Examensarbete
Institutionen för ekologi

Effects of land use on soil microbial community function in western Kenya highlands

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MAGISTERUPPSATS I BIOLOGI, D-NIVÅ, 30 HP

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Examensarbete 2008:6
Uppsala 2008

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Land degradation, agricultural intensification and deforestation may result in a loss of soil microbial community function, detrimental to resilience and to sustained productivity. In order to assess the effects of land use on microbial community function in a tropical soil the following hypotheses were considered: (1) functional capacity measured as substrate-utilization potential decreases with intensification of land use and (2) tree planting - i.e. as used in agroforestry - is a soil conservation and improvement measure which can restore physical, chemical and biological properties of degraded and overused soils.

Samples from protected indigenous forest, forest plantations, agroforestry fields, conventional maize fields and eroded soil were taken on the slopes of Mount Elgon in the Rift Valley province of western Kenya. The agroforestry fields were earlier open agricultural fields. The microbial functional capacity was measured as substrate utilization of soil bacteria studied by using Biolog Ecoplates. Chemical and biological soil properties including pH, extractable P, total N, organic C, nitrate and microbial biomass C and N were also determined.

The results follow a trend with eroded land and conventional maize fields on the low end of the scale and agroforestry, forest plantations and natural forest on the high end regarding pH values, total soil N and C concentration, microbial biomass C (MBC) and microbial biomass N (MCN). Extractable P shows higher levels in agroforestry fields than in indigenous forest, probably due to fertilization. Average well colour development (AWCD) in Biolog Ecoplates shows overall substrate consumption to be higher in land uses with higher tree cover and it is positively correlated with soil properties including pH, total N and C %, moisture, MBC and MBN. Significant differences were found between substrate utilization profiles in the various land uses - as observed in the PCA - indicating the existence of functionally different microbial communities. Additionally, more disparate catabolic responses appear between the samples of less conserved soils, which suggest a loss of functional stability.

Results suggest that microbial functional capacity varies according to the land use and may be restored by increased tree cover and active soil management practices such as agroforestry.

Keywords: soil microbial community function, land use, agroforestry, Biolog Ecoplates, Kenya

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Acknowledgements

For the technical, scientific, logistic and moral support sincere thanks to TSBF-CIAT (Tropical Soil Biology and Fertility - Centro Internacional de Agricultura Tropical), CGIAR (Consultative Group on International Agricultural Research), Moi University (Eldoret), KWS (Kenya Wildlife Service), NMK (National Museums Kenya) - Nairobi and Kitale Museums, ICRAF Headquarters-Nairobi (World Agroforestry Centre), NEMA (National Environmental Management Agency of Kenya) and KARI (Kenya Agricultural Research Institute). To Jan Lagerlöf and to SLU (Sveriges Lantbruksuniversitet), Uppsala, Sweden - all my gratitude for guidance and financial support.

For their cooperation and hospitality, warm thanks to the farmers at Endebess Division; for tireless encouragement and for sharing their expertise thanks to Björn Horváth, Oparah, Okach and All at Vi-AFP in Kitale as well as Matumbei, Mubere and Endebess Divisions; In appreciation to their guidance and support thanks to Marta, Pieter, Didier Lesueur, Chin Ong, Robin, Joan, Lourdes, Sarah.

‘Shukurani zetu pokeeni na dua njema tunawaombea’

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1. Introduction

The effects of different land uses on the biological properties of soils have been considerably investigated, though little is known about the effects on soil microbial functional capacity (Degens and Vojvodic-Vukovic, 1999). This capacity, which has received little attention (Zac et al., 1994; Giller et al., 1997), is based on heterotrophic decomposition functions. This aspect is particularly important because it is likely to be more relevant to the understanding of the role of microorganisms in the functioning of the soil ecosystem than species diversity (Zac et al., 1994; Giller et al., 1997). In any case, links between diversity and function are clearer for functions that are relatively specific, such as decomposition or antibiosis (Giller et al., 2005).

While functional diversity may not be readily interpreted from species diversity in soil microbial communities (Zac et al., 1994), heterotrophic functional diversity in soils can be easily assessed, helping to differentiate microbial communities. Moreover, microorganisms are present in virtually all environments and are typically the first organisms to react to chemical and physical changes in the environment. Because they are at the bottom of the food chain, changes in microbial communities are often a precursor to changes in the health and viability of the environment as a whole. Thus, a question of great concern is whether and how microbial functional capacity changes depending on the land use as well as the reversibility of such changes in soil function, in both natural and productive soils.

Several studies have reported the composition of microbial communities to differ with changing vegetation, land use and management (Bardgett et al., 1996; Bardgett and McAlister, 1999; Buyer and Drinkwater., 1997; Klein et al., 1995; Shutter and Dick, 2001), possibly as a result of differences in the amount and chemical nature of soil organic matter. Land degradation and agricultural intensification have been hypothesised to result in a reduction in soil biodiversity leading to a loss of function detrimental to resilience, and to sustained productivity (Giller et al., 2005). Land uses have been suggested to exhibit patterns of substrate-utilisation potential, which are generalised and independent of the soil type. This is a potentially useful characteristic for assessing whether soils are under adverse pressure from a land use by comparing similar land uses on different soil types (Degens and Vojvodic-Vukovic, 1999). Landscape level patterns of microbial community composition and function have also been suggested in relation to plant community composition (Myers et al., 2001). Moreover, inputs of litter and green manure -i.e. organic matter- have been noted to increase the size and activity of soil microbial communities (Bolton et al., 1985; Fauci and Dick, 1994; Kautz et al. 2004; Kirchner et al., 1993; Martens et al., 1992; Manici et al., 2004) as well as its diversity (Sesstisch et al., 2001) and influencing microbial carbon source utilization profiles (Buyer and Drinkwater, 1997; Lupwayi et al., 1998; Shutter and Dick, 2001). However, other studies have failed to detect any community changes in response to different organic matter input (Wander et al., 1995).

Contrasting results have thus been obtained, but it appears that land use might alter soil microbial communities' composition, size and activity though the impact on soil microbial community function is less well understood. Additionally, knowledge about the functional significance of soil biodiversity has been strongly influenced by emphasis on temperate climates and high-input, intensive agriculture rather than low-input systems (Garrity et al., 2006). Further research is also required on the possibility to reverse possible alterations on soil function through active soil management techniques such as tree planting.

These issues were addressed in the present study focused on the Kenyan highlands, considered to be the food basket of a country where 80% of the land is semi-arid to arid, unsuitable to agriculture. High rural population growth – from 8 to 40 million inhabitants since 1964 - coupled with stagnating urban job growth has accelerated the search for new agricultural land, resulting in a high rate of woodland, forest, grassland and wetland conversion for agricultural use. Deforestation, agricultural use of slopes, poor land management practices and overgrazing have significantly degraded most soils in the highlands, and afforestation has been encouraged since the 80's to control severe, expanding erosion and loss of both fertility and above and below-ground biodiversity. Western Kenya has one of the densest and poorest populations, with up to 1200 persons per km^2 in some rural areas and over 58 per cent of households living below the poverty line -less than 1 US$ per day. At the same time it has unique habitats and biodiversity of local, national and global significance (Tarquis, 2006).

In order to assess the effects of land use on soil biological properties, more specifically the effects of vegetation cover on the functional capacity of microbial communities in tropical soils, the following hypotheses were considered : (1) functional capacity measured as substrate-utilization potential decreases with intensification of land use; (2) land uses with higher vegetation cover -with emphasis on indigenous trees- have physical, chemical and biological properties more suited to supporting below and above-ground life; (3) tree planting is a soil conservation and improvement measure which can also restore to some degree physical, chemical and biological properties of degraded and over-exploited soils.

To test the hypotheses, 40 soil samples were taken in Trans Nzoia District, western Kenya, that would represent five land uses with a gradient of tree cover: protected indigenous forest, in Mt. Elgon National park; woodlots, with trees of interest in agroforestry; farmlands with agroforestry*, implementing alley cropping or growth of crops between rows of trees and application of trees' leafy biomass to crops; conventional maize farming; and eroded lands, completely barren and free of vegetation.

The analysis of the effects of vegetation cover linked to land use was done by investigating the impact on (1) soil physical and chemical properties; (2) microbial

* Agroforestry, is a collective name for land-use systems and technologies, where woody perennials (trees, shrubs, bamboos, etc.) are deliberately used on the same land management unit as agricultural crops and/or animals, either in some form of spatial arrangement or temporal sequence. It is said to have ecological integrity when the habitat structure, natural functions and species composition of the system are interacting in ways that ensure its sustainability in the face of changing environmental conditions as well as both internal and external stresses (ICRAF, 1993).

biomass and (3) soil microbial community function through microbial substrate-utilization potential as determined with the Biolog EcoPlate method. This is a biochemical, physiological profiling method created specifically for community analysis and microbial ecological studies. Such an approach has appeared to be effective at distinguishing spatial and temporal changes in microbial communities, as well as changes based upon the variable introduced, for instance land use.

Research on land use history and inventory of woody species was also carried out in the sampled plots, as described in 'materials and methods' below.

2. Materials and Methods

2.1. Site description and experimental design

The study focused on the area of Mount Elgon, in the Endebess Division of Trans Nzoia district, Rift-Valley province, in western Kenya (1°00′N, 38°00′E). More specifically in the focal areas of Endebess, Matumbei and Mubere (See *Fig. A.* for details). The district has a highland equatorial climate, with average annual precipitation of 1296 mm fairly well distributed throughout the year. To the west, the slopes of Mt. Elgon- the second highest mountain in Kenya with its 4313 m of altitude above sea level- receive the highest amount of rainfall. The Endebess-Kitale plain, at its foot, covers 50% of the district located between 1800-2000 m above sea level. The district has a mean temperature of 18.6 ºC and experiences bimodal rainfall patters, with long rains from April to July and short ones from August to November. Trans Nzoia lies in a basement system with mainly sedimentary rocks or clay stones, as well as andosols and nitosols soil types (Horváth, 2006).

The district, traditionally considered to be the food basket of the country, has a major maize production seconded by beans. Extensive, tree-free monocultures were established during colonial times and agroforestry, woodlots included, has mainly been promoted during the last two decades, following independence in 1964 and a sharp population increase. Naturally forested lands are hardly found out of protected areas. (Horváth, 2006)

As for Mt. Elgon, it is an ancient eroded volcano lying 140 km north east of Lake Victoria and is bisected by the Kenya-Uganda border. Mt Elgon National Park was created in 1968 and covers a narrow transect of 169 km^2 up the North Eastern slopes of the mountain, from lower mountain forest to the caldera edge. The remaining forest and moorland is part of the Mt Elgon Forest Reserve, and the Ugandan side of the mountain is protected within Uganda's Mt Elgon National Park. The mountain is an important water catchment for the Nzoia River, which flows into Lake Victoria and for the Turkwel River, which flows into Lake Turkana (Kenya Wildlife Service, 2007).

The study is concerned with the effect of various land use types –protected indigenous forest, woodlots, traditional maize farmland, farmland with agroforestry, eroded land - on soil physical and chemical properties as well as the functional diversity of microbial communities. To that end, four replicates of the protected natural forest land use in Mt. Elgon National Park were sampled. Nine replicates of the four other land uses were sampled; from Kitale plain to the slopes of Mt. Elgon. The total number

of samples considering all three locations (Mt Elgon National Park, Mt Elgon slopes and Kitale plain) adds up to 40. (See *Figures A and B* below for more details).

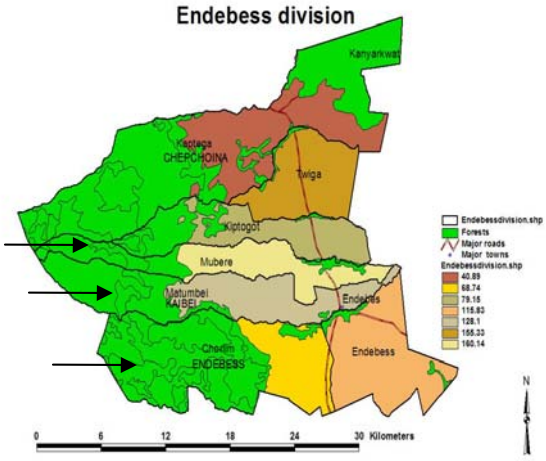

Fig. *A*. Study site

The study focused on the area of Mount Elgon, in the Endebess Division of Trans Nzoia district, Rift-Valley province, in western Kenya (1°00′N, 38°00′E), specifically in the focal areas of Endebess, Matumbei and Mubere (marked by arrows in lower right map).

1

2

3

4

5

Fig. *B*. Land use types
Soil samples were taken in the Trans Nzoia District from 5 land use types along a gradient of woody species cover: protected indigenous forest (1), in Mt. Elgon National park; woodlots with trees of interest in agroforestry (2); farmlands with agroforestry (3), implementing alley cropping or growth of crops between rows of trees and application of trees' leafy biomass to crops; conventional maize farming (4); and eroded lands (5) – mainly gullies - barren and free of vegetation.

2.2. Sampling and analysis of soil

Sampling of soil was carried out twice in March 2007, before sowing of maize and in a period without any rains. A minimum of five soil cores were taken with an auger from each replicate of the different land uses to a depth of 25 cm, and then combined to a composite sample for each replicate. After sampling, the soil was mixed and either air dried or kept fresh at 6ºC prior to transportation to the laboratory according to the requirements of different tests. Analyses of fresh material were done within a maximum of 48 h after collection, and tests were carried out both at MOI University in Eldoret and at TSBF-CIAT (Tropical Soil Biology and Fertility - Centro Internacional de Agricultura Tropical) facilities in ICRAF (World Agroforestry Centre) compound, Nairobi.

Soil moisture (%) was determined by oven-drying the soil at 105ºC for 24 h, and soil pH was determined in water suspensions at a soil/water ratio of 1:2.5. Soil particle size was established by the hydrometer method. Soil total concentrations of N and P as well as that of extractable nitrates were determined on fresh soil samples by colorimetric methods, which are fast and reproducible as well as low-cost ones. The Olsen method involving extraction with 0.5 M solution of sodium bicarbonate at pH 8.5 was used for determination of extractable P, and flame photometry was used in the analyses of extractable K. Organic carbon content was determined by a sulphuric acid and aqueous potassium dichromate mixture (Anderson and Ingram, 1993).

Microbial biomass C (MBC) and N (MBN) was determined by the chloroform fumigation-extraction method involving the extraction of chloroform-fumigated and non-fumigated samples with 0.5 M K2SO4 followed by determination of organic C and N in the extracts (Vance et al., 1987) [35]. The fumigated soil sample is extracted in the same fashion as the non-fumigated sample, and MBC and MBN are calculated as the difference in concentrations of C and N between extracts of fumigated and non-fumigated soil.

Additionally, I made an inventory of the woody species in the sampled plots, and the altitude and geographical coordinates of each sampling location was recorded with a GPS-unit. To get an insight into land use history, farmers were questioned on their background; land area, ownership status and use previous to current farmers' settlement; agricultural practices and use of pesticides and fertilizers; crops currently planted or last planted; first year of implementation of agroforestry, inventory of tree species and their use and observed improvement in yields/fertility following tree planting; constraints; firewood collection related issues and livestock tenure.

2.3. Substrate utilization potential

Potential substrate utilization was determined by using the Biolog EcoPlate (Biolog Inc., Hayward, CA) after the method described by Schutter and Dick (2001). This method is based on plates containing three repetitions of 31 of the most useful carbon sources for soil community analysis, plus three water controls. A mixed culture of microorganisms is inoculated and formation of colour purple occurs when microbes can utilize the carbon source and begin to respire. The respiration of cells in the community reduces a tetrazolium dye that is included with the carbon source. By following the colour change and so measuring the community metabolic fingerprint

over time, characteristics about that community can be ascertained (Garland and Mills, 1991). Despite its limitations as a cultural method it is fast, relatively inexpensive and highly reproducible. It must be taken into account that fungi, a major component in forest ecosystems, are not capable of using the tetrazolium dye incorporated in the wells for colour development resulting from substrate consumption (Grayston et al., 1994). Thus, they don't contribute to the substrate utilization potential measured by this method.

As for the procedure, soil equivalent to 10 g dry soil, was suspended in 90 ml of sterile 0.145 M NaCl and homogenized in a blender at high speed for 1 min. The solution was serially diluted to 10^{-3}, and 125 ml of the 10^{-3} dilution was inoculated into each well of the plate. Plates were incubated for 96 h at 25ºC, and well absorbance was measured at 590 nm using a plate reader every 24 h. All solutions and equipment were sterilised by autoclaving prior to use.

The absorbance values at the start of the incubation were subtracted from the absorbance values of the subsequent readings. In addition, the colour development of the control well at each reading was subtracted from the other wells to correct for any respiratory activity due to carbon added with the inoculum. Negative values were set to zero. The average well-colour development (AWCD) for each land use, i.e. the mean absorbance values of all 31 substrates, and for different substrate categories (carbohydrates, carboxylic acids, amines/amides, miscellaneous, amino acids and polymers) were calculated for every reading. The higher the AWCD, the more substrates can be used by a certain microbial community and the higher is its functional substrate-utilisation potential. The substrate-utilization profile was analysed on well-absorbance values at the last reading.

2.4. Statistical analyses

The significance of differences between soil physical-chemical properties and microbial biomass data means in relation to land use was analysed by means of ANOVA. Tukey's HSD test was used to compare land use means, considered to be significantly different at p-levels <0.05. Means and standard deviations given in tables and figures are untransformed data.

Biolog data was analysed with non parametric tests. Kruskal-Wallis test was used in the first place to assess the significance of the differences between AWCD means for the various land uses. Paired comparisons between AWCD for the different land uses at the last reading (96 h) were then done by means of a Mann-Whitney-Wilcoxon's test, using Bonferroni's correction. ANOVA was not made because of lack of normality of some data and the size of the sample.

A principal component analysis (PCA) was performed on normalized Biolog absorbance values considering the 31 different substrates as variables, and the plates as observations. The normalization of the Biolog values was done by dividing the absorbance values for individual wells by the AWCD for the whole plate, in order to account for differences in inoculum density as suggested by Garland and Mills (1991).

Land use effects on substrate-utilization profiles were assessed on the scores for principal component (PC) 1 and 2 in the same way as AWCD. A matrix correlating PC

1 and 2 as well as AWCD at the last reading with physical and chemical properties of the sampled soils was also produced by means of Pearson correlation coefficients. All statistical analyses were performed using SPSS for Windows version14.0.2 (SPSS Inc. Chicago- EUA).

3. Results

3.1. Land use history

The agroforesty farmers on whose plots soil was sampled are mostly aged 30-50, have a mean number of eight children, the husband as the household head and implement agroforestry techniques with help of an international NGO[†]. They cultivate on average 0.2-1 acre (1 acre = 4047 m^2) of land with an equal number of agroforestry farmers owning the land and having a share of communally owned one. Only a minority were squatters, i.e. occupying non-owned land. Half of the farmers have laboured that land for the last 20 to 30 years, and the other half has been cultivating it for the last 5-15 years, with an observed evolution in land tenure status with time. Squatters were more common some years ago whereas owners with title deeds have become more common recently.

Before current settlers started cultivating it, the land had usually been used for agricultural purposes, with the main production being of maize, beans, onions, sweet potatoes, bananas and, more rarely, coffee. In a third of the current farms it had been rangeland or fallow. Agricultural practices common in all of the sampled farms are crop rotation, intercropping and hand or oxen ploughing usually twice a year. In at least half of the farms heaping and burying of weedy material as well as establishment of trash lines takes place regularly as a way to increase the amount of organic matter in the soil and for erosion control. Short fallows take place in a third of the plots, while irrigation and burning of crop residues and weedy materials is rare. More that half of the farms use organic fertilizers such as manure – a few animals on free range are usually kept on farm. Compost and mulch is complemented to a lesser degree with inorganic fertilizers. Pesticides are used in all but one of the cases, though in an irregular way. The most widespread crops and vegetables include beans, maize, sweet and Irish potatoes, tomatoes, bananas and cassava, while other rather common ones are onions, cowpeas, cabbages and local green leaved vegetables.

Agroforestry was implemented zero to four years after the title deed had been obtained in the case of individual owners, and 13-33 years after settlement in the case of squatters and land share owners. Thus, agroforestry was first implemented 11-15 years ago in four of the plots, and one to seven years ago in the rest, in a region where pests, diseases, weeds, erosion, water logging, destructive wind and loss of fertility are among the main constraints to agriculture. All but two of the farmers, those implementing

[†] The Vi Agroforestry programme (ViAFP) is funded by the Swedish NGO Vi Tree Planting Foundation. ViAFP implements agroforestry practices since 1983 by creating a green belt around LakeVictoria aiming to 'promote an ecologically sustainable environment; economical growth and reduction of poverty among more than 190.000 smallholder families, through sustainable management of natural resources and business development'. Its immediate objectives include increased firewood, fodder and nutritional security at household level by 2008 (Horváth, 2006).

agroforestry for the first time in 2004 and 2006, had observed improvements in yields and fertility after introduction of agroforestry.

A mean number of eight different tree species, mostly indigenous, have been planted in the sampled plots. Nearly all farms include *Sesbania sesban, Grevillea robusta, Cordia africana and Calliandra calothyrsus*, which are mainly used for mulch, soil conservation/improvement, windbreak, nitrogen fixation (ie. *Calliandra sp*.) and other purposes such as fodder, beehives and bee forage, firewood – collecting it on farm avoids collecting it inside Mt Elgon National Park and Reserve -, charcoal, timber, construction, fencing and medicine. Production of avocados (*Persea americana*), passion fruit (*Passiflora edulis*), bananas (*Musa sp*.), guava (*Psidium guajava*) and papaya (*Carica papaya*) is also common.

Conventional, usually bigger scale maize monocultures don't integrate trees neither in space nor in time, and have the burning of crop residues on the field and the use of inorganic fertilizers as a more common practice. Use of herbicides and pesticides may take place, but in a rather irregular, uncontrolled manner. Woodlots are usually small, field-surrounded tree stands out of protected areas and have a mean number of 18 tree species. Most of the tree species found in woodlots are also found in Mt Elgon National Park and/or in agroforestry farms. Woodlots covering less that 4 acres are mostly owned by individuals and were first established 5 to 11 years ago, one of them even 24 years ago. No use of pesticides or fertilizers, either organic or inorganic, is made. Trees are periodically harvested.

As for Mt. Elgon National Park, - i.e. protected indigenous forest - the diversity of tree species decreases with altitude, from a mean ten species in the lower areas to four in higher ones. Some of the species, which may also be used in agroforestry are: *Podocarpus falcatus, Teclea vespris* and *T. nobilis, Olea africana* and *O. capensis* (windbreak), *Croton macrostachyus* (soil conservation and improvement), *Albizia spp.* (nitrogen fixation), *Bersana abyssinica* and *Vangueria madagascariensis*.

3.2. Soil characteristics and nutritional status

The pH values were medium – acid to neutral - and showed only slight variation between the land uses. The lowest values were found in conventional maize farmland (Table 1). A decreasing tendency for total soil N and C concentration, microbial biomass C (MBC), microbial biomass N (MCN) and moisture was found in the following order of land uses: protected indigenous forest, woodlots, farmland with agroforestry, conventional maize farmland and eroded land. An increasing tendency for for MBC/MBN and C/N was found in the stated order of land uses. The C/N ratio, though, had higher values for woodlots than it did for conventional maize farmland.

Significant differences were found between protected forest, agroforestry/woodlot and eroded land for both total N and C concentrations, and between protected forest, woodlot and eroded land or eroded land/conventional maize farmland for MBC and MBN respectively. The MBC/MBN ratio differed significantly between protected forest, agroforestry and eroded land. Moisture in the protected forest land use nearly doubled compared to the other land uses. Nitrates (ppm) and available P (ppm) had the highest values for conventional maize farmland and for agroforestry respectively, though significant differences were only found between agroforestry and eroded/natural forest land uses in the case of P (ppm). Total P concentrations (Pt) were

highest in conventional maize farmland whereas no significant differences were observed for total K concentrations between the various land uses.

As for soil texture, increasing % of clay and decreasing % of sand was found in the following order of land uses: protected indigenous forest, woodlots, farmland with agroforestry, conventional maize farmland and eroded land. Soil texture ranges from loamy sand (mainly found in the protected forest), to sandy clay (the main texture in the eroded land), through sandy clay/ loam/ clay-loam.

Table 1. Concentration (% of dry weight) of total soil carbon (Ct), nitrogen (Nt), C/N ratio, microbial biomass C (MBC: mgC/kg) and biomass N (MBN: mgN/kg), MBC/MBN ratio, total soil K (Kt: %), P (Pt: %), available soil P (ppm), nitrates (ppm), pH, moisture (%) and sand-clay-silt content (%) of the different land uses in March 2007.

Land use	**Ct** (%)		**Nt (%)**		**C/N ratio**		**MBC** (mgC/kg)		**MBN** (mgC/kg)		**MBC/MBN**	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Protected forest	5.93c	1.02	1.06c	.49	6.18a	2.93	655.55 d	104.16	113.9 c	34.7	6.03a	1.30
Woodlots	3.79b	1.50	.62b	.27	7.77a	5.44	282.81 c	51.60	34.58 b	11.6	8.67a	2.05
farmland with agroforestry	3.40b	.28	.46b	.26	8.18a	2.91	211.90 bc	32.54	19.61 ab	9.93	12.83 ab	4.78
Conventional maize farmland	2.34ab	.60	.36ab	.08	6.50a	.98	172.58 ab	34.17	13.03 a	4.48	14.20b	3.48
Eroded land	1.26a	2.40	.09a	.06	16.43b	8.25	118.73 a	30.30	6.55 a	2.79	19.84 bc	5.47

Land use	**Kt** (%)		**Pt** (%)		**P** (ppm)		**Nitrates** (ppm)		**pH**		**Moisture (%)**	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Protected forest	.17a	.06	.03a	.02	14.13a	9.72	7.59a	5.03	6.72ab	.34	45.31b	7.84
Woodlots	.31a	.14	.13b	.03	34.65 ab	40.3	4.89a	3.13	6.78b	.46	26.49a	5.21
farmland with Agroforestry	.33a	.24	.11ab	.05	60.81b	45.2	6.34a	4.51	6.53ab	.50	25.85a	6.67
Conventional maize farmland	.36a	.14	.13b	.06	19.61 ab	10.9	9.14a	3.69	6.04a	.38	23.86a	4.36
Eroded land	.29a	.13	.11ab	.05	8.53a	10.8	8.92a	5.66	6.20ab	.55	20.03a	6.44

Land use	**Sand (%)**		**Clay (%)**		**Silt (%)**	
	Mean	SD	Mean	SD	Mean	SD
Protected forest	78.00c	9.27	8.25a	4.19	13.50a	5.74
Woodlots	70.88 bc	6.25	16.22 ab	5.60	12.88a	2.26
farmland with agroforestry	61.55 ab	9.95	22.77 ab	10.5 6	15.66a	3.31
Conventional maize farmland	53.11a	12.2	28.66 bc	13.6 8	18.22a	9.24
Eroded land	50.88a	9.59	38.55 c	9.42	10.55a	3.12

Means of the replicates for each land use and standard deviations (S.D.) are presented. n = 4 (protected forest), n = 9 (other land uses).
Different letters in the same column indicate significant differences at $P < 0.05$ (Tukey's test).

3.3. Substrate utilization potential

Total average well colour development - AWCD (Absorbance Units) - increased with time and showed decreased values in conventional maize farmland and eroded land as compared to indigenous forest, farmland with agroforestry and woodlots (Figure 1). Significant differences ($P< 0.05$) in total AWCD at the last reading (96h) were found between the uses protected forest and eroded land; woodlot vs. conventional maize farmland and eroded land; farmland with agroforestry vs. conventional maize farmland and eroded; and conventional maize farmland vs. eroded land.

AWCD for the different substrate categories was significantly lower for eroded land and intermediate for conventional maize farmland. The rest of the land uses had higher values for all the substrate categories (Figure 2). Substrate consumption took place in the following decreasing order: carbohydrates, amino acids, amines/amides, polymers, carboxylic acids and miscellaneous. Woodlots, protected forests and farmlands with agroforestry had, for instance, the highest AWCD for carbohydrates and also for amino acids at the last reading. Most importantly, maize farmland and eroded land had distinctively lower AWCD values for all substrate categories – eroded land always had the lowest - and they appeared to clearly differ from one another as well as from the three other land uses in their response to substrates.

The PCA of the absorbance values of the 31 carbon sources indicated significant differences ($P < 0.05$) for the first component between the various land uses, though the size of the sample was not big enough to allow for significance in pairwise statistical comparisons. The first two components explained 34.23% of the variation (18.85% PC1, 15.38% PC2) and the third one explained 11.62%. The PC1 vs. PC2 plot showed negative and close to zero values for the first component in the eroded land use (Figure 3), while the conventional maize farmland land use had rather negative values for the second principal component.

Total AWCD for the last reading was correlated with soil properties including pH, total N and C, % of sand, clay and moisture, MBC and N, MBC/MBN ratio and C/N. The correlation was negative for Clay (%), MBC/MBN and C/N. The first principal component was correlated with soil properties such as Nt, Ct, % of sand and clay, MBC, MBC/MBN and C/N. The correlation was negative when it came to clay (%), MBC/MBN and C/N. The second component was positively correlated with Moisture (%) and negatively with MBC/MBN ratio (Table 2). Thus, the substrates represented by PC1 and PC2 show linear relationships with the physical, chemical and biological soil properties named above, primarily with Ct and C/N in the case PC1 and moisture and MBC/MCN for PC2.

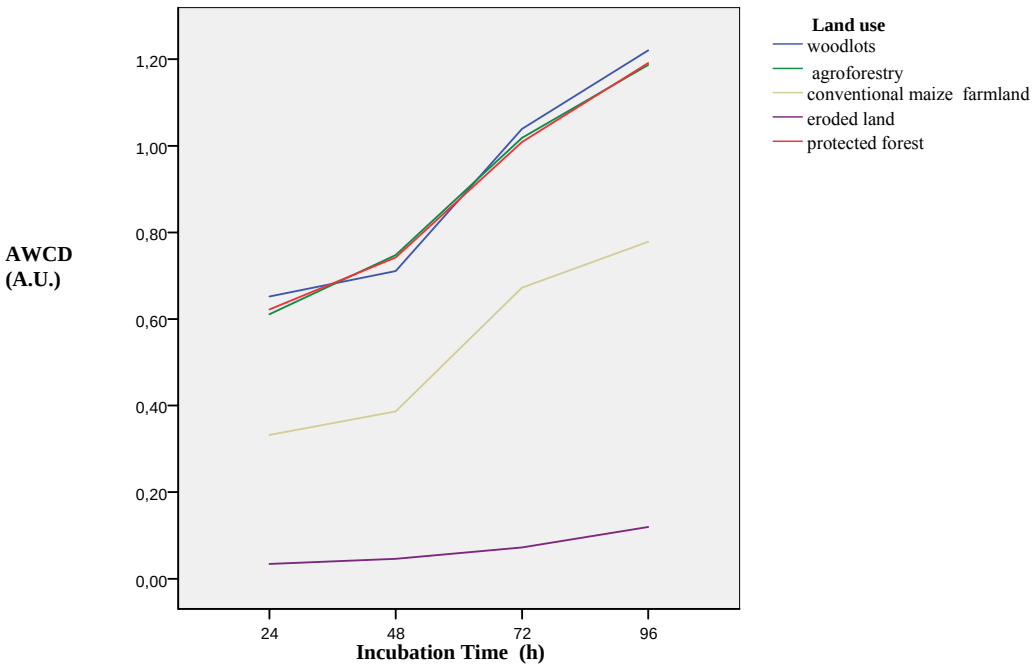

Figure 1. Average well colour development –AWCD (Absorbance Units)- of the respective land uses. Means for each land use are shown. Absorbance read at 590 nm.

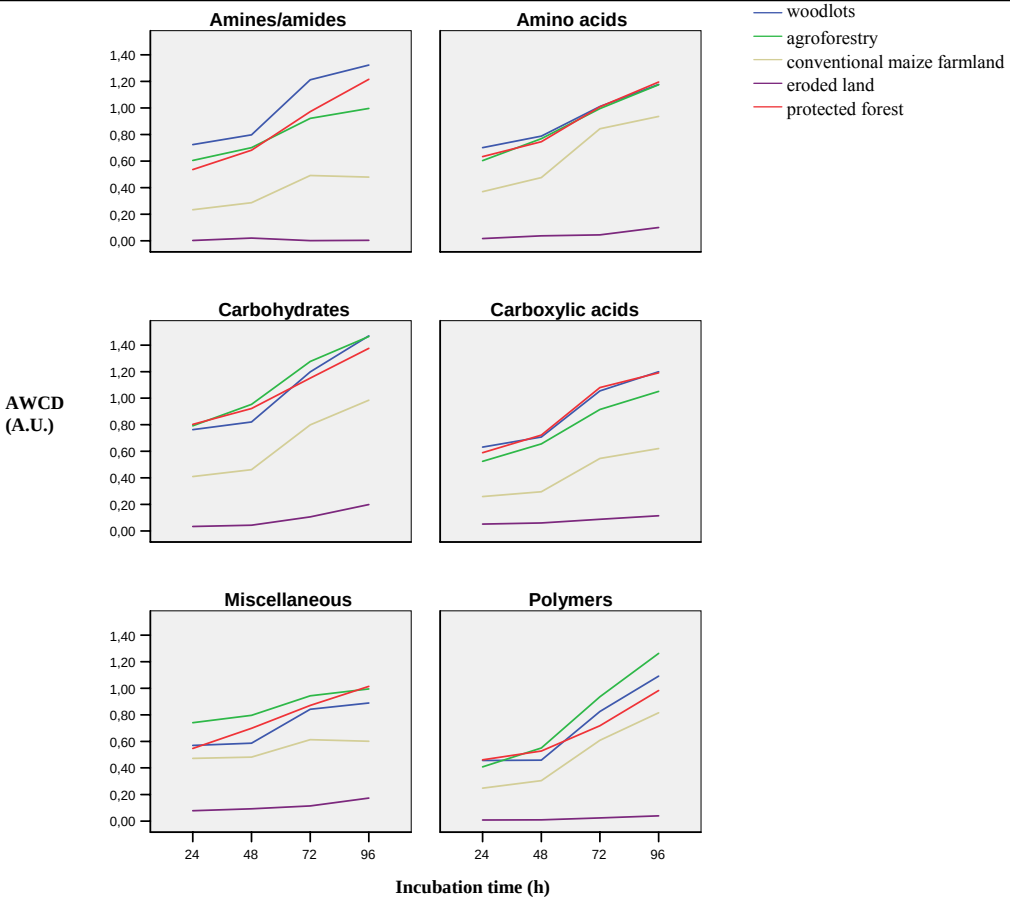

Figure 2. AWCD (A.U.) of the respective land uses for each substrate category. Means of the replicates for each land use. Absorbance values read at 590 nm.

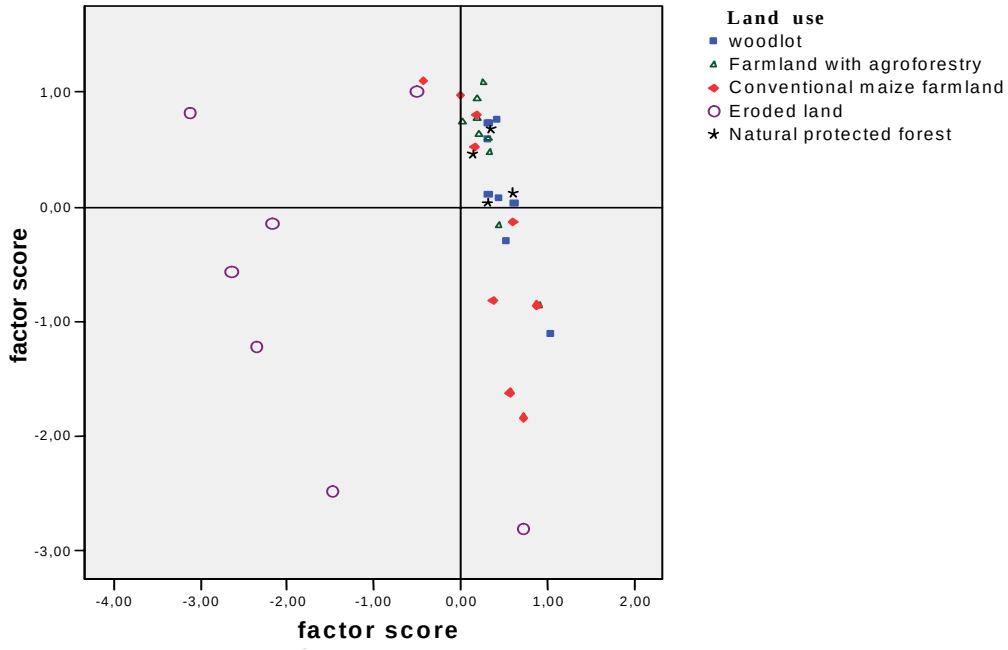

Fig.3. PCA scores for substrate utilization potential in the respective land uses. PCA was performed on normalized absorbance values read at 590 nm after 96 h of incubation. PC1 explains 18.85% of the variation while PC2 explains 15.38% of it.

Table 2
Correlation matrix of soil physical, chemical and biological properties. Pearson r-values calculated from the replicates of each land use.

	AWCD (A.U.)(96 h)	**factor score 1**	**factor score 2**
pH	.322(*)	.233	.107
Nt	.537(**)	.464(**)	.110
Kt	-.081	-.085	-.008
P (ppm)	.308	.277	.056
Pt	-.162	-.095	-.085
Nitrates (ppm)	-.153	.084	-.170
Ct	.608(**)	.466(**)	.254
Sand (%)	.638(**)	.462(**)	.153
Clay (%)	-.640(**)	-.559(**)	-.099
Silt (%)	.025	.218	-.125
Moisture (%)	.457(**)	.121	.398(*)
MBC (mg C/kg)	.497(**)	.325(*)	.229
MBN (mg N/kg)	.471(**)	.241	.253
MBC/MBN	-.738(**)	-.524(**)	-.466(**)
C/N	-.392(*)	-.609(**)	.188

** significant correlation at 0,01 level.
* significant correlation at 0,05 level.
CD: Average well colour development. Biolog substrate utilization potential after 96 h of incubation.

4. Discussion and Conclusions

The results suggest the existence of links between land use, microbial biomass and microbial catabolic activity in agreement with other studies (Archiori and De Melo, 2000; Bossio et al., 2005). The selected land uses showed a gradient in vegetation cover with an emphasis on trees, thought to be an important soil conservation and improvement measure. In effect, a higher vegetation cover generally increased the soil microbial biomass, and microbial biomass C and total soil C concentration were strongly correlated. The latter serves as an energy supply for heterotrophic soil microorganisms and acts as an indicator of the organic matter content of the soil, which was expected to be higher in more forested lands.

Total soil N is mainly organic and is in turn correlated with total soil carbon. Although C/N ratio tended to higher values in farmlands with agroforestry compared to those with conventional maize plantations, the opposite happened for MBC and MBN, suggesting more accessible C and N sources in agroforestry farmlands. Not only was the nutritional status generally more suited to support both microbial and plant communities in less depleted, more vegetation-covered soils, but the moisture and texture were also better. Soils in Endebess division typically have an upper sandy layer with a relatively thick clay layer underneath (Horváth, 2006). Consequently, more eroded soils had higher proportions of clay. Clay is known to complex and immobilize carbon, making it unavailable to organisms. Higher moisture obviously favours microbiological activity facilitating organic matter decomposition and liberation of available nutrients to the biota (Table 1).

As for average well colour development, it showed overall substrate consumption to be higher in land uses with higher – woody - vegetation cover (Fig.1), which were the ones to have better nutrient status, texture and moisture and so higher microbial biomass (Table 1, 2). Consumption patterns and dynamics for each substrate category also varied depending on the land use (Figure 2), with only slight differences among the three land uses including trees but pronounced ones between them and both conventional maize fields and eroded lands. These reaction patterns or metabolic fingerprints are characteristic to each community of microorganisms and show a functional capacity, which is linked to changes in the environment.

Significant differences were found between substrate utilization profiles in the various land uses, indicating the existence of functionally different microbial communities (Figure 3). Additionally, more disparate catabolic responses appear between the samples of less conserved soils – i.e. in eroded land and conventional maize fields- which suggest a loss of functional stability. Anyway, significant differences were not shown in pair wise comparisons between land uses mainly due to an insufficient sample size, which might have been tackled with statistical modelling techniques.

Bonferroni correction was used together with Mann-Whitney Wilcoxon test to do multiple comparisons. In this conservative correction, α that had a value of 0.005, is substituted by α/nc where nc is the total number of comparisons that were done.

Differences were difficult to find as nc=10 and so α=0.005. An alternative to Bonferroni correction is Sidak correction. In Sidak correction, α is substituted by $1 - (1 - \alpha)^{1/nc}$. As a result, α=0.0051162. Unfortunately, the value is very similar and there were no changes in the results.

In any case, it should be born in mind that the Biolog test only gives information regarding the microbial community function for a very narrow range of conditions. A drawback is the selection for species adapted to rapid growth on simple substrates in high concentrations (Smalla et al., 1998). Biolog might fail to separate the land uses since it is primarily fast growing microorganisms and simple C compounds that are tested, for which functional redundancy is believed to be high (Waldrop and Firestone, 2004).

In fact, functional redundancy of soil microorganisms is usually behind structural changes found without the corresponding functional changes. Fungi, a major component in forest ecosystems, are not capable of using the tetrazolium dye incorporated in the wells (Preston-Mafham et al., 2002). Thus, they were not contributing in the substrate utilization potential despite their importance in terms of functional biodiversity. It has also been noted that carbon sources used in the Biolog assay can be highly correlated and, in some cases, contribute little to the discrimination of microbial communities (Campbell et al., 1995; Campbell et al., 1997 Grayston et al., 1994).

Biochemical methods of studying soil microbial capacity such as community-level physiological profiling methods – i.e. Biolog - should be performed along with molecular methods such as DGGE (denaturing and temperature gradient gel electrophoresis). Such procedures offer complementary information, ie., on community composition, while overcoming the limitations of cultivable methods.

The land use history of the sampled plots revealed that most current farmlands with agroforestry and woodlots used to be either conventional, intensive cultures, or deforested land having been turned into fallow land or pastures about 1-15 years ago. Except for the protected forest land use, sampling areas were selected in all locations – Mt Elgon slopes and Kitale plain- so that the four land uses where found in each other's vicinity, sharing a common environment for each replicate. It must also be noted that the protected forest land use had the highest MBC, MBN, Ct and Nt concentrations in spite of being higher up the mountain, where shallower, less fertile soils are to be found as opposed to lower areas (Horváth, 2006).

Despite their diverse background and location, land uses including trees appeared to be better suited to support life - both plants and microorganisms. My results suggest soil physical, chemical and biological properties - microbial biomass and function - to be affected by land use. They also suggest that not only is vegetation cover, especially by woody species, an important soil conservation measure, but also an effective improving and restoring one. Thus, they suggest that degraded and over-exploited land with impoverished physical, chemical and biological properties – i.e. microbial community structure - might well be restored to variable degrees by management techniques such as planting of indigenous trees. In the case of productive lands, agroforestry, which couples good agricultural practices with integration of trees in crops to favour adequate long-term yields while protecting soils and water, could

have a potential value as a functional capacity and biodiversity conservation measure as much as a human development one.

Microorganisms are at the bottom of the food chain and changes in their communities might affect the viability and health of the environment as a whole. Thus, further research on possibly bigger sample sizes and in both natural and productive landscapes is required on several issues. Among them is the role of biodiversity for functional stability in terms of resilience and resistance to change in the face of perturbations. More specifically, more studies are needed on the redundancy of general functions that could be key to such stability and how much soil biodiversity can be lost before soil function is.

When relating biodiversity and ecosystem function it should be taken into account that procaryotes have opportunities for genetic recombination and horizontal transfer of adaptive and useful functions. Other issues to consider are the low openness and therefore highly local connectivity of soils compared to aquatic systems, leading to less inherent recovery capacity (Giller et al., 1998; Wardle, 2002). It must also be noted that effects of disturbances – short-term perturbations caused by management - are much more likely to be reversible than stress – such as effects of long-term chronic toxicities (Giller et al., 2005). Linking the two approaches to soil biology – the organism and the functional - is desirable in view to resolving many of these issues. Other challenges include the development of tools for managing soil biodiversity through manipulation of above-ground vegetation and soil amendments, and understanding the effects of scale –ecosystem processes and functions vary with it- to design land use systems for optimal future conservation of the functional capacity and biodiversity of tropical soils.

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7. Annexes

7.1. Annex 1

Location names, GPS ID and coordinates and altitude

The data is presented for all of the locations where soil was sampled.

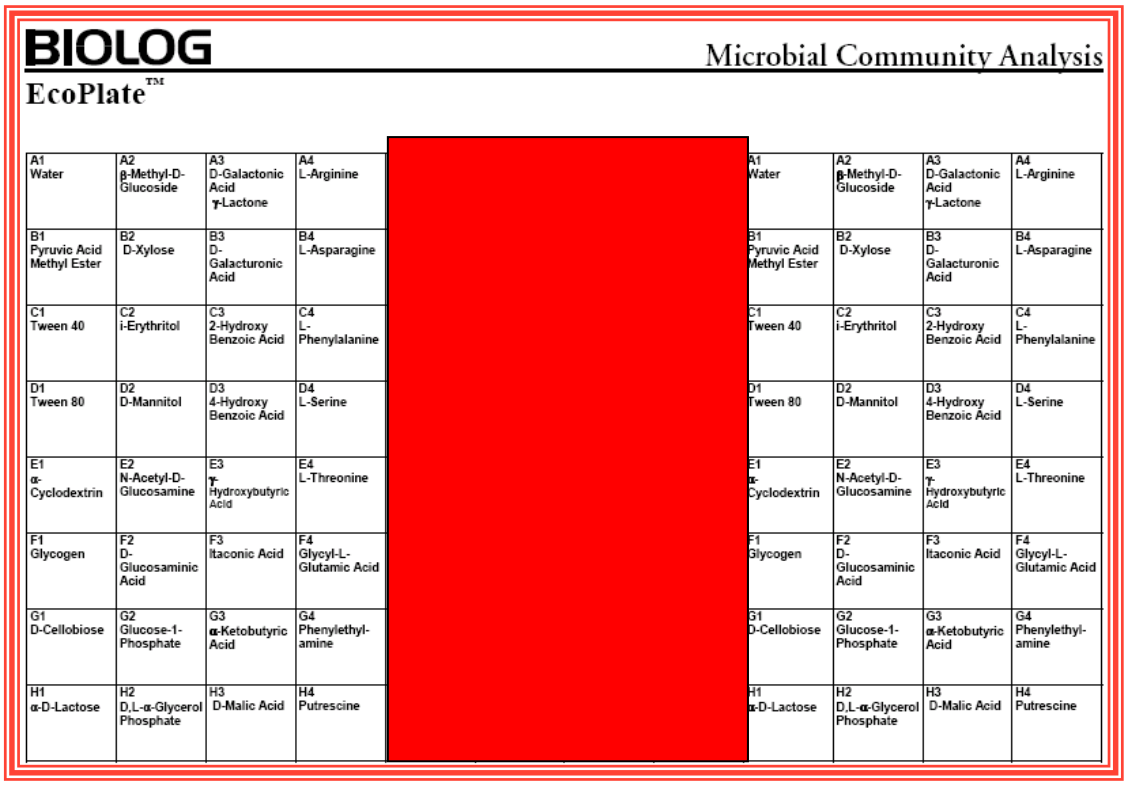
Name	Land use	GPS ID	GPS coordinates	Altitude (m)
Endebess				
Jennifer Lumumba	Farmland with agroforestry	A	N 01°02.102′ E 034°51.769′	1888
Jennifer's neighbour	Conventional maize field	B	N 01°02.113′ E 034°51.757′	1886
Peter Wehesa, Odero	Woodlot	C	N 01°01.658′ E 034°51.224′	1873
Odero's neighbour	Conventional maize field	D	N 01°01.713′ E 034°51.230′	1879
Kitale				
Kitale NM arboretum	Woodlot	Kk	N 01°06.584' E 034°00.225'	1874
OPAC arboretum	Woodlot	Mm	N 01°00.561' E 035°00.324'	1873
OPAC demonstration plot	Farmland with agroforestry	Nn	N 01°00.637' E 035°00.437'	1894
maize field in Milimani	Conventional maize field	i	N 01°00.320' E 034°00.55'	1890
Barren land Kitale	Eroded land	ii	N 01°00.102' E 034°4.384'	1890
Barren land in Milimani	Eroded land	iii	N 01°00.131' E 034°00.254'	1891
Matumbei				
Elijah Joel	Farmland with agroforestry	E	N 01°05.162′ E 034°49.807′	1918
Elijah's neighbour	Conventional maize field	F	N 01°05.025′ E 034°50.784′	1919
Matumbei road plantation	Woodlot	Ll	N 01°04.200' E 034°50.491'	1901
Mubere				

Consolatta Alunga	Farmland with agroforestry	Bb	N 01°06.602' E 034°48.443'	1985
Mr Philipo	Woodlot	Cc	N 01°06.401' E 034°48.242'	1967
Cheptaragai, near church	Eroded	Dd	N 01°06.433' E 034°49.319'	1925
Konga'asis academy	Eroded	Ee	N 01°06.150' E 034°49.368'	1925
Across Mubere primary school	Eroded	Ff	N 01°06.935' E 034°46.847'	1970
Barnabas Magut	Farmland with agroforestry	Ii	N 01°05.977' E 034°51.782'	1968
Barnabas Magut	Conventional maize field	Hh	N 01°06.001' E 034°51.817'	1866
Matumbei 2				
Gabriel Machabe	Woodlot	K	N 01°05.936′ E 034°46.720′	2130
Gabriel's neighbour	Conventional maize field	J	N 01°05.377′ E 034°46.124 ′	2128
Mrs Bisonga	Woodlot	H	N 01°05.037′ E 034°46.493′	2137
Mrs Bisonga's neighbour	Conventional maize field	I	N 01°05.197′ E 034°46.513′	2135
Dickson Masinde	Farmland with agroforestry	L	N 01°05.749' E 034°46.528'	2158
Mubere 2				
Vincent Cheheli Wanyoni	Farmland with agroforestry	N	N 01°06.457' E 034°46.439'	2128
Vincent's neighbour	Conventional maize field	M	N 01°06.517′ E 034°46.448′	2144
Basale after Vincent's place	Eroded land	O	N 01°06.517′ E 034°46.448′	2142
Richard Wamalwa	Woodlot	S	N 01°06.458' E 034°46.685'	2135
Next to Wamalwa's place	Eroded land	Q	N 01°06.452' E 034°46.705'	2131
Wamalwa's neighbour	Conventional maize field	R	N 01°06.443' E 034°46.709'	2129
Mrs Wayongo	Woodlot	T	N 01°06.650' E 034°46.727'	2142

Mrs Wayongo	Farmland with agroforestry	U	N 01°06.652' E 034°46.725'	2137
Khalabana village	Eroded	V	N 01°06.836' E 034°47.012'	2164
Malulingo	Eroded land	Aa	N 01°06.965' E 034°47.295'	2152
Mr Primus'	Farmland with agroforestry	P	N 01°06.380' E 034°46.642'	2115
Mt Elgon National Park				
Highest altitude	Natural protected forest	Ss	N 01°01.952' E 034°44.764'	2519
2nd highest	Natural protected forest	Tt	N 01°01.847' E 034°45.620'	2457
3rd highest	Natural protected forest	Uu	N 01°01.442' E 034°45.498'	2343
Lowest altitude	Natural protected forest	Vv	N 01°01.356' E 034°45.254'	2215

7.2. Annex 2

The Biolog EcoPlate allows determination of substrate utilization potential by means of plates containing 3 repetitions of 31 of the most useful carbon sources for soil community analysis, plus 3 water controls.



BIOLOG Microbial Community Analysis

EcoPlate™

A1 Water	A2 β-Methyl-D-Glucoside	A3 D-Galactonic Acid γ-Lactone	A4 L-Arginine	A1 Water	A2 β-Methyl-D-Glucoside	A3 D-Galactonic Acid γ-Lactone	A4 L-Arginine
B1 Pyruvic Acid Methyl Ester	B2 D-Xylose	B3 D-Galacturonic Acid	B4 L-Asparagine	B1 Pyruvic Acid Methyl Ester	B2 D-Xylose	B3 D-Galacturonic Acid	B4 L-Asparagine
C1 Tween 40	C2 i-Erythritol	C3 2-Hydroxy Benzoic Acid	C4 L-Phenylalanine	C1 Tween 40	C2 i-Erythritol	C3 2-Hydroxy Benzoic Acid	C4 L-Phenylalanine
D1 Tween 80	D2 D-Mannitol	D3 4-Hydroxy Benzoic Acid	D4 L-Serine	D1 Tween 80	D2 D-Mannitol	D3 4-Hydroxy Benzoic Acid	D4 L-Serine
E1 α-Cyclodextrin	E2 N-Acetyl-D-Glucosamine	E3 γ-Hydroxybutyric Acid	E4 L-Threonine	E1 α-Cyclodextrin	E2 N-Acetyl-D-Glucosamine	E3 γ-Hydroxybutyric Acid	E4 L-Threonine
F1 Glycogen	F2 D-Glucosaminic Acid	F3 Itaconic Acid	F4 Glycyl-L-Glutamic Acid	F1 Glycogen	F2 D-Glucosaminic Acid	F3 Itaconic Acid	F4 Glycyl-L-Glutamic Acid
G1 D-Cellobiose	G2 Glucose-1-Phosphate	G3 α-Ketobutyric Acid	G4 Phenylethyl-amine	G1 D-Cellobiose	G2 Glucose-1-Phosphate	G3 α-Ketobutyric Acid	G4 Phenylethyl-amine
H1 α-D-Lactose	H2 D,L-α-Glycerol Phosphate	H3 D-Malic Acid	H4 Putrescine	H1 α-D-Lactose	H2 D,L-α-Glycerol Phosphate	H3 D-Malic Acid	H4 Putrescine

The substrates in the Biolog EcoPlate wells do belong to the following categories (Plate wells where the carbon source is found between brakets) :

1-Amines/amides:

-Phenylethylamine (G4, G8, G12)
-Putrescine (H4, 8, 12)

2-Aminoacids

-L-Arginine (A4, 8, 12)
-L-Asparagine (B4, 8, 12)
-L-Phenylalanine (C4, 8, 12)
-L-Serine (D4, 8, 12)
-L-Threonine (E4, 8, 12)
-Glycyl-L-glutamic acid (F4, 8, 12)

3-Carbohydrates

-D-Cellobiose (G1, 5, 9)
- α-D-Lactose (H1, H5, H9)
-β-Methyl-D-glucoside (A2, 6, 10)
-D-Xylose (B2, 6, 10)
-i-Erythritol (C2, 6, 10)

-D-Mannitol (D2, 6, 10)
-N-Acetyl-D-glucosamine (E2, 6, 10)

4- Carboxylic acids

-D-Galacturonic acid γ-Lactone (A3, 7, 11)
-D-Galacturonic Acid (B3, 7, 11)
-2-Hydroxy benzoic acid (C3, 7, 11)
-4-Hydroxy benzoic acid (D3, 7, 11)
-γ-Hydroxy butyric acid (E3, 7, 11)
-Itaconic acid (F3, 7, 11)
-α-Keto butyric acid (G3, 7, 11)
-D-Malic acid (H3, 7, 11)
-D-Glucosaminic acid (F2, 6, 10)

5-Miscelaneous

-Glucose-1-phosphate (G2, 6, 10)
-D, L- α-Glycerol phosphate (H2, 6, 10)
-Pyruvic acid methyl ester (B1, 5, 9)

6-Polymers

-Tween 40 (C1, 5, 9)
-Tween 80 (D1, 5, 9)
-Cyclodextrin (E1, 5, 9)
-Glycogen (F1, 5, 9)