SOIL AND MOLD INFLUENCES ON Fe AND Zn CONCENTRATIONS OF SORGHUM GRAIN IN MALI, WEST AFRICA

A Dissertation

by

CHERYL L. VERBREE

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

August 2012

Major Subject: Soil Science

Soil and Mold Influences on Fe and Zn Concentrations of Sorghum Grain in Mali, West Africa

SOIL AND MOLD INFLUENCES ON Fe AND Zn CONCENTRATIONS OF SORGHUM GRAIN IN MALI, WEST AFRICA

A Dissertation

by

CHERYL L. VERBREE

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Approved by:

Co-Chairs of Committee,	Jacqueline A. Aitkenhead-Peterson
	William A. Payne
Committee Members,	Richard H. Loeppert
	Joseph A. Awika
Head of Department,	David D. Baltensperger

August 2012

Major Subject: Soil Science

ABSTRACT

Soil and Mold Influences on Fe and Zn Concentrations of
Sorghum Grain in Mali, West Africa.
(August 2012)

Cheryl L. Verbree, B.S., Calvin College; M.S., The Ohio State University

Co-Chairs of Advisory Committee: Dr. Jacqueline A. Aitkenhead-Peterson
Dr. William A. Payne

Iron (Fe) and zinc (Zn) deficiencies affect an estimated 3 billion people worldwide and are linked with cognitive and physical impairments, maternal and child mortality rates, and decreased adult work activity. To combat this “hidden” hunger, plant breeders in Mali are working to increase sorghum grain Fe and Zn concentrations. The objective of this study was to investigate soil and mold influences that affect Fe and Zn uptake and accumulation in sorghum grain. In southern Mali, soils from participatory sorghum variety trials and areas of different parent material and proximity to Shea (*Vitellaria paradoxa*) trees were analyzed for diethylenetriaminepentaacetic acid (DTPA)-extractable Zn and related soil properties, and sorghum grain was analyzed for Zn concentration. An inoculation trial was also performed at College Station, TX to determine if sorghum grain infected by the mold *Curvularia lunata* significantly increased grain Fe concentrations.

DTPA-extractable Zn concentration was highly variable with high concentrations found in soils under Shea tree canopies with high pH and organic carbon and derived from mafic, high Zn-content parent material. However, these high concentrations did not significantly affect grain Zn concentrations in sorghum grown outside of the canopy. Groundnut grown underneath the canopy is likely to be affected and warrants further investigation. In many cases, soil DTPA-extractable Zn concentrations were at deficient levels, thus hampering its correlation to sorghum grain Zn concentration and potentially limiting the expression of genetic Zn biofortification. Knowledge of soil DTPA-extractable Zn concentrations or basic soil properties such as pH, organic carbon, and soil parent material may aid in the location of suitable available Zn fields and overall biofortification efforts.

Grain Fe concentration was not significantly related to *Curvularia lunata* percent recovery or grain mold rating, but instead showed a relatively high variance by panicle, digestion batch, and grain subsample. Additional work is needed to address these sources of Fe variation so as to determine better if mold affects grain Fe concentrations.

DEDICATION

To my husband Dave who always believed that I could do this, and to my son Peter who put up with a mommy who missed some important kindergarten field trips.

To the rural farmers of West Africa who also put up with me and my strange request for finding fields with black rocks.

ACKNOWLEDGEMENTS

I would like to thank my committee chairs, Dr. Jacqueline Aitkenhead-Peterson and Dr. William “Bill” Payne and my committee members, Dr. Richard Loeppert and Dr. Joseph Awika.

I would also like to thank the many researchers who collaborated with me: Dr. Louis Prom (USDA-ARS in College Station, TX), Dr. Kevin Ong (Texas Plant and Disease Diagnostic Laboratory in College Station, TX), Dr. Lloyd Rooney, Cassandra McDonough, and Linda Dykes (Food Science and Cereal Quality Group, Texas A&M University), Dr. William Rooney and Delroy Collins (Sorghum Program, Texas A&M University), Dr. Tony Provin (Soil, Water, and Forage Testing Laboratory, Texas A&M University), Dr. Brendan Roark (Department of Geosciences, Texas A&M University), Dr. Meredith Steele (Duke University), Dr. David Verbree (University of Tennessee), Dr. Eva Weltzien, Dr. Fred Weltzien-Rattunde, and Ibrahima Sissoko (International Crop Research Institution for the Semi-Arid Tropics-Mali).

This research was conducted on behalf of the Soil and Crop Sciences Department and the Norman Borlaug Institute of International Agriculture of Texas A&M University. It was made possible through funding from the McKnight Foundation and an assistantship from the Texas A&M University Department of Soil and Crop Sciences.

Finally, thanks to my husband and son for their patience and support.

TABLE OF CONTENTS

LIST OF FIGURES

Page

LIST OF TABLES

Page

Page

CHAPTER I

INTRODUCTION

Background

Sorghum is an important staple food crop and along with other cereals provides for most of the daily energy intake of West Africans. Sorghum is especially adapted to the high temperature, high rainfall variability, and low soil fertility conditions of highly weathered, poorly buffered, old West African soils. Sorghum is also unique among crops for its phenolic compounds and tannins that serve effectively to combat fungi diseases under hot and humid conditions.

Sorghum research has primarily focused on breeding for improved cultivars with higher yields and disease resistance. Sorghum yields in Africa have not increased in the last 35 years and remain at average low levels of 800 kg ha^{-1} (Olembo et al. 2010). However, besides improving yields so that farmers can feed growing populations, sorghum researchers have also recently been tasked with addressing cereal nutrition, specifically with increasing iron (Fe) and (Zn) in sorghum grain. There is significant evidence that Fe and Zn deficiencies are a large problem for the poor people in West Africa and other areas of the world (Welch 2008). Fe and Zn are essential trace metals required in the human body for proper metabolism and growth. Deficiencies of these metals can cause a host of cognitive and physical problems including anemia as well as decreased adult work productivity (WHO 2008; Slingerland et al. 2005; Hotz and Brown

---

This dissertation follows the style of Plant and Soil.

2004). It is hoped that by increasing Fe and Zn concentrations in sorghum grain that these deficiencies can be alleviated.

Breeding for higher Fe and Zn or "biofortification" involves an understanding of the genetics of sorghum as it relates to Fe and Zn uptake and translocation into the grain. Knowledge of environmental factors such as soil properties and growing conditions are also needed for breeding of higher Fe and Zn. Many soil and plant processes occur that interact with these environmental factors and ultimately determine sorghum grain Fe and Zn concentrations. Although many Fe and Zn studies have been conducted on other crops such as wheat and rice in other areas of the world and have contributed to a better understanding of these soil and plant processes, few studies exist on sorghum in the particular environment of West Africa. Indeed, little is known about plant available Fe and Zn in West African soils other than that some soils have been reported to be Zn deficient (Soumare et al. 2002; Gardestedt 2009). Deficiencies of Zn are thought to result from soil parent materials with relatively low Zn concentrations and soils low in organic matter (Alloway 2008). In addition, there has been little research into the effect of field growing conditions including grain molding or weathering on Fe and Zn grain concentrations, even though it is known that grain molding can potentially decrease grain weight and viability (Bandyopadhyay et al. 2000; Thakur et al. 2006).

Objectives

The objectives of the current study are:

1) To investigate the potential environmental factors that may influence Zn concentrations in sorghum varieties across southern Mali, West Africa by: 1)

determining the variability of available Fe and Zn in soil within and among fields and across locations in southern Mali; 2) evaluating the genetic, environmental, and genetic x environmental interaction effects on grain Zn concentration in sorghum grown in farmers' fields across southern Mali; and, 3) determining the relationship between soil properties, including available Zn, and grain Zn concentration.

2) To determine the influence of soil parent material and tree proximity on soil properties, grain properties, and grain Zn concentrations in sorghum.
3) To examine the effect of molding on grain Fe concentration by: 1) determining whether pathogen attack results in a significant accumulation of Fe in the grain under field conditions; and, 2) determining the natural variation of Fe in grain of different panicles and within a single panicle.

CHAPTER II

GENOTYPE AND ENVIRONMENT EFFECTS ON SORGHUM (*SORGHUM BICOLOR* L. MOENCH) GRAIN ZINC CONCENTRATION IN SOUTHERN MALI, WEST AFRICA

Introduction

Micronutrient deficiencies affect an estimated 3 billion people worldwide, most of whom are poverty-stricken women and children in developing countries (Welch 2008). One of the most important micronutrients is Zn, which is a constituent of over 100 enzymes within the human body and is a key trace element in human metabolism and growth. Zn deficiencies are specifically linked with cognitive and physical impairments such as stunted growth in young children, reduced resistance to disease, and lower neuro-behavioral function (Hotz and Brown 2004). It is estimated that 36 to 48% of the people in West Africa are at risk for inadequate Zn intake and associated health problems.

Many people in West Africa have a diet that consists of a staple cereal crop, legumes, vegetables, or fruit, and rarely any meat. Cereals were found to account for over 75% of Zn intake in a recent village food survey conducted in southern Mali (Tuinsma et al. 2009). One possible solution to this problem is to breed cereals for high Zn concentration which could substantially increase Zn intake (Pfeiffer and McClafferty 2007). Sorghum is the main cereal crop in southern Mali and research has shown genetic variability for Zn in sorghum (Barikmo et al. 2007; Tuinsma et al. 2009; Kayode et al. 2006). It may be possible to breed for this trait; therefore, sorghum breeders at the

International Crop Research Institution of the Semi-Arid Tropics (ICRISAT) in Bamako, Mali have been investigating the variance of grain Zn concentration through variety trials. They have found that approximately 43% of the explained variance in decorticated sorghum Zn concentration was due to a genetic effect while 33% and 24% were due to environmental and genetic x environmental effects, respectively (Tuinsma et al. 2009).

Environmental factors that may lead to Zn variation in sorghum grain are typically soil properties that influence Fe and Zn plant uptake. Zn^{2+} is absorbed through the roots mediated by a family of proteins identified as Zinc and Iron Regulated Transporters or "ZIP" proteins (Palmgren et al. 2008). Fe^{3+} is taken up by the roots of sorghum in the rhizosphere through a plant release of chelating phytosiderophore compounds (Feng 2005). Soil solution properties such as pH, redox potential, and concentration of water-soluble Fe and Zn complexing agents, and Fe oxide solubility characteristics including the rate of dissolution can affect Fe and Zn uptake and its ultimate accumulation in grain (Wissuwa et al. 2008; Briat 2008; Alloway 2008). Diethylenetriaminepentaacetic acid (DTPA) extractant is the most commonly used procedure for measuring Fe and Zn that is considered "plant available" (Loeppert and Inskeep 1996). Plants extract and take up labile forms of Fe^{3+} and Zn^{2+} from soil and DTPA similarly complexes these labile forms.

Several recent studies involving crops other than sorghum have shown that the concentration of Fe and Zn in the grain does not always consistently reflect DTPA-extractable Fe and Zn concentrations in the soil. Wissuwa et al. (2008) found that Zn concentrations in rice grain generally increased with higher concentrations of DTPA-extractable Zn in the 0-15 cm soil layer. Lombaes and Singh (2003) determined a correlation coefficient of 0.69 between DTPA-extractable Zn in soil and Zn concentration in barley and oat leaves. In contrast, Wang et al. (2009) found no significant correlation between DTPA-extractable Fe and Zn in soil and Fe and Zn concentrations in rice grain and strong spatial variation of both DTPA-extractable Fe and Zn and grain Fe and Zn concentrations. Joshi et al. (2010) found a significant difference between DTPA-extractable Zn concentrations in soil at 0-30 cm and 30-60 cm and wheat-grain Zn concentration across a multi-year, multi-location trial in India.

No other study specifically investigating the environmental influences on grain Zn concentrations have been, to my knowledge, published for sorghum. The objective of this study was to: 1) determine the variability of available Fe and Zn in soil within and among fields, and across locations in southern Mali; 2) evaluate the genetic, environmental, and genetic x environmental interaction effects on grain Zn concentration in sorghum grown in farmers' fields across southern Mali; and, 3) determine the relationship between soil properties, including available Zn, and grain Zn concentration.

Materials and Methods

Site Description and Field Design

ICRISAT-Mali performed a sorghum variety trial across 8 villages in southern Mali in 2009. The villages of Keniero, Wacoro, and Tiguere as well as the Field Station were chosen for this study (Figure 2.1). Four fields were established in each village – two for short varieties and two for tall varieties of sorghum.

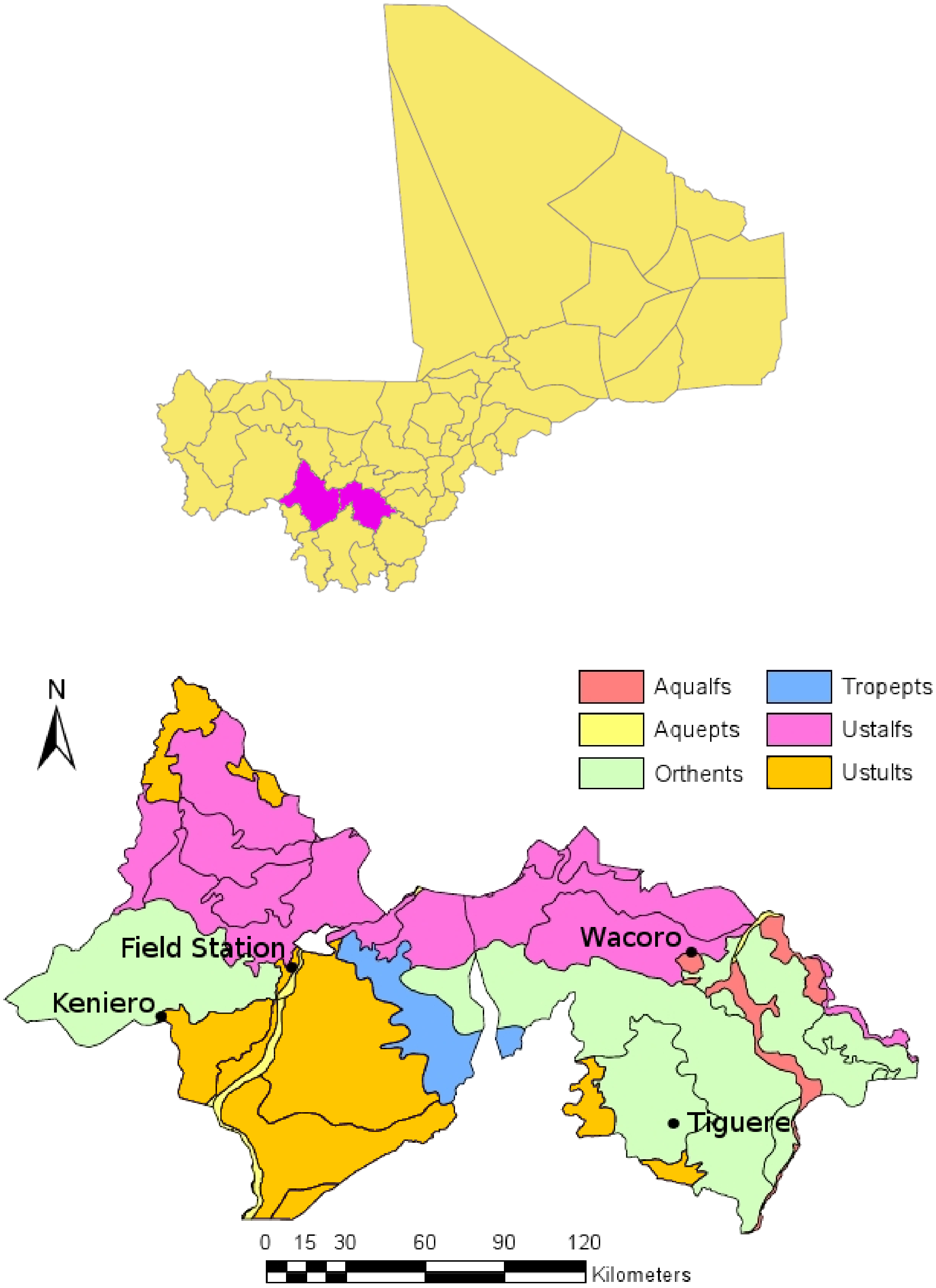


Figure 2.1: Area of southern Mali where the 2009 ICRISAT-Mali sorghum variety trial took place (upper figure). Locations and soil suborders in the area of the trial (lower figure).

Average temperatures for the sorghum growing period (June-September) in 2009 were 33.2 ° C (daily maximum) and 22.6 ° C (daily minimum). Total rainfall for this period was 1,069 mm, which is at the average of 1,098 mm (Climate Temperature Info, 2012). Temperature and rainfall data are from the nearest weather station located at the ICRISAT-Mali research facility in Bamako which is within 200 km of village locations. Actual temperature and rainfall at these locations could vary from those recorded at the Bamako weather station. A description of the geographic and soil information for each site and location was collected during site visits (Table 2.1). In this area of Mali, soil classification data is very limited. Soils in the PIRT (1983) database include Ustalfs, Ustults, and Orthents (soils formed from laterite). Soils in my study were generally observed to be highly weathered with clay, iron oxides, and sometimes iron concretions occurring throughout the profile.

Each field was arranged in a randomized complete block design and consisted of two replications of 15 varieties and a common check (local landrace Tieble). Each plot was 35 x 30 m and planted with six rows of sorghum (rows were 5 m long and 30 cm apart). All varieties were white sorghums with 3 glume colors (purple, tan, red) and classified as thin or thick based on pericarp appearance (thick = low luster, thin = high luster; Belum et al. 2005).

The sorghum trial was planted in late June to early July 2009 at the beginning of the rainy season and harvested the first week of November 2009 approximately one month after the onset of the dry season. All fields except the Field Station fields were traditional farmer “bush” fields located away from village compounds. Bush fields are part of the traditional parkland agroforestry system with Shea (*Vitellaria paradoxa*) trees interspersed within fields approximately 30 m from each other. Fields were cultivated for 5 years and then allowed to fallow for 5 to 15 years depending on population pressure. Fertilizer was added to each field (100 kg ha^{-1} di-ammonium phosphate and 50 kg ha^{-1} urea) to ensure adequate yields. Farmers were interviewed regarding the agronomic history of the field and how they rated productivity, soil, and drainage properties.

Table 2.1: Geographic and soil information for soils sampled at each location-field.

Location-Field	Lat	Long	Elev (m)	GtGp	PM	Geol	Form	Conc
Keniero								
Field 1	N12°22.4'	W8°31.5'	389	Cuir	M	D	A	None
Field 2	N12°22.1'	W8°30.8'	384	Cuir	M	D	A	Surf
Field 3	N12°22.6'	W8°30.7'	385	Cuir	NM	S	R	Surf
Field 4	N12°22.1'	W8°30.9'	384	Cuir	M	D	A	Surf
Tiguere								
Field 1	N12°00.6'	W6°46.9'	342	Cuir	NM	S	R	60
Field 2	N12°59.7'	W6°48.2'	357	Cuir	NM	S	R	Surf
Field 3	N12°59.6'	W6°48.1'	359	Cuir	NM	S	R	Surf
Field 4	N12°00.4'	W6°46.2'	346	Cuir	NM	S	R	Surf
Wacoro								
Field 1	N12°35.5'	W6°43.0'	296	Trop	NM	S	R	30
Field 2	N12°35.3'	W6°43.3'	295	Trop	NM	S	R	30
Field Station								
Field 1	N12°31.9'	W8°04.4'	330	Hal	NM	S	A	None
Field 2	N12°32.0'	W8°04.4'	330	Hal	NM	S	A	None
Field 3	N12°31.7'	W8°04.3'	328	Hal	NM	S	A	None

Lat = latitude, Long = longitude, Elev = elevation, GtGp = soil Great Group classification according to PIRT (1983), Cuir = Cuirorthents, Trop = Tropaqualfs, Hal = Halaquepts, PM = soil parent material group based on observed geology, M = mafic, NM = nonmafic, Geol = type of geology observed at the sites, D = dolerite, S = sandstone, Form = type of soil formation observed at the sites, A = alluvium, R = residuum, Conc = concretions present (cm depth), Surf = surface.

Sample Collection and Laboratory Analysis

Sorghum panicles were harvested from each plot in the first week of November 2009. Each panicle was dried and threshed by hand. A sample subset of 8 short and 8 tall varieties were selected. Approximately 100 g of sorghum from each plot were obtained and further cleaned by hand to remove chaff and gravel. Grain weights were recorded by measuring the weight of 100 randomly selected seeds. Approximately 4 g of clean grain from each sample was mill-ground to 1-mm size (Cyclone Lab Sample Mill, Udy Corporation, Fort Collins, CO, U.S.A.).

Milled grain was placed in Crystal Clear^{TM} plastic bags and scanned on a flatbed scanner for measurement of the three primary colors red, green, and blue by Color Scanning Analysis software (D. Verbree unpublished 2012). Color represents a mixture of the monochromatic spectra of red (700 nm), green (546 nm), and blue (436 nm), and on an 8-bit digital system, these three primary colors are quantified by numeric tristimulus values that range from 0 (darkness) to 255 (whiteness; Viscarra Rossel et al. 2006). The measured red, green, and blue values between 0 and 255 are then converted to a percent out of 255 and reported as a decimal number between 0 and 1 (i.e., 0.856).

Zinc concentrations in grain were quantified by a wet digestion method at Dairy One in Ithaca, NY. Briefly, 1 g of ground grain from the Crystal Clear^{TM} plastic bags was weighed out to the nearest 0.01 g and placed into Xpress Teflon PFA digestion vessels (CEM, Matthews, NC, U.S.A). Eight mL of concentrated nitric acid and 2 mL of concentrated hydrochloric acid were dispensed into the tubes and allowed to predigest at room temperature for 15 min. The tubes were then heated in a closed system under microwave assistance (CEM, Matthews, NC, U.S.A) at 1,600 W to 200 ° C and held there for 15 min. before cooling to room temperature. Vessels were diluted with deionized water to the 50 mL volume mark and transferred to 17 mL polypropylene tubes. Samples were then analyzed by inductively coupled plasma spectrometer (Intrepid ICP Radial Spectrometer, Thermo-Scientific, Waltham, MA, U.S.A.). Concentrations were reported on a dry matter basis.

Five soil cores were taken from each of the four fields in each location (from plots at the center and north, east, south, and east edges of a field) with a 32-mm diameter soil probe to the depth of a lateritic layer or a maximum of 90 cm. Short-variety sorghum fields at Wacoro were not sampled due to flooded conditions. The third field at the Field Station was a combination of short and tall sorghum-variety fields from which 6 total soil cores were taken from plots at the four corners of the field and at plots located intermediate along the west and east sides of the field. Latitude and longitude (World Geographic System 1984) of the soil cores were recorded by global positioning system receiver (Garmin etrex Vista HCx, Olathe, KS, U.S.A.).

Each soil core was divided into 15-cm depths, and the soil samples were placed in cotton cloth sample bags and allowed to air dry for at least 24 hours before shipping to the U.S.A. Soil samples were further air dried under laboratory conditions after arrival in the U.S.A. Air-dried soil samples were lightly ground and passed through a 2-mm sieve. Concretions that did not pass through the 2-mm sieve were weighed and the percent concretion was calculated as concretion weight divided by total sample weight multiplied by 100. Soil samples from the 0-15 cm and 15-30 cm intervals were analyzed for the following chemical properties.

Soil samples were extracted by DTPA (Lindsay and Norvell 1978) and the supernatant was filtered through a 0.45-µm pore-size MFS mixed cellulose ester membrane filter (Advantac, Dublin, CA, U.S.A.). Atomic absorption spectroscopy (AAnalyst 400, Perkin Elmer Instruments, Waltham, MA, U.S.A) was used to quantify Fe and Zn concentrations. Percent soil organic carbon and total nitrogen were measured by catalytic oxidation combustion (Vario Max CN analyzer, Elementar, Mt. Laurel, NJ, U.S.A.). Soil pH was measured using a 1:2 ratio of soil to water or 1 M potassium chloride (TitraLab^{TM} 90, Radiometer, Copenhagen, Denmark). Soil samples with pH_{KCl} < 4.5 were analyzed for exchangeable aluminum (Al) by transferring the 1:2 soil to 1 M KCl slurry to polypropylene centrifuge tubes with the addition of 5 mL of 1 m KCl. Centrifuge tubes were re-shaken 30 min., centrifuged for 20 min. at 29,668 g-force, and filtered through 0.45-µm pore-size MFS mixed cellulose ester membrane filter. Al concentrations were measured by atomic absorption spectroscopy (AAnalyst 400, Perkin Elmer Instruments, Waltham, MA, U.S.A).

Soil color was measured by Color Scanning Analysis software (D. Verbree unpublished software, 2012). Approximately 1-2 g of soil was placed in Crystal Clear^{TM} plastic bags and scanned on a flatbed scanner. Measured red, green, and blue color values were converted to the most commonly used soil color system, that of Munsell hue, value, and chroma (Viscarra Rossel et al. 2006). Red, green, and blue values can also be quantitatively related to various soil properties to produce radiometric indices.

The redness index (Eq. 2.1) was defined using measured red, green, and blue values (Madeira et al. 1997). This is a soil index based on the quantitatively derived relationship between red, green, and blue color with hematite content of soil.

Eq. 2.1 Redness index = $(\text{red}^2)/(\text{blue}*\text{green}^3)$

Statistical Analysis

Summary statistics were calculated for the five soil sample replications at 0-15 cm and 15-30 cm per field and Pearson's HSD correlation analysis was performed between soil properties. Log transformation improved normality and the significance (p value) for percent organic carbon and DTPA-extractable Fe and Zn, exchangeable Al, and WEP concentrations. Therefore, log transformations of these soil properties were used in all statistical analysis. For 13 fields, ANOVA and mean separations by the Tukey-Kramer method were performed to determine if fields were significantly different from each other by soil property. Soil properties and grain-Zn concentrations were averaged by field and Pearson's HSD correlation coefficients were calculated.

Grain Zn concentrations passed or came close to passing the Shapiro-Wilks normality test after log transformation. All subsequent statistical procedures were based on log transformations of grain Zn concentrations. A generalized linear model (GLM) procedure was conducted with variety as a fixed effect and field and block within field as random effects for grain-Zn concentration. A variance component procedure (VARCOMP) with a completely random model was used to partition out variance components (σ^2) and to calculate the percent influence of each factor (g = variety and e = environment). In this study, the field component is defined as the environment (e). From the variance components, broad sense heritability (h^2) estimates were made using equations 2.2 and 2.3 where r = # of replications and t = # of environments (Fehr 1991):

Eq. 2.2 Heritability per entry (h^2) = $\sigma^2_g/(\sigma^2_g + \sigma^2_e/rt + \sigma^2_{g*e}/t)$

Eq. 2.3 Heritability per plot = $\sigma^2_g/(\sigma^2_g + \sigma^2_e + \sigma^2_{g*e})$

All data were analyzed using SAS software version 9.2 (SAS Institute, Cary, N.C., U.S.A.).

Results

Soil Properties

Soil pH_{H2O} means ranged from 5.16+/-0.14 to 6.81+/-0.28 at the 0-15 cm depth and 4.97+/-0.28 to 6.12+/-0.56 at the 15-30 cm depth (Table 2.2). Soil pH_{KCl} values (data not shown) were approximately one unit lower than the soil pH_{H2O} values. Such relatively low soil pH_{H2O} values resulted in 6 out of the 13 fields having measurable exchangeable Al concentrations at the 0-15 cm depth and 10 out of 13 fields at the 15-30 cm depth (Table 2.2). Mean percent soil organic carbon ranged from 0.23+/-0.03 to 1.33+/-0.08% and 0.30+/-0.01 to 1.18+/-0.03% at 0-15 cm and 15-30 cm, respectively, whereas mean mass fraction of soil total nitrogen ranged from 213+/-81.9 to 757+/-70.1 mg kg^{-1} and 395+/-80.8 to 612+/-161 mg kg^{-1} in the 0-15 cm and 15-30 cm depths, respectively. WEP concentrations were generally very low (means < 66.4 μg kg^{-1}), except for Field 3 at the Field Station where the mean was 258+/-68.0 μg kg^{-1}.

Table 2.2: Mean and standard deviation (parentheses) of soil properties by location-field and depth.

Location-Field	Depth cm	pH_{H2O}	Exch Al $cmol_c\ kg^{-1}$	OC %	TN $mg\ kg^{-1}$	WEP $ug\ kg^{-1}$	DTPA Fe $mg\ kg^{-1}$	DTPA Zn $mg\ kg^{-1}$
<u>Keniero</u>								
Field 1	0-15	6.36 (0.55)	--	0.43 (0.13)	468 (58.2)	21.8 (17.1)	3.91 (1.15)	1.50 (0.62)
	15-30	5.42 (0.11)	0.58 (0.23)	0.59 (0.24)	612 (161)	--	1.90 (0.26)	0.88 (0.34)
Field 2	0-15	6.00 (0.26)	--	0.84 (0.11)	710 (51.6)	14.7 (5.4)	8.99 (3.76)	1.68 (1.47)
	15-30	5.46 (0.12)	0.23 (0.14)	0.43 (0.04)	461 (16.2)	--	5.22 (3.10)	0.65 (0.22)
Field 3	0-15	5.57 (0.22)	0.23 (0.19)	0.65 (0.17)	596 (87.1)	8.5 (3.5)	7.35 (2.56)	1.90 (1.72)
	15-30	5.26 (0.04)	0.88 (0.20)	0.39 (0.04)	450 (30.8)	--	3.01 (0.94)	1.01 (0.72)
Field 4	0-15	6.23 (0.45)	--	1.00 (0.27)	755 (178)	22.6 (18.4)	6.60 (3.99)	2.16 (1.20)
	15-30	5.96 (0.54)	0.13 (0.13)	0.69 (0.15)	533 (89.9)	--	5.43 (1.44)	0.95 (0.55)
<u>Tiguere</u>								
Field 1	0-15	6.24 (0.58)	--	1.33 (0.08)	590 (54.6)	12.8 (5.7)	7.75 (4.28)	2.78 (1.77)
	15-30	5.61 (0.47)	0.15 (0.14)	1.18 (0.03)	517 (12.6)	--	3.90 (1.37)	0.87 (0.34)
Field 2	0-15	6.10 (0.08)	--	1.13 (0.45)	757 (70.1)	14.4 (9.6)	6.07 (2.53)	0.67 (0.34)
	15-30	5.85 (0.14)	--	0.69 (0.44)	507 (100)	--	1.82 (0.25)	0.43 (0.31)
Field 3	0-15	5.64 (0.15)	0.02 (0.02)	0.72 (0.14)	699 (108)	7.2 (2.3)	7.68 (1.55)	0.59 (0.26)
	15-30	5.34 (0.20)	0.66 (0.47)	0.56 (0.06)	582 (33.7)	--	3.41 (0.91)	0.75 (0.49)
Field 4	0-15	5.16 (0.14)	0.98 (0.33)	0.56 (0.11)	542 (75.9)	10.7 (4.2)	12.0 (1.67)	1.80 (1.73)
	15-30	4.97 (0.28)	1.68 (0.57)	0.44 (0.08)	480 (54.7)	--	3.94 (0.69)	1.19 (0.44)
<u>Wacoro</u>								
Field 1	0-15	6.81 (0.28)	--	0.54 (0.05)	584 (99.2)	56.0 (22.3)	4.36 (2.37)	3.52 (3.17)
	15-30	6.12 (0.56)	--	0.34 (0.02)	456 (44.8)	--	5.02 (2.45)	0.80 (0.34)
Field 2	0-15	5.76 (0.54)	0.08 (0.08)	0.52 (0.02)	569 (36.5)	66.4 (39.0)	17.0 (7.62)	2.62 (1.94)
	15-30	5.49 (0.59)	0.70 (0.51)	0.36 (0.04)	516 (60.3)	--	7.75 (2.91)	0.62 (0.13)

Table 2.2: (Continued).

Location-Field	Depth cm	pH_{H2O}	Exch Al $cmol_c\ kg^{-1}$	OC %	TN $mg\ kg^{-1}$	WEP $ug\ kg^{-1}$	DTPA Fe $mg\ kg^{-1}$	DTPA Zn $mg\ kg^{-1}$
Field Station								
Field 1	0-15	5.26 (0.10)	0.33 (0.04)	0.23 (0.03)	213 (81.9)	21.9 (8.7)	6.25 (1.90)	1.02 (0.97)
	15-30	5.04 (0.11)	0.62 (0.23)	0.30 (0.01)	395 (80.8)	--	3.47 (0.54)	1.41 (1.05)
Field 2	0-15	5.17 (0.05)	0.49 (0.09)	0.62 (0.24)	364 (94.8)	11.1 (2.9)	11.6 (1.56)	0.42 (0.07)
	15-30	5.09 (0.08)	0.53 (0.25)	0.81 (0.16)	457 (38.0)	--	3.08 (0.64)	0.47 (0.08)
Field 3	0-15	6.09 (0.45)	--	0.36 (0.09)	294 (136)	258 (68.0)	10.5 (3.86)	0.43 (0.24)
	15-30	5.96 (0.40)	--	0.42 (0.14)	423 (53.7)	--	7.43 (3.09)	0.64 (0.13)
By Depth	0-15	5.88 (0.58)	0.16 (0.29)	0.68 (0.35)	545 (190)	43.8 (74.2)	8.49 (4.63)	1.66 (1.60)
	15-30	5.51 (0.48)	0.45 (0.50)	0.55 (0.28)	490 (87.5)	--	4.31 (2.44)	0.77 (0.48)
	p value	<0.0001	<0.0001	0.025	0.034	--	<0.0001	<0.0001

pH_{H2O} = soil pH in water, Exch Al = exchangeable Al, OC = organic carbon, TN = total nitrogen, WEP = water extractable phosphorus, DTPA = diethylenetriaminepentaacetic acid-extractable.

Mean DTPA-extractable Fe concentrations ranged from 3.91+/-1.15 to 16.96+/-7.62 mg kg^{-1} at 0-15 cm and 1.90+/-0.26 to 7.75+/-2.91 mg kg^{-1} at 15-30 cm. Mean DTPA-extractable Zn concentrations ranged from 0.42+/-0.07 to 3.52+/-3.17 mg kg^{-1} at 0-15 cm and 0.47+/-0.08 to 1.41+/-1.05 mg kg^{-1} at 15-30 cm. Soil pH_{H2O} and percent organic carbon, total nitrogen concentration, and DTPA-extractable Fe and Zn concentrations were significantly lower at the 15-30 cm depth than the 0-15 cm depth, whereas exchangeable Al concentration was significantly higher at depth ($p < 0.0001$; Table 2.2).

There were significant differences among fields for pH_{H2O}, percent concretions and organic carbon, and concentrations of exchangeable Al, total nitrogen, WEP, and DTPA-extractable Fe and Zn (ANOVA with Tukey-Kramer means separation; Table 2.3). Mean values for these soil properties were used in correlation analyses with average grain Zn concentrations. In 7 out of 13 fields, there were one or two DTPA-extractable Zn concentrations at 0-15 cm depth that were highly influential (> 3 mg kg^{-1}), thereby limiting any statistical differences between fields because of high within field variance (Table 2.4). Almost all of these influential concentrations for soil DTPA-extractable Zn concentrations were located next to Shea agroforestry trees either at the time of sampling or in recent history based on aerial photographs.

Table 2.3: Mean and standard error (SE) of DTPA-extractable Fe and Zn concentrations (mg kg^{-1}) by location-field and depth. Lowercase letters denote Tukey-Kramer groups resulting from the combined analysis of variance by the general linear model procedure based on log transformations of DTPA-extractable Fe and Zn concentrations and alpha = 0.05.

		DTPA Fe (mg kg $^{-1}$)				DTPA Zn (mg kg $^{-1}$)			
Location-		0-15 cm		15-30 cm		0-15 cm		15-30 cm	
Field	N	Mean	SE	Mean	SE	Mean	SE	Mean	SE
KEN-1	5	3.9d	0.52	1.9c	0.12	1.5ab	0.28	0.9ab	0.15
KEN-2	5	9.0abcd	1.7	5.2ab	1.4	1.7ab	0.66	0.7ab	0.10
KEN-3	5	7.4abcd	1.1	3.0bc	0.42	1.9ab	0.77	1.0ab	0.32
KEN-4	5	6.6abcd	1.8	5.4ab	0.65	2.2ab	0.54	1.0ab	0.25
TIG-1	5	7.8abcd	1.9	3.9abc	0.61	2.8a	0.79	0.9ab	0.15
TIG-2	5	6.1bcd	1.1	1.8c	0.12	0.7ab	0.15	0.4b	0.14
TIG-3	5	7.7abcd	0.69	3.4abc	0.41	0.6ab	0.12	0.8ab	0.22
TIG-4	5	12.0ab	0.75	3.9abc	0.31	1.8ab	0.78	1.2a	0.20
WAC-1	5	4.4cd	1.1	5.0ab	1.1	3.5a	1.4	0.8ab	0.15
WAC-2	5	17.0a	3.4	7.8a	1.3	2.6a	0.87	0.6ab	0.06
FS-1	5	6.3abcd	0.85	3.5abc	0.24	1.4ab	0.47	1.0ab	0.43
FS-2	5	11.6ab	0.70	3.1bc	0.29	0.5b	0.04	0.4ab	0.03
FS-3	6	10.5abc	1.6	7.4a	1.3	0.6ab	0.05	0.4ab	0.10

DTPA = diethylenetriaminepentaacetic acid, N = number of samples, KEN = Keniero, TIG = Tiguere, WAC = Wacoro, FS = Field Station.

Table 2.4: Mean DTPA-extractable Zn concentrations (mg kg^{-1}), number out of 5, and value of influential DTPA-extractable Zn concentration by location-field and suspected parent material. ** denotes locations adjacent to Shea trees, * denote locations adjacent to Shea trees in the past according to historical aerial photographs, # denote locations adjacent to dirt roads.

Location-Field	Parent Material	Mean DTPA Zn mg kg^{-1}	Influentials # out of 5	Influentials mg kg^{-1}
KEN-1	Dolerite	1.50	0	
KEN-2	Dolerite	1.68	1	4.27**
KEN-3	Dolerite	1.89	1	4.87*
KEN-4	Dolerite	2.16	1	3.92**
WAC-1	Sandstone	3.52	2	6.19*, 7.65
WAC-2	Sandstone	2.62	2	$3.98^{\#}$, 5.30*
TIG-1	Laterite (Sandstone)	2.78	2	3.42*, 5.53*
TIG-2	Laterite (Sandstone)	0.67	0	
TIG-3	Laterite (Sandstone)	0.59	0	
TIG-4	Laterite (Sandstone)	1.80	1	$4.70^{\#}$
FS-1	Allluvium (Sandstone)	1.41	0	
FS-2	Allluvium (Sandstone)	0.47	0	
FS-3	Allluvium (Sandstone)	0.64	0	

DTPA = diethylenetriaminepentaacetic acid, KEN = Keniero, TIG = Tiguere, WAC = Wacoro, FS = Field Station.

The soil properties pH_{H2O} and pH_{KCl} were significantly correlated ($p < 0.0001$) at both depths ($r = 0.99$ and $r = 0.95$, respectively, Table 2.5), and exchangeable Al concentration was significantly and negatively correlated ($p < 0.0001$) to pH_{H2O} at both depths ($r = -0.75$ and $r = -0.80$, respectively). Percent organic carbon and total nitrogen concentration were significantly and positively correlated ($p < 0.0001$) at both depths ($r = 0.82$ and $r = 0.57$, respectively).

Table 2.5: Correlation coefficients (r) between soil properties at 0-15 cm and 15-30 cm. ** denotes significance at the alpha = 0.01 level and * at the 0.05 level.

0-15 cm	DTPAFe	DTPAZn	pH_{H2O}	pH_{KCl}	ExchAl	OC	TN	WEP
DTPAFe	--							
DTPAZn	-0.042	--						
pH_{H2O}	-0.618**	0.222	--					
pH_{KCl}	-0.548**	0.111	0.987**	--				
ExchAl	0.325**	-0.151	-0.746**	-0.797**	--			
OC	0.036	0.074	0.230*	0.194	-0.324**	--		
TN	-0.030	0.183	0.286**	0.283*	-0.383**	0.816**	--	
WEP	0.071	0.060	0.376**	0.403**	-0.254*	-0.372**	-0.332**	--

15-30 cm	DTPAFe	DTPAZn	pH_{H2O}	pH_{KCl}	ExchAl	OC	TN	WEP
DTPAFe	--							
DTPAZn	0.104	--						
pH_{H2O}	-0.071	-0.185	--					
pH_{KCl}	-0.121	-0.199	0.947**	--				
ExchAl	0.015	0.268*	-0.796**	-0.878**	--			
OC	-0.139	-0.048	0.087	0.072	-0.057	--		
TN	-0.162	0.045	0.106	0.051	0.025	0.567**	--	
WEP	--	--	--	--	--	--	--	--

DTPA = diethylenetriaminepentaacetic acid-extractable, pH_{H2O} = soil pH in water, pH_{KCl} = soil pH in 1 M KCl, Exch Al = exchangeable Al, OC = organic carbon, TN = total nitrogen, WEP = water extractable phosphorus.

DTPA-extractable Fe and Zn concentrations were not correlated with other soil properties at 15-30 cm; however, DTPA-extractable Fe concentration was significantly ($p < 0.0001$) and negatively correlated with pH_{H2O} ($r = -0.55$) and significantly ($p = 0.008$) and positively correlated with exchangeable Al concentration ($r = 0.33$) at 0-15 cm. There were also some weakly significant correlations between pH_{H2O} and exchangeable Al concentration with soil fertility variables (organic carbon, total nitrogen and water extractable phosphorus).

Grain Zn Concentrations

Grain weights (100 grains) of short varieties (2.02+/-0.28 g) were significantly lower than tall varieties (2.19+/-0.25 g; Table 2.6). For short varieties, the mean grain Zn concentrations across all environments ranged from 12.6 mg kg^{-1} for Siguikumbe to 19.5 mg kg^{-1} for Togotigi (Table 2.7). The mean grain Zn concentrations across all short varieties had a slightly smaller range from 13.6 mg kg^{-1} for field TIG-3 to 18.5 mg kg^{-1} for field FS-3. Short varieties with a thin pericarp and purple glume color (Fada, Lata, and Mara varieties, Table 2.7) were consistently 14 mg kg^{-1} and significantly lower (with grain weight as a covariate) than the thin, red-glumed Tieble variety (17.5 mg kg^{-1}) and thick, tan-glumed cultivars Sawaba, Sewa, Siguikumbe, and Togotigi varieties ($p < 0.0001$). For tall varieties, the mean grain Zn concentrations across all environments ranged from 15.9 mg kg^{-1} for Jamajigi to 19.9 mg kg^{-1} for Sotigui (Table 2.7). Mean grain Zn concentrations across all tall varieties varied similarly from 16.1 mg kg^{-1} at field TIG-1 to 20.7 mg kg^{-1} at fields KEN-5 and WAC-2. Grain Zn concentrations in tall varieties with a thick pericarp were higher, but not significantly so when grain weight was accounted for in the model (p = 0.51).

Table 2.6: Sorghum grain physical properties by variety. Mean values are given with standard deviations in parentheses.

Genotype	Peri	Glume	Color Red	Green	Blue	GW
Short*						
Fada	Tn	P	0.798 (0.05)	0.705 (0.05)	0.655 (0.05)	2.05 (0.20)
Lata	Tn	P	0.783 (0.05)	0.692 (0.06)	0.654 (0.05)	2.20 (0.16)
Mara	Tn	P	0.790 (0.04)	0.699 (0.04)	0.661 (0.04)	2.20 (0.25)
Sawaba	Tk	T	0.887 (0.02)	0.813 (0.02)	0.754 (0.02)	2.00 (0.31)
Sewa	Tk	R/T	0.878 (0.03)	0.800 (0.03)	0.743 (0.03)	1.86 (0.23)
Siguikumbe	Tk	T	0.888 (0.02)	0.813 (0.03)	0.754 (0.03)	1.72 (0.30)
Tieble	Tn	R	0.846 (0.05)	0.758 (0.05)	0.708 (0.05)	2.02 (0.17)
Togotigi	Tk	T	0.892 (0.01)	0.820 (0.01)	0.763 (0.02)	2.13 (0.26)
Tall**						
Babalissa	Tn	T	0.859 (0.02)	0.786 (0.02)	0.722 (0.02)	1.90 (0.16)
Caufa	Tn	P	0.834 (0.05)	0.747 (0.05)	0.704 (0.05)	2.20 (0.21)
Jamajigi	Tn	R	0.887 (0.02)	0.816 (0.02)	0.751 (0.02)	1.99 (0.24)
Keneya	Tk	T	0.881 (0.02)	0.807 (0.02)	0.746 (0.02)	2.28 (0.20)
Omba	Tk	P	0.832 (0.05)	0.742 (0.05)	0.704 (0.04)	2.34 (0.17)
Pablo	Tn	P	0.835 (0.03)	0.746 (0.04)	0.702 (0.03)	2.33 (0.26)
Sotogui	Tn/Tk	R	0.839 (0.03)	0.747 (0.04)	0.698 (0.04)	2.32 (0.23)
Tieble	Tn	R	0.841 (0.04)	0.751 (0.04)	0.702 (0.04)	2.14 (0.15)
By Type	Short		0.847 (0.06)	0.764 (0.07)	0.714(0.06)	2.02 (0.28)
	Tall		0.851 (0.04)	0.768 (0.05)	0.716(0.04)	2.19 (0.25)
	p value		0.47	0.59	0.74	<0.0001

GW = grain weight, * grown in Keniero-Fields 3 and 4, Tiguere-Fields 3 and 4, and Field Station-Fields 2 and 3, ** grown in Keniero-Fields 1 and 2, Tiguere-Fields 1 and 2, Wacoro-Fields 1 and 2, and Field Station-Fields 1 and 3.

Table 2.7: Mean grain Zn concentrations (mg kg^{-1}) for short genotypes (upper table) and tall genotypes (lower table) by environment (location-field).

Genotype	Environment							
	KEN-		TIG-		FS-		G-mean	SE
	3	4	3	4	2	3		
Fada	12	14	15	13.5	15	16.5	14.3	0.58
Lata	14	14.5	11	14.5	16	19	14.3	0.83
Mara	12	12.5	11.5	13	16.5	18.5	14.0	0.87
Sawaba	15	16.5	14	19	20.5	21	17.7	0.91
Sewa	14	12	13.5	12	18	18	14.8	1.0
Siguikumbe	12	12	9.5	12	15	15	12.6	0.65
Tieble	15.5	16	15	18	20	20.5	17.5	0.70
Togotigi	19	18	19	18.5	23	19.5	19.5	0.57
E-mean	14.2	14.6	13.6	15.1	18.1	18.5	15.6	
SE	0.61	0.65	0.80	0.80	0.80	0.67		

Genotype	Environment									
	KEN-		TIG-		WAC-		FS-		G-mean	SE
	1	2	1	2	1	2	1	3		
Babalissa	19	17	16	16	15.5	21	17	18	17.4	0.5
Caufa	22	17	17	17.5	16	22	17	20	18.3	0.6
Jamajigi	20	14	16	16	14.5	17	15	14.5	15.9	0.5
Keneya	20.5	18	16.5	16.5	18.5	21.5	17.5	20.5	18.7	0.50
Omba	22.5	17.5	17.5	16.5	17	21	17	22	18.9	0.7
Pablo	20	16.5	14	15	16	19.5	13	20.5	16.8	0.7
Sotogui	20.5	19.5	16	22.5	17.5	23	16	22.5	19.9	0.8
Tieble	21	18	16	18	17	21.5	19	21	18.9	0.6
E-mean	20.7	17.2	16.1	17.3	16.5	20.7	16.6	19.9	18.1	
SE	0.36	0.49	0.34	0.60	0.34	0.52	0.51	0.64		

G-mean = genotypic mean, E-mean = environmental (location-field) mean, SE = standard error, KEN = Keniero, TIG = Tiguere, WAC = Wacoro, FS = Field Station.

The mean grain Zn concentration of short varieties was lower at 15.6 mg kg^{-1} than for tall varieties, which was at 18.1 mg kg^{-1} (Table 2.7). When a GLM procedure was performed with type of variety in the model and grain weight as a covariate, the results showed that tall varieties are significantly higher in Zn concentration than short varieties ($p = 0.047$, Table 2.8). The correlation coefficients between grain weight and grain Zn concentration for short and tall varieties were 0.404 and 0.378, respectively ($p < 0.0001$). For short varieties, the mean grain Zn concentrations by field within locations varied by less than 1.5 mg kg^{-1} whereas with tall varieties, mean grain Zn concentrations varied 4.2 mg kg^{-1}.

Table 2.8: Results of the combined analysis of variance by the general linear model procedure for Zn concentration (mg kg^{-1}) of whole grain sorghum with type of variety (short or tall) and grain weight in the model. ** denotes significance at the alpha = 0.01 level and * at the 0.05 level.

Zn Concentrations (mg kg^{-1})					
Source	DF	SS	MS	F	p
Variety Type (VT)	1	1.38	1.38	4.00	0.047*
Grain Weight (GW)	1	1.13	1.13	41.94	<0.0001**
VT * GW	1	0.05	0.05	1.92	0.17
Error	214	8.37	0.03		
CV%			5.85		

DF = degrees of freedom, SS = sum of squares, MS = mean squares, F = F test, p = p values, CV = coefficient of variation.

Effect of Genetic, Environmental, and Genetic x Environmental Interaction on Grain Zn Concentration

Grain Zn concentration in short variety sorghum grain was significantly affected by genotype, environment, and block within environment whereas grain Zn concentration in tall variety sorghum was significantly affected by genotype, environment, and genotype x environment (GLM Model; Table 2.9). For short varieties, the mean grain Zn concentration was highest at the two Field Station fields, FS-2 (18 mg kg^{-1}) and FS-3 (18 mg kg^{-1}; Table 2.10). For tall varieties, the mean grain Zn concentration was highest at fields KEN-1 (21 mg kg^{-1}), WAC-2 (21 mg kg^{-1}), and FS-3 (20 mg kg^{-1}; Table 2.10). The highest mean grain Zn concentrations were found for short varieties Togotigi (20 mg kg^{-1}), Sawaba (18 mg kg^{-1}), and Tieble (18 mg kg^{-1}), and for tall varieties Sotogui (20 mg kg^{-1}), Omba (19 mg kg^{-1}), and Tieble (19 mg kg^{-1}; Table 2.10).

Table 2.9: Results of the combined analysis of variance by the general linear model procedure for Zn concentration (mg kg^{-1}) of whole grain sorghum. ** denotes significance at the alpha = 0.01 level and * at the 0.05 level.

	Zn Concentrations (mg kg^{-1})									
Source	Short					Tall				
	DF	SS	MS	F	p	DF	SS	MS	F	p
Geno	7	1.87	0.27	18.95	<0.0001**	7	0.54	0.08	7.65	<0.0001**
Env	5	1.29	0.26	6.46	0.016*	7	1.25	0.18	17.73	<0.0001**
Block	6	0.22	0.04	3.38	0.009**	8	0.03	0.00	0.97	0.47
G x E	35	0.50	0.01	1.31	0.21	49	0.50	0.01	2.45	0.0008**
Error	92	4.40				124	2.59			
CV%	10.4					6.53				

Geno = genotype (G), Env = environment (E), DF = degrees of freedom, SS = sum of squares, MS = mean squares, F = F test, p = p values, CV = coefficient of variation. Environment based on location-field.

Table 2.10: Mean and standard error (SE) of whole grain sorghum Zn concentrations (mg kg^{-1}) by environment (location-field) across genotypes (upper table) and by genotype across environments (location-fields; lower table). Lowercase letters denote Tukey-Kramer groups resulting from the combined analysis of variance by the general linear model procedure based on log transformations of grain Zn concentrations and alpha = 0.05.

Zn concentration (mg kg^{-1})							
Location-Field	Short			Location-Field	Tall		
	N	Mean	SE		N	Mean	SE
FS-3	15	18a	0.67	KEN-1	16	21a	0.36
FS-2	15	18a	0.80	WAC-2	15	21a	0.52
KEN-4	15	15b	0.65	FS-3	16	20a	0.64
TIG-4	16	15b	0.80	TIG-1	16	17b	0.60
KEN-3	16	14b	0.61	KEN-2	16	17b	0.48
TIG-3	16	14b	0.80	WAC-2	16	17b	0.34
				FS-1	14	17b	0.51
				TIG-1	16	16b	0.34

Zn concentration (mg kg^{-1})							
Genotype	Short			Genotype	Tall		
	N	Mean	SE		N	Mean	SE
Togotigi	12	20a	0.57	Sotogui	15	20a	0.77
Tieble	12	18a	0.70	Omba	16	19a	0.66
Sawaba	12	18a	0.91	Tieble	16	19a	0.55
Sewa	11	15b	1.0	Keneya	16	19ab	0.50
Fada	12	14b	0.58	Caufa	15	181b	0.59
Lata	10	14b	0.83	Babalissa	16	17bc	0.49
Mara	12	14bc	0.87	Pablo	16	17cd	0.72
Siguikumbe	12	13c	0.65	Jamajigi	15	16d	0.52

N = number of samples, KEN = Keniero, TIG = Tiguere, WAC = Wacoro, FS = Field Station.

Genotype explained 41 and 19% of the variation in grain Zn concentration in short and tall varieties, respectively (VARCOMP Model; Table 2.11). These percentages were approximately reversed for environment as 30 and 49% of the variance was explained in short and tall varieties. The genotype x environment interaction accounted for 2% and 13% of total variance for short and tall varieties, respectively. Broad sense heritability (h^2) estimates based on entry means for Zn in whole grain sorghum were 90% for short varieties and 84% for tall varieties, whereas the heritability on a per plot basis was 54 and 45%, respectively.

Table 2.11: Results of the combined analysis of variance by the variance component (VARCOMP) procedure for Zn concentration (mg kg^{-1}) of whole grain sorghum.

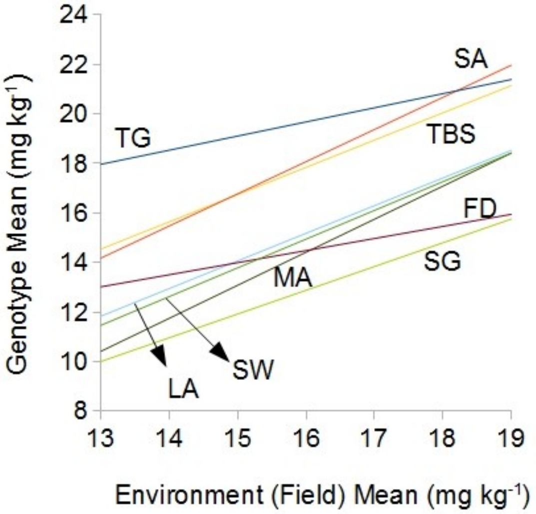
	Zn Concentrations (mg kg^{-1})					
Source	Short			Tall		
	DF	Varcomp	%Total	DF	Varcomp	%Total
Geno	7	5.14	41.33	7	1.47	19.42
Env	5	3.72	29.92	7	3.73	49.28
Block	6	0.66	5.33	8	0.01	0.12
G x E	35	0.26	2.11	49	0.96	12.68
Error	92	2.65	21.31	124	1.40	18.50

Geno = genotype (G), Env = environment (E), DF = degrees of freedom, E based on location-field.

An interaction plot of the two factors, genotype and environment, illustrated which short and tall varieties had cross-over interactions (Figure 2.2). For the short varieties, very little cross-over occurred except for the low-Zn genotype Fada and the high-Zn genotype Togotigi. Among the high-Zn tall varieties, Omba and Caufa were lower ranking than Tieble and Keneya at low environment means. For low-Zn tall varieties, Pablo similarly switched rank below Babalissa and Jamajigi. This indicated that these 3 varieties (Omba, Tieble, and Pablo) may be more responsive to high-Zn soil environments.

Relationship between Sorghum Grain Zn Concentration and Soil Properties

Significant correlations between means of grain Zn concentration and soil property means occurred with total nitrogen concentration (r = -0.919; p = 0.01) and percent concretions (r = -0.915; p = 0.01) at 0-15 cm for short varieties (Table 2.12). A significant negative correlation also occurred for percent concretions (r = -0.966; p = 0.002) at 15-30 cm. No significant correlations occurred at either depth for tall varieties. Correlations of mean concentrations of DTPA-extractable Fe (Figure 2.3) and Zn (Figure 2.4) and grain Zn were not significant for either short or tall varieties (Table 2.12). Correlations between DTPA-extractable Zn and grain Zn concentrations appeared to be hampered by the large standard error occurring in some fields for DTPA-extractable Zn concentration (Figure 2.4).



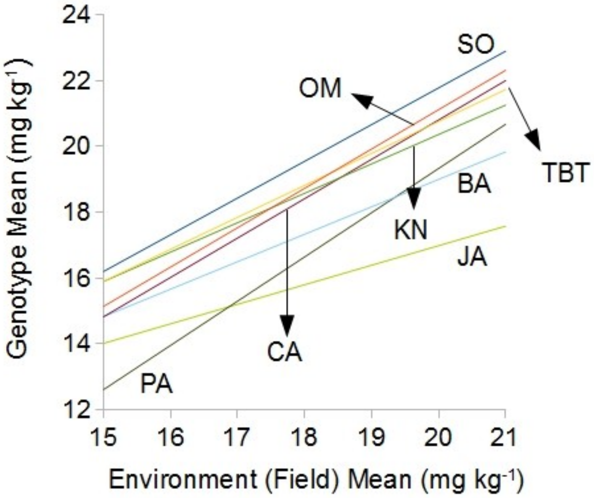
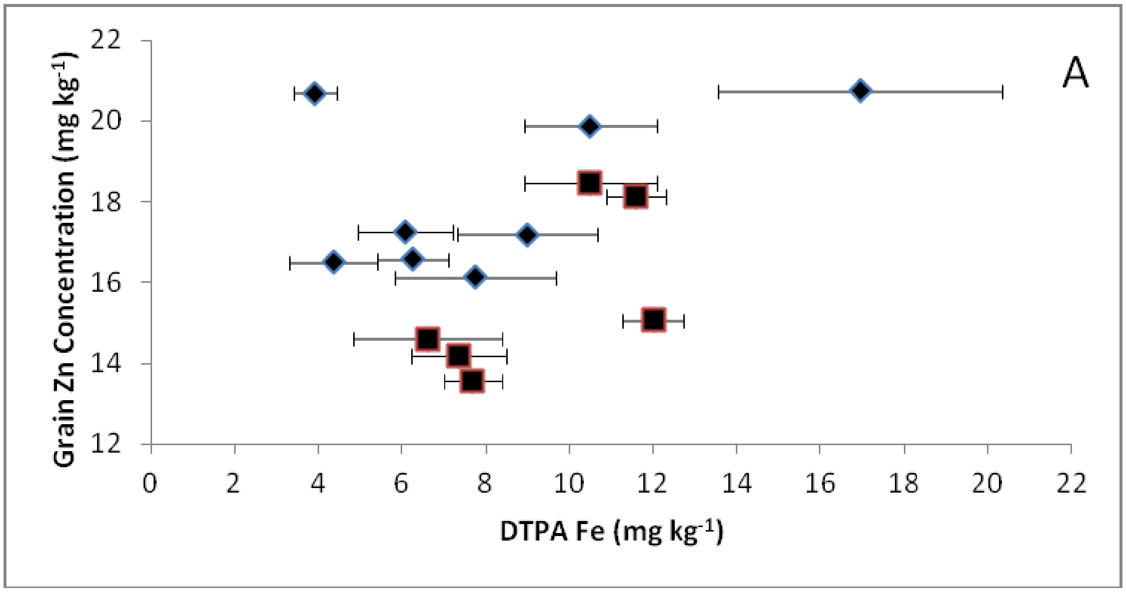


Figure 2.2: Interaction plots of genotype and environment (field) for grain Zn concentrations (mg kg^{-1}) of short varieties (upper figure) and tall varieties (lower figure). FD=Fada, LA=Lata, MA=Mara, SA=Sawaba, SW=Sewa, SG=Siguikumbe, TBS=Tieble-short, TG=Togotigi, BA=Babalissa, CA=Caufa, JA=Jamajigi, KN=Keneya, OM=Omba, PA=Pablo, SO=Sotogui, and TBT=Tieble-tall.

Table 2.12: Correlation coefficients (r) between environment (field) means of grain Zn concentration for short and tall varieties and soil property means at 0-15 and 15-30 cm depth. ** denotes significance at the alpha = 0.01 level and * at the 0.05 level.

	Grain Zn Concentration (mg kg^{-1})			
	Short		Tall	
Soil Property	0-15cm	15-30cm	0-15cm	15-30cm
DTPA Fe	0.615	0.407	0.348	0.178
DTPA Zn	-0.504	-0.771	-0.161	-0.186
pH_{H2O}	-0.017	0.186	-0.034	-0.038
pH_{KCl}	0.090	0.386	0.037	-0.051
ExchAl	0.059	-0.399	-0.189	0.442
OC	-0.691	0.149	-0.355	-0.229
TN	-0.919**	-0.734	-0.204	0.459
WEP	0.685	--	0.472	--
perconc	-0.915*	-0.966**	-0.420	-0.366
hue	-0.748	-0.449	0.177	0.087
value	0.075	-0.477	0.122	-0.003
chroma	0.232	-0.159	-0.142	-0.252
redness	0.035	0.657	-0.052	-0.014

DTPA = diethylenetriaminepentaacetic acid-extractable, pH_{H2O} = soil pH in water, pH_{KCl} = soil pH in 1 M KCl, ExchAl = exchangeable Al, OC = organic carbon, TN = total nitrogen, WEP = water extractable phosphorus, perconc = % concretions, redness = redness index.



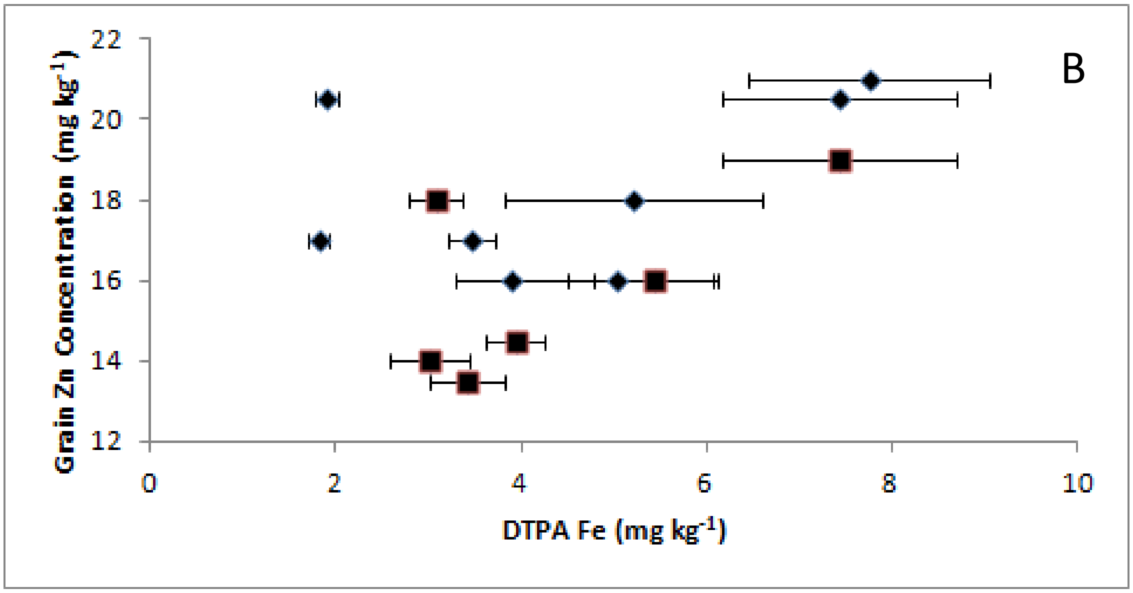


Figure 2.3: Environmental (field) mean grain Fe concentration (mg kg^{-1}) versus mean DTPA Fe (mg kg^{-1}) from: A) 0-15 cm; and, B) 15-30 cm. Error bars are based on the standard error of the mean. Standard error bars for grain Zn concentrations range from 0.34 to 0.80 mg kg^{-1} and are not included in the figures. DTPA Fe = DTPA-extractable Fe concentration, Squares = short varieties, Diamonds = tall varieties.

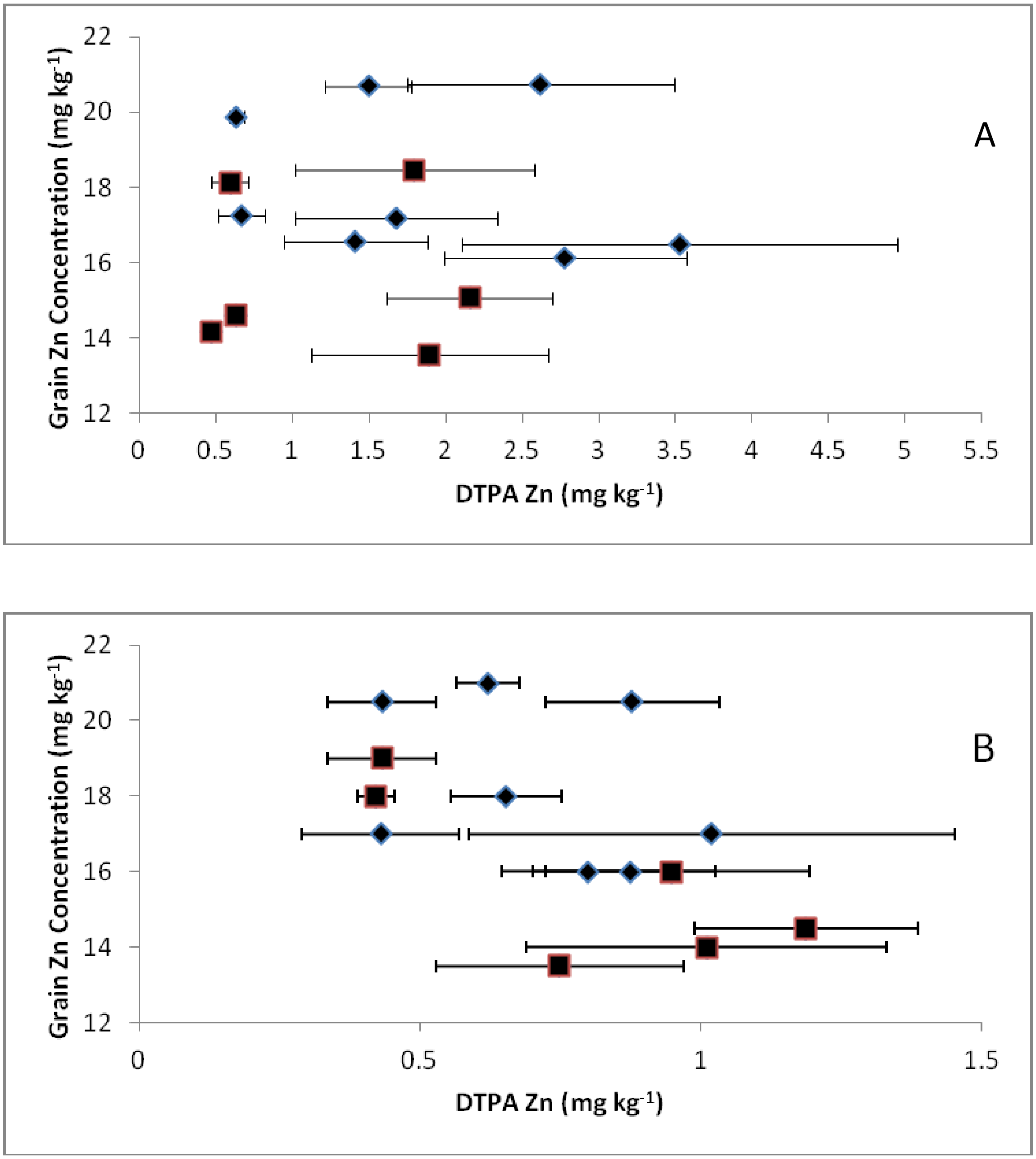


Figure 2.4: Environmental (field) mean grain Zn concentration (mg kg^{-1}) versus mean DTPA Zn (mg kg^{-1}) from: A) 0-15 cm; and, B) 15-30 cm. Error bars are based on the standard error of the mean. Standard error bars for grain Zn concentrations range from 0.34 to 0.80 mg kg^{-1} and are not included in the figures. DTPA Zn = DTPA-extractable Zn concentration, Squares = short varieties, Diamonds = tall varieties.

Discussion

Soil Properties

Soil properties pH_{H2O}, percent organic carbon, and total nitrogen concentration had a similar range to those reported in other studies of highly weathered soils in southern Mali (Soumare et al. 2002; Keita 2000) and the Sudan zone of West Africa (Saiz et al. 2012). Percent organic carbon and total nitrogen concentration were significantly correlated, but with lower r values (0.82 at 0-15 cm and 0.57 at 15-30 cm) than expected which may reflect tillage and fertilizer addition at these fields. The negative correlation between pH_{H2O} and exchangeable Al concentration was expected, as measurable amounts of Al ions are present at soil $pH_{H2O} < 5.5$ (Sumner and Yamada 2002). The weak negative relationship between exchangeable Al concentration and the weak positive relationship between soil pH and soil fertility variables such as percent organic carbon and total nitrogen concentration illustrated the important role organic matter plays in buffering acidic conditions in these soils. Organic matter may increase pH through the specific adsorption of organic anions onto hydrous Fe and Al surfaces and subsequent release of hydroxyl ions (Fageria and Baligar 2008).

Information on DTPA-extractable Fe and Zn concentrations in West African soils is scarce. Mean DTPA-extractable Fe concentrations ranged from 36.6 to 117 mg kg^{-1} from Northern Guinea savanna Alfisols in Nigeria (Agbenin 2003a) that had a variety of cultivation practices including fallow, inorganic fertilization, organic fertilization, and both inorganic and organic fertilization. Other broader soil studies such as a comprehensive study of DTPA-extractable Fe and Zn concentrations from northern

Indian Indo-Gangetic Plain soils across arid and humid zones had DTPA-extractable Fe mean concentrations at 0-15 cm depth ranging from 15.0 to 73.4 mg kg^{-1} (Sidhu and Sharma 2010). The northern Indian study also reported a general decrease in DTPA-extractable Fe concentrations with depth and a negative correlation with soil pH. Mean DTPA-extractable Fe concentrations in the current study generally fell below the reported minimum range (<15 mg kg^{-1}) perhaps because the soils were upland soils that tended to be more well drained and thus more oxidizing and likely to contain less available Fe than poorly drained and more reduced lowland soils (Shenker and Chen 2005). For cultivation purposes, a soil is considered Fe deficient if DTPA-extractable Fe concentration < 4.5 mg kg^{-1} (Lindsay and Norvell 1978). However, field crops such as sorghum have their own mechanisms of Fe uptake by using phtyosiderophore compounds that can dissolve Fe oxides which renders Fe available for plant uptake (Robin et al. 2008).

Buri et al. (2000) reported a range of mean DTPA-extractable Zn concentrations of 0.37-0.99 mg kg^{-1} for soils (0-15 cm) of inland valley swamps and a range of 0.04-3.53 mg kg^{-1} for soils (0-15 cm) of river floodplains across the Sudan savanna in West Africa. Mean DTPA-extractable Zn concentrations ranged from 0.51 to 1.01 mg kg^{-1} from Northern Guinea savanna Alfisols in Nigeria (Agbenin 2003b), and DTPA-extractable Zn mean concentrations ranging from 0.31 to 1.61 mg kg^{-1} with concentrations as high as 8.60 mg kg^{-1} were recorded from soils in northern India (Sidhu and Sharma 2010). Mean DTPA-extractable Zn concentrations in my study fell within and slightly above reported means from previous studies in West Africa and India.

Critical DTPA-extractable Zn concentrations ranged from 0.5 to 1.2 mg kg^{-1} specifically for sorghum grown in different soils of southern India (Takkar et al. 1989); thus, many agricultural soils, including the soils in my study, would be considered Zn-deficient. This is a problem noted in well weathered, acidic, continuously cultivated soils in southern Mali and other areas of the world (Soumare et al. 2002; Alloway 2008).

While it is widely reported that DTPA-extractable Zn concentration is positively correlated with soil percent organic carbon (Buri et al. 2000; do Nascimento et al. 2006; Sidhu and Sharma 2005); no significant correlation was found between soil percent organic carbon and DTPA-extractable Zn concentration in my study probably because of the low range of percent organic carbon present in soils. It has also been reported that pH and DTPA-extractable Zn concentration are negatively correlated for soils with a range of pH (5-9) across northern North Dakota (Wu et al. 2006). The relatively narrow range of pH (5-7) in my study may have also resulted in a lack of correlation between DTPA-extractable Zn concentration and pH.

Grain Zn Concentrations

Average grain Zn concentration of 17.8 mg kg^{-1} were reported for 5 short and tall sorghum varieties grown in 9 locations in southern Mali in 2006 (Tuinsma et al. 2008). The average grain Zn concentration for short varieties (15.6 mg kg^{-1}) and tall varieties (18.1 mg kg^{-1}) in the current study supports grain Zn concentrations reported by Tuinsma et al. (2008). However, average grain Zn concentration in my study was lower than the concentration of 26.5 mg kg^{-1} reported in sorghum grown in the Mali Sahel region (Gardestedt 2009) yet higher than the average grain Zn concentration of 10 mg kg^{-1} in sorghum grown in the far south of Mali (Barikmo et al. 2007). Published concentrations for sorghum grain Zn concentrations in Mali are very limited and comparisons should be viewed in a general way as sampling and analytical methods across studies do vary substantially. In addition, grain Zn concentrations can significantly vary from year to year in sorghum (Kumar et al. 2010).

Negative relationships sometimes occur between sorghum grain Zn concentration and glume color in short varieties (Reddy et al. 2010). Short variety glume color (low = white, high = purple) and grain Zn concentration correlation was reported in a study conducted on 84 sorghum lines from India (Reddy et al. 2010) and my data supports this correlation. However, the significant negative relationship between grain Zn concentration and pericarp thickness in short varieties (low = thick, high = thin) found in my study was not observed in a study of both early and later maturing sorghums from India (Belum et al. 2005). It must be noted that the short varieties with thick pericarps in my study also had light glumes.

Weak, significant positive correlations exist between grain Zn concentration and grain weight (Tuinsma et al. 2009; Kumar et al. 2010). Although grain weight may play a positive role in grain Zn concentration, it does not appear to explain all of the variance in grain Zn concentration between short and tall varieties. The current study showed that while there was no interaction between grain weight and variety, variety was also able to explain some of the variance in grain Zn concentration. Only one study conducted on approximately 3,000 sorghum accessions in India reported a significant, but weak relationship between grain Zn concentration and days to 50% flowering (Reddy et al.

2010). Other studies conducted with substantially less accessions showed non-significant relationships (Kumar et al. 2010; Kumar et al. 2009; Belum et al. 2005). Tall, later maturing varieties may have increased root length and root dry matter than short, early maturing varieties (Bruck et al. 2003a). Zn uptake by roots is dependent upon diffusion of Zn^{2+} to the roots; thus, increased root growth could lead to increased Zn uptake and grain Zn concentrations (Singh et al. 2005) in tall varieties.

Effect of Genetic, Environmental, and Genetic x Environmental Interaction on Grain Zn Concentration

Variation in sorghum grain Zn concentration can be explained by genotype, its local growing environment, and an interaction between the two factors (Tuinsma et al. 2009). The ICRISAT 2007 trial conducted in southern Mali showed that the variation in grain Zn concentration of decorticated sorghum was explained by genotype (39%), environment (17%), and genotype x environment interaction (15%) for short varieties and by genotype (21%), environment (26%), and genotype x environment interaction (16%) for tall varieties (Tuinsma et al. 2009). My results supported the findings at the ICRISAT 2007 trial where genotype explained 41% of the variation in grain Zn concentration of short varieties, environment 30%, and genotype x environment 19%, and genotype explained 19% of the variation in tall varieties, environment 49%, and genotype x environment 13%. The 2007 trial consisted of twice as many varieties and environments compared to my study and thus may have been better able to capture genotype x environment interactions. For both studies, a higher amount of variance was explained by environment rather than genotype for the tall varieties versus short varieties. The longer maturity time for tall varieties may have led to larger root systems and more variable Zn uptake depending on the availability of soil Zn in the root zone (Bruck et al. 2003a).

Broad sense heritability values are a quantification of the genetic influence on the phenotypic trait of grain Zn concentration (Gomez-Becerra 2010). High broad sense heritability, as was found in my study (90 and 84% for short and tall varieties, respectively), indicate a high genetic influence. High broad sense heritability values for grain Zn concentration have been reported in other sorghum studies. Belum et al. (2005) reported a broad sense heritability of 86% for both early and late maturing sorghum in India whereas Tuinsma et al. (2009) reported a broad sense heritability of 96% (short varieties) and 91% (tall varieties) of decorticated sorghum grown in southern Mali. These results show good breeding potential for grain Zn concentration in sorghum. Genotypes that are particularly high in grain Zn concentration and show good stability across environments (i.e. Togotigi, Fada, and Sotigui; Figure 2.2) are most desired for biofortification purposes.

Relationship between Sorghum Grain Zn Concentration and Soil Properties

The relationship between grain Zn concentration and soil DTPA-extractable Zn concentration has not always been reported to be significant (Wang et al. 2009). In my study, grain Zn concentration was not significantly correlated with soil DTPA-extractable Zn concentration. This contrasts to studies conducted with rice (Wissuwa et al. 2008), barley (Lombaes and Singh 2003), and wheat (Joshi et al. 2010). The variation of DTPA-extractable Zn concentration among 5 soil samples collected across a 35 x 30 m field in my study was too high for a good comparison with grain Zn concentration. The variation in many cases stemmed from the presence of 1 or 2 influentially high DTPA-extractable Zn concentrations (up to 7.65 mg kg^{-1}) per field. Both the Buri et al. (2000) West African soil study and the Sidhu and Sharma (2009) north Indian soil study also reported DTPA-extractable Zn soil concentrations > 6 mg kg^{-1} as did studies by Wu et al. (2006) in North Dakota and do Nascimento et al. (2006) in Brazil.

Shea trees are the primary agroforestry tree utilized in the West African parkland agricultural system and have been shown to improve overall soil fertility including organic matter and sorghum production (Traore et al. 2004; Boffa et al. 2000). The presence or past presence of Shea trees appeared to be spatially related to the influentially high soil DTPA-extractable Zn concentrations in my study. Available Zn in soil would likely increase due to the root uptake of Zn and its deposition and accumulation in top soil through Shea tree leaf litter. Little work has been done regarding soil micronutrients within parkland systems of Africa except for one study with *Acacia erioloba* trees in South Africa (Murovhi and Matercechera 2006). Murovhi and Matercechera (2006) reported that both DTPA-extractable soil Fe and Zn and wheat-grain Fe and Zn concentrations were higher in field positions surrounding trees.

In addition, relative to Shea trees, many of the highly influential DTPA-extractable Zn concentrations in my study also occurred in soils with a dolerite parent material. Dolerite is an igneous mafic rock relatively high in Zn that could possibly contribute more Zn to soils than low Zn parent materials such as sandstone (Tardy 1997).

Other soil properties such as total nitrogen concentration and percent concretions were better correlated with short variety grain Zn concentrations than soil DTPA-extractable Zn concentrations in my study. Some of the farmers observed temporary ponding in their fields after hard rain events. Temporary ponding would impede nitrate-N losses through leaching, increase their availability for denitrification, and lead to overall total nitrogen loss. In addition, ponding would cause temporarily reduced conditions which can facilitate Fe oxide solubility and potential release of any specifically adsorbed Zn^{2+} ions from Fe oxide surfaces (Bigham et al. 2005) and may have led to higher Zn uptake and grain Zn concentrations. Percent concretions appeared to negatively affect grain Zn concentrations perhaps as a result of concretions impeding sorghum root growth and limiting diffusion of Zn^{2+} to the roots (Singh et al. 2005).

In southern Mali, soil properties can differ depending on laterite occurrence and erosion and toposequence position. Soils that form from laterite will contain a higher percentage of concretions and clay in its upper layers relative to alluvial or non-lateritic soils that are sandier at the top of the soil profile and have concretions only deeper in the profile. Depending on the toposequence position, a lateritic soil may or may not have its drainage impeded, leading to temporary reducing conditions. It is therefore not surprising that soil properties and crop growth conditions varied spatially in fields located 1-3 km away from each other and that the environmental effect on grain Zn concentrations occurred by field within location.

Most Malian soils have been weathering for a long period of time, thus any particular soil can develop very low pH and high exchangeable Al. It appears that the short varieties in my study were located on such poorer soils with significantly lower pH ($p = 0.0002$), higher exchangeable Al concentration ($p = 0.0003$), and higher percent concretions ($p < 0.0001$) and that this could have led to significantly lower grain Zn concentrations as these soil properties can negatively affect sorghum growth (Doumbia et al. 1993). The selection of fields for breeding variety trials should therefore take into account these “poor soil” properties, particularly drainage properties. Sorghum varieties then can be tested under both poor and good soil Zn conditions, allowing for a better test of varieties that show Zn efficiency or responsiveness.

It is recommended that further studies be conducted on the possible influence of Shea tree and parent material on DTPA-extractable Zn concentration and subsequently on sorghum grain Zn concentration. It would be important to determine how much of an increase in Zn could occur in sorghum grown in certain soils under agroforestry conditions. Also, any further investigation would require soil sampling at a smaller scale (1-2 m) and a one to one correspondence between grain Zn concentration and soil properties.

CHAPTER III

INFLUENCE OF SOIL PARENT MATERIAL AND SHEA (*VITELLARIA PARADOXA* C.F. GAERTN) TREES ON SOIL PROPERTIES AND SORGHUM (*SORGHUM BICOLOR* L. MOENCH) GRAIN Zn CONCENTRATIONS IN SOUTHERN MALI, WEST AFRICA

Introduction

A better understanding of the uptake of Zn and the environmental factors that tend to increase Zn in sorghum grain could aid breeders in their efforts to biofortify sorghum. It is a well-documented problem that many agricultural soils are Zn deficient and present a challenge to genetic biofortification (Cakmak 2008). It becomes necessary then to investigate what soil factors are limiting Zn availability and whether these can be improved by agronomic practices so that biofortified varieties can fully express their ability to increase Zn in grain.

The amount of Zn uptake and ultimate storage in sorghum grain depends on a number of factors including plant available Zn concentration in the soil, which is determined by total Zn concentration as well as several soil properties that influence its availability (Alloway 2008). Total Zn concentration in soil is dependent on the relative Zn composition of the soil parent material (Alloway 2008). Rocks can be mafic and relatively high in Zn concentration (mean = 123 mg kg^{-1}) or nonmafic and relatively low in Zn concentration (mean = 45 mg kg^{-1}; Krauskopf 1967; Wedepohl 1978; Anand and Gilkes 1987). Plant available Zn concentration depends largely on soil pH and organic matter content (Cakmak 2008). Soils that have low amounts of total Zn and organic matter and are calcareous with a high pH can lead to low plant available Zn concentration and concomitant Zn deficiency in crops (Cakmak 2008). Soils in southern Mali are generally non-calcareous with a pH of less than 7 and low amounts of total Zn and organic matter. Continuous cropping and removal of stover material in these soils have led to Zn deficiencies in many locations (Gardestedt et al. 2009; Soumare et al. 2002).

Elevated concentrations of plant available Zn, as measured by diethylenetriaminepentaacetic acid (DTPA) extraction, were found adjacent to Shea (*Vitellaria paradoxa* C.F. Geartn) trees and in areas where mafic parent material (dolerite) was located (Chapter 2). It was hypothesized that the influence of Shea trees and dolerite parent material are responsible for these elevated DTPA-extractable Zn concentrations. A possible mechanism for this influence includes Shea root growth and the uptake of Zn that is present through the weathering of mafic minerals. The decomposition of the Shea leaf litter likely leads to increased organic matter concentration which could then chelate the relatively large amount of Zn, increasing its solubility and plant availability, thus elevating DTPA-extractable Zn concentrations in the soil.

Shea trees are the most popular agroforestry tree in Mali (Bazie et al. 2012) with an estimated distribution area of 22.9 million ha in southern Mali (Boffa 1999). Shea nuts are processed into butter used for cooking oil and cosmetics, fruits are eaten fresh, bark and roots used for medicine, and wood used for fuel (Boffa et al. 2000). In southern

Mali, traditional “bush” fields are located outside village compounds, and crops are grown for 5 years and then allowed to fallow for 10-15 years (Namankan Keita - farmer Keniero village, personal communication). At the end of the fallow time, small trees and bushes are burned and cleared and the field planted again. Large trees such as the Shea tree are grown within the fields at various densities of typically 1.5-24 trees per hectare (Traore et al. 2004).

Shea tree height, bole circumference, and canopy diameter average 11 m, 1.4 m, and 10 m, respectively in southern Mali (Sanou et al. 2006) with some trees growing up to 25 m high. The Shea tree tap root has been estimated to be 1 m long, but can reach up to 2 m deep (Breman and Kessler 1995). Lateral roots extend as much as 20 m out from the tap root and grow downwards to a 2 m depth with most roots concentrated at 10-20 cm depth (Hall et al. 1996). The Shea tree is considered a shallow root tree and is correspondingly grown at the highest densities in the lower part of the soil catena where water availability and fertility are greatest (Traore et al. 2004; Gijsbers et al. 1993). Leaves of the Shea begin to shed in December and through the dry season, and leaf litter production is estimated to range from 0.32 to 1.78 kg m^{-2} (Traore et al. 2004). Decomposition of Shea leaves is relatively slow (Bayala et al. 2005); however, leaf matter was reported to significantly affect soil fertility in a Shea tree and soil catena study conducted in Burkina Faso (Traore et al. 2004). They reported that for Shea trees located in the middle and lower sections of the soil catena, significantly higher pH, carbon, and nitrogen occurred in soils directly under the canopy, whereas lower pH, carbon, and nitrogen occurred in soils outside the canopy.

Increased organic matter from the leaf litter of Shea trees could lead to a corresponding increase in DTPA-extractable Zn concentration; however, very little information is known about the possible extent of improvement in micronutrients such as Fe and Zn in soil due to agroforestry trees. One study has reported the relationship between agroforestry trees and micronutrients in the soil and grain wheat (Murovhi and Materechera 2006). They reported significant increases of DTPA-extractable Fe and Zn concentrations in soil and wheat inside the canopy relative to outside the canopy of *Acacia erioloba* trees in South Africa.

In mixed vegetation communities such as Shea tree-cropland, stable isotope-ratio analysis can describe the origin of soil carbon and nitrogen in soil (Takimoto et al. 2009; Hobbie and Ouimette 2009). $\delta^{13}C$ values express the relative carbon isotope composition (^{13}C and ^{12}C) and are related to photosynthetic pathway. C3 plants such as the Shea tree have average $\delta^{13}C$ values of $-27^{0}/_{00}$ whereas C4 plants such as sorghum average $-13^{0}/_{00}$ (Boutton 1991). $\delta^{13}C$ values of $-20.7^{0}/_{00}$ to $-18.7^{0}/_{00}$ were reported for soils in a Mali Shea tree-cropland study (Takimoto et al. 2009). In addition, $\delta^{15}N$ values express the relative nitrogen isotope composition (^{15}N and ^{14}N) and are related to a host of soil N processes including N transfer from mycorrhizal fungi to its plant host (Hobbie and Ouimette 2009). Only one study has been conducted on the inoculation of Shea trees with arbuscular mycorrhiza, and results showed generally low root colonization in Shea seedlings grown in pots of sandy soil (Dianda et al. 2009).

Elevated concentrations of DTPA-extractable Zn were also found in areas where dolerite rock was observed (Chapter 2). Within southern Mali, there are four types of original rock parent material from different ages: Precambrian greenstones, schists, and granites, Cambrian sandstone, Jurassic dolerite, and Tertiary laterite (Picouet et al. 2001). The rock types considered to be mafic and subsequently relatively higher in Zn are the Precambrian greenstones and schists and Jurassic dolerite. Nonmafic Precambrian granite and Cambrian sandstones are relatively low in Zn (Tardy 1997). Laterite rock generally reflects the original parent material such that laterite formed from mafic rock would retain a relatively higher amount of Zn than laterite formed from nonmafic rock (Anand and Gilkes 1987; Tardy 1997). Soils derived from mafic and mafic-origin laterite should contain higher concentrations of total Zn. Indeed, recently published soil geochemical maps show anomalously high concentrations of total Zn within the 0-30 cm soil layer throughout southern and western Mali in areas of Precambrian greenstones (Feybesse 2006).

Geochemical prospecting studies have shown that sampling various trace metals in the 0-4 cm soil layer adjacent to trees can be an effective marker of buried mineral deposits below (Anand et al. 2007). With tree roots growing into soils with elevated concentrations of total Zn derived from mafic parent material, there may be a significant increase in Zn uptake when compared to trees growing into soils derived from nonmafic parent material which have low concentrations of total Zn.

If an interaction between parent material and tree leads to increased Zn concentration in soils, then it may also lead to increased Zn concentrations in sorghum grain. A Burkina Faso field study showed that sorghum grain Zn concentrations could be significantly improved with Zn fertilization or a combination of inorganic Zn fertilizers and compost (Traore 2006). Other studies have shown that Zn fertilization can alleviate Zn deficiency symptoms in crops as approximately 20% of the applied Zn can become available (Singh et al. 2005). No studies have investigated whether agroforestry trees can similarly increase Zn in crops except for the *Acacia erioloba* study (Murovhi and Materechera 2006) which found that grain Fe and Zn concentrations were significantly higher in wheat planted near the tree.

The objective of this study was to determine the influence of parent material and Shea trees on soil properties, grain properties, and grain Zn concentrations in sorghum.

Materials and Methods

Location and Site Description

Four locations in southern Mali were selected for this study (Figure 3.1). Wacoro and Teneya were previously observed to have nonmafic parent material such as sandstone, whereas dolerite was observed in Keniero. Results of previous sampling and analysis of soils in the region indicated an effect of trees and geology (Chapter 2). Yekelebougou was selected based on geological information from Dars (1962) and field observations of dolerite from outcrops north of Bamako (Figure 3.2).

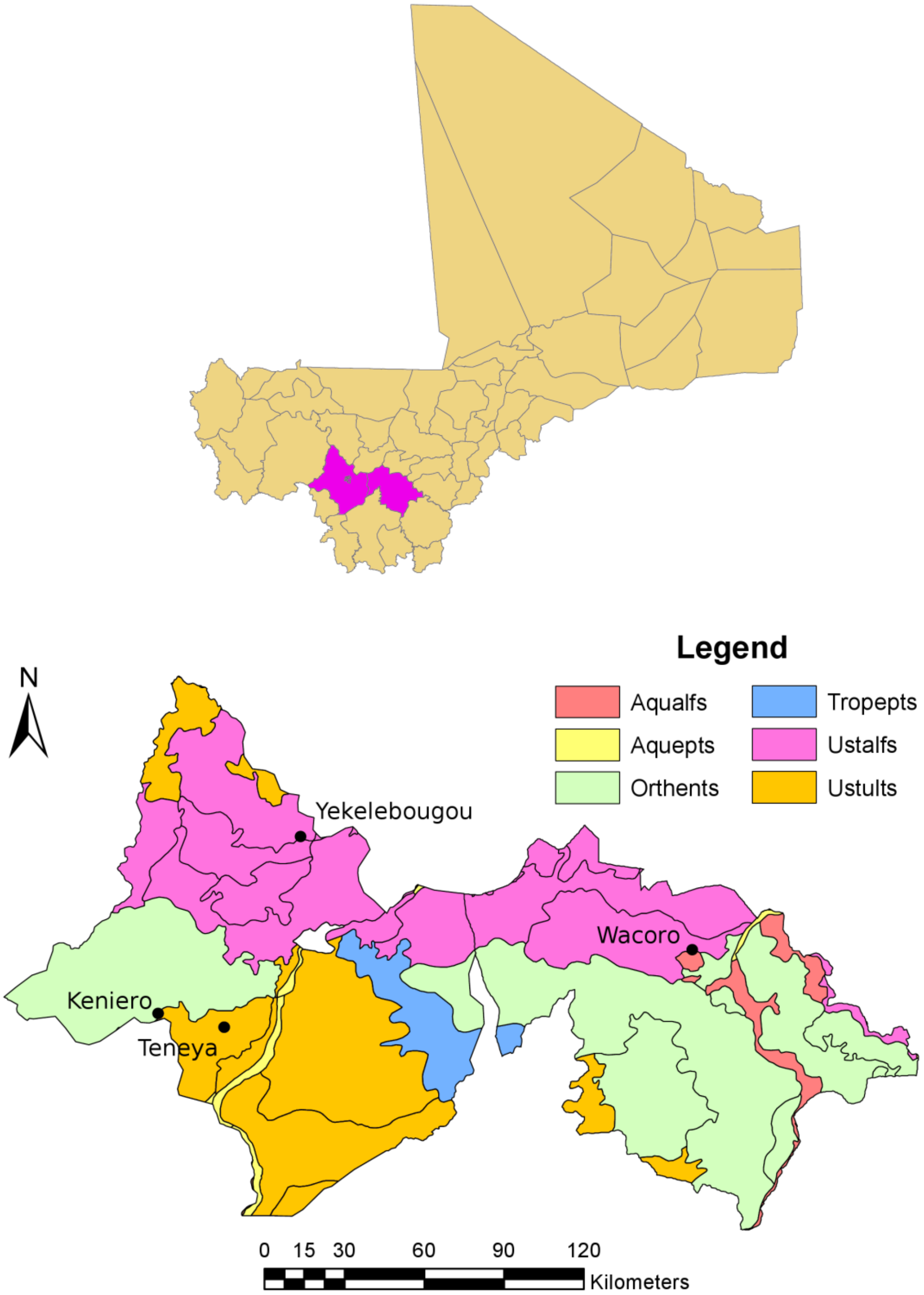


Figure 3.1: General location in southern Mali of soil and grain sampling (upper figure). Village locations and corresponding soil suborders (lower figure).

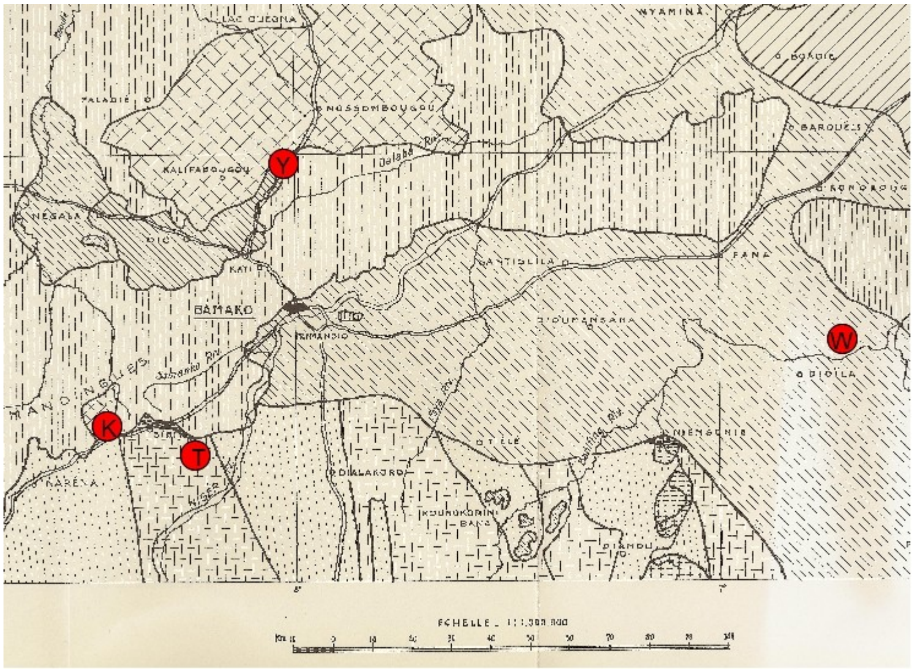


Figure 3.2: Geological formations in southern Mali. K = Keniero, T = Teneya, W = Wacoro, Y = Yekelebougou. Left to right slash marks = Sotuba Series (sandstone), vertical and horizontal lines = granites, and diamonds = dolerite. Source: adapted from Dars (1962).

Average temperatures for the sorghum growing period (June-September) in 2010 were 32.2 ° C (daily maximum) and 22.6 ° C (daily minimum). Total rainfall for this period was 1,231 mm, which is above the annual average of 1,098 mm (Climate Temperature Info, 2012). Temperature and rainfall data are from the nearest weather station located at the ICRISAT-Mali research facility in Bamako which is within 200 km of village locations. Actual temperature and rainfall at these locations could vary from those recorded at the Bamako weather station. In this area of Mali, soil classification data is very limited. Soils in the PIRT (1983) database include Ustalfs, Ustults, and Orthents (soils formed from laterite). Parent material was classified as either mafic or nonmafic based on the geology (Table 3.1). Soils at the site were further classified into two groups according to the formation of parent material. Soils were considered alluvium if observed to have either a few rounded or no Fe concretions and no surficial lateritic debris material (Table 3.1). Residuum soils were observed to be weathering *in situ* evidenced by either non-rounded lateritic debris and Fe concretions or the presence of a highly eluviated zone as in the case of Wacoro soils.

Table 3.1: Geographic and soil information for soils sampled per location-site.

Loc-Site	Lat	Long	Elev (m)	GtGp	Texture*	PM	Gl	Frm
Keniero								
KEN-S-1	N12°22.3'	W8°31.4'	400	CuirE	S Cly Lm	M	D	A
KEN-S-2	N12°21.9'	W8°31.4'	400	CuirE	S Cly Lm	M	Mx	R
KEN-S-3	N12°22.9'	W8°30.6'	391	CuirE	S Cly Lm	M	D	A
Teneya								
TEN-S-1	N12°19.5'	W8°18.1'	350	Hal	S Cly Lm	NM	S	A
TEN-S-2	N12°20.1'	W8°18.3'	357	Hal	S Lm	NM	S	A
TEN-S-3	N12°19.1'	W8°17.4'	349	Hal	S Lm	NM	S	A
Wacoro								
WAC-S-1	N12°35.0'	W6°43.5'	298	Trop	S Cly Lm	NM	S	R
WAC-S-2	N12°35.3'	W6°43.2'	298	Trop	S Lm	NM	S	R
WAC-S-3	N12°35.5'	W6°43.1'	285	Trop	S Cly Lm	NM	S	R
Yek								
YEK-S-1	N12°59.5'	W8°02.2'	415	CuirA	S Cly Lm	M	D	R
YEK-S-2	N12°57.4'	W8°02.7'	413	CuirA	S Cly Lm	NM	Cg	R

Loc-Site = location-site, KEN = Keniero, TEN = Teneya, WAC = Wacoro, YEK = Yekelebougou. S-1 = Site number, Lat = latitude, Long = longitude, Elev = elevation, GtGp = soil Great Group classification according to PIRT (1983), CuirE = Cuirorthents, Hal = Halaquepts, Trop = Tropaqualfs, CuirA = Cuirustalfs, * = soil texture according to Thien 1979. S Cly Lm = sandy clay loam, S Lm = sandy loam, PM = soil parent material based on observed geology, M = mafic, NM = nonmafic, Gl = type of geology observed at the sites, D = dolerite, Cg = conglomerate, Mx = mix of conglomerate, dolerite, and schist, S = sandstone, Frm = type of soil formation of parent material observed at the sites, A = alluvium, R = residuum.

At each location, three sites were chosen. Each site was approximately 1-3 km from its nearest neighbor. Each site was located outside the village center and consisted of sorghum bush fields with Shea trees present. At each site, two Shea trees approximately 30-80 m apart were selected to serve as replicates for each site. Sorghum was planted in late June and early July 2010 at the onset of the rainy season. All fields were mold-board plowed (except at YEK-S-1 which was a boulder field) and the sorghum was sown on top of ridges approximately 80 cm apart. There was a distance of 40 cm between rows. Sites were selected in areas where farmers had not fertilized within the past 5 years except for sites WAC-S-1 and WAC-S-3 where farmers had grown cotton which often requires fertilization. All sorghum grain collected was from local white sorghum landraces with red, purple, or a mix of red and purple glume colors (Table 3.2). The sorghum grain was classified as thin or thick based on pericarp appearance (thick = low luster, thin = high luster; Belum et al. 2005).

Sample Collection

Shea trees were selected based on whether adjacent sorghum panicles were bent over on stalks (ready to be harvested). The bole circumference and canopy radius were recorded for each tree. The tree height was also calculated using a Brunton® compass clinometer. Latitude and longitude (World Geographic System 1984) of the tree, as well as at 12 m from the edge of the canopy, were recorded by global positioning system receiver (Garmin etrex Vista HCx, Olathe, KS, U.S.A.). The recording of latitude and longitude at both the tree bole and 12 m from the edge of the canopy allowed an estimation of soil and grain sample transect direction.

Table 3.2: Sorghum grain characteristics by site (Soil ID).

Soil ID	Pericarp Thickness	Glume Color
KEN-S-1	Thin/Thick	Purple
KEN-S-2	Thick	Red
KEN-S-3	Thin/Thick	Red
TEN-S-1	Thick	Purple
TEN-S-2	Thin	Purple
TEN-S-3	Thin	Red/Purple
WAC-S-1	Thin/Thick	Red/Purple
WAC-S-2	Thick	Red/Purple
WAC-S-3	Thick	Purple
YEK-S-1	Thick	Purple
YEK-S-2	Thin/Thick	Red/Purple

KEN = Keniero, TEN = Teneya, WAC = Wacoro, YEK = Yekelebougou, S-1 = Site number.

At each tree, soil samples were collected along a transect directly out from the tree bole. Samples were collected at 0 m (edge of the canopy) and at 2 m, 4 m, 6 m, 8 m, 10 m, and 12 m from canopy edge. Additional samples were collected under the canopy at -2 m and -4 m from the edge. Depending on the canopy radius, these soil samples were 1-4 m from the tree bole (Table 3.3). Each soil sample was collected from 0-15 cm depth with a 38-mm diameter soil probe and was a composite of 3 soil cores collected from an approximately 10 cm^2 area. Soil samples were placed in paper bags and allowed to air dry for at least 24 hours before shipping to the U.SA. for analysis.

Table 3.3: Tree characteristics by site (Soil ID).

Soil ID	Tree	Bole cm	Canopy m	Height m	Transect
KEN-S-1	1	1.9	5.0	12	S
	2	1.7	7.5	11	W
KEN-S-2	3	0.9	3.0	8	W
	4	1.0	5.0	9	NW
KEN-S-3	5	1.0	5.0	-	NW
TEN-S-1	1	1.0	4.0	8	SE
	2	1.5	4.5	10	NE
TEN-S-2	3	1.7	7.0	13	N
	4	2.1	7.0	11	E/NE
TEN-S-3	5	1.9	8.0	11	NE
	6	2.0	7.5	15	E/SE
WAC-S-1	1	0.8	4.5	8	NW
	2	1.5	5.0	6	W
WAC-S-2	3	1.3	5.0	9	E/NE
WAC-S-3	4	1.3	5.0	7	S/SW
	5	2.0	6.0	9	S/SE
YEK-S-1	1	1.8	5.0	9	SW
	2	1.4	5.0	11	S/SE
	3	1.5	5.0	17	SE
YEK-S-2	4	1.4	4.0	10	N
	5	1.0	4.0	9	SW
	6	1.0	4.0	-	NE

KEN = Keniero, TEN = Teneya, WAC = Wacoro, YEK = Yekelebougou, S-1 = Site number, Bole = Shea bole circumference, Canopy = radius of Shea tree canopy, Height = height of Shea tree, Transect = direction of transect away from the Shea tree.

Approximately 5 sorghum panicles from stalks located around each soil sample location at 0 m, 2 m, 4 m 6 m, 8 m, 10 m, and 12 m were also collected and placed in paper bags. At site KEN-S-3, only one sorghum stalk was collected at these distances because the farmer had intercropped groundnut with sorghum such that spacing between sorghum stalks was approximately 2 m. Collection of soil and sorghum panicles occurred during the week of November 8, 2010 which is when sorghum in southern Mali is usually harvested. Panicles were allowed to dry for over 3 weeks. The number, weight, length, and glume color were recorded for all panicles harvested. The panicles were threshed by hand. Approximately 100-200 g of grain from each sorghum panicle was sent to Texas A&M University for analysis.

Laboratory Analysis

For each sorghum sample, grain weights were recorded by measuring the weight of 100 randomly selected seeds. Approximately 4 g of clean sorghum grain was randomly selected from each sample and mill-ground to 1 mm size (Cyclone Lab Sample Mill, Udy Corporation, Fort Collins, CO, U.S.A.). Milled grain was placed in Crystal Clear^{TM} plastic bags and scanned on a flatbed scanner for measurement of the three primary colors red, green, and blue by Color Scanning Analysis software (D. Verbree unpublished 2012). Color represents a mixture of the monochromatic spectra of red (700 nm), green (546 nm), and blue (436 nm), and on an 8-bit digital system, these three primary colors are quantified by numeric tristimulus values that range from 0 (darkness) to 255 (whiteness; Viscarra Rossel et al. 2006). The measured red, green, and blue values were then converted to the Commission Internationale de l'Eclairage (CIE) standardized color space model which uses a lightness (L) function to describe brightness which ranges from 0 (black) to 1 (white).

Zinc concentrations in grain were quantified by a wet digestion method at Dairy One in Ithaca, NY. Briefly, 1 g of ground grain from the Crystal Clear^{TM} plastic bags was weighed out to the nearest 0.01 g and placed into Xpress Teflon PFA digestion vessels (CEM, Matthews, NC, U.S.A). Eight mL of concentrated nitric acid and 2 mL of concentrated hydrochloric acid were dispensed into the tubes and allowed to predigest at room temperature for 15 min. The tubes were then heated in a closed system under microwave assistance (CEM, Matthews, NC, U.S.A) at 1,600 W to 200 ° C and held there for 15 min. before cooling to room temperature. Vessels were diluted with deionized water to the 50 mL volume mark and transferred to 17 mL polypropylene tubes. Samples were then analyzed by inductively coupled plasma spectrometer (Intrepid ICP Radial Spectrometer, Thermo-Scientific, Waltham, MA, U.S.A.). Concentrations were reported on a dry matter basis.

Air-dried soil samples were lightly ground and passed through a 2-mm sieve. Concretions that did not pass through the 2-mm sieve were immersed in water, dried, and sieved a second time to break up any potential soft aggregates (Joao Herbert-Brazilian Agricultural Research Center), personal communication) and added to the previously sieved soil. The percent of concretions > 2-mm in each soil sample was calculated gravimetrically. Soil samples from TEN-S-3 were noted to have charcoal pieces present throughout the entire soil sample. Care was taken to pick out the larger pieces of charcoal that fell through the sieve, but it was not possible to remove all fragments.

Soil samples were extracted with DTPA (Lindsay and Norvell 1978), and the supernatant was filtered through a 0.45-µm pore-size MFS mixed cellulose ester membrane filter (Advantac, Dublin, CA, U.S.A.). Fe, Zn, Mn, and Cu concentrations were measured by an inductively coupled plasma (ICP) spectrometer (Axial Arcos, Spectro Analytical Instruments, Kleve, Netherlands) at the Soil, Water and Forage Testing Laboratory in College Station, TX. Soil pH was measured using a 1:2 ratio of soil to water (TitraLab^{TM} 90, Radiometer, Copenhagen, Denmark). Soil color was measured by Color Scanning Analysis software (D. Verbree unpublished software, 2012). Approximately 1-2 g of soil was placed in Crystal Clear^{TM} plastic bags and scanned on a flatbed scanner. Measured red, green, and blue color values were converted to the most commonly used soil color system, that of Munsell hue, value, and chroma (Viscarra Rossel et al. 2006).

Soil samples were analyzed for organic carbon and total nitrogen using a NA1500 Carlo Erba elemental analyzer (Thermo Fisher Scientific, Waltham, MA, U.S.A). Carbon (^{12}C and ^{13}C) and nitrogen (^{14}N and ^{15}N) isotopes were analyzed using a Finnigan Delta Plus XP isotope ratio mass spectrometer (Thermo Fisher Scientific, Waltham, MA, U.S.A.) connected with the elemental analyzer by a Conflo III interface. After color analysis was performed on the 1-2 g of soil placed in Crystal Clear^{TM} plastic bags, a small subsample of soil (12-80 mg) for isotope analysis was scooped out of the bag and placed in pre-weighed aluminum boats and weighed to the nearest 10 µg on a M2P microbalance scale (Satorius AG, Goettingen, Germany). Carbon and nitrogen isotopes were reported as $\delta^{13}C$ and $\delta^{15}N$ which are standard calculations of the relative enrichment of ^{13}C to ^{12}C and ^{15}N to ^{14}N, respectively. The relative contribution of organic carbon from C3 plants can be estimated by a mass balance equation (Eq. 3.2: Balesdent and Mariotti 1996).

Eq. 3.2 $\% \text{ C3 plant contribution} = [(\delta^{13}C_{sample} * (-27)]/[(-13) * (-27)] * 100$

Statistical Analysis

Summary statistics including mean and standard deviation of soil and grain variables were calculated for each type and formation of parent material (mafic-alluvium, mafic-residuum, nonmafic-alluvium, nonmafic-residuum) and site number (soil ID). Log transformation improved normality and the significance for percent organic carbon and DTPA-extractable Fe, Zn, Mn, and Cu, and total nitrogen concentrations, soil color hue, value, and chroma, panicle weight, panicle length, grain lightness, and grain Zn concentration. Log transformations of these variables were used in all statistical analysis. A univariate analysis of variance using a generalized linear model (GLM) was applied to the soil and grain data using parent material and tree proximity as independent variables. For soil variables, tree proximity groups were: near = -4 m to 0 m; and, far = 2 m to 12 m. Tree proximity groups were defined differently for grain variables because grain was planted only at 0 m to 12 m (near = 0 m to 4 m and far = 6 m to 12 m). In the GLM model, each tree within parent material was assigned as a random factor while parent material and tree proximity were assigned as fixed factors. Mean separations by the Tukey-Kramer method were performed to determine if soil properties were significantly different from each other by parent material and tree proximity.

Soil DTPA-extractable Fe, Zn, Mn, and Cu concentrations, and percent organic carbon, and total nitrogen mass fraction, $\delta^{13}C$, $\delta^{15}N$, soil pH, hue, value, chroma, grain Zn concentration, and grain weight were plotted against distance from Shea tree for each parent material to illustrate the interaction of parent material and tree proximity. Pearson's HSD correlation analysis was performed for all soil and grain properties. All data were analyzed using SAS software version 9.2 (SAS Institute, Cary, N.C., U.S.A.).

Because there were increased DTPA-extractable Zn concentrations at site TEN-S-3 which can be attributed to charcoal (which was not observed at any other sites) and not to parent material or Shea tree effects, these concentrations were excluded from the analysis. There are several reasons why recent bush fires and charcoal may contribute to higher DTPA-extractable Zn concentrations. First, studies analyzing bush fire aerosols have reported a consistent enrichment in K and Zn (Scholes and Andreae 2000). Second, any Zn contained in aerosols will fall to the soil and potentially be absorbed by the remaining charcoal or biochar fragments (Mishra and Chaudhury 1994). Third, biochar itself has been reported to contain very high Zn concentrations (1,599 mg kg^{-1}; Namgay et al. 2010).

Results

Average Soil Properties at Study Sites

Mean soil color hue ranged from 7.57+/-0.30 to 8.49+/-0.47 YR (Table 3.4). Mean soil color value ranged from 3.66+/0.20 to 5.90+/-0.18, whereas mean soil chroma ranged from 3.13+/0.05 to 4.68+/0.19. Some residuum soils (KEN-S-2, YEK-S-1, and YEK-S-2) contained concretions and these were observed in both mafic and nonmafic derived soils. Mean soil pH ranged from 6.00+/-0.53 to 7.54+/-0.30. Mean percent soil organic carbon ranged from 0.43+/-0.13 to 2.68+/-0.86% and mean percent soil total nitrogen from 0.03+/-0.01 to 0.169+/-0.06%. Soil organic carbon and total nitrogen were very highly correlated (r = 0.982, p = <0.0001). Mean $\delta^{13}C$ ranged from -23.18+/-1.95 to -17.21+/-1.65$^{0}/_{00}$ and mean $\delta^{15}N$ ranged from 4.01+/-0.82 to 6.65+/-0.59$^{0}/_{00}$. The percent C3 contribution ranged from 64 to 86%.

Mean DTPA-extractable Fe concentrations by Soil ID ranged from 9.17+/-1.97 to 46.49+/-13.03 mg kg^{-1} (Table 3.5). The nonmafic-residuum soils from the Wacoro location were light colored with low chroma (Table 3.4). Mafic-residuum soils that were low in DTPA-extractable Fe concentration contained a high percentage of Fe concretions. Mean DTPA-extractable Mn concentrations ranged from 14.39+/-3.98 to 62.02+/-23.46 mg kg^{-1}, whereas mean DTPA-extractable Cu concentrations ranged from 0.44+/-0.11 to 3.93+/-1.06 mg kg^{-1}.

For individual samples, DTPA-extractable Zn concentrations ranged from a low of 0.15 to 12.34 mg kg^{-1}. Mean-site DTPA-extractable Zn concentrations ranged from 0.34+/-0.20 to 2.98+/-2.04 mg kg^{-1}. Soils from TEN-S-3 that contained charcoal fragments had DTPA-extractable Zn concentrations ranging from 1-12 mg kg^{-1} with a mean of 2.75+/-2.51 mg kg^{-1}, whereas soils from TEN-S-1 and TEN-S-2 had mean DTPA-extractable Zn concentrations of 0.51+/-0.22 mg kg^{-1} and 0.34+/-0.20 mg kg^{-1}, respectively. These increased soil DTPA-extractable Zn concentrations at site TEN-S-3 can be attributed to charcoal (which was not observed at any other sites) and not to parent material or Shea tree effects.

Table 3.4: Mean and standard deviations (parentheses) of soil properties by site (Soil ID).

Soil ID	Color			Conc.	pH	OC	$\delta^{13}C$	TN	$\delta^{15}N$
	hue (YR)	value	chroma	%		%	$^{0}/_{00}$	%	$^{0}/_{00}$
KEN-S-1	8.37 (0.16)	5.01 (0.13)	4.68 (0.19)	0.0 (0.0)	6.81 (0.58)	0.80 (0.28)	-19.6 (2.00)	0.06 (0.01)	4.82 (0.47)
KEN-S-2	8.13 (0.58)	4.15 (0.14)	3.86 (0.25)	48.2 (35.5)	6.93 (0.40)	1.04 (0.36)	-17.2 (1.65)	0.07 (0.03)	5.14 (0.69)
KEN-S-3	8.16 (0.14)	4.90 (0.15)	4.09 (0.19)	0.0 (0.0)	7.42 (0.24)	0.93 (0.15)	-23.2 (1.95)	0.06 (0.01)	5.31 0.49)
TEN-S-1	7.82 (0.33)	5.29 (0.13)	4.13 (0.18)	2.0 (4.7)	6.67 (0.24)	0.70 (0.13)	-20.1 (1.80)	0.05 (0.01)	4.91 (0.68)
TEN-S-2	7.68 (0.35)	5.25 (0.30)	4.06 (0.13)	0.0 (0.0)	6.02 (0.40)	0.44 (0.17)	-18.7 (2.06)	0.03 (0.01)	5.14 (0.81)
TEN-S-3	7.57 (0.08)	5.45 (0.14)	4.02 (0.16)	0.0 (0.0)	6.81 (0.29)	0.43 (0.13)	-19.9 (1.64)	0.03 (0.01)	5.68 (0.61)
WAC-S-1	8.37 (0.32)	5.40 (0.15)	3.41 (0.16)	1.0 (2.9)	6.22 (0.57)	0.71 (0.35)	-17.3 (2.80)	0.05 (0.02)	5.22 (0.51)
WAC-S-2	7.83 (0.20)	5.61 (0.10)	3.13 (0.05)	0.0 (0.0)	6.03 (0.47)	0.52 (0.09)	-18.7 (2.15)	0.04 (0.01)	4.98 (0.67)
WAC-S-3	7.80 (0.29)	5.90 (0.18)	3.74 (0.20)	1.1 (3.3)	6.00 (0.53)	0.54 (0.13)	-18.8 (2.16)	0.04 (0.01)	6.65 (0.59)
YEK-S-1	8.49 (0.47)	3.66 (0.20)	3.90 (0.31)	69.4 (70.1)	7.54 (0.30)	2.68 (0.86)	-19.3 (2.68)	0.17 (0.06)	4.01 (0.82)
YEK-S-2	7.57 (0.30)	4.69 (0.20)	4.36 (0.22)	53.3 (34.2)	7.16 (0.28)	1.33 (0.42)	-20.2 (1.90)	0.09 (0.02)	4.75 (0.73)

KEN = Keniero, TEN = Teneya, WAC = Wacoro, YEK = Yekelebougou, S-1 = Site number, Conc. = % concretions, pH = soil pH in water, OC = organic carbon, $\delta^{13}C$ = delta ^{13}C, TN = total nitrogen, $\delta^{15}N$ = delta ^{15}N.

Table 3.5: Mean and standard deviation (parentheses) of soil DTPA-extractable Fe, Zn, Mn, and Cu concentrations by site (Soil ID).

Soil ID	DTPA Fe	DTPA Zn	DTPA Mn	DTPA Cu
	mg kg^{-1}			
KEN-S-1	11.6 (2.84)	0.55 (0.17)	62.0 (23.5)	0.79 (0.13)
KEN-S-2	17.4 (6.37)	1.15 (1.19)	45.6 (13.4)	1.15 (0.46)
KEN-S-3	9.17 (1.97)	2.50 (2.15)	28.5 (7.54)	1.22 (0.43)
TEN-S-1	11.2 (6.28)	0.51 (0.22)	15.9 (4.81)	0.56 (0.17)
TEN-S-2	19.1 (5.76)	0.34 (0.20)	37.0 (8.10)	0.44 (0.11)
TEN-S-3	15.4 (9.49)	2.18 (0.78)	21.2 (4.64)	0.58 (0.14)
WAC-S-1	46.5 (13.0)	0.56 (0.18)	18.8 (5.43)	1.18 (0.29)
WAC-S-2	29.8 (9.29)	0.35 (0.08)	35.3 (9.79)	0.56 (0.06)
WAC-S-3	30.2 (11.2)	0.50 (0.22)	14.4 (3.98)	0.99 (0.21)
YEK-S-1	9.80 (3.53)	2.98 (2.04)	32.7 (7.76)	3.93 (1.06)
YEK-S-2	9.38 (2.90)	1.02 (1.24)	39.3 (9.51)	1.64 (0.61)

KEN = Keniero, TEN = Teneya, WAC = Wacoro, YEK = Yekelebougou, S-1 = Site number, DTPA = diethylenetriaminepentaacetic acid.

DTPA-extractable Zn concentrations from YEK-S-1, a mafic-residuum soil, ranged from 4.38 to 7.58 mg kg^{-1}, which is up to 7.5 times greater than the median concentration of 1.0 mg kg^{-1} for DTPA-extractable Zn in 64 soil samples collected to test the relationship between soil DTPA-extractable Zn concentrations and sorghum grain Zn concentrations from southern Mali (Chapter 2). Other soil sites in my study with mafic-residuum soils had DTPA-extractable Zn concentrations 2 to 4 times higher, and DTPA-extractable Zn from KEN-S-3, a mafic-alluvium soil, had most concentrations 2 to 6.8 times higher than the median concentration of 1.0 mg kg^{-1} as reported in Chapter 2.

Effect of Parent Material, Tree Proximity, and Parent Material x Tree Proximity Interaction on Soil Properties

The statistical analysis using a univariate analysis of variance with parent material and tree proximity yielded some interesting results. Parent material was a significant factor for soil color hue and value, percent concretions, pH, and percent organic carbon and total nitrogen, and DTPA-extractable Zn and Cu concentrations (Table 3.6). Tree proximity was a significant factor for chroma, pH, $\delta^{13}C$, $\delta^{15}N$, and percent organic carbon and total nitrogen, and DTPA-extractable Zn and Mn concentrations. The interaction between parent material and tree proximity was significant for soil color hue, value, and chroma, pH, percent organic carbon and total nitrogen, and DTPA-extractable Fe and Mn concentrations. Tree within parent material was significant for all soil variables.

Soils derived from mafic parent material had significantly lower soil color value than non-mafic parent material (Table 3.7) with mafic-residuum the darkest. Soil pH was significantly higher in soils derived from mafic parent material. Both percent organic carbon and total nitrogen were significantly higher in mafic-residuum soils, followed by mafic-alluvium and nonmafic-residuum soils, and were lowest in nonmafic-alluvium soils. $\delta^{13}C$ was more negative (depleted) in mafic-alluvium soils, whereas $\delta^{15}N$ was significantly lower in mafic-residuum soils. Certain parent material soils were higher in DTPA-extractable Fe and Mn concentration but not significantly so. DTPA-extractable Zn and Cu concentration were significantly higher in mafic-residuum soils.

Table 3.6: Results of the combined analysis of variance by the general linear model procedure (p values) for soil properties. Log transformations were performed on OC, TN, DTPA (diethylenetriaminepentaacetic acid)-extractable Fe, Zn, Mn, and Cu, soil color hue, value, and chroma. ** denotes $p < 0.0001$.

Source	hue	value	chroma	Conc	pH	OC	$\delta^{13}C$	TN	$\delta^{15}N$	Fe	Zn	Mn	Cu
PM	0.002	**	0.07	0.04	0.02	0.0005	0.30	0.0005	0.16	0.18	0.005	0.08	0.0004
Tree(PM)	**	**	**	**	**	**	**	**	**	**	**	**	**
Tprox	0.05	0.76	**	0.59	**	**	**	**	**	0.14	**	**	0.50
PM*TProx	**	0	0.0004	0.65	0.0002	0.04	0.16	0.004	0.36	**	0.06	0.0002	0.12

PM = parent material, Tprox = tree proximity, Conc. = % concretions, pH = soil pH in water, OC = organic carbon, $\delta^{13}C$ = delta ^{13}C, TN = total nitrogen, $\delta^{15}N$ =delta ^{15}N, Fe, Zn, Mn, and Cu = DTPA-extractable.

Table 3.7: Means for soil properties by parent material (PM) and tree proximity (Tprox) factors. Lowercase letters denote Tukey-Kramer groups resulting from the combined analysis of variance by the general linear model procedure based on log transformations of OC, TN, DTPA (diethylenetriaminepentaacetic acid)-extractable Fe, Zn, Mn, and Cu, soil color hue, value, and chroma and alpha = 0.05.

Factor	hue YR	value	chroma	Conc. %	pH	OC %	δ^{13}C ^{0}/00	TN %	δ^{15}N ^{0}/00	Fe	Zn mg kg^{-1}	Mn	Cu
PM													
MfAlv	8.30a	4.97c	4.48a	0.0b	7.02b	0.84b	-20.8a	0.06b	4.99a	10.8a	1.35b	50.8a	0.93c
MfRs	8.36a	3.84d	3.89a	61.5a	7.31a	2.07a	-18.6a	0.13a	4.43a	12.6a	2.30a	37.5a	2.87a
NonMfAlv	7.69c	5.32a	4.07a	0.7b	6.50c	0.52c	-19.6a	0.04c	5.24a	15.3a	0.43d	24.7a	0.53d
NonMfRs	7.87b	5.31b	3.72a	19.1ab	6.46c	0.86b	-18.9a	0.06b	5.40a	27.2a	0.68c	26.9a	1.21b
Tprox													
Near	8.05a	4.97a	3.91b	20.7a	7.01a	1.22a	-20.8a	0.08a	4.87b	20.0a	1.52a	26.3b	1.40a
Far	7.96a	4.93a	4.02a	20.7a	6.62b	0.95b	-18.6b	0.06b	5.18a	17.8a	0.95b	34.7a	1.34a

MfAlv = mafic-alluvium, MfRs = mafic-residuum, NonMfAlv = nonmafic-alluvium, NonMfRs = nonmafic-residuum, Conc. = % concretions, pH = soil pH in water, OC = organic carbon, δ^{13}C = delta ^{13}C, TN = total nitrogen, δ^{15}N =delta ^{15}N, Fe, Zn, Mn, and Cu = DTPA-extractable.

Soil color chroma, δ^{13}C, and DTPA-extractable Mn concentration increased with distance away from tree, whereas soil pH, percent organic carbon and total nitrogen, δ^{15}N, and DTPA-extractable Zn concentration decreased away from the tree (Table 3.7). Percent soil organic carbon and total nitrogen decreased away from the tree particularly in those soils derived from mafic-residuum (from a mean of 3.5% to 1.5% OC and 0.25% to 0.10% TN, respectively), and soils from mafic-alluvium decreased below those of nonmafic-residuum soils at greater than 10 m away from the tree (Figure 3.3). δ^{13}C significantly increased away (by approximately 4-5^{0}/₀₀), whereas δ^{15}N only increased from a mean of 3.7 to 4.7^{0}/₀₀ (i.e. 1^{0}/₀₀) for mafic-residuum soils and showed high variability at each distance (Figure 3.4). Soil pH significantly decreased by 0.6 units away from the tree in all soils except nonmafic-alluvium soils. Nonmafic alluvium soils remained at a steady pH and were higher than the pH in nonmafic-residuum soils at distances greater than 2 m from the edge of the canopy (Figure 3.5). Soil color chroma increased with distance away from the tree in mafic-residuum soils (Figure 3.6).

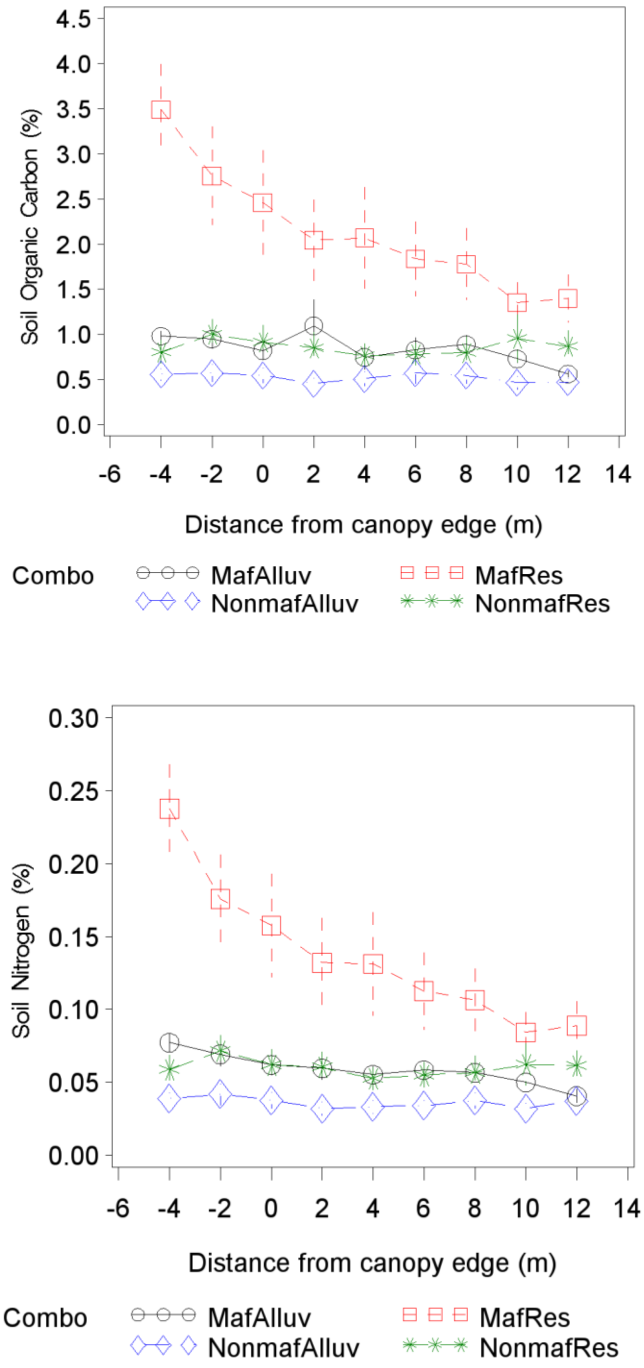
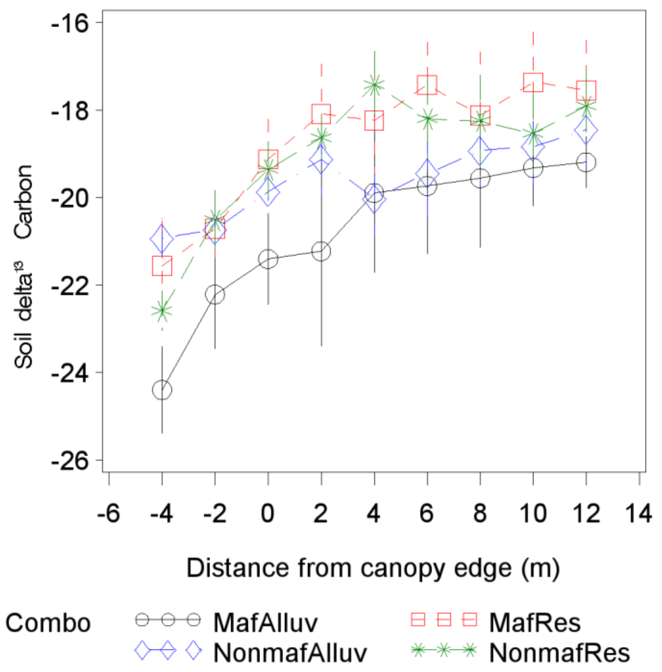


Figure 3.3: Means for soil organic carbon (upper figure) and total nitrogen (lower figure) at each distance from the tree canopy edge (m) by 4 different soil parent materials. Vertical bars are standard errors. MafAlluv = mafic-alluvium, MafRes = mafic-residuum, NonmafAlluv = nonmafic-alluvium, NonmafRes = nonmafic-residuum.



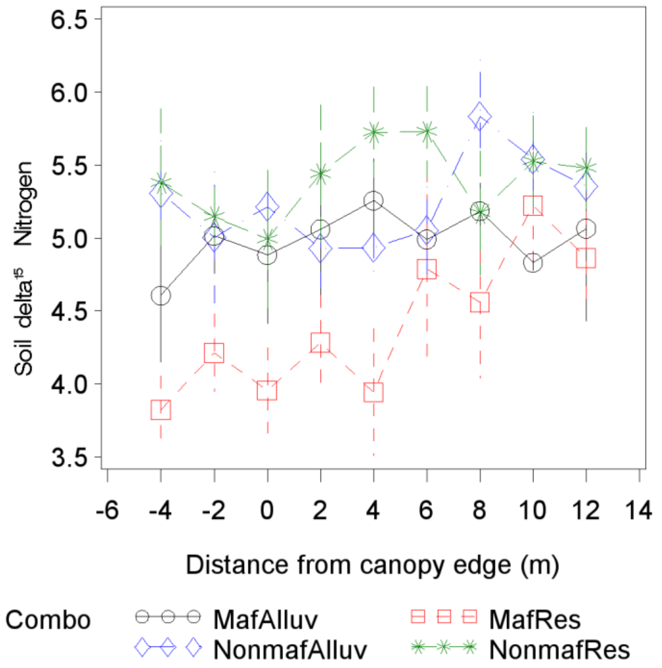
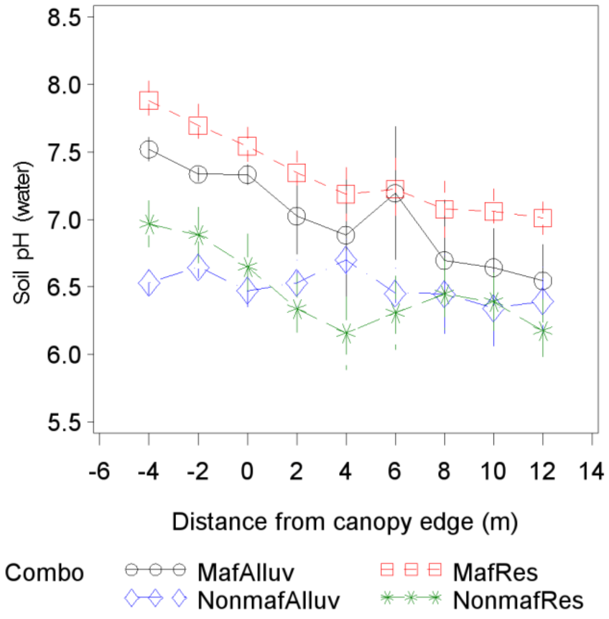


Figure 3.4: Means for soil delta ^{13}C (upper figure) and delta ^{15}N (lower figure) at each distance from the tree canopy edge (m) by 4 different soil parent materials. Vertical bars are standard errors. MafAlluv = mafic-alluvium, MafRes = mafic-residuum, NonmafAlluv = nonmafic-alluvium, NonmafRes = nonmafic-residuum.



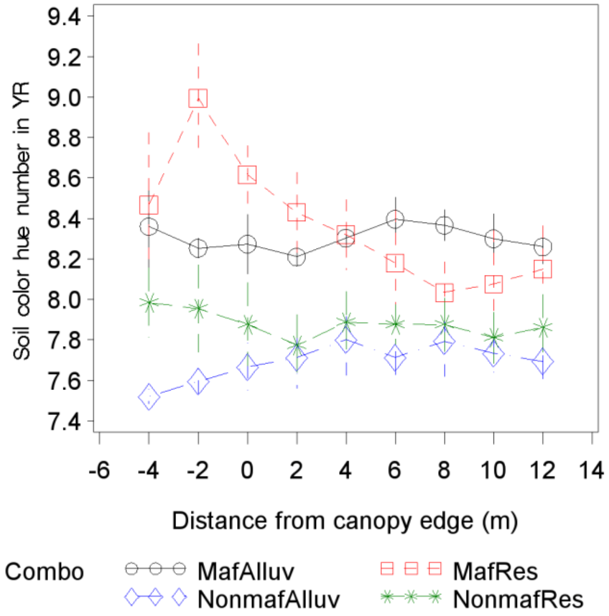
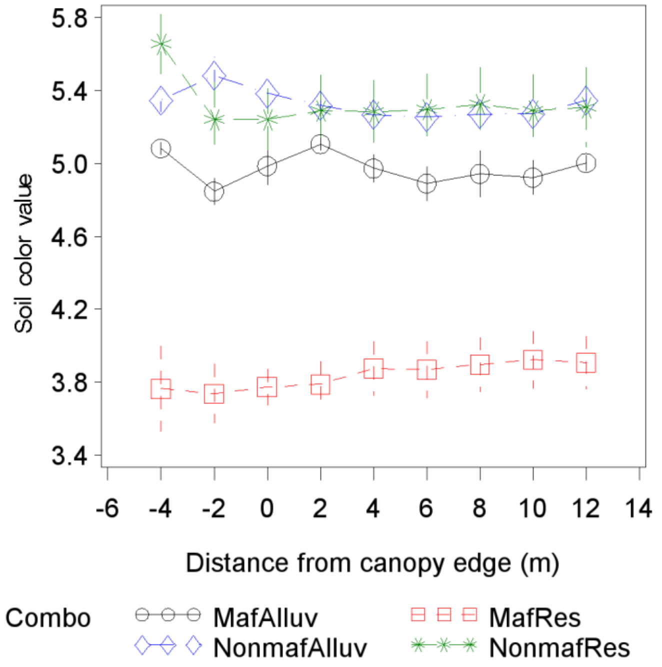


Figure 3.5: Means for soil pH (upper figure) and soil hue number in YR (lower figure) at each distance from the tree canopy edge (m) by 4 different soil parent materials. Vertical bars are standard errors. MafAlluv = mafic-alluvium, MafRes = mafic-residuum, NonmafAlluv = nonmafic-alluvium, NonmafRes = nonmafic-residuum.



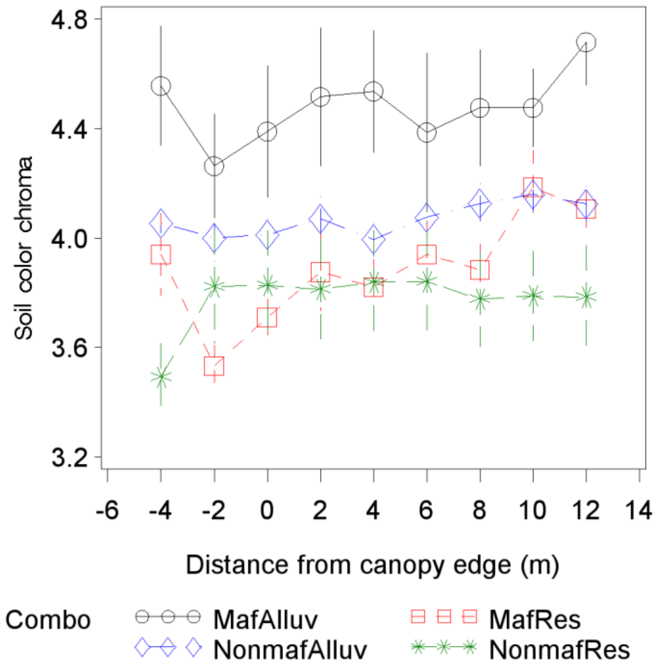


Figure 3.6: Means for soil value (upper figure) and chroma (lower figure) at each distance from the tree canopy edge (m) by 4 different soil parent materials. Vertical bars are standard errors. MafAlluv = mafic-alluvium, MafRes = mafic-residuum, NonmafAlluv = nonmafic-alluvium, NonmafRes = nonmafic-residuum.

DTPA-extractable Fe concentration significantly decreased away from the tree (a mean decrease from 23 to 10 mg kg^{-1}) only in nonmafic-alluvium soils resulting in concentrations that were less than that of soils derived from mafic parent material (Figure 3.7). DTPA-extractable Mn concentration increased significantly in all soils away from the tree except for nonmafic-alluvium. DTPA-extractable Zn concentration decreased in soils derived from mafic parent material for both residuum (mean decrease of 3.9 to 2.0 mg kg^{-1}) and alluvium (mean decrease of 2.1 to 0.8 mg kg^{-1}; Figure 3.8). DTPA-extractable Cu concentration showed no trend with distance from tree, although there was a very high DTPA-extractable Cu concentration at -4 m in mafic-residuum soils.

Correlations among Soil Properties

Soil pH was found to be significantly correlated with all other measured soil variables except DTPA-extractable Mn concentration (Table 3.8). Correlations were positive and high ($r > 0.60$; $p < 0.0001$) between soil pH and concentrations of organic carbon and total nitrogen, and DTPA-extractable Zn (Table 3.8). Correlations were negative and medium to high ($r = 0.50 – 0.65$; $p < 0.0001$) between soil pH and $\delta^{13}C$, $\delta^{15}N$, DTPA-extractable Fe concentration, and soil value. A plot of the relationship between soil pH and DTPA-extractable Zn concentration suggested that high DTPA-extractable Zn concentrations (> 3 mg kg^{-1}) only occur at pH > 6.7 and only in soils derived from mafic parent material (Figure 3.9). Percent organic carbon and total nitrogen were similarly correlated as soil pH to the other soil variables.

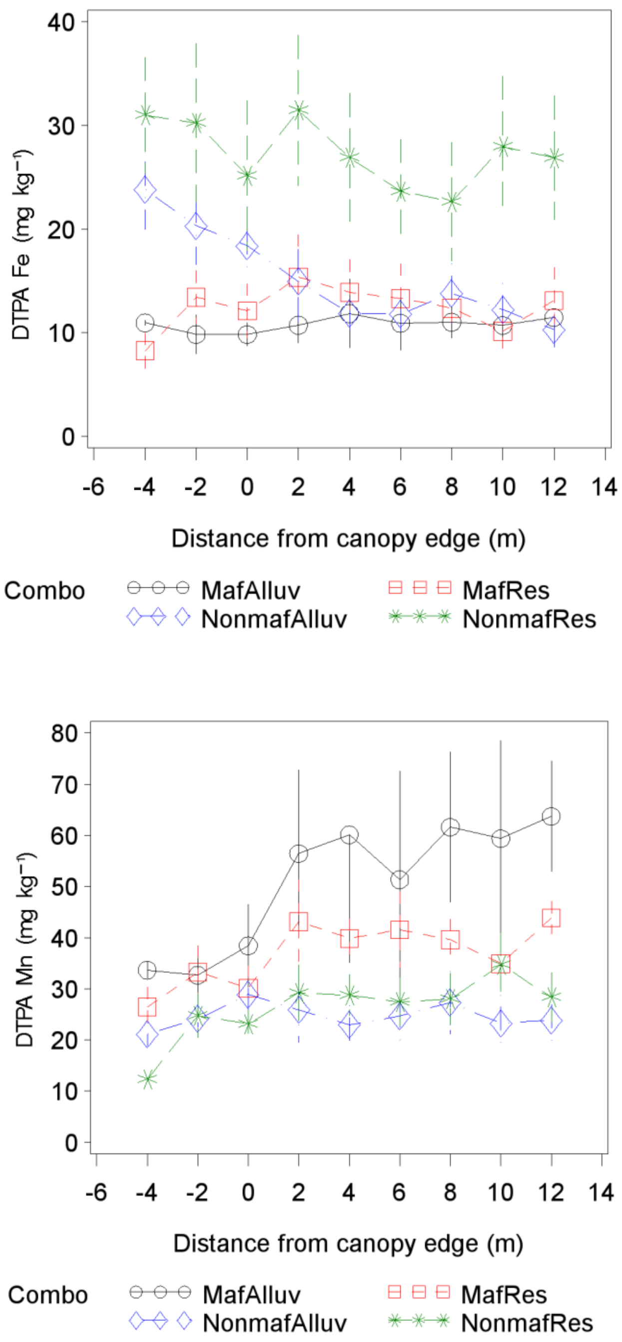
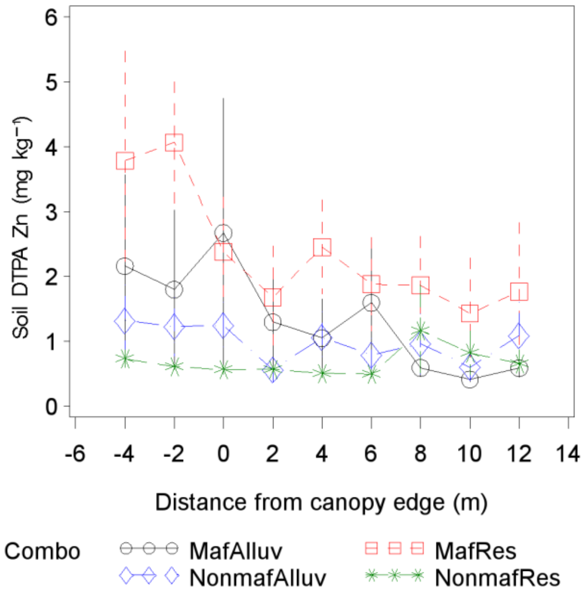


Figure 3.7: Means for soil DTPA-extractable Fe concentration (upper figure) and soil DTPA-extractable Mn concentration (lower figure) at each distance from the tree canopy edge (m) by 4 different soil parent materials. Vertical bars are standard errors. MafAlluv = mafic-alluvium, MafRes = mafic-residuum, NonmafAlluv = nonmafic-alluvium, NonmafRes = nonmafic-residuum.



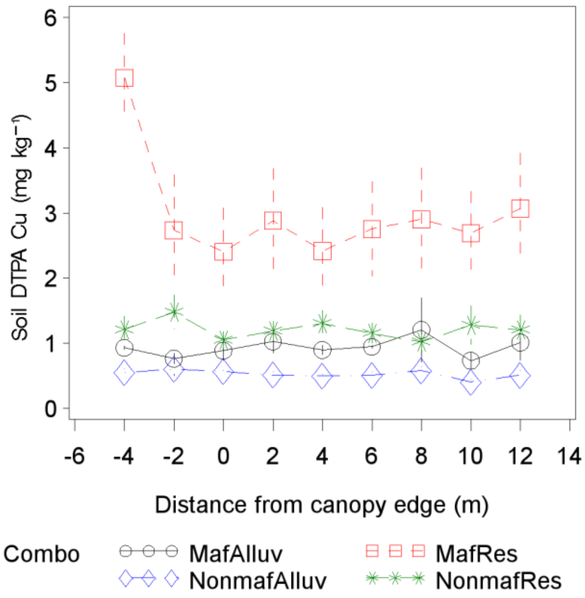


Figure 3.8: Means for soil DTPA-extractable Zn concentration (upper figure) and soil DTPA-extractable Cu concentration (lower figure) at each distance from the tree canopy edge (m) by 4 different soil parent materials. Vertical bars are standard errors. MafAlluv = mafic-alluvium, MafRes = mafic-residuum, NonmafAlluv = nonmafic-alluvium, NonmafRes = nonmafic-residuum.

Table 3.8: Correlation coefficients (r) among soil properties. ** denotes significance at $p < 0.0001$, * at $p < 0.01$.

	pH	OC	$\delta^{13}C$	TN	$\delta^{15}N$	Fe	Zn	Mn	Cu	Conc	Hue	Value	Chrm
pH	--												
OC	0.723**	--											
$\delta^{13}C$	-0.543**	-0.300**	--										
TN	0.734**	0.982**	-0.314**	--									
$\delta^{15}N$	-0.575**	-0.597**	0.351**	-0.572**	--								
Fe	-0.650**	-0.356**	0.338**	-0.350**	-0.397**	--							
Zn	0.710**	0.724**	-0.423**	0.730**	-0.465**	-0.345**	--						
Mn	0.078	0.254*	0.138	0.208*	-0.285**	-0.111	0.061**	--					
Cu	0.547**	0.809**	-0.056	0.816**	-0.336**	-0.149	0.606**	0.182	--				
Conc	0.416**	0.579**	-0.171	0.579**	-0.447**	-0.505**	0.519**	0.255	-0.022	--			
Hue	0.319**	0.486**	-0.057	0.491**	-0.346**	0.052	0.433**	0.110	0.377**	-0.090	--		
Value	-0.645**	-0.816**	0.008	-0.780**	0.546**	0.419**	-0.586**	-0.428**	-0.731**	-0.040	0.427**	--	
Chrm	0.214*	0.001	-0.086	0.001	-0.016	-0.582**	-0.088	0.332**	-0.019	0.091	-0.275*	-0.122	--

pH = soil pH in water, OC = organic carbon, $\delta^{13}C$ = delta ^{13}C, TN = total nitrogen, $\delta^{15}N$ =delta ^{15}N, Fe, Zn, Mn, and Cu = DTPA-extractable, DTPA = diethylenetriaminepentaacetic acid, Conc = percent concretions, Chrm = chroma. Log transformations were performed on OC, TN, DTPA-extractable Fe, Zn, Mn, and Cu, soil color hue, value, and chroma.

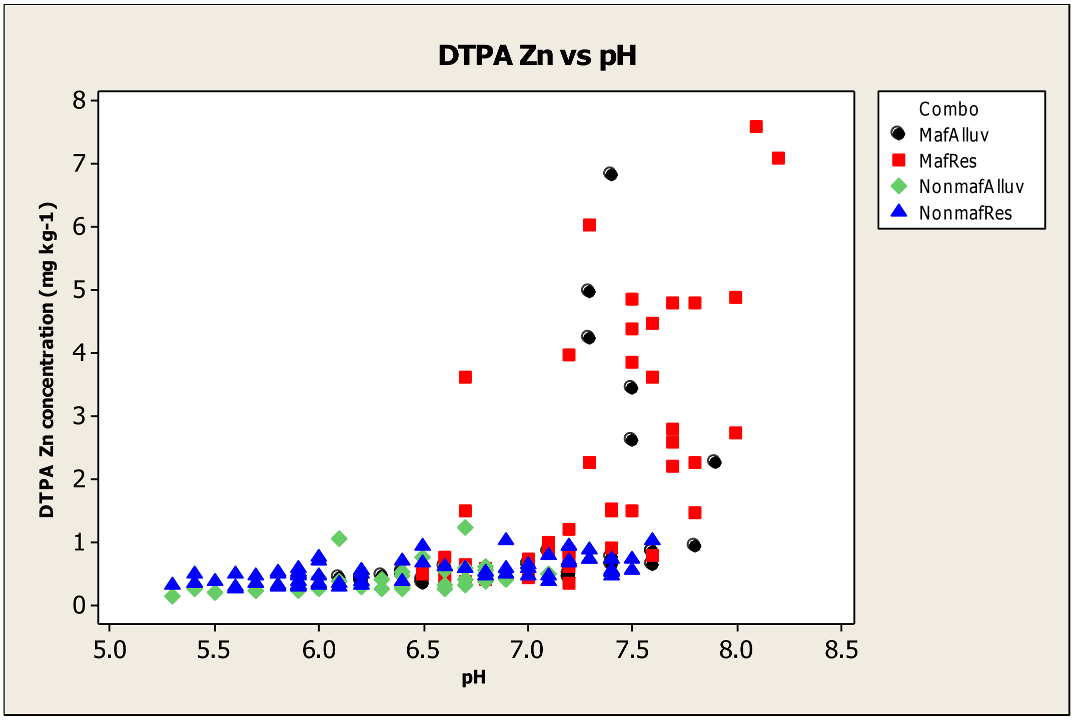


Figure 3.9: Plot of soil DTPA-extractable Zn concentration (mg kg^{-1}) versus soil pH by soil parent material (combo). MafAlluv = mafic-alluvium, MafRes = mafic-residuum, NonmafAlluv = nonmafic-alluvium, NonmafRes = nonmafic-residuum.

Average Grain Properties at Study Sites

Sorghum grain properties differed by soil site with mean grain weight (100 grains) ranging from 1.86+/-0.15 g at site KEN-S-2 to 2.68+/-0.14 g at KEN-S-1 (Table 3.9). Mean grain Zn concentrations ranged from 15.5+/-1.2 mg kg^{-1} (TEN-S-1) to 28.0+/-3.1 mg kg^{-1} (KEN-S-3). Mafic-alluvium parent material was divided into two groups; KEN-S-3 was placed into a group of its own based on its different sorghum planting density and intercropping with groundnut.

Across all sorghum grain, glume color was not related to grain Zn concentration (p value = 0.36) neither was pericarp thickness (p value = 0.60). As differences in grain Zn concentration by grain physical properties may be more a result of field and soil differences, individual grains of thin pericarp and red glume color were separated from grains of thick pericarp and purple glume color from mixed grain samples at site YEK-S-2. Grain Zn concentration was significantly higher (p value = 0.04) and grain lightness lower (darker; p value = 0.01) in thin, red-glumed sorghum than thick, purple-glumed sorghum.

Table 3.9: Mean and standard deviations (parentheses) of sorghum grain properties by site (Soil ID).

Soil ID	GW	PnWgt	PnLngth	Light	Zn
	g	g	cm		mg kg^{-1}
KEN-S-1	2.68 (0.14)	68.3 (22.2)	38.1 (4.7)	0.766 (0.009)	21.5 (1.3)
KEN-S-2	1.86 (0.15)	34.9 (9.3)	34.7 (2.8)	0.788 (0.009)	17.7 (2.4)
KEN-S-3	2.15 (0.25)	94.0 (35.3)	43.7 (4.3)	0.776 (0.014)	28.0 (3.1)
TEN-S-1	2.31 (0.20)	28.5 (6.4)	32.9 (2.9)	0.775 (0.020)	15.5 (1.2)
TEN-S-2	2.25 (0.14)	32.9 (9.3)	33.1 (1.6)	0.766 (0.007)	18.2 (2.3)
TEN-S-3	2.36 (0.09)	53.3 (18.3)	35.6 (3.9)	0.770 (0.013)	16.6 (1.0)
WAC-S-1	2.22 (0.16)	35.5 (12.5)	32.7 (4.6)	0.736 (0.018)	22.2 (2.3)
WAC-S-2	2.08 (0.24)	19.9 (8.3)	28.0 (3.4)	0.748 (0.016)	19.3 (1.6)
WAC-S-3	2.11 (0.15)	28.9 (11.2)	31.9 (2.8)	0.727 (0.020)	21.3 (2.0)
YEK-S-1	2.44 (0.15)	29.7 (8.6)	28.9 (2.3)	0.792 (0.012)	18.1 (1.8)
YEK-S-2	2.37 (0.22)	31.5 (11.5)	31.1 (2.7)	0.771 (0.016)	20.0 (2.3)

KEN = Keniero, TEN = Teneya, WAC = Wacoro, YEK = Yekelebougou, S-1 = Site number, GW = grain weight of 100 seeds, PnWgt = average panicle weight, PnLngth = average panicle length, Light = grain lightness, Zn = grain Zn concentration.

Effect of Parent Material, Tree Proximity, and Parent Material x Tree Proximity Interaction on Grain Properties

Parent material was a significant factor for panicle weight, panicle length, grain lightness, and grain Zn concentration (Table 3.10). Panicle weight and length were significantly higher for both groups of mafic-alluvium soils (Table 3.11). Soil parent material had significantly different grain Zn concentrations with mafic-alluvium group 1 soils having the highest and nonmafic-alluvium soils the lowest concentrations. However, when grouped by geologic parent material alone (mafic versus nonmafic), grain Zn concentration was not significant (p value = 0.33).

Table 3.10: Results of the combined analysis of variance by the general linear model procedure (p values) for sorghum grain properties. ** denotes p < 0.0001.

Source	GW	PnWgt	PnLgth	Light	Zn
PM	0.06	0.0002	0.0005	0.004	**
Tree(PM)	**	**	**	**	**
Tprox	**	0.02	0.79	0.66	0.32
PM*Tprox	0.07	0.12	0.50	0.90	0.22

PM = parent material, Tprox = tree proximity, GW = grain weight of 100 seeds, PnWgt = average panicle weight, PnLngth = average panicle length, Light = grain lightness, Zn = grain Zn concentration. Log transformations were performed on PnWgt, PnLngth, Light, and Zn.

Tree proximity was a significant factor for grain weight and panicle weight (Table 3.10). Panicle weight (Figure 3.10) and grain weight (Figure 3.10) were significantly higher near the tree and decreased away from the tree (Table 3.11). Panicle length (Figure 3.10), grain lightness (data not shown), and grain Zn concentration (Figure 3.11) showed no significant trend with distance from the tree. No sorghum samples were collected from underneath the tree canopy at -4 m and -2 m.

The interaction between the two main factors, parent material and tree proximity, were not significant for any of the grain variables, whereas tree within parent material was significant for all grain variables (Table 3.10).

Table 3.11: Means for sorghum grain properties by parent material (PM) and tree proximity (Tprox) factors. Lowercase letters denote Tukey-Kramer groups resulting from the combined analysis of variance by the general linear model procedure based on log transformations of PnWgt, PnLgnth, Light, and Zn and alpha = 0.05.

Factor	GW	PnWgt	PnLgnth	Light	Zn
	g	g	cm		mg kg^{-1}
PM					
MfAlv1	2.69a	4.18a	3.63b	0.766b	3.07b
MfAlv2	2.17b	4.52a	3.78a	0.776ab	3.32a
MfRs	2.22b	3.42bc	3.43d	0.790a	2.89c
NonMfAlv	2.31b	3.57b	3.52c	0.770b	2.81d
NonMfRs	2.23b	3.33c	3.44d	0.751c	3.03b
Tprox					
Near	2.40a	3.88a	3.56a	0.768a	3.03a
Far	2.25b	3.72b	3.56a	0.766a	3.01a

MfAlv1 = mafic-alluvium (KEN-S-1), MfAlv2 = mafic-alluvium 2 (KEN-S-3), MfRs = mafic-residuum, NonMfAlv = nonmafic-alluvium, NonMfRs = nonmafic-residuum, GW = grain weight of 100 seeds, PnWgt = average panicle weight, PnLngth = average panicle length, Light = grain lightness, Zn = grain Zn concentration.

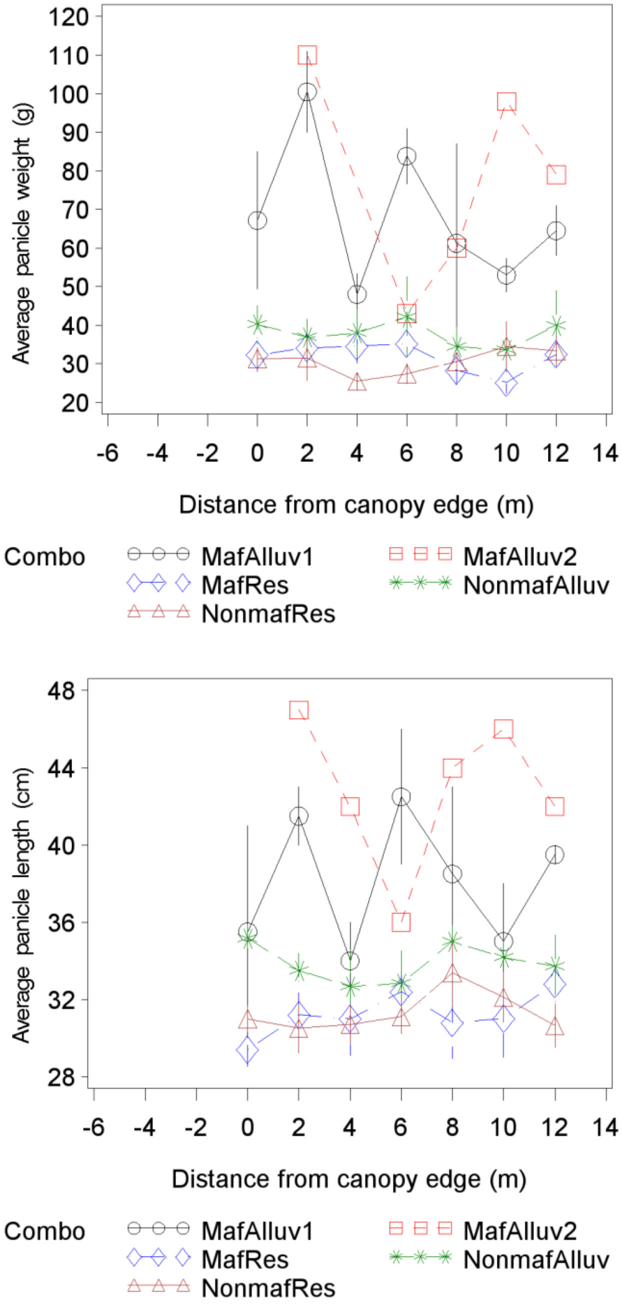
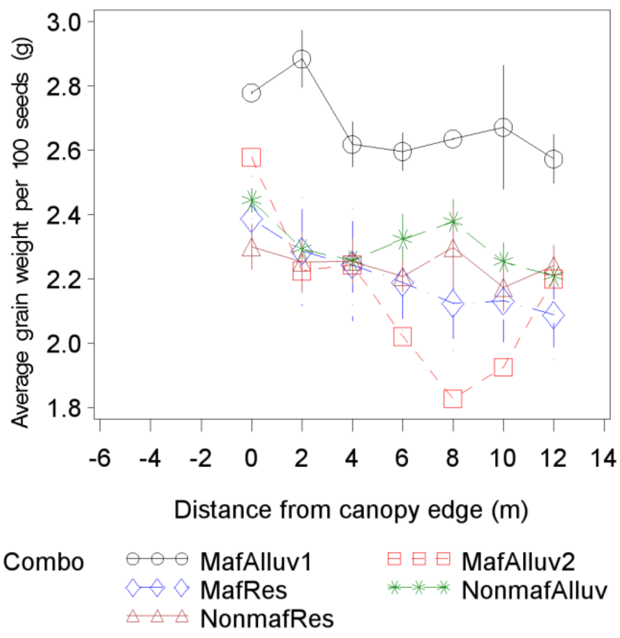


Figure 3.10: Means for panicle weight (upper figure) and panicle length (lower figure) at each distance from the tree canopy edge (0 m) by 4 different soil parent materials. Grain samples were not collected from underneath the canopy (-4 m, -2 m). Vertical bars are standard errors. MafAlluv1 = mafic-alluvium 1 (KEN-S-1), MafAlluv2 = mafic-alluvium 2 (KEN-S-3), MfRs = mafic-residuum, NonMfAlv = nonmafic-alluvium, NonMfRs = nonmafic-residuum.



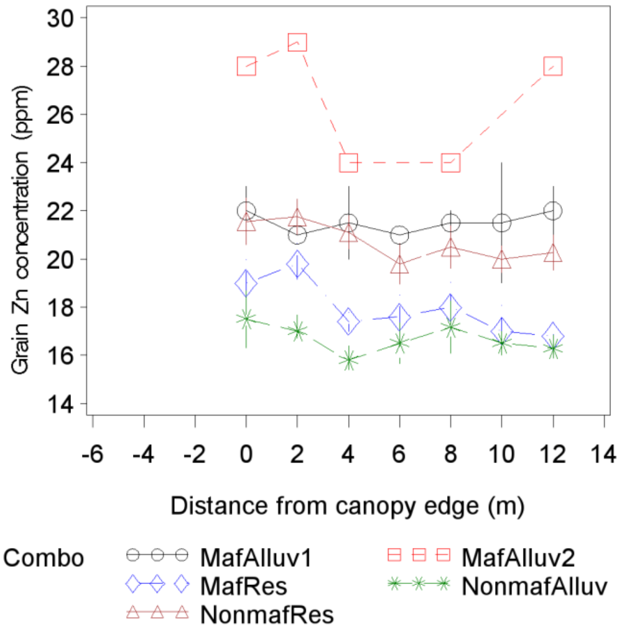


Figure 3.11: Means for grain weight (upper figure) and grain Zn concentration (lower figure) at each distance from the tree canopy edge (0 m) by 4 different soil parent materials. Grain samples were not collected from underneath the canopy (-4 m, -2 m). Vertical bars are standard errors. MafAlluv1 = mafic-alluvium 1 (KEN-S-1), MafAlluv2 = mafic-alluvium 2 (KEN-S-3), MfRs = mafic-residuum, NonMfAlv = nonmafic-alluvium, NonMfRs = nonmafic-residuum.

Correlations among Grain Properties

There were weakly significant relationships between grain weight and tree parameters such as distance from the tree, tree bole circumference, and tree height (Table 3.12). Panicle weight and length were also weakly correlated with tree height and canopy radius. Grain Zn concentration was weakly, but significantly correlated with panicle weight and length and grain lightness.

Table 3.12: Correlation coefficients (r) among sorghum grain properties and between sorghum grain properties and tree properties. ** denotes significance at $p < 0.01$, * at $p < 0.05$.

	GW	PW	PL	Light	Zn	Dist	Circ	Hgt	CRd
GW	--								
PW	0.343**	--							
PL	0.092	0.736**	--						
Light	0.159*	0.062	0.009	--					
Zn	0.098	0.275**	0.229**	-0.325**	--				
Dist	-0.209**	-0.059	0.039	-0.017	-0.142	--			
Circ	0.281**	0.136	0.074	-0.049	-0.105	-0.017	--		
Hgt	0.297**	0.224**	0.147	0.338**	-0.269**	-0.011	0.524**	--	
CRd	0.173	0.284**	0.237**	-0.099	-0.111	-0.025	0.757**	0.508**	--

GW = grain weight of 100 seeds, PW = average panicle weight, PL = average panicle length, Light = grain lightness, Zn = grain Zn concentration. Dist = distance from the edge of the canopy, Circ = tree bole circumference, Hgt = tree height, CRd = canopy radius. Log transformations were performed on PW, PL, Light, and Zn.

Soil-Grain Relationship

Correlation coefficients between grain Zn concentrations and soil properties showed very little significance except for DTPA-extractable Fe concentration and soil hue (Table 3.13). Grain weight was the most influenced by soil properties including pH, chroma, percent organic carbon and total nitrogen, and DTPA-extractable Fe and Zn concentrations. Panicle weight and length were also weakly correlated with other soil properties including percent concretions.

Table 3.13: Correlation coefficients (r) between soil and sorghum grain properties. ** denotes significance at $p < 0.0001$, * at $p < 0.01$.

	Grain Property			
Soil Property	GrainZn	GW	PW	PL
pH	-0.032	0.293**	0.254**	0.073
OC	0.046	0.229**	-0.051	-0.234**
$\delta^{13}C$	-0.155	-0.238**	-0.210**	-0.168
TN	0.063	0.258**	-0.037	-0.244**
$\delta^{15}N$	0.052	-0.311**	0.016	0.142
DTPA Fe	0.248**	-0.306**	-0.168	-0.093
DTPA Zn	0.166	0.209*	0.188*	-0.004
DTPA Mn	0.109	0.179	0.140	0.091
DTPA Cu	0.144	0.146	-0.034	-0.248**
Conc	0.002	-0.026	-0.218**	-0.287**
Hue	0.239**	0.158	0.116	0.045
Value	0.158	-0.111	0.063	0.213**
Chroma	-0.105	0.370**	0.362**	0.323**

GrainZn = grain Zn concentration, GW = grain weight of 100 seeds, PW = average panicle weight, PL = average panicle length, pH = soil pH in water, OC = organic carbon, $\delta^{13}C$ = delta ^{13}C, TN = total nitrogen, $\delta^{15}N$ = delta ^{15}N, DTPA = diethylenetriaminepentaacetic acid-extractable, Conc. = % concretions. Log transformations were performed on OC, TN, DTPA-extractable Fe, Zn, Mn, and Cu, soil color hue, value, and chroma, PW, PL, and GrainZn.

It is apparent that the relationship between soil properties and grain Zn concentration differed by soil parent material (Table 3.14). In mafic-residuum soils, grain Zn concentration was significantly and positively correlated with pH and percent concretions and negatively correlated with $\delta^{13}C$, $\delta^{15}N$ and chroma. Mafic-alluvium group 1 soils were significantly and negatively correlated with value and chroma. Conversely, nonmafic-residuum soils were significantly and positively correlated with DTPA-extractable Fe concentration and soil color hue and negatively correlated with DTPA-extractable Mn concentration while nonmafic-alluvium soils were significantly and positively correlated with DTPA-extractable Fe and Mn concentrations, and negatively correlated with pH and total nitrogen concentration.

Table 3.14: Correlation coefficients (r) between soil properties and grain Zn concentration by soil parent material. ** denotes significance at $p < 0.0001$, * at $p < 0.01$.

	Grain Zn Concentration				
Soil Property	MfRs	MfAlv1	MfAlv2	NonMfRs	NonMfAlv
pH	0.408*	-0.140	-0.609	-0.169	-0.313*
OC	0.152	0.107	-0.089	-0.136	-0.186
$\delta^{13}C$	-0.405*	-0.461	0.217	-0.096	0.137
TN	0.208	0.185	-0.144	-0.096	-0.318*
$\delta^{15}N$	-0.399*	-0.332	-0.137	0.221	-0.112
DTPA Fe	-0.206	0.433	0.288	0.357**	0.455**
DTPA Zn	0.315	0.132	-0.047	0.04	0.049
DTPA Mn	-0.227	0.400	0.153	-0.394**	0.560**
DTPA Cu	-0.046	0.085	-0.486	0.036	0.092
Conc	0.479**	---	---	-0.213	-0.176
Hue	0.228	-0.157	-0.213	0.360**	-0.038
Value	0.030	-0.692**	0.092	0.208	0.050
Chroma	-0.394*	-0.570**	-0.079	-0.169	-0.184

MfAlv1 = mafic-alluvium 1 (KEN-S-1), MfAlv2 = mafic-alluvium 2 (KEN-S-3), MfRs = mafic-residuum, NonMfAlv = nonmafic-alluvium, NonMfRs = nonmafic-residuum, pH = soil pH in water, OC = organic carbon, $\delta^{13}C$ = delta ^{13}C, TN = total nitrogen, $\delta^{15}N$ = delta ^{15}N, DTPA = diethylenetriaminepentaacetic acid-extractable, Conc = % concretions. Log transformations were performed on OC, TN, DTPA Fe, Zn, Mn, and Cu, soil color hue, value, and chroma, and grain Zn concentration.

Discussion

Role of Soil Parent Material on Zn Availability

The mineralogy and geochemistry of parent material can greatly influence soil properties including the type and quantity of clay minerals, cation-exchange capacity, organic-matter retention, pH, and trace-metal abundance and availability. The distinctly different soil chemistry of soils derived from mafic-residuum parent material can be attributed to differences in mineral weathering between mafic and nonmafic rock. Weathered dolerite boulders with a thin layer of soil were observed at YEK-S-1 and pieces of weathered schist at KEN-S-2. Due to the instability of mafic minerals such as pyroxenes, amphiboles, biotite mica, and plagioclase, mafic rock weathers relatively quickly as compared to rocks with nonmafic minerals such as granite (Scott and Pain 2008). These mafic rock derived soils have not gone through the more typical long weathering sequence in Mali that results in 1:1 kaolinitic clays with low cation-exchange capacity and pH. At YEK-S-1, soil cracking was observed, thus indicating the likely presence of 2:1 smectite clays, particularly the Fe^{3+} smectite, nontronite (Galan 2006).

Soil properties such as pH and percent organic carbon are generally higher in soils dominated by smectite clays than kaolinite clays (Reid-Soukup and Ulery 2002). High mean pH (7.31+/-0.45) and percent organic carbon (2.07+/-1.07%) of the mafic-residuum derived soils are not typically found in southern Mali soils. Results from previous sampling and analysis of soils in the same region yielded an average pH of 5.9 and percent organic carbon of 0.68+/-0.35% (Chapter 2). In the absence of carbonates (in my study, there was no evidence of carbonates as determined with dilute hydrochloric acid), the high pH found in mafic-derived soils confirmed the likely presence of smectite clays as proton attack on the silicate structure of mafic minerals can lead to the release of Fe^{3+} and other isomorphically substituted cations, protonation of structural oxygens, and formation of OH^- cations in solution (Reid-Soukup and Ulery 2002). In addition, in the absence of textural differences (soils were similarly sandy loams to sandy clay loams) the high percent organic carbon in the mafic-residuum soils likely resulted from the ability of smectite clays to absorb organic matter plus the particular physical conditions conducive to the addition and protection of organic matter. Bedrock erosion into boulders at site YEK-S-1 led to a very uneven surface which was convenient for leaf litter accumulation and moisture retention. Organic matter was protected from decomposition, especially as it readily absorbed onto the high sorption-capacity smectites present. Moldboard plowing was not conducted at YEK-S-1 and likely contributed further to organic matter preservation (Takimoto et al. 2009).

Parent material can influence other properties of soils including trace metal chemistry. The mafic minerals of pyroxene and amphibole in particular can have a substantial amount of Zn^{2+} substitution in the silicate structure; thus, mafic rocks such as dolerite are reported to have a mean Zn concentration of 123 mg kg^{-1}, whereas nonmafic rocks such as granite (containing very little pyroxene or amphibole) have a mean of 45 mg kg^{-1} of Zn (Anand and Gilkes 1987). As noted earlier, mafic minerals such as pyroxene and amphibole are unstable and weather relatively quickly resulting in the likely release of ions such as Zn^{2+}.

The solubility of Zn in soil systems is low such that only a fraction of the total Zn is soluble with solubility dependent on pH and the availability of Zn bonding sites on Fe oxide minerals and organic matter (Alloway 2008). Low molecular weight organic acids form soluble complexes with Zn and have been shown to prevent adsorption on and occlusion within Fe oxides (Barrow 1993; Agbenin 2003b), making Zn more readily available for plant uptake. It is thought that organic-sulfur functional groups may also play an important role in Zn bonding (Vodyanitskii 2010). Several studies have reported high correlations between DTPA-extractable Zn and organic carbon (Buri et al. 2000; do Nascimento et al. 2007; Siddhu and Sharma 2005). Behera et al. (2011) found significant and positive correlations between organic carbon and both total Zn and DTPA-extractable Zn concentrations. In my study, soil percent organic carbon was significantly and positively correlated with DTPA-extractable Zn concentration. The high percent organic carbon mafic-residuum soils were highest in DTPA-extractable Zn concentration.

The pH-dependent nature of Zn solubility is strongly related to the specific adsorption of Zn on Fe oxides. Variable charge sites, such as on Fe oxides, have a more positive charge at low pH and therefore adsorb less Zn^{2+} thus increasing Zn solubility (Alloway 2008). This theoretical solubility or activity of Zn^{2+} is proportional to the square of the proton activity (Kiekens 1995). However, a negative relationship between soil pH and Zn solubility (as measured by DTPA-extractable Zn concentration) has not always been consistently found and reflects the more complex influences of multiple adsorption mechanisms including adsorption by organic matter.

Studies conducted by Buri et al. (2000) and do Nascimento et al. (2007) reported a significant negative relationship between soil pH and DTPA-extractable Zn concentration. Jiang et al. (2009) also reported a significant negative relationship for a cultivated field, but a significant positive relationship in woodland fields containing higher soil organic matter. Behera et al. (2011) reported a positive relationship from four cultivated acidic soils in India. In my study, a significant positive relationship was found between soil pH and DTPA-extractable Zn which may be explained in two ways. First, it may simply be an outcome of the positive relationship between organic carbon and pH which is found in smectite soils. In this case, the relatively high organic carbon may be controlling Zn solubility instead of pH. This is shown in Figure 3.9 where DTPA-extractable Zn concentration rises rapidly at pH higher than 6.7. Second, at low pH, soils are typically kaolinitic with very low cation exchange capacity and fewer negative sites for Zn^{2+} to adsorb onto. Under these low pH conditions, the solubility of Zn would then be increased which would technically be better for plant uptake; however, in reality, this increased solubility and lack of suitable adsorption sites leads to Zn^{2+} being readily leached or “cropped” away through time (Behera et al. 2011). Thus, in my study, soils from KEN-S-1, TEN-S-1, TEN-S-2, WAC-S-1, WAC-S-2, and WAC-S-3 had mean DTPA-extractable Zn concentrations all below the range of 0.50 to 1.2 mg kg^{-1}, the concentration of DTPA-extractable Zn in soil considered to be “Zn deficient” for sorghum (Takkar et al. 1989). Five out of the six soils were derived from nonmafic parent material which would contribute less Zn originally.

It is unclear whether soil derived from mafic parent material, but transported under alluvial conditions can retain their high DTPA-extractable Zn concentration signature relative to soils weathered in place (residuum). DTPA-extractable Zn concentrations in mafic-alluvium soils were generally lower than mafic-residuum soils, but still were significantly higher than in nonmafic soils. At the mafic-alluvium site, KEN-S-3, DTPA-extractable Zn concentrations were comparable to those of the mafic-residuum soils, but the mafic-alluvium site, KEN-S-1, was more comparable to nonmafic soils. Alluvial material such as silts and clays together with a sufficient amount of organic matter could retain the Zn that has been weathered out from the mafic rocks above; however, one possible reason why DTPA-extractable Zn concentration was lower at KEN-S-1 was that it was quite a distance (approximately 300 m) from the dolerite outcrop (Figure 3.12). Furthermore, the alluvium material here was at least 90 cm thick (Chapter 2).

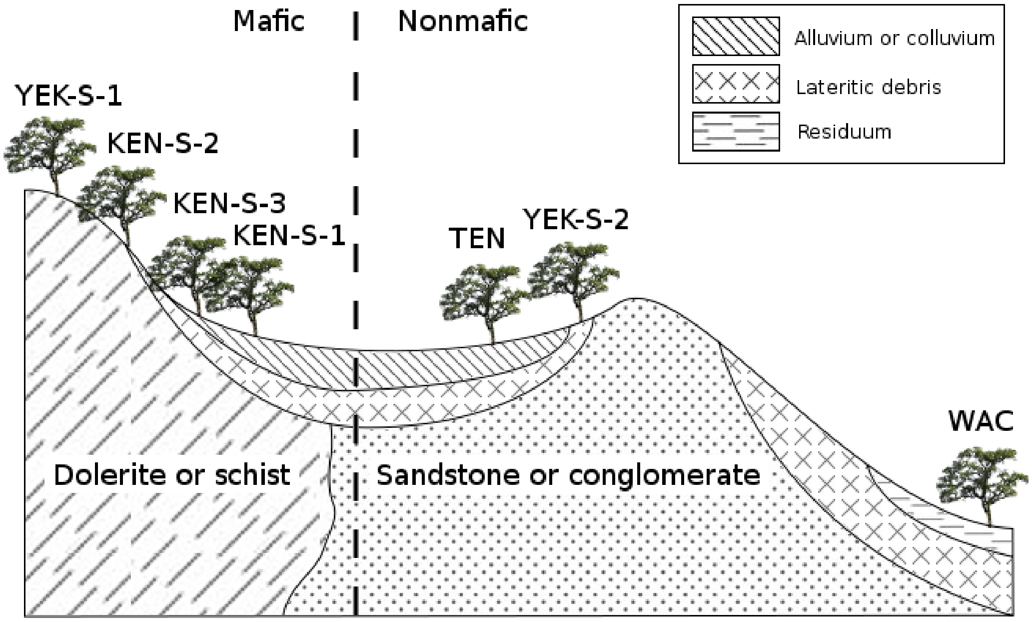


Figure 3.12: Geologic and soil block diagram (after Beauvais et al. 1999). KEN = Keniero, TEN = Teneya, WAC = Wacoro, YEK = Yekelebougou, S-1 = Site number.

Role of Shea Trees on Zn Availability

Soils surrounding Shea trees have been found to be higher in pH and percent organic carbon and total nitrogen (Boffa et al. 2000; Traore et al. 2004). The increase in these soil fertility properties near the tree were also observed in my study at mafic-residuum sites. Leaf litter from the trees can replenish the soil carbon and nitrogen lost to decomposition and cropping in tropical cultivated soils. The organic carbon from the trees also can buffer highly weathered soil, most likely by providing organic anions such as carboxyls that specifically adsorb onto hydrous Fe and Al surfaces (Fagaria and Baligar 2008). These organic anions can then adsorb Al^{3+} and H^+ with a corresponding release of hydroxyls that raise the pH (Wong et al. 1998).

When organic carbon and total nitrogen are at very low concentrations (means less than 1.0%), it is difficult to document relationships with other soil properties including DTPA-extractable Zn concentration and pH (Chapter 2). At such low concentrations, there may be too much sample variation or lack of a suitable range to see a significant decrease in organic carbon or a tree effect. Indeed, at the mafic-alluvium and nonmafic sites with low organic carbon, percent organic carbon and nitrogen decreased very little with increasing distance from the tree. This lack of decrease could also be the result of field plowing extending under the canopy, which can cause significant loss of organic carbon by accelerating carbon mineralization (Takimoto et al. 2009).

Trees have the ability to take up Zn through their roots from within the deeper soil profile, transport Zn to leaves, and deposit Zn to the soil through leaf litter fall (Anand et al. 2007; REngel 2007). At mafic sites, this mechanism, along with higher pH and organic matter, may be responsible for maintaining high DTPA-extractable Zn concentrations near the tree; however, at nonmafic sites, it appears that the Shea tree cannot help maintain DTPA-extractable Zn concentrations in soils surrounding the tree at greater than deficient levels because there is minimal geologic source of Zn. At these nonmafic sites, mean DTPA-extractable Zn concentrations were lower than 1.2 mg kg^{-1} which is in the deficient range for sorghum (Takkar et al. 1989). This suggests that in soils weathered from low Zn parent material, Shea trees with their shallow root systems, cannot contribute to the maintenance of DTPA-extractable Zn concentrations in soil especially if continuous cropping and removal of crops occurs.

An agrofrestry system with a mixture of Shea trees and sorghum show a combined influence of C3 and C4 pathways (Takimoto et al. 2009). Carbon isotope values measured in my study support the published values of Takimoto et al. (2009) for a Mali Shea tree-cropland study (-20.7 to -18.7$^{0}/_{00}$) and reflect the combined influences of both C3 pathway organic carbon (-27$^{0}/_{00}$) and the C4 pathway (-13$^{0}/_{00}$). However, Takimoto et al. (2009) did not find the percent C3 contribution to be significantly higher near the tree as my study showed (78% near the tree; 67% far from the tree). Jonsson et al. (1999) reported a 20% drop in C3 contribution outside the Shea tree canopy in a study conducted in Burkina Faso. No other studies to my knowledge have reported correlations between δ^{13}C and DTPA-extractable Zn concentrations in a Shea agroforestry setting. In my study, there was a weakly negative, but significant correlation between δ^{13}C and DTPA-extractable Zn concentration.

Percent C3 contribution cannot be assumed to solely originate from the C3 Shea tree because the C3 crop groundnut is often grown underneath the canopy of Shea trees adjacent to the C4 sorghum or grown historically in the current C4 sorghum field. The particularly low mean δ^{13}C value of -23.2$^{0}/_{00}$ at site KEN-S-3 illustrates the effect of C3 groundnut as it was the only site to have groundnut intercropped with sorghum from 0 to 12 m out from the edge of the canopy.

Average nitrogen isotope values (δ^{15}N) are 4.6$^{0}/_{00}$ for arbuscular mycorrhizal (AM) soils (broadleaf-evergreen, coniferous, and grassland species) from a compilation study of sites in the U.S., Brazil, and Europe (Hobbie and Ouimette 2009). My δ^{15}N values for bush fields in Mali fell near the average of published values (Hobbie and

Ouimette 2009). Although soil $\delta^{15}N$ values reflect a combination of the various nitrogen biogeochemical processes that occur in the soil, $\delta^{15}N$ values have been estimated to be 0 to $3.5^{0}/_{00}$ for the nitrogen transfer from AM fungi to plant hosts based on differences between AM and non-mycorrhizal plants (Handley et al. 1999). Glomalin-related soil protein (GRSP), a protein produced by arbuscular mycorrhizal fungi, was reported to be highly correlated with DTPA-extractable Zn concentration in soils with sufficient Zn (Cornejo et al. 2008). Again, to my knowledge, no other studies have reported correlations between $\delta^{15}N$ and DTPA-extractable Zn concentrations in a Shea agrofrestry setting, and just as with $\delta^{13}C$ values in my study, $\delta^{15}N$ values were weakly negative, but significantly correlated with DTPA-extractable Zn concentration.

Role of Soil Parent Material and Tree Proximity on Grain Properties

It was hypothesized that soil parent material and tree proximity or their interaction would influence grain Zn concentrations. Despite the increased DTPA-extractable Zn concentrations found in soils from mafic parent material under Shea trees, a corresponding increase in grain Zn concentration was not consistently found. It is likely that sorghum, which was only grown up to the edge of the tree canopy, could not take full advantage of the higher DTPA-extractable Zn concentration in soil directly underneath its canopy. Grain grown in soils from mafic-alluvium group 2 had the highest Zn concentrations, but this could be a result of intercropping with groundnut and the rather large planting space between sorghum (2 m). It seemed to have resulted in longer and heavier panicles with no corresponding increase in grain weight. The larger plant spacing at this site may have led to decreased competition in the subsoil between sorghum plant roots (Bruck et al. 2003b) and hence subsequent higher grain Zn concentrations.

Shea trees do still improve sorghum growth. Boffa et al. (2000) reported a significant decrease in sorghum height and yield away from the edge of the tree canopy. Although not measured in my study, sorghum height was generally observed to decrease with distance away from the Shea tree. In addition, measured grain weight significantly decreased with distance from tree and was weakly, but positively, correlated with tree height, tree bole circumference, pH, and percent organic carbon and total nitrogen, and DTPA-extractable Zn concentration. Shea trees had the overall effect of improving sorghum growth and grain weight, likely due to increased organic matter concentration and soil fertility, as well as improved soil physical structure increasing infiltration (Vetaas 1992) and lowering soil and plant temperatures (Vandenbeldt and Williams, 1992).

Available Soil Zn-Grain Zn Relationship

Various crop studies conducted on the relationship between DTPA-extractable Zn and grain Zn concentrations have yielded conflicting results (Wissuwa et al. 2008; Lombaes and Singh 2003, Wang et al. 2009; Joshi et al. 2010). Many of these studies including a previous sampling and analysis of DTPA-extractable Zn and sorghum grain Zn concentrations in southern Mali (Chapter 2) suffered from the high variability of DTPA-extractable Zn concentrations and lack of one-to-one correspondence between soil DTPA-extractable Zn and grain Zn concentrations. In my study, a similar overall non-correlation between DTPA-extractable Zn and grain Zn concentrations occurred despite a one-to one correspondence of over 160 soil and grain samples.

Soil parent material appears to influence the grain Zn and DTPA-extractable Zn correlation as well as correlations between grain Zn and other soil properties. Soils with high pH, percent organic carbon, and DTPA-extractable Zn concentration from the mafic-residuum sites, had grain Zn concentrations significantly correlated with pH, $\delta^{13}C$, and $\delta^{15}N$ and significantly correlated with DTPA-extractable Zn concentration at a 90% confidence level ($r = 0.336$, $p = 0.07$). Conversely, soils with low pH, low organic carbon, and deficient DTPA-extractable Zn concentrations from nonmafic sites, had grain Zn concentrations significantly correlated with DTPA-extractable Fe concentration. The different correlations at each soil parent material may explain why it has been difficult to establish the expected overall positive relationship between DTPA-extractable Zn and grain Zn concentrations in this study as well as other published studies.

It is in the nonmafic soils with low pH and low organic matter and DTPA-extractable Zn concentrations, that the role of Fe oxides and their solubility in Zn availability and uptake is more readily seen. In my study, the range of soil color hue (7.87+/-0.42 to 8.3+/-0.18) fell within the lower range of goethite mineral hues (Scheinost and Schwertmann 1999) and indicated soils dominated by goethite with a small amount of hematite. These Fe oxides are generally quite insoluble and lead to relatively low DTPA-extractable Fe concentrations especially at mafic sites; however, DTPA-extractable Fe concentrations can be increased when ferrihydrite is present, which is a very small particle-sized Fe oxide that is more soluble (Loeppert and Inskeep 1995; Bigham et al. 2005). With its small size, ferrihydrite can have over 7 times the surface area of crystalline Fe oxides such as hematite and goethite (Cornell and Schwertmann 1996). This increased surface area may allow for more specific adsorption of metal cations such as Zn^{2+}. It may be the case that specific absorption on small-sized Fe oxides such as ferrihydrite is how Zn is retained against leaching in these soils and constitutes the only source of Zn available for plant uptake by sorghum.

Conclusions

Soil parent material and the proximity to Shea trees were significant factors for DTPA-extractable Zn concentration with the highest concentrations measured at mafic-residuum sites under the Shea tree canopy. Mafic-residuum parent material contain mafic minerals of relatively high Zn content that weather quickly to form smectite clays with high organic matter and pH. Both organic matter and pH were found to be significantly and positively correlated with DTPA-extractable Zn concentration.

Despite a significant positive effect of Shea trees on the growth of sorghum just outside of the tree canopy, grain Zn concentrations were not likewise significantly improved. This may be because sorghum was not grown underneath the Shea tree canopy where DTPA-extractable Zn concentrations were the highest. Instead, groundnut is often grown there, and further study on the likely increase in grain Zn concentrations of groundnut grown underneath Shea trees in mafic-residuum soils is recommended.

This study confirms the complex nature and numerous factors that affect grain Zn uptake from soil. Overall, the relationship between soil DTPA-extractable Zn and sorghum grain Zn concentrations was not significant; however, at the high pH and organic carbon mafic-residuum sites, pH and DTPA-extractable Zn concentrations were positively correlated with grain Zn concentrations. In Zn deficient soils with low pH and organic carbon concentrations, grain Zn concentration was weakly related to DTPA-extractable Fe concentration, possibly demonstrating the important role of Fe oxides in Zn availability and uptake.

Zn deficient soils were found in this study, the Chapter 2 study, and other previous Mali soil studies; however, the location and extent of Zn deficiency throughout Mali is not known. Mafic parent material exists throughout western Mali, and it would be of interest to determine whether Zn deficiencies are prevalent there or are limited to soils with nonmafic parent material such that occur in southern Mali and in former cotton zone areas. Also, it appears that Shea trees cannot provide enough Zn to the soil in nonmafic areas as fast as it is leached or cropped away. This has important implications for fallow times and the potential use of Zn fertilizers.

As up to 50% of sorghum grain Zn concentration is dependent upon field and soil effects (Chapter 2), the biofortification of sorghum may be hampered if variety trials occur only under Zn deficient conditions. Knowledge of basic soil properties such as pH and organic carbon and an estimate of the soil parent material may aid in the location of fields likely to be high or low in available Zn. Measurements of DTPA-extractable Zn concentrations may also accomplish this, but it must be kept in mind that these concentrations can be variable over short distances and anomalously high for reasons cited in my study including mafic-residuum soils, Shea trees, and also biochar after vegetative burning.

CHAPTER IV

FE RESPONSE IN TWO *SORGHUM BICOLOR* L. MOENCH CULTIVARS INOCULATED WITH *CURVULARIA LUNATA*

Introduction

Fe is an essential micronutrient in the human body and adequate concentrations of Fe must be maintained for healthy growth, physical and cognitive functioning, and reproduction. Fe is a component of hemoglobin in red blood cells which are responsible for oxygen transport throughout the body (Slingerland et al. 2005). Fe is also present in certain enzymes that synthesize steroid hormones, detoxify the liver, and are involved in neurotransmitter metabolism in the brain (Cockell 2007). Fe deficiency leads to anemia and associated negative health consequences such as impaired cognitive and physical development of children, reduced resistance to disease, and decreased work productivity in adults (WHO 2008; Slingerland et al. 2005). Anemia is a particular concern for pregnant women as it is linked with increased rates of pre-term births, low birth weight babies, and other pregnancy complications that can lead to maternal death (Cockell 2007; Slingerland et al. 2005; Stein 2010).

Anemia is estimated to affect 25-30% of the world's population (approximately 1.5-2 billion people) and is considered to be the most common nutrition problem in the world (WHO 2008, Stein 2010). Although anemia can affect people in industrialized and developing countries, it is particular prevalent among women and children in poor countries. In the West African countries of Burkina Faso, Mali, and Niger, 81 to 92% of children have blood hemoglobin concentrations above the threshold for anemia (WHO 2008). Anemia and the underlying cause of Fe deficiency result from a diet lacking in meat and high in cereals and vegetables which contain anti-nutritional compounds that can decrease Fe absorption to 5% Fe intake (Vasconcelos and Grusak 2006).

Due to the considerable health and work loss that Fe deficiency and anemia bring, development initiatives have sought to bolster Fe intake by breeding staple food crops for increased Fe concentration (Bouis and Welch 2010). Over the past several years, the International Crop Research Institution of the Semi-Arid Tropics (ICRISAT), located in Bamako, Mali, has bred for high Fe sorghum varieties through participatory field trials. In a 2007 trial on decorticated sorghum grain, genetic, environmental, and genetic x environmental effects were documented for Fe (Tuinsma et al. 2009). Fe analysis of *whole* grain sorghum from the 2009 trial yielded no such genetic, environmental, or genetic x environmental effects. An understanding of this unexplained Fe variation in whole grain sorghum could help the breeding of high Fe sorghum go forward more successfully, especially in light of recent studies showing issues with the measurement of Fe concentration in grain (Stangoulis 2010).

There are several possible reasons for the lack of genetic, environmental, or genetic x environmental significance in whole grain sorghum: 1) high natural variation of Fe within a sorghum panicle or between panicles; 2) grain contamination during harvest and subsequent threshing; and, 3) laboratory contamination. Laboratory contamination is always a possibility if procedures are not put in place for the prevention of contamination of samples. It was observed that contamination from dust or rust falling from a laboratory fume hood is possible and warrants careful covering of all sample and digestion vessels. Contamination by dust or soil is thought to contribute to Fe in analyzed grain (Pfeiffer and McClafferty 2008) and would likely occur when harvesting, threshing, or grinding grain (Stangoulis and Sison 2008). Sorghum grain from the 2007 and 2009 trial were harvested by farmers and threshed by hand in the village, likely by wooden sticks on straw mats on the ground, and by mechanical threshers at the research station.

The third possibility is that Fe variability may have a natural explanation. One particular aspect of the plant environment, that of grain molding, is hypothesized to have an effect on grain Fe concentrations. In a similar genetic, environmental, and genetic x environmental study, newly developed genotypes of wheat were grown across 14 locations in India for 3 years. Results showed a strong environmental effect and among the soil and meteorological factors measured, maximum temperature before flowering and rainfall and relative humidity after flowering significantly affected grain Fe concentrations (Joshi et al. 2010). These weather variables, high temperature and relative humidity, are often highly correlated with increased infection by mold fungi (Thakur et al. 2006). In West Africa, long season, photosensitive varieties are typically grown in order to avoid maturity during such warm and humid weather conditions; however, short season varieties that mature earlier in the field often suffer more mold problems (Ratnadass et al. 1999). The varieties planted in the 2009 trial in Mali were both short and tall varieties, and visible mold formation was noted on many of the varieties. Fungal molds can affect grain mass, viability, and quality for food production (Bandyopadhyay et al. 2000; Thakur et al. 2006). Grain molding or grain exposure to climatic conditions can also affect micronutrient density (Pfeiffer and McClafferty 2007), especially if related to reduced mass.

Plant pathologists often see elevated concentrations of the Fe storage protein ferritin in plants attacked by fungus (Dr. Kevin Ong – Director, Texas Plant Disease Diagnostic Laboratory, Texas A&M University, personal communication). Ferritin is the main storage structure for Fe in seeds (de la Fuente et al. 2011). A possible mechanism for increased ferritin and consequential increased Fe upon pathogen attack was shown by Liu et al. (2007). They found an accumulation of reactive Fe^{3+} at the cell wall appositions in wheat leaves attacked by powdery mildew pathogens and further reported that this additional Fe^{3+} mediates the production of reactive oxygen species (ROS), which is one type of plant pathogen defense mechanism that requires Fe for donation of an electron. Recent scanning and transmission electron microscope micrographs of a Fe hyperaccumulator grass plant *Imperata cylindrica* (L.) have confirmed the presence of ferritin in the cell wall (de la Fuente et al. 2011). Plants as well as animals must tightly control and store Fe as too much "free" Fe will lead to oxidative stress and cell death (Robin et al. 2008). The transport of Fe from root to shoot to seed is controlled by various Fe transporter genes (Briat 2008; Kerkeb and Connolly 2006), but it is possible that during an attack of airborne fungi on sorghum grain, plants remobilize Fe to the site of attack where it donates an electron for production of ROS; Fe is then stored as ferritin in the cell wall. The amount of Fe remobilized to the grain may then depend upon the presence of mold fungi and the subsequent plant response to the pathogen. It is hypothesized that the molding of sorghum grain may be responsible for the variable and elevated concentrations of grain Fe noted in the previous studies on sorghum and wheat.

The purpose of this study was to: 1) determine whether pathogen attack results in a significant accumulation of Fe in the grain under field conditions; and, 2) determine the natural variation of Fe in grain of different panicles and within a single panicle.

Materials and Methods

Field Trial

Five sorghum cultivars were grown including two mold resistant (Tx2911 and SC719-11E), a moderately mold resistant (Sureno), and two mold susceptible (Rtx2536 and Rtx430). Cultivars Rtx2536 and Rtx430 are known to be Fe chlorosis resistant (Esty et al. 1980; Peterson and Onken 1992). Cultivars were planted April 18, 2011 in a randomized complete block design at the Texas A&M University Research Farm, near College Station, TX. Each cultivar was replicated 5 times in 12-m rows of sorghum with 75 cm row spacing and plant spacing of 5-7 cm within rows.

The soil is classified as a Ships Clay (0 to 1 percent slopes) which is a clayey alluvium, moderately alkaline (pH = 7.9-8.4) with a maximum $CaCO_3$ content of 20% (Chervenka 2002). Fertilizer applications were as follows: 168 kg ha^{-1} of 10-34-0 and 4.4 kg ha^{-1} Zn applied 2 months before planting. One month after planting, 1,030 L ha^{-1} of 32-0-0 was applied as a side-dressing near the plants.

Two treatments were used in this study: 1) *Curvularia lunata*; and, 2) sorghum panicles sprayed with water as a control. Within each cultivar row, three panicles were inoculated at 50% bloom (half-bloom) by a hand-held spray bottle containing conidial suspensions of C. *lunata* or sterile distilled water. Conidial suspensions were made following the procedure of Prom (2003) where isolates of C. *lunata* were grown in petri plates containing one-fifth strength potato dextrose agar medium at 25 º C for 10-14 days. C. *lunata* conidia suspensions were made by adding approximately 10 mL of distilled water to each of 2 plates and scraping conidia with a rubber spatula into suspension. The suspensions were filtered through 4 layers of cheesecloth into a beaker before being transferred to a plastic spray bottle and diluted with sterile water to a final volume of approximately 1 L.

Each cultivar had a slightly different maturity time for half-bloom, thus resulting in inoculation dates varying from June 28 to July 18, 2011. Panicles were inoculated either in the early morning or late evening by spraying each panicle until water was seen to drip off the bottom of the panicle. They were then tagged and covered with paper bags for 24 hours.

Parameters Measured

Panicles were harvested at maturity the first week of October 2011. They were cut off at the base and immediately placed in new paper bags and allowed to air dry for one week. Seeds were threshed by hand, dechaffed by blowing, and placed in Ziploc® bags. Seed mycoflora analysis was conducted according to Prom (2004). Fifty seeds were randomly selected from each panicle and cleaned by placing them in plastic air-holed vials, washing with a solution of 10% NaOCl for 1 min., and rinsing in sterile water for 1 min. Vials were placed in a hood and allowed to dry overnight. Seeds were transferred to 5 petri plates (10 seeds per plate) using an aseptic technique (by forceps sterilized by alcohol flame) after each sample. Plates contained half-strength potato dextrose agar and were incubated with the seeds at 25 º C for approximately 5 days. Fungal species were identified and counted after this time based on conidia and colony descriptions and figures in Navi et al. (1999).

It became apparent during the course of the mycotoxin analysis, that the C. *lunata* inoculated panicles of the mold resistant varieties Tx2911, SC719-11E, and Sureno contained very few counts of C. *lunata* and were indistinguishable from control samples, whereas the mold susceptible varieties Rtx2536 and Rtx430 showed clear differences in C. *lunata* counts. Thus, the resistant cultivars were dropped from subsequent analysis and only Rtx2536 and Rtx430 were analyzed for grain mold rating, grain color, and grain Fe concentration. Also, of the 60 panicles that were inoculated for Rtx2536 and Rtx430, 15 panicles were lost due to lodging and subsequent consumption by animals.

Harvested seeds from each panicle were assessed for grain mold rating using a 1-5 scale (Isakeit et al. 2008) as follows: 1. seed bright with no mold and no discoloration due to weathering; 2. seed is not as bright and has little or no mold, but has some discoloration; 3. seed is not bright, there is some mold and some discoloration; 4. seed is almost entirely covered in mold and is deteriorating; 5. seed is covered entirely with mold, is deteriorated, and looks dead. Seed weight was determined from the weight of 100 randomly selected seeds from each panicle. Plant height and panicle length were measured at maturity. Plant height was measured from the base of the plant at the soil surface to the top of the panicle and panicle length was measured from the first branch with racemes to the top of the panicle.

Approximately 5 g of grain were selected from each panicle and ground to 1 mm size (Cyclone Lab Sample Mill, Udy Corporation, Fort Collins, CO, U.S.A.). Milled grain was placed in Crystal Clear^{TM} plastic bags and scanned on a flatbed scanner for measurement of the three primary colors red, green, and blue by Color Scanning Analysis software (D. Verbree unpublished 2012). Color represents a mixture of the monochromatic spectra of red (700 nm), green (546 nm), and blue (436 nm), and on an 8-bit digital system, these three primary colors are quantified by numeric tristimulus values that range from 0 (darkness) to 255 (whiteness; Viscarra Rossel et al. 2006). The measured red, green, and blue values were then converted to the Commission Internationale de l'Eclairage (CIE) standardized color space model which uses a lightness (L) function to describe brightness which ranges from 0 (black) to 1 (white).

Fe Digestion Procedure

Fe analysis was performed using a procedure developed by UW-Madison (2005). The digestion of grain samples used a combination of concentrated nitric acid and peroxide. Briefly, 1 g of ground grain from the Crystal Clear^{TM} plastic bags was weighed out to 4 decimal places (and the weight recorded) and placed in Teflon digestion tubes (SCP Science, Champlain, N.Y., U.S.A). Tubes were placed in a DigiPrep MS 48-position graphite digestion block (SCP Science, Champlain, N.Y., U.S.A.) Several (5-6) ultra-pure PTFE boiling stones (Saint-Gobain Performance Plastics, Paris, France) were placed into each Teflon tube which were then covered with clean glass funnels to facilitate refluxing. Ten mL of trace metal grade nitric acid (67-70% HNO_3) was dispensed into the tubes and allowed to predigest at room temperature for 2 hours. The tubes were then heated to 122 ° C (just above the boiling temperature of concentrated nitric acid) and allowed to reflux for 16 hours before cooling to below 70 ° C. Approximately 1 mL of reagent grade hydrogen peroxide (30% solution) was added to the tubes and the temperature raised to 122 ° C again and held there for 30 min. After cooling to below 70 ° C, another 1 mL of hydrogen peroxide was added and the temperature raised again as before. After cooling below 70 ° C, approximately 5 mL of deionized water was added to each tube. Five mL of each sample was transferred to a 25 mL volumetric flask and brought up to volume with deionized water.

Sorghum samples and 3 NIST Rice Flour checks (Standard Reference Material 1568a) were digested in one batch in the 48-position digestion block. The entire Fe digestion procedure from re-selection of 1 g of ground sorghum samples to transference of the liquid samples to 25 mL volumetric flasks was repeated an additional two times for a total of three digestion batches. The first two batches were conducted in a fume hood with a Plexiglas enclosure (DigiPrep MS, SCP Science, Champlain, N.Y., U.S.A.) The third batch was identical to the first two batches except that it occurred on the laboratory workbench in a Plexiglas enclosure with a DigiVac vacuum exhaust system (SCP Science, Champlain, N.Y., U.S.A.) where airflow into the digestion block chamber was filtered. Samples were analyzed for Fe concentration by atomic absorption spectrometry (AAnalyst 400, Perkin Elmer Instruments, Waltham, MA, U.S.A). The average Fe concentration of the 3 batches was obtained for each sorghum sample and used in subsequent comparisons and statistical analysis.

Additionally, a single digestion batch precision test with sample replications was performed on a subset of sorghum samples to determine the within batch and sample variation in Fe concentration. For both Rtx2536 and Rtx430, the 3 control panicles within a particular row were selected and approximately 10 g of each sample was mill ground (Cyclone Lab Sample Mill, Udy Corporation, Fort Collins, CO, U.S.A.). 1 g of ground sample was weighed out to 4 decimal places (and the weight recorded) and placed in Teflon-digestion tubes. This was repeated 5 times for a total of 6 sub-sample replicates per sample. In addition, 6 replications of nitric acid blanks and 6 replications of NIST Rice Flour were also digested. This batch was digested with filtered airflow and vacuum venting on the laboratory workbench. The Fe concentration of these samples was analyzed by AAS.

Statistical Analysis

A univariate analysis of variance using a generalized linear model (GLM) was conducted on cultivar Rtx25365 and Rtx430 for percent C. *lunata* (CL) recovery, grain mold rating, Fe concentration, seed weight, plant height, panicle length, and grain lightness. Factors in the model were treatment, block number (random), and treatment x block number. Mean comparisons were conducted using Tukey-Kramer for sorghum grain and mold variables. Correlations between percent recovery of CL and other measured traits were conducted by Pearson's HSD correlation analysis.

Summary statistics for the Fe concentration of sorghum samples by digestion batch and within batch were calculated. For the within batch digestion, an analysis of variance using the VARCOMP procedure was conducted for each cultivar. The only factor in the model was the panicle number, leaving the error component to include the variation in individual 1 g grain sub-samples selected from the larger sample of ground grain sorghum plus any variation due to the digestion procedure and AAS analysis.

All data were analyzed using SAS software version 9.2 (SAS Institute, Cary, N.C., U.S.A.).

Results

The frequency of recovery of various fungal species including the inoculation species C. *lunata* is presented by cultivar-block and treatment (Table 4.1). In general, C. *lunata* recovery was high for panicles treated with C. *lunata* (67% and 49% for Rtx2536 and Rtx430, respectively) vs. the control (9% and 8% recovery, respectively). Species of *Alternaria, Bipolaris, and Aspergillus* were the most frequently recovered fungi from control panicles as these species are naturally occurring grain molds. They typically do not infect sorghum flower tissues, result from late field weathering or storage of sorghum, and are restricted to the pericarp portion of the grain (Frederiksen and Odvody 2000; Bandyopadhyay et al. 2000). These later stage fungi were also recovered from C. *lunata* treated panicles (Table 4.1).

Table 4.1: Frequency of recovery (%) of various fungal species from two cultivars inoculated with *Curvularia lunata*. Frequency of recovery (%) was based on assays of 50 seeds per cultivar/panicle combination plated on half-strength potato dextrose agar medium.

			Fungal species								
Cultivar-Block	Trt	#Pan	CL	Alt.	Asp.	Bip.	CH	FT	FS	Fsp.	Unk.
Rtx2356-1	CL	2	72	27	0	1	0	0	0	0	0
	CON	3	12	55	16	11	0	3	1	0	3
Rtx2356-2	CL	3	51	33	9	5	0	0	1	0	1
	CON	2	6	60	11	17	0	0	0	0	6
Rtx2356-3	CL	3	85	13	0	1	0	1	0	0	0
	CON	3	9	65	12	7	0	1	3	1	4
Rtx2356-4	CL	3	63	31	0	3	0	2	1	0	1
	CON	3	16	52	3	8	1	2	14	3	1
Rtx2356-5	CL	2	63	26	2	3	0	0	4	2	0
	CON	2	4	65	1	16	0	0	6	8	0
Rtx430-1	CL	2	33	45	15	3	0	2	0	0	2
	CON	2	15	69	4	6	0	0	3	0	3
Rtx430-2	CL	1	54	24	2	10	0	8	0	0	2
	CON	3	4	69	8	12	0	0	1	0	6
Rtx430-3	CL	3	61	29	0	3	2	0	3	0	2
	CON	3	8	68	5	6	0	1	2	9	1
Rtx430-4	CL	1	68	26	0	4	0	2	0	0	0
	CON	2	6	70	0	7	0	3	0	9	5
Rtx430-5	CL	2	31	47	6	12	0	0	1	3	0
	CON	0	--	--	--	--	--	--	--	--	--
Overall Mean											
Rtx2356	CL	13	67	26	2	2	0	1	1	0	0
	CON	13	9	59	9	12	0	1	5	2	3
Rtx430	CL	9	49	34	5	6	0	2	1	1	1
	CON	10	8	69	4	8	0	1	2	4	4

Trt = treatment, #Pan = number of panicles (reps) per cultivar-block, CL = *Curvularia lunata*, CON = control, Alt. = *Alternaria* species, Asp. = *Aspergillus* species, Bip. = *Bipolaris* species, CH = *Curvularia harveyi*, FT = *Fusarium thapsinum*, FS = *Fusarium semitectum*, Fsp. = *Fusarium* species, Unk. = unknown fungal species.

Grain mold rating and percent recovery of CL were the only seed and plant characteristics to be significantly higher in C. *lunata* treated panicles (Table 4.2) based on the GLM analysis (Table 4.3 and Table 4.4). This shows that the C. *lunata* treated panicles were in fact infected with C. *lunata* and that it resulted in an increased grain mold rating compared to the control. None of the seed or plant characteristics was significantly correlated with grain mold rating or percent recovery of CL (Table 4.5).

There was no significant affect ($p < 0.05$) of treatment or block number or interaction between treatment x block number on grain Fe concentration (Table 4.6). There were no significant correlations between other plant parameters except for Fe concentration and grain lightness for Rtx2536 (Table 4.7). For both cultivars, there was inconsistency when comparing grain Fe concentrations for C. *lunata*-treated panicles and control panicles within a block (Figure 4.1). Grain Fe concentration varied considerably by replication (panicle) within block number with standard deviations ranging from 0.2 to 5.4 mg kg^{-1} (Table 4.2). However, the standard deviations of grain Fe concentration by digestion batch also ranged similarly from 0.28 to 6.12 mg kg^{-1} (Table 4.8). Within batch digestion variation was smaller, and standard deviations ranged from 0.55 to 3.29 mg kg^{-1} (Table 4.9). The results of the VARCOMP model for within batch variation showed different results for each cultivar (Table 4.10). For Rtx2536, variation due to panicle was only 26% while for the error factor (variation due to sub-sample and digestion procedure) was 74%. The ratios were opposite for Rtx430 (75% and 25%, respectively).

Table 4.2: Reactions of two sorghum cultivars to grain mold. Values are means with standard deviations in parentheses. Lowercase letters denote Tukey-Kramer groups resulting from the combined analysis of variance by the general linear model procedure for each cultivar.

Cultivar-Block	Trt	#Pan	GMR	FeConc	Seedwt	PltHgt	PnLngth	Light
			(1-5)	mg kg^{-1}	g	cm	cm	
Rtx2356-1	CL	2	2.5	40.1 (2.0)	2.6	40.0	10.5	0.804
	CON	3	1.5	34.9 (5.4)	2.7	43.3	10.0	0.830
Rtx2356-2	CL	3	2.0	36.7 (2.1)	3.0	43.4	10.3	0.814
	CON	2	1.0	35.6 (4.2)	2.6	--	--	0.791
Rtx2356-3	CL	3	2.0	37.8 (1.5)	2.5	43.0	10.5	0.792
	CON	3	1.2	39.7 (3.6)	2.5	43.0	10.8	0.807
Rtx2356-4	CL	3	1.8	39.2 (3.6)	2.6	43.8	11.2	0.791
	CON	3	1.7	39.0 (3.2)	2.5	43.0	9.5	0.795
Rtx2356-5	CL	2	3.5	36.1 (2.0)	2.2	44.5	10.0	0.818
	CON	2	1.5	37.0 (2.9)	2.4	44.3	11.0	0.796
Rtx430-1	CL	2	3.0	42.0 (4.3)	2.9	40.8	11.5	0.750
	CON	2	2.3	38.9 (4.0)	3.3	41.5	11.0	0.762
Rtx430-2	CL	1	3.0	46.4 (4.3)	2.5	43.5	11.0	0.769
	CON	3	2.5	41.0 (2.5)	3.2	--	--	0.768
Rtx430-3	CL	3	3.0	40.7 (2.6)	3.0	42.3	10.0	0.781
	CON	3	2.2	40.8 (4.0)	3.3	40.2	11.3	0.770
Rtx430-4	CL	1	3.0	36.4 (0.2)	2.4	38.0	10.0	0.765
	CON	2	2.0	40.0 (3.5)	1.9	38.8	10.3	0.743
Rtx430-5	CL	2	2.5	39.3 (2.8)	2.7	40.0	11.0	0.756
	CON	0	--	--	--	--	--	--
Overall Mean								
Rtx2356	CL	13	2.3a	37.9a (2.7)	2.6a	43.0a	10.5a	0.801a
	CON	13	1.4b	37.4a (4.3)	2.5a	43.4a	10.3a	0.805a
Rtx430	CL	9	2.9a	40.8a (3.9)	2.8a	41.1a	10.7a	0.765a
	CON	10	2.3b	40.3a (3.4)	3.0a	40.1a	10.9a	0.762a

Trt = treatment, #Pan = number of panicles (reps) per cultivar-block, CL = *Curvularia lunata*, CON = control, GMR = grain mold rating based on a 1 to 5 scale (Isakeit et al. 2008), FeConc = grain Fe concentration (mg kg^{-1}), Seedwt = seed weight of 100 seeds per panicle per cultivar, PltHgt = plant height measured from the soil to the top of the plant, PnLngth = panicle length measured from the first branch with racemes to the top of the panicle, Light = grain lightness.

Table 4.3: Results of the combined analysis of variance by the general linear model procedure for grain mold rating of sorghum. ** denotes significance at the alpha = 0.01 level and * at the 0.05 level.

	Grain Mold Rating									
Source	Rtx2536					Rtx430				
	DF	SS	MS	F	p	DF	SS	MS	F	p
Trt	1	6.4	6.4	12.5	0.02*	1	2.11	2.1	65.9	0.004**
BlkNm	4	2.8	0.70	7.37	0.002**	4	0.46	0.1	0.64	0.64
Trt*BlkNm	4	2	0.5	5.37	0.006**	3	0.10	0	0.18	0.91
Error	16	1.5	0.1			10	1.79	0.2		
CV%			16.79					16.58		

Trt = treatment, BlkNm = block number, DF = degrees of freedom, SS = sum of squares, MS = mean squares, F = F test, p = p values, CV = coefficient of variation.

Table 4.4: Results of the combined analysis of variance by the general linear model procedure for recovery of C. *lunata* (%) of sorghum. ** denotes significance at the alpha = 0.01 level and * at the 0.05 level.

	% Recovery C. *lunata*									
Source	Rtx2536					Rtx430				
	DF	SS	MS	F	p	DF	SS	MS	F	p
Trt	1	4301	4301	309	<0.0001**	1	1854	1854	21.63	0.02*
BlkNm	4	134	34	0.36	0.83	4	290	72	1.84	0.20
Trt*BlkNm	4	56	14	0.15	0.96	3	257	86	2.17	0.15
Error	16	1499	94			10	394	39		
CV%			54.58					46.78		

Trt = treatment, BlkNm = block number, DF = degrees of freedom, SS = sum of squares, MS = mean squares, F = F test, p = p values, CV = coefficient of variation.

Table 4.5: Correlation coefficients (r) among grain mold rating and recovery of C. *lunata* (%) with agronomic traits, grain color, and grain Fe concentration.

Variable	Rtx2536		Rtx430	
	GMR	CL	GMR	CL
Seedwt	-0.250	0.027	-0.211	-0.109
PltHgt	-0.151	-0.015	0.358	0.334
PnLngth	0.027	0.049	-0.200	-0.441
FeConc	0.229	0.129	0.303	-0.040
SeedColor				
Red	-0.094	-0.189	0.095	0.346
Green	-0.095	-0.188	0.079	0.375
Blue	-0.043	-0.110	0.194	0.376
Lightness	-0.070	-0.147	0.157	0.372

GMR = grain mold rating based on a 1 to 5 scale (Isakeit et al. 2008), CL = recovery of C. *lunata* (%), FeConc = grain Fe concentration, Seedwt = seed weight of 100 seeds per panicle per cultivar, PltHgt = plant height measured from the soil to the top of the plant, PnLngth = panicle length measured from the first branch with racemes to the top of the panicle, Lightness = grain lightness.

Table 4.6: Results of the combined analysis of variance by the general linear model procedure for Fe concentration (mg kg^{-1}) of whole grain sorghum. ** denotes significance at the alpha = 0.01 level and * at the 0.05 level.

	Fe Concentration (mg kg^{-1})									
Source	Rtx2536					Rtx430				
	DF	SS	MS	F	p	DF	SS	MS	F	p
Trt	1	7.7	7.7	0.73	0.44	1	9.1	9.1	0.92	0.41
BlkNm	4	44.1	11.0	1.41	0.28	4	48.6	12.2	1.02	0.44
Trt*BlkNm	4	41.7	10.4	1.33	0.30	3	29.8	9.9	0.84	0.50
Error	16	125.2	7.8			10	182.3	11.9		
CV%			7.82					7.76		

Trt = treatment, Blk= block number, DF = degrees of freedom, SS = sum of squares, MS = mean squares, F = F test, p = p values, CV = coefficient of variation.

Table 4.7: Correlation coefficients (r) among measured parameters. ** denotes significance at the alpha = 0.01 level and * at the 0.05 level.

Rtx2536	FeConc	Seedwt	PltHgt	PnLngth	Light
FeConc	--				
Seedwt	0.016	--			
PltHgt	-0.340	-0.264	--		
PnLngth	0.133	0.019	-0.16	--	
Light	-0.414*	0.217	0.236	-0.118	--

Rtx430	FeConc	Seedwt	PltHgt	PnLngth	Light
FeConc	--				
Seedwt	-0.375	--			
PltHgt	0.191	0.282	--		
PnLngth	0.062	0.361	-0.02	--	
Light	-0.084	0.261	0.494	-0.338	--

FeConc = grain Fe concentration, Seedwt = seed weight of 100 seeds per panicle per cultivar, PltHgt = plant height measured from the soil to the top of the plant, PnLngth = panicle length measured from the first branch with racemes to the top of the panicle, Light = grain lightness.

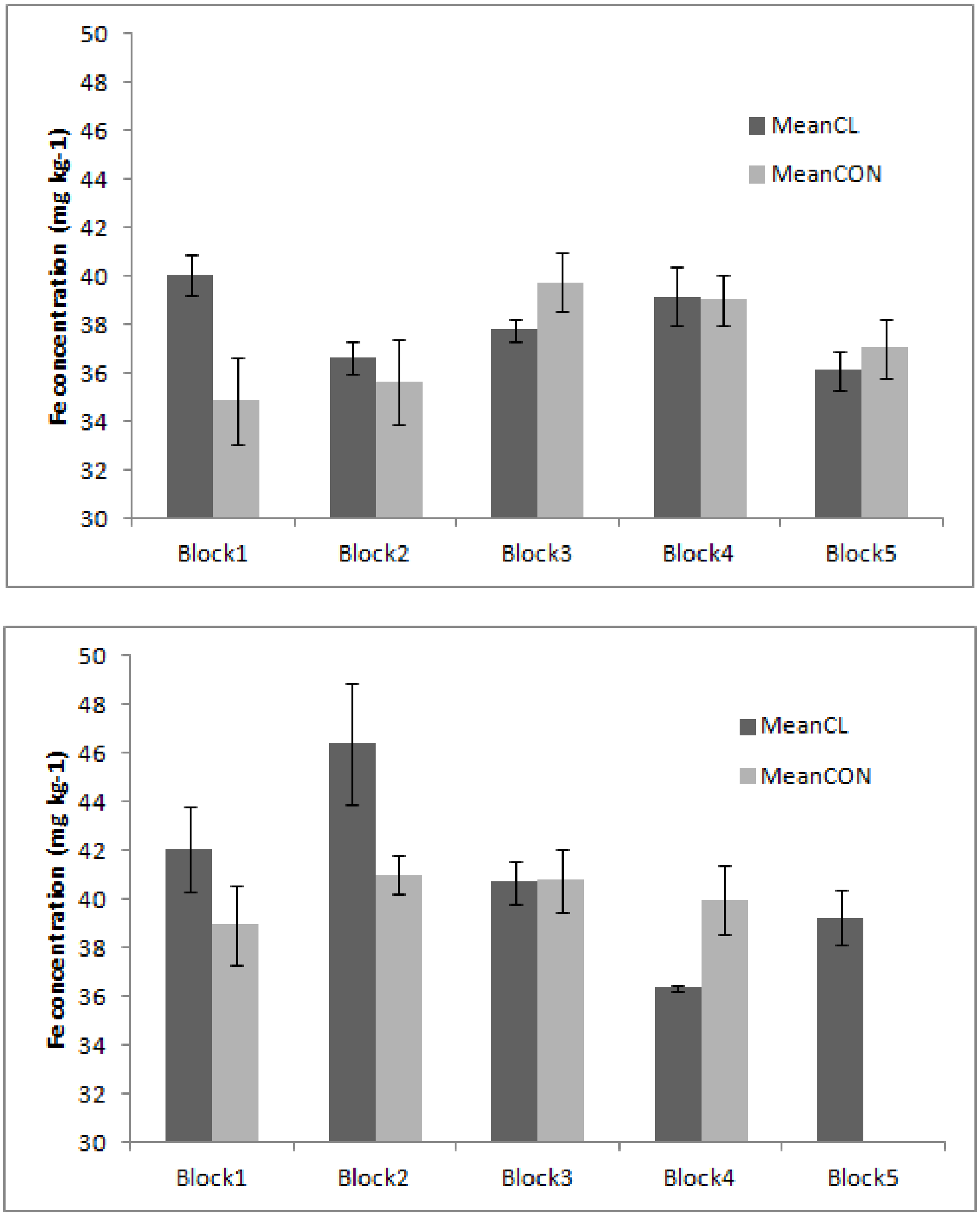


Figure 4.1: Concentration of Fe (mg kg^{-1}) in cultivar RTx2536 (upper figure) and RTx430 (lower figure) by block number and treatment. MeanCL = Fe concentration for C. *lunata* treated panicles, MeanCON = Fe concentration for control panicles. Error bars are based on standard errors.

Table 4.8: Mean, minimum (Min), maximum (Max), and standard deviation (StDev) of Fe concentrations for 3 replications (digestion batches) presented by panicle for each cultivar.

	Fe Concentration (mg kg^{-1})							
Panicle	Rtx2536				Rtx430			
	Min	Max	Mean	StDev	Min	Max	Mean	StDev
Block 1								
CL-1	40.49	41.41	40.97	0.46	42.67	47.90	45.60	2.67
CL-2	--	--	--	--	--	--	--	--
CL-3	36.16	41.47	39.18	2.73	37.97	38.79	38.45	0.43
CON-1	29.97	31.07	30.60	0.57	34.12	42.00	37.39	4.11
CON-2	40.87	43.36	41.78	1.37	36.15	43.99	40.50	3.99
CON-3	30.57	34.09	32.21	1.77	--	--	--	--
Block 2								
CL-1	33.99	37.83	36.41	2.10	41.65	50.07	46.39	4.31
CL-2	37.67	39.86	38.54	1.16	--	--	--	--
CL-3	33.56	35.76	34.95	1.21	--	--	--	--
CON-1	--	--	--	--	41.79	43.16	42.31	0.74
CON-2	32.65	33.87	33.11	0.66	38.99	41.61	40.10	1.36
CON-3	34.32	43.75	38.18	4.94	37.24	45.30	40.61	4.19
Block 3								
CL-1	35.22	39.65	38.09	2.49	38.27	44.11	42.04	3.27
CL-2	36.47	38.37	37.54	0.97	37.40	40.97	38.69	1.98
CL-3	36.88	38.88	37.68	1.06	40.29	43.50	41.38	1.84
CON-1	39.91	40.94	40.40	0.52	34.77	39.86	37.96	2.78
CON-2	35.65	42.45	38.58	3.49	38.32	46.14	41.93	3.95
CON-3	35.59	47.18	40.25	6.12	37.25	46.38	42.42	4.69
Block 4								
CL-1	37.06	43.34	40.16	3.14	36.22	36.48	36.35	0.18
CL-2	34.43	40.07	37.20	2.82	--	--	--	--
CL-3	36.06	46.14	41.92	5.23	--	--	--	--
CON-1	42.16	43.78	43.01	0.81	--	--	--	--
CON-2	39.51	41.47	40.23	1.08	37.22	41.75	39.15	2.34
CON-3	34.13	37.46	35.38	1.82	36.30	45.82	40.85	4.77

CL = C. *lunata*, CON = control.

Table 4.8: Continued.

Panicle	Rtx2536				Rtx430			
	Fe Concentration (mg kg^{-1})							
	Min	Max	Mean	StDev	Min	Max	Mean	StDev
Block 5								
CL-1	--	--	--	--	38.33	41.44	39.74	1.57
CL-2	33.56	37.16	34.80	2.05	34.79	42.95	38.77	4.08
CL-3	37.18	37.74	37.43	0.28	--	--	--	--
CON-1	33.44	36.79	34.89	1.72	--	--	--	--
CON-2	--	--	--	--	--	--	--	--
CON-3	37.15	41.54	39.16	2.22	--	--	--	--
Overall	29.97	47.18	37.67	3.55	34.12	50.07	40.56	3.63

Table 4.9: Results of the within digestion batch experiment.

Rep	Rtx2536			Rtx430		
	Fe Concentration (mg kg^{-1})					
	Pan#1	Pan#2	Pan#3	Pan#1	Pan#2	Pan#3
1	47.54	37.75	--	37.12	41.77	37.86
2	38.85	38.24	36.20	38.36	42.49	38.56
3	38.69	39.21	38.29	41.49	41.57	37.95
4	40.62	36.48	37.61	37.83	43.47	37.70
5	40.43	39.25	35.67	37.36	42.07	38.32
6	40.03	40.06	36.60	38.62	40.17	39.17
Min	38.69	36.48	35.67	37.12	40.17	37.70
Max	47.54	40.06	38.29	41.49	43.47	39.17
Mean	41.03	38.50	36.87	38.46	41.92	38.26
StDev	3.29	1.28	1.06	1.60	1.09	0.55
S/N	12.47	30.08	34.78	24.20	38.39	70.07

Rep = replication of sample, Pan# = panicle number, Min = minimum, Max = maximum, StDev = standard deviation, S/N = mean to standard deviation ratio.

Table 4.10: Results of the combined analysis of variance by the variance, component procedure (VARCOMP) for Fe concentration (mg kg^{-1}) of whole grain sorghum.

		Fe Concentration (mg kg^{-1})			
Source	DF	Rtx2536		Rtx430	
		Varcomp	%Total	Varcomp	%Total
PanNum	2	1.89	25.68	4.01	74.95
Error	15	5.47	74.32	1.34	25.05

PanNum = panicle number.

Discussion

Recent studies in College Station, TX conducted on sorghum accessions inoculated with C. *lunata* showed that percent recovery of C. *lunata* was not always high (Prom et al 2011). In the 2005 growing season, 61% of C. *lunata* was recovered on C. *lunata*-treated accessions while in 2006, just 13% was recovered. For controls, 38% of Alternaria species were recovered in 2005 and 25% in 2006. In a study conducted in 2000 and 2001 in College Station, TX, Rtx2536 and Rtx430 inoculated with C. *lunata* had a percent recovery of 85% and 68%, respectively in 2000 and 59% and 57% in 2001 (Prom 2004). Alternaria species were recovered in the controls at 21% in 2000 and 13% and 27%, respectively in 2001. The percent recovery for C. *lunata* were slightly lower in this study (67% for Rtx2536 and 49% for Rtx430) and may be explained by a small rain event (8.1 mm) that occurred after inoculation in mid-July ensuring adequate C. *lunata* mold formation in the mold susceptible cultivars RTx2536 and RTx430. While the 2011 summer growing season in southeast Texas was one of the hottest and driest on record, the rain event ensured mold formation of the C. *lunata* but not on the mold resistant cultivars which were earlier maturing and were also inoculated before the small rain event.

Grain molding and grain weathering are terms that more properly distinguish the timing of mold attack within a field. It must be noted that the analysis of traits (i.e. Fe concentration, seed weight, etc.) by treatment (C. *lunata* vs. control) was a comparison of the effect of early grain molding with post maturity grain weathering. Inoculating with C. *lunata* at half-bloom resulted in true "grain molding" as infection occurred before the physiological maturity of the grain and resulted in higher grain mold ratings than controls. In the case of the controls, the high percent recovery of species such as Alternaria, Bipolaris, and Aspergillus did not result in high grain mold ratings, indicating that the physiological mature grain was more likely infected due to hot or humid conditions during harvest. This is termed "grain weathering", and the subsequent infection is thought to be limited to the pericarp portion (Bandyopadhyay 2000). It is also likely that any remobilization of Fe to the infected seed would occur in the period before physiological maturity during the grain filling stage of seed formation and that post maturity grain weathering would not affect grain Fe concentrations.

Grain size can be reduced when early infection of the grain interferes with grain filling (Frederiksen et al. 1982) or causes a premature formation of the black layer (Castor 1981). There was no significant correlation between seed weight and grain mold rating or percent recovery of C. *lunata* for Rtx2536 or RTx430. C. *lunata* in particular has been noted to be restricted from further colonization of the endosperm by the peripheral endosperm cells unlike *Fusarium* species which appear to not be restricted

(Castor 1981; Prom 2004). With the endosperm unaffected by C. *lunata*, the seed weight would correspondingly remain unaffected. In my study, field inoculation by *Fusarium thapsinum* was also attempted, but percent recovery of F. *thapsinum* was very low, indicating that the slower growing fungi failed to infect under the dry conditions experienced during the summer of 2011.

In any field study, there is always the possibility of confounding factors that mask the effect of the treatment applied. Fe was highly variable by panicle with none of the measured parameters explaining this Fe variation. Cultivars RTx2536 and RTx430 often grow with two or three tillers, leading to some panicles joined to the same stalk base while other panicles are single with no tillers. A greenhouse study where sorghum can be grown under more controlled conditions with one tiller per pot may lead to less variation per panicle and perhaps a better comparison of molded and non-molded panicles for Fe.

The appreciable difference in Fe concentrations between batches (up to 10 mg kg^{-1}) either shows a poor repeatability of the Fe digestion procedure or that sub-samples of grain were highly variable. Regarding the digestion procedure, it did not appear that performing the digestion with unfiltered fume hood air (Batches 1 and 2) versus vacuum filtered air (Batch 3) had any significant affect on Fe concentrations. Plus, it is not expected that differences in AAS performance would account for this variability given that calibrations were conducted every 5-6 samples to account for instrument drift.

Grain position studies may elucidate why Fe concentrations varied within subsamples from the same grounded sample. A study on grain position affects on micronutrient content of wheat showed that Fe concentrations of seeds from distally versus proximally located on a spikelet can vary from 37 to 44 mg kg^{-1} and that spikelets positioned basally versus apically differed from 40 to 44 mg kg^{-1} (Calderini and Ortiz-Montaserio 2003). It is not known why one particular cultivar had a larger amount of panicle variation versus sub-sample variation than the other; nonetheless, a sub-sample variation as high as 74% seems to indicate that whole grain sorghum is variable in Fe concentration even perhaps down to the individual seed. Also, variation of Fe in seeds from the same panicle may, as hypothesized, stem from differences in mold that can occur within the same panicle. Percent recovery of C. *lunata* in this study was 49% (RTx430) and 67% (RTx2536), meaning that the majority, but not all seed actually had mold from the treatment.

If Fe did indeed vary by seed position and presence of mold, it should not be a problem if random seeds are chosen from all positions on the panicle, and if during grinding, seed and seed parts such as the pericarp were uniformly mixed. It is possible though that grinding to 1 mm leaves larger pieces of the pericarp (which would contain relatively more Fe than the endosperm portion), and that scooping up a 1 g sub-sample may result in a selection of a non-uniform mix of pericarps with more or less Fe resulting in more or less Fe in each sub-sample. As care was taken during harvesting and threshing for seed to not come into contact with metal, any potential sample contamination is less likely perhaps except for the steel Udy Mill. Therefore, a future study investigating the possible effect of the Udy Mill grinding on sub-sample variation in Fe is recommended. A further study utilizing a mortar and pestle to homogenize the grain further is also recommended. The results of this study would be helpful in determining how many sub-samples would be necessary to accurate determine the “true” Fe concentration in the grain, thus avoiding any under or overestimation.

Conclusions

Grain mold was shown to be unrelated to Fe concentrations in sorghum seed and inoculated grain did not have significantly higher Fe than the control grain. However, high variation in Fe occurred by panicle, between digestion batches, and within digestion batch and ground grain sub-samples. Further studies may be able to reduce panicle Fe variance with greenhouse pot studies, and additional work involving sub-sampling may elicit reasons for grain Fe variation within a panicle. It is hoped that these additional studies could improve the precision in measuring grain Fe so that further mold treatments could be tested and its effect on Fe could be known. It is important for this variation in Fe within sorghum and other crops to be understood as plant breeders go forward in their attempts to breed for high Fe in staple crops and alleviate the Fe deficiency and anemia problem so prevalent in the population of poor countries.

CHAPTER V

CONCLUSIONS

The objective of this study was to investigate soil and mold influences that affect Fe and Zn uptake and accumulation in sorghum grain. The 2009 sorghum variety trial showed soils with DTPA-extractable Zn deficiencies and high variability of DTPA-extractable Zn concentrations with a poor relationship with grain Zn concentrations. For sorghum grown under low organic carbon, grain Zn concentrations appeared to be more related to water drainage conditions and poor soil properties such as low pH and high exchangeable aluminum. The results of the 2010 sorghum study found that high DTPA-extractable Zn concentrations at 0-15 cm depth were located under the canopy of Shea trees in soils derived from mafic, high Zn-content rock with high pH and organic carbon. These high DTPA-extractable Zn concentration soils, however, did not affect sorghum grain Zn concentration. Overall, the relationship between soil DTPA-extractable Zn and grain Zn concentrations was not significant. However in mafic-residuum soils, pH and DTPA-extractable Zn concentration were significantly and positively correlated with grain Zn concentration, and in nonmafic soils DTPA-extractable Fe and grain concentrations were significantly correlated. In regard to grain Fe concentrations, the inoculation study yielded no significance of C. *lunata* inoculation on grain Fe concentration or grain mold rating, but instead showed a relatively high Fe variance by panicle, digestion batch, and grain subsample.

There are several important implications of the DTPA-extractable Zn and grain Zn concentration studies conducted in southern Mali. First, DTPA-extractable Zn concentration can be useful for differentiating fields that are deficient in Zn, and it appears that many of the soils sampled were Zn deficient because of relatively low Zn parent material, high weathering, and low organic carbon. Second, it can also be useful to measure simple soil properties such as pH and organic carbon and to observe soil parent material as these measurements and observations can place a field under: 1) the low organic carbon and pH, highly weathered, high Zn solubility regime with Zn deficiency; or, 2) the high organic carbon and pH dominated regime with abundant available Zn. Breeders can then test varieties that are efficient or responsive depending upon their goal for biofortification purposes.

Further research is recommended for the study of grain Zn concentration in groundnut grown in mafic soils in Shea tree fields, specifically to test whether these crops are taking advantage of the high DTPA-extractable Zn concentrations observed in these soils. Additionally, it would be of interest to know the location and extent of Zn deficient soils throughout western and southern Mali and to confirm whether Shea trees are unable to prevent Zn deficiency in intensively cropped nonmafic soils. It must be kept in mind for these future studies, that DTPA-extractable Zn concentrations can be highly variable over short distances and that the presence of biochar can anomalously increase DTPA-extractable Zn concentrations.

The results of the inoculation study, although not significant, leave many unanswered questions regarding the source of variation in grain Fe concentrations; and it is important from a biofortification standpoint that some of these questions be addressed with additional work. It is recommended that further inoculation studies in pots under greenhouse conditions with more panicle replications and grain subsampling be conducted so that variance can be reduced and the mold effect ascertained.

REFERENCES

Agbenin JO (2003a) The distribution and transformation of iron and manganese in soil fractions in a savanna Alfisol under continuous cultivation. Nutr Cycl Agroecosys 66:259-270

Agbenin JO (2003b) Zinc fractions and solubility in a tropical semi-arid soil under long-term cultivation. Biol Fertil Soils 37:83-89

Alloway BJ (2008) Zinc in Soils and Crop Nutrition. IZA Publications. International Zinc Association, Brussels, Belgium.

Anand RR, Gilkes RJ (1987) Iron oxides in lateritic soils from Western Australia. J Soil Sci 38:607-622

Anand RR, Cornelius M, Phang C (2007) Use of vegetation and soil in mineral exploration in areas of transported overburden, Yilgarn Craton, Western Australia: a contribution towards understanding metal transportation processes. Geochemistry: Exploration, Environment, Analysis 7:267-288

Balesdent J, Mariotti A, (1996) Measurement of soil organic matter turnover using ^{13}C natural abundance. In: Boutton TW, Tamasaki SI (eds) Mass spectrometry of soils. Marcel Dekker, New York, NY, pp 83-112

Bandyopadhyay R, Butler DR, Chandrashhekar A, Reddy RK, Navi SS (2000) Biology, epidemiology, and management of sorghum grain mold. In: Chandrashekar A et al (eds) Technical and institutional options for sorghum grain mold management: proceedings of an international consultation, 18-19 May 2000, ICRISAT. International Crops Research Institute for the Semi-Arid Tropics, Patancheru, India, pp 34-71

Barikmo I, Ouattara F, Oshaug A (2007) Differences in micronutrients content found in cereals from various parts of Mali. J Food Compos Anal 20:681- 687

Barrow NJ (1993) Mechanisms of reaction of zinc with soil and soil components. In: Zinc in Soil and Plants. Robson AD (ed) Kluwer Academic Publishers, Dordrecht, the Netherlands, pp. 15-32

Bayala J, Mando A, Teklehaimanot Z, Ouedraogo SJ (2005) Nutrient release from decomposing leaf mulches of karite (*Vitellaria paradoxa*) and nere (*Parkia biglobosa*) under semi-arid conditions in Burkina Faso, West Africa. Soil Biol Biochem 37:533-539

Bazie HR, Bayala J, Zombre G, Sanou J, Ilstedt U (2012) Separating competition-related factors limiting crop performance in an agroforestry parkland system in Burkina Faso. Agroforest Syst 84:377-388

Beauvais A, Ritz M, Parisot JC, Dukhan M, Bantsimba C (1999) Analysis of poorly stratified lateritic terrains overlying a granitic bedrock in West Africa, using 2-D electrical resistivity tomography. Earth Planet Sci Lett 173:413-424

Behera SK, Singh MV, Singh KN, Todwal S (2011) Distribution variability of total and extractable zinc in cultivated acid soils of India and their relationship with some selected soil properties. Geoderma 162:242-250

Belum VS, Reddy BVS, Ramesh S, Longvah T (2005) Prospects of breeding for micronutrients and beta-carotene-dense sorghum. Journal of SAT Agricultural Research 1:1-4

Bigham JM, Fitzpatrick RW, Schulze DG (2002) Iron oxides. In: Soil Mineralogy with Environmental Applications. Dixon JB, Schulze DG (eds) SSSA Book Series no. 7. Soil Science Society of America, Madison, WI, pp 323-366

Boffa JM (1999) Socio-economic benefits of parklands. Agroforestry Parklands in Sub-Saharan Africa, FAO Conservation Guide 34, Food and Agriculture Organization of the United Nations. Rome, Italy, pp 172

Boffa JM, Taonda S, Dickey J, Knudson D (2000) Field-scale influence of karité (*Vitellaria paradoxa*) on sorghum production in the Sudan zone of Burkina Faso. Agroforest Syst 49:153-175

Bouis HE, Welch RM (2010) Biofortification-A sustainable agricultural strategy for reducing micronutrient malnutrition in the Global South. Crop Sci 50:S-20-S-32

Boutton TW (1991) Stable carbon isotope ratios of natural materials II: atmospheric, terrestrial, marine and freshwater environments. In: Coleman DB, Fry B (eds) Carbon Isotope Techniques, Academic Press, San Diego, CA, pp 173-186

Breman H, Kessler JJ (1995) Woody plants in agro-ecosystems of semi-arid regions, with an emphasis on the Sahelian countries. Springer Verlag, Berlin, Germany, pp 340

Briat JF (2008) Iron dynamics in plants. In: Kader JC, Delseny M (eds) Advances in Botanical Research v.46 Incorporating Advances in Plant Pathology, Academic Press, London, England, pp 137-180

Bruck H, Piro B, Sattelmacher B, Payne WA (2003a) Varietal differences in shoot and rooting parameters of pearl millet in sandy soils in Niger. Plant Soil 251:175-185

Bruck H, Piro B, Sattelmacher B, Payne WA (2003b) Spatial distribution of roots of pearl millet on sandy soils of Niger. Plant Soil 256:149-159

Buri MM, Masunaga T, Wakatsuki T (2000) Sulfur and zinc levels as limiting factors to rice production in West African lowlands. Geoderma 94:23-42

Cakmak I (2008) Enrichment of cereal grains with zinc: Agronomic or genetic biofortification? Plant Soil 302:1-17

Calderini DF, Ortiz-Montaserio I (2003) Grain position affects grain macronutrient and micronutrient concentration in wheat. Crop Sci 43:141-151

Castor LL (1981) Grain mold histopathology, damage assessment and resistance screening within *Sorghum bicolor* (L.) Moench lines. Ph.D. Dissertation. Texas A&M University, USA

Chervenka G (2002) Soil Survey of Brazos County, Texas. National Cooperative Soil Survey, United States Department of Agriculture, Washington, D.C.

Climate Temperature Information (2012) 2008-2011 climatetemp.info. http://www.climatetemp.info/mali/bamako.html. Accessed 30 2012

Cockell K (2007) An overview of methods for assessment of iron bioavailability from foods nutritionally enhanced through biotechnology. Journal of AOAC International 90:1480-1491

Cornejo P, Meier S, Borie G, Rillig MC, Borie F (2008) Glomalin-related soil protein in a Mediterranean ecosystem affected by a copper smelter and its contribution to Cu and Zn sequestration. Sci Total Environ 406:154-160

Cornell RM, Schwertmann U (1996) The Iron Oxides. VCH Publishers, Weinheim, Germany

de la Fuente V, Rodriguez N, Amils R (2011) Immunocytochemical analysis of the subcellular distribution of ferritin in Imperata cylindrica (L.) Raeuschel, an iron hyperaccumulator plant. Acta Histochem 114:232-236

Dianda M, Bayala J, Diop T, Ouedraogo SJ (2009) Improving growth of shea butter tree (*Vitellaria paradoxa* C.F. Gaertn.) seedlings using mineral N, P and arbuscular mycorrhizal (AM) fungi. Biotechnol Agron Soc Environ 13:93-120

Dars R (1962) Les Formations Sedimentaires et Les Dolerites du Soudan Occidental (Afrique de L'Ouest). Memoires du Bureau de Recherches Geologieques et Minieres No. 12. Editions Technip, Paris, France

do Nascimento CWA, de Oliveira AB, Ribeiro MR, de Melo ECC (2006) Distribution and availability of zinc and copper in benchmark soils of Pernambuco State, Brazil. Commun Soil Sci Plant Anal 37:109-125

do Nascimento CWA, de Melo EEC, do Nascimento RSMP, Leite PVV (2007) Effect of liming on the plant availability and distribution of zinc and copper among soil fractions. Commun Soil Sci Plant Anal 38:545-560

Doumbia MD, Hossner LR, Onken AB (1993) Variable sorghum growth in acid soils of subhumid West Africa. Arid Soil Res Rehab7:335-346

Esty JC, Onken AB, Hossner LR, Matheson R (1980) Iron use efficiency in grain sorghum hybrids and parental lines. Agron J 72:589-592

Fageria NK, Baligar VC (2008) Ameliorating soil acidity of tropical oxisols by liming for sustainable crop production. In: Sparks DL (ed) Advances in Agronomy, v. 99, Academic Press, San Diego, CA pp 345-399

Fehr WR (1991) Principles of Cultivar Development. v. 1 Theory and Technique. Macmillan Publishing Co., New York, NY

Feng J (2005) Plant root responses to three abundant soil minerals: silicon, aluminum and iron. Crit Rev Plant Sci 24:267-281

Feybesse JL (2006) Bamako Ouest Carte Geochimique Du Zinc. Ministere des Mines de L'Energie et de L'Eau, Republic of Mali, Bamako, Mali

Frederiksen RA, Castor LL, Rosenow DT (1982) Grain mold, small seed and head blight: the *Fusarium* connection in sorghum. Proceedings of the thirty-seventh annual corn and sorghum industry research conference 37:26-36

Frederiksen RA, Odvody GN (2000) Compendium of Sorghum Diseases. The American Phytopathological Society, St. Paul, MN

Galan E (2006) Genesis of clay minerals. In: Bergaya F et al (eds) Developments in Clay Science. v. 1 Elsevier, Amsterdam, the Netherlands, pp 1129-1162

Gårdestedt C, Plea M, Nilsson G, Jacks B, Jacks G (2009) Zinc in soils, crops, and meals in the Niger inland delta, Mali. AMBIO: A Journal of the Human Environment 38:334-338

Gijsbers HJM, Kessler JJ, Knevel MK (1993) Dynamics and natural regeneration of woody species in farmed parklands in the Sahel region (Province of Passore, Burkina Faso). Forest Ecol Manag 64:1-12

Gomez-Becerra HF, Erdem H, Yazici A, Tutus Y, Torun B, Ozturk L, Cakmak I (2010) Grain concentrations of protein and mineral nutrients in a large collection of spelt wheat grown under different environments. J Cereal Sci 52:342-349

Hall JB, Aebischer DR, Tomlinson HF, Osei-Amaning E, Hindle JR (1996) Vitellaria paradoxa: a monograph. Chapter 2-The species-systematics and description. School of Agricultural and Forest Sciences. Univesity of Wales Bangor, UK pp 105

Handley LL, Azcon R, Lozano JMR, Scrimgeour CM (1999) Plant δ^{15}N associated with arbuscular mycorrhization, drought and nitrogen deficiency. Rapid Commun Mass Spectrom 13:1320-1324

Hobbie EA, Ouimette AP (2009) Controls of nitrogen isotope patterns in soil profiles. Biogeochemistry 95:355-371

Hotz. C, Brown K (2004) International Zinc Nutrition Consultative Group (IziNCG) Technical document #1. Assessment of the risk of zinc deficiency in populations and options for its control. Food Nutr Bull 25:S91-S203

Iskeit T, Collins SD, Rooney WL, Prom LK (2008) Reaction of sorghum hybrids to anthracnose, grain mold and grain weathering in Burleson County, Texas, 2007. Plant Management Network www.plantmanagementnetwork.org/pub/trial/PDMR/reports/2008/FC003.pdf. Accessed 28 October 2011

Jiang Y, Zhang YG, Zhou D, Qin Y, Liang WJ (2009) Profile distribution of micronutrients in an aquic brown soil as affected by land use. Plant Soil Environ 55:468-476

Jonnson K, Ong CK, Odongo JCW (1999) Influence of scattered nere and karite trees on microclimate, soil fertility and millet yield in Burkian Faso. Exp Agric 35:39-53

Joshi AK, Crossa J, Arun B, Chand R, Trethowan M, Vargas M, Ortiz-Monasterio I (2010) Genotype x environment interaction for zinc and iron concentration of wheat grain in eastern Gangetic plains of India. Field Crops Res 116:268-277

Kayode APP, Linnemann AR, Hounhouigan JD, Nout MJR, van Boekel MAJS (2006) Genetic and environmental impact on iron, zinc, and phytate in food sorghum grown in Benin. J Agric Food Chem 54:256-262

Keita B (2000) Les Sols du Dominants du Mali. Quatorzieme reunion du Sous-Comite et centre africain de correlation des sols pour la mise en valuer des terres. Food and Agriculture Organization Repository. http://www.fao.org/docrep/005/Y3948F/y3948f00.HTM. Accessed 13 May 2009

Kerkeb L, Connolly E (2006) Iron transport and metabolism in plants. In: Setlow JK (ed) Genetic Engineering Principles and Methods v. 27, Springer Science and Business Media, New York, NY, pp 119-140

Kiekens L (1995) Zinc. In: Alloway BJ (ed) Heavy Metals in Soils (2nd ed), Blackie Academic and Professional, London, England, pp 284-305

Krauskopf KB (1967) Introduction to Geochemistry. McGraw-Hill, New York, NY

Kumar AA, Reddy BVS, Ramaiah B, Reddy PS, Sahrawat KL, Upadhyaya HD (2009) Genetic variability and plant character association of grain Fe and Zn in selected core collection accessions of sorghum germplasm and breeding lines. Journal of SAT Agricultural Research 7:1-4

Kumar AA, Reddy BVS, Sahrawat KL, Ramaiah B (2010) Combating micronutrient malnutrition: identification of commercial sorghum cultivars with high grain iron and zinc. Journal of SAT Agricultural Research 8:1-5

Lindsay WL, Norvell WA (1978) Development of DTPA Soil test for zinc, iron, manganese, and copper1. Soil Sci Soc Am J 42:421-428

Liu G, Greenshields DL, Sammynaiken R, Hirji RN, Selvaraj G, Wei Y (2007) Targeted alterations in iron homeostasis underlie plant defense responses. J Cell Sci 120:596-605

Loeppert RH, Inskeep WP (1996) Iron. In: Sparks DL et al (eds) Methods of Soil Analysis. Part 3 – Chemical methods. SSSA Book Series no.5. Soil Science Society of America, Inc, Madison, WI, pp 639-664

Madeira J, Bedidi A, Cervelle B (1997) Visible spectrometric indices of hematite (Hm) and goethite (Gt) in lateritic soil: the application of a Thematic Mapper (TM) image for soil-mapping in Brasilia, Brazil. Int J Remote Sens 18:2835-2852

Mishra SP, Chaudhury G (1994) Kinetics of zinc adsorption on charcoal. J Chem Technol Biot 59:359-364

Murovhi RN, Materechera SA (2006) Nutrient cycling by Acacia erioloba (syn. Acacia giraffae) in smallholder agroforestry practices of a semi-arid environment in the North West Province, South Africa. Southern African Forestry Journal 208:1, 23-30

Namgay T, Singh B, Singh BP (2010) Influence of biochar application to soil on the availability of As, Cd, Cu, Pb, and Zn to maize (*Zea mays* L.). Aust J Soil Res 48:638-647

Navi SS, Bandyopadhyay R, Hall AJ, Bramel-Cox PJ (1999) A Pictorial Guide for the Identification of Mold Fungi on Sorghum Grain. Information Bulletin no. 59. International Crops Research Institute for the Semi-Arid Tropics, Patancheru, India

Olembo KN, M'mboyi F, Kiplagat S, Sitieney JK, Oyugi FK (2010) Sorghum Breeding in Sub-Saharan Africa: The Success Stories. African Biotechnolgy Stakeholders Forum, Nairobi, Kenya

Palmgren MG, Clemens S, Williams LE, Kramer U, Borg S, Schjorring JK, Sanders D (2008) Zinc biofortification of cereals: problems and solutions. Trends Plant Sci 13:464-473

Peterson GC, Onken AB (1992) Relationship between chlorophyll concentration and iron chlorosis in grain sorghum. Crop Sci 32:964-967

Pfeiffer WH, McClafferty B (2007) HarvestPlus: Breeding crops for better nutrition. Crop Sci 47: S-88-S-105

Pfeiffer WH, McClafferty B (2008) Biofortification: breeding micronutrient-dense crops. In: Kang MS, Priyadarshan P (eds) Breeding Major Food Staples. Blackwell Publishing Ltd., Oxford, UK, pp 61-91

Picouet C, Dupre B, Orange D, Valladon M (2001) Major and trace element geochemistry in the upper Niger river (Mali): physical and chemical weathering rates and CO_2 consumption. Chem Geol 185:93-124

PIRT-Projet d'inventaire des ressources terrestres (1983) Zonage agroecologique du Mali, Ministere de l'Elevage et de l'Environnment, Bamko, Mali.

Prom LK, Waniska RD, Kollo AI, Rooney WL (2003) Response of eight sorghum cultivars inoculated with *Fusarium thapsinum*, *Curvularia lunata* and a mixture of the two fungi. Crop Prot 22:623-628

Prom LK (2004) The effect of *Fusarium thapsinum*, *Curvularia lunata* and their combination on sorghum germination and seed mycoflora. Journal New Seeds 6:39-49

Prom LK, Isakeit T, Peruma, R, Erpelding JE, Rooney WL, Magill CW (2011) Evaluation of the Ugandan sorghum accessions for grain mold and anthracnose resistance. Crop Prot 30:566-571

Ratnadass A, Butler DR, Marley PS, Ajayi O, Bandyopadyhay R, Hamada MA, Hess DE, Assamoi F, Atokple IDK, Beyo J, Cisse O, Daouo D, Diakite M, Dossou-Yovo S, Le Diambo B, Sissoko I, Vopeyande MB, Akintayo I (1999) Final report of the West and Central Africa Sorghum Research Network (WCASRN) 1996 and 1997 regional sorghum head bug and grain mold trial. Presented at the West African sorghum research network meeting, 8-12 March 1999, Lome, Togo

Reddy PS, Reddy BVS, Kumar AA, Ramesh S, Sahrawat KL, Rao PV (2010) Association of grain Fe and Zn contents with agronomic traits in sorghum. Indian J Plant Genet Resour 23:280-284

Reid-Soukop DA, Ulery AL (2002) Smectites. In: Soil Mineralogy with Environmental Applications. Dixon JB, Schulze DG (eds) SSSA Book Series no. 7. Soil Science Society of America, Madison, WI, pp 467-495

Rengel Z (2007) Cycling of micronutrients in terrestrial ecosystems. In: Marschner P, Rengel Z (eds) Soil Biology v. 10 Nutrient Cycling in Terrestrial Ecosystems. Springer-Verlag, Berlin, Germany, pp 93-120

Robin A, Vansuyt G, Hinsinger P, Meyer JM, Briat JF, Lemanceau P (2008) Iron dynamics in the rhizosphere: consequences for plant health and nutrition. In: Sparks DL (ed) Advances in Agronomy, v. 99, Academic Press, San Diego, CA, pp 183-225

Saiz G, Bird MI, Domingues T, Schrodt F, Schwarz M, Feldpausch TR, Veenendaal E, Djagbletey G, Hien F, Compaore H, Diallo A, Lloyd J (2012) Variation in soil carbon stocks and their determinants across a precipitation gradient in West Africa. Glob Change Biol 18:1670-1683

Sanou H, Picard N, Lovett PN, Dembele M, Korbo A, Diarisso D, Bouvet JM (2006) Phenotypic variation of agromorphological traits of shea tree, *Vitellaria paradoxa* C.. Gaertn., in Mali. Genet Resour Crop Ev 53:145-161

Scheinost AC, Schwertmann U (1999) Color identification of iron oxides and hydroxysulfates: use and limitations. Soil Sci Soc Am J 63:1463-1471

Scholes M, Andreae MO (2000) Biogenic and pyrogenic emissions from Africa and their impact on the global atmosphere. AMBIO: A Journal of the Human Environment 29:23-29

Scott KM, Pain CF (2008) Regolith Science. CSIRO Publishing, Collingwood, Australia

Sidhu GS, Sharma BD (2005) Diethylenetriaminepentaacetic acid-extractable micronutrients status in soil under a rice-wheat system and their relationship with soil properties in different agroclimatic zones of Indo-Gangetic Plains of India. Commun Soil Sci Plant Anal 41:29-51

Singh B, Kumar S, Natesan A, Singh BK, Usha K (2005) Improving zinc efficiency of cereals under zinc deficiency. Curr Sci 88:36-44

Slingerland MA, Traore K, Kayode APP, Mitchikpe CES (2006) Fighting Fe deficiency malnutrition in West Africa: an interdisciplinary programme on a food chain approach. NJAS Wageningen Journal of Life Sciences 53:253-279

Soumare M, Demeyer A, Tack FMG, Verloo MG (2002) Nutrient availability in the surface horizons of four tropical agricultural soils in Mali. Tropicultura 20:58-63

Stangoulis J (2010) Technical aspects of zinc and iron analysis in biofortification of the staple food crops, wheat and rice. 19th World Congress of Soil Science, Soil Solutions for a Changing world, 1-6 August 2010, Brisbane, Australia

Stangoulis J, Sison C (2008) Crop Sampling Protocols for Micronutrient Analysis. HarvestPlus Technical Monograph Series 7. http://www.agrosalud.org/descargas/completo_ingles.pdf. Accessed 10 Feb 2012

Stein AJ (2010) Global impacts of human mineral nutrition. Plant Soil 335:133-154

Sumner M, Yamada T (2002) Farming with acidity. Commun Soil Sci Plant Anal 33:2467-2496

Takkar PN, Chibba IM, Mehta SK (1989) Twenty years of coordinated research on micronutrients in soil and plants (1967-1987). ISSS Bulletin 1, Indian Institute of Soil Science, pp 314

Tardy Y (1997) Petrology of Laterites and Tropical Soils. A.A. Balkema Publishers, Rotterdam, the Netherlands, pp 177

Takimoto A, Nair PKR, Nair VD (2008) Carbon stock and sequestration potential of traditional and improved agroforestry systems in the West African Sahel. Agr Ecosyst Environ 125:159-166

Thakur RP, Reddy BVS, Indira S, Rao VP, Navi SS, Yang XB, Ramesh S (2006) Sorghum Grain Mold. Information Bulletin No. 72. International Crops Research Institute for the Semi-Arid Tropics, Patancheru, India, pp 32

Traore K, Ganry G, Oliver R, Gigou J (2004) Litter production and soil fertility in a *Vitellaria paradoxa* parkland in a catena in Southern Mali. Arid Land Research and Management 18:359-368

Traore K (2006) Effects of soil amendments and drought on zinc husbandry and grain quality in Sahelian sorghum. PhD Dissertation. University of Wageningen, the Netherlands

Thien SJ (1979) A flow diagram for teaching texture by feel analysis. Journal of Agronomic Education 8:54-55

Tuinsma M, Nyirandutiye D, Dembele AM, Berthe Z, Rattunde F, Smit M, Sidibe CS (2008) An Be Jigi: use of local diversity for enhancing nutrition (especially Fe/Zn bioavailability) in sorghum/pearl millet consuming communities. Annual Report March 2007-February 2008. The McKnight Foundation, Helen Keller International, International Crops Research Institute for the Semi-Arid Tropics, Institut d'Economie Rurale du Mali. Bamako, Mali

Tuinsma M, Dembele AM, Berthe Z, Rattunde F, Smit M, Sidibe CS, Cisse DF (2009) An Be Jigi: Use of local diversity for enhancing nutrition (especially Fe/Zn bioavailability) in sorghum/millet consuming communities. Annual Report March 2008-February 2009. The McKnight Foundation, Helen Keller International, International Crops Research Institute for the Semi-Arid Tropics, Institut d'Economie Rurale du Mali. Bamako, Mali

UW-Madison (2005) Analysis of major, minor, and trace elements in plant tissue samples with ICP- OES and ICP-MS, Standard Operation Procedure, Soil and Plant Analysis Laboratory. University of Wisconsin-Madison http://uwlab.soils.wisc.edu/lab-procedures. Accessed 15 October 2011

Vandenbeldt RJ, Williams JH (1992) The effect of soil surface temperature on the growth of millet in relation to the effect of *Faidherbia albida* trees. Agr Forest Meteorol 60:93-100

Vasconcelos M, Grusak MA (2006) Status and future developments involving plant iron in animal and nutrition. In: Barton LL, Abadia (eds) Iron Nutrition in Plants and Rhizospheric Microorganisms. Springer, Amsterdam, the Netherlands, pp 1-22

Vetaas OR (1992) Micro-site effects of trees and shrubs in dry savannas. J Veg Sci 3:337-344

Viscarra Rossel RA, Minasny B, Roudier P, McBratney AB (2006) Colour space models for soil science. Geoderma 133:320-337

Wang L, Wu JP, Liu YX, Huang HQ, Fang QF (2009) Spatial variability of micronutrients in rice grain and paddy soil. Pedosphere 19:748-755

Wedepohl KH (1978) Handbook of Geochemistry. Springer-Verlag, New York, NY

Welch RM (2008) Linkages between trace elements in food crops and human health. In: Alloway BJ (ed) Micronutrient Deficiencies in Global Crop Production. Springer Science and Businesss Media, New York, NY, pp 287-309

Wissuwa M, Ismail A, Graham R (2008) Rice grain zinc concentrations as affected by genotype, native soil-zinc availability, and zinc fertilization. Plant Soil 306:37-48

Wong MTF, Nortcliff S, Swift RS (1998) Method for determining the acid ameliorating capacity of plant residue compost, urban waste compost, farmyard manure, and peat applied to tropical soils. Commun Soil Sci Plant Anal 29:2927-2937

World Health Organization/Food and Agriculture Organization (2004) Vitamin and mineral requirements in human nutrition. Report of a Joint FAO/WHO Expert Consultation, Geneva, Switzerland.

World Health Organization. 2008. Worldwide Prevalence of Anaemia 1993-2005 WHO Global Database on Anaemia. de Benoist B et al (eds) WHO Press, Geneva, Switzerland

Wu J, Norvell WA, Welch RM (2006) Kriging on highly skewed data for DTPA extractable soil Zn with auxiliary information for pH and organic carbon. Geoderma 134:187-199

APPENDIX A

CHAPTER II DATA

Appendix A1: Data from soil property analysis by location, field, and rep for depth 0-15 cm. KEN = Keniero, TIG = Tiguere, WAC = Wacoro, FS = Field Station, pH_{H2O} = soil pH in water, pH_{KCl} = soil pH in KCl, ExchAl = exchangeable Al, OC = organic carbon, TotN = total nitrogen, WEP = water extractable phosphorus, Conc = % concretions, DTPA = diethylenetriaminepentaacetic acid.

Field-rep	Block	Plot	pH_{H2O}	pH_{KCl}	ExchAl	OC	TotN	WEP	Conc	DTPA Fe	DTPA Zn
					$cmol_c\ kg^{-1}$	%	%	$ug\ kg^{-1}$	%	$mg\ kg^{-1}$	$mg\ kg^{-1}$
KEN-1-T											
N1	1	4	6.46	5.58	0	0.34	0.04	33.01	0	4.0	2.3
C3	2	4	6.05	5.03	0	0.59	0.05	14.65	0	5.8	1.3
S5	2	13	6.64	5.60	0	0.55	0.06	9.28	0	2.8	0.6
E4	2	8	7.04	5.78	0	0.33	0.04	46.01	0	3.2	1.5
W2	2	1	5.60	4.44	0	0.34	0.04	6.19	0	3.8	1.7
KEN-2-T											
N1	1	4	6.10	5.02	0	0.80	0.07	13.41	37	12.2	4.3
C3	2	4	5.71	4.62	0	0.89	0.07	9.70	3	11.2	1.2
S5	2	13	6.38	5.46	0	0.83	0.07	14.24	49	4.9	1.4
E4	2	8	5.82	4.61	0	0.68	0.06	12.17	34	4.9	0.7
W2	2	1	5.99	4.99	0	0.97	0.08	23.93	8	11.7	0.8
KEN-3-S											
N1	1	4	5.68	4.43	8	0.66	0.06	9.49	22	6.8	1.7
C3	1	13	5.34	4.11	43	0.60	0.05	5.76	9	6.5	4.9
S5	2	13	5.47	4.17	28	0.52	0.06	9.06	12	6.5	1.4
E4	1	16	5.48	4.24	25	0.55	0.05	4.73	17	5.2	0.7
W2	2	8	5.89	4.63	0	0.93	0.07	13.58	6	11.8	0.8
KEN-4-S											
N1	1	4	5.68	4.49	0	0.91	0.07	11.14	18	13.6	2.4
C3	2	4	6.10	4.96	0	0.84	0.07	9.49	26	4.2	1.6

Field-rep	Block	Plot	pH$_{H2O}$	pH$_{KCl}$	ExchAl	OC	TotN	WEP	Conc	DTPA Fe	DTPA Zn
					cmol$_c$ kg^{-1}	%	%	ug kg^{-1}	%	mg kg^{-1}	mg kg^{-1}
KEN-4-S											
S5	2	13	6.07	4.93	0	0.95	0.07	16.92	18	6.0	2.3
E4	2	1	6.90	6.01	0	1.46	0.10	54.47	27	4.8	3.9
W2	2	8	6.40	5.27	0	0.83	0.06	20.84	25	4.3	0.6
TIG-1-T											
N1	2	13	5.60	4.49	0	1.40	0.06	6.81	3	13.2	5.5
C3	2	4	6.62	5.61	0	1.22	0.05	13.82	3	3.9	1.5
S5	1	4	7.03	-	0	1.34	0.06	17.70	3	3.1	2.3
E4	2	8	6.09	4.97	0	1.28	0.05	7.01	6	8.0	1.1
W2	2	1	5.87	4.75	0	1.39	0.07	18.73	3	10.5	3.4
TIG-2-T											
N1	1	4	6.21	5.24	0	0.89	0.07	30.53	3	1.8	1.1
C3	2	4	6.09	5.15	0	1.59	0.08	13.20	9	6.7	0.4
S5	2	13	6.12	5.09	0	0.67	0.07	9.90	11	7.5	0.5
E4	2	8	6.06	4.98	0	1.64	0.08	5.36	3	6.0	0.4
W2	2	1	6.00	4.91	0	0.85	0.07	12.79	6	8.3	1.0
TIG-3-S											
N1	1	4	5.69	4.42	3	0.71	0.07	5.16	23	7.0	1.0
C3	2	4	5.52	-	0	0.58	0.06	7.84	31	6.4	0.3
S5	2	13	5.45	-	0	0.82	0.08	6.19	35	9.5	0.5
E4	2	8	5.82	4.64	0	0.59	0.06	5.98	33	6.3	0.5
W2	2	1	5.72	4.46	3	0.89	0.08	10.93	36	9.2	0.6
TIG-4-S											
N1	1	4	5.05	3.85	94	0.67	0.05	8.46	14	12.7	0.5
C3	2	4	5.09	3.80	125	0.58	0.06	11.14	21	11.2	1.1
S5	2	13	5.07	3.88	82	0.53	0.06	8.87	20	10.8	2.1
E4	2	8	5.21	4.01	53	0.62	0.06	17.74	13	14.6	0.7
W2	2	1	5.38	-	0	0.39	0.05	7.22	32	10.8	4.7

Field-rep	Block	Plot	pH_{H2O}	pH_{KCl}	ExchAl	OC	TotN	WEP	Conc	DTPA Fe	DTPA Zn
					$cmol_c\ kg^{-1}$	%	%	$ug\ kg^{-1}$	%	$mg\ kg^{-1}$	$mg\ kg^{-1}$
WAC-1-T											
N1	1	4	7.10	6.18	0	0.65	0.07	60.92	0	2.6	1.3
C3	2	4	7.06	-	0	0.47	0.05	71.18	0	2.3	7.7
S5	2	13	6.65	5.61	0	0.47	0.05	44.98	3	4.1	6.2
E4	2	8	6.44	5.42	0	0.69	0.07	23.31	0	8.3	1.9
W2	2	1	6.78	6.13	0	0.44	0.05	79.43	0	4.4	0.6
WAC-2-T											
N1	2	13	5.33	4.24	16	0.51	0.05	67.67	0	21.3	1.9
C3	2	4	6.55	5.59	0	0.49	0.05	29.09	0	3.6	1.1
S5	1	4	5.53	4.27	5	0.49	0.06	33.63	0	17.7	0.9
E4	2	1	5.31	4.13	16	0.58	0.06	75.93	0	21.7	4.0
W2	2	8	6.09	4.86	0	0.52	0.06	125.85	0	20.4	5.3
FS-1-T											
G1	-	-	5.36	4.19	28	0.25	0.02	22.84	0	9.5	3.0
G2	-	-	5.36	4.00	30	0.19	0.02	34.78	3	4.8	0.7
G3	-	-	5.17	4.00	33	0.21	0.02	24.29	0	5.3	0.4
G4	-	-	5.22	4.04	25	0.22	0.02	13.79	0	5.4	1.0
G5	-	-	5.17	4.04	35	0.26	0.04	14.00	0	6.2	1.9
FS-2-S											
G1	-	-	5.22	3.95	42	0.52	0.04	10.08	0	14.2	0.4
G2	-	-	5.10	3.99	33	0.56	0.02	11.11	0	11.1	0.4
G3	-	-	5.15	3.96	42	0.81	0.04	10.91	0	11.8	0.6
G4	-	-	5.17	3.96	55	0.91	0.04	15.66	0	10.5	0.4
G5	-	-	5.23	4.00	50	0.32	0.03	7.82	0	10.3	0.6

Field-rep	Block	Plot	pH$_{H2O}$	pH$_{KCl}$	ExchAl	OC	TotN	WEP	Conc	DTPA Fe	DTPA Zn
					cmol$_c$ kg^{-1}	%	%	ug kg^{-1}	%	mg kg^{-1}	mg kg^{-1}
FS-3											
G82	-	-	5.29	4.15	0	0.25	0.02	217.95	3	13.2	0.7
G84	-	-	6.01	5.07	0	0.42	0.04	370.05	3	10.4	0.8
G85	-	-	6.59	5.85	0	0.25	0.02	177.00	3	5.6	0.5
G86	-	-	6.31	5.45	0	0.38	0.02	273.93	0	8.9	0.7
G87	-	-	6.31	5.39	0	0.41	0.04	222.89	3	8.3	0.7
G88	-	-	6.03	5.01	0	0.45	0.04	287.93	3	16.5	0.5

Table A2: Data from soil property analysis by location, field, and rep for depth 15-30 cm. KEN = Keniero, TIG = Tiguere, WAC = Wacoro, FS = Field Station, pH_{H2O} = soil pH in water, pH_{KCl} = soil pH in KCl, ExchAl = exchangeable Al, OC = organic carbon, TotN = total nitrogen, WEP = eater extractable phosphorus, Conc = % concretions, DTPA = diethylenetriaminepentaacetic acid.

Field-rep	**Block**	**Plot**	**pH_{H2O}**	**pH_{KCl}**	**ExchAl**	**OC**	**TotN**	**WEP**	**Conc**	**DTPA Fe**	**DTPA Zn**
					$cmol_c\ kg^{-1}$	%	%	$ug\ kg^{-1}$	%	$mg\ kg^{-1}$	$mg\ kg^{-1}$
KEN-1-T											
N1	1	4	5.34	4.19	53	0.75	0.08	-	0	2.2	1.3
C3	2	4	5.28	4.20	62	0.80	0.07	-	0	1.9	1.0
S5	2	13	5.55	4.24	40	0.73	0.07	-	0	1.8	0.5
E4	2	8	5.47	4.31	25	0.35	0.05	-	0	2.1	0.5
W2	2	1	5.44	4.18	80	0.29	0.04	-	0	1.5	1.1
KEN-2-T											
N1	1	4	5.34	4.21	35	0.42	0.05	-	33	3.0	0.7
C3	2	4	5.33	4.10	30	0.46	0.04	-	28	7.0	0.9
S5	2	13	5.56	4.40	10	0.42	0.05	-	33	3.2	0.6
E4	2	8	5.59	4.47	5	0.37	0.05	-	27	3.0	0.3
W2	2	1	5.50	4.13	25	0.48	0.05	-	10	9.8	0.7
KEN-3-S											
N1	1	4	5.33	4.15	72	0.38	0.04	-	32	2.3	2.1
C3	1	13	5.26	4.05	98	0.39	0.04	-	35	3.6	1.3
S5	2	13	5.23	4.10	95	0.39	0.05	-	15	2.7	0.8
E4	1	16	5.24	4.18	75	0.35	0.04	-	40	2.1	0.3
W2	2	8	5.26	4.06	55	0.45	0.05	-	12	4.4	0.6
KEN-4-S											
N1	1	4	5.64	4.28	13	0.51	0.05	-	41	6.0	0.6
C3	2	4	5.55	4.26	20	0.58	0.05	-	33	5.2	0.9
S5	2	13	5.59	4.17	25	0.75	0.05	-	25	7.6	1.0
E4	2	1	6.78	5.73	0	0.91	0.06	-	49	3.9	1.9
W2	2	8	6.24	5.14	0	0.70	0.06	-	20	4.4	0.4

Field-rep	Block	Plot	pH$_{H2O}$	pH$_{KCl}$	ExchAl	OC	TotN	WEP	Conc	DTPA Fe	DTPA Zn
					cmol$_c$ kg^{-1}	%	%	ug kg^{-1}	%	mg kg^{-1}	mg kg^{-1}
TIG-1-T											
N1	2	13	5.20	3.99	28	1.16	0.05	-	3	4.9	1.4
C3	2	4	5.55	4.31	8	1.19	0.05	-	0	3.7	0.8
S5	1	4	5.72	4.49	5	1.15	0.05	-	3	3.1	0.5
E4	2	8	6.31	4.97	0	1.17	0.05	-	6	2.2	0.8
W2	2	1	5.26	4.05	25	1.22	0.05	-	7	5.6	0.8
TIG-2-T											
N1	1	4	5.92	4.93	0	0.35	0.04	-	3	2.2	0.5
C3	2	4	5.97	4.87	0	1.34	0.06	-	10	1.6	0.3
S5	2	13	5.94	4.80	0	0.94	0.06	-	9	1.7	0.1
E4	2	8	5.62	4.54	0	0.41	0.04	-	3	1.8	0.2
W2	2	1	5.79	4.68	0	0.39	0.05	-	10	1.8	0.9
TIG-3-S											
N1	1	4	5.33	4.12	43	0.50	0.06	-	27	1.9	1.6
C3	2	4	5.23	3.98	89	0.62	0.06	-	29	3.6	0.3
S5	2	13	5.17	3.92	112	0.51	0.05	-	26	3.8	0.6
E4	2	8	5.68	4.46	5	0.56	0.06	-	36	3.5	0.6
W2	2	1	5.30	4.07	46	0.62	0.06	-	18	4.3	0.6
TIG-4-S											
N1	1	4	5.01	3.90	183	0.49	0.05	-	26	4.2	0.7
C3	2	4	4.84	-	0	0.43	0.05	-	3	3.6	1.7
S5	2	13	4.59	3.90	193	0.38	0.04	-	12	3.1	1.4
E4	2	8	5.11	3.89	148	0.55	0.06	-	39	5.0	0.8
W2	2	1	5.32	4.09	79	0.34	0.04	-	39	3.8	1.3

Field-rep	Block	Plot	pH_{H2O}	pH_{KCl}	ExchAl	OC	TotN	WEP	Conc	DTPA Fe	DTPA Zn
					$cmol_c\ kg^{-1}$	%	%	$ug\ kg^{-1}$	%	$mg\ kg^{-1}$	$mg\ kg^{-1}$
WAC-1-T											
N1	1	4	6.91	5.69	0	0.30	0.04	-	0	1.6	0.4
C3	2	4	6.36	4.90	0	0.35	0.04	-	0	5.4	1.1
S5	2	13	6.13	4.79	0	0.35	0.04	-	0	5.2	1.2
E4	2	8	5.49	4.12	10	0.35	0.05	-	8	8.5	0.7
W2	2	1	5.69	4.49	0	0.35	0.05	-	0	4.4	0.6
WAC-2-T											
N1	2	13	5.08	3.85	98	0.35	0.05	-	0	9.4	0.8
C3	2	4	6.51	5.05	0	0.37	0.05	-	3	2.7	0.6
S5	1	4	5.13	3.77	111	0.37	0.05	-	0	9.6	0.5
E4	2	1	5.23	3.81	72	0.30	0.04	-	0	7.9	0.5
W2	2	8	5.50	4.04	35	0.41	0.06	-	0	9.1	0.7
FS-1-T											
G1	-	-	4.99	3.92	78	0.29	0.03	-	0	4.2	2.7
G2	-	-	4.91	3.98	65	0.30	0.04	-	0	3.8	0.5
G3	-	-	5.02	3.97	62	0.29	0.04	-	0	3.2	0.4
G4	-	-	5.08	4.00	52	0.30	0.05	-	0	2.9	0.8
G5	-	-	5.20	4.07	23	0.32	0.04	-	0	3.2	0.6
FS-2-S											
G1	-	-	5.11	4.07	32	0.56	0.04	-	3	2.8	0.4
G2	-	-	4.95	3.89	80	0.75	0.05	-	3	3.2	0.5
G3	-	-	5.14	4.09	25	0.89	0.05	-	0	4.1	0.4
G4	-	-	5.15	4.01	43	0.89	0.04	-	0	2.6	0.4
G5	-	-	5.10	3.99	60	0.97	0.04	-	3	2.6	0.5

Field-rep	**Block**	**Plot**	**pH$_{H2O}$**	**pH$_{KCl}$**	**ExchAl**	**OC**	**TotN**	**WEP**	**Conc**	**DTPA Fe**	**DTPA Zn**
					cmol$_c$ kg^{-1}	%	%	ug kg^{-1}	%	mg kg^{-1}	mg kg^{-1}
FS-3											
G82	-	-	5.22	4.16	0	0.30	0.04	-	0	8.9	0.4
G84	-	-	6.19	5.37	0	0.64	0.05	-	3	8.3	0.9
G85	-	-	5.97	5.05	0	0.32	0.04	-	3	7.2	0.3
G86	-	-	6.27	5.25	0	0.37	0.04	-	3	4.1	0.2
G87	-	-	6.24	5.14	0	0.37	0.04	-	0	3.9	0.3
G88	-	-	5.89	5.10	0	0.55	0.05	-	3	12.1	0.5

Table A3: Data from soil color analysis by location, field, and rep for depth 0-15 cm. KEN = Keniero, TIG = Tiguere, WAC = Wacoro, FS = Field Station, red, green, blue = soil color tristimulus values, hueN = hue number, redidx = redness index.

Field-rep	Block	Plot	red	green	blue	hueN YR	value	chroma	redidx
KEN-1-T									
N1	1	4	0.569	0.468	0.353	8.610	5.080	5.140	8.948
C3	2	4	0.562	0.455	0.337	8.770	4.960	4.730	9.950
S5	2	13	0.586	0.484	0.366	8.710	5.240	5.120	8.275
E4	2	8	0.593	0.485	0.366	8.800	5.270	4.760	8.422
W2	2	1	0.596	0.484	0.359	8.510	5.270	5.160	8.727
KEN-2-T									
N1	1	4	0.589	0.487	0.379	8.450	5.280	4.580	7.925
C3	2	4	0.547	0.463	0.374	9.220	5.000	4.000	8.060
S5	2	13	0.555	0.465	0.371	7.930	5.030	4.910	8.258
E4	2	8	0.592	0.485	0.371	8.650	5.260	4.550	8.280
W2	2	1	0.548	0.465	0.376	9.050	5.010	4.010	7.944
KEN-3-S									
N1	1	4	0.589	0.482	0.374	8.140	5.230	4.650	8.284
C3	1	13	0.620	0.506	0.393	7.690	5.490	5.110	7.550
S5	2	13	0.619	0.512	0.405	8.490	5.540	4.280	7.049
E4	1	16	0.616	0.504	0.394	7.650	5.470	5.080	7.523
W2	2	8	0.600	0.501	0.402	8.180	5.410	4.390	7.121
KEN-4-S									
N1	1	4	0.506	0.424	0.344	9.300	4.590	3.430	9.764
C3	2	4	0.509	0.424	0.340	8.450	4.600	3.750	9.997
S5	2	13	0.516	0.431	0.351	9.170	4.680	3.490	9.475
E4	2	1	0.483	0.407	0.332	8.870	4.410	3.260	10.423
W2	2	8	0.534	0.449	0.364	9.230	4.860	3.820	8.655
TIG-1-T									
N1	2	13	0.583	0.497	0.409	7.760	5.340	4.170	6.769
C3	2	4	0.593	0.506	0.414	8.010	5.430	4.260	6.556
S5	1	4	0.563	0.475	0.382	9.440	5.120	4.060	7.742
E4	2	8	0.603	0.517	0.427	8.790	5.540	3.720	6.162
W2	2	1	0.588	0.500	0.406	8.500	5.380	4.250	6.813
TIG-2-T									
N1	1	4	0.556	0.462	0.367	8.010	5.010	4.570	8.542
C3	2	4	0.569	0.472	0.372	8.160	5.110	4.550	8.277
S5	2	13	0.566	0.469	0.370	8.150	5.080	4.510	8.393
E4	2	8	0.568	0.470	0.373	8.000	5.100	4.440	8.331
W2	2	1	0.567	0.470	0.372	8.130	5.100	4.520	8.324

Field-rep	Block	Plot	red	green	blue	hueN YR	value	chroma	redidx
TIG-3-S									
N1	1	4	0.581	0.487	0.391	8.040	5.260	4.620	7.475
C3	2	4	0.567	0.478	0.378	8.090	5.160	5.230	7.787
S5	2	13	0.573	0.489	0.391	9.280	5.250	4.280	7.181
E4	2	8	0.582	0.485	0.377	8.370	5.240	5.030	7.876
W2	2	1	0.566	0.483	0.386	9.360	5.190	4.220	7.366
TIG-4-S									
N1	1	4	0.586	0.487	0.376	8.490	5.270	5.030	7.907
C3	2	4	0.616	0.509	0.388	8.830	5.500	4.920	7.416
S5	2	13	0.595	0.495	0.388	8.400	5.350	4.710	7.523
E4	2	8	0.560	0.464	0.361	8.230	5.030	4.800	8.696
W2	2	1	0.671	0.566	0.447	8.740	6.060	4.770	5.555
WAC-1-T									
N1	1	4	0.574	0.512	0.449	8.930	5.440	2.590	5.467
C3	2	4	0.605	0.543	0.475	9.090	5.750	2.940	4.813
S5	2	13	0.586	0.521	0.450	9.180	5.540	2.900	5.396
E4	2	8	0.572	0.511	0.446	8.980	5.430	2.870	5.498
W2	2	1	0.635	0.568	0.495	9.270	6.010	2.810	4.445
WAC-2-T									
N1	2	13	0.636	0.576	0.513	8.940	6.070	2.640	4.126
C3	2	4	0.610	0.551	0.489	8.900	5.830	2.780	4.549
S5	1	4	0.669	0.607	0.547	8.260	6.380	2.440	3.658
E4	2	1	0.627	0.567	0.503	8.960	5.980	2.770	4.288
W2	2	8	0.661	0.600	0.541	8.350	6.310	2.470	3.739
FS-1-T									
G1	-	-	0.654	0.527	0.419	6.630	5.720	4.960	6.974
G2	-	-	0.675	0.542	0.432	6.760	5.880	4.570	6.624
G3	-	-	0.648	0.519	0.407	6.760	5.650	5.080	7.380
G4	-	-	0.632	0.500	0.383	6.710	5.460	5.750	8.343
G5	-	-	0.625	0.496	0.379	6.670	5.420	5.900	8.446
FS-2-S									
G1	-	-	0.615	0.497	0.383	6.570	5.410	5.090	8.044
G2	-	-	0.604	0.484	0.376	5.960	5.290	5.960	8.558
G3	-	-	0.620	0.502	0.393	7.270	5.450	5.390	7.732
G4	-	-	0.620	0.488	0.372	6.180	5.350	5.910	8.892
G5	-	-	0.617	0.496	0.381	6.830	5.400	5.370	8.188
FS-3									
G82	-	-	0.568	0.474	0.389	7.340	5.130	4.340	7.788
G84	-	-	0.553	0.467	0.390	7.430	5.050	3.850	7.699
G85	-	-	0.572	0.478	0.392	7.290	5.170	4.340	7.642
G86	-	-	0.556	0.466	0.379	9.140	5.040	4.050	8.060
G87	-	-	0.536	0.451	0.366	9.130	4.880	3.840	8.557
G88	-	-	0.591	0.503	0.429	6.490	5.410	4.560	6.398

Table A4: Data from soil color analysis by location, field, and rep for depth 15-30 cm. KEN = Keniero, TIG = Tiguere, WAC = Wacoro, FS = Field Station, red, green, blue = soil color tristimulus values, hueN = hue number, redidx = redness index.

Field-rep	Block	Plot	red	green	blue	hueN YR	value	chroma	redidx
KEN-1-T									
N1	1	4	0.653	0.510	0.364	7.500	5.590	6.480	8.831
C3	2	4	0.663	0.520	0.373	7.500	5.680	6.570	8.381
S5	2	13	0.646	0.500	0.347	7.560	5.500	6.760	9.621
E4	2	8	0.654	0.507	0.352	7.500	5.560	6.820	9.324
W2	2	1	0.626	0.488	0.343	7.500	5.360	5.350	9.831
KEN-2-T									
N1	1	4	0.658	0.514	0.385	6.080	5.630	6.550	8.281
C3	2	4	0.633	0.523	0.407	8.740	5.650	4.410	6.882
S5	2	13	0.682	0.551	0.422	5.710	5.970	5.940	6.589
E4	2	8	0.663	0.523	0.400	6.990	5.710	5.800	7.682
W2	2	1	0.610	0.506	0.394	8.570	5.470	4.750	7.290
KEN-3-S									
N1	1	4	0.634	0.483	0.356	5.470	5.340	6.100	10.020
C3	1	13	0.643	0.511	0.388	7.340	5.580	5.940	7.986
S5	2	13	0.680	0.554	0.429	7.070	5.980	5.780	6.339
E4	1	16	0.644	0.493	0.363	6.640	5.440	5.470	9.535
W2	2	8	0.672	0.551	0.427	7.550	5.940	4.730	6.322
KEN-4-S									
N1	1	4	0.572	0.474	0.366	8.390	5.130	4.920	8.394
C3	2	4	0.574	0.477	0.383	7.840	5.170	4.520	7.926
S5	2	13	0.541	0.448	0.357	8.200	4.870	4.070	9.118
E4	2	1	0.504	0.418	0.332	8.650	4.540	3.570	10.476
W2	2	8	0.588	0.492	0.396	8.080	5.310	4.470	7.331
TIG-1-T									
N1	2	13	0.674	0.570	0.442	8.950	6.100	5.290	5.550
C3	2	4	0.649	0.544	0.419	8.900	5.840	5.190	6.244
S5	1	4	0.694	0.592	0.465	8.880	6.300	5.310	4.992
E4	2	8	0.662	0.562	0.442	8.690	6.010	5.270	5.586
W2	2	1	0.656	0.553	0.431	8.790	5.930	5.120	5.904
TIG-2-T									
N1	1	4	0.570	0.444	0.326	7.070	4.890	5.540	11.386
C3	2	4	0.591	0.449	0.321	6.980	4.980	6.000	12.021
S5	2	13	0.587	0.449	0.320	6.840	4.970	5.990	11.896
E4	2	8	0.632	0.488	0.354	5.620	5.380	6.510	9.709
W2	2	1	0.587	0.456	0.334	7.020	5.030	5.630	10.880

Field-rep	Block	Plot	red	green	blue	hueN YR	value	chroma	redidx
TIG-3-S									
N1	1	4	0.622	0.499	0.376	8.120	5.440	4.920	8.281
C3	2	4	0.614	0.500	0.375	9.010	5.430	4.620	8.043
S5	2	13	0.661	0.548	0.416	9.150	5.900	4.880	6.382
E4	2	8	0.606	0.494	0.368	9.030	5.360	4.740	8.278
W2	2	1	0.622	0.516	0.389	8.960	5.570	5.230	7.239
TIG-4-S									
N1	1	4	0.668	0.539	0.402	7.780	5.850	5.760	7.089
C3	2	4	0.705	0.572	0.429	9.290	6.180	4.490	6.191
S5	2	13	0.683	0.553	0.415	7.520	5.980	4.050	6.647
E4	2	8	0.631	0.508	0.381	8.090	5.530	5.110	7.972
W2	2	1	0.718	0.602	0.463	8.670	6.430	5.520	5.104
WAC-1-T									
N1	1	4	0.645	0.574	0.498	9.340	6.070	2.790	4.417
C3	2	4	0.662	0.590	0.510	9.360	6.230	3.140	4.184
S5	2	13	0.622	0.547	0.466	8.020	5.820	3.820	5.073
E4	2	8	0.658	0.587	0.505	9.480	6.200	3.160	4.239
W2	2	1	0.717	0.631	0.551	7.500	6.660	4.160	3.714
WAC-2-T									
N1	2	13	0.700	0.627	0.551	8.170	6.590	2.310	3.608
C3	2	4	0.652	0.585	0.511	9.020	6.180	3.090	4.155
S5	1	4	0.709	0.635	0.563	8.000	6.680	2.850	3.487
E4	2	1	0.705	0.632	0.556	8.090	6.640	2.270	3.541
W2	2	8	0.694	0.626	0.559	8.090	6.580	2.420	3.512
FS-1-T									
G1	-	-	0.629	0.497	0.380	6.770	5.440	5.700	8.481
G2	-	-	0.644	0.501	0.380	6.150	5.500	5.640	8.679
G3	-	-	0.648	0.501	0.374	6.350	5.510	5.740	8.928
G4	-	-	0.640	0.488	0.358	5.610	5.390	6.160	9.845
G5	-	-	0.615	0.467	0.337	6.390	5.170	6.090	11.020
FS-2-S									
G1	-	-	0.641	0.493	0.366	6.530	5.440	5.570	9.369
G2	-	-	0.631	0.484	0.358	6.690	5.340	5.240	9.809
G3	-	-	0.642	0.493	0.369	6.470	5.440	5.300	9.322
G4	-	-	0.625	0.469	0.342	6.200	5.210	6.170	11.072
G5	-	-	0.640	0.490	0.361	6.660	5.400	5.370	9.644
FS-3									
G82	-	-	0.570	0.469	0.378	7.410	5.100	4.750	8.332
G84	-	-	0.495	0.417	0.341	8.840	4.520	3.380	9.909
G85	-	-	0.549	0.448	0.355	7.730	4.880	4.100	9.442
G86	-	-	0.549	0.450	0.350	8.430	4.890	4.110	9.450
G87	-	-	0.569	0.475	0.380	8.010	5.140	4.450	7.950
G88	-	-	0.554	0.469	0.387	7.570	5.060	4.000	7.688

Table A5: Data from sorghum whole grain by location, field, and variety. KEN = Keniero, TIG = Tiguere, WAC = Wacoro, FS = Field Station, T = tall variety field, S = short variety field, Zn = grain Zn concentration, GW = 100 grain weight, red, green, blue = grain color tristimulus values, lightness = grain lightness * anomalously high concentration.

Field-variety	**Block**	**Plot**	**Pericarp**	**Glume**	**Zn** **mg kg^{-1}**	**GW** **g**	**red**	**green**	**blue**	**lightness**
KEN-1-T										
Babalissa	1	11	Thin	Tan	19	2.05	0.834	0.761	0.694	0.764
	2	7	Thin	Tan	19	1.95	0.847	0.775	0.704	0.776
Sotigui	1	4	Thin	Red	19	2.20	0.837	0.743	0.692	0.765
	2	8	Thin	Red	22	2.40	0.824	0.728	0.682	0.753
Keneya	1	10	Thick	Tan	22	2.44	0.875	0.801	0.740	0.807
	2	12	Thick	Tan	19	2.48	0.878	0.805	0.743	0.811
Jamajigi	1	15	Thin	Red	20	1.86	0.867	0.795	0.728	0.797
	2	2	Thin	Red	20	1.85	0.885	0.815	0.748	0.816
Pablo	1	9	Thin	Purple	20	2.55	0.823	0.730	0.688	0.756
	2	15	Thin	Purple	20	2.69	0.852	0.765	0.718	0.785
Omba	1	2	Thick	Purple	24	2.47	0.800	0.707	0.670	0.735
	2	10	Thick	Purple	21	2.52	0.787	0.694	0.664	0.726
Caufa	1	16	Thin	Purple	22	2.32	0.802	0.710	0.672	0.737
	2	6	Thin	Purple	22	2.44	0.772	0.679	0.646	0.709
Tieble	1	8	Thin	Red	21	2.35	0.781	0.681	0.638	0.710
	2	11	Thin	Red	21	2.36	0.784	0.687	0.646	0.715
KEN-2-T										
Babalissa	1	16	Thin	Tan	18	1.92	0.876	0.805	0.740	0.808
	2	15	Thin	Tan	16	1.93	0.880	0.808	0.749	0.814
Sotigui	1	14	Thin	Red	21	2.56	0.885	0.804	0.755	0.820
	2	11	Thin	Red	18	2.49	0.893	0.824	0.763	0.828
Keneya	1	5	Thick	Tan	18	2.44	0.903	0.834	0.775	0.839
	2	3	Thick	Tan	18	2.29	0.891	0.823	0.761	0.826
Jamajigi	1	2	Thin	Red	14	2.21	0.900	0.834	0.765	0.832
	2	1	Thin	Red	14	1.96	0.911	0.843	0.783	0.847
Pablo	1	3	Thin	Purple	15	2.47	0.835	0.744	0.698	0.767
	2	9	Thin	Purple	18	2.73	0.859	0.770	0.722	0.790
Omba	1	1	Thick	Purple	17	2.53	0.870	0.785	0.737	0.804
	2	14	Thick	Purple	18	2.47	0.874	0.793	0.749	0.811
Caufa	1	8	Thin	Purple	18	2.37	0.865	0.784	0.732	0.798
	2	6	Thin	Purple	16	2.32	0.876	0.791	0.743	0.809
Tieble	1	6	Thin	Red	20	2.27	0.884	0.810	0.753	0.819
	2	10	Thin	Red	16	2.29	0.877	0.796	0.747	0.812
KEN-3-S										
Sawaba	1	13	Thick	Tan	14	2.21	0.914	0.844	0.790	0.852
	2	7	Thick	Tan	16	2.06	0.896	0.824	0.765	0.831
Togotigi	1	6	Thick	Tan	19	2.22	0.896	0.822	0.765	0.831
	2	6	Thick	Tan	19	2.16	0.895	0.824	0.766	0.831

Field-variety	Block	Plot	Pericarp	Glume	Zn mg kg^{-1}	GW g	red	green	blue	lightness
KEN-3-S										
Sewa	1	1	Thick	Tan	14	1.99	0.877	0.794	0.743	0.810
	2	3	Thick	Tan	14	1.85	0.884	0.808	0.752	0.818
Siguikumbe	1	12	Thick	Tan	11	1.76	0.905	0.834	0.773	0.839
	2	9	Thick	Tan	13	1.93	0.886	0.812	0.748	0.817
Fada	1	16	Thin	Purple	12	2.04	0.819	0.724	0.686	0.752
	2	10	Thin	Purple	12	1.99	0.831	0.734	0.694	0.762
Mara	1	15	Thin	Purple	11	2.56	0.817	0.726	0.688	0.752
	2	1	Thin	Purple	13	2.21	0.871	0.792	0.741	0.806
Lata	1	11	Thin	Purple	13	2.18	0.861	0.774	0.725	0.793
	2	2	Thin	Purple	15	2.15	0.866	0.784	0.734	0.800
Tieble	1	3	Thin	Red	15	2.22	0.888	0.807	0.757	0.822
	2	8	Thin	Red	16	2.25	0.887	0.812	0.757	0.822
KEN-4-S										
Sawaba	1	1	Thick	Tan	16	2.28	0.924	0.854	0.799	0.861
	2	14	Thick	Tan	17	2.17	0.900	0.829	0.768	0.834
Togotigi	1	11	Thick	Tan	18	2.34	0.918	0.848	0.795	0.857
	2	6	Thick	Tan	18	2.24	0.897	0.825	0.768	0.833
Sewa	1	8	Thick	Tan	12	1.92	0.908	0.839	0.780	0.844
	2	13	Thick	Tan	25*	1.85	0.918	0.851	0.793	0.855
Siguikumbe	1	4	Thick	Tan	11	1.78	0.915	0.846	0.789	0.852
	2	3	Thick	Tan	13	1.87	0.935	0.869	0.818	0.876
Fada	1	6	Thin	Purple	12	1.97	0.874	0.793	0.745	0.809
	2	1	Thin	Purple	16	2.18	0.877	0.796	0.742	0.810
Mara	1	3	Thin	Purple	12	2.11	0.806	0.712	0.679	0.742
	2	5	Thin	Purple	13	2.28	0.814	0.718	0.683	0.748
Lata	1	12	Thin	Purple	12	2.24	0.787	0.692	0.660	0.724
	2	16	Thin	Purple	17	2.48	0.769	0.675	0.644	0.706
Tieble	1	13	Thin	Red	16	1.86	0.879	0.797	0.747	0.813
	2	7	Thin	Red	16	2.03	0.866	0.784	0.730	0.798
TIG-1-T										
Babalissa	1	10	Thin	Tan	16	1.79	0.865	0.794	0.735	0.800
	2	1	Thin	Tan	16	1.85	0.861	0.789	0.727	0.794
Sotigui	1	11	Thin	Red	17	2.31	0.799	0.699	0.653	0.726
	2	11	Thin	Red	15	2.52	0.828	0.730	0.686	0.757
Keneya	1	2	Thick	Tan	16	2.28	0.881	0.807	0.748	0.815
	2	4	Thick	Tan	17	2.11	0.877	0.800	0.739	0.808
Jamajigi	1	3	Thin	Red	17	1.96	0.880	0.809	0.743	0.812
	2	10	Thin	Red	15	1.97	0.879	0.808	0.739	0.809
Pablo	1	14	Thin	Purple	14	2.13	0.842	0.749	0.707	0.774
	2	2	Thin	Purple	14	2.22	0.845	0.752	0.710	0.777
Omba	1	1	Thick	Purple	16	2.44	0.787	0.693	0.660	0.724
	2	8	Thick	Purple	19	2.13	0.792	0.697	0.664	0.728

Field-variety	Block	Plot	Pericarp	Glume	Zn mg kg^{-1}	GW g	red	green	blue	lightness
TIG-1-T										
Caufa	1	16	Thin	Purple	16	2.32	0.801	0.708	0.667	0.734
	2	9	Thin	Purple	18	2.29	0.811	0.719	0.677	0.744
Tieble	1	13	Thin	Red	15	2.19	0.831	0.735	0.687	0.759
	2	13	Thin	Red	17	1.98	0.852	0.759	0.709	0.780
TIG-2-T										
Babalissa	1	15	Thin	Tan	15	2.01	0.855	0.779	0.717	0.786
	2	14	Thin	Tan	17	1.86	0.858	0.784	0.719	0.788
Sotigui	1	9	Thin	Red	24	2.27	0.797	0.699	0.653	0.725
	2	8	Thin	Red	21	2.61	0.799	0.700	0.656	0.728
Keneya	1	4	Thick	Tan	16	2.35	0.886	0.812	0.754	0.820
	2	6	Thick	Tan	17	2.29	0.872	0.796	0.733	0.803
Jamajigi	1	1	Thin	Red	17	2.13	0.883	0.812	0.745	0.814
	2	11	Thin	Red	15	2.09	0.894	0.824	0.759	0.827
Pablo	1	7	Thin	Purple	15	2.13	0.840	0.747	0.703	0.772
	2	10	Thin	Purple	15	2.07	0.853	0.762	0.721	0.787
Omba	1	14	Thick	Purple	16	2.25	0.794	0.699	0.667	0.730
	2	1	Thick	Purple	17	2.27	0.810	0.715	0.682	0.746
Caufa	1	16	Thin	Purple	18	2.25	0.809	0.717	0.674	0.742
	2	5	Thin	Purple	17	2.10	0.836	0.742	0.703	0.770
Tieble	1	12	Thin	Red	18	2.22	0.835	0.738	0.695	0.765
	2	4	Thin	Red	18	2.23	0.811	0.711	0.668	0.739
TIG-3-S										
Sawaba	1	8	Thick	Tan	15	1.64	0.871	0.789	0.735	0.803
	2	2	Thick	Tan	13	1.65	0.884	0.806	0.751	0.818
Togotigi	1	4	Thick	Tan	17	1.87	0.892	0.817	0.766	0.829
	2	11	Thick	Tan	21	1.88	0.868	0.793	0.734	0.801
Sewa	1	11	Thick	Tan	16	1.80	0.845	0.753	0.701	0.773
	2	14	Thick	Tan	11	1.47	0.859	0.767	0.719	0.789
Siguikumbe	1	9	Thick	Tan	10	1.28	0.853	0.769	0.713	0.783
	2	16	Thick	Tan	9	1.30	0.868	0.779	0.724	0.796
Fada	1	7	Thin	Purple	15	1.75	0.760	0.664	0.628	0.694
	2	6	Thin	Purple	15	2.10	0.772	0.678	0.643	0.708
Mara	1	13	Thin	Purple	13	2.04	0.775	0.683	0.648	0.712
	2	4	Thin	Purple	10	1.76	0.805	0.712	0.668	0.736
Lata	1	3	Thin	Purple	11	1.98	0.810	0.719	0.672	0.741
	2	1	Thin	Purple	11	1.99	0.763	0.670	0.639	0.701
Tieble	1	10	Thin	Red	16	2.03	0.812	0.715	0.668	0.740
	2	8	Thin	Red	14	1.76	0.850	0.757	0.709	0.780
TIG-4-S										
Sawaba	1	7	Thick	Tan	16	1.55	0.860	0.783	0.718	0.789
	2	11	Thick	Tan	22	1.95	0.882	0.809	0.751	0.816
Togotigi	1	4	Thick	Tan	18	1.57	0.875	0.802	0.744	0.810
	2	1	Thick	Tan	19	2.23	0.896	0.825	0.772	0.834

Field-variety	Block	Plot	Pericarp	Glume	Zn	GW	red	green	blue	lightness
					mg kg^{-1}	g				
TIG-4-S										
Sewa	1	1	Thick	Tan	11	1.60	0.864	0.790	0.730	0.797
	2	12	Thick	Tan	13	1.56	0.884	0.813	0.749	0.817
Siguikumbe	1	5	Thick	Tan	13	1.41	0.881	0.809	0.744	0.813
	2	4	Thick	Tan	11	1.42	0.889	0.819	0.757	0.823
Fada	1	11	Thin	Purple	13	1.71	0.813	0.721	0.672	0.743
	2	2	Thin	Purple	14	2.10	0.822	0.728	0.684	0.753
Mara	1	2	Thin	Purple	11	2.00	0.814	0.723	0.675	0.745
	2	3	Thin	Purple	15	2.01	0.794	0.701	0.659	0.727
Lata	1	12	Thin	Purple	14	2.06	0.744	0.656	0.616	0.680
	2	15	Thin	Purple	15	2.25	0.786	0.693	0.655	0.720
Tieble	1	13	Thin	Red	18	1.74	0.860	0.774	0.718	0.789
	2	16	Thin	Red	18	2.09	0.855	0.763	0.713	0.784
WAC-1-T										
Babalissa	1	13	Thin	Tan	16	1.90	0.880	0.807	0.748	0.814
	2	12	Thin	Tan	15	1.85	0.869	0.796	0.736	0.803
Sotigui	1	1	Thin	Red	17	2.13	0.883	0.792	0.744	0.814
	2	9	Thin	Red	18	2.00	0.857	0.765	0.716	0.787
Keneya	1	3	Thick	Tan	19	2.16	0.893	0.821	0.761	0.827
	2	11	Thick	Tan	18	2.16	0.902	0.831	0.774	0.838
Jamajigi	1	14	Thin	Red	14	2.00	0.906	0.836	0.779	0.843
	2	1	Thin	Red	15	1.83	0.903	0.834	0.771	0.837
Pablo	1	6	Thin	Purple	16	2.00	0.868	0.788	0.739	0.804
	2	4	Thin	Purple	16	2.18	0.872	0.786	0.743	0.807
Omba	1	12	Thick	Purple	16	2.11	0.879	0.795	0.754	0.816
	2	2	Thick	Purple	18	2.30	0.888	0.807	0.765	0.826
Caufa	1	7	Thin	Purple	16	1.82	0.914	0.839	0.796	0.855
	2	6	Thin	Purple	16	1.80	0.877	0.795	0.747	0.812
Tieble	1	2	Thin	Red	16	1.99	0.891	0.809	0.763	0.827
	2	13	Thin	Red	18	2.03	0.870	0.782	0.733	0.802
WAC-2-T										
Babalissa	1	14	Thin	Tan	22	2.06	0.868	0.796	0.732	0.800
	2	3	Thin	Tan	20	2.05	0.855	0.782	0.717	0.786
Sotigui	1	2	Thin	Red	22	2.23	0.862	0.770	0.720	0.791
	2	16	Thin	Red	24	2.12	0.871	0.779	0.730	0.800
Keneya	1	6	Thick	Tan	21	2.22	0.899	0.828	0.770	0.835
	2	8	Thick	Tan	22	2.31	0.890	0.819	0.759	0.825
Jamajigi	1	5	Thin	Red	17	2.00	0.902	0.833	0.770	0.836
	2	14	Thin	Red	17	2.20	0.886	0.816	0.751	0.819
Pablo	1	1	Thin	Purple	21	2.12	0.871	0.791	0.742	0.807
	2	5	Thin	Purple	18	2.33	0.830	0.739	0.696	0.763
Omba	1	10	Thick	Purple	22	2.23	0.873	0.786	0.743	0.808
	2	4	Thick	Purple	20	2.10	0.884	0.799	0.755	0.820

Field-variety	Block	Plot	Pericarp	Glume	Zn mg kg^{-1}	GW g	red	green	blue	lightness
WAC-2-T										
Caufa	1	7	Thin	Purple	30*	2.16	0.879	0.798	0.750	0.815
	2	1	Thin	Purple	22	2.14	0.873	0.791	0.744	0.808
Tieble	1	15	Thin	Red	22	2.02	0.860	0.769	0.717	0.788
	2	2	Thin	Red	21	2.04	0.863	0.775	0.722	0.793
FS-1-T										
Babalissa	1	7	Thin	Tan	16	1.57	0.871	0.798	0.732	0.802
	2	11	Thin	Tan	18	1.57	0.866	0.791	0.726	0.796
Sotigui	2	12	Thin	Red	16	1.90	0.821	0.725	0.678	0.750
Keneya	1	2	Thick	Tan	18	2.04	0.863	0.789	0.723	0.793
	2	9	Thick	Tan	17	1.79	0.876	0.795	0.739	0.807
Jamajigi	1	14	Thin	Red	27*	1.53	0.880	0.807	0.739	0.809
	2	16	Thin	Red	15	1.64	0.882	0.810	0.747	0.815
Pablo	1	6	Thin	Purple	13	2.19	0.831	0.740	0.694	0.762
	2	8	Thin	Purple	13	2.10	0.829	0.738	0.692	0.761
Omba	1	16	Thick	Purple	17	2.25	0.860	0.768	0.723	0.791
	2	10	Thick	Purple	17	2.27	0.865	0.773	0.729	0.797
Caufa	1	13	Thin	Purple	17	2.07	0.856	0.771	0.724	0.790
	2	5	Thin	Purple	17	1.95	0.866	0.777	0.730	0.798
Tieble	1	3	Thin	Red	20	2.00	0.872	0.788	0.739	0.806
	2	6	Thin	Red	18	1.86	0.850	0.759	0.708	0.779
FS-2-S										
Sawaba	1	9	Thick	Tan	21	1.79	0.881	0.807	0.747	0.814
	2	14	Thick	Tan	20	1.88	0.869	0.794	0.733	0.801
Togotigi	1	13	Thick	Tan	24	2.18	0.884	0.810	0.752	0.818
	2	1	Thick	Tan	22	2.02	0.897	0.824	0.766	0.831
Sewa	1	3	Thick	Tan	20	1.88	0.892	0.818	0.760	0.826
	2	15	Thick	Tan	16	2.30	0.907	0.837	0.779	0.843
Siguikumbe	1	2	Thick	Tan	16	1.79	0.881	0.808	0.746	0.814
	2	10	Thick	Tan	14	1.91	0.887	0.814	0.754	0.820
Fada	1	15	Thin	Purple	15	1.97	0.753	0.657	0.625	0.689
	2	13	Thin	Purple	15	2.05	0.761	0.666	0.636	0.698
Mara	1	7	Thin	Purple	18	2.28	0.763	0.671	0.638	0.701
	2	3	Thin	Purple	15	2.08	0.768	0.676	0.643	0.706
Lata	1	16	Thin	Purple	16	2.38	0.744	0.651	0.621	0.683
Tieble	1	1	Thin	Red	21	1.89	0.873	0.788	0.738	0.805
	2	7	Thin	Red	19	2.13	0.867	0.775	0.726	0.796
FS-3-T										
Babalissa	1	3	Thin	Tan	17	1.95	0.832	0.758	0.689	0.761
	2	7	Thin	Tan	19	2.14	0.831	0.754	0.683	0.757
Sotigui	1	18	Thin	Red	22	2.68	0.833	0.742	0.692	0.762
	2	4	Thin	Red	23	2.30	0.799	0.704	0.652	0.725
Keneya	1	9	Thick	Tan	20	2.56	0.858	0.782	0.717	0.787
	2	6	Thick	Tan	21	2.49	0.844	0.769	0.702	0.773

Field-variety	Block	Plot	Pericarp	Glume	Zn mg kg^{-1}	GW g	red	green	blue	lightness
FS-3-T										
Jamajigi	1	8	Thin	Red	14	2.03	0.854	0.780	0.711	0.782
	2	16	Thin	Red	15	2.60	0.875	0.805	0.737	0.806
Pablo	1	10	Thin	Purple	20	2.73	0.759	0.667	0.630	0.695
	2	1	Thin	Purple	21	2.57	0.755	0.663	0.626	0.691
Omba	1	11	Thick	Purple	21	2.62	0.771	0.678	0.646	0.708
	2	17	Thick	Purple	23	2.55	0.778	0.686	0.649	0.714
Caufa	1	16	Thin	Purple	20	2.39	0.770	0.678	0.643	0.707
	2	9	Thin	Purple	20	2.51	0.742	0.649	0.614	0.678
Tieble	1	14	Thin	Red	21	2.16	0.809	0.717	0.666	0.738
	2	3	Thin	Red	21	2.29	0.791	0.696	0.648	0.720
FS-3-S										
Sawaba	1	4	Thick	Tan	21	2.39	0.883	0.810	0.747	0.815
	2	2	Thick	Tan	21	2.48	0.883	0.810	0.749	0.816
Togotigi	1	13	Thick	Tan	19	2.44	0.895	0.821	0.761	0.828
	2	6	Thick	Tan	20	2.43	0.894	0.823	0.764	0.829
Sewa	1	12	Thick	Tan	15	1.95	0.855	0.764	0.712	0.784
	2	11	Thick	Tan	21	2.10	0.844	0.752	0.698	0.771
Siguikumbe	1	1	Thick	Tan	14	2.14	0.868	0.794	0.729	0.799
	2	10	Thick	Tan	16	2.09	0.885	0.808	0.751	0.818
Fada	1	14	Thin	Purple	19	2.41	0.753	0.659	0.624	0.688
	2	18	Thin	Purple	14	2.28	0.738	0.644	0.602	0.670
Mara	1	3	Thin	Purple	18	2.60	0.707	0.619	0.589	0.648
	2	9	Thin	Purple	19	2.43	0.746	0.656	0.625	0.686
Lata	1	15	Thin	Purple	19	2.28	0.695	0.604	0.575	0.635
Tieble	1	5	Thin	Red	22	2.15	0.752	0.656	0.617	0.685
	2	4	Thin	Red	19	2.09	0.763	0.665	0.619	0.691

APPENDIX B

CHAPTER III DATA

Table B1: Data from soil chemical property analysis by location, site, and tree. KEN = Keniero, TIG = Tiguere, WAC = Wacoro, FS = Field Station, S-1 = site number, Dist = distance from the edge of the tree canopy, pH = soil pH in water, C = organic carbon, $\delta^{13}C$ = delta 13C, N = total nitrogen, $\delta^{15}N$ = delta 15N, Fe, Zn, Mn, and Cu = DTPA (diethylenetriaminepentaacetic acid)-extractable Fe, Zn, Mn, and Cu.

ID	**Dist**	**pH**	**C**	**$\delta^{13}C$**	**N**	**$\delta^{15}N$**	**Fe**	**Zn**	**Mn**	**Cu**
	m		%	$^{0}/_{00}$	%	$^{0}/_{00}$	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}
KEN-S-1										
Tree 1	-4	7.63	1.02	-23.99	0.09	3.96	10.58	0.85	36.65	0.95
	-2	7.42	1.15	-22.83	0.08	4.72	13.57	0.64	36.62	0.76
	0	7.42	0.95	-21.85	0.08	4.12	11.99	0.75	51.98	0.84
	2	6.99	1.62	-19.78	0.07	4.51	13.86	0.64	73.42	0.87
	4	6.29	0.78	-18.49	0.06	4.69	17.92	0.48	104.66	0.97
	6	6.23	0.74	-17.39	0.06	4.90	15.12	0.41	93.75	0.96
	8	6.09	0.79	-18.45	0.06	4.89	14.04	0.43	86.57	0.76
	10	6.22	0.69	-18.84	0.05	4.94	14.51	0.45	93.47	0.62
	12	6.20	0.50	-19.40	0.04	-	10.95	0.37	77.38	0.66
Tree 2	-4	7.59	0.88	-22.93	0.07	4.38	10.83	0.65	33.29	0.96
	-2	7.25	0.79	-19.84	0.06	4.79	7.63	0.51	39.53	0.62
	0	7.19	0.64	-19.41	0.05	4.78	9.07	0.44	39.40	0.66
	2	6.56	0.62	-18.42	0.04	4.62	10.38	0.61	72.13	0.83
	4	6.50	0.50	-17.68	0.04	5.62	11.12	0.41	57.40	0.76
	6	7.85	0.65	-19.14	0.05	4.89	6.08	0.94	28.66	0.90
	8	6.43	-	-17.53	0.05	5.55	9.88	0.50	62.44	0.67
	10	6.51	0.68	-18.10	0.05	4.77	10.12	0.35	57.65	0.59
	12	6.36	0.57	-18.08	0.05	5.70	11.55	0.54	71.43	0.82
KEN-S-2										
Tree 3	-2	7.99	2.04	-20.88	0.15	4.87	11.77	2.74	24.43	1.03
	0	7.56	1.53	-18.93	0.11	4.45	22.01	0.79	46.51	1.46
	2	7.03	1.33	-16.54	0.09	4.43	30.73	0.74	73.33	2.16
	4	6.82	0.99	-16.07	0.07	4.21	25.13	0.50	43.45	1.73
	6	6.63	0.96	-15.66	0.05	6.50	24.96	0.77	61.78	1.22
	8	6.73	0.83	-16.36	0.06	5.25	17.95	1.50	35.83	1.64
	10	6.77	0.73	-16.14	0.04	6.16	13.91	0.40	45.54	1.23
	12	6.68	0.62	-15.40	0.04	5.52	25.11	0.65	54.86	1.48

ID	Dist	pH	C	δ^{13}C	N	δ^{15}N	Fe	Zn	Mn	Cu
	m		%	$^{0}/_{00}$	%	$^{0}/_{00}$	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}
KEN-S-2										
Tree 4	-2	7.23	1.03	-19.59	0.07	4.64	15.31	3.96	40.36	1.00
	0	7.20	0.79	-16.68	0.05	4.84	7.79	0.36	20.65	0.34
	2	6.96	0.78	-16.85	0.05	4.94	14.55	0.45	33.20	0.54
	4	6.71	0.74	-16.99	0.05	5.15	16.06	3.62	49.59	0.82
	6	6.74	1.07	-15.54	0.06	6.00	15.43	0.38	56.59	1.02
	8	6.52	1.14	-19.55	0.07	5.39	14.80	0.51	55.50	0.95
	10	6.60	1.02	-16.51	0.06	5.65	13.11	0.43	43.85	1.05
	12	6.78	1.00	-17.72	0.06	4.32	10.14	0.60	43.96	0.73
KEN-S-3										
Tree 5	-4	7.35	1.05	-26.28	0.07	5.48	11.43	4.98	31.07	0.87
	-2	7.34	0.91	-23.97	0.06	5.53	8.28	4.25	21.90	0.91
	0	7.39	0.88	-22.94	0.06	5.74	8.41	6.83	23.95	1.16
	2	7.53	1.04	-25.48	0.07	6.04	7.94	2.63	23.83	1.37
	4	7.86	0.95	-23.50	0.06	5.46	6.49	2.26	18.34	0.97
	6	7.51	1.09	-22.65	0.06	5.18	11.54	3.44	31.66	0.97
	8	7.57	0.99	-22.68	0.06	5.11	9.11	0.85	35.85	2.19
	10	7.20	0.81	-21.00	0.05	4.78	7.46	0.44	27.06	0.98
	12	7.07	0.61	-20.09	0.03	4.43	11.89	0.85	42.38	1.56
TEN-S-1										
Tree 1	-4	6.85	0.82	-20.65	0.05	4.44	10.71	0.53	12.98	0.76
	-2	6.73	0.81	-20.30	0.06	4.22	20.57	1.22	21.76	0.49
	0	6.53	0.78	-19.70	0.05	4.84	18.24	0.77	25.54	0.65
	2	6.92	0.38	-19.51	0.03	3.81	5.57	0.45	12.28	0.37
	4	6.78	0.72	-19.85	0.04	5.15	10.86	0.61	18.17	0.49
	6	6.44	0.83	-20.75	0.04	4.42	13.03	0.52	17.52	0.61
	8	7.07	0.74	-20.63	0.05	4.26	6.37	0.49	16.34	0.59
	10	6.60	0.80	-20.25	0.04	4.83	17.51	0.57	17.32	0.57
	12	6.37	0.49	-15.83	0.04	5.63	5.56	0.26	8.77	0.26
Tree 2	-4	6.68	0.68	-20.75	0.05	5.55	28.27	0.58	24.19	0.58
	-2	6.75	0.79	-22.34	0.06	3.65	9.47	0.57	18.72	1.05
	0	6.84	0.78	-21.51	0.05	4.48	6.50	0.51	16.68	0.51
	2	6.68	0.61	-21.08	0.05	5.67	6.68	0.31	8.52	0.59
	4	6.89	0.75	-23.42	0.05	5.00	5.09	0.40	14.43	0.52
	6	6.41	0.54	-18.11	0.04	5.60	7.94	0.28	14.56	0.41
	8	6.42	0.86	-20.65	0.06	5.48	12.83	0.46	15.46	0.66
	10	6.23	0.55	-16.70	0.04	5.52	7.62	0.28	9.21	0.42
	12	6.77	0.65	-20.44	0.05	5.83	9.55	0.39	14.03	0.57

ID	**Dist**	**pH**	**C**	**δ^{13}C**	**N**	**δ^{15}N**	**Fe**	**Zn**	**Mn**	**Cu**
	m		%	$^{0}/_{00}$	%	$^{0}/_{00}$	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}
TEN-S-2										
Tree 3	-4	6.33	0.42	-18.99	0.03	6.13	15.81	0.25	25.77	0.40
	-2	6.64	0.47	-21.51	0.03	4.76	14.75	0.26	30.89	0.46
	0	6.06	0.43	-19.44	0.03	6.04	24.89	0.38	40.62	0.51
	2	6.02	0.61	-19.65	0.04	4.59	32.69	0.44	49.97	0.65
	4	6.70	0.57	-20.43	0.03	4.28	12.75	0.41	27.05	0.47
	6	6.23	1.00	-22.97	0.04	4.25	18.14	0.49	43.19	0.73
	8	5.74	0.47	-17.35	0.03	5.85	24.55	0.22	49.02	0.50
	10	6.04	0.45	-18.56	0.03	5.21	17.88	0.29	40.20	0.33
	12	5.87	0.47	-19.54	0.03	5.38	13.22	0.23	41.69	0.44
Tree 4	-4	6.34	0.39	-21.52	0.03	3.98	26.77	0.41	22.51	0.38
	-2	6.56	0.36	-19.83	0.02	4.79	27.61	0.33	23.96	0.36
	0	6.15	0.28	-18.11	0.02	5.15	16.92	1.06	37.65	0.51
	2	5.95	0.24	-16.50	0.01	5.04	14.22	0.30	36.28	0.35
	4	5.96	0.27	-16.98	0.02	4.47	15.45	0.26	40.18	0.39
	6	5.75	0.38	-15.85	0.02	5.76	14.08	0.23	34.50	0.33
	8	5.45	0.43	-15.81	0.03	7.09	20.02	0.19	43.90	0.41
	10	5.26	0.30	-16.94	0.02	5.34	18.17	0.15	40.00	0.28
	12	5.45	0.28	-17.17	0.02	4.34	16.11	0.26	38.24	0.39
TEN-S-3										
Tree 5	-4	6.34	0.55	-21.19	0.04	5.69	37.33	2.82*	26.14	0.70
	-2	6.88	0.74	-21.14	0.05	6.06	34.32	3.17*	25.84	0.72
	0	6.87	0.63	-22.12	0.05	5.54	21.87	2.51*	24.01	0.64
	2	6.75	0.55	-20.94	0.04	5.09	21.27	12.34*	29.19	0.70
	4	6.84	0.40	-20.14	0.03	5.22	14.62	2.15*	19.65	0.48
	6	6.82	0.31	-20.03	0.02	5.80	9.23	1.67*	18.90	0.45
	8	6.82	0.30	-19.04	0.03	6.47	7.82	1.23*	17.67	0.43
	10	7.34	0.37	-22.34	0.03	5.25	3.96	1.54*	11.97	0.46
	12	7.10	0.50	-17.13	0.05	5.66	6.11	3.11*	23.55	0.79
Tree 6	-4	6.64	0.48	-22.48	0.04	6.05	23.99	3.32*	15.22	0.50
	-2	6.32	0.25	-19.25	0.02	6.55	15.29	1.80*	23.85	0.55
	0	6.39	0.37	-18.31	0.03	5.22	21.85	2.22*	28.86	0.55
	2	6.86	0.34	-17.07	0.03	5.40	9.31	1.30*	19.24	0.40
	4	7.04	0.33	-19.34	0.03	5.49	12.28	2.50*	18.38	0.63
	6	7.08	0.38	-18.97	0.03	4.50	8.33	1.54*	19.66	0.52

ID	**Dist**	**pH**	**C**	**δ^{13}C**	**N**	**δ^{15}N**	**Fe**	**Zn**	**Mn**	**Cu**
	m		%	$^{0}/_{00}$	%	$^{0}/_{00}$	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}
TEN-S-3										
Tree 6	8	7.19	0.44	-20.05	0.03	5.87	11.10	3.18*	21.61	0.88
	10	6.59	0.32	-18.19	0.03	7.09	8.19	0.77*	21.00	0.36
	12	6.82	0.42	-20.56	0.04	5.31	11.12	2.26*	16.57	0.61
WAC-S-1										
Tree 1	-4	7.57	1.28	-23.66	0.10	4.27	28.86	1.04	11.84	1.74
	-2	7.36	1.86	-23.38	0.13	4.25	35.44	0.74	11.30	1.47
	0	6.96	0.83	-17.44	0.06	4.50	24.72	0.64	14.24	1.20
	2	6.49	0.72	-18.79	0.06	4.91	33.33	0.67	18.48	1.56
	4	5.98	0.57	-15.23	0.05	5.66	40.30	0.48	20.47	1.57
	6	5.73	0.74	-15.53	0.06	5.36	43.96	0.46	23.96	1.32
	8	5.68	0.48	-14.67	0.04	5.56	55.70	0.34	26.82	1.07
	10	6.16	0.67	-18.10	0.05	5.23	64.78	0.56	31.54	1.44
	12	5.89	0.88	-16.09	0.06	4.97	41.63	0.46	20.69	1.29
Tree 2	-4	6.61	0.52	-21.33	0.04	5.28	43.59	0.61	12.81	1.14
	-2	5.91	0.54	-17.62	0.04	5.06	68.49	0.50	23.58	1.02
	0	5.82	0.63	-16.60	0.05	5.78	64.42	0.53	21.30	1.08
	2	6.04	0.50	-15.95	0.04	5.68	57.63	0.70	16.69	0.99
	4	5.78	0.58	-14.49	0.04	6.01	54.92	0.51	19.16	1.03
	6	6.02	0.51	-15.60	0.04	5.64	38.00	0.75	13.69	0.78
	8	5.97	0.45	-15.93	0.03	4.96	35.42	0.33	15.31	0.82
	10	5.89	0.52	-15.20	0.04	5.55	48.57	0.38	16.39	0.86
	12	5.98	0.55	-16.02	0.04	5.39	56.98	0.35	20.05	0.84
WAC-S-2										
Tree 3	-4	7.06	0.57	-21.87	0.04	4.42	10.83	0.37	13.44	0.59
	-2	6.37	0.72	-22.05	0.05	4.55	37.74	0.39	29.62	0.59
	0	6.11	0.60	-19.78	0.04	4.41	39.92	0.36	36.29	0.55
	2	5.89	0.45	-18.43	0.03	4.52	33.97	0.54	36.98	0.61
	4	5.60	0.48	-18.54	0.03	4.64	33.88	0.27	34.75	0.54
	6	5.56	0.43	-16.51	0.03	6.32	36.85	0.28	38.99	0.66
	8	5.89	0.46	-17.79	0.03	4.85	24.08	0.32	35.10	0.51
	10	5.91	0.53	-16.02	0.04	5.60	28.01	0.33	47.75	0.47
	12	5.85	0.46	-17.39	0.04	5.48	23.03	0.30	44.42	0.51

ID	Dist	pH	C	δ^{13}C	N	δ^{15}N	Fe	Zn	Mn	Cu
	m		%	$^{0}/_{00}$	%	$^{0}/_{00}$	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}
WAC-S-3										
Tree 4	-4	6.90	0.73	-22.57	0.06	7.04	38.05	1.04	12.15	1.57
	-2	6.95	0.54	-20.88	0.04	5.98	29.82	0.58	7.32	1.17
	0	6.49	0.58	-19.42	0.04	7.25	29.62	0.95	12.60	1.13
	2	5.58	0.56	-18.78	0.05	7.85	61.34	0.50	14.63	1.06
	4	5.37	0.55	-16.09	0.04	7.02	36.96	0.49	15.37	0.99
	6	6.14	0.69	-19.10	0.04	7.26	19.72	0.29	12.73	0.92
	8	6.24	0.46	-16.22	0.04	6.70	21.48	0.51	10.55	1.14
	10	6.17	0.39	-19.45	0.03	7.13	20.80	0.38	9.82	0.89
	12	5.87	0.49	-16.56	0.04	6.82	37.06	0.59	10.36	1.09
Tree 5	-4	6.69	0.89	-23.54	0.06	5.89	33.65	0.60	11.41	1.00
	-2	6.39	0.63	-21.57	0.05	5.73	46.50	0.71	15.62	1.04
	0	5.79	0.50	-18.72	0.03	6.35	25.66	0.31	21.16	0.66
	2	5.39	0.51	-17.92	0.04	6.94	34.82	0.36	18.05	0.94
	4	5.40	0.45	-16.91	0.03	6.46	23.68	0.36	20.72	0.83
	6	5.46	0.44	-17.15	0.03	6.40	20.72	0.39	17.28	0.89
	8	5.89	0.50	-17.93	0.04	7.04	16.76	0.30	13.78	0.96
	10	5.71	0.45	-19.16	0.03	5.94	25.52	0.36	20.46	0.68
	12	5.26	0.40	-17.12	0.04	5.99	22.24	0.33	14.95	0.80
YEK-S-1										
Tree 1	-4	8.16	3.22	-23.44	0.23	3.36	5.85	7.09	26.00	6.43
	-2	8.09	4.13	-23.14	0.25	3.39	6.24	7.58	20.88	2.30
	0	8.02	3.56	-22.05	0.24	3.36	8.40	4.87	28.97	3.33
	2	7.85	3.11	-22.11	0.22	3.03	6.43	4.78	25.25	2.47
	4	7.53	3.30	-21.21	0.22	2.93	8.82	4.38	35.53	2.66
	6	7.61	2.81	-20.23	0.18	2.64	6.76	4.47	29.76	2.83
	8	7.69	2.59	-23.16	0.18	2.54	6.29	4.78	34.57	3.01
	10	7.51	1.79	-21.82	0.12	4.00	5.67	4.85	37.17	3.55
	12	7.33	1.87	-21.38	0.12	4.19	6.10	6.02	37.49	3.54
Tree 2	-4	7.66	4.47	-21.34	0.29	4.03	11.55	2.80	33.30	4.54
	-2	7.48	3.31	-19.55	0.20	3.87	21.79	3.84	48.67	5.29
	0	7.60	3.79	-19.95	0.23	3.47	10.08	3.61	26.32	2.87
	2	7.46	2.90	-19.04	0.17	4.53	11.54	1.51	38.82	4.09
	4	7.73	3.40	-21.11	0.21	2.96	7.86	2.58	27.58	2.13
	6	7.78	2.82	-19.21	0.17	4.45	12.16	2.27	34.47	4.56

ID	Dist m	pH	C %	δ^{13}C ‰	N %	δ^{15}N ‰	Fe mg kg^{-1}	Zn mg kg^{-1}	Mn mg kg^{-1}	Cu mg kg^{-1}
YEK-S-1										
Tree 2	8	7.09	2.81	-15.32	0.11	4.70	10.70	0.99	36.94	3.38
	10	7.19	1.93	-16.87	0.11	4.86	11.27	0.84	28.05	3.96
	12	7.08	2.03	-18.09	0.12	4.54	12.99	0.81	45.22	4.44
Tree 3	-4	7.83	2.78	-19.91	0.19	4.08	7.41	1.47	20.23	4.27
	-2	7.70	3.28	-20.38	0.21	4.30	12.00	2.21	32.12	4.05
	0	7.34	2.62	-17.98	0.16	3.66	12.23	2.27	28.33	4.04
	2	7.43	2.09	-15.88	0.13	4.49	13.59	0.92	45.09	5.16
	4	7.16	1.92	-15.79	0.11	4.46	11.58	1.20	43.18	4.77
	6	7.37	1.52	-16.44	0.09	4.35	7.25	1.51	25.19	4.13
	8	7.35	1.53	-16.19	0.10	4.92	12.14	1.53	35.29	5.51
	10	7.24	1.29	-15.45	0.09	5.45	6.76	0.63	20.06	3.66
	12	7.18	1.47	-15.11	0.10	5.74	11.18	0.76	38.03	5.14
YEK-S-1										
Tree 4	-2	7.47	1.31	-19.96	0.10	5.13	7.41	0.74	34.93	1.88
	0	7.41	1.04	-20.47	0.07	3.53	4.48	0.55	25.27	1.18
	2	7.02	1.01	-19.12	0.07	4.91	6.71	0.47	34.38	1.30
	4	6.81	0.99	-18.46	0.07	5.26	8.03	0.53	46.54	1.61
	6	7.37	1.35	-22.78	0.10	4.77	6.77	0.53	30.59	1.14
	8	7.35	1.77	-24.06	0.12	3.55	10.92	0.87	52.40	1.41
	10	7.19	2.59	-23.96	0.15	4.14	12.00	0.94	51.91	2.74
	12	7.10	1.19	-23.01	0.09	4.72	10.94	0.79	36.61	2.14
Tree 5	-2	7.40	1.16	-18.29	0.08	5.29	6.46	0.53	39.94	1.67
	0	7.45	2.13	-22.37	0.13	3.61	4.98	0.46	26.52	0.94
	2	7.29	1.88	-20.52	0.12	3.84	13.24	0.74	59.85	1.08
	4	7.09	0.92	-18.45	0.06	6.23	6.85	0.46	36.28	1.49
	6	6.89	0.90	-18.14	0.06	5.42	15.10	0.51	50.85	1.56
	8	7.09	1.26	-18.73	0.09	4.43	9.39	6.09	33.58	0.74
	10	7.26	1.11	-18.19	0.08	4.80	11.45	3.13	44.73	0.78
	12	7.21	1.82	-19.41	0.12	4.43	8.46	2.11	33.48	0.80
Tree 6	-2	7.24	1.25	-20.33	0.08	5.16	10.24	0.67	35.85	2.98
	0	7.16	0.97	-20.01	0.06	4.52	7.58	0.70	27.74	1.68
	2	7.00	1.15	-19.57	0.08	4.87	10.69	0.58	34.77	1.96
	4	7.22	1.55	-21.15	0.09	4.52	10.67	0.95	36.95	2.34
	6	7.30	1.17	-20.77	0.08	4.66	8.04	0.74	31.28	1.96
	8	7.48	0.98	-20.66	0.06	4.27	7.50	0.55	36.68	1.49
	10	6.81	1.37	-18.20	0.09	5.82	12.56	0.48	55.25	2.38
	12	6.24	1.14	-17.60	0.07	6.04	14.67	0.31	46.42	2.18

Table B2: Data from soil physical property analysis by location, site, and tree. KEN = Keniero, TIG = Tiguere, WAC = Wacoro, FS = Field Station, S-1 = site number, Dist = distance from the edge of the tree canopy, Conc = % concretions, Mott = mottles present (Y = yes, N = no, F = few, FSF = few soft mottles, SM = some), red, green, blue = soil color tristimulus values, hueN = hue number, rededx = redness index.

ID	Dist	Conc	Mott	red	green	blue	hueN	value	chroma	redidx
	m	%					YR			
KEN-S-1										
Tree 1	-4	0	N	0.566	0.465	0.356	8.55	5.05	4.78	8.939
	-2	0	N	0.527	0.433	0.337	8.21	4.72	4.35	10.117
	0	0	N	0.538	0.443	0.348	8.07	4.82	4.38	9.554
	2	0	N	0.564	0.467	0.367	8.27	5.06	4.54	8.490
	4	0	N	0.552	0.455	0.354	8.30	4.94	4.56	9.139
	6	0	N	0.544	0.447	0.347	8.22	4.87	4.52	9.530
	8	0	N	0.557	0.459	0.356	8.31	4.98	4.62	9.044
	10	0	N	0.544	0.450	0.351	8.46	4.89	4.46	9.252
	12	0	N	0.555	0.456	0.350	8.41	4.96	4.67	9.282
Tree 2	-4	0	N	0.565	0.465	0.355	8.63	5.05	4.77	8.928
	-2	0	N	0.554	0.458	0.357	8.35	4.97	4.54	8.963
	0	0	N	0.578	0.477	0.366	8.56	5.17	4.81	8.425
	2	0	N	0.582	0.476	0.361	8.23	5.17	4.94	8.717
	4	0	N	0.576	0.471	0.358	8.40	5.12	4.91	8.858
	6	0	N	0.566	0.465	0.355	8.59	5.06	4.81	8.967
	8	0	N	0.574	0.474	0.365	8.52	5.14	4.75	8.471
	10	0	N	0.570	0.470	0.362	8.38	5.10	4.73	8.663
	12	0	N	0.576	0.468	0.351	8.25	5.09	5.01	9.233
KEN-S-2										
Tree 3	-2	101	N	0.431	0.369	0.297	9.69	3.99	3.26	12.458
	0	24	N	0.426	0.353	0.275	8.87	3.84	3.57	15.008
	2	12	N	0.438	0.359	0.277	8.45	3.92	3.71	15.009
	4	23	N	0.463	0.379	0.292	8.23	4.14	3.88	13.505
	6	15	N	0.471	0.383	0.296	7.88	4.19	3.94	13.303
	8	19	N	0.469	0.379	0.292	7.67	4.15	3.98	13.808
	10	37	N	0.488	0.396	0.304	7.81	4.33	4.14	12.665
	12	14	N	0.483	0.391	0.304	7.54	4.28	4.05	12.868

ID	**Dist**	**pH**	**C**	**δ^{13}C**	**N**	**δ^{15}N**	**Fe**	**Zn**	**Mn**	**Cu**
	m		%	$^{0}/_{00}$	%	$^{0}/_{00}$	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}
KEN-S-2										
Tree 4	-2	7.23	1.03	-19.59	0.07	4.64	15.31	3.96	40.36	1.00
	0	7.20	0.79	-16.68	0.05	4.84	7.79	0.36	20.65	0.34
	2	6.96	0.78	-16.85	0.05	4.94	14.55	0.45	33.20	0.54
	4	6.71	0.74	-16.99	0.05	5.15	16.06	3.62	49.59	0.82
	6	6.74	1.07	-15.54	0.06	6.00	15.43	0.38	56.59	1.02
	8	6.52	1.14	-19.55	0.07	5.39	14.80	0.51	55.50	0.95
	10	6.60	1.02	-16.51	0.06	5.65	13.11	0.43	43.85	1.05
	12	6.78	1.00	-17.72	0.06	4.32	10.14	0.60	43.96	0.73
KEN-S-3										
Tree 5	-4	7.35	1.05	-26.28	0.07	5.48	11.43	4.98	31.07	0.87
	-2	7.34	0.91	-23.97	0.06	5.53	8.28	4.25	21.90	0.91
	0	7.39	0.88	-22.94	0.06	5.74	8.41	6.83	23.95	1.16
	2	7.53	1.04	-25.48	0.07	6.04	7.94	2.63	23.83	1.37
	4	7.86	0.95	-23.50	0.06	5.46	6.49	2.26	18.34	0.97
	6	7.51	1.09	-22.65	0.06	5.18	11.54	3.44	31.66	0.97
	8	7.57	0.99	-22.68	0.06	5.11	9.11	0.85	35.85	2.19
	10	7.20	0.81	-21.00	0.05	4.78	7.46	0.44	27.06	0.98
	12	7.07	0.61	-20.09	0.03	4.43	11.89	0.85	42.38	1.56
TEN-S-1										
Tree 1	-4	6.85	0.82	-20.65	0.05	4.44	10.71	0.53	12.98	0.76
	-2	6.73	0.81	-20.30	0.06	4.22	20.57	1.22	21.76	0.49
	0	6.53	0.78	-19.70	0.05	4.84	18.24	0.77	25.54	0.65
	2	6.92	0.38	-19.51	0.03	3.81	5.57	0.45	12.28	0.37
	4	6.78	0.72	-19.85	0.04	5.15	10.86	0.61	18.17	0.49
	6	6.44	0.83	-20.75	0.04	4.42	13.03	0.52	17.52	0.61
	8	7.07	0.74	-20.63	0.05	4.26	6.37	0.49	16.34	0.59
	10	6.60	0.80	-20.25	0.04	4.83	17.51	0.57	17.32	0.57
	12	6.37	0.49	-15.83	0.04	5.63	5.56	0.26	8.77	0.26
Tree 2	-4	6.68	0.68	-20.75	0.05	5.55	28.27	0.58	24.19	0.58
	-2	6.75	0.79	-22.34	0.06	3.65	9.47	0.57	18.72	1.05
	0	6.84	0.78	-21.51	0.05	4.48	6.50	0.51	16.68	0.51
	2	6.68	0.61	-21.08	0.05	5.67	6.68	0.31	8.52	0.59
	4	6.89	0.75	-23.42	0.05	5.00	5.09	0.40	14.43	0.52
	6	6.41	0.54	-18.11	0.04	5.60	7.94	0.28	14.56	0.41
	8	6.42	0.86	-20.65	0.06	5.48	12.83	0.46	15.46	0.66
	10	6.23	0.55	-16.70	0.04	5.52	7.62	0.28	9.21	0.42
	12	6.77	0.65	-20.44	0.05	5.83	9.55	0.39	14.03	0.57

ID	Dist	Conc	Mott	red	green	blue	hueN	value	chroma	redidx
	m	%					YR			
TEN-S-2										
Tree 3	-4	0	N	0.563	0.479	0.395	7.54	5.16	4.08	7.331
	-2	0	N	0.568	0.483	0.402	7.50	5.20	4.04	7.147
	0	0	N	0.567	0.481	0.397	8.05	5.18	4.10	7.289
	2	0	N	0.526	0.447	0.367	8.41	4.82	3.85	8.448
	4	0	N	0.518	0.440	0.362	8.38	4.75	3.79	8.695
	6	0	N	0.557	0.468	0.378	8.06	5.05	4.04	8.027
	8	0	N	0.571	0.482	0.398	7.80	5.20	4.14	7.297
	10	0	N	0.576	0.486	0.402	7.74	5.24	4.16	7.187
	12	0	N	0.531	0.448	0.369	7.94	4.84	3.87	8.497
Tree 4	-4	0	N	0.610	0.523	0.448	7.35	5.60	4.07	5.790
	-2	0	N	0.630	0.545	0.470	7.47	5.82	4.12	5.224
	0	0	N	0.604	0.518	0.445	7.19	5.55	4.03	5.914
	2	0	N	0.623	0.534	0.455	7.42	5.72	4.22	5.603
	4	0	N	0.601	0.512	0.431	7.47	5.50	4.19	6.243
	6	0	N	0.581	0.492	0.408	7.50	5.30	4.16	6.947
	8	0	N	0.582	0.491	0.407	7.50	5.29	4.23	7.011
	10	0	N	0.548	0.464	0.386	7.50	5.00	3.94	7.807
	12	0	N	0.583	0.496	0.417	7.50	5.34	4.03	6.671
TEN-S-3										
Tree 5	-4	0	N	0.572	0.491	0.412	7.57	5.27	3.91	6.716
	-2	0	N	0.582	0.506	0.433	7.79	5.42	3.60	6.031
	0	0	N	0.588	0.507	0.430	7.57	5.43	3.81	6.179
	2	0	N	0.583	0.499	0.419	7.53	5.36	3.97	6.525
	4	0	N	0.588	0.504	0.423	7.56	5.40	4.00	6.391
	6	0	N	0.584	0.497	0.413	7.56	5.34	4.10	6.709
	8	0	N	0.591	0.502	0.418	7.50	5.40	4.15	6.596
	10	0	N	0.612	0.520	0.433	7.50	5.58	4.27	6.137
	12	0	N	0.611	0.522	0.437	7.57	5.60	4.14	5.999
Tree 6	-4	0	N	0.593	0.509	0.428	7.60	5.45	4.00	6.242
	-2	0	N	0.619	0.531	0.448	7.51	5.69	4.10	5.715
	0	0	N	0.615	0.527	0.445	7.56	5.65	4.08	5.785
	2	0	N	0.579	0.494	0.412	7.58	5.31	4.05	6.743
	4	0	N	0.606	0.521	0.442	7.56	5.58	3.86	5.859
	6	0	N	0.582	0.496	0.413	7.55	5.33	4.06	6.704

ID	Dist m	Conc %	Mott	red	green	blue	hueN YR	value	chroma	redidx
TEN-S-3										
Tree 6	8	0	N	0.574	0.491	0.408	7.73	5.27	3.85	6.829
	10	0	N	0.582	0.494	0.409	7.56	5.32	4.15	6.856
	12	0	N	0.617	0.525	0.441	7.50	5.64	4.20	5.948
WAC-S-1										
Tree 1	-4	0	N	0.541	0.476	0.406	8.54	5.09	3.34	6.707
	-2	0	N	0.546	0.486	0.418	9.15	5.18	3.21	6.211
	0	0	N	0.544	0.483	0.414	9.04	5.15	3.24	6.355
	2	7	N	0.575	0.509	0.442	8.26	5.43	3.29	5.672
	4	10	N	0.561	0.493	0.421	8.51	5.27	3.46	6.244
	6	0	N	0.577	0.509	0.442	8.07	5.43	3.31	5.703
	8	0	N	0.568	0.500	0.432	8.02	5.33	3.31	5.982
	10	0	N	0.574	0.508	0.441	8.23	5.41	3.27	5.695
	12	0	N	0.569	0.505	0.438	8.47	5.38	3.26	5.752
Tree 2	-4	0	N	0.600	0.524	0.446	8.14	5.59	3.72	5.609
	-2	0	N	0.605	0.534	0.459	8.42	5.68	3.50	5.227
	0	0	N	0.590	0.515	0.442	7.89	5.50	3.63	5.757
	2	0	N	0.582	0.514	0.442	8.33	5.47	3.41	5.668
	4	0	N	0.583	0.514	0.442	8.23	5.48	3.41	5.674
	6	0	N	0.583	0.510	0.437	8.09	5.45	3.59	5.857
	8	0	N	0.570	0.501	0.430	8.33	5.35	3.39	5.989
	10	0	N	0.588	0.512	0.433	8.33	5.47	3.68	5.922
	12	0	N	0.580	0.515	0.445	8.55	5.48	3.33	5.556
WAC-S-2										
Tree 3	-4	0	N	0.617	0.551	0.487	7.95	5.84	3.24	4.670
	-2	0	N	0.590	0.527	0.466	7.96	5.60	3.12	5.100
	0	0	N	0.584	0.522	0.463	7.86	5.54	3.09	5.193
	2	0	N	0.592	0.529	0.468	7.97	5.62	3.13	5.060
	4	0	N	0.588	0.525	0.465	7.94	5.58	3.12	5.133
	6	0	N	0.593	0.530	0.470	7.93	5.63	3.11	5.018
	8	0	N	0.594	0.531	0.470	7.91	5.64	3.13	5.017
	10	0	N	0.577	0.516	0.465	7.45	5.49	3.07	5.201
	12	0	N	0.589	0.526	0.471	7.54	5.59	3.12	5.050

ID	Dist	Conc	Mott	red	green	blue	hueN	value	chroma	redidx
	m	%					YR			
WAC-S-3										
Tree 4	-4	0	N	0.643	0.574	0.509	7.75	6.07	3.31	4.287
	-2	0	N	0.642	0.570	0.496	8.12	6.04	3.50	4.480
	0	0	N	0.629	0.558	0.483	8.29	5.92	3.51	4.712
	2	10	F	0.650	0.568	0.488	7.62	6.03	3.74	4.733
	4	0	N	0.632	0.559	0.481	8.36	5.93	3.58	4.740
	6	0	N	0.634	0.561	0.484	8.21	5.95	3.57	4.711
	8	0	N	0.657	0.582	0.506	7.98	6.16	3.59	4.333
	10	0	N	0.654	0.578	0.499	8.06	6.12	3.63	4.436
	12	11	SM	0.654	0.574	0.499	7.50	6.09	3.88	4.543
Tree 5	-4	0	N	0.615	0.532	0.456	7.54	5.69	3.85	5.486
	-2	0	N	0.606	0.525	0.449	7.59	5.61	3.82	5.654
	0	0	N	0.606	0.525	0.447	7.71	5.61	3.76	5.675
	2	0	FSF	0.626	0.540	0.458	7.66	5.77	3.92	5.437
	4	0	FSF	0.616	0.532	0.454	7.54	5.68	3.87	5.546
	6	0	FSF	0.632	0.546	0.464	7.63	5.82	3.93	5.285
	8	0	FSF	0.637	0.551	0.471	7.59	5.88	3.94	5.135
	10	0	FSF	0.630	0.547	0.471	7.55	5.83	3.91	5.152
	12	0	FSF	0.657	0.568	0.484	7.74	6.05	4.04	4.852
YEK-S-1										
Tree 1	-4	144	N	0.471	0.383	0.296	7.87	4.19	3.94	13.343
	-2	101	N	0.434	0.356	0.277	8.23	3.88	3.63	15.109
	0	93	N	0.425	0.349	0.273	8.43	3.81	3.56	15.502
	2	151	N	0.422	0.350	0.277	8.53	3.81	3.45	15.026
	4	153	N	0.410	0.338	0.260	9.01	3.68	3.59	16.694
	6	174	N	0.414	0.341	0.264	8.76	3.71	3.57	16.384
	8	170	N	0.444	0.361	0.277	8.08	3.95	3.78	15.107
	10	265	N	0.434	0.351	0.265	8.45	3.84	3.92	16.446
	12	152	N	0.437	0.351	0.261	8.62	3.85	4.19	16.974
Tree 2	-4	27	N	0.382	0.312	0.234	9.11	3.39	3.68	20.601
	-2	30	N	0.377	0.309	0.234	9.35	3.36	3.63	20.531
	0	7	N	0.394	0.322	0.244	9.02	3.51	3.71	19.028
	2	28	N	0.395	0.316	0.229	9.07	3.46	4.08	21.447
	4	7	N	0.424	0.346	0.270	8.08	3.78	3.59	16.017
	6	18	N	0.400	0.325	0.244	8.83	3.54	3.78	19.198

ID	Dist	Conc	Mott	red	green	blue	hueN	value	chroma	redidx
	m	%					YR			
YEK-S-1										
Tree 2	8	97	N	0.416	0.341	0.264	8.41	3.71	3.58	16.549
	10	25	N	0.410	0.330	0.244	8.93	3.61	4.06	19.172
	12	35	N	0.430	0.345	0.258	8.62	3.79	4.10	17.339
Tree 3	-4	9	N	0.424	0.337	0.248	8.42	3.71	4.20	18.819
	-2	32	N	0.377	0.308	0.231	9.18	3.34	3.67	21.231
	0	18	N	0.411	0.331	0.249	8.53	3.62	3.82	18.658
	2	16	N	0.415	0.326	0.237	7.85	3.59	4.27	21.076
	4	24	N	0.402	0.318	0.233	8.14	3.49	4.11	21.631
	6	9	N	0.417	0.328	0.240	7.93	3.61	4.24	20.565
	8	34	N	0.396	0.313	0.227	8.31	3.43	4.12	22.490
	10	11	N	0.422	0.323	0.228	7.69	3.58	4.70	23.077
	12	42	N	0.403	0.314	0.228	7.85	3.46	4.30	23.059
YEK-S-1										
Tree 4	-2	67	N	0.533	0.436	0.342	7.64	4.75	4.36	10.040
	0	66	N	0.555	0.456	0.361	7.61	4.95	4.46	9.035
	2	52	N	0.537	0.438	0.345	7.52	4.78	4.44	9.967
	4	60	N	0.543	0.445	0.352	7.59	4.85	4.38	9.506
	6	81	N	0.535	0.444	0.356	7.84	4.82	4.19	9.220
	8	60	N	0.530	0.442	0.356	8.04	4.80	4.10	9.124
	10	106	N	0.524	0.432	0.344	7.80	4.70	4.16	9.862
	12	52	N	0.527	0.433	0.342	7.77	4.71	4.25	10.028
Tree 5	-2	111	N	0.512	0.414	0.326	7.46	4.53	4.39	11.352
	0	136	N	0.527	0.429	0.342	7.50	4.68	4.31	10.310
	2	83	N	0.522	0.432	0.347	7.82	4.70	4.07	9.718
	4	71	N	0.568	0.467	0.372	7.47	5.07	4.58	8.520
	6	59	N	0.553	0.456	0.359	7.90	4.95	4.47	8.994
	8	43	N	0.557	0.464	0.372	8.03	5.03	4.34	8.351
	10	55	N	0.531	0.437	0.348	7.60	4.75	4.21	9.710
	12	26	N	0.504	0.422	0.342	8.05	4.58	3.72	9.911
Tree 6	-2	30	N	0.520	0.413	0.318	7.29	4.54	4.68	12.051
	0	25	N	0.523	0.418	0.325	7.11	4.58	4.61	11.545
	2	19	N	0.515	0.412	0.324	7.01	4.52	4.50	11.688
	4	0	N	0.499	0.402	0.315	7.45	4.41	4.31	12.158
	6	22	N	0.495	0.391	0.298	7.34	4.31	4.55	13.708
	8	23	N	0.502	0.401	0.313	7.10	4.40	4.41	12.536
	10	0	N	0.513	0.415	0.328	7.46	4.54	4.38	11.250
	12	33	N	0.525	0.418	0.323	7.27	4.59	4.67	11.654

Table B3: Data from sorghum whole grain by location, site, and tree. KEN = Keniero, TIG = Tiguere, WAC = Wacoro, FS = Field Station, S-1 = site number, Dist = distance from the edge of the tree canopy, Glm = glume color (B = black (also purple), R = red, M = mix of black and red), Peri = pericarp thickness, GW = 100 grain weight, PW = average panicle weight, PL = average panicle length, Color = grain color (W = white, T = tan), red, green, blue = soil color tristimulus values, lightness = grain lightness, Zn = grain Zn concentration.

ID	**Dist**	**Glm**	**Peri**	**GW**	**PW**	**PL**	**Color**	**red**	**green**	**blue**	**light**	**Zn**
	m			g	g	cm						mg kg^{-1}
KEN-S-1												
Tree 1	0	B	Thick	2.78	49	30	W	0.841	0.769	0.686	0.764	23
	2	B	Thick	2.97	111	43	W	0.837	0.763	0.684	0.760	21
	4	B	Thin	2.69	53	36	T	0.829	0.753	0.672	0.751	23
	6	B	Thick	2.54	77	39	W	0.846	0.775	0.692	0.769	21
	8	M	Mix	2.64	36	34	TW	0.846	0.773	0.691	0.768	22
	10	B	Mix	2.86	49	38	TW	0.854	0.782	0.701	0.778	24
	12	B	Mix	2.50	58	40	TW	0.834	0.758	0.677	0.756	23
Tree 2	0	B	Thick	2.78	85	41	W	0.845	0.773	0.693	0.769	21
	2	B	Thick	2.80	90	40	W	0.841	0.762	0.682	0.761	21
	4	M	Mix	2.55	43	32	TW	0.855	0.783	0.698	0.777	20
	6	B	Thick	2.66	91	46	W	0.850	0.778	0.697	0.774	21
	8	B	Mix	2.63	87	43	TW	0.845	0.770	0.689	0.767	21
	10	B	Thick	2.48	57	32	W	0.854	0.781	0.698	0.776	19
	12	B	Thick	2.65	71	39	W	0.829	0.752	0.679	0.754	21
KEN-S-2												
Tree 3	0	R	Thick	2.12	43	32	W	0.869	0.808	0.714	0.791	18
	2	R	Thick	2.10	47	37	W	0.866	0.803	0.712	0.789	19
	4	R	Mix	1.98	53	37	TW	0.859	0.798	0.703	0.781	17
	6	R	Thick	1.86	42	35	W	0.855	0.791	0.697	0.776	19
	8	R	Thick	1.79	39	32	W	0.876	0.815	0.720	0.798	18
	10	R	Thick	1.87	29	38	W	0.872	0.811	0.717	0.795	20
	12	R	Thick	1.72	35	36	W	0.857	0.794	0.702	0.780	17
Tree 4	0	R	Thick	2.05	23	30	W	0.873	0.812	0.720	0.797	22
	2	R	Thick	1.78	23	31	W	0.881	0.819	0.725	0.803	21
	4	R	Thick	1.70	29	32	W	0.862	0.799	0.706	0.784	17
	6	R	Thick	1.80	39	38	W	0.872	0.810	0.715	0.794	14
	8	R	Mix	1.72	33	37	TW	0.859	0.795	0.703	0.781	17
	10	R	Thin	1.81	29	34	W	0.868	0.808	0.712	0.790	14
	12	R	Thin	1.68	25	37	W	0.848	0.784	0.692	0.770	15

ID	Dist	Glm	Peri	GW	PW	PL	Color	red	green	blue	light	Zn
	m			g	g	cm						mg kg^{-1}
KEN-S-3												
Tree 5	0	R	Thick	2.58	138	49	W	0.872	0.810	0.715	0.793	28
	2	R	Mix	2.23	110	47	TW	0.853	0.790	0.692	0.773	29
	4	R	Mix	2.24	130	42	TW	0.851	0.785	0.690	0.771	24
	6	R	Mix	2.02	43	36	TW	0.851	0.786	0.686	0.768	31
	8	R	Thin	1.83	60	44	T	0.872	0.807	0.724	0.798	24
	10	R	Mix	1.93	98	46	TW	0.849	0.783	0.687	0.768	32
	12	R	Mix	2.20	79	42	TW	0.838	0.771	0.681	0.760	28
TEN-S-1												
Tree 1	0	B	Thick	2.56	26	36	W	0.854	0.789	0.701	0.778	16
	2	B	Thick	2.32	29	36	W	0.836	0.769	0.680	0.758	17
	4	B	Thick	2.27	37	37	W	0.846	0.781	0.693	0.769	15
	6	B	Thick	2.63	28	30	W	0.844	0.778	0.691	0.767	17
	8	B	Thick	2.61	21	36	W	0.842	0.777	0.687	0.765	18
	10	B	Thick	2.48	33	36	W	0.864	0.802	0.711	0.788	16
	12	B	Mix	2.12	32	30	TW	0.842	0.777	0.690	0.766	15
	12	B	Thick	2.06	32	30	G	0.795	0.709	0.639	0.717	16
Tree 2	0	B	Thick	2.46	35	35	W	0.867	0.804	0.724	0.795	15
	2	B	Thick	2.28	38	31	W	0.864	0.801	0.714	0.789	15
	4	B	Thick	2.21	20	29	W	0.864	0.802	0.713	0.789	14
	6	B	Thick	2.15	19	31	W	0.871	0.810	0.722	0.797	14
	8	B	Thick	2.15	19	32	W	0.858	0.795	0.711	0.785	14
	10	B	Thick	2.04	29	30	W	0.855	0.792	0.705	0.780	16
	12	B	Thick	2.27	30	34	W	0.858	0.794	0.709	0.783	14
TEN-S-2												
Tree 3	0	B	Thin	2.33	39	33	T	0.842	0.773	0.689	0.766	16
	2	B	Thin	2.19	28	32	T	0.846	0.779	0.694	0.770	20
	4	B	Thin	1.96	35	33	T	0.837	0.769	0.674	0.756	36*
	6	B	Thin	2.17	41	35	T	0.848	0.782	0.692	0.770	20
	8	B	Thin	2.27	41	37	T	0.845	0.780	0.694	0.769	19
	10	B	Thin	2.25	27	33	T	0.842	0.775	0.687	0.765	18
	12	B	Thin	2.24	22	32	T	0.854	0.788	0.699	0.777	16
Tree 4	0	B	Thin	2.53	44	34	T	0.845	0.778	0.689	0.767	23
	2	B	Thin	2.28	40	32	T	0.845	0.778	0.690	0.768	17
	4	B	Thin	2.31	25	31	T	0.839	0.773	0.682	0.760	17
	6	B	Thin	2.44	45	32	T	0.839	0.772	0.684	0.762	17

ID	Dist	Glm	Peri	GW	PW	PL	Color	red	green	blue	light	Zn
	m			g	g	cm						mg kg^{-1}
TEN-S-2												
Tree 4	8	B	Thin	2.31	35	33	T	0.846	0.778	0.690	0.768	21
	10	B	Thin	2.17	20	35	T	0.846	0.780	0.690	0.768	15
	12	B	Thin	2.06	18	32	T	0.832	0.763	0.671	0.751	18
TEN-S-3												
Tree 5	0	R	Thin	2.42	61	38	T	0.837	0.758	0.676	0.756	18
	2	R	Thin	2.44	58	34	T	0.833	0.755	0.669	0.751	17
	4	R	Thin	2.40	55	27	T	0.827	0.745	0.660	0.744	17
	6	R	Thin	2.39	91	40	T	0.851	0.777	0.693	0.772	16
	8	R	Thin	2.52	51	35	T	0.846	0.772	0.687	0.766	15
	10	R	Thin	2.26	37	36	T	0.848	0.776	0.690	0.769	18
	12	R	Thin	2.40	83	42	T	0.846	0.769	0.686	0.766	17
Tree 6	0	R	Thin	2.37	37	35	T	0.864	0.795	0.710	0.787	17
	2	R	Thin	2.26	29	36	T	0.864	0.797	0.708	0.786	16
	4	R	Thin	2.39	56	39	T	0.859	0.786	0.700	0.779	16
	6	R	Thin	2.16	30	29	T	0.854	0.779	0.692	0.773	15
	8	R	Thin	2.41	40	37	T	0.863	0.794	0.709	0.786	16
	10	R	Thin	2.33	55	35	T	0.851	0.781	0.692	0.772	16
	12	R	Thin	2.31	63	36	T	0.856	0.785	0.701	0.779	18
WAC-S-1												
Tree 1	0	M	Thin	2.49	33	32	T	0.826	0.755	0.665	0.746	21
	2	M	Thin	2.28	64	31	T	0.827	0.760	0.665	0.746	21
	4	R	Thick	2.52	18	25	W	0.822	0.744	0.661	0.742	23
	6	R	Thick	2.33	17	28	W	0.814	0.731	0.649	0.732	23
	8	R	Thin	2.06	31	28	T	0.814	0.741	0.654	0.734	18
	10	M	Thin	2.02	35	31	T	0.828	0.755	0.667	0.747	19
	12	R	Thin	2.20	47	33	T	0.823	0.749	0.661	0.742	20
Tree 2	0	M	Thick	2.24	39	34	W	0.780	0.694	0.614	0.697	26
	2	B	Thick	2.19	40	38	W	0.826	0.747	0.663	0.745	25
	4	B	Thick	2.04	41	37	W	0.831	0.757	0.675	0.753	25
	6	B	Thick	2.02	37	34	W	0.793	0.708	0.630	0.711	23
	8	B	Thick	2.19	30	41	W	0.798	0.711	0.633	0.716	22
	10	B	Thick	2.26	20	28	W	0.804	0.719	0.642	0.723	23
	12	B	Thick	2.17	45	38	W	0.841	0.768	0.685	0.763	22

ID	Dist	Glm	Peri	GW	PW	PL	Color	red	green	blue	light	Zn
	m			g	g	cm						mg kg^{-1}
WAC-S-2												
Tree 3	0	M	Thick	2.51	35	34	W	0.840	0.769	0.689	0.765	22
	2	R	Thick	1.96	23	30	W	0.832	0.759	0.675	0.753	19
	4	B	Thick	2.23	25	30	W	0.820	0.741	0.665	0.742	21
	6	R	Thick	2.18	16	26	W	0.840	0.769	0.687	0.764	18
	8	M	Thick	1.85	14	26	W	0.832	0.758	0.675	0.754	18
	10	R	Thick	1.84	13	25	W	0.798	0.715	0.642	0.720	19
	12	R	Thick	1.96	13	25	W	0.816	0.736	0.657	0.736	18
WAC-S-3												
Tree 4	0	B	Thick	2.08	27	28	W	0.781	0.695	0.617	0.699	25
	2	B	Thick	1.97	14	26	W	0.810	0.727	0.648	0.729	23
	4	B	Thick	1.91	27	32	W	0.815	0.731	0.653	0.734	23
	6	B	Thick	2.32	28	33	W	0.825	0.743	0.666	0.745	22
	8	B	Thick	2.04	33	33	W	0.799	0.711	0.635	0.717	22
	10	B	Thick	2.13	25	35	W	0.787	0.698	0.623	0.705	22
	12	B	Thick	2.08	60	35	W	0.796	0.710	0.634	0.715	19
Tree 5	0	B	Thick	2.42	36	29	W	0.808	0.721	0.643	0.725	23
	2	B	Thick	2.06	22	32	W	0.792	0.702	0.629	0.710	22
	4	B	Thick	2.24	31	33	W	0.822	0.736	0.662	0.742	21
	6	B	Thick	2.12	30	31	W	0.814	0.727	0.650	0.732	19
	8	B	Thick	2.20	35	36	W	0.794	0.708	0.631	0.713	18
	10	B	Thick	1.87	14	33	W	0.853	0.780	0.698	0.775	19
	12	B	Thick	2.04	22	30	W	0.813	0.728	0.650	0.731	20
YEK-S-1												
Tree 1	0	B	Thick	2.42	23	28	W	0.865	0.800	0.720	0.793	20
	2	B	Thick	2.76	38	30	W	0.850	0.782	0.704	0.777	22
	4	B	Thick	2.41	32	32	W	0.857	0.791	0.712	0.785	19
	6	B	Thick	2.35	29	29	W	0.839	0.769	0.687	0.763	19
	8	M	Thick	2.28	22	31	W	0.854	0.787	0.706	0.780	22
	10	B	Thick	2.21	16	25	W	0.875	0.811	0.727	0.801	19
	12	B	Thick	2.31	43	32	W	0.877	0.811	0.734	0.806	18
Tree 2	0	B	Thick	2.61	41	30	W	0.849	0.779	0.712	0.781	16
	2	B	Thick	2.27	16	26	W	0.876	0.810	0.735	0.806	17
	4	B	Thick	2.49	25	26	W	0.882	0.816	0.735	0.809	17
	6	B	Thick	2.49	33	33	W	0.878	0.813	0.732	0.805	18
	8	B	Thick	2.45	29	27	W	0.868	0.803	0.726	0.797	17
	10	B	Thick	2.29	21	27	W	0.874	0.808	0.729	0.801	16
	12	B	Thick	2.30	26	30	W	0.868	0.805	0.729	0.798	18

ID	Dist	Glm	Peri	GW	PW	PL	Color	red	green	blue	light	Zn
	m			g	g	cm						mg kg^{-1}
YEK-S-1												
Tree 3	0	B	Thick	2.73	32	27	W	0.864	0.799	0.731	0.798	19
	2	B	Thick	2.52	47	32	W	0.872	0.807	0.727	0.800	20
	4	B	Thick	2.64	34	28	W	0.861	0.795	0.715	0.788	17
	6	B	Thick	2.45	33	27	W	0.866	0.800	0.720	0.793	18
	8	B	Thick	2.37	18	27	W	0.853	0.787	0.706	0.780	16
	10	B	Thick	2.48	31	31	W	0.861	0.796	0.711	0.786	16
	12	B	Thick	2.43	34	29	W	0.852	0.781	0.701	0.776	16
YEK-S-2												
Tree 4	0	M	Mix	2.57	45	30	TW	0.841	0.765	0.681	0.761	17
	2	B	Thick	2.36	42	28	W	0.874	0.807	0.727	0.800	22
	4A	M	Thin	2.23	24	31	T	0.856	0.790	0.707	0.781	22
	4B	M	Thick	2.32	24	31	W	0.855	0.788	0.710	0.783	18
	6	M	Thick	2.36	43	35	W	0.852	0.783	0.700	0.776	23
	8	B	Thick	2.49	30	33	G	0.835	0.764	0.682	0.758	25
	10A	R	Thin	2.36	63	32	T	0.848	0.778	0.694	0.771	20
	10B	B	Thick	2.26	63	32	W	0.862	0.797	0.713	0.787	21
	12A	R	Thin	2.26	27	28	T	0.852	0.783	0.697	0.775	24
	12B	B	Thick	2.15	27	28	W	0.852	0.782	0.698	0.775	19
Tree 5	0	M	Thick	1.97	20	30	W	0.813	0.741	0.659	0.736	18
	2	M	Thick	2.52	18	28	W	0.849	0.780	0.701	0.775	19
	4A	R	Thin	2.01	18	28	T	0.848	0.779	0.693	0.771	20
	4B	B	Thick	2.53	18	28	W	0.868	0.804	0.721	0.795	19
	6A	R	Thin	1.96	22	32	T	0.836	0.757	0.677	0.757	16
	6B	B	Thick	2.24	22	32	W	0.837	0.762	0.679	0.758	18
	8	B	Thick	2.72	38	38	W	0.845	0.773	0.688	0.766	20
	10	M	Thick	2.42	44	38	W	0.797	0.727	0.654	0.726	19
	12A	R	Thin	2.27	29	28	T	0.846	0.771	0.687	0.766	24
	12B	B	Thick	2.39	29	28	W	0.856	0.783	0.702	0.779	17

ID	Dist	Glm	Peri	GW	PW	PL	Color	red	green	blue	light	Zn
	m			g	g	cm						mg kg^{-1}
YEK-S-2												
Tree 6	0A	R	Thin	2.21	23	31	T	0.842	0.759	0.675	0.758	21
	0B	B	Thick	2.20	23	31	W	0.862	0.793	0.707	0.785	21
	2	M	Thin	2.68	29	31	T	0.835	0.762	0.674	0.754	23
	4	M	Mix	2.52	29	32	TW	0.853	0.782	0.695	0.774	19
	6A	R	Thin	2.15	30	30	T	0.838	0.765	0.679	0.758	19
	6B	B	Thick	2.41	30	30	W	0.859	0.790	0.704	0.782	17
	8	B	Thick	2.83	33	32	W	0.862	0.793	0.714	0.788	21
	10	M	Thick	2.41	34	35	W	0.845	0.772	0.692	0.769	18
	12A	R	Thin	2.66	34	32	T	0.855	0.787	0.702	0.779	22
	12B	B	Thick	2.48	34	32	W	0.862	0.799	0.720	0.791	18

APPENDIX C

CHAPTER IV DATA

Table C1: Data from sorghum grain mold analysis by cultivar-block and treatment and rep. Cultiv= cultivar, Trt = treatment, CL-1 = treatment-rep #, GMR = grain mold rating, Alt = *Alternaria* species, Bip = *Bipolaris* species, CL = *Curvularia lunata*, CH = *Curvularia harveyi*, FT = *Fusarium thapsinum*, FS = *Fusarium semitectum*, Fsp = *Fusarium* species, Asp = *Aspergillus* species, Unk = unknown, C l % = % recovery CL.

Cultiv- Block	Trt	GMR (1-5)	Alt	Bip	CL	CH	FT	FS	Fsp	Asp	Unk	CL %
							# of 50					
RTx2536												
Block1	CL-1	2.5	22	1	27	0	0	0	0	0	0	54
	CL-3	2.5	5	0	45	0	0	0	0	0	0	90
	Ctrl-1	1	27	1	7	0	0	0	0	14	1	14
	Ctrl-2	2	31	8	6	0	2	1	0	1	1	12
	Ctrl-3	1.5	24	7	5	0	2	0	0	9	3	10
Block2	CL-1	2	15	0	32	0	0	0	0	3	0	64
	CL-2	2	17	4	19	0	0	0	0	9	1	38
	CL-3	2	17	3	26	0	0	2	0	1	1	52
	Ctrl-2	1	23	8	6	0	0	0	0	11	2	12
	Ctrl-3	1	37	9	0	0	0	0	0	0	4	0
Block3	CL-1	2	31	1	5	0	0	12	0	1	0	10
	CL-2	2.15	4	1	45	0	0	0	0	0	0	90
	CL-3	2	9	0	40	0	1	0	0	0	0	80
	Ctrl-1	1.5	39	4	5	0		0	0	1	1	10
	Ctrl-2	1	30	3	2	0	1	3	1	7	3	4
	Ctrl-3	1	28	3	6	0	0	1	0	10	2	12
Block4	CL-1	2	11	0	39	0	0	0	0	0	0	78
	CL-2	2	20	1	25	0	2	1	0	0	1	50
	CL-3	1.5	16	3	30	0	1	0	0	0	0	60
	Ctrl-1	1.5	30	6	7	0	0	4	3	0	0	14
	Ctrl-2	2	27	0	5	0	0	17	0	0	1	10
	Ctrl-3	1.5	21	6	12	2	3	0	1	5	0	24
Block5	CL-2	3.5	6	1	40	0	0	0	2	1	0	80
	CL-3	3.5	20	2	23	0	0	4	0	1	0	46
	Ctrl-1	1	31	8	1	0	0	3	7	0	0	2
	Ctrl-3	2	34	8	3	0	0	3	1	1	0	6

Cultiv-Block	Trt	GMR (1-5)	Alt	Bip	CL	CH	FT	FS	Fsp	Asp	Unk	CL %
							# of 50					
RTx430												
Block1	CL-1	3	22	3	6	0	2	0	0	15	2	12
	CL-3	3	23	0	27	0	0	0	0	0	0	54
	Ctrl-1	2	38	4	2	0	0	0	0	4	2	4
	Ctrl-2	2.5	31	2	13	0	0	3	0	0	1	26
Block2	CL-1	3	12	5	27	0	4	0	0	1	1	54
	CL-3	3.5	32	8	5	0	0	0	0	1	4	10
	Ctrl-1	2	37	4	1	0	0	2	0	2	4	2
	Ctrl-3	2	34	6	0	0	0	0	0	9	1	0
Block3	CL-1	3	18	1	29	1	0	1	0	0	0	58
	CL-2	3	9	2	36	1	0	1	0	0	1	72
	CL-3	3	17	2	26	1	0	2	0	0	2	52
	Ctrl-1	2	37	1	4	0	2	1	3	2	0	8
	Ctrl-2	2	35	4	8	0	0	2	0	0	1	16
	Ctrl-3	2.5	30	4	0	0	0	0	10	5	1	0
Block4	CL-1	3	13	2	34	0	1	0	0	0	0	68
	Ctrl-2	2	32	2	2	0	3	0	9	0	2	4
	Ctrl-3	2	38	5	4	0	0	0	0	0	3	8
Block5	CL-1	2.5	20	4	18	0	0	1	2	5	0	36
	CL-2	2.5	27	8	13	0	0	0	1	1	0	26

Table C2: Data from sorghum grain analysis by cultivar-block and treatment and rep. Cultiv= cultivar, Trt = treatment, CL-1 = treatment-rep #, GW = 100 grain weight, PH = panicle height, PL = panicle length, red, green, blue = grain color tristimulus values, light = grain lightness, Fe1, Fe2, Fe3 = grain Fe concentration for digestion batch 1, 2, and 3.

Cultiv- Block	Trt	GW	PH	PL	red	green	blue	light	Fe1	Fe2	Fe3
		g	cm	cm						mg kg^{-1}	
RTx2536											
Block1	CL-1	2.74	35.5	11.5	0.868	0.788	0.732	0.800	41.41	41.01	40.49
	CL-3	2.45	44.5	9.5	0.876	0.802	0.738	0.807	36.16	39.92	41.47
	Ctrl-1	2.68	44	9	0.930	0.859	0.807	0.869	29.97	30.77	31.07
	Ctrl-2	2.69	42	10	0.879	0.803	0.743	0.811	43.36	41.11	40.87
	Ctrl-3	2.64	44	11	0.881	0.807	0.741	0.811	30.57	34.09	31.98
Block2	CL-1	3.03	43	10	0.886	0.812	0.749	0.818	33.99	36.15	37.83
	CL-2	2.86	43	11.5	0.884	0.810	0.732	0.808	39.86	37.40	37.67
	CL-3	3.00	44	9.5	0.886	0.813	0.745	0.816	35.76	38.10	35.52
	Ctrl-2	2.67	-	-	0.855	0.776	0.713	0.784	32.65	33.56	32.82
	Ctrl-3	2.53	-	-	0.872	0.795	0.725	0.798	36.46	33.87	34.32
Block3	CL-1	2.46	42	10.5	0.879	0.803	0.744	0.812	39.39	37.24	35.22
	CL-2	2.71	41.5	10	0.846	0.769	0.700	0.773	38.37	39.65	37.78
	CL-3	2.32	45.5	11	0.865	0.788	0.718	0.791	36.88	36.47	37.28
	Ctrl-1	2.73	45	12	0.898	0.823	0.766	0.832	40.35	38.88	39.91
	Ctrl-2	2.53	41	9.5	0.860	0.783	0.709	0.785	42.45	40.94	35.65
	Ctrl-3	2.27	-	-	0.875	0.796	0.733	0.804	37.99	37.65	35.59
Block4	CL-1	2.62	42	11.5	0.859	0.782	0.722	0.791	37.06	37.26	43.34
	CL-2	2.36	44.5	11.5	0.865	0.785	0.721	0.793	34.43	40.07	37.17
	CL-3	2.68	45	10.5	0.863	0.785	0.717	0.790	43.54	37.11	36.06
	Ctrl-1	2.47	45	10.5	0.877	0.801	0.725	0.801	43.08	43.78	42.16
	Ctrl-2	2.28	43	8.5	0.860	0.785	0.695	0.777	41.47	39.51	39.71
	Ctrl-3	2.63	43	-	0.878	0.804	0.736	0.807	37.46	34.54	34.13
Block5	CL-2	1.94	45	10.5	0.886	0.809	0.751	0.818	33.67	36.30	33.56
	CL-3	2.43	44	9.5	-	-	-	-	37.38	37.16	37.74
	Ctrl-1	2.44	47	10	0.875	0.800	0.736	0.806	33.44	37.18	34.44
	Ctrl-3	2.35	41.5	12	0.855	0.779	0.714	0.785	37.15	36.79	38.78

Cultiv-Block	**Trt**	**GW**	**PH**	**PL**	**red**	**green**	**blue**	**light**	**Fe1**	**Fe2**	**Fe3**
		g	cm	cm						mg kg^{-1}	
RTx430											
Block1	CL-1	2.82	40	13	0.811	0.725	0.662	0.736	42.67	47.90	46.24
	CL-3	3.07	41.5	10	0.839	0.757	0.689	0.764	38.79	37.97	38.60
	Ctrl-1	3.53	42	11	0.850	0.767	0.704	0.777	36.05	34.79	34.12
	Ctrl-2	3.03	41	11	0.823	0.738	0.670	0.747	43.99	42.00	41.35
Block2	CL-1	2.53	43.5	11	0.842	0.760	0.696	0.769	50.07	43.75	47.44
	CL-3	2.62	-	-	0.838	0.751	0.695	0.767	41.98	41.65	41.79
	Ctrl-1	3.42	-	-	0.836	0.753	0.689	0.763	41.61	43.16	39.70
	Ctrl-3	3.55	-	-	0.846	0.759	0.700	0.773	45.30	38.99	39.30
Block3	CL-1	2.72	41.5	9	0.856	0.772	0.716	0.786	44.11	47.18	43.74
	CL-2	3.33	41.5	10.5	0.858	0.778	0.719	0.789	37.69	38.27	37.40
	CL-3	2.98	44	10.5	0.842	0.759	0.693	0.768	40.34	40.97	40.29
	Ctrl-1	3.70	41	12.5	0.849	0.770	0.700	0.774	39.25	43.50	34.77
	Ctrl-2	3.51	40	11	0.842	0.761	0.696	0.769	41.32	39.86	38.32
	Ctrl-3	2.71	39.5	10.5	0.837	0.752	0.695	0.766	46.38	46.14	43.64
Block4	CL-1	2.43	38	10	0.837	0.754	0.692	0.765	36.48	39.22	-
	Ctrl-2	2.88	38	10.5	0.802	0.713	0.651	0.727	38.49	36.22	37.22
	Ctrl-3	0.99	39.5	10	0.838	0.755	0.679	0.758	40.42	41.75	45.82
Block5	CL-1	2.68	39	11.5	0.831	0.747	0.681	0.756	38.33	41.54	39.46
	CL-2	2.81	41	10.5	0.828	0.745	0.682	0.755	42.95	41.44	38.57
Rice	-	-	-	-	-	-	-	-	8.83	9.44	7.63
	-	-	-	-	-	-	-	-	8.00	8.20	7.20
	-	-	-	-	-	-	-	-	7.17	7.57	6.79
	-	-	-	-	-	-	-	-	-	-	7.89

VITA

Cheryl L. Verbree received her Bachelor of Science degree in geology from Calvin College at Grand Rapids, Michigan in 1995. She entered the Department of Geology at The Ohio State University in September 1995 and received her Master of Science degree in May 1997. Her research interests include soil chemistry and fertility especially in relation to West African soils. She plans to direct agricultural research projects for Verbree International, a non-profit organization she co-founded with her husband in 2004.

Ms. Verbree may be reached at 748 Sugarcreek Trail, Webster, NY, 14580. Her email is cheryl@verbree.org.