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# Preliminary evaluation of nematode community responses to ground covers in jute leaf cultivation

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**Abstract: Jute leaf (*Corchorus olitorius*) is an emerging ethnic crop in the Mid-Atlantic United States. No information is available on nematode associations and nematode community responses to jute leaf grown with ground cover. We conducted a preliminary field study in the summer of 2023 in Beltsville, Maryland to evaluate the responses of endemic nematode communities to three cultivars of jute leaf (Firebird, Molokhia, and USDA PI 404029) and four ground cover treatments (compost, compost and landscape fabric, compost and straw mulch, and compost and wood mulch). We extracted nematodes from soil samples collected before planting, at midseason, and at harvest. By the end of the season, plots with straw had higher counts of *Prismatolaimus, Mononchus* and total plant-parasites and plots with wood chips had higher counts of *Helicotylenchus*. Structure index and maturity index 2-5 were also higher in plots with straw at the end of the season. Cultivar had a relatively small impact on the nematode community with USDA PI 404029 plots having the highest *Plectus* counts and Firebird plots having the highest predator counts at midseason only. The channel and enrichment indexes showed a shift occurred with all four treatments: the soil environment became dominated by bacterial decomposition pathways with nutrient enriched conditions. However, plant biomass was not different between treatments. These results suggest ground covers can influence soil nematode communities in jute leaf production.**

## 1. Introduction

Ethnic foods are increasingly in demand in the United States (US). Metropolitan areas such as Washington, DC are home to growing populations of ethnically diverse residents with a broad range of dietary preferences (Mangan *et al*., 2008; Govindasamy *et al.*, 2022). Many tropical crops from Africa and Asia can be grown as annuals in the summer months in the mid-Atlantic US (Mangan *et al.*, 2010). Local production of ethnic crops on small-scale farms can increase accessibility of products that might otherwise be difficult to obtain at high quality due to long-distance transport (Trobe, 2001). Jute leaf (*Corchorus olitorius*) is an important crop in tropical countries in Africa and Asia and is in demand in the eastern US (Govindasamy *et al.,* 2007). It is primarily grown as a leafy vegetable and the leaves are often used to add flavor and thicken soups and stews (Islam, 2013). Roots can be used for medicinal purposes and stems can also be used for fiber production (Islam *et al*., 2013; Nyadanu *et al*., 2017).

A management practice useful for crop production is using ground covers and mulching. Physical barriers laid on the soil surface can create an impediment for weed development and aids in conserving moisture and regulating soil temperature (Flint, 2012; Richardson *et al.*, 2023, 2024). Sustainable ground covers derived from organic materials used to suppress weeds can contribute to higher soil moisture content and higher yields of vegetables in the northeastern US (Gheshm and Brown, 2018; Larkin, 2020; Richardson *et al*., 2023). Urban centers produce large quantities of organic waste, some of which can be repurposed as compost or ground covers for agricultural use (Arcas-Pilz *et al.,* 2023). Wastes such as biosolids, yard waste, and food waste can provide nutrients to crops as compost, ground covers, or amendments (Wang *et al.,* 2008; Splawski *et al.,* 2016; Shrestha *et al*., 2020).

Ground cover applications can have cascading effects on the soil ecosystem. Nematodes are microscopic worms that are ubiquitous in soil environments and are sensitive to additions of organic matter-based mulches and amendments (McSorley and Gallaher, 1996; Forge and Kempler, 2009; Waldo *et al.,* 2024). Plant-parasitic nematodes feed on plant roots and can negatively affect plant health and reduce yields in leafy vegetable crops, including jute leaf (Atungwu *et al.,* 2013; Mbogoh *et al.,* 2013; Kimaru *et al.,* 2014). Yield of jute leaf in Nigeria and India has been reduced by 52% and 68%, respectively, due to feeding injury from *Meloidogyne incognita* (Saikia and Phukan, 1986; Adepoju and Oluwatayo, 2016). Organic amendments can contribute to suppression of undesirable plant-parasitic nematodes by introducing and enhancing natural antagonistic organisms including predatory/parasitic fungi, collembola, tardigrades, mites and protozoa as well as releasing lethal compounds such as ammonia and organic acids that are byproducts of decomposition (Akhtar and Malik, 2000; Thoden *et al*., 2011; Timper, 2014; Rosskopf *et al.,* 2020). Some microorganisms such as *Bacillus* spp., *Pastueria* spp., *Pochonia* spp, and *Trichoderma* spp. have shown promise at reducing numbers of plant-parasitic nematodes and have been further studied as potential biocontrol agents of plant-parasitic nematodes in cropping systems (Meyer and Roberts, 2002; Pires *et al.,* 2022). Other nematode groups feed on a range of soil microflora and microfauna and can have positive effects on soil health (Yeates *et al.,* 1993; Neher, 2001). Bacterivore nematodes rapidly increase following additions of organic matter in response to bacterial blooms (Ferris and Bongers, 2006). Bacterivores contribute to nutrient cycling by culling bacteria, which releases carbon (C) and nitrogen (N) back into the soil that may otherwise be respired or immobilized during periods of high microbial activity (Akhtar and Malik, 2000; Wang and McSorley, 2005). Omnivorous and predatory nematodes also play important roles in soil ecosystems as biological indicators of food web structure. Increases in predatory nematode abundance are common in response to applications of organic materials, which is desirable in agricultural systems (Forge *et al.,* 2003; Oka, 2010; McSorley, 2011). Predatory nematodes consuming plant-parasitic nematodes and opportunistic nematodes can act as a check on unregulated population growth that could otherwise occur under nutrient enriched conditions (Bongers and Bongers, 1998; Ferris, 2010).

As farmers look for opportunities to diversify their crop production and reach underserved ethnic markets, they need an understanding of best practices for cultivating ethnic crops. However, it is unknown how ground covers influence yield of jute leaf and associated nematode communities in the Mid-Atlantic US. Information on impacts of ground covers on the nematode community in Maryland may help identify the structure of the soil food web and potential risks of plant-parasitic nematodes to jute leaf. To fill this gap in knowledge, we conducted a preliminary investigation to ascertain how ground covers influenced yield of jute leaf and the nematode community. We used four ground cover treatments (compost, compost and landscape fabric, compost and straw, and compost and wood mulch) to grow three cultivars of jute leaf (Firebird, Molokhia, and

USDA PI 404029) in Maryland.

## 2. Materials and Methods

*Study site*

We established experimental plots in May 2023 at the University of the District of Columbia's (UDC) Firebird Research Farm (39°3'11.1492 N, 76°52'52.716 W). The soil was classified as a Russett-Christiana complex, with a fine loamy, mixed, semiactive, mesic Aquic Hapludults for the Russett series, and a fine, kaolinitic, mesic Aquic Hapludults as part of the Christiana series. Soil texture was 39% sand, 36% silt, and 25% clay. Plots were solarized prior to planting to kill weeds. Plots were hand weeded as necessary during the study and no pesticides or fertilizers were applied.

*Treatments*

We used four ground cover treatments and three jute leaf cultivars in the study. Ground cover treatments were applied to 0.9 m by 9 m plots and were arranged as a randomized complete block design in a 4×3 factorial with four replicates (Supplementary materials - SM - Figs. 1S - 2S). Each ground cover plot was subdivided into three 0.9 m by 3 m subplots, with each subplot randomly being assigned one of the three cultivars. Fifteen centimeters of mushroom compost was applied on top of the ground of the entire study area with a C:N ratio of 9:1. The four ground cover treatments chosen were: mushroom compost only (compost), landscape fabric (fabric), straw, or wood chips. Each treatment was placed on top of the 15 cm of mushroom compost by the beginning of June (5 June 2023). Mushroom compost and straw were purchased from Purple Mountain Organics (Takoma Park, MD). Landscape fabric used in the study was Sunbelt Black Ground Cover 3.2oz (DeWitt, Sikeston, MO). Wood chip mulch was produced in 2019 from a mixture of local softwood and hardwood trees felled from Firebird Farm and chipped for use as mulch. A compost sample was sent to Waypoint Analytical Inc. (Leola, PA) to determine C:N content (Peters *et al.,* 2003). The three jute leaf cultivars included Firebird (developed at UDC), Molokhia (Egyptian spinach) (Kitazawa Seed Co, Oakland, CA), and USDA PI 404029 (USDA germplasm repository). Seeds were soaked overnight and planted in Fort Vee potting mix (Vermont Compost, Montpelier, VT) in 50 cell trays and grown under high tunnel conditions for 30 days prior to transplanting.

*Soil sampling and data collection*

Soil samples were collected after a soil solarization (pre-treatment) in early May (8 May 2023). We collected midseason and final samples on 10 August and 29 September, respectively. The experiment lasted a total of 144 days from soil pre-treatment to collection of final samples. We collected six 3 cm × 20 cm soil cores from the center of each plot. The six cores were combined and homogenized into a single composite sample per plot, placed in individual polyethylene sample bags, and then placed into a cooler for transportation to the lab where they were stored at 4.5°C. Soil cores were homogenized and nematodes were extracted from 100 $cm^3$ soil using centrifugal sugar floatation (Jenkins, 1964).

Nematodes were fixed in 2% formalin and the genera of a subset of 100 nematodes were identified from each sample using an inverted microscope (Zeiss, Oberkochen, Germany). Relative abundance was determined by multiplying the proportion of each genus in the sample by the total number of nematodes in the sample. Nematodes were categorized into functional groups, based on their diet, and colonizer-persister (cp) groups (Bongers, 1990; Yeates *et al.,* 1993). The cp numbers assigned to genera reflect life history traits. Numbers near one correspond with *r* strategists that are associated with nutrient enriched and disturbed ecosystems and numbers near five correspond to *K* strategists that are associated with stable ecosystems (Bongers, 1990; Yeates *et al.,* 1993; Ferris *et al.*, 2001). We calculated ecological indexes from nematode counts using the Nematode Indicator Joint Analysis (NINJA) online tool (Sieriebriennikov *et al*., 2014). We also measured fresh aboveground plant biomass at the end of the season by cutting plant stems 7.5 cm above the soil surface and measuring the mass of each plant.

*Statistical analysis*

We compared the effects of ground cover, cultivar, and the interaction of ground cover and cultivar on nematode abundance and index means using analysis of covariance (ANCOVA). $Log_{10}$ transformations were performed on data prior to analysis to improve normality and homogeneity of variance. Mean relative abundance of nematodes from midseason and final sampling dates were each analyzed individually using pre-treatment counts as a covariate. ANCOVA was selected to help account for seasonal variation by including pre-treatment counts as a covariate. Means of plant biomass across ground cover treatments and cultivars were compared using analysis of variance (ANOVA). Significant results ($P \leq 0.05$) were separated with Tukey's HSD. Significant differences presented at ≤0.05 occurred within an individual sampling date (midseason or final). Statistical analyses were conducted in R using 'Agricolae' package (R Core Team, 2019; de Mendiburu, 2021). Graphics were generated using ggplot2 (Wickham, 2016).

## 3. Results

We processed 144 soil samples during the study. Twenty-seven nematode genera were identified, with six classified as plant-parasitic nematodes, ten as bacterivores, five as fungivores, four as omnivores, and two as predators (Table 1). Bacterivores were

Table 1 - Nematode genera and proportion of all 144 soil samples that contain each genus from the study in Beltsville, MD

| Functional group [z] | Genus | cp or pp value [y] | Proportion of samples genus was identified |
|---|---|---|---|
| Plant-parasite | | | |
| | *Criconemella* | 3 | <0.01 |
| | *Helicotylenchus* | 3 | 0.94 |
| | *Heterodera* | 3 | <0.01 |
| | *Hoplolaimus* | 3 | 0.03 |
| | *Paratylenchus* | 2 | 0.28 |
| | *Pratylenchus* | 3 | 0.04 |
| Bacterivore | | | |
| | *Acrobeles* | 2 | 0.03 |
| | *Alaimus* | 4 | 0.15 |
| | *Butlerius* | 1 | 0.45 |
| | *Cephalobus* | 1 | 0.44 |
| | *Diploscapter* | 1 | 0.10 |
| | *Eucephalobus* | 1 | 0.80 |
| | *Panagrolaimus* | 1 | 0.03 |
| | *Plectus* | 2 | 0.26 |
| | *Prismatolaimus* | 3 | 0.60 |
| | *Rhabditis* | 1 | 1.00 |
| Fungivore | | | |
| | *Aphelenchoides* | 2 | 0.22 |
| | *Aphelenchus* | 2 | 0.79 |
| | *Diphtherophora* | 3 | 0.05 |
| | *Ditylenchus* | 2 | 0.44 |
| | *Tylenchus* | 2 | 1.00 |
| Omnivore | | | |
| | *Aporcelaimus* | 5 | 0.04 |
| | *Dorylaimoides* | 4 | <0.01 |
| | *Eudorylaimus* | 4 | <0.01 |
| | *Prodorylaimus* | 4 | 0.02 |
| Predator | | | |
| | *Ironus* | 4 | 0.10 |
| | *Mononchus* | 4 | 0.27 |

[z] Functional groups assigned to genera based on nematode genus primary dietary preference (Yeates *et al*., 1993).
[y] Colonizer-persister (cp) or plant-parasite (pp) number according to (Bongers, 1990). Cp and pp numbers near one correspond with *r* strategists that are associated with nutrient enriched and disturbed ecosystems and numbers near five correspond to *K* strategists that are associated with stable ecosystems (Ferris *et al.*, 2001).

the most abundant functional group followed by fungivores (SM Tables 1S, 2S). The most prominent genera were *Rhabditis* (58% of total nematodes) and *Tylenchus* (25% of total nematodes). *Helicotylenchus* was the most abundant plant-parasitic nematode genus, representing 93% of all plant-parasitic nematodes. *Paratylenchus* was the second most abundant genus, representing 6% of the total plant-parasitic nematodes.

*Ground cover effects*

Nematode counts and ecological indexes were not different among ground cover treatments at midseason sampling, but were during the final sampling period (Figs. 1, 2). Total plant-parasitic nematodes numbers were more than two times as abundant in straw plots than compost only plots ($P$= 0.02). Plots with wood chips had greater *Helicotylenchus* abundance than plots with compost only ($P$= 0.03). Straw plots had the highest counts of *Prismatolaimus* ($P$= 0.02) and cp 3 nematodes ($P$= 0.02) compared to fabric plots as well as the highest counts of *Mononchus* ($P$= 0.03), total predators ($P$= 0.03), and cp 4 nematodes ($P$= 0.04) compared to wood chip plots. Whereas, the range of count means for *Rhabditis* greatly increased from pre-treatment samples (55-150) to midseason samples (671-1044) ($P$= 0.7) and final samples (682-1004) ($P$= 0.6), no differences occurred among treatments (SM Table 1 S).

Maturity index 2-5 ($P$= 0.03) and structure index ($P$= 0.05) were also greater in plots with straw compared to those with fabric at the final sampling date (Fig. 2). Across all treatments at the end of the season, the range of enrichment index means were near the upper limit of possible values at the end of the study (87-91) ($P$= 0.4) and the range of channel index means were approaching the lowest limit of possible values at the end of the study (9-15) ($P$= 0.6), but no differences occurred among treatments for either index (Fig. 2). Mean biomass for the four ground covers ranged 1.39-1.69 kg, but was not statistically different among ground cover treatments ($P$= 0.4).

*Effects of plant cultivars*

*Plectus* and the predator functional group differed across cultivars (Fig. 3). *Plectus* counts at midseason were higher in plots with USDA PI 404029 than Molokhia or Firebird ($P$= 0.01). Predators were more numerous at midseason in Firebird plots than

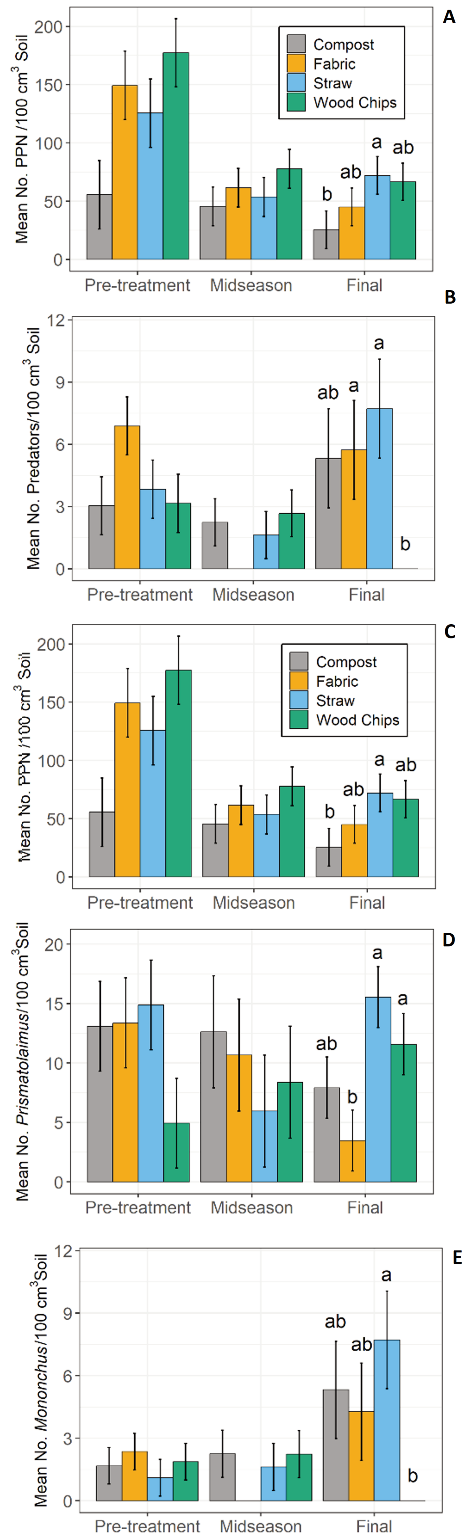


Fig. 1 - Mean number of nematodes categorized by functional group or genus, with standard errors of the mean, across three sampling periods (pre-treatment, midseason, and final) and four ground cover treatments: compost only; compost+fabric; compost+straw; compost+wood chips. Different letters indicate differences between treatments within a sampling date (Tukey HSD, P≤0.05). PPN = plant-parasitic nematodes.

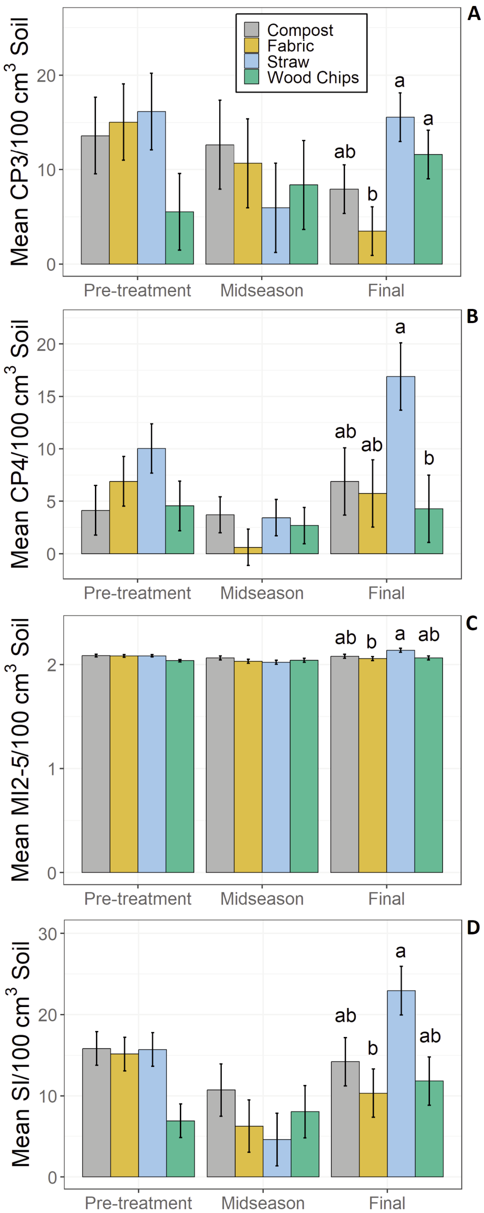


Fig. 2 - Mean number of nematodes that were categorized as colonizer persisters (cp) or ecological indexes. Means, with standard errors of the mean, were presented across three sampling dates (pre-treatment, midseason, and final) and four ground cover treatments: compost only; compost+fabric; compost+straw; compost+wood chips. Different letters indicate differences between treatments within a sampling date (Tukey HSD, P ≤ 0.05). cp 3 = colonizer-persister group 3; cp 4 = colonizer-persister group 4; MI2-5 = maturity index colonizer-persister groups 2-5; SI = structure index. Colonizer-persister groups are based on life history traits with values approaching one representing *r* selection strategists and values approaching five representing *K* selection strategists. MI2-5 is a measure of environmental disturbance with values approaching zero indicating high disturbance and values approaching five indicating low disturbance. SI is a measure of food web complexity with values approaching zero indicating low food web complexity and values approaching 100 indicating high food web complexity.

Molokhia and USDA PI 404029 (*P*= 0.02). No ecological indexes differed across cultivars (*P*>0.05) (SM Table 2S). Mean biomass ranged 1.42-1.72 kg, but the effect of cultivar was not significant (*P*= 0.2).

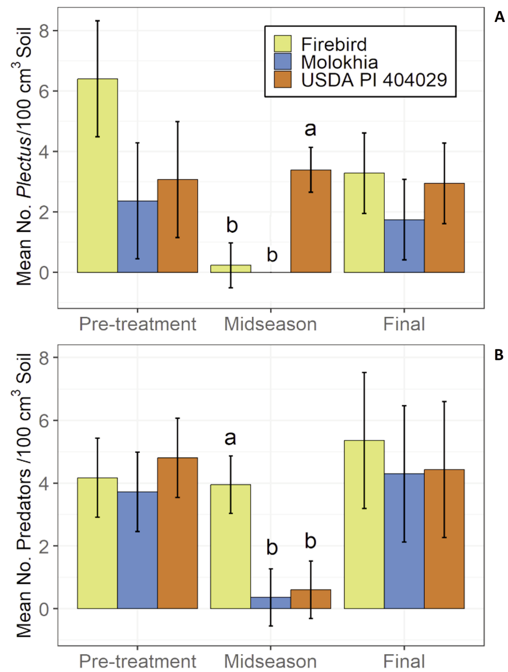


Fig. 3 - Mean relative abundance of nematode Plectus (A) and predatory nematodes (B), with standard errors of the mean, across three sampling periods (pre-treatment, midseason, and final) and three cultivars of jute leaf: Firebird; Molokhia; and USDA PI 404029. Different letters indicate differences between treatments within a sampling date (Tukey HSD, P≤0.05).

## 4. Discussion and Conclusions

In this study we demonstrate how ground cover treatments on jute leaf, has a direct impact on the nematode community. No single ground cover enhanced abundance of all free-living nematodes, but nematode abundance differed across the treatments. Plots with straw had higher counts of the free-living bacterivore *Prismatolaimus* and predator *Mononchus* by the end of the season than the others treatment. Higher counts of *Prismatolaimus* contributed to the increase of cp 3 nematodes and structure index in straw plots at the end of the study. Differences in abundance of *Mononchus* largely contributed to increases in cp 4 nematodes, predators, and maturity index 2-5 in straw plots at the end of the study. Organic mulch applications can stimulate increases of bacterivore and predatory nematode abundances (Ferris and Bongers, 2006;

Wang *et al.,* 2008; Pavao-Zuckerman and Sookhdeo, 2017). *Prismatolaimus* and *Mononchus* are indicators of preliminary structure development and become more common as enriched conditions transition to more stable conditions (Ferris *et al.,* 2001). Use of amendments with high carbon content such as straw can result in greater free-living nematode abundance compared to using nitrogen rich sources such as manures (Liu *et al.,* 2016). Soil amended with products that include straw can have positive effects on physical properties of soil that are favorable for nematodes such as increased moisture retention and enhanced soil porosity as well as providing high levels of carbon that stimulates growth of microbial food sources (Zhao *et al.,* 2009). Elevated nutrient levels occurring following applications of straw mulch can lead to an increase of *Prismatolaimus* abundance and omnivore-predator metabolic activity (Song *et al.,* 2020). Predator abundance has been shown to increase following application of compost mixtures with straw and leaf litter mulches in barley and tomato production systems (Renčo *et al.,* 2010; Petrikovszki *et al.,* 2021).

*Helicotylenchus* was the dominant plant-parasitic nematode in our study in line with what already presented in natural environment by other studies (Babatola, 1983; Atungwu *et al.,* 2013). Abundance of *Helicotylenchus* numerically declined across all ground cover treatments from pre-treatment to midseason, and remained low at final sampling. Compost only plots had lower abundance of *Helicotylenchus* than straw and lower total plant-parasitic nematode abundance than wood chips at the end of the season. Materials with high C:N content such as straw or wood chips can be less effective at suppressing plant-parasitic nematodes than low C:N content mulches like compost or manure (Liu *et al.,* 2016; Hornung *et al.,* 2020). Low C:N content materials may better facilitate growth of nematode antagonists or make soil conditions less favorable by altering pH through soil acidification, though these mechanisms need further evaluation (Liu *et al.,* 2016; Ye *et al.,* 2018; Martinez *et al.,* 2023). Despite the differences in plant-parasite abundance, no differences occurred in plant yield. Higher *Helicotylenchus* abundance in plots with wood chips was accompanied by zero presence of predatory nematodes. The lack of predatory nematodes may have resulted in less top-down regulatory pressure on *Helicotylenchus* reproduction in plots with wood mulch compared to other treatments.

Enriched conditions dominated by bacterial composition with low community structure were observed in this study, which are commonly associated with addition of organic amendments (Thoden *et al.*, 2011). Bacterial feeders in the genus *Rhabditis* greatly increased following applications of all ground cover treatments. The enrichment and channel indexes were strongly influenced by the drastic change in *Rhabditis* abundance. *Rhabditis* nematodes are categorized as cp 1 bacterial feeding nematodes which are opportunistic and respond quickly to nutrient enriched conditions (Bongers and Bongers, 1998; Ferris and Bongers, 2006). Flushes of bacterial growth in response to compost applications provides abundant resources for *r*-strategists like *Rhabditis* which often drives rapid population growth (Bulluck *et al.*, 2002; Ferris and Bongers, 2006; Fengjuan *et al.,* 2020).

In our study, densities of *Plectus* were greatest in USDA PI 404029 plots and predator densities were greatest in Firebird plots at midseason sampling. The counts were very low among the three cultivars at midseason and impacts were temporary and did not last to the end of the season. Plant species diversity can impact nematode abundance and diversity (Yeates 1999; Porazinska *et al.,* 2003; de Deyn *et al.,* 2004). For example, bacterivore abundance generally increases as plant species diversity increases, but responses of predatory nematodes can be more variable and take place more gradually (Viketoft *et al.,* 2011; Kostenko *et al.,* 2015; Cortois *et al.,* 2017; Dietrich *et al.,* 2021). Impacts of intraspecific plant diversity on functional groups of soil invertebrates are less well studied, but increases of plant diversity can influence soil communities, particularly at lower trophic levels (Koricheva and Hayes, 2018; Yan *et al.,* 2021). For example, omnivore-predator abundance varied between individual genotypes of *Phragmites australis*, whereas bacterivore nematode abundance was affected by the overall genetic diversity of *P. australis* genotypes (Yan *et al.,* 2021). The mechanism of how plant genetic diversity impacts free-living nematodes is not clearly understood. Resource quantity (plant biomass) and root quality (C:N ratio) may play a role in influencing nematode communities, especially where levels of organic matter in soil are low (Bezemer *et al.,* 2010; Cortois *et al.,* 2017; Dietrich *et al.,* 2021). Secondary compounds produced by roots may also influence microbial communities and could also shape nematode communities from the bottom up (Bezemer *et al.,* 2010). Cultivar effect in our study could have been more pronounced earlier in the season, but likely had a lesser overall impact on the nematode community. Effects from organic matter decomposition from mulches may have been limited earlier in the season, but became more pronounced as nematode populations may have responded to changes in the availability of resources.

Our findings indicate that ground covers derived from organic materials and urban wastes can influence nematode communities in plots of jute leaf in Maryland. Our study was limited to a single year because of insufficient funding and capacity to continue for additional years. Future research incorporating multi-season trials in a broader geographic area could validate these findings and help growers select a ground cover that promotes nematode communities beneficial to soil health. Information is also needed on ecological impacts of producing other ethnic crops on the soil community in the Mid-Atlantic US.

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