Effects of *Erythrina poeppigiana* pruning residues on soil organic matter in organic coffee plantations

By

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Declaration

This work has not previously been accepted in substance for any degree and is not being concurrently submitted in candidature for any degree.

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This thesis is the result of my own investigations, except where otherwise stated.

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Dedication

To the memory of Monseñor Oscar A. Romero and Eric Eugenio Sanchez Nuñez
Who practiced what they preached.

To my wife Belén and my daughter Adriana
For being companions in this adventure.

To my family
For their permanent support.

Acknowledgements

SUMMARY

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Key words: *Coffea arabica,* organic agriculture, liquid organic amendments, indicators, light fraction, litter
decomposition, soil characteristics.

The effects of *Erythrina poeppigiana* pruning residue effects on soil organic matter were
investigated with three different objectives. Firstly, to study the effects of proximity of this
shade tree on soil characteristics in organic and conventional coffee farms in Costa Rica;
additionally a comparison between soil characteristics in organic and conventional farms
was carried out (Chapter III). Secondly, to analyze the effects of *Erythrina poeppigiana*
pruning residue additions on the size-density fractions, and on other fractions of soil
organic matter (SOM) (Chapter IV); and thirdly, to investigate the effects of the application
of microbial inocula or earthworm treatments on pruning residue decomposition, in order to
evaluate the possibility of improving nutrient release from these pruning residues in the
topsoil of organic coffee farms (Chapter V).

Respect the first objective, the impact of *Erythrina poeppigiana* on soil characteristics, at
three different positions relative to the shade tree and from three different soil depths, were
evaluated in five paired coffee farms (organic and conventional) in central Costa Rica in
2000 and 2004. In both years, C and N concentrations at 0-5 cm were similar for all
positions in the organic system probably due to an even distribution of pruning residues;
but in the conventional system, higher C and N concentrations were found close to the
shade tree *vs.* the positions 2 m from the trunk. This finding highlighted the importance of
E. poeppigiana in maintaining SOM levels. A trend to higher total C and N concentrations
for organic farms in comparison to conventional farms was found, but these differences
were not significant in either of the two study years. No significant temporal changes in soil
C or N concentrations were found between 2000 and 2004. In the 2004 study, significantly
higher soil K and Ca concentrations were found near the shade tree due to the high contents
of nutrients in *E. poeppigiana* pruning residues. Organic systems had similar nutrient
concentrations (P, K, Ca, Mg and Zn and NO_3^-) to conventional farms at 0-5 cm. A balance
of nutrient inputs-outputs in each farm within both systems appears to explain the absence
of differences between systems.

Respect the second objective, two field trials were set up to evaluate the effects of *E.
poeppigiana* pruning residue addition on size-density SOM fractions (2002 and 2004).
Within the first field trial, the effects of the addition of 10 Mg ha^{-1} of *E. poeppigiana*
pruning residues on three size density fractions, were evaluated over a 330-day period. In
the second field trial, the effects of the addition of *E. poeppigiana* pruning residues were
evaluated in a 105-day trial, with soil samplings every 15 days to determine if there were

early effects of treatments on SOM fractions. Additionally, the macroorganic fraction (>150 μm), total C in the 2002 trial and POM (>53 μm) in 2004 were evaluated as indicators of changes due to pruning residue management. All of the analyzed SOM fractions reflected SOM decomposition since they showed higher values when plant residue decomposition in the soil was incomplete (90 or 105 days). All the indicators also showed lower values or tended to have lower values at the end of each decomposition period (180 days). Total soil C was not a good indicator of short-term changes in labile SOM. The amount of C in the light fraction appeared to be the best indicator of changes in labile soil C due to pruning residue management. However, an economic evaluation of the costs of size density-fractionation is recommended because evaluations of macroorganic C or dry weight of LF (as opposed to C content of LF) may be less expensive indicators of short-term labile SOM decomposition. Carbon in macroorganic matter after 330 days was reduced by 33% at CATIE and 44% at Pejivalle in the residue only treatment, and by 62 and 52% in the respective bare soil controls, highlighting the importance of pruning residue additions in organic coffee farms to maintain the active soil C components.

Respect the third objective, in the 2002 and 2004 field trials (see preceding paragraph), the effects of *E. poeppigiana* pruning residue additions on soil K, NO_3^- or NH_4^+ concentrations, with and without microbial inocula or earthworms additions, were tested. An additional greenhouse trial was set up to evaluate the effects on maize seedling growth, of pruning residues with microbial inocula applications. The positive effects of pruning residue additions on soil K concentrations were detected in both the field and the greenhouse trials. No effects of microbial inocula or earthworm additions on soil K, NO_3^- or NH_4^+ concentrations were detected in the field trial. The microbial applications should be considered ineffective in increasing nutrient availability in field conditions and also in greenhouse conditions, due to weak temporary effects on maize seedling growth which were only observed in the first two weeks of the experiment on poor soils (taken from the 10-20 cm layer). Microbial mixture and earthworm applications also should be considered ineffective due to their insignificant and temporary impact on labile SOM in 2002, and because no significant effects were detected when disturbance factors were strictly controlled in the 2004 trial.

Biography

Fidel Payan is a Mexico national who was born in El Salvador in 1960. He obtained an Agronomical Engineering degree at the Universidad Autonoma Metropolitana-Xochimilco in Mexico City in 1984. He worked as an extensionist with indigenous cooperatives in Puebla and Morelos, Mexico (1984-1989). He was awarded his masters degree in Rural Development Studies at Colegio de Postgraduados de Chapingo in 1993 which was funded by a Ford Foundation scholarship. He was a research professor at the Agronomy School (Universidad Autonoma Metropolitana-Xochimilco; 1989-1999). In 1999, he initiated his doctoral studies in agroforestry at CATIE, which he completed in 2005 under the CATIE-UW-Bangor joint PhD program. In 1999, he was awarded a scholarship from the National Council for Science and Technology from the Mexican Government. His main scientific interests are soil organic matter dynamics in agroforestry, integrated productive approaches for small farms, and organic agriculture.

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List of abbreviations and acronyms

Anova   Analysis of variance

CEC   Cation exchange capacity ($cmol^+ l^{-1}$)

CIRAD   French development-oriented agricultural research organization

CONACYT National Council for Science and Technology

DBH   Tree stem diameter (cm) at 1.3 m breast height

HF   Heavy fraction of particulate organic matter

ICAFE   Costa Rican coffee Institute

IFOAM   International Federation of Organic Agriculture Movements

IOAS   International Organic Accreditation System

LF   Light fraction of particulate organic matter

m a.s.l.   Altitude in meters above sea level

MAG   Ministry of Agriculture of Costa Rica

MF   medium fraction of particulate organic matter

POM   particulate organic matter

SDF   Size density fractionation method using Ludox
SOM   Soil organic matter

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Effects of *Erythrina poeppigiana* Pruning Residues on Soil Organic

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Chapter 1   Introduction

1.1. General Introduction and Nature of the Problem

The role of soil organic matter (SOM) in maintaining soil fertility has been recognized since ancient times. In the tropics, SOM is a key component of nutrient recycling particularly in low input cropping systems. Resource-poor farmers usually cannot replace the soil nutrients removed by crop harvest because insufficient chemical or organic fertilizers are applied (Thomas *et al*. 1999, Phiri *et al*. 2001). The use of trees in agroforestry systems, and particularly of their pruning residues, has been studied as an alternative to these problems since the application of tree biomass which is rich in nutrients and organic matter can theoretically help maintain soil fertility (Lehmann *et al*. 1995, Palm 1995, Cobo *et al*. 2002).

Trees can maintain and even improve the surrounding soil fertility by a range of mechanisms, in particular those that directly and indirectly affect nutrient availability. Some tree species fix nitrogen and thus increase the concentration of N in the soil. Other tree species, which develop extensive and deep root systems, not only take up nutrients from a widespread area and from deeper soil layers but also concentrate them in the area under their canopy through litter fall. Further, trees in agroforestry systems promote efficient use and cycling of nutrients and reduce losses due to leaching. In dry zones, some trees recover nutrients from depth which are lost to most annuals and many perennials which are predominantly shallow rooting (Young 1999). Some trees use water complementarily with annual crops. For example, *Faidherbia albida,* a tree which is found in African savannas, loses its leaves during the wet season, allowing crops to grow with little competition for water (Teixeira *et al*. 2003). Trees also increase soil organic matter concentrations through litter and root turnover and generate an appropriate microclimate

and environment for meso and microfauna in the rhizosphere and in the associated mycorrhizosphere (Fisher 1995).

The trees in agroforestry systems contribute to the conservation and in some cases to the increase of SOM by: 1-increasing the biomass inputs to the soil (natural litter fall, pruning residues, root residues); 2- controlling erosive losses of SOM and nutrients by protecting the soil surface with mulch; 3- changing the microclimate under the tree canopy (e.g. reduced soil temperature maximums) which retards the loss of organic material by reducing the decomposition of SOM. In contrast to monocultural systems without trees, older trees in agroforestry systems act as a live biomass and nutrient reservoir. Finally, in high input systems, the combination of higher SOM with other inputs (including fertilizer), may lead to more efficient retention and use of nutrients as the ratio of nutrient recycling to losses is higher for agroforestry than for agricultural systems (Young 1989 and 1999).

Erythrina poeppigiana is a leguminous shade tree commonly grown in organic coffee farms in Costa Rica. This tree is preferred over other shade trees due to the high amount of mulch produced when it is intensively pruned. *E. poeppigiana* pruning residues can contain up to 300 kg N ha^{-1} yr^{-1}, and can provide more than 50% percent of the above ground organic matter deposits in shaded coffee systems (Beer 1988). It has been particularly recommended for farms with low soil fertility and low availability of chemical fertilizer inputs (Beer *et al*.1990).

Organic coffee production has acquired increased economic and ecological relevance due to world market saturation problems for conventional coffee and the ecological problems that conventional management can cause for the flora, fauna and soil in some regions (Boyce *et al*. 1994, Fishersworring and Roßkamp 2001). At the beginning of the last decade, organic agriculture represented only 0.08 % of Costa Rica's coffee production (Boyce *et al*. 1994). The coffee price crisis [*] promoted a noticeable conversion to organic farming because conventional approaches became uneconomic (Varangis *et al*.

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[*]The conventional green coffee prices almost halved between December 1999 and January 2001. Although temporary "high price" years can occur; in the long term a declining price trend is expected (Ponte 2002, Varangis *et al*. 2003).

2003). For 2001, 31.4% of the organic coffee supply from Central America originated from Costa Rica (CEPAL 2002).

Few studies have compared the effects of organic and conventional management strategies on the chemical and biological properties of soil (Reganold *et al.*1993). In addition, few studies have analyzed the provision of nutrients within organic coffee farms without using artificially produced fertilizers, which is one of the greatest limitations to organic coffee production (Lyngbaeck *et al.* 2001). The search for synchrony between the release of nutrients from organic residues at a time of high demand by the crops has been studied as an important scientific issue for organic coffee systems (Haggar *et al.* 1993).

Organic C is the central element of organic agriculture since the organisms that depend on it modulate nutrient cycling, soil structure and even natural crop resistance to many diseases (Lotter 2003). Organic farming is based on the maintenance of natural soil fertility and on the release and cycling of nutrients from SOM mineralization in order to maintain sustainable production (Lampkin 1990). N cycling and availability is linked to microbial activity and is limited by C availability. Likewise, organic material decomposition rates can be limited by N availability (Anderson and Flanagan 1989). Decomposition rates have been used in many models of SOM dynamics to define the active, slow and passive functional C pools which have 1.5, 5-25 and > 25 year turnover times respectively (Paul and Clark 1996). In both C and N cycling processes, the "active" fraction of SOM has an important role. Particulate Organic Matter (POM)[**] and the Light Fraction (LF) have been considered alternatively as the "active fractions" (Cambardella and Elliot 1992, Gregorich and Ellert 1993). The POM fraction consists of partially decomposed sand sized materials which can be free or bound to mineral particles, and has rapid turnover periods. This labile fraction (POM) has been separated into size-density sub-fractions (including LF) by physical methods using the different densities of the sub-fractions. Both, POM and LF can be considered as a bridge between plant materials and the stable humus (Theng *et al.* 1989). Nevertheless, research is needed on the relationships

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[**] In this work, a size-density method for POM fractionation, proposed by Meijboom *et al.* (1995), was used. This method defines 150 μm instead of 53 μm as the limit for obtaining POM, which is called macroorganic matter. Details are given in Chapter IV.

between the microbial biomass and the light fraction as well as on the role of the light fraction as an indicator of changes in soil management (Barrios *et al*. 1996a, Fließback and Mäder 2000).

Considering the aforementioned aspects, the central research problem of this study was the effect of pruning residues of the shade tree *Erythrina poeppigiana* on soil characteristics in organic and conventional coffee farms in Costa Rica. In addition, the effects of *Erythrina poeppigiana* pruning residues on the size-density fractions of SOM were studied. The possibility of improving nutrient release from these pruning residues using biological treatments that enrich the microflora and mesofauna in the topsoil of organic coffee farms was also investigated.

1.2. Objectives

This study comprises three experimental chapters, to address the following main objectives:

1.2.1. (Chapter III) A- to evaluate the effects of *E. poeppigiana* shade trees, at three different positions around the trees, on soil chemical and biological properties: i.e. total C and N, pH, electrical conductivity, soil respiration, soil organic matter size fraction distribution, and major nutrient concentrations (N, P, K, Mg, Ca). These variables were analyzed in comparable organic and conventional systems. B- to analyze differences in soil characteristics between organic and conventional farms.

1.2.2. (Chapter IV) A- to compare three size-density fractions obtained using Ludox™, macroorganic matter (>150 µm) and POM (>53 µm) as indicators that reflect the decomposition of topsoil labile SOM, and hence of changes induced by management. B- to test the effects of microbial or earthworm treatments applied to *E. poeppigiana* pruning residues on the amount of C resulting in three size-density fractions.

1.2.3. (Chapter V) A- to test the effects of microbial inocula or earthworms, added to *E. poeppigiana* pruning residues, on N and K availability in the topsoil of organic coffee farms. B- to test the effects of microbial inocula, added to *E. poeppigiana* pruning residues on the growth and N and K foliar concentrations in maize seedlings.

1.3. Dissertation plan and study strategy

This dissertation is divided into six chapters. Chapter 2 reviews the main theoretical and methodological aspects in regard to the nature of soil organic matter (SOM) and its fractions, the effects of shade trees on soil organic matter, and the management of tree pruning residues in coffee farms. Soil organic matter as a nutrient source for plants and its impact on soil characteristics through natural and induced SOM dynamics are discussed. The management of tree residues as a source of organic material in the search of synchronization of organic input decomposition and nutrient release with plant demands for nutrients is also considered. Furthermore, Chapter 2 addresses SOM fraction classification, especially by physical fractionation methods, and the role of the 'light' fraction in soil nutrient release. Finally, the effects of trees, and particularly of *E. poeppigiana*, on soil characteristics (principally nutrient cycling in shaded coffee agroforestry systems), as well as the methods used to study these effects, are presented. Additionally, in each of the following experimental chapters, a methods section has been included where the procedures and experiment protocols are described in detail.

Chapters III, IV and V contain the results of the experimental work. In Chapter III, a study of the effects of *E. poeppigiana* proximity on soil characteristics in organic and conventional farms is presented. The hypothesis was that the soil close to *E. poeppigiana* trunks offers better chemical and biological conditions where there is greater intensity of tree mediated influences (shade, litter fall, etc). This spatial effect of the trees may be influenced by the management system (organic *vs* conventional coffee farming systems), and hence the effect of distance from tree is compared between these two systems. The influence of proximity to the coffee bushes was also analyzed. Secondly, a comparison

between selected soil characteristics in both systems is presented based on an average of the sampling positions. This soil study was done in 2000 and 2004 and changes in total C and N concentrations in this four-year period are presented. The results of this first experimental chapter highlighted the importance of pruning residues in increasing total soil C and N concentrations. Furthermore, organic coffee farms depend, to a large degree, on *E. poeppigiana* pruning residues for coffee plant nutrition, but these farms produce lower yields in comparison to conventional farms (Lyngbaeck *et al.* 2001). These lower yields may have been partially due to nutrient deficits in organic farms, even though they receive up to 10 Mg ha^{-1} yr^{-1} of *E. poeppigiana* pruning residues which contain important amounts of nutrients (Beer 1988, Beer *et al.* 1990).

The problems observed in organic coffee farms in Chapter III led to an analysis in Chapter IV of the effects of *E. poeppigiana* pruning residue additions on the dynamics of labile fractions of SOM, which are thought to be the most important SOM fractions in nutrient release processes (Barrios *et al.* 1996a). Chapter IV also includes a study of the effects of treatments that enhance soil microbial activity (introduction of earthworms and addition of an indigenous mixed microbial inocula) on the amount of C in different size-density fractions of SOM. Size-density fractions, macroorganic matter (Meijboom *et al.* 1995), and POM (Cambardella and Elliot 1992) were assessed as indicators of changes in organic matter input management. The dynamics of labile fractions, under the effect of biological treatments, were studied in two field trials in 2002 (on the CATIE organic coffee farm and in Pejivalle on a small private organic coffee farm); and in one field trial on CATIE's organic coffee farm in 2004. In the 2004 field trial at CATIE, soil samples were taken every 15 days to study in detail the early stages of the pruning residue decomposition process.

In Chapter V, the practical aspects and measurable effects of the use of microbial mixtures and earthworm inoculation on soil K and N concentrations were studied in greenhouse trials. Organic amendments and treatments recommended in organic farming guidelines for small coffee farms have rarely been tested in controlled studies (Soto and Muschler 2001). Therefore, a greenhouse trial with maize seedlings, *in vitro* tests on

pruning residue CO_2 production, and analyses of samples taken from the 2002 and 2004 field trials were carried out to test the effectiveness of microbial and earthworm addition treatments.

Chapter VI provides a discussion of general themes that emerge from the three preceding experimental chapters. The effects on C, N and nutrient concentrations of evenly distributed and probably higher organic matter inputs in organic farms are discussed. This leads on to an analysis of the problems and consequences in SOM status and of the methods for studying SOM fractions derived from the high quality *E. poeppigiana* residues litter (low lignin, low C:N ratio and low polyphenol contents). Methodological issues related to sampling strategies in small coffee farms on hillsides in the tropics are discussed along with the effectiveness of different fractionation methods for obtaining fractions that reflect changes in SOM due to different pruning residue management techniques. The practical consequences of the application of microbial mixtures or earthworm treatments, for pruning residue management in organic farms, are presented. Finally, general conclusions of the dissertation and recommendations in regard to methodological issues and for the management of pruning residues as well as further research needs in organic coffee farms are suggested in Chapter VII.

1.4. Alternative hypotheses

1.4.1.a (Chapter III) Chemical and biological soil variables differ at different distances (> 2 m and < 1 m) from the shade tree. These soil variables also differ when sampled at a different position with respect to a coffee bush; i.e. under a coffee bush vs. equidistant from two coffee row (both at 2 m from the shade tree).

1.4.1.b (Chapter III) There are significant differences between conventional and organic systems in regard to soil C and N concentrations, nutrient content and other chemical and biological soil properties (e.g. microbial activity expressed in soil respiration).

1.4.2.a (Chapter IV) The C content in each of the three size-density fractions of the soil macroorganic matter obtained using Ludox™ shows temporal differences during the decomposition process of labile soil organic matter in an organic coffee farming system.

1.4.2.b (Chapter IV) The amount of C within each size density (SD) fraction is affected by microbial, earthworm or *in situ* composting treatments under organic farming conditions. Therefore, the three SD fractions are good indicators of changes in soil organic matter management.

1.4.3.a (Chapter V) The amounts of mineralized N and K in the top soil of organic farms are affected by treatments which increase micro and/or mesofauna populations under organic management guidelines. These treatments can also induce higher growth of maize seedlings under controlled environmental conditions in a greenhouse.

Chapter 2   Literature Review

2.1. Introduction

The effects of shade trees on soil organic matter (SOM) and management of tree pruning residues in coffee farms are two of the central themes of the three experimental chapters in this thesis. Topics from each chapter are addressed in the four following sub-chapters in the literature review. Sub-chapter 2.2 focuses on the importance of SOM as a nutrient source for plants and on the impact of SOM on the chemical, biological and physical status of soil in agroecosystems. Also reviewed in this sub-chapter is the classification of SOM fractions as well as the natural and induced changes that can occur with SOM. Sub-chapter 2.3 covers SOM management with an emphasis on trees as a source of organic material and the possibility of synchronization of organic input applications with the plant demands for nutrients within agroforestry systems. A review of the role of the 'light' SOM fraction on soil nutrient status are also considered in this sub-chapter. Methodologies for SOM fractionation, particularly physical fractionation methods are covered in subchapter 2.4. Sub-chapter 2.5 addresses the effects of trees on soil fertility as well as the methods used to study them under three conditions: isolated naturally regenerated trees in degraded soils, trees in monoculture plantations, and trees in agroforestry systems. The role of *Erythrina poeppigiana* in nutrient cycling in shaded coffee agroforestry systems is discussed in Sub-chapter 2.6.

Much is known about the nature of SOM; however, there are gaps in the agronomic and biochemical knowledge of the dynamic and management of SOM and on its role in nutrient cycling. This is especially true for the effects of residue quality from different trees on soil characteristics, the spatial and temporal impact of residues from different tree species, the possibility of synchrony between crop demands and nutrient supply from plant residues, and the impact of these tree residues on soil characteristics within organic coffee cropping systems; therefore, these issues are highlighted in the following sub-chapters. Biochemically, the relationship between SOM fractions and nutrient availability, the identification of an operatively measurable SOM fractions that are directly related to

biological activity in the soil, and finally the search for new methods that show appropriate sensitivity to changes in SOM management are also addressed.

2.2. Characteristic and transformations of soil organic matter (SOM)
2.2.1. The importance and classification of soil organic matter fractions

Soil organic matter (SOM) is defined as the carbon (C) enriched soil fraction that is formed from an intimate mixed combination of plant, animal and microbial residues. These residues are in different decomposition states and include low molecular weight substances and humic substances with high molecular weight compounds (Anderson and Ingram 1993). The energy fluxes and cycling of nutrients in ecosystems are critically dependent on the rate of SOM turnover (Gunapala and Scow 1998, Alvarez and Alvarez 2000). SOM directly and indirectly influences the physical, chemical and biological factors that intervene in soil fertility. The physical factors include enhancing aggregation, water and air permeability, water retention, resistance to erosion and soil compaction as well as color and capture of solar energy. The most important chemical factors include the cation exchange capacity (CEC) and the availability of nutrients and minerals for the plants; the biological factors include diversity and population density of the soil fauna and microflora, as well as the microbial biomass (Brady and Weil 1996).

However, some of these characteristics are not associated with SOM as a whole, but rather with specific fractions or pools of SOM. In terms of relative stability, SOM fractions can be divided into labile and stable fractions. Labile fractions decompose in weeks or months whereas the stable fractions can remain in the soil for years or decades (Alvarez and Alvarez 2000, Duxbury *et al*. 1989). The labile fractions include root residues, above ground plant residues, macroorganisms, microorganisms, particulate organic matter (POM), vegetal and animal litter on the soil surface, non-humic carbon enriched substances such as lignin, cellulose and polyphenols. The stable fractions include humic substances subdivided into humic, and fulvic acid and humines. Organic acids and pigments, which are associated with soil minerals, along with some complex polymers, which come from free amino acids, are also included in the stable fraction category. The stable fractions are resistant to microbial activity not only because of their chemical structure but also because of their

association with soil mineral components. They can also be occluded in the spaces between soil aggregates (Six *et al.* 2002).

Another form of classification of the SOM fractions is to divide them into living and dead components. Within the living components, which only make up approximately 4% of the total organic carbon in the soil, are roots, fauna and microbes. Within the dead components, which contribute to the rest of the total organic soil carbon, are POM which contributes 10-30% and humus that contributes 70-90% of the carbon in this category (Theng *et al.* 1989).

2.2.1.1. Organic matter as a source of negative ion charges in the soil

Values for soil organic matter CEC, which is pH dependent, can usually range from 85 meq 100 g^{-1} organic matter to 400 meq 100 g^{-1}. This CEC depends on soil pH and on the decomposition status of the materials. Organic matter can also retain anions, especially phosphates and sulfates, thus avoiding losses through leaching (Foth and Ellis 1988, Fassbender 1993). In the tropics, some of the SOM negative charges are blocked by Fe and Al cations diminishing the SOM potential for reducing nutrient losses by leaching (Duxbury *et al.*1989).

2.2.1.2. Organic matter as a nutrient source for plants

One of the most important characteristics of organic matter is its role in supplying nutrients, especially N, for plant growth (Palm 1995, Barrios *et al.* 1996b, Schroth 2003). Particularly in the tropics, low input cropping systems rely on nutrients from organic matter residues for soil fertility management (Tian 1998). N, P, and S mineralization and release are controlled by microbiological processes which "open" the structural units of organic matter where they are stored. Interactions in the soil among elements such as Ca, Mg, K, Fe, Cu, Zn and Mn are in part controlled by chemical processes related to the electrical charges in the organic matter structure. Generally 95% of the N and between 20-75% of the P contained in surface soils are found in SOM. The average ratio between C:N:S in

agricultural soils is about 130:10:1.5 and is very constant across a wide range of soil types. This rather constant ratio is related to the similar mineralization processes for these elements (Duxbury *et al*. 1989). For P, recycling processes seem to have different biochemical pathways than C, N, and S, which explains the large variability of the ratios between P and the other nutrients mentioned (Phiri *et al*. 2001).

2.2.1.3. The role of SOM in maintaining physical soil qualities

Organic matter affects soil color which sometimes helps in field assessments of soil quality; therefore, SOM enriched soils are often darker. Some black soils contain up to 10-15% organic matter while yellow soils contain 0-2%. Soil color has an important role in maintaining the thermal balance of the soil. The darker the soil the more infrared waves are absorbed thus warming the soil although energy is also irradiated. The temperature increase enhances the microbial activity for SOM turnover (Fassbender 1993).

High organic matter content can also improve soil structure by permitting the formation of permanent soil aggregates. Fine particles are cemented together by SOM and form larger storage pores, thus improving infiltration along with water and air storage. A better soil structure also permits a higher resistance to erosion due to the formation of stronger aggregates (Feller 1994, Kouakoua 1998). Low soil bulk density is usually associated with higher levels of SOM. A relevant characteristic of SOM enriched soils such as histosols is their lower bulk density. Values of 0.30 Mg m^{-3} can be found in temperate histosols, commonly called organic soils; while other cultivated mineral soils can show values of 1.25-1.45 Mg m^{-3} (Brady and Weil 1996). In particular, organic matter additions to soils commonly produces lower bulk density levels in comparison to control areas where no mulches have been applied. Kimemia *et al*. (2001) found diminutions in soil bulk density within a range of 8-25% after 3 years of mulch applications from seven agroforestry species in Kenyan coffee plantations. The effect of SOM on texture is usually small; however, in some cases clay particles can be cemented into sand-sized particles. This can affect texture tests for sand content (Kass 1996).

2.2.1.4. SOM and biological soil status

In biologically stable soil, there is high biodiversity which is caused by the heterogeneity and abundance of microfauna (bacteria, fungi, actinomycetes, protozoa and nematodes), mesofauna (enchytraeidae and acari) and macrofauna (earthworms and termites) whose weight dominates the total soil biomass. Many of these organisms can regulate other species, limiting severe crop damages. Fauna limit microfloral growth by feeding on them (Atlas and Bartha 1998). In other cases, fauna and microflora have cooperative relationships. Many bacteria are responsible for mineralization of protein compounds; but these processes are facilitated by invertebrates which pulverize and predigest the coarse residues. Microflora and soil fauna integratively decompose organic material. While microbes are ultimately responsible for decomposition, soil fauna contribute by comminuting residues, feeding on microflora and providing an appropriate environment for microbial growth (Tian 1998). In a ten week experiment (Tian *et al*. 1993), earthworms and millipedes contributed on average to 10-28% of the plant residue breakdown. However, the indirect effect of macrofauna in SOM decomposition is larger. Earthworms produce feces covered by a carbon-enriched mucus which feeds bacteria and other microorganisms, thus enhancing the microbial mediated decomposition of SOM (Lavelle 1997). Earthworms (anecic species) and termites also contribute to SOM dynamics not only by bringing organic and weathered mineral materials to the soil surface but also by burying organic materials into the mineral soil horizons (Anderson and Flanagan 1989).

It has been calculated that 95% of the N fixed in nature, which is approximately two hundred million Mg yr^{-1}, is fixed by microorganisms that require C as an energy source and base material for protoplasmic synthesis. Specifically, the addition of organic material increases the number of heterotrophic organisms that generate sub-products which are used by N-fixing bacteria (Ferrara and Santamaría 1996).

Mycorrhizal fungi that are not soil inhabitants but rather root inhabitants produce an abundance of spores in the soil when organic materials are added. Similarly, the non-

mycorrhizal fungi populations increased from 6.6×10^1 to 6.5×10^2 CFU[*] g^{-1} dry soil when 40 Mg ha^{-1} of cow manure was added to a degraded soil in central Mexico (Ferrara and Santamaría 1996). Finally, suppressive effects of the phytopathogenic fungi species *Sclerotium rolfsii* have been observed after applying traditional and earthworm compost rich in antagonistic fungi (Handar and Gorodecky 1991). This indicates that SOM plays an important role in maintaining soil biodiversity and controlling plant diseases.

2.2.2. Natural and induced changes in SOM

SOM is always undergoing change and transformation processes. Even the fractions most resistant to decomposition such as humus, transform and release minerals and C even though it may take six hundred years or more (Duxbury *et al*. 1989).

2.2.2.1. Natural transformation of SOM

The SOM cycle begins with plant and animal residues that fall to or are produced within the soil (e.g. fine roots turnover). The decomposition process through microbial activity converts these materials into humus, labile fractions that include simple carbonated substances such as sugars, and minerals that can be deposited in or released from the soil. In the early stages of decomposition, resistant substances such as lignin remain. The nutrients and C released in the process are used by microorganisms and plants and incorporated into living tissues, thus initiating a new SOM cycle.

In natural ecosystems, these cycles maintain a dynamic equilibrium for nutrients and C that are naturally relocated from one pool to another due to edaphic and climatic conditions (Fassbender 1993). Even though this general concept is very clear, research is still needed to understand the biological, chemical and physical factors that determine SOM transformation (Duxbury *et al*. 1989).

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[*] Colony forming units

Mineralization and immobilization of the SOM chemical components are two main processes for the natural transformation of SOM. They are the net result of sub-processes which are controlled by environmental factors and involve heterogeneous organisms such as insects, microbes and annelids. Temperature is most often the driving factor in SOM transformation. However, soil humidity, soil pH, micro and mesofauna levels as well as soil characteristics such as texture, original chemical composition of the soil and the quality of the original organic materials are also relevant (Anderson and Flanagan 1989).

In tropical agro-ecosystems, the decrease in POM can be accelerated thus affecting soil fertility. Under tropical conditions, the SOM decomposition process can be four times faster than in temperate conditions (Russell 1988). The accelerated turnover rates of labile fractions, including particulate organic matter (POM), is fundamentally important in soil management in the tropics. Intense soil disturbance from some agricultural practices contribute to high nutrient release and loss from agroecosystems. These disturbances can diminish the highly labile POM fraction which is an important nutrient reservoir (Barrios *et al*. 1996a). This is particularly true if the vegetation is removed from the farm (Lundquist *et al*. 1999, Mafongoya 1998, Tian 1998). For these reasons, it is necessary to keep the crop and weed residues on the farm for conserving soil nutrients in tropical conditions.

The conversion of plant residues to stable SOM has been studied both qualitatively and quantitatively. Indices such as "K1" have been proposed which describe the relation between organic matter inputs and remaining residues after one year. Extensive studies have also been done that quantitatively analyze the evolution of shade tree residues in tropical agroforestry systems. Nutrients as well as C inputs and outputs of the SOM reserves have been evaluated. The contribution of each part of the shade tree to the SOM pools has also been quantified. The importance of shade trees in the sustainability of the systems, particularly in C conservation has been highlighted (Beer *et al*. 1990).

Plant residue decomposition is greatly influenced by the quality of the residues. Quality is determined by the C, N and P content, lignin content as well as by concentration of inhibiting elements such as terpenes, alkaloids, polyphenols and tannins. For example

organic materials with high lignin contents are considered of poor quality because lignin acts as an organic C and N protector. On the contrary, material with a low C:N ratio are considered of high quality because they are easily decomposed (Mafongoya *et al*. 1998). The biochemical decomposition process begins with accelerated mineralization rates, along with synthesis of new compounds and formation of complex compounds from microbial metabolites (Russell 1988). The decomposition rates decrease as available sugars are depleted and recalcitrant materials, such as cell walls that are predominant in the total residual mass, remain. In a 443 day trial, soil was incubated to measure the presence of carbohydrate substances. At the end of the experiment, only 2.3% of the xylose, which is an easily decomposed component of the hemicellulose, remained from the original quantity added. On the other hand, the abundance of mannose, a principal component of pigmented fungal cell walls that is resistant to degradation, increased by 450% (Cheshire *et al*. 1974).

Geophagous earthworms, such as the *Pontoscolex* genus, digest organic material. They have bacteria in a digestive tract that is covered with mucus rich in nutrient compounds used by microorganisms. The mucus is expelled along with the digested residues accelerating microbial activity (Barois and Lavelle 1986). Some studies have suggested that earthworms accelerate soil C mineralization. Organic matter decomposition and nutrient release are accelerated because some earthworms species prefer to feed on residues on soil surface. Some researches have suggested that is possible to reach synchrony between crop demands and nutrient availability through earthworm inoculation. For example, Brown *et al*. (1996) studying decomposition rates utilizing earthworm (*Pontoscolex corethrurus*) inoculations found that worm casts had 3.3 times the mineralization rates of the control soils. However, other studies indicated that endogeic earthworms increase SOM-C stabilization by combining organic matter with clay minerals to form soil aggregates and also by incorporating C in their cast into the stable chemically protected SOM, which in turn maintain SOM levels (Anderson and Flanagan 1989).

Termites hydrolyze SOM in an alkaline process (pH 9-11) in their digestive tract. This is followed by the assimilation of nutrients through a bacterial system. As a consequence, nutrients such as available P, are found in concentrations up to 16 times

higher in the fecal mounds than in the control soils. In some cases, termites can negatively affect the cultivated soil by diminishing the accumulation of organic matter around their nests. When nests are eliminated with pesticides, considerable increases in SOM may be observed (Anderson and Flanagan 1989).

2.2.2.2. Induced changes in SOM

Normally, human intervention has induced SOM loss in agro-ecosystems. Forest clear cuts, deforestation, slash and burn practices, the use of herbicides, intensive tilling and monocultures where plant residues are removed from the farm all usually decrease the SOM content. Soil disturbance accelerates the mineralization not only of labile fractions but also protected fractions in soil aggregates. Soil microbial biomass is reduced in quantity and diversity and volatilization of minerals also occurs. Intensive agricultural systems, particularly on steep slopes, produce SOM runoff. The physical loss of nutrients affects the natural nutrient cycle in these systems (Mc Donald *et al.* 2003).

Slash and burn systems are a good example of how SOM decreases as a result of human intervention. Soil fertility can be dramatically reduced in 2-5 years. In Senegal, C, N, pH, and exchangeable cations, which are fertility factors associated with organic matter, were invariably reduced after clear cutting and removing crop residues. After 3 years of cultivation, exchangeable cations decreased from 4 to 2.5 meq 100 g^{-1} and to 1.8 meq 100 g^{-1} after 12 years. The organic C decreased from 3.5% under original forest conditions to 2.5% after 3 years of cultivating (Fassbender 1993). Even though these intensive agricultural practices produced negative impacts on SOM, there are agricultural practices that can counter these effects.

Cover crops with legumes, crop rotation, plant and animal residue additions, incorporation of green manure, mulching, fallow cropping, contour cropping and terrace forming are all agricultural practices which help to reestablish the SOM equilibrium. In particular, some crop rotations which include roots, cereals and grasses can return high amounts of organic residues to the soil and lead to increased soil C equilibrium values

(Russell 1988). These soil conservation practices aim to recover the physical, biological and chemical soil properties. Green manure use, for example, increases organic matter production and the inherent accumulation of nutrients. Terraces and contour cropping reduce leaching and erosion of SOM. In fallow cropping systems, the secondary forest roots may recover nutrients from deep soil layers and return them to the soil as leaf litter. N reserves can be increased and microclimatic conditions that lead to SOM protection can be improved by mulching (Elzakker 1995).

Since the 1980's, some traditional agricultural practices have become popular again and are used for restoring SOM contents in low input systems. One of these techniques is the production of "bokashi" which is a mixture of organic materials and mineral sulfates that are anaerobically fermented. Bokashi increases the soil microbial biomass, slowly releases nutrients, and increases soil organic C contents (Fishersworring and Roβkamp 2001, Restrepo 2000). In a field experiment with maize in Nebraska using maize residue mulching, bacteria, fungi and actinomycete populations increased two- to six-fold after the addition of 7 and 14 Mg ha^{-1} of residues in comparison with the control that did not have residue additions. Nitrifying bacteria increased 2 to 20 fold after surface residue applications. The author attributed the increases not only to the soil C content increase but also to the soil moisture increase under mulch (Doran 1980).

Manipulation of earthworm populations as well as the use of worm manure have been considered interesting techniques for restoring or at least slowing the decrease of SOM in low input cropping systems. In some cases, earthworm casts have been thought to be responsible for labile SOM protection, but contrasting information states that lower levels of overall C stocks can be detected as a result of earthworm activity (Brown *et al*. 1996).

In field trials that lasted 3-7 years with low-input maize and yam crops in Ivory Coast, Peru and Mexico, the hypothesis that earthworm activity reduces the loss of soil C in the soil was not clearly supported. In Ivory Coast, after six continuous cropping cycles, the organic C stored in the soil decreased from 1.62 to 1.24 kg m^{-2} in plots with earthworm

additions and to 1.13 kg m^{-2} in the control plots. There were no significant differences between the treatment and control. Under the treatment with the addition of earthworms, the C content decreased by 28% over three years. A negative net balance was observed between the extra C incorporated into the soil by earthworms (2.9 Mg ha^{-1} in 7 years) and the C used in earthworm metabolism (7 Mg ha^{-1} in 7 years). Nevertheless, in one of the experimental sites in Ivory Coast, the earthworm activity significantly reduced the loss of C by 8% ($0.05 > p < 0.1$). This site had a very low degree of soil aggregation and the protecting effect was due to the formation of soil aggregates which protected the SOM from decomposition. In a 6 year field trial in Yurimaguas, Peru, inoculation with earthworms reduced the decline in soil C only during the second and third years. However, at the end of the experiment, the final result was a net reduction in the C content. In Mexico, soil C decreased 12% after three years of maize cropping and there were no differences between treatments. However, a high immigration of native earthworms into the control plots was observed (Gilot *et al*. 1996).

The incorporation of plant residues into deeper soil layers as the main effect of earthworm activity was studied by Gilot *et al*. (1996). Substituting C3 for C4 (maize) plants to monitor the ^{13}C:^{12}C ratio, the authors observed the proportion of C from maize residues that was incorporated into the soil with and without earthworm addition. In Ivory Coast after a three year experiment, the inoculation of earthworms produced an increase in the incorporation of C from maize residues from 0.24 Mg ha^{-1} in the control to 0.53 Mg ha^{-1}. In seven-year experiments with the inoculation of *Pontoscolex corethrurus*, C mineralization was stimulated and the total soil C content decreased from 35.6 Mg ha^{-1} to 32.4 Mg ha^{-1}. A lower amount of fresh organic material was incorporated into the soil. The earthworm species used for inoculation seemed to play an important role since in Ivory Cost *Millsonia anomala* increased the amount of C from maize residues incorporated into SOM (Brown *et al*. 1996).

Worm manure which consists of earthworm casts can be produced from a large variety of substrates with different earthworm species and in different production conditions. Ruiz *et al*. (1994) studied some chemical characteristics of earthworm manure

produced from cow and rabbit manure. The predominant humic substance present was fulvic acid, the total soluble salts ranged from 4,480 to 19,520 mg kg^{-1}, CEC was 123-188 Cmol kg^{-1}, humus content was between 34.5 and 38.4% and protein content was between 2.99 and 4.37%. Amines, tannins, terpenoids, steroids, quinones and lactones were also detected in the vermicompost. In a study of the effects of adding worm manure to two commercial crops in Cuba, similar yields were observed along with improved chemical soil properties. The study showed similar yields for *Ipomoea batata* crops with up to a 50% reduction in chemical fertilizers (30-20-25 NPK) plus 12 Mg ha^{-1} worm manure (Piedra *et al.* 1994).

Limited positive effects on plant growth have been observed after the introduction of earthworms into agricultural systems. The mechanisms involved in this growth improvement are unclear but include: a- mobilization of nutrients that are not readily available for plants without earthworm presence; b- increase of O_2 supply to roots by excavated galleries; c- the release of hormone-like substances present in earthworm casts; d- synchronization of nutrient release and nutrient uptake by plants (Lavelle 1997).

2.3. SOM management in agroforestry

2.3.1. The possibility of synchrony and synlocation of organic input application in agroforestry systems

Many of the management practices reviewed in the previous subchapter are related to the possibility of using tree residues as a source for increasing SOM contents and quality. Moreover, many crops in agroforestry systems are partially dependent on litter and pruning residues as nutrient sources. In low input and organic farming systems using shade trees, the lack of readily available and cheap nutrient sources is a major problem in addition to the scarcity of organic material for maintaining SOM levels (Giller and Cadisch 1995). However, it has been shown that only around 20% of newly added plant residues remain in the soil in the long term (Paul and Clark 1996) and 10-20% of the N contained in organic residues is taken up by the crops that receive the residue application (Palm 1995).

Furthermore, availability of some key nutrients is reduced due to lower mineralization rates caused by the low organic matter inputs (Haggar *et al*. 1993). Leaching processes and volatilization also contribute to nutrient loss. These two processes are enhanced in tropical regions between 23°N and 23°S latitude. Higher temperatures, usually 15°C hotter than in temperate regions (Theng *et al*. 1989) and heavy rainfall accelerate the biochemical reactions that lead to SOM decomposition and nutrient release. These problems partially explain why low yields are observed in some low input Costa Rican coffee farms. For example, the pruning residues of *E. poeppigiana* (a commonly used shade tree in Costa Rican organic coffee farms) can contain up to 300 kg ha^{-1} of N each year (Beer 1988), but the organic coffee farms that are not using chemical fertilizer usually have yields up to 30% less than conventional farms (Lyngbaeck *et al*. 2001).

Management of organic matter inputs can ameliorate the decrease of soil nutrient availability caused by low organic matter inputs and high leaching. Two management hypotheses have been stated. The "SOM hypothesis" states that if high quality organic matter inputs are added to the soil then the labile pools of organic-bound compounds in the soil can be increased with medium-long term benefits for soil fertility. The second, the synchrony hypothesis, states that the efficiency in which the nutrients from organic residues are used by the crops is highly related to the timing of their application. This hypothesis implies that if the release of nutrients from residues is not in synchrony with the crop demand then large quantities of nutrients can be lost from the system (Anderson and Ingram 1993). This approach can be applied to both annual and perennial crops. In annual crops, there are phenological stages when nutrient demands are critical for crop yield; even though these critical stages are not so precisely defined for perennial crops, synchrony of residue decomposition with the crop demands is still desirable.

The efficiency of nutrient uptake by plants can be improved by the management of both plant residues and SOM and it can be achieved not only by synchrony but also by synlocation which is the allocation of residues to where the plant roots are denser and highly receptive to nutrients (Schroth 2003). Different techniques have been tested to achieve synchrony and synlocation particularly with the use of mulch from tree residues.

Hence, some methods of residue application deal not only with the timing but also with the quality and freshness of the residues as well as the form of residue application. The quality of tree residue materials influences the decomposition rate; plant materials with high N and low lignin and polyphenol contents decompose more readily and release nutrients faster than plant residues containing low N and high lignin and polyphenols. This relationship makes it possible to manage the rate of nutrient release. For example, with annual crops, trees with high quality inputs can be planted in alley cropping systems. High quality *E. poeppigiana* mulches were used as a N source for short term crops like maize and beans. A 44% higher maize yield was obtained, using 20 t fresh weight ha^{-1} applied twice a year, compared to the control with no residue addition (Kass *et al*. 1989). However, such large amounts of plant materials are difficult to obtain in closed farming systems with crops that have low shade tolerance. In addition, the efficiency of tree residue nutrient uptake by plants may be very low (Haggar *et al*. 1993).

Montagnini *et al*. (1993) tested the mulch of four tree species which significantly surpassed the unmulched control in the growth of maize seedlings. In another experiment, Byard *et al*. (1996) observed higher litter accumulation due to low decomposition rates of residues with *Vochysia guatemalensis* that made it suitable for soil protection in the Atlantic lowlands of Costa Rica. In contrast, a very fast decomposition rate was found under *Jacaranda copala* that made it suitable for combination with annual crops where fast nutrient release was required. Decomposition rates have been consistently associated with the quality and nutrient content of tree residues. For example, *Terminalia amazonia* litter disappeared totally after six months of lying on the soil in the Costa Rican lowland climate, while 15% of the residues from *V. koschnyi* still remained on the soil surface after one year (Kershnar and Montagnini 1998). Therefore, *Terminalia amazonia* could be used when a rapid nutrient supply is required.

In some cases, low quality material can be used to increase crop yield if the application timing is appropriate and allows enough time for the residues to decompose. These plant materials can help to maintain a better microclimate for decomposition and can foster better yields in dry conditions (Tian *et al*. 1993). Mixtures of materials with different

qualities and decomposition rates have also been tested for increasing the release of nutrients from low quality materials, but in most of the studies, non-additive effects have been observed. Mineral fertilizers can be added to these poor quality residues to avoid initial immobilization of nutrients and supply some nutrients like P that are usually lacking in tree residues (Schroth 2003).

The freshness of tree residues also influences nutrient release rates. When green residues are applied to the crops, higher N uptake rates in crops are observed. On the contrary, when sun dried residues are applied, in which lignin and polyphenol concentrations have increased, residues are not so easily attacked by microbes, thus reducing the nutrient release and uptake rates by crops (Mafongoya *et al*. 1998).The degree to which the pruning residues are chopped also increases the rate of microbial activity on them, and can accelerate decomposition of low quality material with a high C:N ratio. Nevertheless, grinding residues may not be a practical method particularly in low input small farms. On the contrary, the incorporation of residues into the soil is an easier method than grinding residues to increase the nutrient availability for the crops. Burying the residues (e.g. by ploughing) provides closer contact between microbes and residues and high moisture levels are maintained in the residues, therefore accelerating residue decomposition as well as reducing losses due to surface run off and volatilization (Mafongoya *et al*. 1998).

In contrast to the synchrony hypothesis, the "SOM hypothesis" is related to the long term build up of SOM and the enhancement of mineralization processes. The effects of green manure on soil fertility are often only observed after several years of applications (Mafongoya *et al*. 1998). This was also supported by Haggar *et al*. (1993) using ^{15}N labeled mulch, compared the amount of N taken up by maize under an alley cropping system in comparison with sole crops. He found that the mulch N released during the crop growth season only accounted for about 15% of the total N taken up by maize in the alley cropping system. A possible explanation for the low incorporation of residue N in the crops was the low incorporation of recently applied N to the microbial biomass (3-5%). The remaining difference (~85%) between the sole and alley systems, as in the case of total N in the crop,

was due to the mineralization of native SOM built up over 7 years of residue application. Schroth (2003) also hypothesized that nutrient benefits to the crops, particularly for N, come more from the mineralization of SOM built up during years of residue application rather than directly from the immediate decomposition of tree residues.

Holland and Coleman (1987) studied the role of microorganism biomass in soil C cycling during winter wheat (*Triticum aestivum*) straw decomposition. The study found slower SOM decomposition when the fungi:bacteria biomass ratio increased. This inverse relationship was due to the higher growth efficiency of fungi which had more abundant cell-wall materials than bacterial biomass. However, few studies have addressed the effect of artificially augmented microbial communities in the acceleration of nutrient release and uptake by plants. Schroth (2003) suggested that strategies for managing biomass using the interaction between microbial and mesofauna populations with soil conditions can lead to more efficient nutrient cycling from SOM.

The direct impact of native SOM mineralization on nutrient uptake by crops has to be addressed (Barrios *et al*. 1996a). Synchrony and the build up of readily decomposable SOM are both important methods to assure efficient nutrient supply for crops under agroforestry systems. Although controversy exists as to which is the research priority, the challenge in using tree residues is to find practical and economical methods to manage biomass in order to improve nutrient uptake by crops while conserving the long-term SOM levels (Mafongoya *et al*. 1998).

2.3.2. The role of the light fraction of SOM in soil fertility

2.3.2.1. Definition of light fraction (LF) and isolation methods

Dead components of SOM which contribute to ca. 96% of the soil organic C can be divided into humus and the macroorganic fraction that represents 10-30% of the total organic C. Commonly LF and macroorganic fraction are used synonymously and are mineral free organic matter consisting of plant residues that are not completely

decomposed. However, in strict terms, LF is obtained by the flotation of SOM in high density liquids, e.g. 1.4 – 2.0 g cm^3 while the macroorganic fraction is obtained by soil sieving and is usually comprised of sand-sized materials (>53 µm) (Theng *et al*. 1989, Meijboom *et al*. 1995, Barrios *et al*. 1996a). Both LF and macroorganic matter are considered labile fractions because they are very susceptible to microbial activity as they are sources of available nutrients. Nevertheless, the macroorganic fraction contained different C pools with different qualities and turnover rates. These different pools have distinct microbial and enzymatic activity which is why it should not be considered as a homogeneous substance (Gregorich and Ellert, 1993). More thorough physical and chemical approaches have been proposed to separate macroorganic matter into different fractions including LF (Meijboom *et al*. 1995, Hassink 1995).

The LF generally contributes to 0.1-3% of the total soil weight in arable soils, but in grassland and forest soils it could reach up to 3-10% of the total soil weight. The light fraction is a C and N enriched fraction due not only to its high contents of oligosaccharides, polysaccharides, and hemicelluloses but also fungi hyphae, seeds, spores, charcoal and animal residues. The constituents of LF make it a readily decomposable fraction (Gregorich and Ellert 1993).

2.3.2.2. Light fraction and soil nutrient contents

The importance of LF is based on its role as a reservoir of recyclable nutrients that are readily available to crops. LF is an intermediary between crude plant residues and humic materials that are resistant to degradation. The main factors that determine LF decomposition rates are: material quality and quantity (the C:N ratio of LF is related to that of the original plant residues), aeration, pH and seasonal temperature and moisture changes. The presence of earthworms and other invertebrates is another important factor that influences LF transformation and composition (Theng *et al*. 1989).

The LF contributes to the available N reserves, but it is also the source for large losses of organic C, particularly in agro-ecosystems with intensive tillage. In a study of five

tropical soils, the rate of organic C loss in the LF was two to eleven times higher than in the heavier fractions. One of the main explanations for this lower N mineralization rate in the heavier fractions is the physical inaccessibility of the organic material to microbes (Sollins *et al*. 1984). Therefore, the LF can be used as an indicator of changes in the labile C status due to soil management (Fließbach and Mäder 2000). The LF has been used as an important soil quality indicator because more than half of the microbial population and enzymatic activity in the soil is associated with the LF since it decomposes and releases nutrients easily (Gregorich and Ellert 1993).

2.4. SOM dynamics measurement

The most important variables for quantitative analysis of SOM are: a) soil C content, b) chemical composition and decomposition rates of plant residues in the soil, c) nutrient release rates and mineralized C in SOM fractions, d) microbial biomass and e) content and type of humus as the stable fraction of SOM.

2.4.1. Soil organic C content

Soil organic matter C content has been traditionally measured by wet digestion with potassium dichromate which is known as the Walkey and Black method. This method provides acceptable accuracy and is relatively fast and simple. It is based on SOM oxidation with potassium dichromate and sulfuric acid. The digested solution is then titrated with an acidified ferrous ammonium sulphate solution and phenanthroline monohydrate as an indicator (Nelson and Sommers 1996). The dry digestion method is based on the volatilization of organic C. A very small sample is burned at 1000 °C under a flame fueled by oxygen. The combustion converts the C to CO_2 which passes through an infrared cell to be quantified. The results are expressed on percentage C basis. To transform the C concentration values obtained with these two methods, and considering that SOM is usually composed of 58% organic C, a 1.724 multiplying factor can be applied to obtain a percentage of organic matter in the soil. To avoid variations due to particular soil

conditions, usually the C concentration is reported instead of SOM percentages (Anderson and Ingram 1993).

2.4.2. Decomposition rate of plant residues in the soil

The decomposition of organic materials in the field has been commonly measured using the "litter bag" approach. This method uses plastic mesh bags which contain the material during the experimental period in close contact with the soil surface. Since the decomposition is not linear, a logarithmic regression model is used to calculate the decomposition rate constant "k" at specific times:

$$\log_n (W_t /W_o) = \log_n W_o - kt$$

where W_t is the amount of the initial mass (W_o) remaining at time "t". This method has been criticized because contact to the soil surface and access of soil organisms may be limited and compaction occurs inside the litter bags thus changing the microclimatic conditions in comparison to the natural unconfined litter. Internal moisture in the bags may also be very different from that of the surrounding soil (Anderson and Ingram 1993).

The decomposition rates of organic materials have also been measured in laboratories by confining fixed quantities of organic substrates and mixing them with soil. Measurements are periodically taken for C and nutrient evolution (Francia 1998). Even though this method is simple and direct, it does not allow for extrapolation to changing field conditions. Another disadvantage is that it considers the organic material as a whole. It does not account for the different decomposition and nutrient release rates of the pools that make up organic matter.

Unconfined litter decomposition can be also measured from field plots, but only in the initial phases when the materials are clearly recognizable. This method works best for large plant material such as branches that can be labeled properly. The turnover rate "k" is

then calculated as the quotient of residue inputs (I) divided by the standing material (S) over time (Anderson and Ingram 1993).

2.4.3. Soil organic matter fractionation (in search of the biologically-active SOM fraction)

There is no universally accepted model for C turnover, but all of them consider different C pools. Generally, these models include: organic matter inputs, one or more pools of SOM, and microbial biomass (Evans *et al.* 2001). Within each component, different C pools (which are usually associated with different theoretical turnover rates) are considered. For example, Verberne *et al.* (1990) considered four organic pools for modeling organic matter dynamics: microbial biomass and three fractions of residues. The three fractions include: decomposable materials (carbohydrates and proteins), structural materials (hemicellulose and cellulose), and resistant materials (lignified materials). Frequently, the models assign speculative turnover rates for each pool and difficulties are observed in completely describing the soil C turnover. These problems are associated with the absence of unique standard techniques for measuring the size of the C pools. However, search for "biologically active" C fractions has led to the development of chemical, physical and biological fractionation methods (Evans *et al.* 2001). Most of the methods try to quantify either a labile or a resistant pool, and the other pool is calculated by subtraction from total soil organic C values. A comparative study of four different techniques for separating labile and resistant fractions was carried out on soils from 33 restored grasslands in northwestern Minnesota (McLauchlan and Hobbie 2004). In that study, positive correlations were found between two biological approaches (Microbial biomass C and C respired after soil incubation), one chemical (Acid-hydrolysable C), and one physical (light fraction C using NaI, 1.7 g cm^{-3}). In this dissertation, experimental Chapter IV is focused on a physical fractionation method based on the density of macroorganic matter fractions. Accordingly, a review of chemical and biological methods and their results are presented, but more emphases is placed on physical methods.

2.4.3.1. Chemical fractionation

Evans *et al*. (2001) stated that within the chemical methods, the partitioning of organic matter into dissolved organic carbon (DOC) and water soluble organic matter has been used to characterize labile SOM. Dissolved organic carbon, which is released from microbial activity, root exudates and leaching of organic matter, is the most labile form of C in SOM.

In a Brazilian savanna, a method based on the solubility of labile carbon fractions was used (Westerhof *et al*. 1999). Water extractable carbon fraction (WEOC) was obtained by mixing 5 g of sieved soil with 12.5 ml of distilled water. Samples were shaken for 30 m, and then centrifuged to separate the supernatant which was then analyzed for C concentration. A similar procedure was applied substituting water with a potassium permanganate solution. Soil organic carbon was measured before and after the extraction. The difference was called the permanganate extractable organic carbon fraction (PEOC) and the sediment was called the stable fraction. WEOC and PEOC was significantly correlated with *in vitro* N mineralization of savanna soil samples. In a comparison between different land uses, pasture-based systems had significantly higher PEOC when compared with continuous croplands. Total soil carbon measurements were not useful in detecting differences between land uses. Hydrolysis is another common technique to study the labile components of SOM, particularly carbohydrates (both monosaccharides and polysaccharides). The amount of mannose, for example, were used by Cheshire *et al*. (1974) to study SOM decomposition by measuring the amounts of this sugar (which is common in fungi cell walls) remaining after soil incubation, and comparing this with cellulose which is very labile. Determining lignin contents following the "Goering and Van Soest" method in organic residues has been commonly used to predict the decomposition rates of different tree residues. This method is based on the fact that material with low lignin:N ratio decomposed faster than material with higher lignin:N ratios (Palm 1995). Finally, the sequential chemical extraction of humic and fulvic acids has been widely used. The fractionation takes advantage of the fact that humic acids are soluble in high pH solutions, but they are insoluble in acidic solutions.

Humus is considered a "resistant" SOM pool with very long turnover periods; therefore, its role in soil nutrient release (via decomposition) is limited. Humus can be fractionated into humic and fulvic acids and humins using their solubility properties. Humic acids are soluble in alkaline solutions and fulvic acids in acidic solutions; the humins remain after the humic and fulvic acid fractions have been removed (Bohn *et al*. 1993). The separation of these fractions has been criticized because the use of reagents can affect the nature of SOM in addition to the turnover rates of the fractions, particularly if the macroorganic matter has not previously been separated. Other methods for studying humus properties include pyrolysis/mass spectrometry and nuclear magnetic resonance with ^{13}C (Theng *et al*. 1989).

In the last decade, supercritical fluids have been used for separating organic matter into labile and stable fractions. Different aspects of this technique have recently been reviewed by Evans *et al*. (2001). Supercritical fluids are efficient extractants due to their high diffusion coefficients and low viscosities. The method operates under the assumption that the stable SOM pools have higher molecular weights and are less soluble than labile pools. The low solubility of stable pools is due to stronger Van der Waals forces and dipole-dipole interactions in the predominant functional groups. Therefore, the dissolved fraction obtained after supercritical fluids application to soil samples is considered the labile fraction and the remaining portion is considered the stable fraction. Validation and calibration of this technique in different soils using ^{14}C dating has been suggested.

2.4.3.2. Biological fractionation

Microbial biomass is considered as an important labile SOM fraction even though it contains only 1-5 % of the SOM C and N (Jenkinson and Powlson 1976). When microbial biomass is mineralized, it can act as a nutrient source or sink during immobilization (when microorganisms uptake SOM nutrients). Soil microorganisms not only act as catalyzing agents but also as live C and nutrient reservoirs (Gunapala and Scow 1998). The size and composition of this fraction is an important indicator of changes in land use since it is

associated with the quantity and quality of organic inputs in the soil. However, the use of microbial biomass as an indicator of SOM dynamics has been criticized because of the high sensitivity of this fraction to environmental moisture changes (Borken *et al*. 2003). The most commonly used method to measure microbial biomass is the fumigation-incubation method with chloroform developed by Jenkinson and Powlson (1976). Details of the method with modifications suggested by Anderson and Ingram (1993) are given in the methods section in Chapter IV.

Another important method is the basal respiration rate method that measures microbial activity using the rate of CO_2 evolution from soil. Microbial activity is usually used as an indicator of soil "health" and "quality". Microbial biomass can be also inferred from the CO_2 results (Cheng and Coleman 1989, Vandevivere and Ramírez 1995). This method has been applied to compare microbial activity between different farming systems. Some studies have shown that organic farming systems usually show not only higher microbial activity in comparison with conventional systems but also more diverse and metabolically efficient microbial communities (Lundquist *et al*. 1999, Castillo and Joergensen 2001, Fließbach and Mäder 2000).

One of the most effective methods for measuring the turnover of organic C in the soil is the stable isotope natural labeling method using ^{13}C. The method is based on the calculation of the ^{13}C:^{12}C ratio since ^{13}C is an abundant isotope in C4 plants such as sugar cane and maize. C3 and C4 plants have distinct ^{13}C:^{12}C ratios, therefore, changes in the isotope ratio of the SOM residues can be measured when natural vegetation (C3) is substituted with the C4 plants. The temporal changes in the ^{13}C:^{12}C ratio of the recently applied residues (C4) can be monitored in each SOM fraction and then used to calculate their decomposition rates (Balesdent *et al.* 1987).

Soil organic matter can also be fractionated and turnover rates estimated using mathematical models that combine chemical and biologically obtained data. The residue decomposition rates as well as temperature and moisture measurements permit the accumulation rates of the theoretical fractions to be estimated. The two primary theoretical

fractions are: a) the labile pool (LAB) that is susceptible to decomposition and has an average turnover time of 77 weeks and b) the resistant pool (RPM) which has an average turnover time of 27 to 600 years. Some authors consider POM, which is a physically measured fraction, to be within LAB and COM (chemically protected organic matter), which is the most stable SOM fraction, to be within RPM (Duxbury *et al.* 1989).

A method to measure potentially mineralizable N that is associated with turnover rates of the LAB fraction was developed in the 70's. In this method the aerobically mineralized N accumulated after the incubation of soil in the absence of O_2 in a confined vessel is measured. Environmental variables were also measured and a kinetic model which could be applied to field conditions was proposed. The amount of organic inputs, temperature and soil moisture were used to generate the turnover constants in the model. However, this model has been criticized because it frequently overestimates field N mineralization rates since it does not account for potential N immobilization in the microbial biomass. In addition, the concept of permanent discrete pools is not totally realistic because the quantity and quality of SOM is constantly changing (Duxbury *et al.* 1989). These problems highlight the importance of physical field measurements of the turnover of SOM fractions.

2.4.3.3. Physical fractionation

Soil organic matter has been physically fractionated into discrete components or fractions to study the decomposition and nutrient release processes with greater accuracy. There are two main physical fractionation methods for SOM (Figure 1). The first, called granulometric fractionation, is based on separating fractions by size. The SOM can be physically fractionated by passing the soil through different size sieves using water. The size of the organic or mineral-organic material is related to different decomposition rates and functions within the SOM. In granulometric fractionation, each fraction is comparable with the three textural soil particle types: the sand size (>53 μm[(*)]), called particulate

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[*] Some authors defined 150 μm as the limit to classify the sand size fraction [e.g., Meijboom *et al.* 1995 labeled this fraction as "macrorganic" fraction of SOM]

organic matter (POM) or macroorganic fraction; the silt size fraction (2-53 μm); and the clay size fraction (<2 μm). Besides these fraction sizes, some authors have studied some intermediate size fractions (20–53; 53-200 μm) (Kouakoua 1998).One of the main goals of the granulometric approach is to quantify the amounts (mass) of the different fractions as a result of different soil management regimes. For example, Neufeldt *et al*. (1999) compared croplands, pastures, reforested sites and natural savannas in Brazilian Cerrado oxisols. The study found that continuous cropping systems tended to reduce two size fractions of POM (20-50 and 50-2000 μm) in comparison with pastures and natural savanna. Total C showed few changes after 10-20 years of cropping.

The second approach is based on the fraction densities and it is called Size-Density Fractionation (SDF). After size fractionation, macroorganic matter can be further fractionated by floating the sieved material in high density liquids such as halogenated hydrocarbons. Inorganic salt solutions are usually used to separate less dense materials which are rich in C from denser colloidal materials which are bound to minerals. These denser materials are more or less humified (Strickland and Sollins 1987). Sodium iodide (NaI, 1.7 g cm^{-3}), bromoform-ethanol mixture (2.0 g cm^{-3}) or silica suspension (1.39 g cm^{-3}) has been used to obtain the light and heavy fractions (Gregorich and Ellert 1993, Alvarez and Alvarez 2000, Meijboom *et al.* 1995). In some cases, separation of SOM fractions is obtained only by flotation in NaI (1.7 g cm^{-3}) without previous size classification. For example, Roscoe and Buurman (2003) used three density fractions to analyze the effect of plowing and no-till cropping systems after a 30-year period in a Brazilian Cerrado oxisol (savanna). The three fractions were: a free light fraction (F-LF) with density <1.7 g cm^{-3}, an occluded light fraction (O-LF) with the same characteristics as F-LF but separated after ultrasonic dispersion, and a heavy fraction (HF) which was the sediment after LF recovery with a density of 1.7 g cm^{-3}. Differences between plowing and no-till systems were not detected using density fractions, but the amounts of C in F-LF and HF significantly decrease when natural savanna soils were converted into croplands.

In a comparison between pastureland and native savanna soil in Brazilian savanna oxisols, Freibauer *et al*. (1999) divided floating POM (polytungstate solution 1.6 gcm^{-3}) in

three different pools: free POM, an easily accessible POM which was obtained from water stable aggregates (<2mm) that were crushed during sieving, and an occluded POM that was firmly bound in stable aggregates and had low turnover rates. In contrast to the results of Roscoe and Buurman (2003), free POM showed higher values under croplands in comparison with natural savanna. The study suggested that differences were probably due to incorporation of maize crop residues by plowing, and to the short period of treatment applications (4 years). No differences in soil total C were found between natural savannas and cropland after four years.

The SDF method considers three principal fractions: a- the Light Fraction (LF) which consists of plant residues that are not completely decomposed and still recognizable (density < 1.13 g cm^{-3}); b-The medium (MF) which consists of partially humified materials; c- The heavy fraction (HF) which consists of amorphous organic materials (>1.37 g cm^{-3}). It has been observed that C in the LF is much more labile than the C in the other two fractions (Hasssink 1995, Fließbach and Mäder 2000).

The size and the size-density fractionation methods force soil samples through sieves with different mesh sizes using water. In the case of the granulometric approach, the wash water and fine materials are recovered. The sample is sieved several times through progressively smaller mesh sizes. In the case of the SDF method, the macroorganic fraction (also called particulate organic matter, POM) is separated and the material less than 150 μm is discarded. A nylon sieve containing the POM material is submerged in a plastic container filled with a high density silica suspension, Ludox™ (Dunlop Inc) with a density of 1.37 g cm^{-3}. The sediments are recovered as the HF and the floating materials are recovered and submerged into another container with a Ludox™ density of 1.13 g cm^{-3} to separate the MF and LF (Meijboom et $al.$ 1995, Hassink 1995). Some drawbacks of the method have been reported. A considerable laboratory error risk has to be assumed because the method requires human skills to separate the fractions; therefore, quality control should be maintained. Ludox™ produces an increase in N mineralization rates that can alter the result of studies on N dynamics. Water has been suggested in some cases as a good substitute for Ludox™ (Magid et al. 1996).

The fractions obtained by the SDF method are analyzed for total C and N concentrations and can be used in comparative incubations of plant residues with different C:N and lignin:N ratios. Magid *et al.* (1996) studied the decomposition of *Lolium perenne* plant material grown under two different CO_2 concentrations in order to identify an "active fraction" of SOM. Fractionation methods of labile SOM using both Ludox™ and sodium tungstenate were compared. The SDF method using Ludox™ proved to be more efficient in separating fractions that indicate the decaying process of the materials. However, the three size-density fractions showed similar declining trends, and no different roles were detected for any of the fractions in regard to the decomposition of the different plant materials. More field research is needed in order to discern if SDF can provide information about the "active fraction" of SOM.

- Fig. 1. Soil Organic Matter Fractionation (2 approaches)

1. Granulometric aproach: (by size)
(Feller 1994, Kouakoua1998) >53 μm

2. Size-density approach:
(Meijboom *et al.*, 1995, Hassink 1995, Flieβach and Mäder (2000)

>150 μm
Light fraction*
Density:1.13 g cm³
Intermediate fraction
Density:1.37 g cm³
Heavy fraction

200-2000μm
50-200 μm
20-50 μm
2-20 μm
<2 μm
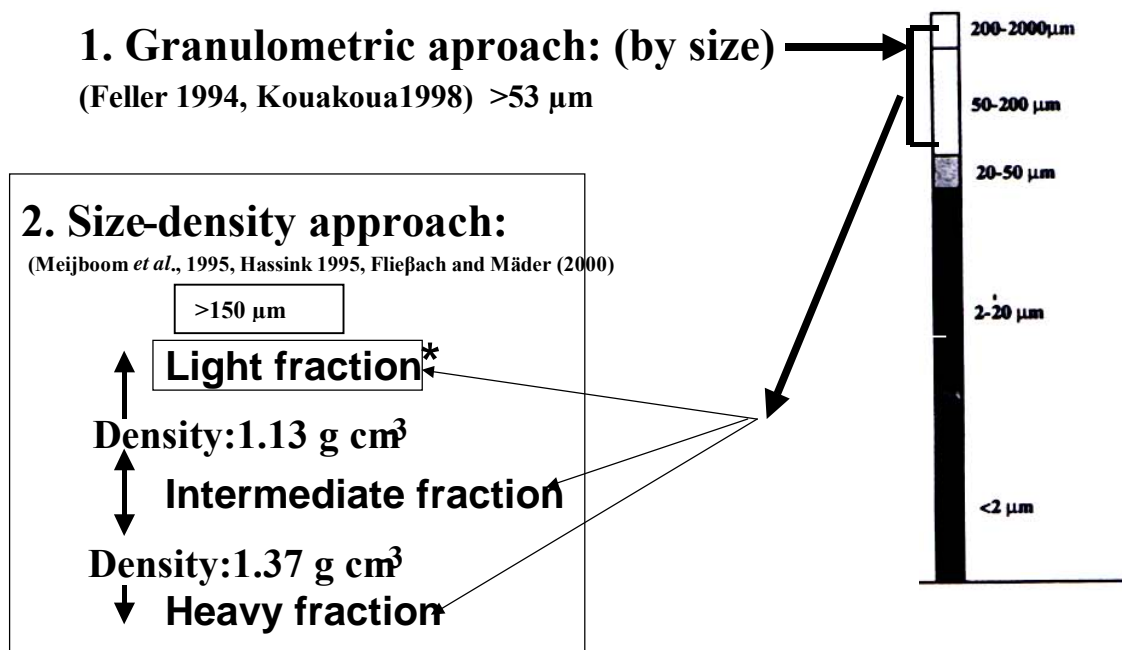
The SD fractions also have been used as indicators of induced changes in soil through different vegetal residue inputs (Hassink 1995), and in comparative studies for the effect of organic and conventional systems on soil organic matter (Fließbach and Mäder

2000) (details are included in Chapter IV). Barrios *et al*. (1996a) suggested that the light fraction of SOM obtained using size-density fractionation methods has an important role in nutrient cycling and supply. They studied SOM under seven maize-legume cropping systems after the eighth cropping season. Some fractions were obtained using the particle-size density method (Cambardella and Elliot 1992) and a size-density fractionation method with two liquids, NaI (density = 1.7 g cm^{-3}) and Ludox (density = 1.39 g cm^{-3}). Total C was also evaluated as an indicator of changes in the SOM state under the different cropping systems. LF-C, but not total soil C, reflected the effect (higher C contents in the fraction) of leguminous residue addition into the soil in the intercropped systems. The effect of three different fallow species, *Tithonia diversifolia, Calliandra calothyrsus* and *Indigofera constricta,* as well as a continuously tilled maize-bean rotation on SOM fraction properties was studied. The Medium SD fraction (LM) dry weight, C, N and P contents showed significant differences among treatments. All the fallow species tended to have higher values for the parameters measured in LF and LM in comparison with the rotation cropping system (Phiri *et al*. 2001). Nevertheless, the small size of LF and lack of information about nutrient mineralization rates in this fraction do not allow for a definitive conclusion about its role as a unique "biological active fraction" (Fließbach and Mäder 2000, Magid *et al.* 1996, Hassink 1995). The SDF method has been proposed as an alternative to the "litterbag" approach for studying decomposition of plant residues because a closer contact between the organic materials and the soil environment can be achieved; in addition, soil nutrients can be available for microbes during the decomposition process and organic residues exposed to faunal activity and natural soil moisture (Magid *et al.* 1996). However, more research is needed to determine the usefulness of this alternative method.

2.5. Effects of trees on the fertility of surrounding soil

The next five sections summarize information about the effects of trees on soil variables. Methods used to study the effects of trees on soil fertility are also reviewed under three conditions (isolated trees of natural regeneration in deteriorated soils; trees in monoculture plantations; and trees in agroforestry systems).

2.5.1. Results on the effects of trees on soil fertility

The effect of isolated *Acacia tortilis* and *Adansonia digitata* trees on chemical soil properties under the crown and in the adjacent open savanna under different grazing intensities was studied in the Kenyan savanna by Belsky *et al*. (1993). In the sites with low grazing intensities, higher P, K, Ca and mineralizable N levels were found below the crowns than in the surrounding areas without trees. However, in the sites with intensive grazing no differences between areas with and without trees were found because of the diminished vegetation strata and soil compaction from trampling.

In a similar study of the beneficial effect of *Parkia biglobosa* on the Burkina Faso and Nigerian savanna soils, six sites were selected with different soil and rainfall patterns. Isolated trees of three DBH (diameter at breast height) categories were studied. Significantly higher N and available K levels were found under the tree crowns; but the soil organic matter levels, available P, and pH did not show significant differences when compared to the open areas far from the trees (Tomlinson *et al*. 1995).

The decomposition and incorporation of organic matter from leaf litter into the soil and its distribution amongst granulometric soil organic matter fractions below clumps of *Acacia seyal* and *Eucalyptus camaldulensis* plantations were compared in Senegal (Berhard-Reversat 1987). The study found higher mineralization in the soil below *A. seyal*. The light fraction (LF) in the soil below *E. camaldulensis* did not contribute N because 80% was associated with the mineral bound fine fraction. In contrast, the LF under *A. seyal* contributed 50-70% of the mineralized N (Berhard-Reversat 1987).

The effect of tree plantations on the fertility of degraded soils has also been studied. One trial measured the effect of introduced and native tree species, established on pastures located in the biological station *La Selva* in northeastern Costa Rica. During the first four

years, soil bulk density decreased under eight of the eleven tree species tested, but the soil organic matter concentration only increased under three species. Nitrogen did not increase considerably under N fixing species; however, available P increased markedly under species of the genus *Vochysia*. The study concluded that an improvement of soil conditions occurred in a very short time (Fisher 1995).

In a similar study in the same region, decreases of P, K, and Mg were found in the soil under fast growing species such as *Jacaranda copaia* and *Vochysia guatemalensis* planted in monoculture. However, under species such as *Terminalia amazonia* and *Virola koschnyi,* with high leaf Ca content and high leaf shed ratios, the soil Ca concentrations were higher than in the control plots without trees. Nutrient decrements were less in areas planted with a mixture of species (Montagnini 2000).

A third type of research was focused on the nutrient trends under trees associated with crops in agroforestry systems. A study conducted in Costa Rica, measured the net N mineralization rate and nitrification in shaded and full sun coffee farms. The study found higher N mineralization rates under shade. The lower rates occurred in the dry season, (January to March). The annual average rate under shade was 15 and in full sun 11 g N m^{-2} yr^{-1}; 95% of the total soil N was oxidized to nitrates in both types of plantations. Even though shaded farms had higher N availability, the amount of N leaching was lower than in full sun plantations. N seems to be conserved better within agricultural systems in which shade trees are present (Babbar and Zak 1994, 1995).

Haggar *et al*. (1993) studied the decomposition of pruning residues, soil N mineralization rates and N transfer from trees to crops in alley cropping systems of corn and beans with *E. poeppigiana* and *Gliricidia sepium* trees in the experimental station of *La Montaña* in CATIE. Soil under tree associated crops had higher N mineralization rates than under monoculture crops. At the beginning of the field trial, low N mineralization rates were found in the area close to the tree trunk, which indicated N immobilization due to the presence of trees. However, this result was not found at other experimental times.

2.5.2. Site selection and experimental designs

Different studies in the African Savanna have analyzed the effects of naturally regenerated trees on the surrounding soil fertility. A variable number of sites, which were supposed to represent the natural tree distribution, and also a variable number of trees were selected in each site. In these studies, the experimental models used varied from comparisons between paired samples to complicated block designs. In order to evaluate the effects on soil quality of isolated clumps of *A. seyal* (Berhard-Reversat 1987) and *Parkia biglobosa* (Tomlinson *et al*. 1995) paired sampling was used. A complete random block designs were used by Fisher (1995) while Belsky *et al*. (1993) used a split plot design.

Berhard-Reversat (1987) studying the incorporation of organic matter from leaf litter into the soil below *Acacia seyal* and *Eucalyptus camaldulensis* selected two sites representing sandy and sandy clay soils and compared average results from ten bulk samples from each of the two sites. Belsky *et al.* (1993) studied the effects of *A. tortilis* and *A. digitata* on soil quality. Four sites with mature trees of these two species were selected. Two of the sites had low grazing intensities with different rainfall patterns (one of moderate grazing and one was only occasionally grazed by wild animals). In the sites with high grazing intensities, two sub-locations were chosen due to the variation in relief and vegetation patterns. He sampled an area below the crown and two pasture areas, 20 or 50 m from the tree depending upon the size of the tree crown so that the control samples were not influenced by tree roots. In the low intensity grazing area, three trees from each species were studied. In the medium and high intensity grazing areas, only two trees from each species were selected since the individuals of any one species were homogeneous. The main factor in the split plot design was the tree species and the subtreatment was the distance from the tree (under the canopy or 20-50 m. from the tree). The replication units were individual trees.

At a larger scale, Fisher (1995) compared the effect of three introduced and eight native species on soil fertility using a complete random block design with eleven treatments, four replications and 2500 m^2 experimental plots. The sites were previously grazed for 25 years and abandoned shortly before the trees were planted. Montagnini (2000) used two plantations, each one with four different tree species. This work used the same experimental design as Fisher (1995): six treatments, four replications and a plot size of 1296 m^2. The control was also the same as in Fisher´s study, a neighboring fallow plot with natural regeneration. Haggar *et al*. (1993) studied the effects on soil of trees with edible crops in agroforestry systems. They used a complete random block design with three replicates and three treatments (single crop, alley cropping with *E. poeppigiana* at 6 m between rows and 3 m between trees, and *G. sepium* at 6 m between rows and 0.5 m between trees). Twelve by 8 m plots were use to evaluate productivity (total crop biomass).

Babbar and Zak (1994, 1995) selected three sites in the Central Valley in Costa Rica. In each site, a pair of farms less than 600 m apart were chosen. N mineralization in shaded and full sun coffee farms was studied using the paired farms as blocks, with one block in each locality (replication). A random complete block design was used to analyze annual average N mineralization. The study consisted of three replications and hence six farms.

2.5.3. Soil sampling

There is a large variation between the sampling designs used in the studies described in this section. Within agroforestry systems, a sampling design that uses progressive distances from the trees, was commonly utilized to evaluate the effect of proximity of the tress on soil N concentrations (Haggar *et al*. 1993). In most studies bulk soil samples are used. The nutrient concentrations within the soil profile are studied at variable depths commonly concentrated in the topsoil (0-30 cm) which is divided in two or three layers. Soil sampling commonly began after 40 days of applying residues when decomposition or nutrient release was studied. Babbar and Zak (1994) studying N mineralization in coffee plantations sampled four sub-zones, defining a 2 m distance as the

optimum distance for observing changes produced by the proximity of shade trees. Details of the different sampling methodologies are presented in Table 1.

Table 1. Examples of sampling designs used to study the effects of trees on soil fertility and productivity

Study type	Variables and source	Sampling characteristics
Effects of isolated trees on soil fertility	Soil textures and chemical properties (Belsky *et al*. 1993)	• Bulk samples from four 5 cm diameter subsamples from the 0-15 cm soil layer • 4 randomly selected sites both below the tree crown and in open savanna
	Vegetative strata composition (percent coverage of all the species) (Belsky *et al*. 1993)	• 1.0 m^2 frame placed at 1 m intervals on a 50 m transect from the trunk toward the pasture area in any direction • Subsamples within 0-8 m from the trunk were mixed into one bulk sample and the 20-50 m subsamples into another
	Soil organic matter fractionation. Leaf litter decomposition and mineralization in the 0-1 cm soil layer (Berhard-Reversat 1987)	• Samples were collected from randomly selected 400 cm^2 squares from areas under both *E. camaldulensis* and *A. seyal* • Material from 3 to 4 squares were combined into one bulk sample (Because it was difficult to sample a 1 cm depth layer, the volume and the theoretical mass of 1 cm $\times$ 400 cm^2 were calculated and compared with the volume and mass of the actual samples. The samples with the highest deviation were discarded). Ten bulk samples were taken from two sites with different soil textures
	Chemical soil characteristics (Tomlinson *et al*. 1995)	• Samples were taken at 30 cm depth from 4 positions: base of the trunk, below the middle of the crown, below the edge of the crown, and 2 m outside of the third position. • Corresponding samples from the four cardinal directions were collected and mixed
Effects of tree plantations on soil fertility	Chemical soil characteristics (Fisher 1995)	• 0.25 ha plots were divided into quadrants • 20 subsamples (0-15 cm) were mixed into one bulk sample per quadrant
	Chemical soil characteristics (Montagnini 2000)	• Bulks samples were collected from each of the replications at 4 soil depths: 0-5, 5-15, 15-30, 30-60 cm
Effects of trees in agroforestry systems	Net mineralization and nitrification Babbar and Zak (1994)	• 4 positions in shaded coffee farms were sampled: under the crown more than 2 m from the trunk of both growing and recently pollarded *E. poeppigiana* trees and below a coffee plant • 3 positions in full sun coffee farms were sampled: below a coffee plant, below a pollarded coffee plant and equidistant between two coffee rows • For both types of farms, a pair of 10 cm diameter samples at to 10 cm depth were taken at four week intervals. One of the two samples was immediately returned to the field for incubation.
	N Concentration in mulch (Haggar *et al*. 1993)	• Three 0.5 m^2 squares were randomly chosen in each experimental plot at 0.5-1.0, 1.5-2.0, and 2.5-3.0 m from the hedgerows, the mulch from corresponding squares was collected and mixed into a bulk sample. • Samples were taken 38 days and 115 days after pruning the trees

	N mineralization. (Haggar *et al.* 1993)	• Samples were taken at 0.5-1.0, 1.5-2.0, and 2.5-3.0 m from the hedgerows. • In each position a pair of 7 cm diameter samples were taken at 25 cm depth. One of the paired samples was analyzed for NO_3^- and NH_4^+, and the other sample was isolated in a PVC tube for field incubation
	N transfer from the mulch to the soil Haggar *et al.* 1993)	• Soil samples were taken five days before the pruning residue application and 40, 70, and 105 days after. • Samples were taken along three randomly selected 3m transects which were perpendicular to the hedgerows. Two 2.5 cm diameter and 0-25 cm depth cores were taken at 75, 150 and 225 cm from the trees on each transect. • 18 corresponding cores in each main plot were taken and mixed in a bulk sample, four 30 g subsamples were then separated for analyses.

2.5.4. Evaluation of field variables

Microclimatic effects, leaf litter decomposition rates and aerial tree biomass are field variables that have been measured to study the relationships between the components of agroforestry systems. In some cases, the investigators adapted the standard methods such as "litterbag" to measure the decomposition rate of leaf litter (Berhard-Reversat 1987). Another standard method that has been used is ^{15}N labeling to evaluate nutrient transfer between different agroforestry system components (Haggar *et al*. 1993).

Microclimatic effects of tree plantations in northeastern Costa Rica were studied by Fisher (1995). He measured soil moisture daily early in the morning for three weeks in January, the dry season, and three weeks in June, the rainy season. Data were collected in the third year after the establishment of the plantation at 10, 20, and 30 cm depths in the center of each of the experimental plot quadrant. In another study, soil temperature was measured at 30 minute intervals at 5 and 10 cm depth in five randomly chosen sites in two study areas around isolated trees in the Kenyan savanna. Statistical analyses using t-tests were used to compare the data (Belsky *et al*. 1993).

Leaf litter decomposition of *E. camaldulensis* and other tree species with slow decomposition rates was measured by collecting fresh and old leaf litter from the soil

surface (Berhard-Reversat 1987). The litter was separated by color. The old litter stayed on the ground for at least one rainy season. In the case of *A. seyal*, fresh litter was collected in square nets, air dried, and then used as old litter. This procedure is questionable since the drying process stops microbial activity and the data can not be compared to the *E. camaldulensis* exposed to the natural conditions. Plastic nets, 1.5-2 mm mesh, were used for the *in-situ* decomposition trials. The nets, which were filled with 20-25 g fresh leaf litter, were left on the ground and collected at 30 day intervals (Berhard-Reversat 1987).

2.5.5. Laboratory techniques and statistical analysis

As in the case of sampling methods, the laboratory techniques are varied, and they are summarized in Table 2.

Table 2. Methods, laboratory equipment and types of statistical analyses to study the effects of trees on soil fertility

Study type	Source, variables and statistical analyses	Methods and laboratory equipment
Effects of isolated trees on soil fertility	Soil temperature: Texture: Total C and N: Bulk density: Water infiltration: NH_4^+, NO_3^-: Microbial biomass: ANOVA and t-tests, SAS-GLM Data were normalized logarithmically (Belsky *et al.* 1993)	Termisthor Omega 450 Bouyoucos hydrometer Dry combustion and mass spectrophotometry Stainless steel rings Metal rings (10 cm diameter x 25 cm long) Steam distillation Jenkinson chloroform fumigation
	Extraction of soluble litter compounds: Organic matter CO_2 production: Fraccionation of SOM: C and N in lixiviates and in fractions: t-test for paired samples (Berhard-Reversat 1987)	Percolation with water NaOH capture with air circulation Sieves with five mesh sizes Kjeldahl
	Total C: Total N: P and K: Mycorrhizal infection: Three way ANOVA: site, tree size, and distance from the tree (Tomlinson *et al.* 1995)	Dry combustion Kjeldahl Olsen and flame photometry Root staining with Trypan blue (0.05%)

Effects of tree plantations on soil fertility	Total C: Total N: K, Ca, Mg: Bulk density: ANOVA, for mean comparisons. Least significant difference test LSD α=0.05 (Fisher 1995)	Walkley and Black Micro-Kjeldahl NH_4OAc extraction at field pH The mean of two 100 cm^3 surface soil cores
	Chemical soil properties: ANOVA, for mean comparisons. Least significant difference test LSD α=0.05 (Montagnini 2000)	Similar to Fisher (1995) but cations measured using an atomic absorption spectrophotometer
Effects of trees in agroforestry systems	Net mineralization and nitrification: ANOVA and LSD α=0.05, t-tests for paired observations. SYSTAT Babbar and Zak (1994)	Colorimetrically, using a Lachat automated ion analyzer
	Net mineralization and nitrification: N transfers from the mulch to the crops Microbial biomass ANOVA and orthogonal contrast for mean comparisons, SAS (Haggar et al. 1993)	Stem distillation and extraction with 2 M KCl ^{15}N measurements with a mass spectrophotometer Sparkling and West fumigation-extraction

2.6. Description of shade coffee agroforestry systems with *Erythrina poeppigiana*

2.6.1. Technical characteristics of the system

The use of shade trees in perennial crops, such as coffee and cacao is considered an ancient agroforestry systems in Central America (Beer *et al*. 1998; Kass *et al*. 1997). Coffee has been cultivated in Costa Rica, utilizing different native and introduced shade tree species, since its introduction in the middle of the XIX Century (Samper 1999).

This study was carried out in conventional and organic coffee plantations with shade trees. In the conventional system, farmers use technology developed by the Costa Rican Instituto Costarricense del Café (ICAFE) based mostly on external chemical inputs. In order to intensify production, conventional farms use high external inputs for plant nutrition and pest and disease control. While farmers in organic farming systems exclude the use of synthetic chemicals, which are not permitted under the certification guidelines of specialized organizations such as the International Federation of Organic Agriculture

Movements (IFOAM) and the International Organic Accreditation System (IOAS) (Soto and Muschler 2001). Exceptions include copper hydroxide, a fungicide, and calcium carbonate, used to increase soil pH. Organic production is based on the main principles of: a- imitation of natural relationships and processes between plants and animals in natural ecosystems; b- increasing (or maintaining) biodiversity; c- nutrient recycling; d- the concept that soil is a living system in which the organic matter plays a very important role; e- minimizing or avoiding any form of pollution from agricultural activities (Lampkin 1990, Soto and Muschler 2001).The main differences between systems are defined by the use of denser shade and higher shade tree populations in organic systems; conventional systems had higher nutrient inputs (chemical fertilizer) and less use of labor. Some principal characteristics for conventional and organic coffee systems in Costa Rica are presented in Table 3.

Table 3. Principal technology characteristics of conventional and organic agroforestry shade coffee systems in Costa Rica

Technical Characteristics	Conventional Systems		Organic Systems
Average yields. "C" Samper (1999) "O" Boyce *et al*. (1994) Lyngbaeck *et al*. (2001)	National average, 1697.4 kg ha^{-1}(*). Sample of 10 farms = 1400±74 kg ha^{-1}		Average of a 17 farm sample = 933.8 Kg ha^{-1}. Sample of 10 farms = 1086±225 kg ha^{-1}
Farm Size "C" Samper (1999) "O" Boyce *et al*. (1994) "O" Lyngbaeck *et al*. (2001)	92% <5 ha and 2% of the farms are >20 ha		41.2% of the farmers between 0.3 and 7 ha
Plant Population Density "C" Bertrand *et al.* (1999) "O" Lyngbaeck *et al*. (2001)	3200-6300 plants ha^{-1}		5000 plants ha^{-1} approximately
Fertilizers "C" Beer (1988) "O" Boyce *et al*. (1994)	Up to 300 kg N ha$^{-1;}$; also complete formulas 18-5-15		Calcium carbonate, cow manure, chicken manure, 500kg compost and bocashi
Weed Control Boyce *et al*. (1994)	Paraquat, Oxyfluofen, Glyphosphate, 2,4 D		Manual weed cutting, self-shading, burning, grazing
Plague Control Boyce *et al*. (1994)	Fungicides	Benomyl, lead arsenate	Copper hydroxide, kilol
	Nematocides	Terbufos, Carbofuran	
	Insecticides	Chlorpyrifos	
Shade Tree Species	*Erythrina poeppigiana, Cordia*		Tree shade used in most cases; species similar

"C" Beer *et al.* (1998) "O" direct observation	*alliodora, Inga spp, Gliricidia sepium*; and others. Most intensive systems no shade	to conventional
Tree Population Density Direct observation	100-300 trees ha^{-1}	100-420 trees ha^{-1}
Tree Pruning "C" Beer (1988) "O" direct observation	1 to 3 times per year. January-February to promote flowering, June-July to promote fruit ripening in the Atlantic zone	0-2 times per year, same timing as conventional
Labor use (excluding harvesting) Boyce *et al.* (1994)	Weed control requires the most labor	40% more labor than conventional due to collecting, processing and application of organic fertilizer 91 days ha^{-1} year^{-1} in total
Production cost, for farms from 0.3 to 7 ha Lyngbaeck *et al.* (2001)	0.59 US$ per pound	0.62 US$ per pound

2.6.2. Nutrient recycling in shaded coffee systems

In coffee systems with N fixing trees, nutrients can be transferred from tree to soil, and hence at least partially to the crops, in pruning residues and natural litter fall, contributing up to 12 Mg ha^{-1} yr^{-1} of dry matter (Beer 1988, Fassbender 1993). The role of leguminous shade trees in nutrient recycling in the soil by providing organic matter (potential source of nutrient mineralization and CEC), may be more important than N fixation alone on many sites. Pruning residues plus natural leaf litter can provide more than 50% of the organic matter added to the system and shade trees such as *E. poeppigiana* can return to the soil up to 90% of the nutrients contained in aerial tree biomass when intensively pruned. This species can provide 300 kg N ha^{-1} yr^{-1} of nitrogen in pruning residues and litter, but the amount of fixed nitrogen does not exceed 60 kg N ha^{-1} yr^{-1} (Beer 1988). Babbar and Zak (1994) found higher N mineralization under shade where both mineralization and nitrification were seasonal. The lowest ratios occurred in the dry season (January to March). Some trees that do not fix N contain more Ca and Mg, but the release rate is slower than in leguminous trees (Fassbender 1993).

Chapter 3   Soil carbon and nitrogen concentrations and other soil characteristics below *Erythrina poeppigiana* in organic and conventional coffee plantations

Key words: *Coffea arabica,* organic agriculture, shade trees, soil fertility, soil organic matter

3.1. Introduction

Shade trees are traditionally planted in association with coffee plants to reduce the impact of adverse climatic conditions, especially high temperatures, and to positively influence the size and the quality of the coffee beans (Muschler 2001). These shade trees also have an important role in the uptake and recycling of nutrients from the soil (Young 1999) and in the input of nutrients; e.g., leguminous trees can fix N_2 which may later be recycled to the coffee via above ground litter, pruning residues and root turnover. The influence of trees on adjacent soil (chemical and physical characteristics) has also been studied in natural ecosystems (Boettcher and Kalisz 1990). Lower soil pH and higher mineral N was measured under the tree crown as a result of chemical properties of needle and branch litter of *Pinus contorta* in a pioneering study (Zinke 1962). This radial pattern of influence can be modified in part by topographically and climatically controlled variables like predominant wind direction, soil creep, and in some cases, litter distribution. However, even in areas with a very steep slope the effect of single trees on surrounding soil pH, nutrient concentrations and bulk density has successfully been measured (Zinke 1962, Boettcher and Kalisz 1990).

On abandoned pasture land in northeastern Costa Rica, Fisher (1995) found that the long term presence of eleven tree species (age 25 years) significantly increased the base cations, available P and organic C content of degraded soils, and also caused a reduction in bulk density. A study conducted in coffee plantations in the Central Valley of Costa Rica

found higher N mineralization rates under leguminous shade trees in comparison to a control site that lacked shade trees (Babbar and Zak 1994, 1995). Although N availability was higher, leaching of N occurred at a lower rate indicating better conservation of N in coffee plantations that included shade trees. Leaf and root residues of the leguminous shade tree *E. poeppigiana* have been studied as an important source of nutrients in coffee plantations (Fassbender 1993). Almost 90% of the nutrients stored in above ground biomass are returned to the soil surface, principally because of pollarding carried out 1-3 times annually; these trees can contribute 5000-12,000 kg of organic material ha^{-1} year $^{-1}$ (Beer 1988).

The use of *E. poeppigiana* pruning residues as a source of nutrients also has been studied in alley cropping systems (Ramírez and Bornemisza 1990; Kass *et al* 1993) and in multistrata agroforestry systems (Szott *et al* 1991; Szott and Melendez 2001). In these studies, higher soil C and nutrient concentrations were found in soils with pruning residue mulching in comparison with soils without mulching. These findings have been attributed to the high K, and Ca contents in the pruning residues and to the high biomass production of this shade tree (Russo and Budowski 1986). Nevertheless, the information on the impact of shade trees on soil characteristics in tropical regions and particularly in low input systems is very limited (Beer *et al.* 1998, Vaast and Snoeck 1999, Fernández and Muschler 1999).

Depressed international coffee prices from 1999 to 2003 have led to a search for new niche markets that offer greater economic premiums. One option that has been adopted by some Costa Rican farmers is the production of organic coffee, particularly in agroforestry systems (Lyngbaeck *et al.* 2001, Boyce *et al.* 1994). Many studies conducted in temperate regions have shown that organic agricultural systems, in comparison to conventional agronomic practices, can have positive impacts on the soil including higher organic matter concentrations, soil respiration, mineralizable N, promotion of a more friable soil structure and enhancing soil biological activity (Lotter 2003, Fließbach and Mäder 2000). For example, in organic farms in New Zealand the number of earthworms found in the topsoil (0-15 cm depth) was ten times higher than those present in

conventional farms. Cation exchange capacity (CEC) and total soil N were also higher in organically farmed soils, but pH, available soil P and S were higher in conventionally farmed soils possibly due to chemical inputs (Reganold *et al.* 1993, Reganold 1995, Wells *et al.* 2000). The possible advantages of organic farming systems must be evaluated in a greater number of farms and soil types, particularly in tropical regions, to determine whether these systems truly represent an ecologically and economically sustainable management strategy.

The central objectives of the research reported in this article were to analyze the impact of the shade tree *E. poeppigiana* on chemical and biological soil variables in organic and conventional coffee farms. The study focused mainly on the effects of this tree species on soil C and N concentrations, macro and micronutrient concentrations, pH, electrical conductivity, CO_2 production and soil organic matter size fraction distribution. These variables were analyzed in comparable organic and conventional systems at three different positions around the shade tree and at three separate depths. Secondarily, differences in soil characteristics between organic and conventional farms were also studied.

3.2. Materials and Methods

In July 2000, a preliminary soil study was done on 14 farms in Central Costa Rica. To choose comparable paired farms, five pairs of farms were selected for this study based on the criteria presented below. Based on the results of the original work in 2000, another soil study was done in 2004 using a more detailed soil characterization of the original farms.

3.2.1. Criteria for farm selection

Five pairs of farms were compared (five organic and five conventional) in the municipalities of Aserrí (Aserrí1 and Aserrí2), Turrialba (CATIE), and Paraíso (Paraíso and Pejivalle) in Costa Rica (Figure 1). The distance between two farms of any one pair was less than 500 m. The farms were chosen so that soil characteristics and management

regimes were as similar as possible for the pair (Table 1) with the main difference being the absence of agrochemical inputs on the organic farm. Soil bulk density was measured for both organic and conventional farms to look for reasonable comparability between paired farms (no significant differences between paired farms were found).
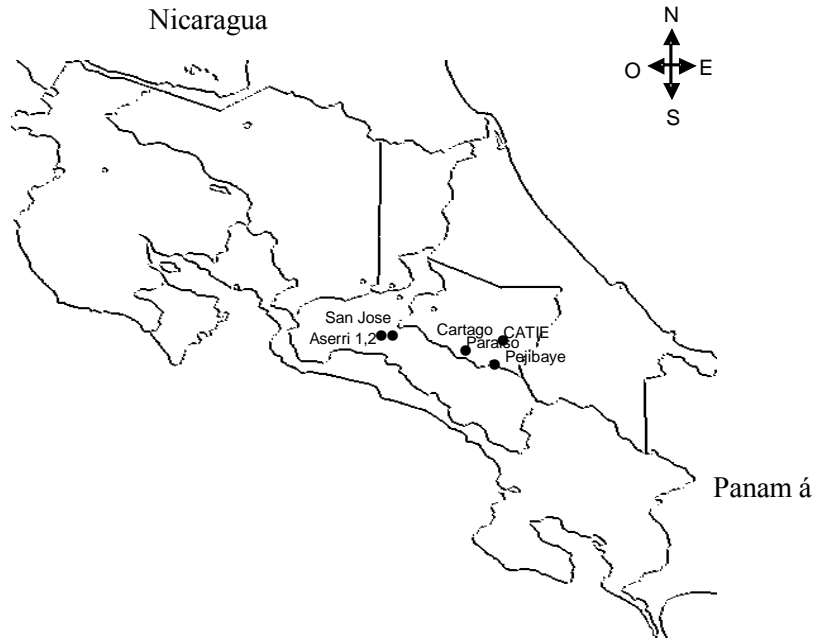
Figure 1. Site location of the paired farms in Central Costa Rica.

Another criterion was that the organic farms had to have been managed organically for at least four years (longest was ten and average was seven years). Preference was given to organic farmers with recognized leadership in their communities, who used standard regional practices and who were associated with a regional organization. A neighboring conventional farm, using standard herbicide and fertilizer doses was then selected to complete each pair. *E. poeppigiana* was the principal shade tree on all farms selected. Tree spacing (approximately 4 m between trees), coffee type (mostly the "Caturra" cultivar), soil type, altitude, slope and farm size (less than 10 ha) were also taken into account when selecting sites. In two pairs of farms (Aserrí2 and Paraíso), the organic farms had denser

Table 1. General soil and management characteristics of five organic and conventional paired farms in Central Costa Rica.

Farm	Subgroup. USDA 2003[a]	Slope (%)	Age of coffee plantation (yr); previous use	Years managed organically in 2004[e]	Liming (kg ha^{-1})	Fertilizers (study 2000) Total amount and kg NPK (ha^{-1} yr^{-1})	Fertilizers (study 2004) Total amount and NPK (kg ha^{-1} yr^{-1})	Weed control and herbicides (l ha^{-1} yr^{-1})	Pruning regime, pollarding height and residue distribution[c]	Yield (kg fresh cherries)[e]
Aserrí 1 Organic	Andic Haplustoll	15-40	30; forest	11	500 in 1997	Chicken manure[b] 2500, 50N, 50P, 25K (once a year in april 1996 only)	Compost (coffee pulp + cow manure and CaCO$_3$ 5:5:2 in volume) 2000 kg in Oct 2003	2-3 hand cutting a year	Partially once a year; 2.5 m; residues are evenly distributed	5950
Aserrí 1 conventional	Andic Haplustoll	15-30	30; forest		0	Formula 18 5 15; 675, 120N, 34P, 100K (twice a year)	Formula 18 5 15; 675, 120N, 34P, 100K (once a year)	Glyphosate (1.0) + Paraquat (1.0) once a year + 3 hand cuttings	Total twice a year; 1.5 m; no distribution of residues	7000
Aserrí 2 Organic	Andic Haplustoll	25	30; forest	9	750 in 1999	Chicken manure; 4000; 80N,80P,40K,once a year in May-June (1999-2003)	Compost (coffee pulp+ chicken manure 1:1 in vol.) 2000 kg in May-03 KMAG(22%K$_2$O); 200, 44K + 100 CaCO$_3$	2-3 hand cutting a year	Partially once a year; 2 to 3 m; residues are distributed	5950
Aserrí 2 conventional	Andic Dystrustept	30	7; pastures and fallow land		500 In 1999	Formula 18 5 15;400, 72N, 20P, 60K, once a year		Glyphosate (1.0) once a year + 3 hand cuttings	Partially twice a year; 2m; no distribution of residues	7000
Aserrí 2 conventional (substitute)	Andic Dystrustept	40-50	10; pastures and fallow land.		0		Formula 18 5 15;400, 72N, 20P, 60K, once a year	Glyphosate (1.0) once a year + 2 hand cuttings	no prunings since 2000	n.a. [f]

Table 1. General soil and management characteristics of five organic and conventional paired farms in Central Costa Rica.

Farm	Subgroup . USDA 2003[a]	Slope (%)	Age of coffee plantation (yr); previous use	Years managed organically in 2004[e]	Liming (kg ha^{-1})	Fertilizers (study 2000) Total amount and kg NPK (ha^{-1} yr^{-1})	Fertilizers (study 2004) Total amount and kg NPK (ha^{-1} yr^{-1})	Weed control and herbicides (l ha^{-1} yr^{-1})	Pruning regime, pollarding height and residue distribution[c]	Yield (kg fresh cherries)[e]
CATIE Organic	Andic Dystrudept/ Typic Hapludand	2-4	30; sugar cane	8	1600 April 2001 and June 2003	KMAG(22%K_2O); 226.5,50K In May	KMAG(22%K_2O); 226.5,50K In May	2-3 hand cutting a year	Partial, twice a year; 2-3 m; residues are distributed	3900
CATIE conventional	Typic Hapludand	7	30; sugar cane		1500 in April 1996	18 5 15;855, 154N, 42.7P, 128.2K partitioned in three applications, annually (Jan,Apr,Aug)	Formula 18 5 15;350, 63N, 17.5P, 52.5K in May 2003	Glyphosate (1.5) Paraquat (1.0); 2,4D 0.25 every 3 months	Total twice a year; 1.5 m; no distribution of residues	6600
Pejivalle organic	Typic Haplohumult	25	30; sugar cane	14	1000 only November 2000	Compost; 1800, (chopped grass) 9N, 9P, 9K. Annually in May	Compost; 1800, (chopped grass) 9N, 9P, 9K in May 2003	2-3 hand cutting a year	Twice a year; 2-3 m; residues are distributed	3050
Pejivalle conventional	Typic Haplohumult	20	15; forest		0	18 5 15, 540, 97N, 27P, 81K partitions in two applications	Formula 18 5 15;350, 63N, 17.5P, 52.5K in May 2003	Glyphosate (1.0) Oxiflurofen (0.25) twice a year	Total twice a year; 1-5m; no distribution of residues.	4900
Paraíso organic	Andic Haplohumult	5-10	>30; forest	12	1500 in August 1999	earthworm manure171+ KMAG(22%K_2O); 143;3.5N, 3.5P, 26K	earthworm manure171 + KMAG (22%K_2O), 143; 3.5N, 3.5P, 26K Compost, 1000kg ha^{-1} (coffee pulp+ chicken manure+$CaCO_3$ 5:5:.2 in vol.) annually in September	2-3 hand cutting	Partial twice a year; 2.5 m; residues are distributed	4900
Paraíso conventional	Andic Haplohumult	15-20	30; pastures		0	18 5 15; 640, 116N, 32P, 96K, once a year	No chemical fertilizers applied since 2001 Chicken manure in part of the farm 1200 kg ha^{-1} Jan /04 + 2 hand cuttings	Paraquat (1.0) + Oxiflurofen (0.25) 0.25 twice a year	Total twice a year; 1-5m; no distribution of residues.	10500

[a] Soil Survey Staff (2003). [b] nutrient concentrations: 2%N ; 2% P_2O_5, 1%K_2O (Fishersworring and Roβkamp 2001).
[c] With partial pruning 2-3 branches remain on the tree. [d] 1.0 qq of fresh cherries = 254 kg
[e] all organic farms were conventional coffee before conversion.. [f] no trustable record was found
[g] Two soil pits were dug in opposite sides of the organic field and two different subgroups were classified.

shade due to less intensive although more frequent pruning regimes. In another case, the organic plantation had a steeper slope than its pair (Aserrí1).

All farmers were interviewed to determine plantation history including: age of the plantation, intensity of herbicide and fertilizer use (conventional farms), shade tree pruning regimes, production levels, lime used as a pH modifier, pest and disease problems, and their general impressions about coffee management. Eight out of the ten farms were private properties and two belonged to CATIE's commercial farms. High internal spatial variability was unavoidable; e.g., it is common to find different plant ages and coffee cultivars within the same plantation as old coffee bushes are usually gradually replaced (Somarriba *et al.* 2000). Topographic conditions and also vegetative components also are variable within small areas in coffee regions.

3.2.2. Selection of sites and sampling positions

Within both organic and conventional plantations, a central plot area (30 × 50 m) was selected to avoid edge effects. Similarity with the corresponding plot of the pair (similar age of shade trees, soil phase and coffee-tree row spacing) was also sought. Within this central plot, three square 4 × 4 m sample sites were selected randomly. Each sample site contained one *E. poepiggiana* tree on each corner and four coffee rows (Figure 2). Within each sample site, soil samples were collected from three different positions with respect to *E. poeppigiana*: 1-in the alley equidistant from two coffee rows and more than 2 m from a shade tree ("alley"); 2- below a coffee plant and more than 2 m from a shade tree ("bc>2"); 3- below a coffee plant and less than 1 m from a shade tree ("bc <1"). The shade tree was selected at random.
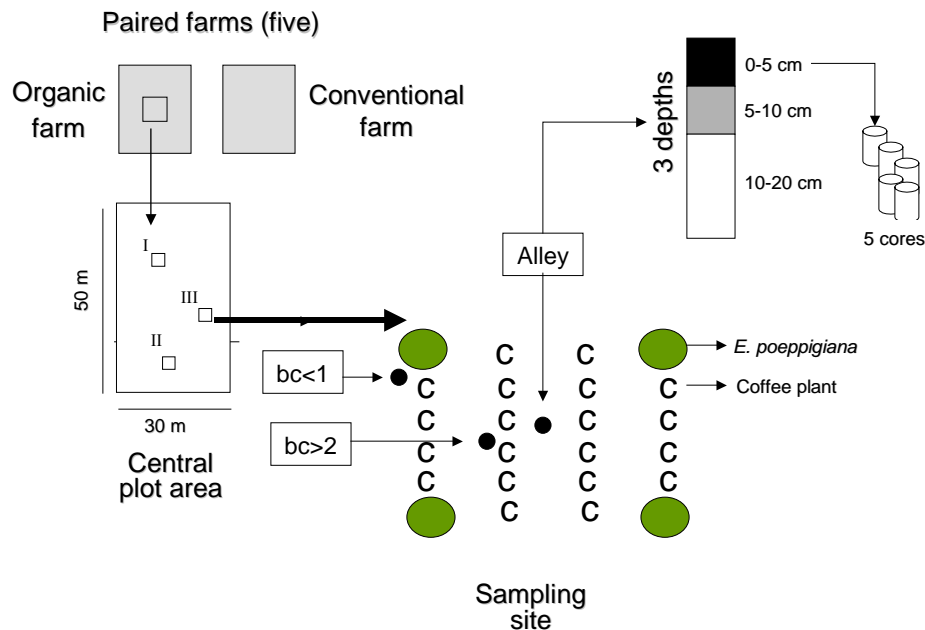
Figure 2. Sampling design to compare soils in organic and conventional coffee farms in central Costa Rica, 2000

3.2.3. Soil sampling in the 2000 study

Soil samples were taken in July 2000 and June 2004. A helicoid-iron auger was used to obtain soil cores at 0-5, 5-10 and 10-20 cm depth. Surface litter was removed before the mineral soil was sampled. At each position, 4-5 cores at each depth were mixed in a bulk sample. The first 1-2 cm of the cores at 5-10 and 10-20 cm depths were separated to avoid contamination between different depths. In positions "bc>2" and "bc <1", cores were taken at 30-40 cm away from the coffee plant trunk; in the "alley" position, cores were taken equidistant from two coffee rows. If structural roots were encountered, a new core was taken beside the previous one. Twenty seven bulk samples per farm were obtained (3 sample sites × 3 positions × 3 depths). The samples from the same depth and position from each of the three sites per farm were later mixed thoroughly to obtain nine compound

samples per farm. Samples were kept in cold boxes with ice, conserved at 4 °C and analyzed at the University of Wales, Bangor.

3.2.4. Methods in the 2004 study

A detailed soil survey and a new soil sampling were carried out between April and June 2004, in all but one of the farms that were studied in 2000. The conventional farm in Aserrí 2 was substituted by a farm with similar soil and management conditions located less than one km from the original because most of the *E. poeppigiana* shade trees had been removed from the original conventional farm. On farms that had irregular topography (both organic and conventional), the plantation was divided into zones with different slopes, and separate soil pits were dug in each slope to assure that comparable areas were sampled. The soil profile was described and samples were taken from the soil horizons to determine total C and N, texture, pH, Fe and Al concentrations in oxalate (to find *Andic* characteristics), Ca, Mg, K and P concentrations. All data were used to classify the soil in the farms following USDA methodology (Soil Survey Staff 2003) and to assure that the pair of conventional and organic farms were comparable (Soil profile descriptions and chemical properties are included in Appendix 1 and 2).

In June 2004, only two positions with respect to the shade tree were sampled: 1- in the alley equidistant from two coffee rows and more that 2 m from a shade tree ("alley"); and 2- below a coffee plant and less than 1 m from a shade tree ("bc<1"). The position "bc>2" (below a coffee plant and more than 2 m from a shade tree) was not sampled in 2004 because the 2000 results indicated no differences to the "alley" position. The soil was sampled using mini soil pits (40 × 40 × 40 cm) to verify that horizon "A" was always sampled. In some places the soil had crept downhill from upper areas burying horizon "A", particularly in the Aserrí farms (slopes between 15-45%). The use of mini soil pits avoided sampling soil that came from other horizons due to mass soil movement caused by steep slopes. The least disturbed wall of the mini soil pits was sampled at 0-5, 5-10, and 10-20 cm depths, and samples from three mini soil pits distributed in comparable areas (according

to the soil profile descriptions) of the paired farms were taken. As in 2000, samples from the same depth and position were mixed to obtained 6 compound samples per farm.

3.2.5. Measurements and analytical methods
3.2.5.1. Analytical methods in the 2000 study

Soil samples were air-dried and passed through a 2 mm stainless steel sieve. For total C and N analysis, approximately 3 g of sieved soil were pulverized; between 0.1 and 0.2 g were wrapped in tinfoil to be analyzed by gasification in a CHN2000 (Leco Corporation St. Joseph, MI, USA). Nitrate and ammonium concentrations were measured colorimetrically with a San-plus auto-analyzer (Skalar Analytical B.V., Netherlands). Microbial respiration was measured using fresh soil (20 g) incubated at 22 °C in an infrared gas analyzer (CIRAS-SC, PP Systems Ltd, Hitchin, UK). K, Ca, Mg and Zn were extracted from the soil with 0.5 M $BaCl_2$ (1:10 soil: solution w/v) and analyzed using a JY-Ultrace Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES; Jobin Yvon Ltd, London, UK). Electrical conductivity and pH (in water at a 1:1 v/v soil:water ratio) were measured with a Jenway 4010 conductivity meter and an Orion 410A pH meter. Organic matter fractions were obtained by the granulometric fractionation method (Kouakoua 1998). Subsamples of air dried soil (10 g) were dispersed in 50 ml sodium hexametaphosphate solution (5 g l^{-1}) by shaking at 120 rpm for one hour. The samples were sieved through three stacked 2000, 200 and 53 μm stainless steel sieves. The samples were washed using tap water until all clay, silt and fine organic materials were separated and the water passing through the sieves became clear. Two particulated organic matter (POM) fractions (200-2000 and 53-200 μm) were collected, dried at 40°C, and weighted for intersystem comparisons. Soil organic matter size fractions and P concentrations (Olsen-Dabin) were obtained for the farming system comparison. Analyses for POM fractions and P were done at the CIRAD soil laboratory in Montpellier, France; and data pertaining to the positions with respect to shade trees had to be previously mixed at CATIE due to the limited time for fractionation work at the lab in Montpellier (complete granulometric analyses took one day per sample).

Bulk density was measured in September 2003, using the "cylinder" method (MacDicken 1997). A measurement was taken in each of the three sampling positions described for the 2000 study, in the three different sample sites in the central plot of each paired farm. A mini soil pit (40 × 40 × 40 cm) was dug at each sampling point and a stainless steel cylinder with a known volume was inserted at 0-5 cm depth using a rubber mallet with minimum soil disturbance. A step in the mini soil pit was built for inserting the second cylinder at 5-10 cm. The cylinder was extracted with a machete and the excess soil removed to have two flat surfaces at both ends of the cylinder. The soil from the cylinder was kept in labeled plastic bags and dried at 105 °C for 24 hours. After cooling, stones were removed and the samples weighed. The bulk density was calculated by dividing mass by the known volume of the cylinder (98.175 cm^3). The weight and volume of the stones were measured separately to adjust the results.

3.2.5.2. Analytical methods in the 2004 study

Soil total C and N were analyzed by gasification as described above for the 2000 study. Soil K, Cu, Zn, Mn and Fe were extracted using Olsen-modified solutions with a pH of 8.5. Calcium and Mg were extracted with KCl 1 N. All nutrients in the 2004 study were analyzed at the soil lab in CATIE, Costa Rica by atomic absorption using an AAnalyst-100 Spectrometer (Perkin Elmer Inc., Boston, MA, USA). Values for pH were measured in water at a 1:1 v/v soil:water ratio (see above). Available P (Olsen modified, pH 8.5) was measured for two positions at 0-5 and 10-20 cm depth. In the 2004 study, nutrient analyses were only made for two depths based on the 2000 results, particularly on total C, which indicated that clear differences will be possibly found in these two layers.

Weed biomass measurements were only made in the Pejivalle paired farms (the great similarity between soil profiles in these paired farms were taken into account for choosing this site). A square meter framework was located randomly on the ground in each of the three sampling sites and all weeds on the surface were collected and dried at 40 °C for 3 days. An average of the three measurements for the conventional and organic farms were calculated.

3.2.6. Statistical analysis

Data was analyzed with ANOVA as a randomized complete blocks (the farm pairs) split-plot (systems as the main treatment and positions as sub-treatment) design with five replications. When significant statistical interactions between system and position were found, a t-test was performed. Data was analyzed using SAS, version 8 (SAS Institute 1999). For comparisons between farming systems, an average value of the positions within each farm was considered. Differences in total soil C and N between 2000 and 2004 were analyzed using ANOVA as a randomized complete block (the farm pairs) split-split-plot (systems as the main treatment and positions and years as sub-treatments) design with four replications due to the substitution of the conventional farm in Aserrí2. Bulk density values were analyzed using a split-plot design for each pair of farms. The two farming systems were the main treatments and positions were the subtreatments.

3.3. Results and discussion
3.3.1. Total C and N concentrations
3.3.1.1. Comparisons between positions

In 2000, the concentrations of C (Figure 3) and N (Figure 4) for the five paired-farms were analyzed for three different positions ("bc >2", "alley" and "bc<1") at three soil depths (0-5, 5-10 and 10-20 cm). Significant differences between the three sampling positions, for the 0-5 and 5-10 cm layer ($p<0.05$), but not for the deepest layer, were found for total C and N concentrations (Figures 3C and 4C).

At a depth of 0-5 cm in the conventional farms, the position nearest the shade tree ("bc<1") had a higher soil C concentration than the positions located 2 m from the trees (5.04 *vs* 4.18 % and 4.16 %). Soil N concentrations in conventional farms for the position closest to the tree ("bc<1") were also higher than those farther away from the tree (0.43 *vs* 0.37 and 0.36 %). In the position "bc<1", no significant differences in soil N content were found when organic farms were compared to those which had been conventionally managed. However, soil C and N concentrations were higher in the organic treatments for

the two positions located 2 m from the tree (Figures 3A and 4A). Soil carbon concentrations for the three positions (0-5 cm layer) within the organic farms were similar (Figure 3A). In the 5-10 cm layer, at the position "bc>2", higher total C and N concentrations were observed in organic farms when compared with conventionally managed farms. Carbon and N concentrations in conventional farms were higher for "bc<1" compared to "bc>2" (Figures 3B and 4B).

The results for soil C concentrations in the 2004 study were similar to the 2000 study. At the 0-5 cm depth within conventional farms, the position nearest to the tree trunk "bc<1" had higher C concentrations than in the "alley" (5.67 vs 4.17 %), but within organic farms these two positions had similar C concentrations. In organic farms, the "alley" positions had higher values than its counterpart in conventional farms. No significant differences were found between organic and conventional farms for the position "bc<1" (Figure 3D). At soil depths of 5-10, 10-20 cm, no differences in soil C content between positions in either organic or conventional systems were found (Figures 3E and 3F). No clear tendencies were found for changes in total C and N concentrations over time between 2000 and 2004 (Table 2, Appendix 3 for N). Split-split plot analysis did not detect significant effect of time for positions in the study period.

The total soil N concentration in the 2004 study at the 0-5 cm soil layer, mirrored the results for C concentrations. In conventional farms, "bc<1" had higher N values than in the "alley" position ($p<0.05$), but no differences were found between "bc<1" and "alley" within organic farms. When conventional and organic systems were compared, similar values were found for "bc<1", but "alley" in organic farms had higher values than its counterpart in conventional farms (Figure 4D). In 2004, at soil depths of 5-10 and 10-20 cm, the same tendencies were observed for the 0-5 cm layer were found but none of the differences, for positions or for system contrasts, were significant (Figures 4E and 4F).

Table 2. Total carbon percentage in 2000 and 2004 in the topsoil at three depths for positions of five organic (Org) and conventional (Con) coffee farms in central Costa Rica. Treatments: alley = equidistant from two coffee rows and more than 2 m from a shade tree; bc>2 = below a coffee plant and more than 2 m from a shade tree; bc <1 = below a coffee plant and less than 1 m from a shade tree

Farms	Aserri1 Org		Aserri1 Con		Aserri2 Org		Aserri2 Con		CATIE Org		CATIE Con		Pejivalle Org		Pejivalle Con		Paraiso Org		Paraíso Con	
Year	2000	2004	2000	2004	2000	2004	2000	2004	2000	2004	2000	2004	2000	2004	2000	2004	2000	2004	2000	2004
Soil depth (cm)										**Alley**										
0-5	4.16	5.60	4.96	3.80	5.54	6.49	3.96	2.47	5.05	5.22	3.35	4.02	4.19	5.51	4.31	3.52	6.04	5.54	4.35	7.06
5-10	3.91	3.89	4.79	3.07	4.67	4.17	4.09	1.36	4.10	3.86	2.87	2.74	2.71	3.79	3.85	2.49	4.51	4.03	3.36	6.21
10-20	3.19	3.33	3.84	3.08	4.34	3.83	3.67	1.07	3.36	2.79	2.47	2.05	2.42	2.95	2.50	2.17	2.94	3.20	2.98	4.93
										bc>2										
0-5	4.96		5.33		6.29		4.13		5.20		3.32		5.16		4.03		5.04		3.99	
5-10	4.54		4.64		5.92		3.66		4.08		2.88		3.23		3.33		4.01		3.05	
10-20	3.82		4.52		5.44		3.69		3.60		2.19		2.78		2.30		3.14		2.71	
										bc<1										
0-5	3.67	5.27	5.54	4.05	5.98	4.74	5.81	2.95	4.94	5.22	3.86	5.37	4.91	5.25	4.33	4.53	5.66	5.94	5.64	8.23
5-10	3.33	4.35	4.50	2.98	5.16	4.48	5.45	2.15	3.94	3.88	3.49	4.14	3.44	3.85	3.68	2.69	4.12	4.45	4.10	6.29
10-20	3.30	3.75	4.32	2.57	5.24	4.48	5.19	1.76	3.46	2.78	2.33	2.65	2.50	3.28	2.30	2.24	3.41	3.63	2.66	4.67
avg for positions at 0-5 cm	4.26	5.44	5.28	3.93	5.94	5.62	4.63	2.71	5.06	5.22	3.51	4.70	4.75	5.38	4.22	4.03	5.58	5.74	4.66	7.65
Difference[1]	1.17		-1.35		-0.32		n.a.		0.16		1.19		0.63		-0.20		0.16		2.99	

[1] Differences represent the percentage change between the average for the positions at 0-5 cm measured in 2000 and again in 2004.

2000
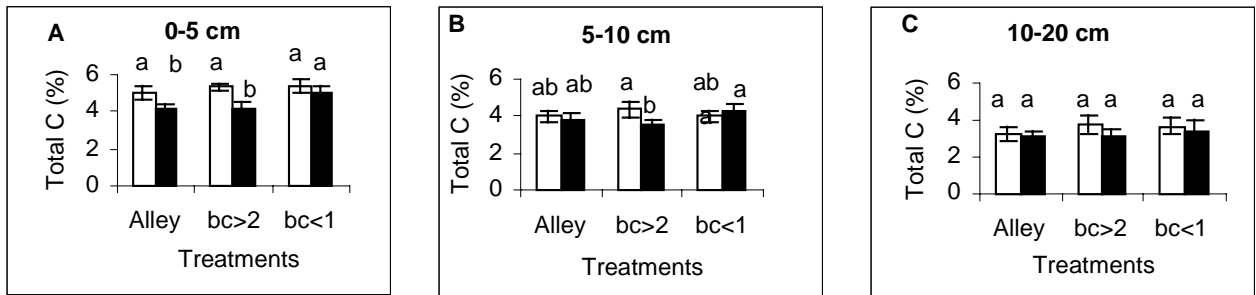
2004
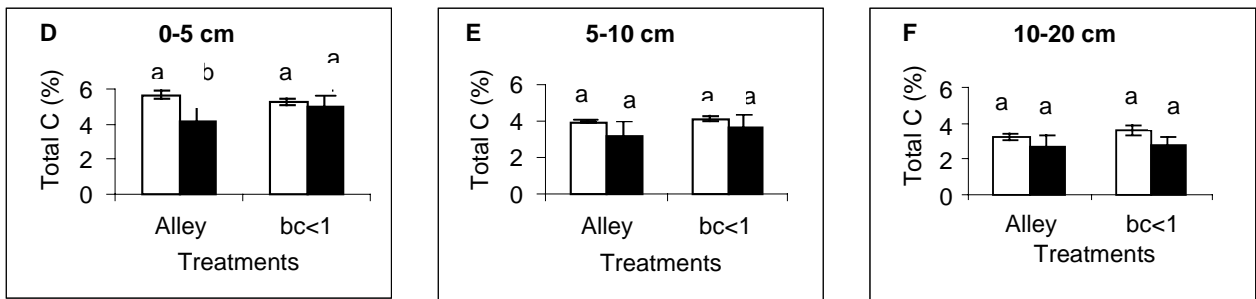
Figure 3 Total soil C concentration for three depths in organic (□) and conventional (■) coffee farms in central Costa Rica. Treatments: Alley = equidistant from two coffee rows and more than 2 m from a shade tree; bc>2 = below a coffee plant and more than 2 m from a shade tree; bc <1 = below a coffee plant and less than 1 m from a shade tree. Columns with the same letter are not significantly different (Lsmean-test *p*<0.05).

For the three positions ("alley", "bc>2" and "bc<1"), in the 2000 study, a frequency analysis of C concentrations was completed at the 0-5 cm depth layer (where the main differences were found); four out of the five organic farms had greater soil C concentrations than the conventionally managed farms in "bc>2" and three out of five at "alley"(Figure 5A and 5B). In the position closest to the tree ("bc<1"), no tendency was observed (N data in Appendix 4b). At this depth, total N followed a very similar pattern to soil C.

2000

2004
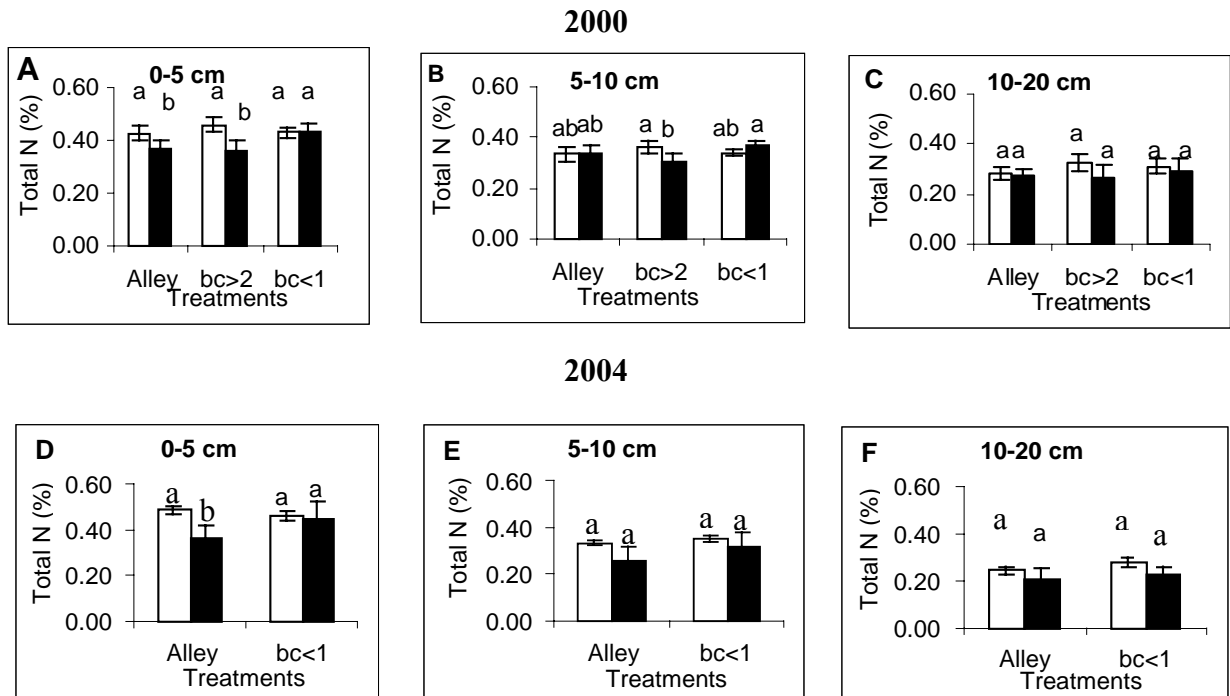
Figure 4. Total soil N concentration for three depths in organic (□) and conventional (■) coffee farms in central Costa Rica. Treatments: alley = equidistant from two coffee rows and more than 2 m from a shade tree; bc>2 = below a coffee plant and more than 2 m from a shade tree; bc <1= below a coffee plant and less than 1 m from a shade tree. Columns with the same letter are not significantly different (Lsmean-test *p*<0.05).
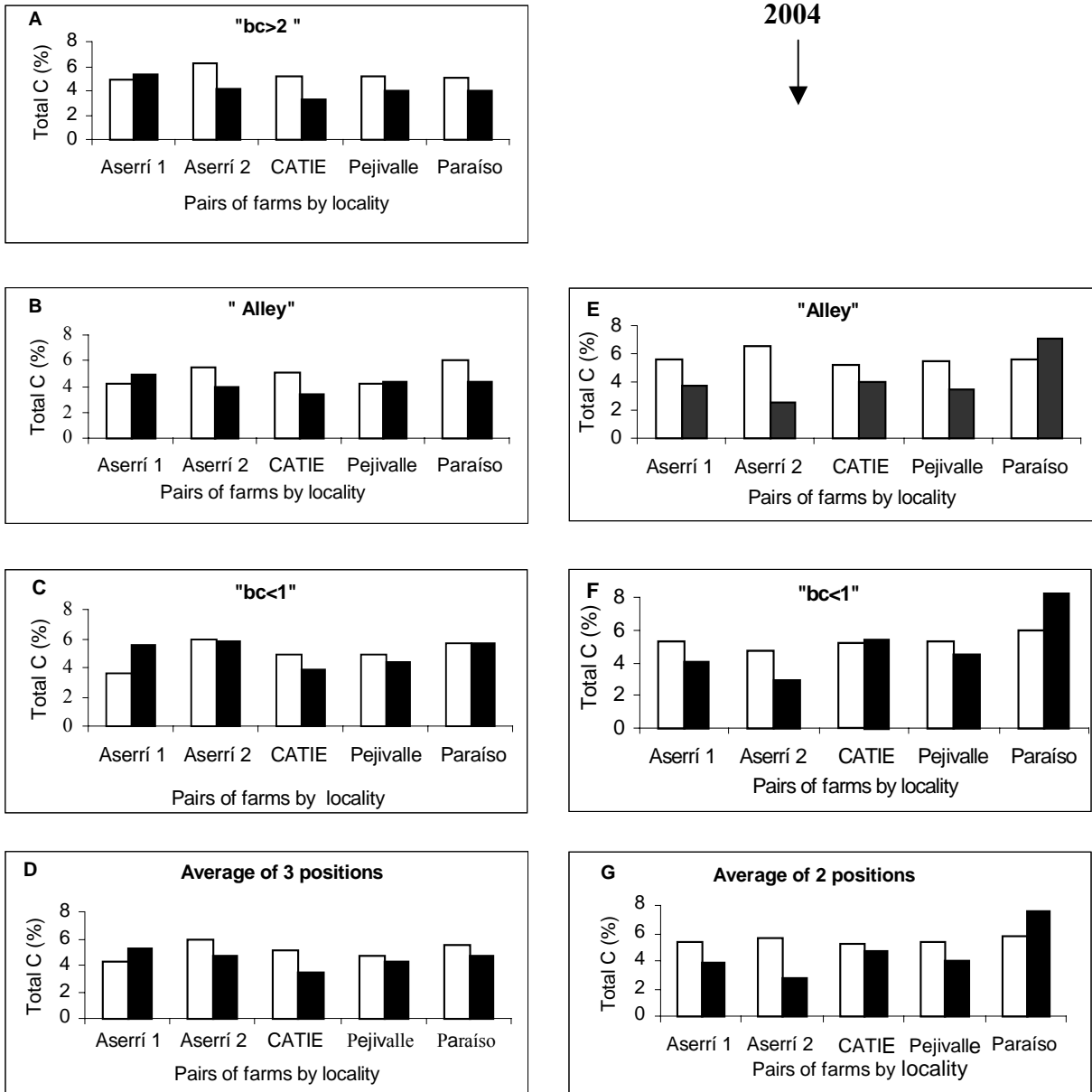
Figure 5. Carbon concentrations in the topsoil (0-5 cm depth) for five pairs of coffee farms, [organic (□) and conventional (■)] in Costa Rica. Treatments: alley = equidistant from two coffee rows and more than 2 m from a shade tree; bc>2 = below a coffee plant and more than 2 m from a shade tree; bc <1 = below a coffee plant and less than 1 m from a shade tree.

In 2004, the overall frequency analysis (0-5 cm depth) showed similar trends to 2000 although in some pairs of farms the relationship changed (e.g. Aserrí1 and Paraíso). In intra-system comparisons for positions, in conventional farms the values for "bc<1" were always higher than in the "alley" position; within organic farms, no tendencies were observed between the two positions (Figure 6). When the same position was compared in the two systems, in the "alley" position, four organic farms had a greater C concentration than the conventional farms (Figure 5E). At the position closest to the tree ("bc<1"; Figure 5F), there were no clear tendencies.
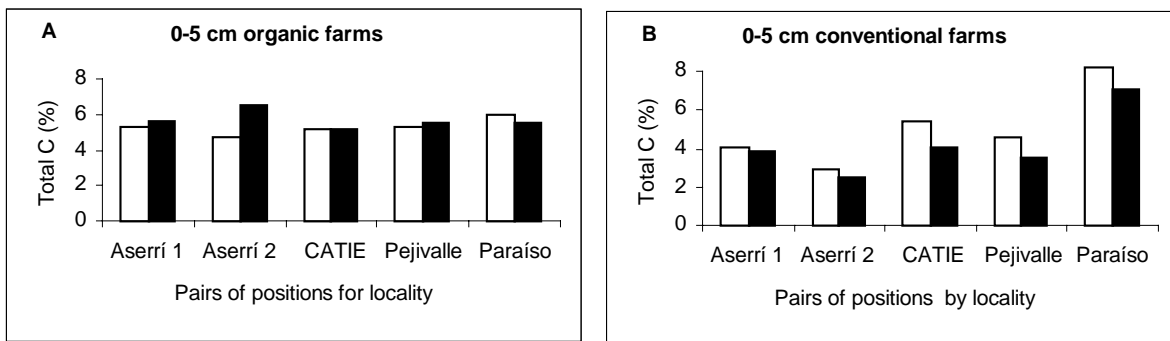
Figure 6. Total soil carbon concentrations at the 0-5 cm depth for two positions in five organic (A) and conventional (B) coffee farms, in Costa Rica, 2004. Treatments: bc <1 (□) = below a coffee plant and less than 1 m from a shade tree and alley (■) = equidistant from two coffee rows and more than 2 m from a shade tree.

The positive impact of shade trees on total C and N concentrations in the soil close to their trunks was more pronounced in the conventional farms compared to the organically managed farms. This was probably due to different management practices in conventional farms such as more frequent and more intensive shade tree pruning, which produce less dense shade cover and lower quantities of pruning residues per year (Beer 1988, Chesney 2000); these also tend to be concentrated around the shade tree trunks in the conventional farms (Table 1). The homogeneous distribution of chopped residues from the trees is only usual in organic farms. Furthermore, a lower height for tree pollarding in conventional

farms (Table 1) leads to a smaller area under the crown receiving litter fall around the tree trunk.

Research has shown that the accumulation of *E. poeppigiana* pruning residues on soil can lead to increases in soil C concentrations due to its high biomass production (Beer 1988, Beer *et al.* 1998). The effect of *E. poeppigiana* litter fall on a *Trofuldalf* soil in Brazil was studied by Santana and Cabala-Rosand (1982). In that study, higher total N concentrations were found under the crown of *E. poeppigiana* than outside the circle of influence (0.32 *vs* 0.24%, respectively) which they conclude was due to litterfall accumulation. Studies on the effects on SOM, of mulching with *E. poeppigiana* pruning residues, showed increased soil C concentrations over short time periods. Ramírez and Bornemisza (1990), on a *Typic Humitropept* soil in Turrialba, Costa Rica, found higher soil C in plots that received 9.2 Mg DM ha^{-1} yr^{-1} (3.73 v*s* 3.11% C in controls) after five years, although high decompositions rates were found due to the absence of a dry season. The application of 3 Mg DM ha^{-1} yr^{-1} of *Leucaena leucocephala* mulch (a legume with a similar residue quality to *E. poeppigiana*), in coffee plantations on a clay soil in Kenya, led to a 15 % increase in soil C concentrations after three years (Kimemia *et al*. 2001). These studies can help explain the higher C concentration in "bc<1" compared to "alley" in conventional farms in the present study.

Part of the explanation for the more pronounced differences in soil C and N concentrations between positions within conventional farms could be that the use of herbicides decreases weed biomass in this system, leaving the topsoil more exposed to POM runoff and with less C inputs from the decomposition of weed residues (root and shoot turnover). Losses of soil and POM were measured by Garzón (1991) in fields near to Turrialba with similar slopes to the fields sampled in the current study (35% angle). The loss of topsoil by run off (surface erosion) in a five month period using 16 Mg ha^{-1} yr^{-1} of *E. poeppigiana* mulch, was five fold lower than in fields without mulching. In organic farms, biomass inputs such as organic amendments, green manure as well as weed and pruning residues were evenly distributed, which should reduce any spatial differences in soil C and N. Muller and Bormann (1976) suggested that herbaceous cover had an important role in

the conservation of N and K in a watershed. *Erythronium americanun* Ker (Liliaceae), although accounting for a small proportion of the biomass production in the ecosystem, acted as a short-term sink for these nutrients. A study on weed biomass production and N reserves in weeds in shaded coffee plantations where no chemical herbicides were used, was undertaken in Masatepe, Nicaragua (500 m a.s.l., average rainfall of 2012 mm yr^{-1}). The results showed that 3.35 Mg ha^{-1} of weed biomass (DM) were added to the system in a seven month period after seven hand cuttings. In contrast, systems using herbicides (two applications of Paraquat + Simazine + 2,4-D; 1.6 l ha^{-1}) and two hand cuttings a year for three years only received 0.79 Mg ha^{-1} weed biomass residues (Aguilar and Staver 1997). Preliminary field sampling of weed biomass taken at Pejivalle, where the most similar soil characteristics between the paired farms were found, also showed much higher weed biomass accumulation in organic than in conventional farms (4.44 *vs.* 1.27 Mg ha^{-1}, respectively).

3.3.1.2. Comparison between management systems

ANOVA showed no significant differences between organic and conventionally managed systems in both the 2000 and 2004 studies. In 2000, at 0-5 cm depth, total C and N concentrations (averaged over all positions) were 5.12 *vs* 4.46% C and 0.44 *vs* 0.39% N for organic and conventional systems, respectively. In 2004, the average values were 5.5 vs 4.6% C and 0.47 vs 0.41% N for organic and conventional farms, respectively; all of which were higher than in 2000 (values for C in 2004 in organic farms were raised by higher values in Aserrí 1 ORG, and by Paraíso CON in conventional farms, (Table 2, Appendix 5a). A frequency analysis was done for the two variables at the 0-5 cm soil depth. In 2000, when an average of the three positions for each farm was compared, four out of five organic farms had higher C concentrations that their conventional counterpart (Figure 5D). and three out of five in the case of N (Appendix 3). In 2004, four of five organic farms had higher C concentrations at the 0-5 cm depth than the conventional farms (Figure 5G).

When data for systems (averaged over all positions) were compared between 2000 and 2004, no clear trends were found for changes in total C and N concentrations (Table 2,

Appendix 3 for N). Split split plot analysis showed no significant effect of time for systems after the four-year study period. Nevertheless in two conventional farms, soil C concentrations (averaged over all positions) increased from 2000 to 2004 (1.19% in CATIE CON and 2.99 in Paraíso CON). Management changes help explain these changes in conventional farms: in CATIE CON a denser shade due to taller shade trees was found and in Paraíso CON, banana plants (625 plants ha^{-1}) and chicken manure applications were found. On the other hand, increases were also observed in two organic farms (1.17% in Aserrí 1 ORG and 0.63% in Pejivalle ORG). However, in 2004 at Aserrí 1 ORG, zones were sampled where soil creeping of a B horizon did not affect the 0-5 cm layer. This can explain the increase of soil C in this farm because no management changes ocurred during the four year period. In the other two pairs of farms where data for the time comparison were available, no important increases were observed. These results indicated that in some cases, conventional farms had a greater response to management changes during the study period, and organic farms had more stable C and N levels. This was in line with the findings in a detailed study of C and N changes in two paired farms at Paraíso (Zuloaga 2004). The study concluded that soil C in the conventional farm was more sensitive to management changes than in its organic counterpart.

Finally, the C-to-N ratio was almost the same for both systems (11.9 and 11.7 for organic and conventional systems, respectively). Similar C-to-N ratios (11.7 on average) were found by Ramírez and Bornemizsa (1990) in plots where *E. poeppigiana* mulch was applied over a five year period. This similarity could be expected since the main source of organic matter in the two studies was *E. poeppigiana* pruning residues with the same level of quality. The absence of differences between organic and conventional suggests that in organic coffee farms, a stable soil characteristic, such as the C-to-N ratio (Russell 1988), has not been modified by management. This result is consistent with Wells *et al.* (2000) who did not find significant differences in the C-to-N ratio between organic and conventional farms after a four year experiment in a loam soil in Australia and Lockeretz *et al.* (1981) who did not find changes after a 25 year observation period on 30 paired farms growing cereals in the midwestern USA.

In 2004, after examining the soil profiles, comparable areas with less influence of soil creeping were identified. In this new study, the C concentration averages for organic and conventional farms confirmed the general trend found in 2000 (in 2004, four out of five organic farms had higher C concentrations than their conventional counterparts). Nevertheless, in the 2000 study, in the Aserrí1 ORG farm an area with lower concentrations, possibly belonging to a B horizon from uphill, sampled in the top soil layer (soil profile No.1; Appendix 1). As a consequence, the C concentrations were lower than in the conventional paired farm. On the other hand, in Paraíso CON, the same situation have occurred in one of the three sampled areas (soil profile, labeled "low in C", N°2; Appendix 1). This was in a low slope position that could have received soil that crept from an uphill B horizon. In this area, a lighter color and lower C concentration were registered in horizon A2 (3-25 cm depth; C concentration of 2.24%) in comparison with the horizon A in other zones in the same farm (4.94% at 0-25 cm depth; profile labeled "high in C", N°1; Appendix 1). The use of the mini soil pits in the 2004 study also permitted more efficient detection of comparable areas that were less disturbed by erosion. A record of A horizon color was also taken at every sampling site. As a result, in 2004 higher C concentrations were found for the organic farm in Aserrí1 ORG than in 2000; and equally for the conventional farm in Paraíso (Table 2). Although a general trend of higher C concentrations in organic farms could be observed, these two cases indicate that the differences between paired farms can sometimes be more related to the original soil conditions than to the recent soil management.

Another factor that has to be considered to interpret the differences in C concentrations (soil organic material) between systems is the previous land use on the farms. Although most of the farms within the study were planted with coffee for an average of 30 years, some locations were planted with sugar cane for many years before coffee was introduced (e.g. CATIE). In other cases, secondary forest was predominant in the area (Aserrí1). This factor introduced extra variability between "blocks" in the ANOVA. In another case, "intra-block" variability could have been produced by locally different previous land use. In Pejivalle for example, the organic farm was under sugar cane before 1970 and the conventional counterpart was covered by secondary forest before that year.

García *et al.* (1990) found a lower soil organic matter and cation exchange capacity, leading to a loss of base cations at 0-30 cm depth, in coffee plantation soils in comparison with secondary forest on Andisols in Veracruz, Mexico. On the other hand, sugar cane has been reputed to negatively affect the organic matter balance due to regular burning each harvest season (Schroth *et al.* 2001). The different previous land use may explain in part why such small differences were found in soil C concentrations at Pejivalle in 2000 (4.75 vs 4.22% for organic –previously sugar cane- and conventional –previously forest-, respectively) even though the Pejivalle organic farm was 10 years old at the sampling date.

During the study period (2000-2004), in conventional farms, low prices of coffee in the international market have resulted in a decreased use of chemical and labor inputs for weed control as well as for fertilization. In some farms, the introduction of bananas as shade plants has been an alternative for diversification (Aserrí1 CON and Paraíso CON). These factors may have diminished the differences between organic and conventional farms (Zuloaga 2004), but this could not be supported quantitatively.

Stockdale *et al.* (2001) reviewed the impact of organic farming on soil quality and reported nine studies which found higher C concentrations for organic systems in comparison to conventionally managed systems. Their review suggested that such increases are a consequence of the incorporation of green manures, composts, and crop residues into the soil. In the same way, Wells *et al.* (2000) compared organic and conventional management effects on soil C in a four year experiment with vegetable rotations. The effects of tillage, crop rotations, pest management and crop nutrition (chemical or organic) on soil characteristics were analyzed. The study concluded that the only factor that could explain higher soil C concentrations in organic farms was the addition of organic amendments like compost (40-60 Mg ha^{-1} yr^{-1}). In New Zealand, a comparison of 16 paired farms in a range of cropping systems that included fruit, citrus, vegetables, dairy and pastures, found consistently higher C concentrations for organic farms (Reganold *et al.* 1993).

In the current study, although a trend for higher C and N values in organic systems was clear in the two studies (2000 and 2004), the differences were not detected using ANOVA. In addition, there is no clear tendency of an increase in C values in organic systems during the four year study period (2004 *vs* 2000). A low coefficient of variation in most of the tested contrasts was found using ANOVA. However, a larger number of farms would improve the sensitivity of this test. As a result of only having five pairs of farms, the fact that one pair in the 2000 (and also in the 2004) studies contradicted the general trend (Aserrí1 in 2000 and Paraíso in 2004 Figure 5D and 5G) led to the absence of statistically significant differences (at the $p < 0.05$ level). Texture differences in "A" horizons between paired farms at Aserrí2, CATIE and Paraíso could also affect the inter-system comparison (Appendix 2). However, intra-system comparisons for positions should not be affected by different textures in the top soil. Finally, in line with Wells *et al.* (2000), the use of organic amendments, higher weed biomass inputs and probably higher C inputs from pruning residues of the shade trees in organic farms due to taller trees (Table 1) can help explain the generally higher C and N in soils of organic farms when compared to those which have been conventionally managed.

3.3.2. Bulk density

Soil bulk density was analyzed independently for each pair of farms in the five localities. At the 0-5 cm depth no clear tendencies for bulk densities were found: in two out of the five pairs (CATIE and Pejivalle), position "bc<1" had a significantly lower bulk density than the positions 2 m from the shade tree; in Aserrí 2 and Paraíso no significant differences were detected; in Aserrí1, "alley" had significantly lower density that the other two positions. At a 5-10 cm soil depth no significant differences were detected for positions in any pair of farms. When systems were compared, no differences were found for any of the five pairs of farms at the two study depths (Table 3).

In the biological station *La Selva* in northeastern Costa Rica, the effects of tree introduction in a degraded pasture soil were measured. After four years, soil bulk density decreased under seven out of eleven tree species, although higher SOM was only found

Table 3 Bulk density in the topsoil at two depths for three different positions in five organic and conventional coffee farms in central Costa Rica, 2003. Treatments: alley = equidistant from two coffee rows and more than 2 m from a shade tree; bc>2 = below a coffee plant and more than 2 m from a shade tree; bc <1= below a coffee plant and less than 1 m from a shade tree.

Locality	Depth (cm)	System	Alley	bc>2	bc<1	Avg/System
Asserri 1	0-5	ORG	0.63 (0.0)[1]	0.86 (0.1)	0.93 (0.0)	0.81
		CON	0.75 (0.0)	0.73 (0.1)	0.81 (0.0)	0.76
		Average for positions	**0.69 b**[2]	**0.80 a**	**0.87 a**	
	5-10	ORG	0.82 (0.1)	0.78 (0.1)	0.86 (0.0)	0.82
		CON	0.79 (0.1)	0.78 (0.1)	0.83 (0.1)	0.80
		Average for positions	0.81	0.78	0.85	
Asserri 2	0-5	ORG	0.82 (0.1)	0.86 (0.1)	0.87 (0.1)	0.85
		CON	0.83 (0.1)	0.87 (0.1)	0.94 (0.1)	0.88
		Average for positions	0.82	0.86	0.91	
	5-10	ORG	0.84 (0.0)	0.86 (0.1)	0.79 (0.0)	0.83
		CON	0.86 (0.0)	0.89 (0.0)	0.93 (0.1)	0.89
		Average for positions	0.85	0.87	0.86	
CATIE	0-5	ORG	0.84 (0.1)	0.84 (0.0)	0.76 (0.0)	0.81
		CON	0.81 (0.1)	0.86 (0.0)	0.71 (0.0)	0.79
		Average for positions	**0.82 a**	**0.85 a**	**0.73 b**	
	5-10	ORG	0.87 (0.0)	0.89 (0.0)	0.86 (0.0)	0.87
		CON	0.90 (0.0)	0.94 (0.0)	0.85 (0.0)	0.90
		Average for positions	**0.88 ab**	**0.91 a**	**0.85 b**	
Paraiso	0-5	ORG	0.76 (0.0)	0.87 (0.1)	0.72 (0.0)	0.78
		CON	0.86 (0.0)	0.76 (0.0)	0.73 (0.1)	0.79
		Average for positions	**0.81 ab**	**0.82 a**	**0.72 b**	
	5-10	ORG	0.83 (0.0)	0.83 (0.0)	0.79 (0.1)	0.81
		CON	0.85 (0.0)	0.79 (0.0)	0.84 (0.0)	0.83
		Average for positions	0.84	0.81	0.82	
Pejivalle	0-5	ORG	0.82 (0.0)	0.72 (0.0)	0.70 (0.0)	0.75
		CON	0.89 (0.0)	0.79 (0.1)	0.78 (0.0)	0.82
		average for positions	0.86	0.75	0.74	
	5-10	ORG	0.85 (0.0)	0.80 (0.0)	0.79 (0.0)	0.81
		CON	0.90 (0.1)	0.82 (0.0)	0.81 (0.1)	0.84
		average for positions	0.87	0.81	0.80	

[1] Standard error [2] values with the same letter within a row are not significantly different (Lsmeans-test $p < 0.05$).

under three of them (Fisher 1995). Kimemia *et al*. (2001) measured bulk density after three years of mulch application in a clay soil in Kenya. The mulch from seven leguminous species that are commonly used in agroforestry systems was applied at a rate of 3000 kg ha^{-1} yr^{-1}. Decreases in lower bulk densities within a range of 19-25% were measured. However, in Turrialba Costa Rica in a *Typic Humitropept*, Kass *et al*. (1989) and Céspedes (1991) applying 9.2 Mg ha^{-1} yr^{-1} of *E. poeppigiana* mulch during five years did not find significant differences in bulk density in comparison with control unamended plots. Our results showed no evidence of bulk density decreasing from the application of *E. poeppigiana* pruning residues. In only two out of five pairs of farms, lower soil bulk densities (0-5 cm) were found close to the trunk where residues tend to be concentrated. The positive effects of trees on soil density can be attributed in part to the increases in SOM from litter and pruning residues which have lower density than mineral soil components thus inducing lower bulk density (Henríquez and Cabalceta 1999). Excavating effect of roots can also induced lower soil bulk density. In the current study, the differences in C concentrations between positions in conventional farms were not large enough to produce detectable differences in bulk density in soils with naturally low bulk density (and where shade tree roots were widely distributed).

Reganold *et al*. (1993), found a lower soil bulk density in organic farms in four out of 7 pairs of farms in a comparison between organic and conventional systems in New Zealand. Stockdale *et al*. (2001) reviewed contradictory results related to the effect of farming systems on bulk density. Changes were associated with organic inputs but also with the tillage regime in both systems. Their study affirms that changes in physical soil conditions take decades to establish. In our case, the maximum number of years under organic farming was fourteen years for Pejivalle ORG. These short periods of organic management may explain why a consistent difference was not found between the two contrasting management systems.

3.3.3. pH and electrical conductivity

Using ANOVA, once again no significant differences were detected for pH (0-5 cm) between positions or for inter-system comparisons in the two study years (Table 4). Therefore, a frequency analysis was done for inter-position and inter-system comparisons. In the 0-5 cm depth in 2000, two out of five conventional farms had a higher pH (differences of more than 0.5 pH units) at the position "bc<1" than in "alley" (CATIE CON and Paraíso CON), and in two other cases, "bc<1" had higher pH than at "bc>2" (Aserrí1 CON and Aserrí2 CON). Within organic farms, in 2000, two out of five farms (Aserrí1 ORG and Paraíso ORG) had a higher pH at "alley" than at "bc<1". The other three farms had very similar values between positions. All the organic farms had similar values between "bc<1" and "bc>2". In the organic farms where differences were found, the influence of liming was suspected. Paraíso ORG, for example, was limed less than one year before the sampling date, and differences were probably due to the way that the lime was spread by hand (banded); thus "alley" received more lime than "bc<1". Furthermore, in the 0-5 cm layer, pH values of 6 were found, but at the 10-20 cm layer a pH of 5 was found showing that the effects of the addition of lime, or other basic amendments like earthworm manure, were limited to the surface soil. The influence of liming in the inter-system comparisons was also observed in the 2000 study. For Aserrí2 and Paraíso, organic farms had a higher average pH than their conventional counterparts, but they also had values of 6 in the first 5 cm of soil and around 5 in the 10-20 cm depth layer; recent applications of chicken manure in Aserri2 ORG and lime in Paraíso ORG were registered (Table 1). In Pejivalle ORG and CATIE ORG, where differences between the first 5 cm and the deepest layer were very small, and no liming was registered in the previous three years before sampling (July 2000), conventional farms had a higher pH than organic farms. These differences were observed in spite of the use of annual chemical fertilizer inputs in the conventional counterparts (Table 1).

In the 2004 study, trends similar to those in 2000 were observed. Only in two conventional farms, position "bc<1" had higher pH values than "alley" (Aserrí1 CON and CATIE CON); the other three conventional farms had similar values. Within organic farms,

Table 4. Values for soil pH in five organic and conventional coffee farms in central Costa Rica at 0-5, 5-10 and 10-20 cm depth in 2000 and 0-5 and 10-20 cm in 2004. Treatments: alley = equidistant fom two coffee rows and more than 2 m from a shade tree; bc>2 = below a coffee plant and more than 2 m from a shade tree; bc <1= below a coffee plant and less than 1 m from a shade tree.

Farms	Aserri1 Org		Aserri1Con		Aserri2 Org		Aserri2Con		CATIE Org		CATIE Con		Pejivalle Org		Pejivalle Con		Paraiso Org		Paraíso Con	
Year	2000	2004	2000	2004	2000	2004	2000	2004	2000	2004	2000	2004	2000	2004	2000	2004	2000	2004	2000	2004
Depth/position									Alley											
0-5 cm	6.1	5.7	5.5	4.9	6.2	6.3	5.2	4.6	4.2	7.1	4.6	5.2	4.5	4.3	4.5	4.7	6.3	5.9	4.4	4.4
5-10 cm	5.6		4.5		5.4		5.0		4.0		4.1		4.4		4.6		6.4		4.3	
10-20 cm	5.1	5.2	5.0	4.8	5.0	5.5	5.0	4.3	4.1	4.6	4.2	4.4	4.4	4.4	4.8	4.8	5.0	5.2	4.3	4.2
									bc>2											
0-5 cm	5.9		4.6		6.4		4.7		4.3		5.5		4.5		4.4		5.3		4.6	
5-10 cm	4.3		5.6		5.0		5.2		4.0		4.3		4.4		4.5		5.0		4.5	
10-20 cm	5.3		4.7		5.2		4.4		4.2		4.4		4.4		4.6		4.5		4.5	
									bc<1											
0-5 cm	5.5	6.0	5.4	5.5	6.1	5.7	5.2	4.4	4.4	6.3	5.5	5.6	4.5	4.2	4.5	4.9	5.5	5.9	4.7	4.6
5-10 cm	5.3		4.9		5.1		4.5		4.1		4.6		4.3		4.5		5.0		4.9	
10-20 cm	5.0	5.0	5.0	5.9	4.8	4.9	4.4	4.2	4.1	4.8	4.4	4.9	4.3	4.3	4.8	4.9	4.6	5.4	4.6	4.3
Average for positions at 0-5 cm	5.8	5.9	5.2	5.2	6.2	6.0	5.0	4.5	4.3	6.7	5.2	5.4	4.5	4.3	4.5	4.8	5.7	5.9	4.6	4.5

Aserri2 ORG and CATIE ORG had higher pH in "alley" than in "bc<1", and again the use of chicken manure in both these organic plantations was registered less than one year before the sampling date (June 2004). In two out of four pairs[*] of farms, (CATIE ORG and Paraíso ORG), organic farms had a higher average soil pH than conventional farms; however, in these two farms, the recent additions of lime or amendments seemed to be the main reason for the observed difference.

No changes in pH (0.5 cm depth) between 2000 and 2004 were observed either for organic or conventional farms, except for CATIE where liming led to the change. In summary, although several sites appear to have a higher pH near the tree, other sites show no effect and some have a lower pH near the trunk. The results did not show rising pH levels from *E. poeppigiana* pruning residue applications near the tree trunk in conventional farms. Ramírez and Bornemisza (1990) reported very small changes on soil pH in alley cropping systems in Costa Rica after five years of applications of 9.2 DM Mg ha^{-1} yr^{-1} of pruning residues (pH = 4.5 with *E. poeppigiana* residue additions and 4.2 in controls with no additions). Our results were in line with these findings.

Trends for higher pH in organic systems in annual crops in New Zealand were found by Reganold *et al*. (1993) and Lotter (2003). Those studies suggested that not using chemical fertilizers was one of the main reasons for higher pH in organic farms. In contrast, Lockeretz *et al.* (1981) did not find significant differences in pH in a comparison of 30 paired organic and conventional wheat, corn and soybean farms. Other studies suggested the influence of a dense herbaceous cover in raising pH (Boettcher and Kalisz 1990). They studied the impact of single-trees on soil properties (*Typic Dystropepts*) and found higher pH under *Lyriodendron tulipifera* when a dense herbaceous cover (26 species) was associated with the trees (pH = 5.6) in comparison to areas under the trees where herbaceous cover was absent (pH = 4.7). In the current study, mean pH values in the 0-5 cm layer were 5.31 *vs* 4.90 and 5.74 vs 4.88 for organic and conventional farms in 2000 and 2004, respectively. However, although a trend for higher values in organic systems was

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[*] The paired farm in Aserri2 had to be taken out from the analysis since Aserrí 2 CON was substituted in the 2004 study.

found also in the frequency analysis for the two years, the results did not permit a reliable conclusion for the influence of organic management on pH. Furthermore, pH values may even have decreased during the study period in both conventional and organic systems. The use of lime but not the use of chemical fertilizers seemed to be the main reason for the differences found.

No significant differences ($p < 0.05$) were found for soil electrical conductivity values at any of the three depths studied when positions were compared probably because of high rainfall in the study areas. Overall average conductivity for organic *vs* conventional systems was very similar: 3.09 *vs* 2.88 dS m^{-1} at 0-5 cm; 2.12 *vs* 2.26 dS m^{-1} at 5-10 cm; and 1.67 *vs* 1.77 dS m^{-1} at 10-20 cm (Appendix 4c).

3.3.4. Nutrient concentrations
3.3.4.1. Phosphorus

In 2004, an ANOVA analysis of positions showed that the proximity of shade trees did not show any influence on P concentrations at either of the two study depths (Table 5). Data for positions in 2000 were not available (as explained in the methods section). In 2000, average P concentrations in soils in organic farms were not statistically different from conventional (257 *vs* 286; 192 *vs* 202 and 157 *vs* 148 mg kg^{-1} for 0-5, 5-10 and 10-20 cm, respectively; Table 5 and Appendix 4h). However, a trend for higher concentrations was observed in the frequency analyses. Four out of five conventional farms had higher values for 0-5 cm than organically managed farms. This trend was also observed in 2004, when again, four out of five conventional farms had higher available soil P concentration at the 0-5 cm depth. At deeper layers (10-20 cm) this trend was less apparent. Only two out of five conventional farms had higher values than their counterparts (Appendix 5d).

Available soil P is a critical issue for agricultural systems but particularly for agroforestry systems. In experiments that tested mulch effects on soils, the mulches were generally found not to provide sufficient amounts of P to fulfill crop demands (Palm 1995).

Table 5. Nutrient concentrations in the topsoil at three depths for three different positions of organic and conventional coffee farms in central Costa Rica, 2000. Treatments: alley = equidistant

from two coffee rows and more than 2 m from a shade tree; bc>2 = below a coffee plant and more than 2 m from a shade tree; bc <1= below a coffee plant and less than 1 m from a shade tree.

		Organic				Conventional			
Positions Element	Depth (cm)	Alley	bc>2	bc<1	Average For three positions	Alley	bc>2	bc<1	Average for three positions
Ca	0-5	1.3 (0.2)[1]	1.3 (0.3)	1.9 (0.4)	1.5	2.1 (0.7)	2.2 (0.7)	1.8 (0.2)	2.0
$(cmol(+)kg^{-1})$	5-10	3.1 (1.3)	1.9 (1.5)	1.7 (1.0)	2.2	2.1 (0.9)	1.2 (0.6)	1.8 (1.0)	1.7
	10-20	1.4 (0.4)	1.7 (0.6)	1.4 (0.3)	1.5	1.9 (0.5)	2.0 (0.6)	1.8 (0.4)	1.9
Mg	0-5	0.2 (0.1)	0.2 (0.1)	0.2 (0.1)	0.2	0.1 (0.1)	0.2 (0.1)	0.2 (0.1)	0.2
$(cmol(+)kg^{-1})$	5-10	0.6 (0.3)	0.4 (0.3)	0.4 (0.3)	0.4	0.5 (0.2)	0.4 (0.2)	0.4 (0.2)	0.4
K	0-5	0.3 (0.1)	0.3 (0.1)	0.4 (0.1)	0.3	0.3 (0.1)	0.3 (0.1)	0.3 (0.2)	0.3
$(cmol(+)kg^{-1})$	5-10	0.2 (0.1)	0.1 (0.1)	0.1 (0.0)	0.1	0.3 (0.1)	0.3 (0.0)	0.3 (0.1)	0.3
	10-20	0.2 (0.1)	0.3 (0.1)	0.1 (0.1)	0.2	0.3 (0.1)	0.3 (0.1)	0.4 (0.1)	0.3
Zn	0-5	5.3 (1.5)	4.9 (1.5)	5.2 (1.3)	5.1	3.6 (1.4)	3.5 (1.2)	3.8 (1.3)	3.6
$(mg\ kg^{-1})$	5-10	0.9 (0.3)	1.2 (0.2)	1.0 (0.3)	1.0	1.4 (0.3)	1.9 (0.6)	1.7 (0.3)	1.7
	10-20	7.1 (0.2)	7.0 (0.2)	6.9 (0.2)	7.0	7.2 (0.2)	7.6 (0.3)	7.3 (0.2)	7.4
Mn	0-5	1.1 (0.2)	4.7 (2.9)	3.7 (1.5)	3.2	3.4 (2.0)	1.5 (0.4)	2.0 (1.2)	2.3
$(cmol(+)kg^{-1})$	5-10	1.4 (0.2)	1.7 (0.6)	1.8 (0.4)	1.6	3.1 (0.7)	4.3 (1.7)	2.5 (0.9)	3.3
P	0-5	n.a	n.a	n.a	258	n.a	n.a	n.a	286
$(mg\ kg^{-1})$	5-10	n.a	n.a	n.a	192	n.a	n.a	n.a	203
	10-20	n.a	n.a	n.a	157	n.a	n.a	n.a	148
Ammonium	0-5	3.6 (0.2)	3.9 (0.2)	3.6 (0.3)	4.0	3.4 (0.8)	4.0 (0.4)	6.5 (1.2)	4.6
$(mg\ kg^{-1})$	5-10	1.8 (0.3)	2.3 (0.7)	2.3 (0.7)	2.1	2.2 (0.8)	2.0 (0.5)	2.6 (0.8)	2.3
	10-20	5.2 (0.4)	6.7 (0.6)	8.1 (1.1)	6.7	7.2 (1.3)	6.0 (0.7)	9.5 (2.0)	7.6
Nitrate	0-5	67.3 (8.0)	81.0 (5.3)	66.9 (11.2)	71.7	59.8 (10.1)	63.5 (7.1)	82.7 (16.4)	68.7
$(mg\ kg^{-1})$	5-10	47.1 (2.9)	55.9 (4.6)	57.3 (7.6)	53.4	50.6 (5.1)	50.4 (10.4)	55.9 (4.1)	52.3
	10-20	36.3 (5.9)	44.3 (6.2)	40.6 (5.1)	40.4	36.2 (4.0)	38.3 (6.1)	50.4 (15.3)	41.6

[1] Standard error (n=5)

The main problem is that most of the agroforestry species have relatively poor P tissue concentrations. Russo and Budowsky (1986) reported 7 kg P ha^{-1} in 4 Mg DM leaves of *E. poeppigiana*. For the same species, Kass *et al.* (1989) found 24 kg ha^{-1} in 9.6 Mg DM ha^{-1}. As a result, no differences between plots mulched with *E. poeppigiana* and control plots were found by Kass *et al.* (1989) on a *Typic Humitropept* soil in Turrialba after five years of mulching.

In other trials, in a *Distropept* soil in Bolivia and in a *Tropofluvent* soil in Yurimaguas, Peru, decreases in available P concentrations were found after three years of addition of 3 and 20 Mg ha^{-1} yr^{-1} of *E. poeppigiana* mulch (Szott *et al.* 1991). The low P concentrations in pruning residues can explained in part by the absence of differences in conventional farms between "bc<1", where pruning residues were concentrated, and "alley". Other reasons are related to the cycling of P in tree-soil relationships. Haggar *et al.* (1991) reported that the shade trees captured much of the available P added in the mulch, therefore, making it unavailable for crops. Kass *et al.* (1989) also suggested that P can be sequestered in the stems of the trees.

Phosphorus fractionation methods were use by Ramírez and Bornemisza (1990), to show differences in organic P when chemical and organic fertilizers were mixed. They found differences in soluble P (Olsen modified) after application of 9.2 Mg DM ha^{-1} yr^{-1} of *E. poeppigiana* pruning residues. This suggests that P fractionation techniques can be useful to understand P cycling effects in agroforestry systems using shade trees e.g. a study by Szott and Melendez (2001) on an *Inceptisol* soil in Turrialba, Costa Rica. Their results showed greater proportions of plant available P (resin extraction using the Hedley P fractionation method; Hedley *et al.* 1982) when multistrata systems were compared to annual cropping.

Phosphorous deficits have frequently been reported in comparative studies between organic and conventional systems. Wells *et al.* (2000), found a two-fold higher Bray P concentration under conventional systems in comparison with organic systems even though 60 Mg ha^{-1} yr^{-1} of compost were added to the soil in organic farms. In the current study, the

differences in P inputs between organic and conventional farms (Table 1) can explain the trends for higher values in conventional farms. Indeed, the only two organic farms where available P was higher than in conventional farms (Aserrí2 ORG in 2000 and Pejivalle ORG in 2004) had received chicken manure or compost applications, recently before the sampling dates (Table 1). Gijsman and Sanz (1998) found that frequent applications of chicken manure increased available P even in strongly P-sorbing soils such as volcanic-ash soils in Colombia. The application of organic amendments was also evident in the soil profiles in Aserrí1 ORG (compost) as well as in Aserrí2 ORG and Paraíso CON which received chicken manure. In these farms, P (Olsen modified) in the A horizon was quite high when compared to deeper horizons (Appendix 2). These examples show how recent management influenced the intersystem comparisons of nutrients.

3.3.4.2. Potassium

In 2004, significantly higher soil K concentrations were found in organic systems (0-5 cm) near the tree trunk in comparison to other positions (0.60 vs 0.41 cmol kg^{-1}, for "bc<1" and "alley", respectively). Higher K values near the trees were also found in conventional systems (0.59 vs 0.38 cmol kg^{-1}; for "bc<1" and "alley", respectively) (Table 6). Although not significant, in 2000, soil K concentrations for the 0-5 cm soil layer were higher close to the shade trees in both farming systems (Table 5). The frequency analysis reinforced this conclusion. For both conventional and organic systems, four out of the five farms had higher K concentration for this depth in "bc<1" than in "alley". This tendency was not observed at deeper layers in either of the two studies.

A study of the effect of the addition of 8 Mg DM ha^{-1} yr^{-1} of *E. poeppigiana* pruning residues on soil characteristics in alley farming was done on a *Typic Humitropept* soil in Turrialba, Costa Rica. After five years of mulch application, even though K is highly mobile in humid conditions, significant increases were found in topsoil K concentration when compared with controls (0.85 vs 0.50 cmol (+) kg^{-1}; Kass *et al.* 1989). Consistent with our results, similar increases were not observed in deeper soil layers. The positive effects of pruning residues on soil K concentrations were attributed to the high K content in *E. poeppigiana* pruning residues. Kass *et al.* (1993) reported a content of 156 kg ha^{-1} of K in an annual biomass production of 9.6 Mg DM ha^{-1} yr^{-1}.

Table 6. Nutrient concentrations in the topsoil at two depths for two different positions of organic and conventional coffee farms in central Costa Rica, 2004. Treatments: alley = equidistant from two coffee rows and more than 2 m from a shade tree; bc <1 = below a coffee plant and less than 1 m from a shade tree.

Positions Element	Depth (cm)	Organic			Conventional		
		Alley	bc<1	average for two positions	Alley	bc<1	average for two positions
Ca	0-5	11.9 (2.9)a[1)2)]	10.4 (2.8)a	11.2	4.8 (1.3)c	7.1 (1.9)b	5.9
(cmol(+)kg^{-1})	10-20	5.1 (1.9)	4.3 (1.5)	4.7	2.8 (1.2)	4.2 (1.7)	3.5
Mg	0-5	2.5 (0.9)	2.8 (1.0)	2.6	2.3 (0.7)	2.8 (0.9)	2.5
(cmol(+)kg^{-1})	10-20	1.2 (0.6)	1.1 (0.4)	1.2	0.9 (0.5)	1.9 (1.1)	1.4
K	0-5	0.4 (0.1)b	0.6 (0.1)a	0.5	0.4 (0.1)b	0.6 (0.2)a	0.5
(cmol(+)kg^{-1})	10-20	0.2 (0.1)	0.3 (0.1)	0.2	0.2 (0.1)	0.4 (0.2)	0.3
P	0-5	19.0 (3.6)	15.9 (1.6)	17.4	28.6 (6.8)	26.2 (7.7)	27.4
(mg kg^{-1})	10-20	10.3 (2.7)	9.2 (1.8)	9.7	12.9 (4.5)	9.3 (2.7)	11.1
Cu	0-5	15.2 (8.6)	12.9 (6.7)	14.1	17.0 (4.9)	12.5 (2.6)	14.8
(mg kg^{-1})	10-20	15.7 (5.8)	15.4 (5.8)	15.6	14.2 (4.2)	14.0 (4.1)	14.1
Zn	0-5	3.0 (1.4)	2.6 (1.1)	2.8	1.9 (0.3)	2.5 (0.6)	2.2
(mg kg^{-1})	10-20	1.3 (0.2)	1.4 (0.4)	1.4	1.6 (0.4)	1.7 (0.5)	1.6
Mn	0-5	62.2 (31.6)	74.3 (41.5)	68.3	115.7 (40.2)	120.3 (53.3)	118.0
(mg kg^{-1})	10-20	85.3 (39.7)	103.9 (45.3)	94.6	122.9 (35.9)	129.9 (49.0)	126.4
Fe	0-5	162.2 (98.4)	141.6 (66.6)	151.9	248.8 (54.5)	231.2 (59.9)	240.0
(mg kg^{-1})	10-20	191.6 (36.0)	203.6 (37.0)	197.6	235.8 (74.0)	215.0 (63.0)	225.4

[1] Standard error (n = 5)

[2] values with the same letter within a row are not significantly different (Lsmeans-test p < 0.05).

Cobo *et al.* (2002), evaluating the decomposition of 12 plant materials including herbaceous and woody species on a *Dystropept* soil in Cauca, Colombia, concluded that K had the highest release rates (k) among five major plant nutrients ($k > 0.085$ d^{-1}). The ability of *E. poeppigiana* to maintain higher soil K concentrations near the trunk seems to be limited to 0-5 cm depth (Figures 7A and B). It is important to note that even though K release rates from pruning residues are very high and K is a very mobile element in rainy conditions (sampling was done in months with high rainfall) differences between positions could be detected.

No significant differences were found in K concentrations between the two farming systems in either of the two study years. Direct comparisons between years could not be done because different laboratory methods for nutrient analyses were applied. In the 0-5 cm layer, similar average K concentrations were found for organic and conventional farming systems in 2000 (0.34 *vs* 0.28 cmol kg^{-1}) and 2004 (0.50 *vs* 0.48 cmol kg^{-1}, respectively). The same situation was found at the 5-10 and 10-20 cm depth layers in 2000 (0.14 *vs* 0.26 cmol kg^{-1}; 0.17 *vs* 0.32 cmol kg^{-1}, for organic and conventionally managed farms, respectively) and in 2004 (0.22 *vs* 0.32 cmol kg^{-1}) (Tables 5 and 6). In the frequency analysis, a trend for higher K concentrations in organic systems was observed in 2000 (four out of five organic farms had higher values than their counterparts) but not in 2004 when only two organic farms had higher values than conventional farms (Appendix 4i and 5e).

The K cycle in agroforestry systems can help explain the similar soil K concentrations between organic and conventional systems. Alpízar *et al.* (1986) and Fassbender (1993) modeled inputs and outputs of K in two multistrata agroforestry systems (coffee with *Cordia alliodora* or with *E. poeppigiana*). Absorption of K by coffee plants was higher when the soil received higher K inputs. For example, in multistrata systems with *E. poeppigiana,* coffee absorbed 104 kg K ha^{-1} from the soil, whereas the systems with *C. alliodora*, absorbed only 30 kg K ha^{-1}. At the same time, systems with *E. poeppigiana* received almost five fold higher amounts of K inputs from pruning residues. In another study on the same site, Imbach *et al.* (1989) found low losses of K (1.8 kg ha^{-1} yr^{-1}) in the percolating soil water at 1 m depth in coffee plantations with *E. poeppigiana* shade trees. Furthermore, K output in coffee cherry yields were also higher in conventional farms since

higher yields were obtained in the systems with *E. poeppigiana*. However, as a result of the input-output balance, relatively similar exchangeable soil K reserves were found in both systems (687 and 630 kg ha^{-1} for *C. alliodora* and *E. poeppigiana*, respectively). In conventional farms of the present study, presumably higher amounts of K are absorbed by coffee plants when compared to organic farms; because they are receiving up to 130 kg K ha^{-1} from chemical fertilizers plus K inputs from pruning residue decomposition (Table1). In contrast, organic farms only receive K inputs from pruning residue decomposition. In addition, in conventional farms, higher amounts of K are exported because fruit yields are from 20-100% higher in conventional farms than in organic farms (Table 1). Considering that coffee cherries have a K concentration of 1.5% (Fassbender 1993), maximum exports of up to 157 kg K ha^{-1} yr^{-1} can be calculated in Paraíso CON farm (average yield of 10500 kg ha^{-1}) while Paraíso ORG exports only 73 kg K ha^{-1} yr^{-1} (average yield of 4900 kg ha^{-1}). Additionally, higher losses of K (applied in chemical fertilizers) due to runoff can be also expected in conventional farms because the areas between coffee rows are usually less protected by mulch and weeds than in organic farms. In summary, available K in the soil of organic and conventional farms may remain at similar levels, as shown in the data collected in the current study, since differences in K inputs and outputs balance.
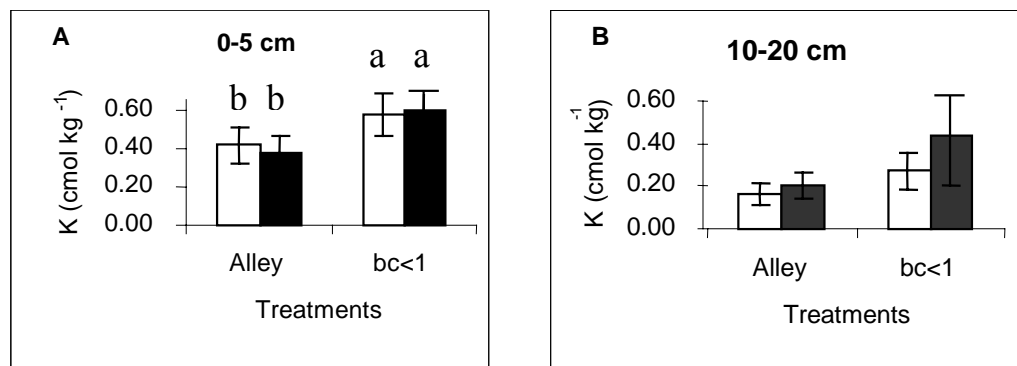
Figure 7. Potassium concentration in the topsoil at two depths for two different positions on organic (□) and conventional (■) coffee farms in central Costa Rica, 2004. Treatments: alley = equidistant from two coffee rows and more than 2 m from a shade tree; bc <1 = below a coffee plant and less than 1 m from a shade tree.

3.3.4.3. Calcium, Magnesium, Zinc and Manganese

Since *E. poeppigiana* has high Ca concentrations in pruning residues (Beer 1988), higher Ca values were expected close to the tree trunk where residues were concentrated, particularly in conventional farms. In 2000, proximity to a shade tree did not influence Ca concentrations in any of the three soil layers studied (Table 5). Nevertheless, in 2004 at the 0-5 cm depth, Ca concentrations were greater near the tree trunk ("bc<1") than 2 m away from the trunk ("alley") in conventional farms. In organic farms, no significant differences were found between these two positions (Table 6). In 2004, "bc<1" and "alley" in organic farms (0-5 cm) had higher Ca concentrations than their counterparts in conventional farms. At 10-20 cm depth, no differences either for positions nor for inter-system comparison were found. In the case of Mg, for both 2000 and 2004 studies, no significant differences were found for any of the comparisons between positions at any depth (Tables 5 and 6 and Appendix 4k and 5g).

Kass *et al.* (1989) found no changes in soil Ca and Mg after five years of applications of 9.2 Mg ha^{-1} yr^{-1} *E. poeppigiana* pruning residues. In the same experiment, after nine years of applications, there were very small differences in soil Ca and Mg concentrations (5.04 *vs* 4.87 cmol kg^{-1} Ca and 1.22 *vs* 1.14 cmol kg^{-1} Mg with *E. poeppigiana* pruning residues. In the same experiment, after nine years of applications, there were very small differences in soil Ca and Mg concentrations (5.04 *vs* 4.87 cmol kg^{-1} Ca and 1.22 *vs* 1.14 cmol kg^{-1} Mg with *E. poeppigiana* residues and control, respectively). However higher Ca and Mg recycling through shade trees were measured (Soto *et al.* 1993). Kimemia *et al.* 2001 found that cations decreased after applications of 3 Mg ha^{-1} yr^{-1} of *L. leucocephala* during three years in coffee plantations in Kenya. A rapid release rate of these nutrients can explain the difficulty in detecting differences between treatments when pruning residues are applied. Montagnini (2000) found higher soil Ca concentrations under species such as *Terminalia amazonia* and *Virola koschnyi,* with high leaf Ca content and high leaf shed ratios, than in control plots without trees.

Szott *et al*. (1991) found high mineralization rates of cations from high quality *E. poeppigiana* residues. Within four weeks, 40% of the initial Ca and 75% of the initial K and Mg were released; and after 20 weeks only 25% of the initial content of these nutrients remained. In a study at Yurimaguas, Peru similar soil cation levels where measured after three rice harvests, when intercropped plots were compare to sole crop plots. Intercropped plots received *Inga edulis* pruning residues (2.5 Mg ha^{-1} per pruning and one pruning per crop) for three years. Three hypothesis were given as explanations for these findings: a- the amount of residues was too low; b- nutrients applied with the residues were retained in organic forms, which are not detectable with usual analytical techniques in routine soil tests; and c- the nutrients were removed by crops or lost through other mechanisms that were not measured (Szott *et al*. 1991). In the current study, hypotheses "b" and "c" can help explain the similarity between positions for Ca, K and Mg in present 2000 study, and for Mg and micronutrients in 2004.

When organic and conventional systems were compared (averages for positions), no significant differences were found in Ca concentrations at a 0-5 cm soil depth in either 2000 or 2004 using ANOVA (1.52 in organic *vs* 2.01 cmol kg^{-1} in conventional farms; 11.17 in organic *vs* 5.93 cmol kg^{-1} in conventional). At 10-20 cm depth, no differences for intersystem comparison were found (Tables 5 and 6). No direct comparison between years was carried out because different laboratory methods for nutrient analyses were applied. High variability between sites (blocks in the ANOVA analysis) also tended to hide statistical differences between systems. However, in a frequency analysis in 2004, in four out of five pairs of farms, organic systems showed higher Ca concentrations than conventional systems, but again the differences were associated with recent applications of lime or chicken manure compost, which usually has added lime[*] (Table 1). For example, in 2004 CATIE ORG had higher values than its counterpart, but lime was applied four months before sampling. In contrast, in 2000 Ca concentrations for CATIE CON (where 1500 kg lime ha^{-1} was applied two years before sampling) were higher than CATIE ORG (Tables 1, 5 and 6). In general, all the organic farms used in the present study applied lime

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[*] Organic farms usually used compost that contains chicken manure, and lime is usually added to the manure in the chicken farms to control bad odors.

at least once between 1999 and 2003 (Table 1). In the case of Mg, for 2000 there were no significant differences between organic and conventional farming systems for 0-5 or 5-10 cm (0.20 *vs* 0.18 and 0.45 *vs* 0.44 cmol kg^{-1}, respectively). In 2004, trends did not change because no significant differences were found in Mg concentrations for system comparisons (Tables 5 and 6). As described above, no direct comparison between years was possible. A larger coefficient of variance was found for this element in ANOVA (56%), due to a high natural Mg concentration between sites. For example, in Aserrí 1 the organic and conventional farms had 5.92 and 4.97 cmol Mg kg^{-1}, respectively. In contrast, the average Mg concentrations for the two paired farms in Aserrí 2 were 2.72 and 0.70 cmol kg^{-1} for organic and conventional farms (Appendix 4k and 5g).

In the 0-5 cm soil layer, no significant differences between systems or positions within a system were found for Zn or Mn in 2000 in any soil layer (Table 5). Serpa and Bornemizsa (1980) studied soil Zn and Mn concentrations on an Inceptisol in Turrialba, comparing a field after four years of maize and bean rotations with a field under pastures. The crop rotations received NPK fertilizers and 2 Mg ha^{-1} yr^{-1} of lime. Pastures did not receive chemical fertilizers or liming. No significant differences between the two cropping systems were found, and the study suggested that the high organic matter content of the top soil in the two compared fields (7.4 and 6.1% organic matter) masked the differences between the systems. This argument can help to explain the absence of differences in soil Zn and Mn concentrations between the systems investigated in the current study. The Zn concentrations in this work were also within the range of their study (2-9 mg kg^{-1}). All contrasts between systems and between positions in 2004 for Cu, Zn, Mn and Fe were not significant at any depth (Table 6 and Appendices 4 and 5).

In a review of the effects of organic farming on chemical soil characteristics Stockdale *et al.* (2001) suggested that changes in nutrient contents in organic farms have to be predicted from the input-output balance in each farm. The variety of organic fertilizers (nutrient contents vary widely and farmers frequently do not have precise information on these and hence about application rates) used by organic farmers in Costa Rican coffee plantations makes this goal very difficult. Reganold *et al.* (1993) found similar aggregated amounts of Ca, Mg, and K in organic and conventional farms in New Zealand. The

similarities were associated with high natural soil fertility in both types of farms. Lockeretz *et al.* (1981) did not find differences in nutrient status in a comparison of 30 organic and conventional paired farms. In both types of farms wheat and soybean rotations were grown. The study suggested that more P and K where removed by the crops when high amounts of nutrients were available, thus producing similar net balances in both organic and conventional farming systems.

3.3.4.4. Nitrate and ammonium

In conventional systems, in 2000, ammonium concentrations for 0-5 cm were not statistically different between positions although values appeared to be higher (6.5 *vs* 4.0 and 3.4 mg kg^{-1} for "bc<1", "bc>2" and "alley", respectively) possibly due to higher organic matter concentrations. In this depth, the conventional system had higher soil ammonium concentrations in comparison to the organic system (4.6 *vs* 3.7 mg kg^{-1}, respectively) presumably because of chemical inputs of N, mainly granular formula that usually included ammonia as a nitrogen source (Tisdale and Nelson 1991) in conventional farms. There were no significant differences between positions nor between systems for the other two layers. Nitrate concentrations were similar for all contrasts for the three study depths presumably because of the high mobility of nitrates under the high rainfall conditions (Table 5; Appendices 4d and 4e).

No differences in nitrate concentrations after a four year period were found by Clark *et al.* (1999) in a comparative experiment in Sacramento Valley, California. Organic, low input and conventional systems on a silt loam were compared over five crop rotations. Conventional systems received N as urea and/or ammonium nitrate at about 170 kg N ha^{-1} yr^{-1}. In their study, a large variation of nitrate concentrations in both systems (5 to 50 mg kg^{-1}) led to the absence of significant differences and consistent trends. Soil NH_4^+ concentrations were even more variable than nitrates.

3.3.5. Soil respiration rates

Soil respiration was measured as an indicator of biological soil quality. Higher soil respiration rates were measured for organic systems in comparison to conventional at 0-5 cm (1.68 vs 1.21 mg CO_2-C kg^{-1} h^{-1} dry soil; respectively) (Table 7). In the deeper layers, both systems had similar respiration rates. Reganold et $al.$ (1993) and Lotter (2003) have reported higher soil respiration rates in organic farms, associated with higher microbial activity and C concentrations in organic systems. In addition, the highly diverse microbial communities present in organic systems also have been associated with a more efficient metabolic quotient qCO_2 (Fließbach and Mäder 2000). No differences were found between the three study positions at any depth for either system. Nevertheless, a similar pattern between soil respiration at 0-5 cm and C concentrations at 0-5 cm suggest a relationship between these two variables (Table 7; Appendix 4g).

Table 7. CO_2 production (mg CO_2-C kg^{-1} h^{-1}) from three soil depths in organic and conventional coffee farms in central Costa Rica, 2000. Treatments: alley = equidistant fom two coffee rows and more than 2 m from a shade tree; bc>2 = below a coffee plant and more than 2 m from a shade tree; bc <1 = below a coffee plant and less than 1 m from a shade tree.

Positions	Organic				Conventional			
Depths	Alley	bc>2	bc<1	Average	Alley	bc>2	bc<1	Average
0-5 cm	1.76 (0.23)[1]	1.66 (0.22)	1.61 (0.11)	**1.68a**[2]	1.16 (0.10)	1.14 (0.11)	1.33 (0.14)	**1.21b**
5-10 cm	1.06 (0.29)	0.99 (0.13)	0.75 (0.02)	0.93a	0.74 (0.01)	0.70 (0.02)	0.88 (0.10)	0.77a
10-20 cm	1.05 (0.11)	1.03 (0.11)	0.93 (0.05)	1.00a	1.04 (0.09)	0.93 (0.07)	1.02 (0.10)	1.00a

[1] Standard error (n=5)

[2] Values with the same letter within a row are not significantly different ($p<0.05$, Duncan test).

Respiration rates were regressed on soil C concentrations. Separate regression procedures were done for each position within each system but only one significant regression model was found (alley in conventional systems; Figure 8). No other significant regression model was fitted. This result indicated that respiration rates were not directly associated with C contents, except for one of the positions far away from the shade tree trunk in conventional farms. Factors others than C concentration, such as fungi:bacteria

biomass rates can influence respiration rates in organic farms and in positions near to the trees, as suggested by Holland and Coleman (1987).
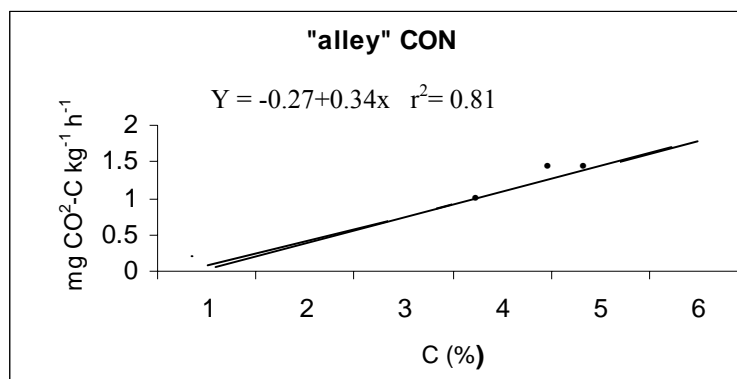
Figure 8 Estimated respiration rates vs. carbon concentrations in "alley" position within conventional farms in central Costa Rica, 2000.

3.3.6. Organic matter size fractions

The amount of macroorganic matter (>200 μm) in 2000 found in organic farm soils was lower than in conventional farms at all depths but was only significant for 10-20 cm (Table 8). At 0-5 and 5-10 cm, in four out of five pairs of farms, conventional farms had larger amounts of macroorganic matter than organic farms (Table 8). High variability between locations was found particularly at 5-10 cm. This fraction could be influenced by recent management or recent movements of organic inputs in the shallowest soil layers; e.g. a recent pruning or run off of macroorganic matter. Barrios *et al*. (1996a) suggested that macroorganic matter (200–2000 μm) can be a better indicator for detecting the effects of different soil management in SOM than the 53-200 μm fraction. In line with this idea, in the current study the amount of the POM fraction (53-2000 μm) was also compared for the two farming systems (Table 9) and although once again all average values for conventional farms were higher than organic, no significant differences were found at any depth.

Soil respiration may be associated with the quantity of POM (Fließbach and Mäder 2000). Consistent with the above finding that soil respiration was higher for organic soil,

greater transformation of macroorganic matter and POM in organic soil can be expected, presumably due to greater biotic activity. Carpenter-Boggs *et al.* (2000) found that a *Ultic Haploxeroll* soil in Washington that received compost for two years was more biologically active than the same soils that received NPK chemical inputs. Unfortunately, in the present study no significant correlations between respiration rates and the amount of macrorganinc matter or POM for any layer were found. In the following chapters the effect of artificially increased biological activity on macroorganic matter is one of the main topics that is discussed.

Table 8. Amount of macroorganic matter (>200um) in 5 pairs of organic (Org) and conventional (Con) coffee farms in central Costa Rica, 2000 at three soil depths.

Farms depth/	Aserri1 Org [1]	Aserri1 Con	Aserri2 Org	Aserri2 Con	CATIE Org	CATIE Con	Pejivalle Org	Pejivalle Con	Paraiso Org	Paraíso Con	average Org	average Con
0-5 cm	2.41	2.01	5.04	6.61	3.31	4.11	1.12	2.11	0.99	2.22	2.5a [2]	3.4a
5-10 cm	5.10	1.19	5.74	11.76	2.90	3.10	0.34	2.45	0.48	2.05	2.9a	4.1a
10-20 cm	1.19	1.96	3.84	5.37	2.90	3.18	0.56	1.45	0.26	1.44	1.7a	2.7b

[1] g 100 g^{-1} dry soil
[2] Values with the same letter within a row are not significantly different ($p<0.05$, Duncan test).

Table 9. Amount of particulate organic matter, POM (53-200 um) in five pairs of organic (Org) and conventional (Con)(coffee farms in central Costa Rica, 2000 at three soil depths.

Farms depth/	Farms depth/	Aserri1 Org [1]	Aserri1 Con	Aserri2 Org	Aserri2 Con	CATIE Org	CATIE Con	Pejivalle Org	Pejivalle Con	Paraiso Org	Paraíso Con	average Org	average Con
0-5 cm	0—5	11.28	10.56	10.73	11.20	8.75	11.43	4.31	10.99	4.93	6.83	8.00	10.20
5-10 cm	5—10	22.23	10.40	10.38	10.53	7.63	9.64	3.74	11.90	3.86	5.90	9.57	9.67
10-20 cm	10—20	8.06	18.89	12.07	11.81	10.40	12.25	5.72	n.a.	5.05	8.73	8.26	12.92

1) g 100 g^{-1} dry soil

3.4. Conclusions

In the two study years (2000 and 2004), surface soil C and N concentrations (0-5 cm) were higher close to *E. poeppigiana* in conventional farming systems but no evidence of this effect was found in organic farming systems. These higher values can be attributed to the concentration of tree pruning residues that farmers leave near to the tree trunks in conventional farms. This positive effect of the shade trees, in raising soil C and N concentrations, could influence up to 20 % of the total plantation area in conventional farms when high populations of *E. poeppigiana* are used (625 trees ha^{-1}, an assumed a circle of influence with a radius of 1 m, affect 1964 m^2 in each hectare). In organic systems, a better spatial distribution of pruning residues and of other organic amendments was observed, thus diminishing the differences between positions respect proximity of shade trees.

A trend for higher total soil C and N soil concentrations in organic systems was detected in 2000 and 2004. We hypothesize that this trend can be due to an even distribution of pruning residues in the field, denser shade and greater organic matter inputs into the soil from weed biomass and organic amendments. The differences between positions and between systems were detected only at the 0-5 cm depth. This suggests that the management period studied is too short to affect deeper layers. Furthermore, the 10-20 cm layer did not show the effect of tree proximity in a four year study period (2000-2004). No significant changes were found in C or N concentrations in either system at any depth between 2000 and 2004. This was particularly true for organic farms which seem to have reached a more stable level of soil C and N concentrations.

Proximity to *E. poeppigiana* and the type of farming system did not affect physical soil characteristics such as soil pH and bulk density at any of the study depths. Literature states that long time periods are needed to observe changes on these soil properties. In the majority of the farms, the proximity of shade trees had no effect on nutrient concentrations. Nevertheless, greater superficial K concentrations close to the shade tree (only 0-5 cm depth) were observed in both systems (not significant in 2000; significant in 2004). Significant differences in Ca concentrations between positions, following a similar pattern

to soil C concentrations, were found in 2004 but not in 2000 at 0-5 cm. These results suggested that tree residues play a role in supplying K and Ca to the soil, but again the effect is limited to the shallowest layer, close to the shade trees.

No differences between organic and conventional systems were detected for soil P, K, Ca, Mg, NO_3^-, and micronutrients in any layer. The only exception was a higher NH_4^+ concentration for conventional farms at 0-5 cm. In some cases, when trends for higher concentrations in one type of farms were found (e.g. P and Ca), recent fertilizer additions (either organic or chemical) were involved. However, the influence of higher organic matter concentrations in the 0-5 cm layer may not be discarded. Individual input-output nutrient budgets are needed for each farm. For example, the lower amount of nutrients that are applied through organic fertilizers can be balanced by lower outputs in yields and lower nutrient absorption by plants within organic systems and vice versa in conventional farms. These budgets can explain the absence of differences in the inter-system comparisons. However, the variety of organic fertilizers of variable and often unknown quality, with irregular application timing, complicates nutrient cycle calculations for organic farms.

Positions less than 1 m and 2 m from the shade trees showed similar respiration rates, but at the 0-5 cm depth, higher respiration rates in organic farms than in conventional were observed. The amount of macroorganic matter (200-2000 μm) was lower in organic farms but the relationship with higher respiration rates in these farms was not detected. The relationship between macroorganic matter dynamics and biological activity in the soils of organic farms will be approached in Chapter IV in this thesis.

Although criteria for the selection of paired farms were strictly followed, the study of original soil conditions though profile descriptions and chemical analysis of samples from each horizon indicated that paired farms were not strictly comparable. This natural variability in soil conditions affected comparisons between organic and conventional systems and impeded definitive conclusions. In this study, the effect of *E. poeppigiana* proximity could be observed in fields with up to 45% slope. However, in the inter-system comparison even small slope differences between the farms could affect the comparison

between farming systems (CON vs ORG). Nevertheless, the frequency analysis and the results from a pair of farms that showed almost identical soil properties in the soil profile (Pejivalle, 2000 and 2004) indicated that the hypothesis of higher soil C and N concentrations in organic farms in comparison to conventional at the 0-5 cm depth can not be discarded.

The use of the mini soil-pit method instead of augers for soil sampling can help to detect differences between treatments in sloping field conditions. A larger number of farms should be included in future studies of this kind due to inevitable inter and intra-site variability in on-farm studies of this kind. Comparative analyses should be concentrated in the 0-5 cm layer, where management and shade tree proximity had the greatest effect. A better distribution of pruning residues in conventional farms can extend beneficial effects of higher organic matter concentrations to larger areas.

3.5. References

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Chapter 4   Carbon contents in size-density fractions of soil macroorganic matter after the addition of *Erythrina poeppigiana* pruning residues in organic coffee farms

Key words: *Coffea arabica*, litter decomposition, liquid organic amendments, shade trees, soil organic matter.

4.1. Introduction

The most labile fraction of soil organic matter (SOM), the so called "active" fraction due to its rapid turnover rate (Duxbury *et al.* 1989), has been linked integrally with nutrient mineralization and subsequent availability to plants (Phiri *et al*. 2001). The light fraction (LF) and particulate organic matter fraction (POM) have been identified as unprotected SOM fractions with similar characteristics, although different isolation methods have been used to obtain them (Gregorich and Ellert 1993). Both fractions (LF and POM) contain fragmented plant residues as a main component, but also microbial debris such as hyphae and spores (Six *et al.* 2002). Light, medium and heavy size-density fractions (LF, MF and HF), as well as POM, have been studied as indicators of changes in soil fertility as a result of changes in land use and management. The LF is a C and N labile pool available for microbial activity and its size is more sensitive to changes in SOM after organic residue inputs than is total SOM (Cambardella and Elliot 1992, Hassink 1995, Meijboom *et al.* 1995, Phiri *et al*. 2001).

Many laboratory methods have been developed to isolate the "active" fraction of SOM since halogenated hydrocarbons were first used in 1964 to separate partially decomposed organic material with different densities (Magid *et al*. 1996). The "active" fraction has been considered as the labile SOM pool possessing a short turnover time, the turnover of which is directly related to the speed of soil nutrient cycling (Phiri *et al*. 2001). A method that separated soil macroorganic matter (defined as the labile organic material >150 μm) into three fractions using a non toxic silica suspension (Ludox^{TM}: light fraction (LF), with a density <1.13 g cm^{-3}; medium fraction (MF), with a density between 1.13 and

1.37 g cm^{-3}; and heavy fraction (HF), with a density >1.37 g cm^{-3}) has been developed by Meijboom *et al*. (1995). In this method, different density fractions can be separated because more humified SOM is associated with the mineral components of soil becoming denser than the less decomposed SOM that is mineral-free (Barrios *et al.* 1996a). The method has been used successfully to compare the effects of different residues on soil macroorganic matter (Hassink 1995) and to compare size-density (SD) fractions between organic and conventional farming systems (Fließbach and Mäder 2000). Nevertheless, more field data is needed to assure that this method effectively measures the "active" fraction (Magid *et al*. 1996).

An evaluation of SOM fractionation by size, density, and size-density methods, using NaI, Na-polytungstate and Ludox™ showed that the LF obtained using Ludox™ was a sensitive indicator of changes in SOM due to soil management (Barrios *et al*. 1996a). However, its small size and the lack of information of N release rates from this fraction raised doubts about its role as the "active" pool in SOM models. In spite of their small size in comparison with total SOM, LF and POM have been studied as important contributors of N (Sierra 1996, Barrios *et al*. 1996b, 1998) and P (Phiri *et al*. 2001; Salas *et al*. 2003) cycling in the soil. Soil moisture and temperature, composition and C to N ratio of residues, earthworm populations and oxygen and N availability in the soil are the main factors determining the size of unprotected SOM (Theng *et al*. 1989).

Traditionally, total soil C has been used as an indicator of the effects of changes in soil management on SOM status. However, some studies have shown that SOM fractions (LF-C, macroorganic matter or POM) but not total soil C better reflect the effects of some practices such as mulching or crop rotations (Barrios *et al.* 1996a; Neufeldt *et al.* 1999; Cambardella and Elliot 1992). For example, the effects of seven maize-legume cropping systems on SOM fractions were studied in Colombian hillsides. Size density and size fractions as well as total C were evaluated as indicators of changes in SOM. Carbon contents in the fractions were compared after eight cropping seasons under the different cropping systems. LF-C, but not total soil C, reflected the effect of residue addition into the soil (Barrios *et al.* 1996a). In the same area in Colombia, dry weight, C, N and P contents in

three SD-fractions were compared after the addition of chopped plant material from three different fallow species (*Tithonia diversifolia, Calliandra calothyrsus* and *Indigofera constricta),* as well as after a continuously tilled maize-bean rotation. Only the medium size-density (SD) fraction showed significant differences among treatments, although a trend of higher values for residue added treatments in comparison with the continuously tilled were also observed for light SD fraction parameters (Phiri *et al.* 2001). However, several researchers have highlighted the need for more field data on the biological characteristics and dynamics of these fractions because of their small proportion in comparison with total soil organic carbon and limited data on their nutrient mineralization rates (Fließbach and Mäder 2000, Magid *et al.* 1996, Hassink 1995).

In practical terms, the importance of these labile SOM fractions resides in their role as source of readily available nutrients that can be provided to crops through decomposition. This is particularly important in organic coffee farms in Costa Rica, which depend greatly on nutrients provided by pruning residues from shade trees such as *E. poeppigiana* (Lyngbaeck *et al.* 2001). In addition, the use of microbial mixtures for improving the soil in organic farms has been widely adopted but seldom tested under controlled conditions (Soto and Muschler 2001). Likewise, the effects of earthworm inoculation on SOM fractions and soil microbiology have been studied but no conclusive results have been obtained (Gilot *et al.* 1996, Syers and Springett 1984, Springett *et al.* 1992). In this study, native microbial strains or earthworms were added to *E. poeppigiana* pruning residues in organic coffee farms with the following objectives: 1) to evaluate dynamics of SD-fractions after the addition of *E. poeppigiana* pruning residues in Costa Rican organic coffee farms; 2) to measure the effects on LF, MF and HF of treatments that enhance microbial activity (microbial mixtures and earthworms) applied on to the *E. poeppigiana* pruning residues; and 3) to evaluate macroorganic matter (>150 μm) and POM (>53 μm), as indicators of changes induced by pruning residue management.

4.2. Materials and methods

4.2.1. Site description

Two simultaneous field trails were conducted from August 2002 until August 2003. Experimental sites were in CATIE´s organic coffee plantations in Turrialba (600 m.a.s.l.) and on a private organic coffee farm in Pejivalle, 25 km south of Turrialba (650 m.a.s.l.). In 2004 a new field trial was set up at the same area in CATIE. The soil at the CATIE experimental site was classified as an *Andic Dystrudept* (Soil Survey Staff 2003). This site has a 0-3% slope and a poorly drained clayey surface layer. The soil of the Pejivalle farm was classified as an *Typic Haplohumult*; a well-drained clayey soil in the middle of a hill with 25- 35% slope, typical of small coffee farms in central Costa Rica. Soil profile description for each site and chemical characteristics of the horizons can be consulted in Appendices 1 and 2, Chapter III. Both sites are located in the tropical premontane wet forest zone (Holdridge 1967) of the Atlantic region of Costa Rica, with annual rainfall of 2650 mm for CATIE and estimated in 3500 mm for Pejivalle (MAG 1990). There is no well defined dry season; the annual mean temperature for both sites was between 21 and 22°C (MAG 1990). Both sites have been planted with coffee for at least 30 years. CATIE plots had been managed organically for seven years and the Pejivalle farm for thirteen years. Selected soil characteristics and fertilization practices are summarized in Table 1. Mean temperature and monthly rainfall (CATIE site only, data not available for Pejivalle) during the field trials are presented in Figures 1 and 2.

Table 1. Selected soil characteristics (0-20 cm depth) and agricultural practices of experimental sites in organic coffee plantations in Turrialba and Pejivalle, Costa Rica.

Soil characteristics	Experimental sites and localities	
(0-20 cm)	CATIE, Turrialba	Pejivalle, Cartago
Texture		
Clay (%)	47	62
Sand (%)	29	19
Silt (%)	25	20
pH (1:1, soil/water)	4.29	4.37
Total C (%)	2.0	2.6
Base saturation (%)	28	30
Fertilizer type; nutrient equivalent (kg ha^{-1}yr^{-1})	226 "KMAG" a commercial organic fertilizer (22%K$_2$O) = 50 K	1800 Compost of grass and leaf litter = 9N;9P;9K
Liming (Mg ha^{-1}yr^{-1})	1.6 every 3 years	1.0 every 3 years
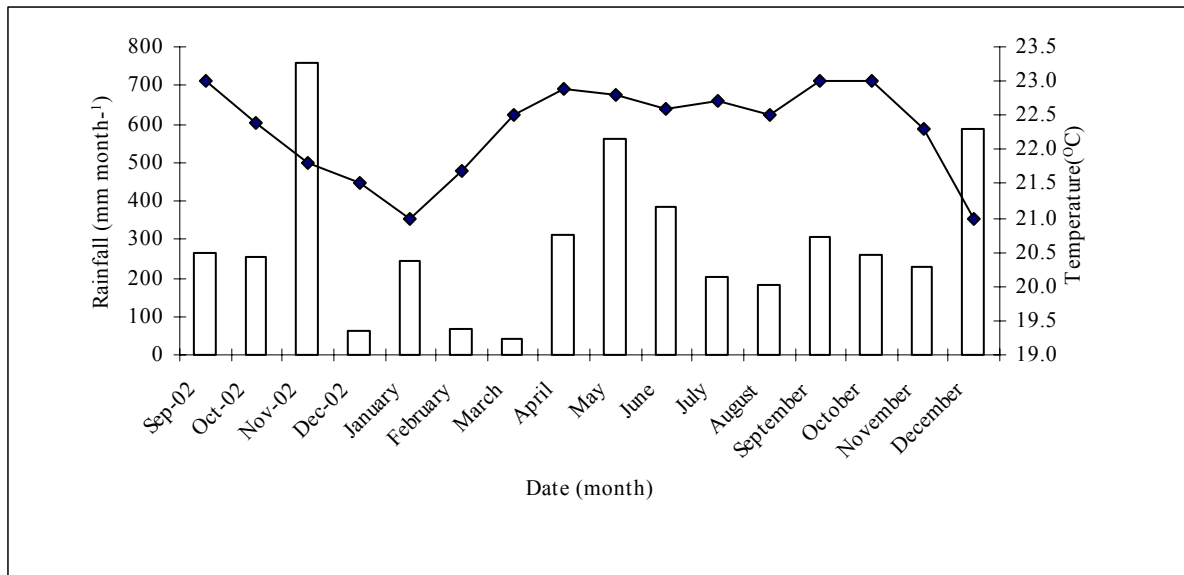
Figure 1. Monthly rainfall (columns) and mean temperature (-♦-♦-♦-) at the CATIE meteorological station from September 2002 to December 2003
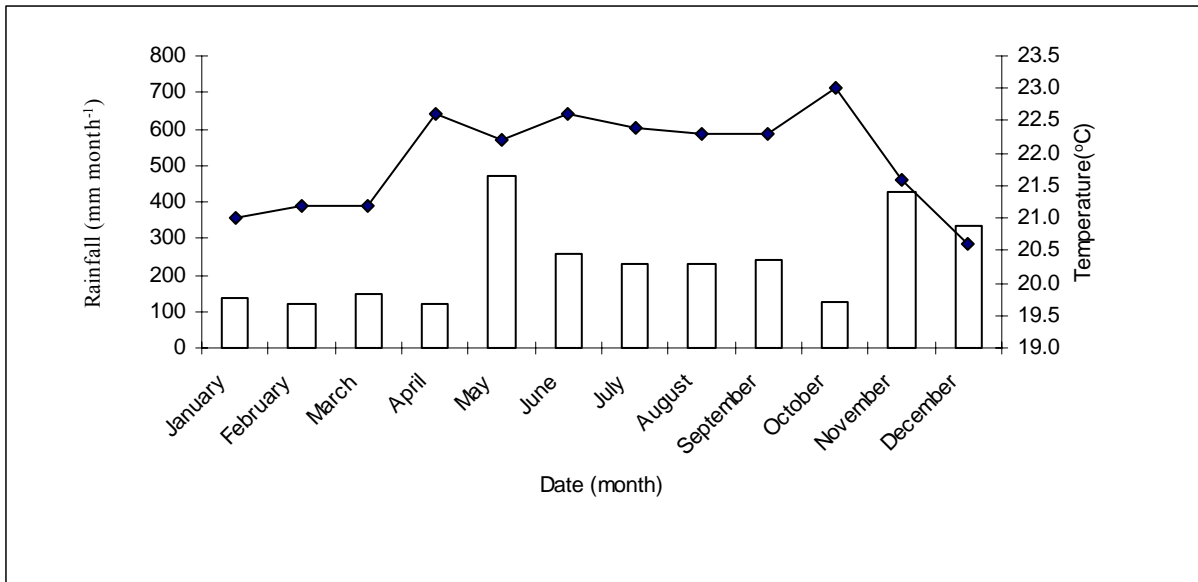
Figure 2. Monthly rainfall (columns) and mean temperature (-♦-♦-♦-) at the CATIE meteorological station in 2004

4.2.2. Field trial design

In 2002, a randomized complete block experimental design, with three replicates, was established at each site. Each block had five 2 × 2 m plots; each plot was surrounded by an untreated 1.0 m buffer zone (i.e. 2 m between the plots) to avoid inter-treatment interference (Figure 3). The plots were marked and cleared of litter and weeds at the end of August 2002. All treatments, except the bare soil controls, received the equivalent of 5 Mg ha^{-1} of dry matter, grossly chopped *E. poeppigiana*, pruning residues (10 kg of fresh residues per 4 m^2 central plot
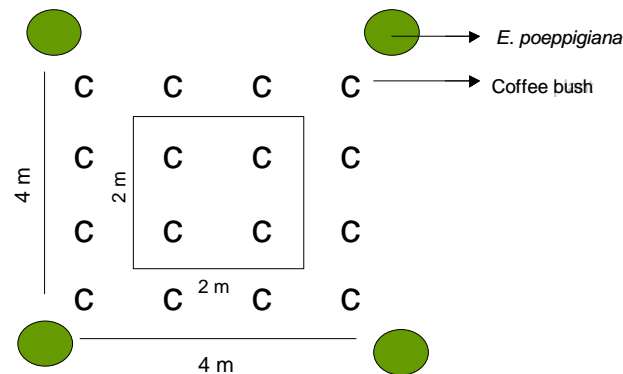
Figure 3. Characteristics of the experimental plots in the field trials, CATIE and Pejivalle, 2002 and CATIE, 2004

including branches up to 2.5 cm diameter, containing an average of 20% dry matter) at the end of September or early October 2002 and again in the middle of March 2003 (total 10 Mg ha^{-1}). The amount and timing of the tree residue input simulated the typical pruning residue inputs in local coffee farms of 10 Mg ha^{-1} yr^{-1} of dry matter of *E. poeppigiana* (Beer 1988). In each block, three treatments that enhance microbial activity [microbial mixture A (MICROB), microbial mixture B + composting (COMPOS), earthworms (EART)], one treatment which only received the pruning residues (RESID) and one bare soil treatment as a control (BARESO) were randomly distributed. The control plots were maintained clear of litter and weeds, leaving the mineral soil exposed, throughout the experiment. All of the experimental plots were cleared every week of new fallen leaves. Weeds were also carefully removed if they succeeded in establishing themselves despite the mulch.

Microbial mixture "A" (MICROB) was produced from 40 ml of cooked rice colonized by native microbial strains from a nearby secondary forest, dissolved in 12 l of water and mixed with 250 ml of molasses, 250 ml of yogurt and 227 g of soybean meal. This was prepared four weeks before application. This primary solution was diluted to a concentration of 2.5% v/v in tap water and 50 ml per plot were sprayed on the recently distributed tree pruning residues. Microbial mixture "B" (COMPOS) was produced from 250 g of molasses, 62.5 g of yogurt and 113 g of soybean meal mixed in 50 l of water with

5 kg of *E. poeppigiana* litter that had been colonized by fungi (collected in the same secondary forest). This was prepared one week before application. Fifty milliliters of this solution were sprayed on the recently distributed tree pruning residues in the corresponding plots. During the experiment, the pruning residues with microbial mixture "B" were turned over monthly to accelerate decomposition imitating a composting process. Microbial mixture preparation procedures were modified from Fishersworring and Roβkamp (2001). The microbial mixtures contained the fungi genera *Aspergillus, Penicillium, Thrychoderma, Gliocladium, Metarrhizium* and *Verticillium*, identified in the phytopathology laboratories at CATIE and the University of Costa Rica after cultivation in PDA (potato dextrose agar) medium and nutritive agar medium at 10^{-8} dilution. Seven strains of bacteria were also isolated (Appendix 1). In the third treatment (EART), 40-50 g m^{-2} of live earthworms (*Eisenia foetida*) were added to the plot to feed on the 10 kg of pruning residues. Zinc sheets, buried along the plot edges to 20 cm depth without disturbing the soil, were used to attempt to keep earthworms within the plot. Nevertheless, during the second month it was observed that most of the earthworms had died or escaped from the plots. In the sixth month of the field trial, 40-50 g m^{-2} of live earthworms were nursed under cover and fed the 5.0 Mg ha^{-1} of pruning residues. Two months later, the earthworms and the partially decomposed material were transferred to the corresponding plots. The doses and preparation methods for the EART treatment were modified from the usual recommendations by "Lombritica" [+], a private earthworm farm in Cartago, Costa Rica. The RESID treatment only received the grossly chopped tree pruning residues, distributed without any other treatment, imitating normal management practice of organic coffee farms in the region. An absolute bare soil control plot (BARESO) was left within each block. In Pejivalle, the experimental plots (blocks) were arranged in lines across the slope to avoid interference or contamination due to the steepness of the slope.

In May 2004, a second field trial was set up in the same organic coffee plantation at CATIE that was used in the original 330-day field trial in 2002. The main goal of this new experiment was to observe the effect of residue additions on SD fractions over 105 days with less time between sampling dates. The BARESO, MICROB and RESID treatments

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[+] Lombritica S.A. Km 35 Carretera Interamericana Sur, Cartago, Costa Rica.

were tested using a randomized complete block design with four replicates. Treatments were prepared and applied in the same way as in the previous field trial. New plots (4 m^2) were marked and cleared of litter and weeds in the second week of May 2004 (two months before residue application). A 6 m^2 plastic shade net (60% shade) was placed 2 m over the plots to homogenize the sunlight the plots received. A surrounding ditch (30 cm wide and 25 cm deep) was dug around each plot to prevent flooding during heavy rainfall. Three more 4 m^2 plots were marked but not cleared to maintain them in undisturbed farm conditions. The plots were sampled every 15 days (six dates including the baseline date; samples could not be taken at day 30) to compare the effects of treatments in the trial to normal farm conditions. Litter fall that was trapped in the shade net of these three undisturbed plots was gathered and scattered on the plots.

4.2.3. Soil sampling

In the 2002 trials, bulk soil samples from four cores from the center of each plot were taken at 0-5 cm depth with an helicoidal iron auger (5 cm in diameter) one week before the first addition of pruning residues (refrigerated at 4 °C until analysis). At 90, 180 and 330 days after the first residue addition, ten cores per plot were taken and mixed in a bulk sample, following a concentric pattern from the external area to the center of the 4 m^2 plot to avoid sampling the same points in different months. Before sampling, all litter, weeds and mulch were removed from the soil surface to expose mineral soil.

In the 2004 field trial, samples were taken at 0 (baseline data, 2 days before residue application on July 12th), 15, 30, 45, 60, 75, 90 and 105 days after residue application. Sampling followed the same procedure as in the 2002 field trial; i.e. an iron auger was used to obtain 10 cores per sampling date from the 0-5 cm layer following a concentric pattern. A wooden stick was placed in each sampling point to avoid sampling the same point twice.

4.2.4. Size-density fractionation

The method used for size-density fractionation of SOM in both the 2002 and 2004 field trials was based on Meijboom *et al*. (1995). Fresh bulked soil samples were mixed thoroughly with a rotary mixer for 30 minutes before separating gravel and roots using a 8 mm stainless sieve. Ten grams of fresh soil were dried at 105 °C to calculate gravimetric moisture of the bulked soil samples. A sub-sample of fresh soil (100 g) was put on the uppermost of three stacked 2000, 250 and 150 μm stainless steel sieves and washed using tap water until all clay, silt and fine organic materials were separated and the water passing through the sieve became clear. Small stones and roots were collected from the 2000 μm sieve and weighed for adjusting the dry weight of each sample. The remaining materials on the 250 and 150 μm sieves [*] were washed into a 3 liter bucket and swirled around for decantation; this suspended material was separated from the mineral material which immediately sank to the bottom of the bucket. This organic material was referred to as macroorganic matter. The macroorganic matter was fractionated in Ludox^{TM} (Du Pont), an aqueous colloidal dispersion of silica particles in dilute NaOH solution (pH 9.1). The fractionation followed two main steps. First, a heavy fraction (HF) was obtained when the macroorganic matter was put in Ludox^{TM} with a density of 1.30-1.37 g cm^{-3}. The mixture was swirled around and then after standing for 3-4 minutes, a floating fraction and a settling "heavy" fraction were recovered. The HF floats in water but sinks in Ludox^{TM}. It can be separated because it is more humified and is associated with mineral components of soil, and hence is denser than the less decomposed LF and MF that are mineral-free (Strickland and Sollins 1987, Barrios *et al* 1996a). This operation was repeated three times until the amount of floating material was negligible. The resulting floating material was put in Ludox^{TM} with a density of 1.13 g cm^{-3}. The same procedure described above produced two new fractions; a settling "medium" fraction (MF) and a floating "light" fraction (LF). The fine roots were separated manually from the LF material and weighed to correct the dry weight of this fraction. All fractions were washed thoroughly with tap water, dried at 55 °C for 48 hours and weighed. Finally, the dried material was ground in ceramic mortars and

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[*] Meijboom *et al.* (1995) defined 150 μm instead of 53 μm as the limit for obtaining the size density fractions in order to hasten the washing procedure by avoiding clogging of the sieves. The 250 μm sieve was only used for hastening the washing procedure.

analyzed for total C and N using a Thermofinigan analyzer (Flash EA 1112 Thermoquest, Milan Italy). Total C for the four experimental dates in the 2002 field trial was also measured using the same equipment.

4.2.5 Size fractionation of SOM

In the 2004 trial, samples from the baseline, 75 and 105 days after residue application also were fractionated following the method proposed by Cambardella and Elliot (1992). Ten grams of fresh soil were dried at 105 °C to calculate gravimetric moisture. A fresh soil sub-sample (12.5 g) from the 0-5 cm depth layer were taken from pre-homogenized field samples. The sub-samples were air dried, passed through a 2 mm iron sieve and 50 ml of sodium hexametaphosphate (5 g l^{-1}) were added to the material that passed through the sieve (< 2 mm). The mixture was shaken for one hour in a horizontal reciprocal shaker at 120 rpm to disperse the macro and micro soil aggregates.

After shaking the samples, the soil was wet-sieved using distilled water and a 53 µm iron sieve. The remaining material in the sieve was refereed to as Particulate Organic Matter (POM). The fine fraction (less than 53 µm) that included clay and silt was collected in previously weighed beakers and POM in separate beakers. Both fractions were oven-dried at 55 °C and weighed. The sand size fraction (53 µm to 2 mm) was analyzed for total C concentration, and the amount of C in 100 g of dry soil was calculated to be compared with C amounts in SD fractions.

4.2.6. CO$_2$ production from plant residues

In 2004, an *in vitro* lab test was performed to compare respiration rates of the RESID, MICROB and BARESO treatments using a modified substrate induced respiration (SIR) method described by Cheng and Coleman (1989). *Erythrina poeppigiana* pruning residues from both MICROB and RESID were taken randomly from the field plots of the SOM fractionation experiment and tested 15 and 70 days after spraying the microbial mixture. Pruning residues sub-samples of each treatment (5 g DM) were tested using the SIR method. The pruning residue samples were placed in one-liter flasks and 0.4 g of

glucose powder was added to stimulate microbial activity. Pruning residue moisture levels were similar between treatments and the materials were mixed with soil in the flasks. Then, the flasks were left undisturbed for 30 minutes and then connected to a constant flow of air, free of CO_2 (2-3 l h^{-1}). The air expelled from the flasks was directed to NaOH traps for one hour (from time 30 minutes). Three empty flasks were also connected to the NaOH traps during the same time (laboratory controls). The solution in the traps was transferred to beakers, and 6 ml of $BaCl_2$ (0.2 N) was added as well as two drops of phenolphthalein as an indicator. The $BaCl_2$ was used to precipitate the calcium carbonate present in the traps. The solution was treated with HCl (0.546 N). The amount of HCl consumed in the titration was subtracted from that consumed by the controls (average of the three empty flaks) and then multiplied by the molarity of HCl and by 6 (mg CO_2 –C/meq of H$^+$) (Vandevivere and Ramírez 1995).

4.2.7. Microbial biomass

In 2002, microbial biomass was measured at 90 and 330 days in CATIE using the chloroform fumigation-incubation technique adapted from Anderson and Ingram (1993). After fumigating a subsample of soil with $CHCl_3$ for five days, fumigated and non-fumigated soil was extracted with K_2SO_4 (0.5 M) and soluble organic C in the extracts was measured using the Nelson and Sommers (1996) method modified for extracts. Microbial biomass-C was calculated as the difference between the fumigated and non-fumigated extracts.

4.2.8. Statistical analysis

Carbon content in the size-density fractions from each site was analyzed with ANOVA as a randomized complete block split-plot design (with dates as the subtreatment). Total C, C content in the size-density fractions, macroorganic matter dry weight, C contents in POM and microbial biomass from each treatment were also compared at each experimental time using ANOVA as a randomized complete block design. Where the analyses showed significance at the $p < 0.05$ level, Duncan tests were used for mean comparisons (GLM procedure, SAS Institute 1999). The CO_2 measurements were considered as a subsidiary unreplicated test.

4.3. Results

4.3.1. Dynamics of SOM fractions

4.3.1.1. Dynamics of C concentrations and C contributions to SOM in Size-density
(SD) fractions

A previous analysis of C concentrations and characteristics of the three SD fractions can help to understand their dynamics. In the 2002 field trial, C concentrations were highest in the light fraction (LF) and much lower in the heavy fraction (HF), with consistently higher values for the Pejivalle sites for all fractions (Table 2). Values for a given site and fraction were similar for different dates; no temporal trends were observed. The LF (<1.13 g cm^{-3}), examined under a stereoscope (18×), showed a characteristic light brown color and the residues had an "intact" appearance (Appendix 2). In the medium fraction (MF; >1.13 and < 1.37 g cm^{-3}), a darker brown color was observed and residues appeared to be more damaged than in the light fraction. The HF (>1.37 g cm^{-3}) had the darkest brown color and residues were not recognizable under the microscope. Mineral particles contaminated the amorphous organic matter residues in the HF.

Table 2. Carbon concentration (average of all treatments) in the light (LF), medium (MF) and heavy (HF) fractions of macroorganic matter (>150 µm) from the organic coffee plantations in CATIE and Pejivalle, Costa Rica, 2002

| Sampling dates (days) | Carbon concentrations in size-density fractions of macroorganic matter | | | | | |
| | LF (%) | | MF (%) | | HF(%) | |
	CATIE	Pejivalle	CATIE	Pejivalle	CATIE	Pejivalle
Time 0 (0) [1]	35.2 (1.1)[2]	39.3 (0.6)	32.6 (0.8)	32.0 (0.8)	6.5 (0.5)	8.6 (0.8)
Time 1 (90)	35.0 (0.8)	35.2 (1.0)	27.2 (0.6)	30.4 (1.0)	8.9 (0.5)	10.7 (0.7)
Time 2 (180)	36.4 (1.3)	41.4 (0.8)	28.6 (1.1)	37.3 (0.6)	6.7 (0.7)	7.8 (0.9)
Time 3 (330)	38.1 (0.4)	38.3 (0.3)	30.3 (0.9)	32.0 (0.5)	6.0 (0.8)	7.6 (0.8)
Average	36.2 (0.7)	38.6 (1.3)	29.7 (1.1)	32.9 (1.5)	7.0 (0.6)	8.7 (0.9)

[1] Days after the first application of *Erythrina poeppigiana* pruning residues
[2] Standard error ($n = 15$)

In the 0-5 cm soil depth, the total soil C concentrations were 5.0 and 4.7% (Table 2, Chapter III) for CATIE and Pejivalle, respectively. The contribution of LF-C to total soil C in CATIE ranged from 0.9 to 1.9% and in Pejivalle from 1.7 to 2.9%. In 2004, similar characteristics and C concentrations were found for the three SD-fractions.

At CATIE in 2002, contributions by each of the three fractions to soil C contents (g C kg^{-1} soil) were very similar for any given sampling time because although C concentrations in HF was much lower, this was compensated by much higher weights of HF compared to LF and MF (Figure 4A). However, values for all three fractions at 180 and 330 days were lower than for 0 and 90 days ($p<0.01$). In Pejivalle, at 90 days, C contents of the three fractions were similar, but at 180 and 330 days, carbon contents of LF and MF were higher than HF (Figure 4B). As in CATIE, C contents at 180 and 330 days were lower than for 0 and 90 days ($p<0.05$).
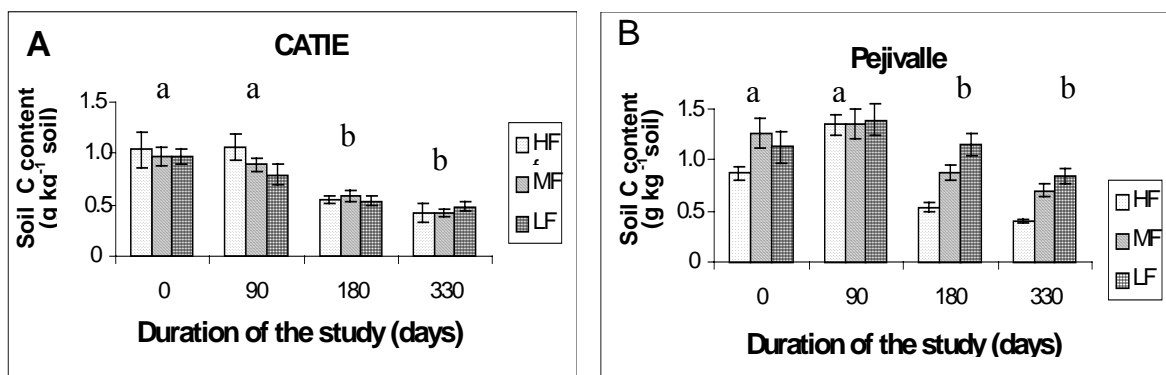
Figure 4. Carbon contents (average of all treatments) in three size-density fractions (LF, MF and HF) in organic coffee plantations in CATIE and Pejivalle, Costa Rica, 2002-2003. Same letters between different times indicate that the average of the three size-density fractions in each time are not significantly different

At the end of the 2002 experiment (330 days after the first addition of pruning residues), the average soil C content provided by each fraction, for all treatments, had decreased by 60, 57 and 50% (with respect to the initial values at 0 days) at CATIE and by 54, 45 and 26% at Pejivalle for HF, MF and LF, respectively ($p<0.01$). In this period, macroorganic matter C (sum of the C provided by these three size-density fractions) decreased by 56% at CATIE and by 41% at Pejivalle (Appendices 3 and 4). The soil carbon contents provided by HF, MF and LF, calculated for each treatment during the 330 day experiment, mostly increased from 0 to 90 days, decreased abruptly between 90 to 180 days and then decreased gradually from 180 to 330 days (Figure 5). In CATIE, the LF-C in the bare soil plots showed a consistent declining trend, and at the end of the experiment,
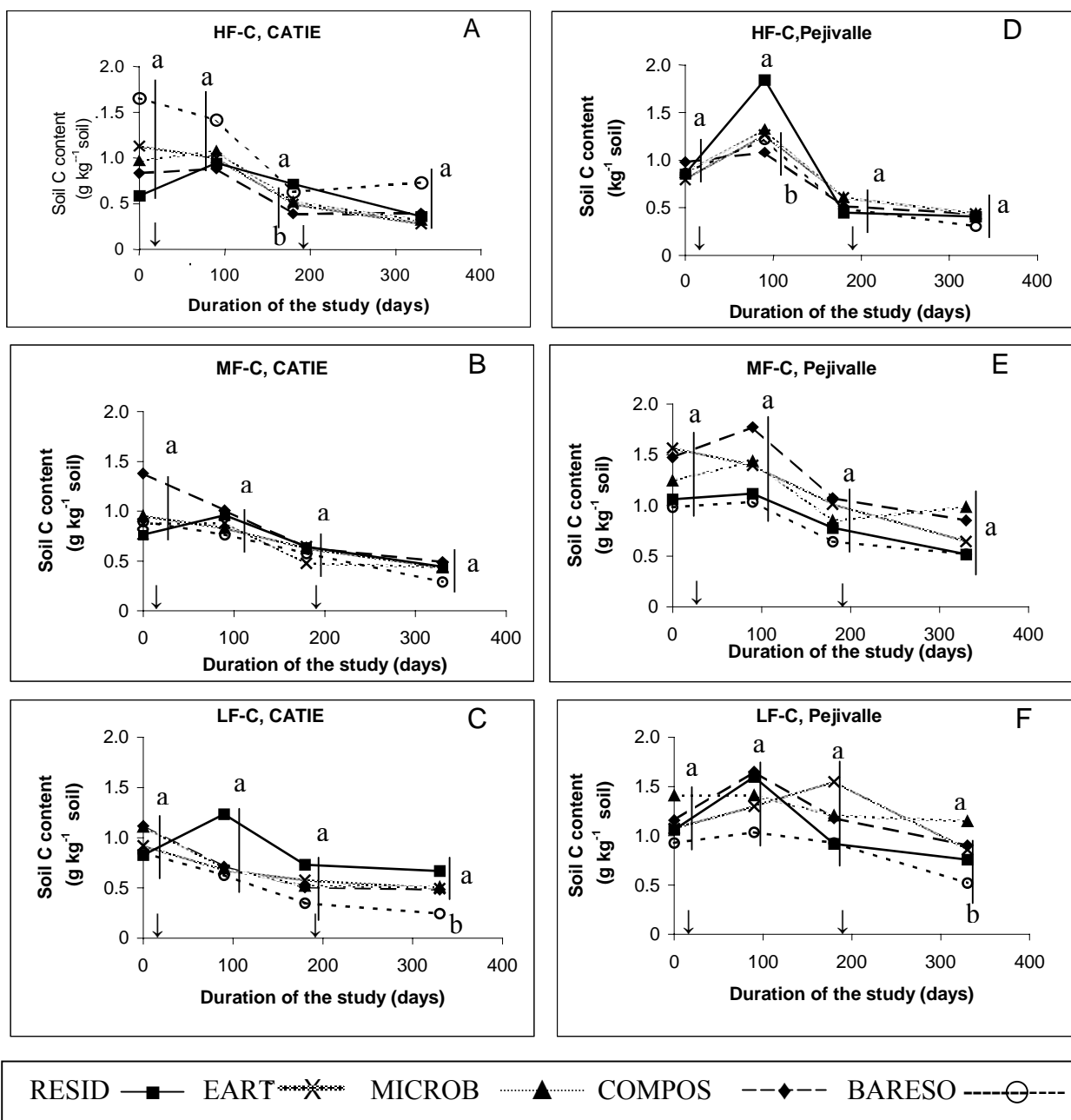
Figure 5. Soil carbon content provided by the heavy (HF), medium (MF) and light (LF) size-density fractions in CATIE (A, B and C) and Pejivalle (D, E and F), Costa Rica, 2002 at 0, 90, 180 and 330 days after initial application of pruning residues of *Erythrina poeppigiana*. Treatments were: RESID, addition of 10 Mg ha^{-1} yr^{-1} of pruning residues only; EART, 10 Mg ha^{-1} yr^{-1} of pruning residues + two times 40-50 g m^{-2} live earthworms; MICROB, 10 Mg ha^{-1} yr^{-1} of pruning residues + microbial mixture "A"; COMPOS, composting of 10 Mg ha^{-1} yr^{-1} of pruning residues with microbial mixture "B"; BARESO, bare soil. The arrows (↓) indicate the moment of residue addition. Within each sampling date, site and fraction, values with the same letter are not significantly different (Duncan $p<0.05$)

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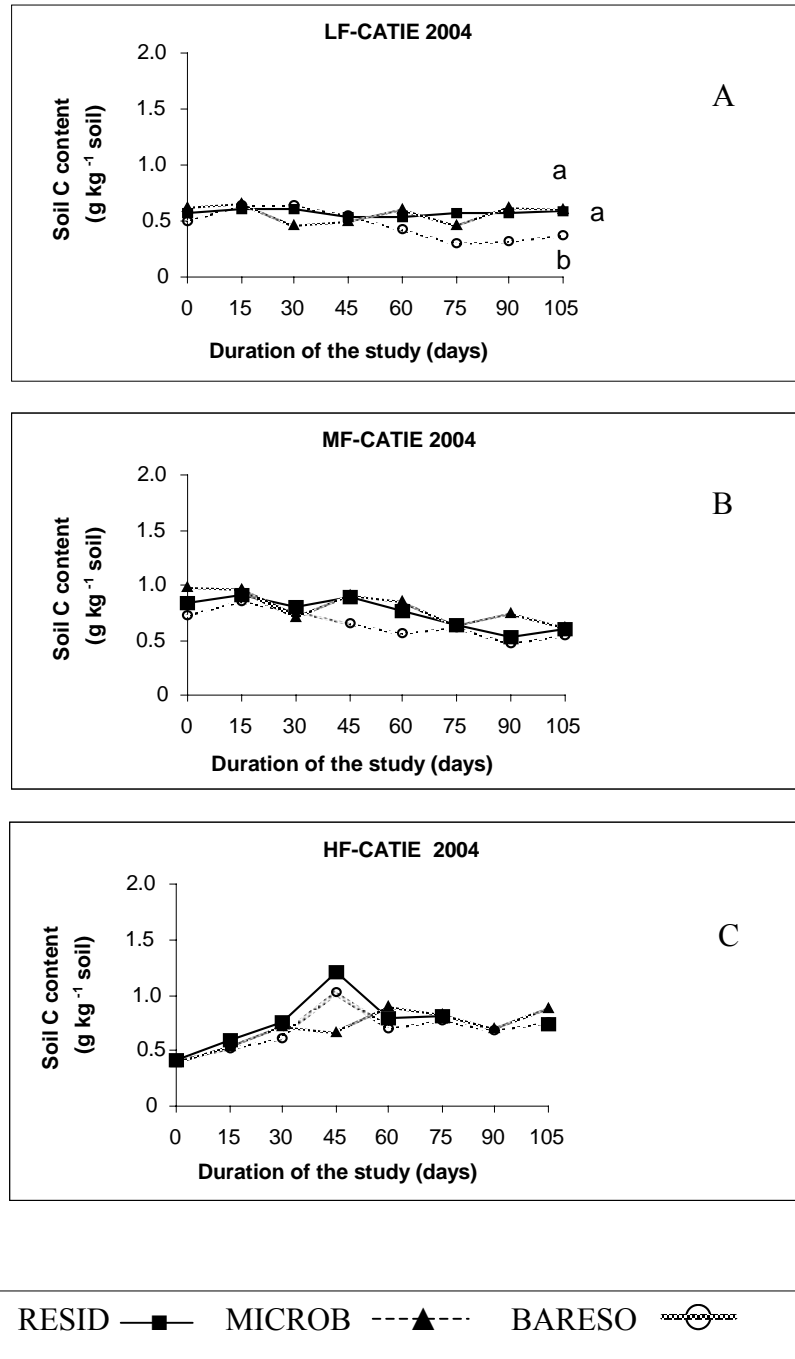
Figure 6. Soil carbon content provided by the light (LF), medium (MF) and heavy (HF) size-density fractions in CATIE, 2004 (A, B and C) during 105 days after application of pruning residues of *Erythrina poeppigiana*. Treatments were: RESID, addition of 10 Mg ha^{-1} yr^{-1} of pruning residues only; MICROB, 10 Mg ha^{-1} yr^{-1} of pruning residues + microbial mixture; BARESO, bare soil. Values with the same letter are not significantly different (Duncan $p<0.05$)

it was 32% of the original value (Figure 5C). In Pejivalle, LF-C in the bare soil plots did not show any decrease during the first 180 days, but after 330 days, the remaining LF-C was only 49% of the original value (Figure 5F).

In the 2004 trial, the line that described LF-C values under RESID was stable during the 105 days of the experiment (Figure 6A). The MICROB line showed fluctuations from day 30 to day 90 but ended (105 days) with a similar value to RESID; the values for RESID and MICROB LF-C at day 105 (0.60 g kg^{-1} soil) were similar to values at 0 days (baseline data). Under BARESO, LF-C showed a decreasing trend from 30 days to 105 days. At this final date, values were 35% lower than values at 0 days. The average for the three treatments at 90 days (0.57 g kg^{-1} soil) was similar to the baseline average (Figure 6A). The amount of C in MF showed a decreasing trend for all the treatments (Figure 6B). The average of the three treatments at day 105 was 33% lower than the average at day 0 (0.08 vs. 0.06 g kg^{-1} soil, $p<0.05$). An increasing trend was observed for the three treatments in HF-C during the experiment (Figure 6C). The average for the three treatments at 105 days was double the baseline average (0.08 vs 0.04 g kg^{-1} soil, $p<0.05$).

In 2004, size-density fractions were monitored in undisturbed plots, to obtain subsidiary information, at six dates during the experiment (Appendix 5). After day 15, a trend to lower values was observed for LF and MF. At 105 days, LF-C and MF-C were 3 and 1.7 fold lower, respectively, than values at 0 days. In contrast, HF-C showed more stable values (except for a possible anomalous peak at 90 days). Final HF-C was 28% lower than the value at 0 days. No statistical analyses could be done because the plots were out of the original experimental design.

4.3.1.2. Total C, macroorganic matter and POM dynamics

No clear trend was found for total soil C in the 2002 trial at CATIE[*] through the four sampling dates (Table 3). Values at 180 and 330 days were not significantly different from the baseline for all four treatments that received pruning residues as well as for the control. It was noticeable that total C under BARESO after 330 days was almost the same as at 0 days. In that year, the amount of C in macroorganic matter (>150 µm; i.e. the sum of C in the three fractions), reduced from 3.0 ± 0.2 (0 days) to 1.3 ± 0.1 (330 days) g kg^{-1}soil in CATIE, and from 4.1 ± 0.3 (90 days) to 1.9 ± 0.1 (330 days) g kg^{-1}soil in Pejivalle (Appendices 3 and 4).Thus in CATIE, macroorganic matter C represented between 2.6 and 5.9% of total soil C in the overall average across all treatments. In Pejivalle, macroorganic matter C, averaged over all treatments, represented between 4.1 and 9.0% of total soil C.

Dry weight of macroorganic matter (average of all the treatments) showed a declining trend over time in both sites from day 90 onward. In CATIE, significant differences were only found between 0 and 180-330 days (Table 4). At Pejivalle, the average of treatments at 90 days was higher than the baseline (0 days). Averages at 180 and 330 days were lower than at 90 days but no differences were found between these dates nor with initial values. These results were similar to those observed in 2002 for average of treatments in the three size-density fractions (Table 5, Figure 4). In 2004, the average for the treatments of dry weight of macroorganic matter showed a significant increase from day 0 to day 15 [**](Table 6). After that date, similar values were measured until day 105 (one possible anomalous value at day 90). It was noticeable that at 105 days macroorganic dry weight under BARESO was 46% higher than at day 0. When the alternative size particle fractionation method was applied at three different sampling times, amounts of C (average over treatments) in POM (>53 µm) at 75 days were significantly higher than the baseline and higher than values at 105 days (Table 7). In the split-plot analysis for the treatments, no differences were detected for BARESO between the baseline and 105 days.

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[*] Total C was only analyzed for CATIE in 2002, since the results in Pejivalle seemed to be affected by steep slope.
[**] Initial values in 2004 were lower than initial values in 2002 because plots in 2004 were cleared of litter during a longer period before pruning residue applications.

Table 3. Total soil C (%) in CATIE, Costa Rica, 2002 at 0, 90, 180 and 330 days after initial application of pruning residues of *Erythrina poeppigiana*. Treatments were: RESID, addition of 10 Mg ha^{-1} yr^{-1} of pruning residues only; EART, 10 Mg ha^{-1} yr^{-1} of pruning residues + two times 40-50 g m^{-2} live earthworms; MICROB, 10 Mg ha^{-1} yr^{-1} of pruning residues + microbial mixture "A"; COMPOS, composting of 10 Mg ha^{-1} yr^{-1} of pruning residues with microbial mixture "B"; BARESO, bare soil

	Time after residue application (days)							
	0		90		180		330	
Treatments	Mean	Std. Error	Mean	Std. Error	Mean	Std. Error	Mean	Std. Error
COMPOS	4.59	(0.27)[1]	4.08	(0.28)	4.28	(0.18)	4.37	(0.16)
EART	4.70	(0.24)	4.17	(0.08)	4.34	(0.05)	4.35	(0.07)
MICROB	4.36	(0.06)	3.93	(0.14)	4.42	(0.11)	4.25	(0.13)
RESID	4.57	(0.38)	4.38	(0.21)	4.74	(0.38)	4.81	(0.35)
BARESO	4.52	(0.35)	3.94	(0.27)	4.42	(0.26)	4.47	(0.25)
Average of treatments	4.55	(0.06)	4.10	(0.08)	4.44	(0.08)	4.45	(0.10)

[1] Standard error ($n = 3$)

Table 4. Mean dry weight (g 100g^{-1} of soil) of macroorganic matter (>150 μm) in CATIE, Costa Rica, 2002 at 0, 90, 180 and 330 days after initial application of pruning residues of *Erythrina poeppigiana*. Treatments were: RESID, addition of 10 Mg ha^{-1} yr^{-1} of pruning residues only; EART, 10 Mg ha^{-1} yr^{-1} of pruning residues + two times 40-50 g m^{-2} live earthworms; MICROB, 10 Mg ha^{-1} yr^{-1} of pruning residues + microbial mixture "A"; COMPOS, composting of 10 Mg ha^{-1} yr^{-1} of pruning residues with microbial mixture "B"; BARESO, bare soil

	Time after residue application (days)			
	0	90	180	330
Treatments				
COMPOS	2.03(0.09)[1]	1.40(0.18)	1.03(0.24)	1.22(0.54)
EART	2.24(0.55)	1.87(0.52)	1.46(0.60)	1.06(0.54)
MICROB	2.32(0.51)	1.79(0.39)	1.38(0.39)	1.18(0.52)
RESID	1.54(0.30)	1.62(0.56)	1.51(0.22)	1.17(0.63)
BARESO	2.99(0.76)	2.44(0.61)	1.16(0.19)	1.39(0.44)
Mean	2.22(0.23)a[2]	1.82(0.17)ab	1.31(0.09)bc	1.21(0.05)c

[1] Standard error ($n = 3$)
[2] Values with the same letter within the row for means are not significantly different ($p<0.05$)

Table 5. Mean dry weight (g 100g $^{-1}$ of soil) of macroorganic matter (>150 µm) in Pejivalle, Costa Rica, 2002 at 0, 90, 180 and 330 days after initial application of pruning residues of *Erythrina poeppigiana*. Treatments were: RESID, addition of 10 Mg ha^{-1} yr^{-1} of pruning residues only; EART, 10 Mg ha^{-1} yr^{-1} of pruning residues + two times 40-50 g m^{-2} live earthworms; MICROB, 10 Mg ha^{-1} yr^{-1} of pruning residues + microbial mixture "A"; COMPOS, composting of 10 Mg ha^{-1} yr^{-1} of pruning residues with microbial mixture "B"; BARESO, bare soil

	Time after residue application (days)			
	0	90	180	330
Treatments				
COMPOS	2.05(0.39) [1]	2.02(0.07)	1.58(0.52)	0.96(0.06)
EART	1.80(0.63)	2.16(0.61)	2.42(0.87)	1.77(0.43)
MICROB	1.68(0.58)	1.96(0.22)	1.60(0.41)	1.85(0.87)
RESID	2.96(1.10)	3.26(0.55)	1.25(0.29)	1.68(0.89)
BARESO	1.44(0.27)	1.74(0.35)	1.02(0.23)	0.65(0.04)
Mean	1.99(0.59)a [2]	2.23(0.60)b	1.58(0.53)a	1.38(0.54)a

[1] Standard error ($n = 3$)
[2] Values with the same letter within the row for means are not significantly different ($p<0.05$)

Table 6. Mean dry weight (g 100g $^{-1}$ of soil) of macroorganic matter (>150 µm) in CATIE, Costa Rica, 2004 at 0, 15, 30, 45, 60, 75, 90 and 105 days after initial application of pruning residues of *Erythrina poeppigiana*. Treatments were: RESID, addition of 10 Mg ha^{-1} yr^{-1} of pruning residues only; MICROB, 10 Mg ha^{-1} yr^{-1} of pruning residues + microbial mixture; BARESO, bare soil

	Time after residue application (days)							
	0	15	30	45	60	75	90	105
Treatments								
BARESO	0.70(0.07) [1]	1.23(0.25)	1.19(0.21)	1.40(0.24)	0.97(0.15)	1.02(0.06)	1.81(0.20)	1.02(0.08)
MICROB	0.71(0.08)	0.92(0.11)	1.12(0.07)	1.40(0.35)	1.02(0.10)	1.00(0.14)	1.90(0.21)	0.94(0.08)
RESID	0.69(0.05)	1.28(0.26)	1.09(0.09)	0.96(0.10)	1.08(0.26)	0.99(0.22)	1.93(0.22)	1.10(0.08)
Mean of treatments	0.70(0.01)c [2]	1.15(0.11)b	1.14(0.03)b	1.25(0.15)b	1.02(0.03)b	1.01(0.01)b	1.88(0.04)a	1.02(0.05)b

[1] Standard error ($n = 3$)
[2] Values with the same letter within a row are not significantly different ($p<0.05$)

Table 7. Amount of C (g 100g^{-1} soil) in POM (>53 µm) obtained with the size fractionation method (Cambardella and Elliot 1992) at the organic coffee farm at CATIE, Costa Rica, 2004.

	Time after residue application (days)		
Treatments	0	75	105
BARESO	1.54 (0.16)[1]b[2]	1.73 (0.22)b	1.55 (0.29)a
MICROB	2.39 (0.38)a	2.51 (0.43)a	2.14 (0.47)a
RESID	1.89 (0.29)b	2.44 (0.32)a	1.85 (0.35)a
Avg over Treatments	1.94 (0.25)A[3]	2.23 (0.25)B	1.85 (0.17)A

[1] Standard error ($n = 3$)
[2] Values with the same letter within a column are not significantly different. Duncan Test $p<0.05$
[3] Values with the same capital letter within a row are not significantly different. Lsmean-test $p<0.05$

4.3.2. Effects of treatments on SOM fractions

4.3.2.1 Effects of treatments on each of the size-density (SD)fractions

In 2002, at CATIE, C content in the LF for the four treatments showed a trend of higher values than the bare soil control ($p<0.01$) 90, 180 and 330 days after the application of pruning residues. When values for LF-C for each date were analyzed separately, under RESID 90 days after the initial addition of residues, there was a consistent increase in soil C content at both sites. Although not significant, this increment was particularly noticeable for LF-C at CATIE (+48%). In contrast, for the same sampling date in CATIE, the microbial (MICROB and COMPOS) and earthworm (EART) treatments showed, on average, an LF-C decrement of 33% (Figure 5C). Carbon content provided by the LF at CATIE, for the four pruning residue treatments, was always higher than bare soil, but the difference was only significant 330 days after the first residue application (Figure 5C). Under RESID after 330 days the percentage of initial soil C provided by LF was double the value for the two microbial treatments and bare soil (Figure 7). However, the differences were not significant due to high variability under the earthworm treatment. Likewise, in

Pejivalle, the LF-C content of bare soil was always the lowest. Significant differences between the earthworm and the two microbial treatments were not found in either CATIE or Pejivalle for the LF-C content for any of the four sampling dates. In the 2002 field trial, the impact of treatments that enhanced microbial activity on LF-C were more apparent in CATIE. At Pejivalle, it is possible that the steep slope affected the amount of C in the lighter fractions (LF and MF) due to soil material movement down the slope, and only the least mobile HF showed some of the effects observed in CATIE.

Using the individual values for the five treatments and the three replicates, a significant negative correlation ($p<0.05$ and $r = -0.84$) between microbial biomass and the amount of C in the LF was detected in CATIE at 90 days but after 330 days, the correlation was not significant (Appendix 6). Correlations of the MF, HF and macroorganic matter C with microbial biomass at 90 and 330 days were not significant.
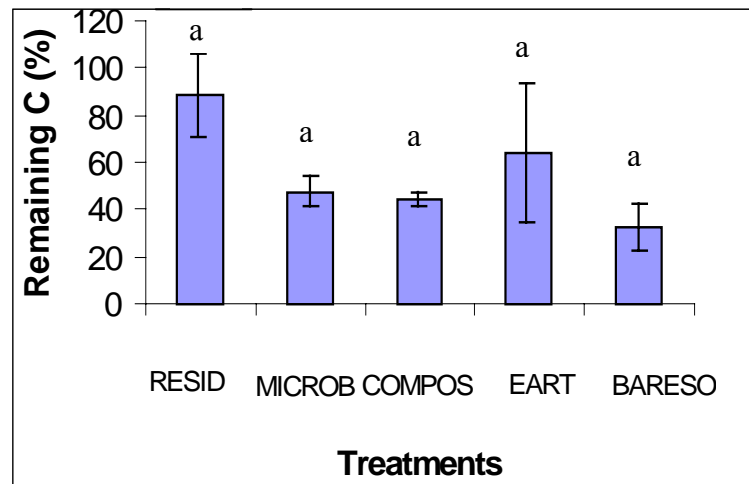
Figure 7. Percentage of initial soil LF-C values 330 days after application of *Erythrina poeppigiana* pruning residues in CATIE, Costa Rica, 2002. Treatments were: RESID, addition of 10 Mg ha^{-1} yr^{-1} of pruning residues; EART, 10 Mg ha^{-1} yr^{-1} of pruning residues + two times 40-50 g m^{-2} live earthworms; MICROB, 10 Mg ha^{-1} yr^{-1} of pruning residues + microbial mixture "A"; COMPOS, 10 Mg ha^{-1} yr^{-1} of pruning residues + microbial mixture "B"+ turnover of the residues; BARESO, bare soil. Standard error bars are presented for *n* = 3. Columns with the same letter are not significantly different (Duncan *p<0.05*)

In the 2004 trial, the amount of C in LF showed significant differences between treatments only at 105 days ($p<0.05$, Figure 6A). On this date, carbon amounts in LF for RESID and MICROB were 38% higher than for the control. No significant differences were found between MICROB and RESID during the experiment. At days 75 and 90, the trend of higher values for MICROB and RESID in comparison to BARESO also was observed but it was not significant.

In 2002, in both sites, the MF-C content did not vary between treatments at any time (Figures 5B and 5E). Likewise, there were no significant differences between treatments in the percentage of initial C remaining in the MF after 90, 180 and 330 days. Consistent downward trends were observed for MF-C content for all the treatments in both sites over time with the exception of Pejivalle at 90 days, when an increase for all the treatments was observed. In 2004, the three treatments had similar C amounts in MF except for two sampling dates (Figure 6B); when the BARESO plots had lower average values than RESID and MICROB at 45 and 60 days but no significant differences were detected.

In 2002, an initial increase in HF-C was observed at 90 days for all treatments on both sites. At this date, increases in HF-C contents under untreated residues (RESID) appeared to be greater than under the rest of the treatments. This increment was extreme for RESID at Pejivalle (116%); in contrast, at this site, the other three treatments only increased by 40% after 90 days (Figure 5D; $p<0.10$). In CATIE, initial HF-C was highly variable before any treatments were applied (time 0 days) affecting the sensitivity of the study. However, as in Pejivalle, at 90 days RESID HF-C showed a larger increase compared to the rest of the treatments. After 180 days, RESID HF-C at CATIE was higher than the other treatments (Figure 5A) and the percentage of initial C remaining in HF after 180 days was more than double the values for the earthworm and the two microbial treatments (Figure 8). In both sites there were consistent downward trends and similar values for all the treatments and the control 180 and 330 days after initial residue addition. No differences were detected between microbial and earthworm treatments for HF-C contents at any time. In 2004 a peak for RESID and BARESO HF-C (not significant) was

observed at 45 days. At the other sampling dates, the three treatments showed similar values (Figure 6C).
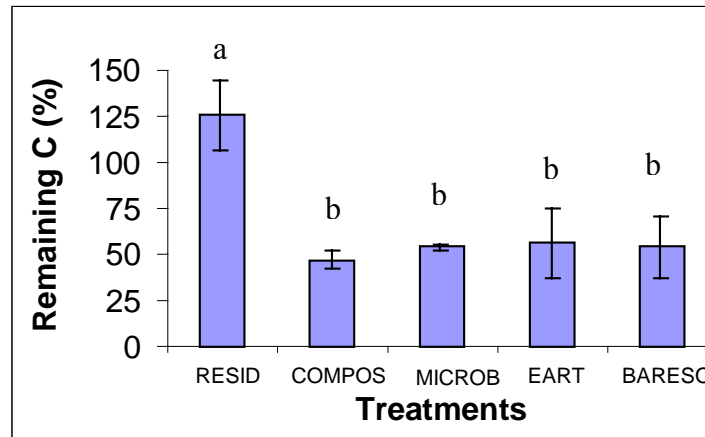
Figure 8. Percentage of initial soil HF-C values 180 days after application of *Erythrina poeppigiana* pruning residues in CATIE, Costa Rica, 2002. Treatments are: RESID, addition of 10 Mg ha^{-1} yr^{-1} of pruning residues; EART, 10 Mg ha^{-1} yr^{-1} of pruning residues + two times 40-50 g m^{-2} live earthworms; MICROB, 10 Mg ha^{-1} yr^{-1} of pruning residues + microbial mixture "A"; COMPOS, 10 Mg ha^{-1} yr^{-1} of pruning residues + microbial mixture "B"+ turnover of the residues; BARESO, bare soil. Standard error bars are presented for *n* = 3. Columns with the same letter are not significantly different (Duncan *p*<0.05)

In 2004, respiration rates, using the SIR method, were measured for pruning residues collected from each plot, to obtain subsidiary information about microbial activity under RESID and MICROB (Table 8). A trend of higher values (unreplicated test) was observed for MICROB than RESID 15 day after spraying the microbial mixtures. For the second measurement (day 70), similar values were recorded for MICROB and RESID. Soil respiration values for BARESO at day 15 were low and similar to the values obtained in other trials for CO_2 production using *E. poeppigiana* pruning residues reported in Chapter V.

Table 8. Production of CO_2 ($mgCO_2$ $100g^{-1}$ h^{-1}) from *E. poeppigiana* pruning residues (unreplicated test), Costa Rica, 2004. Treatments were: MICROB, 12.5 g of fresh residues added with 50 ml 10 kg $^{-1}$ of microbial mixture; RESID,12.5 g fresh pruning residues only.

	Time after residue application (days)	
	15	70
Treatments		
MICROB	6.33(1.07)[1]	1.90(0.23)
RESID	3.43(0.37)	2.05(0.49)

[1] Standard error for laboratory replicates ($n = 3$)

4.3.2.2. Effects of treatments on total C, macroorganic matter and POM dynamics

In 2002, although not significantly different, there was a consistent trend of higher total C under RESID in comparison with the rest of the treatments in 2002 after the addition of pruning residues (90, 180 and 330 days, Table 3). No significant effects of treatments on the dry weight of macroorganic matter were detected in 2002 or 2004 in CATIE (Tables 4 and 6). In Pejivalle, higher values under RESID observed after 90 days (Table 5). The analysis of the amount of C in macroorganic matter (the sum of C in the three density fractions) in either CATIE or Pejivalle showed no significant differences between treatments for any of the four sampling dates. A comparison of the reduction in macroorganic carbon (% of initial values) at day 330 did not show any significant differences for any of the treatments. Nevertheless, in CATIE, at 90 and 180 days, RESID tended to show higher values in comparison with the rest of the treatments. At 330 all the treatments showed similar values (Appendix 7).

The overall carbon percentage in the POM fraction (>53 µm) was 4.52 ± 0.11%. Carbon concentrations of POM were similar at the three sampling dates. Significant differences between treatments were found for the amounts of C in POM at the baseline and at 75 days after residue application. At 0 days, MICROB POM-C, was higher than in the other two treatments; and MICROB and RESID POM-C were higher than BARESO. At 105 days, no clear trend for treatments was found (Table 7). In general terms, the amounts of C in the POM fraction were higher than in LF or in the macroorganic fraction.

4.4. Discussion

4.4.1. Size-density fraction effectiveness for reflecting decomposition of labile SOM

4.4.1.1. Characteristics of the light, medium and heavy fractions of macroorganic matter obtained using Ludox™

Carbon concentrations of 37% for the LF, and 32% for the MF, found in a comparison of organic and conventional crop rotations (potatoes, winter wheat and grass clover) in an alfisol in Switzerland (Fließbach and Mäder 2000), were similar to those obtained in the present study (36-39 and 30-33% for LF and MF, respectively). The C concentrations in the present study were higher than those obtained by Barrios *et al*. (1996a) who studied the effects of leguminous tree residues on SD- fractions in a sandy soil in Kenya (20 and 14% for LF and MF, respectively). The present values for C concentrations in the HF (7.0 and 8.7% in CATIE and Pejivalle, respectively, Table 2) were lower than Fließbach and Mäder (2000) (10-17%) but similar to Barrios *et al.* (1996a) (8.1%). In the current study, during the decantation phase of fractionation, dense mineral particles were observed in HF. Their presence explains the much lower C concentration for HF compared to MF and LF. The appearance of the three size-density fractions was similar to that described by Meijboom *et al.* (1995).

In the current study, similar contributions to soil C contents, from each of the three fractions, were found at a given site for any given sampling date. In contrast, Hassink (1995) found 4-6 fold more C in HF than in LF after applying 10 Mg ha^{-1} yr^{-1} C inputs of wheat chaff over 25 years. However, Meijboom *et al*. (1995), comparing grassland soils in the Netherlands, also found similar contributions of C from the three fractions. In summary, the C concentration (% C in the fractionated materials) and appearance of the three fractions were different, but the contributions of each fraction to soil C (g C kg^{-1} soil, averaged for all treatments) were similar for any given sampling date at CATIE (Figure 4A). A possible explanation for this absence of differences in the contributions of the three

fractions is that the high quality material of *E. poeppigiana* did not lead to the accumulation of humified material in HF.

The quality of plant residues has been associated with C:N ratios of SD fractions (Phiri *et al.* 2001). In a study on pruning residue decomposition of *Gliricidia sepium*, a legume with similar quality to *E. poeppigiana* (C:N ratio 10-20), Lehmann *et al.* (1995) found a 70% mass loss of leaf residues (when used as mulch) after 40 days. Munguía (2003) used litterbags to measure *E. poeppigiana* leaf litter decomposition during a 213 day period in San Isidro, Costa Rica. He reported a loss of 65% DW of residues after 40 days of exposure on the soil surface probably due to the high quality of these plant materials (lignin:N ratio = 2.76). *E. poeppigiana* pruning residues have low amounts of lignin that can be humified to form HF; and even more, these HF materials could be decomposed over time. In contrast, results from the aforementioned Hassink (1995) study, with a more lignified material (wheat chaff) showed higher accumulation of C in HF after 25 years. Another possible explanation for the lack of differences in C contributions from the three SD fractions measured in the present study is the low density limit used for HF (≥ 1.37 g cm^{-3}). Other authors have used ≥ 1.7 g cm^{-3} as the limit for separating the HF fraction (Cambardella and Elliot 1992). It is possible that for the soil conditions studied, the HF was too similar to LF and MF, and a denser liquid should have been used to obtain wider differences between them.

Percentages of total soil C provided by macroorganic matter (1.2 to 3.0%) at CATIE were similar to those reported by Fließbach and Mäder (2000) (2-3.5% of total C). Pejivalle values (2.1 to 4.3%) were slightly higher, possibly due to contamination of the experimental plots with organic materials due to the laminar erosion from uphill. Contamination between adjacent plots was prevented by their cross slope location. The amount of C in LF has been found to contribute, on average, 1.8% (Hassink 1995) and 1.7% (Barrios *et al.* 1996a) to topsoil C. In the current study, similar results were found in CATIE (0.9 to 1.9%) and Pejivalle (1.7 to 2.9%). Phiri *et al.* (2001) found slightly higher values (3.8%) of topsoil C in LF.

4.4.1.2. Size-density fraction dynamics during the labile SOM decomposition

The averaged C values for the treatments, within each of the three SD fractions, reflected the decomposition process of labile SOM since they showed lower values at 180 and 330 days than at 90 days when the labile SOM was probably less decomposed (Figure 5). The SDF method has been proposed as an alternative to the litter bag approach for studying decomposition of plant residues, since a closer contact between the organic materials and the soil is maintained (Magid *et al.* 1997). In decomposition studies using the SDF method, soil nutrients are available to facilitate the decomposition process and the samples are fully exposed to faunal activity and natural soil moisture. The data obtained in the present study, for soil C content provided by SD fractions during plant residue decomposition, suggested that these fractions can reflect effectively the decomposition of labile SOM in organic farms; when treatments were analyzed separately, the initial increments in soil C content contributed by each fraction indicated that the decomposition process was incomplete 90 days after beginning the experiment. This was consistent with the results of the 2004 trial, particularly for LF-C, when the results for RESID after 105 days were similar to the baseline (Figure 6A). As the labile material was decaying with microbial activity, values at 180 days, before the second residue application, were dramatically lower. This was particularly apparent under RESID which represented the natural decomposition process of pruning residues in organic coffee plantations. After the second application of pruning residues, no temporal differences were found (comparing 180 to 330 days); i.e., the second application of 5 Mg ha^{-1} of residues seemed to stabilize the amount of C in the three fractions, but at a lower level than the initial (0 days) C content (Figure 5). The fact that the final level was lower may have been because the initial labile C pool included partially decomposed natural litter residues from *E. poeppigiana* and coffee (without considering pruning residues), which can total 4 and 5 Mg ha^{-1} yr^{-1} (Fassbender 1993), as well as weed and root residues. During the experiment, new natural litter fall and weed biomass were removed from the plots. These data were complemented when size-density fractions were monitored in undisturbed plots in 2004 (Appendix 5). The baseline data for these undisturbed plots represented the steady state of labile SOM in an organic farm. In these plots, the consequences of not adding pruning or weed residues could be

observed after 105 days when values for LF and MF were 3 and 2 fold lower, respectively, than the baseline. The contributions of pruning and weed residues, tree and coffee bush litter, as well as root litter, are probably all necessary to maintain initial values of labile SOM in organic farms.

A comparison after 330 days between the untreated residues (RESID) and the bare soil control showed how pruning residues contribute to maintaining macroorganic matter C levels. At CATIE, macroorganic matter C under RESID decreased by 32% of the initial value while BARESO decreased by 62%. (Appendix 7A). These figures highlighted the importance of shade tree pruning residue inputs (10 Mg ha^{-1} yr^{-1}; Beer 1988) in organic coffee farms. However, the macroorganic matter C under RESID decreased indicating that shade tree pruning residues alone were not enough to maintain original macroorganic matter C levels. Furthermore, the dramatic decrease of the bare soil LF-C in CATIE (72% of the initial value) after 330 days (Figure 5C) compared to RESID (20%) indicated the large decrements of labile SOM that may occur in full sun coffee farming systems where tree residue and weed inputs are absent. This confirms empirical observations of ICAFE (National coffee institute of Costa Rica) extensionists (Beer 2005). The quantification of weed biomass contributions in organic coffee systems and the usefulness of the SD fractionation approach to measure macroorganic matter C losses in full sun coffee farms appears to be of value for further research.

Between 180 and 330 days, most treatments had similar values for any given fraction because most of the labile materials were consumed (Figure 5). That was expected as a new amount of residues (5 Mg ha^{-1} yr^{-1}) was applied after 180 days. Only very resistant materials remained as shown by the declining rates of C loss between the last two measurement dates. These results are partially in agreement with those of Magid *et al.* (1997) who monitored LF and HF-C during a 20 month decomposition period after 0, 4 or 8 Mg ha^{-1} of rape (*Brassica napus L*) straw were incorporated into the soil. Two main stages were observed: during the first four months, a rapid decrease in the amount of LF-C was observed, but in the last 16 months LF-C decreased more gradually and final values were similar for all treatments. HF-C was not affected by the addition of this straw; this was

explained by the dominance of more recalcitrant native SOM-C in HF. This latter finding contrasts with those from the current study since HF-C (average of treatments) decreased over 330 days in both sites; i.e., 60 and 54% from the initial values at CATIE and Pejivalle, respectively (Figures 4A and B). In addition, in the present study, at both sites HF-C in the bare soil control had a similar decreasing pattern. The BARESO results indicated that without external residue inputs, the amount of soil C contributed by HF decreased similarly to the other fractions, and the impact of more recalcitrant native C was not evident. The differences from Magid *et al.* (1997) could be due to the high lignin:N ratio (27.3) and more recalcitrant characteristics of rape straw residues in comparison with the high quality organic material provided by *E. poeppigiana* pruning residues which have a low lignin:N ratio (2.76) (Munguía *et al.* 2003). On the other hand, Magid *et al.* (1997) found high rates of C losses in LF and MF during the first four months of decomposition which was probably due to the location of the residues (buried as green manure). Holland and Coleman (1987) found that plant residues used as green manures, which are usually buried into the soil, tended to have higher decomposition rates in comparison with mulches. In the current study, at 90 days, in both the 2002 and 2004 trials, most of the treatments showed an increasing or stable trend for LF and MF-C (Figures 5C and F; 6A and B). In contrast, HF-C tended to increase indicating that LF and MF decomposed to produce HF (Figure 6C). This contrast with the results of Magid *et al.* (1997) who found little changes on HF-C over time after the addition of rape straw. This can also be due, as stated above, to the low quality of rape residues applied in their experimental soil, which produced important accumulation of highly resistant native SOM.

In Pejivalle, both LF and MF (Figure 5) did not show a clear decreasing trend over 330 days. Results could be affected by organic litter material runoff because the field trial was in the middle of a steep slope (typical condition of coffee farms in the region; this is why this additional trial was established on sloping ground in contrast to the flat CATIE site). Runoff soil losses of about 1 Mg ha^{-1} were measured during one crop season in a mulched maize field (35% slope) near Turrialba (Garzón 1991). Accumulation of runoff residues in the lower part of the zinc sheet frame protecting the earthworm treatments showed that such a disturbance had occurred. Runoff effects were also observed in some

other plots at the Pejivalle site where the mulch was washed away in small areas within the plots. The residues coming from uphill probably mixed with the experimental residues and provoked biases; e.g. higher amounts of C, especially in the lighter fractions. A loss of light material from the plots, due to run off, may also have occurred. However, the increase or stabilization of MF-C and LF-C in the control plots at 90 days (when a decrease was expected as in CATIE) suggests that inputs of light material were more influential than the losses.

In 2002, in Pejivalle, HF-C for all the treatments that received pruning residues increased at 90 days (Figure 5D). This increase was also observed in the 2004 trial at CATIE (Figure 6C). The organic material in HF may be less affected (less movement) by runoff due to its higher density compared to LF and MF. This was speculated because during fractionation, HF sank rapidly even in liquids much denser than water ($>1.37 \text{gcm}^{-3}$). The lower mobility of HF would explain why similar results to CATIE were only observed for this fraction. Although problems with lighter fractions were observed, the results in Pejivalle, which include some significant differences between treatments for HF, are valuable as a source of information on labile SOM dynamics in the sloping fields that are usually used for coffee production in Costa Rica.

4.4.2. Total C, macroorganic matter and POM dynamics during the labile SOM decomposition

In the current study, total C of whole soil samples was not a good indicator of decomposition of labile SOM provided by pruning residues. This was evident when the evolution of total C in BARESO showed similar values to the baseline after one year (Table 3). One reason for the absence of changes in total C over time is the dominating influence of large proportions of protected SOM and the presence of stable fractions which include humic acids, fulvic acid, humines as well as organic acids and pigments. These substances are resistant to microbial activity not only because of their chemical structure but also because of their association with soil mineral components. Important amounts of stable SOM can also be occluded in the spaces between soil aggregates (Six *et al*. 2002). In

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several comparative studies of SOM dynamics, total C has been shown to be useless as an indicator of changes over time due to soil management. For example, total C showed few changes after 10-20 years of cropping in a savanna oxisol in Brazil in comparison with natural savanna C levels (Neufeldt *et al*. 1999) and no differences in total soil C between natural savannas and cropland after four years of conversion were found in a Brazilian "Cerrado" oxisol (Roscoe and Buurman 2003). In a comparison of seven cropping systems including maize and legumes, total soil C did not indicate significant changes after four years of treatment applications (Barrios 1996a).

In the 2002 field trial, macroorganic-C (total of LF+MF+HF) reflected the decomposition process of labile SOM since it showed lower values at 180 and 330 days than at 90 days when the recently added labile SOM was only partially decomposed (Appendices 3 and 4). In 2004, no significant changes for macroorganic-C were observed during the 105-day experimental period (Appendix 7C). In 2002, Macroorganic matter-C decreased less in Pejivalle (40%) than in CATIE (55%) after 330 days (Figure 4). One possible explanation was the higher clay content in Pejivalle (Table 1), since high clay content may contribute to the stabilization of SOM (Hassink 1997). Another possible explanation for the lower reduction in macroorganic-C at 330 days in Pejivalle was related to runoff due to the steep slope as explained in section 4.4.1.2.

The results for dry weight of macroorganic matter were not consistent for the two experimental sites, although in both sites a decreasing trend over time was found. At CATIE, significant differences were found between 0 and 180-330 days as well as between 90 and 330 days (Table 4). In Pejivalle, values at 90 days were significantly higher than for the other measurement dates (Table 5). In the 2004 field trial, amounts of macroorganic matter in BARESO after 105 days (Table 6), that should have shown natural decomposition of SOM (lower values), did not show significant differences in comparison to initial values. In summary, this variable appears to have limited value in showing the effects of pruning residue addition over time. These results are not in line with Barrios *et al*. (1996a) who reported that dry weight of SD fractions indicated differences between treatments over time, and suggested that it is a rapid and economic indicator. The use of dry weight of

unfractionated macrorganic matter was proposed as a substitute for LF-C analysis because of the criticism that SD fractionation consumes more processing time per sample than other approaches for SOM fractionation (Barrios *et al*. 1996a). The high consumption of time by SD fractionation also was observed in our study since three technicians could process only four samples per day.

The data obtained from size fractionation were probably insufficient to evaluate its efficiency as an indicator of labile SOM dynamics because only three sampling dates were analyzed (Table 7). The average over treatments at 75 days reflected the addition of pruning residues since higher values were obtained for POM (>53 μm). However, two unexpected results indicated the low accuracy of this method for showing changes of labile SOM over short periods of time. First, the average POM for treatments showed significantly lower values at 105 days than at 75 days. In contrast, LF and HF as well as C in macroorganic matter showed higher values at 105 than at 75 days in both 2002 and 2004 (Figures 5 and 6, Appendix 7C). The higher values at 105 days under the other indicators were supposed to be a result of incomplete decomposition of pruning residues and labile SOM at 105 days, whereas POM results showed a significant decrement between 75 and 105 days. Secondly, the POM-C values under the BARESO control did not decrease as expected after 105 days (Table 7). The low C average concentrations of POM (4.52±0.11%) in comparison with some SD-fractions (36-39% for LF and 30-33% for MF; Table 2) indicated that a large amount of minerals are included in the POM fraction. An important amount of non-labile C can be associated with these minerals provoking low sensibility of the fraction to short-term changes in labile SOM as suggested by Conant *et al.* (2003).

4.4.3. Potential value of the three size-density fractions as indicators of treatment effects on labile SOM

4.4.3.1. Impact of treatments on C content in size-density fractions

In the 2002 field trial, at 90 days after the first residue application, there were some indications that the microbial and earthworm treatments accelerated the *E. poeppigiana* residue and macroorganic matter decomposition. The C content in the three SD fractions for microbial and earthworm treatments tended toward lower values than RESID which peaked at this time. This probable effect was more apparent for LF at CATIE and significant for HF at Pejivalle at 90 days (Figures 5C and D) as well as for HF at CATIE at 180 days (Figure 5A). In addition, at 90 days the average value of RESID LF-C in CATIE was almost double that of the other three pruning residue treatments, but the high variability eliminated any statistical significance. Another indication of the impact of microbial mixtures on the amount of LF-C was observed in CATIE at 330 days when the remaining LF-C in RESID (% of initial values) was 48% higher than values for the MICROB and COMPOS treatments. Nevertheless, the differences between the treatments were again not significant due to the high variability in the EART treatment (Figure 7). The microbial and mesofaunic treatments after 90 days showed similar amounts of LF-C in CATIE and of HF-C in Pejivalle in comparison to the bare soil control. After 180 days in CATIE, HF-C values under microbial and mesofaunic treatments were also similar to the control values (Figure 5). In Pejivalle, no impacts of earthworm and microbial treatments on the LF and MF-C content were found. As a result of runoff disturbances, many discordant data were found on this site: e.g., a small decrease for MICROB LF-C (0-330 days), an outlying data point for EART LF-C (180 days), no change in BARESO LF-C (0-180 days), and increasing COMPOS and MICROB MF-C (90 days) (Figures 5E and F).

Earthworms and mixtures of fungi and bacteria have been reported to accelerate organic material decomposition in field inoculation and composting processes (Gilot *et al*. 1996, Tian *et al*. 1993, Velikonja *et al*. 2003); hence in the current study, enhanced decomposition of labile SOM by the two microbial and earthworm treatments was

expected. In a three year experiment, a 7-29% depletion of original POM-C (>53 μm) was observed after artificially increasing the earthworm population (Gilot *et al*. 1996). Labile young SOM fractions can be digested by earthworms, through mechanisms that involve ingested microflora in the gut. Associated microflora have a mutualistic relationship in the gut producing enzymes such as cellulase and mannase, which help to degrade SOM components. This process is enhanced by carbohydrate enriched mucus in the first part of the gut (5-38% of the dry weight of ingested soil) that is expelled in the casts, enhancing soil microbial activity (Barois and Lavelle 1986). Soil incubated with ten *Eisenia foetida* worms per liter during an 11 week laboratory trial increased decomposition of clover residues by 26% and significantly enhanced its comminution in comparison to the control (Ruz-Jerez *et al*. 1992). The effect of enhanced faunal activity in accelerating plant decomposition was reported by Tian *et al*. (1993); higher decomposition rates were found when macrofauna was allowed to enter into litterbags using different mesh sizes. Using a microbial inoculant, Shintani and Tabora (2000) obtained mature bokashi-type fertilizer from a mix of bananas and stalks in three weeks. In another study, mass inocula of microorganisms enhanced the mineralization of C in a mixture of organic household wastes and shredded wood after a 198 day composting period; i.e., 38% of initial C was mineralized vs 10% in the non-inoculated control (Velikonja *et al.* 2003).

After the 2002 field trial, it was thought that differences in C content contributed by LF, comparing RESID and the treatments that enhanced microbial activity, would probably be consistently significant soon after the residue application e.g., 20 or 40 days. This assumption was based on two factors. First, high amounts of macroorganic matter C and LF have been measured immediately after the incorporation of plant residues (Schroth *et al.* 2003). Secondly, high initial microbial activity has been reported in experiments on composting with mass inocula. Velikonja *et al.* (2003) found that the temperature induced by microbial activity in a compost began to increase from the second day after inoculation while the temperature in the non-inoculated control only began to increase after seven days. Moreover, they reported that the temperature after mass inoculation was much higher than in the control during the first 20 days of composting (60 $^{\circ}$C inoculated *vs* 35 $^{\circ}$C non-inoculated control). Therefore, early sampling should show a higher amount of LF-C in

RESID than microbial and earthworm treatments, which should have decreased LF-C in a shorter period after the residue application.

For these reasons, a new field trial was set up in 2004 to test MICROB, RESID and BARESO treatments [(*)] with strict control of shade and flooding and shorter sampling periods. Shade variations were diminished using shade nets, and flooding was prevented by using ditches around the plots, which isolated them efficiently. Significant differences between the two treatments that received pruning residues and BARESO were found for LF-C at 105 days (Figure 6A), indicating the influence of RESID on increasing labile SOM levels in the short-term. However, no significant differences were found between MICROB and RESID at any sampling date for any of the three SD-fractions; indicating that during this period the microbial treatment had not accelerated decomposition.

In 2004, fluctuations in the amount of LF-C at 30 and 75 days in comparison with more stable values under the RESID treatment were the only indications of probable microbial impact on LF-C (Figure 6A). Similar to the 2002 trial, after 90 days, the trend of lower LF-C under MICROB, was not consistent through the replicates eliminating any significance. It is possible that hourly changes in soil and residue moisture (dry-wet cycles) negatively affected the viability of microbial mixtures annulling its probable effect on LF-C contents. Early effects of microbial applications were visually observed on pruning residues in the field during the first two weeks of the 2004 field experiment. In MICROB plots, *E. poeppigiana* leaves presented a darker color with white and black spots in comparison with untreated leaves that remained green for almost one week (Appendix 8). White mycelium was also observed growing on the surface of *E. poeppigiana* branches under MICROB but less commonly under RESID. However, at the end of the experiment, the appearance of the pruning residues was very similar in all the plots. These observations are consistent with measurements of *in vitro* CO_2 evolution at day 15. Leaf materials from MICROB plots (unreplicated test without statistical analysis) showed a trend of higher rates of *in vitro* CO_2 production, in comparison with RESID leaf material (Table 8). In contrast, leaf material

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[(*)] In 2004, only the treatments that showed higher apparent impact in 2002 (MICROB and RESID) were tested again.

taken at day 70 presented similar CO_2 production rates. In 2004, treatments did not show any clear effects on the MF and HF-C values (Figure 6B and C). The peak observed at 45 days for HF-C, could be a result of a lab bias, due to a change in the laboratory team for this date. This fraction is frequently the most affected when the duration of decantation differs (Meijboom *et al.* 1995). If this operation is faster than normal, minerals can pass to the Ludox™ containers and can be measured along with HF due to their higher density, thus producing large variability.

Two different processes have been reported in the literature in regard to the effect of dry-wet cycles on soil microbial activity. Plant materials, particularly fragile leaves such as those of *E. poeppigiana*, that are suddenly moistened after having been dried, fracture easily; microorganisms then have more surface area to grow. Additionally, the biomass from microorganisms which died during a preceding dry cycle provides a readily available source of C for growing microbial communities which produce a CO_2 pulse when litter is humidified ("Birch effect"). This effect has been detected in the "O" horizons of forest soils (Borken 2003). The second process occurs when small pieces of leaves are mixed into the topsoil as a result of the fractures (produced by the wet-dry cycle), increasing the LF-C pool without microbial intervention.

Cabrera *et al.* (2005) found that in some cases, the effects of temperature and humidity changes on plant residue decomposition are more important than those on the topsoil. These changes can produce lower litter decomposition rates. Although homogeneous shade of the plots was a selection criteria, microclimatic differences could have occurred in the plots, affecting the temperature and humidity of the pruning residues and hence the activity of microbial mixtures on SD-fractions in the blocks. Cabrera's study also suggested that dry-wet cycles can diminish microbial activity, particularly bacteria populations that are less resistant to dry conditions. The lower resistance of bacteria to changes in litter humidity was also observed by Doran (1980) during a field experiment which measured soil microbial biomass under maize stove mulching. In organic liquid amendments, such as the microbial mixtures used in the current field trial, bacteria are usually predominant since they grow better under low O_2 pressure (Krauss 2004). In the

case of MICROB, seven bacterial strains were isolated in the laboratory (Appendix 1). If bacteria were negatively affected by changes in moisture (hourly dry-wet cycles) in some plots, similar values of LF-C under RESID and MICROB could be expected. As stated above, shade differences could occur within some plots due to the variability of shade tree crown architecture. Although monthly rainfall during the experiment in 2004 was relatively constant in October, at the end of the experiment, when 50% less rainfall occurred than in the other experimental months (Figure 2). During days with no rainfall, dry superficial pruning residues were observed in the afternoon in some plots even under shade nets. Neither the "Birch effect" nor low bacteria resistance can be discarded as explanations of why the evidence of microbial mixture effects on SD fractions was weak and inconsistent in the 2002 and 2004 trials. An effective microbial mixture should resist these changing microclimatic conditions in order to be recommended to farmers.

Another explanation for the absence of significant differences between MICROB and RESID could be that the weak effects of microbial mixtures were annulled by naturally high microbial activity which is usually expected in organic farms (Carpenter-Boggs 2000, Fließbach and Mäder 2000). Samples in the two field trials were taken from the 0-5 cm depth where higher microbial activity is usually found [*]. Other external variables such as the "wash effect" on sprayed leaves by rainfall, armadillo activity (digging the soils) as well as mesofauna (millipedes and centipedes) affected the plots in a random pattern increasing natural variability between the plots. Tian (1998) found that 8 to 24% of the decomposition of *Cajanus cajan* leaves could be attributed to soil fauna activity in a 150-day field trial using litter bags in a Nigerian alfisol.

In contrast to measurements after 90 days, after 330 days C contents in most of the fractions for all of the treatments for both sites were similar (Figure 5). Remaining residues will be lignin enriched and hence resistant to microbial decomposition (Theng *et al.* 1989, Fließbach and Mäder 2000, Mafongoya *et al.* 1998). The trends to lower percentage of initial soil LF-C values at 330 days at CATIE (Figure 7) and the significantly lower

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[*] The effects of treatments on growth of maize seedlings in pots filled with soil from 0-5 and 10-20 cm depths are presented in Chapter V.

percentage of initial HF-C at 180 days at Pejivalle (Figure 8) could be a residual effect of an early "burn" of SOM by MICROB and EART during the first 90 days. At the end of the experiment, all the treatments, including RESID, reached stable levels with respect to the amount of soil C provided by each fraction.

In 2002 at CATIE, the LF and HF-C in the EART and the two microbial treatments behaved similarly. In Pejivalle, these three treatments also gave similar results for HF-C. This similarity is interesting since the EART and the two microbial treatments had different preparation methods and the mixture concentrations applied to the residues between the microbial treatments were also different; i.e COMPOS solution diluted to 2.5% v/v with water and MICROB undiluted. Earthworms feed preferentially on crop residues or undecomposed organic matter present in the soil (Syers and Springett 1984); the decomposition mechanism is different from the treatments COMPOS and MICROB. Earthworms also accelerate SOM decomposition through microbial activity in the intestinal tract; in addition, the earthworm feces have a large microbial charge that enhances microbial activity in the soil (Gilot *et al*. 1996, Ruz-Jerez *et al*. 1992). However, no effects of these microbial and mesofaunic treatments on the final labile C content of the soil (after 330 days) were found.

In summary, under the conditions of this study, the MICROB and EART treatments were not effective in accelerating decomposition of *E. poeppigiana* residues. Significantly lower values were only found in HF-C at CATIE (after 180 days) and in HF-C at Pejivalle (after 90 days). It might be interesting to test the effects of such treatments in conventional farms (assuming that the microbial inocula and earthworms can reproduce under such conditions) where soil microbial activity is supposed to be lower than in organic farms.

4.4.3.2 Light vs medium and heavy fractions as indicators of the treatment impacts in labile SOM

The SDF method was effective in separating macroorganic matter fractions with different C concentrations and microscopic appearance (4.4.1.1.) However, the MF did not

show the effect of treatments (Figures 5B, E and 6B), probably because of its intermediate condition. It is not clear from the existing literature which of the three size-density fractions best indicates differences in soil conditions resulting from organic matter decomposition under different treatments. For example, Magid *et al.* (1996) found that the three SD fractions equally showed the effects of vegetal material quality on the labile SOM decomposition rate. More recently, Magid *et al.* (1997) separated macroorganic matter into only two fractions: a heavy fraction (>1.4 g cm^{-3}) and a combination of the MF and LF into one fraction (<1.4 g cm^{-3}). Fließbach and Mäder (2000) only found significant effects of organic and conventional fertilizer additions on the LF and the MF but not on the HF. Other studies found an insignificant role for HF in nutrient recycling since no differences were found in weight and amount of N in this fraction for different cropping systems (Barrios *et al.* 1996a). The MF and LF dry weight, C, N and P contents showed high sensitivity to land use changes in an experiment where soils under fallow species were compared to soil under crop rotations in a hillside oxisol in Colombia. All the top soils under fallow species tended to have higher values for the parameters measured in LF and MF in comparison with the rotation cropping system (Phiri *et al.* 2001). Roscoe and Buurman (2003) used a free light fraction, which was separated after ultrasonic dispersion (F-LF) and heavy (HF) density fractions to compare plowed maize lands and no till maize croplands in a Brazilian Cerrado oxisol. The two fractions were obtained using NaI (1.7) and no previous size fractionation was done. The light and the heavy fractions were useful in detecting significant differences between natural savanna and 30-year croplands (the percentage of total C in F-LF dropped from 12-18% in natural savanna to 4-5% upon cultivation). However, differences between plowed and no till maize cropping systems were not found, indicating that F-LF and HF were not sensitive to changes in soil management. Barrios *et al.* (1996a) and Phiri *et al.* (2001) in the studies described above did not find any response of HF-C to changes in plant residue management. In the present study, HF effectively showed a response to the addition of residues, and also reflected a decomposition trend in the 330 day experimental period of the 2002 experiment.

In the present study, LF and HF but not MF appeared to offer some resolution with respect to the impact of management practices that affect labile SOM decomposition.

Additionally, LF showed significant differences between treatments that include pruning residues and the control. The amount of C in LF (NaI 1.7 g cm^{-3}) was proposed by Janzen *et al.* (1992) as a good indicator of short term effects of cropping on labile SOM. That study compared fields under cropping, perennial forages and seasonal fallow in three mollisols in Saskatchewan, Canada. McLauchlan and Hobbie (2004) found a significant positive correlation between LF-C, calculated by flotation in NaI (obtained using 1.7 g cm^{-3}), and microbial biomass C (calculated by the Fumigation-incubation method using chloroform). That study compared four different approaches for calculating labile SOM pools, with data from 33 restored pasture lands and it suggested that LF-C is a good indicator of changes in labile SOM. In contrast, microbial biomass and the amount of LF-C (obtained using Ludox™ 1.13 g cm^{-3}) showed a negative correlation in a study of the effects on labile SOM from organic (cow manure as nutrient input) and conventional (legume rotations and chemical fertilizer inputs) farming systems in a 25-year experiment in Basle, Switzerland (Fließbach and Mäder 2000). The result of LF-C in the current study suggests that LF-C is the best indicator for detecting short-term changes in SOM management. The fact that in the present study, a significant negative correlation only was found between LF-C and microbial biomass (appendix 6) indicates that the amount of C in this fraction is associated with the process of microbial degradation of plant residues and SOM. No other SD fraction showed such a significant correlation.

4.4.4. Total C, macroorganic matter dry weight, and POM as indicators of treatment effects on labile SOM

In the current study, total C of whole soil samples did not show significant differences between treatments (Table 3). Only a trend to higher values under RESID in comparison to the other treatments over the three sampling dates in 2002, could be detected. This finding is in line with many results in the literature. Total C is usually useless for indicating the effect of changes in SOM management particularly in the short and medium terms. For example, in the studies from Roscoe and Buurman (2003) and Neufeldt *et al.* (1999), mentioned in section 4.2, total soil C was not useful as an indicator of natural savanna conversion to croplands and pastures after 4 years or 10-20 years,

respectively. Three different fallow species, *Tithonia diversifolia, Calliandra calothyrsus* and *Indigofera constricta,* as well as a continuously tilled maize-bean rotation did not produce significant changes in total C after one year of treatment application in an Oxic Dystropept in southwestern Colombia (Phiri *et al*. 2001).

In some studies, the weight of a fraction and not the amount of C in the fraction was tested as an indicator of changes in land use. In other cases, the weight of unfractionated macroorganic matter was tested as an indicator. For example, the weight of LF was proposed as an indicator of the effects of seven cropping systems which included pruning residues when compared to bare soil in a sandy soil in Kenya (Barrios *et al.* 1996a). Phiri *et al.* (2001) found significant differences in dry weight of the medium fraction, (obtained by flotation in Ludox ™). However, LF dry weight did not correlate with maize yields in a field trial on an Ustic Rhodustalf in Zambia (Barrios *et al.* 1998). For these reasons, in the present study, in 2002 and 2004, the dry weight and amount of C in macroorganic matter were tested, but the result indicated that they were not accurate indicators of the effect of treatment applications. No significant differences were found between treatments at any sampling date (Tables 4, 5 and 6; Appendices 7A, B and C). Nevertheless, at Pejivalle in 2002, C in the macroorganic matter under RESID showed a trend of higher values at 90 days. Likewise, at CATIE in 2002, the amount of C in the macroorganic matter under RESID showed a trend of higher values at 90 and 180 days, while at 330 days all treatments showed similar values (Appendix 7A). In 2004, the weight of the macroorganic matter at 105 days under BARESO was 46% higher than the baseline (Table 6), indicating that this indicator was useless to show differences between treatments because of the variability in the control plot measurements.

Particulate organic matter (POM, >53 μm) has been proposed as a fraction that matches characteristics of the "slow" SOM pool. In a comparison between three tillage treatments (20 yr under cultivation) and an undisturbed grassland in a Pachic Haplustoll in the High Plains in the USA, lower amounts of C in POM were found under tilled fields than in undisturbed soils (Cambardella and Elliot 1992). In Puntarenas, Costa Rica, lower amounts of C in POM were found under secondary forest in a Typic Rhodustalf when

compared with adjacent improved pastures (*Brachiaria brizantha*) associated with trees (Ramos 2003). In contrast, in a comparison between intensive grazing (3-25 yr under short rotation grazing) and hay fields (extensively grazing) in a Typic Hapludult, in Virginia, USA inconclusive results were found. The amount of C in POM (0-10 cm depth) under intensive grazing was higher only in two out of four sampling sites. No differences in POM-C were found when samples from the 0-50 cm depth were compared. Although 22% more total C was found under intensive grazing systems in three of four paired sampling sites, differences in total C could not be associated with differences in POM-C (Conant *et al.* 2003). One of the reasons suggested in that study for the poor sensitivity of POM-C to short-term changes in soil management is that this fraction includes a considerable proportion of SOM which has turnover times of decades. The inclusion of root C as POM-C also was a source of variability that eliminated significant differences in their study.

In the 2004 study, values for MICROB and RESID POM-C obtained with the alternative size fractionation method, were higher than BARESO POM-C at 75 days (Table 7). These results showed the impact of residue addition, but unexpectedly the three treatments showed similar POM-C at 105 days (Table 7) when SD fractions and macroorganic matter showed higher values for RESID POM-C. Another unexpected result was found at 0 days: MICROB POM-C was higher than POM-C under RESID and BARESO but at that date none of the plots had received any treatment. In addition, neither SD fractions, macroorganic matter C nor total C showed significant differences between treatments at 0 days. These results at the baseline (0 days) showed a limitation of this method. At 105 days no differences were found using the POM-C analysis between RESID and BARESO as they were with the LF-C analysis. Although only three experimental dates could be analyzed, these observations allow one to question the efficiency of POM as an accurate indicator of short-term changes in labile SOM as a result of *E. poeppigiana* pruning residue additions. The POM separation includes chemical dispersion with sodium hexametaphosphate which releases C and N enriched labile SOM and other microbial by-products from macroaggregates (250-2000 μm). This release can alter the results (higher percentage of total C and N) since they are not produced by recent additions of plant

materials (Barrios *et al*. 1996a). In summary, in this study the size fractionation of SOM produced highly variable results and hence it was not practical.

4.5. Conclusions

All of the analyzed SOM fractions reflected the labile SOM decomposition since they showed higher values 90 days after pruning residue addition which is when plant residue decomposition appears to have been incomplete. Also, all the indicators showed lower values or tended to have lower values 180 days after residue applications. Total soil C in whole soil was not a good indicator of short-term changes in labile SOM as only trends to higher values at 90 days were observed. The amount of C in LF was the only indicator amongst the three SD fractions that showed significant differences between RESID and BARESO in the 2004 trial clearly reflecting after 105 days the initial addition of 5 Mg ha^{-1} organic pruning residues. The amount of C in LF was also the only SD fraction that could be correlated with microbial biomass suggesting its relation with microbial activity in the topsoil. However, an evaluation of the costs of SD fractionation should be done; macroorganic C or dry weight of LF can be evaluated as less expensive indicators of short-term labile SOM decomposition.

In 2002, higher C content values in LF (after 330 days) and HF (after 180 days) were observed for untreated residues, when compared to bare soil in CATIE. In 2004, significant differences were found between RESID LF-C and BARESO LF-C. These findings highlighted the importance of *E. poeppigiana* pruning residue additions in sustaining labile SOM levels in organic farms. However, in the 2002 field trial, the final macroorganic matter C contents under RESID were lower than the initial values indicating that typical residue additions alone can not totally sustain macroroganic matter C values. Decreasing final SD fraction levels and the evaluation of these indicators in undisturbed plots suggested that the conservation of initial levels of labile SOM is related not only to the contribution of pruning residues from *E. poeppigiana* but also with contributions from weed residues, coffee bushes and tree litterfall as well as coffee pruning residues. A separate evaluation of the contribution of each of these components would be worthwhile.

In the 2002 field trial, in practical terms, the microbial mixture and earthworm applications should be considered ineffective due to their inconsistent and temporary impact on labile SOM. At 90 days, the earthworm and microbial treatments only showed a trend to lower C contents compared to the RESID treatment in LF and HF at CATIE. After 180 days this differences were significant in CATIE. In Pejivalle, HF-C under the microbial and earthworm treatments was significantly lower than under RESID only at 90 days. In the 2004 trial, no differences between the MICROB and RESID treatments were found in any of the three SD fractions. If the microbial mixture and earthworm treatments have any value, they may be annulled by homeostasis (in a soil with naturally high microbial activity) and by their low resistance to the environmental field conditions.

The amount of C in LF-C showed more accuracy as an indicator of changes in organic matter management. This fraction was the only indicator that showed significant differences between the RESID and BARESO treatments at 105 days in the 2004 field trial. Total C, macroorganic matter (>150 µm) C and dry weight of LF, as well as POM (>53 µm) at 75 days showed a consistent trend to higher values under RESID in comparison to the BARESO treatment, but the differences were not significant.

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Chapter 5   Soil nitrogen and potassium availability after addition of *Erythrina poeppigiana* pruning residues to soil alongside applications of microbial inocula or earthworms.

Key words: Litter decomposition, native strains of fungi, liquid organic amendments, shade trees, soil organic matter.

5.1. Introduction

Mulches from residues of agroforestry tree species have been evaluated both in greenhouse and field trials as sources of nutrients for plant growth. High quality mulches from native tree species in lowlands in the Atlantic coast of Costa Rica, which had intermediate decomposition rates, produced higher growth rates in maize seedlings than plants grown with low quality mulches (Kershnar and Montagnini 1998). In the same area, Byard *et al.* (1996) found that the mulch from the N fixing tree *Stryphnodendron microstachyum* had benefitial effects on initial growth and N uptake of maize seedlings. In field experiments, mulches from pruning residues of *Erythrina poeppigiana* have been evaluated as a source of nutrients for maize and beans in alley cropping systems. In a *Typic Humitropept* in Turrialba, Costa Rica, higher soil C and N contents were found in plots that received 9.2 Mg DM ha^{-1}y^{-1} over a five year period (Ramírez and Bornemisza 1990). In a later evaluation of the same experiment, positive effects of *E. poeppigiana* mulches on maize growth over a 10-year period were found, but no significant differences in soil N, P, Ca, and Mg concentrations were found in samples from 0-60 cm depth (Soto *et al.* 1993). Nutrients in *E. poeppigiana* residues are recycled by pruning the trees used in the alley cropping systems (Kass *et al.* 1993). However, in some cases, nutrients (especially N) from the residues are only partially incorporated into the crop biomass (Haggar *et al.* 1993) or may be lost after mineralization.

The main factors that determine the decomposition rates of organic residues are: climate variables, quality of residues, specific characteristics and activity of soil invertebrates and microorganisms as well as live root exudates which enhance microbial

activity (Lavelle *et al*. 1992, Lavelle 1997, Palm 1995). Interaction of the factors involved in plant residue decomposition affects not only SOM dynamics but also soil structural development as well as nutrient release and supply for plants. The search for strategies to manage biomass inputs, using the interaction between microbial and mesofauna populations with soil conditions, has been proposed to find more efficient nutrient cycling processes via SOM decomposition (Schroth 2003). The synchrony between nutrient release (e.g. the decomposition of tree pruning residues) and crop demand may determine adequate nutrition of crops in agroforestry systems. The build up of readily decomposable SOM reserves is also an important method to assure adequate nutrient supply for crops under agroforestry systems (Haggar *et al*. 1993). Controversy exists with respect to the best research focus (synchrony or SOM build up), and it is necessary to find practical and economical methods to manage biomass in order to maximize efficient nutrient uptake by crops while conserving the long-term SOM levels (Mafongoya *et al*. 1998).Controversy exists respect the best research focus (synchrony or SOM build up), and it is necessary to find practical and economical methods to manage biomass in order to have efficient nutrient uptake by crops while conserving the long-term SOM levels (Mafongoya *et al*. 1998).

Several studies have addressed the role of microorganisms during the controlled decomposition of plant residues. For example, slower SOM losses were found in no-till systems where the fungal-to-bacterial biomass ratio was increased, in a study on the role of microorganisms in soil C cycling during winter wheat (*Triticum aestivum*) straw decomposition (Holland and Coleman 1987). This inverse relationship between the fungal-to-bacterial biomass ratio and C losses was due to the higher efficiency of fungi in conserving C, because these organisms possessed more resistant cell-wall materials than bacterial biomass. Clein and Schimel (1993) found that wet-dry cycles diminished microbial activity during plant residue decomposition and nutrient release rates when plant residues were laid on the soil surface. Shintani and Tabora (2000) accelerated the fermentation process of a mixture of bananas and stalks using microbial inoculants to obtain a mature bokashi-type fertilizer in only three weeks while a compost without inoculation took 8 weeks. Velikonja *et al*. (2003) used a mixed inocula of microorganisms

to enhance the mineralization of C in a blend of organic household wastes and shredded wood in a 198-day composting period. At the end of the experiment 38% of initial C in the residues was mineralized versus 10% in the non-inoculated control. However, few studies have addressed the effect of artificially augmented microbial communities in the acceleration of nutrient release and uptake by plants. On the other hand, some studies have suggested that microbial biomass can act as a living nutrient reservoir (Gunapala and Scow 1998, Srivastava and Lal 1994). The possibility of conserving nutrients in living biomass by using microbial mixture applications has not often been addressed.

There are two contrasting ideas about the role of earthworms in SOM decomposition. One hypothesis states that earthworms accelerate C mineralization because microflora in the gut of earthworms digest labile SOM fractions. These microorganisms produce cellulase and mannase in their digestive tract, which contributes to organic residue decomposition. Additionally, carbohydrate enriched mucus, which is expelled along with their casts, enhances soil microbial activity (Barois and Lavelle 1986). Some earthworms preferentially feed on plant residues from the soil surface accelerating SOM decomposition and nutrient release, and hence nutrient availability in the soil for plants. This could be a way to achieve synchronization of the crop nutrient demands and the release of mineral nutrients (Brown *et al*. 1996). The second hypothesis states that endogeic earthworms, particularly in temperate climates, provoke an increase in SOM-C stabilization by mixing organic matter and clay minerals in soil aggregates. In addition, a large proportion of the worm casts left in the soils are incorporated into the COM (chemically protected fraction), which is very stable, and thus contributes to maintaining SOM levels (Anderson and Flanagan 1989).

Low nutrient availability and minimal fertilizer inputs have led to a constant decrease of crop productivity in low-input agroecosystems (Phiri *et al*. 2001). This is particularly true in organic coffee farms in Costa Rica. Yields from organic coffee farms are on average 22% lower than for conventional managed farms. A lack of plant or animal residues to fertilize coffee plants is usually a limiting factor for organic coffee production (Lyngbaeck *et al*. 2001). Organic farming systems depend on natural processes to maintain

soil fertility and sustainable yield levels (Lockeretz *et al*. 1981, Lampkin 1990, Lotter 2003); crop nutrition relies on nutrient liberation when organic materials (e.g. SOM or organic fertilizers) are mineralized by soil microflora (Fließbach and Mäder 2000). Most coffee farmers use shade tree pruning residues as the main source of nutrients. For example, *E. poeppigiana,* a leguminous shade tree commonly used in Costa Rica, is pruned or pollarded two to three times a year to reduce shade and also to provide mulch and nutrients for the coffee plants. Some studies on conventional farms have shown that *E. poeppigiana* residues contain up to 300 kg ha^{-1} of N, due to recycling from the soil, which is a similar amount of nutrients, to those provided by chemical fertilizers used in these farms (Beer 1988). Nutrients (especially N and K) may be lost during decomposition or leached by heavy rainfall. Although high densities of *E. poeppigiana* pruning residues are used, and large amounts of pruning residues are applied in many organic farms, coffee yields are still low compared to yields in conventional farms (Table 1, Chapter III).

The possibility of organic residue management within the farm has been suggested as an alternative in order to have more efficient nutrient cycles within organic farms (Fishersworring and Roβkamp 2001). Earthworm inoculation in field trials has been used to stimulate plant growth through enhanced mineralization of SOM nutrients (Lavelle 1997). In addition, organic farming guidelines frequently recommend using microbial mixtures to enhance microbial activity in the soil and crop nutrition (Fishersworring and Roβkamp 2001) as well as adding earthworms to improve the microbial activity and nutrient release (Syers and Springett 1984, Springett *et al*. 1992). Nevertheless, few experimental tests have evaluated the efficiency of such interventions (Soto and Muschler 2001). Additionally, the idea of better nutrient conservation within microbial biomass through enhanced microbial activity during decomposition of *E. poeppigiana* pruning residues needs to be tested. In this study, a bio-assay with maize seedlings in a greenhouse and a field trial were carried out. Native microbial community mixtures or earthworms (only in the field trial) were added to *E. poeppigiana* pruning residues produced and distributed according to normal practices on organic coffee farms in order to detect probable improvements in soil N and K availability.

5.1.1. Specific objectives

A- to analyze the effects of microbial mixtures added to *E. poeppigiana* pruning residues, on the growth rate of maize seedlings; B- to measure K and N in foliar tissue of maize seedlings grown in pots to which *E. poeppigiana* residues had been added with and without microbial mixture applications; C- to measure *in vitro* CO_2 production from *E. poeppigiana* residues with and without microbial mixtures applications; D- to analyze the effect of earthworms or microbial mixture treatments on soil N and K availability in organic coffee farms.

5.2. Materials and methods

5.2.1. Site description

Two simultaneous field trials were conducted (August 2002 until August 2003) in CATIE´s organic coffee plantations in Turrialba and a private organic coffee farm in Pejivalle, Costa Rica. In 2004, a new field trial was set up in the same area at CATIE. Details of the location, soil and farming characteristics of the two sites are given in the methods section in Chapter IV; i.e. selected soil characteristics and fertilization practices are summarized in Table 1 and mean temperature and monthly rainfall are presented in Figures 2 and 3 (Chapter IV).

5.2.2. Greenhouse experiment

To test the effect of microbial mixture additions to *E. poeppigiana* pruning residues, on maize growth and soil N and K availability, a greenhouse pot trial, with a completely randomized design with three treatments and five replicates was conducted at the CATIE campus. The treatments were arranged in a factorial design with three factors. The first factor was the soil layer used as a source of material to fill the pots; either 0-5 or 10-20 cm depth. The second factor was the method of residue application (8g DM of *E. poeppigiana* pruning residues added to each one-liter pot) which was added to the pots in 2 different

ways: (1) added to the soil surface ("UP"); and (2) mixing the residues in with the soil ("MIXED"). The third factor was the application of microbial solution to the pruning residues and had three treatments: (1) application of a microbial mixture to the pruning residues; (2) pruning residues without the microbial mixture; and (3) a control treatment of soil only (Figure 1).

Type of soil: Soil from 0-5cm depth layer   Soil from 10-20cm depth layer

Method for: UP   MIXED   UP   MIXED

Treatments: BARESO RESID MICROB   BARESO RESID MICROB   BARESO RESID MICROB   BARESO RESID .MICROB

Treatments   **Description**

BARESO   pots filled only with soil from each soil depth

RESID   8g DM of *E. poeppigiana* chopped pruning residues (size $\approx$ 1 cm^2) in each pot

MICROB   50 ml 10 kg^{-1} of fresh residues plus the addition of the same amount of pruning residue as in RESID

Figure 1. Diagram of the factorial design for the greenhouse trial and description of the treatments

        One-liter pots were filled with 500 g of soil (44% gravimetric moisture content) from the 0-5 or 10-20 cm soil layer of the organic coffee plantation in CATIE (thirty pots in both cases). The soil was previously homogenized using an iron sieve (10 mm mesh size) and mixed by hand several times. The pots were left under field conditions for 45 days to stabilize biological activity. The original moisture was maintained but leaching was avoided during the experiment. Maize seeds (hybrid H5) were soaked in distilled water for 48 hours and then three seeds were planted in each pot at 2 cm depth. The pots were placed in a greenhouse with 75% sunlight and the following treatments were applied: only the addition of 8g DM of *E. poeppigiana* chopped pruning residues (size $\approx$ 1 cm^2) in each pot (RESID); the application of the microbial mixture described in Chapter IV (50 ml 10 kg^{-1} of fresh residues) plus the addition of the same amount of pruning residue as in RESID

(MICROB); pots filled only with soil from each soil depth were used as controls (BARESO). The microbial mixture was sprayed on the *E. poeppigiana* pruning residues eight days before planting of the seeds.

After four days, maize shoots appeared and the smallest one was removed if all three seeds germinated. The height of the taller of the remaining two plants was taken daily for the first two weeks and then every other day until day 38. The height was measured from the base of each plant to the tip of the longest leaf when fully extended. The shorter plant in each pot was cut at day fifteen, oven dried, and the dry biomass (leaves and stems) was measured. Foliar K and N concentrations were measured for plants grown in soil from the 10-20 cm depth where height differences between RESID and MICROB were apparent. At the end of the experiment the remaining plant was collected and oven dried. Biomass of the complete plant (leaves, stem and roots) was measured and K and N concentrations in foliar tissue for plants grown in soil from the 10-20 cm layer were analyzed. A growth curve was drawn from height measurements of the taller plant in each pot. Long and short axis were measured at 15 and 28 days using a caliper and the cross section was calculated using the ellipse's area formula:

$$\text{Cross section area (mm}^2) = \pi * (\text{Long axis} / 2)* (\text{Short axis}/2)$$

ANOVA tests were performed at each height measurement date, as well as at 15 and 38 days, for biomass, K and N concentrations.

5.2.3. *In vitro* CO_2 production from pruning residues

Two types of *in vitro* laboratory tests were performed for comparisons of respiration rates among the RESID (pruning residues only), MICROB (RESID + microbial mixtures) and BARESO (soil only) treatments using a modified substrate induced respiration (SIR) method described by Cheng and Coleman (1989) modified by Vandevivere and Ramírez (1995). These tests were carried out at the Phytopathology Laboratory of the Agronomy School at the University of Costa Rica. In the first type, respiration rates for the soil with

the addition of *E. poeppigiana* pruning residues and the microbial mixture described in Chapter IV were compared with those from soil mixed with only pruning residues during a 70-day period (this test was referred as Trial 1). In the second type, respiration rates of pruning residues with no soil, but with the addition of the microbial mixtures, were compared with those of pruning residues alone (Test II) during four weeks.

In the first type (Trial I), three treatments were tested: A- MICROB, in which 12.5 g of chopped *E. poeppigiana* pruning residues (size $\approx$ 1 cm^2, with 62% moisture) were sprayed with the microbial mixture (50 ml 10 kg^{-1} pruning residues) and then mixed with 77.6 g of soil (42% moisture). The soil was taken from the 0-5 cm depth layer in the organic farm at CATIE; B- RESID, which had the same proportion of pruning residues and soil (1:9 DM/DM) but no microbial mixture addition; and C- a control (BARESO), consisting of the same amount of soil with no residue or microbial addition. Four replicates of each treatment for each measurement date were prepared and kept in plastic cups (250ml) at room temperature until respiration was measured. Sixty cups were covered with parafilm™ to allow gas exchange, and then incubated. The weight of the cups was measured weekly, and in case of differences, was adjusted to the initial weight by adding distilled water. The first measurement was taken eight days after the treatment applications, and then every 15 days from the first week of August to the first week of October 2004. The experimental procedures for CO_2 measurements were taken from Vandevivere and Ramírez (1995), and details are given in Section 2.6, Chapter IV.

In the second type of test (Test II), 4 kg of fresh pruning residues were put in each of two plastic bags with aeration. The materials in one bag were sprayed with the microbial mixture used in Trial I at the same proportion and then incubated in the dark at 24 °C (MICROB). After spraying, the pruning residues were mixed in the bag. In the second bag, residues were incubated at the same temperature without spraying (RESID). Both bags were dampened with distilled water to maintain original moisture during the experiment. Readings were taken at 8, 15, 19 and 24 days after the microbial mixture was sprayed. Sub-samples from both treatments (12.5 g fresh weight about 60% moisture) were put in the flasks as in Trial I. Four laboratory repetitions (sub-samples of unreplicated treatment) of

each treatment at each measurement date were used. However, this test was considered supplementary because the bags that received the two treatments were not replicated in the laboratory.

In another variation (Trial II c), a solution of de-chlorinated water and molasses 50:1 mixed with EM™ (effective microorganisms), a consortia of lactic acid bacteria, yeasts, and phototropic bacteria (Shintani and Tabora 2000), sold by EARTH University, was sprayed on pruning residues and tested for CO_2 production. The preparation and measurement procedures were the same as in Trial II.

5.2.4. Field trial design and soil sampling

In 2002, a randomized complete block experimental design, with three replicates, was established at CATIE and another in Pejivalle. Each block had five 2×2 m plots surrounded by an untreated 1.0 m buffer zone to avoid inter-treatment interference. In each block, three treatments that enhance microbial activity [microbial mixture A (MICROB), microbial mixture B + composting (COMPOS), earthworms (EART)], one treatment which only received the pruning residues (RESID) and one bare soil treatment as a control (BARESO) [*] were randomly distributed. All treatments, except the bare soil control, received 5 Mg ha^{-1} DM of fresh coarsely chopped *E. poeppigiana* pruning residues. Selected soil characteristics (0-20 cm depth) and agricultural practices of experimental sites are given in Table 1, Chapter IV. In each site, a soil profile description and classification were carried out (Appendix 1 and 2, Chapter III). In 2004, a new field trial was set up; in CATIE only; soil exchangeable K was tested for MICROB, RESID and BARESO treatments in a randomized complete block design with four replicates. Details of the field trial are given in Chapter IV. Samples from the 0-5 cm layer that were used for macroorganic matter fractionation at days 0 and 30 were also used for soil exchangeable K analyses. In the 2002 and 2004 studies, soil samples were taken as described in Section 2.3, Chapter IV.

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[*] Details of treatment preparations are given in method section in Chapter IV.

5.2.5. Measurements and analytical methods for nitrogen and potassium availability in the field trials

Net N mineralization was estimated only in the CATIE field trial (in 2002) using a modification of an *in situ* incubation method from Raison *et al*. (1988). Two pairs of undisturbed cores (four cores total) were taken in each plot at the 0-5 cm depth using a "Göttingen" auger with a cylinder with an internal diameter of 8 cm. One of each pair (two cores) was extracted with 2 M KCl for 1 h; the extracts were filtered and the solutions analyzed for nitrate and ammonium concentrations using an Alliance colorimetric automatic analyzer. The other two cores were placed into PVC tubes, 10 cm in diameter and 20 cm in length. In order to prevent root in-growth and leaching effects due to rain, the bottoms and tops of the tubes were covered with 50 μm metallic fabric and PVC cups. The tubes were inserted 6 cm into the soil for incubation for approximately four weeks (30-35 days of field incubation). The soil cores that were not incubated were considered to be time 0 (t_0) and those that were incubated were time 1 (t_1). Net mineralization was calculated as the difference in mineral-N (nitrate and ammonium) between t_1 and t_0 for each paired core, averaged to obtain one value per plot. This procedure was carried out at 190, 220 and 270 days after the first residue application (40, 75 and 120 days after the second pruning residue application; the field trial was originally designed for SD fraction evaluation and had two residue applications at 0 and 180 days). Exchangeable soil K concentrations in 2002 and 2004 were analyzed with a modified Olsen solution (pH 8.5) and atomic absorption spectroscopy (AAnalysist 100, Perking Elmer, Boston, MA, USA).

5.2.6. Statistical analysis

In the greenhouse trial, data for plant growth was analyzed using ANOVA as a complete randomized design for each measurement date. Data for *in vitro* CO_2 production in the Trial 1 were also analyzed in a complete randomized design for each measurement date. The CO_2 measurements in Test II were considered a subsidiary unreplicated test and error standard was calculated for laboratory replications within each treatment. The three sets of measurements of N mineralization as well as K concentrations were analyzed in a

randomized complete block design for each sampling date. Means comparisons were carried out using Duncan tests (GLM procedure, SAS Institute 1999).

5.3. Results
5.3.1. Maize seedling bioassay
5.3.1.1. Height of maize plants

From day five on, maize seedlings in pots filled with soil from the 0-5 cm layer were 20 to 24% taller than plants grown in soil from the 10-20 cm layer. In the plants grown in soil from the 0-5 cm layer, no differences were observed among treatments (Figs. 2a and b) during the experiment. From day six onwards, significant differences between treatments were apparent in the plants grown in soil from the 10-20 cm layer. In the soil from the 10-20 cm depth, the two treatments which included pruning residues had greater heights than the control ($p<0.05$). MICROB and RESID were about 35% and 29%, respectively, higher than the control plants from days 13 onwards (Figures 3a and b). On average, in the 10-20 cm soil, plants grown in pots using the "MIXED" method were 9% taller than those grown using the "UP" method from day 8 onwards. Significant differences between MICROB and RESID were detected on five measurement dates, from day 13 until day 17, with soil from the 10-20 cm layer (Figures 3a and b). On these dates 6-12% taller plants were measured under the MICROB treatment in comparison with the RESID treatment for both forms of residue application ($p<0.05$). Nevertheless these differences were temporary and microbial mixtures gave no additional benefit for maize height at any other experimental dates.

5.3.1.2. Plant biomass

The average biomass across treatments at day 15 showed that plants grown in soil from the 0-5 cm layer had 46% more foliar biomass than plants grown with soil from the 10-20 cm layer. At the end of the experiment (day 38) this difference between the two soils was 55%. At 15 days foliar biomass was significantly higher for the MICROB and RESID treatments in comparison with BARESO treatment in soil from the 10-20 cm layer, but no differences were found among treatments with soil from the 0-5 cm layer (Table 1). At day

38, the two treatments that included addition of residues produced higher biomass in both types of soils (Table 1). Only at day 15, the average foliar biomass across treatments was higher under the MIXED treatment in comparison to the UP residue application method; at 38 days both foliar and total biomass were similar.
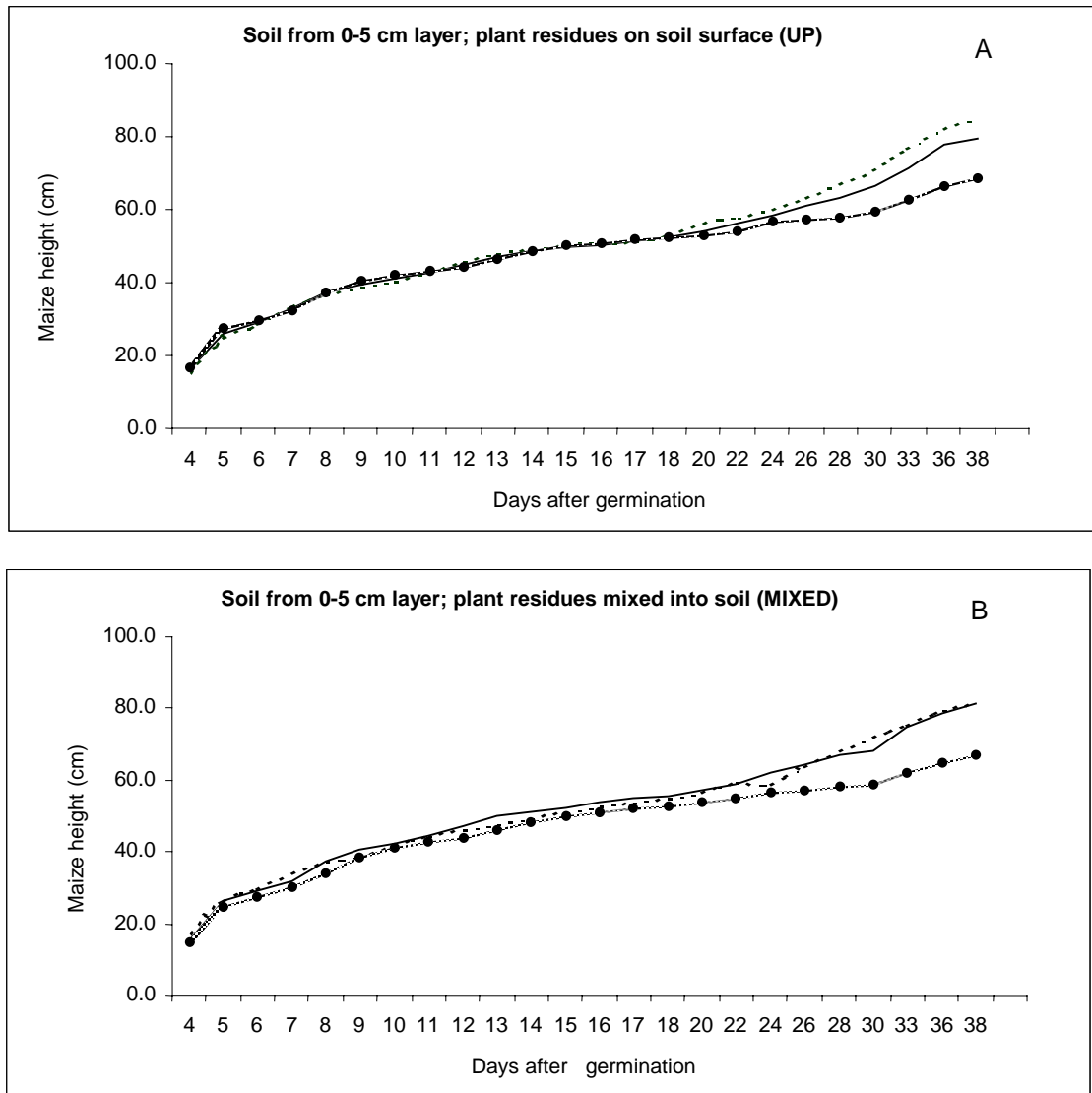
Figure 2. Maize seedling heights in pots to which a microbial inocula and *E. poeppigiana* pruning residues were added to the soil. The treatments were: MICROB (---), 12.5 g of fresh *E. poeppigiana* pruning residues added with 50 ml10 kg $^{-1}$ of microbial mixture to the soil; RESID (——),12.5 g fresh pruning residues added only; and BARESO(·-··), bare soil
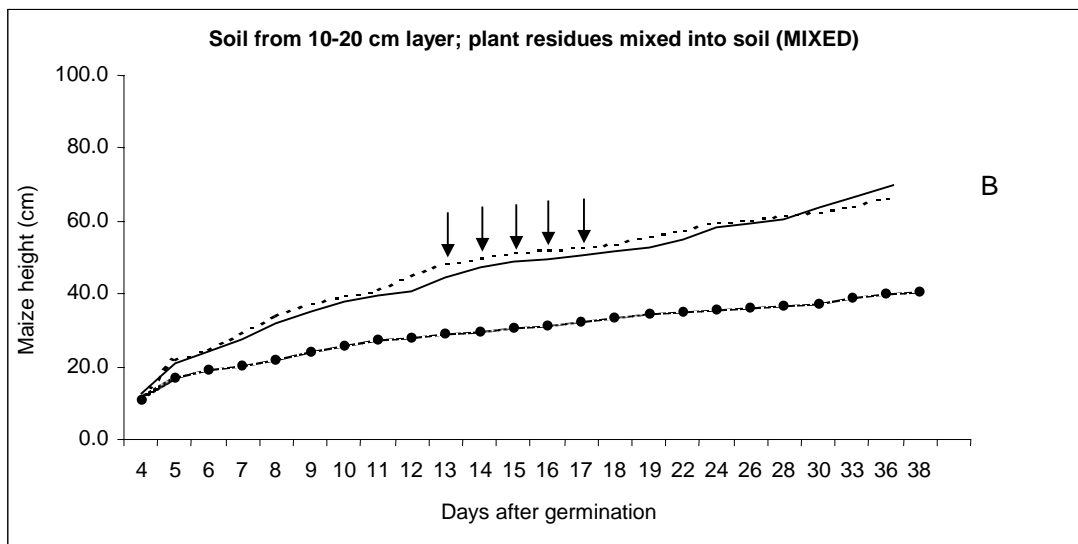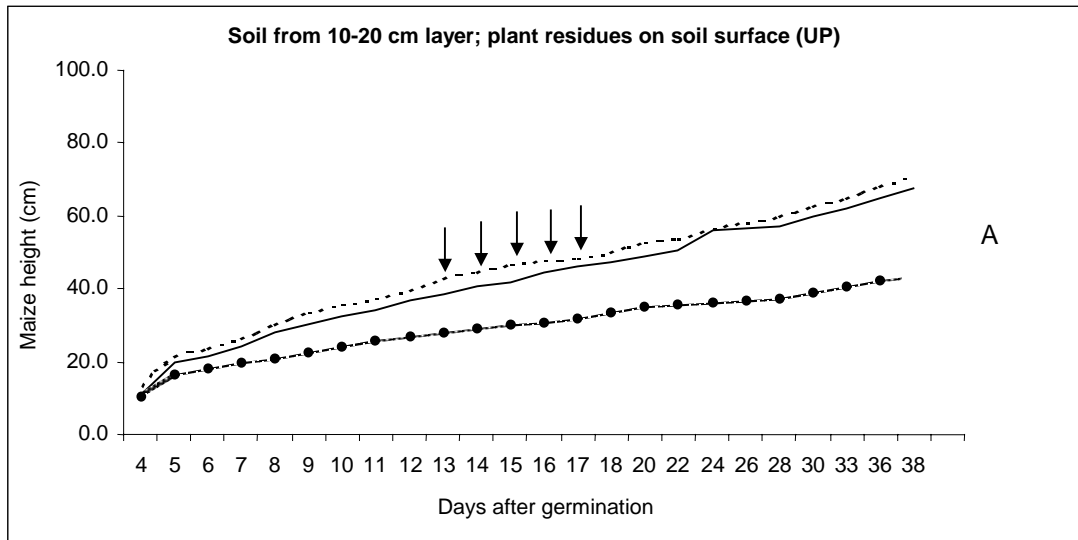
Figure 3. Maize seedling heights in pots to which a microbial inocula and *E. poeppigiana* pruning residues were added to the soil. The treatments were: MICROB (---), 12.5 g of fresh *E. poeppigiana* pruning residues added with 50 ml 10 kg $^{-1}$ of microbial mixture to the soil; RESID (——),12.5 g fresh pruning residues added only; and BARESO(·-··), bare soil. The arrows mark the dates in which significant differences between MICROB and RESID were found. (n=5, Duncan test, $p<0.05$).

No significant differences were found between the RESID and MICROB treatments with any of the two soils at any date when data were sorted by type of soil (Table 1). Only a

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trend of higher values under MICROB was observed at day 15 in the 10-20 cm soil; the differences between the two treatments were less than 15% in the first 15 days and 7-8% at 40 days. On the contrary, in the soil from the 0-5 cm depth there was no clear trend between these two treatments for biomass production (Table 1). When data were sorted by method of application, the MICROB treatment had a 28% higher foliar biomass than RESID within "MIXED" method after 15 days (0.50 vs 0.39 g plant^{-1}) (p <0.01; Table 2). Significant differences were also observed for total biomass at day 38 under MIXED. In this case MICROB had 10% higher biomass than RESID (Table 2).

Table 1. Biomass (g) of maize plants grown in soil taken from two depth layers from an organic coffee plantation. Treatments are: RESID, addition of 8 g DM of pruning residues only; MICROB, 8 g DM of pruning residues + 50 ml 10 kg^{-1} microbial mixture; BARESO, bare soil

	0-5 cm				10-20 cm			
	RESID	MICROB	BARESO	Avg	RESID	MICROB	BARESO	Avg
Variables								
Foliar biomass 15 d[2]	0.39a[1]	0.44a	0.40a	0.41A	0.33a	0.38a	0.12b	0.28B
Foliar biomass 38 d	2.21a	2.23a	1.22b	1.88A	1.55a	1.66a	0.45b	1.21B
Total biomass 38 d	3.83a	3.84a	2.59b	3.41A	3.39a	3.67a	0.99b	2.68B

[1] Values with the same letter within a category or between averages are not significantly different (Lsmean-test p<0.05)
[2] Days after germination

Table 2. Maize plant biomass (g) for two methods of residue application. "UP", plant residues on soil surface; and "MIXED", plant residues mixed into the soil. Treatments are: RESID, addition of 8 g DM of pruning residues only; MICROB, 8 g DM of pruning residues + 50 ml 10 kg^{-1} microbial mixture; BARESO, bare soil

Method	Up				Mixed			
	RESID	MICROB	BARESO	Avg	RESID	MICROB	BARESO	Avg
Variables								
Foliar biomass 15 d[2]	0.33a[1]	0.32a	0.23b	0.29B	0.39b	0.50a	0.29c	0.39A
Foliar biomass 38 d	1.79a	1.84a	0.91b	1.51A	1.97a	2.05a	0.75b	1.59A
Total biomass 38 d	3.70a	3.62a	1.93b	3.00A	3.51b	3.88a	1.65c	3.08A

[1] Values with the same letter within a category or between averages are not significantly different (Lsmean-test p<0.05)
[2] Days after germination

5.3.1.3. Stem cross section [+]

Plants grown with soil from the 0-5 cm depth always had higher stem diameters and cross

      sections than plants grown with soil from the 10-20 cm depth (Table 3). In all cases

      plants under treatments that included *E. poeppigiana* pruning residues had

      significantly higher diameter and cross sections than the control with no plant

      residues. No differences were found among methods of residue application when

      averages for treatments were compared at the two measurement dates (Table 4). No

      differences between the RESID and MICROB treatments were detected for cross

      sections, at any of the two measurement dates.

Long diameter (long axis) did not show any significant differences between the RESID and

      MICROB treatments. Plants grown with soil from the 10-20 cm depth showed

      significantly higher (14%) short diameters (short axis) only at 15 days under the

      MICROB treatment than under the RESID treatment (Table 3). At this date, these

      plants also had higher (12%) short diameters under the MICROB treatment when

      they were grown using the MIXED application method (Table 4). At 28 days no

      significant differences between the RESID and MICROB treatments were found for

      this variable.

Table 3. Diameter and cross section (mm) of plants grown in soil from two depth layers. Treatments are: RESID, addition of 8 g DM of pruning residues only; MICROB, 8 g DM of pruning residues + 50 ml 10 kg^{-1} microbial mixture; BARESO, bare soil

Depth	0-5 cm				10-20 cm			
Treatment	RESID	MICROB	BARESO	Avg	RESID	MICROB	BARESO	Avg

[+] Shorter and longer stem diameters were respectively considered as the short and the long axes of an ellipse for calculating the stem cross section.

	RESID	MICROB	BARESO	Avg	RESID	MICROB	BARESO	Avg
Long axis[2] 15 d[3]	5.3a[1]	5.25a	4.8b	5.1A	4.4a	4.3a	2.8b	3.8B
Short axis[4] 15 d	3.8a	4.0a	3.1b	3.6A	2.9b	3.3a	2.2c	2.8B
Cross section 15 d	15.7a	16.4a	11.7b	14.6A	10.0a	11.0a	4.7b	8.6B
Long axis 28 d	6.9a	7.0a	5.1b	6.3A	6.2a	6.3a	3.8b	5.4B
Short axis 28 d	5.2a	5.1a	4.6b	5.0A	4.5a	4.8a	2.7b	4.0B
Cross section 28 d	27.9a	28.4a	18.7b	25.0A	21.9a	23.6a	8.0b	17.8B

[1] Values with the same letter within a category or between averages are not significantly different (Lsmean-test $p<0.05$)
[2] Longer stem diameter was considered as the long axis of an ellipse
[3] Days after germination
[4] Shorter stem diameter was considered as the short axis of an ellipse

Table 4. Diameter and cross section (mm) of plants for two methods of residue application. Treatments are: RESID, addition of 8 g DM of pruning residues only; MICROB, 8 g DM of pruning residues + 50 ml 10 kg^{-1} fresh residues of microbial mixture; BARESO, bare soil

Method	Up				Mixed			
Treatment	RESID	MICROB	BARESO	Avg	RESID	MICROB	BARESO	Avg
Long axis[2] 15 d[3]	4.6a[1]	4.5a	3.9b	4.3A	5.1a	5.1a	3.6b	4.6A
Short axis[4] 15 d	3.4a	3.5a	2.7b	3.2A	3.3b	3.7a	2.6c	3.2A
Cross section 15 d	12.4a	12.6a	8.9b	11.3A	13.4a	14.8a	7.5b	11.9A
Long axis 28 d	6.3a	6.3a	4.8b	5.8A	6.7a	7.0a	4.1b	5.9A
Short axis 28 d	4.8a	4.7a	3.7b	4.4A	4.9a	5.2a	3.6b	4.6A
Cross section 28 d	23.9a	23.2a	15.3b	20.8A	25.8a	28.8a	11.5b	22.0A

[1] Values with the same letter within a category or between averages are not significantly different (Lsmean-test $p<0.05$)
[2] Longer stem diameter was considered as the long axis of an ellipse
[3] Days after germination
[4] Shorter stem diameter was considered as the short axis of an ellipse

5.3.1.4. Potassium concentrations in foliar tissue

Plants harvested 15 days after germination showed a trend of higher K concentrations (about two fold higher) under the two treatments which included *E. poeppigiana* pruning residues compared to plants under BARESO. This trend remained at 38 days but in less proportion (Table 5). At 15 days plants under the "MIXED" treatment also showed a trend to higher values (10%) in comparison to the "UP" method, but this trend had disappeared after 40 days. Unfortunately, at 15 days, an ANOVA test could not be applied because the size of the plants did not permit enough material for testing, and replicates were combined for analysis. At this date, MICROB showed a trend of higher K

(12%) concentrations than RESID under the "UP" method (Table 5). Forty days after germination no significant differences were found between methods or between the MICROB and RESID treatments. However, a trend of higher values was observed for MICROB in comparison with RESID (7%, 11% for UP and MIXED, respectively; Table 5). From day 15 to day 38, average K concentrations in foliar tissue across treatments dropped 28% and 39% for UP and MIXED, respectively.

5.3.1.5. Nitrogen concentrations in foliar tissue

Fifteen days after germination, plants which received pruning residue additions had higher N foliar concentration than the BARESO plants. These significant differences were also found at 38 days. At day 15, plants under the "UP" method had on average 18% higher foliar N concentrations than under the "MIXED" method ($p<0.05$; Table 6), but these differences disappeared at the end of the experiment. No differences were found in N foliar concentrations between MICROB and RESID for any of the methods of application at any date. Average foliar N concentrations across treatments dropped strongly from day 15 to day 40 (86% and 88% for "UP" and "MIXED", respectively).

Table 5. Potassium concentration (%) in maize seedlings grown in soil from the 10-20 depth layer from the organic coffee farm in CATIE, for two application methods: "UP", plant residues on soil surface; and "MIXED", plant residues mixed into the soil. Treatments are: RESID, addition of 8 g DM of pruning residues only; MICROB, 8 g DM of pruning residues + 50 ml 10 kg^{-1} microbial mixture; BARESO, bare soil

Method:	Up				Mixed			
	RESID	MICROB	BARESO	Avg	RESID	MICROB	BARESO	Avg
15 days after Germination	5.20	5.85	2.48	4.51	6.04	6.09	2.79	4.97
38 days after Germination	3.81a[1]	4.11a	2.69b	3.53A	3.67a	4.09a	2.54b	3.57A

[1] Values with the same letter within a category or between averages are not significantly different (Lsmean-test $p<0.05$)

Table 6. Nitrogen concentration (%) in maize seedlings grown in soil from the 10-20 depth layer from the organic coffee farm in CATIE; for two application methods: "UP", plant residues on soil surface; and "MIXED", plant residues mixed into the soil. Treatments are:

RESID, addition of 8 g DM of pruning residues only; MICROB, 8 g DM of pruning residues + 50 ml 10 kg^{-1} fresh residues of microbial mixture; BARESO, bare soil

Method:	Up				Mixed			
	RESID	MICROB	BARESO	Avg	RESID	MICROB	BARESO	Avg
Nitrogen concentration 15 d[2]	2.85a[1]	2.95a	1.72b	2.51A	2.40a	2.41a	1.59b	2.13B
Nitrogen concentration 38 d	1.28a	1.35a	0.92b	1.18A	1.28a	1.32a	0.81b	1.13A

[1] Values with the same letter between treatments or between averages are not significantly different (Lsmean-test $p<0.05$)
[2] Days after germination.

5.3.2. CO$_2$ production from pruning residues
5.3.2.1. Trial I

After eight days of treatment applications, the two treatments which included pruning residues had maximum CO$_2$ values (Table 7). At this date, RESID and BARESO had notably higher values than BARESO. Overall, BARESO had lower average amounts of CO$_2$ for the five measurement dates (0.60 mg CO$_2$ –C 100g^{-1}). This treatment showed more stable measurements during the experiment although small amounts of CO$_2$ were produced. No significant differences between MICROB and RESID were found at any of the five measurement dates. A day 8, similar values were found for MICROB and RESID. The next three readings (22, 36 and 50 days after application of treatments) showed a trend of higher values for RESID in comparison to MICROB, and both treatments showed high variability between replicates. At the end of the experiment (day 70) these two treatments again had similar values and lower variability between replicates. All measurements are presented in Appendix, Trial I.

5.3.2.2. Test II

The respiration rates measured at 8 and 15 days for both MICROB and RESID were higher than at 19 and 24 days. A peak was observed for the two treatments at 15 days and then CO$_2$ production decreased notably for the last two measurement dates. At 15 days,

variability between laboratory replicates was higher for MICROB than RESID which had relatively low variability for the last three measurement dates. A trend of higher values, on average, was read for MICROB (31%) than RESID at 8 days after spraying. At the end of the experiment (24 days after spraying), the opposite trend was observed with RESID having higher values than MICROB (Table 8). In this test, significance could not be evaluated as the treatments were not replicated in the laboratory.

In the last experiment (Trial II c), there were no differences between the EM and RESID treatments during the four measurement dates. The CO_2 production decreased with time after a peak at day four. The trend of higher values under MICROB observed in test II at day 8 was not observed at the same date using EM (Table 9).

Table 7. Trial I: CO_2 production (mgCO_2/100g h^{-1}) from *E. poeppigiana* residues. Treatments were: MICROB, 12.5 g of fresh residues with 50 ml 10 kg^{-1} of microbial mixture mixed with soil 1:9 ratio (DM/DM); RESID,12.5 g fresh pruning residues only mixed with soil 1:9 ratio (DM/DM); and BARESO, soil only

Days Treatments	8	22	36	50	70
MICROB	12.3 (1.9)a[1][2]	3.4 (1.3)a	4.5 (1.7)ab	4.2 (2.0)a	3.5 (0.6)a
RESID	11.2 (1.3)a	4.1 (1.3)a	7.3 (2.7)a	4.6 (1.4)a	3.7 (0.3)a
BARESO	0.5 (0.2)b	0.7 (0.2)a	0.9 (0.4)b	0.3 (0.1)a	.

[1] Standard error (n=4)

[2] Values with the same letter within a column are not significantly different (Duncan test $p<0.05$).

Table 8. Test II CO_2 production (mgCO_2/100g h^{-1}) from *E. poeppigiana* pruning residues. Treatments were: MICROB, 12.5 g of fresh residues with 50 ml 10 kg^{-1} of microbial mixture; RESID,12.5 g fresh pruning residues only; and BARESO, bare soil

Days Treatments	8	15	19	24
MICROB	1.6 (0.3)[1]	2.3 (0.6)	0.6 (0.1)	0.4 (0.1)
RESID	1.1 (0.2)	2.1 (0.1)	0.8 (0.1)	1.1 (0.1)

[1] Standard error for laboratory replicates (n=4)

Table 9. Test II c: CO_2 production (mgCO_2/100g h^{-1}) from *E. poeppigiana* pruning residues. Treatments were: EM, 12.5 g of fresh residues with 50 ml/10 kg^{-1} of Effective Microorganisms (EM ™); RESID,12.5 g fresh pruning residues only

Days	1	4	8	12

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Treatments				
EM	0.27 (0.05)[1]	2.93 (0.61)	0.74 (0.19)	0.61 (0.14)
RESID	0.32 (0.18)	4.03 (0.22)	0.71 (0.06)	0.85 (0.14)

[1] Standard error for laboratory replicates (n=4)

5.3.3. Nitrogen and potassium availability in the field trial

In 2002, nitrification (only measured in CATIE) was lowest during the 220-250 day period (March 2003) when lower rainfall occurred (Table 10) (Mean temperature and monthly rainfall are presented in Figure 2, Chapter IV). No differences between treatments were found for nitrification and ammonium release for any of the three sampling periods at CATIE (Table 10).

Table 10. Net N mineralization (mg kg^{-1} soil) at 0-5 cm depth in organic coffee plantations in CATIE, Costa Rica, 2002. Treatments were: RESID, addition of 10 Mg ha^{-1} yr^{-1} of pruning residues; EART, RESID + two times 40-50 g m^{-2} live earthworms; MICROB, RESID + microbial mixture "A"; COMPOS, RESID + microbial mixture "B"+ turnover of the residues; BARESO, bare soil

Sampling periods (days)	Nitrate (mg kg^{-1} soil)					Ammonium (mg kg^{-1} soil)				
	COMPOS	EART	MICROB	RESID	BARESO	COMPOS	EART	MICROB	RESID	BARESO
190-220	11.7	10.8	25.3	21.3	19.9	2.4	1.7	2.5	2.5	2.8
	(4.9)[1]	(5.0)	(9.3)	(3.0)	(2.3)	(0.9)	(0.7)	(0.6)	(0.2)	(0.3)
220-250	-9.5	3.7	16.4	8.6	12.0	2.4	1.3	2.6	2.2	1.7
	(3.4)	(2.6)	(10.9)	(9.0)	(5.4)	(0.4)	(0.2)	(0.3)	(0.4)	(0.2)
270-300	26.6	23.9	27.3	34.6	23.9	1.9	1.5	1.3	1.5	1.4
	(5.3)	(5.3)	(5.6)	(6.5)	(1.8)	(0.3)	(0.3)	(0.1)	(0.2)	(0.2)

[1] Standard error (n = 3)

Soil K concentrations at CATIE, were approximately double the values for Pejivalle (Table 11). An increase of 44% in soil K concentration was observed between 0 and 90 days for EART in Pejivalle, but no increase for soil K under EART was observed at

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CATIE. The concentration value under COMPOS after 330 days at CATIE was 32% higher than in BARESO. No other differences between treatments were detected in either site. All soil K values at 330 days in both CATIE and Pejivalle were lower than at 0 days. The bare soil control had consistently lower K concentrations for any given date than treatments that included pruning residue additions, but this occurred before any treatments were applied (time 0 days; but no significance was detected). At CATIE, the bare soil control had larger decreases in soil K concentration than in Pejivalle.

Table 11. Soil K concentration (cmol kg^{-1}) at 0-5 cm depth in organic coffee plantations in CATIE and Pejivalle, Costa Rica (2002). Treatments were: RESID, addition of 10 Mg ha^{-1} yr $^{-1}$ of pruning residues; EART, 10 Mg ha^{-1} yr^{-1} of pruning residues + two times 40-50 g m^{-2} live earthworms; MIC A, 10 Mg ha^{-1} yr^{-1} of pruning residues + microbial mixture "A"; MIC COM, 10 Mg ha^{-1} yr^{-1} of pruning residues + microbial mixture "B"+ turnover of the residues; BARESO, bare soil

Sampling dates (days)	K concentration (cmol kg^{-1})									
	CATIE					Pejivalle				
	COMPOS	EART	MICROB	RESID	BARESO	COMPOS	EART	MICROB	RESID	BARESO
0	0.51(a)[1]	0.54(a)	0.44(a)	0.49(a)	0.42(a)	0.25(a)	0.25(a)	0.24(a)	0.22(a)	0.18(a)
90	0.50(a)	0.51(a)	0.43(a)	0.47(a)	0.39(a)	0.24(b)	0.36(a)	0.25(ab)	0.23(b)	0.20(b)
330	0.44(a)	0.34(b)	0.32(b)	0.36(ab)	0.30(b)	0.16(a)	0.18(a)	0.18(a)	0.19(a)	0.17(a)

[1] Values with the same letter within a row for the same site are not significantly different (Duncan $p<0.05$)

In the 2004 field trial, RESID and MICROB lead to significantly higher soil K concentrations than BARESO 30 days after application, but no significant effect of microbial mixture additions was detected (Figure 4). Although the baseline data for MICROB was higher than RESID, averaged soil K concentrations after 30 days tended to be slightly higher (9%) under MICROB (Figure 4). When data were analyzed block by block, the effect of MICROB was inconsistent (in blocks 2 and 3, higher values for MICROB than for RESID were observed; blocks 4 had the same values and in block 1, RESID had higher K concentration than MICROB) (Table 12).

Table 12. Soil K concentrations 0-5 cm in a field trial at 0 and 30 days after residue application in CATIE, 2004. Treatments are: RESID, addition of 10 Mg ha^{-1} yr $^{-1}$ of pruning residues; MICROB, 10 Mg ha^{-1} yr^{-1} of pruning residues + microbial mixture; BARESO, bare soil

Block	I			II			III			IV		
Treatments/Days	0	30	Difference	0	30	Difference	0	30	Difference	0	30	Difference
BARESO	0.47	0.39	-0.08	0.32	0.31	-0.01	0.31	0.31	0.00	0.45	0.42	-0.03
RESID	0.54	0.66	0.12	0.38	0.37	-0.01	0.42	0.48	0.06	0.35	0.53	0.18
MICROB	0.64	0.69	0.05	0.45	0.48	0.03	0.35	0.47	0.12	0.44	0.62	0.18
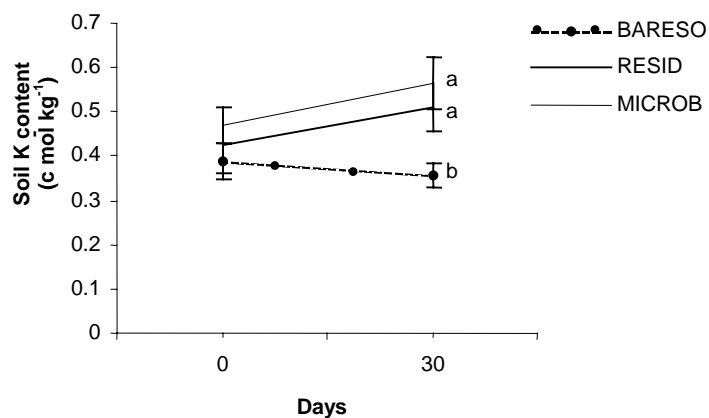
Figure 4. Soil exchangeable K at 0-5 cm in a field trial, CATIE, 2004. Treatments were: RESID (——), addition of 10 Mg ha^{-1} yr $^{-1}$ of pruning residues; MICROB (——), pruning residues + 50 ml 10 kg^{-1} of microbial mixture; BARESO (• -• -•), bare soil

5.4. Discussion

5.4.1. Impact of microbial mixtures on maize seedling growth indicators

The possibility of residue manipulation to obtain enhanced microbial activity and reduced nutrient losses from leaching was suggested by Mafongoya *et al.* (1998). One of the main objectives of this study was to test this approach on maize seedling growth, after the addition of a microbial consortia (MICROB) to *Erythrina poeppigiana* pruning residues.

5.4.1.1. Maize seedling height

In the current study, a positive effect of *E. poeppigiana* mulch was detected on plants grown with soil collected from 10-20 cm depth (Figures 3a and b). In greenhouse experiments, significant increases in growth of maize seedlings have been measured as a result of mulching (Kershnar and Montagnini 1998, Muñoz 2002, Osorio 2004). Heights of maize seedlings during the current greenhouse experiment were comparable to those found by Kershnar and Montagnini (1998) in a 30-day greenhouse trial with *Albizia guachapele* (a legume with high quality pruning residues). Differences in maize growth between treatments with *E. poeppigiana* mulch and bare soil treatments have often been found in field trials and been attributed to the high N, K and Ca content in *E. poeppigiana* pruning residues (Kass *et al*. 1993, Soto *et al*. 1993).

A relatively small but significant difference in height between the MICROB and RESID treatments was detected at five measurement dates from day 13 to day 17 (Figs. 3a and b). The positive effect of the MICROB treatment was probably due to the microbially enhanced decomposition of plant material and nutrient release as a result of increased microbial populations in the first days after microbial mixture application. However, significant differences in plant height were found only within a five day period (age 13-17

days), indicating that the initial higher flux of nutrients under the MICROB treatment was equaled, with no artificial intervention, under the RESID treatment for the last 20 days of the trial. This can be partially explained as a result of homeostasis processes. Usually, after a disturbance on microbial populations, regulating mechanisms related to nutrient reserve depletion and secondary metabolite production, lead to the reestablishment of normal population levels (Atlas and Bartha 1998). In addition, a rapid die-off of microbial inocula added to soil is a common phenomenon.

Another explanation for the low impact of the microbial inocula MICROB is related to the high quality of *E. poeppigiana* residues. In natural field conditions *E. poeppigiana* residues have fast decomposition rates. Mass and remaining C content in litter usually decrease after four weeks of lying on the soil surface; in the same period, only 40% of initial P and Ca content and 25% of initial Mg and K remain in the residues (Munguía *et al.* 2003, Szott *et al.* 1991, Tian 1998). Application of the microbe inocula sprays could have occurred too close in time to the natural major flux of nutrient release (i.e. the initial phase of decomposition). It may be difficult to accelerate a process that is naturally fast due to the high quality of the tree residues. Further studies of microbial inocula addition may focus on their application to pruning residues during the more secondary, more recalcitrant phase of decomposition. Microbial biomass has been studied as a living nutrient reservoir (Gunapala and Scow 1998). Srivastava and Lal (1994) found that increases in soil microbial biomass led to significantly higher productivity in a rice (*Oryza sativa*) field in India through enhanced nutrient release. In the aforementioned study, higher microbial activity was found after applications of cow manure to the soil. Their study found an annual flux of N through the microbial biomass of 30-45 kg ha^{-1}yr^{-1}.

In our experiment, however, the absence of significant differences in seedling heights after day17 (Figures 3a and b), indicated that the probable enhanced nutrient release due to microbial inocula addition (MICROB) was limited to the first two weeks after application of the microbial mixtures. These results also indicated that no immobilization of nutrients from pruning residues in the microbial biomass was obtained with MICROB. If a larger microbial population could have been induced for more than two weeks with

MICROB, nutrients might have been immobilized in microbial biomass. A gradual nutrient release, and thus higher plant growth under MICROB vs RESID, should have been detected as microorganism turnover at later stages of the experiment. The study of Gunapala and Scow (1998) found increases in microbial biomass 50 days after the addition of 120 kg N ha^{-1} from vetch (*Vicia dasycarpa*) green manure in organic farming systems. Similar tomato yields were obtained in conventional and in organic systems although organic systems did not receive chemical fertilizers; and higher nutrients availability from increased microbial biomass turnover in organic systems was hypothesized as one of the causes. In the current study, no later effects on plant responses under MICROB were detected, at least not in the 40 days of the greenhouse trial.

5.4.1.2. Maize seedling biomass

Maize seedling biomass values in the present study were similar to the results obtained by Osorio (2004). That study found higher biomass in maize seedlings in pots mulched with *E. poeppigiana* residues in comparison with plants mulched with *Inga edulis* residues in a 32-day greenhouse trial.

Differences in maize dry matter production between the MICROB and RESID treatments were only apparent in soils from the 10-20 cm layer and were not higher than 15% during the first 15 days reducing to 7-8% at 40 days; thus indicating that the effect of microbe application can only be detected in less nutrient-rich soil (Table 1). The application of microbes had an initial effect (28%; Table 2) when residues previously sprayed with microbes are mixed with the soil in comparison with the UP method. This result indicated that when the pruning residues were mixed into the soil, they created better conditions (e.g. constant soil moisture) for the introduced microbes (MICROB), resulting in an increase in plant growth. However, this effect was lower (10%) at the end of the experiment. A probable explanation for the lack of a response with microbial mixtures under the UP method is the sensitivity of microbial populations, particularly bacteria, to wetting and drying cycles. Soil fungi are more resistant to desiccation and have advantages over bacteria at lower water contents (Alexander 1977, Doran 1980). Holland and Coleman

(1987) found 44% higher fungal biomass-C when straw residues were placed on the soil surface in comparison with treatments where the straw was incorporated- into the soil. Salas *et al.* (2003) found P immobilization in fungal biomass growing on fresh *Crotalaria juncea* residues added to the soil. These findings are important for the current study because usually in aqueous organic amendments, as used in the MICROB treatment, bacteria are predominant due to their higher resistance to low O_2 pressures (Krauss 2004). Further, these organisms probably were not so active in residue decomposition and nutrient release when applied to residues in the UP method. Moreover, if fungi were the predominant microbial population on *E. poeppigiana* residues, P immobilization could have occurred.

In addition, drying and wetting cycles as well as temperature, not only in the soil but in the plant residue materials, have an important influence on microbial activity after the addition of fresh plant residues to the soil surface (Cabrera *et al.* 2005). A reduction in decomposition rates of plant residues was reported by Clein and Schimel (1993) in dried and rewetted birch litter when compared with continuously moist litter due to diminished microbial activity. Schomberg *et al.* (1994) suggested that drying and rewetting events have more intense effects when residues lie on the surface than when they were incorporated into the soil. These findings suggested that the microbial populations tested in the current study found better conditions when residues were incorporated into the soil. Indeed, residues under the "UP" method appeared to have dried out before the daily afternoon irrigation in the greenhouse and this dry-wet cycle could have negatively affected the microorganisms applied in MICROB. Further work is therefore required to determine the sensitivity of different components of the soil microbial community in these two plant residue incorporation methods.

5.4.1.3. Stem cross sections[+]

[+]Shorter and longer stem diameters were respectively considered as the short and the long axes of an ellipse for calculating the stem cross section

One of the main plant growth indicators (stem cross section) showed the effect of *E. poeppigiana* pruning residue additions in comparison to the bare soil control, but no significant differences for cross section between the MICROB and RESID treatments were found at any date (Table 3 and 4). Shorter stem diameter (short axis) measurements showed differences between the RESID and MICROB treatments at 15 days (Tables 3 and 4), However, the influence of long diameter (long axis), which did not show any difference, eliminated differences in the product of the formula for cross section calculation. In summary, the use of the microbial inocula did not have a clear effect in seedling stem width.

In soil from 10-20 cm a positive effect of MICROB was observed in higher foliar biomass and seedling heights but was always limited to the first two weeks after germination. Growth indicators have not always been consistent in detecting effects after mulch application. Different results were found using total biomass and height as response variables when mulches from four tree species were compared (Kershnar and Montagnini 1998). While differences in biomass were found with a mixed species plant mulch and *A. guachapele* mulch, no such differences were found with height. Muñoz (2002) in a study of the effect of coffee-pulp compost and vermicompost on maize seedling growth, found that height and total biomass did not correlate. The higher growth rates found under coffee-pulp compost were not found when total biomass was measured as a response variable. In the current study, a similar situation was observed when responses for foliar biomass at 40 days were not coincident with total biomass at the same dates; similarly, short stem diameters did not show the same results as long stem diameters for the same measurement date. Height and foliar biomass seemed to be more sensitive as response variables.

5.4.2. Nutrient concentrations in foliar tissue of maize seedlings

In the current study, average K concentrations in maize seedlings (Table 5) were within the range found in a study of the effects of soil temperature on nutrient uptake on maize shoots (3.3-6.8%, two weeks after germination) (Lal 1974). The trend of higher K concentrations in maize shoots grown with the MIXED method is in agreement with some of the findings on growth indicators. A possible explanation for the trend of higher residue K release and the positive plant growth response under the MIXED method, is the better

environment for microorganisms when the residues are buried into the soil. Holland and Coleman (1987) reported higher microbial activity when straw residues were incorporated into the soil in a field trial. This higher microbial activity was also detected in a laboratory test with the incorporation of wheat straw into the soil. Higher ^{14}C incorporated into the soil microbial biomass was found when straw residues were buried in the soil in comparison to when residues were applied to the soil surface. The study suggested that the pool of microorganisms involved in SOM decomposition was higher when residues were incorporated into the soil. Lehmann *et al.* (1995) found higher decomposition rates of *Gliricidia sepium* and *Calliandra calothyrsus* pruning residues when they were incorporated into the soil in comparison with surface placement.

The greenhouse trial using pots detected differences for foliar N concentrations between the two treatments that included pruning residues and the control (showing the impact of pruning residues on N release) ; but no differences were found between the MICROB and RESID treatments. However, foliar N concentrations in plants grown under the UP method had higher values than the MIXED method after 15 days but not after 40 days (Table 6). This result can be related with a N immobilization due to higher microbial biomass when residues were buried in the soil. Microorganisms have advantages in comparison to plants in N uptake; therefore, when residues of C:N ratios of about 20 or higher are added to the soil, available soil N is immobilized in the microbial biomass (Paul and Clark 1996). On the contrary, a net N mineralization is usually observed when residues have a C:N ratio lower than 20. Thus, in the case of *E. poeppigiana* with a C:N ratio of 11 (Palm and Sánchez 1991), net mineralization of N was expected. Nevertheless after residues were mixed into the soil, the lower content of N in the foliar tissue of maize plants indicated that in very early stages immobilization could occur and N limitations affected the maize seedlings. Haggar *et al.* (1993) found a net immobilization of N in cores incubated closest to the *E. poeppigiana* trees in alley cropping systems that received *E. poeppigiana* pruning residues. Only two measurements could be taken in the present study (at 15 and at 40 days, when plants could be harvested) and soil N uptake by plants did not explain the differences in growth and biomass that were detected for the MICROB treatment in the first two weeks of the experiment in soil form 10-20 cm. This finding

indicated that nutrients other than N in the *E. poepiggiana* residues are also important in maize seedling growth. This finding concurs with Kershnar and Montagnini (1998), who found that Ca and Mg and not only N can determine the influence of mulch on plant growth. Important amounts of Ca and Mg have been reported in *E. poeppigiana* pruning residues (Palm and Sánchez 1991, Kass *et al.* 1993). Another explanation of the higher foliar N concentrations under the UP incorporation method, could be that a faster decomposition and N release occurred under this method during the first 15 days of the experiment, but this was not the general trend for foliar and total biomass, minor diameter as well as foliar K concentrations, where the MIXED method showed a better performance than UP in the first 15 days of the experiment. Finally, the decreasing values of K and N from day 15 to day 38 were the result of the dilution of these nutrients in a higher biomass. After 23 days the rate of biomass increase was faster than the amount of nutrient taken up, thus leading to lower nutrient concentrations.

5.4.3. *In vitro* evolution of CO_2 from pruning residues and its relationship with maize seedlings growth variables

One of the main results of the biological decomposition of residues, which implies oxidative digestion reactions, is CO_2 production (Lavelle 1997). Measurements of CO_2 using the substrate-induced respiration (SIR) method have been considered as a respiratory response of microbial populations to amendments with a source of C and readably available energy (Anderson and Domsch 1978). The impact of pruning residue additions into the soil on CO_2 production was observed in Trial I because the two treatments which included pruning residues had higher values than the soil-only control during the five experimental dates (Table 7). CO_2 evolution in both the RESID and MICROB treatments had maximum values at day eight and then they decreased to a stable level until day 70 indicating that an early response to plant residue addition was later eliminated by homeostasis among microbial populations (and an exhaustion of labile substrate). This trend to higher CO_2 production in the first fifteen days of the experiment was also observed in Test II for the MICROB treatment. This could also be partly attributable to the death and mineralization of the added microbial consortia. No significant differences were found between the RESID

and MICROB treatments in both Trial I and Test II at any experimental date. A great variability between replicates, indicated by standard error values, was observed in Trial I for all the treatments until day 50 (even when temperature and residue moisture were strictly controlled). High variability in CO_2 production has been observed when organic fertilizers have been tested using SIR; and probably this variability can be attributed to a non-homogeneous colonization of the substrate by microorganisms (Ramírez 2004). Although significance in Test II was not evaluated (unreplicated test, Table 8), at eight days MICROB showed a trend of higher values than RESID when pruning residues were tested without being mixed with soil. This was the only date when this occurred, and at 24 days RESID showed the opposite trend. This result indicated a reduced impact of MICROB in microbial activity (CO_2 production) that was limited to the first week after applications.

The results for CO_2 production in the Test II concurred with Hadas *et al.* (1996). In that study, evolution of CO_2 was monitored during a 33 week laboratory trial testing the effect of organic material additions on two soils (one that had received cattle manure for 30 years and a control soil). Higher CO_2 levels in treated soil were only detected in the first week and values tended to converge with time. Respiration rates in the present work were near the lowest limit of the range obtained by Beare *et al.* (1991). Beare's study correlated the SIR rates from different quality plant residues with their decomposition rates and found values from 10 to 200mg CO_2-C $100g^{-1} h^{-1}$. The lower CO_2 production in the current study was due to different preparation of plant material (leaves were chopped and not blended, lower concentration of glucose added to the material).

In the current study, CO_2 production tended to be higher when residues were mixed with soil (using the SIR method in Trial 1) than when they were put alone in the testing flasks (Test II). This probable higher activity in the laboratory trials and associated nutrient release can help explain the higher biomass of plants under the MIXED method and also the better performance of microbial mixtures in comparison to the RESID treatment under this method. The constant moisture and contact of plant residues with soil nutrients help to explain these better results under the MIXED treatment.

The lowest impact of the EM on CO_2 production in Trial II c (Table 9), particularly at day 8 when MICROB show a trend of higher values than RESID in Test II, indicated that indigenous microbial strains used in Trials I and II had better response than exotic strains (a trend of higher amounts of CO_2 after residue addition *in vitro*) when *E. poeppigiana* residues were used as a substrate for microbial growth. MICROB was prepared with mycelium growth on *E. poeppigiana* litter collected near CATIE's organic farm. The advantage of indigenous microbial strains in occupying ecological niches has been addressed by Atlas and Bartha (1998) and should lead to more studies on the use of these microbial mixtures based on "on farm" microbial collections.

The higher values for maize seedling growth under MICROB between days 13 and 17 in the 10-20 cm soil, concurred with a trend to higher CO_2 values at day 8 in Test 2. Probable higher microbial activity during the first week of the greenhouse experiment could lead to a higher nutrient release, and thus to a response in seedlings growth. After 15 days respiration values tended to be similar or higher for RESID. Concurrently, after day 17, the differences between MICROB and RESID in maize seedling heights were not significant any more. These results again indicated that homeostatic processes limited the weak impact of MICROB to the first two weeks after the spraying date.

5.4.4. Soil K and N availability in the field trial

The positive effect of *E. poeppigiana* pruning residues on soil K concentrations have been reported by Beer (1988) and Munguía *et al*. (2003). In the 2002 field trial there were indications that the addition of residues provided more K to the soil when compared to a bare soil control. In CATIE and Pejivalle at 90 days, although not significant, all treatments that had residue additions had a trend of higher values than the control (Table 11). Furthermore, in the 2004 field trial in CATIE, at 30 days significant differences were found between the two treatments that included pruning residues in comparison with the BARESO treatment (Figure 4). In a field trial in a *Typic Humitropept* in Turrialba, Costa Rica, Soto *et al*. (1993) found higher exchangeable K in the 0-60 cm depth of mulched soil

in comparison to an unmulched control (0.58 vs 0.35 cmol kg^{-1}). Soil K concentrations in the current study are in line with their findings.

It was noticeable that after 330 days, K concentrations in both sites in all the treatments had reduced in comparison to the day 0 values, indicating that the amount of residues added to the soil were insufficient to maintain K concentrations, mirroring the trends found for soil C contents in the size density fractions in Chapter IV. In experiments using plastic mesh bags ("litter bags") for measuring tree pruning residue decomposition, Szott *et al.* (1991) found that after four weeks only 25% of the initial K in *E. poeppigiana* residues remained. The study suggested that the use of high quality plant residues such as those from leguminous trees can lead to asynchrony between nutrient release and plant demand in critical phenological stages. This idea was sustained by the results in the current study because in Pejivalle higher amounts of soil K were found in the plots that included residue additions at 90 days, but not at 330 days (Table 11). In addition, in CATIE, significant differences between residue-added plots and controls were found at 30 days (Figure 4). However, as indicated above, the importance of *E. poeppigiana* pruning residue additions in providing K to the soil was evident in CATIE (at 90 days in 2002 and at 30 days in 2004), and in Pejivalle (90 days in 2002), where values for residue added treatments showed a consistent trend of higher values than in the bare soil control.

At 90 days, K concentrations under the earthworm treatment (EART) at Pejivalle was significantly higher than RESID indicating a positive effect of earthworm inoculation in providing K to the soil. However, at 330 days (180 after the second residue addition) no effect in the EART treatment was detected in comparison to the other treatments. At this date, either loss of exchangeable K by leaching due to heavy rainfall, or protection within the earthworm casts could have occurred, or the earthworms had died, and the differences, if any, were not detected. The results at 90 days were noticeable because K has a very fast release rate. Cobo *et al.* (2002) found the highest release rates for K in a comparison of the nutrient release and decomposition rates of 12 plant materials ($k = 0.028$ d^{-1}) on hillsides in Cauca, Colombia. Lavelle (1997) has suggested that the introduction of earthworms can trigger a flush of biological activity that cannot be sustained in the long term due to the

limitation of food supply for the increased populations; i.e. the quality and amount of organic inputs can limit earthworm activity. These suggestions were sustained by the results in the current study because 180 days after inoculation no effect could be detected. In addition, no significant effect for soil K concentrations under EARTH was detected at CATIE at any sampling date, probably because the CATIE organic farm received annual doses of a K based fertilizer (Table 1, Chapter III) thus raising the baseline levels. Soil K values in CATIE were almost double the values at Pejivalle at 90 days because an annual commercial organic fertilizer (Kmag 22% K_2O) was applied in the CATIE organic farm five months before the trial was set up.

Earthworms are thought to participate in SOM dynamics either by accelerating C mineralization or increasing SOM-C stabilization in macro-aggregates. Earthworms can accelerate the decomposition of organic matter and nutrient release through comminution of plant residues on which they feed (Syers and Springett 1984). In addition, earthworm casts, formed from digested soil, incubated for 420 days had C mineralization rates 3.3 times higher than the control soils (Brown *et al.* 1996). On the other hand, endogeic earthworms can also induce SOM-C and nutrient stabilization by mixing organic matter and clay minerals in resistant soil aggregates and thus contribute to maintaining SOM and nutrient levels (Anderson and Flanagan 1989). In field experiments in the Ivory Coast, C stock losses were reduced by 8% after 3 years of yam cultivation when they were inoculated with earthworms (*Millsonia anomala*) (Gilot *et al.* 1996). In a field experiment at the National Agricultural Research Institute station, at Yurimaguas, Peru, significantly higher soil K was also measured after earthworm inoculation. These higher values were detected when compared with soil in non inoculated plots in the last year of a seven year field trial after a continuous maize cropping period (Gilot *et al.* 1996). In the current study, the effect of earthworm inoculation was not consistent in the two experimental sites, and no effect was observed 180 days after inoculation. Nevertheless, in Pejivalle an increased release of K after 90 days of inoculation was in line with the findings of Gilot *et al.* (1996).

No significant differences in soil K were detected between the MICROB and RESID treatments in 2002, except for a weak trend of higher values under the COMP

treatment at 330 days at CATIE (Table 11). In 2004, no significant differences were found between the MICROB and RESID treatments during a 30-day study period. The inconsistency of the effects of microbial additions in the field trial was observed when data were sorted by blocks. Microbial consortia addition (MICROB) worked in some blocks but not in others (Table 12). This situation indicated that the activity of the added microbial populations is highly sensitive to micro-environmental field conditions because when environmental and soil conditions were homogenized (greenhouse trial) the small effect of MICROB was statistically detected. Usually, in liquid organic amendments, bacteria are predominant in the microbial community (Krauss 2004), and these organisms are very sensitive to temperature, moisture and O_2 pressure changes (Holland and Coleman 1987). In the greenhouse experiment, plants under the MIXED method (residues had constant high moisture levels) showed indications of higher biomass using microbial mixtures, but not under the UP method (residues had variable moisture). In the field trial, pruning residues were applied to the surface (UP method) and there was no response or it was inconsistent in the different plot conditions and was not statistically detected. Further work is required to determine the temporal changes in the population density of the added microbial inocula. This result indicated that under field conditions MICROB, COMP and EART are ineffective in practical terms.

The absence of effects from the microbial and earthworm treatments on soil K in the 2002 trial was thought to be due to the fast liberation of K and its great mobility in the soil, i.e., it was probably that any initial difference had disappeared when the first measurement was made after 90 days. However, in 2004, soil K was measured 30 days after treatment application, and again no significant effect from MICROB was detected. This result indicated that sampling timing was not the cause for the absence of differences between MICROB and RESID. It is more probable that a strong effect of accumulated organic residues from previous years masked any changes. The fact that samples were taken in the 0-5 cm layer, which had relatively high organic matter content (5.0%, Table 2, Chapter III), could have masked some effects of microbial addition. Furthermore, in the greenhouse trial, effects were only detected in pots filled with soil from the 10-20 cm layer (Figures 2 and 3).

Unlike K, no differences were found between treatments for soil NO_3^- and NH_4^+ (Table 10; only measured at CATIE), possibly because of the high initial N and SOM concentrations at this site (0.48% N and 5.0% total C, at 0-5 cm depth). Arana (2003) found no effects of pruning residue addition on mineralized soil N, in organic farms and suggested that the high initial soil N concentrations hid the effects of the residues in the short term (9 months). However, some studies have calculated the mineral N released through earthworm activity as being between 25-150 kg mineral N ha^{-1} in tropical grasslands (Lavelle *et al*. 1992). Gilot *et al*. (1996) measured higher mineralized N with earthworm inoculation in the previously mentioned experiment at Yurimaguas, Perú. However, Haggar *et al*. (1993) found that the higher N availability under *E. poeppigiana* mulched plots in comparison with a bare soil control was the result of long term accumulation of SOM, and hence soil organic N that took years to build up. Haggar *et al*.'s study concluded that recently added residues contribute only a small proportion of mineral N uptake by maize plants. Soto *et al*. (1993), in the field experiment mentioned above, comparing mulched soil vs unmulched control, found similar values for total soil N (2.2 g kg^{-1} for mulch and control treatments, respectively) after nine years of mulch application. However, higher maize yields were registered compared to unmulched controls (3200 vs 2100 kg ha^{-1}).

Several studies have reported that in the first 30 days of the decomposition process of high quality litter material, 25-65% of the original N (3.5-5.7%) is released (Mafongoya *et al*. 1998, Palm and Sánchez 1991, Munguía *et al*. 2003). Berhard-Reversat (1987) reported that the light fraction (LF) under *Acacia seyal,* which also has easily decomposable residues with a similar lignin:N ratio to *E. poeppigiana,* contributed 50-70% of the mineralized N in the early decomposition stages. In the 2002 field trial, the soil was sampled 40, 75 and 120 days after the second pruning residue application. It is probable that the residues had already lost an important part of the N content contributed by these applications prior to sampling. Nitrogen could then be lost by leaching or volatilization. Since *E. poeppigiana* has a fast decomposition rate with a lignin:N ratio of 2.76 and 3.5% of N (Palm and Sánchez 1991), maybe soil sampling should be done earlier. Once again, the absence of differences was thought to be the delayed sampling time. However, the

effects of treatments on N availability were not detected in the greenhouse trial during a 40-day period with first measurements 15 days after residue application. Effects were not detected either on soil from the 0-5 cm layer or from the less fertile 10-20 cm layer. The suggestion that soil N availability and plant response did not depend on recently added residues, but rather on the accumulated soil N in SOM (Haggar *et al.* 1993), seems to be the better explanation for the lack of differences between treatments in the present study.

On the last sampling date, for nitrates and ammonium, some of the plots were inundated due to high rainfall. Hence, an important amount of nitrate may have been lost when the tubes were removed from the ground. He *et al.* (2000) found that between 60 and 80% mineralized N was leached from a compost six months after application in a sandy soil in an area with 1458 mm annual rainfall. Lixiviation of mineralized N in areas with high rainfall levels has been cited as a major drawback of the *in situ* mineralization method (Anderson and Ingram 1993).

5.5. Conclusion and recommendations

The beneficial effects of *E. poeppigiana* pruning residues on soil K concentrations were shown in both the greenhouse experiment and in the field trials, especially in soils with poor nutrient levels. However, the effects of treatments on mineralized N and N uptake were not detected in any of the trials. The effects of microbial mixtures (increasing seedling growth and K uptake by seedlings) were detected in controlled environmental conditions in the greenhouse, but only to a very small degree in soils from the 10-20 cm layer, and during the first two weeks of residue decomposition. A trend to higher microbial activity, measured through CO_2 production, was also detected in the first week of an *in vitro* test and could be associated with what was observed in the bioassay.

An increase of 9% in maize seedling growth on nutrient poor soils suggests a limited effect of the addition of microbial mixtures. Even if there was such a temporary increase in maize growth (age 13-17 days), which had disappeared by age 38 days, a recommendation to use these microbial mixtures on pruning residues for improving crop

nutrition are not justified. In soils with medium or high soil nutrient levels (0-5 cm depth), no positive effects were observed and therefore the use of these microbial mixtures definitely cannot be recommended.

In field conditions, practically no effect of the microbial mixture treatment was found on soil K or N concentrations. The main reason could be the sensitivity of microorganisms to changing environmental field conditions, or because of methodological problems such as the sampling of soils only from the 0-5 cm depth layer. In the 0-5 cm layer microbial activity is already high in organic farms, and the addition of microbial inocula had no additional benefit on soil K and N availability from pruning residue decomposition. More research is needed to understand the fungal:bacterial ratio and its relationship with the survival and/or effect of microbial mixtures in field conditions.

In the greenhouse trial, the probably enhanced activity under the microbial inocula treatment (MICROB) and corresponding effects on plant residue decomposition and seedlings growth (foliar biomass and minor stem diameter) were detected at 15 days when residues were buried, and not when they were applied on the soil surface (UP). This was probably due to the environment under the soil surface (higher and constant humidity and darkness), being favorable for microorganism growth, particularly bacteria. Changes during the day in temperature and humidity apparently affected the activity of microorganisms, canceling any positive effects of the microbial spray applications in the field experiment. In the greenhouse as well as in the laboratory test, the small effect of MICROB at the beginning of the experiments when the mixtures were more active tended to disappear at the end of the experiments when no differences appeared This indicated that the idea of conserving nutrients through enhancing microbial activity is not supported by the data in the present study.

The application of microbial sprays could have occurred temporally too close to the natural major flux of nutrient release, masking some of the effects of microbial addition in the greenhouse trial. The same problem could have affected both the nitrogen mineralization trial and the field experiment for both nutrients. When lower proportions of nutrients still remained in the residues, the activity of microbes also decreased as shown by

the results of the respiration trials. Thus, the effectiveness of microbial additions was considerably diminished. Therefore microbial applications should be considered ineffective in increasing nutrient availability in field conditions.

Conservation and delayed release of nutrients through microbial applications was not detected in our results until 42 days after residue applications. The EART effects can be interpreted as a delayed release of nutrient in only one case (Pejivalle 90 days), when K values were significantly higher than those in the other treatments that included residues. It would be worth continuing these experiments using native earthworm species. The explanation for the larger plants observed under MICROB at early stages of the experiment was not associated with any trend in N concentrations in foliar tissue, but higher K (and possibly other nutrients released during enhanced decomposition and not measured in this work) may be involved based on its trend of higher concentrations in maize seedlings 15 days after germination. In the pot trial, a probable immobilization of N was detected at 15 days even though *E. poeppigiana* has been reported as a high quality plant material.

Repeated doses of microbial mixtures and different ingredient concentrations can be tested to look for real and prolonged effects after initial stages of the greenhouse and field experiments. Although positive early effects of "MIXED" were detected in the pot experiment, the use of microbial mixtures with buried residues is probably not economically attractive because the application of these mixtures is rather labor intensive. In addition, the use of "MIXED" is not highly recommended for high quality residues because it would provoke a too rapid nutrient release.

Furthermore, the probably enhanced decomposition of residues may produce negative effects such as enhanced nutrient losses due to leaching. More research is still needed to reach better control of decomposition processes to synchronize decomposition of pruning residues with plant nutrient demands. This study does not permit us to recommend the use of treated *E. poeppigiana* residues as a nutrient reservoir that can be easily and economically managed to provide N and K through microbial manipulations in organic coffee farms.

5.6. References

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Appendix 1

Trial I: CO_2 production (mgCO_2/100g h^{-1}) from *E. poeppigiana* pruning residues when added to soil. Treatments were: MICROB, 12.5 g of fresh residues mixed with 50 ml 10 kg^{-1} of microbial mixture and mixed with soil $1:9$ ratio (DM/DM); RESID,12.5 g fresh pruning residues only and mixed with soil $1:9$ ratio (DM/DM; and BARESO, bare soil.

Days	8	22	36	50	70
Treatments					
MICROB	15.0	2.5	2.7	10.0	3.3
	9.7	0.5	7.0	3.4	5.0
	15.8	6.3	7.7	2.3	2.8
	8.5	4.2	0.6	1.1	2.7
Average	12.3 (1.9)a[1)2)]	3.4 (1.3)a	4.5 (1.7)ab	4.2 (2.0)a	3.5 (0.6)a
CV	0.3	0.7	0.8	0.9	0.3
RESID	9.3	7.7	6.7	4.7	3.7
	9.8	2.0	9.6	1.6	2.8
	10.9	2.4	12.7	8.3	4.4
	14.9	4.4	0.3	3.8	3.8
Average	11.2 (1.3)a	4.1 (1.3)a	7.3 (2.7)a	4.6 (1.4)a	3.7 (0.3)a
CV	0.2	0.6	0.7	0.6	0.2
BARESO	0.4	0.5	0.6	.	[3)].
	0.8	0.5	0.7	0.4	.
	0.6	0.5	1.5	0.2	.
	0.2	1.3	0.8	0.3	.
Average	0.5 (0.2)b	0.7 (0.2)a	0.9 (0.4)b	0.3 (0.1)a	..
CV	0.5	0.5	0.4	0.4	.

[1)] Standard error (n=4)

[2)] Values with the same letter within a line are not significantly different (Duncan test $p<0.05$).

[3)] Data not available

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Chapter 6   General discussion

6.1. The positive impact of *Erythrina poeppigiana* pruning residues on SOM and on other soil characteristics in conventional and organic coffee farms

Comprehension of tree-soil interactions is crucial for designing agroforestry systems which are economically sustainable (Rhoades 1997). The use of pruning residues has an increasing importance for small farmers due to the scarcity and high costs of chemical inputs (Schroth *et al*. 2001). The use of *E. poeppigiana* pruning residues has been suggested as a good source of nutrients in farms without external fertilizer inputs in Costa Rica (Beer *et al*. 1990). In the current dissertation, the most evident impact induced by *E. poeppigiana* pruning residue inputs was the increase of soil C (Chapters III and IV).

Higher total soil C and N was found near the trees (<1 m away) in conventional farms, presumably due to the accumulation of residues near the tree trunks (Chapter III). In addition, the more distant areas are probably more exposed to POM erosion since herbicide use reduces the weed cover in the whole area, and the distant areas have less protective mulch from the pruning residues. A higher concentration of soil C and N under leguminous trees in coffee farms has previously been reported by Beer (1988) and Fassbender (1993); under isolated trees in African savannas by Belsky *et al*. (1993) and Tomlinson *et al*. (1995); and under some tree species in plantations by Fisher (1995). Maintaining SOM or even increasing it, is one of the most important positive effects of trees on soil, given the multiple roles of SOM as a nutrient source and sink, as a chemical substrate for the exchange of plant nutrient bases, and also in maintaining physical soil properties. Lotter (2003) stated that organic C is the center of many of the biological processes that permit organic agriculture to be sustainable. For these reasons soil organic matter is one of the few universally accepted key measures of soil quality (Reeves, 1997). However, the spatial differences in C and N concentrations on conventional farms indicated that these benefits are not always evenly distributed throughout the farm. The scarcity of sources of organic material in organic farms obligates the farmers to efficiently use pruning residues; i.e., their

distribution is more homogeneous than on conventional farms (Table 1, Chapter III). *E. poeppigiana* residue inputs are important due to their role in maintaining soil C levels in low chemical input farms (Beer *et al*. 1990). The finding that these benefits are not spread uniformly in the conventional farms as in organic farms, is an important finding of the current work. Practical recommendations for improving the application of pruning residues and organic materials in conventional coffee farms, derived from this finding, are presented in section 6.7

Hypothesis 1.4.1.a was partially supported by the results; i.e., there is a positive effect of tree proximity on soil C and N concentrations but this was only evident in the conventional systems in the topsoil (0-5 cm layer). The second part of the hypothesis 1.4.1.a, which suggested that samples from the two positions ("alley" position equidistant from two coffee rows *vs* "bc>2" under coffee plants) located at the same distance (2 m) from the shade tree in a given system (organic or conventional) have differences in chemical and biological soil properties could not be accepted. No differences were found between these two positions for soil C, N, pH, electrical conductivity, available nutrients, or basal soil respiration in either organic or conventional systems despite the higher coffee plant litter in "bc>2" and the greater impact of management practices such as liming, fertilization or weeding as well as possible greater soil compaction in the "alley". Further studies on pruning residue effects on coffee farms might be focused on the 0-5 cm depth layer, and in only two contrasting positions ("bc<1" and "alley").

The importance of tree pruning residue additions in organic farms was also evident in Chapter IV where labile SOM dynamics were analyzed. After 330 days, the macroorganic matter C decrement with a tree pruning residue only treatment (RESID) was half of that in bare soil control plots (BARESO). In particular, the LF-C decrement under RESID was almost a third of that for BARESO at the end of the experiment. After 90 days from the first pruning residue application, the impact of residues was also shown by a temporary trend of increased LF and HF-C when compared to bare soil. Similarly, this impact also was shown in the 2004 field trial, at day 105, when the two treatments that included pruning residues had higher amounts of LF-C than the bare soil control.

The reduction of C available within macroorganic matter under RESID indicated that higher amounts of organic inputs than those used in the present field trial are necessary to maintain original labile C levels in organic farms. Although tree pruning residues are probably the most important source of organic C for maintaining soil C levels (Beer 1988, Beer *et al*. 1990), the influence of other organic materials (organic amendments, green manure, weeds, natural coffee and shade tree litter fall and coffee pruning residues) also appears to have been important in all study sites. For example, Aguilar and Staver (1997) showed that 3.35 Mg ha^{-1} of weed biomass (almost a third of the average *E. poeppigiana* pruning residue inputs) were added to the system in a seven month period after seven hand cuttings of weeds in shaded coffee plantations in Masatepe, Nicaragua. The extra C inputs could explain the absence of differences between positions in organic farms (Chapter III). It is clear that further work is needed to determine the optimal amount and combination of residue addition to enable maintenance of critical levels of soil organic matter in coffee farms.

Soil K concentrations and C in size density (SD) fractions under RESID, after 10 Mg ha^{-1} yr^{-1} DM of pruning residue application, were lower than initial values (Chapter V). These decreasing values indicated that nutrient status cannot simply be sustained only with the addition of the shade tree pruning residues. The RESID treatment in Chapter V represented the farm condition where pruning residues are the only nutrient source to the soil. Low coffee bean yields (Table 1, Chapter III) in organic farms could be a reflection of this declining soil quality problem. Low soil C levels in labile fractions in unshaded conventional coffee farms can be inferred from the data obtained in the present study for bare soil LF-C; i.e. in CATIE, 72% losses over 330 days under bare soil (BARESO) compared to RESID (20%). It would be interesting to see the results obtained with the SD fractionation approach on POM-C levels in conventional unshaded coffee farms.

Indications of soil C balance between C inputs and natural C mineralization are presented in Chapter III. The maximum soil C values found near the shade tree trunks at 0-5 cm, in both organic and conventional farms were similar, indicating that higher C inputs

in organic farming did not increase SOM. The small increases in soil C concentrations in organic farms four years after the first soil sampling in 2000 was another indication of balance between C inputs and outputs in organic farms (Table 2, Chapter III). This result was in line with Zuloaga (2004), who found few changes in C contents in organic farms after a four-year study period in Paraíso, Costa Rica.

The positive influence of residue additions on soil K concentrations was found in two studies presented in this dissertation. In the first study in 2000 and 2004, indications (significant in 2004) of higher soil K concentrations near the shade trees were found at 0-5 cm in both organic and conventional farms. However, the effects were limited to the areas close to the trees (Chapter III). In the second study in 2002 and 2004, all the plots which included residues tended to have higher K concentrations than the bare soil plots throughout the observation period (Chapter V). Furthermore, in the 2004 study, significantly higher K concentrations were found between the two treatments that included pruning residues and the bare soil control 30 days after residue applications. This finding was consistent with several studies which have reported the importance of *E. poeppigiana* pruning residues in supplying K within shaded coffee farming systems (Beer 1988, Kass *et al*. 1993, Soto *et al*. 1993 and Munguía 2003). However, as stated above, 10 Mg ha^{-1} of pruning residues were insufficient to maintain original soil K levels, mirroring labile SOM trends. The fact that benefits (more available K) were only temporary was presumably due to the high mobility of K in the soil or its rapid uptake by plants (Cobo *et al*. 2002).

The positive impact of pruning residues on plant growth was also observed in greenhouse conditions (Chapter V), particularly in poor soil (10-20 cm depth); both treatments that included pruning residues led to taller maize seedlings from day 6 of 105 day study period. These results are similar to those of Byard *et al*. (1996) and Kershnar and Montagnini (1998) with other legume species, and with Osorio (2004) and Gutiérrez (2002), who also used *E. poeppigiana* residues. However, in the greenhouse trial differences in N uptake by maize seedlings were not found, even in poorer soil from the 10-20 cm layer. This result suggested that the recently added *E. poeppigiana* residues did not have an immediate effect on the nutrient uptake by plants as was suggested by Haggar *et al*.

(1993) using ^{15}N markers. The amount of N in the pruning residues that organic farms received did not seem to improve yields although they were evenly distributed in the field (Chapter III). The high quality of *E. poeppigiana* plant residues does not lead to an increase of the more humified HF (Chapter IV). Thus, the lack of build up of decomposed organic matter in organic farms could be one of the reasons for the incongruence between the high N contents in *E. poeppigiana* and low crop yields in these farms.

6.2. Problems associated with the use of high-quality plant materials from *E. poeppigiana*

E. poeppigiana pruning residues, which have a low C:N ratio, low lignin and low polyphenols, have been useful for plant nutrition in both conventional (Beer *et al*. 1990) and organic farms (Lyngbaeck *et al*. 2001). Nevertheless, in the present studies, problems were found in relation to the high quality of pruning residues. In Chapter III, the positive effect of higher amounts of pruning residues on the soil surrounding shade trees was limited mostly to the 0-5 cm depth layer. In some cases, this reduced effect can be related to the young age of shade trees, but in many conventional farms, in which the trees have been present for at least 30 years, the explanation may be the low amount of resistant humic substances that these high quality residues produce (Szott 1991, Mafongoya *et al*. 1998). This was hypothesized because although coffee farming systems are practically no till systems (no mechanical residue incorporation), other natural factors such as endogeic earthworm activity could help transfer humic substances to deeper soil layers (Anderson and Flanagan 1989, Lavelle 1997). However, the accumulation of C near the tree trunks in conventional farms was not observed beyond 10 cm depth. Therefore, one probable explanation is the lack of accumulation of humified substances due to high *E. poeppigiana* quality residues.

The hypothesis that little resistant organic matter is formed from *E. poeppigiana* pruning residues was supported by the results in Chapter IV. The amount of C in the HF, supposedly the most humified fraction (Barrios *et al*. 1996a), decreased at comparable rates to MF and LF (the less humified fractions) one year after residue application (Table 1,

Chapter IV). Hassink (1995), in a comparison of LF and HF twenty-five years after wheat chaff (0.8% N, 42.5% C) or lucerne (2.5% N, and 43.6% C) application, found that the amount of HF was almost 57% higher under the wheat chaff treatment. This indicated that material with a high C:N ratio can lead to higher amounts of humified substances in HF. Another indication that *E. poeppigiana* pruning residues are not helping accumulate C in resistant forms, is the fact that low increases in total C were observed in organic farms where management of C inputs was maintained at a constant level in the four year study period (Table 2, Chapter III). Another drawback of the use of high quality residues from *E. poeppigiana* is the very fast release of nutrients such as K, which only permits the detection of the effect of residue addition at 30 days but not at 90 days in the field trial reported in Chapter 3. This problem has been reported widely by Kass *et al.* (1993), Szott *et al.* (1991) and Munguía (2003) amongst others.

Finally, the positive effect of pruning residue mulches on bulk density, observed in coffee farms in Kenya (Kimemia *et al.* 2001), could not be detected in farms at positions close to the tree trunks where pruning residues are accumulated in conventional farms. The low lignin content (fragility) of *E. poeppigiana* pruning residues could not influence soil bulk density in soils which naturally have low values for this variable due to their volcanic origin (Alvarado *et al.* 2001). Considering the problems derived from the high quality of E. poeppigiana pruning residues, the strategy of mixing plant residues of low and high quality (Beer 1993, Rhoades 1997, Mafongoya *et al.* 1998) could be useful to obtain higher impacts on soil properties in coffee farms.

6.3. Soil characteristics in organic *vs.* conventional farming systems

No significant differences in total soil C concentrations were found between organic and conventional systems in either of the two study years (2000 and 2004). Several factors influenced the absence of significant differences between these systems. Both organic and conventional are multistrata shaded systems with high organic inputs from shade trees. In addition, economical problems during this period (exceptionally low coffee prices) led to conventional farms using less chemical inputs, and management practices

between organic and conventional became more similar. Finally, due to contradictory results for C and N concentrations in one of the five pairs of farms, no significant differences between systems were found[1].

In 2000, a trend of higher total C and N concentrations was found at 0-5 cm for organic farms in comparison to conventionally managed farms. In 2004, soil profile examinations were performed in both types of farms to ensure that comparable areas were studied within farm pairs. Similar trends as in the 2000 study were found (four out of five organic farms had higher total C in the topsoil than their corresponding conventional pair). In a four year experiment with vegetable rotations comparing organic and conventional systems, Wells *et al.* (2000) concluded that the only factor that could explain higher soil C concentrations in organic farms was the addition of organic amendments such as compost ($40 \ Mg \ ha^{-1} \ yr^{-1}$). In the current study, organic farms received higher weed biomass inputs and probably higher biomass from pruning residues of taller trees as well as from organic amendments. These factors can explain the trend of higher topsoil C in organic farms in comparison with conventional farms. The trends of higher total C under organic systems in the current study were in line with results of several comparisons between organic and conventional farms (Lockeretz 1981, Reganold *et al*. 1993, and Wells *et al*. 2000). Although there was a tendency of higher C and N concentrations in organic farms, no statistical differences were found. In two farms, the differences between paired farms were more associated with original soil conditions than with recent soil management (Section 4.3.1.2., Chapter III). At the 5-10 and 10-20 cm depths, no differences were found between systems; therefore, a definitive conclusion about hypothesis 1.4.1.b which suggests that there are significant differences between conventional and organic systems in regard to soil C and N concentrations, was impeded.

No significant differences for pH between organic and conventional farms were found at any study depth. This result differed with other studies which reported significantly higher pH under organic systems (Lockeretz 1981, Reganold *et al.* 1993, Clark 1999). This may be due to the shorter period of organic farming management in our

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[1] Farm characteristics for this pair of farms are discussed in Chapter III.

study farms (7 year average) when compared to other authors' study farms (25 year average). Wells *et al*. (2000) obtained similar observations to ours, with small differences in pH between organic and conventional systems during a four-year study.

Higher soil respiration is associated with higher microbial activity and C availability in organic systems (Lotter 2003, Lundquist *et al*. 1999). In addition, highly diverse microbial communities in organic systems have been associated with a more efficient metabolic quotient for CO_2 (qCO_2) (Gunapala and Scow 1998, Fließbach and Mäder 2000). In 2000 in the current study, higher respiration rates were measured for 0-5 cm in organic farms in comparison to conventional farms (Chapter III). This finding showed that higher microbial activity could be found in the topsoil of organic coffee farms in Costa Rica as reported in the literature. In the deeper layers, respiration rates were similar for soils of organic and conventionally managed farms (Table 7, Chapter III).

The higher soil respiration rates concurred with lower levels of POM (>200 μm) in organic coffee farms at 0-5 cm (Table 4, Chapter III). These results indicated that presumably higher microbial activity (reflected in soil respiration) may reduce the amount of labile POM-C. Some studies have suggested that as a result of higher microbial activity in the soil, labile SOM (a readily available source of C) should be consumed at higher rates in organic farms in comparison with conventional farms (Fließbach and Mäder 2000). However, in the current study, no significant (negative) correlation was found between soil respiration and POM (>200 μm). Further laboratory work focused on the 0-5 cm depth layer and on a higher number of organic and conventional coffee farms is needed to understand the relationship between these variables.

Recently added organic amendments led to higher Ca and P in some farms within the compared pairs. In other cases, the balance between nutrient input and outputs probably led to similar soil nutrient concentrations in farms with different farming systems. Stockdale *et al*. (2001) suggested that the NPK balance in organic farms is not a result of system conditions but depends on a particular nutrient budget in each farm. However, different types of organic fertilizers, with irregular application times, complicate nutrient

balance calculations for organic farms. Summing up, the varying behavior of each nutrient at different depths did not permit generalizations about hypothesis 1.41.b which refers to differences in soil nutrient concentrations between organic and conventional systems. As a result of these factors, no definitive conclusion could be stated on soil nutrient differences between organic and conventional systems. In the current study, definitive positive impacts of organic coffee systems on soil characteristics (total soil C and N, and major nutrient concentrations, pH, bulk density) were not detected. More field data are needed to evaluate the consequences of chemical input substitutions in organic coffee farms. Our results showed the necessity of debating and critically assessing with controlled experiments the real benefits of organic coffee farming considering the lower yields obtained in these farms.

Methodologically, the use of a central area within study farms had important drawbacks in the current study. In one of the Aserrí farms, the steep slope provoked differences in topsoil characteristics even within a short range (<100 m). Somarriba *et al.* (2000) criticized the use of the "central plot" approach due to the probable bias when the whole area, with its natural differences, is taken out of the study. Ryan *et al.* (2001) also found differences in soil nitrate adsorption within small distances in coffee farms due to differences in clay composition. The "central plot" approach did not show major problems in four out of five pairs of farms. However, the use of mini soil-pits and previous soil sampling to identify comparable areas within pairs of farms are recommended, particularly in steeply sloping areas. The identification of comparable areas is also necessary for transversal studies (pseudo time scale; not following the evolution of soil characteristics over long periods of time).

6.4. Usefulness of SD fractions for evaluation of changes in labile SOM

6.4.1. Labile SOM decomposition reflected by size-density fractions

One of the main hypotheses of this thesis was that the SOM size-density (SD) fractions should show temporal differences during a 330 day study period, reflecting the decomposition process of labile soil organic matter (hypothesis 1.4.2.a, Chapter IV). The LF, MF and HF, as well as the amount of C in macroorganic matter and POM-C did reflect the decomposition process of pruning residues, particularly at 180 and 330 days after pruning residue applications, when values for all three fractions were lower than for 0 and 90 days in both sites (Figures 4A and B, Chapter IV). Therefore, hypothesis 1.4.2.a was supported in the current study. These results were in line with Roscoe and Buurman (2003) who found that LF and HF decrease significantly as a result of less organic C inputs when natural savanna soils were converted into croplands in a Brazilian Cerrado oxisol. In the current study, C concentrations in the three SD fractions were similar to those obtained in long-term field trials in Switzerland (Fließbach and Mäder 2000) and in crop rotation trials in Colombia (Barrios *et al*. 1996a). The initial increments in C content of the fractions, when treatments were analyzed separately (Figure 5, Chapter IV), indicated that the decomposition process was incomplete 90 days after applying pruning residues. This result was also obtained at 105 days in the 2004 field trial at CATIE (Figure 5, Chapter IV). Other density fractions have also been shown to be sensitive to organic inputs into the soil. In a comparison between different land uses, pasture-based systems (high C inputs) had significantly higher PEOC (permanganate extractable organic carbon fraction) when compared with continuous croplands which had lower C inputs (Westerhof *et al*. 1999). In a four year study, Freibauer *et al*. (1999), using a floating POM (>53 μm, polytungstate solution 1.6 g cm^{-3}), found higher values under croplands in comparison with natural savanna. The study suggested that differences were probably due to incorporation of maize crop residues by plowing. However, few studies in the literature have focused on very short periods of observation (15 days) for changes in SD fractions as our study did.

At 180 days after residue incorporation, the SOM size-density (SD) fractions values were lower than after 90 days because the labile material had decayed. The natural decomposition process of pruning residues was observed in the data for the (RESID) treatment. This result supported hypothesis 1.4.2.a, which suggested that the C content of the SD fractions shows temporal differences during the decomposition process of labile SOM in an organic coffee farming system. Comparing 180 to 330 days, no temporal differences were found. This indicated that the second residue application (5 Mg ha^{-1} of residues at 190 days) stabilized the C in the three fractions, but at a lower level than the initial C content (0 days). This reduction could be explained by the absence in these study plots of any contribution from natural litter fall from *E. poeppigiana* and coffee, nor coffee pruning and weed residues, that can provide an additional (to tree pruning residues) 4 and 5 Mg ha^{-1} yr^{-1} of organic material inputs (Fassbender 1993).

Magid *et al*. (1997) have proposed the SD fractionation method as an alternative or complement to the litter bag approach for studying decomposition of plant residues applied to the soil. In the litterbag method, the residues are placed on the soil or introduced into the soil in mesh bags; soil entering the bags may offset the residue weight loss in the bags. The confinement of residues within the bags can produce a different microenvironment from the natural conditions affecting residues that are not in direct contact with the soil. In contrast, in the SDF method, residues can be introduced or applied to the soil surface and macroorganic matter C is obtained directly from the soil (after natural incorporation of residues into the soil); i.e., because the organic materials and the soil are in close contact, soil nutrients are available for the decomposition process and faunal activity, and soil moisture can interact freely with the organic material. However, the SDF method is focused only on macroorganic matter (>150 μm) and fine fractions are discarded, which can be a drawback of the method.

In summary, in the current study, C content in SD fractions during plant residue decomposition suggested that SD fractions can reflect the labile SOM decomposition in organic farms as proposed by hypothesis 1.4.2.a. Additionally, the results indicated that the

SD fractionation method using Ludox™ can be used as a complement or an alternative to the litter bag approach to study the dynamics of organic matter decomposition.

6.4.2. Effects of treatments on C content in size-density fractions

The study hypothesis 1.4.2.b postulated that the amount of soil C provided by each SD fraction is affected by earthworm and microbial treatments under organic farming conditions. In 2002, significant differences between these treatments and untreated pruning residue treatment (RESID) were found only in a few cases. In CATIE, the amount of C provided by the LF under treatments enhancing the microbial and mesofauna activity, tended to be lower than under the RESID treatment at early stages. The differences between the averages of RESID and the other three treatments at 90 days for LF in CATIE were almost two fold, but the high variability eliminated any significant differences between treatments. The amount of HF-C under the earthworm and microbial treatments was significantly lower than the RESID treatment in CATIE and Pejivalle at 180 and at 90 days respectively (Figures 5A and D, Chapter IV). The MF did not show the impact of these treatments in any of the two sites, probably due to its intermediate condition. In the 2004 field trial, no significant differences of LF-C under the MICROB treatment in comparison to RESID were found. The absence of differences occurred in spite of the use of shade nets which provided more uniform exposure of soil to the sunlight in all of the plots. However, the LF was the best indicator of microbial activity in the soil because it was the only fraction that showed a significant correlation with microbial biomass, and also was the only fraction that clearly showed the effect of residue addition in comparison with BARESO control (Figure 6 Chapter IV). These results partially supported hypothesis 1.4.2.b. However, the effects of treatments were only temporary (found after 180 days at CATIE and after 90 at Pejivalle); final C values after 330 days tended to be similar for all treatments.

Shintani and Tabora (2000) and Velikonja *et al*. (2003) found that decomposition of inoculated organic residues usually begins sooner than non-inoculated residues. Schroth *et al*. (2003) also stated that high LF values are usually found at the beginning of natural

residue decomposition. Considering these findings, it was thought that an earlier measurement (e.g., 20 days after residue application) would detect statistical differences in C content between the RESID treatment *vs.* earthworm as well as microbial treatments. However, in 2004, 30 days from the inoculation of pruning residues, no significant differences between the MICROB and RESID treatments were found (Figure 6, Chapter IV). Methodologically, the use of high quality residues could be a problem in the detection of the probable effects of microbial residue addition or earthworm inoculation since the decomposition rates of these plant materials are naturally fast (Tian *et al*. 1993).

Earthworm and microbial treatments never led to final lower C values for the three SD fractions than bare soil controls. This was an indication that there is a limit to their influence on the decomposition of organic residues. Further research on the effects of mixed treatments (microbes plus earthworms) on bare soil plots should be performed to observe if they can diminish the amount of C contained in SD fractions to a level below the bare soil threshold (with no microbial additions). Another interesting conclusion derived from the effects of microbial and earthworm treatments was that they modified soil SD fractions in some cases, even under organic management, which should already provide high natural microbial activity and diversity (Lundquist *et al*. 1999, Fließbach and Mäder 2000). In conventional farms, the effects of such treatments should be more apparent, assuming that the added organisms survived long enough in these less favorable conditions.

In order to be effective in improving nutrient supply to plants, microbial mixtures should have significantly increased microbial activity in the soil and enhance the decomposition of the labile fractions. Particularly decomposition of LF, is thought to be directly related to nutrient availability in soils (Barrios *et al* 1996a). None of these conditions were definitively and consistently observed in Chapters IV and V. In addition, CO_2 production from pruning residues was not significantly increased and SD fractions did not decrease in the 2005 field trial at any sampling date during the experiment. Positive impacts on maize seedling growth were not observed for more than two weeks after MICROB applications. These results are not in line with suggestions of the possibility of using the microbial biomass as a living reservoir of nutrients for plants (Gunapala and

Scow 1998; Srivastava and Lal 1994). Research is still needed to study in greater detail the relationship between C dynamics in labile SOM fractions and microbial activity in organic coffee farms.

The present study, only found weak indications that it is possible to modify the amount of C in the SD fractions after tree pruning residue addition, using earthworm and microbial inoculation treatments. In contrast, the addition of untreated pruning residues produced higher amounts of C in LF in comparison to the bare soil treatment in both 2002 (after 330 days) and in 2005 (after 105 days) (Figures 5 and 6, Chapter IV). The trends of lower C contents in SD fractions under microbial and earthworm treatments were limited to the initial stages after applications. Moreover, the impacts of these treatments on soil N and K availability could not be detected; and the impacts on maize seedling growth were also limited and temporary (observed in soils from the 10-20 cm layer, Figure 3, Chapter V). The use of the microbial mixtures used in this work (based on indigenous microbial strains) to reach a controlled decomposition of plant residues is not justified in practical terms. However, the search for a controlled decomposition of labile SOM (managing shade tree pruning residues) for efficiently providing nutrients in synchrony with the temporal needs of crops, remains an important issue for further research.

6.4.3. Methodological issues (contributions to the study of labile SOM fractionation methods)

The relative value of the different methods for studying labile SOM requires more comparative studies. Six *et al*. (2002) reviewed how total POM (>53 μm) and density fractions have been used alternatively to study SOM transformations. Salas *et al*. (2003) used unfractionated macroorganic matter to study soil P cycling and found that *Sorghum bicolor* gave higher POM-P contributions to the soil at different decomposition stages than *Crotalaria juncea*. Several recent studies have proposed SDF using Ludox™ as a useful method in searching for the "biologically active" fraction of SOM and as an effective approach for observing changes in labile SOM over time (Hassink 1995, Barrios *et al*. 1996a). Barrios *et al.* (1996b) used SD fractions as better predictors of soil N

mineralization compared to total soil C. Higher LF-C and LF-N were found in plots that received *Gliricidia sepium* pruning residues in comparison with bare soil plots. The amount of LF-N obtained using Ludox™ correlated with soil N mineralization, while the amount of N in the light fraction obtained by flotation in NaI did not correlate with N mineralization in the soil.

The accuracy of the Ludox™-SDF method has been questioned in regard to the possible laboratory (operator) bias due to manual decantation before density fractionation (Meijboom *et al*. 1995). In the same way, the use of Ludox™ as a flotation medium has been criticized due to its high viscosity which can alter the fine particle separation. Another criticism has been that it consumes more processing time per sample than other approaches for SOM fractionation (Magid *et al*. 1996). Another limitation is that no common criteria for sample weights has been defined for the POM fractionation: e.g., 500 g fresh soil (Meijboom *et al*. 1995); 10 g dry soil (Cambardella and Elliot 1992) and 250 g of air dried soil (Phiri *et al.* 2001) have been used. In the current study, a mid-range of 100 g fresh soil was used.

In the literature, contrasting findings about the role of LF as an indicator of land and soil management changes have been reported. LF dry weight, the amount of LF-C, and LF-N in soil have been shown to be good indicators after different cropping rotations (Fließbach and Mäder 2000, Barrios *et al.* 1996a, 1996b and 1998). In contrast, Phiri *et al.* (2001) found that the medium fraction but not LF was the best indicator of changes in SOM due to plant residue additions. In other studies, good correlations between dry weight of LF and maize yields were found on a *Ustic Rodhustalf* in Zambia (Barrios *et al.* 1998), but in an *Oxic Dystropept* in Colombia, this correlation was not found (Phiri *et al.* 2001). In the current study, LF was the only one of the three SD fractions in the 2004 field trial that reflected the impact of the pruning residue additions in a 105-day experimental period. Many works have studied different soil management effects on SD fractions with different residue additions. Alternatively, Chapters IV and V in the current dissertation focused on the effects on size-density fractions of only one factor (microbial activity) on the same type of plant residue and in the same type of soil. Comparative studies between different soil

types, or between different coffee management systems with the same type of residues, might be done in regard to these SOM fractions; i.e. clay *vs.* sandy soils or organic *vs.* conventional coffee farms. In addition, different quality residues from other shade trees used in coffee systems in the same soil and climatic conditions could also be tested.

The value of LF and HF in the present study was somewhat different than that reported in the literature: Magid *et al.* (1996) reported that the effect of vegetal material quality on labile SOM decomposition rate was shown equally by the three SD fractions. In a subsequent study they used only two fractions: an HF (>1.4 g cm^{-3}) and a combination of the MF and LF into one fraction (<1.4 g cm^{-3}) to study rape straw decomposition, as MF did not seem useful (Magid *et al.* 1997). Fließbach and Mäder (2000) only found significant effects of organic and conventional fertilizer additions to the soil on the LF and MF, but not on HF. In the current study in 2002, LF and HF reflected similarly the decomposition process of *E. poeppigiana* pruning residues. The amount of HF-C could be affected by the high quality of the pruning residues. Nevertheless, without using isotopic labeled material, there is a grade of uncertainty as to the percentage of recently added organic matter that is influencing the size of each SD fractions. The use of two different controls (RESID and BARESO) was an alternative to reduce the uncertainty, but more accurate results can be obtained if isotopic markers can be used.

In the current study, no significant differences were found between treatments when the soil C contents contributed by the fractions were either aggregated in total macroorganic matter C or evaluated with POM (>53 μm). Additionally, analysis of the remaining C in macroorganic matter (% of the initial values) at the end of the experiment did not show any significant differences. This indicated that neither the unfractionated macroorganic matter SOM nor POM fraction was as useful as the LF and HF for showing the impact of different pruning residue managements on labile SOM.

The results in the current study were in agreement with several works in the literature: Magid *et al.* (1996) obtained better results using Ludox™ -SDF than using sodium tugstenate and total POM to discriminate the early effects of material quality on

SOM decomposition. Hassink (1995) successfully used SD fractions as indicators of N mineralization in different soil textures. However, some studies have questioned the usefulness of the SD fractionation method: Six *et al*. (2002) suggested that the advantage of the SDF method in defining an "active" pool of SOM is not definitive. Magid *et al*. (1996) were unable to define an "active" SOM fraction using the SD fractions to trace ^{14}C labeled plant residues during a 200 day trial. Cambardella and Elliot (1992) successfully characterized POM as the SOM fraction that is most easily depleted as a result of long term cultivation of grassland soils, and Salas *et al*. (2003) used unfractionated macroorganic matter as a good indicator to study soil P cycling after plant residue addition. Although significant effects of treatments on LF and HF-C at CATIE and on HF-C at Pejivalle were found (Chapter IV), they were not found on macroorganic matter or POM-C (>53 µm), suggesting that both of them are less sensitive indicators than the SD fractions. Nevertheless, more field data is necessary to support the use of the SD fractionation method, which requires more resources and time than size fractionation methods.

6.5. Evaluation of strategies for pruning residue management: soil K and N availability after management of pruning residues

Asynchrony between nutrient release from pruning residues and plant nutrient needs in different phenological stages has been highlighted as a major problem in agroforestry systems (Szott *et al*. 1991). The search for strategies to manage biomass inputs, using the interaction between microbial and mesofauna populations with soil conditions, has been proposed to find more efficient nutrient cycling processes via SOM decomposition (Schroth 2003). The build up of readily decomposable SOM reserves is also an important method to assure adequate nutrient supply for crops under agroforestry systems (Haggar *et al*. 1993). Controversy exists with respect to the best research focus (synchrony or SOM build up), and it is necessary to find practical and economical methods to manage biomass in order to have efficient nutrient uptake by crops while conserving long-term SOM levels (Mafongoya *et al*. 1998).

In the present study, soil K and mineralized N concentrations in the 2002 field trial showed a trend of higher values under treatments that included *E. poeppigiana* pruning residues 90 or 180 days after pruning residue applications (hypothesis 1.4.3.a) (Table 11, Chapter V). Evidence of the positive impact of *E. poeppigiana* pruning residue inputs on soil K has previously been reported. However, differences in soil K content under residue treatments in comparison to bare soil control were not significant in the 2002 field trial. This was partially due to the delayed sampling time; i.e., 90 days after the residue addition, a large proportion of K in residues was already released and lost by leaching. Munguía (2003) and Szott *et al*. (1991) reported that about 40% of the K in *E. poeppigiana* residues had been released during the first 24 days of decomposition. Another probable explanation of the absence of significant differences between treatments was the masking effect of the existing organic residues in both study farms which had accumulated in the soil in these organic farms, and in the case of CATIE, the masking effect of previous applications of organic K based fertilizer five months before the beginning of the experiment. Analysis of soil K concentrations after 330 days (always lower than initial values in both sites) indicated that the 10 Mg ha^{-1} of pruning residues alone were not enough to preserve the initial soil K concentrations as occurred for macroorganic matter C contents.

Microbial and earthworm treatments did not produce significant differences on soil K in the 2002 trial (Table 11, Chapter V). It was thought that the main reason for the absence of differences was the fast liberation of K irrespective of such treatments and its great mobility in the soil (Cobo *et al*. 2002); i.e., it was probably that any initial difference if it had existed, had disappeared when the first measurement was made after 90 days. To test this idea, in the 2004 field trial, soil K was measured 30 days after treatment application, but again the MICROB treatment did not have any effect on existing rates of nutrient release from *E. poeppigiana* under field conditions (Figure 4, Chapter V). In the 2004 greenhouse trial, small impacts were only detected in pots filled with soil from the 10-20 cm layer (Figures 2 and 3, Chapter V). The soil samples taken in the 0-5 cm layer, which had relatively high organic matter content (5.0%, Table 2, Chapter III), and the naturally high microbial activity could have annulled probable weak effects of microbial mixture additions.

In the 2002 field trial, no significant differences in soil NO_3^- and NH_4^+ values were found between MICROB, COMPOS and EART when compared to RESID. It was also thought that the sampling delay affected the detection of probable differences in soil NO_3^- and NH_4^+ values. The N release during the litter decomposition process of materials with low lignin:N ratios is relatively fast; e.g., during the first 30 days of decomposition of these kinds of organic materials, 25-65% of the original N is released (Mafongoya et al. 1998, Palm and Sánchez 1991, Munguía 2003). Berhard-Reversat (1987) reported that the LF under A. seyal, which has a similar lignin:N ratio to E. poeppigiana, provided higher mineralized N than controls in the early decomposition stages. The first soil sample in this present study, was taken 40 days after the second 5 Mg ha^{-1} addition of pruning residues; at this date, the residues probably had already lost an important part of their N content, and hence treatment effects, if they occurred, were not found (Chapter V). However, in the greenhouse trial in 2004, 15 days after MICROB application, maize seedling did not show any difference in comparison to the control in regard to uptaken N, indicating that MICROB did not produce any effect on N availability even in this short term.

In a study of the effects of E. poeppigiana residue additions on mineralized soil N in organic farms, Arana (2003) suggested that the high initial topsoil N concentrations hid the effects of the residues in the short term (9 months). The high original N and SOM concentrations could have masked any possible effects of microbial and earthworm treatments on the soil NO_3^- and NH_4^+ concentrations. The large native N stock, accumulated in the soil over many years of continuous residue additions (with corresponding N mineralization), was not increased by the 5 Mg ha^{-1} of residues added in each of the two study decomposition cycles. In line with this idea, no significant differences in mineralized N were found between RESID and BARESO treatments in 2002. Some other studies have suggested that earthworm inoculations in tropical grasslands can increase mineralized N stocks in the soil (Lavelle et al. 1992, Gilot et al. 1996). This effect was not observed in any case in the 2002 field trial (Table 10, Chapter V).

In conclusion, data obtained in the present study did not support the hypothesis of a positive effect of microbial and earthworm treatments on the amount of available N and K in the soil (hypothesis 1.4.3.a, Chapter V). Furthermore, the theoretical influence of LF dynamics on soil N and K concentrations could not be observed, since the amount of C in LF did not decrease significantly using microbial treatments in 2004 (Chapter IV). Therefore, the practical use of MICROB or EART on *E. poeppigiana* residues to provide N and K through microbial manipulations cannot be recommended for organic coffee farms based on the present results.

In practical terms, the study of timing of enhanced decompositions should be targeted on gaining higher fluxes of nutrients near the critical phenological phase for coffee (after the higher flowering flux). Currently, in organic farms in the Atlantic coffee zones of Costa Rica, the criteria for pruning is shade control (January) and maturity of cherries (August, Table 1, Chapter III). An approach that tries to synchronize pruning dates and rapid fluxes of nutrients from *E. poeppigiana* residue decomposition with critical moments of plant necessities, particularly in May-June when nutrient demands of coffee plants for foliar and cherry growth are higher (Alvarado-Soto and Rojas-Cubero 1994), could be useful to improve nutritional levels in organic farms. Until new research leads to practical and effective microbial methods for improving the utilization of high nutrient amounts in *E. poeppigiana* pruning residues, changes in pruning dates could be tested. Kass *et al.* (1993) have suggested that the pruning management would be adopted by farmers only if the labor cost of maintaining and pruning shade tress is not higher than the cost of using chemical inputs. However, research on tree biomass production and nutrient content after changes in pruning dates will be needed. Examples of new approaches for different pruning timing, adapted to tomato cropping, have been proposed by Chesney (2000).

Chapter 7   Conclusions and recommendations for further research

The increase of soil C induced by *Erythrina poeppigiana* pruning residue inputs in coffee plantations was the most evident impact in this study. Higher total soil C and N contents were found near the trees in conventional farms; due to the accumulation of residues near the tree trunks. In organic systems, the proximity of shade trees did not have a significant impact on total soil C and N concentrations. A more homogenous distribution of pruning residues would explain the absence of differences between positions within the organic farms. In addition, lower reductions in the size of labile SOM fractions (from initial values) were found under treatments that included *E. poeppigiana* pruning residues in comparison to bare soil treatments.

Shade tree proximity did not affect neither soil conditions, such as bulk density and pH, nor nutrient concentrations, except for K and Ca in the 0-5 cm depth layer, which showed higher concentrations near the tree, particularly in the 2004 study. In the 2002 field trial on organic farms, the addition of 10 Mg ha^{-1} yr^{-1} of pruning residues also led to a trend to higher soil K content in comparison to the bare soil control 90 days after treatment application. In the 2004 field trial, significantly higher soil K values were found at day 30 under the treatments which included pruning residue additions.

Indications of higher soil C and N concentrations in organic coffee farms in comparison to conventional farms were detected. The difference may be due to greater overall biomass inputs in organic farms (taller shade trees and greater organic matter inputs into the soil from weed biomass, and organic amendments), and also to an even distribution of pruning residues in the field. An analysis of the frequency of higher total C under organic farms (four of the five pairs of farms), and the analysis of the most homogeneous pair of farms at Pejivalle in the 2000 and 2004 study, suggests that although differences in overall averages are not statistically significant, there is a benefit for soil C and N from organic management of coffee. Soil carbon and nitrogen concentrations did not change significantly in either system at any depth between the 2000 and 2004 soil samplings. However, in the organic farms the final labile macroorganic matter levels (after an eleven

month period) were lower than initial values, in spite of the addition of 10 Mg ha^{-1} yr^{-1} of *E. poeppigiana* pruning residues to the soil. Along with the observation of lower final labile SOM levels in undisturbed plots in the 2004 field trial, these results indicated that the shade tree pruning residues alone were not enough to maintain stable levels of labile SOM. Weed biomass, natural coffee and *Erythrina poeppigiana* litter fall, coffee pruning residues and organic amendments are also important to maintain original POM-C levels in organic farms. There were no differences between organic and conventional systems in regard to soil nutrient concentrations. However, a study of original soil conditions, through profile descriptions and chemical analysis of samples from each horizon, indicated that not all the paired farms were strictly comparable and definitive conclusions were therefore impeded.

The 0-5 cm layer is more appropriate than the 5-10 and 10-20 cm layers for studying the effects of management on soil in organic *vs.* conventional coffee farming systems, as well as the spatial differences within conventional farms. Most differences between positions and between systems were only found for the 0-5 cm soil layer; the effects of shade trees therefore appear to be only superficial. In 2000, higher soil respiration rates were measured in organic systems in comparison to conventional systems at 0-5 cm; in the deeper layers, both systems had similar respiration rates. Although in organic systems higher respiration rates concurred with lower amounts of POM (>200 μm) in the 0-5 cm depth, no significant correlation between the two variables was found.

The analysis of C content in SD fractions during two decomposition periods of *E. poeppigiana* pruning residues suggested that the SD fractions do reflect the decomposition process of labile organic materials in the soil. However, an economic study on the costs of the SD fractionation method would be worthwhile since macroorganic C and dry weight of LF (as opposed to C content of LF) also appear to be good indicators of the decomposition process of pruning residues. In the 2000 field trial, in a few cases, evaluation of SD fractions suggests that earthworm and microbial treatments increased decomposition of tree pruning residues 90 days and 180 days after treatment application, but this effect was temporary and inconsistent. In 2004, no differences were observed between inoculated and non-inoculated treatments in a 105 day field experiment. When the impacts of the pruning

residue addition as well as earthworm and two microbial treatments were evaluated, LF-C and HF-C offered better resolution than other labile SOM fractions [MF-C, unfractionated macroorganic matter, POM-C (>53 µm) as well as dry weight of LF]. LF was the only fraction that consistently showed the effect of pruning residue addition and a significant correlation with microbial biomass.

In practical terms, the microbial mixture and earthworm treatments used in this study should be considered ineffective in increasing soil nutrient availability due to their inconsistent impact on labile SOM as well as on soil N and K availability. Moreover whatever impact they had was limited to the two weeks following application. The impact of introducing large amounts of microbial inoculants is probably of little value as their population density rapidly returns to pretreatment levels in soils with a naturally high microbial activity; i.e. (1) the inoculated strains are out competed by other members of the soil microbial community, and (2) their population will decline after the labile source of C becomes exhausted and the population can no longer be sustained. No significant effects of microbial or earthworm treatments on soil K and mineralized N concentrations were detected either in the 2000 field trial (11 months) or in the 2004 field trial (105 days). In controlled environmental conditions in the greenhouse, a small impact of microbial inocula (increased maize seedling growth and a trend of higher K uptake by maize seedlings) was detected, but only in pots filled with poor soil from the 10-20 cm depth layer. Therefore, the use of these microbial mixtures to accelerate decomposition of pruning residues for improving crop nutrition is not justified.

Recommendations for further research

Chapter III

1. Total soil C may not always reflect the impact of soil management changes because the labile SOM, which is affected by these changes represents no more than 3% of total soil C. Nevertheless, this labile fraction is very important for plant nutrition (Schroth *et al.* 2003, Barrios *et al.* 1996a, Phiri *et al.* 2001). It would be worthwhile analyzing if labile SOM changes occur between positions or between farming systems when no differences in total C appear; i.e., to apply the methodology of Chapter IV to the problems approached in Chapter III. For example, the amount of LF-C can be useful for comparing the effects of soil management in organic *vs.* conventional farms. In further studies on soil C and N concentrations, there is no need to include the comparison of the "bc >2" (under coffee plant) and "alley" (between coffee rows) positions when studying soil C since their results were very similar.

2. Due to the relatively recent introduction of organic coffee farming in Costa Rica, the number of organic coffee farms is relatively small compared to the number of conventional farms. In addition, many of the coffee farms in Costa Rica are found on steep slopes. These two factors make it very difficult to find comparable paired organic and conventional farms. If such transversal studies, using sampling to detect effects of preceding management, have to be done (when long term field trials are not feasible), previous soil profile descriptions and the use of mini soil-pits instead of augers are necessary to find comparable areas within the paired farms. When comparing farming systems, more paired farms should be studied to increase the sensitivity of the ANOVA due to high intra-site and inter-site variability commonly found in this type of on-farm research.

3. Changes in pruning residue management in conventional farms could induce more positive effects on soil characteristics. A more even distribution of pruning residues (from shade trees and coffee bushes) as well as crop residues (coffee pulp) can lead to

overall improvements in soil characteristics in the whole plantation area without affecting the economic budgets of farms; e.g. higher total C concentrations, soil K concentrations and biological activity as well as soil protection. Weed biomass input may also have a significant role in increasing organic C levels in organic farms (especially for positions far from the shade trees). A more detailed study, which includes an economic analysis, should be done on all the benefits and cost of replacing herbicides by manual or mechanical weed control.

4. In organic coffee farms in Costa Rica, substitution of chemical nutrient sources by organic amendments may have led to lower coffee yields. In the short-term which was analyzed in the present work (7 years of organic management on average[*]), this substitution apparently had no significant positive effects on some soil characteristics (major nutrient concentrations, pH, bulk density). Few evaluations of the effects of organic systems on soil characteristics have been done in tropical conditions, and even fewer in organic coffee plantations. Therefore, more field data are needed to evaluate the consequences of substituting chemical input, as has been done in these organic coffee farms, using soil chemical analyses Additional research may also wish to focus on the long-term sustainability of organic coffee farms in terms of calculating a nutrient budget for the farms where inputs (e.g. organic residues, mineral weathering, dry and wet deposition etc) and outputs (e.g. crop offtake, leaching losses, volatilization, erosional losses etc) are quantified.

5. No correlation between the amounts of POM (> 200 μm) and biological activity (soil respiration rates) in the topsoil was found in the present study. More detailed laboratory studies focused on the correlation between the amount of C in different size-density fractions of POM and soil respiration rates in the soil of organic coffee farms, could contribute valuable information to understand this relationship.

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[*] In the 2004 study the average was 11 years.

Chapter IV:

1. It is important to follow the movement of N from *E. poeppigiana* pruning residues in organic farms to add new information to help explain low yields in these farms (compared to conventional coffee farms in similar shade and climatic conditions[*]) under *E. poeppigiana* shade trees. It has been hypothesized that most mineral N derived from *E. poeppigiana* pruning residues is only taken up by plants from the build up of old soil organic matter (Haggar *et al.* 1993). Therefore, the application of isotopic marker techniques (e.g. ^{15}N) might be applied in organic coffee farms to follow the N liberation and absorption in recently added pruning residues and their contribution to coffee bush nutrition.

2. Quantitative studies on weed C inputs are necessary to determine their contribution to maintaining stable labile SOM levels in organic coffee farms. It is also important to quantify the proportional contribution of shade tree and coffee bush litter fall, as well as coffee pruning residues, in maintaining the labile SOM levels in organic coffee farms.

3. A hypothesis has been proposed from the current work suggesting that the similarity of LF and HF values, in reflecting the impact of microbial and earthworm treatments, is related to the high quality (labile) of *E. poeppigiana* residues. As a result, these residues may not have contributed to the formation of recalcitrant materials that contributes to HF as reported in the case of more lignified materials. Thus, the impact on the size of the SD fractions, from decomposition of low quality shade tree residues (with higher C:N ratios and higher lignin contents such as *I. edulis*) needs to be tested in organic coffee farms. If this hypothesis is correct, the impact of long application periods of *I. edulis* should be found in larger proportions of HF than LF and MF-C after controlled residue applications.

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[*] In some cases light may be a more limiting factor than soil nutrient availability

4. Some comparative studies between clay and sandy soils, using the same type of residues, might be done in regard to the size of SD fractions in organic coffee farms. This comparative research could add new information about the role of clay in protecting SOM from decomposition in tropical conditions.

5. The SD fractionation method using Ludox™ has been proposed as a complement and possibly an alternative to the litterbag approach (Magid *et al* 1997). The potential usefulness of this method to study labile SOM decomposition was shown by the current study. Therefore, comparative studies between the litterbag approach and the size density fractionation method should be designed to evaluate their relative value for obtaining more realistic data for SOM decomposition and about the contribution of organic residues to soil nutrient contents in tropical regions.

6 Longer term field trials (more than three years) might be done using bare soil plots in organic coffee farms to measure if decomposition of native labile SOM falls to a level below the bare soil threshold found in the present study. The information obtained could be used to evaluate if a protected POM-C stock exists as suggested by Six *et al*. (2002).

Chapter V:

1. Since only weak and temporary impacts of the microbial inocula treatment (MICROB) were found in the current work, further research investments in this area are questionable. Nevertheless, field and greenhouse trials might be set up with different doses, concentrations of sugar and N sources as well as different fungal-to-bacterial ratios, to adapt the use of indigenous microbial mixtures to different plant residues (particularly low quality) in low-input small farms. This new research could determine if such microbial treatments may have any practical value for coffee farmers (both conventional and organic).

2. Further research is needed to determine the sensitivity of fungi and bacteria to different plant residue incorporation methods. Antibiotics (stretptomycin and cycloheximide) have been used for selective microbial activity analyses (Beare *et al*. 1991). This type of analysis (although very expensive) would be useful for measuring the fungal:bacteria biomass ratio in the liquid organic amendments (MICROB) used in this study and their survival rates after application. The results would help advance the understanding of the absence of real and/or consistent effects of indigenous microbial solutions observed in field, greenhouse and *in vitro* trials. In addition, molecular approaches such as fatty acid profiling, and PCR-based denaturing gradient gel electroforesis (DGGE) could also be used to identify the role of pruning residues and inoculants on the dynamics, activity and structure of the soil microbial community. Some authors have also suggested that the effects of temperature and moisture changes on decomposition of plant materials should be measured on plant residues that receive the microbial solutions (Cabrera *et al*. 2005) to detect the impact of wet-dry cycles on plant residue decomposition.

3. The results observed in Chapter V for the MIXED treatment suggested that this method could lead to real effects on plant growth. In organic coffee farms, the use of high quality plant material such as from E. poeppigiana, may negatively affect long-term nutrient absorption by coffee bushes. Rapid fluxes of nutrients from decomposition of residues are usually asynchronous with phenological needs of crops (Szott et al. 1991). The use of mixtures of low quality and high quality material can lead to a more gradual (not pulsed) nutrient release over time, as suggested by Beer (1993) and Rhoades (1997). Although this strategy was not approached directly in this dissertation, it seems to be a more economical and efficient strategy than the use of microbial inocula to manipulate plant residue decomposition.

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