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Opposing effects of different soil organic matter fractions on crop yields

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Abstract

Soil organic matter is critical to sustainable agriculture because it provides nutrients to crops as it decomposes and increases nutrient- and water-holding capacity when built up. Fast- and slow-cycling fractions of soil organic matter can have different impacts on crop production because fast-cycling fractions rapidly release nutrients for short-term plant growth and slow-cycling fractions bind nutrients that mineralize slowly and build up water holding capacity. We explored the controls on these fractions in a tropical agroecosystem and their relationship to crop yields. We performed physical fractionation of soil organic matter from 48 farms and plots in western Kenya. We found that fast-cycling, particulate organic matter was positively related to crop yields, but did not have a strong effect, while slower-cycling, mineral-associated organic matter was negatively related to yields. Our finding that slower-cycling organic matter was negatively related to yield points to a need to revise the view that stabilization of organic matter positively impacts food security. Our results support a new paradigm that different soil organic matter fractions are controlled by different mechanisms, potentially leading to different relationships with management outcomes, like crop yield. Effectively managing soils for sustainable agriculture requires quantifying the effects of specific organic matter fractions on these outcomes.

Keywords: Africa; agroforestry; carbon; extracellular enzymes; legumes; microbes; soil organic matter

1. Introduction

Soil organic matter (SOM) provides many benefits to people and ecosystems (e.g., Banwart et al. 2014). Organic matter is a particularly important component of soils in agricultural systems because it provides nutrients to crops when decomposed and increases nutrient holding capacity and water-holding capacity when built-up (Lal 2004). Despite these clear benefits, there are different viewpoints on how to manage SOM to support livelihoods. On the one hand are proponents of "using" SOM: encouraging decomposition of organic matter to liberate nutrients that can be taken up by crops in the short term (Janzen 2006). On the other hand are proponents of "saving" SOM by prioritizing the formation and long-term stabilization of organic matter to serve as a store for elevated atmospheric carbon (C) and enhance long-term soil quality through nutrient retention and water holding capacity (Lal 2004).

Although the ideas of "using" versus "saving" SOM seem, at the surface, to be at odds, an emerging paradigm of SOM dynamics suggests that these opposing processes occur in distinct components of SOM. Specifically, the fast-cycling particulate organic matter (POM) fraction serves as a source of nutrients to be "used" by plants, while the slow cycling, mineral-associated C fraction (MIN C) serves as a long term storehouse of C, "saved" in the soil for hundreds or thousands of years (Schmidt et al. 2011, Lehmann and Kleber 2015). Under this emerging paradigm, nutrients for crop production are released from the decomposition of the fast-cycling, POM fraction, dominated by partially decomposed OM. Conversely, the slow-cycling, mineral-

associated SOM fraction is where C stabilizes for storage of atmospheric CO_2, nutrient retention, and water holding capacity. This mineral-associated fraction is formed primarily from C compounds that are assimilated by soil microbes, converted into microbial biomass, and stabilized on the charged surfaces of mineral soil particles in the form of microbial necromass and microbial exudates (Cotrufo et al. 2013). These microbial products may be the primary pathway through which plant inputs become bound to mineral surfaces and thus stabilized into long-term stores of SOM (Schmidt et al. 2011, Lehmann and Kleber 2015).

In addition to their role in the formation and stabilization of SOM, microbes also drive the decomposition of SOM. However, most of the compounds in SOM cannot be directly assimilated by microbes; rather, they must first be depolymerized by extracellular enzymes that microbes release into the soil environment (Burns et al. 2013). Microbial production of enzymes incurs both energetic and nitrogen (N) costs, and thus the allocation of resources is responsive to the elemental stoichiometry of available substrates (Sinsabaugh et al. 2008, Allison et al. 2011). As a result, enzyme activities can be interpreted as indicators of the relative availability of accessible C and nutrients, and should not be responsive to substrates that are minerally protected and therefore less vulnerable to enzymatic attack.

Given the important roles of fast- and slow-cycling SOM fractions in agriculture, it is important to understand how agricultural management practices control the stocks of these two SOM fractions, and how in turn these stocks affect agroecosystem services such as crop productivity (Oldfield et al. 2015). This need is especially relevant to farming systems that have low external inputs, such as tropical smallholder agriculture, and thus depend in part on

ecological processes, such as decomposition of organic matter that makes nutrients available to crops, to maintain soil fertility. Tropical smallholder agriculture supports the livelihoods of over 900 million of the world's poorest people (Wiggins et al. 2010). These farmers are clustered in regions such as sub-Saharan Africa, where more than 90% of farms belong to smallholders (GRAIN 2014). Smallholder farms can contribute the majority of food production in some countries—in Kenya, for example, small farms produced 73% of agricultural output in 2004 (Binswangher-Mkhize et al. 2009).

The purpose of our research is to examine how mineral and organic nutrient addition to smallholder farms in western Kenya impacts fast- and slow-cycling SOM fractions, the microbial extracellular enzymes responsible for OM decomposition, and resulting changes in crop yields. Because the degrading effects of continuous cultivation on soil quality can be attenuated by organic inputs (Moebius-Clune et al. 2011), we expected that recent organic inputs would be an important control on SOM fraction sizes, the microbial mechanisms of decomposition of those fractions, and resulting yields. We hypothesized that the inclusion of recent, labile organic inputs in a farm's management leads to lower activity of enzymes involved in degradation of C-rich compounds because of abundant C and, thus, greater investment in enzymes targeted to N- and P-rich substrates. Soils with greater amounts of slow-cycling, mineral-associated, C should also have lower microbial investment in C-degrading enzymes, but for a different reason. Because mineral-associated C is bound to clay surfaces and often held within clay aggregates, it should be less sensitive to microbial attack. Thus, we expect both MIN and POM C to be negatively associated with C-degrading enzymes, but for different reasons: POM C because microbes need to invest less in enzymes when C is abundant in soil, MIN C because microbial enzyme attack is

less effective on C that is bound to clays and held within aggregates. We also hypothesized that recent organic inputs would be positively associated with the size of POM fractions, but that the longer-term land use history, such as the vegetation history, would dictate the size of mineral-associated fractions, which respond over longer time scales (Lajtha et al. 2014).

For relationships between the SOM fractions and yield, we hypothesized that POM stocks would be positively related to crop yields because POM represents a fast-cycling fraction from which nutrients for crop growth can be liberated (Haynes 2005). We also expected higher crop yields with a larger stock of the slower-cycling, mineral-associated fraction because of its capacity to retain plant-available water and nutrients in the soil that mineralize slowly to release nutrients for crop growth over time (Lal 2004, Haynes 2005).

2. Materials and Methods

2.1 Study site and sample collection

Soils were collected from 24 experimental fertilization plots in Yala, Kenya and from 24 maize farms in the project area of the Millennium Villages Project (MVP), an international development project that provides access to agricultural inputs and extension services, among other services. The fertilization plots were located at the field office of MVP (34.511 E, 0.101 N). They include mineral fertilizer additions of 0, 50, 75, 100, 150, and 200 kg N ha^{-1} with four plots per fertilizer level and maize grown on the plots. Actively managed farms were distributed across the ~20 km^2 MVP project area. They are clustered by treatment on similar underlying soils (Wood et al. 2015a, 2015b). The area was originally part of the moist montane broadleaf forest zone in eastern and central Africa, but is now a mixed-maize agricultural system, with

most farmers cultivating a maize crop in both the long and short rainy seasons. Some farmers, however, replace the short-rain maize crop with a seasonal legume rotation that fixes N, the leaves are often returned to the soil as an organic input (and is thus expected to build SOM). This treatment is henceforth referred to as legume rotation. Actively managed farms were grouped into three categories: low fertilizer (<10 kg mineral N ha^{-1}); high fertilizer (>60 kg mineral N ha^{-1}); legume rotation (>60 kg mineral N ha^{-1} paired with legume rotation). Fertilizer is typically applied to the base of maize stalks with about 1/3 of N added at planting as diammonium phosphate and 2/3 applied 5-8 weeks later as urea. Greater detail of farms and selection criteria is given in Wood et al. (2015a, 2015b) and Tully et al. (2015). Fertilization plots and actively managed farms have broadly different land use histories. Fertilization plots are situated on land owned by the Kenya Broadcasting Corporation and leased to MVP. The land was originally converted to agriculture in the 1960s or 1970s, but was left fallow in the 1980s and from 1994-2007. Fertilization plots were established in 2011. In contrast, the actively managed farms have been in production for at least 50 years. Greater detail of fertilization plots is given in Hickman et al. (2015).

The mean annual temperature and precipitation for the study region are 24°C and 1800 mm, respectively. Annual precipitation is distributed bi-modally with 1120 mm in a long rainy season from March to June and 710 mm in a short rainy season from September to December. The soils are classified as Kandiudalfic Eutrodox (USDA classification) and are well-drained sandy clay loams (on average 54% sand, 13% silt, 34% clay) with a mean pH of 5.45 and C:N of 11.52 (0-20 cm; Table 1).

We sampled soils for this study in June 2013, in the middle of the long rains, two weeks after fertilizer application and when maize plants were nearing the pollination stage. For farm fields, we took 20 2-cm dia. soil cores from the top 20 cm of bulk soil. We targeted bulk soil to avoid changes in microbial communities that could be due to direct impacts from crop root exudation as well as recent fertilizer application, which is targeted to the root zone. Cores were taken at regular intervals throughout an entire field area and homogenized and aggregated to a composite sample. Cores were spaced evenly across the field; the spacing of intervals thus depended on total field size. Because fertilization plots were significantly smaller than farms, we took nine 2-cm diameter cores to 20-cm depth per plot, and aggregated to a composite sample. At each core location we recorded volumetric soil moisture content using a HydroSense moisture probe (Campbell Scientific, Logan, UT, USA).

2.2 General soil properties and crop yield

Subsamples of sieved (2-mm screen) field soil were stored at 4°C and used to determine pH, gravimetric soil moisture, and water holding capacity. Gravimetric soil moisture and water holding capacity (after wetting soils to field capacity) were determined by drying field moist and field capacity moistened soil (respectively) at 105°C for 24 h. Soil pH was determined using a benchtop meter and a 1:1 slurry of soil:H_2O by volume. We measured bulk density with a slide hammer (Core Sampler Complete, AMS Idaho, USA) by inserting a 5.08-cm diameter plastic tube into the corer, followed by a 10.12-cm depth aluminum tube (volume = 205.9 cm^3). The corer was driven into the soil to 20 cm; the aluminum tube was then removed, the soil leveled with a knife, and the whole tube was wrapped in aluminum foil. Aluminum tubes were weighed and oven-dried at 105°C until a constant weight was attained (Tully et al. 2015). Bulk density

was used to calculate SOM fraction size (g cm^{-3}) to 20 cm depth. A subsample of sieved soil was air-dried and used to determine soil texture by the hydrometer method (Bouyoucous 1962). Because experimental plots are on a field with the same soil type (and treatments are randomized within the field), texture was only measured on plots with 0 kg N ha^{-1}.

We estimated microbially available C with a 30-day C mineralization assay (henceforth referred to as *cumulative respiration*) following Bradford et al. (2008) and Oldfield et al. (2014). Briefly, we measured CO_2-efflux four times across thirty days (days 1, 4, 15, 30). For each measurement, 4 g soil slurries were placed in 50 mL centrifuge tubes. Tubes were flushed with CO_2-free air and incubated for 24 h before determination of headspace CO_2 concentrations on an infrared gas analyzer (IRGA; Li-Cor Biosciences, Lincoln, NE, USA, Model LI-7000). Samples were maintained at 60% water holding capacity. Cumulative carbon mineralized was determined by integrating CO_2-efflux rate values across each measurement during the 30 days. Yields were measured by harvesting aboveground maize biomass in a 3 m x 3 m sub-plot on actively managed fields and by harvesting the entire plot on the experimental plot. Harvested plants were separated into stalks and cobs and weighed in the field. Subsamples were taken from the field, cobs separated into core and grain, and all materials weighed fresh and oven-dried (60°C until constant mass was obtained). Plot yields were estimated based on dry grain per plant and the total number of plants per plot; yields were then scaled to a per hectare basis. Further description is given in Tully et al. (2015).

2.3 Soil organic matter fractions

We used a size-based physical fractionation method to differentiate between the faster-cycling particulate organic matter (POM) and slower-cycling mineral-associated (MIN) soil C and N fractions (Schlesinger and Lichter 2001), using the method described in Bradford et al. (2008). Briefly, air-dried soil (10 g) from each plot was dispersed with sodium hexametaphosphate via shaking (18 h) to break apart aggregates, and then passed through a 53-μm sieve to physically fractionate the soil. Mineral-associated (i.e., silt and clay minerals) material is considered <53 μm and POM material is >53 μm. Both soil fractions were dried (65°C until constant mass achieved) and ball-milled to a fine powder. Carbon and N contents were measured on a Costech ESC 4010 Elemental Analyzer (Costech Analytical Technologies Inc., Valencia, CA) in the Earth Systems Center for Stable Isotopic Studies at the Yale Institute for Biospheric Studies. We present estimates of the different soil C and N fractions per m^2 to a depth of 20 cm using bulk density (g cm^{-3}) measurements described above. Stable isotope data for soils and crops are given in Wood (2015).

2.4 Extracellular enzyme potential assays

Sub-samples of soil for enzymatic assays were frozen immediately after sampling and transported within one week to the United States where they were frozen at -20°C and transported on dry ice to the Natural Resource Ecology Laboratory at Colorado State University. While freezing can reduce measured enzyme activities, relative differences among treatments and sites persist (German et al. 2011). All enzyme assays were conducted within one month of sampling. We measured the rate of potential activity of seven hydrolytic enzymes (Table S1) using a fluorometric approach described in German et al. (2011) and Bell et al. (2013). Four of

the measured enzymes (BG, CB, XYL, AG) are involved in degrading C-rich substrates, two (NAG and LAP) are involved in degrading N-rich substrates, and one is involved in degrading P-rich substrates (PHOS).

Sub-samples of 2.75 g of fresh soil were homogenized and aliquots were pipetted into 96-well plates. Soils were inoculated with a non-limiting amount (200 μL) of each fluorescently labeled substrate (Table S1) dissolved in deionized water. The plate was inverted several times to mix soil samples and substrates and placed in an incubator at 25°C. Reference standards were prepared in a similar manner as the soil samples. In the standard plates, we added fluorescent standards, instead of the substrates, in seven concentrations: 0, 2.5, 5, 10, 25, 50, 100 μM. We used two types of fluorescent standards, 7-amino-4-methylcoumarin (MUC) and 4-methylumbelliferone (MUB); MUC standards were used for LAP, and MUB for the other enzymes.

After incubation, the plates were centrifuged and 250 μL of supernatant was removed from each well and pipetted into a corresponding well of a 96-well black plate. Fluorescent activities were immediately measured using an Infinite M500 spectrofluorometer (Tecan, Männedorf, Switzerland). Readings of the fluorescent activities from standards were used to calculate potential enzyme activities for each sample in units of nmol activity g^{-1} dry soil h^{-1}. We calculated three enzymatic stoichiometric ratios C:N, C:P, and N:P based on the main substrates related to each enzyme. The ratios are calculated by summing the log of enzyme potential activities for each enzyme within a given nutrient category (e.g., C) and dividing by the sum of the log of nutrients in the other category (e.g., N).

2.5 Data analysis

2.5.1 Soil organic matter fractions and extracellular enzyme potential activities

To determine the factors controlling the sizes of fast- and slow-cycling SOM fractions, we used a linear modeling approach with soil and treatment covariates as predictor variables and fraction size as a response variable. Initial predictor variables included pH, percent clay, N addition, legume rotation (a binary variable that captures the effect of legume rotation independent of N addition), and a binary variable indicating whether an observation came from an experimental plot or an actively managed farm. To select for a final model, we first assessed the normality of a given response variable using the Shapiro-Wilk test. In the case of significant non-normality, we transformed data using a Box-Cox transformation before performing model selection (Mateu 1997). The lambda parameter of the transformation was determined by computing and plotting the log-likelihood profile for the Box-Cox power transformation using the *MASS* package in R (Venables and Ripley 2002).

We identified a full model of likely predictor variables for a given response variable. To avoid cases of unclear causality (such as respiration as a predictor of enzyme potential), we did not do an exhaustive search on all potential predictor variables, but only those that we identified as likely to be causally important. To select a final model, we used an exhaustive regression subsets search using the *leaps* package (Lumley 2004). The best model was selected based on Schwartz's information criterion (BIC) score. After fitting this model, we checked for highly influential points using Cook's influence score. We tested for assumptions of constant variance and we report robust standard error estimates in cases where this assumption was violated. Validation of linear model assumptions was done using the *gvlma* package (Pena and Slate

2014). We standardized model coefficients using a z-transformation in which we converted all model variables to a common mean and standard deviation by subtracting the mean and dividing by the standard deviation for all independent model variables (Gelman 2008). The approach gives model coefficients that describe the standardized slopes, which, unlike partial correlation coefficients, are comparable in magnitude within models because variables are expressed in common units (Schielzeth 2010). Standardization was done using the *arm* package (Gelman and Su 2013). For all statistical tests, we considered coefficients with $P < 0.05$ significant and coefficients with $P < 0.10$ marginally significant (Hurlbert and Lombardi 2009).

To assess the determinants of extracellular enzyme potential activity we used the same procedure described above. In addition to including soil properties and farm characteristics in the full, initial model we also included particulate and MIN C fraction sizes as potential predictors of enzyme potential activity.

2.5.2 Organic matter relationship with crop yields

We used both linear and non-linear models to assess the relationship between yields, SOM fractions, farm management (fertilization and legume rotation) and other soil properties. Because the relationship between SOM fractions and yield visually appeared to be non-linear, we fit and compared both linear and non-linear models. We defined the non-linear model to be an exponential model given by the equation:

$$y = a^{(k*x_1)} + BX$$

where x_1 is the particular soil fraction (either POM or MIN C), *k* is the growth/decay parameter, B is a vector of coefficients, and X is a vector of control variables corresponding to B, including

percent clay, N addition, a binary variable for legume rotation, and a binary variable for whether an observation is from fertilization plots or actively managed farms. For a positive relationship between yield and SOM, an exponential growth model was fitted with a positive value for *k* and for a negative relationship an exponential decay model was fit with a negative value for *k*. Final variables in the model were selected using a stepwise approach equivalent to the one described above. To assess whether there was a significant difference in the model fit of linear vs. non-linear models, we performed a likelihood ratio test (Canham & Uriarte, 2006; Hobbs & Hilborn, 2006; Johnson & Omland, 2004). If the chi-square statistic indicated significant differences between models, we selected the model that minimized residual sum of squares. Where there was no evidence for a difference between models, we selected the linear model for parsimony. As another, more subjective, indicator of model fit, we used the final statistical model to predict new data based on the parameters generated for the model. We visualized predicted values on top of observed data to indicate whether the functional form and spread of the data was close to the original data; high overlap between original and predicted data is suggestive of an adequate model.

2.5.3 Structural equation modeling

Because of conceptual linkages among the drivers of SOM and the drivers of yield, we used structural equation modeling to simultaneously represent relationships among models and model variables. Colinearity among predictor variables in a least squares modeling framework is well known to bias coefficient estimates and make results invalid. Structural equation modeling is not subject to the same problems of colinearity because it estimates coefficients by generating implied data from the specified model and comparing the implied data with the observed data

(Grace 2006). We therefore chose to use structural equation models to simultaneously estimate each of the pathways among farm management, soil C fractions, and yields while accounting for correlations between multiple response variables.

We report standardized path estimates that allow for comparison of the relative magnitude of variables within the same model (Grace and Bollen 2005). For model goodness-of-fit, we report X^2 and root mean square error of approximation (RMSEA). These measures assess the similarity between the covariance matrix of the observed data and the covariance matrix implied by the specified model. A X^2 P-value greater than 0.05 implies significant overlap between the observed and implied data, and thus adequate model fit. Because the X^2 test is based on large sample theory, we also report RMSEA, which is a goodness-of-fit measure weighted by sample size. We use an RMSEA value below 0.1 to represent good model fit because for sample sizes less than 50, the conventional RMSEA cut-off value of 0.05 is overly conservative (Chen et al. 2008). Individual paths were estimated using maximum likelihood. Insignificant paths were excluded from models unless they significantly improved overall model fit, based on X^2 and RMSEA values as well as assessment of modification indices, which indicate important variables to add to, exclude from, or retain in the model (Grace 2006). Soil covariates (percent clay and pH) were included in models where significant, but were not visualized in the results path diagram to minimize the number of arrows and improve readability; percent clay and pH are included in reported model results. All models were fitted using the *lavaan* package (Rosseel 2012).

3. Results

3.1 Soil organic matter

3.1.1 Soil C fractions and cumulative respiration

Cumulative respiration, an indicator of the microbially available C fraction, was 32% higher on actively managed farms (3.77 g C m^{-2} to 20 cm) than on experimental fertilization plots (2.85 g C m^{-2} to 20 cm). Nitrogen addition—across both farms and plots—was positively related to cumulative respiration ($P < 0.05$, Figure 1C, Table S3). The standardized coefficient for N addition and plot identity (indicating farm or experimental plot) were similar in absolute magnitude (0.05 and -0.08, respectively), indicating that their relative impact on cumulative respiration was roughly equal.

On average, POM C:N was 17.71 (Table 1). Particulate OM C was significantly related to legume rotation, pH, and N addition (Figure 1A, Table S3). POM C fractions were 22% greater on legume rotation fields (86.26 g C m^{-2} soil to 20 cm) than on fields not using legume rotation (70.42 g C m^{-2} soil to 20 cm, including both non-legume rotation farms and fertilization plots). Legume rotation had the greatest effect on POM C ($P < 0.05$), with a standardized coefficient 1.12 times greater than pH and 1.13 times greater than N addition, both of which were also significantly, positively related to POM C fraction size ($P < 0.01$) (Figure 1A, Table S3).

On average, MIN C:N was 10.68 (Table 1). Mineral-associated C was significantly higher on experimental plots and significantly positively related to pH (Figure 1B; Table S3). Mineral-associated C fraction sizes were 17% higher on fertilization plots (345.16 g C m^{-2} soil to

20 cm) than actively managed farms (295.90 g C m^{-2} soil to 20 cm). Whether an observation came from an experimental plot (captured by the 'plot' binary variable) was the strongest relative predictor of MIN C ($P < 0.001$), with an effect 2.31 times the size of the effect of pH, which was also positively related to MIN C fraction size ($P < 0.05$) (Figure 1B, Table S3).

3.1.2 Relationship with yields

We used linear- and non-linear least squares regressions to determine the relationship between crop yield and soil organic matter pools. We found that the POM C fraction was positively related to yield, but the relationship was only mildly significant ($P < 0.1$) and the magnitude of the effect of POM on yield was low (Figure 2, Table 2). By contrast MIN C fractions were significantly negatively associated with yield ($P < 0.05$) and had a relatively strong impact on yields (Figure 2, Table 2). We use structural equation modeling to quantitatively evaluate the relative impact of POM-vs.-MIN C on yields (results described below). Both percent clay and 'experimental plot' were significantly negatively related to yields ($P < 0.05$, Table 2). Because the MIN C model is non-linear, we were unable to compare standardized coefficients to determine relative importance of variables. Relative importance was instead assessed using structural equation modeling (Section 3.3).

3.2 Extracellular enzyme potential

We found that the C-degrading enzyme potential was negatively associated (mildly significantly) with MIN C ($P < 0.10$), fitting with our first hypothesis that MIN C would be negatively correlated with C-degrading enzymes. Carbon-degrading enzyme potential, however, were not negatively associated with POM C, as was predicted under our first hypothesis. The

potential activity of all C-related enzymes was most significantly impacted by percent clay (Figure 3A, individual enzyme activities reported in Table S2). The relative importance of MIN C and percent clay—as indicated by standardized regression coefficients—was approximately the same (Figure 3A, Table S3).

In addition to the negative relationship between C-degrading enzymes and MIN C, we observed a significant negative relationship between enzymatic stoichiometry and mineral-associated C pools, which also supports our first hypothesis. The ratio of total C-to-total N degrading enzymes was lower on experimental plots ($P < 0.05$, Figure 3B, Table S3), which were also associated with greater MIN C pool sizes, though this model had low overall explanatory power (adj. $R^2 = 0.10$). Carbon-to-N degrading enzymes, however, were not negatively associated with POM C, as was predicted under our first hypothesis. Both percent clay and MIN C were negatively related to the investment in C- vs. P-degrading enzymes ($P < 0.01$ and $P < 0.05$, respectively), but POM C was not a significant predictor. The magnitude of the effect of percent clay was one-and-a-half times greater than that of MIN C (Figure 3C, Table S3). The model of N- vs. P degrading enzymes also had low explanatory power (adj. $R^2 = 0.07$) and only percent clay was significantly related (negatively) to enzyme stoichiometry (Figure 3D, Table S3).

3.3. Structural equation models

We used least squares regression to perform our hypothesis testing (results reported above), but this approach cannot simultaneously evaluate relationships between farm management, SOM fractions, and crop yields because of high co-linearity between variables. To

simultaneously fit models of SOM and yield response we used structural equation modeling. Our structural equation modeling confirmed results from the linear and non-linear models. We found that yields were positively and significantly associated with POM, but negatively on MIN C. The SEM allowed us to assess the relative importance of these variables. We found that the plot binary variable had the strongest effect on yield, followed by MIN C, percent clay, and POM C (Table 2). POM C was the only variable that was significantly positively related with yields. The effect of the plot variable was between 1.5 and 2 times stronger than the other variables. The effect of MIN C on yield (negative) was 1.37 times greater than the positive effect of POM C on yield.

Particulate OM C fractions were significantly positively related to pH, N addition—on both plots and farms, and legume rotation, in order of effect magnitude. The effect of pH was similar to that of N addition (1.1 times greater); the effect of N addition on POM C was 1.3 times greater than that of legume rotation. As in the linear model, MIN C fractions were positively related to the plot variable and pH, with the plot effect being 3.5 times larger. The structural equation model metrics indicated that crop productivity was also an important predictor of MIN C. We included yield in the model and its effect magnitude was 70% as strong as the plot variable and 2.4 times stronger than the pH effect (Table 3).

Though yields were impacted by POM and MIN C, and POM C was impacted by N addition and legume rotation, N addition and legume rotation did not significantly affect yields other than through POM. Structural equation model modification indices for N addition and legume rotation were greater than 3, indicating that they are important to retain in the model for

overall model fit. Their individual coefficients (relationship to yields), while positive, were not statistically significant.

4. Discussion

The objectives of our study were to identify the effect of organic and mineral nutrient addition on (1) microbial enzyme activities—the proximate mechanism of SOM transformation and degradation—and (2) SOM fractions and their relationship to yield within a smallholder tropical agroecosystem. In support of our first hypothesis, we found that the inclusion of recent organic inputs through legume rotation resulted in higher levels C in POM fractions. Land-use history—specifically the long-term presence of continual vegetation in the past on the experimental plots—was the dominant control of mineral-associated fractions, which turn over on longer time scales. In support of our second hypothesis, we found that soils with higher POM had elevated yields, independent of mineral nutrient addition. However, the effect of POM was minor compared to other variables, such as the amount of MIN C present in the soil and whether a soil came from an experimental plot. Based on Lal (2004), we also hypothesized that yields would be positively related to MIN C because of greater long-term retention of nutrients and higher water-holding capacity. Counter to this hypothesis, we found a negative relationship between MIN C and yields. This finding is thus in conflict with the notion that the long-term build up of SOM can contribute to food security (Lal 2004 but see Janzen 2006). Our finding suggests a need to improve understanding of how SOM impacts crop yield by identifying and quantifying the effect of specific OM fractions on plant production.

4.1 Changes in enzyme stoichiometry

Previous findings have shown that the stoichiometry of extracellular enzyme acquisition is responsive to nutrient availability (Sinsabaugh et al. 2008). For instance, N addition to N-limited systems has been found to stimulate OM decomposition through changes in enzymatic ratios (Keeler et al. 2009). We did not find that enzyme ratios—either C:(N or P) or N:P—were dependent on N addition. We did find, consistent with our first hypothesis, that C-degrading enzymes, overall, were correlated with stable (MIN), but not labile (POM), C pools. Also consistent with the first hypothesis, we found that enzyme stoichiometry was related to MIN C—specifically, we observed less investment in C- relative to N- and P-degrading enzymes in soils with more MIN C. The negative relationship between C-degrading enzymes (and C:N/P of enzyme potential) may be because, as we hypothesized, MIN C is bound to mineral surfaces and more resistant to enzymatic attack (MIN C does mineralize, but does so more slowly). We did not find that the C:N ratio of either particulate or mineral-associated OM was a significant predictor of enzyme stoichiometry, further suggesting our finding is related to the availability, not the quality, of organic matter to microbes.

4.2 Organic matter fractions and crop yields

In support of our first hypothesis, we found that short-term organic inputs (legume rotation) were positively related with the POM fraction and that the longer-term fallow on fertilization plots was strongly positively related to the mineral-associated fraction. We found, contrary to our expectations, that mineral N addition was positively related to the size of the POM fraction. This effect may be due to N addition increasing plant biomass and, thus, the amount of particulate inputs. Nitrogen addition has been shown to stimulate total soil C in

agricultural soils, largely by stimulating low molecular weight carbon inputs from plant roots (Lu et al. 2011, but see Khan et al. 2007). These root-derived inputs often pass through a microbial filter and become stabilized as soil C on mineral surfaces (Bradford et al. 2013). This supports the expectation that N addition would be a significant driver of MIN C. However, the quantity of MIN C was most significantly determined by whether a soil came from an experimental plot. We attribute this pattern to the fact that soils on experimental plots have a long history of fallow and native vegetation before recent conversion to cultivation, while actively managed farms have been cultivated for many decades. Thus, regular plant inputs to experimental plots over the long term should have created greater stable (mineral-associated) C pools—that mineral-associated C could be directly associated with mineral surfaces, held in secondary organo-mineral complexes where it is bound to other organic compounds that are held by mineral surfaces (Chenu and Plante 2006), or held in micro-aggregates. Long-term cultivation should decrease soil C through the oxidation of organic matter and break up of aggregates.

Though N addition should also contribute to stable C, we may not be observing that effect for three potential reasons. First, N addition is a fairly recent—and interannually variable—management strategy on farms and its effect may be small relative to the large background stable C pool already in the soil. Second, experimental plot soils, which have significantly higher total soil C than farm fields, because of a long history of no tillage and high plant inputs from the long vegetative fallow, making them potentially close to C saturation—it is harder to build up more C even with extra N (Castellano et al 2015). Third, the impact of N addition on mineral C may be indirect and mediated by plant productivity. When fitting the structural equation model, we discovered that crop yield was a strong predictor of MIN C (70%

as important as the plot indicator). Thus, mineral N inputs that drive plant productivity may be indirectly contributing to stable C through plant inputs, which may also explain the positive relationship between mineral N and POM C.

To test our second hypothesis regarding the effects of different soil C pools on maize yields, we used structural equation modeling to control for nutrient addition and soil properties and found that MIN C was negatively associated with yields, while POM C was positively related to yields (though both variables were weaker predictors than the effect of experimental plot). This finding supported our hypothesis that POM would be positively related to crop yields because it represents a faster-cycling fraction that can liberate nutrients for crop growth, and/or contribute to soil aggregate formation and retention (Haynes 2005). However, the relative weakness of POM as a predictor suggests that its effect, while significant, may be less important in magnitude than other drivers of yield.

The finding that MIN C was significantly negatively related to crop yield conflicts with the current paradigm that long-term build up of SOM is important for food security (Lal 2004). Instead, our findings suggest that the effect of OM on productivity depends on the specific fraction of OM considered: build-up of short-term OM pools may have a positive relationship to yields (though the magnitude can be weak), but build-up of long-term pools may be negatively related. One potential reason that MIN OM is not positively related to yields—as we predicted—is that the stabilized OM may delay the release of nutrients into the plant-available pool, thus not contributing to yields (Janzen 2006). Though this may explain the lack of positive relationship, it does not explain the negative relationship between yield and MIN C. The mechanism explaining

the negative yield-MIN C relationship is not clear. One possible mechanism is that soils with greater MIN C are locking up more nutrients in secondary organo-mineral complexes. Primary organo-mineral complexes are the associations between organic matter and the mineral environment of the soil (Chenu and Plante 2006). Our data suggest higher organo-mineral complexes (e.g. MIN C) in the plot soils because of long-term plant inputs. There is evidence that organic matter can also be stabilized indirectly onto mineral surfaces through bridges with other organic complexes (Clarholm et al. 2015; Keiluweit et al. 2015). In this case, soils with greater MIN C may be locking up additional nutrients from plant-available pools through these secondary organo-mineral complexes, which could potentially explain the negative relationship with yields. Fallow vegetation in Western Kenya is often dominated by *Lantana camara*, which is high in polyphenols and tannins (Palm et al. 2001) that can strongly bind mineral N and could be held in secondary organo-mineral complexes. Additionally, soils in Western Kenya can have high levels of micro-aggregate formation, which can protect OM from mineralization (Verchot et al. 2011). More work, however, needs to be done to confirm the mechanism of our observation. Also, the negative relationship between MIN C and yields could be offset by nutrient addition through POM or mineral sources. This is supported by our finding on legume rotation farms that MIN C does *not* negatively impact yields; instead, yields are slightly positively related to POM C pools.

Also contrary to our expectations, we found that short-term nutrient addition through legume rotation and N addition did not directly impact crop yield. Though counterintuitive, this finding is consistent with other results from this system which show that crop yields were not significantly impacted by N addition in 2013, but were impacted by nutrient input in 2012 (Tully

et al. 2015). One possible explanation for this finding is poor rain conditions in 2013. In 2013, weather was variable and dry spells were frequent at important crop growth stages (Tully et al. 2015), which may have acted as an overall control on crop productivity, masking the effect of nutrient addition. In 2012 there was more favorable weather and yield was responsive to nutrient addition (Tully et al. 2015).

5. Conclusions

Some researchers have argued strongly that the long-term build up of SOM will have co-benefits for food security and climate change mitigation (Lal 2004). Our results challenge this perspective by showing a negative relationship between yield and long-term stabilized OM. Current focus on the benefits of SOM rarely distinguishes between different fractions (e.g., Banwart et al. 2014). Our results suggest that different soil C pools may have differential impacts on crop yield. This is a key finding because an emerging paradigm in SOM research suggests that different soil C pools may have different underlying drivers and potentially effects (Janzen 2006, Schmidt et al. 2011). Thus, understanding the controls on each of these pools and quantifying their impacts on different ecosystem properties will be essential to the management of SOM for ecosystem services.

Because our results are based on observation, and not experimental test of mechanism, future work should clarify the mechanisms behind our findings and assess whether they are generalizable across different types of production systems and soil types. Nevertheless, our findings highlight that different SOM fractions may have differential relationships to key crop-

related outcomes (and potentially other ecosystem services) and that this context dependency may need to be taken into account when managing for soil-based ecosystem services.

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Author Contributions

S.A.W conceived research, conducted fieldwork, analyzed data, and wrote the manuscript; S.A.W, M.A.B, C.A.P, and S.N designed research; S.A.W, C.W.B, and N.S conducted lab work; M.A.B and M.D.W contributed materials and reagents; M.A.B, C.A.P, and N.S contributed significantly to manuscript revisions; all authors contributed to data interpretation and provided feedback on the manuscript.

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Data Availability

Data: https://dx.doi.org/10.6084/m9.figshare.3083554.v2
Code: https://dx.doi.org/10.6084/m9.figshare.3083557.v2

Tables

Table 1. Soil properties, organic matter fractions, and enzyme stoichiometry by treatment. Individual enzyme activity is presented in Table S2. Values are means with standard deviation in parentheses.

					Soil Organic Matter						**Enzyme Stoichiometry**		
	Soil properties				*C pools (g C m^2 $soil^{-1}$ to 20 cm)*			*C:N*			*nmols g dry $soil^{-1}$ h^{-1}*		
	pH	% Silt	% Sand	% Clay	Microbially-available	Mineral	Particulate	Mineral	Particulate	Total	C:N	C:P	N:P
Managed Farms													
Low Fertilizer (n=9)	5.54	14.23	53.92	31.74	2.93	308.03	65.94	10.65	17.02	11.39	1.64	0.46	0.27
<10 kg N ha-1	[0.40]	[7.13]	[5.29]	[5.93]	[0.54]	[47.68]	[20.09]	[0.54]	[1.62]	[0.41]	[0.99]	[0.33]	[0.04]
High Fertilizer (n=9)	5.31	10.48	55.81	33.63	4.70	292.37	66.81	10.56	16.86	11.33	1.08	0.32	0.31
> 60 kg N ha-1	[0.28]	[6.17]	[2.99]	[6.93]	[1.15]	[33.61]	[15.26]	[0.35]	[1.67]	[0.43]	[0.28]	[0.03]	[0.06]
High + Legume Rotation (n=6)	5.32	10.08	58.48	31.33	4.04	284.83	86.26	10.49	17.61	11.57	1.22	0.33	0.30
	[0.62]	[4.28]	[1.86]	[4.59]	[0.89]	[42.07]	[24.62]	[0.34]	[1.66]	[0.59]	[0.45]	[0.07]	[0.09]
Experimental Plots													
(kg N ha^{-1})													
0 (n=4)	5.97	13.45	51.60	34.80	2.47	338.18	66.59	10.72	17.50	11.44	1.22	0.34	0.29
	[0.26]	[2.20]	[4.87]	[5.31]	[0.45]	[50.86]	[18.88]	[0.59]	[1.55]	[0.45]	[0.47]	[0.11]	[0.08]
50 (n=4)	5.64	13.45	51.60	34.80	2.59	330.94	77.32	10.63	18.66	11.56	1.01	0.31	0.33
	[0.46]	[2.20]	[4.87]	[5.31]	[0.32]	[24.27]	[37.47]	[0.66]	[1.05]	[0.91]	[0.14]	[0.11]	[0.17]
75 (n=4)	5.67	13.45	51.60	34.80	2.65	350.22	72.26	10.82	18.99	11.69	0.96	0.26	0.28
	[0.30]	[2.20]	[4.87]	[5.31]	[0.52]	[34.56]	[5.30]	[0.23]	[1.38]	[0.19]	[0.18]	[0.05]	[0.04]
100 (n=4)	5.53	13.45	51.60	34.80	2.65	353.63	68.53	10.70	18.09	11.46	0.86	0.26	0.32
	[0.44]	[2.20]	[4.87]	[5.31]	[0.37]	[26.93]	[3.60]	[0.36]	[0.10]	[0.29]	[0.10]	[0.08]	[0.14]
150 (n=4)	5.16	13.45	51.60	34.80	4.31	357.09	75.60	10.93	19.36	11.83	1.17	0.27	0.25
	[0.29]	[2.20]	[4.87]	[5.31]	[2.98]	[21.73]	[11.12]	[0.62]	[0.60]	[0.53]	[0.49]	[0.06]	[0.05]
200 (n=4)	5.05	13.45	51.60	34.80	2.55	325.45	84.41	10.42	17.63	11.38	1.01	0.29	0.30
	[0.10]	[2.20]	[4.87]	[5.31]	[0.42]	[17.76]	[12.70]	[0.34]	[0.75]	[0.29]	[0.21]	[0.05]	[0.09]

Table 2. Nonlinear and linear models of crop yield responses to different organic matter fractions. In the model formula x_1 is the variable of interest (the soil organic matter fraction) and *k* its parameter. X is a vector of control variables and B its associated parameter vector. Linear and non-linear models are selected based on likelihood ratio test. Where models are significantly different the model with the lowest residual sum of squares was selected, where not different, the linear model was selected for parsimony. A * next to the response variable indicates the model in that column was selected as the best model. Coefficient estimates are presented with standard error in parentheses. Model results are visualized in Figure 2. * p < 0.1; ** p < 0.05; *** p < 0.001; **** p < 0.0001

Response variable	*Yield*	*Yield**	*Yield**	*Yield*
Model type	POM NLS	POM Linear	MIN NLS	MIN Linear
Model equation	$y = a^{(k*x_1)}+B*X$	$y = a+k*x_1+B*X$	$y = a^{(k*x_1)}+B*X$	$y = a+k*x_1+B*X$
Parameters				
a	5.077***	4.841***	12.248****	10.667****
	(1.418)	(1.496)	(2.916)	(2.055)
POM C (k)	0.002	0.016*		
	(0.002)	(0.009)		
MIN C (k)			-0.002**	-0.012**
			(0.001)	(0.005)
Plot vs. Farm	-1.879****	-1.889****	-1.121**	-1.160***
	(0.331)	(0.331)	(0.408)	(0.406)
Percent clay	-0.081**	-0.079**	-0.116***	-0.115***
	(0.038)	(0.038)	(0.037)	(0.038)
Model evaluation				
Adj. R^2		0.51		0.54
Log likelihood	-70.092	-69.968	-68.251	-79.889
RSS	52.134	51.865	48.286	78.417
P-value	P > 0.1		P < 0.000	

Table 3. Parameter estimates and model statistics for structural equation model of the relationship between crop yield and soil organic matters. Variables are ordered by absolute value of standardized coefficient, a higher value of which indicates a greater relative impact on the response variable. X^2 statistics represent overlap between observed- and model-implied data; P > 0.05 thus indicates that the model adequately represents the data. Root mean square error of approximation (RMSEA) is a sample-size weighted measure of model fit. A 90% confidence interval is reported; P values below 0.1 indicate good model fit.

	Standardized Estimate	Estimate	SE	P
Yield ~				
Plot	-0.40	-0.40	0.08	0.00
MIN C	-0.26	0.00	0.00	0.00
Percent Clay	-0.22	-0.02	0.00	0.00
POM C	0.19	0.00	0.00	0.01
Legume rotation	0.08	0.09	0.09	0.28
N Addition	0.04	0.00	0.00	0.65
POM C ~				
pH	0.44	19.71	7.69	0.01
N Addition	0.40	0.13	0.04	0.00
Legume rotation	0.32	17.82	5.75	0.00
MIN C ~				
Plot	1.18	98.35	38.75	0.01
Yield	0.83	88.98	62.67	0.16
pH	0.34	34.65	13.20	0.01
Structural Equation Model Metrics				
n		48		
df		6		
χ^2		5.15		
$P_{\chi 2}$		0.52		
RMSEA		[0.00,0.17]		
P_{RMSEA}		0.6		

Figure Headings

Figure 1. Coefficient plots for different soil organic carbon pools. Standardized regression coefficients are visualized. The dotted centerline represents no effect, points to the right have a positive effect on the response variable, and points to the left have a negative effect. Distance of coefficient point from the dotted centerline represents the absolute effect size. Thus, variables further from center have a stronger effect on the response variable. Full model results are presented in Table S3.

Figure 2. Relationship between yield and particulate organic carbon (A), and yield and mineral-associated organic carbon (B). Filled circles are actual data points (n=48) and size is adjusted by percent clay content to visualize an important co-variate. Unfilled circles are data points predicted by the statistical model; good overlap between the two indicates adequate model fit. The given equation is the equation of the line defining the relationship between each soil C pool and yield, controlling for co-variates. Statistical model results are reported in Table 2.

Figure 3. Coefficient plot for enzymes. Standardized regression coefficients are visualized. The dotted centerline represents no effect, points to the right have a positive effect on the response variable, and points to the left have a negative effect. Distance of coefficient point from the dotted centerline represents the absolute effect size. Thus, variables further from center have a stronger effect on the response variable. Full model results are presented in Table S3.

Figure 4. Structural equation modeling of the relationship between crop yield and soil organic matter fractions. Hypothesized relationships are shown in panel A. Solid lines (Panel B) represent fitted relationships in the model that are statistically significant at $P < 0.05$, dotted lines are insignificant at $P < 0.05$, but were retained in the model based on model selection criteria. Shade represents sign of the relationship (black = positive, gray = negative). Path widths are proportional to standardized regression coefficients, which are shown in Table 3 with P values and model statistics. To visualize farm influence on organic matter and crop yield, models control for, but do not visualize, effects of pH and percent clay.