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Natural Abundance of Foliar ^{15}N as an Early Indicator of Nitrogen Deficiency in Fertilized Cotton

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ABSTRACT

Information on the contribution of various soil nitrogen (N) sources to plant N uptake is often needed for the implementation of sustainable or site-specific management practices in agriculture. Considering the limitations of traditional methods in meeting these needs, this study investigated the potential of leaf $\delta^{15}N$ as an early indicator of nutrient deficiency in cotton. The spatial and temporal natural abundance of ^{15}N was measured in the soil and leaves of a fertilized cotton field located near the village of Moschochori (Larissa, Greece). The isotopic signal of the leaves was interpreted in the context of the relative contribution of fertilizer to cotton N uptake,as has been demonstrated in the past for other agricultural crops such as wheat (Triticum aestivam L.) and corn (Zea mays). Spatial variability of leaf $\delta^{15}N$ was high early in the growing season (June), reflecting differences in fertilizer N availability and uptake between the east and west side of the field, as well as differences resulting from soil denitrification in depressions. The west side of the field appears to have lost significant amounts of fertilizer N, due to leaching during the rainy period in May, that accumulated in depressions near the waterway. In the subsequent months, the isotopic signal of the leaves was consistently high and indicated reduced fertilizer N uptake on the west side that resulted in deficiencies of N as well as of phosphorus (P) and potassium (K). The significant correlations of mid-square leaf $\delta^{15}N$ with late-season nutrient content and soil elelctrical conductivity(EC) provided evidence that the natural abundance of ^{15}N was a sensitive indicator of soil and plant nutrient status in this fertilized cotton field.

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INTRODUCTION

Nitrogen (N) is a major crop nutrient, but the contributions of various soil N sources to plant N uptake in the field have been difficult to assess by traditional methods. Earlier observations that the natural abundance of ^{15}N in plants exhibits large within-site variability (Sutherland et al., 1991) has offered hope that plant $\delta^{15}N$ can be used as a tracer of crop N sources and needs. However, the relationship between plant $\delta^{15}N$ and an external N source may be complex because plants are integrators of the $\delta^{15}N$ of available N sources that vary in amount and ^{15}N composition (nitrate, ammonium, organic N, on N_2 from diazotrophic prokaryotes). Isotopic composition of the source sometimes depends on the season and local soil conditions, such as the enrichment of nitrate-^{15}N during denitrification under anaerobic conditions (Hogberg, 1997). In addition, metabolic fractionations during plant uptake (N assimilation) and mixing of assimilated and unassimilated N pools between roots and shoots after N uptake may distort the $\delta^{15}N$ signal of the source (Handley and Raven, 1992; Hogberg, 1997; Robinson et al., 1998).

Despite these difficulties, certain crops exhibit a strong preference for a specific soil N source, i.e., nitrate. When an inorganic N fertilizer is applied, usually with a lower ^{15}N content than soil-derived nitrate, its isotopic signal may become distinct enough to reveal the contribution of fertilizer N in the plant tissue. Although there were early doubts about the ability of natural ^{15}N abundance to trace sources of fertilizer N (Hauck et al., 1972), this principle has been used to show that the $\delta^{15}N$ of soil nitrate decreased significantly after the addition of fertilizer in a number of central Illinois soils (Feigin et al., 1974). In the same soils, other researchers showed that ^{15}N of corn grain and leaves (Kohl et al., 1973) and whole-wheat samples (Shearer and Legg, 1975) decreased systematically as the rate of N fertilizer application increased. Because some soils have low $\delta^{15}N$ values, it may not be possible to detect fertilizer N in this way (Bremner and Tabatabai, 1973). The potential value of using fertilizer N as a natural tracer in soil-plant systems has not been fully investigated in these studies, and more data are needed on the relation between plant ^{15}N, phenology, nutrient status, and yield.

Cotton (Grossypium hirsutum), like wheat and maize, requires high levels of N, particularly under irrigation, with measured uptakes as much as 230 kg N/ha (Constable and Rochester, 1988). Soil nitrate is the principal form in which the cotton plant assimilates the N required for growth and reproduction (Breitenbeck, 1990; Ebelhar, 1990). This paper reports on the spatial and temporal variability of ^{15}N natural abundance of cotton leaves following inorganic N additions in a field with two distinct productivity regions. The changes in leaf

^{15}N were followed during the summer and compared to a number of soil and plant chemical properties, biomass, and yield. The objective of this study was to assess, through a series of spatial and temporal measurements, the potential for using leaf δ^{15}N content as an indicator of crop nutrient status and related growth patterns.

MATERIALS AND METHODS

Site Description and Management Practices

The experimental field was located near the village of Moschochori in the municipality of Nikea (Larissa, Greece) (lat 39°29′ 51.18″ N, long 22°32′ 36.42″ E) and covers an area of 7 ha. The east and west side of the field were separated by a waterway running in a south-to-north direction. The soil on the east side is classified as a Typic Xerochrept, and on the west side as a Typic Xerorthent, both with a clay texture. The soil near the waterway was heavier in texture and was classified as Vertic Xerochrept.

The field was ploughed to a depth of 25 cm in the fall of 2000. Row spacing was 95 cm and plant density was ∼140,000 plants ha^{-1} (12–15 plants m^{-1}). Basic N and phosphate fertilization was applied at rates of 120 kg N ha^{-1} (96 kg ammonium and 24 kg nitrate) and 60 kg P_2O_5 ha^{-1} 10 d before sowing in mid-April 2001. The following pesticides and herbicides were applied to the crop: phorate to the seed (10 kg ha^{-1}), prometryne (10 kg ha^{-1}) on the soil surface after sowing, endosulfan (3 kg ha^{-1}) at first bloom on July 20, and two to three sprays of pyrethrine in combination with acaricides thereafter. Groundwater was supplied to the plants by drip irrigation and on occasion by spraying with a mobile unit. With the exception of a rainy period in May, the summer period was dry and warm, with only 140 mm of rainfall in July and August, a mean temperature of 27°C (19°C–36°C average daily minimum and maximum), and a mean relative humidity of 44% (20%–70%).

Experimental Design, Sampling, and Analysis

Six strips were chosen across the length of the field in an east-west direction in order to include different soil colors as indicated by color aerial photography taken in May. Soil coloration has been shown to be related to organic-matter content and soil productivity (Francis and Schepers, 1997). Following preliminary soil sampling, a total of 33 sampling positions were selected along the strips, based on a grid-sampling design (30 × 30 m). The coordinates of each position were recorded by a differential GPS (OmniStar Fugro 3000L, OmniSTAR/Europe BV, Leidschendam, the Netherlands).

Soil and leaf samples were taken randomly from within a 5 m radius around each sampling position. Six composite surface soil samples (0–30 cm) were

taken with an Oakfield-type soil sampler at the end of June and September 2001. At the end of June, July, and August 2001, leaf samples from ~25 plants were taken from each sampling position (the fourth healthy unfolded leaf from the top; Reddy et al., 2001). Five plants were randomly chosen from each sampling position in mid-September for the determination of aboveground biomass, open/closed bolls, and lint production.

Soil samples sealed in plastic bags and leaf samples in paper bags were transported to the laboratory in a portable cooler. Prior to packing, the chlorophyll content was estimated for each leaf blade with a Minolta SPAD-502 portable meter and, after visual examination, leaf pest damage was qualitatively categorized as healthy, moderately damaged, or extensively damaged. Soil samples were weighed, mixed, and passed through a 2 mm sieve. Plant samples were dried at 65°C and leaf samples were subsequently ground to a fine powder. Standard soil-quality analysis (Doran and Parkin, 1996) included water content (w/w), bulk density, pH, electrical conductivity (EC), and nitrate and ammonium content in soil extracts of 1 *M* potassium chloride as measured using an ion chromatograph (Dionex DX-120, Dionex Corporation, Sunnyvale, CA) adjusted to a 1:1 soil-water ratio. Carbonate content was determined using GC analysis (Micro-GC P200 equipped with a thermal conductivity detector, Hewlett Packard, Wilmington, DE) of evolved CO_2 within sealed containers upon addition of hydrochloric acid (Kettler and Doran, 1995). Macronutrients and trace elements [calcium (Ca), potassium (K), magnesium (Mg), phosphorus (P), sulfur (S), and zinc (Zn)] were determined by inductively coupled plasma spectrometry (Jarrell Ash Iris Advantage, Thermo Jarrell Ash Corporation, Franklin, MA) after extraction of soil samples with a multiple-extraction solution (Soltanpour and Schwab, 1977) and after digestion of leaf samples with concentrated nitric acid in a microwave system (Mars 5, CEM Corporation, Matthews, NC). Total N and carbon (C) content, as well as isotopic composition (δ^{15}N, δ^{13}C) of soil (20 $\pm$ 0.1 mg) and leaf (2.8 $\pm$ 0.1 mg) samples, were measured by an automated-combustion elemental analyzer interfaced with a triple-collector isotope ratio mass spectrometer (IRMS, PDZ Europa, Cheshire, UK). Samples were prepared as described by Schepers et al. (1989). Ground Eucalyptu globulus leaves ($\delta^{15}N = 3.12‰$, $\delta^{13}C = -29.71‰$) was used as the plant working standard, and Sharpsburg silty clay loam ($\delta^{15}N = 10.647‰$, $\delta^{13}C = -17.251‰$) was used as the soil working standard. Working standards were previously calibrated against the international standard Pee Dee belemnite (PDB) for δ^{13}C and atmospheric N_2 ($\delta^{15}N = 0$) for δ^{15}N. Overall precision (machine error plus sample preparation error) for carbon isotopic composition was 0.05‰–0.2‰ and for nitrogen was 0.3‰–0.8‰. Organic carbon was also measured by IRMS after removal of inorganic carbon by the addition of 0.5 *N* hydrochloric acid and subsequent rinsing with distilled water as described by Midwood and Boutton (1998), as well as by the method of wet oxidation by Walkley-Black (Nelson and Sommers, 1982).

Statistical Analysis

Data analysis included analysis of variance (general linear models) and correlation analysis. A completely randomized design was used for the analysis of variance, with two classification variables: landscape position and growth stage with three levels each. Neuman-Keuls post-hoc comparison of means was used at the level of $P < 0.05$. All employed procedures are reported in the Statistical Analysis System (SAS Institute, 1990).

RESULTS AND DISCUSSION

Preliminary soil sampling indicated, on average, a higher organic-matter content on the east side and near the waterway (1.9%–2.4%) compared with the west side (0.6%–2.2%) of the waterway. Analysis of regular soil samples (0–30 cm depth) further showed that the soil on the east side had significantly higher water content, EC, and nitrate-N and K concentrations and lower pH and carbonate content in June (Table 1). The average amount of nitrate-N on the west side was about half that required for ideal N uptake by cotton (27 mg kg^{-1}, or about 100 kg ha^{-1} NO_3-N for uptake of 108 kg N ha^{-1}) in the top 30 cm of unfertilized brown clay soils of Illinois (Constable and Rochester, 1988).

Table 1
Spatial variability of selected soil properties (0–30 cm depth) across the cotton field in June 2001

	Landscape position		
Soil properties	East side n = 12	Waterway n = 4	West side n = 17
Physical			
Bulk density, g cm^{-3}	1.20	1.33	1.23
Water content, g g^{-1}	0.22a	0.19b	0.17b
WFPS, %	48	53	40
Chemical			
pH	8.03b	7.90c	8.17a
EC, μS cm^{-1}	871b	1017a	556c
NO_3-N, kg ha^{-1}	92.2b	139.0a	52.1c
CO_3-C, %	0.55b	0.78b	1.44a
N, kg ha^{-1}	2516	2662	2473
$\delta^{15}N$, ‰	8.563	8.730	8.801
K, kg ha^{-1}	1570a	1123b	1112b
P, kg ha^{-1}	5.1	4.6	4.8

Means within rows followed by different letter(s) are significantly different at $P < 0.05$.

The soil around the waterway dividing the two areas had the highest EC and nitrate-N concentration and lowest pH (Table 1). This finding is indicative of more intense nitrification processes near the waterway, which can be explained by lateral leaching of applied N fertilizer and long-term surface erosion from higher elevations. Similar findings were reported by Stamatiadis et al. (1999), with highest values for soil EC, total N, biomass N, and basal respiration in the waterway of a terraced landscape in Lincoln, NE.

Despite the low soil nitrate-N content on the west side of the field, plants appeared to have an adequate soil N supply during vegetative growth, as leaf N content was similar in all areas of the field in June (N $\sim$ 4.4%, Table 2). Differences between the east and west side of the field started to appear at peak bloom and increased rapidly during the boll development period. During this period, leaf N content on the west side declined from 3.4% in late July to 2.4% in late August (Table 2). These values are within critical (N = 3.3%) or marginal values (N = 2.5%–3.0%) reported for the period between peak bloom and first boll formation (Reuter et al., 1997). Leaf N deficiency during boll development indicated exhaustion of the soil mineral-N supply. As expected, significant

Table 2
Temporal variability of selected foliar elements and yield across the cotton field

		Landscape position		
Variable	Growth stage	East side n = 12	Waterway n = 4	West side n = 17
Leaf δ^{15}N, ‰	Mid-square[1]	1.682c	5.573a	3.228b
	Peak bloom[2]	1.669c	2.696b	3.428a
	Boll opening[3]	4.522	4.743	4.285
Leaf N, %	Mid-square[1]	4.40	4.38	4.36
	Peak bloom[2]	3.74	3.74	3.39
	Boll opening[3]	3.30a	3.21a	2.43b
Leaf P, %	Mid-square	0.27	0.30	0.28
	Peak bloom	0.27	0.23	0.23
	Boll opening	0.22a	0.24a	0.14b
Leaf K, %	Mid-square	2.13	2.16	1.89
	Peak bloom	2.02	1.62	1.80
	Boll opening	1.43a	1.40a	0.92b
Aboveground biomass, kg ha^{-1}	Boll maturation[4]	18908a	21135a	11588b
Yield, lint kg ha^{-1}	Boll maturation	8863a	11278a	5172b
Closed bolls, %	Boll maturation	39a	24b	6c

[1]late June, [2]late July, [3]late August, [4]late September.

Means within rows followed by different letter(s) are significantly different at $P < 0.05$.

correlations of leaf N content with soil properties were evident only at this period of development in late August, i.e., with EC ($r = 0.76$, $n = 33$), pH ($r = -0.65$, $n = 33$), nitrate-N ($r = 0.64$, $n = 33$), and K ($r = 0.64$, $n = 33$).

Plants integrate ^{15}N supplied from available soil N sources, although $\delta^{15}N$ in foliage may also be affected by discrimination against ^{15}N during and after plant uptake of inorganic N (Hogberg, 1997). In this respect, the variability of $\delta^{15}N$ in the leaves was greater than in the soil and reflected the more dynamic nature of available N than total N. Cotton leaves had lower $\delta^{15}N$ than that of the total soil N (Table 1), as in many agricultural systems (Hogberg, 1997; van Kessel et al., 1994), indicating the uptake of nitrate N by the crop under predominantly aerobic conditions.

The lower leaf $\delta^{15}N$ values on the east side than the west side of the field in mid-square and peak bloom (Table 2) indicated increased plant uptake of fertilizer N, assuming that the $\delta^{15}N$ of the fertilizer N was lower than that of soil-derived nitrate. This assumption is supported by data obtained by Shearer et al. (1974) and Feigin et al. (1974), who found that the ^{15}N content of a number of fertilizers was significantly lower than that of nitrate produced by soils in central Illinois. The mean soil $\delta^{15}N$ value in our experimental field ($\delta^{15}N = 8.7$) is even higher than that reported by Kohl et al. (1973), for the central Illinois soils ($\delta^{15}N$ of about 8). The same researchers found a negative correlation of $\delta^{15}N$ in the total N in wheat plants (Shearer and Legg, 1975) and of $\delta^{15}N$ of grain and leaf tissue in corn (Kohl et al., 1973), with fertilizer N at similar and lower application rates that support our assumption. In contrast, the significantly higher leaf $\delta^{15}N$ values on the west side of the field in mid-square and peak bloom (Table 2) indicated a greater contribution of organic-derived N due to limited availability of fertilizer N. This interpretation is consistent with the low soil-nitrate content at mid-square (Table 1) and the appearance of N deficiency during boll development on this side of the field. The leaf $\delta^{15}N$ increased and was strikingly similar in all field positions during boll opening in late August (Table 2). These data indicated either a general exhaustion of the fertilizer N supply or root penetration beyond the depth of fertilizer N availability. It is also feasible that more ^{14}N assimilates were translocated to the bolls, thus leaving proportionately more ^{15}N in the leaves.

The highest leaf $\delta^{15}N$ values were recorded near the waterway early in mid-square (Table 2) and particularly in two sampling positions that were located in depressions in the northern part of the waterway (7.436‰ and 10.403‰, respectively). These values appear to be associated with denitrification activity, as has been found to be the case in depressions of an irrigated field with durum wheat (Triticum durum) (Sutherland et al., 1991). The soil near the waterway (Vertic Xerochrept) had higher bulk density, water-filled pore space, and nitrate content (Table 1). These conditions favor denitrification under anaerobic conditions that were likely to occur during the rainy period in May 2001. Under these conditions, more rapid changes in $\delta^{15}N$ will take place in the active inorganic N pools when NO_3 (although ^{15}N-depleted due to fractionation against $\delta^{15}NH_4$ during

nitrification) will become enriched (heavier) during denitrification (Hogberg, 1997) and will transfer its signature to the plant.

Early season $\delta^{15}N$, after excluding the values of the waterway positions, was correlated to crop-nutrient status and soil EC. Increased soil EC was associated with more intense nitrification effects on the east side of the field because EC was strongly correlated to the level of soil nitrates and pH (Figure 1). Thus,

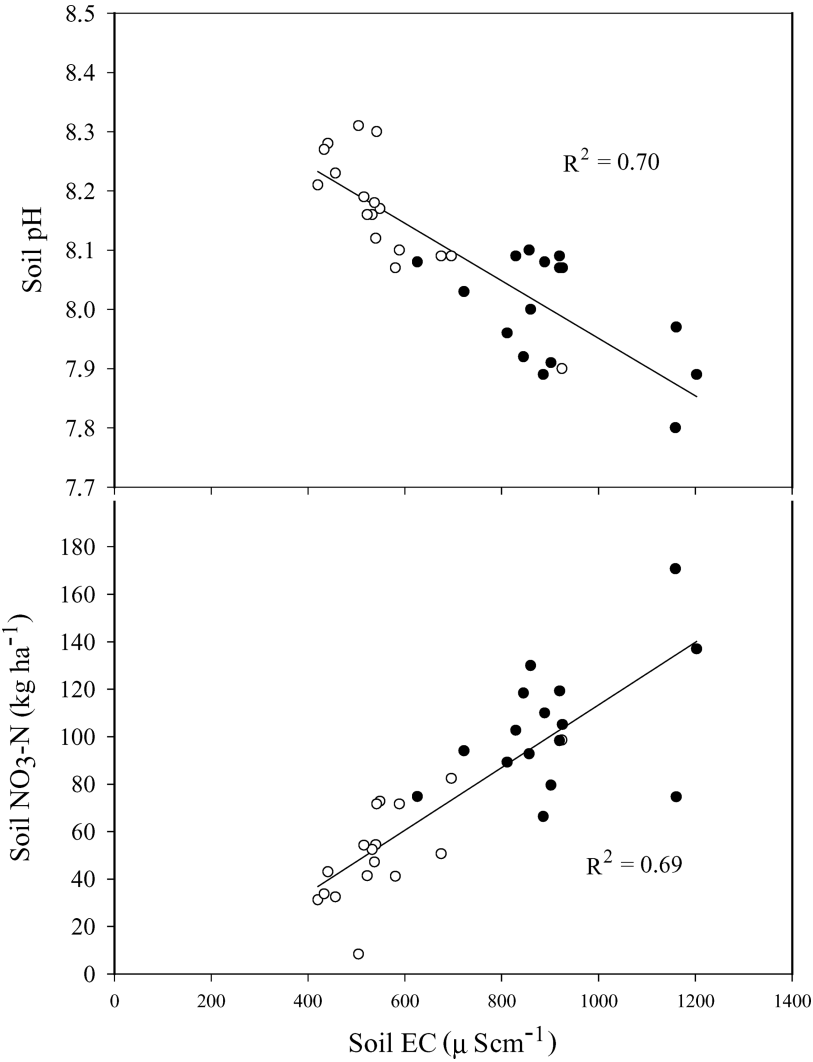

Figure 1. The linear relationship of soil EC with soil pH and NO_3-N. Open circles represent sampling positions on the west side and closed circles on the east side of the field.

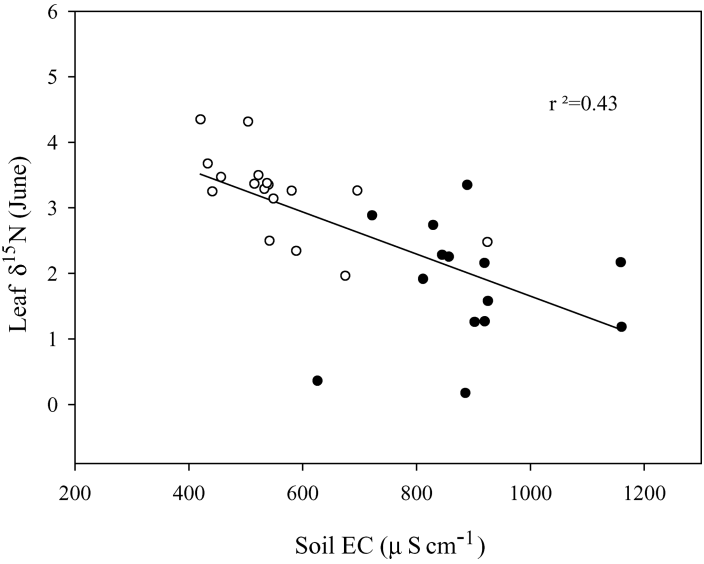

Figure 2. The inverse relationship between leaf δ^{15}N at mid-square and soil EC measured in the laboratory. Open circles represent sampling positions on the west side and closed circles on the east side of the field.

the negative correlation between EC and leaf δ^{15}N at mid-square (Figure 2) provides evidence that fertilizer N was the main cause of amplification of the differences in these soil chemical properties between the two sides of the field. Consequently, the negative correlation between early-season leaf δ^{15}N and late-season leaf N content (Figure 3a) represents the relative contribution of fertilizer N to plant N uptake. The elevated ^{15}N values were predominantly found on the west side of the field and corresponded to N deficiency between peak bloom and first open boll, as leaf N content was below the minimum adequacy level reported in the literature for these growth stages (Reuter et al., 1997). Similar negative correlations were found between leaf δ^{15}N and late-season leaf K (Figure 3b) and P (Figure 3c). Like fertilizer N, both soil-extractable P and K had lower concentrations on the west side of the field (Table 1) that may be explained by increased erosion and leaching events. Fertilizer N availability and uptake may also explain the negative, but weaker, correlation between foliar ^{15}N and boll weight ($R^2 = 0.36$), which was greater for plants on the east side of the field (data not shown). Larger fruit is known to be produced in response to N in cotton (Constable and Rochester, 1988).

The ability of early-season leaf ^{15}N to predict late-season nutrient deficiencies in this fertilized cotton field may find applications in site-specific management where corrective measures need to be taken at an early stage during the growing season. The foliar ^{15}N signatures obtained at mid-square are

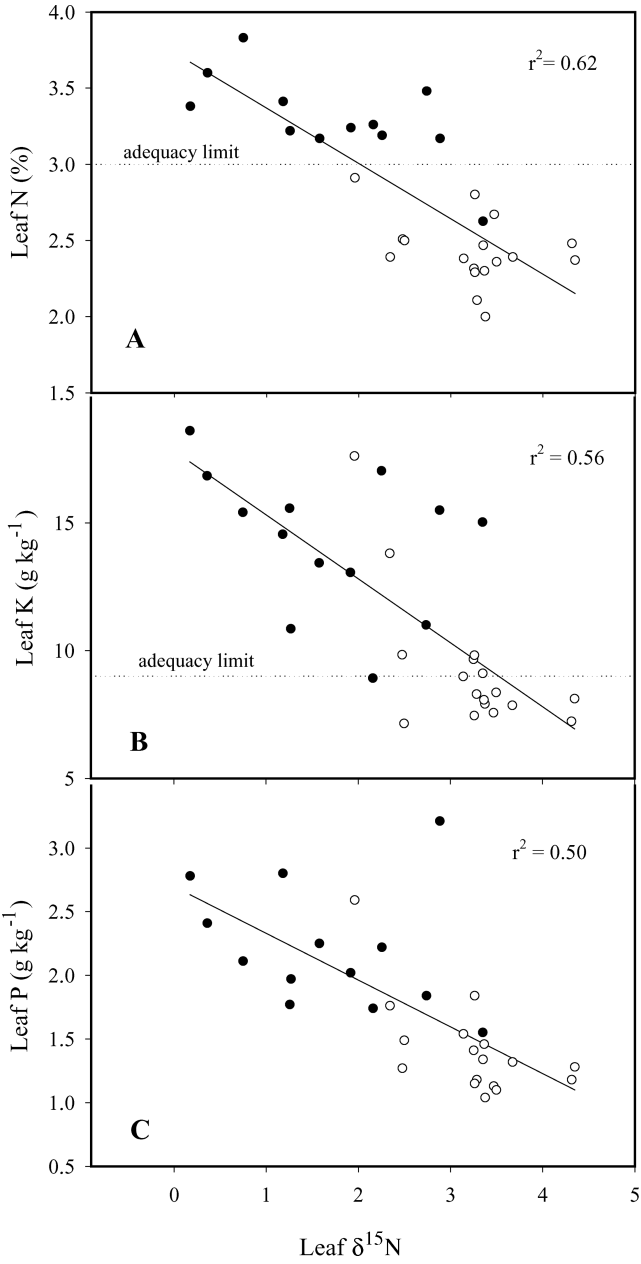

Figure 3. The inverse relationship between leaf $\delta^{15}N$ at mid-square and (a) leaf N, (b) leaf K, and (c) leaf P during boll development. Open circles represent sampling positions on the west side and closed circles on the east side of the field. Dotted lines represent the lowest reported adequate concentration in the leaves between peak bloom and first open boll (Reuter et al., 1997).

well in advance of the final nutrient/herbicide applications, which are typically completed at early-to-mid bloom (July) in cotton.

CONCLUSIONS

The high spatial variability of leaf ^{15}N early in the growing season reflected differences of fertilizer N cycling and plant uptake in the cotton field. Standard quantitative analysis of soil and plant samples provided supplementary evidence that fertilizer N acted as an isotopic tracer of plant N sources and allowed significant relationships to be revealed. Mid-square leaf δ^{15}N was a predictor of nutrient deficiency at the period of boll opening and showed that it has the potential to be used as an effective monitoring tool in site-specific management of cotton under the management practices followed in this fertilized field.

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