

The impacts of surface mining restoration efforts on soil dwelling nematode communities in the Appalachian region

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Dedication

This work is dedicated to my mother for teaching me patience, my father for teaching me to be stubborn,
and Jack for teaching me to use both wisely.

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Abstract

Traditional restoration of post-mining sites in the southeastern U.S. utilizes methods that discourage above and below-ground succession of native floral and faunal communities. Recent advances in mining restoration techniques proposed by the Appalachian Regional Reforestation Initiative (ARRI) have improved above-ground plant succession and growth rates in reclaimed sites by developing the Forestry Reclamation Approach (FRA), a process that discourages substrate compaction and encourages native plant succession. The below-ground component of these reclaimed areas remains undescribed, however. Below-ground invertebrate communities in mining restoration sites have been largely ignored, despite their potential importance to the long-term recovery of these profoundly disturbed sites. Nematode communities were measured in both traditional and FRA reclaimed sites using a chronosequence to measure community shifts over time, as well as intact un-mined forests. Nematodes were identified to morphospecies and by trophic groups. This study demonstrated that species richness, Shannon diversity, and abundance measures became similar to forested sites in reclaimed sites between nine and fifteen years since reclamation. Less coarse analyses, including abundance of indicator morphospecies and trophic abundances showed that a deficit of nematode taxa remained, even in older sites. Bacteria-feeding nematodes increased over time in both FRA and traditional reclamation sites, while plant-feeding nematodes increased later in the chronosequence of both treatments. Genera such as *Ogma*, *Xiphinema*, *Teratocephalus*, and *Acrobeles* were indicative of intact forest sites and were under-represented in disturbed sites. Nematode communities may have bottom-up effects on plants, and can have inhibitory effects on both the nutrient status and biomass of plants when nematode abundances are low or plant parasites abundances are high.

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Chapter 1

Introduction and General Information

The status of post-mining restoration in the southeastern United States

Coal power generates 41% of electricity worldwide, and 49% of electricity in the United States (World Coal Association 2012). Surface coal mining accounts for 67% of coal production within the United States (World Coal Institute 2005). This method requires the removal of plant and soil using land-moving equipment. Underlying rock and coal is removed using explosives and moved to an adjacent area.

The Office of Surface Mining estimates that from 1978-1999, in the United States, over 500,000 hectares were disrupted by surface mining techniques (Office of Surface Mining 1999) and it is estimated that an equivalent area of land was disturbed prior to this time. In 2009, surface mining disturbed over 42,000 hectares in Appalachia (Office of Surface Mining Reclamation and Enforcement 2009).

Prior to 1977, restoration requirements of surface mined sites in the United States were left to the discretion of each state. Numerous human health and safety issues were created by a lack of regulation of abandoned mine sites. Some of these issues included water quality degradation, landslides, esthetic problems, decrease in economically viable land for forage and timber, as well as loss of environmental services (Angel *et al.* 2005). These issues disproportionately affected impoverished communities in the Appalachian region.

In 1977, the Surface Mining Control and Reclamation Act (SMCRA) was passed, creating an agency to oversee the required restoration of former surface mine sites as well as creating a fund for the restoration of abandoned sites. The Office of Surface Mining enforces the

law, which requires mine operators to put money into a bond which would be relinquished in three phases once established reclamation criteria were met.

The phases are as follows:

Phase I: Phase I constitutes return of topography to its original contour or an approved variation thereof.

Phase II: Verifies that plant cover or other erosion control methods have been used at the site to stabilize substrate and safeguard against erosion. Additionally, the site must not contribute to stream sedimentation.

Phase III: Certifies that the site has met remediation standards set by the Office of Surface Mining in the areas of restoration and site stabilization (Office of Surface Mining Reclamation and Enforcement 2007).

While the SMCRA had immediate positive impacts on communities by decreasing the number of mine wall collapses and landslides, the act's emphasis on erosion control led to mechanical compaction of substrate using land-moving equipment, and planting of fast growing, aggressive, non-native ground covers. The SMCRA does not prioritize restoration of ecosystem processes such as native plant succession, soil formation or nutrient cycling. In the process of achieving phase requirements, negative effects, such as failure to establish native plant communities, were further exacerbated by the execution of methods to meet the requirements, such as soil compaction.

Traditional SMCRA mining techniques remove topsoil and plant material along with the overburden. The overburden, typically shale and un-decomposed limestone (or gray limestone), is returned following the conclusion of commercial mining on a site. Mechanical compaction is utilized to decrease short-term landslide danger, which decreases porosity and water infiltration. The site is then planted with agricultural grasses and herbaceous cover such as *Festuca arundinacea*, *Sericea lespedeza* and other, often non-native, plants to further avoid erosion and runoff.

Traditional restoration techniques undertaken to meet SMCRA standards discourage return of above and below-ground communities to pre-mining conditions in the following ways:

- a. Compaction of soils decreases the pore-space that is conducive to soil fauna habitat and native plant succession. Compacted soils increase damage from drought and nutrient problems. (Dittmer & Schrader 2000; Bassett *et al.* 2005; Groninger *et al.* 2007; Wang *et al.* 2008);
- b. Soils devoid of soil organic matter (SOM) harbor considerably less soil fauna, and low nutrient environments will depress growth of native persistent plant species (Abdul-Kareem 1984);
- c. Non-native plants foster depauperate soil communities and change nutrient dynamics within that system (Reinhart & Callaway 2006; Scharfy *et al.* 2009; Gandhi & Herms 2010).

Recently, the Appalachian Regional Reforestation Initiative (ARRI), which is comprised of researchers in association with the Office of Surface Mining, proposed new approaches for

reclamation of coal-mined land, which have been shown to increase the rate of native plant succession in post-mining areas. This method is referred to as the Forestry Reclamation Approach (FRA). The FRA is a cost-effective approach to mining restoration with the purpose of developing wildlife habitat, watershed protection, and other ecosystem services (Angel *et al.* 2005).

The techniques proposed by ARRI include:

- a. Create a loosely graded planting medium, comprised of soil or decayed limestone (also referred to as brown limestone) to avoid compaction.
- b. Plant ground covers that are compatible with native tree succession.
- c. Plant early-establishing native tree species as well as later successional tree species (Angel *et al.* 2005).

ARRI methods have been shown to be highly successful in increasing native plant growth and diversity (Angel *et al.* 2005; Groninger *et al.* 2007). While ARRI methods create an above-ground plant community that is more similar to pre-disturbed conditions, there are few studies on below-ground community composition and succession, especially within the Appalachian region (Frouz *et al.* 2008; Helingerova *et al.* 2010). Most studies looking at nematodes as a proxy for ecosystem function do not identify nematodes beyond their trophic group.

Below-ground and above-ground communities are intrinsically linked and the restoration of below-ground communities is vital if site function is to be restored (Table 1). Though the two communities are interdependent, they do not necessarily mirror each other in structure, rates of

succession, or complexity (Kardol & Wardle 2010). Therefore, a diverse plant community may lack a functioning below-ground component. With below and above-ground systems so intrinsically linked, it is unlikely that above-ground succession will continue on a path similar to that of an intact system without similarly functioning below-ground dynamics and food webs (van der Putten *et al.* 2009). Secondly, there are numerous other ecosystem services performed by soil communities that may be lost or diminished from post-mining sites.

Table 1: Microfaunal and microfloral above and below-ground interactions.

Interactions	References
<i>Impacts of below-ground communities on above-ground flora</i>	
Dispersal of plant pathogens by soil mesofauna	(Van der Putten 2001)
Root grazers impact on above-ground terpenoid production	(Van der Putten 2001)
Nematode parasitism of plant root grazers	(Van Tol 2001, Reynolds 2003, De Deyn 2004)
Above-ground nutrient translocation due to below-ground grazing	(Stanton 1988, Ingham 1985)
Increase of ammonium nitrate due to nematode diversity	(Ingham 1985, Villenave 2004)
Soil mesofauna contributes up to 30% of N mineralization	(Verhoef and Brussaard 1990)
Soil mesofauna diversity increases seedling nutrient status, biomass	(Laakso & Setälä 1999)
<i>Impacts of above-ground flora on below-ground communities</i>	
Plant root exudate impacts rhizosphere microbial community	(Verhoef and Brussaard 1990, Villenave 2004)
Plants create spatial variations in microbial populations	(Yeates 1999, Miniaci 2007)
Certain root exudates may select for beneficial microbes beneficial to plant growth	(De Deyn 2004, Yeates 2007)
Plants alter microbial communities, making soils hospitable to other soil fauna and plants	(Yeates 1999, Kardol 2007)
Plant litter quality impacts microbial growth and mesofaunal communities	(Yeates 1999)

Impacts of nematodes on above and below-ground dynamics

Nematodes are early colonizers of disturbed sites, and colonization has been seen in coal-spoils in about 20 days (Wanner 1998). Nematodes are ubiquitous in the soil environment, with abundances reaching up to $2.9 \times 10^7/\text{m}^2$ in a mixed hardwood forest type (Sohlenius 1980). Nematodes typically appear earlier than larger mesofauna species such as earthworms (Hutle 2001).

Nematodes are the most abundant metazoans in the soil (Bernard 1991) and have roles at numerous trophic levels. All nematodes feed on living organisms; they may be root parasites, fungal grazers, bacterial feeders, or predators of other soil fauna. Some species are omnivores, and trophic groups may vary based on age group. For instance, some mononchid nematodes feed on bacteria in early juvenile stages, and later become predators on other nematodes as they mature.

Free-living soil-dwelling nematodes play important roles in nutrient dynamics by consuming soil fauna and microflora, expediting nutrient transfer within soil systems into plant-available forms (Clarholm 1985). Several studies have shown that a high diversity in soil microfauna, such as nematodes and Collembola, increases seedling survival and plant growth in nutrient-limited sites by increasing ammonium in soils, and in turn increasing the nitrogen content of plants (Ingham *et al.* 1985). Therefore, a diverse and abundant soil biotic system should increase plant growth and survival in nutrient-limited environments, such as surface coal mine restoration sites.

Following initial disturbances in soil, bacteria are generally the first organisms to respond, followed soon after by fungi and nematodes. Early successional nematodes are opportunistic feeders, typically bacterivores, but in certain conditions plant parasites may arrive

immediately following the first plants (Bernard 1991). Later successional trophic groups typically include plant root feeders and predators, including the long-lived omnivorous *Dorylaimida* (Bongers 1990). Some species are sensitive to site traits, such as soil moisture, pH or soil chemistry, and nematodes frequently have been used as indicators of heavy metal contamination.

Nematodes are useful bio-indicators in several ways. Their semi-permeable cuticle is hygroscopic and ensures direct contact with chemicals dissolved in soil-water (Bongers 1990). Some species of nematodes can live for over a year, while others live only a few days, making dating of disturbances possible (Schloter *et al.* 2003). Life-cycle length is closely correlated with R- and K-reproductive strategies. Some species appear early successional while others only appear in a more developed system, and can be used easily as an indicator of site quality and development following disturbance succession (Bongers 1990). Additionally, the structure of a nematode assemblage is indicative of the abundance and variety of their food sources, which can lead to hypotheses about other soil quality traits such as bacterial, root, or fungal biomass (Sanchez-Moreno *et al.* 2008).

The maturity index was created as an approach for using various life strategies of nematode species to value nematode families according to several life characteristics using a scale referred to as the colonizer-persister scale (c-p scale). These characteristics include: presence or absence of dauer lifestage, reproduction rate, length of life cycle, gonad: body ratio, ecological amplitude, egg size, and placement within the food web. Nematode families that are true r-strategists, respond rapidly after disturbance, and are poor competitors are given a c-p value as low as to 1, while k-strategists with longer lives, niche specificity, and more complex food web interactions may have c-p value of up to 5 (Bongers 1990; Ferris & Bongers 2006).

It is clear that nematode assemblages play important roles in below-ground communities, and can increase nitrogen uptake by hardwoods (a limited nutrient in sites with low soil organic matter inputs), as well as act as indicators of soil qualities and soil communities in sites that have thus far not been measured or established. However, no study has established the utility of nematode communities to indicate below-ground soil food web health, or as a biological remediation tool to increase native plant growth in post-mining sites. Additionally, restoration techniques have failed to integrate recent studies that show the positive effects soil organisms can have on native succession (Kardol 2007).

This study has the following objectives:

- a. Compare below-ground assemblages of sites that use various remediation techniques in order to understand which remediation techniques increase the similarity of communities post-mining to intact systems;*
- b. Develop index of morphospecies indicative of specific site traits, including age of site, forested sites, and disturbed sites;*
- c. Establish the contribution of soil-dwelling nematodes as soil bio-remediators to increase native seedling survival in post-mining soils.*

Chapter 2

Methods

Three mines were selected that had used both the Appalachian Restoration Reforestation Initiative methods and the post-Surface Mining Control and Reclamation Act methods between one and three years ago, four and eight years ago, and between nine and fifteen years ago. Mine sites were selected for study in Kentucky, Tennessee, and Virginia.

Kentucky Mine site description:

The Kentucky mine is located on Bent Mountain and operated by Appalachian Fuels (Latitude N 37° 35' 49", Longitude W 82°24' 19"). Bent Mountain is located near the community of Meta on the Cumberland Plateau. The predominant soil order is ultisols (USDA 1998). The predominant geology composition, in order of largest component, is sandstone, shale, siltstone and coal (Wolcott & Jenkins 1966). Average annual temperature is 13° C. Average annual precipitation is 114 cm.

Virginia mine site description:

The Powell River Project Education Center is a mining reclamation research facility located near Wise, VA (Latitude 37 °01'92", Longitude W 82°52'99"). The site is comprised of sandstone, shale, siltstone and coal (Daniels & Amos 1985). The site's average annual temperature is 11.7° C. Average annual precipitation is 118 cm.

Tennessee mine site description:

The Tennessee mine is located near Jellico, TN(Latitude 36°31'59", Longitude W 83°59'56"). The predominant geology is sandstone and sandy shale. The site's average annual temperature is 13.6. Average annual precipitation is 130 cm.

Objective 1 and 2 methods

Three age groups were sampled in each restoration treatment at all mines: 1-3 years since restoration, 4-9 years since restoration, and 10 years or more since restoration. Samples from nearby intact forest sites were collected as well (Table 2). Forest sites were selected without any obvious disturbance such as cut tree stumps or logging roads. Field data collected at each plot included dominant plant species, slope, and distance to intact forest.

Twenty-meter transects were laid through each plot along a random compass bearing. Soil was collected at five points every five meters along the transect. Four subsamples were taken at each sampling point in order to decrease microsite variation due to the tendency of nematodes to pool in high numbers around a food source. At each sampling point, four subsamples were collected 30 cm away from the center, and 90 degrees from each subsample. Soil was collected with a shovel to a depth of 15 cm, or until rock limited soil depth. Subsamples for each point were bulked, resulting in five samples per plot. Soils were transported on ice to the University of Tennessee and stored at 4° C until extraction within 72 hours.

Distance from plots to intact forest was measured using a distance tape. Site slope was measured using a clinometer. Plant species data was collected by identifying cover plant species at each soil collection point along the transect.

Soil pH was measured in the lab. Soil was air dried for one week. Five grams of air-dried soils was added to ten ml deionized water and mixed thoroughly, and pH probe used to measure pH (Eutech Instruments, Vernon Hills, IL).

Nematodes were extracted with a sugar flotation-centrifugation method (Jenkins 1964) by filtering nematode soils through a 177- μm -pore sieve, and collecting them on a 38- μm -pore sieve. Sieved material was mixed with a sucrose solution (454 g table sugar in 1 liter of water) and centrifuged at 400 $\times$ g to separate nematodes from soil. Nematodes were killed and fixed with hot formalin and preserved in glycerin (Seinhorst 1959). Nematodes were permanently slide-

Table 2: Sampling design of the field study, where FRA is Forestry Reclamation approach and SMCRA is Surface Mining Control and Reclamation Act.

<i>State</i>	<i>Plot</i>	<i>Reclamation technique</i>	<i>Years since mining</i>	<i>Number of samples</i>
TN	1	FRA	1	5
	2	FRA	9	5
	3	FRA	15	5
	4	SMCRA	1	5
	5	SMCRA	9	5
	6	SMCRA	15	5
	7	Unmined Forest	not mined	5
KY	1	FRA	1	5
	2	FRA	6	5
	3	FRA	12	5
	4	SMCRA	2	5
	5	SMCRA	5	5
	6	SMCRA	10	5
	7	Unmined Forest	not mined	5
VA	1	FRA	2	5
	2	FRA	4	5
	3	FRA	10	5
	4	SMCRA	2	5
	5	SMCRA	4	5
	6	SMCRA	10	5
	7	Unmined Forest	not mined	5

mounted in glycerin and identified to morphospecies, with trophic group, genus, and species determined when possible.

Shannon diversity index, total morphospecies abundance, trophic abundance, and maturity index were calculated. Multiple analysis of variance (MANOVA) was performed to analyze effects of block, reclamation, and age on total nematode abundance, morphospecies diversity, morphospecies richness, and trophic abundance using a general linear mixed model. Within blocks, ANOVAs were performed to determine effects on dependent variables. Tukey's test was used to measure statistical differences at a confidence level of $P = 0.05$.

The maturity index was calculated, $\sum v(i)f(i)$, as defined by Bongers (1990) where $v(i)$ is the colonizer-persister value assigned by to the family, and $f(i)$ is the frequency of the family within a sample.

Ecological interactions were analyzed with canonical correspondence analysis (CCA) (Canoco, Plant Research International Wageningen, Netherlands). Factors analyzed included management technique (FRA, Un-mined forest, and SMCRA plots), site age, nematode community measures, and morphospecies identity.

Objective 3 methods

Soils from the Tennessee site were used for the greenhouse study. A full factorial design was used, with each soil type having each nematode community added to defaunated soils. Two controls were used as well: an intact soil which was not defaunated, nor were nematodes added, and a defaunated soil that did not have any nematodes added back. Each manipulation was replicated five times (Table 3).

Table 3: Full factorial design of objective two in which each soil type received each nematode community treatment and was replicated five times.

<i>Soil Type</i>		<i>Nematode Community</i>
1 year old SMCRA		1 year old SMCRA
1 Year old FRA		1 Year old FRA
15 Year old SMCRA	×	15 Year old SMCRA
15 Year old FRA		15 Year old FRA
Unmined Forest		Unmined forest
		Intact control
		Defaunated control

Soils for the greenhouse study were collected from the Tennessee mine in the 1-year-old FRA site, 1-year-old SMCRA site, 15-year-old FRA site, 15-year-old SMCRA site, and intact forest site. Approximately 20 kg of soils were collected to a depth of 15 cm. Soils were transported on ice to the University of Tennessee where they were stored at 4° C until processing, which occurred within 48 hours.

Soils for the greenhouse study were defaunated by cooling to -15° C for 8 hours then heating to 50° C for 4 hours. This method limited impacts on bacterial community, which can occur at -20° C (J. Debruyne, personal communication) while limiting nitrogen volatilization which occurs above 64° C. After treatment, no viable nematodes were observed in extracted soils prior to the beginning of the greenhouse study. Six hundred grams of each defaunated soil was placed in 500-ml size D-40 cone-tainers (Stuewe and Sons, Tangent, OR). Nematodes were extracted from an equivalent volume of soil, with the sugar extraction method mentioned previously, and kept in suspension in water by constant mixing. This inoculum was added to their assigned soils, excluding the intact soil control and defaunated control. To each control, an equivalent amount of nematode-free water was added.

In order to add any microbial species that may have been killed in the defaunation process, for each soil type, microbial inoculum was added by placing 100 g of non-defaunated soil on a 38- μ m-pore sieve and rinsing the soil with 100 ml tap water, in order to extract microbes but exclude nematodes. The rinse water was collected in a beaker, then added equally to each Cone-tainer. This method was repeated for each soil type.

Seeds of *Cercis canadensis* L. (Eastern redbud), a non-nitrogen-fixing tree species common in early successional hardwood forests, were obtained (Sheffield Seed, Locke, NY). Seeds were stratified in boiling water and soaked for 24 hours, then placed in moist peat moss in a sealed plastic bag and stratified at 4° C for 30 days. The stratified seeds were then moved to trays of vermiculite in the greenhouse and allowed to germinate. Seedlings were grown to the two true-leaf stage (approximately three weeks), then one seedling was planted in each Cone-tainer. After fourteen weeks, seedlings were harvested and cut above the root collar. Plants were dried in a 60° C oven for 72 hours (Cornelissen, 2003) and weighed to the nearest 0.01 g.

Above-ground biomass and intact control soils were assessed for carbon and nitrogen. Dried leaves were collected and ground for analysis with using a PowerGen high through-put homogenizer (Fisher scientific). Samples were tested for total carbon and nitrogen using a Flash 2000 Combustion CHNS/O analyzer (CE Elantech Lakewood, NJ).

Multiple analyses of variance were used to measure impact of nematode community, soil treatment, soil age, and interactions between these independent variables on plant biomass (above and below), leaf nitrogen content, and leaf C:N ratio. Tukey's test was used to measure statistical differences at a confidence level of $P=0.05$.

Chapter 3

Results

A total of 7,264 individual nematodes across all blocks were identified to morphospecies: 2,100 from Virginia plots, 3,164 from Tennessee plots, and 2,000 from Kentucky. Tennessee had the largest number of identified morphospecies with 117, followed by 79 in Kentucky and 76 in Virginia. A total of 156 morphospecies were observed (Table 4, Appendix).

Nematode total abundance among soil treatments and over time were not significantly different, although young Surface Mining Control Act (SMCRA) soil had the lowest average abundances in all three states. Total abundances were highly variable over time (Fig. 1). The Kentucky block had lower total abundances than either the Tennessee or Virginia blocks at the same age and treatment levels. Unmined Kentucky forests averaged 40 nematodes per 100 cm³ of soil, while Tennessee and Virginia forests average 151 and 150 nematodes per 100 cm³ sample, respectively.

Trophic Abundance

Younger sites were dominated by bacterivores, dominating total abundance in young sites across all blocks. Bacterial abundance was similar between Forestry Reclamation Approach (FRA) and un-mined forest plots in Kentucky, with Surface Mine Control and Reclamation Act (SMCRA) plots containing fewer bacterial nematode species. Additionally, bacterivore abundance increased with significantly age in all sites (Fig. 2).

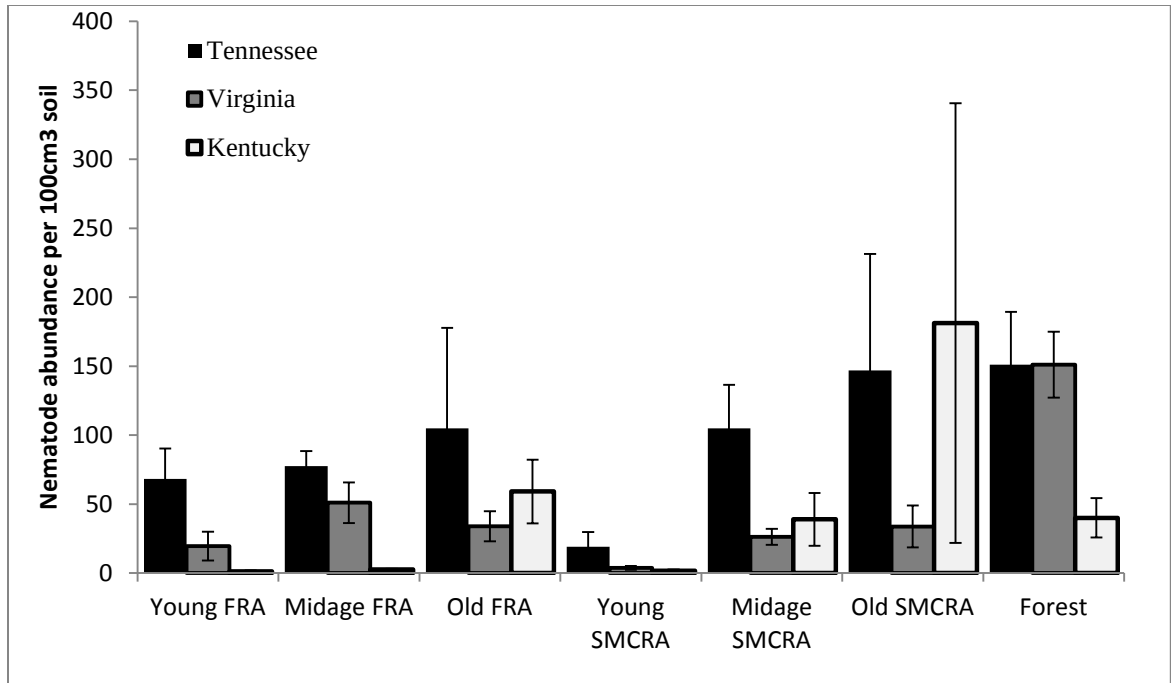


Fig. 1: Nematode abundance in Forestry Reclamation Approach (FRA) soils and Surface Mine Control and Reclamation Act soils (SMCRA) over a chronosequence, as well as an un-mined forest soil control. Bars represent mean values $\pm$ standard error.

Plant parasites increased with time since reclamation in both the Tennessee and Kentucky sites. Predator abundance was greatest in intact forest as well as mid-aged treatments in Tennessee, and lowest in abundance in both young and older sites. Fungivore and omnivore nematode abundance was not affected by treatment or age.

Richness

Average species richness was highest in Tennessee (mean value of 19.4 morphospecies, across all treatments), with a similar species richness in both Kentucky (mean value of 11.7 morphospecies across all treatments) and Virginia (mean 13.2 morphospecies across all treatments) blocks (Fig. 3). The Tennessee un-mined forest site had the highest richness, with 39.6 species on average.

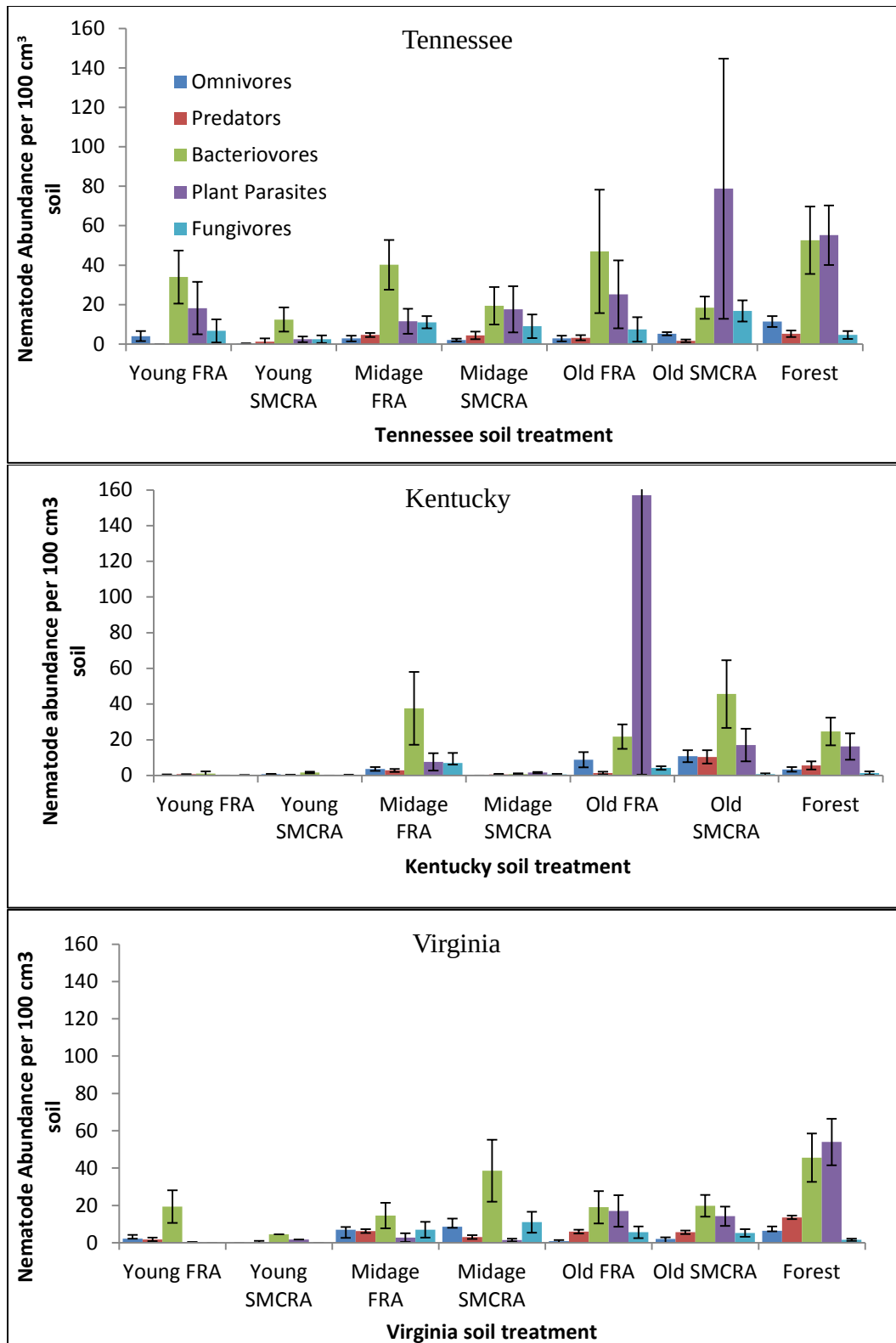


Fig. 2: Trophic abundance among Forestry Reclamation Approach (FRA), Surface Mining control and Reclamation Act (SMCRA), and forest soils based on block (TN, VA, and KY). Bars represent mean values $\pm$ standard error.

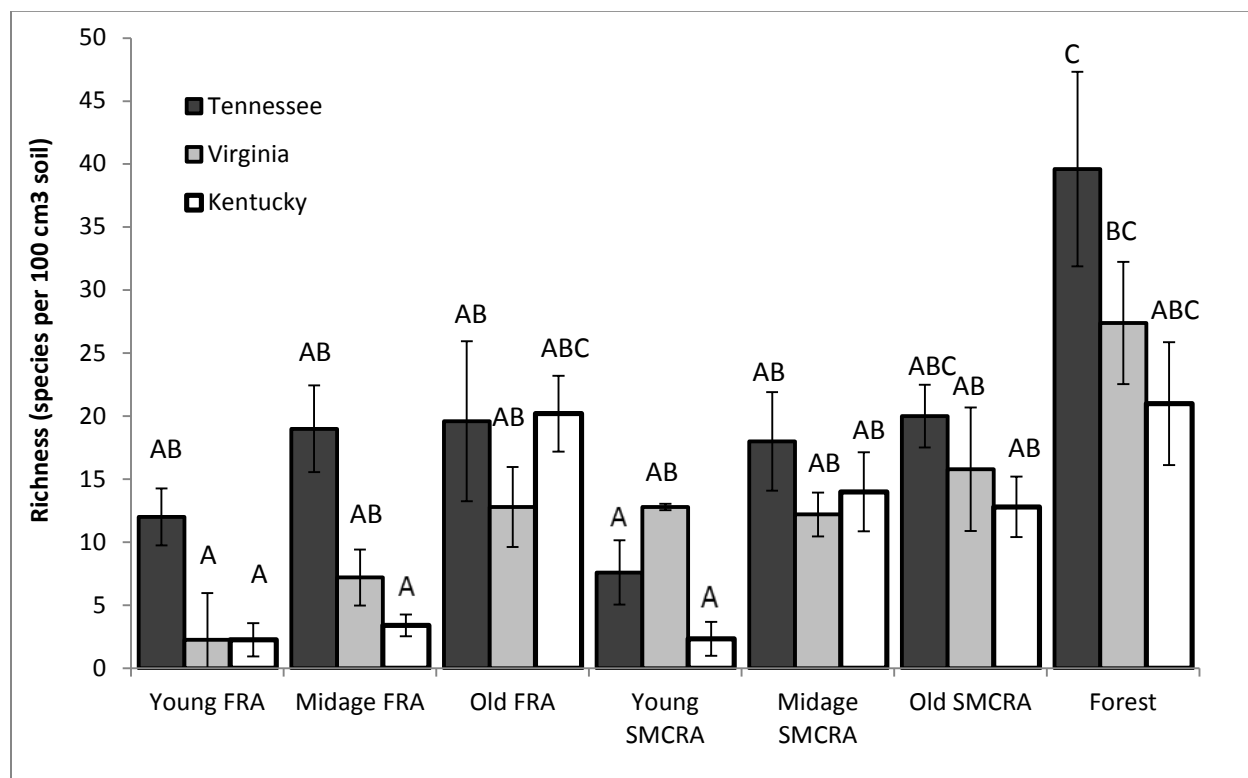


Fig. 3: Nematode richness in Forestry Reclamation Approach (FRA) soils, and Surface Mining Control and Reclamation Act soils (SMCRA) over a chronosequence, as well as an un-mined forest control. Bars represent mean values $\pm$ standard error. Means with different letters are significantly different according to a Tukey's test at $P=0.05$

Species richness was statistically similar between treatment groups of the same age in Virginia and Tennessee, however, the middle age SMCRA plot in Kentucky had similar richness to the un-mined forest, while middle age FRA site had lower richness than un-mined forest (Fig. 4).

Bacteria-feeding nematode richness increased across all states with age, while management technique had no effect on bacterial-feeder richness (Fig. 5). Plant parasites species richness increased with time in Tennessee plots. Predator species richness was not influenced by management technique or age.

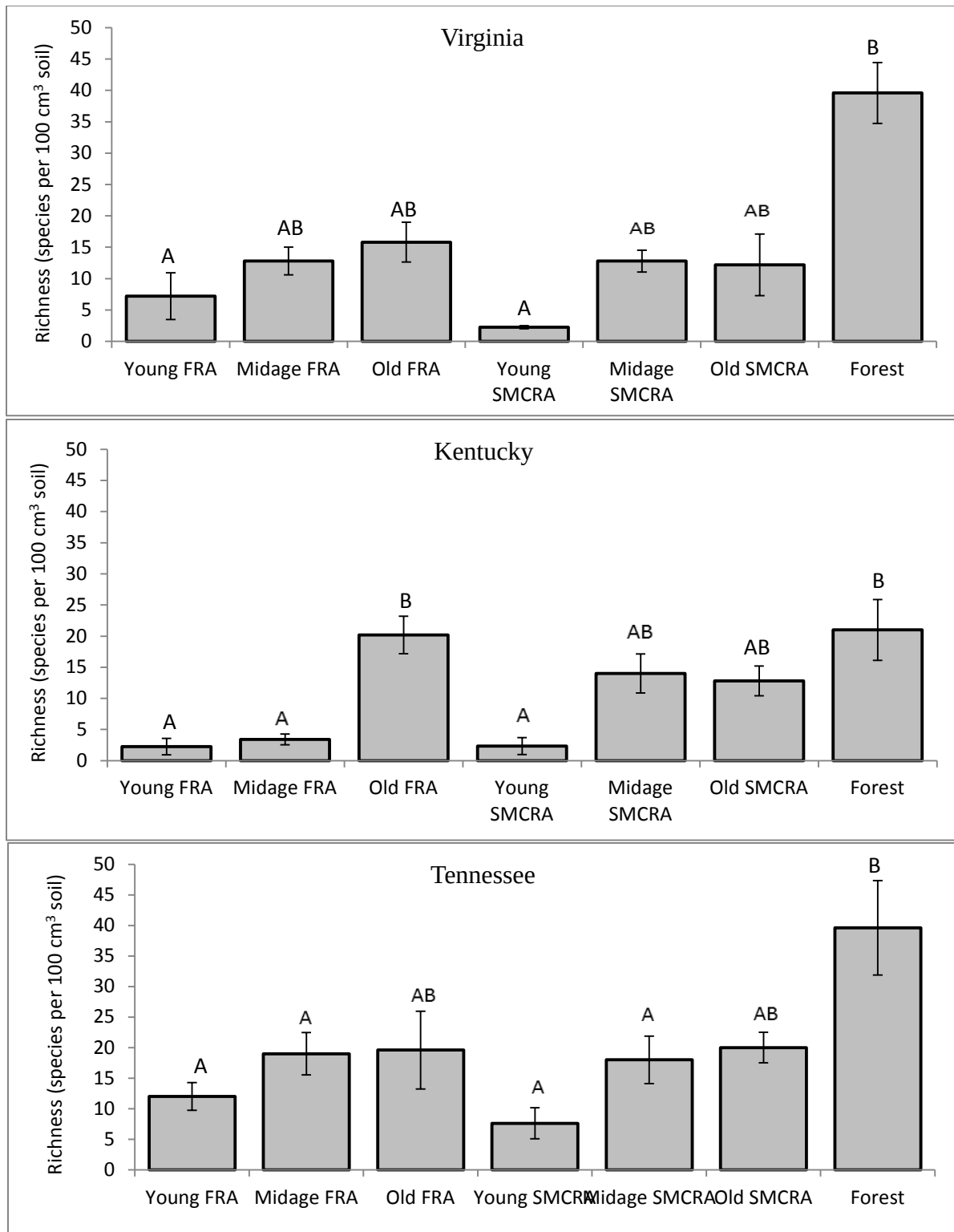


Fig. 4: Morphospecies richness among Forestry Reclamation Approach (FRA), Surface Mining Control and Reclamation Act (SMCRA), and un-mined soils based on block (TN, VA, and KY). Bars represent mean values $\pm$ standard error. Means with different letters are significantly different according to a Tukey's test at $P=0.05$

Omnivore richness was lowest in youngest sites averaging less than one omnivorous species per sample in the young SMCRA and FRA plots in all blocks, excluding the young FRA Tennessee site, with an average richness of 1.6 morphospecies per sample. Omnivores increased in number in older and un-mined plots. Fungivore richness was not influenced by management technique or age, but tended to be low in all plots across all blocks, as compared with richness levels of other feeding groups.

Shannon Diversity

Diversity ranged from 0.62 (young FRA Kentucky site) to 3.13 (un-mined Tennessee forest) (Fig. 6). Average Shannon diversity increased over time in both FRA and SMCRA treatments across all blocks (Figs. 7, 8 & 9). Old FRA and un-mined forest plots were statistically similar in Virginia. Older FRA plots across all blocks had higher average Shannon diversities than their corresponding SMCRA plots. Shannon diversity of older FRA and both older SMCRA plots and middle aged SMCRA plots were similar to un-mined forests in Kentucky.

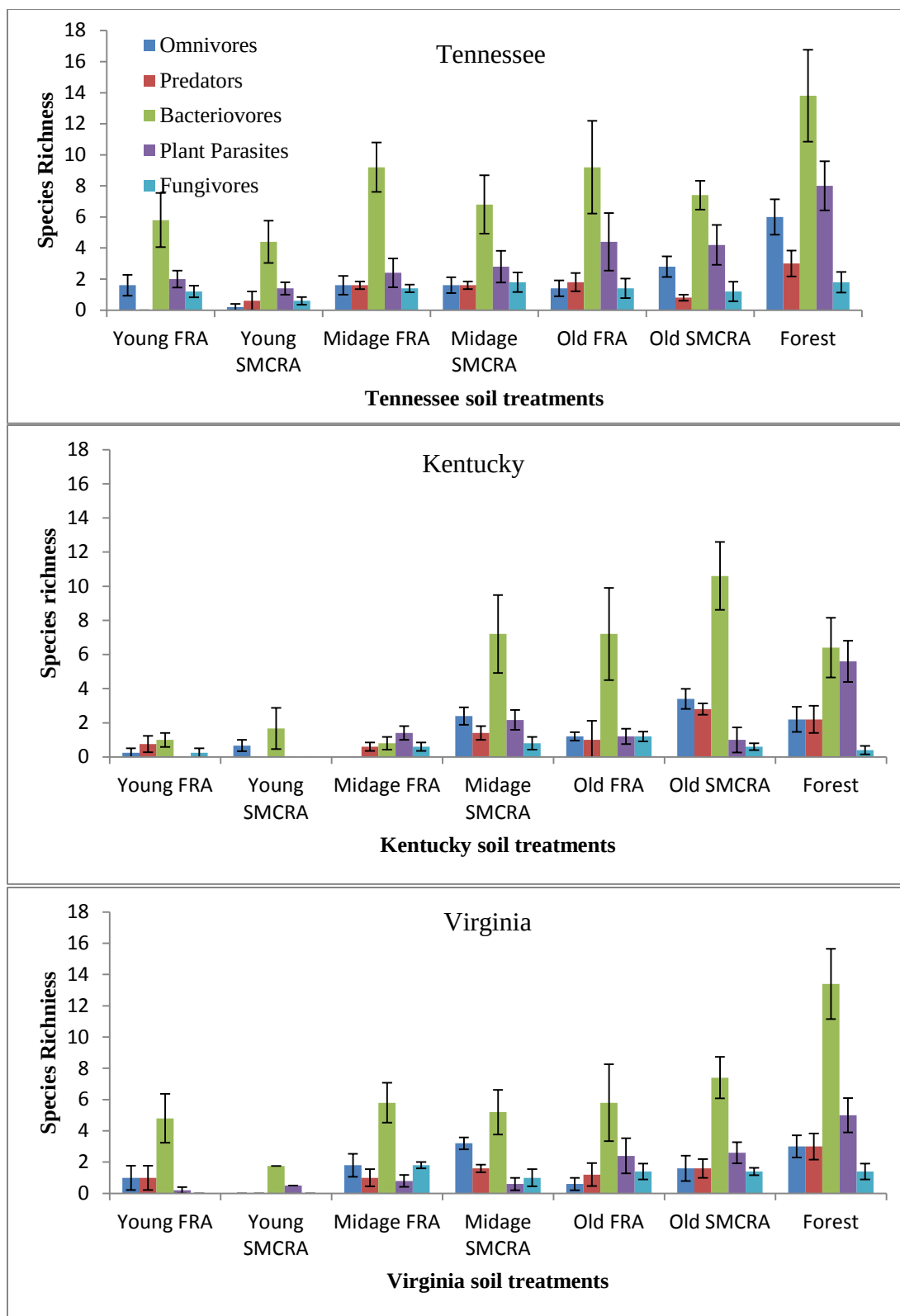


Fig. 5: Trophic richness among Forestry Reclamation Approach (FRA), Surface Mining Control and Reclamation Act (SMCRA), and forest soils based on block (TN, VA, and KY). Bars represent mean values $\pm$ standard error. Means with different letters are significantly different according to a Tukey's test at $P=0.05$

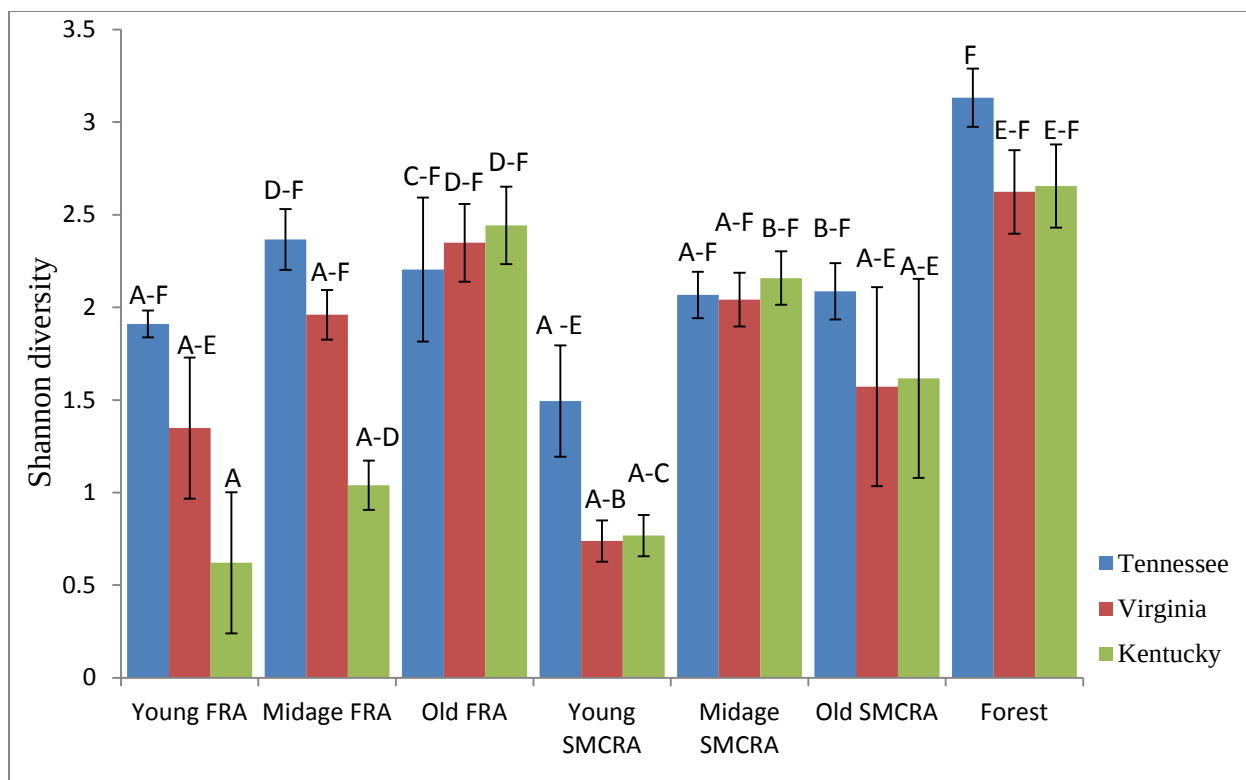


Fig. 6: Shannon Diversity of nematode morphospecies in Forestry Reclamation Approach (FRA) soils, and Surface Mining Control and Reclamation Act soils (SMCRA) over a chronosequence, as well as an un-mined forest control. Bars represent mean values $\pm$ standard error. Means with different letters are significantly different according to a Tukey's test at $P=0.05$

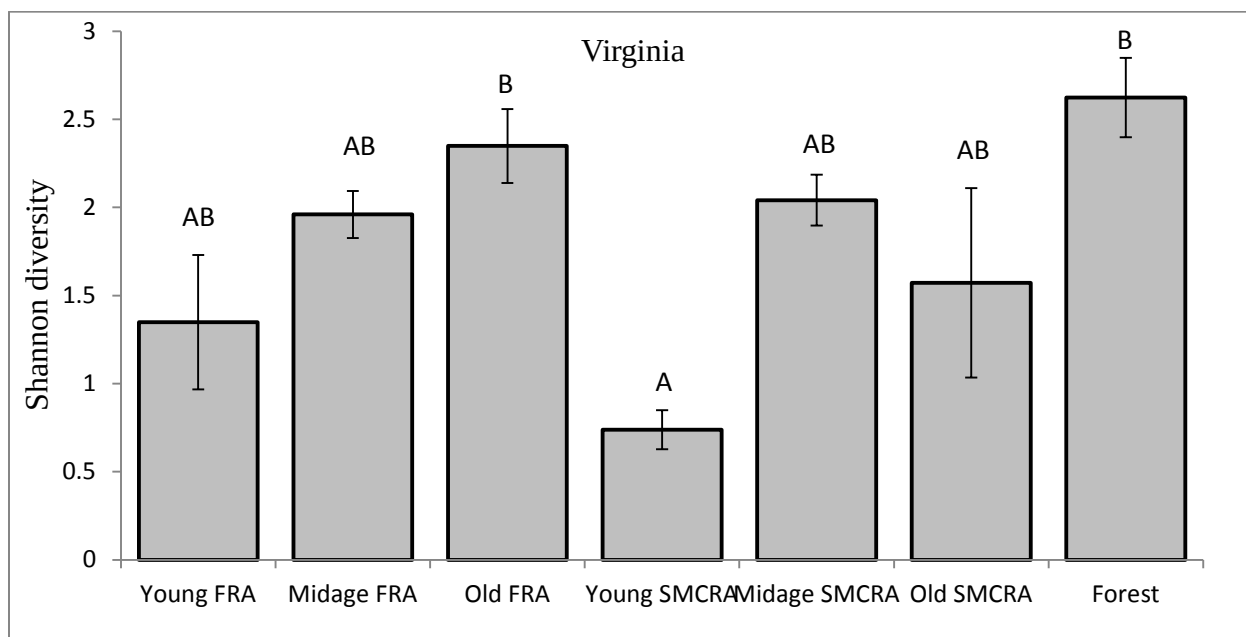


Fig. 7 Shannon diversity of nematodes among Forestry Reclamation Approach (FRA), Surface Mining Control and Reclamation soils (SMCRA), and intact forest soils in Virginia. Bars represent mean values $\pm$ standard error. Means with different letters are significantly different according to a Tukey's test at $P=0.05$.

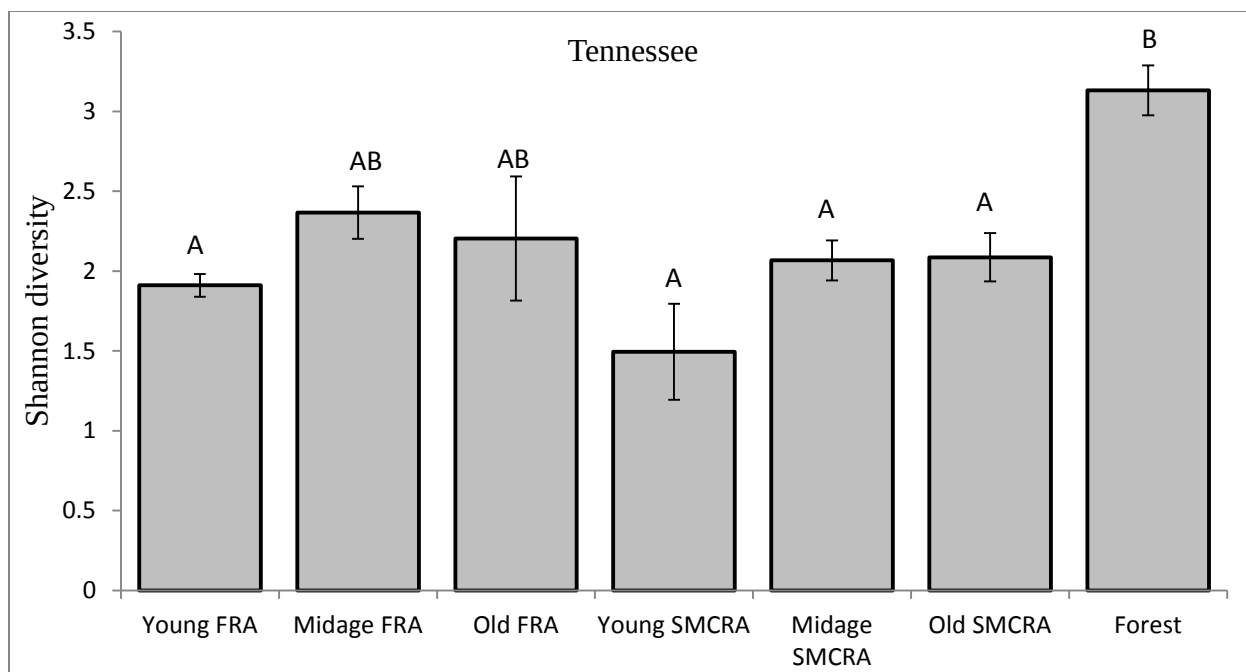


Fig. 8: Shannon Diversity of nematodes among Forestry Reclamation Approach (FRA), Surface Mining Control and Reclamation Act soils, and intact forest soils in Tennessee. Bars represent mean values $\pm$ standard error. Means with different letters are significantly different according to a Tukey's test at $P=0.05$.

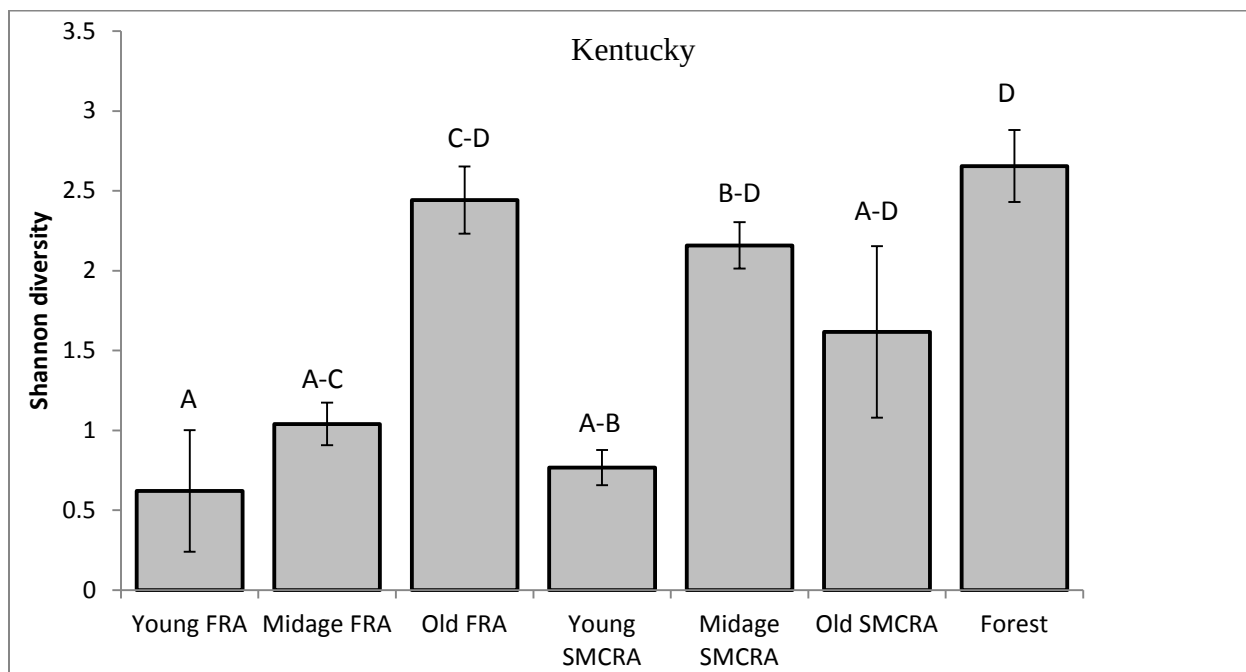


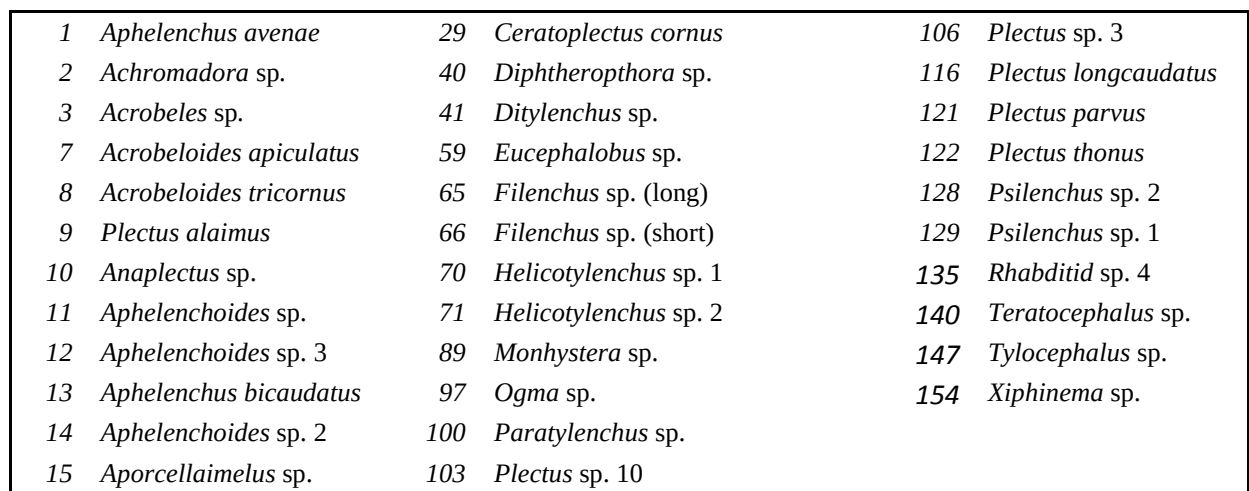
Fig. 9: Shannon Diversity of nematodes among forestry Reclamation Approach soils(FRA), Surface Mining Control and Reclamation Act soils (SMCRA), and intact forest soils in Kentucky. Bars represent mean values $\pm$ standard error. Means with different letters are significantly different according to a Tukey's test at $P=0.05$.

Numerous interactions were detected by means of canonical correspondence analysis among nematode morphospecies and environmental traits (Fig. 10). Shannon diversity and richness were positively associated with intact forest plots and FRA plots. Plot slope and SMCRA sites were negatively associated with Shannon diversity and richness. Several nematode morphospecies were found to be indicative of intact forest based on proximity (Table 5). Additionally, several morphospecies were associated with SMCRA sites and negatively associated with un-mined forest sites are described (Table 6).

All blocks had lower abundance and richness of forest indicator species in disturbed sites under both management treatments (excluding older FRA Kentucky sites) (Fig. 11). Tennessee forests had no *Xiphinema* sp. present. In Virginia sites, *Xiphinema* species were the first indicator species to respond after disturbance. Richness of forest indicator species began to increase in the older disturbed sites. Kentucky plots were the only location where nematode indicator species was higher in FRA sites than SMCRA sites. In older FRA sites, *Prismatolaimus*, *Mylonchulus*, and *Teratocephalus* sp. abundance was higher than un-mined forest.

Native plant species had the strongest association with nematode morphospecies (Fig. 12). Native plants that seeded into plots naturally were most strongly associated with Shannon diversity, including *Cornus florida* and *Juglans nigra*. *Eleagnus umbellata*, a non-native invasive, had a negative association with species diversity. Other plant species (Table 6, appendix) were not found to influence nematode species distributions.

Maturity indices across all three sites showed older FRA sites with a higher average value than both forest soils and older SMCRA soils (Fig. 13). The ratio of nematodes with a c-p value



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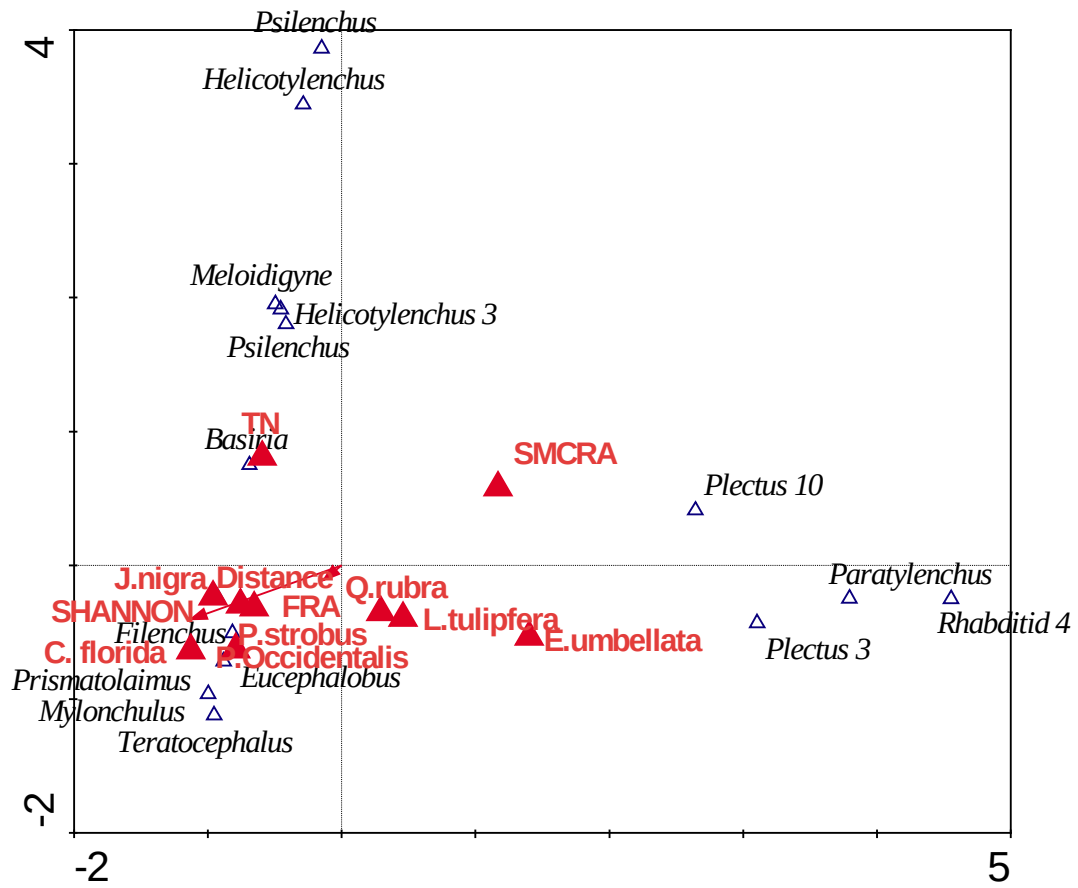


Fig. 11: Canonical correspondence analysis of environmental traits and tree species presence in relation to nematode morphosecies abundance. Axes 1 and 2 are presented. Eigenvalues for axis 1: 0.699; axis 2: 0.480. Monte Carlo test of all axes was significant ($p < 0.005$).

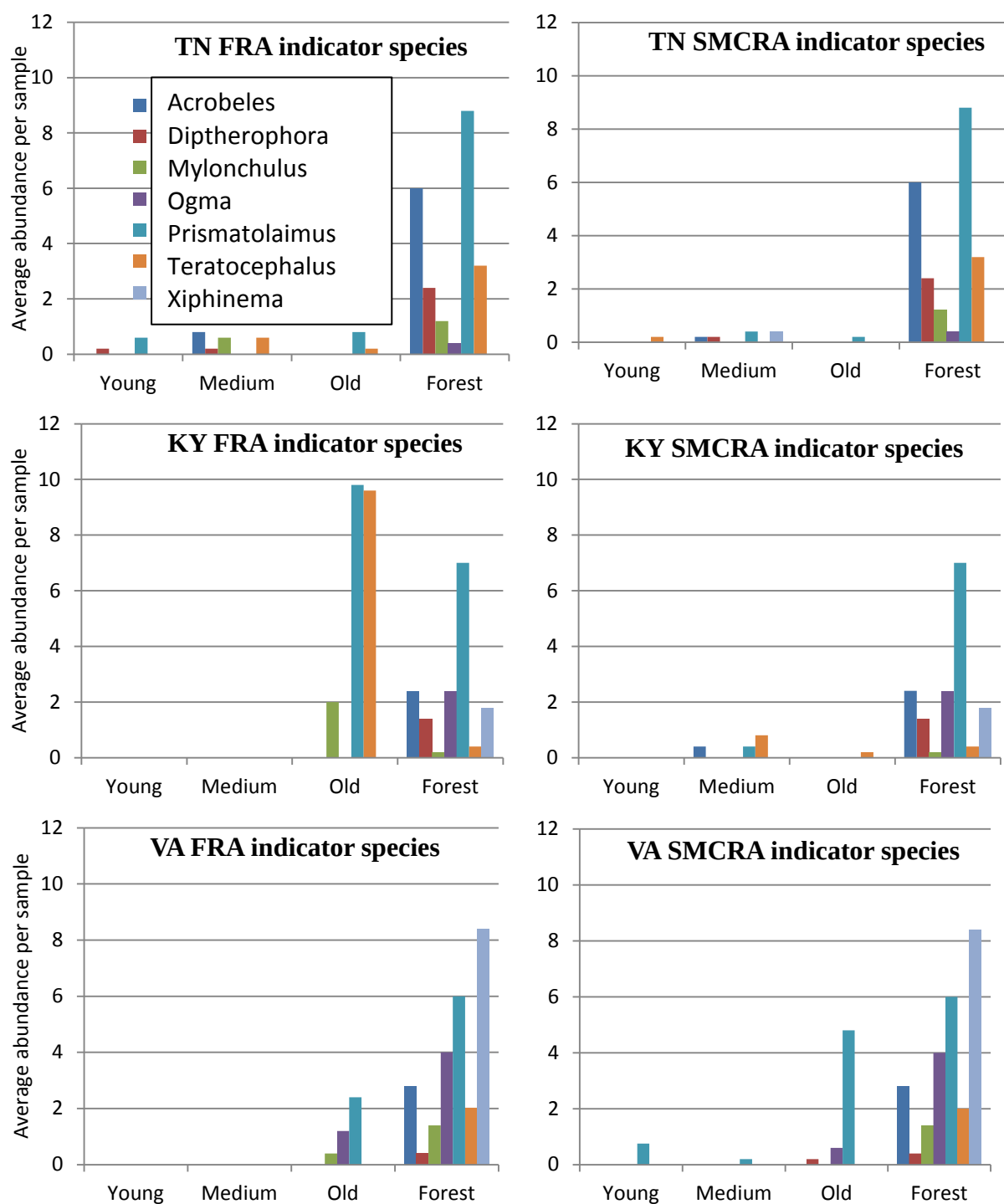


Fig. 12: Nematode species abundance indicative of native forest as they change over time in both Forestry Reclamation Approach management regimes (FRA), Surface mining control and reclamation act management regimes (SMCRA), as compared to un-mined forest sites in Tennessee, Kentucky, and Virginia.

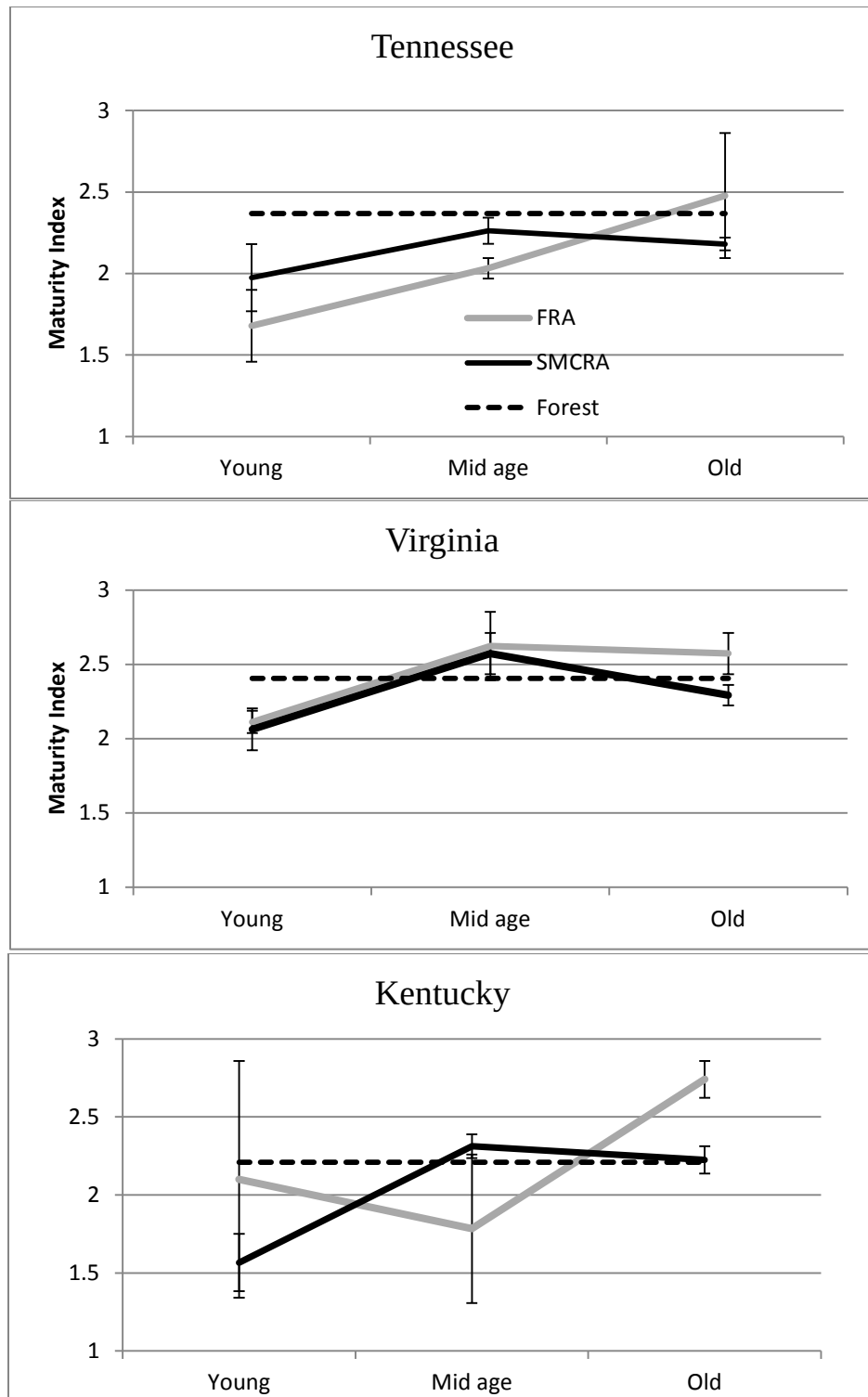


Fig. 13: Maturity index of nematode communities from three mine sites across time based on management technique.

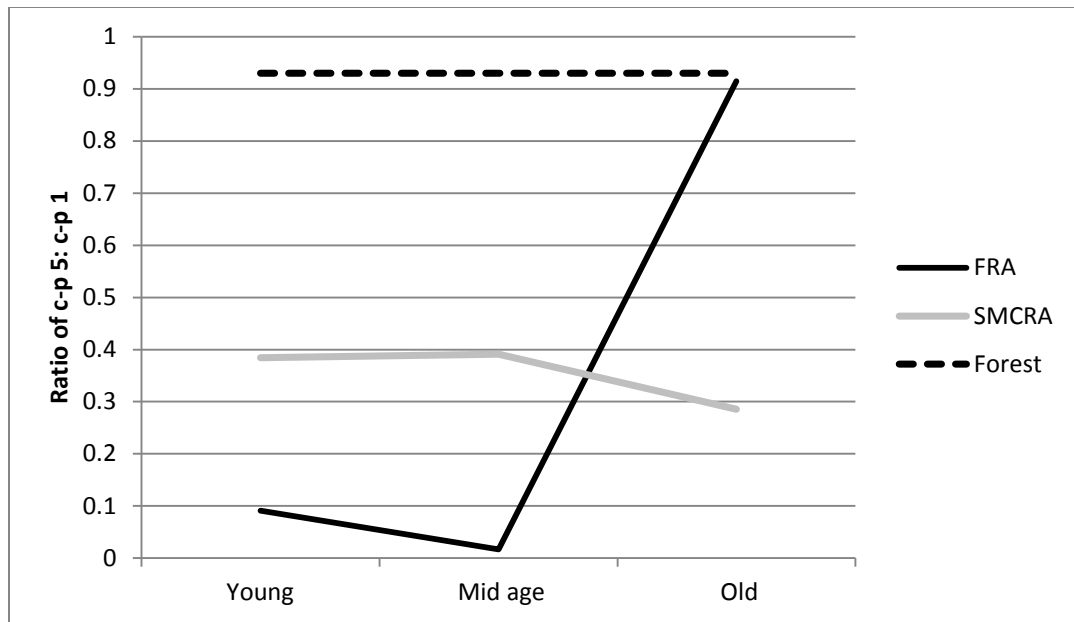


Fig. 14: Ratio of c-p 5 to c-p 1 nematodes within samples based on management treatment across a chronosequence among three location blocks.

Greenhouse Results

Above and below-ground plant biomass of intact soils (control soils that had not been defaunated) was similar for all soils (Fig. 15). Plants grown in intact 15 SMCRA soil and intact forest soil had highest leaf level nitrogen content (Fig. 16), while 1 FRA, 15 FRA, and young SMCRA soils had lower leaf level nitrogen content. Additionally, leaf level C:N content of *C. canadensis* leaves was lowest in forest and old SMCRA soils, but similar to both old FRA soils and young SMCRA soils. Seedlings from intact young FRA soils had the highest leaf level C:N content (Fig. 17).

Above and below-ground plant biomass was similar among all nematode community treatments and control in FRA 1 soils (Fig. 18). Additionally, leaf level nitrogen levels and C:N ratios did not vary based on treatment in FRA 1 soils (Figs. 19,20).

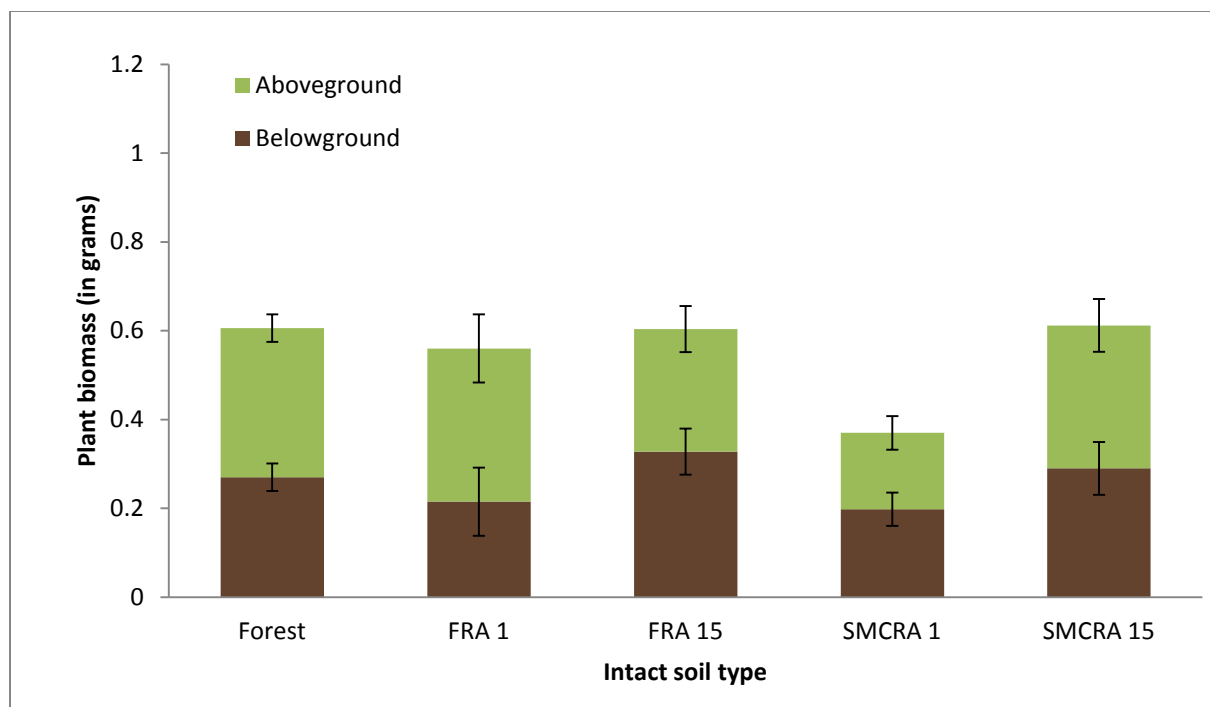


Fig. 15: Above and below-ground plant biomass of *C. canadensis* seedlings grown in intact soils from Tennessee site. Bars represent mean values $\pm$ standard error.

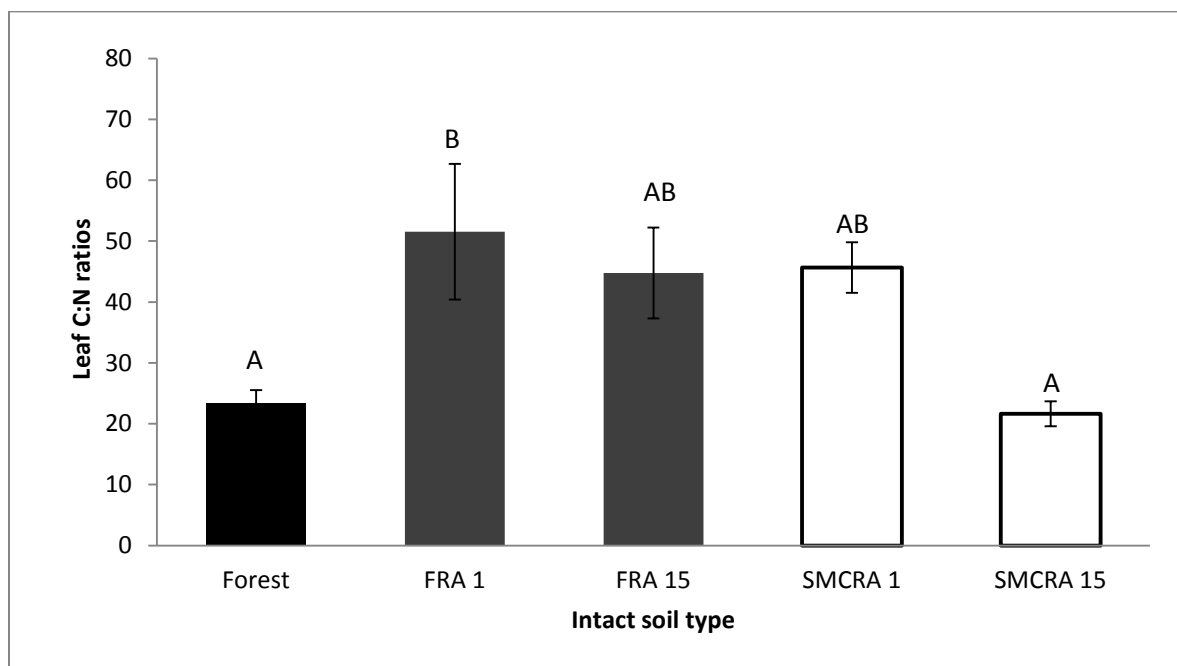


Fig. 16: Leaf Carbon: Nitrogen ratio of *C. canadensis* seedlings grown in intact soils from a Tennessee surface mine and neighboring forest. Bars represent mean values $\pm$ standard error. Means with different letters are significantly different according to a Tukey's test at P=0.05.

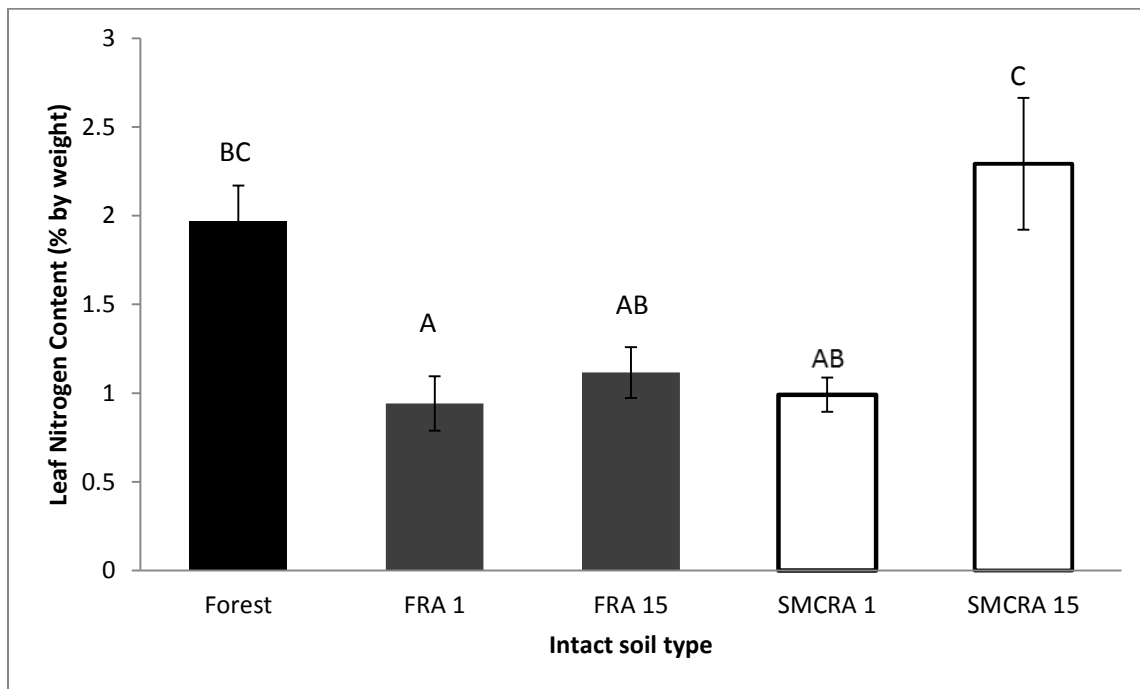


Fig. 17: Leaf Nitrogen content of *C. canadensis* seedlings grown in intact soils from a Tennessee surface mine and neighboring forest. Bars represent mean values $\pm$ standard error. Means with different letters are significantly different according to a Tukey's test at $P=0.05$.

Above and below-ground plant biomass was similar among all nematode community treatments and control in FRA 1 soils (Fig. 18). Additionally, leaf level nitrogen levels and C:N ratios did not vary based on treatment in FRA 1 soils (Figs. 19, 20).

Within SMCRA 1 soils, above and below-ground plant biomass was not influenced by nematode community addition (Fig. 21). Carbon: nitrogen ratios were influenced by nematode community. SMCRA 15 and SMCRA 1 soils had the lowest C:N ratios (38.83 and 40.2, respectively), and the forest nematode addition was the highest (66.32) (Fig. 22). Leaf nitrogen content was not influenced by nematode addition (Fig. 23).

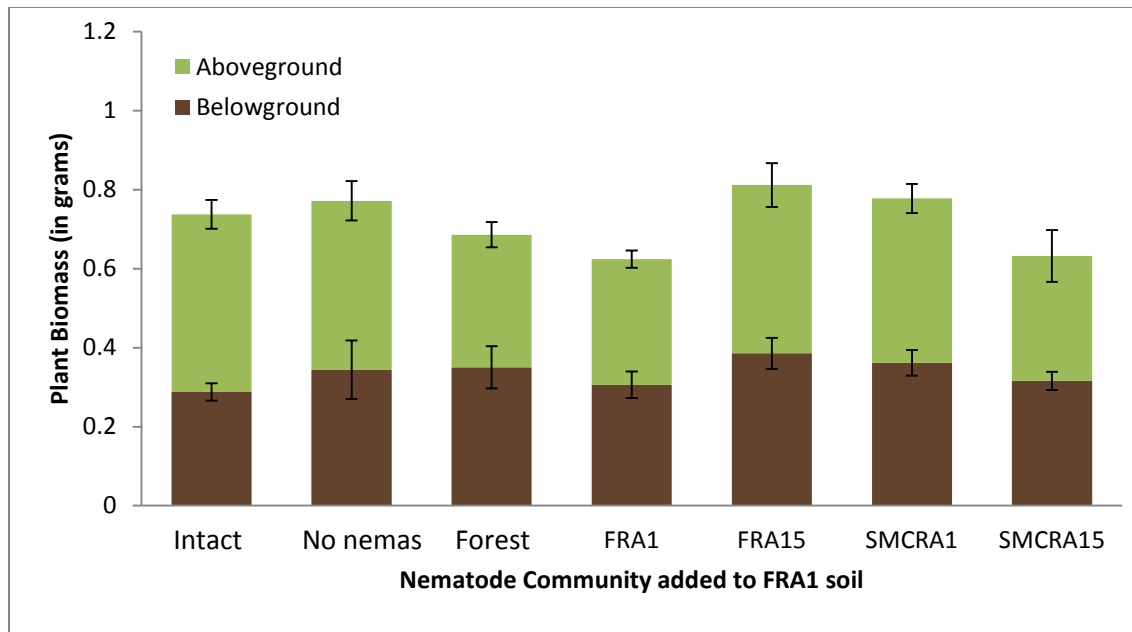


Fig. 18: Above and below-ground plant biomass of *C. canadensis* seedlings grown in soils from a one-year-old Forestry Reclamation Approach with the indicated nematode community added intact control nematodes, defaunated control nematodes, forest nematodes, FRA one- year-old nematode communities, FRA fifteen-year-old communities, Surface Mine Control and Reclamation Act (SMCRA) one-year-old nematode communities, SMCRA fifteen-year-old nematode communities. Bars represent mean values $\pm$ standard error.

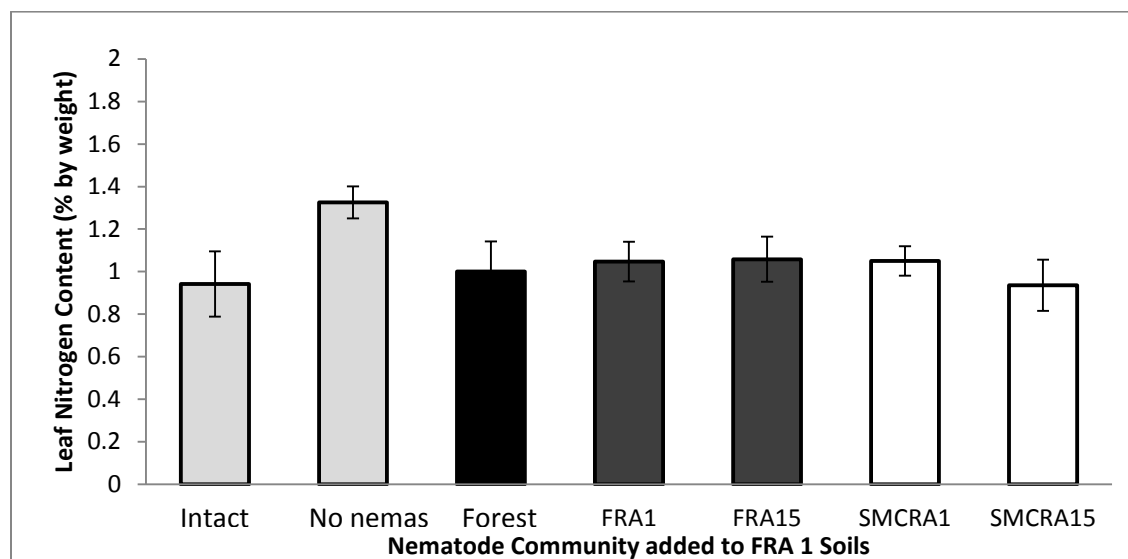


Fig. 19 Leaf nitrogen content of *C. canadensis* seedlings grown in soils from a one-year-old Forestry Reclamation Approach site with the indicated nematode community added: intact control nematodes, defaunated control nematodes, forest nematodes, FRA one- year-old nematode communities, FRA fifteen-year-old communities, Surface Mine Control and Reclamation Act (SMCRA) one-year-old nematode communities, SMCRA fifteen-year-old nematode communities. Bars represent mean values $\pm$ standard error.

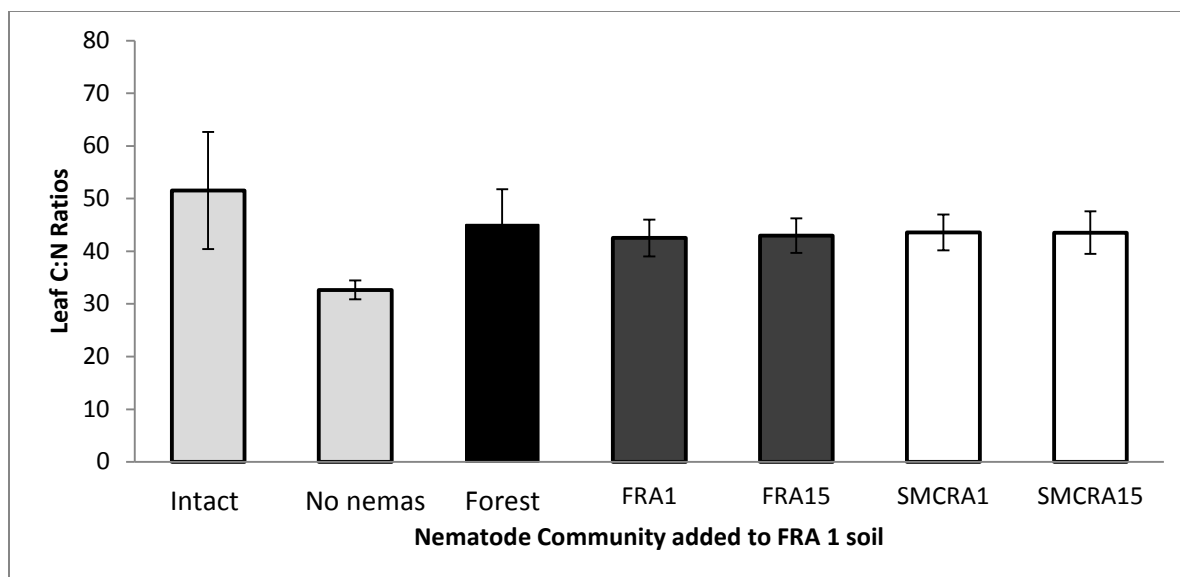


Fig. 20: Leaf carbon:nitrogen ratio of *C. canadensis* seedlings grown in soils from a one-year-old Forestry Reclamation Approach site (FRA) with the indicated nematode community added: intact control soils, defaunated control, forest nematodes, FRA one- year-old nematode communities, FRA fifteen-year-old communities, Surface Mine Control and Reclamation Act (SMCRA) one-year-old nematode communities, SMCRA fifteen-year-old nematode communities. Bars represent mean values $\pm$ standard error.

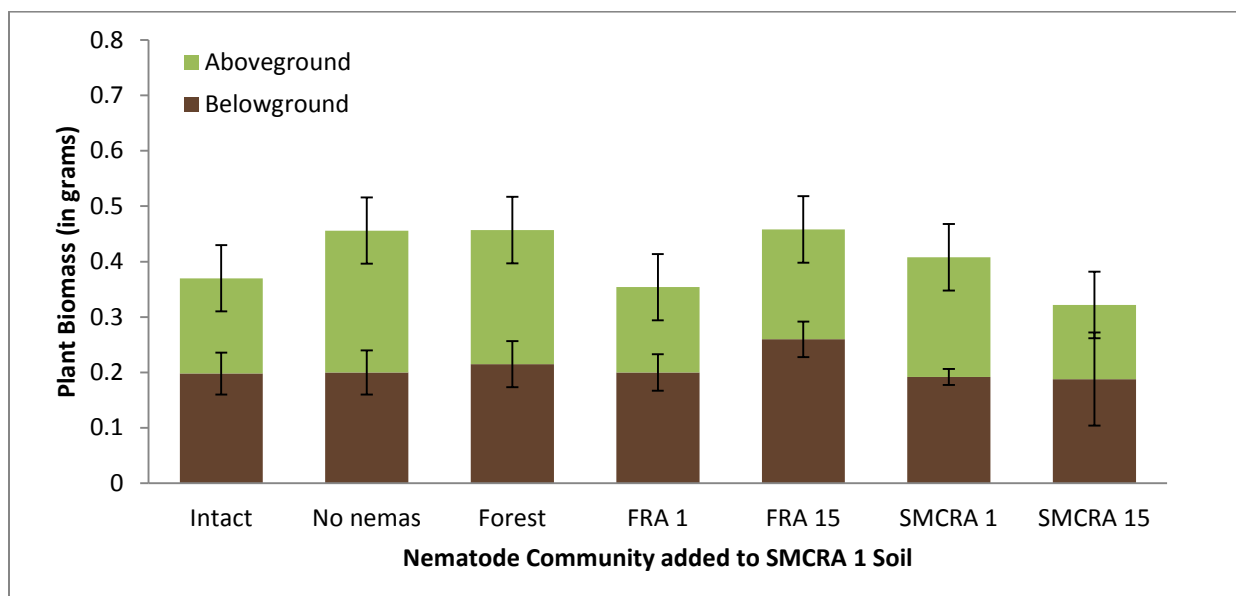


Fig. 21: Above and below-ground plant biomass of *C. canadensis* seedlings grown in soils from a one-year-old Surface Mining Control and Reclamation act site with the indicated nematode community added: intact control nematodes, defaunated control nematodes, forest nematodes, FRA one- year-old nematode communities, FRA fifteen-year-old communities, Surface Mine Control and Reclamation Act (SMCRA) one-year-old nematode communities, SMCRA fifteen-year-old nematode communities. Bars represent mean values $\pm$ standard error.

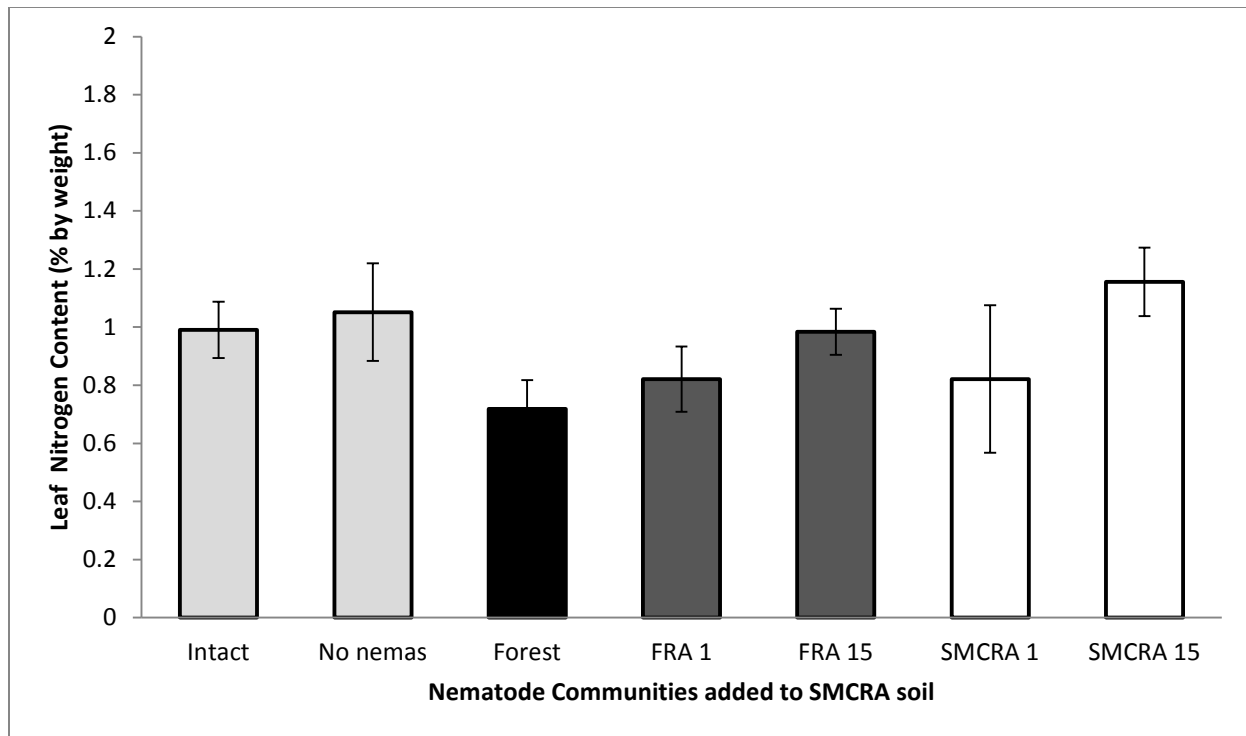


Fig. 22: Leaf nitrogen content of *C. canadensis* seedlings grown in soils from a one-year-old Surface Mine Control and Reclamation Act site with the indicated nematode community added: intact control nematodes, defaunated control nematodes, forest nematodes, FRA one- year- old nematode communities, FRA fifteen-year-old communities, Surface Mine Control and Reclamation Act (SMCRA) one-year-old nematode communities, SMCRA fifteen-year-old nematode communities. Bars represent mean values $\pm$ standard error.

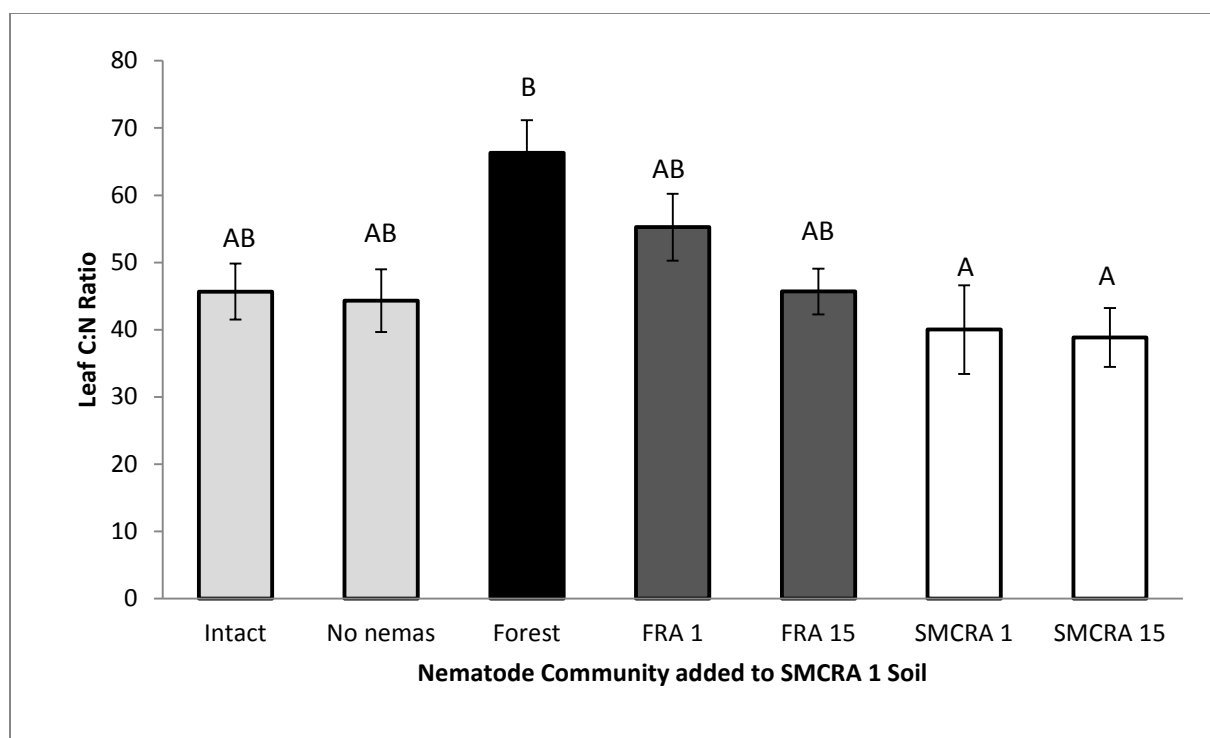


Fig. 23: Leaf carbon:nitrogen ratio of *C. canadensis* seedlings grown in soils from a one-year-old Surface Mining Control and Reclamation Act site with the indicated nematode community added: intact control nematodes, defaunated control nematodes, forest nematodes, FRA one- year-old nematode communities, FRA fifteen-year-old communities, Surface Mine Control and Reclamation Act (SMCRA) one-year-old nematode communities, SMCRA fifteen-year-old nematode communities. Bars represent mean values $\pm$ standard error. Letters indicate significant differences between groups.

Above and below-ground biomass of seedlings was similar among FRA 15 soils under all nematode community addition treatment (Fig. 24). Additionally, C:N and nitrogen levels did not vary based on treatment (Figs. 25 and 26).

Above and below-ground biomass of seedlings was similar among all SMCRA soils under all nematode community addition treatment (Fig. 27). Leaf nitrogen content (Fig. 28) was highest in intact SMCRA 15 soils, and lowest in SMCRA 15 soils that had been defaunated. Similarly, carbon:nitrogen leaf levels were lowest in seedlings grown in intact control soils, and highest in defaunated soils (Fig. 29).

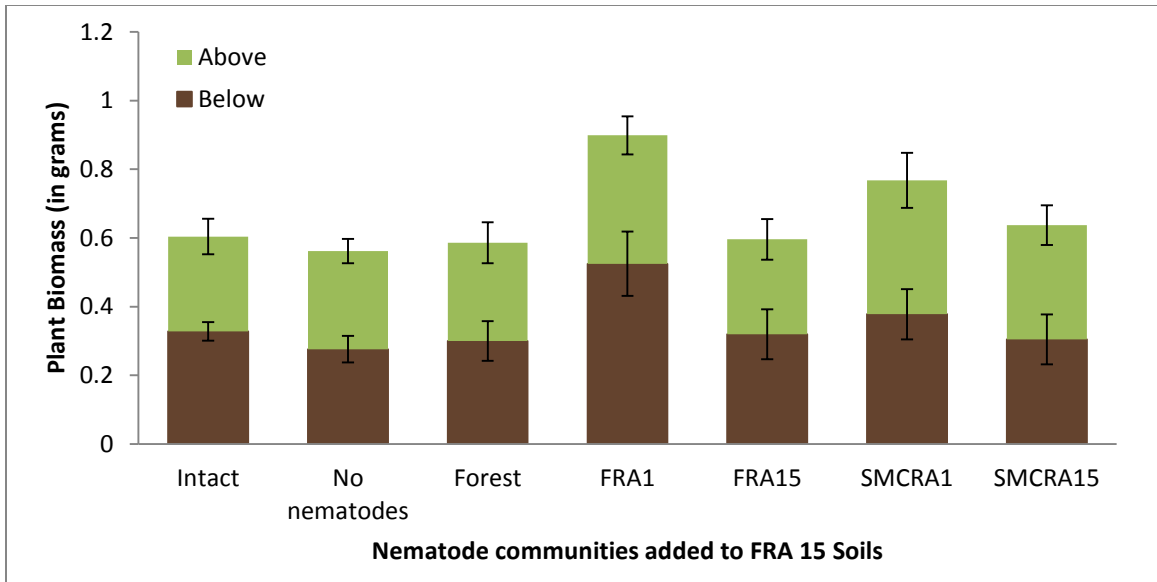


Fig. 24: Above and below-ground plant biomass of *C. canadensis* seedlings grown in soils from a fifteen-year-old Forestry Reclamation Approach site with the indicated nematode community added: intact control nematodes, defaunated control nematodes, forest nematodes, FRA one- year-old nematode communities, FRA fifteen-year-old communities, Surface Mine Control and Reclamation Act (SMCRA) one-year-old nematode communities, SMCRA fifteen-year-old nematode communities. Bars represent mean values $\pm$ standard error. Letters indicate significant differences between groups.

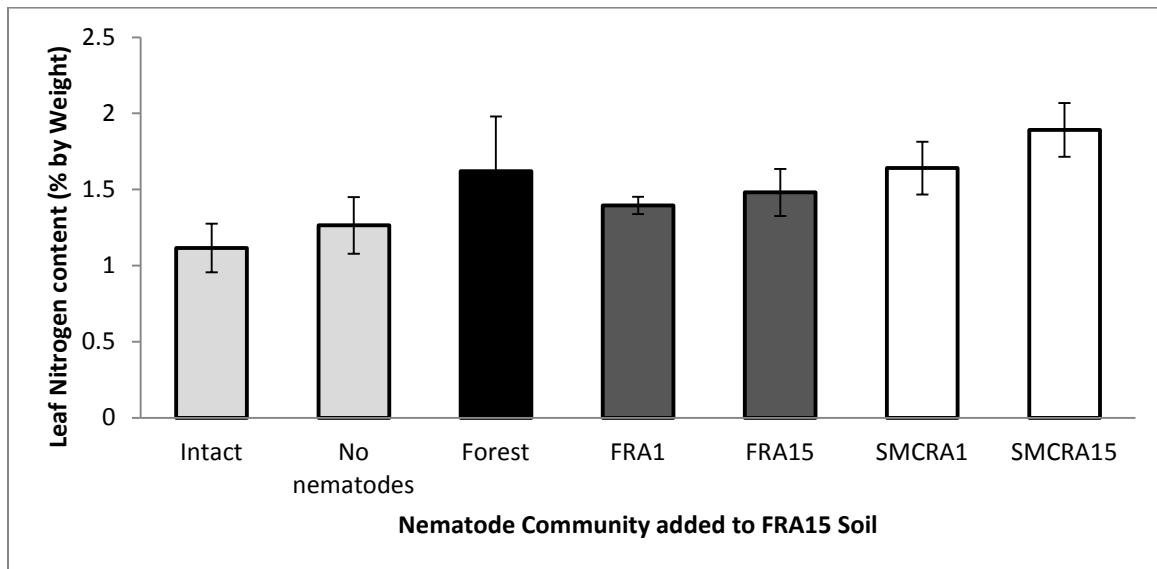


Fig. 25: Leaf nitrogen content of *C. canadensis* seedlings grown in soils from a fifteen-year-old Forestry Reclamation Approach site with the indicated nematode community added : intact control nematodes, defaunated control nematodes, forest nematodes, FRA one- year-old nematode communities, FRA fifteen-year-old communities, Surface Mine Control and Reclamation Act (SMCRA) one-year-old nematode communities, SMCRA fifteen-year-old nematode communities. Bars represent mean values $\pm$ standard error. Letters indicate significant differences between groups.

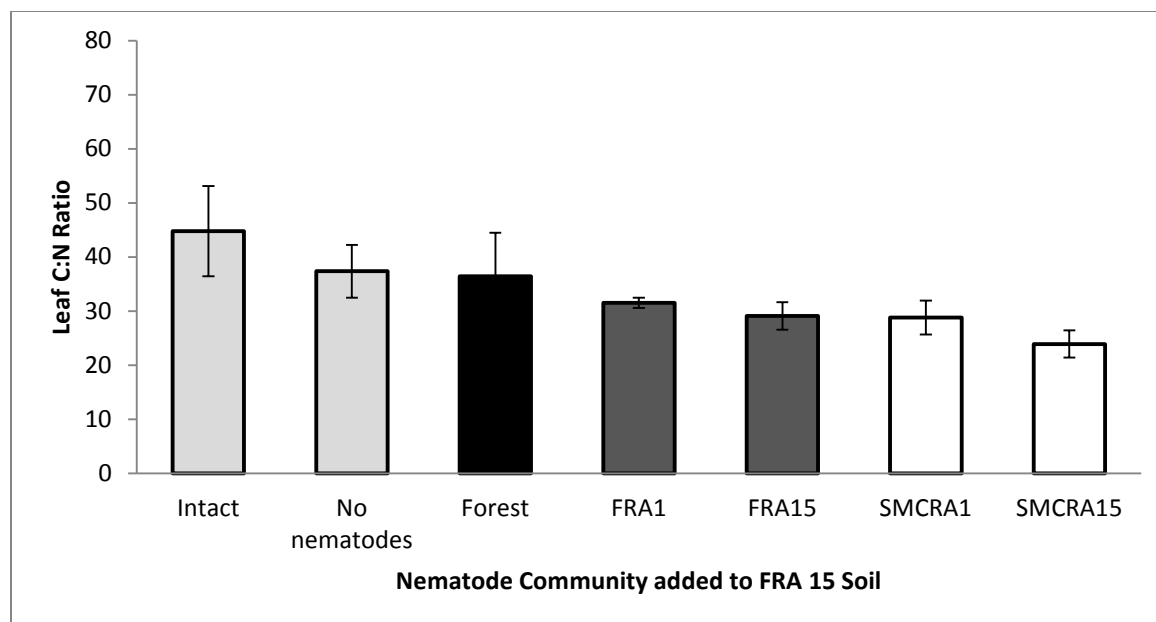


Fig. 26: Leaf carbon:nitrogen ratio of *C. canadensis* seedlings grown in soils from a fifteen-year-old Forestry Reclamation Approach site with the indicated nematode community added: intact control nematodes, defaunated control nematodes, forest nematodes, FRA one- year-old nematode communities, FRA fifteen-year-old communities, Surface Mine Control and Reclamation Act (SMCRA) one-year-old nematode communities, SMCRA fifteen-year-old nematode communities. Bars represent mean values $\pm$ standard error. Letters indicate significant differences between groups. Bars represent mean values $\pm$ standard error.

Below-ground biomass was influenced by nematode community, with defaunated soils, forest nematode addition, SMCRA 1, and intact control having lower root biomass, while nematode additions FRA 15, SMCRA 15, and FRA 1 had higher root biomass (Fig. 30). Leaf nutrient content of seedlings grown in forest soils were not influenced by nematode community treatment (Figs. 27, 28).

The diversity of nematode community additions, as measured by the Shannon diversity index was not related to biomass measures or nutrient content of leaves in any soil.

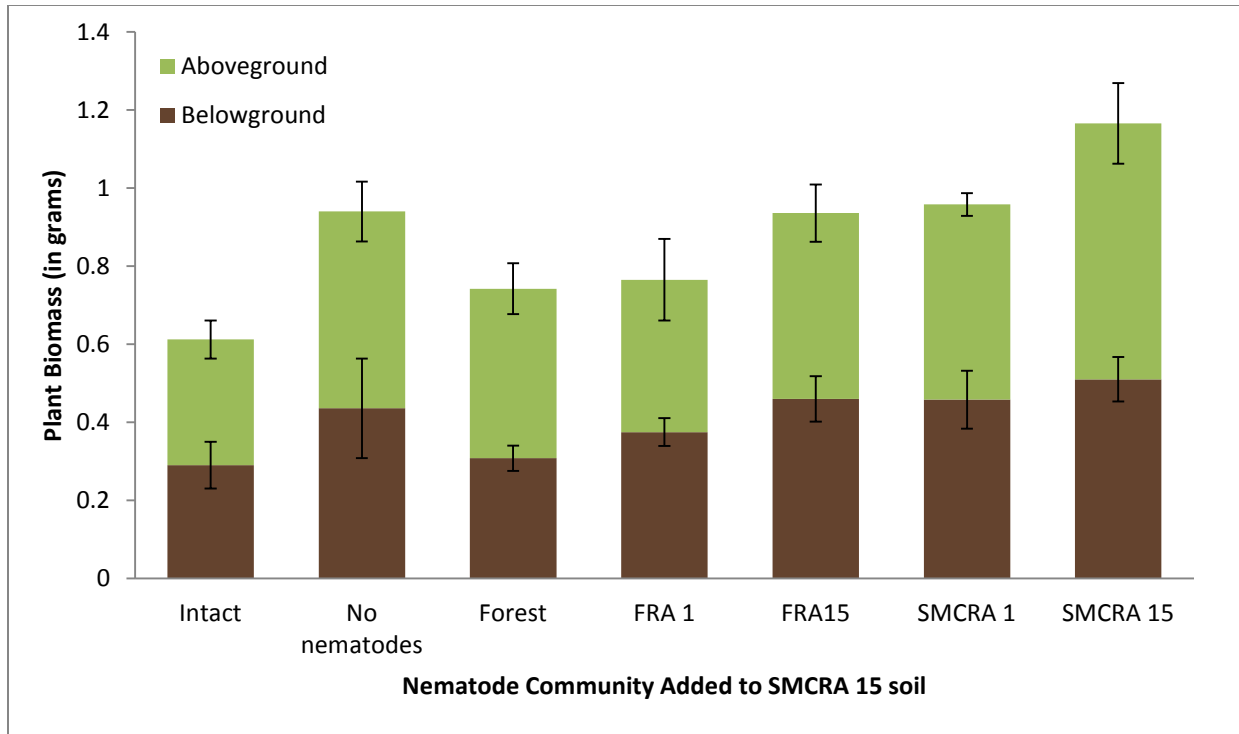


Fig. 27: Above and below-ground plant biomass of *C. canadensis* seedlings grown in soils from a fifteen-year-old Surface Mining Control and Reclamation Act site with the indicated nematode community added: intact control nematodes, defaunated control nematodes, forest nematodes, FRA one- year-old nematode communities, FRA fifteen-year-old communities, Surface Mine Control and Reclamation Act (SMCRA) one-year-old nematode communities, SMCRA fifteen-year-old nematode communities. Bars represent mean values $\pm$ standard error.

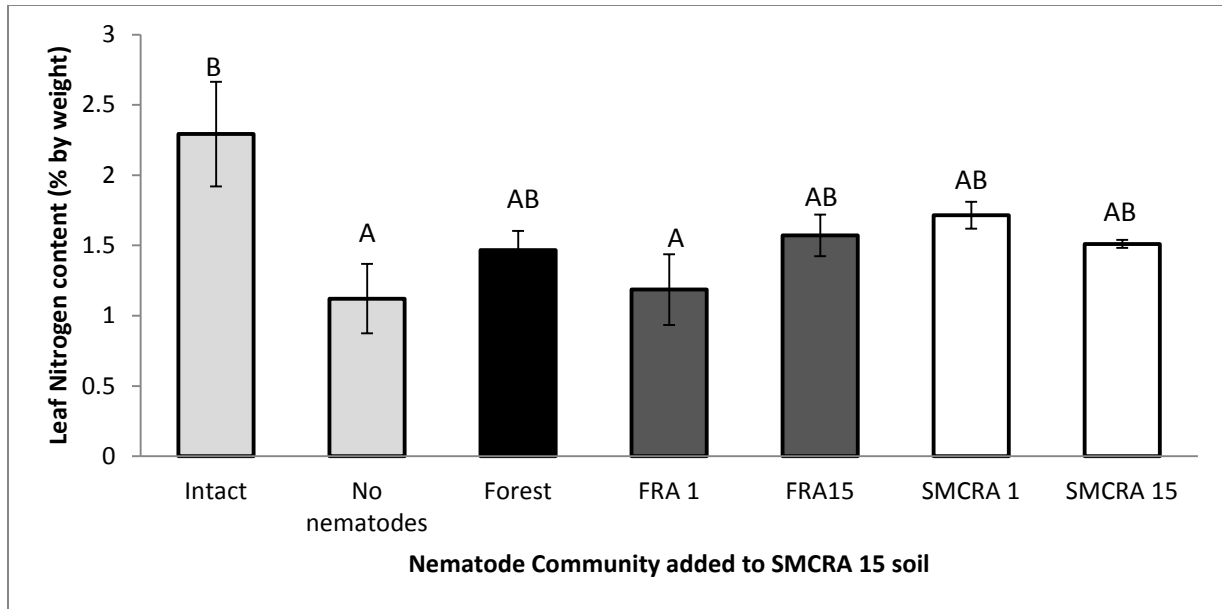


Fig. 28: Leaf nitrogen content of *C. canadensis* seedlings grown in soils from a fifteen-year-old Surface Mining Control and Reclamation Act site with the indicated nematode community added: intact control nematodes, defaunated control nematodes, forest nematodes, FRA one- year-old nematode communities, FRA fifteen-year-old communities, Surface Mine Control and Reclamation Act (SMCRA) one-year-old nematode communities, SMCRA fifteen-year-old nematode communities. Bars represent mean values $\pm$ standard error. Letters indicate significant differences between groups.

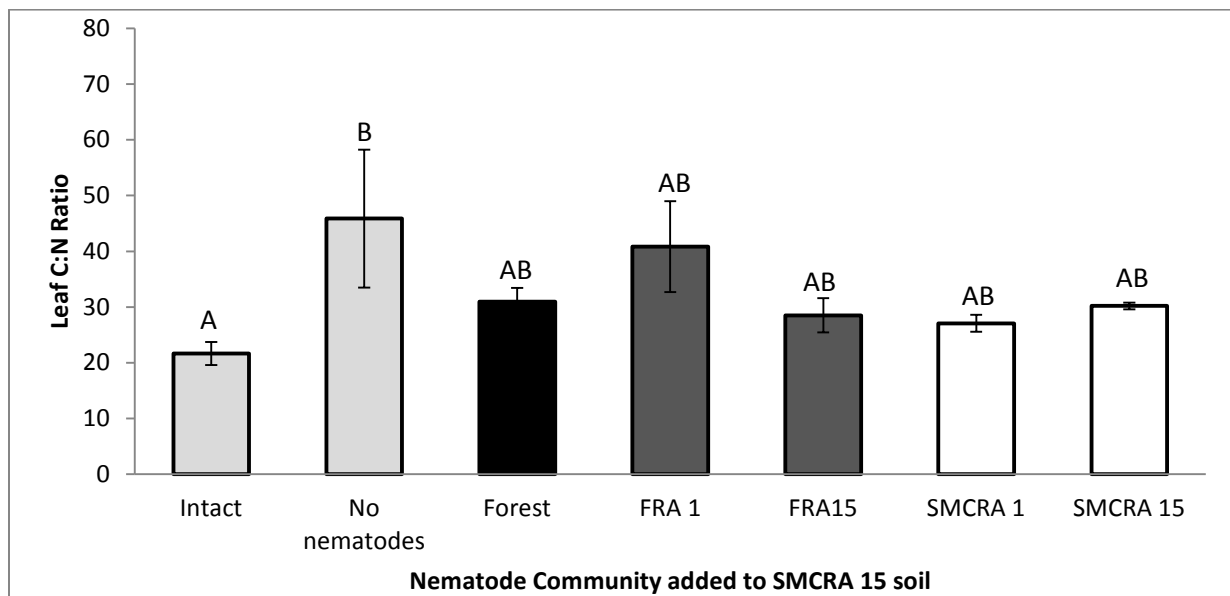


Fig. 29: Leaf carbon:nitrogen ratio of *C. canadensis* seedlings grown in soils from a fifteen-year-old Forestry Reclamation Approach site with the indicated nematode community added: intact control nematodes, defaunated control nematodes, forest nematodes, FRA one- year-old nematode communities, FRA fifteen-year-old communities, Surface Mine Control and Reclamation Act (SMCRA) one-year-old nematode communities, SMCRA fifteen-year-old nematode communities. Bars represent mean values $\pm$ standard error. Letters indicate significant differences between groups.

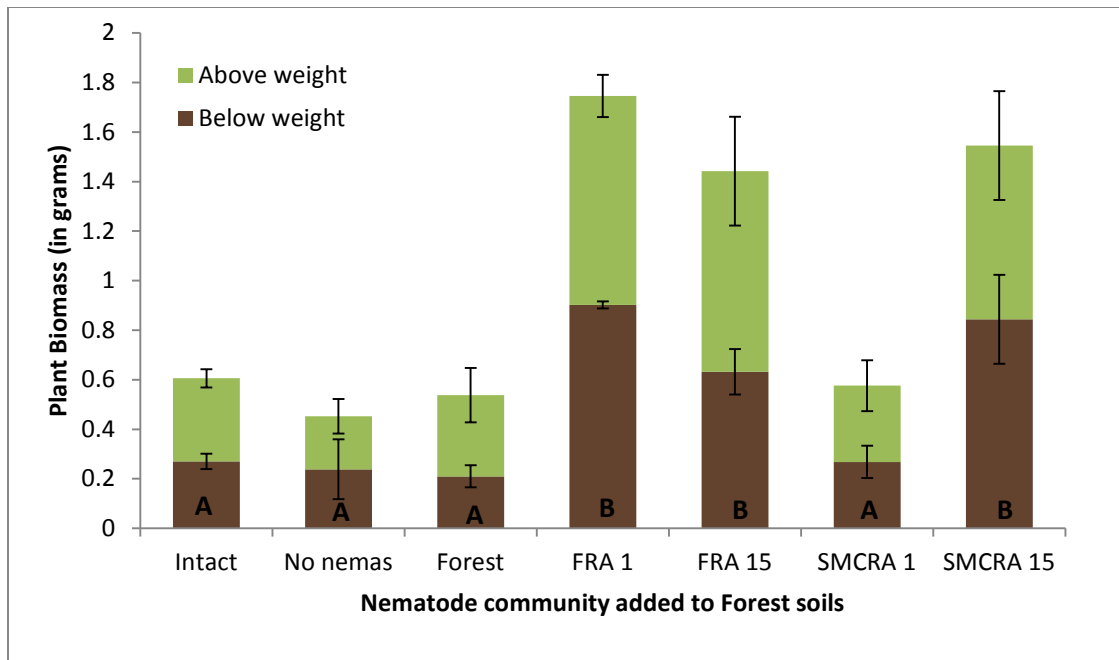


Fig. 30: Above and Below-ground Plant Biomass of *C. canadensis* seedlings grown in soils from an un-mined forest site with the indicated nematode community added: intact control nematodes, defaunated control nematodes, forest nematodes, FRA one- year-old nematode communities, FRA fifteen-year-old communities, Surface Mine Control and Reclamation Act (SMCRA) one-year-old nematode communities, SMCRA fifteen-year-old nematode communities. Bars represent mean values $\pm$ standard error. Letters indicate significant differences between groups.

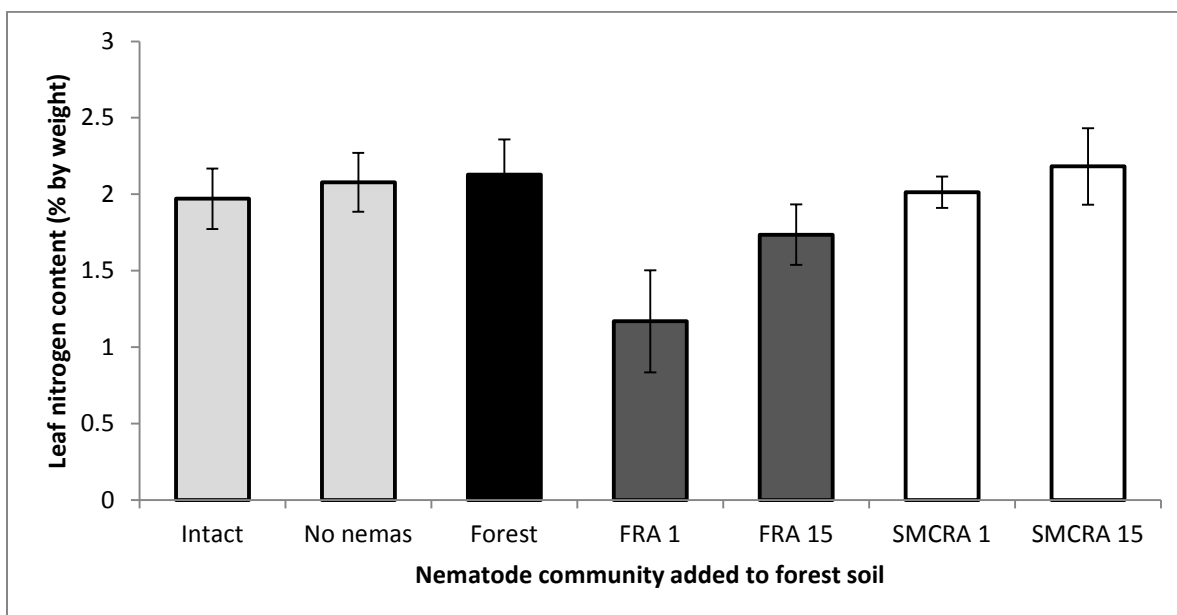


Fig. 31: Leaf nitrogen content of *C. canadensis* seedlings grown in soils from an un-mined forest site with the indicated nematode community added: intact control nematodes, defaunated control nematodes, forest nematodes, FRA one- year-old nematode communities, FRA fifteen-year-old communities, Surface Mining Control and Reclamation Act (SMCRA) one-year-old nematode communities, SMCRA fifteen-year-old nematode communities. Bars represent mean values $\pm$ standard error.

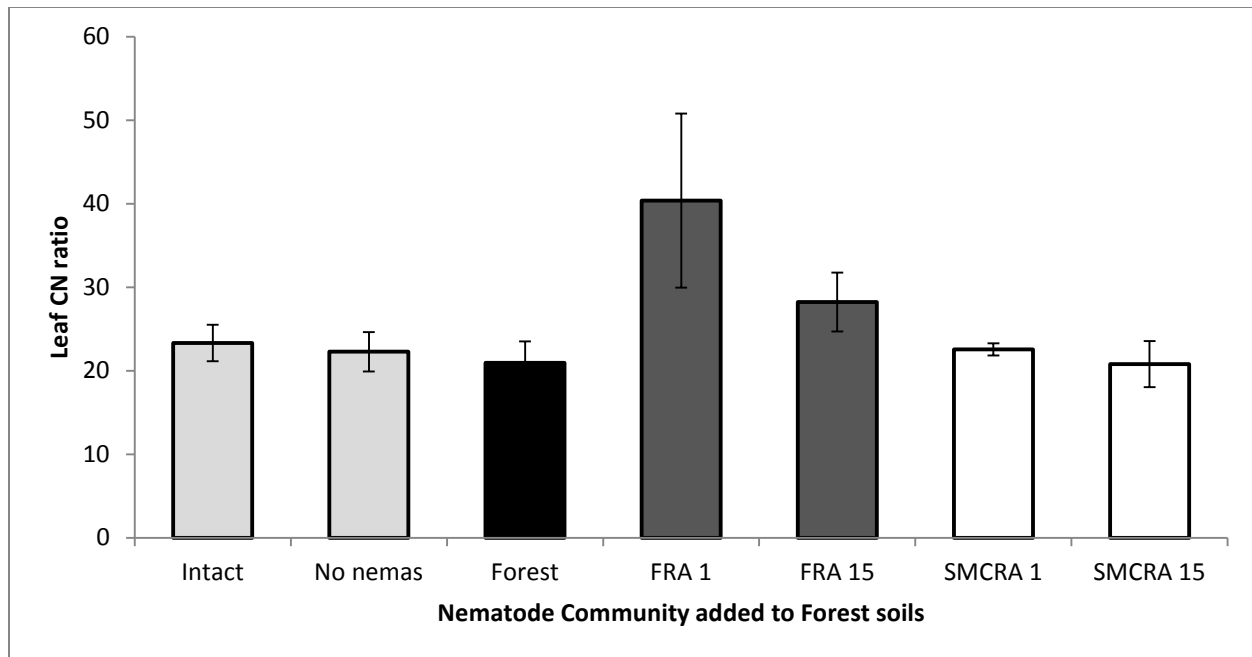


Fig. 32: Leaf carbon:nitrogen ratio of *C. canadensis* seedlings grown in soils from un-mine forest site with the indicated nematode community added: intact control nematodes, defaunated control nematodes, forest nematodes, FRA one- year-old nematode communities, FRA fifteen-year-old communities, Surface Mine Control and Reclamation Act (SMCRA) one-year-old nematode communities, SMCRA fifteen-year-old nematode communities. Bars represent mean values $\pm$ standard error.

Chapter 4

Discussion

Nematode abundance varied widely across samples, often due to a few dominant morphospecies. Soil communities are highly spatially variable, often due to limited mobility of soil organisms and high nutrient variability within soils. Nematodes tend to non-random distributions due to unevenly distributed food resources across the landscape, explaining the wide variation of several morphospecies across these plots

Shannon diversity increased over time across all sites. FRA sites become more similar to unmined forests in Shannon diversity at earlier ages than did SMCRA management in Tennessee and Virginia sites ($p < 0.05$). Higher diversity of nematodes can help to stabilize ecosystem functions during disturbances. Various species performing the same ecosystem function can increase likelihood of at least one of the species surviving disturbance. FRA sites in Virginia and Tennessee will be more likely to continue nitrogen mineralization during disturbances, such as drought, for this reason.

Nutrient cycling in soil systems can occur through the fungal or bacterial pathway (Sylvain & Wall 2011). All plots in this study seem to be utilizing the bacterial pathway to a greater extent than the fungal pathway, as indicated by the increased abundance of bacteria feeding nematodes over time, and continued suppression of fungal feeders across all plots. This may explain low levels of fungal feeders as well as omnivores that may be outcompeted by plant parasites. Bacteria more rapidly convert nitrogen to plant available forms than do fungi, potentially cycling substrate more rapidly than would occur in fungus dominated sites (Wardle 2003, Bardgett 2005).

Early soil communities tend to be unstable and unpredictable, and shaped by nutrient inputs, which are extremely limited in early mining restoration sites. Detrital input quality can lead to long term stability of food webs (Yeates *et al.* 1999). Fungal and bacterial communities are shaped by organic input quality, with more labile plant material (such as leaf litter) decomposed by bacterial pathways, and more recalcitrant material (such as twigs and bark) decomposed by fungi (Bardgett & Wardle 2003; Sylvain & Wall 2011). In early successional sites, there are few inputs, due to low plant biomass.

The low fungivorous nematode abundance found in the forested plots sampled in this study may be due to the high labile leaf inputs associated with diverse temperate hardwoods during fall. Additionally, plants in high nutrient soil, such as the un-mined intact forest sampled for this study, tend to limit mycorrhizal associations to avoid potential parasitism (Nilsson *et al.* 2005).

Early successional nematodes (cp-1 and cp-2) tend to be bacteria feeders (Villenave, Ettema and Bongers 1993), such as rhabditids and *Acrobeloides*. Bacterial grazing makes up a large portion of nutrient cycling and nitrogen mineralization, up to 30% (Villenave *et al.* 2004). In this study, bacterial feeders dominate the early successional sites, and plant parasites increase at the older restoration sites, possibly due to increased biomass associated with bacterial priming (Ingham *et al.* 1985; De Deyn *et al.* 2004).

Root exudate leads to increases bacteria in the rhizosphere (De Deyn *et al.* 2004; Kardol 2007), and bacterial grazing increases plant available nitrogen. This perpetuating top-down, bottom-up feedback loop can be vital in nutrient deficient systems, such as restored mines.

Increased nutrient status in older mine restoration sites appears to be defending plants from potential nutrient impacts from plant parasitism, as seen in the greenhouse plants grown in

youngest SMCRA soils with forest nematode populations added. In this study, the forest nematode community contained the highest abundance of plant parasitic nematodes. Adding a large number of plant parasites to the seedlings planted in the youngest SMCRA soil (which was presumably already nutrient-limited), resulted in poor plant nutrient status. Other nematode populations, which had fewer plant parasites, had no negative impacts of nitrogen content, or increasing C:N status in plants, including the SMCRA 1 soils. This indicates that nematode populations with high plant parasites may actually hinder survival of seedlings in soils with low nutrient status.

While nutrient status in young SMCRA soils may have impacted nutrient quality of seedlings, forest soils may have experienced inhibitory factors from younger nematode populations. *C. canadensis* seedling grown in nematode communities from SMCRA 1 plots, forest plots, as well as the intact and defaunated controls experienced reduced below-ground plant biomass. The defaunated control and SMCRA 1 added to forest soils had reduced nematodes for nutrient cycling, possibly limiting nitrogen mineralization rates.

Lower nutrient status and decreased root biomass associated with either depauperate soil populations (from SMCRA 1 soils) or high numbers of plant parasites (from forest soils) indicate nematode populations may be uniquely capable of cycling nutrients and preventing severe plant growth limitations within their own soils. Nematode populations interact with soil nutrients to direct plant growth.

Diverse nematode populations have been shown to exert controls on dominant plant species (or in this case, the only plant species) (De Deyn *et al.* 2004). The high number of plant parasites in the forest nematode community may have decreased root biomass in forest soils. In the landscape, this decrease would have the impact of allowing for an increase in competition for

alternative plant species. This root biomass impact was not observed in other soils for unknown reasons; but potentially, due to structural limitations associated with poor soil development, plant parasitic nematodes may have not been as effective at parasitizing plants in younger soils, to the point that structural changes occurred in roots.

The maturity index between middle age and old age plots of both SMCRA and FRA treatments was similar to the maturity index of the un-mined forest plots in both Virginia and Tennessee. FRA plots exceeded maturity index of intact forest sites in all three locations, possibly due to the increase in plant biomass in forests leading to an increase in abundance of plant parasitic nematodes, many of which have a low MI value. Many bacterial feeders remained present in forest ecosystems and increase in abundance as well in comparison to disturbed soils that lacked the nutrient input to support a large bacterivore community.

While the maturity index was relatively similar between treatments, the ratio of c-p 5 to c-p 1 values was over three times higher in older FRA soils than older SMCRA soils (0.98 and 0.29, respectively). Bacterial feeders and plant feeders with c-p values of two increased over time, and were highest in abundance in un-mine forest soils. This increase in nematodes with lower c-p values caused the increase in c-p 5 nematodes to be overshadowed when maturity indices was calculated. The maturity index itself may lack the resolution required to be an effective bioindicator. It has been suggested by the original authors of the maturity index that a finer resolution may be necessary in order to overcome issues such as population spatial heterogeneity, variations in life strategies within families, and microenvironment effects (Ferris & Bongers 2006).

Indicator species show that differences in species composition still remain between old restored sites and un-mined forests, although Shannon diversity was similar between these sites

(Fig. 11). These indicator species represent a sensitive measure by which to assess restoration. This method of assessing management success is site specific, as seen by the lack of *Xiphinema* sp. in un-mined Tennessee forests. Many of the species most strongly associated with intact un-mined forests had c-p values of 2, 3, or 4. The assignment of c-p values may need to be assessed on a geographic or ecosystem basis.

Plant species tended to have a positive association with nematode diversity. The plant species with the strongest positive effect on nematode diversity was *Cornus florida*. *Cornus florida* may be highly associated with nematode diversity due its common presence in the midstory of closed canopy forests. A strong midstory plant presence in closed forests indicates increased plant inputs, which can influence local nematode communities. *Eleagnus angustifolia* was the only non-native plant to yield a strong enough impact on nematode populations to be included in the canonical analysis. The negative association of *E. angustifolia* with diversity may be due to the presence of *Eleagnus angustifolia* exclusively on SMCRA sites. It was commonly found in lower plant diversity sites, and was often associated with agricultural grasses in relatively homogeneous plant communities. Homogeneous plant communities may limit niche, diversity which rare nematode species could occupy.

Chapter 5

Conclusions

The abundance of indicator species, as defined by the canonical correspondence analysis, was much lower in all treatments and age groups of reclaimed sites. This lower abundance indicates that while basic community measures, such as abundance, diversity, and even trophic ratios, may be similar between restored sites and forests, species level differences still exist after several years of reclamation. While canonical correspondence shows a higher degree of similarity of nematode species identity between forested sites and FRA sites than forested sites and SMCRA sites, rare species are still missing from disturbed sites. The use of a few sensitive species or families as an indicator of reclamation success may be more valuable than other common community structure analyses. Trophic abundance may have strong ties to reclamation as well, based on the development of plant parasites in the forested sites being higher than in disturbed sites. The maturity index failed to find differences within these sites in the basis of the colonizer-persister scale.

Though FRA reclamation is considered more successful for above-ground development of young forests, the same dramatic effects do not appear to occur below-ground, where community structure and development are similar in many ways between FRA and SMCRA plots.

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Appendix

Table 4: List of nematode morphospecies in all plots, order, feeding group, and c-p value

Order	Morphospecies	Feeding group	c-p value
Alaimida			
	<i>Alaimus</i> sp. 1	B	4
	<i>Alaimus</i> sp. 2	B	4
Araeolaimida			
	<i>Anaplectus</i> sp.	B	2
	<i>Araeolaimida</i> sp.	P	3
	<i>Bastiania</i> sp.	B	3
	<i>Ceratoplectus armaidus</i>	B	2
	<i>Ceratoplectus cornus</i>	B	2
	<i>Ceratoplectus elongatus</i>	B	2
	<i>Plectus</i> sp.	B	2
Chromadorida			
	<i>Achromadora</i> sp.	B	3
	<i>Teratocephalus</i> sp. 1	B	2
	<i>Teratocephalus</i> sp. 2	B	2
Triplonchida			
	<i>Diphorophora</i>	H	3
Dorylaimida			
	<i>Aporcelaimellus</i> sp. 1	P	5
	<i>Aporcelaimellus</i> sp. 2	P	5
	<i>Aporcellaimellus</i> sp. 3	P	5
	<i>Aporcellaimellus</i> sp. 4	P	5
	<i>Dorylaimida</i> sp. 1	O	4
	<i>Dorylaimida</i> sp. 1	O	4
	<i>Dorylaimida</i> sp. 10	O	4
	<i>Dorylaimida</i> sp. 11	O	4
	<i>Dorylaimida</i> sp. 12	O	4
	<i>Dorylaimida</i> sp. 13	O	4
	<i>Dorylaimida</i> sp. 14	O	4
	<i>Dorylaimida</i> sp. 15	O	4
	<i>Dorylaimida</i> sp. 2	O	4
	<i>Dorylaimida</i> sp. 3	O	4
	<i>Dorylaimida</i> sp. 4	O	4
	<i>Dorylaimida</i> sp. 5	O	4
	<i>Dorylaimida</i> sp. 5	O	4
	<i>Dorylaimida</i> sp. 7	O	4
	<i>Dorylaimida</i> sp. 8	O	4
	<i>Dorylaimida</i> sp. 9	O	4
	<i>Dorylaimida thornia</i>	O	4
	<i>Enchodelus</i> sp.	O	4

Order	Morphospecies	Feeding group	c-p value
	<i>Epidorylaimus</i> sp.	O	4
	<i>Eudorylaimus</i> sp. 1	O	4
	<i>Eudorylaimus</i> sp. 2	O	4
	<i>Eudorylaimus</i> sp. 3	O	4
	<i>Eudorylaimus</i> sp. 4	O	4
	<i>Qudsianematidae</i> sp. 1	O	4
	<i>Microdorylaimus</i> sp.	O	4
	<i>Thonus</i> sp.	P	2
	<i>Tylencholaimus</i> sp.	PP	5
	<i>Xiphinema</i> sp.	PP	5
Monhysterida			
	<i>Geomonhystera</i> sp.	B	3
Mononchida			
	<i>Clarkus</i> sp.	P	4
	<i>Prionchulus</i> sp.	P	4
Enoplida			
	<i>Oncholaimidae</i> sp.	B	4
Plectida			
	<i>Anaplectus</i> sp.	B	2
	<i>Araeolimida</i> sp.	P	3
	<i>Bastiania</i> sp.	B	3
	<i>Ceratoplectus armatus</i>	B	2
	<i>Ceratoplectus cornus</i>	B	2
	<i>Ceratoplectus elongatus</i>	B	2
	<i>Odontolaimid</i> sp.	B	4
	<i>Plectus armatus</i>	B	2
	<i>Plectus elongatus</i>	B	2
	<i>Plectus longicaudatus</i>	B	2
	<i>Plectus parvus</i>	B	2
	<i>Plectus</i> sp. 1	B	2
	<i>Plectus</i> sp. 10	B	2
	<i>Plectus</i> sp. 11	B	2
	<i>Plectus</i> sp. 12	B	2
	<i>Plectus</i> sp. 2	B	2
	<i>Plectus</i> sp. 3	B	2
	<i>Plectus</i> sp. 3b	B	2
	<i>Plectus</i> sp. 4	B	2
	<i>Plectus</i> sp. 5	B	2
	<i>Plectus</i> sp. 6	B	2
	<i>Plectus</i> sp. 6b	B	2
	<i>Plectus</i> sp. 7	B	2

Order	Morphospecies	Feeding group	c-p value
Rhabditida	<i>Plectus</i> sp. 8	<i>B</i>	2
	<i>Plectus</i> sp. 9	<i>B</i>	2
	<i>Plectus varians</i>	<i>B</i>	2
	<i>Tylocephalus</i>	<i>B</i>	2
	<i>Wilsonema</i> sp. 1	<i>B</i>	2
	<i>Wilsonema</i> sp. 2	<i>B</i>	2
	<i>Acrobeles</i> sp.	<i>B</i>	2
	<i>Acroboloides</i> sp. 2	<i>B</i>	2
	<i>Acroboloides</i> sp. 3	<i>B</i>	2
	<i>Acroboloides apiculatus</i>	<i>B</i>	2
	<i>Acroboloides</i> sp. 1	<i>B</i>	2
	<i>Acroboloides tricornis</i>	<i>B</i>	2
	<i>Bunonema</i> sp.	<i>B</i>	1
	<i>Cephalobid</i> sp.	<i>B</i>	2
	<i>Cervidellis</i> sp.	<i>B</i>	2
	<i>Chiloplacus</i> sp.	<i>B</i>	2
	<i>Cruznema</i> sp.	<i>B</i>	1
	<i>Diplogasteridae</i> sp.	<i>B</i>	1
	<i>Eucephalobus</i> sp.	<i>B</i>	2
	<i>Hemicyclophora</i> sp.	<i>PP</i>	3
	<i>Panagrolaimus</i> sp.	<i>B</i>	4
	<i>Psilenchus</i> sp. 1	<i>PP</i>	2
	<i>Psilenchus</i> sp. 2	<i>PP</i>	2
	<i>Rhabditid</i> sp. 1	<i>B</i>	1
	<i>Rhabditid</i> sp. 2	<i>B</i>	1
	<i>Rhabditid</i> sp. 3	<i>B</i>	1
	<i>Rhabditid</i> sp. 4	<i>B</i>	1
Triplonchida	<i>Diptherophora</i> sp.	<i>H</i>	3
	<i>Prismatolaimus</i> sp.	<i>B</i>	3
	<i>Prismatolaimus</i> sp. 2	<i>B</i>	3
	<i>Trichodorus</i> sp.	<i>PP</i>	4
	<i>Tripyla</i> sp.	<i>P</i>	2
Tylenchida	<i>Ogma</i> sp. 1	<i>P</i>	3
	<i>Aphelenchoides</i> sp. 1	<i>H</i>	2
	<i>Aphelenchoides bicaudatus</i>	<i>H</i>	2
	<i>Aphelenchoides</i> sp. 2	<i>H</i>	2
	<i>Aphelenchus avenae</i>	<i>H</i>	2
	<i>Aphelenchus</i> sp. 3	<i>H</i>	2

Order	Morphospecies	Feeding group	c-p value
	<i>Basiria</i> sp. 1	PP	2
	<i>Basiria</i> sp. 2	PP	2
	<i>Boleodorus</i> sp.	PP	2
	<i>Criconema</i> sp.	PP	3
	<i>Criconemella</i> sp.	PP	3
	<i>Ditylenchus</i> sp.	PP	2
	<i>Filenchus</i> sp. 1	PP	2
	<i>Filenchus</i> sp. 2	PP	2
	<i>Filenchus</i> sp. 3	PP	2
	<i>Gracilacus</i>	PP	2
	<i>Gymnotylenchus</i> sp.	PP	2
	<i>Helicotylenchus</i> sp. 1	PP	2
	<i>Helicotylenchus</i> sp. 2	PP	2
	<i>Helicotylenchus</i> sp. 3	PP	2
	<i>Helicotylenchus</i> sp. 4	PP	2
	<i>Helicotylenchus</i> sp. 5	PP	2
	<i>Meloidigyne</i> sp.	PP	4
	<i>Nothotylenchus</i> sp.	H	2
	<i>Ogma</i> sp. 1	P	3
	<i>Paraphelenchus</i> sp.	F	2
	<i>Paratylenchus</i>	PP	2
	<i>Seriespinula</i> sp.	PP	2
	<i>Tylenchorynchus</i> sp.	PP	5
	<i>Xenocriconamella</i> sp.	P	3
	<i>Xenocriconemella macrodora</i>	P	3
	<i>Xenocriconemella macrodora</i>	P	3
	<i>Xenocriconemella</i> sp. 1	P	3

*Based on Yeates 1993, Bongers 1990.

H: Hyphal feeding, B: Bacteria feeding, PP: Plant Parasitic, B: Bacteria feeding

PP: Plant Parasitic, O: Omnivore, PR: Predatory feeding

'b' indicates similar morphospecies

Table 5: Plant species in Surface Mining Control and Reclamation Act sites (SMCRA), Forestry Reclamation Approach sites (FRA), and unmine forest sites across a chronosequence in three mine loacations, Tennessee, Virginia, and Kentucky.

Plant Species in plots					
Management type	Age group	Scientific name	Common name	Native status	
Tennessee sites					
SMCRA	Young	Trifolium sp.	clover	native	
		Lolium multiflorum	annual ryegrass	non-native	
		Lotus corniculatus	birdsfoot trefoil	non-native	
		Eragrostis curvulata	weeping lovegrass	non-native	
FRA	Young	Robinia pseudoacacia	black locust	native	
		Quercus rubra	northern red oak	native	
		Fraxinus americana	white ash	native	
		Dactylis glomerata	orchard grass	non-native	
SMCRA	Middle-age	Sericea lespedeza	Chinese lespedeza	non-native	
		Fraxinus americana	white ash	native	
		Quercus rubra	northern red oak	native	
		Liriodendron Tulipifera	yellow poplar	native	
FRA	Middle-age	Fraxinus pennsylvanica	green ash	native	
		Fraxinus americana	white ash	native	
		Pinus strobus	white pine	native	
		Quercus rubra	northern red oak	native	
SMCRA	Old	Sericea lespedeza	Chinese lespedeza	non-native	
		Festuca Arundinaceae	tall fescue	non-native	
		Lotus corniculatus	birdsfoot trefoil	non-native	
		Robinia pseudoacacia	black locust	native	
FRA	Old	Fraxinus americana	white ash	native	
		Quercus rubra	borthern red oak	native	
		Liriodendron Tulipifera	yellow poplar	native	
		Pinus virginiana	Virginia pine	native	
		Acer rubra	red maple	native	
		Juglans nigra	black walnut	native	
Unmined Forest		Fraxinus americana	white ash	native	
		Quercus rubra	northern red oak	native	
		Liriodendron Tulipifera	yellow poplar	Native	

	Management type	Age group	Scientific name	Common name	Native status
Virginia sites			<i>Acer rubra</i>	red maple	native
			<i>Juglans nigra</i>	black walnut	native
	SMCRA	Young	<i>Sericea lespedeza</i>	Chinese lespedeza	non-native
			<i>Lotus corniculatus</i>	birdsfoot trefoil	non-native
			<i>Eragrostis curvulata</i>	weeping lovegrass	non-native
			<i>Lolium multiflorum</i>	annual ryegrass	non-native
			<i>Quercus rubra</i>	northern red oak	native
			<i>Platanus occidentalis</i>	American sycamore	native
	FRA	Young	<i>Trifolium sp.</i>	clover	native
			<i>Lolium multiflorum</i>	annual ryegrass	non-native
			<i>Lotus corniculatus</i>	birdsfoot trefoil	non-native
			<i>Fraxinus americana</i>	white ash	native
			<i>Pinus strobus</i>	white pine	native
			<i>Quercus rubra</i>	northern red oak	native
			<i>Platanus occidentalis</i>	American sycamore	native
			<i>Q. Prinus</i>	chestnut oak	native
			<i>Dactylis glomerata</i>	orchard grass	non-native
	SMCRA	Middle-age	<i>Lolium multiflorum</i>	annual ryegrass	non-native
			<i>Dactylis glomerata</i>	orchard grass	non-native
			<i>Lotus corniculatus</i>	birdsfoot trefoil	non-native
			<i>Cercis canadensis</i>	eastern redbud	native
			<i>Platanus occidentalis</i>	American sycamore	native
			<i>Eleagnus Umbellata</i>	autumn olive	non-native
	FRA	Middle-age	<i>Trifolium sp.</i>	clover	native
			<i>Lolium multiflorum</i>	annual ryegrass	non-native
			<i>Cercis canadensis</i>	eastern redbud	native
			<i>Quercus rubra</i>	northern red oak	native
			<i>Platanus occidentalis</i>	American sycamore	native
	SMCRA	Old	<i>Trifolium sp.</i>	clover	native
			<i>Lolium multiflorum</i>	annual ryegrass	non-native
			<i>Pinus strobus</i>	White pine	native
	FRA	Old	<i>Solidago canadensis</i>	goldenrod	native
			<i>Festuca Arundinaceae</i>	tall fescue	non-native
			<i>Liriodendron Tulipifera</i>	yellow poplar	native

Management type	Age group	Scientific name	Common name	Native status
		<i>Eleagnus Umbellata</i>	autumn olive	non-native
Unminded forest		<i>Fraxinus pennsylvanica</i>	green ash	native
		<i>Fraxinus americana</i>	white ash	native
		<i>Quercus rubra</i>	northern red oak	native
		<i>Betula Americana</i>	American beech	native
		<i>Robinia pseudoacacia</i>	black locust	native
		<i>Cornus florida</i>	flower dogwood	native
		<i>Acer rubra</i>	red maple	native
		<i>Juglans nigra</i>	black walnut	native

Kentucky Sites

SMCRA	Young	<i>Festuca Arundinaceae</i>	tall fescue	non-native
		<i>Platanus occidentalis</i>	American sycamore	native
		<i>Cercis canadensis</i>	eastern red bud	native
		<i>Solidago canadensis</i>	goldenrod	native
FRA	Young	<i>Solidago canadensis</i>	goldenrod	native
		<i>Festuca Arundinaceae</i>	tall fescue	non-native
		<i>Prunus serotina</i>	black cherry	native
		<i>Cercis canadensis</i>	eastern redbud	native
		<i>Platanus occidentalis</i>	American sycamore	native
SMCRA	Middle-age	<i>Sericea lespedeza</i>	Chinese lespedeza	non-native
		<i>Dactylis glomerata</i>	orchard grass	non-native
		<i>Festuca Arundinaceae</i>	tall fescue	non-native
		<i>Eleagnus Umbellata</i>	autumn olive	non-native
FRA	Middle-age	<i>Solidago canadensis</i>	goldenrod	native
		<i>Quercus rubra</i>	northern red oak	native
		<i>Liriodendron Tulipifera</i>	yellow poplar	native
		<i>Lotus corniculatus</i>	birdsfoot trefoil	non-native
		<i>Acer rubra</i>	red maple	native
SMCRA	Old	<i>Eleagnus Umbellata</i>	autumn olive	non-native
		<i>Festuca Arundinaceae</i>	tall fescue	non-native
		<i>Solidago canadensis</i>	goldenrod	native
		<i>Lolium multiflorum</i>	annual ryegrass	non-native

Management type	Age group	Scientific name	Common name	Native status
FRA	Old	<i>Prunus serotina</i>	black cherry	native
		<i>Liriodendron Tulipifera</i>	yellow poplar	native
		<i>Robinia pseudoacacia</i>	black locust	native
		<i>Acer rubra</i>	red maple	native
Unmined forest		<i>Cercis canadensis</i>	eastern red bud	native
		<i>Quercus rubra</i>	northern red oak	native
		<i>Liriodendron Tulipifera</i>	yellow poplar	native
		<i>Platanus occidentalis</i>	American sycamore	native
		<i>Betula Americana</i>	American beech	native
		<i>Acer rubra</i>	Red maple	native
		<i>Juglans nigra</i>	black walnut	native

Vita

Haley Smith had the good sense to be born in Stillwater, Oklahoma. She attended high school in Edmond, Oklahoma, prior to returning to Stillwater to pursue a B.S. in forestry at Oklahoma State University. During her tenure in her Bachelor's program, she attended Humboldt State University in Arcata, California for an exchange program. After graduation in 2008, she worked as a field technician in the Cascade Mountains in southern Oregon, and also in the Mojave Desert in southern California. She moved to Knoxville Tennessee to work as a lab manager in an ecosystem ecology lab from 2009-2010. In January of 2011, she enrolled in the Entomology and Plant Pathology program at the University of Tennessee.