Distribution of Nematodes in Pasture Soil Across Seasons and in Combination with Plant Species and Fertiliser Application

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Abstract

The intensification of pastoral land use has increased with the use of fertilisers and the selection of highly productive pasture plant species to increase pasture production. When choosing a ryegrass cultivar infected with selected endophyte fungus, ploidy choices are vital for determining pasture production. Modern perennial ryegrass cultivars and White clover plant species mixtures and nitrogen fertiliser with seasonal climatic characteristics can influence the soil biodiversity and soil functions in a pastoral system. Pasture soils have unique systems with different soil faunal and microbial communities, including soil-dwelling nematodes and plant species composition. Nematodes have the potential to be indicators of soil processes and condition because of their abundance and ubiquity; they are simple multicellular animals, and highly diverse, taxonomically or functionally or both. They occupy multiple trophic positions in the soil food web and play a significant role in the decomposition of soil organic matter, the mineralisation of plant nutrients and nutrient cycling. The distribution of the nematode community can be influenced directly and indirectly by various management activities, including nitrogen fertiliser application and plant species, and the changing climatic conditions during seasons. The magnitude of these biotic and abiotic impacts was studied through analysis of the nematode community. Our study examined the impact on the nematode community of combinations of low and high rates of nitrogen application, clover presence and absence, and two different ranges of ryegrass cultivars, One50 (diploid AR37) and Base (tetraploid AR37), during two seasons. Sampling was carried out twice a year (autumn and spring) for two years (2013 and 2014) to investigate the distribution pattern between the seasons when host plants are actively growing and nematode activity is high. Soil samples were collected in the North Island of New Zealand from two sites in the Waikato and the Manawatu. Soil nematode assemblages are different between these chosen sites. Soil nematodes were extracted from soil samples, identified, enumerated, and assigned to the total nematode population, the plant parasitic group and trophic groups.

A total of 24 taxa of nematodes were found in this study, comprising 9 plant feeders, 3 fungal feeders, 8 bacterial feeders, 3 predators, and 1 omnivore. However, the range of taxon richness per sample collection period was 12 to 14. Overall, plant feeders (Tylenchids, *Paratylenchus*, *Pratylenchus*), fungal feeders (*Aphelenchus*, *Aphelenchoides*), and bacterial feeders (Cephalobids and *Panagrolaimus*) were the most common nematodes found in both regions. *Meloidogyne* abundance in the Waikato site was relatively high compared with the Manawatu. In contrast, *Longidorous* and *Diplogaster* were more abundant in Manawatu than Waikato. In both study sites, predators and omnivores were relatively low compared with plant, fungal and bacterial feeders; however, c-p 1 taxon of Panagrolaimdae and Rhabditide are most common in autumn.

Significant differences in the nematode community were observed with substantial impact changes (season), especially in the total nematode and Maturity Index (MI) measures. Interestingly, a relatively small difference in nitrogen fertiliser (N) input (in on-farm terms) resulted in a consistent, although not significant, decrease in MI at a high rate of N application. This reflects a proportional increase in resource opportunist nematodes (c-p 1 and 2), probably in response to the pulse of microbial growth post-fertilisation. Ryegrass cultivar/ploidy impacts were observed on the plant-feeding component of the nematode community (Plant Parasitic Index (PPI)). An interesting seasonal contrast between MI (greatest in spring) and PPI (greatest in autumn) shows how these two components of the nematode community respond to food resources and seasonal conditions with PPI relying directly on plant growth. We have used a range of different statistical properties to interpret the complex datasets generated from the soil community and environmental interactions. The analysis approaches taken here is often used for analysis of among-species relationships, but are also increasingly used for functional interpretation of ecosystem networks, including those generated by molecular approaches.

We conclude that the addition of even moderate rates of N to the soil has implications for soil functioning and sustainable use given that a more structured (i.e., greater MI) nematode community is a desirable indicator of soil health. The ability to graphically represent the range of the strength of interactions amongst the members of the nematode community adds new insights to these datasets and facilitates bringing together the results of morphological and molecular studies within a common framework of representation and hence understanding the function of ecosystem networks.

Preface

This thesis is written as a paper, following the format of the international journal, Pedobiologia. This thesis has three chapters: the first chapter contains a general introduction; the second chapter contains a research paper which has an abstract, introduction, materials and methods, results, discussion and references sections; and the third chapter covers the general discussion.

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DEDICATED TO MY LATE FATHER

Mr N P Markandu

List of Publications

The following works are very relevant to this study, and were either published in the refereed journal or presented at the conference during this study period.

Journal Papers

Rohan, T. C., Aalders, L. T., Bell, N. L., and Shah, F. A. (2016). First report of *Meloidogyne fallax* hosted by *Trifolium repens* (white clover): implications for pasture and crop rotations in New Zealand. Australasian Plant Disease Notes11, 1–3. doi: 10.1007/s13314-016-0201-x

Page-Weir NEM, Jamieson LE, Bell NL, **Rohan, T. C**, Chhagan A, Clare G, Kean AM, Davis VA, Griffin M, Connolly PG 2013. Interceptions and hot water treatment of mites and nematodes on root crops from the Pacific Islands. New Zealand Plant Protection 66, 17-28.

Jamieson, L.E., Chhagan, A., Redpath, S.P., Griffin, M. J., **Rohan, T. C.,** Tunupopo, F., Tugaga, A., Connolly, P.G., & Woolf, A. B. (2016) Development of a hot water disinfestation treatment for taro exported from the Pacific Islands New Zealand Plant Protection, 69, 200-206

Conferences

Rohan, T.C., Aalders, L.T., and Bell, N.L. (2013). Determining the propagule size required for *Meloidogyne* species to establish in managed and native soil in New Zealand, *New Zealand Plant Protection 66 (2013): 377* (poster abstract).

Reports

Chhagan A, Woolf AB, Tyson JL, Griffin M, **Rohan, T. C**, Jamieson L (2015) *Development of risk management treatments for root crops from the Pacific Islands: hot water treatments of taro,* Plant & Food Research SPTS No11058. *Science Solutions for Better Border Biosecurity*; www.b3nz.org

Wilson, M., **Rohan, T. C**, Tourna, M., Bennett, K., & Hogg, I. (2014). *Caenorhabditis elegans associated with slugs in New Zealand* (Environmental Protection Authority).

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Chapter 1

General Introduction

1.1 Nematodes

Nematodes, which are unsegmented, unpigmented roundworms or eelworms, are the most numerous multicellular, ancient, and diverse group of invertebrates on Earth (Watson & Mercer, 2000; Wilson & Kakouli-Duarte, 2009, p 44) they are found in almost every conceivable moist habitat, including marine, freshwater or terrestrial habitats, as well as being parasites of humans and other vertebrates, and insects, other invertebrates and plants. They range in size from 0·2mm to over 6m, and, on a global scale, approximately 25,000 nematode species have been described (Yates 2010; Hodda, 2011), but it is estimated that there may be more than one million, not reported (Heywood and Watson, 1995). Many different types of nematodes inhabit soil in large numbers, but they are often unnoticeable due to their cryptic habits and microscopic size, usually 0.5-2.0mm in length.

The nematodes belong to the phylum Nematoda and are divided into two classes, Adenophorea and Secrenentea, which are traditionally divided into 16 orders (Pionar, 1983). The new phylogeny was systematised by De Ley and Blaxter (2002, 2004) and they proposed a new classification for the phylum Nematoda, based primarily on phylogenetic hypotheses resulting from a small subunit rDNA sequence analysis. According to their classification, Nematoda comprises the classes Enoplea and Chromadorea and the subclasses Enoplia, Dorylaimia and Chromadoria. The most traditional analyses suggested mainly two divisions of the phylum, into 'Adenophorea' (largely marine, but also including terrestrial plant and animal parasites) and 'Secernentea' (largely terrestrial, and including many animal and plant parasites); however, molecular analyses show three major divisions. The 'Adenophorea' are split into three divisions and 'Secernentea into one subgroup, according to De Ley and Blaxter (2004). They can be divided into 14 orders (Wilson and Kakouli-Duarte, 2009, p43). Hodda (2011) classified Nematoda into 3 classes, 31 orders, 267 families, 2829 genera and 24,783 species, with fossil taxa represented in 2 genera by 10 species. There are 7 genera and 7 species known only as fossils.

The name Nematoda is derived from the Greek word ‘nema’ meaning ‘thread like’. They are often fusiform like thin rods shape and tapered at each end, or filiform like a thread shape. They are collectively called vermiform and the majority of nematode species are vermiform (having a worm-like appearance) throughout their lives. They exhibit bilateral symmetry and are usually circular in cross-section, but some are vermiform only in the early stages and become swollen as adult females with spherical, pear-shaped or intermediate forms with characteristic organs. Upon swelling these nematodes become sedentary (Viglierchio, 1991).

Nematodes have a cuticular tube within a tube bauplan which is their intestinal tract, with a mouth leading to the stoma, then connected to muscular lumen and oesophagus that lead to a tubular intestine, a rectum and anus with a reproductive system situated in the body cavity. The tail beyond the anus varies in length. Reproductive or copulatory structures can be used to distinguish male and female. They have complete digestive, nervous, excretory and reproductive systems as well as longitudinal muscles. They do not have respiratory and circulatory systems. The sexes are separate, although some species have intersexes. Reproduction is mostly sexual and amphimictic, although some species are parthenogenetic or hermaphroditic (Pionar, 1983; Bird and Bird, 1991).

The head structure of the nematodes has a vast range which facilitates food ingestion and indicates the feeding habits. The head anatomy, characteristic of stoma has some needle-like stylets for feeding (mouth region), but it is not common for all nematodes. The oesophagus can be used to discriminate among different nematode taxa. The typical life cycle of the nematodes consists of an egg, four juveniles and the adults. There is moulting but no metamorphosis. The length of the life cycle varies from a few days to more than a year. The most common plant parasitic nematode life cycle duration is 3 to 6 weeks, but the life cycle of some free-living nematodes is only 1 to 2 weeks. Nematodes are poikilothermic (cold blooded) organism and their physiological activity depend upon an appropriate environmental temperature range, with the optimum between somewhere extremes. The temperature threshold for major functions of many nematodes is about 15^0 C (Viglierchio, 1991). Nematode communities exhibit a seasonal dynamic where the dominant species differs through time as season changes the temperature and quantity and quality of plant litter as a food resource (Neher, 2010).

1.2 Soil biodiversity

Soil is a static and non-living medium which is inhabited by a wide range of living organisms, including bacteria, fungi, algae, protozoa, nematodes, mites, insects, annelids, molluscs and plant roots. The vast range of microbes, and animals live in the soil and constitute part of the soil food web, which contributes to an important role in the decomposition of organic matter and the mineralisation of the plant nutrients it contains (Coleman, 1983; Wardle, 1999; Bardgett 2005; Wall, 2004). Soil nematodes are usually 0.4mm to 1.0mm in length, and are classified either as microfauna or mesofauna (Neher, 2010). Bardgett (2005) categorised soil nematodes as microfauna if their body width is less than 1.0mm.

The role of nematodes in the soil system is based on their feeding habits, and direct excretion of nutrients into the soil, and indirectly through their size, composition and activity in the microbial community. Also, the inoculation of a new substrate takes place through phoretic transport or excretion of viable microbes; plant parasitic nematodes have direct effects on plant communities (Bardgett, 2005).

Increases in fertiliser input, and plant species' composition and richness can affect the biodiversity of soil fauna and nutrient cycling in pastures. Increased use of nitrogen (N) and phosphorus (P) fertilisers on pastures may decrease above-ground plant and below-ground soil faunal diversity, despite greater net primary production and improved litter quality (Ettema et al., 1999; Donnison et al., 2000).

1.3 Environmental disturbance

Neher (2005) suggested that disturbance on invertebrates can be divided into different categories: by types, chemical, physical, and biological; by characteristic, intensity, frequency, regularity, and magnitude; by seasonal or cyclical or by larger scale or smaller scale. Environmental disturbance may be specific to an ecosystem, geographic location or local climate. Nematodes at the genus level are ideal for interpreting disturbances in the system, but it is generally expensive and impractical to perform a complete community description so identifying suitable key taxa is needed to represent critical functional and trophic positions for each ecosystem and region to represent the contrasting soil types and climate (Neher, 2005). Nematode community indices can be interpreted with these disturbances as they represent between five and eight trophic groups (Yeates et al., 1998), and they occupy primary, secondary and/or tertiary consumer levels in soil food webs. Abiotic ecological factors influencing nematodes in the soil environment include soil structure, soil water, soil aeration, chemical properties of soil and soil temperature (Norton, 1978).

1.4 Nematode as indicators

Soil nematodes comprise a key group in soil processes and are considered as indicators of soil conditions (Yeates, 1979, 2003; Yeates and Bongers, 1999; Wall, 2004; Bargett, 2005). Wilson and Kakouli-Duarte, (2009) believed that nematodes are the most ideal organism to use as an environmental indicator compared to any other group of organisms. Nematodes are easily extracted from soil and can then be counted. The feeding groups are identified under a microscope; with substrate lighting, the nematodes appear transparent due to the internal anatomical and morphological characteristics; they can be easily examined and grouped according to the type of food they consume depending on the morphology of the mouth parts. There are five trophic groups, which are the most common feeding groups of nematodes: plant feeders/herbivores, bacterial feeders/bacterivores, fungal feeders/fungivores, predators and omnivores (Yeates et al., 1993). Nematodes are one of the most abundant and diverse groups of soil organisms, and they occupy key trophic positions in the soil detritus food web. Nematodes have, therefore, been proposed as useful indicators of the pastoral soil environment, because they respond to changes in the soil system and enable understanding of the impacts of abiotic and biotic factors. The most common feeding group in the soil are plant feeders and bacterial feeders. Looking at the whole nematode community and the balance between different feeding types, it is possible to assess the health and functioning of the soil. Use of soil nematodes in environmental studies, because they have a permeable cuticle, allows them to respond to a range of reactions to pollutants corresponding with the restorative capacity of soil ecosystems; some nematodes have resistant stages, such as cryptobiosis or cysts that allow them to survive inactively during environmental conditions unfavorable to growth and/or development. Further, nematodes have heat shock proteins that are highly conserved (Hashmi et al., 1997); expression of these proteins is enhanced when exposed to stresses, such as heat, metal ions, or organic toxins (Kammenga et al., 1998). These proteins could act as biomarkers for ecotoxicological assessment of soils (Guven et al., 1994, 1999; Kammenga et al., 2000). Although nematodes represent a relatively small amount of biomass in the soil, occupying multiple trophic levels are important in the soil environment (Barker and Koenning, 1998), since their feeding habits are closely related to oral structure, and their trophic roles are easily inferred. There are benefits of using nematodes as bioindicators in environmental studies, because they occupy a high diversity and density in every soil, live in the soil capillary water, respond rapidly to disturbances, perform a vital role in soil food-webs, are easily assigned to trophic groups and are in direct contact with dissolved compounds in the soil water through their permeable cuticle (Bongers, 1999).

1.5 Nematode community Indices

Bongers (1990) classified nematode trophic groups as a colonizer-persister (c-p) ranging from 1 to 5. Nematodes with a c-p value equal to one are short-lived, have high fecundity, and feed on enriched media, whereas those of c-p value five have a large body size, longer lifespan, low fecundity, and are susceptible to disturbance (Bongers, 1990). There are many general indices of biological diversity; for nematodes, specific tools have been developed, which are Maturity Index (MI) (Bongers,1990; Bongers et al., 1991), the sigma-MI (Yeates, 1994), the MI (2-5) (Bongers & Korthals, 1994), the PPI/MI-ratio (Bongers et al., 1997) and the 'guild' concept (Bongers & Bongers, 1998). All these indices are established on the allocation of taxa based on life strategy, from colonizers (r-strategists in the broad sense) to persisters (K-strategists).

1.6 New Zealand agriculture and pasture nematodes

New Zealand is a set of islands in a remote location in the mid-latitude westerlies of the South Pacific Ocean. The two main islands stretch across a wide span of latitudes (34–47° south), with mountain ranges affecting the patterns of wind and rain. This varied geography contributes to marked variations in climate across the country, particularly between the east and west, and to extreme weather events, such as flooding and droughts.

The combination of relatively high rainfall and warm temperature areas are suitable for dairy farming in New Zealand. The northern, western and central parts of the North Island are mainly dairying areas; drier areas in the eastern part of both islands are suitable for sheep farming.

The New Zealand pastoral agriculture sector is the country's largest exporter and one of the largest users of New Zealand's natural resources. Hence it is a major contributor to the economy of the country, and it has a considerable effect on the condition of the land and fresh water in New Zealand. The area of livestock grazing on pastoral farms in New Zealand remained relatively stable between 1996 and 2012. Over 40% of the total land area (10.4 million ha) is in pasture and supports arable cropping; the pasture land consists of low producing and high producing grasslands. Over recent decades, the area of dairy farming in New Zealand has increased by 28% through converting from sheep and beef farms; this is due to the huge demand for increased dairy production driven by strong commodity prices (Ministry for the Environment & Statistics New Zealand., 2015).

In some areas, especially in Canterbury, Otago, and Southland, the farming nature and its intensity has changed. The changes have put more pressure on the land, and on freshwater and marine environment. Ninety soil types are found in New Zealand (Molly 1998): mainly South Island, and the southern and northern parts of the North Island have sedimentary soils, whereas central North Island soil are pumices, and Waikato and Taranaki north-central and western North Island soils are derived from volcanic ash. Soils are differentiated based on climatic effects on the parent material. Some soil types depend on local variation in rainfall and topography. In the northern parts of New Zealand, the soil is weathered more rapidly by temperature which increases the rate of chemical reaction. Based on colour, texture, parent material, climate or topography, New Zealand soils are grouped into 16 main classes (Molly, 1998).

Pastoral ecosystems have a unique soil food web with groups of soil-inhabiting organisms, such as bacteria, fungi, protozoa, nematodes, arthropods and earthworms. Grazed grasslands have unique soil systems with abundant and diverse soil faunal and microbial communities, including soil inhabiting nematodes (Bardgett and Cook, 1998). The biological status of pasture soils can be influenced by various management activities, including rate of nitrogen fertiliser application and plant species interactions, and the magnitude of these impacts can be studied through analysis of the nematode community. The pastoral sector is this country's largest export sector and one of the largest users of New Zealand's natural resources. A better understanding of the impacts of agricultural land usage will help improve the sustainable use of resources. In New Zealand pastures, agricultural productivity has recently increased through intensification of pasture systems. Increased use of nutrient inputs and selection of highly productive pasture plant species has led to increased pasture production. In the pastoral system, some nematode species are parasites of plants (e.g., root-knot and cyst nematodes) or farm animals (e.g., Ostertagia) and they cause considerable reductions in plant and animal performance. However, the majority of nematode species feed on microbes or other nematodes and have beneficial effects on the soil by increasing the availability of plant nutrients, acting as biological controls for insect and slug pests, and transporting beneficial microbes to root surfaces. Apart from that, all non-parasites nematodes are collectively referred to as free-living nematodes.

Yeates (2010) summarised that from New Zealand and the southern hemisphere, the nematode fauna has a total of 708 species, including soil free living, 151, and plant parasitic nematodes, 157. He made a checklist of New Zealand species based on that of Maggenti et al. (1988). The first free-living nematodes were described in New Zealand by N. A. Cobb in 1904. Hoy (1954) described an insect pathogenic nematode. In New Zealand pastures, most are less than 2mm long and barely visible to the naked eye. Most nematodes associated with pastures live in the soil and are either beneficial or neutral to human interests, and the majority of these are species that feed on bacteria or fungi (Yeates, 1999). Other nematodes include those that are predators on small soil organisms, including other nematodes, or are parasites of larger animals, including insects, slugs and snails. All of these nematodes together commonly comprise 80–90% of nematodes in pasture soil. Typical pasture soils in New Zealand may contain up to six genera of plant-feeding nematodes, usually comprising exotic species. Some nematode species feed only on grasses (e.g., the pin nematode species, *Paratylenchus nanus*), some on clovers and not grasses (e.g., clover cyst nematode *Heterodera trifolii*, and the root-knot nematodes, *Meloidogyne hapla and M. trifoliophila*), while others have mixed plant hosts (e.g., the lesion nematodes, *Pratylenchus crenatus, P. penetrans,* and stubby root nematode, *Paratrichodorus minor*) (Watson & Mercer, 2000). Other genera occur on a much more localised basis. These include four species of ectoparasitic nematode, *Helicotylenchus canadensis, Subanguina radicicola, Tylenchorhynchus maximus* and *Geocenamus nanus* (Mercer, 1986; Yeates & Wouts, 1992; Yeates, 1992). Other localised genera include *Ditylenchus, Mesocriconema, Longidorus* and Xiphenema. The plant feeding nematode fauna in New Zealand pasture is generally less diverse than in comparable situations elsewhere (Cook & Yeates, 1993). Rohan et al. (2016) found that the root-knot nematode, *Meloidogyne fallax*, is reported for the first time on white clover roots in New Zealand and they have mixed plant hosts. Yeates (2010) estimated there were 18 alien and 47 endemic species of PPN in New Zealand. Clearly, if a PPN is globally cosmopolitan then it is likely, but not definite, to be exotic to New Zealand. Additionally, the full host range of PPN species is often unknown and this presents a major problem in determining their status as native or alien (Singh, 2013). We know, for example, that *M. fallax* is found in agricultural situations in a number of temperate countries, including New Zealand, which may suggest that it is alien to New Zealand. However, this nematode has also been found associated with native tussock grassland in New Zealand (N Bell pers comm). It is unclear, then, if this is a New Zealand native PPN that has spread to agriculture and thence overseas or is an alien PPN that has invaded New Zealand and spread to our native estate. Knowledge of prior and current community composition is required to determine native or invasive status of species (Litchman, 2010). Unfortunately, information on nematode communities is rarely available because most of the world has not been thoroughly surveyed. Even where there are surveys, many species are not identified to species level, because of dwindling taxonomic expertise (Coomans, 2002).

Zydenbos et al. (2011) considered that plant-parasitic nematodes affect the establishment and persistence of New Zealand pastures, particularly the clover portion. The root-knot (*Meloidogyne spp.)* and cyst *(Heterodera sp.)* genera of nematodes are the most damaging to clover, with two species of root-knot (*M. trifoliophila and M. hapla*) and a single species of cyst nematode *(H. trifolii)*, which are predominant in New Zealand pastures. While both genera are found in many North Island pastures, in South Island pastures it is more common to find only cyst nematodes (Mercer & Woodfield, 1986; Skipp & Christensen, 1983). The impacts of nematodes and research to mitigate their effects on pasture production and persistence have been reviewed by Mercer et al. (2008). Plant damage is caused by disruptions of root function, including nitrogen fixation. A region-wide series of plots of pasture swards were chemically treated to remove nematodes and showed an increase in clover yield of 40% and a nitrogen fixation of 55% (Watson et al., 1985); similar results have been found in other field and glasshouse studies around New Zealand (Mercer et al., 2008).

Abiotic factors (e.g. drought) that impact on nematode damage include soil temperature, with yield loss increasing with increasing temperature (Mercer et al. 2008), and soil moisture, which interacts with soil temperature to exacerbate damage. Nematodes are one of the biotic factors influencing damage to clovers, traditional plant breeding has been used to develop nematode-resistant or -tolerant plants to improve field performance (Mercer et al., 2008) Some microbes may have an impact on nematode populations (Hay & Skipp, 1993) in established pastures, although there has been little research to quantify this.

1.7 Mixed cropping

Before 1990, New Zealand dairy farming was based on low N fertiliser applications, and mainly relied on N inputs from clover N fixation (Ledgard & Steele, 1992). White clover benefits from animal performance (Harris et al*.,* 1997), as well as environmental and cost benefits. Mixed swards of ryegrass and white clover cultivars have the potential to influence each other when in association (Camlin, 1981). New Zealand soils were originally low in nutrients and fertility, and enhanced by P fertilizer application together with N fixation by clovers (Ledgard et al., 1996). White clover is generally sown in a pasture mix at 3-4 kg/ha, usually a mix of two cultivars based on leaf size and stolon growing point density. White clover rarely reaches its potential yield because of the various abiotic (particularly drought) and biotic restrictions. Chief among the nematode parasites are clover root-knot nematode (*Meloidogyne trifoliophila*—CRKN) and clover cyst nematode (*Heterodera trifolii*—CCN) (Watson & Mercer, 2000).

One of the sustainable grassland management measures is mixed cropping, that is, introduction of white clover due to its ability to fix atmospheric nitrogen and reduce the reliance on inorganic nitrogen fertiliser (Van Eekeren et al., 2009). White clover is an important component of New Zealand pastures because of its contributions to N fixation, feed quality and complementary growth patterns (Caradus et al., 1995). Species mixtures reveal that in mixed cropping, there are many potential advantages under various conditions, both in temperate and tropical agriculture, such as higher overall productivity, better pest and disease management, improved ecological services and greater economic profitability.

1.8 Nitrogen (N) fertiliser, clover and Pasture soil fertility

Pasture soil fertility is improved with nitrogen fixation by legumes and by applying urea fertiliser. A big issue in recent years is the over usage of nitrogen fertiliser leading to nitrogen leaching into other ecosystems, particularly waterways. Another effect of increased nitrogen fertiliser use is pasture swards changing to become grass dominated. Increases in nitrogen fertiliser usage activities have greatly changed the composition and functioning of soil communities. These factors can influence the soil biodiversity including soil nematode fauna and nutrient cycling in pastures. To date, there have been few studies on the effects of modern perennial ryegrass cultivars-clover mixtures and nitrogen fertiliser on nematode fauna. N fertiliser use in New Zealand has increased seven-fold from 1991 to 2009, with N use on dairy farms in 2009 averaging 120 kg /ha (Ministry of Primary Industries, 2012). More than 400 kg /ha is used on some farms. In recent years, there has been an increasing use of N fertiliser on hill country sheep and cattle farms, as well as on dairy farms (Campbell, 2005). Increased use of nutrient inputs and selection of highly productive pasture plant species has led to increased pasture, undoubtedly leading to higher levels of production (Clark et al., 1996). Although clovers can support reasonable levels of pasture and hence animal production from N fixation, N fertiliser use will undoubtedly lead to higher levels of production. The best results would be achieved with clover content of 30–40% and nitrogen fertiliser rates of 100–200 kg/ha/year (Clark et al., 1996). In 2000, it was estimated that the average dairy farm was applying between 80 and 100 kg /ha/year (Caradus and Clark, 2001), but since then it has increased dramatically. Annual rates no greater than 150-200 kg/ha are recommended to achieve economic efficiency, and to limit leaching losses and negative environmental impacts. These nitrogen sources contribute to the nitrogen cycle, pasture production and nitrate leaching. Urea is the most commonly used form of N fertiliser in pasture-based systems within New Zealand. Although application of N fertiliser increased the total herbage production, numerous studies have shown a continuous decline in clover content with increasing amounts of N fertiliser (Ball & Field, 1982; Harris & Clark, 1996; O'Connor, 1982).

On the other hand, increased food supply associated with increased soil fertility often stimulates an abundance of soil invertebrates (Yeates, 1976; Cole et al., 2005; Curry et al., 2008), particularly bacterial-feeding, plant feeding, and plant-associated nematodes (Yeates and Bongers, 1999).

In recent years in New Zealand, pastoral agriculture culture has increased production through more intensively grazed pastures that have been managed to increase production through increases in nutrient inputs (Haynes and Williams, 1993; MacLeod and Moller, 2006). Increases in fertiliser inputs and livestock numbers affect plant species composition and richness, and this can affect the biodiversity of soil fauna and nutrient cycling in pastures (Parfitt et al., 2010).

Clover plants, nitrogen fertiliser and imported supplementary feed are the main sources of nitrogen (N) in New Zealand clover-based pastures. These nitrogen sources contribute to the nitrogen cycle, pasture production and nitrate leaching;

nitrate leaching increases environmental pressure on waterways. The nitrogen fertiliser losses from the farm system can be considered as “direct” or “indirect”. Pasture dry matter production and consequent N consumed and N excreted by the grazing animals are considered as indirect losses. Ammonia volatilisation, nitrous oxide emissions (and N_2 gas) and nitrate leaching are direct losses from nitrogen fertiliser application (Shepherd and Lucci, 2011). The nitrogen cycle in grazed pasture systems were studied by McLaren and Cameron (1996). N surplus can accumulate in the soil organic matter in some cases, is lost from the farm as gaseous emissions, or leached (mainly as nitrate) below the plant root zone into sub-surface waters, and eventually enters groundwater and surface water (Glassy et al., 2013).

1.9 Sustainable intensification

Food security is an urgent global challenge. The global population will continue to grow and global demand for food will increase to feed a predicted 9 billion people by roughly the middle of this century (Garnett et al., 2013). There is higher consumption and a greater demand for processed food, meat, dairy, and fish, all of which add pressure to the food supply system. At the same time, food producers are experiencing greater competition for land, water, and energy, and there is a need to consider the many negative effects of food production on the environment. Growing competition for land, water, and energy, in addition to the overexploitation of fisheries, will affect food production, and there will be an impact of the food system on the environment. The effects of climate change are a further threat. However, the world can produce more food and can ensure that it is used more efficiently and equitably (Godfray et al., 2010). Understanding the future environmental impacts of global crop production and how to achieve greater yields with lower impacts requires quantitative assessments of future crop demands, and how different production practices affect yields and environmental variables.

Sustainable intensification has been defined as a form of production wherein “yields are increased without adverse environmental impact and without the cultivation of more land” or producing more food from the same area of land while reducing the environmental impacts, but it includes a range of farming practices, from specific agro-ecological methods and practices used in commercial agriculture, to biotechnology. Sustainability of pastures can be considered that require relatively few inputs, retain and cycle nutrients efficiently, produce forage of sufficient quality and quantity to support animal gain or hay production and economic goals, and are relatively resilient to climate variability, pest pressure and outbreaks (Scott et al., 2000). They may also contribute to preserving biodiversity, maintaining water quality and quantity, and potentially mitigating future changes in atmospheric CO2 concentrations by sequestering carbon in the soil (Kemp et al., 2000; Franzluebbers, 2005).

In New Zealand, the area of pastoral farming which is grazed by livestock remained relatively stable between 1996 and 2012, but in some regions the nature of farming and its intensity have changed. These changes have put more pressure on land, and on our freshwater and marine environments**.** Intensive farming is involved with higher stocking rates, repeated cultivation, and increased inputs including fertilisers, supplementary feed, water for irrigation and livestock, pesticides, herbicides, and energy to produce more food from the same area of land.

1.10 Pasture species

The New Zealand pasture system is mainly a mixed pasture with perennial ryegrass and white clover. Selection of forage is important and can determine pasture production. Ryegrass, clover, cocksfoot, and tall fescue are the main pasture species in New Zealand. Choosing a ryegrass cultivar is important and can determine pasture production. Endophyte selection, heading dates, aftermath heading, ploidy choice and seasonal productivity are the main selecting criteria to choose the cultivar. Perennial ryegrass adapts to a wide range of temperate climates and fertile soils throughout the world (Jung et al., 1996). It is valid for its fast establishment, palatability, high yields of quality herbage, and tolerance to a range of conditions and grazing management. It is well suited to mild, moist climates and free draining highly fertile soil, which conditions are very common in New Zealand and some other countries (Peeters, 2004; Chapman et al., 2011). Ryegrass plants in pastures in New Zealand are usually infected with a fungal endophyte which provides protection from different insect pests, and discourages overgrazing by stock, thus enhancing ryegrass persistence. The ability of *Neotyphodium* endophyte-infection for pest resistance on valuable forage grasses has resulted in the incorporation of new strains into a range of cultivars, such that they are currently the most widely distributed plant biotechnology product in New Zealand pasture land (Bell et al., 2009). Novel or selected endophytes have been introduced into the New Zealand market in four stages: Endosafe in the early 1990s, AR1 in 2001, NEA2 and Endo5 in 2005, and AR37 in 2007. Currently, AR1 novel endophyte has the largest percentage in the New Zealand market. To maintain protection from insects and to minimise livestock health issues, novel strains of endophytes, such as AR1 and NEA2, have been developed. These endophytes do not contain neurotoxic lolitrem and ergovaline, but contain high levels of peramine, an alkaloid to which the Argentine stem weevil is very sensitive. Ryegrass plants in pastures in New Zealand are usually infected with a fungal endophyte which provides protection from different insect pests, and discourages overgrazing by stock, thus enhancing ryegrass persistence.

The recently released AR37 endophyte does not produce peramine, lolitrem or ergovaline alkaloids. However, it does produce epoxy-janthitrem compounds which confer pest deterrence to a wider range of invertebrate pests than the AR1 endophyte. Some staggers may still occur in livestock grazing on AR37 infected ryegrass. Farmers in the warmer areas of New Zealand, where there is the greatest pest challenge to ryegrass productivity and persistence are encouraged to sow AR37 ryegrasses. The intensification of pastoral land use in New Zealand has increased with the use of fertilisers and the selection of highly productive pasture plant species to increase the pasture production. Choosing a ryegrass cultivar with the criteria of endophyte selection, ploidy choice, heading dates, aftermath heading and seasonal productivity is vital and determines pasture production. Ploidy refers to the number of chromosomes per cell in a plant; a diploid ryegrass has two, while a tetraploid ryegrass has four. These differences create differing plant characteristics giving both advantages and disadvantages for each type. Diploid plants have more tillers per plant and are more tolerant of over grassing due to the lower water content per cell. Generally, diploids are recommended in higher stocking rate systems where overgrazing and pugging may occur, and attack by the Argentine stem weevil is likely. Drawbacks are lower metabolizable energy (because of lower ratio of soluble carbohydrates to fibre) and less clover friendly (due to lower, denser growth habit can shade clover). Tetraploids grow more upright, are clover-friendly plants, more palatable, and are preferred by grazing animals. However, tetraploid ryegrasses are less robust and require more careful management under stressful conditions, especially wet and drought conditions. They have a bigger cell size and have a higher ratio of cell contents (soluble carbohydrates) to cell wall (fibre), indicating that they have a higher water content per cell. Tetraploids are preferred by the Argentine stem weevil.

1.11 Forage Value Index

Dairy NZ Forage Value Index (FVI) is an independent, profit-based and region-specific evaluation system for perennial ryegrass cultivars. It gives more information to farmers to select the best cultivar with more confidence for their region and requirements to make more profit. This rating system has basically been developed on grass-based dairy production systems when cultivars were sown in ryegrass monocultures and under high levels of soil fertility with high nitrogen (N) fertiliser inputs and with defoliation by mowing or grazing. However, sowing perennial ryegrass in a mixture with white clover is a standard practice in New Zealand. Independent information on the relative rating of economic performance of pasture cultivar is important for farmers to select the cultivars according to different options. DairyNZ has developed the forage evaluation system to rate perennial ryegrass cultivars according to a forage value index (FVI; $/ha) based on the economic value of seasonal dry matter (DM) production in New Zealand dairy production systems. FVI is an evaluation tool, giving economically-based ranking indices for perennial ryegrass cultivars in New Zealand. Indices to rank perennial ryegrass cultivars based on their relative economic benefit to grass-based dairy production systems have been developed in Ireland and New Zealand during the last three years: Pasture Profit Index (PPI) in Ireland (McEvoy et al., 2011), and Forage Value Index (FVI) in New Zealand (Chapman et al*.,* 2012). FVI currently includes only seasonal dry matter (DM) yield, which is one of the three primary traits of grasses, and other primary traits, such as nutritive value and persistence / stability will be included progressively. Phenotypic data of dry matter yield is collected from the National Forage Variety Trial (NFVT) operated by the

New Zealand Plant Breeding and Research Association (NZPBRA). NZPBRA was formed by a group of seed companies and is involved in developing and marketing improved plant varieties for the New Zealand pastoral and arable sectors (Dairy NZ). These cultivars evaluating trials are short-term trials (up to 3 years), and are conducted using only perennial ryegrass monoculture and high nitrogen fertilizer inputs.

Ryegrass species vary from their persistence or stability as to how long they live or persist. Annual, Italian, short rotation, and perennial can persist less than one year, 1-2 years, 2-5 years and 5 years respectively, under suitable conditions (Dairy NZ). Perennial ryegrass (PRG) is the most widely-sown species in New Zealand pastures and has been intensively researched since the 1920s (Hunt and Easton., 1989). However, as late as 1980, the discoveries were made that the endophytic fungus, *Neotyphodium lolii*, caused ryegrass staggers (Fletcher & Harvey, 1981), and also protected the plant from the Argentine stem weevil (ASW) (Prestidge et al., 1982). Perennial ryegrass is the most widely sown grass in New Zealand as it grows well in a wide range of conditions, is easy to establish and manage, and provides high animal performance; generally, it is mixed with white clover. Unfortunately, some of the antiherbivore alkaloid compounds produced by the symbiosis also impair animal performance (Stuedemann and Hoveland, 1988; Fletcher et al., 1999).

There are, however, some compounds which are found in roots and are thought to be responsible for deterring root-feeding pests (Patchett et al*.,* 2008) including insects (Popay & Gerard., 2007) and nematodes (Elmi et al., 2000; Timper et al., 2005). Given that many compounds in roots are exuded into the soil around the roots (rhizosphere) as part of normal root functioning (Bais et al., 2006), and that these have been shown to affect the structure and activity of microorganisms in the rhizosphere of many plant species (Costa et al., 2006), it is possible that endophytes could influence organisms other than the targeted pests that inhabit this important and dynamic habitat. Various nematodes may affect ryegrass growth in pasture (Yeates & Barker, 1986; Watson et al., 1986) but their real importance has not been established yet. However, Tozer et al. (2014) found that lesion nematodes (*Pratylenchus spps*) is higher in tetraploid cultivars.

1.12 Environmental impact

N surplus from N inputs to a farm by N fertiliser, N fixation, and imported feed can accumulate in the soil organic matter in some cases, or is lost from the farm as gaseous emissions or leached mainly as nitrate below the plant root zone into sub-surface waters and eventually enters groundwater and surface water (Glassy et al., 2013). Land-use intensification is generally associated with the biological community in soils becoming dominated by species with shorter generation times, smaller body sizes, rapid dispersal, and a higher incidence of asexual reproduction (Schon et al., 2008).

1.13 Monitoring soil environmental factors

Land use and management practices influence three key factors, such as food resource, physical disturbance, and habitable space (soil pores), all of which have a major influence on the diversity and abundance of the soil's biological community.

Soil nematodes are abundant and omnipresent in the ecosystem and play an important role in the soil ecological process. Monitoring their activity in response to natural or human-induced impacts has become a key function of soil quality monitoring. Soil nematodes occupy a central position in the soil food web occurring at multiple trophic levels, and, therefore, they have the potential to provide insights into the condition of the soil food webs. N fertiliser inputs and plant species composition can influence the ecological process in the pasture ecosystem. These effects on the composition of plant parasitic and free-living soil nematode communities are poorly known. Thus, nematodes can be used as bioindicators to study the effects of the intensification of pasture systems. A better understanding of the impacts of agricultural land usage will help improve the sustainable use of resources.

1.14 The effects of seasonality and pasture management practices

Climate is the average weather conditions over a period of time, and includes atmospheric conditions, temperature, precipitation and humidity, air pressure, wind velocity and direction, sunlight and other radiation. Whether directly or indirectly, it significantly determines the distribution and diversity of organisms.

The climate and weather around New Zealand are influenced by a number of factors. These include geography position on the globe, the time of year, natural drivers of variability, and human activities (Ministry for the Environment & Stats NZ.,2017) Studying the impacts of a changing climate is difficult in natural and human systems that are typically variable and complex. Average rainfall varies around the country and with the season: the annual pattern of increases in the west and south of New Zealand and decreases in the north and east of the North Island. Droughts increase in severity and frequency in most areas, except for Taranaki, Manawatu, the West Coast and Southland (Ministry for Primary Industries, 2013 and Ministry for the Environment, 2016). Soil moisture or drought is one indicator of a range of potential future impacts for agriculture and other primary industries. Impacts are regionally and locally specific and may be both positive and negative. Alongside drought, for example, impacts may include: change in the yield and quality of pasture, trees, broad-acre crops such as wheat, barley, and oats, and pasture species; changes in pressure from weeds, pests, and diseases; stress on animals and plants from increased warm days (above 25^0C); and water shortages and increased irrigation demand (Clark et al., 2012). Warming is greatest at higher elevations and during the summer/autumn. The average land surface temperature has increased by 1°C since 1909. The 2012–13 drought was one of the most extreme New Zealand had experienced in the previous 41 years and was unusual for being especially widespread, affecting the entire North Island and the west coast of the South Island (Ministry for Primary Industries, 2013). Like many plant-microbe relationships (Compant et al., 2010), grass-endophyte symbioses have been shown to be sensitive to climate change factors, such as elevated atmospheric CO_2 concentrations, elevated temperatures, and altered water availability. The majority of this symbiosis work has been performed on tall fescue, although perennial ryegrass pastures have also been shown to be sensitive to climate change (Newton et al., 2010). As global climate changes, geographical distribution of plant-parasitic nematodes can expand into cooler regions when temperatures increase. Yeates & Boag (2003) described that plant-parasitic nematodes and their host would migrate together. Increasing soil temperatures affect the nematode community leading to a shift to a higher percentage of fungivores and plant parasitic genera (Ruess et al., 1999), because the nematode life cycle is temperature dependent. Also, the life cycle would move more rapidly due to that extra generation per year in some areas having a short growing season, and the plant-parasitic nematodes typical of those crops will follow (McSorley, 2011).

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Chapter 2

Distribution of Nematodes in Pasture Soil Across Seasons and in Combination with Plant Species and Fertiliser Application

2.1 Introduction

New Zealand's pasture-based livestock farming system utilises mainly perennial ryegrass *(Lolium perenne L)* and white clover *(Trifolium repens* L) for grazing ruminant animals, including dairy cattle, sheep, beef and deer, to graze fresh pastures throughout the year in open paddocks (Kemp et al., 1999; Zaman et al., 2008). The pastoral sector is this country's largest export sector and one of the largest users of New Zealand's natural resources. In New Zealand, pastoral productivity has recently increased through intensification of pasture systems. Increased usage of nutrient inputs and selection of highly productive pasture plant species has led to increased pasture production. Thus, pasture soil fertility is improved by nitrogen fixation by legumes and by applying fertiliser. A big issue in recent years is the increased usage of nitrogen fertiliser leading to nitrogen leaching into other ecosystems, particularly waterways. Another issue is changing pasture swards to grass-dominated pasture. Therefore, grassland management practices are likely to be directly and indirectly impacting on the soil biota and their functions (Bardgett, 2005).

Grazed grasslands have unique soil systems with abundant and diverse soil faunal and microbial communities, including soil inhabiting nematodes (Bardgett and Cook., 1998). They are an important component of the pasture soil ecosystem, because they occupy multiple trophic positions in the soil food web, including root herbivores, bacterivores, fungivores, as well as omnivores and predators, nematodes play a significant part in the decomposition of soil organic matter, mineralisation of plant nutrients and nutrient cycling (Griffiths, 1994), and they occupy a key position in the detritus food web (Ingham et al., 1985; Freckman, 1988). They are sensitive to soil environmental change via direct contact with dissolved compounds in the soil water through their permeable cuticle. Nematodes can be easily extracted from soil and assigned to trophic groups (Yeates et al., 1993). All these characteristics make nematode sensitive indicators that provide a measure of the biotic and functional status of soil (Bongers & Ferris, 1999; Neher, 2001).

Soil nematodes rely almost entirely on plant-derived resources so that the plant community changes may significantly influence the availability of soil resources, thus altering the soil nematode communities (Yeates, 1999; Pollierer et al., 2007). Soil microhabitat factors like soil pH and moisture affect soil nematode communities, which are very sensitive to changes in soil properties (Chen et al., 2013). Clearly then, changes in food resources and habitat with environmental conditions in seasons and other management practices, like the selection of plant species, fertiliser application and biological interactions may affect soil nematode community composition and structure in the pasture soil (Yeates, 2006).

The spatial and temporal distribution of nematode populations are patchy (Nannipieri et al., 2002) and depend on various abiotic and biotic factors including their seasonal variations and foraging strategies. Studying nematode populations in a sequence in which the climate, soil, or vegetation changes are useful for illustrating the effect of these factors on population composition. A series of samplings is necessary to describe the pattern of development of soil nematode populations, but its results vary with sampling frequency. More frequent sampling reveals the life stages of genera like *Meloidogyne* (Yeates, 1973a); however, pre- and post-cropping samples may be adequate for some economically important species like cyst nematodes (Southey, 1978). For wider ecological studies, quarterly (Yeates, 1971) or monthly samples are used (Yeates, 1967; Yeates, 1978a, b) and these studies explain distinct seasonal cycles in the most abundant genera. Most sampling programmes are carried out in less than a 12-month period. In contrast, Yeates (1982) carried out 36 consecutive monthly samplings and observed month to month changes and year to year differences related to primary production in the sampling area. He observed annual cycles of populations of many genera, which gave better correlations with plant herbage production than soil temperature or moisture, and year to year differences with differing patterns of plant production. Neher's (2005) research suggests that mid-summer is an optimal month to detect any soil disturbance impacts on nematode communities but other studies indicated autumn or spring as optimal (Neher, 1999; Neher et al.,2003) so that different times of the year may be suited to different groups of bio-indicators.

Understanding how nitrogen addition affects taxonomic richness and composition of soil nematodes is challenging because of the complex interactions between the direct effects of nitrogen addition on soil biota, and indirect effects mediated by altered plant community composition (Wardle, 2002). Liang et al. (2009) and Sarachandra et al. (2001) have documented that nitrogen addition to soil can inhibit nematodes. Generally, nitrogen addition decreased total nematode abundance and diversity, but responses vary among trophic groups, such as reduced numbers of root herbivores, fungivores and omnivores-predators, but increased numbers of some opportunistic bacterivores in forests (Murray et al., 2006; Xu et al., 2007; Sohlenius et al., 1984,) grasslands (Sarachandra et al., 2001; Murray et al., 2006; Wei et al., 2012; Zhao et al., 2010) and croplands (Liang et al., 2009; Hu et al., 2010). Schon et al. (2011) found that plant-feeding and bacterial-feeding nematodes stimulated with the use of N fertiliser through increased pasture production, lifted the quantity and quality of plant residue inputs. Total mesofauna and nematode abundances were, on average, higher at high N concentrations, such as nematodes, *Panagrolaimus* and *Heterocepholobus*, were more abundant at high N, and the nematodes, *Diplogaster* and Rhabditidae, were more abundant with low soil bulk density and high N combination. Differential responses of the soil nematode community composition and diversity to nitrogen addition often vary with time after application. For example, in a long-term fertilisation experiment in northern China, Liang et al. (2009) found that nitrogen fertilisation initially decreased the abundance of root-feeding nematodes, but increased their abundance the following season. However, another study (Van der Wal et al., 2009) reported that the diversity of soil nematodes increased with increasing plant productivity after nitrogen addition. Nitrogen addition can affect soil nematode communities through changing the patterns of below ground C allocation to soil biota in forest ecosystems (Ruess, 1999). It must also be noted that responses of soil nematodes to nitrogen addition often vary with application time, soil type, sampling depth, and ecosystem type. Song et al.’s (2016) study results showed decreases in generic richness but unchanged abundance of nematodes, and increased bacterivores and decreased fungivores and omnivores-predators, in grassland and agriculture field studies (Wei et al., 2012; Azpilicueta et al., 2014; Pan et al., 2015).

One of the important factors inhibiting soil nematode abundance after nitrogen addition is soil acidification following nitrogen addition (Zhao et al., 2010; Li et al., 2010). In other studies, Nitrate (NO_3^{2}-N) and Ammonium (NH_4^{+}-N), which are two of the available forms of N for plant uptake, were found to be negatively correlated with root herbivores and fungivores (Liang et al., 2009; Rodriguez-Kabana, 1986), suggesting direct effects of nitrogen addition on soil nematodes. Importantly, soil nematodes may be directly influenced by changes in physicochemical soil conditions, but also indirectly by shifts in plant community composition (De Deyn et al., 2004). Altered plant community composition and widespread species loss after nitrogen addition have been observed across the world (Xia et al., 2008; Bai et al. 2010). Schon et al. (2011) stated that the clover-cyst nematode (*H. trifolii*) declined, as the application of N fertiliser reduced legume content, which was supported by Gillingham et al. (2008); as clover content of the pasture declined, the N fertiliser application rate increased, probably a shading effect by the responsive grass component.

Little is still known about the extent to which the introduction of legumes affects soil biota under grasslands and the functioning of the soil–plant system. Van Eekeren et al. (2009) described a greater proportion of bacterivorous nematodes (as dauer larvae), and a lowered proportion of herbivorous nematodes under clover, and they suggested this higher proportion of dauer larvae in white clover-only plots was probably related to the easily decomposable litter in the rhizosphere to which bacterivorous nematodes with a short generation time can quickly respond. Various researchers have reported more bacterivorous nematodes under legumes (Sohlenius et al., 1987; Viketoft et al., 2005) versus more herbivorous nematodes under grass (Sarathchandra et al., 2001; Baars, 2002; Viketoft et al., 2005). Viketoft (2008) found a lower number of nematode taxa in white clover than in other legumes, forbs and grasses where legume addition increased abundance. The presence of *Heterodera trifolii* and *Meloidogyne* spp reflects the presence of white clover, with which these genera are particularly associated in most of the pastures sampled (McLeish et al., 1997; Stirling and Lodge, 2005; Mercer et al., 2008). Kostenko et al. (2015) showed that legumes positively affected entomopathogenic nematodes' (EPNs) infectivity, and plant diversity indirectly affected the infectivity of EPNs via effects on the abundance of soil insects. In this case, the abundance of prey (soil insects and root-feeding, bacterivorous, and fungivores nematodes) increased with higher plant diversity. Maturity index and structural index were increased by legume addition into a forest ecosystem (Zhao et al., 2014). Nicholas (1984) demonstrated the recovery of bacteria-feeding nematodes from red clover roots and showed that bacteria-feeding nematodes can thrive on legume-symbiotic *Rhizobium* spp. bacteria. Bardgett et al. (1999) found that infestation of white clover roots by plant-parasitic nematodes increase the amount of N that leaks into the soil from the host plant and it is transferred to the neighbouring ryegrass, thereby benefiting neighbouring crops.

The presence of fungal endophyte inside the above-ground parts of pasture grasses is reported to have inconsistent effects on nematodes in roots and soil (Yeates and Prestidge, 1986; West et al., 1988; Elmi et al., 1990; Latch, 1993). Yeates and Prestidge (1986) counted fewer nematodes in plots of endophyte-infected 'Grasslands Ruanui' compared with endophyte-free 'Grasslands Ruanui', even though the level of endophyte-infection was only 10%. Conversely, they also found more nematodes in 'Ellet' plots with a higher (95%) endophyte infection level than on the lower (58%) infection level.

Seasonal variation in the nematode population dynamics, including nematode activity and their hatchability and mortality, is correlated with various factors, such as moisture, temperature, and plant growth. Temperature directly affects hatching of eggs, moulting between stages, reproduction, length of the life cycle, survival of plant-parasitic nematodes without a host and indirectly affects host plant responses like growth and stress. Soil moisture content affects nematode movements, feeding and reproduction (Bell and Watson, 2001). Plant and soil nematodes are an important part of the soil food web and they are directly dependent on primary producers for their energy so that primary production is the essential driving force in the soil food web as indicated by Yeates (1982), Norton (1978), and Wallace (1971, 1973). Yeates (1982) stated that plant-feeding nematode populations correlated with the growing season of annual crops and concluded that they exhibited distinct annual cycles with herbage production. He substituted pasture production measurements for root production estimation to understand the biological interaction of nematodes in the soil.

The DairyNZ Forage Value Index (FVI) is a region-specific index for short-term and perennial ryegrass cultivars devised with the aim of allowing farmers to make more informed decisions when choosing ryegrass cultivars for their pasture renewal programme (Chapman et al., 2012). The index is based on calculated Economic Values (EV) and Performance Values (PV) for seasonal dry matter production. Performance value (dry matter production by season in winter, early spring, late spring, autumn and summer) and the associated attributes (ploidy, heading date, endophyte) act as a cultivar selector tool to provide decision support based on regional star ratings. The Forage Value Index results are divided into the four main dairy regions of New Zealand: The Upper North Island; Lower North Island; Upper South Island; and the Lower South Island. The index was initially calculated using data from cultivar evaluation trials conducted using perennial ryegrass monocultures and high nitrogen (N) fertiliser inputs (>300 kg N/ha/annum) (Easton et al., 2001; DAFM, 2010). However, sowing perennial ryegrass in a mixture with white clover is a standard practice in New Zealand and is becoming more widespread in other countries, due to potential benefits for animal performance (Harris et al., 1997). To test some of these interactions, DairyNZ and AgResearch have established large-scale grazed species testing sites. There are currently four such sites established where plots include not only ryegrass but also clover and nitrogen treatments to investigate if FVI ryegrass ratings change when ryegrass and clover are grown together, and what impact additional nitrogen application has on ratings. The sites are in Waikato, Manawatu, Canterbury and Southland to allow for regional differences to be observed; these have been maintained for five years allowing for longer-term agronomic assessments to be made.

The aim of the current study is to examine the impact on the nematode community of the combinations of nitrogen fertiliser application and clover presence or absence, and a range of ryegrass cultivars, with seasons. Results from this study are useful for assessing the impacts of plant-feeding nematodes on plant productivity within these treatments, and also understanding the impact on the ecosystem by these treatments. Total nematodes community analysis is useful to achieve these goals (Wilson and Kakouli-Duarte, 2009). This provides for evaluating the soil health changes with plant species, and application of nitrogen levels and seasons, which will add another layer to decision making about ryegrass ratings and indicate which combinations of treatments are most desirable from a long-term persistence and sustainability perspective. This study focused on nematode communities, conducted in two consecutive years (2013 and 2014), and in two sites (Waikato and Manawatu) with contrasting climates and soil types.

2.2 Materials and methods

2.2.1 Study sites and geographical location

The study sites were on the DairyNZ Scott Farm, Hamilton in the Waikato region of New Zealand (37.845^{0}S, 175.821^{0}E) and Palmerston North in the Manawatu region of New Zealand (40.40^0). The Waikato trial site is at an altitude of 30 m and has an average air temperature of 14.8°C and annual rainfall of 1200 mm. These sites were part of an existing Species Interaction Trial established by DairyNZ, which was a DairyNZ /Lincoln University-Teagasc-AgResearch collaborative project begun in 2012. This Species Interaction Trial contributes to the development of the Forage Value Index, a joint initiative of DairyNZ and the New Zealand Plant Breeding and Research Association. Two sites in the North Island in NZ were chosen for this study. The Waikato site is managed by DairyNZ and the Manawatu site by AgResearch (Cosgrove et al., 2014). The selected sites have different climates, soil types and nematode community. Sampling was carried out twice a year in autumn and spring (selection of those two periods was discussed in Section 2.1, Neher, 1999; Neher et al., 2003) to investigate contrasts between seasons when host plants are actively growing and nematode activity is high. Year to year variability was researched over two years, in 2013 and 2014.

2.2.2 Layout and experimental design

Experimental design and treatments at each site of the Species Interaction Trial consists of two levels of nitrogen (N) fertiliser application(low 100 kg /ha/yr or high 325 kg /ha/yr as the form of Urea) × two levels of white clover (plus or minus) × eight cultivars of perennial ryegrass × five replications, laid out in a split-plot design giving 160 plots in total (Cosgrove et al., 2014; Rossi et al., 2014). For the study described here, two cultivars were selected for cultivar comparison, both infected with AR37 endophyte, which is widely used by farmers in the northern part of the North Island to assist in pasture production and persistence. The two cultivars were Base and One 50 which have differing sets of chromosomes (tetraploid vs diploid) and notable differences in their growth characters. Base AR37 tetraploids represent open, low tiller density/broad leaf material with heading date being mid-season, while One50 AR37 diploid is late-season flowering. In the with-clover treatments, the ryegrass cultivars were sown with a 50:50 mixture of Kopu II and Tribute clover cultivars. This meant that of the 160 plots of each plant species interaction trial, 40 plots were sampled for this study.

The experimental plots were grazed by dairy cows following standard farm management practices for each site, and nine grazing events occurred on both the high and low N plots in the experiment. The main plots were individually fenced but cultivars within plots were grazed in common.

2.2.3 Nematode sampling, extraction and identification

Soil samplings were performed in mid-autumn (April) and late spring (November) in consecutive years of 2013 and 2014, i.e. one year after the establishment of the experimental plots (Cosgrove et al., 2014). For each sampling campaign, ten soil samples were taken per plot by using a soil corer with a diameter of 25 mm, depth of 10 mm, with cores pooled by plot and stored at 4°C for <1 week.

A 100g subsample of soil was taken from each hand-crumbled, thoroughly mixed bulk soil sample and nematodes were extracted from this field moist soil from each plot by the method described by Bell and Watson, (2001). Extracted nematodes were allowed to sediment with water to 10 ml, and then nematode suspension was transferred to a Doncaster dish (Doncaster, 1962) and nematodes were counted under a transmitted light dissection stereo microscope at 40–80× magnification while they were alive.

Total nematodes and plant parasitic nematodes were counted. Soil moisture was determined by oven drying at 80°C for 48 hours; a separate 100 g subsample of the hand crumbled soil and results were used to calculate the number of nematodes per 100 g dry soil. After extraction and fixation in 4% formalin each nematode sample was reduced to 1 ml by aspiration and centrifugation, and a 100 µl subsample taken for nematode counts and identification as described in O'Callaghan et al. (2008). The taxonomic diversity of each sample was determined to genus level or feeding group at 100–400x magnification by examining at least 100 individuals which were identified to genus according to Bongers (1999) or trophic group classification following (Yeates et al., 1993) (e.g., as plant feeders or plant parasites, bacterial feeders / bactivores, fungal feeders / fungivores, predators and omnivores) according to Yeates et al. (1993) and Bongers and Bongers (1998). Nematode genera were also assigned to colonizer persister (c-p) groups according to Yeates et al. (1993) and Bongers (1999). Bongers (1990) defined life-history characteristics for families of nematodes and allocated them to a colonizer–persister (c-p) scale. The scale ranges from one, indicating early colonizers of new resources to five, explaining mainly persisters in undisturbed habitats. This scale is used to calculate a number of indices (Wilson and Kakouli-Duarte., 2009), for example, the maturity index (MI) is the weighted mean of non-plant feeders using c-p. MI values for soil subjected to varying levels of disturbance range from less than 2.0 in nutrient-enriched disturbed systems to 4.0 in undisturbed, pristine environments.

Results were analysed by ANOVA of untransformed and of natural log-transformed nematode data (nematode data transformed to normalise distribution). Nematode density was expressed as individual numbers per 100 g of dry soil. Meteorological data were obtained from a summary of climate statistics for each site which were extracted from the NIWA Virtual Climate Station network (Tait et al., 2006).

2.3 Data analysis

To understand the ecosystem health (disturbance / enrichment) and function, various indices are often calculated using nematode counts. Computation of involved indices and their interpretations are discussed below (Maturity Index – MI; Maturity Index with 2 to 5 - MI2-5; Total maturity index - ΣMI; Plant Parasite Index – PPI; Ratio PPI versus MI – PPI/MI).

2.3.1 Computation of Indices for Ecosystem Health

Ecosystem health is measured by various indices. For example, maturity index (MI) evaluates the ecosystem disturbance while the plant parasite index (PPI) assesses ecosystem enrichment.

Maturity Index (MI)

MI is calculated with the following formulae:

$$MI = \frac{\sum_{i=1}^{5} Cp_{BF,i} \times f_{BF,i} + \sum_{i=1}^{5} Cp_{FF,i} \times f_{FF,i} + \sum_{i=1}^{5} Cp_{PF,i} \times f_{PF,i} + \sum_{i=1}^{5} Cp_{OF,i} \times f_{OF,i}}{\sum_{i=1}^{5} (f_{BF,i} + f_{FF,i} + f_{PF,i} + f_{OF,i})} \qquad (1)$$

where $Cp_{AA,i}$ is the value of *i*, indicating the *i*th group of c-p in feeder *AA*; and $f_{BF,i}$, $f_{FF,i}$, $f_{PF,i}$, and $f_{OF,i}$ are frequency of bacterial feeders (BF), fungal feeders (FF), predator feeders (PF) and omnivores feeders (OF) in the *i*th group of c-p respectively. We see from the MI computation that MI is calculated based on nematodes numbers with all feeders except plant feeders. High MI indicates that bacteria, fungus, predators and omnivores are available in the ecosystem. This situation is considered as a stable ecosystem (Bongers, 1990). In contrast, low MI indicates a poor ecosystem or frequently disturbed ecosystem.

Maturity Index with c-p 2 to 5 (MI2-5)

MI2-5 is exactly same as (1), but the value *i* goes from 2 to 5 in the summation notation. That is, only c-p groups 2 to 5 are considered for this computation. Nematodes in the group 1 of c-p respond rapidly to increases in soil resources (e.g. decomposing organic material) (Wilson & Kakouli-Duarte, 2009), hence MI calculation without group 1, known as MI2-5, provides a better indicator of long-term ecosystem health.

Total Maturity Index (ΣMI)

All five feeders, including plant feeders, are included for this calculation. That is, the components $\sum_{i=1}^{5} Cp_{PlF,i} \times f_{PlF,i}$ and $f_{PlF,i}$ are added to numerator and denominator of the equation (1) respectively, where $Cp_{PlF,i}$ is the value of *i*, indicating the *i*th group of c-p in plant feeder and $f_{PlF,i}$ is the frequency of plant feeder (PIF) in the *i*th group of c-p. This index is a useful tool to assess the impact of extra nutrients added to the soil ecosystem.

Plant Parasite Index (PPI)

Plant parasite index is calculated based on the plant feeder nematodes and given in (2) below.

$$PPI = \frac{\sum_{i=1}^{5} Cp_{PlF,i} \times f_{PlF,i}}{\sum_{i=1}^{5} f_{PlF,i}} \qquad (2)$$

Note that ΣMI is not equal to the sum of MI and PPI. A high PPI indicates an unhealthy ecosystem, while low PPI indicates a stable ecosystem.

Ratio PPI vs MI (PPI/MI)

This ratio between PPI and MI is a good measure to assess the ecosystem health. If the ratio is < 1, particularly close to zero, the ecosystem is defined as a healthy. If the ratio is > 1 particularly a large value, the ecosystem may be described as unhealthy.

2.3.2 Statistical Analysis

Details of environmental factors, such as soil moisture, soil temperature and rainfall, are provided based on the information of four weeks prior to the sample collection date for each location. Details are also given of the identified total number of taxa of nematodes including feeding groups with colonizer-persister (c-p) scale. To describe the large number of relationships between taxa, we present the correlation coefficient diagrams in two formats: a network diagram for clear visual representation of relationships between taxa, clustered by feeding type; and a “heat map” to show quantified associations between taxa, including grouping by closely associated taxa, regardless of feeding type. These presentations are novel for nematological ecological data and provide an improvement to the way the inter-relationships with a large set of variables can be described for future studies.

Our primary interest in this study was the distribution of changes in nematodes with eight treatments and eight various stages of sampling, as given in Table 1.

Table 1: Notations for treatments and sampling stages

Treatments	Notations	Sampling Stages	Notations
Base, High Nitrogen and Clover absent	BHN	Manawatu, 2013 and Autumn	M_13_A
Base, High Nitrogen and Clover present	BHY	Manawatu, 2013 and Spring	M_13_S
Base, Low Nitrogen and Clover absent	BLN	Manawatu, 2014 and Autumn	M_14_A
Base, Low Nitrogen and Clover present	BLY	Manawatu, 2014 and Spring	M_14_S
One50, High Nitrogen and Clover absent	OHN	Waikato, 2013 and Autumn	W_13_A
One50, High Nitrogen and Clover present	OHY	Waikato, 2013 and Spring	W_13_S
One50, Low Nitrogen and Clover absent	OLN	Waikato, 2014 and Autumn	W_14_A
One50, Low Nitrogen and Clover present	OLY	Waikato, 2014 and Spring	W_14_S

To recognize the nematode changes with various conditions, the mean and standard deviation in table format plus graphical presentation of box plots are provided for all outcome measures. For the rest of the analyses, we concentrate on the primary outcome variables of the total number of nematodes (sum of all the taxa of nematodes) and ecosystem health indices. The study was originally designed as a split-plot design, so that the split-plot analysis for total nematodes and indices were done to examine the treatment effects, such as nitrogen level, cultivars and clover presence and absence, and seasonal effects. Past research showed that the nematode communities under pasture in Waikato and Manawatu are different (Yeates, 1977), and environmental conditions, including temperature and soil moistures, were different for 2013 and 2014. For simplification, four independent analyses for Waikato 2013, Waikato 2014, Manawatu 2013 and Manawatu 2014 were conducted. To recognise the significant and non-significant effects of treatments and seasons, p-values are displayed in table form for all four scenarios. Statistical significance is defined if the p-value is less than 0.05.

To reduce the current 24 taxa to interpretable number groups, and to identify the similar experimental units, represented by two locations, two seasons, two data collection years and all three treatments, a principal component analysis was performed. Overall 64 (= 2^6) experimental units were considered for the analysis. Means of each taxon from five replications of each experimental condition were computed and those values used for the principal component analysis. Consequently, the second principal component was plotted against the first principal component for identifying any possible clusters. In addition, similar taxa within clusters were reported.

2.4 Results

Table 2 presents mean/total values of the environmental factors computed based on the four weeks prior to the sampling period. Air temperature with maximum and minimum, soil temperature at 9 am, vapour pressure at 9 am, relative humidity at 9 am, solar radiation and wind speed values were averaged over the four weeks period and given in Table 2. In addition to this, total rainfalls and potential evapotranspiration for the four weeks period was calculated and given in Table 2. The soil wetness, given in the third row of Table 2, was computed as a difference between the total rainfall values and the total potential evaporation. If evapotranspiration values are greater than rainfall values, soil wetness becomes negative and indicates a drying stage.

In the Waikato, the 2014 autumn and spring were slightly cooler and much wetter than the corresponding seasons in 2013, which led to much greater soil drying occurring in spring 2013. A similar pattern occurred in spring in the Manawatu, whereas autumn 2014 was only slightly wetter and warmer than autumn 2013 at that site.

Table 2: Mean and totals for environmental factors over the four weeks prior to each seasonal sampling. Note potential evapotranspiration (mm) is calculated by the Penman method (see Tait et al., 2006).

	Waikato				**Manawatu**			
	2013		**2014**		**2013**		**2014**	
	Autumn	**Spring**	**Autumn**	**Spring**	**Autumn**	**Spring**	**Autumn**	**Spring**
Rainfall (mm)	117.7	41.4	284.7	89.0	91.7	34.1	93.6	83.3
Potential evapotranspiration	60.8	103.6	243.3	89.1	47.4	103.4	52.8	93.2
Soil wetting	56.9	-62.2	41.4	-0.1	44.3	-69.3	40.8	-9.9
Min air temperature (°C)	11.1	8.7	10.5	9.0	11.0	10.2	11.4	8.6
Max air temperature (°C)	22.7	20.1	20.7	18.3	19.2	19.5	21.4	17.3
Soil temperature (°C) at 9 am	16.2	15.5	15.8	14.7	14.0	15.4	15.4	13.6
Solar radiation (MJ /m2)	12.2	20.5	15.9	19.0	8.6	19.3	9.3	17.6
Relative humidity (%) at 9 am	86.5	76.6	84.6	80.3	84.7	75.6	87.0	76.8
Vapour pressure (hPa) at 9 am	14.8	13.0	14.4	12.9	13.5	12	14.5	11.3
Wind speed (m /s) estimated	2.0	2.9	2.7	3.1	4.1	4.1	3.7	4.6

A total of 24 taxa of nematodes were found in this study comprising 9 plant feeders, 3 fungal feeders, 8 bacterial feeders, 3 predators, and 1 omnivore. However, the range of taxon richness per sample collection period was 12 to 14 (Table 3).

Overall, plant feeders (Tylenchids [Based on the practice and brevity, Tylenchinae are hereafter referred to as Tylenchids in this thesis], *Paratylenchus*, *Pratylenchus*), fungal feeders (*Aphelenchus*, *Aphelenchoides*), and bacterial feeders (Cephalobids and *Panagrolaimus*) were the most common nematodes found in both regions (Appendix 1). *Meloidogyne* abundance in Waikato was relatively high compared with Manawatu (Appendix 1). In contrast, *Longidorous* and *Diplogaster* were more abundant in Manawatu than Waikato (Appendix 1). In both study sites, predators and omnivores were relatively low compared with plant, fungal and bacterial feeders (Appendix 1).

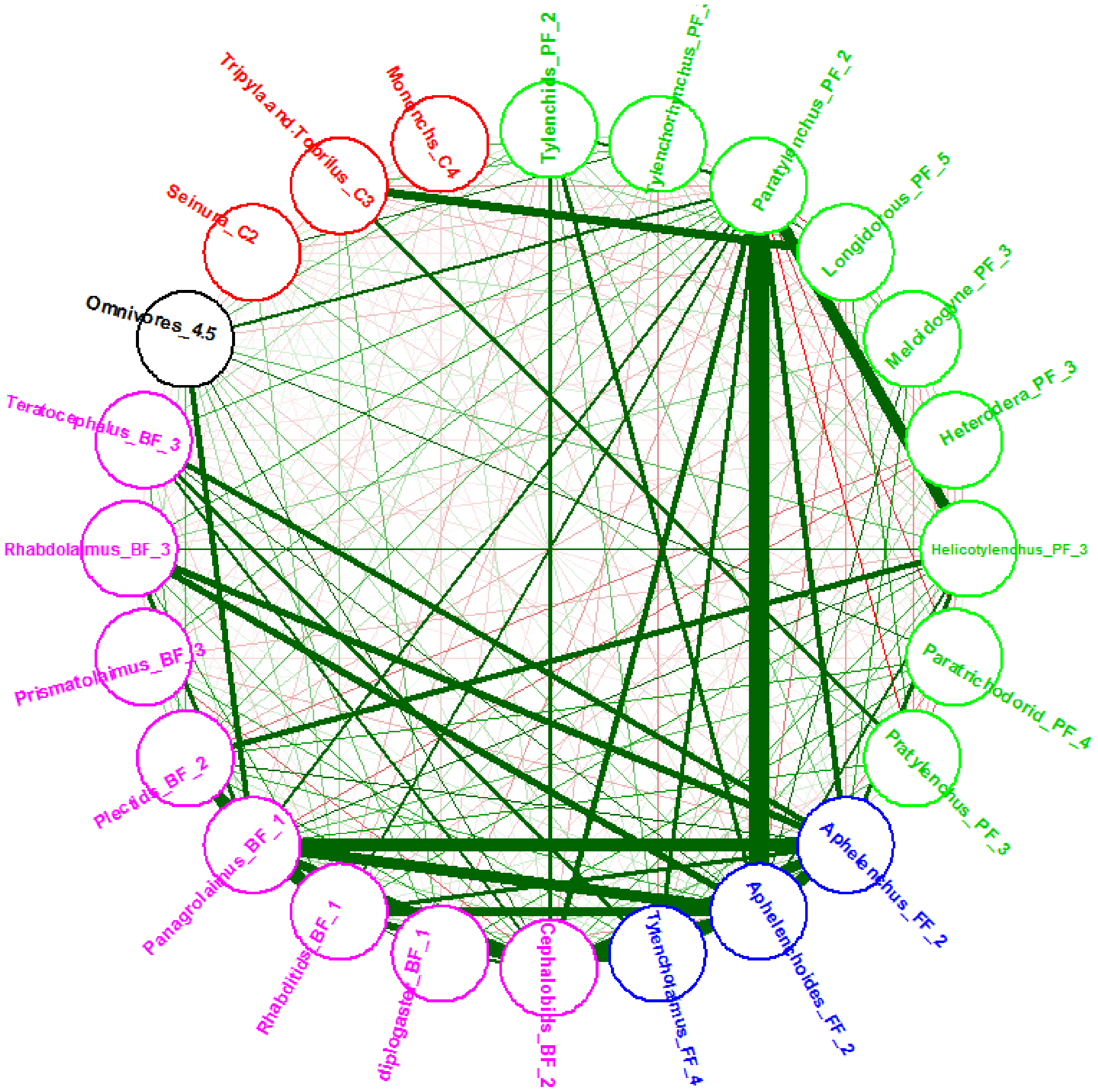
Table 3: Taxon richness at each sampling for each site

Sample collected	Location	Location × season identifier	Identified taxa
07 - 05 – 2013	Manawatu	Manawatu 2013 Autumn	13
14 - 11 – 2013	Manawatu	Manawatu 2013 Spring	13
28 - 05 – 2014	Manawatu	Manawatu 2014 Autumn	12
14 - 11 – 2014	Manawatu	Manawatu 2014 Spring	13
03 - 05 – 2013	Waikato	Waikato 2013 Autumn	13
15 - 11 – 2013	Waikato	Waikato 2013 Spring	14
23 - 04 – 2014	Waikato	Waikato 2014 Autumn	14
21 - 11 – 2014	Waikato	Waikato 2014 Spring	14

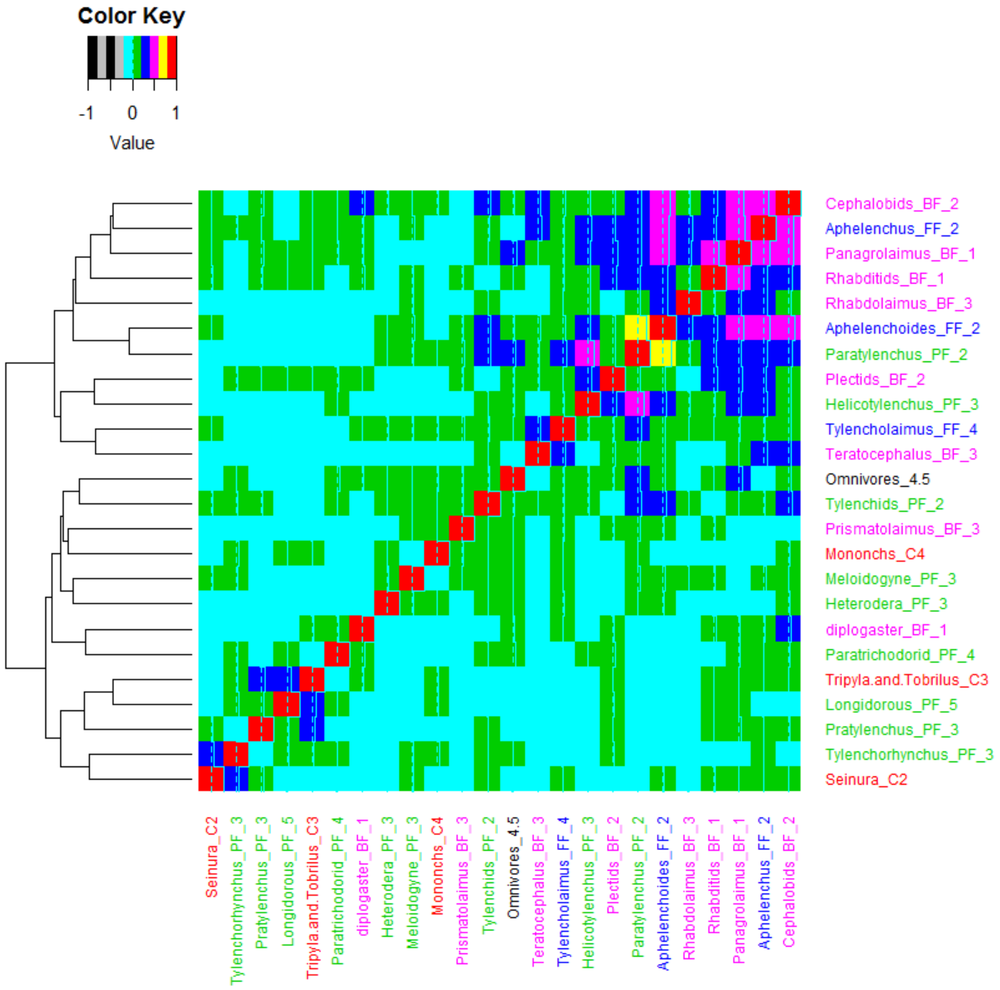
Due to the large number of taxa (n = 24), calculating all pairwise correlation coefficients gave a total of 276 (= ${}^{24}C_2$) comparisons. For easy visualization and understanding all the pairwise correlations, correlation coefficients are presented in graphical formats. In Fig 1 (a) and (b), plant feeders, fungal feeders, bacterial feeders, omnivores and predators are indicated with colour codes of green, blue, purple, black and red, respectively. The range for all coefficients (r) is –0.18 to 0.65. Note that, 239 pairs out of 276 had an absolute correlation coefficient with less than 0.20 (Figure 1(b)) indicating that most taxa had little interaction with other taxa. For example, some taxa (*Meloidogyne*, *Heterodera*, *Paratrichodorus*, Plectids, *Prismatolaimus*, Mononchs) are uncommon in this study (Appendix 1), but could survive without other taxa (Figure 1(a)), and the correlation coefficient is almost zero (Figure 1(b)). Figure 1(a) shows that fungal feeders (FF) such as *Aphelenchus* and *Aphelenchoides* are associated with plant feeders (PF) and bacterial feeders (BF). For example, the correlation coefficient for: (i) *Aphelenchoides* (FF) and *Paratylenchus* (PF) is 0.65 (Figure 1(b) with yellow colour); (ii) *Aphelenchoides* (FF) and Cephalobids (BF) is 0.51; (iii) *Aphelenchus* (FF) and *Panagrolaimus* (BF) is 0.47; (iv) *Aphelenchus* (FF) and *Aphelenchoides* (FF) is 0.47; (v) *Aphelenchoides* (FF) and *Panagrolaimus* (BF) is 0.44; (vi) *Aphelenchus* (FF) and Cephalobids (BF) is 0.43; (vii) *Aphelenchoides* (FF) and Rhabditids (BF) is 0.37; (viii)

Aphelenchus (FF) and *Rhabdolaimus* (BF) is 0.33; and (ix) *Aphelenchus* (FF) and *Paratylenchus* (PF) is 0.31. *Paratylenchus* abundance was associated with plant feeders (*Helicotylenchus*), fungal feeders (*Aphelenchus*, *Aphelenchoides*), bacterial feeders (Cephalobids, Rhabditids and *Panagrolaimus*) and omnivores (Figure 1(a)). In addition, the very uncommon predator feeder taxa *Tripyla* and *Tobrilus* in this study had some association with plant feeders (*Longidorous* and *Pratylenchus*). All these mentioned associations are positive (Figure 1(a) in green line), which show that if we find one of the associated taxa, there is an increased chance of finding the other taxa in the same soil. However, some are negative associations with small magnitude. For example, there is a rare chance of some plant feeders inhabiting together (*Paratylenchus* and *Pratylenchus*, Figure 1(a) in red thin line).

In addition, many taxa with cp scale 1 and 2 are associated (Figure 1(a)). Additionally, some are associated between cp scale with 2 and 3 (*Helicotylenchus* and Plectids; *Aphelenchoides* and *Rhabdolaimus*; etc.), 3 and 3 (*Paratylenchus*, and *Tripyla* and *Tobrilus*), and 1 and 4.5 (*Panagrolaimus* and omnivores). Many taxa with more than 2 in cp-scale are unlikely to be associated with other taxa (Figure 1(a)).



(a) Association between taxon with *c-p* scale



(b) Correlation coefficients between taxon with dendrogram

Figure 1: (a) Diagram for pairwise association between taxon. Green and red colours indicate positive and negative association between taxa, and the thickness of the line indicates the strength of the association (thick line – good association; thin line – almost no association between taxa). (b) Heat plot provides the pairwise correlation coefficient of taxon indicated by colour key with dendrogram.

Table 4: Mean (sd) for primary outcomes by treatments and sampling stages

Primary outcomes	Location	Year	Seasons		BHN	BHY	BLN	BLY	OHN	OHY	OLN	OLY	Over All mean (sd)
Total Nematodes	Manawatu	2013	Autumn	M_13_A	5481.7	5416.63	5824.87	5929.29	5562.9	4245.63	6037.68	4973.08	**5433.97 (2201.5)**
	Manawatu	2013	Spring	M_13_S	2249.97	2307.07	2782.89	2322.38	2190.97	2283.27	2975.23	2170.95	**2410.34 (755.4)**
	Manawatu	2014	Autumn	M_14_A	2712.92	3272.31	3333.72	2593.81	4101.19	4104.5	4212.62	2800.93	**3391.5 (1548.8)**
	Manawatu	2014	Spring	M_14_S	1889.93	1685.61	2372.58	2114.8	1964.2	1701.48	2073.99	2281.69	**2010.53 (522.1)**
	Waikato	2013	Autumn	W_13_A	7755.81	8224.28	8383.89	7248.08	9354.22	6922.51	6082.68	5582.66	**7479.18 (3247.2)**
	Waikato	2013	Spring	W_13_S	3361.42	3415.66	3112.62	2546.56	3606.68	3450.88	2925.42	3429.65	**3238.95 (960.6)**
	Waikato	2014	Autumn	W_14_A	3561.28	4090.3	3129.13	3606.33	3788.62	5788.61	3489.74	4245.29	**3974.53 (1307.2)**
	Waikato	2014	Spring	W_14_S	2866.32	2987.49	3437.94	3286.14	2459.83	2832.81	4008.82	2919.64	**3076.57 (669)**
			Over All	**Mean**	**3734.92**	**3924.92**	**4047.2**	**3705.92**	**4128.58**	**3916.21**	**3959.01**	**3550.49**	
				(sd)	**(2550.8)**	**(2325.5)**	**(2346.5)**	**(2008.9)**	**(3199.3)**	**(2255.7)**	**(2199.4)**	**(1586.5)**	
MI	Manawatu	2013	Autumn	M_13_A	1.51	1.57	1.6	1.6	1.41	1.63	1.58	1.6	**1.56 (0.13)**
	Manawatu	2013	Spring	M_13_S	1.88	1.87	1.95	1.91	1.85	1.79	1.9	1.79	**1.87 (0.12)**
	Manawatu	2014	Autumn	M_14_A	1.63	1.55	1.5	1.64	1.45	1.47	1.38	1.51	**1.52 (0.17)**
	Manawatu	2014	Spring	M_14_S	1.84	1.94	1.99	1.96	1.87	1.86	1.89	1.75	**1.89 (0.12)**
	Waikato	2013	Autumn	W_13_A	1.6	1.7	1.63	1.68	1.58	1.75	1.75	1.67	**1.67 (0.12)**
	Waikato	2013	Spring	W_13_S	1.95	1.93	1.9	1.89	1.93	1.97	1.99	1.92	**1.93 (0.09)**
	Waikato	2014	Autumn	W_14_A	1.72	1.71	1.79	1.8	1.69	1.6	1.84	1.71	**1.73 (0.12)**
	Waikato	2014	Spring	W_14_S	1.99	1.86	2.14	2.01	2.01	1.78	2.05	1.95	**1.97 (0.15)**
			Over All	**Mean**	**1.76**	**1.77**	**1.81**	**1.81**	**1.72**	**1.73**	**1.79**	**1.74**	
				(sd)	**(0.2)**	**(0.19)**	**(0.24)**	**(0.18)**	**(0.24)**	**(0.18)**	**(0.23)**	**(0.17)**	

Table 4 (contd)

Primary outcomes	Location	Year	Seasons		BHN	BHY	BLN	BLY	OHN	OHY	OLN	OLY	Over All mean (sd)
MI25	Manawatu	2013	Autumn	M_13_A	2.04	2.01	2.11	2.03	2.09	2.06	2.09	2.05	**2.06 (0.08)**
	Manawatu	2013	Spring	M_13_S	2.03	2.04	2.1	2.03	2.06	2.03	2.05	2.02	**2.04 (0.06)**
	Manawatu	2014	Autumn	M_14_A	2.04	2.02	2.03	2.03	2.02	2.02	2.02	2.08	**2.03 (0.05)**
	Manawatu	2014	Spring	M_14_S	2.02	2.09	2.04	2.09	2.05	2.06	2	2.1	**2.06 (0.07)**
	Waikato	2013	Autumn	W_13_A	2.01	2.02	2.09	2.03	2.03	2.05	2.08	2.03	**2.04 (0.05)**
	Waikato	2013	Spring	W_13_S	2.07	2.06	2.07	2.06	2.05	2.1	2.12	2.05	**2.07 (0.07)**
	Waikato	2014	Autumn	W_14_A	2.01	2.02	2.01	2.07	2.01	2.02	2.07	2.03	**2.03 (0.04)**
	Waikato	2014	Spring	W_14_S	2.08	2.09	2.24	2.11	2.15	2.03	2.15	2.09	**2.12 (0.11)**
			Over All	**Mean**	**2.04**	**2.04**	**2.09**	**2.06**	**2.06**	**2.05**	**2.07**	**2.06**	
				(sd)	**(0.06)**	**(0.06)**	**(0.1)**	**(0.07)**	**(0.07)**	**(0.06)**	**(0.08)**	**(0.07)**	
PPI	Manawatu	2013	Autumn	M_13_A	2.79	2.8	2.76	2.8	2.74	2.76	2.82	2.75	**2.78 (0.15)**
	Manawatu	2013	Spring	M_13_S	2.76	2.76	2.71	2.76	2.61	2.77	2.8	2.72	**2.74 (0.14)**
	Manawatu	2014	Autumn	M_14_A	2.71	2.67	2.64	2.68	2.71	2.56	2.58	2.67	**2.65 (0.14)**
	Manawatu	2014	Spring	M_14_S	2.55	2.4	2.58	2.64	2.48	2.53	2.39	2.53	**2.51 (0.13)**
	Waikato	2013	Autumn	W_13_A	2.22	2.26	2.29	2.36	2.19	2.18	2.15	2.28	**2.24 (0.14)**
	Waikato	2013	Spring	W_13_S	2.43	2.36	2.43	2.31	2.35	2.35	2.25	2.34	**2.36 (0.16)**
	Waikato	2014	Autumn	W_14_A	2.32	2.55	2.3	2.45	2.26	2.28	2.25	2.28	**2.34 (0.17)**
	Waikato	2014	Spring	W_14_S	2.25	2.39	2.26	2.3	2.18	2.28	2.19	2.3	**2.27 (0.13)**
			Over All	**Mean**	**2.5**	**2.52**	**2.5**	**2.54**	**2.44**	**2.47**	**2.45**	**2.48**	
				(sd)	**(0.25)**	**(0.22)**	**(0.23)**	**(0.24)**	**(0.25)**	**(0.25)**	**(0.3)**	**(0.24)**	

Table 4 (contd)

Primary outcomes	Location	Year	Seasons		BHN	BHY	BLN	BLY	OHN	OHY	OLN	OLY	Over All mean (sd)
SigmaMI25	Manawatu	2013	Autumn	M_13_A	2.44	2.45	2.48	2.41	2.35	2.33	2.42	2.33	**2.4 (0.14)**
	Manawatu	2013	Spring	M_13_S	2.44	2.49	2.52	2.48	2.36	2.36	2.51	2.39	**2.44 (0.12)**
	Manawatu	2014	Autumn	M_14_A	2.34	2.35	2.4	2.37	2.36	2.23	2.26	2.32	**2.33 (0.13)**
	Manawatu	2014	Spring	M_14_S	2.28	2.25	2.37	2.4	2.24	2.29	2.22	2.33	**2.3 (0.09)**
	Waikato	2013	Autumn	W_13_A	2.11	2.13	2.18	2.16	2.09	2.1	2.1	2.12	**2.12 (0.06)**
	Waikato	2013	Spring	W_13_S	2.25	2.21	2.24	2.19	2.19	2.23	2.19	2.21	**2.21 (0.08)**
	Waikato	2014	Autumn	W_14_A	2.19	2.23	2.18	2.23	2.12	2.15	2.17	2.13	**2.17 (0.09)**
	Waikato	2014	Spring	W_14_S	2.17	2.22	2.24	2.2	2.17	2.15	2.17	2.18	**2.19 (0.08)**
			Over All	**Mean**	**2.27**	**2.29**	**2.33**	**2.3**	**2.24**	**2.23**	**2.27**	**2.25**	
				(sd)	**(0.15)**	**(0.15)**	**(0.17)**	**(0.17)**	**(0.13)**	**(0.11)**	**(0.15)**	**(0.13)**	
SigmaMI	Manawatu	2013	Autumn	M_13_A	1.97	2.08	2.09	2.04	1.68	1.94	1.97	1.91	**1.96 (0.22)**
	Manawatu	2013	Spring	M_13_S	2.35	2.39	2.45	2.42	2.23	2.16	2.41	2.22	**2.33 (0.16)**
	Manawatu	2014	Autumn	M_14_A	1.99	1.97	1.98	2.06	1.82	1.71	1.67	1.81	**1.87 (0.22)**
	Manawatu	2014	Spring	M_14_S	2.15	2.17	2.35	2.3	2.12	2.15	2.16	2.07	**2.18 (0.14)**
	Waikato	2013	Autumn	W_13_A	1.8	1.9	1.88	1.9	1.76	1.86	1.86	1.83	**1.85 (0.11)**
	Waikato	2013	Spring	W_13_S	2.17	2.13	2.11	2.09	2.12	2.16	2.11	2.13	**2.13 (0.09)**
	Waikato	2014	Autumn	W_14_A	2.01	1.98	2.05	2.03	1.9	1.84	2.05	1.91	**1.97 (0.13)**
	Waikato	2014	Spring	W_14_S	2.13	2.06	2.2	2.14	2.1	1.99	2.12	2.08	**2.1 (0.1)**
			Over All	**Mean**	**2.07**	**2.08**	**2.14**	**2.12**	**1.97**	**1.98**	**2.05**	**1.99**	
				(sd)	**(0.2)**	**(0.2)**	**(0.22)**	**(0.21)**	**(0.23)**	**(0.19)**	**(0.24)**	**(0.19)**	

Table 4 (contd)

Primary outcomes	Location	Year	Seasons		BHN	BHY	BLN	BLY	OHN	OHY	OLN	OLY	Over All mean (sd)
PPI/MI	Manawatu	2013	Autumn	M_13_A	1.85	1.81	1.73	1.76	1.96	1.7	1.79	1.72	**1.79 (0.19)**
	Manawatu	2013	Spring	M_13_S	1.49	1.48	1.4	1.45	1.42	1.55	1.48	1.52	**1.47 (0.12)**
	Manawatu	2014	Autumn	M_14_A	1.68	1.73	1.77	1.66	1.89	1.77	1.88	1.78	**1.77 (0.24)**
	Manawatu	2014	Spring	M_14_S	1.38	1.23	1.3	1.35	1.32	1.37	1.26	1.46	**1.33 (0.11)**
	Waikato	2013	Autumn	W_13_A	1.39	1.33	1.43	1.4	1.38	1.25	1.24	1.38	**1.35 (0.14)**
	Waikato	2013	Spring	W_13_S	1.25	1.23	1.29	1.23	1.22	1.2	1.14	1.22	**1.22 (0.11)**
	Waikato	2014	Autumn	W_14_A	1.36	1.49	1.29	1.37	1.34	1.43	1.22	1.33	**1.36 (0.13)**
	Waikato	2014	Spring	W_14_S	1.13	1.29	1.06	1.14	1.08	1.29	1.07	1.18	**1.16 (0.12)**
			Over All	**Mean**	**1.44**	**1.45**	**1.41**	**1.42**	**1.45**	**1.44**	**1.41**	**1.45**	
				(sd)	**(0.26)**	**(0.25)**	**(0.26)**	**(0.24)**	**(0.32)**	**(0.24)**	**(0.33)**	**(0.23)**	

Table 4 displays the mean and standard deviation (sd) for all primary outcomes with various scenarios, and results with p-values from the split-plot analysis are given in Table 5. Next, we deliver the results for each primary outcome separately.

2.4.1 Total nematodes

Over all pooled sample times and sites, the total number of nematodes varied with treatments, but within a small range (from 3550 at One50 + Low nitrogen + Clover presence to 4128 at One 50 + high nitrogen+ No clover, Table 4). This indicates that the nematode number may vary with nitrogen levels and the presence / absence of clover. We found that the average nematode numbers were extremely high at the first sampling in autumn 2013 in both locations (Autumn 2013 in Manawatu – 5434; and Autumn 2013 in Waikato – 7479, Table 4) with a large variation in the nematode numbers among the sampling times (from 2011 nematodes in the spring of 2014 in Manawatu to 7479 nematodes in the autumn of 2013 in Waikato, Table 4). Seasons had the largest consistent impact on total nematode numbers with the largest populations occurring in autumn at both sites (p-value < 0.001, Table 5).

It is possible to have different changing patterns between feeding groups and within feeding groups (taxa), so a summary of results is given in Appendix 1. Plant feeders' nematode numbers across all eight treatments are very similar (ranging from 1210 to 1909, Appendix 1). Similar results were observed for bacterial feeders (1517 – 2068, Appendix 1) and fungal feeders (572 – 695, Appendix 1). Seasonal variations, especially peak in autumn, with locations reported for plant feeders, fungal feeders and bacterial feeders (Appendix 1). Highest number omnivore and predator nematodes were found in One50 cultivars and base in autumn 2013 in Manawatu when nitrogen level was high without clover (42, Appendix 1), and when nitrogen level was low with presence of clover (38, Appendix 1) respectively.

To satisfy the normality assumption for the split-plot analysis, the total number of nematodes was transformed to a log scale with base 10. As mentioned earlier, four independent (Manawatu 2013, Manawatu 2014, Waikato 2013 and Waikato 2014) analyses were performed, and the results are displayed in Table 5.

In 2013 in Waikato, the total number of nematodes in autumn (mean = 7479, Table 4) is significantly higher than spring (mean = 3239, Table 4 & p-value < 0.001, Table 5). There is a significant impact on total nematode population with application of nitrogen levels (mean with low nitrogen = 4140, mean with high nitrogen = 4966 & p-value = 0.04, Table 5), and with interactions of treatment conditions (Table 4 & p-value = 0.033, Table 5).

In 2014 in Waikato, autumn nematode numbers (mean = 3802) are significantly higher than spring (mean = 3034 & p-value < 0.001, Table 5). In addition, presence and absence of clover with season (mean of no clover with autumn = 3373 , mean of clover present with autumn = 4285 , mean of no clover with spring = 3141, mean of clover present with spring = 2931 & p-value = 0.006, Table 5), and nitrogen changes with season (mean of low nitrogen with autumn = 4074 , mean of high nitrogen with autumn = 3556 , mean of low nitrogen with spring = 2735 , mean of high nitrogen with spring = 3365 & p-value = 0.002, Table 5) have a significant impact on total nematode numbers.

In 2013 in Manawatu, there is a significant difference between autumn (mean = 5047) and spring (mean = 2275) in nematode numbers (p-value < 0.001, Table 5).

In 2014 in Manawatu, nematode numbers are significantly changing with seasons (mean at autumn = 3112, mean at spring = 1945 & p-value < 0.001, Table 5). It is interesting to see that nematode numbers in cultivars One50 (mean = 2582) are significantly greater than Base (mean = 2344 & p-value = 0.04, Table 5).

Colonizer-Persister (c-p)

Regarding c-p values, ranges from a colonizer (c-p1) to a persister (c-p5) were present in the studied samples. Group c–p2 and c-p3 were dominant in both locations, indicating nematodes with a short to medium generation time, reasonable reproduction rate, and sensitivity to disturbances. Opportunistic (c-p1) with three taxa were studied, but reported only two taxa in Waikato and three taxa in Manawatu. A c-p5 taxon, indicating long-term information about environmental conditions, was only found in the Manawatu.

2.4.2 Maturity Index (MI)

The Maturity Index (MI) was always greatest in low N treatments, and this was significantly so on two occasions (Table 5). The cultivar Base was always associated with nematode populations that had a greater MI than One50, except for Waikato 2013 when differences were least apparent. MI increased from autumn to spring so that spring MI was always significantly greater.

Maturity indices (1.72 [One50, high nitrogen & no clover] to 1.81 [Base, Low nitrogen & clover present; Base, low nitrogen, clover absent], Table 2) are very consistent with all eight treatments. This suggests the ecosystem was a nutrient-enriched disturbed system (Bongers & Ferris, 1999), but it was not changed by the treatments. We found that maturity indices are changing from 1.52 (Manawatu, autumn & 2014, Table 2) to 1.97 (Waikato, spring & 2014, Table 2). Again, it is a nutrient-enriched disturbed system (Bongers & Ferris, 1999), but there is a trend of ecosystem improvement from autumn (low MI, Table 2) to spring (high MI, Table 2).

In 2013 in Waikato, the MI in autumn (mean MI = 1.68) is significantly lower than spring (mean MI = 1.94, & p-value < 0.001, Table 5). MI values are significantly changing with interaction of presence/absence of clover and high/low nitrogen levels (mean MI in clover present with low nitrogen = 1.79, mean MI in clover present with high nitrogen = 1.84, mean MI in clover absent with low nitrogen = 1.84, mean MI in clover absent with high nitrogen = 1.76, & p-value = 0.047, Table 5).

In 2014 in Waikato, MI in spring (mean MI = 1.97) is significantly greater than autumn (mean MI = 1.73, & p-value < 0.001, Table 5). The main effects of clover (mean MI with clover = 1.80, mean MI without clover = 1.90 & p-value < 0.001, Table 5), nitrogen (mean MI with high nitrogen = 1.80, mean MI with low nitrogen = 1.91 & p-value < 0.001, Table 5) and cultivars (mean MI with One50 = 1.82, mean MI with Base = 1.88 & p-value = 0.031, Table 5) have a significant impact on MI.

In 2013 in Manawatu, the MI has a significant variation with seasons (mean MI with autumn = 1.56, mean MI with spring = 1.87, & p-value < 0.001 Table 5). MI is significantly high when low nitrogen (mean MI with low nitrogen = 1.74) compared with high nitrogen (mean MI with high nitrogen = 1.70, p-value = 0.02, Table 5). Two way interactions of clover and nitrogen (mean MI with clover and low nitrogen = 1.73, mean MI with clover and high nitrogen = 1.72, mean MI without clover and low nitrogen = 1.66, mean MI without clover and high nitrogen = 1.76, & p-value = 0.047, Table 5), and clover and season (mean MI with clover and autumn = 1.60, mean MI with clover and spring = 1.84, mean MI without clover and autumn = 1.53, mean MI without clover and spring = 1.90, & p-value = 0.046, Table 5) have significant impact on MI.

In 2014 in Manawatu, MI in autumn (mean MI = 1.52) is significantly lower than spring (mean MI = 1.89, p-value < 0.001, Table 5). MI in base (mean MI = 1.77) is significantly higher than One50 (mean MI = 1.65, p-value = 0.002, Table 5). Three way interactions of season, clover and nitrogen (mean MI with clover and low nitrogen in autumn = 1.85, mean MI with clover and low nitrogen in spring = 1.57, mean MI with clover and high nitrogen in autumn = 1.51, mean MI with clover and high nitrogen in spring = 1.90, mean MI without clover and low nitrogen in autumn = 1.44, mean MI without clover and low nitrogen in spring = 1.94, mean MI without clover and high nitrogen in autumn = 1.54, mean MI without clover and high nitrogen in spring = 1.86) have significant impact on MI (p-value = 0.014, Table 5).

2.4.3 Maturity Index 2 to 5 (MI 2-5)

Maturity indices 2 to 5 (MI 2-5) are very similar with sampling stages (2.03 [Manawatu, autumn & 2014; Waikato, autumn & 2014] to 2.12 [Waikato, spring & 2014], Table 2), and with treatments (2.04 [Base, high nitrogen & clover absent; Base, high nitrogen & clover present] to 2.09 [Base, low nitrogen & clover present], Table 2). Overall, MI 2-5 moved to around 2 after removing *c-p*1 from MI. As for MI, MI2-5 was greater in spring than autumn, except for Manawatu 2013 when the differences were small.

In 2013 in Waikato, MI 2-5 is marginally different with seasons (mean MI 2-5 with autumn = 2.05, mean MI with spring = 2.08, & p-value = 0.032, Table 5). Two-way interactions of nitrogen and clover (mean MI 2-5 with clover and high nitrogen = 2.06, mean MI 2-5 with clover and low nitrogen = 2.04, mean MI 2-5 without clover and high nitrogen = 2.04, mean MI 2-5 without clover and low nitrogen = 2.10) have a significant impact on MI 2-5 (p-value =0.033, Table 5).

In 2014 in Waikato, MI 2-5 in spring (mean MI 2-5 = 2.12) is significantly higher than autumn (mean MI 2-5 = 2.03, p-value < 0.001, Table 5). MI 2-5 with low nitrogen (mean MI 2-5 = 2.09) is significantly greater than high nitrogen (mean MI 2-5 = 2.05, & p-value = 0.035, Table 5).

In 2013 in Manawatu, none of the variables including seasonal variation impact on MI 2-5.

In 2014 in Manawatu, MI 2-5 is marginally varied with two way interactions of season and clover (mean MI 2-5 with clover in autumn = 2.04, mean MI 2-5 with clover in spring =2.08, mean MI 2-5 without clover in autumn = 2.03, mean MI 2-5 without clover in spring = 2.03, & p-value = 0.044, Table 5).

Table 5: p-values obtained from the split-plot design analysis with primary outcome measures of four data sets (Waikato 2013, Waikato 2014, Manawatu 2013 and Manawatu 2014).

Primary Outcomes	Main effects	Two-way Interactions	Three and four way interactions	Waikato 2013	Waikato 2014	Manawatu 2013	Manawatu 2014
Total nematodes in log10 scale	WClover			0.803	0.127	0.424	0.195
	Nitrogen			**0.043**	0.519	0.143	0.364
		WClover.Nitrogen		0.816	0.067	0.299	0.343
	Cultivar			0.257	0.336	0.364	**0.037**
		Cultivar.WClover		0.624	0.736	0.452	0.519
		Cultivar.Nitrogen		0.233	0.5	0.954	0.28
			Cultivar.WClover.Nitrogen	**0.033**	0.084	0.619	0.796
	Season			**<.001**	**<.001**	**<.001**	**<.001**
		Season.Cultivar		0.204	0.065	0.495	0.17
		Season.WClover		0.694	**0.006**	0.684	0.693
		Season.Nitrogen		0.983	**0.002**	0.964	0.128
			Season.Cultivar.WClover	0.263	0.133	0.888	0.449
			Season.Cultivar.Nitrogen	0.227	0.545	0.737	0.683
			Season.WClover.Nitrogen	0.925	0.489	0.318	0.123
			Season.Cultivar.WClover.Nitrogen	0.866	0.816	0.84	0.635

Table 5 (contd)

Primary Outcomes	Main effects	Two-way Interactions	Three and four way interactions	Waikato 2013	Waikato 2014	Manawatu 2013	Manawatu 2014
MI	WClover			0.741	**<.001**	0.543	0.665
	Nitrogen			0.655	**<.001**	**0.02**	0.967
		WClover.Nitrogen		**0.047**	0.400	**0.047**	0.768
	Cultivar			0.149	**0.031**	0.108	**0.002**
		Cultivar.WClover		0.516	0.174	0.766	0.568
		Cultivar.Nitrogen		0.273	0.777	0.843	0.303
			Cultivar.WClover.Nitrogen	0.108	0.465	0.439	0.644
	Season			**<.001**	**<.001**	**<.001**	**<.001**
		Season.Cultivar		0.992	0.797	0.405	0.514
		Season.WClover		0.066	**0.05**	**0.046**	0.194
		Season.Nitrogen		0.328	0.711	0.712	0.568
			Season.Cultivar.WClover	0.628	0.409	0.261	0.158
			Season.Cultivar.Nitrogen	0.786	0.314	0.71	0.261
			Season.WClover.Nitrogen	0.205	0.436	0.481	**0.014**
			Season.Cultivar.WClover.Nitrogen	0.69	0.304	0.637	0.596

Table 5 (contd)

Primary Outcomes	Main effects	Two-way Interactions	Three and four way interactions	Waikato 2013	Waikato 2014	Manawatu 2013	Manawatu 2014
MI 2-5	WClover			0.237	0.117	0.096	0.101
	Nitrogen			0.21	**0.035**	0.503	0.578
		WClover.Nitrogen		**0.033**	0.895	0.482	0.376
	Cultivar			0.321	0.382	0.546	0.996
		Cultivar.WClover		0.909	0.468	0.707	0.603
		Cultivar.Nitrogen		0.9	0.284	0.061	0.762
			Cultivar.WClover.Nitrogen	0.27	0.306	0.2	0.255
	Season			**0.032**	**<.001**	0.225	0.054
		Season.Cultivar		0.913	0.395	0.222	0.508
		Season.WClover		0.705	**0.019**	0.722	**0.044**
		Season.Nitrogen		0.467	0.33	0.645	0.569
			Season.Cultivar.WClover	0.944	0.808	0.799	0.31
			Season.Cultivar.Nitrogen	0.394	0.241	0.939	0.461
			Season.WClover.Nitrogen	0.832	0.636	0.799	0.89
			Season.Cultivar.WClover.Nitrogen	0.282	**0.025**	0.658	0.619

Table 5 (contd)

Primary Outcomes	**Main effects**	**Two-way Interactions**	**Three and four way interactions**	**Waikato 2013**	**Waikato 2014**	**Manawatu 2013**	**Manawatu 2014**
PPI	WClover			0.632	**0.002**	0.65	0.858
	Nitrogen			0.851	0.294	0.705	0.69
		WClover.Nitrogen		0.422	0.702	0.507	**0.05**
	Cultivar			**0.016**	**<.001**	0.481	0.168
		Cultivar.WClover		0.146	0.13	0.809	0.467
		Cultivar.Nitrogen		0.355	0.334	0.22	0.254
			Cultivar.WClover.Nitrogen	0.213	**0.071**	**0.087**	0.931
	Season			**0.002**	**0.009**	0.246	**<.001**
		Season.Cultivar		0.713	0.123	0.946	0.791
		Season.WClover		0.205	0.929	0.655	0.424
		Season.Nitrogen		0.142	0.685	0.819	0.172
			Season.Cultivar.WClover	0.311	**0.069**	0.614	**0.073**
			Season.Cultivar.Nitrogen	0.857	0.926	0.767	**0.049**
			Season.WClover.Nitrogen	0.601	0.849	0.648	0.824
			Season.Cultivar.WClover.Nitrogen	0.999	0.856	0.496	0.152

2.4.4 Plant Parasite Indices (PPI)

Plant parasite indices (PPI) are very similar with sampling stages, but different between Waikato and Manawatu (2.51 [Manawatu, spring & 2014] to 2.78 [Manawatu, autumn & 2013] and 2.24 [Waikato, autumn & 2013] to 2.36 [Waikato, spring & 2013], Table 2). This indicates that the Waikato soil is healthier than Manawatu. Again, it is consistent with treatments (2.44 [One50, high nitrogen & clover absent] to 2.54 [Base, low nitrogen & clover present], Table 2). Overall, PPI is around 2.5, suggesting it is not a well-developed, stable ecosystem.

White clover increased PPI in the Waikato in 2014 but this was not consistent across years or sites. However, PPI was consistently greater for plots sown to Base than One 50 ryegrass over all sites and years with the Waikato site producing the differences. In 2013 at the Waikato site, the PPI was greatest in spring but at all other samplings PPI was greatest in autumn, in contrast to MI.

In 2013 in Waikato, the PPI in spring (mean PPI = 2.35) is greater than autumn (mean PPI =2.24 & p-value = 0.002, Table 5) and changes in PPI were observed with cultivars (mean PPI with Base = 2.33, mean PPI with One50 = 2.26, & p-value = 0.016, Table 5).

In 2014 in Waikato, the main effects of seasons (mean PPI with autumn = 2.33, mean PPI with spring = 2.26, & p-value = 0.009, Table 5), clover (mean PPI with clover = 2.35, mean PPI without clover = 2.24, & p-value = 0.002, Table 5) and cultivars (mean PPI with Base = 2.35, mean PPI with One50 = 2.24, & p-value < 0.001, Table 5) have a significant impact on PPI.

In 2013 in Manawatu, none of the treatments, including seasons, have an impact on PPI. In 2014 in Manawatu, a significant seasonal effect on PPI was found (mean PPI in autumn = 2.65, mean PPI in spring = 2.51, p-value < 0.001, Table 5).

2.4.5 Ratio PPI/MI

The ratio between PPI and MI by treatments ranged from 1.41 (Base, low nitrogen & clover absent, Table 2) to 1.45 (Base, high nitrogen & clover present; One50, high nitrogen & clover absent: One50, low nitrogen & clover present, Table 2), and by sampling stages ranged from 1.16 (Waikato, spring & 2014, Table 2) to 1.79

(Manawatu, autumn & 2013). The ratio for Manawatu (around 1.5, Table 2) is higher than Waikato (around 1.25 Table 2). That means, plant feeder activities for Manawatu and Waikato are 1.5 and 1.25 times more than other feeder activities, such as bacterial feeders, fungal feeders, predators and omnivores respectively.

2.4.6 Sigma MI (ΣMI)

The total maturity index (ΣMI) by treatments ranged from 1.97 (One50, high nitrogen & clover absent, Table 2) to 2.14 (Base, low nitrogen & clover absent, Table 2) and by sampling stages ranged from 1.85 (Waikato, autumn & 2013, Table 2) to 2.33 (Manawatu, spring & 2013, Table 2), and the greatest ΣMI was always found in spring (Table 2).

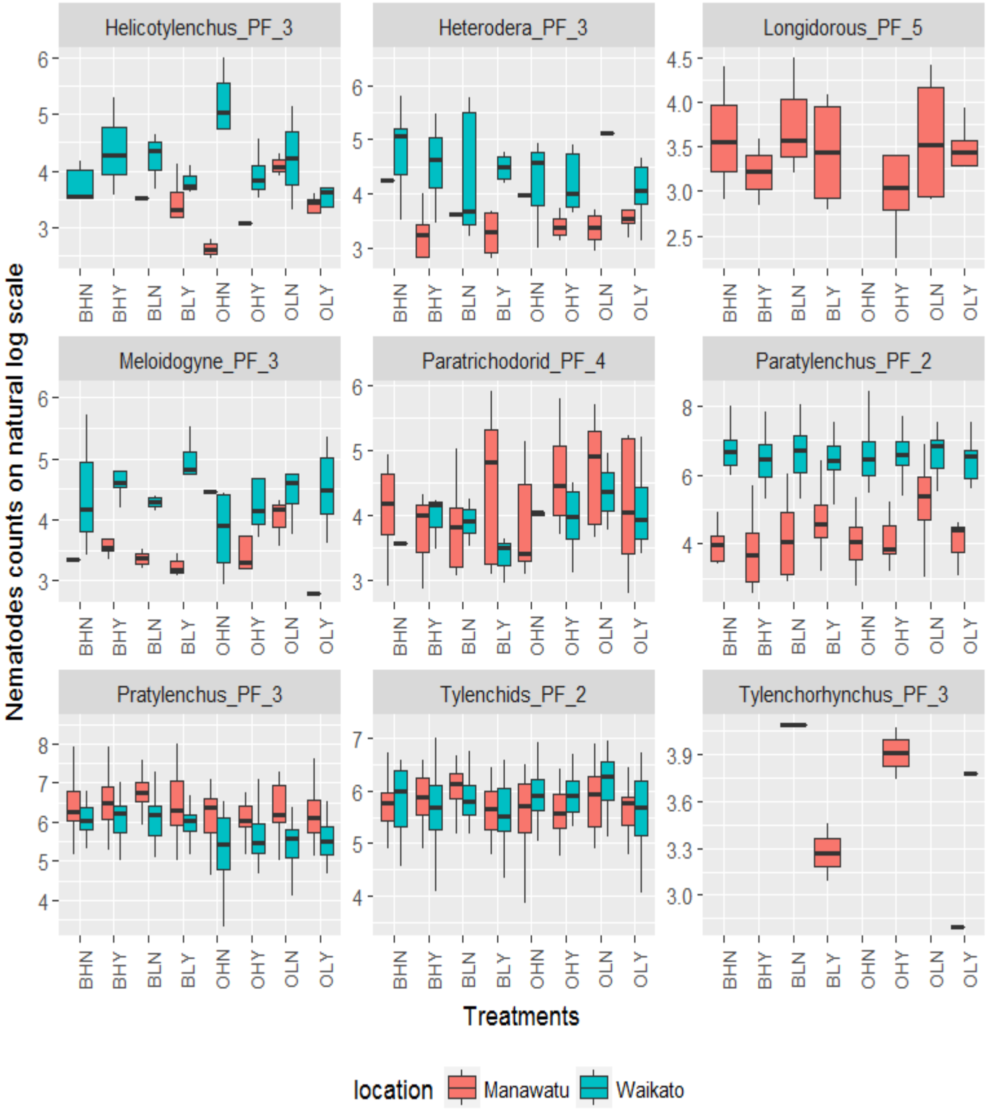


Figure 2: Boxplots for plant feeders' taxon counts on natural log scale versus treatments (BHN – Base high nitrogen and no clover; BHY – Base high nitrogen and present clover; BLN – Base low nitrogen and no clover; BLY – Base low nitrogen and clover present; OHN – One50 high nitrogen and no clover; OHY – One50 high nitrogen and present clover; OLN – One50 low nitrogen and no clover; OLY – One50 low nitrogen and clover present).

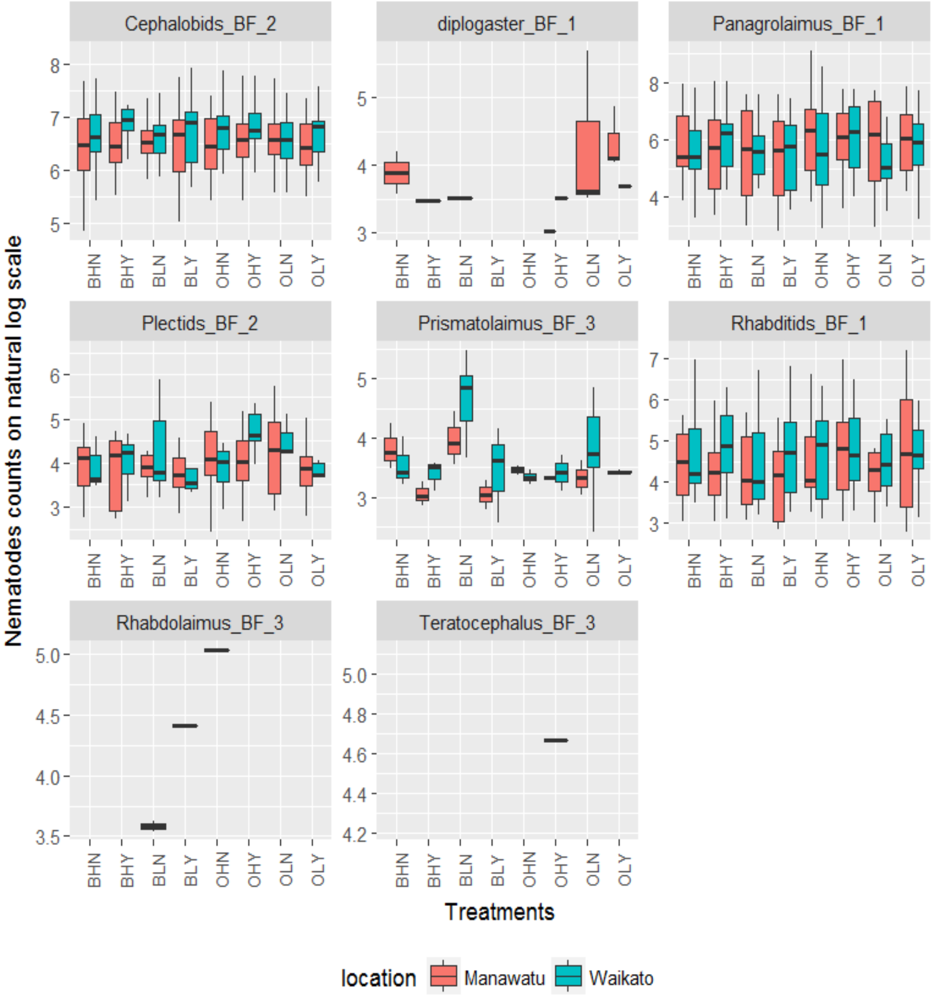


Figure 3: Boxplots for bacterial feeders' taxon counts on natural log scale versus treatments.

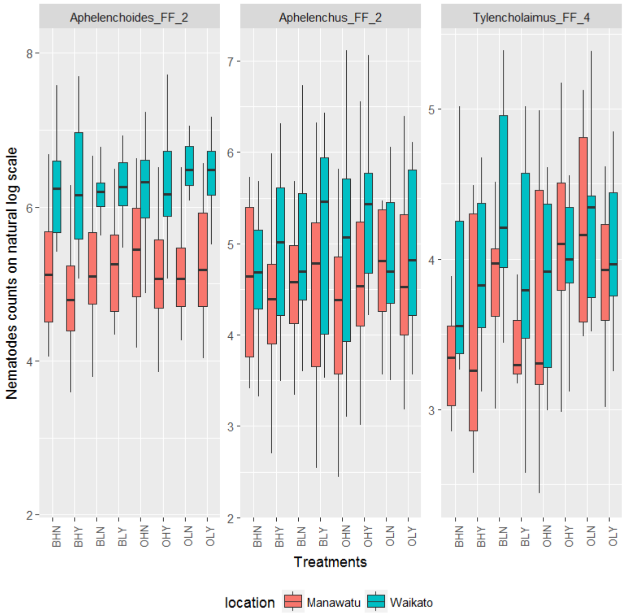


Figure 4: Boxplots for fungal feeders' taxon counts on natural log scale versus treatments.

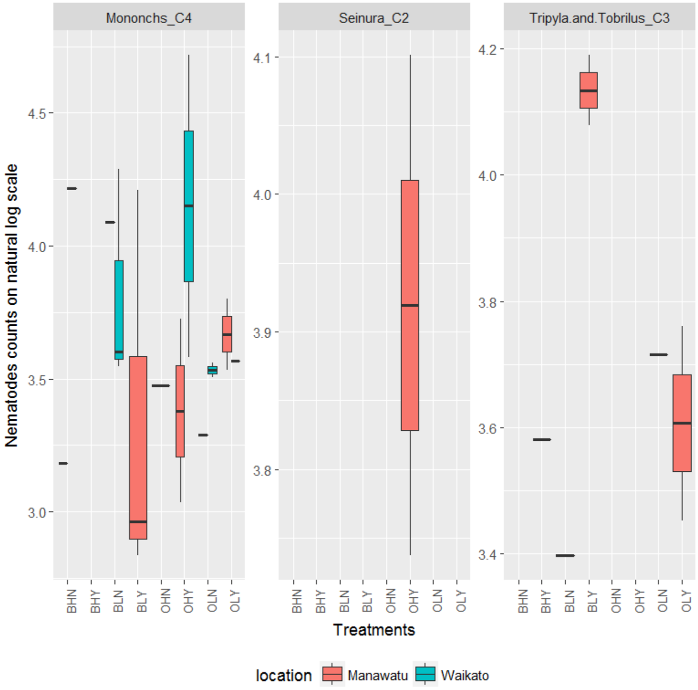


Figure 5: Boxplots for predator feeders' taxon counts on natural log scale versus treatments.

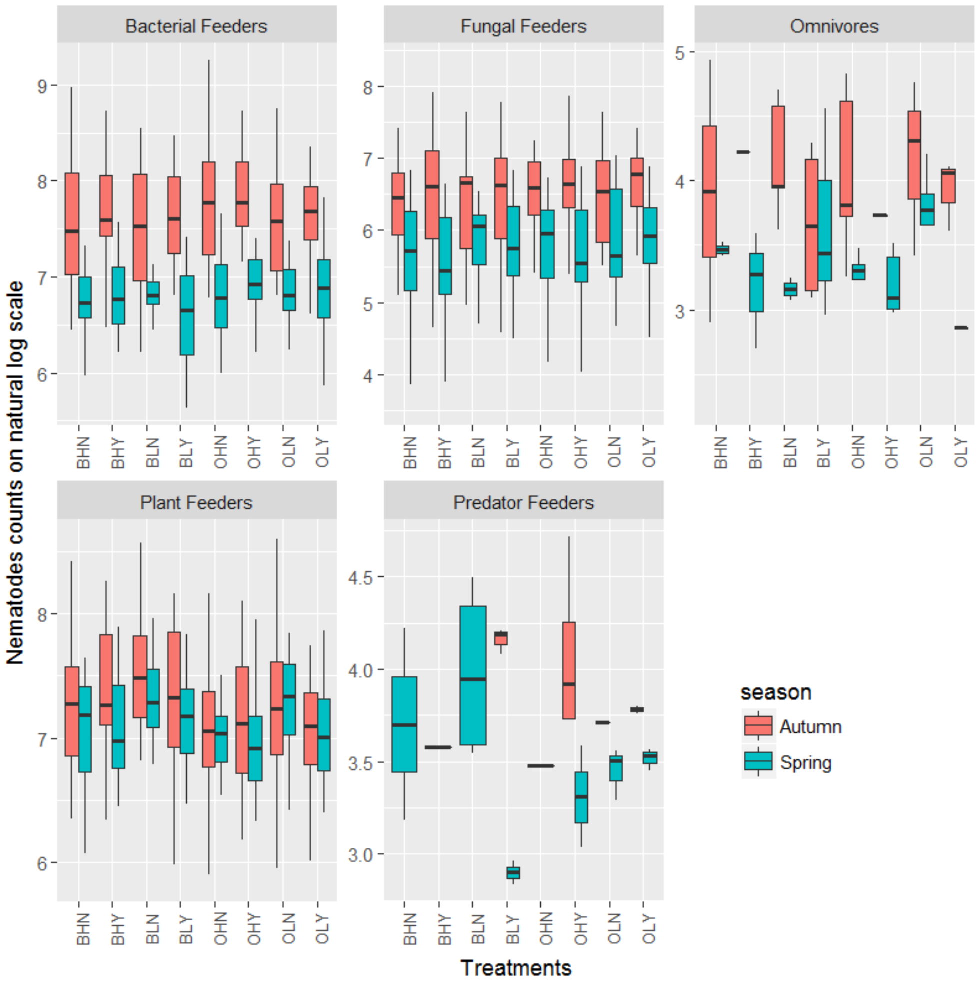


Figure 6: Nematodes changes in the feeder group with treatments and seasons.

Table 6: Results from principal component analysis of all taxon using all data.

Feeding Group	**Taxa**	**PC1**	**PC2**	**PC3**	**PC4**
Plant Feeders	Tylenchids	0.071	**-0.191**	**-0.276**	0.008
	Tylenchorhynchus	-0.043	0.129	0.018	**-0.422**
	Paratylenchus	**0.337**	**-0.261**	-0.045	0.065
	Longidorous	-0.114	**0.327**	**-0.212**	-0.162
	Meloidogyne	0.146	**-0.188**	**0.218**	-0.326
	Heterodera	-0.030	**-0.210**	-0.093	-0.020
	Helicotylenchus	**0.311**	0.019	0.072	**0.173**
	Paratrichodorid	-0.105	**0.257**	-0.090	0.156
	Pratylenchus	-0.036	**0.360**	**-0.168**	0.156
Fungal Feeders	Aphelenchus	**0.335**	0.112	0.086	**-0.321**
	Aphelenchoides	**0.395**	-0.140	-0.019	-0.046
	Tylencholaimus	0.089	**-0.235**	**-0.392**	0.033
Bacterial Feeders	Cephalobids	**0.343**	0.104	**-0.244**	-0.079
	Diplogaster	0.006	**0.266**	**-0.234**	0.065
	Rhabditids	**0.322**	0.143	0.071	-0.041
	Panagrolaimus	**0.315**	**0.274**	0.045	0.053
	Plectids	0.178	**0.263**	0.106	0.115
	Prismatolaimus	-0.011	**-0.236**	**-0.299**	**0.244**
	Rhabdolaimus	**0.269**	0.054	0.079	**0.224**
	Teratocephalus	0.115	-0.052	**-0.260**	**-0.252**
Omnivores		0.145	0.072	-0.163	0.327
Predator Feeders	Seinura	-0.002	0.107	-0.038	**-0.367**
	Tripyla and Tobrilus	-0.004	**0.279**	**-0.265**	-0.075
	Mononchs	-0.020	-0.085	**-0.473**	**-0.228**
	Eigen values	5.135	3.353	2.417	1.884
	Proportion of explained variance	0.211	0.138	0.099	0.077
	Cumulative variance	0.211	0.349	0.448	0.525

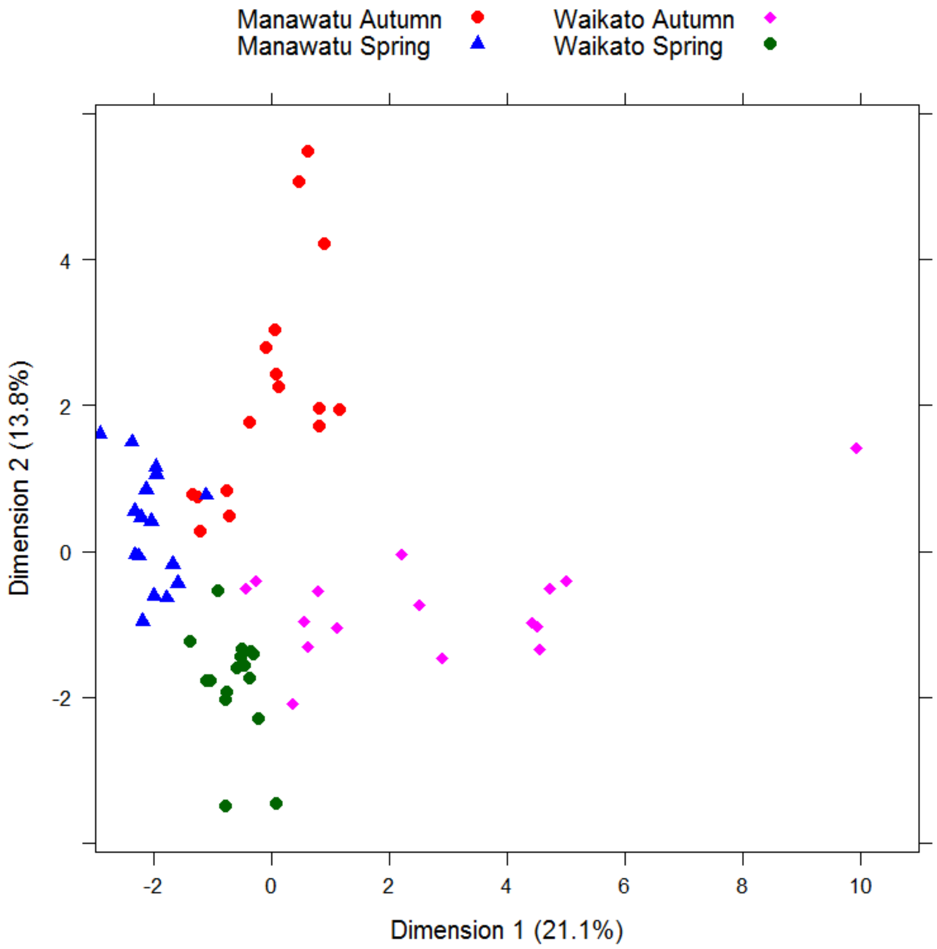


Figure 7: Scatter plot for first principal component versus second principal component.

Before performing principal component analysis, the data was standardised. Table 6 shows the first four principal components, which explained 52.5% of the total variation. The first principal component (PC1) mainly depends on *Paratylenchus* (PF 2), *Helicotylenchus* (PF 3), *Aphelenchus* (FF 2), *Aphelenchoides* (FF 2), Cephalobids (BF 2), Rhabditids (BF 1), *Panagrolaimus* (BF 1), and Rhabdolaimus (BF 3). The second principal component (PC2) describes positively with *Longidorous* (F 5), Paratrichodorid (PF 4), *Pratylenchus* (PF 3), *diplogaster* (BF 1), *Panagrolaimus* (BF 1), *Plectids* (BF 2), and *Tripyla* and *Tobrilus* (C3), and negatively with Tylenchids (PF 2), *Paratylenchus* (PF 2), *Meloidogyne* (PF 3*), Heterodera*(PF 3), *Tylencholaimus* (PF 3), and *Prismatolaimus* (BF 3). The third principal component indicates that *Meloidogyne* (PF 3) prefer to live alone, particularly without taxa, such as Tylenchids (PF 2), *Longidorous* (PF 5*), Pratylenchus* (PF 3), *Tylencholaimus* (FF 4), Cephalobids (BF 2), *diplogaster* (BF 1), *Prismatolaimus* (BF 3), *Teratocephalus* (BF 3), *Tripyla and Tobrilus* (C3), and Mononchs (C4). The fourth principal component describes positively with *Helicotylenchus* (PF 3), *Prismatolaimus* (BF 3), and *Rhabdolaimus* (BF 3), and negatively with *Tylenchorhynchus* (FF4), *Meloidogyne* (PF 3), *Aphelenchus* (FF2), *Teratocephalus* (BF 3), Seinura (C2), and *Mononchs* (C4).

Figure 7 shows the prediction of PC 2 (explained 13.8% of the total variation) versus PC1 (explained 21.1% of the total variation). We found four major differences in the data collections, indicating sites and seasons are the main factors to describe the distribution of taxon. The variations within clusters were found due to the treatment impact.

2.5 Discussion

This study was focused on the nematode populations in New Zealand pasture soil with sixteen experimental conditions, mentioned in Table 1, and analysis was performed with four independent sampling stages. Yeates (1982) did a similar study on one site, but used an observational study to find the relationship between nematode population changes and environmental factors, particularly with seasonal changes. He found 28 genera, but restricted to report 24 nematodes genera in Manawatu soil. In our Manawatu sample, we found 21 nematode taxa, most of which are in common with Yeates' (1982) identifications, with one prominent difference being the presence of the large plant feeding nematode *Longidorous*, which has been reported in Manawatu soil (Yeates et al., 2008). The presence of *Longidorous* indicates that soil at the Manawatu site must be either fine sandy loam or have large available soil pore spaces (Yeates et al., 2008).

The average nematode numbers were extremely high at the first sampling in autumn 2013 in both locations. There are doubts about the stability of the ecosystem in the study area at the beginning of the experiment. Such nematode abundance or diversity means that simple effects of soil and management can often be demonstrated in long-term grasslands. In young pastures, nematodes will not be in equilibrium with local conditions (Wasilewska, 1994). Similar observation was found in Tozer et al., (2014) study and they described that the youngest age category pastures where total nematode populations were higher than old pastures, and possibly soil disturbance effects from nutrient pulses to soil which will change the soil food web.

The natural abundance of soil nematodes in Waikato is still poorly understood. However, Sarathchandra et al. (2001) found 32 nematode genera in Waikato soil with the application of different levels of fertiliser. More than one-third of the identified genera were reported as a rare taxon in Waikato soil. In our Waikato samples, we found 16 nematode taxa, mostly similar to Sarathchandra et al., (2001). However, most common taxa of Tylenchids, Paratrichodorid and Mononchs found in our sample were not reported in Sarathchandra et al., (2001) while some taxa (Diplogaster, Ditylenchcus, etc.) were not found in our samples, but reported previously (Sarathchandra et al., 2001). The differences between studies indicate that some nematodes taxa may sensitive to either prior host and management or soil type or time of sampling.

Based on all outcome measure results, except MI 2-5, the nematode population is greater in autumn compared with spring. This finding agrees with Yeates' (1982) conclusion of nematode population being affected by seasonal changes. This result may have been due to (i) the wetter and cooler period being favourable for new plant root growth resulting in an increase in food supply for nematodes (Elstrand et al., 2008); (ii) the life cycle of the organism; (iii) depth distribution of populations so that maximum populations were below the sample depth we used; (iv) nematode population development rates being directly impacted by changes in soil temperature and moisture (Yeates., 1978).

The correlation coefficient between nematode genera was used to determine the association between taxa. Novel graphical displays of correlation coefficients in ecological science are presented in this study. We found most of the fungal feeders are associated with some plant feeders and bacterial feeders. For example, the strong positive relationship between *Paratylenchus* and *Apheleneholdes* (Figure 1) may be interpreted as positive functional activities between *Paratylenchus* and *Aphelenechoides* (availability food sources for fungi seem high due to damage on plant roots made by plant feeders (Wardle et al., 2003). This result clearly reflects in our samples. For example, *Paratylenchus* numerically dominant in Waikato compared with Manawatu (Figure 2), and *Apheleneholdes* in Waikato are numerically greater than Manawatu (Figure 4). It is a clear evidence of the relationship between plant feeders and fungal feeders. In contrast, a very weak negative relationship between *Paratylenchus* and *Pratylenchus* was found in this study (Figure 1) due to the competition for the same food resources (Bell and Watson, 2001). Figure 2 shows that the dominant residences for *Paratylenchus* and *Pratylenchus* are Waikato and Manawatu respectively, indicating competition for resources. In addition, some association between plant feeders and predator feeders plus between bacterial feeders of *Panagrolaimus* and Omnivores were found (Figure 1).

Based on the total nematodes, the mean nematode numbers marginally varied with nitrogen levels and the presence/absence of clover. The reasons for this result are: (i) nematodes are associated with clover (Mercer et al., 2008); (ii) clover fixes nitrogen (Mercer et al., 2008; Yeates, 1982); (iii) the structural changes of the community pattern of nematodes is possible with nitrogen levels. For example, increased contribution in bacterial feeders and decreased contribution in fungi, predators and omnivores were found with a nitrogen addition (Song et al., 2016; Wei et al., 2012; Azpilicueta et al., 2014; Pan et al., 2015; Sarathchandra et al., 2001; Rodriguez-Kabana, 1986). In some cases, we found significant changes in nematode population with main treatments (nitrogen levels, presence/absence of clover and cultivars), and some two or three way interactions. However, in most of the cases, non-significant results were found. It is hard to interpret the non-significant results at this stage, because the remarkable treatment effect may be seen after a longer period post established. This was discussed in many previous studies (Yeates et al., 2003; Wardle et al., 1999; Yeates et al., 2000).

As we mentioned before, maturity index (MI) and plant parasite index (PPI) are used to evaluate the state of succession of the ecosystem and the enrichment of the ecosystem respectively. This study can describe the short to medium stability of the ecosystem, because most taxona with c-p2 to c-p3 were studied here (Wilson and Kakouli-Duarte., 2009). An MI value below 2 indicates that the eco-system was a nutrient-enriched disturbed system. Overall, low MI found in this study (Table 2) suggests the unhealthy eco-system or nutrient-enriched disturbed system (Bongers & Ferris, 1999). However, the greatest MI was always found to have a low nitrogen level (Table 2). This finding partially agrees with a suggestion that high MI may be observed with the lowest organic matter content (Ürkmez et al., 2014)**,** since MI values are low in autumn and high in spring. This result was opposite to the total nematode numbers, including plant feeders. As a result of this, we may consider that activities of shorter life nematode in autumn is considerably high, since MI 2-5 values are consistent with around 2 after removing c-p1 taxa from MI. This means that the observed small seasonal effect on MI disappears with MI 2-5. This shows that the small seasonal effect on MI was caused by c-p1 taxa. In addition, MI values are lowest in autumn, showing that the c-p1 taxa are very active in autumn. In our samples, c-p1 taxon of Panagrolaimdae and Rhabditide are very predominant in autumn. Higher values of PPI show that the unstable ecosystem and plant feeders seem to be the dominant influence on the sampling soil. The ratio between PPI and MI in autumn is high compared with spring. That means greater plant feeder activities compared with other feeders in autumn are relatively high compared with spring.

Figure 7 shows clear clustering based on sampling sites and seasons. For this sample, the population of nematode taxa may be described with locations and seasons rather than treatments.

2.6 Conclusion

This study analysed the use of the nematode population and community structure as a tool for determining impacts of some biotic and abiotic factors on soil functioning. The factors ranged from those with large, obvious differences during the two-year study period (e.g. season, and associated temperature and moisture changes) to those likely to have more subtle impacts such as plant cultivar/ploidy. Unsurprisingly, but encouragingly, large differences in the nematode community were observed for the large impact changes (season), especially in total nematode and MI measures. A relatively small difference in fertiliser N input (in on-farm terms) resulted in a consistent, although not always significant, decrease in MI at the high rate of application. This reflects a proportional increase in resource opportunist nematodes (c-p 1 and 2), probably in response to the pulse of microbial growth post-fertilisation. This finding suggests the addition of even moderate rates of N to soil have implications for soil functioning and sustainable use given that a more structured (i.e. greater MI) nematode community is a desirable indicator of soil health.

Even more encouragingly, ryegrass cultivar/ploidy impacts were observed on the plant-feeding component of the nematode community (PPI). It is not clear what the mechanism for this is but likely candidates are the quantity or quality of roots beneath the respective ryegrasses. An interesting seasonal contrast between MI (greatest in spring) and PPI (greatest in autumn) shows how these two components of the nematode community, the latter which relies directly on plant growth, respond to food resources and seasonal conditions.

This study has employed a range of different statistical properties to interpret the complex datasets generated from the soil community and environmental interactions. The analysis approaches taken here is often used for analysis of among-species relationships, but are also increasingly used for functional interpretation of ecosystem networks, including those generated by molecular approaches. The ability to graphically represent the range of strength of interactions amongst the members of the nematode community adds new insights to these datasets. Few other researchers to date have used this form of graphical representation for morphological nematode community analyses, but it could well be a means of bringing together the results of morphological and molecular studies within a common framework of representation and hence understanding.

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2.9 Appendix 1: Mean nematode numbers for feeding groups and genera by treatments and sampling stages

Feeding Group	Taxa	Location	Year	Seasons	BHN	BHY	BLN	BLY	OHN	OHY	OLN	OLY	Over-All mean
Plant Feeder		Manawatu	2013	Autumn	1811.41	2251.69	2576	2233.65	937.19	1253.36	2073.77	1329.08	**1808.27**
		Manawatu	2013	Spring	1026.57	1414.21	1762.69	1386.87	1017.45	863.98	1745.7	995.46	**1276.62**
		Manawatu	2014	Autumn	817.87	1020.7	1305.71	980.23	1102.48	769.09	1127.76	688.08	**976.49**
		Manawatu	2014	Spring	843.22	868.04	1421.7	1095.41	787.6	736.3	1100.82	893.29	**968.3**
		Waikato	2013	Autumn	2471.14	2944.65	3364.45	2435.8	2871.48	1793.65	2211.44	1432.72	**2446.54**
		Waikato	2013	Spring	1596.85	1714.34	1243.09	1327.05	1685.25	1700.77	1275.5	1630.13	**1527.93**
		Waikato	2014	Autumn	1752.19	1250.44	1655.13	1282.85	1452.85	1851.95	1831.7	1487.06	**1563.82**
		Waikato	2014	Spring	1642.22	1184.13	1939.87	1573.27	1311.63	1249.98	2064.41	1222.92	**1509.68**
			Over All mean		**1495.18**	**1581.02**	**1908.58**	**1539.39**	**1395.74**	**1277.38**	**1660.35**	**1209.84**	
	Tylenchids	Manawatu	2013	Autumn	330.83	358.46	407.56	287.18	268.23	303.81	380.34	321.6	**332.25**
		Manawatu	2013	Spring	273.71	355.38	423.8	320.75	378.36	234.59	429.08	337.89	**344.2**
		Manawatu	2014	Autumn	241.33	302.4	423.05	223.74	285.89	336.36	315.3	188.51	**289.57**
		Manawatu	2014	Spring	390.51	508.59	623.09	383.98	362.05	364.94	537.05	432.92	**450.39**
		Waikato	2013	Autumn	428.11	502.09	428.58	641.97	391.98	444.6	386.38	375.24	**451.5**
		Waikato	2013	Spring	424.11	501.48	386.18	228.55	524.47	457.71	540.78	373.87	**426.79**
		Waikato	2014	Autumn	282.98	207.07	252.93	190.76	363.63	444.64	542.23	420.22	**332.82**
		Waikato	2014	Spring	463.68	253.89	476.01	371.3	442.17	266.38	619.65	252.86	**387.44**
			Over All mean		**354.41**	**373.67**	**427.65**	**331.03**	**377.1**	**356.63**	**462.92**	**337.89**	

Appendix 1 (contd)

Feeding Group	**Taxa**	**Location**	**Year**	**Seasons**	**BHN**	**BHY**	**BLN**	**BLY**	**OHN**	**OHY**	**OLN**	**OLY**	**Over-All mean**
	Tylenchorhynchus	Manawatu	2013	Autumn	0	0	0	0	0	8.4	0	0	**1.05**
		Manawatu	2013	Spring	0	0	0	6.32	0	0	0	0	**0.79**
		Manawatu	2014	Autumn	0	0	0	4.39	0	11.76	0	0	**2.02**
		Manawatu	2014	Spring	0	0	11.96	0	0	0	0	3.29	**1.91**
		Waikato	2013	Autumn	0	0	0	0	0	0	0	0	**0**
		Waikato	2013	Spring	0	0	0	0	0	0	0	0	**0**
		Waikato	2014	Autumn	0	0	0	0	0	0	0	8.72	**1.12**
		Waikato	2014	Spring	0	0	0	0	0	0	0	0	**0**
			Over All mean		**0**	**0**	**1.49**	**1.34**	**0**	**2.52**	**0**	**1.5**	
	Paratylenchus	Manawatu	2013	Autumn	52.54	81.32	160.02	195.83	3.19	54.38	129.57	28.81	**88.21**
		Manawatu	2013	Spring	0	37.41	9.28	187.78	38.19	36.89	85.58	21.94	**52.13**
		Manawatu	2014	Autumn	19.62	46.55	49.34	58.38	73	45.12	322.29	48.01	**82.79**
		Manawatu	2014	Spring	27.97	17.23	31.22	52.02	32.91	0	154.29	24.99	**42.58**
		Waikato	2013	Autumn	1511.55	1749.13	2077.65	954.16	2053.09	1049.66	1661.58	673.84	**1461.33**
		Waikato	2013	Spring	519.83	632.39	330.59	758.69	612.3	606.03	408.9	747.66	**581.36**
		Waikato	2014	Autumn	924.44	335.85	887.94	506.7	738.54	900.23	836.72	664.41	**721.47**
		Waikato	2014	Spring	784.6	496.47	1001.47	760.65	657.53	693.61	1098.67	682.39	**763.54**
			Over All mean		**480.07**	**424.55**	**568.44**	**434.28**	**526.09**	**423.24**	**541.17**	**361.51**	

Appendix 1 (contd)

Feeding Group	**Taxa**	**Location**	**Year**	**Seasons**	**BHN**	**BHY**	**BLN**	**BLY**	**OHN**	**OHY**	**OLN**	**OLY**	**Over-All mean**
	Longidorous	Manawatu	2013	Autumn	16.07	0	0	11.81	0	15.28	16.42	10.11	**8.71**
		Manawatu	2013	Spring	0	7.26	4.91	0	0	4.44	0	6.19	**2.85**
		Manawatu	2014	Autumn	3.63	0	0	0	0	0	11.83	0	**1.93**
		Manawatu	2014	Spring	6.94	3.43	25.06	16.98	0	5.81	7.47	9.75	**9.43**
		Waikato	2013	Autumn	0	0	0	0	0	0	0	0	**0**
		Waikato	2013	Spring	0	0	0	0	0	0	0	0	**0**
		Waikato	2014	Autumn	0	0	0	0	0	0	0	0	**0**
		Waikato	2014	Spring	0	0	0	0	0	0	0	0	**0**
			Over All mean		**3.33**	**1.34**	**3.75**	**3.6**	**0**	**3.19**	**4.96**	**3.25**	
	Meloidogyne	Manawatu	2013	Autumn	0	0	0	0	0	25.2	0	0	**3.15**
		Manawatu	2013	Spring	0	6.96	4.96	6.32	0	0	7.07	0	**3.16**
		Manawatu	2014	Autumn	5.74	18.03	6.66	9.16	0	15.67	13.05	0	**8.54**
		Manawatu	2014	Spring	0	6.86	0	0	17.15	0	14.91	3.29	**5.28**
		Waikato	2013	Autumn	60.93	64.76	0	73.45	0	0	29.13	19.04	**30.96**
		Waikato	2013	Spring	0	35.33	45.54	0	6.46	25.34	0	12.77	**16.08**
		Waikato	2014	Autumn	0	103.26	12.53	66.04	104.81	81.16	31.59	96.49	**62.76**
		Waikato	2014	Spring	19.12	20.16	0	26.27	25.68	0	29.19	43.9	**20.32**
			Over All mean		**10.72**	**31.92**	**8.71**	**22.65**	**19.26**	**18.42**	**14.86**	**21.94**	

Appendix 1 (contd)

Feeding Group	Taxa	Location	Year	Seasons	BHN	BHY	BLN	BLY	OHN	OHY	OLN	OLY	Over-All mean
	Heterodera	Manawatu	2013	Autumn	13.85	0	7.49	0	0	8.29	8.21	0	**4.73**
		Manawatu	2013	Spring	0	22.08	0	15.47	10.59	4.62	7.07	17.97	**9.72**
		Manawatu	2014	Autumn	0	0	0	0	0	0	0	6.92	**0.86**
		Manawatu	2014	Spring	0	6.69	0	7.15	0	5.8	8.79	6.86	**4.41**
		Waikato	2013	Autumn	0	39.18	0	14.54	0	19.06	0	29.8	**13.15**
		Waikato	2013	Spring	156.11	100.88	118.36	59.31	50.97	145.63	42.17	63.87	**93.44**
		Waikato	2014	Autumn	0	6.4	14.23	0	0	8.22	0	36.16	**8.33**
		Waikato	2014	Spring	0	73.54	0	86.28	0	43.37	0	4.56	**26.63**
			Over All mean		**21.24**	**31.1**	**17.51**	**22.84**	**7.69**	**29.37**	**8.03**	**20.77**	
	Helicotylenchus	Manawatu	2013	Autumn	0	0	0	20.57	0	0	0	0	**2.57**
		Manawatu	2013	Spring	0	0	6.69	10.44	2.3	4.39	14.83	13.55	**6.52**
		Manawatu	2014	Autumn	0	0	0	9.55	0	0	11.83	0	**2.67**
		Manawatu	2014	Spring	0	0	0	6.82	3.3	0	9.94	9.61	**3.71**
		Waikato	2013	Autumn	37.21	39.26	36.3	20.38	185.86	27.45	0	15.51	**46.41**
		Waikato	2013	Spring	6.79	7.18	0	0	3.98	0	0	5.82	**3.05**
		Waikato	2014	Autumn	26.31	0	7.97	7.55	0	10.42	49.36	0	**11.76**
		Waikato	2014	Spring	0	14.33	0	0	0	6.69	0	11.63	**4.19**
			Over All mean		**8.79**	**7.6**	**6.37**	**9.41**	**24.43**	**6.12**	**10.57**	**7.02**	

Appendix 1 (contd)

Feeding Group	Taxa	Location	Year	Seasons	BHN	BHY	BLN	BLY	OHN	OHY	OLN	OLY	Over-All mean
	Paratrichodorid	Manawatu	2013	Autumn	27.69	14.74	39.07	57.57	24.94	24.86	62.7	41.87	**36.68**
		Manawatu	2013	Spring	29.88	14.36	9.22	123.89	5.3	65.82	108.69	73.92	**53.88**
		Manawatu	2014	Autumn	15.11	0	0	4.39	39.87	11.76	44.32	16.06	**16.44**
		Manawatu	2014	Spring	14.46	0	12.07	10.31	10.09	8.14	9.94	3.29	**8.54**
		Waikato	2013	Autumn	7.04	13.6	0	0	0	0	0	35.98	**7.26**
		Waikato	2013	Spring	0	0	23.87	3.83	0	28.8	0	12.88	**8.89**
		Waikato	2014	Autumn	0	0	0	7.55	0	10.33	0	0	**2.29**
		Waikato	2014	Spring	0	19.26	6.74	6.57	33.03	28.93	46.05	14.09	**18.65**
			Over All mean		**11.77**	**7.75**	**11.37**	**26.76**	**14.15**	**22.33**	**36.46**	**24.76**	
	Pratylenchus	Manawatu	2013	Autumn	1370.44	1797.17	1961.85	1660.69	640.83	813.14	1476.53	926.69	**1330.92**
		Manawatu	2013	Spring	722.98	970.76	1303.84	715.9	582.7	513.24	1093.37	524	**803.35**
		Manawatu	2014	Autumn	532.45	653.72	826.66	670.61	703.71	348.41	409.12	428.59	**571.66**
		Manawatu	2014	Spring	403.34	325.23	718.31	618.16	362.1	351.61	358.44	399.3	**442.06**
		Waikato	2013	Autumn	426.3	536.63	821.92	731.3	240.54	252.88	134.35	283.3	**435.94**
		Waikato	2013	Spring	490.01	437.07	338.55	276.67	487.07	437.25	283.64	413.25	**398.31**
		Waikato	2014	Autumn	518.45	597.87	479.53	504.25	245.86	396.94	371.8	261.07	**423.26**
		Waikato	2014	Spring	374.82	306.49	455.64	322.2	153.22	211	270.85	213.48	**288.91**
			Over All mean		**604.85**	**703.12**	**863.29**	**687.47**	**427.01**	**415.56**	**581.38**	**431.21**	

Appendix 1 (contd)

Feeding Group	Taxa	Location	Year	Seasons	BHN	BHY	BLN	BLY	OHN	OHY	OLN	OLY	Over-All mean
Fungal Feeder		Manawatu	2013	Autumn	652.12	424.95	669.73	649.21	554.86	773.04	537.41	773.18	**629.31**
		Manawatu	2013	Spring	189.04	153.43	224.32	178.51	216.9	190.04	191.58	229.18	**196.63**
		Manawatu	2014	Autumn	357.11	368.01	247.7	307.58	482.55	486.5	449.75	368.75	**383.49**
		Manawatu	2014	Spring	159.34	160.68	253.04	236.84	304.15	196.52	252.45	273.17	**229.52**
		Waikato	2013	Autumn	1573.22	1800.42	1360.35	1545.73	1778.96	1437.97	1419.18	1533.24	**1559.64**
		Waikato	2013	Spring	584.77	461.01	514.48	413.02	631.33	634.27	597.23	601.29	**553.58**
		Waikato	2014	Autumn	552.02	987.08	679.73	1036.7	826.73	1279.39	862.52	1161.46	**924.76**
		Waikato	2014	Spring	505.32	465.39	537	695.98	414.71	387.36	868.71	622.12	**554.21**
			Over All mean		**571.62**	**602.62**	**560.79**	**632.95**	**651.27**	**673.13**	**615.18**	**695.3**	
	Aphelenchus	Manawatu	2013	Autumn	184.17	144.91	151.25	290.38	113.53	306.74	168.65	303.34	**207.87**
		Manawatu	2013	Spring	51.31	41.19	44.67	65.97	56.17	44.67	44.22	79.16	**53.42**
		Manawatu	2014	Autumn	176.75	226.34	99.78	115.51	164.94	256.94	176.28	136.41	**169.12**
		Manawatu	2014	Spring	6.94	30.11	30.83	32.46	34.26	37.44	23.8	51.72	**30.95**
		Waikato	2013	Autumn	358.11	267.12	140.36	389.5	371.71	460.98	264.13	286.69	**318.69**
		Waikato	2013	Spring	93.92	65.47	68.58	85.47	67.37	113.56	69.83	105.34	**84.05**
		Waikato	2014	Autumn	125.95	252.7	353.31	330.1	337.35	275.94	170.34	295.48	**270.14**
		Waikato	2014	Spring	59.73	80.62	47.42	81.4	26.26	78.1	32.35	75.22	**60.85**
			Over All mean		**132.11**	**138.56**	**117.03**	**173.85**	**146.45**	**196.8**	**116.98**	**166.67**	

Appendix 1 (contd)

Feeding Group	Taxa	Location	Year	Seasons	BHN	BHY	BLN	BLY	OHN	OHY	OLN	OLY	Over-All mean
	Aphelenchoides	Manawatu	2013	Autumn	461.31	265.3	481.05	358.84	416.39	436.08	312.92	433.64	**395.69**
		Manawatu	2013	Spring	120.84	108.76	151.7	112.54	121.52	136.49	139.95	137.64	**128.68**
		Manawatu	2014	Autumn	176.44	136.47	125.99	187.3	317.61	194.24	266.94	221.36	**203.29**
		Manawatu	2014	Spring	144.11	110.04	218.19	189.16	261.87	155.13	228.65	190.42	**187.2**
		Waikato	2013	Autumn	1184.93	1520.22	1166.95	1139.71	1387.12	915.45	1115.46	1220.52	**1208.63**
		Waikato	2013	Spring	459.74	352.09	423.82	311.03	548.79	484.46	496.82	454.86	**440.03**
		Waikato	2014	Autumn	419.08	716.48	320.15	655.79	484.06	993.03	650.61	839.28	**634.4**
		Waikato	2014	Spring	428.72	331.92	376.5	553.93	333.55	298.06	741.3	496.58	**437.47**
			Over All mean		**424.4**	**442.66**	**408.04**	**438.54**	**483.86**	**451.62**	**465.53**	**499.29**	
	Tylencholaimus	Manawatu	2013	Autumn	6.64	14.74	37.43	0	24.94	30.23	55.84	36.2	**25.75**
		Manawatu	2013	Spring	16.89	3.48	27.95	0	39.21	8.88	7.41	12.37	**14.52**
		Manawatu	2014	Autumn	3.92	5.21	21.93	4.78	0	35.33	6.53	10.98	**11.08**
		Manawatu	2014	Spring	8.29	20.53	4.02	15.23	8.02	3.94	0	31.03	**11.38**
		Waikato	2013	Autumn	30.18	13.09	53.04	16.52	20.13	61.53	39.59	26.03	**32.33**
		Waikato	2013	Spring	31.12	43.46	22.08	16.52	15.17	36.25	30.58	41.09	**29.51**
		Waikato	2014	Autumn	6.99	17.9	6.26	50.81	5.32	10.42	41.56	26.7	**20.21**
		Waikato	2014	Spring	16.87	52.85	113.07	60.65	54.9	11.2	95.06	50.33	**55.89**
			Over All mean		**15.11**	**21.41**	**35.72**	**20.56**	**20.96**	**24.72**	**32.67**	**29.34**	

Appendix 1 (contd)

Feeding Group	Taxa	Location	Year	Seasons	BHN	BHY	BLN	BLY	OHN	OHY	OLN	OLY	Over-All mean
Bacterial Feeder		Manawatu	2013	Autumn	2990.48	2739.99	2541.9	2995.6	4028.58	2182.18	3403.42	2854.84	**2967.12**
		Manawatu	2013	Spring	1034.36	732.18	791.57	744.35	956.62	1224.86	1022.71	946.32	**931.62**
		Manawatu	2014	Autumn	1534.3	1883.61	1780.31	1296.84	2510.95	2848.91	2635.12	1722.94	**2026.62**
		Manawatu	2014	Spring	882.55	653.91	679.89	761.08	862.89	754.54	720.73	1098.62	**801.78**
		Waikato	2013	Autumn	3711.45	3465.61	3626.72	3252.03	4683.66	3660.15	2415.34	2605.15	**3453.47**
		Waikato	2013	Spring	1159.44	1233.12	1342.95	799.39	1271.95	1088.81	1012.38	1198.23	**1141.51**
		Waikato	2014	Autumn	1257.05	1852.79	794.28	1286.78	1509.05	2657.27	795.52	1596.77	**1485.95**
		Waikato	2014	Spring	712.72	1332.68	939.15	997.81	722.82	1188.79	1056.02	1063.97	**1000.35**
			Over All mean		**1660.29**	**1736.74**	**1562.1**	**1516.74**	**2068.32**	**1950.69**	**1667.42**	**1635.85**	
	Cephalobids	Manawatu	2013	Autumn	1077.71	1228.31	1022.58	1444.64	794.34	944.81	1574.51	1154.68	**1155.2**
		Manawatu	2013	Spring	861.3	556.99	602.75	602.7	698.37	865.94	811.91	647.6	**705.95**
		Manawatu	2014	Autumn	673.06	745.78	677.79	600.53	723	1026.39	557.98	465.77	**683.79**
		Manawatu	2014	Spring	682.33	529.31	641.01	594.3	635.29	539.66	593.38	567.86	**597.89**
		Waikato	2013	Autumn	1353.29	1647.99	1296.8	1600.86	1678.91	2163.91	1131.65	1022.2	**1496.06**
		Waikato	2013	Spring	951.16	975.89	965.75	605.87	997.61	842.47	792.08	932.75	**885.28**
		Waikato	2014	Autumn	714.48	996.64	451.53	712.94	746.24	1085.95	443.57	743.51	**744.38**
		Waikato	2014	Spring	566.87	916.16	761.95	813.79	565.87	778.68	816.58	831.77	**754.92**
			Over All mean		**860.03**	**949.63**	**802.52**	**871.95**	**854.95**	**1030.97**	**845.12**	**795.77**	

Appendix 1 (contd)

Feeding Group	Taxa	Location	Year	Seasons	BHN	BHY	BLN	BLY	OHN	OHY	OLN	OLY	Over-All mean
	Diplogaster	Manawatu	2013	Autumn	13.28	0	0	0	0	0	66.22	37.29	**14.6**
		Manawatu	2013	Spring	7.14	0	0	0	0	0	7.41	0	**1.82**
		Manawatu	2014	Autumn	0	0	0	0	0	0	0	0	**0**
		Manawatu	2014	Spring	0	0	0	0	0	4.07	0	12.07	**2.02**
		Waikato	2013	Autumn	0	0	0	0	0	0	0	0	**0**
		Waikato	2013	Spring	0	0	0	0	0	0	0	0	**0**
		Waikato	2014	Autumn	0	0	0	0	0	0	0	8.06	**1.03**
		Waikato	2014	Spring	0	6.44	6.74	0	0	6.69	0	0	**2.55**
			Over All mean		**2.55**	**0.81**	**0.84**	**0**	**0**	**1.35**	**10.23**	**7.18**	
	Rhabditids	Manawatu	2013	Autumn	99.77	106.18	160.85	159.01	176.05	188.12	240.09	273.34	**175.42**
		Manawatu	2013	Spring	25.59	30.37	34.41	10.24	51.5	76.22	50.27	46.91	**40.69**
		Manawatu	2014	Autumn	83.49	117.19	49.79	63.39	206.67	440.31	156.68	226.99	**168.06**
		Manawatu	2014	Spring	43	13.84	0	25.31	44.38	11.42	19.2	369.97	**65.89**
		Waikato	2013	Autumn	545.42	205.23	400.12	334.74	397.48	75.48	185.61	183.95	**293.71**
		Waikato	2013	Spring	49.78	28.08	58	45.5	92.02	88.04	90.69	68.93	**64.47**
		Waikato	2014	Autumn	111.02	154.15	45.02	126.76	85.53	291.99	90.01	192.85	**138.38**
		Waikato	2014	Spring	27.75	175.47	41.64	107.57	98.52	167.75	61.44	72.57	**94.93**
			Over All mean		**123.23**	**103.81**	**98.73**	**109.06**	**144.02**	**167.42**	**112.28**	**179.44**	

Appendix 1 (contd)

Feeding Group	Taxa	Location	Year	Seasons	BHN	BHY	BLN	BLY	OHN	OHY	OLN	OLY	Over-All mean
	Panagrolaimus	Manawatu	2013	Autumn	1733.23	1380.36	1333.18	1359.56	3033.25	1015.88	1460.95	1285.43	**1575.23**
		Manawatu	2013	Spring	109.81	113.78	98.63	103.55	176.39	254.58	130.9	224.02	**151.46**
		Manawatu	2014	Autumn	723.83	980.59	1006.82	588.04	1423.82	1241.74	1760.8	961.95	**1085.95**
		Manawatu	2014	Spring	142.6	88.91	38.88	111.23	134.4	170.56	92.39	115.34	**111.79**
		Waikato	2013	Autumn	1805.7	1577.82	1810.12	1281.1	2406.96	1357.06	1072.89	1336.99	**1594.11**
		Waikato	2013	Spring	143.35	198.39	247.95	145.39	150.26	125.44	85.11	184.91	**162.02**
		Waikato	2014	Autumn	411.99	684.54	280.07	426.4	677.28	1222.12	251.46	635.58	**581.94**
		Waikato	2014	Spring	74.18	220.72	68.06	57.37	44.02	231.17	109.26	156.08	**120.39**
			Over All mean		**643.09**	**655.64**	**610.46**	**509.08**	**1005.8**	**702.32**	**647.22**	**612.54**	
	Plectids	Manawatu	2013	Autumn	52.65	25.14	17.8	32.39	24.94	33.37	61.65	104.11	**44.01**
		Manawatu	2013	Spring	24.03	27.57	14.78	27.87	24.48	28.13	14.81	27.79	**23.68**
		Manawatu	2014	Autumn	45.35	35.92	45.91	44.88	157.46	140.47	159.66	68.22	**87.23**
		Manawatu	2014	Spring	14.63	16.59	0	21.57	41.93	23.2	11.61	21.01	**18.82**
		Waikato	2013	Autumn	7.04	34.57	71.81	18.82	169.57	42.47	16.8	62.01	**53.81**
		Waikato	2013	Spring	15.14	26.26	45.49	0	25.6	32.86	41.67	11.64	**24.4**
		Waikato	2014	Autumn	19.55	17.46	17.67	13.14	0	48.98	0	16.77	**17.12**
		Waikato	2014	Spring	21.66	0	7.34	6.36	3.75	0	17.58	3.54	**7.27**
			Over All mean		**25.01**	**22.94**	**27.6**	**20.63**	**55.97**	**43.68**	**42.86**	**39.39**	

Appendix 1 (contd)

Feeding Group	Taxa	Location	Year	Seasons	BHN	BHY	BLN	BLY	OHN	OHY	OLN	OLY	Over-All mean
	Prismatolaimus	Manawatu	2013	Autumn	13.85	0	0	0	0	0	0	0	**1.73**
		Manawatu	2013	Spring	6.5	3.48	34.09	0	5.88	0	7.41	0	**7.17**
		Manawatu	2014	Autumn	8.57	4.13	0	0	0	0	0	0	**1.59**
		Manawatu	2014	Spring	0	5.26	0	8.67	6.9	5.63	4.14	12.36	**5.37**
		Waikato	2013	Autumn	0	0	47.87	0	0	0	8.4	0	**7**
		Waikato	2013	Spring	0	4.51	25.76	2.63	6.46	0	2.83	0	**5.33**
		Waikato	2014	Autumn	0	0	0	7.55	0	8.22	10.48	0	**3.1**
		Waikato	2014	Spring	22.25	13.89	53.42	12.72	10.66	4.51	51.17	0	**20.31**
			Over All mean		**6.39**	**3.91**	**20.14**	**3.95**	**3.74**	**2.29**	**9.7**	**1.54**	
	Rhabdolaimus	Manawatu	2013	Autumn	0	0	7.49	0	0	0	0	0	**0.94**
		Manawatu	2013	Spring	0	0	6.91	0	0	0	0	0	**0.86**
		Manawatu	2014	Autumn	0	0	0	0	0	0	0	0	**0**
		Manawatu	2014	Spring	0	0	0	0	0	0	0	0	**0**
		Waikato	2013	Autumn	0	0	0	16.52	30.73	0	0	0	**6.06**
		Waikato	2013	Spring	0	0	0	0	0	0	0	0	**0**
		Waikato	2014	Autumn	0	0	0	0	0	0	0	0	**0**
		Waikato	2014	Spring	0	0	0	0	0	0	0	0	**0**
			Over All mean		**0**	**0**	**1.8**	**2.06**	**3.84**	**0**	**0**	**0**	

Appendix 1 (contd)

Feeding Group	Taxa	Location	Year	Seasons	BHN	BHY	BLN	BLY	OHN	OHY	OLN	OLY	Over-All mean
	Teratocephalus	Manawatu	2013	Autumn	0	0	0	0	0	0	0	0	**0**
		Manawatu	2013	Spring	0	0	0	0	0	0	0	0	**0**
		Manawatu	2014	Autumn	0	0	0	0	0	0	0	0	**0**
		Manawatu	2014	Spring	0	0	0	0	0	0	0	0	**0**
		Waikato	2013	Autumn	0	0	0	0	0	21.23	0	0	**2.72**
		Waikato	2013	Spring	0	0	0	0	0	0	0	0	**0**
		Waikato	2014	Autumn	0	0	0	0	0	0	0	0	**0**
		Waikato	2014	Spring	0	0	0	0	0	0	0	0	**0**
			Over All mean		**0**	**0**	**0**	**0**	**0**	**2.65**	**0**	**0**	
Predator Feeder		Manawatu	2013	Autumn	0	0	0	38.46	0	28.76	8.21	8.6	**10.5**
		Manawatu	2013	Spring	0	0	0	0	0	0	5.36	0	**0.67**
		Manawatu	2014	Autumn	0	0	0	0	0	0	0	8.95	**1.12**
		Manawatu	2014	Spring	4.82	0	17.93	7.28	0	4.15	0	13.17	**5.92**
		Waikato	2013	Autumn	0	0	0	0	0	22.36	0	0	**2.87**
		Waikato	2013	Spring	13.57	7.18	6.94	0	6.46	7.19	8.34	0	**6.16**
		Waikato	2014	Autumn	0	0	0	0	0	0	0	0	**0**
		Waikato	2014	Spring	0	0	21.93	0	0	0	8.79	7.09	**4.62**
			Over All mean		**2.3**	**0.9**	**5.85**	**5.72**	**0.81**	**7.81**	**3.79**	**4.73**	**0**

Appendix 1 (contd)

Feeding Group	Taxa	Location	Year	Seasons	BHN	BHY	BLN	BLY	OHN	OHY	OLN	OLY	Over-All mean
	Seinura	Manawatu	2013	Autumn	0	0	0	0	0	20.48	0	0	**2.56**
		Manawatu	2013	Spring	0	0	0	0	0	0	0	0	**0**
		Manawatu	2014	Autumn	0	0	0	0	0	0	0	0	**0**
		Manawatu	2014	Spring	0	0	0	0	0	0	0	0	**0**
		Waikato	2013	Autumn	0	0	0	0	0	0	0	0	**0**
		Waikato	2013	Spring	0	0	0	0	0	0	0	0	**0**
		Waikato	2014	Autumn	0	0	0	0	0	0	0	0	**0**
		Waikato	2014	Spring	0	0	0	0	0	0	0	0	**0**
			Over All mean		**0**	**0**	**0**	**0**	**0**	**2.56**	**0**	**0**	
	Tripyla and Tobrilus	Manawatu	2013	Autumn	0	0	0	25.01	0	0	8.21	8.6	**5.23**
		Manawatu	2013	Spring	0	0	0	0	0	0	0	0	**0**
		Manawatu	2014	Autumn	0	0	0	0	0	0	0	0	**0**
		Manawatu	2014	Spring	0	0	5.98	0	0	0	0	6.32	**1.54**
		Waikato	2013	Autumn	0	0	0	0	0	0	0	0	**0**
		Waikato	2013	Spring	0	7.18	0	0	0	0	0	0	**0.92**
		Waikato	2014	Autumn	0	0	0	0	0	0	0	0	**0**
		Waikato	2014	Spring	0	0	0	0	0	0	0	0	**0**
			Over All mean		**0**	**0.9**	**0.75**	**3.13**	**0**	**0**	**1.14**	**1.86**	

Appendix 1 (contd)

Feeding Group	Taxa	Location	Year	Seasons	BHN	BHY	BLN	BLY	OHN	OHY	OLN	OLY	Over-All mean
	Mononchs	Manawatu	2013	Autumn	0	0	0	13.45	0	8.29	0	0	**2.72**
		Manawatu	2013	Spring	0	0	0	0	0	0	5.36	0	**0.67**
		Manawatu	2014	Autumn	0	0	0	0	0	0	0	8.95	**1.12**
		Manawatu	2014	Spring	4.82	0	11.96	7.28	0	4.15	0	6.86	**4.38**
		Waikato	2013	Autumn	0	0	0	0	0	22.36	0	0	**2.87**
		Waikato	2013	Spring	13.57	0	6.94	0	6.46	7.19	8.34	0	**5.24**
		Waikato	2014	Autumn	0	0	0	0	0	0	0	0	**0**
		Waikato	2014	Spring	0	0	21.93	0	0	0	8.79	7.09	**4.62**
			Over All mean		**2.3**	**0**	**5.1**	**2.59**	**0.81**	**5.25**	**2.65**	**2.86**	
Omnivores		Manawatu	2013	Autumn	27.69	0	37.25	12.36	42.27	8.29	14.87	7.38	**18.77**
		Manawatu	2013	Spring	0	7.26	4.31	12.63	0	4.39	9.88	0	**4.81**
		Manawatu	2014	Autumn	3.63	0	0	9.16	5.2	0	0	12.2	**3.78**
		Manawatu	2014	Spring	0	2.97	0	14.18	9.56	9.97	0	3.43	**5.01**
		Waikato	2013	Autumn	0	13.6	32.36	14.54	20.13	8.38	36.72	11.56	**16.66**
		Waikato	2013	Spring	6.79	0	5.15	7.09	11.69	19.84	31.98	0	**9.76**
		Waikato	2014	Autumn	0	0	0	0	0	0	0	0	**0**
		Waikato	2014	Spring	6.07	5.29	0	19.08	10.66	6.69	10.89	3.54	**7.7**
			Over All mean		**5.52**	**3.64**	**9.88**	**11.13**	**12.44**	**7.2**	**12.28**	**4.76**	

Chapter 3

General Discussion

This thesis investigated and revealed that some biotic and abiotic factors impact on the pasture nematode community. The distribution of the nematode community can be influenced directly and indirectly by various management activities, including nitrogen fertiliser application and plant species, and the changing climatic conditions during seasons. Our study examined the impact on the nematode community of combinations of low and high rates of nitrogen application, clover presence and absence, and two different ranges of ryegrass cultivars, One50 (diploid AR37) and Base (tetraploid AR37), during two seasons (Autumn, Spring) in two regions in the North Island of New Zealand in two years, 2013 and 2014.

To date there have been few studies on the effects of modern perennial ryegrass cultivars-clover mixtures and nitrogen fertiliser on nematode fauna. We used an observational study in sixteen experimental conditions to find the relationship between nematode population changes and environmental factors, particularly with seasonal changes. Collection of 320 samples of soil at a depth of 10cm over the study period, and 24 nematode taxa of five feeders were found and studied. A sample from an agroecosystem generally may contain more than 50 nematode taxa in varying proportions. Thus, in agroecosystems soil texture, soil moisture and the availability of suitable food are critical for determining the diversity of the nematode fauna. We found 21 taxa in the Manawatu site, most of which are the same as in a study by Yeates (1982), who found 24 nematode taxa in Manawatu; also, the presence of the large plant feeding nematode *Longidorous* has been reported in Manawatu soil (Yeates et al., 2008). The presence of *Longidorous* indicates that the soil at the Manawatu site must be either fine sandy loam or have large available soil pore spaces (Yeates et al., 2008). Yeates and Bongers (1999) described that the soil texture has a major impact on nematode diversity under pasture. Texture itself is important in affecting the movement, feeding and reproduction of all nematodes; there are also management effects on a given soil via plant species, irrigation, pesticide, and fertilizer application. Soil nematodes are basically worm-like in all stages; the length of adult nematodes ranges from under 0.3mm (*Bunonema*) to over 5mm(*Longidorus*), and, as aquatic animals, they must be able to move freely though water to feed and complete their life cycles with available pore size. *Longidorus elongatus* was influenced by significant changes in the turnover and longevity of ryegrass roots, and feeding by *L. elongatus* led to root senescence and a greater resource for microbial populations and an increase in bacterial-feeding nematodes (Yeates & Stirling, 2008).

We found 16 taxa in the Waikato site, but Sarathchandra et al. (2001) found 32 taxa in Waikato soil. The most common taxa found in our sample (Tylenchids, Paratrichodorids, and Mononchus) were not reported in Sarathchandra et al. (2001) while the reverse was true for other taxa eg. *Diplogaster* and *Ditylenchcus* (Sarathchandra et al., 2001).

Over all pooled sample times and sites, the total nematode numbers varied with treatments, but within a small range from 3550 at One50 + Low nitrogen + Clover presence to 4128 at One 50 + high nitrogen+ No clover. This indicates that the nematode numbers may vary with nitrogen levels and the presence/absence of clover. Some plant-feeding nematodes were associated with legumes, and in some situations, they will cause root damage and reduce N fixation.

We found that the average nematode numbers were extremely high at the first sampling in autumn 2013 in both locations, with a large variation in the nematode numbers among the sampling times. There are doubts about the stability of the ecosystem in the study area. Such nematode abundance or diversity means that simple effects of soil and management can often be demonstrated in long-term grasslands. In young pastures, nematodes will not be in equilibrium with local conditions (Wasilewska, 1994). In long-term grasslands, the seasonal changes in nematode populations over several sampling times have a more stable value than results from single sampling events (Yeates & Bongers, 1999). Sampling frequency may be considered at least monthly for all the seasons and for more than two years, because some nematodes have only one generation per year, while some have up to eight generations per year and some may even have a very short generation, like within a week.

Seasons had the largest consistent impact on total nematode numbers, with the largest populations occurring in autumn at both sites. It is possible to have different changing patterns between feeding groups and within feeding groups of genera. Yeates (1987) observed a positive relationship between total nematode abundance and increase in herbage production in grasslands. Abiotic factors like soil moisture and temperature in autumn, while over spring may be favoured for pasture composition.

We found most of the fungal feeders are associated with some plant feeders and bacterial feeders. The strong positive relationship between *Paratylenchus* and *Apheleneholdes* may be interpreted as positive functional activities between *Paratylenchus* and *Aphelenechoides* availability; food sources for fungi seem high due to damage on plant roots made by plant feeders (Wardle et al. 2003). Yeates and Stirling. (2008) described that some plant-feeding nematodes were associated with legumes, and in some situations, they will cause root damage, reduce N fixation and may also increase the rate at which fixed N becomes available to the soil microbes; this will have flow-on effects within the soil biological community (Yeates et al., 1977, 1999). Little is known about parasitic nematodes on pasture grasses (Cook and Yeates, 1993), but these effects were observed on *Pratylenchus* which is damaging to other grasses.

Bouwman and Zwart (1994) also associated dry conditions with stimulation of *Aphelenchoididae* possibly because this group of nematodes can readily survive as anhydrobiotes. Mechanisms underlying the predominance of the fungal channel in dry conditions act as a ‘fungal hyphal bridge’, by which nutrients are translocated through the soil via the hyphal network (Holland and Coleman, 1987), and factors favouring plants which allocate C to defence rather than growth (Wardle, 2005).

Paratylenchus are mainly grass feeders. On the other hand, *Aphelenchoides* may feed directly on the endophyte Fungus, as they have different feeding preferences (Ruess et al., 2000) and they can be found in leaf tissues or leaf sheath. Although root from endophyte infected grasses do not generally bear hypae, but secondary metabolites from leaves and sheath can be transferred to root (Cook and Lewis., 1991; Cook et al., 1991).

*c-p*1 activities are high in autumn. (c-p1) groups are Enrichment opportunists; they colonize food-enriched conditions which ranks them along a colonizer–persister continuum on a one to five scale. The most rapid colonizers are bacterial-feeding, enrichment opportunists with short generation times, large gonad volume, high rates of egg production, high rates of mobility and metabolic activity, and almost constant ingestion of the microbial suspension in soil solution. These nematodes can enter a non-feeding, inactive 'dauerlarva' survival stage when resources are limited or under stressed conditions, for example, Rhabditidae, Panagrolaimidae, and Diplogasteridae (Bongers and Ferris, 1999).

The season and associated temperature and moisture changes contrast between MI (greatest in spring) and PPI (greatest in autumn); this shows how these two components of the nematode community, the latter of which relies directly on plant growth, respond to food resources and seasonal conditions. Bongers et al. (1995) demonstrated that under certain conditions the PPI and MI behave in opposite manners and suggested that an increase in the PPI/MI ratio might reflect ecosystem enrichment. Bongers (1999) accepted that the MI decreases and the PPI increases with increasing soil fertility. After enrichment of a habitat, the opportunistic bacterial feeders will react rapidly, followed by fungal feeders; if fungi develop, omnivores and predators will follow. Plant feeders, however, are dependent on roots for their nourishment, so a short-term action cannot be expected. Moreover, Tylenchidae among the plant feeders (group 1) of (Yeates et al., 1993) are extremely tolerant to pollutants, possibly having an impermeable cuticle (Korthals et al., 1996a). The reproduction rate of these plant feeding Tylenchidae are intermediate on the cp-scale, but based on tolerance to pollution they behave like colonizers. Recently it has been confirmed that their original exclusion from MI-calculation (Bongers et al., 1997) but further study is needed to review PPI/MI ratio increases if the PPI is based on the Tylenchidae as plant feeders only.

Using different statistical properties to interpret the complex data is important to reveal nematode composition, community structure and environmental interaction. Graphically, these represent the range of strength of interactions amongst the members of the nematode community, which could be easily studied. This ecological network interprets the biological interactions where the nodes species/biological components are connected to the strength of association. These are often used for analysis of among-species relationships, but are increasingly used for functional interpretation of ecosystem networks, including those generated by molecular approaches (Deng et al, 2012).

Due to climate change, the 2012–13 drought was one of the most extreme New Zealand had experienced in the previous 41 years and was unusual for being especially widespread, affecting the entire North Island and the west coast of the South Island (Ministry for Primary Industries, 2013) (Ministry for the Environment, 2017). It may have had some effects on the soil environment during the study period.

In the future to achieve a greater level of precision, better techniques may need to be considered to extract all soil inhabiting nematodes, including big and small nematodes and dauer stages. Also, from the plant roots at the sampling time, identification techniques need to be used at species level by using morphological and molecular methods. A balanced molecular and morphological taxonomic approach is required to deal with large numbers of samples or with species mixtures. Sampling frequency may be at least monthly for all the seasons and for a longer period than two years. In addition, knowledge of the number of generations per year, collecting samples from deeper in the soil, plant herbage production data all need to be studied to find the relationship with life stages of nematodes and their genera with seasonal patterns. Further work needs to be done to assess the quality and quantity of plant litter/roots, because these food resources differ by season and different site sampling; also, proper identification of stressors and assessment of Duare stages, c-p1, c-p5 groups needs to be done, as well as more detailed study for identification and research on Tylenchinae in the soil conditions. Proper statistical properties and reference criteria need to be studied for easy interpretation of community structure in the ecosystem function.

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