Sveriges lantbruksuniversitet
Swedish University of Agricultural Sciences

This is an author produced version of a paper published in
Applied Soil Ecology.
This paper has been peer-reviewed but may not include the final publisher proof-corrections or pagination.

Citation for the published paper:
Lagerlöf, Jan; Adolfsson, Lena; Börjesson, Gunnar; Ehlers, Knut; Palarès Vinyoles, Glòria; Sundh, Ingvar. (2014) Land-use intensification and agroforestry in the Kenyan highland: impacts on soil microbial community composition and functional capacity. *Applied Soil Ecology*. Volume: 82, pp 93-99.
http://dx.doi.org/10.1016/j.apsoil.2014.05.015.

Access to the published version may require journal subscription.
Published with permission from: Elsevier.

Epsilon Open Archive http://epsilon.slu.se

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

Jan Lagerlöf, Lena Adolfsson, Gunnar Börjesson, Knut Ehlers Glòria Palarès Vinyoles and Ingvar Sundh.

Published 2014 in *Applied soil ecology* volume 82, pages 93-99

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

3

4 Jan Lagerlöf[1], Lena Adolfsson[1], Gunnar Börjesson[2], Knut Ehlers[3] Glòria Palarès Vinyoles[1]
5 and Ingvar Sundh[4].
6 [1] SLU, Department of Ecology, P.O.Box 7044, SE-750 07 Uppsala, Sweden
7 Corresponding author: jan.lagerlof@slu.se, tel +46 (0)18 672422, fax +46 18 (0)672890
8 [2] SLU, Department of Soil and Environment, P.O.Box 7014, SE-750 07 Uppsala, Sweden
9 [3] German Federal Environment Agency, Wörlitzer Platz 1, 06844 Dessau-Roßlau, Germany
10 [4] SLU, Department of Microbiology, P.O.Box 7025, SE-750 07 Uppsala, Sweden

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 (Tscharntke 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,

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

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

2. Materials and Methods

2.1. Study area

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

2.2. Sampling

Soil samples were taken in four different habitat types representing increasing land-use intensity: planted forest or woodlots consisting of many different species of trees (FO); agroforestry fields (AF); agricultural fields (AG = harvested and not replanted maize fields); and eroded land (ER= bare, uncultivated land often used as pathways). Together with staff from Vi AFP, farms on the chosen altitudes and cultivation systems were selected. There were nine replicates of each land use; five replicates were situated on altitudes between 1900 and 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^2.

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: $H' = -\Sigma p_i \ln p_i$,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^{-1}) 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, 18:1ω7 (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 18:1ω9) 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

382 **Acknowledgements**

383 We are grateful to Björn Horvath and the whole Vi Agroforestry team in Kitale for inviting us
384 to work with the project and for excellent co-operation and hospitality. We also thank
385 personnel at CIAT-TSBF and ICRAF laboratory in Nairobi for help with laboratory work and
386 for great hospitality. The study was financially supported by a Minor Field Study scholarship
387 from Sida/Internationella programkontoret and by a research grant from Formas/Sida for work
388 at CIAT - a CGIAR Institute. We thank Ulf Olsson, SLU and Joseph Mungatu, Nairobi
389 University for statistical advice.

390

391

392 **References**

393 Anderson, J.M., Ingram, J.S.I., 1993. Tropical Soil Biology and Fertility: a Handbook of
394 Methods. CAB International, Wallingford, UK.

395 Biolog, 2007. URL: www.biolog.com. Accessed January 2007.

396 Börjesson, G., Sundh. I., Tunlid, A., Svensson, B.H., 1998. Methane oxidation in landfill
397 cover soils, as revealed by potential oxidation measurements and phospholipid fatty acid
398 analyses. Soil Biol. Biochem. 30, 1423-1433.

399 Börjesson, G., Menichetti, L., Kirchmann, H., Kätterer, T., 2012. Soil microbial community
400 structure affected by 53 years of nitrogen fertilisation and different organic amendments.
401 Biol. Fertil. Soils 48, 245-257.

402 Bossio, D.A., Girvan, M.S., Verchot, L., Bullimore, J., Borelli ,T., Albrecht, A., Scow, K.M.,
403 Ball, A.S., Pretty, J.N., Osborn, A.M., 2005. Soil microbial community response to land
404 use change in an agricultural landscape in Western Kenya. Microb. Ecol. 49, 50-62.

405 Brady, N.C., Weil, R.R. 2002. The Nature and Properties of Soils, 13th Edition. Pearson
406 Education Inc., Upper Saddle River, NJ.

407 Dharani, N., 2002. Field Guide to Common Trees and Shrubs of East Africa. Struik
408 Publishers, Cape Town, South Africa.
409 Elfstrand, S., Hedlund, K., Mårtensson, A., 2007. Soil enzyme activities, microbial
410 community composition and function after 47 years of continuous green manuring. Appl.
411 Soil Ecol. 35, 610-621.
412 Fairhurst, T., 2012. African soil health consortium: Handbook of integrated soil fertility. CAB
413 International, Pondicherry, India.
414 Fierer, N., Schimel, J.P., Holden, P.A., 2003. Variations in microbial community composition
415 through two soil depth profiles. Soil Biol. Biochem. 35, 167-176.
416 Fierer, N., Jackson. R., 2006. The diversity and biogeography of soil bacterial communities.
417 Proc. Natl. Acad. Sci. USA 103, 626-631.
418 Frostegård, Å., Bååth, E., 1996. The use of phosoholipid fatty acid analysis to estimate
419 bacterial and fungal biomass in soil. Biol. Fertil. soils 22, 59-65.
420 Frostegård, Å., Tunlid, A., Bååth, E., 2011. Use and misuse of PLFA measurements in soils.
421 Soil Biol. Biochem. 43, 161-1625.
422 Gachene, C.K.K., Kimaru, G., 2003. Soil fertility and land productivity. Regional Land
423 Management Unit (RELMA) and Swedish International Development Agency (Sida),
424 Nairobi, Kenya.
425 Griffiths, B.S., Ritz, K., Wheatley, R., Kuan, H.L., Boag, B., Christensen, S., Ekelund, F.,
426 Sørensen, S.J., Muller, S., Bloem, J., 2001. An examination of the biodiversity-ecosystem
427 function relationship in arable soil microbial communities. Soil Biol. Biochem. 33, 1713-
428 1722.
429 Huising, J., Okoth P., 2011. Conservation and sustainable management of belowground
430 biodiversity in Kenyan agroecosystems. Trop. Subtrop. Agroecosyst. Special Issue 13, i-
431 iv .118 pp.

432 Insam, H. ,1997. A new set of substrates proposed for community characterization in
433 environmental samples. In: Insam, H., Rangger, A., (Eds.) Microbial Communities.
434 Springer, Berlin, pp. 259-260.
435 Jasper, D.A., 2007. Beneficial soil microorganisms of the Jarrah forest and their recovery in
436 bauxite mine restoration in Southwestern Australia. Restor. Ecol. 15,74-84.
437 Jose, S., 2009. Agroforestry for ecosystem services and environmental benefits: an overview.
438 Agroforest. Syst. 76, 1-10.
439 Ketterings, Q.M., van Noordwijk, M., Bigham, J.M., 2002. Soil phosphorus availability after
440 slash-and-burn fires of different intensities in rubber agroforests in Sumatra, Indonesia.
441 Agric. Ecosyst. Environ. 92, 37-48.
442 Lacombe, S., Bradley, R., Hamel, C., Beaulieu, C., 2009. Do tree-based intercropping systems
443 increase the diversity and stability of soil microbial communities? Agric. Ecosyst.
444 Environ. 131, 25-31.
445 Matson, P.A., Parton, A.G., Swifth, M.J., 1997. Agricultural intensification and ecosystem
446 properties. Science 277, 504-509.
447 Maundu, P., Tengnäs, B. (Eds.), 2005. Useful trees and shrubs for Kenya. World Agroforestry
448 Centre, Nairobi, Kenya.
449 Mutuo, P.K., Cadisch, G., Albrecht, A., Palm, C.A., Verchot, L., 2005. Potential of
450 agroforestry for carbon sequestration and mitigation of greenhouse gas emissions from
451 soils in tropics. Nutr. Cycl. Agroecosyst. 71, 43-54.
452 Peacock, A.D., Mullen, M.D., Ringelberg, D.B., Tyler, D.D., Hedrick, D.B., Gale, P.M.,
453 White, D.C., 2001. Soil microbial community responses to dairy manure or ammonium
454 nitrate applications. Soil Biol. Biochem. 33, 1011-1019.

455 Preston-Mafham, J., Boddy, L., Randersson, P.F., 2002. Analysis of microbial community
456 functional diversity using sole-carbon-source utilisation profiles- a critique. FEMS
457 Microbiol. Ecol. 42, 1-14.
458 Ros, M., Goberna, M., Pascual, J.A., Klammer, S., Insam, H., 2008. 16S rDNA analysis
459 reveals low microbial diversity in community level physiological profile analysis. J.
460 Microbiol. Meth. 72, 221-226.
461 Smith, J., Pearce, B. D., Wolfe, M.S., 2012. Reconciling productivity with protection of the
462 environment: Is temperate agroforestry the answer? Renew. Agric. Food Syst. 28, 80-92.
463 Sharma, K.L., Ramachandra Raju, K., Das, S.K., Prasad Rao B.R.C., Kulkarni, B.S., Srinivas,
464 K., Kusuma Grace, J., Madhavi, M., Pravin Gajbhiye, N., 2009. Soil fertility and quality
465 assessment under tree-, crop-, and pasture-based land-use systems in a rainfed
466 environment. Commun. Soil Sci. Plan. 40, 1436-1461.
467 Steger, K., Jarvis, Å., Smårs, S., Sundh, I., 2003. Comparison of signature lipid methods to
468 determine microbial community structure in compost. J. Microbiol. Meth. 55, 371-382.
469 Susilo, F.X., Neutel, A.M., van Noordwijk, M., Hairiah, K., Brown, G., Swift, M.J., 2004.
470 Soil biodiversity and food webs in below-ground interactions in tropical agroecosystems.
471 CAB International, Wattingford.
472 Tornquist, C.G., Hons, F.M., Feagley, S.E., Haggar, J., 1999. Agroforestry system effects on
473 soil characteristics of the Sarapiquí region of Costa Rica. Agric. Ecosyst. Environ. 73, 19-
474 28.
475 Tscharntke, T., Clough, Y., Wanger, T.C., Jackson, L., Motzke, I., Perfecto, I., Vandermeer,
476 J., Whitbread, A., 2012. Global food security, biodiversity conservation and the future of
477 agricultural intensification. Biol. Conserve. 151, 53-59.
478 Vincenti, B., Termote, C., Ickowitz, A., Powell, B., Kehlenbeck, K., Hunter, D., 2013. The
479 contribution of forests and trees to sustainable diets. Sustainability 5, 4797-4824.

480 Waldrop, M.P., Firestone, M.K., 2004. Altered utilization patterns of young and old soil C by
481 microorganisms caused by temperature shifts and N addition. Biogeochemistry 67, 235-
482 248.
483 Yan, F., McBratney, A.B., Copeland, L., 2000. Functional substrate biodiversity of cultivated
484 and uncultivated A horizons of vertisols in NW New South Wales. Geoderma 96, 321-
485 343.
486
487

488

489 Text to figures

490

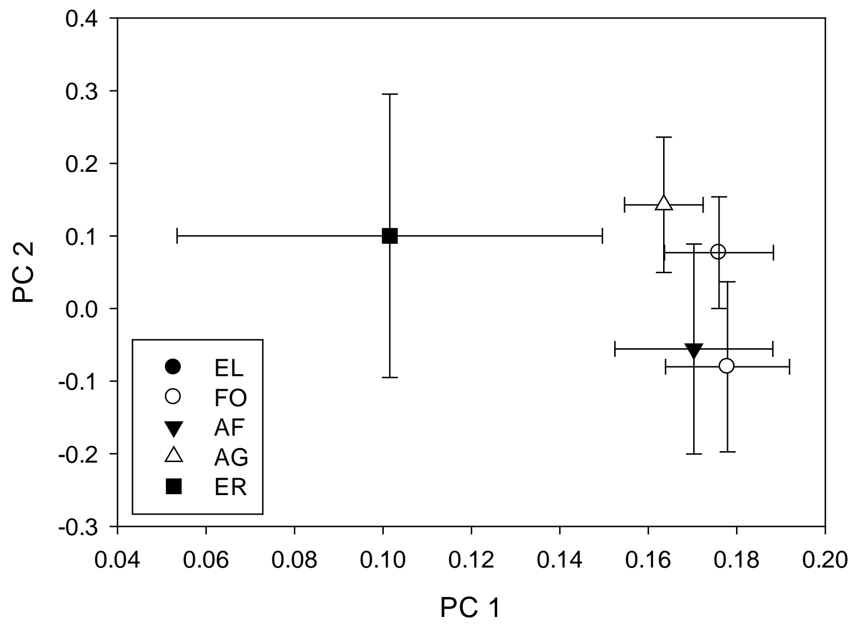
491 **Fig. 1**

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

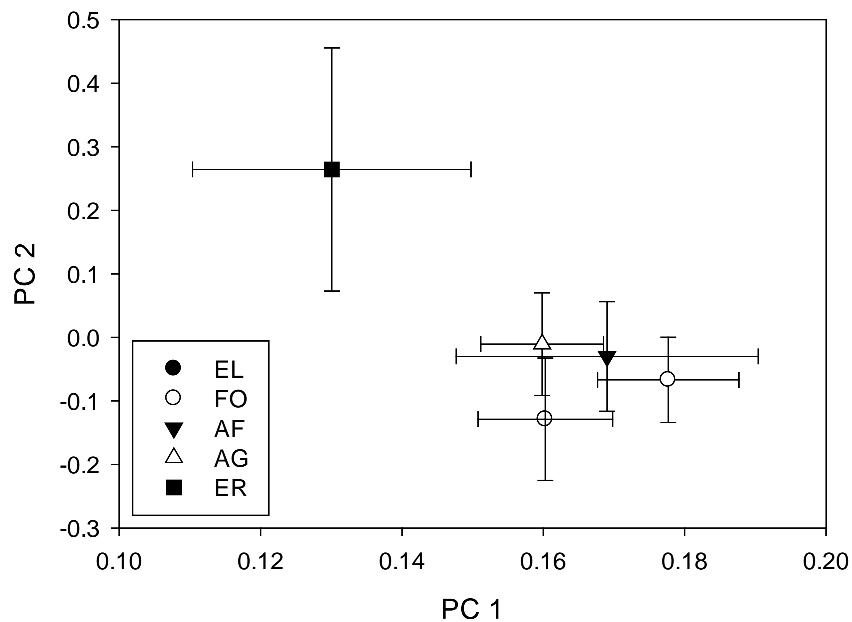
507 five land uses. See Table 1 for explanation of land uses. Error bars are standard deviation; for

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.

518

519

520

521

522

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 (±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_2O)	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 (µg/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*) a	< *0.001*	114 (*34.7*)
AWCD	0.48 (*0.64*) b	0.85 (*0.49*) ab	1.26 (*0.36*) a	1.25 (*0.34*) a	*0.002*	1.23 (*0.29*)
Silt (%)	10.5 (*3.1*) b	18.2 (*9.2*) a	15.6 (*3.3*) ab	12.8 (*2.3*) ab	*0.031*	13.5 (*5.7*)
Sand (%)	50.8 (*9.6*) b	53.1 (*12.2*) b	61.5 (*9.9*) ab	70.8 (*6.3*) a	<*0.001*	78.0 (*9.3*)
Clay (%)	38.5(*9.4*) a	28.7 (*13.7*) ab	22.7 (10.5) bc	16.2 (*5.6*) c	<*0.001*	8.25 (*4.2*)
Moisture (%)	20.0 (*6.4*)	23.8 (*4.3*)	25.8 (*6.6*)	26.5 (*5.2*)	*0.083*	45.3 (*7.8*)

537

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

544

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

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

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

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

	ER	**AG**	**AF**	**FO**	ANOVA	**EL**
Total PLFA						
nmol per g soil	22.6 c	56.5 b	71.7 b	118.7 a	<0.000	281.8
nmol per g soil C	1780.6b	2415.4ab	2101.4b	3125.8a	<0.001	4752.7
Relative abundance of PLFA markers* (% of total PLFA)						
Sum Bacteria	49.5 b	58.2 a	56.6 a	56.7 a	<0.000	59.8
Sum G+ Bact**	19.6 b	26.7 a	25.4 a	25.3 a	<0.001	24.2
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

*: Markers for certain organism groups:

Sum bacteria: i15:0, a15:0, i16:0, 16:1 □7, 16:1 □5, 10Me16:0, i17:0, a17:0, Cy17, 17:0,

18:1 □7, i19:0, 10Me18:0, cy19:0, 19:1

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

Actinobacteria: 10Me18:0

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

Sum fungi: 18:2+18:3, 18:1 □9

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

**: Excluding Actinobacteria

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).

PLFA	Relative abundance – % of total PLFA					
	ER	AG	AF	FO	ANOVA	EL
i14:0	0.30 c	0.47 bc	0.58 ab	0.74 a	<0.000	0.7
14:0	0.91 c	1.00 bc	1.08 ab	1.07 a	<0.000	1.22
i15:0	6.32 b	9.63 a	9.27 a	9.47 a	<0.000	8.66
a15:0	3.63 b	4.88 a	4.86 a	5.20 a	<0.000	4.88
15:0	0.56	0.43	0.58	0.47	0.74	0.71
br16a	0.31 b	0.48 a	0.51 a	0.59 a	<0.000	0.73
br16b	0.032 b	0.21 a	0.24 a	0.23 a	<0.000	0.19
i16:0	5.19	4.78	4.45	4.47	0.264	5.21
16:1ω9	0.32 b	0.70 a	0.80 a	0.72 a	<0.000	0.47
16:1ω7	3.88 b	4.87 ab	5.17 a	5.43 a	<0.001	6.045
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: 1ω9	5.2	4.77	5.03	5.02	0.508	5.33
601	18: 1ω7	4.34 c	5.32 bc	6.24 ab	7.86 a	0	9.78
602	18: 1ω6	0.28	0.42	0.37	0.28	0.577	0.2
603	18: 1ω5	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	**Sum PLFA**	*100*	*100*	*100*	*100*		*100*

616

617