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A comparison of community composition analyses for the assessment of responses to wood-ash soil amendment by free-living nematodes

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A thesis submitted in partial fulfillment of the requirements for the Master of Science degree in Biology

A Comparison of Community Composition Analyses for the Assessment of Responses to Wood-ash Soil Amendment by Free-living Nematodes

(Thesis format: Monograph)

by

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Abstract

Land-use changes can have far-reaching consequences for resident communities and ecosystem functioning. Developing appropriate assessment methods to observe and quantify this change is an important application of community ecology. Here I compare four methods of community assessment for free-living soil nematodes under forest harvesting disturbance and wood ash application. Neither morphological assessment (richness, abundance, diversity) nor molecular assessment (morpho-richness using T-RFLP) was responsive to experimental treatments. Trait-based approaches (Maturity Index (MI) and Body Size Spectra (BSS)) were more sensitive to forest harvest and wood-ash amendment treatments. The efficacy of these methods was also qualitatively compared. Of all methods, the BSS were found to be the most informative and easiest to implement. Morphological assessment and the MI rely strongly on rare taxonomic expertise and T-RFLP requires considerable optimisation to be effective. The use of trait-based approaches for soil fauna is advocated as an accessible tool for community ecologists, especially those interested in taxonomically difficult groups.

Keywords

Soil fauna, nematodes, community ecology, taxonomy, body size, Maturity Index, T-RFLP, forestry

Co-Authorship Statement

This thesis in-part outlines and assesses the use of community assessment metrics known as body size spectra (BSS) in a novel system. This notion was first put forward in a review paper co-authored by Matthew S. Turnbull, myself, and Dr. Zoë Lindo (Turnbull *et al.*, 2014). All co-authors were significantly involved in the literature review, theoretical discussions, writing, and editing of this paper. Ideas put forth in this paper are invoked in Chapter 1 and its applications can be found in Chapters 3 and 4. Data following up on this paper – appearing in Chapter 3 – is currently in submission in a paper co-authored by myself and Dr. Lindo (George & Lindo, *In Submission*). I plan to adapt more data from Chapter 3 for publication with Dr. Lindo.

Acknowledgments

I must first give my heartfelt thanks Dr. Zoë Lindo for welcoming me into her lab. She has been patient, encouraging, and incredibly helpful. Such support allowed me to explore my interests during my time at UWO, and made this experience all the more special. This whole process has helped me build towards a future pursuing science that will both excite and challenge me. I could not have asked for a better supervisor.

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My research was part of a collaborative effort between government agencies, the forestry industry, and community groups. Dr. Paul Hazlett was instrumental in allowing me to join the project. Thanks to Natural Resources Canada for the accommodation in Chapleau.

Starting school mid-year, I was worried that it would be difficult to make friends having missed the previous semester. I could not have been more wrong. Matthew Turnbull, Danielle Griffith, and Catherine Dieleman welcomed me warmly to our lab. I hope to carry their friendships along with all those I made with my fellow graduate students, for a lifetime.

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List of Abbreviations

c-p: colonizer-persistor

MI: Maturity Index

ΣMI: summed Maturity Index

EI: Enrichment Index

SI: Structure Index

BSS: Body Size Spectra

LSDR: Local Size Density Relationship

ISD: Individual Size Density

H': Shannon-Weiner's diversity index

J': Pielou's evenness index

T-RFLP: Terminal Restriction Fragment Length Polymorphism

OTU: Operational Taxonomic Unit

1 Introduction

1.1 Effects of forestry practices on soil systems

Throughout history, human activities have altered natural systems to suit societal needs. Activities such as urban development, forest clearing, agriculture, and silviculture have altered nutrient and hydrologic cycling, increased global carbon dioxide emissions, degraded and fragmented habitats, and ultimately led to a loss in biodiversity (Foley *et al.*, 2005). The effects of land-use change are well documented, particularly with regard to forest clearing and silviculture practices on soil systems (e.g. Huhta *et al.*, 1967; Keenan & Kimmins, 1993) as well as their invertebrate communities (Huhta *et al.*, 1967; Niemelä, 1997). In particular, communities of micro-invertebrates living within soils have been shown to respond to land-use change in agricultural land (e.g. Ou *et al.*, 2005) as well as under various forestry regimes (Huhta *et al.*, 1967; Panesar *et al.*, 2000; Háněl, 2004).

Forest harvest methods in particular have varying effects on invertebrate communities. Nematode abundance, for example, was only marginally affected or unchanged following clear-cutting in Finnish forests (Huhta *et al.*, 1967), whereas this disturbance caused a distinct decrease in nematode abundance in a Canadian temperate rain forest (Panesar *et al*., 2000). The causes of declines in soil invertebrates under various forestry practices are often unclear as they occur in conjunction with other abiotic factors (i.e. site variability, climate, landscape changes). Further, responses to forest harvesting are not always consistent among groups of soil organisms, both taxonomic (Háněl, 2004) and trophic (Forge & Simard, 2001). For example, abundances of nematodes have been shown to drop following clear-cutting (Huhta *et al*., 1967; Panesar *et al*., 2000), whereas this practice may increase the abundance other taxa like molluscs and Collembola (Marshall, 2000).

The boreal forest extends from Canada's Atlantic coast to its border with Alaska, making up 90% of the country's productive forest (Bose *et al.*, 2014). This forest is characterised by extreme seasonal and diurnal temperature fluctuations (Bose *et al.*,

2014), with frequent fire (Bergeron *et al.*, 2002a) and cyclic outbreaks of insect pathogens (Volney & Fleming, 2000) as the prominent drivers of tree species composition. Harvesting within the Boreal zone has a lengthy history across the country (Volney & Fleming, 2000) and has generally consisted of clear-cutting followed by short-rotation, even-aged plantations (Bose *et al.*, 2014). However, forestry practices have lately become more focused on ecosystem management practices (Attiwill, 1994; Bergeron *et al.*, 2002b) as it is thought that these approaches will help support endemic species and increase ecosystem resilience (Drever *et al.*, 2006). Ecosystem management practices can be broadly grouped together as partial or selective cutting methods including shelterwood harvesting (leaving remnant patches), commercial thinning (strip cutting), and diameter-limit cutting (minimum size) amongst others (Bose *et al.*, 2014).

Forestry interests are also looking to increase their annual timber yield whilst simultaneously implementing better management practices. Previous use of clear-cutting has in many cases resulted in the removal of nutrients including: carbon (C) (Grand & Lavkulich, 2012), nitrogen (N), phosphorous (P), potassium (K), and calcium (Ca) (Hornbeck & Kropelin, 1982). This has led to research for possible amendments to reintroduce these nutrients or mitigate the effects of their removal. Wood ash has been identified as one such amendment and has been applied successfully in both agriculture and silviculture (Augusto *et al.*, 2008). Indeed, since as early as 1935, wood ash has been applied to forest soils in attempts to restore biodiversity in acidified soils (Pitman, 2006). Wood ash amendment used in silviculture is generally produced through the combustion of coniferous and deciduous stems, slash, or refuse generated in paper production. The use of wood ash amendment is common across Scandinavia and is growing in popularity in some parts of the United States (Pitman, 2006). However, despite its substantial use in Canadian agriculture (Arshad *et al.*, 2012; Jaramillo-Lopéz & Powell, 2013) wood ash has rarely been applied in Canadian forests (see McDonald *et al.*, 1994).

The composition of wood ash source material can greatly influence the resulting effects on soil properties (Pitman, 2006). Werkelin *et al.* (2005), for instance, found that ash derived from bark and foliage was more nutrient-rich than ash generated from stem wood. This is because nutrient concentrations differ within various parts of the tree; bark

typically has a greater concentration of Ca for example (Pitman, 2006). The most beneficial effects of wood ash amendment are an increased ability to retain soil moisture (Pitman, 2006) and increase pH (Arshad *et al.*, 2012). These effects stem from the high neutralising capacity of ash (Deymeyer *et al.*, 2001) and the subsequent increase in dissolved organic C post-amendment. Vance (1996) proposed that wood ash could fill the role of commercial NPK fertilisers, despite containing lower percentages of these nutrients than traditional products (Naylor & Schmidt, 1989). However, the fertilising effect of ash is likely negligible or minor at best as the majority of both P and K are immobilised in ash (Pitman, 2006) and N is not present in ash (Augusto *et al.*, 2008). However, it should be noted that amendment can increase N availability indirectly through an increase in pH (Vance, 1996).

Wood ash may also have harmful effects on the soil as it can contain substances such as heavy metals and polyaromatic hydrocarbons (Pitman, 2006; Augusto *et al.*, 2008). Wood ash application has been linked to changes in plant (Pitman, 2006; Augusto *et al.*, 2008), microorganism (Pitman, 2006), and animal communities (Nieminen, 2011). However, these changes vary in their magnitude, potentially due to abiotic factors stemming from soil-wood ash interactions (Pitman, 2006). For example the meta-analysis of Augusto *et al.* (2008) found wood ash had no effect on tree growth in mineral soils but that growth was positively affected in organic soils. Herbaceous plants (Pitman, 2006) and grasses (Arvidsson *et al.*, 2002) also respond positively to amendment whereas bryophytes (Kellner & Weibull, 1998), shrubs, and lichens (Jacobson & Gustafsson, 2001) commonly respond negatively. Soil fungi have also been shown to display positive (Pitman, 2006) and negative (Nieminen & Setälä, 2001) responses.

Responses to wood ash amendment in animal communities are perhaps the least understood. Enchytraeid (potworm) communities have been studied most frequently (Pitman, 2006), but only a few studies on soil arthropod and nematode responses exist (Nieminen, 2011). Microarthropods (mites and springtails) are thought to be tolerant to the effects of wood ash amendment (Nieminen, 2011), whereas enchytraeids have been negatively affected with regard to abundance (Lirri *et al.*, 2007) and biomass (Lirri *et al.*, 2002), resulting in decreases in the average community body size (Nieminen, 2009).

Studies quantifying the response of the nematode communities are generally indirect as they are commonly quantified in conjunction with the enchytraeid community and/or used as indicators of microorganism responses (Nieminen & Setälä, 2001; Lirri *et al.*, 2007). In these cases it was determined that nematode abundances were altered indirectly via changes in food sources; Lirri *et al.* (2007) found that the biomass of ectomycorrhizal fungi was reduced in wood ash amended mesocosms compared to controls, with a corresponding reduction in the number of fungivorous nematodes. This result is similar to those of Nieminen and Setälä (2001) although they suggested that nematode feeding preference may have influenced the results. It should also be noted that both of these experiments took place in *ex situ* mesoscosms, whose fidelity to the natural state can be limited by factors like extreme nutrient limitations, loss of natural functions (Nieminen, 2011), and loss of uncommon species (Verhoef, 1996). Bååth *et al.* (1995) suggest that bacterivorous nematodes are more likely to increase in abundance than fungivores following wood ash amendment as fungi appear to be generally less tolerant of ash amendment. This suggestion has been supported in mesoscosm experiments that found limed soils support greater bacterial abundances than unlimed controls and thereby a larger bacterivorous nematode community (Räty & Huhta, 2003). Wood ash amendment in forest soils *in situ* has shown that total nematode abundance initially increased with a brief spike in fungivores immediately after amendment, while the proportion of bacterivores is sustained (Lirri *et al.*, 2002). However, Huhta *et al*. (1983) found that populations of all soil invertebrates declined after three weeks of exposure to wood ash in a mesocosm study, despite an initial increase in nematode abundance. The effects of wood ash amendment on other nematode feeding groups, including predators, remain unclear.

1.2 Nematode functional traits and the Maturity Index

Nematodes are ubiquitous members of the interstitial communities of marine, freshwater, and terrestrial substrates. In soils, they can sometimes number in the millions per square metre (Yeates *et al.*, 2009) and include a number of trophic and functional groups (Bongers & Bongers, 1998). Nematodes are recognised as good indicators of soil quality (Neher, 2001) and effective environmental indicator taxa for these reasons as well as their

ease of sampling (Ferris *et al.*, 2001). However, nematode taxonomic expertise is becoming increasingly rare and has a steep learning curve (Chen *et al.*, 2010). This makes quantifying nematode diversity and interpreting community changes and their consequences difficult.

The study of functional traits has become popular in modern ecological theory. Functional traits are life history characteristics of organisms can alter an ecosystem's functions (effect traits) or respond to environmental changes (response traits), in particular, anthropogenic disturbance (Suding *et al.*, 2008). The study of functional traits has been instrumental in allowing researchers to investigate both how organisms influence and respond to changes in the environment. Functional traits influence an individual's growth, reproductive ability, and survival imparting an overall effect on its fitness (Violle *et al.*, 2007). They have received much more attention in plants than animals (Violle *et al.*, 2007; Suding *et al.*, 2008), and even less so in soil invertebrates. There has been little exploration of effect traits in animals; however, studies focused on response traits such as body size are more common in the literature (e.g. Mulder & Elser, 2009).

The use of functional traits in nematology has been established for over 20 years (Bongers, 1990), predating the current rush to functional measures. In 1990, Bongers pioneered the Maturity Index (MI) to assess changes in soil quality using the free-living nematode community following disturbance. The MI uses a combination of traditional taxonomy and functional traits, allowing changes in both the nematode community and general soil conditions to be tracked over time (Bongers, 1999). With the MI, nematode taxa are assigned to one of five categories along a coloniser-persister (c-p) scale based on functional traits generally related to reproductive strategy. Nematodes classified as colonisers (c-p 1) are generally r-strategists with extremely high fecundity and short life-cycles. They quickly dominate their communities in favourable conditions. Persisters are found on the other end of the scale (c-p 5), and are considered K-strategist taxa, which have longer life spans and invest resources in producing fewer but more competitive offspring. Persisters are never dominant in soils due to their narrower niche requirements and high likelihood of extirpation following disturbance. Their presence indicates soil

stability and thus 'maturity' through succession. The majority of nematode species have traits that fall within the r/K continuum, and are classified as c-p levels 2 through 4. Body size has also been shown to generally correlate with the progression of the c-p scale with an increase in body size following the r/K-selection continuum (Vonk *et al.*, 2013).

Using the c-p scale is advantageous as it incorporates both response traits (e.g. body size), which predict how a taxon will react to disturbance, and effect traits (e.g. trophic level) that influence processes including decomposition (Adl, 2003) and trophic transfer efficiency (Lindo *et al.*, 2012). The c-p groups are also related to feeding preferences (Bongers & Bongers, 1998), which are also typically related to body size. Generally, nematodes can be assigned directly to c-p groups at the family-level but lower units (i.e. genus) may be different enough from related taxa to warrant membership to a different c-p group. Many authors who use the MI will include the c-p rankings of families and genera that they study (e.g. Bongers, 1990; Bongers & Bongers, 1998; Ferris & Matute, 2003; Mills & Adl, 2011), which is helpful, but the c-p designation of undescribed or previously unassigned species, is still required (Bongers, 1999).

The MI is calculated as follows:

(1)

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

where *f*(i) represents the frequency of taxon *i* (of *n* taxa) in a sample and *v*(*i*) is the c-p value of taxon *i* (Bongers, 1990). The MI uses the relative proportions of different functional groups within the nematode community to classify a soil as: *basal*, *enriched*, or *structured* (Bongers & Bongers, 1998; Ferris *et al.*, 2001). Structured soils are typically undisturbed and host a great diversity of trophic groups including larger bodied taxa (highest proportion of c-p 3-5 taxa). When soils are disturbed, they become *basal*, meaning they are dominated by high numbers of small fungivorous and bacterivorous taxa (c-p 2) that can quickly exploit the change in the physical state of their environment. If there is a nutrient enrichment, the community will become *enriched* and thereby

dominated almost exclusively by generalist bacterivores (c-p 1). Over time, soils will become increasingly structured from either the basal or enriched state, as niche space will slowly open up for c-p 3 through c-p 5 taxa, which add trophic links to the community and increase its diversity (Ferris *et al.*, 2001). The MI has produced many derivatives over the past 25 years. When it was originally created, the plant-feeding nematodes of the community were excluded from the calculation (Bongers, 1990), but Yeates (1994) has included this trophic group in the MI by utilising their c-p values and abundances (denoted as the ΣMI with the inclusion of plant-feeding nematodes). Additional indices developed by Ferris *et al.* (2001) have allowed the community to be further explored by representing the expected responsiveness of the dominant feeding-groups of structured and enriched soils (the SI and EI, respectively). Such derivatives allow for finer-scale details of a community's composition to be understood.

1.3 Body size as a response-effect functional trait

As mentioned previously, with some exceptions, body size increases with c-p level (Vonk *et al.*, 2013) making it a component of MI values. Recently, Turnbull *et al.* (2014) postulated that body size might be used independently as a response trait metric in free-living soil nematodes. This notion works on the framework that during community disassembly, species loss is determined by the presence or absence of traits (Zavaleta *et al.*, 2009), and that larger species are more likely to go extinct after habitat disturbance (Leck, 1979; Gonzalez & Chaneton, 2002; Cardillo, 2003). Furthermore, studies of soil community responses to disturbance have shown body size as a predictor of extinction risk, and therefore a response to environmental change (Mulder *et al.*, 2008; Mulder & Elser, 2009), as well as trophic interactions and resource utilisation (Mulder *et al.*, 2009; Mulder *et al.*, 2011). Community wide body size measures can be shown for any given system by using abundance-by-body size plots called body size spectra (BSS) to observe community-level responses to disturbance. Indeed, the use of BSS is common in assessing the effects of disturbance in aquatic systems (Sprules & Munawar, 1986; Transpurger & Bergtold, 2006; White *et al.*, 2007; Petchey & Belgrano, 2010), whilst several studies have also shown the value of BSS in studying soil invertebrate communities (Reuman *et al.*, 2008; Lindo *et al.*, 2012; Hocking *et al.*, 2013).

White *et al.* (2007) review two methods of assessing BSS, both of which can be applied to soil communities. The first is the local size-density relationship (LSDR) model, in which a species' average body size is plotted against its population density (Turnbull *et al.*, 2014) on a log-log scale. The second model works without species identification and is known as the individual size distribution (ISD) model. This method groups body size values into classes and plots them against the log population densities of individuals per size class (Turnbull *et al.*, 2014). Both methods have been applied in soil systems (Mulder & Elser, 2009; Lindo *et al.*, 2012).

The use of BSS to visualise changes in nematode and other soil invertebrate communities following disturbance, and as a community-wide metric of change or perturbation was recently proposed by Turnbull *et al.* (2014). Here, they demonstrate how a BSS approach could be used to demonstrate the changes in nematode communities observed using the MI. As body size generally scales with c-p level (Ferris *et al.*, 2001), it is expected that an overall reduction of large-bodied species would be observed under disturbance in a *basal* MI community, and an overall increase in the abundance of small-bodies species following nutrient addition in an *enriched* MI community. Visually this would manifest in the BSS plot as differences in intercept and slope of the regression from the log-abundance by log-body size plot (LSDR model), where the *structured* MI BSS would have a shallow negative slope, the *basal* MI community would demonstrate a steepening in slope, and the *enriched* MI community would have a steepened slope and higher intercept (Figure 1).

The slope of the LSDR BSS model, whilst indicating change in the relative abundance of body sizes following non-random species loss (i.e. body size as a response trait), has also been proposed to reflect the trophic transfer efficiency (TTE) of a community. The TTE describes the proportional transfer of energy from one trophic level to the next (Jennings & Mackinson, 2003), and is often ventured to be 10%. Sheldon *et al*. (1972) proposed that size distribution models such as the BSS could be used to indicate TTE in size-structured communities (i.e. where predators are larger than their prey), and therefore body size may represent a functional effect trait. This notion has

been useful and corroborated in aquatic and marine systems, but has not yet been examined for terrestrial systems.

1.4 Molecular markers of community composition

Currently, many biological researchers feel that the use of unique DNA markers is needed to gain a better understanding of biodiversity. Indeed, there has been a popular push to compile unique gene sequence to form the Barcode of Life (Herbert & Gregory, 2005), and a number of other molecular-based methods for community analysis have also been developed. These approaches have allowed researchers to identify quickly and accurately the constituents of communities that can be difficult to ascertain via traditional taxonomic means (Donn *et al.*, 2008). This is especially true of cryptic species (Trewick, 2000) as well as microorganisms (Moreira & López-García, 2002). This method often relies on use of the cytochrome *c* oxidase 1 gene, which is underreported in nematodes with researchers favouring use of the 18S rRNA gene (Chen *et al*., 2010). There is also a strong push towards the use of next generation sequencing techniques for community analyses (Taylor & Harris, 2012). Yet, for nematology, next generation sequencing is still in early development (Chen *et al.*, 2010; Martin *et al.*, 2012).

One method that has proven useful for the study of whole nematode communities is terminal restriction fragment length polymorphism (T-RFLP). This method of analysis was developed for studying microbial community composition using a combination of PCR and restriction enzyme techniques. In essence, a target sequence of DNA is amplified via PCR with a fluorescently labeled primer from the extracted DNA of the entire community. This mixed PCR product is then digested with a restriction enzyme, which cuts the amplified DNA at specific target sites that differ on PCR products for different taxa. The terminal restriction fragments differ in size that is mostly unique for each constituent member of the community; subsequently, species-level identity can be ascertained (Liu *et al.*, 1997). Data generated from T-RFLP can be analysed for presence-absence as well as proportional abundance at high volumes, and can be used concurrently with taxonomy-based analyses. For this reason, T-RFLP is considered a cost effective and time efficient molecular method of community analyses (Chen *et al.*, 2010).

The T-RFLP method has been applied successfully to nematode communities in agricultural, dune, forest, and wetland soils (Donn *et al.*, 2008; Donn *et al.*, 2012). However, despite their popularity, molecular methods can prove challenging for beginners, difficult to troubleshoot (Maurer, 2011), and in some cases not ideal for identifying certain taxa (e.g. Cephalopoda, see Strugnell & Lindgren, 2007). Prakash *et al.* (2014) describe a number of potential areas of concern specifically for T-RFLP analyses including biased cell lysis, incomplete enzyme digestion, and variation in sample size. These problems can become especially apparent when molecular methods are applied to a new system. A full comparison of morphological, trait-based and molecular-based approaches to understand a change in nematode communities under disturbance has not been performed.

1.5 Objectives

This study had three objectives. **(1)** quantify the effects of wood ash amendment on nematode abundance and diversity. This was done by identifying and enumerating nematodes at the finest level of taxonomic resolution possible and utilising the Shannon-Weiner Index and the MI to detect differences in taxonomic and functional diversity. **(2)** use BSS to evaluate changes in nematode community structure in response to forest harvest disturbance and subsequent amendment as a trait-based approach. This was determined by comparing the responses of the community via changes in the c-p groups for the MI, and changes in body size using LSDR and ISD models of BSS. **(3)** quantify changes in diversity and community structure using the molecular T-RFLP approach. These objectives all come together under the goals of assessing the overall impacts of wood ash amendment on free-living nematodes whilst also comparing the efficacy of the four methods (morphotaxa identifications, MI and BSS functional traits, and T-RFLP analysis) used to quantify diversity in the study.

1.6 Hypotheses & Predictions

It is predicted that forest harvesting will negatively affect morphological species richness, abundance, and diversity, the average T-RFLP richness, and alter values of the MI and the slope and intercept of the BSS. It was hypothesised that wood ash amendment would

enrich the soil, resulting in an increased proportion of c-p 1 nematodes, further altering the MI values, which would be amplified with increasing wood ash load. For the BSS, I predict that in the LSDR model a lowered intercept under forest harvesting and a steeper slope and increased intercept to be seen under wood ash application. In the ISD model, I expect that there will be a reduction in the abundances of larger size classes following harvest and an increase in smaller classes under wood ash application. Lastly, I predict that T-RFLP analyses will show similar trends in the reduction of morpho-richness to the morphological assessments. Since comparisons between these methods cannot be empirically calculated, each method's effectiveness was qualitatively assessed based on the three following *a priori* criteria. **(1)** *Is the method informative?* In this case, an informative metric will provide information on the community's sensitivity to treatment effects and give some insight into the mechanisms behind them. **(2)** *Is the method feasible?* Here, the metrics were assessed based on the relative costs/benefits of their use, namely: expertise, time, and resolution. **(3)** *Can the results be compared with other studies?* This criterion was assessed theoretically, as some values, such as diversity indices, cannot be compared between separate studies. It was thought that there would be qualitative differences between the methods used in this study. The body size spectra were predicted to be the most informative, feasible, and comparable method.

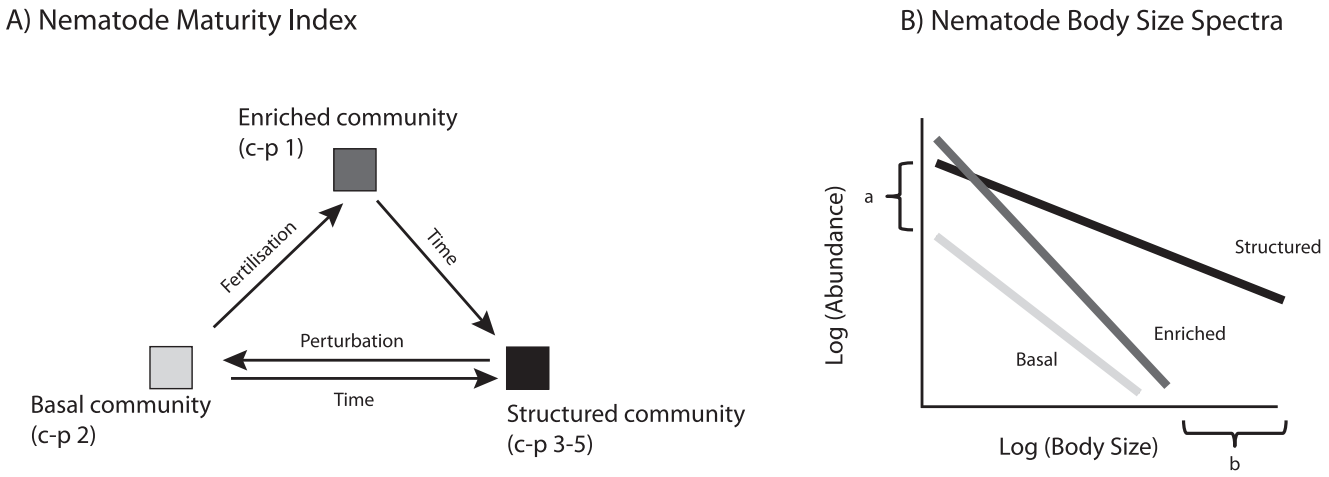


Figure 1.1: A theoretical representation of the continuum of soil states determined by A) the Maturity Index (modified from Ferris *et al.* (2001) and B) their expected representation in a Local Size Density Relationship model BSS. A) Basal nematode community is dominated by c-p 2 taxa, generally small-bodied fungivores and bacterivores in a recently disturbed food-web. With fertilization, the proportion of c-p1 taxa (exclusively bacterivores) increases following an influx of resources post-disturbance to create an Enriched community. Both of these states will mature into Structured communities given time without disturbance and increased resource availability, where larger-bodied and greater diversity of trophic groups exist. Under B) initial Structured communities have a shallow BSS slope; following perturbation, as the community shifts to the Basal state, we observe loss in overall abundance (a) and a disproportionate loss in large-bodied species (b). This results in an overall steepening of slope. Following post-disturbance fertilisation, the increase in small-bodied species increases overall abundance (Enriched), but the BSS slope remains steep compared to that of the Structured community. Figure reproduced with permission from Turnbull *et al.* (2014).

2 Methods

2.1 Site description and experimental design

Sampling took place at the Island Lake Biomass Harvest Research and Demonstration area located in the Martel Forest near Chapleau, Ontario (47°50'N, 83°24'W). This site was developed through collaboration between forestry companies (Tembec, FPInnovations), provincial (Ontario Ministry of Natural Resources) and federal (Canadian Forestry Service) governments, as well as First Nations (Northeast Superior Chief's Forum) and other community supporters (Northeast Superior Forest Community). Consisting of sandy, glaciofluvial soil, the area was previously a jack pine (*Pinus banksiana* Lamb.) plantation, which was harvested in 1959. Currently, both jack pine and black spruce (*Picea mariana* (Mill.) Britton, Sterns & Poggenb.) are being replanted as part of other ongoing experiments. Forest plots were covered in moss carpets and supported a population of approximately 40-year-old jack pine. Clear-cut and ash amended plots were sparsely covered in vegetation in June but vegetation cover was noticeably greater in August. In these plots grasses, small forbs, and shrubs, especially blueberry (*Vaccinium* sp.), were the most common plants.

The experiment had a randomised block design (Map 2.1). Replicate plots were established within a 41.5 ha area that was clear-cut in winter 2011, followed by site preparation (summer 2011) and hand ash application in fall 2011. Ash was generated from branches, bark and other slash collected during harvesting in Tembec's Kapuskasing cogeneration plant using air scrubbers and collection trays below the grates to collect the ash. Wood ash produced at this site contains ~ 20% Calcium (Ca) (for further explanation see Kwiaton *et al.*, 2014). Three ash treatments were applied to each of four replicate 25 x 25 m plots equating to the addition of: 1) one-half of Ca removed through harvest (100 kg/ha) 2) equivalent Ca (200 kg/ha), and 3) twice the Ca removed through the harvest of full-tree biomass (400 kg/ha). The ash treatment plots were compared to four equivalently harvested but unamended clear-cut plots (clear-cut) as well as four adjacent replicate undisturbed forest plots (forest) (5 treatments x 4 plots = 20 experimental units).

A preliminary assessment of soils was performed in June 2013. Few differences in variables were found and are therefore summarised here as site information only (Table 2.1). Soil pH ranged from 5.04 - 5.22 with no significant difference among treatment plots, but was lowest in clear-cut plots and highest in twice Ca amended plots. Soil moisture content was determined for each plot using the formula:

(2)

$$Moisture\ content = \left(\frac{FW - DW}{FW}\right) \cdot 100\%$$

where *FW* is the fresh weight of soil samples before drying and *DW* is the dry weight of the soil after it has reached a constant weight (i.e. all moisture has evaporated) following 24 hours of drying at 60°C. Soil moisture ranged from 41.15% in the forest plots to 44.67% in the twice Ca amended plots; no significant soil moisture conditions were observed among treatments. The organic layer of the soil across the harvested plots was quite thin (35.46 ± 1.28 mm); however, it was significantly deeper in forest plots (55.30 ± 6.18 mm). Acute toxicity of wood ash was tested for using an International Standard Operation with the Collembola species *Folsomia candida* (Environment Canada, 2007). There was no evidence of toxicity. Nutrient analyses provided by the Canadian Forest Service did not show significant differences in K, total C, total N, exchangeable P, cation exchange capacity, or C : N ratio. Interestingly, there were no significant differences in Ca between treatments; however, soil Ca content did follow the expected trend of being lowest in forest plots and highest in twice Ca amended plots.

2.2 Sampling regime

Sampling occurred in June and August 2013. At each plot, eight subsamples of approximately 15 cm in depth – including the organic layer – were collected with 5 cm diameter soil corers (5 treatments x 4 replicate plots (blocks) x 8 subsamples = 160 cores). Subsamples were pooled and homogenized then divided into four aliquots for morphological and molecular identification (June and August), as well as chemical analysis and toxicity assays (June only), totaling 20 pooled-samples. Following collection, samples were kept in coolers in the field and returned to the University of

Western Ontario for nematode extraction within 72 hours. Upon arrival at Western, soil samples were kept at 4 °C until extractions and assays were run.

Nematodes for morphological and molecular analyses were extracted from soil cores using the Baermann funnel technique (Forge & Kimpinski, 2008). For morphological analyses, nematodes were extracted and fixed in 4% formalin solution, stained with Rose Bengal, and mounted with Permount® prior to microscopic observation for body size measurements, identification, and enumeration under 400X magnification. This process involved taking a fixed and stained sample and pouring it into a watch glass under a dissecting microscope at 5x magnification. As nematodes were observed they were collected using a 10 µL pipette to move them in large numbers from the sample liquid to the Permount medium on a microscope slide. Ten to 20 specimens were mounted per slide. For molecular analyses, nematodes were extracted from separate aliquots into water, centrifuged, and stored at -20 °C until DNA extraction and the terminal restriction fragment length polymorphism (T-RFLP) process.

2.3 Morphological analyses

Slides were scanned visually with a compound microscope at magnifications of 100-400X. When a nematode was observed it was identified to morphotaxa (i.e. morophologically distinguishable species types) at the genus and family level based on keys from Bongers (1994) and the University of Nebraska – Lincoln (Tarjan *et al.*, 1977) under 100-400X magnification. The taxonomic richness of each 25 g wet soil weight sample was estimated by summing the number of morphotaxa in each sample. Similarly, the abundance of each morphotaxon was estimated by enumerating the total number of individuals from each morphotaxon in every 25 g wet soil weight sample. These data were used to calculate Shannon-Weiner's diversity index (H') for each sample through the equation:

(3)

$$H' = -\Sigma(p_i)(\ln(p_i))$$

where p_i is the relative proportion of each morphotaxon's abundance in terms of total abundance (Shannon, 1948). This value was subsequently used to find Pielou's evenness for each sample using the formula:

(4)

$$J' = \frac{H'}{\ln(S)}$$

where *S* is the number of morphotaxa in the community (Pielou, 1975). Community composition of the samples also was assessed using morphotaxa identities, richness and abundance of each species. For these indices, unknown individuals were dropped from the analyses.

2.4 Trait-based analyses

2.4.1 Maturity Index

Nematode families and genera were assigned to the c-p scale following their identification as prescribed by Bongers and Bongers (1998). These values were then used to calculate the ΣMI as described by Yeates (1994). This metric uses the equation of the original MI (Bongers, 1990) (Equation 1). However, the MI as described by Bongers (1990) excludes plant-feeding nematodes. Yeates' ΣMI is different in that plant-feeding nematodes and their c-p values are permitted in the equation (1994). The ΣMI was further broken down into the structure index (SI) and enrichment index (EI). These values are presented as percentages and reflect the position of the community along the gradient of soil conditions posited by the MI (Ferris *et al.*, 2001). These were calculated using the formulae from Ferris *et al.* (2001):

(5)

$$SI = 100\% \cdot \left(\frac{s}{s+b}\right)$$

(6)

$$EI = 100\% \cdot \left(\frac{e}{e+b}\right)$$

These calculations incorporate the importance of feeding groups of bacterivore, fungivore, omnivore, plant-feeder, and predator into the c-p scale. Groups that are more indicative of a structured or enriched community receive higher weights than basal groups. For this study the weights of each feeding group were derived from Ferris *et al.* (2001). In both cases *b* denotes the basal component of the community calculated as the sum of the basally weighted taxa using the formula:

(7)

$$b = \sum kb \cdot nb$$

where, *k* is the weight assigned to the basal feeding groups assigned by Ferris *et al*. (2001) and *n* is the total number of individuals in that each basal group. Similar equations for *s* and *e* are used (i.e. instead of *b*), which utilise the weights (*k*) associated with structure and enrichment (Ferris *et al*., 2001).

2.4.2 Body Size Spectra

Following morphological identification, the length and width of each nematode were measured on slide-mounted specimens. For June samples, this was done by digitally capturing the nematode specimen as an image and making calibrated measurements of body length and width using ImageJ® software. For August samples, length and width measurements were made through a digital camera mounted on the microscope and the automated image analysis software program NIS - Elements that can measure calibrated lengths of objects (Nikon Corporation, 2013). This digital imaging system reduced processing times for body size measurements to about 20% of those measured in June.

Nematode length and width measurements were used to approximate nematode body size using the equation of Tita *et al.* (1999):

(8)

$$Dry\ weight\ (\mu g) = \frac{((530 \cdot L \cdot W^2) \times 1.084)}{4}$$

where *L* is the total length (mm) and *W* width (mm). This volume is then converted to wet weight (µg) using the specific gravity of 1.084 (Weiser, 1960) and then to dry weight (µg) assuming a dry/wet weight ratio of 0.25 (Juario, 1975). Dry weights were used as body size in two types of BSS. First a Local Size Density Relationship (LSDR) model was created. This model is a regression between the $\log_{10}$ average abundance of each species and the $\log_{10}$ value for the average body size of that species. In this case, average taxon-specific dry weight was determined from 10 randomly selected individuals (or as many as possible when abundances were less than 10). These data were visualised using a scatter plot with regression lines that were determined in the 75th quartile using the package "quantreg" (Koenker, 2005) in R version 2.14.1 (R Development Core Team, 2014). This method was use to reduce the influence of rare taxa.

The other type of BSS used is the Individual Size Distribution (ISD) model *sensu* White *et al.* (2007). This method uses individual body sizes (x) (without species identities) binned into $\log_2$ (x + 0.5) size classes plotted by the average abundance of each class. This method was used to observe purely qualitative trends and as a result, no statistical analyses were conducted.

2.5 Molecular analyses

The process of extracting DNA for T-RFLP analysis began with breaking up individual nematodes using bead-beating in conjunction with PureLink® genomic DNA extraction kits. This was followed by further purification using a Zymo DNA clean and concentrator kit® and then PCR using the forward primer Nem_SSU_F74 (5' AARCYGCGWAHRGCTCRKTA 3') with the fluorescent label 6-fluorescein amidite (6-FAM), the reverse primer SSU_R_81 (5' TGATCCWKCYGCAGGTTCAC 3') (Donn *et al.*, 2011), and AccuStart II PCR ToughMix®. These were combined with whole community DNA and nuclease-free water in a 25 µL reaction with the following amounts: 12.5 µL AccuStart II PCR ToughMix, 5 µL nuclease-free water, 1.25 µL

forward primer, 1.25 µL reverse primer (both at 20 pMolar concentration), and 5 µL DNA template. A positive control for the PCR was derived from a commercial culture of the nematodes *Heterorhabditis bacteriophora* and *Steinernema carpocapsae.* The PCR reaction was conducted in a thermocycler following the method of Donn *et al.* (2011): 94 °C, 2 min; then 35 cycles of 94°C, 30s; 51 °C, 1 min; 68 °C, 2 min and a final extension step of 68 °C for 10 min. This process yielded a product of approximately 1750 base pairs that was subsequently digested with HinfI restriction endonuclease (Donn *et al.*, 2012) in a 32 µL reaction consisting of: 10 µL PCR reaction mixture, 18 µL nuclease-free water, 2 µL 10X buffer R, and 2 µL Hinf1. The digestion products were sent to the Advanced Analysis Centre at the University of Guelph where they were processed using a 500 LIZ size standard and returned for statistical analyses.

Restriction fragment analyses were conducted using GeneMarker (Softgenetics), which produces an output that displays bands as peaks. This allowed for the quantification of the number of operational taxonomic units (OTU) (i.e. peaks) into a richness value for each 25 g wet soil weight sample. These data were used to conduct community comparisons as described below.

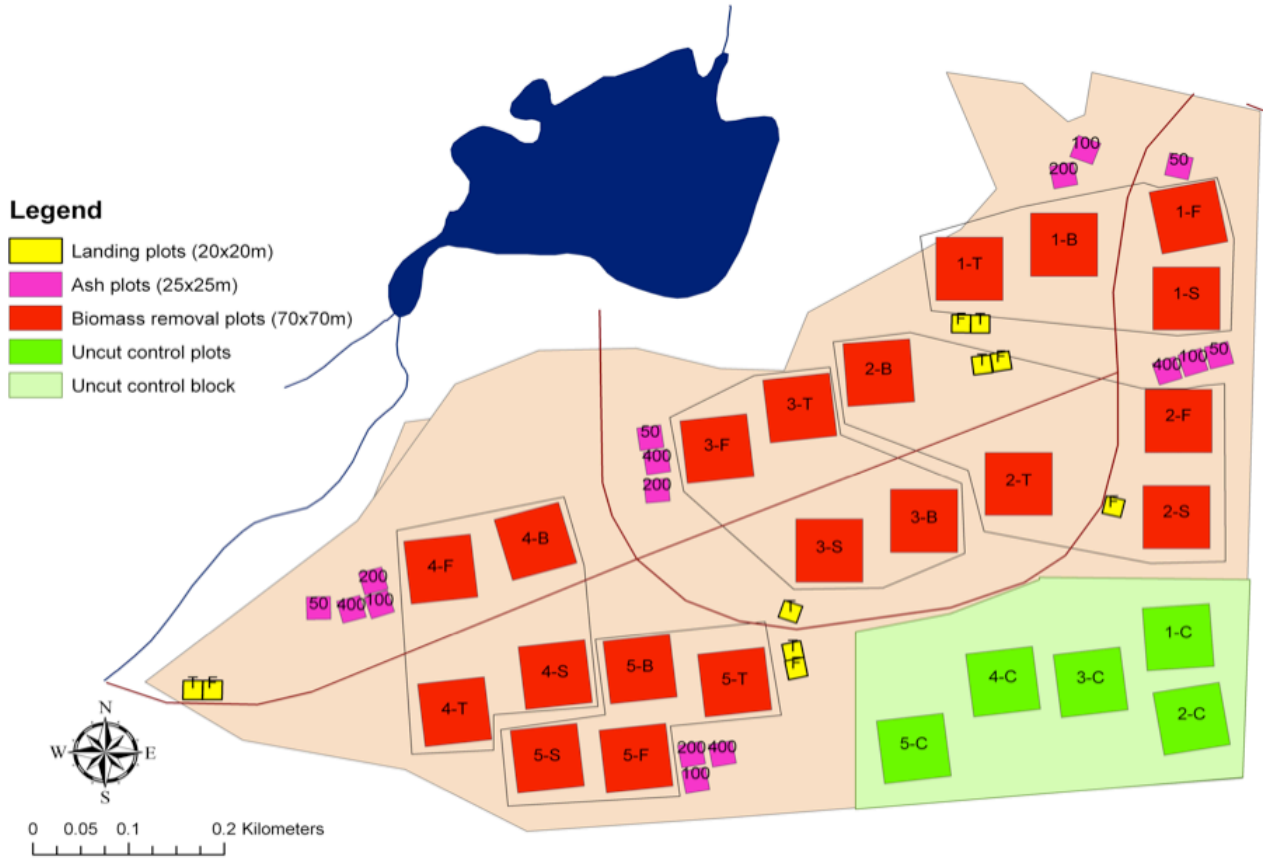
2.6 Statistical analyses

Species richness, abundance, H', and J' from morphological assessments were compared among treatments using repeated measures analysis of variance (RM-ANOVA) to look for the effects of season as well as season by treatment interactions; these were followed by Tukey *post hoc* testing where applicable. These analyses were conducted using Statistica 7.0 (StatSoft, Inc., 2004). Community composition was compared among treatments using non-metric multidimensional scaling (NMDS), followed by analysis of similarity (ANOSIM) test using Primer 5 (Primer-E Ltd., 2001). Here species abundances were square-root transformed and similarity among samples was based on Bray-Curtis percent similarity; NMDS was performed with 10 permutations, and ANOSIM with 10,000 random permutations. For the ΣMI, SI, and EI, effects of treatment, season, and season by treatment interactions were explored by using RM-ANOVA followed by Tukey *post hoc* testing.

In the LSDR model, the slopes and intercepts of the body size spectra for each treatment were calculated using 75% quantile regression and compared using analysis of covariance (ANCOVA). The ISD model of body size spectra was assessed visually without further statistical analyses. Body size spectra were analysed (or visualized for the ISD model) separately for June and August samples. The richness and relative abundance of OTU's based on the molecular assessment of the nematode communities (T-RFLPs) were compared using RM-ANOVA followed by Tukey *post hoc* testing where appropriate. Analyses of community composition of the molecular data (i.e. NMDS followed by ANOSIM) were conducted following the same method previously outlined for morphological analyses.

Table 2.1: Results of nutrient analyses performed by the Canadian Forest Service – Sault Ste. Marie. Mean values are expressed as ppm for K, exchangeable P, and Ca, as me/100g for CEC, as percentage for total C and N, and as a ratio for C : N. Standard errors are listed in parenthesis.

Treatment	K	P	Ca	CEC	Total C	Total N	C : N
Forest	53.75 (±7.70)	6.13 (±0.46)	132.34 (±23.45)	6.13 (±0.79)	3.46 (±0.35)	0.15 (±0.01)	22.59 (±0.37)
Clear-cut	68.01 (±6.81)	7.16 (±0.86)	267.37 (±59.05)	5.37 (±0.53)	3.83 (±0.49)	0.16 (±0.01)	24.37 (±0.96)
Half Ca	65.06 (±3.69)	9.82 (±1.84)	287.52 (±53.74)	6.71 (±0.55)	4 (±0.45)	0.16 (±0.01)	24.96 (±1.21)
Equivalent Ca	75.67 (±8.77)	7.92 (±0.6)	427.06 (±96.44)	6.79 (±1.01)	4.37 (±0.54)	0.17 (±0.02)	24.82 (±0.93
Twice Ca	80.84 (±10.16)	13 (±3.12)	397.59 (±94.82)	6.78 (±0.94)	3.96 (±0.65)	0.16 (±0.02)	24 (±0.91)



Map 2.1: A map of the Island Lake Biomass Research and Demonstration Area, near Chapleau, Ontario. Numbers on the ash plots denote the amount of Ca (kg/ha) used for wood ash amendment. Forested control plot 1-C and biomass removal plot 2-F were not used in the present study. Map provided courtesy of the Canadian Forest Service.

3 Results

3.1 Wood ash and the nematode community: morphological assessment

Samples collected in June and August 2013 yielded a total of 5377 nematode individuals that could be identified into a total of 26 morphotaxa. In June samples, 3437 individual nematodes were enumerated of which, 2933 were identified using morphological characteristics from Tarjan *et al.* (1977) and Bongers (1994). These individuals were classified to 20 genera and 2 families that could not be further subdivided for a total of 22 morphotaxa (Table 3.1). The most abundant groups at this time were: *Plectus* sp., *Acrobeloides* sp., and Rhabditidae sp. August sampling yielded a total of 1940 nematodes, of which, 1741 were identified to 22 genera and 2 families from which no further identifications could be made (24 total morphotaxa) (Table 3.2). The most common taxa in the August samples were: *Acrobeloides* sp., *Plectus* sp., and Rhabditidae sp.

The RM-ANOVA for morphotaxa richness did not suggest any differences among treatments ($F_{4, 15} = 0.675$, $p = 0.620$), sampling time (June versus August) ($F_{1, 15} = 0.004$, $p = 0.953$), nor a time by treatment interaction ($F_{4, 15} = 1.309$, $p = 0.311$). In June, the forest and one-half treatments were equally the most species rich, supporting on average 13.75 species per 25 g wet weight soil, whereas the twice Ca plots were the lowest (11.00 species / 25 g wwt soil). August samples showed a much different trend, with clear-cut plots hosting an average of 13.75 species but only a mean of 10.25 species / g wwt soil were present in the forest treatment.

Overall sampling densities ranged between 400 individuals/25 g wet soil in the equivalent Ca plots and 1084 individuals/25 g wet soil in the one-half Ca plots in June, and 190 individuals/25 g wet soil in the forest plots to 552 individuals/25 g wet soil in the twice Ca plots in August (Table 3.3). However, repeated measures ANOVA found no significant differences in mean abundance between the five treatments ($F_{4, 15} = 0.335$, $p = 0.85$), sampling time ($F_{1, 15} = 3.214$, $p = 0.093$), or time by treatment interaction ($F_{4, 15} =$

0.384, p = 0.817). Mean values of H' (Shannon diversity) were highest in the forest treatment and reached their low point in the twice Ca amended treatment. Yet again, there were no significant differences observed through RM-ANOVA between treatments ($F_{4, 15}$ = 1.518, p = 0.247), sampling time ($F_{1, 15}$ = 0.338, p = 0.570) or time by treatment interaction ($F_{4, 15}$ = 0.703, p = 0.602). The equivalent Ca treatment had the highest average J' (Pielou's evenness index) value, the lowest was observed in the clear-cut in June samples. This trend was different in the August sampling where mean J' was highest in the equivalent Ca and lowest in the twice Ca. As with other morphological variables, there were no significant differences observed in J' between treatments ($F_{4, 15}$ = 1.27, p = 0.325), sampling time ($F_{1, 15}$ = 0.231, p = 0.638) or time by treatment ($F_{4, 15}$ = 0.878, p = 0.500) (Table 3.3). Non-metric multidimensional scaling revealed no distinct groupings of treatment communities; this was confirmed by an analysis of similarity tests in June (global R = -0.080, p = 0.810) and August (global R = 0.045, p = 0.247).

3.2 Wood ash and the nematode community: trait-based measures

3.2.1 Maturity Indices

The taxa identified ranged the entire breadth of the c-p scale (Table 3.1, 3.2); however, c-p 5 taxa were only observed in August samples. Repeated measures ANOVA found no significant differences among the ΣMI or EI indices between treatments ($F_{4, 15}$ = 1.925, p = 0.158; $F_{4, 15}$ = 1.726, p = 0.197, respectively) sampling time ($F_{1, 15}$ = 0.151, p = 0.703; $F_{1, 15}$ = 0.223, p = 0.643, respectively) or from time by treatment interactions ($F_{4, 15}$ = 0.564, p = 0.693; $F_{4, 15}$ = 0.336, p = 0.844, respectively) (Table 3.4). The SI was the only Maturity Index to show a significant treatment effect: the highest mean SI values occurred in the forest treatments in both June and August, which were significantly greater than the August twice Ca treatment. Although there was no significant difference for SI between sampling times ($F_{1, 15}$ = 0.334, p = 0.572), the difference between the forest samples and the twice Ca treatment from August suggests a main treatment effect ($F_{4, 15}$ = 3.617, p = 0.030), driven by the interaction season and treatment ($F_{4, 15}$ = 3.068, p = 0.049) (Figure 3.1).

3.2.2 Body Size Spectra

Both the LSDR and ISD models of BSS showed similar patterns for change between the forest and clear-cut treatments at the June sampling. This is seen in the LSDR model via the regression lines, whose slopes were not significantly different from each other (forest slope = -0.579; clear-cut = -0.592) nor were the y-intercepts (forest = 1.934; clear-cut = 1.785); however data did show a slight reduction in the overall mean abundance (Figure 3.2a). In the ISD model, this trend was observed via the slight reduction in both small and large-bodied taxa (Figure 3.3a). Trends in the LSDR model of ash treatments were unclear. There was an increase of small-bodied taxa in the half Ca treatment (slope = -0.708, intercept = 2.015). However, this did not carry over into the equivalent and twice Ca treatments, which show shallower slopes than all other treatments (slope = -0.305, intercept = 1.512; slope = -0.411, intercept = 1.860, respectively) (Figure 3.2c). Overall, there was a statistically significant difference between treatments in slope ($F_{8, 100} > 100$, $p < 0.001$) but not intercept ($F_{4, 100} = 0.944$, $p = 0.441$). In the ISD model, an increase in all body size classes, not just the smaller ones, was observed in the half Ca treatment, whereas a shift towards larger-bodied individual was seen in the other two Ca treatments (Figure 3.3c).

In August samples, the LSDR model produced much different results. The forest community was very low in abundance (intercept = 1.23) and had the regression line with the shallowest slope (slope = -0.093). Abundance in the clear-cut was unexpectedly high as well and the slope was representative of a community with more large-bodied constituents than the forest community (intercept = 1.66, slope = -0.601) (Figure 3.2b). Results from the ash treatments were also not as expected. The twice Ca community seemed to show an unexpected enrichment effect, with the highest recorded intercept and steepest slope (intercept = 1.673, slope = -0.698). Half and equivalent Ca regressions had shallower slopes indicating the greater presence of larger taxa (intercept = 1.60, slope = -0.428; intercept = 1.387, slope = -0.309, respectively). There was a significant difference between treatment levels in slope ($F_{8, 88} = 2.321$, $p = 0.026$) but not intercept ($F_{4, 88} = 0.724$, $p = 0.578$) (Figure 3.2d). In the ISD model, middle body size classes dominate the uncut forest with a wider breadth than the June samples. The clear-cut shares this

distribution, but similar to the June samples, it shows a reduction in the largest and smallest size classes (Figure 3.3b). However, in the Ca amended soils, the opposite trend was seen in August when compared to June. Smaller body size classes dominated the equivalent and twice Ca treatments, whereas the half Ca treatment was shifted towards larger individuals (Figure 3.3d).

3.3 Wood ash and the nematode community: molecular assessment using T-RFLP

Richness of OTUs was quantified based on the presence/absence of peaks at each band size. There were 92 OTUs observed in June and 80 in August (1204 total). Operational taxonomic units were accepted based on having a minimum of 60 base pairs. A RM-ANOVA found differences between treatments were not statistically significant ($F_{4, 15} = 0.138$, $p = 0.966$). The RM-ANOVA did find that OTU richness was significantly greater in June than in August ($F_{1, 15} = 6.562$, $p = 0.022$) (Table 3.5). However, the interaction between time and treatment was not significant ($F_{4, 15} = 2.020$, $p = 0.143$). When treatments were compared using NMDS there were no distinct groupings; analysis of similarity found no significant differences between the treatment communities when sampled in June (global $R = 0.191$, $p = 0.607$) or August (global $R = 0.217$, $p = 0.402$).

Table 3.1: Total abundance (per 25 g soil) of each taxon of free-living nematodes collected under forest, clear-cut and the three different wood ash applications from June 2013. Taxa are listed in order of lowest to highest c-p rank.

Taxon (c-p rank)	Forest	Clear-cut	Half Ca (100g/ha)	Equivalent Ca (200g/ha)	Twice Ca (400g/ha)
Rhabditidae (1)	86	76	62	66	75
Panagrolaimidae (1)	67	34	103	33	42
Acrobeloides (2)	66	130	114	80	92
Cephalobus (2)	0	2	1	1	0
Chiloplacus (2)	0	0	0	1	0
Eucephalobus (2)	2	0	6	0	0
Plectus (2)	54	62	200	86	128
Wilsonema (2)	29	20	101	24	85
Criconema (3)	1	0	0	0	2
Criconemoides (3)	1	0	0	0	0
Hemicycliophora (3)	10	3	16	0	6
Prismatolaimus (3)	1	0	5	2	3
Teratocephalus (3)	57	21	73	5	34
Trichostoma (3)	0	0	1	0	0
Tripyla (3)	5	27	22	10	20
Tylolaimophorus (3)	3	1	1	6	4
Alaimus (4)	72	61	76	22	64
Clarkus (4)	6	4	4	8	11
Epidorylaimus (4)	1	1	2	1	1
Eudorylaimus (4)	38	9	26	14	20
Paramphidelus (4)	27	39	94	9	31
Thonus (4)	4	6	7	2	6
Unknown	113	75	170	30	116

Table 3.2: Total abundance (per 25 g soil) of each taxon of free-living nematodes collected under forest, clear-cut and the three different wood ash applications from August 2013. Taxa are listed in order of lowest to highest c-p rank.

Taxon (c-p rank)	**Forest**	**Clear-cut**	**Half Ca (100g/ha)**	**Equivalent Ca (200g/ha)**	**Twice Ca (400g/ha)**
Rhabditidae (1)	19	53	37	25	56
Panagrolaimidae (1)	18	31	30	20	13
Acrobeloides (2)	7	39	37	43	222
Plectus (2)	35	83	84	59	51
Cephalobus (2)	0	29	17	39	73
Wilsonema (2)	10	15	19	7	14
Eucephalobus (2)	1	2	0	5	24
Fungiotonchium (2)	0	0	3	0	1
Hemicycliophora (3)	10	10	16	7	9
Teratocephalus (3)	4	9	17	3	4
Tylolaimophorus (3)	3	5	1	7	1
Prismatolaimus (3)	0	3	2	9	0
Bastiania (3)	2	5	5	0	1
Tripyla (3)	2	4	2	3	0
Macroposthonia (3)	1	0	0	0	0
Eudorylaimus (4)	13	23	70	23	10
Paramphidelus (4)	16	21	31	9	16
Alaimus (4)	7	26	18	8	5
Thonus (4)	5	9	5	11	2
Clarkus (4)	12	4	7	7	1
Epidorylaimus (4)	1	1	7	2	0
Paravulvus (5)	4	0	0	0	0
Sectonema (5)	1	0	0	0	0
Unknown	19	59	48	24	49

Table 3.3: Mean abundance, richness, diversity and evenness values by treatment from June and August samples. Standard errors are listed in parenthesis.

Sampling time: June 2013				
Treatment	**Abundance (# individuals / 25 g wwt soil)**	**Richness (# taxa / 25 g wwt soil)**	**Shannon diversity (H')**	**Evenness (J')**
Forest	160.75 (±61.40)	13.75 (±0.63)	2.18 (±0.04)	0.83 (±0.02)
Clear-cut	142.50 (±66.52)	11.50 (±0.96)	1.94 (±0.11)	0.80 (±0.04)
Half Ca	271 (±187.63)	13.75 (±1.18)	2.16 (±0.07)	0.83 (±0.01)
Equivalent Ca	100 (±24.96)	11.50 (±0.87)	1.98(±0.02)	1.98 (±0.02)
Twice Ca	185 (±145.51)	11 (±1.96)	1.86(±1.13)	1.85 (±0.04)
Sampling time: August 2013				
Treatment	**Abundance (# individuals / 25 g wwt soil)**	**Richness (# taxa / 25 g wwt soil)**	**Shannon diversity (H')**	**Evenness (J')**
Forest	42.75 (±19.79)	10.25 (±2.14)	1.93 (±0.17)	0.86 (±0.02)
Clear-cut	94.50 (±34.91)	13.75 (±1.65)	1.99 (±0.10)	0.77 (±0.02)
Half Ca	104.75 (±21.44)	13.50 (±0.87)	2.04 (±0.10)	0.79 (±0.02)
Equivalent Ca	71.50 (±26.88)	12.25 (±1.89)	2.10 (±0.18)	0.85 (±0.04)
Twice Ca	124 (±70.15)	11.50 (±1.32)	1.83 (±0.15)	0.76 (±0.06)

Table 3.4: Mean values of the trait-based indices, ΣMI, SI, and EI, as well as abundance by treatment from samples collected in June and August 2013 from the Island Lake Biomass and Harvesting Demonstration area near Chapleau, Ontario. Indices SI and EI are expressed as percentages. Standard errors are listed in parenthesis. Values followed by the same letter in the same column are not significantly different based on Tukey *post hoc* test among treatments.

	Sampling time: June 2013		
Treatment	**ΣMI**	**SI**	**EI**
Forest	2.50 (±0.12)	80.82 (±2.73)a	61.37 (±11.36)
Clear-cut	2.21 (±0.15)	63.16 (±7.70)ab	54.29 (±16.12)
Half Ca	2.29 (±0.11)	68.52 (±2.80)ab	57.9 (±5.60)
Equivalent Ca	2.11 (±0.08)	58.63 (±1.21)ab	60.81 (±5.08)
Twice Ca	2.21 (±0.19)	72.17 (±3.01)ab	57.52 (±4.74)
	Sampling time: August 2013		
Treatment	**ΣMI**	**SI**	**EI**
Forest	2.49 (±0.18)	82.44 (±3.26)a	64.86 (±5.91)
Clear-cut	2.04 (±0.18)	67.54 (±4.10)ab	55.28 (±13.52)
Half Ca	2.26 (±0.14)	73.35 (±7.74)ab	60.51 (±4.31)
Equivalent Ca	2.33 (±0.22)	65.99 (±12.47)ab	55.43 (±3.50)
Twice Ca	2.03 (±0.12)	42.60 (±10.18)b	39.49 (±7.31)

Table 3.5: Mean richness values of OTUs obtained from T-RFLP analyses in June and August sampling. Standard errors are listed in parenthesis. Letters followed by the same letter in the same column are not significantly different based on Tukey *post hoc* test among treatments (lower case) and time (upper case).

Treatment	**June samples**	**August samples**
Forest	26.75 (±7.32)a	25.00 (±7.61)a
Clear-cut	28.50 (±6.30)a	31.50 (±4.19)a
Half Ca	36.50 (±14.31)a	18.75 (±1.31)a
Equivalent Ca	38.50 (±12.22)a	20.00 (±5.67)a
Twice Ca	34.25 (±10.19)a	30.00 (±5.12)a
Total	32.90 (±3.66)A	25.05 (±2.38)B

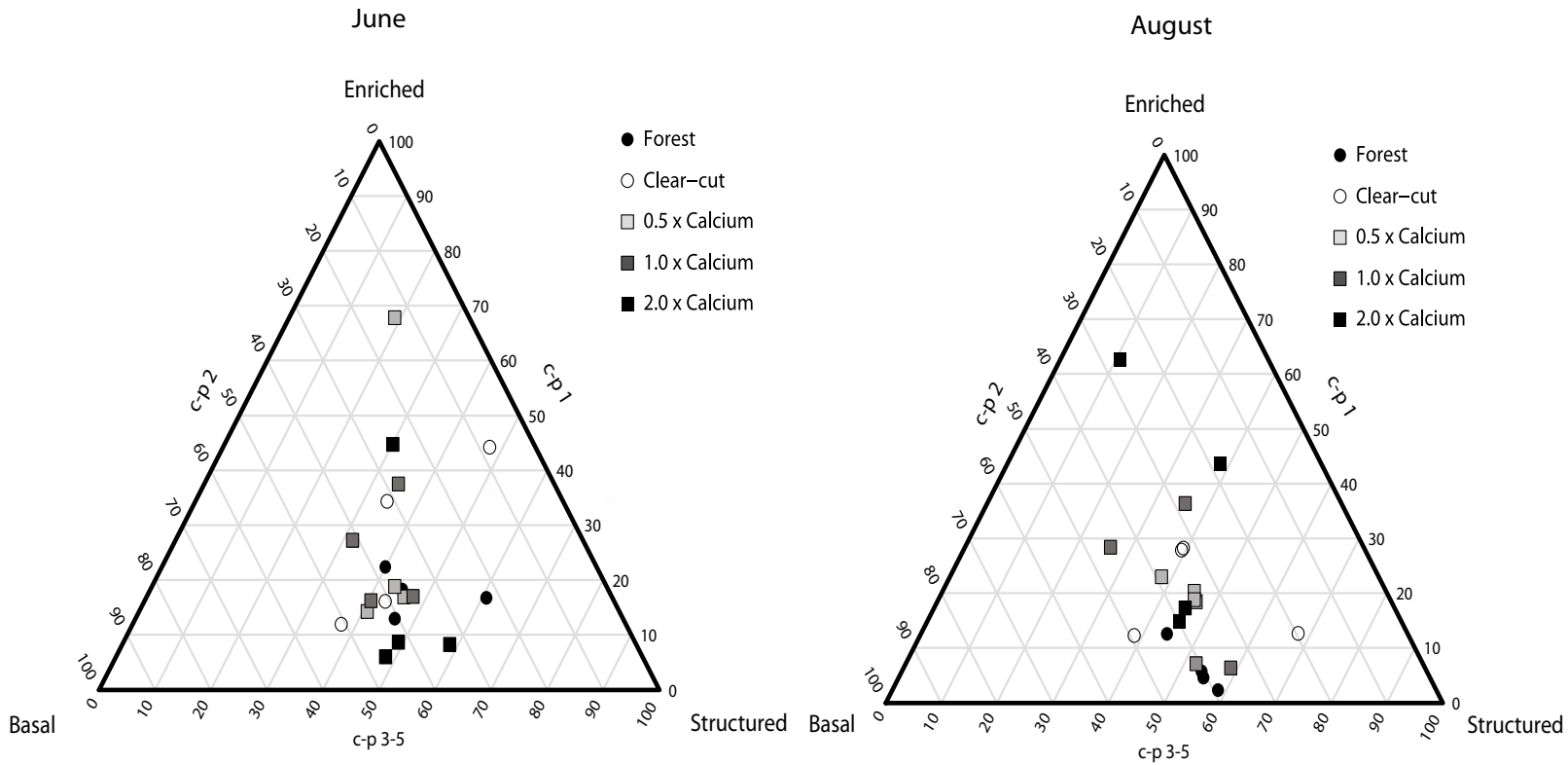


Figure 3.1: Weighted c-p triangles (ternary plots) showing the proportional distribution of c-p groups from each replicate of the forest, clear-cut, and ash-amended treatments in relation to the three soil states identified by the MI from June and August samples.

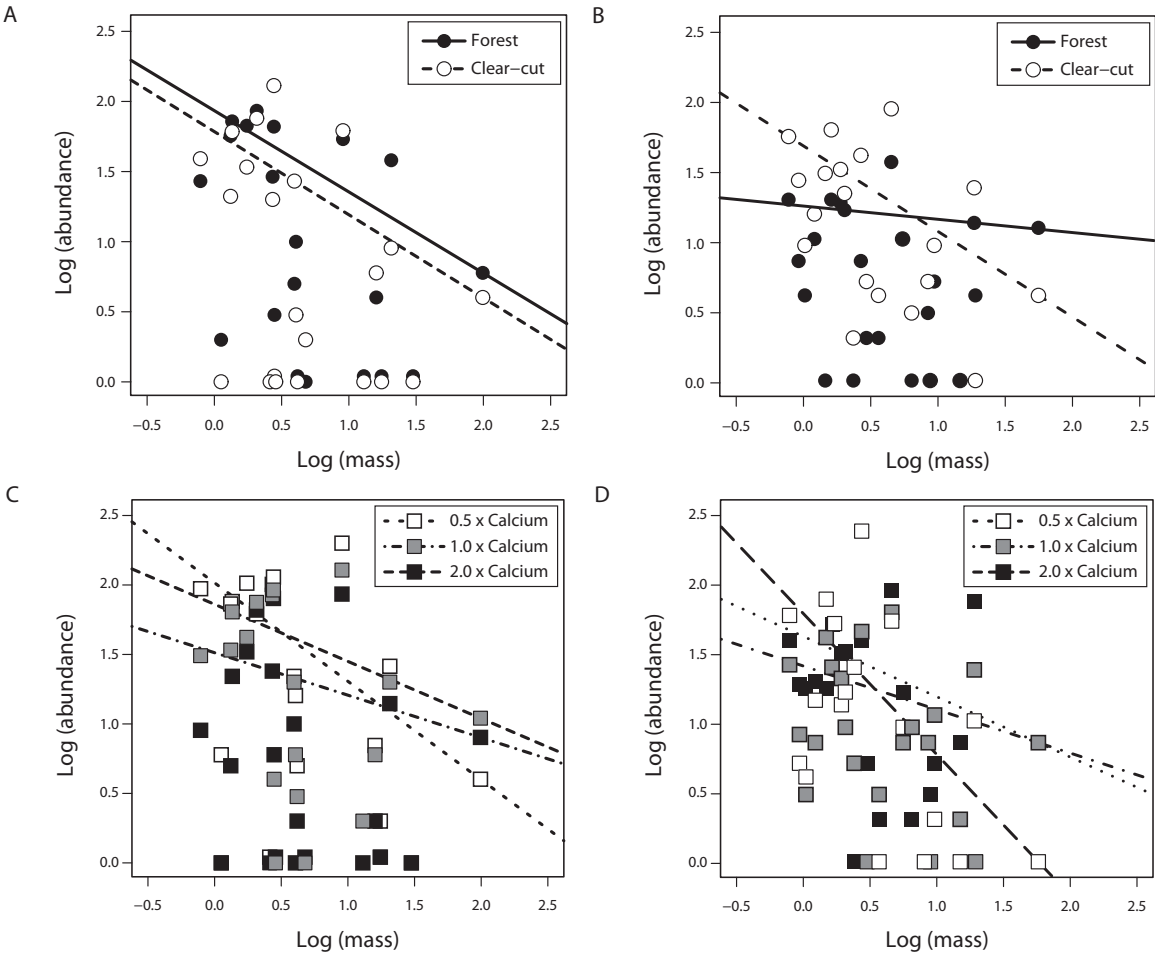


Figure 3.2: The nematode community as seen through the LSDR model of BSS using dry weight (ng) and abundance on a $\log_{10}$ scale of forest and clear-cut treatments in A) June and B) August, and one-half, equivalent, and twice Ca amendment in C) June and D) August. Regression lines were fit using the 75th quartile.

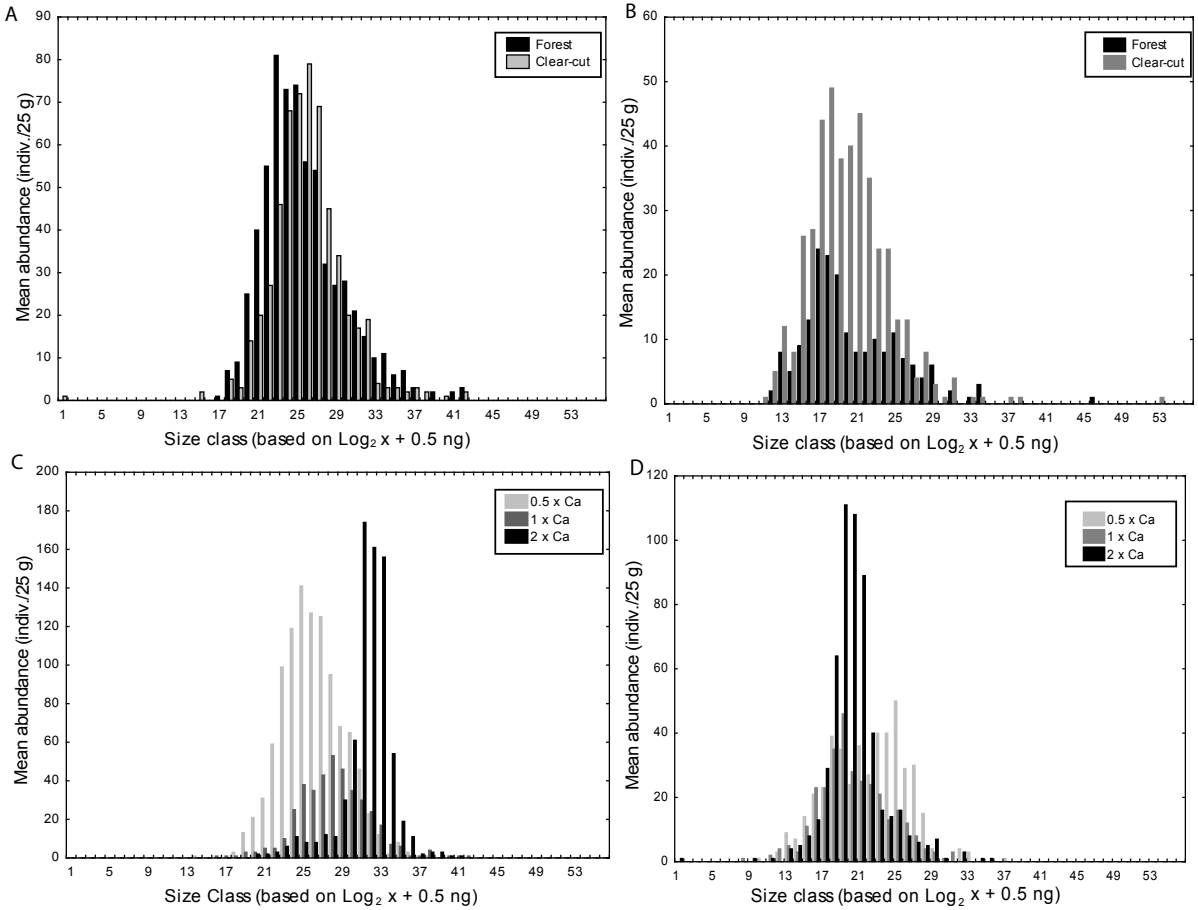


Figure 3.3: The nematode community as seen through the ISD model of BSS with mean body size classes based on Log_2 plus 0.5 ng and abundance of forest and clear-cut treatments in A) June and B) August, and one-half, equivalent, and twice Ca amendment in C) June and D) August.

4 Discussion

4.1 Response of the nematode community to clear-cutting

4.1.1 Morphological measures of nematode communities

The nematode community appeared to be resistant to clear-cutting disturbance in this study. Although nematodes have been frequently observed to negatively respond to clear-cutting, these responses are hardly uniform. Changes in the nematode community after clear-cutting, if they are present, likely stem from alterations in the physical characteristics of the soil, including moisture and temperature regimes, as well as alterations in physical structure (Marshall, 2000; Sohlenius, 2002). Gross abundances of nematodes often decrease following clear-cutting (Huhta *et al.*, 1967; Panesar *et al.*, 2000) yet species richness (Háněl, 2004) and diversity indices are often unaffected, especially in the time shortly following harvesting (Panesar *et al.*, 2000; Forge & Simard, 2001). Such a situation often arises following significant reductions in relative abundances of the most abundant taxa, thereby increasing diversity and evenness values (Forge & Simard, 2001), yet richness stays roughly the same. This was not apparent in the present study with the same three groups (*Plectus* sp., *Acrobeloides* sp., Rhabditidae sp.) remaining the most abundant at both sample times, and diversity indices not changing significantly. Rather I found that lower relative abundances of some groups (e.g. *Alaimus* sp.) were countered with greater abundances in other groups (e.g. *Acrobeloides* sp.), which did not lead to significant changes in community composition, richness, total nematode densities or the diversity index values. More natural disturbance has triggered similar responses as recorded in Slovakia where a spruce forest had been harvested following severe windfall. In this instance, abundances and diversity indices were generally unaffected by tree removal (Čerevková & Renčo, 2009; Čerevková *et al.*, 2013).

Though not expected, a lack of major change in richness and abundance of nematodes following forest harvesting is not altogether unusual; it has been observed with some frequency in the literature (Marshall, 2000; Háněl, 2004), and there are several factors that may contribute to this lack of change. For instance, Huhta *et al.* (1967), who are often cited as evidence for the negative effects of clear-cutting on nematodes, suggest that non-significant changes can arise due to greater pressure from the so-called "prevailing situation" of the system than disturbance itself. Though vague, this phrase can be understood to mean the host of abiotic factors present in a system that leads to high variability and obscures treatment effects. Indeed, Huhta *et al.* (1967) later note that the nematode communities they studied displayed a greater response to seasonality than to clear-cutting itself. Although the present study did not show a significant seasonality of the nematode communities, this could be due to sampling occurring at the beginning and middle of the growing season, which generally are not much different from one another (Panesar *et al*., 2000).

Though microclimate variation following clear-cutting has been extensively considered in the succession of soil fauna (Siira-Pietikäinen & Haimi, 2009) there were no apparent differences in the physical properties of the soil at the time of June sampling. However, this was early in the season. Removal of the canopy within the clear-cut would increase solar radiation and precipitation, and thereby increase soil temperature and temperature fluctuations (Keenan & Kimmins, 1993), as well as soil moisture fluctuations later in the season. Surprisingly, differences in soil organic layer between clear-cut and forest did not appear to influence nematode communities, as nematodes are usually less abundant in mineral soils. However, disturbance can drive nematodes deeper into the soil, so deeper sampling may have uncovered a different community (Marshall, 1974; Ou *et al*., 2005). It should be noted, however, that the unharvested forest is a previously cut and replanted site. The age of this rotation was only 40 years, and the site was donated to the experimental system because it was not considered a 'productive' site (P. Hazlett, Pers. Comm.). Therefore, it may that the 'prevailing situation' of this forest is one of continuous heat stress in a spatially constrained environment.

It is also important to consider the early successional stage of the harvesting treatment sites. As the samples collected in this study were only 1.5 years post-harvest, the forest succession process has only just begun, with pioneer plant species dominating the unmanaged parts of the landscape. Furthermore, the site preparation included the removal of most coarse woody debris in the system. This debris is an important food source for microbes, especially fungi (Zhang & Zak, 1998), and previous research has shown that the proportional abundance of bacterivorous nematodes increases when compared to fungivores after clear-cutting (Háněl, 2001; Sohlenuis, 2002). There was a noticeable lack of fungivorous nematodes encountered in the present study. Although fungi are sensitive to both clear-cut and wood ash amendment processes (Bååth *et al.*, 1995) low fungivorous nematode populations were also seen in the undisturbed forest treatment. This can likely be attributed a combination of the fact that soil fungi are less abundant than bacteria, living almost exclusively in litter and organic layers (Berg *et al.*, 1988), which were already very thin in both the clear-cut and the undisturbed forest site. If harvesting debris was left on site as 'slash' following forest clear-cutting, this in itself may have created an 'enriched' nematode community state as previously observed (Sohlenuis 1996; Sohlenuis, 1997). Therefore, removal of harvest debris for use in wood-ash production may have negated the beneficial nutrient inputs, and the resultant changes in nematode community structure may have been missed due to this removal of biomass for ash production.

4.1.2 Trait-based measures of nematode communities

The above factors also likely influenced the ΣMI and associated SI and EI values found. In general the forest sites had the most structured nematode communities, whereas the clear-cut sites showed the most variable response in maturity index values, but included some very basal MI values. Other studies using trait-indices have similarly shown mixed responses to clear-cutting. For example, Čerevková and Renčo (2009), Čerevková *et al.* (2013), Panesar *et al.* (2000), and Háněl (2004) all report insignificant differences between clear-cut and standing forest treatments for the Maturity Index. Forge and Simard (2001) interestingly observed year-to-year variation between significant and insignificant MI results. Regardless, the suite of MI values shows a greater, and

consistent, treatment effect between forest and clear-cut than morphological (richness, abundance, diversity) indices do.

For the BSS there were large differences in the trends between June sampling and August sampling. This was likely driven by the large reductions in overall abundance in August samples and the resulting overall greater variability in those samples. The June BSS showed only a minor response associated with clear-cut harvesting as a disturbance with both models showing an overall reduction in abundance following forest harvesting. Surprisingly, when visualised in the ISD model this loss was concentrated in smaller nematodes, whereas disturbance theory expects larger organisms to be most susceptible to change (Brose *et al.*, 2012). When thought of in the context of an already stressed soil however, this pattern makes sense. There is evidence that some types of disturbance can cause bottom-up effects, meaning that changes in lower trophic levels will dictate changes in the system, but can require some time before higher consumers are affected (Brose *et al.*, 2012). This trend was not observed in August likely because of the extremely low abundance (9 individuals/25 g) in one forest sample.

4.1.3 Molecular measures of nematode communities

Data from T-RFLP analyses was used to assess OTU richness. Although there was an effect of sampling time found through statistical analysis, no effect of treatment was observed. There are several reasons why this could have occurred. Firstly, although established in the literature (Donn *et al*., 2008; Chen *et al*., 2010; Donn *et al*., 2012), the protocols were being trialed for the first time in this laboratory and indeed in this system. Thus, the method presented here may have suffered from a lack of optimisation that is present in more specialised research groups. Furthermore, the size of soil sample may have impeded the success of T-RFLP analyses. Wiesel *et al*. (2015) have shown that soil samples should weigh 200 g or greater for this method. They further have shown that using samples of less than 100 g will not reflect the true community composition with significant variation appearing at lower weights.

4.2 Response of the nematode community to wood ash amendment

None of the wood ash amendment levels led to any significant changes in the nematode community when compared to each other or to the clear-cut and forest treatments using numerical measures except for the SI. In this case the forest treatments in both July and August were more structured than the twice Ca amended soil when sampled in August. I attribute this to the unsurprising result of the forest treatment being less *basal* rather than some inherent change in the twice Ca plots *per se*. In fact, contrary to my predictions based on the expectations of the MI and previous experiments on wood ash-nematode interactions (Nieminen, 2011), none of the amended sites showed any trend towards being an *enriched* community. Therefore, it is again more likely that heterogeneity was responsible for the broad MI results within the amended plots.

One of the reasons for using wood ash as an amendment is the liming affect it has on soil pH and the subsequent enhancement in soil nutrient availability (Pitman, 2006; Augusto *et al.,* 2008). Indeed, the liming effects of wood ash amendment are known to increase the availability of dissolved organic C (Augusto *et al*., 2008) and available N (Vance, 1996) within soils. However, although a greater pH associated with soil liming has been shown to support higher c-p level taxa (Bongers, 1999), Hyvönen and Persson (1990) have shown that soil liming that increased pH from 4 to 6 did not impact the nematode community. As pH in this study ranged from 5.04 - 5.22 these soils may be considered already less acidic compared to other Boreal systems, and therefore further 'liming' would not induce changes in the nematode community.

Another possibility is that the enrichment of the community occurred within a very short time following wood ash application, and that the enrichment effect had already subsided when sampling was performed 1.5 years post application. Previous studies of wood ash amendment have generally found increased abundances of nematodes following amendment (Nieminen, 2011). For example, wood ash has been observed to significantly affect the soil community for up to 152 weeks after amendment (Lirri *et al.*, 2002), yet such an effect was not observed here. The efficacy of wood ash amendment is heavily influenced by the natural characteristics of the soil it is added to

(Pitman, 2006). In addition, the application rate of wood ash is very important in determining the length of time that the effects of amendment will be observable. Ash application rates of 6 Mg ha^{-1} $CaCO_3$ in a forest with acidic, sandy soils produced noticeable effects for only 7 months, whereas at a rate of 20 Mg ha^{-1} effects were still observable 20 months after application (Kahl *et al.*, 1996). In this study, wood ash was applied at a much lower application rate (although not entirely comparable, estimated orders of magnitude lower in this study). Therefore, it may not be surprising for the effects of wood ash to have completely dissipated 1.5 years after amendment. Disparity between the weights of ash applied also highlights a problem with comparing studies of wood ash amendment; the target effects of application vary greatly between studies as to the standardizations of application rates. Indeed in the present case ash amendment was based on Ca removal and replacement, whilst studies that have found responses to amendment in the nematode community were based on total mass of wood ash (Lirri *et al.*, 2002; Lirri *et al.*, 2007).

Deeper insights into the community compositions were gleaned from BSS analyses. Unfortunately, with the exception of the equivalent Ca amendment treatment, such insights did not present a consistent trend with amendment. Although wood ash might be expected to impart an enrichment effect on the community, this was seen only once in the one-half Ca amendment sampled in August. The marked increase in large-bodied nematodes seen in June sampling through both BSS methods was lost by August, which could be a seasonal effect, possibly related to vegetative cover. Vegetative cover was greater in August than June. Plant abundance and diversity has been known to influence nematode communities as seen in negative associations with forbs (de Deyn *et al.*, 2004) and positive associations with legumes (Viketoft *et al.*, 2005). Legumes are especially important as their nitrogen-fixing abilities support increased populations of bacteria, which have been shown to support greater densities of bacterivorous nematodes (Sohlenius *et al.*, 1987; Viketoft *et al.*, 2005). However, plants were not characterised in this study. Rather, studies of the plant community at this site are ongoing and their results may better inform the conclusions of this project in the future.

4.3 Evaluation of assessment methods by *a priori* criteria

An overarching goal of this study was to compare morphological, trait-based, and molecular methods of community analysis. This was based on the *a priori* criteria of: (1) *Is the method informative*? (2) *Is the method feasible*? and (3) *Can the results be compared with other studies?* To this end, a qualitative assessment of the application of these methods and their actual data output was made (Table 4.1). The assessment methods are considered in the following order of efficacy: T-RFLP < morphological methods < ΣMI < BSS.

4.3.1 Evaluation of molecular T-RFLP assessment

Restriction fragment analysis was used to assess richness of OTUs, which are considered analogous to species-level identifications. *Is OTU data informative*? OTU data can only be comparable to species richness values obtained from morphological methods when T-RFLP fragment length is fully cross-validated for each species in the community. This is time consuming and was not performed in this study. Rather OTU richness was compared to morphotaxa richness and found to be greater, but still did not reveal any treatment effects. Furthermore, as with morphological changes in richness measures, OTU data cannot indicate the mechanisms behind any observed changes. Therefore, community assessment by T-RFLP can only be considered moderately informative. *Are OTU data feasible*? In a specialised lab, there is little cost associated with the expertise and time required to run the specialised T-RFLP protocols. Optimised methodologies can make T-RFLP expedient, providing accurate and informative data sets. However, without expertise, standardised protocols and infrastructure the method can be problematic, as it possesses a steep learning curve involving extensive troubleshooting. In this light, T-RFLP is not considered especially feasible in my assessment. *Can T-RFLP results be compared with those of similar studies?* Richness of OTUs can be compared to other instances in the literature, much like richness values generated from morphological methods. However, single bands representing presence in these data may in fact be made up of sequences from different taxa. Consequently, comparisons to the literature may be impossible without confirming their identity(s)

through parallel DNA sequencing. As this is not always possible, T-RFLP is only considered moderately comparable among different studies.

4.3.2 Evaluation of morphological community assessment

Richness, abundance, and diversity measures were obtained using morphological methods. *Are morphological data informative?* While morphological methods provide accurate estimates of species richness and abundance, they were not sensitive to treatments; further they cannot provide any indications of the mechanisms behind observed changes. For this reason, they are considered, like T-RFLP analyses, to be only moderately informative. *Are morphological data feasible?* The morphological assessment methods also fail the feasibility criterion: a high degree of taxonomic expertise is necessary for these methods to be effective and provide a high resolution of where changes occur. This expertise can be attained, but can only come with time. Lots of time is needed to train in taxonomic identifications, especially in micro-invertebrates so that a lower taxonomic status (i.e. species, or even genus) can be assigned to individuals. Furthermore, actually identifying individuals is a time consuming process even for an expert. That said, a taxonomic expert can efficiently and cheaply (other than time) generate these data without associated costs of many consumables. *Can morphological results be compared with those of other studies?* When based on taxonomic identification, abundance and richness can be considered as morphological measures that are readily compared between disparate studies; however, measures of diversity and evenness are not always comparable as they represent proportional comparisons within a system but do not explicitly account for the reasons for their values.

4.3.3 Evaluation of trait-based community assessment

Are trait-based assessments informative? Both the MI and the BSS trait-based methods were considered informative as they not only demonstrate whether the nematode community is changing in response to treatments, but provides information on which functional groups are changing. For the MI, the use of the c-p scale and feeding group rankings can reveal the mechanisms behind these changes, as changes in the abundance

or presence/absence of c-p groups reflect differences in species ability to respond reproductively or trophically to environmental change.

Both the LSDR and ISD body size spectral models showed sensitivity to treatments and presented data in such a way that searching for potential mechanisms for change was intuitive. However, trends in BSS for this study were not consistently observed between June and August sampling. Predictions based on body size are based on the literature (Zavaleta *et al.*, 2009; Wilson *et al.*, 2010; Brose *et al.*, 2010) where large bodied species are demonstrated to be more extinction prone (Leck, 1979; Gonzalez & Chaneton, 2002; Cardillo, 2003) due to having smaller local population sizes, and often being predators. These organisms are often considered to be K selected (Damuth, 1981; Romanuk *et al.*, 2011; Brose *et al.*, 2012). Similarly, smaller-bodied species are often considered part of the r-select suite of traits that denote fast reproductive cycles, which facilitate rapid population growth under increased resources. Inconsistency in seasonal patterns of body size could arise because large-bodied predators and large-bodied root feeders are confounded in the BSS. Though the general concept of BSS relating to trophic position has been shown in soils, the organisms that are often compared differ in size by several orders of magnitude (Postma-Bloouw *et al.*, 2010). When these effects are investigated within groups of soil fauna, the basic assumption that predators are larger than their prey does not necessarily hold true. First, current knowledge of soil trophic interactions shows a great degree of opportunistic feeding in what were thought to be rigid trophic groups (Crotty *et al.*, 2012). In nematodes this can be seen in the case of the predatory family Mononchoidea. During development, early developmental stages of this family have been known to ingest bacteria and agar in laboratory observations (Bilgrami *et al.,* 1984; Yeates, 1987a). Bacterivorous behaviour may continue in later life stages in the absence of animal prey (Yeates, 1987a) or competition from specialised bacterivores (Yeates, 1987b). This scenario also challenges the notion that larger animals are specialised and therefore more susceptible to disturbance.

Are trait-based assessments feasible? Using the MI has similar problems to morphological assessments, as there is a need for taxonomic expertise to identify and

classify species to c-p groups. Designation of c-p level is generally conserved at the family or genus level (Bongers & Bongers, 1998), so less time and expertise is needed to make this level of identification compared to morphological assessments. As a result, however, resolution may suffer with a loss of species-level identifications, but the conservation of traits at higher taxonomic levels still allows for important conclusions to be made from the data. The BSS methods excel in the feasibility criteria. Whilst the LSDR method still requires taxonomic expertise, it requires only a fraction of body sizes to be obtained for each taxon, thus this method can be thought of as a supplement for traditional morphological assessments by increasing the information produced in a study with low time costs. Conversely, the ISD model ignores taxonomic identifications negating the need for taxonomic expertise and time spent identifying individuals. However, as it requires that every individual be measured, this process can be incredibly time consuming unless more advanced imaging software is used. Here for example, processing of June samples took six months using ImageJ software (5377 individuals) that needed to have each digital image calibrated, whereas the use of automated microscope-associated imaging software allowed me to process 3437 individuals in a single month.

Can data trait-based assessments be compared to those of other studies? Indices like the MI are readily compared between systems (e.g. Panesar *et al.*, 2000). However, comparing trait-index values alone may obscure their implications much like with standard diversity indices. Indeed when MI values are compared without regard to the proportions or knowledge of their constituent groups, valuable insights into the communities are lost. For this reason trait indices can be considered comparable at the same level as morphological methods. For the BSS, the ISD model is most readily used as a qualitative assessment, which negates comparisons among studies. Further, there is a difference between the BSS models: the LSDR model can offer a high degree of resolution as to what taxa are most affected, whilst the ISD model will not offer any. Yet, both of these models have enjoyed considerable use in other systems and therefore there is a wealth of literature for comparisons to be made. There has been extensive work linking the body size of soil organisms to environmental characteristics like nutrient stoichiometry (Mulder & Elser, 2009) and trophic linkages (Mulder, 2006), from which

extrapolations can be made using LSDR data. Again it should be remembered that in the case of the ISD such comparisons will be qualitative, but the results of these models may illuminate trends otherwise missed when dealing with purely numerical data. Use of the ISD model may even facilitate comparisons of nematode communities in vastly different environments. Indeed, I used previous work by Tita *et al.* (1999), to generate body sizes and size classes in this study. This work looks at the interaction of body size and sediment composition in an intertidal nematode community. Indeed the use of body size classes can be seen in a huge array of studies of nematode communities including animal parasites (Monard & Poulin, 2002), benthic freshwater (Traunspurger & Bergtold, 2006), and even those of deep-sea sediments (Gambi *et al.*, 2003). All in all, the BSS provided the most information with minimal costs and offers a degree of comparability unmatched by the other methods used (Table 4.1).

4.4 Summary of results & recommendations

The nematode community did not show significant changes between standing forest, clear-cut, or any of the three wood ash treatments for most of the assessment methods used. This result is likely due to a combination of factors, and indeed, the resistance of the nematode community to disturbance in forestry is not uncommon, and the effects of soil heterogeneity must be considered. It is most likely that no changes were observed in this study due to the overall poor quality of the site prior to treatments, and the low amount of wood ash used in amendment.

However, utilizing different methods of community assessment provided varying degrees of information, which led to a better of understanding of what changes were occurring in the community and why their effects were not significant. When the morphological, trait-based, and molecular methods were compared they showcased their relative merits and shortcomings. Although the assessment is wholly qualitative and indeed largely a personal experience, it is hoped that it will provide some degree insight into how to best utilise methods for a given system. The results of the present study are meant to follow-up on work proposed by Turnbull *et al.* (2014) where the authors (myself included) put forward the notion that BSS are underused in soil systems but could provide similar information to previously existing trait-indices (such as the MI). Despite

the lack of significant differences between treatments, the use of BSS provided a great deal more information than other methods of analysis. I believe that the explicit test of whether BSS can show the same trends predicted by standard trait-indices was passed in this study. I feel that this validates its use in future studies of soil community change both building on this work with nematode-based environmental assessments and moving into other less-studied groups (i.e. Collembola, Rotifera, Tardigrada).

Table 4.1: Simplification of the results of qualitative assessment of each method of community analysis based on *a priori* criteria. Note: + = "yes", +/– = "somewhat", and – = "no".

Criteria	Morphology	ΣMI	BSS	T-RFLP
Information	+/–	+/–	+/–	+/–
Feasibility	–	+/–	+	–
Comparability	+/–	+/–	+/–	–

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