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Farm management, not soil microbial diversity, controls nutrient loss from smallholder tropical agriculture

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33 **Abstract**:
34 Tropical smallholder agriculture supports the livelihoods of over 900 million of the world's
35 poorest people. This form of agriculture is undergoing rapid transformation in nutrient cycling
36 pathways as international development efforts strongly promote greater use of mineral fertilizers
37 to increase crop yields. These changes in nutrient availability may alter the composition of
38 microbial communities with consequences for rates of biogeochemical processes that control
39 nutrient losses to the environment. Ecological theory suggests that altered microbial diversity
40 will strongly influence processes performed by relatively few microbial taxa, such as
41 denitrification and hence nitrogen losses as nitrous oxide, a powerful greenhouse gas. Whether
42 this theory helps predict nutrient losses from agriculture depends on the relative effects of
43 microbial community change and increased nutrient availability on ecosystem processes. We
44 find that mineral and organic nutrient addition to smallholder farms in Kenya alters the
45 taxonomic and functional diversity of soil microbes. However, we find that the direct effects of
46 farm management on both denitrification and carbon mineralization are greater than indirect
47 effects through changes in the taxonomic and functional diversity of microbial communities.
48 Changes in functional diversity are strongly coupled to changes in specific functional genes
49 involved in denitrification, suggesting that it is the expression, rather than abundance, of key
50 functional genes that can serve as an indicator of ecosystem process rates. Our results thus
51 suggest that widely used broad summary statistics of microbial diversity based on DNA may be
52 inappropriate for linking microbial communities to ecosystem processes in certain applied
53 settings. Our results also raise doubts about the relative control of microbial composition
54 compared to direct effects of management on nutrient losses in applied settings such as tropical
55 agriculture.
56
57 **Key words**:
58 Carbon mineralization; denitrification; fertilization; GeoChip; microbial diversity; smallholder
59 agriculture; tropics

60 **1. Introduction**
61 Agricultural management, such as mineral nutrient addition, can lead to marked changes in the
62 taxonomic composition of soil microbial communities (Ramirez et al., 2010; Fierer et al., 2011;
63 Ramirez et al., 2012; Wood et al., in revision). The pairing of mineral and organic nutrient
64 addition to agriculture can significantly impact the ability of soil microbial communities to
65 catabolize a range of carbon (C) substrates as well as affect the abundance of microbial
66 functional genes involved in multiple aspects of C, nitrogen (N), and phosphorus (P) cycling
67 (Wood et al., in revision). Some of the microbially driven processes associated with these
68 changes in functional capacity, such as denitrification and decomposition, determine the
69 retention and loss of nutrients in ecosystems and are thus important to managing agriculture for
70 crop production while minimizing nutrient losses to the environment (Vitousek et al., 2009).
71 There is thus keen interest in whether changes in microbial community composition can directly
72 impact rates of ecosystem processes (e.g. Wessén et al., 2011; Wallenstein and Hall, 2012;
73 Philippot et al., 2013; van der Heijden and Wagg, 2013; Krause et al., 2014).
74
75 Certain ecosystem processes are likely to be more sensitive to changes in microbial community
76 composition than others. Narrow processes are most likely to be affected by changes in
77 community composition because they require a specific physiological pathway and/or are carried
78 out by a phylogenetically clustered group of organisms (Schimel and Schaeffer, 2012). Thus,
79 processes can be either physiologically narrow, phylogenetically narrow, or both. In this
80 manuscript we use the term "narrow" to refer to physiologically narrow processes that require
81 specific physiological pathways, regardless of their distribution in the microbial phylogeny. For
82 instance, we refer to denitrification as a narrow process because it requires particular genes that
83 code for enzymes capable of reducing various forms of nitrogen. Because a relatively small
84 proportion of microorganisms carry these genes, changes in community composition that lead to
85 a shift in the relative abundance of denitrifiers—or changes in the abundances of the relevant
86 functional genes—should have significant impacts on rates of denitrification (Pett-Ridge and
87 Firestone, 2005; Philippot et al., 2013; Powell et al., 2015). Mineralization of soil C to CO_2, by
88 contrast, is a broad process because the ability to mineralize and respire C substrates is relatively
89 simple and shared by many microbial taxa (Schimel and Schaeffer, 2012). We thus expect that
90 carbon mineralization would not respond strongly to changes in the composition of microbial
91 communities.
92
93 Whether this framework of broad and narrow processes helps predict nutrient losses from
94 agriculture depends on the relative importance of the multiple potential drivers of ecosystem
95 process rates, including microbial community composition, nutrient availability, and soil and
96 environmental properties. Though several studies have found support for microbial influence on
97 narrow processes, such as denitrification, such studies often focus on identifying whether
98 microbial community composition is related to ecosystem processes, but stop short of
99 quantifying the relative contribution of the multiple controls on ecosystem processes (e.g.,
100 Philippot et al., 2013). Understanding the importance of biodiversity requires assessing the
101 influence of composition relative to other biotic and abiotic controls (Laliberté and Tylianakis,
102 2012; Bradford et al., 2014).
103
104 Following theory (Schimel, 1995; Schimel and Schaeffer, 2012), we hypothesize that changes in
105 microbial diversity will have a stronger effect on denitrification than will the direct effect of

106 nutrient addition—measured as both N addition and the inclusion of seasonal legume rotations
107 (henceforth *agroforestry*) to increase soil C—if changes in diversity correspond with changes in
108 the relative abundance of denitrifying taxa and the abundances of functional genes involved in
109 denitrification. Because C mineralization is a broad process, we expect that nutrient addition will
110 have a stronger effect on process rates than changes in the microbial community.
111
112 **2. Material and methods**
113 *2.1 Site selection*
114 We examine our hypotheses on 24 smallholder farms in western Kenya participating in the
115 Millennium Villages Project (MVP) site in Sauri, Kenya (Figure 1; Wood et al., in revision). The
116 center of the study area is located at 0°06'04.88 N, 34°30'40.12 E at an elevation of 1450 m. The
117 mean annual temperature and precipitation for the study region are 24°C and 1800 mm,
118 respectively. Annual precipitation is distributed bi-modally with 1120 mm in a long rainy season
119 from March to June and 710 mm in a short rainy season from September to December. The soils
120 are classified as Oxisols and are well drained sandy clay loams (on average 53.75% sand,
121 12.59% silt, 33.54% clay) with a mean pH of 5.45 and C:N of 11.52 (0-20 cm). The study zone
122 was originally part of the moist broadleaf forest area in eastern and central Africa, but is now a
123 mixed-maize agricultural system, with most farmers cultivating maize in both the long and short
124 rainy seasons. Some farmers, however, replace the short rain maize crop with a seasonal legume
125 rotation that fixes nitrogen and builds soil organic matter.
126
127 The MVP was designed to meet the Millennium Development Goals at the village scale in Sub-
128 Saharan Africa and includes an agricultural component that focuses on increasing crop yields
129 through mineral and organic nutrient addition to redress negative soil nutrient balances (Sanchez
130 et al., 2007). This is primarily achieved by subsidizing mineral fertilizers (primarily
131 diammonium phosphate and urea). Farmers are also trained in seasonal legume rotations to fix
132 nitrogen and build soil organic matter. In Sauri, rotational legume trainings have been promoted
133 since the early 1990s (Kiptot et al., 2007) and fertilizer subsidy programs were active from 2005-
134 2008.
135
136 We selected farms to participate in the study based on two years of household surveys. We
137 determined nutrient inputs and outputs for each of these farms through a combination of
138 interviews, on-farm crop harvests, and biomass estimations. Farms were classified into three
139 categories: *low fertilizer*, *high fertilizer*, and *high fertilizer + agroforestry* (specifically, seasonal
140 legume rotations). Low fertilizer farms have applied less than 10 kg mineral N ha^{-1} y^{-1} since
141 2005; high fertilizer farms have applied at least 60 kg N ha^{-1} y^{-1} over the same time period. High
142 fertilizer + agroforestry farms (henceforth *agroforestry*) apply amounts of mineral N comparable
143 to *high fertilizer* farms, but also use agroforestry techniques to build soil organic matter. These
144 agroforestry techniques replace short-rain maize crops with fast-growing leguminous tree, shrub,
145 or herbaceous species that are planted from seed and cut each year for organic inputs to crop
146 fields. These techniques are referred to generally as agroforestry, though agroforestry is a general
147 term that captures different practices not studied here (e.g., wind breaks, live fencing, etc.). Our
148 results, therefore, apply to agroforestry strategies that seasonally incorporate legume rotations.
149
150 We estimated the amount of N added to farms with farmer-reported data on the quantity of N
151 added through mineral and organic sources (diammonium phosphate, urea, biological N_2-

152 fixation, and manure). For agroforestry farms, we also estimated the amount of N added through
153 N_2-fixation based on both literature-reported values and field-reported biomass estimates. To
154 estimate the amount of N added through N_2-fixation we collected data on legume species
155 planted, original planting density, thinning practices, wood harvesting, and legume management.
156 We used plant density to estimate the amount of aboveground biomass N for each species
157 present and then used literature data on the percent of total N derived from biological N_2-fixation
158 for each species to calculate the amount of N derived from fixation (Gathumbi et al., 2002a;
159 2002b; Ojiem et al., 2007). Because farmers tend to remove woody stems but incorporate fresh
160 leaves, we removed the amount of N stored in woody biomass from this value to estimate the net
161 N contribution from the legume species to the farm fields. We conservatively estimate that N_2-
162 fixation contributed between 30 to 50 kg N ha^{-1} $year^{-1}$ during the short rain fallow (Gathumbi et
163 al., 2002), up to 30 kg of which may be due to the presence of *Mucuna pruriens*, an annual
164 climbing legume (Ojiem et al., 2007). Planting densities, however, can vary widely from year-to-
165 year with low-density years being as low as an order of magnitude less than those assumed in
166 this estimate. Thus, depending on the year, actual fixation rates may be as low as 5 to 30 kg N ha^{-}
167 ^{1} short rainy $season^{-1}$. We use the term 'nutrient addition' to refer to both N addition on low- and
168 high-fertilizer and agroforestry farms as well as C addition through agroforestry. The final farms
169 included in the study are distributed across the Sauri village cluster, but are clustered by
170 treatment (Figure 1) on similar underlying soils.
171
172 *2.2 Sample collection and measurement*
173 Soil sampling was conducted in June 2012, in the middle of the long rains, two weeks after
174 fertilizer application. On the farm fields, we took 15 2-cm diameter soil cores from the top 20 cm
175 of bulk soil. Cores were taken at regular intervals throughout the entire farm field and
176 homogenized and aggregated to a composite sample. At each core location we recorded
177 temperature and volumetric soil moisture content using a soil thermometer and a HydroSense
178 moisture probe (Campbell Scientific, Logan, UT, USA). We sieved soils to 2 mm and stored soil
179 for DNA extraction at -20° C. Soils for DNA extraction were transported to the U.S. within one
180 week of sampling. Subsamples of sieved field soil were stored at 4° C, transported to the U.S.
181 within one week of sampling, and used to determine pH, gravimetric soil moisture, and water
182 holding capacity. Gravimetric soil moisture and water holding capacity (after wetting soils to
183 field capacity) were determined by drying soil at 105°C for 24 h. Soil pH was determined using a
184 benchtop meter of a 1:1 slurry of soil:H_2O by volume.
185
186 A subsample of sieved soil was air-dried and used to determine total C and total N by
187 combustion with an Elementar Vario Macro CNS analyzer. Total extractable P was assessed by
188 combining a 5-g soil sample with 20 mL of Mehlich I extraction solution and shaking for 5 min
189 followed by inductively coupled plasma spectrometry (Varian Vista MPX Radial ICP-OES). Soil
190 nutrient assays were performed at the Auburn University Soil Testing Laboratory (AL, USA).
191 Sieved, air-dried soil was also used to determine soil texture using the hydrometer method that
192 uses sodium hexametaphosphate to complex the anions that bind to clay and silt particles into
193 aggregates and suspend organic matter in solution. The density of the soil suspension is
194 determined using a hydrometer after the sand particles settle and then after the silt particles settle
195 (Bouyoucous method).
196
197 Denitrification and C mineralization assays were performed in Kenya on fresh soils at the MVP

198 regional office in Kisumu, Kenya. Denitrification potential was estimated based on N_2O
199 emissions during denitrifying enzyme activity (DEA) assays (Smith and Tiedje, 1979). In a 125-
200 mL flask, we combined 20 g of soil with 20 mL of a 1-mM sucrose and 1-mM KNO_3^- solution.
201 We fit each flask with a #5 stopper, which was inserted with a 22G needle capped with a
202 stopcock. We then brought the headspace of the flask to 10% acetylene (C_2H_2) concentration by
203 volume (to inhibit the reduction of N_2O to N_2 via denitrification). At the beginning of the
204 incubation we closed the stopcocks and placed the flasks onto a shaker table at 125 rpm; flasks
205 were only removed from the table for sampling. We sampled the headspace five times: at 30,
206 150, 210, and 270 min, by removing 30 mL of gas from the headspace and then replacing the
207 volume of headspace that was removed with 30 mL of 10% C_2H_2 room air (fluxes were corrected
208 for N_2O molecules removed at each sampling period). DEA headspace samples were stored in
209 pre-evacuated vials.
210
211 Water-amended soil incubations were used to measure CO_2 efflux and, thus, actual C
212 mineralization. These incubations were performed identically to the DEA incubations with three
213 exceptions: (1) 20 mL of deionized water was added to soil in place of the sucrose and KNO_3^-
214 solution; (2) no C_2H_2 was added to the headspace; and (3) headspace samples were taken at only
215 two time points (240 and 360 min). We also sampled room air at the beginning and end of each
216 incubation and included travel standards to accompany samples, in order to correct for any
217 sample loss during transport and storage. DEA and CO_2 headspace samples were transported to
218 the U.S., where we determined N_2O and CO_2 concentrations by gas chromatography using a
219 Shimadzu GC-14 GC with electron capture (for N_2O) and thermal conductivity (for CO_2)
220 detectors at the Cary Institute (Millbrook, NY).
221
222 To measure taxonomic diversity, we performed 16S rRNA amplicon sequencing of bacteria and
223 archaea following standard protocols of the Earth Microbiome Project using an Illumina MiSeq
224 instrument (www.earthmicrobiome.org/emp-standard-protocols/; Gilbert et al., 2010; Caporaso
225 et al., 2012). Briefly, we extracted DNA using a MoBio PowerSoil 96-well extraction kit and we
226 amplified the 16S rRNA V4 gene from bacterial and archaeal genomes using the primers 515F
227 (forward) and 806R (reverse) (Caporaso et al., 2012). The 16S rRNA gene is a well-conserved
228 gene in bacteria and thus captures evolutionary relationships among bacterial taxa. Quality
229 filtering was performed by comparing input sequences with Phred scores ($Q >= 20$). Sequences
230 shorter than 75% of the Phred score were discarded as well as sequences with ambiguous base
231 call characters. All quality filtering and demultiplexing were performed using the
232 split_libraries_fastq.py algorithm in QIIME and its associated default parameters
233 (www.earthmicrobiome.org/emp-standard-protocols/; Caporaso et al., 2010). Sequence reads
234 were were binned into operational taxonomic units (OTUs) at a 97% similarity threshold. OTUs
235 were then compared to GenBank to identify bacterial lineages. A total of 3,462,835 bacterial
236 sequences were generated across all samples, representing 29,195 OTUs. Sequence lengths
237 averaged 150.63±2.93 per sample. Rarefaction was used to compare samples at depth of 40
238 sequences per sample. We calculated taxonomic diversity as Shannon diversity (H') of all OTUs.
239 We calculated other diversity metrics, such as Faith's PD, and found similar patterns. All data
240 checks and processing were done using QIIME (Caporaso et al., 2010).
241
242 To estimate microbial functional diversity, we measured the abundance of key functional genes
243 using GeoChip 4.0 to analyze DNA samples that were extracted following the protocol for

244 taxonomic assessment. GeoChip is a functional gene array of bacteria, archaea, and fungi
245 covering 401 gene categories involved in major biogeochemical and ecological processes, as
246 previously described (He et al., 2007; Yang et al., 2013; Tu et al., 2014). GeoChip examines the
247 abundance of thousands of functional gene variants simultaneously through a fluorescent
248 procedure. DNA samples were labeled with a fluorescent dye and purified using a QIA quick
249 purification kit (Qiagen, Valencia, CA, USA) following He et al. (2007) and Tu et al. (2014).
250 DNA was then dried in a SpeedVac (ThermoSavant, Milford, MA, USA) and labeled DNA was
251 resuspended in a hybridization solution before hybridization of DNA was carried out on a MAUI
252 hybridization station (BioMicro, Salt Lake City, UT, USA). GeoChip microarrays were scanned
253 by a NimbleGen MS200 scanner (Roche, Madison, WI, USA). Poor quality spots were removed
254 when flagged as one or three by ImaGene (Arrayit, Sunnyvale, CA, USA) or with a signal-to-
255 noise ratio of less than 2.0. Signal-to-noise ratio indicates the amount of luminescence from the
256 sample compared to background noise. Average signal-to-noise ratios are often greater than 50
257 (He et al., 2007), so 2.0 represents high noise to signal. Processed data were subjected to the
258 following steps: (i) normalize the signal intensity by dividing the signal intensity by the total
259 intensity of the microarray followed by multiplying by a constant; (ii) transform by the natural
260 logarithm; (iii) remove genes detected in only one out of three samples from the same treatment.
261 Signal intensities were quantified and processed using a previously described data analysis
262 procedure (He et al., 2007; Yang et al., 2013). We calculated functional diversity as Shannon
263 diversity (H') of the signal intensity for all of the genes reported from the array. We also
264 analyzed the response of individual denitrification genes to changes in functional diversity.
265 These include genes involved in nitrite reduction (*nirK*, *nirS*), nitrate reduction (*narG*), and nitric
266 oxide reduction (*norB*). GeoChip also includes *nosZ*, which is involved in nitrous oxide
267 reduction, but we do not analyze this gene because it is involved in a later stage of denitrification
268 than represented by the denitrification potential assay.
269
270 *2.3. Data analysis*
271 We used structural equation models to simultaneously estimate each of the pathways among
272 nutrient addition, soil and environmental properties (pH, texture, and moisture), microbial
273 communities, and ecosystem processes while accounting for correlations between multiple
274 response variables (Grace, 2006). Structural equation modeling is increasing used in ecology and
275 environmental sciences to assess the relative impacts of multiple variables on each other and a
276 set of response variables (Grace, 2006). This technique has been applied to a wide range of
277 issues in ecology and environmental sciences (e.g., Byrnes et al., 2011; Flynn et al., 2011;
278 Laliberté and Tylianakis, 2012). Relevant to our study, it was used by Colman and Schimel
279 (2013) to determine the drivers of microbial respiration and N mineralization at continental
280 scales.
281
282 To test our hypotheses about the relative importance of nutrient addition and microbial
283 composition, we first fitted models including both nutrient addition and microbial diversity
284 variables. Soil pH was the only significant environmental control and was thus the only
285 environmental variable retained in the final models. We then fitted models to optimize goodness-
286 of-fit and do not include variables that do not contribute strongly to model goodness-of-fit.
287 Different models were fitted for each of the two response variables (denitrification potential and
288 C mineralization). For each response variable, constrained (microbial + nutrient addition) and

289 unconstrained models were compared based on change in AIC values. The final, unconstrained
290 model retained nutrient addition and pH, but did not include microbial diversity.
291
292 We report standardized path estimates that allow for comparison of the relative magnitude of
293 variables within the same model (Grace and Bollen, 2005). For model goodness-of-fit, we report
294 X^2 and root mean square error of approximation (RMSEA). These measures assess the similarity
295 between the covariance matrix of the observed data and the covariance matrix implied by the
296 specified model. A X^2 P-value greater than 0.05 implies significant overlap between the observed
297 and implied data, and thus adequate model fit. We report Sartorra-Bentler X^2 correction factors
298 to improve estimates based on violations of multivariate normality. Because the X^2 test is based
299 on large sample theory, we also report RMSEA, which is a goodness-of-fit measure weighted by
300 sample size. We use an RMSEA value below 0.1 to represent good model fit because for sample
301 sizes less than 50, the conventional RMSEA cut-off value of 0.05 is overly conservative (Chen et
302 al. 2008). Individual paths were estimated using maximum likelihood and we considered paths to
303 be significant at $P < 0.05$ and marginally significant at $P < 0.10$ (Hurlbert and Lombardi, 2009).
304 Insignificant paths were excluded from models unless they significantly improved overall model
305 fit, based on X^2 and RMSEA values as well as assessment of modification indices (Grace, 2006).
306 All models were fitted using the *lavaan* package in R (Rosseel, 2012).
307
308 **3. Results**
309 We hypothesized that changes in microbial diversity would have a stronger effect on
310 denitrification than would the direct effect of nutrient addition if changes in diversity correspond
311 with changes in the relative abundance of denitrifying taxa and/or the abundance of associated
312 genes involved in denitrification. We also hypothesized that nutrient addition would be a
313 stronger predictor of C mineralization, a broad process, than microbial diversity.
314
315 We find that farm management—through N addition and agroforestry—impacts the taxonomic
316 and functional diversity of soil microbial communities. Specifically, taxonomic diversity
317 decreases by 2.40% from low-to-high N addition (Table 1), though this effect is weaker than the
318 effect of pH, which is also associated with lower taxonomic diversity (Figure 2A, B). We did not
319 find that these changes in taxonomic diversity were coupled with changes in the relative
320 abundance of select groups of denitrifying taxa (Figure 3). Agroforestry was the strongest driver
321 of functional diversity, which increased 1% between high fertilizer and agroforestry farms and
322 2% between low fertilizer and agroforestry farms (Table 1; Figure 2A, B). We did find that
323 greater functional diversity is significantly related to greater abundances of several genes
324 involved in denitrification: *nirK*, *nirS*, *norB*, and *narG* (Figure 4).
325
326 We did not, however, find that changes in taxonomic and functional diversity were related to
327 rates of either denitrification or C mineralization. Instead, ecosystem process rates were most
328 strongly linked to the direct effect of farm management. Denitrification decreased by 21.31%
329 from low-to-high N and increased by 63.93% from low N to agroforestry (Table 1). The path
330 estimate for agroforestry on denitrification (0.63) is three times greater than the coefficient for
331 either taxonomic diversity (-0.24) or functional diversity (-0.18). The agroforestry coefficient is
332 also twice the magnitude of the coefficient for N addition (-0.33). We find support for our
333 hypothesis that C mineralization will be more influenced by nutrient addition than microbial
334 community composition. C mineralization rates were 4.81% lower on high-vs.-low N farms and

335 22.12% greater under agroforestry (Table 1). The path coefficient for the effect of agroforestry
336 on C mineralization (0.47) is more than twice as great as the coefficient for taxonomic diversity
337 (-0.23) and N addition (0.16) and around five times the effect of functional diversity (-0.08).
338
339 **4. Discussion**
340 Our results reveal that shifts in microbial taxonomic and functional diversity due to farm
341 management are not significantly related to either denitrification or C mineralization on
342 smallholder farms in western Kenya. This finding supports our hypothesis that C mineralization
343 would not be sensitive to changes in microbial communities because it is a broad process that can
344 be carried out by many microbial taxa. However, we did not find support for our hypothesis that
345 denitrification would be sensitive to community change because it is a narrow process carried out
346 by relatively few taxa.
347
348 This unexpected result may be explained by the fact that changes in taxonomic diversity were
349 not coupled with decreases in the relative abundance of denitrifying taxa. Our hypothesis was
350 built on the expectation that diversity would relate to denitrification rates if changes in diversity
351 were paired with changes in the relative abundance of taxa able to carry out denitrification.
352 Because denitrifying taxa are found widely through the microbial phylogeny, it is difficult to
353 identify groups of taxa that are all denitrifiers. However, we found that groups that broadly
354 contain denitrifiers do not change in relative abundance with changes in diversity. This finding
355 may explain why taxonomic diversity was not a significant predictor of denitrification.
356
357 We also expected that functional diversity would be a significant control on denitrification if
358 changes in functional diversity were coupled with changes in the abundances of key denitrifying
359 genes. We did find a strong coupling between our functional diversity metric (Shannon diversity
360 of all functional genes from GeoChip) and the abundances of four particular genes key to
361 denitrification: *nirK*, *nirS*, *narG*, and *norB*. Thus, our finding that functional diversity was not
362 significantly related to rates of denitrification was unexpected. However, the finding fits with
363 recent meta-analysis showing that microbial functional gene abundances are rarely strongly
364 correlated with corresponding process rates (Rocca et al., 2014). Our lack of observed
365 relationship between gene abundances and process rates may be explained by the fact that our
366 measure of functional diversity is based on the presence of functional genes using DNA. Because
367 DNA only indicates the presence of a gene, rather than whether that gene is expressed, our
368 measure of functional diversity only represents a coarse picture of microbial functional capacity.
369 Our results thus suggest that rates of denitrification are more strongly controlled by the
370 expression of functional genes, rather than their overall abundance. This finding suggests that
371 coarse measures of microbial communities based on DNA—whether taxonomic or functional—
372 may be insufficient to understanding the changes in the functional contributions of these
373 communities under certain types of land management (Rocca et al., 2014).
374
375 Though understanding when microbial communities should impact ecosystem process rates is
376 well established, we show that actual changes in microbial communities observed in a tropical
377 agroecosystem are not a strong predictor of denitrification rates because changes in microbial
378 communities are relatively minor in magnitude. Our findings, however, do not invalidate the
379 hypothesis that narrow processes are sensitive to community composition and broad processes
380 are not, which has been supported in previous work (e.g., Philippot et al., 2013; Powell et al.,

381 2015; Salles et al., 2012; Schimel and Schaeffer, 2012). Instead, our findings raise doubts about
382 the relative importance of microbial community composition compared to direct effects of
383 nutrient addition on nutrient losses in agricultural settings. This is because the magnitude of
384 change in microbial diversity induced by land management was not large enough to significantly
385 impact ecosystem process rates. As a result, the direct effect of farm management (rather than
386 the indirect effect through changes in microbial communities) was the dominant control of both
387 denitrification and C mineralization. Whether changes in microbial community composition
388 translate into changes in rates of ecosystem processes controlled by soil microbes is of great
389 interest in soil ecology (e.g., Torsvik and Øvreås, 2002; Philippot and Hallin, 2005; Van Der
390 Heijden et al., 2008), but remains an ongoing debate (Schimel and Schaeffer, 2012). Our study is
391 unique, however, in that few studies have connected changes in microbial communities to
392 ecosystem process rates in a framework that assesses the relative importance of the multiple
393 drivers of these ecosystem processes.
394
395 Although we focus on smallholder farms in western Kenya, there is a widespread effort to
396 increase crop yields across sub-Saharan Africa and in tropical smallholder agriculture more
397 generally (Wiggins et al., 2010). Because seventy-five percent of the world's 1.2 billion poorest
398 people are engaged in smallholder, making up 500 million farms of less than 2 ha (Wiggins et
399 al., 2010), our findings may help inform understanding of drivers of nutrient loss in tropical
400 smallholder agriculture due to increased attention from international development organizations.
401
402 It is becoming widely recognized that agricultural sustainability requires agricultural practices
403 that maximize multiple ecosystem services while minimizing nutrient losses to the environment
404 (Foley et al., 2011; Bommarco et al., 2013). This is particularly important in tropical ecosystems
405 that are undergoing large-scale modifications of nutrient cycling pathways due to intense efforts
406 by the international development community to increase fertilizer use by tropical smallholder
407 farmers. Further work should focus on understanding how management-induced shifts in
408 microbial communities impact not just potential nutrient loss, but the multiple ecosystem
409 services provided by soil and how such understanding can be integrated into sustainable
410 agricultural strategies.
411
412 **Author contributions**
413 SAW and MA conceived research and performed lab and field work; SAW, MA, MAB, KLM,
414 SN, CN, CAP and KLT designed the study; JZ performed GeoChip analyses; SAW analyzed
415 data and wrote the first draft of the manuscript; all authors contributed to interpretation of results
416 and commented on the manuscript. The authors declare no conflicts of interest.
417
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426 public through the data portal (www.microbio.me/emp).

427
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548 **Figure legends**
549
550 **Figure 1.** Maize production in western Kenya mainly occurs on smallholder farms of around 1
551 hectare (A). Map (B) shows the study farms and their distribution across the Millennium
552 Villages Project site area in western Kenya. Farm types are coded by color.
553
554 **Figure 2.** Path diagrams for structural equation models of the relationship between farm
555 management, microbial diversity, and (A) denitrification enzyme activity or (B) carbon
556 mineralization. Models (A, B) show the relative effect of management and microbial diversity.
557 Solid paths are statistically significant at $p < 0.10$. Dashed paths are insignificant, but were
558 included for hypothesis testing or overall model fit. Line color represents effect direction (light
559 green = positive, deep red = negative). Path widths are proportional to standardized regression
560 coefficients, which are shown next to each path. Results and model statistics are in Table 2.
561
562 **Figure 3.** Taxonomic diversity is not related to changes in the relative abundances of select
563 denitrifying taxa. These groups do not represent all categories of denitrifying taxa and not all
564 taxa within these categories are able to carry out denitrification. These groups were selected
565 because they broadly represent evolutionary lineages that are capable of denitrification and had
566 relatively high relative abundances in our samples.
567
568 **Figure 4.** Functional diversity is positively correlated with changes in the abundances of specific
569 genes involved in denitrification. These genes are involved in nitrite reduction (A: nirK, C: nirS),
570 nitrate reduction (B: narG), and nitric oxide redunction (D: norB). We did not analyze nosZ
571 because it is involved in a later stage of denitrification than included in our potential assay
572 (nitrous oxide reduction).

573 **Tables**
574
575 **Table 1.** Means and standard deviation for variables included in structural equation models among the three categories of nutrient
576 addition: low fertilizer, high fertilizer, and agroforestry. All soil properties are to a depth of 20 cm. Because of unbalanced design
577 statistical comparisons between groups are not valid; instead the effect of Farm Type is represented by the path coefficients of
578 Agroforestry and N Addition in the structural equation models. Further detail on changes in soil properties is presented in Wood et al.
579 (in revision).
580

Farm Type	Denitrification	C mineralization	Taxonomic diversity	Functional diversity	Sand	Silt	Clay	pH	C	N	P
	(ng N g dry soil^{-1} h^{-1})	*(ug C g dry soil^{-1} h^{-1})*	*H'*			*%*		*log [H^{+}]*	*%*	*%*	*ppm*
Low Fertilizer	0.61 [0.49]	1.04 [0.24]	10.02 [0.31]	8.88 [0.07]	53.76 [5.64]	14.40 [7.61]	31.74 [6.34]	5.41 [0.35]	1.83 [0.20]	0.20 [0.03]	16.63 [9.15]
High Fertilizer	0.48 [0.09]	0.99 [0.41]	9.78 [0.45]	8.99 [0.08]	56.00 [3.13]	9.71 [5.91]	34.15 [6.57]	5.06 [0.37]	1.95 [0.16]	0.22 [0.03]	19.13 [10.30]
Agroforestry	1.00 [0.58]	1.27 [0.13]	9.79 [0.30]	9.05 [0.09]	58.58 [2.06]	10.46 [4.67]	30.86 [4.96]	5.47 [0.72]	1.72 [0.27]	0.18 [0.02]	7.00 [2.55]

581

582 **Table 2.** Model results and goodness of fit statistics for structural equation models. We report robust X^2 statistics for model fit. P > 0.05 indicates that estimated models have covariance matrices among variables that are not strongly different from observed values and that the model, therefore, adequately represents the data. Root mean square error of approximation (RMSEA) is a sample-size weighted measure of model fit. Values below 0.1 indicate good model fit.
583
584
585
586

Denitrification			**C Mineralization**		
	Standardized Estimate	*P*		*Standardized Estimate*	*P*
Denitrification~			C mineralization~		
Agroforestry	0.63	0.00	Agroforestry	0.47	0.00
Functional diversity	-0.18	0.31	Functional diversity	-0.08	0.72
N addition	-0.33	0.10	N addition	-0.01	0.95
Taxonomic diversity	-0.24	0.18	Taxonomic diversity	-0.23	0.35
Taxonomic diversity~			Taxonomic diversity~		
N Addition	-0.35	0.06	N Addition	-0.31	0.18
pH	-0.41	0.00	pH	-0.40	0.01
Functional diversity~			Functional diversity~		
Agroforestry	0.50	0.01	Agroforestry	0.48	0.03
Structural Equation Model Metrics			**Structural Equation Model Metrics**		
	n	21		*n*	21
	df	5		*df*	5
	χ^2	2.14		χ^2	2.62
	$P_{\chi 2}$	0.83		$P_{\chi 2}$	0.76
	RMSEA	0.00		RMSEA	0.00
	P_{RMSEA}	0.85		P_{RMSEA}	0.75

587

A

B

0 0.5 1 2 3 4 km

To Uganda

To Kisumu

Farm Type

Agroforestry

High Fertilizer

Low Fertilizer

Laterite Road

Paved Road

MVP Project Area

Figure 2.TIF

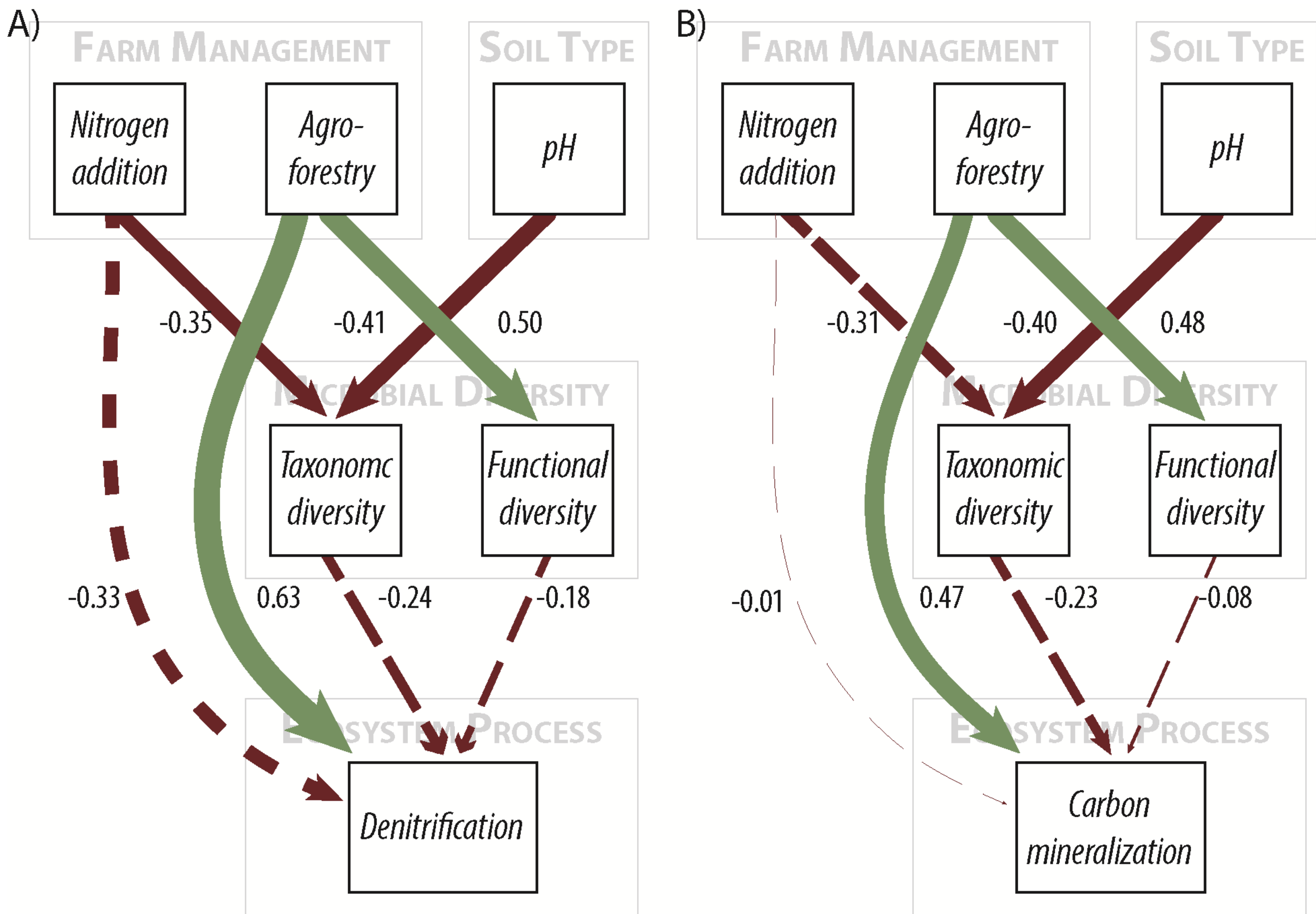

Figure 3.TIF

A) Rhizobiales

$y = 0.014 + 0.0022 \cdot x$

$r^2 = -0.039, \quad P = 0.66$

Relative abundance

0.06
0.05
0.04
0.03

9.5 10.0 10.5

Taxonomic Diversity

B) Legionellales

$y = 0.0054 + -0.00025 \cdot x$

$r^2 = -0.046, \quad P = 0.79$

Relative abundance

0.009
0.007
0.005
0.003

9.5 10.0 10.5

Taxonomic Diversity

C) Burkholderiales

$y = 0.093 + -4e{-}04 \cdot x$

$r^2 = -0.05, \quad P = 0.98$

Relative abundance

0.125
0.100
0.075
0.050

9.5 10.0 10.5

Taxonomic Diversity

D) Pseudomonadales

$y = 0.014 + -0.001 \cdot x$

$r^2 = -0.0086, \quad P = 0.38$

Relative abundance

0.008
0.006
0.004
0.002

9.5 10.0 10.5

Taxonomic Diversity

Figure 4.TIF