Nematode soil community structure and function as a bio-indicator of soil health in Fynbos and deciduous fruit orchards

by
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Thesis presented in fulfilment of the requirements for the degree of Master of Agricultural Sciences in the Faculty of Conservation Ecology and Entomology at Stellenbosch University

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March 2013

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March 2013

Acknowledgements

I wish to express my heartfelt appreciation and gratitude to the following persons and institutions:

My supervisors Dr. A.P. Malan and Mrs. S.G. Storey for their guidance, interest, support and constructive criticism during the course of this study.

N. Ntushelo and M. Booyse, Biometry division Agricultural Research Council Infruitec-Nietvoorbij, Stellenbosch, for assistance with statistical advice and analyses.

Dr. A. Swart, Dr. E. Marais and Dr. E van den Berg from the National Collection of Nematodes, Biosystematics Division, Agricultural Research Council, Plant Protection Research Institute, Pretoria for training in the identification of nematodes.

Mr. B. Gilson, Mr. J. le Roux and the Du Toit group for allowing me to collect samples on their farms.

South African Apple and Pear Producers' Association (SAAPPA) and South African Stone Fruit Producers' Association (SASPA) for funding of the project.

Gerhard Malan for Fynbos identification and sample collection.

S. Niekerk for technical assistance.

My family and friends for unrelenting love, support and motivation throughout.

"Be lions roaring through the forests of knowledge" – Baha'i Scriptures.

Abstract

Soil is a fundamental, non-renewable resource in any ecosystem. To uphold food production for increasing global human populations, it is imperative to develop ways in which to sustain healthy biological productivity and sustainability of agricultural soils. Nematodes are one of the most abundant groups of Metazoa occurring in all soils, and form an integral part of the soil food web at several trophic levels. They respond rapidly to changes within their environments, and can easily be extracted from soil, identified and characterised into functional guilds. Nematodes thus have the potential to impart insight into the condition of the soil food web.

This study aims to establish whether nematodes will be suitable bio-indicators of soil health for the deciduous fruit industry in the Western Cape. Three different objectives have been set to determine the practical use of nematode community structures as a tool for the measurement of soil health. The objectives include describing the nematode community structure, biodiversity and functionality within Fynbos soils; the characterization of organic and conventional orchards; and the differences in nematode soil communities in differently managed soils in an apple orchard. The number of nematodes in each soil sample was quantified and identified to family level. The nematode biodiversity and functionality for each site was determined by evaluating the nematode food webs for trophic group distribution, as enumerated by the Maturity Index (MI), the Enrichment Index (EI), the Structure Index (SI), the Basal Index (BI) and the Channel Index (CI), based on the weighted abundance of coloniser-persister guilds.

The functional guild analysis of Fynbos samples indicated that the enrichment and the structure of any given sample were not bound to a certain area, but it was representative of each of the four quadrats within the faunal analysis. Different geographic areas were found to differ in nematode diversity and functionality, which was mainly associated with dominant plant families and species (such as strong associations between Fynbos families Fabaceae, Solanaceae and Celestraceae with the nematode family Pratylenchidae). The most abundant nematode families present in the Fynbos were Tylenchidae, which are plant-feeding nematodes, and Cephalobidae which are bacterial-feeding nematodes. Despite Tylenchidae and Cephalobidae both having coloniser-persister values (cp-

values) of two, they are split up into different feeding types. Cp-2 nematodes are tolerant to disturbances, and occur in all environments. Only one omnivorous family, the Dorylaimida, was identified in Fynbos samples. The average MI value for Fynbos was found to be very low, with a mean value of 1.26. The value obtained indicated the presence of taxa with tolerance to disturbance, which, in turn, indicated the presence of a disturbed soil, in general. The number of plant-parasitic nematodes within the Fynbos soils was low, which was supported by the low plant-parasitic index (PPI) of 0.85. The diversity, richness and evenness values were low, indicating low nematode diversity, but a distribution of abundances amongst the families. The average Hill's N_0 index value was 8.0, indicating that, in general, eight nematode families would have been present in a Fynbos soil sample.

A study was done to determine the biodiversity and the functionality of the nematodes associated with deciduous fruit orchards that were conventionally, or organically, managed. Herbivores were dominant in all the orchards. The organic apple orchard had the lowest numbers of herbivores and fungivores, with the highest number of carnivores. When comparing organic and conventional apricot orchards, higher numbers of plant-parasitic nematodes were found in the organic orchard. Criconematidae occurred in higher numbers in conventional apricot orchard soil. When comparing organic apricots and apples, higher numbers of Criconematidae occurred within the organic apple soil. Overall, higher levels of plant-parasitic nematodes occurred in the organic apricot orchard. The MI indicated that all orchard soils had values below 1.5, indicating disturbance. Conventionally managed apricot orchard soil had the highest MI value of 1.48. The PPI value was highest in organically managed apricot orchards. All orchard soils were located within Quadrat B of the faunal analysis, indicating enrichment and structure. Regarding the diversity, richness and evenness of the distribution, conventional apricot soil had the highest species richness, while organic apple soil had the most even family distribution. Different management practices did not show marked differences in community composition and structure. The species richness of Fynbos soils was comparable to those of deciduous fruit orchards.

Soil samples from eight different soil surface treatments were collected from an apple orchard in the Grabouw area. Treatments were combined according to the soil surface treatments received (chemical control of cover crops and weeds, mulch and mulch + effective micro-organism spray). Bacterivores were dominant in all soil treatments, with the least number being present in the chemical

control (of cover crops and weeds) treatment. Sites which received chemical control of cover crops and weeds had higher levels of fungivores, compared to the levels at the other sites. High numbers of Rhabditidae occurred within mulch and mulch + effective micro-organism (EM) treatment sites, while high numbers of Aphelenchidae occurred in chemical control sites. The carnivorous family Ironidae only occurred in mulch + EM sites. Strong associations were found between soil surface applications and nematode families present within the soil. Chemical control (of cover crops and weeds) applications had the highest MI value, while values were equal for the other soil applications. The faunal analysis indicated that the mulch and the chemical control fell within Quadrat A, indicating enriched, but unstructured, soil, while the mulch + EM treatment fell within Quadrat B, indicating enrichment and structure, as well as good overall soil conditions. All the systems were dominated by bacterial decomposition pathways. Controversially, sites that received chemical control of the cover crops and weeds had the highest species richness of all three applications, as well as the highest level of diversity, according to the Simpson Index. As only the cover crops and the weeds were chemically controlled, the soil can be regarded as undisturbed, which explains the results obtained in this study. Clear differences in nematode community structure and composition were observed between the different soil applications in the apple orchard.

Uittreksel

Grond is 'n fundamentele, nie-hernubare hulpbron binne enige ekosisteem. Ten einde voedselproduksie vir die toenemende globale menslike bevolking te handhaaf, is dit noodsaaklik om maniere te ontwikkel om gesonde biologiese produktiwiteit en volhoubaarheid van landbougrond in stand te hou. Nematodes is een van die volopste Metazoa in alle gronde en vorm 'n integrale deel van die grond voedsel-web op verskeie trofiese vlakke. Hulle reageer vinnig op veranderinge binne hul omgewings, kan maklik ekstraheer en identifiseer word; en ook maklik in funksionele gildes ingedeel word. Nematodes het ook die potensiaal om insig oor te dra ten opsigte van die toestand van die grond voedsel-web.

Hierdie studie het ten doel om vas te stel of nematodes geskikte bio-indikatore van grond gesondheid kan wees spesifiek vir die sagtevrugte bedryf in die Wes-Kaap. Drie verskillende doelwitte is gestel om die praktiese gebruik van nematode populasie samestelling as 'n instrument vir die meting van grond gesondheid te gebruik. Die doelwitte sluit in die nematode populasie samestelling, biodiversiteit en funksionaliteit binne natuurlike Fynbos; organies verboude versus konvensionele boorde; en die verskil in nematode populasie samestelling tussen verskillend behandelde en bestuurde grondpersele binne ’n appel boord. Die nematode biodiversiteit en funksionaliteit vir elke perseel was bepaal deur die evaluering van die nematode voedselweb vir trofiese groep verspreiding en enumerering deur die Maturity Index (MI) en die Enrichment Index (EI), Strukturele Indeks (SI), Basale Indeks (BI) en Channel Index (CI) wat gebaseer is op die geweegde oorvloed van koloniseerder-persister gildes.

Die funksionele gilde vir Fynbos monsters het aangedui dat die verryking en struktuur van enige gegewe perseel nie gebonde is aan ’n bepaalde gebied nie, aangesien dit verteenwoordig was in elk van die vier kwadrante van die Fauna Analiseerder. Daar is gevind dat verskillende areas verskil in nematode diversiteit en funksionaliteit, wat hoofsaaklik geassosieer was met die dominante plant families en spesies in die omgewing. Die volopste nematode familie wat teenwoordig was in die Fynbos was Tylenchidae, wat plant-voedende nematodes is, en Cephalobidae, wat bakterie-voedend is. Tylenchidae en Cephalobidae het beide ’n cp-waarde van twee, maar is verdeel in verskillende

tipes voedingsgroepe. Die cp-2 nematodes is verdraagsaam vir versteurings en kom in alle omgewings voor. Slegs een omnivoor familie is geïdentifiseer in Fynbos monsters, nl. die Dorylaimidae. Die gemiddelde MI waarde vir Fynbos was laag, met 'n gemiddelde waarde van 1.26. Hierdie waarde is 'n aanduiding van die teenwoordigheid van taxa met verdraagsaamheid tot versteuring, wat op sy beurt 'n versteurde grond in die algemeen aangedui het. Die aantal plant-parasitiese nematodes binne die Fynbos-gronde was laag, wat ondersteun word deur die lae PPI-waarde van 0.85. Die waardes vir die diversiteit, spesie-rykheid en egaligheid was laag, wat dui op 'n lae nematode diversiteit, maar 'n egalige verspreidings onder families. Die Hill's N_0 indeks waarde was gelyk aan 'n gemiddelde van 8.0 en dui daarop dat in die algemeen agt nematode families teenwoordig sal wees in 'n Fynbos grondmonster.

'n Studie is gedoen om die biodiversiteit en funksionaliteit van nematodes wat verband hou met vrugteboorde wat organies en konvensioneel bestuur is te bepaal. Herbivore was dominant in alle boorde. Organiese appels het die laagste aantal herbivore en fungivore gehad en die hoogste aantal karnivore. Wanneer organiese en konvensionele appelkoosboorde met mekaar vergelyk is, was hoër getalle van plant-parasitiese nematodes gevind in die organiese boord. Criconematidae was teen 'n groter aantal in die grond van die konvensionele appelkoosboord gevind. Met die vergelyking van organiese appelkose en appels word, is 'n hoër aantal Criconematidae binne die organiese appelgrond aangetref. In die algemeen was hoër vlakke van plant-parasitiese nematodes gevind in die organiese appelkoosboord. Die MI het getoon dat alle boord waardes laer as 1.5 gehad het, wat daarop dui dat die gronde versteurd is. Die konvensioneel bestuurde appelkoosboord het die hoogste MI waarde gehad met 'n vlak van 1.48. Die waarde vir die PPI was die hoogste in organies bestuurde appelkoosboorde. Alle boord gronde is geleë binne kwadrant B van die Fauna Analiseerder, wat dui op verryking met struktuur. Met betrekking tot die diversiteit, spesie-rykheid en egaligheid van die verspreiding van families, het konvensionele appelkoos grond die hoogste spesierykheid, terwyl die gronde van die organiese appelboord die mees egalige familie verspreiding vertoon het. Verskillende bestuurspraktyke nie toon nie merkbare verskille in die gemeenskap samestelling en struktuur nie. Die spesie-rykheid van Fynbos gronde is vergelykbaar met dié van sagtevrugte-boorde.

Monsters is geneem van agt verskillende grondeoppervlak-behandelings in 'n appelboord in die Grabouw area. Die behandelings is gekombineer volgens die grond toediening wat dit ontvang het (chemiese beheer van dekgewasse en onkruid, deklaag en 'n deklaag + effektiewe mikro-

organismes). Bakterievoedende nematodes was dominant in elke grondoppervlak-behandeling, met die minste teenwoordig in die behandelings wat chemiese beheer van die dekgewasse en onkruid ontvang het. Behandelings wat chemiese beheer van die dekgewasse en onkruid ontvang het, het ook hoër vlakke van fungivore in vergelyking met die ander behandelings. ’n Hoë aantal Rhabditidae het voorgekom in grondmonsters van die deklaag en die deklaag + EM (effektiewe mikro-organismes) behandeling persele, terwyl ’n groot aantal Aphelenchidae voorgekom het in chemiese beheer persele. Die predatoriese familie, Ironidae, het slegs voorgekom in persele wat die deklaag + EM behandeling ontvang het. Sterk assosiasies bestaan tussen grond behandeling en nematode families wat in die grond teenwoordig was. Die chemiese beheer behandeling het die hoogste MI waarde getoon, terwyl waardes vir die ander behandelings laer en gelyk was. Die fauna analise het daarop gedui dat die deklaag en chemiese beheer binne kwadrant A is en dus verryk, maar ook ongestruktureerd is. Die deklaag + EM behandeling het binne kwadrant B geval wat aandui op toestande van verryking en struktuur wat 'n goeie algehele grondtoestand tot gevolg gehad het. Al die stelsels is oorbruggingsweë wat oorheers was deur bakteriële ontbinding. Kontroversieel, het persele wat chemiese beheer van die dekgewasse en onkruid ontvang het, die hoogste spesierykheid van al drie behandelings getoon asook die hoogste vlak van diversiteit volgens die Simpson-indeks. Slegs die dekgewasse en onkruid is chemies behandel, nie die grond nie, en dus kan die grond as onversteurd beskou word en die resultate wat verkry is in die studie verklaar. Duidelike verskille in die nematode gemeenskap struktuur en samestelling is waargeneem tussen die verskillende grond behandelings in appel boorde.

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Introduction

The narrow soil stratum covering the earth's surface is, in one way or another, responsible for the continuing existence of all land-based life forms. Natural processes that uphold the global ecosphere and, essentially, life on earth are under grave threat due to environmental degradation, social volatility, diminishing resources and escalating human populations (Doran & Safley, 1997). Modern agriculture is facing the pressing situation where strategies have to be devised to preserve soil as a non-renewable resource. The utilisation of such non-renewable resources, such as agricultural soil has to be improved, with the processes concerned needing to be in harmony with those biological processes that maintain the existence of life on earth. The stratagems in question have to maintain the long-term sustainability of agriculture by means of eternalising ecological principles (Doran & Safley, 1997).

The concept of soil health, which is becoming more widely used within agricultural circles, can be utilised as a tool to educate producers, especially regarding several of the less apparent possibilities of soil degradation due to inadequate management practices. The education process will in turn, lead to the promotion of more sustainable farming practices. The good health of agricultural soil is the key to the production of healthy food for generations to come.

In order to sustain crop production in the long term, soil must perform certain functions. Such functions include the infiltration and storage of water, the recycling and retention of nutrients, weed and pest suppression, the detoxification of damaging chemicals, the sequestering of carbon, and, lastly, the production of food and fibre (Gugino *et al*., 2009). The disposable income, as well as the sustainable production of a farm, is ultimately at risk if the soil does not function optimally, due to soil restraints existing for a period of time. Improving and maintaining the health of the soil has vast economic benefits for agriculture. Such benefits include improved plant growth, reduced risk of yield

loss, reduced input costs due to the decreased amount of tillage required, and a reduction in input costs, due to a reduction in fertiliser, pesticide and herbicide requirements (Gugino *et al*., 2009).

Healthy soils are an economic and natural asset, as they support sustainable farming practices, as well as the production of various minerals. Healthy soils contribute to environmental health, can aid in enhancing the condition of water resources, sustain soilborne organisms, incorporate and integrate waste products, and store carbon.

What is soil health?

The term 'soil health' has become increasingly popular since the mid 1990s (Nielsen & Winding, 2002), even though the concept itself is not new. The significance of soil health for agricultural affluence was already acknowledged over 2 000 years ago by Roman and Greek philosophers. The necessity for a clear definition for soil health became apparent when it was realised that the terms 'soil health' and 'soil quality' have essentially been regarded as synonyms by many previous researchers.

Soil health is defined as: "the continued capacity of soil to function as a vital living system, within ecosystem and land-use boundaries, to sustain biological productivity, maintain the quality of air and water environments, and promote plant, animal and human health" (Doran *et al*., 1996). Rather than depicting soil as an inanimate mixture of sand, silt and clay, soil health provokes an impression of soil being a dynamic, living organism that functions holistically and that is essentially dependant on ecological characteristics (Van Bruggen & Semenov, 2000). Soil quality relies more on the use of the quantitative characteristics of soil, being the physical, chemical and biological qualities thereof (Doran *et al*., 1996).

According to Gugino *et al*. (2009), authors of the *Cornell Soil Health Assessment Training Manual*, the characteristics of a healthy soil are as follows: "A healthy soil has good soil tilth (which is the overall physical characteristics of the soil when seen in perspective of its suitability for crop production); sufficient depth to enable sufficient root growth; sufficient but not excess supply of nutrients; small populations of plant pathogens and insect pests; good soil drainage; low weed pressure; large population of beneficial organisms; resistance to degradation; resilience to unfavourable conditions; soil [that] is free of chemicals and toxins that may harm the crop."

Within the South African fruit-producing industry, pressure exists for the sustainable production of high-quality fruit to satisfy our local and export markets. Such pressure has prompted great interest in soil health, which is bound also to promote sound production management practices, which, in turn, will serve to enhance soil characteristics. The interest expressed has prompted research in South Africa to be focused on the use of mulches and biological control agents to enhance soil health, within integrated pest management systems.

Overall, however, a major problem is how to measure soil health. The ultimate bio-indicator for soil health should consistently indicate problems that are present within the soil, should work with uniform efficiency in all environments and should be capable of being measured simply (Elliot, 1997). Bio-indicators should preferably not only indicate problems within the environment after the fact, but also be able to predict forthcoming problems, or shortcomings, within the soil environment.

Bio-indicators used to indicate soil health

In some measure, the condition of an ecosystem can be described by soil bio-indicators, which are essentially biological properties or processes within the soil fraction of any given ecosystem. According to Elliot (1997), soil health can never be proved, but only the lack of measurable disease. Time plays an important factor in the establishment of the health of a system. When a system has been functioning normally for an equitable period of time, it can be deduced that the system is healthy, and, alternatively, if a fault has been perceived, it can be deduced that the system is unhealthy.

A whole host of different components within soil affects its health, thus it is improbable that a single measurement can be implemented to measure the soil ecosystem's health. For the reason specified, research, ranging from the impact of heavy metal contamination on microbial biomass and activity (Angle *et al*., 1993; Brendecke *et al*., 1993; Chander & Brookes, 1993; Yeates *et al*., 1994; Speir *et al*., 1995; Valsecchi *et al*., 1995; Frostegård *et al*., 1996) to the responses of soil microbial organisms to pesticides (Harden *et al*., 1993) and industrially contaminated soils (Rowell & Florence, 1993), has been conducted. Key processes and components are mainly used to formulate measures that are symptomatic of disease. Extrapolation of the measures to systems that are known to be dysfunctional can be implemented to determine their effectiveness as measures of disease (Elliot, 1997).

Micro-organisms in the soil are composed of microfauna and are defined as microscopic interstitial animals living in the soil, while microflora can be defined as bacteria and microscopic algae and fungi, especially those living in a particular site or habitat (Oxford Dictionary, Weiner, E.S.C. & Simpson, J.A., 1989). In the current study, mesofauna are defined as nematodes, protozoa, mites and small-sized *Collembola* (Gupta & Yeates, 1997), while macrofauna are defined as animals that are large enough to be seen with the naked eye (Doube & Schmidt, 1997). Potential soil health indicators are discussed below.

Microbial biomass, activity and nutrient cycling

In order for any soil to function normally and healthily, an immense number of micro-organisms, which naturally inhabit and execute an extensive array of essential activities in soil, are needed. Microbes within soil are responsible for the decomposition of organic matter, for the degradation of toxic residues and for the release of nutrients in plant-available forms. Additionally, soil microbes function as adversaries to pathogens, establish symbiotic relations with plant roots, play a role in agglomeration and composition of soil, and encourage solubilisation and deterioration of minerals (Sparling, 1997). The role that microbes perform in soil processes, in addition to maintaining a moderately elevated rate of turnover (0.2-6 years) regarding microbial metabolism, indicate that the microbial fraction could be a conceivable and responsive indicator, as well as an initial predictor of changes within processes concerning soil organic matter (Powlson & Jenkinson, 1981; Powlson *et al*., 1987).

Soil microbial respiration (Anderson, 1982; Insam, 1990) and the microbial biomass (determined by biochemical methods) are the two microbial indices that have been proposed (Jenkinson & Ladd, 1981; Sparling & Ross, 1993; Martens, 1995). Microbial mineralisation of soil organic nitrogen is already deemed as an example of a nutrient alteration procedure that is being applied as an index of soil health (Sparling, 1997). The Cornell Soil Health Assessment Training Manual promotes the implementation of potentially mineralisable nitrogen (µgN/ gdwsoil/week) as a biological indicator of soil health (Gugino *et al*., 2009).

The absolute microbial biomass content of a soil has only provided a significant measurement of soil health in a few cases. Conflicting tendencies in relation to soil fertility and plant production, as well as the natural array in microbial biomass contents within diverse soil types, are known to impede

interpretation. An additional problem is the absence of reference values. Sparling (1997) stated that no clear activation points have been identified either above or below which microbial biomass indices could serve as a reasonable indicator of the relative state of health of a soil. Alternatively, soil microbial biomass can provide a more sensitive measure of change and can demonstrate trends over different periods, although a reference soil of a similar type is required as well as a specific minimum data set for specific end points (Nielsen & Winding, 2002). It has been revealed that the BIOLOG™ assay is more responsive to impacts of sewage sludge amendments to soil and soil management practices than respiration measurements and microbial biomass (Bending *et al*., 2000; Burgess *et al*., 2001; Nielsen & Winding, 2002)

The mineralisation of nitrogen (N) from soil organic matter can supply a valuable incorporation of biological, physical and chemical facets of soil health, since it combines both accumulation of N through previous activities and current N mineralisation activity of soil micro-organisms (Sparling, 1997).

Soil enzyme activities

Soil-inhabiting micro-organisms and fauna are key instruments for countless processes transpiring within the soil. As reported by Dick (1997), soil enzymes are the intermediaries and catalysts of significant functions in the soil. Dick proposed that the evaluation of enzymes in soils can possibly offer an integrative index for the biological status of a soil or for the ability of a soil to perform unobtrusive, enzyme-catalysed processes.

In the past two to three decades, progress has been made in the advancement of techniques aimed at measuring the activity of more than 50 enzymes found in the soil. Various enzymes, which are substrate-specific, can also be selected from assorted functional groupings, allowing for the prospect of determining the potential of a soil to perform an entire range of reactions that might be critical for the functioning of an ecosystem. Enzyme activity could also possibly be implemented to determine whether a degraded or stressed soil is too weakened to perform particular biochemical processes (Dick, 1997).

Dick (1997) also states that soil enzyme activities have not been found to correlate consistently with crop productivity in an agro-ecosystem. In addition, choosing the right enzyme bioassay is also of vital importance. Only when a reference value is available for comparison, has soil

enzyme activities been successfully utilised to distinguish a wide range of soil management practices (Pankhurst *et al*., 1997). Calibration of soil enzyme activities is essential across an extensive range of soil types, ecosystems and soil management practices. The development of relative soil enzyme indices that are easily interpreted and that are similarly independent of soil type and environment is also needed (Pankhurst *et al*., 1997).

Soil microflora

Fungi, algae, actinomycetes and bacteria form part of the assemblage of micro-organisms within a soil environment. These soil microflora potentially possess the capability of being utilised as important indicators of soil health (Van Bruggen & Semenov, 2000). Practically all nitrogen and carbon conversions that take place within the soil environment, as well as the decomposition and modification of organic matter, can be ascribed to micro-organisms (Alexander, 1977; Apsimon *et al*., 1990). By means of the decomposition of carbon compounds by such organisms, energy is supplied to heterotrophic micro-organisms, which, in turn, are responsible for other nutrient transformations.

The transformation of a considerable amount of minerals within soil is dependent on micro-organisms. All the processes performed by the organisms affect the availability of nutrients within the soil environment, which ultimately influences the health and quality of the soil concerned. The microbial fraction plays a crucial role in the functioning of an ecosystem. Consequently, in addition to being a practical tool for determining instability and disruptions in the ecosystem, it can potentially be an available sensitive biological marker (Turco *et al*., 1994).

One of the problems with the use of microflora as an indicator of soil health is the poor cultivability of most organisms concerned (Pankhurst *et al*., 1997). Many such organisms have also not yet been identified (Roper & Ophel-Keller, 1997). Spatial and temporal heterogeneity also makes it practically impossible to use absolute values of microfloral populations or processes as direct bio-indicators of soil health. For the measuring of the impact of chemical pollution on soils, changes in microbial populations and changes within specific functional groups have been utilised. The development of new techniques, such as DNA and GC-FAME techniques, to measure the structural, as well as the functional, diversity of microbial communities will offer innovative, and mostly novel, components for applying micro-organisms as bio-indicators (Pankhurst *et al*., 1997).

Plant root pathogens

Elevated levels of soilborne plant pathogens are considered to be an indication of reduced soil health (Pankhurst, 1994). In all probability, the reason for such a presumption is due to the possibility or threat of disease. A root pathogen is, thus, an indicator of the problem for which a specific pathogen is the cause. According to Hornby and Bateman (1997), the bio-indicator would also, in such instances, be an organism requiring suppression. The presence of a root pathogen does not automatically imply an environmental problem that could cause a decline in the health of the soil concerned, but its presence does indicate the existence of disease in the affected soil.

The occurrence of root pathogens in soil is more of an indication of a problem with certain host plants, rather than being the cause of poor soil health that could negatively influence the majority of organisms present (Hornby & Bateman, 1997). Root pathogens are not regarded as a bio-indicator of soil health (Pankhurst *et al*., 1997).

Soil mesofauna

Nematodes, protozoa, mites and small-sized *Collembola* form part of the group of organisms within the soil mesofauna (Swift *et al*., 1979). Mesofauna form a crucial link connecting the primary decomposers (specifically microflora) and the larger macrofauna in the detritus food-web within the soil. Nutrients that are immobilised by soil microflora are released by the mesofauna, which act as the primary agents in the overall process (Gupta & Yeates, 1997).

As reported by Gupta and Yeates (1997), mesofauna are implicated in an assortment of ecosystems (Stout & Heal, 1967; Yeates, 1981; Freckman & Caswell, 1985; Old, 1986; Gupta & Germida, 1989; Henkinet *et al*., 1990; Gupta & Roper, 1993; Darbyshire, 1994). The nutrient uptake of plants may be directly or indirectly influenced by nematodes, in terms of their effect on plant health. Such effects include plant-parasitic nematodes feeding on the roots of their host plants (Norton, 1978) and symbiotic bacteria (e.g. mycorrhizae and rhizobia) (Westcott & Barker, 1976) serving as a source of food for free-living nematodes (Hussey & Roncadori, 1981), as well as biocontrol bacteria (Bird & Ryder, 1993) and other microflora, such as plant-pathogenic bacteria (Chantanao & Jensen, 1969; Freckman & Caswell, 1985).

Soil mesofauna, especially nematodes and protozoa, are generally very adaptable within their environment to the health and condition of the soil that they inhabit (Gupta & Yeates, 1997). Such organisms conform to the general characteristics required of a bio-indicator, as indicated by Elliot (1997). Nematodes and protozoa, which are omnipresent, abundant and diverse within soil ecosystems, have various measurable qualities and are closely involved in the regulation of decomposition, as well as in plant nutrient cycling, which makes them prospective bio-indicators (Gupta & Yeates, 1997). Because their enumeration in soils is significantly influenced by spatial and temporal variability, serious consideration must be given during sampling to ensure that the soil samples are representative of the entire system.

One of the most useful attributes of the above-mentioned organisms is trophic diversity; as such diversity is directly influenced by the food sources that are available in the soil (Gupta & Yeates, 1997; Pankhurst *et al*., 1997).

Soil arthropod community structures

Collembola, mites, Oribatida, Isopoda and Diplopoda are examples of soil arthropods. These micro-arthropods have complex associations with their niches within the soil, thus their community structures could be exploited as a bio-indicator of soil health. The above-mentioned soil arthropods are very inactive organisms, and consequently tend to reveal the condition of a native habitat better than do other insects with a high dispersal rate (Van Straalen, 1997).

The soil ecological research group at the Staatliches Museum für Naturkunde, Karlsruhe conducted a study in which the soil fauna from 11 different forest sites were examined in order to establish a system of soil quality, based on biological criteria (Ruf, 1998). As part of the study, the incidence of predatory mesostigmatid mites was studied to test whether a maturity index for such mites (Mesostigmata: Gamasina) could be used as an indicator of the environmental impact of pollution on soils within a forest. Predatory soil mites appear to be a good indicator only when their life history traits are taken into consideration.

Collembola and mite species distribution have been effectively used as an indicator of soil health in a number of studies. Their use has been especially accurate in studies related to soil pH (Van Straalen & Verhoef, 1997), soil carbon/nitrogen (C/N) ratio and heavy-metal contamination of

soils (Bengtsson & Tranvik, 1989). Single species analysis is a less useful soil health indicator than is community composition analysis (Dick, 1997; Gupta & Yeates, 1997; Pankhurst *et al*., 1997).

Collembola or springtails are some of the most plentiful arthropods within temperate cultivable farmland. Framptom (1999) investigated the effects of the insecticides pirimicarb, chlorpyrifos and cypermethrin on *Collembola* in winter wheat. *Collembola* were found to be susceptible to organophosphate insecticides, but not to cypermethrin, which limits their use as indicators of pesticide usage. An additional undesired characteristic of *Collembola* is their spatial variability (Framptom, 1999).

Abundance and activity of soil macrofauna

The soil macrofauna fragment is defined as organisms that are larger than 2 mm. They are visible to the naked eye and are comprised of ants, amphipods, termites, centipedes, isopods, millipedes, earthworms, enchytraeid worms, slugs, snails and adult, plus larval, stages of root-feeding insects (Doube & Schmidt, 1997). They redistribute organic residues and, in doing so, increase the extent of microbial activity. This, in turn, enhances nutrient availability throughout the whole root zone and the decomposition of organic matter, which results in improved soil structure (Linden *et al*., 1994). Of the macrofauna, earthworms were identified as being best suited as a potential bio-indicator.

Earthworms influence an infinite assortment of chemical, physical and biological soil factors in the soil environment (Doube & Schmidt, 1997). Through such factors, they increase plant growth in tropical (Tian *et al*., 1993; Kang *et al*., 1994) and temperate (Lee, 1985; Hendrix, 1995; Edwards & Bohlen, 1996) environments. Earthworms are, however, not ubiquitous and do not always respond consistently to treatments (Doube & Schmidt, 1997; Neher, 2001). Their abundance also varies greatly with climate and soil type. A number of studies have indicated that earthworms offer only limited potential to act as a bio-indicator of soil health (Samoiloff, 1987; Freckman, 1988; Doran *et al*., 1996), although they have greater potential for use as bio-accumulators of environmental contaminants (Pankhurst *et al*., 1997).

Nematode communities as bio-indicators of soil health

Within the boundaries of using soil mesofauna as bio-indicators, three different groups of mesofauna, namely *Collembola* (Framptom, 1999), mites (Ruf, 1998) and nematodes (Neher, 2001),

have been measured for their potential use as biological indicators of soil health. Nematodes (both plant-parasitic and free-living) have been most frequently assessed for their use as indicators and are amongst the simplest metazoa (Bongers & Ferris, 1999). The organisms concerned have been determined as the most practical group for community indicator analysis, since information regarding their feeding behaviour and taxonomy is easily obtained.

Nematodes are advantageous as ecological and biological indicators, because they possess numerous useful characteristics. Soil nematodes inhabit a fundamental position in the detritus food web, and can be placed in at least five trophic or functional groups (Neher, 2001). They also feed on most soil organisms and are also a food source for many other organisms. Nematodes are omnipresent in all soil environments that provide a supply of organic carbon. Some species are able to outlive all other animal species in disturbed or polluted environments (Bongers, 1990; Yeates *et al*., 1993; Ferris & Bongers, 2001; Yeates, 2003). They occur in habitats that vary from highly polluted to unspoiled, in all types of soil and under any climatic condition.

Nematodes have diverse feeding behaviours and life strategies ranging from colonisers to persisters (Bongers & Ferris, 1999; Ritz & Trudgill, 1999). Predominantly, morphological structures correlated with diverse feeding behaviours are used to separate and classify nematodes. Nematodes can be identified without implementing biochemical procedures. Since they are transparent, it is possible to distinguish their internal morphological characteristics.

Sampling and extraction of nematodes from soil samples is relatively simple and is more cost effective than are the techniques that are employed to extract other soil organisms, due to the size and relative profusion of the former organisms (Neher, 2001). The structure and function of the nematode mouth cavity and oesophagus makes it possible to deduce their feeding behaviour easily (Yeates & Coleman, 1982; Freckman, 1988; Bongers & Ferris, 1999). Nematodes control nutrient mineralisation and the degree of decomposition by influencing the growth and metabolic activities of microbes and by regulating the behaviour of the microbial community (Neher, 2001). Nematodes have been established as performing a direct role in the distribution of biomass within plants and in nitrogen mineralisation.

The utilisation of nematodes as bio-indicators is reinforced by many of their biological characteristics. Their permeable cuticle allows nematodes to react with a wide range of responses to

changes in the environment (e.g. pollutants, etc.), since they are in immediate contact with their micro-environment. Furthermore, nematodes react speedily to any enrichment and disturbance, leading to an increase in microbial activity, which will sequentially cause changes in the proportion of bacterial feeders in a community (Bongers & Ferris, 1999).

During times of environmental conditions that are unfavourable for development and growth, certain species of nematodes experience resistant stages, such as cyst formation, cryptobiosis and anhydrobiosis, which allows them to survive inactively (Neher, 2001). Many species can also survive oxygen stress, freezing and dehydration (Bongers & Ferris, 1999). Within the soil horizon that is inhabited by nematodes, the condition of the soil is indicated by the nematode community structure.

Nematodes also possess highly conserved heat shock proteins (Hashmi *et al*., 1997). When nematodes are exposed to stresses (such as organic toxins, heat or metal ions) the expression of such proteins is enhanced (Kammenga *et al*., 1998; Neher, 2001). The proteins concerned also have the potential to function as biomarkers during eco-toxicological evaluations of soils (Guven *et al*., 1994; Guven *et al*., 1999; Kammenga *et al*., 2000).

Indices used for the analysis of nematode assemblages

Diversity indices

In order to assess the biodiversity of ecosystems, numerous indices have been developed and utilised. The indices that are used for soil nematodes are not applied to the ecosystem in absolute terms, but are applied to numbers of taxa. Community diversity can be computed at three levels of certitude: 1) diversity founded on the profusion of individuals within each genus/group; 2) trophic diversity, founded on the profusion of individuals within each trophic group or family; and 3) the diversity of genera within each family, which is a form of trophic richness. The relative abundance and evenness of the occurrence of nematode trophic groups is described by means of trophic diversity indices (Ferris & Bongers, 2009).

Diversity indices that are useful for nematodes as bio-indicators of soil health (Ferris & Bongers, 2009) include: Shannon's diversity index (H') (Shannon & Weaver, 1949); species richness (S), which is also referred to as Hill's N_0 index (Hill, 1973); Simpson's diversity index (D) (Simpson,

1951); Hill's N_1 index (Hill, 1973); Hill's N_2 index (Hill, 1973); and Pielou's J' evenness index (Pielou, 1966).

Indices of ecosystem function

Cp values

The coloniser-persister (cp) scale or series is the assignment of taxa of soil and freshwater nematodes to a 1-5 linear scale according to their *r* (opportunists) and *K* (competitor) characteristics (Ferris & Bongers, 2001). The series can vary from extreme *r*- to extreme *K*-strategists (Ferris & Bongers, 2009). Resource availability is denoted by coloniser nematodes, which are regarded as enrichment opportunists, at the lower end of the cp scale. Food web complexity and connectance, as well as system stability is revealed by persister nematodes at the high end of the cp scale.

Bongers (1990) introduced cp scaling, which presents the life-history ordinate for the functional guild matrix of nematodes, with the abscissa being provided by known or inferred feeding habits (Ferris & Bongers, 2006). An individual nematode taxon, especially at family level, is grouped into one of the five cp classes (Ferris & Bongers, 2009). The same cp value is assigned to genera and species within a taxon, as is assigned to the family. For this reason, identifying nematodes to genera and species level for the purpose of the current study is superfluous. As reported by Ferris and Bongers (2009) the relevance of family-level assignments has been justified, on the basis that nematodes with similar life history traits have a high probability of having similar sensitivity and responsiveness to environmental change. Bongers and Bongers (1998) provide the most recent descriptions of the cp assignments for terrestrial nematode families.

Maturity index (MI)

The coloniser-persister scale described in the previous section forms the basis for the MI. Quintessentially, the MI is an ecological indicator of the condition of progression of a system by means of which disturbance, and its resulting enrichment responses, result in an impediment of succession to an earlier state (Odum, 1985). The MI is defined as the weighed mean of the individual cp values for the nematodes in a sample, and, in practice, varies from 1, under conditions of extreme enrichment (such as in cow pats or following heavy maturing), to a value of between 3 or 4 under undisturbed conditions (Bongers & Bongers, 1998; Bongers & Ferris, 1999). The nematode MI, which

is one of the key indices of soil health, is regarded as a gauge for environmental disturbance and is based on non-plant feeding taxa (Bongers & Ferris, 1999), meaning that plant-parasitic nematodes, as listed by Bongers (1990), are excluded. Entomopathogenic nematodes, animal parasites and dauer larvae of enrichment opportunists are also not included in the calculation of the MI. Their exclusion from the calculation is due to the fact that the incidence of the previously mentioned nematodes within a soil community imparts no additional enlightenment regarding the current performance of the soil food web. The presence of a profusion of dauer larvae within a soil is an indication that a system has declined to a less enriched state after a period of enrichment (Ferris & Bongers, 2009).

Enriched and disturbed environments are indicated by low MI values, whereas environments that are stable are indicated by high MI values. Compared to colonisers, persisters demonstrate a greater sensitivity to pollutants and other disturbances. Consequently, the MI additionally functions to evaluate the influence of various assortments of contaminants, identified and unidentified, encompassing their multifaceted interactions with the abiotic and biotic environment.

Plant-parasitic index (PPI)

The plant-parasitic index (PPI), which is analogous to the MI, is calculated only for plant-feeding nematodes, with the *raison d'être* for the profusion of these nematodes being contingent on the dynamism of their host plants, which is influenced sequentially by system enrichment (Bongers, 1990; Bongers *et al*., 1997; Ferris & Bongers, 2009). The PPI increases with the augmentation of soil fertility, while the MI diminishes (Bongers & Ferris, 1999). Under particular circumstances, the PPI is inversely related to the MI.

Other indices that form part of the MI family are: the PPI/MI (Bongers & Korthals, 1995; Bongers *et al*., 1997); the MI2-5 (Bongers & Korthals, 1993); the ∑MI (Yeates, 1994); and the ∑MI2-5 (Neher & Campbell, 1996).

CP triangles

Cp triangles are equilateral triangles that are graphical descriptions of faunal composition. Such triangles are accommodated by two enrichment axes (percentage cp 1 and cp 2) and one axis that indicates ecosystem complexity (percentage cp 3-5), which is founded on the un-weighted

proportions of the nematode fauna within each assemblage (De Goede *et al*., 1993; Ettema & Bongers, 1993). The graphical depictions advance the connection that cp classes are indicators of ecosystem function and structure, which are not necessarily associated on a general trajectory (Ferris & Bongers, 2009). An increase along one of the axes is complemented by a decrease along one of the other axes, owing to each axis of the triangles signifying a proportion of the whole nematode fauna.

The problem with the cp triangles is that the un-weighted data do not provide adequate resolution to changes in the fauna, in addition to the points along the enrichment and structure axes lacking independence (Ferris & Bongers, 2001; Ferris *et al*., 2004).

Indicators of ecosystem function

The advancement in various perceptions, as well as the justification of models and research associated with the improvement of the MI and associated indices, led to the development of a functional guild classification of nematodes, which forms a foundation for the examination and evaluation of ecosystem processes (Bongers & Bongers, 1998; Bongers & Ferris, 1999). The existence of such a guild is a dependable indicator of lack of disturbance, or of recovery from disturbance within a system.

Nematodes that are classified as enrichment opportunists indicate the flow of resources into the soil food web system; furthermore, the occurrence and profusion of organisms higher up within the trophic level indicates the trophic connectance of a system. The two factors mentioned are very important attributes that are provided by the nematode fauna within the soil, with regards to the soil environment that is present along with resident communities (Ferris & Bongers, 2009).

Both the enrichment trajectory and the structure trajectory are based on the indicator consequence of functional guilds of nematodes and are descriptors of the condition of the food web (Ferris & Bongers, 2009). The trajectories allow for the quantification of the state of the soil food web by means of various indices. These indices are the enrichment index (EI), the structure index (SI) and the channel index (CI). The EI is a measure of the presence of opportunistic bacterivore and fungivore nematodes, whereas the SI is an indicator of the food web state being affected by stress or disturbance, and the CI is an indicator of the predominant decomposition pathways. According to Bongers and Ferris (1999) and Ferris *et al*. (2009) functional guilds are defined as a matrix of

nematode feeding habits, with the biological, ecological and life history characteristics being embodied in the cp classification. Consequently, cp-3 bacterivores (for instance Teratocephalidae or Prismatoloaimidae) comprise the Ba_3 functional guild. All nematodes classified as having cp-2 feeding habits are regarded as basal to both the structure and the enrichment trajectories (Ferris & Bongers, 2009). Enrichment indicators are bacterial feeding cp-1 and fungal feeding cp-2 nematodes, while all nematodes with feeding habits in cp 3-5 are considered to be indicators of structure. On the enrichment axis, functional guild indicators are weighted according to growth and metabolic rates or resource consumption, while the structure trajectory is weighted according to sensitivity to disturbance (Ferris & Bongers, 2001).

Commercial use of nematodes as bio-indicators

Nematodes have been utilised as environmental bio-monitors for aquatic systems since the 1970s. The nematode *Panagrellus redivivus* (Linnaeus, 1767) Goodey, 1945 provides a rapid bioassay at 10% of the costs of a *Salmonella* bioassay. The nematode detects toxin concentrations that affect moulting and nematode size through stimulation, inhibition or lethality, enabling it to be used as a bio-monitor (Neher, 2001). It has also been used to ascertain toxic effects of approximately 400 single chemicals (Neher, 2001).

Another commercial use for nematodes in the 1970s was in the nematode: copepod ratio (Amjad & Gray, 1983). Copepods are small, shrimplike crustaceans occurring in seas, lakes and ponds, which are very important in the food web, since many animals feed on them. The ratio concerned was generally used to monitor the condition of aquatic ecosystems. Nematodes are less sensitive to environmental stress factors and pollution, and, consequently, a high nematode: copepod ratio is indicative of pollution and increasing levels of enrichment. However, the ratio is burdened with problems, one of which is that pollution has already transpired by the time that a shift in the ratio becomes obvious. Another problem is that copepods consume nematodes, which leads to an unreliable ratio. Copepod populations are also positively correlated with sand grain size, making it almost impossible to determine whether population changes are due to pollution or are due to the particle size of sediment (Neher, 2001).

The nematode *Aphelenchus avenae* Bastian, 1865, which is commercially used to test for nematicidal activity within soil, feeds on a wide variety of fungi. In agricultural soils, a micro-organism

population can evolve to utilise the active ingredients within organophosphates and carbamates as a source of carbon for energy, due to the frequent use of such nematicides. High populations of said microbial complexes in the soil can shorten the residual activity of nematicides from weeks to a few hours, in a phenomenon that is known as accelerated microbial degradation (AMD). When the number of *A. avenae* per sample tested increases dramatically in comparison to that in a control (i.e. in untreated soil), the assumption is that microbial populations that are responsible for AMD are present in the soil (Stirling *et al*., 1992).

Pattison *et al*. (2008) developed a minimum set of key soil health indicators for the Australian banana industry aimed at integrating the properties (physical, biological and chemical) of soil. The indicators were also developed to allow banana growers, extension workers and researchers to improve soil health management practices. Biological properties of soil were determined by using the soil nematode community as a bio-indicator of soil health. Various nematode community composition indices were calculated from the nematode populations extracted from the soil. The Shannon-Weiner index was used to determine the diversity of the nematode community present, and dominance was calculated by means of the Simpson's index of dominance.

The health of our soils is imperative for the sustainable production of food and fibre. Practices that improve soil health will ultimately lead to improved soil characteristics. Healthy soils created by optimised farm management practices will lead to greater cost-effectiveness on farms, which is critical for the financial crisis in which the world currently finds itself. Healthy soils will also add value to properties and benefit the countries' food exports. Healthier soil will mean a healthier future for generations to come. This study will provide a building block by which South African producers can potentially measure and improve the health of their soil. It can be regarded as the first step in the establishment of key soil health indicators for the deciduous fruit industry within the Western Cape province of South Africa.

The use of nematodes as bio-indicators of soil health cannot be a stand-alone measure for the determination of soil health in deciduous fruit orchards, but may form an important part of an integrated system of measurements.

Aims and objectives of the study

The overall aim of the study was to establish if the nematode community structure and function can be used as a bio-indicator of soil health within deciduous fruit orchards in the Western Cape of South Africa.

The objectives of this study are:

- to determine the biodiversity and functionality of soil nematode communities in the Fynbos ecosystem;
- to determine the structure and function of nematode soil communities in organic and conventional apple and apricot orchards; and
- to determine the difference in the structure and function of nematode soil communities after different treatments were applied to the soil surface in an apple orchard.

The chapters of this study have been written as separate publishable papers, and, for this reason, some repetition in the different chapters has been unavoidable.

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Chapter 2

Soil nematode community structure

Materials and Methods

Introduction

In order to determine the community structure of nematodes in the soil, such as soils from natural Fynbos or organic and conventional orchards, techniques used for sampling, extraction, quantification, identification and fixation of nematodes were crucial to ultimately obtain comparable results. The techniques used in the current study were based on those with a solid foundation that have been effective for decades, mainly for plant-parasitic nematodes.

In the analysis of the results, different maturity and diversity indices were used. Each index formulation was discussed as to its applicability to analyse information concerning the composition and structure of nematode communities into a particular measurement. Indices used in the study were also utilised for numerous other studies (Neher, 1999; Yeates & Bongers, 1999; Ferris *et al*., 2001; Neher *et al*., 2005; Baniyamuddin *et al*., 2007; Pattison *et al*., 2008; Čermák *et al*., 2011) and organisms (eg. mites) in order to determine diversity and ecosystem function. The allocation of nematodes to feeding groups, according to Yeates *et al*. (1993), and the assignment of coloniser-persister values (cp-values) to various nematode families, according to Bongers and Ferris (1999), were extensively used and described in detail.

The taxonomic classification of nematodes within samples was to family level. Identification at family level can lead to a better understanding of the functioning of soil, due to the considerable amount of information that can be gained from it. Identification of nematodes at species level will unquestionably divulge more information concerning redundancy and biodiversity, as well as other ecological perceptions, but is hampered by a current lack of identification keys (Bongers & Bongers, 1998). Given that the analyses were of community structures, the nematodes concerned had only to be identified to family level. Identification to such level was the more feasible, plausible and practical

approach, given the prevailing conditions. Since the identification comprises the most important part of the current study, the morphological characteristics of each family are given in detail. Light microscopic photographs and line drawings have also been included for clarification.

Sampling equipment

A range of tools was implemented when collecting soil samples for nematode extraction. Due to the hard, rocky soils of the Western Cape, it was not possible to use some of the more commonly utilised soil sampling tools. Tools used for the collection of samples were spades, garden trowels and a brick hammer (800g Lasher Brick Hammer with a wooden handle). The brick hammer was used to break through the hard crust on the surface and to break up any large clods. Other sampling equipment included plastic bags, twine ropes, flags made from wooden rods, cooler boxes, elastic bands, a Garmin handheld global positioning system (GPS) device and permanent markers. In the case of farms, producers were also given a questionnaire to provide relevant information concerning the orchard blocks that were sampled.

Fig. 2.1. Sampling tools, on the left the brick hammer, and on the right a small garden trowel.

Sampling pattern

Fynbos

Fynbos is the natural vegetation of the Western Cape, the most southern province within the Republic of South Africa. Fynbos areas are exceptionally hard to sample, since the areas concerned form part of an extremely mountainous terrain with rocky soils. Owing to the above-mentioned factors, it was decided that, within any given locality suitable for sampling, 3-5 of the most dominant, well-

established perennial plants would be sampled. Doing so led to a completely random sampling pattern and also ensured that the Fynbos species sampled would still be traceable should further sampling be required. The sampling pattern remained consistent throughout the entire survey.

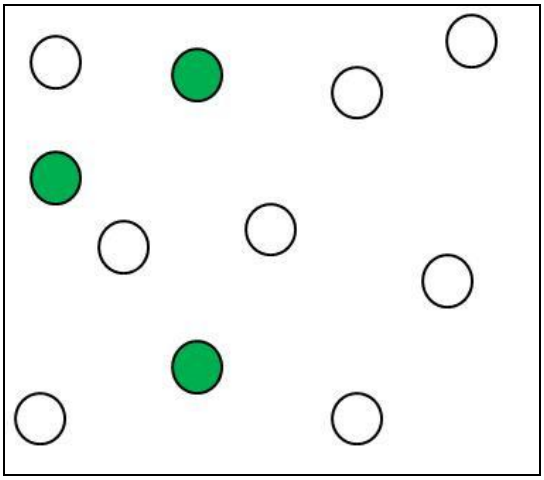

Fig. 2.2. Diagram illustrating the random sampling pattern implemented within the Fynbos sites. The green dots indicate the most dominant, well-established perennial plants within a given area (Coyne *et al*., 2007).

Organic and commercial orchards

The sampling technique for organic and conventionally produced orchards was the same. A systematic sampling pattern was used for orchard sampling. Within any given orchard, a 1-hectare area was measured using 100-m lengths of twine attached to flags. The flags made the sampling area visible, and also served as the borders of the sampling area. The first two outside rows of an orchard, as well as the first four trees within a row, were not sampled. The one-hectare areas were then divided up into quadrants. Subsequently, five subsamples were taken from each quadrant from five different trees to make one sample. In total, 25 trees per quadrant were sampled, which comprised the 5 samples. Trees that were sampled were located diagonally across from each other. Samples were collected on the ridge, on alternate sides, 30 cm from the tree trunks. Sampling holes were dug to a depth of 20 cm to include feeder roots. Collection of samples took place mid-season and after harvest. The accumulated samples were contained within marked plastic bags, labelled with the name of the farm, the GPS location and the block or quadrant number. Samples were transported back to the laboratory in insulated containers and subsamples were mixed together to culminate in four samples per orchard. The subsamples were gently mixed by means of a Sputnik hand-driven

washing-machine. The circular motion of the spinning drum, which was rotated in a clockwise and an anti-clockwise direction, ensured adequate mixing of the samples. The samples were stored at 14^{0}C until processing.

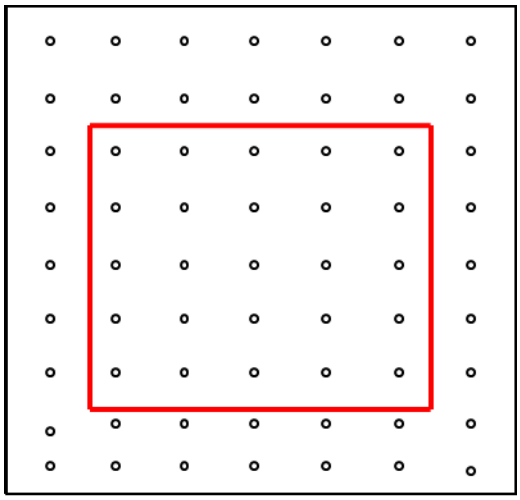

Fig. 2.3. Diagram to illustrate the one-hectare area sampled within an orchard.

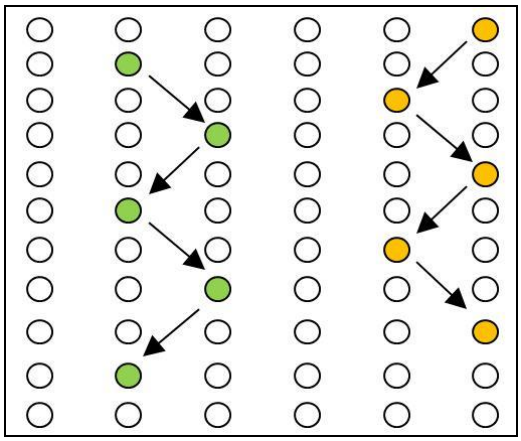

Fig. 2.4. Systematic soil sampling within orchard rows. The green and orange dots are an indication of two different subsamples, as well as of the trees that were sampled.

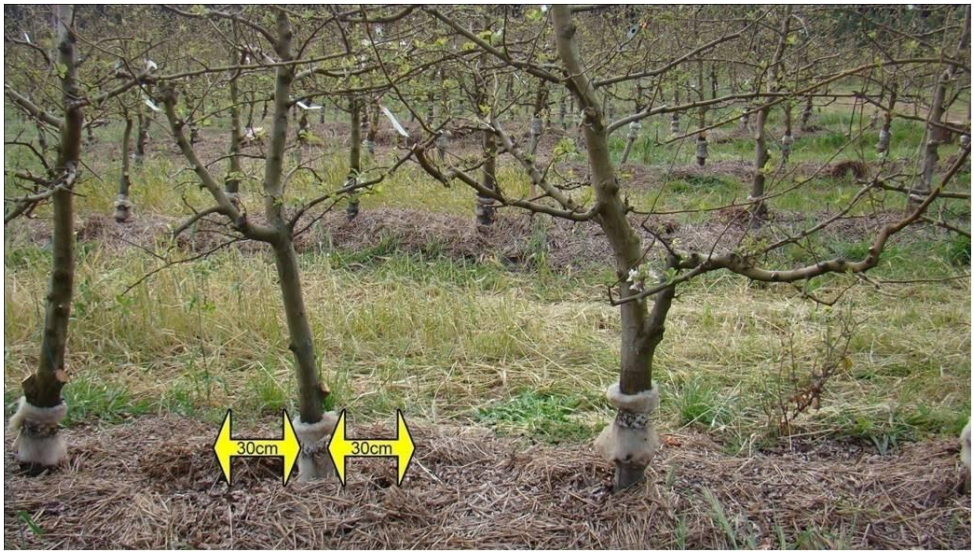

Fig. 2.5. Sampling holes were dug on opposite sides, at 30 cm distance from the trunk.

Nematode extraction

Extraction of nematodes from soil was accomplished by means of Cobb's decanting and sieving method (Cobb, 1918). A 250-mℓ volume of soil was washed through a coarse mesh sieve with an aperture of 2 mm into a 5-ℓ bucket. The sieve served to remove stones and plant material from the samples and was also used to break up clods. Water was added to the bucket to increase the volume to 5 ℓ, and the soil was brought into suspension by stirring and allowed to settle for 60 seconds. Thereafter, the suspension was poured through a bank of sieves consisting of the following apertures from top to bottom: 90 µm, 2 x 53 µm, and 45 µm. The residue collected on each sieve was transferred to a 250-mℓ beaker. The 5-ℓ bucket was filled for a second time and the process repeated, but with a settling time of 30 seconds. Subsequently, the suspension was poured through the sieves and the residue transferred to the same 250-ml beaker previously mentioned. Samples were cleared by means of a modified Baermann funnel (Cobb, 1918). The technique used required samples to be poured onto a watch glass through a two-ply paper towel supported on a coarse-meshed plastic screen. The plastic screen was contained within a metal dish. Water was added to the container until the residue on the paper towel was thoroughly wet, but not immersed. The modified funnel was left undisturbed for 48 hours, after which the filter was removed and discarded, with the suspension being poured into a 250-mℓ beaker for examination.

Nematode suspensions extracted from soil were allowed to settle for one hour in the 250-mℓ beaker, after which the volume was adjusted to 50 mℓ, and then transferred to a 100-mℓ beaker. The nematodes were once more allowed to settle for 60 min, after which the excess water was siphoned off to 20 mℓ, by using a thin plastic tube. A small fish pump was used to blow air through the nematode suspension to agitate the sample. A pipette was used to add a 1mℓ suspension to a 1-mℓ graduated slide for counting. Two slides were counted and the mean number of nematodes for each sample determined. Nematodes were counted using a compound microscope.

Identification of nematodes

Preparation for identification

Temporary slides were made for identification of the nematodes. A 1-mℓ nematode suspension was placed on a microscope slide with a grid pattern (0.5 × 0.5 mm) (Fig. 5) drawn on the back of the slide, using a permanent marker. Use of such a grid pattern facilitated the identification process, by preventing confusion as to which nematodes on the slide had been identified. The suspension was then covered by a 52 × 22 mm rectangular cover slide, which was held over a gentle flame for several seconds to heat kill the nematodes. Clear nail-polish was used to seal the cover slide. The first 100 nematodes were identified to family level. The process was repeated for each sample.

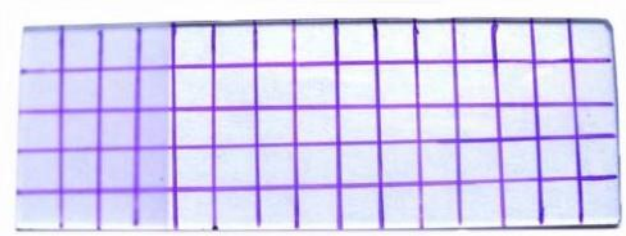

Fig. 2.6. Microscope slide, onto which the 0.5 × 0.5 mm grid was drawn.

Identification

Identification to family level was done using the following books: *A Guide to Plant and Soil Nematodes of South Africa* (Heyns, 1971); *Soil and Freshwater Nematodes* (Goodey, 1963); and *The Nematodes of the Netherlands* (Bongers, 1994). The 'Interactive Diagnostic Key to Plant-parasitic, Free-living and Predaceous Nematodes' from the Nematology laboratory of the University of

Nebraska Lincoln, which is available from their identification website (http://nematode.unl.edu/konzlistbutt.htm), was also used as an aid in the identification.

Conventionally, identification of nematodes is accomplished mainly by the morphology of the oesophagus, in combination with other characteristics. Nematodes were identified with the aid of a compound microscope.

Characteristics of nematode families

Rhabditidae Oerley, 1980 (Order: Rhabditida)

The family is characterised by three or six lips, or by paired oral hooks and an absence of a stylet or spear. The cuticle can be smooth or have cross-banded or blocked patterns. The stoma is a fairly long barrel-shaped conduit. The formation of the stoma occurs when the pro- and mesorhabdions fuse. The anterior part of the oesophagus is relatively cylindroid and, in rare cases, the corpus is dilated to form a median bulb with a valvated bulb. The valves of the ubiquitous basal bulb are plate-like. Female nematodes within the family can have paired ovaries, or ovaries that are prodelphic, opposed or reflexed (Heyns, 1971). Ovaries are seldom single (Goodey, 1963). Within South African genera, all males possess a notable bursa. The bursa is supported by nine to ten pairs of ribs (Bongers, 1994), and a gubernaculum is present (Heyns, 1971). The nematodes concerned can be free-living or parasites of molluscs, vertebrates, earthworms and arthropods (monogenetic) (Goodey, 1963).

Diplogasteridae Micoletzky, 1922 (Order: Rhabditida)

The family has longitudinal striations bearing transverse striae often visible on the cuticle of nematodes. The head consists of six closed, rounded lips, with an apical papilla apiece. Males have four supplementary lateral head papillae (Goodey, 1963). The shape of the stoma varies greatly from long and narrow to broad and short. No stylet or spear is present. Within the oesophagus, the median bulb usually possesses crescentic valve plates, which are also referred to as tri-radiate suction valves, a narrow isthmus and a basal bulb in which valves are absent (Goodey, 1963; Heyns, 1971). Female ovaries are rarely single, typically being in pairs. Males rarely possess a bursa. A pair of spicules is present, in addition to a gubernaculum and seven to twelve pairs of striking caudal papillae arranged in a characteristic pattern. The last group of caudal papillae are dorso-lateral, while the trio

group of the last four pairs are latero-ventral, being frequently situated in the origin of the bursa (Goodey, 1963).

Bunonematidae Micoletzky, 1922 (Order: Rhabditida)

Bunonematidae fall in the order Rhabditida. The cuticle of the nematodes is highly adorned and the body is asymmetrical. The right part of the body exhibits a fine network of structures and/or a series of protruding bristles and lamellae, while the left half is characterised by five longitudinal ribs (Bongers, 1994). The lip region is also asymmetrical, with lamellae and hair-like appendages. As with the Rhabditidae, the oral cavity is tubular and no stylet or spear is present. The anterior part of the oesophagus is swollen, with a narrower isthmus finally terminating in a valvated basal bulb. Nematodes within the family have a long rectum. Gonads of the female nematodes are paired. The male bursa is reduced and asymmetrical, not enveloping the tail. Spicules are long and thin (Bongers, 1994).

Panagrolaimidae Thorne, 1937 (Order: Rhabditida)

Panagrolaimidae possess simple lips that are devoid of appendages, but with small papillae. A buccal cavity, in addition to a cheilostom, prostome and, occasionally, a mesostome that moulds into a single, capacious chamber (lacking stylet or spear), occasionally with an inclination to narrow in the direction of the meta- and telostome is present. The telostome is small and narrow, while the anisoglottid and anisomorphic metastome is somewhat more tapered than the anterior part. The pro-, meso- and telorhabdions are usually thickened, which makes them very visible. Mesorhabdions are not thickened, but the metastome segments often bear small teeth and the cheilostom is occasionally thickened. The frontal part of the oesophagus is tubular, or consists of an enlarged but valveless median bulb. Said part is followed by a narrow isthmus and a basal bulb exhibiting plate-like valves. Females possess a prodelphic gonad that extends forward, and which is then reflexed straight back past the vulva, devoid of any supplementary flexures, typically swollen and reaching adjacent to the anus. A short post-vulval sac is present at times. The tip of the male gonad is habitually reflexed. Male tails lack a bursa, but do have five to seven pairs of caudal papillae. A gubernaculum is present. Panagrolaimidae have fine annules that are less than 2 µm wide (Goodey, 1963; Heyns, 1971).

Cephalobidae Filipjev, 1934 (Order: Rhabditida)

In the Cephalobidae the stoma has separate, yet distinct, rhabdions, with a wide cheilostom. The rest of the oesophagus is narrower. The oesophagus is cylindrical in front, with a valvated terminal bulb. Pro- and mesorhabdions are fused. The metastome is divided into two parts; the upper part is occasionally not sclerotised, while the bottom part has a dorsal tooth. Rhabdions may or may not be present in the telostome. A stylet is not present. Females have a single ovary that is directed anteriorly, but which is reflexed lower down the body past the vulva, with a supplementary double flexure towards the end of the ovary. At the anterior flexure, a spermatheca is present. A relatively developed post-vulval sac is typically present. The tips of the male testes are reflexed, spicules are paired and a gubernaculum is present. Male tails lack a bursa, but do have approximately eight pairs of caudal papillae arranged in a characteristic pattern (Goodey, 1963; Heyns, 1971).

Tylenchidae Filipjev, 1934 (Order: Tylenchida)

Males and females are actively vermiform. The cephalic framework is delicate to reasonably well-developed. The stoma possesses a stomatostylet, typically with basal knobs. The oesophagus has a median bulb that is well-developed, typically ovate with internal, sclerotised, cresentric valve plates (Goodey, 1963). Oesophageal musculature is not strongly developed. The posterior end of the oesophagus ends in a pyriform basal bulb and, in a few cases, lobes overlap the intestine. The nematodes have a thin cuticle with particularly insipid striations. Incisures are regularly visible on the lateral fields. Males have a bursa that is adanal or subterminal. At times, an enveloping bursa can occur. Female nematodes can exhibit one or two ovaries (Goodey, 1963; Heyns, 1971).

Hoplolaimidae Filipjev, 1934 (Order: Tylenchida)

The spear is very robust (longer than 20µm) with large basal knobs. The sclerotised framework of the head is very strong. The shape of the lip region can vary from convex to conical. The median oesophageal bulb is ovate to spheroid, with crescentic valve plates, enclosed by very powerful musculature. The intestine is overlapped by the oesophageal glands. The cuticle 3is visibly striated, typically with incisures and a lateral field. Females typically have two ovaries, but may also have only one. The male bursa can be adanal, completely surrounding the tail, or absent. Scutella (enlarged shield-like phasmids) are present on the tail. Small phasmids may also occur (Bongers, 1994; Goodey, 1963).

Pratylenchidae Thorne, 1949 (Order: Tylenchida)

Nematodes within said family have a very well-developed cephalic framework. The spear is short and fully developed, with strong basal knobs. Phasmids are visible behind the anus. The tail is more than double the length of the anal body diameter. The intestine is overlapped by oesophageal glands. One or two ovaries can be present. A bursa, which can either be sub-terminal or enveloping the tail, can be present on male nematodes (Bongers, 1994; Heyns, 1971).

Heteroderidae Filipjev, 1934 (Order: Tylenchida)

Heteroderidae are sexually dimorphic: males are vermiform, while the females are swollen and pear-shaped. In both of the sexes, the spear and cephalic framework is strongly developed. The oesophageal glands are located within a lobe overlapping the intestine, and the median oesophageal bulb is ovate. Females are swollen, with a subterminal vulva and two ovaries. Vermiform males have very short tails that are bluntly rounded, lacking a bursa. Males also have one or two testes and terminally situated spicules (Goodey, 1963; Heyns, 1971).

Criconematidae Taylor, 1936 (Order: Tylenchida)

Nematodes are small, thick, slow-moving and heavily annulated with an exceptionally long stylet. Basal knobs of the stylet are generally located within the anterior part of the median bulb. The procorpus progressively widens towards the large median oesophageal bulb, with which it is joined to form a continuous structure with well-developed valves. The posterior part of the oesophagus is a spathulate or rounded terminal bulb, while the isthmus is often reduced or absent. The diminutive basal bulb seems to develop into a stem-like projection of the median bulb. With the exception of one genus (*Belonolaimus*), the oesophageal glands are located within the small basal bulb. The vulva is situated posteriorly, with a single anteriorly positioned ovary. Criconematidae are sexually dimorphic. Males frequently exist with reduced or completely absent oesophagus and stylet (Goodey, 1963; Heyns, 1971; Bongers, 1994).

Paratylenchidae Raski, 1962 (Order: Tylenchida)

Said nematodes are rather small, with a fairly long spear. Heat fixation causes the body to curve ventrally. They exhibit a pro- and metacarpus that is swollen and fused into a single large bulbus with valves. The male nematodes are degenerated and the spear and oesophagus are rudimentary or absent. A bursa is absent or rudimentary, in which case it is more a cuticular fold than

a bursa. The cuticle is finely striated. The vulva of the females is far rearward in the body, and the posterior gonads are reduced, with the post-vulvar uterine branch being absent (Bongers, 1994).

Neotylenchidae Thorne, 1941 (Order: Tylenchida)

The median oesophageal bulb is not present or is, at most, fusiform, although crescentic valves are lacking. The cephalic framework consists of six to eight segments. A spear is present, with or without basal knobs or flanges. The dorsal part of the oesophagus, which connects directly to the intestine, is very variable. The dorsal part can sporadically overlap the intestine for a short distance, or an elongated lobe can overlap the intestine for a substantial distance, or it can possess a basal bulb with a stem-like protrusion into the intestine. Females possess a single, prodelphic, outstretched ovary. Male tails have a bursa that can be adanal, subterminal or surrounding the tail. The shape of the tail is similar for both males and females; it can be conoid to elongate-conoid, have an acute terminus or be blunt (Goodey, 1963; Heyns, 1971).

Dolichodoridae Chitwood and Chitwood, 1950 (Order: Tylenchida)

Dolichodoridae have a poorly developed head region and oesophageal glands that do not overlap the intestine. The cuticle is conspicuously annulated. The lip region is definitely offset by an intense constriction. Stylet length varies from 11 to 70 µm, and it is not very well developed, but does exhibit stylet knobs. Valves occur in the median bulb. Females have two gonads, of which the hind one is occasionally reduced. Phasmids are present on the tail and the tail is two to three anal body diameters long, and can be cone-shaped or cylindrical with a rounded tip. The tails of male nematodes are enveloped by a bursa (Bongers, 1994).

Aphelenchidae (Fuchs, 1937) (Order: Tylenchida)

The lobe-like oesophageal glands are enclosed in a lobe that is positioned separately in the body and which joins the alimentary canal behind the bulb. A stylet without knobs is present. Male nematodes have a bursa that is supported by four pairs of ribs or caudal rays. A gubernaculum is present, and the spicules are long and narrow (Goodey, 1963; Heyns, 1971).

Aphelenchoididae Skarbilovich, 1947 (Order: Tylenchida)

The oesophageal glands are lobe-like in shape and situated dorsally within the body, connecting to the alimentary canal behind the first bulb. The stylet possesses small knobs. Male tails

typically are without a bursa, but sometimes a terminal bursa or slight bursal folds are present. One to three pairs of subventral caudal papillae and occasionally pairs of glandular subventral papillae can be present. A gubernaculum is absent and spicules are essentially rose thorn-like in shape (Goodey, 1963; Heyns, 1971).

Paraphelenchidae Goodey, 1951 (Order: Tylenchida)

The oesophageal glands are located within a pyriform basal bulb that does not overlap the intestine. The lip region is continuous and the spear does not have any basal knobs. No bursa is present on the male tails. Four or five pairs of caudal papillae are present, as well as a gubernaculum and long, narrow spicules (Goodey, 1963; Heyns, 1971).

Monhysteridae de Man, 1876 (Order: Monhysterida)

Monhysteridae have amphids that are either circular or flattened ovally. A spear is absent. The stoma, which is commonly small, thin-walled and relatively cup-shaped; is never styletiform. Normally, ocelli are absent. A terminal bulb is usually absent within the oesophagus. The radial muscles of the oesophagus are distributed without cuticular attachments. Generally, an excretory gland is absent. The ovaries can be paired or single, and are typically extended. Males have ventromedian supplements (Goodey, 1963; Heyns, 1971).

Plectidae Oerley, 1880 (Order: Areolaimida)

The cuticle of Plectidae is striated. The oesophagus is cylindroid, along with a muscular basal bulb with plate-like valves. A spear is not present. Terminal ducts and caudal glands are present (Goodey, 1963; Heyns, 1971).

Trichodoridae Thorne, 1935 (Order: Dorylaimida)

The spear and the spear extension are joined to form an elongated, curved, dorsal, mural tooth. The spear is situated behind the spear extension, and is also linear, straight and smaller than the extension. The spear extension is arcuate, joined with the spear to form a tripartite structure and divided anteriorly. The anterior part of the stoma is tubular with cuticularised walls. The walls are somewhat splayed posteriorly, most probably acting as a guide for the spear. Two to three head widths from the anterior end; there is a fixed guiding ring that surrounds the spear. Gonads are typically paired and reflexed, but can also be single. Males may, or may not, have caudal alae, and

some species may have a small bursa. A gubernaculum is present, and spicules are virtually straight. Tails are extremely short (Goodey, 1963; Heyns, 1971).

Leptonchidae Thorne, 1935 (Order: Dorylaimida)

Leptonchidae has an oligocytous intestine, meaning that its oesophagus has only two cells in its circumference. The arrangement of muscle cells within the nematodes is also meromyarian. The axial spear is well developed, with basal extensions. The extensions may exhibit basal knobs or flanges. Large, stirrup-shaped amphids occur. Apertures are narrow in some species. The oesophagus is a slim tube, with either a short cylindroid basal bulb or a pyriform basal bulb. The bulbs are seldom more than one-third of the total length of the oesophagus. A distinct constriction may offset the basal bulb. The lateral cords are broad, predominantly with obvious pores arranged in two lines. The subcuticle has prominent transverse striae, while the cuticle is smooth, or only finely striated. A pre-rectum is present, and males have lateral guiding pieces. A gubernaculum is not present (Goodey, 1963; Heyns, 1971).

Dorylaimidae de Man, 1876 (Order: Dorylaimida)

Dorylaimidae have a stoma with an axial spear. The axial spear generally has simple rod-like extensions, and is seldom modified by basal knobs. Stirrup-shaped, generally broad amphids are present. The oesophagus, which is characteristically dorylaimoid, expands in its posterior a third or more. Dorylaimidae have a polycytous intestinum. A guiding ring, which can either be single or double, is present, occasionally lacking a sclerotised fixed ring. Tails can be sexually dimorphic in certain Dorylaimidae, and are inconsistent in shape. Lateral pores may be indistinct or distinct. Lateral cords can be uniformly granular, or with a series of glandular organs. Males have no gubernaculum, but spicules may be connected to lateral guiding pieces (Goodey, 1963; Heyns, 1971).

Longidoridae Thorne, 1935 (Order: Dorylaimida)

Longidoridae are large, slim nematodes, which can be up to 10 mm long. The stoma has a strong axial spear that is elongated and thin. The spear may or may not possess flanges. The basal third of the typical dorylaimoid oesophagus is expanded. A polycytous intestinum exists. The posterior of the oesophagus is reduced in length, and has only three gland nuclei. Amphids are either pouch- or stirrup-shaped, and occur behind the lip region. The pouch-shaped amphids have an aperture with an indistinct pore. Stirrup-shaped amphids have slit-like apertures. Tails can be sexually dimorphic, or

can vary greatly in length and shape. The lateral pores, which are very apparent, can occur in two lines. Females usually have two gonads. Males have one series of supplements, an adanal pair of papillae and a lateral pair of guiding apparatus (Bongers, 1994; Heyns, 1971).

Mononchidae Chitwood, 1937 (Order: Dorylaimida)

The head is not clearly offset. The amphids, which are small and cup-shaped, have openings that are short, narrow slits that are located at the bases of the lateral lips. The stoma, which is a reasonably large barrel-shaped cavity, has sclerotised walls displaying an immovable dorsal tooth, plus, typically, one or more smaller subventral teeth. Rows of denticles may also occur. The cuticle does not exhibit any clear striations. The area where the oesophagus and intestine are joined can either be tuberculate or non-tuberculate. Female ovaries are normally paired, extended and opposed. Three caudal glands may occur. When such glands are present, they open terminally or subterminally through a channel or a spinneret. Tails are predominantly conical, short or long and are mostly similar for both sexes (Goodey, 1963; Heyns, 1971).

Ironidae de Man, 1876 (Order: Enoplida)

Dorsal and subventral glands open in close proximity to the stoma. The stoma is cylindrical, strongly developed and heavily sclerotised. Setae may, or may not, be present on the body. Within the base of the stoma, small denticles may be present. In some males, a single pre-anal ventromedian supplement may be present. Stirrup-shaped amphids occur (Goodey, 1963; Heyns, 1971).

Alaimidae Micoletzky, 1922 (Order: Enoplida)

Alaimidae have a vestigial stoma without a spear. The amphid openings vary, from minute pore-like openings to crescentic slits that are located far back from the lip region. An excretory pore is present. The oesophagus is dorylaimoid, with the posterior third enlarged. Males have a solitary testis. Male nematodes also lack adanal supplements, a gubernaculum and a pre-rectum. The spicules are straight or arcuate (Goodey, 1963; Heyns, 1971).

Allocation of nematodes into feeding groups

All identified nematode families are allocated to feeding groups according to Yeates *et al*. (1993) (Appendix 1). After the initial nematode identifications were completed, the feeding types were

condensed into five feeding groups, including plant-, hyphal-, bacterial-feeding, predators and omnivores. Yeates *et al*. (1993) suggest that omnivores be divided into the seven other feeding types, but the feeding type of the Dorylaimidae present is not known, thus the current grouping will have to suffice.

Table 2.1. Summary of feeding types of nematodes in plant and soil systems (Yeates et al., 1993).

Feeding type	Summary of definitions
1. Plant feeding	Feed on vascular plants, plant-parasitic. Tylenchoid-stomatostyle or dorylaimoid-odontostyle always present. Post-hatching life stages of most species are migratory. Feeding sites of some sedentary species' females may be undifferentiated, uninucleate, or polynucleate. Males of sedentary species occasionally with degenerated stylet or reduced oesophagus. Plant feeders may show host specificity, or be polyphagus. Migratory species could be classified as endo- or ectoparasites. Root-hair, epidermal, cortical or vascular feeding sites are possible. Six different specialised groups exist.
a. Sedentary parasites	E.g. females of Heterodera, Globodera, Meloidogyne
b. Migratory parasites	E.g. Pratylenchidae
c. Semi-endoparasites	E.g. Hoplolaimidae
d. Ectoparasites	E.g. Criconematidae, Dolichodoridae, Longidoridae, etc.
e. Epidermal cell and root-hair feeders	E.g. Tylenchidae, Psilenchidae
f. Algal, lichen or moss feeders	Feed by piercing, e.g. *Tylenchus*
2. Hyphal feeding	Stomatostyle or odontostyle used to penetrate fungal hyphae for feeding. Obligate fungal feeders.
3. Bacterial feeding	Narrow- or broad-mouthed species feeding on prokaryote food sources. Broad-mouthed species could possibly ingest other types of food. Soil stages of parasitic nematode species of vertebrate and invertebrates, which feed on bacteria, should be included in this group.
4. Substrate ingestion	*Daptonema* sp. and diplogasterids are known for this type of feeding. More than a pure food source is ingested. May include incidental substrate ingestion, bacterial feeding, unicellular eukaryote feeding and predation.
5. Animal predation a) Ingesters b) Piercers	Feed on such invertebrates as enchytraeids, rotifers, nematodes and protozoa. Narrow stylet is present, through which bodily fluids are sucked. Contains no distinct remnants of prey within the intestine
6. Unicellular eukaryote feeding	Ingests fungal spores and whole yeast cells. May also feed on diatoms and other algae.
7. Dispersal or infective stages of animal parasites	Stages of animal parasitic nematodes when not within their animal hosts. Only entomogenous species are included. If feeding does occur during this stage, the nematodes should be placed within a different group.
8. Omnivorous	Feed on a wide range of foods. Preferably nematodes should be classified in groups 1-7.

Indices used for the analysis of nematode assemblages

Maturity and diversity indices (so-called 'community indices') concentrate information concerning the composition and structure of communities into a particular measurement. When it is assumed that communities with dissimilar composition and structure function differently, soil health can be deduced from such indices.

Diversity indices

Shannon's diversity index (H')

$$H' = -\sum P(\ln P_i)$$

P_i = the proportion of family (trophic group) *i* in the total nematode community.

The Shannon index is sensitive to rare species.

Species richness (S) / Hill's N_0 index

The Hill's N_0 index is equal to the number of families present in a sample. N_0 is the number of all families that are present in a sample, regardless of their abundance (Ferris & Bongers, 2009). If there are, for example, eight different nematode families present within a sample Hill's $N_0 = 8$.

Pielou's evenness (J')

An evenness index, which is an essential constituent of diversity indices, is a measure of biodiversity. How evenly individuals are distributed among the different species is expressed by means of an evenness index. The index also numerically quantifies how equal a community is. The evenness of a community can be expressed by means of Pielou's evenness. A J'-value close to 1 is an indication of a very even distribution of abundances amongst species, whereas values close to 0 indicate very uneven distributions (Khan, 2008).

J' = (Hill's N_0)/100

Hill's N_1 index

$$N1 = e^{H'} = \exp\left[\sum P_i (\ln P_i)\right]$$

P_i = the proportion of family (trophic group) *i* in the total nematode community.

Hill's N_2 index

$$N2 = \frac{1}{\lambda} = \frac{1}{\sum \left(\frac{n_i}{N}\right)^2}$$

n_i = the number of individuals in a family (or trophic group) i.

N = the known total number of all individuals in the community.

Simpson's diversity index (D)

$$\lambda = \sum \left(\frac{n_i}{N}\right)^2$$

n_i = the number of individuals in a family (or trophic group) i.

N = the known total number of all individuals in the community.

The Simpson index weights common species or families (Ferris & Bongers, 2009).

Indices of ecosystem function

Allocation of cp-values

Families are assigned coloniser-persister values (cp-value) according to Bongers (1999). In Table 2 below the cp-value of each nematode family is indicated, as well as the feeding group to which the nematode families have been allocated.

Cp-1

Nematodes have a laconic generation time. A considerable part of the body is dominated by gonads, which yield scores of small eggs. In food-rich environments, the population escalation is explosive and feeding is continuous. Said nematodes have a high fecundity, and are predominantly

bacterial feeders. Metabolic activity is elevated. Such enrichment opportunists demonstrate tolerance towards pollutants, as well as products that are produced by means of the decomposition of organic matter. Dauer larvae are formed when microbial blooms abate (Ferris & Bongers, 2009).

Cp-2

Nematodes demonstrate a longer generation time, with reduced fecundity compared to those of cp-1. The nematodes also do not react as readily to situations where environments are enriched. Dauer larvae are not formed, but nematodes can become cryptobiotic. Cp-2 nematodes occur in environments where resources are limited, as well as in environments where resources are plentiful. They are extremely tolerant of pollutants and other unfavourable situations. Feeding is more deliberate (Ferris & Bongers, 2001), persisting even as resources deteriorate. Primarily nematodes consist of fungal and bacterial feeders and some predators (Ferris & Bongers, 2009).

Cp-3

These nematodes have a greater sensitivity to unfavourable circumstances and a longer generation time, compared to cp-2 nematodes (Ferris & Bongers, 2001, 2009). Several predators are included in this group, as well as fungal and bacterial feeders.

Cp-4

Cp-4 nematodes have a longer generation time than do nematodes with lower cp-values, as well as a lower reproductive rate. Large non-dorylaimids with a small gonad : body volume ratio, as well as small dorylaims, fall into this group (Ferris & Bongers, 2009). Nematodes possess a permeable cuticle and greater sensitivity to disturbances and pollutants than do other nematodes (Ferris & Bongers, 2001, 2009). Carnivorous nematodes within the group actively seek out their prey, whereas the non-carnivorous nematodes are moderately sessile (Ferris & Bongers, 2009). The group is comprised of smaller omnivore species and of larger carnivores, as well as some bacterial feeders (Ferris & Bongers, 2001, 2009).

Cp-5

These nematodes have the longest life spans of all the groups (Ferris & Bongers, 2009). Nematodes in the group also demonstrate the lowest fecundity, lowest metabolic rates and sluggish movement (Ferris& Bongers, 2009). Apart from larger omnivores and carnivores, large dorylaimid

nematodes form part of the group. Compared to the body volume, the gonads are small and only a meagre number of large eggs is produced (Ferris & Bongers, 2009). The nematodes are particularly sensitive to toxins, as well as to other disturbances within their environments, due to their permeable cuticles.

Table 2.2. Functional guild classification matrix of soil nematodes. The coloniser-persister (cp) classification system introduced by Bongers (1990) supplies the life-history ordinate for the functional guild matrix, while the known or inferred feeding habits defined by Yeates *et al*. (1993) supply the abscissa. Example taxa of each guild that are indicated are rooted in current knowledge of life-history characteristics, as well as in feeding behaviour (Ferris & Bongers, 2006).

Life-history characteristics (cp-classification)					
Feeding habit	1	2	3	4	5
Plant-feeding	Pf1	Pf2 Tylenchidae	Pf3 Pratylenchidae	Pf4 Trichodoridae	Pf5 Longidoridae
Hyphal-feeding	Fu1	Fu2 Aphelen-chidae	Fu3 Diptherophoridae	Fu4 Tylencholaimidae	Fu5
Bacterial-feeding	Ba1 Panagrolaimidae	Ba2 Cephalobidae	Ba3 Teratocephalidae	Ba4 Alaimidae	Ba5
Animal predation	Ca1 Diplogasteridae	Ca2 Seinuridae	Ca3 Some Tripylidae	Ca4 Mononchidae	Ca5 Discolaimidae
Unicellular-feeding	Un1 Monhysteridae	Un2 Microlaimidae	Un3 Achromadoridae	Un4	Un5
Omnivorous	Om1 Diplogasteridae	Om2	Om3	Om4 Qudsianematidae	Om5 Thornenematidae
Selected key features	Enrichment opportunists High fecundity Small eggs Short lifecycle Dauer stage High gonad:body ratio	Basal fauna Small nematodes Anhydrobiosis Lower metabolic activity Feeding adaptions	Rudimentary food web structure Sensitivity to chemical stressors More food web links (omnivores and predators)	Greater food web structure Greater sensitivity to disturbance Larger body size Fewer eggs Longer life-cycle	Highest food web structure Undisturbed conditions Large nematodes Long-lived Narrow ecological amplitude Lowest body:gonad ratio

Computation and application of Maturity Index family

The ecological successional status of a soil is measured by means of maturity indices. The status is based on the principle that, due to the life-history characteristics of various taxa, the taxa concerned comprise contrasting sensitivities to stress or disruption of the successional sequence.

Maturity Index (MI)

The Maturity Index (MI) was developed by Bongers (1990). The MI is expressed as follows (Ferris & Bongers, 2009):

$$MI = \sum \frac{v_i \times f_i}{n}$$

Where v_i = coloniser persister value (cp-value) allocated to family; f_i = the frequency of family *i* in the sample; and n = total number of individuals in a sample.

This equation is similar for the following indexes: PPI, MI2-5, ∑MI and ∑MI2-5.However, it is important to incorporate the parameters that have been set for each index when indices are calculated.

Plant-parasitic index (PPI)

The PPI is expressed as follows (Bongers & Ferris, 1999):

$$PPI = \sum \frac{v_i \times f_i}{n}$$

Where v_i = coloniser persister value (cp-value) allocated to family; f_i = frequency of family *i* in the sample; and n = total number of individuals in a sample. Only plant-parasitic nematodes are included, thus excluding free-living nematodes.

PPI/MI

Under conditions of nutrient deprivation, the PPI/MI ratio is lower than it is under conditions of nutrient abundance (Ferris & Bongers, 2009). In agro-ecosystems, the PPI/MI is a very sensitive indicator of enrichment (Bongers & Korthals, 1995; Bongers *et al*., 1997).

MI2-5

The MI2-5 index is indistinguishable to the MI, but omits the cp-1 enrichment opportunists (which react to the presence of decomposing organic material) from the calculation (Ferris & Bongers, 2009). The index is used to measure the impact of pollutants under agricultural conditions, since it overcomes any nutrient enrichment undertaken that could eclipse the effect of stress (Bongers, 1999). In some instances, pollutants can be converted into a resource for a constituent of the microbial community that can function consecutively as a resource for cp-1 nematodes (Bongers & Korthals, 1993).

∑MI

Yeates proposed the ∑MI, while Wasileska suggested the Total MI in 1994 (Wasilewska, 1994; Yeates, 1994). The Total MI and the ∑MI equate each other (Ferris & Bongers, 2009). The ∑MI index is the MI for all nematodes, including plant-feeding nematodes, within a soil environment. The index is based on the belief that the entire nematode assemblage imparts vital information pertaining to disturbance and the condition of the environment. Plant-parasitic nematodes do not react suddenly to increases in resources due to nutrient input, as do the bacterial- and fungal-feeding nematodes (Ferris & Bongers, 2009). However, plant-feeding nematode populations may increase later, due to the resulting increase in plant vigour caused by the nutrient input. Many of the plant-parasitic nematodes are cp-3 and higher. The expected decrease in the MI due to the enrichment response, is counter-balanced by the addition of plant-parasitic nematodes in the ∑MI (Ferris & Bongers, 2009). The tolerance to stress caused by contaminants that is demonstrated by some of the plant-feeding nematodes (e.g. cp-3 Pratylenchidae) (Korthals *et al*., 1996a, 1996b) in the ∑MI compensates for the influence that pollutants have on the the MI and MI2-5 (Bongers & Bongers, 1998; Bongers, 1999).

∑MI2-5

The ∑MI2-5 calculates the MI for all nematodes, with cp-values ranging from 2-5 (Neher & Campbell, 1996). Higher value plant-parasitic nematodes provide information regarding environmental stress, with said index acknowledging that. It also endures some of the ∑MI load in situations where nutrient enrichment occurs (Ferris & Bongers, 2009).

Cp-triangles

Cp-triangles can be applied to indicate changes in nematode communities across time (Ettema & Bongers, 1993). Cp-triangles expound the reaction of the various nematode communities by discriminating between persisters (cp-3-5), general opportunists (cp-2) and enrichment opportunists (cp-1). Stress is indicated by an increase in cp-2 (with a consequent decrease in cp-1 and cp-3-5). Enrichment is signified by the domination of cp-1 types. An increase in type cp-3 to cp-5 is indicative of natural succession, which is expressed by amplified environmental stability (Bongers & Ferris, 1999).

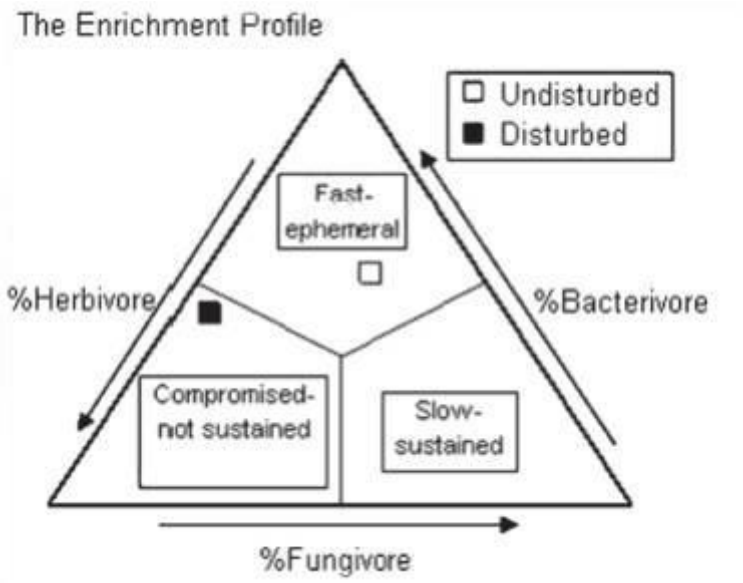

Fig. 2.7. A graphic representation of the enrichment profile (cp-triangle) and interpretation of the nature of food web enrichment in a disturbed and undisturbed environment (Ferris & Bongers, 2006).

Indicators of ecosystem function: basal, structure, enrichment and channel indices

Basal (b), enrichment (e) and structural (s) components encompass the nematode fauna (Ferris & Bongers, 2009):

$b = (Ba_2+Fu_2)\times W_2$, where $W_2 = 0.8$ (W is the enrichment weightings).

$e = (Ba_1\times W_1)+ (Fu_2\times W_2)$, where $W_1 = 3.2$ and $W_2 = 0.8$.

$s = (Ba_n\times W_n)+(Ca_n\times W_n)+(Fu_n\times W_n)+(Om_n\times W_n)$, where n = 3-5, $W_3 = 1.8$, $W_4 = 3.2$ and $W_5 = 5.0$.

The Enrichment, Structure, Basal and Channel Indices are calculated from the weighted faunal components. The indices are calculated as follows (Ferris & Bongers, 2001):

Enrichment Index (EI)

$$EI = 100 \times \frac{e}{(e+b)}$$

Structure Index (SI)

$$SI = 100 \times \frac{s}{(s+b)}$$

Basal Index (BI)

$$BI = 100 \times \frac{b}{(e+s+b)}$$

Channel Index (CI)

$$CI = 100 \times \frac{Fu_2 \times W_2}{(Ba_1 \times W_1 + Fu_2 \times W_2)}$$

According to the CI, the enrichment trajectory can be divided further to establish flow down fungal and bacterial decomposition channels. The CI includes weighting parameters for the size and metabolic rates of the nematode indicators (Ferris & Bongers, 2009).

The EI, SI, and BI are representative of the evolution of the cp-triangles mentioned previously (De Goede *et al*., 1993). These indices offer better outcomes to the effects of enrichment, disturbance and pollutants on the ecosystem. Calculations for the EI and CI do not include dauer larvae, in order for the indices to provide information regarding the present state in which a system is functioning (Ferris & Bongers, 2009).

Graphic representation of the faunal profile gives an indication of whether the soil community is enriched but unstructured (Quadrat A), enriched and structured (Quadrat B), resource-limited (Quadrat C), or resource-depleted with minimal structure, as is indicated by Quadrat D (Ferris & Bongers, 2001) (Fig. 8). The functional guilds of soil nematodes are categorised by trophic group or feeding habit, along with life history traits after Bongers and Bongers (1998). Fungivores (Fu_x), bacterivores (Ba_x), omnivores and carnivores/predators (Ca_x) embody various functional guilds (where the value of x is equivalent to a value of 1-5 on the cp-scale). Indicator guilds of the soil food web condition (basal, enriched and structured) are allocated and weightings of the guilds along the enrichment and structure trajectories are given for determination of the Structure Index (SI) and the

Enrichment Index (EI) of the soil food web (Ferris & Bongers, 2001; Ferris *et al*., 2004; Briar, 2007; Ferris & Bongers, 2009).

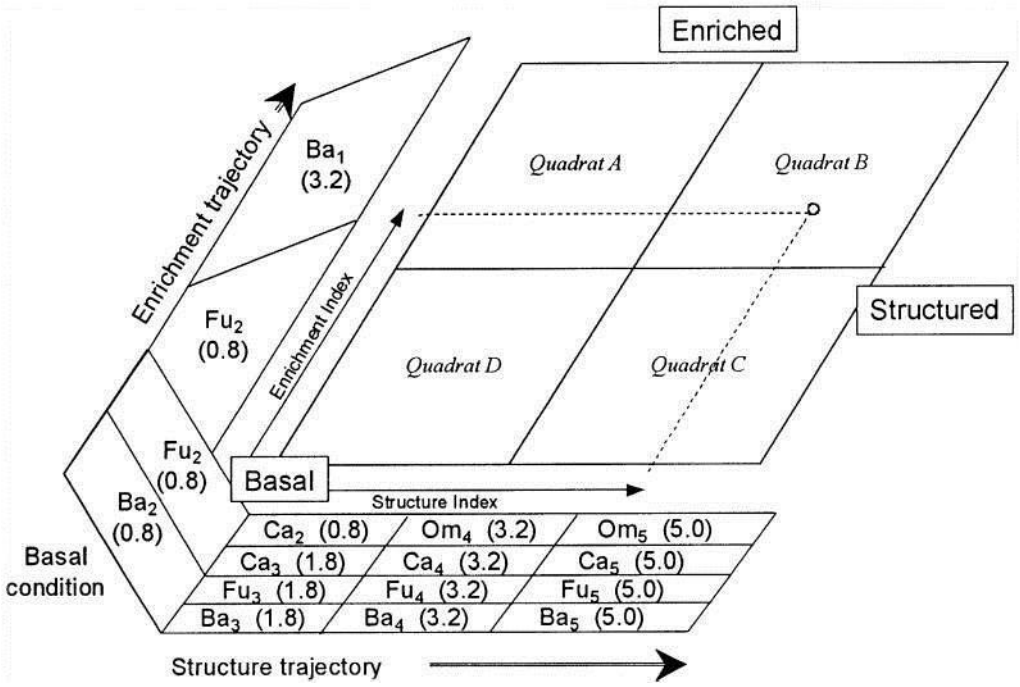

Fig. 2.8. An illustrative depiction of the nematode faunal profile reveals whether the soil is enriched but structured (Quadrat A), enriched and structured (Quadrat B), resource-limited and structured (Quadrat C), or resource-depleted, with minimal structure (Quadrat D) (Ferris & Bongers, 2001).

Table 2.3. Deduced state of the soil food web and its environment, based on weighted nematode faunal analysis. The quadrats denote faunal ordination in the faunal profile illustrated by Figure 2.8 (Ferris & Bongers, 2009).

General diagnosis	Quadrat A	Quadrat B	Quadrat C	Quadrat 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-medium	High
Food web condition	Disturbed	Maturing	Structured	Degraded

Fixing nematodes

Subsequent to counting and identifying the nematodes from the concentrated 20-mℓ sample, 10 mℓ was removed from each sample and placed in test tubes to be permanently fixed. The suspension in the test tube was allowed to settle for approximately one hour, after which time the

excess water was removed, using a pipette, till approximately 2 mℓ of fluid was left in each tube. The test tubes were placed in heated (85°C) water in a beaker for a few seconds to kill the nematodes. Double FAA (Formaldehyde, glacial acetic acid and distilled water) in a test tube was also heated and 2 mℓ was poured into the test tube containing the nematodes. The suspension was transferred to 25-mℓ glass bottles with screw tops for storage for future use.

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Chapter 3

The biodiversity and functionality of soil nematode communities within the natural Fynbos ecosystem of the Western Cape province

Abstract

A survey to determine the biodiversity and functionality of soil nematodes associated with Fynbos was undertaken by taking soil samples from different Fynbos areas in the Western Cape province. For each soil sample, the number of nematodes was quantified and identified to family level. All five trophic groups in the natural soil food web were found to be represented in the Fynbos soils. A total of 19 different nematode families were identified, of which seven (39.09%) were herbivorous (plant-feeding or plant-associated nematodes), six were bacterivorous (36.66%), four fungivorous (17.93%), one omnivorous (6.09%), and one predatory (or carnivorous) (0.23%). Tylenchidae were found to be the most dominant nematode family present within the Fynbos, followed by Cephalobidae and Paraphelenchidae. The average Maturity Index (MI) value for the Fynbos data was found to be very low, with a mean value of 1.26. The value indicated the presence of taxa with tolerance to disturbance, which, in turn, indicated a disturbed soil in general. The average Plant-parasitic Index (PPI) value for the Fynbos soils was also low, with a value of 0.85, as was expected, since the number of true plant-parasitic nematodes was low in comparison to that which tends to be present in agriculturally managed soils. To determine the existing basal, structure and enrichment conditions, the nematode faunal analysis was applied. Each sample showed great variation, indicating Fynbos to be highly area-dependent, as each sample rendered a unique faunal analysis representation. With regard to diversity, richness, and evenness, the values were found to be low, which indicated low nematode diversity, but a distribution of abundances amongst families. Hill's N_0 index gave a value of 8.0, indicating that, in general, there would be eight nematode families present within a Fynbos sample. The results indicated that natural Fynbos from a specific area should be compared to soil in an adjacent area, to which a specific agricultural activity has been applied. Different areas have been

found to differ greatly in nematode diversity and functionality, because of climatic conditions, soil conditions and dominant plant families and species, which greatly affect the nematode community structure present within the soil.

Introduction

Fynbos is not only the natural vegetation of the southern part of the Western Cape province, but also forms part of the Cape Floristic Kingdom, which is the smallest of the world's six botanical kingdoms. It covers roughly 0.04% of the entire land surface of the earth. The Cape Floristic Kingdom does not exist as an uninterrupted line, but occurs in patches from Vanrhynsdorp to the Cape Peninsula and eastwards towards Grahamstown and Port Elizabeth (Van Rensburg, 1987). The name 'Fynbos' is derived from the word Fijnbosch, which was the name given by the Dutch settlers in the Cape to the predominant vegetation that was too fine for use as timber.

Fynbos represents a large group of perennial plants with small, hard leaves. The areas in which Fynbos occur usually experience a dry, warm summer season. Fynbos is also exemplified by the following qualities: in excess of 9000 plant species occur, of which over 6000 species are endemic to particular areas. Thus, there is great species biodiversity, with Fynbos being unique at a plant community level. Within exceptionally small sections of land, an extremely extensive number of species may occur (Van Rensburg, 1987).

Although the distribution pattern of Fynbos can be characterised by typical vegetation factors, there is a deficiency in overall dominance by particular species. Nevertheless, certain species or genera may prevail, including a restoid, ericoid and proteoid constituent. The ericoid constituent consists of evergreen plants with diminutive rigid and rolled leaves. Proteoids are relatively high shrubs, mostly consisting of proteas, with hard, tough leaves. The restios are thin, reed-like, leafless plants that exist in evergreen clumps (Van Rensburg, 1987).

According to Neher (2010), bacterivorous and plant-parasitic nematodes have been found to be most abundant in agricultural soil in Poland. Omnivorous nematodes have been found to be more frequent within grasslands compared to in agriculturally managed soils, and fungivores have been found to be more profuse in forest soils than they are in agricultural soils. The comparative nematode numbers within each functional group have been found to differ in Swedish soils (Neher, 2010), which

is an indication that nematode numbers and functional groups differ according to each distinctive environment and soil type.

In the natural forest soils of Arunachal Pradesh in India, predators have been established to be the most dominant functional group present, followed by bacterivores, fungivores, herbivores or plant parasitic-nematodes, and, lastly, omnivores. When examining individual abundance, it was determined that fungivores were the most dominant feeding group. Subsequent feeders were, in order of dominance, predators, bacterivores, herbivores, and omnivores (Baniyamuddin *et al*., 2007).

Meyer (1999) studied the nematode populations of undisturbed Fynbos soil compared to those in soils within adjacent vineyards and pine plantations. In the study, it was hypothesised that the rich diversity of plant-parasitic nematodes in the rhizosphere of grapevines had two potential origins. Plant-parasitic nematodes could have been present in the soil at the time at which the vines were established, or might have been introduced through planting material that could have been infested with the nematodes in question. The experimental sites consisted of original Fynbos vegetation, an adjoining 10-year-old vineyard and a pine plantation (> 16 years old). Samples were collected from the rhizosphere of *Protea repens*, vineyard rootstocks and *Pinus radiata*. No significant difference was found in the populations of plant-parasitic nematodes between Fynbos and vines, but a significant difference was observed in the populations between the vegetation types and the pine plantations. From the study, it was concluded that proteas were most likely the original hosts for *Meloidogyne* spp. (root-knot nematode), which is an important pest in vineyards globally. It was also concluded that Fynbos was the source of initial infestation for other serious plant-parasitic nematodes, such as *Criconemoides xenoplax* and *Pratylenchus* spp. Such nematodes flourish in vineyards (Meyer, 1999) and protea plantations (Stirling *et al*., 2002; Marais *et al*., 2004) where they cause much damage. The pine trees were found not to maintain plant-parasitic nematode species (Meyer, 1999).

Except for the study of Meyer (1999), no other information about the nematode community structure and diversity within Fynbos soils exists. However, surveys have been done on the plant-parasitic nematodes associated with Fynbos (Marais *et al*., 2004). It is important to note that Fynbos soils are usually poor and infertile (Van Rensburg, 1987). Grasslands, forests and coastal dune systems are to date the only natural ecosystems that have been fairly well studied in this respect (Neher, 2010).

The overall aim of the current study was to obtain information on the soil nematode community structure of undisturbed soil, which could be used as a future reference against the nematode community structures of agricultural soil in the Western Cape province. This was done by determining the abundance, diversity and functionality of the naturally occurring free-living and plant-parasitic nematodes in different areas of the Fynbos concerned.

Materials and methods

Field sampling

A total of 48 Fynbos soil samples were collected during the spring of 2011 throughout different regions in the Western Cape. In a specific area, indigenous, dominant, perennial Fynbos plant families were randomly sampled by taking subsamples in the root zone of up to five plants. Approximately 1 kg of soil was collected, to a depth of 20 cm from each site, and transported in a cooler box back to the laboratory. The samples were then kept in cold (± 14°C) storage until analysed.

Nematode extraction and identification

Nematodes were isolated from a 250 ml sub-sample from each of the samples collected, by means of the Cobb's (1918) decanting and sieving method. Following extraction, the nematodes were stored in culture flasks at 14°C until use (Chapter 2).

To enumerate, nematodes were concentrated in 20ml of water by allowing them to settle for 60 min, after which the supernatant was siphoned off from the suspension. After counting and identification, the remaining nematodes were heat-fixed, using double FAA at 85°C (Chapter 2).

All identified nematode families were allocated to one of five different feeding groups, based on their method of feeding, according to Yeates *et al*. (1993), consisting of plant- (herbivores), hyphal- (fungivores or fungal feeders), and bacterial-feeding groups, as well as predators (carnivores) and omnivores (Yeates *et al*., 1993; Yeates, 1998; Yeates & Bongers, 1999). Families were assigned coloniser-persister values (cp-values), according to Bongers (1990).

Maturity indices

Communities were characterised by various indices, including the Maturity Index (MI), the Plant-Parasitic Index (PPI), and the MI2-5, ∑MI and ∑MI2-5 indices. The nematode families were classified on the cp-scale, according to Bongers (1990).

Trophic diversity indices

To determine the diversity of the nematodes from the different sites, Shannon's Diversity Index (Shannon & Weaver, 1949) was used, while the evenness of the population was calculated using Pielou's J' Evenness Index (Ferris & Bongers, 2009). The Simpson's Diversity Index (Simpson, 1951) was also calculated for each site, in addition to Hill's N_0, Hill's N_1, and Hill's N_2 indices (Ferris & Bongers, 2009).

Basal, structure and enrichment conditions

The weighted faunal analysis concept was employed to determine the basal, structure and enrichment conditions of the soil food web within each sample (Pattison *et al.*, 2008). The conditions were determined by excluding plant-feeding nematodes. The indices calculated to determine these conditions were the Enrichment Index (EI), the Basal Index (BI), and the Structure Index (SI). Additionally, the Channel Index (CI) for each site was also determined.

Soil analysis

Soil samples from each site were analysed by BEMLAB, a SANSAS-accredited testing laboratory, for the analysis of the soil's physical and chemical components. A total of 500 g of soil, taken from each site, was analysed. The following parameters were measured: a mechanical-3-fraction analysis was done to determine the soil classification, as well as the percentage of sand, clay and silt within each soil; the pH (KCl) and exchangeable cations (Na, K, Ca and Mg), and the base saturation (Na%, K%, Ca% and Mg%, with the t-value in cmol/kg); the total carbon was determined by means of the Walkley-Black method and the inorganic nitrogen (NO_3-N and NH_4-N, measured in mg/kg).

Data analysis

Descriptive data analyses were done using Microsoft Excel 2010. Multivariate analyses were performed, using canonical correspondence analysis (CCA) in XLSTAT 2010 and R (R version 2.13.0 Development Core Team, 2007. *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria).

Results and discussion

Nematode families associated with Fynbos and trophic group dynamics

A total of 48 different soil samples were collected from different Fynbos areas in the Western Cape, of which the nematode density, diversity and species richness within each sample was determined (Fig. 3.1).

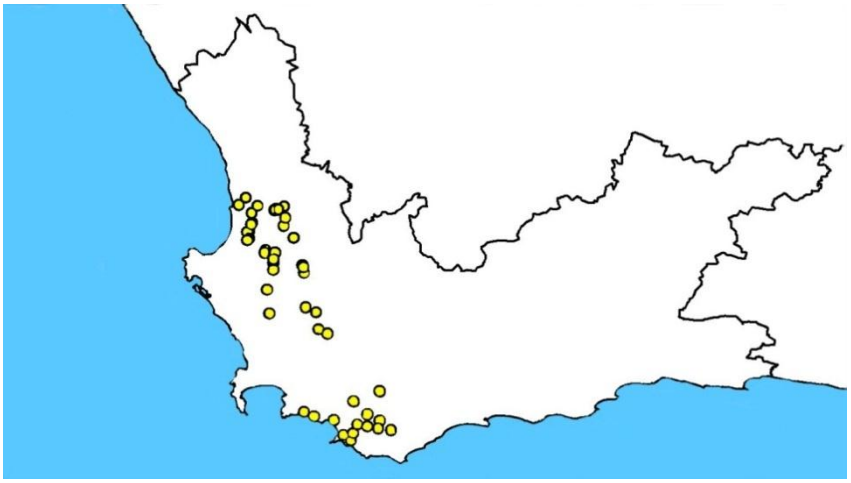

Fig. 3.1. Sampling sites of Fynbos in the Western Cape province of South Africa.

A total of 19 different nematode families were identified. Of the 19 families, seven (39.09%) were herbivorous (plant-feeding or plant-associated nematodes), with six being bacterivorous (36.66%), four fungivorous (17.93%), one omnivorous (6.09%) and one predatory (or carnivorous) (0.23%) (Yeates *et al*., 1993) (Fig. 3.2).

All five trophic groups in the natural soil food web were represented in the Fynbos soils. Herbivores or plant-associated nematodes feed on vascular plants, by means of an onchiostyle, a tylenchid stylet or a dorylaimid stylet (Yeates, 1998). Most life stages are migratory, but sedentary

forms do occur, in which the feeding site of the female can be uninucleate, polynucleate or undifferentiated. In some species, males and migratory infective juveniles may be non-feeding (Yeates, 1998).

Hyphal- or fungal-feeding nematodes feed by penetrating fungal hyphae with a small, thin stylet. Said nematodes are obligate fungal feeders, and possess a narrow lumen (Yeates, 1998), whereas bacterial feeding nematodes feed on any prokaryotic source of food that is available within the soil environment (Yeates, 1998). They are broad-mouthed nematodes that are adapted to feeding on bacteria.

Omnivorous nematodes, which are usually restricted to some dorylaimids, seem to attain nourishment from an assortment of available food resources (Yeates, 1998). Carnivores, or predators, obtain nourishment through feeding on invertebrates, for instance enchytraeids, Protista, rotifers and nematodes. Predation is not always obligate. The group concerned is split up into a number of different subdivisions, the first of which consists of ingesters. Nematodes that fall into this subdivision (such as Mononchidae) have a large anterior opening that is sometimes armed with a tooth or with rows of small teeth, and conspicuous parts of prey may be located in the intestine. The other subdivision consists of piercers. The nematodes concerned suck the contents of their preys' bodies out through a fairly narrow stylet (Yeates, 1998).

In natural soils, omnivores, bacterivores and carnivores are commonly found in low numbers (Neher, 2001b). Except for the high numbers of bacterivores that are present in Fynbos soils, the natural soil of the Western Cape seems to conform to said norm. The high numbers of bacterivorous nematodes that are present in Fynbos soils could be ascribed to the fact that the soils are very dry during the long, hot summers. The feeding and reproduction of the nematodes are also influenced by the type, nature and number of bacteria that are available as a food source. The temperature also influences the reproduction and feeding of bacterivores (Yeates, 1998).

The high temperatures within Fynbos soils are probably the reason why nematodes with higher cp-values, the persisters, perish sooner than the colonising nematodes with low cp-values, such as the bacterivores. It takes longer for persisters than colonisers to reach high population densities, due to the former's sensitivity to stress in the environment. The colonisers, in contrast, reproduce quickly, and population levels can increase rapidly, even if environmental conditions are

not favourable (Bongers *et al*., 1991). Soil moisture has a significant influence on the movement and the persistence of nematodes in soil (Evans, 1969; Griffiths *et al*., 1995; Young et al., 1998). Larger nematodes, such as the omnivorous dorylaimids (cp-5), are not able to move around in dry conditions and so gain access to food sources. Smaller nematodes with lower cp-values (such as bacterivores, and some of the plant- and hyphal-feeders) would still be able to move around in the thin layers of free water that are available in the soil for longer periods of time than would the larger nematodes, which could lead to higher populations. The nematodes that remain after unfavourable conditions are those that can reproduce rapidly and restore the population. Such nematodes also have certain survival traits, such as the ability to form dauer larvae, which aid in the ability to increase population numbers swiftly, with said population having a short life cycle and high reproduction capacity.

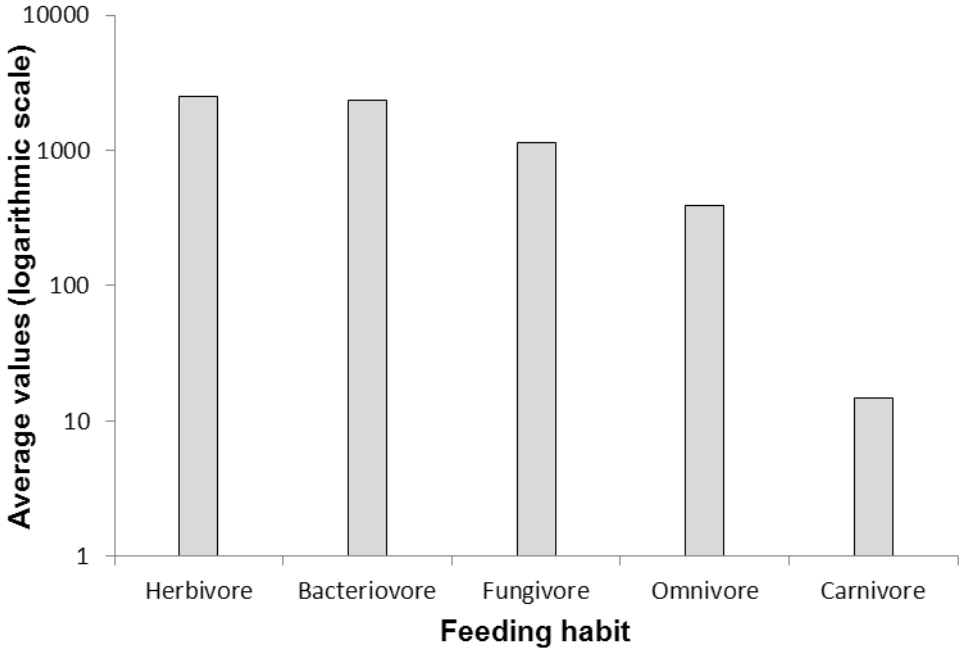

Fig. 3.2. Nematode trophic diversity within Fynbos soil.

In Figure 3.3 below, the total number of nematodes within the Fynbos samples is plotted against the number of nematode families that were found to be present in all the samples. Tylenchidae (sedentary, root-feeding nematodes) were found to be the most dominant nematode family present within the Fynbos, followed by the Cephalobidae (bacterial-feeding nematodes) and the Paraphelenchidae (fungal- or hyphal-feeding nematodes).

Nematodes associated with vascular plants were found in all Fynbos samples and were allocated to feeding group 1, plant-feeding, according to Yeates *et al*. (1993). The highest number of nematodes was found in the family Tylenchidae (Fig. 3.3). They were included in feeding group 1e, with a cp-2 value (Bongers, 1990). Members of feeding group 1e are classified as epidermal-cell and root-hair feeders (Yeates *et al*., 1993), and are regarded as basal fauna (Neher, 1999b; Neher, 2001a). They are small nematodes that have the ability to enter into a state of anhydrobiosis, with lower metabolic activity and feeding adaptations. The nematodes concerned are considered to be plant-associated nematodes. They exist in high concentrations in the rhizosphere of plants, which are devoid of any obvious damage to roots, to pathogenicity, or to decline in yield. Plant-associated nematodes may play an important role in the closed cycling of nutrients within the rhizosphere of particular plants (Yeates, 1998).

In the Fynbos soils, the family Tylenchidae were followed by the Hoplolaimidae (1c semi-endoparasites, cp-3), Criconematidae (1d ectoparasites, cp-3), Pratylenchidae (1b migratory endoparasites, cp-3), Longidoridae (feeding group 1d, cp-5), Trichodoridae (feeding group 1d, cp-4), and Heteroderidae (feeding group 1a, cp-3) (Fig. 3.2). The families are associated as parasites of higher vascular plants. Each of the families contains plant-parasitic nematodes that are of importance to agriculture in the Western Cape. For instance, in the family Criconematidae, *Criconemoides xenoplax* Loof & De Grisse (Raski, 1952) is one of the most important pests in peaches, plums and grapevines. In the family Pratylenchidae, the lesion nematodes or *Pratylenchus* species were found, which are especially important for apple and grapevine production. In the family Longidoridae, species of *Xiphinema* are of importance to deciduous fruit and grapevine production (Meyer, 1982; Smith, 1982) These results corroborate those of Meyer (1999), who concluded that the high diversity of plant-parasitic nematodes in agricultural soil, such as those being used for grapevine and deciduous fruit production, have their origin in the Fynbos itself. For this reason, virgin, or undisturbed, soil cannot be treated as being free of plant-parasitic nematodes especially where new grapevines, deciduous orchards and new Proteaceae orchards for the cut flower industry, are to be established. The plant-parasitic nematodes present in the soil will flourish under such monocultures, if sufficient care is not taken to keep their numbers under the threshold level for damage. During a survey for the presence of plant-parasitic nematodes that was undertaken by Marais *et al*., (2004), most of the economically important plant-parasitic nematodes were identified in undisturbed Fynbos.

Bacterivorous nematodes, of which Cephalobidae and Rhabditidae were the most abundant, were present in all the samples. The family Cephalobidae was assigned a cp value of 2 (Bongers, 1990, 1999), and to the feeding type 4, as bacterial feeders, because of the shape of the stoma, that is without a stylet (Yeates *et al*., 1993). The allocation of the cp-value of 1 to Rhabditidae by Bongers (1990) indicates the nematodes concerned have a very rapid generation rate. Under conditions of extreme food enrichment, population growth tends to occur at an alarming rate (Ferris & Bongers, 2009). Both of the families concerned feed on any prokaryotic food source that is available, and they transpire through either a narrow or broad stoma. They occur in environments that are poor or plentiful in resources, and are extremely tolerant of pollutants and of unfavourable conditions, such as those that can occur in the case of Fynbos.

Cephalobidae and Rhabditidae were followed by Monhysteridae (feeding group 3, cp-2), Panagrolaimidae (feeding group 3, cp-1), Diplogasteridae (feeding group 3, cp-1) and Plectidae (feeding group 3, cp-2). Fungivorous nematodes (armed with a stylet) were also present at each site, and were predominantly Paraphelenchidae, followed by Aphelenchidae, Neotylenchidae and Aphelenchoididae (Fig. 3.3). The families were assigned coloniser-persister values of 2 (Bongers, 1990), and regarded as hyphal feeders that fell into feeding group 2 by Yeates *et al*. (1993). Such nematodes are, in general, small, and are regarded as secondary consumers of fungi within the soil food web, having the ability to enter a state of anhydrobiosis, with low metabolic activity. Aphelenchoididae were found to be rare and to be present only in a small number of the sites (Fig. 3.2).

Omnivorous nematodes, such as Dorylaimidae, were not found in high numbers, but were found to occur in almost every site. The Dorylaimidae were the only family of omnivorous nematodes that was represented within the Fynbos soil. They were assigned to feeding group 8 by Yeates *et al*. (1993) and to a cp-value of 4 by Bongers (1990). This group of nematodes is sensitive to disturbance, having a relatively large body size, with a longer life cycle than is usual among nematodes, and with the ability to produce fewer eggs than is common among such species.

Predatory nematodes, such as Mononchidae, which feed on other nematodes or on invertebrates in the soil, were considered rare, and were only found in very low numbers at a few sites. Mononchidae were the only predatory family identified within Fynbos soils. They are a very sensitive group of nematodes, and are not able to adapt quickly to environmental disturbance. They

were assigned a cp-4 value in the coloniser-persister classification by Bongers (1990) and to feeding group 5 by Yeates *et al*. (1993).

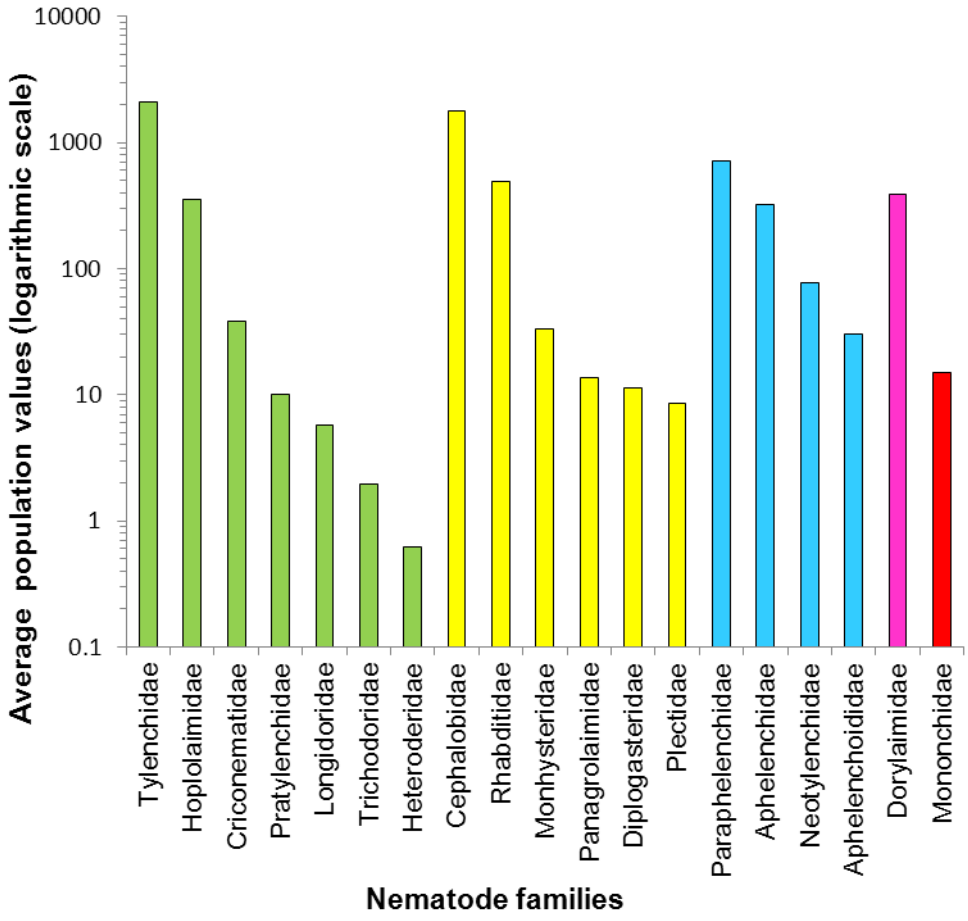

Fig. 3.3. A graphical representation of the different nematode families that is present within Fynbos soils, and the total number of nematodes present**.** Feeding groups are indicated in different colours: herbivores or plant-parasitic nematodes (green); bacterivores (yellow); fungivores (blue); omnivores (pink); and predators (red).

A CCA was done to determine whether any associations between nematode families and Fynbos plant families exist (Fig. 3.4). Results showed a negative association between the nematode family Longidoridae and the Fynbos families Malvaceae and Asteraceae. Longidoridae is also negatively associated with the nematode families Panagrolaimidae, Neotylenchidae, Paraphelenchidae, Aphelenchidae and Cephalobidae. The implication is that these nematode families will most likely not be found to occur in the same sample in which Longidoridae occurs. In the current study, the nematode family Pratylenchidae had a strong association with Fabaceae, Solanaceae and Celestraceae. The Fynbos families Malvaceae and Asteraceae had strong associations with

Paraphelenchidae, Panagrolaimidae, Neotylenchidae, Aphelenchidae and Cephalobidae. Trichodoridae demonstrated an association with Anacardiaceae and Rhamnaceae, but the short vector could be an indication that the association took place by chance. Hoplolaimidae had a strong association with Asparagaceae and Rutaceae, but, once again, the vectors were quite short, which could be an indication of a coincidental association. Plectidae and Rhabditidae had an association with Geraniaceae. The nematode families Longidoridae, Tylenchidae, Dorylaimidae, Diplogasteridae, Heteroderidae, Criconematidae and Mononchidae were not significantly associated with any Fynbos families. The vectors for nematode families Paraphelenchidae, Panagrolaimidae, Neotylenchidae, Aphelenchidae, Cephalobidae and Pratylenchidae were close together, which indicates a positive correlation between the families concerned and that they were, thus, likely to occur together.

The strong associations between nematode and Fynbos families indicate that, when sampling in areas where the Fynbos families occur, it is highly probable that the nematode families concerned will be extracted from the soil sample taken.

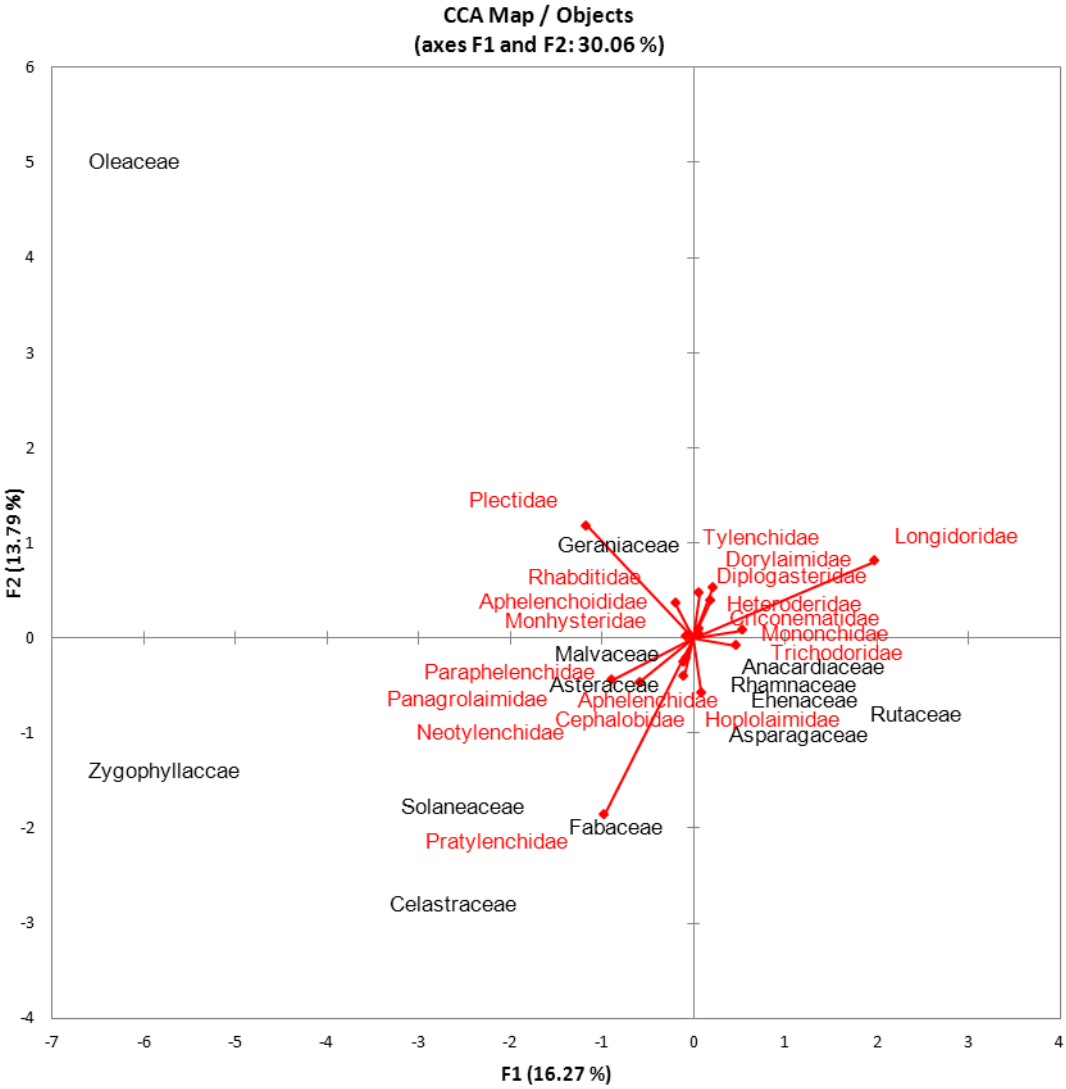

Fig. 3.4. CCA of data, indicating the associations between nematode families (red) and Fynbos families (black).

The CCA of nematode feeding types and Fynbos families indicated that the carnivores and the bacterivores were not significantly associated with each other (Fig. 3.5). The finding was not in agreement with the conclusions drawn by Yeates *et al.* (1993), Yeates and Bongers (1999) and Baniyamuddin *et al.* (2007). The Fynbos families Iridaceae, Fabaceae and Rutaceae indicated a strong association with herbivores (or plant-feeding nematodes), but a weak association with omnivorous nematodes. Malvaceae was strongly associated with fungivores, but weakly associated with plant-feeding nematodes. Sites that had a high number of fungivores, also seemed to have high numbers of omnivorous nematodes. According to Baniyamuddin *et al*. (2007), a possible association

exists between omnivores and nematodes in other feeding groups (except for that of plant-feeding nematodes). An escalation in the omnivore population would, thus, affect populations of other nematodes unfavourably. Oleaceae, Apocynaceae, Geraniceae and Zygophyllaceae express a high association with omnivores, yet the opposite is true regarding herbivores. The same is true for Geraniceae. Aspargaceae seems to have a low affinity for all three dominant feeding groups: omnivores, fungivores and herbivores.

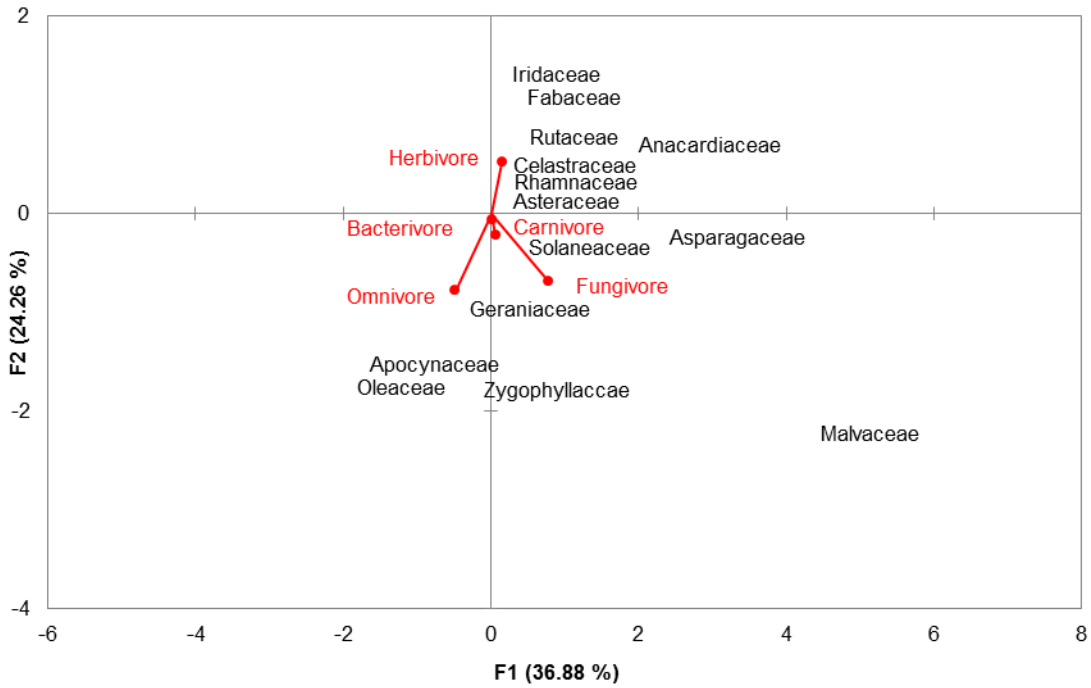

Fig. 3.5. CCA of feeding types (red) and Fynbos families (black).

No relationships were found between the feeding groups and the soil properties that were measured (Fig. 3.6). Therefore, certain soil parameters (physical and chemical) were selected to determine whether associations occurred between such parameters and the nematode families that were present within the soil environment (Fig. 3.7). The parameters were percentage silt, sand and clay (physical characteristics) (Pattison *et al*., 2008), as well as chemical properties that included pH (KCl), phosphorous (P mg/kg determined by means of the Bray II technique), carbon percentage (C%), total nitrogen percentage (N%), nitrate (NO3-N mg/kg) and ammonium (NH4-N mg/kg) (Neher, 1999a; Janvier *et al*., 2007; Pattison *et al*., 2008).

The CCA indicated a strong association between Pratylenchidae and the sand fraction of soil. It also indicated a strong association between the sand fraction and Longidoridae and Trichodoridae.

Monhysteridae, Diplogasteridae and Rhabditidae (all bacterivorous nematodes) were found to have a low association with both the sand and the silt percentage of soil. They also had a low association with phosphorous (P Bray II). They were, however, influenced by the clay fraction within the soil, but, due to the short length of the vector representing the clay percentage, the association detected could have occurred by chance. Criconematidae and Aphelenchidae were associated with phosphorous (P Bray II). Paraphelenchidae, Tylenchidae, Heteroderidae and Aphelenchoididae were negatively associated with the percentage sand. An association between pH and Heteroderidae (a herbivore) and Aphelenchoididae (a fungivore) was also very apparent. The nematode families concerned were, thus, strongly influenced by changes in the soil pH. Paraphelenchidae, Tylenchidae, Heteroderidae and Aphelenchoididae indicated a high association with N% (nitrogen), pH (KCl), C% (carbon) and ammonium (NH_4-N), but, due to the length of the vectors involved, the associations were not significant and had, possibly, only occurred by chance. The nematode families also had a negative association with nitrate (NO_3-N mg/kg). A slight association was also present between nitrate (NO_3-N) and Dorylaimidae (omnivores). The vectors for sand and silt were close together, indicating a good correlation between them. Such a finding might indicate that the nematodes that were found in high numbers in the sandy soil might also occur in high numbers in silt soils.

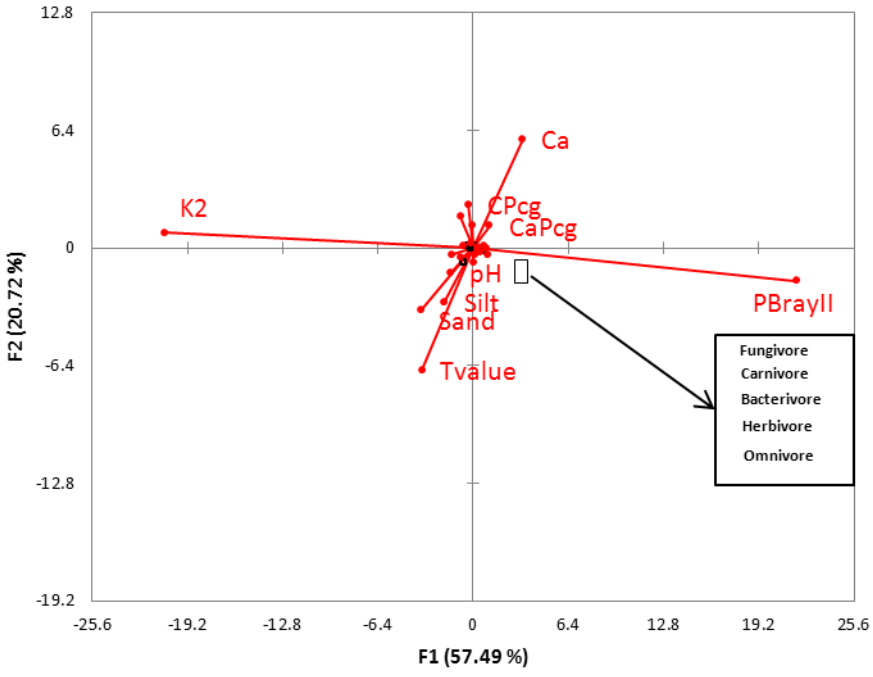

Fig. 3.6. CCA of nematode feeding types and soil properties.

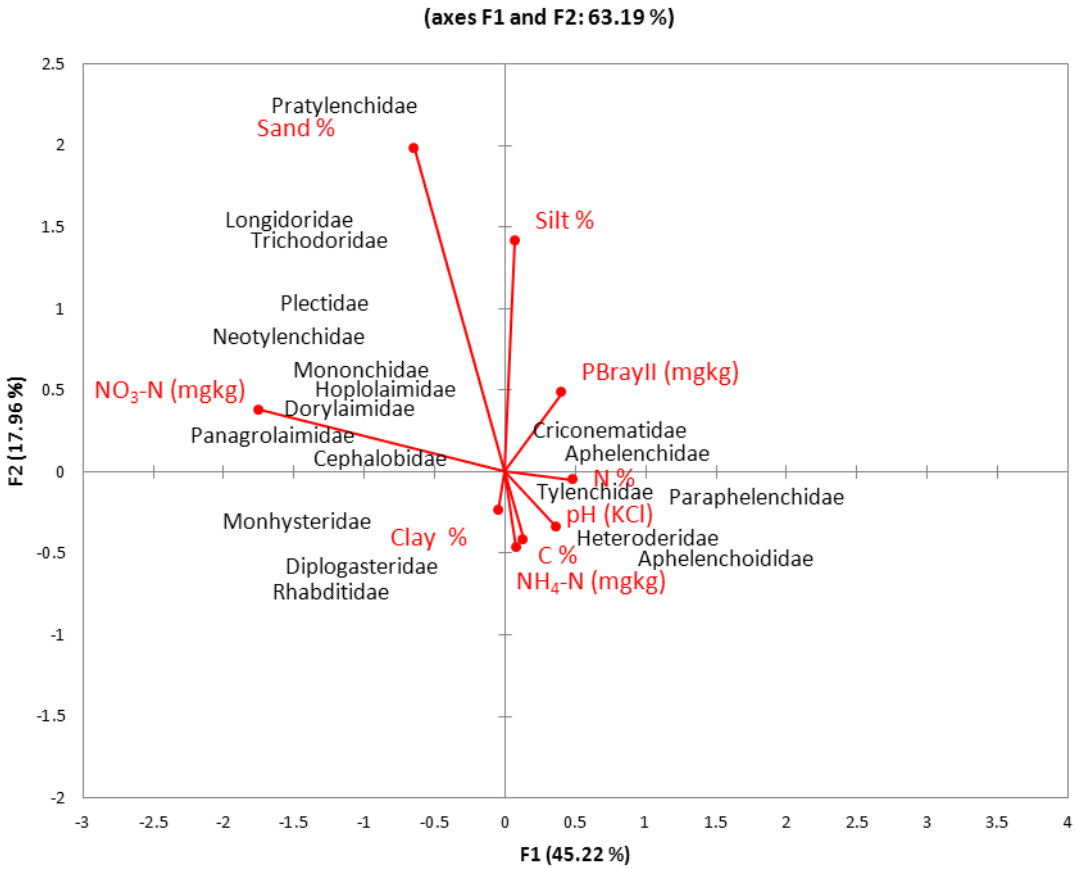

Fig. 3.7. CCA indicating associations between physical and chemical soil properties and nematode families present within the soil food web.

Maturity indices

The values of the five maturity indices (MI, MI2-5, PPI, ∑MI2-5, ∑MI) that were calculated for Fynbos are indicated in Figure 3.8 below (including PPI/MI). From said indices, Neher (2001b) found the MI to be the most valuable for determining nematode communities as the ecological indicators of agroecosystem health.

The average MI value (excluding plant-parasitic nematodes) for the Fynbos data was found to be low, with a mean value of 1.26 (Fig. 3.8). The MI represents a weighted mean frequency of taxa assigned with weights from 1 to 5, with smaller weights being assigned to taxa with tolerance to disturbance, and larger weights representing taxa that are more sensitive to disturbance (Bongers, 1990). Smaller values indicate greater disturbance, and larger values less disturbance (Freckman & Ettema, 1993). The value of 1.26 indicates the Fynbos system to be nutrient-enriched and disturbed. Pristine, undisturbed environments have values of ± 4.0 (Bongers & Ferris, 1999; Baniyamuddin *et al*., 2007).

In the current study, the MI value of the Fynbos indicated the presence of taxa with tolerance to disturbance, which, in turn, indicated a disturbed soil in general, based on the findings of Bongers (1990), Freckman and Ettema (1993) and Neher (2001a). The reason for this low MI value for Fynbos could mainly be ascribed to environmental factors. Fynbos occurs in areas with long, warm summers and sandy soil, which would have a profound effect on the nematode population in the first 20 cm of soil depth. Sandy soil especially tends to become hot and dry in the summer, causing the nematode populations, especially the numbers of taxa that are sensitive to heat and moisture, to be negatively affected. Such disturbance could cause high levels of colonising nematodes, as opposed to persisters in Fynbos soils.

The average value of the MI2-5 was found to be 1.18 (Fig. 3.8), and was of little use in determining the maturity of Fynbos soil, since the MI2-5 indicated the same as did the MI, except for the cp-1 values, which were excluded. The MI2-5 value is of use in the presence of decomposing organic material in agricultural soil, as it excludes the enrichment opportunists (Ferris & Bongers, 2009). It can also be used to measure pollutants in agricultural soils (Bongers & Korthals, 1993).

The average PPI value for Fynbos soils was low, with a value of 0.85. The finding was expected, since the number of true plant-parasitic nematodes present was low in comparison of the number present in the agriculturally managed soil. This was expected, as the soil in which the Fynbos grows is regarded as undisturbed. According to a study done by Meyer (1999), no difference in plant-parasitic nematode diversity was found in undisturbed Fynbos and in an adjacent vineyard. According to Neher (2001a), when using a perennial system as a base of comparison, the time of undisturbance must be considered (Wasilewska, 1979; 1994). The conclusion was drawn that a long-term perennial crop (> 10 years) can be closely related to an undisturbed site.

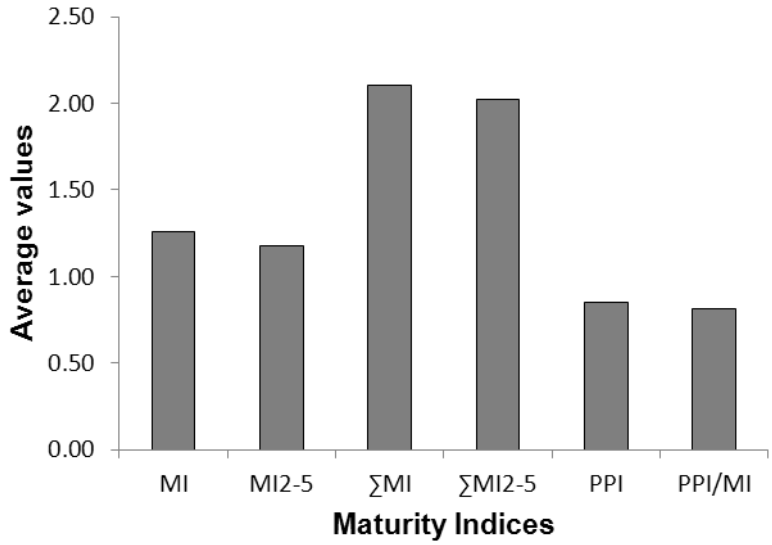

Fig. 3.8. Maturity Index values of nematodes for Fynbos samples as a whole.

Basal, structure and enrichment conditions

The BI, SI, EI and CI signify an assemblage of functional guilds with cp values that range from 3 to 5. Conforming to Pattison *et al*. (2008), the SI is a measure of the number of trophic layers within the soil food web, along with the potential for regulation by predators. A low SI expresses a disturbed or degraded soil ecosystem, whereas a high SI identifies structured or matured conditions (Baniyamuddin *et al*., 2007). Furthermore, the EI gauges the resources available to the soil food web, as well as the responses by primary decomposers to said resources (Pattison *et al*., 2008). Nutrient-enriched soil ecosystems have high EI values, whereas systems that are nutrient depleted have low EI values (Baniyamuddin *et al*., 2007). The level of fungal participation in the primary decomposition channels of soil food webs is signified by the CI (Ferris & Bongers, 2001).

The nematode faunal analysis (Ferris & Bongers, 2001) was applied for each Fynbos sample taken (Fig. 3.9). For the total Fynbos area, data points were found to be spread throughout the plot area. The graphical representation illustrates that Fynbos soils range from structured (Quadrat A), through enriched and structured (Quadrat B) and resource-limited, as well as structured (Quadrat C), to resource-depleted, with minimal structure (Quadrat D). The results indicate that the faunal analysis for Fynbos was highly area-dependent. Each area where Fynbos was sampled showed a unique faunal analysis representation.

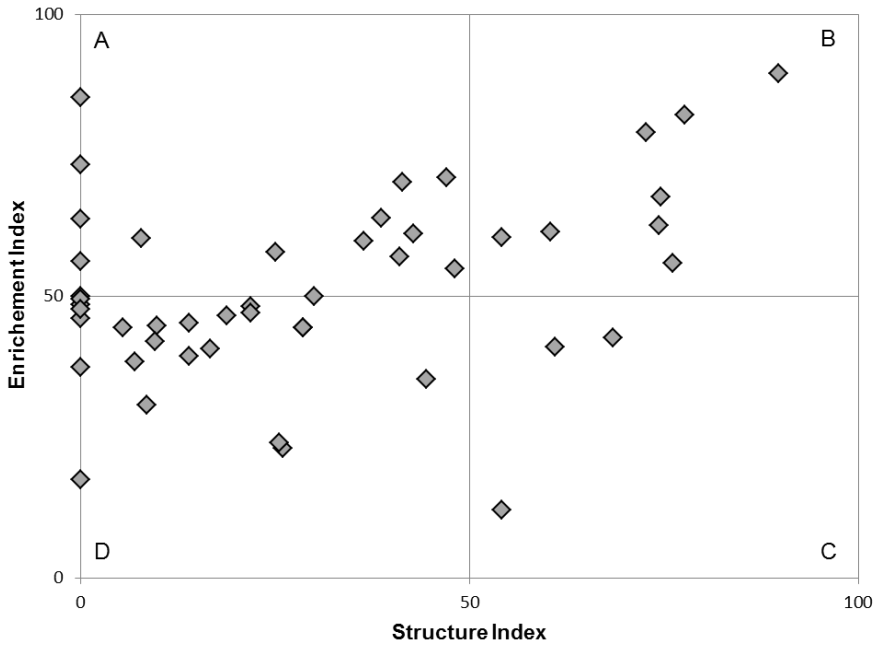

Fig. 3.9. The nematode faunal analysis for the total number of Fynbos samples collected throughout different regions in the Western Cape.

The CI, BI, EI and SI values are graphically represented in Figure 3.10 below. The CI supplies a method of separating the flow of resources through bacterial and fungal channels of decomposition (Ferris & Bongers, 2009). In other words, it indicates the decomposition channel of nutrients (Pattison *et al*., 2008). A high CI value indicates a nematode community that is dominated by fungal decomposers, whereas a low value is an indication of a bacterial-dominated decomposer community (Ferris & Bongers, 2001; Hohberg, 2003; Pattison *et al*., 2008). The CI value for Fynbos was 42.4%, which is regarded as low. The value concerned indicates a nematode community that predominantly consists of bacterial decomposers.

The BI is the relative quantity of the basal, or cp-2, component of the fauna of all the nematodes that are present in the soil. The Fynbos soils had a BI value of 39.9%, which is regarded as low. The cp-2 component of the Fynbos soils was found mostly to consist of fungivores, and, from Figures 3.2 and 3.3, it is clear that they comprised only 17.93% of the total nematode population. Thus, a low BI value was to have been expected.

The EI is signified by the weighted proportion of all cp-1 and cp-2 nematodes. The nematodes that fall into such categories are cp-1 bacterivores and cp-2 fungivores. A high value is an indication that a soil ecosystem is nutrient-enriched, with a low value signifying that an ecosystem is nutrient-

depleted (Baniyamuddin *et al.*, 2007). The EI value for the Fynbos soils was 51.5%, which indicated the presence of a significant number of the nematodes in the soil, and that the ecosystem involved was neither completely nutrient- enriched, nor was it completely depleted either, with the result that it could be regarded as being in balance. The nematodes concerned are primary decomposers within the soil food web, and the value is an indication of the response of these decomposers to the available resources. It was established, when looking at the number of fungivores and bacterivores reflected in Figure 3.2, that the total number of nematodes was significant.

The SI is founded on the relative contribution that is made by the weighted nematodes with cp values of 3-5, in relation to the cp 2-5 grouping (Ferris & Bongers, 2009). The SI is a measure of the number of trophic layers that are present in the soil food web and of the possibility of control by predators (Pattison *et al.*, 2008). The SI value for the Fynbos soil concerned was 29.6%, which was regarded as low. In Figures 3.2 and 3.3, it was established that very few predatory nematodes were present in the Fynbos soil. Thus, Fynbos soil was found to have a low possibility for control by predators. The low SI values obtained also denote whether a soil ecosystem is disturbed or degraded, whereas high values denote matured or structured systems. Since the SI value for the Fynbos soil was low, conditions of disturbance and degradation were indicated.

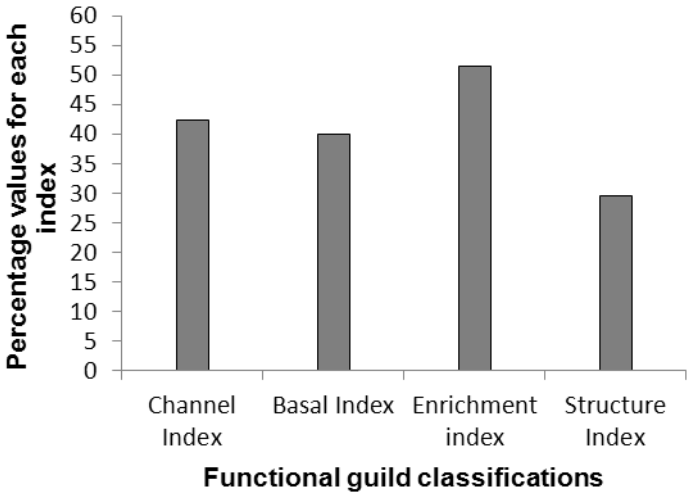

Fig. 3.10. A graphical representation of the Channel, Basal, Enrichment and Structure Index values for Fynbos soil.

Diversity, richness, and evenness

The diversity, richness and evenness of all the Fynbos samples were determined by calculating the diversity indices of the occurrence of the five nematode trophic groups (Fig. 3.11). The diversity indices, Shannon (H') and Simpson (D), indicated values of 1.5 and 0.3, respectively. The values concerned were low, indicating low nematode diversity. Pielou's J' or Evenness Index registered a value of 0.7, which is very close to 1. Such a finding indicates that, on average, there is an even distribution of abundances amongst families within the Fynbos soil. Hill's N_0 Index had a value of 8.0, which indicates that, on average, eight nematode families were present within one Fynbos sample. Hill's N_1 Index was equal to 4.6, and Hill's N_2 Index had a value of 3.8.

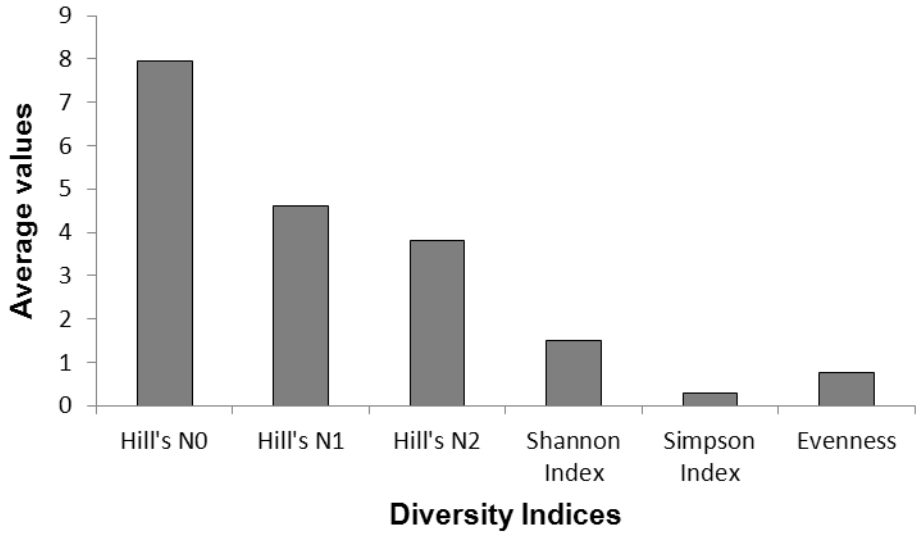

Fig. 3.11. Diversity indices indicative of nematode trophic group diversity, richness and evenness for Fynbos.

Conclusion

The Fynbos soils surveyed were found to be dominated by herbivorous and bacterivorous nematodes. These feeding types were followed by fungivores, omnivores and predators. Predatory and omnivorous nematodes were found to occur in extremely low numbers within the natural soils which was in accordance within other natural vegetation types. The presence of high numbers of bacterivores, however, differed from what was expected, and was attributed to climatic conditions in the Fynbos biome, where the summers are long, hot and dry. The most abundant bacterivores were found to be Cephalobidae, followed by Rhabditidae. Bacterivores have a high fecundity, which could

explain why, even during unfavourable conditions, the population remained high. Due to the winters being short and wet, persisters with long life cycles did not have the opportunity to establish themselves within the Fynbos soils.

Tylenchidae were the nematode family that was found to be the most abundant within the Fynbos soils. Such nematodes are classified as epidermal-cell and root-hair feeders, besides also being known as plant-associated nematodes. The reason for such nematodes being found in such high numbers within the Fynbos soils could have been due to the structure of the Fynbos root systems, which often consist of fine and hairy top roots.

Within fungivores, Paraphelenchidae and Aphelenchidae were the nematode families with the highest population values, followed by Neotylenchidae and Aphelenchoididae. The presence of such nematodes indicated the high levels of fungi that were found to be present within the rhizosphere of Fynbos families, with said fungi acting as an adequate source of food for the nematodes concerned.

Dorylaimidae were the only omnivorous nematode family and Mononchidae the only predaceous nematode family that were present within the Fynbos soils surveyed. The reason for the low numbers of these nematodes could be due to the unfavourable conditions existing during the summer months, which are not conducive to high nematode populations of nematodes with cp-4-5 values, as was the case with the families concerned.

Fynbos families are attractive to certain nematode families. Aphelenchidae, Paraphelenchidae and Neotylenchidae are all fungivores that are associated with different Fynbos families. Their association could indicate that the fungi populations that are present within the rhizosphere of the Fynbos families appeal to specific nematode families. The carnivorous nematodes were not associated with plant families, which indicated that the nematodes were more influenced by the nematode populations than by the plant families concerned. The carnivores were also not significantly associated with the bacterivores, thus an increase in the latter population would not lead to an increase in the population of carnivores. The above could also indicate that the dominant bacterivores were not adequate food sources for the predatory nematode family present within the Fynbos soils.

No associations were found between the nematode feeding types and the soil analysis variables, which could indicate that the nematode feeding-type composition was more strongly

influenced by the Fynbos families present within the soil, than by the soil's physical and chemical properties. Nematodes seem to be more strongly influenced by such biological factors as temperature, moisture, and bacterial and fungal populations present within the soil.

Pratylenchidae were strongly associated with sites that had a high percentage of sand, whereas Longidoridae and Trichodoridae were also associated with sandy soils. The bacterivorous nematodes, Monhysteridae, Diplogasteridae and Rhabditidae, were found to exhibit a low association with the sand and silt percentage of soil, as well as with the phosphorous within the soil. Future studies could examine the effect of phosphorous on local nematode populations.

The MI levels were low for natural vegetation, which could have been due to the lack of high numbers of such higher feeding group nematodes as carnivores and omnivores. The soils were dominated by colonising nematodes, and not by persisters, which resulted in the low MI levels. The MI values obtained indicated nutrient enrichment and disturbance. The values were strongly influenced by such factors as climate and soil moisture. A low PPI value was recorded, which was due to the low levels of truly plant-parasitic nematode present within the soils.

The faunal analysis for Fynbos soils indicated that Fynbos soil structure and enrichment are very variable and site-specific, as they differed widely for each sample taken. The finding made in this regard confirmed the fact that more samples per location and per area needed to be collected to establish adequate average values for an area, which could then act as a base value, for comparison with agriculturally managed soils. The average EI for Fynbos soils did not indicate overly enriched conditions, or conditions of severe disturbance. Conversely, the SI did indicate conditions of disturbance. The CI value was low, which indicated that decomposition within the soils concerned was dominated by bacterial decomposition pathways. This was in agreement with the nematode population values, since the bacterivores dominated the fungivores.

The data obtained during the current study showed the structure and function of the nematode community within Fynbos soils. Great scientific importance could be attached to doing area-wide sampling and data collection in a future study in order to establish base values for specific areas. The current study, however, forms a good base value for the comparison of soil nematode communities in Fynbos and cultivated agricultural land soils, such as in the deciduous fruit orchards in the Western Cape.

For future studies, it would be more important to sample adjacent natural Fynbos soils from a specific area to compare with soils to which a specific agricultural activity has been applied. As the above results showed, different areas differ greatly in nematode diversity and functionality, because climatic conditions, soil conditions and dominant plant families and species can greatly affect the nematodes that are present in soil.

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Chapter 4

The structure and function of nematode soil communities in organic and conventional deciduous orchards

Abstract

A study was done to determine the biodiversity and functionality of soil nematodes associated with deciduous fruit orchards with different management practices. The orchards studied were neighbouring organic and conventional apricot orchards, and a an organic apple orchard. For each orchard, all nematodes were quantified and identified to family level. The five nematode-classified trophic groups were found in each sample, while 14 families were identified in each orchard, respectively. Herbivores were dominant in all orchards. Organic apples had the lowest numbers of herbivores and fungivores, with the highest number of carnivores. When comparing organic and conventional apricot orchards, higher numbers of plant-parasitic nematodes were found within the organic orchard soil. Higher numbers of Criconematidae occurred within the conventional apple orchard. When comparing organic apricots and apples, higher numbers Criconematidae were found within the organic apple orchard, but more plant-parasitic nematodes, overall, were found within the organic apricot orchards. The Maturity Index (MI) indicated that all orchard soils had values below 1.5, and were, thus, rated as disturbed. The conventionally managed apricot orchard had the highest MI value of 1.48. The Plant Parasitic Index (PPI) value was highest in the organically managed apricot orchard. In order to determine the existing enrichment, structural and basal conditions, the nematode faunal analysis was applied to each site. All the orchard soils were located within Quadrat B, indicating enrichment and structure. With regard to the diversity, richness and evenness of the distribution, conventional apricot soil had the highest species richness, while organic apples had the most even distribution of families. Different management practices in fruit orchards did not show marked differences in community composition and structure.

Introduction

Global increases in human population are creating a need for ever-greater amounts of food to be produced. Bad soil management practices have degraded soil to such an extent that if management practices are not rapidly improved, the demand for food will not be met in future. The issues have augmented the need for an intensified awareness of soil ecology and of the significance of attaining and maintaining soil health within agro-ecosystems. Concerns regarding the permanence and quality of the world's natural resources are mounting.

Nematodes are among the most varied soil organisms, typically being the most profuse soil metazoans, and generally the most vital secondary consumers within the soil mesofauna (Sánchez-Moreno *et al*., 2008). They are now seen as an integral part of soil systems (Neher, 2010), as a wide range of trophic groups within the soil food web is encompassed by nematodes. They possess a central role within the soil food web, and can have either a direct or an indirect effect on ecological processes, therefore performing an essential function within an ecosystem.

The knowledge of free-living or beneficial nematodes is significantly less than that of plant-parasitic nematodes and nematodes within agricultural ecosystems (Neher, 2010). Nematodes might well provide an indication of the overall density, maturity or stability of ecosystems, owing to the diversity of families of free-living nematodes and plant-parasitic nematodes, in addition to their disparity in terms of sensitivity to environmental disturbance (Neher & Campbell, 1996). Diversity indices (DI) or the Maturity Index (MI) can be used as measures for enumerating the ecological attributes of soil nematode communities, which give an indication of comparative soil biological or ecological health (Neher & Campbell, 1996). Nematode community structure and composition can also be described in terms of indices of evenness, diversity, similarity, and ecological succession (Neher, 2010).

Sánchez-Moreno *et al*. (2008) reported that organic farming often increases the diversity and/or abundance of crops, soil organisms, insects, plants, and types of labour, and reduces fertiliser requirements and energy inputs. The biological activity in organically farmed soils is increased by the large quantity of organic matter and soil amendments that are typically added to the soil (Sánchez-Moreno *et al*., 2008).

Conventional farming relies more heavily on the addition of artificial fertilizers to the soil, in addition to applying chemical soil drenches to prevent or minimise the effects of pest insects. Currently, there is resistance toward conventional farming practices, which, it is believed, diminish levels of biodiversity within soil. Management practices, in agricultural soils, focusing on eliminating or reducing cultivation (including the utilisation of general biocides and heavy machinery) would more narrowly resemble a natural ecosystem (Neher, 2010). Many studies have been done on annual crops, whereas very few have been done on such perennial crops as fruit trees.

The aim of the current study was to establish the soil nematode community structure and function of organically and conventionally managed deciduous fruit orchards. The establishment was done by determining the abundance, the diversity and the functionality of the naturally occurring free-living and plant-parasitic nematodes in deciduous fruit orchards within the Western Cape province. Ultimately, the objective of the study was to form the basis of using nematodes as future indicators of soil health within fruit orchard soils.

Materials and methods

Sampling sites

The orchards sampled were located in different areas in the Western Cape province of South Africa. Samples were taken during the spring of 2011 at three locations, including Spaarkloof (organically managed), Noree (conventionally managed), which are both in the Robertson area, and Tandfontein (organically managed), which is on the outskirts of Ceres.

Spaarkloof (S33°43’51”/E19°47’29”) and Noree (S33°44’52”/E19°47’26”) are neighbouring farms. The apricot orchard on Spaarkloof has been under cultivation for the past eight years, whereas the apricot orchard on Noree has been cultivated for the past 17 years. The annual rainfall and irrigation, by way of micro-sprinklers, were the same for both farms. No mulch layers were applied in either of the orchards. The orchard on Noree is planted with Bulida apricot variety, grafted on Marianna rootstock. The rootstock for the Spaarkloof orchard is unknown, but the apricot varieties Sungold and Imperial are produced on said farm. The spacing for both orchards was 4 m between the rows, and 1 m between the trees.

Tandfontein (S32°45'59"/E19°13'44"), has been organically managed since 2001, and has an annual rainfall of 650 mm. The orchard contained Royal Gala apple grafted on M793 rootstock, and was irrigated by means of a micro-sprinkler system. The spacing between the rows was 4 m, and that between the trees in the rows was 1 m.

Soil samples were taken according to the technique described in Chapter 2. Approximately 1 kg of soil was collected at each site, and transported back to the laboratory in a cooler box, where it was stored at 14°C until used.

Nematode extraction and identification

Nematodes were extracted from 250 mℓ of soil by means of the Cobb's decanting and sieving method, as well as the modified Baermann funnel technique, over a period of 48 hours (Cobb, 1918). Samples were stored at 14°C in horizontally placed culture flasks until used. By allowing the nematode suspension to settle to the bottom and siphoning off excess water to a level of 20 mℓ, a representative suspension were extracted for counting and identification. After counting, the rest of the nematodes within the suspension were heat- fixed in double FAA at 85°C (Chapter 2).

Identified nematodes were allocated to five different feeding groups, according to Yeates *et al*. (1993). Said feeding groups included herbivores (plant-feeding nematodes), fungivores (hyphal- or fungal-feeding nematodes), bacterivores (bacterial-feeding nematodes), omnivores, and carnivores (predatory nematodes).

Coloniser-persister (cp-values) values were assigned to each identified family (Bongers, 1990; Bongers, 1999; Bongers & Bongers, 1998; Ferris & Bongers, 2009) (Chapter 2).

Nematode maturity indices

Communities were characterised by using various maturity indices, such as the MI, the Plant Parasitic Index (PPI), and the MI2-5, ∑MI and ∑MI2-5. The nematode families were classified in terms of the cp-scale, according to Bongers (1990), Bongers (1999), Bongers & Bongers (1998), and Ferris and Bongers (2009).

Nematode diversity indices

Nematode diversity was determined by means of the Shannon and the Simpson DI. Biodiversity was measured by means of Pielou's evenness (J'). Species richness was calculated in terms of the Hill's N_0 index. Other indices calculated for each site were Hill's N_1 and Hill's N_2 (Ferris & Bongers, 2009) (Chapter 2).

Nematode basal, structure and enrichment conditions

The basal, structure and enrichment conditions of the soil food web within each sample were determined by means of the weighted faunal analysis concept (Pattison *et al*., 2008), excluding the plant-feeding nematodes. The conditions were determined for each site by means of assessing the following indices: the Enrichment Index (EI); the Basal Index (BI); the Structure Index (SI); and the Channel Index (CI) (Chapter 2).

Soil analysis

A representative soil sample (500 g) from each site was sent to BEMLAB, a SANSAS-accredited testing laboratory, for the analysis of the physical and chemical soil components. The parameters which were measured for each site included: a mechanical-3-fraction analysis to determine the soil classification, as well as the percentage sand, clay and silt within each soil; the pH (KCl); the exchangeable cations (Na, K, Ca, and Mg); the base saturation (Na%, K%, Ca% and Mg%, with the T-value in cmol/kg); the total carbon (determined by means of the Walkley-Black method) and the inorganic nitrogen (NO3-N and NH4-N, quantified in mg/kg).

Data analysis

The descriptive analysis of data was done using Microsoft Excel 2010. Multivariate analyses were performed, using correspondence analysis (CA) techniques in XLSTAT 2010.

Results and discussion

Population and trophic group dynamics of nematode communities

A total of 14 nematode families were identified in each of the three orchards, in comparison to 19 in the natural vegetation (Chapter 3). In all soil types, herbivores were the most dominant, with seven families in natural vegetation (Table 1).

The different families were allocated to feeding types, according to Yeates *et al*. (1993). All five trophic groups were represented in each of the three orchard soils. With regard to the number of nematodes in each trophic group, the main difference was found in organic apple soil. The orchard concerned had lower numbers of herbivores and fungivores, compared to the other orchards and natural vegetation (Fig. 4.1).

Table 4.1. Number and percentage of nematode families found in the different orchard types.

Trophic groups	**Organic apricot**	**Organic apple**	**Conventional apricot**	**Natural vegetation (Chapter 3)**
Total number of families	14	14	14	19
Herbivores	6 (57.00%)	5 (28.13%)	5 (38.86%)	7 (39.09%)
Fungivores	3 (26.84%)	1 (3.06%)	3 (28.04%)	4 (17.93%),
Bacterivores	3 (12.06%)	6 (43.61)%	4 (19.77%)	6 (36.66%),
Omnivores	1 (3.86%)	1 (15.76%)	1 (12.63%)	1 (6.09%)
Carnivores	1 (0.25%)	1 (9.44%)	1 (0.71%)	1 (0.23%)

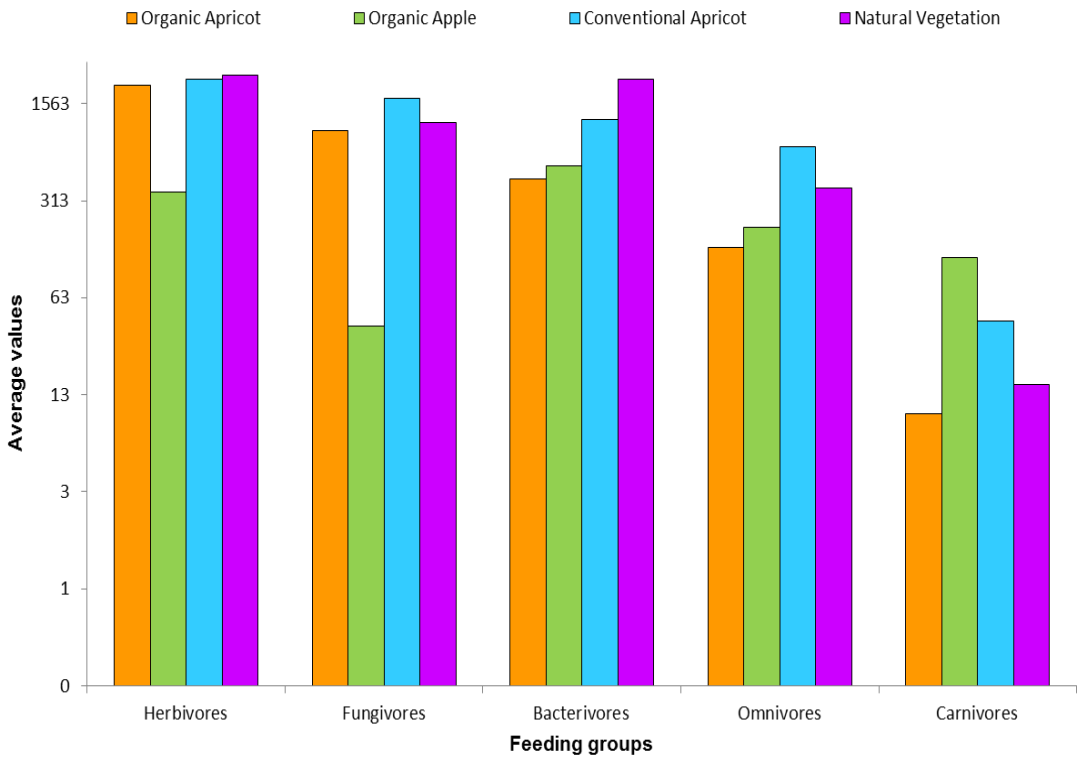

Fig. 4.1. Soil nematode trophic diversity comparison between an organic apricot, an organic apple orchard, a conventional apricot orchard, and natural vegetation.

The nematode families identified within the organic and conventional apricot orchards are indicated in Figure 4.2 below. More plant-parasitic nematodes were found within the organic orchard (6 families) compared to the conventional orchard (5 families).

Criconemoides xenoplax is the only species in the family Criconematidae remaining after soil has been disturbed in cultivation (Meyer, 1982; Nycziper & Halbrendt, 1993). Said nematode is currently regarded as one of the most serious pests of deciduous fruit in orchards in the Western Cape. It is interesting to note that the conventional orchard had higher numbers of Criconematidae than did the organic orchard.

The nematode families Paratylenchidae and Heteroderidae only occurred within the organic apricot orchard, whereas Trichodoridae only occurred within the conventional apricot orchard. The bacterivorous nematode family, Panagrolaimidae, only occurred within the conventional orchard, with also slightly higher numbers of omnivorous and carnivorous nematodes being found to be present than in the other soil.

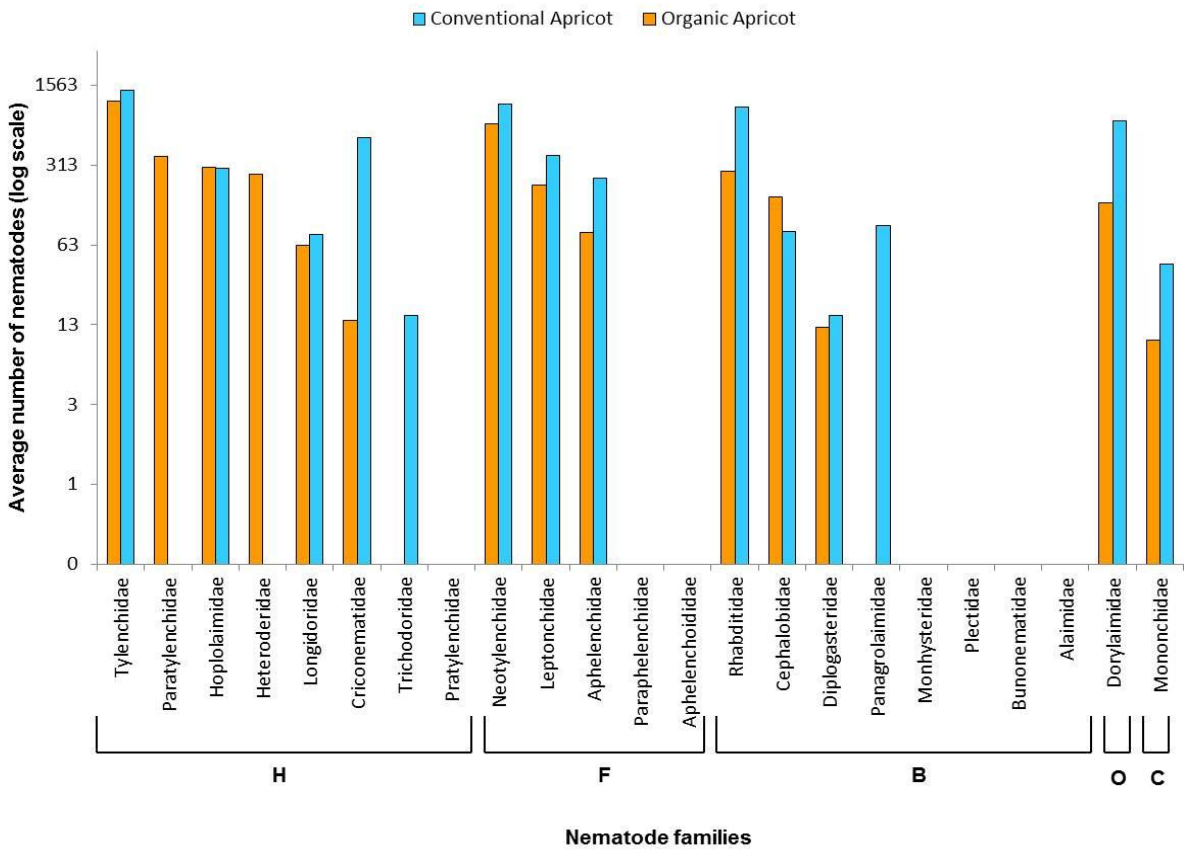

Fig. 4.2. A graphical representation of the different nematode families established in an organic apricot and a conventional apricot orchard (H: herbivores; F: fungivores; B: bacterivores; O: omnivores; C: carnivores).

The numbers of nematodes differed in the organic apricot orchard versus the numbers found in the organic apple orchard (Fig. 4.3). There were higher numbers of plant-parasitic nematodes in the organic apricot soil. The plant-parasitic nematode family Paratylenchidae occurred only in organic apricot soil, whereas the family Pratylenchidae only occured within the organic apple orchard. With regard to Pratylenchidae, the results supported current problems encountered with *Pratylenchus* spp. in apple nurseries and orchards (S. Storey, Pers. Communication).

The organic apricot orchard maintained higher numbers of fungivorous nematodes (3 families) compared to the organic apple orchard (1 family) (Fig. 4.3). The fungivore nematodes that were identified in the organic apricot orchard were also not present in the organic apple orchard, and vice versa. Higher numbers overall, as well as families (6) were found to subsist within the organic apple orchard, versus the organic apricot orchard (3). Carnivorous nematodes, the Mononchidae, were

found to occur in higher numbers within the organic apple orchard than within the organic apricot orchard.

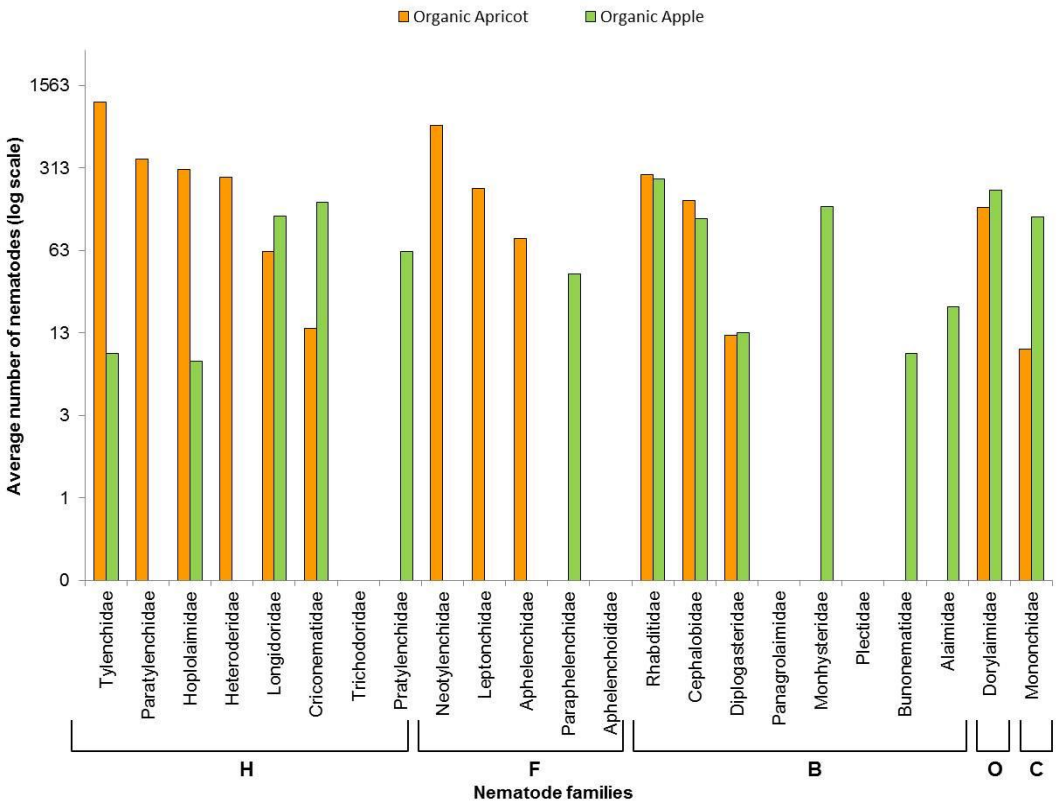

Fig. 4.3. A graphical representation of the different nematode families observed within an organic apricot and an organic apple orchard. (H: herbivores; F: fungivores; B: bacterivores; O: omnivores; C: carnivores).

Cultivation disturbs the soil nematode community structure and function, as well as do applications of chemicals, such as pesticides and fertilizers (Neher & Campbell, 1994; Neher, 1995; Neher, 2001a). Such practices are usually the norm within a conventionally managed orchard. For the above reason, the nematode community structure present within a conventional apricot orchard was compared to that within natural vegetation (Fig. 4.4). More families were identified in the natural soil, which showed the greater diversity present in that soil than in the conventional soil. High numbers of plant-parasitic nematodes occurred in both, but the composition was slightly different, as can be seen in Figure 4.4 below. Natural vegetation indicated the presence of seven different plant-parasitic nematode families, whereas there were only five in the conventional apricot orchard. The nematode families Heteroderidae and Pratylenchidae and the fungivorous nematodes Paraphelenchidae and

Aphelenchidae only occurred in the natural vegetation soil, while Leptonchidae only occurred in the conventional apricot orchard soil.

Natural vegetation had a higher number of bacterivorous nematodes (6) compared to conventional soil (4) (Table 1). Cephalobidae occurred in higher numbers within the natural soil than in conventional soil, while the families Monhysteridae and Plectidae only appeared in the natural vegetation soil.

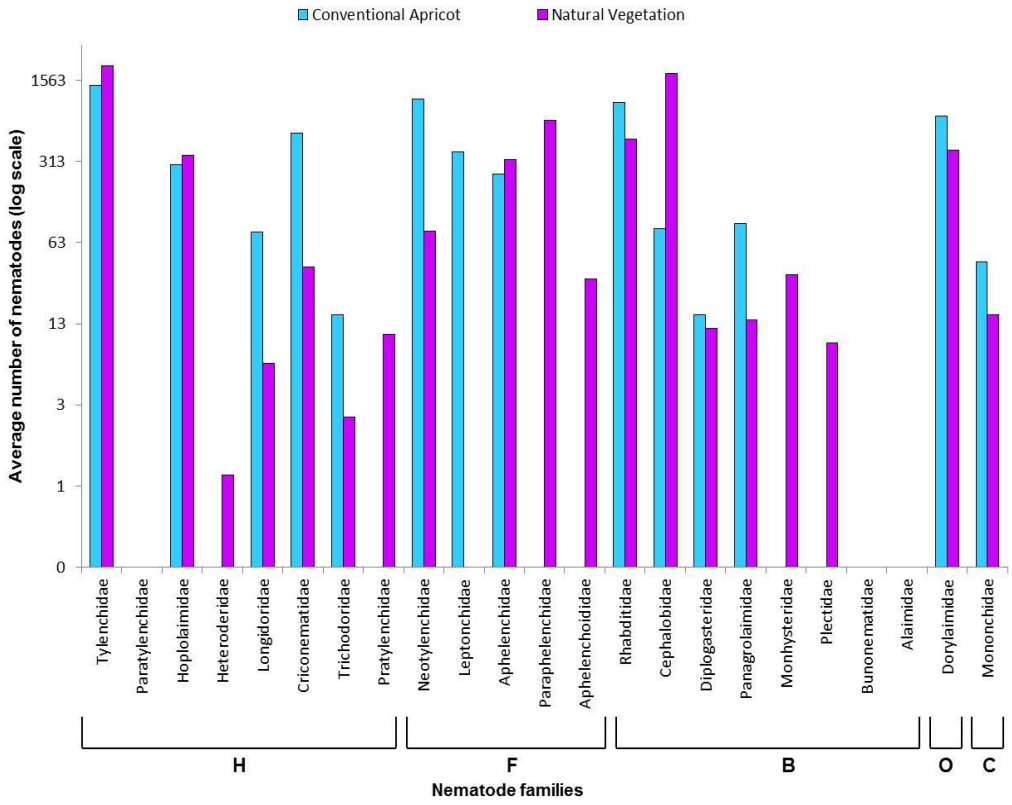

Fig. 4.4. Log number of nematodes in different families perceived within a conventional apricot orchard and natural vegetation (H: herbivores; F: fungivores; B: bacterivores; O: omnivores; C: carnivores).

In order to establish whether specific nematode families were associated with management practices or natural vegetation, a CA was performed, which is graphically presented in Figure 4.5 below. The results showed the organic apple orchard to differ from the other sites, whereas the conventional and organic apricot orchard seemed to be related to each other. Differences in grouping between the nematode families distinguished those that were found in the natural vegetation soil from the rest.

The natural vegetation soil was strongly associated with the nematode families Paraphelenchidae, Plectidae, Cephalobidae and Aphelenchoididae. An association also existed with Aphelenchidae, Tylenchidae and Hoplolaimidae. In the case of the conventionally managed apricot sites, there was a strong association with the following nematode families: Panagrolaimidae, Trichodoridae, Leptonchidae and Neotylenchidae. Such sites were also associated with Rhabditidae, Dorylaimidae, Diplogasteridae and Criconematidae. There were strong associations between the organic apricot sites and the nematode families Heteroderidae and Paratylenchidae. It was notable that the nematode families that had a strong association with the conventional or organic apricot sites were also associated with either the organic or the conventional apricot sites as well. The nematode families Aphelenchidae, Hoplolaimidae and Tylenchidae, which were associated with natural vegetation, might also be found in conventional and organic apricot sites.

Organic apple sites seem to be strongly associated with the nematode families Alaimidae, Bunonematidae, Pratylenchidae and Monhysteridae. They were also associated with Mononchidae. The nematode family, Longidoridae, seems to be more associated with the organic apple site than with the conventional apricot site. The results were comparable to those of Hugo and Meyer (1995), who found *Xiphinema* spp. able to reach damaging levels in apple orchards.

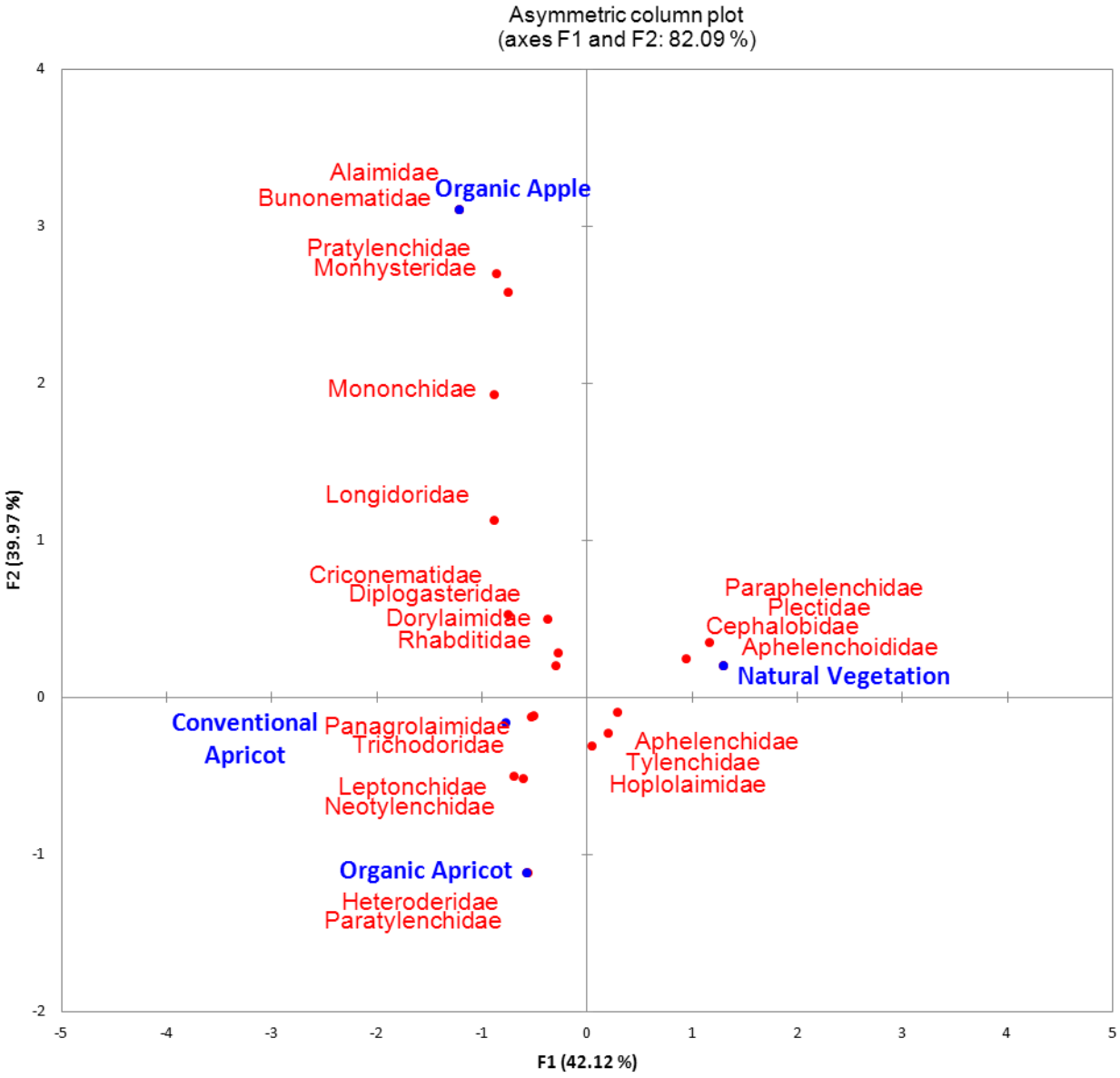

Fig. 4.5. Correspondence analysis of management practices and natural vegetation associations with nematode families.

Maturity indices

The five maturity indices (MI, MI2-5, PPI, ∑MI, ∑MI2-5), as well as the ratio of PPI/MI, were calculated for each of the sites (Fig. 4.6). According to Neher (2001b), the MI was found to be the most valuable tool for determining nematode communities as the ecological indicators of agro-ecosystems.

The MI (excluding the plant-parasitic nematodes) for the orchards and the natural vegetation was found to be below 1.5. In comparison, the natural vegetation had a value of 1.26. The MI for the

organic apricot orchard sites was found to be 0.98, which was low and indicated more seriously disturbed conditions. The MI for the conventional sites was found to be 1.48, whereas the MI for the organic apple orchard was 1.01. These values indicated conditions of disturbance, which were found to be more prominent in the organic sites. Of the four different values, the conventional sites had the highest MI value. Such a finding implied that the conventional orchards, though still disturbed, were less disturbed than were the other sites.

The MI2-5 value was found to be 0.90 in organic apricot sites, 1.27 in conventional sites, 1.18 in natural vegetation, and 0.84 in organic apple sites. The MI2-5 was the same as the MI, but excluded all the cp-1 nematode groups. Said index is of use in the presence of decomposing material, since it excludes the enrichment opportunists (Ferris & Bongers, 2009). The MI2-5 was slightly lower than the MI in the organic and conventional apricot sites, as well as in the organic apple site, but it was higher than was the MI in the natural vegetation. The above could indicate that the bacterivores that were present in the natural soil were secondary bacterivores, and not enrichment opportunists, which could be the case in the other sites, since higher numbers of, for example Rhabditidae, were recorded in said sites.

The organic apple site had the lowest values for each MI used. The site had been under organic production for more than ten years, and thus, according to Neher (2001a), should be closely related to the natural vegetation site. In this case, the conventional apricot site was more closely related to the natural vegetation site than it was to the organically managed sites. Neher (1999) states that agricultural soils would resemble soils within natural ecosystems more strongly, if management practices that were implemented within the soils were to diminish or eliminate cultivation, the use of heavy machinery and the general usage of biocides.

The PPI was lower in the organically managed apple site than in the organically managed apricot site and in the natural vegetation. Neher (2001a) established that the PPI was greater in organically than it was in conventionally managed systems. Comparable results were found in this study when comparing the organic and conventional apricot sites.

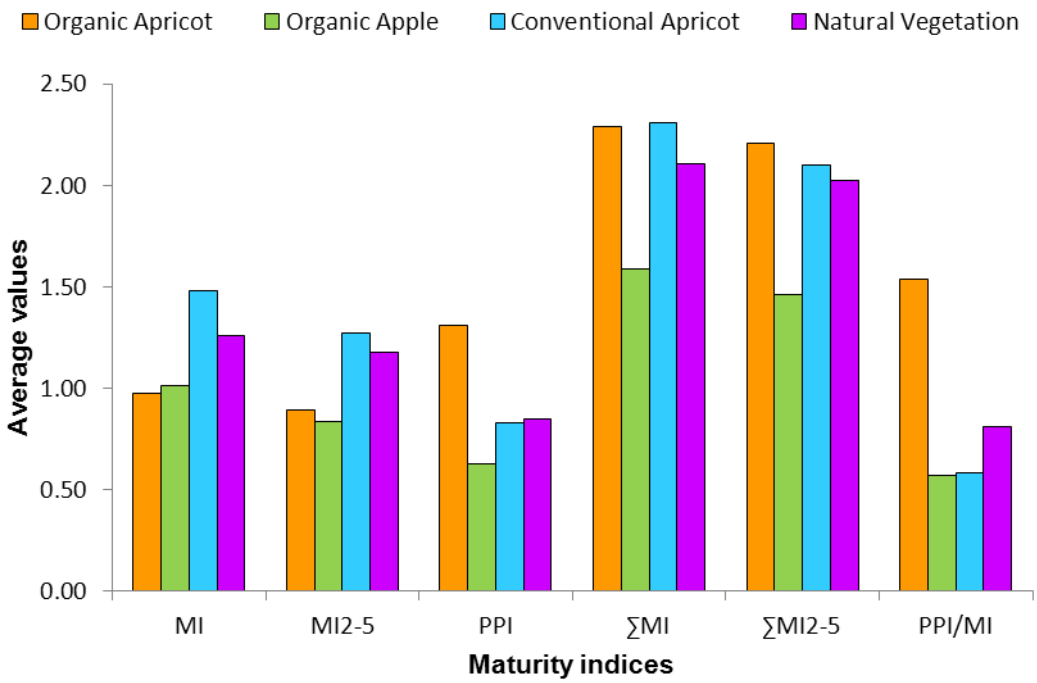

Fig. 4.6. Maturity indices for organic apricot and apple orchards, conventional apricot orchard and natural vegetation.

Basal, structure and enrichment conditions

Indices to determine ecosystem function were calculated for each of the sites. The nematode faunal analysis (Ferris *et al*., 2001) was applied to each site (Fig. 4.7). The data points for the organic apple and for the conventional apricot sites were very close together, with both being located within Quadrat B, which was indicative of the conditions within the soil that was enriched and structured. The data point for the organic apricot site was also found to be within said quadrat, but closer towards the enriched side. The soils mentioned also indicated conditions that were maturing. Natural vegetation was located within Quadrat A, which indicated that the natural soils, although enriched, were structured and disturbed.

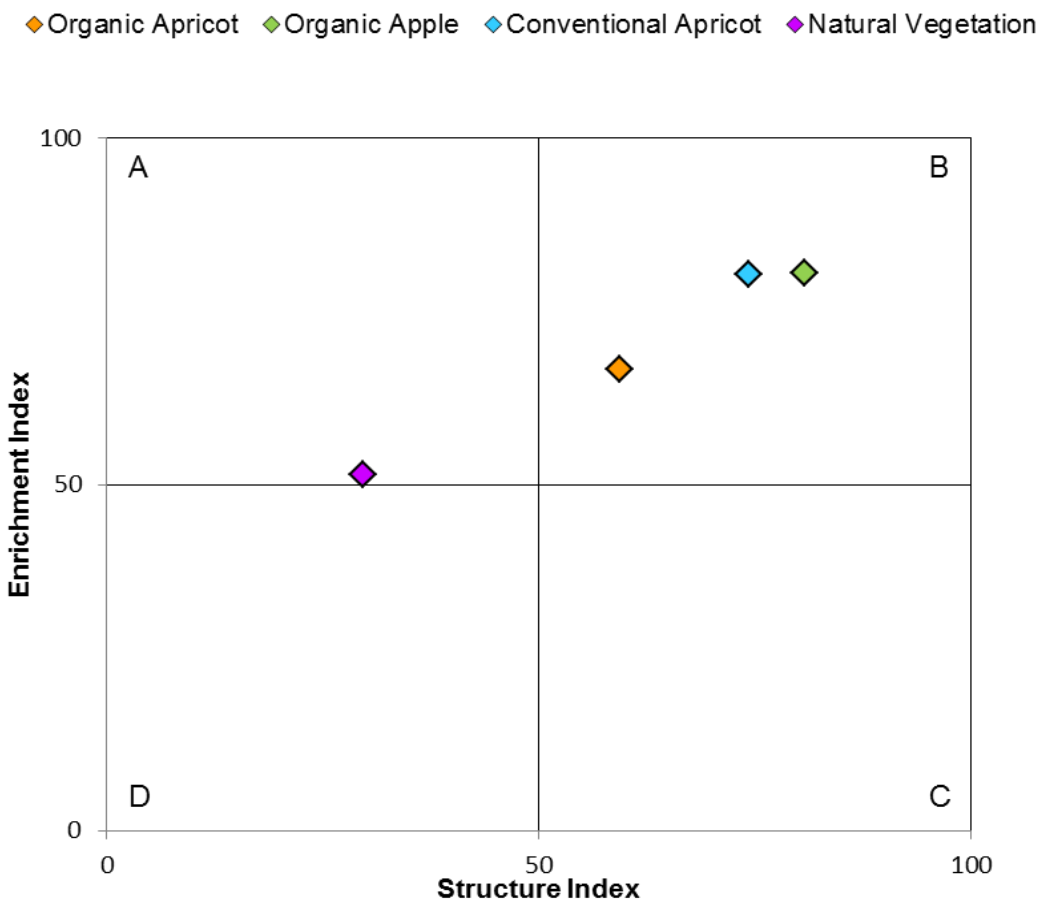

Fig. 4.7. The nematode faunal analysis for an organic apple and an organic apricot site, a conventional apricot site, and a natural vegetation site.

The CI, BI, EI and SI values are graphically represented in Figure 4.8 below. The CI is a method that indicates the decomposition channel of nutrients within a system (Pattison *et al*., 2008). It separates the flow of resources through bacterial or fungal decomposition channels. A low value is indicative of a bacteria-dominated decomposer community, whereas high values denote a nematode community that is dominated by fungal decomposers (Ferris *et al*., 2001; Hohberg, 2003; Pattison *et al*., 2008). The CI for the organic apricot orchard site was a low 40.70%, which indicated that, within the system, decomposition was dominated by bacterial decomposer nematode communities. The CI value for the organic apple site was 3.10%, whereas the conventional apricot site had a value of 23.37%. The natural vegetation had the highest value, with a CI of 42.40%. All the sites indicated a bacterially dominated decomposer community. The organic apricots most closely resembled natural vegetation.

The BI was indicative of the relative quantity of the basal or cp-2 component of the nematode fauna present within the soil ecosystem. The natural vegetation site had the highest BI value of 39.95%, followed by 22.11% for the organic apricot site, 12.06% for the conventional apricot site, and 10.67% for the organic apple site. The latter site had the lowest number of fungivores (the cp-2 component) of all the sites (Fig. 4.2), with only one family being present. Conversely, the natural vegetation had the largest proportion of the cp-2 component.

The EI registered the implied weight of the proportion of cp-1 and cp-2 nematodes, consisting of the bacterivores and fungivores. The EI for the organic apple site was highest, with a value of 80.57%, followed closely by EI of the conventional apricot site, with a value of 80.38%. The organic apricot site had the next highest value (66.59%), followed by the natural vegetation site's EI of 51.49%. High values (>50%) indicate that a soil ecosystem is nutrient-enriched, whereas low values indicate nutrient depletion. All of the values were above 50%, indicating that all the systems were nutrient-enriched. The natural vegetation was borderline, but conditions at the time of sampling inclined towards the nutrient-enriched category.

The SI registered the relative contribution of the weighted nematodes, with cp-values of 3-5 for the grouping 2-5 (Ferris & Bongers, 2009). The values achieved also indicate the possibility of control by predators within the soil food web (Pattison *et al*., 2008). The natural vegetation had the lowest SI value, with 29.64%, whereas the organic apple site had the highest value of 80.64%. The conventional apricot site had a value of 74.32%, whereas the organic apricot site had a value of 59.31%. SI values lower than 50% denote whether a soil ecosystem is disturbed or degraded, which was the case with the natural vegetation site in the current study. High SI values indicate matured or structured systems, with all agriculturally managed soils having values above 50%. The organic apple site, according to the SI, was the most matured and structured system,

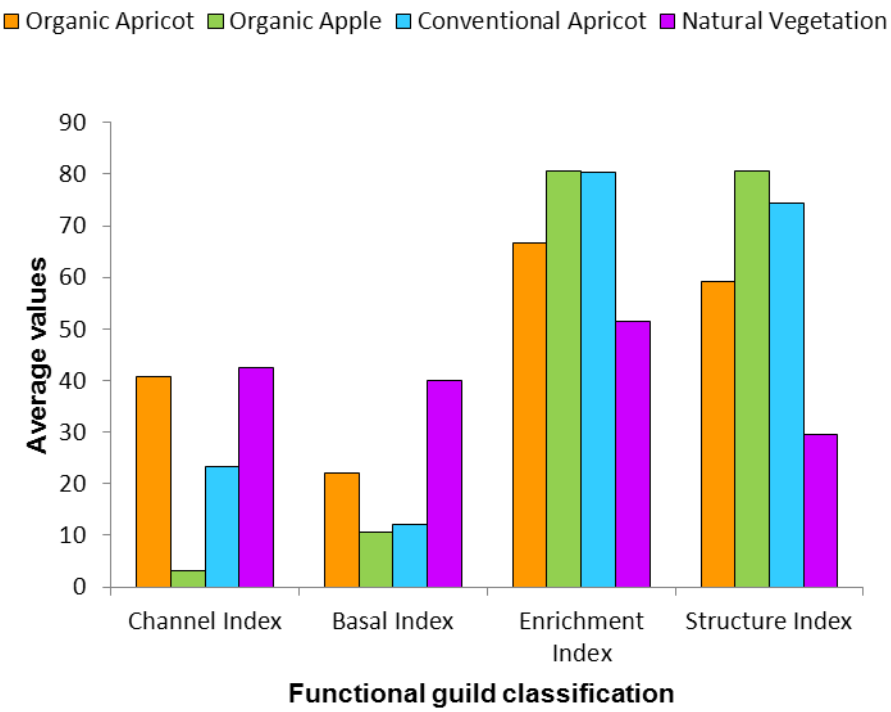

Fig. 4.8. A graphical representation of the Channel, Basal, Enrichment and Structure Index values for an organic apricot and an organic apple site, a conventional apricot site and a natural vegetation site.

Diversity, richness and evenness

The diversity, richness and evenness calculated for each site are represented graphically in Figure 4.9 below. The diversity (Simpson Index and Shannon Index) within all the sites was hardly distinguishable from one another. The natural vegetation site had the highest value for the Simpson DI, which weights common families.

The species richness, or Hill's N_0, index indicates that the conventional apricot site had the highest species richness, followed by the organic apricot, organic apple, and natural vegetation sites.

Pielou's J, or evenness, values, which were close to 1, indicated that the distribution of abundances amongst the families at the different sites was even, whereas the values close to zero indicated an uneven distribution. The organic apple site had a value close to 1 of 0.98, whereas the values for the organic and conventional apricot sites were equal, with a value of 0.84. The natural vegetation site had a J-value of 0.75. Out of the four different sites, the natural vegetation site was found to be the least evenly distributed.

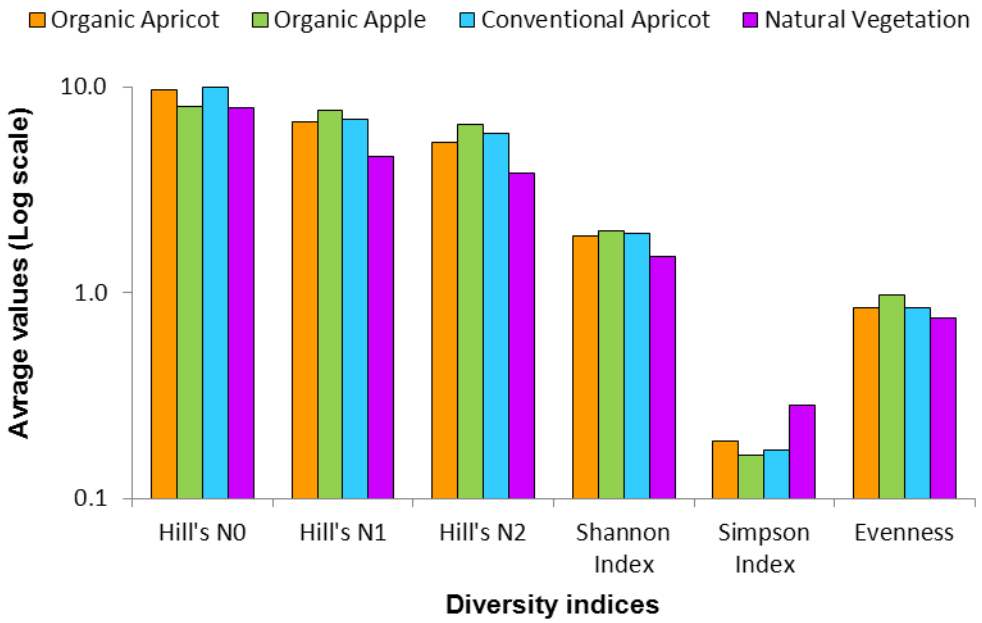

Fig. 4.9. Diversity indices indicative of nematode trophic group diversity, richness and evenness, for each site.

Soil analysis

The soil samples from the three orchards were analysed, with the results obtained being summarised in Table 4.2 below. The soil classification for each orchard was found to be different. The organic apricot orchard was classified as loamy sand soil, whereas the conventional apricot soil was classified as sandy loam, and the organic apple orchard soil as sand. The pH varied from 7 in the organic apricot orchard to 6.2 in the organic apple orchard.

Table 4.2. Soil analysis results for an organic apricot, a conventional apricot and an organic apple orchard.

		Organic apricot	Conventional apricot	Organic apple
Depth (cm)		20	20	20
Soil		Loam	Clay	Sand
pH (KCl)		7.0	6.7	6.2
Resistance (Ohm)		460	350	440
H^{+} (cmol/kg)				
Stone (Vol %)		34	41	34
P Bray IImg/kg		8	103	510
Potassium (K) mg/kg	K	157	117	222
Exchangeable Cations	Na	0.26	0.59	0.17

		Organic apricot	Conventional apricot	Organic apple
(cmol(+)/kg)	K	0.4	0.3	0.57
	Ca	14.2	8.81	23.78
	Mg	1.69	2.79	3.22
Carbon	C%	1.3	1.45	7.06
Chlorine (mg/kg)	Cl (mg/kg)	19.77	105.93	28.25
Nitrogen	N%	0.149	0.164	0.428
Inorganic Nitrogen (mg/kg)	NO3-N (mg/kg)	108	49.82	188
	NH4-N (mg/kg)	5.53	8.15	18.82
Cation Exchange Capacity cmol(+)/kg	CEC (pH7) cmol(+)/kg	2.83	2.51	8.09
Base Saturation	Na %	1.58	4.75	0.62
	K%	2.43	2.4	2.05
	Ca%	85.76	70.51	85.74
	Mg%	10.23	22.34	11.59
	T-Value (cmol/kg)	16.56	12.5	27.74
Mechanical 3-fraction	Clay %	8	14	2
	Silt %	10	22	2
	Sand %	82	64	96
	Classification	LmSa	SaLm	Sa

Conclusion

The orchards that were used in the current study were chosen because of their management status and availability. Fruit orchards that had been organically farmed for eight years or longer were difficult to find, and played an important role in the site selection. The shortage of farms under organic production was an unforeseen hurdle in the research. It is also important to note that limited research has been done regarding perennial crops, which makes the interpretation of the faunal analysis and diversity difficult, in the light of the comparison of annuals and perennials.

The current study does not emphatically prove that organic orchard farming practices are better than are conventional practices, or vice versa, using nematodes as bioindicators. Within this study, the two apricot orchards on the neighbouring farms, Spaarkloof (organic) and Noree (conventional),

were compared. It was expected that the organically managed apricot orchard would have better results regarding the nematode faunal analysis profile and the species richness index, yet in this study the conventionally managed farm showed better results than did the farm under organic production. Tandfontein also had a high species richness index and a desired nematode faunal analysis profile.

The organic apple orchard had the lowest number of herbivores, compared to the other three orchards. This could be due to the natural suppression of pathogenic nematodes and fungi within the root zone of the apple, due to the organic management practices applied within said orchard. It could also be attributed to the fact that nematodes within the feeding groups might not utilise apple trees as a suitable host as much as they do apricot trees. The type of management in the organic apple orchard should be investigated.

Compared to the other soils, the organic apricot orchard had the lowest number of persisters (omnivores and predators). Cultural management practices, such as intensive tillage within rows, the removal of weeds, or the application of organic fertilizers within the orchard, were seen as disturbances. Such practices could influence the nematode populations just as much as do conventional chemical sprays (consisting either of fertilizers or of chemical control agents). This could lead to a reduction in the persistence of such nematodes as predators and omnivores. The reason for the low number of persisters could have led to a major disturbance in the organic management practice, which should be further investigated.

Tylenchidae were found to be the most abundant nematode family within the organic and conventional apricot orchards. Such nematodes feed on root hairs. Certain nematode families within the herbivores were specific to the management practice. Paratylenchidae and Heteroderidae only occurred within the organic apricot orchard, whereas Trichodoridae only occurred within the conventional orchards. The above could have been due to the management practices or the propagation material used. The possibility also exists that the nematodes concerned could have been introduced to the orchard.

The same fungivorous and bacterivorous families, except for Panagrolaimidae (which were found in the conventional orchard), occurred within the apricot orchards. Both orchards also had the

same omnivorous and carnivorous families present. The above implies that the species composition for both orchards was very similar.

Differences were found between the number of nematodes present and the family composition within the organic apricot and the organic apple orchards. The organic apricot orchard had very high numbers of plant-parasitic nematodes in comparison to the numbers that were present in the organic apple orchard. Paratylenchidae only occurred within the organic apricot orchard, whereas Pratylenchidae only occurred within the organic apple orchard. Pratylenchidae is an important economic pest in apple orchards, despite not causing damage to apricot orchards (Storey, 2007). The organic apricot orchard had higher numbers of fungivorous nematodes present, belonging to three different families. The organic apple orchard only had Paraphelenchidae associated with the orchard, which could indicate a higher abundance of fungi in the apricot orchard than in the apple orchard.

The organic apple orchard had a higher abundance of bacterivorous nematode families (6) compared to the number of families that were present in the organic apricots. Three families, Monhysteridae, Bunonematidae and Alaimidae, only occurred within the organic apple orchard. The nematodes concerned fed on eukaryotic cells present in the soil. The presence of certain families in the apple orchard could have been because there were higher numbers of appropriate bacterial food sources available for them in said orchard. The climates might have varied between the two regions, which would have influenced the composition of the families involved.

Both orchards had Dorylaimidae (omnivores) and Mononchidae (carnivores) present in the soil. The higher number of Mononchidae present in the organic apple orchard could have been due to differences in the management of the orchard. The management practices implemented in the organic apple orchard might have been more conducive to the presence of high numbers of predatory nematodes.

The Tylenchidae was the nematode family with the highest numbers in the conventional orchard and in the natural vegetation. The family composition of plant-parasitic nematodes within the two soils was very similar. Heteroderidae and Pratylenchidae only occurred within the natural soil. Criconematidae occurred in very high numbers within the orchard environment, compared to how many there were in the natural soil environment. Said nematodes thrived within the monoculture orchard environment.

Neotylenchidae and Aphelenchidae were the only fungivorous nematode families to occur in both soils. Leptonchidae only occurred in the conventional apricot orchard, which could mean that it was introduced to the orchard, since it did not occur naturally within the natural vegetation environment. Paraphelenchidae and Aphelenchoididae only occurred in the natural soil, which could perhaps imply that the orchard environment did not favour the presence of such nematodes. More bacterivorous nematodes were found to occur in the natural vegetation environment (6), compared to the number that were found in the orchard environment (4). Monhysteridae and Plectidae only occurred within the natural vegetation, with Cephalobidae also occurring in high numbers in natural vegetation. The natural vegetation environment seemed to be more favourable for the fungivorous nematodes. The omnivores (Dorylaimidae) and carnivores (Mononchidae) occurred in similar numbers (Fig. 4.4).

The CA of the various orchards and natural vegetation sites indicated that the sites concerned clearly differed from one another. The organic and the conventional apricot orchards were related to each other, whereas the organic apple orchard and the natural vegetation sites differed from all the other sites. Certain nematode families (Paraphelenchidae, Plectidae, Cephalobidae and Aphelenchoididae) were clearly associated with natural vegetation, which could indicate that the nematodes concerned preferred filling that niche within the natural vegetation.

Nematode families that were associated with the conventional apricot site were also associated with the organic apricot site. This could indicate that the plant species influences nematode occurrence more than does the management practice. Organic apples also had very specific families associated with them.

The MI value was highest in the conventional apricot orchard, whereas it was low in both the organic orchards. The above indicates that the management practices within said orchards were more disruptive to the nematode communities present in the soil, and led to a disturbed environment. The PPI was lowest within the organic apple orchard, which might have indicated that the soil concerned was more suppressive towards such nematodes, that they did not relish the environmental conditions, or that they preferred the apricot more as a host plant.

The faunal analysis for the orchard nematode communities indicated that said soils were all enriched and structured, whereas the natural vegetation soil was enriched and unstructured. The

application of soil amendments and management practices could play a role in the improvement of the soil structure and enrichment. All the soils were dominated by a bacterial decomposition pathway, which was to have been expected, since bacterivorous nematodes were more abundant than were the fungivores.

The nematode family distribution within all the soils was even. The SI, which weights common families, had the highest value within the natural vegetation. Said fact indicates that natural vegetation had the highest number of common families within the soil, compared to the numbers that were present in the orchard environments. The common families were the families that occurred within all the samples.

The aim of the current study was to gain knowledge with regard to the nematode soil community structure and function of organically and conventionally managed deciduous fruit orchards. Future studies should be aimed at establishing the change in nematode community structure in an orchard over time, both before and after a specific management practice has been applied.

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Chapter 5

The structure and function of soil nematode communities after apple orchard floor manipulation

Abstract

Samples from the ridge of the tree row (between the trees) of eight different soil surface application treatments were collected from an apple orchard in the Grabouw area. The treatments were combined according to the soil applications received (chemical control of cover crops and weeds, mulch, and mulch + EM). A study was undertaken to determine the biodiversity and the functionality of soil nematodes associated with the soil surface applications. For each treatment, the nematodes present were identified to family level, in addition to being quantified. All five trophic groups were present within the soil of each of the soil surface applications. Twelve to thirteen families per soil surface application were identified. Bacterivores were dominant in every soil surface treatment, with the least number being present in the chemical control of weeds and cover crop. Chemical control (of weeds and cover crop) sites also had higher levels of fungivores present within the soil, compared to the other sites. High numbers of Rhabditidae occurred within the mulch and within the mulch + EM sites, whereas high numbers of Aphelenchidae occurred within the chemical control sites. The carnivorous family Ironidae occurred only in the mulch + EM sites. Strong associations were found to exist between the soil applications and the nematode families present within the soil. Chemical control of cover crops and weeds had the highest MI value, whereas values were equal for the other soil applications. The faunal analysis indicated that the mulch and chemical control fell within Quadrat A, and were enriched but unstructured, whereas the mulch + EM fell within Quadrat B, indicating enrichment, structure and good overall soil conditions. All the systems were dominated by bacterial decomposition pathways. The sites that received chemical control had the highest species richness of all three applications, as well as the highest level of diversity, according to the Simpson Index. Differences in nematode community structure and composition occurred between the different soil surface applications.

Introduction

The soil nematode community within a deciduous orchard environment can be utilised as a tool for assessing ecological hypotheses, as well as for interpreting biological processes in soil, owing to the fundamental role that is played by such a community in the soil food web, and its connection to ecological processes and mechanisms (Neher, 2010). By establishing links to ecosystem processes (Debruyn, 1997), the determination of the hierarchy of geographical scales (Neher *et al*., 1998), and the gauging of their value across ecosystem boundaries, the value of soil communities as ecological indicators is likely to increase (Neher *et al*., 2005).

Increasing concerns concerning the degradation of soil, water and air quality linked to existing crop production have supported the advancement of such alternative cropping systems as organic farming (Dupont *et al*., 2009). Concerns regarding the quality and permanence of natural resources are also intensifying (Neher, 2010). According to Willer and Yussefi (2001), more than 31 million hectares in 120 countries globally are under organic production. The management of nutrients is not only important in organic farming, but it is also important in conventional systems. The addition of organic matter to soil drives the dynamics within the soil food web, the availability of nitrogen in the soil, and plant growth (Ferris & Matute, 2003). The above provides yet another reason for farmers to know what effect organic amendments to the soil within their orchards really have, by means of using the change in nematode assemblages within the soil as a measurement tool. The addition of certain organic amendments, including green manure, composts and slurries, plays a critical role in soil quality (Reeves, 1997). The amendments have a direct positive effect on the fertility of soil, on the soil structure, and on the biology of the organisms concerned. Amendments also affect the activity of soilborne pathogens, including plant-parasitic nematodes (Thoden *et al*., 2011).

Producers are relying more on biological and cultural approaches to enhance the habitats and effectiveness of natural enemies within the orchard environments, as well as to enhance soil health. The approaches concerned include the addition of a soil mulch layer. The effect of a mulch layer on the nematode community structure within South African deciduous fruit orchards is not yet known.

Forge *et al*. (2003) studied the effects of organic mulches on the soil microfauna in the root zone of apple, focusing on the implications for nutrient fluxes and functional diversity of the soil food web. The mulches used consisted of different combinations of shredded office paper and municipal

biosolids, alfalfa hay and black polyethylene fabrics. The mulches were applied on the ridge tree rows, while the weeds between the tree rows were chemically controlled.

The aims of the current study were to establish the differences in soil nematode community structure and function between various soil surface and mulch applications within an apple fruit orchard.

Materials and Methods

Experimental sampling sites

The different treatments that are indicated in Table 5.1 below were used at Bellevue Experimental Farm (S30°0'491"/E19°01'16"), which is situated in Grabouw. Each treatment was replicated four times within a randomised block design.

The mulch, consisting of compost and straw, was approximately 50 mm thick and1.5 m wide in a tree row and supplemented each year during bud break. In Treatment 1, chemical weed control was applied in the tree row. Treatment 2 was the same as Treatment 1, except that cover crops (wheat and legumes, alternatively) were planted in the working rows during winter. Treatment 3 consisted of mulch within the tree row, and a cover crop in the working row. For Treatment 4, a full mulch layer was applied. Treatment 5 consisted of a mulch layer within the tree row, and a cover crop within the working row. A mulch layer was applied to the tree row of Treatment 6, onto which effective micro-organisms (EM) were then sprayed. For Treatment 7, a mulch layer was applied within the tree row. Treatment 8 also had a mulch layer within the tree row, which received spraying with effective EM.

Table 5.1. Different treatments applied to the orchard floor on the Bellevue organic farm.

Treatment number	Working row		Ridge tree row	Combined Treatment
	Growing season	Winter season		
1	Slash weeds when necessary	Slash weeds	Chemical control	Chemical control
2	Chemical control of cover crop in spring, during growing season	Cover crop[Ω]	Chemical control	
3	Chemical control of cover crop in spring, during growing season	Cover crop	Mulch[#]	Mulch
4	Mulch	Mulch	Mulch	
5	Flatten cover crop with roller in spring; slash weeds when necessary	Cover crop	Mulch	
7	Slash weeds when necessary	Slash weeds	Mulch	
6	Flatten cover crop with roller in spring; slash weeds when necessary	Cover crop	Mulch, spray EM*	Mulch + EM
8	Slash weeds when necessary	Slash weeds	Mulch, spray EM	

*****Effective micro-organisms
[#]Consisting of compost and straw
[Ω]Planting of wheat and legumes in alternate years as cover crops

The orchard was irrigated by means of micro-sprinklers. Approximately 1 kg of soil was collected from the tree ridge row of each treatment site (32 samples) at each sampling site, and transported back in a cooler box to the laboratory, where it was stored at 14°C, until used. Since nematode samples were collected only from the ridge of the tree row, treatments with similar applications were combined in order to provide a single value for analysis. The treatments were evaluated both individually, and in combination. Within an apple orchard, various soil amendments were applied, including chemical control of cover crops and weeds (chemical control); a compost and straw application (mulch); and a mulch application, together with the application of effective micro-organisms (mulch + EM). Thus, Treatments 1 and 2, in combination, formed the cover crop and the weed chemical control application, Treatments 3, 4, 5 and 7 were combined and formed the mulch application, with Treatments 6 and 8 forming the mulch + EM soil surface application. Only the combined treatments were reported on in the current study.

Nematode extraction and identification

The Cobb's decanting and sieving method, as well as the modified Baermann funnel technique, were utilised for the extraction of nematodes from 250 mℓ of soil over 48 hours (Cobb, 1918). After extraction, the samples were stored in horizontally placed culture flasks and left at 14°C until use. Nematode suspensions were allowed to settle, and excess water was siphoned off to a level of 20 mℓ. From the above, a representative suspension was extracted for purposes of counting and identification. The remainder of the suspension was heat fixed in a solution of double FAA at 85°C, in order to preserve the samples (Chapter 2).

Nematodes, after being identified by means of morphological techniques (Chapter 2), were then allocated to five different feeding groups, according to Yeates *et al*. (1993). To each identified family, coloniser-persister (cp-values) values were assigned (Bongers, 1990; Bongers & Bongers, 1998; Bongers, 1999; Ferris and Bongers, 2009) (Chapter 2).

Nematode maturity indices

Various maturity indices were utilised in order to characterise nematode communities. The indices consisted of the Maturity Index (MI), the Plant-Parasitic Index (PPI), and the MI2-5, ∑MI and ∑MI2-5. All nematode families were categorised on the cp-scale conforming to Bongers (1990), Bongers and Bongers (1998), Bongers (1999), and Ferris and Bongers (2009).

Nematode diversity indices

The biodiversity of nematode communities was measured by means of Pielou's evenness index (J'), while nematode diversity was established by means of the Shannon and Simpson diversity indices. The richness of the nematode families within the soil was determined by means of Hill's N_0 index. Supplementary indices, including Hill's N_1, and Hill's N_2, were calculated for each site (Ferris & Bongers, 2009) (Chapter 2).

Nematode basal, structure and enrichment conditions

Within each treatment, the basal, structure and enrichment conditions of the soil food web were determined by means of the weighted faunal analysis concept (Pattison *et al*., 2008), which excludes

plant-feeding or herbivorous nematodes. The indices used were: the Enrichment Index (EI); the Basal Index (BI); the Structure Index (SI); and the Channel Index (CI) (Chapter 2).

Soil analysis

From each treatment, a representative soil sample (500 g) was extracted, in order to establish certain soil physical and chemical components. The analysis was performed within a SANSAS-accredited laboratory, BEMLAB. The following parameters were measured for each treatment: the mechanical-3-fraction analysis to determine the soil classification, as well as the percentage sand, clay and silt within each soil; exchangeable cations (Na, K, Ca and Mg); base saturation (Na%, K%, Ca% and Mg%, with the T-value in cmol/kg); the pH (KCl); the total carbon (determined by means of the Walkley-Black method), and the inorganic nitrogen (NO_3-N and NH_4-N, quantified in mg/kg).

Data analysis

A descriptive analysis of data was done, using Microsoft Excel 2010. Multivariate analyses were done with XLSTAT 2010, using correspondence analysis techniques (CA).

Results

Nematode population and trophic group dynamics

Altogether, 16 different families were identified in the different treatment plots in the apple orchard. Both the chemical control and the mulch had 13 nematode families present, while the mulch + EM application had 12 families present (Table 5.2).

Table 5.2. Number and weighted percentage of nematode families per feeding group found within the different soil amendments in an apple orchard.

Trophic groups	Chemical control	Mulch	Mulch + EM spray
Total number of families	13	13	12
Herbivores	3 (17.23%)	2 (14.79%)	2 (10.43%)
Fungivores	5 (43.49%)	4 (2.77%)	4 (1.52%)
Bacterivores	3 (36.48%	5 (79.14%)	3 (86.51%)
Omnivores	1 (2.15%)	1 (2.90%)	1 (0.67%)
Carnivores	1 (0.66%)	1 (0.40%)	2 (0.86%)

All five trophic groups were present within each of the three soil amendments (Fig. 5.1). Fungivores dominated within the chemical control of cover crops and weeds, while bacterivores dominated the mulch and mulch + EM treatments. Omnivores were also more prevalent within the mulch treatments, while the number of carnivores was slightly higher in the mulch + EM treatments.

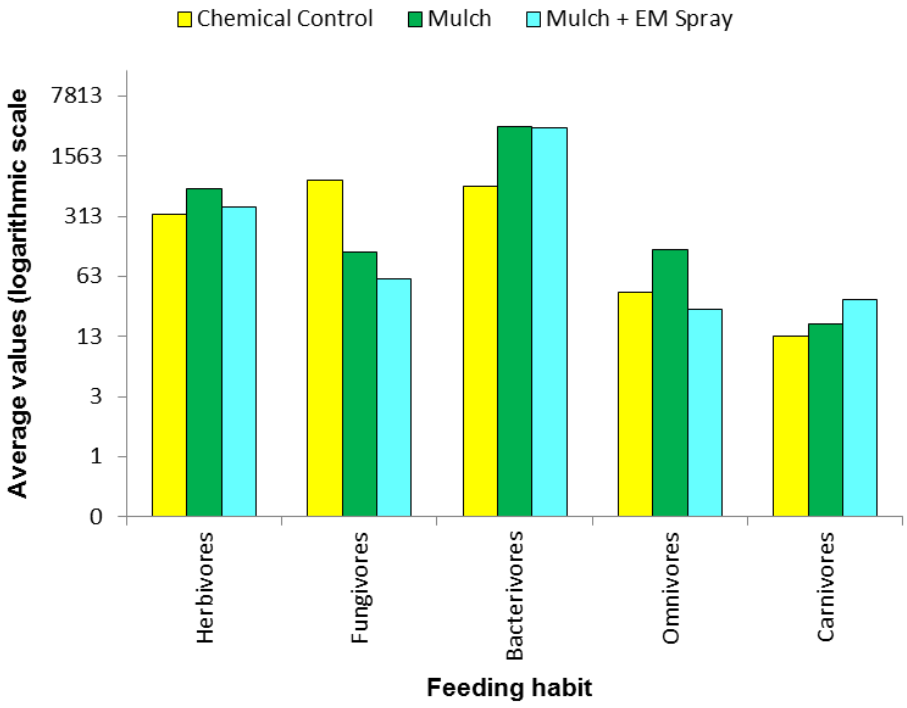

Fig. 5.1. Soil nematode trophic diversity comparison between different soil amendments within an apple orchard.

The collection of nematodes identified within each amendment type is represented in Figure 5.2 below, and indicated Tylenchidae as the only plant-parasitic nematode family that was present within all three treatments. Hoplolaimidae did not occur in plots treated with mulch + EM. Pratylenchidae were only observed within the chemically controlled (weeds and cover crop) plots.

Plots which received chemical control of weeds and cover crops had higher numbers of fungivorous nematodes (5 families) compared to the other amendments. The Neotylenchidae dominated within the mulch application, while they were not present within the mulch + EM sites. The nematode families Leptonchidae, Aphelenchidae, Aphelenchoididae and Paraphelenchidae dominated within the chemically controlled plots. Leptonchidae did not occur within plots with mulch application.

Mulch plots had more bacterivorous families present (5) than did the other amendment applications (both had only 3 families present). The mulch and mulch + EM plots had the same number of Rhabditidae present. Diplogasteridae and Plectidae only occurred in plots that received the mulch application. Cephalobidae dominated in plots in which the weeds and cover crops were chemically controlled. These plots or treatments contained the lowest numbers of Monhysteridae. The omnivorous family Dorylaimidae occurred within all three soil surface treatment plots, with the highest numbers being within the mulch plots. The carnivorous family Mononchidae occurred in all three treatment plots. The family Ironidae was only observed within the mulch + EM treatment plots (consisting of Treatment 6 and 8). Ironidae was also only observed within Treatment 8 when looking at the individual treatments contained within the mulch + EM treatment (Table 5.1).

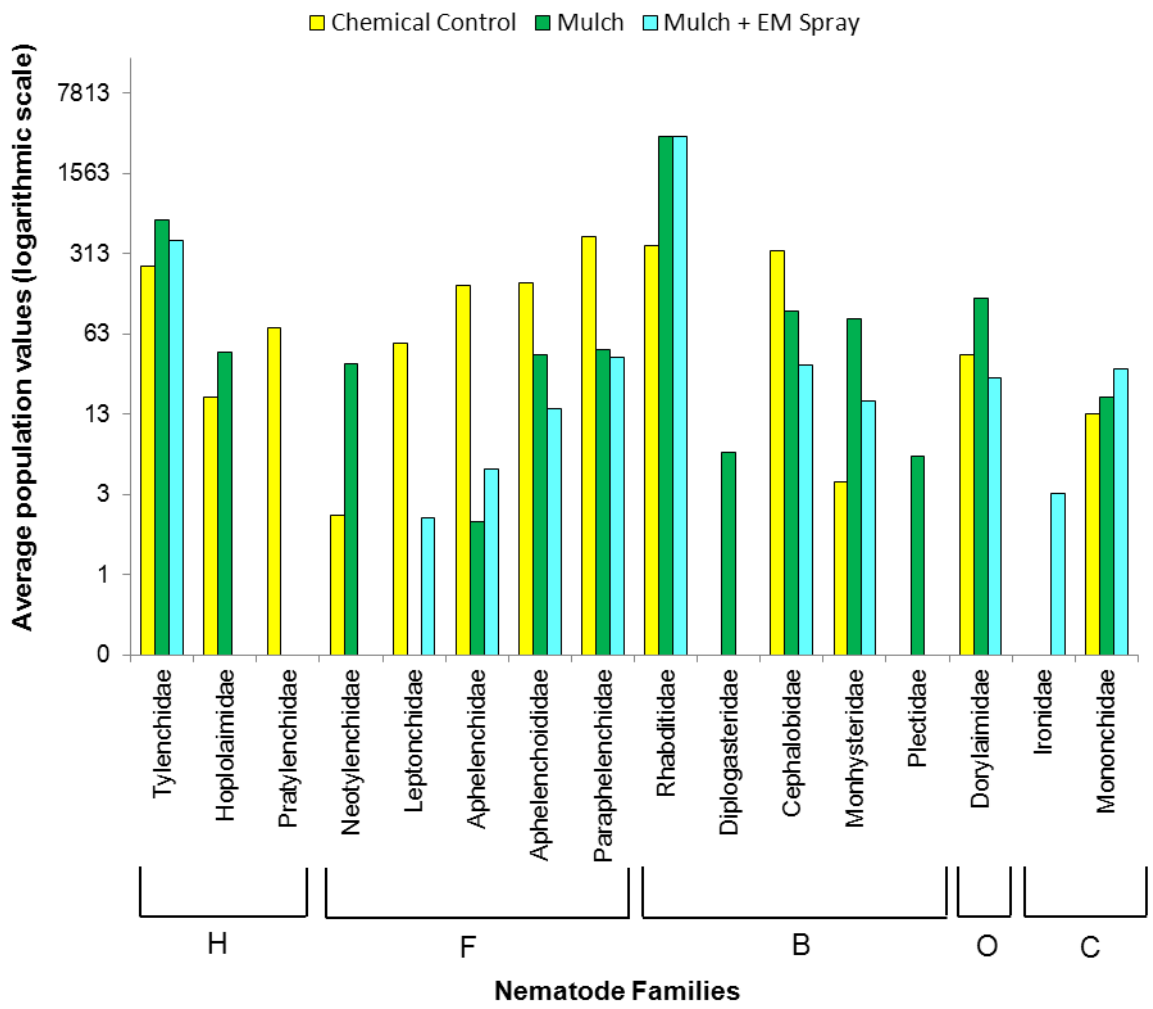

Fig. 5.2. A graphical representation of the different nematode families present within the soil of each soil amendment. (H: Herbivore; F: Fungivore; B: Bacterivore; O: Omnivore; C: Carnivore)

A correspondence analysis (CA) was performed to establish whether associations existed between the various mulch applications and the nematode families (Fig. 5.3). Ironidae was only associated with the mulch applications in combination with the EM spray (mulch + EM). In contrast to the above, Rhabditidae were found to associate strongly with all three applications. It was observed that the mulch + EM applications were very similar only to the mulch applications. The above implies that nematode families that are associated with the one application might well be associated with the other application as well. The nematode families Plectidae, Diplogasteridae, Neotylenchidae and Monhysteridae were only found to associate with the mulch-only application.

The nematode families Paraphelenchidae, Leptonchidae, Aphelenchidae, Pratylenchidae, Aphelenchoididae and Cephalobidae were strongly associated with the chemical control of the weeds

and cover crops. Mononchidae was associated with chemical control of the weeds and cover crop, and with the mulch + EM applications. Chemical control (of cover crop and weeds) and mulch applications both indicated an association with Hoplolaimidae, Dorylaimidae and Tylenchidae.

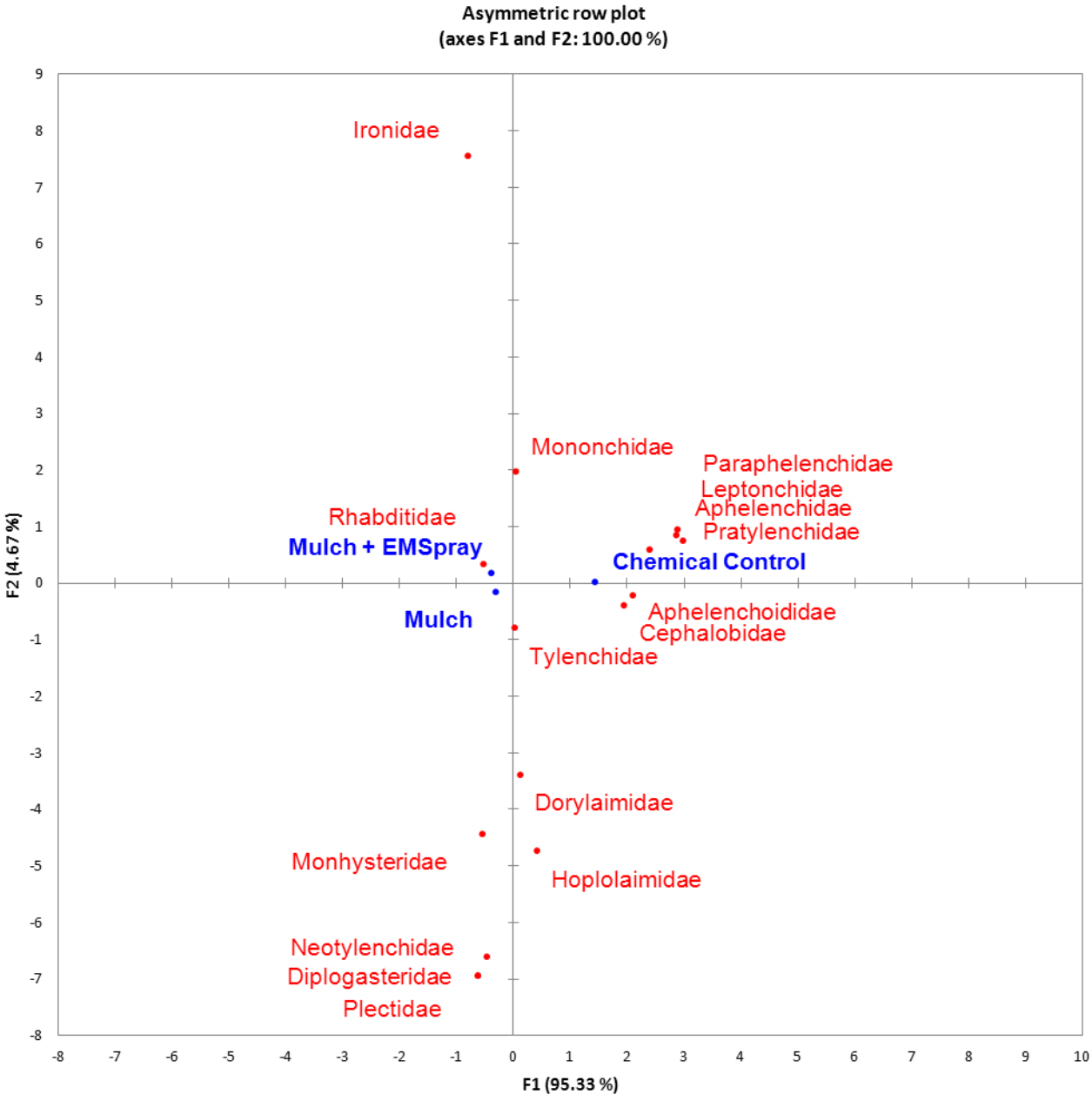

Fig. 5.3. Correspondence analysis of soil amendments applied within an apple orchard.

Maturity Indices

The various Maturity Indices (MI, MI2-5, PPI, ∑MI, ∑MI2-5 and PPI/MI) were calculated for each of the soil amendments (Fig. 5.4). The MI for all of the amendments was below 1.5, which indicated

conditions of disturbance within the soil nematode community. The chemical control of the cover crops and weeds appeared to have the highest MI value (1.2), whereas the mulch and the mulch + EM treatments were equal (0.5). The MI2-5 value was the highest in the plots that were subject to chemical control (1.1), and was the lowest in plots receiving applications of mulch + EM (0.2). The plots that received chemical control (of weeds and cover crops) applications also had the highest values for the ∑MI and ∑MI2-5 values. The ratio of the PPI/MI was lowest within these plots.

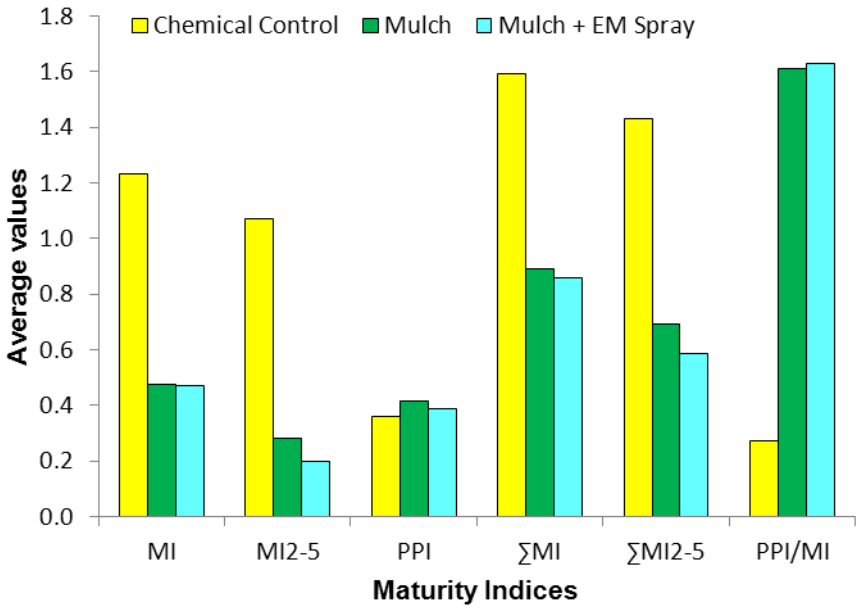

Fig. 5.4. Maturity indices for each soil amendment within an apple orchard.

Basal, structure and enrichment conditions

The indices that were utilised to determine the ecosystem function (BI, CI, EI and SI) were calculated for each treatment, as was the nematode faunal analysis (Ferris *et al.*, 2001). The data points for the chemical control (of cover crops and weeds) and mulch applications were located close together in Quadrat A, while the data point for the mulch + EM application was located in Quadrat B (Fig. 5.5).

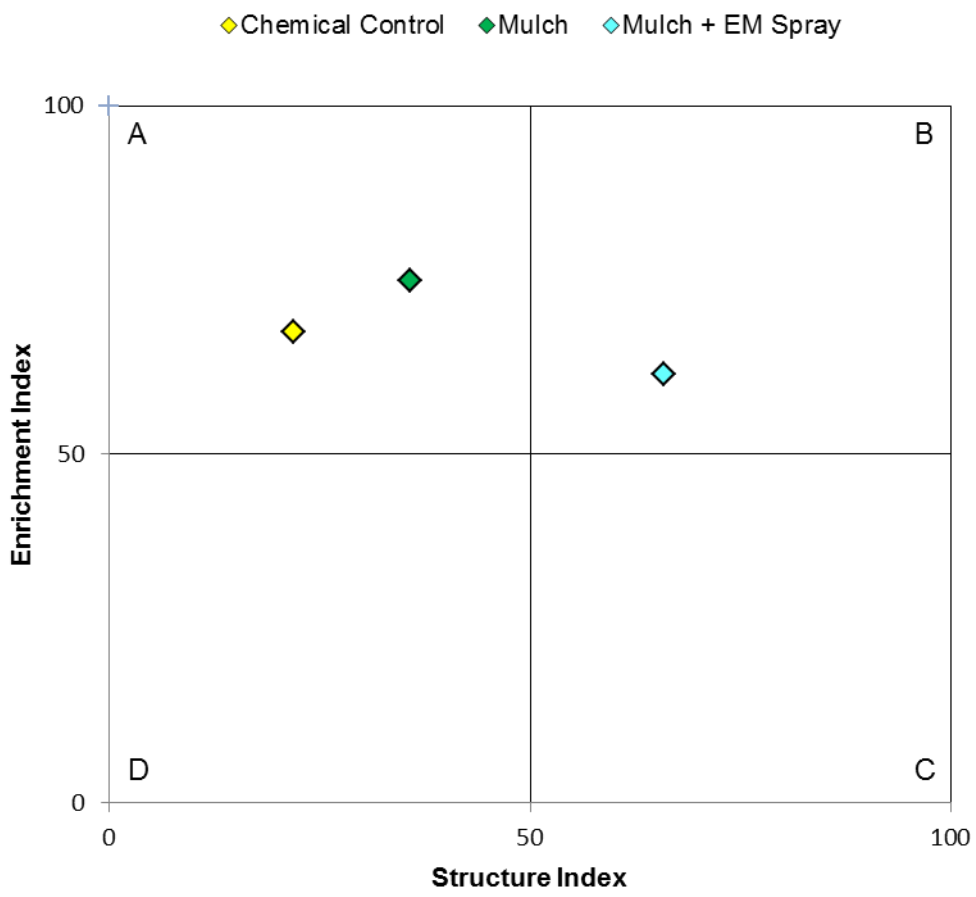

Fig. 5.5. The nematode faunal analysis profile for each soil amendment applied within an apple orchard.

The values obtained for the CI, BI, EI and SI are presented in Figure 5.6 below. The CI is a method that is employed to specify the decomposition channel of nutrients within a soil environment. The flow of resources can be separated into either bacterial or fungal decomposition. Values less than 50% indicated that the decomposer community was dominated by bacterial decomposition channels, whereas values above 50% indicated that decomposition was dominated by fungal decomposers. The CI values for all three soil amendments were below 50%, indicating that the decomposition was dominated by bacterial pathways. The chemical control had the highest CI value (33.7%).

The relative quantity of the basal, or cp-2, component (fungivores) of the nematode community within the soil environment was signified by the BI. The chemical control had the highest BI value, with 29.6%, whereas mulch + EM had the lowest (18.5%).

An EI value is indicative of the proportion of bacterivores and fungivores present within the soil ecosystem. The EI value for all the plots was higher than 50%, which indicated that the soil ecosystem was nutrient-enriched, with mulch having the highest EI value, namely 74.8%.

The SI is the relative contribution of nematodes with higher cp-values (2-5). The value can also specify the possibility of control by predators within a system. The mulch + EM had the highest SI value (65.9%), whereas the chemical control (21.8%) had the lowest value.

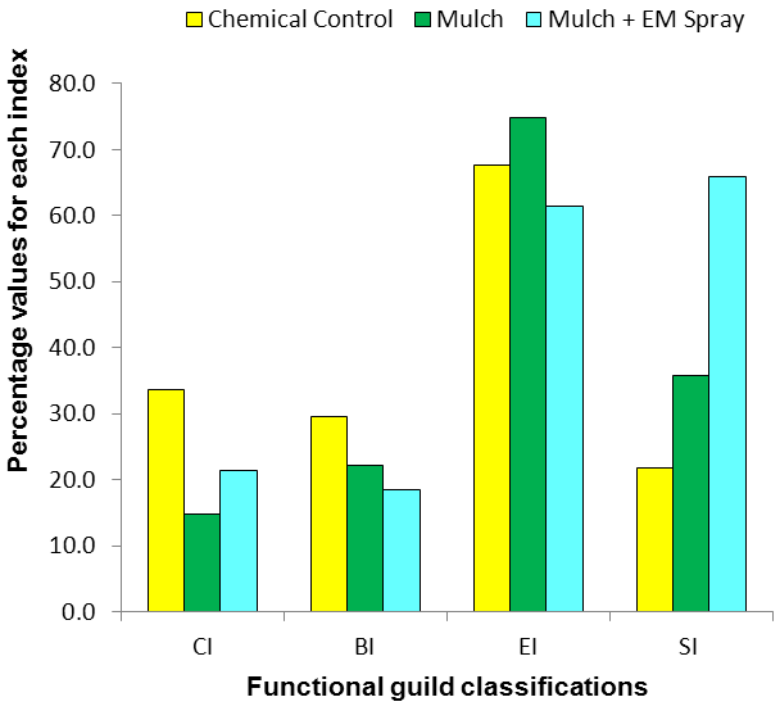

Fig. 5.6. A graphical representation of the Channel, Basal, Enrichment and Structure Indices for each soil amendment applied within an apple orchard.

Diversity, richness and evenness

The evenness, diversity and richness for each of the soil surface amendments was calculated and is represented graphically in Figure 5.7 below. The diversity index (i.e. the Simpson and Shannon Index) expressed a difference between the chemical control of cover crops and weeds and the other two treatments. The chemical control had the highest Shannon Index value (1.7).

The species richness, or Hill's N_0, suggested that the plots that received the chemical control had the highest species richness (7.6), followed by those that received the mulch, and the mulch + EM. Pielou's J (or the evenness) for both the chemical control (0.9) and the mulch (0.7) indicates and

even distribution of nematode families, whereas the mulch + EM had a value of 0.5 also indicating an even distribution but only just. The chemical control had the highest values of Hill's N_1 and N_2, whereas the mulch and the mulch + EM treatment values were almost equal.

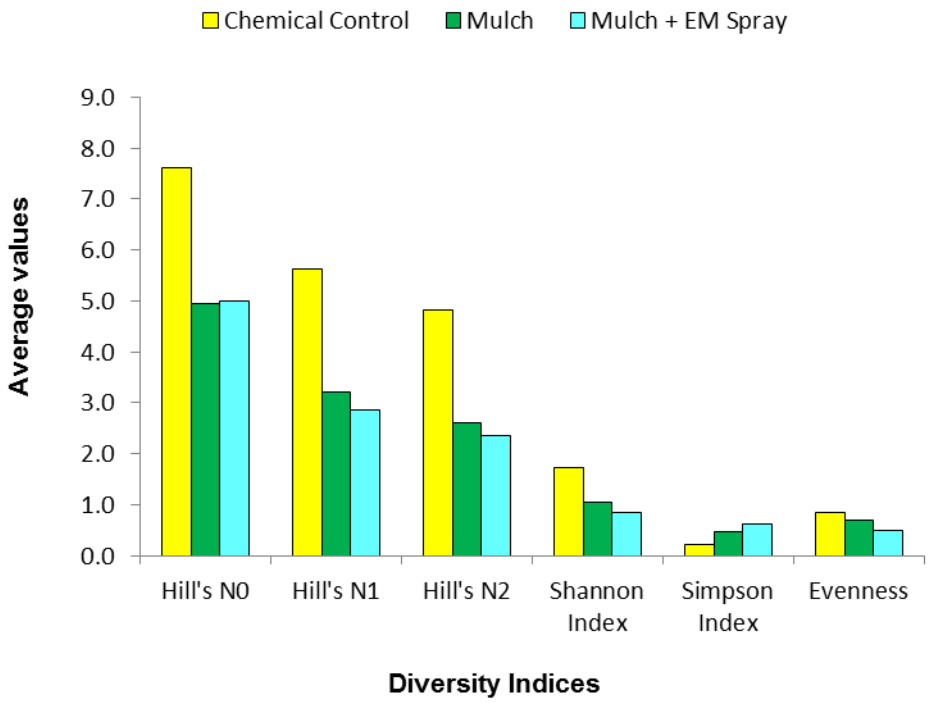

Fig. 5.7. Diversity indices indicative of nematode trophic group diversity, richness and evenness for each soil surface application applied within the orchard.

Soil analysis

The soil samples for each treatment were analysed, with the results being summarised in Table 5.3 below. The soil classification for all treatments was sandy loam soil. The pH varied from 4.43 to 6.50. Treatments 1 and 2, which had the lowest pH values, were the treatments that received chemical control from within the tree ridge row. The highest pH value was recorded in Treatment 5. The treatment concerned received mulch within the tree ridge row, and a cover crop within the working row, which was flattened during spring.

Table 5.3. Soil analysis data for the eight treatments applied within the apple orchard.

Treatment	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Depth (cm)	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00

Treatment		1	2	3	4	5	6	7	8
Soil		Loam	Loam	Loam	Loam	Loam	Loam	Loam	Loam
pH (KCl)		4.43	4.55	6.08	6.20	6.23	6.50	6.30	6.38
Resistance (Ohm)		2827.50	2750.00	1455.00	1172.50	1407.50	1240.00	1442.50	1445.00
H^{+} (cmol/kg)		2.10	1.94	0.25	0.12	0.12	0.00	0.00	0.12
Stone (Vol %)		61.50	60.75	55.50	58.75	59.00	57.25	54.25	54.50
P Bray II mg/kg		17.25	47.25	124.25	211.50	269.25	252.75	148.75	130.75
Potassium K (mg/kg)	K	209.25	218.50	320.50	367.75	304.75	417.00	328.00	317.00
Exchangeable cations (cmol(+)/kg)	Na	0.14	0.16	0.22	0.37	0.32	0.37	0.24	0.20
	K	0.54	0.56	0.82	0.94	0.78	1.07	0.84	0.81
	Ca	2.46	3.10	10.67	12.80	13.83	14.96	12.74	11.47
	Mg	0.55	0.63	1.94	2.35	2.28	2.61	2.38	1.90
Carbon	C %	1.84	1.79	2.67	3.44	3.95	4.21	4.05	2.98
Inorganic nitrogen	NO3-N (mg/kg)	15.75	13.63	22.22	182.38	175.19	172.66	173.19	173.26
	NH4-N (mg/kg)	7.02	7.64	16.44	30.52	43.99	5.52	14.36	14.91
Cation exchange capacity	CEC (pH7) cmol(+)/kg	3.74	3.89	6.09	7.42	9.51	7.65	7.90	8.01
Base saturation	Na %	1.77	1.83	1.61	1.45	1.54	1.64	1.56	1.90
	K %	5.14	3.85	4.38	4.64	3.47	4.81	3.90	4.75
	Ca %	67.84	71.56	69.33	73.74	68.48	61.35	81.72	68.98
	Mg %	10.37	11.51	11.13	7.71	10.43	11.25	9.61	10.90
	T-Value (cmol/kg)	5.77	6.38	13.90	13.90	16.57	17.33	18.99	16.20
Mechanical 3-fraction	Clay %	12.20	11.50	10.40	11.50	10.30	11.95	13.05	11.50
	Silt %	23.70	22.15	20.95	24.30	20.65	25.20	23.15	24.80
	Sand %	64.10	66.35	68.65	66.35	69.05	62.85	67.45	64.50
Classification		SaLm	SaLm	SaLm	SaLm	SaLm	SaLm	SaLm	SaLm

Discussion

Different treatments were applied to the apple orchard floor for a period of nine years. Soil samples from the treatments were analysed to establish the possible effects of mulch and soil surface management on the nematode community structure. The effect of such additions within deciduous

fruit orchards on nematode population structure and function in South Africa has not yet been established. Due to nematode samples in the current study being taken after the treatment applications were completed, results report only on the nematode families and community parameters as they were at that point in time.

Regarding the soil amendments, all five nematode trophic groups were represented within each of the three combined applications. The number of fungivores was notably higher in the chemical control (of cover crops and weeds) treatments, with the lowest number of bacterivores being present. Such numbers could possibly be ascribed to the chemical application, which could have stimulated the growth of certain fungi, which were then utilised as a food source for the fungivorous nematodes. The numbers concerned could also have been due to the bacterial populations being suppressed by chemical application.

Omnivores were predominant in the mulch applications, and carnivores were predominant in the mulch + EM applications. The high numbers of carnivores present within the mulch + EM application could have been due to the high numbers of bacterivores that were utilised as a food source. As reported by Neher (1999), the advantage of the simultaneous addition of organic matter and the exclusion of general biocides had led to an increase in the frequency of the omnivorous and predaceous species. The same could be said for the omnivores that utilised various nematode families from a number of feeding groups as their food sources. Forge *et al*. (2003), who measured the various nematode community parameters, said that they reported a greater abundance of protozoa and bacterivorous nematodes, as well as greater estimated fluxes of N and P through micro-fauna under all combinations of biosolids or municipal compost and shredded office paper than under the plastic mulch application.

According to Thoden *et al*. (2011), amendments with a low C/N-ratio (such as legumes) are exceptional sources of food for bacteria, which consequently leads to an increase in the populations of bacterial-feeding nematodes, classified as enrichment opportunists (Ferris & Matute, 2003). They reported that bacterial- and fungal-feeding nematodes respond to organic matter inputs, yet the response varies in terms of functional guild, and was dependent on the quantity and quality of the organic matter involved. The abundance of bacterivores commonly escalates with the incorporation of

cover crop residues, which is ascribed to the increase in bacterial biomass, following the addition of the cover crop (Ferris *et al*., 1996; 2004).

Treatment 4 (which fell within the mulch application) which consisted of an overall application mulch within the working row and within the ridge of the tree row, lacked omnivorous and carnivorous nematodes. Treatment 6, which received a mulch layer on the tree ridge row, also had no omnivorous nematodes present. It is also important to note that the faunal analysis for Treatment 4 indicated that said treatment had no SI value, and was, thus, only enriched. This treatment also had the lowest values for species richness and diversity. This indicated that the treatment was not contributing to the establishment of a good nematode community structure.

Within all the soil surface applications, Rhabditidae were the most dominant family, followed by Tylenchidae. Hoplolaimidae were only present in the chemical control and mulch applications, with Pratylenchidae only being present in the chemical control (of cover crops and weeds) applications. The fungivorous families were dominated by Paraphelenchidae, followed by Aphelenchoididae.

The bacterivorous nematode families, Diplogasteridae and Plectidae, only occurred within the mulch applications. This could indicate that the nematodes preferred the conditions created by the addition of the straw mulch to an orchard environment. Dorylaimidae, which were the only omnivorous family identified, were present within all three soil surface applications. Overall, the mulch + EM application did not perform better than did the other two soil surface applications. It would be expected that effective microbes would be a suitable food source for bacterivorous and/or fungivorous nematodes, yet the applications that consisted of only a mulch application had higher numbers of fungivores, and almost the same number of bacterivores. Members of the carnivorous nematode family Ironidae were only present in Treatment 8 (mulch + EM). This treatment was included in the mulch + EM treatment which was the reason for the family being present within this specific treatment combination. Porazinska *et al.* (1999) reported that, within a citrus orchard, which was monitored for three years, lower species richness was recorded in mulch-treated plots, due to an absence of mononchid and chromadorid genera.

Baniyamuddin *et al*. (2007) stated that dorylaimid nematode communities are very sensitive to disturbances, including such agricultural practices as ploughing, fertiliser and pesticide applications, within their environment. An elevated proportion of dorylaimids within a system (consisting of more

than 25%) indicates scarce human intervention, whereas low ratios indicate the contrary (Gomes *et al*., 2003; Baniyamuddin *et al*., 2007). Other nematode groups, such as carnivorous families, are not as sensitive to disturbance-induced and physiochemical changes within the soil environment as are the mononchids and omnivorous families (Forge & Simmard, 2001; Baniyamuddin *et al*., 2007).

Wasilewska (1979) indicated that, in agricultural soils, greater diversity of trophic groups is associated with an escalation in the incidence of the existence of less abundant trophic groups (i.e. the fungal-feeding, omnivorous and predatory nematodes). Such an escalation can be compared to the trophic groups that are generally more abundant (i.e. the bacterial-feeding and plant-parasitic nematodes). The above was not observed in the current study, since the bacterial and plant-parasitic nematodes dominated the functional groups concerned.

The correspondence analysis of nematode family associations to the three different soil surface applications indicated that Ironidae are only associated with the mulch + EM treatment, which reinforces previous comments made in this respect. The mulch and mulch + EM treatments were very similar, which is an indication that nematode families that are associated with the one application might also be associated with the other.

The highest MI was calculated for chemical cover crop and weed control applications, indicating the soil to be the maturest with regard to the nematode community structure contained therein. The mulch and the mulch + EM applications had equal MI values, which indicated conditions of disturbance. The chemically controlled (cover crops and weeds) plots were, thus, found to be less disturbed than were the plots receiving mulch and mulch + EM applications. The observation concerned should be further investigated within the context of the entire project.

With regards to the MI2-5, the values did not differ greatly from the MI values that indicated that the bacterivores that were present were not dominated by enrichment opportunists. The PPI indicated that all the applications had similar numbers of plant-parasitic nematodes present.

The faunal analysis profile for the three applications indicated that the mulch and chemical control (of cover crops and weeds) were designated by conditions of enrichment and minimal structure, as well as disturbance. In contrast, the data point for the mulch + EM applications indicated conditions of enrichment and structure within a maturing soil system. Of the three applications,

according to the faunal analysis profile, the mulch + EM performed the best. All three soil surface applications were dominated by bacterial decomposition pathways that confirmed the dominance by bacterivores, as opposed to fungivores.

Mulch applications, followed by the chemical control (of cover crops and weeds) and the mulch + EM, indicated the highest EI values. In the study conducted by Forge *et al*. (2003), the abundance of enrichment opportunists and the EI were consistently greater under combinations of municipal compost or biosolids and shredded paper.

The SI values for mulch and chemical control (of cover crops and weeds) denote that the systems concerned were disturbed or degraded, since both values were below 50%, whereas the mulch + EM system was matured or structured. Forge *et al*. (2003) reported that the SI was highest under shredded paper, and shredded paper applied over compost, and the lowest under municipal biosolids and alfalfa hay. The mulch applied in the current study consisted of straw, which was comparable with alfalfa hay. In the research, the mulch application had a low SI value, which was comparable with the results obtained by Forge *et al*. (2003). They reported that, after a compost amendment, the SI decreased, the CI decreased, and the EI increased. They also reported that the number of bacterial feeders and enrichment opportunists increased after the application of composted yard waste as well as biosolids, shredded office paper, and shredded office paper applied over the compost, as well as after the biosolid layer mulches were applied in an apple orchard (Forge *et al*., 2008).

The values for the nematode family distribution for all three soil applications were close to 1, implying that the distribution of abundances amongst families in the plots was evenly distributed. Mulch + EM had a value of 0.5, which also indicated an even distribution, but also that, of the three, it was the least evenly distributed.

The indication of the highest diversity and species richness under conditions of chemical control of the cover crops and weeds was an unexpected result, since it was previously thought that chemicals destroy the diversity and species richness within a soil ecosystem. But, since only the cover crops and weeds were chemically controlled the soil can be regarded as undisturbed, which could explain the results obtained in this study. Neither the chemicals, nor the remnants of the weeds and cover crops were incorporated into the soil and the soil structure stayed intact. Porazinska *et al*.

(1999) reported that the lower trophic diversity that was found in treatment plots that received a mulch application stemmed from nematode communities being dominated by bacterial and fungal feeders.

The effect of the different treatments on the soil abiotic and biotic factors was studied in detail during the time period of the experiment. The effect on the individual treatments should be combined with the results obtained from the individual nematode composition results (which are not reported on in the current thesis). The soil pH in an agroecosystem normally ranges between 4 and 8, which would normally not influence the presence of nematodes. Only a pH of higher than 10 has been found to have a detrimental effect on the presence of nematodes (Kung *et al.*, 1990).

Clear differences in the various soil applications with regard to the nematode community structure were established. For the purpose of the current study, the eight different treatments were combined. The results from the individual treatments should be further analysed, in combination with all the other measurements taken during the nine-year study. The effect of various soil amendments, such as compost, wood chips, straw mulch, compost tea and organic fertilisers on the soil nematode community structure need to be established over a shorter time period. The current research will provide farmers with a scientific way in which to measure the health of their soil, in order to improve the sustainability thereof.

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Chapter 6

Conclusion

The aim of this study was to determine whether soil nematode communities could serve as a biological indicator of soil health, within deciduous fruit orchards in the Western Cape. The objectives of the study were, firstly, to determine the biodiversity and functionality of soil nematode communities in the Fynbos ecosystem. The second objective was to determine the structure and function of the nematode soil communities in organic and conventional deciduous fruit orchards, with the third and final objective being to determine the differences in structure and function of nematode communities between different soil surface treatments in an apple orchard.

The functionality and biodiversity of soil nematode communities in the Fynbos ecosystem was determined by collecting 48 Fynbos soil samples in natural Fynbos areas throughout the Western Cape. Nematodes were extracted from each of the samples, quantified, and identified to family level, in order to analyse the functionality and biodiversity of each sample. The study, conducted in Chapter 3, indicated that Fynbos soils tend to be dominated by herbivorous and bacterivorous nematode families. Low numbers of omnivorous and predaceous nematodes were found to occur within the Fynbos soil, which was in keeping with the other natural vegetation types investigated. The results indicated Cephalobidae as being the most abundant bacterivorous nematode family, followed by Rhabditidae. Heteroderidae occurred least frequently, with Tylenchidae being the most abundant family overall. A canonical correspondence analysis (CCA) indicated that associations between specific Fynbos families and nematode families existed. Associations between the nematode feeding groups and Fynbos plant families were also established. This analysis indicated that carnivorous nematodes are not influenced by plant families, indicating that the presence of adequate food sources is the driver for the presence of such nematodes within a system. Associations between nematode feeding groups and soil analysis variables were not found. Specific soil analysis variables analysed with the nematode families, indicated that few associations occur, mostly regarding the soil sand fraction.

Results indicated that the Maturity Index (MI) for natural Fynbos soils was low, which was due to the lack of persister nematodes within the system. The PPI value was also low, indicating that, within a natural environment, the plant-parasitic nematodes do not proliferate as effectively as they do in agricultural monocultures. The results for the faunal analysis for Fynbos soils suggest that the structure and enrichment of Fynbos soils varies greatly between different areas. Fynbos soils are old, and are naturally unstructured. This study forms a basis for the comparison of the status of agriculturally managed soils to natural vegetation. It is important to note that, in future studies, Fynbos samples should be collected from around the orchard environment being sampled, in order to establish adequate baseline values.

For the second objective, two different studies were conducted. The first study aimed to establish the soil nematode community structure of organically and conventionally managed deciduous fruit orchards by determining the abundance, diversity and functionality of the naturally occurring free-living and plant-parasitic nematodes. The objective of this study was to form the foundation for the use of nematodes as bio-indicators of soil health in orchards.

Results from the above-mentioned study indicated that that neither organic nor conventional orchard management practices could unequivocally be proven to be better. The organic apple orchard had lower numbers of plant-feeding nematodes than did the organic and conventional apricot orchards. The organic orchard had very low numbers of omnivorous and predatory nematodes present, consisting mostly of the persister nematodes. Such nematodes are sensitive to disturbances within their environment. In organic and conventional apricot orchards, the most dominant nematode family was the Tylenchidae. The same fungivorous nematode assemblages (besides Panagrolaimidae) occurred in the organic and conventional apricot orchards. Differences were observed between the nematode family composition of organic apple and organic apricot orchards, proving that nematode families were more affected by plant species and less affected by management practice. Higher levels of fungivorous nematodes occurred in the organic apricots, compared to organic apple orchards. Bacterivorous nematodes were more prevalent in the organic apple, than they were in the organic apricot orchards. A comparison between the conventional apricot orchard and the natural vegetation (Fynbos) sites was conducted, indicating the Tylenchidae to be more prevalent in the conventional apricot orchard than in the natural vegetation sites. Certain plant-parasitic nematodes were found to thrive within the orchard environment, since higher populations

occurred within the orchard, compared to within the natural vegetation site. Bacterivorous nematodes were more ubiquitous in the natural vegetation site than they were in the orchard sites.

A CA was performed to determine possible associations between nematode families and orchard environments. Results indicated that the organic and the conventional apricot orchards were related to each other, which implied that the nematodes that were present within the one would be present within the other as well. The above findings support the statement that nematode families are influenced by plant family and by species presence, rather than by management practice. The organic apple site had specific nematode family associations. The results indicated that the conventional apricot orchard had the highest MI value, suggesting that, even though the orchard was managed conventionally, the organic management practices led to higher levels of disturbance within the orchard environment than might otherwise have been present. The Plant-parasitic Index (PPI) indicated that the organic apple orchards had the lowest value, which could indicate a suppressive environment within the orchard. The results of the faunal analysis of the different orchards indicated that all the soils were both structured and enriched.

The third objective involved the determination of differences in soil nematode community structures, diversity, functionality and abundance between various soil surface and mulch applications within an apple orchard. The results suggest that treatments which chemically controlled weeds and cover crops experienced notably higher populations of fungivorous nematodes compared to those in the mulch treatment and in the mulch + effective micro-organisms (EM) application. Omnivorous nematodes were prevalent in mulch applications, whereas carnivores were most dominant in mulch + EM (effective micro-organisms) applications. The most dominant nematode family within the three soil applications was the bacterivorous family, Rhabditidae. Tylenchidae was the second most abundant family. Mulch + EM treatments had a higher incidence of carnivorous nematodes, since both Mononchidae and Ironidae were present. The results for the CA indicated that the mulch and mulch + EM treatments were very similar. The addition of EM might, therefore, have been unnecessary. Chemical control treatments of the cover crops and weeds, reported the highest MI value, implying that, of all the treatments, this was the least disruptive to the maturity of the system. These treatments did not disturb the soil and can be the reason for the results obtained in this study. Results for the faunal analysis denote that, of the three applications, mulch + EM applications had the most desirable faunal composition, since the conditions were enriched, as well as structured. The outcome of the

diversity and species richness calculations indicated that the chemical control of the cover crops and weeds had the highest values.

The overall aim was to establish whether nematodes are a suitable bio-indicator for soil health within deciduous fruit orchards. No empirical study was undertaken to establish the correctness of the above, since the results from Chapter 4 and 5 could be implemented to determine it. The study proved that, by implementing various indices, including the MI, the Channel Index (CI), the Enrichment Index (EI), the Structure Index (SI), the Basal Index (BI), the Shannon and Simpson diversity indices, and the faunal analysis that the condition of the soil could be established by means of the nematode families and their composition present within it. The nematode communities within a soil describe the state of the soil, which ultimately indicates whether a soil is disturbed (which, at extremes, indicates an unhealthy state), enriched or structured, which are the proxies for soil health. Nematodes are, thus, a sufficient bio-indicator of the status, and, thus, of the health, of soil at a specific time within a deciduous orchard.

In future studies, more indices could be implemented within the current system to include carbon flow analysis, predator:prey footprints, labile enrichment status, metabolic footprints, fungivore footprints and fungivore:bacterivore ratios. Future research should address issues concerning the optimisation of the mineralisation of such minerals as N, P and K by free-living nematodes, as well as the determination of appropriate types of organic amendments that are likely to nurture the most effective mineralisers. The mineralising nematodes concerned should be established, since this could lead to the addition of these nematodes to organic amendments, which would greatly improve the efficacy thereof. Studies also need to be undertaken to determine whether mutualistic relationships exist amongst particular nematode species and the beneficial rhizobacteria that are present in soil. Nematodes could also be implemented as bio-indicators of the maturity of organic compost.

The sheer volume of information regarding soil structure and, ultimately, soil health that can be gained from the nematode community structure within soil proves that nematodes are a viable biological indicator of soil health. They have also been utilised as such in other countries, mostly targeting annual crops. With more research, a suitable soil health assessment manual could be established for the deciduous fruit industry in South Africa, not only utilising nematodes, but also incorporating soil physical and chemical properties as well.

Appendix 1

Yeates *et al.* (1993) allocation of nematodes into feeding groups.

TABLE 1. Feeding types in nematode genera. Numbers refer to the eight feeding types outlined in the text; numbers in parenthesis indicate a tentative assignment. The main food source is given first. For genera maintained for several generations under defined conditions, unqualified reference is made to that publication by number; for genera where the publication number is preceded by "see," a more general (or less specific) support for allocation to the feeding type is indicated; for genera where we have not obtained a direct reference to feeding activity, the family name is given.

Genus	Feeding type	Family or literature citation
Ablechroiulus	3	Rhabditidae
Achromadora	(6)	Achromodoridae
Acontylus	1b	(66)
Acrobeles	3	(111,126)
Acrobeloides	3	(71,104,126)
Acrobelophis	3	Cephalobidae
Acrolobus	3	Cephalobidae
Acromoldavicus	3	Cephalobidae
Acrostichus	3	Diplogasteridae; (88)
Actinca	5, 8	Actinolaimidae
Actinolaimus	5, 8	(56); (see 31,100)
Aetholaimus	5	Nygolaimidae
Aglenchus	1e	Tylenchidae; (122)
Alaimus	3	(see 77)
Allantonema	7 or 2	(119)
Allodorylaimus	8	Dorylaimidae
Allotrichodorus	1d	Trichodoridae; (see Decraemer in 76)
Amphidelus	3	Alaimidae
Amplimerlinius	1d	(14,36); (see Anderson & Potter in 76)
Anaplectus	3	(122)
Anatonchus	5a	(18); (see 100)
Anguina	1a or b	(22); (see Krall in 76)
Anomyctus	2?	(see Nickle & Hooper in 76)
Antarctylus	1c	Hoplolaiminae
Aorolaimus	1c	Hoplolaiminae
Aphanolaimus	3	Halaphanolaimidae
Aphasmatylenchus	1c	(34); (see Fortuner in 76)
Aphelenchoides	2 or 1b, 1e or 1f	(68); (see 122)
Aphelenchus	2 or 1e	(17,23,40,65); (see 122)
Aporcelaimellus	5, 8	(117,122); (see 31)
Aporcelaimium	8	Aporcelaimidae
Aporcelaimus	5, 8	(114,122); (see 31,100)
Apratylenchoides	1b	Pratylenchidae
Aprutides	2	Aphlenchoididae
Aquatides	5	(10); (see 100)
Atylenchus	1d	Tylenchidae
Aulolaimus	3	Cryptonchidae
Axonchium	1, 8?	(see 100)
Basiria	1e	Tylenchidae
Bastiania	3	Bastianiidae
Bathyodontus	3	Bathyodontidae; (see 12)
Belonolaimus	1d	(15)
Bicirronema	3	Chambersiellidae
Bitylenchus	1d	Tylenchorhynchidae; (see Anderson & Potter in 76)
Boleodorus	1e or 2(?)	Tylenchidae
Brevibucca	3	Brevibuccidae
Brittonema	5, 8	Actinolaimidae
Bunonema	3	(77,128)
Bursaphelenchus	2	(32,63); (see Nickle & Hooper in 76)
Bursilla	3	Rhabditidae
Butlerius	3, 5	(28,89,101); (see 100)
Cacopaurus	1a	Paratylenchidae; (112); (see Raski in 76)
Cactodera	1a	(19,98); (see Baldwin & Mundo-Ocampo in 76)
Caenorhabditis	3	(25,78,97); (see 107)

TABLE 1. *Continued*

Genus	Feeding type	Family or literature citation
Caloosia	1d	Hemicycliophoridídae
Campydora	8?	Campydoridae
Carcharolaimus	5	(28); (see 100)
Cephalenchus	1d	(37,109); (see 122)
Cephalobus	3	(81,88); (see 72,122)
Ceratoplectus	3	Plectididae
Cervidellus	3	(122)
Chambersiella	3	Chambersiellidae
Cheilorhabditis	3	Rhabditidae
Chiloplacus	3	(118,122)
Choanolaimus	5a	Choanolaimidae
Chondronema	7, 2?	(16)
Chromadorina	3, 6?	Chromadoridae; (116)
Chromadorita	6	(49)
Chronogaster	3	Leptolaimidae
Chrysonemoides	?	Chrysonematidae
Clarkus	5a	(67,106); (see 101,130)
Coarctadera	3	Rhabditidae; (see 107)
Cobbonchus	5a	(see 100)
Coomansus	5a	(31)
Coslenchus	1e	(as *Aglenchus*); (122)
Craspedonema	3	Butonematidae
Criconema	1d	(see 26,93)
Criconemoides	1d	(see 26,93)
Crossonema	1d	(see 26,93)
Cruznema	3	(108)
Cryphodera	1a	(see 8,60)
Cryptonchus	3	Cryptonchidae
Curviditis	3	Rhabditidae
Cuticonema	3	Breviibuccidae
Cuticularia	3	Rhabditidae
Cylindrolaimus	3	Diplopeltidae
Daptonema	3, 4, 5 or 6	(13)
Deladenus	2	(9,129); (see 62)
Demaniella	3	(88)
Deontolaimus	3	Leptolaimidae
Desmodora	3, 6	Desmodoridae; (74)
Desmolaimus	3	Linhomoeidae
Desmoscolex	3	Desmoscolecidae
Dichromadora	3, 6?	(13,120)
Diphtherophora	2	Diphtherophoridae; (see 4)
Diplenteron	3?	(64,125); (see 100)
Diplogaster	3, 5a, 6 or 8	(see 72,100)
Diplogasteritus	3	(124)
Diplogasteroides	3	Diplogasteroididae
Diploscapter	3	(42,88)
Discolaimium	5?	(see 100)
Discolaimus	5	(28,56); (see 31,100)
Ditylenchus	2, or 1b	(55,122); (see Sturhan & Brezeski in 76)
Dolichodorus	1d	(82,83,85); (see Smart & Nguyen in 76)
Dolichorhabditis	3	Rhabditidae
Dolichorhynchus	1d	Dolichodoridae
Domorganus	3	Diplopeltidae
Dorydorella	8	Dorylaimidae
Dorylaimellus	1, 2?	Belondiridae; (see 131)
Dorylaimoides	8?	Leptonchidae
Dorylaimus	8	(56); (see 77,100)
Doryllium	2?	Leptonchidae
Drilocephalobus	3	Osstellidae

TABLE 1. *Continued*

Genus	Feeding type	Family or literature citation
Durinema	5	(see 77,100)
Ecphyadophora	1d or 2(?)	Ecphyadophoridae
Ecumenicus	8	Dorylaimidae
Elaphonema	3	Elaphonematidae
Enchodelus	8 or 6?	Nordiidae
Epidorylaimus	8	Dorylaimidae
Ereptonema	3	Ereptonemidae
Ethmolaimus	3, 6?	Ethmolaimidae
Eucephalobus	3	(122)
Eudorylaimus	5, 8	(29,44,117); (see 31,100)
Eurystomina	(5)	Eurystominidae
Eumonhystera	3, 4	Monhysteridae
Euteratocephalus	3	Teratocephalidae
Fictor	3, 5, 6	(88,103); (see 100)
Filenchus	1e	Tylenchidae
Funaria	2?	Leptonchidae
Fungiotonchium	2	Iontonchiidae (see 99)
Geocenamus	1d	Dolichodoridae
Geomonhystera	3, 4	Monhysteridae
Glauxinema	6	Neodiplogasteridae
Globodera	1a	(53); (see Baldwin & Mundo-Ocampo in 76)
Goffartia	3	Diplogasteridae
Goodeyus	3	Cylindrocorporidae
Gracilacus	1d	Paratylenchidae; (see Raski in 76)
Granonchulus	5a	Mononchidae; (35)
Haliplectus	3	Haliplectidae
Helicotylenchus	1c	(51); (see Fortuner in 76)
Hemicriconemoides	1d	Criconematidae; (see 93)
Hemicycliophora	1d	Criconematidae; (see 93)
Heterocephalobellus	3	Cephalobidae
Heterocephalobus	3	Cephalobidae
Heterodera	1a	(see Baldwin & Mundo-Ocampo in 76)
Heterorhabditis	7, 3	(90); (see Wouts in 76)
Hexatylus	2	(24); (see 72)
Hirschmanniella	1b	(7); (see Loof in 76)
Hoplolaimus	1c	Hoplolaimidae
Hoplotylus	1b	Pratylenchidae
Howardula	7 or 2	Allantonematidae
Huntaphelenchoides	2	Aphelenchoididae
Iotonchus	5a	(67,100,101)
Ironus	5a or 6	(47); (see 100)
Isolaimium	(3)	Isolaimidae
Kirjanovia	3	Cephalobidae
Kochinema	8	Nordiidae
Labronema	5, 8	(28,30,88,122); (see 31,100)
Laimaphelenchus	5b, 1f, 2	(see 100)
Laimydorus	8	(see 100)
Lelenchus	1e	Tylenchidae
Leptolaimus	3	(13)
Leptonchus	2	(see 31)
Longidorella	1d	Nordiidae
Longidorus	1d	Longidoridae
Loofia	1d	Hemicycliophoridae
Macrotrophurus	1d	Dolichodoridae
Malenchus	1e	Tylenchidae
Meloidodera	1a	(see Baldwin & Mundo-Ocampo in 76)
Meloidoderita	1a	(see 76)
Meloidogyne	1a	(see 27,96)
Merlinius	1d	(see Anderson & Potter in 76)

TABLE 1. *Continued*

Genus	Feeding type	Family or literature citation
Mesocriconema	1d	(see 93)
Mesodiplogaster	3, 5	(102,103); (see 100)
Mesodorylaimus	8	(29); (see 31,95,100)
Mesorhabditis	3	(107,122,126)
Metacrobeles	3	Cephalobidae
Metadiplogaster	3	Diplogasteridae
Metateratocephalus	3	Teratocephalidae
Miconchus	5a	(see 100)
Microdorylaimus	8	Nordiidae
Microlaimus	3	Microlaimidae
Monhystera	3, (4)	(122); (see 77,115)
Monhystrella	3, 4	(13)
Monobutlerius	3	Diplogasteridae
Mononchoides	3, 5a	(89); (see 100)
Mononchulus	3	(see 31)
Mononchus	5a	(38,88); (see 31,100)
Monotrichodorus	1d	Trichodoridae; (see Decraemer in 76)
Morulaimus	1d	Belonolaimidae; (see Smart & Nyguen in 76)
Mylonchulus	5a	(48,67); (see 31,100)
Myolaimus	3	Myolaimidae
Nacobbus	1a	(see Jatala in 76)
Nagelus	1d	Dolichodoridae
Namibinema	3	Cephalobidae
Neoactinolaimus	5, 8	(101); (see 100)
Neoaplectana	7, 3	(see 76)
Neodiplogaster	3, 4 or 5	Neodiplogasteridae
Neopsilenchus	1e	Tylenchidae
Neothada	1e or 2(?)	Tylenchidae
Nothacrobeles	3	Cephalobidae
Nothotylenchus	2	Anguinidae
Nullonchus	5a	(see 100)
Nygolaimium	5	(see 100)
Nygolaimoides	5	Nygolaimidae
Nygolaimus	5	(113,122); (see 31,100)
Odontolaimus	3 or 6?	Odontolaimidae
Odontopharynx	3, 5a	Odontopharyngidae
Odontorhabditis	3	Odontorhabditidae
Ogma	1d	(see 93)
Oionchus	3	Mononchulidae
Onchulus	5a or 6	Onchulidae
Opisthodorylaimus	8	Dorylaimidae
Orrina	1a or b	(see Krall in 76)
Osstella	3	Osstellidae
Oxydirus	1, 8?	Belondiridae
Panagrellus	3	(21)
Panagrobelium	3	Panagrolaimidae
Panagrobelus	3	Panagrolaimidae
Panagrocephalus	3	Cephalobidae
Panagrolaimus	3	(88,126)
Paracrobeles	3	Cephalobidae
Paractinolaimus	5	(46) (see 100)
Paracyatholaimus	6?	(13)
Parahadronchus	5a	(see 100)
Paralongidorus	1d	(see 11,54)
Paramphidelus	3	Alaimidae
Paraphanolaimus	3	Halaphanolaimidae
Paraphelenchus	2	(110); (see Nickle & Hooper in 76)
Paraplectonema	3	Leptolaimidae
Pararotylenchus	1c	(see Fortuner in 76)

TABLE 1. *Continued*

Genus	Feeding type	Family or literature citation
Paratrichodorus	1d	Trichodoridae; (see Decraemer in 76)
Paratripyla	5a	Tripylidae
Paratrophurus	1d	Dolichodoridae
Paratylenchus	1d	(94,122); (see Raski in 76)
Paravulvus	5?	Nygolaimidae
Paraxonchium	5, 8	Aporcelaimidae
Pareudiplogaster	6, 3	(13)
Paroigolaimella	3	(88)
Paurodontus	2, 7	(as *Neotylenchus*); (40)
Pelliodítis	3	(107)
Pelodera	3	(88,107,122)
Peltamigratus	1c	(see 76)
Phasmarhabditis	3	(107)
Placodira	3	Cephalobidae
Plectonchus	3	Brevibuccidae
Plectus	3	(77,88,122)
Pleurotylenchus	1d	Tylodoridae
Pratylenchoides	1b	(see Loof in 76)
Pratylenchus	1b	(see Loof in 76)
Prionchulus	5a	(5,61,69); (see 100)
Prismatolaimus	3?	(see 77)
Pristionchus	3, 5a	Neodiplogasteridae
Prochromadora	3, 6?	Chromadoridae
Prodesmodora	3	Desmodoridae
Prodorylaimium	8	Dorylaimidae
Prodorylaimus	8	Dorylaimidae
Proleptonchus	8?	Leptonchidae
Protocylindrocorpus	3	Cylindrocorporidae (88)
Protorhabditis	3	Rhabditidae
Pseudacrobeles	3	Cephalobidae
Pseudhalenchus	2	Anguinidae
Pseudoaulolaimus	3	Cryptonchidae
Psilenchus	1e	Psilenchidae
Pterotylenchus	1a or b	Anguinidae
Pterygorhabditis	3	Pterygorhabditidae
Punctodera	1a	(see Baldwin & Mundo-Ocampo in 76)
Punctodora	3, 6?	Chromadoridae
Pungentus	1d, 5, 8	(see 31,100)
Quinisulcius	1d	(see 76)
Radopholus	1b	(see Loof in 76)
Rhabditis	3	(88,102,107,122)
Rhabditoides	3	Rhabditidae
Rhabditophanes	3	Alloionematidae
Rhabdolaimus	3	(see 77)
Rhabdontolaimus	3	(88)
Rhadinaphelenchus	1b	(see Nickle & Hooper in 76)
Rhodolaimus	3	Bunonematidae
Rotylenchulus	1a	(57)
Rotylenchus	1c	Hoplolaiminae; (see Fortuner in 76)
Scottnema	3	Cephalobidae; (80)
Scutellonema	1c	Hoplolaiminae; (see Fortuner in 76)
Scutylenchus	1d	Dolichodoridae
Sectonema	5, 8	(see 100)
Seinura	5b	(28,41,43,101,123); (see 100)
Seleborca	3	Acrobelidae
Sphaerolaimus	5a	Sphaerolaimidae
Sphaeronema	1a	(see Raski in 76)
Sphaerularia	7, 2	(91)
Sporonchulus	5a	(see 100)

TABLE 1. *Continued*

Genus	Feeding type	Family or literature citation
Stegelleta	3	Cephalobidae
Stegelletina	3	Cephalobidae
Steinernema	7, 3	(see 76)
Stomachoglossa	(5)	(see 100)
Subanguina	1a or b	(see 76)
Sulphuretylenchus	7 or 2	(see 76)
Synonchium	5	(see 100)
Telotylenchus	1c	Dolichodoridae
Tenunemellus	1d or 2(?)	Ecphyadorphoridae
Teratocephalus	3	(see 77)
Teratolobus	3	Cephalobidae
Teratorhabditis	3	Rhabditidae
Theristus	3, 4 or 6	(see 77)
Thonus	5, 8	(31)
Thornenema	8	Dorylaimidae
Thornia	8?	(see 100)
Tobrilus	5a or 6	(see 100)
Torumanawa	8	Aporcelaimidae
Tricephalobus	3	Panagrolaimidae
Trichodorus	1d	Trichodoridae; (see Decraemer in 76)
Tripius	7, 2	(see 76)
Tripyla	5a	(77,100)
Trischistoma	5a	Tripylidae; (see 100)
Trophonema	1a	Tylenchulidae; (see Raski in 76)
Trophotylenchus	1a	Tylenchulidae
Trophurus	1d	Dolichodoridae
Turbatrix	3	(70,89)
Tylencholaimellus	2	(122)
Tylencholaimus	2	(29,105,122); (see 31)
Tylenchorhynchus	1d	(see Anderson & Potter in 76)
Tylenchulus	1a	(see Raski in 76)
Tylenchus	1f, 2?	Tylenchidae; (122)
Tylocephalus	3	(see 77)
Tylodorus	1d	Tylodoridae
Tylolaimophorus	2	Diphtherophoridae
Tylopharynx	3	Tylopharyngidae
Verutus	1a	(see Baldwin & Mundo-Ocampo in 76)
Westindicus	(5)	(see 100)
Wilsonema	3	(77,122)
Xiphinema	1d	Longidoridae
Ypsylonellus	3	Acrobelidae
Zeldia	3	(126)
Zygotylenchus	1b	(see Loof in 76)

Appendix 2

CP-values of terrestrial and freshwater nematodes in the subclass Secernentea. Feeding group according to Yeates *et al*. (1993) and (Bongers, 1999).

Scaling of Secernentea			Feeding group	Coloniser-Persister value
Order	Sub Order	Family		
Tylenchida	Tylenchina	Tylenchidae	1e	(2)
		Dolichodoridae	1d	(3)
		Hoplolaimidae	1c	(3)
		Pratylenchidae	1b	(3)
		Heteroderidae	1a	(3)
		Meloidogynidae	1a	(3)
	Criconematina	Criconematidae	1d	(3)
		Hemicycliophoridae	1d	(3)
		Paratylenchidae	1d	(2)
	Hexatylina	Neotylenchidae	2,7	2
		Anguinidae	2,7	2
		Iotonchidae	2,7	2
Aphelenchida		Aphelenchidae	2	2
		Aphelenchoididae	2	2
		Paraphelenchidae	2	2
Rhabditida	Rhabditina	Rhabditidae	3	1
		Bunonematidae	3	1
	Cephalobina	Cephalobidae	3	2
		Panagrolaimidae	3	1
	Diplogasterina	Diplogasteridae	3	1
		Tylopharyngidae	3	1
	Teratocephalina	Teratocephalidae	3	3

Appendix 3

CP-values of terrestrial, freshwater and marine nematodes in the subclass Adenophorea. Feeding group according to Yeates *et al*. (1993) and (Bongers, 1999).

Scaling of Adenophorea			Feeding Group	Coloniser-Persister value
Order	Sub Order	Family		
Monhysterida	Monhysterina	Monhysteridae	3,4,6	2
		Xyalidae	3,4,6	2
Desmoscolecida	Desmoscolecina	Desmoscolecidae	3	3
Araeolaimida	Araelolaimina	Plectidae	3	2
		Leptolaimdae	3	2
		Halaphanolaimidae	3	3
Enoplida	Tripylina	Odontolaimidae	3	3
		Bastianiidae	3	3
		Prismatolaimidae	3	3
		Ironidae	5	4
		Tobrilidae	5,6	3
		Tripylidae	5	3
		Alaimidae	3	4
Dorylaimida	Mononchina	Mononchidae	5	4
		Anatonchidae	5	4
	Dorylaimina	Nygolaimidae	5	5
		Dorylaimidae	8	4
		Chrysonematidae	8	5
		Thornenematidae	8	5
		Nordiidae	1d,8	4
		Qudsianematidae	8	4
		Aporcelaimidae	5,8	5
		Longidoridae	1d	(5)
		Belonderidae	1d,8	5
		Actinolaimidae	5	5
		Discolaimidae	5	5
		Leptonchidae	2	4
	Diphterophorina	Diphterophoridae	2	3
		Trichodoridae	1d	(4)