

NEMATODES AS SOIL QUALITY INDICATORS IN COFFEE SYSTEMS



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PREFACE

Along both my academic formation and my non-academic life I was always inspired by farming. This word, although with a broad range of meanings, I considered it as a way of living in nature and together with nature. In 2010 I had the great opportunity to go to Argentina to work with the “National Peasant and Indigenous Movement” (MNCI) as a formation period for Engineers Without Borders, the NGO I am volunteering in. Living with peasants in remote areas in the Chaco forest, I got to know the real essence of farming, the holistic understanding of farming and the elements involved in it: soil, water, climate, moon cycles, animals and the spiritual connexion of human beings with all those elements. Unfortunately, I had to experience also the greed of multinational companies and big landlords, blinded by the dream of money and power, considering land only as a production unit. During my stay in Argentina, I accompanied my comrades in their daily struggles to stop the transgenic soy frontier to enter in their ancestral territories and destroy the Chaco forest, not always succeeding, as two partners got murdered that year in the hands of land grabbers. The MNCI members embraced agroecology as their way of living and as the only alternative against the capitalist and imperialist model. They, with their way of living, planted the seed of agroecology in me and, since then, it did not stop growing, strengthening myself to fight for agroecology and food sovereignty. As part of my MSc thesis Organic Agriculture at Wageningen University, I had another great opportunity to go to the Zona da Mata region, Brazil, and work with coffee farmers, to learn and participate in, to my opinion, the strongest agroecology movement in the world. I am still amazed about the involvement of Brazilian students, professors, farmers, syndicates, NGO’s... in making agroecology happen, in doing and living agroecology. Indeed, to understand coffee agroforestry in Zona da Mata is essential to know the historic and political context, and how these and the natural context are linked, not just locally but globally. These 5 months in Brazil, working with coffee agroforestry, have given me a broader knowledge about farming and the beauty of it. As well, through the research developed there, I got to learn a lot about above-belowground relationships between shade trees, coffee management and soil life. Agroforestry farmers showed me that another way of farming is possible, feasible and socially fair. I am glad to be part of a movement which cares about nature, food production, people and their cultures, and which promotes feminism as an essential part for human equality. I hope with this thesis I can contribute to increase the knowledge about coffee management and soil quality, and hopefully I can contribute to spread the agroecology seed in many more people.

“The ultimate goal of farming is not the growing of crops, but the cultivation and perfection of human beings”. -

Masanobu Fukuoka; The One-Straw Revolution.-

SUMMARY

Coffee family farmers in Zona da Mata, Brazil, have embraced agroecology as a way to fight against soil degradation and loss of soil fertility; some of the consequences derived from the adoption of the green revolution model. Some family farmers, together with a local NGO (CTA-ZM), and the Federal University of Vicosa (UFV), started to implement agroforestry systems, focusing on the restoration of soil quality. The impact on soil quality of two agroecological coffee agroecosystems, one of them considered as mature, and two conventional coffee agroecosystems was studied and compared to a fragment of Atlantic rainforest, taken as a reference of highest soil quality. Soil quality was assessed using physical and chemical characteristics, together with nematodes as biological indicators. Soil quality through nematode indices was evaluated using: Maturity Index (MI), Plant Parasitic Index (PPI), Channel Index (CI), Enrichment Index (EI) and Structure Index (SI). Nematode diversity was assessed using the Shannon-Wiener Index (H'). Most soil physical characteristics were similar for all systems but for the Atlantic rainforest that had the lowest soil density and highest macro and total porosity. Agroecological coffee agroecosystems improved soil chemical characteristics compared to conventional agroecosystems, showing higher contents in organic matter, phosphorus, potassium, calcium, magnesium, base saturation and cation exchange capacity. MI and PPI pointed out to the Atlantic rainforest as the most mature system, but provided contradictory results for coffee agroecosystems. CI was higher in the Atlantic rainforest and one conventional system, showing a predominant fungal decomposition channel, and was not sensitive enough to account for differences between coffee agroecosystems. The Atlantic rainforest had the most structured soil food web (SI) and was the least nutrient enriched (EI) of all study systems. Diversity of nematodes was higher in the Atlantic rainforest and in mature agroecological systems. These results indicate the Atlantic rainforest as a great example of high soil quality and suggest that mature agroecological agroecosystems have the potential to achieve multifold objectives like increasing soil fertility, enhancing soil diversity and maintaining a structured soil food web, proper of reference high quality soils.

Key words: agroecology, agroforestry, diversity, family farming.

RESUMO

Agricultores familiares produtores de café na região da Zona da Mata, Brasil, adotam a agroecologia como uma forma de lutar contra a degradação e perda de fertilidade do solo; algumas das consequências decorrentes do modelo de produção baseado na revolução verde. Entre as estratégias adotadas por agricultores familiares visando a restauração dos solos, destaca-se a implementação de sistemas agroflorestais, desenvolvidos em parceria com uma ONG local (CTA-ZM) e a Universidade Federal de Viçosa (UFV). No estudo, o impacto do manejo sobre a qualidade do solo foi avaliado em diferentes agroecossistemas da região. Dois agroecossistemas agroecológicos cafeeiros, um deles considerado maduro, e dois agroecossistemas cafeeiros convencionais foram comparados com um fragmento florestal de Mata Atlântica, considerado como referência de máxima qualidade do solo. Além de propriedades físicas e químicas do solo, nematóides foram utilizados como indicadores biológicos para avaliar a qualidade do solo. Diversos índices de nematóides foram utilizados na avaliação: Índice de Maturidade (MI), Índice de Parasitas de Plantas (PPI), Índice Canal (CI), Índice de Enriquecimento (EI) e Índice de Estrutura (SI). A diversidade de nematóides foi avaliada usando o índice de Shannon-Wiener (H'). A maioria das propriedades físicas do solo foram semelhantes para todos os sistemas, exceto para o fragmento florestal, que apresentou menor densidade do solo e maior macro porosidade e porosidade total. Os agroecossistemas agroecológicos melhoraram as características químicas do solo em comparação com agroecossistemas convencionais, apresentando maior teor de matéria orgânica, fósforo, potássio, cálcio, magnésio, saturação por bases e capacidade de troca catiônica. MI e PPI apontaram a Mata Atlântica como o sistema mais maduro, mas forneceram resultados contraditórios para agroecossistemas cafeeiros. CI foi maior no fragmento florestal e em um sistema convencional, mostrando um canal de decomposição fúngica predominante, e não foi sensível o suficiente para explicar as diferenças entre agroecossistemas cafeeiros. O fragmento florestal apresentou uma cadeia alimentar do solo mais estruturada (SI), e o menor enriquecimento de nutrientes (EI) de todos os sistemas incluídos no estudo. A diversidade de nematóides foi maior no fragmento florestal e em sistemas agroecológicos maduros. Estes resultados indicam que fragmentos florestais da Mata Atlântica podem ser considerados como exemplo de alta qualidade do solo e sugerem que agroecossistemas agroecológicos maduros têm o potencial para alcançar múltiplos objetivos como aumento da fertilidade do solo, aumento da diversidade de organismos do solo, e manutenção de uma cadeia alimentar estruturada do solo, características próprias de sistemas referentes de solos de alta qualidade.

Palavras-chave: agroecologia, sistemas agroflorestais, diversidade, agricultura familiar.

1. INTRODUCTION

Coffee farmers in Zona da Mata, Brazil, have been fighting during the past two decades to control soil degradation and the consequent loss of soil fertility (Cardoso, 2001; de Souza, 2012). For maximizing yield, the great majority of farmers adopted full-sun coffee systems based in the green revolution model; which depend on high levels of external inputs and are often associated with soil degradation and environmental negative impacts (DaMatta, 2004). The green revolution implements a model based on agronomical practices that exploit land-use systems, maximizing yields without considering environmental consequences. The advance of this model greatly contributed to the reduction of the Atlantic rainforest that dominated this region, and which currently remains in just 12 percent of its original extension (de Souza et al., 2012). Increasing concern about land degradation, agricultural production and system functioning, encouraged farmers to develop alternative agricultural systems. In 1994, some family farmers started to implement agroforestry systems (SAF) together with a local NGO, Centre for Alternatives Technologies of the Zona da Mata (CTA-ZM), and the Federal University of Viçosa (UFV). Coffee agroforestry systems in Zona da Mata are managed according to agroecological principles (Cardoso et al., 2001) including: recycling of nutrients, enhancing soil organic matter and biological activity, diversifying plant species and genetic resources, and productivity of the whole farming system rather than individual species (Gliessman, 1998). Moreover, agroforestry systems are known to conserve soils, preserve natural resources, require low external inputs (Calle et al., 2014) and provide a more stable income due to the diversification of cash revenues, primarily derived from timber and fruits. Agroecological systems are diverse and heterogeneous, ranging from young to mature within the agroecological spectrum. The different management practices in agroecological and conventional coffee systems, such as shade density or type and quantity of fertilizers, have an impact on soil quality which might influence productivity and ecosystem functioning. The importance of assessing soil quality lies in achieving sustainable land use and management systems, to balance productivity and environmental sustainability.

Soil quality, in its shortest version, is defined as “the capacity of a soil to function”. Depending on the authors and the research focus, the highest soil quality is attributed to: i) Soils able to maintain a high agricultural productivity without negative environmental impacts and, ii) climax soils developed under their climax vegetations (Gil-Sotres et al., 2005). The former assumption is taken by researchers with a more agricultural scope rather than environmental, and/or the lack of possibilities to compare with climax soils because of the inexistence or high disturbance of those climax systems nowadays. In this thesis the latter assumption is taken, and the highest soil

quality is attributed to the soils developed under the Atlantic rainforest. To measure soil quality, physicochemical characteristics have been broadly used. However they just provide limited information about the soil system and do not always reflect the differences observed in the field (Shepherd, 2004). Biological indicators such as microbial activity and biomass, structural microbial diversity (Schloter et al., 2003), enzymatic activity (Garcia Ruiz et al., 2012) and meso and macrofauna composition (Didden et al., 1994; Pauli et al., 2012), have contributed to expand the knowledge about soil functioning. Among biological indicators, nematodes have qualities that make them perfect candidates to assess soil quality (Bongers, 1990).

Several indices have been developed to assess soil quality through the assessment of the nematode population in the soil system. The maturity index (MI) (Bongers, 1990) gives an indication of the condition or disturbance of an ecosystem based on the composition of the nematode community. The maturity index is calculated using colonizer-persister (cp) values assigned to nematode families in relation to their ability to resist disturbances. Therefore, higher MI values correspond to higher soil quality ratings which will be represented by a higher number of persisters than colonizers, while in soils with lower quality, colonizers will dominate, and MI values will be lower. Plant parasitic nematodes are not included in the MI, but in the plant parasitic index (PPI). The rationale behind excluding plant parasitic nematodes from the MI is that a larger abundance of this feeding guild is directly linked with the vigor of the plants they feed in, which in turn depends on the level of enrichment of the system. Therefore, in less enriched natural systems, some plant feeding nematode families, such as *tylenchidae*, dominate and the PPI and MI have an inverse response (Bongers, 1997). Relative abundances of nematode trophic groups affect nutrient cycling in decomposition and primary production channels (Verhoef & Brussaard, 1990). In more intense agricultural fields, such as those managed under conventional systems, the strategy followed by farmers, of adding nutrients easily taken up by plants, allows to increase crop production. In these environments, the C/N ratio is generally low and bacteria dominate soil decomposition processes. In less intense agricultural fields, such as agroecological systems, and natural systems, like forest areas, there are more recalcitrant compounds, which are not easily broken down by bacteria. In these environments, the C/N ratio is generally higher and fungi dominate soil decomposition processes (Ferris et al., 2004; Hodge et al., 2000). Therefore, in undisturbed ecosystems, resource decomposition is dominated by the fungal channel and, consequently more fungi feeding nematodes are present. On the other hand in more disturbed ecosystems the bacterial channel dominates and the nematode community will be dominated by bacterial feeding nematodes. Bacterial and fungal feeding nematodes influence the turnover of the soil microbial biomass and therefore the availability of plant nutrients (Ferris

& Bongers, 2006). The relative contribution of bacterial and fungal feeding nematodes to decomposition channels can be expressed using the Channel Index (CI) (Yeates, 2003).

Furthermore, nematodes reflect changes in ecological structure and function of soils in a predictable and efficient way (Fiscus & Neher, 2002). Several types of soil disturbance such as the disturbances created by the application of nutrients, the frequency of application, the chemical form in which they are applied, and the amount used, affect the structure and successional status of nematode communities (Fiscus & Neher, 2002). The Structure Index (SI) and the Enrichment Index (EI) calculated through the assessment of nematode taxa, provides useful information about the structure, the function and most probably the soil food web resiliency to disturbances (Ferris et al., 2001). Nematode metabolic footprints (Ferris, 2010) are calculated based on estimations of the amount of carbon used for biomass production and respiration by different groups of nematodes. Nematode metabolic footprints give important information about how different nematode groups contribute to ecosystem functioning (Ferris, 2010). Metabolic footprints are derived from the combination of enrichment and structure footprints, which are estimated using the EI and SI. In addition, diversity of nematodes has been reported to be greater in less disturbed systems (Yeates et al., 2009).

Nematode diversity is commonly assessed using the Shannon-Wiener index (H') (Yeates & Bongers, 1999). The use of nematodes as biological indicators, together with chemical and physical parameters, is a potential tool to assess the impact caused by the implementation of agronomic management practices. To monitor if agronomical practices have a positive or negative impact on soil quality is of great importance for assessing their sustainability.

The objectives of this thesis are:

- 1) To determine and compare the soil quality of the Atlantic rainforest with conventional and agroecological coffee agroecosystems, in terms of soil physical and chemical properties.
- 2) To identify the nematode populations of the Atlantic rainforest and the different coffee agroecosystems, at Pedra Redonda, in the Zona da Mata region.
- 3) To assess and compare the soil quality in different coffee agroecosystems and the Atlantic rainforest through nematode-base indices (MI, PPI, CI, SI and EI) and nematode metabolic footprints.
- 4) To assess nematode diversity in the studied systems through the Shannon-Wiener index.

I do hypothesize that:

- 1) Physical and chemical properties are improved in agroecological coffee agroecosystems when compared with conventional agroecosystems.
- 2) There is no hypothesis for objective 2.
- 3) A gradual increase of soil quality regarding maturity (MI, PPI), structure and enrichment (SI and EI) of the soil food web is expected from conventional to agroecological agroecosystems; and with the Atlantic rainforest as the highest soil quality. In the Atlantic rainforest and the agroecological agroecosystems, decomposition processes are dominated by the fungal channel and in conventional agroecosystems by the bacterial channel, expressed through the CI.
- 4) The Atlantic rainforest presents the highest nematode diversity, followed by the agroecological agroecosystems and lastly by the conventional agroecosystems.

2. THEORETICAL FRAMEWORK

2.1. Coffee: Agroecological Revolution and Green Revolution

The green revolution model promotes the mechanization of agriculture, the substitution of locally adapted by genetically improved varieties, the adoption of monocultures and the use of external chemical inputs. Contrary to the green revolution approach, agroecology works with principles which are flexible in relation with local socio-economic and natural biophysical circumstances. The main principles of agroecology include: recycling and closing energy and nutrient cycles on the farm, enhancing soil organic matter and soil biological activity, diversifying plant species and genetic resources in agroecosystems, integrating crops and livestock –using native seeds and local breeds-, and generating synergies for the optimization of the whole farming system (Gliessman, 1998). Agroecology-based production systems are biodiverse, resilient, efficient, socially just and comprise the basis of an energy, productive and food sovereignty strategy (Altieri, 2002). Furthermore, agroecology is knowledge intense, and is based on the capability of local and indigenous communities to experiment, validate, evaluate and scale-up innovations through farmer-to-farmer research and grassroots extension approaches in a horizontal participatory manner. The green revolution and the agroecological model have been adopted globally for the production of commodities and crops.

Coffee is the most important tropical commodity worldwide accounting for almost half of the total net exports of tropical products. Brazil is the undisputed major coffee producing country since records began, with the state of Minas Gerais leading the ranking (Hallam, 2003). Zona da

Mata region, in Minas Gerais is, at the same time, the largest coffee producer region in Brazil, which is recognized for producing a coffee of maximum quality (Silva, 2015). Zona da Mata is characterized by a scattered landscape of coffee, pastures and eucalyptus plantations where scarce Atlantic rainforests remains mainly on top of the hills. Two important fragments of the forest are preserved in the Brigadeiro State Park and Caparaó National Park (De Souza et al., 2012). The tradition for coffee growing in Brazil and Zona da Mata region is based on full sun coffee monocultures, either for large scale plantations or for the predominant small family farms. In Brazil, environmental conditions allowed to grow coffee in open ground to maximize production. This way of management was not possible in some other countries where coffee was introduced contemporaneously, like Ceylon or Venezuela, where shade trees were necessary to protect coffee from excessive heat or too long dry seasons (Thurber, 1889). Coffee plantations in Zona da Mata date from the mid-19th century where large-scale landowners and slave holders exploited the available resources without major qualms; both natural and human. The arrival of coffee into this hilly region shaped the landscape as well as the social relationships (Ferrari, 2010). In the words of a family farmer of the region: “We black people and descendants of Puri Indians occupy the higher parts of the hills, working in steep and small areas. The best lands located in the valley bottoms are owned by 5 or 6 white landowners”.

In the 1960's, with the general adoption of the green revolution model fostered by the military government, agrochemicals and genetically improved varieties were implemented to coffee monocultures. Public policies were the main driver of spearheading the “modernization recipe”. Family farmers could just partially adopt the green revolution package because of socioeconomic constrains, such as lack of access to financial programs and low incomes, and environmental constrains, such as the hilly topography making difficult the implementation of mechanization (Ferrari, 1996). The adoption of the green revolution model significantly contributed to the continuum of deforestation of the Atlantic rainforest, the consequently loss of biodiversity, the pollution of soils and water bodies, the loss of self-sufficiency and independency of farmers, the precariousness of farming as a way of living, and the disconnection of the human-nature bond (Botelho et al., 2015). Soil erosion and loss of fertility prominently accompanied the adoption of coffee plantations based on the green revolution model. The hilly topography of this region contributes to accelerated soil degradation processes. Simultaneously to the general adoption of the green revolution model, the development of some important events created a perfect breeding ground to make agroecology soak through really strong in Brazil, and specifically in Zona da Mata, and are the pillars of what has been called “Agroecological revolution” (Altieri & Toledo, 2011). In a broader scope, the success of the Cuban revolution – with an anti-capitalist

and socialist program- inspired most of the social struggles and insurrections happening in Latin America for the coming 50 years (Löwy, 2009). Narrowing down the scope, the arrival of liberation theology co-headed the agroecological revolution (Altieri & Toledo, 2011). Liberation theology considers the poor as the main actor of her/his liberation, a fact that empowered family farmers to fight against their oppressions and oppressors. Liberation theology started in Brazil in the 1960s lead by the philosopher and educator Rubem Alves, at went hand in hand with the internal transformation of the Catholic Church. Liberation theology got materialized with the creation of basic ecclesial communities, (CEB's as referred in Portuguese). Coffee family farmers in Zona da Mata strongly committed to the CEB's, in which they were empowered to engage political discussions and, later on, to participate in the rural worker's syndicate movement in the 1980's. Some of the people participating in the rural worker's syndicate movement would also participate in the foundation of the Worker's Party (PT) (Botelho et al., 2015).

Simultaneously, in the Federal University of Viçosa (UFV) -the most important agricultural university of the state-, an alternative student movement started, led by students who felt the need to connect the academic sphere to the agronomic reality of the region. The educator Paulo Freire, who joined the PT in 1980 and author of "Pedagogy of the Oppressed" and "Extension or communication", was of great inspiration for the student alternative movement in the UFV. In 1987, the NGO "Centre for Alternative Technology of Zona da Mata" (CTA-ZM) was created with the participation of some of the UFV students and aiming to promote, in the beginning alternative agriculture and later on, agroecology in the region. Some family farmers, who had expressed the decline of soil fertility, and their difficulties to access financial and technical support, started to work together with CTA-ZM, and the UFV. In 1994, farmers started implementing agroforestry systems (AFS), in a participatory experimentation, and which were found a viable alternative to the predominant full sun conventional systems, as well as a strategy to control land degradation and erosion (Cardoso et al., 2001). Farmers and scientist who implemented the AFS's, expressed their need to know more about soils and about how different management practices, like shade density or type and quantity of fertilizers, have an impact on soil quality which, in turn, might influence productivity and ecosystem functioning. Since then, several studies have been developed using quality indices as a promising tool. Nonetheless, further research need to be done in order to better understand such complex systems.

2.2. Soil Quality Indices

Over the past 20 years soil quality has become of great interest, yet there is a lack of consensus on a definition of the concept and the way to assess it (Bastida et al., 2008). Some authors consider the highest soil quality of those climax soils developed under climax vegetation, whereas others attribute the highest quality to those soils capable to maintain a high productivity without causing environmental distortions (Gil-Sotres et al., 2005). Beyond this lack of consensus, the term of soil quality defined by Karlen (1997) as the “capacity of a soil to function within natural or managed ecosystem boundaries, to sustain plant and animal productivity, maintain or enhance water and air quality, and support human health and habitation” has been generally accepted. To assess soil quality, physicochemical properties have been broadly used in a more agronomical perspective; however these properties used as soil quality indicators do not always support the differences observed in the field. For assessing soil quality the integration of static and dynamic chemical, physical, and biological factors need to be defined in order to better understand such a complex system, thereby developing an effective method for evaluating the environmental sustainability of the land use and management practices (Nortcliff, 2002). Also, the consequences of any decline in soil quality may not be immediately experienced because of the soil buffering capacity or resilience to the effects of potentially damaging or beneficial conditions. The implementation of soil biological parameters has contributed to better understand the complexity of soils and systems functioning (Mäder et al., 2015), due to their higher sensitivity, in short and long term, to changes and disturbances. Among biological parameters, nematodes have several attributes that make them excellent candidates as bio-indicators for assessing the quality of terrestrial ecosystems (Bongers, 1997).

2.3. Nematode-base soil quality indices

Nematodes used as soil quality indicators are a promising tool to deepen the knowledge about soil systems. Nematodes often exceed a million of individuals per square meter of soil and account for about 80 percent of all individual animals on earth. They present a great diversity of life cycles and are present at various trophic levels, playing a major role in many ecosystems. They occur in any environment, in every soil type, under all climatic conditions and in a wide range of habitats, from extremely polluted to pristine (Bongers, 1990). Their community structure is an indicative of the condition of the soil horizon they inhabit; they respond rapidly to disturbance and enrichment; due to the clear relation between their feeding behavior and the structure of the mouth cavity and pharynx, they can be easily identified and characterized in

feeding guilds - plant feeders, bacterial feeders, fungal feeders, omnivorous and predators-, and they occupy key positions in the soil food web (Bongers & Ferris, 1999). Several nematode-base indices are used to assess soil quality.

2.3.1. The Maturity Index (MI)

The maturity index (MI) developed by Bongers in 1990 expresses the condition of an ecosystem based on the composition of the nematode community, and has been recognized as an efficient tool to assess the quality of terrestrial ecosystems. Most studies carried out using nematodes as indicators are focused in how agricultural and soil management practices modify the soil quality (Fiscus & Neher, 2002; DuPont et al., 2009; Santiago et al., 2012). The maturity index is calculated based on colonizer-persister (cp) values (Table 1) assigned to nematode families (Table 2) in relation to their ability to resist environmental and agricultural disturbances.

Table 1 Cp values assigned to nematode taxa depending on r-k characteristics (From Ferris et al., 2001)

Cp	Characteristics
1	Short generation time, small eggs, high fecundity, mainly bacterivores, feed continuously in enriched media, form dauerlarvae – state in which nematodes can survive harsh conditions- as microbial blooms subside
2	Longer generation time and lower fecundity than the cp-1 group, very tolerant of adverse conditions and may become cryptobiotic. Feed more deliberately and continue feeding as resources decline. Mainly, bacterivores and fungivores
3	Longer generation time, greater sensitivity to adverse conditions. Fungivores, bacterivores and carnivores
4	Longer generation time, lower fecundity, greater sensitivity to disturbance. Besides the other trophic roles, smaller omnivore species
5	Longest generation time, largest body sizes, lowest fecundity, greatest sensitivity to disturbance. Predominantly carnivores and omnivores

Nematodes with high colonization ability, high reproduction rate and high tolerance to disturbances are considered colonizers and have low cp values. Their populations increase rapidly under favorable conditions, present short life cycles, and are often numerically dominant in samples. Colonizers are generally considered r-strategists (Bongers, 1990) based on the r-k ecology selection theory, which categorizes as r-strategists those organisms with abundant offspring in detriment of parental investment. On the other hand, nematodes with low colonization ability, low reproduction rate and sensitive to disturbances and enrichment are

considered persisters. Opposite to colonizers, persisters present long life-cycles, have few off spring, live in less disturbed habitats and have high cp values (Bongers, 1990).

Table 2 Cp values for nematode families: 1=colonizer, 5=persister (From Bongers, 1990)

Rhabditidae	1	Teratocephalidae	3	Diphtherophoridae	3
Alloionematidae	1	Linhomoedidae	3	Choanolaimidae	4
Diploscapteridae	1	Leptolaimidae	3	Ironidae	4
Bunonematidae	1	Halaphanolaimidae	3	Alaimidae	4
Panagrolaimidae	1	Diplopeltidae	3	Bathyodontidae	4
Diplogasteridae	1	Rhabdolaimidae	3	Mononchidae	4
Neodiplogasteridae	1	Chromadoridae	3	Anatonchidae	4
Diplogasteroididae	1	Hypodontolaimidae	3	Dorylaimidae	4
Tylopharyngidae	1	Achrnadoridae	3	Nordiidae	4
Odontopharyngida	1	Ethmolaimidae	3	Qudsianematidae	4
Monhysteridae	1	Cyatholaimidae	3	Leptonchidae	4
Neotylenchidae	2	Desmodoridae	3	Nygolaimidae	5
Anguinidae	2	Microlaimidae	3	Chrysonematidae	5
Aphelenchidae	2	Odontolaimidae	3	Thornenematidae	5
Aphelechoiidae	2	Aulolaimidae	3	Aporcelaimidae	5
Cephalobidae	2	Bastianiidae	3	Belondiridae	5
Ostellidae	2	Prismatolaimidae	3	Actinolaimidae	5
Myolaimidae	2	Tobrilidae	3	Discolaimidae	5
Xyalidae	2	Onchulidae	3		
Plectidae	2	Trypilidae	3		

Persisters are generally considered K-strategists (Bongers, 1990) with less offspring but more parental investment. Colonizers and persisters are placed in both extremes of a 1 to 5 scale, respectively. Families with intermediate values have also intermediate characteristics (Table 2). Thereby, higher soil quality will be represented by a higher number of persisters and higher MI values, while in soils with lower quality colonizers will dominate and the MI value will be lower. To calculate the MI the weighted mean of the individual cp values is used:

$$MI = \sum_{i=1}^n v(i) * f(i)$$

Where:

MI=maturity index

- v(i)=cp value of taxon i as given in Table 2
- f(i) = the frequency of that taxon in a sample

The fact that nematode genera form the same families have the same cp values makes the MI a straight forward tool, low time consuming and easier to use than other methods based on genera

or species identification. Plant feeding nematodes are not included in the MI but in the Plant Parasite Index (PPI)

2.3.2. The Plant Parasite Index (PPI)

The PPI is the MI version for plant parasitic nematodes. Plant parasitic nematodes, also called plant feeding nematodes, depend on the vigour of their host plants for their establishment. The vigour of the plant hardly influences bacterial, fungal, omnivore and predator nematodes, but it is of great importance for the establishment of obligate plant feeding nematodes. The nematode capacity to colonize disturbed systems is different between plant parasitic nematodes and non-parasitic nematodes. The vigour of the plant it is at the same time determined by the system enrichment. Therefore, the PPI behaves opposite to the MI, being lower under soils of natural ecosystems with lower nutrient availability, while in those soils under enrichment or disturbed conditions due to fertilization, the PPI would be higher (Bongers, 1997). Cp values for the PPI are shown in Table 3.

Table 3 Cp values for plant parasitic nematodes: 2=colonizer, 5=persister (From Bongers, 1990)

Tylenchidae	2	Anguinidae	2	Meloidogynidae	3
Psilenchidae	2	Dolichodoridae	3	Criconematidae	3
Tylodoridae	2	Hoplolaimidae	3	Hemicycliophoridae	3
Ecphyadophoridae	2	Pratylenchidae	3	Trichodoridae	4
Paratylenchidae	2	Heteroderidae	3	Longidoridae	5

2.3.3. Combined indices: Enrichment (EI), Structure(SI),and Channel (CI)

The state of the soil food web can be evaluated through the analysis of its structure and function. Different agricultural management practices can cause perturbations and disturbances that may affect the structure and function of the soil food web. Structural analysis of the soil food web present quite some challenges because of the amount of data needed, the high cost of genetic tests, or the difficulties for sampling, identification and assessment of some faunal taxa. Functional analysis of the soil food web does not lag behind regarding constraints. Ferris et al. (2001) introduced an alternative to complete structural analysis based on the assessment of the presence and abundance of different nematode feeding guilds. Nematode guild indicators of food web conditions take as a base that: i) taxa within the same cp value response similarly to disturbances and, ii) Each functional guild represents a grouping of taxa with similar biology. The authors consider 3 types of food webs – basal, structured and enriched- depending on the presence of different nematode taxa, represented in a faunal profile (Figure 1), and attribute to

them different characteristics (Table 4). Basal food webs are those diminished due to stress, scarce availability of resources, contamination or other harsh environmental conditions. Nematode guilds present in basal food webs might be adapted to stress and be more tolerant to polluted conditions than other taxa. Nematode present in this food webs are represented by bacterial and fungal taxa from the cp2 class of the MI. Structured food webs are those recovering from stress and with more resources available. The degree of structure is represented with the amount of trophic links; therefore more structured food webs have a greater amount of trophic links. Nematode taxa from cp3 class would be present in less structured food webs, while structure in the community will be greater when nematode taxa from cp4 and cp5 class are present. When the latter nematode taxa are present the community structure is considered to be in environmental stability. Enriched food webs are those with plenty availability of resources due to the occurrence of a disturbance event. Opportunist bacterial feeding nematodes from the cp1 class are predominant in these food webs. Fungal feeding nematodes from the cp2 class might increase when more complex resources –higher C:N ratio- become available, or when fungal activity is enhanced in detriment to bacterial activity. The increase of complexity in the food web indicated by the presence of nematode taxa with high cp values does not imply the disappearance of low cp nematode taxa however they are proportionally less dominant. The same applies for less complex food webs where nematode taxa of high cp values are less dominant or not present when compare with taxa from low cp classes (Ferris et al., 2001).

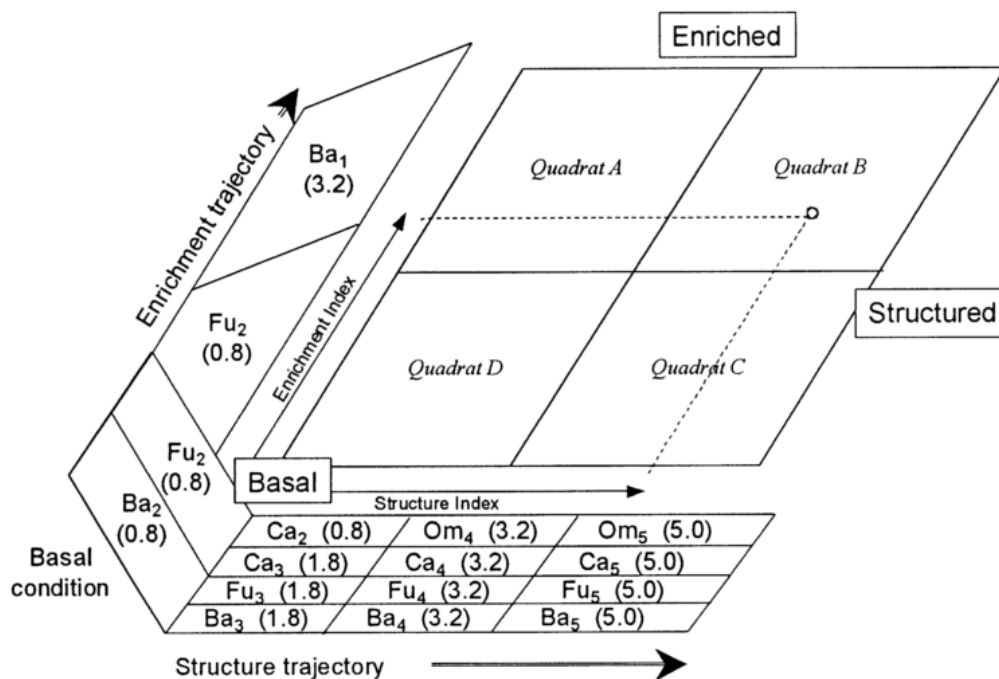


Figure 1 Faunal profile: Functional guilds of soil nematodes characterized by feeding habit (trophic group) and by life history characteristics expressed along a colonizer–persister (cp) scale (after Bongers and Bongers, 1998). Indicator guilds of soil food web condition (basal, structured, enriched) are designated and weightings of the guilds along the structure and enrichment trajectories are provided, for determination of the enrichment index (EI) and structure index (SI) of the food web. (From Ferris et al., 2001)

The structure of the soil food web based on trophic linkages is represented by the structure trajectory, calculated as the structure index (SI). It expresses how probable are regulatory effects on the guilds considered opportunistic, through exploitation or competition of niches. The enrichment trajectory, calculated as the enrichment index (EI), expresses the increase or decrease of primary consumers after an enrichment event (Ferris et al., 2001). The Channel Index (CI) indicates the predominant decomposition channel of the system: either bacterial, fungal or balanced. Lower CI values correspond to a higher proportion of energy transformed through the “fast” bacterial decomposition channel, while the fungal decomposition channel dominates when higher CI values are obtained. To calculate these indices, the following formulas developed by Ferris (2010) are used:

$$EI = 100 * e / (e + b);$$

$$SI = 100 * s / (s + b);$$

$$CI = 100 Fu_2 * w_2 / (Ba_1 * w_1 + Fu_2 * w_2)$$

SI, EI and CI are calculated based on weighted faunal components (b, s and e) of the nematode assemblage, in which:

$$b = (Ba_2 + Fu_2) * w_2;$$

$$e = (Ba_1 * w_1) + (Fu_2 * w_2);$$

$$s = (Ba_n * w_n + Fu_n * w_n + Pr_n * w_n).$$

Where:

- Ba= abundance of bacterial nematodes
- Fu= abundance of fungal nematodes
- Pr = abundance of omnivores and predator nematodes
- n =cp value assigned to the nematode taxa
- w = Weighting assigned to nematodes in each functional group

The combination of SI, EI and CI gives a remarkable amount of information about the quality of the soil system, which can be used to adapt management practices towards more sustainable systems.

Table 4 Inferred condition of the soil food web and its environment based on weighted nematode faunal analysis. Quadrants refer to faunal ordination in the faunal profile (from Ferris et al., 2001)

General Diagnosis	Quadrant A	Quadrant B	Quadrant C	Quadrant D
Disturbance	High	Low to Moderate	Undisturbed	Stressed
Enrichment	N-enriched	N-enriched	Moderate	Depleted
Decomposition Channels	Bacterial	Balanced	Fungal	Fungal
C/N ratio	Low	Low	Moderate to high	High
Food web condition	Disturbed	Maturing	Structured	Degraded

2.3.4. Nematode metabolic footprints

Nematode metabolic footprints are derived from the structure and the enrichment indices. Nematode metabolic footprints are composed by a respiration component and a production component. The respiration component assesses the C utilization of nematodes used for metabolic activity, while the production component assesses the amount of C in the lifetime of nematodes partitioned into growth and egg production (Ferris, 2010). Metabolic footprints provide an estimation of the contribution of nematodes from different taxa, to different ecosystem functions and services related to C and nutrient utilization. The enrichment footprint is the metabolic footprint of nematodes with low cp values that respond fast to enrichment events, these are bacterial nematodes from the cp1 and fungal nematodes from the cp2. The size of the enrichment footprint indicates the dominant trophic levels involved in C utilization. Large enrichment footprints indicate that lower trophic levels are dominating C utilization, mainly as a consequence of an enrichment event, while small enrichment footprints indicate C utilization at higher trophic levels. The structure footprint is the metabolic footprint of nematodes with high cp values -cp3, cp4 and cp5-. Nematodes from higher trophic levels are assumed to have a regulatory function in the soil food web, suppressing opportunistic organisms and regulating nutrient cycles. The functional metabolic footprint is obtained summing the areas of the structure and the enrichment footprints, and can be represented as a rhomboid (Hodson et al., 2014). The shape of the rhomboid shows the balance between the enrichment and the structure footprint. The area of the rhomboid maximizes when its shape is closer to a square. The square

shape suggests that the system is in metabolic balance, and C and nutrients in the system (enrichment) are enough to support organisms of higher trophic levels (structure).

2.4. Shannon-Wiener Index of Diversity

Several indices are used to calculate diversity. The Shannon-Wiener index has been commonly used to assess nematode diversity in different land uses, and in the same land uses managed differently (Yeates, 2003). To calculate the Shannon-Wiener index (H') the following formula is used:

$$H' = - \sum_{i=1}^s p_i \text{Log}_e p_i$$

Where:

S= The number of taxa identified; a given taxon is regarded as the i th taxon.

p= The proportion of individuals in the i th taxon.

3. MATERIAL AND METHODS

3.1. Location

The study systems are located in Pedra Redonda (Figure 2), in the surroundings of Brigadeiro State Park. The landscape is dominated by Atlantic rainforest, coffee and pastures with some spots of eucalyptus plantations. Family farming is predominant in the region in which farmers are oriented either on cattle and therefore pasture production, or on coffee production. Family farmers also have highly diverse vegetable gardens in which they grow cassava, taro, collar greens, tomatoes and several other crops. Vegetable gardens occupy a small space of the total farm area however they contribute importantly to the food supply and the food sovereignty of the families. Predominant soils are Latosols (referred as Oxisols by USDA soil taxonomy). These soils are naturally deep and well drained, acidic and low in nutrient availability (de Souza et al., 2012). Nevertheless due to the heterogeneity of the region, other soils are found, in the case of the studied locations, soils are characterized as humic red-yellow latosol, red-yellow latosol and humic cambisol. The climate of Zona da Mata region is tropical highland climate. The average temperature is 18°C, with a rainy season in summer and a dry season in winter, with average precipitation of 1500mm. In general slopes range from 20 to 45%, while altitude ranges from 200 to 1800masl (Valverde, 1958).



Figure 2 Map of Zona da Mata region. (From de Souza et al., 2012)

3.2. Dialogues with farmers

Information on the history, the characteristics of the farms and the forest fragments, the management of the coffee systems –shade, quantity and type of amendment use, pruning, and cherry picking, etc- was obtained through dialogues kept with farmers. These dialogues were conducted during two stays with coffee farmers in Pedra Redonda. During the first stay of 7days, the goal was to get to know and experience the reality of coffee farmers. In a second stage of 3days, the aim was to do soil collection and to gather more specific information about coffee tree density, specific amount of amendments and other inputs used.

3.3. Coffee systems

The farms are located at the north of the municipality of Araponga, facing Pedra Redonda, a high stone, which gives name to this area (Figure3). The study systems include: 2 agroecological farms (AGR1 and AGR2), 2 conventional farms (CNV1 and CNV2) and one fragment of Atlantic rainforest (RFO) (Figure 4). AGR1 and RFO are east oriented, while CNV1, CNV2 and AGR2 are south oriented. All farmers produce Arabica coffee of the variety catuai. In one of the agroecological farms (AGR1) 3 subsystems can be differentiated according to the type of amendment the farmer uses. In the other agorecological farm (AGR2), two subsystems can be differentiated according to shade regime, in full sun and shaded coffee. Both conventional farmers have a similar type of system management characterized by the absence of shade trees and the use of chemical fertilizers (Table 5).

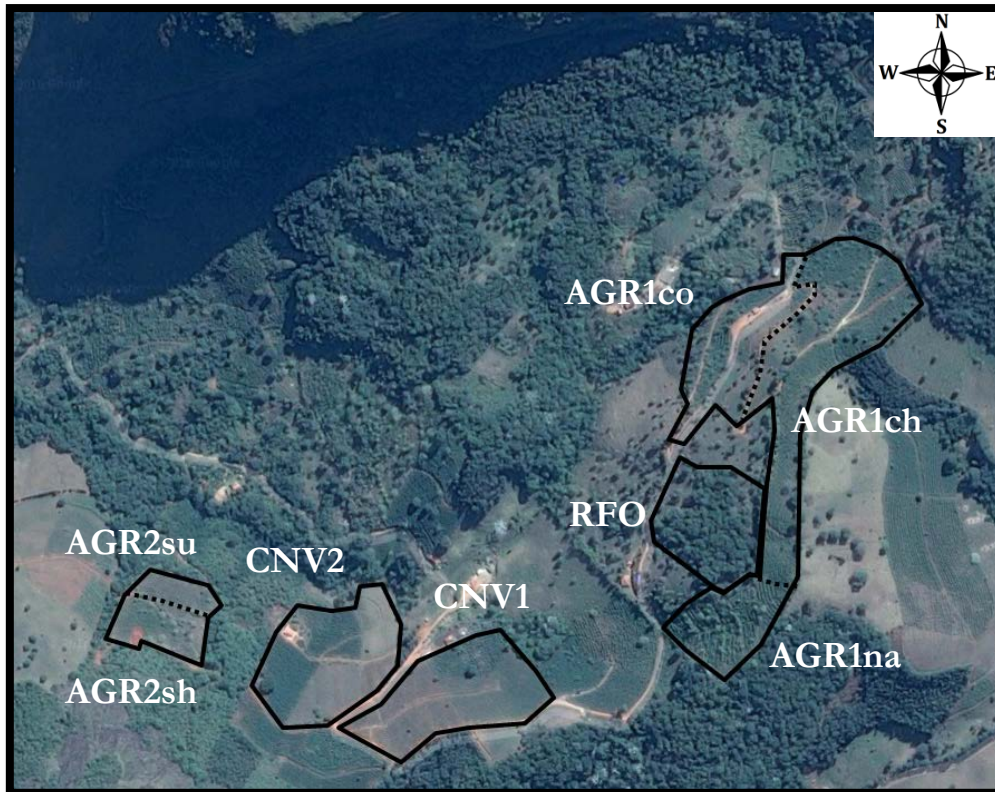


Figure 3 Location of the study sites. Continuous lines demarcate each study site while dotted lines frame sub-systems within the study sites. Each acronym represents one study system: AGR1co=agroecological farm 1 with shade and cow amendments, AGR1ch= agroecological farm 1 with shade and chicken amendments, AGR1na=agroecological farm 1with shade and natural amendments, AGR2sh= agroecological farm 2 with shade and chicken amendments, AGR2su=agroecological farm 2 with no shade and chicken amendments, CNV1=conventional farm 1 with no shade and chemical fertilizers, CNV2=conventional farm 2with no shade and chemical fertilizers and, RFO=Atlantic rainforest.

The study systems have the following characteristics:

- **AGR1:** This system is considered a mature agroecological system with fully grown and diverse shade trees: native and some exotic trees. Some of the native trees are from the leguminous family while exotic trees are fruit trees. Some young shading trees are still in development; thereby a higher shade density is expected in the coming years. The higher coffee density found is 2000 plants per hectare, with an average of 1159 coffee plants per hectare. Amendments are strictly organic, which come from cow (**AGR1co**), chicken (**AGR1ch**) and natural amendments (**AGR1na**). Natural amendments refers to plant residues from leaves and branches from the shading intercropped trees, litter from the neighbouring forest patch, and creeping forage peanut (*Arachis glabrata*) used as a green cover. All three amendments –cow, chicken and natural- are applied together with coffee husk, leaves and pruning remains from diverse shading trees. Weed management

is done using a grass trimmer during the rainy season to control the overgrowth of spontaneous weeds, whereas after the rainy season, the farmer uses a hoe to control the growth of spontaneous plants. Coffee pruning in the farm is done selectively. In the coffee plant, high pruning “descope” is done to rejuvenate the coffee trees that need it, while low pruning “ressepa” - in which coffee plants are cut at 25-35 cm height- is only done in old, unproductive, diseased or really high plants to stimulate the production of new sprouts. Cherry picking is done manually to discriminate between ripe (red) and unripe (green and yellow) coffee cherries. Lime is added approximately every 2 years to buffer soil acidity.

- **AGR2:** In this system two areas can be distinguished according to shade regime. In around 60 percent of the area there are no shade trees, therefore this part can be considered a full sun coffee system (**AGR2su**), while in the other 40 percent of the area the coffee is shaded by trees (**AGR2sh**). Shade is provided mainly by banana trees and capoeiras brancas (*Solanum mauritianum*). Amendments are organic, coming from chicken manure and coffee husk. Coffee density is close to most conventional systems with around 4000 plants per hectare. Weeding is done using a grass trimmer during the rainy season and, when the rainy season is over, the farmer uses a hoe to control the growth of weeds. Pruning is done in a general way in the farm (pruning of the lot), to rejuvenate all coffee trees at the same time. Coffee picking is done without discriminating between ripe and unripe coffee cherries. Lime is added every 3 years.

- **CNV1:** This system is managed in a conventional way, characterized by the use of chemical fertilizers in full sun coffee monoculture. Plant density is considered high, with about 4000 plants per hectare. Weed management is done using a grass trimmer in the rainy season and a hoe when the rainy season finishes, and there are no recordings about the use of agrotoxics in the farm, such as round-up or other herbicides. Pruning in the farm is done in a general way (pruning of the lot). Low pruning “ressepa” in the total area was done 14 years ago. Cherry picking is done without discrimination between ripe and unripe cherries. Lime is added every 2 years.

- **CNV2:** This system is managed in a conventional way. Regarding weeding, pruning, coffee shading, cherry picking and other management practices, this system is comparable to CNV1.

- **RFO:** The Atlantic rainforest fragment is considered a secondary forest. Activities regarding wood for furniture, timber, fences and fire wood stopped since at least 30 years as mentioned by the interviewed farmer. Currently, by law, family farmers are allowed to use 2m³ of forest wood per year.



Figure 4 Study systems: AGR1 (up left), AGR2sh above and AGR2su below in the picture (up right), CNV1 and CNV2 (down left), and Atlantic rainforest, RFO (down right).

Table 5 Characteristics of the studied coffee systems and classification based on amendment type and shade regime.

System	Shade	Amendment type	Area (ha)*	Plants (#/ha)	Amendment applied (kg/plant)	Pruning in the farm	Cherry picking
AGR1ch	Yes	Organic -Chicken & coffee husk	4	1000	4.0	Selective	Selective & manual
AGR1co	Yes	Organic - Cow & coffee husk	1.5	1300	6.0	Selective	Selective & manual
AGR1na	Yes	Organic - Plant residues & coffee husk	0.50	2000	10.0	Selective	Selective & manual
AGR2sh	Yes	Organic - Chicken & coffee husk	0.2	4000	4.0	General	General & with machine
AGR2su	No	Organic - Chicken & coffee husk	0.3	4000	4.0	General	General & with machine
CNV1	No	Chemical - NPK (20,5,20)	3.5	4000	0.5	General	General & with machine
CNV2	No	Chemical - NPK (20,5,20)	3.0	4000	0.5	General	General & with machine

* Area of sampled locations based on farmer estimations

3.4. Soil sampling

Field work was conducted from the 31 of August to the 9 of September of 2015 to characterize the coffee systems and to do soil collection. Soil of Atlantic rainforest was sampled and used as a reference of equilibrium of the nematode population. All study systems are at the same altitude. For nematode identification a total of 5 composed samples per system were collected using a metal ring and a hammer, following a zigzag pattern, with a distance of 10 x 2 meters between samples. The sampling depth and width was 10 x 5 cm respectively. In every sampling location we took 13 samples -sampling points- located as follows: 1 sample close to the coffee trunk, 4 samples at 1 meter distance of the coffee trunk, and 8 samples at 2 meters distance of the coffee trunk (Figure 5). Five soil samples per system were collected from 0-5cm to determine physical characteristics, including: humidity, soil texture, soil bulk density, macroporosity, microporosity and total porosity. A composed sample of every system was brought to the lab for soil chemical analysis, including: Soil pH (pH in 2:1 water extract), exchangeable cations (Ca, Al, Mg) measured after extraction with 1 mol/L KCL, potential acidity (H+Al), K and P extracted by Mehlich⁻¹, cation exchange capacity (CEC), base saturation (%BS) calculated using the concentrations of the exchangeable cations, soil organic matter (OM) and total nitrogen (N), assessed according to EMBRAPA (1997). The points sampled were geo-referenced using a GPS. Soils were stored at 4 degrees immediately after sampling.

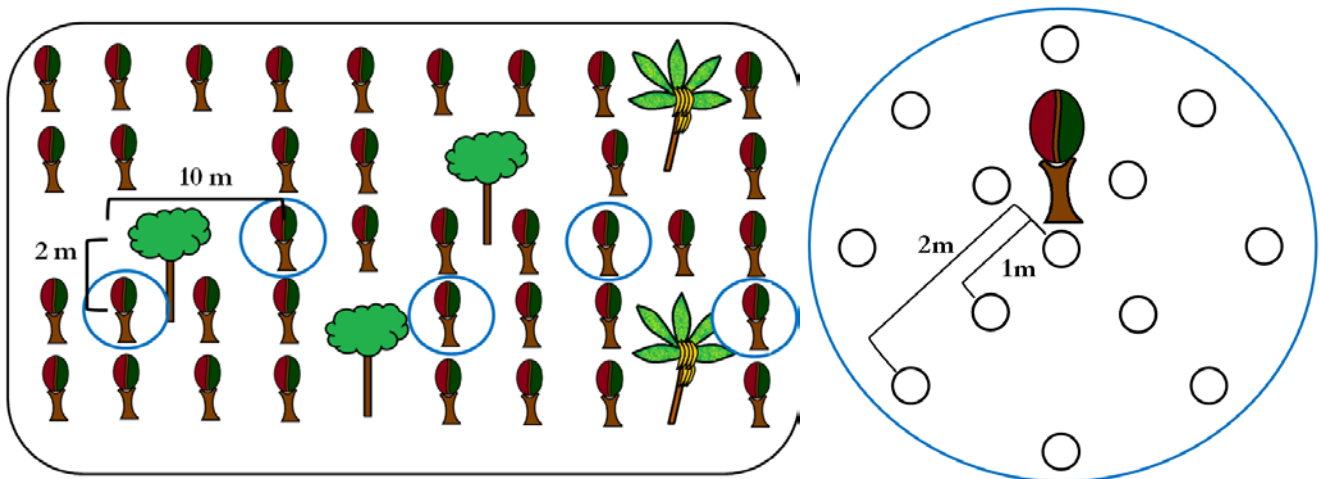


Figure 5 Sampling pattern per study system (left), and per sampling location (right). Blue circles represent each sample location while black circles represent every sampling point.

3.5. Nematode preparation

3.5.1. Nematode extraction from soils

Extraction of nematodes was executed following the methodology developed by Jenkins (1964) also known as “sucrose centrifuge method”. Soil samples were homogenised using a 2 mm sieve to remove stones and roots. From each sample, 100cm³ of soil was washed throughout a sieve of 1mm into a baker with 1liter of water. The solution was stirred gently and left to stand for 20 seconds for all big particles to settle down. The solution was filtered using a 400 mesh sieve. The soil remaining in the sieve was collected using tap water into a 50 ml centrifuge tube. All samples were centrifuged at 3000 rpm for 5 minutes. After centrifuging, the supernatant was discarded and the tubes were filled up with sucrose solution (454g/l). The samples with sucrose were stirred and centrifuged once more at 3000 rpm for 5 minutes. After centrifuging the sucrose solutions the nematodes remained in the sucrose suspension while the soil settled down in the bottom of the tube. The sucrose solution was filtered using a 400 mesh sieve to collect the nematodes. The nematode samples were washed out from the mesh of the sieve to 50 ml pots with the help of a funnel. Samples were stored at 4 degrees Celsius until killing.

3.5.2. Killing and fixing

Samples were levelled up to 10 ml of water. Nematodes were killed by gradually warming up the samples in an oven at 65 degrees Celsius. To fix the samples, 2 ml of formalin were added to every sample.

3.5.3. Glass slides mounting “Embedding”

For facilitating nematode identification, we picked “fished” 100 nematodes randomly of each sample using a binocular stereo microscope for identification. The “fishing’ needle we used is an eyelash hair mounted into a wooden stick and glued with nail polish (Van Bezooijen, 2006). We mounted the nematode samples in ordinary glass slides. We put a droplet of the water-formalin solution in both sides of the glass slide and place 10 of the picked nematodes in them. We sealed the glass slide using a double layer varnish ring and sealing the cover slip with 2 layers of nail polish.

3.5.4. Nematode identification

Nematode counting and identification (Figure 6) to family, or genus when possible, was carried out using a microscope of 100x and 400x magnifications, and with the help of an identification guide of plant parasitic nematodes and the interactive keys for nematode identification of the University of Davis http://plpnemweb.ucdavis.edu/nemaplex/_vti_bin/shtml.dll/index.htm and the University of Nebraska <http://nematode.unl.edu/key/nemakey.htm>.



Figure 6 Nematodes identified from the study sites: *Acrobeles* (left) and *Aphelenchus* (right). (Source: Compiled by the author)

3.6. Calculation of Nematode indices: NINJA

Maturity Index (MI), Plant Parasitic Index (PPI), Channel Index (CI), Enrichment Index (EI), Structure Index (SI) and nematode metabolic footprints were calculated using the NINJA Programme (Sieriebriennikov et al., 2014). The indices were calculated using the nematode families identified in every system, and nematode genus when possible.

3.7. Calculation of the Shannon-Wiener Index of diversity

The Shannon-wiener Index was calculated to assess the different diversity of nematode taxa for every study site.

3.8. Statistical Analysis

One-way ANOVA and multiple comparison tests (Tukey's Honestly Significant Difference) were performed to explore differences in soil physical properties, nematode-base indices and Shannon

diversity index between all study systems. Assumptions of homoscedasticity and normality for ANOVA were verified and data was log transformed when needed. The statistical analysis was carried out using Genstat 18th Edition.

4. RESULTS

4.1. System effect in soil chemical characteristics

The results obtained from the different study systems on soil chemical characteristics are shown in Table 6. Both conventional systems, CNV1 and CNV2, had the highest pH values compared to all agroecological systems and the Atlantic rainforest (RFO). Agroecological systems and the Atlantic rainforest had pH values of between 0.28 and 0.67 units lower than conventional systems. The soil nitrogen content in all agroecosystems was similar. All agroecological systems had higher phosphorus contents when compared with both conventional systems and the Atlantic rainforest. Available phosphorus and remaining phosphorus are particularly high in AGR1ch and AGR2sh followed by AGR2su. Available phosphorus in AGR1ch was almost 3 times higher than in AGR2sh and more than 10 times higher than in AGR2su. Potassium (K), Calcium (Ca^{2+}) and Magnesium (Mg) were higher for all agroecological systems when compared to the conventional systems and to the Atlantic rainforest. Potassium, calcium and magnesium values were the highest for AGR1ch.

The organic matter content was higher for all agroecological systems and the Atlantic rainforest compared with both conventional systems. AGR1ch had the largest amount of organic matter followed by the Atlantic rainforest, AGR1na and AGR1co. Both systems within AGR2 had intermediate organic matter values, and are placed in between the systems with highest OM content, and the conventional systems, which had the lowest OM contents. Aluminium was only present in CNV1 and RFO, the later being 6 times higher. Potential acidity ($\text{H} + \text{Al}$) was the largest in the Atlantic rainforest being about three times larger than in AGR1co. Aluminium saturation followed the same pattern as aluminium content, with a value zero in most systems, except by the CNV1 and RFO systems. Cation exchange capacity was higher in all agroecological systems when compared to the conventional systems and the Atlantic rainforest. AGR1ch had the largest CEC value. Base saturation followed the same pattern as CEC.

Table 6 Means of chemical parameters for all study systems

	pH (H ₂ O)	N (%)	P-av (mg/dm ³)	P-rem (mg/dm ³)	K (mg/dm ³)	Ca ²⁺ (mg/dm ³)	Mg (cmolc/dm ³)	Al (cmolc/dm ³)	H + Al (cmolc/dm ³)	CEC (cmolc/dm ³)	BS (%)	Al Sat (%)	OM (dag/kg)
AGR1ch	5.49	0.28	705.60	42.5	977	9.53	6.83	0.00	2.5	18.87	88.3	0.0	28.69
AGR1co	5.55	0.27	46.70	19.4	329	5.92	2.26	0.00	6.1	9.02	59.7	0.0	14.02
AGR1na	5.80	0.27	15.40	21.1	319	7.30	3.79	0.00	5.0	11.91	70.4	0.0	15.32
AGR2sh	5.45	0.24	242.60	26.4	259	5.81	2.83	0.00	4.5	9.30	67.4	0.0	9.46
AGR2su	5.66	0.28	66.30	24.4	299	6.45	3.45	0.00	2.3	10.67	82.3	0.0	7.83
CNV1	6.08	0.27	10.70	11.0	73	1.65	0.46	0.40	4.5	2.70	33.8	14.8	5.48
CNV2	6.16	0.28	3.50	18.5	141	3.93	1.10	0.00	5.8	5.39	48.2	0.0	6.39
RFO	5.67	0.29	3.00	12.0	60	0.46	0.22	2.40	18.2	3.23	4.4	74.3	22.17

N= Total nitrogen, P-av=Available phosphorus, P-rem= Remaining phosphorus K=Potassium, Ca=Calcium, Mg=Magnesium, Al=Aluminium, H+Al=Potential acidity, BS=Base saturation, CEC= Cation exchange capacity, Al Sat=Aluminium saturation, OM= Organic matter.

4.2. System effect in soil physical characteristics

The effects of the different study systems on soil physical characteristics are shown in Table 7. Moisture was higher for AGR1co compared to AGR1na and AGR2sh. There were no differences with all the other systems, having moisture contents between 26 and 29 percent. Texture was similar for all soils, with no differences in clay content and in which all were categorized as clay soils based on the USDA soil textural triangle, with the exception of AGR1ch, which was categorized as sandy clay, due to a higher proportion of sand compared to all the systems. AGR1ch had the highest soil density of all systems, although not significantly higher when compared to AGR1co and AGR1na, and CNV2. Soil density was the lowest in the Atlantic rainforest compared to all coffee agroecosystems. Additionally, the Atlantic rainforest showed the highest values for macroporosity and total porosity. Regarding total porosity coffee agroecosystems did not differ. AGR1co had higher microporosity when compared to AGR1na and AGR2sh (Table 7).

Table 7 Means of soil physical parameters for all study systems

	Moisture (%)	Clay (%)	Silt (%)	Sand (%)	Texture	Soil density (g/cm ³)	Part. Density(g/cm ³)	Microporosity	Macroporosity	Total porosity
AGR1ch	27.02 ab	45 a	12 c	43 ab	Clay	1.16 d	2.35 a	0.29 ab	0.21 ab	0.51 a
AGR1co	30.36 b	43 a	3 a	54 b	Sandy clay	1.11 cd	2.23 a	0.33 b	0.17 a	0.51 a
AGR1na	23.48 a	49 a	9 abc	42 a	Clay	1.11 cd	2.26 a	0.27 a	0.24 abc	0.51 a
AGR2sh	23.58 a	48 a	7 abc	45 ab	Clay	0.92 b	2.26 a	0.27 a	0.32 bc	0.59 ab
AGR2su	26.19 ab	48 a	10 bc	42 a	Clay	0.97 bc	1.96 a	0.29 ab	0.21 ab	0.50 a
CNV1	28.89 ab	50 a	9 abc	41 a	Clay	0.97 bc	2.35 a	0.32 ab	0.26 abc	0.58 ab
CNV2	27.90 ab	50 a	8 abc	42 a	Clay	1.07 bcd	2.17 a	0.30 ab	0.24 abc	0.54 ab
RFO	25.79 ab	52 a	4 ab	44 ab	Clay	0.68 a	1.94 a	0.29 ab	0.36 c	0.65 b

Means with different letters within the same column shows significant differences (p -value <5%) established by the Tukey's HSD test

4.3. System effect in nematode populations

The Atlantic rainforest had the highest maturity index when compared to all agroecosystems, and did not differ from AGR1na. All coffee agroecosystems did not differ among them regarding maturity (Figure 7). Coffee agroecosystems did not differ in the PPI from the Atlantic rainforest. Nevertheless, AGR1co got the lowest PPI value, together with the Atlantic rainforest, AGR1ch and CNV1 (Figure 8).

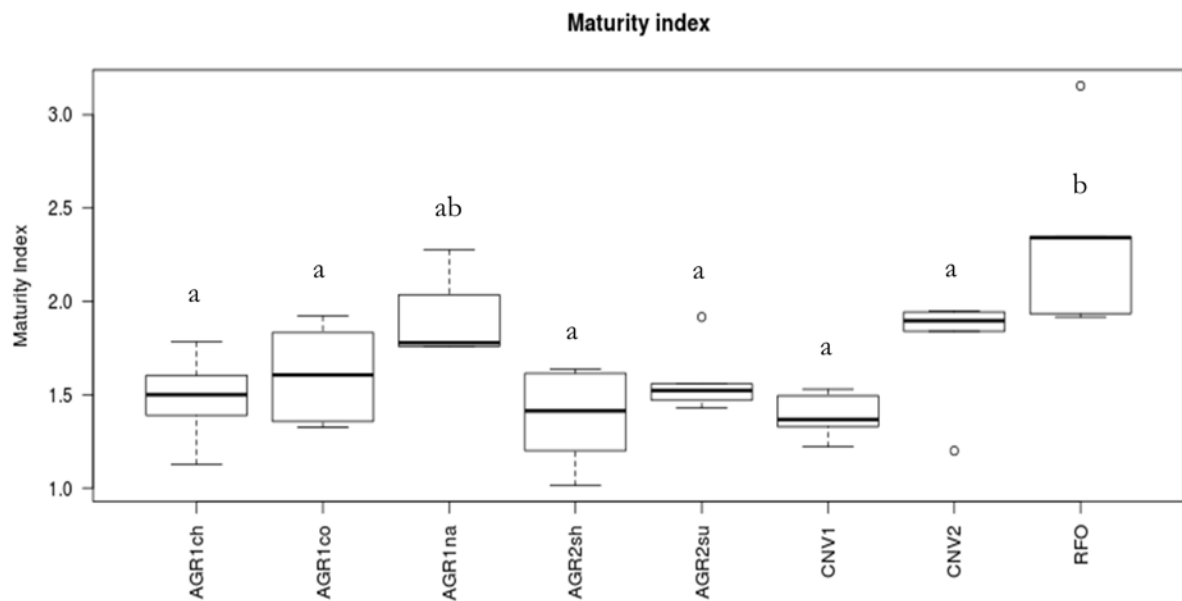


Figure 7 Maturity Index box plots for all study systems.

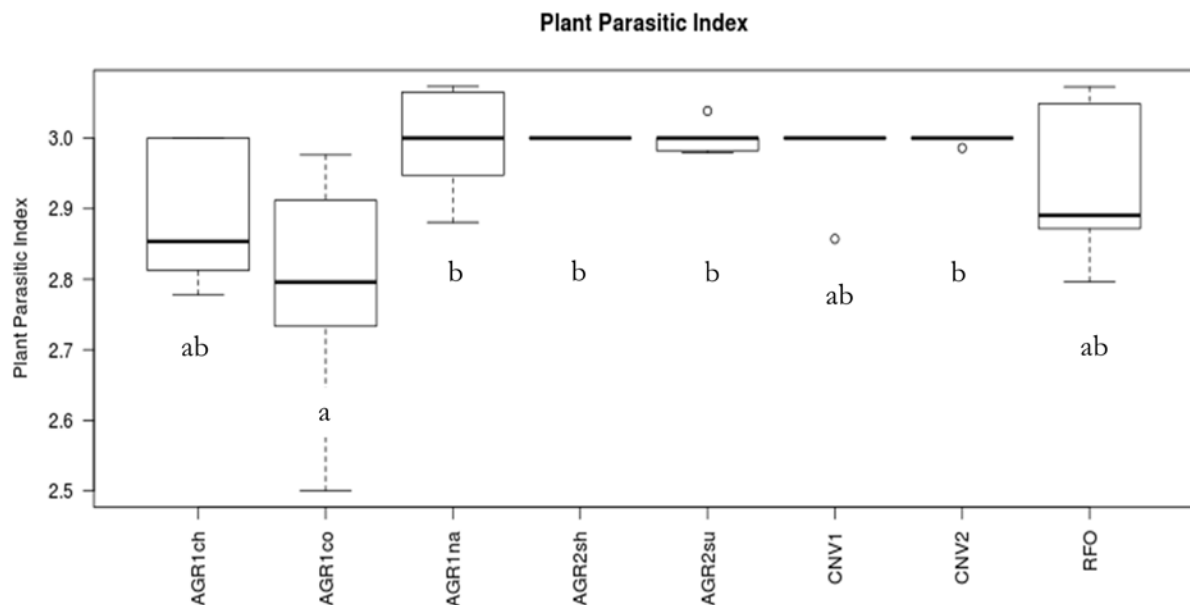


Figure 8 Plant Parasitic Index box plots for all study systems.

Coffee agroecosystems did not differ between them neither from the Atlantic rainforest, with the exception of AGR1ch, which got the lowest CI value, and differed from CNV2 and RFO which had the highest CI values (Figure 9).

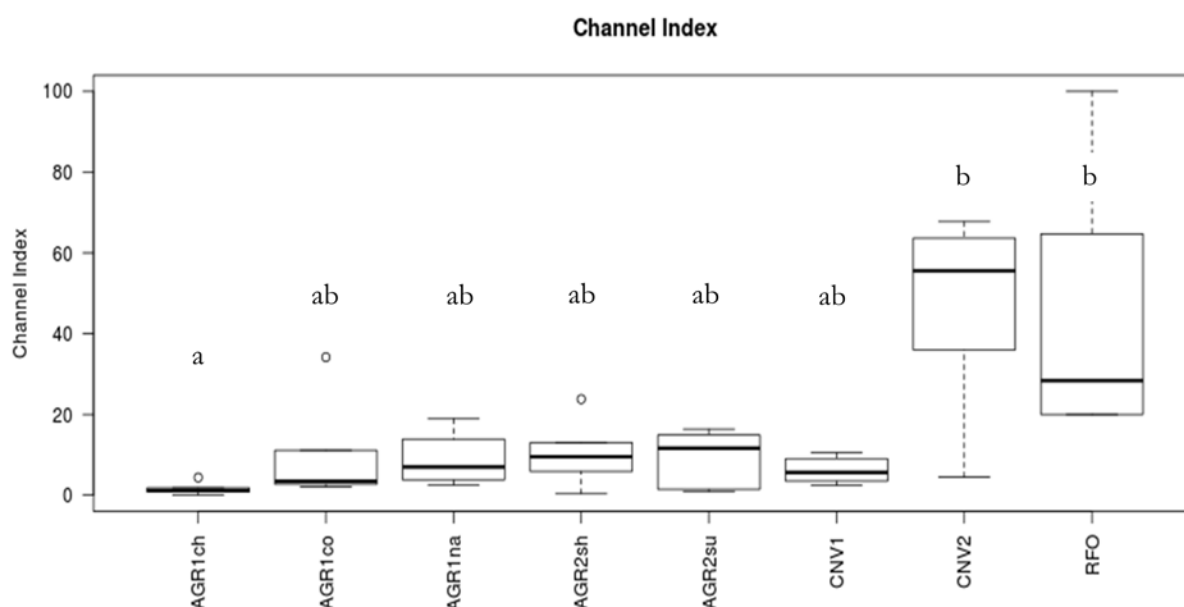


Figure 9 Channel Index box plots for all study systems.

Table 8 Means of nematode-base soil quality indices for all study systems

	Enrichment Index (EI)	Structure index (SI)
AGR1ch	89.6 c	51.3 ab
AGR1co	83.8 bc	45.5 ab
AGR1na	80.2 abc	51.3 ab
AGR2sh	86.7 c	6.2 a
AGR2su	88.3 c	44.7 ab
CNV1	87.6 c	12.8 a
CNV2	62.9 ab	4.0 a
RFO	61.2 a	58.0 b

Means with different letters within the same column shows significant differences (p-value <5%) established by the Tukey's HSD test

The Atlantic rainforest had the lowest EI, but did not differ from AGR1na and CNV2, although these systems showed an increasing trend. The EI of all agroecological systems was similarly high and there were no differences among them. Additionally, the Atlantic rainforest also got the highest value for the SI, and did not differ from agroecological systems, with the exception of AGR2sh. The latter together with both conventional systems got the lowest SI (Table 8). From the interaction of EI and SI, soil food web condition and metabolic footprints of the study systems were calculated and represented (Figure 11). To help with the interpretation of results check Figure 10.

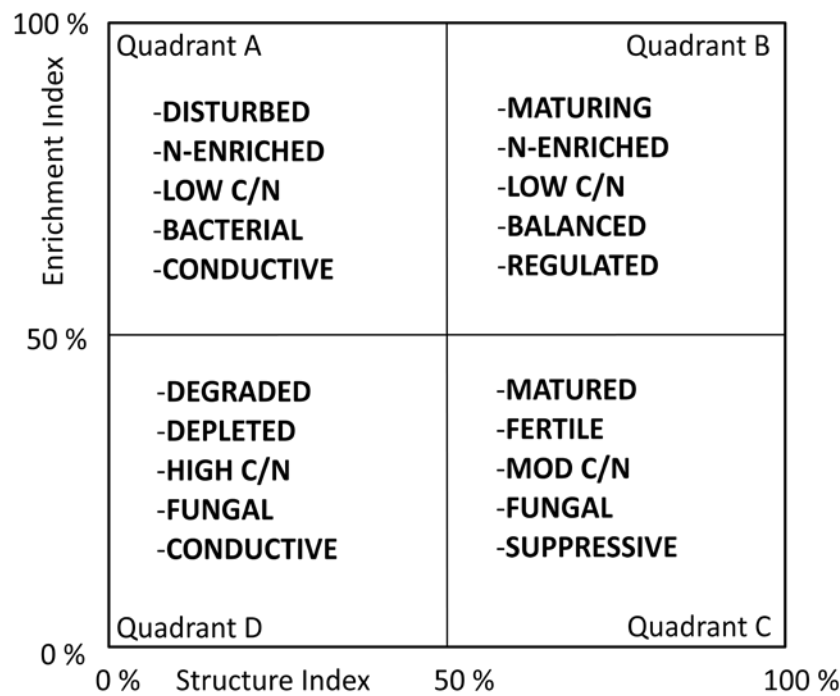


Figure 10 General diagnosis of the food soil web organized in quadrants based on SI and EI.

The Atlantic Rainforest, AGR1na, and AGR1ch fitted in the same quadrant (quadrant B) which represents an enriched environment characterized by a low C/N ratio with a balanced decomposition channel although with bacterial decomposition prevalence. The soil system of the Atlantic rainforest has a moderate disturbance with a maturing soil food web. In addition to this, the Atlantic rainforest is closely situated to quadrant C, which represents the highest standard of quality, with moderate enrichment and moderate to high C/N ratio, thereby with a fungi dominated decomposition channel. The soil system is considered undisturbed and the food web well-structured. Both conventional systems, AGR1co and both AGR2 systems fitted in quadrant A which represents an enriched environment characterized by a low C/N ratio with a decomposition channel dominated by bacteria. The soil system is highly disturbed as well as the soil food web. However AGR1co and AGR2su are located quite close to quadrant B which framed the Atlantic rainforest, AGR1na and AGR1ch. Regarding metabolic footprints, enrichment footprints were larger than structure footprints in all systems and there was not a clear pattern showing differences in enrichment footprints between the Atlantic rainforests and all coffee agroecosystems.

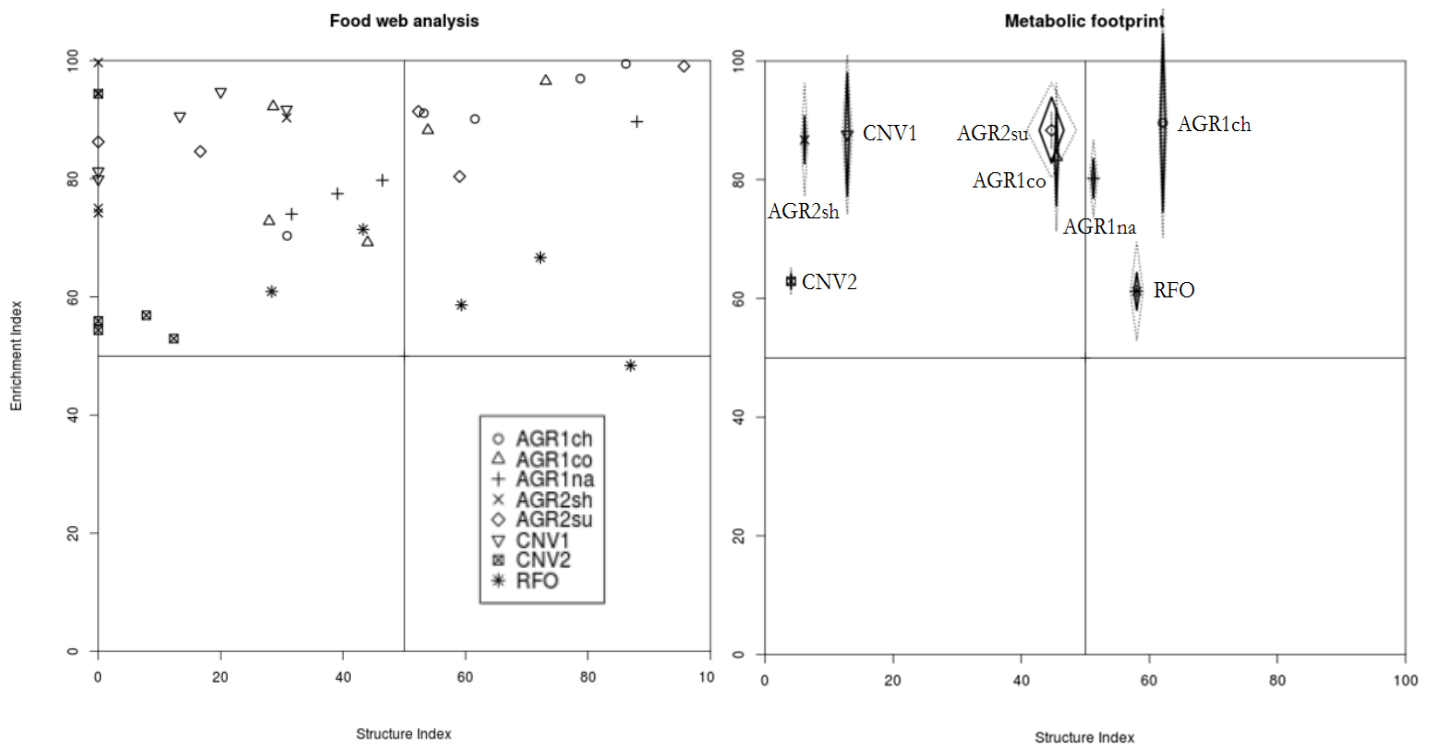


Figure 11 Food web analyses –basal, enriched and structured- based on the food soil web conditions of every study system (left). Metabolic food prints represent the carbon utilisation by different food web components (right). The symbol in the middle of a rhombus corresponds to the intersection between the EI and the SI. The length of the vertical axe corresponds to the enrichment footprint, while the width of the horizontal axe corresponds to the structure footprint.

4.4. System effect in nematode diversity

The Atlantic rainforest together with all AGR1 systems had the highest diversity; expressed by the Shannon index. Both conventional systems, together with both AGR2 systems, had the lowest diversity values, and did not significantly differ among them. AGR1ch does not differ from CNV1 and AGR2su, but shows a tendency towards the high diversity systems – AGR1na, RFO, and AGR1co- (Table 9).

Table 9 Means of Shannon diversity index for all study systems

	AGR1ch	AGR1co	AGR1na	AGR2sh	AGR2su	CNV1	CNV2	RFO
Shannon (H')	1.67bc	2.09c	2.35c	0.68a	1.26ab	1.14ab	0.94a	2.29c

Means with different letters showed significant differences (p-value <5%) established by the Tukey's HSD test

All AGR1 systems got the highest values in diversity together with the Atlantic rainforest. Nevertheless the Shannon index takes into account the number of nematode families in every system but not the distribution of families by feeding guild. AGR1na is the most similar system to the Atlantic rainforest, regarding diversity but also distribution of nematode families within feeding guilds. Both conventional systems and AGR2 systems had a fewer number of nematode families representing each feeding guild, compared to AGR1 systems and RFO (Figure 12). At the same time, predator nematodes, which are considered persisters regarding maturity cp values, are mostly present in the Atlantic rainforest and AGR1 systems, with the exception of AGR2su, which also has some representation in this feeding guild.

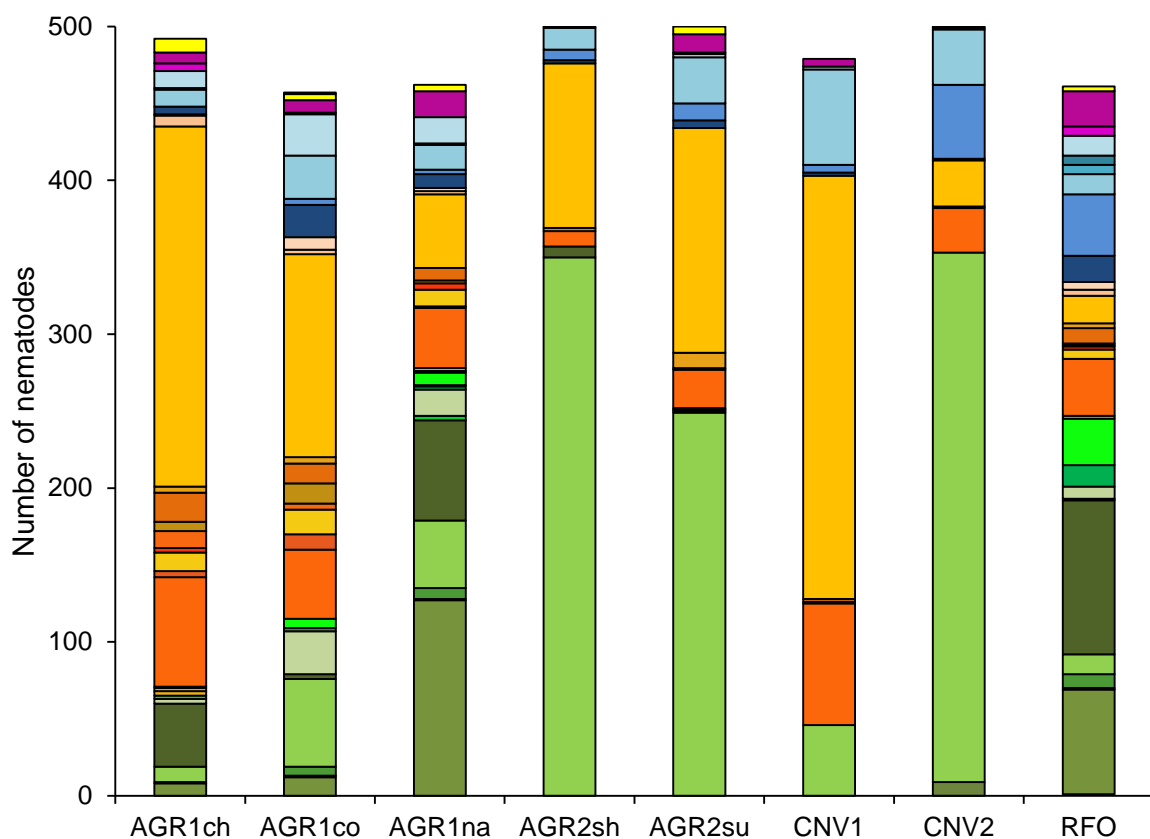


Figure 12 Diversity of nematode families in every study system. Each colour represents each feeding guild: green=plant feeders, orange=bacterial feeders, blue=fungal feeders, purple=omnivore nematodes, yellow=predators. Each tonality within one colour represents one family.

5. DISCUSSION

This research shows that mature agroecological coffee systems can improve soil chemical properties, which is translated into higher soil fertility and therefore leads to highly productive systems, and at the same time can hold a diverse and mature soil food web proper of their reference climax soils: the soils of the Atlantic rainforest. Although results were not conclusive regarding MI, PPI and CI, the results obtained from the interaction of EI and SI about the soil food web condition, showed a clear range of systems regarding soil food web structure and function, from conventional systems to more mature agroecological systems, and with the Atlantic rainforest as reference of highest soil quality. Less mature agroecological systems shared characteristics with conventional and mature agroecological systems, fact that shows the large room for improvement for these systems to achieve more sustainable systems.

5.1. Coffee management in physicochemical soil quality

Management practices of agroecological coffee farmers are focussed in, among others, preserve soils, maintain or increase soil fertility and avoid soil degradation and erosion. In order to achieve the previously mentioned goals, farmers minimize soil disturbance, which leads to the improvement of soil physicochemical characteristics and the enhancement of ecosystem services and functions provided by the soil system. Some soil physical properties are difficult to modify, as texture or particle density, which indeed, for all coffee agroecosystems and the Atlantic rainforest were similar. Coffee agroecosystems did not show differences regarding most soil physical properties. Nevertheless, sustainable practices such as shading coffee and the addition of organic matter from leaves, branches, and organic animal amendments, can improve some soil physical characteristics. In the Atlantic rainforest, which soil is rich in organic matter due to the continuous addition of organic residues from leaves and branches, and which is barely disturbed, soil density was the lowest, while macroporosity and total porosity was the highest compared to all coffee systems. Soil physical structure, is influenced by soil density and porosity, which in turn are influenced by soil texture and type of aggregates. Since the soil texture of all study systems is similar, aggregates are assumed to play a major role influencing soil structure. Indeed, the Atlantic rainforest showed the best structure, regarding porosity and soil density, compared to all coffee systems. Aggregate formation is promoted by the presence of organic matter, which in fact was the highest in the Atlantic rainforest.

Regarding soil chemical properties, I found clear differences between agroecological and conventional coffee systems. High pH values can result in the improvement of other soil

chemical properties, such as cation exchange capacity and base saturation. Increasing organic matter can lead to higher pH values. Although pH values were higher in both conventional systems when compared to the Atlantic rainforest and the agroecological systems, the latter presented higher CEC and base saturation compare to the other systems. Since organic matter was the lowest in conventional systems, higher pH in conventional systems might be obtained due to the addition of lime by farmers to reduce soil acidity. Regarding macro and micronutrients, the same pattern was repeated: agroecological systems had the highest macro and micronutrient contents followed by conventional systems and lastly by the Atlantic rainforest. At the same time, the most mature agroecological systems, referred as those systems within AGR1, presented higher contents in Potassium, Calcium and Magnesium. Both available phosphorus and remaining phosphorus contents were higher in agroecological systems compared with conventional systems, and the highest phosphorus contents were obtained for those systems where chicken manure was applied: AGR1ch, AGR2su and AGR2sh. In fact, chicken manure has been reported as a phosphorus rich manure source.

The Atlantic rainforest and the mature agroecological systems, showed the highest amounts of organic matter, followed by AGR2 systems, and by the conventional systems, accounting with the lowest soil organic matter. Low values obtained in both conventional systems can be explained because of the lack of organic matter added by the farmers, besides the organic matter from coffee leaves and pruning remains. At the same time, high values in the Atlantic rainforest and the mature agroecological system are the consequence of a frequent addition of organic matter coming from leaves and branches from shade trees and, in the case of the coffee system, because of the farmer addition of animal organic amendments together with coffee husk. Furthermore, the higher soil organic matter content in the Atlantic rainforest and the mature agroecological system, led to a higher cation exchange capacity and base saturation. Higher cation exchange capacity and base saturation can be translated into a higher nutrient retention capacity; therefore higher fertility, and higher buffer capacity. The Atlantic rainforest showed a large potential acidity. Potential acidity has been reported much greater in Atlantic rainforest fragments than in coffee agroecosystems (Reiners et al., 1994; de Souza et al., 2012). Low potential acidity values in all coffee agroecosystems might be caused because of the addition of lime that neutralizes toxic elements, such as aluminium, and reduces acidity, and because of the low disturbance in the Atlantic rainforest. Regarding physicochemical properties my first hypothesis is partially confirmed, with a clearer improvement of soil chemical characteristics in agroecological coffee systems compared to conventional systems, while there is not such an evidence for physical characteristics.

5.2. Coffee management in the soil food web condition

Maturity index gives information about the disturbance condition of an environment. High MI values indicate a more mature and therefore less disturbed system, while low values account for less mature and more disturbed systems. The Atlantic rainforest, assumed to be the least disturbed and most mature of the study systems, had the highest MI, followed by AGR1na. Nevertheless the MI was not sensitive enough to show differences between conventional and agroecological coffee systems. Bloemers et al. (1997) showed similar results regarding forest disturbance in tropical soils, in which there were no significant MI differences between undisturbed forests and the most extreme forest disturbances with active slash and burn and complete mechanical forest clearance. For the PPI, the systems AGR1co and AGR1na, together with the Atlantic rainforest and CNV1 showed the lowest values. MI and PPI are supposed to behave opposite to each other, with low values of PPI showing high maturity and the opposite accounts to high values. However, MI and PPI showed contradictory trends. The systems that had high maturity for the MI turned out to be disturbed for the PPI and vice versa. Just for the Atlantic rainforest, MI and PPI pointed out in the same direction, presenting this system as the most mature a least disturbed, compared to all coffee agroecosystems. Similar results, in which MI and PPI showed opposite trends and were not sensitive enough to account for differences, were obtained when different agricultural systems were compared with a heavily polluted system (Urzelai et al., 2000).

High CI values correspond to soils dominated by fungal decomposition channels, also known as “slow” decomposition channels. Materials decomposed by fungi are commonly lignin and carbon rich, containing complex recalcitrant compounds. These compounds are predominant in woody materials like those present in forest areas, therefore a higher proportion of fungi feeding nematodes are expected. Coffee agroecosystems had lower CI values than the Atlantic rainforest, with the exception of CNV2, which had the highest CI value. The CI merely takes into account the ratio between fungal and bacterial nematodes. The soils of CNV2 were heavily infested by *Meloidogyne exigua* in its juvenile stage (J2). *M. exigua* is a plant parasitic nematode known to be the most widespread nematode pests in coffee fields in Brazil, and particularly in Minas Gerais (Souza, R. 2008). A single female of *M. exigua* can produce over 1000 eggs which will pass through J1 stage within the egg and hatch into the infective J2 stage. Staver et al (2001) pointed out that the intensification of coffee production under full sun creates favourable conditions for pests and diseases which are uncommon in shade coffee. At the same time, the reduction of shade implies a reduction in organic matter inputs, which has been linked to increasing nematode damage in coffee (Villain et al, 1999). The infestation of *M. exigua* in CNV2 affects the proportion

of other nematode feeding guilds present in the system, and creates confusing results for the CI, in which CNV2 showed the highest CI value, higher even than the expected Atlantic rainforest. In turn, CNV1, which was not heavily infested by *M. exigua*, had a greater proportion of Rhabditidae nematodes, which are bacterial feeders, and that was also expected to happen for CNV2. Bacterial feeding nematodes were expected to occur mainly in disturbed environments, which are commonly dominated by the bacterial decomposition channel. Nevertheless, there were no differences between coffee agroecosystems regarding CI. As assumed, nematodes were more sensitive indicators than physicochemical properties to changes and disturbances happening in the coffee systems. The study of the nematode population helped to better understand the impact of coffee management practices.

SI and EI pointed out to the Atlantic rainforest as the system with highest soil quality. Agroecological systems had higher SI than conventional systems, although most coffee agroecosystems were equally enriched and there were not clear differences between agroecological and conventional systems. The Atlantic rainforest had a structured and maturing soil food web, proper of regulated soils with a balanced bacterial-fungal decomposition channel. The fact that the rainforest fragment fitted in quadrant B, and not in quadrant C, which is more typical for pristine forests, might be the cause that the chosen forest fragment was a secondary Atlantic forest. At the same time, the mature agroecological systems -AGR1na, AGR1co and AGR1ch- with well-developed trees, and an equilibrated system achieved during more than two decades of agroecological management, showed similar characteristics to the Atlantic rainforest regarding soil food web condition. These results show that improving soil chemical characteristics does not implies negatively impact soil life, as AGR1 had the “best” soil chemical properties for agricultural production compared to all study systems and, at the same time, a soil food web condition comparable to the reference Atlantic rainforest. In contrast, conventional systems showed a disturbed soil food web with a bacterial dominated decomposition channel proper of highly enriched systems of conductive soils. CNV2 showed a tendency towards the category that represents soils under harsh environments, lot of stress and degraded soil food webs. AGR2sh and AGR2su systems did not show a clear pattern, with AGR2su closer to AGR1 systems, which had higher soil quality based on the soil food web condition, while AGR2sh is placed in between CNV1 and CNV2, represented in a lower quality category. AGR2sh and AGR2su have similar nematode communities, however in AGR2sh there were fewer nematodes with higher cp values than in AGR2su. This fact makes AGR2sh a system with less structured soil food web based on the amount of trophic links.

Regarding metabolic footprints, in a study comparing different farming systems in California, enrichment footprints were generally greater in those systems with nutrient resources supplied by organic amendments than by mineral fertilizers. Opposite results were found in another experiment in which conventionally managed systems had a greater enrichment footprint than organically managed grasslands (Ferris, 2010). In this current study, there were not clear differences between organic and conventional systems regarding enrichment footprints; however enrichment footprints were much larger than structure footprints in all systems. This results hold to the hypothesis developed by Ferris (2010) that in a system which is in metabolic balance the productivity and turnover rates of the enrichment indicators should be sufficient to maintain the needs of predators. Larger enrichment footprints indicate that in the study systems C utilization is greater at lower trophic levels of the soil food web -often due to nutrient additions-, which is also supported by the scarce amount or absence of nematodes from higher cp classes present in most of the systems. Regarding soil food web condition my third hypothesis is partially confirmed, with the highest quality represented by the Atlantic rainforest and followed by the mature coffee agroecosystems. However, all agroecological systems were expected to have similar soil quality, and this was not always the case, with AGR2 systems closer to the conventional systems, and AGR1 systems closer to the Atlantic rainforest. Regarding MI, PPI and CI, these indices were only sensitive enough to account for differences between the Atlantic rainforest and the coffee agroecosystems, but did not show differences between coffee agroecosystems.

5.3. Coffee management in nematode diversity

Although the diversity of soils tremendously contributes to global biodiversity, not much attention has been paid to how conservation activities and farming management practices affect soil communities and their relations with soil quality. This fact sounds paradigmatic since up to 90 % of the terrestrial primary production is decomposed in the soil, and nematodes, occupying key positions in the soil food web, largely contribute to the decomposition processes. Agricultural intensification negatively affects soil diversity at different levels within the soil food web. Nematode diversity has been reported to decrease as a result of agricultural intensification (Yeates & Bongers, 1999), and to increase as a result of agricultural abandonment (Háněl, 2010). Also, nematode diversity was found to decrease in conventional agriculture compared to organic systems (Poostma et al, 2010), and after cultivation and clearance of a tropical rainforest (Bloemers et al., 1997). Incorporation of plant residues generally increases the number of free living nematodes (Oka, 2010). In accordance with these findings, the results obtained in the current study pointed out in the same direction.

The Atlantic rainforest together with AGR1, accounted with the highest nematode diversity, not just expressed by the Shannon index, but also by the number of families present in the different nematode feeding guilds. The niche diversity in these systems is supposed to be greater due to the higher diversity of organic inputs and of soil environments, therefore favouring nematode diversity. AGR2 systems and the conventional systems showed lower diversity when compared to the most diverse systems. Even though higher diversity of AGR2 systems over CNV systems was expected, the fact that AGR2shhad lower diversity than CNV1 and CNV2 might be explained by the scarce shade, the low diversity of shade trees and of organic inputs and the high coffee plant density in this system. My fourth hypothesis is also partially confirmed, with the highest diversity represented by the Atlantic rainforest and the mature agroecological coffee system and the lowest by the conventional systems, but there was not a clear pattern in AGR2 systems, and the Atlantic rainforest ranked second in diversity, after AGR1na.

6. CONCLUSIONS

Climax soils developed under climax environmental conditions, such as the soils developed under the Atlantic rainforest, are great examples of high soil quality. Management practices, like the use of shade trees and application of organic amendments, play a major role on achieving high quality soils. Mature agroecological coffee agroecosystems have the potential of achieving multifold objectives like increasing soil fertility, enhancing soil diversity and maintaining a structured soil food web, proper of regulated soils with a balanced decomposition channel. The Atlantic rainforest and the mature agroecological coffee agroecosystems hold the highest nematode diversity and the highest soil quality regarding soil food web condition. Less mature agroecological systems and conventional systems, have a large room for improvement regarding soil physicochemical and biological properties; in order to achieve substantial changes in soil quality towards more sustainable systems. Nematode base indices provide a large amount of information of the soil condition and can be combined with more traditional soil physicochemical analyses to have a more detailed picture of the soil status. Nevertheless, it is biased to assume that base on just one link of the soil food web we can make statements about the state of such a complex system. Complementary information might be needed to have more accurate results regarding functions and condition of soils and soil food webs. Some disturbances, such as coffee infestation by plant parasitic nematodes, can interfere in the calculation of nematode indices, showing confusing results that are difficult to interpret. In addition, contradictory results and lack of sensitivity regarding MI and PPI, obtained in the current study

and in accordance to the literature, raise the need of further studying and validation of the effectiveness of these indices in different agroecosystems.

7. FUTURE RESEARCH RECOMMENDATIONS

Recommendations for further research would be the inclusion of more study sites to have a greater representation of coffee agroecosystems as well as primary Atlantic rainforests. As well it would be recommended to carry out multiple comparison analysis including more variables of the systems and check their influence in soil quality. Furthermore, it would be desirable to have chemical analyses per sampling point to be able to carry out correlation analyses between physicochemical properties and the presence/absence of different nematode taxa, as these soil properties play an essential role in defining the nematode community, and could help to better understand the mechanisms shaping their structure.

In terms of nematode indices, I suggest the validation of the PPI to specific agroecosystems, for example, excluding or adapting the cp values of some nematode families according to the study context. Further research could be done with a focus on how the design of the farming system, besides influencing soil quality, can also help to avoid the presence of some undesirable coffee parasitic nematodes.

As a whole, this study has been an attempt to better understand the influence of different management practices of coffee agroecosystems in soil quality, including static and dynamic soil properties. Adding to the several socioeconomic and environmental benefits that agroforestry systems provide, from this thesis it gets highlighted the potential of coffee agroforestry systems to improve soil quality.

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