

## Changes in Physical and Biological Soil Quality Indicators in a Tropical Crop System (Havana, Cuba) in Response to Different Agroecological Management Practices

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**ABSTRACT** / The objective of our study was to assess the response of physical (aggregate stability and bulk density) and biological (enzyme activities and microbial biomass) soil quality indicators to the adoption of agroecological management practices, such as the planting of forage species (forage area) and the rotation of local crops (polycrop area), carried out in a

representative tropical pasture on an integrated livestock-crop farm. The pasture system was used as control (pasture area). In all three areas, the values of water-soluble C were higher in the rainy season compared to the dry season. Pasture and forage areas had the highest percentage of stable aggregates in the rainy season, while polycrops developed soils with less stable aggregates. Soil bulk density was lower in the pasture and forage areas than in the polycrop area. In the pasture area, the microbial biomass C values, dehydrogenase, urease, protease-BAA, acid phosphatase, and  $\beta$ -glucosidase activities were higher than in the forage and polycrop areas, particularly in the dry season. The highest increase in the microbial biomass C in the rainy season, with respect to the dry season, was recorded in the pasture area (about 1.2-fold). In conclusion, the planting of forage species can be considered an effective practice for carrying out sustainable, integrated livestock-crop systems, due to its general maintenance of soil quality, while the adoption of polycrop rotations appears to be less favorable because it decreases soil quality.

In the semihumid tropics, the lack of rainfall during the dry period (lasting from 5 to 7 months) seriously limits pasture production and quality. Likewise, the majority of soils in these zones have low fertility, especially those dedicated to cattle breeding, where improved pasture establishment requires high levels of fertilizers and soil improvement. Since the early 1990s, integrated small- and medium-scale agroecological crop-livestock-tree systems have been established in an effort to improve Cuban agriculture (Funes-Monzote and Monzote 2000). Practices like the planting of forage crops and trees such as *Leucaena leucocephala* for animal feeding, recycling of manure, and composting of organic waste are followed. Studies carried out by Funes-Monzote and Monzote (2000) showed that integrated agroecological systems can provide sufficient

capacity and potential to sustain increased productivity in terms of animal and crop production, based on available natural resource management alternatives.

Maintenance of soil quality is an integral part of agricultural sustainability (Stenberg 1999). Hence, to carry out sustainable farming systems, it is necessary to apply soil management practices that improve or maintain soil quality. Thus, there is a need to select soil properties that respond rapidly to changes in soil quality while a particular farming system is being carried out, in order to ascertain whether that system can be recommended or not. Soil organic matter influences a wide range of physical, chemical, and biological properties of soil and is considered by some authors as the most important indicator of soil quality (Bolinder and others 1999). The extent to which organic matter contributes to soil quality depends not only on organic matter quality, but also on soil fauna activity and environmental conditions, in particular temperature and humidity, which condition mineralization processes through their effects on microbial activity in the soil

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(Ouédraogo and others 2001). Early changes in total soil organic matter (SOM) may be small and detectable only by monitoring the active fractions of SOM such as the labile C fractions and microbial biomass C. Hence, the study of soil microbial activity is required to assess improvements in soil quality (Jimenez and others 2002). In this way, soil enzyme activities and microbial biomass have been shown to be sensitive indicators of differences between reforestation methods (Caravaca and others 2002) and of changes produced by management practices, crops, additions of fertilizers or environmental conditions (Kandeler and others 1999). However, there are relatively few studies regarding the use of such parameters as indicators of soil quality in integrated agroecological systems.

Stable soil structure is an important requirement for the productivity of cultivated soils. Soil organic matter has a well-established role in the formation and stabilization of soil aggregates. However, changes in aggregate stability following land use changes have been observed without changes in total soil organic matter content, which indicates that only a small fraction of soil organic matter was involved (Puget and others 1999). In order to develop cropping systems that maintain or improve soil organic matter and soil structure, information is required regarding the specific effects of various crops on these properties; such information is surprisingly sparse, however.

The objective of our study was to assess the response of physical (aggregate stability and bulk density) and biological (enzyme activities and microbial biomass) soil quality indicators to the adoption of agroecological management practices, such as the planting of forage species and the rotation of local crops, carried out in a tropical representative pasture area located at an integrated livestock–crop farm. For this, the pasture system managed conventionally was used as control. A secondary objective was to determine whether these indicators can provide a good assessment of the system's performance and sustainability.

## Materials and Methods

### Study Sites

This research was conducted at an experimental farm located in Cangrejas in the Province of La Havana (Northwest Cuba) (coordinates: 23°02'W and 82°31'N), belonging to the Cuban Pasture and Forage Research Institute (IIPF). The experimental farm, about 30,000 m<sup>2</sup> in area, was a ranch dedicated to pasture and milk production for more than 20 years and was grazed by Zebu cattle. The vegetation was

mainly dominated by *Panicum maximum*, *Cynodum nlemfuensis*, and *Teramnus uncinatus*. The regional climate is subtropical, with a mean annual temperature of 24.6°C and an average annual rainfall of 1300 mm, mostly distributed from May to October. The dominant soil type is Eutri-Rhodic Ferralsol (FAO 1988), a clay-textured soil developed from Neogene limestones and slightly acidic in pH. The main minerals in the clay fraction are oxides of iron and kaolinite.

### Compost

The vermicompost used in this experiment was produced from a mixture of crop residues and castings by *Eisenia andrei* Bouché 1972 earthworms, using the Beltsville Aerated Pile Method (Willson and others 1980). *E. andrei* was used because it is well adapted to the humid, subtropical Cuban climate. Earthworm castings contained (on dry weight basis), on average, 1.9% nitrogen, 2.0% phosphorus, 1.3% potassium, and 68% organic matter.

### Land Use Changes

In 1994, the pasture ranching system was converted into an integrated agroecological livestock–crop farm. Two cropping systems (forage and polycrop) were established in a area of 2500 m<sup>2</sup> on the pasture site following a full randomized design with five replication plots. The forage and polycrop systems occupied areas of 1000 and 500 m<sup>2</sup>, respectively, and the remaining area (1000 m<sup>2</sup>) was maintained with the pasture system managed conventionally to be used as control. Each plot of pasture and forage area measured 200 m<sup>2</sup>, and each plot of polycrop area measured 100 m<sup>2</sup>. The forage area was cropped continuously with annual crops of leucaena (*Leucaena leucocephala*) followed by king grass (*Penisetum* sp.). Four successive crops of yucca (*Manihot esculenta*), ayocote bean (*Phaseolus vulgaris* L.), tomato (*Lycopersicon esculentum* Mill.), and spinach (*Spinacia oleracea* L.) were grown in the polycrop area. The vermicompost was applied before planting each crop in the forage and polycrop areas, at a rate of 4 t/ha.

After eight years of the above systems, two samplings were carried out, one in April corresponding to the dry season (from November to April), and the other in October, corresponding to the moist season (from May to October). In each sampling, five soil samples (one per plot) from each area were collected. Each sample consisted of six bulked subsamples (150 cm<sup>3</sup> cores), collected randomly at 0 to 15-cm depth. Field-moist soil samples were divided into two subsamples. One subsample was sieved at 2 mm and stored at 2°C for biological and biochemical analyses, and the other subsample was allowed to dry at room temperature. One

aliquot of air-dried soil was sieved to < 2 mm for physicochemical and chemical analyses and another aliquot was sieved to collect 0.2 to 4-mm aggregates for stability measurements.

#### Physical-Chemical, Biological, and Biochemical Analyses

Soil pH and electrical conductivity were measured in a 1:5 (w/v) aqueous extract (Page and others 1982). The total organic carbon (TOC) was determined by oxidation with potassium dichromate in a concentrated sulfuric medium, and measurement of the excess dichromate using Mohr's salt (Yeomans and Bremner 1988). Total N (TN) was determined by Kjeldahl digestion. Extractable (with ammonium acetate) K was determined by flame photometry.

In aqueous soil extracts, water-soluble carbon (WSC) was determined by wet oxidation with  $K_2Cr_2O_7$  and measurement of the absorbance at 590 nm (Sims and Haby 1971). Water-soluble carbohydrates and total carbohydrates were determined by the method of Brink and others (1960).

Dehydrogenase activity was determined by the reduction of 2-*p*-iodophenyl-3-*p*-nitrophenyl-5 phenyltetrazolium chloride (INT) to idonitrotetrazolium formazan (INTF) using 1 g of soil at 60% of field capacity, exposed to 0.2 ml of 0.4% INT in distilled water for 20 hours, at 22°C in darkness (García and others 1997). The INTF formed was extracted with 10 ml of a mixture of 1:1.5 tetrachloroethylene-acetone by shaking vigorously for 1 min. INTF was measured spectrophotometrically at 490 nm.

Urease and *N*- $\alpha$ -benzoyl-*L*-argininamide (BAA) hydrolyzing protease activities were determined in 0.1 M phosphate buffer at pH 7; 1 M urea and 0.03 M BAA were used as substrates, respectively. Two milliliters of buffer and 0.5 ml of substrate were added to 0.5 g of sample, which was incubated at 30°C (for urease) or 39°C (for protease) for 90 min. Both activities were determined as the  $NH_4^+$  released in the hydrolysis reaction (Nannipieri and others 1980).

Acid phosphatase activity was determined using *p*-nitrophenylphosphate disodium (PNPP, 0.115 M) as substrate. Two milliliters of 0.5 M sodium acetate buffer, adjusted to pH 5.5 using acetic acid (Naseby and Lynch 1997), and 0.5 ml of substrate were added to 0.5 g of soil and incubated at 37°C for 90 min. The reaction was stopped by cooling at 2°C for 15 min. Then, 0.5 ml of 0.5 M  $CaCl_2$  and 2 ml of 0.5 M NaOH were added, and the mixture was centrifuged at 4000 rpm for 5 min. The *p*-nitrophenol (PNP) formed was determined in a spectrophotometer at 398 nm (Tabatabai and Bremner 1969). Controls were made in the

same way, although the substrate was added before the  $CaCl_2$  and NaOH.

$\beta$ -Glucosidase was determined using *p*-nitrophenyl- $\beta$ -*D*-glucopyranoside (PNG, 0.05 M) (Masciandaro and others 1994) as substrate. This assay is based on the release and detection of PNP. Two milliliters of 0.1 M maleate buffer (pH 6.5) and 0.5 ml of substrate were added to 0.5 g of sample and incubated at 37°C for 90 min. The reaction was stopped with Tris hydroxymethyl aminomethane (THAM), according to Tabatabai (1982). The amount of PNP was determined in a spectrophotometer at 398 nm (Tabatabai and Bremner 1969).

For all enzyme assays, controls were included with each soil analysed. The same procedure as for the enzyme assay was followed for the controls, but the substrate was added to the soil after incubation but prior to the analysis of the reaction product. All data are expressed on an oven-dry soil basis.

Microbial biomass C was determined using the fumigation-extraction method (Vance and others 1987).

#### Physical Analysis

The percentage of stable aggregates was determined by the method of Lax and others (1994). A 4-g aliquot of sieved (0.2 to 4-mm) soil was placed on a small 0.250-mm sieve and wetted by spray. After 15 min the soil was subjected to an artificial rainfall of 150 ml with an energy of 270 J/m<sup>2</sup>. The remaining soil on the sieve was put in a previously weighed capsule (*T*), dried at 105°C, and weighed (*P1*). Then, the soil was soaked in distilled water and, after 2 hours, passed through the same 0.250-mm sieve with the assistance of a small stick to break the remaining aggregates. The residue remaining on the sieve, which was made up of plant debris and sand particles, was dried at 105°C and weighed (*P2*). The percentage of stable aggregates with regard to the total aggregates was calculated by  $(P1 - P2) \times 100 / (4 - P2 + T)$ . The four soil samples of each treatment were analyzed in triplicate for percentage of stable aggregates.

Bulk density was determined by the paraffin method described by Barahona and Santos (1981), after maintaining soil moisture at 60% of field capacity for one month.

#### Statistical Analysis

The effects of land use and sampling season, and their interaction, on measured variables were tested by a two-way analysis of variance and comparisons among means were made using the Least Significant Difference (LSD) test calculated at  $P < 0.05$ . Statistical procedures were carried out with the software package SPSS for Windows.

Table 1. Changes in physical-chemical properties of soil in response to land use and sampling season ( $n = 5$ )<sup>a</sup>

|   | Dry season |        |          | Rainy season |        |          |
|---|------------|--------|----------|--------------|--------|----------|
|   | Pasture    | Forage | Polycrop | Pasture      | Forage | Polycrop |
| pH (H <sub>2</sub> O)                                   | 5.92a*     | 5.80a  | 5.60a    | 5.95a        | 5.82a  | 5.76a    |
| Electrical conductivity (1:5, $\mu\text{S}/\text{cm}$ ) | 233a       | 274b   | 339c     | 349a         | 376a   | 314a     |
| Total organic C (g/kg)                                  | 26.1b      | 21.1a  | 20.1a    | 27.6b        | 20.1a  | 21.2a    |
| Total carbohydrates ( $\mu\text{g}/\text{g}$ )          | 2465b      | 1702a  | 1700a    | 2331b        | 1490a  | 1425a    |
| Water-soluble C ( $\mu\text{g}/\text{g}$ )              | 246b       | 164a   | 135a     | 284c         | 206b   | 151a     |
| Water-soluble carbohydrates ( $\mu\text{g}/\text{g}$ )  | 36b        | 18a    | 12a      | 49c          | 36b    | 11a      |
| Total N (g/kg)  | 3.3a       | 2.9a   | 3.2a     | 3.2a         | 2.9a   | 2.8a     |
| Extractable K ( $\mu\text{g}/\text{g}$ )                | 160a       | 100a   | 159a     | 104a         | 114a   | 107a     |
| Aggregate stability (%)                                 | 86.1b      | 80.5a  | 82.3a    | 87.6b        | 86.6b  | 67.6a    |
| Bulk density ( $\text{g}/\text{cm}^3$ )                 | 1.26a      | 1.33a  | 1.37b    | 1.28a        | 1.31a  | 1.36b    |

<sup>a</sup>For each sampling season, values in rows sharing the same letter do not differ significantly ( $P < 0.05$ ) as determined by the LSD test.

Table 2. Two-factor ANOVA (sampling season and land use) for all parameters studied:  $P$  significance values

|                             | Sampling season (S) | Land use (U) | Interaction (S $\times$ U) |
|-----------------------------|---------------------|--------------|----------------------------|
| pH (H <sub>2</sub> O)       | 0.204               | 0.105        | 0.629                      |
| Electrical conductivity     | 0.005               | 0.322        | 0.002                      |
| Total organic C             | 0.465               | < 0.001      | 0.330                      |
| Total carbohydrates         | 0.031               | < 0.001      | 0.816                      |
| Water-soluble C             | 0.006               | < 0.001      | 0.572                      |
| Water-soluble carbohydrates | 0.001               | < 0.001      | 0.011                      |
| Total N                     | 0.061               | 0.056        | 0.078                      |
| Extractable K               | 0.073               | 0.379        | 0.191                      |
| Aggregate stability         | 0.010               | < 0.001      | < 0.001                    |
| Bulk density                | 0.109               | < 0.001      | < 0.001                    |
| Biomass C                   | 0.036               | < 0.001      | 0.205                      |
| Dehydrogenase               | < 0.001             | < 0.001      | < 0.001                    |
| Urease                      | < 0.001             | < 0.001      | 0.006                      |
| Protease-BAA                | < 0.001             | < 0.001      | < 0.001                    |
| Acid phosphatase            | < 0.001             | < 0.001      | 0.001                      |
| $\beta$ -Glucosidase        | 0.013               | 0.002        | 0.964                      |

## Results and Discussion

### Changes in Physical-Chemical Parameters

The lowest values of soil electrical conductivity were recorded in the area of pasture followed by forage area in the dry season, which increased significantly in the rainy season (Table 1). Neither land use nor sampling season, nor the interaction of land use and sampling season, had any significant effect on soil pH (Tables 1 and 2).

In both sampling seasons, the concentrations of total organic C and all the C fractions (total carbohydrates, water-soluble C, and water-soluble carbohydrates) were higher in the area under pasture than in those under

forage species or polycrop (Table 1). Sampling season had no significant effect on total organic C (Table 2). Higher soil organic matter in the pasture area could be due to the higher input of root exudates and plant remains and a lower mineralization rate of soil organic matter. The highest losses of organic matter in the areas of forage and polycrop (2.54 and 2.92 C-CO<sub>2</sub>g/m<sup>2</sup>/day, respectively, compared to 1.77 C-CO<sub>2</sub>g/m<sup>2</sup>/day for pasture area), which were assessed by Rodríguez and others (2002), were not compensated for by plant remains or by the addition of composted residue.

The water-soluble C fraction is an important pool with respect to soil organic matter turnover in agricultural soils, since it acts as a readily decomposable substrate for soil microorganisms and as a short-term reservoir of plant nutrients (Gregorich and others 1994). Moreover, the water-soluble C fraction indicates the soil's potential microbial activity (Ceccanti and Garcia 1994) and hence is strongly influenced by environmental conditions, in particular temperature and humidity. In fact, both sampling season and land use had a significant effect ( $P = 0.006$  and  $P < 0.001$ , respectively) on water-soluble C and water-soluble carbohydrates (Table 2). Except for the polycrop area, where water-soluble carbohydrates did not vary with sampling season, the increases observed in the values of soluble C fractions (water-soluble C and water-soluble carbohydrates) in the rainy season, with respect to the dry season, could be due to increases in soil microbial activity (dehydrogenase activity in Table 3).

### Changes in Physical Parameters

Soil structural stability plays an important role in the control of erosion in degraded areas and in the improvement of the water infiltration rate (Altieri 1999). The mechanisms involved in stabilizing aggregates are based on the enmeshment of soil particles by hyphae

Table 3. Changes in biochemical properties in response to land use and sampling season ( $N = 5$ ).

|  | Dry season |        |          | Rainy season |        |          |
|--|------------|--------|----------|--------------|--------|----------|
|  | Pasture    | Forage | Polycrop | Pasture      | Forage | Polycrop |
| Microbial biomass C ( $\mu\text{g/g}$ )          | 564b*      | 466a   | 438a     | 690b         | 488a   | 464a     |
| Dehydrogenase ( $\mu\text{g INTF/g soil}$ )      | 79b        | 26a    | 22a      | 45b          | 32b    | 16a      |
| Urease ( $\mu\text{mol NH}_3/\text{g/h}$ )       | 1.84b      | 1.03a  | 0.97a    | 2.38b        | 2.46b  | 1.43a    |
| Protease-BAA ( $\mu\text{mol NH}_3/\text{g/h}$ ) | 6.37c      | 3.95b  | 2.51a    | 3.79b        | 2.45a  | 1.93a    |
| Acid phosphatase ( $\mu\text{mol PNP/g/h}$ )     | 3.00c      | 2.70b  | 1.75a    | 2.00b        | 1.57ab | 1.33a    |
| $\beta$ -glucosidase ( $\mu\text{mol PNP/g/h}$ ) | 0.73b      | 0.50a  | 0.49a    | 0.59b        | 0.33a  | 0.35a    |

\*For each sampling season, values in rows sharing the same letter do not differ significantly ( $P < 0.05$ ) as determined by the LSD test.

and roots, and the exudation of polysaccharides (Bearden and Petersen 2000). According to Roldán and others (1994), the binding effect of polysaccharides is short-lived and the maintenance and increase of aggregate stability is attributable to the increases in microbial populations, particularly to the proliferation of fungal mycelia. In fact, the observed improvement in soil structural stability of the forage area in the rainy season, with respect to the dry season, could be related to the increase in the concentrations of water-soluble C and water-soluble carbohydrates (Table 1), which can be used as carbon and energy sources for soil microflora and may also have a structural function (Lu and others 1998, Caravaca and others 2002).

Plant cover contributes to the formation and stability of soil aggregates by supplying organic matter from plant remains. As shown by Cerdà (1998), the present study also confirmed the influence of plant cover on soil aggregate stability. The areas of pasture and forage had the highest percentage of stable aggregates in the rainy season, while polycrops developed soils with less stable aggregates in both sampling seasons. According to Altieri (1999), annual crops with forages disturb soil structure less than crops of short cycle, mainly due to frequent and continuous rotation of crops in the polycrop area. Pasture develops soils with high aggregate stability because this area is dominated by plant species with a graminoid-type, very dense root system (Cerdà 1997). The amount of binding and gluing compounds exuded by the roots is closely related to the total root mass. As a result of this rhizodeposition of C, a large active microbial biomass develops in the rhizosphere. Thus, crops with the greatest root mass often produce the greatest improvement in aggregation (Haynes and Beare 1997).

Low soil bulk density means high soil porosity, mainly involving the percentage of transmission and storage pores (Cox and others 2001). Decreased soil bulk density can improve plant root growth and development, which in turn permits increased root penetration and exploration of a greater volume of soil. In both

sampling seasons, soil bulk density was lower in the pasture and forage areas than in the polycrop area (Table 1). Thus, only land use had a significant effect ( $P < 0.001$ ) on soil bulk density (Table 2). The organic matter coming from plant remains may affect the bulk density of a soil by improving its structural stability. This would explain the low bulk density measured in the pasture and forage areas in comparison with the polycrop area.

#### Changes in biological and biochemical parameters

It has been suggested that improvement in soil physical properties, particularly structural stability, may affect soil biological and biochemical activities, including enzymatic activities (Giusquiani and others 1995). In fact, in the pasture area, the microbial biomass C values were higher than in the forage and polycrop areas (Table 3). Likewise, the highest increase in the microbial biomass C in the rainy season, with respect to the dry season, was also recorded in the pasture area (about 1.2-fold). De Luca and Keeney (1993) defined the water-soluble C content as a reflection of soil microbial activity, and in our experiment there was a clear relationship between microbial biomass C and water-soluble carbon fractions. In the polycrop area, the quantity of these easily biodegradable compounds was low in comparison with the forage area, and hence the microbial activity was less marked.

Dehydrogenase activity ranged from 16 to 79  $\mu\text{g INTF/g soil}$ , the highest value being for the pasture area in the dry season (Table 3). The high dehydrogenase activity found in the pasture area was likely due to the high concentration of labile C substrates (water-soluble C and water-soluble carbohydrates). Dehydrogenase activity expresses the biological oxidation processes of soil microorganisms (García and others 1997). As a respiratory activity measurement, dehydrogenase activity should be strongly representative of the size and the activity of the viable microbial community. However, some authors have criticized this approach (Nannipieri and others 1990), because the activity of this

enzyme is affected by numerous factors (soil type, pH, moisture, etc.). In the rainy season, the highest values of dehydrogenase activity were recorded in the pasture and forage areas, although these values were lower than (pasture area) or similar to (forage area) those recorded in the dry season in the corresponding areas.

With one exception (urease in pasture and forage in the rainy season), the overall levels of hydrolytic enzymes involved in the N (urease and protease-BAA), P (acid phosphatase), and C ( $\beta$ -glucosidase) cycles were higher in the pasture area than in the forage and polycrop areas, in both sampling seasons (Table 3). In all three areas, the activities of protease-BAA, acid phosphatase, and  $\beta$ -glucosidase were lower in the rainy season than in the dry season. However, the values for urease, a hydrolase related to the terminal N cycle in which organic N is transformed into plant available ammonia, were higher in the rainy season, with respect to the dry season, in the pasture (about 1.3-fold), forage (about 2.4-fold), and polycrop (about 1.5-fold) areas. This enzyme can be considered greatly dependent on microbial activity, indicating that the stimulation of soil metabolism in the rainy season affected, at least in part, the biological transformation of N.

In conclusion, our results confirm that the application of agroecological management practices in an integrated livestock-crop farm has caused changes in the soil physical-chemical and biological properties. In particular, the planting of forage species can be considered an effective practice for carrying out sustainable, integrated livestock-crop systems, due to its general maintenance of soil quality, while the adoption of rotations of local polycrop appears to be less adequate, because it decreases soil quality.

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