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# Land-use intensification and agroforestry in the Kenyan highland: impacts on soil microbial community composition and functional capacity

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1 **Land-use intensification and agroforestry in the Kenyan highland: impacts on soil**  
2 **microbial community composition and functional capacity**

3

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11

12 **Abstract**

13

14 This study investigates microbial communities in soil from sites under different land use in  
15 Kenya. We sampled natural forest, forest plantations, agricultural fields of agroforestry farms,  
16 agricultural fields with traditional farming and eroded soil on the slopes of Mount Elgon,  
17 Kenya. We hypothesised that microbial decomposition capacity, biomass and diversity 1)  
18 decreases with intensified cultivation; and 2) can be restored by soil and land management in  
19 agroforestry. Functional capacity of soil microbial communities was estimated by degradation  
20 of 31 substrates on Biolog EcoPlates™. Microbial community composition and biomass were  
21 characterised by phospholipid fatty acid (PLFA) and microbial C and N analyses.

22 All 31 substrates were metabolised in all studied soil types, i.e. functional diversity did  
23 not differ. However, both the substrate utilisation rates and the microbial biomass decreased  
24 with intensification of land use, and the biomass was positively correlated with organic matter  
25 content. Multivariate analysis of PLFA and Biolog EcoPlate™ data showed clear differences  
26 between land uses, also indicated by different relative abundance of PLFA markers for certain

27 microorganism groups. In conclusion, our results show that vegetation and land use control  
28 the substrate utilisation capacity and microbial community composition and that functional  
29 capacity of depleted soils can be restored by active soil management, e.g. forest plantation.  
30 However, although 20 to 30 years of agroforestry farming practises did result in improved soil  
31 microbiological and chemical conditions of agricultural soil as compared to traditional  
32 agricultural fields, the change was not statistically significant.

33 Key words: Biolog EcoPlate™, PLFA, soil nutrients, agriculture, forest plantation, soil C

34

## 35 **1. Introduction**

36

37 The high growth in human population in Sub-Saharan Africa has led to intensification of  
38 agriculture, deforestation and use of less suitable land for agriculture. The traditional  
39 techniques often leave the soil open to erosion by wind and rain, which result in depletion of  
40 soil organic matter and nutrients, in turn leading to lowered fertility.

41 In addition to decreased productivity, these conditions lead to loss of biodiversity both in  
42 flora and fauna (Tschardt et al., 2012; Matson et al., 1997). The above-ground loss of  
43 biodiversity is well documented and of great concern, while the below-ground effects have  
44 been less studied. There is now, however, an increasing number of studies that have  
45 documented changes in soil microorganisms and fauna biodiversity of tropical soils in relation  
46 to intensified land use (e.g. Bossio et al., 2005; Huising et al., 2011).

47 The traditional agricultural maize mono-cropping often results in removal of the  
48 aboveground biomass of stover after harvest. The consequences for soil fertility are reduced  
49 carbon and nutrient supplies (Mutuo et al., 2005). The tillage of crop fields also disturbs the  
50 habitats of soil organisms that in most cases show lower numbers and biomass in cultivated  
51 land than in undisturbed soil (Brady et al., 2002).

52 This study examines the structure and functional capacity of the microbial community in  
53 soil under different land uses on the slope of Mount Elgon in western Kenya, which is  
54 intensively used for agriculture. For more than 20 years, implementation of agroforestry in the  
55 region has been promoted with help of the NGO Vi Agroforestry Project (Vi AFP) and this  
56 type of land use is now wide spread. The aim is to conserve and restore soil fertility and to  
57 diversify agricultural production for improvement of farmers' economy and increase of wood  
58 production for fuel and construction. The agroforestry systems mainly include inter-cropping,

59 trees scattered on farm, trees along conservation structures, hedgerow planting and woodlots  
60 (Gachene et al., 2003; Maundu and Tengnäs, 2005; Dharani, 2002). Mulching with leaves  
61 from the trees to the agricultural fields of agroforestry system farms is a recommended  
62 practice as well as other forms of returning crop residues to the soil. The beneficial effects of  
63 agroforestry on several ecosystem services and its capacity to restore soil structure and  
64 function have been assumed and have in many cases been supported by empirical studies  
65 (Jose, 2009; Sharma, 2009; Smith et al., 2013; Vincenti et al., 2013). However, not all studies  
66 have been able to demonstrate consistently significant effects on soil conditions and in  
67 particular not on soil microbial communities (e.g. Lacombe et al., 2009; Tornquist et al.,  
68 1999).

69 In this study the following hypotheses were tested: 1) Deforestation, agricultural  
70 cultivation and overuse of land resources lead to decrease of microbial biomass, change in  
71 community composition and decreasing functional capacity of soil microorganisms. 2) The  
72 microbial communities can be restored by active soil management, including implementation  
73 of agroforestry. The functional capacity of soil microorganisms was estimated by measuring  
74 the degradation of 31 different substrates on Biolog EcoPlates™. Microbial community  
75 composition and biomass were characterised by phospholipid fatty acid (PLFA) analysis and  
76 by determination of microbial C and N. The microbial community was also analysed in  
77 relation to chemical and physical soil conditions.

78

## 79 **2. Materials and Methods**

80

### 81 2.1. Study area

82

83 The study took place on the slopes of Mt Elgon, west of Kitale in Rift Valley province, Kenya  
84 (coordinates of area centre: 1°04'N, 34°04'E). The areas surrounding Mt Elgon National Park  
85 have been almost completely deforested due to agriculture and fuel wood collection, but to a  
86 great extent, trees have now been reintroduced in agroforestry systems and in forest  
87 plantations and wood lots. The climate is highland equatorial with a mean annual temperature  
88 of 18 °C (Kitale town, 1900 m a.s.l.) and average annual precipitation of around 1300 mm,  
89 most falling during April-July (long rains) and October-November (short rains). The soils on  
90 the mountain slopes are reddish sandy clay loams developed from basalt and ashes and rich in  
91 organic matter. By the foot of the mountain the soils are dark brown andosols and nitosols.  
92 Maize and sunflowers are the most favoured crops, while *Acacia* spp., *Grevillea robusta*,  
93 *Sesbania sesban*, *Calliandra calothyrsus*, *Passiflora edulis*, *Cordia africana*, *Markhamia*  
94 *lutea* and *Persea americana* are the most commonly planted tree species. Mt Elgon National  
95 Park covers the area of the mountain above ca 2200 m and up to the summit at 4321 m a.s.l.  
96 The vegetation is composed of a zonation of mountain forest and afro-alpine vegetation above  
97 the tree line.

98

## 99 2.2. Sampling

100

101 Soil samples were taken in four different habitat types representing increasing land-use  
102 intensity: planted forest or woodlots consisting of many different species of trees (FO);  
103 agroforestry fields (AF); agricultural fields (AG = harvested and not replanted maize fields);  
104 and eroded land (ER= bare, uncultivated land often used as pathways). Together with staff  
105 from Vi AFP, farms on the chosen altitudes and cultivation systems were selected. There were  
106 nine replicates of each land use; five replicates were situated on altitudes between 1900 and  
107 2000 m and four replicates on altitudes between 2000 and 2200 m. All sampling sites were on

108 small-scale farms, except one that was situated on Olof Palme Agroforestry Centre (OPAC,  
109 1900 m a.s.l.). AF soil samples were collected in small agricultural fields surrounded by  
110 planted trees. For comparison, soil sampling (four replicates) was also performed in  
111 indigenous forest of Mt Elgon National Park (EL, 2200-2400 m a.s.l.). Dominating trees in  
112 the EL sites were *Okotea usambarensis*, *Olea africana* and *Juniperus procera*. In total, this  
113 added up to 40 sampling locations spread over an area of more than 100 km<sup>2</sup>.

114 Samples for soil physical and chemical analyses were collected on all 40 locations during  
115 February – March 2007. Five soil cores from 0-25 cm depth were randomly collected with an  
116 auger from a ca. 25 m x 25 m area. The cores were bulked into one soil sample, put in plastic  
117 bags and tagged and then carefully mixed. The bags were left open to air-dry for a minimum  
118 of five days before transport to the lab at Moi University in Eldoret where soil physical and  
119 chemical analyses were done.

120 Samples for soil microbial analyses were taken at the same 40 locations as for physical  
121 and chemical analyses and by the same sampling technique, but at a later date during March  
122 2007. These samples were transported in cool boxes and with minimal disturbance within 46  
123 h to the Tropical Soil Biology and Fertility Programme of International Centre for Tropical  
124 Agriculture/World Agroforestry Centre laboratory in Nairobi (TSBF-CIAT/ICRAF) where  
125 total microbial C and N and Biolog EcoPlate™ analysis were done on the fresh soil.

126 Thereafter the remaining soil was stored in freezer at -20 °C for later transport to SLU in  
127 Uppsala, Sweden, where the PLFA analysis was done.

128

### 129 2.3. Physical and chemical analyses

130

131 All physical and chemical analyses were performed according to Okalebo et al. (1993) and/or  
132 Anderson and Ingram (1993). Briefly, soil particle size analysis was performed using the



133 hydrometer method. Sand, silt and clay content of the soil was measured as percentage of  
134 weight of oven-dry and organic matter-free soil. Extractable nitrate was determined by a  
135 colorimetric method. Soil samples were extracted with potassium sulphate after which  
136 salicylic acid and sodium hydroxide were added and then analysed by the molybdenum blue  
137 method. After colour development, absorbance was read at 419 nm. Plant available P was  
138 analysed by the Olsen method. Air-dried soil was extracted with sodium bicarbonate at pH  
139 8.5. The solution was filtered and the absorbance was measured at 880 nm. The organic  
140 carbon content was determined by complete oxidation by heating after addition of sulphuric  
141 acid and aqueous potassium dichromate mixture. The remaining potassium chromate that was  
142 titrated against ferrous ammonium sulphate gave the measure of organic carbon content. For  
143 total nitrogen, samples were completely oxidised by treating with hydrogen peroxide,  
144 selenium and sulphuric acid. After the acid digestion, sodium reagents were added and the  
145 absorbance was measured at 650 nm.

146

#### 147 2.4. Microbial analyses

148

149 Microbial C (MBC) and N (MBN) was analysed by chloroform fumigation-extraction.  
150 Fumigated and non-fumigated soil was extracted with potassium sulphate and the difference  
151 in concentration gave the amount of microbial biomass C and N in soil (Anderson and  
152 Ingram, 1993).

153 Microbial metabolic activity was measured using 96-well Biolog EcoPlates™ where soil  
154 microbes are cultured in different substrates. The assay is based on the capacity of  
155 microorganisms to utilise different substrates and thus generating a metabolic fingerprint  
156 providing information on functional biodiversity in the soil (Insam, 1997; Preston-Mafham et  
157 al., 2002). Out of the 31 carbon sources, seven were carbohydrates, two amines/amides, six

158 amino acids, nine carboxylic acids, three miscellaneous and four polymers (Biolog, 2007).  
159 From each of the 40 samples, 10 g of fresh soil was suspended in 90 ml of 0.145 M NaCl  
160 (dilution to  $10^{-1}$ ). With additional NaCl solution, the suspension was subsequently diluted to  
161  $10^{-3}$ , transferred to the wells and the plates were incubated for four days at 25 °C. The colour  
162 development was followed over time by measuring the absorbance at 595 nm every 24 h up to  
163 96 h. Average well colour development (AWCD) was calculated both as the total sum and for  
164 the different groups of substrates (Elfstrand et al., 2007). The diversity in colour development  
165 for all substrates after 96 h incubation was calculated with the Shannon-Weaver Diversity  
166 Index, according to the formula:  $H' = -\sum p_i \ln p_i$ , where  $p_i$  is the proportion of AWCD of a  
167 particular substrate to the AWCD of all substrates of a certain land use (Yan et al., 2000).

168 Extraction and methylation of PLFAs were done as described by Börjesson et al. (1998).  
169 Fatty acid methyl esters (FAME) were subsequently quantified by gas chromatography  
170 (Hewlett–Packard model 6890 GC) as described by Börjesson et al. (1998) and Steger et al.  
171 (2003). 37 peaks were identified as PLFAs using a gas chromatograph with a mass-  
172 spectrometer (Hewlett-Packard HP 6890 equipped with a HP 5973 mass selective detector).  
173 Fatty acids were used as markers for different general groups of microorganisms (Börjesson et  
174 al., 2012; Elfstrand et al., 2007; Frostegård and Bååth, 1996, Frostegård et al., 2011).

175

## 176 2.5. Statistical analyses

177

178 Data for soil physical, chemical and biological factors were analysed with a General Linear  
179 Model with the land uses (FO, AF, AG and ER) and altitudes as model components (Minitab  
180 15 or PASW Statistics 17.0). When significant effects were found ( $P < 0.05$ ) Tukey's pairwise  
181 comparisons was used to compare treatment means. Since the sampling locations of Mt Elgon  
182 natural forest (EL) was situated on higher altitude than the other sampling sites, the difference

183 between EL and the other four land uses cannot be attributed only to land use, an altitude  
184 effect cannot be excluded. Therefore, EL was not included in the statistical analysis but the  
185 results are shown for comparative purposes. Data were tested for normal distribution and if  
186 needed log transformation was done in order not to violence the assumptions of normality and  
187 equal variances. Correlation analysis among all soil variables was performed. A principal  
188 component analysis (PCA) was done on correlation matrix for Biolog-data (Minitab 15) and  
189 covariance matrix for PLFAs converted to mole percentage (JMP ver., SAS Institute, Cary,  
190 U.S.A.). In this case the EL data were also included.

191

192

### 193 **3. Results**

#### 194 3.1. Altitude effects

195

196 There were no significant differences between the two altitudes for any of FO, AF, AG and  
197 ER land uses, in any of the tested variables, except for PLFA. Therefore, results of the lower,  
198 1900-2000 m a.s.l., and the higher altitude level, 2000-2200 m a.s.l., will in most cases be  
199 presented together. Results from EL (only on altitudes 2200-2400 m a.s.l.) are presented as a  
200 reference but not included in the statistical analysis.

201

#### 202 3.2. Soil physics, chemistry and microbial biomass

203

204 The clay content was significantly higher in ER than in AF and FO while AG was not  
205 significantly different from ER. Sand and silt content showed the opposite trend with the  
206 highest percentage in FO and AF (Table 1).

207 Land use generally affected most soil chemical parameters in similar direction, with an  
208 increasing trend from ER to AG and AF, FO and EL the highest (Table 1). The mean pH, C  
209 and N significantly separated FO from AG and ER, but not from AF, and mean of EL was the  
210 highest. The MBC and MBN showed similar patterns; the concentrations increased with  
211 decreasing disturbance. The concentration of plant available phosphorus was significantly  
212 higher in AF than in ER and AG, while EL had the lowest value.

213

### 214 3.3. Decomposition capacity of soil microorganisms and functional diversity

215

216 The substrate utilisation capacity of soil microbes followed the same pattern as for soil  
217 physical and chemical variables. Thus, total AWCD was highest in EL, FO and AF,  
218 intermediate in AG (not significantly different from the land uses with trees) and significantly  
219 lower in ER (Table 1). Utilisation patterns were similar also when split into the different  
220 substrate groups, i.e. carbohydrates, amines/amides, amino acids, carboxylic acids,  
221 miscellaneous and polymers (data not shown). Total AWCD was, however, not significantly  
222 correlated with any of the other variables, except a positive correlation to plant available  
223 phosphorus (Table 2).

224 All 31 substrates were utilised in soil from all habitat types, but at different rate, and  
225 decomposition rates of each substrate were lower in the more disturbed land uses. The  
226 functional diversity measured as Shannon-Weaver index did not differ among land uses  
227 (range of  $H'$ : 3.313-3.356). The PCA score plot analysis (Fig. 1) clearly separates ER from  
228 the rest of land uses. It also shows a slight separation of tree-based systems from AG, which is  
229 the most similar to ER.

230

### 231 3.4. Microbial community biomass and composition

232  
233 Total PLFA concentration (Table 3), which reflects total microbial biomass, differed  
234 significantly among land uses and was the highest in EL (281 mmol g<sup>-1</sup>) followed by FO, AF,  
235 AG and ER in decreasing order. However, the difference between AG and AF was not  
236 significant. This is the same trend as for microbial N and C. Total PLFAs per g soil C (Table  
237 3) also show significant differences between the land uses (P<0.001) with the amounts in EL  
238 2.7 times those in ER. However, the differences were smaller than when calculated per g soil.  
239 The total PLFA content was positively correlated with most of the chemical and biological  
240 soil properties (Table 2). Exceptions are nitrate and clay that were significantly negatively  
241 correlated with total PLFA, while the correlation with AWCD was only weakly positive.

242 Similar to Biolog EcoPlate™ data, the PCA of PLFA data showed ER to form a distinct  
243 cluster separated from the other sites (Fig. 2). In the loading plot (figure not shown), the main  
244 drivers for PC 1 were monounsaturated PLFAs (16:1ω7, 18:1ω5 and 18:1ω7) on the positive  
245 side and 20:4 together with branched PLFAs (i16:0, i17:0, a17:0, br18:0) on the negative side;  
246 while PLFA 16:0 dominated strongly in the positive direction of PC 2. Thus, the position of  
247 ER samples in Fig. 2 can be explained by relatively high proportions of branched PLFAs  
248 compared to the other sites, but also low proportions of the mentioned mono-unsaturated  
249 PLFAs (Table 4).

250 Looking at single PLFAs, most had highest concentrations per g soil (as well as per g  
251 microbial biomass C) in EL with a decreasing trend towards ER, like the values for total  
252 PLFA. One exception is 20:4 (a marker for Protozoa) that had markedly higher values per  
253 gram soil and per g C in ER than in the other land uses.

254 Expressed as relative contributions, the sum of PLFA markers for bacteria constituted 55-  
255 60 % of the PLFA, except in ER, where it was 49.5 % (Table 3). PLFAs typical of G+  
256 bacteria (except actinobacteria) also had lower share in ER (19.6 %) than in the other land

257 uses (24.2-26.7 %) while the markers for actinobacteria had the highest relative abundance in  
258 AG (3.3 %) and the lowest in EL (1.8 %). Signature fatty acids for G- bacteria had the highest  
259 percentage in EL (28.5 %) and significantly lower in ER (20 %), while markers for fungi had  
260 a share of around 7 % in all land uses.

261 Table 4 shows the relative abundance of each PLFA as percentage of total PLFA. The  
262 most abundant fatty acid in all land uses was 16:0 followed by i15:0, the former ubiquitous in  
263 all living matter, the latter a marker for G+ bacteria. A number of PLFAs are sticking out with  
264 different values for certain land uses. For example, 18:1 $\omega$ 7 (a marker for G- bacteria) had  
265 higher relative contribution in EL than in the other habitats. Moreover, 10Me18:0 (marker for  
266 actinobacteria) and 10Me16:0 (marker for sulphur reducing bacteria) were especially  
267 abundant in AF and AG. ER stood out from the other habitats by significantly lower  
268 contributions of several PLFAs, e.g. markers for sulphur reducing bacteria, G-, G+ and  
269 actinobacteria. Conversely, the contribution of the Protozoa marker 20:4 was substantially  
270 higher in ER than in the other habitats. Relative values of markers for fungi (18:2, 18:3 and  
271 18:1 $\omega$ 9) did not separate among land uses.

272

273

#### 274 **4. Discussion**

275

276 Since EL only occurs on higher altitudes than the other studied land uses it was not included  
277 in the statistical analysis. However, data from EL can give a quite realistic indication of what  
278 conditions could be expected in undisturbed forest soils also on lower elevation. Also among  
279 the other four land uses, initial conditions were probably not completely identical. Difference  
280 in soil particle size distribution with higher clay and lower sand contents in ER and AG than  
281 in FO (with AF intermediate) indicate that sites with more sandy and probably less fertile

282 soils have been selected for forest plantation while the soils with more clay and higher fertility  
283 have been used for agriculture and agroforestry.

284 The high clay contents in ER could possibly be explained by the fact that the upper,  
285 sandier soil horizons (with more organic matter) have been washed away while soils richer in  
286 clay in lower horizons have been exposed. With this in mind, our results demonstrate a  
287 decrease in soil organic matter, microbial C and N and microbial biomass with increasing  
288 disturbance related to cultivation, which supports our first hypothesis.

289 Although the relative abundance of PLFAs typical for microbial groups differed to some  
290 extent among the studied habitats (Fig. 2, Table 3 and 4), all PLFAs were detected in all of  
291 them. Also all 31 tested substrates were decomposed in all habitats (Table 1, Fig.1). This  
292 indicates that on microbial community level, probably no vital functional and structural  
293 features of the soils were lost by intensified land use. However, the capacity (decomposition  
294 rate) and microbial biomass did decrease substantially. Thus, our first hypothesis is supported  
295 regarding decreased biomass and activity, but less obviously regarding microbial diversity.  
296 However, in the case that some organisms disappear, as long as there is a broad spectrum of  
297 organisms remaining, others can take advantage of the changed conditions of altered land use  
298 and decomposition can be sustained (Griffiths et al., 2001; Susilo et al., 2004).

299 The Biolog method, based on incubations, has been found to favor just a few genera;  
300 those that can grow on the substrates and those that are fast growing (r-strategists), while the  
301 majority of oligotrophic species (K-strategists) are out-competed. Still, the Biolog EcoPlate™  
302 contains a high number of substrates that are relevant for the soil environment and thereby  
303 expresses the action of a large number of enzymes. Therefore, it has been useful for  
304 comparing microbial communities in contrasting environmental samples. It has been  
305 recommended to combine the Biolog method with other techniques for analysis of the  
306 functional ability of the entire soil microbial community (Ros et al., 2008) but for this study

307 further analyses were not feasible. We also employed the PLFA analysis, which gives a clear  
308 picture of the structure of the microbial community. With knowledge of the ecology of the  
309 different microorganism groups this also gives some insight into the functional ability of the  
310 community.

311 Although microbial biomass and decomposition capacity in AF were slightly higher as  
312 compared to AG, this difference was not significant. This shows that agroforestry in 20 to 30  
313 years was not sufficient to restore the chemical and biological conditions of agricultural soil  
314 to the recommended levels, but forest plantations on the same farms are useful for restoration  
315 of soil biodiversity and function (decomposition capacity) of degraded soils. It should be kept  
316 in mind that AF samples were taken in arable fields at a certain distance from trees and other  
317 perennial vegetation. It is possible that if we had sampled under such vegetation components  
318 we could have been able to demonstrate improvement as compared to AG. Microbial  
319 community development depends on the growth of the aboveground vegetation either directly  
320 (symbionts and decomposers) or indirectly via supply of carbon (autotrophic microbes)  
321 (Jasper, 2007).

322 Bossio et al. (2005) studied soil microbial community response to land use changes in  
323 different soil types at several locations in Western Kenya. Soil type was the key determining  
324 factor for the microbial community composition (determined by denaturing gradient gel  
325 electrophoresis - DGGE and PLFA analyses), with secondary variation between forested and  
326 agricultural soils, with highest total abundance and diversity in forested sites. Their data from  
327 Biolog Ecoplate™ and enzyme activity analyses demonstrated that substrate utilisation  
328 profiles were more weakly related to soil type and land use than the microbial community  
329 structure was. They concluded that substrate utilisation profiles can be similar in spite of  
330 genetically different and less diverse microbial communities, because redundancy among  
331 communities will promote soil stability and protect soil processes. This is in agreement with



332 our results, showing low correlation between total AWCD and microbial biomass, measured  
333 as total microbial C and N or PLFA.

334 Soils of the indigenous forest in EL had lower levels of plant available P than the other  
335 habitats except ER. One possible explanation can be that the agricultural soils may have been  
336 fertilized with inorganic P, or P has been added by supplementing mulch and other organic  
337 matter, especially in the agroforestry system. Burning of crop residues in AF may have  
338 supplemented P-rich ash and increased availability of P (Ketterings et al., 2002). Additionally,  
339 in the forest soil there certainly was a great competition for P leaving very low plant available  
340 amounts in the soil. P deficiency is likely to occur in tropical agricultural soil when levels of  
341 plant available P is less than 15 mg per kg soil (Fairhurst, 2012).

342 Differences among soil types were smaller when relating PLFA to g soil C than to g soil  
343 (Table 3). This indicates that not only the concentration of organic matter is decisive for  
344 microbial biomass but also substrate quality as well as other biotic and abiotic factors. We  
345 can assume that the organic matter in ER was older than in the other habitats since input of  
346 fresh organic matter is low.

347 Mono-unsaturated PLFAs, indicating G- bacteria, some of which are known to be  
348 involved in nitrogen cycling, showed lower relative abundance in ER than in the other soil  
349 types. It is likely that this reflects the nutritional status, since G- bacteria are known to  
350 increase after nitrogen fertilisation, both through mineral fertilisers and manure (Peacock et  
351 al., 2001).

352 In ER, higher values were found for the Protozoa marker 20:4 and for unk17a, br18, 18:0  
353 and i19:0. Branched PLFAs (e.g. br18 and i19:0) may be used as signatures for G+ bacteria.  
354 These bacteria may preferentially utilise old organic matter (*e.g.* Waldrop and Firestone,  
355 2004), but since the abundances of these branched PLFAs were rather low in our samples, any  
356 conclusion about this would be very speculative. We are unable to explain the high

357 contribution of 20:4 in ER, and have not found any support in the literature concerning  
358 relatively high density of Protozoa in depleted soil. On the contrary, Fierer et al. (2003) found  
359 that Gram+ bacteria and actinomycetes tended to increase in proportional abundance with  
360 increasing soil depth, while the abundances of Gram- bacteria, fungi, and protozoa were  
361 highest at the soil surface and substantially lower in the subsurface where C and nutrient  
362 content was lower.

363

364 Most abiotic and biotic variables were positively correlated with pH. Fierer and Jackson  
365 (2006) studied the diversity of bacterial communities across North and South America. They  
366 found that the composition of bacterial communities was unrelated to site temperature,  
367 latitude and other variables that typically predict plant and animal diversity. On the local  
368 scale, however, differences in communities could largely be explained by soil pH, which is  
369 similar as in our data.

370 In conclusion, we found that Biolog EcoPlate™ and PLFA analyses give useful  
371 information on structure and function of soil microbial communities, complementary to  
372 estimates of organic matter content and total microbial biomass. Our results indicate that the  
373 substrate utilisation capacity and microbial community composition is similar in land with  
374 similar vegetation and that the functional capacity and microbial biomass of the soil can be  
375 restored by active soil management, such as forest plantation. However, 20 to 30 years of  
376 agroforestry practices was not enough to significantly change the conditions of agricultural  
377 soil as compared to conventional agriculture. It seems that the well documented positive  
378 effects that agroforestry has on above-ground harvest yield, other ecosystem services and  
379 socio economy (Jose, 2009; Smith et al., 2013; Sharma, 2009; Vincenti et al., 2013) are not as  
380 easily demonstrated for soil biology, which in this study was more resistant to change.

381

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390

391

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489 **Text to figures**

490

491 **Fig. 1**

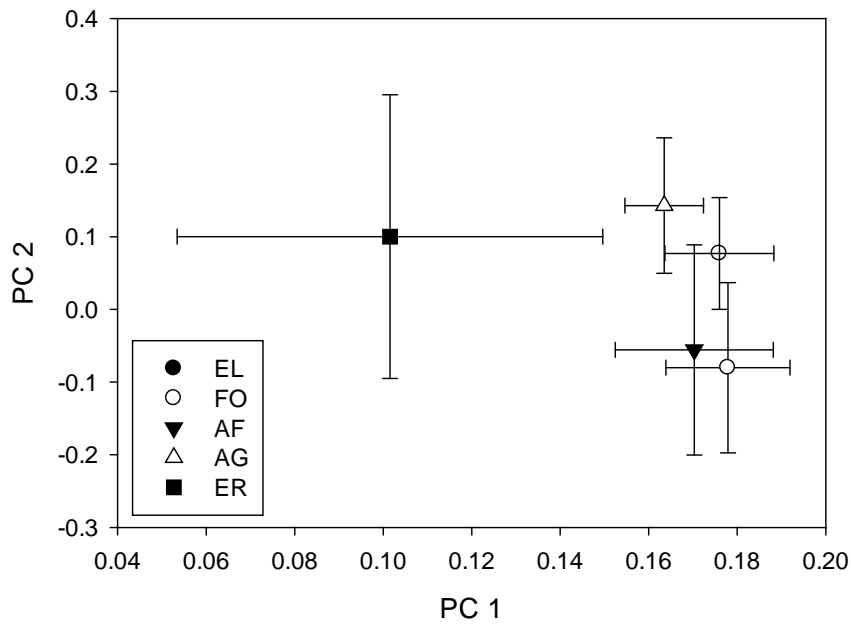
492 PCA multivariate analysis of functional capacity of the soil microorganism community  
493 measured with Biolog EcoPlate™ analysis of decomposition of 31 substrates. Comparison of  
494 five land uses. See Table 1 for explanation of land uses. Error bars are standard deviation; for  
495 EL n=4, for others n=9. One AG sample was removed as an extreme outlier. PC 1 explains  
496 58.9% and PC2 6.9 of the variation.

497 **Fig. 2**

498 PCA multivariate analysis of PLFA composition of the soil microorganism community.  
499 Comparison of five land uses. See Table 1 for explanation of land uses. Two of the samples  
500 were left out in the analysis, due to more than 14 of the PLFAs missing. Error bars are  
501 standard deviation. PC 1 explains 90.5 % and PC2 3.8 % of the variation.

502





503

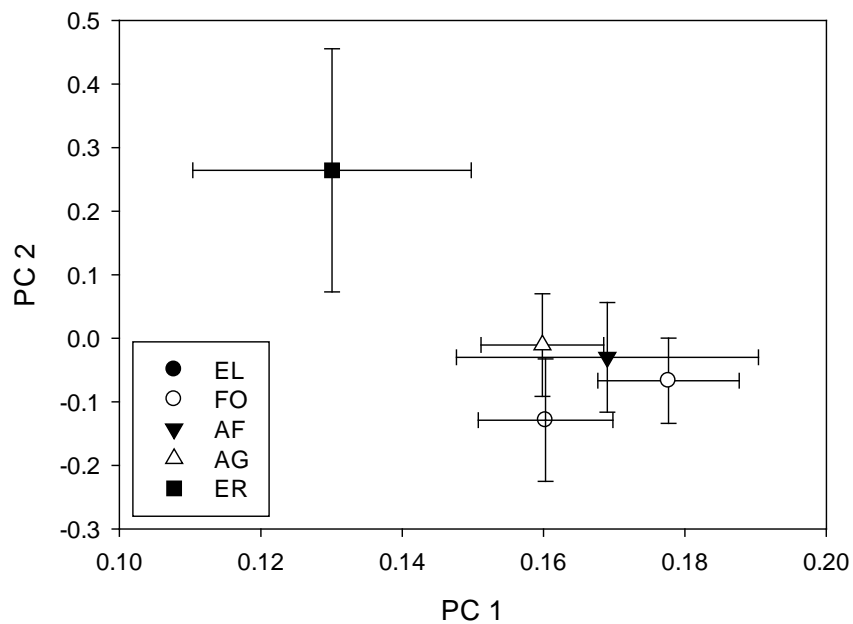
BIOLOG - AWCD

504 **Fig. 1**

505 PCA multivariate analysis of functional capacity of the soil microorganism community  
 506 measured with Biolog EcoPlate™ analysis of decomposition of 31 substrates. Comparison of  
 507 five land uses. See Table 1 for explanation of land uses. Error bars are standard deviation; for  
 508 EL n=4, for others n=9. One AG sample was removed as an extreme outlier. PC 1 explains  
 509 58.9% and PC2 6.9 of the variation.

510

511



512

PLFA

513 **Fig. 2**

514 PCA multivariate analysis of PLFA composition of the soil microorganism community.  
515 Comparison of five land uses. See Table 1 for explanation of land uses. Two of the samples  
516 were left out in the analysis, due to more than 14 of the PLFAs missing. Error bars are  
517 standard deviation. PC 1 explains 90.5 % and PC2 3.8 % of the variation.

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523 **Microbial community composition and functional capacity in Kenyan highland soil**  
 524 **under land-use intensification and agroforestry**

525 Tables Lagerlöf et al. 2014

526 **Table 1**

527 Chemical, biological and physical soil parameters of habitats with different land uses in Mt  
 528 Elgon area, Kenya. The mean ( $\pm$ standard deviation) of pH, total soil nitrogen (N), total soil  
 529 carbon (C), plant available phosphorus (P), nitrate, microbial biomass carbon (MBC),  
 530 microbial biomass nitrogen (MBN), substrate utilisation capacity by Biolog EcoPlates™  
 531 (AWCD, average absorbance at 590 nm after 96 h incubation), silt, sand, clay and moisture.  
 532 ER= Eroded land, AG=Agricultural fields, AF=Agroforestry fields, FO=Planted forest and  
 533 EL=Mt Elgon NP natural forest. n=9, except for EL where n=4. One-way ANOVA did not  
 534 include EL. Different letters in the same row for the mean values indicate significant  
 535 differences at  $P < 0.05$  (Tukey's test).

536

Parameter	ER	AG	AF	FO	ANOVA P-values	EL
pH (H <sub>2</sub> O)	6.2 (0.6) b	6.0 (0.4) b	6.5 (0.5) ab	6.8 (0.5) a	0.006	6.7 (0.3)
N (%)	0.09 (0.07) c	0.37 (0.09) b	0.46 (0.27) ab	0.62 (0.28) a	< 0.001	1.06 (0.49)
C (%)	1.27 (0.61) c	2.34 (0.28) bc	3.41 (1.51) ab	3.80 (1.02) a	< 0.001	5.93 (2.41)
P(mg/kg)	8.53	19.6 (11.0) b	60.8 (45.2)	34.7 (40.3)	0.003	14.1 (9.7)

	(10.8) b		a	ab		
Nitrate ( $\mu\text{g}/\text{kg}$ )	8.9 (5.7)	9.2 (3.7)	6.3 (4.5)	4.9 (3.1)	0.126	7.6 (5.0)
MBC (mg C/kg)	119(30) c	173 (34) b	212 (33) b	283 (52) a	< 0.001	656 (104)
MBN (mg N/kg)	6.6 (2.8) c	13.0 (4.5) bc	19.6 (9.9) b	34.6 (11.6)	< 0.001	114 (34.7)
AWCD	0.48	0.85 (0.49)	1.26 (0.36)	1.25 (0.34)	0.002	1.23 (0.29)
	(0.64) b	ab	a	a		
Silt (%)	10.5 (3.1)	18.2 (9.2) a	15.6 (3.3)	12.8 (2.3)	0.031	13.5 (5.7)
	b		ab	ab		
Sand (%)	50.8 (9.6)	53.1 (12.2) b	61.5 (9.9)	70.8 (6.3) a	<0.001	78.0 ( 9.3)
	b		ab			
Clay (%)	38.5(9.4) a	28.7 (13.7)	22.7 (10.5)	16.2 (5.6) c	<0.001	8.25 (4.2)
		ab	bc			
Moisture (%)	20.0 (6.4)	23.8 (4.3)	25.8 (6.6)	26.5 (5.2)	0.083	45.3 (7.8)

538 **Table 2**

539 Correlation matrix on chemical, physical and biological soil properties for land uses (see tab. 1 for explanation of land uses) ER, AG, EF and FO:  
540 pH, total soil nitrogen (N), plant available soil phosphorus (P), nitrates (Nitr.), total soil carbon (C), moisture, sand, clay, silt, Microbial Biomass  
541 carbon (MBC), Microbial Biomass nitrogen (MBN), altitude (Alt), substrate utilization capacity by total Average Well Colour Development  
542 from Biolog EcoPlates™ (AWCD, 96 h) and total PLFA. The P-values are shown in italics.

543

	<b>CORRELATION COEFFICIENT/ <i>P-values</i></b>												
	<b>pH</b>	<b>N</b>	<b>P</b>	<b>Nitr.</b>	<b>C</b>	<b>Moist</b>	<b>Sand</b>	<b>Clay</b>	<b>Silt</b>	<b>MBC</b>	<b>MBN</b>	<b>Alt</b>	<b>AWCD</b>
<b>N</b>	0.619												
	<i>0</i>												
<b>P</b>	0.362	0.705											
	<i>0.03</i>	<i>0</i>											
<b>Nitr</b>	-0.375	-0.385	-0.457										
	<i>0.024</i>	<i>0.02</i>	<i>0.005</i>										
<b>C</b>	0.605	0.882	0.596	-0.4									
	<i>0</i>	<i>0</i>	<i>0</i>	<i>0.016</i>									
<b>Moist</b>	0.159	0.412	0.367	-0.25	0.54								
	<i>0.354</i>	<i>0.012</i>	<i>0.027</i>	<i>0.141</i>	<i>0.001</i>								

<b>Sand</b>	0.499	0.636	0.219	-0.18	0.638	0.169						
	<i>0.002</i>	<i>0</i>	<i>0.2</i>	<i>0.293</i>	<i>0</i>	<i>0.325</i>						
<b>Clay</b>	-0.512	-0.7	-0.334	0.128	-0.696	-0.27	-0.893					
	<i>0.001</i>	<i>0</i>	<i>0.047</i>	<i>0.456</i>	<i>0</i>	<i>0.112</i>	<i>0</i>					
<b>Silt</b>	0.079	0.205	0.276	0.097	0.192	0.239	-0.135	-0.325				
	<i>0.646</i>	<i>0.23</i>	<i>0.104</i>	<i>0.575</i>	<i>0.262</i>	<i>0.16</i>	<i>0.433</i>	<i>0.053</i>				
<b>MBC</b>	0.349	0.566	0.121	-0.215	0.66	0.355	0.634	-0.57	-0.077			
	<i>0.037</i>	<i>0</i>	<i>0.48</i>	<i>0.208</i>	<i>0</i>	<i>0.033</i>	<i>0</i>	<i>0</i>	<i>0.657</i>			
<b>MBN</b>	0.51	0.587	0.147	-0.187	0.697	0.382	0.615	-0.574	-0.027	0.895		
	<i>0.001</i>	<i>0</i>	<i>0.393</i>	<i>0.274</i>	<i>0</i>	<i>0.021</i>	<i>0</i>	<i>0</i>	<i>0.875</i>	<i>0</i>		
<b>Alt</b>	0.397	0.409	0.54	-0.241	0.278	0.304	0.167	-0.308	0.327	-0.149	-0.12	
	<i>0.016</i>	<i>0.013</i>	<i>0.001</i>	<i>0.156</i>	<i>0.101</i>	<i>0.072</i>	<i>0.329</i>	<i>0.067</i>	<i>0.051</i>	<i>0.386</i>	<i>0.485</i>	
<b>AWCD</b>	0.248	0.305	0.328	0.096	1.237	-0.025	0.021	-0.111	0.199	0.137	0.162	0.148

<b>PLFA</b>	<i>0.171</i>	<i>0.07</i>	<i>0.05</i>	<i>0.577</i>	<i>0.164</i>	<i>0.885</i>	<i>0.902</i>	<i>0.521</i>	<i>0.245</i>	<i>0.425</i>	<i>0.346</i>	<i>0.389</i>	
	<i>0.486</i>	<i>0.715</i>	<i>0.279</i>	<i>-0.223</i>	<i>0.717</i>	<i>0.366</i>	<i>0.599</i>	<i>-0.573</i>	<i>0.004</i>	<i>0.902</i>	<i>0.865</i>	<i>0.011</i>	<i>0.221</i>
	<i>0.003</i>	<i>0</i>	<i>0.099</i>	<i>0.19</i>	<i>0</i>	<i>0.028</i>	<i>0</i>	<i>0</i>	<i>0.982</i>	<i>0</i>	<i>0</i>	<i>0.948</i>	<i>0.195</i>



545 **Table 3**

546 Total PLFA (EL n=4, others n=9) and relative abundance of markers for certain  
 547 microorganism groups in soil samples from different land uses in the Kitale area. See table 1  
 548 for explanation of land uses. EL not tested in ANOVA. Different letters in the same row for  
 549 the mean values indicate significant differences at P<0.05 (Tukey's test).

550

551

	<b>ER</b>	<b>AG</b>	<b>AF</b>	<b>FO</b>	<b>ANOVA</b>	<b>EL</b>
<b>Total PLFA</b>						
nmol per g soil	22.6 c	56.5 b	71.7 b	118.7 a	<0.000	281.8
nmol per g soil	1780.6b	2415.4ab	2101.4b	3125.8a	<0.001	4752.7
<b>C</b>						
<b>Relative abundance of PLFA markers* (% of total PLFA)</b>						
Sum Bacteria	49.5 b	58.2 a	56.6 a	56.7 a	<0.000	59.8
Sum G+	19.6 b	26.7 a	25.4 a	25.3 a	<0.001	24.2
Bact**						
Actinobact.	2.6 bc	3.3 a	3.0 ab	2.2 c	<0.000	1.8
Sum G- Bact	20.0 b	22.3 ab	21.8 ab	24.6 a	<0.01	28.5
Sum Fungi	8.3 a	6.8 a	7.2 a	7.6 a	0.306	8.0
Sum Protozoa	6.6 a	2.1 b	1.8 b	1.5 b	<0.000	2.4

552

553 \*: Markers for certain organism groups:

554 Sum bacteria: i15:0, a15:0, i16:0, 16:1 7 10Me16:0, i17:0, a17:0, Cy17, 17:0,

555 18:1 19:0, 10Me18:0, 19:1

556 Sum G+ bacteria excl. Actinobacteria: i15:0, a15:0, i16:0, 10Me16:0

557 Actinobacteria: 10Me18:0

558 Sum G- bacteria: 16:1 7, 16: cy17, 17:0, 18:1

559 Sum fungi: 18:2+18:3, 18:1 9

560 Sum Protozoa: 20:4, 20:0, 22:0

561

562 \*\*: Excluding Actinobacteria

563

564

565

566 **Table 4**

567 Relative abundance of individual PLFAs (EL n=4, others n=9) in soil samples from different  
 568 land uses in the Kitale area. See table 1 for explanation of land uses. EL not tested in  
 569 ANOVA. Different letters for mean values in the same row indicate significant differences at  
 570 P<0.05 (Tukey's test).

571 **PLFA**      **Relative abundance – % of total PLFA**

572	<hr/>						
573	<hr/>						
574	<b>ER</b>	<b>AG</b>	<b>AF</b>	<b>FO</b>	<b>ANOVA</b>	<b>EL</b>	
575	<hr/>						
576	<hr/>						
577	i14:0	0.30 c	0.47 bc	0.58 ab	0.74 a	<0.000	0.7
578	14:0	0.91 c	1.00 bc	1.08 ab	1.07 a	<0.000	1.22
579	i15:0	6.32 b	9.63 a	9.27 a	9.47 a	<0.000	8.66
580	a15:0	3.63 b	4.88 a	4.86 a	5.20 a	<0.000	4.88
581	15:0	0.56	0.43	0.58	0.47	0.74	0.71
582	br16a	0.31 b	0.48 a	0.51 a	0.59 a	<0.000	0.73
583	br16b	0.032 b	0.21 a	0.24 a	0.23 a	<0.000	0.19
584	i16:0	5.19	4.78	4.45	4.47	0.264	5.21
585	16:1ω9	0.32 b	0.70 a	0.80 a	0.72 a	<0.000	0.47
586	16:1ω7	3.88 b	4.87 ab	5.17 a	5.43 a	<0.001	6.045
587	16: 1ω5	1.33 c	2.50 b	2.78 ab	3.20 a	<0.000	3.29

588	16:00	16.65	13.57	14.88	15.54	0.554	12.66
589	unk17a	1.60 a	1.20 ab	1.07 ab	1.01 b	<0.05	0.9
590	br17	1.55 b	3.15 a	3.14 a	2.88 a	<0.000	2.78
591	10Me16	4.55 b	7.46 a	6.87 ab	6.20 ab	<0.05	5.56
592	unk17b	0.9	0.94	0.54	0.53	0.42	0.42
593	i17:0	3.62 a	2.75 b	2.74 b	2.60 b	0.001	2.57
594	a17:0	3.7	3.12	3.14	2.98	0.214	2.6
595	cy17	1.71 b	2.69 a	2.70 a	2.64 a	0	2.26
596	17:0	0.6	0.63	0.71	0.52	0.432	0.56
597	br18	4.15 a	2.78 ab	2.54 b	2.19 b	<0.01	1.86
598	10Me17	1.22	1.67	1.48	1.17	0.06	1.2
599	18:2+18:3	3.14	2.04	2.15	2.57	0.307	2.64
600	18: 1 $\omega$ 9	5.2	4.77	5.03	5.02	0.508	5.33
601	18: 1 $\omega$ 7	4.34 c	5.32 bc	6.24 ab	7.86 a	0	9.78
602	18: 1 $\omega$ 6	0.28	0.42	0.37	0.28	0.577	0.2
603	18: 1 $\omega$ 5	1.63	1.53	1.93	1.92	0.736	1.69
604	18:0	3.85 a	3.13 ab	2.94 b	2.67 b	<0.01	2.65
605	br19	0.00 b	0.08 ab	0.05 ab	0.13 a	<0.05	0.55
606	10Me18:0	2.58 bc	3.35 a	3.03 ab	2.28 c	<0.000	1.87
607	i19:0	3.32 a	2.07 ab	1.57 b	0.92 b	<0.000	0.9
608	unk19a	1.08	0.63	0.55	0.58	0.05	0.8

609	unk19b	0	0.41	0.12	0.37	0.156	0.02
610	cy19:0/19:1	4.82 a	4.23 ab	3.14 b	4.06 ab	<0.05	5.67
611	20:4	4.13 a	0.40 b	0.37 b	0.28 b	<0.000	0.47
612	20:0	1.87 a	1.01 ab	0.99 ab	0.84 b	<0.05	1.13
613	22:0	0.6	0.69	0.4	0.37	0.348	0.84
614							
615	<b>Sum PLFA</b>	<i>100</i>	<i>100</i>	<i>100</i>	<i>100</i>		<i>100</i>
616							
617							