

ABSTRACT

HASHIMOTO, YOHEI. Soybean Root Growth in Acid Subsoils in Relation to Magnesium Additions and Soil Solution Chemistry. (Under the direction of T. Jot Smyth.)

Aluminum tolerance of soybean [*Glycine max* (L.) Merr.] by citrate secretion from roots, leading to external complexation of toxic Al species in solution, is enhanced by addition of $\mu\text{M Mg}^{2+}$ to hydroponic solutions. The objectives of this dissertation were to assess ameliorative effects of $\mu\text{M Mg}$ additions on soybean root growth in acidic subsoils and to relate the soil solution ionic compositions to soybean root growth. Roots of soybean cultivar Plant Introduction 416937 extending from a limed surface soil compartment grew for 28 days into a subsurface compartment containing acid subsoils [Cecil (oxidic and kaolinitic), Creedmoor (montmorillonitic) and Norfolk (kaolinitic)]. The three Mg treatments consisted of the native equilibrium soil solution concentrations in each soil (50 or 100 μM) and MgCl_2 additions to achieve 150 and 300 $\mu\text{M Mg}$ (Mg150, Mg300, respectively) in the soil solutions. Root elongations into Mg-treated subsoils were compared with a CaCO_3 treatment limed to achieve a soil pH value of 5.5. Subsoil root length for the treatments without added Mg or lime decreased in the order of the Cecil followed by Norfolk and Creedmoor subsoils, and corresponded to the increasing order of percent Al saturation (27, 61 and 83%, respectively). Subsoil root growth and dry matter responses to the Mg treatments were less than the lime treatments, and there were no differences for the Mg150 and Mg300 treatments as compared to the treatments without added Mg or lime. Citrate adsorption experiments found that over 66% citrate added in the subsoils were adsorbed and biodegraded, suggesting that root secreted citrate in the soil might readily be unavailable to complex Al for ameliorating its rhizotoxicity. Root length relative to the limed treatments for

all subsoils (RRL) was poorly related to the activity of soil solution Al species (Al^{3+} and Al-hydroxyl species) and Mg^{2+} . However, the RRL values were more closely related to the parameters associated with soil solution Ca activity including Ca^{2+} , $\text{Al}^{3+}/\text{Ca}^{2+}$, and $\text{Al}^{3+}/(\text{Ca}^{2+} + \text{Mg}^{2+})$, suggesting that Ca could be a primary factor ameliorating Al and H^+ rhizotoxicity in these subsoils. Increased tolerance to Al rhizotoxicity of soybean by μM Mg additions to hydroponic solutions, inducing citrate secretion from roots to externally complex toxic Al species, may be less important in the acid subsoil with a poor native Ca available to root growth.

**SOYBEAN ROOT GROWTH IN ACID SUBSOILS IN RELATION TO
MAGNESIUM ADDITIONS AND SOIL SOLUTION CHEMISTRY**

By

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DEDICATION

For my parents

BIOGRAPHY

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CHAPTER 1

General Introduction

Soil acidity constraints to plant growth are a principal factor limiting crop production. Acid soils occupy approximately 30% of the ice-free land (von Uexkull and Mutert, 1995) and 40% of the agricultural land in the world (Wright, 1989). A major problem for plant growth in acid soils is the reduced availability of macro- and micronutrients because of their decreased solubility in low soil pH conditions (Lucas and Davis, 1961). Aluminum toxicity also affects the plant roots growing in the acid soil, primarily resulting in damage to the root cap cells (Bennet and Breen, 1991).

Lime application alleviates Al toxicity and acidity in the surface soil layer. However, liming is less effective in alleviation of subsoil acidity and Al toxicity unless the downward movement of Ca or neutralizing anions occurs (Ritchey et al., 1980). Subsoil acidity is not completely corrected by the surface incorporation of liming material, thus, limiting the downward root elongation. Therefore, plant growth may be inhibited due to the restricted access to nutrients and available water reserves in the subsoil (Carter and Rufty, 1993). Reclamation of acidic soil could be an alternative for increasing food production demands of the future growth in population (Borlaug and Dowsell, 1997).

Development of Acid Soils

Pedogenesis processes of soil acidification generally occur where rainfall exceeds evapotranspiration. Leaching of Ca and Mg via excess rainfall decreases the base saturation. The vacated sites are initially replaced with protons to form H⁺-clays that eventually decompose to Al-clays (Kamprath and Smyth, 2005). Among agricultural processes,

continuous application of ammonia fertilizers contributes to soil H^+ accumulation via nitrification (Mahler et al., 1985). Plants also excrete H^+ from roots when their uptake of cations exceeds that of anions (Tang and Rengel, 2003). Net H^+ efflux contributing to the rhizospheric acidification typically occurs with N_2 -fixing plants that take up more cations than anions to maintain internal cell electroneutrality (Bolan et al., 1991; Coventry and Slattery, 1991). Another factor contributing to soil acidification is acid deposition generated through the processes of fuel combustion and fertilizer synthesis, which give rise to atmospheric nitrogen and sulfur compounds (Alewell, 2003). The nitrogen and sulfur compounds react with water to form nitric acid and sulfuric acid.

Soil Acidity Complex and Plant Growth

Soil acidity constraints are a complex interaction of several growth-limiting factors for plants. The soil acidity complex restricting plant growth can include H, Al or Mn toxicities, as well as deficiencies of Ca, Mg, P, or Mo (Foy, 1992; Kamprath, 1984). Additional side effects can include reduced mineralization, nitrification and nodulation (Foy, 1984). Failure to plant root development by hydrogen toxicity occurs at pH values below 4.3 for soybean (Sanzonowicz et al., 1998) and below 3.5 for sunflower (Blamey et al., 1982). Manganese toxicity is often observed in the aboveground plant tissues with symptoms of chlorosis followed by necrosis in older leaves (Foy, 1984). This can be explained by the increased solubility of Mn into the soil solution and by high mobility of Mn from roots to aboveground tissues (Nable and Loneragan, 1984). Critical concentration ranges for Mn toxicity to plants are from 0.2 to 12 mM in the hydroponic solution including cotton, sorghum, sweet potato and wheat (Kennedy and Jones, 1991; Mgema and Clark, 1995;

Mortley, 1993; Taylor et al., 1991).

Aluminum toxicity is often cited as the most important component of the soil acidity complex. This is based on the following observations: (1) Al toxicity occurs at μM concentration ranges (Jones et al., 1995; Zhang and Rengel, 1999) that are relatively lower than that of Mn toxicity that occurs at mM concentration ranges in the hydroponic solution (Kennedy and Jones, 1991; Mgema and Clark, 1995; Mortley, 1993; Taylor et al., 1991); (2) it inhibits root growth and subsequently limits water and nutrient acquisitions (Rengel, 1996); and (3) it occurs at higher and more frequently encountered soil pH values (below 5.0) than that for H toxicity in most acidic mineral soils (Kamprath and Smyth, 2005). Aluminum damage to the root tip limits root elongation. The reduction of root elongation generally occurs within 1-2 hours after roots are exposed to Al (Jones et al., 1995; Sivaguru and Horst, 1998). The inhibition of root cell expansion initially reduces root elongation (Kochian, 1995), but the continuous inhibition of root growth also results from reduced rates of cell division (Lazof and Holland, 1999). Recent studies have clarified that the rapid absorption of Al into cell symplast causes intracellular lesions (Silva et al., 2001a; Taylor et al., 2000). Additionally, Al toxicity may damage the apoplast-plasma membrane-cytoskeleton continuum (Horst et al., 1999).

Toxic effects of Al on root growth are primarily influenced by the aqueous Al species, which in turn, is dependent on the solution pH. The two major groups of rhizotoxic Al are the monomeric or polymeric species. The monomeric species of Al^{3+} , AlF^{2+} , AlF_2^+ , and polymeric species of $\text{AlO}_4\text{Al}_{12}(\text{OH})_{24}(\text{H}_2\text{O})_{12}^{7+}$ (Al_{13}) have been identified as rhizotoxic (Kinraide, 1997). The ionic species with a higher charge are generally more toxic than low charged ions, and the relative toxicity of Al ions to wheat roots follows the order of: $\text{Al}_{13} >$

$\text{Al}^{3+} > \text{AlF}^{2+} > \text{AlF}_2^+$ (Kinraide, 1997). However, rhizotoxicity of polymeric Al_{13} is unlikely to occur in the soil solution, because the high affinity to a negatively charged soil surface limits the phytoavailability of Al_{13} (Bertsch and Parker, 1996). Trivalent Al (Al^{3+}) is a predominant species in most acid soils with soil solution pH values below 5 (Lindsay, 1979). Aluminum complexes with sulfide or organic compounds have been determined as nontoxic species (Kinraide, 1991; Menzies, 2003; Parker et al., 1989). Rhizotoxicity of Al-hydroxyl species, $\text{Al}(\text{OH})^{2+}$ and $\text{Al}(\text{OH})_2^+$, is unclear (Kinraide, 1997) because the apparent toxicity of Al-hydroxyl species cannot be distinguished from Al^{3+} toxicity that may be ameliorated by H^+ effects competing for Al^{3+} binding sites on the plasma membrane (Grauer and Horst, 1990; Kinraide and Parker, 1990).

Clay Mineralogy in Acid Soils

Soil Al occurs in primary and secondary phyllosilicate minerals, crystalline Al oxide, hydroxide or oxyhydroxide minerals, and poorly-crystalline Al minerals. Crystalline and poorly-crystalline Al hydroxide clay minerals can impact plant growth because they are a source of phytotoxic Al^{3+} in the acidic soils, and serve as an adsorbent for oxyanion nutrients such as PO_4 and MoO_4 (Huang et al., 2002). In crystalline Al hydroxides, gibbsite [$\text{Al}(\text{OH})_3$] is commonly found in many soils while other minerals such as bayerite, nordstrandite, and doyleite are rare (Huang et al., 2002). Gibbsite often appears as thick, roughly rectangular crystals with 50 to 100 nm length in the clay fraction of Oxisols (de Brito Galvao and Schulze, 1996; de Mesquita Filho and Torrent, 1993).

Gibbsite is abundant in highly weathered soils (Oxisols and Ultisols) and those derived from volcanic materials (Andisols). In tropics where Oxisols are most abundant, the

formation of gibbsite results from strong desilication of primary minerals that is dependent on the intensity of leaching (Huang et al., 2002). Gibbsite is a major clay component in association with kaolinite, goethite and hematite in Oxisols of central Brazil and Costa Rica (Curi and Franzmeier, 1984; Macedo and Bryant, 1987). The typical concentration of gibbsite in these soils reach 600 g kg^{-1} , but some Brazilian Oxisols had more than 950 g kg^{-1} (da Motta and Kampf, 1992). Gibbsite often concentrates in the upper horizons of Oxisols with low Si concentration and decreases with depth (Macedo and Bryant, 1987). This is consistent with thermodynamic predictions of gibbsite and kaolinite concentrations as a function of Al^{3+} and H_4SiO_4^0 by Sposito (1989). Gibbsite is also found in Ultisols that form by weathering of igneous and metamorphic rocks, and Andisols formed by rapid weathering of volcanic ash (hundred to thousand years).

Aluminum hydroxides [$\text{Al}(\text{OH})_3$] are important in sorption of essential nutrient cations and anions for plant growth. The edges in crystalline structure of gibbsite are abundant in uncoordinated oxygen atoms that are the active site for sorption reactions controlled by pH-dependent charge (Johnston and Tombacz, 2002). Aluminum hydroxide minerals tend to have high pH values for their point of zero charge (PZC), such as pH 9.5 to 10 for gibbsite and boehmite and $\text{pH} < 8.5$ for poorly-crystalline Al hydroxides (Goldberg et al., 1996). Therefore, Oxisols and Ultisols abundant in Al hydroxide minerals with a high PZC value are positively charged under acid soil pH conditions, strongly adsorbing organic acids (e.g. humic acid and fulvic acid) and oxianions (e.g. PO_4 and MoO_4). Violante and Pigna (2002) evaluated PO_4 sorption capacity of various pure minerals and soils and found that the amount of Al and Fe oxides was more closely related to sorption capacity of PO_4 than the amount of phyllosilicate clay minerals. The nutritionally important oxianions such as

PO₄ and MoO₄ form inner-sphere complexes with Al hydroxides, and these forms are not readily available to plants.

Ion exchange and hydrolysis reactions associated with soil organic and inorganic components have been studied in acid soils (Thomas and Hargrove, 1984). In subsoils, organic matter content is lower than in topsoils, the chemical reactions associated with mineral substances are of primary importance. For the diagnosis of soil acidity, exchangeable acidity and Al saturation of effective cation exchange capacity (ECEC: sum of exchangeable Al, Ca, K and Mg) have been widely used (Kamprath, 1984). Exchangeable acidity of soils represents a labile Al pool that can enter soil solution via the exchange reactions with other ions. The fraction of exchangeable Al is dependent on cation exchange capacity (CEC) and the degree of Al saturation of ECEC (Menzies, 2003). In North Carolina Ultisols, the CEC and exchangeable Al are mainly associated with the phyllosilicate clay minerals of kaolinite and dioctahedral vermiculite (Thomas and Hargrove, 1984). Additionally, a pH dependent charge associated with Fe and Al oxides coating phyllosilicate minerals should be considered for the reclamation of soil acidity in Ultisols. For example, Al and Fe hydroxides contribute to pH buffer capacity, and these ions are quantitatively more significant than exchangeable Al (Coleman and Thomas, 1964; Volk and Jackson, 1964). Therefore, clay mineralogy is closely related to the ion exchange and pH buffering capacity, which affects Al toxicity in acidic soils.

Aluminum-Calcium Interactions in Al Rhizotoxicity

One of the major physiochemical mechanisms for expressing Al rhitoxicity is the disruption of cytoplasmic Ca²⁺ homeostasis. Aluminum tends to be highly concentrated in

the apoplast of root cells. In an acid soil ($\text{pH} < 5$) where Al^{3+} is the dominant species in the soil solution, up to 99.9% of the total Al may be found in the apoplast of root cells (Reid et al., 1996; Rengel and Reid, 1997). Trivalent Al that strongly binds to the negatively-charged plasma membrane has a 560-fold higher affinity for phosphatidylcholine (Akeson et al., 1989) and a 660-fold higher affinity for plasma membrane vesicles (Yermiyahu et al., 1997) than Ca^{2+} . The high affinity of Al^{3+} to displace essential nutrient elements such as Ca^{2+} from the apoplast is presumed to be a primary mechanism of Al toxicity (Blamey and Dowling, 1995).

Occurrence of aluminum rhizotoxicity was presumed to be due to the blockage of Ca^{2+} channel in plasma membrane. The degree of Al sensitivity of wheat genotypes was inversely proportional to their Ca^{2+} uptake (Rengel et al., 1995), leading to the hypothesis that the blockage of Ca^{2+} channels may be the primary mechanism of Al toxicity. Ryan and Kochian (1993) suggested that inhibitory effects of Al^{3+} on Ca^{2+} influx would be interrelated to the following factors: 1) direct blockage of Ca^{2+} channels; 2) reduction of Ca^{2+} concentration near transport proteins on the root plasma membrane reduces membrane negative potential and inhibits Ca^{2+} uptake; 3) changes in cytoplasmic concentration of Ca^{2+} functioning as signaling molecules; and 4) disruption of normal activity of the plasma membrane due to increased Al^{3+} binding.

Physiological interferences such as Ca^{2+} displacement and Ca^{2+} channel blockage by Al lead to the homeostatic interference of cytoplasmic Ca^{2+} (Huang et al., 1996), which functions primarily as a second messenger in cell biology (Sanders et al., 1999). The increase of cytoplasmic Ca^{2+} ($[\text{Ca}^{2+}]_{\text{cyt}}$) may be a cause of Al toxicity by disrupting the cell homeostasis. For example, increased $[\text{Ca}^{2+}]_{\text{cyt}}$ was observed for an Al sensitive wheat (isoline

ES8) but not for an Al tolerant wheat (isoline ET8) when the root was exposed to 2.6 μM Al for 1 hour (Zhang and Rengel, 1999). A mechanism in increasing $[\text{Ca}^{2+}]_{\text{cyt}}$ induced by Al^{3+} may involve extracellular Ca^{2+} influx (apoplasm) and intracellular Ca^{2+} efflux (vacuole and endoplasmic reticulum: ER), corresponding to Al^{3+} stress (Zhang and Rengel, 1999).

Homeostatic disruption resulting from Al^{3+} leads to the interference of the inositol-phospholipid signaling pathway that is involved in the control of $[\text{Ca}^{2+}]_{\text{cyt}}$ via Ca^{2+} channels. In the inositol-phospholipid signaling pathway, phospholipase C (PLC), an effector enzyme, catalyzes phosphatidylinositol 4,5-bisphosphate (PIP_2) and a second messenger inositol 1,4,5-*triphosphate* (IP_3) is formed. The inhibition of root growth by Al may be attributed to a delay of the PLC activity followed by the interference of IP_3 (Jones and Kochian, 1995; Kopka et al., 1998) because Al^{3+} has a high binding affinity for PIP_2 (Jones and Kochian, 1997).

Amelioration of Al Rhizotoxicity

The addition of Ca or Mg containing materials such as calcite (CaCO_3) or dolomite [$\text{CaMg}(\text{CO}_3)_2$] to acid soils has been used to alleviate the Al toxicity. The frequent explanations of the Ca and Mg effect on reducing Al activity are the increase in the ionic strength of the soil solution, and the reduction in the activity of rhizotoxic Al species, resulting in the alleviation of Al rhizotoxicity (Brady et al., 1993; Wheeler and Edmeades, 1995). The ameliorative effect of Ca or Mg on rhizotoxicity can be found, however, when Al activity in solution is maintained constant (Lazof and Holland, 1999; Silva et al., 2001a; Silva et al., 2001c). Silva et al. (2001c) found that Mg^{2+} , as opposed to Ca^{2+} , had no cationic effect on reducing Al activity or increasing the electrical potential at the surface of the

plasma membrane. These results suggested that electrostatic mechanisms are involved in Ca affects on the reduction of Al activity in solution whereas Mg amelioration of Al rhizotoxicity may involve physiological mechanisms.

Magnesium may be more effective than Ca in the amelioration of Al rhizotoxicity to soybean. Silva et al. (2001a) found that amelioration of Al rhizotoxicity of several soybean cultivars occurred in the μM range of Mg concentrations while mM concentrations of Ca were required for the alleviation of Al toxicity. When Ca concentration was in the μM range, root elongation was inhibited by the presence of Al in solution (Silva et al., 2001c). Results indicate that small amounts of Mg were more effective in the amelioration of Al rhizotoxicity compared to Ca. An ameliorative effect of Mg is associated with a physiological mechanism that enables soybean to alleviate Al toxicity by producing and excreting organic acid from roots (Silva et al., 2001d; Yang et al., 2000). The Mg-treated soybean cultivars enhanced citrate production in the root tip and citrate secretion into solution, compared to soybean cultivars treated with Ca (Silva et al., 2001b). The increase of citrate production in the root tip enhanced secretion of citrate that formed non toxic Al-chelating complexes. The formation of Al-citrate complexes reduces the rhizosphere concentration of free ionic Al^{3+} , which mitigates Al rhizotoxicity.

General Objectives

Studies evaluating the specific effect of Mg^{2+} on Al alleviation of soybean root growth have been conducted in hydroponic systems rather than in the soil environment. Therefore, the effect of Mg^{2+} on alleviation of Al constraints to soybean root elongation is unclear in a soil system. In this study, the amelioration effect of Mg^{2+} on Al rhizotoxicity was

evaluated in Ultisols with mineralogically different acidic subsoils. The objectives of this study were 1) to assess the ameliorative effects of μM soil solution Mg^{2+} concentration on soybean root growth in acidic subsoils with different mineralogies; and 2) to relate the Al ionic speciation and Mg^{2+} equilibria in the soil solution-exchange complex with root elongation responses in the presence of Mg^{2+} .

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CHAPTER 2

Amelioration of Subsoil Acidity Constraints to Soybean Root Elongation with Magnesium Amendments: Preliminary Studies

ABSTRACT

The goal of this dissertation study was to investigate the ameliorative effects of Mg on soybean root growth in acidic subsoils by employing a vertical-split root system. In this chapter, the results of preliminary studies on container capacity of soil water, subsoil lime requirement, and equilibrium soil solution Mg concentration for establishing the conditions and treatments for the complete root growth study are discussed. Three subsoils (Cecil, Creedmoor and Norfolk) with high Al saturation and low pH values were selected from North Carolina Ultisols. The container capacity of soil water content relative to soil weight was 26.0% for the Cecil, 9.5% for the Creedmoor and 7.5% for the Norfolk subsoils. The lime (CaCO_3) required to adjusting soil pH values to 6 was 2.12 (Cecil), 1.03 (Creedmoor) and 0.31 (Norfolk) cmol kg^{-1} . Three Mg treatments were established: native equilibrium state, 150 and 300 μM concentrations in the soil solution. The amount of Mg addition to adjust the soil solution concentrations to 150 μM was 229.5, 43.9 and 50.5 $\mu\text{mol kg}^{-1}$ for the Cecil, Creedmoor and Norfolk subsoils, respectively. Magnesium additions to achieve 300 μM soil solution concentrations for the same order of subsoils was 911.3, 116.1 and 120.0 $\mu\text{mol kg}^{-1}$. A preliminary greenhouse root growth experiment based on these laboratory incubation studies suggested that container capacity of the Cecil subsoil needed to be readjusted to 30% soil weight, and the root growth period should be extended to 28 days to enhance growth into the subsoil.

INTRODUCTION

Plant roots grown in acid soils are inhibited by the low pH, low availability of Ca, Mg and P, and high Al concentration (Carter and Rufty, 1993; Spehar, 1994). Although liming ameliorates Al and H⁺ toxicities in the surface soil layer (Dierolf et al., 1997), subsoil acidity constraints are not completely corrected by the surface incorporation of liming material (Ritchey et al., 1980). Therefore, soil acidity and Al toxicity in subsoils can restrict plant root access to the subsoil water and nutrient reserves and predispose plants to drought.

Recent investigations in hydroponic systems have shown that μM Mg additions can ameliorate Al rhizotoxicity of soybean. Evidence for the role of Mg indicates that ameliorative responses occur in the μM concentration range and support a physiological ameliorative role that involves increased production and exudation of citrate in the root tips, leading to external complexation of toxic Al species in solution (Silva et al., 2001a; Silva et al., 2001b; Silva et al., 2001c). The beneficial effects of Mg on Al rhizotoxicity to soybean in hydroponic systems support field observations that the soybean yield response is often better with dolomitic than with calcitic lime applications (Kamprath and Foy, 1985). Based on these previous investigations, we hypothesized that the beneficial effects of Mg (from dolomite) application on improving the field-grown soybean yield could result from improved root growth into the acid subsoils.

In this chapter, the results of preliminary studies on laboratory and greenhouse experiments are reported. These investigations established the conditions and treatments for the complete root growth study that was conducted afterward. A modified vertical split-root system developed by Sanzonowics et al. (1998) was employed to assess root growth from a limed and fertilized topsoil to acid subsoils. To investigate root growth in untreated subsoils

relative to subsoils amended with either different levels of MgCl_2 or one level of CaCO_3 (neutralization of all exchangeable Al), the preliminary laboratory studies conducted are described in the following four subjects: (1) container capacity of topsoil and subsoil water contents, (2) lime requirement of topsoil and subsoil to adjust the soil pH value to 6, (3) equilibrium soil solution Mg concentrations, and (4) calibration of time domain reflectrometry (TDR) to adjust the topsoil water content to the 90% container capacity. After the experimental conditions and treatments were determined based on these laboratory studies, a preliminary root growth study employed with a modified vertical-split root system was conducted in a greenhouse.

MATERIALS AND METHODS

Soil Collections and Characterization

Considering that clay mineralogy plays important roles in controlling the soil solution Al^{3+} concentration and pH buffer capacity (Coleman and Thomas, 1964; Volk and Jackson, 1964), the subsoils selected for the split-root experiment were dominated by different clay minerals. The soil materials were collected from the subsoil at a 15 to 40 cm depth. Soil materials with low Mg concentrations were chosen. Jallah (1994) has reported that the dissolved Mg concentrations of subsoils varied from 100 to 700 $\mu\text{mol L}^{-1}$ in cultivated fields of Ultisols in North Carolina. Sampling area of each soil dominated with the targeted clay mineralogy was determined by existing soil survey maps. Three soil series of North Carolina Ultisols were selected: Cecil (fine, kaolinitic, thermic Typic Kanhapludults), Creedmoor (fine, mixed, semiactive, thermic Aquic Hapludults) and Norfolk (fine-loamy, kaolinitic,

thermic Typic Kandiudults). All subsoils were collected from a forested area located near Raleigh (Cecil), Apex (Creedmoor) and Clayton (Norfolk) in the North Carolina Piedmont.

The field subsoil samples for the greenhouse experiment were air-dried and ground to pass a 4-mm sieve. After thoroughly mixing each subsoil, sub-samples were air dried and ground to pass a 2-mm sieve prior to physical and chemical characterization. Soil texture was determined by a hydrometer (Gee and Bauder, 1986). Organic carbon content was determined by combustion at 550 °C (Nelson and Sommers, 1996). Soil pH (1:2.5 soil-water ratio) was measured with a glass-electrode after stirring the sample for 10 minutes. Soil exchangeable Ca and Mg were extracted with 1M KCl solution, and soil exchangeable K was extracted with the Mehlich 3 solution (Mehlich, 1984). These exchangeable cations were extracted with 1:10 soil to solution ratio with a 10-minute stirring period and were determined by atomic absorption spectrometry. Exchangeable acidity (Al and H), also extracted with 1 M KCl, was determined by a phenolphthalein endpoint titration that was performed on filtered samples extracted using 1:10 soil to solution ratio. The filtrate was first titrated with a 0.1 M NaOH solution to the first pink endpoint that remained stable for several minutes (Al + H; exchangeable acidity). After adding 10 mL of a 1 M KF solution, a 0.1 M HCl solution was then used to back-titrate to the clear endpoint for the determination of exchangeable Al. Exchangeable H⁺ was calculated by subtracting the exchangeable Al from the exchangeable acidity.

Preparations for a Root Growth Study with the Vertical Split-Root System

Container Capacity of Soil Water

Water content of surface and subsurface compartments for the vertical split-root experiment would be maintained below container capacity to minimize the transfer of solutes between soil compartments. Container capacity of each subsoil and the Wagram topsoil (loamy, siliceous, thermic, Arenic Kandiucludults) common to surface compartments of all treatments was determined by loading air-dried soil with minimal compaction into a modified PVC pipe (10 cm diam.). The pipe consisted of 10 stacked segments of 2.5 cm in length for a total length of 25 cm. Deionized water was slowly added through a surface layer of filter paper to minimize sealing soil pores. The amount of water added about 5% (w/w) for Creedmoor and Norfolk soils and 20% (w/w) for Cecil soil loaded into the PVC pipe. After equilibration for 24 hours at room temperature, the modified PVC pipe was separated into 2.5-cm segments and the soil water content (% by weight dry soil) was determined after oven-drying samples at 105 °C in a forced-draft oven. Container capacity was determined as the average and nearly-identical soil water content of soil segments near the surface above underlying segments where excess water had drained into dry soil through gravitational tension.

Lime Requirement

A lime incubation study was conducted to determine the amount of lime required for the neutralization of exchangeable Al in each subsoil. The targeted value was a soil pH of 6.0. The limed subsoil serves as a reference treatment devoid of Al rhizotoxicity that is used to compare root growth among soils and between Mg treatments within a given soil. An air-

dried soil (200 g) was loaded into a 1 L glass jar and amended with different levels of CaCO_3 ranging from 0 to 3 cmol kg^{-1} . The soil water content was adjusted to container capacity with deionized water. After 7 days of incubation at $25 \text{ }^\circ\text{C}$, the soil was dried at room temperature and soil pH (1:2.5 soil-water ratio) was measured with a glass-electrode.

Soil Solution Mg Concentration (Subsoil compartment)

The amount of Mg added for treatments was determined by a laboratory incubation study. The purpose of the Mg incubation study was to determine the amount of MgCl_2 which should be added to each soil to achieve targeted equilibrium concentrations of Mg in the soil solution of each soil. The soil solution was collected by a modified centrifugation method developed by Jallah (1994). The centrifuge tube with a 2.8-cm diameter and 11-cm length were divided into two compartments separated by a plastic screen (0.7 cm) with filter paper. The top 7 cm compartment contains air-dried soil samples (25 g for Cecil, and 35g for Creedmoor and Norfolk subsoils) with minimal compaction. The MgCl_2 solutions (0, 50, 100, 200, 500 and $1000 \text{ } \mu\text{M}$) prepared with deionized water (18 mmho cm^{-1}) were added to achieve container capacity. After the incubation period for 40 hours at $25 \text{ }^\circ\text{C}$, the soil solution was extracted via the bottom compartment by centrifugation for 15 minutes at 7000 rpm. Soil solutions were pooled from 10 to 15 replicates of each MgCl_2 solution. All extracts were filtered through $0.45 \text{ } \mu\text{m}$ membrane and analyzed for Mg by atomic absorption spectrometry.

Calibration of Time Domain Reflectometry (Surface soil compartment)

Time domain reflectometry (TDR) measures the effective dielectric permittivity of the soil over the probe length (Ferre et al., 1998). Adequate soil water content for plants in

the surface compartment of the vertical-split root system was adjusted daily to 90% container capacity based on TDR readings of permanent probes in each pot. A soil water content of 90% container capacity was chosen to minimize solute movement to the subsoil compartments. Calibration of TDR readings to measure soil water content in the surface compartment was conducted with this compartment filled with 1.25 kg Wagram soil. The soil water content was initially adjusted to 60% container capacity and subsequent adjustments up to 100% in 10% increments. After the incubation for 24 hours at the room temperature at each soil water content, two stainless-steel rods (10 cm length, 1 mm diam.) vertically inserted in the soil were connected to a TDR and readings were recorded.

Preliminary Evaluation of Root Growth with the Vertical Split-Root System

Based on the results collected from preliminary laboratory studies, a trial greenhouse experiment for soybean root growth was conducted at North Carolina State University, Raleigh, NC from April to May 2005. Soybean [*Glycine max* (L.) Merr. cv Plant Introduction 416937] was grown with a modified vertical-split root system similar to that used in prior investigations containing hydroponic treatments in the subsurface compartment (Sanzonowicz et al., 1998). The surface 12 cm compartment was filled with 1.25 kg of Wagram soil amended with CaCO₃ to achieve a soil pH value of 6. Roots from pre-germinated seedlings elongated into the surface soil media with no Al constraint, before penetrating the root permeable membrane into the acid subsoil in the subsurface compartment. The subsurface compartment was filled with acid subsoil collected from each of the three Ultisols (Cecil, Creedmoor and Norfolk). Bulk densities of the subsurface compartment were 0.98 g cm⁻³ for the Cecil, 1.65 g cm⁻³ for the Creedmoor, and 1.60 g cm⁻³

for the Norfolk soil. Replicates of each subsoil were amended with either MgCl_2 or lime. The Mg treatment was prepared by the addition of MgCl_2 solution to achieve $300 \mu\text{M}$ Mg in the soil solution, based on the preliminary incubation results. The lime treatment received CaCO_3 to achieve a soil pH value of 6. Water content of the subsurface soil compartments were adjusted to 90% container capacity. Water and Mg solution were added to each soil seven days before the transplanting pre-germinated seedlings to allow equilibration of the soil solution ionic composition.

Seeds were pre-germinated after surface-sterilization with ethanol for 1 minute, rinsed with deionized water, and placed in the dark at 28°C in paper towels moistened with a $0.4 \mu\text{M}$ CaSO_4 solution (Ramirez et al., 1997). After three days, five seedlings with a radical length of ~ 3 cm were transplanted to the surface soil compartment. Water content of surface soil compartment was adjusted daily to 90% container capacity based on TDR readings and the calibration curve previously developed in the laboratory with the Wagram soil. Seedlings were thinned to two plants per pot after 5 days.

Plants were harvested at 21 days after transplanting. Roots were separated from the surface and subsurface soils using a 0.5 mm wet sieve and refrigerated in 25% ethanol solution for subsequent measurement of length. Root length was determined by an edge discrimination method (Pan and Bolton, 1991) using a desktop scanner preset to a resolution of $29.5 \text{ dots cm}^{-1}$ ($75 \text{ dots inch}^{-1}$). Roots were cut into 1 cm pieces and stained with an ethanol solution containing 5% methyl blue for 2 minutes to improve the contrast of scanned images.

RESULTS AND DISCUSSION

Soil Characterization

All subsoils were characterized as acidic soils with high Al saturation and low pH values (Table 1). Creedmoor subsoil had the most exchangeable Al and greatest Al saturation, suggesting the highest acidity constraints to root elongation among three subsoils.

Exchangeable Ca, Mg and K were low in the Creedmoor and Norfolk subsoils as compared to the Cecil subsoil. These acidic chemical properties would detrimentally contribute to plant root growth and to the response of each subsoil to the lime and Mg amendments. The Creedmoor and Norfolk subsoils had a sandy loam texture with 1.9 and 1.5% OM, respectively. The Cecil subsoil had a clay texture with 11.4% OM.

Container Capacity of Soil Water

Maximum and relatively constant water capacity was found in the two uppermost 2.5-cm segments of the tubes (Fig. 1a). Decreasing water content in lower segments was associated with drainage of added water that exceeded the soil's container capacity. Therefore, average values of soil water content from the two surface segments of the tubes (2.5 and 5.0 cm depth) were determined as container capacity of each subsoil (% w/w). Similar values of container capacity were found for the Creedmoor (9.5%) and Norfolk (7.5%) subsoils. The value of container capacity for the Cecil subsoil was markedly higher (26.0%) than the other subsoils. In addition to these subsoils, the container capacity of Wagram topsoil was determined as 19.6%.

Lime Requirement

The pH values of all subsoils increased linearly with lime additions (Fig. 2). The Cecil subsoil required the most lime to raise the soil pH, followed by the Creedmoor and Norfolk subsoils. Based on the regression equations for predicting the pH increase as a function of added lime, the soil pH value of each subsoil was adjusted to 6 by adding the following lime amounts: 2.12 (Cecil), 1.03 (Creedmoor) and 0.31 cmol kg^{-1} (Norfolk).

Soil Solution Mg Concentration

Magnesium concentration of soil solution for all subsoils increased linearly with added Mg (Fig. 3). The predicted soil solution Mg concentration at the native equilibrium state (0 $\mu\text{mol Mg kg}^{-1}$ addition) was 40.8 μM for Norfolk, 58.5 μM for Creedmoor and 99.5 μM for the Cecil subsoils. The similar slope values of regression equations for the Creedmoor and Norfolk subsoils indicate that both subsoils have a similar Mg sorption affinity. In contrast, the slope value of the regression equation for the Cecil subsoil was approximately one tenth of other subsoils, suggesting a high Mg sorption affinity relative to other subsoils. Based on the regression equations, three levels of Mg treatment were determined: native equilibrium state, 150 and 300 μM Mg concentration in the soil solution. The amount of Mg addition to adjust the soil solution Mg concentration to 150 μM was 229.5, 43.9 and 50.5 $\mu\text{mol kg}^{-1}$ for the Cecil, Creedmoor and Norfolk subsoils, respectively. Magnesium additions to achieve 300 μM soil solution Mg concentration for the same order of subsoils was 911.3, 116.1 and 120.0 $\mu\text{mol kg}^{-1}$.

Calibration of Time Domain Reflectometry

The percent of container capacity of Wagram topsoil water was proportional to the TDR readings (Fig. 4). Based on the regression equation, the targeted 90% container capacity of Wagram topsoil was predicted by a TDR reading value of 0.310. To adjust the Wagram topsoil water content to the targeted 90% container capacity, a TDR reading value measured every watering was subtracted from a value of 90% container capacity (0.310) and the obtained value is converted to the water amount by this regression equation.

Preliminary Evaluation of Root Growth with the Vertical Split-Root System

The lime treatment of the Cecil and Creedmoor subsoils had 2.1-fold and 1.4-fold greater root length than the Mg treatment (Fig. 5). In contrast, the Norfolk subsoil treated with Mg had a greater root length than the lime treatment. Poor root growth found in the limed Norfolk subsoil and topsoil probably resulted from a failure in transplanting of soybean seedlings. The root growth among the Mg amendments was highest in the Norfolk subsoil followed by the Cecil and Creedmoor subsoils. A distinctive tendency found in all soil series and treatments was a significant root growth in the topsoil, which is indicated by the low value of subsoil:total root ratio. The ratio of subsoil:total root length was less than 0.32 for all subsoils.

The preliminary root growth study suggests the reconsideration in (1) container capacity of subsoil water content, and (2) root growth period. The bottom 10 cm of the Cecil subsoil was dried at the end of the root growth period, indicating that container capacity was underestimated by the preliminary laboratory study. By conducting a second laboratory incubation study, the container capacity of the Cecil subsoil was found to be 30% of soil

weight (Fig. 1b). For the same reason, the redetermined container capacity of Creedmoor subsoil was 9.5% soil weight.

The 21 days of root growth period was deemed too short because the roots growing in the subsoil were thin and poorly distributed. To enhance subsoil root growth while avoiding onset of soybean N-fixation that may physiologically influence the root elongation response to subsoil acidity and lime/Mg treatments, the experimental period for the following complete root growth study was extended to 28 days.

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Table 1. Exchangeable cations, Al saturation and pH of subsoil samples used for this study

Soil	Exchangeable cations					ECEC	Alsat	Sand	Silt	Clay	SOM	pH
	Ca	Mg	K	Al	H							
	cmol _c kg ⁻¹							%				
Cecil	0.72	0.52	0.47	1.72	0.41	3.43	50	28	12	60	11.4	4.7
Creedmore	0.05	0.08	0.04	2.48	0.36	2.64	94	64	17	19	1.9	4.7
Norfolk	0.04	0.02	0.02	0.75	0.11	0.83	91	76	10	14	1.5	5.2

ECEC: Effective cation exchange capacity (= Exchangeable Ca + Mg + K + Al)

Alsat: Al saturation = 100 x (Exchangeable Al)/(ECEC)

SOM: Soil organic matter

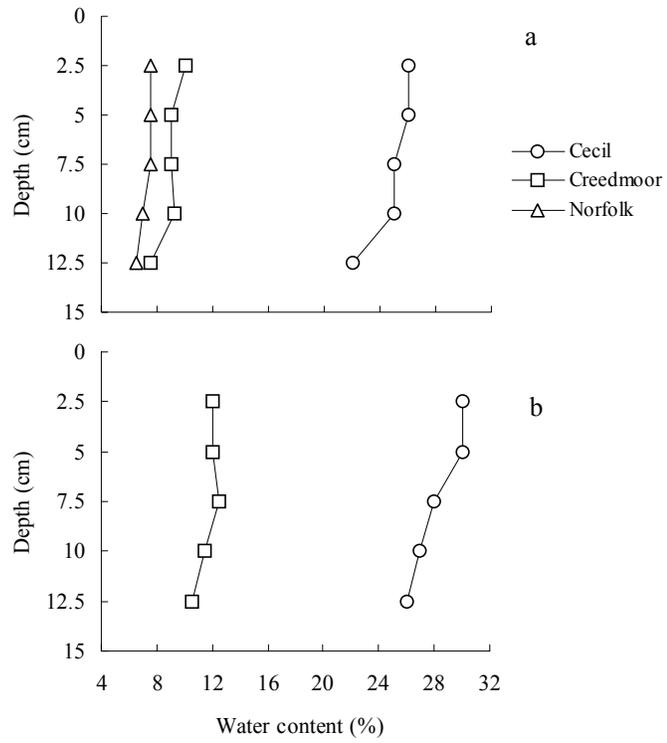


Fig. 1. Container capacity of soil water content for the Cecil, Creedmoor and Norfolk subsoils, determined before (a) and after (b) the greenhouse root growth study.

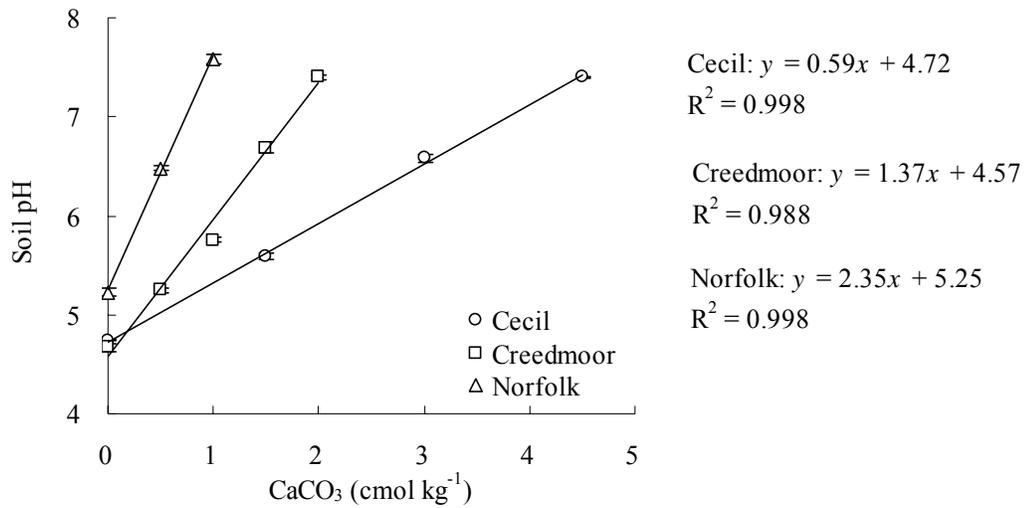


Fig. 2. Observed (symbols) and predicted (lines) soil pH as a function of soil-amended CaCO₃ amount. The error bars represent the standard error of means (n = 3).

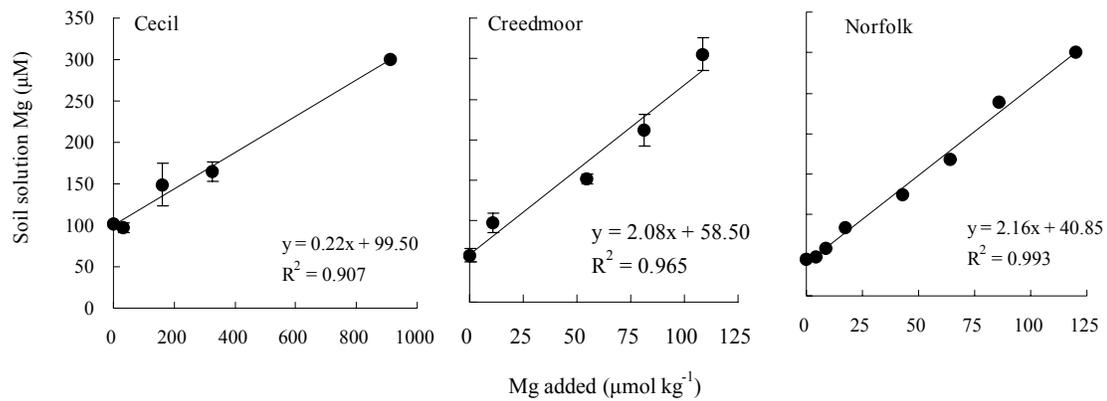


Fig. 3. Observed (symbols) and predicted (lines) Mg sorption capacity of Cecil, Creedmoor and Norfolk subsoils. The error bars represent the standard error of means (n = 3).

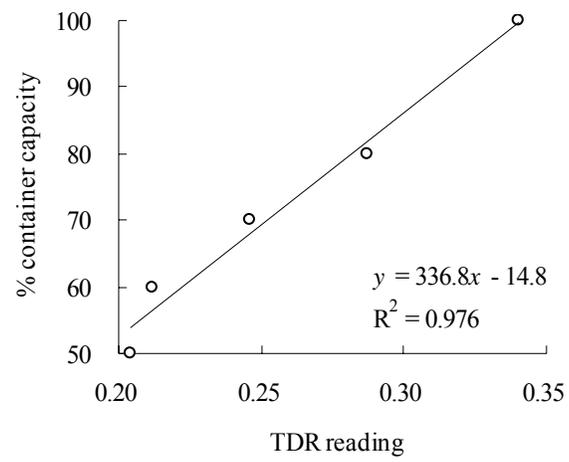


Fig. 4. Observed (symbols) and predicted (line) percent container capacity of Wagram soil as a function of TDR readings.

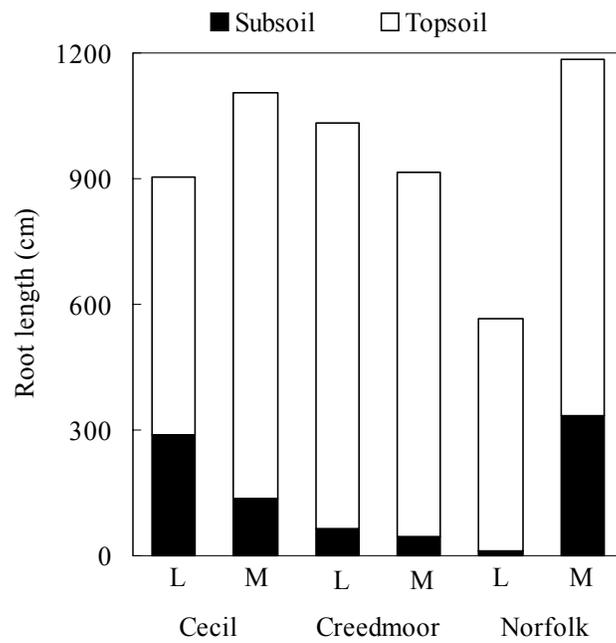


Fig. 5. Soybean root lengths of limed topsoil compartment and subsoil compartment treated with lime (L) with CaCO_3 to achieve the soil pH value of 6, and treated with MgCl_2 solution (M) to achieve 300 μM soil solution Mg concentrations after 21 days of growth period.

CHAPTER 3

Soybean Root Elongation in Response to Micromolar Additions of Magnesium to Acid

Subsoils

ABSTRACT

Aluminum tolerance of soybean [*Glycine max* (L.) Merr.] is enhanced by additions of $\mu\text{M Mg}^{2+}$ to hydroponic solutions. This is associated with enhanced citrate secretion from roots and external complexation of toxic Al species in solution. The objective of this study was to assess the ameliorative effect of $\mu\text{M Mg}$ additions on soybean root elongation into acid subsoils by using a modified split-root system. Roots of soybean cultivar Plant Introduction 416937 extending from a limed surface soil compartment grew for 28 days into a subsurface compartment containing acid subsoil samples from Cecil (oxidic and kaolinitic), Creedmoor (montmorillonitic) and Norfolk (kaolinitic) soils. The three Mg treatments consisted of the native equilibrium soil solution concentrations in each soil (50 or 100 μM) and MgCl_2 additions to achieve 150 and 300 $\mu\text{M Mg}$ (Mg150 and Mg300, respectively) in the soil solutions. Root elongations into Mg-treated subsoils were compared with a CaCO_3 treatment limed to achieve a soil pH value of 6. Subsoil root length for the treatments without added Mg or lime decreased in the order of the Cecil followed by Norfolk and Creedmoor subsoils, and corresponded to the order of increasing percent Al saturation (27, 61 and 83%, respectively). Subsoil root growth and dry matter responses to the Mg treatments were less than the lime treatments, and there were no differences between the Mg150 and Mg300 treatments as compared to the treatments without added Mg or lime. Citrate adsorption experiments revealed that over 66% of citrate added to the subsoils were adsorbed and/or

biodegraded, suggesting that most of the root secreted citrate in the soil might readily be unavailable to complex Al and ameliorate rhizotoxicity.

INTRODUCTION

Amendment of Ca or Mg containing lime materials to acidic soils has been used for the alleviation of Al toxicity. The frequent explanation of the Ca and Mg effect on reducing Al activity is the increase of soil solution ionic strength (Brady et al., 1993; Wheeler and Edmeades, 1995). The ameliorative effects of liming to acidic soils can also result from the decrease of aluminum saturation of the effective cation exchange capacity that is a widely used index for estimating a potential Al toxicity (Kamprath, 1984).

In Oxisols and Ultisols where soil acidity problems are common, Al and H⁺ constraints to plant growth are closely related to soil mineralogical properties. Crystalline and poorly-crystalline Al hydroxide minerals are abundant in these soil orders (Buol et al., 1997) and provide a source of phytotoxic Al. Trivalent Al (Al³⁺) is the most toxic species to root growth (Kinraide, 1997) and is present in most soils when soil solution pH values are below 5 (Lindsay, 1979). Plant nutrient deficiency is another problem in acid soils with abundant Al hydroxides, because they serve as an adsorbent for oxianion nutrients like PO₄ and MoO₄ (Huang et al., 2002; Violante and Pigna, 2002). Additionally, a pH dependent charge associated with Al and Fe oxide coatings of phyllosilicate minerals should be considered in the reclamation of soil acidity. For example, Al and Fe hydroxides contribute to pH buffer capacity and Al and Fe from minerals are quantitatively more significant than exchangeable Al (Coleman and Thomas, 1964; Volk and Jackson, 1964). Therefore, clay

mineralogy is closely related to the ion exchange and pH buffering capacity, which affects Al toxicity in acidic soils.

Recent studies in hydroponics showed that the ameliorative effect of Ca^{2+} and Mg^{2+} on Al rhizotoxicity can occur when Al activity in solution remains constant (Lazof and Holland, 1999; Silva et al., 2001a). Silva et al. (2001b) found that, in contrast with Ca^{2+} additions, μM Mg^{2+} additions had no effect on reducing Al activity or increasing the electrical potential at the surface of the plasma membrane. These results suggested that Ca affects the reduction of Al activity in solution through electrostatic mechanisms, whereas Mg amelioration of Al rhizotoxicity may involve physiological mechanisms such as organic acid secretion from the root tip.

The objective of this study was to assess the ameliorative effect of μM Mg additions on soybean root elongation into acid subsoils of different soil series in North Carolina.

MATERIALS AND METHODS

Greenhouse Experiment

A greenhouse experiment was conducted at North Carolina State University, Raleigh, NC from June to July 2005. Soybean [*Glycine max* (L.) Merr. cv Plant Introduction 416937] was grown with a modified vertical-sprout root system described by Sanzonowicz et al. (1998). A plastic cylinder with 10 cm diameter and 52 cm length was divided into two vertical compartments separated by with a root permeable membrane. The surface 12-cm compartment was filled with 1.25 kg of limed and fertilized Wagram soil (loamy, siliceous, thermic, Arenic Kandiudults). Thus, roots in the surface compartment grow into a media with no Al constraint before extending into the acidic subsoil media.

The subsurface compartment was filled with acid subsoil samples from soil series of three NC Ultisols: Cecil (fine, kaolinitic, thermic Typic Kanhapludults), Creedmoor (fine, mixed, semiactive, thermic Aquic Hapludults) and Norfolk (fine-loamy, kaolinitic, thermic Typic Kandiudults). Each subsoil was amended with different levels of MgCl_2 or lime. The three Mg treatments consisted of the native equilibrium soil solution concentrations in each soil [50 μM Mg (Mg50) for Creedmoor and Norfolk, and 100 μM Mg (Mg100) for Cecil] and MgCl_2 additions to achieve 150 and 300 μM Mg (Mg150, Mg300, respectively) in the soil solutions. A subsoil treatment with CaCO_3 added to raise soil pH to a value of 6 was also included to compare root elongation response with the Mg treatments. Water to achieve 90% container capacity and Mg solutions were added to each soil seven days before transplanting seedlings to allow equilibration of the soil solution ionic composition.

Experimental treatments were arranged in a randomized complete block design with six replicates. Three of the six replicates were prepared for obtaining soil samples for physical and chemical analyses at the end of the experiment, and the other three replicates were used to determine root length after wet-sieve separation from soil. The experimental design was a factorial arrangement of 3 subsoils and 4 amendments. Five pre-germinated seedlings were initially transplanted to the surface soil compartment whose water content was adjusted to 90% container capacity. Seedlings were thinned to two after 5 days.

Plant and Soil Analyses

Plants were harvested 28 days after transplanting. Aboveground tissues of plants were harvested and dried at 60 °C in a forced-draft oven, and the dry weights were measured. Dried plant material (0.5 g) was digested with 3 mL of 6 M HCl and 2 mL of concentrated

HNO₃ in a hot water bath. After digestion, the solution was filtered, diluted with deionized water, and analyzed for Al, Ca, and Mg by atomic absorption spectrometry.

When plants were harvested, soil samples were also collected from each subsurface compartment (0-10 cm, 10-20 cm, 20-30 cm, and 30-40 cm depths) in Al containers to determine the residual water content of soil profile. Soils were oven dried at 105 °C for 48 hours and the water content was determined by the weight difference upon drying. Plant water uptake by roots growing in the subsoil was calculated as the difference in soil water content between container capacity at planting and moisture at harvesting. Roots were separated from the surface and subsurface samples using a 0.5 mm wet sieve. Root length was determined by an edge discrimination method (Pan and Bolton, 1991) using a desktop scanner preset to a resolution of 29.5 dots cm⁻¹ (75 dots inch⁻¹).

Soil samples were air-dried and ground to pass a 2 mm sieve prior to physical and chemical characterization. Soil texture was determined by a hydrometer (Gee and Bauder, 1986), and organic matter content was determined by a loss-on-ignition method (Nelson and Sommers, 1996). Soil pH was measured at a 1:2.5 soil-water ratio. Exchangeable Al, Ca and Mg were extracted with 1M KCl solution, and exchangeable K was extracted with the Mehlich 3 solution (Mehlich, 1984) at a 1:10 soil-solution ratio. All exchangeable cations were determined by atomic absorption spectrometry. Acid ammonium-oxalate extractable Al and Fe (dry soil weight: 0.8 g) and citrate-bicarbonate-dithionite (CBD) extractable Al and Fe were determined for all subsoils with three replicates (dry soil weight: 1.0 g for Cecil and 3.0 g for Creedmoor and Norfolk) based on Jackson et al. (1986). All extracts were filtered through a 0.45 µm membrane and analyzed for Al and Fe by atomic absorption spectrometry.

Citrate Adsorption and Biodegradation Experiment

A batch experiment was conducted to assess the possible fates (adsorption and biodegradation) of citrate in the acid subsoils under microbial-active and inactive conditions. The experimental methods and procedures used were a modification of that by Hutchison and Hesterberg (2004). Sieved soil samples (3.0 g) collected from the Mg50/100 treatments of the subsurface compartment were weighed into tared 40 mL polycarbonate centrifuge tubes. After the addition of 10 mM KCl solution with or without 0.6 mM sodium (Na) azide, the samples were incubated at 25 °C for 24 hours. Potassium citrate monohydrate solution, whose pH value was adjusted to 4.5 by adding 10 mM HCl, was added to each duplicate tube yielding final citrate concentrations between 0 and 1111 µM. After the samples were equilibrated for 12 hours by shaking, suspension pH of each sample was adjusted to a value of 4.5 with 10 mM HCl or KOH and shaking continued for another 12 hours. After the 24-hour equilibration period, the samples were centrifuged at 16000 rpm for 10 minutes. Suspensions passed through a 0.2 µm membrane filter were analyzed for the citrate concentration by ion chromatography. Concentration-dependent adsorption isotherms of citrate were modeled for each subsoil by the Freundlich equation that is normally written as:

$$x = ac^{1/n}$$

where x is the amount of adsorbed citrate ($\mu\text{mol kg}^{-1}$), c is the equilibrium solution citrate concentration (mM), and a and $1/n$ are constants (Fitter and Sutton, 1975).

Statistical Analysis

For each soil type, analysis of variance for a full factorial treatment arrangement was performed on exchangeable cation concentrations, root length, plant dry weight and root

water uptake followed by mean separation with Fisher's LSD by SAS[®], Version 9.1 (SAS Institutes, Cary, NC). When no interactions were found between subsoils and treatments, main effects of each treatment were compared (applied for plant dry weight). Analysis of variance was also used for mean separation of citrate concentration/adsorption in the citrate adsorption and biodegradation experiment. The correlation procedure was performed to compute the correlation between subsoil and topsoil root lengths. The regression procedure was used to determine the regression curve of plant water uptake as a function of relative root length. For assessment of diagnostic indices among the subsoils, the subsoil root length was expressed as a percentage of the root length in the lime treatment of the same subsoil (relative root length). Regression equations were determined on the relative root length expressed as a function of soil exchangeable cations.

RESULTS AND DISCUSSION

Soil Characterization

All subsoils without Mg or lime additions were characterized as acidic with pH values below 4.6 (Table 1). The Creedmoor subsoil had the highest exchangeable Al and Al saturation values, suggesting the strongest acidity constraints to root elongation among the three subsoils. Exchangeable Ca, Mg and K were low ($< 0.1 \text{ cmol}_c \text{ kg}^{-1}$) in the Creedmoor and Norfolk subsoils as compared to the Cecil subsoil. The Creedmoor and Norfolk subsoils had a sandy loam texture with 1.9 and 1.5% OM, respectively. The Cecil subsoil had a clay texture with 11.4% OM.

Exchangeable Mg of Creedmoor and Norfolk subsoils was not different among the treatments ($p > 0.05$), whereas the Mg300 treatment of the Cecil subsoil presented a higher

value than other treatments ($p < 0.05$). There were no differences in exchangeable Al among Mg-amended treatments (Mg150 and 300) as compared to the treatment without added Mg or lime (Mg50/100) for all subsoils ($p > 0.05$). The absence of changes in exchangeable Al and Mg between treatments with or without Mg amendments indicates that μM additions of Mg did not influence the exchanger-phase composition with respect to these cations. Although exchangeable Mg tended to increase slightly with 150 and 300 μM Mg in all subsoils, there were no changes in Al saturation as compared to the Mg50/100 treatments ($p > 0.05$), except a slight decrease in the Mg300 treatment of the Norfolk subsoil. Liming of all subsoils reduced Al saturation and raised exchangeable Ca and pH values above 5.5, where concentrations of the rhizotoxic Al^{3+} species in the soil solution would be nil (Kamprath and Smyth, 2005).

The concentrations of CBD-extractable Fe were typically higher in the Cecil subsoil than other subsoils (Table 2), resulting from more clay and abundant Fe-oxide minerals in the Cecil subsoil. The greater oxalate- to CBD-extractable ratio (0.30) for the Creedmoor subsoil as compared to other subsoils indicated a greater proportion of poorly crystalline Fe-oxides in the Creedmoor subsoil (Schwertmann, 1993). The concentrations of CBD-extractable Al were typically higher in the Cecil than in other subsoils, corresponding to the concentrations of CBD-extractable Fe.

Root Growth

Subsoil root length for the treatments without added Mg or lime (Mg50/100) decreased in the order of Cecil followed by Norfolk and Creedmoor subsoils ($p < 0.05$), and corresponded to the decreasing order of percent Al saturation (Fig. 1). There were interaction

effects between subsoils and amendments ($p < 0.05$). Limed Creedmoor and Cecil subsoils had 1.3-fold and 4.5-fold more subsoil root length relative to the Mg50/100 treatments. Root elongation response to lime increased with subsoil exchangeable Al. However, the subsoil root length of the limed treatment in the Norfolk subsoil was not different from the other treatments ($p > 0.05$). There were no statistical differences in subsoil root length for the Mg150 and Mg300 treatments as compared to the Mg50/100 treatments in each subsoil ($p > 0.05$) although several Mg150 and Mg300 treatments tended to have a greater subsoil root length.

Relative root length among subsoil treatment clearly corresponded to soil exchangeable Al and Al saturation (Table 3), consistent with Al being a primary factor inhibiting root growth in these acid subsoils. Aluminum saturation is often used as an important index to estimate Al rhizotoxicity of plants in the acidic soil (Kamprath, 1984). For example, soybean growth in the acidic soil is generally restricted when Al saturation exceeds 15% (Osmond et al., 2002). The lime treatment in all subsoils reduced the Al saturation below 6%, whereas Al saturation of the Mg150 and Mg300 treatments was similar to that of the Mg50/100 treatments. Since exchangeable Mg in all subsoils was virtually unchanged with the μM Mg additions (Table 1), Al saturation values remained at rhizotoxic levels, leading to less root length in the Mg treatments than that of the limed treatments. Although our study found a strong relationship between exchangeable Al and relative root length (Fig. 2), some previous studies conducted in Australian acid soils reported a poor relationship between these parameters (Bruce et al., 1988; Menzies et al., 1994). In addition to exchangeable Al, over 87% of the variability associated with relative root length can be explained by the exchangeable concentration ratios of Al/Ca and Al/(Ca + Mg) (Fig. 2). This

result indicates that exchangeable Ca, as well as Al, may be a factor determining root growth in the acid subsoils examined here. Bruce et al. (1988) proposed that exchangeable Ca would be an important parameter to predict relative root length in acid soils with a low Ca concentration as found in the subsoils used in our study. Regressions of exchangeable Mg or the ratio of Al/Mg on relative root length gave a low R^2 value (Table 3). This result supports the previous observation on the absence of improvement in root growth in the Mg150 and 300 treatments as compared to the Mg50/100 treatments for all subsoils ($p > 0.05$).

There were significant main effects of soil and amendment on the topsoil root length ($p < 0.05$). Because no interactions were found between soil and amendment, averaged topsoil root length across these treatments (soil or amendment) was used for the following discussion. Topsoil root length of lime treatments was less than other treatments ($p < 0.05$). When compared to the lime treatment, topsoil root length increased by approximately 170 cm in the Mg150 treatment, 190 cm in the Mg300 treatment, and 200 cm in the Mg50/100 treatment. Reduced topsoil root length in the lime treatment indicates a preferential root proliferation in the limed subsoil. This tendency was also explained by the evidence that the subsoil root length was negatively correlated to the topsoil root length ($r = 0.82$; $p < 0.01$).

Topsoil root length averaged across all treatments had the highest value in Creedmoor (2544 cm) followed by Norfolk (2384 cm) and Cecil (1799 cm) ($p < 0.05$). Although subsoil root length tended to show the lowest value in Creedmoor followed by the Norfolk and Cecil subsoils, the topsoil root length increased with decreasing the subsoil root length. More specifically, the subsoil with higher acidic constraints (Al saturation and pH) had the least subsoil root length but the greatest topsoil root length. These relations among topsoil and subsoil root length and soil acidity constraints corresponded to a field study by Smyth and

Cassel (1995) who found that over 70% of corn root growth occurred in the top 5 cm of an Oxisol profile when no lime was applied. Contrastingly, the lime applications of 2 and 4 t ha⁻¹ reduced the root growth in the surface soil by 45% and increased the root distribution to the subsoil layers.

Plant Dry Weight

Plant dry weight was not different among the subsoils, but there were differences among the soil amendments ($p < 0.01$). There was no subsoil \times amendment interaction; therefore, the data were averaged across the soil treatments (Fig. 3). The plant dry weight of the lime treatment had the highest value among all treatments ($p < 0.05$). When compared to the lime treatment, plant dry weight decreased to 91% in the Mg150 treatment, 89% in the Mg300 treatment, and 85% in the Mg50/100 treatments. There were no differences in plant dry weight among the Mg50/100, Mg150 and Mg300 treatments ($p > 0.05$), which corresponded to the trends found in the subsoil root length.

Plant Water Uptake

Plant water uptake was different among the subsoils and amendments ($p < 0.01$), and there were interactions between these treatments ($p < 0.01$; Fig. 4). When averaged across the amendments, more water was taken up from the Cecil subsoil (360 g; $p < 0.01$), and there was no difference between the Creedmoor and Norfolk subsoils (225 g each). Liming of the Creedmoor and Norfolk subsoils increased root water uptake as compared to Mg treatments (Mg50, 150 and 300; $p < 0.05$). There were no differences in plant water uptake among the Mg treatments for the Creedmoor and Norfolk subsoils ($p > 0.05$). When compared to the

lime treatment, averaged plant water uptake decreased by approximately 80 g in the Mg50/100, 86 g in the Mg150, and 81 g in the Mg300 treatments for all subsoils (Fig. 4). In the Cecil subsoil, there were no differences in plant water uptake among the amendments ($p > 0.05$). Plant water uptake from the subsoil increased linearly with increasing subsoil root length relative to the lime treatment (Fig. 5). Increased subsoil root length improved the accessibility to the subsoil water, therefore resulting in increased water uptake.

Cation Accumulation in Aboveground Plant Tissues

Calcium accumulation in plant tissues was different among the treatments ($p < 0.01$) but not different among the subsoils. There were interactions between treatments for the plant Ca accumulation ($p < 0.01$). Liming of the Cecil and Creedmoor subsoils increased Ca accumulation as compared to other treatments within each soil (Fig. 6). When compared to the Mg50 treatment, the plant Ca accumulation with the lime treatment increased by 1.7-fold in the Cecil and 1.6-fold in the Creedmoor subsoils. Increased plant Ca accumulation was apparently attributed to the Ca supply by liming. There were no differences in plant Ca accumulation among Mg50/100, Mg150 and Mg300 treatments within each subsoil of Cecil and Creedmoor. In contrast to the Cecil and Creedmoor subsoils, plant Ca accumulation of the Norfolk subsoil was not different among the soil amendments ($p > 0.05$). This result could be related to the absence of differences in root length among treatments for the Norfolk subsoil.

Magnesium accumulation in plant tissues was different among the amendments and soils, and interaction effects of these treatments were also found ($p < 0.05$). For the Mg accumulation in the Cecil subsoil, the lime treatment had the highest value, followed by

similar values for the Mg150 and Mg300 treatments. In the Creedmoor subsoil, more Mg accumulation was observed in the lime treatment, and there were no differences among the other treatments. When compared to the lowest values within each subsoil in the Mg50 treatment, the plant Mg accumulation of the lime treatments increased by 1.2-fold in the Cecil and 1.5-fold in the Creedmoor subsoils.

Although the lime treatment had no additional Mg supply, increased plant Mg accumulation could be associated with the improved root growth in the Al-neutralized subsoil, which facilitated the nutrient acquisition. Baligar et al. (1993) found that both Ca and Mg concentrations in sorghum tissues increased from 0.01 to 0.23 mmol plant⁻¹ when Al saturation of acidic soil was reduced from 64 to 2 %, respectively. In contrast to the Cecil and Creedmoor subsoils, the largest plant Mg accumulation for the Norfolk subsoil value was found in the Mg300 treatment ($p < 0.05$). When averaged across the soils, plant Mg accumulation (mg) increased linearly by 0.06% for each μM increase in Mg concentration of the subsoils (up to 300 μM). Because there were no apparent differences in the subsoil root length among the Mg treatments, increased plant Mg accumulation in the Mg300 treatment was likely related to the addition of Mg in the subsoil.

While Al accumulation in plant tissues was not different among soils and amendments ($p > 0.05$), it tended to be lower in all limed subsoils and in the Mg300 treatment. For example, plant Al accumulation of the lime treatment was reduced by 24% each as compared to the Mg50 and Mg100 treatments in the Creedmoor and Cecil subsoils, respectively. A tendency of decreasing Al accumulation in aboveground tissues with increased Ca concentration would be consistent with the findings of Ferrufino et al. (2000) that Al accumulation in the aboveground tissues of soybean PI 416937 grown in a

hydroponic solution was decreased by 40% when the Ca concentration was increased from 2 to 10 mM. Kinraide and Parker (1987) suggested that competitive effects of Ca reduce the access of Al to binding sites at the plasma membrane. Results showed that plant Al concentration of the limed Norfolk subsoil was similar to the Mg50 treatment. Although Al saturation and pH of the limed Norfolk subsoil were in the range where Al rhizotoxicity rarely occurs, the exchangeable Al/Ca cation ratio for the lime treatment (0.07) was higher than for the Cecil (0.02) and Creedmoor (0.05) subsoils. The higher Al/Ca ratio suggest less competitive effects by Ca on Al binding to external sites of root plasma membrane (Kinraide, 1998), possibly increasing Al accumulation in tissues.

Citrate Adsorption and Biodegradation in Subsoils

Unlike hydroponic systems, organic acids secreted from the root to the soil solution suffer the potential fates of sorption on clay minerals. The concentration-dependent adsorption isotherms were determined to characterize the citrate adsorption capacity of subsoils (Fig. 7a). Citrate adsorption isotherms for all subsoils were adequately described by the Freundlich equation. The amount of citrate adsorption among subsoils generally followed the order of Cecil > Norfolk > Creedmoor. Across the concentration range used in this study, the Cecil subsoil had the highest citrate adsorption among the subsoils when the citrate addition exceeded 27 μM ($p < 0.05$). The Cecil subsoil had the highest affinity for citrate adsorption among the subsoils with 99% adsorption observed throughout the citrate concentration range. The high adsorption of citrate for the Cecil subsoil resulted from its clayey texture with abundant crystalline and poorly-crystalline Al and Fe hydroxides (Table

2). Citrate adsorption capacity for all subsoils did not correlated with ECEC and exchangeable Al, which agreed with the study conducted by Jones and Brassington (1998).

The amount of citrate adsorption on the Norfolk subsoil was greater than that of the Creedmoor subsoil when the citrate addition was over 270 μM ($p < 0.05$), despite subsoil similarities in clay, OM contents, and amount of crystalline Al and Fe and poorly-crystalline Al. The different characteristics in citrate adsorption for the Creedmoor and Norfolk subsoils could possibly be associated with differences in the predominant clay minerals (montmorillonitic for Creedmoor and kaolinitic for Norfolk). With an acid pH (4.5), kaolinite possesses more positive net charge due to a higher point of zero-charge (PZC) value than montmorillonite (Stumm and Morgan, 1981), which possibly resulted in more citrate adsorption for the Norfolk subsoil. Soils dominated by a kaolinite often showed a higher adsorption capacity for citrate and dissolved organic carbon than those dominated with 2:1 clay minerals including mica, illite and montmorillonite (Jones and Edwards, 1998; Kahle et al., 2004; Lackovic et al., 2003). The difference in citrate adsorption between these minerals increased when the solution contained a high equilibrium citrate concentration (Jones and Edwards, 1998; Lackovic et al., 2003), and similar adsorption characteristics were found in our study.

At low solution citrate concentration ranging from 11 to 111 μM , however, there was more citrate adsorption by the Creedmoor than by the Norfolk subsoil ($p < 0.05$; Fig. 7b). This result indicates that poorly-crystalline Al and Fe hydroxides, which were more abundant in the Creedmoor than the Norfolk subsoils, would be a primary adsorption site for citrate (Jones and Edwards, 1998; Jones et al., 1996) and these clay minerals may have a significant role in controlling the citrate concentration in the soil solution at the lower citrate

concentrations. A high adsorption capacity of Fe hydroxide for organic acids was reported by Jones and Brassington (1998), who found that the adsorption maximum of citrate onto ferrihydrite is approximately 650 mmol kg^{-1} at a pH value of 5.0. Thus, we might expect that citrate secreted from roots to soils, which ranges from nano- to micromolar levels (Jones, 1998), would initially be adsorbed onto poorly-crystalline Al and Fe.

Biodegradation can be an alternative fate of citrate secreted from the root into the soil. The citrate concentration in solution for a given input concentration under the microbial inhibited condition (with Na-azide addition) was higher than that under the microbial active condition (without Na-azide addition) when the citrate concentration exceeded $30 \text{ }\mu\text{M}$ for the Creedmoor and $10 \text{ }\mu\text{M}$ for the Norfolk subsoils ($p < 0.05$; Fig. 8). The concentration of citrate in solution increased from 0 to $40 \text{ }\mu\text{M}$ (without Na-azide) and 0 to $80 \text{ }\mu\text{M}$ (with Na-azide) for the Creedmoor subsoil, and from 0 to $20 \text{ }\mu\text{M}$ (without Na-azide) and 0 to $90 \text{ }\mu\text{M}$ (with Na-azide) for the Norfolk subsoil.

The difference of citrate concentrations in solution between the treatments with and without Na-azide additions (shown as a shaded area in Fig. 8) was attributed to the amount of microbially-degraded citrate. The amount of microbially-degraded citrate was apparently higher in the Norfolk (0 - 63% of citrate) than the Creedmoor (0 - 44% of citrate) subsoils, gradually increasing with the citrate concentration. The amount of microbially-degraded citrate across the whole concentration is shown as the shaded area, which is greater in the Norfolk ($3510 \text{ }\mu\text{M}$) than the Creedmoor ($2220 \text{ }\mu\text{M}$) subsoils. Because the microbial activity in an acidic Al toxic soil is generally inhibited (Amonette et al., 2003; Illmer et al., 2003), the severe acidic constraint (exchangeable Al) of the Creedmoor subsoil relative to the Norfolk subsoil could result in less microbial degradation of citrate in the former soil. In the Cecil

subsoil, all citrate was absorbed from solution both with and without Na-azide additions, indicating the predominance of mineralogical adsorption rather than biological degradation as the potential fate of citrate. These results suggest that the low concentration of citrate in solution for the Cecil subsoil is attributable to mineralogical adsorption, and we could expect that root-secreted citrate in the soil might readily be unavailable to complex Al^{3+} for ameliorating its rhizotoxicity.

Implications of Ameliorative Effects of Mg on Al Rhizotoxicity

Liming of acidic subsoils generally improved subsoil root growth, dry matter production and plant water uptake, whereas Mg amendments in μM soil solution concentrations were not as effective on improving these growth parameters. In addition, Mg ameliorative effects on Al rhizotoxicity were not clearly shown among the subsoils with different mineralogical and soil acidic properties. Two basic mechanisms have been proposed for Ca amelioration of Al rhizotoxicity (Kinraide, 1998; Kinraide et al., 2004; Rengel, 1992): (1) reduction of the negativity of the plasma membrane surface electrical potential (Mechanism I); and (2) restoration of Al-displaced Ca to non-limiting levels (Mechanism II). In addition, a physiological ameliorative mechanism that cannot be explained by Mechanisms I and II has been proposed as Mechanism III (Kinraide, 1998). The Ca and Mg are equally effective ions for alleviation of Al rhizotoxicity in soybean (Mechanism I) when added in mM concentrations (Silva et al., 2001b). The differences between Ca and Mg in ameliorative effects are mainly attributable to Mechanism III (Kinraide et al., 2004). In our study, because the Mg concentration in the soil solution concentration was adjusted to μM

levels, ameliorative effects of Mg on Al-rhizotoxicity would rely theoretically on Mechanism III rather than Mechanism I.

In hydroponic studies, ameliorative responses of Mg to soybean rhizotoxicity occur in the μM concentration range where Mechanism I for the Al-detoxification is effective (Silva et al., 2001b). Evidence for the role of Mg indicated a physiological ameliorative effect that involves increased production and exudation of citrate in the root tips, leading to external complexation of toxic Al species in solution (Silva et al., 2001c; Yang et al., 2000). As opposed to hydroponic experimentations, our study showed that addition of μM levels of Mg in the soil solution did not improve subsoil root growth. Our findings suggest that the addition of μM Mg in the soil solution may be ineffective in amelioration of Al rhizotoxicity by inducing physiological defense mechanism such as citrate secretion from the root tip.

We hypothesized that the lack of improvement in root growth of the Mg-treated subsoils could be associated with soil mineralogical and biological effects, which may potentially reduce the availability of root-secreted citrate to form non-toxic Al complexes. As suggested by our citrate adsorption and biodegradation study (Figs. 7 and 8), the abundance of clay adsorption sites could be a primary factor lowering the citrate concentration in the soil solution, as evidenced by a negligible amount of citrate detected in the Cecil subsoil. When the soil has a sandy texture (low clay content) such as the Creedmoor and Norfolk subsoils, microbial activity and clay mineral type (Al and Fe hydroxides) play important roles in controlling soil solution citrate concentration. Van Hee et al. (2002) found that oxalate mineralization in a organic soil occurred at approximately 4-fold the rate of that in a soil with low organic content, indicating that the organic soil with high microbial activity can generally increase the decomposition rate of an organic acid in the soil solution. The acidic

subsoils examined in our root growth study are classified as Ultisols, that are generally characterized as a moderately weathered soil enriched with Al and Fe hydroxide minerals (Buol et al., 1997). Chemisorption of citrate by Al and Fe hydroxides has shown that organic acid sorption is primarily pH dependent, with increasing sorption capacity in an acidic soil (Jones and Brassington, 1998; Karlum, 1998). Therefore, adsorption (chemisorption) of citrate can be an important phenomenon especially in acid Ultisols with abundant Al and Fe hydroxide minerals commonly found in the eastern U.S. Our study suggests that the possible fates of root-secreted citrate into acid soils, including mineralogical chemisorption and biological degradation, are not reproducible in a hydroponic system. This could explain why root growth in the subsoils treated with μM soil solution Mg concentrations was not improved as compared to the limed subsoils.

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Table 1. Exchangeable cations, Al saturation and pH of subsoils treated with different amounts of Mg and lime after harvesting soybean.

Soil	Treatment	Exchangeable cation					Al saturation	pH _(water)
		Ca	Mg	K	Al	ECEC		
		cmol _c kg ⁻¹					%	
Cecil	Mg100	0.52	0.46	0.28	0.46	1.72	27	4.4
	Mg150	0.51	0.45	0.28	0.46	1.70	27	4.5
	Mg300	0.49	0.52	0.25	0.46	1.72	27	4.5
	Lime	2.32	0.38	0.22	0.05	2.97	2	5.9
	Mean	0.96	0.45	0.25	0.35	2.03	20	4.8
Creedmore	Mg50	0.08	0.08	0.09	1.22	1.47	83	4.3
	Mg150	0.08	0.08	0.09	1.14	1.39	82	4.4
	Mg300	0.07	0.09	0.07	1.15	1.38	83	4.4
	Lime	3.18	0.07	0.08	0.18	3.51	5	5.6
	Mean	0.85	0.11	0.08	0.92	1.97	61	4.7
Norfolk	Mg50	0.07	0.01	0.06	0.22	0.36	61	4.6
	Mg150	0.07	0.02	0.05	0.21	0.35	60	4.6
	Mg300	0.07	0.02	0.05	0.19	0.33	58	4.8
	Lime	0.56	0.01	0.04	0.04	0.65	6	5.7
	Mean	0.19	0.02	0.05	0.16	0.42	45	4.9
		Treatment means						
	Mg50/100	0.22	0.20	0.14	0.63	1.19	56	4.4
	Mg150	0.22	0.19	0.13	0.60	1.16	55	4.6
	Mg300	0.21	0.22	0.12	0.59	1.16	54	4.5
	Lime	2.01	0.16	0.11	0.08	2.38	4	5.7
LSD _{0.05}								
	Soil	0.03	0.01	0.01	0.03	0.06	1	0.04
	Treatment	0.03	0.02	0.01	0.04	0.07	1	0.05
	Soil x Treatment	0.07	0.04	0.02	0.08	0.12	2	0.10

LSD_{0.05}: Fisher's least significant difference (LSD) values of means determined at $p = 0.05$. Statistical differences for mean values of each parameter were found within soils or treatments at $p < 0.01$.

Table 2. Crystalline and poorly-crystalline Al and Fe in the subsoils used for the soybean root growth study.

Soil	Crystalline and poorly crystalline Fe and Al				
	Fe-ox	Fe-CBD	Fe _o /Fe _c	Al-ox	Al-CBD
	mg kg ⁻¹			mg kg ⁻¹	
Cecil	59	1853	0.03	105	303
Creedmore	33	112	0.30	41	61
Norfolk	14	113	0.12	31	61

Fe-ox, Al-ox: oxalate extractable Fe and Al, respectively.

Fe-CBD, Al-CBD: citrate-bicarbonate-dithionite extractable Fe and Al, respectively.

Fe_o/Fe_c: ratio of oxalate extractable Fe-ox to Fe-CBD.

Table 3. Regression equations and regression coefficients (R^2) for relative root length of soybean roots in the subsoils (y) as a function of exchangeable cations (x).

Predictor (x)	Regression equation	R^2
Al	$y = 100 - 59.4x^{1.2}$	0.92
Ca	$y = 41.3 + 59.7(1 - e^{-3.1x})$	0.51
Mg	-	-
K	-	-
Al saturation	$y = 100 + 0.21x + 0.01x^2$	0.87
Al / Ca	$y = 89.7 - 0.34x^{1.8}$	0.87
Al / Mg	$y = 95.1 + 6.1x^{0.6}$	0.27
Al / (Ca + Mg)	$y = 96.4 - 7.6x^{1.1}$	0.91

Al saturation: exchangeable Al expressed as a percent of sum of exchangeable Al, Ca, K and Mg.

Relative root length was unable to be regressed by exchangeable Mg and K with any functional equations.

Table 4. Citrate adsorption isotherms of subsoils predicted by the Freundlich equation and regression coefficients (R^2) as shown in Fig. 7(a).

Subsoil	Freundlich equation	R^2
Cecil	$x = 18.6c^{\frac{1}{2.9}}$	0.91
Creedmoor	$x = 9.1c^{\frac{1}{0.3}}$	0.93
Norfolk	$x = 15.1c^{\frac{1}{0.7}}$	0.96

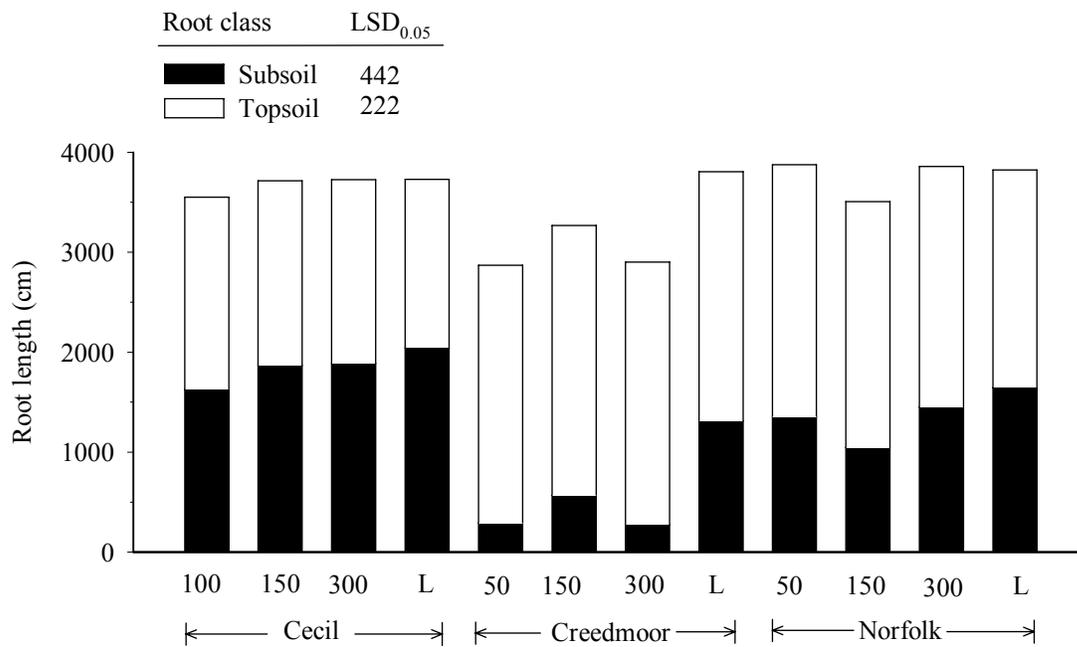


Fig. 1. Root length in the limed and fertilized compartment, and the subsurface compartment (Cecil, Creedmoor and Norfolk subsoils) with either native soil Mg levels (50 or 100 μM), treated with MgCl_2 solution to achieve 150 and 300 μM Mg in the soil solution, or limed with CaCO_3 to neutralize exchangeable Al. The least significant difference (LSD) for each mean represents the soil x amendment interaction which was significant at $p = 0.05$.

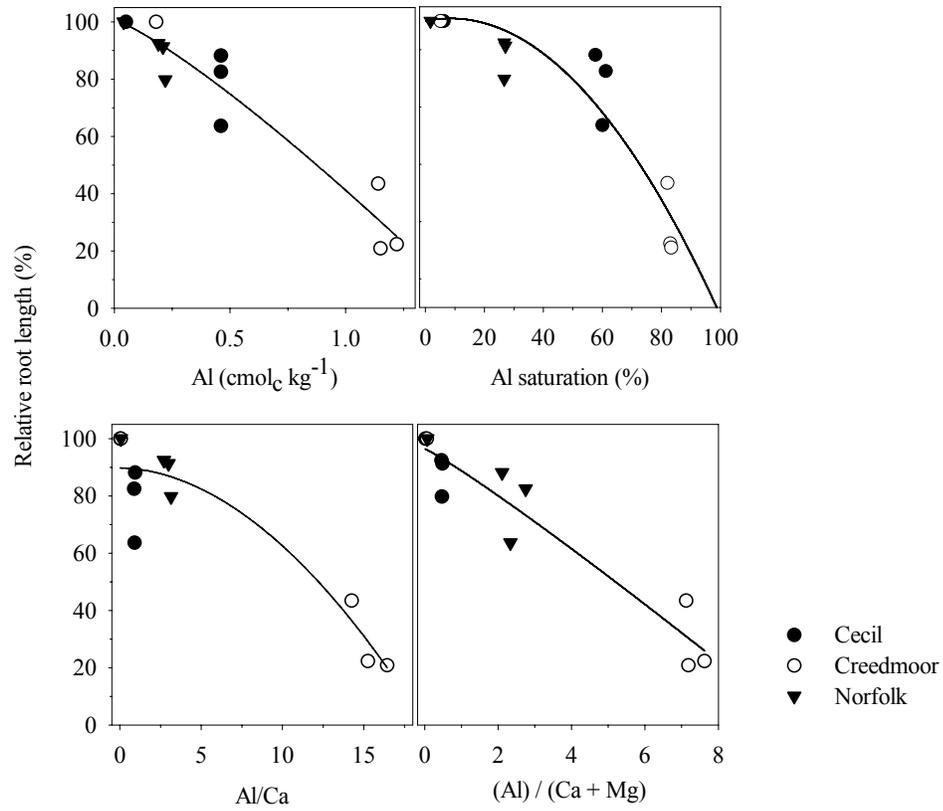


Fig. 2. Observed (symbols) and predicted (lines) relative root length of soybean grown in three subsoils treated with different Mg concentrations expressed as a function of parameters associated with soil exchangeable cations. Relative root length is expressed as a percentage of the root length in the lime treatment of each soil (100% = lime treatment).

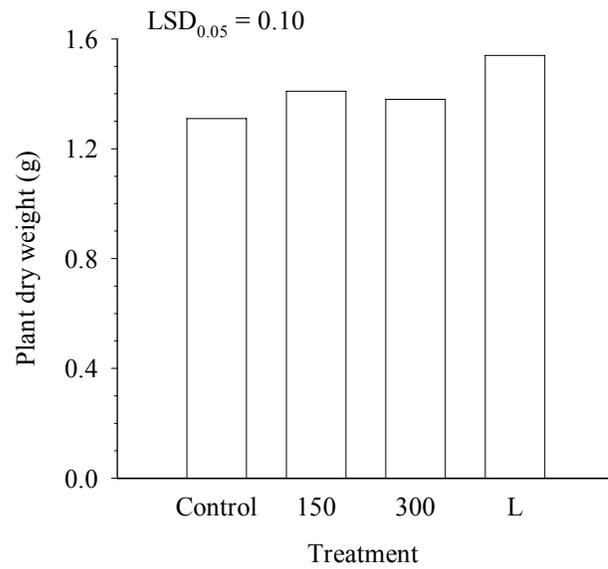


Fig. 3. Plant dry weight averaged across the soil treatments (Cecil, Creedmoor and Norfolk) for each soil amendment, Mg50 and Mg100 (control), Mg150 (150), Mg300 (300), and lime (L). The least significant difference (LSD) for each mean was computed for the significant treatment effect at $p = 0.05$.

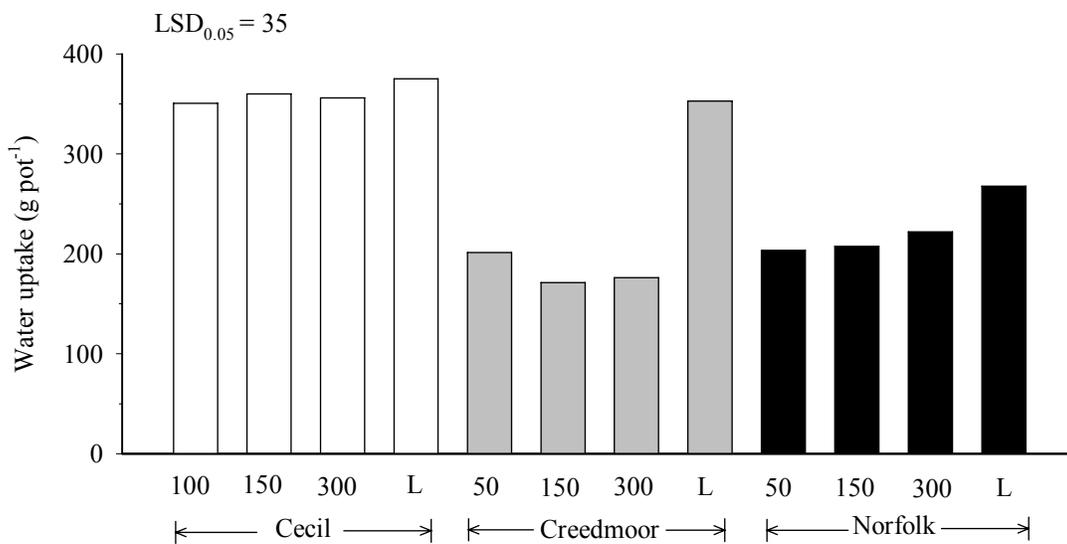


Fig. 4. Plant water uptake from the subsoils (Cecil, Creedmoor and Norfolk) treated with Mg50 and 100 (control: 50 or 100), Mg150 (150), Mg300 (300) and lime (L). The least significant difference (LSD) for each mean was computed for the significant soil x amendment interaction at $p = 0.05$.

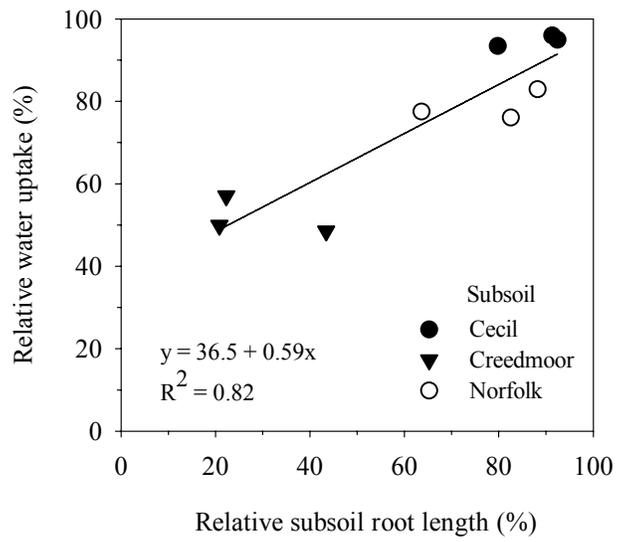


Fig. 5. Observed (symbols) and predicted (line) relative plant water uptake from the subsoil as a function of relative subsoil root length. Both relative values are expressed as % of limed treatments.

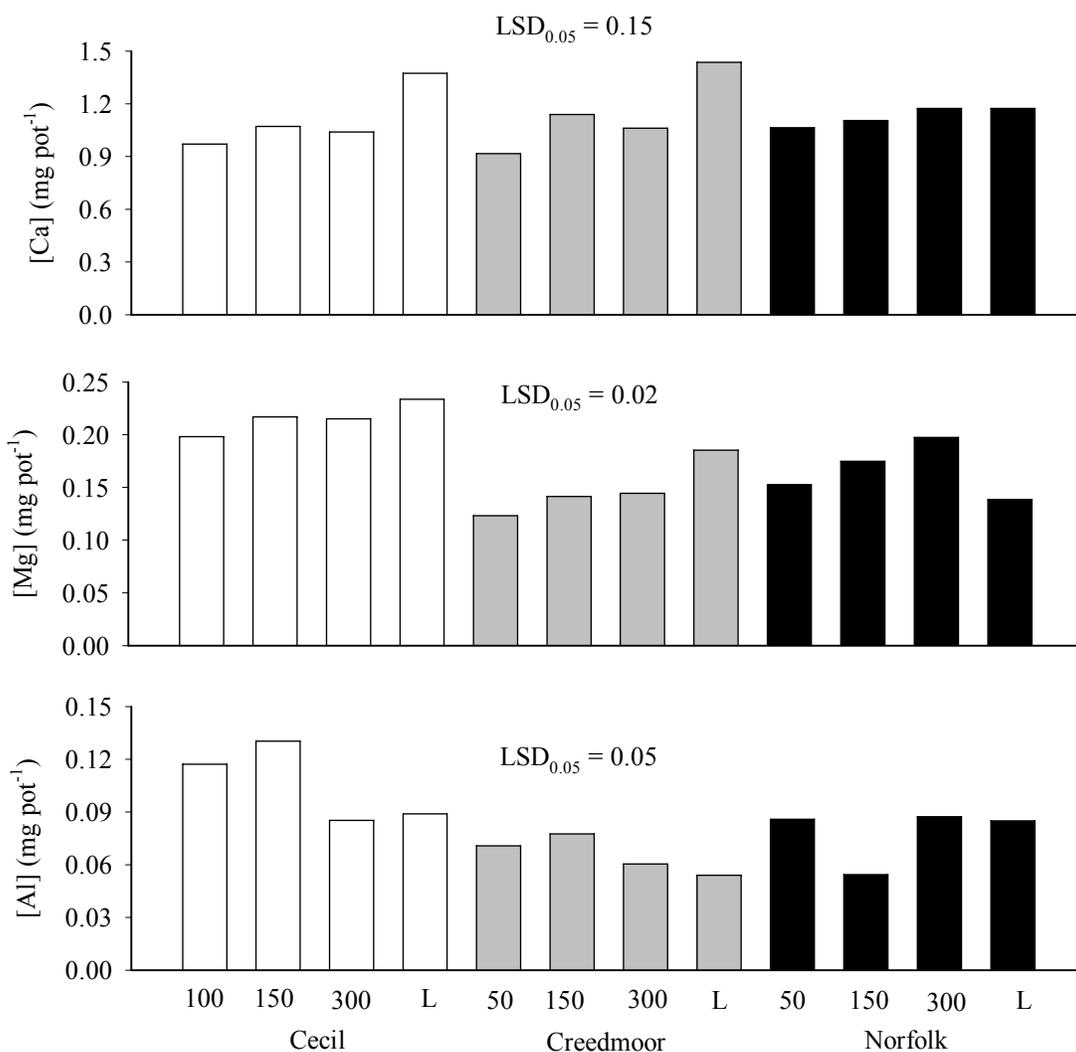


Fig. 6. Cation accumulation (Al, Ca, Mg) in aboveground plant tissues for the subsoils (Cecil, Creedmoor and Norfolk) treated with Mg50 and 100 (control: 50 or 100), Mg150 (150), Mg300 (300) and lime (L). The least significant difference (LSD) for each mean was computed for the soil x amendment interaction at $p = 0.05$.

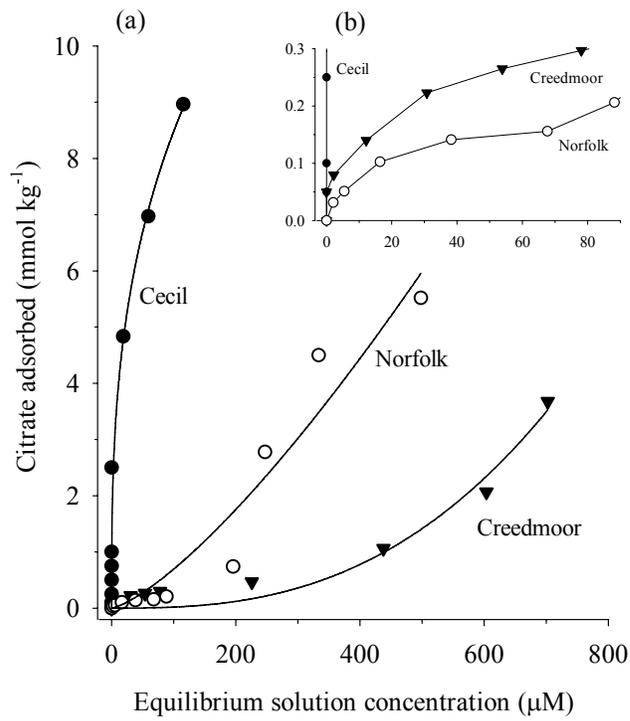


Fig. 7. Concentration-dependent adsorption isotherm of citrate for the Cecil, Creedmoor and Norfolk subsoils (Mg50/100 treatment) determined at a solution pH value of 4.5 with microbial inhibition by Na-azide additions. Citrate adsorption isotherms of all subsoils were predicted by the Freundlich equation (lines). A magnified figure shows a scale from 0 to 90 μM citrate solution concentration..

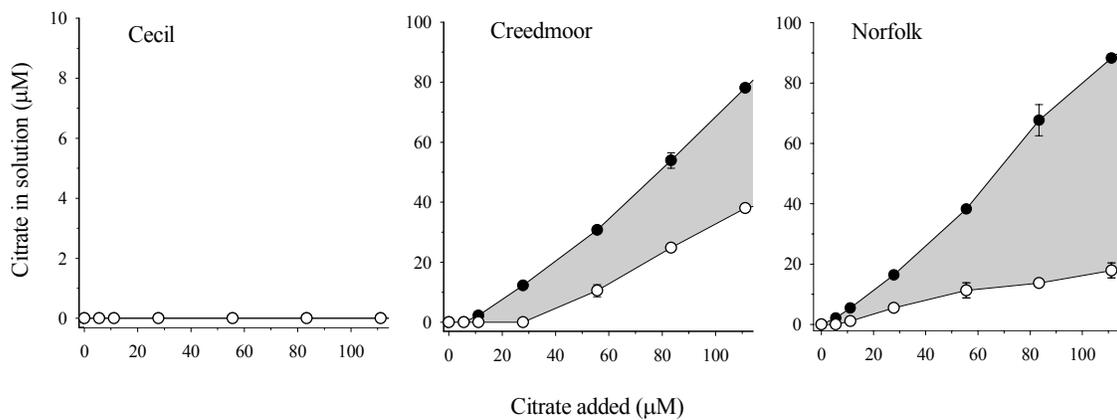


Fig. 8. Citrate concentration in solution after 24 hour equilibrium as influenced by increased concentration of citrate additions with (filled symbols) or without (open symbols) Na-azide additions for the Cecil, Creedmoor and Norfolk subsoils (Mg50/100 treatment) at a solution pH value of 4.5. Data of Na-azide additions (filled symbols) for the Cecil subsoil were not presented because these are equal to the values that were obtained in the treatment without Na-azide additions. The shaded area in the plot (Creedmoor and Norfolk) indicates the amount of biodegraded citrate.

CHAPTER 4

Soybean Root Growth in Relation to Soil Solution Ionic Activities in Magnesium-amended Acid Subsoils

ABSTRACT

Hydroponic studies with soybean [*Glycine max* (L.) Merr.] have shown that μM additions of Mg^{2+} were as effective in ameliorating Al rhizotoxicity as additions of Ca^{2+} in the mM concentration range. The objectives of this study were to assess ameliorative effects of Mg on soybean root growth in acidic subsoils and to relate the soil solution ionic compositions to soybean root growth. Roots of soybean cultivar Plant Introduction 416937 extending from a limed surface soil compartment grew for 28 days into a subsurface compartment containing acid subsoils from the Cecil (oxidic and kaolinitic), Creedmoor (montmorillonitic) and Norfolk (kaolinitic) series. The three Mg treatments consisted of the native equilibrium soil solution concentrations in each soil (50 or 100 μM) and MgCl_2 additions to achieve 150 and 300 μM Mg (Mg150, Mg300, respectively) in the soil solutions. Root elongations into Mg-treated subsoils were compared with a CaCO_3 treatment limed to achieve a soil pH value of 6. Subsoil root growth responses to the Mg treatments were less than for the lime treatments. Root length relative to the limed treatments for all subsoils (RRL) was poorly related to the activity of soil solution Al species (Al^{3+} and Al-hydroxyl species) and Mg^{2+} . However, the RRL values were more closely related to the parameters associated with soil solution Ca activity including (Ca^{2+}) , $(\text{Al}^{3+})/(\text{Ca}^{2+})$, and $(\text{Al}^{3+})/[(\text{Ca}^{2+}) + (\text{Mg}^{2+})]$, suggesting that Ca could be a primary factor ameliorating Al and H^+ rhizotoxicity in these subsoils. Increased tolerance to Al rhizotoxicity of soybean by μM Mg additions to

hydroponic solutions, inducing citrate secretion from roots to externally complex toxic Al species, may be less important in the acid subsoil with low native Ca levels.

INTRODUCTION

Toxic effects of Al on root growth are primarily influenced by the aqueous Al species that are dependent on the solution pH and complexing ligands. Among the mononuclear Al-hydroxyl species, trivalent Al (Al^{3+}) is a predominant species in most soils with solution pH values below 5 (Lindsay, 1979) and is highly correlated with rhizotoxicity (Kinraide, 1997; Kinraide and Parker, 1989; Parker et al., 1988). Aluminum species complexing with ligands are less rhizotoxic than Al^{3+} . Fluoride forms strong complexes with Al and is not toxic to barley and wheat (Cameron et al., 1986; Parker et al., 1989). Sulfate-complexed Al is also less toxic to root growth of barley and mungbean as compared to Al^{3+} (Cameron et al., 1986; Menzies et al., 1994). Root growth of wheat and red clover was insensitive to increased activity of AlSO_4^+ species whereas exposure to a solution with increased Al^{3+} activity decreased root growth (Parker et al., 1989). Aluminum complexed with organic acids such as fulvate, humate and citrate is considered non-rhizotoxic or significantly less toxic than Al^{3+} (Bessho and Bell, 1992; Hue et al., 1986; Shamshuddin et al., 2004).

Plants can avoid Al toxicity by secreting organic acids from roots in response to Al stress. Zhao et al. (2003) reported a positive correlation between the concentration of organic acid secreted from the root and relative root elongation in nine *Poaceae* plants exposed to a solution with 30 μM Al. Organic acids secreted from roots under Al stress are primarily citrate and malate (Delhaize et al., 2001; Li et al., 2000; Miyasaka and Hawes, 2001), although the species and their concentrations differ among the plant species. For soybean,

citrate has been reported to be secreted from roots in response to Al stress (Yang et al., 2000; Yang et al., 2001). Aluminum tolerance of soybean by citrate secretion from roots is enhanced by addition of micromolar Mg^{2+} to hydroponic solutions (Silva et al., 2001b; Silva et al., 2001c). The physiological mechanistic roles of Mg^{2+} in inducing citrate secretions could be associated with stimulation of the entire citric acid cycle and/or citrate release by regulating the putative citrate channel that may be modulated by Mg-dependent G-proteins.

The objectives of this study were to assess ameliorative effects of Mg on soybean root growth in acidic subsoils and to relate the soil solution ionic compositions to soybean root growth.

MATERIALS AND METHODS

Greenhouse Experiment

A greenhouse experiment was conducted at North Carolina State University, Raleigh, NC from June to July 2005. Soybean [*Glycine max* (L.) Merr. cv Plant Introduction 416937] was grown with a modified vertical-sprir root system described by Sanzonowicz et al. (1998a). A plastic cylinder with 10 cm diameter and 52 cm length is divided into two vertical compartments separated by with a root permeable membrane. The surface 12-cm compartment was filled with 1.25 kg of limed and fertilized Wagram soil (loamy, siliceous, thermic, Arenic Kandiuldults). Thus, roots in the surface compartment grow into a media with no Al constraint before extending into the acidic subsoil media.

The subsurface compartment was filled with acid subsoil samples from soil series of three NC Ultisols: Cecil (fine, kaolinitic, thermic Typic Kanhapludults), Creedmoor (fine, mixed, semiactive, thermic Aquic Hapludults) and Norfolk (fine-loamy, kaolinitic, thermic

Typic Kandiudults). Each subsoil was amended with different levels of MgCl_2 or lime. The three Mg treatments consisted of the native equilibrium soil solution concentrations in each soil [50 μM Mg (Mg50) for Creedmoor and Norfolk, and 100 μM Mg (Mg100) for Cecil] and MgCl_2 additions to achieve 150 and 300 μM Mg (Mg150, Mg300, respectively) in the soil solutions. A subsoil treatment with CaCO_3 added to raise soil pH to a value of 6 was also included to compare root elongation response with the Mg treatments. Water and Mg solutions were added to each soil seven days before the transplanting seedlings to allow the soil solution ionic compositions to equilibrate.

Experimental treatments were arranged in a randomized complete block design with six replicates. Three of the six replicates were prepared for obtaining soil samples for physical and chemical analyses at the end of the experiment, and the other three replicates were used to determine root length after wet-sieve separation from soil. The experimental design was a factorial arrangement of 3 subsoils and 4 amendments.

Five pre-germinated seedlings were initially transplanted to the surface compartment soil whose water content was adjusted to 90% container capacity. Seedlings were thinned to two after 5 days. After 28 days, roots were separated from the surface and subsurface soils using a 0.5 mm wet sieve. Root length was determined by an edge discrimination method (Pan and Bolton, 1991) using a desktop scanner preset to a resolution of 29.5 dots cm^{-1} (75 dots inch^{-1}).

Soil and Soil Solution Analyses

Soil samples were air-dried and sieved to 2 mm prior to physical and chemical characterization. Soil texture was determined by a hydrometer, and organic matter (OM)

content was determined by a loss-on-ignition method (Nelson and Sommers, 1996). Soil pH was measured at a 1:2.5 soil-water ratio. Exchangeable Al, Ca and Mg were extracted with 1M KCl solution and exchangeable K was extracted with the Mehlich 3 solution (Mehlich, 1984). All exchangeable cations were determined by atomic absorption spectrometry.

Soil solution from subsoil container was extracted by a modified centrifugation method described by Jallah (1994). Deionized water (18 mmho cm^{-1}) was added to the 40 mL centrifuge tube filled with 25 g of the Cecil subsoil and 35 g of the Creedmoor and Norfolk subsoils to achieve container capacity, and soils were incubated at 25 °C for 40 hours. Soil solution was extracted by centrifugation at 7000 rpm for 10 minutes. Soil solution extracted from 16-20 centrifuge tubes were pooled for each soil treatment replicate and filtered through 0.45 μm membrane filters. Each sample was stored in glass vials for the analysis of pH, EC and dissolved organic carbon (DOC), and in polypropylene plastic containers for the analysis of cations and anions. Electrical conductivity of the soil solution was measured by a temperature compensated conductance meter. Soil solution cations (Al, Ca, Cu, Fe, K, Mg, Mn, Na, Zn) were determined by inductively couple plasma optima emission spectrometry (ICP-OES) and anions (Cl, F, NO_3 , PO_4 , SO_4) by ion chromatography. Dissolved organic carbon (DOC) was determined by an organic carbon analyzer.

Statistical Analysis and Solution Al speciation

Multiple linear regression models were developed to predict soil solution ionic concentrations (Al, Ca, K and Mg) as a function of their soil exchangeable phase and soil solution EC or pH. Based on a predictive model proposed by Benians (1985), a modified multiple linear regression model was developed as follows:

$$\ln M_c = a + b \ln EC + cpH + \ln M_e ,$$

where M_c is the cation concentration in the soil solution, and M_e is the exchangeable cation or cation saturation (exchangeable cation expressed as a percent of sum of all exchangeable cations). Predictive models were developed by using means of each parameter. Multiple linear regression was performed by using the stepwise and backward procedures programmed by SAS[®], Version 9.1 (SAS Institutes, Cary, NC). Based on the statistical analysis performed at $\alpha = 0.15$ probability level, the predicted models with regression coefficient (R^2) of the model, and partial regression coefficients (pR^2) of each parameter were reported. Additionally, the correlation procedure was performed to compute the correlation between cations on soil solution and exchangeable phases.

Ionic compositions and pH of soil solution were used to predict Al speciation with the program, Visual MINTEQ, ver. 2.30 (KTH, Stockholm, Sweden). Thermodynamic reactions and equilibrium constants ($\log K$) at 25 °C and zero ionic strength used for the aqueous speciation analysis are summarized in Table 1. Because all subsoils had negligible F and PO₄ in the soil solution, Al complexes with these ligands were excluded from the speciation model. Assumptions for the speciation analysis were exclusion of precipitation, and an open system with fixed pH and pCO₂ (0.0035 atm) at 10 times the atmospheric concentration (Alexander, 1977). Complexation of DOC with Al was based on the NICA-Donnan Model provided in the program, which assumes that 70% of the DOC is comprised of fulvic acid with 50% carbon content.

To allow the assessment of diagnostic indices among the subsoils, the subsoil root length was expressed as a percentage of the root length in the lime treatment of the same subsoil (relative root length). Regression equations were evaluated for relative root length

expressed as a function of soil solution ionic activity.

RESULTS AND DISCUSSION

Soil and Soil Solution Properties

All subsoils without Mg or lime additions were characterized as acidic soil with pH values below 4.6 (Table 2). The Creedmoor subsoil had the highest exchangeable Al and Al saturation values, suggesting the strongest acidity constraints to root elongation among the three subsoils. Exchangeable Ca, Mg and K were low ($< 0.1 \text{ cmol}_c \text{ kg}^{-1}$) in the Creedmoor and Norfolk subsoils as compared to the Cecil subsoil. The Creedmoor and Norfolk subsoils had a sandy loam texture with 1.9 and 1.5% OM, respectively. The Cecil subsoil had a clay texture with 11.4% OM.

Exchangeable Mg of the Creedmoor and Norfolk subsoils was not different among the treatments ($p > 0.05$), whereas the Mg300 treatment of the Cecil subsoil presented a higher value than other treatments ($p < 0.05$). There were no differences in exchangeable Al among Mg-amended treatments (Mg150 and 300) as compared to the treatment without added Mg (Mg50/100) for all subsoils ($p > 0.05$). The absence of changes in exchangeable Al and Mg between treatments with or without Mg amendments indicates that additions of μM Mg did not influence the exchangeable ionic phases of soil Al and Mg. Although exchangeable Mg and soil pH values tended to increase slightly with 150 and 300 μM Mg in all subsoils ($p < 0.05$), there were no changes in Al saturation as compared to the Mg50/100 treatments ($p > 0.05$), except a slight decrease in the Mg300 treatment of the Norfolk subsoil. Liming of all subsoils reduced Al saturation and raised exchangeable Ca and pH values above 5.5 where concentrations of the rhizotoxic Al^{3+} species in the soil solution would be

nil (Kamprath and Smyth, 2005).

The soil solution ionic composition of all subsoils was dominated by Al, Ca, K, Mg, SO₄ and DOC (Table 3). Soil solution Al concentrations of the Cecil and Norfolk subsoils were not different among the treatments ($p > 0.05$). The soil solution collected from the Creedmoor subsoil had the highest Al concentration in the Mg300 treatment, followed by Mg150, Mg50 and lime treatments ($p < 0.05$). The order of soil solution Al concentration in the Mg50/100 treatments found among the subsoils (Cecil < Creedmoor < Norfolk) did not correspond to the values of soil exchangeable Al (Norfolk < Cecil < Creedmoor). Relative to the Mg50/100 treatments of each subsoil, Al concentration of the limed treatment decreased for the Creedmoor ($p < 0.05$) and was not different from the Cecil subsoils ($p > 0.05$). For the limed Norfolk subsoil, Al concentrations in the soil solution remained high, although exchangeable Al was not determined in the limed treatment (Table 2), indicating a presence of weak forms of hydroxy-Al species (Sparks, 1995).

The soil solution Ca concentration was not different among the Mg treatments (Mg50, 150 and 300) of the Creedmoor and Norfolk subsoils ($p > 0.05$), but the Mg300 treatment of Cecil had a higher Ca concentration than the other Mg treatments ($p < 0.05$). The lime treatment of all subsoils had the highest soil solution Ca concentration among the treatments ($p = 0.05$). Addition of micromolar Mg to all subsoils (Mg150 and 300 treatments) increased Mg concentrations in the soil solution ($p < 0.05$) despite no change in exchangeable Mg concentrations relative to the Mg50/100 treatments.

Magnesium added to the Cecil subsoil was mostly retained on the exchangeable phase. To achieve 300 μM Mg in the soil solution of the Cecil subsoil, for example, the required amount of Mg (911 $\mu\text{mol kg}^{-1}$) was approximately 8-fold more than that of the Creedmoor

and Norfolk subsoils (Fig. 2 in CHAPTER 2). The Cecil subsoil with a clayey texture had a high affinity for Mg, which can also be explained by the increased exchangeable Mg of the Mg300 treatment ($p < 0.05$; Table 1). In contrast, most of Mg added to the sandy Creedmoor and Norfolk subsoils remained in the soil solution rather than on the exchangeable complex (Fig. 2 in CHAPTER 2).

Although the required amount of Mg to achieve three levels of soil solution Mg concentrations (Mg50/100, 150 and 300 μM) was previously determined by the laboratory incubation study, the soil solution ionic compositions determined after soybean harvest did not correspond to the predetermined treatment values. Soil, root and microbial processes constant moist conditions during plant growth might have influenced the native soil Mg solubility and Mg adsorption/desorption properties, resulting in an inconsistency between the values determined at pre- and post-cultivation.

The soil solution SO_4 concentration of all subsoil treatments showed a similar trend as found in the soil solution Ca concentration. The concentrations of SO_4 in the soil solution of all subsoils increased in the lime treatment, as was also found by Jallah (1994), resulting from anionic desorption with increasing pH and/or $\text{HCO}_3\text{-SO}_4$ competition. Although the soil solution pH value tended to decrease in Mg150 and 300 treatments, there were no differences in the pH values among the Mg treatments ($p > 0.05$) of all subsoils, except for the Mg300 treatment of the Creedmoor subsoil. All limed treatments had the highest pH value among the treatments within each subsoil ($p < 0.05$). Increased DOC concentration in all subsoils except for the Creedmoor subsoil treated with lime could be associated with increased solubility and desorption of SOM with increasing pH (Andersson et al., 2000; Impellitteri et al., 2002; Shen, 1999; You et al., 1999).

Cation Relations between Soil Exchangeable and Solution Phases

Soil solution chemical parameters including ionic strength and cation concentrations are often related to the EC values. A strong linear relationship ($R^2 = 0.91$) was found between ionic strength and EC (Fig. 1). The linear coefficient value ($10.5 \mu\text{M} = 0.01 \text{ mM}$) computed in this study was similar to that determined by other studies (Griffin and Jurinak, 1973; Jallah and Smyth, 1995; Ponnampetuma et al., 1966). The soil solution Ca and Mg concentrations of all subsoils combined were related to an exponential function of EC (Fig. 1). Because Ca and Mg were two major cations in the soil solutions, the variability associated with these cations was directly related to EC.

In contrast to EC, the exchangeable cationic phase and soil solution pH were poorly correlated with the soil solution cation concentrations. For example, a relatively weak correlation was found between pH and soil solution Al ($r = 0.12$), Mg ($r = -0.25$) and K ($r = -0.54$). Similar to EC, the soil solution Ca concentration was correlated with pH ($r = 0.70$). The soil solution Al and Mg were poorly correlated with their exchangeable phase ($r < 0.53$). However, the soil solution Al was related to Al saturation of the ECEC ($R^2 = 0.69$) that is a widely used index for estimating a potential Al toxicity (Kamprath, 1984). Although the soil solution Ca and K concentrations showed a relatively high correlation with their exchangeable phases ($r > 0.63$), unique regression relationships were not found between these variables. The difficulties of regressing the soil solution cation concentrations only by parameterizing their exchangeable phases have also been reported in previous studies (Benians, 1985; Jallah and Smyth, 1995)

The soil solution cations can be predicted by a multiple linear regression parameterized by their exchangeable attributes and EC or pH. The soil solution Al

concentration was best regressed by Al saturation and pH values ($R^2 = 0.69$), but the EC value was not statistically significant as a variable for this model (Table 4). The increase of EC, and therefore ionic strength, should be accompanied with increased Al concentration in the soil solution. Liming will decrease Al solubility in the soil solution by increasing pH. Although the EC value increases, this would result in an inconsistent trend for predicting the soil solution Al concentration. The Al saturation value was also a better predictor for the soil solution Al concentration than exchangeable Al (Jallah and Smyth, 1995).

The soil solution Ca concentration was best regressed by exchangeable Ca, pH and EC values ($R^2 = 0.95$). As compared to Ca, the soil solution K and Mg showed a lower value of R^2 and were significantly regressed only by their exchangeable attribute (Table 4). Although EC has often been employed as an important predictor for the soil solution cations (Benians, 1985; Gilliam and Summer, 1987; Pearson, 1971), our study showed that EC was an insignificant variable for predicting K and Mg concentrations in the soil solution. The reduced predictability of soil solution Mg and K as compared to Ca was also reported by Jallah and Smyth (1995). Unlike Ca, the increase of soil solution K and Mg was not accompanied with increased EC among the treatments, which may result in less contribution of EC on predicting K and Mg concentrations in the soil solution.

Chemical Speciation and Activity of Soil Solution Aluminum

Over 75% of Al in the soil solution of all subsoils was predicted as organically complexed forms including organic- and colloidal-Al species (Fig. 2). In the Mg50/100 treatments of all subsoils, the Al^{3+} speciation comprised 1% for the Creedmoor (0.9 μM) and Norfolk (1.1 μM) subsoils and 4% (0.1 μM) of the total solution Al species for the Cecil

subsoil. The Mg300 treatment for all subsoils tended to have a higher proportion of Al^{3+} in the soil solution, which may be associated with increased soil solution Al concentration and decreased soil solution pH values as compared to the Mg50/100 treatments (Table 3).

Aluminum hydroxyl species were scarcely found in most of the soil solutions except for the lime treatment of Norfolk subsoil whose soil solution was predicted to contain approximately 4% of Al-hydroxyl species due to the pH rise. The AlSO_4^+ was typically found in the soil solution of Creedmoor and Norfolk subsoils. In contrast, less than 1.8% of AlSO_4^+ was found in the soil solutions of the Cecil subsoil because of a high concentration of DOC relative to SO_4 . The DOC has a strong affinity for Al in solution, which leads a preferential formation of Al-DOC complexes (David and Driscoll, 1984; Nordstrom and May, 1996).

Molar activity of free ions (Al^{3+} , Ca^{2+} , Mg^{2+}) in the soil solution was related to their ionic concentrations (Table 5). In the Mg50/100 treatment, the Creedmoor and Norfolk subsoils had a similar Al^{3+} activity, but the Cecil subsoils had 1 order lower magnitude of Al^{3+} activity than other subsoils. The Al^{3+} activity tended to increase with Mg additions in all subsoils, and it increased by over 10-fold in the Mg300 treatment of all subsoils as compared to those of Mg50/100 treatments. In contrast to other subsoils, the limed treatment of the Norfolk subsoil had a higher Al^{3+} activity than that of other subsoils, resulting from the increased Al concentration in the soil solution (Table 3). Both Ca^{2+} and Mg^{2+} activities increased with Mg additions in all subsoils. The $(\text{Ca}^{2+})/(\text{Al}^{3+})$ molar activity ratio exceeded 5000 in all subsoils. Ferrufino et al. (2000) found that Al rhizotoxicity for the soybean cultivars (N93, PI, Ransom and Young) was nil when the $(\text{Ca}^{2+})/(\text{Al}^{3+})$ molar activity of hydroponics solutions exceeded 3000. Ionic strength of soil solutions from all subsoils tended to increase with Mg and lime amendments.

Root Growth Relations with Aluminum Speciation and Activity

The relative root length in the Mg treatments differed among the subsoils (Fig. 3). In the Mg50/100 treatments, the relative root length of the Creedmoor subsoil corresponded to 22% of the lime treatment, whereas the relative root length of the Cecil and Norfolk subsoils was 79 and 82% of their respective lime treatment. The relative root length response to applied Mg was not consistent among the subsoils. In the Cecil subsoil, the relative root length increased up to 92% of the lime treatment as the Mg application increased. In the Mg150 treatment, the relative root length of the Creedmoor and Norfolk subsoils increased or decreased as compared to their Mg50 treatments, respectively. To understand the treatment effects on root growth, the relative root length of subsoils was related to soil solution ionic compositions.

Relative root length of all subsoils was poorly related to the soil solution pH or ionic strength ($R^2 < 0.32$). Several studies have also reported that the soil solution pH was not related to root growth in acidic soils (Adams and Lund, 1966; Evans and Kamprath, 1970), whereas Bruce et al. (1988) found a close relationship between soil solution pH and cotton root growth in highly weathered soils. Increased soil solution ionic strength, which leads to the reduction of Al^{3+} activity, is often described as a major mechanism for the alleviation of Al rhizotoxicity. The soil solution ionic strength tended to increase with the Mg application rate and approached values of the lime treatment in the Mg300 treatment in the Creedmoor and Norfolk subsoils (Table 5), but their relative root length was not improved as compared to the Mg50/100 treatments. Our result suggests that improved root length in acid soils cannot be always explained by the increased soil solution ionic strength.

Phytotoxicity of metals is often related to activity of ionic species rather than concentration (e.g. Allen and Hansen, 1996). Our predictive models for relative root length determined higher R^2 values when regressed by activity of ionic species than by concentration; therefore, the latter will not be reported herein. Root growth under the acidic soil constraints is often predicted by the function of Al^{3+} activity in the soil solution because Al^{3+} is most rhizotoxic (Kinraide, 1997; Kinraide and Parker, 1989; Kochian, 1995; Parker et al., 1988) and the predominant species in the acid solution pH (Lindsay, 1979). In our study, however, the relative root length was not clearly related to the activity of soil solution Al^{3+} and other Al species (Table 6). In the Cecil and Norfolk subsoils, the greatest value of relative root length was found in the Mg300 treatment, but Al^{3+} concentration and activity increased over 10-fold more than their Mg50/100 treatments. In the Mg150 treatment of Creedmoor subsoil, the relative root length was improved as compared to the Mg50 treatment although the Al^{3+} concentration and activity were over 7-fold more than the Mg50 treatment. The relative root length of all subsoils combined was poorly regressed by the activity of soil solution Al^{3+} ($R^2 = 0.30$). Similarly, regressions for the relative root length of all subsoils on the activity of other Al species such as $AlOH^+$, $AlSO_4^+$, and organically complexed Al did not account for an appreciable proportion of the variability ($R^2 < 0.29$). Unlike the clear relationships often shown in hydroponic studies assessing Al rhizotoxicity (e.g. Kinraide and Parker, 1990), poor relationships between the relative root length and toxic Al species including Al^{3+} and possibly Al hydroxyls may result from the abundance of DOC in the soil solution (occupied 75% of total Al speciation). Because the organically complexed Al controls the Al^{3+} activity of the soil solution in the acid soil (Cronan et al., 1986; de Wit et al., 1999; Mulder et al., 1989; Walker et al., 1990), soil solution with highly concentrated DOC

relative to Al concentration could mask Al^{3+} toxicity, and therefore prediction of Al rhizotoxicity was rendered more difficult by a single Al species.

Regardless of the increased Al^{3+} concentration and activity as compared to the Mg50/100 treatments, the relative root length of the Mg300 treatment was not severely decreased (not different at $p = 0.05$; Fig. 3). This is indicated by the relative root length of the Mg300 treatment of the Cecil subsoil that had 13% more than that of the Mg100 treatment. In the Mg150 and 300 treatments of all subsoils, increased concentration of Ca in the soil solution, and increased Ca^{2+} activity may be a factor mitigating the Al toxicity to root growth. This could be explained by the appreciable R^2 value of the equation for the relative root length as a function of the parameter associated with solution Ca^{2+} (Table 5). Over 60% of the variability associated with relative root length can be explained by the concentration and activity of soil solution Ca^{2+} (Fig. 4). Runge and Rode (1991) proposed that Al rhizotoxicity is more closely related to molar activity ratios including Al and Ca rather than individual ionic activities in solution. The molar activity ratios involving Al^{3+} and Ca^{2+} served as a good predictor of Al rhizotoxicity to soybean (Ferruffino et al., 2000; Sanzonowicz et al., 1998b) and wheat (Carr and Ritchie, 1993). The molar activity ratio of (Al^{3+}) to [$(\text{Ca}^{2+}) + (\text{Mg}^{2+})$] was also related to relative root length although the R^2 value was lower than that predicted by (Ca^{2+}) or (Al^{3+})/(Ca^{2+}). In our study, the molar activity ratio of (Al^{3+})/(Ca^{2+}) was most strongly related to the relative root length for all subsoils ($R^2 = 0.64$, Fig. 4) although the R^2 value was lower than that of computed by a hydroponic study with the same soybean cultivar used in our study (Ferruffino et al., 2000).

Regressions of Mg^{2+} activity in the soil solution on relative root length had a low R^2 value (Table 6), suggesting that increasing soil solution Mg^{2+} in micromolar ranges did not

relate to root growth in these acidic subsoils. A mechanistic role of micromolar Mg^{2+} in the hydroponic solution for ameliorating Al rhizotoxicity to soybean is based on enhanced citrate secretion from roots, which subsequently forms non-toxic Al-citrate complexes (Silva et al., 2001b; Silva et al., 2001c). The micromolar Mg^{2+} role for ameliorating Al rhizotoxicity could mainly be attributed by physiological effects (e.g. citrate secretion) because the micromolar Mg^{2+} in solution had little cationic effect on increasing the electrical potential and subsequently reducing Al^{3+} activity at the plasma membrane surface of root cells (Silva et al., 2001c). Our study showed that addition of micromolar Mg^{2+} in the soil solution did not change the concentrations of exchangeable Al and other cations in all subsoils, indicating that little cationic effects of Al displacement between the soil solid and solution phases occurred by the micromolar Mg^{2+} additions.

Implications on Root Citrate Ameliorating Subsoil Acidity Constraints

Citrate production in the root tip and secretion may be enhanced by micromolar additions of Mg when the soil solution has sufficient levels of Ca. Silva et al. (2001a) found that root growth was not improved with increased Mg^{2+} concentration when grown in a solution with less than $300\mu M Ca^{2+}$, suggesting that there may be an essential level for Ca^{2+} to express the protective effect of Mg (e.g. citrate secretion). Most of the previous studies reporting citrate secretion from the soybean roots exposed to toxic levels of Al were conducted in a hydroponic solution with sufficient levels of Ca^{2+} to avoid a nutrient deficiency and H^+ toxicity. For example, an Al tolerant soybean cultivar Suzunari secreted citrate from the roots when the solution contained $15\mu M Al^{3+}$ with $325\mu M Mg^{2+}$ and $500\mu M Ca^{2+}$ (Yang et al., 2001). Other studies also confirmed that the addition of $50\mu M Mg^{2+}$ to

a solution containing 800 μM Ca^{2+} induced the citrate production and secretion from root tips of soybean cultivar PI and ameliorated Al rhizotoxicity when the solution had below 2.9 μM Al^{3+} activity (Silva et al., 2001a; Silva et al., 2001b; Silva et al., 2001d). As suggested by Silva et al. (2001a), these studies detected increased root citrate production and secretion induced by micromolar Mg^{2+} in the solutions with sufficient amounts of Ca^{2+} .

In contrast to these studies, our study showed little increase of relative root length of the additions of 150 and 300 μM Mg to soil solutions where the Ca^{2+} concentration was typically low in Creedmoor and Norfolk subsoils ($< 350 \mu\text{M}$). Such solutions with low Ca^{2+} concentration relative to Al^{3+} could detrimentally affect plant root growth due to H^+ and Al^{3+} toxicities in the acidic subsoils. Sanzonowicz et al. (1998b) found that increased H^+ concentration damages root cell division, and Ca^{2+} has an important role in ameliorating H^+ toxicity. Ferrufino et al. (2000) reported that the Ca/Al^{3+} molar activity ratio of 3000 is required to ameliorate Al rhizotoxicity of soybean to achieve 90% root growth of a non Al-stressed condition at a solution pH value of 4.6. The soil solution of the Creedmoor and Norfolk subsoils for the Mg50, 150 and 300 treatments had low pH values (< 4.7) and $\text{Ca}^{2+}/\text{Al}^{3+}$ molar activity ratios (< 52), suggesting that rhizotoxicity by Al and H^+ likely occurred and therefore, inhibited root growth in these treatments as compared to the lime treatment. The micromolar Mg supply to the soil solution of these subsoils with a low Ca^{2+} did not apparently compete with H^+ and Al toxicities due possibly to damage resulting from the severe acidic constraints. As indicated by the relatively close relation between the relative root length and soil solution Ca factors (e.g. Ca^{2+} activity and $\text{Al}^{3+}/\text{Ca}^{2+}$ activity ratio), increased Ca concentration in the soil solution is important to improve a root growth under the constraints of acidic subsoil with a low Ca concentration.

Although the mechanism of Al tolerance has often been explained by chelation and exclusion of extracellular Al by root organic acid exudation (e.g. Kochian et al., 2004; Ma, 2000; Ma et al., 2001), this ameliorative mechanism may not be effective in acid soils as compared to hydroponic solutions. There are possible alternative fates of root exuded citrate in the soil. Several studies have reported that apparent Al tolerance with specific plant genotypes in hydroponics does not always relate to better growth and yield in acidic and Al toxic soils. For example, a soybean cultivar PI 416937 that showed one of the highest rates of root citrate production and secretion among the 8 soybean cultivars tested (Silva et al., 2001d) has been identified as a highly Al tolerant cultivar in hydroponic screenings (Bianchi-Hall et al., 1998; Campbell and Carter, 1990). However, bioassay of the PI 416937 in field or greenhouse pot studies has found a lower potential of Al tolerance than that found in hydroponic examinations (Bushamuka and Zobel, 1998; Hanson and Kamprath, 1979; Ritchey and Carter, 1993). Villagarcia et al. (2001) reported that examination of PI 416937 in a sandculture media showed less root length than the cultivar Perry that showed approximately 50% root tip citrate concentration of PI 416937 (Silva et al., 2001d). These inconsistencies between root growth and root citrate production demonstrated by soil and hydroponic examinations suggest that root citrate production and secretion may not be a primary factor ameliorating Al rhizotoxicity in acid subsoils. A possible reason for inconsistent results between hydroponic and soil studies is that availability of root-secreted citrate to chelate Al is considerably lower in the soil. Citrate secreted from roots growing in the soil suffers from microbial degradation and adsorption on clay minerals (described in CHAPTER III), which reduces the availability of citrate to complex toxic Al species in the soil solution.

Our study showing little ameliorative effects of micromolar Mg addition to acid subsoils has implications to root citrate amelioration of Al rhizotoxicity in the soil. As root growth occurs in the soil, the root tip where citrate synthesis occurs is exposed continuously to the soil media where citrate degradation and adsorption can occur. Therefore, intracellular complexation of Al with citrate rather than exudation of citrate in the rhizosphere for Al complexation would be more effective for root avoidance of Al toxicity in the soil. This is also supported by the previous observations that the cortex of root tips is the most sensitive site for Al accumulation and expression of toxicity as compared to the less Al sensitive root surface cells such as epidermis (Ahn et al., 2002; Kollmeier et al., 2000; Ryan et al., 1993; Silva et al., 2000). Although previous hydroponic studies have examined root citrate efflux into solution to characterize/screen plant tolerance to Al, it would have less importance in ameliorating Al rhizotoxicity in the acid subsoil. Furthermore, examination of the root citrate as a component of Al tolerance has been commonly conducted in hydroponic solutions containing sufficient levels of Ca. In the acid subsoil, however, the soil solution Ca concentration is often poor and lower by several orders of magnitude (Bruce et al., 1988; McCray and Summer, 1990) than the hydroponic settings. Thus, it remains unclear whether root citrate production can be enhanced by the micromolar Mg addition to the acid Al toxic subsoil with low levels of solution Ca.

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Table 1. Thermodynamic data for reactions involving Al complexes and log K values corrected to zero ionic strength at 25 °C used in Al speciation modeling performed with Visual MINTEQ, ver. 2.30.

Al aqueous complexation reactions	log K ($I = 0$)	References
$\text{Al}^{3+} + \text{H}_2\text{O} \rightarrow \text{AlOH}^{2+} + \text{H}^+$	-4.99	Smith et al. (2003)
$\text{Al}^{3+} + 2\text{H}_2\text{O} \rightarrow \text{Al}(\text{OH})_2^+ + 2\text{H}^+$	-10.29	Smith et al. (2003)
$\text{Al}^{3+} + 3\text{H}_2\text{O} \rightarrow \text{Al}(\text{OH})_3^0 + 3\text{H}^+$	-16.69	Smith et al. (2003)
$\text{Al}^{3+} + 3\text{H}_2\text{O} \rightarrow \text{Al}(\text{OH})_4^- + 4\text{H}^+$	-23.00	Wesolowski (1992)
$2\text{Al}^{3+} + 2\text{H}_2\text{O} \rightarrow \text{Al}_2(\text{OH})_2^{4+} + \text{H}^+$	-7.69	Smith et al. (2003)
$3\text{Al}^{3+} + 4\text{H}_2\text{O} \rightarrow \text{Al}_3(\text{OH})_4^{5+} + 4\text{H}^+$	-13.88	Smith et al. (2003)
$\text{Al}^{3+} + \text{SO}_4^{2-} \rightarrow \text{AlSO}_4^+$	3.84	Xiao et al. (2002)
$\text{Al}^{3+} + 2\text{SO}_4^{2-} \rightarrow \text{Al}(\text{SO}_4)_2^-$	5.58	Xiao et al. (2002)
$\text{Al}^{3+} + \text{Cl}^- \rightarrow \text{AlCl}^{2+}$	-0.39	Smith et al. (2003)
$2\text{Al}^{3+} + \text{CO}_3^{2-} + 2\text{H}_2\text{O} \rightarrow \text{Al}_2(\text{OH})_2\text{CO}_3^{2+} + 2\text{H}^+$	4.31	Smith et al. (2003)

Table 2. Exchangeable cations, Al saturation and pH of subsoils treated with different amounts of Mg and lime after harvesting soybean.

Soil	Treatment	Exchangeable cation					Al saturation	pH _(water)
		Ca	Mg	K	Al	ECEC		
		—————			cmol _c kg ⁻¹	—————		%
Cecil	Mg100	0.52	0.46	0.28	0.46	1.72	27	4.4
	Mg150	0.51	0.45	0.28	0.46	1.70	27	4.5
	Mg300	0.49	0.52	0.25	0.46	1.72	27	4.5
	Lime	2.32	0.38	0.22	0.05	2.97	2	5.9
	Mean	0.96	0.45	0.25	0.35	2.03	20	4.8
Creedmore	Mg50	0.08	0.08	0.09	1.22	1.47	83	4.3
	Mg150	0.08	0.08	0.09	1.14	1.39	82	4.4
	Mg300	0.07	0.09	0.07	1.15	1.38	83	4.4
	Lime	3.18	0.07	0.08	0.18	3.51	5	5.6
	Mean	0.85	0.11	0.08	0.92	1.97	61	4.7
Norfolk	Mg50	0.07	0.01	0.06	0.22	0.36	61	4.6
	Mg150	0.07	0.02	0.05	0.21	0.35	60	4.6
	Mg300	0.07	0.02	0.05	0.19	0.33	58	4.8
	Lime	0.56	0.01	0.04	0.04	0.65	6	5.7
	Mean	0.19	0.02	0.05	0.16	0.42	45	4.9
		————— Treatment means —————						
	Mg50/100	0.22	0.20	0.14	0.63	1.19	56	4.4
	Mg150	0.22	0.19	0.13	0.60	1.16	55	4.6
	Mg300	0.21	0.22	0.12	0.59	1.16	54	4.5
	Lime	2.01	0.16	0.11	0.08	2.38	4	5.7
LSD _{0.05}								
Soil		0.03	0.01	0.01	0.03	0.06	1	0.04
Treatment		0.03	0.02	0.01	0.04	0.07	1	0.05
Soil x Treatment		0.07	0.04	0.02	0.08	0.12	2	0.10

LSD_{0.05}: Fisher's least significant difference (LSD) values of means determined at $p = 0.05$. Statistical differences for mean values of each parameter were found within soils or treatments at $p < 0.01$.

Table 3. Selected chemical properties and ionic concentrations in the soil solution collected from three subsoils at the end of experiment.

Soil	Treatment	pH	Al	Ca	K	Mg	SO ₄	DOC
			μM					mg L ⁻¹
Cecil	Mg100	3.8	4	307	401	274	55	113
	Mg150	3.7	4	408	423	372	48	101
	Mg300	3.7	9	746	586	796	42	100
	Lime	5.5	4	2817	226	641	2602	180
	Mean	4.1	5	1069	408	520	685	123
Creedmoor	Mg50	4.2	81	78	94	176	195	132
	Mg150	4.1	122	121	121	317	160	144
	Mg300	3.9	159	147	136	423	131	131
	Lime	4.9	22	894	65	61	574	127
	Mean	4.2	95	310	104	244	265	133
Norfolk	Mg50	4.5	125	144	166	149	213	155
	Mg150	4.7	115	221	181	279	155	152
	Mg300	4.3	128	365	164	617	129	149
	Lime	5.5	135	1650	101	120	1100	267
	Mean	4.7	125	595	168	291	399	180
			Treatment means					
	Mg50/100	4.1	69	176	220	199	154	133
	Mg150	4.1	80	250	241	322	121	132
	Mg300	3.9	98	419	316	612	98	126
	Lime	5.2	53	1786	130	273	1425	191
LSD _{0.05}								
Soil		0.1	7	130	21	37	169	11
Treatment		0.1	9	151	24	42	195	13
Soil x Treatment		0.2	9	261	42	74	338	22

DOC: Dissolved organic carbon.

LSD_{0.05}: Fisher's least significant difference (LSD) values of means determined at $p = 0.05$.

Statistical differences for mean values of each parameter were found within soils or treatments at $p < 0.01$.

Table 4. Best regression models to predict soil solution cations by exchangeable cation, pH and EC parameters.

Dependent variable	Predictive variables				Model R ²
	Alsac or ln(Me)	pH	ln(EC)	Intercept	
————— Coefficients (standard errors, n = 3) and partial regression coefficients (pR ²) —————					
ln Al	0.04 (0.01); pR ² = 0.41*	1.48 (0.51); pR ² = 0.28*	NS	-4.83 (2.52)	0.69
ln Ca	0.39 (0.06); pR ² = 0.69**	0.48 (0.14); pR ² = 0.07**	1.20 (0.22); pR ² = 0.19**	3.14 (0.71)	0.95
ln Mg	0.24 (0.14) [#]	NS	NS	4.92 (0.46)	0.22
ln K	0.68 (0.18)**	NS	NS	2.65 (0.70)	0.57

Alsac: 100 x (exchangeable Al)/(exchangeable Al, Ca, Mg and K) used for the regression model predicting ln Al

ln(Me): Natural log of exchangeable Ca, Mg and K values used for the regression model predicting lnCa, lnMg, and lnK, respectively.

[#], *, **: Statistical significance of predictive variable determined at $p = 0.1$, 0.05, and 0.01, respectively.

NS: Not statistically significant predictive variable determined at $p > 0.15$.

Table 5. Molar activities of selected cations in the soil solution collected from three subsoils at the end of experiment.

Soil	Treatment	(Al ³⁺)	(Ca ²⁺)	(Mg ²⁺)	(Ca ²⁺)/(Al ³⁺)	I
			μM			mM
Cecil	Mg100	0.08	152	136	1900	2.4
	Mg150	0.1	234	212	2340	3.3
	Mg300	0.8	454	482	568	6.4
	Lime	< 0.01	1240	285	> 5000	11.2
Creedmoor	Mg50	0.6	18	41	30	1.4
	Mg150	4.6	55	145	12	2.4
	Mg300	14	85	245	6	3.4
	Lime	0.2	394	26	> 5000	2.9
Norfolk	Mg50	0.7	30	31	43	1.9
	Mg150	1.4	73	92	52	2.7
	Mg300	9.6	197	330	21	4.8
	Lime	0.7	565	40	> 5000	4.7

I: Ionic strength of soil solution determined by Visual Minteq ver. 2.30.

Table 6. Regression equations and regression coefficients (R^2) for relative root length of soybean roots in the subsoils (y) as a function of ionic activity of soil solution compositions (x).

Predictor (x)	Regression equation	R^2
(Al^{3+})	$y = 77.5 - 8.0 \ln x$	0.30
($AlOH_2^+$)	$y = 65.8 - 3.1 \ln x$	0.05
(Al-OH)	$y = 76.4 - 6.0 \ln x$	0.12
($AlSO_4^+$)	$y = 69.7 - 5.4 \ln x$	0.13
(Al_{mono})	$y = 76.1 - 5.7 \ln x$	0.12
(Al-org)	$y = 100.0 - 9.6 \ln x$	0.29
(Ca^{2+})	$y = 31.4 + 71.1(1 - e^{0.006x})$	0.62
(Mg^{2+})	$y = 70.2 + 0.72 \ln x$	0.01
(Al^{3+})/(Ca^{2+})	$y = 19.3 + 74.0e^{-15x}$	0.64
(Al^{3+})/(Mg^{2+})	$y = 92.6e^{-9.9x}$	0.32
(Al^{3+})/($Ca^{2+} + Mg^{2+}$)	$y = -1.8 + 95.0e^{-28.3x}$	0.57

(Al-OH): sum of Al hydroxyl species; (Al_{mono}): sum of monomeric Al species; (Al-org): sum of Al-organic complexes.

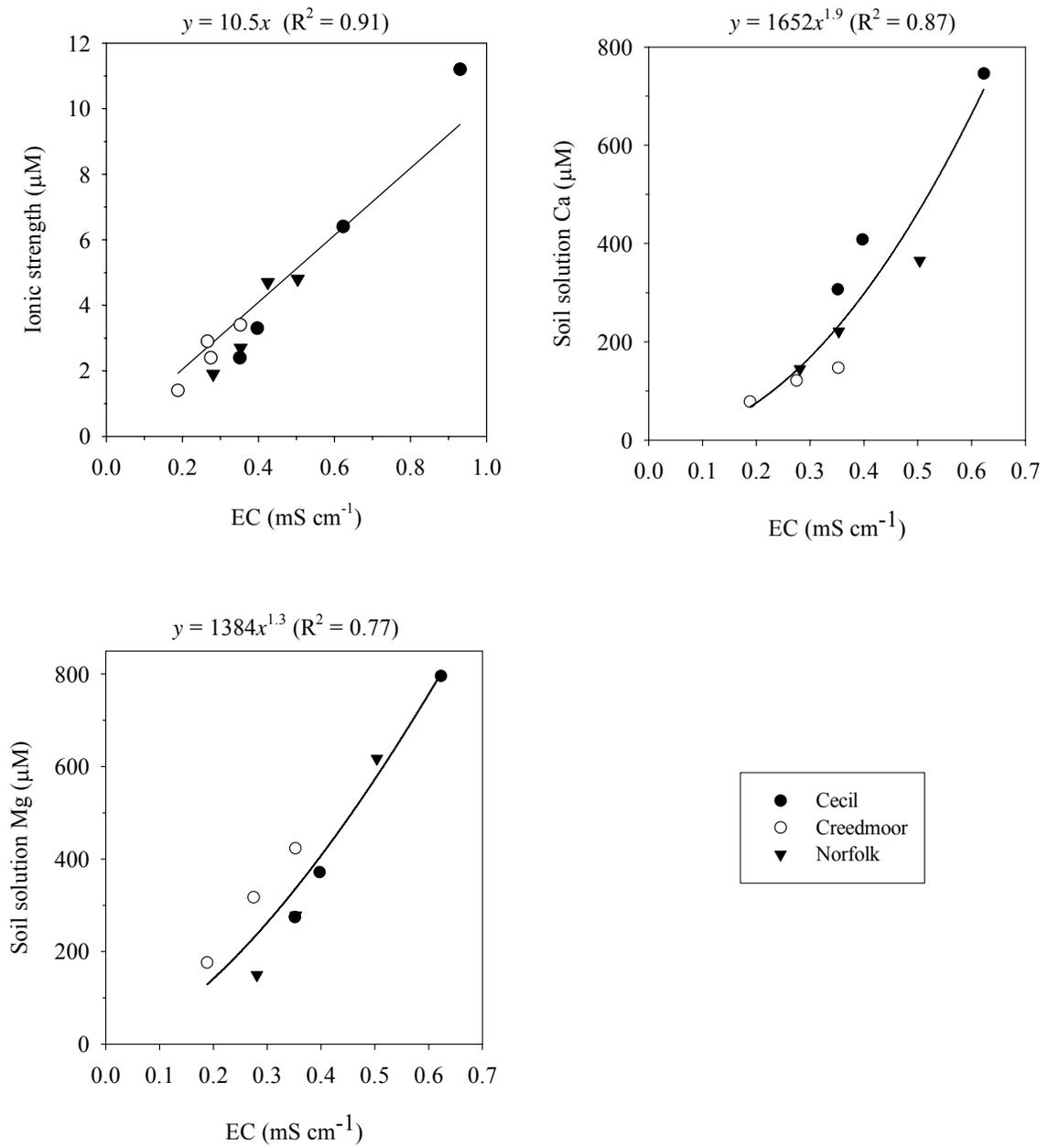


Fig. 1. Observed (symbols) and predicted (lines) ionic strength, and Ca and Mg concentrations measured in the soil solution collected from three subsoils treated with different Mg concentrations and lime expressed as a function of soil solution EC.

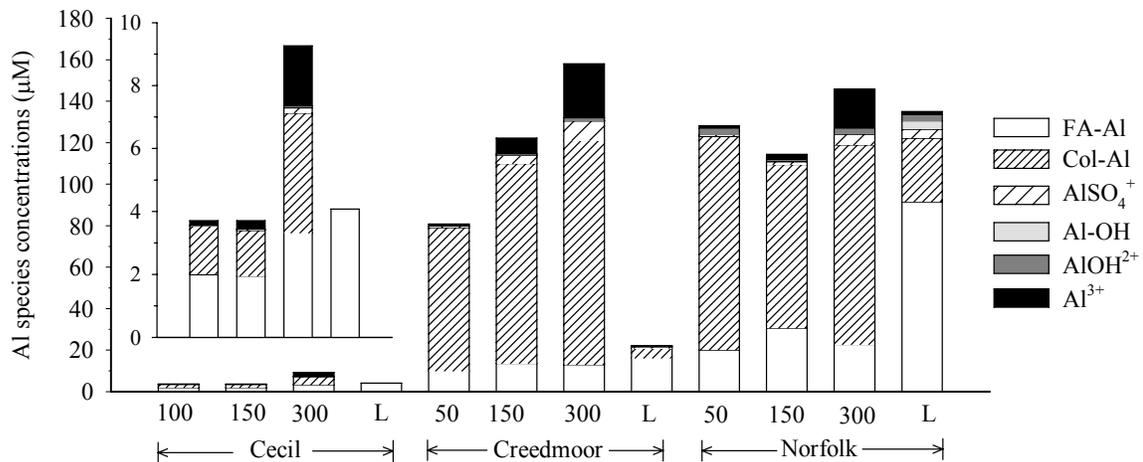


Fig. 2. Concentrations of Al species in the soil solution collected from Cecil, Creedmoor and Norfolk subsoils treated with three Mg levels (μM) and lime (L) after soybean growth. Abbreviated Al species were indicated as follows: FA-Al (Al bound with fulvic acid), Col-Al (colloidal forms of Al), Al-OH [Al hydroxide species predominated with $\text{Al}(\text{OH})_2^+$ and $\text{Al}_2(\text{OH})_2\text{CO}_3^{2+}$ (determined in the limed Norfolk subsoil only)].

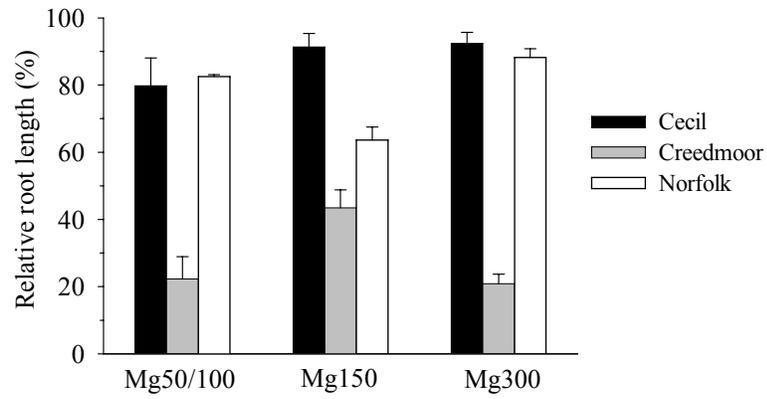


Fig. 3. Relative root length of soybean grown in Cecil, Creedmoor and Norfolk subsoils treated with 50/100, 150 and 300 μM soil solution Mg after 28 days of growth period. Relative root length is expressed as a percentage of the root length in the lime treatment of each soil (100% = lime treatment). Error bars represent standard errors of mean ($n = 3$).

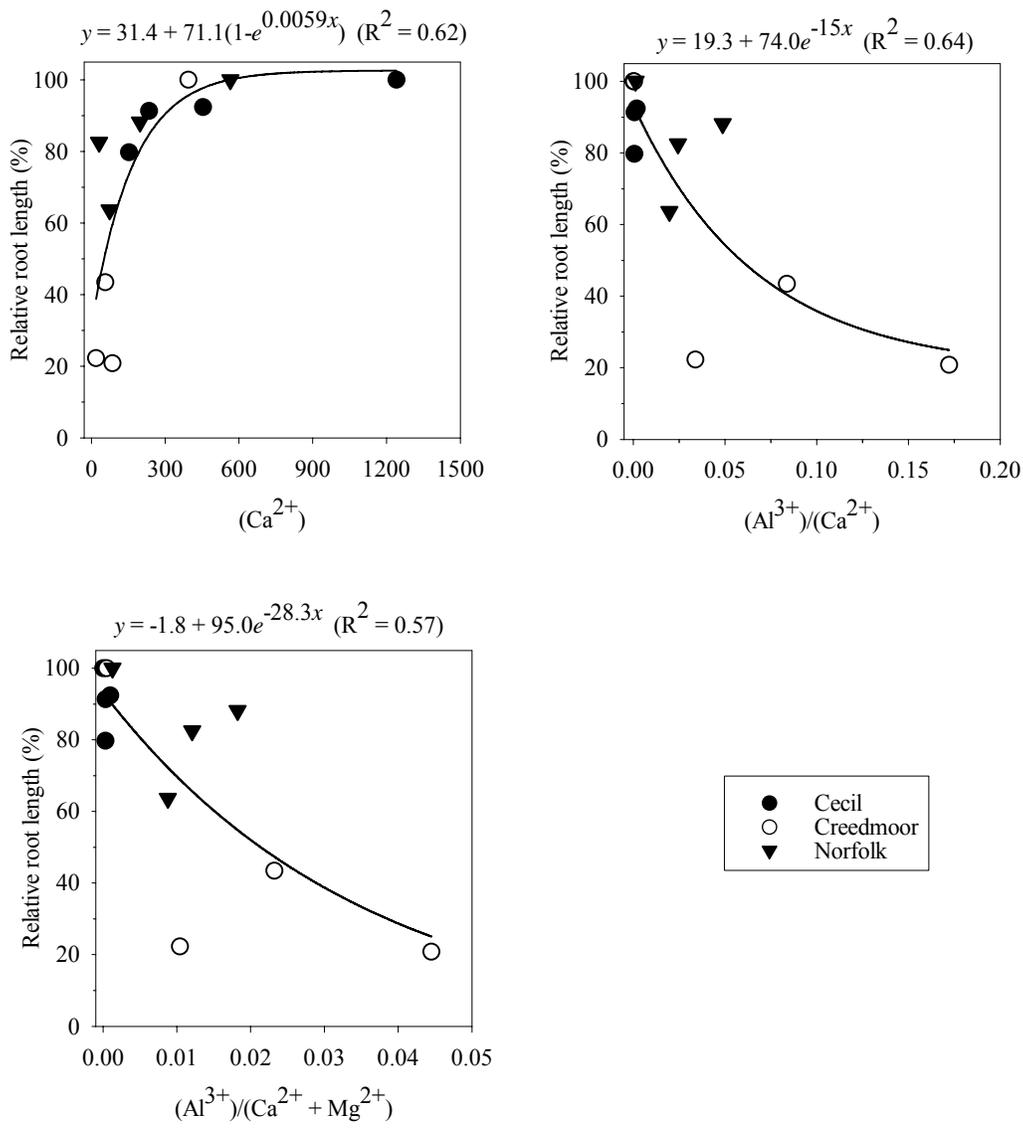


Fig. 4. Observed (symbols) and predicted (lines) relative root length of soybean grown in three subsoils treated with different Mg concentrations expressed as a function of soil solution ionic activities. Relative root length is expressed as a percentage of the root length in the lime treatment of each soil (100% = lime treatment).

APPENDIX

Table A1. Subsoil chemical properties and plant water uptake from subsoils.

Soil	trt	blo	Ca	Mg	K	Al	ECEC	Al sat.	Soil pH	plant water uptake
			cmol _c /kg					%		g/pot
Cecil	0	2	0.52	0.51	0.29	0.47	1.80	26	4.39	362.4
Cecil	0	4	0.52	0.41	0.28	0.42	1.64	26	4.44	364.8
Cecil	0	5	0.51	0.47	0.25	0.48	1.71	28	4.44	324.5
Cecil	1	2	0.51	0.44	0.32	0.47	1.74	27	4.50	343.1
Cecil	1	4	0.50	0.47	0.27	0.44	1.67	26	4.47	364.1
Cecil	1	5	0.53	0.45	0.26	0.48	1.72	28	4.58	372.7
Cecil	2	2	0.50	0.49	0.26	0.44	1.69	26	4.46	366.2
Cecil	2	4	0.52	0.55	0.23	0.47	1.77	27	4.49	328.5
Cecil	2	5	0.47	0.54	0.24	0.47	1.72	27	4.48	375.6
Cecil	L	2	2.24	0.40	0.23	0.06	2.94	2	5.83	378.1
Cecil	L	4	2.45	0.38	0.21	0.05	3.09	2	5.80	374.2
Cecil	L	5	2.26	0.36	0.23	0.03	2.88	1	5.92	373.1
Creedmoor	0	2	0.09	0.13	0.12	1.29	1.63	79	4.38	180.8
Creedmoor	0	4	0.07	0.10	0.08	1.13	1.38	82	4.30	220.4
Creedmoor	0	5	0.08	0.12	0.07	1.24	1.50	82	4.32	202.8
Creedmoor	1	2	0.08	0.12	0.07	1.17	1.44	81	4.45	199.0
Creedmoor	1	4	0.11	0.13	0.07	1.22	1.53	80	4.42	172.8
Creedmoor	1	5	0.05	0.10	0.11	1.05	1.31	80	4.46	141.7
Creedmoor	2	2	0.08	0.14	0.07	1.21	1.50	81	4.40	153.1
Creedmoor	2	4	0.07	0.12	0.07	1.07	1.33	81	4.44	196.8
Creedmoor	2	5	0.07	0.13	0.07	1.16	1.43	81	4.42	178.6
Creedmoor	L	2	3.17	0.11	0.08	0.26	3.62	7	5.50	347.5
Creedmoor	L	4	3.14	0.09	0.08	0.14	3.44	4	5.68	361.4
Creedmoor	L	5	3.22	0.10	0.07	0.14	3.53	4	5.60	349.9
Norfolk	0	2	0.07	0.02	0.07	0.24	0.40	60	4.52	197.6
Norfolk	0	4	0.07	0.02	0.06	0.23	0.37	61	4.58	201.8
Norfolk	0	5	0.06	0.02	0.05	0.20	0.33	60	4.62	211.4
Norfolk	1	2	0.07	0.03	0.05	0.21	0.36	59	4.54	223.9
Norfolk	1	4	0.06	0.02	0.04	0.18	0.31	59	4.62	235.1
Norfolk	1	5	0.07	0.03	0.05	0.23	0.37	61	4.72	163.4
Norfolk	2	2	0.07	0.03	0.06	0.21	0.37	58	4.76	231.1
Norfolk	2	4	0.07	0.04	0.04	0.19	0.33	57	4.70	224.4
Norfolk	2	5	0.09	0.01	0.05	0.17	0.31	54	4.80	210.4
Norfolk	L	2	0.56	0.01	0.05	0.03	0.65	5	5.72	284.9
Norfolk	L	4	0.54	0.01	0.04	0.03	0.62	5	5.70	244.5
Norfolk	L	5	0.58	0.04	0.04	0.04	0.69	6	5.73	273.8
Wagram	0	2	-	-	-	-	-	-	5.66	-
Wagram	0	4	-	-	-	-	-	-	5.69	-
Wagram	0	5	-	-	-	-	-	-	5.69	-

Table A2. Subsoil and topsoil root lengths, root fresh weight and cation concentrations in aboveground plant tissues.

soil	trt	blo	Subsoil	Topsoil	Total	Fresh weight	Al		Ca		Mg	
							mg/g	mg/pot	mg/g	mg/pot	mg/g	mg/pot
			cm			g	mg/g	mg/pot	mg/g	mg/pot	mg/g	mg/pot
Cecil	0	1	1603.4	2007.9	3611.3	2.650	0.051	0.068	0.646	0.866	0.106	0.142
Cecil	0	3	1479.0	2084.1	3563.2	2.573	0.084	0.108	0.733	0.941	0.154	0.198
Cecil	0	6	1808.2	1667.8	3476.0	3.055	0.113	0.183	0.668	1.082	0.145	0.236
Cecil	1	1	2228.9	1516.1	3745.0	3.916	0.108	0.121	0.727	0.814	0.169	0.189
Cecil	1	3	1696.7	2022.0	3718.7	2.446	0.060	0.103	0.737	1.265	0.137	0.234
Cecil	1	6	1673.3	2008.4	3681.7	3.371	0.088	0.120	0.744	1.014	0.148	0.202
Cecil	2	1	2294.1	1818.4	4112.5	3.297	0.054	0.078	0.732	1.055	0.158	0.228
Cecil	2	3	1856.6	1695.5	3552.0	2.855	0.060	0.094	0.690	1.083	0.145	0.227
Cecil	2	6	1515.2	1999.1	3514.3	2.443	0.055	0.071	0.722	0.932	0.149	0.193
Cecil	L	1	1783.4	1676.3	3459.7	2.784	0.060	0.087	0.904	1.319	0.167	0.244
Cecil	L	3	2301.4	1612.2	3913.6	3.307	0.054	0.083	0.780	1.198	0.151	0.232
Cecil	L	6	2046.3	1770.8	3817.1	3.245	0.053	0.076	0.906	1.300	0.148	0.212
Creedmoor	0	1	389.6	2685.4	3075.1	1.251	0.066	0.107	0.694	1.124	0.100	0.161
Creedmoor	0	3	373.1	2676.9	3050.0	0.744	0.051	0.053	0.829	0.863	0.104	0.108
Creedmoor	0	6	115.0	2372.5	2487.5	0.174	0.051	0.053	0.819	0.845	0.101	0.104
Creedmoor	1	1	460.4	2748.7	3209.1	0.970	0.068	0.088	0.938	1.204	0.110	0.141
Creedmoor	1	3	564.9	2771.6	3336.6	1.307	0.059	0.093	0.732	1.157	0.097	0.153
Creedmoor	1	6	686.6	2568.4	3255.0	1.702	0.060	0.088	0.824	1.209	0.118	0.174
Creedmoor	2	1	191.7	2815.5	3007.2	0.347	0.082	0.113	1.060	1.462	0.107	0.147
Creedmoor	2	3	328.7	2375.3	2703.9	0.759	0.053	0.071	0.720	0.972	0.113	0.152
Creedmoor	2	6	300.5	2688.4	2988.9	0.607	0.032	0.037	0.759	0.890	0.102	0.120
Creedmoor	L	1	1272.0	2560.3	3832.3	2.182	0.026	0.044	0.889	1.518	0.108	0.185
Creedmoor	L	3	1396.1	2552.6	3948.8	2.108	0.044	0.079	0.931	1.666	0.113	0.203
Creedmoor	L	6	1271.3	2366.9	3638.2	2.210	0.035	0.055	0.891	1.395	0.119	0.186
Norfolk	0	1	1418.6	2197.9	3616.5	2.903	0.034	0.043	0.766	0.965	0.129	0.163
Norfolk	0	3	847.8	2873.6	3721.5	1.434	0.029	0.042	0.794	1.147	0.113	0.163
Norfolk	0	6	1805.6	2478.4	4284.0	3.178	0.107	0.155	0.830	1.204	0.112	0.162
Norfolk	1	1	1228.9	2205.5	3434.4	1.956	0.044	0.068	0.729	1.121	0.118	0.181
Norfolk	1	3	901.6	2529.4	3431.1	1.536	0.033	0.042	0.796	1.006	0.117	0.148
Norfolk	1	6	1009.1	2641.4	3650.6	1.315	0.183	0.268	0.717	1.051	0.113	0.166
Norfolk	2	1	1369	2013.6	3382.6	2.075	0.077	0.095	0.895	1.107	0.144	0.178
Norfolk	2	3	1470.9	2573.9	4044.8	2.163	0.061	0.092	0.793	1.199	0.157	0.237
Norfolk	2	6	1512.2	2635.6	4147.8	2.489	0.063	0.086	0.796	1.088	0.140	0.192
Norfolk	L	1	2048.8	1790.2	3839.0	3.478	0.056	0.086	0.812	1.249	0.099	0.152
Norfolk	L	3	1245.9	2319.9	3565.8	1.921	0.076	0.102	0.806	1.082	0.096	0.129
Norfolk	L	6	1639.8	2424.2	4064.1	2.101	0.062	0.074	1.062	1.258	0.109	0.130

Table A3. Fresh and dry weights of aboveground plant tissues.

soil	trt	blo	Fresh weight	Dry weight
			g	
Cecil	L	1	6.26	1.46
Cecil	0	1	5.09	1.34
Cecil	1	1	4.48	1.12
Cecil	2	1	5.70	1.44
Cecil	L	2	6.73	1.63
Cecil	0	2	5.75	1.41
Cecil	1	2	6.14	1.46
Cecil	2	2	6.54	1.65
Cecil	L	3	6.62	1.54
Cecil	0	3	5.33	1.29
Cecil	1	3	7.11	1.72
Cecil	2	3	6.25	1.57
Cecil	L	4	6.56	1.71
Cecil	0	4	5.98	1.28
Cecil	1	4	6.02	1.48
Cecil	2	4	5.63	1.30
Cecil	L	5	7.15	1.79
Cecil	0	5	5.23	1.17
Cecil	1	5	6.51	1.70
Cecil	2	5	5.54	1.44
Cecil	L	6	5.77	1.44
Cecil	0	6	6.53	1.62
Cecil	1	6	5.79	1.36
Cecil	2	6	5.12	1.29
Creedmoor	L	1	6.63	1.71
Creedmoor	0	1	6.53	1.62
Creedmoor	1	1	4.95	1.28
Creedmoor	2	1	5.29	1.38
Creedmoor	L	2	5.73	1.47
Creedmoor	0	2	4.53	1.16
Creedmoor	1	2	4.42	1.17
Creedmoor	2	2	4.97	1.32
Creedmoor	L	3	7.39	1.79
Creedmoor	0	3	4.40	1.04
Creedmoor	1	3	6.37	1.58
Creedmoor	2	3	5.42	1.35
Creedmoor	L	4	7.17	1.62
Creedmoor	0	4	4.95	1.14
Creedmoor	1	4	5.75	1.28
Creedmoor	2	4	5.38	1.26
Creedmoor	L	5	7.27	1.71
Creedmoor	0	5	5.75	1.36
Creedmoor	1	5	5.24	1.25
Creedmoor	2	5	5.98	1.42
Creedmoor	L	6	6.63	1.57
Creedmoor	0	6	4.47	1.03
Creedmoor	1	6	6.10	1.47
Creedmoor	2	6	5.04	1.17
Norfolk	L	1	5.83	1.54
Norfolk	0	1	5.17	1.26
Norfolk	1	1	6.32	1.54
Norfolk	2	1	4.94	1.24
Norfolk	L	2	5.78	1.51
Norfolk	0	2	5.98	1.53
Norfolk	1	2	6.86	1.72
Norfolk	2	2	6.89	1.69
Norfolk	L	3	5.55	1.34
Norfolk	0	3	5.87	1.44
Norfolk	1	3	5.22	1.26
Norfolk	2	3	6.16	1.51
Norfolk	L	4	5.12	1.18
Norfolk	0	4	5.63	1.29
Norfolk	1	4	5.43	1.25
Norfolk	2	4	5.49	1.23
Norfolk	L	5	6.83	1.67
Norfolk	0	5	5.44	1.31
Norfolk	1	5	5.93	1.43
Norfolk	2	5	5.55	1.36
Norfolk	L	6	5.08	1.19
Norfolk	0	6	6.22	1.45
Norfolk	1	6	6.35	1.47
Norfolk	2	6	5.75	1.37

Table A4. Cation concentrations in the soil solution collected from three subsoils.

Soil	trt	blo	Al	Ca	Fe	K	Mg	Mn	Na	Zn
			µM							
Norfolk	0	2	117.49	154.69	6.63	188.80	185.19	10.56	530.90	3.67
Norfolk	0	4	127.50	147.95	8.06	160.14	139.09	9.65	574.41	4.89
Norfolk	0	5	130.47	130.74	8.95	148.89	123.46	8.19	491.73	4.74
Norfolk	1	2	109.34	206.34	5.37	172.17	272.84	15.11	535.25	3.67
Norfolk	1	4	113.79	234.78	5.55	177.03	281.89	15.84	530.90	3.52
Norfolk	1	5	120.46	222.55	6.81	192.38	281.07	17.29	617.93	12.85
Norfolk	2	2	121.20	319.36	5.37	216.17	510.29	23.48	574.41	12.08
Norfolk	2	4	134.17	406.69	5.55	232.03	687.24	30.77	587.47	7.49
Norfolk	2	5	128.98	369.26	6.27	229.73	654.32	29.49	639.69	7.19
Norfolk	L	2	162.00	1439.62	32.95	81.09	98.77	2.91	331.16	0.92
Norfolk	L	4	114.16	2143.21	49.07	121.00	146.50	4.19	465.62	2.14
Norfolk	L	5	128.98	1367.27	31.88	102.33	113.58	3.64	364.66	2.75
Creedmoor	0	2	76.35	81.09	6.09	95.68	174.90	13.65	396.00	2.60
Creedmoor	0	4	81.54	76.85	6.45	92.10	176.13	12.93	419.93	2.75
Creedmoor	0	5	84.88	76.35	6.27	95.17	177.78	13.65	384.25	2.60
Creedmoor	1	2	111.56	115.77	6.98	118.70	304.12	20.57	513.49	5.35
Creedmoor	1	4	127.50	127.50	7.34	129.19	321.40	24.94	535.25	5.35
Creedmoor	1	5	127.87	119.76	13.25	116.14	325.93	31.86	487.38	3.36
Creedmoor	2	2	162.34	157.19	8.78	137.38	444.44	29.13	522.19	4.89
Creedmoor	2	4	156.41	139.72	7.34	131.24	401.65	28.95	535.25	4.28
Creedmoor	2	5	156.78	144.46	7.70	140.19	423.87	30.40	517.84	4.43
Creedmoor	L	2	21.50	848.30	8.06	45.54	63.37	2.55	232.38	1.84
Creedmoor	L	4	24.46	1012.97	13.43	47.58	62.55	2.55	229.77	2.29
Creedmoor	L	5	20.39	820.86	10.21	102.33	56.79	2.18	289.38	1.07
Cecil	0	2	5.19	284.43	1.61	447.68	235.39	6.19	530.90	5.20
Cecil	0	4	3.34	294.41	1.07	355.59	299.18	6.01	683.20	7.03
Cecil	0	5	2.97	341.82	0.90	399.08	288.48	7.65	735.42	8.11
Cecil	1	2	2.22	389.22	0.90	406.75	337.04	8.56	722.37	7.34
Cecil	1	4	4.08	429.14	0.90	388.85	403.70	9.28	713.66	7.03
Cecil	1	5	5.19	406.69	1.61	473.27	374.49	9.10	644.04	7.95
Cecil	2	2	8.15	711.08	1.43	547.45	769.55	17.11	644.04	13.30
Cecil	2	4	8.52	741.02	1.07	603.73	790.12	16.38	765.88	12.39
Cecil	2	5	10.75	785.93	1.79	606.29	827.16	17.66	748.48	9.94
Cecil	L	2	3.71	3151.20	1.07	239.19	732.51	4.01	644.04	3.67
Cecil	L	4	4.08	2714.57	0.90	226.40	633.74	4.37	678.85	7.49
Cecil	L	5	4.45	2584.83	1.43	212.33	555.56	3.09	809.40	4.28

Table A5. Anion and DOC concentrations and pH and EC determined in the soil solution collected from three subsoils.

Soil	trt	blo	Cl	NO ₃	SO ₄	NH ₄	DOC	pH	EC
			mM				mg/L		mS/cm
Norfolk	0	2	1.77	0.14	0.18	0.51	150.0	4.72	0.279
Norfolk	0	4	1.61	0.19	0.25	0.52	168.5	4.35	0.3
Norfolk	0	5	1.40	0.11	0.21	0.47	145.5	4.51	0.264
Norfolk	1	2	2.57	0.11	0.14	0.54	142.0	4.88	0.342
Norfolk	1	4	2.58	0.11	0.15	0.52	157.5	4.81	0.328
Norfolk	1	5	3.01	0.06	0.17	0.62	155.0	4.53	0.39
Norfolk	2	2	4.19	0.09	0.10	0.59	128.5	4.43	0.46
Norfolk	2	4	5.35	0.11	0.15	0.66	165.0	4.40	0.525
Norfolk	2	5	4.98	0.11	0.13	0.64	154.5	4.13	0.526
Norfolk	L	2	1.03	0.03	0.73	0.34	273.5	5.43	0.352
Norfolk	L	4	1.40	0.04	1.86	0.59	270.5	5.44	0.555
Norfolk	L	5	1.31	0.04	0.72	0.37	256.5	5.50	0.368
Creedmoor	0	2	1.02	0.01	0.19	0.23	122.0	4.14	0.194
Creedmoor	0	4	0.96	0.04	0.21	0.25	141.5	4.33	0.183
Creedmoor	0	5	0.97	0.08	0.19	0.24	131.0	4.23	0.188
Creedmoor	1	2	1.98	0.04	0.16	0.29	132.5	4.03	0.264
Creedmoor	1	4	2.22	0.06	0.17	0.30	136.0	4.05	0.284
Creedmoor	1	5	2.15	0.01	0.16	0.29	163.0	4.10	0.276
Creedmoor	2	2	3.35	0.04	0.13	0.33	124.0	3.87	0.36
Creedmoor	2	4	3.09	0.04	0.13	0.33	138.5	3.93	0.347
Creedmoor	2	5	3.17	0.04	0.13	0.31	130.0	3.87	0.35
Creedmoor	L	2	1.14	-	0.51	0.17	126.5	4.85	0.263
Creedmoor	L	4	0.98	-	0.65	0.19	143.5	4.87	0.277
Creedmoor	L	5	1.00	0.02	0.56	0.19	111.5	4.96	0.258
Cecil	0	2	1.33	1.83	0.06	0.46	85.5	3.77	0.348
Cecil	0	4	1.06	1.93	0.05	0.34	128.0	3.77	0.345
Cecil	0	5	0.94	2.36	0.05	0.36	126.5	3.72	0.361
Cecil	1	2	1.75	1.81	0.04	0.38	77.0	3.75	0.386
Cecil	1	4	2.17	1.81	0.05	0.39	104.5	3.75	0.405
Cecil	1	5	2.25	1.51	0.05	0.37	120.5	3.62	0.401
Cecil	2	2	5.11	1.22	0.04	0.45	101.5	3.67	0.605
Cecil	2	4	4.56	2.07	0.04	0.48	95.0	3.74	0.629
Cecil	2	5	5.45	1.49	0.04	0.47	103.5	3.65	0.634
Cecil	L	2	2.74	2.79	2.76	0.35	193.5	5.22	0.977
Cecil	L	4	2.44	2.99	2.40	0.36	175.5	5.59	0.899
Cecil	L	5	2.41	2.91	2.64	0.35	169.5	5.78	0.916

Table A6. Citrate adsorption experiments for subsoils with and without a Na-azide solution.

Soil	Citrate added	Citrate in soln	Citrate adsorbed	Soil	Citrate added	Citrate in soln	Citrate adsorbed
with Na-azide				without Na-azide			
	$\mu\text{mol/kg}$	μM	μM		$\mu\text{mol/kg}$	μM	μM
Cecil	50	0	50	Cecil	50	0	50
Cecil	50	0	50	Cecil	50	0	50
Creedmoor	50	0	50	Creedmoor	50	0	50
Creedmoor	50	0	50	Creedmoor	50	0	50
Norfolk	50	2	48	Norfolk	50	0	50
Norfolk	50	2	48	Norfolk	50	0	50
Cecil	100	0	100	Cecil	100	0	100
Cecil	100	0	100	Cecil	100	0	100
Creedmoor	100	2	98	Creedmoor	100	0	100
Creedmoor	100	2	98	Creedmoor	100	0	100
Norfolk	100	5	95	Norfolk	100	1	99
Norfolk	100	6	94	Norfolk	100	1	99
Cecil	250	0	250	Cecil	250	0	250
Cecil	250	0	250	Cecil	250	0	250
Creedmoor	250	13	237	Creedmoor	250	0	250
Creedmoor	250	11	239	Creedmoor	250	0	250
Norfolk	250	16	234	Norfolk	250	6	244
Norfolk	250	17	233	Norfolk	250	5	245
Cecil	500	0	500	Cecil	500	0	500
Cecil	500	0	500	Cecil	500	0	500
Creedmoor	500	31	469	Creedmoor	500	24	476
Creedmoor	500	30	470	Creedmoor	500	26	474
Norfolk	500	39	461	Norfolk	500	10	490
Norfolk	500	38	462	Norfolk	500	13	487
Cecil	750	0	750	Cecil	750	0	750
Cecil	750	0	750	Cecil	750	0	750
Creedmoor	750	52	698	Creedmoor	750	12	738
Creedmoor	750	56	694	Creedmoor	750	9	741
Norfolk	750	64	686	Norfolk	750	13	737
Norfolk	750	71	679	Norfolk	750	14	736
Cecil	1000	0	1000	Cecil	1000	0	1000
Cecil	1000	0	1000	Cecil	1000	0	1000
Creedmoor	1000	78	922	Creedmoor	1000	67	933
Creedmoor	1000	78	922	Creedmoor	1000	9	991
Norfolk	1000	88	912	Norfolk	1000	20	980
Norfolk	1000	88	912	Norfolk	1000	16	984
Cecil	2500	0	2500	Cecil	2500	0	2500
Cecil	2500	0	2500	Cecil	2500	0	2500
Creedmoor	2500	224	2276	Creedmoor	2500	57	2443
Creedmoor	2500	227	2273	Creedmoor	2500	83	2417
Norfolk	2500	197	2303	Norfolk	2500	48	2452
Norfolk	2500	194	2306	Norfolk	2500	57	2443
Cecil	5000	19	4981	Cecil	5000	0	5000
Cecil	5000	18	4982	Cecil	5000	0	5000
Creedmoor	5000	433	4567	Creedmoor	5000	272	4728
Creedmoor	5000	442	4558	Creedmoor	5000	242	4758
Norfolk	5000	243	4757	Norfolk	5000	154	4846
Norfolk	5000	251	4749	Norfolk	5000	167	4833
Cecil	7500	57	7443	Cecil	7500	0	7500
Cecil	7500	61	7439	Cecil	7500	0	7500
Creedmoor	7500	604	6896	Creedmoor	7500	426	7074
Creedmoor	7500	604	6896	Creedmoor	7500	435	7065
Norfolk	7500	333	7167	Norfolk	7500	257	7243
Norfolk	7500	334	7166	Norfolk	7500	280	7220
Cecil	10000	106	9894	Cecil	10000	2	9998
Cecil	10000	125	9875	Cecil	10000	2	9998
Creedmoor	10000	718	9282	Creedmoor	10000	573	9427
Creedmoor	10000	687	9313	Creedmoor	10000	562	9438
Norfolk	10000	494	9506	Norfolk	10000	320	9680
Norfolk	10000	502	9498	Norfolk	10000	362	9638