Impacts of agricultural land use histories on soil organic matter dynamics and related properties of Savannah soils in North Cameroon

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3. MATERIALS AND METHODS

3.1 Field procedures

3.1.1 Site selection and soil profile description

Diagnostic studies were conducted on the Diamare plain, which represents the area most exploited for agricultural production in North Cameroon. This plain also contains the largest area of land containing marginal and degraded soils (Brabant and Gavaud, 1985) as shown by figure 1.2. Sites were selected to represent the main land use histories (LUH) on the main soils between latitudes 10 and 11°N. These considerations were deemed necessary to provide a basis of inference for the development and transfer of appropriate soil organic matter management technologies applicable at regional scale.

Soil maps and available literature on farming systems in North Cameroon (USAID, 1974; Brabant and Gavaud, 1985) were used to identify potential sites. In general, the medium and fine textured soils on the inselberg peneplain are shallow, highly exploited and much more susceptible to degradation than the deep fine textured soils on the lacustrine plains (USAID, 1974; Brabant and Gavaud, 1985). The criteria for selecting potential sites included uniformity in soil type and parent material and diversity in land use types.

Field studies in collaboration with agricultural extension agents and local farmers ensued to characterize the sites and land use histories. Principles of participatory learning and action (PLA) were used. We discussed with the local population who established participatory maps on which the soils, current cropland, pastures, degraded soils, seasonal rivers and village quarters were delimited.

For the diagnostic studies we selected uniformly sloping land of an average area of about one square kilometre with relatively homogenous soil and having clusters of the main land use histories. The dominant LUH based on duration and spatial extension was chosen for the comparative study of the impact of land use history on organic matter and related soil properties. An area of 0.25 hectare was delimited on each LUH for use in the comparative studies, representing the average field size in the region. The selected LUHs on each soil, were generally non-contiguous and very close (<50 m) to each other. On each LUH, soil samples were collected from three depths in the surface layer (0-5, 5-15 and 15-30 cm). On the same plots, soil profiles were described in one meter deep pits, according to standards recommended by FAO Guidelines for Soil Profile Descriptions (FAO, 1966). The depth of one meter was chosen because our interest was to study soil organic matter dynamics in the surface and the rootzone layer of annual crops.

3.1.2 Sampling schemes

The field research comprised two stages connected with the two stages in the overall project; the general characterisation and a detailed analytical research.

Sampling scheme for general characterisation

The general characterisation was based on 24 profiles of the main land use types distributed within seven villages. The 24 profiles were all on bush farms where compost or manure was not added into the agricultural soils as farmers explained that they had difficulties in transporting manure from their homes to the distant bush farms. The objective was to determine the sites with evidence of impact of land use on the properties of the soils and to select the most suitable sites for detailed studies during the second stage of the research.
Within the 0.25 hectare of land delimited on each LUH four spots were randomly selected and on each spot soil samples were collected from three close points within a circle of less than 2 metres radius. The sampling depths were 0-5, 5-15 and 15-30 cm. From each depth three sub samples were bulked into one composite and mixed, from where one replicate sample was taken. In this manner, four replicates representing 12 sub-samples were collected for each depth from the four randomly selected spots. We describe this as micro-scale composite sampling (figure 3.1). The statistical significance of differences in the impacts of land use on between group means of the soil properties was therefore determined separately for each soil type, using one-way ANOVA (SPSS version 9). All soil samples were collected in the field in November 1996; two months after the rainy season when the soils were quite dry, and organic matter levels in the surface soil layers were considered to be relatively high.

Within the same land area, the most representative spot was chosen and a pit dug for soil profile description. From each horizon, bulk soil samples from two opposite sides were collected, mixed and a composite sample collected for use in determining particle size distribution and chemical properties. Chemical analyses were done for soil samples from all the horizons. Some samples were analysed for their mineralogical composition.

The results of the general diagnostic study lead to the inventory of spatial and temporal distribution of current land use types and land use histories. Four sites were selected (figure 2.1) over a stretch of about 120 km, along the South-North direction between latitudes 10 and 11°N, for the detailed analytical studies. These sites are along a climatic and geologic gradient. From South to North the names of the sites and soil types are: Garey Chromic Vertisol and Garey Eutric Planosol, located at latitude 10°N and longitude 14° 20' E, 15 km south-west of Kaele; Mouda Chromic Luvisol, located at latitude 10° 23' N and longitude 14° 12' E, 28 km south-west of Maroua and Djapai Hydromorphic Vertisol, located at 10° 26' N and longitude 14° 19' E, 32 km southeast Maroua. The characteristics of the main LUH chosen for the detailed analytical study are shown in appendix 1a.

**Soil sampling for detailed analysis**

Each of the four sites selected (figure 2.1) had a relatively homogeneous soil type with the representative land use histories. The number of land use histories on each site varied between 2 and 3 constituting ten soil profiles in all. Soil samples from the 0-5 cm soil layer were collected for the detailed analytical studies that consisted of assessing the impact of land use history on: a) the stability of macro aggregates to water drop impacts; b) the dynamics of organic matter fractions; c) aggregate size distribution and micromorphology; d) the 13Carbon
abundance. The detailed studies were limited to the 0-5 cm soil layers because significant impacts of land use changes have been demonstrated to occur particularly in the topsoil rather than in the subsoil (Beare et al. 1994).

**Soil samples for macro aggregate stability, organic matter size fractions and \(^{13}\)Carbon abundance**

In November 1997 soil samples were collected from the 0-5 cm surface layer using a flat-bottomed spade. On each land use history within the same area of 0.25 hectares of land, used for previous sampling, four random spots were selected. On each spot, a soil sample of about 500 g was collected avoiding crushing of the aggregates. Four replicates were thus collected from each field. The soil samples were air-dried at 25 to 35 °C and sieved to obtain 4-4.8 mm macro aggregates that were used to assess the stability to water drop impacts.

Within the same four spots and on same day soil samples were collected by a micro-scale composite sampling procedure. These samples were air dried, ground and sieved over a 2 mm sieve. About 200 g of each fine soil fraction was put in a plastic bag, which was sealed and perforated. The samples were transported to the University of Amsterdam and stored in the cold room at less than 4 °C for subsequent use for the determination of organic matter size fractions and \(^{13}\)Carbon abundance in the organo-mineral size fractions.

**Soil samples for thin section analysis (micromorphological studies)**

The samples were collected from the cultivated and fallow land use histories on the Chromic Luvisol and Eutric Planosol in December 1997. Within the less than 2 metre circle on two of the four random spots where micro-scale composite sampling was executed during the same month, two soil blocks of 5 x 3 x 5 cm were carefully cut from the top 0-5cm soil layer using a saw. These hardened blocks were carefully trimmed to fit into locally fabricated Kubiena boxes, each 6 x 4 x 6 cm. Two soil replicates were collected from each land use history for thin section analysis.

**Soil samples for aggregate size distribution**

Samples were collected on the same cultivated and fallow land use histories on the Chromic Luvisol and Eutric Planosol in a similar manner as samples for thin section analysis. These were collected during the dry season in November 1998. Within the same 0.25 hectare area on each soil used for the previous samplings 4 spots were selected randomly and soil samples collected from 0-5 cm depth using a flat-bottomed spade. In the period of sampling the soils were quite dry. We collected dry soil blocks 5 x 5 x 5 cm to prevent crushing of samples. The samples were further air dried at 25 to 35 °C in the laboratory and later transported to Amsterdam for analysis.

3.2 Analytical Methods used during the general characterisation stage

**Sample Preparation**

The samples collected from surface layers and deeper horizons of the soil profiles were placed in plastic bags and subsequently air dried in the laboratory at 25 to 35 °C air temperatures. The dried soils were ground, sieved through 2 and 1 mm sieves for the soil horizons and surface layers, respectively.

Particle size distribution of samples from soil horizons was determined by the standard pipette method in the Soils and Plant Analytical Laboratory of the ‘Institut de la Recherche Agronomique pour le Development’ (IRAD) at Ekona. Chemical analyses of the soil samples from surface layers and horizons were executed in the Soil Chemistry Laboratory of the
International Institute for Tropical Agriculture (IITA) at Yaounde.

Particle Size Distribution
The Particle size distribution of the soil samples from the various horizons was determined by the standard pipette method in which organic matter was oxidised using hydrogen peroxide (Van Reeuwijk, 1995).

Bulk Density (gcm⁻³)
Dry bulk densities were determined in triplicate for the 0-10 and 10-20 cm surface layers. Undisturbed soil cores were collected using 100cm³ steel rings (Head, 1984). The samples were oven dried at 105 °C for 24 hours and the weight measured using a 0.001 precision balance. Bulk density (gcm⁻³) was calculated from the oven dry weight and volume of the ring.

Soil pH and Electrical Conductivity
The soil pH and electrical conductivity (µS/cm) were determined in 1:2.5 (w/v), soil/distilled deionised water ratio, using the pH meter with a glass electrode and an EC meter respectively (Van Reeuwijk, 1995).

Total Organic Nitrogen (%)
Total organic nitrogen was determined using the simple digestion procedure for estimating nitrogen in soils and sediments by Nelson and Sommers, (1972).

Total Organic Carbon (%)
Total organic carbon (%) was determined using an improvement (Heanes, 1984) of the Walkey-Black wet digestion method for the determination of organic carbon over the range 0.2 - 5.5% in air dry soil samples.

Extractable Bases
Extractable bases (calcium, magnesium, sodium, potassium) were determined using the Mehlich 3 extraction procedure. Calcium, magnesium were determined using the Atomic Absorption Spectrophotometer and potassium and sodium by flame emission spectrometry (Anderson and Ingram, 1989).

Cation Exchange Capacity (CEC)
The cation exchange capacity (cmolc/Kg of soil) was determined using the sodium acetate method (Polemio and Rhoades, 1977), known to be suited for semi-arid and arid land soils.

3.3 Special analytical methods during the detailed analytical stage

3.3.1. Stability of macro aggregates to water drop impacts (WDI)

The methodology for the water drop impact test by Low, (1967) improved by Imeson and Vis (1984) was adopted to assess the stability to impacts of water drops of macro aggregates from the 0-5 cm soil layer of selected land use histories. Bulk, air dried soil aggregates free from gravel were subjected to a pre-treatment involving gentle sieving the 4-4.8 mm fraction of soil aggregates from the bulk samples. The 4-4.8mm macro aggregates obtained were slowly moistened at pH 1 for 24 hours with distilled water.

In this drop test, a supply system with a constant head was fitted to a burette. Water drops 0.1 g in weight (5.8 mm diameter) were obtained by fitting silicon tubing to the burette nozzle.
The water drops were allowed to fall 1 m through a 15 cm diameter polythene pipe with an impact velocity of 4.27 m\textsuperscript{s}\textsuperscript{-1} on an aggregate of 4.0 to 4.8 mm diameter placed on a 2.8 mm metal sieve.

The test procedure simply involved Counting the Number of water Drop (CND) impacts required to disrupt the aggregate sufficiently for it to pass through the 2.8 mm sieve. A reduction of 30\% in aggregate size was considered as an adequate definition of breakdown (Imeson and Jungerius, 1976; Grieve, 1979, as quoted by Farres and Cousen, 1984). This was used to calculate the macro aggregate stability index $\text{ASI}_{50}$, which is the kinetic energy of drop impacts necessary to disintegrate 50\% of the aggregates out of a sample of 20 aggregates. From each land use history, two sets of aggregates were collected for the stability test. From each of these sets, four subsets were used for the stability test. The test was replicated for 20 aggregates from each subset. Mean values for the eight subsets, of the \% aggregates (out of 20 macro aggregates) surviving drop impacts (at 5, 10, 15, 20, 25, 30, 35, 40, 45 and 50 WDI) were used for graphical presentation of the results, calculation of the macro aggregate stability index $\text{ASI}_{50}$ and for statistical analysis. During the water drop test, mechanisms of aggregate breakdown were observed and described. The extent of aggregate hierarchy in the soils tested was inferred from these observations.

The Kruskal-Wallis one-way analysis of variance (SPSS version 9) was used separately in each of the soil types to test for the significance of differences in the stability of macro aggregates from the various land use histories to water drop impacts. This non-parametric test was recommended (Cammeraat and Imeson, 1998) since the 20 replicates of the aggregate stability determinations were non-normally distributed. The significance of the differences between group mean values of the proportion of aggregates surviving drop impacts was determined at 15, 20 and 25 WDI, because results showed that 50\% of the aggregates that survived the WDI generally occurred within this range.

### 3.3.2 Soil organic matter fractionation

The physical method, combining particle size fractionation of the sand sized (53-2000 \text{\textmu}m) organo-mineral fraction with sedimentary fractionation of the finer (0-53, 0-20 and 0-2 \text{\textmu}m) ‘aliquot’ fractions (Gavinelli et al. 1995), was used in this study (figure 3.2). Ultrasonic dispersion was applied to the 0-53 \text{\textmu}m soil suspension to disperse the silt and clay micro aggregates. The fractionation method consists of three stages (figure 3.2):

- a dispersion treatment to disintegrate the 53-2000 \text{\textmu}m aggregates.
- wet-sieving to obtain the sand sized (53-2000 \text{\textmu}m) fraction.
- dispersion of the <53 \text{\textmu}m aggregates followed by sedimentation and sampling of aliquots, which were centrifuged to obtain the 0-53, 0-20 and 0-2 \text{\textmu}m fractions.

**Dispersion and wet sieving**

The air dried soil (<2 mm) was dispersed in two steps to separate the 53-2000 \text{\textmu}m and the less than 53 \text{\textmu}m sized fractions (Gavinelli et al. 1995).

#### a) Separation of the sand (53-2000 \text{\textmu}m) size fraction.

20 g of air dried <2 mm soil for the Vertisols and 40 g for Luvisol and Planosol were each pre-soaked overnight at 4 °C in 240 ml of deionized water with 0.5 g of sodium hexametaphosphate (HMP) as dispersing agent. It was shaken with 5 agate balls on a mechanical shaker model ‘Gerhardt Laboshake LS 2/5 RO 2/5’ at 120 revolutions per minute for 3 hours (Vertisols) or 1 hour (Planosol and Luvisol). The soil suspension was wet sieved through the 53 \text{\textmu}m sieve. The sand fraction (53-2000 \text{\textmu}m) obtained was freeze-
b) Separation of the <53 μm fractions by sedimentation ('Aliquot Method').

The fractions remaining on the sieves were washed with deionised water and the washings added to the 0-53 μm suspension. This suspension was made up to 1L in a glass beaker and ultrasonicated at 60 watts, with a probe type ultrasound generating unit 'Sonifier Model B-12 Branson Ultrasonics 1975' with maximum power output of 350 watts. The probe was placed at 3 cm from the bottom of the beaker, operated for 7 minutes at a setting of 7 on the intensity dial (ranging from 0 to 10).

Each dispersed 0-53 μm suspension was immediately transferred into the 1 litter glass cylinder, shaken by hand (30 end over end tumblings) and 100 ml of the suspension withdrawn immediately from the centre of cylinder. This constituted an aliquot of the entire 0-53 μm fraction. After 5 minutes of settling, a second aliquot representing the 0-20 μm fraction was collected by siphoning about 10 cm from the top of the suspension depending on its temperature. After a settling time of 6.5 hours, a third aliquot was removed by siphoning about 7 cm from the top of the suspension depending on its temperature. This constituted the 0-2 μm fraction. Each of these fractions was flocculated with 100 μl of a saturated solution of 0.01M calcium chloride per 100 ml of organo-mineral suspension. The excess chloride in the suspension was removed by washing several times with de-ionised water and centrifugation at 2000 revolutions per minute for ten minutes. The supernatant was tested with silver nitrate solution and washing was stopped when no white precipitate was formed. The floccules were freeze-dried, oven dried at 60 °C for 24 hours, weighed and finely ground. The freeze-dried sand fraction was also oven dried at 60 °C for 24 hours, weighed and finely ground. Air dried soil (<2 mm) samples were also oven dried at 60 °C for 24 hours and finely ground. The soil (<2 mm) samples were tested (acid test) for carbonates and the results indicated that carbonates were absent.

**Determination of total C and N contents in size fractions and whole soil (<2 mm)**

Total C and N in organo-mineral size fractions and whole soil samples were determined using an EL Micro Elemental Analyser. The total carbon measured, represented total organic carbon as these soil samples showed a negative test for carbonates.

Each soil sample was dried at 105 °C overnight (16 hours) and 50 mg put in a tin cup, was weighed on an electronic balance. The tin cup was folded and inserted by means of a sample feeder into a vertically positioned quartz glass combustion tube containing helium and oxygen. The total organic matter in the sample was oxidised in a highly oxygenated helium atmosphere, to carbon dioxide and nitrogen oxides. Other compounds produced by the combustion were chemically bound to suitable absorbents and removed from the gas flow. The remaining gas mixture of CO₂ and N₂ was guided to an adsorption column in which the CO₂ was temporarily bound while nitrogen was flushed with helium into the detector (thermal conductance detector TCD). When the measuring of the nitrogen was completed, the adsorption column charged with CO₂ was heated to 130 °C, causing the CO₂ to be rapidly desorbed and then flushed with helium into the TCD.

The measuring signals of the detector caused by the components were compared with the signals of a standard material of which the carbon and nitrogen contents were known exactly (thus calibration). For this calibration, acetic acid was used. The resulting total organic carbon and nitrogen contents were expressed as percentage (%).

**Statistical analysis**

For each soil type, the level of significance of the differences in between group means of organic carbon and nitrogen in each size fraction of soil samples from the land use histories
was determined. Additionally the significance of differences between organic carbon or nitrogen in the sand fraction and fine fractions was determined separately for each land use

\[ X_g < 2\text{mm soil in 240 ml of deionised water + 0.5g HMP} + 5 \text{ agate balls} \]

Vertisol: \( X = 20 \text{g} \)
Luvisol, Planosol: \( X = 40 \text{g} \)
24 hours overnight at 4°C

Y hours of mechanical dispersion of the 240 ml of 0-2mm soil suspension

Vertisol: \( Y = 3 \text{ hours} \)
Luvisol, Planosol: \( Y = 1 \text{ hour} \)

Sieving 0-2000μm suspension through 53 μm sieve

53-2000μm size fraction

Freeze dried

0-53μm suspension. Ultrasoundication of 1000ml of suspension at 60 watts for 7 minutes

Sedimentary fractionation of 0-53 μm suspension

Aliquot of 0-53 μm size fraction

Flocculation by 100μl 0.01M CaCl₂/100ml suspension

Centrifuge washing

Freeze dried

Aliquot of 0-20 μm size fraction

Flocculation

Centrifuge washing

Freeze dried

Aliquot of 0-2 μm size fraction

Flocculation

Centrifuge washing

Freeze dried

Figure 3.2: Schematic presentation of the Particle Size Fractionation by Aliquot Method (Gavinelli et al. 1995) used in this study.
history. The data was analysed by one-way ANOVA, followed by multiple comparisons of the LSD test at 0.05 level of significance using the SPSS version 9.

3.3.3 Mineralogical analysis

X-ray diffraction, the most widely used technique for the identification and characterisation of clay minerals, was used in this study. The diffractometer was used to obtain the diffraction pattern from the clay minerals.

X-ray Diffraction

X-ray analyses were carried out on disoriented clay. Diffractograms were made of well-oriented clay samples saturated with Mg, Mg-ethylene glycol, K, K heated to 300 °C and K heated to 550 °C respectively. The mineralogical composition was of the samples was estimated from the height of the peaks in the diffractograms and by reflection intensities on the Guinier-de Wolff camera films.

The sample specimens were examined in the following order for identification of the mineral types:

1. Mg-saturated, air dried at 55% relative air humidity orientated sample (scan from 2° 2θ to 30° 2θ).
2. Mg-saturated, glycol solvated orientated sample (scan from 2° 2θ to 30° 2θ).
3. K-saturated, air-dried at 55% relative humidity orientated sample (scan from 2° 2θ to 30° 2θ).
4. K-saturated, heated in oven at 300°C orientated sample (scan from 2° 2θ to 30° 2θ).
5. K-saturated, heated in oven at 550°C orientated sample (scan from 2° 2θ to 15° 2θ).

The peaks on the diffraction patterns were identified, d spacing assigned and the minerals identified by comparing and interpreting diffractograms (Borchardt, 1989).

3.3.4 Aggregate size distribution and mean weight diameter of water stable aggregates

In the work on macro aggregate stability and aggregate size distribution by wet sieving Yoder's method (1936) or a modification of it has been applied, as shown in table 7.1.

Three main approaches are used to characterise the stability of aggregates to slaking and to determine the aggregate size fractions based on specific aggregate size or whole soil analysis (Feller et al. 1996). The most commonly used method for this approach is based on single or multiple sieve techniques, with considerable variation in the sieve sizes and number as shown in table 7.1. 250 μm has been considered as the boundary between macro (>250 μm) and micro (<250 μm) aggregates (Edwards and Bremner 1967; Tisdall and Oades, 1982; Angers, 1992; Beare and Bruce, 1993).

The second method to characterise WSA is used in situations where soils are high in swelling clays and exchangeable sodium. It is based on the measurement of the 'dispersed' fractions (0-2 or 0-20 μm) (Oliveira et al., 1983; Goldberg et al. 1988; quoted by Feller et al., 1996; Dalal, 1989). The third method to characterise WSA distribution consists of whole aggregate size analysis from the macro to the microaggregates (Albrecht et al., 1992b quoted by Feller et al., 1996).

In North Cameroon, soils often remain saturated for hours between two or more rainfall events during which slaking occurs. Additional rainfall causes transport and reorganisation of soil particles by vertical infiltration and erosion by lateral flow. This causes separation of the weakly bound micro aggregates and primary particles that are transported vertically clogging interparticle and interaggregate pores, which on drying form surface crusts.
(less than 20 mm) or hard-set layers (20 to 200 mm thick) in sandy loam soils. Simulation of field conditions is therefore difficult.

**Fractionation Method**

In this study, we used whole soil samples to separate six aggregate size fractions, in four replicates. The instrument based on Yoder's method and fabricated by the University of Wageningen (Martinez, 1995) had a set of five sieves each 10 cm in diameter, attached on each side of a balance arm. It was regulated to make 9 vertical strokes (up and down count as one stroke) in water per minute with a stroke length of 3.5 cm. During the oscillations, the 2.8 to 0.3 mm sieves were permanently in water, thus preventing entrapment of air bubbles.

100 grams of whole soil were oven dried at 60°C for 24 hours, cooled in a desiccator and broken manually into aggregates that were sieved carefully through 8 mm square holes with the liberated primary particles. All the soil material was placed on the top 4.75 mm sieve of a set of sieves (4.75, 2.8, 2.0, 1.0, and 0.3 mm) and immersed until the soil aggregates were completely immersed in deionised water in a transparent cylinder. After 30 minutes of wetting, the sample was sieved with two replicates being run simultaneously for 5 minutes.

The sieves were removed and the material in each sieve was washed into a 75 ml evaporating basin and dried on a water bath at 70 °C until all water evaporated. Then dried in an oven at 60 °C for 24 hours, allowed to cool in desiccators and weighed. Suspensions of micro aggregates smaller than 300 μm and primary particles were flocculated by adding calcium chloride solution (100 μl 0.01 M CaCl₂ per 100 ml of suspension), washed using a centrifuge, freeze dried and weighed.

Aggregate size in each of the six size fractions (< 300, 300-1000, 1000-2000, 2000-2800, 2800-4750 and 4750-8000 μm) was expressed as the percentage of the original 100 g of bulk soil used. Additionally, the results were also expressed as a mean weight diameter in mm (MWD), which is the sum of the percentage of soil remaining on each sieve after sieving for 5 minutes multiplied by the mean diameter (Haynes and Swift, 1990; Besnard et al., 1996).

**Statistical analysis**

The significance of the differences in percentage of aggregates in each size class between fallow and cultivated samples was determined separately for each soil type using analysis of variance (one-way ANOVA) technique (SPSS Version 9) to analyse the data.

**3.3.5 Micromorphological analysis**

**Preparation of thin sections**

The method is that used to prepare thin sections in the micromorphology laboratory of the department of Physical Geography and Soil Science of the University of Amsterdam. It is based on the general method described by Murphy (1986). The samples were air dried and impregnated with polyester resin. The thin sections were polished to about 30 μm.

**Description of microstructure**

The microstructure of the 0-5 cm soil layer was described and interpreted by microscopic examination of thin sections using the Leitz Polarised microscope. The descriptions were made following the procedures outlined by Brewer (1976), and Bullock et al., (1985). Each thin section was mounted on the Leica M420 research microscope and the microstructure was photographed using the digital camera model Leica DC 200 (Leica Microsystems) that was connected to a PC. The photos were edited and printed.
3.3.6 Carbon abundance

Organo-mineral size fractions (53-2000, 0-53, 0-20, and 0-2 μm) were obtained using the physical methodology described in 3.3.2. The organic carbon contents of these size fractions were measured at the IBED-FGB laboratory of the University of Amsterdam and at the Centre for Isotope Research (CIO). The $^{13}$C isotopic analysis was performed at the Centre for Isotope Research (CIO), University of Groningen. All sub-samples as mentioned above were analysed using a Carlo Erba 1500 Elemental Analyser in combination with a Micromass Optima continuous flow Isotope Ratio Mass Spectrometer. The $^{13}$C abundance in an equivalent mass of sample containing at least 300 μg C was determined in the CO$_2$ obtained by combustion of the organic matter in sealed quartz tubes with CuO at 900 °C. The evolved CO$_2$ was purified and analysed on the Isotope Ratio Mass Spectrometer. The local reference material GS-7, sucrose, was used as both elemental and isotopic standard material. This GS-7 is well-calibrated using calibration and reference materials provided by the International Atomic Energy Agency (Gonfiantini et al., 1995). The $^{13}$C in each sample size fraction was determined in quadruples.

The results are expressed as δ$^{13}$C(%o) units versus a VPDB standard according to international agreement (Coplen, 1995).

$$
\delta^{13}C\%o = (13R_{\text{sample}} / 13R_{\text{standard}} - 1), \quad \text{where} \quad R = ^{13}C/^{12}C.
$$

In total 160 samples were analysed in five batches. Each of the batches contained samples, references and blanks. The ratio number of samples: references were typically 5:1. Since the organic carbon content was known beforehand, the amount of sample could be chosen such that the amount of carbon present was about the same for all samples and references, i.e. around 300 μg. Only for those samples with extremely low carbon content (below 0.3 %) the maximum amount of sample (100 mg) did not allow for this amount of carbon. In the batch containing these samples, the amount of reference material was also reduced.

The standard deviation of a single measurement was better than 0.15 %. This makes the analytical error negligible compared to natural variability in the soil samples. As a side result, the Groningen analysis provided again the carbon percentage of the samples.

**Statistical Analysis**

For each soil type, the level of significance of the differences in between group means of $^{13}$C values in each size fraction of soil samples from the land use histories was determined. Additionally the significance of differences in $^{13}$C values between sand sized and fine sized fractions was determined separately for each land use history. The data was analysed by one-way ANOVA, followed by multiple comparisons of LSD test at 0.05 level of significance using the SPSS version 9.