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The impact of agroforestry and other landuses on soil functional capacity

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ABSTRACT

Deforestation, agricultural cultivation and overuse of land resources can lead to decreasing functional capacity of soil microorganisms, e.g. decreasing decomposition and mineralisation capacity and rate. Agroforestry systems have long been considered to have positive effect on soil conditions and there is a need to investigate if the functional capacity of the soil can be recovered by these practices to reverse land degradation in the tropics. The objective of this study was to compare the functional capacity and diversity of soils under different land use, measured as substrate utilization potential of the soil bacteria community. Samples along an intensification gradient from undisturbed forest, forest plantations, agroforestry fields, agricultural fields and the most disturbed eroded soil were taken from farms on the slopes of Mount Elgon in the Rift Valley province of western Kenya. The microbial substrate utilization was studied by using Biolog EcoPlates™ and chemical and biological soil properties including pH, extractable P, total N, organic C, nitrates and microbial biomass C and N were determined.

In general, the bacterial substrate utilisation potential, pH and N and C content follow a pattern with values from eroded and agricultural land lower than agroforestry and planted as well as natural forest. Microbial biomass C and N also show increasing values with decreasing disturbance with the levels for natural forest two to three times higher than the other land uses. PCA for chemical properties show a significant difference between natural forest and the other land uses, while agroforestry overlap with both agriculture and forest plantation. PCA and the metabolic response rates calculated from average well colour development in Biolog EcoPlates clearly shows that natural and planted forest are equalled by agroforestry but not by agriculture or eroded land. The results indicate that the microorganism community composition is similar in land with similar vegetation and thus that the functional capacity of the soil can be restored by active soil management such as agroforestry practices on earlier overused agricultural land.

INTRODUCTION

In several areas in sub-Saharan Africa, the high human population growth has led to intensification of agriculture and also use of less suitable land for agriculture. The traditional techniques often leave the soil open to erosion by wind and rain and deplete the soil of organic matter. Grazing cattle can also affect the cover and composition of plants (Makokha et al., 1999). These conditions can lead to loss of biodiversity both in flora and fauna and regeneration of surviving species may be compromised. Wetlands and rivers can be threatened both in quality and quantity by draining, encroachment and human activities in the catchment areas and riverbanks, causing flooding due to vegetation cover depletion (Landon, 1991). The nutrients in the soil can easily wash away down streams and pollute the fresh water supplies and also lead to low organic matter content and reduced draining qualities of the soil. The expanding human population also increases the need for fuelwood, which leads to higher pressure on the remaining forests and, in many cases, to total deforestation. Without vegetation to hold the top soil in place, wind and water can carry it off and render remaining soil low in nutrients and less fertile. The resources of the land are stretched thin as it is and a further reduction of productivity would minimize the possibilities to support the human population (Maundu et al., 1999; Noad and Birnie, 1989).

The traditional agricultural maize cropping often means removal of the aboveground biomass of stover after harvest. The consequences for soil fertility are reduced carbon and nutrient supplies in the soil (Mutuo et al., 2005). The tillage of crop fields also disturbs the habitats of soil organisms that show lower numbers and biomass in cultivated land than in undisturbed soil (Brady et al., 2002). Soil organisms can be divided in functional groups according to their roles, the rhizosphere surrounding the plant roots include bacteria, mycorrhiza fungi, nematodes and insects. Earthworms, ants and termites are ecosystem engineers, fungi and bacteria decompose and transform litter, while the grazers include protozoa, beetles and arachnids (Susilo et al., 2004). This paper will mainly look at the microbial community of bacteria.

Microbial life is present everywhere and will proliferate under appropriate conditions (O'Malley, 2007). With a high biodiversity, the ecological niches are filled, functions like decomposition and nitrification are performed well and a great proportion of the available resources are utilised. The complex communities are more likely to contain highly competitive and productive species and some species even facilitate each other's growth (Begon et al., 2006; Bardgett et al., 2005). When environmental conditions are altered after changed land use management, the soil organism composition changes with it (Susilo et al., 2004; Bolton et al., 1985). The functional consequences that this may have can be mitigated if the soil contains a broad spectrum of species that can maintain processes like denitrification and decomposition (Griffiths et al., 2001). It seems however, that there is no direct relationship between biodiversity and ecosystem functioning. According to Bardgett et al. (2005), the exact soil species composition is not vitally important for functions, since there is generally a redundancy of species. But for stability, resilience and resistance to perturbation, diversity can be of major importance.

The two most prominent groups of decomposers are fungi and bacteria. They mineralize organic material into inorganic compounds essential for plant growth through decomposition. In addition, certain microorganisms provide plant available nitrogen through N-fixation (Coleman & Whitman, 2005; Brady et al., 2002; Campbell et al., 1999; Begon et al., 2006). Coleman and Whitman (2005) state, that many studies on ecosystem processes show that great diversity in microorganisms below ground positively affect above ground biomass. Heterogeneity of litter quality and type from high plant diversity can in fact increase species richness of decomposers and detritivores. The process of decomposition is donor controlled but there seem to be a strong link between certain species or functional groups above and below ground (Moore et al., 2004; Griffiths et al., 2001). Mineralization and decomposition processes involve a broad spectrum of micro-organisms so the presence of particular species or groups are likely not crucial. Instead, the manner in which below ground diversity affects above ground biota is in the specific relations like symbiosis, diseases and their antagonists (Susilo et al., 2004; Griffiths et al., 2000; Tate, 1995). Even if the exact plant composition, climate and soil type differs within grassland or a forest, the respective microbial communities remain similar in composition. Changing from one system to the other will change root structure, chemical nature of the soil organic matter and the root exudates (Stevenson et al., 2003; Bossio et al., 2005). This will in turn affect the mycorrhizal interface between plant roots and soil and alter the composition and function of the microbial communities (Jefwa et al., 2004). Study of the functional diversity should complement taxonomic studies to understand the processes of decomposition better (Okoth, 2004). Functional diversity can be described as the composition of microbial communities needed to perform and maintain ecosystem processes in the soil such as decomposition and mineralization. Microorganisms in the soil are often growth limited and the functional capacity would be the potential rate at which these processes would take place if resources where unlimited. This can be measured as substrate utilization potential, i.e. the capability of the microorganisms to metabolise the substrates available. The rate of the metabolism is the substrate utilisation capacity (Bloem et al., 2006).

Agroforestry is a new name for a set of old practices and can shortly be described as a combination of forestry and agriculture with several outputs according to World Agroforestry Centre, formerly known as International Centre for Research in Agroforestry (ICRAF). A more scientific definition of agroforestry would be the deliberate growing of woody perennials and agricultural crops and/or animals on the same unit of land, either in a spatial mixture or in sequence. There are significant ecological and economical interactions (positive and negative) between the woody and non woody components of the system, making the cycle of the system longer than one year. There are always two or more outputs and even the simplest agroforestry system is more complex ecologically, structurally and economically than any monocropping system (www.worldagroforestrycentre.org, 2008-01-11). The implementation of practices such as agroforestry can improve and diversify the agricultural produce and thereby also bring food security, fuel wood and extra income to the families. Research has found agroforestry practices such as improved fallows promising in sequestering carbon and reducing soil degradation (Mutuo et al. 2005; Amadalo et al., 2003; Swinkels et al., 1996). Plant residues and farmyard manure added to the soil improve fertility and

maintain microbial activity (Kautz et al., 2004; Bloem et al., 2006; Berglund, et al., 2006) and legume based cropping systems can increase N-fixation (Giller, 2001).

The Vi Agroforestry Programme (ViAFP) implements agroforestry practices as a means to improve living conditions and reduce poverty among families on smallholder farms in Eastern Central Africa. The main area of operation is the Victoria Lake basin in Kenya, Rwanda, Uganda and Tanzania. The program started in 1983 and is financed by the Foundation *Vi planterar träd*, which is an international non-profit, non governmental organisation based in Stockholm, Sweden. The ViAFP works in several areas in the Rift Valley province in west Kenya, with headquarter of the district in Kitale, at the Olof Palme Agroforestry Demonstration Centre (OPAC). The centre is a demonstration farm for agroforestry practices such as intercropping, grass strips, hedge barriers, tree lines and contour strips. Soil improvement practices such as composting, use of green manure and the use of N-fixing trees are taught here. There are also examples of zero-grazing, bee keeping and an arboretum where many common tree species used in agroforestry can be found. Beside the organisational work, ViAFP promotes agroforestry by education, information and conferences at the centre. This also includes general crop management and harvesting as well as production and management of tree seeds and seedlings. Together with Kenya Wildlife Service (KWS) and the Department of Forestry, ViAFP is also working to protect the environment around Mt Elgon National Park. By implementing agroforestry practices among the farmers to increase the vegetation cover outside the park and thereby increasing people's access to firewood and timber, the risk of encroachment of the park decreases (Horvath, 2006).

Aim of the study and tested hypothesis

The aim of this study was to compare soil conditions and vegetation in areas with different landuse; from the undisturbed natural forest of Mt Elgon National Park, to the very disturbed agricultural system of mono cropped maize. The main aspect of soil condition studied was the functional capacity and diversity of the soils under different land use, measured as substrate utilization potential of the soil bacteria community.

The hypotheses tested were:

1. Deforestation, agricultural cultivation and overuse of land resources lead to decreasing functional capacity of soil microorganisms.
2. Functional capacity of soil organism communities can be restored by active soil management, including addition of organic matter and implementing of agroforestry.

MATERIALS AND METHODS

Study area

The study took place on the slopes of Mount Elgon west of Kitale in Rift Valley province, Kenya. The slopes of this mountain outside Mount Elgon National Park are

almost completely deforested due to agriculture and to the need for fuelwood. The hilly nature of the region and lack of vegetation cover cause much surface runoff of water that washes the soil away and often renders roads practically impassable during the rain seasons. The rainfall pattern is bimodal with the long rains normally falling from April to July and the short rains from August to November. Mt Elgon is the main water catchment area for the Nzoia River that flows into Lake Victoria in the south and for Turkwel River flowing into Lake Turkana in the north. The most favoured crops in the area are maize and sunflowers. The climate is highland equatorial with a mean annual temperature of 18 ^{o}C and average annual precipitation of around 1300 mm with most rain during April-May and October-November. 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. In the area bordering the Mt Elgon National Park, small-scale farms were chosen on altitudes between 1800-2200 m above sea level (Horvath, 2006). The most commonly used tree species for firewood and also most preferred in the area include *Acacia* spp., *Grevillea robusta, Sesbania sesban, Calliandra calothyrsus, Passiflora edulis, Cordia africana, Markhamia lutea* and *Persea americana.* The most used agroforestry systems 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).

We conducted interviews with farmers concerning their living standard, types of crops, access to natural resources, land use and agricultural practices. We chose to use semi-structured questionnaires to encourage free expression of farmers' views (Kephas Okach, 2007, personal communication). The result would provide a base for the continuing work to protect the environment around Mt Elgon and help the local population to implement changes in landuse practices in order to reduce erosion problems and increase yields (Appendix).

Sampling

To decide where to collect the soil samples we prepared a sampling scheme in a block design (Table 1). It included two altitudes: 1900-2000 m and 2000-2200 m above sea level. We decided on four different landuses:
EL – indigenous natural forest of Mt Elgon National Park.
FO – planted forest or woodlots on farms consisting of many different species of trees.
AF – agroforestry; fields with different types of agroforestry systems currently in use.
AG – agricultural fields; currently harvested and not replanted maize fields.
ER – eroded land; bare, uncultivated land such as pathways and ditches.
There were four replicates of each landuse per altitude. This means we planned for eight blocks with one replicate of each land use in each block. Four blocks at 1900-2000 m and four at 2000-2200 m a.s.l. In addition to those eight blocks, there were one block with the four land uses on and in the vicinity of OPAC at 1900 m above sea level and one block with four replicates of natural forest at Mt Elgon National Park called EL, above 2200 m. This added up to a total of 40 sampling locations.

Pictures of a plant school outside Kitale on the left and soil sampling eroded land on the right.

Table 1. Sampling scheme showing at which altitudes the different land uses where sampled.

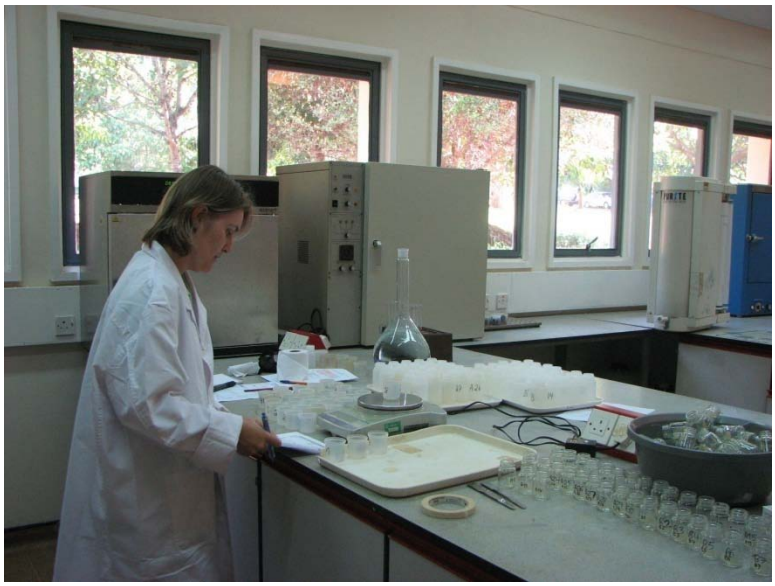
Sampling scheme				
Elevation	**Land uses**			
Mt Elgon >2200 m	EL	EL	EL	EL
2000-2200 m	FO	ER	AF	AG
	AG	FO	AF	ER
	AF	AG	FO	ER
	ER	AF	AG	FO
1900-2000 m	FO	AG	AF	ER
	ER	AF	AG	FO
	FO	AG	ER	AF
	AF	ER	FO	AG
OPAC 1900 m	AF	AG	FO	ER

Together with staff from ViAFP, farms on the chosen altitudes and with cultivation systems according to the sampling scheme were selected. In Mt Elgon National Park, four sampling locations were chosen at 2200-2400 m a.s.l. For soil physics and chemical analyses samples were taken on all 40 locations. Five soil cores at the depth of 0-25 cm were randomly collected with an auger from a ca 25 m x 25 m area that was also randomly selected in the chosen field. The cores were bulked into one soil sample, put in plastic bags and tagged and then carefully mixed. The bags were left open to air-dry for a minimum of five days before transport to the lab. These samples were taken to Moi University in Eldoret where soil Physical and Chemical tests were performed. The auger was cleaned between every location. The sample locations were marked with GPS-coordinates. The dominating vegetation on every location was described and main species of trees and shrubs were identified to allow comparison with the vegetation in Mt Elgon National Park (Maundu et al., 1999; Dharani, 2002; Lötschert and Beese, 1983).

Soil chemical and biological analyses

A soil particle size analysis was performed using the hydrometer method. Sand, silt and clay content of the soil was measured as percentage of weight of oven-dry and organic matter-free soil. The hydrometer method is based on the differential settling velocities of different sized particles in water. Soil pH was measured with a pH-meter in a 2.5:1 deionised water to soil suspension. The amount of extractable nitrates was determined colorimetrically. Soil samples were extracted with potassium sulphate after which salicylic acid and sodium hydroxide were added. After colour development the nitrates were determined colorimetrically at 419 nm. The amount of nitrates in the soil is given in µg/kg soil. To determine the amount of plant available phosphorus in the soil samples, the Olsen method was used. Air-dried soil was extracted with sodium bicarbonate at pH 8.5. The solution was filtered and the absorbance was measured at 880 nm. The concentration of P in the samples is given in mg/kg soil. The content of organic carbon was determined by complete oxidation by heating after adding of sulphuric acid and aqueous potassium dichromate mixture. The remaining potassium chromate that was

titrated against ferrous ammonium sulphate gave the measure of organic carbon content of the soil in % of weight. To retrieve the total content of nitrogen in the soil, samples were completely oxidised by treating with hydrogen peroxide, selenium and sulphuric acid. After the acid digestion, sodium reagents were added and the absorbance was measured at 650 nm. The total nitrogen concentration was given in % of weight. All analyses described above were performed according to Okalebo et al. (1993) and/or Anderson and Ingram (1993).



The analyses for Microbial Biomass carbon and nitrogen were done at TSBF in Nairobi on fresh soil. The method used was chloroform fumigation-extraction. Samples of chloroform fumigated and non-fumigated soil was extracted with potassium sulphate and the difference in concentration gave the amount of microbial biomass C and N in mg/ kg soil (Anderson and Ingram, 1993).

Picture of soil analyses from TSBF in Nairobi.

Biolog EcoPlate™

To study the bacterial community composition we used Biolog EcoPlates where microorganisms such as bacteria are cultured in different substrates. The Biolog EcoPlates are based on the capacity of bacteria to utilise different substrates and thus leaving a metabolic fingerprint providing information on functional biodiversity in the soil over time (Preston-Mafham et al., 2002). Each plate contains three replicates of 31 wells with different substrates and one control well with distilled water, all together 96 wells. The wells also contain an indicator substance; tetrazolium dye, that changes colour with substrate consumption. The reduction of the tetrazolium dye due to cell respiration turns the contents of the wells purple. Out of the 31 carbon sources there are 7 carbohydrates, 2 amines/amides, 6 amino acids, 9 carboxylic acids, 3 miscellaneous and 4 polymers (www.biolog.com, 2007; Schutter and Dick, 2001). Fresh soil was collected from every location during the last 48 h before transport to the Tropical Soil Biology and Fertility programme (TSBF) lab in Nairobi. The samples were kept in coolers and to avoid disturbance of the microbes no mixing, sieving or conditioning was done. From each of the 40 samples, 10 g of fresh soil was suspended in 90 ml of 0.145 M Sodium chloride (dilution to 10^{-1}) With additional Sodium chloride the suspension was

subsequently diluted to 10^{-2} and 10^{-3}. The 10^{-3} dilution was dropped into the wells and the plates were incubated for four days. One EcoPlate was used for each one of the 40 locations and the microbial community analysis showed the characteristic reaction patterns. The increasing strength in purple colour was followed over time by measuring the absorbance at 595 nm every 24 h. AWCD (Average Well Colour Development) was calculated and also AWCD for the different groups of substrates; carbohydrates, amino acids, amines and amides, carboxylic acids, polymers and miscellaneous and for each single substrate (Elfstrand, 2007). The diversity in colour development for all substrates after 96 h incubation was calculated with the Shannon-Weaver Diversity Index. The formula used was: $H = -\Sigma\, p_i \ln p_i$,where p_i in this case is the proportion of AWCD of a particular substrate to the AWCD of all substrates of a certain land use (Fowler et al., 2006; Harch et.al., 1997; Olsson et al., 2005; Yan et.al., 2000).

Statistical analysis

Soil biological, chemical data and physical data as well as Biolog EcoPlate data were analysed with a General Linear Model using Block and Land use as model components. Score plots and loading plots were made with Multivariate Principal Component Analysis. ANOVA General Linear Model, multivariate PCA, correlations, means and standard deviations were performed using Minitab 15. Diversity index and AWCD was calculated in Microsoft Excel.

RESULTS

Soil chemistry and microbial biomass

The values from Mt Elgon (EL) could not be tested statistically in the ANOVA concerning differences between land uses since the sampling locations of EL were not arranged in such a block design as the sampling locations of the other land uses. The EL values will still be compared with the other values but without statistical testing of the differences.

Table 2. The means of pH, concentrations (% of dry weight) of total soil nitrogen (N), total soil carbon (C), plant available soil phosphorus (P), microbial biomass carbon (MBC), microbial biomass nitrogen (MBN) for the investigated land uses. ER= eroded land, AG=maize fields, AF=agroforestry fields, FO=planted forest and EL=natural forest. For means and standard deviations (SD) n=9, except for Mt Elgon NP where n=4. One-way ANOVA not calculated for means of Mt Elgon. The different letters in the same column for the mean values indicate significant differences at $P< 0.05$ (Tukey's test). The ANOVA was done in Minitab 15.

	Measurements											
Land -use	pH (H_2O)		N (%)		C (%)		P (ppm)		MBC (mg C/kg soil)		MBN (mg N/kg soil)	
	Mean	*SD*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*
ER	6.21b	0.56	0.09c	0.07	1.27c	0.61	8.53b	10.8	119c	30.3	6.57c	2.79
AG	6.05b	0.38	0.37b	0.09	2.34bc	0.28	19.6b	11.0	173b	34.2	13.0bc	4.48
AF	6.53ab	0.51	0.46ab	0.27	3.41ab	1.51	60.8a	45.2	212b	32.5	19.6b	9.92
FO	6.79a	0.46	0.62a	0.28	3.80a	1.02	34.7ab	40.3	283a	51.6	34.6a	11.6
ANOVAs	*P = 0.006*		*P = 0.000*		*P = 0.000*		*P = 0.003*		*P = 0.000*		*P = 0.000*	
EL	6.72	0.34	1.06	0.49	5.93	2.41	14.1	9.72	656	104	114	34.7

Table 2 continued with nitrates, silt, sand, clay and moisture.

	Measurements									
Land-use	Nitrates (µg/kg soil)		Silt (%)		Sand (%)		Clay (%)		Moisture (%)	
	Mean	*SD*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*
ER	8.93a	5.67	10.56b	3.13	50.89b	9.60	38.56c	9.42	20.04a	6.44
AG	9.15a	3.69	18.22a	9.24	53.11b	12.18	28.67bc	13.68	23.86a	4.36
AF	6.34a	4.52	15.67ab	3.32	61.56ab	9.95	22.78ab	10.57	25.86a	6.68
FO	4.89a	3.14	12.89ab	2.26	70.89a	6.25	16.22a	5.61	26.50a	5.22
ANOVAs	*P=0.126*		*P=0.031*		*P=0.000*		*P=0.000*		*P=0.083*	
EL	7.60	5.04	13.50	5.74	78.00	9.27	8.25	4.19	45.32	7.85

The different types of landuse generally affects most soil chemistry measurements in similar ways with values from eroded and agricultural land lower than agroforestry and planted as well as natural forest (Table 2). The mean values of pH, C and N significantly separate FO from AG and ER, but not from AF (Table 2). Microbial Biomass C (MBC) and N (MBN) show similar patterns; the proportions increase with decreasing disturbance and the values for EL are two to three times higher than those for the second highest; FO (Table 2). Concerning the contents of phosphorus, the variation is large and the standard deviations sometimes higher than the mean. Still, significantly higher P concentrations was found in AF than in ER and AG, and EL had the lowest value.

The first factor of the PCA analysis of the soil chemical data (Fig. 1) accounts for 51.1 % of the total variation between pH, altitude (height), moisture, sand and MBC and MBN as opposed to clay and nitrates. The second factor explains 14.4 % of the variance and shows a weak relationship between moisture, MBC and MBN as opposed to silt and P. The values of ER are grouped and significantly separated from EL but not from the other land uses (Fig 2). Chemical data from AF, AG as well as FO are spread and intermingled with each other. FO chemical data however, are second to EL when it comes to C, N, MBN and MBC (Table 2), which is the reason why FO data lies somewhat closer to EL on the Score Plot. The score plot of the chemical data divided into the different land uses (Fig. 2) clearly shows that EL is separated from the rest. The MBC and MBN in EL are at least three times as high (Table 2) as in any of the other land use measurements, which

explains this separation. With all soil chemical and microbial biomass data taken in account in the PCA, the score plot show separation of the land uses with increasing values with decreasing disturbance.

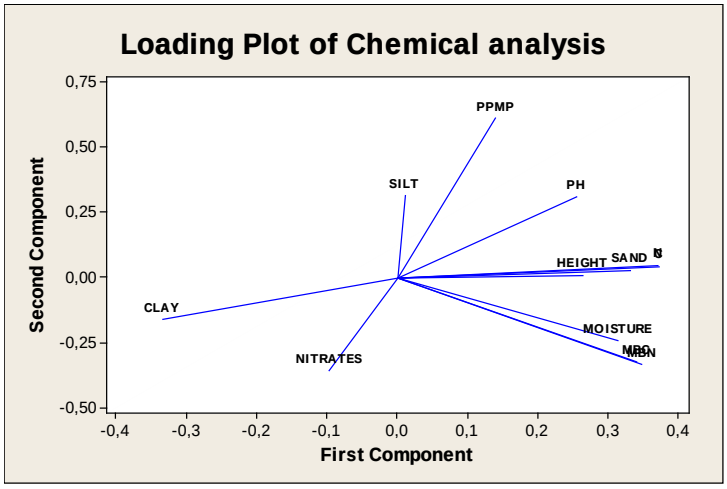


Fig. 1. Loading Plot of the chemical analysis. The first component explains 51.1 % of the total variation and the second component explains 14.4 %. Score plots and loading plots were made with Multivariate Principal Component Analysis in Minitab 15.

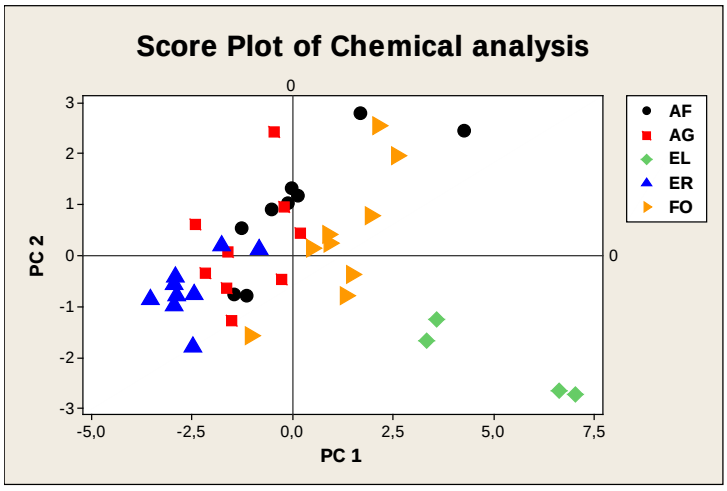


Fig. 2. Score Plot of chemical analysis divided in different land uses.

Substrate utilization potential and functional biodiversity

In Fig. 3 the AWCD per land use for all substrates show the same pattern as for the other chemical and biological analyses with the values of EL, FO and AF consequently higher than AG and ER. The AWCD figures in Fig. 3 and Table 3 reflect substrate utilization capacity.

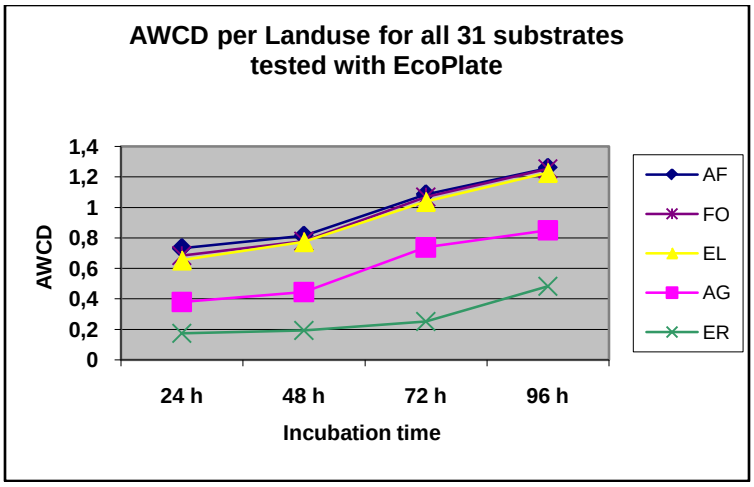


Fig. 3. Relative Average Well Colour Development (AWCD) calculated from absorbance values of Biolog EcoPlate for different land uses over incubation time. The figure shows mean values for four replicates in EL and nine replicates in all other land uses. General Linear Model ANOVA shows *P= 0.002* at 95% confidence level (Tukey's test).

Table 3. AWCD data for Fig 3 and the average number of substrates that reacted; richness.

	24h		48h		72h		96h	
	AWCD	**richness**	**AWCD**	**richness**	**AWCD**	**richness**	**AWCD**	**richness**
AF	0,733	30,44	0,814	30,56	1,085	30,78	1,259	30,89
FO	0,685	30,11	0,779	30,11	1,069	30,67	1,253	30,78
EL	0,654	29,50	0,775	29,75	1,039	29,75	1,229	30,25
AG	0,381	30,33	0,445	30,33	0,738	30,56	0,850	30,67
ER	0,174	29,11	0,192	28,22	0,252	29,33	0,482	29,67

The average substrate utilisation follows the pattern also when divided into substrate groups (Fig. 4 a-f). Values of FO, AF and EL are not significantly different when looking at carbohydrates, amino acids and carboxylic acids. The curves diverge a little with amines/amides and polymers, suggesting that the microbial community in AF can utilise polymers to a higher degree than the microbes in other land uses and that the natural and planted forest of EL and FO have slightly higher utilisation potential than AF when it comes to amines/amides. AG has lower microbial activity than any land with tree cover throughout but differs the least on carbohydrates, amino acids and polymers. Land without vegetation; ER, has lowest utilisation potential for all substrate categories.

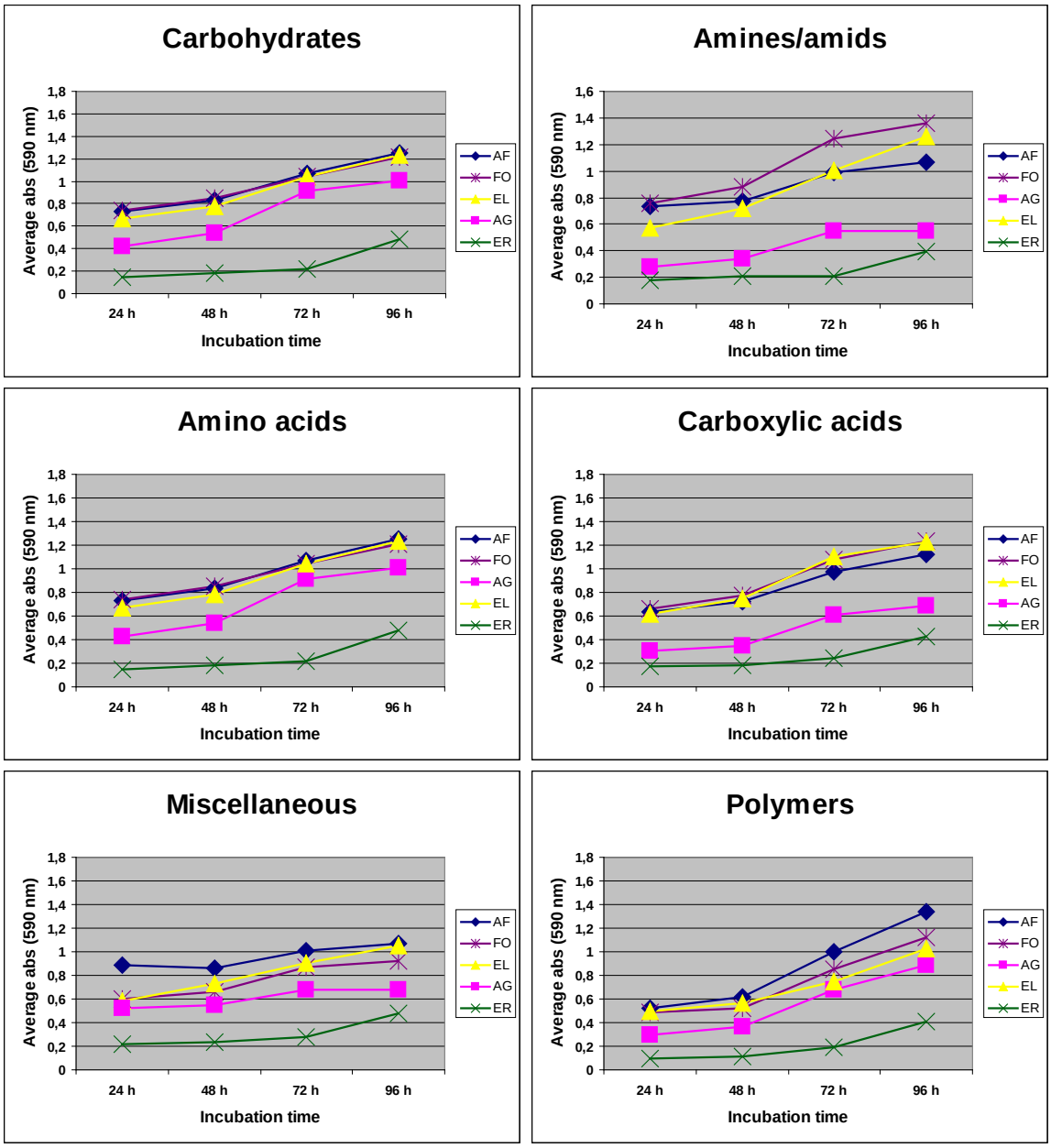


Fig. 4 a-f. The average absorbance values over time per group of substrate, divided in land uses. Relative Average Well Colour Development (AWCD).

Table 4 shows that the levels of utilisation of the substrates generally are similar in EL, FO and AF, while the levels are lower or much lower in AG and ER. One exception is α-Ketobutyric acid with low AWCD in FO, ER and EL and four times that level in AG and AF. Other examples are the polymers α-Cyclodextrin and Glycogen with considerably higher AWCD in AF than in all the other land uses.

The variation in average well colour development (AWCD) is high for the substrates in ER (average SD 0.637) and considerably lower in EL (average SD 0.289). SD for AG, AF and FO lies in between with 0.487, 0.356 and 0.340 respectively.

Table 4. The 31 substrates on Biolog EcoPlate. Average well colour development (AWCD) after 96 h incubation divided in the different land uses Eroded land (ER), Agriculture (AG), Agroforestry (AF), Planted forest (FO) and Mt Elgon NP (EL), (n=9 for all land uses except EL where n= 4). Standard deviations (SD) calculated on AWCD for each land use. Mean AWCD and SD for each land use.

Substrate	**ER**		**AG**		**AF**		**FO**		**EL**	
	AWCD	***SD***	***AWCD***	***SD***	***AWCD***	***SD***	***AWCD***	***SD***	***AWCD***	***SD***
β-Methyl-D-Glucoside	0.568	0.677	0.945	0.437	1.429	0.324	1.349	0.409	1.536	0.080
D-Galacton-acid γ-Lactone	0.494	0.702	0.995	0.455	1.282	0.315	1.535	0.219	1.321	0.142
L-Arginine	0.495	0.823	1.425	0.601	1.725	0.187	1.796	0.164	1.747	0.364
Pyruvic acid Methyl Ester	0.757	0.673	1.087	0.473	1.563	0.221	1.346	0.312	1.619	0.342
D-Xylose	0.544	0.757	0.801	0.601	1.606	0.533	1.416	0.604	0.609	0.566
D-Galacturonic acid	0.701	0.662	0.997	0.622	1.517	0.424	1.619	0.318	1.603	0.256
L-Asparagine	0.679	0.855	1.605	0.609	1.800	0.111	1.842	0.141	1.872	0.111
Tween 40	0.507	0.800	1.284	0.479	1.572	0.331	1.778	0.196	1.716	0.140
i-Erythritol	0.371	0.573	0.606	0.408	1.109	0.517	0.923	0.384	0.896	0.346
2-Hydroxi-Benzoic acid	0.204	0.398	0.143	0.165	0.201	0.299	0.457	0.469	0.351	0.481
L-Phenylalnine	0.517	0.848	0.761	0.559	1.080	0.321	1.066	0.606	0.984	0.537
Tween 80	0.399	0.586	1.112	0.454	1.331	0.337	1.536	0.345	1.295	0.334
D-Mannitol	0.809	0.810	1.667	0.603	1.881	0.174	2.017	0.136	1.993	0.134
4-Hydroxy Benzoic acid	0.453	0.771	0.925	0.538	1.722	0.362	1.823	0.109	1.636	0.341
L-Serine	0.586	0.798	1.414	0.609	1.676	0.192	1.691	0.286	1.788	0.266
α-Cyclodextrin	0.380	0.654	0.594	0.597	1.353	0.480	0.745	0.658	0.615	0.509
N-Acetyl-D-Glucosamine	0.773	0.706	1.230	0.537	1.694	0.250	1.823	0.154	1.883	0.059
γ-Hydroxybyturic acid	0.324	0.546	0.593	0.563	1.363	0.403	1.149	0.526	1.377	0.151
L-Threonine	0.232	0.281	0.436	0.422	0.463	0.201	0.306	0.244	0.377	0.097
Glycogen	0.348	0.417	0.552	0.377	1.085	0.540	0.444	0.420	0.465	0.607
D-Glucosaminic acid	0.562	0.773	1.146	0.546	1.631	0.316	1.590	0.467	1.689	0.137
Itaconic Acid	0.502	0.689	0.652	0.633	0.980	0.657	1.513	0.440	1.614	0.369
Glycyl-L-Glutamic acid	0.381	0.453	0.411	0.326	0.769	0.373	0.572	0.257	0.640	0.185
D-Cellobiose	0.784	0.727	1.235	0.660	1.716	0.347	1.765	0.402	1.844	0.209
Glucose-1-Phosphate	0.498	0.680	0.670	0.647	1.247	0.451	1.164	0.357	1.286	0.118
α-Ketobutyric acid	0.065	0.075	0.208	0.235	0.232	0.365	0.049	0.075	0.079	0.148
Phenyl ethylamine	0.472	0.869	0.301	0.470	1.196	0.677	1.560	0.463	1.472	0.506
α-D-Lactose	0.569	0.783	0.932	0.495	1.319	0.490	1.228	0.583	1.141	0.769
D,L- α-Glycerol Phosphate	0.167	0.209	0.266	0.227	0.388	0.135	0.256	0.064	0.255	0.104
D-Malic acid	0.491	0.590	0.561	0.348	1.156	0.510	1.340	0.531	1.349	0.332
Putrescine	0.324	0.556	0.794	0.413	0.938	0.190	1.154	0.210	1.040	0.216
Mean AWCD and SD	0.482	0.637	0.850	0.487	1.259	0.356	1.250	0.340	1.229	0.289

The metabolic response diagram shows that the group of carbohydrates are the most utilized followed by carboxylic acids and polymers. (Fig 5)

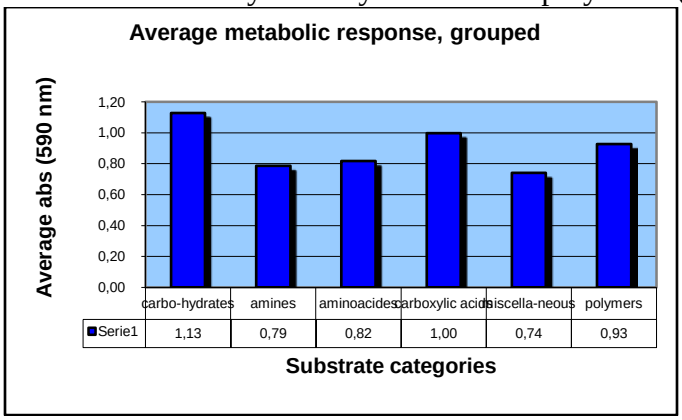


Fig.5. The average relative metabolic response of all land uses together at 96 h incubation, divided in six substrate categories and their respective number; Carbohydrates (7), Amines/amides (2), Amino acids (6), Carboxylic acids (9), miscellaneous (3) and Polymers (4).

Fig. 6 a-e show the metabolized substrates sorted in descending order with the most metabolized substrate to the left. The higher the bars, the quicker the decomposition and therefore higher abundance and activity of bacteria that can break down that particular substrate. The decomposition is slower in ER than for instance FO but all substrates can be utilised in all land uses in roughly the same proportion. The Shannon-Weaver Diversity Index (Table 5) considers the number of substrates utilised and their proportion of the total AWCD. The Index number differs very little between land uses, so the functional diversity is roughly the same in all soils tested.

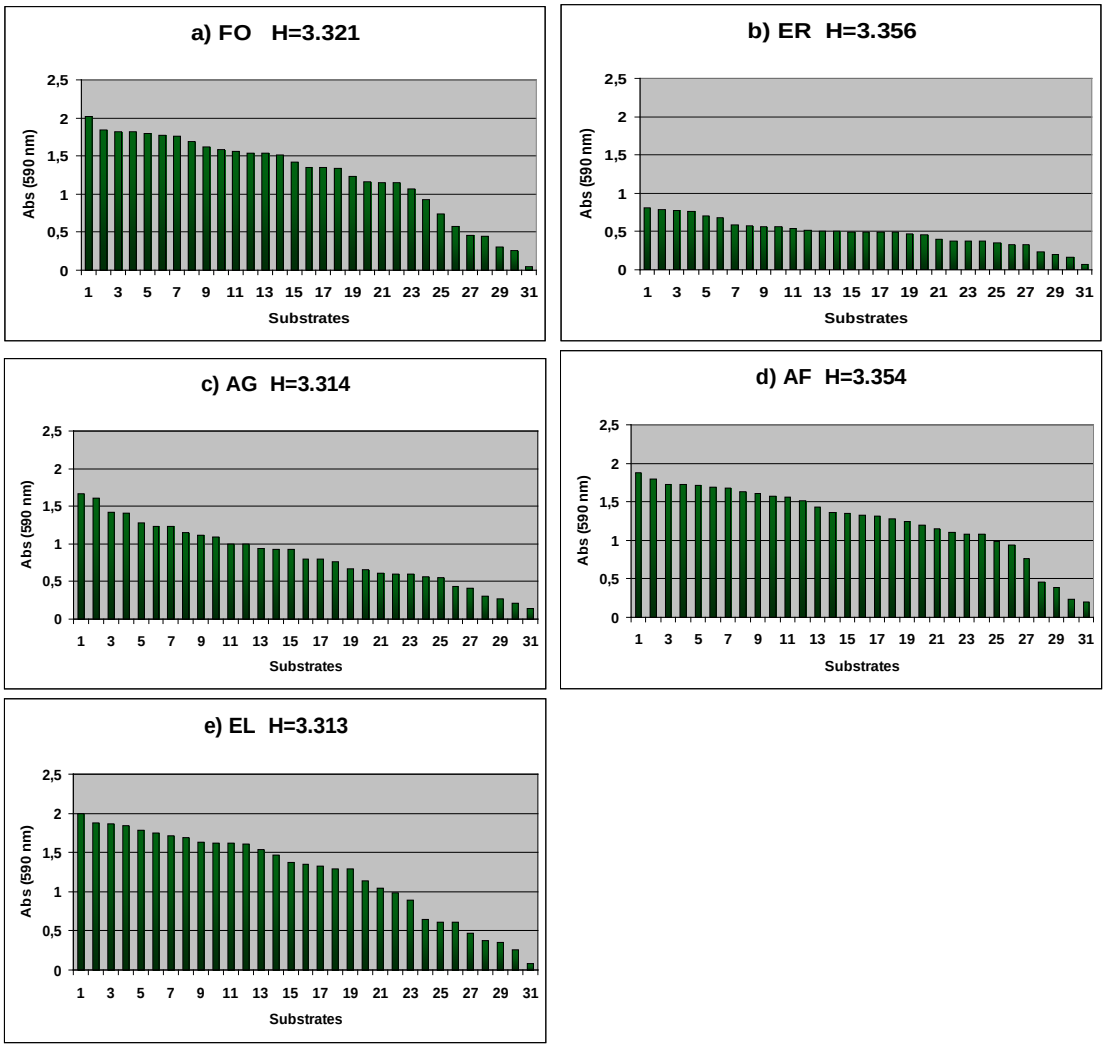


Fig. 6 a-e. All substrates after 96 h incubation, sorted in descending order with the most metabolized substrate to the left.

Land use	H′	SD
AF	3.354	0.032
AG	3.314	0.039
EL	3.313	0.039
ER	3.356	0.031
FO	3.321	0.038
Tot	3.354	0.032

Table 5. Shannon-Weaver Diversity Index (H′) and its standard deviation (SD). H′ is calculated on values for every single substrate after 96 h incubation. Shannon-Weaver Diversity Index was calculated according to Fowler *et al.* (2006) : $H' = -\Sigma\, p_i \ln p_i$ where p_i in this case is the proportion of AWCD of a particular substrate to the AWCD of all substrates of a certain land use (Fowler et al., 2006; Olsson et al., 2005).

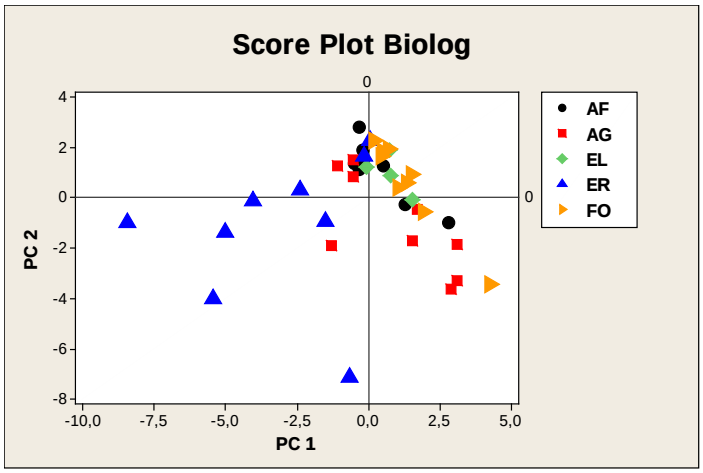


Fig. 7. PCA Score plot of all 31different substrates of Biolog EcoPlate divided in the different land uses. (n=9 for AF, AG, ER and FO, n=4 for EL) The first component explains 19.0% of the total variation and the second component 14.3%. The score plot was made with Multivariate Principal Component Analysis.

The score plot in Fig. 7 shows that ER is separated from the other land uses by the first and second PCA component of the substrate profile. AF, FO and EL are grouped closely together. The variability explained by the first two PCA components was 33.3 %. AWCD was significantly correlated with P, while pH was correlated to almost all variables (Table 6). Total N was significantly correlated to P, C, MBC, MBN, and Altitude but not with pH. Microbial N and C were significantly correlated with each other.

Table 6. Correlation matrix on chemical and biological soil properties: pH, total soil nitrogen (N), extractable soil phosphorus (P), Nitrates, total soil carbon (C), moisture, sand, clay, silt, Microbial Biomass carbon (MBC), Microbial Biomass nitrogen (MBN), altitude (Alt), and Average Well Colour Development from Biolog EcoPlates (AWCD). Their respective *p-values* are shown in italics.

	CORRELATION VALUES/ *P-values*					
	pH	**N**	**P**	**Nitrate**	**C**	**Moist**
N	0.592					
	0.000					
P	0.411	0.359				
	0.008	*0.023*				
Nitrate	-0.328	-0.252	-0.361			
	0.039	*0.116*	*0.022*			
C	0.566	0.907	0.407	-0.226		
	0.000	*0.000*	*0.009*	*0.161*		
Moist	0.291	0.643	0.041	-0.202	0.732	
	0.069	*0.000*	*0.802*	*0.211*	*0.000*	
Sand	0.521	0.701	0.312	-0.101	0.725	0.473
	0.001	*0.000*	*0.050*	*0.535*	*0.000*	*0.002*
Clay	-0.532	-0.711	-0.404	0.079	-0.721	-0.476
	0.000	*0.000*	*0.010*	*0.628*	*0.000*	*0.002*
Silt	0.040	0.039	0.228	0.042	0.007	0.017
	0.807	*0.811*	*0.158*	*0.796*	*0.000*	*0.919*
MBC	0.339	0.726	-0.016	-0.068	0.711	0.785
	0.032	*0.000*	*0.921*	*0.676*	*0.000*	*0.000*
MBN	0.398	0.771	-0.040	-0.044	0.757	0.816
	0.011	*0.000*	*0.804*	*0.676*	*0.000*	*0.000*
Alt	0.356	0.567	0.235	-0.079	0.524	0.496
	0.024	*0.000*	*0.144*	*0.630*	*0.001*	*0.001*
AWCD	-0,221	-0,155	-0,362	0,102	-0,226	-0,004
	0,171	*0,339*	*0,022*	*0,533*	*0,161*	*0,980*

Table 6 continued.

	CORRELATION VALUES/ *P-values*					
	Sand	**Clay**	**Silt**	**MBC**	**MBN**	**Alt**
Clay	-0.908					
	0.000					
Silt	-0.186	-0.244				
	0.252	*0.129*				
MBC	0.626	-0.589	-0.079			
	0.000	*0.000*	*0.629*			
MBN	0.615	-0.569	-0.100	0.966		
	0.000	*0.000*	*0.539*	*0.000*		
Alt	0.423	-0.487	0.155	0.527	0.520	
	0.007	*0.001*	*0.339*	*0.000*	*0.001*	
AWCD	-0,155	0,229	-0,179	0,030	0,027	-0,104
	0,339	*0,155*	*0,269*	*0,855*	*0,870*	*0,522*

DISCUSSION

The aim of this study was to compare soil conditions and substrate utilisation capacity of the bacterial community in soils under different land use regimes. This study shows that all land types where trees grow, naturally or planted, have higher substrate utilisation capacity than plain maize fields. The eroded land, not vegetated at all, has significantly lower capacity than all the other land uses. The difference between AG and AF was not significant throughout but there was a trend that AG was always slightly separated from AF. Many similarities in substrate utilisation of carbohydrates, amino acids and polymers could also be found, shown in Fig.4. There was a correlation between substrate utilisation capacity and microbial C and N indicating that higher microbial population densities and biomass give higher substrate utilisation capacity, i.e., faster decomposition of organic matter. The Shannon-Weaver Diversity Index can be used to quantify diversity, i.e., the distribution of carbon source utilisation by soil microbial communities (Harch et.al., 1997). The small differences in Shannon-Weaver Diversity Indexes show that all 31 substrates of Biolog EcoPlate were metabolised in all soils with approximately the same relative relationships in speed and colour development. This gives similar indexes for H' in all land uses. The total microbial activity differed, which is shown in AWCD. The small differences in the Index can also mean that this particular index is an insensitive way to measure the microbial activity.

When forested land is cultivated, it doesn't seem to eliminate microbial function even if the microbial composition will change. As long as there is a broad spectrum of species available, others can take advantage of the changed conditions of altered land use and the process of decomposition can continue (Griffiths et al., 2001). The stability and sustainability of an ecosystem may not come from a certain array of species, but from the presence of redundancy to ensure resilience (Loreau et al., 2003; Susilo et al., 2004). The range of microbes omnipresent in the soil is huge but reacts negatively to disturbance.

When changes occur and new vegetation comes in place, root exudates become different and the microbial community changes with it (Tate, 1995).

The trees used in agroforestry systems are often multipurpose trees that give a variety of outputs such as: fruits and nuts, fodder, fuel wood, building material, honey, windbreaks, shade and medicine. This can provide an extra income for small-scale farmers and improve food security but also raise food nutritional content and thus reducing the risk for diseases. The drawback with trees and crops on the same land unit can be competition for light, water and nutrients. Mostly, the tree roots go deeper than any roots of field crop and draw nutrients and water from a depth that plants can never reach. But trees do also have shallow roots (www.worldagroforestrycentre.org, 2008-01-11). The work performed by ViAFP and other similar organisations is aimed at providing help for the human population, not just now but for generations to come. The spread of knowledge will assist in providing food security and fight malnutrition and hunger. The diversity that is built into the agroforestry concept provides a sustainable and resilient cultivating system that can also improve fertility and functional capacity and biodiversity. It seems that agroforestry practices have great potential to increase the organic carbon content both above and below ground and thus reduce soil degradation and erosion. The largest gain would be to turn pastures and cropland, like maize fields into tree-based agroforestry systems. Improved fallows with leguminous trees or herbaceous shrubs can add 100-200 kg of nitrogen $ha^{-1}y^{-1}$. N-fixing trees, green manure and adding compost can increase the humus and carbon content of the soil and moisture retention and improve soil fertility and productivity (Mutuo et al. 2005; Amadalo et al., 2003; Swinkels et al., 1996). In a comparative study of agroforestry on farms connected to ViAFP in Uganda, analysis of fatty acid composition was used. This method analyses abundance of different systematic groups of prokaryotic and eukaryotic organisms. In that study, no changes in microbial community composition were detected after changing management methods from traditional to agroforestry practices. The different management systems studied in Uganda was more similar than those that we studied in Kenya. It seems that trees are more frequently incorporated in crop fields by old practices in Uganda. The differences found were more of socioeconomic and educational character (Ulfsax, 2007).

According to my findings there is a significant difference in functional capacity between land with and without trees. This confirms my first hypothesis that deforestation, agricultural cultivation and overuse of land resources leads to decreasing functional capacity of soil microorganisms. Even though it isn't significant throughout, the difference between AG and AF follows a clear pattern with the values of AF closely related to FO and EL. The ability to metabolise the 31 substrates tested remained also in the eroded soil, though with lower speed. I think that agroforestry practices and similar management techniques definitely can improve soil fertility and restore the soil functional capacity, giving support for my second hypothesis. A study by Bossio et al. (2005) also found specific differences between wooded and agricultural soil where management practices affected both microbial comunity composition and function.

One of the drawbacks with cultivating plates like Biolog is that only a small fraction, as little as 1 %, of the soil microbial community actually can utilise the carbon sources on

the plates. Many bacteria cannot reduce tetrazolium dye or be cultivated at all and that must be considered when using methods like Biolog EcoPlate.Those bacteria that cannot adapt to using the substrates provided will be out competed or eliminated by bacteria capable of growing fast in the high nutrient environment (Preston-Mafham et al., 2002). However, many species have the ability to adapt their metabolism to the substrates available and therefore the composition and size of the soil microbial community undergo changes over time (Susilo et al., 2004) To widen the examination several types of Biolog plates can be used that also can detect the activity of fungi. In this case we used Biolog EcoPlates because they provide a lot of data fast and easy, an advantage since available time in the lab was short. Another analysis that would have been a good complement was Denaturated Gradient Gel Electrophoresis (DGGE) to get DNA samples. Fatty acid analysis would also be useful. We planned to do it and soil samples that can be used for this are stored in the TSBF freezer for possible future analysis.

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APPENDIX

Substrates, name and type		
A2N	β-Methyl-D-Glucoside	Carbohydrates
A3N	D-Galactonic-acid γ-Lactone	Carboxylic acid
A4N	L-Arginine	Amino acids
B1N	Pyruvic acid Methyl Ester	Miscellaneous
B2N	D-Xylose	Carbohydrates
B3N	D-Galacturonic acid	Carboxylic acid
B4N	L-Asparagine	Amino acids
C1N	Tween 40	Polymers
C2N	i-Erythritol	Carbohydrates
C3N	2-Hydroxi-Benzoic acid	Carboxylic acid
C4N	L-Phenylalnine	Amino acids
D1N	Tween 80	Polymers
D2N	D-Mannitol	Carbohydrates
D3N	4-Hydroxy Benzoic acid	Carboxylic acid
D4N	L-Serine	Amino acids
E1N	α-Cyclodextrin	Polymers
E2N	N-Acetyl-D-Glucosamine	Carbohydrates
E3N	γ-Hydroxybyturic acid	Carboxylic acid
E4N	L-Threonine	Amino acids
F1N	Glycogen	Polymers
F2N	D-Glucosaminic acid	Carboxylic acid
F3N	Itaconic Acid	Carboxylic acid
F4N	Glycyl-L-Glutamic acid	Amino acids
G1N	D-Cellobiose	Carbohydrates
G2N	Glucose-1-Phosphate	Miscellaneous
G3N	α-Ketobutyric acid	Carboxylic acid
G4N	Phenyl ethylamine	Amines/amides
H1N	α-D-Lactose	Carbohydrates
H2N	D,L- α-Glycerol Phosphate	Miscellaneous
H3N	D-Malic acid	Carboxylic acid
H4N	Putrescine	Amines/amides

Interviews of landusers

Questions asked:
Who owns the land you live on?
What was the land used for before your time?
Which agricultural practices do you implement?
Do you use pesticides or fertilizers?
Which crops have you planted?
Which tree species do you have on the land and what do you use them for?
Do you experience constraints and if, which?
Do you have access to firewood and how?
Which livestock do you have if any?
How often do you have contact with a ViAFP extension worker?