Simulating Infertile Acid Soils with Nutrient Solutions: The Effects on *Brachiaria* Species

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ABSTRACT

Aluminum toxicity limits plant growth in acid soils. Because of their advanced state of weathering, acid soils of the tropics also tend to be deficient in nutrients. A realistic assessment of plant adaptation to these soils would therefore require Al-toxic conditions under which growth is simultaneously limited by nutrient deficiency. We developed and tested a nutrient solution for this purpose. We analyzed soil solutions of two Oxisols from the Colombian savannas. Nutrient concentrations were extremely low (ionic strength <1.7 mM). Nitrification during incubation of soil samples acidified soil solutions, resulting in a release of cations from the exchange phase, an increase in the activity of Al^{3+}, and a decrease in that of $H_2PO_4^-$. Predicted ion activities were taken as guidelines for designing a nutrient solution that simulates these soil solutions. Growth of well-adapted signalgrass (*Brachiaria decumbens* cv. Basilisk) and less-adapted ruzigrass (*Brachiaria ruziziensis* cv. Common) in this solution mirrored the interspecific difference in forage yield that had previously been observed in a field close to where one of the soils originated. This suggests that the designed solution may be a realistic approximation to chemical soil properties that limit forage productivity. The different growth response of the two grasses was apparently due to increased Al sensitivity of less-adapted ruzigrass under low nutrient supply; neither Al toxicity nor nutrient deficiency alone resulted in a comparable growth difference between the grasses. These data highlight the importance of taking into account interactions among stress factors that occur in parallel in infertile acid soils.

IN ACID SOILS, exchange sites vacated by cations leached out of the soil profile are occupied by Al ions solubilized from decomposing primary minerals. The result of this natural acidification process is a complex syndrome of Al (and sometimes Mn) toxicity, deficiencies in Ca, Mg, and Mo, and a frequently low availability of P as a result of chemical fixation. In addition, K and N tend to be deficient in many acid mineral soils of the tropics because they are usually highly weathered or have low organic matter content (Sánchez, 1976; Rao et al., 1993). Since Hartwell and Pember (1918) recognized Al toxicity as being associated with soil acidity, most studies investigating the adaptation of plants to acid soils have focused on the effect of Al^{3+} ions, the

principal phytotoxic Al species (Foy, 1992; Kinraide, 1997).

Many of these studies were conducted with solution culture rather than with soil to circumvent the problem that several soil properties change simultaneously when soil acidity is modified. Initially, solutions contained nutrient levels far in excess of those required for maximum plant growth rates (see critiques by Blamey et al. [1991] and Edmeades et al. [1995]). In such solutions, Al toxicity is alleviated as a result of physicochemical interactions between Al and other ions, including the formation of nontoxic complexes with OH^-, SO_4^{2-}, and silicate ions; precipitation of Al as hydroxide or phosphate; and high ionic strength per se (Blamey et al., 1983; Blamey et al., 1991; Wheeler and Edmeades, 1995; Kinraide, 1997; Ma et al., 1997). In addition, high concentrations of divalent and, to a lesser extent, monovalent cations can ameliorate Al toxicity, presumably because they reduce cell-surface negativity (Kinraide and Parker, 1987; Kinraide et al., 1992; Kinraide, 1998).

Recognition of these interactions led to the use of low-ionic-strength nutrient solutions to avoid artifacts caused by unrealistic Al speciation (Blamey et al., 1983, 1991; Wheeler et al., 1992; Edmeades et al., 1995). These solutions were designed to simulate soil solutions of acid soils because the composition of these determines the bioavailability of nutrients (Pearson, 1971; Parker and Norvell, 1999). Nutrient concentrations, about one order of magnitude lower than those of earlier solution-culture techniques, are at the minimum levels required to achieve close-to-maximum plant growth rates (Asher, 1978, 1986; Edmeades et al., 1995; Wheeler, 1996). As a consequence of these more realistic conditions, Al^{3+} activities ($\{Al^{3+}\}$) required for inhibiting root growth are at least one order of magnitude lower than those of earlier studies. They are comparable with values inferred from studies relating root-growth inhibition to $\{Al^{3+}\}$ measured in soil solutions (Pavan et al., 1982; Wright et al., 1989; Blamey et al., 1991).

Nutrient deficiencies associated with soil acidity may be particularly severe in South American savannas, because the soils of these areas tend to be highly weathered and more acidic than comparable regions on other continents (Eswaran et al., 1997). Hence, currently used low-ionic-strength nutrient solutions supporting close-to-maximum plant growth rates may not reflect the various nutritional constraints under which plants are exposed

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Abbreviations: AN, nutrient solution with adequate nutrient levels; AN + Al, nutrient solution with adequate nutrient levels containing Al; Al_{inorg}, inorganic monomeric Al; LA, leaf area; LN, nutrient solution with low nutrient levels; LN + Al, nutrient solution with low nutrient levels containing Al; RDW, root dry weight; RL, total root length; SDW, shoot dry weight; [ ], concentration of an ion or an ion complex; { }, activity of an ion or an ion complex.

to Al in these soils. We hypothesized that an adequate assessment of Al resistance and edaphic adaptation of crops targeted for these areas requires a solution with low concentrations of those nutrients that typically limit plant growth on these soils.

Large areas of the South American savannas have been sown to pastures based on forage grasses of the *Brachiaria* genus. A field study in an area representing the highly weathered acid soils of the Colombian and Venezuelan savannas had previously shown that acid soil-adapted signalgrass produces a higher forage yield than less-adapted ruzigrass (CIAT, 1995). We therefore used these two species, as well as soils from this area, as a model to develop a solution-culture technique for rapidly evaluating the edaphic adaptation of crops targeted for these areas.

The initial objective of this multi-tiered study was to examine the nutrient status of the highly weathered acid soils of the Colombian savannas, and to design a nutrient solution that simulates chemical factors (Al toxicity, nutrient deficiencies) that limit plant growth in these soils. To test whether the designed solution was a realistic approximation to the chemical properties of these soils, we then examined whether its growth-inhibitory effect resulted in a similar growth difference between signalgrass and ruzigrass as had been observed in the field experiment mentioned above. Finally, to investigate whether interactions among the various stress factors must be taken into account for an accurate assessment of edaphic adaptation, we dissected the stress syndrome into Al toxicity and nutrient deficiency and examined whether they inhibited growth of the two grasses in a synergistic manner.

MATERIALS AND METHODS

Extracting and Analyzing Soil Solutions

Collecting Soil Samples

The soils used in this study were collected from topsoil (0 to 20 cm) under native savanna vegetation at two sites (Alegría and Pista) in the Carimagua Experimental Station (Department of Meta, Colombia; 4° 37′ N lat., 71° 19′ W long; 150 m altitude; 26°C mean temperature; 2300 mm annual rainfall distributed from April to November; December to March almost completely dry). Both were Oxisols (kaolinitic, isohyperthermic, Typic Haplustox), but had either a sandy-loam (Alegría) or clay-loam texture (Pista). Samples were taken at the end of the wet season, air-dried, sieved (2 mm), homogenized by mixing, and stored for routine use in the greenhouse at CIAT headquarters, near Cali, Colombia (3° 30′ N lat.; 76° 21′ long. W, 965 m altitude).

The characteristics of the sandy loam (clay loam) before fertilization, previously determined according to Salinas and García (1985), were as follows: 170 (370) g kg^{-1} clay and 650 (180) g kg^{-1} sand, pH 5.1 (5.0) at a soil/water ratio of 1:1, 9 (34) g kg^{-1} organic matter, 24 (72) mmol kg^{-1} total N, 2.0 (2.1) mg kg^{-1} Bray II-extracted P, 0.03 (0.06) $cmol_c$ kg^{-1} Bray II-extracted K, 0.7 (2.6) $cmol_c$ kg^{-1} KCl-extracted Al, 0.13 (0.21) $cmol_c$ kg^{-1} KCl-extracted Ca, and 0.08 (0.10) $cmol_c$ kg^{-1} KCl-extracted Mg. Aluminum saturation was 77 (89) % and the effective cation-exchange capacity 0.94 (2.97) $cmol_c$ kg^{-1}.

Applying Fertilizer and Rewetting Soil Samples

About 4 mo after collecting and air-drying the soils, a 20-kg aliquot of each soil type was taken. Fertilizer, consisting of (in milligrams of element per kilogram of soil): 20.6 N (urea), 25.8 P (triple superphosphate), 51.6 K (KCl), 34.0 Ca (dolomitic lime), 18.0 Ca (triple superphosphate), 14.6 Mg (dolomitic lime), 10.3 S (elemental sulfur), 1.0 Zn ($ZnCl_2$), 1.0 Cu ($CuCl_2$), 0.05 B (H_3BO_3), 0.05 Mo ($Na_2MoO_4 \cdot 2 H_2O$) was applied to one-half of each sample (10 kg). This mixture is routinely used to raise the fertility of Colombian savanna soils in field studies; addition of 1 mg of element per kg soil corresponds to a surface-area-based application rate of 1.94 kg of element ha^{-1} (Rao et al., 1992, 1996). The four soil samples, two with and two without fertilizer, were rewetted to 90% (w/w) field capacity by adding water at 144 mL kg^{-1} of dry soil for the sandy loam or 225 for the clay loam.

Incubating Soil Samples and Extracting Soil Solutions

Ten 333.3-g aliquots were taken from each of the four rewetted soil samples and transferred into 0.02-mm thick polyethylene bags (Bartlett, 1965). The bags, in upright position with their tops rolled back, were arranged in a single layer and incubated at 28°C. On Day 1, 2, 4, 8, 16, 32 and 48, one bag per soil type and fertilizer level (four aliquots in all) was removed and weighed. Soil solutions were extracted by centrifuging each aliquot, in several rounds, at 2000 × *g* for 45 min (Aitken and Outhwaite, 1987). On Day 64, three bags of each soil type and fertilizer level (12 aliquots in all) were processed to quantify the variability among aliquots (extraction of replicate aliquots for all time points was not possible because the process was too time-consuming). The average loss of soil moisture after 64 d of incubation was 12.5% ± 4.7 (mean ± SD) of the initial water content.

Analyzing Soil Solutions

The electrical conductivity and pH of each soil solution were determined in a 2.5-mL aliquot immediately after extraction. The remainder was filtered (0.22 μm; polycarbonate), and the concentration of inorganic monomeric Al (Al_{inorg}) was estimated, using a modified version of the short-term colorimetric method described by Kerven et al. (1989): 300 μL of soil solution were mixed with 50 μL of 0.1% (w/v) 1,10-phenanthroline, 0.5% (w/v) ascorbic acid, 20 μL of 0.11% (w/v) pyrocatechol violet, and 100 μL of 1 *M* imidazole-HCl (pH = 5.9). Absorbance at 585 nm was measured exactly 1 min after adding the imidazole buffer. Aluminum chloride was used for calibration.

The remaining aliquot of each soil-solution sample was stored at −80°C for later analysis of nutrient concentrations. Ammonium was determined colorimetrically (Kandeler and Gerber, 1988). Other cations, including Ca^{2+}, Mg^{2+}, K^+, and Na^+, were measured by atomic-absorption spectroscopy (Unicam 969 spectrophotometer; Unicam, Cambridge, UK). Anions, such as phosphate, NO_3^-, SO_4^{2-}, and Cl^-, were quantified by HPLC, using a Dionex AS-11 column (Dionex, Sunnyvale, CA) and gradient elution (0.5–37.5 mM NaOH in 18 min at a flow rate of 2 mL min^{-1} and 30°C). Suppressed conductivity was used for detection.

Simulating the Ionic Speciation of Soil Solutions

The ionic speciation of each soil-solution sample was simulated with GEOCHEM 2.0 software using the thermodynamic constants for the formation of Al complexes compiled by Nordstrom and May (1989) (Parker et al., 1987). The measured

ion concentrations (Al_{inorg} in the case of Al) were used as input values.

GEOCHEM simulations were performed at the measured pH values (3.9–5.9), disallowing precipitation of $Al(OH)_3$ and other solids and permitting CO_2 to penetrate the solution at a partial pressure of 3×10^{-4} atm (using the program's options "pH fixed," "open system," and "ionic strength calculated"). The program computed the activities of ions and ion complexes, using the Davies equation and an ionic strength that had been corrected for ion pairing predicted based on ionic-strength-adjusted stability constants. Throughout this paper, the parentheses [ ] and { } denote concentration and activity of a given ion, respectively.

Preparing the Designed Nutrient Solutions

A low-ionic-strength nutrient solution simulating the chemical properties of the highly weathered acid soils from the Colombian savannas was designed based on the data obtained in the first part of this study (LN + Al, low nutrient levels plus Al). In addition, three other treatments were designed: an Al-free reference treatment containing sufficient nutrients for close-to-maximum plant growth rates (AN, adequate nutrients) and two treatments for exposing plants to Al toxicity (AN + Al) or nutrient deficiency (LN) separately (see *Discussion* and Table 1 for the design criteria).

For each of these four treatments, we simulated the ionic speciation of a series of seven different nutrient solutions with a varying NO_3^- to NH_4^+ ratio so that they would be suitable for plant species with different preferences for the two forms of mineral N (Table 2; a ratio of 10 was chosen for this study). For these simulations, the logarithm of the solubility constant of $Al(OH)_3$ was set at 8.8 (Kinraide and Parker, 1989), and precipitation of all solids was permitted. Carbon dioxide was allowed to penetrate the solution at a partial pressure of 3×10^{-4} atm. To facilitate routine preparation of solutions, the amount of HCl required for pH adjustment was computed from the charge-balance deficit predicted by the program when pH was fixed at 4.2. Nutrient solutions were prepared using varying quantities of the following salts (ACS reagent grade; Sigma, St. Louis, MO): NH_4NO_3, KNO_3, $Ca(NO_3)_2 \cdot 4 H_2O$, $CaSO_4 \cdot 2 H_2O$, $NaH_2PO_4 \cdot H_2O$, $MgCl_2 \cdot 6 H_2O$, $MgSO_4 \cdot 7 H_2O$, Na_2–$EDTA \cdot 2 H_2O$, $Na_2SiO_3 \cdot 9 H_2O$, H_3BO_3, $MnSO_4 \cdot$ H_2O, $ZnSO_4 \cdot 7 H_2O$, $CuSO_4 \cdot 5 H_2O$, $Na_2MoO_4 \cdot 2 H_2O$, $FeCl_3 \cdot 6 H_2O$, NaCl, Na_2SO_4, and $AlCl_3 \cdot 6 H_2O$. When prepared with the predicted amounts of HCl, their pH values ranged from 4.15 to 4.23. The predicted activities of the main Al species and selected nutrients in the four treatments are listed in Table 3.

Growing *Brachiaria* Seedlings in the Designed Nutrient Solutions

Seeds of signalgrass (Agrosemillas, Medellín, Colombia) and ruzigrass (FundeAgro, Pucallpa, Peru) were surface-sterilized with 70% (v/v) ethanol for 1 min, followed by treatment with a solution containing 2% (w/v) NaOCl and 0.1% (v/v) Triton X-100 for 15 min. They were then spread on mosquito nets glued to styrofoam frames, which floated on the surface of a solution containing 200 μM $CaCl_2$ (pH = 4.2). After 5 d of germination, uniform seedlings were transferred to the four designed nutrient solutions (AN $\pm$ Al and LN $\pm$ Al; NO_3^- to NH_4^+ ratio = 10; Table 2), which were placed either in a growth chamber or greenhouse. The solutions were continuously aerated to prevent anaerobiosis in roots (aeration did not change the pH of the nutrient solutions).

Growth Chamber

Seedlings were grown in containers holding 110 mL of nutrient solution per individual plant. Nutrient solutions were prepared by adding an acidified Al-stock solution (16 mM $AlCl_3$, 4 mM HCl) and a 50× concentrated nutrient mixture (containing all nutrients plus the remainder of the HCl required to adjust the pH to 4.2) to vigorously stirred deionized water (MilliQ, 18 MΩ). The solutions were changed daily. Their pH tended to increase slightly over a 24-h period; the highest value in Al-free solutions, measured at harvest, was 4.45 (LN). The highest value in Al-containing solutions was 4.38 (LN + Al), a pH at which the Al^{3+} activity is predicted to be 40 μM (= 93% of its original value at pH 4.20; GEOCHEM).

Growth conditions were 24°C, a 12-h diurnal cycle, and a photon-flux density of photosynthetically active radiation of 110 μmol m^{-2} s^{-1}. Because growth was slow under these low-light conditions, groups of three individual plants were treated as an experimental unit. This experiment consisted of two

Table 1. Criteria used to design a low-ionic-strength nutrient solution that simulates soil solutions of the highly weathered acid soils in the Colombian savannas (LN + Al, low nutrient levels plus Al).

Parameter adjusted	Rational
pH = 4.2	• Low as in highly weathered acid soils • Leaves sufficient room to increase {Al^{3+}} beyond the adaptive range of most plants (up to 150 μM), without causing Al precipitation (Kinraide and Parker, 1989)
[Al^{3+}] = 80 μM	• Resulting solution ({Al^{3+}} = 43 μM) slightly oversaturated with respect to crystalline gibbsite, similar to soil solutions in this and other studies (Fig. 4; Bruce et al., 1989)
[$H_2PO_4^-$] = 1 μM	• Resulting activity well above the solubility limit in the two soils (approximately 0.2 μM at pH 4.2; Fig. 4) to compensate for the rapid depletion due to plant uptake in intermittently renewed solution culture (previously established empirically for *Brachiaria* seedlings)
[Ca^{2+}] = 60 μM, [Mg^{2+}] = 30 μM, and [K^+] = 60 μM	• Resulting activities simulating those in soil solutions of soils without fertilizer before the onset of nitrification (see values at {NO_3^-} < 1 mM in Fig. 3) • Resulting K^+ activity slightly lower to reflect its typically stronger depletion in rhizosphere soil (Fritz et al., 1994; Yanai et al., 1995)
[NO_3^-] + [NH_4^+] = 110 μM	• Simulating soil solutions depleted in mineral N as a result of plant uptake • Set of solutions with varying NO_3^- to NH_4^+ ratios designed to match nutritional requirements of different plant species and to minimize pH changes (Table 2; Asher, 1978)
[SO_4^{2-}] = 100 μM	• Resulting activity at the lower end of the range of values in soil solutions extracted from the sandy loam (without fertilizer) before the onset of nitrification, thus minimizing alleviation of Al toxicity due to formation of the non-toxic $AlSO_4^+$ complex (Fig. 3; Kinraide, 1997)
[Na^+] = 160 μM	• Resulting activity simulating that in soil solutions of soils without fertilizer before the onset of nitrification (Fig. 3)
[Cl^-] = 232.4–1671.4 μM	• Used as balancing ion to achieve electroneutrality, resulting in Cl^- activities comparable to those in soil solutions of soils without fertilizer (Fig. 3)

Table 2. Concentrations of Al and nutrients in the four treatments designed in this study. The concentration of Al was higher under adequate than under low nutrient supply to adjust the activity of Al³⁺, the principal phytotoxic species, to identical values (Table 3). For each treatment, seven alternative nutrient solutions with varying NO₃⁻ to NH₄⁺ ratios were designed (separated by "/"). The concentration of HCl required to adjust the pH to 4.2 was predicted by simulating the ionic speciation of each solution (see *Materials and Methods*).

	Concentrations, μM			
	Adequate nutrient levels (AN)		Low nutrient levels (LN)	
Ion	−Al	+Al	−Al	+Al
Al^{3+}	–	113	–	80
NO_3^-	550/500/400/275/150/50/–	550/500/400/275/150/50/–	110/100/80/55/30/10/–	110/100/80/55/30/10/–
NH_4^+	–/50/150/275/400/500/550	–/50/150/275/400/500/550	–/10/30/55/80/100/110	–/10/30/55/80/100/110
Ca^{2+}	300	300	60	60
Mg^{2+}	150	150	30	30
K^+	300	300	60	60
$H_2PO_4^-$	5	5	1	1
SO_4^{2-}	286	286	100	100
H_3BO_3	6	6	6	6
Fe^{3+}	5	5	5	5
Zn^{2+}	1	1	1	1
Mn^{2+}	1	1	1	1
Cu^{2+}	0.2	0.2	0.2	0.2
SiO_3^{2-}	5	5	5	5
H_2EDTA^{2-}	5	5	5	5
MoO_4^{2-}	0.001	0.001	0.001	0.001
Na^+	160	160	160	160
Cl^-	232.4/332.4/532.4/782.4/ 1032.4/1232.4/1332.4	571.4/671.4/871.4/1121.4/ 1371.4/1571.4/1671.4	88.4/108.4/148.4/198.4/ 248.4/288.4/308.4	328.4/348.4/388.4/438.4/ 488.4/528.4/548.4
HCl (pH = 4.2)	67.8/67.8/67.9/68.0/68.0/ 68.1/68.1	54.7/54.8/54.8/54.9/55.0/ 55.2/55.2	65.4/65.4/65.5/65.5/65.6/ 65.6/65.6	55.4/55.4/55.5/55.5/55.5/ 55.7/55.7

independent harvests, each comprising six experimental units (18 seedlings) for each of the two grasses and four treatments.

Greenhouse

Seedlings were grown in containers holding 350 mL of nutrient solution per individual plant. Nutrient solutions were prepared and changed daily as outlined for the growth chamber. The highest pH value in Al-free solutions, measured at harvest, was 4.53 (LN). The highest value in Al-containing solutions was 4.33 (LN + Al), a pH at which the Al³⁺ activity is predicted to be 41 μM (= 95% of its original value at pH 4.20; GEOCHEM).

The greenhouse was located at CIAT headquarters. Typical diurnal maximum and minimum temperatures in the greenhouse were 36 and 19°C. The typical maximum photon-flux density of photosynthetically active radiation during the day was 1100 $\mu mol\ m^{-2}\ s^{-1}$. This experiment consisted of two independent harvests, each comprising six individual plants for each of the two grasses and four treatments.

Measuring Plant Growth and Nutrient Uptake

Plants were harvested 20 d (growth chamber) or 13 d (greenhouse) after the seedlings had been transferred to the nutrient solutions. All plants from the growth chamber and half of the plants from the greenhouse were used to measure growth. Roots were stained in a solution containing 0.1% (w/v) methylene blue [3,7-bis(dimethylamino)phenothiazin-5-ium chloride] and 0.1% (w/v) neutral red [$N^{8,N8}$ 3-trimethyl-2,8-phenazinediamine monohydrochloride] for 24 h, and scanned with a flatbed scanner, which was also used to scan leaves. Root-image-analysis software (WinRHIZO, Regent Instruments Inc., Quebec, Canada) was used to determine total root length (RL) and leaf area (LA). The dry weights of roots (RDW) and shoots (SDW) were determined after drying plant material at 70°C for 2 d.

Half of the plants from the greenhouse, selected at random during harvest, were used to measure nutrient concentrations in tissues. Shoots and roots of individual plants were dried at 70°C for 2 d. They were then weighed and digested in a 2:1 mixture of 65% (w/w) HNO₃ and 70% (w/w) HClO₄ at 200°C for 2 h. The concentrations of Ca, Mg, K, and Al were determined, using atomic-absorption spectroscopy (Salinas and García, 1985).

Statistical Analysis

All growth data were log-transformed (Sokal and Rohlf, 1995) before being subjected to Model I ANOVA to test for interaction between the effects of Al toxicity and nutrient supply on plant growth. Relative growth parameters were computed using weighted jackknifing (Buonaccorsi and Liebhold, 1988), and compared with t tests, based on separate variance estimates if heteroscedasticity was detected with Levene's test. ANOVA of nutrient-concentration data was per-

Table 3. Activities of selected ions and ion complexes in the four treatments designed in this study as predicted by GEOCHEM software. For those cases where activities varied for solutions with different NO₃⁻ to NH₄⁺ ratios, the predicted range of values is shown.

	Activity, μM			
	Adequate nutrient levels (AN)		Low nutrient levels (LN)	
Ion or complex	−Al	+Al	−Al	+Al
Al^{3+}	–	42–44	–	43
$AlOH^{2+}$	–	6.6–8.6	–	6.7–6.9
$AlSO_4^+$	–	26–28	–	10
$AlPO_4$	–	2.5–2.6	–	0.5
K^+	283–285	282–283	58	58
Ca^{2+}	231–236	228–233	52–53	51
$CaSO_4$	9.9–10.3	8.8–9.1	0.9	0.8
Mg^{2+}	116–119	115–117	26	25–26
$MgSO_4$	4.0–4.1	3.5–3.6	0.4	0.3
$H_2PO_4^-$	4.7	2.1–2.2	1.0	0.4
$(Fe\text{-}EDTA)^-$	4.7	4.7	4.8	4.8
Mn^{2+}	0.8	0.8	0.9	0.9
Zn^{2+}	0.8	0.8	0.9	0.9
Cu^{2+}	0.14	0.15	0.17	0.17
H_3BO_3	6.0	5.9	6.0	5.9
SO_4^{2-}	216–220	191–194	86–87	75–76

formed without data transformation, and differences between group means were tested for significance using the Spjotvoll/Stoline test.

RESULTS

Composition of Soil Solutions

The charge balance in soil solutions extracted from the clay loam was close to neutrality (1 ± 9% excess of anions; mean ± SD), while those from the sandy loam contained a slight excess of cations (9 ± 14%; mean ± SD). Coefficients of variation for ion concentrations, pH and electrical conductivity of soil solutions, extracted from separate soil aliquots after 64 d of incubation, ranged from 6 to 26%.

Effect of Fertilizer Application

Nutrient concentrations in soil solutions from soil samples without fertilizer, extracted shortly after rewetting, were very low. Their ionic strength varied between 1.3 and 1.7 mM. Fertilizer application significantly increased the concentrations of cationic macronutrients (micronutrients were not measured). This effect was more pronounced in the sandy loam. The cation-activity ratio, that is, $-\log [\{K^+\}/(\{Ca^{2+}\} + \{Mg^{2+}\})^{0.5}]$, was between 1.9 and 2.2 in soil solutions of samples without fertilizer (Moss, 1963). Fertilization decreased these values to between 1.2 and 1.3 (sandy loam) or between 1.5 and 1.6 (clay loam).

Fertilizer application also increased the levels of anions in soil solutions, except phosphate, the increase being, again, more pronounced in the sandy loam. In soil solutions extracted from the clay loam, SO_4^{2-} levels were one to two orders of magnitude lower than for solutions from the sandy loam, even if fertilizer had been added.

Soil-Solution pH and Nitrogen Mineralization

The pH of soil solutions increased by 1 to 1.5 units during the first 16 d after rewetting, and dropped to a similar extent between Days 16 and 64. The amplitudes of pH fluctuations were larger in the sandy loam than in the clay loam (Fig. 1A). The acidification of soil solutions from Day 16 onward was clearly associated with the onset of nitrification, which led to a pronounced accumulation of NO_3^-, particularly in soil solutions from sandy loam + fertilizer (Fig. 1B). By contrast, ammonium concentrations in soil solutions remained virtually constant throughout the incubation of soil samples. They were higher in the soil samples + fertilizer, presumably because the urea in the fertilizer was partly mineralized (Fig. 1C).

The Effect of Nitrification on Cation and Anion Levels

The substantial increase in the concentration of NO_3^- was balanced by a simultaneous increase in the concentration of Ca, Mg, K, and, to a lesser extent, Na. In soil solutions from the soil samples without fertilizer, the concentration of NO_3^- was close to the sum of cat-
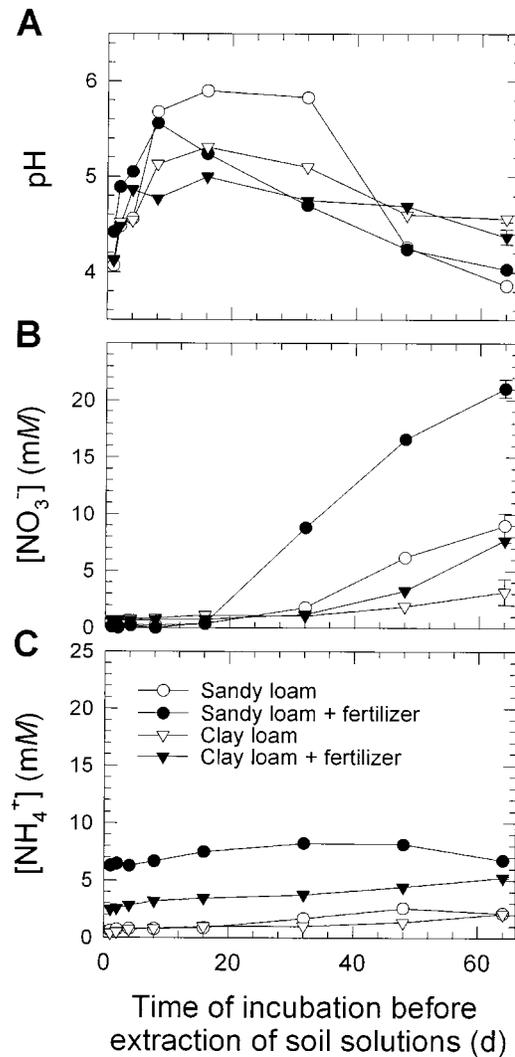

Fig. 1. Nitrogen mineralization and pH changes in soil solutions. The (A) pH and (B) concentrations of NO_3^- and (C) NH_4^+ were determined in soil solutions extracted from soil samples of sandy loam and clay loam (incubated for up to 64 d after rewetting).

ionic equivalents (Fig. 2). Before the onset of nitrification (at NO_3^- concentrations smaller than 1 mM), however, there was a significant excess of cations over NO_3^- (data not shown). In the soil samples + fertilizer, other anions, including Cl^- (both soils) and SO_4^{2-} (sandy loam), contributed to balancing the positive charges of
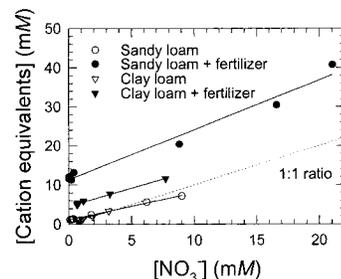

Fig. 2. Relationship between the concentrations of NO_3^- and cations in soil solutions extracted from soil samples of sandy loam and clay loam (incubated for up to 64 d after rewetting). The dotted line designates a hypothetical equimolar ratio between the two.
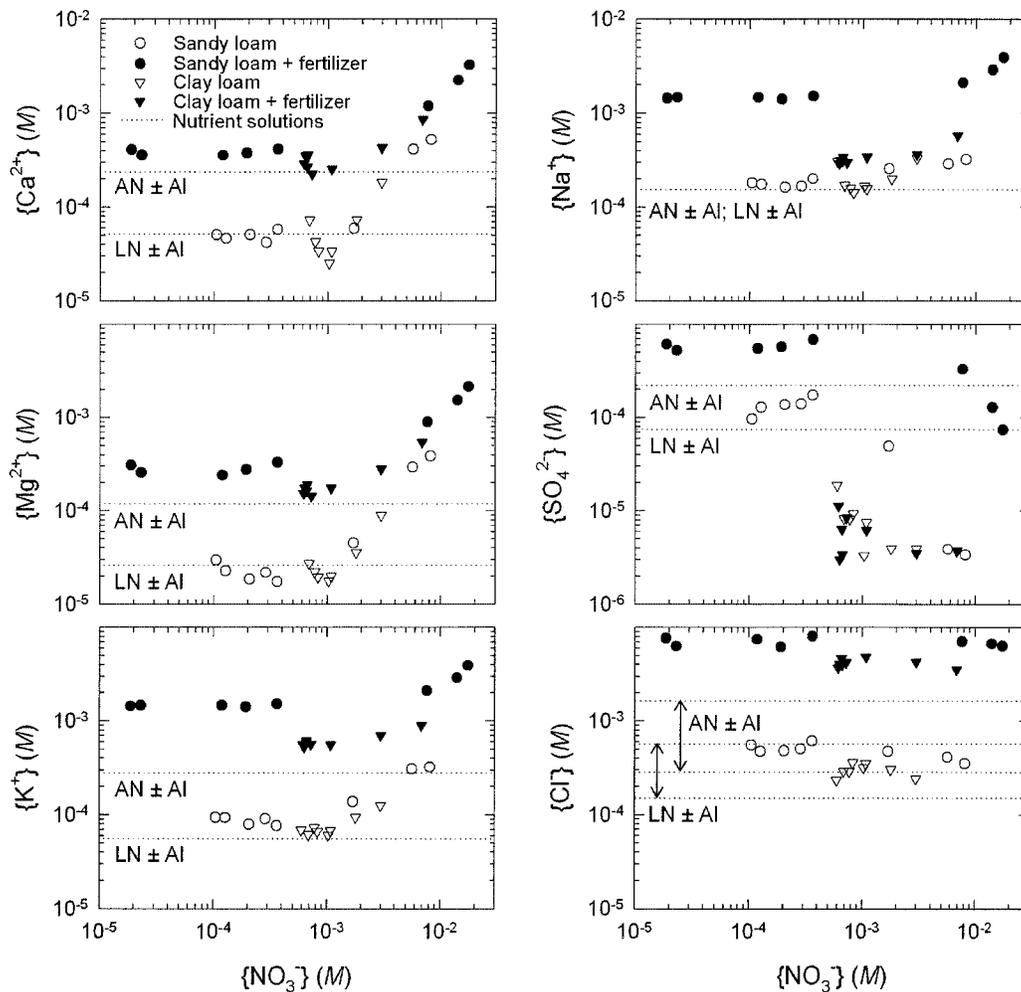
Fig. 3. Activities of major cations (Ca^{2+}, Mg^{2+}, K^+, Na^+) and anions (SO_4^-, Cl^-) in soil solutions extracted from soil samples of sandy loam and clay loam (incubated for up to 64 d after rewetting). Activities were plotted against that of NO_3^-, which increased substantially during nitrification. Dotted lines indicate the activities of the same ions in the four designed treatments for comparison (AN ± Al, LN ± Al).

cations. This can be deduced from their higher levels and the significant excess of cationic charge equivalents over NO_3^- in soil solutions from the soil samples + fertilizer (Fig. 2 and 3).

When the activity of each cation was plotted against that of NO_3^-, a linear relationship with two distinct phases became apparent. At {NO_3^-} < 1 mM, slopes were close to zero, but at greater activities they became positive (Fig. 3). Despite the substantial increase of cation levels, cation-activity ratios in soil solutions of all four soil samples remained approximately constant (data not shown). In contrast to cations, anion activities tended to decrease as {NO_3^-} increased in the course of nitrification (Fig. 3).

The acidification associated with nitrification increased the solubility of Al. A plot of log {Al^{3+}} versus pH suggested that Al solubility was controlled by a form of gibbsite that was slightly more soluble than the crystalline form, which often puts an upper limit to Al solubility in acid soils (Lindsay and Walthall, 1989). Only soil solutions extracted shortly after rewetting were significantly undersaturated with respect to crys-

talline gibbsite (Fig. 4). There was no evidence that kaolinite decreased Al solubility below that of gibbsite.

The activities of $H_2PO_4^-$ in soil solutions indicated that variscite in equilibrium with gibbsite imposed an upper limit to P solubility (Barber, 1984). Only shortly after rewetting, $H_2PO_4^-$ activities exceeded significantly this solubility limit (Fig. 4).

Growth of *Brachiaria* Seedlings in the Designed Nutrient Solutions

Based on the composition of soil solutions analyzed in the first part of this study, a treatment was designed to simulate, in liquid culture, the chemical properties of the highly weathered acid soils of the Colombian savannas (LN + Al, low nutrient levels plus Al; Table 1).

Effect of the Treatment Simulating Chemical Properties of Savanna Soils

Dry matter production of signalgrass and ruzigrass under unstressed conditions was 152 and 180 mg plant^{-1} in the greenhouse and 52.8 and 85.8 mg (3 plants)$^{-1}$ in
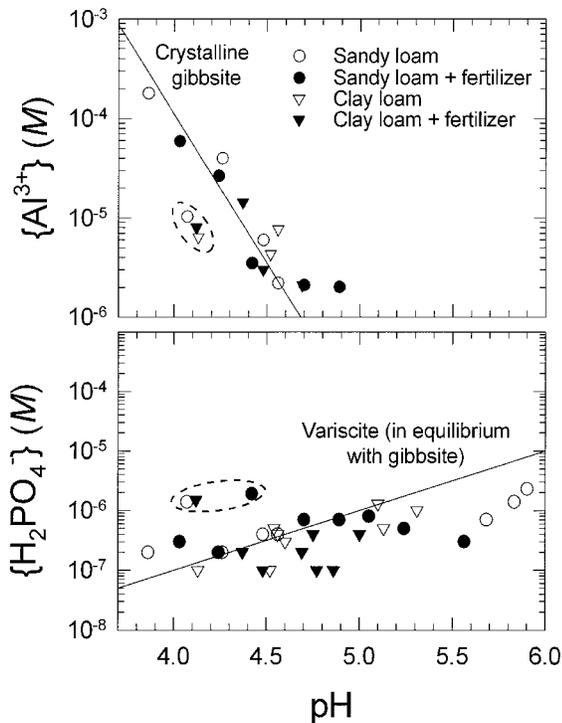
Fig. 4. Solubilities of Al and phosphate in soil solutions as a function of pH. The activities of Al^{3+} and $H_2PO_4^-$ in soil solutions extracted from soil samples of sandy loam and clay loam (incubated for up to 64 d after rewetting) were plotted against soil-solution pH. Lines show the upper solubility limits of crystalline gibbsite and variscite in equilibrium with gibbsite. Data points within dotted ellipses are from soil solutions extracted 1 d after the soil samples were rewetted. Because of the limited sensitivity of the colorimetric assay used to quantify Al_{inorg}, only $\{Al^{3+}\}$ values greater than 1 μM are shown in the top plot.
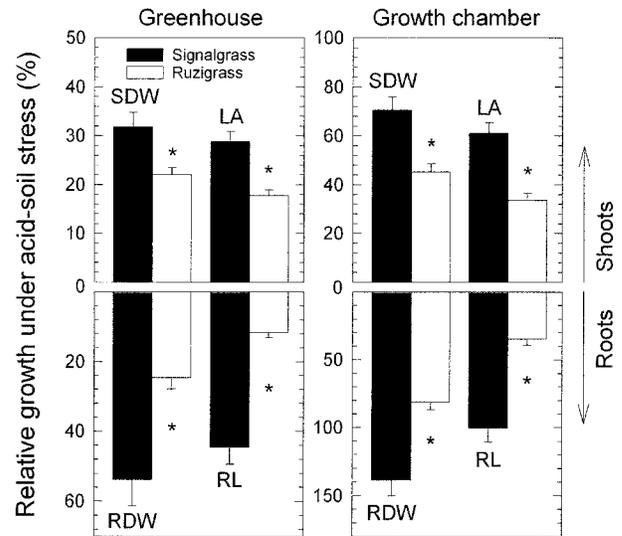

Fig. 5. Growth-inhibitory effect of the nutrient solution simulating chemical properties of infertile acid soils (LN + Al). Signalgrass and ruzigrass were grown in the greenhouse (left panel) and growth chamber (right panel). Growth was measured relative to the Al-free reference treatment containing adequate nutrient levels (i.e., [LN + Al]/AN). Values of growth parameters for plants from the AN treatment in the greenhouse were: 125.8 mg (shoot dry weight, SDW), 26.3 mg (root dry weight, RDW), 40.5 cm^2 (leaf area, LA), and 12.1 m (root length, RL) for signalgrass, and 139.3 mg (SDW), 40.5 mg (RDW), 52.5 cm^2 (LA), and 18.8 m (RL) for ruzigrass. Corresponding values in the growth chamber were: 47.5 mg (SDW), 5.3 mg (RDW), 23.4 cm^2 (LA), and 1.43 m (RL) for signalgrass, and 76.0 mg (SDW), 9.8 mg (RDW), 39.9 cm^2 (LA), and 2.81 m (RL) for ruzigrass. Error bars designate standard errors computed by weighted jackknifing, and asterisks indicate statistically significant differences between the grasses ($P < 0.05$).

the growth chamber, respectively (AN, adequate nutrient levels; Table 2). The effect of the LN + Al treatment, simulating soil solutions of the Colombian savanna soils, was evaluated by quantifying growth relative to the AN treatment. The less-adapted ruzigrass was considerably more affected than the well-adapted signalgrass, irrespective of whether plants were grown in the greenhouse or growth chamber (Fig. 5). Statistically significant interspecific differences were observed for the relative SDW and RDW, LA, and RL ($P < 0.05$ to $P < 0.001$ for the various growth parameters).

Interaction between the Effects of Aluminum Toxicity and Nutrient Deficiency

The growth-inhibitory effects of two important stress components in low-fertility acid soils of the Colombian savannas—nutrient deficiency and Al toxicity—were evaluated separately. Nutrient deficiency decreased the total dry weight of both *Brachiaria* species to a similar extent, by about 57% in the greenhouse ($P < 0.001$) or 20% in the growth chamber ($P < 0.05$) (computed from the LN/AN ratio). The two grasses also responded similarly to Al toxicity: total dry weight decreased by 23% in the greenhouse ($P < 0.05$) and was unaffected by Al in the growth chamber (computed from the ratio [AN + Al]/AN).

The analysis of variance of growth data from the plants grown in the greenhouse, however, indicated a statistically significant interaction between the effects of Al toxicity and nutrient deficiency in ruzigrass but not in signalgrass (Table 4). Corresponding data ob-

Table 4. Direct and interaction effects of Al toxicity and nutrient deficiency on the growth of signalgrass and ruzigrass in the greenhouse.†

| Stress factor | Parameter‡ | Statistical significance | |
		Signalgrass	Ruzigrass
Nutrient deficiency	SDW	***	***
	LA	***	***
	RDW	***	***
	RL	***	***
Al toxicity	SDW	**	***
	LA	***	***
	RDW	*	***
	RL	**	***
Nutrient deficiency × Al toxicity	SDW	ns§	***
	LA	ns	***
	RDW	ns	**
	RL	ns	*

* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
*** Significant at the 0.001 probability level.
† Results of a two-way Model I ANOVA for each grass; data from the experiment in the growth chamber displayed a similar trend, although they were complicated by the slightly stimulatory effect of Al toxicity on root elongation of signalgrass under low nutrient supply.
‡ SDW, shoot dry weight; LA, leaf area; RDW, root dry weight; RL, root length. For mean values see Fig. 5 and 7, including their legends.
§ ns, not significant.

Signalgrass

Ruzigrass
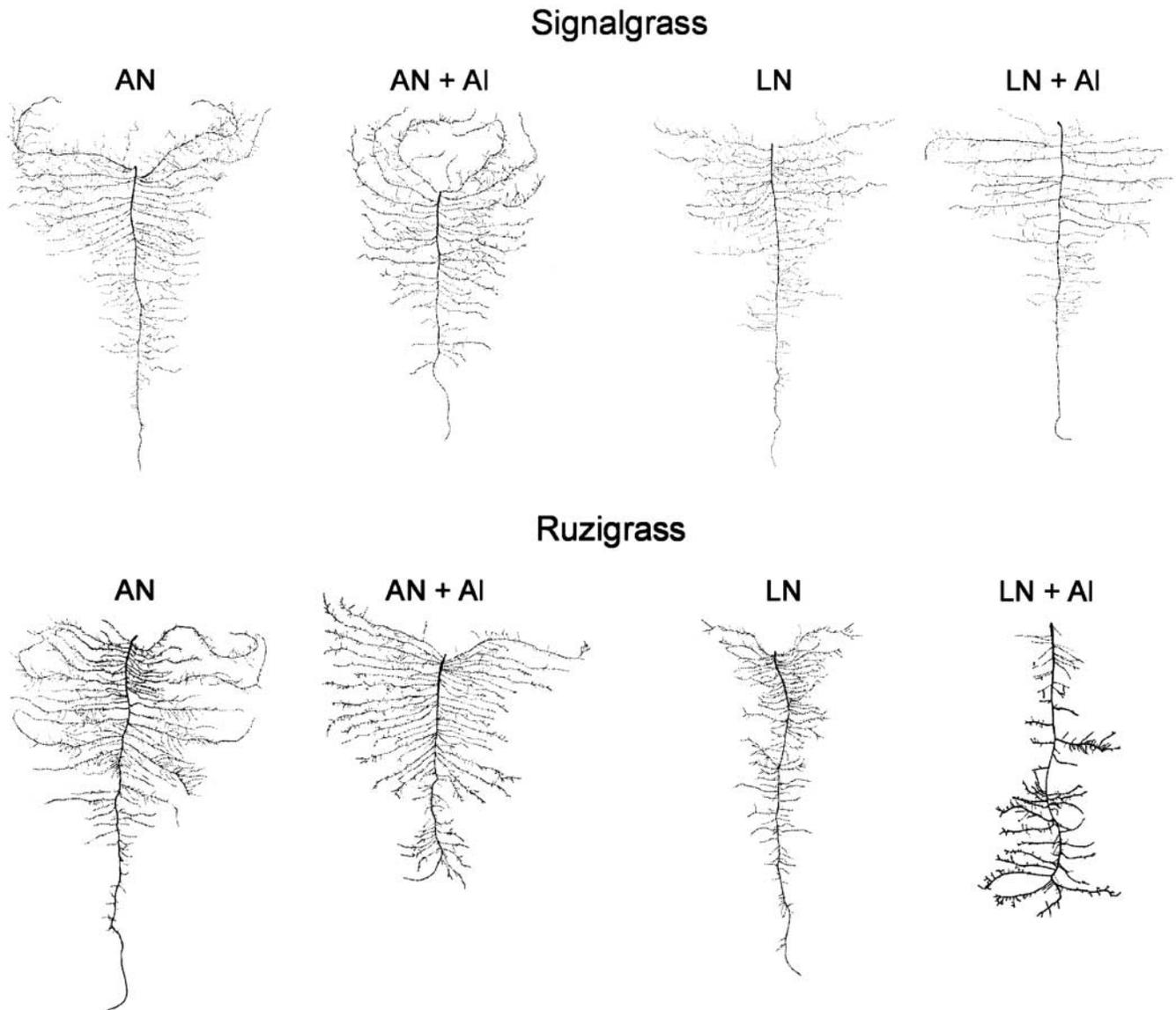
Fig. 6. Interaction between the effects of Al toxicity and nutrient deficiency on root architecture of plants grown in the greenhouse. The images display primary root systems of plants grown under adequate (AN) and low (LN) nutrient supply and in the presence or absence of Al.

tained from the plants grown in the growth chamber displayed essentially the same trend, although they were complicated by a slightly stimulatory effect of Al on root growth of signalgrass under low nutrient supply (data not shown). This interaction was associated with an alteration of the morphology and architecture of the root system of ruzigrass. Roots of plants simultaneously exposed to both stress factors were thicker and had more laterals close to the tip of the main axis, both typical symptoms of Al injury (Fig. 6).

We therefore evaluated the effect of Al toxicity at both levels of nutrient supply. As indicated previously, hardly any difference was found between the two grasses at adequate nutrient supply ([AN + Al]/AN). (Only the total length and dry weight of roots of ruzigrass grown in the greenhouse were somewhat more affected than those of signalgrass; $P < 0.05$, Fig. 7B). In contrast, there was a pronounced difference in Al resistance be-

tween the two grasses under nutrient-deficient growth conditions ([LN + Al]/LN), irrespective of whether the plants were grown in the greenhouse or growth chamber ($P < 0.05$ to $P < 0.001$ for the various growth parameters; Fig. 7A).

Effects on Nutrient Uptake

The mineral composition of shoots displayed a similar trend to that of roots (correlation coefficients for shoot vs. root values ranged from 0.52 for K to 0.95 for Mg; computed with data from both grasses and all treatments). The only exception to this trend was Al, which accumulated in the roots of both grasses without being translocated to shoots. The inhibitory effects of Al toxicity and nutrient deficiency on nutrient acquisition were therefore evaluated based on nutrient concentrations in shoots, which were unaffected by the contributions of
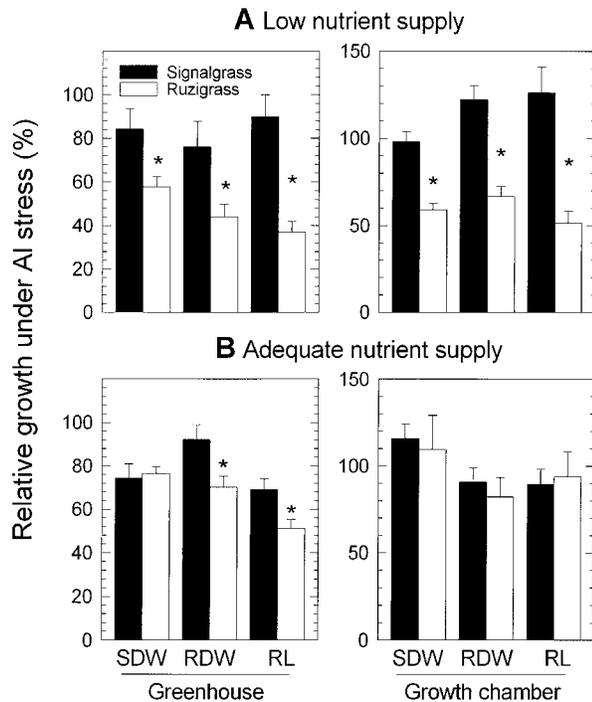
Fig. 7. Aluminum resistance of signalgrass and ruzigrass and its dependence on nutrient concentrations in the growth medium. Resistance to Al was measured at (A) low and (B) adequate nutrient supply, by quantifying its effect on plant growth relative to the corresponding Al-free treatment ([LN + Al]/LN and [AN + Al]/ AN, respectively). Values of growth parameters for plants from the LN treatment in the greenhouse were 47.3 mg (shoot dry weight, SDW), 18.3 mg (root dry weight, RDW), 15.3 cm² (leaf area, LA), and 5.98 m (root length, RL) for signalgrass, and 52.8 mg (SDW), 22.7 mg (RDW), 19.1 cm² (LA), and 5.92 m (RL) for ruzigrass. Corresponding values in the growth chamber were 34.5 mg (SDW), 6.2 mg (RDW), 13.8 cm² (LA), and 1.15 m (RL) for signalgrass, and 58.3 mg (SDW), 11.8 mg (RDW), 21.2 cm² (LA), and 1.89 m (RL) for ruzigrass. The values for plants from the AN treatment are listed in the legend to Fig. 5. Error bars designate standard errors computed by weighted jackknifing, and asterisks indicate statistically significant differences between the two grasses ($P <$ 0.05).

ions passively adsorbed to cation-exchange sites in root cell walls. An ANOVA showed that a low supply of nutrients during growth reduced uptake of N, P, and K in both grasses to a similar extent (Table 5). In the case of Ca and Mg, this inhibitory effect was amplified by Al toxicity, as indicated by the statistically significant interaction between the effects of nutrient supply and Al toxicity (Table 5). No difference in nutrient acquisition between the two grasses was detected.

Aluminum-stressed plants grown at low nutrient levels (LN + Al) differed from their counterparts grown at adequate nutrient levels (AN + Al) only in their P and Mg content ($P < 0.05$). Shoot concentrations of other nutrients were statistically indistinguishable (Fig. 8).

DISCUSSION
Oxisols of the Colombian Savannas Are Extremely Poor in Nutrients

Soil solutions extracted from soils without fertilizer before the onset of nitrification were more dilute than those from most other soils reported in the literature (ionic strength <1.7 mM; Altman and Dittmer, 1966; Edmeades et al., 1985; Menzies and Bell, 1988; Parker and Norvell, 1999). They contained fewer nutrients than soil solutions from acidic topsoils from Australia and New Zealand, which had previously been used as a model to design low-ionic-strength nutrient solutions for Al-toxicity studies (Gillman and Bell, 1978; Bruce et al., 1989). The poor nutrient status of the Colombian samples presumably reflects an advanced state of soil weathering. Hence, both Al toxicity and nutrient deficiency might limit plant growth in these savanna soils.

Adding fertilizer increased the ionic strength of soil solutions from 1.3–1.7 mM to values similar or greater than those of soil solutions used to design low-ionic-strength nutrient solutions (5.4–13.4 mM) (Gillman and Bell, 1978; Edmeades et al., 1985; Bruce et al., 1989).

Rewetting Soils Stimulates Nitrification

The seasonal pattern of soil drying and rewetting induces microbially mediated fluctuations of pH and mineral N in tropical soils (Sánchez, 1976; Gigou et al., 1985; Sylvester-Bradley et al., 1988; Mueller-Harvey et al., 1989). Studies in rice fields in the Colombian savannas, for example, have shown that soil solutions become increasingly acidic over the course of the wet season (CIAT, 1994; Okada and Fischer, 2001).

After an initial rise in pH, perhaps caused by slow equilibration of the soils with the added water, we found a similar acidification of soil solutions, starting 2 to 4 wk after rewetting (Fig. 1A). This acidification was presumably caused by nitrification, well known for its acidifying effect, because it was associated with a substantial increase in the concentration of NO_3^- (Fig. 1B). This suggests that rewetting the soil samples, which had been collected at the end of the wet season and subsequently air-dried, might have stimulated nitrification in a similar way as the onset of the wet season presumably does in the field.

The NO_3^- produced during nitrification may not have been derived from ammonium, because ammonium levels in soil solutions did not decrease as nitrification set in (Fig. 1C). This finding is similar to the results of previous experiments with soil cores collected in the Colombian savannas (Sylvester-Bradley et al., 1988). Together, they are consistent with the suggestion that heterotrophic nitrifiers might have produced the NO_3^- directly from organic substrates. Heterotrophic nitrification often dominates in acid soils because autotrophic nitrifiers are inhibited at low pH (Haynes, 1986).

Nitrification Affects Soil-Solution Composition

Before the onset of nitrification, cation levels in soil solutions were not correlated with that of NO_3^- (see {NO_3^-} <1 mM; Fig. 3). Chloride appeared to balance most of the positive charges of cations because it was more abundant than NO_3^- and SO_4^{2-}. Sodium was the most abundant cation in soil solutions from soils without fertilizer, but not in soils + fertilizer (Fig. 3). Dominance

Table 5. Inhibitory effects of Al toxicity and nutrient deficiency on the uptake of selected nutrients by signalgrass and ruzigrass. (The two grasses did not differ.)†

Nutrient	Range of concentrations in shoots‡	Stress factor	Statistical significance
	g kg^{-1}		
N	34–48	Nutrient deficiency	***
P	1.8–4.5	Nutrient deficiency	***
K	47–68	Nutrient deficiency	***
Ca	1.3–5.2	Nutrient deficiency	***
		Al toxicity	***
		Nutrient deficiency × Al toxicity	**
Mg	0.7–4.1	Nutrient deficiency	***
		Al toxicity	***
		Nutrient deficiency × Al toxicity	*

* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
*** Significant at the 0.001 probability level.
† Results of a three-way Model I ANOVA (species × nutrient supply × Al toxicity).
‡ For individual values of plants grown in Al-containing solutions, see Fig. 8.

of Na has been reported for soil solutions of subsoils, but only occasionally for surface soils (Bruce et al., 1989).

The activities of other cations (before nitrification) were in the order of $K^+ \geq Ca^{2+} > Mg^{2+}$, irrespective of soil type or fertilizer addition (Fig. 3). Yet cation-activity ratios in soil solutions from soils without fertilizer were higher than those from fertilized soils, indicating a comparatively low abundance of K relative to divalent cations in native soils (see *Results*).

The acidification associated with nitrification had a profound impact on the composition of soil solutions. Above a NO_3^- activity of approximately 1 mM, cation activities increased in parallel to the activity of NO_3^-, the latter being a measure of the amount of protons produced by nitrification (Fig. 3). The stoichiometric relationships between the amounts of NO_3^- and cations accumulating in soil solutions, and the approximately constant cation-activity ratios (see *Results* for a definition), together suggest that the protons produced during nitrification desorbed cations from pH-dependent cation-exchange sites in proportion to their abundance on the exchange phase (Fig. 2; Moss, 1963; Curtin and Smillie, 1995). A similar mechanism of cation release appears to exist in the field, because the progressive acidification of soil solutions in rice fields during the wet season, ob-served in a previous field study in the Colombian savannas, was also accompanied by a marked increase in cation concentrations (CIAT, 1994; Okada and Fischer, 2001).

These data suggest that nitrification may be an important factor affecting the soil-solution composition in these soils if levels of organic N and/or NH_4^+ are high enough. In contrast to our soil-incubation experiments, leaching and uptake of NO_3^- by plants counteract the impact of seasonal peaks of nitrification on soil-solution composition in the field (Sánchez, 1976; Gigou et al., 1985; Sylvester-Bradley and Mosquera, 1985; Mueller-Harvey et al., 1989). In established pastures NO_3^- salts are therefore not expected to accumulate to the same extent as in this study. The acidifying effect of nitrification, however, allowed us to establish that the solubilities of Al^{3+} and $H_2PO_4^-$ were controlled by gibbsite and variscite (in equilibrium with gibbsite), respectively (Fig. 4; Barber, 1984; Lindsay and Walthall, 1989).

A Nutrient Solution Simulating Chemical Properties of Highly Weathered Acid Soils

One of the goals of this study was to design a nutrient solution that simulates soil solutions of highly weathered acid soils (LN + Al, low nutrient levels plus Al). We used the composition of soil solutions from soils without fertilizer before the onset of nitrification as a guideline to adjust the activities of Al^{3+} and macronutrients to values typical for the Colombian savanna soils. For micronutrients, the concentrations in the low-ionic-strength nutrient solution described by Blamey et al. (1991) were adopted. No attempts were made to adjust their concentrations to lower levels because maintaining such low levels would have required a flowing solution culture system. Manganese toxicity was not considered in this study because its occurrence depends on the soil's parent material, and other South American savanna soils have been shown to contain lower-than-world-average levels of Mn (de Sá e Melo Marques, 2000). Table 1 details the criteria used to design the LN + Al solution based on the results of the first part of this study.

The concentrations of most macronutrients in the LN + Al treatment are lower than in other low-ionic-strength nutrient solutions that have been used for Al-toxicity studies (Blamey et al., 1983, 1991; Wheeler et
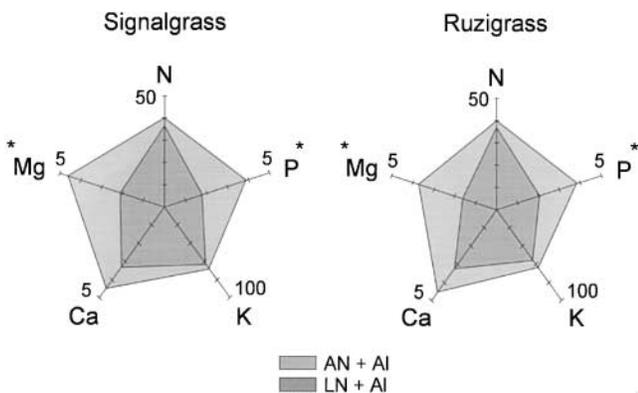
Fig. 8. Mineral composition of shoots of plants grown at two levels of nutrient supply in the presence of Al (AN + Al and LN + Al treatments). Nutrient concentrations are shown in grams per kilogram (g kg^{-1}) of dry weight. Numbers designate the maximum values of the scales of five different axes, each used to plot the concentration of a single nutrient. Asterisks indicate statistically significant differences between the two treatments ($P < 0.05$).

al., 1992; Edmeades et al., 1995). Plant growth is likely to be limited as a result of such low nutrient levels (Asher, 1978, 1986).

To accurately quantify the growth-inhibitory effect of the LN + Al treatment, growth has to be referenced against the plants' inherent growth potential, measured under unstressed conditions (AN, adequate nutrients without Al). Nutrient concentrations in soil solutions from soils + fertilizer were similar or greater than those in the low-ionic-strength nutrient solution designed by Blamey et al. (1991) to support close-to-maximum growth rates, while minimizing problems with Al speciation. Because it would be desirable to measure the effect of Al at adequate (but not excessive) nutrient levels, we did not adjust nutrient concentrations of the AN solution above their levels in that solution. Only the concentration of P was raised from 2 to 5 μM to compensate for depletion of such a small quantity in intermittently renewed solution culture (Table 2). Previous greenhouse experiments at variable P and N levels, but otherwise identical conditions, had confirmed that their concentrations in the AN solution resulted in 84 to 94% of maximum yield for the two grasses (Lasso, 1998).

To disentangle the effects of Al toxicity and nutrient deficiency, we also designed two solutions that exhibited either an identical activity of the principal phytotoxic Al species (Al^{3+}; AN + Al) or identical nutrient concentrations (LN) as the LN + Al treatment (Tables 2 and 3).

The Growth Response of two *Brachiaria* Species in the Designed Nutrient Solution Mirrors the Difference in Forage Yield in the Field

To test the effectiveness of the LN + Al treatment in simulating the chemical properties of the Colombian savanna soils, we evaluated growth of two contrasting *Brachiaria* species relative to unstressed conditions ([LN + Al]/AN). Results showed that the acid soil-adapted signalgrass was significantly less affected by simultaneous Al stress and nutrient deficiency than was the less-adapted ruzigrass. This growth difference was similar to the differences in forage yield measured in a previous field evaluation at the Pista site, where the samples of clay loam had been collected for this study (CIAT, 1995).

Comparable results were obtained whether plants were grown in the greenhouse or growth chamber, suggesting that physiological responses to this treatment were reproducible between very different growth regimes (Fig. 5). Hence, this technique should be a robust method to identify *Brachiaria* genotypes adapted to infertile acid soils. Relative growth of *B. brizantha* cv. Marandú, a *Brachiaria* species of intermediate edaphic adaptation, was intermediate between the other two species in all experiments (data not shown). These data are consistent with the suggestion that the LN + Al treatment provides a realistic approximation to the stress factors that limit plant growth in the highly weathered acid soils of the Colombian savannas.

Nutrient Deprivation Affects Aluminum Resistance of the Less-Adapted *Brachiaria* Species

Having tentatively established the effectiveness of the LN + Al treatment in simulating chemical factors that limit plant growth in these savanna soils, we dissected the stress syndrome into two of its components. This was accomplished by evaluating the relative effects of nutrient deficiency and Al toxicity in separate treatments (LN/AN and [AN + Al]/AN, respectively).

A low supply of nutrients reduced growth in both signalgrass and ruzigrass to the same extent (see *Results*). A similar result was obtained for Al toxicity, except for a moderate difference in the dry weight and total length of roots of plants grown in the greenhouse (Fig. 7B). The pronounced growth difference between the two grasses under simultaneously Al-toxic and nutrient-deficient conditions, therefore, was the result of an interaction between the effects of the two stress factors on growth of less adapted ruzigrass (Fig. 5, Table 4). In principle, either inhibition of nutrient uptake by Al or an increase in Al susceptibility under nutrient deprivation could account for this interaction. The distinct root morphology and architecture of ruzigrass grown in the LN + Al solution, reminiscent of the effects of Al toxicity, suggested that its level of Al resistance was affected by the low nutrient concentrations in the medium (Fig. 6).

We therefore compared Al susceptibility of the grasses at the two different levels of nutrient supply. Only under nutrient-limited growth conditions did a clear interspecific difference in Al resistance appear (Fig. 7A). Again, *B. brizantha* cv. Marandú ranked between the other two *Brachiaria* species, in accordance with its intermediate level of edaphic adaptation (data not shown). This increase in Al sensitivity under low nutrient supply may help explain the poor persistence of ruzigrass in South American savannas. It may also explain why a root-elongation assay in simple salt solutions had revealed a marked difference in Al resistance between signalgrass and ruzigrass, although we could not detect this difference in a previous experiment with fertilized soil samples exhibiting varying degrees of Al saturation (Rao et al., 1996; Wenzl et al., 2001).

To identify a particular nutrient, whose deficiency could account for an increased sensitivity of ruzigrass to Al, we analyzed nutrient uptake by plants. There was no difference between the two grasses in the way nutrient deficiency and Al-toxicity inhibited nutrient acquisition (Table 5). This appears to indicate that the adverse effect of nutrient deficiency on Al resistance of ruzigrass was not due to an inefficient uptake of nutrients. Instead, it may have been the result of a less effective use of scarce nutrient(s) in physiological processes that prevent or alleviate Al phytotoxicity.

We therefore identified the nutrients whose tissue concentrations decreased most strongly when nutrient deficiency was superimposed on Al stress, because a lower tissue concentration is likely to affect the efficiency with which a particular nutrient can be employed to counteract Al phytotoxicity. As shown in Fig. 8, the uptake of P and Mg was affected the strongest. Differ-

ences in the way either of these nutrients is employed to counteract Al toxicity may therefore be responsible for the marked difference in Al resistance between the two grasses under nutrient-deficient growth conditions.

CONCLUSIONS

This study has confirmed the poor nutrient status of the highly weathered Oxisols of the Colombian savannas, thus highlighting the likelihood that plant growth in this area is simultaneously limited by Al toxicity and multiple nutrient deficiencies. Based on the similarity of the growth response of signalgrass and ruzigrass in the field and in solution culture, we suggest that the nutrient solution designed to simulate soil solutions is a realistic approximation to the stress syndrome in these soils.

The poorer growth of less-adapted ruzigrass in this solution appeared to be the result of an insufficient capacity simultaneously to counteract Al toxicity and nutrient deficiency, even though these stresses could be successfully dealt with separately. Clearly, experimental isolation of individual stress components of highly weathered acid soils is a prerequisite for mechanistic studies. The data presented, however, highlight the need for a complementary holistic approach that conceptualizes adaptation to infertile acid soils as a coordinated physiological adjustment to several stress factors. Analysis of growth in the designed nutrient solution may serve to rapidly test this holistic aspect of a plant's adaptive capacity.

REFERENCES

Aitken, R.L., and R.J. Outhwaite. 1987. A modified centrifuge apparatus for extracting soil solution. Commun. Soil Sci. Plant Anal. 18: 1041–1047.

Altman, P.L., and D.S. Dittmer. 1966. Environmental biology. Federation of American Societies for Experimental Biology, Bethesda, MD.

Asher, C.J. 1978. Natural and synthetic culture media for spermatophytes. p. 575–609. In M. Rechcigl Jr. (ed.) CRC Handbook series in nutrition and food. Vol. III. Section G: Diets, culture media, food supplements. CRC Press, Cleveland, OH.

Asher, C.J. 1986. Effects of nutrient concentration in the rhizosphere on plant growth. Trans. XIII Congr., Int. Soc. Soil Sci. (Hamburg) 5:208–216.

Barber, S.A. 1984. Soil nutrient bioavailability. A mechanistic approach. John Wiley & Sons, New York.

Bartlett, R.J. 1965. A biological method for studying aeration status of soil in situ. Soil Sci. 100:403–408.

Blamey, F.P.C., D.C. Edmeades, C.J. Asher, D.G. Edwards, and D.M. Wheeler. 1991. Evaluation of solution culture techniques for studying aluminium toxicity in plants. p. 905–912. In R.J. Wright et al. (ed.) Plant-soil interactions at low pH. Kluwer Academic Publishers, Dordrecht.

Blamey, F.P.C., D.G. Edwards, and C.J. Asher. 1983. Effects of aluminum, OH:Al and P:Al molar ratios, and ionic strength on soybean root elongation in solution culture. Soil Sci. 136:197–207.

Buonaccorsi, J.P., and A.M. Liebhold. 1988. Statistical methods for estimating ratios and products in ecological studies. Environ. Entomol. 17:572–580.

Bruce, R.C., L.A. Warrell, L.C. Bell, and D.G. Edwards. 1989. Chemical attributes of some Queensland acid soils: I. Solid and solution phase compositions. Aust. J. Soil Res. 27:333–351.

CIAT. 1994. Annual report 1994. Rice Program. CIAT, Cali, Colombia.

CIAT. 1995. Biennial report 1994–1995. Tropical Forages. Working Document No. 152. CIAT, Cali, Colombia.

Curtin, D., and G.W. Smillie. 1995. Effects of incubation and pH on soil solution and exchangeable cation ratios. Soil Sci. Soc. Am. J. 59:1006–1011.

Edmeades, D.C., F.P.C. Blamey, and M.P.W. Farina. 1995. Techniques for assessing plant responses on acid soils. p. 221–233. In R.A. Date et al. (ed.) Plant-soil interactions at low pH. Kluwer Academic Publishers, Dordrecht.

Edmeades, D.C., D.M. Wheeler, and O.E. Clinton. 1985. The chemical composition and ionic strength of soil solutions from New Zealand topsoils. Aust. J. Soil Res. 23:151–165.

Eswaran, H., P. Reich, and F. Beinroth. 1997. Global distribution of soils with acidity. p. 159−164 In A.C. Moniz et al. (ed.) Plant-soil interactions at low pH: Sustainable agriculture and forestry production. Brazilian Soil Science Society, Campinas, Viçosa, Brazil.

Foy, C.D. 1992. Soil chemical factors limiting plant root growth. Adv. Soil Sci. 19:97–149.

Fritz, E., D. Knoche, and D. Meyer. 1994. A new approach for rhizosphere research by X-ray microanalysis of microliter soil solutions. Plant Soil 161:219–223.

Gigou, J., F. Ganry, and J. Pichot. 1985. Nitrogen balance in some tropical agrosystems. p. 247–268. In B.T. Kang (ed.) Nitrogen management in farming systems in humid and subhumid tropics. Institute for Soil Fertility and IITA, Haren, Netherlands.

Gillman, G.P., and L.C. Bell. 1978. Soil solution studies on weathered soils from tropical north Queensland. Aust. J. Soil Res. 16:67–77.

Hartwell, B.L., and F.R. Pember. 1918. The presence of aluminum as a reason for the difference in the effect of so-called acid soils on barley and rye. Soil Sci. 6:259–279.

Haynes, R.J. 1986. Mineral nitrogen in the plant-soil system. Physiological ecology—A Series of monographs, texts and treatises. Academic Press, Orlando, FL.

Kandeler, E., and H. Gerber. 1988. Short-term assay of soil urease activity using colorimetric determination of ammonium. Biol. Fertil. Soils 6:68–72.

Kerven, G.L., D.G. Edwards, C.J. Asher, P.S. Hallman, and S. Kokot. 1989. Aluminium determination in soil solution: II. Short-term colorimetric procedures for the measurement of inorganic monomeric aluminium in the presence of organic acid ligands. Aust. J. Soil Res. 27:91–102.

Kinraide, T.B. 1997. Reconsidering the rhizotoxicity of hydroxyl, sulphate, and fluoride complexes of aluminium. J. Exp. Bot. 48:1115–1124.

Kinraide, T.B. 1998. Three mechanisms for the calcium alleviation of mineral toxicities. Plant Physiol. 118:513–520.

Kinraide, T.B., and D.R. Parker. 1987. Cation amelioration of aluminum toxicity in wheat. Plant Physiol. 83:546–551.

Kinraide, T.B., and D.R. Parker. 1989. Assessing the phytotoxicity of mononuclear hydroxy-aluminum. Plant Cell Environ. 12:479–487.

Kinraide, T.B., P.R. Ryan, and L.V. Kochian. 1992. Interactive effects of Al^{3+}, H^+, and other cations on root elongation considered in terms of cell-surface electrical potential. Plant Physiol. 99:1461–1468.

Lasso, N.L. 1998. Efecto del nitrógeno, fósforo y aluminio en la estructura del sistema radical de tres especies del género Brachiaria. (In Spanish.) B.Sc. thesis, Universidad del Valle, Cali, Colombia.

Lindsay, W.L., and P.M. Walthall. 1989. The solubility of aluminum in soils. p. 221–239. In G. Sposito (ed.) The environmental chemistry of aluminum. CRC Press, Boca Raton, FL.

Ma, J.F., M. Sasaki, and H. Matsumoto. 1997. Al-induced inhibition of root elongation in corn, *Zea mays* L. is overcome by Si addition. Plant Soil 188:171–176.

Menzies, N.W., and L.C. Bell. 1988. Evaluation of the influence of sample preparation and extraction technique on soil solution composition. Aust. J. Soil Res. 26:451–464.

Moss, P. 1963. Some aspects of the cation status of soil moisture: Part I. The ratio law and soil moisture content. Plant Soil 18:99–113.

Mueller-Harvey, I., A.S.R. Juo, and A. Wild. 1989. Mineralization of nutrients after forest clearance and their uptake during cropping. p. 315–324. *In* J. Proctor (ed.) Mineral nutrients in tropical forest and savanna ecosystems. Spec. Pub. No. 9 of the British Ecological Society. Blackwell Scientific Publications, Oxford.

Nordstrom, D.K., and H.M. May. 1989. Aqueous equilibrium data for mononuclear aluminium species. p. 29–53. *In* G. Sposito (ed.) The environmental chemistry of aluminum. CRC Press, Boca Raton, FL.

Okada, K., and A.J. Fischer. 2001. Adaptation mechanisms of upland rice genotypes to highly weathered acid soils of South American savannas. p. 185–200. *In* N. Ae et al. (ed.) Plant nutrient acquisition. New perspectives. Springer, Tokyo.

Parker, D.R., and W.A. Norvell. 1999. Advances in solution culture methods for plant mineral research. Adv. Agron. 65:151–213.

Parker, D.R., L.W. Zelazny, and T.B. Kinraide. 1987. Improvements to the program GEOCHEM. Soil Sci. Soc. Am. J. 51:488–491.

Pavan, M.A., F.T. Bingham, and P.F. Pratt. 1982. Toxicity of aluminum to coffee in Ultisols and Oxisols amended with $CaCO_3$, $MgCO_3$, and $CaSO_4 \cdot 2H_2O$. Soil Sci. Soc. Am. J. 46:1201–1207.

Pearson, R.W. 1971. Introduction to symposium—The soil solution. Soil. Sci. Soc. Am. Proc. 35:417–420.

Rao, I.M., P.C. Kerridge, and M.C.M. Macedo. 1996. Nutritional requirements of *Brachiaria* and adaptation to acid soils. p. 53–71. *In* J.W. Miles et al. (ed.) *Brachiaria*: Biology, agronomy, and improvement. CIAT, Cali, Colombia.

Rao, I.M., W.M. Roca, M.A. Ayarza, E. Tabares, and R. García. 1992. Somaclonal variation in plant adaptation to acid soil in the tropical forage legume *Stylosanthes guianensis*. Plant Soil 146:21–30.

Rao, I.M., R.S. Zeigler, R. Vera, and S. Sarkarung. 1993. Selection and breeding for acid-soil tolerance in crops. Upland rice and tropical forages as case studies. BioScience 43:454–465.

de Sá e Melo Marques, J.J.G. 2000. Trace element distributions in Brazilian Cerrado soils at the landscape and micrometer scales. Ph.D. thesis, Purdue University, West Lafayette, IN.

Salinas, J.G., and R. García. 1985. Métodos químicos para el análisis de suelos ácidos y plantas forrajeras. (In Spanish.) CIAT, Cali, Colombia.

Sánchez, P.A. 1976. Properties and management of soils in the tropics. John Wiley & Sons, New York.

Sokal, R.R., and F.J. Rohlf. 1995. Biometry. Freeman, New York.

Sylvester-Bradley, R., and D. Mosquera. 1985. Nitrification and responses to *Rhizobium* inoculation in tropical savannas as affected by land preparation. p. 167–183. *In* B.T. Kang (ed.) Nitrogen management in farming systems in humid and subhumid tropics. Institute for Soil Fertility and IITA, Haren, Netherlands.

Sylvester-Bradley, R., D. Mosquera, and J.E. Méndez. 1988. Inhibition of nitrate accumulation in tropical grassland soils: effect of nitrogen fertilization and soil disturbance. J. Soil Sci. 39:407–416.

Wenzl, P., G.M. Patiño, A.L. Chaves, J.E. Mayer, and I.M. Rao. 2001. The high level of aluminum resistance in signalgrass is not associated with known mechanisms of external aluminum detoxification in root apices. Plant Physiol. 125:1473–1484.

Wheeler, D.M. 1996. Solution concentrations required for near maximum yield in ryegrass and white clover when grown in a low ionic strength solution: preliminary results. J. Plant Nutr. 19:565–572.

Wheeler, D.M., and D.C. Edmeades. 1995. Effect of ionic strength on wheat yield in the presence and absence of aluminium. p. 623–626. *In* R.A. Date et al. (ed.) Plant-soil interactions at low pH. Kluwer Academic Publishers, Dordrecht.

Wheeler, D.M., D.C. Edmeades, R.A. Christie, and R. Gardner. 1992. Effect of aluminium on the growth of 34 plant species: A summary of results obtained in low ionic strength solution culture. Plant Soil 146:61–66.

Wright, R.J., V.C. Baligar, K.D. Ritchey, and S.F. Wright. 1989. Influence of soil solution aluminum on root elongation of wheat seedlings. Plant Soil 113:294–298.

Yanai, J., S. Araki, and K. Kyuma. 1995. Effects of plant growth on the dynamics of the soil solution composition in the root zone of maize in four Japanese soils. Soil Sci. Plant Nutr. 41:195–206.