the largest amount occurring in May and June (> 200 mm). 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 19

mm of irrigation water at the end of June. This was the only irrigation event for the entire study period recorded at the Oxford Research Station.

Table 2-4. Monthly rainfall amounts recorded at the Oxford Research Station for April thru September, 2009. (Source: NC State Climate Office CRONOS network).

Month	Rainfall Amount
	- mm -
April	32
May	90
June	126
July	73
August	64
September	88
Mean=	78.8
Total=	473

2.7.2.2 Soil Water Content and Bulk Density

An indirect measure of soil moisture content during the study was monitored using soil tensiometers. This device provides 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.

Clusters of tensiometers were installed in all of the plots to three depths. Because the tensiometers were installed in the beds of soil containing the tobacco plants, the plane of reference for the depth measurements is the top of the beds, not the original soil surface. Typically the beds 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 beds was ~ 15 cm below the original soil surface. The reported depth measurements therefore are referenced to the top of the beds 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 bed, 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 beds. The tensiometers were in place for the second sidedress application of either potassium nitrate or calcium nitrate.

Soil tensiometer readings in Plot 9-10 (Treatment 2), bare soil, as a function of date and soil depth are shown in Figure 2-21. Initial readings in early June reflect high soil water content under well drained conditions (~ -0.1 Bar). This status did not change despite a large rain event during the week of June 18th. However, the surface 15 cm (soil bed) began to dry noticeably following the week of June 18th. More rainfall during the weeks of July 10th and 17th appear to maintain the bed water content, but then the soil continued to dry till late September.

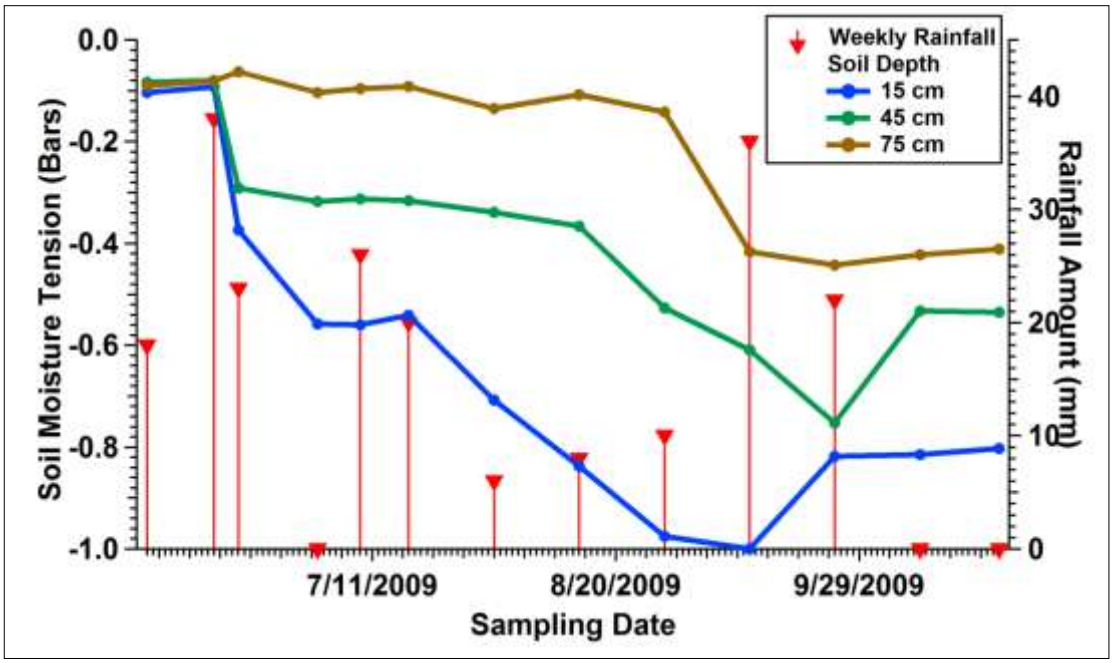

Figure 2-21. Mean soil tensiometer readings for Treatment 2 - Bare soil, Oxford Research Station, as a function of date and soil depth together with weekly rainfall amounts. Soil depths are referenced to the top of the row beds.

The next soil depth increment (45 cm) appears to mimic the pattern at the 15 cm depth somewhat with a noticeable decline after August 1st. 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. Treatment 2 contained no plants, thus the increase in soil tension over time reflected 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 moved into the dry soil. A similar pattern in soil tension readings was observed in Plot 1-8 (Treatments 1 and 3), which contained tobacco as shown in Fig. 2-22.

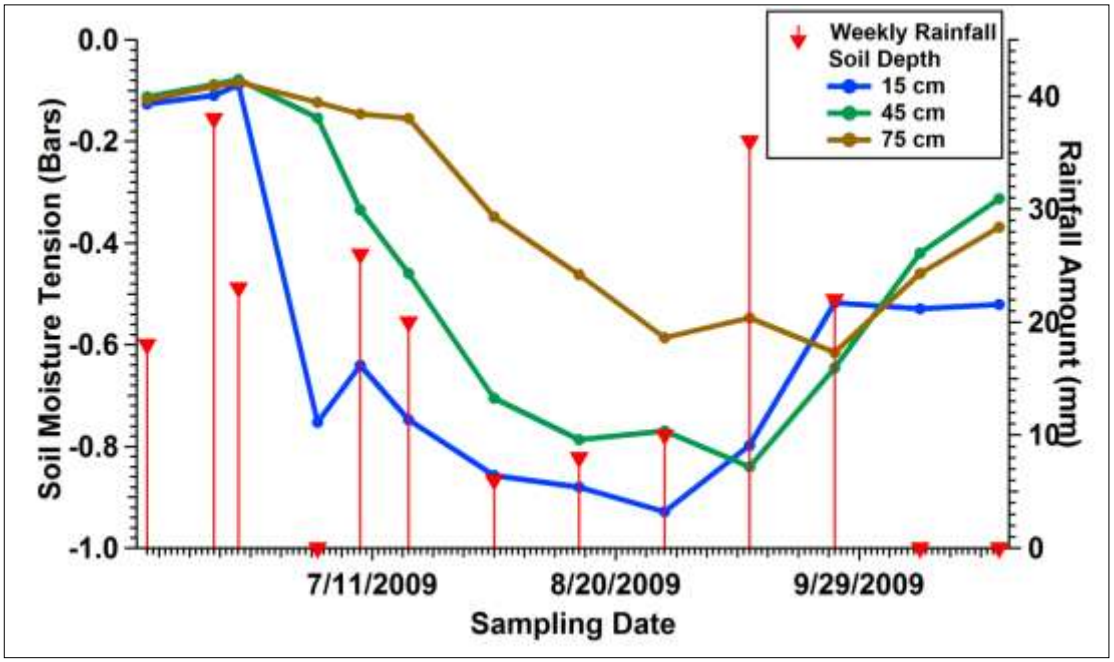

Figure 2-22. Mean soil tensiometer readings for Treatments 1 and 3, Oxford Research Station, as a function of date and soil depth together with weekly rainfall amounts. Soil depths are referenced to the top of the soil mounds.

With the presence of tobacco plants, the soil in Treatments 1 and 3 dried relatively faster than the bare soil, with the 75 cm depth beginning to show an increase in soil tension by the second week in July. Driest conditions were reached in early to mid-August across all three-soil depths monitored. As illustrated in Fig. 2-19 and 2-20, 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 September 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 became wet enough by September 25th that the second substantial rain event during this period begins to promote slight recovery of soil water content at the 75 cm depth relative to the top of the soil bed (Fig. 2-22).

Both Figs. 2-21 and 2-22 illustrate that despite the recorded rainfall during the summer of 2009 at the Oxford Research Station, the soil within the research plots began drying down 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 bed 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 possibly 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 Plots 1-10 (treatments 1-3). Samples were taken with reference to the top of the soil bed. Soil bulk density values as a function of plot and soil depth relative to the top of the soil bed are shown in Table 2-5. The vertical profile of soil bulk density as a function of soil depth relative to the top of the soil bed is shown in Fig. 2-23.

Table 2-5. Soil bulk density as a function of plot and soil depth relative to the top of the soil bed, and mean soil porosity as a function of soil depth. Oxford Research Station.

Depth range	Plot 1	Plot 2	Plot 3	Plot 4	Plot 5	Plot 6	Plot 7	Plot 8	Plot 9	Plot 10	Mean	Mean Soil Porosity
cm	---------- g/cubic centimeter----------											----%---
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 plots. Lowest soil bulk density was measured in the soil bed, which is consistent with being formed by excavation of the surrounding soil earlier in the season. Maximum soil density was observed at a depth of approximately 30 cm below the original surface of the soil (Fig. 2-23). 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 2-21 and 2-22. Thus the presence of this more dense soil layer would also act to restrict vertical transport of soil nitrate (and perchlorate) in these soils.

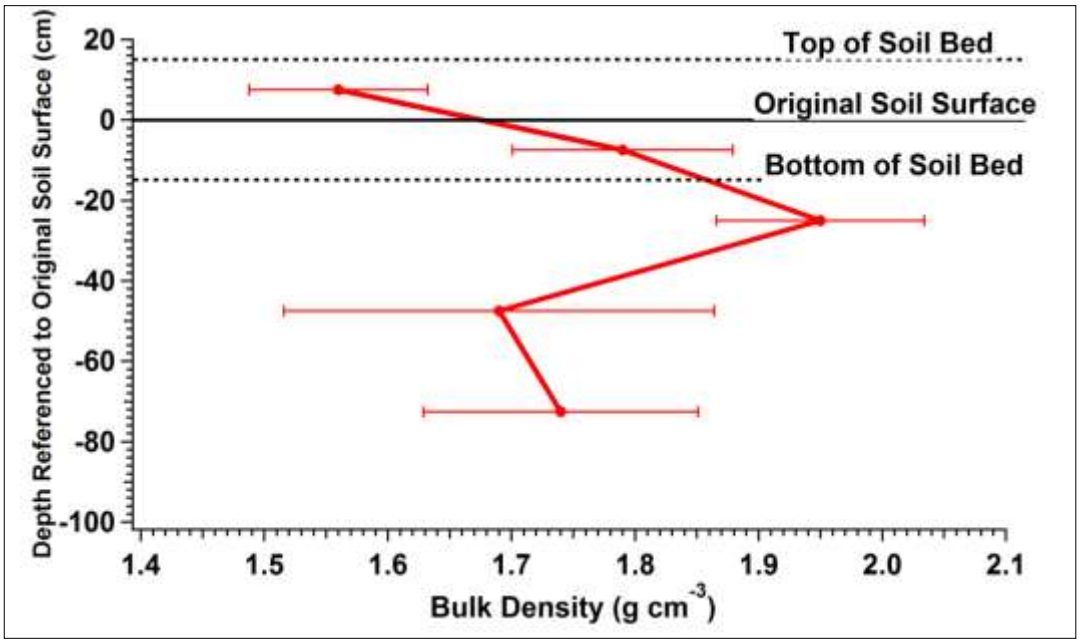

Figure 2-23. Mean soil bulk density and standard deviation for Plots 1-10 as a function of soil depth relative to the top of the soil bed at the Oxford Research Station.

Another indication of the presence of a plow-pan or at least a restrictive layer present in the soil at ~ the 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 Research Stations, 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).

2.7.2.3 Groundwater and Shallow Wells

Shallow groundwater wells were installed in the center of both ends of each plot on 16 April 2009. The wells consisted of PVC pipe with a slotted end section 15 cm (6 in) long. As noted in Table 2-5 and Fig. 2-23, a clay-rich dense layer was encountered at the Oxford Research site at ~ the 30 cm depth. Shallow wells were installed just above this restrictive clay layer, so as to intersect any water that might remain 'perched' on top

of the clay. Thus, while the actual final depth for the shallow wells varied slightly for any given plot, they averaged just 30 cm (~1 foot) deep. The top of each well pipe was cut close to the ground surface and sealed with an orange-painted cap so that farm equipment could more easily avoid running over and damaging the wells.

Unfortunately, these wells remained dry throughout the entire sampling process as no water detected perched above the shallow clay layer. This is consistent with the soil tension readings throughout the growing season as illustrated in Figs. 2-21 and 2-22. It appears that any rainfall on the site that may have quickly percolated through the upper more permeable soil (i.e. not already lost through surface runoff) may have moved laterally out of the plots once it encountered the restrictive clay layer at 30 cm. However, this would only take place if the soil above the restrictive layer reached near zero soil water tension. There is no indication this happened throughout much of the growing season given the consistent low readings in soil water tension at the 15 cm depth (Figs. 2-21 and 2-22), unless such lateral movement was restricted to the inter-rows.

Deeper monitoring wells were installed between May 11th and 14th 2009 by Parratt-Wolff, a professional well drilling company under the direction of a Geosyntec Consultant employee. The 6 deeper PVC wells were installed at each end of plots 3, 6, and 9, in the middle between the plot edges. The installation crew drilled down until they reached the regional rock layer (presumed bedrock) and set the wells at the top of this confining rock layer. Depth to this rock layer varied by plot from 3 m (10ft) to 8 m (27ft); the average depth for all three plots was 6 m (20 ft). Each well had a 1.5 m (5ft) screened end section at the bottom and was backfilled with 1.5 m of sand, then bentonite clay up to the 30 cm (1ft) below the soil surface, then finally capped with a 30 cm layer of concrete. Each well had a rubber compression plug to fully seal the well opening in addition to a hard plastic cap to protect it from accidental damage. Most of the wells filled with water within a day, and all contained water two weeks later when sampled.

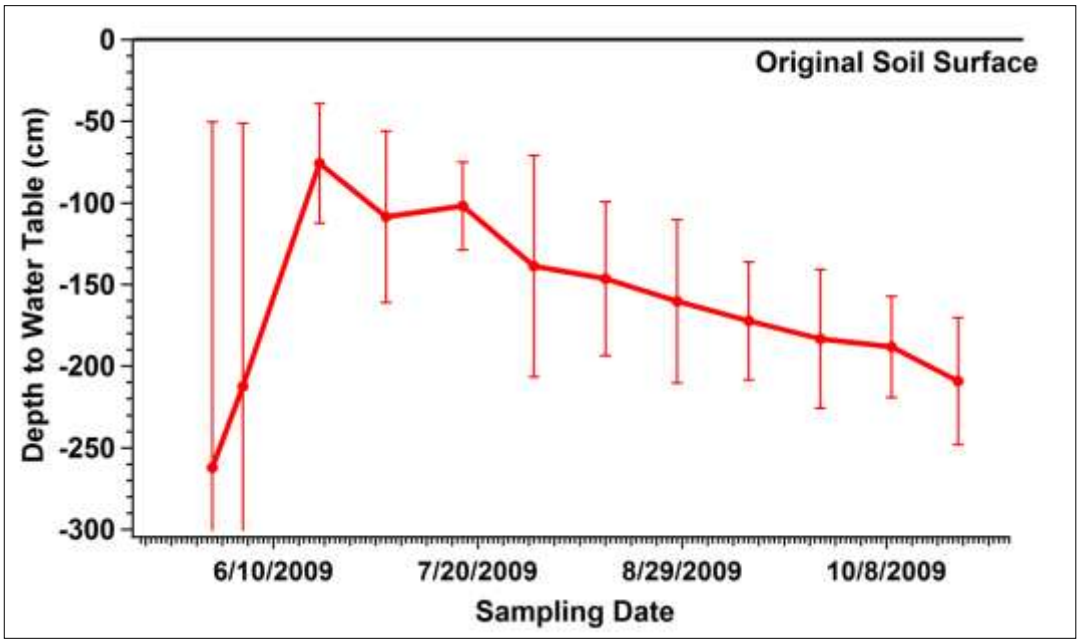

Figure 2-24. Average depth to groundwater as a function of date for wells installed at the ends of Plots 3, 6 and 9. Location: Oxford Research Station. Vertical lines represent one standard deviation.

Averaged across all the wells at the Oxford Research Station, 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 Figs. 2-21 and 2-22. The soil water tension readings indicate that the soil was not saturated but at near field capacity with enough distance between the surface of the groundwater 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. The rise in groundwater suggests some interactions with rainfall events, even given the presence of the dense clay layer at 30 cm. Whether water movement within the field (soil plots) occurred via potential large cracks in the underlying soil clay layer or presence of discontinuities in the soil clay layer 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 movement 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, i.e. relatively low in ionic strength and near neutral pH value.

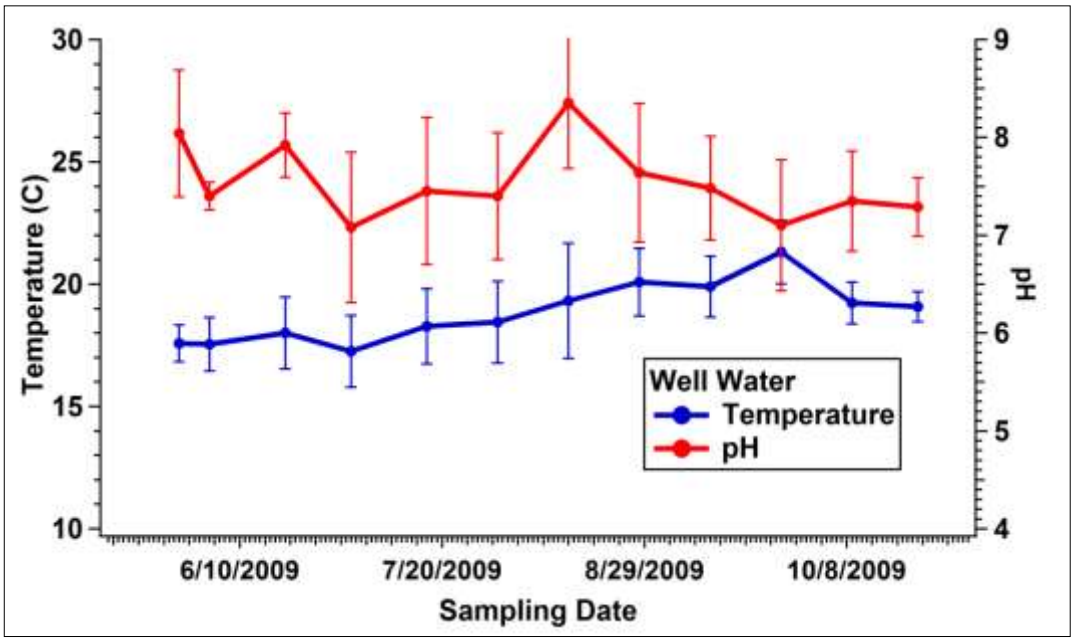

Figure 2-25. Average pH and temperature as a function of date for groundwater from wells installed at the ends of Plots 3, 6 and 9. Location: Oxford Research Station. Vertical lines represent one standard deviation.

Average groundwater temperature gradually increased throughout the summer from 18° C in the early spring to about 21° C by the late summer. This is 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 down to 19° C.

At Oxford Research Station, both redox potential and dissolved oxygen followed the same trend throughout the growing season (Fig. 2-26). 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, 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 depth.

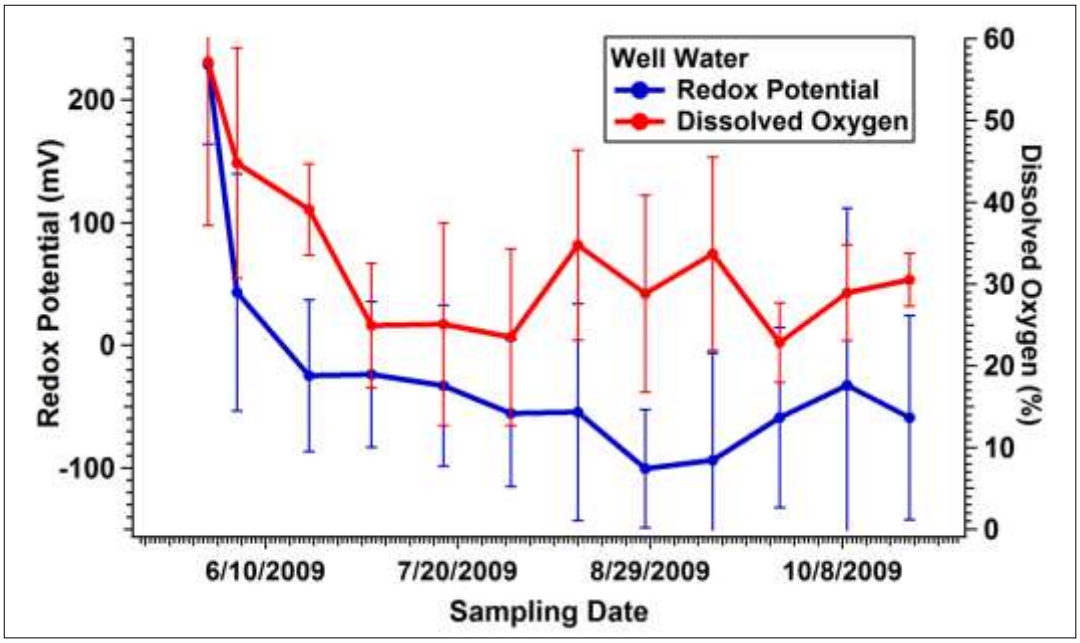

Figure 2-26. Average redox potential and dissolved oxygen content as a function of date for groundwater from wells installed at the ends of Plots 3, 6 and 9 at the Oxford Research Station. Vertical lines represent one standard deviation

Failure of the dissolved oxygen content to reach values of near zero suggests that nitrate may still be present in the groundwater, although the redox potential readings suggest 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 well water samples collected, although the presence of other anions in the well water samples was more variable than expected. Fig. 2-27 illustrates a typical result of for anion analyses (chloride, nitrate, phosphate and sulfate) of the well water samples.

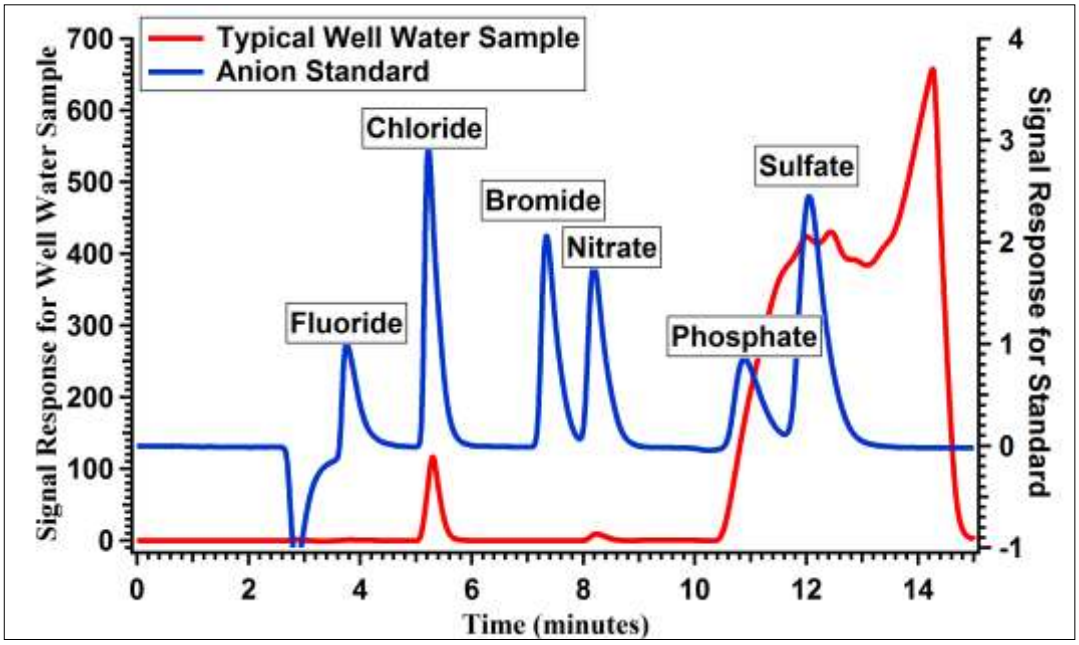

Figure 2-27. Standard anion analysis of a typical well water sample compared to an anion standard using ion chromatography. Oxford Research Station. (50 microliter sample loop, AG-22+AS-22 columns, 4.5 mM sodium carbonate+1.4 mM sodium bicarbonate, 1 mL/min, conductivity detection after suppression with an ARSR-300 in recycle mode, 50 mA).

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 Fig. 2-27 vary by almost a factor of 200, 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 Research Station and the Whiteville Research Station) had shapes similar to that illustrated in Fig. 2-27. The presence of phosphate/sulfate and other anions may be an artifact related to the installation of the wells, although only shallow groundwater wells were installed at the Whiteville Research Station by project staff. If the presence of such large amounts of phosphate/sulfate and other anions were due to well installation, it would have to arisen from a material common to the installation of the wells at both sites.

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; ppb = parts per billion), in part because the presence of relatively high salts or other anions required a reduction in 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 (Fig. 2-28).

As noted, no perchlorate was detected in the well water samples collected during the study using ion chromatography. A small subset of well water samples was sent to the Columbia Analytical Services Laboratory to check analyses of the well water samples by ion chromatography (Table 2-6). The stated detection limit for analyses by the Columbia Services Laboratory was 0.2 ppb (parts per billion = micrograms per liter). 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 2-6). 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 consistent with the elevated detection limit using ion chromatography due to the presence of relatively high concentrations of salts

in the well water samples. It is not known to what extent these salts may have impacted the analyses conducted by the Columbia Analytical Services Laboratory.

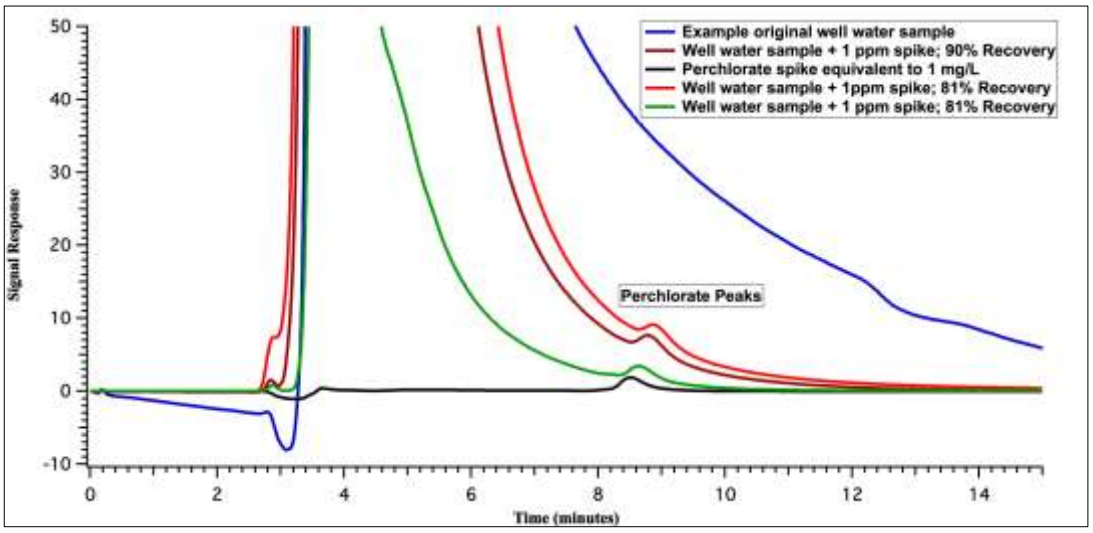

Figure 2-28. Example chromatogram and spiked recoveries for several well water samples for perchlorate. Oxford Research Station. (500 microliter sample loop, AG-16+As-16 columns, 40 mM NaOH, 1 mL/min, conductivity detection after suppression with a ARSR-300 in recycle mode, 100 mA).

Table 2-6. Results of well water sample analyses for perchlorate by Columbia Analytical Services Laboratory. Oxford Research Station.

SAMPLE	DATE	MATRIX	COMPOUND	MRL	Adjusted MRL	RESULT	FLAG
				---------- µg/L----------			
OX-P9DB	06/04/09	WATER	PERCHLORATE	0.2	0.20	0.20	U
OX-P3DB	06/19/09	WATER	PERCHLORATE	0.2	0.20	0.20	U
OX-P3DA	07/02/09	WATER	PERCHLORATE	0.2	0.20	1.4	
OX-P6DA	07/31/09	WATER	PERCHLORATE	0.2	0.20	0.20	U
OX-P9DA	08/28/09	WATER	PERCHLORATE	0.2	0.20	36	E
OX-P9DA-Dup	08/28/09	WATER	PERCHLORATE	0.2	2.0	32	D
OX-P6DA	09/25/09	WATER	PERCHLORATE	0.2	0.20	0.20	U
OX-P6DB	10/22/09	WATER	PERCHLORATE	0.2	0.20	0.20	U
OX-P3DB	10/22/09	WATER	PERCHLORATE	0.2	0.20	0.20	U
OX-P9DB	10/22/09	WATER	PERCHLORATE	0.2	0.20	0.20	U

U = indicates compound was analyzed for but not detected.

E = indicates that the sample concentration exceeded the calibration range

D = deep well

A = up-gradient position

B = down-gradient position

Only two of the groundwater wells were found to consistently contain nitrate (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^{-1}), or nitrate was detected at concentrations < 1 mg NO_3-N L^{-1} only during the first several sampling dates. For well OX-P3DA, the concentration of nitrate consistently detected was always low, averaging 0.3 mg NO_3-N L^{-1}, with the majority of observations being closer to 0.1 mg NO_3-N L^{-1}. The highest nitrate concentration observed for well OX-P3DA was 0.9 mg NO_3-N L^{-1} 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 NO3-N L^{-1}. The highest nitrate concentrations (~ 8 mg NO_3-N L^{-1}) 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^{-1}. There were no apparent temporal trends in the nitrate concentrations in either well OX-P3DA or OX-P9DA.

Unlike nitrate, chloride was found to be present in the groundwater from all 6 wells (Table 2-7). 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. Lack in temporal trends in chloride concentrations, as for the two wells with detectable nitrate, suggests that the groundwater was not directly influenced by the surface applied fertilizers used in this study. The consistency in the observed concentrations suggests that the groundwater being sampled is controlled by a local source in the immediate vicinity of each well.

Table 2-7. Chloride concentration in groundwater as a function of sampling date. Location: Oxford Research Station.

	Well Designation					
Date	OX-P3DA	OX-P3DB	OX-P6DA	OX-P6DB	OX-P9DA	OX-P9DB
	----------mg Cl L^{-1}----------					
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
%RSD=	3	15	5	16	6	7

2.7.2.4 Tobacco Production

Tobacco seedlings (variety K326) were originally grown in a greenhouse and transplanted to the field along 61 cm (~ 2 foot) intervals into prepared, raised beds about 30 cm (12 inches) high and 1.2m (4 feet) 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 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 sidedress of an all-nitrogen fertilizer is applied 225 kg/ha (200 lbs/ac) of 15.5-0-0, in the same position with regards to the plants as the complete fertilizer.

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 plots 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 Plots 1-8 are summarized in Table 2-8.

Table 2-8. Mass of harvested tobacco leaves from Plots 1-8. Results are for cured tobacco (moisture content 12-15%). Oxford Research Station.

Plot	Harvest 1 8/21/2009	Harvest 2 9/11/2009	Harvest 3 10/9/2009	Totals
	----------kg/ha----------			
	Nitrogen Source: Potassium Nitrate – Treatment 1			
Plot 1	665	1029	3345	5038
Plot 2	770	1020	3098	4888
Plot 3	885	1002	2616	4503
Plot 4	930	1107	2352	4390
Means	813	1040	2853	4705
	Nitrogen Source: Calcium Nitrate – Treatment 3			
Plot 5	720	982	3043	4745
Plot 6	819	1120	2961	4900
Plot 7	984	995	2746	4725
Plot 8	710	1099	3228	5037
Means	808	1049	2994	4852

The total mass of tobacco leaves produced appeared not to be influenced by the source of fertilizer used for the sidedress application (potassium nitrate versus calcium nitrate). The mean of the amount harvested from the sets of four plots per treatment were essentially identical for the first two harvests, with the mean removed from Plots 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.

2.7.2.5 Soil Chemical Analysis – Perchlorate

Pre-planting soil samples were obtained from Plots 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 plots. 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 Research Station location (Table 2-9). The samples were air-dried, processed as outlined in the Materials and Methods section and analyzed for extractable perchlorate.

Table 2-9. Date, depth interval, and number of soil samples collected during 2009 field experiment at the Oxford Research Station.

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

Extractable soil perchlorate was determined using EPA Protocol 314.0. The only modification was the use of a relatively dilute 0.001M $CaCl_2$ solution instead of deionized water as the extractant. The $CaCl_2$ solution was used to aid settling of fine soil clays during centrifugation and to facilitate filtering of the extract before analysis using ion chromatography. The desired detection limit for the analysis of the soil extracts was 3 micrograms perchlorate L^{-1} (ppb) perchlorate. Figure 2-29 illustrates the instrument response obtained in this study for the range 3 – 200 micrograms perchlorate L^{-1}. Figure 2-30 provides an expanded view of the 3 ppb perchlorate standard illustrating that the signal to noise range for the instrument easily exceeded 3:1 at this concentration.

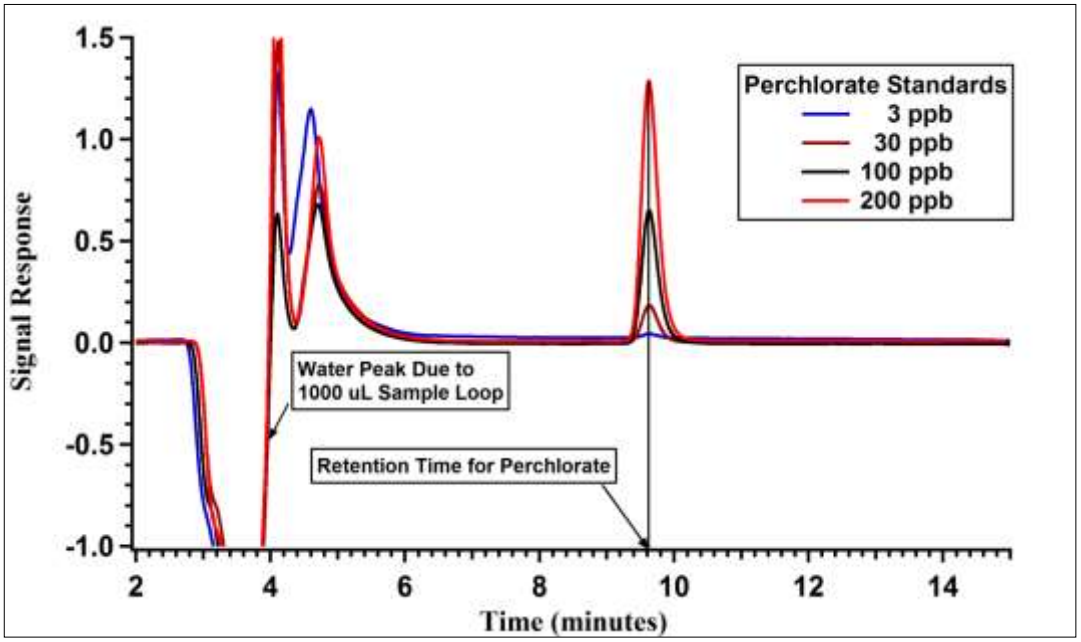

Figure 2-29. Example chromatograms for perchlorate standards in deionized water. (1000 microliter sample loop, AG-16+AS-16 columns, 40 mM NaOH, 1 mL/min, conductivity detection after suppression with a ARSR-300 in recycle mode, 100 mA).

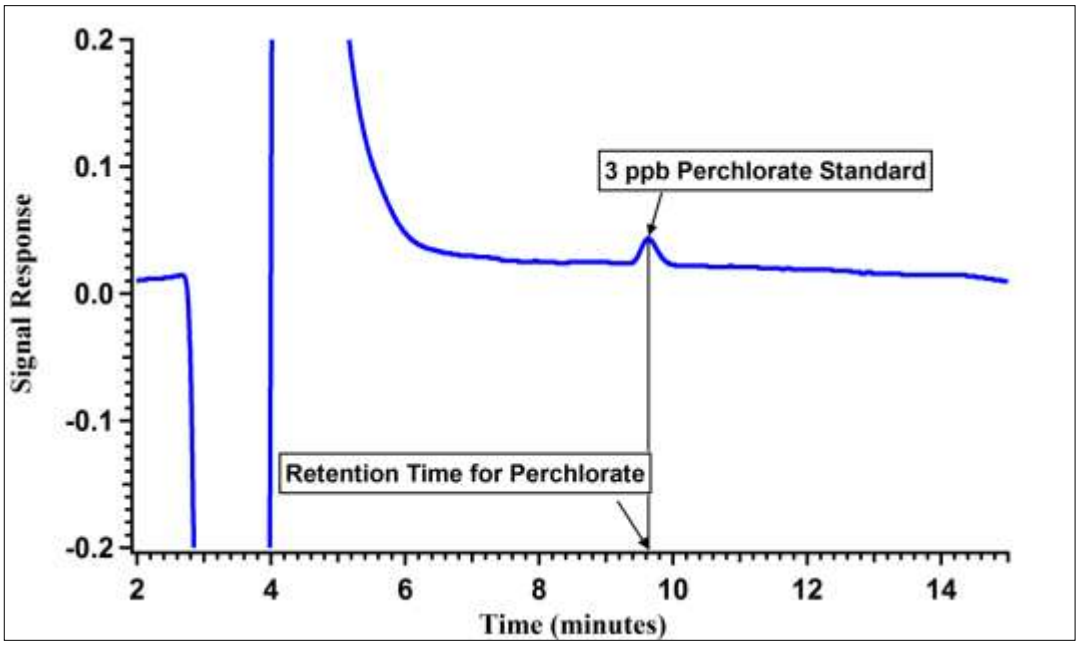

Figure 2-30. Expanded chromatogram for 3 micrograms perchlorate L^{-1} (ppb) standard illustrating signal to noise ratio > 3:1. (1000 microliter sample loop, AG-16+AS-16 columns, 40 mM NaOH, 1 mL/min, conductivity detection after suppression with a ARSR-300 in recycle mode, 100 mA).

Use of $CaCl_2$ for the soil extracting solution shifted the baseline of the resulting chromatograms but did not degrade the detection limit of the procedure. Portions of three chromatograms for three soils picked at random are illustrated in Fig. 2-31. A chromatogram for a 3 ppb perchlorate standard is also provided. Fig. 2-31 demonstrates that 3 ppb of perchlorate in the soil extracts would have been detected if perchlorate were indeed present at sufficient amounts in the original soil sample.

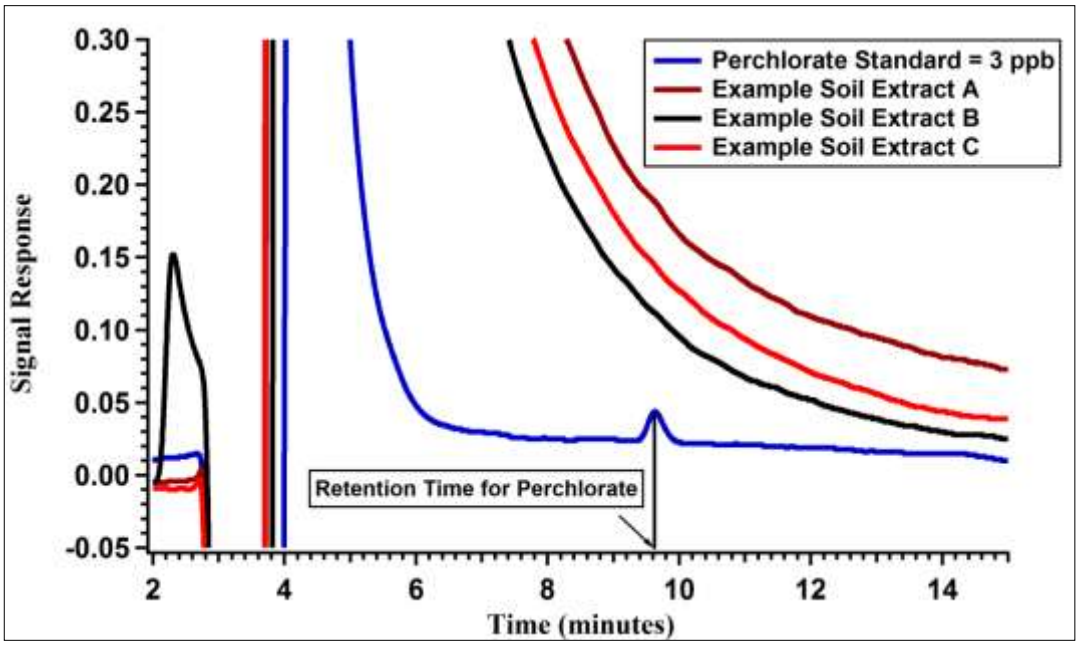

Figure 2-31. Portions of the chromatograms for three soil extracts (0.001 M $CaCl_2$) compared to the for 3 micrograms perchlorate L^{-1} (ppb) standard. (1000 microliter sample loop, AG-16+AS-16 columns, 40 mM NaOH, 1 mL/min, conductivity detection after suppression with a ARSR-300 in recycle mode, 100 mA).

Recovery of perchlorate in the $CaCl_2$ soil extracts was tested using spiked additions to yield and equivalent concentration of ~ 140 micrograms perchlorate L^{-1}. Figure 2-32 compares the original soil extracts to an equivalent perchlorate concentration of 140 micrograms perchlorate L^{-1}. Figure 2-33 demonstrates the recovery of the spiked perchlorate for three soil extracts. Recoveries for these three soil extracts ranged from 98.2 – 100.2%. Recoveries tested for other soil extracts yielded similar results.

All 454 soil samples obtained from the field experiment at the Oxford Research Station location 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 by hand 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 sidedress 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.

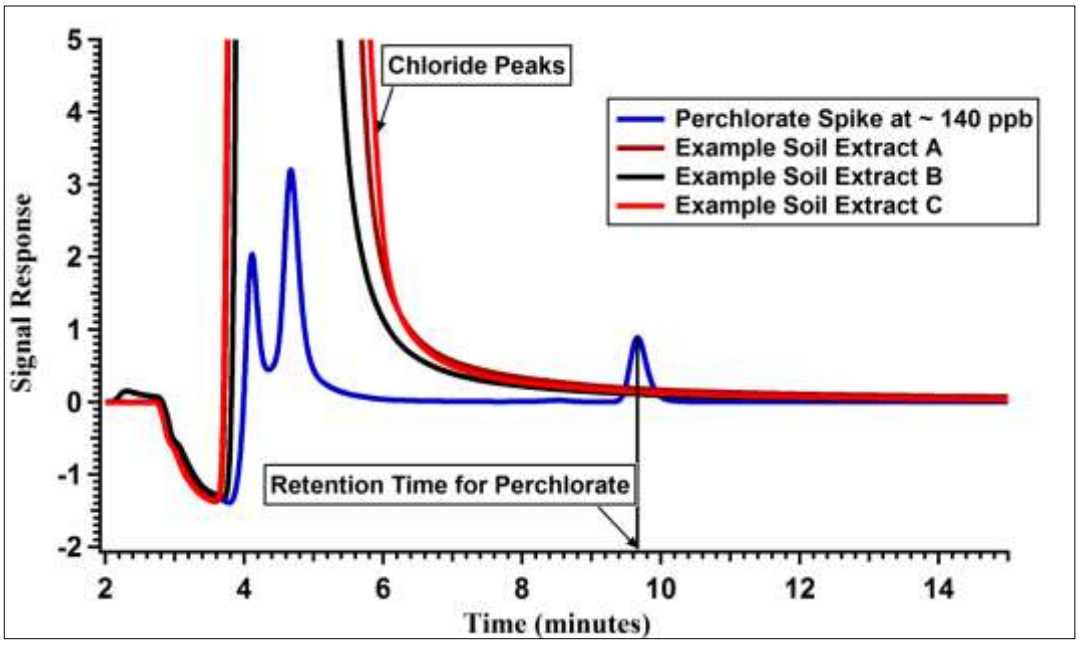

Figure 2-32. Portions of the chromatograms for three soil extracts (0.001M $CaCl_2$) compared to ~140 micrograms perchlorate L^{-1} (ppb). (1000 microliter sample loop, AG-16+AS-16 columns, 40 mM NaOH, 1 mL/min, conductivity detection after suppression with a ARSR-300 in recycle mode, 100 mA).

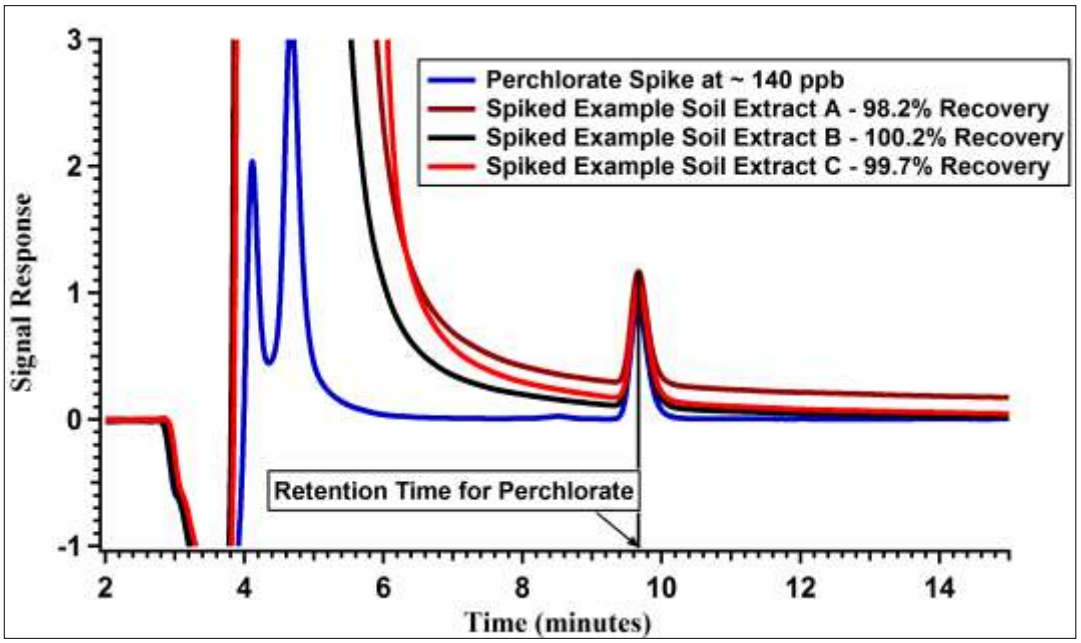

Figure 2-33. Portions of the chromatograms for three soil extracts (0.001M $CaCl_2$) demonstrated recovery of spiked perchlorate at equivalent concentration of ~140 micrograms perchlorate L^{-1} (ppb). (1000 microliter sample loop, AG-16+AS-16 columns, 40 mM NaOH, 1 mL/min, conductivity detection after suppression with a ARSR-300 in recycle mode, 100 mA).

A total of 6 soil samples were submitted to Columbia Analytical Services Laboratory for analysis of extractable perchlorate (Table 2-10). The soil samples were chosen at random from the various plots and sampling dates. Perchlorate was below the detection limit 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 ppb perchlorate. These results confirm the inability to detect perchlorate by ion chromatography, as the levels of perchlorate present are well below the stated detection limit using ion chromatography and the cited EPA protocol.

Table 2-10. Results of soil analyses for perchlorate by Columbia Analytical Services Laboratory. Oxford Research Station.

SAMPLE	DATE	MATRIX	COMPOUND	MRL	Adjusted MRL	RESULT	FLAG
				----------µg/L----------			
OX-P3-50-100	06/01/09	SOIL	PERCHLORATE	2.0	2.0	2.0	U
OX-P1-0-15	06/19/09	SOIL	PERCHLORATE	2.0	2.1	4.7	
OX-P8-30-50	07/02/09	SOIL	PERCHLORATE	2.0	2.0	2.0	U
OX-P2-30-50	07/31/09	SOIL	PERCHLORATE	2.0	2.0	2.0	U
OX-P3-15-30	08/28/09	SOIL	PERCHLORATE	2.0	2.0	2.0	U
OX-P9-30-50	09/25/09	SOIL	PERCHLORATE	2.0	2.1	2.1	U

Notes: Report Qualifiers for the FLAG column:

U = indicates compound was analyzed for but not detected.

2.7.2.6 Soil Chemical Analysis – Ammonium and Nitrate

All 454 soil samples obtained from the Oxford Research 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 2-34). This was consistent throughout the 0-200 cm sampling depth. Extractable NO_3-N was on average higher in Plots 9-10 (Treatment 2) where plots were bare, but still < 1 mg NO_3-N/kg. Overall extractable NH_4-N was higher than NO_3-N, with the lowest amounts of extractable NH_4-N observed for Plots 5-8 (Treatment 3). The highest concentration of extractable NH_4-N was observed in the 150-200 cm depth increment from Plots 9-10 (~ 2.2 mg NH_4-N/kg). 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 Plots 1-10 (Treatment 1-3) as a function of depth increment sampled was used to compare to results from post-planting soil samples.

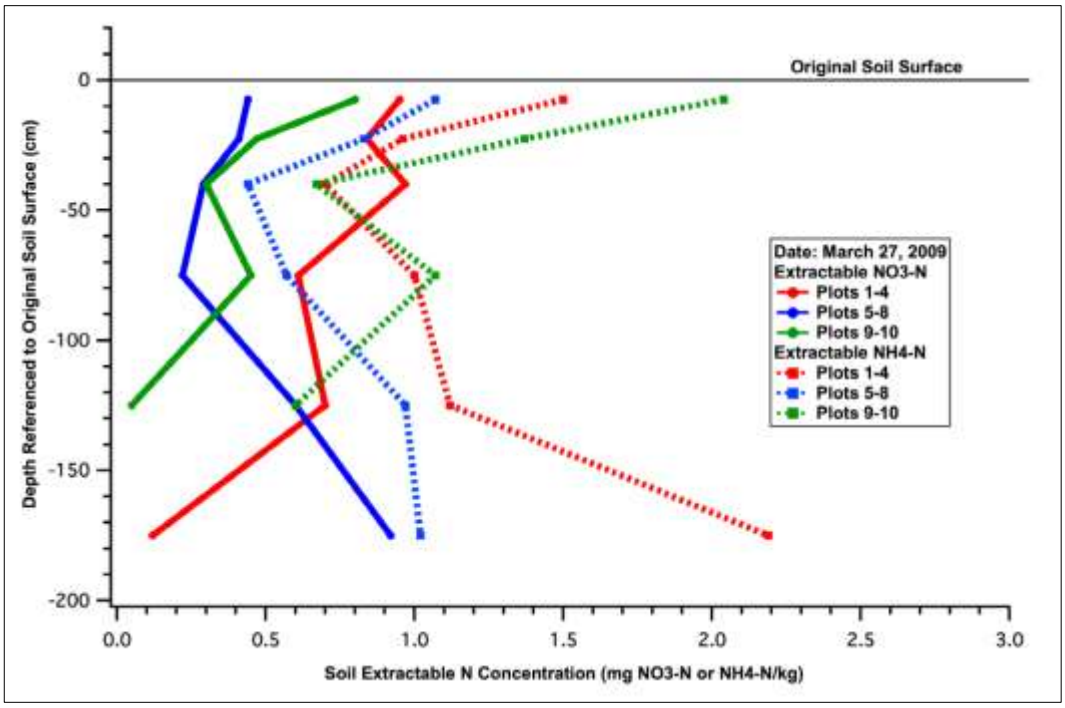

Figure 2-34. Mean soil extractable NO_3-N and NH_4-N concentrations as a function of sampled depth increment for Plots 1-10 at the Oxford Research Station. Date: March 27, 2009. Mean values are plotted at mid-point of depth increment sampled.

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 are provided in Table 2-11. 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 on vertical transport and the soil sampling protocol adopted focused on perchlorate transport.

Soil sampling was conducted by compositing soil cores taken from the center of the constructed soil beds that received the tobacco transplants as a function of specified depth increments (see Table 2-11). Fertilizer was applied as a narrow band adjacent to and below the transplanted tobacco plants. As illustrated in Figure 2-34, 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 beds. The distribution of this soil NO_3-N was not uniform and in fact 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 beds would increase, as NO_3-N moved and dispersed both laterally within the bed and vertically into 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.

In general, this expected trend in extractable soil NO_3-N concentrations is evident in Table 2-11, where the highest concentrations of soil NO_3-N were not observed with the first soil samples collected on June 19, 2009. The highest soil NO_3-N concentrations were observed in early and the middle of July, followed by a slight increase in NO_3-N at the lower soil depth increments sampled. These trends are more evident in Figures 2-35, 2-36 and 2-37.

Table 2-11. Mean soil concentrations of extractable NO_3-N as a function of depth increment sampled collected from June 19, 2009 to September 25, 2009 at the Oxford Research Station. Values in parentheses are calculated standard deviation for plots sampled. Depth increments are referenced to top of soil beds.

Depth Increment	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
-----cm-----	----------mg NO_3-N/kg----------							
Plots 1-4 (Potassium Nitrate plus Tobacco) – Treatment 1								
0-15	18.3	41.5	25.1	19.5	10.3	5.2	2.1	0.6
	(11.2)	(19.4)	(15.9)	(13.8)	(7.4)	(3.0)	(3.0)	(1.2)
15-30	12.4	24.9	14.9	36.0	24.6	3.5	2.9	1.8
	(5.6)	(13.6)	(9.2)	(29.1)	(21.0)	(2.1)	(2.1)	(2.1)
30-50	7.0	5.4	4.0	3.1	2.5	2.0	1.6	1.0
	(3.9)	(3.2)	(2.1)	(1.7)	(2.3)	(1.2)	(1.2)	(0.8)
50-75	2.8	3.7	3.4	2.4	1.9	1.7	1.4	0.5
	(1.3)	(2.1)	(2.8)	(1.2)	(1.6)	(1.1)	(1.1)	(0.7)
75-100	1.4	3.0	2.2	2.2	2.1	2.1	1.3	0.5
	(1.0)	(2.2)	(1.1)	(1.4)	(1.9)	(1.8)	(1.8)	(0.7)
Plots 5-8 (Calcium Nitrate plus Tobacco) – Treatment 3								
0-15	23.9	66.8	41.9	35.4	28.4	14.9	10.8	2.9
	(18.4)	(7.5)	(26.0)	(16.4)	(11.6)	(7.9)	(7.9)	(1.4)
15-30	23.2	24.2	29.9	56.6	24.0	14.4	9.4	2.0
	(5.7)	(9.3)	(23.2)	(30.2)	(10.4)	(12.3)	(9.8)	(0.5)
30-50	7.8	3.0	2.6	8.2	5.5	4.6	4.9	2.7
	(2.0)	(0.8)	(1.1)	(6.3)	(5.9)	(5.6)	(6.7)	(3.7)
50-75	3.3	1.5	1.8	2.8	2.4	2.5	1.3	1.3
	(1.4)	(0.4)	(0.9)	(1.3)	(2.3)	(1.3)	(0.2)	(0.9)
75-100	1.4	1.2	1.2	2.0	2.5	2.8	1.2	1.0
	(0.4)	(0.6)	(0.2)	(1.3)	(2.2)	(1.9)	(0.4)	(0.4)
Plots 9-10 (Potassium Nitrate minus Tobacco) – Treatment 2								
0-15	41.0	73.3	49.0	53.5	39.6	35.3	20.4	10.3
	(10.9)	(1.7)	(7.5)	(18.9)	(29.5)	(12.6)	(8.8)	(10.1)
15-30	16.7	20.1	32.4	59.6	21.4	18.6	28.2	13.5
	(7.1)	(6.9)	(12.0)	(19.5)	(7.5)	(11.9)	(0.8)	(8.2)
30-50	8.1	14.2	13.4	5.6	3.3	11.5	2.9	2.5
	(0.5)	(14.7)	(1.0)	(1.3)	(2.5)	(6.2)	(2.4)	(2.9)
50-75	3.0	1.9	2.5	0.7	2.4	2.3	1.9	0.5
	(1.4)	(0.5)	(1.4)	(0.4)	(2.0)	(0.5)	(0.9)	(0.4)
75-100	3.7	2.2	1.5	0.5	0.9	1.5	0.9	0.5
	(0.9)	(0.7)	(0.1)	(0.3)	(0.1)	(1.2)	(0.1)	(0.3)

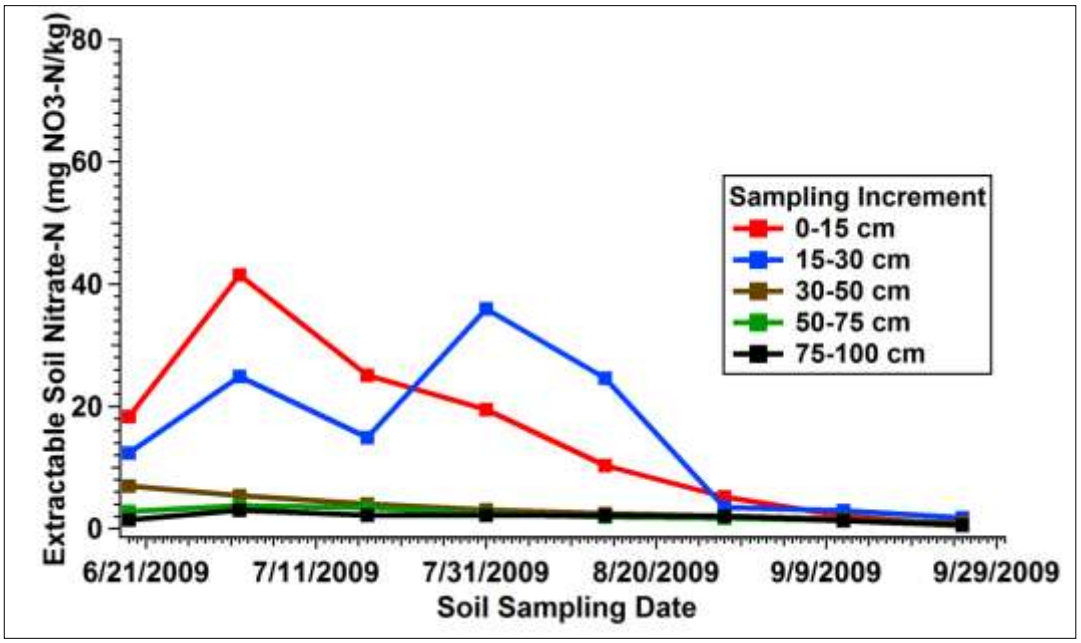

Figure 2-35. Mean concentrations of soil extractable NO_3-N for Plots 1-4 (Treatment 1) as a function of sampling date and depth increment at the Oxford Research Station. Sampling depths are referenced to top of soil beds.

Closer inspection of Figure 2-35 suggests a number of reactions occurring with soil NO3-N throughout the growing season in Plots 1-4. The first is that the soil volume in the top 15 cm of the bed 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 thoroughly explores the volume of the beds.

Figure 2-35 however, also suggests the possibility of very rapid transport of a small fraction of NO_3-N that occurred prior to the June 19, 2009 sampling as evident by the elevated NO_3-N concentrations in the 30-50 cm sampling increment. A similar occurrence of possible rapid transport of a fraction of the applied NO_3-N is evident for Plots 5-8 (Figure 2-36).

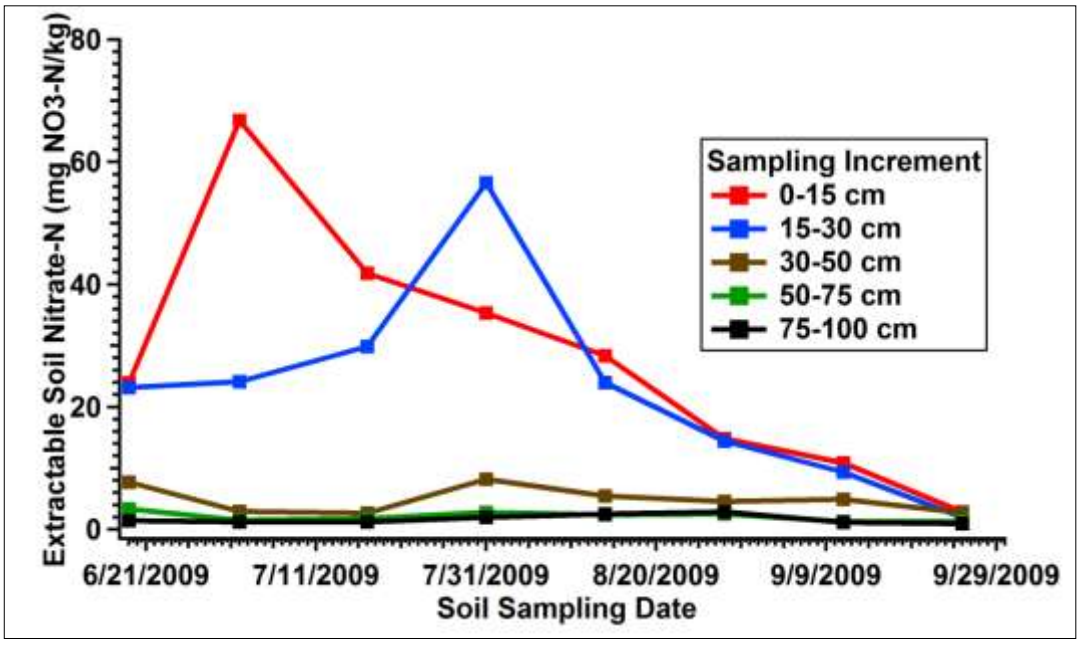

Figure 2-36. Mean concentrations of soil extractable NO_3-N for Plots 5-8 (Treatment 3) as a function of sampling date and depth increment at the Oxford Research Station. Sampling depths are referenced to top of soil beds.

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 Plots 1-4 and 5-8. Plots 5-8 exhibited the same trend in the concentration of NO_3-N in the 0-15 cm sampling increment (top of the bed) as Plots 1-4, and also exhibited the maximum concentration in soil NO_3-N in the 15-30 cm sampling increment (bottom of the soil bed) 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 Plots 1-4, but not to the same extent, with about twice the concentration of

NO_3-N remaining across the 0-30 cm depth in Plots 5-8 than Plots 1-4. The concentration of NO_3-N in the 30-50 cm depth increment also remained constant and somewhat higher than observed in Plots 1-4, although the difference is probably not significant given the variation in observed concentrations (Table 2-9).

The similar patterns observed in soil NO_3-N concentration as a function of date and sampling depth support the conclusion that nitrate supplied by the two different sources used in this study (potassium nitrate for Plots 1-4 and calcium nitrate for Plots 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 depth for Plots 1-8 were therefore calculated and plotted in Figure 2-37 to facilitate comparison to the soil NO_3-N patterns observed for the bare soil plots (Plots 9-10; Figure 2-38).

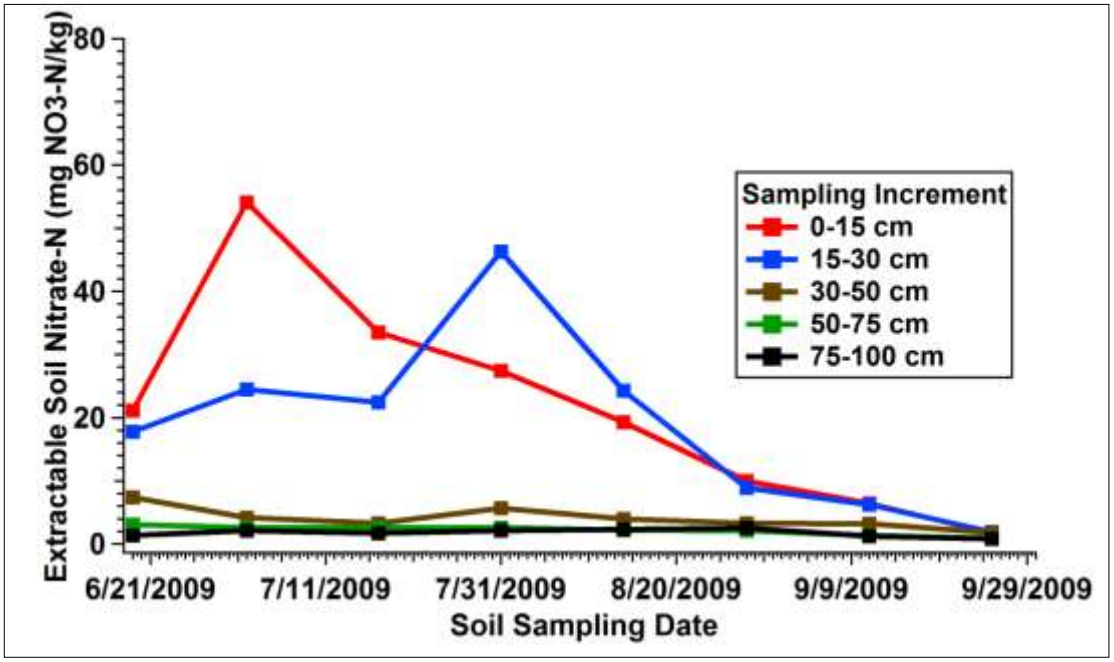

Figure 2-37. Mean concentrations of soil extractable NO_3-N for Plots 1-8 as a function of sampling date and depth increment at the Oxford Research Station. Sampling depth increments are referenced to top of soil beds.

Comparison of Figures 2-37 and 2-38 indicates similar patterns in NO_3-N in the presence of tobacco as in the absence of tobacco (Plots 9-10). Soil NO_3-N increased in the top of the bed (0-15 cm sampling increment) and reached a maximum on July 2, 2009. There then was a steady somewhat linear 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 bed) was also observed on July 31, 2009. In comparing plots with tobacco (1-8) versus bare soil (Plot 9-10), the major difference is the relative magnitude of the soil NO_3-N concentrations, with relatively higher NO_3-N concentrations found in bare soil throughout the growing season. The rapid increase in NO_3-N in the 30-50 cm depth increment is duplicated under non-planted conditions (Plot 9-10) and remains elevated throughout the growing season.

A consistent theme across all 10 plots was the apparent very modest to no increase in the soil NO_3-N concentration at the 50-75 and 75-100 depth increments. The NO_3-N concentrations at these deeper soil depths remained constant or decreased slightly during the growing season (Table 2-11, Figures 2-37 and 2-38).

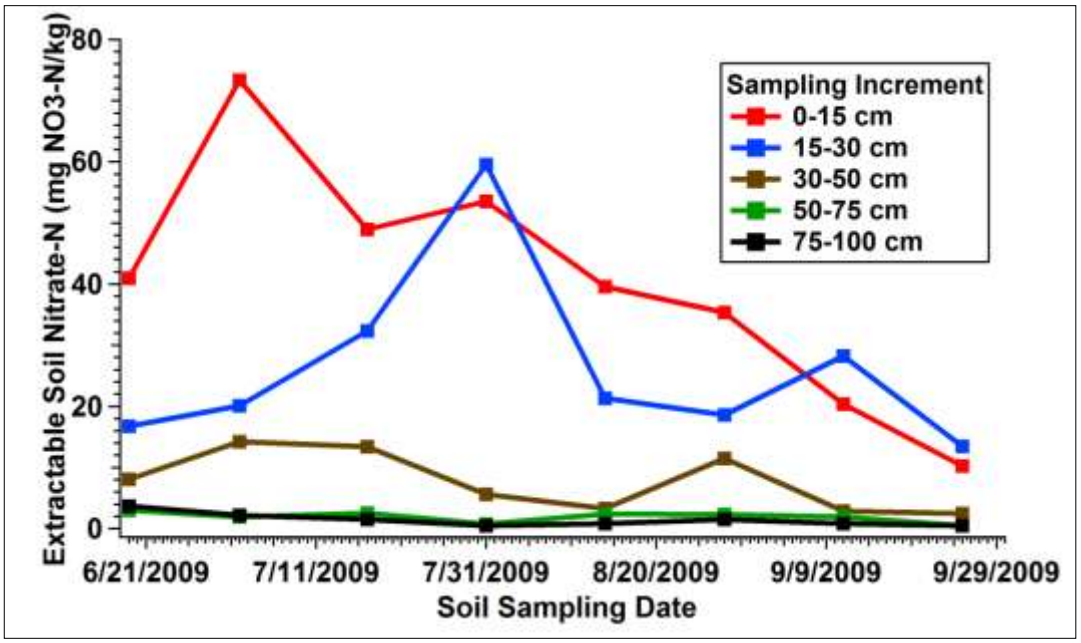

Figure 2-38. Mean concentrations of soil extractable NO_3-N for Plots 9-10 (Treatment 2) as a function of sampling date and depth increment at the Oxford Research Station. Sampling depth increments are referenced to top of soil beds.

The vertical distribution of extractable soil NO_3-N from Plots 1-10 as a function of date of sampling are shown in Figures 2-39, 2-40 and 2-41. These figures illustrate again how the concentration of extractable NO_3-N initially increased in the top of the beds after application of the fertilizer sidedress, reaching a maximum concentrations around ~July 2, 2009 time period. Then there is a shift of the mass of extractable NO_3-N downward into the lower 15 cm of the bed. The presence of a restrictive layer, as suggested by the soil bulk density values in Table 2-5, is also apparent as the concentration of extractable NO_3-N substantially decreases below the 45 cm depth relative to the top of the soil bed.

However, there is elevated NO_3-N below the 45 cm depth relative to the assumed background concentration of NO_3-N as determined on March 27, 2009. As discussed above for Figures 2-37 and 2-38, the vertical distribution of NO_3-N strongly suggests

there was relatively rapid movement of nitrate into the soil profile by the June 19, 2009 sampling date in all 10 plots (Figs. 2-39, 2-40, 2-41). Extractable NO_3-N continued to be detected at the lower soil depths for the entire growing season, but for Plots 1-8, the concentration of NO_3-N never exceeded the concentration values observed for the first soil sampling on June 19, 2009 (Figs. 2-39, 2-40). As the mass of soil NO_3-N moved into the bottom 15 cm of the bed, there is not an apparent corresponding increase in extractable NO_3-N at lower soil depths, suggesting the presence of the tobacco crop acted to retard further movement, perhaps through decreases in soil water content (Fig. 2-22). In the absence of the tobacco crop, there is evidence of some further movement in the mass of soil NO_3-N deeper into the soil, but not beyond the 50 cm depth as referenced to the original soil surface. This may reflect the relatively wetter conditions in Plot 9-10 during the growing season (Fig. 2-21), but also probably illustrates the ability of the restrictive soil zone evident in Table 2-5 to limit vertical transport.

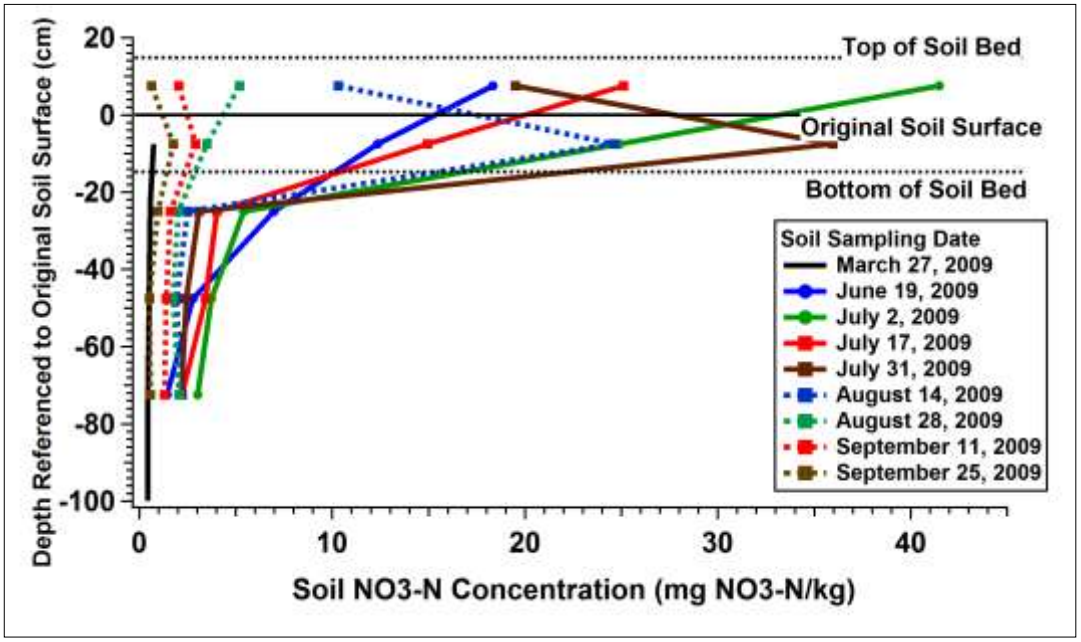

Figure 2-39. Vertical distribution of extractable NO_3-N from Plots 1-4 (Treatment 1) as a function of date of sampling at the Oxford Research Station. Soil samples obtained on

March 27, 2009 are referenced to the original soil surface, all other samples referenced to the top of soil bed.

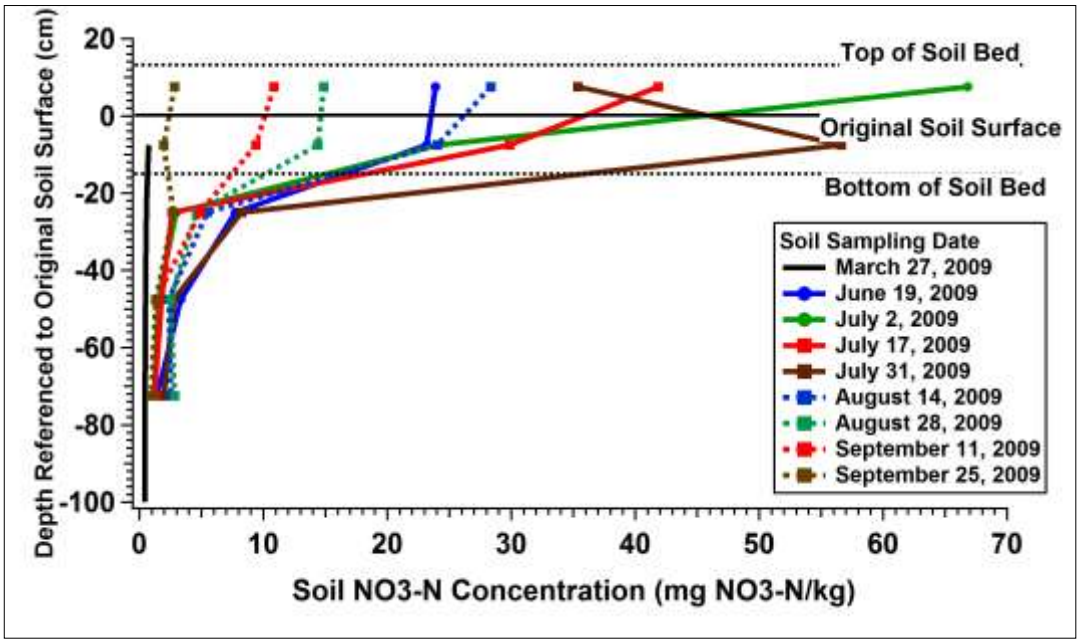

Figure 2-40. Vertical distribution of extractable NO_3-N from Plots 5-8 (Treatment 3) as a function of date of sampling at the Oxford Research Station. Soil samples obtained on March 27, 2009 are referenced to the original soil surface, all other samples referenced to the top of soil bed.

Comparison of Figures 2-39, 2-40 and 2-41 suggests the bulk of extractable soil NO_3-N movement in the soil bed 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 2-5). The absolute magnitude of NO_3-N in the bed 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 of the bed. By September 25, 2009, NO_3-N concentrations where tobacco was planted (Plots 1-8) were approaching those observed in the background soil

samples collected on March 27, 2009 (Figs. 2-39 and 2-40), while concentrations of NO_3-N in nonplanted conditions (Plots 9-10) remained relatively elevated, at least to the 50 cm depth as referenced to the original soil surface (Fig. 2-41).

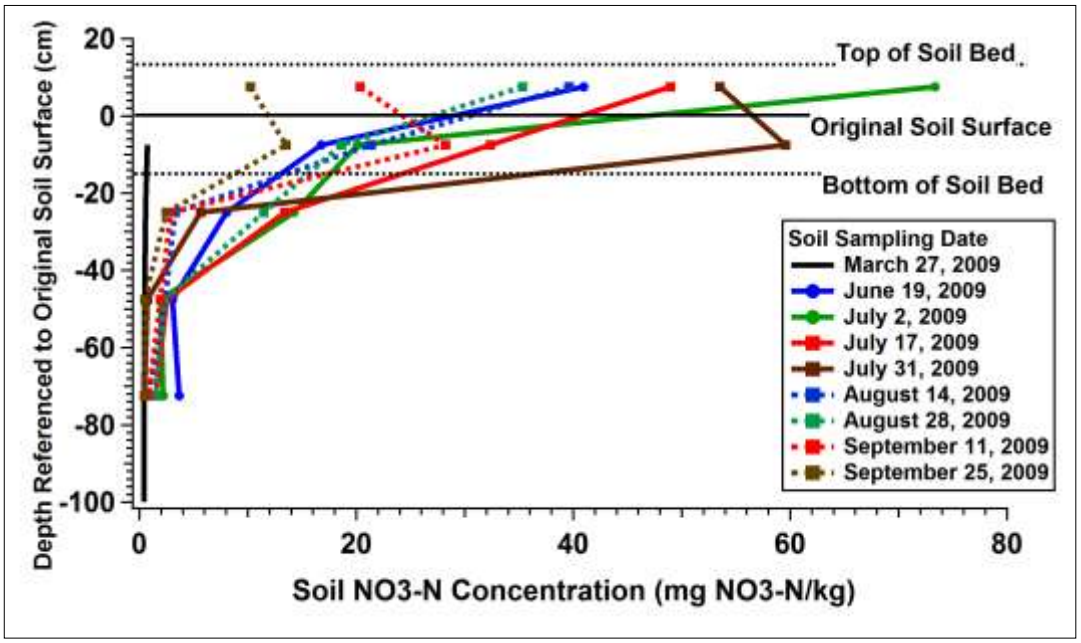

Figure 2-41. Vertical distribution of extractable NO_3-N from Plots 9-10 (Treatment 2) as a function of date of sampling at the Oxford Research Station. Soil samples obtained on March 27, 2009 are referenced to the original soil surface, all other samples referenced to the top of soil bed.

Overall, patterns in the distribution of extractable soil NH_4-N were similar to those for NO_3-N, although the concentration of NH_4-N was substantially less than those of NO_3-N. The highest concentrations of extractable NH4-N occurred during the July 31, 2009 sampling date, but beyond this date, there was little pattern in the NH_4-N data and values typically oscillated between 2 – 5 mg NH_4-N kg^{-1} across the total soil depth sampled. Part of this distribution in NH_4-N may be due to fertilizer applications, but the NH_4-N data may also have been influenced by mineralization of soil carbon, decaying roots or leachate from the tobacco crop that infiltrated the soil. The mean soil

concentrations of extractable NH_4-N as a function of depth increment sampled collected from June 19, 2009 to September 25, 2009 at the Oxford Research Station are summarized in Table 2-12. The mean soil concentrations of NH_4-N across all soil depths and plots was < 1 mg NH4-N kg^{-1} for background soil samples collected on March 27, 2009.

Table 2-12. Mean soil concentrations of extractable NH_4-N as a function of depth increment sampled collected from June 19, 2009 to September 25, 2009 at the Oxford Research Station. Values in parentheses are calculated standard deviation for plots sampled. Depth increments are referenced to top of soil beds formed for tobacco transplants.

Depth Increment	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
----cm-----	----------mg NH4-N/kg----------							
Plots 1-4 (Potassium Nitrate plus Tobacco) – Treatment 1								
0-15	2.4	4.7	4.1	4.3	3.6	3.7	3.6	3.4
	(0.8)	(0.5)	(0.8)	(0.7)	(0.7)	(1.1)	(0.6)	(0.2)
15-30	2.0	4.8	3.2	5.6	4.2	3.6	2.9	3.9
	(0.5)	(0.3)	(0.7)	(1.1)	(2.1)	(0.7)	(0.8)	(0.9)
30-50	2.6	3.4	3.5	3.1	2.8	3.5	2.7	3.8
	(0.9)	(0.7)	(1.5)	(0.5)	(0.4)	(1.0)	(0.4)	(1.1)
50-75	1.2	2.5	3.9	3.0	2.4	3.0	2.5	3.8
	(0.1)	(0.7)	(1.3)	(1.1)	(0.7)	(1.0)	(0.5)	(2.0)
75-100	1.1	3.0	3.2	3.5	2.8	3.0	3.2	3.3
	(0.1)	(0.4)	(0.4)	(1.0)	(0.8)	(0.4)	(0.6)	(0.8)
Plots 5-8 (Calcium Nitrate plus Tobacco) – Treatment 3								
0-15	2.3	6.4	4.9	6.9	4.3	4.6	3.1	3.4
	(0.6)	(0.9)	(2.4)	(1.3)	(0.2)	(1.3)	(0.3)	(0.5)
15-30	2.6	4.6	3.9	11.1	4.2	2.7	2.9	2.6
	(1.6)	(1.2)	(1.5)	(3.3)	(1.4)	(0.6)	(0.4)	(0.3)
30-50	2.1	3.4	3.2	3.5	2.6	2.9	2.7	2.5
	(0.5)	(1.3)	(0.8)	(0.6)	(0.9)	(1.5)	(0.4)	(0.5)
50-75	1.5	2.3	3.0	3.6	2.4	3.0	2.3	1.9
	(0.6)	(0.3)	(0.1)	(0.2)	(0.8)	(0.6)	(0.4)	(0.1)
75-100	1.1	3.7	3.5	4.0	2.8	3.3	2.4	2.6
	(0.1)	(1.0)	(0.6)	(0.8)	(0.4)	(0.9)	(0.2)	(0.2)
Plots 9-10 (Potassium Nitrate minus Tobacco) – Treatment 2								
0-15	2.4	5.0	3.2	4.5	2.5	3.0	2.6	3.2
	(0.0)	(0.2)	(0.1)	(1.5)	(0.2)	(0.0)	(0.6)	(0.4)
15-30	2.1	3.6	3.0	5.6	3.1	2.6	2.9	3.2
	(0.1)	(0.4)	(0.2)	(1.8)	(0.8)	(0.8)	(0.2)	(0.1)
30-50	2.6	2.4	4.4	4.0	2.1	3.0	2.3	3.2
	(0.5)	(0.3)	(2.6)	(0.2)	(0.1)	(0.1)	(0.7)	(1.3)
50-75	1.3	2.7	3.9	3.0	2.1	3.8	2.5	2.3
	(0.1)	(0.4)	(0.7)	(0.5)	(0.7)	(0.2)	(0.0)	(0.1)
75-100	1.7	3.1	3.6	3.8	2.2	3.3	3.6	3.0
	(0.2)	(0.3)	(0.3)	(0.5)	(0.5)	(0.0)	(0.0)	(0.3)

2.7.2.7 Tobacco Nitrogen Content and Removal

Leaf punches were obtained from tobacco plants in Plots 1-8 throughout the growing season. Percent moisture content ranged from 67 to 87 % regardless of age of the leaf (Table 2-13). Percent N content did vary with age of leaf, with highest N content found in the youngest leaves, and the lowest N content found in harvested leaves. Overall, the N content between harvests and plots showed similar trends reinforcing the conclusion that there was no significant difference in tobacco growth at the Oxford Research Station using either potassium nitrate or calcium nitrate as the N source for the sidedress with N.

An estimate of N removal by the harvested tobacco leaves is presented in Table 2-14, based on the provided mass of leaves harvested reported by the Oxford Research Station staff (Table 2-7), and the analyses of leaf punches obtained from the harvested leaves (Table 2-13). An average of 13.5% moisture content was assumed for the cured-tobacco (Table 2-7) in order to express the calculated results on an oven-dry basis.

The calculated removals of N added averaged 91.7 and 96.3 kg N ha^{-1} for Plots 1-4 (Treatment 1) and 5-8 (Treatment 3), respectively. While such high recoveries are consistent with the patterns of NO_3-N observed in the soil beds 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 bed with relatively little downward leaching of N), crop removal approaching nearly 100% of the N fertilizer added are not typically observed. More typical N uptake recoveries vary from 30 to possibly 50% (MacKown and Sutton, 1997). One possible explanation for the results in Table 2-14 may be because of the way leaf sampling was conducted during the study.

Table 2-13. Nitrogen and moisture content (expressed on a wet-weight basis) of tobacco samples during 2009 field experiment at the Oxford Research Station.

		Lower Leaves		Upper Leaves	
Date	Sample Type	N-Content Wet-Wt. Basis	Moisture Content Wet-Wt. Basis	N-Content Wet-Wt. Basis	Moisture Content Wet-Wt. Basis
		---------- % ----------			
Plots 1-4 Nitrogen Source: Potassium Nitrate - Treatment 1					
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
Plots 5-8 Nitrogen Source: Calcium Nitrate – Treatment 3					
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

Leaf samples used to make the calculations in Table 2-14 are based on leaf punchouts and not total leaf samples gathered each time the crop was sampled. Leaf punches will favor 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 2-14 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 data in Table 2-14 is that there was another source of N available to support plant growth during the growing season. This additional source of N may be the result of mineralization of soil organic matter with the resultant release of N that is available to support plant growth. This option is discussed further in Section 2.8.

Table 2-14. Mass of nitrogen removed in harvested tobacco at the Oxford Research Station. All calculations normalized to an oven-dry weight basis.

Harvest Date	Harvested Leaf Mass	Percent N Concentration	Mass of N Removed	
	- kg/ha-	------ % ------	- kg N/ha-	- lbs N/Ac-
Plots 1-4 Nitrogen Source: Potassium Nitrate – Treatment 1				
21-AUG-09	703	2.12	14.9	13.3
11-SEP-09	899	2.20	19.8	17.6
9-OCT-09	2468	2.31	57.0	50.7
Totals	4070	-	91.7	81.7
Plots 5-8 Nitrogen Source: Calcium Nitrate – Treatment 3				
21-AUG-09	699	2.19	15.3	13.6
11-SEP-09	907	2.07	18.8	16.7
9-OCT-09	2590	2.40	62.2	55.4
Totals	4197	-	96.3	85.7

2.7.3 Whiteville Tobacco Research Station

Site selection for the study was conducted in early 2009 upon consultation with the Whiteville Tobacco Research Station Superintendent. A suitable location was identified on a approximately level uniform research field that allowed the positions of Plots 1-10 in sequence in one row. Initial soil sampling to a depth of 100+ cm was

conducted on March 9, 2009 to determine background levels of soil extractable nitrate and ammonium as well as survey for the possible presence of perchlorate. Tobacco seedlings (flue-cured variety K 326) where transplanted to the prepared research plots on April 20, 2009. On April 28, 2009, the complete fertilizer with the formulation of 6-6-18 was applied in each bed adjacent to the tobacco plants. The target application rate was 47 kg N/ha (42 lbs N/acre), or 1.3 kg N/plot (2.78 lbs N/plot).

One June 2, 2009, the sidedress of N-containing fertilizer was applied. The potassium nitrate source material was applied at a rate of 40-42 kg N/ha (36-37 lbs N/acre) or 1.1 kg N/plot (2.38-2.45 lbs N/plot) to Plots 1-4 (Treatment 1), which were designated as the perchlorate treatment plots. The non-perchlorate treated Plots 5-8 (Treatment 3) were sidedressed using ammonium nitrate at a rate of 36-38 kg N/ha (32-34 lbs N/acre) or 0.96-1.0 kg N/plot (2.12-2.25 lbs N/plot). Plots 9-10 (Treatment 2) were treated exactly the same as plots 1-4, except these plots were kept bare (no tobacco plants). Plots 9-10 were sprayed several times during the season with a herbicide to prevent weed growth. Every attempt was made to place both applications of fertilizer in the same position in the soil relative to the tobacco plant. As with the Oxford research site, it was assumed that following application of the fertilizer, the majority of nitrate (or perchlorate) would remain in the volume of soil composing the bed, and would move vertically into the underlying soil over time.

Soil and foliar sampling started following the sidedress with N fertilizer. A visual representation of the development of the tobacco crop at the Whiteville Tobacco Research Station from April to September 2009 is given in Figures 2-42 to 2-46.

2.7.3.1 Intensity and Temporal Distribution of Rainfall

Rainfall amounts at the Whiteville Tobacco Research 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 (Table 2-15), the largest amount occurring in April and May (~ 290 mm). As at the Oxford Research Station, a substantial portion of this rainfall occurred between the first and second application of fertilizer. June was relatively dry receiving only 61 mm of rainfall. Rainfall totals in July and August were more normal averaging ~ 120 mm. September was again relatively dry. Because of the adequate amount of rainfall during the study period, no supplemental irrigation was used at the Whiteville Tobacco Research Station.

Figure 2-42. April 10, 2009, Whiteville Tobacco Research Station. Prepared soil beds across research plots. (Photo by Scott King, Dept. of Soil Science, NCSU)

Figure 2-43. May 27, 2009, Whiteville Tobacco Research Station. Tobacco plants following transplanting and approximately 30 days after application of complete fertilizer. (Photo by Scott King, Dept. of Soil Science, NCSU)

Figure 2-44. June 27, 2009, Whiteville Tobacco Research Station. Tobacco plants approximately 9 weeks since transplanting and 3 weeks following application of sidedress of nitrogen fertilizers. (Photo by Scott King, Dept. of Soil Science, NCSU)

Figure 2-45. August 31, 2009, Whiteville Tobacco Research Station. Tobacco plants ~ 17 weeks since transplanting and after first harvest. (Photo by Scott King, Dept. of Soil Science, NCSU)

Figure 2-46. September 28, 2009, Whiteville Tobacco Research Station. Representative picture of research plots after final harvest, demonstrating soil beds are still intact at the end of the sampling period. (Photo by Scott King, Dept. of Soil Science, NCSU)

Table 2-15. Monthly rainfall amounts recorded at the Whiteville Tobacco Research Station for April through September, 2009. (Source: NC State Climate Office CRONOS network).

Month	Rainfall Amount
	----- mm -----
April	116
May	171
June	61
July	101
August	144
September	41
Average	106
Total	634

2.7.3.2 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 bed and not the original soil surface. The height of the soil beds 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 bed, 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 beds. The tensiometers were in place for the sidedress application of either potassium nitrate or ammonium nitrate.

Soil tensiometer readings in Plots 9-10, bare soil plots, as a function of date and soil depth are shown in Figure 2-47 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 bed 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 bed 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 bed continued despite a large rain event between July 22 and 29, 2009. The top 15 cm of the soil bed eventually became very dry in mid-August and remained so through September.

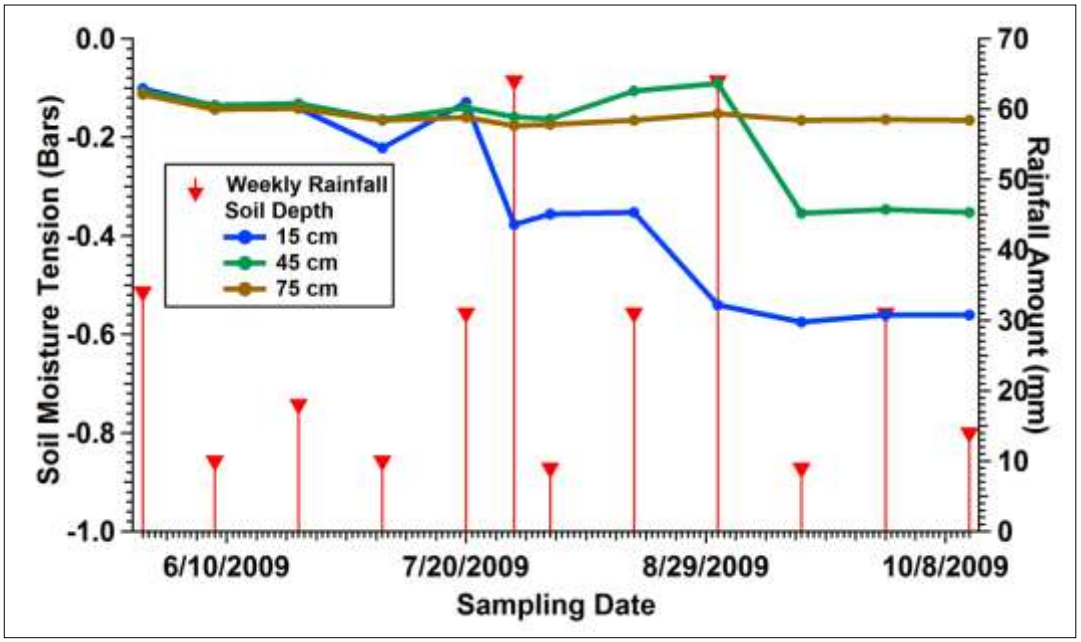

Figure 2-47. Mean soil tensiometer readings for Plots 9-10 (Treatment 2-bare soil) at the Whiteville Tobacco Research Station as a function of date and soil depth together with weekly rainfall amounts. Soil depths are referenced to the top of the soil beds.

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, Plots 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 Plots 9-10 to control weeds, so some water loss evident in the top 15 cm of the bed may have been due to weed growth.

The presence of the tobacco plants altered the observed pattern in soil moisture content for Plots 1-8 as compared to Plots 9-10, at least for the 15 and 45 cm depths (Figure 2-48). 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 bed. This dip in soil moisture content is also present in Plots 9-10 for the 15 cm depth (Figure 2-47). 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 moisture content at 45 cm depth seemed to first decline and then subsequently recover for the remainder of the growing season.

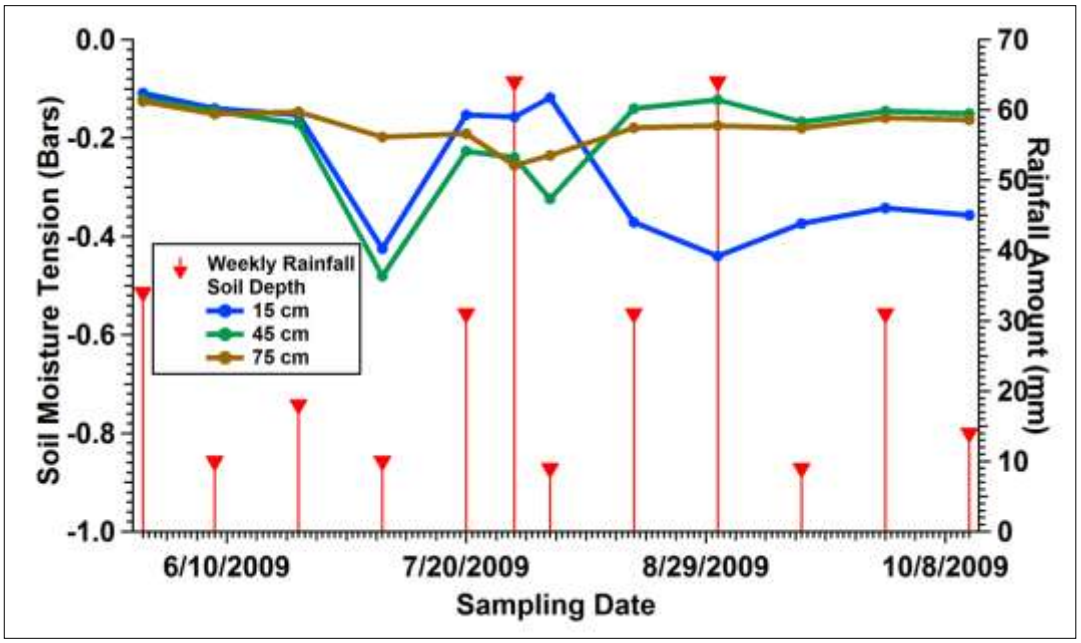

Figure 2-48. Mean soil tensiometer readings for Plots 1-8, Whiteville Tobacco Research Station, as a function of date and soil depth together with weekly rainfall amounts. Soil depths are referenced to the top of the soil beds.

After August 5, 2009, the moisture content in 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.

The data in Figure 2-48 suggests 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 beds as well. Presence of the tobacco crop would act to both direct rainwater to or shield the top of the bed from direct contact with rainfall. Rainwater contacting the center of the plants would drain downward along the stalk and infiltrate the soil at the base of the plant at the top of the bed. However, as the plants grow and the older leaves elongate, they would redirect the rainfall to the inter rows, where lateral infiltration of water would occur at the base of the beds. This lateral infiltration would move both across the bed 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 bed.

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 bed, 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 drawn down of soil moisture from the 45 and 75 cm depths. Under such conditions, it can be assumed there was probably 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 Plots 1-10. Samples were taken with reference to the top of the beds. Soil bulk density values as a function of plot and soil depth relative to the top of the bed are shown in Table 2-16. The vertical profile of soil bulk density as a function of soil depth relative to the top of the bed is shown in Fig. 2-49.

Table 2-16. Soil bulk density as a function of plot and soil depth relative to the top of the bed, and mean soil porosity as a function of soil depth at the Whiteville Tobacco Research Station.

Depth range	Plot 1	Plot 2	Plot 3	Plot 4	Plot 5	Plot 6	Plot 7	Plot 8	Plot 9	Plot 10	Mean	Mean Soil Porosity
--cm--	----------g/cubic centimeter----------											--%--
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 Plots 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 bed. 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 Research Station, the restricted layer is still rather porous and probably not a major impediment to vertical transport of water.

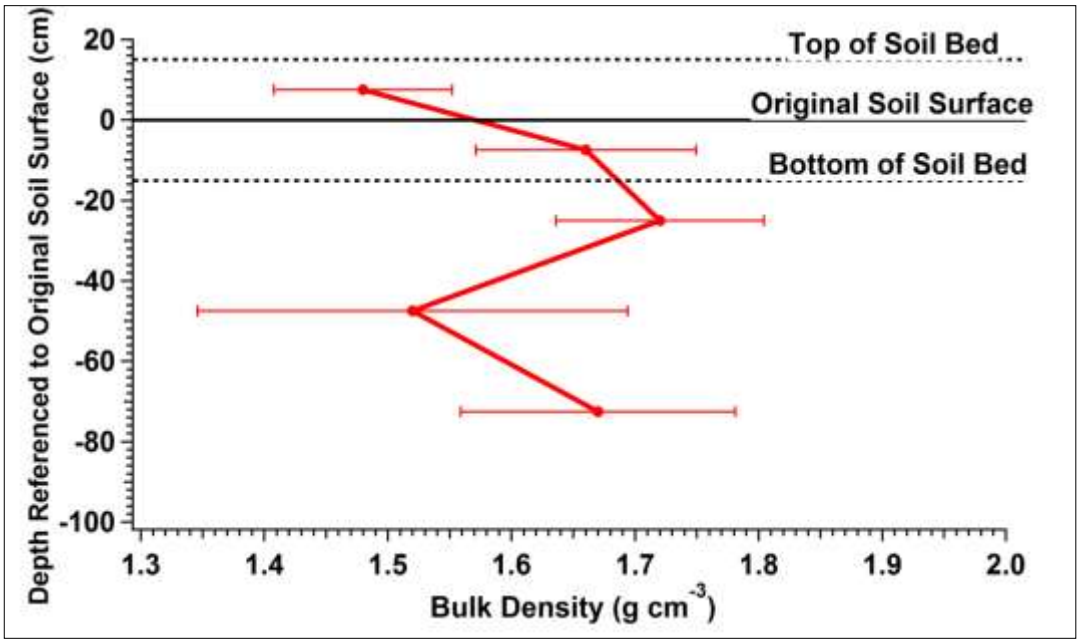

Figure 2-49. Mean soil bulk density and standard deviation for Plots 1-10 as a function of soil depth relative to the top of the soil bed at the Whiteville Tobacco Research Station.

2.7.3.3 Groundwater and Shallow Wells

One set of shallow wells were installed at the Whiteville Tobacco Research Station. The shallow wells (designations P1A to P9B; A = upslope, B = downslope) were placed into position on March 26, 2009, and were located at either end of the plots (Figure 2-50). The wells consisted of PVC pipe with a slotted end section 30 cm (~1foot) long and were installed so that the slotted end was buried approximately 1m (~3ft) deep, 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 eventually leads to a higher-clay sublayer, which acts as a confining layer and often supports a 'perched' water table above it. Each shallow well was set so that the slotted section was just above this clay layer, resulting in the final depth for the wells varied slightly for any given plot. The top of each well pipe was cut close to the ground surface and sealed

with an orange-painted cap so that farm equipment could more easily avoid running over and damaging the wells.

Water levels were checked on every site visit for soil sampling and/or leaf tissue collection. No water was ever detected in this first set of installed 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 2-48), and soil porosity for this location (Table 2-16).

A second set of shallow wells were installed further downslope of the plots as indicated in Figure 2-50 (well designations 11A – 13B; A = upslope, B = downslope). These 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. Total distance to the field edge was ~ 30 m (~100ft). The assumption was made that subsurface lateral flow from the experimental plots would be toward the lower-elevation marsh area. The second set of shallow wells were installed using the same design as the first set with the exception that the average depth of each well was ~120 cm deep.

Figure 2-50. Location of shallow wells installed at the Whiteville Tobacco Research Station. (Photo modified by Scott King, Dept. of Soil Science, NCSU)

Sampling of the second set of shallow 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 meter (www.solinst.com).

Average water depth in the second set of shallow wells (designations 11A-13B) 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 event on May 26, 2009, (data not shown) and a 30 mm event on August 30, 2009 (Figure 2-48). There is a suggestion of an impact from a relatively large event at the end in June, 2009 (Figure 2-48) as well.

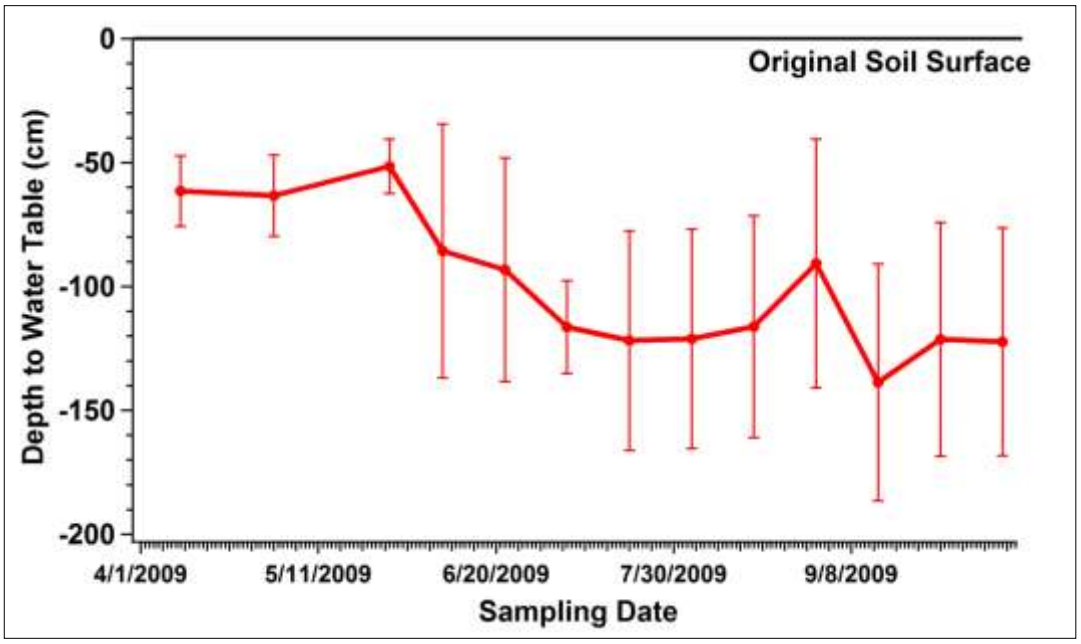

Figure 2-51. Average depth to groundwater as a function of date for wells installed downslope of field site at the Whiteville Tobacco Research Station. Vertical lines represent one standard deviation.

The transient rise in water depth observed for the two rain events suggests that the shallow water table at these locations does respond 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 Figure2-51 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 2-

48). 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 total.

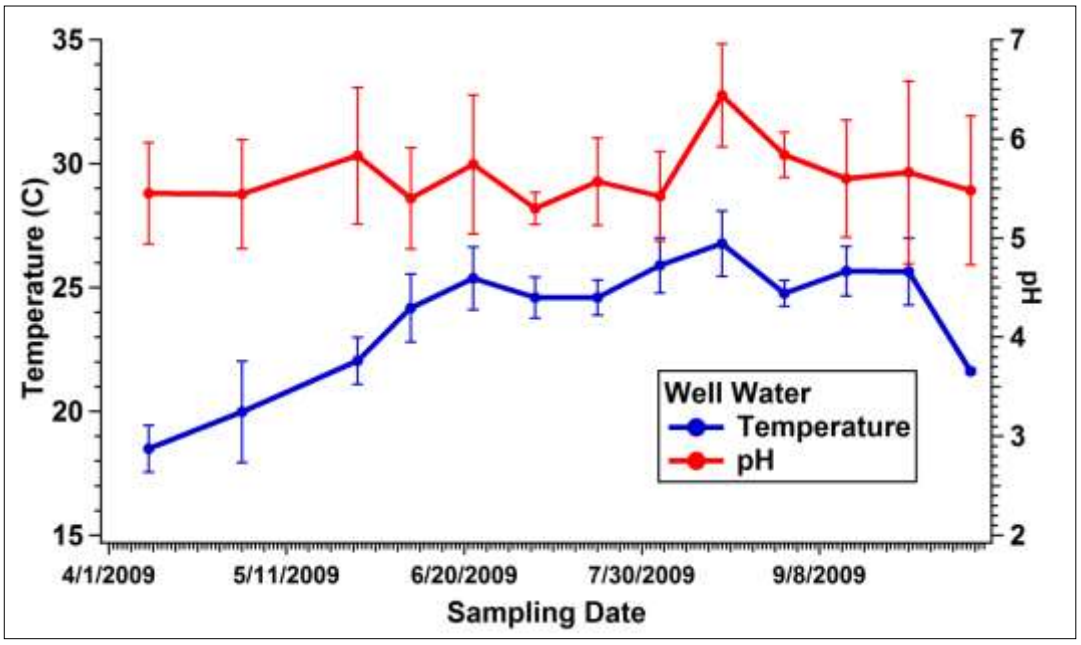

Figure 2-52. Average pH and temperature as a function of date for wells installed downslope of field site at the Whiteville Tobacco Research Station. Vertical lines represent one standard deviation.

Temperature of the shallow water table gradually increased during the growing season from ~ 20 °C to a maximum of ~ 27 °C on August 17, 2009 (Figure2-52). The maximum of 27 °C appeared to be related to a short term rise on water table height (Figure 2-51) and provides further support for direct interaction between water infiltrating through upper surface layers of soil and eventually reaching the underlying

water table. Overall, the water table temperature remained relatively constant at 25 ºC from late June to the end of September. There was a noticeable decline in water temperature at last sampling date in October.

Groundwater 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 the impact of soil water draining from upper soil layers and coming in contact with the underlying water table. The pH decreases back to the range 5.5 – 6.0 probably because of the dominance of nearby soil material surrounding the wells.

The measured ORP for the shallow water table was variable and displayed somewhat of a cyclic pattern during the study period (Figure 2-53). The highest ORP was observed right after well installation. In general, ORP tends to decline with time but subsequent events appear to raise the ORP, and then the values decline again. There is no consistent trend between the cyclic pattern in the ORP readings and rainfall events (Figure 2-51).

Dissolved oxygen (DO) in the shallow water table declined during the growing season, eventually reaching near zero values after mid-July 2009. 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 again to the bi-weekly nature of sampling the shallow water table, as well as time of transport of water through the soil profile. Overall, the data in Figure 2-53 suggest that denitrification of nitrate may have been taking place in the shallow water table located down slope of the field site but it would be equally likely to find nitrate present in the water table as well.

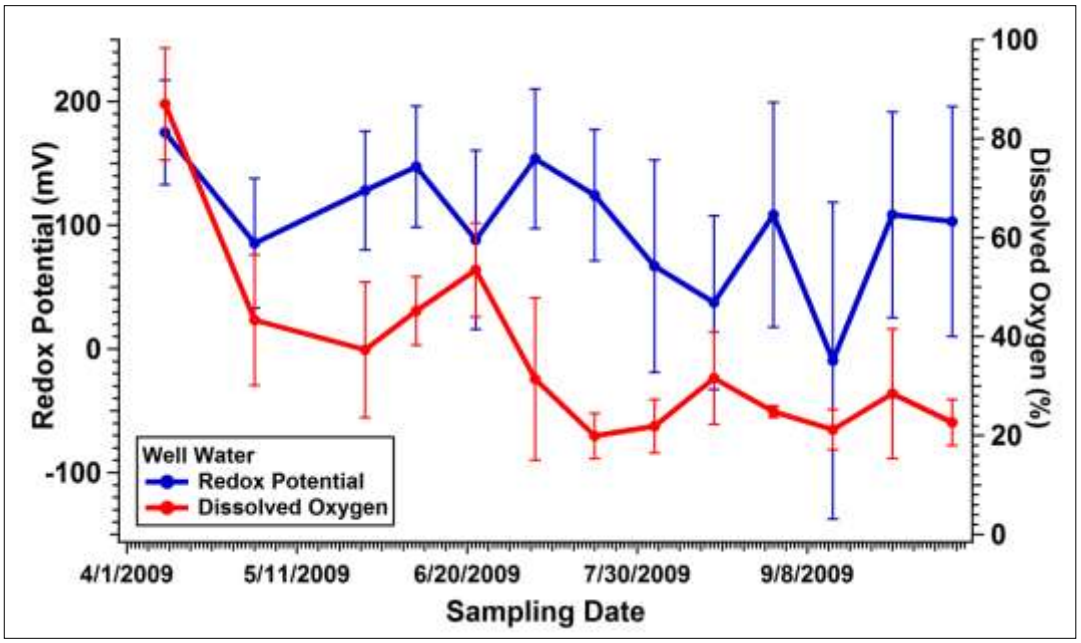

Figure 2-53. Average redox potential and dissolved oxygen content as a function of date for wells installed downslope of field site at the Whiteville Tobacco Research Station. Vertical lines represent one standard deviation.

Analysis of water samples from the shallow water table wells (designations 11A-12B) 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 Research Station, no perchlorate was detected in any of the well water samples collected using ion chromatography, although the presence of other anions in the well water 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 2.7.3.3 and will not be repeated here.

Upon further discussion with the superintendent at the Whiteville Tobacco Research Station, and consultation of station records, it was determined that the field location used for this study at the Whiteville site 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 water table 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 2.7.3.3, the presence of sulfate in the shallow water table raised the effective detection limit for perchlorate via ion chromatography.

A small subset of well water samples was sent to the Columbia Analytical Services Laboratory to check analyses of the well water samples by ion chromatography (Table 2-17). The stated detection limit for analyses by the Columbia Services Laboratory was 0.2 ppb (parts per billion = micrograms per liter). 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 2-17).

Table 2-17. Results of well water sample analyses for perchlorate by Columbia Analytical Services Laboratory at the Whiteville Tobacco Research Station.

SAMPLE	DATE	MATRIX	COMPOUND	MRL	Adjusted MRL	RESULT	FLAG
				----------µg/L----------			
WV-12B	05/01/09	WATER	PERCHLORATE	0.2	0.20	0.20	U
WV-13A	06/22/09	WATER	PERCHLORATE	0.2	0.20	1.3	
WV-13B	07/20/09	WATER	PERCHLORATE	0.2	0.20	0.27	
WV-12B	08/17/09	WATER	PERCHLORATE	0.2	0.20	0.20	U
WV-12B	09/14/09	WATER	PERCHLORATE	0.2	0.20	0.20	U
WV-11B	10/12/09	WATER	PERCHLORATE	0.2	0.20	0.20	U

Notes: Report Qualifiers for the FLAG column:
U = indicates compound was analyzed for but not detected.
E = indicates that the sample concentration exceeded the calibration range.

The detection of perchlorate in the shallow water table samples is consistent with timing of fertilizer applications at the Whiteville site, 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 shallow water table wells installed along the field edge. Well 11A always had nitrate present if there was water present in the well (mean NO_3-N concentration ~ 12 mg NO_3-N L^{-1}). At somewhat reduced concentrations, well 11B also was observed to contain nitrate in the water, 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^{-1}. 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 NO3-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 that may have resulted in denitrification.

Chloride was found to be present in the shallow water table sampled from all 6 wells (Table 2-19). 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 also observed for nitrate, suggests that the shallow water table sampled 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 did contain potassium chloride. The appearance of the chloride coincides with a preceding relatively large rainfall event.

Table 2-18. Nitrate concentration in shallow water table as a function of sampling date at the Whiteville Tobacco Research Station. DW = dry well.

	Well Designation					
Date	WV-11A	WV-11B	WV-12A	WV-12B	WV-13A	WV-13B
	----------mg NO3-N L^{-1} ----------					
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
%RSD	36	67	143	88	102	106

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 2-19. Chloride concentration in shallow water table as a function of sampling date at the Whiteville Tobacco Research Station. DW = dry well.

	Well Designation					
Date	WV-11A	WV-11B	WV-12A	WV-12B	WV-13A	WV-13B
	---------- mg Cl L^{-1} ----------					
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

2.7.3.4 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 upon the station superintendent'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 plots 1-8 were typical of harvests in the area that year according to the station superintendent (Table 2-20). 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 Plots 1-4 versus Plots 5-8. This difference was consistent throughout the growing season, especially for Plot 1-3 where smaller plant heights were observed for the entire plots over the course of the season. There was no obvious explanation for this reduction in plant growth in Plots 1-4. Consultation with the station superintendent indicated that there were no problems during transplanting or with fertilizer applications for these plots. 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 Plots 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 that Plots 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 sidedress of nitrogen. Results at the Oxford Research Station indicated that the potassium nitrate product does not have a negative impact on tobacco growth (Table 2-8).

Unlike the Oxford Research Station harvests, the mass of tobacco leaves harvested 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 research station is greater because of the difference in moisture contents between freshly harvested leaves (typically 70-80%) versus cured leaves (12-15%).

Table 2-20. Mass of harvested tobacco leaves from Plots 1-8. Results are for field-harvested tobacco (moisture content 70-80%). Whiteville Tobacco Research Station.

Plot	Harvest 1 7/27/2009	Harvest 2 8/17/2009	Harvest 3 9/17/2009	Harvest 4 9/24/2009	Totals
	----------kg/ha----------				
Nitrogen Source: Potassium Nitrate– Treatment 1					
Plot 1	2834	4502	3751	3835	14922
Plot 2	2401	2551	3501	2501	10954
Plot 3	2851	3535	4001	3835	14222
Plot 4	3335	3251	5085	4502	16172
Means	2855	3460	4085	3668	14067
Nitrogen Source: Ammonium Nitrate – Treatment 3					
Plot 5	3601	3668	4918	5168	17356
Plot 6	3234	3968	4251	5335	16789
Plot 7	3168	3718	5168	6336	18390
Plot 8	3284	4285	4335	4168	16072
Means	3322	3910	4668	3252	17152

2.7.3.5 Soil Chemical Analysis – Perchlorate

Pre-planting soil samples were obtained from Plots 1 – 10 on March 9, 2009 and March 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 Tobacco Research Station location (Table 2-21) and processed as outlined in the Materials and Methods section.

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 2.7.2.5

Table 2-21. Date, depth interval, and number of soil samples collected during 2009 field experiment. Location: Whiteville Tobacco Research Station.

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

A total of 5 soil samples were submitted to Columbia Analytical Services Laboratory for analysis of extractable perchlorate (Table 2-22). The soil samples were chosen at random from the various plots and sampling dates. No perchlorate above the stated method reference limit of 2 micrograms per kilogram by Columbia Analytical Services Laboratory was detected in the soils. These results further confirm the inability to detect perchlorate by ion chromatography, as the levels of perchlorate present are well below the stated detection limit using ion chromatography and the cited EPA protocol.

2.7.3.6 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 2-54). Ammonium-N appeared to be relatively uniform with depth, with Plots 1-8 have extractable NH_4-N from 1.5 to 2 mg NH_4-N kg^{-1}. The surface and deepest depth sampled had extractable NH_4-N values > 3 mg NH_4-N

kg^{-1} for Plots 9-10, but the mid-depth concentrations of NH_4-N were similar to values for Plots 1-8.

Table 2-22. Results of soil analyses for perchlorate by Columbia Analytical Services Laboratory at the Whiteville Tobacco Research Station.

SAMPLE	DATE	MATRIX	COMPOUND	MRL	Adjusted MRL	RESULT	FLAG
				----------µg/l----------			
WV-P1-15-30	03/09/09	SOIL	PERCHLORATE	2.0	2.0	2.0	U
WV-P6 – 0- 15	06/22/09	SOIL	PERCHLORATE	2.0	2.0	2.0	U
WV-P9-15-30	07/20/09	SOIL	PERCHLORATE	2.0	2.1	2.1	U
WV-P2-75-100	08/17/09	SOIL	PERCHLORATE	2.0	2.0	2.0	U
WV-P4-30-50	09/14/09	SOIL	PERCHLORATE	2.0	2.1	2.1	U

Notes: Report Qualifiers for the FLAG column:

U = indicates compound was analyzed for but not detected.

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^{-1}), and then soil concentrations increasing up to 3.5 – 5 mg NO_3-N kg^{-1} at the 150 cm depth. Below this depth, the range in concentrations across the plots was more variable but still exceeded 5 mg NO_3-N kg^{-1} for Plots 1-4. The overall distribution of soil NO_3-N is consistent with a possible restricted layer to water movement at the 100-150 cm depth. As noted in Section 2.7.3.3, installation of the shallow wells associated directly with Plots 1-10 (Figure 2-50) were positioned to a depth of ~ 100 cm, which was just above a shift in soil texture to clayey subsoil. Thus the pattern in soil NO_3-N with depth suggests fairly rapid vertical movement of soil water to the 100-150 cm depth, where a restricted layer would attenuate salt migration through the soil.

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 2-23. 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 Research Station (Table 2-11). This suggests that there had been a rapid diffusion/dispersion of NO_3-N away from the initial fertilizer bands, perhaps the result of rain event occurring right before the first soil sampling (Figure 2-47).

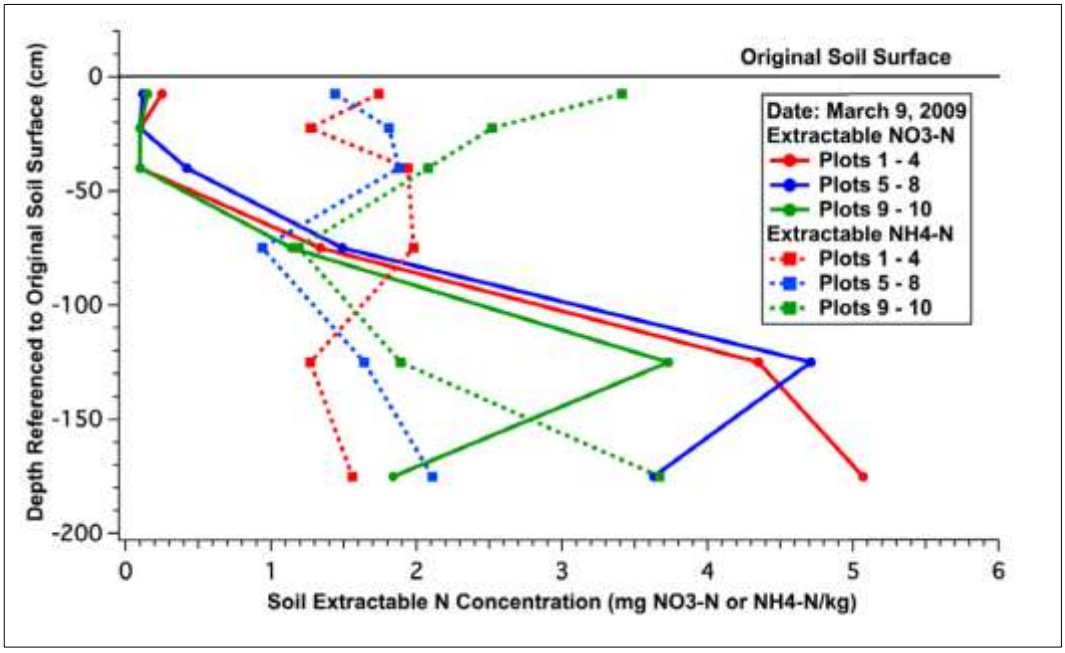

Figure 2-54. Mean soil extractable NO_3-N and NH_4-N concentrations as a function of depth increment for Plots 1-10 at the Whiteville Tobacco Research Station. Date: March 9-11, 2009. Mean values are plotted at mid-point of depth increment sampled.

Table 2-23. Mean soil concentrations of extractable NO_3-N as a function of depth increment sampled collected from June 8, 2009 to September 14, 2009 at the Whiteville Tobacco Research Station. Values in parentheses are calculated standard deviation for plots sampled. Depth increments are referenced to top of soil beds formed for tobacco transplants.

Depth	Sampling Date							
Increment	6/08/09	6/22/09	7/06/09	7/20/09	8/03/09	8/17/09	8/31/09	9/14/09
--cm--	----------mg NO_3-N/kg----------							
Plots 1-4 (Potassium Nitrate plus Tobacco) – Treatment 1								
0-15	4.4	0.74	2.0	0.54	1.6	2.1	0.92	0.72
	(2.8)	(0.7)	(0.6)	(0.7)	(1.1)	(0.9)	(0.8)	(0.3)
15-30	3.7	4.2	3.0	0.75	0.86	1.6	1.8	0.52
	(1.1)	(4.4)	(1.6)	(0.5)	(0.3)	(0.9)	(1.2)	(0.4)
30-50	6.5	4.5	5.2	1.1	0.70	0.69	0.72	0.50
	(3.0)	(2.1)	(1.6)	(0.2)	(0.3)	(0.5)	(0.9)	(0.4)
50-75	3.3	6.8	3.7	2.4	1.6	0.91	0.21	0.45
	(1.2)	(2.7)	(0.7)	(1.4)	(1.7)	(0.8)	(0.1)	(0.4)
75-100	1.6	3.5	2.7	1.1	1.5	1.9	0.36	1.7
	(0.6)	(0.8)	(0.8)	(0.7)	(1.0)	(1.6)	(0.5)	(1.6)
Plots 5-8 (Ammonium Nitrate plus Tobacco) – Treatment 3								
0-15	5.1	2.4	3.0	0.54	1.1	1.6	2.2	4.6
	(5.0)	(1.6)	(1.5)	(0.5)	(0.2)	(0.5)	(1.4)	(1.5)
15-30	7.1	5.0	4.7	2.0	0.76	1.3	2.9	5.9
	(5.6)	(0.5)	(2.4)	(1.8)	(0.5)	(0.2)	(1.1)	(1.4)
30-50	8.9	5.8	4.6	1.8	0.75	0.55	1.6	2.6
	(3.2)	(0.4)	(2.2)	(0.6)	(0.3)	(0.3)	(0.7)	(0.1)
50-75	2.8	3.4	2.4	2.5	0.76	0.79	0.59	0.17
	(0.7)	(0.7)	(1.5)	(2.7)	(0.8)	(0.5)	(0.8)	(0.1)
75-100	2.6	1.6	1.9	1.5	1.0	0.97	0.44	0.15
	(0.9)	(0.5)	(0.8)	(1.3)	(0.2)	(0.8)	(0.6)	(0.1)
Plots 9-10 (Potassium Nitrate minus Tobacco) – Treatment 2								
0-15	6.4	15.1	11.4	4.3	0.65	0.77	1.7	2.8
	(3.1)	(9.7)	(1.2)	(2.0)	(0.2)	(0.4)	(1.0)	(1.6)
15-30	15.5	8.2	5.8	7.8	2.0	2.9	5.3	4.7
	(11.0)	(0.6)	(0.6)	(2.4)	(1.1)	(0.5)	(3.7)	(2.7)
30-50	12.9	9.5	9.3	9.5	6.5	5.5	9.8	5.4
	(8.7)	(3.8)	(0.2)	(1.8)	(0.4)	(1.2)	(3.3)	(1.3)
50-75	4.7	8.3	5.3	6.5	5.4	9.7	8.8	3.0
	(0.9)	(2.0)	(0.3)	(4.0)	(1.3)	(7.7)	(7.5)	(1.7)
75-100	2.5	5.0	4.2	3.1	1.6	7.0	6.0	2.3
	(0.1)	(3.5)	(0.8)	(1.4)	(0.7)	(5.2)	(3.9)	(0.4)

As discussed briefly in Section 2.7.2.6, soil samples were obtained through the center of the soil mounds, while the fertilizer bands put down by the research staff were located to the side of the tobacco plants. Soil sampling was performed to avoid the fertilizer band. Thus the observed soil concentrations of extractable NO_3-N (and NH_4-N, especially for Plots 5-8) are a function of the degree of movement and/or diffusion of fertilizer N away from the fertilizer bands. In sandy surface soils, such as those at the Whiteville Tobacco Research Station, this movement could be relatively rapid given a significant rainfall event.

The mean concentration of extractable NO_3-N for Plots 1-4 as a function of sampling date is provided in Figure 2-55. For the 0-15 cm depth (top of the bed), 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 bed or, more likely, the majority of fertilizer N had moved vertically away from the top of the bed or was removed by plant uptake.

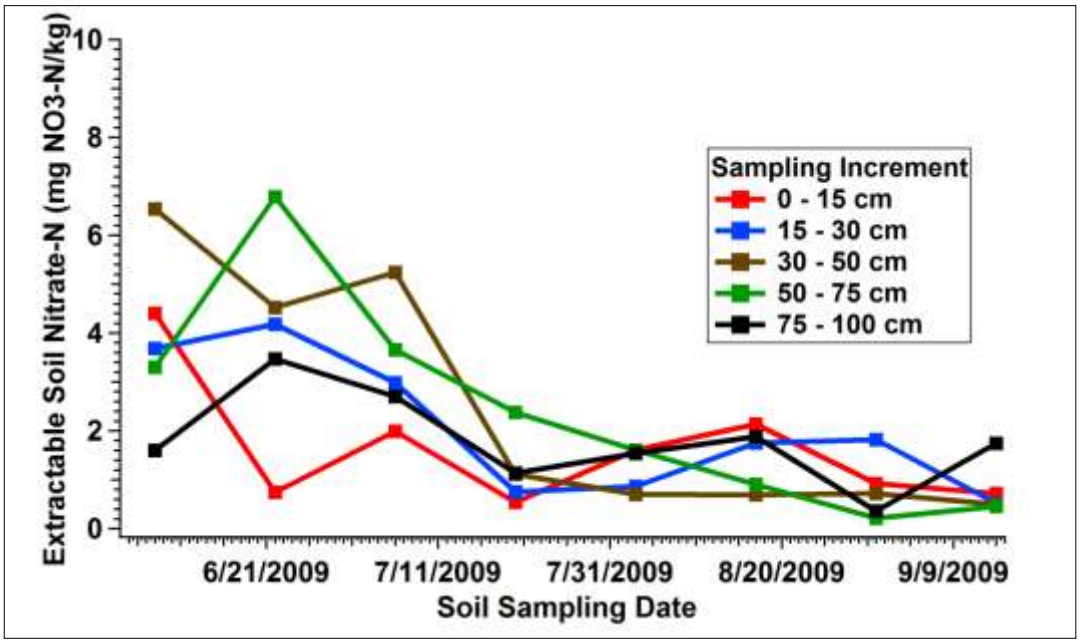

Figure 2-55. Mean concentrations of soil extractable NO_3-N for Plots 1-4 as a function of sampling date and depth increment at the Whiteville Tobacco Research Station. Sampling depth increments are referenced to top of soil beds.

For the remaining soil depths sampled, the patterns in extractable NO_3-N with soil sampling date suggest fairly rapid downward movement of NO_3-N through the soil, with even an increase in soil NO_3-N concentration being detected at 75-100 cm depth (relative to the top of the bed) by the second sampling date, June 22, 2009. For the remaining sampling dates, extractable NO_3-N declined in a similar pattern across the deeper depths sampled with values reaching < 2 mg NO_3-N kg^{-1} by July 31, 2009.

The mean concentration of extractable NO_3-N for Plots 5-8 as a function of sampling date is provided in Figure 2-56. By the first soil sampling date, the mean concentrations of extractable NO_3-N were already higher in the 15-30 (bottom of the bed) and 30-50 cm depths than in the top of the bed (0-15 cm depth). There is also a suggestion of elevated NO3-N at the 50-75 and 75-100 cm depths. As for Plots 1-4, the data in Figure 2-56 suggest that there was rapid movement of NO_3-N through the soil

profile substantially impacting soil concentrations at least to the 50 cm depth, relative to the top of the bed.

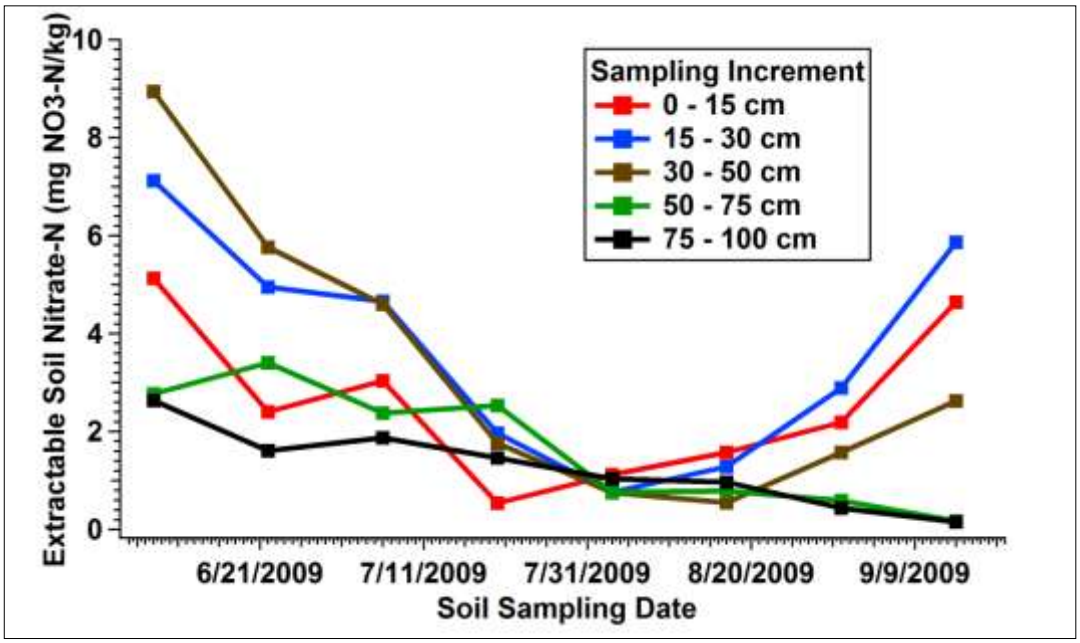

Figure 2-56. Mean concentrations of soil extractable NO_3-N for Plots 5-8 as a function of sampling date and sampling depth increment at the Whiteville Tobacco Research Station. Sampling depth increments are referenced to top of soil beds.

Soil extractable NO_3-N declined uniformly with depth and sampling date in Plots 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 2-20). 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 bed. It is not known why a

similar pattern was not observed for Plots 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 Plots 9-10 as a function of sampling date is provided in Figure 2-57. In the absence of tobacco plants, the pattern in NO_3-N in the top of the bed (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. Rapid interaction with the deeper depths sampled, however, 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 deeper within the soil profile, especially after the end of July, 2009. Across the 50-100 cm depth, relative to the top of the bed, 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.

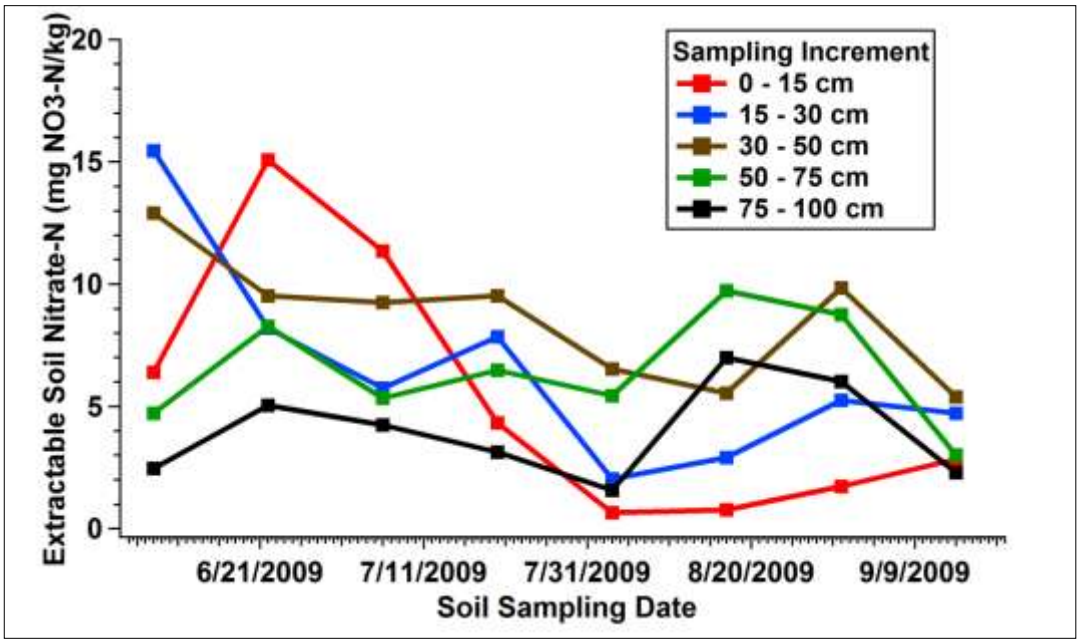

Figure 2-57. Mean concentrations of soil extractable NO_3-N for Plots 9-10 as a function of sampling date and sampling depth increment at the Whiteville Tobacco Research Station. Sampling depth increments are referenced to top of soil beds.

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 Plots 5-8. There is no such trend for the 30-50 cm depth. This increase cannot be attributed to mineralization of dead tobacco roots. Weeds were present on Plots 9-10 and herbicide was used several times during the growing season to control weed growth. The increase in soil NO_3-N may, therefore, be due to the mineralization of weed roots, which may have penetrated only the 0-30 cm depth of the soil bed.

The vertical distribution of extractable soil NO_3-N from Plots 1-10 as a function of date of sampling are shown in Figures 2-58, 2-59 and 2-60. Figure 2-58 illustrates that for Plots 1-4, by the first sampling date (June 8, 2009) a substantial portion of the soil NO_3-N appears to have moved below the bottom of the bed. Subsequent sampling dates

suggest continued movement downward, although the data also indicate considerable variability in NO_3-N concentrations as the plume moved vertically. In general, the majority of extractable NO_3-N not taken up by plant growth appears to have passed the 100 cm depth, relative to the top of the bed, by August 3, 2009.

For Plots 5-8, the trend in extractable soil NO_3-N is more consistent over time, but also still demonstrates the apparent rapid movement of a substantial amount of NO_3-N out of the bottom of the bed by the first sampling date (Figure 2-59). Soil NO_3-N concentration continues to decline in the soil throughout the growing season, reaching a minimum on August 3, 2009. Beyond this date, there is a consistent increase of soil NO_3-N in the upper 50 cm of the soil profile. Figure 2-59 suggests the source of this new NO_3-N began to release or form NO_3-N after August 3, 2009.

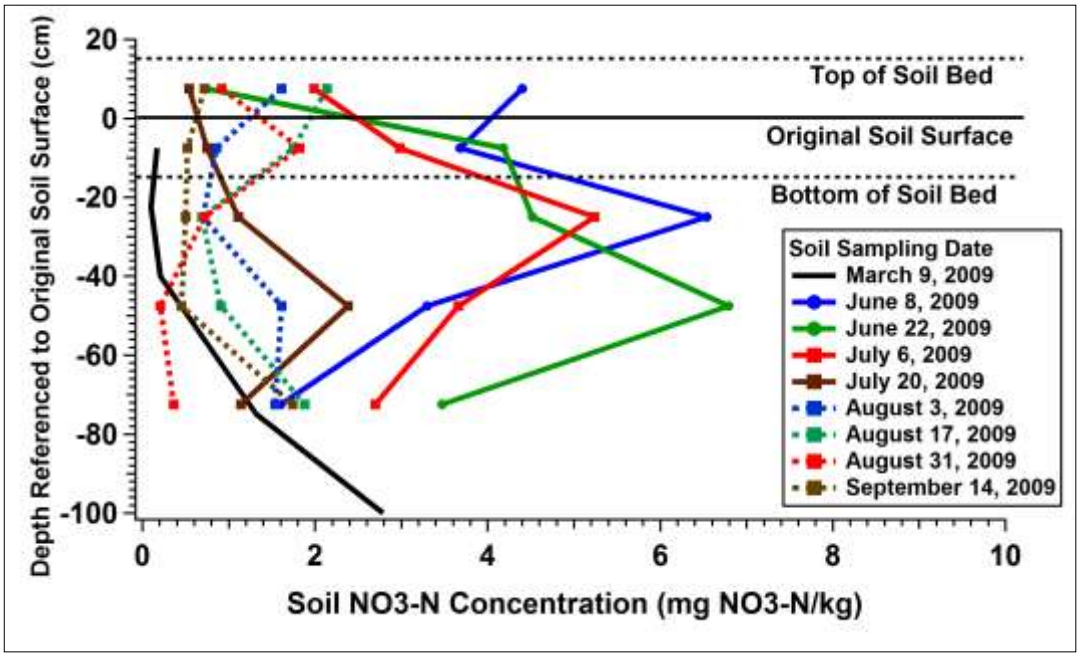

Figure 2-58. Vertical distribution of extractable NO_3-N from Plots 1-4 (Treatment 1) as a function of date of sampling at the Whiteville Tobacco Research Station. Soil samples obtained on March 9-11, 2009 are referenced to the original soil surface, all other samples referenced to the top of soil bed.

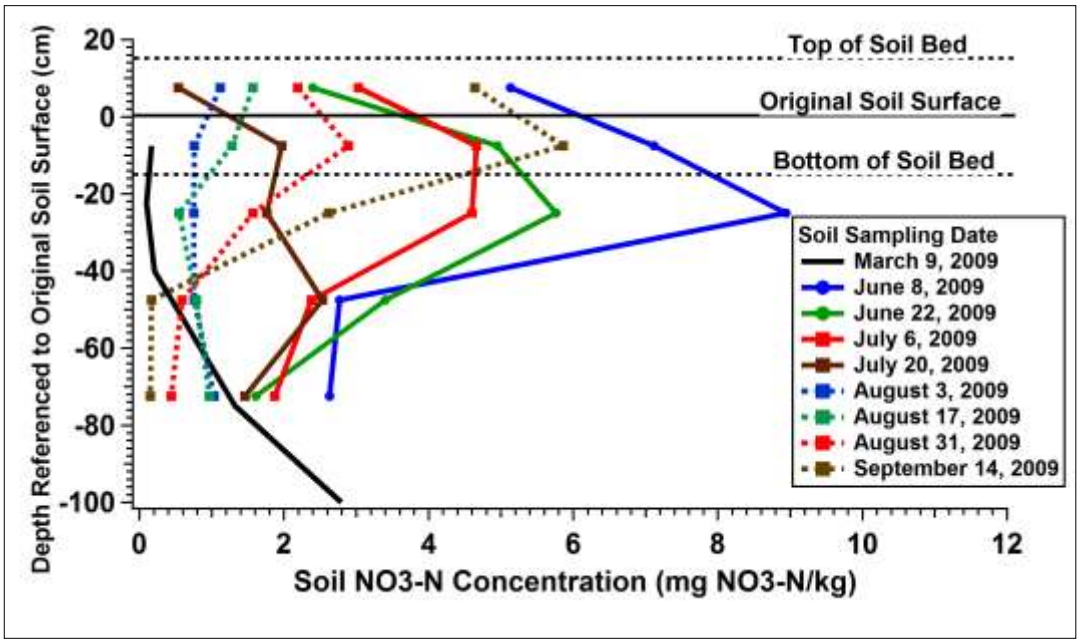

Figure 2-59. Vertical distribution of extractable NO_3-N from Plots 5-8 (Treatment 3) as a function of date of sampling at the Whiteville Tobacco Research Station. Soil samples obtained on March 9-11, 2009 are referenced to the original soil surface, all other samples referenced to the top of soil bed.

As for Plots 5-8, the trend in extractable soil NO_3-N is more consistent over time in Plots 9-10 than in Plots 1-4 (Figure 2-60). By the first sampling date, there is a significant concentration of NO_3-N in the bottom portion of the bed. This peak in soil NO_3-N continued to move vertically with subsequent increases in extractable NO_3-N below the bottom of the soil bed at deeper depths. By August 17, 2009, the NO_3-N plume has reached the 75 cm depth, relative to the top of the bed, with the majority of NO_3-N moving past the 100 cm depth by September 14, 2009.

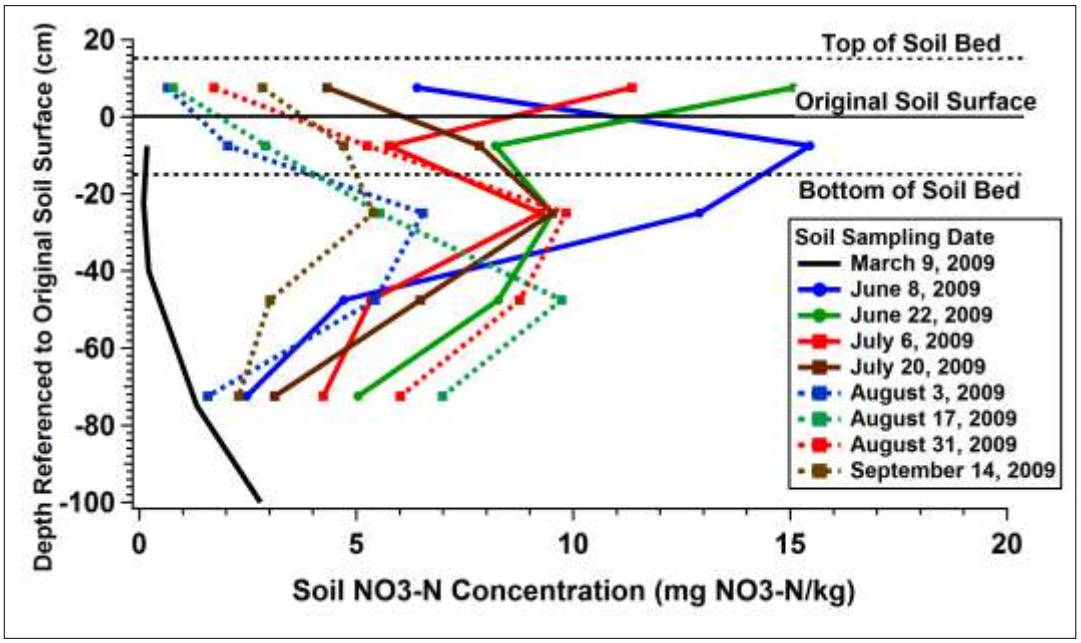

Figure 2-60. Vertical distribution of extractable NO_3-N from Plots 9-10 (Treatment 2-Bare Soil) as a function of date of sampling at the Whiteville Tobacco Research Station. Soil samples obtained on March 9-11, 2009 are referenced to the original soil surface, all other samples referenced to the top of soil bed.

Figures 2-58, 2-59 and 2-60 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 bed 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 bed. 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 of second wave of NO_3-N that would move or diffuse away from the fertilizer band. This may explain why there is more apparent

variability if NO_3-N in the top of the bed in Figures 2-58, 2-59 and 2-60. 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.

The mean soil concentrations and calculated standard deviations of extractable NH_4-N grouped by treatment plots for the soil samples collected post-planting as a function of depth increment sampled are provided in Table 2-24. The majority of calculated mean concentrations of extractable soil NH_4-N for Plots 1-10 were in the same range as first observed for the pre-plant soil sampling in March 9, 2009. Relative higher concentrations of NH_4-N were observed at the first and second soil sampling for Plots 5-8, but this was expected given that ammonium nitrate was used for the sidedress application of N when the potassium nitrate was applied to Plots 1-4 and Plots 9-10. The general absence of extractable NH_4-N in the soil profile supports the assumption that fertilizer N supplied in the form of NH_4-N is rapidly assimilated by plant uptake or converted to NO_3-N in the soil.

Higher concentrations of extractable NH_4-N were observed for Plots 1-10 for the August 17, 2009, soil sampling, and for Plots 1-4 and Plots 9-10 for the September 14, 2009 sampling. As noted in the discussion for NO_3-N, the reason for the appearance of this relatively "new" N late in the growing season is not immediately evident. One possible explanation is that production of NH_4-N was stimulated by the harvesting of the tobacco that occurred on July 27, 2009. This however, does not explain the presence of similar amounts of extractable NH_4-N in Plots 9-10. Stimulation of NH_4-N through mineralization of dead plant roots also does not explain why elevated NH_4-N was detected on the September 14, 2009 sampling date for Plots 1-4 and Plots 9-10, but not Plots 5-8.

Table 2-24. Mean soil concentrations of extractable NH_4-N as a function of depth increment sampled collected from June 8, 2009 to September 14, 2009 at the Whiteville Tobacco Research Station. Values in parentheses are calculated standard deviation for plots sampled. Depth increments are referenced to top of soil beds formed for tobacco transplants.

Depth	Sampling Date							
Increment	6/08/09	6/22/09	7/06/09	7/20/09	8/03/09	8/17/09	8/31/09	9/14/09
--cm--	----------mg NH4-N kg^{-1} ----------							
Plots 1-4 (Potassium Nitrate plus Tobacco) – Treatment 1								
0-15	2.0	1.8	1.2	1.3	1.3	2.4	1.0	4.0
	(0.7)	(0.0)	(0.1)	(0.1)	(0.3)	(0.5)	(0.1)	(1.4)
15-30	1.4	1.5	1.2	1.2	1.3	2.8	1.1	4.5
	(0.3)	(0.1)	(0.2)	(0.1)	(0.1)	(0.8)	(0.1)	(1.9)
30-50	1.4	1.0	0.9	1.0	1.2	2.5	1.0	3.6
	(0.8)	(0.2)	(0.1)	(0.2)	(0.1)	(0.6)	(0.3)	(1.3)
50-75	1.4	0.9	0.7	0.8	1.0	2.1	1.1	3.5
	(0.2)	(0.4)	(0.1)	(0.3)	(0.5)	(0.2)	(0.6)	(0.3)
75-100	0.9	1.1	0.8	1.0	1.4	2.9	1.9	4.1
	(0.6)	(0.1)	(0.2)	(0.3)	(0.4)	(1.0)	(0.8)	(1.5)
Plots 5-8 (Ammonium Nitrate plus Tobacco) – Treatment 3								
0-15	4.2	2.8	2.0	1.2	1.6	2.4	1.3	1.7
	(3.7)	(1.0)	(0.7)	(0.2)	(0.3)	(0.2)	(0.2)	(0.5)
15-30	2.8	1.4	1.8	2.1	1.4	2.1	1.1	1.6
	(2.8)	(0.3)	(0.3)	(2.0)	(0.1)	(0.2)	(0.2)	(1.2)
30-50	1.3	1.1	1.0	1.3	1.2	1.8	1.0	1.6
	(0.3)	(0.2)	(0.1)	(1.1)	(0.3)	(0.2)	(0.1)	(1.4)
50-75	1.0	1.1	0.9	0.6	1.1	2.3	1.6	3.8
	(0.2)	(0.3)	(0.2)	(0.2)	(0.3)	(0.3)	(1.0)	(0.5)
75-100	2.0	1.1	1.3	0.8	2.2	2.1	2.7	3.1
	(1.3)	(0.1)	(0.5)	(0.1)	(1.2)	(0.2)	1.4	(0.6)
Plots 9-10 (Potassium Nitrate minus Tobacco) – Treatment 2								
0-15	1.5	1.2	1.4	0.9	1.1	2.7	0.9	3.2
	(0.0)	(0.1)	(0.5)	(0.0)	(0.0)	(0.1)	(0.1)	(1.0)
15-30	1.3	1.3	1.3	0.7	1.2	2.1	1.0	3.3
	(0.0)	(0.1)	(0.3)	(0.0)	(0.1)	(0.0)	(0.1)	(0.6)
30-50	1.1	1.2	1.0	0.7	1.1	2.1	0.8	2.2
	(0.1)	(0.3)	(0.5)	(0.0)	(0.2)	(0.1)	(0.1)	(0.6)
50-75	1.3	1.0	1.2	0.6	1.6	3.4	1.0	3.4
	(0.4)	(0.2)	(0.8)	(0.0)	(0.2)	(0.5)	(0.6)	(1.3)
75-100	1.6	1.5	1.1	1.2	1.3	3.0	1.8	4.7
	(0.3)	(0.9)	(0.1)	(0.0)	(0.0)	(0.6)	(0.4)	(1.0)

In fact, elevated NH_4-N was observed for the 50-75 and 75-100 cm depths for Plots 5-8 and there was elevated NO_3-N observed in the upper soil depth increments sampled (Figure 2-56). These results suggest that no increase in NH_4-N was readily evident for Plots 5-8 on the September 14, 2009 sampling date because the majority of NH_4-N in the upper soil depths had already been converted to NO_3-N. The mineralization of NH_4-N in Plots 1-4 and Plots 9-10 was delayed relatively to Plots 5-8 and thus the NH_4-N was found to be present in these soils. A similar delay may have occurred at the deeper soil depths in Plots 5-8. This speculation is consistent with the trends observed in NO_3-N late in the growing season in the upper soil depths of the soil profile.

The above explanation regarding the appearance of NH_4-N in Plots 9-10 is only valid if it can be assumed that roots from dead weeds was the source of the relative increase in NH_4-N. If this assumption is not valid, then an alternative explanation is that the NH_4-N detected, and thus the additional NO_3-N detected, was derived as an artifact of soil sampling late in the growing season. Since soil samples were taken from the center of the soil bed, the proportion of root material captured in the soil samples should increase as the growing season progresses, especially later in the growing season when root mass should reach a maximum. It was not possible to immediately freeze the soil samples during soil sampling. Thus formation of NH_4-N and also possibly NO_3-N may have occurred in the soil samples before they were air-dried. This explanation, however, still requires that a similar amount of root material had to be present in Plots 9-10 in order to produce NH_4-N in the soil samples.

2.7.3.7 Tobacco N Content and Removal

A total of 4 harvests were obtained from the field site at the Whiteville Tobacco Research Station in 2009 (Table 2-25). The moisture content ranged from 70-80% in the harvested leaves and the % N concentration ranged from 0.46 to0.64%, with the majority

of values across Plots 1-8 < 0.5 % N in the harvested leaves. No significant differences between Plots 1-4 and 5-8 were noted in moisture content or % N concentration in the harvested leaves.

Table 2-25. Nitrogen and moisture content (expressed on a wet-weight basis) of tobacco samples during 2009 field experiment at the Whiteville Research Station.

		Lower Leaves		Upper Leaves	
Date	Sample Type	N-Content Wet-Wt. Basis	Moisture Content Wet-Wt. Basis	N-Content Wet-Wt. Basis	Moisture Content Wet-Wt. Basis
		---------- % ----------			
Plots 1-4 Nitrogen Source: Potassium Nitrate - Treatment 1					
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
Plots 5-8 Nitrogen Source: Ammonium Nitrate - Treatment 3					
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 removal by the harvested tobacco leaves is presented in Table 2-26, based on the provided mass of leaves harvested as determined by the Whiteville Tobacco Research Station staff in the field (Table 2-20), and the analyses of leaf punches obtained from the harvested leaves (Table 2-25). The percent moisture content for each harvest (Table 2-25) was used to express the calculated results on an oven-dry weight basis.

As for the calculated N removal for the Oxford Research Station (Table 2-7), the calculated removal of fertilizer N at the Whiteville Station approached the total amount of fertilizer N added as complete fertilizer and via the sidedress application. Expressed as a percentage, the amount of N removal in the harvested leaves was 83% for Plots 1-4, and 106% for Plots 5-8.

Table 2-26. Mass of nitrogen recovered in harvested tobacco leaves at the Whiteville Tobacco Research Station. All calculations normalized to an oven-dry weight basis.

Harvest Date	Harvested Leaf Mass	Percent N Concentration	Mass of N Recovered	
	-- kg/ha --	-- % --	- kg N/ha-	- lbs N/Ac-
Plots 1-4 Nitrogen Source: Potassium Nitrate – Treatment 1				
28-JUL-09	868	2.03	17.6	15.7
17-AUG-09	1111	1.74	19.3	17.2
17-SEP-09	1013	1.99	20.2	18.0
24-SEP-09	767	2.22	17.0	15.2
Totals	3758	-	74.1	66.0
Plots 5-8 Nitrogen Source: Ammonium Nitrate – Treatment 3				
28-JUL-09	1036	2.01	20.8	18.6
17-AUG-09	1310	1.96	25.7	22.9
17-SEP-09	990	2.02	20.0	17.8
24-SEP-09	1113	2.23	24.8	22.1
Totals	4449	-	91.3	81.3

Leaf punchout samples taken for chemical analyses used the same protocol as at the Oxford Station, therefore calculations based on these samples would be subject to the same potential positive bias as discussed for the calculated N removal at the Oxford Station (Section 2.7.2.7). Removal of N in the harvested leaf tissue exceeding 100% of the fertilizer N added strongly suggests an additional source of N was available at the Whiteville Station to support tobacco growth. As noted above, the possible source of this additional N is discussed further in the following section (Section 2.8).

2.8 **Conclusions and Implications for Future Research & Implementation**

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. Prior to the mid-1980's, 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 on whether the current issue of wide spread perchlorate contamination to the underlying groundwater or other shallow aquifers 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 behavior of perchlorate resulting from the application of perchlorate-containing fertilizer to tobacco crop 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). However, due to reasons explained above, it was decided to use potassium nitrate provided by the SQM Corporation as an alternative due to the unavailability of nitrate of

soda product. Hence, the expected perchlorate concentration in soil and water samples was expected to be less pronounced as the approximate concentration of perchlorate in potassium nitrate is at least an order magnitude less than the original nitrate of soda product.

The study was designed to encompass all the accessible routes for possible perchlorate contamination, involving the underlying groundwater or shallow aquifers, soil and soil water, as well as the potential uptake by plants. We also considered nitrate as the possible surrogate for predicting the movement of perchlorate should the amount of perchlorate applied using the potassium nitrate product prove too low for a quantitative assessment.

The experiments were conducted according to standard procedures for analysis of perchlorate as described in the Materials and Methods section. All the necessary actions were taken to ensure the credibility of the results obtained. An external laboratory was employed to provide confirmation on the data collected. Further internal assessments were done by standard addition method or spike addition.

The analysis of potassium nitrate fertilizer performed at North Carolina State University found no detectable perchlorate and the subsequent analysis done by Columbia Analytical Service 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.

Perchlorate and nitrate is 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 depth of 0-75 cm (referenced to the top of the beds) of each plot for Oxford Tobacco Research 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.

The soil moisture data for Whiteville Tobacco Research Station showed that the vertical movement was prominent compared to Oxford Tobacco Research 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 Tobacco Research 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 Tobacco Research Station versus the Oxford Tobacco Research Station. However, no water was ever detected in shallow wells at the Whiteville site 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.

Deep groundwater wells (wells extending to bedrock) could only be installed at the Oxford Tobacco Research Station. The average temperature and pH of groundwater of Oxford Tobacco Research Station ranged from 18 ^{0}C to 21 ^{0}C and 7.0 to 8.3 respectively. Both the redox potential and dissolved oxygen measurements indicated an oxygenated environment. The average temperature and pH of groundwater of Whiteville Tobacco Research Station ranged from ~20 ^{0}C to ~27 ^{0}C and 5.5 to 6.5 respectively. The measured oxidation reduction potential for the shallow water table was variable, and the dissolved oxygen concentrations steadily decreased during the latter half of the growing seasons suggesting that denitrification of nitrate possibly occurred in the shallow water table. Rivett et al. (2008) noted that in a review of the literature, denitrification would be favored in the pH and temperature ranges observed for the groundwater wells at both study sites. However, denitrification typically will not occur when dissolved oxygen contents are > 2-4 mg O_2 L^{-1}. Therefore, conditions at the Oxford site did not appear to favor denitrification during the 2009 growing season, while denitrification may have occurred at the Whiteville site during late summer and early fall.

The no perchlorate was detected in well water samples from the Oxford Tobacco Research Station analyzed using ion chromatography. Other anions such as nitrate, chloride, sulfate and phosphate were present. A lack in temporal trends in chloride concentrations, as well as for the two wells with detectable nitrate, suggested that the groundwater being sampled by the six groundwater wells was not directly influenced by the surface applied fertilizers used in this study. However, it should be noted that chloride content of fertilizers used for tobacco production are low and fixed by state law

to protect the quality of the product. The only other source of chloride would be the fumigants used before transplanting to prepare the soil beds.

There was also no perchlorate detected in well water samples from Whiteville Tobacco Research Station and the data obtained showed that the presence of other anions such as nitrate, chloride, phosphates and sulfates cannot be linked entirely to the fertilizer applied for the study. These results suggest that if the fertilizer used in this study had a slightly higher perchlorate concentration, there is still the possibility that the perchlorate will not reach the underlying groundwater. If the perchlorate is present in the groundwater, it has to be within a reasonable quantitative range to be detected via ion chromatography as the detection limit of perchlorate was substantially increased due to the relatively high amounts of dissolved salts in the well water samples.

The results from the well water sampling in this study support the conclusion that there is not a reservoir of perchlorate in groundwater underlying areas in NC historically used for tobacco production, especially flue-cured tobacco. The extent to which perchlorate may have been destroyed through redox processes in the groundwater is not known. Shrout and Parkin (2006) have demonstrated that bacteria degradation of perchlorate is dependent primarily on the concentration of the electron donor (typically dissolved carbon) and is not a simple function of redox potential. They observed destruction of perchlorate in the range from -220 to +180 mV, which is within the range of most of the redox measurements in this study. They also noted the apparent continued destruction of perchlorate even in the presence of some dissolved oxygen, provided a sufficient alternative supply of an electron donor was present. More oxidized conditions did inhibit destruction of perchlorate. The range in redox and dissolved oxygen observed for groundwater in this study suggest further research under more controlled conditions is justified to determine to what extent perchlorate derived through the historical use of nitrate of soda products was destroyed in the underlying groundwater via redox processes.

No perchlorate was 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 (Columbia Analytical Service). Extractable NO_3-N data obtained for soil samples from Oxford Tobacco Research Station showed almost similar patterns for Plots 1-4 and Plots 5-8 regardless the different type of fertilizers used: potassium nitrate versus calcium nitrate. Plots 9-10 displayed trends similar to Plots 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 Tobacco Research Station were in general an order of magnitude less than those observed at the Oxford Research Station and the data showed different patterns for Plots 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 Plots 5-8 after the first and second harvest dates tended to increase relative to Plots 1-4. No immediate explanation is available, although overall tobacco yields were also lower for Plots 1-4. Lower yields may be due to differences in drainage across the plots 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 denitification but on a time scale not detectable with the bi-weekly observations used in this study. The data for Plots 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.

Throughout the study, soil extractable NH_4-N was relatively low at both research stations, even though ammonium nitrate was the source of fertilizer used for the sidedress N application in Plots 5-8 at the Whiteville station. One explanation for these observations is the rapid assimilation of NH_4-N by plant uptake and/or conversion to NO_3-N in the soil. However, the presence of NH_4-N throughout the growing season

could also be indicative of mineralization of soil organic matter resulting in a supply of additional N for plant growth.

Yields of tobacco at the Oxford Tobacco Research Station from Plots 1-8 were comparable and the high end of the expected range for flue-cured tobacco in NC (4700-4850 kg ha^{-1} at). The total mass of tobacco leaves produced and the N content between harvests and plots appeared not to be influenced by the source of fertilizer used for the sidedress N application (potassium nitrate versus calcium nitrate).

Yields of tobacco collected from Whiteville Tobacco Research Station averaged 14100 kg ha^{-1} for Plots 1-4 and 17200 kg ha^{-1} for Plots 5-8 (70-80% moisture content). Expressed on a flue-cured basis (~ 12-15% moisture content) these fresh weights correspond to 4100 kg ha^{-1} and 4970 kg ha^{-1}, respectively. Yields from Plots 5-8 were comparable to those observed at the Oxford Tobacco Research Station. As noted, poor drainage may account for the lower yields observed for Plots 1-4, although even at 4100 kg ha^{-1}, the yield is still relatively high compared to typical expectations of 3600 kg ha^{-1} from farmer fields (Dr. Loren Fisher, Tobacco Extension Specialist, Department of Crop Science, NC State University, personal communication).

Removal of N by the tobacco crop must be considered in using NO_3-N as a surrogate for fate and transport of perchlorate. The amounts of N removed by the tobacco crops was higher than expected based on the assumption that typical fertilizer N uptake recoveries vary from 30 to possibly 50% for the amount of fertilizer N applied. Taken at face value, the calculated N removal by the tobacco crop would suggest little or no NO3-N loss/leaching from the upper surface of the soils. As a surrogate for perchlorate this would imply all the applied perchlorate was incorporated into the tobacco plants, leaving none for leaching to groundwater. Such an interpretation, however, is unrealistic based on common knowledge of N use efficiency for row crops such as tobacco.

A more suitable explanation for the calculated amounts of N removed by the tobacco crops is the presence of a second source of available N in the soils to support plant growth. Soil sampling prior to planting in March 2009 suggested there was little residual N in the soils with extractable concentrations typically ~ 1 mg kg^{-1} or less. For the range in soil bulk densities in this study, this corresponds to ~ 5 kg N ha^{-1}. Tobacco roots are capable of extending deeper into the soil, provided there is no restrictive zone to root growth, or the restrictive zone has been altered through deep tillage. In addition, soil extractable NH_4-N was consistently measured at ~ 2 mg NH_4-N kg^{-1} throughout the growing season. This corresponds to a value of ~ 10 kg N ha^{-1}. Thus it is reasonable to assume that mineralization of soil organic matter maintained a supply of ~ 10 kg N ha^{-1} to support plant growth throughout the growing season, and that the tobacco crops took advantage of this additional N source. Such a conclusion provides a reasonable explanation for the N removal calculated for the tobacco crops, while still preserving the use of soil nitrate as a surrogate for transport of perchlorate through the soil during the growing season. No zero N control was included in this study, as the emphasis was on perchlorate fate and transport. Hardy (1986) reported yields of 1690 and 2430 kg ha^{-1} for zero N treatments from two sites located on coastal plain soils similar to those found at the Whiteville Tobacco Research Station, and observed optimum yields at only 50 kg N ha^{-1} as opposed to the rates of near 90 kg N ha^{-1} in this study. These results support the conclusion that the combination of soil mineralization of N during the growing season, combined with the potential deeper root development by the tobacco plants if soil conditions allow, explain the amounts of N removal calculated.

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 source of perchlorate cannot be completely rejected. The lack of data availability on the historical records regarding experiments and sources and amounts of fertilizer applied at both research stations, can be taken as

another reason to maintain the precaution measure on the potential impact of long term Chilean nitrate fertilizer application to agricultural sites.

Based on the application rate of potassium nitrate fertilizer for both sites, the potential concentration of perchlorate applied to the sites in Oxford Tobacco Research Station and Whiteville Tobacco Research Station can be projected as within the range of 208 - 347 mg ClO_4^- /plot (7910- 13232 mg ClO_4^- ha^{-1}) and 238 - 397 mg ClO4/plot (9092- 15204 mg ClO_4^- ha^{-1}), respectively, assuming the perchlorate content of the potassium nitrate ranged from 0.003 to 0.005%.

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 could probably be useful to compensate the lack of available historical data for the assessment on perchlorate contamination and for further understanding on the fate of perchlorate in the environment.

As mentioned earlier, perchlorate is very soluble. Thus the perchlorate concentration is most likely to be associated with leaching, rainfall, plant uptake and other environmental actions. Current limited understanding on the behavior of perchlorate including the possible natural attenuation of perchlorate degradation provide more challenges toward the establishment of promising approaches to quantify the potential impact of perchlorate contamination resulted from agricultural practices.

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 more historical levels and/or the use of other anion tracers such a bromide whose presence and transport though the soil will not be confounded by other soil processes.

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3. CHAPTER 3: LITERATURE REVIEW (CADMIUM)

3.1 **Introduction**

Cadmium has been identified as one of the toxic elements causing worldwide environmental problems over the past several decades. A note-worthy early discovery of cadmium toxicity effect to humans occurred in Japan more than 40 years ago. There cadmium was found to be responsible for a disease called “itai-itai” that resulted in kidney failure and osteoporosis due the consumption of contaminated rice. The origin of cadmium was industrial wastes (Van Kauwenbergh S.J. (2002); (Krishnamurti et al., 2005)). Cadmium contamination was also reported in the United States; in 1998, more than 700 hazardous waste sites with high levels of cadmium were identified (NRC, 2005).

Exposure of cadmium to humans are mostly due to consumption of foods containing cadmium and smoking tobacco (Moulis and Thévenod, 2010; Unkiewicz-Winiarczyk et al., 2009). Various levels of cadmium have been found in edible plants such as cocoa, rice and wheat grown on various soils (Kirkham, 2006). Other than plants, cadmium has also been found in mollusks and crustaceans such as oysters (Copes et al., 2008). High levels of cadmium exposure to humans have caused damage to the liver, as well as the kidney. Recent studies have suggested possible links to cancer and cardiovascular diseases (Moulis and Thévenod, 2010).

Cadmium is a soft, ductile silver white metal located in the transition metals group in the periodic table. It exists primarily as a divalent species which forms complexes with other compounds such as ammonia and cyanide or precipitates in the presence of anions such as carbonates and phosphates (Evanko and Dzombak, 2000). Cadmium is widely used in many products such as batteries and automobile parts. It is mainly produced as a byproduct during the smelting of other metals (Mead, 2010).

However other sources contribute to the overall cadmium found in the environment such as from soil-applied phosphate fertilizer and sewage sludge (Lugon-Moulin et al., 2006; McBride and Cherney, 2004). Although most significant sources of cadmium contamination are based on anthropogenic activities, the natural occurrence of cadmium due to certain types of rocks or materials found in the soil such as basalt and shale can also be accounted towards total cadmium contamination although the contribution is less compared to other inputs noted here (Van Kauwenbergh S.J., 2002).

Various guidelines and regulations have been put forth to limit exposure to cadmium. The World Health Organization (WHO) suggested the daily intake for cadmium in drinking water at 3µg/kg of live body mass in 1993. The current recommended limit is 1 µg/kg (WHO, 2010). Cadmium has been classified in Group A2, which is defined as substances that are probably carcinogenic to humans by International Agency for Research on Cancer (IARC, 1993). In 1985, the United States Environmental Protection Agency (US EPA) established the Reference Dose for Chronic Oral Exposure (RfD) to be 0.0005 and 0.001 mg Cd/kg/day from water and food, respectively. The EPA also listed cadmium as a probable human carcinogen in the Group B1 category (US EPA, 1994).

The adverse effect of cadmium on humans is the major impetus for a continued study of its behavior and fate in environment. This chapter covers general information pertaining to the occurrence cadmium of in the environment, with more emphasis on cadmium accumulation in tobacco due to the application of phosphate fertilizer.

3.2 **Analytical methods for analysis of cadmium**

There are a variety of methods used for the analytical determination of cadmium. The choice of the technique used for cadmium analysis is mainly dependent of the nature of the sample matrix (soils, sediments, rocks and plants) in which the cadmium exists.

Selection of a suitable digestion method is thus a critical first step in the analysis of cadmium (Bradl et al., 2005).

After sample preparation (drying, grinding and sieving), solid samples are digested usually either via dry combustion or using strong acid solutions and a heat source (hot plate, boiling device or microwave system). Digestion may be established for total heavy metal contents using a total digestion or for partial metal content using a partial digestion technique, depending on the need of the study. Total digestion dissolves heavy metals in samples completely, including fractions bound to silicates, while partial digestion usually only dissolves heavy metals not associated with silicate fractions of solid samples.

Since heavy metals incorporated within the bulk-silicate matrix are unlikely to impose health risks (Thomas, 2003), partial digestion to measure metal content is more widely used and accepted over total digestion to estimate the level of contaminant availability to humans and for predicting plant uptake. Common partial digestion techniques involve the use of strong acid such as nitric acid or a mixture of strong acids with a certain ratio. For example, aqua regia uses a mixture of concentrated nitric acid and concentrated hydrochloric acid with a ratio of 1 to 3. Total digestion techniques are less desirable compared to partial digestion techniques because of the potential danger associated with the use of highly hazardous materials such as the hydrofluoric and perchloric acids (Bradl et al., 2005).

The analysis of heavy metals including cadmium generally involves the use of atomic absorption spectrometry (AAS), and inductively coupled plasma optical emission spectrometry (ICP-OES). The choice of analytical instrument to be used depends on the types of samples, the element of interest and the expected range of concentration. The AAS technique determines the concentration of analyte based on the amount of light (energy) absorbed at a specific wavelength by ground state atoms which are elevated to

an excited state. Analysis by (ICP-OES), determines the concentration of elements based on the intensity of the emitted light at a certain wavelength(s) as related to the element of interest, by introducing the samples to the plasma for it to be desolvated, dissociated, atomized, and excited.

Several standard methods have been provided by US EPA to be used for cadmium analysis in the United States: EPA Method 6010 and EPA Method 7130. EPA Method 6010 involves the use of ICP-OES and digestion techniques using concentrated nitric acid and a hot plate. This method can be applied on various types of samples such as groundwater, soils, sludge, sediments and other solid wastes with instrument detection limit estimated to be 4µg L^{-1}. EPA Method 7130 has been recommended to be used for cadmium determination in drinking water, surface water and wastewater. This method uses AAS with a minimum detection limit of 5µg L^{-1} (Anonymous, 1996).

3.3 **Cadmium and Tobacco**

As related to humans smoking tobacco, it is well documented that cadmium in tobacco can cause health concerns via inhalation. Cadmium has a long half-life in the human body (about 10-35 years) and can accumulate in the kidney (Lugon-Moulin et al., 2004; WHO 2010). Smokers have been found to have high cadmium levels in their hair as compared to the non smokers (Unkiewicz-Winiarczyk et al., 2009).

Soil-applied phosphate fertilizer is one possible source of cadmium in tobacco production. Since it is an often applied fertilizer to enhance production, its continued use may result in substantial cadmium accumulation in the soil (Gray et al., 1999; Lugon-Moulin et al., 2006) Most P fertilizers are produced from phosphate rocks. The range of cadmium in sedimentary phosphate rock is between 0.5 to 150 ppm with the varying concentrations of cadmium found in different types of fertilizers depending on the

manufacturing process (Van Kauwenbergh S.J., 2002). Lugon-Moulin et al., (2006), reported that the concentration of P fertilizer from Central Florida, USA used for tobacco production to be 51 ± 5 mg/kg.

The amount of cadmium found to accumulate in leaves and roots depends on the variety and maturity of tobacco (Wagner and Yeargan, 1986). The cadmium concentration in field-grown tobacco leaves was found to be less than 0.5 µg/g or more than 5 µg/g (Lugon-Moulin et al., 2004). Although the leaves of tobacco seem to be less affected by higher level exposure of cadmium, the tobacco seedlings are proven to be susceptible (Maaroufi Dguimi et al., 2009).

Other than plant factors such as plant genotype, cadmium uptake by tobacco is highly influence by several soil properties. These include soil pH, redox conditions, soil mineralogy, the sorptive capacity of soil including soil organic matter content and interactions between cadmium and other metals such as zinc, iron and copper (Katayama et al., 2010; Holmgren et al., 1993; Tsadilas, 2000; Golia et al., 2009).

Although cadmium uptake by plants, particularly tobacco, can be affected by various factors as mentioned above, it is crucial to understand the bioavailability of cadmium in soil that leads to possible plant accumulation. By referring to the context of a soil contaminant, bioavailability can be defined as the fraction of elements in soil that has the ability to interact with other organisms within the environment (Katayama et al., 2010). Therefore, it is important to be able to determine the total concentration and also the bio-available fraction of metals in soil (Kirkham, 2006).

The available fraction of metals also known as the exchangeable or mobile fraction, is mostly present in soil solution as free ions and has high influence on plant uptake (Krishnamurti et al., 2005). The concentration of the available fraction can be correlated with the concentration of total metals contents, i.e., higher total metals

contents generally results in higher amount of the available fraction (Prokop et al., 2003) However, the correlation between the heavy metal concentrations in plants and the available metal contents in soil is still an active area of scientific investigation. Several studies report that by using certain extraction methods such as diethylenetriaminepentaacetic acid (DTPA), a correlation can be established that better predicts plant availability of heavy metals (Kirkham, 2006)(Golia et al., 2009; Tsadilas, 2000).

Soil pH is the most important factor to assess the metal availability since as soil acidity increases the mobility of heavy metals including cadmium in soils increases. Thus, at low pH, the amount of exchangeable cadmium for plant uptake will be higher (Tsadilas, 2000). Furthermore, redox conditions paired with certain soil pH levels affected the mobility of heavy metals in soil. The mobility of metals in soil was found to increase in aerobic soil with low soil pH while under anaerobic conditions with high pH, increased the immobilization of metals (Peijnenburg, 2004).

The sorptive capacity of soil for metals is influenced by various factors such as organic matter content, clay fraction, and cation exchange capacity (CEC) (Holmgren et al., 1993). However, cadmium is less affected by organic matter as compared to other metals since cadmium complexation with OM is weak due to the competition with other elements in soil for binding sites (Bradl et al., 2005). Several studies suggest the plant uptake and mobility of cadmium in soil can be affected by the interaction of cadmium with other divalent cations in soil such as calcium, zinc and lead as these cations will compete with cadmium for adsorption sites in the soils (Appel and Ma, 2002; Lambert et al., 2007). The mobility of cadmium due to various factors mentioned earlier may also increase its movement from soil to groundwater (Peijnenburg, 2004), although actual transport may not be substantial in most soils (Keller et al., 2002; Krishnamurti et al., 2005).

3.4 **Conclusion**

Although the ability of tobacco to accumulate cadmium has been proven and cadmium accumulation in soil via application of phosphate fertilizers is likely, further investigation to better understand the interactions between soil factors and the available fraction of cadmium in soils, including mechanisms involving plant uptake, is needed. This can potentially be achieved via field experiments for better assessment on the behavior of cadmium in the environment. Extraction methods also need to be improved for a better estimation of cadmium bio-availability irrespective of differences in soil conditions and type of plants.

3.5 **References**

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