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EXAMINING ENVIRONMENTAL DRIVERS OF SPATIAL VARIABILITY IN AFLATOXIN ACCUMULATION IN KENYAN MAIZE: POTENTIAL UTILITY IN RISK PREDICTION MODELS

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ABSTRACT

Maize, a staple food in most African countries, is prone to contamination by aflatoxins, toxic secondary metabolites mainly produced by *Aspergillus flavus* and *A. parasiticus*. Aflatoxins are known to cause liver cancer, and chronic exposure has been linked to other adverse health outcomes including growth faltering in children. To mitigate exposure in maize-dependent populations, there is need to identify the factors associated with aflatoxin contamination. This is difficult, however, because of high sampling cost and lack of affordable and accurate analytical methods. Publicly available, remotely-sensed data on vegetation, precipitation, and soil properties could be useful in predicting locations at risk for aflatoxin contamination in maize. This study investigates the utility of publicly available remotely-sensed data on rainfall, vegetation cover (indicated by normalized difference vegetation index or NDVI), and soil characteristics as potential predictors of aflatoxin contamination in Kenyan maize. Aflatoxin was analyzed in maize samples (n=2466) that were collected in 2009 and 2010 at 243 local hammer mills in eastern and western Kenya. Overall, 60% of maize samples had detectable aflatoxin. Global positioning system coordinates of each mill location were linked to remotely-sensed, spatially explicit indicators of average monthly NDVI, total monthly rainfall, and soil properties. Higher rainfall and vegetation cover during the maize pre-flowering period were significantly associated with higher prevalence of aflatoxin contamination. Conversely, higher rainfall and vegetation cover during the maize flowering and post-flowering periods (not including harvest) were associated with lower prevalence of aflatoxin contamination. Water stress throughout the growing season may cause increased plant susceptibility to fungal colonization and aflatoxin accumulation. Soil organic carbon content, pH, total exchangeable bases, salinity, texture, and soil type were significantly associated with aflatoxin. In conclusion, this study shows that remotely-sensed data can be regressed on available aflatoxin data highlighting important potential predictors that could reduce the cost of data collection and the cost of aflatoxin risk forecasting models.

Key words: Aflatoxin, GIS, NDVI, soil characteristics, rainfall, mycotoxins, East Africa, Kenya

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INTRODUCTION

It is estimated that approximately 4.5 billion people, predominantly those living in developing countries, are at risk of being exposed to dietary aflatoxins, with many chronically exposed at high levels [1]. Aflatoxins B1 (AFB1), B2, G1 and G2 are toxic secondary metabolites mainly produced by *Aspergillus flavus* and *A. parasiticus*, and are common contaminants of staple foods such as maize and groundnuts [2]. Aflatoxins have been most widely studied as causative in the etiology of liver cancer [2]. Chronic exposure has been linked to other adverse health outcomes, including growth faltering in children and immunomodulation, and acute exposure through ingestion of highly contaminated food can cause death [2–5]. Aflatoxin contamination is particularly concerning in developing countries, such as Kenya, where mycotoxins are not effectively controlled in the food system and consumption of high-risk foods, such as maize, is high. In Kenya, 477 cases of acute aflatoxicosis were reported between 2004 and 2011, 40% of which were fatal [6, 7].

To deliver effective reduction in human aflatoxin exposure, there is need to identify the geographical locations at high long-term and seasonal risk. Since aflatoxin accumulation in food is highly dependent on environmental factors such as moisture, temperature, nitrogen availability, and plant density [8], it may be possible to identify locations at risk through models that predict aflatoxin contamination based on these variables. These factors may change drastically from year to year. Aflatoxin accumulation predictive models which are based on laboratory experiments and field trials have been used in identifying the conditions for biosynthesis of the toxin [9, 10], but because of multiple environmental factors, such models may not be useful in natural field conditions. There is need to utilize field data from non-experimental sources in establishing aflatoxin predictive models. Publicly available, remotely-sensed, spatially-explicit indicators of vegetation, precipitation, and soil properties could be used in establishment of aflatoxin risk prediction models to facilitate surveillance and timely interventions.

The extent of aflatoxin accumulation varies both spatially and temporally, depending on biotic and abiotic factors that affect interactions between the maize host and the aflatoxigenic fungi. Differential resistance to fungal infection and aflatoxin contamination in maize can be attributed to genetic components associated with resistance *per se* and with resistance-associated traits (for example, kernel characteristics, stress tolerance, pest resistance), but these genetic resistances are highly influenced by the environment [11–16]. Aflatoxigenic fungi populations are present in soils worldwide and their strain composition and aflatoxigenicity are also highly sensitive to environmental conditions [17, 18]. Drying and threshing practices can also influence the further growth of existing fungal colonists, as well as exposing grain to new sources of inoculum [19]. Improper grain storage has been associated with aflatoxin contamination in maize grain [7, 20, 21].

Water and nutrient availability greatly affect the maize-fungi pathosystem. Drought stress, especially during the flowering and early grain-filling stages, has been associated with increased aflatoxigenic fungal infection and aflatoxin accumulation in maize [22–25]. After physiological grain maturity, excess moisture in the field and in storage can

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also lead to increased fungal colonization and aflatoxin production [26]. Sub-optimal nitrogen availability and high planting density have been associated with aflatoxin contamination [27–29]. Soil properties can affect water retention and nutrient availability, thereby influencing plant health and susceptibility to fungal colonization and aflatoxin production [27–29].

Previous research describing spatial variability of aflatoxin levels in Kenyan maize used agroecological zone (AEZ) as an explanatory factor associated with variability in aflatoxin levels [7]. Agroecological zone provides only a coarse classification based on broad, aggregated features such as climate patterns and soil quality; the authors hypothesized that finer analysis of potential drivers of aflatoxin accumulation could be of greater utility in identifying risk zones. Here the authors used publicly available metrics from geographic information systems (GIS) that contribute to AEZ classification to further dissect spatial and temporal variability in aflatoxin contamination of Kenyan maize, specifically normalized difference vegetation index (NDVI), rainfall, and soil characteristics. In addition to being efficiently measured via remote sensing, NDVI is an accurate predictor of vegetative cover [30] and has been suggested to be an indicator of plant stress, although it may be less accurate at the canopy level [31, 32]. Rainfall and soil quality are known determinants of crop vigor and fungal growth [23, 33]; this study used rainfall estimates as indicators of environmentally-driven water availability and soil properties to characterize soil quality.

METHODS

Field sampling data

The aflatoxin data that were used in this analysis were from maize samples (n=2466) collected in 2009 in eastern Kenya and in 2010 in western Kenya at local hammer mills (n=243) (locally called *posho* mills). *Posho* mills are the last point before human consumption in the value chain, and are a reasonable proxy for human exposure because maize is milled shortly before consumption [7]. Methods for sample collection and initial results were published previously [7, 34]. Briefly, sample sites and hubs were identified based on a GIS overlay of administrative locations and AEZ data. Approximately 30-45 maize flour samples were collected from patrons of local hammer mills, and quantification of aflatoxin was conducted at the Biosciences eastern and central Africa (BecA) laboratory in Nairobi using enzyme-linked immunosorbent assay (ELISA, Helica Biosystems Inc., Santa Ana, CA). The ELISA kit used has 1-20 ppb quantification limits, and samples with aflatoxin concentration above 20 ppb in the first assay were diluted and retested. Analysis was limited to maize that was reported to be self-grown from nearby the mill. Values of aflatoxin greater than 1 ppb were considered detectable in analyses.

Retrieval and synthesis of remotely-sensed data

Global positioning system coordinates of each *posho* mill location were linked to remotely-sensed, spatially-explicit indicators of vegetation, precipitation, and soil properties.

Normalized Difference Vegetation Index (NDVI) is a measure of the density of chlorophyll contained in vegetative cover and is defined as (NIR-RED)/ (NIR+RED),

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where NIR is the near-infrared reflectance and RED is the visible-red reflectance. Normalized Difference Vegetation Index data for Kenya in 2009 (eastern Kenya) and 2010 (western Kenya) were obtained from the Famine Early Warning System Net Africa data portal (FEWS NET, http://earlywarning.usgs.gov/fews/africa/index.php) as raster files containing five-day average NDVI at 250 m^2 resolution (six files per month). Rainfall estimates for 2009 (eastern Kenya) and 2010 (western Kenya) were also downloaded from FEWS NET, as raster files containing 10-day total rainfall at 8 km^2 resolution (three files per month). Rainfall rasters were re-projected into the longitude/latitude coordinates system for further analysis. Responses were extracted as the average values for a 5-km buffer around each individual mill location, a distance determined to be representative of the geographic dispersion of the customer base of each mill [7]. Individual NDVI values were aggregated as monthly averages and rainfall values aggregated as monthly rainfall totals. Monthly values were further aggregated into values for periods of agronomic interest (pre-flowering, flowering, post-flowering, flower + post-flowering, and the total growing season) according to the location specific timing reported by in each study location (Supplemental Table S1). Geographic information systems data were extracted from the raster files using the raster package in R (v. 2.0-12 in R. v. 2.15.1, http://www.r-project.org/). Aggregation of variables was performed with an R script.

Soil data were obtained from the Harmonized World Soil Database (HWSD, [35]), which used a 2003 FAO published Digitized Soil Map of the World as the source data for Kenya. Soil properties for the HWSD were contained in a Microsoft Access database that is linked to a world raster file according to a Soil Mapping Unit (SMU) value for each pixel. Each SMU corresponds to a series of 1-10 records in the database table, each record comprising a component of the aggregate soil, with the proportions of each component identified in the database as the 'share' of each component in the SMU. Each component record has unique soil properties. Soil Mapping Unit values were extracted for each pixel in a 5-km radius around each mill and used to calculate aggregate soil properties values for each location as follows: (a) for properties with continuous values, the average property value over all soil components listed was taken for each pixel, weighted by the share of each component, and (b) for properties with categorical values, the majority class after adding the class values was taken for each pixel weighted for the share of each component. Food and Agriculture Organization of the United Nations (FAO) soil type (FAOSOIL) categories were extracted from the Digital Soil Map of the World (DSMW, FAO, ver. 3.6, January 2003) corresponding to the coordinates of each mill.

Due to high correlation between many of the soil variables, the following soil variables were chosen for subsequent analyses: topsoil organic carbon content (% wt), topsoil pH (measured in H_2O), topsoil cation exchange capacity (CEC), topsoil total exchangeable bases (TEB), topsoil salinity (dS/m), soil texture, and soil type. These variables were chosen because they encompass a wide range of soil characteristics and are relatively common soil measurements. Soil organic carbon content can influence several soil characteristics that are relevant for plant health, including soil structure, water dynamics, aeration, nutrient holding capacity, nutrient availability, and biological activity. Soil pH affects nutrient availability and is a very common soil measurement. Cation exchange capacity represents the soil's potential nutrient holding capacity; TEB is a measure of the

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total base cations that are actually present and can be exchanged from the soil. Salinity can influence soil structure, water availability, and plant uptake of nutrients and toxic ions.

Statistical analysis

Maize samples were collected from the patrons of each mill, whilst the GPS coordinates were for the mill, requiring the analysis to adjust for the cluster effect of the mill. First, the environmental variables (NDVI, rainfall and soil characteristics) were examined across the mills (n=243) to assess whether there were differences between highly contaminated areas and less contaminated areas (defined as the percent of samples with detectable aflatoxin). The percent of maize with detectable aflatoxin was calculated for each mill. Tertiles of contamination were calculated (T1=10-44%; T2=45-71%; T3=72-100%). None of the continuous variables were normally distributed, as indicated by a Shapiro-Wilk test of normality. Therefore, association between the predictor and the severity of mill contamination was assessed using a Kruskal-Wallis test. Categorical variables (soil type and soil texture) were tested for association with the binary predictor using a chi-squared test. Although these tests do not take the trend (low contamination to high contamination) into account, it is possible that the relationship between predictors and aflatoxin contamination would be non-linear, and this test allows analysis of both linear and non-linear trends.

Secondly, multivariable analysis was conducted using multi-level modeling to assess the variability in aflatoxin contamination that could be explained by location (mill) and by environmental predictor variables. Multi-level modeling was used to account for the non-independence of maize samples collected from the same mill, and allow separation of the relative explained variance within the mill from the variance between mills. Multi-level models have significant advantage over complete pooling of data (aggregation to mill level) because they recognize the appropriate hierarchical structure of the data and give more appropriate effect estimates and standard errors [36]. To analyze the relationship between aflatoxin contamination and rainfall, NDVI and soil characteristics, a two-level model was used with mill identified as a random effect at the second level with independent maize samples nested within the mill. Standardized coefficients were reported for continuous variables. Initially, separate models were run for samples collected in eastern and western Kenya respectively, but relationships were consistent; therefore, in reported models, samples were pooled with east vs. west included as a covariate. Effect estimates, odds ratios (OR) and confidence intervals (CI) are reported in Table 3. All statistical analyses were performed in STATA 12.0. Multilevel-mixed-effects models were built with xtmelogit and xtmixed commands in STATA 12.0.

To illustrate how these GIS analyses could be used to determine regions with higher and lower risk of aflatoxin presence and levels, we plotted the survey results for each mill on a map of the flowering and post-flowering rainfall during the growing season using map and raster packages in R (v. 2.0-12 in R. v. 2.15.1, http://www.r-project.org/).

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RESULTS

Descriptive statistics of the aflatoxin data

Overall, 60% of samples analyzed for aflatoxin had detectable levels of the toxin. Considering the regulatory limits of 10 ppb, 28% of samples exceeded regulatory limits. Toxin levels were non-normally distributed, with heavy skew towards higher concentrations. To take account of the negative samples, we conducted a two-part analysis: (1) detectable (>1ppb) vs. non-detectable and (2) log transformed toxin level if aflatoxin>1ppb.

Relationship between rainfall, NDVI and aflatoxin contamination

Univariate analyses of remotely-sensed data suggest that many plant stress and soil properties were associated with aflatoxin occurrence (Tables 1 and 2). In areas with greater aflatoxin contamination (percent contaminated per mill), there were slightly higher rainfall (6 mm difference between low and high tertiles) and NDVI (4% difference between low and high tertiles) during the pre-flowering time period, whereas these areas had lower rainfall (139 mm difference between low and high tertiles) and NDVI (6% difference between low and high tertiles) during the flowering and post-flowering time periods.

The overlay of aflatoxin contamination with the flowering and post-flowering rainfall during the growing season illustrates that areas with lower rainfall showed higher prevalence of aflatoxin; this effect was roughly correlated with the known lower rainfall in eastern Kenya relative to western Kenya (Figure 1).

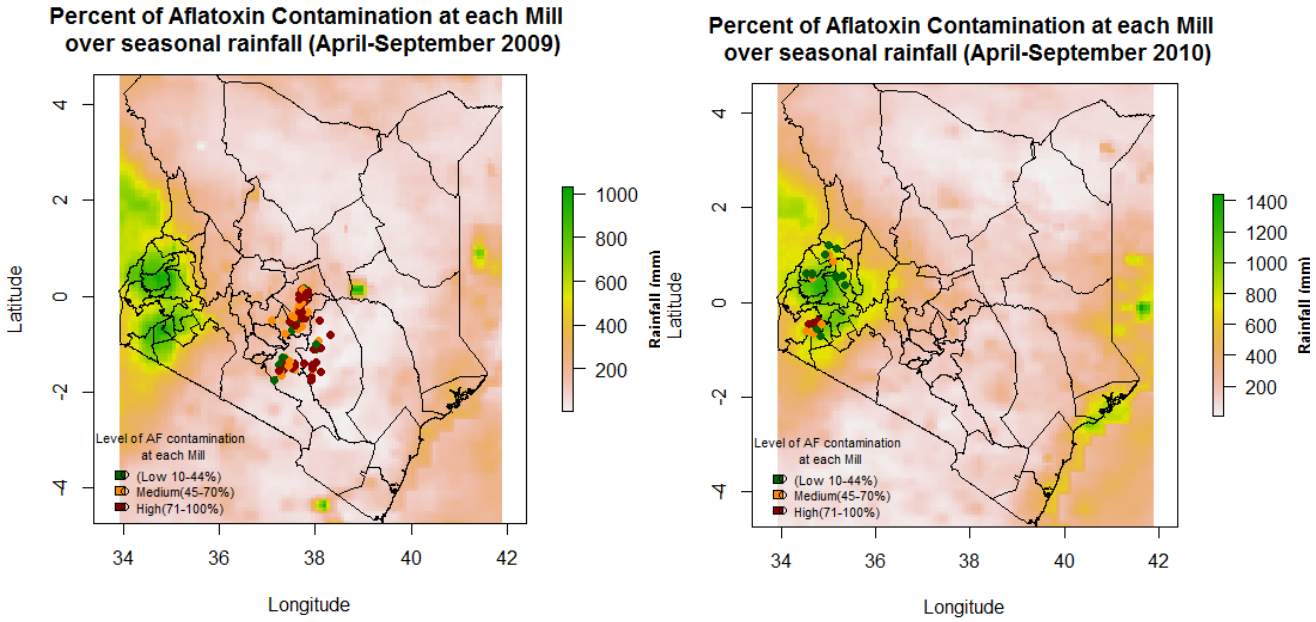

Figure 1: Flowering and post-flowering rainfall during the growing seasons for the maize collected in eastern Kenya in 2009 (top) and western Kenya in 2010 (bottom). Points are mill locations colored by the prevalence of aflatoxin in samples collected at those mills

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Relationship between soil characteristics and aflatoxin contamination

Univariate analysis showed that several topsoil characteristics were significantly associated with aflatoxin (Table 1; results for subsoil characteristics were similar and are not reported here). In areas with high prevalence of aflatoxin, soils had higher pH and total exchangeable bases (TEB) (P<0.001). In areas with low prevalence of aflatoxin, soils had higher organic carbon content (P<0.001). Cation exchange capacity (CEC) was not significantly associated with aflatoxin. Fine and coarse textured soils were associated with lower prevalence of aflatoxin contamination, whereas medium textured soils were associated with higher prevalence of aflatoxin contamination (Table 2; P<0.0001). Of the FAO soil classification types, there were significant differences in the prevalence of aflatoxin contamination and soil type with a chi squared test (P<0.0001). This association was likely driven by Orthic Ferralsols and Humic Nitosols, which were negatively associated with aflatoxin contamination, and Rhodic Ferralsols and Eutric Nitosols, which were positively associated with aflatoxin contamination (P<0.0001).

The association between rainfall, NDVI, soil characteristics and aflatoxin contamination

Overall, 25% of variation in the level of aflatoxin and 32% of the variability in the odds of aflatoxin presence was due to the mill location, while 28% of the variance in aflatoxin levels and presence between mills was explained by the chosen predictor variables. Vegetation cover (NDVI) was significantly associated with the presence of aflatoxin and level of aflatoxin if detected. Pre-flowering NDVI was associated with increased aflatoxin presence (for an increase in one standard deviation OR=1.66, P<0.01) and level (coeff=0.16, P<0.01). Increased flowering and post-flowering NDVI was protective, associated with decreased presence (OR=0.60, P=0.04) and level (coeff=-0.14, P=0.05). Rainfall was not significantly associated with aflatoxin in the combined models. The NDVI and rainfall were highly correlated, and it is likely that NDVI captures the effect of rainfall observed in univariate analyses. Multi-level regression analysis showed that soil texture was not significantly associated with aflatoxin contamination (Table 3). Organic carbon content was negatively associated with aflatoxin contamination (for increase in one standard deviation OR=0.64, P=0.03). The CEC was positively associated with aflatoxin contamination (one standard deviation OR=1.55, P<0.01).

DISCUSSION

There is significant variation in aflatoxin accumulation across time and space, driven by a host of environmental conditions as well as harvest and post-harvest practices. This analysis explores potential relationships between remotely-sensed environmental data and aflatoxin accumulation to examine its utility in future risk prediction models. This analysis is limited to discrete geographical areas in eastern and western Kenya during two calendar years, but provides the foundation for developing further prediction models.

One important finding is that the timing of rainfall, rather than the total amount of rainfall, might be important in determining spatial risk of aflatoxin accumulation. Normalized Difference Vegetation Index was positively associated with aflatoxin during the pre-flowering stage and negatively associated with aflatoxin at the flowering and

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post-flowering stages. The NDVI represents vegetative cover and could be a proxy for both plant health and plant density. However, reduced NDVI could also indicate decreased plant health due to biotic and abiotic stress, which has been linked to aflatoxin accumulation in maize [37, 38]. In univariate analyses, rainfall was also positively associated with aflatoxin levels before flowering and negatively associated with aflatoxin at the flowering and post-flowering stages; this effect was attenuated with the addition of NDVI to the multivariate models. Days to maturity varied among the sampling areas (6 months in Uasin Gishu and Trans Nzoia and 3-4 months in other study areas), which could contribute to some of the unexplained variability in the models because different types of maize have different susceptibility to aflatoxin contamination [37]. Additionally, NDVI and rainfall was based on a monthly aggregation. Flowering occurs over a period shorter than a month and we were unable to determine the exact week in the month that flowering occurred, potentially introducing error into the effect estimates. However, we examined the correlation between total rainfall in each month (used in our estimates) and rainfall during each 10-day period within the month, and found these values were highly correlated (r=0.60-0.94), indicating that total rainfall for the month is a suitable proxy for rainfall during the growing season. The NDVI is relatively stable within a month period with an average of a 3% coefficient of variation among the 5-day periods within each month, further indicating that data aggregated by month are suitable in these models.

The significant association between NDVI and aflatoxin, controlling for rainfall, suggests that NDVI was also influenced by other environmental factors; soil organic carbon content may mediate the effect of rainfall on NDVI because it affects the water-holding capacity of the soil [39]. These relationships among rainfall, NDVI, and aflatoxin suggest that the timing of precipitation may affect maize susceptibility to aflatoxigenic fungal infection and subsequent aflatoxin production. Excess rainfall during the vegetative growth period (pre-flowering) may lead to high canopy density, which is favorable for fungal growth [40]. Drought stress (represented by low rainfall conditions in this study) during the flowering and early grain-filling stages has previously been associated with increased aflatoxigenic fungal infection and aflatoxin accumulation [23–25].

Except for NDVI, the remotely-sensed environmental data examined are only associated with the presence of aflatoxin, not the level of aflatoxin, which may be more greatly influenced by harvest and post-harvest practices. The models explain 28% of the variation in aflatoxin across mill location; although this is lower than the variance explained in simulation models [9, 10], this type of model has utility. One limitation of the environmental data reported here is they are only representative of environmental conditions during the maize growing season while the maize samples were collected at the mill. Aflatoxin can continue to accumulate in grain post-harvest. The majority of maize samples in this study were collected from smallholder farmers who have limited and suboptimal grain drying and storage options. For example, excess, unregulated moisture in storage has been linked to increased post-harvest aflatoxin accumulation in maize [41–43]. Further work could investigate the impact of environmental conditions on the accumulation of aflatoxin in maize post-harvest. Although modeling environmental drivers of aflatoxin accumulation does not take into account farming, harvest and storage practices, it could be used to target geographically riskier areas.

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This analysis also explored soil characteristics that may influence plant stress including indicators of texture, nutrient availability, organic carbon content and pH because plant stress is thought to favor pre-harvest fungal infection and aflatoxin production. Findings indicate that total exchangeable bases (TEB), organic carbon content and pH as well as soil types may be useful in contributing to prediction of areas at risk for aflatoxin accumulation. It should be noted, however, that the soil data used here represent a relatively narrow range of values and that the differences between tertile median values within most variables are so small that they may not represent agronomically meaningful changes. Furthermore, these data do not reflect site-specific management practices, which influence soil fertility, water-holding capacity, and other important characteristics.

Previous studies have linked pre-harvest aflatoxin levels with suboptimal nitrogen fertilization, drought conditions, and increased soil temperatures [22, 24, 27]. Univariate analyses showed that other soil characteristics were also significantly associated with aflatoxin contamination, but there were few clear trends. For example, soil organic content (SOC)—an important component of soil fertility—was negatively associated with aflatoxin, potentially supporting the hypothesis that increased soil fertility is protective. However, increased pH (from 5.86 to 6.03, which is still acidic but slightly more optimal for maize) and increased TEB (from 9.9 to 10.9, representing a slight increase in available nutrients) were associated with a higher percent of contaminated maize samples, contradicting this hypothesis. The role of soil texture was also somewhat equivocal—fine and coarse textured soils were negatively associated with aflatoxin prevalence, while medium textured soils were positively associated with aflatoxin prevalence. Once rainfall, NDVI, and select soil characteristics were accounted for in a mixed model, soil texture was no longer significantly associated with aflatoxin contamination. In this model, SOC was still protective against aflatoxin, and greater CEC was not, confirming that these characteristics may be useful in aflatoxin risk assessments, and that the relationship between soil fertility and aflatoxin accumulation is complex.

CONCLUSION

In conclusion, publicly available, spatially-explicit environmental data on rainfall, NDVI and soil properties could provide a foundation for future models to predict areas at risk of aflatoxin exposure (Figure 2). This analysis was limited to regions in eastern and western Kenya during 2009 and 2010 and provides the methodological foundation for building more robust models to predict risk in other locations across time. Future work should include long-term sampling strategies over greater geographical areas.

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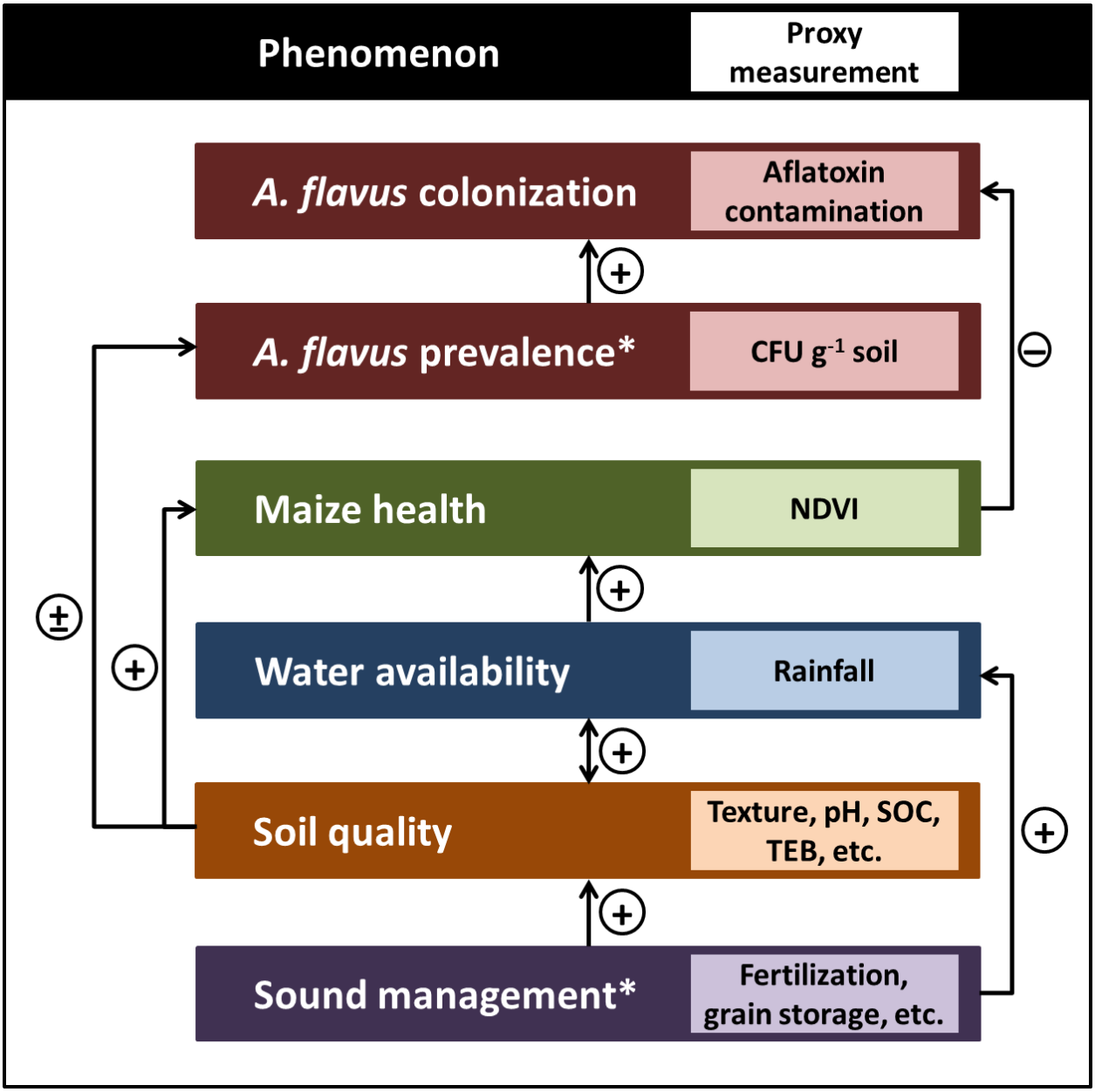

Figure 2: Conceptual framework of interactions between indicators of sound management practices, soil quality, water availability, maize health, and *A. flavus* prevalence and colonization. *Phenomena not assessed in this study; CFU=colony forming units; +Positive relationship; -Negative relationship; ±Positive or negative relationship; NDVI= normalized difference vegetation index; SOC= soil organic content; TEB= total exchangeable bases

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Table 1: Rainfall, NDVI and soil characteristics of mills by low, medium and high aflatoxin contamination

Variable	Units	Percent of Contaminated maize in each mill (Tertiles)			
		Low (10-44%)	Medium (45-71%)	High (71-100%)	
		Median (IQR)			p-value
Average NDVI for the pre-flowering period	% dmnl	56.5 (50.3, 62.3)	59.4 (53.9, 67.8)	61.0 (58.1, 68.7)	<0.001**
Total rainfall for the pre-flowering period	Mm	226 (206, 259)	224 (176, 259)	232 (203, 324)	<0.001**
Average NDVI for flowering + post-period	% dmnl	59.8 (57.4,65.4)	59.2 (52.0, 65.0)	54.5 (49.4, 63.0)	<0.001**
Total rainfall for flowering + post-period	Mm	201 (56, 288)	79 (57, 205)	62 (39, 80)	<0.001**
Topsoil organic carbon	% wt	1.17 (0.96, 1.58)	1.29 (0.82, 1.79)	1.03 (0.67, 1.4)	<0.001**
Topsoil pH (H_2O)	-log[H^+]	5.86 (5.42, 6.00)	5.90 (5.60, 6.09)	6.03 (5.86, 6.12)	<0.001**
Topsoil CEC	cmol(+)/kg	16 (14, 19)	18 (14, 25)	16 (15, 24)	0.202
Topsoil TEB	cmol(+)/kg	9.9 (8.7, 12.3)	10.7 (8.4, 12.8)	10.9 (9.4, 12.8)	<0.001**
Topsoil salinity	dS/m	0.00 (0.00, 0.002)	0.00 (0.00, 0.00)	0.00 (0.00, 0.009)	<0.001**

* 0.05>P>0.01; ** *P*<0.01; CEC=cation exchange capacity; TEB=total exchangeable bases; dmnl=dimensionless; NDVI= normalized difference vegetation index; IQR= Interquartile range

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Table 2: Soil classifications of mills by low, medium and high aflatoxin contamination

Variable	Percent of contaminated maize in each mill (Tertiles)			
	Low (10-47%)	Medium (45-71%)	High (71-100%)	
	Percent (n)			Chi square p-value
Dominant soil type				<0.0001**
Chromic Cambisols	6.5 (160)	5.3 (130)	6.9 (170)	
Orthic Ferralsols	10.6 (260)	9.7 (240)	3.6 (90)	
Rhodic Ferralsols	3.0 (74)	6.6 (163)	6.0 (171)	
Lithosols	1.6 (40)	0.8 (20)	2.4 (60)	
Ferric Luvisols	0 (0)	1.2 (30)	1.2 (30)	
Eutric Nitosols	2.8 (70)	7.3 (180)	9.7 (240)	
Humic Nitosols	8.4 (206)	1.6 (40)	0 (0)	
Eutric Gleysols	0.4 (10)	1.2 (30)	0.4 (10)	
Mollic Andosols	0.4 (10)	0.8 (20)	0 (0)	
Pellic Vertisols	0.4 (10)	0 (0)	0 (0)	
Topsoil texture				<0.0001**
Coarse	3.4 (84)	3.7 (91)	1.4 (34)	
Medium	4.9 (120)	4.1 (100)	6.5 (160)	
Fine	25.8 (636)	26.9 (662)	23.4 (557)	

** $P<0.01$

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Table 3: Multilevel regression models of aflatoxin level in maize samples for rainfall (100mm increase), NDVI (1% increase) and soil characteristics

Predictor		Parameter estimate (95% CI) for Aflatoxin	
		Presence [>1ppb] OR (N=936)	Level [log(ppb)] | Presence [>1ppb] (N=589)
Rainfall	Total rainfall for the pre-flowering period (std)	1.03 (0.80, 1.33)	0.032 (-0.046, 0.109)
	Total rainfall for flowering and post-flowering period (std)	1.03 (0.61, 1.74)	-0.025 (-0.200, 0.140)
NDVI	Avg. NDVI for the pre-flowering period (std)	**1.66** (1.15, 2.4)**	**0.016** (0.051, 0.274)**
	Avg. NDVI for the flowering and post-flowering period (std)	**0.60* (0.34, 0.97)**	**-0.143* (-0.287, 0.000)**
Soil Texture (ref Coarse)	Medium	0.69 (0.22, 2.21)	-0.150 (-0.507, 0.206)
	Fine	0.79 (0.27, 2.30)	-0.065 (-0.397, 0.267)
Soil Organic Carbon Content (std)		**0.64* (0.42, 0.97)**	-0.101 (-0.226, 0.024)
Cation Exchange Capacity (std)		**1.55* (1.04, 2.32)**	0.104 (-0.019, 0.228)
Region (ref= West)	East	1.75 (0.50, 6.04)	0.261 (-0.084, 0.606)

* 0.05>*P*>0.01; ** *P*<0.01
NDVI= normalized difference vegetation index

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Supplemental Table S1: Maize growing seasons in locations where maize was sampled for aflatoxin analysis between 2009 and 2010

Sub-county (formerly known as district)	Month						
	March	**April**	**May**	**June**	**July**	**August**	**September**
Embu	Planting	Planting	Flowering	Flowering	Harvest		
Mbeere	Planting	Planting	Flowering	Flowering	Harvest		
Meru south	Planting	Planting	Flowering	Flowering	Harvest		
Meru central	Planting	Planting	Flowering	Flowering	Harvest		
Meru north	Planting	Planting	Flowering	Flowering	Harvest		
Machakos	Planting	Planting	Flowering	Flowering	Harvest		
Kathiani	Planting	Planting	Flowering	Flowering	Harvest		
Mwingi	Planting	Planting	Flowering	Flowering	Harvest		
Mwala	Planting	Planting	Flowering	Flowering	Harvest		
Kitui	Planting	Planting	Flowering	Flowering	Harvest		
Bungoma	Planting	Flowering	Flowering	Harvest	Harvest		
Rachuonyo	Planting	Flowering	Flowering	Harvest	Harvest		
Kisii	Planting	Flowering	Flowering	Harvest	Harvest		
Homa Bay	Planting	Flowering	Flowering	Harvest	Harvest		
Trans Nzoia		Planting	Planting	Flowering	Flowering	Harvest	Harvest
Uasin Gishu		Planting	Planting	Flowering	Flowering	Harvest	Harvest

Planting
Flowering
Harvest

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