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Agricultural intensification and the functional capacity of soil microbes on smallholder African farms

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Summary

1. Fertilization may impact ecosystem processes that sustain agriculture, such as nutrient cycling, by altering the composition of soil microbial communities that regulate such processes. These processes are crucial to low-input, smallholder tropical agriculture, which supports 900 million of the world's poorest people. Yet little is known about how efforts to increase crop yield on such farms will affect the capacity of soil microbial communities to carry out ecosystem processes.

2. We studied the diversity and functional capacity of microbial communities on smallholder farms in western Kenya. We measured functional capacity as the abundance of functional genes involved in several components of nutrient cycling as well as catabolism of multiple carbon substrates; taxonomic diversity was measured using metagenomic sequencing. Diversity and functional capacity were measured on short-term, experimental mineral fertilizer addition plots and on actively managed farms that have maintained for at least seven years a management strategy of low mineral fertilization, high mineral fertilization, or high fertilization combined with legume rotations.

3. Soil bacterial diversity decreased with mineral fertilizer addition, with a community shift towards taxa that thrive in high-resource conditions. This taxonomic response did not correspond with decreased microbial functional capacity. Instead, functional capacity was increased, along with yields, when fertilizers were combined with legume rotations that add organic matter to soil.

4. *Policy implications*. Mineral fertilizer use is associated with lower soil microbial diversity on smallholder farms, but not associated with changes in microbial functional capacity. Functional capacity is highest, along with yields, when mineral fertilizers are paired with legume rotations. Our findings suggest that this type of agroforestry can be an important strategy for maintaining the long-term functional capacity of soil microbes as well as increasing crop yields on smallholder farms. These observations support proposals to achieve long-term food production targets in sub-Saharan Africa by combining mineral fertilizers with organic inputs.

Key-words: African Green Revolution, agroforestry, fertilization, functional diversity, GeoChip, microbial diversity, smallholder agriculture

Introduction

Intensive agriculture has driven increases in crop production, but is responsible for environmental damage, such as water pollution and greenhouse gas emissions (Vitousek *et al*. 2009). Intensification may also impact the ecosystem processes that sustain agriculture, such as soil nutrient cycling, by altering the composition of soil microbial communities that mediate these processes. The composition of microbial communities is controlled by land management, such as fertilizer addition (Ramirez *et al*. 2010; Fierer *et al*. 2012; Ramirez, Craine & Fierer 2012), yet little is

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known about whether management-induced changes in community composition will feed back on microbial capacity to control the ecosystem processes on which agriculture depends.

Soil nutrient cycling processes, such as carbon (C) and nitrogen (N) cycling, are especially crucial to low-input smallholder tropical agriculture, which supports 900 million of the world's poorest people on 500 million farms of <2 ha (Wiggins, Kirsten & Llambi 2010). Nutrient budgets on these farms are undergoing rapid change due to increases in mineral (Vitousek *et al.* 2009) and organic fertilizer use (Glover, Reganold & Cox 2012) promoted to increase yields and decrease poverty – often referred to as the African Green Revolution. It remains unknown how such modifications to nutrient economies of smallholder farms will impact the functional capacity of soil microbial communities.

Fertilization decreases the diversity of plant communities (Bobbink *et al.* 2010) and causes shifts in microbial community composition (Ramirez *et al.* 2010; Fierer *et al.* 2012; Ramirez, Craine & Fierer 2012). We thus hypothesize that fertilization on tropical smallholder farms will be associated with decreased microbial taxonomic diversity and a shift in community composition towards taxa that perform well in high-resource environments (e.g. copiotrophs), thus resulting in lower microbial functional capacity (*diversity-functioning hypothesis*; Bell *et al.* 2005). Lower taxonomic diversity should lower functional capacity by creating a community that has a lower range of functionally distinct taxa in similar abundances. A community shift towards copiotrophic taxa should also decrease functional capacity by producing a community with a lower ability to use recalcitrant C that makes up the bulk of the soil C pool and, thus, lower heterotrophic respiration and microbial standing biomass (Fierer *et al.* 2012), which is a key control of microbially mediated processes.

Microbial functional capacity is also constrained by nutrient availability (Drake *et al.* 2013). We therefore alternatively hypothesize that fertilization will increase functional capacity by allowing microbes to overcome nutrient limitation and thus increase their potential contribution to ecosystem processes (*limitation release hypothesis*). The addition of mineral and organic nutrients, which colimit microbial activity, should build functional capacity by increasing the ability of microbes to produce extracellular enzymes that drive organic matter decomposition (Drake *et al.* 2013). As a result, soil microbes should increase their active biomass (Drake *et al.* 2013). If the *limitation release hypothesis* is supported then we would expect microbial functional capacity to increase with fertilization, along with fertilizer-induced increases in crop productivity.

To test these hypotheses, we collected data on microbial taxonomic diversity and community composition, functional capacity and crop yield from experimental plots and actively managed farms in western Kenya (see Fig. S1 in Supporting Information). Actively managed farms are categorized as low fertilizer use, high fertilizer use and high fertilizer use plus legume rotations. Experimental plots only include mineral fertilizer addition. The study was conducted in the Sauri village cluster of the Millennium Villages Project (MVP) in western Kenya (Fig. S1, Supporting information). The MVP agriculture strategy aims to implement an African Green Revolution strategy through high-yielding crop varieties, mineral fertilization and combining fertilizer use with organic inputs.

Materials and methods

SITE DESCRIPTION

The study zone is mixed maize agriculture with maize production usually occurring twice annually, during a long rainy season (March–June: 1100 mm) and a short rainy season (September–November: 700 mm). Soils are Kandiudalfic Eutrodox (U.S.D.A) and are well-drained sandy clay loams derived from volcanic parent materials.

We sampled soil from both a controlled fertilizer addition experiment and a actively managed farms with at least 7 years of low or high fertilizer use or high fertilizer use paired with seasonal legume rotations. On experimental plots, we sampled from five levels of mineral fertilization (0, 50, 75, 100 and 200 kg N ha^{-1}). Each treatment has four replicates. Plots are 6 × 3 m and arranged in two rows, separated by 0·5 m within a row and by a 10 m buffer between rows. Fertilizer is added in a split application with one-third added at planting as diammonium phosphate and the remainder added as urea at top-dressing (4–6 weeks after planting). Because diammonium phosphate is 18% NH_4 and 46% P_2O_5, which is 44% P, the fertilization treatment also adds: 0, 3·35, 5·02, 6·69 and 13·38 kg P ha^{-1}. This management was maintained for 2 years prior to sampling, before which the land was unplanted fallow. There is no legume rotation treatment on the experimental plots. Experimental plots are located approximately in the middle of the study zone at 0°06′04·88 N, 34°30′40·12 E at an elevation of 1450 m.

Actively managed farms were selected to represent three broad management approaches: low fertilizer, high fertilizer and high fertilizer + legume rotation. In the long rainy season, high-fertilizer farms received 60 kg N ha^{-1} or more, but often closer to 60 kg N ha^{-1} since this is the recommended application amount, and low-fertilizer farms received <10 kg N ha^{-1} (Table 1). On legume rotation farms, farmers replace short-rain maize crops with fast-growing leguminous tree, shrub or herbaceous species (Table 1) that are planted from seed and cut each year for organic inputs to crop fields. These legume rotation techniques were initially promoted in Sauri in the early 1990s as a low-cost option for improving soil fertility.

Farm selection was based on 2 years of household surveys on 42 candidate farms to identify N inputs (from reported inputs of diammonium phosphate, calcium ammonium nitrate and urea), maize yield and crop choice over the past 10 years. The 21 farms included in the final list reported management strategies that were not highly variable over the 10-year reporting period, had inputs and outputs that roughly agreed and had farmer-reported yields that were demonstrative of their reported fertilizer levels. Based on reported N_2 fixation rates in the region, we conservatively estimate that N_2 fixation contributed between 30 and 50 kg N ha^{-1} $year^{-1}$ (Gathumbi, Cadisch & Giller 2002). Plant-

Table 1. Farm selection criteria for high fertilizer, low fertilizer and high fertilizer + legume rotation farms

	High fertilizer	Low fertilizer	High fertilizer + legume rotation
Short rain activity	Maize	Maize	Legume rotation
Management history	Fertilizer since 2005	Patchy fertilizer use	Annual fallow for at least last 7 years
N amount	~60 kg N ha^{-1} $year^{-1}$	<10 kg N ha^{-1} $year^{-1}$	65–110 kg N ha^{-1} $year^{-1}$
Farm area	Mean: 0·4 Min–Max: 0·1–3·7 ha	Mean: 0·3 ha Min–Max: 0·2–1·6 ha	Mean: 0·2 ha Min–Max: 0·1–1·3 ha
Species present			*Calliandra calothyrsus* *Crotalaria grahamiana* *Crotalaria paulina* *Crotalaria ochroleuca* *Mucuna pruriens* *Tephrosia candida*
Species density			2000 plants ha^{-1}

ing densities can vary widely from year to year with low-density years being as low as an order of magnitude less than those assumed in this estimate. Thus, actual fixation rates may be as low as 5–30 kg N ha^{-1}.

In the final farm sets, all treatment types were clustered spatially into *farm sets* (Fig. S1, Supporting information) to control for differences in elevation and texture across the landscape. Farms in a set are all situated within 200 m of one another along the same contour or slope. Sample size was limited by the fact that farmers who live in close proximity tend to have similar farming approaches. To partially address this issue of sample size, we added pairs of high- and low-fertilizer farms, which was a more common treatment than long-term legume rotation. The final list includes 21 farms grouped into five sets of the three farm types plus three high–low pairs, totalling eight high-fertilizer farms, eight low-fertilizer farms and five legume rotation farms.

SOIL SAMPLING

Soil sampling was conducted in June 2012, in the middle of the long rains, 2 weeks after fertilizer application. On the farm fields, we took 15 2-cm-diameter soil cores from the top 20 cm of bulk soil. Cores were taken at regular intervals throughout the entire farm field and homogenized and aggregated to a composite sample. Because experimental plots were significantly smaller than farms (18 m^2 compared to 0·1–3·7 ha), we took nine 2-cm cores per plot and aggregated to a composite sample. Soils were sieved to 2 mm using a UV-sterilized sieve. Soils for catabolic assays were immediately refrigerated and transported to the laboratory within 1 week of sampling where they were stored at 4 °C. Soils for DNA extraction were immediately frozen and transported to the laboratory within 1 week of sampling where they were stored at −20 °C.

CROP AND SOIL PROPERTIES

A subsample of sieved soil was air-dried and used to determine total C and total N by combustion with an Elementar Vario Macro CNS analyser. Extractable P and micronutrients were assessed by inductively coupled plasma spectrometry (Varian Vista MPX Radial ICP-OES). Soil texture was determined using the standard hydrometer method. Yields were measured by harvesting above-ground biomass in a 3 × 3 m subplot on actively managed farms and by harvesting the entire plot on the experimental farm, less the border rows. 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.

Subsamples of sieved field soil (stored at 4 °C for 1 month) were used to determine pH, gravimetric soil moisture and water-holding capacity using standard methods. Active microbial biomass was determined using modified substrate-induced respiration (West & Sparling 1986). Microbially available C was estimated using a 30-day C mineralization assay (Bradford, Fierer & Reynolds 2008) by measuring CO_2 efflux across 30 days (days 1, 4, 15, 30). For each measurement, 4 g of soil was placed in 50-mL centrifuge tubes that were fitted with gas-tight lids. Tubes were flushed with CO_2-free air and incubated for 24 h. Headspace CO_2 concentrations were measured using infrared gas analysis (Li-COR model LI-7000, Lincoln, NE, USA). Samples were maintained at 60% water-holding capacity across the 30-day period.

MICROBIAL TAXONOMIC DIVERSITY

To classify soil bacterial communities, we extracted DNA, amplified the 16S rRNA V4 gene, and sequenced the gene using an Illumina MiSeq instrument at Argonne National Laboratory (Gilbert *et al.* 2010). The 16S rRNA gene is a well-conserved gene in bacteria that captures evolutionary relationships among bacterial taxa. Sequence reads were binned into operational taxonomic units (OTUs) based on a 97% similarity threshold. OTUs were then compared to GenBank to identify bacterial lineages. All procedures were performed using the standard protocols of the Earth Microbiome Project (www.earthmicrobiome.org/emp-standard-protocols/; Gilbert *et al.* 2010). A total of 3 462 835 bacterial sequences were generated across all samples, representing 29 195 OTUs. Sequence lengths averaged 150·63 ± 2·93 per sample; samples were compared at a depth of 40 sequences per sample.

MICROBIAL FUNCTIONAL CAPACITY

To assess the abundance of key functional genes, we used GeoChip 4.0 to analyse DNA samples that were extracted following the protocol for taxonomic assessment. GeoChip is a functional gene array that examines the abundance of thousands of functional gene variants simultaneously through a fluorescent procedure. DNA samples were labelled with a fluorescent dye and

purified following Yang *et al.* (2013). Labelled DNA was suspended in a hybridization solution before hybridization on a MAUI station (BioMicro, Salt Lake City, UT, USA). GeoChip microarrays were scanned by a NimbleGen MS200 scanner (Roche, Madison, WI, USA). Signal intensities were quantified and processed using a previously described data analysis procedure (Yang *et al.* 2013). We analysed the following: ammonification; assimilatory N reduction; C fixation; cellulose, chitin; hemicellulose; lignin, pectin and starch degradation; denitrification; dissimilatory N reduction; methane oxidation; methane production; N fixation; N limitation; nitrification; phosphate limitation; and phosphorus utilization. Some categories are aggregates of specific genes, such as 'denitrification', which includes *narG*, *nirK*, *nirS*, *norB* and *nosZ*.

We assessed the ability of microbial communities to degrade C substrates using a catabolic profiling assay that measures microbial respiration on a range of C substrates that represent key plant inputs to the soil system, including root exudates (labile) and structural parts of plants and fungi (recalcitrant) (Degens & Harris 1997). Included substrates are sucrose, glucose, glycine, citric acid, oxalic acid, yeast, chitin and cellulose. 8 mL of each of the eight different substrates (plus a control) was added separately to 4 g of soil (dry wt equivalent) in a 50-mL centrifuge tube to make a slurry and shaken for 1 h. Soils were capped and flushed with CO_2-free air and incubated at 20 °C for 4 h (labile substrates) or 24 h (recalcitrant substrates). Net CO_2 production was measured by injecting 5 mL of centrifuge headspace into an IRGA (Li-COR model LI-7000).

DATA ANALYSIS

We calculated Shannon diversity of OTUs and Faith's phylogenetic diversity (PD) from unweighted UniFrac differences in OTUs among samples (Lozupone *et al.* 2011). Faith's PD constructs a phylogenetic tree and calculates the sum of all branch lengths in the portion of the tree connecting a given set of OTUs. For experimental plots, we fitted visually weighted regression models of changes in microbial diversity and community composition. Visual weighting adjusts the colour saturation and contrast of bootstrapped regression lines proportional to an estimate's variance; ranges of the data where the confidence interval is dark and sharply contrasted with the regression line indicate high confidence in that local data region (Hsiang 2013). Since this approach is nonparametric and cannot be used for hypothesis testing, we used piecewise linear regression for hypothesis testing (Muggeo 2003). Piecewise linear regression identifies thresholds in the microbial response to fertilization and fits separate linear regressions for the separate segments of the data.

For all models on experimental plots, we included fertilization treatment, soil pH, % C, and % N as control variables and selected a final model that optimized adjusted R^2. The terms 'control variables' and 'covariates' are interchangeable; we use the former to highlight that we are interested in the effect of farm management on microbial diversity and functional capacity, controlling for broad soil properties. We did not include texture as a control because of lack of variation among the experimental plots. We standardized independent model coefficients using a z-transformation that produces coefficients representing standardized slopes, which are comparable in magnitude within models because variables are expressed in common units (Schielzeth 2010). Because the response of community composition to fertilization on experimental plots was linear, we used a conventional linear modelling approach, rather than piecewise regression as used for the diversity variables.

For active farms, we fit generalized least squares models assuming a Gaussian error distribution. As on experimental plots, we included fertilization treatment, soil pH, % C, % N and texture as control variables. Because of the spatial distribution of farms, we tested for spatial autocorrelation using Moran's I. When present, we controlled for autocorrelation by weighting residuals by the semivariogram of autocorrelation; when not present, we used linear mixed effects (LME) models with farm set – the spatial cluster to which each farm belongs – as a random effect (Bates, Maechler & Bolker 2012). The final LME models were selected to minimize AIC and adjusted R^2 values are reported as a measure of model goodness-of-fit (Tables S3 and S4, Supporting information). The reported R^2 value represents the amount of variance explained only by the fixed effects and is calculated by adapting a previous approach for calculating non-adjusted R^2 values for LMEs (Nakagawa & Schielzeth 2013). The F-statistic is not considered valid for the 'LME4' package (Baayen, Davidson & Bates 2008); we therefore estimated *P*-values and coefficients following the Satterthwaite approach to estimating denominator d.f. (Kuznetsova, Christensen & Brockhoff 2012).

To assess functional genetic capacity of GeoChip data, we used the same modelling framework of community composition described above. To assess catabolic potential, we applied an approach designed to assess multiple ecosystem processes (Byrnes *et al.* 2014); respiration of each substrate was considered analogous to a separate ecosystem process. Although this approach has been criticized when applied to processes that are individually important and have context-dependent underlying drivers (Bradford *et al.* 2014a), this approach is well suited to determining the mean response of multifunctionality when individual processes, such as respiration of different C substrates, are not highly informative individually, but together broadly represent functional capacity. Our response variable was the number of substrates with respiration rates exceeding a given threshold of maximum respiration, for thresholds ranging from 5% to 99%. We calculated the maximum respiration rate for each substrate across all samples as the mean of the $n + 1$ highest measurements, where n is the smallest sample size of a single treatment. To model the effect of farm management on catabolism, we used a generalized LME model with a quasi-poisson error distribution. This model was iterated for each threshold level between 5% and 99% to assess at which thresholds the relationship between treatment and catabolism is significant. For farm sets, models were fit with set identifier as a random effect. For all statistical tests, we considered coefficients with $P < 0{\cdot}05$ significant and coefficients with $P < 0{\cdot}10$ marginally significant (Hurlbert & Lombardi 2009).

Results

CROP AND SOIL PROPERTIES

On experimental fertilization plots, crop yield increased from $1{\cdot}52 \pm 0{\cdot}23$ t ha^{-1} on control plots to $2{\cdot}17 \pm 0{\cdot}38$ t ha^{-1} on plots receiving 200 kg N ha^{-1} (Table S1, Supporting information). On actively managed farms, yield increased from $0{\cdot}86 \pm 0{\cdot}40$ t ha^{-1} on low-fertilizer plots to $2{\cdot}67 \pm 1{\cdot}22$ t ha^{-1} on high-fertilizer plots. The combination of legume rotation with mineral fertilizers increased yields further to $3{\cdot}25 \pm 1{\cdot}02$ t ha^{-1} (Table S1,

Supporting information). However, fertilizer and legume rotation management did not affect broad measures of soil quality, such as total C, N and P (Table S1, Supporting information). The legume rotation treatment did increase a fine-resolution fraction of soil C, specifically microbially available C (Table S1, Supporting information), which is a proxy for the size of the labile C pool (Bradford, Fierer & Reynolds 2008).

MICROBIAL TAXONOMIC DIVERSITY

Supporting the assumptions of the *diversity-functioning hypothesis* that fertilization will lower diversity, taxonomic diversity was significantly lower on fertilizer addition plots, with the strongest decrease occurring between 0 and 75 kg N ha^{-1} (Shannon: 2·15% decrease, $P < 0·05$; Faith's PD: 6·12% decrease $P < 0·1$; Fig. 1; Table S2, Supporting information). We observed a qualitatively similar decrease on active farms receiving high vs. low fertilization (Shannon: 2·14% decrease, Faith's PD: 1·41% decrease; decrease NS; Fig. 1; Table S2, Supporting information).

Supporting the assumption of the *diversity-functioning hypothesis* that fertilization will shift communities towards copiotrophic dominance, we found that *Gammaproteobacteria*, which broadly represent copiotrophic taxa (Ramirez *et al*. 2010; Ramirez, Craine & Fierer 2012), significantly increased in relative abundance with fertilization on experimental plots (117% from 0 to 200 kg N; $P < 0·01$) and with legume rotation (29%; $P < 0·1$; Fig. 2 and Tables S3 and S4, Supporting information). *Deltaproteobacteria* are broadly considered oligotrophic, and thus are expected to have greater relative abundance under low-resource conditions (Ramirez *et al*. 2010; Ramirez, Craine & Fierer 2012). Consistent with this, we found that the relative abundance of *Deltaproteobacteria* significantly decreased with fertilization (19% from 0 to 200 kg N; $P < 0·05$) and legume rotation (29%; $P < 0·05$; Tables S3 and S4, Supporting information). We also found a 577% increase in the coefficient of variation (CV) of Shannon diversity and a 99% increase in the CV of Faith's PD between 0 and 200 kg N ha^{-1} (Fig. S2A,C, Supporting information). We also found a 271% increase in the CV of *Gammaproteobacteria* and a 20% increase in the CV of *Deltaproteobacteria* between 0 and 200 kg N ha^{-1} (Fig. S2B,D, Supporting information).

MICROBIAL FUNCTIONAL CAPACITY

Despite support for the assumptions of the *diversity-functioning hypothesis* (lower diversity, community shift towards copiotrophic taxa), we found little evidence that decreases in taxonomic diversity and altered community composition due to mineral fertilization were associated with altered functional capacity (Fig. 3, Table 1 and S5, Supporting information). On experimental plots, there was no significant change in either the relative abundance of genes in key functional categories or measured C catabolism (Fig. 3, Tables 1 and S5, Supporting information). Legume rotation, by contrast, was nearly always a significant positive predictor of functional capacity (Tables 1 and S5, Supporting information), consistent with the *limitation release hypothesis*. On legume rotation farms, genes related to C cycling, degradation and fixation had significantly elevated abundances (Tables 1 and S5, Supporting information). Legume rotation was often twice (or more) as strong of a predictor of C-related functional gene abundances as fertilizer use without legume rotation, as shown by stan-

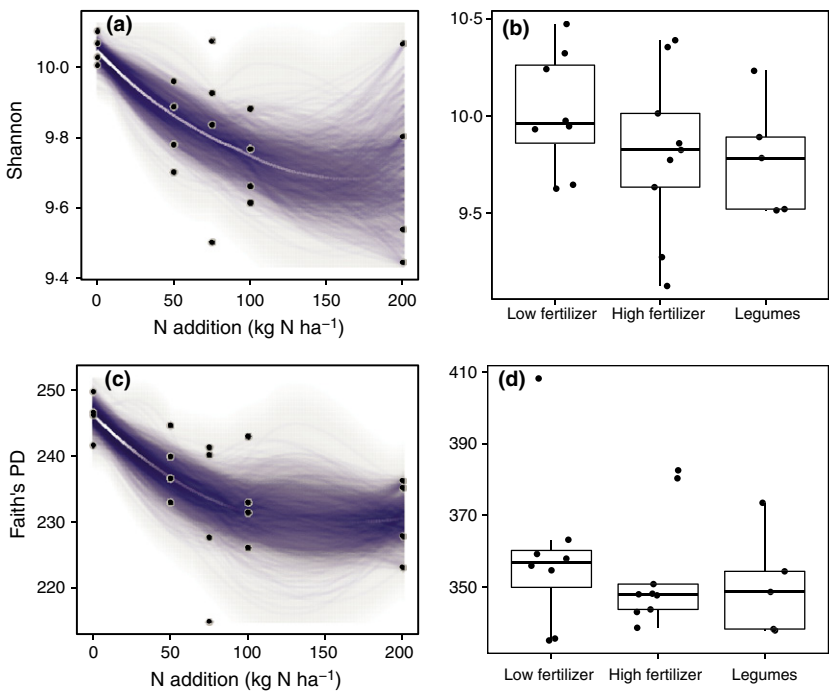

Fig. 1. Diversity of operational taxonomic units (OTUs) decreases with fertilization. Mean regression line is white, individual bootstrapped regressions are darker. Darker areas represent higher confidence. Data points (b, d) are jiggered for visibility.

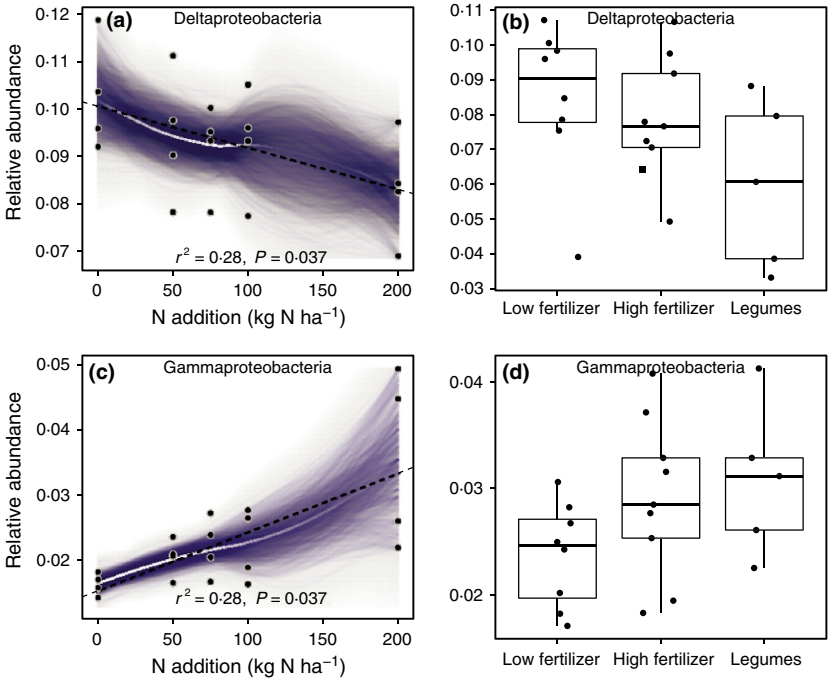

Fig. 2. Microbial communities shift towards copiotrophic dominance with fertilization. Mean regression line is white, individual bootstrapped regressions are darker. Darker areas represent higher confidence. Black line shows the slope of the full model. Data points (b, d) are jiggered for visibility.

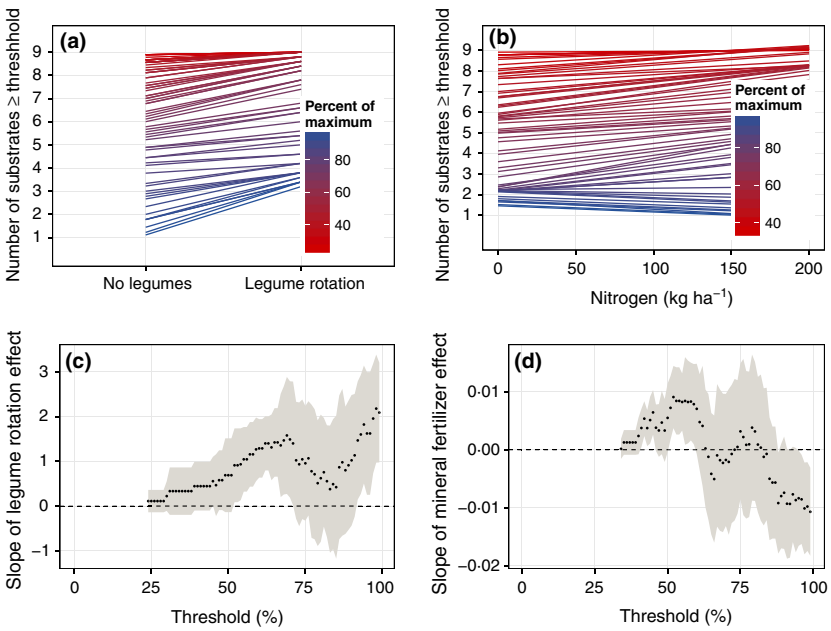

Fig. 3. Catabolic capacity is highest under legume rotation at high levels of functioning. Capacity for a farm or plot is the number of substrates whose observed CO_2-efflux rate is greater than or equal to a threshold of the maximum value for each substrate. Individual regressions were run for each threshold (a, b) and coefficients plotted across all thresholds (c, d).

dardized regression coefficients (Tables 1 and S5, Supporting information). Legume rotation also contributed to the ability of microbes to catabolize a range of C substrates, with the contribution being greatest at highest thresholds of maximum catabolism (>85%) and consistently less important at lower thresholds (<50%; Fig. 3).

The total abundances of genes related to N and P cycling were also significantly impacted by legume rotation (Tables 1 and S5, Supporting information). Genes coding for denitrification, assimilatory N reduction, dissimilatory N reduction and P use were significantly more abundant on legume rotation farms (Table 2). Genes coding for N_2 fixation were significantly less abundant with greater resources (Table 2). The inclusion of legume rotation practices had a greater relative impact on all N and P cycling genes than high fertilizer use, except for N_2 fixation genes, which were more impacted by fertilization (Table 2).

Discussion

CROP AND SOIL PROPERTIES

We found experimental mineral fertilizer use to significantly increase crop yields, but that the highest yield increases were observed when mineral fertilizer use was paired with legume rotation practices. These yield data

Table 2. Results from models of functional gene abundances on experimental plots and actively managed farms. Results for other genes are reported in Table S4 (Supporting information). Standard error is reported in parentheses $*P < 0{\cdot}1$; $**P < 0{\cdot}05$; $***P < 0{\cdot}01$; $****P < 0{\cdot}001$

Gene categories	Fertilization (kg N ha^{-1})	High fertilizer	High fertilizer + legume rotation	pH	Carbon (%)	Nitrogen (%)	Texture	Adj. R^2
Managed farms								
C cycling		75·35 (55·08)	245·03**** (56·46)	−28·23 (48·93)	82·52 (56·71)	36·53 (58·19)	29·34 (45·89)	0·34
C degradation		40·80 (42·55)	167·13*** (43·71)	−18·67 (38·21)	62·48 (44·46)	22·38 (45·61)	22·56 (36·17)	0·24
C fixation		32·45*** (10·57)	68·86**** (10·84)	−5·82 (9·39)	16·53 (10·88)	11·24 (11·17)	5·96 (8·81)	0·60
N and P cycling								
Assimilatory N reduction		11·13** (4·17)	20·66**** (4·31)	−3·76 (3·67)	7·50* (3·78)			0·50
Denitrification		22·51 (15·22)	71·08**** (15·60)	−5·26 (13·52)	22·72 (15·67)	6·90 (16·08)	12·35 (12·68)	0·37
Dissimilatory N reduction		−0·18 (1·53)	5·01*** (1·65)		3·38** (1·40)		4·61*** (1·32)	0·44
N fixation		−26·04**** (4·85)	−9·53* (5·25)		3·35 (5·26)	10·02* (5·54)	4·85 (4·24)	0·46
Phosphorus utilization		17·27** (7·86)	32·06*** (8·11)	−8·63 (6·78)	13·77 (8·16)	5·00 (8·36)		0·42
Experimental plots								
C cycling	95·16 (74·95)			305·58* (151·78)		−239·07 (139·58)		0·15
C degradation	71·60 (56·68)			235·52* (114·78)		−184·12 (105·56)		0·16
C fixation	18·97 (14·82)			58·13* (30·02)		−45·32 (27·61)		0·14
N and P cycling								
Assimilatory N reduction	4·69 (3·29)			13·07* (6·66)		−11·47* (6·13)		0·18
Denitrification	23·85 (19·14)			89·45** (38·76)		−72·04* (35·65)		0·22
Dissimilatory N reduction	5·36 (3·98)			18·28** (8·51)	−8·40 (7·80)			0·09
N fixation	−1·10 (7·43)			28·64* (14·86)				0·13
Phosphorus utilization	12·10 (9·69)			38·65* (19·62)		−31·92* (18·04)		0·15

support the proposal from proponents of an African Green Revolution that to maintain yields over time, mineral fertilizer use should be paired with inputs that increase soil organic matter (Glover, Reganold & Cox 2012). Consistent with previous work from smallholder African agroecosystems, fertilizer and legume rotation did not affect broad measures of soil quality, such as total soil C (Barrios, Buresh & Sprent 1996). These observations are consistent with results suggesting that once particulate organic matter has decomposed (as would be the case in low-C, arable, sub-Saharan African soils), N addition has little effect on total soil C (Brown *et al*. 2014), although there may still be differences in specific soil C fractions. In support of this latter possibility, the legume rotation treatment increased a labile C pool (Table S1, Supporting information). The legume rotation treatment might then be expected to increase total C and N contents in the future, given that responses of these pools tend to be detected only over relatively long time-scales (Conant *et al*. 2011).

MICROBIAL TAXONOMIC AND FUNCTIONAL RESPONSE

We show that changes in microbial communities on smallholder farms in Kenya are predictable based on life-history traits (e.g. copiotroph vs. oligotroph). The specific taxonomic group responses we observed are also consistent with fertilization effects in temperate systems (Ramirez *et al*. 2010; Ramirez, Craine & Fierer 2012). Non-significant decreases in diversity with mineral fertilization on actively managed farms may be due to the fact that the range of fertilizer addition between the low- and high-fertilizer treatments (10–60 kg N ha^{-1}) is narrower than on experimental plots (0–200 kg N ha^{-1}), as well as year to year variability in environmental conditions and farm management.

We find that fertilization-induced losses in diversity and altered composition of microbial communities do not correspond with losses in the functional capacity of the soil microbiota, in contrast to what is predicted by the *diversity-functioning hypothesis*. Efforts to increase yield that combine mineral fertilization with legume rotation to build up soil organic matter have much stronger effects on microbial functional capacity than mineral fertilization alone. This suggests that legume rotation can be an important strategy for both increasing crop yields on smallholder farms and maintaining the long-term functional capacity of the soil microbiota. Our finding that the contribution of legume rotations to catabolic capacity was greatest at high thresholds of maximum catabolism suggests that the importance of legume rotation as a management strategy to promote microbial C use may depend on the level of catabolic capacity targeted.

In most cases, legume rotation was associated with higher abundances of genes related to C, N and P cycling. In some cases, however, fertilization and legume rotation were associated with decreases in functional gene abundances. For instance, N_2 fixation genes were significantly lower with resource addition. Concurrent with this finding, symbiotic N_2 fixation in the tropics can downregulate under high soil N conditions (Barron, Purves & Hedin

2011). Our results suggest that changes in microbial communities may help explain this downregulation, though common explanations often focus on plant physiology (Arrese-Igor *et al.* 1999).

TEMPORAL DECOUPLING OF TAXONOMIC AND FUNCTIONAL RESPONSES

Because taxonomic diversity decreased with mineral N over the short and longer term, but functional capacity was only affected over the longer term (i.e. on farms and not plots), there appears to be a temporal decoupling between taxonomic and functional responses to mineral N addition. In other words, effects of nutrient addition on taxonomic composition emerge faster than effects on functional capacity. Commensurate with this apparent decoupling, we observed in the experimental plots an increase in the coefficient of variation of taxonomic diversity and community composition between 0 and 200 kg N ha^{-1} (Fig. S2, Supporting information). Theory and evidence suggest that increased variability in ecological communities can be an important precursor to shifts in alternative states (Scheffer *et al.* 2009). In our system, the observed increase in variability of diversity and community composition with fertilization over shorter time-scales may, thus, help explain shifts in the functional capacity of communities that we observed over longer time-scales.

SYNTHESIS AND APPLICATIONS

Agricultural research has long focused on the direct influence of farm management on soil nutrient cycling processes. Evidence has begun to emerge that microbial communities can also act as an ultimate control of ecosystem processes. Bradford *et al.* (2014b) show that microbial communities at local scales can act as a stronger control on decomposition than broad-scale factors that were previously thought to be dominant controls of nutrient cycling. Thus, management-induced changes to microbial communities may have important consequences for agroecosystem functioning. Research in temperate systems has shown positive correlations between taxonomic composition and catabolic capacity under fertilization (Fierer *et al.* 2012). In contrast, we find that in tropical smallholder agroecosystems that taxonomic changes under fertilization are not necessarily coupled with changes in functional capacity. Instead, functional capacity was generally increased, along with yields, when fertilizers were combined with legume rotation practices.

Our results demonstrate that legume rotation can be an important management strategy for both increasing short-term crop yields and building the ability of microbial communities to contribute to ecosystem processes that are crucial to agricultural sustainability over the long-term. This will be important for sustainable agriculture when enhanced microbial functional capacity – through, for instance, increased ability to decompose litter and convert nitrogen to plant-available forms – leads to greater nutrient availability for crops. This increased functioning, paired with the additional N added from legume rotation, could reduce the need for farmers to invest in costly synthetic fertilizers when net N balances are positive.

Increased functional capacity could also play an important role in predicting changes in soil organic matter stocks. Because organic matter pools change over long time periods (Conant *et al.* 2011), indicators of the success of farm management to improve soil quality are needed at shorter, management-relevant time-scales. Increased soil microbial functional capacity may serve as such an indicator. While classical paradigms of soil organic matter turnover suggest that greater microbial functional capacity could deplete soil C pools through elevated mineralization of soil C to CO_2, emerging paradigms suggest that greater microbial activity may instead build up and stabilize soil organic matter pools (Schmidt *et al.* 2011). Increased functional potential may therefore be an indicator of soil quality and, as a result, crop production.

Trade-offs, however, may occur with increased microbial functional capacity, such as greater conversion of soil nutrients and organic matter to greenhouse gases. Future work will need to connect changes in microbial functional capacity with ecosystem process rates and to assess the potential trade-offs associated with these multiple processes (Wood *et al.* 2015a). Despite potential trade-offs, our findings support the notion that agricultural development strategies that are based on ecological principles, such as legume rotations, can both increase yields and build the capacity of the soil microbiota to contribute to soil nutrient cycling processes that are important to agricultural sustainability. Measures of microbial functional capacity might also serve as indicators of changes to soil organic matter stocks before actual changes are detected.

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Data accessibility

All amplicon and metadata have been made public through the data portal (www.microbio.me/emp). Taxonomic, GeoChip and catabolic data: DRYAD entry doi: 10.5061/dryad.7d5g4 (Wood *et al.* 2015b).

Author contributions

S.A.W conceived research, performed fieldwork, non-sequencing laboratory work, analysed data and wrote the manuscript. S.A.W, M.A.B, K.L.M, C.A.P and S.N designed the research. S.A.W, M.A.B, J.G, K.L.M and J.Z determined laboratory methods. S.A.W, C.A.P and K.L.T identified, established and maintained research locations. J.G conducted taxonomic sequencing. J.Z conducted GeoChip assay.

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

Fig. S1. Map of study area.

Fig. S2. Variability in taxonomic diversity and community composition.

Table S1. Soil properties and crop yield.

Table S2. Regression model results of taxonomic diversity.

Table S3. Regression results for relative abundance of microbial taxa on experimental plots.

Table S4. Regression results for relative abundance of microbial taxa on actively managed farms.

Table S5. Model results of key functional gene abundances.