NITRIFICATION IN SANDY SOILS OF THE ATLANTIC COASTAL PLAIN AND ITS

RELATIONSHIP TO THE DEVELOPMENT OF SUBSOIL ACIDITY

by

JASON E. MOWRER

(Under the Direction of David E. Kissel)

ABSTRACT

The process of nitrification is known to exacerbate the development of soil and subsoil acidity, particularly in weathered and coarsely textured soils such as those often found in the Atlantic Coastal Plain in the Southeastern United States. The purpose of this research was to elucidate the relationship between typical soil properties in this region and the pattern of nitrification. The research shows that nitrification rates in these soils were found to be at or near the low end of the scale as compared with other reported rates. Lengths of lag phases were found to be at or near the high end of the scale. Rates and lag phases were closely related to both soil CEC and relative water content. This study also shows that the relationship between nitrification and change in soil pH is not simple and that buffering mechanisms related to the pattern of pH change in these soils need further study.

INDEX WORDS: Nitrification, subsoil acidity, ammoniacal fertilizer

NITRIFICATION IN SANDY SOILS OF THE ATLANTIC COASTAL PLAIN AND ITS

RELATIONSHIP TO THE DEVELOPMENT OF SUBSOIL ACIDITY

by

JASON E. MOWRER

B.S.E.H., University of Georgia 1995

A Thesis Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment

of the Requirements for the Degree

MASTER OF SCIENCE

ATHENS, GEORGIA

2009

NITRIFICATION IN SANDY SOILS OF THE ATLANTIC COASTAL PLAIN AND ITS

RELATIONSHIP TO THE DEVELOPMENT OF SUBSOIL ACIDITY

by

JASON E. MOWRER

Major Professor: David E. Kissel

Committee: Miguel Cabrera
William Miller

Electronic Version Approved:

Maureen Grasso
Dean of the Graduate School
The University of Georgia
May 2009

DEDICATION

This thesis is dedicated first to my wife Martha and our children, Olen and Ana. Without their

support, I would not have found the strength or discipline to pursue this degree. Also, I am

indebted to my grandparents, Herman and Lottie Kimmich for buying my books.

ACKNOWLEDGEMENTS

Thank you to Dr. David E. Kissel for sharing his wealth of knowledge and perspective on soil science with me. His immense stature in the field is enough in itself to lend credibility to my future pursuits in soil chemistry. I can only hope to return the favor by making my own small contribution to the field as I mature as a scientist and researcher. Thank you also to the entire staff at the University of Georgia Soil, Plant, and Water Testing Laboratory and the Agricultural and Environmental Services Laboratories. Thank you to Dr. Miguel Cabrera for his patience with frequent questions. Thank you also to Dr. Harry Mills and his entire family for their support during this process.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION AND REVIEW OF LITERATURE

I. Introduction

In a study of cotton plants grown on the cabin field in Crisp county Georgia, it was determined that plants on the North side of the field were unable to efficiently extract soil water at depths greater than 20", whereas identical plants on the South side of the same field were significantly better able to access all available water to depths of 28" (Table 1.1). The cotton plant tops reflected this restrictive situation with respect to plant available soil water and exhibited less plant mass above ground on the North side of the field than observed on the plants at the South end (personal communication, Kissel, D.E.). After eliminating nematodes and subsoil compaction as the causative agents, it was determined that an acidic subsoil condition was most likely responsible for the reduction in plant water uptake (Table 1.2).

Subsoil acidity is a widely identified problem limiting crop yields in agricultural production. It can reduce a plant's ability to explore subsoil horizons for water and nutrients by constraining root growth due to conditions that promote aluminum toxicity and/or reduced calcium availability. In addition, low pH can have direct effects on the availability of other nutrients even when Al toxicity is not a growth limiting factor. For instance, while iron and other micronutrients may become more available at lower pH values, phosphorus and molybdenum are examples of essential nutrients that less available under acid conditions. Soil acidity is most frequently countered with applications of liming agents. These may include agricultural lime, a calcium carbonate product obtained by mining and crushing limestone or dolomitic limestone, calcium hydroxide, etc. The effects of liming are not far reaching, though, as surface applications of liming agents not incorporated into the soil have been shown to be

most effective in the top few centimeters of soil. This is due to a combination of factors: 1) the slow rate of dissolution of calcium carbonate and 2) the rapidity of reaction of dissolved bicarbonate anions with protons in solution. Correction of subsoil acidity, however, has often been approached with prohibitively costly deep incorporation methods. In light of this, some research has shown that given sufficient time, subsoil acidity can be positively affected simply through regular surface applications of lime (Gascho and Parker, 2001). Unfortunately, this process can take many years to raise subsoil pH to desired levels.

Sumner and Noble (2003), assert that ammoniacal fertilizers provide the major acidifying input to soils under intensive agricultural production. Ammoniacal fertilizers include products such as urea, anhydrous ammonia, urea-ammonium-nitrate, and various other ammonium salts. These are popular nitrogen sources as they are less expensive than nitrate salts, yet are oxidized to NO_3^- by microorganisms ubiquitous in soils. This is important as it has been shown that plants supplied with both NH_4^+ and NO_3^- produce higher yields than those receiving only one or the other (Haynes, R.J., 1986b).

The two-stage process by which ammonium is oxidized ultimately to nitrate is termed nitrification. Nitrification is carried out in soils largely by two types of chemolithoautotrophic bacteria. One group (*Nitroso-* spp.) derives energy from the oxidation of NH_4^+ to NO_2^-, while another group (*Nitro-* spp.) derives energy from the oxidation of NO_2^- to NO_3^-. In these two reactions, a total of two moles of acidity (H^+) are produced for every mole of ammonium oxidized. The general chemical reaction is classically summarized as follows:

$$NH_4^+ + 2O_2 \rightarrow NO_3^- + H_2O + 2H^+$$

Depending upon the acid buffering capacity of a soil, acidification resulting from this process can progress rapidly. If these reactions proceed at a relatively vigorous rate, nearly all of

the ammonium will be oxidized in the tillage zone of the soil where the acid products can be more easily ameliorated with surface application of lime. If the nitrification rate is slow, however, ammonium may be allowed to migrate downwards and the reaction can lessen pH values in lower horizons where it is not so easily countered by conventional liming.

While the mobility of ammonium is not considered significant in soils that contain 2:1 clays, the coarse sandy soils of Georgia's Coastal Plain are more susceptible to ammonium leaching. Additionally, excessive plant uptake of cations (NH_4^+) must be accompanied by the uptake of an equivalent anion or otherwise must result in the release of protons or organic acids as a method of maintaining cellular electrochemical balance. Subsoil acidity is further promoted when NO_3^- is leached below the root zone resulting in a permanent charge imbalance (Bouman, 1995). As long as nitrate remains in the root zone, however, there exists the potential for its uptake and subsequent release of bicarbonate which can counteract acidification. Finally, tillage practices have been shown to influence a soil's ability to reduce ammonium mobility as no-till soils promote increased immobilization of mineral nitrogen over conventionally tilled soils (Kitur et al., 1984).

Factors that most influence the rate of nitrification in soils when substrate is not limiting have been identified by investigators as temperature, soil moisture content, pH, soil texture, osmotic potential, and aeration. Additionally, it has been shown that there are geographical and/or climatic correlations with optimum temperature as well as interactions between optimum soil moisture and temperature.

With respect to the problem observed with the growth of cotton plants in the cabin field, the question would seem to be: How does subsoil acidity develop on one side of a field and not another when relatively uniform management practices have been in place for many years? We

know that naturally occurring, within-field variability accounts for a wide range of values for various soil physical and chemical properties. Therefore, uniform applications on a field scale of fertilizers and liming agents based on test results for composite soil samples will result in some portions of a field receiving deficient amounts of amendment while other areas receive excessive amounts.

Within-field variability of topography, soil physical, chemical, and biological properties will affect the physical, chemical, and biological fate of ammoniacal fertilizers following application. For example, coarser soil will allow for more rapid downward transport of ammonium (NH_4^+). Differences in soil pH buffering capacity on the field scale will also result in differential responses to pH affecting inputs such as the aforementioned ammoniacal fertilizers and liming agents.

Thus, our goal should be to facilitate the evolution of agricultural management practices along with our understanding of field scale variability to include less uniform application of inputs. As it has been shown that maintenance of topsoil pH at a level of 6.0 or above can prevent the development and even facilitate a gradual remediation of subsoil acidity (Gascho and Parker, 2001) precision agriculture approaches will lead to better targeting of sub-field scale soil fertility requirements and a realization of that goal.

The data I have collected from soils typical of those under intensive cropping in the Coastal Plain will hopefully shed some light on the factors affecting nitrification rates in these soils. Analysis of the data may further suggest management practices, particularly in the area of tillage, liming, and application of ammoniacal fertilizers, which can most effectively ameliorate and prevent acidification of the subsoil in the Coastal Plain of Georgia.

The purpose of the review that follows is to provide empirical evidence for the description of a model wherein the development of subsoil acidity results from the application and subsequent nitrification of ammoniacal fertilizers with particular attention paid to more coarsely textured soils such as those typically found under intensive row crop production in the Southeastern Coastal Plain of the United States. Responses of chemolithoautotrophic nitrifiers included in the framework of this model with respect to activity, ecology, and enzymology will be considered in detail as necessitated by the central role these organisms are proposed to fulfill within the system.

Table 1.1. Soil available water (%) remaining with depth on the North and South ends of the cabin field at Crisp County, Georgia, illustrating the more efficient removal of soil available water in lower horizons on the South end.

Date of Measurement	South 12"	South 20"	South 28"
6/12	85	90	92
6/22	70	72	72
7/1	48	2	0
7/8	25	0	0
7/18	78	11	0
7/29	55	0	0

Date of Measurement	North 12"	North 20"	North 28"
6/12	100	99	100
6/22	64	93	95
7/1	0	62	69
7/8	0	1	37
7/18	93	93	87
7/29	37	84	84

Table 1.2. pH with depth on the North and South ends of the cabin field at Crisp County, Georgia, demonstrating a probable causative relationship with water extraction by cotton plants.

Depth (inches)	pH North End	pH South End
0-12"	5.5	5.5
12-24"	5.2	6.2
24-36"	5.2	6.6
36-48"	5.4	6.1
48-60"	5	6.1

References

Bouman, O.T., D. Curtin, C.A. Campbell, V.O. Biederbeck, and H. Ukrainetz. 1995. Soil acidification from long-term use of anhydrous ammonia and urea. Soil Sci. Soc. Am. J. 59:1488-1494.

Gascho, G.J., and M.B. Parker. 2001. Long-term liming effects on coastal plain soils and crops. Agron. J. 93:1305-1315.

Haynes, R.J. 1986a. Nitrification. *In* Mineral Nitrogen in the Plant-Soil System (Kozlowski, T.T. ed.), pp. 127-157. Academic Press, Inc. Orlando.

Haynes, R.J. 1986b. Uptake and assimilation of mineral nitrogen by plants. *In* Mineral Nitrogen in the Plant-Soil System (Kozlowski, T.T. ed.), pp. 303-358. Academic Press, Inc. Orlando.

Kitur, B.K., M.S. Smith, R.L. Blevins, and W.W. Frye. 1984. Fate of 15N-depleted nitrate applied to no-tillage and conventional tillage corn. Agron. J. 76:240-242

Sumner, M.E., and A.D. Noble. 2003. Soil Acidification: The World Story. *In* Handbook of Soil Acidity (Rengel, Z., ed.), pp. 1-28. Marcel Dekker, Inc. New York.

II. Soil and Subsoil Acidity

Overview

Acid soil conditions have long been recognized as being detrimental to agricultural crop yields and are characterized by pH values < 7.0, with most crops expressing symptoms of stress at values below pH 5.5 (Vitorello et al, 2005; Islam, et al. 1980). It is estimated that 40% of the world's arable land contains acidic soils (von Uexküll and Mutert, 1995). Soils may naturally become acid as a result of parent materials originally deficient in base cations such as calcium (Ca^{2+}), magnesium (Mg^{2+}), and potassium (K^+) or may similarly result from prolonged weathering wherein the same base cations are removed via leaching by normal rainfall (Foy, 1984). Anthropogenic sources of soil acidity include the combustion of fossil fuels which acidify rainfall, the application of elemental sulfur-containing and/or ammoniacal fertilizers, and the loss of base cations through crop plant material removal.

If left untreated, the development of soil acidity will render land unfit for agricultural production. Treatment most often involves the application of agricultural liming agents such as crushed calcitic or dolomitic limestone, though symptoms of acid stress in crops have been alleviated with the application of gypsum (Toma et al. 1999; Farina et al. 2000) and organic materials such as manures or composts (Hue and Licudine, 1999; Inoue et al. 2001). It has been demonstrated that applications of agricultural lime can as much as double crop yields over those produced on unlimed soil (Gascho and Parker, 2001; Sumner et al. 1986).

Application of agricultural lime is typically achieved by incorporating the material into the topsoil layer, although in the case of no- or minimal-tillage practices, surface application is necessitated. Both methods are effective at ameliorating acidity and/or maintaining a topsoil pH appropriate for crop production (Sumner and Yamada, 2002). However, remediation of subsoil

acidity may require methods that are more costly in terms of money, energy, and time. These

approaches include (but need not be confined to) deep mechanical incorporation and deep

injection of liming agents (Farina and Channon, 1988; Edmeades and Ridley, 2003; Sumner et

al. 1986). Given time, though, it has been shown that a proper maintenance of topsoil pH

(Gascho and Parker, 2001) and the accumulation of organic matter (Foy, 1984; Hue and

Licudine, 1999) can prevent or even effect a slow positive change in subsoil horizons with

respect to acidity.

The fertility of acid soils is reduced primarily due to a combination of 1) the toxicities of

aluminum, manganese, and iron, and 2) deficiencies of calcium, magnesium, phosphorus,

molybdenum, and potassium (von Uexküll and Mutert, 1995; Foy, 1984). These mineral

toxicities and deficiencies will result in depressed crop yields and diminished whole plant health.

When considering the effects on plant growth specific to acid subsoils, however, it is a severe

constraint on the ability of roots to proliferate throughout lower horizons that dominates the list

of issues. If prevented from exploring lower horizons, a vast storehouse of potentially available

nutrients and water will remain out of reach for the plant. This scenario then becomes one in

which a crop will be less able to weather drought and other stresses throughout the growing

season. Larger and more frequent inputs will be required to maintain the viability of the land for

agricultural production as the volume of soil utilized by the plants will be confined to the

uppermost horizons.

Depth of Root Penetration

Maximum rooting depths for herbaceous and crop plants have been reported as being

approximately 2 meters (Canadell et al. 1996). Herron et al. (1971), found corn (Zea mays) roots

as deep as 1.5 m. Li et al. (2001) measured cotton (Gossypium hirsutum L.) root penetration to a

depth of 0.9 m in a sandy soil (sand content = 810 g kg^{-1}) with the main distribution of root material occurring in the top 0.3 m. This asymmetrical distribution is very common as topsoil is typically much richer in mineral nutrients than subsoil, although some nutrients such as the anionic nitrate (NO_3^-) are known to be highly mobile in soil profiles. Conversely, and particularly in between rainfall or irrigation events, the bulk of the water will be found in subsoil horizons. An ability to explore these depths allows a plant some advantage in times when water supply is restricted.

There is considerable ecological significance attached to the ability to set deep roots in the areas of water, carbon, and nutrient cycling. Gregory et al. (1978) reported that the 3% of the total root weight of winter wheat found below 1 m supplied 20% of the transpired water during dry periods. Caldwell and Richards (1989) showed that hydraulic lift, a process wherein deep rooted plants take up water from lower soil layers and release it from shallow roots back into upper soil layers, was responsible for as much as a 50% increase in daytime canopy water flux that in turn supplied water to less deeply rooted neighbors. Deep roots are also critical in the recovery of nitrate from lower profiles. Access to stores of NO_3^- below 30 cm prevents contamination of groundwater supplies and economic waste from lost nitrogen input. Finally, deep rooting places carbonaceous organic matter into the lower horizons. Acidic subsoils have the potential therefore to prevent recovery of nitrate, sequestration of carbon, and utilization of water otherwise available to the entire canopy.

Inhibition of Root Growth

In contrast to the plethora of mineral toxicities and nutrient deficiencies responsible for the decreased fertility of acid soils affecting whole plant health and crop yields, the mechanisms by which root growth specifically is inhibited are confined almost entirely to aluminum toxicity

and/or calcium deficiency (Adams and Moore, 1983, Adams and Hathcock, 1984, Howard and Adams, 1965).   It should be noted that calcium deficiencies are rarer in managed soils than aluminum toxicity. Nonetheless, there exists a potential for calcium leached below the root zone in acid subsoils even under managed conditions.

Root injury as a direct result of H^+ concentration in soil is expected for most plants only at pH values at or below 3.3 (Islam et al., 1980). Some more sensitive species have been shown to be inhibited by H^+ at pH 4.0 (Howard and Adams, 1965). Islam et al. (1980) reported that hydrogen ion-injured root morphology is characterized by short, thickened roots that are comparatively few in number and discolored brown or dull grey. Lateral root growth under these conditions was severely inhibited. These researchers further reported that at a pH value of 4.8, in solution culture absent of aluminum toxicity, roots exhibited no signs of injury. Root morphology was normal with a fine, light colored and profusely branched structure. It is fair to say, however, that pH levels in the range of 3.3 to 4.0 represent severe extremes in agricultural and natural soil systems and so will not be explored further within this review.

Aluminum in Soil

Aluminum comprises about 7 percent of the Earth's mass (Delhaize and Ryan, 1995). It is the most abundant metal and the third most abundant element in the Earth's crust, the bulk of which occurs as aluminosilicate soil minerals (Rengel, 1992; Kochian, 1995). At neutral pH values, aluminum is primarily in the form of insoluble aluminosilicate, hydroxy-aluminum, or aluminum oxide minerals (Kochian, 1995), but under acid conditions aluminum in soil is made soluble and mobile in levels that adversely affect root and plant growth.

The primary form of aluminum thought to be responsible for toxicity to plant root tissue has been the focus of much study. There is a general agreement that it is the mononuclear

species of aluminum that are the most phytotoxic. One interesting suggestion has been that the trivalent cationic form Al^{3+} may be the primary phytotoxic species to monocots (Kinraide, 1991; Parker et al. 1988) while dicots are more susceptible to the $Al(OH)^{2+}$ and $Al(OH)_2^+$ forms (Alva et al.1986; Kinraide and Parker, 1990). From a solution chemistry standpoint, this information would lead one to believe that dicots would experience symptoms of aluminum stress at higher soil solution pH levels than monocots. Unfortunately, the existing literature does not provide a basis for supporting such a broad and convenient statement.

To put this into perspective, in solutions of pH < 5.0 aluminum exists as the octahedral hexahydrate $(Al(H_2O))_6^{3+}$ which is conventionally symbolized as Al^{3+}. The high ionic charge associated with aluminum (z = 3+) coupled with the small ionic radius (r = 0.054 nm) strongly polarizes water molecules in its hydration shell (Vitorello et al. 2005). As pH rises, $Al(H_2O)_6^{3+}$ undergoes successive deprotonations to form $Al(OH)^{2+}$, $Al(OH)_2^+$, and $Al(OH)_3$ at neutral pH (equations 1 – 3, Table 1.3). It should be clear from equations 1 – 3 that free aluminum in soil solutions represents a profound source resistance that must be overcome prior to imposing an effective positive change in soil pH.

Polymeric aluminum has also been reported to exert some inhibitory effects on plant root growth in pure solution culture; although its significance in the soil systems has been downplayed (Bertsch and Parker, 1996; Parker et al. 1988) as the high charge associated with polymeric species results in an enhanced affinity for negatively charged soil particles. Furthermore, polymeric species may be too large to gain access to the interior of the root cell and are thought to simply exist as intermediates in the formation of gibbsite that do not persist for long periods of time. Free aluminum activity in soil solution is known to be significantly affected by the activities of several inorganic anions such as sulfate (SO_4^{2-}), fluoride (F^-),

phosphate (PO_4^{2-}), and silicate (SiO_4^{4-}) by precipitation and/or complexation (Menzies, 2003). Complexation may also occur between monomeric aluminum species and organic acids due to strong interactions with oxygen donor ligands (Kochian, 1995; Hargrove and Thomas, 1981).

Mechanisms of Al Toxicity

Much work has been performed in the area of uncovering the specific mechanism/s by which aluminum exerts inhibitory effects on plant roots. Hecht-Buchholz and Foy (1981) characterized aluminum toxicity as resulting in stunted brown root tips, inhibition of newly emerging lateral roots, destruction of root cap cells, and swelling and destruction of epidermis and cortex cells. Adams and Lund (1965) established a relationship between root length and molar activity of aluminum in solution. Generally speaking, aluminum is known to interfere with the cell wall, the plasma membrane, and the cytoskeletal structure of plant root cells (Vitorello et al. 2005). This leads to the potential efflux of cellular solutes and increased access for aluminum to intracellular target sites.

Clarkson (1965) was the first investigator to show the correlation between aluminum inhibition of root elongation and cessation of mitotic Figures in the root meristem. In this study, he explained the aluminum induced morphological abnormalities of *Allium cepa* (garden onion) roots as arising from a constraint on either cell division or cell extension. These abnormalities were observed to include (1) a reduction or inhibition of the growth of the main axis of the root and (2) initiation of numerous lateral roots, the growth of which were subsequently reduced or inhibited. He showed that other metals from the same column of the periodic table (i.e. same 3^+ valence charge) such as gallium, indium, and lanthanum produced similar results. Clarkson concluded from this study that some mechanism associated with cell division is highly sensitive

to aluminum and is permanently damaged by short exposures. Furthermore, he noted that the observable effect of aluminum exposure in *Allium Cepa* roots was rapid.

In a study of the influence of aluminum ions on sugarbeet roots, Keser et al. (1975) observed extraneous material in the apical meristem, root cap, and cortical region of roots exposed to aluminum that were not observed in normal roots. The apical regions did not exhibit a typical organization and cells divided in an irregular pattern. Keser detailed the differences between normal and abnormal roots in this study. In normal roots, the root cap and epidermis arose from a single tier of initials 5 to 7 cells wide, while abnormal roots developed larger cells in the area between the root cap and epidermis that contained a red staining precipitate composed of $Al(PO_4)$, a substance thought to interfere with cell division. In normal cells, the tissue structure of lateral roots was similar to primary root structure and was initiated in the pericycle. In abnormal roots, the root cap and cortex disintegrated and nuclei were not observed. Furthermore, the root cap separated with time and cells divided with an irregular pattern in which the root tip was curved. A higher number of lateral roots that ceased to grow soon after emergence was a phenomenon once again reported as being a result of aluminum exposure. These were explained to be initiated at or near the apical region of the primary root. One interesting note resulting from this study is that some plants grew better at minor levels of aluminum in solution than when aluminum was completely absent, an observation supported by Hackett (1962).

The effects of aluminum on cell elongation and expansion has been attributed to a reduction in cell wall extensibility in wheat roots (Ma et al. 2004). These authors confirmed the rapidity of effect for Al on root elongation. Inhibition occurred within hours and most of the

aluminum was bound to the cell wall. Longer exposures allowed for greater infiltration of Al into other cell structures.

Aluminum uptake mechanisms and effects at the sub-cellular level have been documented by several researchers. Though negatively charged outer surfaces of plasma membranes will attract positively charged ions, trivalent cations such as aluminum are virtually insoluble in lipid bilayers and are therefore unable to cross the plasma membrane through passive transport. However, aluminum was reported by Delhaize and Ryan (1995) as being present in the symplasm of root apices shortly after exposure. It has been suggested that aluminum may cross the plasma membrane via active transport channels as Al^{3+} (r = 0.054 nm) has a similar ionic radius to other biologically important cations such as Fe^{3+} (r = 0.065 nm) and Mg^{2+} (r = 00.072 nm) (Sigel and Sigel, 1988).

Rengel (1992) noted that aluminum need not enter the cytoplasm for toxic effects to occur. Free aluminum has been shown to inhibit initial adsorption of calcium on negatively charged surfaces of root cells by competitive reduction of short term Ca^{2+} uptake. Also, root hair formation in leguminous species is reduced in the presence of free aluminum, subsequently inhibiting the initial nodulation step of rhizobial attachment. Smit et al. (1987) demonstrated that calcium binding proteins (e.g. rhicadhesin) are necessary for adsorption of Rhizobia to root hairs in peas.

Al-Ca Interactions

Interactions between calcium availability and aluminum toxicity have been reported within plant root cells as well as external surfaces. Ryan et al. (1993) placed the site of Al^{3+} root growth inhibition at the meristem and root cap by comparing exposures of only the root cap to Al with exposure to the remaining portion of the root. However, Schofield et al. (1998) measured

aluminum and calcium distribution in root tips via PIXE (particle-induced X-ray emission) in a study that compared root growth with and without the root cap structure in the presence of toxic concentrations of aluminum. They showed that aluminum was present only in fractional amounts when compared to calcium and concluded that aluminum does not directly inhibit growth in the interior of the apical root meristem as the root growth rate was unaffected by root cap removal. The authors instead suggested that growth inhibition by aluminum was mediated by a signal initiated or disrupted by excess Al^{3+} in the periphery of the meristem and was related to calcium distribution in the cell.

Aluminum disruption of the root plasma membrane interferes with such essential cellular processes as the regulation of transmembrane potential and pH gradients and may possibly result in the formation of non-bilayer lipids (Hargrove and Thomas, 1981). Calmodulin, a small acidic protein present at high levels in root caps, regulates intracellular calcium concentrations, integrates calcium-dependent processes, and has been shown to be adversely affected by aluminum present in the interior of plant root cells (Haug and Caldwell, 1985). Membrane bound ATPases are involved in the transport of calcium ions in plant cells. Complexes between these enzymes and aluminum are key lesions in the syndrome of aluminum toxicity, as the protein is denatured and rendered incapable of exercising its regulatory properties.

Other mechanisms important to the phytotoxicity of aluminum discussed in the available literature include binding to the phosphate groups of DNA molecules and the imposition of oxidation stress (Vitorello et al. 2005). The latter must be an indirect effect as aluminum is a non redox-reactive metal, and may involve some influence on the substrates of oxidation. Finally, Delhaize and Ryan (1995) summed their study on aluminum toxicity and tolerance in plants with speculations that interactions between aluminum and the cell wall include possible binding to

pectic residues or proteins that decrease extensibility, displacement of other cations from critical sites on the cell wall and/or membrane, binding to membrane-bound proteins involved in nutrient transport, or disruption of intracellular metabolism by triggering secondary messenger pathways.

It is clear then that the soil solution aluminum-plant root interface sub-system of a model describing subsoil acidity effects is complex and includes interactions both within and without the root cell. These reactions may be classed under any of the following broadly defined mechanisms: those affecting physical root expansion and division, those affecting the transcription and/or signaling of DNA, RNA, or mRNA, disruption of the plasma membrane, cell wall, or cytoskeleton, those that interfere with the uptake and/or intracellular regulation of calcium, oxidative stress, and those external processes related to interface with the soil environment including mychorrizal attachment and the availability of essential cations for plant nutrition. Understanding the important mechanisms involved not just at the soil solution level, but also at the cellular and sub-cellular level, will help in predicting plant responses, choosing the most appropriate ameliorative program, and selecting the most aluminum tolerant cultivars for agricultural production.

Calcium in Soil

Calcium accounts for roughly 3.5% of the Earth's crust with typical ranges in non-calcareous soils around 0.7% to 1.5% (Havlin et al. 2005). Expected fates of calcium in or applied to soils include; 1) leaching by drainage water, 2) absorption/immobilization by soil organisms, 3) adsorption to cation exchange sites, and 4) precipitation as secondary calcium compounds. Only that calcium existing as the cationic form (Ca^{2+}) in solution or adsorbed phase is considered plant available. Typical temperate region soils (non-calcareous) exhibit soil

solution concentrations of 30 to 300 ppm while a paltry 15 ppm (375 μM) is considered
sufficient to supply growth (Havlin et al. 2005).

As previously noted, calcium deficiency is less common in managed soils than aluminum
toxicity. However, in highly weathered soils in humid regions with soils formed from low
calcium containing parent materials, the possibility of deficiency is very real, and so its inclusion
into a model describing the development and effects of subsoil acidity must be considered. The
coarse sandy soils often found under agricultural production in the Southeastern U.S. Coastal
Plain fit this description and have been documented as being deficient in plant available calcium
(Howard and Adams, 1965; Adams and Moore, 1983). In acid examples of these soils, the
potential for calcium deficiency is markedly increased either through increased dissolution
coupled with percolation and loss through the root zone (Terry and McCants, 1970) or induced
by interactions between free aluminum and calcium uptake by roots (Schofield et al. 1998;
Rengel, 1992). Loss of Ca^{2+} may be countered by lime which raises the pH and provides
exchangeable calcium. However, the low solubility of lime unfortunately confines its zone of
effectiveness to the topsoil. Acid subsoils will require other approaches to increase available
calcium at depth to promote subsoil penetration by crop plant roots. These approaches may
include the physical placement of lime at depth via deep incorporation or deep injection, and/or
alternative inputs such as gypsum or organic manures, which contain more soluble forms of
calcium (Hue and Licudine, 1999; Alva et al. 1990). The two latter examples of inputs will
increase subsoil exchangeable calcium more rapidly, though without directly addressing the
problem with pH.

Role of Calcium in Plant Roots

Calcium is classified as an essential macronutrient and has many functions in plants. These functions include contributions to cell elongation and division as well as cell wall membrane integrity and permeability. Calcium is also necessary for the translocation of sugars and starches throughout the plant. While the whole plant effects of aluminum toxicity are mainly expressed as a limitation of nutrient and water uptake due to constrained root growth, calcium is involved in many physiological functions and effects of root inhibition are compounded by reduced uptake of calcium as a nutrient.

Calcium starved roots exhibit an abnormal morphology characterized by straight, small-diameter primary roots with brown tips (Howard and Adams, 1965). Recent research confirms the role of calcium in root cell elongation and growth. Much of this work was done with *Arabidosis thaliana*, or Thale Cress, often used as a model organism in plant biology. Šamaj et al. (2004) reported that signaling enzymes such as NADPH oxidase and pholpholipase D are crucial for root hair growth and development by activating calcium ion channels in the apical plasma membrane leading to a tip-focused calcium gradient. The behavior of this gradient was studied by Bibikova et al. (1997). These investigators induced a forced redirection of root hair tip growth by rapidly producing a localized Ca^{2+} gradient. Their results demonstrated that some sensing mechanism in root hair tips can signal for a change in root hair growth. Removal of the gradient source resulted in the restoration of root hair growth oriented parallel to the root. This behavior must aid plants in the uptake of non-homogenously distributed, more localized supplies of calcium.

Calcium as a nutrient (Ca^{2+}) may not cross suberized tissue in plants (Havlin et al. 2005), and so uptake by roots is limited to meristematic areas where a continuous endoderm has not yet

formed. The root apex has significantly higher rates of cell division, cell elongation, and calcium uptake relative to the mature root (Beemster and Baskin, 1998). Kiegle et al. (2000) identified calcium specific hyper-polarization activated uptake channels that may play a large role in the supply of Ca^{2+} received by the plant. These channels were identified only in the elongation zone of roots, where cells are rapidly growing at the root apex and were shown to be activated at membrane potentials of less than -120mV. Interestingly, the authors reported that these channels were irreversibly inhibited by small concentrations of Al^{3+}. Demidchick et al. (2002) described voltage-independent, non-selective cation uptake channels that coexist with the hyper-polarization activated uptake channels. These were found to be inhibited by the trivalent cations Gd^{3+} and La^{3+}, though effects specific to aluminum were not mentioned. They proposed a calcium acquisition system for plant roots in which the non-selective cation uptake channels dominate in more mature epidermal cells while the hyper-polarization activated Ca^{2+} selective channels predominate in the rapidly expanding cells of the root apex where a more rapid influx of calcium is needed to stabilize cells and facilitate elongation.

Koyama et al, (2001) studied the growth of *A. thaliana* roots in Al-free acidic solution culture. At pH ~4.5, they found that the elongation zone of growing roots lost viability within 1 to 2 hours. This damage was reported to be irreversible after only 1 hour. Growing lateral roots were similarly affected but non-growing lateral roots remained entirely viable, an observation that strengthens the concept of meristematic tissue as the locus of sensitivity to soil acidity-induced calcium deficiency. In this study it was determined that, at pH 5.0, growing roots required 25 µM of calcium to maintain elongation. At pH 4.8 and pH 4.5, the growing roots required 250 µM and 750 µM respectively (Figure 1.1). Havlin et al. (2005) set the concentration in soil solution necessary to provide sufficient levels for whole plant nutrition at

375 μM under non-stress conditions. Damage done by hydronium ions in solution was found to be ameliorated by divalent cations in the order Ba^{2+} (r = 1.34 Å) $\approx$ Sr^{2+} (r = 1.12 Å) $\geq$ Ca^{2+} (r = 0.99 Å) > Mg^{2+} (r = 0.66 Å). In this study, potassium (K^+) was ineffective at ameliorating proton damage. This order not only follows the order of ionic radii from largest to smallest in efficacy of ameliorating proton damage, but also the order of selectivity coefficients for pectin from largest to smallest. This relationship led the authors to conclude that the primary target of proton toxicity may be linked to a disturbance in the stability of the pectic polysaccharide network, where calcium fulfills a key role in plant roots.

What should be clear from the existing research is a general condition wherein calcium is an essential requirement for whole plant function, but especially necessary for new growth and stability of root cells. Trivalent cations (primarily Al^{3+}) are directly and indirectly phytotoxic through a number of mechanisms, all of which seem to target the most sensitive meristematic tissue. Probable direct effects include destruction of cell walls, plasma membranes, and cytoskeletons, and interference with the normal function of DNA and crucial enzymatic processes such as nutrient transport and regulation of trans-membrane potentials. Indirect effects include competitive inhibition at nutrient uptake sites, displacement of cationic nutrients adsorbed to extracellular walls, and inhibition of mychorrizal and rhizobial attachment. Divalent base cations have been shown to ameliorate these effects. Increases in soil organic matter or application of inorganic salts containing Ca^{2+} or Mg^{2+} provide good sources for such ameliorative materials.

Overcoming Subsoil Acidity

Raising the pH (> 5.5) of soil by application of liming agents will ameliorate the toxic effects of aluminum by causing it to precipitate out of solution as gibbsite (equations 1 – 3, Table

1.3). Unless deeply incorporated at great cost, however, the effect of carbonaceous liming

materials will be slow to affect exchangeable aluminum in subsoils. Successful amelioration of

aluminum toxicity following surface-applied or topsoil-incorporated gypsum is well documented

(Caires et al. 2002; Carvalho and van Raijj, 1997; Alva et al. 1990). Gypsum is much more

soluble than agricultural lime and will move downward through the soil profile much more

rapidly. This approach is explained to be more successful by Alva et al (1990) in soils

dominated by pH dependent charge, such as the soils of the Southeastern Coastal Plain, than in

those dominant in permanent charge characteristics and its effect is imposed by increasing

exchangeable calcium, and decreasing exchangeable aluminum.

Cation amelioration of the effects of aluminum on plant roots has been described in the

literature as well. Kinraide and Parker (1987) showed that increased calcium in the presence of a

static concentration of aluminum in solution resulted in an increase in root growth. The

mechanism the authors suggested in this study was improved calcium competition for external

cellular binding sites. This mechanism was also suggested by Rengel (1992) as being the result

of competition at the apoplasmic mouth of Ca^{2+} channels. Rengel (1992) also described an effect

of decreased ionic strength of aluminum in solution as other (base) cations become more

concentrated. Hue and Licudine (1999) attributed this ionic strength phenomenon to the

reduction in aluminum toxicity following the application of organic manures.

Additions of certain anions may also reduce the toxic effects of aluminum. Root

elongation of sunflower (helianthus annuus L.), soybean (glysice max Merr.), subterranean

clover (trifolium subterraneum L.), and alfalfa (medicago sativa L.) was shown to increase with

increases in the molar ration of phosphorus/aluminum (Alva et al. 1986). Sulfate induced

surface charge development in variable charge soils is credited by Alva et al. (1990) with the

reduction in free aluminum in soil solutions following application of gypsum. The presence of fluoride and phosphate can remove aluminum from solution by precipitation, although use of fluoride is not recommended due to its own phytotoxic properties.

Finally, it has been shown in many studies that soil organic materials are effective at removing aluminum from soil solution. Haug and Caldwell (1985) and Adams and Moore (1983) described chelation and immobilization of Al^{3+} by soil humiferous substances rich in carboxylate ligands. Plant exudates that can chelate Al^{3+} in the rhizosphere such as malic acid and citric acid are a natural defense against aluminum toxicity (Delhaize et al. 1993; Miyasaka et al. 1991). Hecht-Buchholz and Foy (1984) ascribed Al resistance in barley to a mucigellic substance covering the root cap cell. The effect of organic acid exudates and other defense mechanisms should be considered to be extremely localized and not significant in the pH improvement of the larger soil volume. Perhaps selection of cultivars that are similarly resistant and/or exhibit an increased ability to generate exudates will be a successful approach to overcoming aluminum toxicity, particularly in areas where vast tracts of land and/or remote locations make lime applications a less feasible option.

Modeling Subsoil Acidity

The system of interest in an agricultural soil acidity model can be thought of to include all living and dead plant material above, as well as the partial pressure of all gases in contact with the soil surface as the upper boundary extending downward to include the entire volume of soil and all its components to a depth that corresponds to the maximum rooting depth of the plant species growing in that soil as the lower boundary. The scale of this model may be adjusted to fit a plot, field or watershed of interest.

Not all inputs, exports, and/or accumulations that could be considered in this system will contribute to the production (or consumption) of acidity. The list of processes pertinent to the development of subsoil acidity, though not small, is reasonably finite and includes some components of the carbon, nitrogen, and sulfur cycles as well as certain redox reactions involving iron and manganese. Helyar and Porter (1989) asserted that soil acids are largely derived from the carbon and nitrogen cycles as effects from the oxidation of sulfur are minimal in comparison while the effects of Fe and Mn redox reactions are more important on a geological time scale. De Vries and Breeuwsma (1987) stressed that actual acidification is result of the weathering of soils, specifically the leaching of cations (regulated by the mobility of some major anions) from the soil. Reconciling these two views is not difficult if one considers that it is the openness of the system of interest as defined above with respect to the loss or exportation of materials (existing and input) that leads to the uncoupling of the nitrogen and carbon cycles and subsequent proton accumulation.

That the carbon and nitrogen cycles, as well as the cation cycle described by De Vries and Breeuwsma (1987), contain the important processes accounting for nearly all of the soil acid production/consumption in an agricultural system over the course of a growing season is supported by Helyar and Porter (1989), Donnelly et al, (1997), and Verburg et al, (2003). This review will therefore confine itself to discussion of those processes within the carbon, nitrogen, and cation cycles that contribute to the change in soil pH germane to the range of agricultural production practices common to the Southeastern U.S. Coastal Plain soils. A comprehensive subsoil acidity model should also consider differential rates of pH change from horizon to horizon and so must account for mass flow and diffusion of protons, aluminum, base cations, and mobile anions as well as the influence of root uptake and excretion on the system with depth.

Quantifying soil acidity is achieved in the following way:

Net acid addition (mol unit volume soil^{-1} time^{-1}) =

nitrogen cycle effects

+ net root excretion (cation cycle)

+ net effect of organic anion reactions (carbon cycle)

- lime dissolution (carbon cycle)

+ net mass flow of acidity (cation cycle)

Actual changes in soil pH as a rule do not reflect absolute changes in total soil acidity.

Due to the buffering capacity of the system, this change is always less than the absolute value of

net acid addition.

pH change (ΔpH_i) = (moles of H$^+$ added unit soil volume^{-1})$_i$(pHBC$_i$ x mass$_i$)

Where: i = soil horizon

pHBC = pH buffer capacity

And the total acidification rate is calculated thusly:

Acidification rate (moles of H$^+$ added unit soil volume^{-1}) =

$$\Sigma^n_{i=1}\Delta pH_i \text{ x pHBC}_i \text{ x BD}_i \text{ x V}_i$$

Where: BD = bulk density

V = soil volume

The Carbon Cycle

Soil water contains carbonic acid (H_2CO_3) in equilibrium with CO_2 in the soil air (Equation 1, Table 1.4). The partial pressure of CO_2 in soil air varies from 0.0015 to 0.0065 atm (Russel, 1973). Bicarbonate (HCO_3^-) and H^+ are the dissociation products when this reaction involves absorption of CO_2 into the soil system and so acid accumulation results. In the reverse situation (i.e. the reaction trends toward liberation of CO_2), protons are consumed. Helyar and Porter (1989) minimized the role of bicarbonate fluxes in acid soils without high rates of leaching.

In the Coastal Plain, soils are known to undergo high rates of leaching and solute movement (Terry and McCant, 1970) yet the rapidity with which bicarbonates react with protons may limit the movement of these particular anions. For instance, carbonates released into the soil solution following the application of agricultural lime will consume protons in a soil volume located very near the initial placement of the material. The rate of lime dissolution is variable (De Vries and Breeuwsma, 1987) and will depend on the amount applied, temperature, water flow, and soil pH. Furthermore, Stevens and Blanchar (1992) have demonstrated a difference in the solubility of and pH gradients surrounding particles of calcitic and dolomitic sources of lime which may prove important to modeling efforts.

Organic anions similarly influence the development of acidity (Equation 2, Table 1.4). Association/dissociation reactions can be involved in the production or consumption of acidity and as such contribute strongly to soil pH buffer capacity. Additionally, complexation/chelation of free aluminum by organic anions is a mechanism by which soil acidity can be reduced (Equation 3, Table 1.4). In fact, soil organic matter reactions are credited as playing the largest role in soil pH buffer capacity in soils containing 2:1 clay minerals (Magdoff and Bartlett, 1985).

However, when considering sandy subsoils of the Southeastern U.S. Coastal Plain, soil organic matter contents are generally low.

Nitrogen Cycle

Nitrogen may be taken up by plants in either of two forms: the anionic nitrate (NO_3^-) or the cationic ammonium (NH_4^+). Mineral nitrogen is introduced into the soil system in meaningful amounts either through the mineralization of organic matter, or application as fertilizer. Small amounts may be included in normal precipitation events as NH_4NO_3. The importance of rainfall deposition in lands under agricultural production is likely dwarfed by the contributions of ammonification and the application of nitrogenous fertilizers. It should be noted that the practice of applying nitrate salts to agricultural lands is prohibitively expensive and therefore highly uncommon.

The hydrolysis of organic nitrogen compounds leads to the consumption of one proton per mole of ammonium produced, as will the application of fertilizers containing anhydrous ammonia or urea (Equations 4, 7, & 12, Table 1.4). Ammonia resulting from these processes or applied as ammonium salts will then likely, though not always, be converted to nitrate via a bacterially mediated oxidation process termed nitrification. In this process, two moles of acidity are produced for every mole of ammonium oxidized. No matter the source of ammoniacal nitrogen, it should be clear that some amount of acidity will result from its introduction into the soil system.

This net production of acidity can be reversed, however, if the nitrate produced from nitrification is taken up by plant roots (Equation 10, Table 1.4). Because nitrogen is the mineral nutrient taken up in the greatest amounts by plants, the form taken up (NH_4^+ or NO_3^-) will likely hold the greatest sway over the contribution to soil acidity provided by plant roots. Typical

concentrations of nitrogen in plants average around 1.5%. Potassium (K^+) is second at around 1% of plant tissue by weight. Calcium (Ca^{2+}) follows at 0.5%. Phosphorus (PO_4^{2-}), magnesium (Mg^{2+}), and sulfur (SO_4^{2-}) average 0.2% of plant weight each (Havlin et al, 2005). If one were to exclude nitrogen uptake, it is clear that cations dominate the electrolytic distribution of nutrient uptake by plants. Plants must maintain an electrochemical balance at the cellular and organism levels, so excessive cation uptake (i.e. uptake of nitrogen as NH_4^+) will result in root excretion of protons into the soil in the interest of maintaining this balance (Equation 9, Table 1.4). However, if nitrate is the largest form of nitrogen taken up, then the potential exists to reverse this process either through the accompanying uptake of protons or the excretion of hydroxyl (OH^-) or bicarbonate (HCO_3^-) ions. Adams and Pearson (1969) demonstrated this effect with the application of calcium nitrate ($Ca(NO_3)_2$) and ammonium nitrate (NH_4NO_3) as nitrogen sources. Subsoil acidity was corrected to depths of 60-80 cm with $Ca(NO_3)_2$ within 4 years in the presence of active root uptake whereas lime application to the surface had only a marginal effect in the 15-30 cm range. It is the loss of nitrate through leaching below the root zone that uncouples this cycle and allows the rapid acidification of subsoils.

Other processes that may play a small role in the nitrogen cycle as it applies to soil acidity are immobilization by soil organisms and denitrification. Immobilization leads to the release of one proton per mole of nitrogen absorbed while denitrification of nitrate to gaseous nitrogen (N_2) consumes one proton per mole of nitrate. Denitrification however, is unlikely to play a significant role within the soil system as defined due to the necessitation of anaerobic conditions. Such conditions most often exist below the root zone in coarse, well drained soils, placing its effects outside the system.

Cation Cycle

The cation cycle described by De Vries and Breeuwsma (1987) is regulated by the activities and mobilities of primarily the following anions: HCO^-, $RCOO^-$, NO_3^-, and SO_4^{2-}, and actual soil acidification is largely imposed by the leaching of base cations form the soil.

H^+ consumption (cation cycle) = $M^+_{out} - M^+_{in} + M^+_{uptake}$.

Where: M+ refers to any base cation

     Out is exported out of the system

     In is input into the system

     Uptake refers to plant uptake

According to these authors, removal of vegetation also causes soil acidification because it leads to a continuous removal of cations from the soil. Furthermore, removal of vegetation temporarily leads to proton production when mineralization is not balanced by uptake.

Cations that are evenly distributed throughout the profile in this model were considered to be taken up by roots in proportion to the mass of roots within a given horizon. In the case, however, where a nutrient is distributed in a more localized fashion, this assumption should not be employed. This may lead to a localization of acid production or consumption which should be addressed by the model. Marschner and Römheld (1983) demonstrated this phenomenon in a beautifully designed split-root experiment involving 4 different species of plants (maize, wheat, chickpea and white clover). All plants were grown in soil containers with separated compartments into which different forms of nitrogen were supplied. The experiments clearly showed that NH_4^+-N uptake and uptake by N_2 fixation decreased the rhizosphere soil pH by as much as 2 units while NO_3^--N uptake resulted in a 1.5 pH unit increase. As only the soil pH in

the respective compartments was affected, the authors concluded that plant root excretion of acid or alkali components was localized and not expressed throughout the root system.

The Role of Roots in Subsoil Acidification

Studies by Marschner and Römheld (1983) and Adams and Pearson (1969) have previously been cited as examples in which plant roots can affect their soil environment with respect to pH in highly significant ways. Nitrate is normally incorporated into crops in greater amounts than any other anionic nutrient and so its uptake prior to its leaching through the root zone is of paramount importance to preventing the development of subsoil acidity. If this can be affected in the lower portion of the root zone without incurring significant losses, the potential for an increase in subsoil pH exists. Implementation of a winter cover crop program is recommended to maximize this potential (Verburg et al., 2003; Helyar and Porter, 1989). Uptake of nitrate may be inhibited in the presence of ammonium (Ayling, 1993; Howitt and Udvardi, 2000) and this fact should not be ignored in modeling uptake when both N-sources are available. Other studies have shown root excretions of protons in response to the uptake of calcium (Demidchick et al. 2002; Kiegle et al. 2000) or to phosphorus deficiency (Neumann and Römheld, 1999) or as a result of simple normal root growth (Versel and Mayor, 1985; Weisenseel et al. 1979).

Summary

The development of acid subsoils (and acid soil in general) probably reflects the natural trend of soils to weather with age and exposure to water. This progression is hastened by the use of land for agricultural production mainly through the increased removal of base cations through plant uptake and leaching as well as the input of ammoniacal fertilizers. Acid subsoils should be

of particular concern in soils that are already weathered to some degree and/or experience high rates of leaching through the entire profile.

The main effect of acid subsoils on crop production is to diminish root proliferation to lower horizons where water and nutrients are more available in times of water stress. This inhibition of root growth is primarily caused by aluminum toxicity and/or calcium deficiency. Of the two, aluminum toxicity more frequently causes inhibition of root growth in managed soils that receive periodic lime treatments. Treatment of subsoil acidity has traditionally been approached in the short term by deep incorporation or injection of liming agents. These methods involve high costs and specialized machinery and could be prevented through simple long-term management practices. Some acid subsoils have responded favorably to treatments involving gypsum, which is more soluble than lime. Although it is another expensive option, fertilization with nitrate salts has proven effective in the relatively short term.

As soils are not a homogenous medium spatially, temporally, or with respect to depth, it may be unwise to treat them as such when modeling response to acid and alkali inputs. There are many components to the soil system involved in soil acidity models. These include elemental cycles, mass flow of solutes, and plant soil interactions. Two software-based soil acidity models, GRAZPLAN and AP-SIM (Donnelly et al. 1997: McCown et al. 1996) have been published in recent years. Verburg et al. (2005) have compared the success of each of these models' components and approaches.

Table 1.3. Some equilibrium reactions of importance to acid soils.

Equilibrium Reaction (25°C)	K (stability constant)
Hydrolysis of Al³⁺	
(1)[1] $Al^{3+} + H^2O \leftrightarrow AlOH^{2+} + H^+$	9.55×10^{-6}
(2)[1] $Al^{3+} + 2H_2O \leftrightarrow Al(OH)_2^+ + 2H^+$	5.01×10^{-10}
(3)[1] $Al^{3+} + 3H_2O \leftrightarrow Al(OH)_3^0 + 3H^+$	1.02×10^{-15}
Solubility of Gibbsite	
(4)[1] $Al(OH)_3^0 + 3H^+ \leftrightarrow Al^{3+} + 3H_2O$	1.10×10^8
Aluminum Complexes	
(5)[1] $Al^{3+} + F^- \leftrightarrow AlF^{2+}$	9.55×10^6
(6)[1] $Al^{3+} + SO_4^{2-} \leftrightarrow AlSO_4^-$	1.58×10^3
(7)[4] $Al^{3+} + HPO_4^{2-} \leftrightarrow AlHPO_4^+$	1×10^8
(8)[2] $Al^{3+} + Citric\ Acid \leftrightarrow Al\text{-}Citrate$	7.41×10^7
(9)[2] $Al^{3+} + Malic\ Acid \leftrightarrow Al\text{-}Malate$	1×10^6
(10)[3] $Al^{3+} + ATP \leftrightarrow Al\text{-}ATP$	6.31×10^9
(11)[3] $Al^{3+} + EDTA \leftrightarrow Al\text{-}EDTA$	1.35×10^{16}
Solubility of Calcium Minerals	
(12)[1] $CaSO_4 \cdot 2H_2O\ (gypsum) + H_2O \leftrightarrow Ca^{2+} + SO_4^{2-} + 2H_2O$	2.29×10^{-5}
(13) $CaCO_3\ (calcite) + H_2O \leftrightarrow Ca^{2+} + CO_2(g)$	3.8×10^{-9}

[1] Lindsay, W.L., Chemical Equilibria in Soils, 1979. John Wiley & Sons, NY.

[2] Jones, D.L., 1998. Organic acids in the rhizosphere - a critical review. Plant and Soil 205:25-44.

[3] Furia, T.E., 1972. Sequestrents in Foods. *In* CRC Handbook of food additives, 2nd ed. CRC Press.
[4] Stumm, W., Morgan, J.J., 1981. Aquatic Chemistry, ed 2. John Wiley & Sons, NY.

Table 1.4. Some major processes involved in soil acidification and their chemical reactions.

	Process	Reaction Equation	Proton Contribution (+/-)
		Carbon Cycle	
(1)	Dissociation of CO_2	$CO_2 + H_2O \leftrightarrow HCO_3^- + H^+$	+1
(2)	Dissociation of Organic Acids	$RCOOH \leftrightarrow RCOO^- + H^+$	+1
(3)	Organo-Aluminum Complexation	$3RCOOH + Al(OH)_3 \leftrightarrow (RCOO)_3Al + 3H_2O$	-3
		Nitrogen Cycle	
(4)	Ammonification	$RNH_2 + H_2O + H^+ \leftrightarrow ROH + NH_4^+$	-1
(5)	Immobilization	$NH_4^+ + ROH \leftrightarrow RNH_2 + H_2O + H^+$	+1
(6)	Dissociation of Nitric Acid	$HNO_3 \leftrightarrow H^+ + NO_3^-$	+1
(7)	Dissociation of Ammonia	$NH_4^+ \leftrightarrow NH_3 + H^+$	+1
(8)	Nitrification	$NH_3 + 2O_2 \leftrightarrow NO_3^- + 2H^+ + H_2O$	+2
(9)	Plant Uptake (NH_4^+)	$(Plant)ROH + NH_4^+ \leftrightarrow RNH_2 + H_2O + H^+$	+1
(10)	Plant Uptake (NO_3^-)	$(Plant)ROH + NO_3^- + H^+ + 2CH_2O \leftrightarrow RNH_2 + 2CO_2 + 2H_2O$	-1
(11)	Denitrification	$5CH_2O + 4NO_3^- + 4H^+ \leftrightarrow 2N2 + 5CO_2 + 3H_2O$	-1
(12)	Urea Hydrolysis	$(NH_2)_2CO + H_2O + H^+ \leftrightarrow 2NH_4^+ + CO_2$	-1
		Cation Cycle	
(13)	Cation Uptake	$RCOOH + Cat^+ \leftrightarrow RCOOCat + H^+$	+1
(14)	Cation Adsorption to Clay Surface	$M^+ + H+ex \leftrightarrow M+ex + H^+$	+1
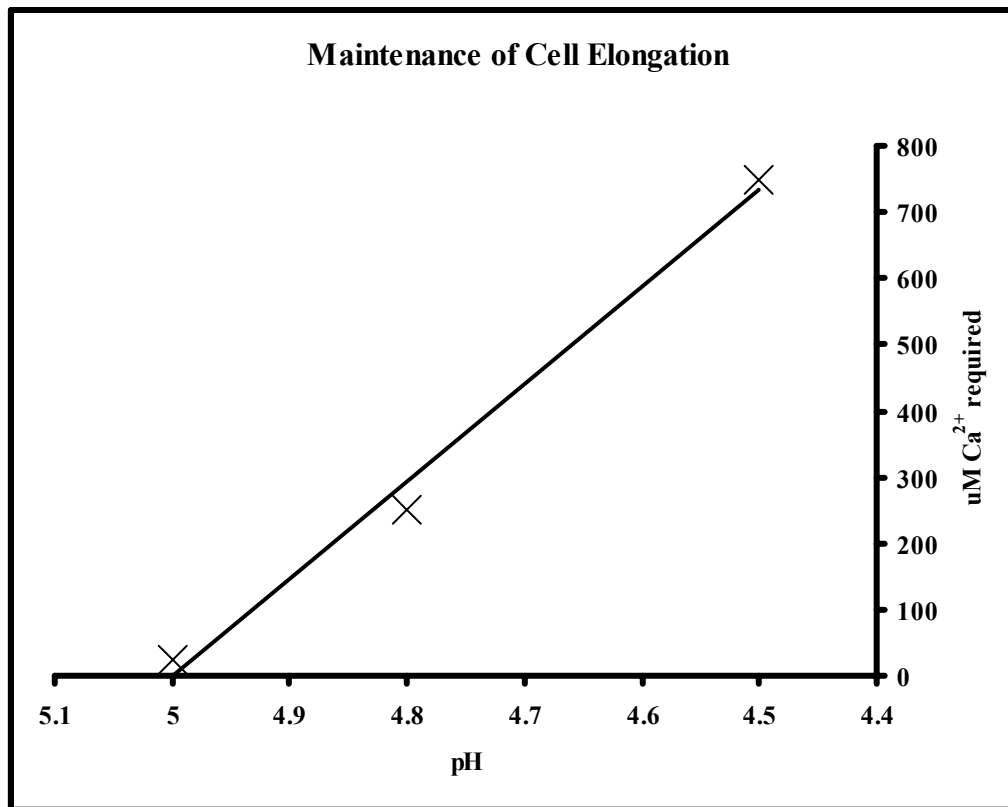
Figure 1.1. Calcium required for plant root cell elongation. (Data from Koyama et al. 2001)

References

Adams F., and Z.F. Lund. 1965. Effect of chemical activity of soil solution aluminum on cotton root penetration of acid subsoils. Soil Sci. 101:193-198.

Adams, F., and P.J. Hathcock. 1984. Aluminum toxicity and calcium deficiency in acid subsoil horizons of two coastal plains soil series. Soil Sci. Soc. Am. J. 48:1305-1309.

Adams F., and B.L. Moore. 1983. Chemical factors affecting root growth in subsoil horizons of coastal plain soils. Soil Sci. Soc. Am. J. 47:99-102.

Adams, F., and R.W. Pearson. 1969. Neutralizing soil acidity under bermudagrass sod. Soil Sci. Soc. Amer. Proc. 33:737-742.

Alva, A.K., D.G. Edwards, C.J. Asher, and F.P. Blamey. 1986. Relationships between root length of soybean and calculated activities of aluminum monomers in nutrient solution. Soil Sci. Soc. Am. J. 50:959-962.

Alva, A.K., M.E. Sumner, and W.P. Miller. 1990. Reactions of gypsum or phosphogypsum in highly weathered acid subsoils. Soil Sci. Soc. Am. J. 54:993-998.

Ayling, S.M. 1993. The effect of ammonium ions on membrane potential and anion flux in roots of barley and tomato. Plant, Cell and Environment 16:297-303.

Beemster, G., and T. Baskin. 1998. Analysis of cell division and cell elongation underlying the developmental acceleration of root growth in *Arabidosis thaliana*. Plant Phys. 116:1515-1526.

Bertsch, P., and D.R. Parker. 1996. Aqueous polynuclear aluminium species. *In* the Environmental Chemistry of Aluminum. (Sposito, G., ed.) Lewis Publishers, Boca Raton, Fl. Pp117-168.

Bibikova, T.M., A. Zhigilei, and S. Gilroy. 1997. Root hair growth in *Arabidopsis thaliana* is directed by calcium and an endogenous polarity. Planta 203:495-505.

Caires, E.F., I.C. Feldhaus, G. Barth, and F.J. Garbulo. 2002. Lime and gypsum application of the wheat crop. Scientia Agricola 59:357-364.

Caldwell M.M., and J.H. Richards. 1989. Hydraulic lift: water efflux from upper roots improves effectiveness of water uptake by deep roots. Oecologia 19:1-5.

Canadell, J., R.B. Jackson, J.R. Ehleringer, H.A. Mooney, O.E. Sala, and E.D. Schulze. 1996. Maximum rooting depth of vegetation types at the global scale. Oecologia 108:583-595.

Carvalho, M.C.S., and B. van Raij. 1997. Calcium sulphate, phosphogypsum and calcium carbonate in the amelioration of acid subsoils for root growth. Plant and Soil 192:37-48.

Clarkson, D.T. 1965. The effect of aluminium and some other trivalent metal cations on cell division in the root apices of allium cepa. Annals of Botany, N.S. 29:309-315.

De Vries, W., A. Breeuwsma. 1987. The relation between soil acidification and element cycling. Water, Air, and Soil Pollution 35:293-310.

Delhaize, E., P.R. Ryan, and P.J. Randall. 1993. Aluminum tolerance in wheat (triticum aestivum l.) II. Aluminum-stimulated excretion of malic acid from root apices. Plant Physiol. 103:695-702.

Delhaize, E., and P.R. Ryan. 1995. Aluminum toxicity and tolerance in plants. Plant Physiol. 107:315-321.

Demidchik, V., H.C. Bowen, F.J.M. Maathuis, S.N. Shabala, M.A. Tester, P.J. White, and J.M. Davies. 2002. *Arabidopsis thaliana* root non-selective cation channels mediate calcium uptake an are involved in growth. The Plant Journal 32:799-808.

Donnelly, J.R., A.D. Moore, and M. Freer. 1997. GRAZPLAN: decision support systems for Australian grazing enterprises. I. Overview of the GRAZPLAN project, and a description of the MEtAccess and LambAlive DSS. Agric. Syst. 54:57-76.

Edmeades, D.C., and A.M. Ridley. 2003. Using Lime to ameliorate topsoil and subsoil acidity. *In* Handbook of soil acidity (Rengel, Z., ed.) Marcel Dekker, Inc. New York.

Farina, M.P.W., and P. Channon. 1988. Acid-subsoil amelioration: I. A comparison of several mechanical procedures. Soil Sci. Soc. Am. J. 52:169-175.

Farina, M.P.W., P. Channon, and G.R. Thibaud. 2000. A comparison of strategies for ameliorating subsoil acidity: I. Long-term growth effects. Soil Sci. Soc. Am. J. 64:646-651.

Foy, C.D. 1984. Physiological effects of hydrogen, aluminum, and manganese toxicities in acid soil. *In* Soil Acidity and Liming (Adams, F. ed.) 2nd ed. Agron. Monogr. 12 SSSA, Madison, Wi.

Furia, T.E. 1972. Sequestrents in Foods. *In* CRC Handbook of food additives, 2nd ed. CRC Press, Orlando.

Gascho, G.J., and M.B. Parker. 2001. Long-term liming effects on coastal plain soils and crops. Agron. J. 93:1305-1315.

Gregory, P.J., M. McGowan, P.V. Biscoe, and B. Hunter. 1978. Water relations of winter wheat. I. Growth of the root system. J. Agric. Sci. 91:91-103>

Hackett, C. 1962. Stimulative effects of aluminum on plant growth. Nature 195:471-472.

Hardy, D.H., C.D. Raper, Jr., and G.S. Miner. 1990. Chemical restrictions of roots in ultisol subsoils lessened by long-term management. Soil Sci. Soc. Am. J. 54:1657-1660.

Hargrove, W.L., and G.W. Thomas. 1981. Effect of organic matter on exchangeable aluminum and plant growth in acid soils. ASA Spec. Publ. (Chem. Soil Environ.) 40:151-166.

Haug, A.R., and C.R. Caldwell. 1985. Aluminum toxicity in plants: The role of the root plasma membrane and calmodulin. *In* Frontiers of membrane research in agriculture, 359-381. St. John, et al (eds.) Rowman & Allanheld, Totowa, N.J.

Havlin, J.L., J.D. Beaton, S.L. Tisdale, and W.L. Nelson. 2005. Soil Fertility and Fertilizers, an Introduction to Nutrient Management 7th ed. Pearson/Prentice Hall, Upper Saddle River, NJ.

Hecht-Buchholz, C.H., and C.D. Foy. 1981. Effect of aluminium toxicity on root morphology of barley. *In* Structure and Function of Plant Roots, 343-345. R.Bouwer et al. (eds.) Martinus Nijhoff/Dr W. Junk Publishers, The Hague/Boston/London.

Helyar, K.R., and W.M. Porter. 1989. Soil acidification, its measurement and the processes involved, *In* Soil Acidity and Plant Growth (Robson, A.D., ed.) Academic Press, Sydney. pp. 61-101.

Herron, G.M., A.F. Dreier, A.D. Flowerday, W.L. Colville, and R.A. Olson. 1971. Residual mineral N accumulation in soil and its utilization by irrigated corn, Agron. J. 63:322-327.

Howard, D.D., and F. Adams. 1965. Calcium requirement for penetration of subsoils by primary cotton roots. 29:558-562.

Howitt, S.M., and M.K. Udvardi. 2000. Structure, function and regulation of ammonium transporters in plants. Biochimica et Biophysica Acta 1465:152-170.

Hue, N.V., and D.L. Licudine. 1999. Amelioration of subsoil acidity through surface application of organic manures. J. Environ. Qual. 28:623-632.

Inoue, K., S. Kondo, Y. Tamano, and H. Yokota. 2001. Amelioration of subsoil acidity in a nonallophanic andosol by surface application of organic calcium salts. Soil Sci. Plant Nutr. 47:113-122.

Islam, A.K.M.S., D.G. Edwards, and C.J. Asher. 1980. pH optima for crop growth. Plant and Soil 54:339-357.

Jones, D.L. 1998. Organic acids in the rhizosphere - a critical review. Plant and Soil 205:25-44.

Keser, M., B. F. Neubauer, and F.E. Hutchinson. 1975. Influence of Aluminum Ions on Developmental Morphology of Sugarbeet Roots. Agron. J. 67:84-88.

Kiegle, E., M. Gilliham, J. Haseloff, and M. Tester. 2000. Hyperpolarisation-activated calcium currents found only in cells from the elongation zone of *Arabidopsis thaliana* roots. The Plant Journal 21:225-229.

Kinraide, T.B. 1991. Identity of the rhiz-toxic aluminium species. Plant Soil 134:167-178.

Kinraide, T.B., and D.R. Parker. 1990. Apparent phytoxicity of mononuclear hydroxy-aluminum to four dicotyledonous species. Physio. Plant. 79:283-288.

Kochian, L.V. 1995. Cellular mechanisms of aluminum toxicity and resistance in plants. Annual review of plant physiology and plant molecular biology. 46:237-260.

Koyama, H., T. Toda, and T. Hara. 2001. Brief exposure to low-pH stress causes irreversible damage to the growing root in Arabidopsis thaliana: pectin Ca interaction may play an important role in proton rhizotoxicity. Journal of Experimental Botany 52:361-368.

Li, H., R.J. Lascano, J. Booker, T.L. Wilson, and K.F. Bronson. 2001. Cotton lint yield variability in a heterogeneous soil at a landscape scale. Soil and Tillage Research 58:245-258.

Lindsay, W.L. 1979. Chemical Equilibria in Soils. John Wiley & Sons, NY.

Ma, J.F., R. Shen, S. Nagao, and E. Tanimoto,. 2004. Aluminum targets elongating cells by reducing cell wall extensibility in wheat roots. Plant Cell Physiol. 45:583-589.

Magdoff, F.R., and R.J. Bartlett. 1985. Soil pH buffering revisited. Soil Sci Soc. Am J. 49:145-148.

Marschner, H., and V. Römheld. 1983. *In vivo* measurement of root-induced pH changes at the soil-root interface: Effect of plant species and nitrogen source. Z. Pflanzenphysiol. Bd. 111:241-251.

McCown, R.L., G.L. Hammer, J.N.G. Hargreaves, D.L. Holzworth, and D.M. Freebairn. 1996. AP-SIM: a novel software system for model development, model testing, and simulation in agricultural systems research. Agric. Syst. 50:255-271.

Menzies, N.W. 2003. Toxic elements in acid soils: chemistry and measurement. *In* Handbook of Soil Acidity (Rengel, Z., ed.), pp. 1-28. Marcel Dekker, Inc. New York.

Miyasaka, S.C, J.G. Buta, R.K. Howell, and C.D. Foy. 1991. Mechanism of aluminum tolerance in snapbeans. Root exudation of citric acid. Plant Physiol. 96:737-743.

Neumann, G., and V. Römheld. 1999. Root excretion of carboxylic acids and protons in phosphorus-deficient plants. Plant and Soil 211:121-130.

Parker, D.R., T.B. Kinraide, and L.W. Zelazny. 1988. Aluminum speciation and phytotoxicity in dilute hydroxyl-aluminum solutions. Soil Sci. Soc. Am. J. 52:438-444.

Rengel, Z. 1992. Role of calcium in aluminium toxicity. New Phytol. 121:433-413.

Russell, E.W. 1973. Soil conditions and plant growth. 10th ed. Longman, London.

Ryan, P.R., J.M. Ditomaso, and L.V. Kochian. 1993. Aluminium toxicity in roots: an investigation of spatial sensitivity and the role of the root cap. J. Exp. Bot. 44:437-446.

Šamaj, J., F. Baluška, and D. Menzel. 2004. New signalling molecules regulating root hair tip growth. Trends in Plant Science 9:217-220.

Schofield, R.M.S., J. Pallon, G. Fiskesjö, G. Karlsson, and K.G. Malmqvist. 1998. Aluminum and calcium distribution patterns in aluminum-intoxicated roots of Allium cepa do not support the calcium-displacement hypothesis and indicate signal-mediated inhibition of root growth. Planta 205:175-180.

Sigel, H., and A. Sigel. eds. 1988. Metal ions in biological systems: aluminum and its role in biology, Vol. 24. New York: Marcel Dekker.

Smit, G., J.W. Kijne, and B.J.J. Lugtenberg. 1987. Involvement of both cellulose fibrils and a Ca-dependent adhesion in the attachment of rhizobium leguminosarum to pea root hair tips. Journal of Bacteriology 169:4294-4301.

Stevens J.J., and R.W. Blanchar. 1992. Soil pH gradients near calcite and dolomite particles. Soil Sci Soc. Am. J. 56:967-972.

Stumm, W., and J.J. Morgan. 1981. Aquatic Chemistry, ed 2. John Wiley & Sons, NY.

Sumner, M.E., H. Shahandeh, J. Bouton, and J.E. Hammel. 1986. Amelioration of an acid soil profile through deep liming and surface application of gypsum. Soil Sci. Soc. Am. J. 50:1254-1258.

Sumner, M.E., and T. Yamada. 2002. Farming with acidity. Comm. in Soil Sci. and Plant Anal. 33:15-18.

Terry, D.L., and C.B. McCants. 1970. Quantitative prediction of leaching in field soils. Soil Sci. Soc. Am. Proc. 34:271-276.

Toma, M., M.E. Sumner, G. Weeks, and M. Saigusa. 1999. Long-term effects of gypsum on crop yield and subsoil chemical properties. Soil Sci. Soc. Am. J. 39:891-895.

Verburg, K., J. Braschkat, Z. Hochman, A.D. Moore, K.R. Helyar, N.E. Prober, J.N.G. Hargreaves, and R.J. Simpson. 2003. Modeling acidification processes in agricultural systems. *In* Handbook of Soil Acidity (Rengel, Z., ed.), pp. 135-187 Marcel Dekker, Inc. New York.

Versel, J-M. and G. Mayor. 1985. Gradients in maize roots: Local elongation and pH. Planta 164:96-100.

Vitorello, V.A., F.R. Capaldi, V.A. Stanuto. 2005. Recent advances in aluminum toxicity and resistance in higher plants. Braz. J. Plant Physiol. 17:129-143.

von Uexküll, H.R., and E. Mutert. 1995. Global extent, development and economic impact of acid soils. Plant Soil 171:1-15.

Weisenseel, M.H., A. Dorn, and L.F. Jaffe. 1979. Natural H+ currents traverse growing roots and root hairs of barley (*Hordeum vulgare* L.) Plant Physiol. 64:512-518.

III. Chemolithoautotrophic Nitrification in Sandy Soils

Overview

Nitrification is the term used to describe the biologically mediated oxidation of ammonia to nitrate, a conversion from the most reduced to the most oxidized form of nitrogen (see table 1.5). This process is largely performed by chemolithoautotrophic bacteria in soil and aquatic environments and is central to the global nitrogen cycle. Other documented pathways for the oxidation of ammonia include heterotrophic bacteria, fungi, and actinomycetes (Tate, 2000; Haynes, 1986), methanotrophs (De Boer, and Kowalchuk, 2001; Bedard and Knowles, 1989) and the anaerobic oxidation of ammonium directly to N_2 (annamox) by plantomycetes (Thamdrup et al., 2006). Archaeal organisms similar to bacteria but given their own domain are also currently being studied for their contribution to ammonia oxidation (Leininger et al. 2006; Tourna et al., 2008). Chemical photooxidation has also been proposed as a possible pathway (Bartlett, 1981). Under the range of conditions encountered in soils under agricultural production in the Southeastern U.S. Coastal Plain, however, none of these alternative pathways are expected to make meaningful contributions to the overall oxidation of ammonia. In fact, it is only under extreme conditions which severely restrict or inhibit the activity of soil chemolithoautotrophic nitrifiers (i.e. substrate limitation, pH < 4.5, the presence of inhibitory allelopathic substances, anaerobic environment, etc.) that any of these alternative pathways becomes significant in oxidizing mineral nitrogen. The scope of this review will therefore remain confined to the subject of chemolithoautotrophic nitrification and its importance with respect to coarse textured sandy soils such as those common to the Southeastern U.S. Coastal Plain.

Chemolithoautotrophs, so named because of their utilization of mineral ammonia (NH_3) as the sole source of energy (chemotrophy) and reductant (lithotrophy) and the use of CO_2 as the

sole carbon source (autotrophy), fulfill a monumentally important role in the global nitrogen cycle. These organisms are the primary reason that the Earth's atmosphere, rather than its litho- or hydrosphere, is the global sink for nitrogen. Without the oxidation of ammonia to nitrate, ammonia/ammonium concentrations would continue to rise without providing substrate for denitrification and subsequent production of gaseous nitrogen (N_2, N_20, etc.). Some well known adverse environmental effects that have driven much of the research into nitrification are the acidification of soils and the accumulation and leaching of nitrates into groundwater. The former can lead to a decline in soil fertility while the latter is associated with human and animal health concerns. In acknowledgement of this fact, the U.S. Environmental Protection Agency has limited the permissible level of nitrates in water to 10 ppm NO_3^--N (40 CFR 141.11). Infiltration of nitrate into freshwater systems may also lead to eutrophication of aquatic environments and stimulation of phototrophic and/or heterotrophic organisms resulting in decreased biodiversity and anoxification of the ecosystem.

The oxidation of ammonia by soil nitrifiers has been classically summarized by equation [1].

$$NH_3 + 2O_2 \rightarrow NO_3^- + H^+ + H_2O \qquad\qquad [1]$$

This equation can be broken down into two separate steps carried out by two phylogenetically distinct groups of bacteria that collectively complete the process. The first group is often termed ammonia-oxidizing bacteria (AOB). The organisms that belong to this group obtain their energy by catabolizing ammonia (Suzuki et al., 1974; Hyman and Wood, 1985) and in soils include the genera *Nitrosomonas, Nitrosospira, Nitrosococcus, Nitrosolobus, and Nitrosovibrio* (Table 1.6. Nitrifiers and Morphology). The end product of the utilization of ammonia for energy by AOB

is nitrite. The second group is designated as nitrite-oxidizing bacteria (NOB) and contains the

single genus *Nitrobacter.*

All of the aforementioned genera of nitrifiers belong to the family Nitrobacteraceae, a

designation based on physiological properties such as the use of ammonia or nitrite oxidation as

an energy source and fixation of CO_2 for organic carbon (Prosser, 1989). They are prokaryotic

organisms of the beta subclass Proteobacteria. All members of the family Nitrobacteraceae are

gram negative, yet exhibit a wide range of morphologies (Table 1.6). They share a common and

narrow phylogeny, probably originating from a single ancestor in common with methanotrophs

(Bedard and Knowles, 1989), and although some *Nitrobacter* spp. may be facultative anaerobes,

nitrification is an obligately aerobic process.

Enzymology of Ammonia Oxidation

The overall oxidation of ammonia to nitrate as given by equation [1] above is partitioned

between the two groups of bacteria as follows.

$$\text{AOB} \qquad NH_3 + 1\tfrac{1}{2}O_2 \rightarrow NO_2^- + H_2O + H^+ + 84 \text{ kcal mol}^{-1} \qquad\qquad [2]$$

$$\text{NOB} \qquad NO_2^- + \tfrac{1}{2}O_2 \rightarrow NO_3^- + 17.8 \text{ kcal mol}^{-1} \qquad\qquad\qquad [3]$$
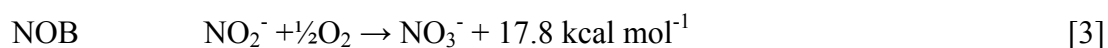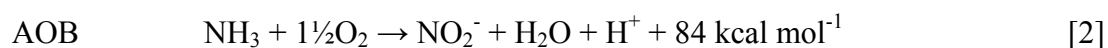
Equation [2] produces roughly 4 times the amount of energy as equation [3] and involves a

compound reaction wherein a hydroxylamine intermediate is produced and further oxidized to

nitrite. The process described by equation [2] requires two separate enzymes, ammonia mono-

oxygenase (AMO) and hydroxylamine oxygenase (HAO) (Figure 1.2). AMO is a membrane-

bound copper protein that initiates ammonia catabolism by oxidizing ammonia to

hydroxylamine. Hydroxylamine produced in reaction [4] is delivered to HAO, which operates in

the periplasm.

AMO $\quad\quad\quad$ $NH_3 + O_2 + 2H^+ + 2e^- \rightarrow NH_2OH + H_2O + 0.7\ kcal\ mol^{-1}$ $\quad\quad\quad$ [4]

HAO $\quad\quad\quad$ $NH_2OH + H_2O \rightarrow NO_2^- + 5H^+ + 4e^- + 83.3\ kcal\ mol^{-1}$ $\quad\quad\quad$ [5]
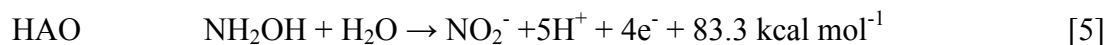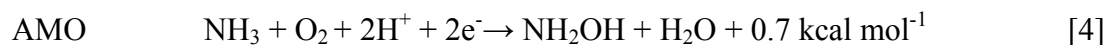
According to Arp et al. (2007), the core metabolic process is the extraction of electrons from hydroxylamine to generate proton-motive force and reductant in AOB. The extension of AMO provides a mechanism to feed this process using ammonia and oxygen but produces a negligible amount of power in and of itself. Four specialized proteins have been identified as being required to facilitate the aerobic use of ammonia as the sole source of energy and reductant. These are AMO, HAO, and cytochromes c554 and c_M552. Two of the electrons produced in reaction [5] are returned to AMO via a reduced c554 to regenerate the enzyme. The remaining two electrons pass down the electron-transport chain (ETC) via c_M552 generating a proton motive force (PMT) (Prosser, 1989). Two of the protons produced in reaction [5] are returned to the electron donor resulting in a net of 1 mole H^+ produced per mole of NH_3 consumed (Figure 1.2). Hooper et al., (1997) suggested that, as other known monooxygenases activate oxygen (rather than the substrate) by reaction with a 2 electron-reduced metal containing enzyme followed by release of water, this is probably the mechanism by which AMO also operates.

Hydroxylamine can serve directly as a reduced substrate for the production of energy for biosynthesis but it is toxic at fairly low concentrations. When ammonia is absent and only hydroxylamine is present, ATP will continue to be synthesized and energy dependent transport activity will continue. In a study designed to separate the two steps in the oxidation of ammonia to nitrite performed by AOB, Frijlink et al. (1992) reported that the maximal rates of ammonia and hydroxylamine oxidation were very similar. These authors suggested that the rate limiting step must be the cytochrome mediated electron transport back to AMO. The same authors also

reported the discovery that hydroxylamine oxidation may occur at much lower pH values (< 5.0) than the oxidation of ammonia. Hydroxylamine (as an intermediate in the oxidation of ammonia) was not observed to accumulate under acid conditions. This is important as it is well known that nitrifiers are highly sensitive to acidic environments (De Boer and Kowalchuk, 2001; Darrah et al., 1986b; Morrill and Dawson, 1967). In light of the report on the properties of hydroxylamine oxidation, this sensitivity may well be due to some form of acid inhibition to reaction [4].

Enzymology of Nitrite Oxidation

In contrast to the oxidation of ammonia to nitrite by AOB, the oxidation of nitrite to nitrate by NOB is a simple one-step reaction (equation [3]). Nitrate production from nitrite in NOB is catalyzed by the soluble, molybdenum-containing protein nitrite oxidoreductase (NXR) (Starkenburg et al., 2006; Hooper, 1989). This enzyme is apparently reversible, allowing for anaerobic growth of NOB by dissimilatory nitrate reduction (Freitag et al., 1987). In the oxidation reaction (equation [3]) nitrite functions as an electron donor for the reduction of NAD via reverse electron flow and the generation of ATP by oxidative phosphorylation. 2 moles of electrons are produced per mole of nitrite oxidized in this fashion. Unfortunately, much more research currently exists concerning the enzymology, electron transport, and energy generation of AOB than exists for NOB.

Habitat and Ecology of Soil Nitrifiers

In soils, nitrifiers (AOB and NOB) are typically found in the greatest numbers in the top few centimeters nearest the surface, though nitrification activity has been documented to occur in the subsoil at depths as low as 230 cm below the surface (Swensen and Bakken, 1997). Nitrification is photo-inhibited, and so the exposed surface of the soil itself will not provide a

growth-conducive environment as copper-containing proteins such as AMO have been proven to be inactivated in the presence of UV light (Bedard and Knowles, 1989).

In soils under agricultural production, these organisms exist in an environment characterized by cycles involving periods of high substrate availability accompanied by robust growth and activity followed by periods of starvation and rest. Temporal variations in soil water potential, soil solution pH, temperature, and osmotic pressure are also environmental 'norms' for nitrifiers that impact their patterns of growth and activity. For instance, Singh and Kashyap (2007) documented the seasonal population dynamics of AOB and NOB at several sites that varied in soil properties and seasonal rainfall. These authors reported a correlation between soil moisture and nitrification rates resulting from a higher number of free-living cells of nitrifying bacteria during the wet period as compared to the dry period. They found further correlations between sites with the highest amount of year round moisture and overall nitrifier populations as well as positive relationships between increased AOB and NOB populations. The concept of seasonal variability in nitrifier activity is supported by Breuer et al. (2002) and Badía (2000). Nitrifiers are also reported in this study as being among the soil microorganisms most sensitive to moisture stress.

Soil nitrifiers are, in fact, more sensitive than most other soil bacteria to many types of environmental stresses. The low thermodynamic favorability and relative inefficiency of the oxidation reactions carried out by AOB and NOB (equations [4] and [5]) for metabolic energy makes them poor competitors with heterotrophs for substrate and habitat (Verhagen and Laanbroek, 1991). Their growth rate is slower and their activity is lower than most other prokaryotic bacteria. Total numbers have been measured as constituting less than 0.01% of the total population of soil bacteria (Phillips et al., 2000). Despite these seemingly unfavorable

properties, or perhaps because of the relatively unique ecological niche occupied by chemolithoautotrophic nitrifiers, they are reported as being ubiquitous wherever supplies of ammonium or nitrate are found (Walker, 1975).

Such ubiquity, when considered against the range of climate and soil types nitrifiers have been observed to proliferate in, may be attributed to the ability or quality of these organisms to adapt (albeit slowly) to environmental change. Many investigators have noted either the presence of multiple strains of chemolithoautotrophic nitrifiers or the adaption of an existing population to changing environmental conditions over time (Avrahami and Bohannan, 2007; Avrahami and Conrad, 2003; Hankinson and Schmidt, 1989; De Boer et al., 1989). It is suggested here that the population ecology of soil nitrifiers is complex enough with respect to strains of both AOB and NOB that a range of optimum conditions may exist such that, as environmental conditions change, a more suitably adapted strain of nitrifier will slowly predominate both in numbers and activity. This concept may well apply to many of the factors known to affect nitrification rates.

Generally speaking, greater numbers of AOB and NOB (both in the growing and resting states) are known to exist in soils that contain higher contents of clay (Hoffman et al., 2007; Smith, 1964; McDowell and Smith, 1958) and organic matter (Chu et al., 2008; Weier and Gilliam, 1986). This phenomenon is a result of increased capacity of clay particles ($< 2\mu m$) over larger soil constituents to adsorb ammonium through cation exchange, increased surface area for nitrifier attachment, and a greater amount of micro-pore space less than 2 μm. That soil bacteria inhabit soil pore spaces is well established (Foster, 1988; Hattori and Hattori, 1976). This ability in nitrifiers provides protection against predation by protozoa (Rutherford and Juma, 1991). Pore space openings of $< 2\mu m$ present the restrictive limit for entrance by the smallest of

protozoa. Nitrifiers, however, are smaller than many other prokaryotic organisms and are better able to occupy these smaller pores. For instance, the reported sizes for *Nitrosomonas* sp. are around 0.5 μm in diameter and *Nitrobacter* sp. are placed in the range 0.6 – 0.8 μm wide and 1.0 μm in length with a pear-like shape (Tate, 2000; Hankinson and Schmidt, 1989).

The most favorable environment may well be the small diameter pores in soil aggregates that also retain water longer in a drying soil, thereby providing additional protection against desiccation. Hoffman et al. (2007) elaborated on this concept by reporting the results of micro-scale measurements of nitrification rates in soil aggregates. In their study, it was determined that the external surface layers of the aggregates showed higher potential nitrification rates than the interior fractions of the aggregates. Observed differences were more pronounced in clayey soils than in sandy soils leading the authors to suggest that oxygen and nutrient diffusion may be more efficient in coarsely textured soils and limiting in soils that are more finely textured. The conclusions reached in this study included a statement that, overall, nitrifiers in the interior of aggregates contributed little to the measurable nitrification rates. Also, the authors indicated that there were a significantly lower number of nitrifiers in the aggregate interior as compared to the exterior. This was determined by physically separating the aggregates with a scalpel and performing assays on slurries containing these portions. It is possible however, that this rapid assay may have suffered from a lag time effect leading to less than concretely conclusive results. Had the assay been of a longer duration, the population of the interior may have had a chance to overcome its resting state and establish a more robust level of activity.

Organic matter in soils leads to increased aggregation and also provides increased pore space, water holding capacity and exchange capacity. Even in coarsely textured sandy soils, such as those found in the Southeastern U.S. Coastal Plain, increases in organic matter are

associated with an increased capacity to support soil bacteria. Hissett and Gray (1976) showed that in a sandy soil, 64% of the bacteria were associated with organic particles even though these comprised a mere 15% of the total soil volume. This is certainly also a result of the increase in organic substrate for decomposition by soil organisms.

It is known that reduced and no-till practices have a profound effect on the amount of organic matter content, soil aggregation and microbial ecology of the topsoil under agricultural management. Accordingly, a great deal of research exists contrasting nitrification in conservation and conventional tillage regimes. In general, it is agreed upon that nitrifier numbers and activities are higher in no-till and reduced-till soils as compared to soils that are turned over and mixed (Carpenter-Boggs et al., 2003; Malhi et al., 2006). Stearman and Matocha (1992) have reported higher numbers of nitrifying organisms in no-till soils supplied with fertilizer during the course of , though not immediately prior to the growing season. Phillips et al., (2000) demonstrated that AOB diversity and nitrification rates are reduced in conventionally-tilled soils. These authors suggested that, as no-till soils maintain pore structure and continuity, greater hydraulic conductivity and infiltration rates, they may support more stable and active AOB communities than tilled soils. This supports the conclusions of Rice and Smith (1983) and Groffman (1985) who noted that the physical properties of no-till soils allow for greater water holding capacity.

The above findings must be tempered with the knowledge that almost all of the microbial activity, organic matter, and placement of substrate occur in the topmost centimeters of the profile of no-till managed soils. Kandeler and Böhm (1996) found that overall potential nitrification (0-30 cm) was not significantly different between conventional and minimum-till treatment plots, but the location of the respective highest activities was. In the minimum-till soil,

activity was far higher in the 0-10 cm depth range while the conventionally tilled treatment

exhibited higher nitrification rates in the 20-30 cm range. Both were reported to be equivalent at

the intermediate 10-20 cm depths. Similar results have been described by Groffman (1985) and

Fuentes et al. (2006). The combination of high microbial activity, organic material, and substrate

concentration in such a small volume of soil portends some interesting patterns unique to soils

under conserved and no-tillage management. This volume of soil will be more buffered against

pH change due to the high organic matter content, yet it will be subjected to more rapid changes

in pH due to increased nitrification and location of NH_4^+-N substrate. However, the localization

of changes in soil pH allows for more effective treatment with lime. Furthermore, there may

exist a reduced potential for nitrate to leach below the root zone, particularly in cases where

conservation tillage is coupled with the planting of winter cover crops.

Nitrifiers as Community Organisms

Nitrifiers may be found free in soil solution but find many advantages to attached and

aggregated growth, including protection from environmental stress and predation by larger soil

organisms as well as improved physical proximity to substrate (Foster, 1988; Rutherford and

Juma, 1992; De Boer et al., 1991). Aggregation and surface attachment of nitrifiers to soil

surfaces may in fact provide both AOB and NOB with advantages in times of 'feast' and in times

of 'famine'. Albrecht and McCalla (1937) demonstrated very early on that ammonium adsorbed

to colloidal clay is preferentially nitrified to nitrite and that calcium adsorbed to soil colloidal

material stimulated nitrification. Lees and Quastel (1946) later presented the results of a series

of controlled experiments concerning the concept that adsorbed NH_4^+ is preferentially oxidized

by AOB over free solution ammonium. By controlling the amount of adsorbed ammonium

through cation competition or increased exchange capacity, they arrived at the following

conclusions. (1) Nitrification in soils occurs on surfaces and not in solution. (2) The rate of nitrification in soil is proportional to the fraction of the total NH_4^+ adsorbed on exchange complexes independent of soil solution concentration and increases when adsorbed ammonium concentration increases. (3) The rate of nitrification can be increased by the addition of sterile soil proportional to the base exchange capacity of the added soil. This implies that AOB are able to increase use of space by proliferating onto surfaces that offer increased adsorbed substrate. Addition of sand did not increase nitrification rates in the study. Finally, soils that have been enriched with nitrifying bacteria prior to the addition of substrate fail to exhibit a lag period, and as long as substrate is not limiting, will instead show a linear rate of NO_3^- accumulation. In contrast to the study by Albrecht and McCalla (1937), Lees and Quastel (1946) found that adsorbed calcium resulted in a decrease in nitrification rates and attributed this to a reduction in available substrate (i.e. adsorbed ammonium) due to competitive adsorption by calcium.

Underhill and Prosser (1987) reported that AOB (*N. europaea*) colonized much more extensively on negatively charged exchange resins while NOB (*Nitrobacter*) colonized almost exclusively on anion exchange resins. Results also showed that attachment of nitrifiers to charged resin beads was associated only with growth and not with stationary phase cultures as significant adsorption onto ion exchange resins was always associated with substrate oxidation. These researchers concluded that a major factor affecting colonization is the site of substrate adsorption. However, typical agricultural soils offer far more cation exchange capacity than anion exchange capacity. If substrate location is the determining factor then it may be that NOB operate better than AOB in solution.

De Boer et al. (1991) discovered that NOB existed as single cells and as aggregates in soil. Aggregates were characterized as being spherical in appearance with a diameter of 3 to 20

μm. Some aggregated communities of nitrifiers contained both *Nitrosospira* and *Nitrobacter* sp., though the different organisms occupied separate portions of the aggregate complex without 'intermingling'. Such close proximity between the two organisms on soil surfaces would facilitate access by NOB to nitrite produced by AOB. This observation is supported by Stein and Arp (1998) who suggested that nitrite toxicity to AOB would also be alleviated by physical proximity between the two populations. Additionally, De Boer et al. (1991) reported evidence of a stabilizing polymer matrix holding the aggregates together.

Batchelor et al. (1997) attributed the prolonged growth of nitrifiers under limiting conditions such as low pH or the presence of inhibitors to the production of extracellular polymeric substances (EPS) which also protect against desiccation. The authors suggest that EPS contain anionic components which may adsorb ammonium to be released gradually within the biofilm, maintaining cells at low levels of activity. This study showed that starved biofilm populations recovered much more rapidly than freely suspended cells. Recovery of activity by suspended cells following starvation (absence of substrate) was markedly improved upon treatment with N-(3-Oxohexanoyl)-L-homoserine lactone (OHHL), a synthesized lactone similar to the naturally produced N-acyl-homoserine lactone (AHL), even at the lowest dose tested. These investigators concluded that a signaling system termed 'quorum sensing' may be involved in community response to severe substrate limitation and subsequent recovery. The mechanisms of this signaling system are reported to be dependent on the accumulation of signal molecules such as AHL. The results of this study provide evidence that cell density signaling mediated by AHL has a role in activating cell growth in biofilms.

It may be that extracellular polymeric material allows surface attached nitrifiers to remain active longer than suspended cells in acid soil conditions. Allison and Prosser (1993) induced a

decrease in pH from 6 to pH 5 and reported a gradual decrease in activity rather than an abrupt cessation. This finding is in contrast with the general impression that nitrification is very often not initiated or severely depressed in soils exhibiting a pH <5.5 (De Boer and Kowalchuk, 2000; Morrill and Dawson, 1961). Such a slow decrease in activity may represent the consumption of metabolic energy for cell maintenance and the cessation of growth, even as the oxidation of ammonia continues. The authors also suggested that cells do not rely on passive diffusion but use active transport to absorb NH_4^+.

Urea Hydrolysis by AOB

Urea hydrolysis by ammonia oxidizers has been reported by Swensen and Bakken (1997) and Burton and Prosser (2001). The study performed by Burton and Prosser (2001) examined the growth of Nitrosospira sp. in a poorly buffered liquid media supplied with urea at pH values ranging from 4 to 7.5. Growth was observed at all pH values. Hydrolysis of the supplied urea was followed by an exponential increase in ammonium and nitrite which subsequently stopped after exhaustion of the urea supply. Lag phases were reported as being shorter than those observed when ammonium was supplied as the nitrogen source. This indicates that at least some AOB are urealytic. It is possible that the observed shorter lag phase would support a model in which neutral internal cellular pH must be maintained (at the expense of metabolic energy) to oxidize ammonia. If a chemical reaction such as the one given by equation [6] enhances this mechanism then the organism spends less of its own energy on the process of pH maintenance and lag times are thus shortened. In this case the consumption of one proton during hydrolysis would provide considerable 'enhancement'.

Urea Hydrolysis $(NH_2)_2CO + H_2O + H^+ \leftrightarrow 2NH_4^+ + CO_2$ [6]
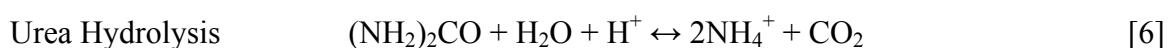
Inhibition of Nitrification

Nitrification is known to be inhibited whenever necessary conditions veer into the severely sub-optimal range. This applies to highly acidic (or alkaline) soil conditions, extremely dry environments, and/or prolonged periods of extreme heat or cold. High salt concentrations contributing to elevated osmotic potentials have been demonstrated to inhibit nitrification (Rosenberg et al., 1986; Darrah et al., 1987). Inhibition has been suggested to occur as the result of naturally-occurring allelopathic substances found particularly in acid forest soils (Rice, 1984). The list of potential allelopathic inhibitors includes phenolic acids, flavonoids and terpenoids, antibiotic compounds (De Boer and Kowalchuk, 2000). Monoterpenes have been included in the group of allelopathic substances known to inhibit nitrification (White, 1994). Monoterpenes are volatile compounds that share some commonalities with other known AMO inhibitors such as nitrapyrin.

Nitrapyrin (N-serve) is an example of a substance often deliberately applied to agricultural soils to inhibit nitrification. Other commercially available inhibitors include thiourea, dicyandiamide (DNDN), and the reformulated nitrapyrin product Stay-N 2000 (Rovita and Killorn, 2007; Hauck, 1980). Also, many currently available herbicides, fungicides and insecticides are known to inhibit nitrification (Hauck, 1980). The reason for such application is to prevent the loss of applied N fertilizer over the winter months. This practice takes advantage of the adsorption of ammonium by clays and organic matter to prevent the leaching of applied N by inhibiting the production of the much more highly mobile nitrate.

In the Southeastern U.S. however, due to the warm climate, deep and easily drained sandy soils in the Coastal Plain, and high rainfall in the spring, the use of nitrification inhibitors is not generally recommended as a method of improving N efficiency in agri-production.

Comparatively warm winter temperatures can stimulate nitrification even in the presence of common inhibitors such as nitrapyrin, which itself degrades as a function of time and temperature (Touchton and Boswell, 1980). Fall-applied anhydrous ammonia has been shown to completely nitrify by January, well ahead of planting (Kyverga et al., 2004). In deep sandy soils, a single 2.5 cm rainfall event can result in the downward movement of nitrates as much as 30 cm (Touchton and Boswell, 1980).

Measuring Nitrification

Rates of nitrification in soils may be characterized as the amount of substrate (NH_4^+-N or NO_2^--N) oxidized or as amount of product (NO_2^--N or NO_3^--N) formed per unit soil per unit time. This is most often expressed as mg N kg^{-1} soil $time^{-1}$. Under most conditions, differences between substrate disappearance and product accumulation rates have been reported as being very small (Hadas et al., 1986; Weier and Gilliam, 1986). As measurement in situ is nearly impossible at this point due to the need to perform measurements in laboratory settings on soil material that is often prepared in some way (drying, crushing, sieving, etc.) to minimize heterogeneity, quantifications of nitrification rates are often based on laboratory experiments that allow for greater control by the experimenter but relate little to realities in the field. It should be noted that Eno (1960) described a method to measure rates in situ using polyethylene bags buried in the field that is still in use today (Singh and Kashyap , 2007). In this way, the experimenter is allowed to expose the treatments to the same environmental temperature fluctuations as the surrounding soil of interest, while maintaining isolation from other chemical and physical interactions.

Several approaches to laboratory measurement of nitrification rates have emerged in the last century. As a result, the term 'nitrification potential' has generally come to be accepted as

the descriptor of choice to label net rates as measured under a set of operationally defined experimental conditions set forth by a researcher or researchers (Hart et al., 1994). Net rates are often very similar to gross rates under conditions when interfering processes such as competition for substrate or product (i.e. heterotrophic immobilization and/or nitrification by non-autotrophic organisms), denitrification, or interlayer clay mineral fixation are minimal. Some of the most commonly used laboratory approaches to the measurement of nitrification rates include soil perfusion columns (McLaren, 1971; Lees and Quastel, 1946), incubated soil slurries (Wheatley et al., 2001; Low et al., 1997), and aerobic soil incubations (Rovita and Killorn, 2007; Robertson and Vitousek, 1981; McDowell and Smith, 1958). Any of these may be used in combination with N^{15} tracer techniques (Russell et al., 2002, Sahrawat, 1985) or reaction specific inhibitors (De Boer and Kowalchuk, 2000). Such combined approaches are most useful when the aim of the researcher is to separate effects of interferences on the measurement of gross nitrification rates or when it is the aim to eliminate individual component(s) of the overall process of nitrification by AOB and NOB.

Hart et al. (1994) recommend the use of soil slurries over other methods to characterize potential nitrification rates. These authors cite a superior ease in interpretation and reproducibility over other types of nitrification assays as well as a relative linearity in approximating the maximum nitrification rate (V_{max}) under the stated conditions over a relatively short period ($\leq$ 24 hrs). This seems an excellent suggestion when only maximum rates are of interest. However, maximum rates under any set of conditions are at best only a fraction of the overall activity of nitrifiers when measured over time. As outlined earlier, soil nitrifiers exist in an environment characterized by fluctuations in temperature, moisture, substrate availability, etc. The nitrifying population in soil is relatively small but it increases rapidly upon addition of

substrate (NH_3) to the soil (Hadas et al., 1986). The increase in nitrifier numbers leads to an increase in the rate of substrate decomposition and/or product accumulation. As the depletion of substrate nears, rates and organism numbers decline. The sum of these processes yields a sigmoidal curve when cumulative nitrate production is plotted against time (Figure 1.3).

This sigmoidal curve has been described by many researchers as displaying three distinct phases (Sabey et al., 1959; Morrill and Dawson, 1967; Hadas et al., 1986; Laubscher et al., 1990). The first is the initial 'lag phase' wherein nitrifier numbers are low but increasing due to a recent increase in substrate or alleviation of some inhibiting factor. The second is the 'maximal rate phase' in which the rate of nitrification can be said to be relatively linear for some period of time following the end of the lag phase and prior to the onset of the effects of substrate limitation. The final phase is the 'retarded' or 'reduced rate' phase wherein rates decline to near zero as the availability of substrate declines sharply. Observation of the differential response of each of these phases when making comparisons across soil types or as a function of some environmental condition such as pH or soil moisture potential on a single soil over time is more appropriately achieved when perfusion columns or aerobic incubations are the laboratory method employed. There are difficulties and advantages reported in using either method. Soil perfusion columns require the attainment of steady state (or accounting for non-steady state) conditions before conclusions can be drawn concerning measurements, but allow for the measurement of solute transport characteristics (McLaren, 1971; Grundmann et al., 1995). Aerobic incubations require attention and maintenance of soil moisture potential and aeration status in experimental units throughout the duration of the study, but can be set up more easily than perfusion columns and results may be easier to interpret and compare to field studies (Shi and Norton, 2000; Weier and Gilliam, 1986; Laubscher et al., 1990).

A recent call was made (Sahrawat, 2008) for a more systematic way of comparing nitrification potentials in differing soils as a function of the factors that influence nitrification. A characterization of all three phases of nitrification following the introduction of substrate must be provided in any such system. It has been shown that each of the three phases can be closely correlated with one or more of the physical and chemical soil factors that affect soil nitrification potentials (Sabey et al., 1959; Sabey et al., 1969; Morrill and Dawson, 1967; Laubscher et al., 1990; Rovita and Killhorn, 2007). In the interest of providing a more complete understanding of the process of nitrification, it is recommended here that aerobic incubations be employed toward this end. Experimental conditions are easier to control than with perfusion columns and more experimental units can be observed per treatment combination allowing for greater statistical interpretation of results. It is believed that results will be more closely related to field conditions as well. The need for such an improved understanding is important and has been reiterated often (Subbarao et al., 2006, Hadas et al., 1986; Malhi and McGill, 1982), especially in reference to agricultural systems that receive very high inputs of fertilizer nitrogen. The potential for loss in agricultural systems can be massive and depends largely on the process of nitrification. Recent estimates of nitrogen fertilizer use efficiency have placed losses at or near 70% of applied fertilizer-N through nitrification and associated processes (Glass, 2003). The need for a better understanding and systematic way of interpreting potentials is apparent and has rightly made the process of nitrification in agricultural soils a subject of ongoing investigation. However, when solute transport is an area of interest, then aerobic incubations cannot provide the same insight as soil perfusion columns.

Factors Affecting Nitrification Potential in Soils

There are a number of environmental factors known to affect all bacteria in soil. As previously stated, soil nitrifiers (AOB and NOB) are unusually sensitive to most of these. Environmental factors that have been identified by researchers as affecting nitrification potentials in agricultural soils include some physical, climatic, chemical, and geographic conditions. The factors considered to be the most important to the process of nitrification in agricultural soils have been reported in the existing literature as: pH, soil moisture potential, temperature, substrate availability, osmotic potential, aeration, soil texture, and soil organic matter (concentration and C/N ratio). The influence of each of these factors is complex and often interactive with some or all other factors during any given time interval. The response of nitrification potentials to changes in most of these factors often resembles a bell shaped curve where nitrification ceases at some low and high extreme and approaches a maximum at some factor level in between. What follows is intended to be a thorough discussion of the effect of each factor on nitrification potential with respect to all three phases of the nitrification process as outlined previously.

Factors: Substrate Effects

Without substrate (NH_3) there can be no production of nitrite by AOB, and without nitrite there can be no production of nitrate by soil nitrifiers. At very low concentrations, substrate can be said to be limiting and nitrate accumulation usually proceeds at a linear rate. For a population of nitrifiers to experience growth, substrate must be supplied in amounts that result in a production of metabolic energy in excess of that necessary for maintenance requirements (Shi and Norton, 2000). When supplied in sufficient amounts, substrate utilization, nitrifier numbers, and nitrate accumulation increase exponentially until substrate nears exhaustion and activity

slows resulting in the characteristic sigmoidal curve. As substrate concentrations increase beyond sufficiency, no increase in activity is observed and inhibition eventually occurs (Bouman et al., 1995; Malhi and McGill, 1985; Boon and Laudelot, 1962; Justice and Smith, 1962). In agricultural soils, it is not only the concentration but the source of fertilizer-N (e.g. ammonium sulfate, urea, anhydrous ammonia, MAP, DAP, organic manures, and animal wastes) that has been documented to affect nitrification rates as well (Russell et al., 2002; Shi and Norton, 2000; Whitehouse and Leslie, 1973; Eno and Blue 1954).

Inhibition at high substrate concentrations has been attributed to toxic levels of ammonia at high pH and nitrous acid at low pH (Anthonisen et al., 1976; Malhi and McGill, 1985; Justice and Smith, 1962), osmotic inhibition (Darrah et al., 1987; Malhi and McGill, 1985; Laura, 1977), and pH changes imposed by the form of fertilizer-N. Urea and anhydrous ammonia are known to initially raise the soil solution pH resulting in inhibitory pH values and/or toxic levels of ammonia while ammonium sulfate depresses the soil pH. Eno and Blue (1954) found that ammonium sulfate lead to more rapid nitrification rates in neutral sandy soils (as compared to anhydrous ammonia) while anhydrous ammonia applications resulted in higher rates in acidic sandy soils. These authors also noted a lethal effect of anhydrous ammonia to bacteria and fungi within the retention zone. Whitehouse and Leslie (1973), however, reported that in a clayey soil (alkaline black earth) ammonium sulfate nitrified more slowly than anhydrous ammonia. These authors concluded that the possibility of restricted diffusion and greater concentration of acidic effect produced in clay soil was the reason for the contradictory results.

Some optimum concentrations of nitrogen sources found in the literature are as follows. Bouman et al. (1995) reported that 90 kg N ha^{-1} (112 mg N kg^{-1} soil) resulted in the highest rates of nitrification in a field study with no increase in nitrate accumulation at twice that rate supplied

as anhydrous ammonia and/or urea. Justice and Smith (1962) reported a maximum rate of nitrate accumulation when 150 mg N kg^{-1} soil was supplied as $(NH_4)_2SO_4$. However, substrate depletion and nitrite accumulation were more rapid at the 450 mg N kg^{-1} soil treatment suggesting inhibition of NOB by ammonia and or osmotic pressure at this level. Malhi and McGill (1985) documented a rapid nitrification rate when 200 mg N kg^{-1} soil was supplied as $(NH_4)_2SO_4$ with inhibition occurring at 300 mg N kg^{-1}. These authors reported no nitrite accumulation at either level. Finally, NOB have been reported to be inhibited at nitrite solution concentrations exceeding 14.5 mM (667 mg NO_2^- L^{-1}; 203 mg NO_2^--N L^{-1}) and limited below this value (Boon and Laudelout, 1962).

Factors: pH

The response of nitrification potentials as a function of pH is one of the factors that follow a bell shaped curve. Ranges for minimum soil pH values at which nitrification will occur have been reported as being pH 3.5 - 5.5 (Frederick and Boadbent, 1965; Frijlink et al., 1990; Hankinson and Schmidt, 1989). Maximum values at which overall process of nitrification will occur have been reported between pH 8.5 - 9.6 (Frederick and Boadbent, 1965; Morrill and Dawson, 1967; Boon and Laudelout, 1962). Optimal pH values have been placed between pH 5.5 – 8.8 (Hankinson and Schmidt, 1989; Darrah et al., 1986b; Rosenberg et al., 1986; Quastel and Scholefield, 1951).

There are differences in response to pH between ammonia oxidation by AOB and nitrite oxidation by NOB. Nitrite oxidizers are generally more sensitive to pH changes than ammonium oxidizers and occupy a more narrow range of pH values within which activity has been documented. These differences with respect to pH between AOB and NOB were illustrated very well by Morrill and Dawson (1967) in a strong presentation of experimental data which should

always be considered whenever attempting to draw conclusions based on results of nitrification assays. In this paper, four reasonably distinct patterns were observed and described concerning the results of assays on 116 soil samples ranging in pH from 4.4 to 8.8 (Figures 1.4 – 1.6).

Pattern I: (pH range = > 7.3) at the highest pH values observed, ammonium is rapidly oxidized to nitrite which accumulated for some time before eventually being oxidized to nitrate.

Pattern II: (pH range = 4.5 – 7.3) Ammonium is rapidly oxidized with some small amount of nitrite accumulation early on which is then rapidly oxidized to nitrate with no further detectable nitrite.

Pattern III: (pH range = 4.5 – 6.4) Ammonium is more slowly oxidized to nitrate with no nitrite accumulation.

Pattern IV: (pH range = < 4.5 – 5.9) No ammonium oxidation is observed by either nitrite or nitrate formation at the lowest pH values observed.

The above patterns were highly correlatable with pH and pH related soil properties including Calcium, Iron, and Aluminum content. Correlation coefficients relating growth rate constants with soil properties are as follows: r^2_{pH} = 0.768; $r^2_{Calcium}$ = 0.679; $r^2_{pH\ and\ Calcium}$ = 0.782. The authors reported the results of organism number studies in this experiment. Patterns I (highest pH) and II both show very high activities for *Nitrosomonas* sp. Pattern I exhibits a longer lag time for *Nitrobacter* sp. than Pattern II, though the relative maximum rates are similar until substrate (NO_2^-) becomes limited by the reduced activity of AOB. Low numbers of *Nitrosomonas* sp. were measured in Pattern III soils which in turn controlled NOB populations. Pattern IV soils (lowest pH) tended to support little or no measurable nitrifier numbers from either AOB or NOB.

An attempt was made by these researchers to account for the mechanism by which these patterns were established. They reported that type II and III could be converted to exhibit type I patterns via upward adjustment of soil pH through $CaCO_3$ additions. Also, a type I pattern soil was converted to a type II pattern soil by pre-percolating the perfusion column with nitrite to "pre-activate" the *Nitrobacter* sp. present. This approach was more successful than inoculating the column with NOB contained in the perfusion solution. The authors' interpretation that this change was affected by an increase in NOB population (which circumvented the lag time otherwise necessary to establish high active organism numbers) rather than a change in pH seems very plausible. Another interpretation of this phenomenon might be that pH has less to do with NOB maximum rates than it does with optimum growth conditions. Metabolic processes associated with cell growth, reproduction, and maintenance may be the pH-restricted reactions that limit overall nitrite oxidation. It should be understood also that some if not most of the lag time associated with the growth and activity of nitrite oxidizers across all pattern types in this study can be attributed to their dependence on the product of ammonia oxidation for substrate. Morrill and Dawson (1967) further concluded that NH_3 has an inhibitory effect on NOB that results in an initial accumulation of nitrite and that NOB can be affected by the presence of nitrite (NO_2-N) and nitrate (NO_3-N), and by pH independently of the pH effect on ammonia concentration.

That the highest rates of oxidation of ammonia to nitrite by AOB occur in higher pH soils than the highest rates of nitrite oxidation to nitrate by NOB has been supported by other investigators. Eno and Blue (1954) noted an inhibitory effect of high pH in their study concerning ammoniacal fertilizer source effects on nitrification. They reported that neutral soils exhibited higher rates of nitrification when supplied with $(NH_4)_2SO_4$ while acid soils responded

better to applications of anhydrous ammonia. The pH elevating effects of anhydrous ammonia were credited with both results. The inhibitory effect of high pH on nitrification (both AOB and NOB) has been credited to the increased presence of NH_3 in soil solution resulting from its equilibrium relationship with NH_4^+ (Anthonisen et al., 1976). This inhibition was reported to be impermanent in that its effects were overcome following adjustment of pH or dilution of NH_3 concentrations. It is interesting to note that the findings of Anthonisen et al. (1976) illuminated a major difference in the sensitivities of AOB and NOB to inhibition by ammonia. AOB inhibition by ammonia was observed at solution concentrations from 10 to 150 mg l^{-1} while NOB inhibition can was observed at concentrations from 0.1 to 1.0 mg l^{-1}.

Dancer et al. (1973) observed that a general increase in soil pH was associated with a decrease in the length of delay period and an increase in maximum rate of nitrate accumulation. They calculated a linear relationship between pH and nitrate accumulation after 15 days as $y = 41.5x - 168.5$ ($r^2 = 0.92$) and concluded that the data support previous observations that there is an inverse relationship between initial numbers of nitrifiers and delay or lag period. Kyverga et al. (2004) reported that high pH values stimulated nitrification even in the presence of very low temperatures and inhibitory substances. These investigators also observed a good linear relationship between increased pH and rates of nitrification. Justice and Smith (1962) reported very high rates of nitrate accumulation in a calcareous loamy soil (pH = 7.8) at 22°C and 1 to 0.3 bars soil moisture tension. At all levels of NH_4-N added (0 – 450 mg N kg^{-1} soil) the amount of NO_3-N accumulated met or exceeded the same amount after 21 days. Such rapid nitrification rates should be expected in calcareous soils that feature high pH values and tremendous acid buffering capacity. Broadbent et al. (1957) reported rates as high as 350 ppm NO_3-N produced per week in a calcareous Salinas clay (pH = 8.1).

The generally positive linear aspect to the relationship between soil pH and nitrification rates (up to the point of inhibition) has continued to be supported by more recent studies (Weier and Gilliam, 1986; Page et al., 2002; Islam et al., 2006). The nature of this relationship mandates that nitrification may not proceed or may proceed only very slowly in soils with low pH values. This scenario is potentially detrimental to plant nutrition and can promote soil acidification through the unbalanced uptake of nitrogen primarily in the cationic form (NH_4^+) and in sandy or gravelly soils may lead to the leaching loss of ammonium. The adverse effect of low pH on nitrification is compounded by the fact that the oxidation of ammonium to nitrite produces acidity. In poorly buffered soils, the production of protons as a natural byproduct of nitrification can have a dynamic impact on the expected sigmoidal shape of the nitrate accumulation curve. A continually diminishing nitrification potential as a result of decreasing soil pH has been successfully addressed and predicted by Darrah et al. (1986b) in their attempts at modeling nitrification in soils. They reported an improved predictive accuracy over a model that does not include a term accounting for pH change resulting from nitrification. If the buffer capacity of a soil is known, and a total of 1 mole of H^+ are produced per mole of ammonia (NH_3) oxidized following dissociation of one proton from ammonium (NH_4^+), then the pH change can be calculated. A reduction of the μ_{max} term in Monod kinetics was applied via a fourth order function (equation [7]).

$$RNR_{pH} = 35.019 - 24.538\ pH + 6.147\ pH^2 - 0.647 pH^3 + 2.435 \times 10^{-2}\ pH^4 \qquad [7]$$

Anthonisen et al. (1976) asserted that the inhibition of nitrite oxidizers at low pH may be largely due to the increased presence of nitrous acid (HNO_2). Their study observed inhibition of NOB to be initiated at solution concentrations between 0.22 and 2.8 mg l^{-1}. Though the profound depression of nitrification potentials at low soil pH values has long been reported in the

existing literature (Hall et al., 1908; Morrill and Dawson, 1967; Weier and Gilliam, 1986), some research from the last two decades reports the findings of unexpectedly high rates of nitrification in low pH soils. These findings have been attributed to protection afforded by aggregation (De Boer et al., 1991; Allison and Prosser, 1993), the ability of some AOB to hydrolyze urea (Burton and Prosser, 2001) and the existence of 'microsites' (Hankinson and Schmidt, 1989: Burton and Prosser, 2001) arising from the natural heterogeneity of a soil or from tiny regions of soil that may be protected somehow from the chemical influence of the surrounding soil such as is the case with micro-pores or other areas that experience stagnant flow. Islam et al. (2006) dismissed the influence of microsites and suggested instead that the phenomenon of nitrification in acid soils is more likely the result of adaptation of (or structural change in the composition of) the AOB population. However, Hankinson and Schmidt (1989) also described the discovery of an acidophilic strain of *Nitrobacter* sp. isolated along with a neutrophilic strain from the same acid forest soil. The pH optimum for nitrite oxidation by the acidophilic strain was reported to be 5.5, well below the pH 7.2 optimum of the neutrophile. The existence of acid tolerant strains of both AOB and NOB is supported by De Boer et al. (1989).

Factors: Soil Water Potential

Nitrifiers are perhaps more sensitive to small changes in soil water potential than to any other factor affecting their growth and activity. Sabey (1969) showed that small reductions in water potential (< - 0.02 MPa) substantially reduced the rate of nitrification. Changes in soil water affect the availability of substrate and osmotic potential through changes in diffusive tortuosity and dilution/concentration of soil solution respectively (Papendick and Campbell, 1978). Soil water content effects on nitrification rates have also been shown to be temperature dependent (Grundmann et al., 1995; Macduff and White, 1985). When one considers that

fluctuations in soil water content can be rather extreme and may occur very rapidly due to precipitation, irrigation, and evaporation/transpiration, it should be clear that the response of chemolithoautotrophic nitrifiers to such changes can be complex and difficult to correctly interpret. Further complicating the issue is the fact that soil water content has been expressed in many ways. These include soil water potential (Ψ), soil water content (determined either gravimetrically or volumetrically), and percent of water-filled pore space (%WFP). The relationship between these different approaches to expressing soil water content is inconsistent and often dependent upon soil texture and organic matter content. For instance, matric potential at any given relative water content is a function of soil pore space. Coarse textured soils range in porosity from 0.3 to 0.4 (volume pore space volume soil^{-1}), while more clayey soils and organic soils may exhibit porosities in excess of 0.6 (Papendick and Campbell, 1978).

Soil water potential is the sum of the matric potential (including both adsorption and capillary effects) and osmotic potential (Papendick and Campbell, 1978). Both are always negative. Nitrification rates, when plotted against soil water potential, do not appear as a neat bell shaped curve as is found with other factors that affect rates. Such plots are frequently presented with soil water potential as the independent variable (x-axis) on a logarithmic scale and the response of nitrification rates on the y-axis. The general trend is for rates to increase from non-detectible at or below the permanent wilting point (often set at -15 bar tension) to a maximum somewhere around the field capacity (-0.1 to -0.5 bar) of a given soil (Miller and Johnson, 1964, Sabey, 1969; Sabey and Johnson, 1971; Malhi and McGill, 1982; Macduff and White, 1985, Rodrigo et al., 1997).

Theoretically, the expression of soil water content in terms of matric or soil water potential allows for the comparison of soils of differing textures. However, Papendick and

Campbell (1978) asserted that water content rather than potential is more appropriate for relating

functions concerning solute diffusion, and therefore substrate availability, to microbial activity in

systems which are diffusion limited. These authors offer a theoretical physical equation

(equation [8]) based on the Michaelis-Menten relationship to describe the flux (J) of nutrients to

microorganisms,

$$J = \frac{c_o + K + rJ_m - \{(c_o + K + J_mr)^2 - 4rc_oJ_m\}^{1/2}}{2r}$$
[8]

where c_o is the substrate concentration at the microorganism surface, K is the Michaelis-Menten

rate constant, r is the resistance to diffusion at the microorganism surface which increases with

proximity to soil surfaces as a function of the diffusion rate coefficient D_o, J_m is the maximum

possible process rate when the substrate is not limiting. The dependence of r on the diffusion

rate coefficient D_o is greatly affected by the thickness of the water film on soil surfaces as

thinner films exhibit reduced cross-sectional area and are prone to more drag and resultant

decreases in diffusive rates. Values for J_m and K can be found in the literature (Malhi and

McGill, 1982; McLaren, 1971; Sabey, 1969; Ardakani et al., 1973; Ardakani et al., 1974; Low et

al., 1997; Rovita and Killhorn, 2007), however, these values are highly variable with soil type

and experimental condition set.

According to Linn and Doran (1984) aerobic microbial activity increases with soil water

content until a point is reached where water displaces too much air, restricting the diffusion and

availability of oxygen. These authors suggested that %WFP is a better indicator for soils than

water tension or water holding capacity when determining aerobic vs. anaerobic microbial

activity as water contents approach field capacity. The value at which aerobic activity becomes

restricted was found to be 60% water filled pore space (%WFP). The function the authors use to relate aerobic activity to %WFP is as follows (equation [9]):

$$\text{Relative Aerobic Activity} = \%WFP / 60\% \text{ (for WFP} < 60\%) \qquad [9]$$

$$= 60\% / \%WFP \text{ (for WFP} > 60\%)$$

This finding concerning the critical value separating maximum aerobic activity and the onset of anaerobic activity in soil microorganisms is supported by Miller and Johnson (1964), Grundmann and Rolston (1987), and Grundmann et al. (1995). The latter reported optimal water contents for nitrification to occur at a water status wherein aggregates only were saturated with water and no water existed in the interaggregate spaces. Breuer et al. (2002), however, found that nitrification was severely reduced or not detectible at a WFP of ~60%. The authors of this study attributed this contradictory finding to the high organic matter content of the soils studied. They concluded that, as mineral soils have a generally lower pore space and bulk density than soils containing high amounts of organic matter, these soils held a higher relative water content resulting in a restricted diffusion of O_2 into the areas of nitrifier activity.

Stark and Firestone (1995) reported observations concerning the mechanisms for soil moisture effects on nitrifiers. As soils lose water through evaporation or drainage, the soil solution becomes more concentrated with regard to solutes. In general, microorganisms in this environment must increase intracellular solutes to maintain cellular integrity. This osmotic potential maintenance is done at some cost in energy. As soil pores drain, water films on soil surfaces become thinner. This condition will increase the tortuosity of diffusion of a substrate. In the case of nitrifiers, rates of NO_3^- and NO_2^- production may be depressed by either increases in osmotic gradients or reduced substrate flux. Substrate that would otherwise be available may even become locked in smaller unconnected pores at lower matric potentials.

Stark and Firestone (1995) investigated the two effects of reduced water conent on nitrification rates (dehydration and substrate limitation) by comparing the results of two studies. In one study, soil slurries of different osmotic potentials were supplied with more than sufficient amount of substrate as $(NH_4)_2SO_4$ and rates at each osmotic potential recorded. The other study supplied a moist soil at various water potentials with increasing amounts of gaseous NH_3 to test the contribution of substrate diffusional limitations on nitrification rates. The conclusions drawn from these investigators were that dehydration effects account for almost all reduction in nitrification rates at lower water potentials (< -2.7 MPa), while substrate limitations due to diffusional path tortuosity became more important with increasing water potentials. At the higher water potentials (> -0.6 MPa), substrate limitation accounted for more than 50% of the decline in nitrification rates in the experimental soil. This helps explain the lower maximum rates (V_{max} values) in courser textured soils as they hold less water at the same potential as finer soils, and thus the diffusional limitations are more pronounced. The resultant effect is that, at the same substrate concentration, there is a significant reduction in real or relative availability to the nitrifying organism.

Sabey (1969) and Sabey and Johnson (1971) proposed a reduction term for modeling nitrifier response to changes in soil moisture content. The Moisture Rate Index term (R_M) was arrived at by placing a value of 1 at the experimentally observed soil water level corresponding to maximum activity. Soil water was expressed as soil moisture tension on a logarithmic scale and nitrifier activity was measured as cumulative nitrate nitrogen (NO_3-N) production. Nitrification rates at lower moisture tension values were assigned a fraction of the optimum index value. This approach yielded very good correlation between observed values and the equation used to fit them (Figure 1.7), but has not been further examined by subsequent

researchers. This review presents results of the application of Moisture Rate Index (R_M) to two other published data sets found in the literature in the interest of elucidating the relationship between nitrification rates and soil moisture tension in other soils. Garrido et al. (2000) reported an exponential increase in nitrification with increases in soil moisture (Figure 1.8). Miller and Johnson (1964) found that the maximum amount of NO_3-N accumulated in soils occurred at approximately the same moisture tensions as the maximum evolution of CO_2 (Figure 1.9). The results of this exercise demonstrate a strong relationship between nitrification rates and their response to changes in soil moisture across many different soil types incubated at temperatures ranging from 22 to 30°C. Correlation coefficients (r^2) for the equations used to fit the data ranged from 0.815 to 0.983. Slopes for the logarithmic equations ranged from -0.1221 to -0.2964 and compared favorably among all silt loams examined.

Factors: Temperature

The response of nitrifiers (as measured by either NH_4^+-N depletion or NO_3^--N accumulation) follows a bell shaped curve model. Optimum temperatures for the overall process of nitrification when all other conditions are favorable have been reported between 15°C and 40°C (Boon and Laudelout, 1962; Mahendrappa et al., 1966; Malhi and McGill, 1982; Breuer et al., 2002). The wide range of values for temperature optima can be attributed to climatic selection for nitrifier communities with the prevailing community temperature optimum at any soil's geographic location being closely related to the mean annual temperature for that location (Malhi and McGill, 1982; Breuer et al., 2002; Avrahami and Conrad, 2003). The same trend has been observed for temperature minimum and maximum values. Minimum values at which nitrification will occur have been reported at or below freezing for agricultural soils furthest from the equator to 10°C in a tropical rainforest (Malhi and McGill, 1982; Justice and Smith, 1962;

Breuer et al., 2002). Maximum values at which nitrification will occur have been reported to be between 30°C and 50°C (Malhi and McGill, 1982; Stark, 1996; Avrahami and Conrad, 2003, Avrahami and Bohannan, 2007).

Most laboratory studies on the effect of temperature on nitrification in soil have maintained constant temperature throughout. However, Frederick (1956) induced a controlled fluctuation in the temperature of incubated soils to study the effect on nitrification rates, observing that the rate of nitrification was influenced by both the maximum temperature and the amount of time the soil was held at that temperature. Temperatures below freezing for short periods had the effect of halting nitrification but did not impose any irreversible effects. Overall, at temperatures above 15°C the nitrification rate at a constant temperature was as high as or higher than the rate with fluctuating temperatures. Frederick concluded that fluctuations in temperature made the accurate prediction of nitrate production impossible in this study.

It is important to note that temperature has a significant effect on both gas and solute diffusion with respect to soil solution therefore exerting a differential effect on the response of nitrifiers to soil water content (Grundmann et al., 1995). These authors modeled the combination of water and temperature conditions that sustained favorable O_2 concentrations for nitrification using four temperature dependent parameters: (1) Maximum nitrification rate ($Nr_{max}(T)$), (2) Optimal relative water content ($\Theta_{opt}(T)$), (3) Minimum relative water content ($\Theta_{min}(T)$), and (4) Maximum relative water content ($\Theta_{max}(T)$). The equation for nitrification rate as a function of relative water content is as follows:

$$Nr(\Theta) = \frac{(\Theta - \Theta_{min})(Nr_{max})\exp^{[((\Theta_{max}-\Theta_{opt})(\Theta-\Theta_{opt}))/((\Theta_{opt}-\Theta_{min})(\Theta-\Theta_{max}))]}}{(\Theta_{opt} - \Theta_{min})} \qquad [10]$$

The Θ_{min} and Θ_{max} values were not included in the range of relative water contents within the study. However, using research by MacDuff and White (1985), the minimum was set at 0.22 or the permanent wilting point. Also the maximum value was set at 0.75 as this corresponds to denitrification conditions where nitrification is likely inhibited by insufficient aeration (Skopp et al., 1990).

A more recent study performed by Avrahami and Bohannan (2007) found an increase in temperature from 20° to 30°C resulted in an increase in nitrification potential as measured by nitrate plus nitrite accumulation, yet the higher temperature treatments exhibited a significantly lower cell density of nitrifiers as measured by DGGE and T-RFLP. The same effect was observed when temperature was correlated with soil %WFP. Numbers of nitrifying organisms were higher in the samples with factor combinations of 20°C x 60%WFP and 30°C x 30%WFP than at 30°C x 60%WFP. Nitrification potential was highest however, in the 30°C x 60%WFP treatment. This phenomenon may hold the key to understanding the differential effect of temperature and soil moisture content. The authors' conclusions concerning these observations included speculation that the diffusion of ammonia into the cells of AOB may be more rapid at higher temperatures. They further hypothesized that the uncoupling of the relationship between abundance and activity might provide a short term advantage through increased cell maintenance and survival under high temperatures or a decrease in the lag time prior to growth when conditions improve. Finally, they proposed that archaeal ammonia oxidizers may have contributed to some of the enhanced activity observed. This presence and contribution to nitrification in soils by archaeal bacteria has been investigated in some recent studies (Leininger et al., 2006; Tourna et al., 2008).

In general, the response of nitrification rates has been described as being linear over narrow temperature ranges (Breuer et al., 2002; Macduff and White, 1985) while the Van't Hoff function or the Arrhenius relationship has proven more applicable over slightly broader temperature ranges (Rodrigo et al., 1997; Stark, 1996; Addiscott, 1983; Russell et al., 2002). Still, these researchers have reported all of these approaches to be inadequate over the entire range of temperatures expected over the course of a year in most temperate climes. Stark (1996) found moderate success over a wide range of temperatures with a Poisson density function, though it may prove more appropriate to model the temperature response of nitrifiers using quadratic, exponential or multiple temperature functions when considering a temperature range of -5°C to 50°C. Q_{10} values for nitrification ({nitrification at t} {nitrification at t-10}$^{-1}$) have been reported to be from <1.6 to 3.6 (Macduff and White, 1985; Breuer et al., 2002).

Mahandrappa et al. (1966) and Russell et al. (2002) observed that nitrite accumulated in soils at some temperatures but not at others. This phenomenon was attributed in both cases to a differing sensitivity of NOB to temperature as compared to AOB. While there may exist differing temperature optima for NOB and AOB, the authors of both studies neglected to consider the effect of pH on nitrite accumulation (Morrill and Dawson, 1967). In both studies, the soil in question was characterized by a pH >7.5 and so should be expected to accumulate NO_2-N regardless of temperature. Two of the soils studied by Mahandrappa et al. (1966) did not accumulate nitrite at any temperature (Yolo, pH=6.8; Walla Walla, pH=6.1). More work should be done in this area before drawing conclusions concerning the differential response of AOB and NOB to temperature.

Finally, Stark (1996) concluded in his study that the difference in optimum temperatures for growth and nitrification might indicate that the energetic cost for the organism in these soils

of operating at or near the optimum nitrification temperature may exceed the benefit produced.

Other cellular processes are likely depressed as higher temperatures can denature enzymes and

lead to increased maintenance energy requirements. One reason suggested by the investigator

here for the difference is that the enzyme responsible for oxidation of ammonia may have

reached an evolutionary maximum that failed to change as the organism adapted to the

temperature range of this specific environment. It is worth considering that the often unique

properties of the AMO enzyme may result in similar disparities between optimum conditions for

nitrification and those required for cell growth and reproduction with respect to other factors

such as pH and/or substrate (NH_3) concentration.

Factors: Osmotic potential

It is a general characteristic of lipid membranes that they allow the diffusion of water

molecules (solvent) into or out of cells while preventing the transport of most solutes. Naturally,

the osmotic properties of bacterial cells arise from the semipermeable nature of these lipid cell

membranes. Water will diffuse across a membrane in response to an electrochemical gradient.

When the direction of this diffusion is into the cell, the resultant expansion of the cytoplasmic

volume is limited at some point by a rigid cell wall. Any further influx of water beyond this

point results in an increase in cytoplasmic, or turgor, pressure. Cytoplasmic shrinking, or

plasmolysis, represents the opposite condition wherein water exits the cell leaving the remaining

cellular fluid more concentrated with respect to solutes and smaller with respect to volume. At

some point in between, nitrifier cell turgor pressure can be said to be at an optimum level for the

oxidation of ammonia to nitrate. As osmotic potential trends away from this point in either

direction nitrification will decrease to a negligible rate or, in extreme cases, cell death may occur.

The response curve for nitrifier activity to changes in osmotic pressure will appear to rise steeply

toward a maximum on the side where potentials are nearest to zero and fall more gradually as solute concentrations increase. Osmotic stress in bacteria has also been reported to result in a depression of other normal cellular functions such as nutrient uptake and DNA replication (Csonka, 1989). Also, Jin et al. (2007) have demonstrated that NOB may be more sensitive to osmotic stress than AOB.

Darrah et al. (1985) predicted that no inhibition of nitrification will be observed at soil osmotic pressures of less than 1.9 atm, a critical value supported by the data published in that study. The osmotic pressure resulting from the sum of salts in soil solution can be estimated from the measurement of a soil's electrical conductivity (equation [11]) (McBride, 1994).

$$\text{Osmotic Pressure (atm)} = EC \text{ (mS/cm)} \times 0.36 \qquad [11]$$

A summary of the results of electrical conductivity measurements on some soils in Georgia as reported by the University of Georgia Soil Plant and Water Testing Laboratory (2400 College Station Road, Athens, Ga. 30602) between July 2007 and September 2008 reveals an average value of 0.09 mmhos/cm (unpublished data) (mmhos/cm = mS/cm). EC was measured at the on the filtrate of an equilibrated 1:2 ratio of soil to water (w/v). Given that normal soil water contents may be closer to 0.10 g H_2O g^{-1} soil, conversion of this value by equation [11] gives an average estimated osmotic pressure for these soils of 0.65 atm, well below the predicted value at which inhibition of nitrifier activity occurs. It is unlikely then that many soils in the Southeastern Coastal Plain will be inhibitory to nitrifiers with respect to osmotic pressure. An exception to this assumption must be made for the time period following fertilizer application. The osmotic pressure imposed by highly concentrated band or surface applications of fertilizers is temporary but potentially severe if fertilizer is banded.

Interpretation of the effects of osmotic pressure is complicated by the existence of specific ion effects. For example, the chloride anion (Cl⁻) has been credited with inhibition of nitrification at concentrations too low to cause osmotic inhibition (Darrah et al., 1985; Darrah et al., 1987; Rosenberg et al., 1986). Furthermore, osmotic potential has been reported to be lower for chloride salts than for sulfate salts (Rosenberg et al., 1986). Also, changes in the salt content of soil solutions will cause a shift in the equilibrium between adsorbed and solution ammonium, potentially decreasing available substrate (Low et al., 1997). The potassium cation (K⁺) has been observed to alleviate osmotic stress in nitrifiers and other bacteria (Csonka, 1989; Jin et al., 2007). The study performed by Jin et al. (2007) focused on nitrification in an airlift bioreactor. Though no comparable study was found relating this effect to soil nitrifiers, it seems reasonable to expect a similar result in soils as potassium ions are the most prevalent cations in bacterial cytoplasm and serve as one of the major intracellular osmolytes that maintain turgor (Csonka, 1989).

According to Low et al. (1997), when modeling nitrification based on Michaelis-Menten kinetics, the effect of osmotic potential on V_{max} can be described as follows;

$$K = a + be^{c\psi s} \qquad [12]$$

where K is the N transformation rate (mg N kg^{-1} d^{-1}). a, b, and c are empirically determined parameters and e is the base of the natural logarithm. Insertion of this modified term into the Michaelis-Menten equation gives the following;

$$N = [(a + be^{c\psi s}) \times S]/ (K_m + S) \qquad [13]$$

The authors of this study offered a K_m value of 39.7 μM NH$_4^+$ and concluded that the effect of osmotic stress on nitrifiers was insignificant when substrate was limiting, suggesting that substrate effects predominate over osmotic effects.

Factors: Soil Texture

Texture is a physical property of soils that refers to the particle size distribution of the sand, silt, and clay fractions. Generally, nitrification can be expected to proceed more vigorously in soils that contain higher amounts of clay particles (size fraction < 2 μm) and less rapidly in soils that contain high amounts of sand particles (size fraction > 50 μm) (Albrecht and McCalla, 1937; Smith, 1964; Rovita and Killorn, 2007). The mechanisms attributed to this observation include increased negatively charged surface area with increasing clay content for attachment by AOB (Underhill and Prosser, 1987), increased CEC resulting in more available substrate at soil particle surfaces (Albrecht and McCalla, 1937; Smith, 1964), better aggregation of soil particles (Hoffmann et al., 2007), and protection from predation by protozoa afforded by a greater number of micro-pore spaces (Rutherford and Juma, 1992).

Strong et al. (1999) reported a contradictory finding to the generalization that increased clay content is positively correlated with nitrification rates. The possibility of low pH was eliminated and the investigators attributed the effect to the tortuosity of NH_4^+ diffusive path length, stating that a colony may exhaust its local supply of substrate before fresh ammonium can diffuse to the vicinity. Another explanation is possible. The water potential here was reported to be 10 kPa (0.01 MPa). According to Stark and Firestone (1995), at such a water potential, differences in clay (matrix effect on soil moisture potential) may have a greater effect on substrate limitation than at lower potentials. One factor not considered by the author of this study is insufficient aeration. Conditions for saturation are nearly fulfilled at such a high potential (-0.01MPa) and oxygen is possibly limiting. Furthermore, as clay content increases, more water will be held as a percentage of total soil volume than at lower clay contents. Thompsen et al. (2003) noted a similar decrease in net nitrification with increasing clay content

at -0.1 MPa matric potential regardless of organic matter content. These authors concluded that the water content was well above optimum and that gaseous N losses were probably occurring. Furthermore, it was reported that the optimum matric potential decreased with increasing clay content.

Modeling Nitrification in Soils

Many approaches have been used to model nitrification rates. Zero order rate equations (independent of substrate concentration) are useful generally only when substrate or some other factor is serious limiting to nitrifier growth such as is the case at very low temperatures, low soil water content, or when substrate is slowly supplied solely through the mineralization of organic matter. First order rate equations which take into account the effect of dwindling substrate concentrations do not however consider the effect of population growth and decline with changes in substrate concentration. Neither of these are appropriate for approximating the sigmoidal response characteristic of NO_3 accumulation or NH_3 consumption in agricultural soils following the application of more than sufficient amounts of substrate to stimulate nitrifier population growth.

Many researchers have advocated the use of the nonlinear Michaelis-Menten equation (Kirk and Kronzucker, 2005; Kremen et al., 2005; Prosser, 1989; Malhi and McGill, 1985; Van Veen and Frissel, 1981: Boon and Laudelout, 1962) which accounts for changes in substrate concentration but not for population dynamics.

$$v = \frac{V_{max}[S]}{K_m + [S]}$$ [14]

The rate (mass time^{-1}) of ammonia degradation (-v) or nitrate accumulation (v) is a function of the maximum potential rate (V_{max}) when all conditions are set at optimum, the concentration of substrate [S] at any time, and the parameter K_m, an indicator of the affinity that the enzyme has

for the substrate that can be interpreted as the concentration of substrate at which the reaction occurs at half the maximum rate. Some important underlying assumptions to consider when employing the Michaelis-Menten equation are that the substrate and the enzyme are in solution. These assumptions must be challenged or ignored if credence is given to research indicating the membrane bound nature of the AMO enzyme, the periplasmic location of the HAO enzyme in AOB (Arp et al., 2007; Hooper, 1997; Frijlink, 1992), and the likewise intercellular location of the NXR enzyme in NOB (Starkenburg et al., 2006; Hooper, 1989). Furthermore, many researchers have indicated that adsorbed, rather than solution ammonium (NH_4^+) may be the preferred source of substrate ammonia (NH_3) taken up by AOB (Albrecht and McCalla, 1937; Lees and Quastel, 1946; Underhill and Prosser, 1987; De Boer et al., 1991; Batchelor et al., 1997). It is interesting to note that no model yet published has attempted to utilize the concentration of ammonia (NH_3) in solution as the direct source of substrate, nor has any model been based solely on adsorbed ammonium (NH_4^+) as the source of substrate despite the mounting evidence of AOB preference for surface adsorbed substrate and the non-polar compound affinity of the AMO enzyme.

Another approach to modeling nitrification uses the Monod and/or modified Monod equations which account for both substrate and population change and may therefore provide more accurate predictive power for modeling nitrification in agricultural soils (Alexander, 1994; Darrah et al., 1986a). The Monod equation has been used to relate the specific growth rate (μ) to a growth-limiting substrate [S] concentration in bulk solution,

$$\mu = \mu_{max} \frac{[S]}{K_s + [S]}$$

[15]

where μ_{max} is the maximum specific growth rate and K_s is the Monod constant. This equation looks almost exactly like the Michaelis-Menten equation relating substrate concentration to

population growth rather than substrate degradation rate. K_s could easily be interpreted then to be a growth rate equal to half the maximum rate, yet according to Liu et al. (2003), Monod intended no theoretical or mechanistic base for this equation. These authors proposed that the magnitude of K_s represents the equilibrium position of a microbial growth process, and μ_{max} represents the limit of microbial growth. Relating growth and substrate concentration to the rate of substrate decomposition or product accumulation is clearly not accomplished by the Monod equation as given by equation [15].

A modified version of the Monod equation can be used to predict substrate decomposition or product accumulation. However, nitrifier biomass density and biomass yield have yet to be measured satisfactorily (Avrahami and Bohannan, 2007; De Boer and Kowalchuk, 2001) and so their estimated values must be modeled when this approach is used (Simkins and Alexander, 1984). The equation for calculating the rate of substrate decomposition (-v) or product accumulation (v) as a function of time can be written as follows:

$$v = \mu_{max} \frac{[S]([S_0] + (B_0 / Y) - [S])}{K_s + [S]} \qquad [16]$$

μ_{max}, [S], and K_s remain the same as in equation [15]. $[S_0]$ is the initial concentration of substrate in solution. B_0 is the initial density of active microbial biomass and Y is the yield coefficient relating the amount of biomass produced per amount of substrate consumed. Simkins and Alexander (1984) demonstrated that when K_s is sufficiently larger than $[S_0]$, a logistic sigmoidal curve can be generated that closely fits the nitrate accumulation patterns observed during the course of nitrification in soil when the addition of substrate follows a period of starvation or growth-limiting substrate concentration. Any equation used to approximate the nitrate accumulation curve typically resulting from the process of nitrification can be modified to

reflect reductions in nitrifier activity resulting from a less than optimal level of any of the previously described factor, as described earlier.

Conclusion

Nitrifiers exist in an environment marked by a continual change in temperature, soil water potential, pH, salinity, substrate availability, and aeration. These organisms are highly sensitive to such changes in environmental conditions while at the same time being very slow to respond or adapt in order to maintain activity and/or growth. These qualities inherent in both AOB and NOB are exacerbated in the coarse, sandy soils of the Southeastern U.S. Coastal Plain region that provide little protection for these organisms from predation. Low surface area and a low volume of micro-pore space when compared to more clayey soils likewise do not support high densities of resting biomass in times of low substrate availability. Such coarse textured soils are often characterized by a low water holding capacity which results in rapid changes in soil water content and osmotic pressure that serve to depress nitrifier activity and if severe enough, may result in desiccation or lysis. The minimal cation exchange capacity of these soils may reduce the availability of adsorbed substrate sources while the marginal acid buffering capacity offers little protection against inhibition arising from the protons produced as a natural byproduct during the oxidation of ammonia.

This production of protons during the process of nitrification and its implications for the sustainability of agricultural production in the Southeastern U.S. is well known, but less understood in detail as it could be. The development of soil acidity in the subsoil horizons is difficult to remediate and has been documented to depress cotton yields in this region due to a reduction in root exploration of this portion of the soil volume. Important trends in subsoil acidity may be overlooked when samples for soil fertility testing are taken from the plow layer or

topsoil only. The ability of roots to proliferate in the subsoil greatly increases a crop's potential

to withstand water and nutrient stress, while the recovery of highly mobile nitrate (and possibly

ammonium) from the subsoil also serves to protect groundwater from contamination and reduce

'waste' from applied fertilizer by tightening the nitrogen cycle.

Areas of research identified as showing emerging promise or need in building a better

understanding of the process of nitrification in soils are numerous. One of the most important is

the development of better, more accurate methods for determining nitrifier cell densities in soil,

both at rest and during periods of positive and negative growth. Identification of the many

species and strains of nitrifiers that make up the population ecology within a given soil system is

advancing through recently developed molecular techniques such as PCR (polymerase chain

reaction), DGGE (denaturing gradient gel electrophoresis), and T-RFLP (terminal restriction

fragment length polymorphism); while the most probable number (MPN) approaches have been

shown to be largely inappropriate for identification and enumeration (Avrahami and Bohannan,

2007; De Boer and Kowalchuk, 2001). The mechanism(s) by which nitrification is stimulated

and/or inhibited by the presence of organic matter needs to be better illuminated as more interest

continues to be developed in the area of conservation tillage in agricultural production,

particularly in the Southeastern U.S. A systematic way of comparing the process of nitrification

across soil types is long overdue. It is suggested here that the landmark work of Sabey (1959)

and the more recent work of Laubscher et al. (1990) be used to correlate the effects of any

nitrification factor on each of the three phases of the sigmoidal nitrate accumulation curve. For

example, 'what are the effects of temperature on the lag phase?' or 'how does soil texture impact

the concentration of remaining substrate at which the terminal phase commences?' Finally,

process and organism based models must be improved to give better predictive accuracy in the

field based on real knowledge of nitrifier biology and enzymology and less on a 'black box'

approach in order to create an agricultural legacy of lower input and higher sustainability.

Table 1.5. N oxidation states.

Compound	N Oxidation State
NH_4^+	-3
NH_2	-1
N_2	0
N_2O	+1
NO	+2
NO_2^-	+3
NO_3^-	+5

Table 1.6. Some soil nitrifiers and their morphology.

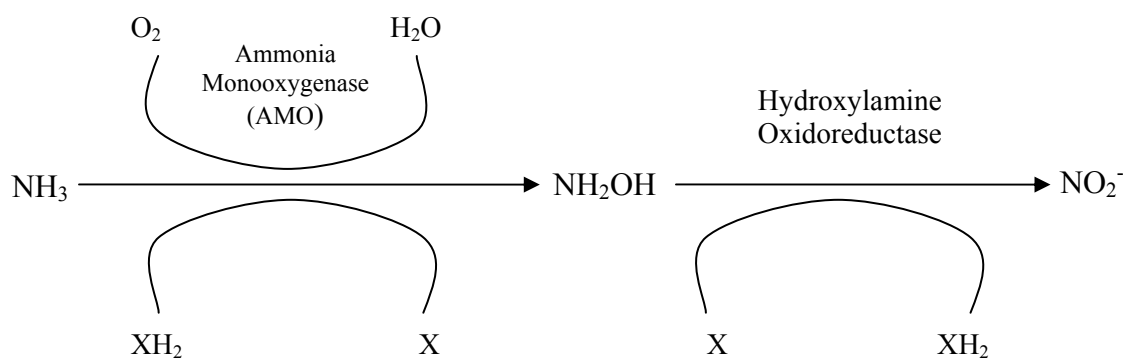| Bacterial Species	Morphology
AOB	
Nitrosomonas europea	Ellipsoidal, short rods
Nitrosospira briensis	Spiral
Nitrosolubus multiformis	Lobate, pieomorphic
Nitrosovibrio tenuis	Comma shaped
Nitrosococcus nitrosus	spherical
NOB	
Nitrobacter winogradski	Short rods
Nitrobacter agilis	Short rods

from Tate (2000).

Figure 1.2. Enzymatic pathway for Equation (2): Oxidation of ammonia to nitrite. X and XH_2 are the oxidized and reduced forms of the electron donor. NH_4^+ is oxidized for energy for the fixation of CO_2 via the Calvin cycle (Bedard and Knowles. 1989).

Cumulative Nitrate Production
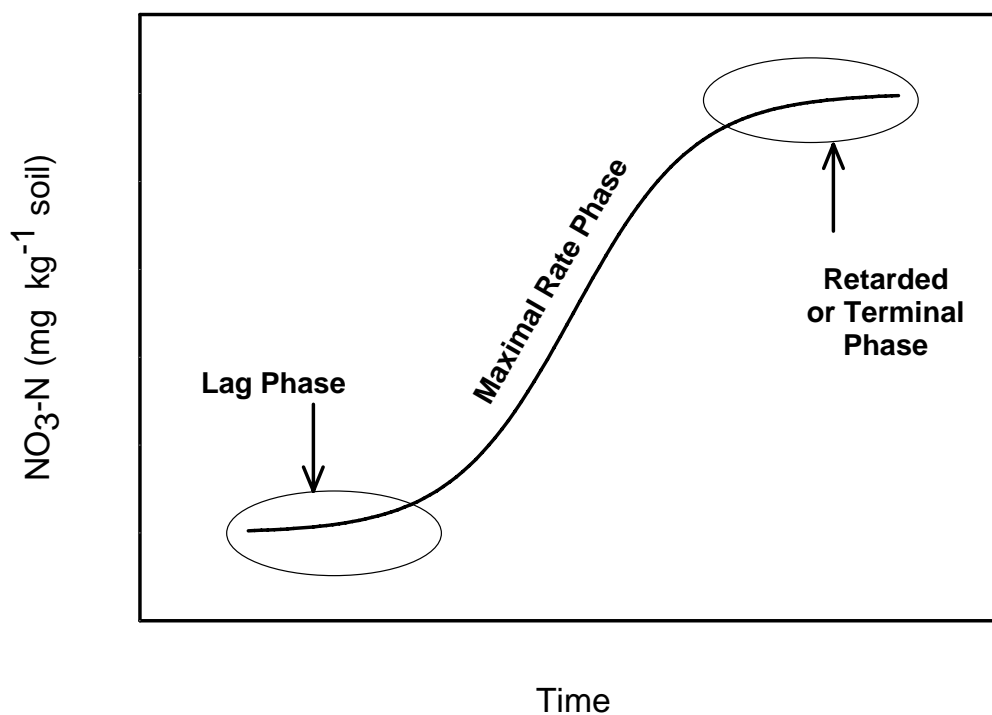
Figure 1.3. Three distinct phases of nitrification in soils following the addition of substrate (NH$_3$-N): The Lag Phase is characterized by slow growth and a gradual increase in the rate of product (NO$_3^-$-N) accumulation. The Maximal Rate Phase follows as numbers and/or activities of soil nitrifiers have reached the maximum supported by the set of environmental or experimental conditions. The final phase represents the near depletion of substrate and the subsequent rapid drop in activities and/or numbers of nitrifiers approaches the minimum supported by the set of conditions. This final phase is the Retarded or Reduced Rate Phase.

Figure 1.4. Nitrification in Type I Pattern Soils.
(Data from Morrill and Dawson, 1967)
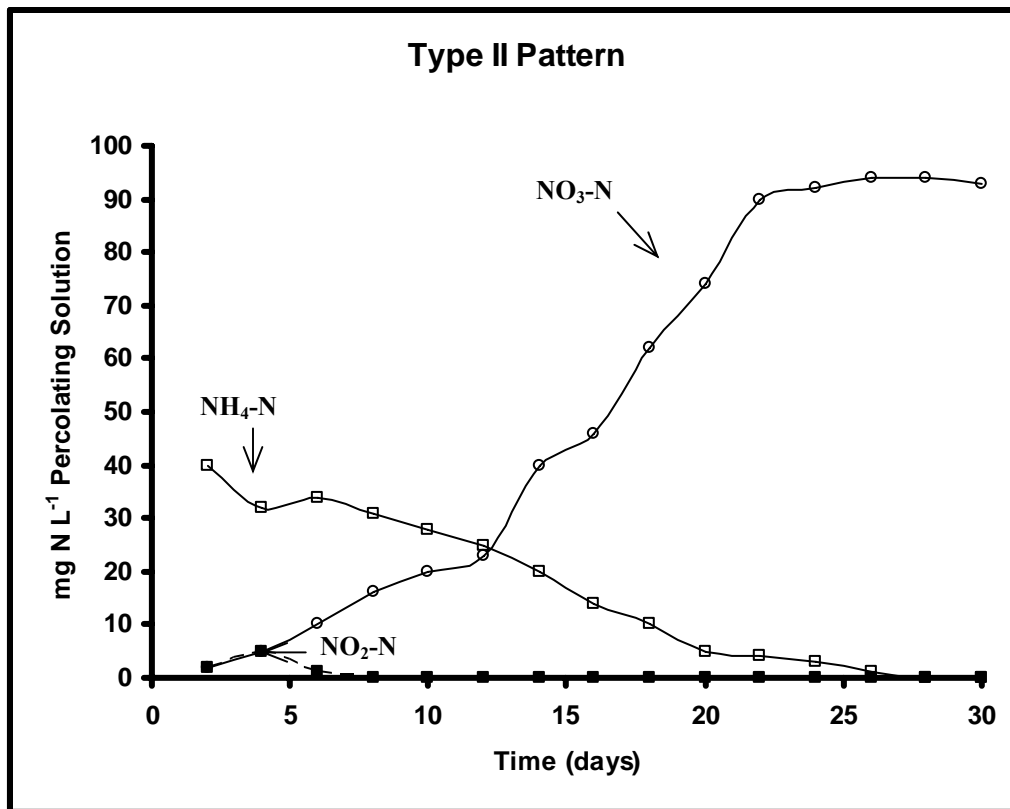
Figure 1.5: Nitrification in Type II Pattern Soils.
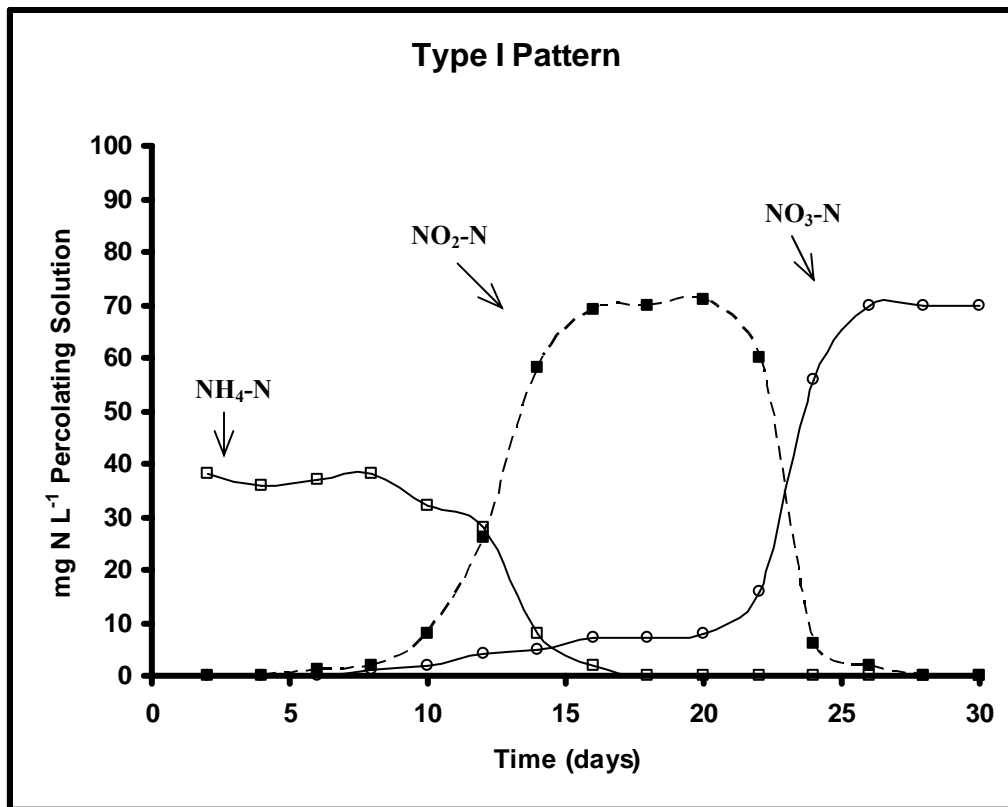(Data from Morrill and Dawson, 1967)
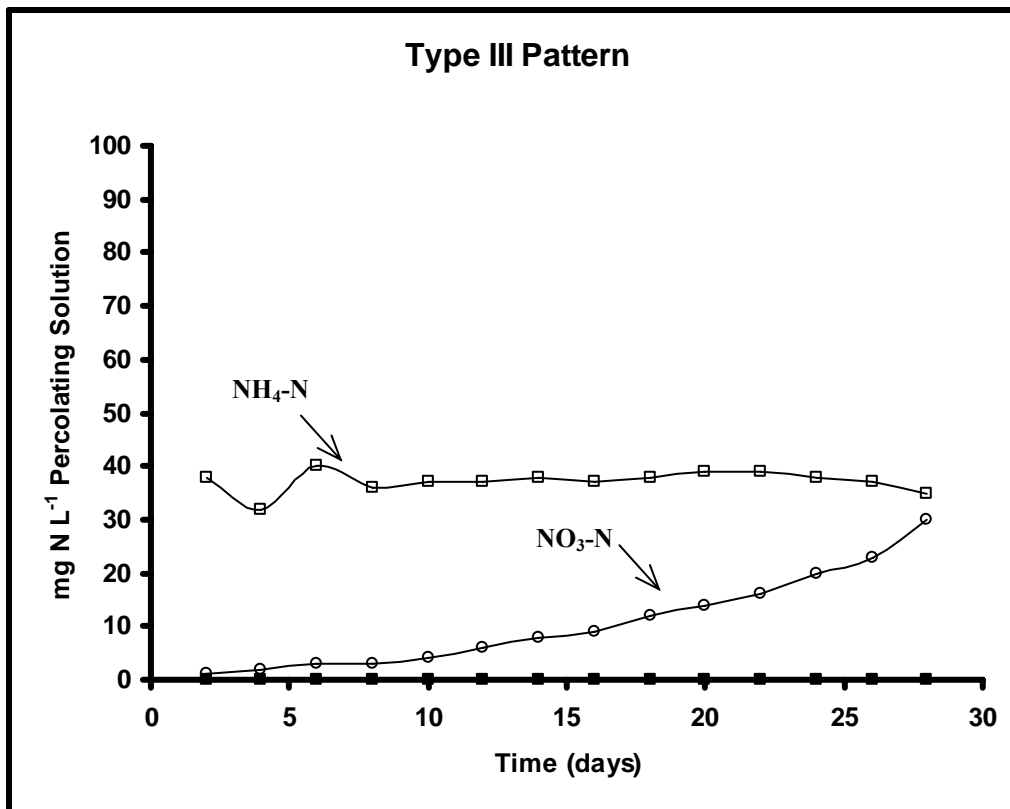
Figure 1.6: Nitrification in Type III Pattern Soils.
(Data from Morrill and Dawson, 1967)
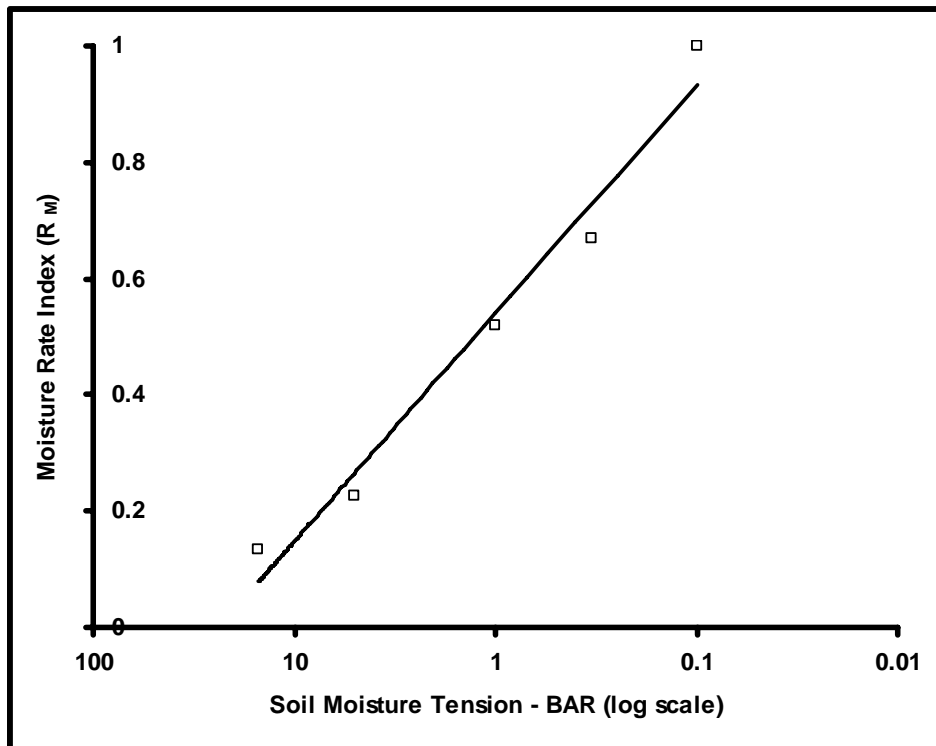
Figure 1.7. Influence of soil moisture tension on the moisture rate index (R_M) in Midwestern silty clay loam and silty loam soils @ 25°C.
$y = -0.1706Ln(x) + 0.5409$ ($r^2 = 0.9727$)
Combined data from Sabey and Johnson (1971) and Sabey (1969).
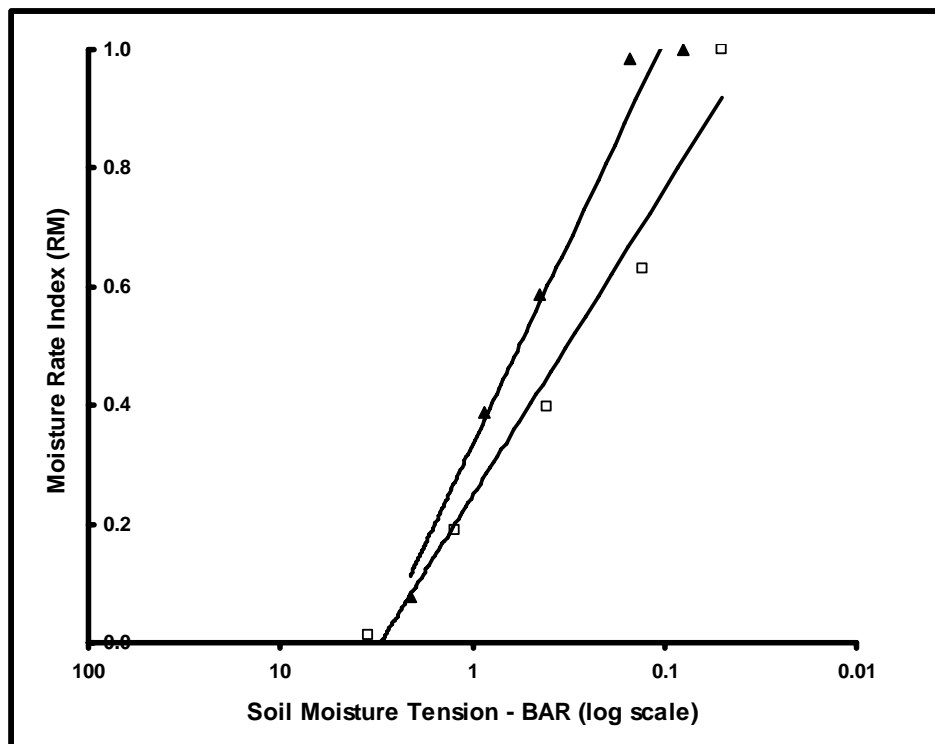
Figure 1.8. Influence of soil moisture tension on the moisture rate index (R_M) in two soils
incubated @ 20°C.
Rendosol[□] - hypercalcareous w/ 32.4% clay, 2.03% organic C, pH 8.1;
$y = -0.2161Ln(x) + 0.262$ ($r^2 = 0.9746$).
Luvisol[▲] - 20% clay, 1.08% organic C, pH 6.0;
$y = -0.2964Ln(x) + 0.3339$ ($r^2 = 0.9826$).
Data adapted from Garrido et al. (2000).
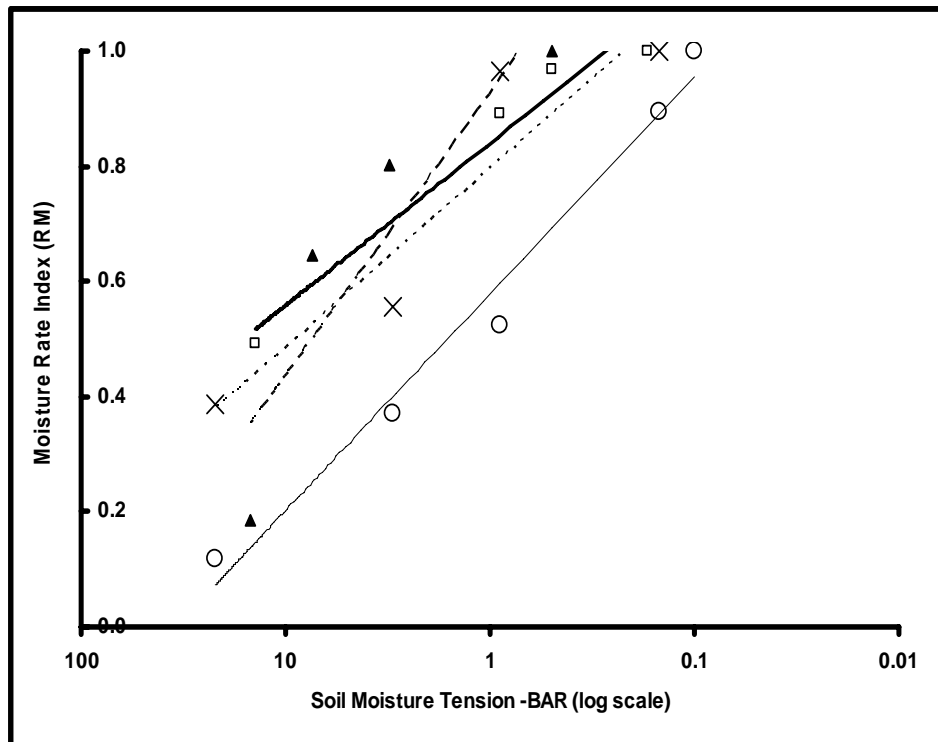
Figure 1.8. Influence of soil moisture tension on the moisture rate index (R_M) in four soils incubated @ 30°C.

Blackstock silt loam$^{▲——}$ - y = -0.2132Ln(x) + 0.9325 (r^2 = 0.8153);

Dunlop silt loam$^{□—}$ - y = -0.1221Ln(x) + 0.8399 (r^2 = 0.9568);

Fort Collins clay loam (calcareous)$^{X---}$ - y = -0.1351Ln(x) + 0.8003 (r^2 = 0.8737);

Fort Collins clay loam (non-calcareous)$^{○—}$ - y = -0.1634Ln(x) + 0.5784 (r^2 = 0.9812);

Data adapted from Miller and Johnson (1964).

References

Addiscott, T.M. 1983. Kinetics and temperature relationships of mineralization and nitrification in Rothamsted soils with differing histories. European Journal of Soil Science 2:343-353.

Albrecht, W.A., and T.M. McCalla. 1937. Nitrification of ammonia adsorbed on colloidal clay. Soil Sci. Soc. Am. Proc.

Alexander, M. 1994. Biodegradation and bioremediation, p. 71-98. Academic Press, A division of Harcourt Brace & Company, New York.

Allison, S.M., and J.I. Prosser. 1993. Ammonia oxidation at low pH by attached populations of nitrifying bacteria. Soil Biol. Biochem. 25:935-941.

Anthonisen, A.C., R.C. Loehr, T.B.S. Prakasam, and E.G. Srinath. 1976. Inhibition of nitrification by ammonia and nitrous acid. Journal Water Pollution Control Federation 48:835-852.

Ardakani, M.S., J.R. Rehbock, and A.D. McLaren. 1973. Oxidation of nitrite to nitrate in a soil column. Soil Sci. Soc. Amer. Proc. 37:53-56.

Ardakani, M.S., R.K. Schulz, and Mclaren. 1974. A kinetic study of ammonium and nitrite oxidation in a soil field plot. Soil Sci. Soc. Amer. Proc. 38:273-277.

Arp, D.J., P.S.G. Chain, and M.G. Klotz. 2007. The impact of genome analyses on our understanding of ammonia-oxidizing bacteria. Ann. Rev. Micro. 61:503-528.

Avrahami, S., and B.J.M. Bohannan. 2007. Response of *Nitrosospira sp.* strain AF-like ammonia oxidizers to changes in temperature, soil moisture content, and fertilizer concentration. App. Environ. Microbiol. 73:1166-1173.

Avrahami, S., and R. Conrad. 2003. Patterns of community change among ammonia oxidizers in meadow soils upon long-term incubation at different temperatures. App. Environ. Microbiol. 69:6152-6164.

Badía, D. 2000. Potential nitrification rates of semiarid cropland soils from the Central Ebro Valley, Spain. Arid Soil Research and Rehabilitation 14:281-292.

Bartlett, R.J. 1981. Nonmicrobial nitrite-to-nitrate transformations in soils. Soil Sci Soc. Am. J. 45:1054-1058.

Batchelor, S.E., M. Cooper, S.R. Chhabra, L.A. Glover, G.S.A.B. Stewart, P. Williams, and J.I. Prosser. 1997. Celldensity-regulated recovery of starved biofilm populations of ammonia-oxidizing bacteria. App. Environ. Microbioil. 63:2281-2286.

Bedard, C., and R. Knowles. 1989. Physiology, biochemistry, and specific inhibitors of CH4, NH4+, and CO oxidation by methanotrophs and nitrifiers. Microbiological Reviews. 53:68-84.

Boon, B., and H. Laudelout. 1962. Kinetics of nitrite oxidation by *nitrobacter winogradskyi*. Biochem. J. 85:440-447

Bouman, O.T., D. Curtin, C.A. Campbell, V.O. Biederbeck, and H. Ukrainetz. 1995. Soil acidification from long-term use of anhydrous ammonia and urea. Soil Sci. Soc. Am. J. 59:1488-1494.

Breuer, L., R. Kiese, and K. Butterbach-Bahl. 2002. Temperature and moisture effects on nitrification rates in tropical rain-forest soils. Soil Sci. Soc. Am. J. 66:834-844.

Broadbent, F.E., K.B. Tyler, and G.N. Hill. 1957. Nitrification of ammoniacal fertilizers in some California soils. Hilgardia 27:247-267.

Burton, S.A.Q., and J.I. Prosser. 2001. Autotrophic ammonia oxidation at low pH through urea hydrolysis. App. Environ. Microbiol. 67:2952-2957.

Carpenter-Boggs, L., P.D. Stahl, M.J. Lindstrom, and T.E. Schumacher. 2003. Soil microbial properties under permanent grass, conventional tillage, and no-till management in South Dakota. Soil and Tillage Research 71P:15-23.

Chu, H., T. Fujii, S. Morimoto, X. Lin, and K. Yagi. 2008. Population size and specific nitrification potential of soil ammonia-oxidizing bacteria under long-term fertilizer management. Soil Biol. Biochem. 40:1960-1963.

Csonka, L.N. 1989. Physiological and genetic responses of bacteria to osmotic stress. Microbiological Reviews 53:121-147.

Dancer, W.S., L.A. Peterson, and G. Chesters. 1973. Ammonification and nitrification of N as influenced by soil pH and previous N treatments. Soil Sci. Soc. Amer. Proc. 37:67-69.

Darrah, P.R., P.H. Nye, and R.E. White. 1985. Modelling growth responses of soil nitrifiers to additions of ammonium sulphate and ammonium chloride. Plant and Soil 86:425-439.

Darrah, P.R., R.E. White, and P.H. Nye. 1986a. Simultaneous nitrification and diffusion in soil. II. The effects at levels of ammonium chloride which inhibit nitrification.

Darrah, P.R., R.E. White, and P.H. Nye. 1986b. Simultaneous nitrification and diffusion in soil V. The effects of pH change, following the addition of ammonium sulphate, on the activity of nitrifiers. Journal of Soil Science, 37:479-484.

Darrah, P.R., R.E. White, and P.H. Nye. 1987. The effect of high solute concentrations on nitrification rates in soil. Plant Soil 97:37-45.

De Boer, W., P.J.A. Klein Gunnewiek, M. Veenhuis, E. Bock, and H.J. Laanbroek. 1991. Nitrification at low pH by aggregated chemolithotrophic bacteria. App. Environ. Microbiol. 57:3600-3604.

De Boer, W., P.J.A. Klein Gunnewiek, S.T. Troelstra, and H.J. Laanbroek. 1989. Two types of chemolithotrophic nitrification in acid heathland humus. Plant and Soil 119:229-235.

De Boer, W., and G.A. Kowalchuk. 2001. Nitrification in acid soils: micro-organisms and mechanisms. Soil biology and Biochemistry 33:853-866.

Eno, C.F. 1960. Nitrate production in the field by incubating the soil in polyethylene bags. Soil Sci. Soc. Am. Proc. 24:277-279.

Eno, C.F., and W.G. Blue. 1954. The effect of anhudrous ammonia on nitrification and the microbiological population in sandy soils. Soil Sci. Soc. Am. Proc. 18:178-181.

Foster, R.C. 1988. Microenvironments of soil microorganisms. Biol. Fertil. Soils 6:189-203.

Frederick, L. and F.B. Broadbent. 1965. Biological Interactions. *In* Agricultural Anhydrous Ammonia Technology and Use. (McVickar, M.H., ed.) Ammonia Institute, Memphis, TN, pp. 198–212.

Frederick, L.R. 1956. The formation of nitrate from ammonium nitrogen in soils. I. Effect of temperature. Soil Sci. Soc. Amer. Proc. 20:496-500.

Freitag, A., and E. Bock. 1987. Growth of *Nitrobacter* by dissimilatoric nitrate reduction. FEMS Microbiol. Lett. 48:105-109.

Frijlink, M.J., T. Abee, H.J. Laanbroek, W. de Boer, amd W.N. Konings. 1992. The bioenenrgetics of ammonia and hydroxylamine oxidation in *Nitrosomonas europaea* at acid and alkaline pH. Arch. Microbiol. 157:194-199.

Fuentes, J.P., D.F. Bezdicek, M. Flury, S. Albrecht, and J.L. Smith. 2006. Microbial activity affected by lime in a long-term no-till soil. Soil and Tillage Research 88:123-131.

Garrido, F., C. Hénault, H. Gaillard. and J.C. Germon. 2000. Inhibitory capacities of acetylene on nitrification in two agricultural soils. Soil Biol. Biochem. 32:1799-1802.

Glass, A.D. 2003. Nitrogen use efficiency of crop plants: Physiological constraints upon nitrogen absorption. Crit. Rev. Plant. Sci. 22:453-470.

Groffman, P.M. 1985. Nitrification and denitrification in conventional and no-tillage soils. Soil Sci Soc. Am. J. 49:329-334.

Grundmann, G.L., and D.E. Rolston. 1987. Water function approximation to degree of anaerobiosis associated with denitrification. Soil Sci. 144:437-441.

Grundmann, G.L., P. Renault, L. Rosso. and R. Bardin. 1995. Differential effects of soil water content and temperature on nitrification and aeration. Soil Sci. Soc. Am. J., 59:1342-1349.

Hadas, A., S. Feigenbaum, A. Feigin, and R. Portnoy. 1986. Nitrification rates in profiles of differently managed soil types. Soil Sci. Soc. Amer. J. 50:633-639.

Hall, A.D., N.H.J. Miller, and C.T. Gimingham. 1908. Nitrification in acid soils Proc. Roy. Soc. (London), Ser. B., 80:196-212.

Hankinson, T.R., and E.L. Schmidt. 1989. An acidophilic and a neutrophilic Nitrobacter strain isolated from the numerically predominant nitrite-oxidizing population of an acid forest soil. App. Environ. Microbiol. 54:1536-1540.

Hart, S.C., J.M. Stark, E.A. Davidson, and M.K. Firestone. 1994. Nitrogen mineralization, immobilization, and nitrification. *In* Methods of Soil Analysis: part 2 Microbiological and biochemical properties (managing ed. Mickelson, S.H.) Soil Sci. Soc. Am., Inc. Madison, WI.

Hattori, T., and T. Hattori. 1976. The physical environment in soil microbiology: An attempt to extend the principles of microbiology to soil microorganisms. Crit. Rev. Microbio. 4:423-461.

Hauck, R.D. Mode of action of nitrification inhibitors. ASA special publication No. 38. Amer. Soc. Agron. and Soil Sci. Soc. Amer, Madison, Wi. 1980.

Haynes, R.J. 1986. Nitrification. *In* Mineral Nitrogen in the Plant-Soil System (Kozlowski, T.T. ed.), pp. 127-157. Academic Press, Inc. Orlando.

Hissett, R., and T.R.G. Gray. 1976. Microsites and time changes in soil microbe ecology. *In* The role of terrestrial and aquatic organisms in decompostioin processes (Anderson, J.M., MacFadyen, A., eds.) Blackwell, Oxford, pp 23-39.

Hoffmann, H., M. Schloter, and B-M Wilke. 2007. Microscale-scale measurement of potential nitrification rates of soil aggregates. Biol Fertil. Soils 44:411-413.

Hooper, A.B. 1989. Biochemistry of the nitrifying lithoautotrophic bacteria. *In* Autotrophic Bacteria (Shlegel, H.G., Bowien, B., eds.) Science Tech, Madison, WI, pp.239-265.

Hooper, A.B. 1997. Enzymology of the oxidation of ammonia to nitrite by bacteria. Antonie van Leeuwenhoek 71:59-67.

Hyman, M.R., and P.M. Wood. 1985. Suicidal inactivation and labeling of ammonia mono-oxygenase by acetylene. Biochem. J. 227:719-725.

Islam, A., R.E. White, and D. Chen. 2006. Nitrification activity in acid soils of North-Eastern Victoria, Australia, as affected by liming and phosphorus fertilization. Australian Journal of Soil Research 44:739-744.

Jin, R.-C., P. Zheng, Q. Mahmood, B-L. Hu. 2007. Osmotic stress on nitrification in an airlift bioreactor. Journal of Hazardous Materials 146:148-154.

Justice, J.K., and R.L. Smith. 1962. Nitrification of ammonium sulfate in a calcareous soil as influenced by combinations of moisture, temperature and levels of added nitrogen. Soil Sci. Soc. Proc. 26:246-250.

Kandeler, E., and K.E. Böhm. 1996. Termporal dynamics of microbial biomass, zylanase activity, N-mineralisation and potential nitrification in different tillage systems. Applied Soil Ecology, 4:181-191.

Kirk, G.J.D., H.J. Kronzucker. 2005. The potential for nitrification and nitrate uptake in the rhizosphere of weland plants: A modeling study. Annals of Botany 96:639-646.

Kremen, A., J. Bear, U. Shavit, and A. Shaviv. 2005. Model demonstrating the potential for coupled nitrification denitrification in soil aggregates. Environ. Sci. Technol. 39:4180-4188.

Kyverga, P.M., A.M. Blackmer, J.W. Ellsworth, and R. Isla,. 2004. Soil pH effects on nitrification of fall-applied anhydrous ammonia. Soil Sci. Soc. M. J. 68:545-551.

Laubscher, K.J., J.M. Van Zyl, and C.C. du Preez. 1990. Equations to calculate the approximate duration and termination of the maximal rate phase of nitrification in soil. Commun. Soil Sci. Plant Anal., 21:611-621.

Laura, R.D. 1977. Salinity and nitrogen mineralization in soil. Soil Biol. Biochem. 9:333-336.

Lees, H., and J.H. Quastel. 1946. Biochemistry of nitrification in soil. II. The site of soil nitrification Biochem. J. 40:815-823.

Leininger, S., T. Urich, M. Schloter, L. Schwark, J. Qi, G.W. Nicol, J.I. Prosser, S.C. Schuster, and C. Schleper. 2006. Archaea predominate among ammonia-oxidizing prokaryotes in soils. Nature 442:806-809.

Linn, D.M. and J.W. Doran. 1984. Effect of water filled pore space on carbon dioxide and nitrous oxide production in tilled and nontilled soils. Soil Science Society of America Journal 48:1267–1272.

Liu, Y., Y-M. Lin, and S-F. Yang. 2003. A thermodynamic interpretation of the monod equation. Current Microbiology 46:233-234.

Low A.P., J.M. Stark, and L.M. Dudley. 1997. Effects of soil osmotic potential on nitrification, ammonification, N-assimilation and nitrous oxide production. Soil Science 162:16-27.

Macduff, J.H., and R.E. White. 1985. Net mineralization and nitrification rates in a clay soil measured and predicted in permanent grassland from soil temperature and moisture content. Plant and Soil 86:151-172.

Mahendrappa, M.K., R.L. Smith, and A.T. Christiansen. 1966. Nitrifying organisms affected by climatic region in Western United States. Soil Sci Soc. Amer. Proc., 30:60-62.

Malhi, S.S., R. Lemke, Z.H. Wang, and B.S. Chhabra. 2006. Tillage, nitrogen and crop residue effects on crop yield, nutrient uptake, soil quality, and greenhouse gas emissions. Soil and Tillage Research 90:171-183.

Malhi, S.S., and W.B. McGill. 1982. Nitrification in three Alberta soils: Effect of temperature, moisture and substrate concentration. Soil Biol. Biochem. 14:393-399.

McBride, M.B. 1994. Environmental chemistry of soils. Oxford University Press, N.Y.

McDowell, L.L., and G.E. Smith. 1958. The retention and reactions of anhydrous ammonia on different soil types. Soil Sci. Soc. Amer. Proc. 22:38-42.

McLaren, A.D. 1971. Kinetics of nitrification in soil: Growth of the nitrifiers. Soil Sci. Soc. Amer. Proc. 35:91-95.

Miller R.D., and D.D. Johnson. 1964. The effect of soil moisture tension on carbon dioxide evolution, nitrification, and nitrogen mineralization. Soil Sci. Soc. Am. J. 28:644-647

Morrill, L.G., and J.E. Dawson. 1961. Growth rates of nitrifying chemoautotrophs in soil. Bacteriol. 83:205-206.

Morrill, L.G., and J.E. Dawson. 1967. Patterns observed for the oxidation of ammonium to nitrate by soil organisms. Soil Sci. Soc. Amer. Proc. 31:757-760.

Page, K.L., R.C. Dalal, N.W. Menzies, and W.M. Strong. 2002. Nitrification in a vertisol subsoil and its relationship to the accumulation of ammonium-nitrogen at depth. Aust. J. Soil Res. 40:727-735.

Papendick, R I., G.S. Campbell. 1978. Theory and measurement of water potential. *In* Water potential relations in soil microbiology. (Kral, D. ed.) SSA special publication no. 9. Madison, Wis: Soil Science Society of America; 1978. pp. 1–21.

Phillips , C.J., D. Harris, S.L. Dollhopf, K.L. Gross, J.I. Prosser, and E.A. Paul. 2000. Effects of agronomic treatments on structure and function of ammonia-oxidizing communities. Appl. Environ. Microbiol. 66:5410-5418.

Prosser, J.I. 1989. Autotrophic nitrification in bacteria. Advances in Microbial Physiology 30:125-181.

Quastel, J.H., and P.G. Scholefield. 1951. Biochemistry of nitrification in soil. Bacteriol. Rev. 15:1-53.

Rice, C.W., and M.S. Smith. 1983. Nitrification of fertilizer and mineralized ammonium in no-till and plowed soil. Soil Sci. Soc. Am. J. 47:1125-1129.

Rice, E.L. 1984. Allelopathy. 2nd ed. Academic Press, London.

Robertson, G.P., and P.M. Vitousek. 1981. Nitrification potentials in primary and secondary succession. Ecology 62:376-386.

Rodrigo, A., S. Recous, C. Neel, and B. Mary. 1997. Modelling temperature and moisture effects on C-N trasformations in soils: comparison of nine models. Ecological Modelling 102:325-339.

Rosenberg, R.J., N.W. Christensen, and T.L. Jackson. 1986. Chloride, soil solution osmotic potential, and soil pH effects on nitrification. Soil Sci. Soc. Am. J. 50:941-945.

Rovita, D. and Killorn, R. 2007. Kinetics of Nitrification in Selected Iowa Soils Treated with Stay-N 2000. Commun. Soil Sci. Plant Anal. 38:13,1949-1963

Russell, C.A., I.R.P. Fillery, N. Bootsma, and K.J. McInnes. 2002. Effect of termperature and nitrogen source on nitrification in a sandy soil. Commun. Soil Sci. Plant Anal. 33:1975-1989.

Rutherford, P.M., and N.G. Juma. 1992. Influence of texture on habitable pore space and bacterial-protozoan populations in soil. Biol. Fertil. Soils 12:221-227.

Sabey, B.R., and L.R. Frederick, and W.V. Bartholomew. 1959. The formation of nitrate from ammonium nitrogen in soils: III Influence of temperature and initial population of nitrifying organisms on the maximum rate and delay period. Soil Sci. Soc. Amer. Proc.23:462-465

Sabey, B.R., L.R. Frederick, and W.V. Bartholomew. 1969. The formation of nitrate from ammonium nitrogen in soils: IV. Use of the delay and maximum rate phases for making quantitative predictions. Soil Sci. Soc. Amer. Proc. 33:276-278.

Sabey, B.R. 1969. Influence of soil moisture tension on nitrate accumulation in soils. Soil Sci. Soc. Amer. Proc. 33:263-266.

Sabey, B.R., and D.D. Johnson. 1971. Effect of soil moisture tension on nitrate accumulation in Drummer silty clay loam. Soil Sci. Soc. Am. J. 35:848-850.

Sahrawat, K.L. 1988. Use of 15N in nitrification inhibitor studies with special reference to indigenous materials. Jounal of Nuclear Agriculture and Bioilogy 17:238-242.

Shi, W., and J.M. Norton. 2000. Microbial control of nitrate concentrations in an agricultural soil treated with dairy waste compost or ammonium fertilizer. Soil Biology and Biochemistry 32:1453-1457.

Simkins, S., and M. Alexander. 1984. Models for mineralization kinetics with the variables of substrate concentration and population density. App. Env. Micro. 47:1299-1306.

Singh, S.S., and A.K. Kashyap. 2007. Contrasting pattern of nitrifying bacteria and nitrification in seasonally dry tropical forest soils. Current Science 92:1739-1744.

Skopp, J., M.D. Jawson. and J.W. Doran. 1990. Steady-state microbial activity as a function of soil water content. Soil Sci. Soc. Am. J. 54:1619-1625.

Smith, J.H. 1964. Relationships between soil cation-exchange capacity and the toxicity of ammonia to the nitrification process. Soil Sci. Soc. Am. Proc. 28:640-644.

Stark, J.M. 1996. Modeling the temperature response of nitrification. Biogeochemistry. 35:433-435.

Stark, J.M., M.K. Firestone. 1995. Mechanisms for soil moisture effects on activity of nitrifying bacteria. Applied and environmental microbiology. 61:218-221.

Starkenburg, S.R., P.S.G. Chain. L.A. Sayavedra-Soto. L. Hauser. M.L. Land, F.W. Larimer, S.A. Malfatti, M.G. Klotz, P.J. Bottomley, D.J. Arp, and W.J. Hickey, W.J., 2006. Genome sequence of the chemolithoautotrophic nitrite-oxidizing bacterium *Nitrobacter winogradskyi* Nb-255. Appl. Environ. Microbio. 72:2050-2063.

Stearman, G.K., J.E. Matocha. 1992. Soil microbial population and nitrogen-transforming bacteria from 11-year sorghum tillage plots. Soil and Tillage Research 22:299-310.

Stein, L.Y., and D.J. Arp. 1998. Loss of ammonia monooxygenase activity in *Nitrosomonas europaea* upon exposure to nitrite. App. Environ. Microbiol. 64:4098-4102.

Strong, D.T., P.W.G. Sale, and K.R. Helyar. 1999. The influence of the soil matrix on nitrogen mineralization and nitrification. IV. Texture. Aust. J. Soil Res., 37:329-344.

Subbarao, G.V., M. Rondon, O. Ito, T. Ishikawa, I.M. Rao, K. Nakahara, C. Lascano, and W.L. Berry. 2006. Biological nitrification inhibition (BNI) – is it a widespread phenomenon? Plant and Soil 294:5-18.

Suzuki, I., U. Dular, and S.C. Kwok. 1974. Ammonia or ammonium ion as substrate for oxidation by *Nitrosomonas europaea* cells and extracts. Journal of bacteriology 120:556-558.

Swensen, B., L.R. Bakken. 1997. Nitrification potential and urease activity in a mineral subsoil. Soil Biol. Biochem. 30:1333-1341.

Tate, R.L. III. 2000. Soil Microbiology, 2nd ed. John Wiley & Sons, Inc. New York.

Thamdrup, B., T. Dalsgaard, M.M. Jensen, O. Ulloa, L. Farías, and R. Escribano. 2006. Anaerobic ammonium oxidation in the oxygen-deficient waters off northern Chile. Limnol. Oceonagr. 51:2145-2156.

Thompson, S.P., H.W. Paerl, and M.C. Go. 1995. Seasonal patterns of nitrification and denitrification in a natural and a restored salt marsh. Estuaries 18:399–406.

Touchton, J.T., and F.C. Boswell. Performance of nitrification inhibitors in the Southeast. *In* Nitrification Inhibitors- Potentials and Limitations. ASA special publication No. 38. Amer. Soc. Agron. and Soil Sci. Soc. Amer, Madison, Wi. 1980.

Tourna, M., T.E. Freitag. G.W. Nicol, and J.I. Prosser. 2008. Growth, activity, and temperature response of ammonia oxidizing bacteria in soil microcosms. Environ. Microbiol. 10:1357-1364.

Underhill, S.E., and J.I. Prosser. 1987. Surface attachment of nitrifying bacteria and their inhibition by potassium ethyl xanthate. Microb. Ecol. 14:129-139.

Van Veen, J.A., and M.J. Frissel. 1981. Simulation model of the behavior of N in soils. *In* Simulation of nitrogen behaviour of soil-plant systems. (Frissel, M.J., and Vav Veen, J.A. eds.) Centre for Agricultural Publishing and Documentation, Wageningen, The Netherlands.

Verhagen, F.J.M., and H.J. Laanbroek. 1991. Competition for ammonium between nitrifying and heterotrophic bacteria in dual energy-limited chemostats. App. Environ. Microbiol. 57:3255-3263.

Walker, N. 1975. Nitrification and nitrifying bacteria. Ch. 7 *In* Soil Microbiology (Walker, N. ed.) John Wiley & Sons, N.Y.

Weier, K.L., and J.W. Gilliam. 1986. Effect of acidity on nitrogen mineralization and nitrification in Atlantic Coastal Plain soils. Soil Sci. Soc. Amer. J. 50:1210-1214.

Wheatley, R.E., K. Ritz, D. Crabb, and S. Caul. 2001. Temporal variations in potential nitrification dynamics in soil related to differences in rates and types of carbon and nitrogen inputs. Soil Biol. Biochem. 33:2135-2144.

White, C.S. 1994. Monoterpenes: their effects on ecosystem nutrient cycling. Journal of Chemical Ecology 20:1381-1406.

Whitehouse, M.J., and J.K. Leslie. 1973. Movement, pH effect and nitrification of band-applied anhydrous ammonia, urea and sulphate of ammonia in an alkaline black earth. Queensland journal of Agricultural and Animal Sciences 30:301-310.

CHAPTER 2

NITRIFICATION RATES IN SANDY SOILS OF THE SOUTHEASTERN COASTAL PLAIN AS AFFECTED BY SOIL TEXTURE, ORGANIC MATTER, AND pH

Abstract

Nitrification is known to proceed more slowly in sandy soils than in clayey soils. This fact holds implications for the fate of ammoniacal fertilizers applied to agricultural land. The fate of ammoniacal fertilizers is closely tied to the pattern of acid soil and subsoil development, a persistent problem with moderately weathered and coarse textured soils. Incubation experiments were conducted on four sandy soils typical of those under agricultural production in the Coastal Plain region of the Southeastern United States. The soils were chosen for study based on their differing properties with respect to soil texture and organic matter content. Each soil was additionally adjusted to three different target pH values (5.5, 6.0, and 6.5) to study the effect of initial soil pH on nitrification rates. The results of the study show that nitrification rates are strongly and positively related to soil cation exchange capacity (CEC) of these soils and that the lag phase (time from introduction of ammonia substrate to the onset of measureable nitrification) is negatively related to CEC. No significant relationship between nitrification rates and initial soil pH was found, though the multiple regression analysis model was strengthened when initial soil pH was included. Finally, it was shown that soil matric potential is a poor reference index for comparing soils with respect to nitrifier activity. Evidence is presented here to support the concept of using percent water-filled pore space (%WFP) as a measurement of soil water content to compare such activity.

Introduction

Nitrification is the process wherein ammonia (NH_3) is oxidized first to nitrite (NO_2^-) and ultimately to nitrate (NO_3^-). This process is of great interest in agricultural production as it has implications both environmental and economic. The end product of nitrification is nitrate, an anion easily leached through the root zone leading to losses of applied or native nitrogen, ecological imbalance, and/or human toxicities. Furthermore, protons (H^+) are a by-product of the oxidation of ammonia. One mole of H^+ ions is produced for every mole of ammonia oxidized to nitrate. When ammonia exists as the ionic form (NH_4^+), dissociation of one additional proton must occur prior to oxidation. This production of protons, coupled with the leaching of nitrates (accompanied by base cations) makes nitrification a highly significant contributor to the acidification of soils and subsoils (Helyar and Porter, 1989; Bouman et al., 1995). For these reasons, Sumner and Noble (2003) have asserted that ammoniacal fertilizers provide the major acidifying input to soils under intensive agricultural production.

Nitrification is performed in soils by a number of microorganisms including fungi, heterotrophic bacteria, and archaea (Hayatsu et al., 2008); but is dominated in normal (non-extreme) soil environments by chemolithoautotrophic bacteria. The two groups of nitrifying chemolithoautotrophs can be generalized as ammonia oxidizing bacteria (AOB) and nitrite oxidizing bacteria (NOB). Under soil conditions considered typical for agriculture in the Southeastern United States Coastal Plain (i.e. temperature 100° - 40°C, pH < 7.0), nitrite is consumed by NOB and converted to nitrate as quickly as it is produced (Morrill and Dawson, 1967). Therefore, net nitrification rates are frequently measured as nitrate accumulation or as ammonium consumption. The pattern of nitrate accumulation or ammonia consumption by soil nitrifiers under non-limiting conditions will appear as a sigmoidal curve when plotted against

time on the x-axis. Three distinct phases of the curve have been described as the lag phase, maximal rate phase, and the retarded (reduced rate) phase (Sabey et al. 1959; Hadas et al. 1986). Using either measurement approach, a sigmoidal curve can be fit to the observed data using a non-linear least squares regression of the Verhulst equation $\{dN/dt = kN(a-N)\}$ related to substrate or product rather than population. Parameters describing the phases of the sigmoidal curve can then be calculated using the method suggested by Sabey et al. (1969) and expanded on by Hadas et al. (1986) and Laubscher et al. (1990). Often there is good agreement between equation parameters when fitting to data for NO_3^- accumulation or NH_3^+ consumption (Hadas et al., 1986), though significant differences have been reported when other processes such as nitrogen mineralization, immobilization, and denitrification interfere with measurement of net rates (Weier and Gilliam, 1986; Justice and Smith, 1962). In these instances, net nitrification rates may fail to represent actual or gross nitrification rates leading to less accurate interpretations of experimental data.

Some soil properties known to affect nitrification rates (net and gross) include soil texture (Strong et al. 1999), pH (Islam et al., 2006; Page et al., 2002), organic matter content (Chu et al., 2008; Rice and Smith, 1983), temperature (Avrahami and Conrad, 2003; Russell et al., 2002), osmotic pressure (Low et al., 1997), substrate availability/concentration (Shi and Norton, 2000; Malhi and McGill, 1982; Anthonisen et al., 1976), aeration (Grundmann et al., 1995), and water content (Stark and Firestone, 1995; Doran et al., 1990). These soil properties have been well studied and are related to nitrification rates closely enough to be applied to the process as factors whose individual and interactive effects been estimated with some success (Grundmann et al. 1995, Darrah et al., 1989). Generally, nitrification rates increase with increasing soil clay content and decrease with increasing sand content. Nitrification rates are also positively related

to soil organic matter content. With respect to soil pH, ammonia oxidation is typically optimized at neutral to slightly alkaline pH with rates decreasing with increasing alkalinity or acidity. Similarly, there exists an optimum value for temperature, water content, substrate availability, osmotic pressure, and aeration. When nitrification rates are plotted on a y-axis against pH, temperature, water content, substrate availability, osmotic pressure and/or aeration, the result often appears as an imperfect or in some cases heavily skewed parabola. The optimum level of each factor may be considered to be the apex of the parabola. As this value increases or decreases along the x-axis, that factor value is less than optimal and nitrification rates will therefore decrease.

Soils under agricultural production in the Southeastern United States Coastal Plain contain mostly 1:1 clay, aluminum- and iron-oxide minerals, are well weathered, often coarse in texture (> 50% sand), and poorly buffered against changes in pH (Adams and Hathcock, 1984) . This combination of soil properties should be expected to result in lower potential nitrification rates than those observed in the more clayey soils of the Western, Midwestern, or Northeastern United States (Weier and Gilliam, 1986). Clay content is positively associated with soil surface area and cation exchange capacity. Soil AOB are reported to perform best when attached to positively charged surfaces and NOB perform best when attached to negatively charged surfaces (Allison and Prosser, 1993). Therefore, sandy soils will provide less habitat and support smaller populations of resting and active AOB (Rutherford and Juma, 1992; Underhill and Prosser, 1987). The poor pH buffering capacity of these soils can allow for large changes in pH resulting from the production of protons during the oxidation of ammonia. This in turn will potentially lead to acid inhibition of the entire nitrification process (Morrill and Dawson, 1967). The overall pattern of ammonia consumption and nitrate production, when considered along with downward

ion (solute) transport, plant root uptake, and proton production, will in large part determine the pattern of soil and subsoil acidity development (Helyar and Porter, 1989).

The development of soil and subsoil acidity has been observed to be more rapid than in soils found in other regions of the U.S., and therefore deserves attention (Weier and Gilliam, 1986; Eno and Blue, 1957). Towards this end, some coarsely textured soils typical of those under agricultural production in the Southeastern U.S. Coastal Plain were selected for study. Four representative soils were sampled from different locations in the Cabin Field (Crisp Co., Georgia) and incubated with added ammonium substrate source. These soils were selected for their differing properties with respect to clay and organic matter content to study the effect(s) these properties exert on the oxidation of ammonia. Additionally, each of the four soils were adjusted to three pH values (pH 5.5, 6.0, and 6.5) representing the range of soil pH values often found in the field at the time of nitrogen fertilizer application in this region. It was hypothesized that the data from this experiment could be used to generate values for the duration of lag phase, maximal rate, and duration of maximal rate phase that would differ significantly from each other based on the soil properties selected for and from those reported in other regions of the U.S. For instance, it was expected that lag times would be longer and maximal rates would be lower. These biotic properties related to nitrification in the highly-weathered, coarsely textured soils of the Southeastern Coastal Plain should be closely related to certain abiotic properties of soil, including texture, organic matter content, CEC, and initial incubation pH.

Materials and Methods

Soils and Experimental Treatments

All four soils were sampled from Northwest Crisp County, Georgia ($83^{o}56'20.510''$ to $83^{o}56'51.944''$ W; $32^{o}00'16.994''$ to $32^{o}01'24.675''$ N). Some selected properties of each soil are

presented in Table 2.1. The soils included a Bonifay soil from an upper slope or ridge area of the

field as well as a Bonifay soil from a depressional area of the field to contrast the effect of

organic matter on nitrification within the same soil series. The latter soil will be referred to as

Bonifay depressional while the former will simply be designated Bonifay. A Norfolk series soil

from a depressional area which contained the highest amount of organic matter and clay was also

chosen for the experiment along with an Orangeburg soil containing a low amount of organic

matter and intermediate clay content. Prior to the incubation experiment, each soil was adjusted

to three different target pH values using additions of $Ca(OH)_2$. The target pH values were pH

5.5, 6.0, and 6.5 (1:1 soil:0.01 M $CaCl_2$ *w/v*). Also, nitrate was leached from all soils prior to

incubation (to reduce measurement error) using at least three pore volumes of deionized water

through a Buchner funnel.

The actual initial pH values following addition of substrate $((NH_4)_2PO_4 + NH_4HPO_4)$

differed slightly from the intended target pH (Tables 2.2 – 2.5). The combination of mono-

ammonium- and di-ammonium-phosphate as sources of substrate for the experiment were chosen

for the minimization of this substrate-induced pH effect. Chloride (Cl-) was specifically avoided

for its inhibitory effects. All experimental treatments received the addition of 100 mg NH_3^+-N

kg^{-1} soil. Four replicates of each soil experimental unit received substrate (treatments) and four

replicates of each soil received no substrate (control). Soil moisture for all soils was adjusted to -

0.033 MPa (approximately field capacity) and monitored and maintained throughout the course

of the experiment.

Soil Incubation Conditions

50 grams (oven-dry weight) of air dried soil was placed into specimen cups with screw-

top lids. 0.95 cm holes were drilled into the screw caps using a paddle bit to allow diffusion of

oxygen into the incubation units. Cylindrical pieces of sponge 1.3 cm in diameter and 1.9 cm long were inserted into the holes and wet (not saturated) with deionized water to reduce moisture loss without preventing the diffusion of oxygen into the unit. Substrate was added to deionized water in amounts calculated to deliver 100 mg NH_3-N kg^{-1} soil when the appropriate volume of water was added to each soil after subtracting the air-dry soil water content. The soils were not packed but were gently tamped against the benchtop at this time and following each sampling period to reduce any irregularities in the soil surface. Gravimetric moisture values for air-dry soils and soils at -0.033 MPa are provided in Table 2.1. The soils were incubated at a temperature of 31°C for 20 days. This is a temperature reported to be at or very near to the optimum for a temperate area such as Southern Central Georgia (Malhi and McGill, 1982). Samples were taken for extraction at days 0, 5, 8, 11, 14, 17, and 20 except in the case of the Norfolk depressional soil where sampling times were similar at 0, 5, 7, 10, 13, 16, and 19 days.

Soil Moisture

Soil gravimetric moisture contents (θ_g) corresponding to soil water potentials of -0.1 and -0.033 MPa were determined by placing each soil on a pressure plate apparatus followed by oven drying (Equations [1,2]). Measurements were made in triplicate and replicate measurements generally agreed very well.

(mass of soil @ -0.033 MPa) – (mass of oven-dry soil) = (mass of soil water)   [1]

θ_g = (mass of soil water) / (mass of oven-dry soil)   [2]

A soil water potential of -0.033 MPa was maintained throughout the experiment by weighing at every sampling period and adding as necessary. Sponges were re-wet daily with deionized water to prevent their drying out.

Percent water filled pore space (%WFP) was determined on the experimental soils by

first placing 50 cm^3 of soil into a 100 mL class A graduated beaker. Deionized water was added

until the soil appeared to be just saturated. Prior to the final volume measurement the graduated

cylinders were then gently tamped to simulate the settling that occurred during incubation,

covered with parafilm to prevent evaporation, and allowed to equilibrate for 24 hours. It was

hoped that, by tamping the soils in this way, a bulk density matching that of the incubation soils

could be simulated. Following the equilibration period, more deionized water was added if the

sheen had disappeared to ensure displacement of all entrapped air. The equilibration step and

addition of water was repeated until the sheen remained on the soils for 24 hours and

displacement of all entrapped air was ensured. 100% water filled pore space was considered to

be satisfied at the point of soil water saturation. Water volume (cm^3) was considered to be

equivalent to water mass (g). Bulk densities for the Bonifay, Bonifay Depressional, Orangeburg

and Norfolk Depressional soils were 1.53, 1.45, 1.43, and 1.30 g cm^{-3} respectively.

$$\text{Soil Bulk Density (g cm}^{-3}) = \text{Soil Oven Dry Weight / Soil Volume} \qquad [3]$$

$$\text{\% Soil Pore Space = Saturated Soil Water Volume / Soil Volume} \qquad [4]$$

$$\text{Volumetric Water Content } (\theta_v) \text{ (-0.033 MPa)} = \theta_g \times \text{Soil Bulk Density} \qquad [5]$$

$$\text{\%WFP (-0.033 MPa)} = 100\% * \theta_v \text{ (-0.033 MPa) / \% Soil Pore Space} \qquad [6]$$

Sampling, pH Measurement, and Extraction of Soil Mineral Nitrogen

5 grams (oven-dry weight) of soil was taken from each incubation unit and placed into

50-mL centrifuge tubes with screw top caps. A solution of 0.01 M CaCl$_2$ was added to each tube

so that the volume of solution when added to the volume of soil water totaled 5 mL. The

centrifuge tubes containing the soil and salt solution were shaken (side to side) for 30 minutes

prior to pH measurement. Measurement of pH was performed using a semi-micro electrode in conjunction with a standard benchtop pH meter.

Following pH measurement, 20 mL 2 M KCl was added to the centrifuge tubes for extraction of NH_3 and NO_3^-. The extraction solution to soil ratio was 5:1 (v/w). All centrifuge tubes were then shaken (side to side) for 30 minutes followed by centrifugation 30 minutes. The supernatant was frozen ($< 0°C$) until analysis. Extracted NO_3^--N and NH_3-N were quantified by flow injection colorimetry and a dilution factor of 5x was applied. Nitrate and ammonia measured in the control samples (no added substrate) was subtracted from that of the treatments. Nitrite (NO_2-N) was not detected in any of the extracts.

Measurement of Some Selected Soil Properties

Soil organic matter and total nitrogen were measured by the combustion (Dumas) method and expressed as mg carbon kg^{-1} soil or mg nitrogen kg^{-1} soil (Nelson and Sommers, 1996; Bremner, 1996). Soil cation exchange capacity (CEC) was measured using the unbuffered method of Sumner and Miller (1996). The saturation solution from CEC determination process was collected and analyzed by ICP-OES for exchangeable cations. Soil texture was determined using the hydrometer method (Bouyoucos, 1962).

Non-linear Parameter Estimation

Verhulst equation parameters were estimated by non-linear least squares regression using SAS software (version 9.1, SAS Institute). The values for duration of lag phase (t'), maximal rate (K_{max}), and onset of retarded or reduced rate phase (t_s) were subsequently calculated by the method of Sabey et al. (1969) later expanded by Hadas et al. (1986), and Laubscher et al. (1990).

Results and Discussion

Net Nitrification

The results of measurements for soil nitrate nitrogen (NO_3^--N) and ammonium nitrogen (NH_3-N) at each sampling period are presented in Tables 2.2 – 2.5. All soils showed at least some evidence of nitrification as measured either by substrate (NH_3-N) consumption or product (NO_3^--N) accumulation. At the end of the 20 day incubation period, the Bonifay soil at the initial target pH 5.5 exhibited the lowest rate of nitrification (10.2 mg NO_3^--N kg^{-1} soil; -24.9 mg NH_3-N kg^{-1} soil). The Bonifay also soil exhibited the greatest effect of pH on nitrification rates. However, the same soil series at the same initial target pH from a nearby depressional area exhibited the highest amount of nitrate accumulation and ammonium consumption at the end of the 20 day incubation period (89.1 mg NO_3^--N kg^{-1} soil; -131.033 mg NH_3-N kg^{-1} soil). Soil pH had very little effect on rates in this soil. The clay and sand contents were very similar, though the soil organic matter contents were markedly different for the Bonifay and Bonifay depressional.

This greater amount of soil organic matter was in all probability the reason for the large difference in gravimetric moisture contents (θ_g) at -0.033 MPa. The soil organic matter also contributed an increase in CEC (> 3x) in the Bonifay depressional compared to the Bonifay. There is evidence that both percent water filled pore space (%WFP) (Doran et al., 1990) and cation exchange capacity are closely related to nitrification rates in agricultural soils supplied with non-limiting substrate (Underhill and Prosser, 1987). It is believed, then, that the increased organic matter in the Bonifay depressional soil is almost solely responsible for the dramatic difference in observed nitrification rates in two soil samples that differ very little otherwise.

Of the two other soils incubated during the experiment, the nitrification rate of the Orangeburg soil was very similar to the Bonifay depressional. Soil pH treatments had very little effect on net nitrification rates in the Orangeburg soil, which contained considerably more clay than the Bonifay soils. Nitrification rates are often positively related to clay content when aeration is not limiting (Smith, 1964) and negatively related when aeration becomes limiting (Strong et al., 1999). The Norfolk Depressional contained the largest percent clay (33.2%) as well as the highest concentration of organic matter (23,387 mg C kg^{-1} soil). However, the Norfolk depressional soil nitrified less than the Orangeburg soil.

The reduced nitrification rates in the Norfolk Depressional soil are believed to be related to insufficient aeration (excessive WFP). Although all soils were incubated at -0.033 MPa, the gravimetric moisture contents at this potential were very different (Table 2.1). This observation prompted a measurement of water filled pore space for each soil by the method described previously. Maximum respiration by aerobic bacteria in coarse textured soils was described by Doran et al. (1990) as occurring at 54.1 %WFP. A quadratic fit of the experimental data from Doran et al. study provided the following formula for relative aerobic activity as a function of %WFP.

$$\text{Relative Activity} = 5.44 \, (\text{WFP}) - 5.03 \, (\text{WFP})^2 - 0.492 \quad\quad\quad [7]$$

The %WFP for the Norfolk depressional soil was determined to be 88.07% by equations [2-6], a value well above the optimum of 54.1%. Application of equation [7] to this value indicated a 40% relative activity in the Norfolk depressional soil when incubated at -0.033 MPa as compared to 80% (Bonifay), 98% (Orangeburg), and 92% (Bonifay depressional) relative activity. It is interesting to note that the soils exhibiting the most rapid nitrification rates during

the experiment were, according to Doran et al. (1990), functioning at 90 to 100% relative activity with respect to %WFP (Table 2.10).

Calculation of Delay, Maximal Rate, and Terminal Phases

Integration of the Verhulst equation $\{dN/dt = kN(a-N)\}$ as outlined by Hadas et al. (1986) yields the following equation for expressing NO_3^--N accumulation with time (t) quantitatively,

$$NO_3^--N = a \ / \ \{1 + (a/[NO_3^--N]_0 - 1)\exp(-ak[t - t_0]\} \quad\quad [8]$$

where a and $[NO_3^--N]_0$ are the asymptotic and initial values of NO_3^--N, respectively (mg N kg^{-1} soil). The coefficient k is a constant, and t is time. In this experiment, t_o is set at zero days. The parameters a, k, and $[NO_3^--N]_o$ were calculated by the least-squares fit of equation [8] to the experimental data for nitrate accumulation. The equation parameter a depends on the amount of substrate added to the soil. However, the maximal rate (K_{max}) is expected to depend on soil properties as long as substrate is not limiting (Sabey et al., 1959). K_{max} was calculated as the maximal slope of equation [8] at the inflection point where NO_3^--N = a/2.

$$K_{max} = k \ x \ (a^2 \ / \ 4) \quad\quad [9]$$

The delay period (t'), or lag phase was calculated as the value of t when the maximal slope was extrapolated to $[NO_3^--N]_0$ (Sabey et al., 1959; Hadas et al., 1986).

$$t' = (1 \ / \ ak)\ln[a \ /[NO_3^--N]_0 - 1] + ([NO_3^--N]_0 - a/2) \ / \ K_{max} \quad\quad [10]$$

According to Laubscher et al. (1990), the time to the termination of the maximal rate phase (t_s) and the onset of the retarded phase, can be estimated by first calculating the time (t_{mx}) to the inflection point at NO_3^--N = a/2.

$$t_{mx} = (1/ak) \ \ln(a \ / \ [NO_3^--N]_0 - 1) \quad\quad [11]$$

$$t_s = t_{mx} + (a/2 - [NO_3^--N]_0)/K_{max} \quad\quad [12]$$

The duration of the maximal rate phase (Δt) is then calculated as the difference between the time to termination of the maximal rate phase and the time to the onset of same.

$$\Delta t = t_s - t' \qquad [13]$$

Termination of the maximal rate phase is initiated when substrate becomes too limiting to support respiration at the rate observed during the maximal rate phase. This limitation may arise from insufficient substrate for maximum respiration, which may be affected by the resupply of substrate to the organism. The mechanism of resupply may be ammonification (mineralization) of organic nitrogen when available and/or diffusion. During this phase nitrification does not altogether cease, but rather proceeds at a lower rate that can be described by a first order linear equation of the general form $y = ax + b$ (Sabey et al. 1969; Darrah et al. 1989). This slope of the equation fitting all observations at time $\geq t_s$ when such observations exist should give some indication of how ammonification and diffusional limitation combine to supply substrate to and subsequently support the AOB population following the onset of substrate limitation. As some soils have been reported to nitrify very nearly 100% of the added substrate prior to onset of the retarded phase while others have been observed to nitrify much less than this, it is believed that measurements beyond the onset of the reduced rate phase could prove useful in illuminating the rate and or mechanism of substrate resupply.

Hadas et al. (1986) suggested a method based on substrate decomposition/consumption for estimating the same parameters of $[NH_3\text{-}N_0]$, K_{max}, t', t_s, and Δt. The initial value of substrate $[NH_3\text{-}N_0]$ can be considered equivalent to parameter $a - [NO_3^-\text{-}N_0]$. The concentration of substrate as a function of time is obtained in the following manner.

$$NH_3\text{-}N = \frac{a \times [NH_3 - N_0] \times \exp(-akt)}{a - [NH_3 - N_0] + [NH_3 - N_0] \times \exp(-akt)} \qquad [14]$$

Again the parameters a, k, and [NH$_3$-N$_0$] were calculated from the experimental observations

using least squares regression independently of those derived from the NO$^-_3$-N data. The

maximal rate of substrate decomposition (K$_{max}$) is the slope at [NH$_3$-N$_0$] = a/2.

$$K_{max} = -ka^2/4 \hspace{4cm} [15]$$

The delay period (t') at the time when the maximal slope was extrapolated to the initial value of

[NH$_3$-N$_0$] is derived with the following equation.

$$t' = \frac{1}{ak} \ln \frac{[NH_3 - N_0]}{a - [NH_3 - N_0]} + \frac{[NH_3 - N_0] - a/2}{K_{max}} \hspace{2cm} [16]$$

Calculation of the remaining rate parameters is as above.

All Verhulst equation parameters for the NO$^-_3$-N and NH$_3$-N data are presented in Tables

2.6 and 2.7 with approximate standard errors for each parameter. Estimations of goodness of fit

for equations to data are often supported by r^2 values which require an intercept term in the

equation. Non-linear equations such as the Verhulst do not intercept the x axis and so the

goodness of fit for this procedure with each data set was estimated through the use of a pseudo r^2

term (pseudo r^2 = [1 - SS$_{residual}$] / SS$_{total\ corrected}$) (Introduction to SAS, 2009). Net nitrification rate

parameters calculated using equations [9-13, 15, 16] for both substrate consumption and product

accumulation are presented in Tables 2.8 and 2.9.

The pseudo r^2 values for all least-squares regression fits of the Verhulst equation to the

observations were very high (0.9275 – 0.9993) indicating very good fits for all data sets and

standard errors for each parameter are relatively low. This was true regardless of the application

to nitrate or ammonia data. Figures 2.1 – 2.8 show the relationship between the observations and

the regression curves. For those soils where nitrification reached the reduced rate phase by the

end of the incubation time period (Bonifay, Orangeburg and Bonifay depressional soils), all three phases are clearly evidenced.

Comparison of maximal rates and the duration of phases as estimated by either NO^-_3-N or NH_3-N data is presented in Tables 2.8 and 2.9. The highest calculated rates were those belonging to the Bonifay depressional followed closely by the Orangeburg soil. The lowest rates belonged to the Bonifay soil from the upslope/ridge area, which contained the most sand, the least organic matter and the lowest CEC of all soils incubated. The delay or lag phase (t') was most brief in the Bonifay depressional and Orangeburg soils and longest in the Norfolk depressional soil. Durations of the maximal rate phase (Δt) when estimated using nitrate measurements were closely grouped between 7 to 11 days with only two values outside this range. Δt estimated from the ammonia measurements varied much more widely (~7 to 37 days). Maximal rates (K_{max}) for the Bonifay soil compared fairly well when estimated using either substrate or product data. The relationship between K_{maxNO3} and K_{maxNH3} for the other three soils was not very close, indicating interference in measurement of net rates from some other process(es). Because the maximal rates calculated from the NH_3-N depletion data are greater than those calculated from the NO_3^--N accumulation data, it is likely that ammonia and/or ammonium nitrogen was being consumed by these other processes. As these coarsely textured soils contain very little 2:1 clays, interlayer fixation can be excluded as a possibility. The most likely source of interference in all four soils is immobilization by competing soil organisms, though it is also likely that some nitrate was lost to denitrification in the Norfolk depressional soil (> 62% WFP) (Linn and Doran, 1984). The effect of this competition for nitrogen should be more pronounced in ammonia measurements as heterotrophs are reported to preferentially immobilize ammonia/ammonium over nitrate when both are available (Puri and Ashman, 1999).

This scenario is supported by the discrepancy between initial and final mineral nitrogen measurements observed in all treatments for all soils (Tables 2.2 – 2.5). The difference between total initial and final mineral nitrogen was greatest in those soils with the highest concentrations of organic matter and the widest C:N ratios (Norfolk depressional and Bonifay depressional) and lowest in the remaining two soils containing low amounts of organic matter and C:N ratios less than 13:1. For this reason it is recommended that NO_3^--N accumulation measurements be used when estimating net nitrification rates in sandy soils where aeration is not a limiting factor.

The values for K_{max} at -0.033 MPa and those adjusted for the effects of water-filled pore space are in the same range (~2 to ~15 mg NO_3^--N kg^{-1} soil day^{-1}) as those observed by Laubscher et al. (1990) in a study that included soils similarly low in clay content and organic C, though the substrate was supplied at a rate of 50 mg N kg^{-1} soil and the soils were wet to "approximately field capacity" and incubated at 31°C. Lag times (t') were similar as well and ranged from 1.1 to 7.3 days. Rovita and Killorn (2007) observed maximal rates of 13 and 26 mg kg^{-1} day^{-1} and t' values of less than 3 - 5 days. Experimental conditions in this study included incubation at 20°C, 60% water-holding capacity (method of determination not specified), and 200 mg N kg^{-1} soil. Both Sabey et al. (1959) and Hadas et al. (1986) observed much higher maximal rates and shorter lag phases than the values of K_{max} and t' reported in the current study (Tables 2.8 and 2.9). The values reported here appear to fall near the low and high end of the scale respectively when compared to those found in the literature, even when incubated under what are presumed to be optimum temperature and water content, indicating that these soils have relatively slow nitrification rates when compared to their counterparts under agricultural production around the world.

Regression analysis using the SAS multiple regression procedure (SAS Institute, version 9.1) revealed a strong negative relationship (p < 0.0005) between the lag phase (t') and soil CEC (Table 2.13).   There was also a positive interactive effect on the lag phase when the co-factors organic matter and CEC were entered into the model ($R^2 = 0.8450$). This relationship between CEC and the lag phase provides more evidence to support previous assertions placing the location of AOB activity and substrate uptake at soil adsorption sites (Albrecht and McCalla, 1937; Lees and Quastel, 1946; Allison and Prosser, 1993). Multiple regression analysis of the relationship between the maximal rate (K_{max}) and soil properties was performed on the values calculated at -0.033 MPa as well as the maximal rate values adjusted to 100% relative activity by the method of Doran et al. (1990) using equation [7].   Results of the regression procedure revealed a strong positive relationship (p < 0.05) between the maximal rate at -0.033 MPa ($K_{max-0.033}$) and CEC and initial pH ($R_2 = 0.7638$). When the same model was regressed on the adjusted rates ($K_{max\ adjusted}$) the relationship with CEC (p < 0.001) and initial pH were strengthened ($R^2 = 0.8084$) indicating that effects often attributed to texture and organic matter are mechanistically related to the proliferation of sites for attachment and substrate uptake (CEC) and also to the capacity of a soil to hold water relative to its pore volume.

Effect of pH on Nitrification

The lone soil observed to exhibit distinct differences in nitrification rates between treatments (initial pH) was the Bonifay soil containing the least amount of clay and organic matter. Low contents of clay mineral fraction and soil organic matter are associated with lowered capacity for holding water and reduced CEC. If the number of active nitrifiers and substrate availability are indeed positively associated with adsorption sites and clay content (Rutherford and Juma, 1992; Underhill and Prosser, 1987) then soils with low clay content and

low permanent charge characteristics (low CEC) will support lower numbers of nitrifiers with access to less substrate than soils higher in clay content. The lack of pronounced differences in rates among the other experimental soils may indicate that pH may have an interactive effect on soils where microorganisms are already stressed by low substrate availability, available habitat, and/or limitations arising from less than optimal soil water content. This effect of pH on soils may exert itself at or below some critical level of clay, CEC, or %WFP and remains to be studied in depth. One interesting phenomenon observed is that two of the four soils exhibited higher K_{max} values in the target pH 6.0 treatment rather than the pH 6.5 treatment. This observation was also noted by Weier and Gilliam (1986) in other Atlantic Coastal Plain soils contrary to the proliferation of reports that neutral to alkaline conditions favor the highest nitrification rates (Kyverga et al., 2004; Morril and Dawson, 1967). This may represent a local adaptation of the dominant strain of AOB to long-term soil acidity.

As the development of soil and subsoil acidity is closely related to the application of ammoniacal fertilizers (Sumner and Noble, 2003), it was thought that the change in pH of the incubated soils receiving ammonium additions would closely match the rate of nitrification tempered by the lime buffer capacity as follows:

$$\Delta \mathrm{pH} = \frac{mgN}{kgSoil} \times \frac{2meq(H^+)}{14mgN} \times \frac{100mgCaCO_3}{2meq(H^+)} \times \frac{kgsoil \times unitpH}{mgCaCO_3} \qquad [17]$$

where [(kg soil x unit pH)/mg CaCO$_3$] = 1/LBC and mg N = mg substrate consumed or product accumulated measured as mg nitrogen kg^{-1} soil. Prediction of pH change in these soils based on the proton production associated with the disappearance of NH$_3$-N or accumulation of NO$_3^-$-N and lime buffer capacity was unsuccessful (Figures 2.9 – 2.16). A rise in pH was observed in some soils during the lag phase and in some cases continued to be observed for a short time following the onset of the maximal rate phase. A pH rise was also observed in some cases

during the reduced rate phase even as nitrate continued to appear. One possible source of pH

elevation may be the mineralization (ammonification) of native organic nitrogen in these soils.

However, without monitoring by-products such as CO_2 evolution or the use of ^{15}N isotopic

dilution, quantification of the effect of ammonification is impossible. Another potential for pH

elevation resides in the high iron oxide content of these soils. Oxidation/reduction reactions

associated with iron minerals are known to exert a considerable influence on soil pH as shown by

equation [18] (McBride, 1994).

$$Fe(OH)_3(s) + 2H^+ = Fe^{2+} + 1/4\ O_2(g) + 5/2\ H_2O \qquad [18]$$
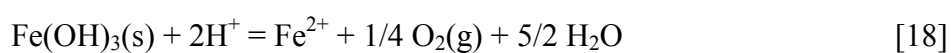
Although this is more often considered a phenomenon of anaerobic conditions, recent research

with aerobically incubated soils may also point in this direction (Thompson, 2008).

Furthermore, this phenomenon may not be strictly chemical. There is some evidence that

acidophilic heterotrophic bacteria can solubilize ferric oxides through enzymatic catalysis of the

reaction given in equation [18] (Bridge and Johnson, 1998; Johnson and McGuinness, 1991)

Regardless of the above postulations, the degree to which the prediction of pH change was

unsuccessful indicates that nitrification alone is a highly insufficient indicator for modeling

changes in soil acidity in these soils when incubated under these conditions.

Conclusions

First, the results of this study provide good evidence that soil CEC is important to the

nitrification process. Mechanistically, cation exchange sites are presumed to be the preferred

location for AOB surface attachment as well as the site of substrate uptake. This may provide

some survival advantage and reduce energy spent by the organism on acquiring substrate for

respiration. Second, this study has reaffirmed the appropriateness of expressing soil water

content with respect to microbial activity as percent water-filled pore space over soil water

potential. Past conclusions by investigators concerning the relationship between soil texture and or organic matter and nitrification rates may have been clouded by adhering to the concept of expressing soil water content as potential without regard to its effects on aeration.

There were some problems encountered in this study as well. The determination of net nitrification rates via the measurement of product (NH_3-N) disappearance and/or substrate (NO_3^--N) accumulation in these soils suffers from a lack of agreement between approaches. Furthermore, there was a discrepancy between total initial and final mineral nitrogen measured during the incubation period. The disappearance of mineral nitrogen measured during the experiment indicates some immobilization likely occurred in the soils that were maintained at aerobic conditions. One of the four soils (Norfolk depressional) was incubated at a water content (~88% WFP) that would promote loss of nitrate through denitrification as well and accordingly exhibited the greatest discrepancy in total mineral nitrogen measured. Ammonia loss through volatilization, although not measured, was considered minimal to insignificant at these pH values. Estimation of the effect of immobilization and/or denitrification on net nitrification rates as determined under the experimental conditions is impossible and so it is recommended that future incubation studies include efforts to quantify the contribution of these processes to the overall pattern of nitrogen flux between the available forms and the forms that have been immobilized or lost from the experimental system. This may be achieved through the use of ^{15}N isotopic pool enrichment and/or monitoring of CO_2 evolution by heterotrophic activity. When unable to employ these techniques for the estimation of gross nitrification rates, it is recommended that net rates be based on the measurement of nitrate accumulation as the interferences are suspected to be less influential (under aerobic conditions) than on the disappearance of ammonia.

Finally, the failure to predict pH change in these soils as a result of the nitrification process contradicts opinions that the fate of ammoniacal fertilizers is the dominant force in the acidification of soils. The spectacular degree to which the prediction equation failed to agree with observations indicates that there is some significant part of the soil acidification system not accounted for in this study. A likely source of pH buffering in this case may be an artifact of the conditions under which the incubation study was conducted. Specifically, soil moisture was maintained at a relatively constant level and oxygen consuming processes were stimulated by the presence of high levels of nitrogen. This may have resulted in anoxic 'hot spots' that promoted the reduction of ferric materials leading to a rise in pH that would not have been observed under normal 'field' conditions where drying and wetting cycles are environmental norms.

Table 2.1. Some selected soil properties of the incubated soils.

Some Selected Soil Properties

Soil	θ_g -0.01 MPa	θ_g -0.033 MPa	Texture Sand	Texture Silt	Texture Clay	Organic Matter	Total Nitrogen	LBC	CEC
	———g g^{-1}———		———%———			———mg kg^{-1}———		mg CaCO$_3$ kg^{-1}	cmol kg^{-1}
Bonifay	0.129	0.076	84.03	4.76	11.21	3,456	480	208.2	0.86
Bonifay Depressional	----	0.110	75.53	9.26	15.21	12,843	432	291.8	2.83
Norfolk Depressional	0.310	0.286	44.03	22.76	33.21	23,387	1,419	379.0	6.82
Orangeburg	0.203	0.148	69.53	8.86	21.61	3,016	233	226.3	1.75

Table 2.2. Soil pH, NH_3-N, NO_3^--N as measured at each sampling period (Bonifay Soil).

Bonifay Soil: pH, NH_3-N, and NO_3^--N

Sampling Time (day)	pH 5.5	NH_3-N 5.5 (mg kg^{-1})	RSD*	NO_3^--N 5.5 (mg kg^{-1})	RSD*	pH 6.0	NH_3-N 6.0 (mg kg^{-1})	RSD*	NO_3^--N 6.0 (mg kg^{-1})	RSD*	pH 6.5	NH_3-N 6.5 (mg kg^{-1})	RSD*	NO_3^--N 6.5 (mg kg^{-1})	RSD*
0	5.48	104.9	7.15	0.0	----	5.81	98.7	6.13	-0.1	----	6.03	107.6	3.12	0.2	----
5	5.66	102.2	5.22	-1.2	----	5.81	89.3	10.65	1.8	27.44	6.12	99.5	2.24	0.7	9.01
8	5.71	97.4	4.35	-1.8	----	5.64	81.6	2.45	11.6	8.06	5.99	88.5	1.82	4.0	12.01
11	5.77	96.4	5.84	0.0	----	5.51	61.3	5.58	28.1	17.10	5.89	84.4	1.69	13.5	6.44
14	5.67	90.9	14.61	1.7	157.06	5.20	42.1	8.89	44.2	14.28	5.59	60.2	3.53	27.4	5.97
17	5.64	84.4	17.61	7.8	141.20	4.94	27.6	13.84	64.9	4.71	5.36	55.9	35.37	43.4	7.03
20	5.51	80.0	26.44	10.2	122.58	4.99	16.7	27.95	65.7	8.92	5.25	38.0	14.76	45.0	37.25

• *RSD- Relative Standard Deviation (100*(stdev / mean of observations))
• ---- RSD not reported. Standard deviation is inflated when measurements approach the lower detection limit of the analytical method.

Table 2.3. Soil pH, NH$_3$-N, NO$_3^-$-N as measured at each sampling period (Bonifay Depressional Soil).

Bonifay Depressional Soil: pH, NH$_3$-N, and NO$_3^-$-N

Sampling Time (day)	pH 5.5	NH$_3$-N 5.5	RSD*	NO$_3^-$-N 5.5	RSD*	pH 6.0	NH$_3$-N 6.0	RSD*	NO$_3^-$-N 6.0	RSD*	pH 6.5	NH$_3$-N 6.5	RSD*	NO$_3^-$-N 6.5	RSD*
		mg kg^{-1}		mg kg^{-1}			mg kg^{-1}		mg kg^{-1}			mg kg^{-1}		mg kg^{-1}	
0	5.59	131.0	8.77	-0.1	----	5.86	120.0	10.44	0.9	138.69	6.23	122.5	18.76	0.0	----
5	5.26	80.8	11.35	21.9	13.87	5.43	73.1	13.70	24.3	21.67	5.91	83.9	9.15	8.1	21.91
8	5.14	50.6	7.96	46.5	6.14	5.19	34.8	20.82	60.7	13.62	5.52	47.5	15.00	43.4	13.67
11	4.75	20.8	8.96	63.9	1.54	4.91	4.6	48.06	67.6	32.72	5.26	6.4	50.14	55.9	17.78
14	4.84	0.3	----	73.5	26.53	5.04	0.0	----	86.7	23.12	5.34	0.0	----	72.3	38.82
17	4.94	0.0	----	81.9	18.50	5.16	0.4	----	78.4	22.78	5.58	0.0	----	78.8	14.42
20	4.93	0.0	----	89.1	8.67	5.16	0.0	----	87.8	28.99	5.51	0.0	----	79.2	32.78

- *RSD- Relative Standard Deviation (100*(stdev / mean of observations))
- ---- RSD not reported. Standard deviation is inflated when measurements approach the lower detection limit of the analytical method.

Table 2.4. Soil pH, NH$_3$-N, NO$_3^-$-N as measured at each sampling period (Orangeburg Soil).

Orangeburg Soil: pH, NH$_3$-N, and NO$_3^-$-N

Sampling Time (day)	pH 5.5	NH$_3$-N 5.5	RSD*	NO$_3^-$-N 5.5	RSD*	pH 6.0	NH$_3$-N 6.0	RSD*	NO$_3^-$-N 6.0	RSD*	pH 6.5	NH$_3$-N 6.5	RSD*	NO$_3^-$-N 6.5	RSD*
		mg kg^{-1}		mg kg^{-1}			mg kg^{-1}		mg kg^{-1}			mg kg^{-1}		mg kg^{-1}	
0	5.86	104.7	7.55	-0.1	----	6.07	101.0	13.42	-0.2	----	6.29	108.5	7.22	-0.4	----
5	5.72	95.6	1.00	13.3	16.73	5.89	86.4	15.55	13.6	27.74	6.06	85.6	5.07	17.7	7.79
8	5.26	62.9	10.47	38.2	8.73	5.45	59.2	24.66	41.4	14.15	5.62	54.7	5.66	50.5	7.38
11	4.88	35.0	9.75	53.9	2.89	4.95	24.0	8.33	65.2	3.06	5.09	15.7	9.63	69.5	3.54
14	4.59	16.7	8.11	67.2	6.42	4.81	9.7	89.81	71.1	3.63	4.98	3.8	78.53	76.4	23.02
17	4.54	8.6	21.06	83.7	3.23	4.84	2.6	92.61	87.5	1.89	5.06	-0.2	----	77.4	17.85
20	4.48	2.6	22.54	82.2	4.47	4.80	-0.1	----	81.9	10.41	5.07	-0.1	----	84.4	4.66

• ***RSD- Relative Standard Deviation (100*(stdev / mean of observations))**
• **---- RSD not reported. Standard deviation is inflated when measurements approach the lower detection limit of the analytical method.**

Table 2.5. Soil pH, NH$_3$-N, NO$_3^-$-N as measured at each sampling period (Norfolk Depressional Soil).

Norfolk Depressional Soil: pH, NH$_3$-N, and NO$_3^-$-N

Sampling Time (day)	pH 5.5	NH$_3$-N 5.5 (mg kg^{-1})	RSD*	NO$_3^-$-N 5.5 (mg kg^{-1})	RSD*	pH 6.0	NH$_3$-N 6.0 (mg kg^{-1})	RSD*	NO$_3^-$-N 6.0 (mg kg^{-1})	RSD*	pH 6.5	NH$_3$-N 6.5 (mg kg^{-1})	RSD*	NO$_3^-$-N 6.5 (mg kg^{-1})	RSD*
0	5.57	107.7	19.30	-0.8	----	5.96	121.8	7.90	-0.6	----	6.32	92.0	6.08	0.0	----
5	5.73	108.4	21.98	-6.3	----	6.30	104.3	14.03	-0.4	----	6.72	98.1	8.32	-3.0	----
7	5.63	99.6	6.10	-6.0	----	5.96	94.0	8.64	-2.3	----	6.58	95.0	5.31	-1.1	----
10	5.34	67.6	33.58	7.4	110.74	5.65	72.3	31.06	9.5	91.06	6.36	87.3	4.07	4.6	55.97
13	5.20	44.5	61.53	13.1	84.77	5.68	65.4	56.30	15.7	121.85	6.14	72.6	10.82	13.9	34.80
16	5.17	30.8	84.63	22.7	52.19	5.48	45.7	57.11	21.9	43.49	6.09	55.0	5.48	23.8	14.94
19	5.05	13.9	87.22	38.8	26.49	5.58	27.1	66.48	39.4	44.03	5.84	29.0	15.03	43.8	12.08

• *RSD- Relative Standard Deviation (100*(stdev / mean of observations))
• ---- RSD not reported. Standard deviation is inflated when measurements approach the lower detection limit of the analytical method.

Table 2.6. Verhulst equation parameters calculated by least-squares regression (SAS NLIN procedure) fit of NO_3^--N accumulation data.

Soil	pH_i	a (approx. std. err.)	N_0 (approx. std. err.)	k (approx. std. err.)	Pseudo r^2
	5.5	10.3662	0.00000398	0.0902	0.9636
		(1.3298)	(0.000023)	(0.0429)	
Bonifay	6.0	70.3258	0.505	0.00573	0.9936
		(3.7519)	(0.3142)	(0.00104)	
	6.5	47.8257	0.0909	0.00998	0.9964
		(1.9093)	(0.0588)	(0.00142)	
	5.5	85.0411	3.3181	0.00408	0.9882
		(4.6249)	(1.6336)	(0.000873)	
Orangeburg	6.0	83.1822	1.9253	0.00545	0.9873
		(3.5797)	(1.2812)	(0.00117)	
	6.5	79.9708	1.5278	0.00686	0.9986
		(1.9522)	(0.7832)	(0.000994)	
	5.5	86.44463	4.7787	0.00412	0.9899
		(3.6448)	(1.9255)	(0.000767)	
Bonifay Depressional	6.0	83.4997	2.87	0.00607	0.9763
		(3.9603)	(2.4564)	(0.00174)	
	6.5	78.4794	1.5833	0.00595	0.9821
		(4.0387)	(1.33)	(0.00154)	
	5.5	49.3642	0.0671	0.00835	0.9275
		(27.4082)	(0.2407)	(0.00979)	
Norfolk Depressional	6.0	67.0867	0.3803	0.0043	0.9605
		(52.7737)	(0.605)	(0.00526)	
	6.5	67.7784	0.1187	0.00537	0.9827
		(29.6626)	(0.1856)	(0.00401)	

Table 2.7. Verhulst equation parameters calculated by least-squares regression (SAS NLIN procedure) fit of NH_3-N consumption data.

Soil	pH_i	a (approx. std. err.)	N_0 (approx. std. err.)	k (approx. std. err.)	Pseudo r^2
Bonifay	5.5	111.3	105	0.000856	0.9898
		(3.7558)	(0.993)	0.000197)	
	6.0	103.9	99.2533	0.00233	0.9752
		(2.8061)	(1.7927)	(0.000204)	
	6.5	116.1	107.8	0.0014	0.9792
		(8.9482)	(3.9917)	(0.000375)	
Orangeburg	5.5	110.8	107	0.00334	0.9948
		(4.805)	(3.4878)	(0.000472)	
	6.0	103	101.1	0.00448	0.9993
		(1.4983)	(1.2339)	(0.000235)	
	6.5	109.3	107.3	0.00469	0.9981
		(2.7648)	(2.2951)	(0.000437)	
Bonifay Depressional	5.5	145.3	130.1	0.00251	0.9941
		(10.6183)	(4.7243)	(0.000511)	
	6.0	126.6	119.6	0.00391	0.9972
		(4.83283)	(3.0417)	(0.000509)	
	6.5	125.4	121.3	0.00407	0.9939
		(6.2706)	(4.6863)	(0.000715)	
Norfolk Depressional	5.5	116.5	113.1	0.00256	0.9818
		(7.7116)	(5.6379)	(0.000578)	
	6.0	140.4	121.5	0.00118	0.9895
		(12.2675)	(3.9427)	(0.000268)	
	6.5	96.3611	96.0538	0.00359	0.9896
		(2.2759)	(2.1245)	(0.000486)	

Table 2.8. Maximal rates and duration of phases of nitrification calculated from NO_3^--N accumulation data.

Soil	pH_i	K_{max}	t'	t_{max}	t_s	Δt
		mg kg^{-1} d^{-1}		——————day——————		
	5.5	2.42	13.66	15.80	17.94	4.28
Bonifay	6	7.08	7.34	12.23	17.12	9.78
	6.5	5.71	8.95	13.12	17.30	8.35
	5.5	7.38	3.92	9.23	14.55	10.63
Orangeburg	6	9.43	4.05	8.26	12.46	8.41
	6.5	10.97	3.67	7.18	10.69	7.01
	5.5	7.70	2.98	7.97	12.96	9.99
Bonifay Depressional	6	10.58	2.91	6.58	10.26	7.35
	6.5	9.16	4.21	8.32	12.43	8.22
	5.5	5.09	11.17	16.01	20.85	9.68
Norfolk Depressional	6	4.84	11.06	17.91	24.77	13.71
	6.5	6.17	11.96	17.43	22.91	10.95

Table 2.9. Maximal rates and duration of phases of nitrification calculated from NH_3-N consumption data.

Soil	pH_i	K_{max}	t'	t_{max}	t_s	Δt
		mg kg^{-1} d^{-1}		day		
	5.5	-2.65	10.91	29.53	48.15	37.23
Bonifay	6	-6.29	5.12	12.65	20.17	15.05
	6.5	-4.72	5.23	15.77	26.32	21.09
	5.5	-10.25	3.99	9.02	14.05	10.07
Orangeburg	6	-11.88	4.44	8.61	12.79	8.35
	6.5	-14.01	4.01	7.77	11.53	7.52
	5.5	-13.25	1.55	5.89	10.22	8.67
Bonifay Depressional	6	-15.67	2.14	5.73	9.33	7.19
	6.5	-16.00	2.97	6.64	10.30	7.32
	5.5	-8.69	5.44	11.75	18.07	12.63
Norfolk Depressional	6	-5.82	2.41	11.23	20.05	17.64
	6.5	-8.33	10.86	16.61	22.35	11.49

Table 2.10. Percent water filled pore space and relative nitrifier activity for experimental soils.

Soil	Water-Filled Pore Space	Relative Nitrifier Activity
	————————%————————	
Bonifay	35.13	80
Orangeburg	55.54	98
Bonifay Depressional	42.93	92
Norfolk Depressonal	88.07	40

Table 2.11. Kmax (NO_3^--N) values adjusted to 100% relative nitrifier activity.

Soil	pH_i	K_{max} -0.033 MPa	K_{max} Adjusted
		——mg kg^{-1} d^{-1}——	
Bonifay	5.5	2.42	3.03
	6	7.08	8.86
	6.5	5.71	7.13
Orangeburg	5.5	7.38	7.53
	6	9.43	9.62
	6.5	10.97	11.19
Bonifay Depressional	5.5	7.70	8.37
	6	10.58	11.50
	6.5	9.16	9.96
Norfolk Depressional	5.5	5.09	12.72
	6	4.84	12.10
	6.5	6.17	15.42

Table 2.12. Exchangeable cations. (extracted as per Miller and Sumner, 1996 and analyzed by ICP-OES). *ND = Not Detected

Soil	P	K	Ca	Na	Mg	Mn	Fe	Al
				—mg kg^{-1}—				
Bonifay	4.70	36.17	348.70	2.89	36.55	20.42	2.53	8.24
σ (N = 3)	0.092	3.142	3.305	0.297	2.317	0.523	0.291	0.246
Orangeburg	ND*	259.38	570.20	4.43	109.28	44.61	3.50	9.82
σ (N = 3)	NS	11.977	29.717	0.398	5.766	0.952	0.380	1.356
Bonifay Depressional	5.77	196.72	987.10	3.43	113.87	30.18	2.61	9.90
σ (N = 3)	1.257	4.590	6.338	0.223	2.030	0.262	0.122	0.541
Norfolk Depressional	4.94	561.60	1813.90	29.83	323.50	107.03	3.45	11.76
σ (N = 3)	0.773	2.563	20.712	3.038	2.851	0.580	0.432	0.682

Table 2.13. Model parameters from regression analysis of response nitrification rate parameters to soil factors. (NS – Not Significant; $p > 0.1$)

Nitrification Parameter	Intercept	Initial pH	Sand	Clay	Cation Exchange Capacity	Organic Matter	Clay * Organic Matter	CEC * Organic Matter	R-Square
				Soil Nitrification Factor					
t'	16.49112 $p > 0.0001$	NS	NS	NS	-8.65933 $p > 0.0005$	NS	NS	0.0003372 $p > 0.0005$	0.8450
$K_{max (-0.033 MPa)}$	-20.42642 $p > 0.1$	4.22379 $p > 0.05$	NS	NS	2.76933 $p > 0.05$	NS	-0.0000235 $p > 0.01$	NS	0.7638
$K_{max\ adjusted}$	-23.33375 $p > 0.05$	5.08317 $p > 0.05$	NS	NS	0.98353 $p > 0.001$	NS	NS	NS	0.8084

NO$_3$ Accumulation (Bonifay Soil)
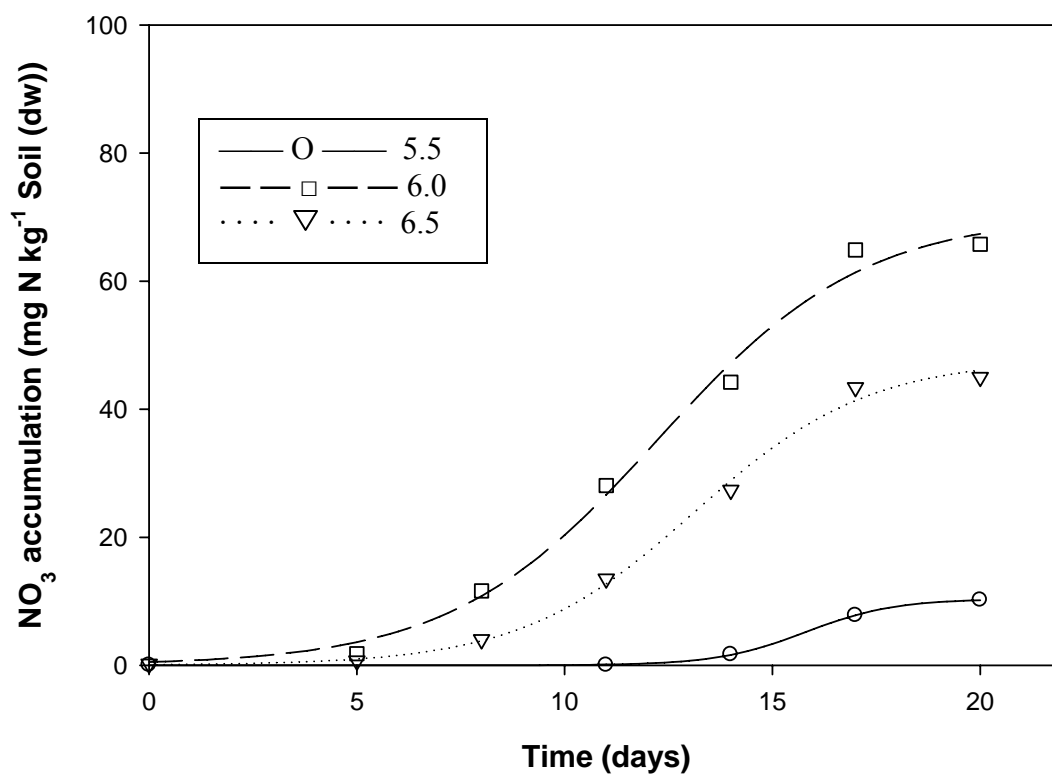
Figure 2.1. Bonifay soil NO$_3^-$ accumulation observations. Lines represent fits of Verhulst equation to data.

NO$_3$ Accumulation (Bonifay Depressional Soil)
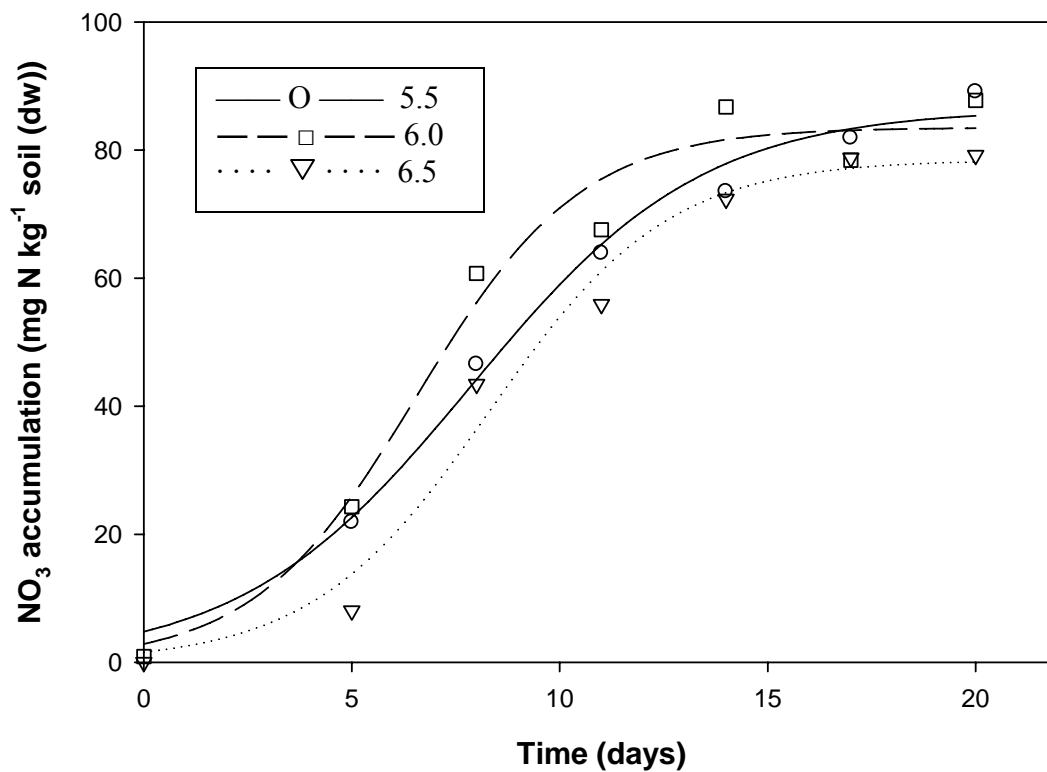
Figure 2.2. Bonifay Depressional soil NO$_3^-$ accumulation observations. Lines represent fits of Verhulst equation to data.
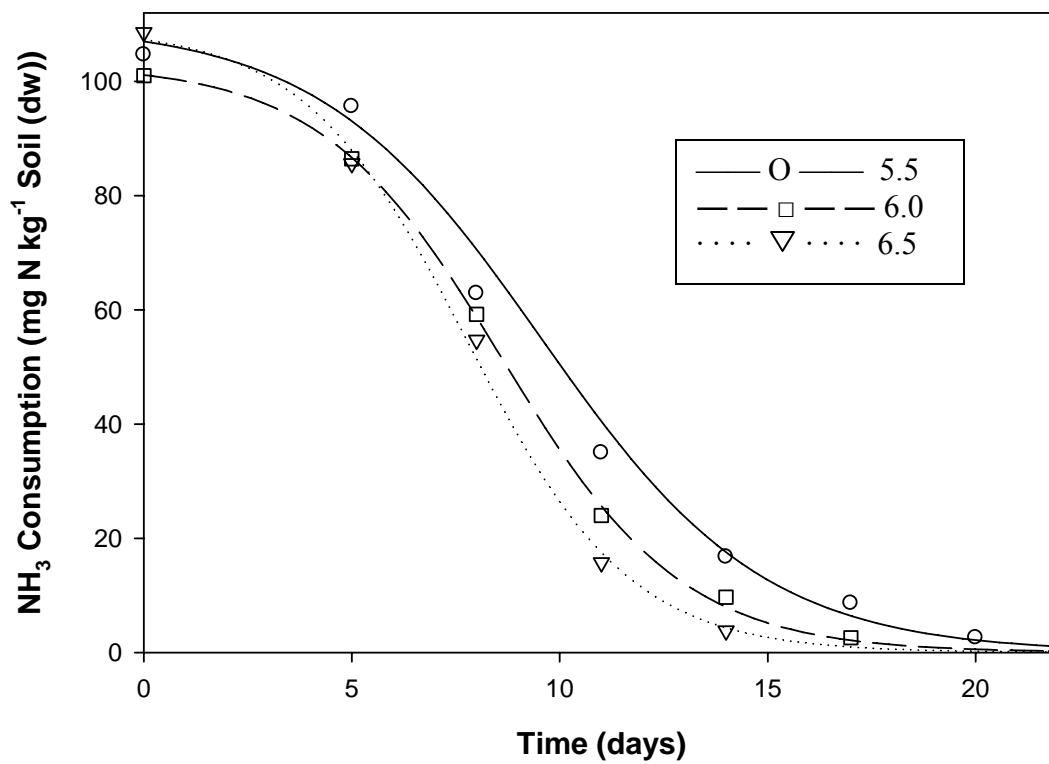
NO$_3$ Accumulation (Orangeburg Soil)
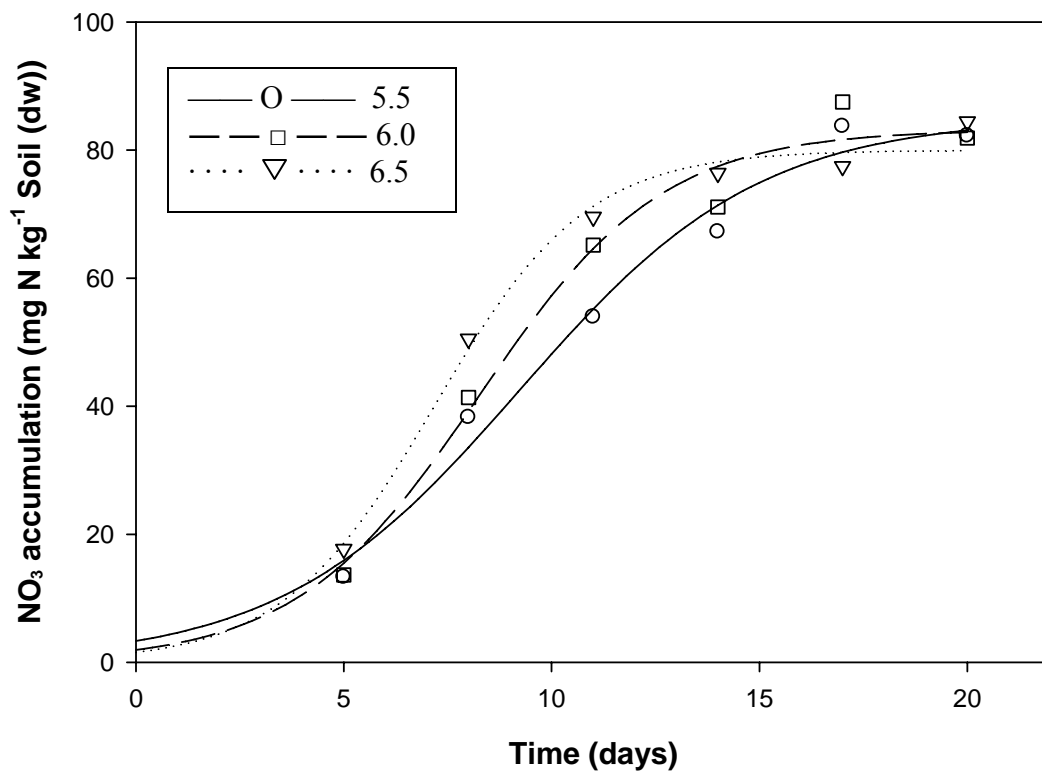
Figure 2.3.   Orangeburg soil NO$_3^-$ accumulation observations. Lines represent fits of Verhulst equation to data.

NO$_3$ Accumulation (Norfolk Depressional Soil)
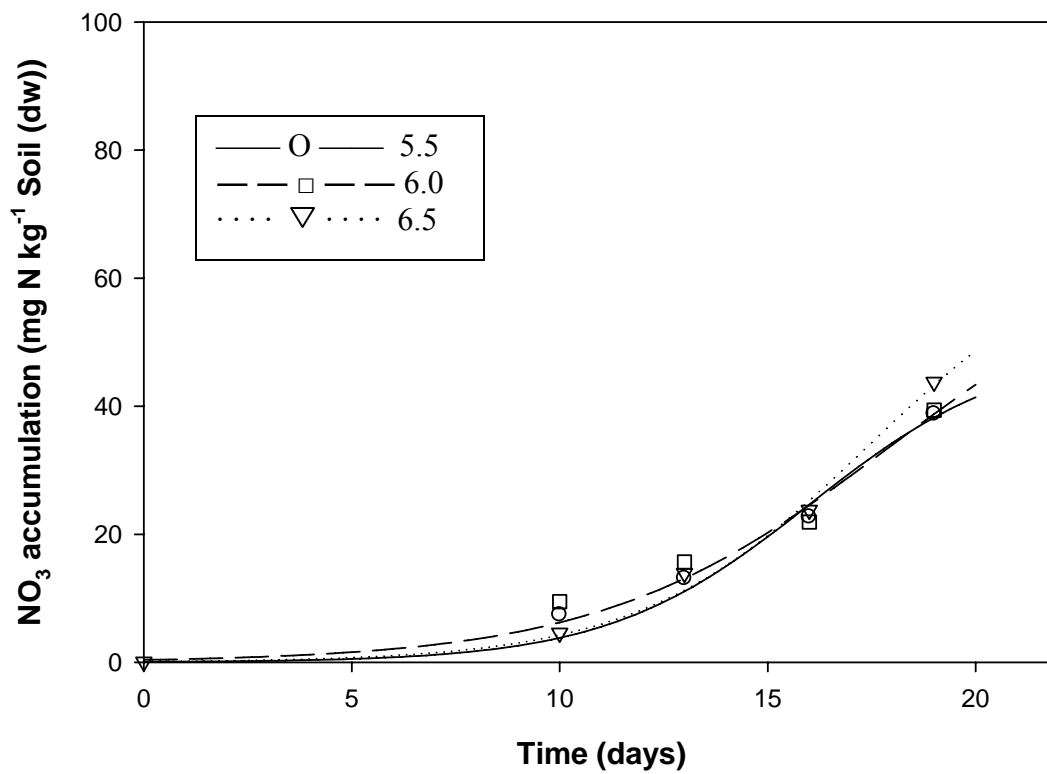
Figure 2.4. Norfolk Depressional soil NO$_3^-$ accumulation observations. Lines represent fits of Verhulst equation to data.

NH$_3$ Consumption (Bonifay Soil)
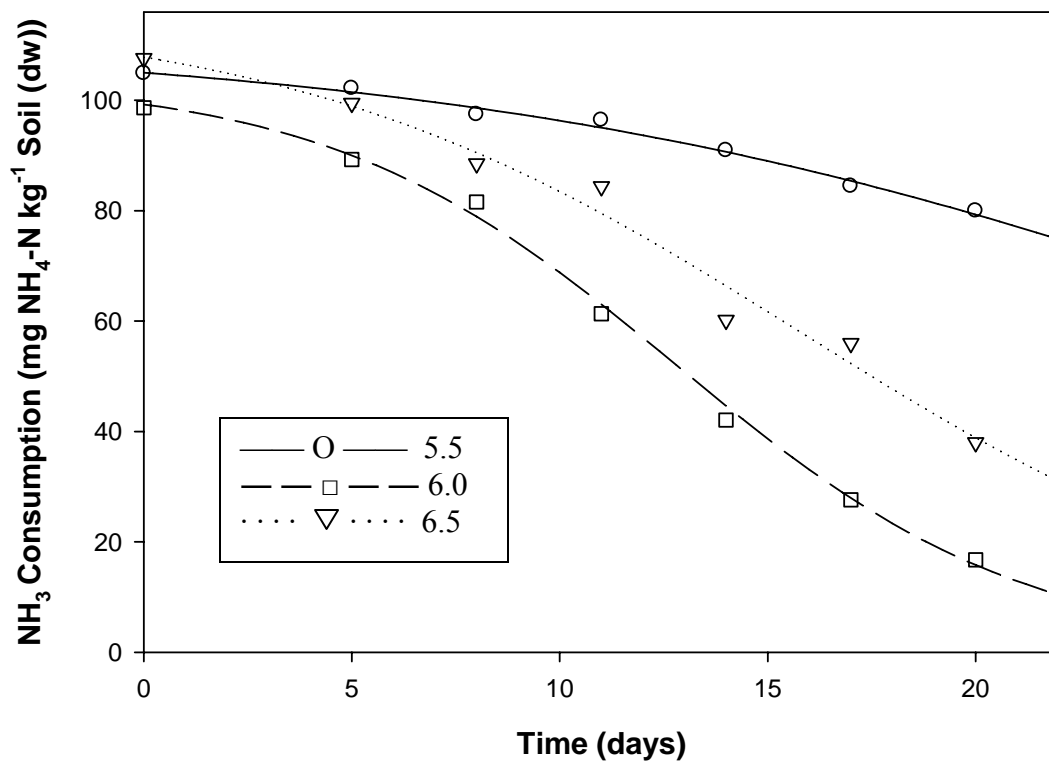
Figure 2.5. Bonifay soil NH$_3$ consumption observations. Lines represent fits of Verhulst equation to data.

NH$_3$ Consumption (Bonifay Depressional Soil)
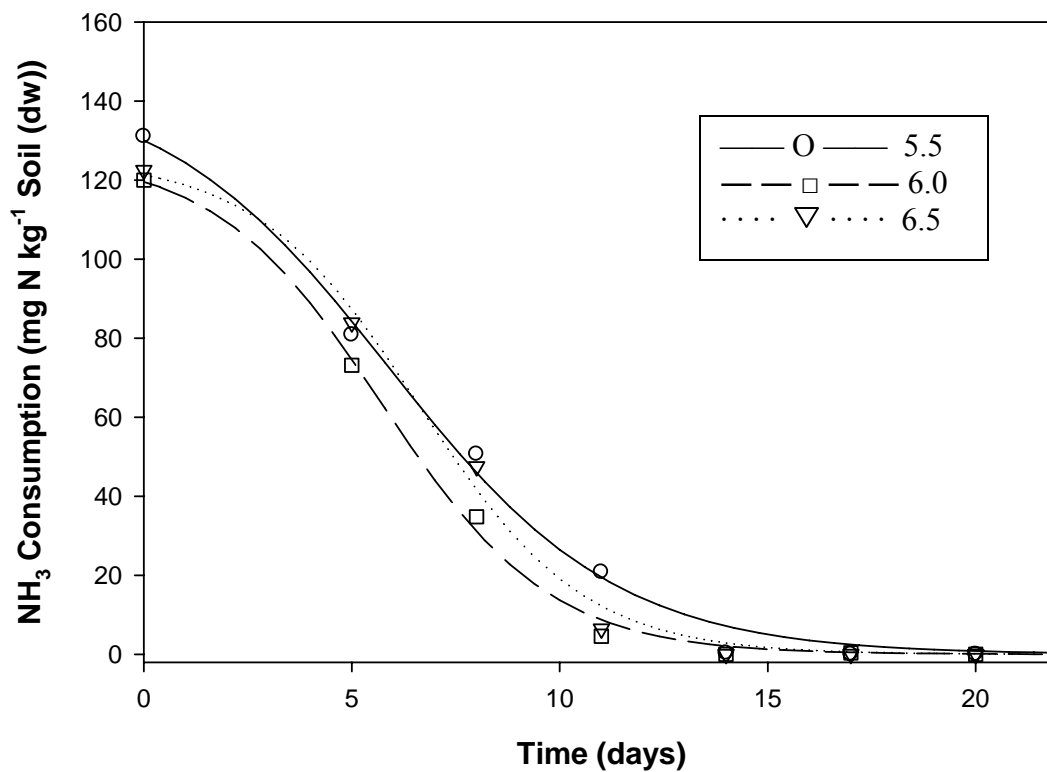
Figure 2.6. Bonifay Depressional soil NH$_3$ consumption observations. Lines represent fits of Verhulst equation to data.

NH$_3$ Consumption (Orangeburg Soil)

Figure 2.7. Orangeburg soil NH$_3$ consumption observations. Lines represent fits of Verhulst equation to data.

NH$_3$ consumption (Norfolk Depressional Soil)
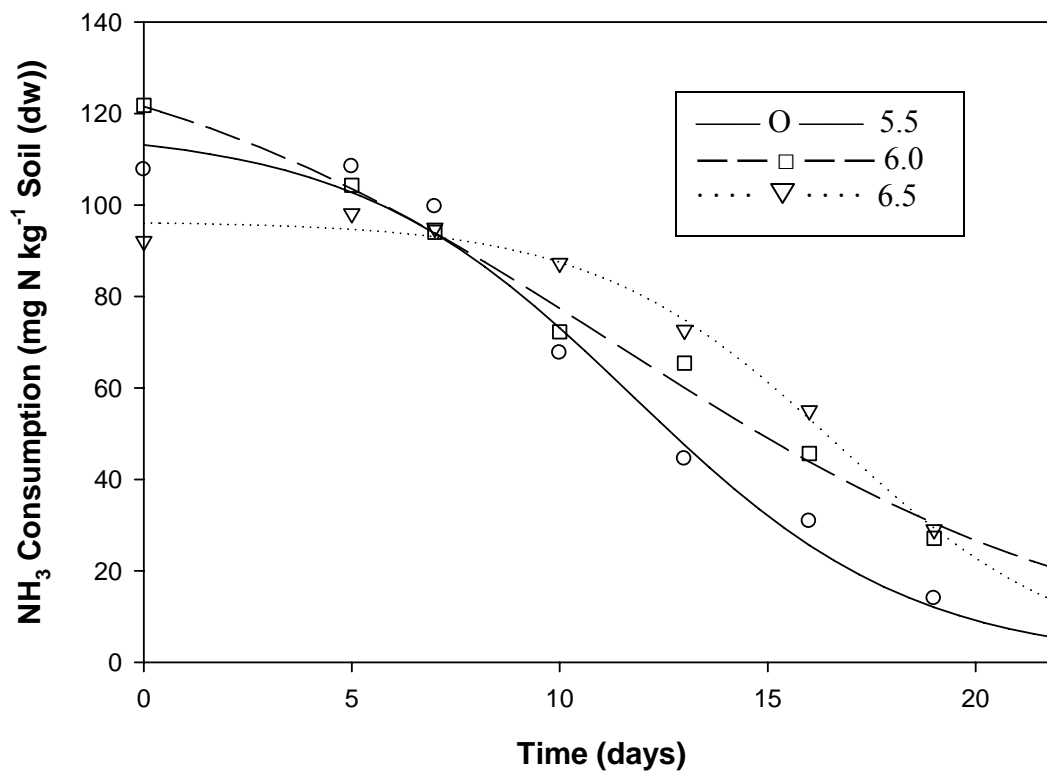
Figure 2.8. Norfolk Depressional soil NH$_3$ consumption observations. Lines represent fits of Verhulst equation to data.
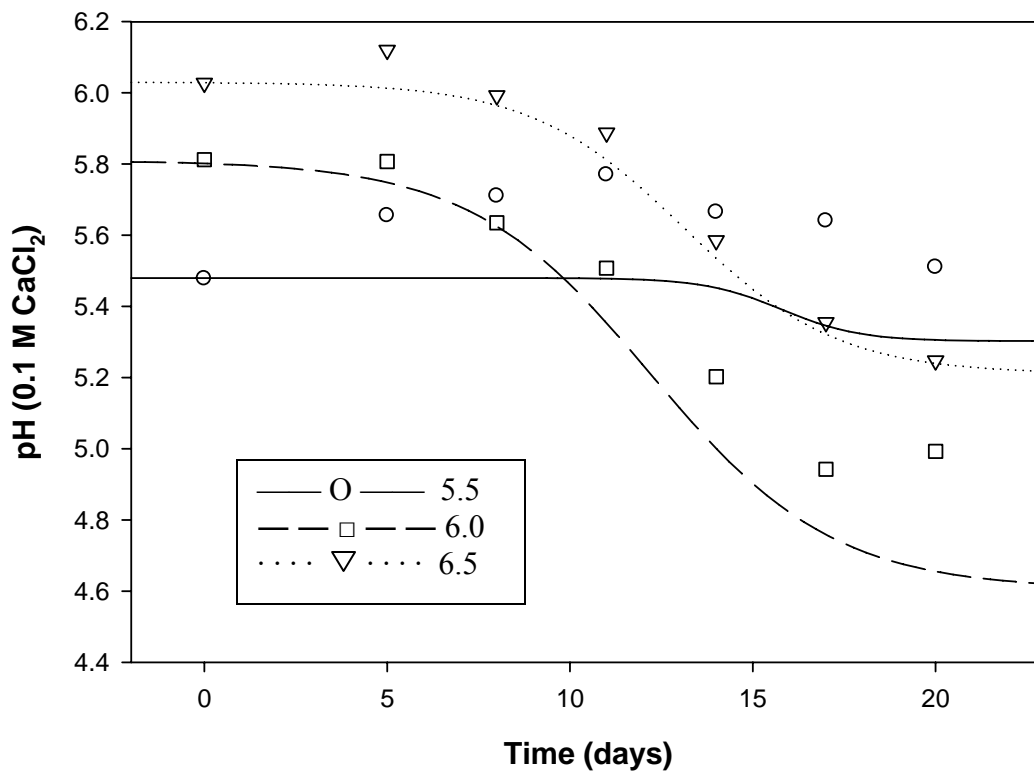
Figure 2.9. Bonifay soil pH observations with pH prediction lines calculated from NO_3^- data.
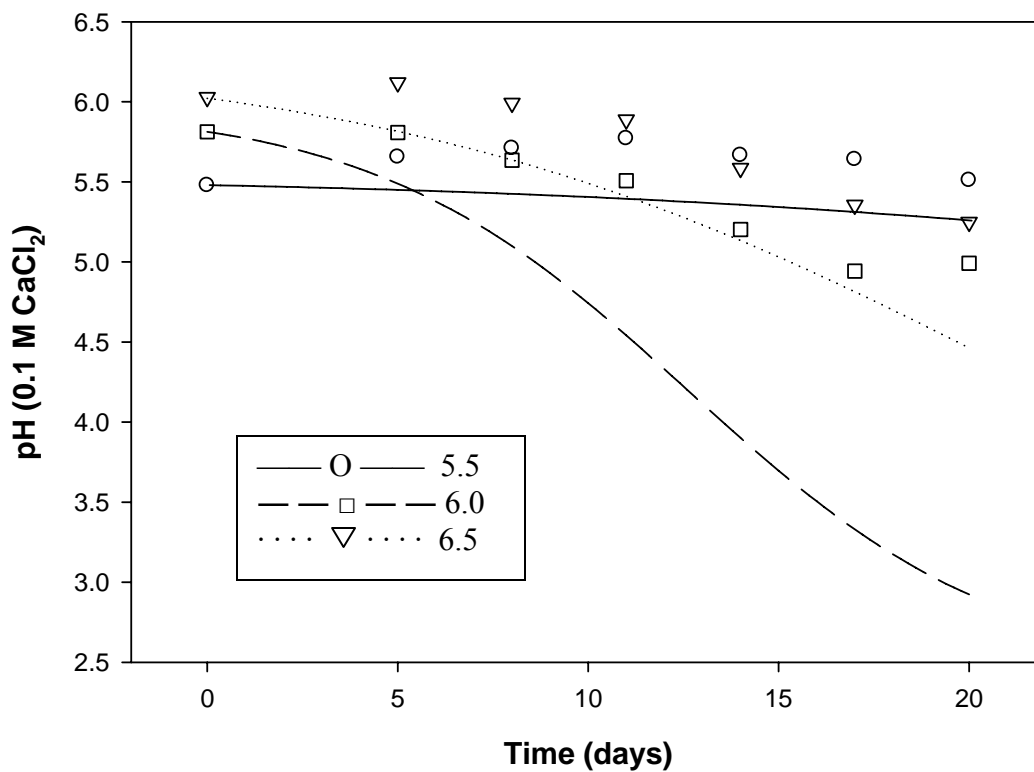
Figure 2.10. Bonifay soil pH observations with pH prediction lines based on NH₃ data.

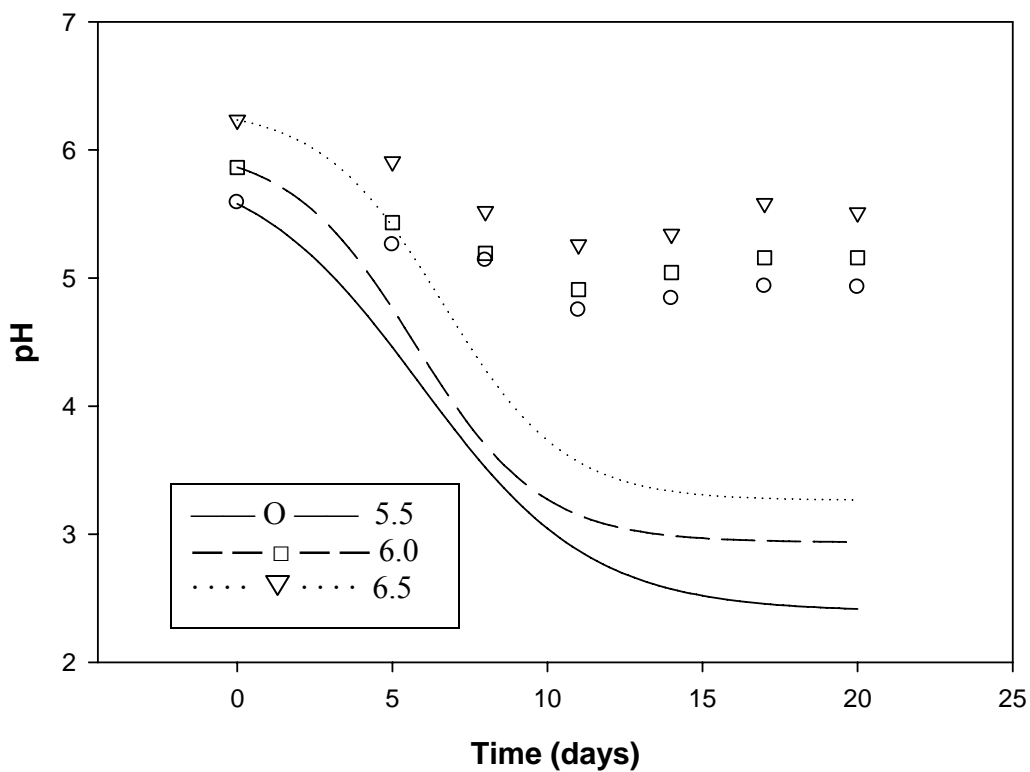pH Change During Incubation
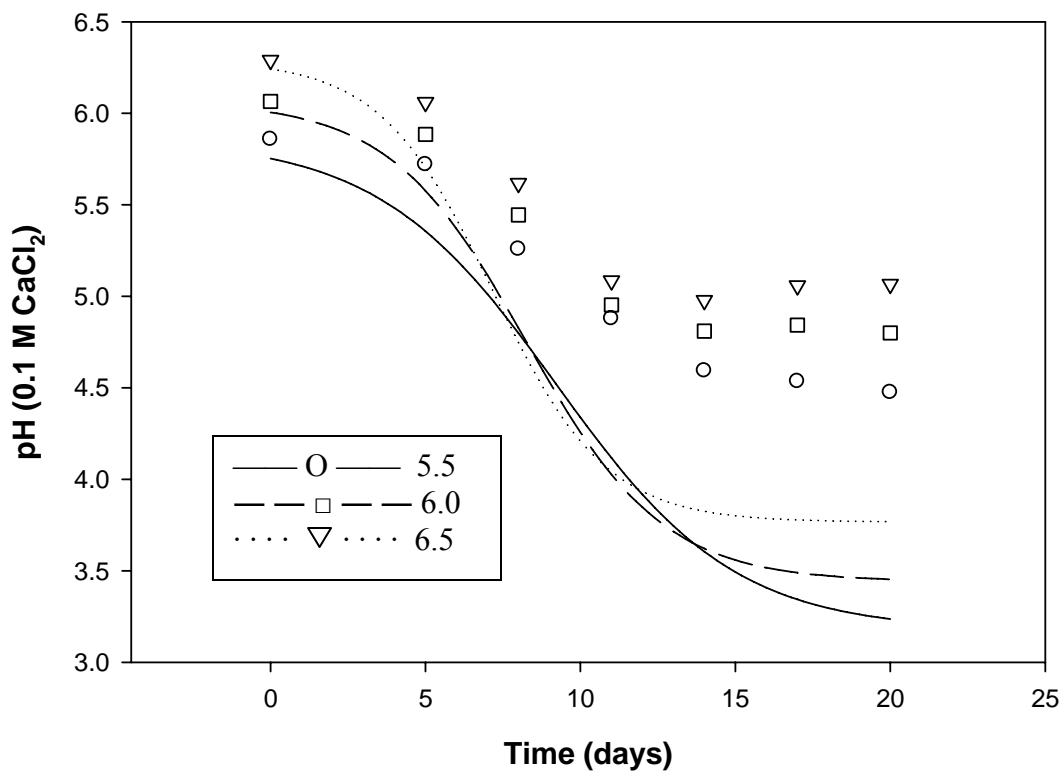(Bonifay Depressional Soil - NO$_3$ data)
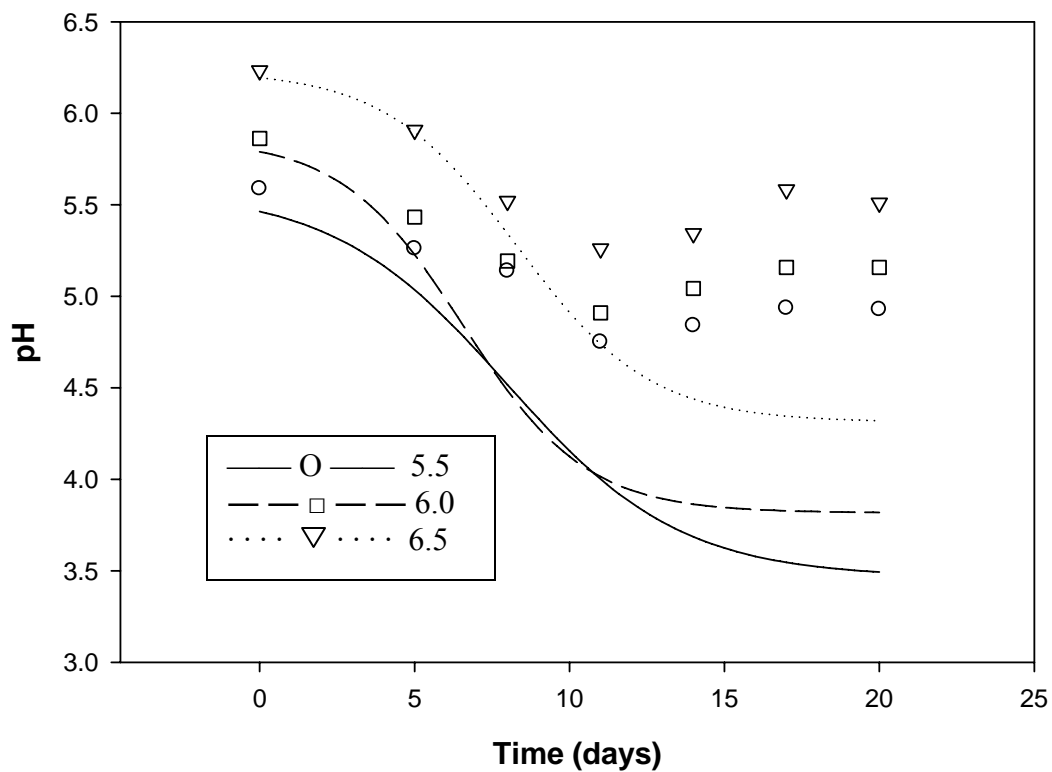
Figure 2.11. Bonifay Depressional soil pH observations with pH prediction lines calculated from NO$_3^-$ data.

.

pH Change During Incubation
(Bonifay Depressional Soil - NH$_3$ data)

Figure 2.12. Bonifay Depressional soil pH observations with pH prediction lines based on NH$_3$ data.

pH Change During Incubation
(Orangeburg Soil - NO_3 data)

Figure 2.13. Orangeburg soil pH observations with pH prediction lines calculated from NO_3^- data.

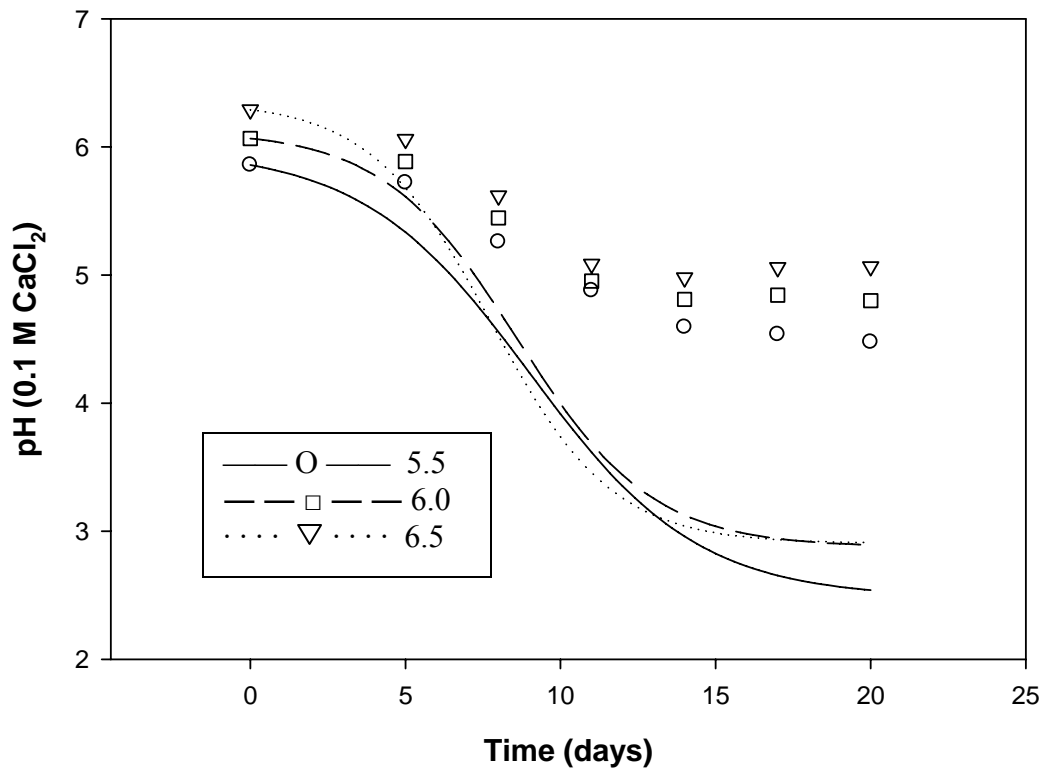pH Change During Incubation
(Orangeburg Soil - NH$_3$ data)

Figure 2.14. Orangeburg soil pH observations with pH prediction lines based on NH$_3$ data.

pH Change During Incubation
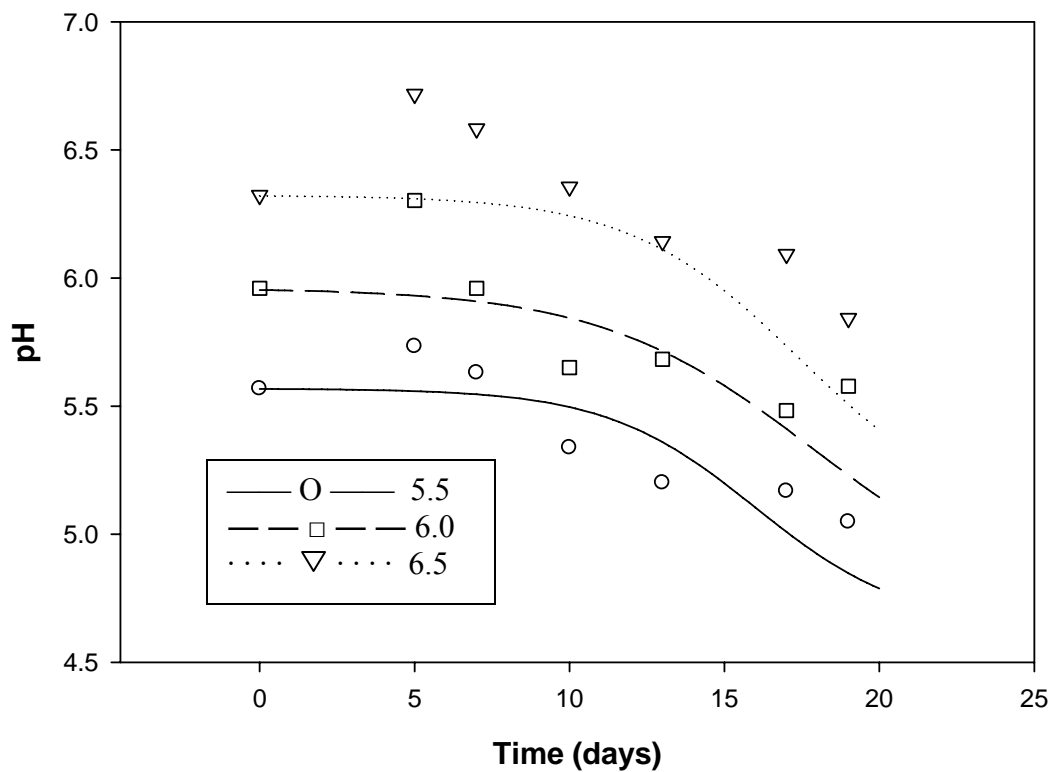(Norfolk Depressional Soil - NO_3 data)

Figure 2.15. Norfolk Depressional soil pH observations with pH prediction lines calculated from NO_3^- data.
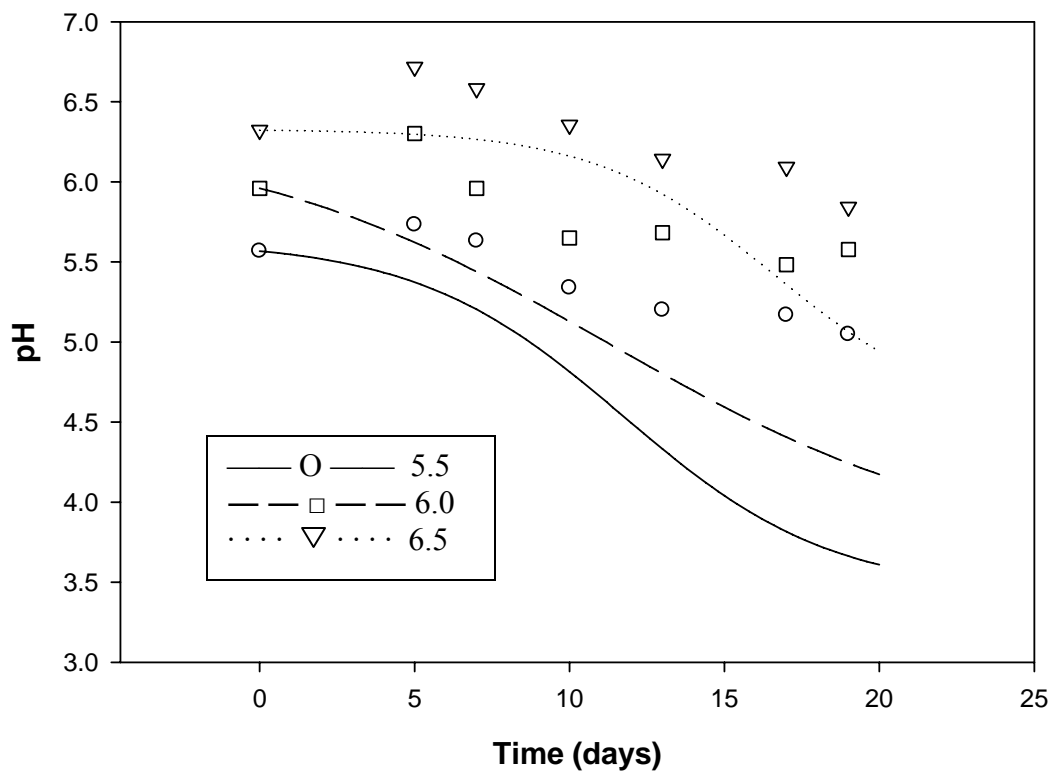
.

Figure 2.16. Norfolk Depressional soil pH observations with pH prediction lines based on NH_3 data.

References

Adams, F., and P.J. Hathcock. 1984. Aluminum toxicity and calcium deficiency in acid subsoil horizons of two coastal plains soil series. Soil Sci. Soc. Am. J. 48:1305-1309.

Albrecht, W.A., and T.M. McCalla. 1937. Nitrification of ammonia adsorbed on colloidal clay. Soil Sci. Soc. Am. Proc.

Allison, S.M., and J.I. Prosser. 1993. Ammonia oxidation at low pH by attached populations of nitrifying bacteria. Soil Biol. Biochem. 25:935-941.

Anthonisen, A.C., R.C. Loehr, T.G.S. Prakasam, and E.G. Srinath. 1976. Inhibition of nitrification by ammonia and nitrous acid. 48:835-851.

Avrahami, S., and R. Conrad. 2003. Patterns of community change among ammonia oxidizers in meadow soils upon long-term incubation at different temperatures. App. Environ. Microbiol. 69:6152-6164.

Bouman, O.T., D. Curtin, C.A. Campbell, V.O. Biederbeck, and H. Ukrainetz. 1995. Soil acidification from long-term use of anhydrous ammonia and urea. Soil Sci. Soc. Am. J. 59:1488-1494.

Bouyoucos, G.J. 1962. Hydrometer method improved for making particle size analysis of soils. Agron. J. 54:464-465.

Bremner, J.M. 1996. Nitrogen-Total. p. 1085-1121. *In* D.L. Sparks et al. (ed.) Methods of Soil Analysis, Part 3. Chemical methods. SSSA Book Ser. 5. SSSA and ASA, Madison, WI.

Bridge, T.A.M., D.B. Johnson. 1998. Reduction of soluble iron and reductive dissolution of ferric iron-containing minerals by moderately thermophilic iron-oxidizing bacteria. Appl. Environ. Microbiol. 64:2181-2186.

Chu, H., T .Fujii, S. Morimoto, X. Lin, and K. Yagi. 2008. Population size and specific nitrification potential of soil ammonia-oxidizing bacteria under long-term fertilizer management. Soil Biol. Biochem. 40:1960-1963.

Darrah, P.R., R.E. White, and P.H. Nye. 1989. Simultaneous nitrification and diffusion in soil. I. The effects of the addition of a low level of ammonium chloride. Journal of Soil Science 36:281-292.

Doran, J.W., L.N. Mielke, and J.F. Power. 1990. Microbial activity as regulated by soil water-filled pore space. In. Transactions, 14th International Congress of Soil Science, Kyoto, Japan. p. 94-99.

Eno, C.F., and W. G. Blue. 1957. The comparative rate of nitrification of anhydrous ammonia, urea, and ammonium sulfate in sandy soils. Soil Sci. Soc. Am. Proc. 21:392-396.

Grundmann, G.L., P. Renault, L. Rosso, and R. Bardin. 1995. Differential effects of soil water content and temperature on nitrification and aeration. Soil Sci. Soc. Am. J., 59:1342-1349.

Hadas, A., S. Feigenbaum, A. Feigen, and R. Portnoy. 1986. Nitrification rates in profiles of differently managed soil types. Soil Sci. Soc. Am. J. 50:633-639.

Hayatsu, M., K. Tago, and M. Saito. 2008. Various players in the nitrogen cycle: Diversity and functions of the microorganisms involved in nitrification and denitrification. Soil Science and Plant Nutrition. 54:33-45.

Helyar, K.R., and W.M. Porter. 1989. Soil acidification, its measurement and the processes involved, *In* Soil Acidity and Plant Growth (Robson, A.D., ed.) Academic Press, Sydney. pp. 61-101.

Introduction to SAS. UCLA: Academic Technology Services, Statistical Consulting Group. from http://www.ats.ucla.edu/stat/sas/library/SASNLin_os (accessed February 12, 2009).

Islam, A., R.E.White, and D. Chen. 2006. Nitrification activity in acid soils of North-Eastern Victoria, Australia, as affected by liming and phosphorus fertilization. Australian Journal of Soil Research 44:739-744.

Johnson, D.B., and S. McGinness. 1991. Ferric iron reduction by acidophilic heterotrophic bacteria. Appl. Environ. Microbiol 57:207-211.

Justice, J.K., and R.L. Smith. 1962. Nitrification of ammonium sulfate in a calcareous soil as influenced by combinations of moisture, temperature, and levels of added nitrogen. Soil Sci. Soc. Proc. 26:246-250.

Kyverga, P.M., A.M. Blackmer, J.W. Ellsworth, and R. Isla. 2004. Soil pH effects on nitrification of fall-applied anhydrous ammonia. Soil Sci. Soc. Am. J. 68:545-551.

Laubscher, K.J., J.M. Van Zyl, and C.C. du Preez. 1990. Equations to calculate the approximate duration and termination of the maximal rate phase of nitrification in soil. Commun. Soil Sci. Plant Anal., 21:611-621.

Lees, H., and J.H. Quastel. 1946. Biochemistry of nitrification in soil. II. The site of soil nitrification Biochem. J. 40:815-823.

Leininger, S., T. Urich, M. Schloter, L. Schwark, J. Qi, G.W. Nicol, J.I. Prosser, S.C. Schuster, and C. Schleper. 2006. Archaea predominate among ammonia-oxidizing prokaryotes in soils. Nature, 442:806-809.

Linn, D.M. and Doran, J.W., 1984. Effect of water filled pore space on carbon dioxide and nitrous oxide production in tilled and nontilled soils. Soil Sci. Soc. Am. J. 48:1267–1272.

Low A.P., J.M. Stark, and L.M. Dudley. 1997. Effects of soil osmotic potential on nitrification, ammonification, N-assimilation and nitrous oxide production. Soil Science 162:16-27.

Malhi, S.S., and W.B. McGill. 1982. Nitrification in three Alberta soils: Effect of temperature, moisture and substrate concentration. Soil Biol. Biochem. 14:393-399.

McBride, M. 1994. Environmental chemistry of soils. Oxford University Press. N.Y., New York.

Morrill, L.G., and J.E. Dawson. 1967. Patterns observed for the oxidation of ammonium to nitrate by soil organisms. Soil Sci. Soc. Amer. Proc. 31:757-760.

Nelson, D.W., and L.E. Sommers. 1996. Total carbon, organic carbon, and organic matter. p. 961-1010. *In* D.L. Sparks et al. (ed.) Methods of Soil Analysis, Part 3. Chemical methods. SSSA Book Ser. 5. SSSA and ASA, Madison, WI.

Page, K.L., R.C. Dalal, N.W. Menzies, and W.M. Strong. 2002. Nitrification in a vertisol subsoil and its relationship to the accumulation of ammonium-nitrogen at depth. Aust. J. Soil Res. 40:727-735.

Puri, G., and M.R. Ashman. 1999. Microbial immobilization of ^{15}N-labelled ammonium and nitrate in a temperate woodland soil. Soil Biology and Biochemistry 31:929-931.

Rice, C.W., and M.S. Smith. 1983. Nitrification of fertilizer and mineralized ammonium in no-till and plowed soil. Soil Sci. Soc. Am. J. 47:1125-1129.

Russell, C.A., I.R.P. Fillery, N. Bootsma, and K.J. McInnes. 2002. Effect of temperature and nitrogen source on nitrification in a sandy soil. Commun. Soil Sci. Plant Anal. 33:1975-1989.

Rutherford, P.M., and N.G. Juma. 1992. Influence of texture on habitable pore space and bacterial-protozoan populations in soil. Biol. Fertil. Soils 12:221-227.

Sabey, B.R., L.R. Frederick, and W.V. Bartholomew. 1959. The formation of nitrate from ammonium nitrogen in soils: III. Influence of temperature and initial population of nitrifying organisms on the maximum rate and delay period. Soil Sci. Soc. Am. Proc. 23:462-465.

Sabey, B.R., L.R. Frederick, and W.V. Bartholomew. 1969. The formation of nitrate from ammonium nitrogen in soils: IV. Use of the delay and maximum rate phases form making quantitative predictions. Soil Sci. Soc. Am. Proc. 33:276-278.

Shi, W., and J.M. Norton. 2000. Microbial control of nitrate concentrations in an agricultural soil treated with dairy waste compost or ammonium fertilizer. Soil Biology and Biochemistry 32:1453-1457.

Smith, J.H. 1964. Relationships between soil cation-exchange capacity and the toxicity of ammonia to the nitrification process. Soil Sci. Soc. Am. Proc. 28:640-644.

Stark, J.M., and M.K. Firestone. 1995. Mechanisms for soil moisture effects on activity of nitrifying bacteria. Applied and environmental microbiology. 61:218-221.

Strong, D.T., P.W.G. Sale, and K.R. Helyar. 1999. The influence of the soil matrix on nitrogen mineralization and nitrification. IV. Texture. Aust. J. Soil Res., 37:329-344.

Sumner, M.E., and W.P. Miller. 1996. Cation exchange capacity and exchange coefficients. p. 1201-1229. *In* D.L. Sparks et al. (ed.) Methods of Soil Analysis, Part 3. Chemical methods. SSSA Book Ser. 5. SSSA and ASA, Madison, WI.

Thompson, J. S. 2008. Master's Thesis, University of Georgia.

Underhill, S.E., and J.I. Prosser. 1987. Surface attachment of nitrifying bacteria and their inhibition by potassium ethyl xanthate. Microb. Ecol. 14:129-139.

Weier, K.L., and J.W. Gilliam. 1986. Effect of acidity on nitrogen mineralization and nitrification in Atlantic Coastal Plain soils. Soil Sci. Soc. Amer. J. 50:1210-1214.

CONCLUSIONS AND IMPLICATIONS

The results of this study show that nitrification rates in the sandy soils of the Southeastern Atlantic Coastal Plain are among the slowest reported in the existing literature. Slow nitrification rates allow ammonium nitrogen to persist in the soil for longer periods of time, increasing the potential for its downward transport via mass flow into lower soil horizons. The presence of ammonium nitrogen in lower horizons will change the balance of anion:cation uptake by plants and promote subsoil acidification through the release of protons by plant roots in an effort to maintain the plant's electrochemical balance.

Nitrification rates in these soils were shown to be strongly affected by soil water content and the results of this study confirm the appropriateness of expressing soil water as percent water-filled pore space (%WFP) over soil water potential (ψ). This study also strengthened the existing evidence that soil cation exchange capacity (CEC) is mechanistically related to nitrifier activity. It is proposed that soil CEC serves both as a source of substrate in the ionized form for uptake by ammonium oxidizers and as a location for organism attachment and aggregation. Increases in soil clay and organic matter content are positively associated with soil water content and CEC. Nitrification rates were not affected by pH difference in the 5.5 to 6.5 range in soils where the water content was near or above the optimum %WFP and CEC was determined to be 1.75 cmol kg^{-1} or above.