# Chapter 10

# Nematodes as indicators of soil health

*Deborah A. Neher, University of Vermont, USA; and Krisztina N. Mosdossy, Cornell University, USA*

## 1 Introduction

The sheer abundance of nematodes, their biodiversity, and occupation of every trophic niche and ecosystem type make them one of the most useful ecological indicators amongst all soil microinvertebrates (Freckman, 1988; Neher, 2001a). Globally, there are an estimated 4.4 ± 0.64 × $10^{20}$ nematodes (total biomass of approximately 0.3 Gt) inhabiting surface soils, with greater abundance in sub-Arctic regions (38%) than in temperate (24%) or tropical (21%) (van den Hoogen et al., 2019). Nematodes are not only ubiquitous but also serve as key indicators of important soil ecosystem functions, in part because they occupy at least five functional or trophic groups, ranging from grazers of primary decomposers to predators of other nematodes and plant-parasites (Yeates et al., 1993). Their central position in the detrital food web allows them to infer soil carbon (C) and nutrient cycling (Moore and de Ruiter, 1991). All nematodes release excess ammonium ($NH_4^+$) into soil, a form readily available to plants. Bacterivores and fungivores graze upon and disperse bacteria and fungi, respectively, which tends to maintain microflora populations in the rhizosphere at a 'youthful' state

http://dx.doi.org/10.19103/AS.2025.0159.11

with fast rates of growth, selectively shaping the microbial community in ways that promote plant growth (Ingham et al., 1985). The breadth of ecological function that nematodes influence makes them excellent soil health indicators.

The concept of nematodes as indicators of soil health and their predictive use in soil management represents a major shift in emphasis in the science of nematology (Beaumelle et al., 2021). Formerly a science focused on the control of parasitic and harmful species, now nematodes are recognized for their contribution to biological processes in the soil. By the 1980s, nematologists were beginning to recognize and promote the use of nematode community indicators, including free-living (beneficial) nematodes, as metrics for assessing terrestrial soil health (Freckman, 1988). Nematodes make effective indicators because they occupy a trophic level above microbes in the food chain and integrate physical, chemical, and biological properties related with their food resources (Neher, 2001a). Thus, soil chemical properties (sand, clay, pH) and fungal: bacterial biomass ratio account for only 23% of the variation explained by nematode communities (Neher and Campbell, 1994). Initial attempts explored various ratios and individual trophic groups (Freckman and Ettema, 1993) but it was really Bongers' (1990) paper that introduced the 'maturity index' that was a major advance; the index, which based on family level assessment, was more practical than indices based on species (Ritz and Trudgill, 1999). Through time, improvements to calculating nematode indices of soil health evolved into a sophisticated and rigorous system that reflects resource availability, disturbance, and ecological succession.

Ecological succession is a helpful concept in understanding the maturity stage of ecosystems, including agroecosystems. In nematode ecology, the use of family-based 'maturity' indices provides insights into the ecological successional status of a soil community, which can reflect its history of disturbance (Neher, 2010). A major disturbance, such as fumigation or tillage, pushes the community back to early stages of succession, where recovery can be tracked with increased values of maturity indices (Ettema and Bongers, 1993). In these early successional stages, nematode communities are dominated by organisms with short generation times, small body sizes, rapid dispersal, and generalist feeding habits, such as bacteria and bacterivorous nematodes (Beare et al., 1992). If left undisturbed, the soil community transitions to include fungi and fungivorous nematodes and eventually omnivores and higher-level predators.

Originally, the maturity index (MI) was based solely on free-living nematodes, while plant-parasites were separated into a separate plant-parasitic maturity index (PPI). Both MI and PPI are univariate indices, distilling the community composition into a single numeric value, but the concept can be partitioned into dimensions that represent structural complexity (SI) and nutrient enrichment (EI) (Ferris et al., 2001). Since 2014, the popularity of EI and SI has increased, likely because of their associated graphic and suggested interpretations (Du Preez et al., 2022). This faunal analysis diagram (Fig. 1) is particularly useful for explaining differences between locations, cropping systems, or ecosystems but not changes in management in a single location (Du Preez et al., 2022). Finally, approaches focusing on biomass as a metric of a metabolic footprint (MF) have been introduced (Ferris, 2010). The footprint approach assigns biomass units to published widths and lengths of adult nematodes (e.g. Ferris, 2010; Neher and Weicht, 2013). Once converted to carbon units, estimates of C fluxes can be estimated and linked to decomposition and climate change models or incorporated into energetic food web models (Buchkowski et al., 2023).

In this chapter, we will cover several key topics: methods for sampling and extraction to aid standardization, identification techniques (comparing both morphological and molecular approaches), index calculations and interpretation. For each main topic, we provide recommendations based on current practices and highlight approaches for improving the detection of changes that are crucial for quantifying soil health, such as the role of sentinel

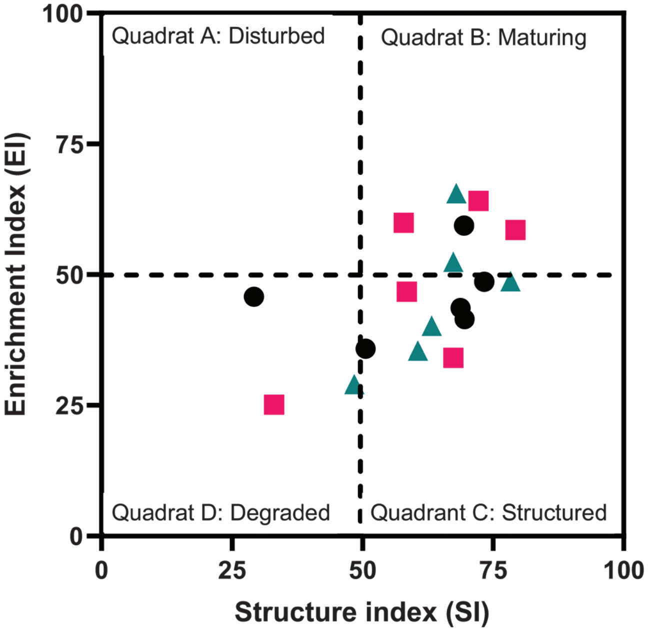


**Figure 1** Plot of enrichment vs. structure indices of different management practices of field corn (*Zea mays* L.) at harvest. Experimental plots varied in EI and SI but these differences were not explained by corn treatment. Black circle: a Coleopteran Bt hybrid expressing the Cry3Bb1 protein, Red square: a non-Bt isoline treated with insecticide tefluthrin as positive control; Teal triangle: a non-Bt Isoline without insecticide as negative control. Data from Neher et al. (2014) *Soil Biol. Biochem.*, 76, 127–135.

species, ecological interactions, and links to ecosystem function. Finally, example case studies will be described to illustrate the application of nematode indicators as metrics of soil health.

## 2 Developments in nematode detection

Most commercial and public laboratories only process plant-parasitic nematodes for agronomic and quarantine purposes. However, indices of nematode communities rely on free-living nematodes that constitute the majority of nematodes found in most soil samples. Commercial laboratories that handle large numbers of samples for identification of free-living nematodes are rare (Neher, 2010). Successional indices necessitate the identification of entire communities, at least to the family level, and an agricultural sample may contain over 25 families and 50 genera of free-living nematodes (e.g. Neher et al., 1995; Neher et al., 2014; Neher et al., 2019). Unfortunately, morphological identification to an adequate taxonomic level is time-consuming and poses a high barrier to entry for novices. The challenge is to simplify and expedite sample processing while enhancing the accuracy and precision of detecting changes in management practices and environmental conditions. Several approaches can address these challenges and yield synergistic results. For instance, focusing on sentinel taxa and developing molecular tools can significantly streamline the process.

Successful use of nematodes as indicators requires careful attention to sampling (Barker, 1985a; Neher and Campbell, 1996) and extraction methods (Barker, 1985b; Neher et al., 1995). The primary goal of these processes is to collect and extract a sample that accurately represents the nematode community at any given time without bias related to specific locations where cores are taken. Importantly, no method of collecting or extracting nematodes can be used in all situations (Freckman and Baldwin, 1990). Regardless of the method employed, consistency is key: samples should be taken at the same time of year, from the same depth and volume, and using the same technique.

### *2.1 Sampling*

Nematode communities shift dynamically with the seasons and plant phenology (cropping cycle), reflecting climatological variations more accurately than calendar dates. A single sample taken at one point in time will not capture the temporal dynamics and should be interpreted within its specific context rather than generalized (Freckman and Baldwin, 1990). For annual crops, the ideal sampling times are spring before planting or pre-plant field operations; mid-season, around anthesis when root growth stops; and fall, near harvest. If only one sample can be taken in a year, and the focus is on plant-parasitic nematodes, the best time to sample is near harvest when plants are mature and potentially more susceptible to pathogens.

Distribution of nematodes varies both horizontally and vertically, influenced by plant roots, litter, and soil type. Therefore, it is essential to collect multiple cores across the area of interest to ensure representativeness and obtain sufficient volume for extraction. These cores are then combined to form a composite sample, which is subsampled before extraction and identification. The minimum number of cores to be collected for one soil sample is as follows: 10 cores for small plots (1–5 $m^2$), 20+ cores for medium plots (5–100 $m^2$), and 30+ cores for large plots (>100 $m^2$). Mixing the cores within a composite sample is best done by hand because mechanical mixing can damage nematodes (Neher et al., 1995; van Bezooijen, 2006).

Given the spatial aggregation or clustering of biological and abiotic soil characteristics, systematic sampling yields more reliable results than random sampling (Barker et al., 1985a). The importance of a systematic pattern, combined with a random starting point, lies in providing a representative sample and reducing bias. A systematic approach means collecting cores at multiple locations equal distance apart. The pattern can be a line, zigzag, serpentine, or points on a grid (Neher et al., 1995). The placement of cores should depend on the specific research question. For instance, studies focused on plant productivity may require sampling in the plant rhizosphere, whereas regional monitoring programs necessitate sampling across different soil types, vegetation, and both rhizosphere and bulk soil (Neher et al., 1998). Another useful approach is to use a stratified random pattern where strata represent different soil textures, vegetation type, or management practices.

Soil samples for nematode analysis are typically collected using an Oakfield core (2.0 cm diameter) to a depth of 15–20 cm (van Bezooijen, 2006). This depth was historically chosen to represent the plow pan, indirectly reflecting rooting depth. Nematodes reside in soil wherever there are roots or decaying detritus. Therefore, in ecosystems with deep-rooting vegetation, such as grasslands or forests, sampling may extend deeper, e.g. to 40 cm.

Samples should be stored in plastic bags to avoid dehydration and kept in an insulated container in the shade to maintain the temperature they were at in the field prior to sampling. Extreme temperatures will damage the samples. The standard practice is to store samples at 4°C (van Bezooijen, 2006); however, there are valid reasons to store samples at 10°C (Coyne et al., 2014) or 15°C (Neher et al., 1995). At 15°C, nematodes may reproduce, but the community structure remains relatively constant (Barker et al., 1969).

For regional and national scale environmental monitoring programs, special considerations regarding sampling design and number of samples are crucial. To enable quantitative inferences about the proportion of land area, the distribution of selected fields across the region should correspond to the hectarage of each cropping system from the previous year (Neher et al., 1998). Based on this design, Neher and Campbell (1996) established recommendations for how many composite samples (transects) to collect per field and the number of subsamples to assay for each transect to achieve a high degree of sample reliability.

### *2.2 Extraction and their biases*

Extraction should occur as soon as possible, but it is important to note that nematodes tend to store better in soil than after extraction. Samples can be stored in soil for up to 6 months without significant effects on nematode populations (van Bezooijen, 2006). Once extracted, sample integrity has a limited shelf life unless nematodes are preserved with a fixative.

Both morphological and molecular identification techniques necessitate the extraction of nematodes from soil to obtain a clean sample. Attempts to extract DNA directly from soil samples often lead to the amplification of non-target taxa such as fungi, plants, and other metazoans, which can reduce the sequence reads for nematodes (Sapkota and Nicolaisen, 2015).

No extraction method guarantees 100% efficiency. It is essential to choose the most appropriate method for each situation while maintaining consistency and standardization. Extraction methods vary in their efficacy for retrieving nematodes of differing mobility and trophic groups (Table 1). Additionally, variations in extraction methods can be compounded by differences between laboratories. Therefore, results should be interpreted in the context of laboratory and extraction method interactions rather than considering the extraction method alone.

Extraction methods were developed first for plant-parasitic nematodes focusing on high throughput, possible with elutriation methods that rely on size, shape, and sedimentation rate differences between nematodes and soil particles. Unfortunately, these elutriation methods, while efficient for extracting plant-parasitic nematodes, tend to overlook important ecological groups. Specifically, they extract omnivores and predators an average of 5.5 and 6.6 times less frequently, respectively, than the Cobb's sifting and gravity method when applied to subsamples of the same composite soil sample (Neher et al., 1995).

Ecological studies have largely neglected the extraction of endoparasites from roots, because this often necessitates a separate method and longer incubation period (Freckman and Baldwin, 1990). Nematodes can be extracted from litter/roots using a mist extraction (Ayoub, 1980) or funnel spray (van Bezooijen, 2006) method. While these methods yield clean samples, they require the construction of a misting apparatus (Ayoub, 1980).

Most extraction methods are indirect, relying on various properties to separate nematodes from their surrounding medium, such as weight and settling rate, size and shape, and mobility. The Baermann method has been adopted by international programs and global surveys (Guerra et al., 2021) due to its simplicity and low cost. However, the method relies on the active mobility of nematodes, which can result in under-sampling slow-moving or sedentary species. In this technique, samples are either wrapped and placed in a funnel (Baermann) or placed on top of a filter in a collection tray or pan. Nematodes must then swim through the material into free water, where they settle.

Flotation methods involve using an extraction fluid with a higher specific gravity than nematodes, allowing the nematodes to float during centrifugation. The supernatant is then passed through a fine sieve (25–35 μm) to concentrate the sample. Common extraction fluids include sugar, $MgSO_{4,}$ or $ZnSO_4$, all of which have their unique challenges and can distort the shape of nematodes making identification difficult (van Bezooijen, 2006). For example, sugar is inexpensive but sticky to work with and can create an osmotic gradient that may cause nematodes to shrivel if not rinsed properly.

Generally, Cobb's method and centrifugal flotation with pre-extraction is more efficient than Baermann or centrifugal flotation without pre-extraction (van Bezooijen, 2006). Pre-extraction is typically performed using a sieving method. Sieves and flotation techniques capture both mobile and sedentary nematodes, whether dead or alive.

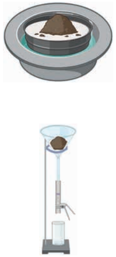
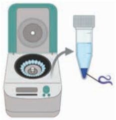
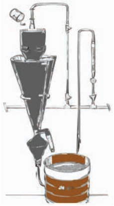
Sieving approaches are effective for extracting of all types of nematodes but require slightly more specialized equipment and skill. The approach is also known as the Modified Cobb's decanting and sieving method or Cobb's sifting and gravity method (Ayoub, 1980). Although more time consuming, the extraction efficiency increases significantly (up to 90–95% efficiency) when more sieves are used and each sieve is passed through three times (Neher et al., 1995). However, sieves with finer mesh sizes can be expensive and are prone to damage. After the final pass, the sample can be concentrated and cleaned using centrifugal flotation (e.g. Bekker et al., 2022) or a cotton-wool filter extraction tray, where the sample is left for 48 h (e.g. Darby et al., 2007).

The final step in all extraction methods is to create a suspension of nematodes in water. Because nematodes settle at a rate of approximately 1″ (2.54 cm) per hour, it is crucial to remix the suspension before counting and identification to ensure a representative sample. One effective way to suspend and mix the aqueous samples is by using an aquarium bubbler.

### *2.3 Morphology*

Fresh specimens are valuable for identification because movement patterns are a key feature. However, tracking a moving target can be challenging, and

**Table 1** Extraction method comparison

| | Baermann  | Centrifugal flotation  | Sieving  | Misting  | Elutriator  |
|---|---|---|---|---|---|
| Principal | Nematodes crawl out of material into the water; use facial tissue, milk filters, napkins, or cheesecloth to contain sample | Based on differential specific gravity so nematodes float; supernatant through fine sieve | Wet slurry containing nematodes passed through series of sieves to progressively smaller mesh opening | Nematodes crawl out of material into water; Every 10 min sprinkler switched on for 1.5 min. | Makes use of difference in size, shape, and sedimentation rate between nematodes and soil particles |
| Variations | Funnel, pie pan, tray, filter, Whitehead tray, cotton-wool extraction tray | Sugar, magnesium sulfate, zinc sulfate | Cobb's sifting and gravity; Modified Cobb's decanting and sieving 500–1000 µm, 350–375 µm, 175 µm, 100 µm, 45 µm | Synonym: funnel spray; means to handle roots & litter | Oostenbrink |
| Bias | Mobile, standardized time (72 h; harvest every 24 h to avoid decay and deterioration) | Both mobile and inmobile | Both active and inactive | Active only | Omnivores and predators under-represented |

| | | | | | |
|---|---|---|---|---|---|
| Sample size | 25–50 g | | 100–500 g | | ≤ 250 mL |
| Advantages | Simple, inexpensive, fairly clean sample | Immobile nematodes; ~ 1 h and relatively clean suspension | High extraction efficiency using only small set of sieves and little water; tapwater okay | Avoids oxygen depletion, clean samples | Efficient and easy to standardize |
| Disadvantages | Can go anaerobic; 72 hours (harvest every 24 hr); avoid tapwater; kimwipes have a mesh that reduce efficiency; Poor extraction of large & non-mobile, temperature and oxygen dependent | Requires swinging bucket centrifuge | Nematodes can be easily damaged; sieves can be expensive and require an experienced worker; relatively time consuming | May take a few weeks for all nematodes to crawl out of roots or litter | Expensive equipment requires large amounts of water |
| Van Bezooijen, J. (2006) | Figure 9 | Figure 19 | Figure 11 | Figure 11 | Figures 16 and 17 |

Van Bezooijen, J. (2006) *Methods and Techniques for Nematology*. Wageningen University, The Netherlands. https://www.scribd.com/document/234069625/Methods-and-TechniquesforNematology_ the shelf-life of samples is limited. To prevent decay in aqueous solutions, samples are typically fixed in a preservative, such as formalin or a DMSO/EDTA/saturated NaCl solution (DESS; Yoder et al., 2006). While temporary mounts are typically used for ecological studies, permanent mounts in anhydrous glycerol are essential for archiving samples (Freckman and Baldwin, 1990). Temporary mounts can be created using an 8% formalin solution (Darby et al., 2007). Formalin is a known carcinogen so good ventilation or use in a fume hood is recommended. Individual nematodes can be mounted on microscope slides, but mass mounts of mixed taxa onto slides is a time-saving step. The paraffin wax ring method (van Bezooijen, 2006) is recommended, utilizing a round cover slip with edges sealed with fingernail polish.

Ecologists typically identify at least 10% or up to 150 representative individuals per sample to the genus level (Darby et al., 2007). If fewer than 100 nematodes are present in a sample, all individuals should be identified (Neher et al., 2017). When counting a subsample, it is necessary to determine the total number of nematodes within a sample to convert subsample identifications into proportions of the full sample. Any count values should be adjusted to reflect numbers per unit dry weight or volume of soil.

### *2.3.1 Resolution*

Identifying nematodes to at least the family or genus level requires use of a compound microscope. Optical filters, such as differential interference contrast (DIC), can increase visibility by increasing contrast, making it easier to distinguish fine features in these transparent organisms. Additionally, specific stains like lactophenol cotton blue, acid fuchsin, or Lugol’s solution can further enhance certain features. Freckman and Baldwin (1990) provide a comprehensive key for classifying classes and orders of terrestrial nematodes; however, this resolution is often insufficient for computing community indices. Beginners may benefit from a simpler pictorial key that classifies nematodes into eight groups based on the presence a stylet and the nature of the stylet or stoma (Fig. 2 and Fig. 3 in Neher, 2023).

### *2.3.2 Useful keys*

Unfortunately, there is no single key available to identify all groups of free-living nematodes within a sample. Within the Nematoda phylum there are 5 classes, 39 orders, and 241 families (Hodda, 2007). About 14 000 species of free-living, invertebrate- and plant-associated nematode species are known, described and accepted. There are an additional 12 000 species of nematode parasites on vertebrates (Hodda et al., 2009). Different keys specialize in different taxa and geography. Some popular keys include Jairajpuri and Ahmad (1992) or Andrássy (2009) for Dorylaimida; Hunt (1993) for Aphelenchida, Longidoridae, and Trichodoridae; de Ley et al. (2003) for Secernentea; and Siddiqi (2000) for Tylenchida. There are keys that focus on specific countries such as The Netherlands (Bongers, 1994), Hungary (Andrássy, 2005; Andrássy, 2007; Andrássy, 2009), or northern Great Plains of the US (Thorne, 1974). General keys are also available, such as Nickle (1991) for agriculture and Abebe et al. (2006) for freshwater and soil nematodes. However, there is a pressing need for a comprehensive key that covers free-living nematodes across all orders, which would eliminate the necessity of consulting multiple keys.

## *2.4 Molecular*

### *2.4.1 Universal barcode*

While morphological identification provides real-time, genus-specific, and accurate community-level results, nematodes exhibit phenotypic plasticity which complicates the identification process. In contrast, molecular methods hold promise for characterizing entire communities using standard polymerase chain reaction (PCR) techniques. However, these methods face limitations due to the scarcity of DNA probes and primers (Table 2). For example, primers designed for *Caenorhabditis elegans* do not work effectively on other bacterivorous nematodes, including members of the families Cephalobidae and Panagrolaimidae, as well as the genus *Plectus* (Neher and Stürzenbaum, 2006). The mitochondrial protein coding gene, cytochrome *c* subunit I (COX-1), emerged during the quest for a universal barcode marker for most animals (Ratnasingham and Hebert, 2007); however, as with most animals, it fails to recover many nematode taxa (Ahmed et al., 2019). The most commonly used DNA barcodes for family, genus, and sometimes species-level identification are 18S rRNA and 28S rRNA genes, targeting the hypervariable V4 – V8 and D2 – D3 regions, respectfully (Porazinska et al., 2009; Sapkota and Nicolaisen, 2015). However, amplifying 18S and 28S rRNA genes offer limited taxonomic resolution, particularly when samples contain closely related species (Powers, 2004). In contrast, mitochondrial protein coding genes, such as COX-1, offer higher resolution. The lack of comprehensive DNA libraries hinders the recovery of many taxa, thus calling for a need to expand DNA libraries regardless of whether using mitochondrial protein coding genes or rRNA genes (Porazinska et al., 2009; Powers et al., 2021). Nevertheless, both molecular techniques are capable of detecting all life stages (Derycke et al., 2007; Powers et al., 2021), an improvement from morphology, which relies mainly on vermiform and adult life stages.

Consequently, most molecular methods currently only enable accurate characterization of nematode communities to the family level. Individual nematodes can be identified to the species level using single primers (Powers, 2004), but this approach has low throughput (Gendron et al., 2024). Extensive

**Table 2** Table of commonly used and emerging primer pairs

| Target genes | Primer | Nucleotide sequence (5′-3′) | Fragment length (bp) | Reference |
|---|---|---|---|---|
| *COX-1* | JB5GED | ACCTAAACTTARWACRTARTGAAAATG | 420 | Bowles et al., 1992 |
| | JB3 | TTTTTTGGGCATCCTGAGGTTTAT | | |
| *COX-1* | JB4.5 | TAAAGAAAGAACATAATGAAAATG | 442 | Bowles et al., 1992 |
| | JB3 | TTTTTTGGGCATCCTGAGGTTTAT | | |
| *COX-1* | COIFGED | CCTTTGGGCATCCNGARGTNTAT | 450 | Ren et al., 2024 |
| | JB5GED | ACCTAAACTTARWACRTARTGAAAATG | | |
| *18S* | 18Sr2b | GCCTTGCCAGCCCGCTCAGTACAAAGGGCAGGGACGTAAT | 380 | Porazinska et al., 2009 |
| | NF1 | GCCTCCCTCGCGCCATCAGGGTGGTGCATGGCCGTTCTTAGTT | | |
| *28S* | | CTGGTGCCAGCAGCCGCGGYAA | | Machida and Knowlton, 2012 |
| | | TCCGTCAATTYCTTTAAGTT | | |
| *28S* | | GGGAAAGAAGACCCTGTTGAG | | Machida and Knowlton, 2012 |
| | | GCTTGGCBGCCACAAGCCAGTTA | | |

efforts are underway to develop metabarcoding techniques that allow for the detection of entire nematode communities, thereby generating sufficient information to use these organisms as indicators of soil health.

Results from morphological and molecular techniques can sometimes yield inconsistent findings. For example, one study reported only weak correlations between morphology-based abundance and DNA biomass metrics derived from COX-1 methods (Gendron et al., 2024). Conversely, another study using the nematode MI found that the two methods produced similar results (Herren et al., 2020). The comparability of results between morphological and molecular methods often hinges on the analytical approach used. When examining trophic groups, bacterivorous and plant-parasitic nematodes showed relatively consistent results between morphology and COX-1 detection. In contrast, omnivores and predators were only detected through morphological methods and not with COX-1 (Gendron et al., 2024). Further, when analyzing across replicates and sites, the nematode trophic groups identified through COX-1 exhibited inconsistencies. Using both molecular and morphological methods on the same sample to characterize nematode communities likely produces the most accurate results compared to one or the other method.

### 2.4.2 New, mitochondrial-based metagenomics

Mitometagenomics (mtMG) offers an alternative to shotgun sequencing, allowing for high throughput analysis while bypassing the need for taxon-specific primers and overcoming the poor phylogenetic resolution of early methods (Gendron et al., 2023). The mtMG approach can recover up to 95% of all expected species across trophic groups with a mean 99% sequence identity in mock communities *in vitro*. This method was further validated in an agricultural field study under strawberry production, where mtMG was particularly effective in detecting crop-specific plant-parasitic nematodes and evaluating species-level effects of fumigants (Gendron et al., 2023).

While mtMG is proving to increase the accuracy and depth of information on nematode taxa, the scarcity of primer libraries remains a challenge. The most common reference database for nematodes, SILVA, contains only about 5600 nematode sequences from the 18S rRNA gene (Hodda, 2022). Major efforts are underway to synthesize existing primers and curate nematode-specific databases, such as the open-source 18S-NemaBase, drawing from broader databases like SILVA that encompass all living taxa (Gattoni et al., 2023). Although mtMG provides good coverage for some taxa, like Rhabditidae (Gendron et al., 2023), even the most recently designed primers fail to amplify other important taxa, such as *Merlinius*, a globally distributed and significant plant-parasite. Specialized primers remain necessary for these taxa (Ren et al., 2024).

In addition to the scarcity of primer libraries, unexplained discrepancies between mtMG and traditional morphological techniques hinder the widespread adoption of mtMG. For example, the diversity of trophic groups at various depths varies greatly depending on whether mtMG or morphological methods are used (Gendron et al., 2024). Moreover, mtMG tends to yield a simpler and more homogenous nematode community composition across samples compared to morphology-based approaches. Notably, mtMG often fails to detect omnivores, predators, and some key plant-parasites, suggesting that further refinement is needed before this technique can be fully relied upon for accurate bioindication of soil health.

## *2.5 Limitations and future research*

Many nematologists are shifting from traditional morphological methods to molecular methods, finding advantages such as speed and cost-effectiveness, though concerns over the accuracy of these newer techniques persist. Despite these advancements, expertise in nematode taxonomy remains essential, as molecular methods should complement, not replace, morphological approaches to achieve a comprehensive understanding of nematode communities (Geisen et al., 2018). While morphological identification is labor intensive, it delivers immediate data, unlike molecular methods which involve multiple steps, including DNA extraction and sequencing, that can delay results (e.g. Powers et al., 2009). Empirical studies that evaluate differences in time and resources required for molecular and morphological methods are urgently needed (Du Preez et al., 2022).

Molecular techniques offer semi-quantitative estimates of diversity but fall short of measuring absolute abundances or biomass, key metrics needed to link nematodes to ecosystem functions and global biogeochemical cycles (van den Hoogen et al., 2019; Schenk et al., 2019). Factors like body size and amplicon size vary among nematode taxa, which biases read counts and skews taxon-specific abundances (Darby et al., 2013). Additionally, the number of rRNA gene copies correlates with genome size in nematodes, like other eukaryotes (Prokopowich et al., 2003; Bik et al., 2013). However, biomass correlates with read abundances when using 18S metabarcoding, while variation in primer binding sites for COX-1 results in low recovery, even for large nematodes like *Eudorylaimus* and *Mylonchulus* genera (Ren et al., 2024).

Molecular techniques also face challenges in distinguishing between living (active or dormant) and dead organisms, limiting their ability to quantify the active components of nematode communities. Furthermore, rRNA polymorphism is common in nematodes and can be shaped by genomic factors like mutation, genetic drift, and selection or environmental conditions. For example, rRNA copy numbers may vary with ecological factors like food availability (e.g. Bik et al., 2013). Despite these limitations, the ongoing refinement of molecular methods is improving the accuracy of nematode community identification. These advances are increasing the utility of nematodes as indicators for assessing ecosystem properties and soil health.

## 3 Developments in community analysis

### *3.1 Ecology*

Nematode community indices are limited by the incomplete understanding of many nematode taxa's ecology (du Preez et al., 2022). Ecological traits of free-living nematodes are often inferred from morphological structures, particularly mouthparts which help predict their diet and lifestyles (Yeates et al., 1993; Hodda, 2022) (Table 3). These traits are central to the colonizer-persister (*cp*) scale, a framework used to categorize nematodes based on their responses to environmental disturbances (Bongers, 1999). The *cp* scale, ranging from 1 to 5, aligns with the *r-K* type strategist scale in ecology, which describes organisms' life history strategies along a gradient from rapid colonizers (*r*-strategists) to long-term persisters (*K*-strategists). Nematodes with lower *cp* values (1–2) are early colonizers, such as Rhabditidae, whose generation time span only 2 weeks to a year. Populations of *cp1* nematodes are resilient to disturbances due to their dauer larva stage, a non-feeding phase that allows them to survive unfavorable conditions. As 'enrichment opportunists,' their

**Table 3** Colonizer-persister (*cp*) value assignments for select nematode families (Bongers and Bongers (1998) unless a different year in parentheses where 1995 or 1991 refer to Bongers et al., (1995), or Bongers et al., (1991), respectively

| *cp* value | Family |
|---|---|
| 1<br>Enrichment opportunists[b] | Rhabditidae[a], Diplogasteridae[a] (*s.l.*), Panagrolaimidae[a], Bunonematidae Myloaimidae (Bongers, 1995), Monhysteridae (FB) |
| 2<br>Basal fauna[b]<br>General opportunists | Cephalobidae, Plectidae, Monhysteridae (Bongers, 1995), Aphelenchoididae, Leptolaimidae (Bongers, (1991), Aphelenchidae (FB), Seinuridae (FB), Microlaimidae (FB) |
| 3<br>Rudimentary food web structure[b] | Teratocephalidae, Chromadoridae, Diphtherophoridae, Prismatolaimidae, Tripylidae (FB), Achromadoridae (FB) |
| 4<br>Greater food web structure[b] | Alaimidae, Mononchidae, Leptonchidae, Qudsianematidae, Dorylaimidae, Tylencholaimidae (FB) |
| 5<br>Highest food web structure[b] | Aporcelaimidae, Actinolaimidae, Thornenematidae, Belondiridae, Discolaimidae (FB) |

[a] Omit dauer larvae because they are non-feeding or inactive stages, indicating something happened in the 'past' (survival stage)
Those with FB or [b] are from Ferris and Bongers (2006).

populations can spike rapidly after a disturbance like a nutrient influx but often decline just as quickly (Freckman, 1988; Bongers et al., 1995). Nematodes with *cp*2 values are 'generalist opportunists' exist in low-resource environments and can adapt to moderate environmental fluctuations without dauer larvae. They reflect ecosystems in intermediate states, making them useful indicators of moderate disturbances. Nematodes at the higher end of the *cp* scale (3–5) include *K*-strategist omnivores and predators, longer generation times (sometimes spanning several years), larger body sizes, fewer offspring, and sensitivity to disturbance. These nematodes indicate more stable, undisturbed environments.

### *3.2 Calculations*

To compute nematode community indices, the first step is assigning a *cp* value (Table 3) and a trophic group (Yeates et al., 1993). The following is a summary of how the most common indices are calculated, providing a numerical basis and general interpretation. For more detailed explanations, including options and associated risks, refer to Neher and Darby (2006; 2009; 2025) and Schmidt et al. (2025).

There are a family of maturity indices that generally serve as an indicator of disturbance, with lower values reflecting more disturbed environments and higher values indicating less disturbance or stable environments. All versions use identical *cp* assignments and math. They vary in whether plant-parasitic

**Table 4** Maturity index variations

| Index | Trophic groups | *CP*[a] | Interpretation |
|---|---|---|---|
| MI | Free-living (i.e. bacterivores, fungivores, omnivores, predators) | 1–5 | Smaller values represent environments disturbed by nutrient enrichment or pollution-induced stress; larger values represent recovered or stable environments |
| MI25 | Free-living | 2–5 | Same as MI but avoids spikes of ephemeral *cp*1 nematodes that spike after applications of fertilizers or heavy metals; less temporal fluctuation |
| PPI | Plant-parasites | 2–5 | Associated with plant growth vigor; Sensitive to whether *cp*2 Tylenchidae nematodes are included (as plant-parasites) or excluded (as fungivores) |
| ΣMI | Free-living and plant-parasites | 1–5 | Hybrid of MI and PPI and avoids confusion of trophic assignment of Tylenchidae. Acknowledges that complete nematode communities provide integral information |
| ΣMI25 | Free-living and plant-parasites | 2–5 | Same as ΣMI except less temporal fluctuation |

[a] CP = colonizer-persister values (Bongers, 1990).
These indices all reflect the state of succession of a system whereby disturbance and its consequent enrichment effects result in a setback of succession to an earlier state.

nematodes or *cp*1 groups are included (Table 4). The original MI represents free-living taxa only and excludes plant-parasites. MI tends to decrease with increasing microbial activity and pollution-induced stress. One can complement the MI by computing a separate plant parasite index (PPI) or a version that combines both free-living and plant-parasites (ΣMI25). All variations of maturity indices are computed as a weighted mean frequency,

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

where $v_i$ = *cp* value assigned to family, $f_i$ = frequency of family *i* in sample, *n* = total number of individuals in a sample. Given that populations of *cp*1 nematodes increase rapidly but briefly after intense disturbance, index values are considered more stable if one excludes *cp*1 whether for free-living nematodes alone (MI25) or combined with plant-parasites (ΣMI25). Combining MI and PPI is logical if they both correlate to disturbance in the same direction (Neher and Campbell, 1994), which is not always the case (Bongers et al., 1997). The benefit of doing so avoids any confusion of trophic assignment.

The fungivore to bacterivore ratio (F:B) is a simple yet effective index that also reflects ecological succession (Neher and Campbell, 1994). Early stages of colonization are dominated by bacteria and their predators, followed by fungi and fungivores. The recommended method for calculating this ratio is F/(F+B), where the numerals represent actual abundance rather than proportions (Neher and Campbell, 1994). Reporting this equation in publications is essential for consistency, as other ratios like F/B or B/F are not true proportions and can lead to misinterpretation. This index can be used to infer decomposition rates and pathways but that aspect can be better portrayed with the Channel Index (CI).

The CI is analogous to the F:B ratio but is interpreted as reflecting the predominant pathway or rate of decomposition, being either bacterial or fungal (Du Preez et al., 2022). Small values of CI indicate bacterivores are prevalent, indicating fast decomposition and nutrient turnover because of readily available C sources, such as root exudates and simple sugars. Whereas large CI values indicate fungivores are relatively prevalent and indicate slower decomposition and less readily available C such as woody plant litter with high lignin content. Recent studies challenge the simplistic 'slow vs. fast' decomposition model (de Vries et al., 2016), urging caution in interpreting index values. For example, plant photosynthesis and the release of labile C as root exudates can stimulate populations of arbuscular and ectomycorrhizal fungi, which in turn may boost fungivore nematode populations (de Vries et al., 2016). The CI is calculated as follows:

$$Channel\,Index\left(CI\right) = 100 \times \frac{0.8Fu_2}{3.2Ba_1 + 0.8Fu_2}$$

where $Fu_2$, $Ba_1$, and $Ba_2$ represent the percentage of *cp*2 fungivores, *cp*1 bacterivores, and *cp*2 bacterivores, respectively.

The Enrichment Index (EI) represents the flow of resources into the food web by enrichment opportunistic bacterivore and fungivore nematodes. The EI weights are the inverse of *cp* values, focusing on the colonizers or *cp* values of 1 and 2 for bacterivores and fungivores representing the portion of food web that responds ephemerally to bursts of food availability and EI (Du Preez et al., 2022). The EI is calculated as follows:

$$Enrichment(e) = 0.8 * Fu_2 + 3.2 * Ba_1$$

where $Fu_2$ and $Ba_1$ represent the percentage of *cp*2 fungivores and *cp*1 bacterivores, respectively.

The Structural Index (SI) infers food web structure or complexity (Du Preez et al., 2022), thus analogous to the persister portion of the *cp* scale. The SI focuses on the larger *cp* values or more like the MI35 axis of *cp* triangles, a precursor to SI (de Goede et al., 1993). The SI is calculated as follows:

$$Structure(s) = 0.8 * Ca_2 + 1.8cp_3 + 3.2cp_4 + 5.0cp_5$$

where $Ca_2$ represents the percentages of carnivores (predators) with a *cp*2, and the other variables represent the percentage of free-living nematodes weighted as *cp*3, *cp*4, and *cp*5.

Graphing the enrichment trajectory as a function of the structural direction creates a graphical representation of food web condition (Ferris et al., 2001). This graphic is an evolution of the original *cp* triangle precursor, which featured three axes: *cp*1, *cp*2, and *cp*3–5 (De Goede et al., 1993). Each quadrant of the graphic represents a different state of condition (Fig. 1). Each axis is computed relative to *cp*2 bacterivores (basal opportunists):

$$Enrichment\,trajectory = 100 \times \frac{e}{e+b}$$

$$Structure\,trajectory = 100 \times \frac{s}{s+b}$$

$$Basal(b) = 0.8 * Fu_2 + 0.8 * Ba_2$$

There are a variety of other graphic illustrations that are less popular, but useful for various applications related to basic ecology or assessment of soil health across regions. For example, multivariate approaches illustrate community assembly in static (canonical correspondence analysis or redundancy analysis) or time series (principal response curves), illustrated in Fig. 2 and Fig. 3,

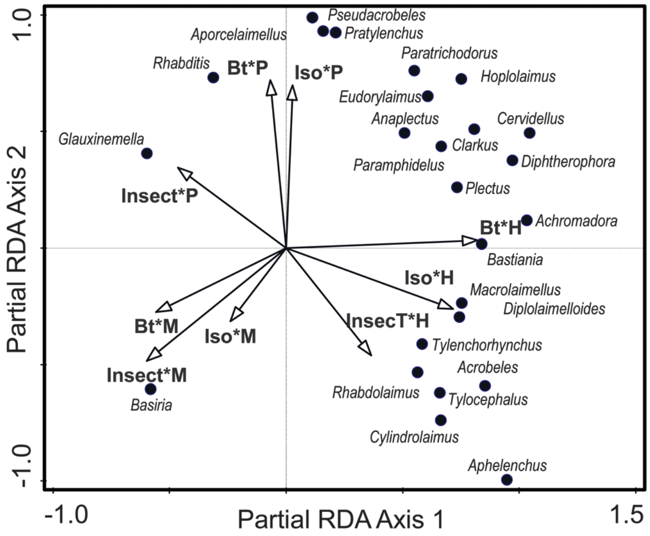


**Figure 2** The overall community structure and nematode genera varied more by seasonal phenology than corn treatment. Constrained-Partial redundancy analysis (RDA) biplot of nematode genera in soil with the two-way interaction of treatment and phenology as explanatory variables and year as a covariable treated as a block (Bt: Bt hybrid, Iso: non-Bt isoline without insecticide, Insect: non-Bt isoline with insecticide tefluthrin; P: pre-plant, M: midseason at anthesis, and H: harvest phenology). Circles represent each of the 25 of 73 nematode genera that explained the most variation. Eigenvalues (lambda) are 0.0785 (pseudo-F = 4.7, $P = 0.006$), 0.0504, 0.0223, and 0.0142 for the first (horizontal), second (vertical), third and fourth axes respectively. The first two axes represent 64.48% of the fitted variation. Sample size was 18 per treatment after averaging subsamples (3 phenology times x 2 years x 3 replicates). The analysis was performed using Canoco software, version 5 (Microcomputer Power, Ithaca, New York, United States). Reprinted from Soil Biology and Biochemistry, vol 76, Neher, D.A., Muthumbi, A.W.N. and Dively, G.P., Impact of coleopteran-active Bt corn on non-target nematode communities in soil and decomposing corn roots, pp. 127–135., Copyright (2014), with permission from Elsevier.

respectively. Cumulative Distribution Functions are tools used to illustrate the distribution of index values on a land area basis (Neher et al., 1998, Fig. 4). Co-correspondence (Neher et al., 2017) or canonical correlation (Neher and Campbell, 1994) are tools useful to examine multivariate associations between groups of plants or microbes in relationship to nematode communities. Tools such as Principal Components Analysis (Neher et al., 2012) or Factor Analysis (Martin et al., 2022) can be useful to create new and fewer variables that represent communities with plethora taxa. Computational techniques such as Artificial Neural Networks (Lek and Guegan, 1999) and null models (Gotelli, 2001) in conjunction with current multivariate statistical techniques may help to link individual taxa with habitat conditions.

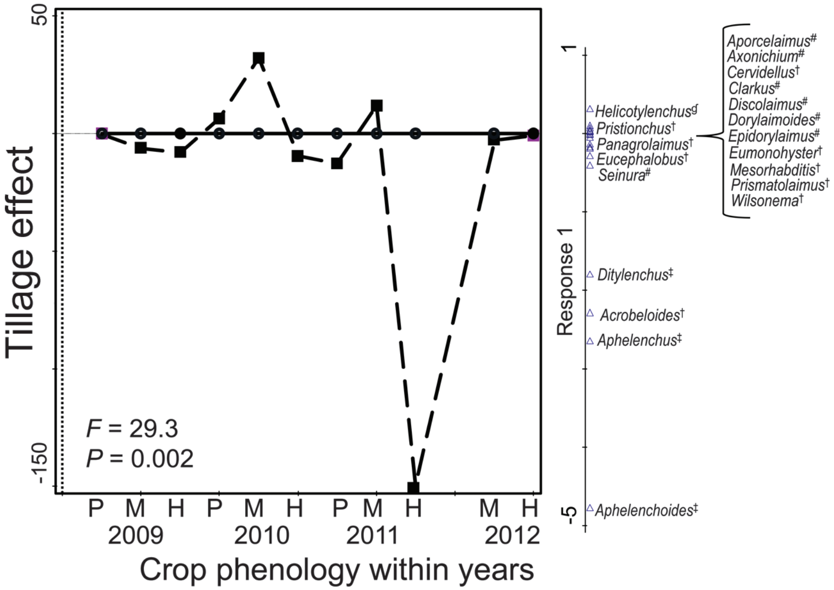


**Figure 3** Compared to no-till, conventional tillage increased abundances of *Aphelenchoides*, *Aphelenchus*, *Acrobeloides*, and *Ditylenchus*, especially at harvest in the first three years of the experiment. Principal response curve biplot of PRC (*y*-axis, till) and crop phenology (P: planting, M: midseason, H: harvest in 2009 to 2011, and M and H in 2012) are shown. Dashed line represents conventional tillage and solid line for no tillage as reference line. Genus names are coded by trophic group (†: bacterivores, ‡: fungivores, ɡ: plantparasites, #: omnivores-predators). For simplicity, the biplot is restricted to illustrate only the 20 genera that explained the most variation. Statistical significance was computed by Monte Carlo permutation of both first ordination axis and all axes together. Reprinted from Applied Soil Ecology, vol 140, Neher, D.A., Nishanthan, T., Grabau, Z.J. and Chen, S.Y., Crop rotation and tillage affect nematode communities more than biocides in monoculture soybean', pp. 89–97, Copyright (2019), with permission from Elsevier.

Standard diversity index values (e.g. Shannon, Simpson) have limited utility because they do not retain taxon identity, thus incapable of distinguishing native and introduced species. In nematode communities, diversity indices are typically calculated at the genus or trophic level. Genus is usually the finest resolution used for free-living nematodes. Trophic diversity reflects the complexity of food webs, with a maximum value equal to the number of trophic groups present. Higher values represent food webs containing more omnivores and predators that reside relatively high in the food chain (Neher and Campbell, 1994)

Neher conducted a series of experiments to evaluate the statistical reliability of a range of indices, based on their ability to detect a 10% change between two time points and 80% statistical power (1-β) where β is Type 2

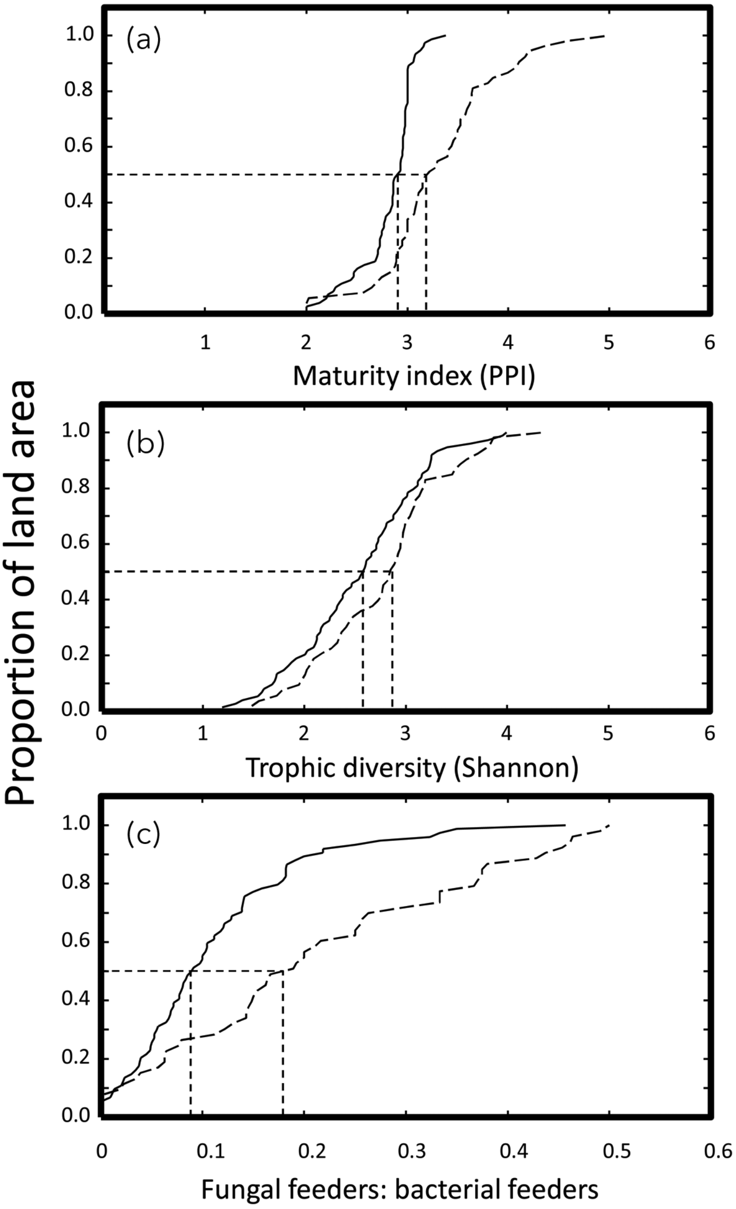


**Figure 4** Values of PPI and F:B were greater in perennial than annual crops, but there was no difference in trophic diversity values. Cumulative distribution function of the (a) maturity index of plant-feeding nematodes, (b) Shannon trophic diversity, and (c) fungal-to bacterial-feeding (= fungivores to bacterivores) nematodes for soybean (solid line) and perennial (dashed line) crops sampled in North Carolina. Dotted lines represent median values. Reprinted from Applied Soil Ecology, vol 1, Neher, D.A. and Campbell, C. L., Nematode communities and microbial biomass in soils with annual and perennial crops, pp. 17–28, Copyright (1994), with permission from Elsevier.

error (Neher et al., 1995; Neher and Campbell, 1996). Based on those studies, maturity (ecological succession) indices differentiated among sampling sites better and more efficiently than diversity indices and are more appropriate for a regional and/or national monitoring program (Neher et al., 1995). Better performing indices require fewer samples which save time and cost.

### 3.3 Sentinel taxa

Maturity indices depend on accurate assignment of *cp* values, as these classifications form the basis for understanding nematode community responses to environmental changes. Specific nematode taxa may behave or respond differently than expected based on their assigned trophic or *cp* group (Table 5). Inaccurate *cp* assignment clouds the interpretation of indices. Empirical confirmation of hypothesized ecology has potential to greatly improve the sensitivity and interpretation of community indices.

The solution is to validate these assignments and identify sentinel taxa. Potential sentinel taxa can be screened first by using meta-analysis of published data (e.g. see Table 6) to determine which taxa respond consistently to specific type(s) and magnitude of disturbance (e.g. Fiscus and Neher, 2002, Zhao and Neher, 2013). Candidate taxa can be tested empirically by natural history experiments (e.g. Li et al., 2005) or independent assessments of sensitivity to disturbance that do not rely on life history characteristics, e.g., general biomarker approach such as DNA adducts (Neher and Stürzenbaum, 2006). Adducts are a complex formed by the chemical binding of DNA to pollutants such as polycyclic aromatic hydrocarbons. If mis-repaired, they can lead to deleterious mutations. This approach was tested on *C. elegans* as a model system but needs to be extended to a full range of nematodes within and among *cp* categories.

**Table 5** Discrepancies between *CP* values and response to disturbance

| Genus | *CP* | Trophic group | Sensitive | Tolerant | Reference[a] |
|---|---|---|---|---|---|
| *Eumonhystera* | 1 | Bacterivore | √ | | 2 |
| *Acrobeles* | 2 | Bacterivore | √ | | 1 |
| *Plectus* | 2 | Bacterivore | √ | | 1 |
| *Clarkus* | 4 | Predator | | √ | 2 |
| *Enchodelus* | 4 | Omnivore | | √ | 2 |
| *Tylencholaimus* | 4 | Fungivore | | √ | 2 |
| *Aporcelaimus* | 5 | Predator | | √ | 2 |

[a] 1: Korthals et al. 1998, 2: Fiscus and Neher 2002.

**Table 6** Sentinel taxa that consistently or directly respond to specific types of disturbance

| Type of disturbance | Decrease | Increase |
|---|---|---|
| Cultivation | *Diphtherophora*[4], *Eucephalobus*[2], *Eudorylaimus*[2], *Heterocephalobus*, *Prismatolaimus*[4], *Tylenchorhynchus*[4], *Wilsonema*[2] | *Anatonchus*[2], *Chiloplacus*[2], *Clarkus*[2], *Ditylenchus*[4], *Dorylaimoides*[4], *Psilenchus*[4], *Tylencholaimellus*[2] |
| Synthetic chemical fertilizers | *Helicotylenchus*[3], *Plectus*[4] | |
| Organic fertilization | *Cephalobus*[3] | *Cruznema*[4], *Mesodorylaimus*[4], *Mesorhabditus*[4], *Nygolaimus*[4], *Odontolaimus*[2], *Prismatolaimus*[2], *Pratylenchus*[3] |
| Herbicides/ insecticides | *Alaimus*[2] | *Diphtherophora*[2], *Eucephalobus*[2], *Monhystera*[2] |
| Cadmium | *Heterocephalobus*[4] | *Diploscapter*[4], *Eucephalobus*[4], *Meloidogyne*[4], *Pratylenchus*[4], *Prismatolaimus*[4] |
| Chromium | *Aporcelaimellus*[4] | *Criconemoides*[1], *Paratylenchus*[1] |
| Copper | *Heterocephalobus*[4] | *Aphelenchoides*[4], *Chiloplacus*[1], *Pratylenchus*[1], *Seinura*[4] |
| Nickle | *Cephalobus*[4] | *Plectus*[1], *Wilsonema*[4] |
| Zinc | | *Aphelenchoides*[4], *Cephalobus*[1], *Filenchus*[4], *Malenchus*[4], *Prismatolaimus*[4], *Tylenchus*[1], *Tylenchorhynchus*[4] |

[1] Ekschmitt and Korthals (2006).
[2] Fiscus and Neher (2002).
[3] Neher and Olson (1999).
[4] Zhao and Neher (2013).

### *3.4 Limitations and future research*

Trophic assignments are relatively coarse and can be ambiguous because genera within a trophic group or family can respond differently to the same disturbance (Fiscus and Neher, 2002). Additional tools such as stable isotope analysis have proved useful to study nematode trophic interactions, especially in complex ecosystems where direct observations are difficult. For example, Darby and Neher (2012) used stable isotopes to demonstrate that nematodes in biological soil crusts were consuming biologically fixed N from cyanobacteria.

Feeding habits and reproductive potential are known to vary within families (Yeates 2003). In Tylenchidae, e.g., there are genera (e.g. *Tylenchus*) that are often considered plant associates (Yeates 2010) and other genera (*Filenchus*) that are fungivores (Okada et al., 2005). Whether Tylenchidae is grouped with plant-parasitic or free-living nematodes in maturity indices can heavily influence the results and interpretations of soil health and ecosystem disturbance.

Subsequent studies are needed to verify the sentinel status of specific taxa and to determine the geographic or ecological range of their utility. These experiments should be conducted to quantify impacts of multiple and interacting management practices on biodiversity, nutrient cycling, pest populations, and plant productivity (Neher, 2010). Once sentinel taxa are validated, one can disregard the inconsistent and more cosmopolitan taxa from index calculations. Including them just makes it more difficult to detect true changes ('signal') separate from natural variation ('noise'). The result is fewer taxa that need to be identified and indices that have greater precision and accuracy. Furthermore, this information is necessary to improve molecular kits, so they are approachable across a broader spectrum of scientists, complete with interpretation tools. There could even be designer kits based on ecosystem type and by major land resource region (Neher et al., 1998).

## 4 Relating analyses to soil health

Healthy soils typically exhibit a high abundance and biodiversity of free-living nematodes in complex food webs with long chains and feedback loops, while containing a relatively low proportion of herbivores or pathogens (Neher, 2001a). The utility of using nematodes as indicators of soil health is contingent on their ability to reflect ecological processes such as N mineralization (Neher, 2010) or decomposition. There have been modeling attempts to link flows of C and N to predict function (Hunt et al., 1987, Ferris et al. 2001), as well as experiments with empirical measures of nematode communities and function (Neher et al., 2012).

Calibration and interpretation of nematode community indices require some sort of relatively 'undisturbed' reference or healthy soil when used for environmental monitoring, conservation, and restoration. The choice of baselines for intensively managed agricultural practices on soil health should reflect the native vegetation of a region. For example, consensus is building for use of long-term pastures or managed grasslands as baselines for agricultural fields in the USA (Neher et al., 1998), South Africa (Girgan et al., 2020), and New Zealand (Yeates and Bongers, 1999). In addition, soil properties such as texture, salinity, pH, and organic matter influence nematode community assemblages. If ignored, they can inflate inherent variability and thus interfere with detection of treatment differences. However, quantifying them and treating them as covariables in statistical analysis can increase the likelihood of detecting treatment effects (Barbercheck et al., 2009; van den Hoogen et al., 2019).

### *4.1 Linking to N mineralization*

Nematodes make N available to plants by excreting excess $NH_4^+$ and indirectly by grazing decomposers including bacteria and fungi (Ingham et al., 1985; Mosdossy et al., 2004b). Under field conditions, bacterivorous and predatory nematodes are estimated to contribute (directly and indirectly) about 8% and 19% of N mineralization in conventional and integrated farming systems, respectively (Beare, 1997). These estimates were confirmed in a regional study (Neher et al., 2012). Net mineral N is regulated by a multi-trophic relationship among C:N of plant litter, microbial biomass, and the successional status of nematode communities. More immobilization occurred when *cp*3 and *cp*4 nematodes were more abundant than *cp*1 or *cp*2 nematodes regardless of abundant microbial biomass (Mosdossy et al., 2024a). Intensive disturbance decouples the contribution of available N to plants by nematodes (de Ruiter et al., 1993).

Notably, there are time lags between relative abundance of nematodes and subsequent availability of N. For example, negative correlations were found with 1, 2, or more months of lag time between nitrate or $NH_4^+$concentrations and nematode indices (e.g. Neher, 1999; Neher et al., 2012). Nematode community structure may thus depend more on the past than current environmental conditions. Understanding these time lags is crucial for developing effective sampling schedules to assess grazer-food resource relationships. Ignoring them may lead to misinterpretations, suggesting an opposite or negligible relationship.

Analyses of N mineralization in food webs typically focus on bottom-up effects. For example, abundances of bacterivores are correlated positively with N fertility (e.g. Zhang et al., 2017), as are plant-parasites (Todd, 1996). However, top-down interactions influence N mineralization and energy flux (Thakur and Geisen, 2019). For example, predators may eat bacterivores through a 'trophic cascade' (Wardle and Yeates, 1993), which explains why they are responsible for a notable amount of N mineralization. Indeed, inclusion of network topology in food web analysis can drastically affect interpretation of functional indicators, including nematode trophic groups (Potapov et al., 2022).

### *4.2 Linking to decomposition and climate change models*

Given their abundance and ubiquity, nematodes are major contributors to C cycling in soil and general ecosystem function (van den Hoogen et al., 2019). Studying nematodes in the context of the soil food web under different agroecological management systems can indicate soil C levels, plant productivity, and effects on processes that drive function (Zhang et al., 2017; Jochum and Eisenhauer, 2022). Indeed, decomposition of litter in cultivated soil can result in litter-specific fungal and nematode communities that affect bioavailable N for crops depending on whether litter was surface-mulch or incorporated into the soil (Mosdossy et al., 2024a, 2024b).

Historically, climate change models relied mostly on soil bacteria and fungi and litter chemistry to estimate carbon pools and flows and have largely ignored the contribution of soil fauna. However, soil fauna are increasingly identified as important drivers of soil organic matter formation, C mineralization, and soil health in general. Therefore, new ways of incorporating them into C models, particularly through the use of food web models are becoming apparent (Filser et al., 2016; Grandy et al., 2016; Buchkowski et al., 2022; Barreto et al., 2024). Recently, there has been an effort to map the biogeography of nematodes at a global scale as the first steps toward global biogeochemical models and predicting elemental cycling under current future climate scenarios (van den Hoogen et al., 2019).

Climate change can come in the form of increased temperature or altered precipitation leading to water stress. In desert experiments, nematodes are sensitive to increased precipitation frequency and elevated temperatures (more than protozoa) so climate change could change their relative contribution to N mineralization (Darby et al., 2011). Nematodes can survive temperatures up to 60°C as long as they are allowed to go into an anhydrobiotic state (Darby, 2008). If forced to be active because of recent precipitation, their sensitivity to elevated temperatures increases (Darby, 2008). Thus, nematodes are valuable indicators of changes in soil ecosystem processes as climate extremes become more common.

The EI, CI, SI approach can be translated from numbers to units of C creating a MF which can be used to estimate C flux within food webs (Ferris, 2010). MF defines metabolism as the sum of production and respiration derived from biomass estimates expressed in C units. Body mass is established based on weight, length, and diameter of the vermiform body shape using a formula proposed by Andrássy (1956). Body mass estimates are available through publications (e.g. Ferris et al., 2001; Neher and Weicht, 2013) that populate the Nemaplex database, available online (see Section 8). Flux can then be estimated with tools such as the R package, *fluxweb* allowing relatively easy analysis of energetic food webs (Gauzens et al., 2019). Estimates of nematode C fluxes can be linked to decomposition and climate change models and can be a powerful approach to quantify ecosystem services by accounting for C that is being transferred through trophic interactions (Barnes et al., 2018). For example, herbivory may be detected by loss of root biomass but C flux from the plant is not accounted for comprehensively compared to quantifying C assimilation and respiration by plant-parasitic nematodes (Barnes et al., 2018). Carbon and nitrogen fluxes of nematodes and the broader soil food web can be modeled using the R package, *soilfoodwebs* (Buchkowski et al., 2023). This is a true energetic food web model (sensu Moore and de Ruiter 2012), thus improving upon *fluxweb* to more accurately quantify C flux through nematodes, which can allow for more accurate predictions of ecosystem function in the face of climate change.

## 5 Impacts of particular agronomic practices (crops/soil and water management)

Nematode community indices are sensitive to management practices (Neher and Olson, 1999). For example, nematode MF fluctuate based on management techniques (e.g. litter mulching, tillage) and seasonal changes (Ferris, 2010), making them strong indicators of soil organic C, microbial biomass, and respiration, especially in agricultural systems (Schmidt et al., 2020; Luo et al., 2021). Bacterivorous nematode MF tends to increase with litter amendments, particularly in fall, reflecting accelerated C flux (Ewald et al., 2020). In contrast, the MF of fungivorous nematodes remains stable regardless of litter amendments, indicating a constant C flux (Ewald et al., 2020). However, organic amendments and mineral fertilizers can push the soil food web toward earlier successional stages (Ferris and Bongers, 2006). Greater diversity and abundance often correspond to greater C utilization and more efficient energy transfer within the nematode food web (Gauzens et al., 2019).

There are two major types of disturbances in agricultural ecosystems, physical and chemical, which have contrasting effects on the soil food web.

### *5.1 Physical*

Cultivation is more damaging to the soil food web than chemical applications (Neher, 2010; Bongiorno et al., 2019). Mechanical tillage shreds organic matter, increasing its surface area for colonization by decomposer bacteria and fungi, which accelerates decomposition. Decomposition rates are 1–1.4 times faster in tilled than in no-till systems, releasing nutrients more quickly and decreasing food web complexity (Beare, 1997). Tillage also disrupts soil structure and faunal habitats, decreasing populations of fungi and fungivores (Neher, 2010). In contrast, no-till practices support later ecological succession and greater trophic diversity within nematode communities (Neher, 2010).

In a case study, no-tillage and a monoculture of susceptible soybeans developed natural suppression to soybean cyst nematode (*Heterodera glycines*) (Chen, 2007). However, once the soil was cultivated or rotated to corn, this suppression was lost (Neher et al., 2019, Fig. 3). Mechanisms of suppression have been attributed to nematode-trapping fungi (*Hirsutella rhossiliensis*) (Chen, 2007) and disruption of a free-living nematode community containing a common core of bacterivores (*Wilsonema*) and omnivore-predators (*Aporcelaimus* or *Aporcelaimum*, *Clarkus*, *Dorylamoides*, *Eudorylaimus*, and *Paraxonchium*) (Neher et al., 2019). Traditionally, monocultures of a susceptible host typically promote disease. In response, rotation to a non-host is recommended. Rotation may decrease populations of the plant-parasitic nematode, but it also reduced food web complexity (trophic diversity, SI), and increased values of EI compared to a naturally suppressive soil (Neher et al., 2019). Relatively later stages of succession in the nematode community are congruent with hypotheses about soils with high organic matter content (e.g. 6.64 ± 0.97% in Neher et al., 2019) supporting high densities or diversities of soil microbes and most notably, high abundance and diversity of antagonistic populations (Weller et al., 2002; Bonanomi et al., 2010). Numerous studies have found that organic matter correlates directly with increased numbers of nematode antagonists (Oka, 2010).

### *5.2 Chemical*

Chemicals can be in the form of nutrients (e.g. synthetic fertilizers, organic matter) or pollutants (e.g. pesticides, metals, petroleum products). Application of either can be disruptive to soil food webs and result in a rapid increase in bacteria and *cp*1 bacterivorous nematodes, thus increasing EI values (De Goede et al., 1993; Ferris et al., 2001). This enrichment affect is short-lived (e.g. Ettema and Bongers, 1993; Yeates and Bird, 1994), having minimal impact on nematode communities over the long term (Dmowska and Ilieva, 1995).

The type of fertilizer and pest management strategies are factors that differentiate conventional from organic agriculture, but these differences might not be reflected in nematode indices if the frequencies of disturbance are similar in both management systems. For example, similar values of MI25, ΣMI, and F:B were observed between soils managed organically or conventionally with cultivation and annual crops (Neher, 1999). Another study also observed similar proportions of *cp*1, *cp*4, and *cp*5 in organic or conventional cropping systems (Yeates et al., 1997).

Understanding the individual and combined effects of cultivation and chemical applications on nematode communities is essential, as these factors often produce contradictory results. In an 18-year farming trial in southeast Nebraska, chemical treatments were organized into a disturbance hierarchy: O (manure only), F (mineral fertilizer only), HF (mineral fertilizer plus herbicides), and HFI (mineral fertilizer, herbicides, and insecticides) (Neher and Olson, 1999). All fields were cultivated but varied in organic inputs and pesticide use. The relative abundance of bacterivores, plant-parasites, and PPI were greater and MI values smaller after organic than synthetic fertilizer applications. It appears that herbicide and insecticide applications shift nematode communities to earlier successional stages more than cultivation or organic amendments do. This study highlights the distinct effects of pesticides and nutrient sources on nematode community composition.

### *5.3 Limitations and future research*

Caution is needed when using MF as indicators of ecosystem function, as online databases rely on the size and biomass of adult females, without accounting for age-structured populations of variability in size or ecology. This is true for the user-friendly online tool, The Nematode Indicator Joint Analysis (NINJA), for calculating various nematode community indices (Sieriebriennikov et al., 2014). However, NINJA-computed MF reflects the potential lifetime C utilization, not the C utilization or estimate of ecosystem function at one point in time. Furthermore, Nemaplex is the most comprehensive database available to retrieve biometrics data as well as respiration and production rates and other life history traits, all of which are used to model C flux. Yet, this database contains a limited number of species.

Nematodes are excellent candidates for monitoring energy flux dynamics because they occupy multiple trophic levels (Moore and De Ruiter, 2012). Yet community and ecosystem ecology have not fully embraced the use of nematodes as indicators of energy flux, which links food web theory with biodiversity-ecosystem functioning theory (Jochum et al., 2021). Embracing ubiquitousness, diversity, and positioning in the soil food web by nematodes could lead to a more comprehensive understanding of energy flux in agroecosystems, thus informing best practices for soil health.

## 6 Future trends

Metabarcoding is an exciting new technique showing promise of solving the conundrum of not currently having barcodes or universal markers for free-living nematodes. Tailoring the technique to nematodes could overcome barriers that prevent nematodes from being utilized widely in soil health programs and monitoring programs.

Empirical confirmation of hypothesized ecology has potential to greatly improve the sensitivity and interpretation of community indices. For example, reports of taxa that do not respond to disturbance as predicted necessitate empirical testing of *cp* assignments. Metadata analyses of existing data identify potential sentinel taxa that can be verified by natural history experiments and general biomarkers.

The multitrophic nature of nematode communities make them good candidates for testing ecological theory initially limited to above-ground systems. For example, they also make good candidates for energy flux models which can link food web theory with biodiversity-ecosystem functioning theory (Jochum et al., 2021).

## 7 Summary

The concept of nematodes as indicators of soil health represents a major shift in emphasis in the science of nematology. Nematodes make good indicators because they reside a trophic level above microbes in the food chain and integrate physical, chemical, and biological properties. The best performing indices are based on successional maturity and food web complexity. There is a paucity of laboratories that can morphologically identify free-living nematodes at least to family. Therefore, many nematologists are moving away from traditional morphological methods and toward molecular methods citing benefits like speed and cost-effectiveness, but some important challenges need to be overcome before those methods are readily available. For example, we need metabarcoding that detects all nematode genera and aligns with morphological identification and ecological traits. The precision of the indices would be improved with empirical verification to generate a proven set of sentinel taxa. Community indices are useful as indicators of soil health because they are responsive to management practices, including cultivation, fertilization, and pest management practices. Beyond applications in soil health, nematode communities can make good test subjects for applications including ecological theory and global climate change models.

## 8 Where to look for further information

The following articles provide a good overview of the subject:

- Du Preez, G., et al. (2022), ‘Nematode-based indices in soil ecology: application, utility, and future directions’, *Soil Biol. Biochem.*, 169, 108640. https://doi.org/10.1016/j.soilbio.2022.108640.
- Ferris, H. (1993), ‘New frontiers in nematode ecology’, *J. Nematol.*, 25(2), 374–382.
- Hodda, M. (2022), ‘Phylum nematoda: feeding habits for all valid genera using a new, universal scheme encompassing the entire phylum, with descriptions of morphological characteristics of the stoma, a key, and discussion of the evidence for trophic relationships’, *Zootaxa*, 5114(1), 318–451. https://doi.org/10.11646/zootaxa.5114.1.3.
- Kakouli-Duarte, T. Korthals G.W., Sánchez Moreno, S., du Preez, G. and de Goede, R. (2025) *Nematodes as Environmental Indicators: From Theory to Practice*, 2nd edition. CABI, Wallingford, UK.
- Neher, D.A. (1999), ‘Soil community composition and ecosystem processes: comparing agricultural ecosystems with natural ecosystems’, *Agroforest. Syst.,* 45, 159–185. https://doi.org/10.1023/A:1006299100678.
- Neher, D.A. (2001a), ‘Role of nematodes in soil health and their use as indicators’, *J. Nematol.,* 33(4), 161–168.
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*Sustainability: Developing Practical Strategies*. CRC Press, Boca Raton, FL, pp. 105–120.

- Neher, D.A. (2010), 'Ecology of plant and free-living nematodes in natural and agricultural soil', *Annu. Rev. Phytopathol.,* 48(1), 371–394. https://doi.org/10.1146/annurev-phyto-073009-114439.
- Neher, D.A. and Powers, T.O. (2023), 'Nematodes', *Encyclopedia of Soils in the Environment*, 11, 105–111. https://doi.org/10.1016/B978-0-12-822974-3.00038-0.
- Neher, D.A. (2023), 'Moving up within the food web: protists, nematodes and other microfauna'. In N. Uphoff and J. Thies (Eds), *Biological Approaches to Regenerative and Resilient Soil Systems*, 2nd edition. CRC, Boca Raton, FL, pp. 157–168.
- Yeates, G.W. (2010), 'Nematode in ecological webs'. In Wiley (Ed), *Encyclopedia of Life Sciences.* John Wiley & Sons, Chichester, UK. https://doi.org/10.1002/9780470015902.a0021913.

Following are seminal articles or books which have shaped the subject:

- Bongers T. (1990), 'The maturity index: an ecological measure of environmental disturbance based on nematode species composition', *Oecologia,* 83(1), 14–19. https://doi.org/10.1007/BF00324627.
- Ettema C.H. and Bongers T. (1993), 'Characterization of nematode colonization and succession in disturbed soil using the maturity index', *Biol. Fert. Soils* 16, 79–85. https://doi.org/10.1007/Bf00369407.
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- Yeates G.W., et al. (1993), 'Feeding habits in soil nematode families and genera—an outline for soil ecologists', *J. Nematol.,* 25(3), 315–331.

References that provide useful methods are as follows:

- Buchkowski, R.W., et al. (2023), 'soilfoodwebs: an R package for analyzing and simulating nutrient fluxes through food webs', *Eur. J. Soil Biol.*, 119, 103556. https://doi.org/10.1016/j.ejsobi.2023.103556.
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- Van Bezooijen, J. (2006), *Methods and Techniques for Nematology*. Wageningen University, The Netherlands. https://www.scribd.com/document/234069625/Methods-and-TechniquesforNematology; accessed October223, 2025.

Key journals are as follows:

- Applied Soil Ecology
- Soil Biology and Biochemistry
- European Journal of Soil Biology
- Pedobiologia
- Journal of Nematology, https://sciendo.com/journal/JOFNEM
- Nematology (Brill), https://brill.com/view/journals/nemy/nemy-overview.xml?language=en
- Nematropica, https://journals.flvc.org/nematropica

Key conferences are as follows:

- Soil Ecology Society, https://www.soilecologysociety.com/
- European Society of Nematologists, https://www.esn-online.org/
- ONTA: Organization of Nematologists of Tropical Americas, https://ontaweb.org/
- SON: Society of Nematologists, https://www.nematologists.org/
- Soil Ecology section of Ecological Society of America, https://www.esa.org/soilecology/home/

Major international research projects are as follows:

- Global Soil Biodiversity Initiative (https://www.globalsoilbiodiversity.org/)
- NETSOB, International Network on Soil Biodiversity (van der Putten et al., 2023)
- SoilBON (Guerra et al., 2021)
  - https://soilbonfoodweb.org/ – protocols & Manuals https://soilbonfoodweb.org/protocols-and-manuals/
  - https://soilbonfoodweb.org/wp-content/uploads/2024/02/SBF-Team-Nematode-extraction_v1.6.pdf
  - https://youtu.be/8Ennbh0KkUQ?si=-I4TV7sapPuRzMuC

Top research center to keep up with research trends are as follows:

- Ghent University – Belgium
- Wageningen University – The Netherlands
- University of Florida – Gainesville, USA
- University of California – Davis, USA
- University of California – Riverside, USA

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