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## Tropical agricultural land management influences on soil microbial communities through its effect on soil organic carbon

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## ABSTRACT

We analyzed the microbial community that developed after 4 years of testing different soil-crop management systems in the savannah–forest transition zone of Eastern Ghana where management systems can rapidly alter stored soil carbon as well as soil fertility. The agricultural managements were: (i) the local practice of fallow regrowth of native elephant grass (*Pennisetum purpureum*) followed by biomass burning before planting maize in the spring, (ii) the same practice but without burning and the maize receiving mineral nitrogen fertilizer, (iii) a winter crop of a legume, pigeon pea (*Cajanus cajan*), followed by maize, (iv) vegetation free winter period (bare fallow) followed by maize, and (v) unmanaged elephant grass-shrub vegetation. The mean soil organic carbon (SOC) contents of the soils after 4 years were: 1.29, 1.67, 1.54, 0.80 and 1.34%, respectively, differences that should affect resources for the microbial community.

From about 290,000 sequences obtained by pyrosequencing the SSU rRNA gene, canonical correspondence analysis showed that SOC was the most important factor that explained differences in microbial community structure among treatments. This analysis as well as phylogenetic ecological network construction indicated that members of the *Acidobacteria* GP4 and GP6 were more abundant in soils with relatively high SOC whereas *Acidobacteria* GP1, GP7, and *Actinobacteria* were more prevalent in soil with lower SOC. Burning of winter fallow vegetation led to an increase in Bacillales, especially those belonging to spore-forming genera. Of the managements, pigeon-pea cultivation during the winter period promoted a higher microbial diversity and also sequestered more SOC, presumably improving soil structure, fertility, and resiliency.

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## 1. Introduction

The soil carbon cycle consists of inputs such as plant residues and exudates and outputs such as harvest, carbon dioxide released by microbial decomposition and soil carbon leaching (Trumbore

and Czimczik, 2008). The carbon cycle equilibrium is often disturbed by the conversion of natural ecosystems to agriculture, and particularly so in tropical regions. This is mainly due to soil structure disturbances by cultivation and decreased carbon input as a result of typical practices such as burning or crop residue removal. These practices elevate soil organic carbon (SOC) loss by increased rates of decomposition, organic matter oxidation, leaching, and erosion (Lal, 2008). Such management practices typically result in rapid SOC depletion followed by a slower rate of decrease for several decades, before a new SOC steady-state is reached (Scholes et al., 1997). These losses can range between 20% and 70% of the original SOC content (Mann, 1986), but can be remediated with the

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111 use of cover crops and minimum tillage, when the residue is not  
112 removed (Mann et al., 2002; Lal et al., 2004). Reduced tillage in-  
113 creases SOC retention through macroaggregate preservation  
114 (Grandy and Robertson, 2007), and has been proposed as a primary  
115 method for optimizing SOC in fine-textured soils (Chivenge et al.,  
116 2007). In recent years, agricultural practices common in tropical  
117 regions have been re-evaluated with the goal of gaining benefits  
118 from developing a winter fallow crop that would provide food,  
119 sequester more soil carbon and improve soil fertility and structure.  
120 Since deforestation rates are greater in the tropics than rates of  
121 current or historical land use changes in any other region  
122 (Houghton, 1994), management practices that preserve soil carbon  
123 are expected to have a greater impact on reducing atmospheric CO<sub>2</sub>,  
124 and in doing so, potentially provide monetary incentives for  
125 farmers from the developing carbon markets (Sandor et al., 2002).

126 While much study has focused on the chemical and physical  
127 changes to soil due to different cropping systems, the associated  
128 shifts in soil microbial community structure and function remain  
129 largely unknown. Soil microbial communities are responsible for  
130 organic matter mineralization and are thus an integral component  
131 of soil productivity and the global carbon and nitrogen cycles. Here,  
132 we investigated microbial community shifts using plots under  
133 different land managements where the treatment effects on carbon  
134 mineralization rates (Adiku et al., 2008), crop yields and soil carbon  
135 (Adiku et al., 2009) and on microbial biomass (Asuming-Brempong  
136 et al., 2008) are documented. Soil carbon declined from 55 to 19%  
137 and microbial biomass by up to 50% for the different treatments  
138 over the 4-year period. Microbial biomass was also strongly  
139 correlated with SOC. Hence, this study provided a unique oppor-  
140 tunity to evaluate if different microbial taxa were more sensitive to  
141 this major carbon resource shift and to determine if crop and/or  
142 management practice altered the microbial community over the  
143 relatively short time period. We used pyrosequencing of the SSU  
144 rRNA gene (Sogin et al., 2006; Sul et al., 2011) to determine com-  
145 munity structure as it provides sufficient information depth so that  
146 community responses could be quantified under the contrasting  
147 soil management schemes.

## 149 2. Materials and methods

### 151 2.1. Study sites, experimental design and sampling method

153 The research site (50 m by 80 m) was located at the Kpeve  
154 Agricultural Experimental Station (KAES) in the Volta Region,  
155 Ghana (coordinates 6° 43.15'N, 0° 20.45'E). Classified as a savanna  
156 to forest transitional zone, the area is dominated by Haplic Lixisols  
157 (sandy clay loams), Haplic Acrisols and Leptic Haplic Acrisols. Soil  
158 samples were taken as ten sub-samples with a 2.5 cm diameter  
159 corer to 18.5 cm depth from each of four replicate plots (10 m by  
160 10 m) in a randomized complete block design of the same annual  
161 rotations at the end of the 4th year's maize harvest. In this study,  
162 the annual rotations (and notations) were 1) EbM: growth of  
163 elephant grass (*Pennisetum* sp.) in the fallow period with its residue  
164 burned followed by maize crop (native practice), 2) PM: winter  
165 pigeon pea (*Cajanus cajan*) (N<sub>2</sub>-fixing) crop, minimal tillage of  
166 fallow (winter) period residues followed by maize crop, 3) EfM:  
167 winter growth of elephant grass with no burn and followed by  
168 fertilized maize crop, 4) BF: bare fallow, i.e. no fallow season plant,  
169 followed by maize crop and 5) Eu: regrowth of the native elephant  
170 grass–shrub vegetation left unmanaged for 4 years (native condi-  
171 tion). Each replicate sample was a homogenized composite of the  
172 ten random sub-samples from each replicated plot (Asuming-  
173 Brempong et al., 2008), with the exception of Eu, for which the  
174 composites consisted of two sub-samples. After collection, soil  
175 samples were immediately placed on ice and then stored at –20 °C

176 until DNA extraction. Samples were also obtained from agricultural  
177 soils in Iowa (IA) and Michigan (MI) to serve as outgroups. These  
178 sites had been under no-till management and samples were also  
179 collected immediately after maize harvest.

### 181 2.2. SSU rRNA gene pyrosequencing

182 Soil DNA was extracted with the Mobio PowerSoil DNA Isolation  
183 Kit (Mobio, Carlsbad, CA) and the V4 region of the SSU rRNA gene  
184 was amplified using the previously described primer set 1 (Sul  
185 et al., 2011). Amplification conditions were as follows: initial in-  
186 cubation for 3 min at 95 °C; 30 cycles of 95 °C for 45 s, 57 °C for 45 s,  
187 and 72 °C for 1 min; and a final 4 min incubation at 72 °C.  
188 Sequencing was performed using the Genome Sequencer FLX Sys-  
189 tem (454 Life Sciences, Bradford, CT), by Michigan State University's  
190 Research Technology Support Facility.

### 192 2.3. Pyrosequencing data analysis

193 Raw reads were trimmed, quality-filtered, aligned, and clustered  
194 (Sul et al., 2011) at 97% to generate Operational Taxonomic Units  
195 (OTUs) using programs from the RDP Pyrosequencing Pipeline  
196 (Cole et al., 2009), and assigned to bacterial taxa using the RDP  
197 Classifier set at the recommended bootstrap threshold of 50%  
198 (Wang et al., 2007). Bias-corrected Chao1 species richness esti-  
199 mates and Shannon index (H') were obtained using EstimateS  
200 ([www.purl.oclc.org/estimates](http://www.purl.oclc.org/estimates)).

### 202 2.4. Statistical and network analyses

203 Canonical correspondence analysis (CCA) and non-metric  
204 multidimensional scaling (NMDS) were performed using the R  
205 statistical program (R Development Core Team, [http://www.R-  
206 project.org](http://www.R-project.org)) running the vegan package (Oksanen et al., 2007).  
207 The Random Matrix Theory (RMT)-based network approach (Luo  
208 et al., 2006, 2007) was used to construct pMEN (phylogenetic  
209 molecular ecological network), which defines the co-occurrence  
210 (positive or negative interaction) among OTUs (Zhou et al.,  
211 2010). The pMEN construction was performed with OTUs  
212 ( $n = 1273$ ) present in the non-carbon stressed samples (4 bio-  
213 logical replicates of BF, EfM, EbM, and PM). Based on the threshold  
214 detected by the RMT method ( $s_t = 0.83$ ), 476 OTUs (nodes)  
215 remained. The average geodisc distance, average clustering coef-  
216 ficient and modularity of the pMEN were used as values to test the  
217 significance of the difference from random networks. All pMEN  
218 construction and analyses were performed by a pipeline written  
219 in Java and Perl scripts (Zhou et al., 2010). Network graphs were  
220 visualized using Cytoscape 2.6.0 software (Cline et al., 2007).  
221 Except where otherwise indicated, processing software was  
222 written in Java (API v1.5.0) and executed on the Macintosh  
223 (OS 10.4) or Linux (2.4.23) operating systems running Java virtual  
224 machines from Apple or Sun, respectively. Processed sequences  
225 and project are deposited in MG-RAST server (Meyer et al.,  
226 2008) under MG-RAST ID:4522002.3–4522002.4.3 and [http://](http://metagenomics.anl.gov/linkin.cgi?project=4416) Q1  
227 [metagenomics.anl.gov/linkin.cgi?project=4416](http://metagenomics.anl.gov/linkin.cgi?project=4416).

## 231 3. Results

### 232 3.1. Microbial community diversity differed by agricultural 233 management

234 The experimental sites were developed to investigate the effect  
235 of seven different agricultural managements between 2003 and  
236 2006 (Adiku et al., 2008); we selected four of the most contrasting  
237 treatments for our study. Over a 4-year period, SOC declined under  
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239  
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